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Received: 2019-06-06 ; Accepted: 2019-08-01

POSSIBILITY OF LIMITING THE NUMBER OF BLACK CHERRY APHID (*Myzus cerasi* F.) WITH BASIC SUBSTANCES FOR ORGANIC SWEET CHERRY

Summary

The effectiveness of basic substances in controlling back cherry aphid (Myzus cerasi F.*) was evaluated under laboratory and field conditions during the seasons 2018-2019. The following compounds were tested in the trials: a water extract from dandelion plants (Taraxacum officinale F.H. Wigg.), an extract from neem seeds (Azadirachta indica* L.*), a suspension made from grounded bark of cinnamon (Cinnamonum cassia L.) added with potassium soap, organic silicon in the form of diatomaceous earth or orthosilicic acid, unpasteurized whole cow milk water diluted, anise oil obtained from the ripe fruits of Pimpinella anisum (*L.*). The tested substances showed an efficacy rate in controlling the aphid species in the range of 0-100%, depending on the product, the dose (in case of the organic silicon) and the time elapsed after the treatment. Milk, cinnamon + potassium soap and the dandelion extract showed the best performance in terms of efficacy, with a level comparable to azadirachtin. Field trials performed with the organic silicon showed a higher efficacy when the compound was added with an essential oil.*
Keywords: *Myzus cerasi, control of aphid, organic sweet cherry growing*

MOŻLIWOŚCI OGRANICZANIA LICZEBNOŚCI MSZYCY CZEREŚNIOWEJ (*Myzus cerasi* F.) Z WYKORYSTANIEM SUBSTANCJI PODSTAWOWYCH W EKOLOGICZNEJ UPRAWIE CZEREŚNI

Streszczenie

Skuteczność podstawowych substancji do zwalczaniu mszycy czereśniowej (Myzus cerasi F.*) została oceniona w warunkach laboratoryjnych i polowych w sezonach 2018-2019. W badaniach przetestowano następujące substancje: ekstrakt wodny z mniszka lekarskiego (Taraxacum officinale FH Wigg.), ekstrakt z nasion neem (Azadirachta indica* L.*), zawiesina wykonana ze zmielonej kory cynamonu (Cinnamonum cassia* L.*) z mydłem potasowym, krzem organiczny w postaci ziemi okrzemkowej lub kwasu ortokrzemowego, niepasteryzowane mleko krowie rozcieńczone wodą, olejek anyżowy uzyskany z dojrzałych owoców Pimpinella anisum (L.). Badane substancje wykazały wskaźnik skuteczności w zwalczaniu tego gatunku mszyc w zakresie od 0 do 100%, w zależności od produktu, dawki (w przypadku krzemu organicznego) i czasu, który upłynął od stosowania produktu. Mleko, cynamon + mydło potasowe i ekstrakt z mniszka lekarskiego wykazały najlepszą skuteczność przy poziomie porównywalnym do azadirachtiny. Próby polowe przeprowadzone z krzemem organicznym wykazały większą skuteczność, gdy dodano olejek eteryczny z pomarańczy.*
Słowa kluczowe*: Myzus cerasi, zwalczanie mszyc, ekologiczna uprawa czereśni*

1. Introduction

One of the leading and commonly occurring pests in organic cherry cultivation is the black cherry aphid *Myzus cerasi*. This pest is widespread in Poland [27] as well as in Europe, Asia, New Zealand, Australia and North America [9]. The black cherry aphid is a homocyclic species having the biological cycle and two-host species [27]. It is estimated that under Polish climatic conditions, the species may have up to several generations during one growing season [21]. Beside the damage on flower buds due to larvae feeding, the main damage derives from their feeding on leaves and from the honey dew secreted, on which fungi are developing reducing the leaves' assimilation process and fruits' commercial value. The black cherry aphid is also considered a vector of several agents of viral diseases [9, 17, 27]

