TOXICITY OF SEVERAL FUNGICIDES FOR ORANGE WHEAT BLOSSOM MIDGE, *SITODIPLOSIS MOSELLANA* (GÉHIN) (DIPTERA: CECIDOMYIIDAE)

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SUMMARY

The orange wheat blossom midge, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae), can cause severe losses in wheat grain yield and quality. This pest is known to be susceptible to many insecticides, but various field observations have suggested that some fungicides could also significantly affect *S. mosellana*. In order to confirm these field observations, the effect on adult midges of several fungicides commonly applied to wheat crops was investigated in the laboratory and in small plots in the field. In each experiment, the fungicides were compared with a positive (insecticide) and a negative control (water). Four fungicides were assessed in the laboratory, each with five doses based on basis of a tenfold dilution starting at the field-recommended dose. The mortality rate was evaluated after 24 hours and the lethal dose 50% (LD50) was determined for each product. In the field, six fungicides were tested at the recommended dose. The effect of each product was compared on the basis of the number of *S. mosellana* adults caught alive with an insect vacuum sampler (Vortis®) on the morning after the treatments. Both experiments showed a significant effect of several fungicides tested on *S. mosellana* adults. Chlorothalonil was not toxic for *S. mosellana*, but tebuconazole, fluxapyroxad and azoxystrobin all induced significant mortality rates.

Key words: midge, *Sitodiplosis mosellana*, wheat, fungicides, toxicity

INTRODUCTION

The orange wheat blossom midge, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae) is a common pest of wheat (*Triticum aestivum* L.) in all cereal-growing regions in the northern hemisphere. This univoltine species overwinters in the soil as cocooned larvae. Each spring, a proportion of the larvae leave their cocoon, pupate and then emerge (Barnes 1956). After mating at the emergence site, the females fly in search of a wheat crop at the susceptible growth stages (i.e., from ear emergence until the end of flowering) to lay their eggs on the spikes (Ding and Lamb 1999, Oakley *et al.* 1998). The eggs hatch a few days later and the larvae feed on the developing kernels, causing damage and shrivelled grain (Reeher 1945). After the feeding period, the L3 stage-larvae drop onto the soil with rainfall, where they spin a cocoon and enter into diapause until at least the following spring (Barnes 1956).
Recent damaging outbreaks have occured in Europe (Gaafar and Volkmar 2010, Jacquemin *et al.* 2014, Oakley *et al.* 2005), North America (Knodel and Ganehiarachchi 2008, Vera *et al.* 2013) and Asia (Wu *et al.* 2013). Attacks by *S. mosellana* can significantly reduce wheat yield (Chavalle *et al.* 2015, Olfert *et al.* 1985) and grain quality (Miller and Halton 1960). In order to reduce *S. mosellana* infestation levels, one of the methods currently used is chemical control. Several studies have been carried out and have shown that this pest is susceptible to many insecticides when the treatment is correctly timed (Chavalle *et al.* 2015, El-Wakeil *et al.* 2013, Olfert *et al.* 1985). These insecticide treatments target the adult midges during the susceptible

growth stages of wheat. During these growth stages, fungicide treatments may also be applied. Several field observations suggested that some fungicides could also significantly affect *S. mosellana*. On the day after farmers' fungicide treatments many dead midges were found on the wheat leaves and on the ground.

The current study was conducted to assess the effect on adult midges of several fungicides commonly applied in wheat at the heading stage, where they could be in contact with *S. mosellana*. The studies were carried out in the laboratory and in the field via two distinct and complementary experiments.

MATERIALS AND METHODS

Laboratory experiment

The experiment was conducted in the laboratory at 20°C using newly emerged *S. mosellana* adults that were less than 24 h old. The adult midges were produced following the method described by Chavalle *et al.* (2014). The midge larvae were collected in July 2013 before they dropped onto the soil and were put into baskets filled with soil. The baskets spent the winter outside and were placed in cold storage at the end of winter. They were then placed in a room at 20°C and watered to simulate inductive rainfall, triggering the emergence of the adult midges as described by Jacquemin *et al.* (2014).

