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ORIGINAL ARTICLE

Monitoring and possibilities of controlling nematodes and fruit damaging pests of *Rosa* spp. with microbial-derived products

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Abstract

Interest in growing roses in Poland is related to the production of cut flowers as ornamentals and of petals and hips for cosmetics or food products. However, recently there has been an increasing number of reports of pest damage on rose plantations. In the case of fruits the damage has been attributed to flies (*Rhagoletis alternata*) or moths (*Cydia tenebrosana*), while nematodes have been implicated for growth reduction even on plantations grown under soil-less conditions. Field trials and laboratory experiments to test the possibility of controlling *R. alternata* larvae or pupae with entomopathogenic fungi and nematodes resulted in a lack of parasitism. On the other hand, the use of *Bacillus thuringiensis* subsp. *kurstaki* or *Cydia pomonella* granulovirus effectively controlled *C. tenebrosana*. *Meloidogyne incognita* infestation of roses growing on rock wool substrate was drastically reduced by *Arthrobothrys oligospora* or abamectin. Factors such as the method of product application or pest susceptibility to the used microbial-based products accounted for the observed differences in efficacy.

Keywords: *Arthrobothrys oligospora, Beauveria bassiana*, *Cydia tenebrosana,* entomopathogenic nematodes, *Rhagoletis alternate*, roses

Introduction

The flower trade is an ever-growing world market and roses are the most important species in this regard with trade worth about 800 million euro (Anonymous 2018). Rose production in Poland for cut flowers is about 27% of total ornamental production (Marosz 2013). However, an increasing interest in growing roses in Poland is also related to the production of petals and hips for cosmetics or food products, for which the rugosa rose (*Rosa rugosa* Thunb.) is one of the most commonly grown species (Maciąg and Kalemba 2015; Patel 2017). Nevertheless, for both ornamental and food products, the market wants farmers to pursue more environmentally friendly production processes to reduce the risk related to the presence of pesticide residues (Kumar *et al.* 2004) or to gain an entrance into specific market segments, such as that of organic products (Willer and Meredith 2016). As a result, biological control agents (BCAs) based on microorganisms are increasingly being used (Chandler *et al.* 2011; Chattopadhyay *et al.* 2017; Lacey 2017).

Reports of fruit damaged by pests have increased along with an enlarged land area planted with rugose rose in Poland. *Rhagoletis alternata* (Fallen) was reported to be present on rugosa rose in northern regions of Poland (Klasa *et al.* 2011) and to damage plantations in the vicinity of Lublin (Winiarska 1998). The deep-brown piercer (*Cydia tenebrosana* Dup., syn. *Grapholita tenebrosana* Duponchel [1843]) is a specific pest for the rugosa rose of the Tortricidae family and its presence in Poland has been confirmed by an ecological study (Chrzanowski *et al.* 2015). Damage caused by these two species was observed after farmers requested advice concerning fruit losses (Tartanus pers. comm.).

The soil-less cultivation method normally utilized for the production of cut roses is expected to avoid the risk of damage from soil-borne pests. However, the use of uncertified planting material or the contamination of irrigation water could represent a source of plant parasitic nematodes such as species of *Meloidogyne* (Hänisch *et al.* 2005), which can survive and reproduce also on soil-less substrates (Lehman 1987).

Several species of each major group of entomopathogens (i.e. viruses, bacteria, fungi, and nematodes) have been investigated for application as biological control agents (BCAs) and used in classic, conservation, and augmentative biological control of different crops (Lacey 2017). BCAs could thus be a possible solution for pests also in minor crops such as roses. We carried out several field trials and laboratory experiments to test different BCAs for the control of soil- or air-borne pests affecting rose plants.

Materials and Methods

Two sets of trials were carried out to assess the effect of BCAs on rose pests. The first set was performed to control fruit pests of rugosa rose, specifically *R. alternata* and *C. tenebrosana,* along with the monitoring of these species in the field. In the case of *R. alternata*, the field trial was coupled with laboratory experiments using the same BCAs applied in the fields. The second trial was set to assess the possibility of controlling *Meloidogyne incognita,* a soil-borne pest, affecting rose plants for flower production (*Rosa* L.) grown in a glasshouse, on a soil-less substrate.

Trials to control rugosa rose fruit pests with biological control agents (BCAs)

Monitoring of fruit pests

In order to monitor the occurrence of *R. alternata*, yellow chromotropic traps (classic sticky traps used for monitoring or mass trapping) were deployed in several fields of rugosa rose located in different regions of Poland. Traps were deployed at the end of May and the presence of flies on them was checked on a weekly basis for about four months.

