

Article Integrated Control of Scales on Highbush Blueberry in Poland

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Abstract: In the past decade, the development of highbush blueberry production in Poland has been followed by the occurrence of new pests in the plantations, including scales. Since both the assessment of the populations of natural enemies present in a territory and the knowledge of the scale species present in the crop are crucial for the correct application of IPM strategies, a study was carried out to address these aspects and evaluate the efficacy of several active substances in controlling Parthenolecanium spp. in several highbush blueberry plantations. Specimens of adult larvae collected on several plantations were phylogenetically closely linked to two species, P. corni and P. fletcheri. However, considering the ecology and behavior of these species, it was concluded that the pest population was more likely to belong to P. corni. Analyzing the scale parasitoids' community present in the different locations, it emerged that it was quite diversified, including species affecting both the initial and adult biological phases of the scales, with differences also in the population size and diversity, including both general or specialized parasitoids and predators. The different active substances tested in the efficacy trials, which included both synthetic and bio-based compounds, were suitable for controlling the scale infestation. However, the different efficacy observed between them, depending on season and location, could be interpreted taking into consideration the initial level of infestation. It is concluded that applying an IPM strategy that combines agronomical practices with the application of insecticides with different mechanisms of action, attentive to the benefit of protecting natural enemies, can result in satisfactory control of *P. corni* in highbush blueberry plantations.

Keywords: IPM; natural enemies; Parthenolecanium spp.; phylogenetic analysis; Vaccinium corymbosum

1. Introduction

Highbush blueberry production in Poland has been rapidly developed in the past two decades, allowing the country to become the second largest producer in the EU after Spain, with about 24% of the total EU harvest [1]. This production trend has been followed by the occurrence of "new" pests in the plantations across the country [2,3], including *Parthenolecanium* spp. (Hemiptera: Coccoidea) [4]. Among *Parthenolecanium* spp., several species have been recorded in Poland [5], such as the European fruit lecanium (*P. corni* [Bouchè]), a scale species that is damaging crops either directly or indirectly [6], normally observed on a wide range of fruit host plants (EPPO Global Database, https://gd.eppo.int/taxon/LECACO, accessed on 10 May 2023). The control of *Parthenolecanium* spp. is made difficult by their biological cycle and phenology, requiring that insecticide applications target crawler (mobile instar) emergence to achieve an effective reduction of the scale insect population [7].

Integrated Pest Management (IPM), which is compulsorily applied in the European Union since January 2014, can benefit from the populations of natural enemies present in a territory [8,9]. Important natural enemies of soft scales include predators, particularly members of the Anthribidae and Coccinellidae (Coleoptera) [10], as well as parasitoids, mainly belonging to the hymenopterous family Encyrtidae [11]. Outbreaks of scale insects



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have been explained by the reduction of natural enemies [12] and eventually as an effect of pesticide application [13].

Scale insects management is complicated by difficulties in their morphological identification [14]. This condition could result in less effective control by using methods or products not fully appropriate for the species present in the crop. However, the recent development of DNA barcoding techniques has also provided useful information for classifying scale insects [15,16]. Recently, specimens of a soft-scale insect were found on *Vaccinium corymbosum* cultivar 'Bluecrop' in Poland, which seemed to be close to *Parthenolecanium corni* (Bouché) based on their morphology but showed significant differences in their life cycle and in their settlement sites.

Therefore, a study was performed to (i) evaluate the efficacy in the control of *Parthenolecanium* spp. under field conditions in several locations and seasons with products having different mechanisms of action; (ii) assess the composition of natural enemies' populations in these orchards; and (iii) determine whether specimens collected in these orchards should be considered congeneric with *P. corni* or whether they formed a separated (undescribed) species.

