

# Assessing cultivar resistance to *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae) using a phenotyping method under semi-field conditions

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## Abstract

The orange wheat blossom midge, *Sitodiplosis mosellana* (Géhin), can significantly reduce wheat yield. Growing resistant wheat cultivars is an effective way of managing this pest. The assessment of cultivar resistance in field trials is difficult because of unequal pressure of *S. mosellana* caused by differences in cultivar heading dates relative to the flight period of *S. mosellana* adult females and huge variations of egg laying conditions from 1 day to another. To overcome these hurdles and to expose all cultivars homogeneously to the pest, an assessment method of cultivar resistance was developed under semi-field conditions. In 2015, the resistance of 64 winter wheat cultivars to *S. mosellana* was assessed. Few or no larvae developed in the ears of resistant cultivars, but in susceptible cultivars, large numbers of larvae developed. Seventeen cultivars proved to be resistant, whereas 47 were susceptible. The identification of new resistant cultivars offers more opportunities to manage *S. mosellana*. The phenotyping method is easy, cheap, efficient and reliable. It can be used to guide the breeding of new resistant wheat cultivars. Using specific midge populations, this method could also be used in research on new resistance mechanisms in winter wheat or in other cereal species.

## KEYWORDS

cultivar resistance, host plant resistance, orange wheat blossom midge, *Sitodiplosis mosellana*, wheat

## 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. This univoltine insect overwinters in the soil, as larvae inside a cocoon. After leaving their cocoon, the larvae move towards the soil surface where they pupate. Adults of both sexes emerge during the spring and mate immediately at the emergence site, after which the females fly off in search of host plants to lay their eggs on the ears. Eggs hatch a few days later, and the young larvae feed on the developing kernels. When the feeding period is over, the larvae leave the ears with rainfall and burrow into the soil, where they form a cocoon and enter into diapause (Barnes, 1956; Oakley, 1994).

Recent damaging outbreaks have occurred in Europe (Chavalle, Censier, San Martin y Gomez, & De Proft, 2015; Gaafar & Volkmar, 2010; Oakley et al., 2005), North America (Knodel & Ganehiarachchi, 2008; Smith et al., 2014) and Asia (Duan et al., 2013). Attacks by *S. mosellana* can significantly reduce yield and the quality of harvested wheat grain (Chavalle, Censier et al., 2015; Miller & Halton, 1961; Olfert, Mukerji, & Doane, 1985). The damage from this pest is often underestimated because most damaged seed is not retained at harvest (Smith et al., 2014). The damage varies between fields and from year to year. The main reason for this irregularity is the required coincidence of three elements: the susceptible phase of wheat, the presence of adult midges and weather conditions conducive to adult midge flight and egg laying (Basedow & Gillich, 1982; Oakley et al., 1998; Pivnick & Labbé, 1993).

The susceptible phase of wheat starts when the ears emerge from the leaf sheath and continue until the end of flowering (Barnes, 1956; Ding & Lamb, 1999). Kernel damage is higher in ears exposed to oviposition during heading (Zadoks growth stages 51-59) than during flowering (Zadoks growth stages 61-69) (Elliott & Mann, 1996; Zadoks, Chang, & Konzak, 1974). This difference results from the level of larval survival, which declines when oviposition occurs after the start of anthesis, as well as from the impact of larval feeding on kernel, which declines with kernel development (Ding & Lamb, 1999; Elliott & Mann, 1996). To forecast the presence of adult midges during this susceptible phase of wheat, several models of *S. mosellana* emergence have been proposed in Europe (Basedow & Gillich, 1982; Jacquemin, Chavalle, & De Proft, 2014; Kurppa, 1989; Oakley et al., 1998) and North America (Ellis et al., 2009; Knodel & Ganehiarachchi, 2008; Lamb, Smith, Wise, & McKenzie, 2016). The model recently proposed by Jacquemin et al. (2014) starts with a period of cold weather sufficient to break diapause. In northern Europe, these requirements are always met by the end of the calendar year (Oakley et al., 1998). The next phase starts 1 January and consists of a temperature accumulation of 250 degree-days (DD) above 3°C. The third phase begins once the second phase is completed and continues until a rise in the mean daily temperature up to 13°C, followed by rainfall. This "inductive rainfall" triggers the fourth phase, which consists of a temperature accumulation of 160 DD above 7°C. At the end of this last phase, the adults emerge. After emergence, adult midge flights and egg laying are strongly dependent on temperature and relative humidity and limited by rainfall and wind (Pivnick & Labbé, 1993).

