

# EVALUATION OF THE EFFICACY OF SEX PHEROMONES AND FOOD ATTRACTANTS USED TO MONITOR AND CONTROL *SYNANTHEDON MYOPAEFORMIS* (LEPIDOPTERA: SESIIDAE)

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## Abstract

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*Synanthedon myopaeformis* (Borkhausen, 1789) (Lepidoptera: Sesiidae) is a prominent pest of commercial apple orchards in Europe. the sex pheromones of *S. myopaeformis* and food attractants based on apple juice, beer and red wine were evaluated as tools for monitoring and control the populations of *S. myopaeformis* in apple orchards in the Czech Republic. For monitoring *S. myopaeformis* flight activity, trap designs were also evaluated, and the results indicated that wing traps were more suitable than delta traps because of their high efficacy even at low population densities of *S. myopaeformis*. the flight activity patterns of *S. myopaeformis* showed high intrapopulation variability and variability between years. the use of pheromones as a mating disruption technique led to a decrease of tree injury in comparison to untreated controls during the three years of the experiment. the reduction of the number of *S. myopaeformis* larvae per tree on a 14-ha plot treated subjected to the mating disruption technique reached 56 % in the third year of the experiment. In the three-year experiment using food attractants for the mass trapping of *S. myopaeformis*, catches of *S. myopaeformis* in traps using a combination of beer and apple juice (50:50) at a density of 4 traps/ha on a 4-ha plot increased more than 4-fold.

Keywords: apple clearwing moth, apple orchards, mass trapping, mating disruption

## INTRODUCTION

The apple clearwing moth, *Synanthedon myopaeformis* (Borkhausen, 1789) (Lepidoptera: Sesiidae), is a prominent pest of commercial apple orchards in Europe (Dickler, 1976), Mediterranean countries (Ateyyat, 2006), Canada (Philip, 2006) and the USA (LaGasa, 2009). the larvae bore into bark tissues and leave deposits of brown debris with feces on the branches and trunks of the trees. the injuries result in the weakening of the trees and the subsequent attack of diseases. In the Czech Republic, damage caused by *S. myopaeformis* is frequent and occurs mainly in old orchards with intensive pruning practices.

Chemical control (Judd *et al.*, 2015) is difficult and usually ineffective; therefore, non-chemical means are preferred to control *S. myopaeformis*. Potential non-chemical control strategies against *S. myopaeformis* include mating disruption (Stüber and Dickler, 1987), mass trapping (Trematerra, 1993) and coating or wrapping the damaged bark (Ateyyat and Al-Antary, 2006).

Mating disruption, based on deploying dispensers of synthetic sex pheromones to cause communication disruption between the sexes, is a widely used control strategy in a variety of ecosystems (Witzgall *et al.*, 2010). Mating disruption in orchards has been achieved against *Cydia pomonella*

(Angeli *et al.*, 2007) and *Grapholita molesta* (Il'ichev *et al.*, 2006).

Mass trapping relies on the attraction of both sexes to a lure, usually in combination with an insecticide-impregnated target. This method is widely used to control pests in palm and banana plantations, stored products and households (Witzgall *et al.*, 2010). In the EU, mass trapping is commercially used to control pests in olive fields (Noce *et al.*, 2009) and vineyards (Vacas *et al.*, 2011). For the control of *S. myopaeformis*, studies of mass trapping using pheromone and juice baits (Aurelian *et al.*, 2012) and using female-targeted attractants (Tóth *et al.*, 2012) have been published. Aurelian *et al.* (2016) studied the diversity and abundance of beneficial arthropod by-catch in semiochemical-baited traps targeting *S. myopaeformis* and noted the necessity of a risk-to-benefit approach before mass-trapping technology is widely implemented. Until now, no study has been conducted to compare the efficacy of pheromone delta and wing traps by monitoring the flight activities of *S. myopaeformis*. Studies concerning mass trapping using food attractants or mating disruption techniques in the areas of Central Europe are missing.

The aim of this study was to i) evaluate the efficacy of different types of pheromone traps in monitoring flight activity; ii) evaluate the variability of the flight activity of *S. myopaeformis* in different years; iii) evaluate the efficacy of different mixtures of food attractants used for mass traps targeting *S. myopaeformis* moths; and iv) evaluate the efficacy of mating disruption on decreasing the population density.

