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Developing a method to test the repulsive effects of pesticides for the parasitic wasp *Aphidius rhopalosiphi*

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Abstract: Methods recommended to test the side effects of plant protection products on beneficial arthropods are mainly based on the assessment of lethal effects after a prolonged contact with a treated surface, inert or natural. The effects of this exposure on reproduction, parasitisation ability or feeding capacity of the surviving organisms are usually followed to detect any sublethal effects. For most of the species, these assessments are performed on untreated units. There is however an increasing interest to also detect sublethal effects in the presence of the pesticide to take into account possible interference of the pesticide residues on the ability of beneficial to control pests.

The aim of this work was to develop a methodology to detect possible repulsive effects of plant protection products on the aphid parasitic wasp *Aphidius rhopalosiphi* and their possible impact on aphid parasitisation and aphid parasitoid efficacy. In a first test, adult wasps were confined on exposure units with two glass plates, one treated and the other one left untreated, in order to identify products to be used in a second set of tests: a product with a strong repellent effect and a lack of direct toxicity to the wasp. a candidate. Five different products were tested and two of them, Stop-Insect and Baïa were considered as repulsive, with significantly more wasps found on the untreated glass plate compared to the treated one.

A second test was performed by confining mated female wasps on plants treated with the insecticidal soap Stop-Insect. The experiments were realized in parallel on plants infested with aphids, where mortality, position of the wasps and parasitisation efficiency were assessed on treated plants and on plants treated with sucrose, according to the classical method recommended for *Aphidius*, where mortality and position of the wasps were assessed on treated plants and parasitisation efficiency later on untreated plants. Results were indicating the repulsive effects of the insecticide with plant infested with aphids, with 30% less wasps observed on plants and 30% less aphid mummies produced. With the plants treated with sucrose, no differences between control and insecticide were observed. Stop-Insect has also an slight effect on the survival of *A. rhopalosiphi* on aphid infested plants with 19.0% corrected mortality, while no effects were detected on sucrose treated plants.

These results are indicating that a plant protection product, because of its possible repellence, can reduce the activity of a parasitic wasp and its efficacy in controlling aphids, despite an apparent "harmless" profile.

Key words: *Aphidius rhopalosiphi*, repellence, sublethal effects

Introduction

The parasitic wasp *Aphidius rhopalosiphi* (Destefani-Perez) (Hym.; Aphidiidae) is an important specific aphid parasite. This species is highly specialised in the parasitism of cereal aphids and can be really effective to control these pests, especially at the beginning of aphid infestation, when the aphid populations are low and spread out (Ankersmit, 1983; Holler, 1990; Hagvar et Hofsvang, 1991). *A. rhopalosiphi* is also a species highly sensitive to pesti-cide and is used as a standard species to detect possible unacceptable effects on non-target arthropods community in the context of the registration process in Europe (Candolfi *et al.*, 1999).

There are several validated methods to assess the effects of pesticide on this species (Mead-Briggs *et al.*, 2000, 2010). These methods included an assessment of lethal effects, by contact with treated glass plates or plants and the assessment of sublethal effects by the evaluation of the reproductive capacity of the female that survived to the lethal assessment phase.

Several authors have emphasized on the possible sublethal effects of pesticides on beneficial arthropods (Desneux *et al.*, 2007). With *A. rhopalosiphi*, several fungicides that had no or nearly no lethal effects on the survival of the wasps reduced significantly the aphid parasitism on plants. These reductions were linked to repellent effects of the products, the wasps spending less time on treated plants than on untreated and therefore were not able to parasitise aphids as in the control (Jansen, 1999). In the test designed for registration purpose on plants, several observations are performed to detect the possible repellence of pesticide for parasitic wasps but these observations are only performed during the first lethal phase of the tests, to check the exposure of the wasps to the products (Mead-Briggs *et al.*, 2010). The assessment of aphid parasitism ability of the wasps is performed later on untreated plants. Therefore, the possible negative impact of pesticide repellence on aphid parasitism cannot be detected with this method. For regulatory purposes, these effects were not considered as critical, as the main aim of the process was to protect non target arthropods populations, regardless their efficiency controlling pests. In the context of IPM, the repellence of pesticides can have an important impact on aphid control by parasitic wasps and there is a need to focus on this subject, especially for products that have no or little lethal effects.