Pheromone traps used to attract males of *C. funebrana* were deployed to monitor *C. tenebrosana* in the same fields where monitoring of *R. alternata* was performed. The traps were positioned at the end of May and systematically controlled for about four months on a weekly basis.

Field trial to control *Rhagoletis alternata* with biological control agents (BCAs)

A trial was set up on the plantations that presented a high population of flies (Dolice I and II, see Results). The following treatments were applied:

- *Beauveria bassiana* strain BB59, 10^7 cfu · g^{-1} (CCS Aosta, I) – dose equivalent to 100 kg · ha^{-1},
- *Heterorhabditis bacteriophora* (Nemasys G, BASF, D) – dose equivalent to 1 million nematodes · m^{-2},
- *Steinernema kraussei* (Nemasys L, BASF, D) – dose equivalent to 1 million nematodes · m^{-2}.

The selected doses were determined on the basis of the high level of infestation and the lack of specific data about the efficacy of these products against *R. alternata.* BCAs were applied to the soil before the flies started to appear on the chromotropic traps (first half of July) in order to inoculate the soil with the entomopathogenic microorganisms at the moment the larvae reached the soil for pupation.

The effect of the treatment was assessed by determining the number of living or dead pupae present in soil samples collected from the ground below the plants. To do this, four soil samples were randomly collected from each treatment. Each sample contained the soil from 1 m^2 to 5 cm depth.

Laboratory trials to control *Rhagoletis alternata* with biological control agents (BCAs)

The trial was carried out in a glasshouse with two independent experiments. Flat, 5 l pots were filled with a substrate made of a mixture of sand and sandy-loam soil (50 : 50 v : v) which was treated with the following BCAs (same products as above):

- *Beauveria bassiana* – dose equivalent to 100 kg · ha^{-1},
- *Heterorhabditis bacteriophora* – dose equivalent to 1 million nematodes · m^{-2},
- *Steinernema kraussei* – dose equivalent to 1 million nematodes · m^{-2}.

Each treatment consisted of four pots (repetitions) containing 18 or 20 rose hips each (first and second experiment, respectively), which were collected from the same orchard where the field trial was performed. All these hips showed some symptoms of damage due to *R. alternata*. The rose hips were laid on a net positioned over the substrate to avoid direct contact of the larvae present on hips with the soil. The first experiment was started on August 24, 2017 and was assessed five weeks later; the second experiment was started on September 26, 2017 and the assessment was made three weeks later. Fruits were checked for the presence of larvae and the pupae in the soil were counted assessing their vitality and colonization by BCAs.

Field trial to control *Cydia tenebrosana* with biological control agents (BCAs)

Two BCAs were used: *Bacillus thuringensis* subs. *kurstaki* (Dipel WG, Koppert – Poland; dose equivalent to 1 kg · ha^{-1}) and *Cydia pomonella* granulovirus (Madex Max, Biocont – Poland; dose equivalent to 0.1 l · ha^{-1} with the addition of sugar 0.5% to the solution). Both products were applied with a Stihl motor-backpack type sprayer with a volume of 750 l · ha^{-1} of water. A randomized block design was established with four replications, totalling 2500 m^2 per treatment. The products were applied three times, from the beginning of June till the beginning of July, every ten days, according to monitoring data. Assessments of damaged fruits were made on July 19 and August 21, on 100 fruits from each replicate and checked for *C. tenebrosana* larvae in the fruit or their damage. Efficacy was calculated according to Abbott (1925).

Trial to control root-knot nematodes with biological control agents (BCAs)

The trial was carried out in a glasshouse of a rose producing farm located in Boguchwala (49.9847°N, 21.9451°E, Rzeszów county, southeastern Poland) where plants were grown on rock wool substrate. The plants showed visible symptoms of strong growth reduction and, after an initial sampling, it was found that they were infested by *M. incognita* nematodes. The nematode was identified on the basis of the perineal pattern of mature females (Eisenback 1985).

About 1150 plants positioned in a row about 70 m long, were treated with one of the following products dissolved in 100 l water and applied using the fertigation system:

Untreated control: received water treatment at the time of application of other products,

Arthrobothrys oligospora strain AO1 NCAIM 153/2012 – $5 \cdot 10^5$ cfu $\cdot$ g^{-1} (Artis, Kwizda Agro, HU): the first application was performed with a 1% solution and it was followed by daily application of a 0.01% solution for 3 months. The product, dissolved in water at room temperature (on average 25°C) 24 hours before application, was applied with an evening irrigation, to increase the time of contact with the root system;

Abamectin 18 g $\cdot$ l^{-1} (Vertimec 018 EC, Syngenta) at a dose of 2 mg $\cdot$ l^{-1} was applied twice during the trial by irrigation. The second treatment was done 15 days after the initial application.

