

# Protection of winter wheat against orange wheat blossom midge, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae): efficacy of insecticides and cultivar resistance

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## Abstract

**BACKGROUND:** In 2012 and 2013, *Sitodiplosis mosellana* (Géhin) flights occurred during the susceptible phase of wheat development in Belgium. The protection against this midge afforded by various insecticides was assessed in infested fields on four winter wheat cultivars (susceptible or resistant, and early or late heading).

**RESULTS:** The insecticides sprayed at the right time reduced the number of larvae in the ears by 44–96%, depending on the product. For Julius, the cultivar (cv.) most exposed to *S. mosellana* in 2013, the mean yield gain resulting from insecticide use was 1558 kg ha<sup>-1</sup> (18%). In the same year, insecticide use resulted in a yield gain of 780 kg ha<sup>-1</sup> (8%) for the cv. Lear, in spite of its resistance to this pest. The link between yield and number of larvae counted in the ears was a logarithmic relationship, suggesting an important reduction in yield, caused either by the damage inflicted by young larvae that died at the start of their development or by the activation of costly reactions in plants.

**CONCLUSION:** The study showed that, in cases of severe attack, the timely application of insecticide treatments can protect wheat against *S. mosellana*, and that even resistant cultivars can benefit from these treatments.

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**Keywords:** midge; wheat; *Sitodiplosis mosellana*; chemical control; insecticides; cultivar resistance

## 1 INTRODUCTION

The orange wheat blossom midge, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae), is a common pest of wheat (*Triticum aestivum* L.) in the northern hemisphere. During the past decade in Europe there have been serious outbreaks resulting in important damage in the United Kingdom,<sup>1,2</sup> Germany,<sup>3</sup> France<sup>4</sup> and Belgium.<sup>5</sup> Attacks by *S. mosellana* can significantly reduce harvest yield and grain quality,<sup>6,7</sup> and can facilitate secondary fungal infections.<sup>1</sup> In the United Kingdom, crop losses exceeded £30 million in 1993<sup>1</sup> and £60 million in 2004, in spite of more than 500 000 ha of wheat having been sprayed with insecticides.<sup>2</sup> Owing to the difficulties in detecting *S. mosellana*, the degree of damage to crops is often underestimated.

The orange wheat blossom midge is a univoltine species. The midge larvae overwinter in the soil inside a cocoon, and each spring some of them pupate and emerge. Shortly after emergence, females emit a sexual pheromone that attracts males,<sup>8,9</sup> identified as (2S,7S)-nonadiyl dibutyrate.<sup>10</sup> After mating at the emergence site, female midges fly off in search of a host plant.<sup>11</sup> The mated females lay eggs on the spikes mainly in the evening and early morning.<sup>12</sup> Oviposition is inhibited below 11 °C and when wind speed exceeds 10 km h<sup>-1</sup>.<sup>13</sup> The eggs hatch a few days later, and the midge larvae feed on the surface of newly developing kernels for 3–4 weeks.<sup>14</sup> When the feeding period is completed, the larvae

at L3 stage encased in ecdysal sheath enter a quiescent phase until triggered by rainfall to drop to the soil and burrow into the soil where they form a cocoon.<sup>15</sup>

High infestations are not often observed because this insect fails to multiply if the emergence of adult midges does not coincide with susceptible growth stages of the host plant.<sup>16</sup> In wheat, the susceptible phase starts when the ear emerges from the leaf sheath, and it lasts until the end of flowering.<sup>14,15</sup> Kernel damage is much higher in ears exposed to oviposition during early heading (Zadoks growth stages 51 to 59)<sup>17</sup> than in those exposed during flowering (growth stages 61 to 69).<sup>18</sup> This difference is due to the level of larval survival declining after the onset of anthesis.<sup>14,18</sup>

In the case of heavy infestations in the soil, two methods are commonly used to manage *S. mosellana* populations: growing resistant wheat cultivars (cv.) and chemical control. Some wheat

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cultivars have shown varying vulnerability to this pest, whereas others have proved to be resistant.<sup>2,19</sup> The sources of cultivar resistance to *S. mosellana* are linked to antixenosis or antibiosis. Antixenosis deters oviposition on the wheat spikes,<sup>20</sup> whereas antibiosis, conferred by the *Sm 1* gene, deters larvae feeding, leading to their death through the enhanced production of phenolic acids.<sup>21</sup> Several studies on the chemical control of *S. mosellana* have been carried out. They show that applying an insecticide treatment at the right time protects the kernel and results in higher yields.<sup>22–24</sup> In order to provide good control, an insecticide treatment must be applied promptly before the larvae reach the grain.<sup>2</sup> A useful tool for determining the relevance of an insecticide treatment and the best moment for its application is the pheromone trap, which allows midge emergences and flights to be monitored.<sup>25</sup> In the United Kingdom, an insecticide application is recommended when a pheromone trap catches more than 120 midge adults per day or when it catches more than 30 midge adults per day and at least one adult midge per six ears is observed in the field in the evening.<sup>19</sup>

The potential high economic costs of *S. mosellana* damage make it important to be able to control this pest effectively. Using four winter wheat cultivars, this study compared the effectiveness of some insecticides on the basis of the number of *S. mosellana* larvae that developed in the ears. It then compared the yields and yield gains resulting from the different treatments and analysed the relationship between yield and number of larvae.

