Pesticides and Beneficial Organisms
IOBC/wprs Bulletin Vol. 23 (9) 2000
pp. 73 - 80

Toxicity of fungicides to adults of *Aphidius rhopalosiphi* DeStefani-Perez (Hymenoptera: Aphidiidae) in laboratory tests with field - treated wheat

Jansen, J.-P.
Laboratoire d'Ecotoxicologie, Ministry of Agriculture, Agricultural Research Centre, Gembloux, Department of Biological control and Plant genetic resources, Gembloux, Chemin de Liroux 2, 5030 Gembloux, Belgium

Abstract
Effects of twelve fungicides used in wheat were assessed on adults of *A. rhopalosiphi* in the laboratory using plants treated in the field. Plots of wheat were treated at earring in the field and plants were brought back in the laboratory directly after treatment. Sections of stem with last leaves and ears were used to form exposure units. More than 100 *S. avenae* aphids were added in each unit and 3 males and 3 females adults wasps were released in it 1h later. Wasps were observed during exposure to detect repellent effects of products and mortality was checked 24h later. Aphids were harvested and put on uninfested barley seedlings. Mummies that developed were counted 10-12 days later and reduction of beneficial capacity calculated according to fertility performance.

Amistar pro, Bufalo, Duett, Libero, Mentor, Punch SE, Sportak delta, Stereo, Triumph and Zenit were harmless to adult wasps, aphid parasitism being not reduced by more than 25 % compared with the control. Boscor was classified as slightly harmful, with a parasitism reduction of 26.2 % and Matador was moderately harmful, with a reduction of 50.5 %.

These effects were much less severe than those obtained with glass plate tests or with plants treated in the laboratory till run-off. Exposure of wasps to plants treated in the field with fungicides showed that these products, with few exceptions, did not reduce the ability of females to parasite cereal aphids. Therefore, side effects of these fungicides on adults of *A. rhopalosiphi* are not expected in the field.

Keywords: *A. rhopalosiphi*, fungicides, extended laboratory test, wheat.

Introduction

Aphidiid wasps are considered as a major aphid natural enemy in numerous crops. Their action is one of the factors contributing to the control of aphid populations (Stary, 1988). In wheat, several authors have reported their beneficial activity. As aphidiid wasps can be active at low temperature (Ankersmit, 1983) and as they can parasitize aphids at very low aphid/shoot densities (Höller, 1990, Dedryver *et al.*, 1991), their action on cereal aphid populations can be important, especially at the beginning of aphid infestation (Hagvar & Hofsvang, 1991). Different aphidiid species are found in cereals and *A. rhopalosiphi* is one of the most commont found in wheat in Belgium (Latteur, 1973, Latteur & Destain, 1980) and one of the most important in Western Europe (Stary, 1970, Dedryver *et al.*, 1985).

During periods when activity of *A. rhopalosiphi* wasps is especially important, wheat generally receives at least one fungicide application at earring (GS 59, Zadoks *et al.*, 1974) with sometimes a previous treatment at first node (GS 31) or last leaf (GS 39), depending on disease pressure and climatic conditions (Meuus, 1995). Different studies have shown that several fungicides can be toxic for aphidiid wasps in the laboratory on glass plates, on natural

substrate (Hassan *et al.*, 1988, 1991 & 1994, Kühner *et al.*, 1985) or in the field (Sotherton *et al.*, 1987). In particular, toxicity of fungicides used in wheat in Belgium was assessed in the laboratory on glass plates and on plants on *A. rhopalosiphi* (Jansen, 1998).