In order to assess the toxicity of the fungicides under controlled conditions, the methods used were adapted from the IRAC method No. 11 (IRAC 2009). Four fungicides, each containing only one active substance, were chosen because they represented four different families. They were assessed on the basis of five doses, using a tenfold dilution starting at the field-recommended dose (R) and compared with a positive (lambda-cyhalothrin, insecticide) and a negative control (control, water) (Table 1). Glass vials (h: 4.7 cm, Ø: 2.5 cm) were used for the test. The vials were treated by filling them with the tested solution. The solution was then removed and the vials were rotated for 1 minute in order to homogenise the remaining treatment deposit. Finally, the vials were dried at room temperature for 1 h. Three replicates were performed. Apart from the control, one replicate consisted of five vials (one vial per tested dose) for each treatment. For the control, one replicate consisted of three vials. Ten adult midges were placed in each vial using an insect mouth aspirator. The vials containing the insects were placed upside down on damp squares of towelling to keep the moisture at levels necessary for the survival of the *S. mosellana* adults.

Table 1. Description of treatment used in the laboratory to assess the tested solutions. For the fungicides (tebuconazole, chlorothalonil, fluxapyroxad, azoxystrobin), the tested solutions consisted of five doses based on a tenfold dilution starting at the field-recommended dose (R). For the insecticide (lambda-cyhalothrin), the tested solutions consisted of five doses based on a tenfold dilution starting at a dose 10,000 times lower than the field-recommended dose (R).

Treatment (a.s.)	Trade name	Concentration (g a.s./L)	Formulation	Field recommended dose (L/ha)
control	-	-	-	-
lambda-cyhalothrin	Karate Zeon	100	CS	R = 0.05
tebuconazole	Horizon	250	EW	R = 1
chlorothalonil	Bravo	500	SC	R = 2
fluxapyroxad	Imtrex	62,5	EC	R = 2
azoxystrobin	Amistar	250	SC	R = 1

The number of adult midges that were dead or moribund in each vial was scored after 24 h in order to determine the mortality rate. The toxicity of the fungicides on *S. mosellana* was evaluated on the basis of the mortality rates after 24 h and the lethal dose 50% (LD50) was determined for each product on the basis of the corrected mortality rates.

Winter wheat field experiment

The experiment was conducted in 2014 at Gembloux which is located in an important cereal-growing region in Belgium (latitude 50°33'46'' N, longitude 4°42'32'' E). The selection of the winter wheat field was based on the level of infestation by *S. mosellana*. A randomised complete block design (four blocks) was used, with eight plots of 8 × 2 m (16 m²) within each block. The first two blocks were sown with the cultivar Expert and the other two with the cultivar Tabasco. These two cultivars are susceptible to *S. mosellana*, and vary in terms of time taken to reach heading, Expert being the earlier.

In order to determine the date for treatment application, *S. mosellana* flights were monitored using Delta pheromone traps as described by Bruce *et al.* (2007), with removable sticky inserts and rubber septum lures that released the sex pheromone of *S. mosellana*. These traps were obtained from Suterra® (Suterra Europe Biocontrol Espana SL, Gavà, Barcelona, Spain). Two pheromone traps were set up 20 m apart and 0.20 m above ground level in the field around trial area. Early each afternoon, the sticky inserts were replaced and the insects caught were identified using the identification key for the Cecidomyiidae family (Skuhravá 1997) and counted using a stereomicroscope.

Six fungicides applied at the field-recommended dose were compared with a positive (lambda-cyhalothrin, insecticide) and a negative control (control, water) (Table 2). The treatments were sprayed using a backpack sprayer fitted with a 2 m-long boom, at a volume of 200 L/ha of the mixture. The plots were sprayed on the evening of 6 June at growth stage 65 for Expert and 60 for Tabasco.

The toxicity of the fungicides on *S. mosellana* was evaluated on the basis of the number of adult midges caught alive with an insect vacuum sampler (Vortis®) on the morning after the treatments. In each plot, sampling was performed by sliding the tip of the vacuum sampler at 0.20 m between two seed rows on 2 x 8 m (i.e., a round trip between two seed rows in each plot).

Table 2. Description of treatments applied in the field

Treatment (a.s.)	Trade name	Concentration (g a.s./L)	Formulation	Dose (L/ha)
control	-	-	-	-
lambda-cyhalothrin	Karate Zeon	100	CS	0.05
tebuconazole	Horizon	250	EW	1
azoxystrobin + chlorothalonil	Olympus	80 + 400	SC	2.5
fuxapyroxad + metconazole	Librax	62.5 + 45	EC	2
chlorothalonil	Bravo	500	SC	2
fluxapyroxad	Imtrex	62,5	EC	2
azoxystrobin	Amistar	250	SC	1

Statistical analysis

Statistical analyses were performed using R 3.1.1 (R Development Core Team 2014). The data from each experiment were analysed separately.

