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ORIGINAL ARTICLE

Assessment of applying an integrated pest management strategy to control the raspberry leaf and bud mite, *Phyllocoptes gracilis* (Nal.) and its effect on the raspberry leaf metabolites

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Abstract

In the years 2018–2020, the effectiveness of three synthetic active substances (acequinocyl, fenpyroximate, spirodiclofen), one substance derived from *Streptomyces* spp. (abamectin), a plant extract (orange oil) and silicone polymers in controlling *Phyllocoptes gracilis* in two Polish raspberry plantations (v. 'Glen Ample') was assessed. All the substances showed high and comparable efficacy against the tested pest, significantly reducing its population. However, their effects occurred at different times after the application. The strongest immediate control was shown by silicone polymers, followed by abamectin and spirodiclofen. The full effect of fenpyroximate application was visible after approx. 2 weeks, while acequinocyl was effective 3–4 weeks after the application. Moreover, the content of phenolic compounds, sterols and triterpenoids was determined in leaves of plants treated with spirodiclofen, orange oil and silicone polymers. The observed increase in the content of salicylic acid and changes in the content of triterpenoids in leaves may indicate a stimulating effect of the substances to the natural defense processes of plants.

Key words: active ingredients, Eriophyoidea, pest control, salicylic acid, triterpenoids

Introduction

The raspberry leaf and bud mite (*Phyllocoptes gracilis* Nalepa, 1891), belonging to the Eriophyidea family, is a dangerous pest of raspberry plantations in several major production countries such as Serbia (Milenković and Marčić 2012), Hungary (Szántóné Veszelka and Fajcsi 2003), Switzerland (Linder *et al.* 2008) or the Scandinavian countries (Trandem *et al.* 2010). In Poland, the economic losses caused by this mite are increasing, probably also as a result of its capacity to transmit the raspberry leaf blotch virus (RLBV) (McGavin *et al.* 2012; Cieślińska and Tartanus 2014). Besides the variety 'Glen Ample', the most frequently infested by the mite, it also occurs on the varieties 'Polka', 'Polana', 'Laszka', 'Malling Promise' (Cieślińska and Tartanus 2014).

Several chemical active substances have been tested and found to reduce raspberry leaf and bud mite infestation (Gordon and Taylor 1977; Tartanus *et al.* 2015). However, the development of effective strategies to control this pest has become urgent within a scenario of decreasing availability of registered active substances and the need to implement integrated pest management (IPM) approaches and reduce the use of pesticides (EU Commission 2020).

Even though research on insecticides focuses primarily on their effectiveness in controlling pests or on their side effects on beneficial entomofauna and food safety (Carvalho 2017), a recent area of study concerns their effect on plant metabolic and physiological processes to better understand and exploit possible mechanisms of plant tolerance or resistance against pests. Modification of primary metabolic pathways (Xia *et al.* 2006; Sharma *et al.* 2013) or the expression of antioxidant enzymatic activities (Sharma *et al.* 2018; Homayoonzadeh *et al.* 2022) as well as the production of secondary metabolites (Chauhan *et al.* 2013) have been reported in various crops as a result of pesticide treatments. Nevertheless, information about the impact of active substances of natural origin is still limited.

The aim of this study was to assess the effectiveness of some active substances suitable for integrated and organic production systems to control the raspberry leaf and bud mite. The effect of some substances on raspberry leaf secondary metabolites related to the plant defense response was also evaluated.

Materials and Methods

Trials set up

A semifield trial was performed in 2018 on raspberry potted plants at the National Institute of Horticultural Research in Skierniewice (Łódź Voivodeship, 51°96' N 20°15' E). Field trials were performed on raspberry plantations located in Tarczyn (Masovian Voivodeship, 51°59' N 20°50' E) in 2019, and in Cielądz (Łódź Voivodeship, 51°42 N' 20°20' E) in 2020. The summer-fruiting variety 'Glen Ample' was used in all trials.

The trials were set up with a randomized block design and four replicates: in the case of the pot trial, four potted plants formed a replicate, while for the field trials each replicate consisted of a plot of 17.5 m^2 (2.5 × 7 m each) with spacing 0.5 × 2.5 m, containing about 30 plants.

