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Selectivity and efficiency: evaluation of synthetic *Grapholita molesta* sexual pheromones

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Abstract: The effectiveness of pheromone lures for *Grapholita molesta* (Busck 1916) from Propher (Czech Republic) and Pherobank (Netherlands), the attractiveness of green and transparent pheromone traps and the representation of non-target species were evaluated in three study areas of southern Moravia (Czech Republic) in 2019–2021 (24 traps per year). A total of 6 536 *Grapholita molesta* and 946 individuals of 17 non-target species were collected. *Grapholita molesta* was frequently captured in green delta traps with a Pherobank pheromone lure. Therefore, pheromone lures from different manufacturers have different efficacy and require the independent setting of harmfulness thresholds. An overview of non-target species recorded by other authors was compiled. Among the non-target species, morphologically similar species of Tortricidae dominated, causing considerable problems in the signalling of control measures of the target species.

Keywords: Lepidoptera; Tortricidae; pests; monitoring; pheromone traps; non-target species

Grapholita molesta (Busck, 1916) is a species originally from Central up to East Asia (Laštůvka 2010). It has been introduced to many parts of the world and is considered an important pest in areas where *Prunus persica*, other *Prunus* species, and local *Malus* and other rosewoods are grown (Blomefield & Geertsema 1990; Bellerose et al. 2007; Lo & Walker 2016). The species was recorded in Europe in 1920 (Paoli 1922) and in the Czech Republic (southern Moravia) in 1951 (Hrdý & Krampl 1977). It is possible that the species occurred earlier in Europe but had not been differentiated from the very similar *Grapholita funebrana* Treitschke, 1835. Hrdý et al. (1979a, 1994) analysed the distribution of *G. molesta* in the Czech Republic using sexual pheromones. Additionally, non-target species responding to the *G. molesta* pheromone have been monitored by Sziráki (1978), Hrdý et al. (1979b), Velcheva (2000), Hrudová (2003) and Jakubíková et al. (2016). Synthetic sexual pheromones are crucial in the occurrence and abundance monitoring of this species. They are also used in male disorientation methods (Hrdý et al. 1990; Evenden & McLaughlin 2004; Witzgall et al. 2008). The chemical structure of the *G. molesta* sexual pheromone was identified by Roelofs et al. (1969). It was later discovered that additional components are necessary for optimal male trapping (Beroza et al. 1973; Roelofs & Carde 1974). Several authors have noted the high number of non-target species caught in pheromone traps for *G. molesta*, which can distort results and make it difficult to determine the need for control measures (Sziráki 1978; Jakubíková et al. 2016).

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Table 1. Characteristics of study areas Soběšice, Starý Lískovec, Kyjov

Study area	Soběšice	Starý Lískovec	Kyjov
Coordinates	49°16'9.640"N, 16°37'47.925"E	49°09'314"N 16°34'25.1"E	49°00'33.3"N 17°08'29.5"E
Faunistic square	6 765	6 865	6 968
Altitude	390 m	270 m	192 m
Area	1.2 ha	2 ha	1.8 ha
Dominant fruit tree species	*Malus domestica* Borkh.	*Prunus domestica* L.	*Prunus armeniaca* L.
Surroundings	mixed forests	apricot orchard	gardens
Pest control	without	synthetic pesticides	ferrous sulphate

The objectives of this study were to evaluate the effectiveness of pheromone lures for *G. molesta* from two different manufacturers, to assess the effectiveness of traps in different colours, and to recognise the spectrum of non-target species and their possible influence on monitoring results.

MATERIAL AND METHODS

Study areas. *Grapholita molesta* was monitored at three study locations in southern Moravia, Czech Republic: Soběšice (S), Brno-Starý Lískovec (SL) and Kyjov (K) in 2019–2021. Table 1 lists the characteristics of each location, with the maximum distance between them being 45 km.

Monthly temperatures at the study locations were not significantly different in 2019–2021 due to their proximity, so we only report them specifically for the Starý Lískovec location. Clearly, the coldest year was 2021, with the beginning of spring significantly delayed; the spring and summer periods of the other two study years were similar in temperature (Figure 1).

Monitoring, species identification and evaluation of results. Commercially available pheromone lures from two manufacturers, Propher (Czech Republic) and Pherobank (Netherlands), were used. Transparent and green plastic delta traps were used. Four green and four transparent traps with two pheromone lures from both manufacturers were placed in each study area. This made a total of 24 pheromone traps per year.