## 2 MATERIALS AND METHODS

### 2.1 Winter wheat trials

The experiments were conducted in 2012 and 2013 at Gembloux in Belgium (latitude 50° 34' N, longitude 4° 44' E, 152 m amsl). Gembloux is located in an important cereal-growing region in Belgium and in an area of loamy soil. In each year the trial was conducted in a winter wheat field selected according to the level of infestation by *S. mosellana*. Nine insecticide treatments were compared with a control. A randomised complete block design (four blocks) was used, with 10 plots of 8 × 8 m within each block. Each plot was composed of four subplots measuring 8 × 2 m (16 m<sup>2</sup>) sown with four different winter wheat cultivars: Julius (susceptible and late heading), Lear (resistant and late heading), Premio (susceptible and early heading) and Altigo (resistant and early heading). Resistant and susceptible cultivars were proven in biotests carried out by Jacquemin.<sup>26</sup> However, the type of resistance involved for both resistant cultivars was unknown. All around the trial area, the experimental field was sown with the cv. Julius.

### 2.2 Monitoring *S. mosellana* flights using pheromone traps

In order to determine the optimum date for insecticide application, *S. mosellana* flights were monitored using pheromone traps as described by Bruce *et al.*<sup>25</sup> These delta traps had removable sticky inserts and rubber septum lures that released the sex pheromone of *S. mosellana*. The traps and lures were obtained from Sutterra® (Suterra Europe Biocontrol Espana SL, Gavà, Barcelona, Spain). Two pheromone traps were set up each year in the experimental field around the trial area; the traps were 20 m apart and 0.20 m above the soil. In both traps the same rubber septum lure remained in place for a full season. Early each afternoon, the sticky inserts were replaced and the insects captured were identified on the basis of the identification key of family Cecidomyiidae given by Skuhravá,<sup>27</sup> and counted using a stereomicroscope.

### 2.3 Chemical control

Ten insecticide treatments applied once on the same date were compared (Table 1). The control was sprayed only with water. The insecticides were sprayed using a backpack sprayer fitted with a ramp of 2 m, at a volume of 200 L mixture ha<sup>-1</sup>. In 2012, the plots were sprayed on 30 May at growth stages 55, 45, 59 and 60 for Julius, Lear, Premio and Altigo respectively. In 2013, the plots were sprayed on 14 June at growth stage 55 for Julius and Lear and at growth stage 65 for Premio and Altigo.

### 2.4 Evaluating *S. mosellana* infestation

The number of midge larvae was assessed by randomly collecting 50 ears per plot and per cultivar. The ears were collected before the larvae dropped onto the soil at the L3 stage. To ensure that no larvae had dropped from the ears before the collection of ears, water traps placed at the foot of the plants were daily checked. The first L3 larvae of *S. mosellana* were observed in the water traps on 5 July 2012 and 24 July 2013. Ear samples were collected on 26 June 2012 and 12 July 2013. The samples were frozen at –18 °C until the larvae could be extracted. In order to facilitate this extraction, the samples were divided into two groups of 25 ears. The spikelets were detached from the ear rachis after cutting the awns in the case of awned cultivars (Premio and Altigo). The spikelets were immersed in 1 L of water and shaken for 20 min. Then, the water, still containing the spikelets, was poured through three successive sieves with mesh sizes of 2.8, 1.0 and 0.3 mm. The spikelets were placed on the sieves and kneaded manually under a continuous stream of water for 20 min. Only the sieve with the 0.3 mm mesh size retained larvae. These larvae were collected, identified and counted using a stereomicroscope. The number of *S. mosellana* larvae for each subplot was expressed as number of larvae per 100 ears.

### 2.5 Checking the presence of other pests

In order to bring out an eventual effect of other pests on yield, the presence not only of *S. mosellana* but also of thrips was checked in the winter wheat trials. The presence of thrips in the ears was checked, together with determination of the *S. mosellana* development stage: at least 20 ears were carefully examined by opening all the spikelets. This control was performed each week until detection of the first L3 larvae of *S. mosellana*, and daily after this stage. Examining ears during the whole relevant period showed clearly that thrips populations were very low and unable to affect yield in the trial fields.

As regards aphids [*Sitobion avenae* (F.) and *Metopolophium dirhodum* (Wlk.)] and cereal leaf beetles [*Oulema melanopus* (L.) and *Oulema lichenis* (Voet.)], as observed everywhere in Wallonia, populations were very low in the trial fields in both years (CADCO, <http://www.cadcoasbl.be>).

### 2.6 Yield measurements

The yield from each subplot was measured at full maturity of the cultivar with the latest heading on 14 August and 17 August in 2012 and 2013 respectively. The subplots were harvested using a Haldrup experimental combine harvester fitted with a cutter bar of 2 m. The harvest from each subplot was weighed immediately in the field. A sample (1.5 kg) of grain from each subplot was put into a plastic bag for moisture measurement. Yields and yield gains were expressed in kg grain ha<sup>-1</sup> (15% humidity). The yield gain was taken as the difference between the yield of a cultivar under each insecticide treatment and the yield of the same cultivar in the same block under the control treatment.