## MATERIALS AND METHODS

### Study site

The experiments were conducted from 2001–2008 in a portion of a 41-ha apple orchard under integrated pest management (IPM) in Velké Bílovice (48°52' N, 16°53' E) and in 2002 in a 15-ha apple orchard under IPM in Roudnice nad Labem (50°26' N, 14°16' E). the experimental plots in Velké Bílovice were composed of dwarf Golden Delicious, Jonagold, Idared and Jonagored varieties of high-density super spindle plantings with an average of 3,333 apple trees/ha<sup>-1</sup>. the age of the trees was 8 years old at the beginning of experiment. the orchard was surrounded by old plantings with a high population density of pests. the experimental orchard in Roudnice nad Labem was composed of semi-dwarf Champion and Golden Delicious varieties with an average density of 800 apple trees/ha<sup>-1</sup>. the age of the trees was 15 years old. the orchard was isolated without a source of pest infestation.

Velké Bílovice is a high-population orchard (HPO), and Roudnice nad Labem is a low-population orchard (LPO).

## Sampling and control methods

### Comparison of pheromone traps with different designs

The efficacy of two pheromone trap designs for monitoring the flight activity of *S. myopaeformis* was evaluated in 2002 in 4-ha plots of both the HPO and LPO. the two trap designs used were the wing trap (Intercept-wing, IPM Tech., Inc., Portland, OR) and the delta trap (Biolatrap-delta, CZ). Both of the traps were green in color. the traps were placed in orchards in three replicates and in five replicates in the LPO and HPO, respectively. At each site, the traps were deployed on 9 May and placed 40 m apart from one another and at least 40 m from the edge of the experimental plot. the traps were equipped with a female pheromone lure filled with 10 mg of 3Z, 13-18:Ac (Pherobank, Wageningen, the Netherlands); the traps were inspected weekly. *S. myopaeformis* males caught on the traps were removed, and the sticky bottoms of the traps were replaced when needed.

### Flight activity of *S. myopaeformis*

The wing trap was used to monitor the flight activity of *S. myopaeformis* in the HPO from 2001–2006, and the data were used to evaluate the variability in flight activity of *S. myopaeformis* in particular years. Maximum (t max) and minimum (t min) temperatures were obtained from the weather station at Velké Bílovice. a lower developmental threshold (LDT) of 10 °C was used for calculating the sum of day degrees (DD), which is the accumulation of daily effective temperatures. the daily effective temperature was calculated as ((t min + t max)/2) – LDT. For calculating biological time, the daily effective temperatures were summed from January 1. Weekly trap catches of *S. myopaeformis* were expressed as the percent of total trap catches.

### Attractants for mass trapping

The efficacy of different mixtures of food attractants for the mass trapping of *S. myopaeformis* was evaluated in 2002 in a 4-ha plot in the HPO. Four mixtures of food attractants were used: A) 50 % beer + 50 % apple juice; B) 50 % red wine + 50 % apple juice; C) 70 % red wine + 30 % apple juice; and D) 70 % beer + 30 % apple juice. the non-commercial trap was composed of a 5-L plastic yellow bucket filled with 1 L of attractant and was covered with a plastic cap placed 10 cm above the opening of bucket. the cap enabled *S. myopaeformis* to fly into the bucket and prevented dilution of the attractant by rain. the traps were deployed in the orchard on 9<sup>th</sup> May in four replicates. the traps were placed 40 m apart from one another and at least 40 m from the edge of the experimental plot; the traps were positioned within the tree canopy ca. 1.5 m above the ground. the catches were inspected, and the attractants were changed weekly.

In 2006–2008, variant a of the food attractant (50 % beer + 50 % apple juice) was used in the HPO for the mass trapping of *S. myopaeformis* in a 4-ha plot. the traps were placed uniformly in the experimental plot at a density of 4 traps per 1 ha. In 2007 and 2008, the number of males and females caught in the traps using food attractant was evaluated.