The traps were installed at the end of April and remained in place until the end of September every year. Each trap was placed in the treetop at a height of approximately 160 cm. The glue plates were checked weekly and replaced if necessary. The pheromone lures were replaced every four weeks. During the season, the traps were moved in one position and rotated around the site each week during regular checks.

Captured specimens were determined in the laboratory by genitalia morphology (Razowski 2001). Regression of abundance data was used to compare and statistically evaluate differences between sites, trap types (colour), and pheromone lures from different manufacturers (Agresti 2007).

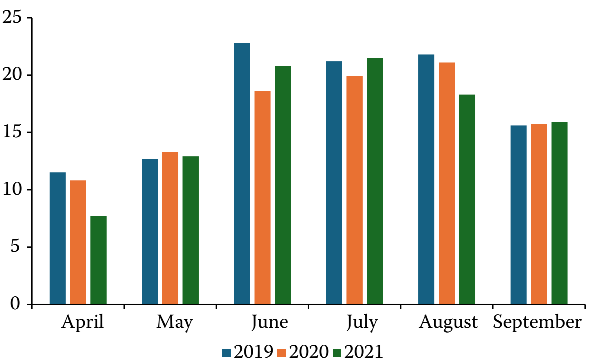

Figure 1. Average monthly temperatures in Starý Lískovec in 2019–2021

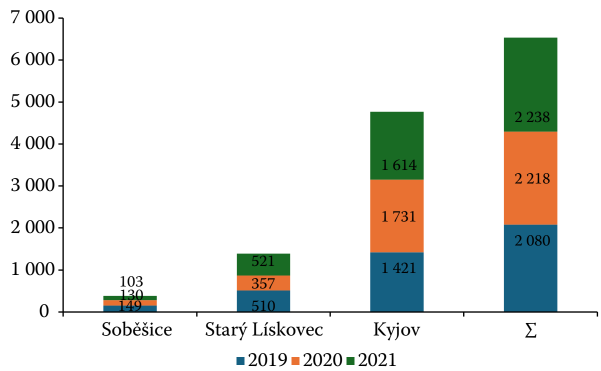

Figure 2. Total numbers of *Grapholita molesta* captured in all study areas

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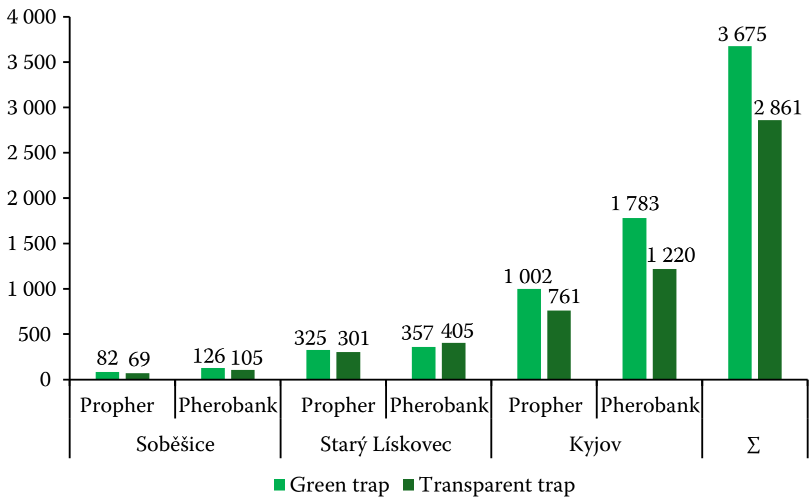

Figure 3. Numbers of *Grapholita molesta* caught in green and transparent traps in the three study areas over three years

RESULTS

Occurrence and abundance of adults in monitored areas. *G. molesta* was recorded in numbers in all study plots, with 6 536 adults caught over three years. The highest total abundance was in the warmest site of Kyjov (4 763 adults), and the lowest was in the coldest site of Soběšice (382 adults). The highest abundance was in 2021 (2 238 adults), the lowest in 2019 (2 080 adults) (Figure 2).

Numbers of adults in different traps. *G. molesta* was more abundant in the green delta traps, where 3 675 adults were captured over three years. A total of 2 861 adults were caught in transparent traps. Only at the Starý Lískovec site were slightly more *G. molesta* caught in transparent traps (Figure 3).

Efficiency of pheromone lures from different manufacturers. Pherobank pheromone lures were more attractive, with 3 996 adults recorded. Proper pheromone lures attracted 2 540 adults (Figure 4).

