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Susceptibility of cocooned pupae and adults of the parasitoid *Microplitis mediator* to selected insecticides

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Abstract The braconid parasitoid *Microplitis mediator* (Haliday) is a key natural enemy of the cabbage moth, *Mamestra brassicae* (L.), in Europe. In the context of an Integrated Pest Management approach, the use of selective insecticides is essential for the conservation of naturally occurring beneficial arthropods. The present laboratory study investigated the side effects of six insecticides applied at recommended field rates on adults and cocooned pupae of *M. mediator*. Male and female parasitoids were paired in drum cells contaminated with dry residues of insecticides. Besides lethal effects after 24 h, parasitization capacity and longevity of the surviving parasitoids was evaluated. Lethal effects on cocooned pupae were also investigated by assessing adult emergence from treated cocoons. Pirimicarb caused 100% adult mortality after 24 h, whereas the other tested insecticides caused no direct toxic effects. However, sub-lethal effects in terms of reduced parasitization activity, percentage of parasitism or female longevity were found for flonicamid, pymetrozine, spinosad and thiacloprid. Spirotetramat shortened only male longevity. Adult emergence from treated cocoons was reduced only by flonicamid and pymetrozine.

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The cabbage moth, *Mamestra brassicae* (L.), is a key pest of *Brassica* crops and frequently causes serious economic damage (Pfiffner *et al.* 2009). Several parasitoids have been reported to attack *M. brassicae* in European cabbage crops. The braconid *Microplitis mediator* (Haliday) is an endoparasitoid of *M. brassicae* larvae (Lauro *et al.* 2005) and reportedly the species with the highest constancy and abundance in central Europe (Turnock and Carl 1995). Although natural control exerted by *M. mediator* may be substantial, additional insecticide treatments remain necessary to control *M. brassicae* and other key pests in *Brassica* crops. Knowledge of the side effects of chemical pesticides on beneficial arthropods is therefore crucial. Assessment of a compound's non-target effects should include both direct toxicity and sub-lethal effects (Desneux *et al.* 2007). Little is known about the toxicity of spinosad and several novel aphicides registered for use in Brassica crops to *M. mediator*. The present study examined the lethal and sub-lethal effects of four biorational (flonicamid, pymetrozine, spinosad, spirotetramat) and two conventional (thiacloprid and pirimicarb) insecticides on this parasitoid. Effects of these pesticides were tested both on adult parasitoids and cocooned pupae.

Microplitis mediator was collected in Brassica fields in Belgium and cultured using *M. brassicae*