The control or limitation of the aphid populations is particularly difficult in organic orchards of different fruit species. The difficulty lies not only in the determination of the optimal period for a control treatment, but particularly in the selection and application of an effective substance. At present, vegetable and mineral oils, acids obtained from *Quassia amara*, some species of fungi (e.g. *Beauveria bassiana*, *Lecanicillium muscarium*), natural pyrethrins or azadirachtin are recommended in the EU to control aphids in organic orchard crops [13, 26], but there is the need to find additional active substances that could be used in various crops [1, 10]. Studies have been carried out for the control of aphid species typical for a given crop, e.g. *Dysaphis plantaginea* (Pass.) on apple trees with extracts from calamus (*Acorus calamus* L.), garlic (*Allium sativum* L.), black poplar (*Populus nigra* L.) [7], comfrey (*Symphytum officinale* L.), calendula (*Calendula officinalis* L.), or with the use of other plant-derived compounds (e.g. cinnamic acid [23], azadirachtin, natural pyrethrins [5, 22], kaolin clay [12]) or microbial inocula based on *Beauveria bassiana* or *Paecilomyces fumosoroseus* [5, 22]. In case of the foxglove aphid (*Aulacorthum solani* Kalt.) the use of essential oils of lavender (*Lavandula officinalis* L.), pine (*Pinus silvestris* L.), thyme (*Thymus vulgare* L.) or peppermint (*Mentha piperita* L.) were also tested [19]. To control the black cher-

ry aphid, other authors have tested, with contrasting results, infusions of horsetail (*Equisetum arvense* L.), garlic (*Allium sativum* L.), white mustard (*Sinapis alba* L.) and *Brassica juncea* L., as well as the extract obtained from *Quassia amara* (L.) and azadirachtin [15]. We have thus carried out several laboratory and field trials testing different compounds, considered to have different mechanisms of activity, including some basic substances verifying their efficacy on the control of the black cherry aphid.

2. Material and Methods

The study was carried out in the years 2018-2019 under laboratory conditions (Experiments I and II), where cherry shoots with aphid colonies or aphid larvae alone taken in an organic cherry orchard were used as research material. Field studies were conducted in an organic orchard on fourteen-year-old fruiting cherry trees of 'Summit', 'Bürlat' and 'Karesova' cultivars growing at a spacing of 7x4.5 m (Experiment III and IV) under a randomized block design with 3-4 replications. The list and characteristics of the substances used in all experiments are presented in Table 1.

In Experiment I (starting day 28.05.2018), Petri dishes with cherry leaves were used, on which larvae of black cherry aphids were feeding. Each treatment consisted of 3 dishes (each treated as a repetition). Before performing the treatment, the number of live larvae was counted on each replicate. The application of each product was made using a Potter tower, in order to evenly distribute the product on the leaves, using 1 ml/dish of the prepared solution.

Experiment II was carried out in breeding cages, in which properly protected vessels with water were placed and 3 cherry shoots with aphid colonies were placed in them. Each shoot represented a repetition. The experiment was performed in 4 replications. The application of the products was carried out using a hand sprayer with 250 ml of each product' solution per treatment (22.05.2018).

The field trials III and IV were performed in 2018 and 2019, respectively. The experimental set up consisted of 3 replicates each with 3 trees, from which 15 shoots in total (3 trees x5 shoots) with aphid colonies were collected. The application of the products was carried out three times using a standard orchard sprayer equipped with an auxiliary air stream using 700 l/ha of the working solution. The first application was carried out within 2 to 3 days after the presence of the black cherry aphid was detected on the leaves, i.e. on 12.05, 26.05 and 1.06.2018 (trial III) and on 28.05, 6.06 and 14.06.2019 (trial IV).

The evaluation of the efficacy of the substances used in the experiments was performed by counting the aphids alive 3 and 8 days (Experiment I) or 7 days (Experiment II) after the application of the products. In case of the field trials (III and IV), the observation was performed after the first and third treatment (18.05 and 11.06.2018; 03.06 and 20.06.2019, respectively).

Specimens of dead aphids were observed with a light microscope (Zeiss Axiolab A1 m) and by scanning electron microscopy (SEM), using a ZEISS EVO 50 XVP with LaB6 source, in high vacuum, collecting secondary electrons (SEs). The potential difference used was equal to 10 keV and the working distance maintained at 8 mm.

The results were statistically analysed by ANOVA performed on raw data. The significance of differences between means was assessed using the Duncan multi-range test at $p = 0.05$ using the Statistica v.13 program. The efficacy was calculated according to Abbott's formula.

3. Results and Discussions

The impact of the active substances used was very diverse under laboratory conditions. In Experiment I, compared with control, all treatments resulted in a significant reduction in aphid numbers, irrespective of the time of observation (Table 2). Three days after the products' application, the highest efficacy in reducing the number of aphids was recorded with milk and cinnamon with potassium soap. The anise oil and azadirachtin showed an intermediate reduction.