#### **Efficacy of mating disruption**

Mating disruption of *S. myopaeformis* was conducted in a 14-ha plot in the HPO in 2006–2008. a comparable untreated 14-ha control plot was adjacent to the treated plot. Isomate P dispensers (Shin-Etzu Chemical Co., Tokyo, Japan) containing 180 mg of *S. myopaeformis* sex pheromone were deployed from late April to early September at a rate of 300 dispensers/ha<sup>-1</sup>. Evaluation of the efficacy of mating disruption in reducing the population density of *S. myopaeformis* in the treated plot was done in early spring of 2007, 2008 and 2009 by inspecting the average number of larvae per tree. the numbers of *S. myopaeformis* larvae per tree were evaluated by inspecting the tree trunks of 5 trees in 5 replications in each plot, so that, in total, 25 trees were evaluated in both the treated and untreated plots.

#### **Statistical analysis**

The numbers of *S. myopaeformis* trap catches in the two types of pheromone traps were analyzed using the nonparametric Mann-Whitney test. the numbers of *S. myopaeformis* trap catches in the traps with four variants of food attractants were analyzed using the nonparametric Kruskal-Wallis test. the numbers of *S. myopaeformis* larvae per tree in the plots treated with mating disruption, as well as those in the untreated control plot, were analyzed by Mann-Whitney test. a nonlinear regression with the equation  $y = pr3 / (1 + \exp(-pr1 - pr2 * x))$  was fitted into a plot of the cumulative percentage of catches of males and day-degrees (DD), where  $y$  is the cumulative percentage of captured moths,  $x$  is DD, and  $pr1$ ,  $pr2$  and  $pr3$  are the parameters of the model. All the analyses were performed using XLSTAT 2011 (Addinsoft Inc., New York, USA).

### **RESULTS**

#### **Comparison of pheromone traps with different designs**

According to the total number of catches in the delta and wing pheromone traps, a significantly higher number of *S. myopaeformis* males were caught in the pheromone traps in the HPO than in those of the LPO ( $p < 0.0001$ ) (Tab. I). At both locations, significantly higher numbers of males per trap were caught in the wing traps than in the delta traps ( $p < 0.0001$ ) (Tab. I). In the LPO, only 1 male was caught per delta trap per season, but 92 males were caught per wing trap per season. In the HPO, 49 and 206 males were caught per delta trap and wing trap per season, respectively.

Both the delta and wing traps detected the beginning and end of the flight activity in the same week. In contrast, the low number of catches in the delta traps in the LPO did not enable the accurate monitoring of the beginning or end of *S. myopaeformis* flight activity.

#### **Flight activity of *S. myopaeformis***

The monitoring of *S. myopaeformis* flight activity in the HPO in 2001–2006 by wing traps showed high variability in flight activity between years (Tab. II). the beginning of flight activity was recorded between 27<sup>th</sup> May (180 DD) in 2005 and 28<sup>th</sup> June (391 DD) in 2004. the termination of flight activity was recorded between 14<sup>th</sup> August (1203 DD) in 2003 and 15<sup>th</sup> September (1173 DD) in 2005 (Tab. II). the total catches in the pheromone traps per season varied from 94 males in 2004 to 343 males in 2006 (Tab. II). the general flight patterns of *S. myopaeformis* were established from all the data of the cumulative catches and DDs from 2001 to 2006 (Fig. 1). However, regarding the high variability in flight in particular years (Fig. 1), the data are not suitable for the estimation of the beginning, peak or termination of *S. myopaeformis* flights.

#### **Attractants for mass trapping**

All four of the tested mixtures of food attractants proved to be effective for the mass trapping of *S. myopaeformis* moths in the HPO in 2002. No significant difference ( $p < 0.0001$ ) was found in the mean catch per week per trap between the four variants of the food attractant mixtures (Tab. III). However, the highest mean catch of 145.0 moths was recorded in variant a (based on 50 % beer and 50 % apple juice). Hence, this variant was used for the mass trapping of *S. myopaeformis* in the HPO in 2006–2008. With a density of 4 traps ha<sup>-1</sup>, the average catch per trap per season increased from 789 moths in 2006 to 1851 moths in 2007 and to 3592 moths in 2008. Significantly lower numbers of females than males were caught in the traps with food attractant in both 2007 ( $p = 0.297$ ) and 2008 ( $p = 0.147$ ). In 2007 and 2008, the percentages of females from the total catch were 38.7 % and 25.9 %, respectively.