A total of six active substances (Tab. 1) were tested and compared to an untreated control (sprayed with water). A "Stihl" motorized backpack sprayer was used for the treatment, using the equivalent of 750 l of water solution per hectare. In all trials, only one treatment was performed after detecting the presence of the raspberry leaf and bud mite on leaves: in 2018 – on June 6, in 2019 – on May 11, and in 2020 – on June 1. In each year of the study, the date of treatment was decided based on the pest occurrence, which varied between seasons. In turn, the different intervals and frequency of sampling stemmed from the need to determine the time dynamics of the substance efficacy, useful in defining alternative control strategies. The day of application was selected to fulfill EPPO standards related to weather conditions, i.e., avoiding extreme temperatures or windy conditions or closeness to precipitation events. Other necessary plant protection treatments were performed in accordance with IPM principles.

Samples of 10 leaves from each replicate were collected randomly from the entire length of the shoots to assess the efficacy of the active substances. From each leaf a disc with a diameter of 1.4 cm was cut out with a cork borer and the alive mites on it were counted under a stereoscopic microscope. In 2018, the assessment was performed twice – 7 and 14 days after the treatment (DAT), in 2019 once – 30 DAT, and 7, 14 and 21 DAT in 2020. The efficacy of the active substance was calculated according to Abbott's formula (Abbott 1925).

Determination of leaf metabolites

The effect of three active substances (spirodiclofen, silicon polymers and orange oil), characterized by a diverse mechanism of action and origin, on leaf primary

Table 1. Characteristics and doses of the active substances applied to control *Phyllocoptes gracilis* in the trials

Active substance	IRAC classification	Mode of action	Trade name, producer	Dose
Abamectin	avermectins, milbemycins (6)	contact and gastrointestinal	Vertigo 018 EC, Nufarm Polska Sp. z o.o.	1.0 l · ha^{-1}
Acequinocyl	acequinocyl (20)	contact	Kanemite 150 SC, Sumi Agro Poland Sp. z o.o.	1.0 l · ha^{-1}
Fenpyroximate	METI acaricides and insecticides (21)	contact and gastrointestinal	Ortus 05 SC, Sumi Agro Poland Sp. z o.o.	1.5 l · ha^{-1}
Spirodiclofen	tetronic and tetramic acid derivatives (23)	contact	Envidor 240 SC, Bayer CropScience AG	0.4 l · ha^{-1}
Orange oil	botanical essence including synthetic, extracts and unrefined oils with unknown or uncertain MoA – E (UN)	contact	Limocide, Vivagro	0.1%
Silicone polymers	no classification	physical	K-Pak, Synthos Agro Sp. z o.o.	0.2%

and secondary metabolites was determined in 2020. Raspberry leaves collected at 2 and 8 DAT, were air-dried and stored at room temperature until analysis.

Extraction and fractionation of diethyl ether extracts from raspberry leaves

Air-dried raspberry leaves were ground in a mortar to a fine powder and extracted in a Soxhlet apparatus for 8 h with diethyl ether. The obtained extracts were evaporated to dryness under reduced pressure on a rotary evaporator. Evaporated extracts were fractionated by adsorption preparative thin layer chromatography (TLC) on 20 × 20 cm glass plates coated manually with silica gel 60H (Merck, Darmstadt, Germany) in a solvent system (chloroform:methanol 97:3 v/v) into two fractions: (i) the neutral triterpenoids and steroids, and (ii) triterpenoid acids. The individual fractions were localized on plates by comparison with standards of sitosterol and α-amyrin for the fraction (i), and oleanolic acid for the fraction (ii); the fractions were visualized by spraying the appropriate area on the plate with 50% H_2SO_4, followed by heating with a hot-air stream. Fractions were eluted from the gel with at least 10 times the volume of diethyl ether relative to the volume of the isolated gel. The fractions containing neutral triterpenoids and steroids (R_F 0.3-0.9) were directly analyzed by gas chromatography-mass spectrometry (GC-MS), whereas fractions containing triterpenoid acids (R_F 0.2–0.3) were first methylated with diazomethane.