The aim of this work is to investigate in the development of specific methods to detect the repellence of pesticide for adult of the parasitic wasp *A. rhopalosiphi*, with experiments conducted both on glass plates and on plants.

Material and methods

Selection of products and glass plates test

Several pesticides were reselected to be tested first on glass plates for their possible repellent effects for the wasps, on basis of previous experiments. Products that could be toxic for aphids or for wasps were rejected to facilitate the interpretation of the results. They were tested at their recommended field rate. The products tested are listed in Table 1.

Table 1. List of products assessed for repellence for *A. rhopalosiphi* on glass plates.

Trade name	Active ingredient	Formulation type and a.i. content	Tested rate	Possible repellence origin
Baia	Etephon	SL, 480g/l	1.5 l/ha	Oily/viscous formulation
Cupravit forte	Copper oxychlorid	WP, 500g/kg	5 kg/ha	Abrasive surface
Hermovit	Sulfur	WG, 800g/kg	5 kg/ha	Abrasive surface
Ortiva	Spiroxamine + tebuconazole	EC, 250 + 133 g/l	1 l/ha	Highly volatile solvent
Stop-Insect	Lineseed oil	Oil, nearly pure	12 l/ha	Oily/viscous formulation

The units were similar to those used in standard tests with this species (Mead-Briggs *et al.*, 2000) but the products were applied with the help of a Burgerjon spray tower only on one of the two glass plates. For the control, two untreated glass plates were used, with one randomly considered as treated. According to the glass plate and the frame surface, one glass plate corresponded to 36% of the arena surface. 1 to 2 hours after treatment, when the pesticide residues were dried, the units were assembled and 10 *Aphidius* (5 males, 5 females) were released in each arena. There were 5 replicates per product and for control. The position of the wasp (treated glass plates or untreated glass plates and frame) were observed 10 times in each unit 1 hour, 24 hours and 48 hours after release. The percentage of the wasps on the treated part of the arena was calculated, not taking into account dead or missing wasps. The tests were performed in a climatic room at 20°C ± 2°C, 60-90% RH with a maximum of 1000 lux on basis of a 16/8 L/D photoperiod.

The percentage of wasps on the treated glass plates were then transformed (arcsin) before being compared with the help of a one-way ANOVA analysis followed by a Tukey Test at $p = 0.05$.

Test on plants

The organic insecticide Stop-Insect was tested on plants for effects on *A. rhopalosiphi* according two different methods. The first was the classical method designed for registration (Mead-Briggs *et al.*, 2010). This method included a first assessment of lethal effects on treated plants and secondly an assessment of reproduction ability on untreated plants. The second methods was an adaptation of the classical method with the two phases performed in a same time on the treated plants, to be able to put in evidence the effects of the repellence on the aphid parasitism.

For the classical test, the exposure units were made of a barley seedlings (5-8 plants, 8-10cm high) covered with a ventilated perspex cage with a fine layer of sand for the observation. A 25% w/v sucrose solution was applied till run-off 2-3 hours before product application to simulate aphid-honeydew, feed the wasp and attract them on the plants. This concentration was selected from a previous experiment to maximise wasp attraction. After product application, 5 female wasps were released in each unit. The position of the wasps (treated plants or untreated unit walls and sand) was noted 5 times for each replicate 1 hour, 24 hours and 48 hours after release. After 48 hours of exposure, the units were dismantled, the percentage of survival assessed and the surviving wasps released in fertility units, made of barley seedlings infested with 60 *S. avenae* aphids. All surviving wasps from one exposure unit were released in one fertility units. This is a modification of the classical methods in order to compare the results obtained with the adapted method on basis on the same test design. After 48 hours, wasps were removed from the fertility units and aphid mummies were left to developed. 10 to 12 days later, the fertility units were dismantled and the aphid mummies were counted.