#### **Efficacy of mating disruption**

According to the evaluation of the average number of *S. myopaeformis* larvae per tree, Isomate P significantly decreased the number of larvae in comparison with that of the untreated control plot. the evaluation in spring 2007 after the first year of mating disruption application did not reveal differences between the plot treated with mating disruption and the untreated plot (Mann-Whitney test,  $U = 38$ ,  $n_{1(\text{mating disruption})} = 8$ ,  $n_{2(\text{untreated})} = 9$ ,  $\alpha = 0.05$ ,  $p = 0.885$  two-tailed). However, the next evaluations in spring 2008 showed a significant decrease in the average number of larvae per tree in the plot treated with mating disruption (Mann-Whitney test,  $U = 1$ ,  $n_{1(\text{mating disruption})} = 5$ ,  $n_{2(\text{untreated})} = 8$ ,  $\alpha = 0.05$ ,  $p = 0.007$  two-tailed). In spring 2009, the evaluation

showed no differences between the plot treated with mating disruption and the untreated plot (Mann-Whitney test,  $U = 5$ ,  $n_{1(\text{mating disruption})} = 5$ ,  $n_{2(\text{untreated})} = 7$ ,  $\alpha = 0.05$ ,  $p = 0.051$  two-tailed). the average number of larvae per tree in the plot treated with mating disruption decreased from 3.78 in 2007 to 2.20 in 2009 after 3 years of mating disruption application (Fig. 2). However, the decrease of the average number of larvae per tree was not statistically significant (KW- $H_{2,8} = 1.703$ ,  $\alpha = 0.05$ ,  $p = 0.427$ ). In the untreated control plot, the average number of larvae per tree ranged from 3.21 to 7.75 (Fig. 2). the average number of larvae per tree in 2007 was significantly lower than in 2008 (KW- $H_{2,9} = 12.235$ ,  $\alpha = 0.05$ ,  $p = 0.002$ ).

## DISCUSSION

The efficacy of the different types of pheromone traps for monitoring the flight activity of Sesiidae has been presented in numerous papers (Yonce *et al.*, 1976; Trematerra, 1993). Kutinkova *et al.* (2006) did not find significant differences in the catches of *S. myopaeformis* moths when comparing delta sticky and dry funnel traps used for monitoring flight activity.

According to our results, wing pheromone traps can be recommended as reliable and effective traps for the monitoring of *S. myopaeformis* flight activity in localities with high or low population densities of this pest. Delta traps can be recommended for the monitoring of *S. myopaeformis* flight activity only in localities with a higher population density. In contrast to this, Kutinkova *et al.* (2006) found that delta traps provided accurate information on the seasonal flight dynamics of *S. myopaeformis* despite a very low number of catches in some orchards.

Monitoring by wing traps showed high variability in the flight activity of *S. myopaeformis* from year to year. For example, the beginning of flight varied 34 days, and the termination of flight varied 32 days in particular years. Similarly, Bąkowski *et al.* (2013) found in western Poland a high variability (exceeding 30 days) in the beginning of *S. myopaeformis* flight from year to year. In contrast, Kutinkova *et al.* (2006) found a very low variability in the beginning of flight from year to year in the Plovdiv region in Bulgaria. From a practical point of view, it is necessary to monitor the flight activity of *S. myopaeformis* every year using pheromone traps to evaluate the efficacy of different control measures used against *S. myopaeformis*.

Based on our experiments, the mass trapping method based on food attractants may be an efficacious method to catch *S. myopaeformis* moths. In our experiments, 4 traps/ha ensured catches of over 100 *S. myopaeformis* moths per trap. According to Aurelian *et al.* (2012), juice baits were more efficient in mass trapping of *S. myopaeformis* than pheromone-based traps. However, high catches (over 1000 moths per trap) were obtained using

the mass trapping method based on pheromone lures (Trematerra, 1993).