Damage by *S. mosellana* is also influenced by the vulnerability of cropped cultivars to this pest. Whereas most cultivars have proved to be susceptible, others have proved to be resistant (Chavalle, Jacquemin, & De Proft, 2014; Ellis et al., 2009; Gaafar, El-Wakeil, & Volkmar, 2011; Jacquemin, 2014; Lamb et al., 2016; Robert et al., 2015). Resistance to *S. mosellana* is linked to antixenosis or antibiosis: the antixenosis deters oviposition (Lamb, Smith, Wise, & Clarke, 2001; Lamb et al., 2002), whereas antibiosis, conferred by the *Sm1* gene, leads to the death of larvae through a rapid increase in the production of phenolic acids in response to larval feeding on the kernel surface (Ding, Lamb, & Ames, 2000). Growing resistant cultivars enables *S. mosellana* to be managed effectively because it greatly restricts insect multiplication and eliminates the need to apply insecticide treatments whose broad-spectrum can affect beneficial insects, especially the natural enemies of aphids and *Macroglenes penetrans* (Kirby), the principal parasitoid of *S. mosellana* (Chavalle, Buhl, Censier, & De Proft, 2015).

The assessment of cultivar resistant to *S. mosellana* is difficult in field trials because the heading dates between the earliest and latest wheat cultivars are spread over 2-3 weeks. It is very uncommon to observe gravid female midges and weather conditions conducive to egg laying during this whole period, and cultivars are almost never uniformly exposed to the pest. To expose all cultivars homogeneously to this pest, a method of assessing cultivar resistance to *S. mosellana* under semi-field conditions was developed. In this study, the resistance of 64 winter wheat cultivars to *S. mosellana* was assessed. The assessment of cultivar resistance was based on the number of larvae that developed in the ears.

## 2 | MATERIALS AND METHODS

### 2.1 | Winter wheat cultivars

The experiment was conducted in 2014-15 at Gembloux in Belgium (latitude 50° 33' N, longitude 4° 42' E). The 64 assessed winter wheat cultivars came from the Belgian post-registration evaluation network and represented the most commercialized cultivars in Belgium at this time.

On 9 December 2014, the winter wheat cultivars were sown (nine seeds per 7 × 7 cm square pots), in a mixture of soil taken from a field (loamy soil) and potting soil. On 6 January 2015, the plants were transplanted into plastic pallet boxes (1,110 × 710 × 425 mm). At the bottom of boxes, holes were punched and a 5-cm argex layer covered with a geotextile cloth was put in place to allow good water drainage. The pallet boxes were then filled with loamy soil and placed in a cold glasshouse. The 64 winter wheat cultivars were put in 16 pallet boxes using a randomized complete block design (two blocks), with eight pallet boxes for each block. In each pallet box, eight cultivars were put in small rectangular plots of 54 plants, closely spaced in order to limit tillering and to avoid producing several stems of the same genome. On 13 January 2015, the pallet boxes were placed outdoors to ensure that the growth conditions were close to those in a wheat field. Herbicides, fertilizers, growth regulators and fungicides were applied using the same parameters as those used in the wheat fields. When heading was imminent, on 19 May 2015, the pallet boxes were put into a glasshouse and covered with anti-insect netting to constitute two cages, each cage corresponding to one block. In each cage, the number of ears exposed to *S. mosellana* (i.e., emerged from the flag leaf sheath) was counted for each cultivar twice a week. The watering was applied to the base of the plants in the morning. The water quantity was adjusted as necessary to keep the soil surface humid. The cages were examined daily and cleaned of cereal leaf beetles, aphids and others pests, as well as of spiders so as to prevent cobweb formation that could catch *S. mosellana* adults.

### 2.2 | Establishment of an *S. mosellana* larvae reserve

To produce young adult midges during the susceptible phase of all assessed cultivars in 2015, an *S. mosellana* larvae reserve was established in 2014, that is, the year before the assessment of cultivar resistance.