Identification and quantification of triterpenoids and steroids

An Agilent Technologies 7890 A gas chromatograph equipped with a 5975C mass spectrometric detector was used for qualitative and quantitative analyses. Samples dissolved in diethyl ether:methanol (5 : 1, v/v) were applied (in a volume of 1–4 μl) using 1 : 10 split injection. The column used was a 30 m × 0.25 mm i.d., 0.25 µm, HP-5MS UI (Agilent Technologies, Santa Clara, CA, USA). Helium was used as the carrier gas at a flow rate of 1 ml · min^{-1}. The separation was made at the temperature programed: an initial temperature of 160°C held for 2 min, then increased to 280°C at 5°C for 1 min and the final temperature of 280°C was held for a further 44 min. The other parameters of the instruments were set as follows: inlet and FID (flame ionization detector) temperature 290°C; MS transfer line temperature 275°C; quadrupole temperature 150°C; ion source temperature 230°C; EI 70 eV; m/z range 33–500; FID gas (H_2) flow 30 ml · min^{-1} (hydrogen generator); and air flow 400 ml · min^{-1}. Individual compounds were identified by comparing their mass spectra with library data from Wiley 9th Ed. and NIST 2008 Lib. SW Version 2010 or previously reported data and by comparison of their retention times and corresponding mass spectra with those of authentic standards, when available. Quantification was performed using an external standard method based on calibration curves determined for the compounds belonging to representative triterpenoid classes: α-amyrin for triterpenoid alcohols, oleanolic acid methyl ester for triterpenoid acid methyl esters, and sitosterol for steroids.

Preparation of soluble phenolics

Extraction of soluble phenolics was performed according to Solecka and Kacperska (2003). Air-dried raspberry leaves were subjected to an 8-hour extraction with 80% ethanol containing 2% ethyl ether. The obtained fraction of free phenolic compounds and their glycosidic and ester derivatives was further determined.

Determination of total phenolic content

Total phenolic content in the cytoplasmic fraction was determined spectrophotometrically at 750 nm (Shimadzu 160A, Shimadzu) using the Folin-Ciocalteu method (Forrest and Bendall 1969). The total content of phenolic compounds was calculated using a calibration curve made with ferulic acid in the range 0.5–100 µg. The result was given in µg · g^{-1} dry weight of the sample.

Anthocyanin extraction

Anthocyanins were extracted for 3 hrs from dry leaf samples (1 g each) with 1% HCl in methanol, centrifuged at 10 000 g for 20 min and determined spectrophotometrically at 570 nm, as described by Solecka *et al.* (1999). The anthocyanin content was calculated using a standard curve for peonin (in the range 5–50 µg). The result was expressed as µg anthocyanins · g dry weight $^{-1}$ of the sample.

Identification of phenolic compounds

The free phenolic compounds obtained by acid hydrolysis were analyzed by HPLC (Shimadzu LC-20AD chromatograph). Separation was carried out on a COSMOSIL(R) Cholester Packed Column 4.6 mm, I.D. × 250mm with a Bionacom Filter Column Protector, 316 Stainless Steel, 2 microns. The separation temperature was 30°C.

For free phenolic acids the mobile phase was a mixture of acetonitrile/acetic acid/water (10/2/88, v/v/v) (Merck) and the flow rate was 2 ml · min^{-1}. Phenolic acids were detected at 254 nm using a UV/VIS spectrophotometric detector and identified by comparison with authentic standards (Sigma).

For flavonoids and anthocyanins a different mobile phase was used: eluent A (0.1% formic acid in water) and B (acetonitrile) in a system: 90–75% up to 30 min; then 75–40% from 30 to 45 min, at a flow rate of

1 ml · min^{-1}. Compounds were detected at 430/525 nm using a UV/VIS spectrophotometric detector and identified by comparison with authentic standards (Sigma).

Statistical analyses

The data from the trials were analyzed by one-way ANOVA; efficacy data were transformed by log(x) + 1 in order to assure a normal distribution. The significance of differences between means was assessed with Tukey multiple range test at $p \leq 0.05$ using the package Statistica v.6.1.