2. Materials and Methods

2.1. Taxonomic Identification of Scales Sampled from Blueberry Plantations

2.1.1. DNA Extraction, Amplification, and Sequencing

Young adult females of *Parthenolecanium* sp. were collected on *Vaccinium corymbosum* cv. Bluecrop in various locations (Table 1) and stored at -20 °C until analysis. The DNA extraction was performed using the CTAB method [17], with slight modifications. The DNA concentration was estimated on a 1.5% agarose gel and compared with GeneRuler 100 bp DNA Ladder Plus (Thermo Fisher Scientific, Waltham, MA, USA). Next, the DNA samples were diluted to give a concentration of 20 ng/µL and stored at -20 °C for downstream analyses.

To study the phylogenetic relationships among specimens, a fragment of DNA containing the mitochondrial cytochrome oxidase subunit I (COI) gene was amplified. The PCR amplifications were performed in a total volume of 25 μ L containing: 20 ng/ μ L of template DNA, 2.5 μ L 10 × Buffer Taq (750 mM Tris HCl pH 8.8; 200 mM (NH₄)₂SO₄, 0.1% Tween 20) (Thermo Fisher Scientific Waltham, MA, USA), MgCl₂ 1500 μ M, dNTP mix 800 μ M, 0.2 μ M of each primer, and 1.0 U Taq polymerase (Fermentas AB, Vilnius, Lithuania). The COI (mtDNA) was amplified with the primer pairs PcoF1 and HCO (Table 1). PCR conditions for COI were set as follows: an initial denaturation at 94 °C for 2 min, followed by 40 cycles of denaturation, annealing, and extension, and a final extension step at 72 °C for 10 min. For the COI amplification, the 35 cycles consisted of 30 s at 95 °C, 50 s at 50 °C, and 1 min at 72 °C. A negative control was used for PCR reactions.

PCR products with an addition of fluorescent dye were separated electrophoretically in a 1.5% agarose gel at 80 V for 1.5 h in $1 \times$ TBE buffer containing 0.01% EtBr and visualized under UV light. After checking and determining the size of the resulting PCR product, the DNA was subjected to purification using an agarose gel of low melting point. The sequencing was conducted by Genomed S.A. (Warsaw, Poland) using the PCR primers together with a Big Dye[®] Terminator Cycle Sequencing Kit V. 3.1 of Applied Biosystems (Life Technologies, Warsaw, Poland) and separated using a capillary sequencer 3730XL DNA Analyzer.

2.1.2. DNA Sequence Alignment and Phylogenetic Analysis

DNA COI fragments of five *Parthenolecanium* species (*P. corni*, *P. fletcheri*, *P. persicae*, *P. pomeranicum*, and *P. pruinosum*) (Table 2) were included in the sequence alignment to compare genetic diversity with the specimens collected on *V. corymbosum*. Sequences were assembled and edited using BioEdit v. 7.2.5. Related DNA sequences of COI were compared using the BLAST function of GenBank. Multiple sequence alignments were performed with Clustal W using BioEdit v. 7.2.5 [18].

Scale Insect Species	Host	Locality	Isolate	GenBank No. Sequences
Deuthanalaaninn aani (Darrah i)	No data	Chile	24245	KY085297
Partnenoiecanium corni (bouche)	No data	China	S3_499	KP189846
Dauthan al acquirum flat al ani	No data	No data	PARFLE	MZ 567176
(Cockerell)	Platycladus orientalis	Chungcheongbuk-do, Korea		MK543920
Parthenolecanium persicae	No data	Australia	WIL	KY362203
(Fabricius)	No data	China	S4_079c	KP189853
Parthenolecanium pomeranicum (Kawecki)	<i>Taxus</i> sp.	Poland	PAR_POM	MN603162
Parthenolecanium pruinosum	<i>Vitis</i> sp. L.	Australia	Mudgee_2a	KC784924
(Coquillett)	<i>Vitis</i> sp. L.	Australia	Gumeracha_b	KC784923
Parthenolecanium sp.	Vaccinium corymbosum 'Bluecrop'	Piskórka, Poland	CBr3	ON899817
	Vaccinium corymbosum 'Bluecrop'	Piskórka, Poland	CBr5	ON899818
	Vaccinium corymbosum 'Bluecrop'	Piskórka, Poland	CBr9	ON899819
	Vaccinium corymbosum 'Bluecrop'	Piskórka, Poland	CBr10	ON899820
	Vaccinium corymbosum 'Bluecrop'	Maciejowice, Poland	CBm11	ON899821
	Vaccinium corymbosum 'Bluecrop'	Maciejowice, Poland	CBm13	ON899822
	Vaccinium corymbosum 'Bluecrop'	Maciejowice, Poland	CBm14	ON899823

Table 1. Samples of Parthenolecanium species used in the molecular analyses.