At the beginning of July 2014, ears highly infested by *S. mosellana* were collected from several winter wheat plots cropped with the susceptible cultivar Tabasco (Jacquemin, 2014) and protected by anti-insect nettings. The anti-insect netting prevented the dispersion of adult midges released in the cage, improved the conditions for flight and egg laying by reducing wind and increasing temperature and, above all, protected the midge eggs from parasitoids. To obtain a high level of ear infestation, adult midges produced from rearing, as described below, were released in large numbers in the cages during the susceptible phase of wheat. The ears were collected when the larvae reached the L3 stage and before they left the ears. They were arranged

on wire grills placed on collecting trays and were sprayed with a fine mist continuously overnight to stimulate the larvae to leave the ears. When the larvae had sunk to the bottom of the collecting trays, they were collected with a sieve with a mesh size of 0.02 mm.

About 300,000 larvae were collected and put in batches of about 3,500 in plastic baskets containing about 4 dm<sup>3</sup> of loamy soil, into which they burrowed. After the burying of larvae, the baskets were placed outside in a trench sheltered from direct light and exposed to prevailing outdoor conditions until after the winter, apart from being watered during the summer drought.

### 2.3 | Production of *S. mosellana* adults and infestation of cultivars

To apply the emergence model described by Jacquemin et al. (2014) for the production of *S. mosellana* young adults, the air temperature around the baskets was recorded using ThermoPuce<sup>®</sup> (Waranet Solutions SAS, Auch, France).

On 11 March 2015, the baskets were put in a cold room at 5°C to slow down the first phase of temperature accumulation of 250 DD above 3°C. In the cold room, the moisture of the soil in each basket was maintained by cold light watering three times per week. Starting 23 April 2015, several baskets were removed from the cold room every 2 days and placed in a room at 20 ± 2°C to attain 250 DD above 3°C. Five days later, each basket was immersed gently in water at room temperature for 2 min to trigger the phase of temperature accumulation of 160 DD above 7°C, after which the emergence of *S. mosellana* adults occurred. Shortly before the start of emergence, each basket was placed in an emergence cage covered with a black cloth and topped with a clear plastic trap for collecting emerging insects. The *S. mosellana* adults were released in the cages with cultivars from the time of the flag leaf sheath opening of the earliest cultivars (19 May 2015) through to the end of the flowering of the latest cultivars (13 June 2015). Each day, the number of young adults emerging from the baskets was estimated and the midges were released in the cages containing the tested varieties.

### 2.4 | Cultivar resistance assessment

The assessment of cultivar resistance to *S. mosellana* was based on the number of larvae that developed in the ears. To ensure that cultivars had been sufficiently exposed to the pest, several, well known for their susceptibility to *S. mosellana*, were included in the experiment. These reference cultivars were chosen to cover the entire early heading spectrum. Among the 64 cultivars, Barok, Boregar, Farandole, Lyrik, Renan and Rubisko were known to be resistant to *S. mosellana*, whereas Bergamo, Cellule, Edgar, Expert, KWS Ozon, Sahara and Terroir were known to be susceptible (ARVALIS, 2014; Chavalle et al., 2014; Jacquemin et al., 2014).

The ears were collected on 9 and 10 July 2015, when the midge larvae at the L3 stage had finished feeding. None of the larvae could have spontaneously left the ears because the plants were watered at their base and so larvae were not stimulated to leave the ears. In each

cage (block), 20 ears per cultivar were collected and arranged in pairs (10 pairs of two ears) vertically over small funnels placed in glass tubes for collecting the larvae. This device was sprayed with a fine mist continuously overnight to stimulate the larvae to leave the ears. The larvae were collected from the bottom of each glass tube, identified and counted under a stereomicroscope using the identification key for the Cecidomyiidae family (Harris, 1966).

## 3 | RESULTS

### 3.1 | Infestation of cultivars by *S. mosellana* adults

The estimated number of adults released during the susceptible phase of winter wheat cultivars was the same in each cage (block) ( $n = 7,320$ ) (Figure 1). The wide range in heading dates between the early and late wheat cultivars meant that it took nearly 1 month to release the midges. Over the whole experiment, 2.5 *S. mosellana* adults were released on average per ear. The number of ears exposed was similar in each block, and at the end of ear emergence, 2,873 ears had been exposed in block A and 2,885 in block B.