Plant samples were taken three times during the trial. The first sample was taken 7 days after the initial treatment, while the last was collected at the end of the treatment period. Three plants were uprooted at random along the row at each sampling time. The substrate adhering to the root system was removed by gentle agitation. Root gall severity was assessed using a 0–5 rating scale according to the percentage of galled tissue (0 – 0%; 1 – 1–15%; 2 – 16–25%; 3 – 26–50%; 4 – 51–75%; and 5 – 76–100%). Roots were then carefully separated from the rock wool and aliquots of 20 g of roots were left in water for 2 days. Nematodes were then gathered using a collecting mesh of 36 μm and were examined and counted under a dissecting microscope. Their density was expressed as the number of nematodes per gram of fresh root.

Statistical analysis

Data were analysed by ANOVA, and means separated by the Tukey's test for $p \leq 0.05$. Prior to statistical analysis, percentages data and nematode count were transformed by $\log^{(x+1)}$; non-transformed data are shown.

Results

Population size and control of rugosa rosa fruit pests

Assessment of the population size and fruit damage from *Rhagoletis alternata* and *Cydia tenebrosana* in rugosa rose plantations in different regions of Poland

Three out of four monitored plantations were found to be infested by *R. alternata* (Table 1). The presence of the fly was also recorded in an additional plantation located in the western part of Poland (Krzyżowniki near Poznań, 52.4064° N, 16.9252° E, Poznan district), where no infested fruits were found (data not shown). However, the population size of the insect, as assessed by the average number of captured adults per trap during the whole monitoring period, and the resulting level of damage to fruits in the plantations were quite different: very high (about 75%) in the two plantations located in the north western part of Poland (Dolice, Stargard Szczeciński district), quite limited (1–13%) in the two plantations located in central Poland (Żurawieniec, Kutno district) and north eastern Poland (Ostrów Nowy, Sokółki district).

The population size of *C. tenebrosana* was also different in the plantations and was reverberated in hip damage (Table 1). Parallel to a high number of captured moths, about 30% of fruits were infested by larvae of this pest in the two fields located in north western Poland. On the other hand, almost no damaged fruits were observed in the fields of the other two locations where no moths were attracted to the traps. No adults were captured and no symptoms of damage by either *R. alternata* or *C. tenebrosana* were recorded in the additionally monitored plantation located in western Poland (Krzyżowniki near Poznań).

Effect of soil treatment with biological control agents (BCAs) on *Rhagoletis alternata*

The number of pupae found in the samples from the plantation Dolice I was more than double that of the second plantation (Dolice II) (Table 2). However, very few of them were infected or damaged by the BCAs in both plantations, with no significant differences between BCA-treated and untreated control.

On average, about 30% of the fruits collected on the first date (August 24) and incubated under

Table 1. Average total number of *Cydia tenebrosana* and *Rhagoletis alternata* adults captured by traps and of their larvae found in the hips of rugosa rose on plantations located in different regions of Poland, 2017

Location	*Rhagoletis alternata*			*Cydia tenebrosana*		
	flies captured by trap [n.]	fruits sampling date	hips infested by larvae [%]	moths captured by trap [n.]	fruits sampling date	hips infested by larvae [%]
Dolice field 1 (northwestern Poland)	–	23.06.17	76	–	23.06.17	24
Dolice field 2 (northwestern Poland)	–		70	–		30
Dolice field 1	219	13.07.17	69	79	13.07.17	31
Dolice field 2	92		28	129		21
Żurawieniec (central Poland)	0	14.07.17	1	0	14.07.17	1
Ostrów Nowy near Sokółki (northeastern Poland)	32	23.08.17	13	0	23.08.17	0

Table 2. Effect of soil treatment with entomopathogenic fungi or nematodes on the survival of *Rhagoletis alternata* pupae (Dolice 2017)

Treatment	Dolice I number of pupae		Dolice II number of pupae	
	living	dead or damaged	living	dead or damaged
Control	10 ± 1.1	0	4 ± 0.7	1
Beauveria bassiana	7 ± 2.0	1	1 ± 1.1	0
Heterorhabditis bacteriophora	6 ± 0.9	0	3 ± 0.7	0
Steinernema kraussei	10 ± 2.1	3	4 ± 0.7	0

means ± SD, *n* = 4

Table 3. Average percent of fruits infested by *Rhagoletis alternata* larvae and of pupae collected from the substrate, Skierniewice 2017