Table 1. List of basic substances and formulated commercial products used to control *Myzus cerasi* on cherry leaves
Tab. 1. Wykaz substancji podstawowych i gotowych produktów handlowych zastosowanych do zwalczania mszycy czereśniowej Myzus cerasi na czereśni

Basic substance (product trade name)	Dose or concentration	Preparation method or biological agent
Milk + water	In a 1: 1 ratio	Unpasteurized milk with a fat content of 3.2% – formulated product
Organic silicon (AdeSil)	1.5%; 3%	Amorphous diatomaceous earth containing organic silicon – formulated product
Cinnamon + potassium soap	Without dilution	Cinnamon derived from the ground bark of Chinese cinnamon *Cinnamonum cassia* with the addition of potassium soap dissolved in water (100 g of cinnamon + 200 g of grey soap + 1 l of warm water)
Azadirachtin (NeemAzal-T/S)	0.5%	Neem seeds extract (*Azadirachta indica*) – formulated product
Extract of dandelion *Taraxacum officinale* (F.H. Wigg.)	Undiluted water extract	Extract prepared from 400 g fresh dandelion leaves in 10 l of water, macerated for 55 hours.
Anise oil	0.1%	The oil obtained from ripe fruits of the Anise *Pimpinella anisum* (L.) – formulated product
Organic silicon (ZumSil)	0.1 %	Orthosilicic acid obtained from amorphous diatomaceous earth – formulated product
Adjuvant (Wetcit)	0.1%	Auxiliary for reducing the surface tension of water containing ethoxylated alcohol and orange oil – formulated product

Source: own work / Źródło: opracowanie własne

Table 2. Effect of different substances on the survival of aphid larvae under in-vitro conditions
Tab. 2. Wpływ różnych substancji na przeżycie larw mszyc w warunkach in vitro

Combination	Average number of live aphids		
	Experiment I		Experiment II
	After treatment		
	3 days	8 days	7 days
Control	93.0 d	47.7 b	55.3 c
Milk + water	12.7 a	0.0 a	18.8 a
Organic silicon 1.5%	31.0 ab	5.7 a	26.0 ab
Organic silicon 3%	71.0 cd	5.7 a	23.0 ab
Cinnamon + potassium soap	21.0 a	8.3 a	20.8 a
Azadirachtin	36.7 abc	0.3 a	25.5 ab
Extract of dandelion	60.0 bcd	0.0 a	16.0 a
Anise oil	32.3 ab	6.7 a	35.0 b

Source: own work / Źródło: opracowanie własne

Table 3. Effect of different substances on the survival of aphid larvae under field conditions
Tab. 3. Wpływ różnych substancji na przeżycie larw mszyc w warunkach polowych

Combination	Average number of live aphids			
	Experiment III		Experiment IV	
	Treatment n.			
	1	3	1	3
Control	393.4 b	7.8 b	6920.0 c	8.25 b
Organic silicon 1.5% + adjuvant	-	-	1456.5 a	0.0 a
Organic silicon 3%	336.8 b	3.2 a	5037.5 b	0.0 a
Orthosilicic acid	202.8 a	3.2 a	1010.0 a	0.0 a

Source: own work / Źródło: opracowanie własne

Interestingly, the lower dose of the organic silicone was more effective in reducing the aphids population with respect to the higher dose. The dandelion extract was also showing a low effectiveness. However, 8 days after products' application, the different substances showed a similar efficacy. All treatments resulted in a significant reduction in aphid numbers in comparison to the untreated control also in Experiment II, when the observation was performed 7 days after the products' application. All products were similarly effective, with only the anise oil showing a slightly lower reduction in the number of individuals in the colonies in comparison to the other products (Table 2).

As a result, efficacy in both experiments varied and ranged in Experiment I, on average for both terms, from 56% to 93%, with milk with water, cinnamon with potassium soap, azadirachtin, organic silicon (1.5% dose) and anise oil showing an acceptable value (Fig. 1). In Experiment II, the efficacy was slightly lower in comparison to the first experiment, ranging from 38% of the anise oil to 71% of the dandelion extract and with all other substances having an intermediate value (Fig. 2).