Results

Effect of the different active substances on the control of *Phyllocoptes gracilis*

Application of abamectin, spirodiclofen and silicone polymers significantly reduced the number of mites on the leaves of potted raspberry plants already 7 DAT (Tab. 2). All tested active substances, except acequinocyl, reached 100% efficacy at 14 DAT, compared to the control which presented an increased number of mites with respect to the first assessment. However, the assessment of the same substances in 2019 after a longer period from the treatment (30 DAT) showed a high efficacy also of acequinocyl and confirmed that of the other substances (Tab. 2).

To better define the efficacy of the substances in relation to the length of the period, and to verify the control dynamics of orange oil, three assessments were carried out in 2020. Abamectin and spirodiclofen confirmed better efficacy in controlling the raspberry leaf and bud mite in a short time compared to fenpyroximate and the silicone polymers and also the orange oil (Tab. 2). However, at 14 DAT all synthetic substances were able to fully control the mites on the raspberry leaves. Orange oil required 1 week more to reach the same efficacy level as the other substances.

Effect of diverse active substances on raspberry leaf metabolites

The three selected active substances induced diverging changes in the plant secondary metabolism. Even though there was an increasing trend in the content of the triterpenoid alcohols (α- and β-amyrins) in leaves at 8 DAT after application of spirodiclofen and a decreasing trend after orange oil application, they were not confirmed by the statistical analysis (Fig. 1). On the other hand, all substances induced a significant increase of the content of the triterpenoid acids (oleanolic and ursolic acids compared to the control,