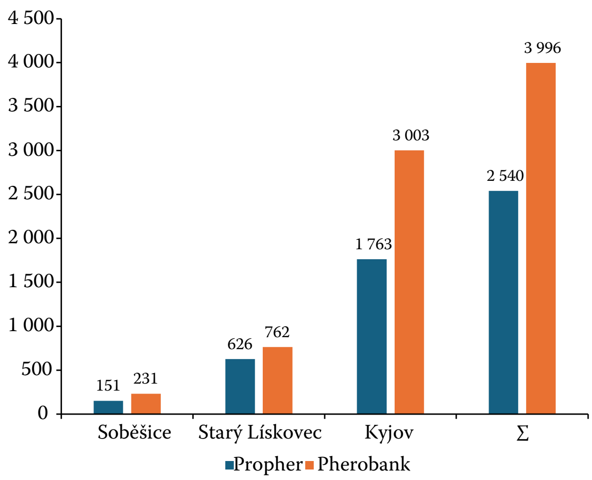

Figure 4. Numbers of *Grapholita molesta* caught on Propher and Pherobank pheromone lures

Statistical analysis of differences between the experimental area – year – trap – pheromone lures. Data, as seen in Figure 5, are highly skewed within each location (Kyjov, Starý Lískovec, Soběšice), type of the trap (transparent, green) and pheromone lure (Pherobank, Propher).

The generalised linear model (GLM) was applied, and the negative binomial regression was selected (see Figure 6) since the data were over-dispersed (the unconditional mean was lower than its variance).

The GLM equation is:

$$\log(N) = b_0 + b_1 I(trap = transparent) + b_2 I(lure = Propher) + b_3 I(place = Sobesice) + b_4 I(place = Stary\ Liskovec) + b_5 year \quad (1)$$

where: N – the number of caught moths; I – the indicator function [i.e., $I(A) = 1$ if A is true, $I(A) = 0$ if A is false].

However, the variable year was not found to be statistically significant, so it was dropped, and the new model has the following equation:

$$\log(N) = b_0 + b_1 I(trap = transparent) + b_2 I(lure = Propher) + b_3 I(place = Sobesice) + b_4 I(place = Stary\ Liskovec) \quad (2)$$

where: N – the number of caught moths; I – the indicator function.

The resulting coefficients of the new model are summarised in Table 2.

The model shows that the change from green to transparent trap results in a 16% decrease in number of caught moths. Similarly, using the Propher lure instead of the Pherobank one leads to a decrease of 32%. The places differ as well; taking the number of moths caught in Kyjov as a base-

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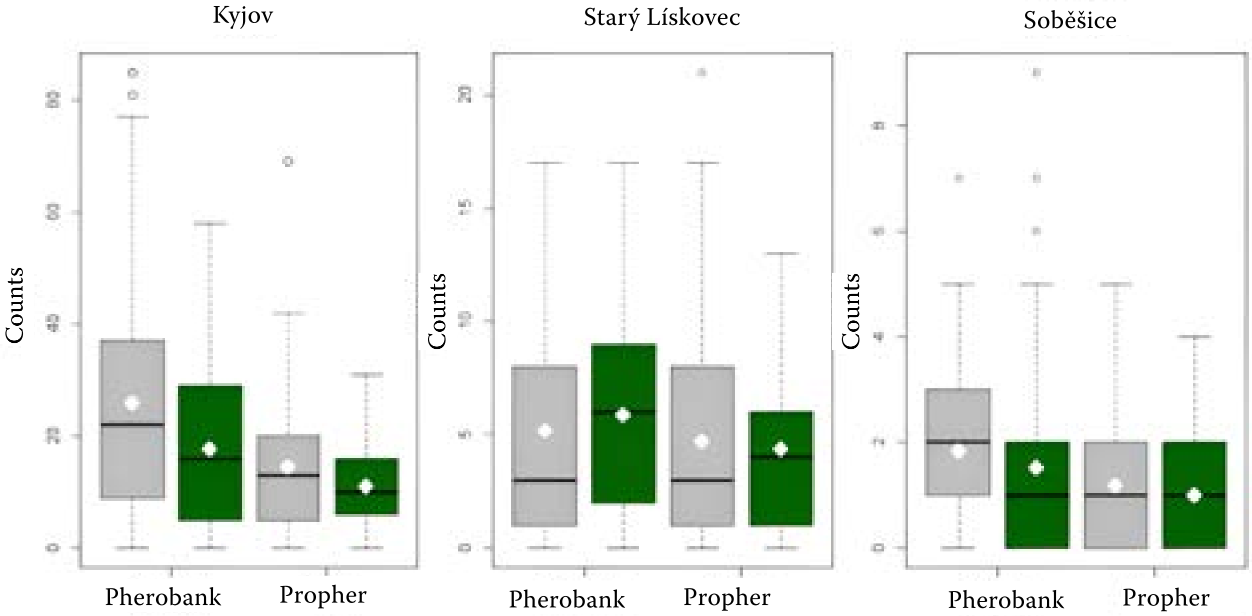

Figure 5. Boxplots of counts of the caught moths with mean values denoted by white points

line, Soběšice and Starý Lískovec have lower numbers of moths by 92% and 70%, respectively.