Gupta et al. [20] showed that the percentage of rose aphids (*Macrosiphum roseiformis*) mortality could reach up to 70% 48 hours after rose plants were treated with whole cow milk diluted to 50% with water. Interestingly, behaviour response of aphids via choice–no choice test revealed that about 40 per cent reached the untreated rose leaves after two hours. Increased mortality of *Brevicoryne brassicae* was observed after milk treatment of kale (*Brassica oleracea* L. var. *acephala* D.C.) plants both in vivo and in vitro (about 55%), but the same treatment was effective on *Myzus persicae* only in vitro (62%) [25].

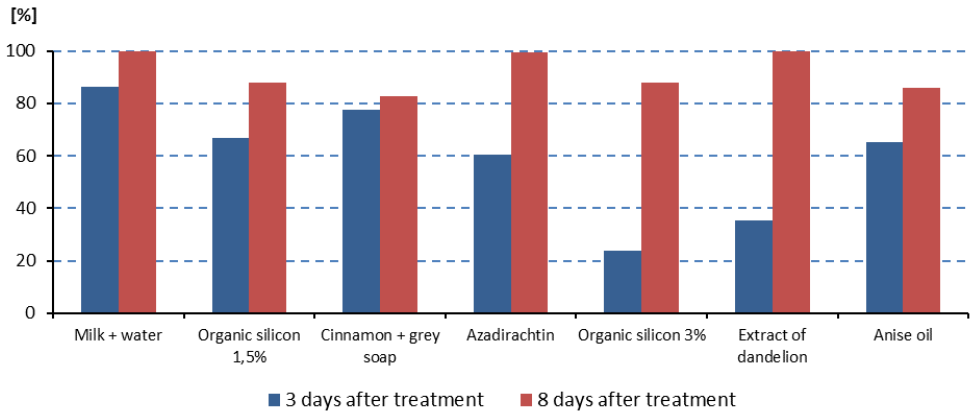

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Fig. 1. Efficacy of the different substances on the control of aphid larvae in Petri dishes conditions
Rys. 1. Skuteczność różnych substancji w zwalczaniu larw mszyc w doświadczeniu in vivo

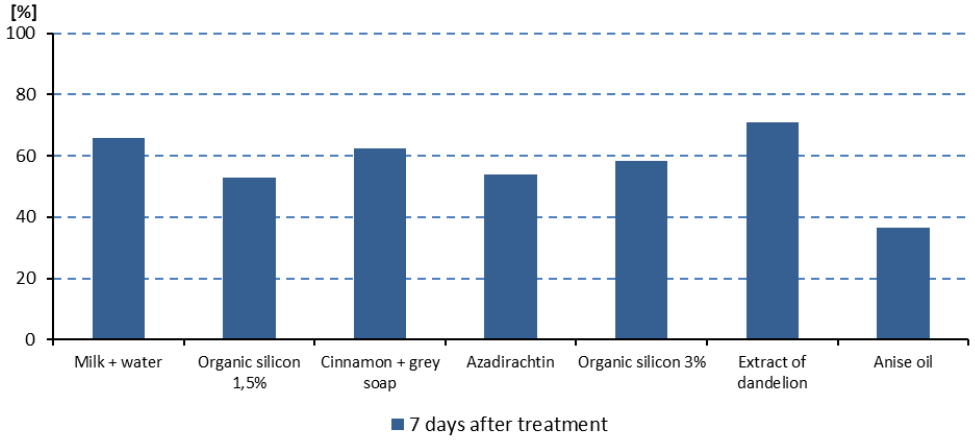

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Fig. 2. Efficacy of the different substances on the control of aphid larvae feeding on shoots under breeding cages conditions
Rys 2. Skuteczność różnych substancji w zwalczaniu larw mszyc żerujących na pędach w klatkach hodowlanych