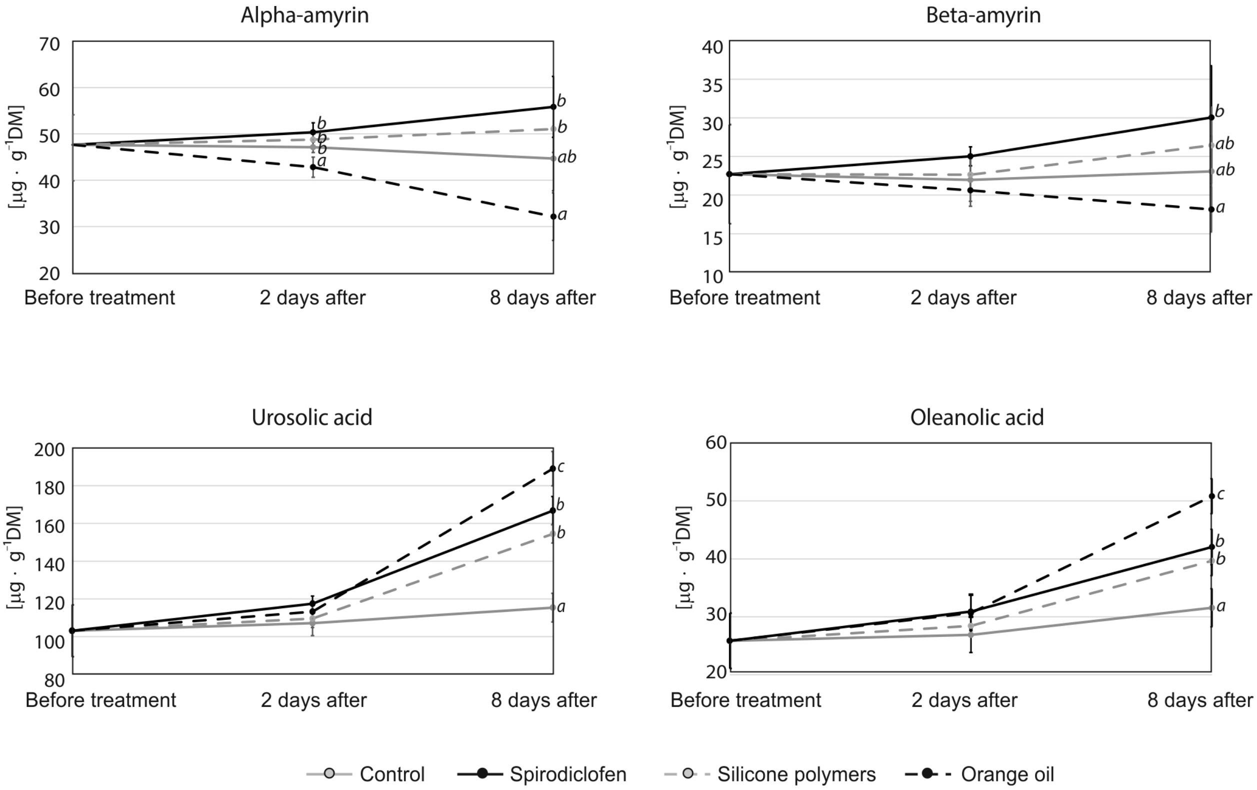

Fig. 1. The effect of active substances applied against *Phyllocoptes gracilis* on the content of triterpenoids in raspberry leaves. Means marked with different letter(s) on each sampling date are significantly different at $p \leq 0.05$

Table 2. The efficacy of synthetic and natural active substances on *Phyllocoptes gracilis* control (mean ±SD, n = 40)

Treatment	2018				2019		2020					
	7 DAT		14 DAT		30 DAT		7 DAT		14 DAT		21 DAT	
	NM	EF [%]	NM	EF [%]	NM	EF [%]	NM	EF [%]	NM	EF [%]	NM	EF [%]
Control	2.3 ± 5.4 c	–	5.8 ± 17.0 c	–	1.4 ± 2.0 b	–	± 7.8 b	–	2.2 ± 6.2 c	–	1.5 ± 1.2 b	–
Abamectin	0.1 ± 0.5 a	97.8	0.0 ± 0.0 a	100.0	0.1 ± 0.0 a	92.7	0.0 ± 0.4 a	98.9	0.0 ± 0.4 a	99.1	0.0 ± 0.0 a	100.0
Acequinocyl	1.4 ± 7,9 bc	39.1	1.7 ± 0.4 b	70.7	0.0 ± 0.4 a	98.5	nd	nd	nd	nd	nd	nd
Fenpyroximate	1.0 ± 9.0 b	56.5	0.0 ± 0.0 a	100.0	0.0 ± 0.0 a	100.0	0.4 ± 4.2 ab	77.8	0.0 ± 0.0 a		0.1 ± 0.5 a	96.7
Spirodiclofen	0.1 ± 1.3 a	95.7	0.0 ± 0.0 a	100.0	0.1 ± 0.5 a	96.4	0.0 ± 0.4 a	98.9	0.0 ± 0.0 a		0.0 ± 0.0 a	100.0
Orange oil	nd	nd	nd	nd	nd	nd	1.8 ± 6.3 b	0.0	0.4 ± 1.9 b	81.8	0.0 ± 0.0 a	100.0
Silicone polymers	0.0 ± 0.0 a	100.0	0.0 ± 0.0 a	100.0	0.0 ± 0.4 a	98.5	0.1 ± 0.5 ab	94.4	0.0 ± 0.0 a		0.0 ± 0.0 a	100.0

NM – number of mites per 1.5 cm^2 of leaf surface; EF – efficacy according to Abbott's formula; nd – no data. Means followed by a different letter(s) in each column are significantly different at $p \leq 0.05$

with orange oil inducing the highest increase, significantly higher than the other two substances).

An increasing trend of the salicylic acid content in leaves was observed at 8 DAT after the application of each active substance, but it was only significant for orange oil compared to the control (Fig. 2).

The three substances did not affect the total content of polyphenols and anthocyanins in leaves. Only ellagic acid and chlorogenic acid were affected among the six major polyphenolic compounds determined in the leaves (tab. 3). Ellagic acid was reduced by all three substances, significantly by spirodiclofen and the silicon polymers, while chlorogenic acid was increased by the silicon polymers.