Seasonal flight activity. In all study areas, the flight (sexual) activity of the study species was recorded from the beginning of May to the end of September. Only minor differences in abundance during the year and at the onset of the first adult generation were observed between plots and years, depending on the spring weather (temperature). As a specific example, we present the situation at the Starý Lískovec site in 2021 (Figure 7). Due to the cold spring, the arrival of more nu-

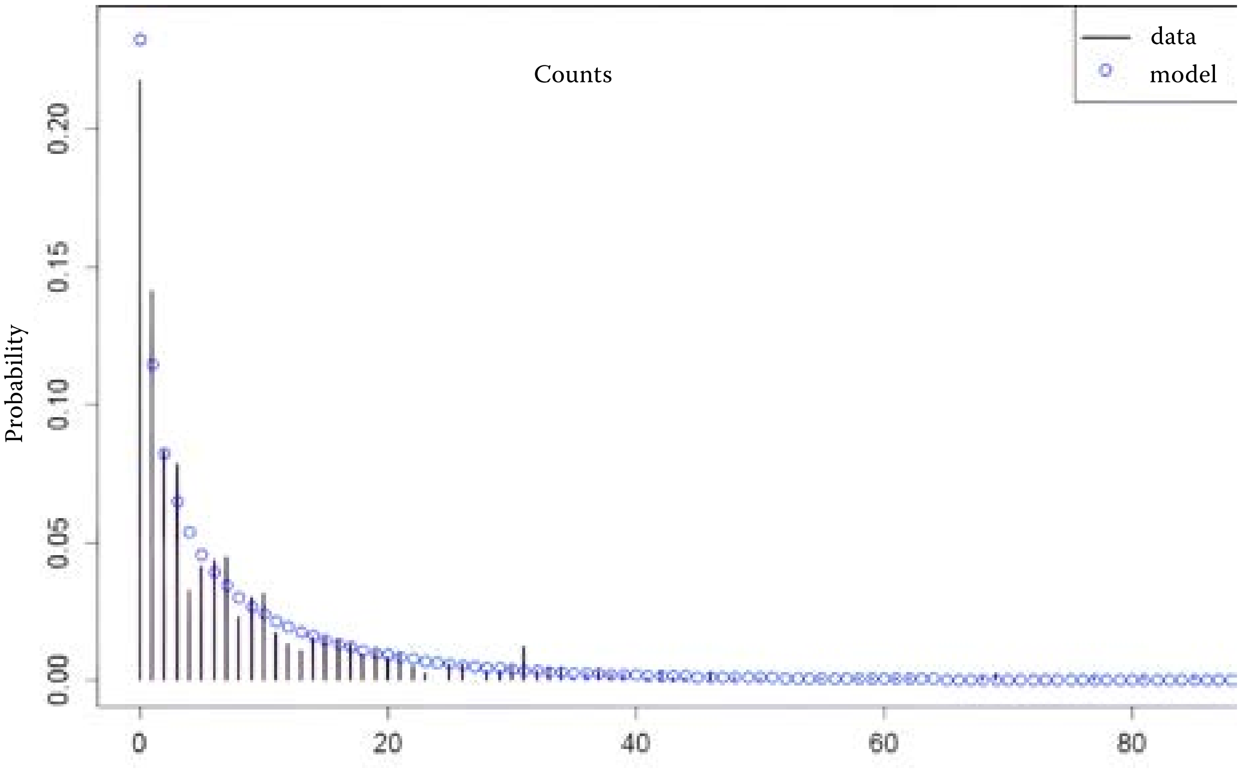

Figure 6. Negative binomial model (blue) of the measured count data (black)