larvae as a host. The caterpillars were reared on an artificial diet modified from Poitout and Bues (1974). For parasitization, four 1- to 4-day-old mated females of *M. mediator* were placed in a petri dish (90 mm Ø and 15 mm high) containing 20 3- to 4-day-old (first instar) larvae of *M. brassicae*. After a 4-h exposure, host larvae were transferred to plastic larval rearing cages (9 cm Ø and 10 cm high) with vented lids and fed *ad libitum* on artificial diet until parasitoid cocoons were formed. One-day-old cocoons were removed and placed in 30×30×30 cm rearing cages (Bugdorm-1, MegaView Science Education Services Co., Taiwan). Each adult rearing cage contained a plastic cup (3 cm Ø) filled with cotton wool soaked with honey-water (20% honey in tap water). Cultures of both insects were maintained and experiments conducted at 23±1°C, 60±10% r.h. and a 16:8 hL:D photoperiod. The tested insecticides were formulated materials of flonicamid (Teppeki, 50% WG, ISK Biosciences Europe S.A., Diegem, Belgium), pirimicarb (Pirimor, 50% WG, Syngenta Crop Protection N.V., Seneffe, Belgium), pymetrozine (Plenum, 50% WG, Syngenta Crop Protection N.V.), spinosad (Tracer, 480 gl^{-1} SC, Dow Agrosciences B.V., Antwerp, Belgium), spirotetramat (Movento, 100 gl^{-1} SC, Bayer CropScience N.V., Gent, Belgium) and thiacloprid (Calypso, 480 gl^{-1} SC, Bayer CropScience N.V.). Fresh solutions in distilled water were prepared at doses corresponding to the maximum recommended field rate, which was 80, 200, 200, 96, 75 and 96 g a.i. ha^{-1}, for the respective compounds. Pirimicarb was used as a toxic reference. For the adult assay, one-day-old virgin wasps were exposed to dry residues of insecticides on glass plates (90 mm Ø). One side of each glass plate was sprayed with pesticide by means of a Cornelis spray tower (1 bar pressure) (Van Laecke and Degheele 1993). In the control, glass plates were sprayed with distilled water. The spraying resulted in a homogeneous spray coverage of 1.58±0.06 mg aqueous solution deposit per cm^2. The plates were left to dry for 1 h, after which they were joined with a Plexiglas cylinder (90 mm Ø and 12 mm high) to form a drum cell. Each cylinder had seven holes (5 mm Ø) covered with nylon gauze allowing ventilation. A plastic tube filled with cotton wool connected to a water reservoir and led through the cylinder served as a source of free water for the parasitoid. Then, one virgin female and male adult were confined together in a drum cell and offered a droplet of honey on the nylon gauze as food. Twelve drum cells were set up for each product and for the water control. One day after exposure, the mortality of the parasitoids was recorded. Females surviving exposure after 24 h were placed in individual petri dishes containing 20 first instars of *M. brassicae* and allowed to oviposit for 4 h. During the first hour, the number of stings by the female parasitoids (*i.e.*, parasitization activity) was recorded as an indication for the host handling behavior of the wasp. After the 4 h exposure period, female wasps were returned to the treated drum cells and fed honey and water as described above. Honey and water were changed weekly. Survival of both female and male wasps was recorded daily and longevity was determined as an indication for the continuous sub-lethal impact of the insecticides. Each group of exposed caterpillars was placed together in larval rearing cages and reared as described above. Percentage of parasitism was calculated as the number of parasitoid cocoons formed divided by the number of host larvae exposed and multiplied by 100. For assessing effects on cocooned pupae, five 1- to 2-day-old cocoons were stuck on a piece of cardboard (4×4 cm^2) using honey-water (50% honey in tap water). The cardboards with cocoons were sprayed with the same concentration of each pesticide as in the adult assay. In the control, cardboards were sprayed with distilled water. After treatment, cardboards with cocoons were left to dry for 2 h and placed in individual petri dishes (9 cm Ø) up to adult emergence. Parasitoids that had not emerged from cocoons after 14 days were considered dead. In each treatment and in the control, 30 or 40 cocoons were exposed to insecticides, divided over six or eight replicates. Data of sub-lethal impacts (parasitization activity, parasitism percentage and male and female longevity) and percent mortality of cocooned pupae were analyzed with the Independent Samples *T*-test (P=0.05). Each time, means were compared with the control group. Percentage data were transformed by arcsine square root before analysis (SPSS Inc. 2006).

Mortality percentages of adult *M. mediator* exposed to dry residues of the selected insecticides are presented in Table 1. Only pirimicarb affected adult survival when applied on glass plates, causing 100% mortality after 24 h. There was no difference in susceptibility to the tested compounds between the sexes. The impact of the different insecticides to

Table 1 Effects of selected insecticides on cocooned pupae (% mortality) and adults (% mortality, parasitization activity [number of stings per female per h],% parasitism and longevity) of *Microplitis mediator*

Active ingredient	Cocooned pupae	Adults					
	% Mortality[y]	% Mortality		Parasitization activity[y]	% Parasitism[y]	Longevity (days)[y]	
		♂	♀			♂	♀
Control	5.79±1.83	0	0	13.56±1.19	42.65±3.20	31.38±0.83	37.85±0.88
Flonicamid	22.17±9.20*	0	0	4.92±1.67*	27.08±5.59	28.09±1.34	32.55±2.06*
Pirimicarb	6.67±4.23	100	100	–	–	–	–
Pymetrozine	20.00±7.30*	0	0	3.00±1.34*	22.50±6.17*	28.83±1.62	34.00±2.45
Spinosad[z]	10.00±6.83	0	0	5.20±2.60*	28.00±7.68	4.00±0.54*	4.17±0.49*
Spirotetramat	13.33±6.67	0	0	11.33±2.26	36.25±5.41	25.36±2.28*	33.58±2.48
Thiacloprid	7.50±5.26	0	0	5.42±2.19*	32.50±6.61	27.80±2.67	30.80±2.62*

[z] Parasitization activity and % parasitism were monitored for only five female adults; in all other treatments, 12 females were monitored

[y] Means±SEM. Within columns, means followed by an asterisk are significantly different from the control (Independent Samples *T*-test, $P<0.05$)

cocooned pupae was relatively low, with no treatment yielding mortality over 23% (Table 1). Development time of treated and control pupae ranged from 4.7 to 6.3 days and did not differ among treatments. After 24 h, 41.67% of the adult wasps exposed to spinosad were unable to right themselves. Therefore, activity and parasitism rate were assessed for only five of the 12 adult females. As no adults survived exposure to residues of pirimicarb, the sub-lethal effects of this compound could not be assessed. Except for spirotetramat, residues of all tested insecticides had a negative impact on the parasitization activity of the surviving females. Male and female longevity were affected mainly by exposure to spinosad.