For the adapted method, the exposure units were similar except the fact that the barley seedlings were infested with 60 *S. avenae* aphids introduced 24 hours before the application of the product in order to produce honeydew and attract the wasps on the plants, instead of the sucrose application. After product application, 5 female wasps were released in each unit and the position of the wasps (treated plants or perspex walls and sand) was noted 5 times per replicate 1 hour, 24 hours and 48 hours after release. After 48 hours of exposure, the wasps were harvested. The plants were then kept and 10 to 12 days later, the aphids mummies that developed were counted.

Both experiments were performed in a same time, with the same batch of wasps. Stop-Insect was applied in 400l water/ha with the help of a boom sprayer with AZO 110 flat fan nozzles. Plants for the two experiments were treated together. Each experiment included a water-treated control. There was a total of 20 units of 5 wasps, with 5 replicates for Stop-Insect and 5 control with the classical and adapted method.

The tests were performed in the same conditions than with the glass plates, exept the light, provided by Sodium lamp (250W Son-T Agro) with about 8-10.000 lux on basis of a 16/8 L/D photoperiod. Statisitcal analysis was similar.

Results and discussion

Test on glass plates

The percentage of wasps found on the treated glass plates in the exposure units are illustrated in Figure 1. Hermovit, Cupravit and Orca did not differ from the control 1 hour and 24 hours after product application, with Aphidius wasps found in the range of 20% to 37% on the treated glass plate or considered as treated for the control. With Stop-Insect and Baia, the percentage of wasps was around or less than 10% and significantly different to control for the three observation periods.