Treatment	Experiment I		Experiment II	
	fruits with larvae [%]	larvae forming pupae in the substrate [%]	fruits with larvae [%]	larvae forming pupae in the substrate [%]
Control	33	53.6	21	75.0
Beauveria bassiana	26	54.5	14	69.2
Heterorhabditis bacteriophora	31	50.0	22	76.2
Steinernema kraussei	25	52.4	13	76.9

controlled conditions were infested by *R. alternata* larvae (Table 3). However, only about 50% of the larvae were able to complete the growth cycle and form pupae in the soil substrate. In the second experiment, utilizing fruits collected about a month later, about 20% of the fruits contained larvae, and on average 60% of them were able to form a pupa in the substrate (Tab. 3). Nevertheless, similar to the field trials, all pupae collected in the BCAs-treated substrate from both experiments did not show any symptom of infection.

Effect of biological control agents (BCAs) on the control of *Cydia tenebrosana*

The application of BCAs containing *B. thuringensis* or the *C. pomonella* granulovirus (CpGV) resulted in a significant reduction in the number of damaged hips by *C. tenebrosana* (Table 4). The efficacy of the treatments appeared to be increased after the second assessment.

Effect of biological control agents (BCAs) on the control of root-knot nematodes

The product based on *A. oligospora* reduced the number of nematodes by 10–25% in comparison to the control, with a final efficacy of 66% (Table 5). Abamectin efficacy was higher than *A. oligospora*, though its effect on limiting the number of root-parasitizing nematodes fluctuated during the trial period. The reduced number of nematodes was paralleled by a reduction in the gall index (Table 5).

Table 4. Efficacy of biological control agents (BCAs) in controlling *Cydia tenebrosana* on rugosa rose

Treatment	Damaged fruits [%] date of assessment		Efficacy* [%] date of assessment	
	July 19	August 21	July 19	August 21
Control – water	29.9 ± 5.2 a	14.1 ± 1.7 a	–	–
Bacillus thuringiensis	11.5 ± 5.8 b	3.5 ± 0.9 b	61.5	75.2
Cydia pomonella granulovirus	16.7 ± 1.1 b	4.0 ± 1.9 b	43.9	71.7

Means followed by different letters in a column are significantly different ($p \leq 0.05$) according to Tukeys' test, means ± SD, $n = 4$
*efficacy calculated according to Abbot (1925)

Table 5. Effect of biological control agents (BCAs) on root colonization by root-knot nematodes of roses

Treatments Assessment date	N. nematodes · g^{-1} root			Efficacy* [%]	Gall index
	June 7	June 27	August 30		
Control	115.0 a	61.0 a	89.0 a	–	4
Arthrobothrys oligospora	11.5 b	16.0 b	30.0 b	66	2
Abamectin	1.5 c	13.0 b	7.0 c	92	1

Means followed by different letters in a column are significantly different ($p \leq 0.05$) according to Tukeys' test
*efficacy calculated according to Abbot (1925)

Discussion

Control of air-borne pests with biological control agents (BCAs)

Rhagoletis alternata is a common tephritid fly in central Europe, whose larvae feed on the hypanthium of rose hips. However, the monitoring in plantations of rugosa rose showed that different levels of infestation also depended on the geographical location of the fields. As expected, a high presence of adults in the traps, from June to August, but particularly in July, was associated with a high percentage of damaged fruits. It is interesting that the start of the flight period of the same species differed in various regions of the country (Tartanus *et al.* 2018a).

The high risk of damage and fruit loss derive from the resource (i.e. fruits) exploitation strategy by *R. alternata*, which apparently is not opposed by defence mechanisms in the host plant, and the optimization of the number of infested hips, due to the female marking pheromone which ensures an even distribution of eggs among the hips (Bauer 1998). Therefore, as for other species of the tephritid fly family, considering their biological cycle (Headrick and Goeden 1998), it is quite difficult to find an efficient method to control *R. alternata*. Our trials and experiments were based on the assumption that it could be possible to target both the pupae and the larvae at the moment when they exit from the fruit and begin to develop into pupae in the soil. The selected BCAs were chosen because of their wide range of hosts (Zimmermann 2007; Lacey and Georgis 2012). Furthermore, there have been positive reports about fly species control (Yee and Lacey 2003; Cossentine *et al.* 2010). However, no pupae were found infected after both field and lab experiments, even though a high dosage was applied for all three BCAs. A possible explanation for this result could be the low susceptibility of *R. alternata* to the strains used in our trials (Yee and Lacey 2003; Ekesi *et al.* 2012) and/or an insufficient dose applied (Cossentine *et al.* 2010; Ekesi *et al.* 2012). However, with the aim of applying an IPM-based strategy to control this pest, there are plans to test the use of BCAs to target flying adults, considering the positive results of other similar species (Dimbi *et al.* 2003; Yee and Lacey 2003). Integration of this strategy with mass traps or soil mulching (Tartanus *et al.* submitted) or inter-row tillage (Slauta 1984) could further reduce the damage of rugosa rose fruits by *R. alternata*.