The susceptibility of adult *M. mediator* differed greatly between the tested insecticides. Whereas pirimicarb was highly toxic to adults by residual contact, flonicamid, pymetrozine, spinosad and thiacloprid revealed only sub-lethal impacts. These findings indicate that besides studying lethal effects, it is warranted also to assess sub-lethal effects to judge the compatibility of an insecticide with a natural enemy (Desneux *et al.* 2007).

Little is known about the side effects of the selective homopteran feeding blocker, flonicamid, on parasitoids. Cloyd and Dickinson (2006) reported that adults of the parasitoid *Leptomastix dactylopii* (Howard) exposed to flonicamid residues on glass plates for 72 h suffered no toxicity. Furthermore, exposure to residues on leaves did not influence the parasitization rate or sex ratio of *L. dactylopii*. In contrast, Jansen *et al.* (2011) reported a diminished parasitization rate of *Aphidius rhopalosiphi* (DeStefani-Perez) females exposed to residues on glass plates. Our study also revealed sub-lethal effects in terms of a reduced parasitization activity and female longevity. Furthermore, adult emergence from treated cocoons was reduced, suggesting that flonicamid also has an impact on the pupal stage of *M. mediator*.

Several studies indicated that pymetrozine, another selective homopteran feeding blocker, is harmless for a number of hymenopteran parasitoids (Acheampong and Stark 2004; Medina *et al.* 2007; Torres *et al.* 2003). However, Van Driesche *et al.* (2008) and Jansen *et al.* (2011) found direct toxic effects when adults of *Aphidius colemani* (Viereck) or *A. rhopalosiphi*, respectively, were exposed to residues on glass plates. Sub-lethal effects in terms of reduced female fertility were reported by Jansen *et al.* (2011), confirming the findings in the present study. Additionally, we observed reduced adult emergence after treatment of *M. mediator* cocoons. Thus, susceptibility towards this insecticide may vary depending on the species.

Spinosad has repeatedly been reported to cause harmful effects on adult parasitoids in laboratory trials (Carmo *et al.* 2010; Haseeb *et al.* 2004; Xu *et al.*

2004). Cordero *et al.* (2007) found that the LC_{50}-value of spinosad for two diamondback moth parasitoids was only a fraction (<2%) of the actual field rate concentration, indicating the high toxicity of the compound. Mason *et al.* (2002) showed that the mortality of *M. mediator* due to exposure to spinosad gradually increased up to 96.8% after 7 days. The latter finding is consistent with the delayed mortality in our study as indicated by the short longevities of the exposed males and females. Furthermore, the parasitization activity was significantly reduced. However, as only five adults were available in our study to assess parasitism of *M. mediator,* further research on the sub-lethal effects of spinosad on *M. mediator* is warranted.

Little is known about the side effects of spirotetramat, a lipid biosynthesis inhibitor, on parasitoids. Hall and Nguyen (2010) reported more than 68% mortality when adults of the eulophid parasitoid *Tamarixia radiata* (Waterson) were directly sprayed with spirotetramat. Furthermore, exposure to residues on leaf disks yielded a mortality of 53.1% after 48 h. In our study, however, only male longevity was shortened by exposure to dry residues on glass plates. Differences among studies may be attributed to the different experimental set-up and the limited contact activity of spirotetramat. According to Brück *et al.* (2009), spirotetramat becomes biologically active only after uptake by the plant and transformation to the spirotetramat-enol. The susceptibility of hymenopteran parasitoids to the neonicotinoid thiacloprid reportedly varies with species. Several egg parasitoids (Bastos *et al.* 2006; Wise *et al.* 2010), *Encarsia formosa* (Gahan) (Van de Veire and Tirry 2003) and *A. rhopalosiphi* (Mead-Briggs 1992) were found to be susceptible in laboratory trials, whereas the braconid *Dolichogenidea tasmanica* (Cameron) was not (Newman *et al.* 2004). Our results revealed a significant decline in parasitization activity and longevity of exposed females.