According to these studies, several fungicides, especially associations of two active ingredients, were still toxic and further testing in semi-field or field conditions need to be undertaken for an appropriate assessment of their effects. However, semi-field test with aphidiid wasps are rather difficult and laborious. Furthermore, it is not always possible to check exposure of wasps on treated plants in the field and this particular topic strongly limits the value of such semi-field experiments. It can lead to a false decision, e.g. by classifying harmless a harmful product, because there was no real exposure in the field, wasps staying most of the time on untreated cages or frame. Thus, it was decided to test fungicides in the laboratory with plants treated in the field. In this way, exposure of wasps to the test substance can easily be checked and insects were exposed to treated plants in a similar way as in a field or semi-field test. Another advantage of this particular method was that results obtained can be compared to an extended lab-test previously performed with the same products, using wheat seedlings treated till run-off in the laboratory instead of plants treated in the field (Jansen, 1998).

Material and methods

Chemicals

Fungicides tested in this study were all used in wheat to control foliar and ear diseases. They contained each two active ingredients and were tested at the maximum recommended field rate for Belgium. Details of trade name, formulation, actives ingredients and tested rate are given in table 1. These fungicides were selected because they were toxic for adults of *A. rhopalosiphi* on glass plates test and/or on extended-lab test on plants (Jansen, 1998).

Table 1. Fungicides applied in the field and tested on *A. rhopalosiphi*.

trade name	formulation	active ingredients	tested rate
Amistar Pro	SE	Azoxystrobine + fenpropimorph	200g + 560g
Boscor	EC	Fenpropidin + fenpropimorph	188g + 562g
Bufalo	EC	Fenpropidin + tebuconazole	300g + 200g
Duett	SC	Carbendazim + epoxiconazole	125g + 125g
Libero	SC	Carbendazim + tebuconazole	200g + 300g
Matador	EC	Tebuconazole + triadimenol	250g + 125g
Mentor	SE	kresoxym-methyl + fenpropimorph	105g + 210g
Punch SE	SE	Carbendazim + flusilazol	100g + 200g
Sportak Delta	SC	Cyproconazole + prochloraz	60g + 400 g
Stereo	EC	Cyprodinil + propiconazole	500g + 125g
Triumph	SC	Chlorothalonil + flusilazole	500g + 200g
Zenit	SE	Fenpropidin + propiconazole	375g + 125g

Application of chemicals
Fungicides were applied at earring (GS 59) in the field on rectangular wheat plots (3m x 10m). Plots were delimited in a wheat field (cv „Pajero") conducted according Good Agricultural Practice and left untreated with fungicides. Test products were applied with the help of a 3 m ramp bearing 6 Azo 110° nozzles at 50 cm spacing, connected to a knapsack sprayer. Work pressure was 2.5 b and products were diluted and applied in a volume equivalent to 300 l of water/ha. Products were applied in 3 sets of 4, at 2-3 days interval, with an untreated control plot in each group. One plot was treated per product.

Exposure units
30 minutes to 1h after treatment, when residue of product has dried, 30 to 40 stems of wheat with ear, first and second leaves were selected randomly in treated plots, avoiding first and last meter and two lateral 50 cm-wide areas of plots as border. Plants were put in a plastic tray and immediately brought back in the laboratory distant of about 500m from the field.

In the laboratory, wheat plants were carefully inspected and aphids and aphid predators (syrphid eggs or larvae, ladybirds) were removed and terminal sections of wheat stem, with last leaf and ear were thus immediately used to form exposure units. Exposure units were made of 4 stems of about 30cm length planted in pots (Ø: 12cm) containing moistened vermiculite (Sibli, grade 5). 5 units were assembled for each product and for control. Vermiculite was directly covered with sand (1cm height) to form an uniform untreated surface and a cylindrical Perspex cage (Ø: 12cm, H: 25 cm) was added on each unit. Top of cage and two rectangular cutaway were covered with nylon netting for ventilation. Ear and last leaves of wheat were selected to represent a worst-case field exposure in the laboratory.