For the laboratory experiment, we used the corrected mortality rates after 24 h in order to estimate the LD50 of each fungicide. As the total number of midges was not exactly 10 in all the vials, the Henderson and Tilton formula (1955) was used to calculate these corrected mortality rates. The resulting rates were not all positive values, so the negative values were set at 0. For lambda-cyhalothrin, one replicate at the lowest dose produced an exceptionally high mortality rate that seemed to be abnormal. For this reason, the data were analysed first with this outlier value ('lambda-cyhalothrin') and then without this abnormal value ('lambda-cyhalothrin bis').

For each product, three types of model were fitted: (1) probit analysis assuming a normal distribution (binomial GLM with probit link); (2) probit analysis assuming a lognormal distribution (binomial GLM with probit link function and log-transformed doses); (3) probit analysis assuming a Weibull distribution (binomial GLM with complementary log-log link function and log-transformed doses). The model with the best fit (highest likelihood) was used to determine the LD50.

For the field experiment, statistical analyses were conducted to evaluate the effect of the treatments on the number of *S. mosellana* caught alive. This dependent variable was analysed using a mixed model with a Poisson distribution. The treatment was used as a fixed explanatory variable and the blocks as a random effect. The conditions of application for the model were checked using residual plots. The significance of differences among treatments was tested using likelihood ratio (LR) tests (analysis of deviance). When the LR test was significant, all pairwise post-hoc comparisons were performed using a generalisation of Tukey's test (Bretz *et al.* 2011).

RESULTS

Laboratory experiment

Mortality rates after 24 h

The number of *S. mosellana* adults that were dead or moribund after 24 h in each vial was scored in order to determine the mortality rate (Figure 1). A mortality rate of 7-13% after 24 h was recorded in the three replicates of the control. The results showed that lambda-cyhalothrin was very toxic for *S. mosellana,* with a 79% mortality rate with a dose 10,000 times lower than the field-recommended dose. Among the tested fungicides, tebuconazole and fluxapyroxad were very toxic, with a 100% mortality rate at the field-recommended dose. Azoxystrobin was moderately toxic, with a 53% mortality rate at the field-recommended dose. Chlorothalonil was not toxic.