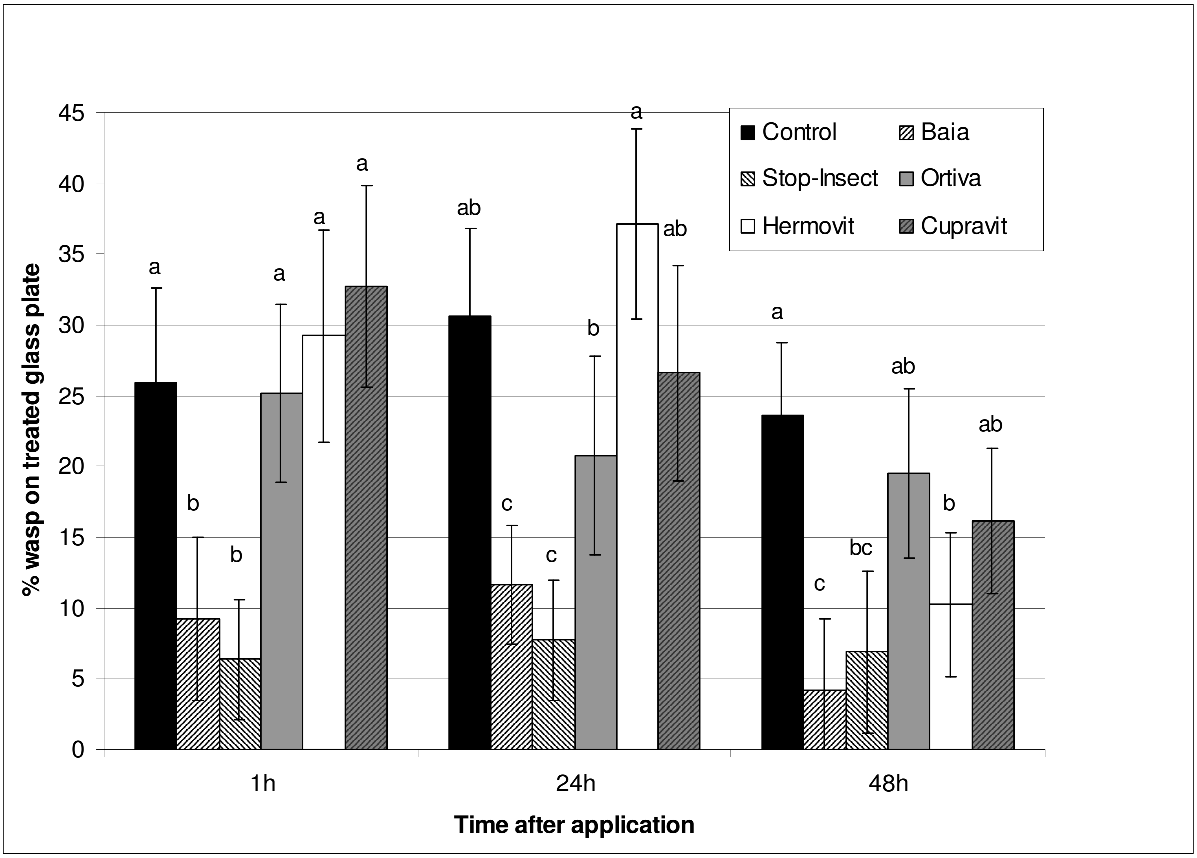

Figure 1. Percentage of wasps found on the treated glass plates (10 observations of 5 replicates/ product at each occasion).

Test on plants

The results of the tests performed on plants with the insecticide Stop-Insect obtained with the two different methods are illustrated in Figure 2 (mortality assessments), Figure 3 (% of wasps observed on the plants during the exposure) and Figure 4 (number of aphid mummies produced).

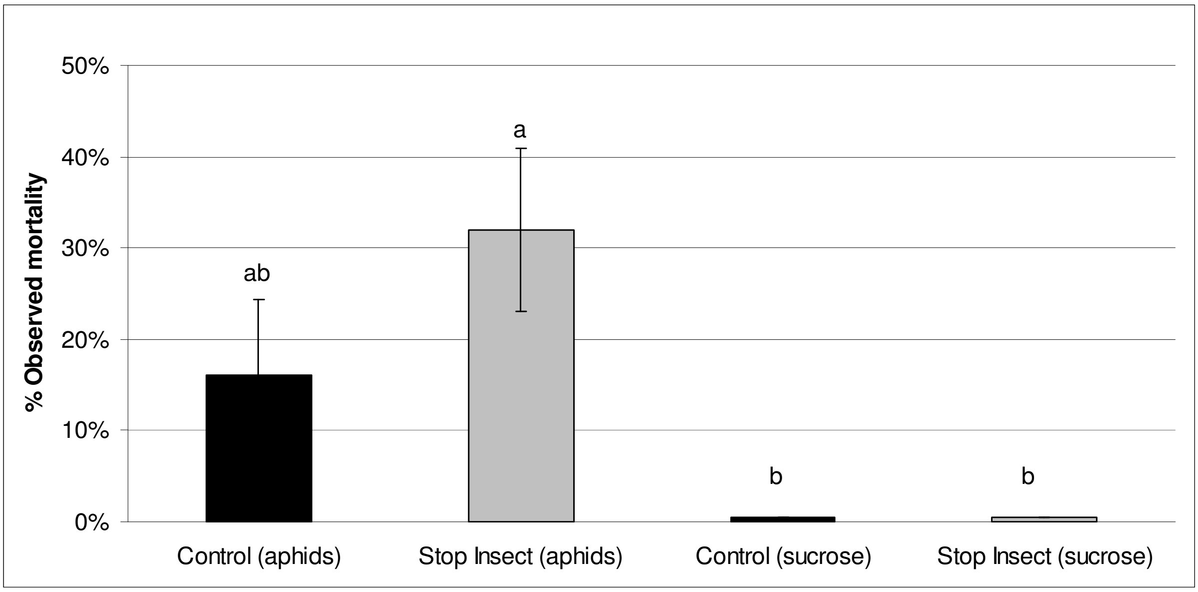

Figure 2: Mortality of *A. rhopalosiphi* on plants treated with Stop-Insect according to two different methods for exposure: plants infested with aphids (adapted method) or plants treated with sucrose (classical method).