**Table 1.** Description of applied treatments

Treatment	Trade name	Concentration (g L <sup>-1</sup> )	Formulation	Dose (L ha <sup>-1</sup> )
control	–	–	–	–
lambda-cyhalothrin	Karate Zeon	100	CS	0.050
lambda-cyhalothrin (1/2)	Karate Zeon	100	CS	0.025
cypermethrin	Cytox	100	EC	0.250
deltamethrin	Decis 2.5 EC	25	EC	0.200
esfenvalerate	Sumi-alpha	25	EC	0.200
cyfluthrin	Baythroid 050 EC	50	EC	0.300
zeta-cypermethrin	Fury 100 EW	100	EW	0.100
chlorpyrifos + cypermethrin	Nurelle D 550	500 + 50	EC	0.500
thiacloprid	Biscaya 240 OD	240	OD	0.300

## 2.7 Statistical analysis

Statistical analyses were performed using R 3.0.1.<sup>28</sup> The data from each year were analysed separately.

An initial series of analyses was conducted to evaluate the effect of insecticides and cultivars on three dependent variables: number of larvae per 100 ears; yield (kg ha<sup>-1</sup>); yield gain (kg ha<sup>-1</sup>). These variables were analysed using linear mixed models with a Gaussian distribution. The treatments and cultivars were used as fixed explanatory variables, whereas the blocks were defined as a random effect. A log<sub>10</sub>(x + 1) transformation was applied to the number of larvae per 100 ears in order to limit problems of heteroscedasticity. For all models, the conditions of application 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, *post hoc* multiple comparisons were performed using a generalisation of Tukey's test provided by the multcomp package.<sup>29</sup> For models testing the number of larvae per 100 ears and the yield, all treatments for a cultivar were compared with the control of the same cultivar. For models testing the yield gain, only relevant comparisons were conducted: between the two late heading cultivars (Julius and Lear); between the two early heading cultivars (Premio and Altigo); between the two susceptible cultivars (Julius and Premio) and the two resistant cultivars (Lear and Altigo).

A second series of analyses was conducted to evaluate the direct relationship between number of larvae per 100 ears and yield (kg ha<sup>-1</sup>). Yield was the dependent variable and was analysed using a mixed linear model with a Gaussian distribution of residuals. The susceptible cultivars (Julius and Premio) and the number of larvae per 100 ears were used as fixed explanatory variables, whereas the blocks were defined as a random effect. A log<sub>10</sub>(x + 1) transformation was applied to the number of larvae per 100 ears in order to linearise the relationship between number of larvae per 100 ears and yield.

## 3 RESULTS

### 3.1 Monitoring *S. mosellana* flights using pheromone traps

The flight patterns of *S. mosellana* (Fig. 1) and the susceptible period of wheat varied from year to year, depending on meteorological conditions. In both 2012 and 2013, the coincidence between *S. mosellana* flights and the susceptible growth stages of wheat allowed egg laying on all four tested cultivars. These four cultivars, however, were not exposed to the insect in the same way. In 2012, the early heading cultivars were the most exposed, whereas in 2013 the late heading cultivars