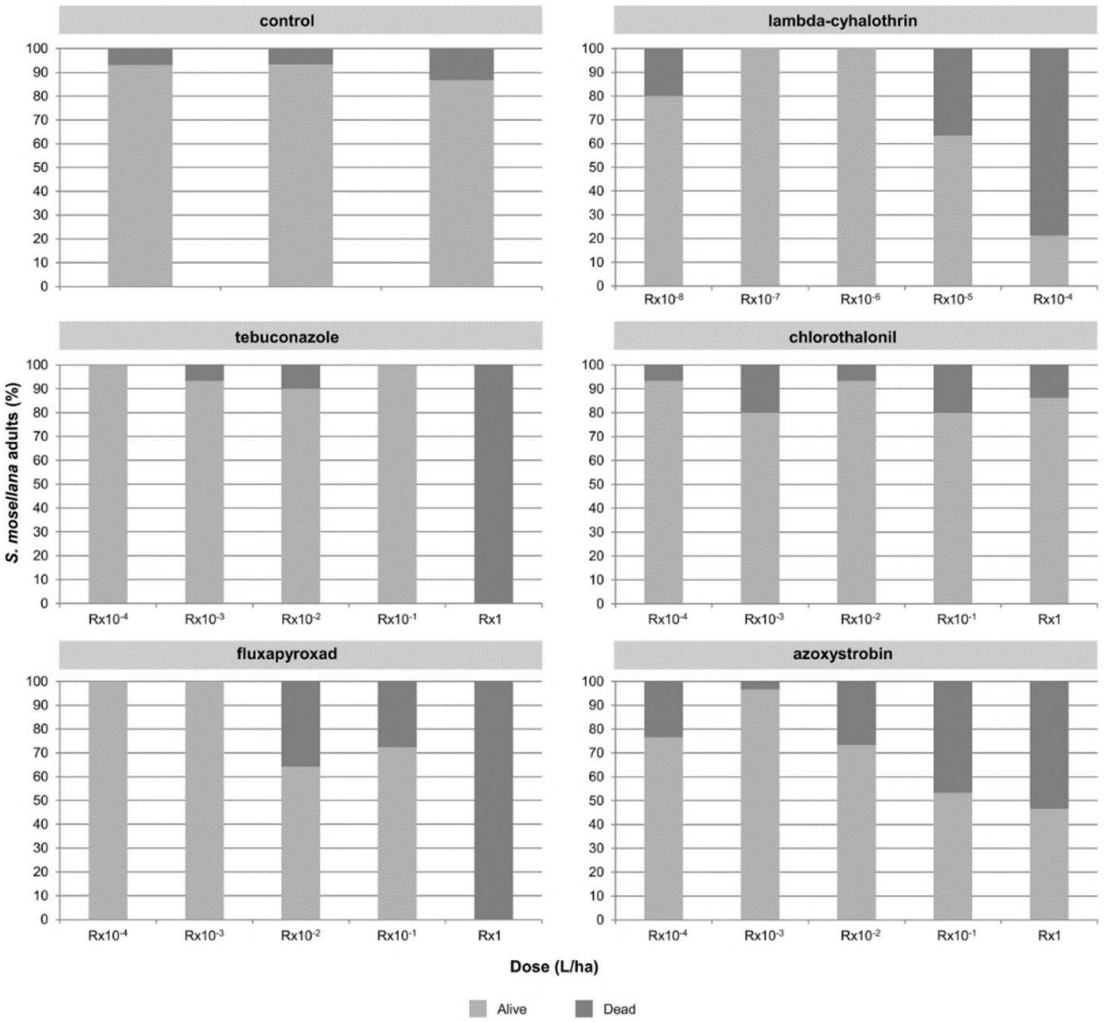

Figure 1. Percentage of *S. mosellana* adults alive or dead in relation to the dose under each treatment after 24 h. R is the field-recommended dose for each active substance (Table 1).

Toxicity of fungicides under controlled conditions

For each treatment, the LD50 was determined using the model with the best fit (Figure 2). For chlorothalonil, the mortality rate observed at the highest tested dose was less than 50% and the LD50 was considered to be greater than this highest tested dose, which corresponded to the field-recommended dose.