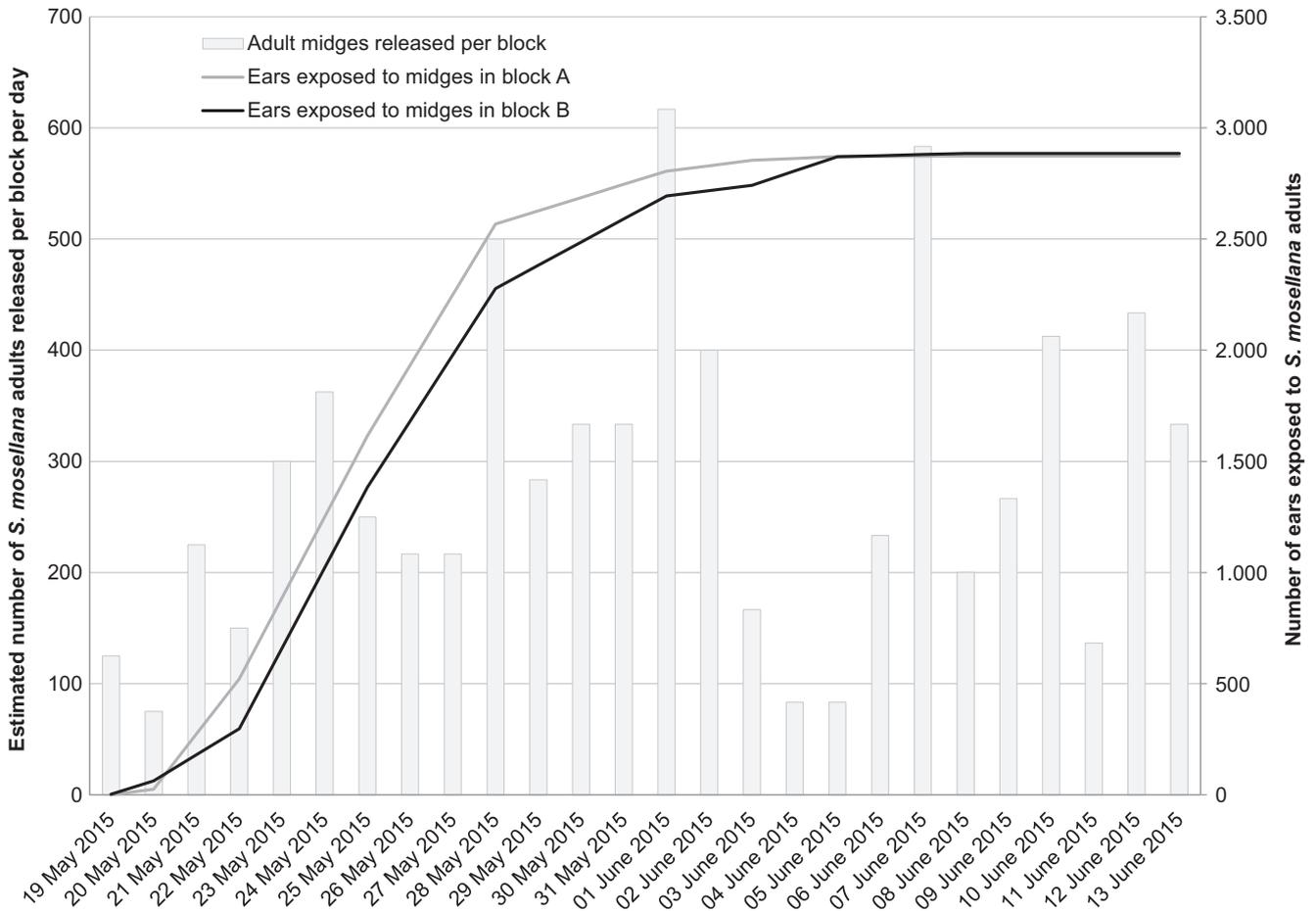
### 3.2 | Cultivar resistance assessment