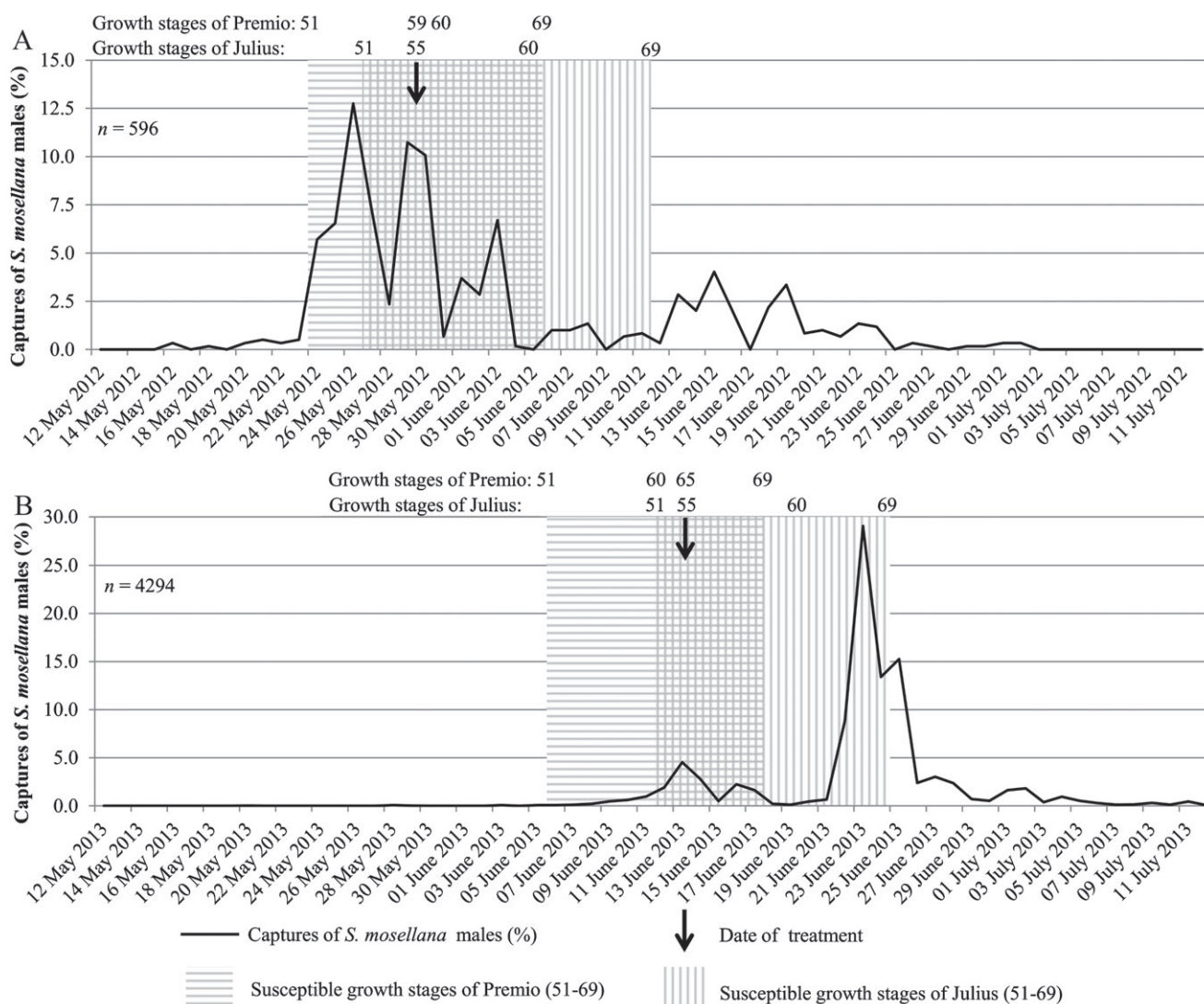
were the most exposed. In addition, because of the small size of plots (8 × 2 m), the preference for ears at the best growth stage could also have enhanced *S. mosellana* pressure differences between cultivars. Finally, flights were more abundant in 2013 (*n* = 4294) than in 2012 (*n* = 596). This difference in abundance was linked to the reproductive success of the midges in 2012.

### 3.2 Effectiveness of some insecticides, deduced from the number of larvae per 100 ears

The relationships between number of *S. mosellana* larvae per 100 ears and insecticide treatments for each tested cultivar in 2012 and 2013 are presented in Fig. 2. Regardless of treatment, there was a significant difference among the cultivars (2012: LR = 328.10, *df* = 3, *P* < 0.0001; 2013: LR = 380.8, *df* = 3, *P* < 0.0001). Low numbers of *S. mosellana* larvae were observed in the ears of the resistant cultivars, Altigo and Lear, whereas significant numbers were observed for the susceptible cultivars, Premio and Julius, in 2012 and 2013. Attack levels were higher in the second year owing to abundant flights (2012: *n* = 596; 2013: *n* = 4294). Premio was more heavily attacked than Julius in 2012, and vice versa in 2013. This result is consistent with the coincidence between flights and the susceptible growth stages (51 to 69) of Premio and Julius (Fig. 1). In 2012, the susceptible phase of Premio coincided with more flights than there had been during the susceptible phase of Julius. In 2013, the susceptible phase of Julius coincided with two 'small' peaks (about 700 adult midges), as in the case of Premio, but also with one 'big' peak (about 3000 adult midges) which occurred at the end of the susceptible phase (growth stages 67 to 69) of Julius. The relative exposure of early and late heading cultivars to the pest could be measured through the relative abundance of larvae in the ears of untreated susceptible cultivars. In 2012, there were, on average, 1.5 times more larvae in Premio, the early heading cultivar (131 larvae per 100 ears), than in Julius, the late heading cultivar (86 larvae per 100 ears). In 2013, however, there were, on average, 4.1 times fewer larvae in Premio (491 larvae per 100 ears) than in Julius (2037 larvae per 100 ears).

The effect of the treatments differed among the cultivars in both years (treatment × cultivar interaction significant: 2012: LR = 56.46, *df* = 27, *P* = 0.0008; 2013: LR = 47.92, *df* = 27, *P* = 0.0078). The effect of the treatments on the number of larvae that developed in ears depended on the nature (susceptible or resistant) of the cultivars. In the susceptible cultivars, the treatments were far more likely to reduce larva production than was the case with the resistant cultivars. In the resistant cultivars, Altigo and Lear, none of the





**Figure 1.** *Sitodiplosis mosellana* males caught by pheromone traps in relation to susceptible growth stages of susceptible cultivars and the date of treatment in 2012 (A) and 2013 (B).

treatments reduced the *S. mosellana* infestation compared with the control, which was hardly infested.