Table 2. Primers and PCR protocols used.

Gene	Primer	F or R	Primer Sequence 5' to 3'	References
COL	PcoF1	F	CCTTCAACTAATCATAAAAATATYAG	[19]
COI —	HCO	R	TAAACTTCAGGGTGACCAAAAAATCA	[20]

2.2. Assessment of Parthenolecanium spp. Parasites

Parasitism of *Parthenolecanium* spp. Larvae was determined on sampled shoots (three shoots from four sites in each plantation) that showed the presence of scale females, which were collected during three observation periods (April, May, and June). The healthy females and those with clear parasite damage or parasites inside were separately counted.

Specimens of parasites/parasitoids were obtained by keeping the sampled shoots with the female scales in isolators until the hatched parasites were visible and systematically collected. The specimens were then sent to the Natural History Museum of London for identification.

2.3. Trials of Parthenolecanium sp. Control on Blueberry Plantations

Trials testing different products and strategies for the control of *Parthenolecanium* spp. Were conducted in 2017–2019 on several plantations of highbush blueberry cv. Bluecrop located in four locations in Mazovian Voievodship (Central Poland). The application of control products was carried out during the period of the overwintering larvae's migration.

The experimental field design in each trial consisted of four replications arranged in randomized blocks. Each plot (replication) covered 52.55 m² (three rows of 15 m length, for a total of 45 plants). Tested and reference products having different mechanisms of action (Table 3) were applied with a motorized knapsack sprayer ("Stihl SR 420") with a spray volume of 750 L/ha.

Active Substance	Product	Mechanism of Action According to IRAC	Activity on the Pest
Camelina oil (Camelina sativa (L.) Crantz)	Emulpar 940 EC	N/A	Mechanical action and suffocation
Spinosad	SpinTor 240 EC	Nicotinic acetylcholine receptor (nAChR) allosteric modulators (Site I)	Contact and stomach poisoning, and ovicidal
Silicon polymers	Insect Control	N/A	Mechanical action and suffocation.
Silicon polymers	Siltac EC	N/A	Mechanical action, suffocation
Acetamipryd	Mospilan 20 SP Stonkat 20 SP	Nicotinic acetylcholine receptor (nAChR) competitive modulators	Contact and stomach poisoning
Flonicamid	Tepekki 50 WG	Chordotonal organ and Modulators—undefined target sites	Systemic
Spirotetramat	Movento 100 SC	Inhibitors of acetyl CoA carboxylase	Systemic

 Table 3. List of active substances utilized in the field trials for the control of highbush blueberry scales.

The scale population density was estimated just before the treatment and approximately 2–3 weeks after spraying. The number of alive scale larvae was counted under a stereoscopic microscope on three stems (each 30 cm long) taken randomly from each plot/replication on each counting date.

2.4. Statistical Analysis

2.4.1. Phylogenetic Analysis

The phylogenetic analysis was performed with MEGA11 software [21], and the evolutionary history was inferred using the Maximum Likelihood method based on the Tamura-Nei model [22]. The bootstrap consensus tree was inferred from 1000 replicates [23]. Branches corresponding to partitions reproduced in less than 50% of bootstrap replicates collapsed. The initial tree(s) for the heuristic search were obtained automatically by applying the Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model and then selecting the topology with the superior log likelihood value. This analysis involved 16 nucleotide sequences. Codon positions included were 1st, 2nd, 3rd, and noncoding. All positions containing gaps and missing data were discarded. There were a total of 378 positions in the final dataset. The nucleotide sequences were compared with sequences collected in the NCBI GenBank databases using BLAST software [24] (http://www.ncbi.nlm.nih.gov/BLAST/, accessed on 15 January 2023).