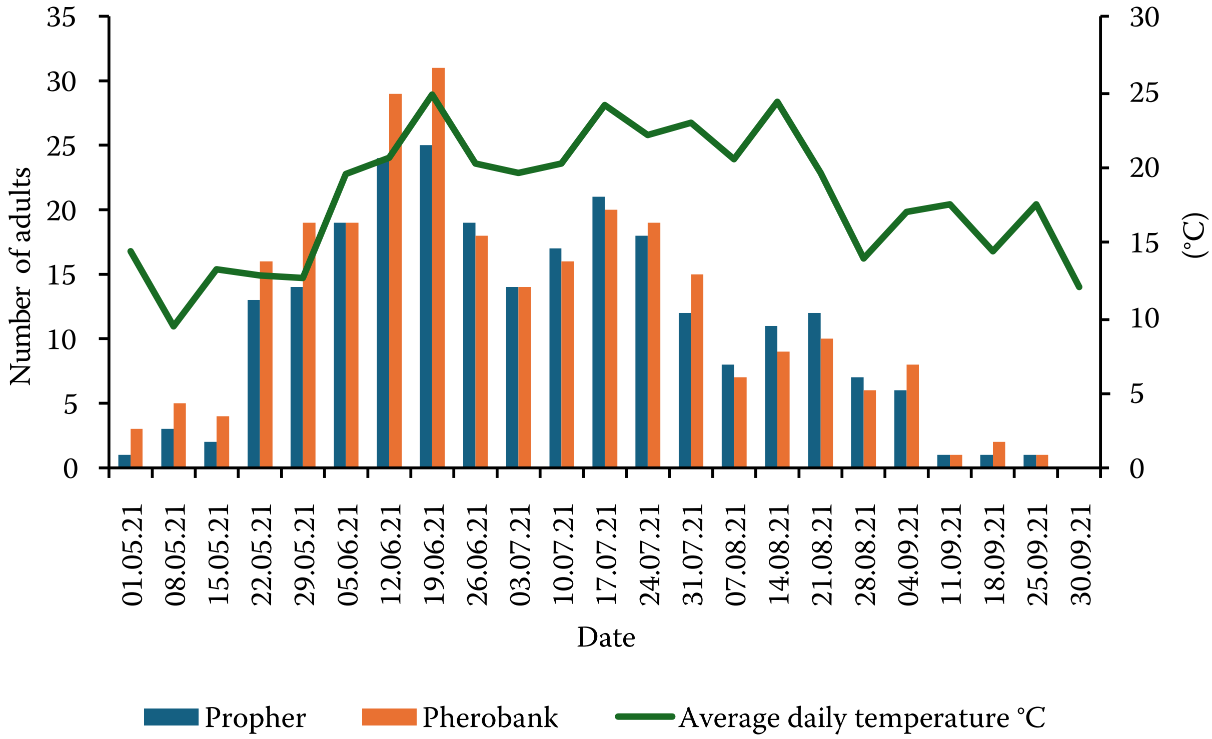

Figure 7. Numbers of *Grapholita molesta* males captured at Starý Lískovec in 2021

merous people was postponed to the second half of May. The number of specimens largely followed the temperature pattern in the subsequent period from early June to early September. It was, therefore, difficult to assess whether the decline in numbers in early July and early August could indicate a gap between generations or simply a result of reduced male activity due to lower temperatures.

Non-target species. A total of 17 non-target species of moths from 6 families (10 Tortricidae,