In 2012, all the insecticide treatments were significant in the susceptible cultivars ( $P < 0.05$ ), apart from the lambda-cyhalothrin treatment at the lowest rate and the thiacloprid treatment for Julius, whereas for Premio none was significant apart from the chlorpyrifos + cypermethrin treatment. In 2013, all the insecticide treatments were efficient and significant ( $P < 0.05$ ), apart from the thiacloprid treatment.

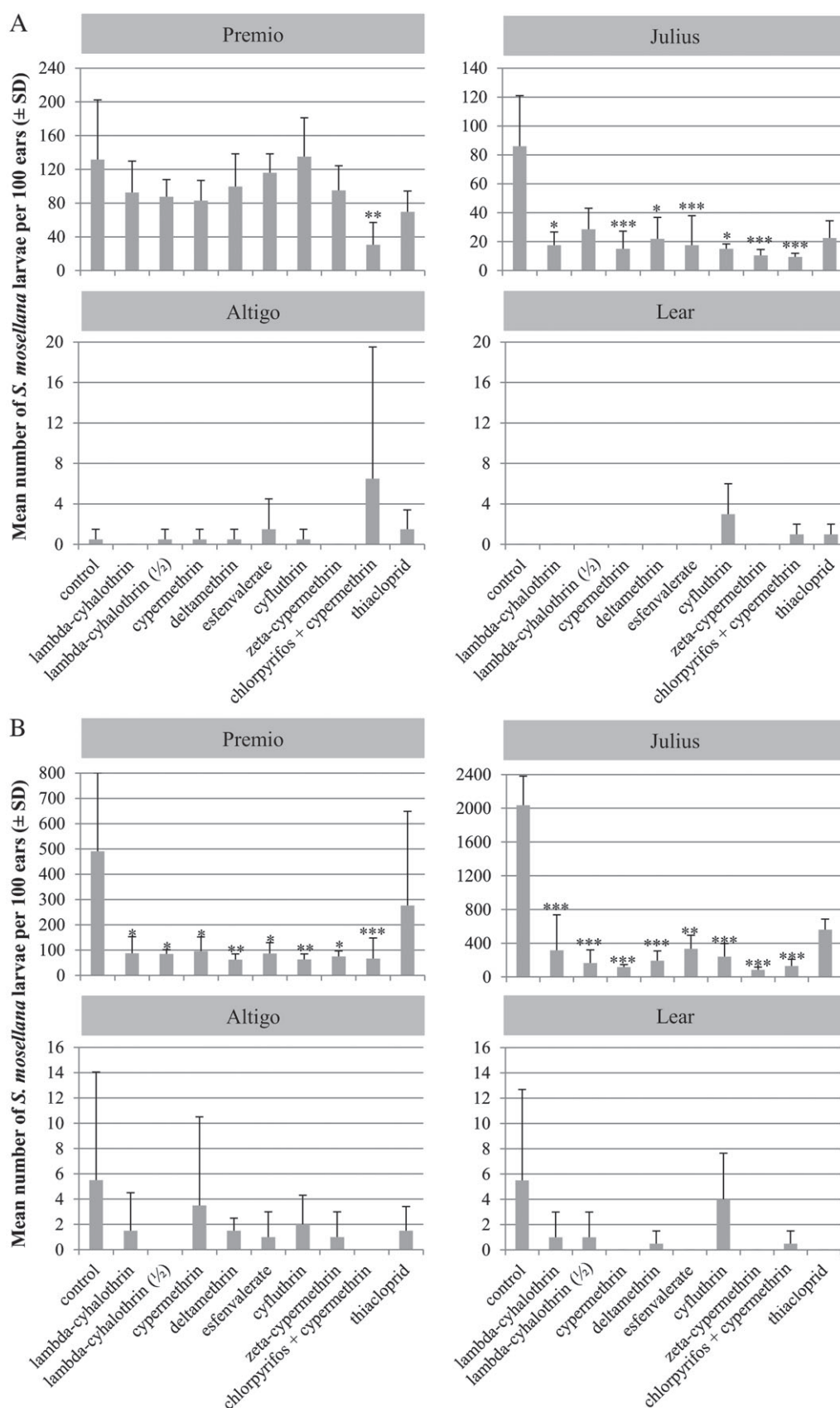
### 3.3 Effect of insecticides on yield

The yield results are given in Fig. 3. In 2012, the mean yields of untreated plots were 7798, 8163, 8302 and 8134 kg ha<sup>-1</sup> for Julius, Lear, Premio and Altigo respectively. In 2013, the corresponding figures were 8659, 9979, 10 061 and 10 120 kg ha<sup>-1</sup> respectively.