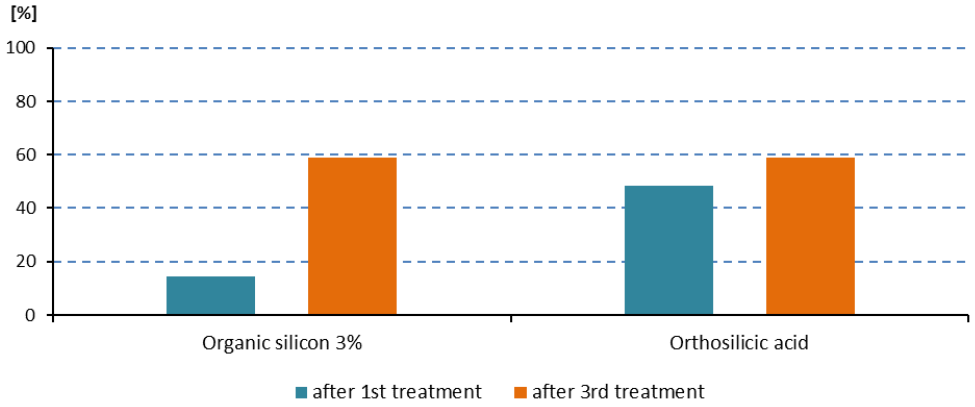

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Fig. 3. Efficacy of the different substances on the control of aphid larvae under field conditions (2018)
Rys. 3. Skuteczność różnych substancji w zwalczaniu larw mszyc w warunkach polowych (2018)

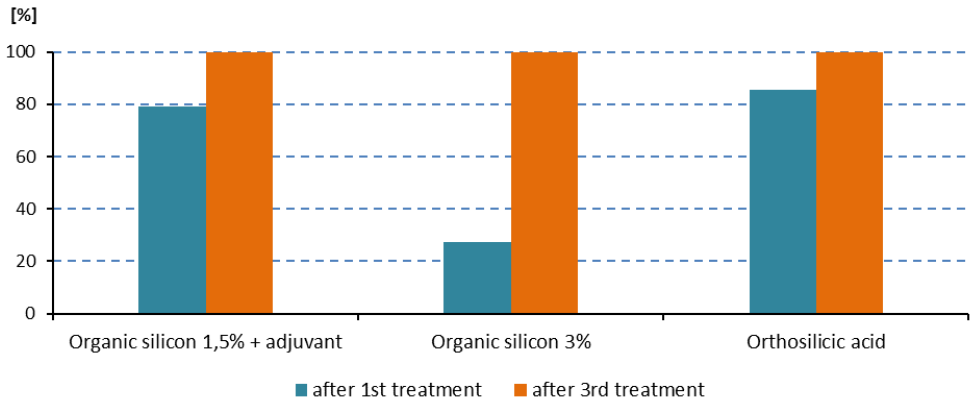

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Fig. 4. Efficacy of the different substances on the control of aphid larvae under field conditions (2019)
Rys. 4. Skuteczność różnych substancji w zwalczaniu larw mszyc w warunkach polowych (2019)

Surprisingly, the dead aphids were parasitized by a fungus identified as *Cladosporium cladosporioides*, a fungus reported as having entomopathogenic activity [8]. In our microscopic observations, fungal-like structures were found on the milk-treated aphids (Fig. 5A). It is noteworthy that milk was not showing any synergistic effect on the aphid mortality when applied in association with the fungus [25], suggesting that the build-up of the *Cladosporium* sp. population on the aphid might be a consequence of its mortality by milk application followed by its colonization by the fungus. The possibility of using dandelion extracts for the control of black cherry aphid was described by Legutowska [24]. Our laboratory tests confirm those results and the efficacy observed (about 70%) indicates the possibility of using this extract in the field. The positive effect of dandelion could also be associated to the development of entomopathogenic fungi, as found in the specimens observed at the microscope (Fig. 5B). This fact may point to the need of further re-

search on the use of these substances and the role of fungi in controlling aphids not only on cherries.

The efficacy in controlling cherry aphid with cinnamon was higher than that reported by Laznik et al. [23] on *Aphis pomi* with cinnamic acid applied in various concentrations. Products containing cinnamic acids are known for the effect on different arthropods [11, 28], also as an additive to other substances [2, 29].

Anise oil efficacy was not consistent among the different experiments, ranging from 37% to 75.5%. Even though Dancewicz et al. [14] found a positive effect of using anise oil against *M. persicae*, it was pointed out that the solvent used for the oil extraction could have an impact on the insecticide efficacy [3]. Furthermore, a different efficacy of anise oil at different doses was observed when administered against *Acyrthosiphon pisum* or *M. persicae* [16], likely as an effect of the differences in host range, which is associated to the ability to overcome several types of plant defences.