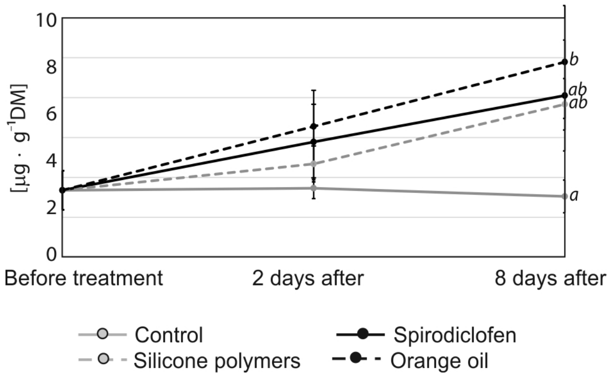

Fig. 2. The effect of three active substances applied against *Phyllocoptes gracilis* on the content of salicylic acid in raspberry leaves. Means marked with different letter(s) in each term are significantly different at $p \leq 0.05$

Table 3. The effect of active substances applied for the control of *Phyllocoptes gracilis* on the concentration of sterols and phenolic compounds in raspberry leaves at 8 DAT (mean ±SD, n = 3)

	Control	Spirodiclofen	Orange oil	Silicone polymers
	[μ · g^{-1} DM]			
Sterols				
Campesterol	40.0 ± 8.5 a	33.3 ± 7.0 a	39.2 ± 3.5 a	42.8 ± 5.4 a
Sitosterol	1286.2 ± 84.3 b	1057.9 ± 94.2 a	1188.9 ± 59.5 ab	1244.6 ± 60.5 b
Stigmasterol	664.9 ± 52.6 b	510.1 ± 60.3 a	663.7 ± 51.8 ab	630.3 ± 64.4 b
Polyphenols				
Total phenols	307.0 ± 31.7 a	344.1 ± 73.0 a	353.4 ± 72.4 a	386.3 ± 117.8 a
Anthocyanins	21.9 ± 1.5 a	32.0 ± 5.1 a	32.9 ± 10.0 a	27.0 ± 12.2 a
Chlorogenic acid	3.2 ± 0.7 a	3.1 ± 0.5 a	3.2 ± 0.2 a	4.6 ± 0.6 b
Ellagic acid	16.6 ± 0.9 b	11.4 ± 1.7 a	12.7 ± 2.3 ab	10.8 ± 2.0 a
Ferulic acid	1.0 ± 0.4 a	1.3 ± 0.3 a	2.1 ± 0.7 a	0.9 ± 0.5 a
Kaempferol	4.7 ± 0.5 a	3.9 ± 1.0 a	4.7 ± 1.0 a	4.5 ± 1.3 a
Quercetin	6.9 ± 1.9 a	7.7 ± 0.9 a	9.0 ± 2.5 a	8.2 ± 1.7 a
Quercitrin	37.2 ± 5.5 a	35.4 ± 3.8 a	40.6 ± 4.0 a	40.5 ± 10.1 a

Means followed by different letters in each row are significantly different at $p \leq 0.05$

With regards to sterols, only spirodiclofen affected their content, inducing a significant reduction of both sitosterol and stigmasterol content in the raspberry leaves compared to the control (Tab. 3). No differences were observed for the other two substances in their content, nor for any substance in the case of campesterol content.

Discussion

Efficacy of diverse active substances in controlling *Phyllocoptes gracilis*

All tested active substances were effective in the control of *P. gracilis* in raspberry crops, irrespective of their synthetic or natural origin, as well as of the mechanism of action. Even though the consistency of the results between seasons and locations supports the benefits of the substances, it should be mentioned that the full efficacy in controlling the pest could be different under different conditions. These could include climatic conditions (i.e., temperature and humidity) affecting the development time of *P. gracilis*, the incidence of the pest population in nearby plantations, as well as rates of product application and the plant coverage. Indeed, the approach of sampling leaves randomly from the entire length of the shoots applied in this study to verify the efficacy of the products under field conditions was also derived from the observation of symptoms of the presence of the pest along the shoots on different parts of the bushes, including also their lower part, which is commonly more difficult to reach with the sprayed product, resulting in potentially higher pest abundance. Even though *P. gracilis* can be observed in all organ types and along all bush/shoot heights, a prevalence of mites on the upper two-thirds of the canes was observed (Minguely *et al.* 2019), suggesting to direct sampling for monitoring the pest between 60–180 cm above collar level, according to crop development. Therefore, our approach was to consider both the need of verifying the efficacy of the products on all plant parts, useful from an agronomical point of view, and the correct monitoring of the pest presence.