The Bayesian inference analysis was carried out using the Markov Chain Monte Carlo (MCMC) algorithm with the mrbayes program ver. 3.2.2 [25], using Biolinux 8.0 as OS, and applying the default parameters.

2.4.2. Field Data Analysis

The data from field trials were analyzed by ANOVA performed on values transformed by log(x) + 1 in order to assure a normal distribution. The significance of differences between means was assessed with the Tukey multiple range test at $p \le 0.05$ using the package Statistica v.6.1. The value of actual mortality was calculated according to Abbott's formula [26].

3. Results

3.1. Taxonomic Identification of Highbush Blueberry Scale Specimens

A phylogenetic tree was constructed based on the sequence of the COI gene fragments obtained from the *Parthenolecanium* sp. specimens collected in the study and the sequences of the genus *Parthenolecanium* deposited in GenBank (Figure 1). Two main clades can be distinguished in the trees obtained with both bootstrap and Bayesian analyses. The first clade is formed by the species *P. prunoisum*, while the second is formed by *P. pomeraniucum*, *P. persicae*, *P. fletcheri*, and *P. corni*, including the specimens from *V. corymbosum* as well. Two branches can be distinguished within the second clade, one of which contains *P. pomeranicum*. The specimens collected from blueberry plants were located on the second branch, positioned close to *P. fletcheri* and *P. corni*, indicating a close relationship between these taxa.



Figure 1. Dendrograms showing the phylogenetic relationship of the adult females scale specimens collected on *V. corymbosum* with isolates of different *Parthenolecanium* species based on the analysis of molecular data by the maximum likelihood method (**A**) and Bayesian inference analysis (**B**). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test is shown below the branches.

3.2. Level of Parasitism of Parthenolecanium sp. under Field Conditions

The number of larvae found during the assessment was, on average, about 240 per shoot. The dynamic of the population and the percentage of parasitized larvae varied depending on the location (Figure 2). However, a clear difference in the dynamic was observed comparing the plantation in Piskórka with the other three: the increase (more than doubled) in the population size in Piskórka from April to May was paralleled by an increase of the parasitized larvae, while in the other two locations the population size was either steady (Rokotów) or decreased (Prazmów) in a similar pattern for healthy or parasitized larvae (Figure 2). The Piskórka plantation was also characterized by having almost 20% parasitized larvae during the whole season, ranging from 15% up to six times that of the other three plantations throughout the whole period of assessment (Figure 3).

3.3. Identification of Parthenolecanium spp. Parasites

The number of parasitic species or genera found in the four plantations ranged from four to nine (Table 4). The plantation located in Prażmów was characterized by the highest biodiversity in this respect, also including a predator (*Anthribidae* spp.) not found in other locations. On the other hand, three species (*Blastothrix brittanica, Coccophagus lycimnia,* and *Encyrtus infelix*) were common to all sites, and two (*Syrphophagus taeniatus* and *Metaphycus insidiosus*) were found in three sites. One genus, *Scelionidae* spp., was found only in one location (Jakubów).



Figure 2. Dynamic impact of natural parasites on the infestation of highbush blueberry plantations by *Parthenolecanium* spp.: number of healthy (**A**) and parasitized (**B**) larvae. Bars represent SD, n = 12.



Figure 3. Percent of parasitized *Parthenolecanium* larvae in the sampled population. Mean \pm SD, n = 12. Different letters at each time point represent the statistical difference for $p \le 0.05$.

3.4. Control of Parthenolecanium sp. on Blueberry Plantations

The infestation by the scales varied depending on the season and the location, averaging from a few individuals to some hundreds (Tables 5–7). However, interestingly, the same location, Rokotów, recorded both the lowest (on average 2.5 individuals per shoot) and the highest (on average about 190 individuals per shoot) infestations in 2018 and 2019, respectively.