The number of *S. mosellana* larvae that developed in the ears varied with the cultivar (Figure 2). Few or no larvae developed in the ears of resistant cultivars, whereas in susceptible cultivars, large numbers of larvae developed. The control cultivars known to be resistant (Barok, Boregar, Farandole, Lyrik, Renan and Rubisko) were confirmed as being resistant, and those known to be susceptible (Bergamo, Cellule, Edgar, Expert, KWS Ozon, Sahara and Terroir) were confirmed as being susceptible. These results for the control cultivars showed that the exposure of all the cultivars to the pest had been sufficient and homogeneous. Of the 64 tested cultivars, 17 were resistant, and 47 were susceptible. All the analysed ear pairs of susceptible cultivars contained a large number of midge larvae. These numbers varied among ear pairs, explaining the standard deviations observed. For example, the number of larvae varied from 107 to 335 larvae per ear pair for the cultivar Cellule in block A.

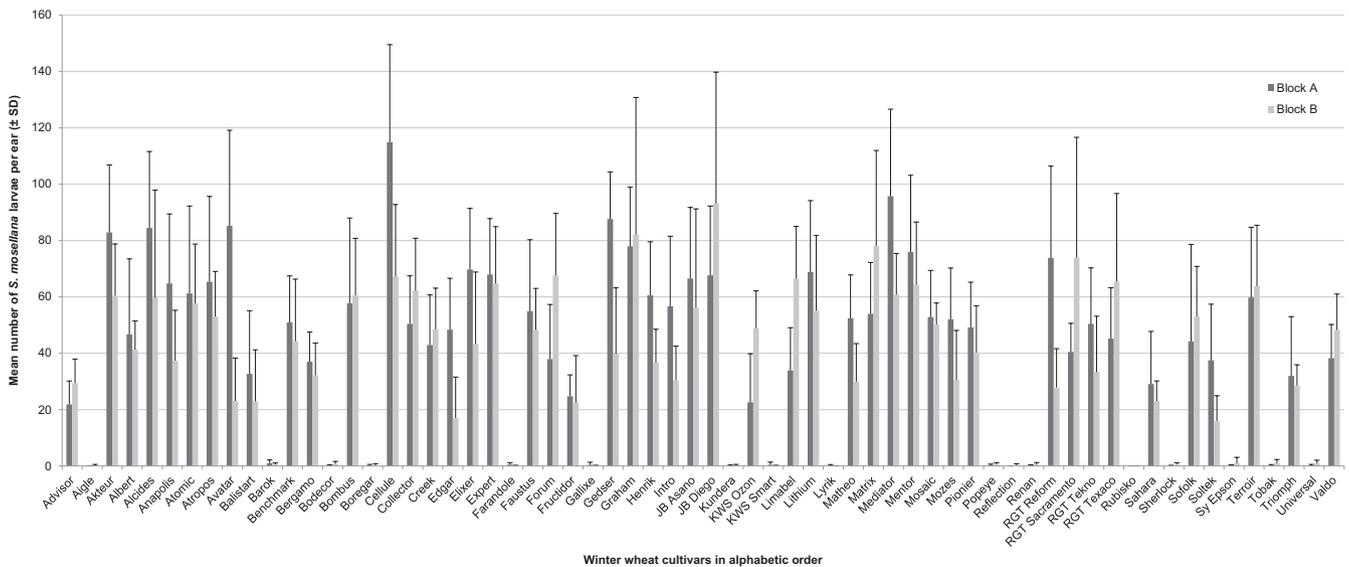
## 4 | DISCUSSION

The sufficient and homogeneous exposure of wheat cultivars to *S. mosellana* during their susceptible phase is key to assess their resistance to this pest. The use of control varieties with different heading dates allowed us to verify whether the exposure to adult midges had been sufficient. The results of these experiments were validated by the large numbers of larvae produced in all the susceptible control varieties, from the earliest ones, such as Cellule, to the latest ones, such as Sahara, proving that cultivars of the whole range of earliness were well exposed to the pest.