With the methods where the plants used for exposure were infested with aphids, the observed mortality reached 32% with Stop-Insect and was significantly higher than with plants treated with the sucrose solution where no mortality was observed. This higher mortality is probably the consequence of a longer exposure period of the wasps to the product (Figure 3), as indicated by the percentage of wasps found on the treated plants with aphids (30%) compare to the treated plants with sucrose (5.0%). There was also large difference of the plants attractiveness between the control aphid infested plants and the control sucrose plants, with 43% of the wasps found on plants with aphids compare to 11% only with sucrose. The analysis of the intermediate observations at 1, 24 and 48 hours were indicating that these differences between the two systems were not so important for the first observations performed after 1 hour but there was an increase with the time, indicating that the female wasps were attracted to the plants by the sucrose for feeding reasons at the beginning of the exposure but were not attracted later when they were satiated. With the aphids, the attractiveness seemed to be more constant during the time and this could explain the higher the percentage of wasps found on plants. Unfortunately, the statistical analysis of the intermediate results did not give any significant differences, probably because of the too low numbers of intermediate observations and the high variability of such observations.

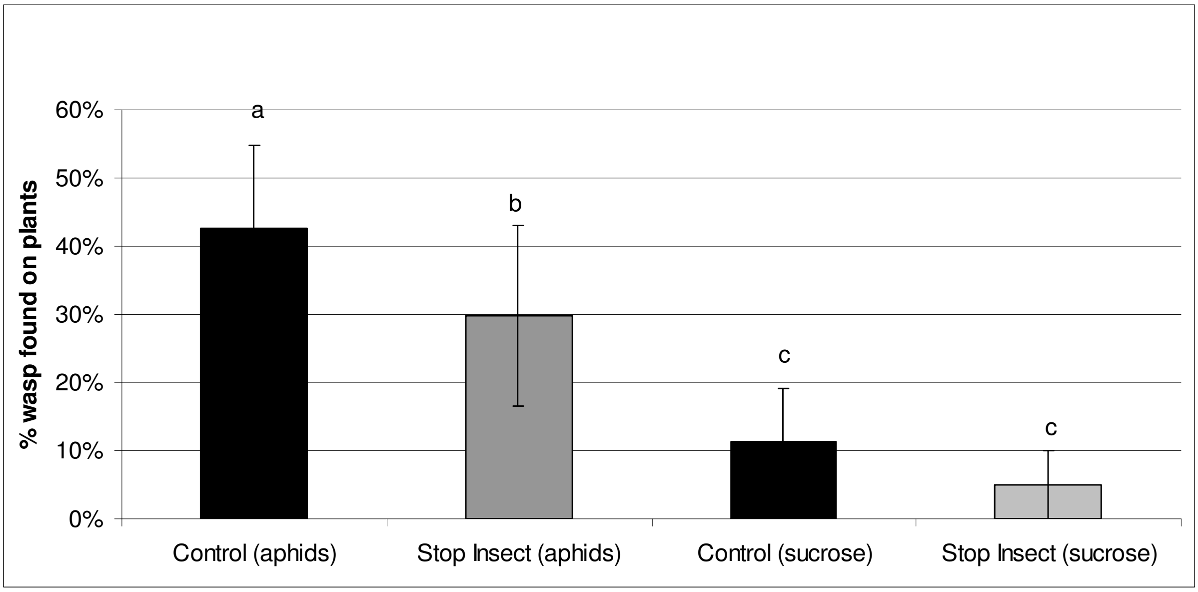

Figure 3: Percentage of *A. rhopalosiphi* wasps found on plants treated with Stop-Insect according to two different methods for exposure: plants infested with aphids (adapted method) or plants treated with sucrose (classical method). 15