It should be underlined that not all substances applied in the study are currently registered for the specific control of *P. gracilis*. Therefore, these results showed that their use against other mites, for which they are authorized, will also effectively control the population of raspberry leaf and bud mite.

The impact of the treatments on the overwintering population of the pest was not assessed in the current study. However, it could be hypothesized that the drastic reduction of the summer population would negatively affect the number of the overwintering females. Nevertheless, the impact of the control treatments could also affect the predators of *P. gracilis*, as shown with other plant parasitic mites (Stavrinides and Mills 2009), making it necessary to consider further studies in this respect for each tested active substance. Similarly, it could also be worthy to evaluate the interaction of these active substances with entomopathogenic fungi (Minguely *et al.* 2021) to verify possible synergic or detrimental effects in view of a more complex integrated control approach.

It should be mentioned that the number of assessments in the different years were derived from the observation of the actual occurrence of the pest. Moreover, to reduce the effect of seasonal conditions, the assessments were conducted considering the phenological phase of the plant, which was expected to assure a sufficient degree of comparison between years. Nevertheless, the control of the pest occurred with different time laps after the application of the various active substances. Those with the strongest immediate effect included silicone polymers, abamectin and spirodiclofen, which drastically reduced the mite population in a few days irrespective of the season and location. A longer (2 to 3 weeks) period was necessary for the other substances to achieve the same result. This aspect shall be taken into consideration in view of the infestation level of the mite. Fenpyroximate, abamectin and spirodiclofen were found effective in controlling *P. gracilis* also in previous works with high initial levels of mites' infestation (Milenković and Marčić 2012; Tartanus *et al.* 2015). The silicone polymers, when applied to plants, spread on the treated surface creating a three-dimensional polymeric grid structure with sticky properties (Somasundaran *et al.* 2006) blocking the pest's physical functions (Tartanus and Malusà, unpublished observations). The main ingredient of the product based on silicone polymers is modified trisiloxane, a compound which belongs to a broad category of molecules characterized by different features, including surfactants (Cheng *et al.* 2022). For this reason, the formulation can be considered to be a suitable alternative to mineral oils, which are also applied against mites, but in contrast to them, it is not expected to induce phytotoxicity.

Integration of the different tested active substances can also be a suitable approach considering the long harvest period of raspberry and in relation to the different withdrawal periods they have. Considering that, these substances belong to different classes of the IRAC classification and have different mechanisms of action, their alternate application shall also reduce the risk of developing resistance in the mite population (Sparks and Nauen 2015).

The use of alternative, non-synthetic, active substances is gaining importance due to the development of organic horticultural production and the pressure from policies and consumers to minimize the use of chemical compounds in agriculture. Essential oils from different *Citrus* species were effective in controlling different mite species, without negatively affecting predatory mites (Pimentel Farias *et al.* 2020; Brito *et al.* 2021). The positive results obtained with the orange oil in the trials can thus support their application within IPM strategies as well as provide a suitable control in organic raspberry crops.

Interaction of pesticide applications and leaf mites on metabolites of the raspberry leaves

The analysis of the short-term impact (at 2 DAT) of the compounds on secondary metabolites was carried out since it would be interesting to verify the triggering of chemical signals through secondary metabolites that could be associated to defence-related responses (Khare *et al.* 2020; Jha and Mohamed 2022). However, even though we could not completely exclude some changes occurring earlier than 2 DAT, the results of the study would suggest that the modification under field conditions, if any, could occur at a later stage.

At 8 DAT all three active substances increased the content of salicylic acid in the raspberry leaves, particularly the orange oil. Salicylic acid is a signaling molecule that induces the expression of genes involved in the defense reactions of plants against pathogens including synthesis of PR proteins and phytoalexins (Filgueiras *et al.* 2019; Osei *et al.* 2021). Studies have shown that the application of insecticides with different modes of action (e.g. spirotetramat, imidacloprid, chlotianid) may result in increased synthesis of salicylic acid (Ford *et al.* 2010; Szczepaniec *et al.* 2013; Homayoonzadeh *et al*. 2022). Interestingly, exogenous salicylic acid reduced the feeding of spider mite on strawberry (Favaro *et al.* 2019). In addition, under biotic stress, salicylic acid may be converted to its volatile methyl ester (MeSA), a signal compound that induces a defense response in plants that have not yet been attacked by the pest (Baldwin *et al.* 2006) and that can also be a cue for natural enemies to locate potential host colonies (Filgueiras *et al.* 2019; Li *et al.* 2020). The increase in salicylic acid content in raspberry leaves observed as a result of the application of the orange oil and its possible role for the protection against mites would be worthy of additional studies.