Grapholita tenebrosana was among the most frequent fruit pests of rugosa rose found in a previous survey (Tartanus *et al.* 2018b). In the current monitoring the different levels of infestation depended on the location of the plantations. It is considered to be a species of the Tortricidae family affecting small crops, but it was also recently reported in Siberia in a survey on stone fruit orchards using pheromone traps for *G. molesta* (Akulov *et al.* 2014).

The use of formulations based on *B. thuringensis* subsp. *kurstaki* or CpGV resulted in an efficient limitation of damage caused by *C. funebrana* on the fruits. However, it should be emphasized that the product based on CpGV is a mixture of different strains that was developed to overcome risks of resistance in

C. pomonella populations (Gebhardt *et al.* 2014). Therefore, an alternation of products based on both bacteria and virus would be the most suitable way to reduce the risk of resistance development in this species. Indeed, different strains of *B. thuringiensis* secrete different types of δ-endotoxins, which vary greatly in structure, mode of action and specificity, and can thus be useful in overcoming resistance development (Chattopadhyay and Banerjee 2018). Furthermore, considering that the use of entomopathogenic nematodes to control *C. pomonella* has shown a good efficacy (Georgis *et al.* 2006), this third kind of BCAs could also be included in an IPM strategy for the control of *C. funebrana*.

Control of soil-borne pests with biological control agents (BCAs)

Application of both *A. oligospora* and abamectin resulted in a significant decrease in the infestation of the roots of rose plants grown on a soil-less substrate (rock wool). It is worth noting that the effect on the plants' health status and growth was noticeable at the end of the experiment and was also acknowledged by the owner of the plantation. *A. oligospora* is the most common nematode-trapping fungus found on the rhizosphere of different plants. It is able to form adhesive trapping nets when in contact with nematodes, but also to colonize endophytically the epidermis and cortex regions of monocotyledon and dicotyledon plant roots, but not the vascular tissues (Bordallo *et al.* 2002). Due to its production of several secondary metabolites that appear to be associated with its nematicidal properties, it has been proposed as a possible tool for biological control (Niu and Zhang 2011). However, in this specific case, the unsatisfactory efficacy could be related to the soil-less substrate (rock wool), since this BCA had never been tested on such a growing medium, but was always used on soil (Peter Endes, pers. comm.). The application methodology recommended by the manufacturer involved dissolving the product in lukewarm water about 24 hours before the solution was applied. This was perceived by the farmer as labour and technically demanding.

Abamectin is a blend of B1a and B1b avermectins, macrocyclic lactones produced by *Streptomyces avermitilis*, with low toxicity to non-target beneficial arthropods, and is thus feasible for use under IPM (Khalil 2013). The efficacy of abamectin in controlling root-knot nematodes has been reported for several crops grown under glasshouse conditions (Khalil *et al.* 2012; Huang *et al.* 2014). However, few studies have focussed on the control of plant-parasitic nematodes in soil-less substrates of a growing crop (López-Pérez *et al.* 2011). Interestingly, on a rock wool substrate abamectin was highly effective only when applied as a drench at the time of nematode inoculation, while application after nematode inoculation was largely ineffective (López-Pérez *et al.* 2011). The short-lived, non-systemic activity of the compound was thus overcome in our trial by repeated treatment, which showed the importance of the application method to achieve a good efficacy of the treatment, frequently underestimated in the case of BCAs (Malusá *et al.* 2017).

Conclusions

The application in rugosa rose plantations of different BCAs based on a bacterium and baculovirus or entomopathogenic fungi and nematodes to control *C. tenebrosana* or *R. alternata*, respectively, was not always effective. Factors such as application period and method or pest susceptibility to the used strains can account for such diverse behaviour.

Root-knot nematodes affecting rose plants grown on a soil-less substrate can be successfully controlled by BCAs of fungal origin. However, also in this case, the method of application can play a key role in the level of efficacy and the farmers' willingness to use these tools for the biological control of pests.

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