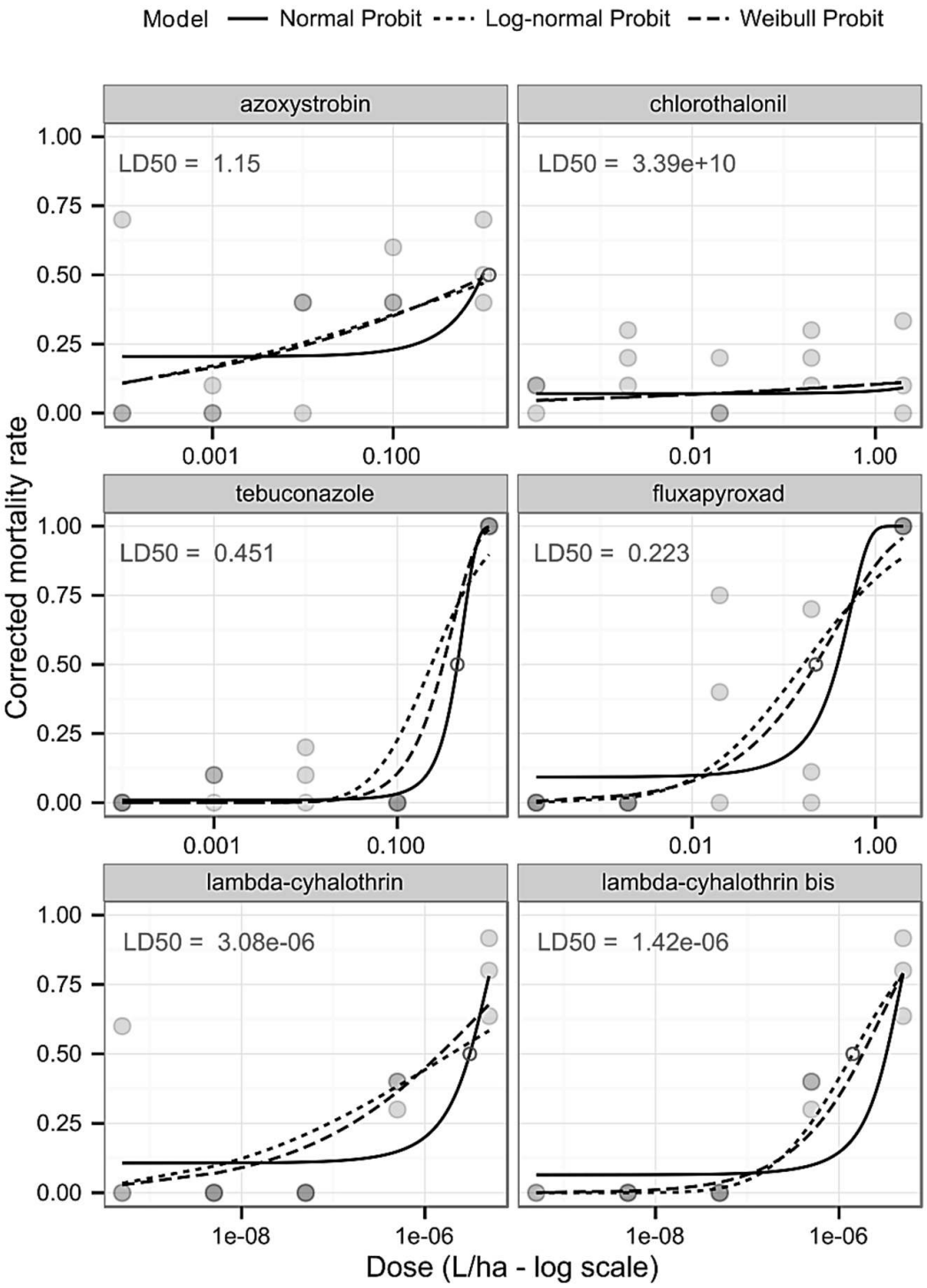

Figure 2. Corrected mortality rates observed after 24 h in relation to the dose (L/ha – log scale) for each treatment (closed circles) with three types of probit analyses and the LD50 determined with the best model (open circle). The 'lambda-cyhalothrin bis' panel presents the analysis of lambda-cyhalothrin after the removal of one outlier.

Winter wheat field experiment

Monitoring S. mosellana flights

A large number of *S. mosellana* adults (*n* = 1957) were caught by pheromone traps in the winter wheat field (Figure 3). In 2014, the timing of the *S. mosellana* flights coincided with the susceptible growth stages of the wheat cultivars.

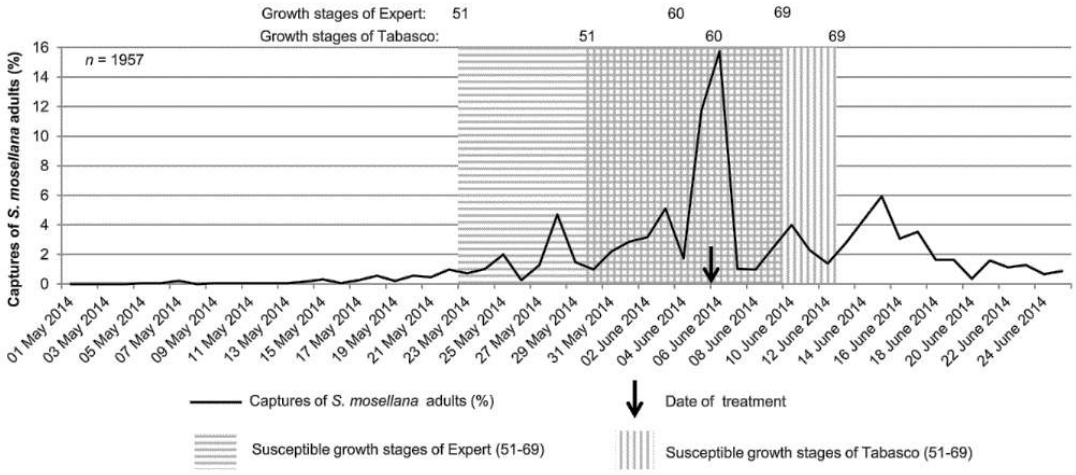

Figure 3. Sitodiplosis mosellana adults caught by pheromone traps in relation to susceptible growth stages of wheat cultivars and the date of treatment

Toxicity of fungicides based on S. mosellana adults caught alive

Live *S. mosellana* adults were caught with an insect vacuum sample (Vortis®) in each treatment (Figure 3). Important standard deviations were observed because of the wide variation in the number of midges caught alive in the replicates. There was a significant difference among the treatments (LR = 171.4, df = 7, $P < 0.0001$): chlorothalonil was not toxic for *S. mosellana*, but the other fungicides were toxic at a level equal to lambda-cyhalothrin (insecticide).