The three active substances did not induce significant modifications of the leaf polyphenolic and anthocyanins total content. However, spriodiclofen and the silicon polymers reduced the content of ellagic acid. This compound is a strong antioxidant related to the ellagitannins, a group of condensed tannins present as a structural component of the plant cell wall and the cell membrane (Vattem and Shetty 2005). The active substances could thus have some impact on the antioxidative processes associated to the cell wall and membrane, the significance of which requires further studies. The lack of significant changes in the level of anthocyanins observed in raspberry leaves may be related to the low level of stress induced by both mite infestation and active substances (Chalker-Scott 1999; Duran *et al.* 2015).

The applied substances also induced a minor impact on the content of sterols and pentacyclic triterpenoids, limiting their effect on few compounds. These two groups of compounds, considered primary and secondary (specialized) metabolites, respectively, are derived from a common precursor, squalene. Therefore, the changes in their proportions are often regarded as a reflection of the balance between primary and secondary metabolism, that might be triggered by stress conditions or substances acting as elicitors (Rogowska *et al.* 2022). Only spirodiclofen caused an increase in the content of amyrins and a simultaneous decrease in the content of sterols, pointing to the possible competition between primary and secondary metabolic pathways. However, this effect did not seem strong enough to substantially modify the metabolism of the plant, and influence the processes of plant growth and normal development. Indeed, the ratio between sitosterol and stigmasterol was not changed in response to the application of spirodiclofen indicating that the sterol metabolism was not profoundly modified. Sterols are structural components of cell membranes and have diverse and essential functions in plants including an important role in plant response to stress, signaling, and plant-pathogen interactions (Tapken and Murphy 2015; Rogowska and Szakiel 2020). The observed consistent reduction of the two most common sterol molecules (sitosterol and stigmasterol) could be associated with the conversion occurring in the cell (Aboobucker and Suza 2019).

Orange oil and the silicone polymers slightly modified the content of triterpenoids, while they did not affect the sterol content. Therefore, it can be hypothesized that they could stimulate specialized metabolic pathways that might lead to the enhancement of plant defense potential without affecting the primary metabolism. Oleanolic acid and its isomer, ursolic acid, as well as α- and β-amyrin, are among the triterpenoid compounds that widely occur in nature (Liu 1995; Hernández-Vázquez *et al.* 2012). Although the biological function of the majority of the triterpenes is yet to be determined, some are suggested to play specialized/secondary functions as defense compounds (Ghosh 2016; Cárdenas *et al.* 2019). The observed decrease in the content of both amyrins after orange essential oil treatment might be due to the sharply enhanced biosynthesis of respective acids, since α- and β-amyrin are precursors of ursolic and oleanolic acid, respectively.

Thus, orange oil appears to be an effective elicitor of triterpenoid acid biosynthesis, including fast conversion of alcohols into acids.

Conclusions

The synthetic active substances (fenpyroximate, spirodiclofen and acequinocil) as well as the microbially-derived abamectin and the alternative compounds (essential orange oil and silicon polymers), effectively controlled the raspberry leaf and bud mite, and can thus be considered for the development of a control strategy based on IPM principles. Considering that some of them are not specifically authorized for the control of *P. gracilis*, their exploitation within an IPM strategy could rely on their use against other pests present in the plantation against which their application is allowed. The implementation of possible derogations foreseen by the current EU legislation could also serve for the purpose of widening the authorization scope of the specific substance. The modifications of the content of different plant compounds, some of them also related to the plant defense system, could be a direct effect of the active substances, but also as a result of the changes induced by them on the raspberry leaf and bud mite population. Further studies are needed to clarify the mechanisms and impact of these factors on the plant metabolism.

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