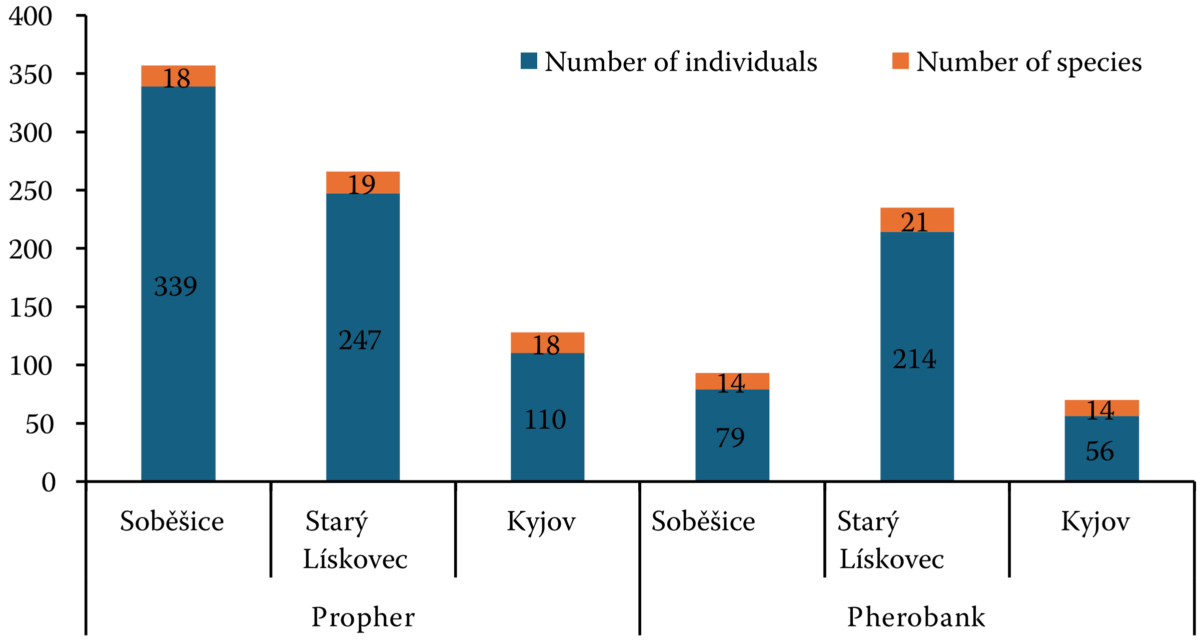

Figure 8. Numbers of non-target species and individuals captured by the Propher and Pherobank *Grapholita molesta* pheromone lures in all areas

Table 2. Estimated coefficients of the regression model with their exponential values, their corresponding *P*-values and confidence intervals

Coef.	Estimate	Exp (estimate)	*P*-value	Confidence interval
b_0 (intercept)	3.0872	21.92	< 0.0001	(2.9430; 3.2357)
b_1	−0.1711	0.84	0.0196	(−0.3152; −0.0271)
b_2	−0.3888	0.68	< 0.0001	(−0.5328; −0.2446)
b_3	−2.5067	0.08	< 0.0001	(−2.6932; −2.3212)
b_4	−1.1929	0.30	< 0.0001	(−1.3582; −1.0276)

3 Noctuidae, one species each from Autostichidae, Crambidae, Depressariidae and Erebidae) were captured on *G. molesta* pheromone lures during the study, totalling 946 individuals.

Of these, 697 individuals of 16 species from 6 families were captured on Propher pheromone lures and 249 individuals of 12 species from 3 families were captured on Pherobank pheromone lures (Figure 8). In Table 3, non-target species are ranked by constancy of occurrence). *G. funebrana* was the most frequent species (540 individuals) (Figure 9).

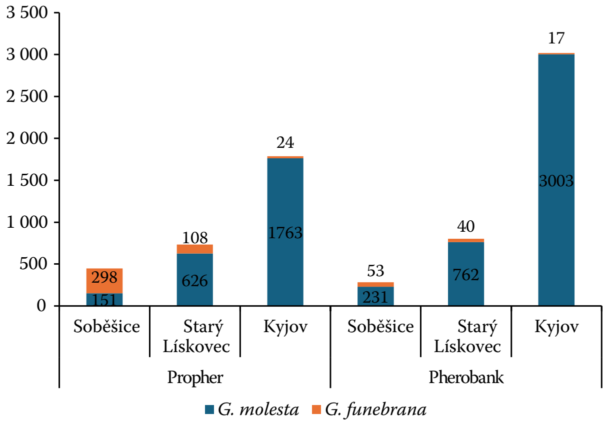

Figure 9. Numbers of adults of *Grapholita molesta* and *G. funebrana* captured in traps with *G. molesta* pheromone lures from Propher and Pherobank

DISCUSSION

G. molesta was recorded in relatively high abundance in all study plots during the study period. This indicates a long-term, gradual increase in the abundance of this species compared to the past and, thus, an increase in its phytosanitary impor-

Table 3. Non-target moth species captured on Propher and Pherobank pheromone lures

Non-target moth species	Propher									Pherobank									
	2019			2020			2021			2019			2020			2021			
	S	SL	K	S	SL	K	S	SL	K	S	SL	K	S	SL	K	S	SL	K	Σ
Grapholita funebrana	172	44	12	57	25	3	69	39	9	27	15	8	16	11	3	10	14	6	540
Cnephasia stephensiana	1	9	7	1	3	15	3	5	13	2	10	–	2	3	2	–	4	–	80
Epiblema scutulana	–	4	12	–	1	2	–	9	15	–	7	7	–	2	4	–	5	6	74
Oegoconia novimundi	5	–	–	–	–	1	2	–	1	1	–	8	4	–	3	2	–	5	32
Pammene gallicolana	2	10	–	–	11	–	1	10	–	4	6	–	1	1	–	5	2	–	53
Pammene suspectana	7	13	–	4	8	–	5	9	–	1	6	–	3	2	–	–	4	–	62
Hedya nubiferana	–	12	3	–	14	–	–	17	1	–	9	–	–	2	1	–	7	1	67
Agrotis segetum	–	1	–	–	3	–	–	–	–	–	–	–	–	1	–	–	2	–	7
Hedya pruniana	–	–	3	–	–	1	–	–	2	–	–	–	–	–	–	–	–	–	6
Oligia latruncula	–	–	–	1	–	–	6	–	–	–	–	–	–	–	–	–	–	–	7
Celypha striana	–	–	–	–	–	9	–	–	–	–	–	–	–	–	1	–	–	–	10
Cnephasia alticolana	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	2
Cnephasia ecullyana	–	–	–	–	–	1	–	–	–	–	–	–	–	–	1	–	–	–	2
Cydalima perspectalis	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	1
Depressaria chaerophylli	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
Hypena rostralis	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
Xestia xanthographa	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	1