Conduct of trials
Immediately after being assembled, about 100 to 120 cereal aphids (*S. avenae*) of all growth stages, picked up in aphid mass rearing on barley seedlings, were added to each unit. They were left to get established on the plants for 1h. Six 2 - 48h old adults of *A. rhopalosiphi* (3 males, 3 females) were released in each unit for a 24 h period. Wasps were observed 10 times in each unit and number of wasps found on plants or on sand or perspex cage (untreated) were noted. Percentage of wasps found on plants were calculated and compared to control to detect possible repellent effects of products. Percentages of wasps found on plants were compared with the control with the help of a Student *t*-test (Dagnelie, 1974).

24 h after being released, units were disassembled and living wasps counted in each unit. Wasps not found were considered as dead. Observed mortalities were calculated for each treatment and corrected with the mortalities of the corresponding control according to Abbott (Abbott, 1925). Aphids were removed from the plants and put on barley seedlings. Mummies were left to develop and counted 10-12 days later. Mean number of mummies produced in each unit were compared to control with the help of a Student *t*-test (Dagnelie, 1974). Reduction in beneficial capacity was calculated on basis of mean mummy production, with the help of the following formula :

$$E(\%) = 100 - 100*(Ro/Rc)$$

Ro = mean number of mummy/unit, test product , Rc = mean number of mummy/unit, control.

According to the E value, products were classified in one of the four IOBC category for extended-lab test (Hassan, 1994):

1 – „harmless", E < 25%
2 – „slightly harmful", 25% < E < 50%
3 – „moderately harmful", 50 % < E < 75 %
4 – „harmful", E > 75 %

Results

Results of the 3 sets of experiment are listed in table 2. Control mortalities ranged from 12.4 to 13.3 %. With plants treated in the field with fungicides, the maximum corrected mortalities obtained was 46.0 % (Libero), but a majority of corrected mortalities were around or below 30%.

Table 2. Exposure in the laboratory of adults of *A. rhopalosiphi* to wheat plants treated in the field with fungicides. Observed and corrected mortalities (5 x 6 wasp/product), observation of wasps during exposure (10 observations x 5 units/product), mummies production/unit (5 replicates/product), IOBC rating for extended-lab test.

Trade Name	observed mortality	corrected mortality	% wasp on plants	mummies/ unit	E	IOBC class
set 1						
Control	12.4%	-	32.6a	21.0a	-	
Boscor	41.7%	34.0%	22.8b	15.5a	26.2%	2
Bufalo	43.3%	36.7%	25.6a	21.8a	-3.8%	1
Matador	36.7%	27.1%	28.0a	10.4a	50.5%	3
Sportak delta	37.3%	28.3%	39.8a	16.5a	21.4%	1
set 2						
Control	13.3%	-	28.6a	21.6a	-	
Amistar pro	20.0%	7.3%	30.1a	29.2a	-35.2%	1
Libero	50.0%	46.0%	30.6a	21.2a	1.8%	1
Mentor	40.0%	32.3%	23.7a	22.6a	-4.6%	1
Punch SE	30.0%	18.3%	28.3a	27.0a	-25.0%	1
set 3						
Control	12.5%	-	35.4a	16.4a	-	
Duett	29.2%	16.5	22.8b	19.6a	-19.5%	1
Stereo	12.5%	-0.7	37.6a	17.6a	-7.3%	1
Triumph	27.8%	16.8	17.5b	13.6a	17.1%	1
Zenit	22.2%	11.6	27.3b	17.4a	-6.1%	1

Numbers followed by the same letter are not different in a significant way (Student *t*-test at p=0.05 level).

Observations of wasps during exposure showed that some of the fungicides tested had repellent effects on adult wasps. With plants treated in the field with Boscor (set 1), Duett, Triumph and Zenit (set 3), a significant reduction of percentage of wasps found foraging or staying on treated plants was observed compared to control.

With Boscor and Matador, production of mummies was reduced by more than 25 % and 50%, respectively, resulting in classifying these products as slightly and moderately harmful according to the IOBC classification for extended-lab test. However, values obtained were not different from control in a significant way ($p=0.05$) and reduction of beneficial capacity was closed to the limits of class 1 – harmless (25%) and 2 – slightly harmful (50 %).