Barasita Spesies or Conora	Location of the Plantation				
rarasite Species of Genera –	Jakubów	Piskórka	Prażmów	Rokotów	
Blastothrix spp. including B. brittanica (Girault, 1917)	Х	Х	X *	Х	
Coccophagus lycimnia (Walker, 1839)	Х	Х	Х	Х	
Encyrtus infelix (Embleton, 1902)	Х	Х	Х	Х	
<i>Metaphycus</i> spp. including <i>M. insidiosus</i> (Mercet, 1921)		Х	X *	Х	
<i>Syrphophagus</i> spp. including <i>S. taeniatus</i> (Förster, 1861)		X *	Х	Х	
Anthribidae spp.			Х		
Scelionidae spp.	Х				
Total	4	5	6	5	

Table 4. List of the parasites/parasitoids identified from larvae of *Parthenolecanium* spp. collected from different highbush blueberry plantations.

* Location where specimens classified both at species and genus levels were found.

Table 5. Effect of different products on the control of *Parthenolecanium* sp. on different blueberry plantations in 2017. Mean \pm SD, n = 12. Different letters in columns represent statistical differences for $p \leq 0.05$.

Treatment	Dose Applied	Living Larvae per Shoot (n)		
	_	Piskórka	Maciejowice	
Before treatment	-	9.3 ± 26.9	10.0 ± 8.5	
Control	-	$7.9\pm2.2~\mathrm{b}$	$34.3\pm16.6~\text{b}$	
Spinosad	0.4 L/ha	$5.0\pm 6.7~\mathrm{b}$	17.3 ± 17.3 a	
Silicon polymers	0.2%	$1.7\pm1.1~\mathrm{ab}$	$11.0\pm15.4~\mathrm{a}$	
Spirotetramat	0.75 L/ha	1.0 ± 2.1 a	$12.7\pm11.4~\mathrm{a}$	

Table 6. Effect of different products on the control of *Parthenolecanium* sp. on different blueberry plantations in 2018. Mean \pm SD, n = 12. Different letters in columns represent statistical differences for $p \leq 0.05$.

Treatment	Dose Applied	Living Larvae per Shoot (n)				
ireament		Rokotów	Piskórka I	Piskórka II (Tunel)	Prażmów	
Before treatment	_	2.5 ± 3.0	19.5 ± 18.2	20.7 ± 14.2	79.2 ± 33.2	
Control	_	$1.4\pm1.3~\text{b}$	$9.3\pm5.1b$	$4.7\pm3.1~\text{b}$	$15.2\pm\!6.6\mathrm{b}$	
Camelina oil	1.2%	$0.6\pm0.8~ab$	$1.0\pm2.1~\mathrm{a}$	1.2 ± 3.5 a	$6.6\pm13.5~ab$	
Acetamipryd	0.2 kg/ha	$0.5\pm1.5~\text{ab}$	$1.3\pm6.4a$	$1.3\pm5.8~\mathrm{a}$	$8.7\pm\!20.9$ ab	
Flonicamid	0.14 kg/ha	$1.6\pm4.2b$	$1.4\pm1.5~\mathrm{a}$	$1.5\pm7.8~\mathrm{a}$	$4.7\pm9.3~ab$	
Spirotetramat	0.75 L/ha	$0.0\pm0.0~\mathrm{a}$	$0.9\pm3.7~\mathrm{a}$	$0.7\pm2.9~\mathrm{a}$	$2.1\pm6.8~\text{a}$	

Treatment	Dose Applied	Living Larvae per Shoot (n)		
		Piskórka	Rokotów	
Before treatment	-	187.5 ± 36.7	336.0 ± 32.0	
Control	-	$15.5\pm7.4~\mathrm{c}$	$248.2\pm30.4~\mathrm{c}$	
Spinosad	0.4 L/ha	$6.1\pm2.9~{ m bc}$	5.5 ± 9.1 a	
Silicon polymers	0.15%	$4.8\pm7.8~{ m bc}$	$17.3\pm20.6~\mathrm{b}$	
Acetamipryd	0.2 L/ha	$1.2\pm2.9~\mathrm{a}$	$8.0\pm12.5~\mathrm{ab}$	
Flonicamid	0.14 kg/ha	$6.7\pm5.4~\mathrm{bc}$	$12.9\pm9.1~ab$	

Table 7. Effect of different products on the control of *Parthenolecanium* sp. on different blueberry plantations in 2019. Mean \pm SD, n = 12. Different letters in columns represent statistical differences for $p \le 0.05$.