S – Soběšice; SL – Starý Lískovec; K – Kyjov

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tance (see, e.g. Hrdý et al. 1979a; Rauleder 2007). The differences in the numbers of captured individuals between the study areas are due to their overall character, i.e. climatic conditions and the presence of suitable (various) host plants. The annual differences were due to weather; in particular, the cold spring of 2021 caused the first generation's phenological shift (delay) compared to the other two years. Minor differences in the effectiveness of the two colour types of pheromone traps evaluated do not significantly affect their use for monitoring or signalling of this species. The effectiveness of pheromone lures from different manufacturers is probably mainly due to the amount of pheromone (active substance) in the lure. It is important that as the amount of the active substance in a pheromone lure increases, so does its price. In relation to the price of the pheromone lure, the lower efficiency (fewer individuals caught) is not negative. However, adjusting the harmfulness thresholds (number of males captured) for lures from different manufacturers is necessary, which signals the need for a control intervention.

The very long period of adult emergence and oviposition, combined with the endophagous development of the caterpillars, poses a problem for effectively controlling *G. molesta*. During our study of flying activity, we did not observe any significant peaks in abundance that would have signalled appropriate times for control interventions. Male disorientation has been suggested as an ecologically acceptable but economically relatively expensive method (Kyparissoudas 1989; Falta et al. 2008).

A major problem in monitoring this species using synthetic sexual pheromones is the lack of selectivity of the pheromones used. This has not improved significantly after 50 years of use. There are reports of 63 non-target species being caught by the *G. molesta* pheromone. We recorded a further eight species during our research. Of these 71 species, 51 belong to the family Tortricidae, one to the families Autostichidae, Crambidae, Depressariidae, Pterophoridae, two belong to the families Erebidae, Geometridae, Pyralidae and Yponomeutidae, and eight belong to the family Noctuidae (Sziráki 1978; Hrdý et al. 1979a, b, 1989, 1994; Velcheva 2000; Hrudová 2003, 2005; Jakubíková et al. 2016) (Table 4). Of these species, 46 were recorded in a single research, usually in one or a few individuals. Their presence in traps was likely entirely accidental (they may have used them as shelters). The same may be true for other species, such as *Agrotis segetum* and *Oligia latruncula*, although they were captured in several individuals.

On the contrary, about 10 species of the family Tortricidae have been captured in pheromone traps for *G. molesta* by most of the listed authors in higher numbers. In addition to *G. funebrana*, also *Cnephasia stephensiana*, *Epiblema scutulana*, *Pammene gallicolana*, *P. suspectana* and, among species from other families, *Oegoconia novimundi* were recorded in particularly high numbers. In our study, non-target species represented about 15% of the captured individuals, but depending on the nature of the orchard environment, their proportion may reach up to 90% (our unpublished observations). These species are often more or less similar to *G. molesta*. They are not distinguished in practice (and cannot be distinguished with the technical capabilities of the assessor), which subjectively 'increases' the need for regulation of the target species and may lead to unnecessary control interventions.

Grapholita molesta is also attracted by pheromone lures for *G. funebrana* (Hrdý et al. 1979b; Hrudová 2003; Jakubíková et al. 2016) and has also been reported in traps with lures for *G. janthinana* (Hrdý et al. 1997; Jakubíková et al. 2016) and *G. lobarzewskii* (Jakubíková et al. 2016). The proportion of *Grapholita molesta* and *G. funebrana* in the pheromone lures for these two species varies depending on the composition of the pheromone and, to a significant extent, also on the environment of the trap (presence of different species or cultures of fruit trees and wild Rosaceae species).

The relatively small number of replications is likely the main reason the results show a statistically significant degree of variability (Figure 5). The year-to-year differences were not statistically significant, although the weather was relatively different year to year, with the particularly cold spring of 2021 causing a more pronounced phenological shift. As expected, the statistically significant differences between the locations were caused by their different character. The small difference in the effectiveness of the different trap colours was also statistically significant but is unlikely to have a major impact on the practical use of the traps. The clear difference between the number of individuals captured by the Propher and Pherobank pheromone lures was also statistically confirmed and needs to be considered in practical monitoring.