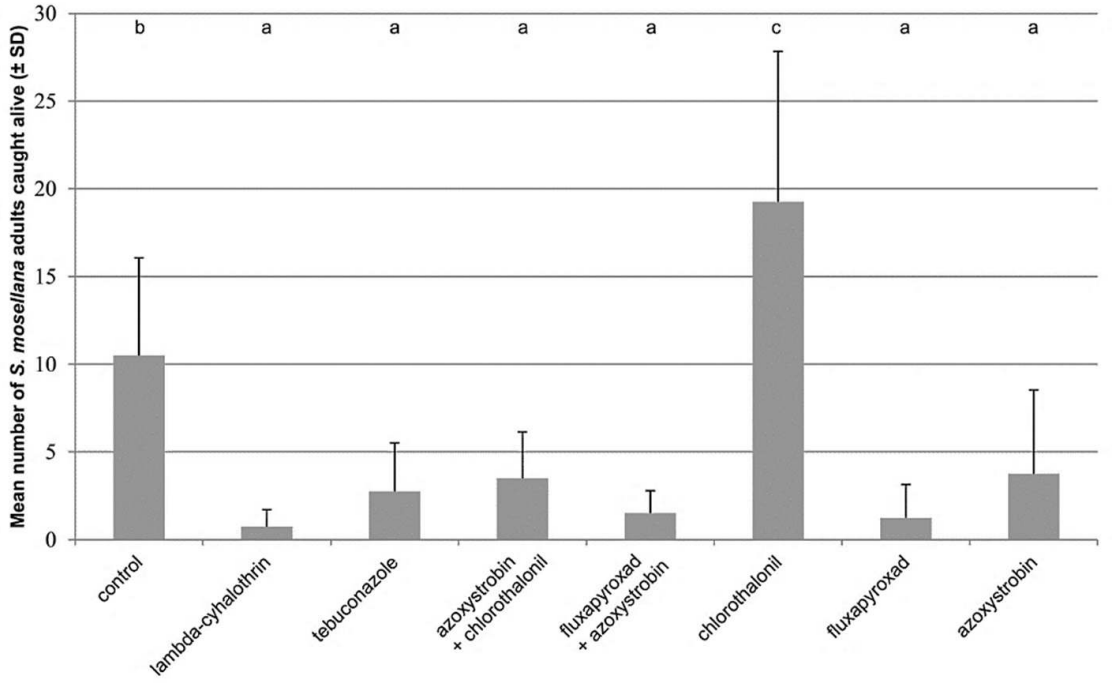

Figure 4. Mean number of *S. mosellana* adults caught alive with an insect vacuum sampler (Vortis®) in relation to treatment applied in the winter wheat field. Means with at least one common letter are not significantly different based on the Tukey-like test (α = 0.05).

DISCUSSION

The experiments conducted in the laboratory and in the field both showed a significant effect on *S. mosellana* adults of several fungicides commonly applied to wheat.

In the laboratory, the method used to assess the toxicity of the fungicides proved satisfactory. A relatively low mortality rate was observed after 24 h in the control (7-13%), whereas experiments conducted with another midge species, *Dasineura brassicae* (Winnertz), resulted in higher values, e.g. 31% after 24 h (Heimbach *et al.* 2006). Several studies have shown that fungicides have a toxic effect on insects at the field-recommended dose (Hassan *et al.* 1987, Jansen 1999, Sterk *et al.* 1999). These studies have demonstrated that the toxicity of each fungicide depends on the active substance, the insect species and the methods used. In our study, chlorothalonil did not prove to be toxic for *S. mosellana* at the field-recommended dose either in the laboratory or in the field. This active substance is described in the IOBC toxicity classes (Hassan 1992) as 'harmless' for several insects (Hassan *et al*. 1987) and as 'slightly harmful' for *Aphidius rhopalosiphi* DeStefani-Perez, an aphid parasitoid considered to be an indicator species of beneficial insects (Jansen 1999). Tebuconazole and fluzapyroxad were very toxic for *S. mosellana* at the field-recommended dose, whereas azoxystrobin appeared to be moderately toxic. Tebuconazole and fluxapyroxad are classed as 'harmful' for *A. rhopalosiphi* and azoxystrobin as 'slightly harmful'. The tested fungicides seemed to affect *S. mosellana* and *A. rhopalosiphi* in the same way. The LD50 for *S. mosellana* was determined for the three fungicides (tebuconazole, fluxapyroxad and azoxystrobine) and the insecticide (lambda-cyhalothrin). In order to determine the LD50 of each product more precisely, however, additional tests with a lower range of dilution rates need to be performed.