The effectiveness of the mating disruption technique is affected by many factors, such as the shape, size and isolation of the orchard (Charmillot *et al.*, 2000). In a 3-year experiment with mating disruption against *C. pomonella*, mating disruption decreased fruit damage in plots larger than 1 ha (Angeli *et al.*, 2007); in smaller plots, mating disruption was sufficient only when the population density of *C. pomonella* was low (Howell *et al.*, 1992; Vickers *et al.*, 1998). High efficacy of mating disruption against *S. myopaeformis* has been demonstrated in a 2-year experiment on 3.5 ha plots; in which, an 82.1 % reduction in the number of larvae per tree was found (Stüber and Dickler, 1987). In the second year of that experiment, 4.2 larvae per tree were found in the treated plot compared with 23.3 larvae per tree in the untreated area (Stüber and Dickler, 1987). In our experiments, by the third year there was a 56 % reduction in *S. myopaeformis* larvae per tree in the plot treated with mating disruption compared with the untreated plot. After three years, the numbers of larvae per tree were reduced from 3.78 to 2.20 in the plot treated with mating disruption, and the numbers of larvae per tree increased from 3.21 to 5.01 in the untreated plot. However, reductions of the population density to 2 larvae per tree on slim spindle apple trees do not prevent economic losses caused by *S. myopaeformis*. Beginning a mating disruption technique against *S. myopaeformis* on slim spindle apple trees is recommended at a population density of *S. myopaeformis* of lower than 3 larvae per tree. a significant reduction of population density can be achieved after more than 3 years of using the mating disruption technique on the same plot.



I: Mean catch of *S. myopaeformis* males per trap, per week, by locality (delta and wing together); total catch per trap design, per season; and mean catch per week, per trap design  $\pm$  SD in delta and wing pheromone traps monitored in two localities in 2002.

Locality	Mean catch/week	Trap design	Total catch/trap/season	Mean catch/trap/week
Roudnice n. Labem	$3.475 \pm 8.88^a$	Delta	1	$0.05 \pm 0.22^a$
		Wing	92	$7.08 \pm 11.69^b$
Velké Bílovice	$15.938 \pm 16.39^b$	Delta	49	$3.08 \pm 5.43^a$
		Wing	206	$12.86 \pm 14.77^b$

Values of mean catch/week in each locality with a different small letter are significantly different (Mann-Whitney test,  $U = 5036.50$ ,  $n_1 = n_2 = 80$ ,  $\alpha = 0.05$ ,  $p < 0.0001$  two-tailed). Values of mean catch/trap/week in delta and wing traps in each locality with a different small letter are significantly different (Roudnice n. Labem: Mann-Whitney test,  $U = 248.00$ ,  $n_1 = n_2 = 39$ ,  $\alpha = 0.05$ ,  $p < 0.0001$  two-tailed, Velké Bílovice: Mann-Whitney test,  $U = 4602.50$ ,  $n_1 = n_2 = 80$ ,  $\alpha = 0.05$ ,  $p < 0.0001$  two-tailed).

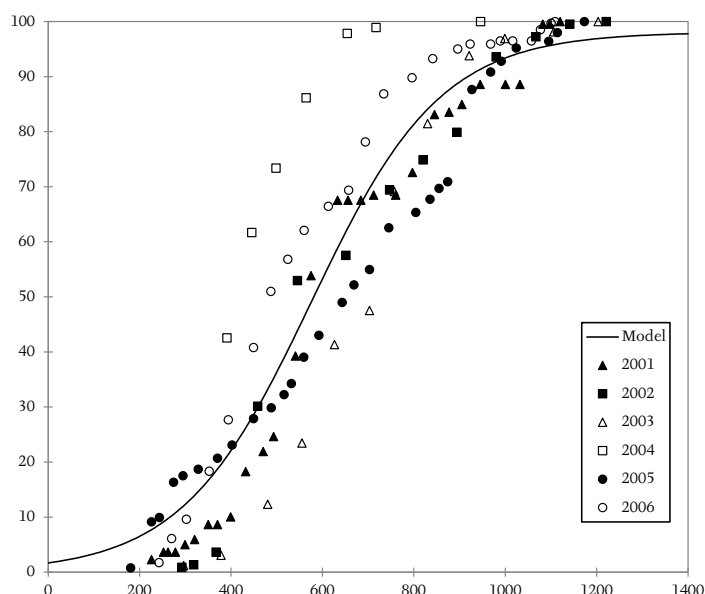
II: Date of first and last catch of *S. myopaeformis* in pheromone wing traps and total catches per trap in Velké Bílovice in 2001–2006.