The impact of pirimicarb on aphid parasitoids is well studied. Again, toxicity varies considerably with the experimental set-up and species. High mortality rates have been reported when adults were exposed to dry residues or directly sprayed (Borgemeister *et al.* 1993; Desneux *et al.* 2004; Jansen 1996;), but no effects (Jansen 1996) or only sub-lethal effects (Umoru and Powell 2002) were shown when the parasitoid was allowed to attack and/or develop in treated aphids. Little is known, however, about the effect of pirimicarb on non-aphid parasitoids. The outcome of our worst case laboratory trials indicates that pirimicarb is highly toxic for adult *M. mediator* and should therefore be tested further under more realistic conditions to judge its harmfulness.

The findings of this study may be useful for the conservation of *M. mediator* in cabbage fields, which are characterized by a broad pest complex. The selective features of spirotetramat can make this insecticide a valuable management tool in situations where both sucking pests and caterpillars have to be controlled and when *M. mediator* populations are present. However, care must be taken when extrapolating the results of our laboratory study to the field, since only a limited number of routes of exposure were tested, which did not take into account the systemic properties of the compound. In this respect, multi-step studies like that of Desneux *et al.* (2006) may also be useful for *M. mediator*. The other tested insecticides showed either lethal or sub-lethal effects which may affect the population growth of *M. mediator* and hence lead to a reduced natural control of the cabbage moth. Further studies need to be done under more realistic semi-field and field conditions to assess more precisely the potential impact of these pesticides on *M. mediator*.

References

Acheampong, S., & Stark, J. D. (2004). Effects of the agricultural adjuvant Sylgard 309 and the insecticide pymetrozine on demographic parameters of the aphid parasitoid, *Diaeretiella rapae*. *Biological Control, 31*, 133–137.

Bastos, C. S., de Almeida, R. P., & Suinaga, F. A. (2006). Selectivity of pesticides used on cotton (*Gossypium hirsutum*) to *Trichogramma pretiosum* reared on two laboratory-reared hosts. *Pest Management Science, 62*, 91–98.

Borgemeister, C., Poehling, H. M., Dinter, A., & Holler, C. (1993). Effects of insecticides on life-history parameters of the aphid parasitoid *Aphidius rhopalosiphi* [Hym, Aphidiidae]. *Entomophaga, 38*, 245–255.

Brück, E., Elbert, A., Fischer, R., Krueger, S., Kuhnhold, J., Klueken, A. M., *et al.* (2009). Movento (r), an innovative ambimobile insecticide for sucking insect pest control in agriculture: biological profile and field performance. *Crop Protection, 28*, 838–844.

Carmo, E. L., Bueno, A. F., & Bueno, R. C. O. F. (2010). Pesticide selectivity for the insect egg parasitoid *Telenomus remus*. *BioControl, 55*, 455–464.

Cloyd, R. A., & Dickinson, A. (2006). Effect of insecticides on mealybug destroyer (Coleoptera: Coccinellidae) and parasitoid *Leptomastix dactylopii* (Hymenoptera: Encyrtidae), natural enemies of citrus mealybug (Homoptera: Pseudococcidae). *Journal of Economic Entomology, 99*, 1596–1604.

Cordero, R. J., Bloomquist, J. R., & Kuhar, T. P. (2007). Susceptibility of two diamondback moth parasitoids, *Diadegma insulare* (Cresson) (Hymenoptera; Ichneumonidae) and *Oomyzus sokolowskii* (Kurdjumov) (Hymenoptera; Eulophidae), to selected commercial insecticides. *Biological Control, 42*, 48–54.

Desneux, N., Decourtye, A., & Delpuech, J. M. (2007). The sublethal effects of pesticides on beneficial arthropods. *Annual Review of Entomology, 52*, 81–106.

Desneux, N., Rafalimanana, H., & Kaiser, L. (2004). Dose-response relationship in lethal and behavioural effects of different insecticides on the parasitic wasp *Aphidius ervi*. *Chemosphere, 54*, 619–627.

Desneux, N., Ramirez-Romero, R., & Kaiser, L. (2006). Multi step bioassay to predict recolonization potential of emerging parasitoids after a pesticide treatment. *Environmental Toxicology and Chemistry, 25*, 2675–2682.

Hall, D. G., & Nguyen, R. (2010). Toxicity of pesticides to *Tamarixia radiata*, a parasitoid of the Asian citrus psyllid. *Biocontrol, 55*, 601–611.

Haseeb, M., Liu, T. X., & Jones, W. A. (2004). Effects of selected insecticides on *Cotesia plutellae*, endoparasitoid of *Plutella xylostella*. *Biocontrol, 49*, 33–46.

Jansen, J.-P. (1996). Side effects of insecticides on *Aphidius rhopalosiphi* (Hym.: Aphidiidae) in the laboratory. *Entomophaga, 41*, 37–43.