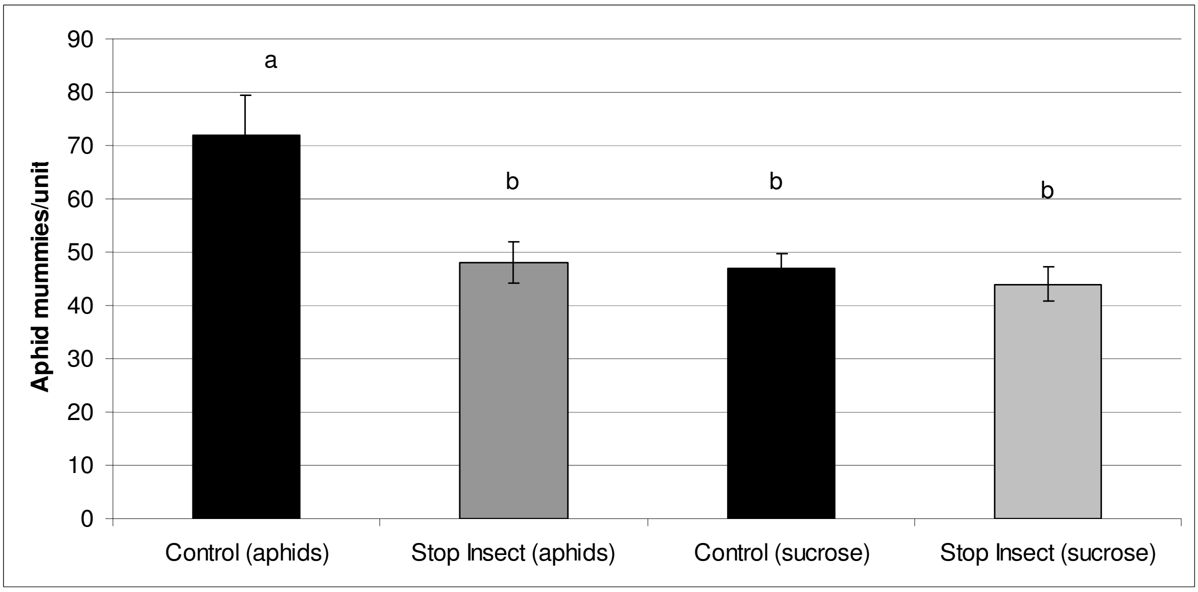

Figure 4: Fertility performance of *A. rhopalosiphi* wasps exposed to Stop-Insect according to two different methods: plants infested with aphids (adapted method) or plants treated with sucrose (classical method).

When the effects on the reproduction were assessed after the 48h-exposure on plants treated with the insecticide, according to the methods developed for registration and using untreated aphid-infested plants (Figure 4), no effects were detected, indicating that Stop-Insect has no impact on the reproduction ability of the wasps. When the parasitic wasps were confined on plants treated with Stop-Insect and infested with aphids, a reduction of 30% of the aphid parasitism was observed compared to the control. As the product was proven to be harmless on reproduction for adult wasps, this reduction was probably linked to the repellence of the product and the diminution of the foraging time of the wasps on the plants.

The results obtained in this study have shown that one product, Stop-Insect, could reduce the ability of *A. rhopalosiphi* wasps to parasite aphids because of a probable repellent effects. No significant effects on mortality and reproduction were detected when the product was tested according to the methods recommended for registration. However, when the fertility assessments were performed on plants treated with the insecticides, which is not performed with the recommended method, significant effects were detected on reproduction and these effects were correlated with a modification of the wasp behavior, with a reduction of 30% of the time spent on the treated plants compared to the control. The exposure was also found to be higher with aphid infested plants than with sucrose treated plants and the mortality was also higher with aphid infested plants than with sucrose treated plants.

Even if the effects observed with Stop-Insect were limited and obtained in the laboratory, these results suggested that sublethal effects could have a measurable impact on *Aphidius* aphid control efficacy, despite an harmless profile when tested according to the methods recommended for registration.

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