The different products applied, expressing different mechanisms of action, including some allowed in organic farming (i.e., camelina oil and spinosad), were in general effective in reducing the number of living larvae (Figure 4), but their efficacy was also influenced by the location, season, and initial level of infestation (Tables 5–7). For example, in the case of the synthetic active substances, during the three years of trials at the Piskórka plantation, only spirotetramat consistently reduced the number of living larvae significantly compared to the control, reaching an efficacy of 85–100%. Flonicamid or acetamiprid instead had a more variable effect, both when comparing the same location across the years (Piskórka) or confronting different locations (Piskórka and Rokotów) or the initial level of infestation (Tables 5–7), resulting in a lower efficacy than spirotetramet (Figure 4). The products allowed in organic farming (camelina oil and spinosad), as well as those based on silicon polymers, also had a variable effect, influenced by the same factors as the synthetic substances (Tables 5–7). However, in some cases, their efficacy was similar to the synthetic molecules, even for the protected crops, thus showing good control potential.



■ Movento 100 SC ■ SpinTor 240 SC ■ InsectControl ■ Mospilan 20 SP ■ Emulpar 940 EC ■ Teppeki 50 WG ■ Siltac EC

Figure 4. Efficacy rate of the different active substances in controlling larvae of *Parthenolecanium* spp. on different blueberry plantations in 2017–2019. Mean \pm SD, n = 12.

4. Discussion

4.1. Phylogenetic Identification of the Specimens

The specimens collected from the highbush blueberry orchards were grouped together, thus indicating a common genetic relationship, even though they were collected from orchards located in different regions. All specimens were phylogenetically closely linked to two other species, *P. corni* and *P. fletcheri*, which practically did not show genetic distance between them and the collected specimens. The dendrograms visually suggested a closer relationship between the specimens and *P. fletcheri* compared to the *P. corni* samples analyzed, but the genetic distance resulted in similar results. Both species have been recorded in Poland [5]. However, *P. fletcheri*, even though it was recorded for the first time in Poland in 1935, is still considered an alien species [27]. *P. corni* and *P. fletcheri* are morphologically very similar and share high variability in many morphological characters that are normally used in classification [28]. Moreover, considering that *P. corni* is a widespread species normally found on fruit trees, while *P. fletcheri* tends to be found mainly on a few forest tree species, it is more likely that the specimens analyzed belonged to the former species.

4.2. Ecology of Parthenolecanium sp. Parasites

Genera and species found to parasitize *Parthenolecanium* spp. in Polish highbush blueberry plantations are known natural enemies, either as parasitoids (*Blastothrix, Coccophagus, Encyrtus,* and *Metaphycus*) or predators (*Anthribus*) [29–31]. *Coccophagus lycimnia* and *Blastothrix* species are cosmopolitan, generalist parasitoid taxa that attack many scale species [30,32], so it was not surprising to find them in all the highbush blueberry plantations. Moreover, *Blastothrix longipennis, Metaphycus insidiosus,* and *Coccophagus lycimnia* were reported to be common and important parasitoids of *P. corni* in Poland [33]. Interestingly, the number of parasitoid genera found in association with *Parthenolecanium* spp. under Polish conditions was similar to that found in a fruit-producing region of Bulgaria [34]. Under those conditions, *C. lycimnia* and *B. confusa* were found to have the greatest importance in regulating the population density of the pest, as they have different targets: *C. lycimnia* parasitizing overwintering larvae and *B. confusa* parasitizing adult females.