Results obtained with plants treated in the field were compared in table 3 with those previously published on glass plates and wheat seedlings (Jansen, 1998) except for new results with Stereo, Matador and Mentor. Products were tested with the different methods at the same rates, corresponding to the maximum recommended field rate. Comparison of results showed that exposure of adult wasps to plants treated in the field resulted in lower effects compared to exposure on glass plates or extended-lab tests carried out with wheat seedlings treated till run-off. Differences between glass plate test results and extended-lab test results can be explained by a repellent effect of fungicides on adult parasitoid, causing female wasps to spend less time on plants treated with Duett or Stereo compared to control and subsequently reducing their ability to parasite aphids. In a general way, there are some questions about the possible effects on the behaviour of beneficial arthropods with products that were harmless on glass plates and don't need any further test according to the IOBC testing scheme. Anyway, repellent effect of Duett and Stereo was not observed in the laboratory with plants treated in the field.

Table 3. Comparison of IOBC toxicity class obtained in the laboratory with wheat plant treated in the field (extended lab test 2) with those previously obtained with the same products on glass plates or on wheat seedlings treated till run-off (extended-lab test 1).

trade name	actives ingredients	glass plate	extended lab test 1	extended lab test 2
Amistar pro	azoxystrobine + fenpropimorph	3	3	1
Boscor	fenpropidin + fenpropimorph	-	4	2
Bufalo	fenpropidin + tebuconazole	-	4	1
Duett	carbendazim + epoxiconazole	2	4	1
Libero	carbendazim + tebuconazole	-	4	1
Matador	tebuconazole + triadimenol	-	4	3
Mentor	kresoxym-methyl + fenpropimorph	3	3	1
Punch SE	carbendazim + flusilazol	4	4	1
Sportak delta	cyproconazole + prochloraz	4	1	1
Stereo	cyprodinil + propiconazole	2	4	1
Triumph	chlorothalonil + flusilazole	4	3	1
Zenit	fenpropidin + propiconazole	-	4	1

Discussion

Results obtained in these experiments show that when adults of *A. rhopalosiphi* were exposed in the laboratory to plants treated in the field with fungicides, no or little effects are observed. With fungicides that were toxic on previous tier test, including an extended-lab test on wheat seedlings, and tested at the same rates, results were much less severe with plants treated outside. In fact, methods used in the extended lab-test and with plants treated in the field were quite similar, except two important parameters that can explain such differences: application of products and development of parasite larvae inside aphids.

With methods used in this study, plants were treated at maximum recommended field rate with technical equipment's close to those used by farmers and with pesticide repartition on plants close to real field situation. In previous extended-lab tests, wheat seedlings were treated till run-off with fungicides. Several studies have shown that repartition of pesticides on wheat or barley plants treated in the field greatly varied between different part of plants and were much less than recommended field rate. Chemical analysis of pesticides residues applied on cereals have shown that ear and last leaf receive more or less 20 to 25% of the field rate, the rest being distributed on stem, other part of plants and soil (Cilgi & Jepson, 1992, Longley & Jepson, 1997). If it is supposed that seedlings treated till run-off receive more or less the full field rate, it can be concluded that with this kind of pesticide application, tested rates were overestimated in the extended lab test 1 compared with field practice.

Other differences between the two methods concern the development of parasitised aphids. With plants treated in the field, as it was not assumed that plants that were cut and inserted in moistened vermiculite could be an appropriate food source for aphids during parasitoid larvae development, aphids were removed at the end of the exposure period and transferred to untreated barley seedlings. Thus, contact between aphids and test products was limited in time and probably in magnitude. With previous extended-lab test, aphids were put on plants before fungicide application and parasitised aphids were left to develop on treated plants. Thus, toxicity of fungicides on parasitoid larvae could interfere with assessment of side-effects on adults. As most of tested fungicides were systemic, parasitoid larvae can be in contact with these products when aphids feed on treated plants and ingest phloem sap contaminated by fungicides. Unfortunately, activity of pesticides and especially fungicides on larval stages of parasitoid is not well known. According to Delorme (1976) and Krespi *et al.* (1991), survival of aphidiid larvae was strictly dependant on survival of host. The case of a product that killed a parasitoid larvae inside the aphid without killing the aphid has never been reported. However, this topic has also never been intensively studied and specific studies need to be undertaken.