Azadirachtin is a compound having a wide spectrum of activity and commonly applied in organic fruit production [13]. Its effect found in our experiments confirmed the usefulness of this substance for controlling the black cherry aphid obtained in previous work of ours under field conditions [6, 15] or by other authors in laboratory [4] with similar efficacy levels, as well as for the control of other aphid species such as *Myzus persicae* [18].

The results obtained with the application of the organic silicon, either as diatomaceous earth (field trials) and orthosilicic acid (laboratory tests) are in line with those reported by El-Wakeil and Saleh [18], where the average efficacy of the substance provided as diatomaceous earth was about 60%. Despite this, scanning electron microscopy showed that silicon particles covered aphid bodies in a very large percentage (Fig. 6), suggesting a physical mode of action of this substance (e.g. suffocation). It is noteworthy to mention that the general higher efficacy observed from the field trials in 2019 was obtained by adding orange oil as an adjuvant, underlining the importance of the formulation for the results particularly of non-synthetic pesticides. Indeed, a higher effectiveness of diatomaceous earth treatments was obtained in combination with azadirachtin [18] in comparison to the single compound.

a) b)

Source: own work / Źródło: opracowanie własne

Fig. 5. Fungal structures developing on the body of the aphid after applying: a) milk, b) dandelion extract
Rys. 5. Struktury grzybowe rozwijające się na ciele mszycy po zastosowaniu: a) mleka, b) wyciągu z mniszka

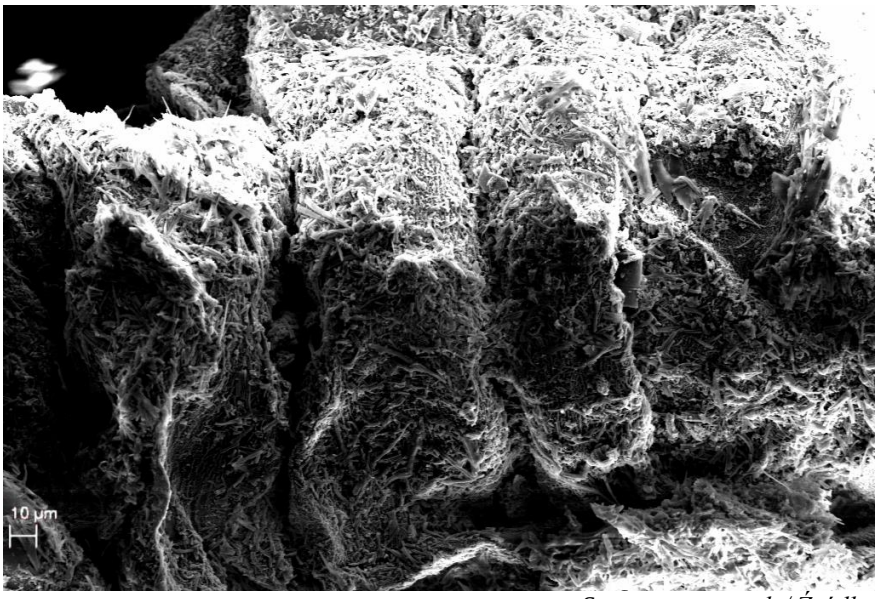

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Fig. 6. Particular of the aphid body covered with silicon microparticles
Rys. 6. Ciało mszycy dokładnie pokryte mikrocząsteczkami krzemu

4. Conclusions

- The basic substances used for the control of black cherry aphid showed a diverse efficacy, but at a level similar to that of known pesticides allowed in organic farming (i.e. azadirachtin).
- The field tests confirmed the potential use of some of the basic substances (namely milk, cinnamon with potassium soap and dandelion extract) emerged from laboratory tests.
- Organic silicon applied in various forms required the addition of an essential oil in the formulation to achieve under field conditions an efficacy similar to that obtained under laboratory conditions.

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Acknowledgments

The research was carried out under the Multi-Annual Program for Instytut Ogrodnictwa „Actions to improve the competitiveness and innovation of the horticultural sector, including food quality and safety, and environmental protection; Task 3.4 - Improving organic horticultural production" (field trials), as well as from the project „Organic fruit production - research on basic substances used in the protection of organic fruit crops" (laboratory experiments) financed by the Polish Ministry of Agriculture and Rural Development.

Publikacja przygotowana na 21. Konferencję ROL-EKO w Poznaniu w dniu 10.10.2019 r.