Of the 64 tested cultivars, the resistance status of 11 cultivars of these cultivars had been unknown before the experiment. Although



**FIGURE 1** Estimated number of *Sitodiplosis mosellana* adults released per block (cage) per day, and number of ears exposed to *S. mosellana* adults during the susceptible phase of winter wheat cultivars



**FIGURE 2** Mean number of *Sitodiplosis mosellana* larvae per ear ( $\pm$ SD) observed for each winter wheat cultivar (listed in alphabetic order)

the number of larvae developed by ear pairs varied for a same cultivar, the susceptible or resistant character was consistent among ear pairs. Large numbers of midge larvae developed in the ears of the

susceptible cultivars, whereas small numbers were present in the ears of the resistant cultivars. This last phenomenon, already observed in previous studies (Lamb, McKenzie, Wise, Barker, & Smith, 2000; Lamb

et al., 2015; Smith, Wise, & Lamb, 2007; Wise, Fox, & Smith, 2015), may have been artificially amplified by particular conditions of this method. The excellent growing conditions in these tests may have favoured development of larvae beyond what would have been possible under most field conditions. Nevertheless, this last observation is consistent with the hypothesis that a virulence allele allowing an adaptation to *Sm 1* is present in the *S. mosellana* population (Smith et al., 2007). This hypothesis raises the question of resistance management of this pest in wheat. Although growing resistant cultivars is an effective way of managing *S. mosellana*, its widespread use could lead to resistance breakdown (Smith, Lamb, Wise, & Olfert, 2004; Smith et al., 2007). If resistant wheat cultivars were planted over a wide area, the immigration of midges from susceptible wheats with avirulence alleles would be greatly reduced. Without this influx into midge populations developing on resistant wheats, the frequency of the virulence allele would increase in the population, allowing *S. mosellana* to overcome the *Sm1* resistance gene. This phenomenon of resistance breakdown was illustrated by the *H3* gene, which was completely broken down within 9 years by another midge, the Hessian fly, *Mayetiola destructor* (Say) (Foster, Ohm, Patterson, & Taylor, 1991). The use of interspersed refuges in wheat in the form of cultivar blends, as practiced in Canada, is a possible approach for protecting the effectiveness of the *Sm1* gene and delaying the evolution of virulence in *S. mosellana* populations (Smith et al., 2004; Vera et al., 2013).

The phenotyping method developed under semi-field conditions allowed a large number of cultivars to be assessed efficiently and reliably using a few ear numbers, but did not determine the type of resistance. The presence of the *Sm1* gene could be detected using molecular markers (Ellis et al., 2009). The phenotyping method is complementary to the molecular method for assessing cultivar resistance to *S. mosellana* and to avoid the false positives or negatives observed with the molecular markers (Robert et al., 2015). Phenotyping is the only means to identify new sources of resistance. The semi-field method proposed seems well adapted for phenotyping resistance against *S. mosellana*. It can be used very early in the development process of cultivar creation because it needs very few grains. This technique could be used for developing specific midge populations using a two-step screening method. In a first step, larvae collected from *Sm1* cultivars would be isolated from avirulent midge and reared for multiple generations on a *Sm1* cultivar to produce a homozygous virulent-midge colony. In a second step, young adults could be released on *Sm1* cultivars. If larvae do not develop on so infested cultivars, it would indicate the presence of a new resistance mechanism. Moreover, the infestation variation among ears of a same plant or among plants could be determined. This information of high precision can be used to guide the breeders in the selection of their breeding lines to make new resistant wheat cultivars. The semi-field method could be adapted to study oviposition deterrence, as well as resistance damage. The latter, illustrated by field trials (Chavalle, Censier et al., 2015), needs an extra step where plots could grow to maturity. The phenotyping method could be also applied to cereals other than winter wheat to find other resistances to *S. mosellana*. With some adjustments of the phenotyping method, it could be adapted to others midge pests like the saddle gall midge, *Haplodiplosis*

*marginata* (von Roser), and the yellow wheat blossom midge, *Contarinia tritici* (Kirby), two pests of wheat in Europe for which no resistance is currently known.

The identification of 11 new resistant cultivars among those commercialized in Belgium provides farmers with greater choice in the management of *S. mosellana*. This method could be used to identify other resistant cultivars commercialized in other countries. Using resistant cultivars, it should be possible to reduce reliance on insecticides which will help protect non-target organisms including those that are parasites or predators of *S. mosellana*.

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