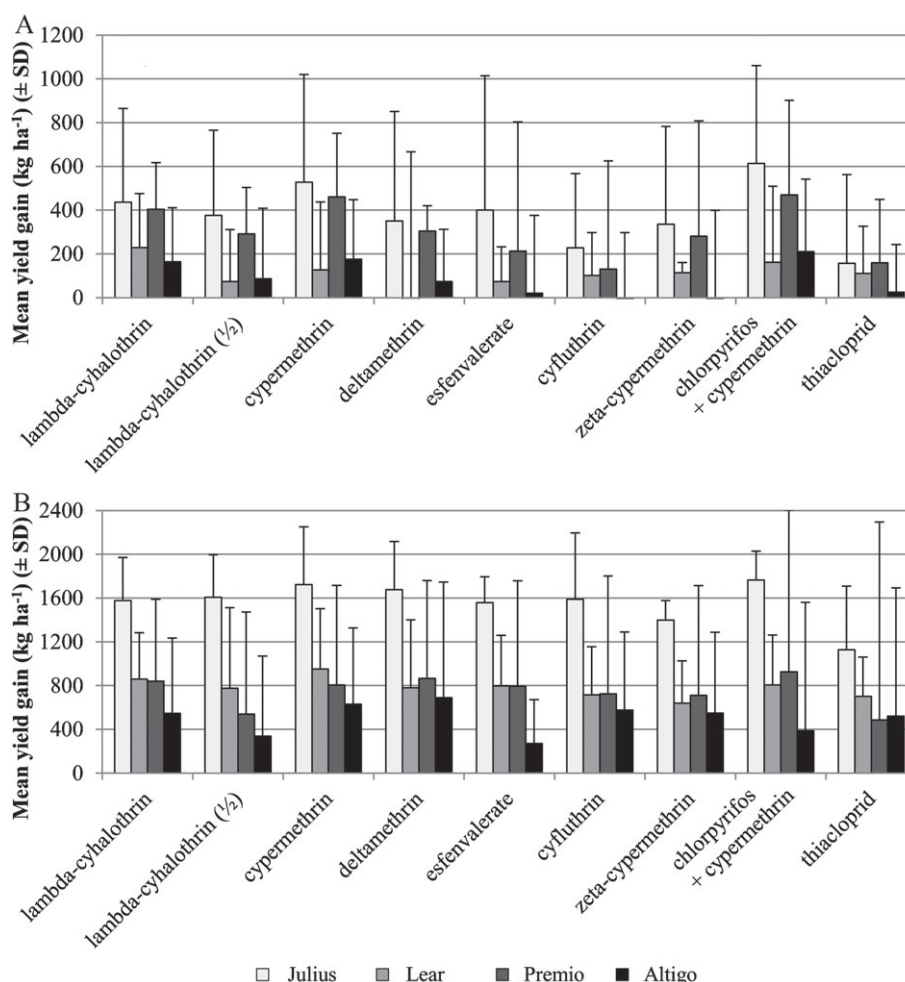
In order to determine the impact of treatments, the measured yield of each plot was compared with the yield from the untreated plot of the same cultivar in the same block. There was a significant difference among cultivars (2012: LR = 66.65, df = 3,  $P < 0.0001$ ; 2013: LR = 66.24, df = 3,  $P < 0.0001$ ) and among treatments (2012: LR = 30.46, df = 9,  $P = 0.0004$ ; 2013: LR = 69.46, df = 9,

$P < 0.0001$ ). The effect of treatments did not depend on the cultivar, and the effect of cultivars did not depend on the treatment (treatment × cultivar interaction not significant: 2012: LR = 12.35, df = 27,  $P = 0.9928$ ; 2013: LR = 27.07, df = 27,  $P = 0.4602$ ). All the insecticide treatments ( $P < 0.001$ ) led to a significant yield increase for all cultivars compared with their controls in 2013, but in 2012 only the lambda-cyhalothrin ( $P < 0.01$ ), cypermethrin ( $P < 0.01$ ) and chlorpyrifos + cypermethrin ( $P < 0.001$ ) treatments had a significant effect.

In order to compare the efficacy of the different treatments and identify the cultivars that derived more benefit from the insecticide treatments, yield gain was calculated. In 2012, all the insecticide treatments resulted in a positive mean yield gain compared with the control: 380 (5%), 180 (1%), 301 (4%) and 77 (1%) kg ha<sup>-1</sup> for Julius, Lear, Premio and Altigo respectively. In 2013 the mean yield gains were 1558 (18%), 780 (8%), 743 (8%) and 500 (5%) kg ha<sup>-1</sup>. There were no significant differences in yield gain among the insecticide treatments in 2013 (LR = 4.528, df = 8,  $P = 0.8067$ ). In 2012, however, the difference in yield gain among the insecticide treatments was significant (LR = 16.410, df = 8,  $P = 0.0369$ ), but



**Figure 2.** Mean number of *S. mosellana* larvae per 100 ears ( $\pm$ SD) in relation to treatment applied to the four wheat cultivars in 2012 (A) and 2013 (B). Means with star(s) are significantly different from the control of the same cultivar (\*:  $P < 0.05$ ; \*\*:  $P < 0.01$ ; \*\*\*:  $P < 0.001$ , *post hoc* tests with corrected  $P$ -values).



**Figure 3.** Mean yield gain (kg ha<sup>-1</sup>) (±SD) compared with the control in relation to treatment applied to the four wheat cultivars in 2012 (A) and 2013 (B).

none of the *post hoc* tests proved to be significant after correction for multiple testing.