In the field, the results corresponded with those obtained in the laboratory. The effects of treatments with a mix of two active substances were consistent with the effect of the active

substance alone determined in the laboratory. No apparent synergistic or antagonistic effects were observed.

This study demonstrated that several fungicides are toxic for *S. mosellana*. Spraying some fungicides during heading or flowering in wheat (i.e., the susceptible growth stages at which the cereal is susceptible to *S. mosellana*) has a significant effect on this pest. Spraying a fungicide active against *S. mosellana* could obviate the need to spray an insecticide. This fungicide treatment, however, could also affect parasitoids such as *Macroglenes penetrans* (Kirby) and other pests and beneficial insects. Greater knowledge of the toxicity of fungicides against pests and parasitoids is needed to improve integrated pest management.

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REFERENCES

BARNES H.F. (1956). Gall Midges of Economic Importance. Vol. VII. Gall midges of cereal crops. Crosby Lockwood and Sons Ltd, London, UK.

BRUCE T.J.A., HOOPER A.M., IRELAND L., JONES O.T., MARTIN J.L., SMART L.E., OAKLEY J.N. AND WADHAMS L.J. (2007). Development of a pheromone trap monitoring system for orange wheat blossom midge, *Sitodiplosis mosellana*, in the UK. *Pest Management Science*, **63**(1):49–56.

BRETZ F., HOTHORNT T. AND WESTFALL P. (2011). Multiple comparisons using R. Chapman and Hall/CRC.

CHAVALLE S., CENSIER F., SAN MARTIN Y GOMEZ G. AND DE PROFT M. (2015). Protection of winter wheat against orange wheat blossom midge, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae): efficacy of insecticides and cultivar resistance. *Pest Management Science*, **71**(5):783–790.

CHAVALLE S., JACQUEMIN G. AND DE PROFT M. (2014). Evaluation en conditions contrôlées de la résistance variétale du blé tendre d'hiver à la cécidomyie orange du blé, *Sitodiplosis mosellana* (Géhin). AFPP-10ème Conférence Internationale sur les Ravageurs en Agriculture, Montpellier, France, 22-23 October 2014, 575–584.

DING H. AND LAMB R.J. (1999). Oviposition and larval establishment of *Sitodiplosis mosellana* (Diptera: Cecidomyiidae) on wheat (Graminae) at different growth stages. *The Canadian Entomologist,* **131**(4):475–481.

EL-WAKEIL N.E., ABDEL-MONIEM A.S.H., GAAFAR N. AND VOLKMAR C. (2013). Effectiveness of some insecticides on wheat blossom midges in winter wheat. *Gesunde Pflanzen*, **65**(1):7–13.

GAAFAR N. AND VOLKMAR C. (2010). Evaluation of wheat ear insects in large scale field in central Germany. *Agricultural Sciences*, **1**(2):68–75.

Hassan S.A., Albert R., Bigler F., Blaisinger P., Bogenschütz H., Boller E., Brun J., Chiverton P., Edwards P., Englert W.D., Huang P., Inglesfield C., Naton E., Oomen P.A., Overmeer W.P.J., Rieckmann W., Samsøe-Petersen L., Stäublii A., Tuset J.J., Viggiani G. and Vanwetswinkel G. (1987). Results of the third joint pesticide testing programme by the IOBC/WPRS-Working Group 'Pesticides and Beneficial Organisms.' *Journal of Applied Entomology*, **103**(1-5):92–107.

HASSAN S.A. (1992). Meeting of the working group 'Pesticides and Beneficial Organisms', University of Southampton, UK, September 1991. *IOBC/WPRS Bulletin* **1192/XV/3**:1-3.