Conclusions

According to the results obtained in this study, it can be concluded that exposure of adults of *A. rhopalosiphi* to wheat plants treated in the field using a field sprayer with fungicides found toxic in lower tier tests did not result in marked toxic effects. The tested fungicides will probably be without effects on parasitoid populations in the field. However, the impact of these products on other growth stages than adults, especially larval stages, must also be assessed and specific methods need to be developed.

References

Abbott, S. W. 1925: A method of computing the effectiveness of insecticides. Journal of Economic Entomology, 18: 265-267

Ankersmit, G. W. 1983: Aphidiids as parasites of the cereal aphids *Sitobion avenae* and *Metopolophium dirhodum*. In "Aphid antagonists, proceedings of a meeting of the EC experts group", Portici, Italy, 1983. Ed. by R. Cavalloro, Balkema, Rotterdam

Cilgi, T. & Jepson, P. 1992: The use of tracers to estimate the exposure of beneficial insects to direct pesticide spraying in cereals. Annals of Applied Biology 121: 239-247.

Dagnelie, P. 1974: Théorie et méthodes statistiques, Vol. 2, ed. by Duculot SA, J., Gembloux, Belgium.

Dedryver, C. A., Ankersmit, G. W., Basedow, T., Bode, E., Carter, N., Castanera, P., Chambers, R., Dewar, A., Keller, S., Latteur, G., Papierok, B., Powell, W., Rabbinge, R., Sotherton, N. W., Sunderland, K., Wilding, N. & Wratten, S. D. 1985: Rapport de synthèse sur les activités du sous-groupe "Ecologie des pucerons des céréales". IOBC/WPRS Bulletin 1985/VIII/3: 57-104.

Dedryver, C. A., Creach, V., Rabasse, J-M. & Nenon, J-P. 1991: Spring activity assessment of parasitoids in western France by experimental exposure of cereal aphids on trap plants in a wheat field. IOBC/WPRS Bulletin 1991/XIV/4: 82-93.

Delorme, R. 1975: Evaluation en laboratoire de la toxicité pour *Diaeretiella rapae* (Hym.; Aphidiidae) des pesticides utilisés en traitements des parties aériennes des plantes. Entomophaga 21: 19-29.

Hagvar, E. B. & Hofsvang, T. 1991: Aphids parasitoids (Hymenoptera, Aphidiidae): biology, host selection and use in biological control. Biocontrol News and Information 12: 13-41.

Hassan, S. A. 1994: Activities of the IOBC/WPRS Working group "Pesticides and Beneficial Organisms". IOBC/WPRS Bulletin 17 (10), 1-5.

Hassan, S. A., Bigler, F., Bogenschütz, H., Boller, E., Brun, J., Chiverton, P., Edwards, P., Mansour, F., Naton, E., Oomen, P.A., Overmeer, W. P. J., Polgar, L., Rieckmann, W., Samsøe-Petersen, L., Stäubli, A., Sterk, G, Tavares, K., Tuset, J. J., Viggiani, G. & Vivas, A. G.,1988: Results of the fourth joint pesticide testing programme by the IOBC/WPRS-Working Group "Pesticides and Beneficial Arthropods". Journal of Applied Entomology 105: 321-329.