In 2012 and 2013, the cultivars did not derive the same level of benefit from the treatments (2012: LR = 32.503, df = 3,  $P < 0.0001$ ; 2013: LR = 60.61, df = 3,  $P < 0.0001$ ). A comparison of the two early heading cultivars showed that yield gain for Premio (susceptible cultivar) was significantly better than for Altigo (resistant cultivar), whatever the insecticide treatment, but only in 2012 ( $P < 0.05$ ). For the two late heading cultivars, the yield gain for Julius (susceptible cultivar) was significantly better than for Lear (resistant cultivar) in both years, whatever the insecticide treatment ( $P < 0.001$ ). A comparison of the two susceptible cultivars with the two resistant cultivars showed that the susceptible cultivars benefited more from the insecticide treatments than the resistant cultivars ( $P < 0.001$ ). This observation reflected the effect of insecticide treatments on the level of damage by *S. mosellana* in these experiments.

### 3.4 Relationship between yield and number of larvae per 100 ears

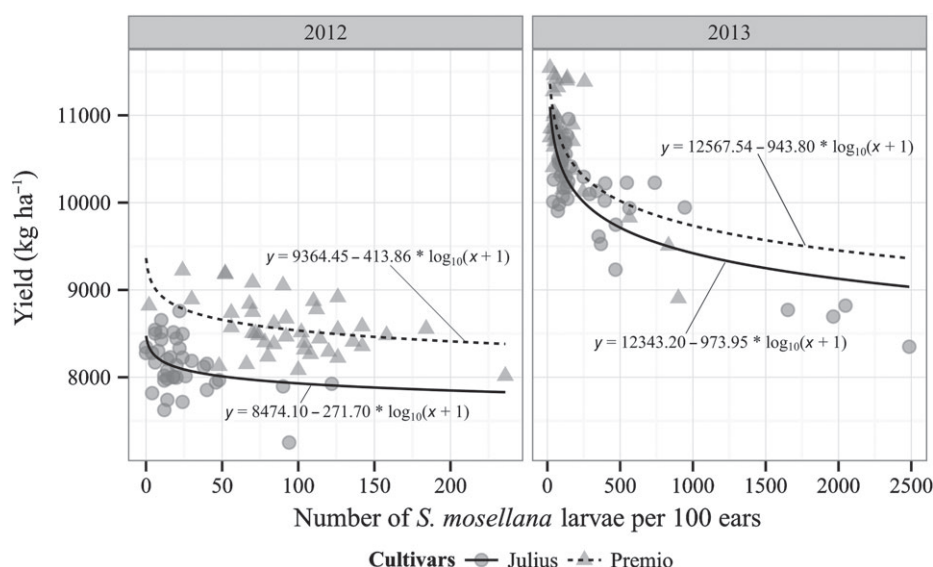
The relationship between number of *S. mosellana* larvae per 100 ears and yield of the susceptible cultivars, Julius and Premio, in 2012 and 2013 was described by a logarithmic relationship (Fig. 4). The curves for the two years had a similar appearance when examined at the same scale. In the case of low *S. mosellana* infestation in the ears, each developed larva caused greater yield

loss than was the case with high infestation. For example, in 2012 for Premio, when the number of larvae per 100 ears increased from 0 to 100, the yield fell from 9364 to 8535 kg ha<sup>-1</sup> (i.e. 9%), and when the larvae increased from 100 to 200 the yield fell from 8535 to 8411 kg ha<sup>-1</sup> (i.e. 1%). In 2013, all the plots were infested. It was therefore impossible to predict, without extrapolation, the yield loss corresponding to an increase from 0 to 100 larvae per 100 ears. The minimum number of larvae per 100 ears was 20, for Julius. When the number of larvae per 100 ears in this cultivar increased from 20 to 120, the yield fell from 11 055 to 10 315 kg ha<sup>-1</sup> (i.e. 7%), and when they increased from 400 to 500 the yield fell from 9808 to 9714 kg ha<sup>-1</sup> (i.e. 1%).

The results showed an evident effect of cultivar on yield in both years (2012: LR = 50.077, df = 1,  $P < 0.0001$ ; 2013: LR = 8.833, df = 1,  $P = 0.003$ ). The number of larvae per 100 ears (2012: LR = 15.290, df = 1,  $P = 0.0001$ ; 2013: LR = 56.57, df = 1,  $P < 0.0001$ ) had a similar influence on the yield of both cultivars (cultivar × number of larvae interaction not significant: 2012: LR = 0.754, df = 1,  $P = 0.3852$ ; 2013: LR = 0.02405, df = 1,  $P = 0.8768$ ).

## 4 DISCUSSION

The coincidence between *S. mosellana* flights and the susceptible growth stage of wheat is a key element in the infestation levels of wheat cultivars. This coincidence was observed in each year of the



**Figure 4.** Number of *S. mosellana* larvae per 100 ears in relation to the yield for the susceptible cultivars Premio and Julius in 2012 and 2013. The dots correspond to the observed values and the curves correspond to the predicted values.

study, but the infestation of susceptible cultivars was clearly higher in 2013 than in 2012. This difference was explained by a higher intensity of flights in 2013 than in 2012, which was measured by pheromone trap catches.