Jansen, J.-P., Defrance, T., & Warnier, A. M. (2011). Side effects of flonicamide and pymetrozine on five aphid natural enemy species. *Biocontrol*. doi:10.1007/s10526-011-9342-1.

Lauro, N., Kuhlmann, U., Mason, P. G., & Holliday, N. J. (2005). Interaction of a solitary larval endoparasitoid, *Microplitis mediator*, with its host, *Mamestra brassicae*: host acceptance and host suitability. *Journal of Applied Entomology, 129*, 567–573.

Mason, P. G., Erlandson, M. A., Elliott, R. H., & Harris, B. J. (2002). Potential impact of spinosad on parasitoids of *Mamestra configurata* (Lepidoptera: Noctuidae). *Canadian Entomologist, 134*, 59–68.

Mead-Briggs, M. A. (1992). A laboratory method for evaluating the side-effects of pesticides on the cereal aphid parasitoid *Aphidius rhopalosiphi* (Destefani-Perez). *Aspects of Applied Biology, 31*, 179–189.

Medina, P., Morales, J. J., Budia, F., Adan, A., Del Estal, P., & Vinuela, E. (2007). Compatibility of endoparasitoid *Hyposoter didymator* (Hymenoptera: Ichneumonidae) protected stages with five selected insecticides. *Journal of Economic Entomology, 100*, 1789–1796.

Newman, I. C., Walker, J. T. S., & Rogers, D. J. (2004). Mortality of the leafroller parasitoid *Dolichogenidea tasmanica* (Hym: Braconidae) exposed to orchard pesticide residues. *New Zealand Plant Protection, 57*, 8–12.

Pfiffner, L., Luka, H., Schlatter, C., Juen, A., & Traugott, M. (2009). Impact of wildflower strips on biological control of cabbage lepidopterans. *Agriculture, Ecosystems & Environment, 129*, 310–314.

Poitout, S., & Bues, R. (1974). Elevages des chenilles de vingt-huit espèces de lepidoptères Noctuidae et de deux espèces d'Arctiidae sur milieu artificiel simple. *Annales de Zoologie Ecologie Animale, 6*, 431–441.

SPSS Inc. (2006). SPSS 15.0 for Windows. Chicago, IL, USA.

Torres, J. B., Silva-Torres, C. S. A., & de Oliveira, J. V. (2003). Toxicity of pymetrozine and thiamethoxam to *Aphelinus gossypii* and *Delphastus pusillus*. *Pesquisa Agropecuaria Brasileira, 38*, 459–466.

Turnock, W. J., & Carl, K. P. (1995). Evaluation of the palearctic *Eurithia consobrina* (Diptera, Tachinidae) as a potential biocontrol agent for *Mamestra configurata* (Lepidoptera, Noctuidae) in Canada. *Biocontrol Science and Technology, 5*, 55–67.

Umoru, P. A., & Powell, W. (2002). Sub-lethal effects of the insecticides pirimicarb and dimethoate on the aphid parasitoid *Diaeretiella rapae* (Hymenoptera: Braconidae) when attacking and developing in insecticide-resistant hosts. *Biocontrol Science and Technology, 12*, 605–614.

Van de Veire, M., & Tirry, L. (2003). Side effects of pesticides on four species of beneficials used in IPM in glasshouse vegetable crops: "Worst case" laboratory tests. *IOBC/WPRS Bulletin, 26*(5), 41–50.

Van Driesche, R. G., Lyon, S., Sanderson, J. P., Bennett, K. C., Stanek, E. J., III, & Zhang, R. (2008). Greenhouse trials of *Aphidius colemani* (Hymenoptera: Braconidae) banker plants for control of aphids (Hemiptera: Aphididae) in greenhouse spring floral crops. *Florida Entomologist, 91*, 583–591.

Van Laecke, K., & Degheele, D. (1993). Effect of insecticide synergist combination on the survival of *Spodoptera exigua*. *Pesticide Science, 37*, 283–288.

Wise, J. C., Jenkins, P. E., Vander Poppen, R., & Isaacs, R. (2010). Activity of broad-spectrum and reduced-risk insecticides on various life stages of cranberry fruitworm (Lepidoptera: Pyralidae) in highbush blueberry. *Journal of Economic Entomology, 103*, 1720–1728.

Xu, Y. Y., Liu, T. X., Leibee, G. L., & Jones, W. A. (2004). Effects of selected insecticides on *Diadegma insulare* (Hymenoptera: Ichneumonidae), a parasitoid of *Plutella xylostella* (Lepidoptera: Plutellidae). *Biocontrol Science and Technology, 14*, 713–723.