HEIMBACH U., MULLER A. AND THIEME T. (2006). First steps to analyze pyrethroid resistance of different oilseed rape pests in Germany. *Nachrichtenblatt Deutscher Pflanzenschutzdienst*, **58**(1):1–5.

HENDERSON C.F. AND TILTON E.W. 1955. Tests with acaricides against the brown wheat mite. *Journal of Economic Entomology*, **48**(2):157–161.

IRAC (2009). IRAC (Insecticide Resistance Action Committee) Susceptibility Test Methods Series, Methods No 11: Pollen Beetle, *Meligethes aeneus*, Adults, Synthetic Pyrethroids. http://www.irac-online.org/methods/meligethes-aeneus-adults/ (10/05/2014).

JACQUEMIN G., CHAVALLE S. AND DE PROFT M. (2014). Forecasting the emergence of the adult orange wheat blossom midge, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae) in Belgium. *Crop Protection*, **58**(1):6–13.

JANSEN J.P. (1999). 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**(4):217–224.

KNODEL J. AND GANEHIARACHCHI M. (2008). Integrated pest management of the wheat midge in North Dakota - E1130. NDSU Extension Service.

MILLER B.S. AND HALTON P. (1960). The damage to wheat kernels caused by the wheat blossom midge (*Sitodiplosis mosellana*). *Journal of the Science of Food and Agriculture*, **12**(5):391–398.

OAKLEY J.N., CUMBLETON P.C., CORBETT S.J., SAUNDERST P., GREEN D.I., YOUNG J.E.B. AND RODGERS, R. (1998). Prediction of orange wheat blossom midge activity and risk of damage. *Crop Protection*, **17**(2):145–149.

Oakley J.N., Talbot G., Dyer C., Self M.M., Freer J.B.S., Angus W.J., Barrett J.M., Feuerhelm G., Snape J., Sayers L., Bruce T.J.A., Smart L.E. and Wadhams L.J. (2005). Integrated control of wheat blossom midge: variety choice, use of pheromone traps and treatment thresholds. HGCA Project Report No. 363.

OLFERT O.O., MUKERJI M.K. AND DOANE J.F. (1985). Relationship between infestation levels and yield loss caused by wheat midge, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae), in spring wheat in Saskatchewan. *The Canadian Entomologist*, **117**(5):593–598.

R DEVELOPMENT CORE TEAM (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. *http://www.R-project.org*.

REEHER M.M. (1945). The wheat midge in the Pacific Northwest. US Department of Agriculture.

SKUHRAVÁ M. (1997). Family Cecidomyiidae. In: Papp, L., Darvas, B. (eds.), Contributions to a Manual of Palaearctic Diptera. Vol. 2. Science Herald, Budapest, Hungary, pp. 71–204.

Sterk G., Hassan S.A., Baillod M., Bakker F., Bigler F., Blümel S., Bogenschütz H., Boller E., Bromand B., Brun J., Calis J.N.M., Coremans-Pelseneer J., Duso C., Garrido A., Grove A., Heimbach U., Hokkanen H., Jacas J., Lewis G., Moreth L., Polgar L., Rovesti L., Samsøe-Peterson L., Sauphanor B., Schaub L., Stäubli A., Tuset J.J., Vainio A., Van De Veire M., Viggiani G., Viñuela E. and Vogt H. (1999). Results of the seventh joint pesticide testing programme carried out by the IOBC/WPRS-Working Group 'Pesticides and Beneficial Organisms.' *BioControl,* **44**(1):99–117.

VERA C.L., FOX S.L., DEPAUW R.M., SMITH M.A.H., WISE I.L., CLARKE F.R., PROCUNIER J.D. AND LUKOW O.M. (2013). Relative performance of resistant wheat varietal blends and susceptible wheat cultivars exposed to wheat midge, *Sitodiplosis mosellana* (Géhin). *Canadian Journal of Plant Science,* **93**(1):59–66.

WU Y.-Q., DUAN A.-J., ZHANG Z.-Q., LIU C.-Y., LIU S.-T., JIANG Y.-L., MIAO J., DUAN Y., GONG Z.-J. AND LI T. (2013). Resistance grading method and evaluation in wheat varieties to orange wheat blossom midge, *Sitodiplosis mosellana* (Diptera: Cecidomyiidae) in China. *Acta Agronomica Sinica*, **12**(39):2171–2176.