In the case of coincidence, an efficient protection of susceptible cultivars needs a well-chosen date of insecticide treatments. In 2012, the treatments were applied too late in relation to the *S. mosellana* flights for Premio to be well protected, apart from the chlorpyrifos + cypermethrin treatment. Premio was more extensively attacked by *S. mosellana* than Julius, which benefited from a better-timed insecticide treatment. In 2013, insecticide treatments were applied at the start of flights and therefore helped to protect the two susceptible cultivars throughout their susceptible growth stages. These treatments also proved to be beneficial when applied at the end of the susceptible growth stage (65), as was the case for Premio. During the two years of the study, the thiacloprid treatment was consistently the least effective treatment in reducing the number of *S. mosellana* larvae in the ears. This finding that thiacloprid (a neonicotinoid insecticide) was less effective than the pyrethroid insecticides confirmed observations from trials conducted in Germany in 2012.<sup>30</sup> The greater effectiveness of the chlorpyrifos + cypermethrin treatment on Premio could be explained by a partial curative effect of chlorpyrifos (organophosphate) on the larvae. Elliott showed in earlier studies<sup>22,23</sup> that chlorpyrifos effectively controlled *S. mosellana* eggs and young larvae because of its high vapour pressure, which allows it to penetrate different parts of the spikelet. In spite of the potential control offered by chlorpyrifos, treatment against *S. mosellana* eggs and larvae is not recommended because it could cancel the benefits of egg–larval parasitoids such as *Macroglanes penetrans* (Kirby).<sup>23</sup>

As expected, the insecticide treatments led to a yield increase. There was a significant increase in 2013 for Altigo and Lear, although they are resistant to *S. mosellana*. This increase might have resulted from an effect of the insecticide treatments on other pests or even on fungal diseases. In Belgium, however, 2013 was particularly remarkable for the almost total absence of aphids, thrips, cereal leaf beetles and other wheat pests, apart from the orange wheat blossom midge.<sup>31</sup> The year 2013 was also

remarkable for the exceptionally low impact of fungal diseases.<sup>32</sup> An effect of insecticides on *S. mosellana* as a vector of *Fusarium* spp. could be dismissed because an assessment of the ears showed that there was no difference in *Fusarium* infection between the treated and untreated plots. The yield increase could more probably derive from the effectiveness of insecticides in protecting wheat against kernel damage caused by the initial feeding of larvae on resistant kernels.<sup>33</sup> In addition, the resistance conferred by the *Sm 1* gene induces a chemical defence through the production of phenolic acids.<sup>21</sup> This chemical defence probably has costs for the plant.<sup>34</sup> The physiological mechanisms associated with this defence are probably not activated in the absence of midges. In the case of severe infestation, as in 2013, however, the cost of this defence is perceptible on the yield through the effect of insecticide treatments on resistant cultivars.

In order to assess the relationship between yield and number of larvae per 100 ears, a non-experimental and correlational study was carried out. A limitation of this approach is that the number of larvae per 100 ears depended partly on the insecticide treatments, and therefore the *S. mosellana* effect was partly due to the treatment effects.

Yield losses caused by *S. mosellana* can be important in the case of high infestation, as in 2013. Based on the idea that the development of larvae in ears results in yield loss, several authors have tried to estimate the harmfulness of *S. mosellana* by linking yield losses and/or the proportion of infested kernels with the numbers of larvae (L3) in the ears.<sup>35,36</sup> Several studies have measured a yield loss of 100 kg ha<sup>-1</sup> for one larva per ear. In 1983 in Canada, Olfert *et al.*<sup>36</sup> recorded a decrease in yield of 100 kg ha<sup>-1</sup> for one or two larvae per ear. In 1994 in England, Oakley *et al.*<sup>11</sup> recorded a mean yield loss of 68 kg ha<sup>-1</sup> for an attack level of one larva per ear. In 2005 in France, yield losses of between 300 and 800 kg ha<sup>-1</sup> for an attack level of 3–10 larvae per ear were recorded by Rouillon *et al.*<sup>37</sup>

The present study showed that there was no linear relationship between yield loss and number of larvae per 100 ears. Each larva that developed in the ears induced greater yield loss when there was low infestation than when there was high infestation. Three non-exclusive hypotheses could explain this result. The first is that some of the damage is inflicted on the kernel by very young



larvae, which die shortly after the start of their development. These dead larvae are not taken into account in the measurement of the 'infestation level of the ears', as defined. A second hypothesis relates to costly reaction of plants to attacks by *S. mosellana*: by activating specific mechanisms in response to the presence of the pest, plants might have to pay for their defence. A third hypothesis could explain these results through intraspecific competition for resources. When more than one larva feed to the detriment of a same kernel, they are smaller because they are competing with each other, and therefore each one inflicts less damage.<sup>38</sup> These hypotheses need to be confirmed in order to improve the integrated pest management of this important pest.

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