Analyzing the parasitoid community attacking *P. corni* at different stages of its development, it emerged that it was quite diversified in its range, affecting both initial and adult biological phases, but also in its occurrence in different locations, as more species were present in Prażmów compared to Jakubów or Rokotów. Moreover, Prażmów was also the only location where a predator of the *Anthribidae* genera was observed. The presence of natural coniferous woods surrounding the plantation at Prażmów could suggest a possible explanation for this finding, as scales are frequently found on such and other forest plants under Polish conditions [35,36], thus giving the possibility of developing a complex population of natural enemies. This would also support the only finding in this location of *Anthribidae* spp., a predator species, as ladybirds can be an effective natural enemy of coniferous scales [37]. *Anthribus nebulosus* is an effective natural enemy of scale insects, including *P. corni*, as its larvae can act as parasitoids of adult scale individuals, while adults are able to act as predators in all stages of their hosts [9,38,39]. The efficacy of *A. nebulosus* in reducing the populations of *P. corni* was about 22% in Serbia [40].

The dynamic of the healthy or parasitized scale populations recorded in Jakubów, a location densely filled with fruit orchards, could be considered a classic example of the relation between pest and parasite populations [41], with the latter mainly formed by generalistic species, including scelionids. Most scelionids are solitary, and all are egg parasitoids that utilize the eggs of a wide variety of insects [42]. However, species of this family have been used successfully in classical biological control programs [43]. The recent release of *Trissolcus japonicus* to control *Halyomorpha halys* [44] is also an example of the potential biocontrol from using specific parasitoids of this family for *Partenolecanium* spp. control. Even though the level of parasitization was much higher in Piskórka, a site characterized by a natural environment rich in coniferous woods, compared to the other plantations in all three sampling periods, the parasitization rate found in the highbush blueberry plantations was in general low compared to other reports from other fruit growing areas [34].

4.3. Is It Possible to Develop a Strategy for the Integrated Control of Parthenolecanium sp. in Highbush Blueberry Orchards with Low Environmental Impact?

The different active substances tested in the trials proved suitable for controlling the scale infestation. However, the different efficacy observed between them should be interpreted taking into consideration the initial level of infestation. The high efficiency of Spinosad in the Rokotów 2019 trial was obtained when the untreated control had a high infestation level, almost unchanged from the assessment before the treatments. The same substance was less effective when the population in control plots was drastically reduced during the assessment period (Piskórka in 2019), likely as a result of the impact of natural enemies. In the latter case, only acetamiprid, a chloropyridinyl neonicotinoid, was highly effective. However, even though it was consistent in its efficacy, acetamiprid was sometimes less effective than the other synthetic substances (e.g., Rokotów and Prazmów in 2018). The use of acetamiprid for the control of the 1st instar crawler induced almost 97% mortality 21 days after the first treatment [45].

Spinosad, a bio-insecticide derived from the soil actinomycete *Saccharopolyspora spinosa*, is considered a valuable bioactive substance to control several pests [46]. It was found to be effective in controlling *P. corni* in grapes, but only at the beginning of the infestation [47]. Even though spinosad is classified as a substance with reduced environmental and toxicological risk [48,49], its toxicity on several hymenopteran parasitoids has been reviewed recently [50], and it was shown to cause 100% mortality on *C. lycimnia*, a specific parasitoid of *P. corni*, just 24 h after the treatment [51].

The silicon polymer showed intermediate efficacy in all trials. However, when the infestation in the control was high (Maciejowice in 2017 or Rokotów in 2019), the reduction of the number of larvae was significant. When applied to plants, the silicone polymer spreads on the treated surface, creating a three-dimensional grid structure with sticky properties that blocks the insect's physical functions [52]. The efficacy of this kind of substance can thus depend on the application method and on the environmental conditions, as was also previously shown [4].