Hassan, S. A., Bigler, F., Bogenschütz, H, Boller, E., Brun, J., Calis, J. N. M., Chiverton, P., Coremans-Pelseneer, J., Duso, C., Lewis, G. B., Mansour, F., Moreth, L., Oomen, P. A., Overmeer, W. P. J., Polgar, L., Rieckmann, W., Samsøe-Petersen, L., Stäubli, A., Sterk, G, Tavares, K., Tuset, J. J.& Viggiani, G. 1991: Results of the fifth joint pesticide testing programme carried out by the IOBC/WPRS-Working Group "Pesticides and Beneficial Arthropods". Entomophaga 36: 55-67

Hassan, S. A., Bigler, F., Bogenschütz, H., Boller, E., Brun, J., Calis, J. N. M., Coremans-Pelseneer, J., Duso, C., Grove, A., Heimbach, U., Helyer, N., Hokkanen, H., Lewis, G. B., Mansour, F., Moreth, L., Polgar, L., Samsøe-Petersen, L., Sauphanor, B., Stäubli, A., Sterk, G, Vainio, A., Van de Veire, M., Viggiani, G.& Vogt, H. 1994: Results of the sixth joint pesticide testing programme of the IOBC/WPRS-Working Group "Pesticides and Beneficial Arthropods". Entomophaga 39: 107-119

Höller, C. 1990: Overwintering and hymenopterous parasitism in autumn of the cereal aphid *Sitobion avenae* (F.) in northern FR Germany. Journal of Applied Entomology 109: 21-28

Jansen, J-P. 1998: Effects of wheat foliar fungicides on the aphid endoparasitoid *Aphidius rhopalosiphi* DeStefani-Perez (Hym.; Aphidiidae) on glass plates and on plants. Journal of Applied Entomology 123: 217-223

Krespi, L., Rabasse J. M., Dedryver, C. A. & Nenon J. P., 1991: Effect of three insecticides on the life cycle of *Aphidius uzbekistanicus* Luz. (Hym.: Aphidiidae). Journal of Applied Entomology 111: 113-119

Kühner, C., Klingauf, F. & Hassan, S. A. 1985: Development of laboratory and semi-field methods to test the side-effect of pesticides on *Diaeretiella rapae* (Hym : Aphidiidae) Med. Fac. Landbouww. Rijksuniv. Gent 50/2b: 531-538.

Latteur, G. 1973: Etude de la dynamique des populations des pucerons des céréales - Premières données relatives aux organismes aphidiphages en trois localités différentes. Parasitica 29: 134-151.

Latteur, G. & Destain, J. 1980: Etude de l'action des champignons entomopathogènes et des hyménoptères parasites inféodés aux populations de *Sitobion avenae* (F.) et de *Metopolophium dirhodum* (Walk.) dans le champ expérimental de Milmort en 1978 et 1979. IOBC/WPRS Bulletin, 1980/III/4: 11-17.

Longley, M. & Jepson, P. 1997: Effects of life stage, substrate, and crop position on the exposure and susceptibility of *Aphidius rhopalosiphi* De Stefani-perez (Hymenoptera: Braconidae) to deltamethrin. Environmental Toxicology and Chemistry,16(5): 1034-1041.

Meuus, P. 1995: Analyse de l'incidence de la protection fongicide sur les rendements du froment et de l'orge d'hiver en 1994 par rapport aux résultats obtenus au cours des dix dernières années. Parasitica 51: 81-92.

Sotherton, N. W., Moreby, S. J. & Langley, M. G. 1987: The effects of the foliar fungicide pyrazophos on beneficial arthropods in barley fields. Annals of Applied Biology 111:75-87.

Stary, P. 1988: Natural enemies: Parasites. In: World Crop Pests, Aphids, their biology, natural enemies and control. Ed. By A. K. Minks and P. Harrewijn, Vol. 2B: 171-188.

Stary, P. 1970: Biology of Aphid parasitoids (Hymenoptera: *Aphidiidae*) with respect to integrated control. Series Entomologica, Dr W. Junk. Den Haag

Zadoks, J. C., Chang, T. T. & Konzak, C. F. 1974: A decimal code for the growth stages of cereals. Weed Research 14: 415-421.