Year	Total catch	First catch		Last catch	
		Calendar date	DD	Calendar date	DD
2001	219	28 <sup>th</sup> May	226	3 <sup>rd</sup> September	1120
2002	206	3 <sup>rd</sup> June	292	29 <sup>th</sup> August	1121
2003	162	29 <sup>th</sup> May	296	14 <sup>th</sup> August	1203
2004	94	28 <sup>th</sup> June	391	23 <sup>rd</sup> August	946
2005	251	27 <sup>th</sup> May	180	15 <sup>th</sup> September	1173
2006	343	8 <sup>th</sup> June	242	31 <sup>st</sup> August	1109

III: *S. myopaeformis* moth catch (mean per week per trap  $\pm$  SD) in traps with different mixtures of food attractants monitored in Velké Bílovice in 2002: (A) 50 % beer + 50 % apple juice; (B) 50 % red wine + 50 % apple juice; (C) 70 % red wine + 30 % apple juice; (D) 70 % beer + 30 % apple juice.

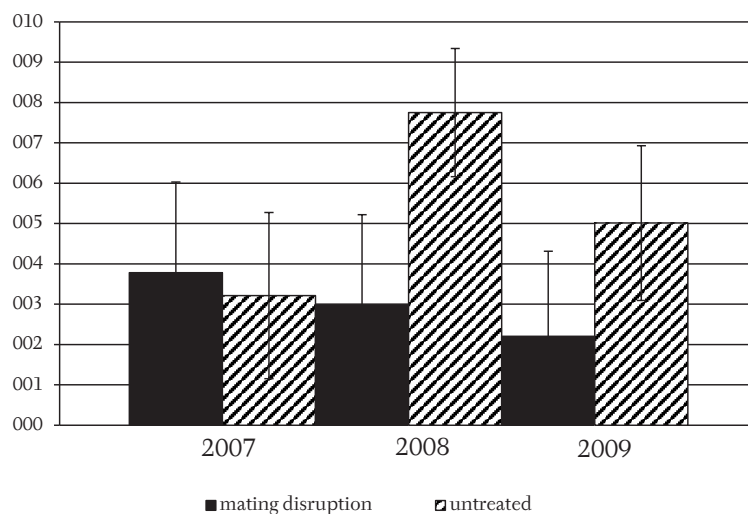
Mixture of food attractant	Catch
A	$145.0 \pm 134.94^a$
B	$132.56 \pm 149.04^a$
C	$123.54 \pm 136.91^a$
D	$135.52 \pm 140.76^a$

Values with the same small letter are not significantly different (Kruskal-Wallis test,  $KW-H_{3,48} = 0.445$ ,  $\alpha = 0.05$ ,  $p = 0.931$ ).



1: General flight patterns of *S. myopaeformis* developed as a nonlinear regression of cumulative catches dependent on DDs in 2001–2006 (model) and data of cumulative catches dependent on DDs in particular years between 2001–2006.

Equation of the model:  $y = pr3 / (1 + \exp(-pr1 - pr2 * x))$ , where  $y$  is the cumulative percentage of captured moths,  $x$  is the DD, and  $pr1$ ,  $pr2$  and  $pr3$  are the parameters of the model.



2: Number of *S. myopaeformis* larvae (mean per tree  $\pm$  SD) in plots with mating disruption and in untreated control plots in Velké Bílovice in 2007–2009

## CONCLUSION

To evaluate the efficacy of different control measures used against *S. myopaeformis*, it is necessary to monitor the flight activity of *S. myopaeformis* every year using pheromone traps. the wing pheromone trap can be recommended as a reliable and effective trap for monitoring *S. myopaeformis* flight activity in all localities, irrespective of the population density of this pest. Mixtures of apple juice, beer or red wine are effective food attractants for the mass trapping of *S. myopaeformis*. the mating disruption technique using Isomate P can be recommended as an effective control strategy to decrease the population density of *S. myopaeformis*, mainly in cases when mating disruption has started early enough at low population densities of the pest.

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