Camelina oil, characterized by a high content of poly-unsaturated and saturated fatty acids [53], was also more effective with a high infestation rate, including when the crop was grown under protected conditions. The mode of action for many oils is suffocation and water loss [54], and therefore they are unlikely to induce resistance in insect populations [55]. A canola oil treatment resulted in very high efficacy with concentrated sprays (90% with 20 L/ha and 99% with 30 L/ha), similar to that obtained with a tank of the same oil mix (1 L/ha) with chlorpyrifos-methyl [56]. Paraffin oil reduced the number of P. corni larvae by an average of about 80% in trials under different pedo-climatic conditions [57]. Other non-synthetic substances suitable for scale control under organic management of highbush blueberries were reported to have different efficacy. A mixture of vegetal amino acids and fatty acids or treatment with Quassia amara extract 50% and potassium soap showed insufficient efficacy [56]. On the other hand, a product based on polysaccharides and also containing chitosan showed a satisfactory level of efficacy [4]. The combined and alternated use of this kind of substance for the control of scales on highbush blueberries could thus represent a good strategy to reduce the environmental impact and improve the overall control efficacy.

The difficulty in controlling scales can be assessed considering the results from the treatments with synthetic active substances. The consistent and high efficacy shown by spirotetramat across locations and seasons, confirming previous results [4], can allow us to consider this product as a reference standard. However, spirotetramat efficacy changed significantly across seasons in a 2-year trial in Slovenia, from 98% in the first year to 70% in the second [58]. The efficacy of flonicamid, a selective systemic pesticide that interferes with the alimentary behavior of several insect species [59,60], was somehow inconsistent throughout the seasons and locations in the present study; its efficacy was significant only in two out of six trials where it was tested. However, as it was shown to have a small impact

on parasitoids of other Coccidae species, causing the lowest reduction in their parasitism rate [61], its application and efficacy should be further verified.

Scale populations could remain below the economic threshold thanks to natural biological control, and it is believed that this has been the case for highbush blueberry plantations in Poland in the past, as no major need for their control was raised by producers. However, it could be argued that the need to assure protection against *D. suzuki* in recent years also in Poland, with increased use of pesticides, might have disrupted the natural control in highbush blueberry orchards, similarly to other cases [47,62], requiring the performance of some control measures against scales.

The presence of several genera and species of parasitoids in the highbush blueberry plantations concerned by the research allows us to envision their contribution to *P. corni* scale control even when pesticides are applied. Indeed, while insecticides are generally toxic to adult scelionids, it appears that preimaginal parasitoids within host eggs could escape high mortality in the field even when formulations based on insect growth regulators are applied [63]. Nevertheless, in addition to direct mortality, pesticides sublethal effects on beneficial insects' physiology and behavior can also modify the population dynamics and biological control potential of scale parasitoids [11], thus requiring a careful selection of substances to be applied.

Control of scales is a major challenge because adult females and eggs are protected from pesticides, with only the first-instar nymphs being susceptible due to their mobility, making the timing of pesticide applications targeting them critical to achieving significant efficacy. A good integrated strategy to manage scales includes dormant pruning of old, weak canes and scale-infested wood, which prevents increasing their population density. Applying a complex strategy, combining the application of several synthetic insecticides with different MoAs and timings, allowed us to achieve an efficacy above 90% [64]. A good control was also possible by introducing a control based on non-synthetic substances (this report; [4]). The importance of *P. corni* control is also derived from its function as a vector of several viruses [65,66]. Recently, it was shown that larval stages and adult females of *P. corni* feeding on highbush blueberry plants infected by the blueberry red ringspot virus carried the virus, even though it was not proven they were able to transmit it [67].

5. Conclusions

The substances tested for the control of *P. corni* scales on highbush blueberry during several years and in different locations were suitable, but the level of efficacy depended on various factors and should be interpreted taking also into consideration the initial level of infestation. It is argued that the infestation level could have been affected by the population composition of natural enemies, which was diversified at the studied sites. Therefore, applying an IPM strategy that combines agronomical practices with the application of insecticides with different mechanisms of action, attentive to the benefit of protecting natural enemies, can result in satisfactory control of *P. corni* in highbush blueberry plantations. Even though the scales present in the highbush blueberry plantations presented some uncommon morphological characteristics, the comparison based on COI sequences of their DNA with those of other accessions from *P. corni* or *P. fletcheri* did not show significant differences.

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