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Table 4. Non-target species of moths caught in pheromone traps for *Grapholita molesta* by different authors; species recorded during our research are shown in bold, and species recorded for the first time are underlined

Species	Sziráki (1978)	Hrdý et al. (1979b)	Hrdý et al. (1979a)	Hrdý et al. (1989)	Velcheva (2000)	Hrdý et al. (1994)	Hrudová (2003)	Hrudová (2005)	Jakubíková et al. (2016)
Tortricidae									
Adoxophyes orana (Fischer von Röslerstamm, 1834)							×	×	
Apotomis lineana (Dennis & Schiffermüller, 1775)	×								
Argyrotaenia ljungiana (Thunberg, 1797)		×							
Archips rosana (Linnaeus, 1758)							×	×	
Archips xylosteana (Linnaeus, 1758)		×							
Cacoecimorpha pronubana (Hübner, 1799)									×
Celypha lacunana (Denis & Schiffermüller, 1775)	×								
Celypha rosaceana (Schläger, 1848)									×
Celypha rurestrana (Duponchel, 1843)	×								
***Celypha striana* (Denis & Schiffermüller, 1775)**		×	×			×			
***Cnephasia alticolana* (Herrich-Schäffer, 1851)**									
***Cnephasia ecullyana (*Réal, 1951)**									
*Cnephasia genitalana (*Pierce & Metcalfe, 1915)			×			×			
***Cnephasia stephensiana* (Doubleday, 1849)**		×	×	×	×	×			×
Cydia pomonella (Linnaeus, 1758)									×
Dichrorampha petiverella (Linnaeus, 1758)					×				
Enarmonia formosana (Scopoli, 1763)			×			×		×	
Epiblema cirsiana (Zeller, 1843)									×
***Epiblema scutulana* (Denis & Schiffermüller, 1775)**	×	×	×		×	×		×	
Eupoecilia ambiguella (Hübner, 1796)		×							
***Grapholita funebrana* (Treitschke, 1835)**	×	×		×	×		×	×	×
Grapholita janthinana (Duponchel, 1835)	×								×
Grapholita lobarzewskii (Nowicki, 1860)									×
Grapholita tenebrosana (Duponchel, 1843)	×	×	×		×	×			
***Hedya nubiferana* (Haworth, 1811)**	×								
***Hedya pruniana* (Hübner, 1799)**		×		×	×				×
Isotrias hybridana (Hübner, 1817)	×			×					
Notocelia roborana (Denis & Schiffermüller, 1775)								×	
Notocelia rosaecolana (Doubleday, 1850)							×	×	
Pammene albuginana (Guenée, 1845)	×				×				×
Pammene amygdalana (Duponchel, 1843)					×				×
Pammene argyrana (Hübner, 1799)	×				×				×
Pammene aurana (Fabricius, 1775)	×								×
Pammene fasciana (Linnaeus, 1761)	×			×	×	×			×
Pammene gallicana (Guenée, 1845)	×								
***Pammene gallicolana* (Lienig & Zeller, 1846)**	×				×				×
Pammene giganteana (de Peyerimhoff, 1863)	×				×				
Pammene insulana (Guenée, 1845)	×				×				
Pammene querceti (Gozmány, 1957)	×								

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Table 4. to be continued...

Species	Sziráki (1978)	Hrdý et al. (1979b)	Hrdý et al. (1979a)	Hrdý et al. (1989)	Velcheva (2000)	Hrdý et al. (1994)	Hrudová (2003)	Hrudová (2005)	Jakubíková et al. (2016)
Pammene regiana (Zeller, 1849)	×								
***Pammene spiniana* (Duponchel, 1843)**	×				×				×
Pammene splendidulana (Guenée, 1845)	×								
***Pammene suspectana* (Lienig & Zeller, 1846)**	×	×			×				×
Pandemis cerasana (Hübner, 1786)							×	×	
Pandemis dumetana (Treitschke, 1835)	×								
Paramesia gnomana (Clerck, 1759)	×								
Philedonides lunana (Thunberg, 1784)					×				
Pristerognatha fuligana (Denis & Schiffermüller, 1775)					×				
Spilonota ocellana (Denis & Schiffermüller, 1775)							×	×	
Tortricodes alternella (Denis & Schiffermüller, 1775)	×								
Tortrix viridana (Linnaeus, 1758)		×							
Autostichidae									
***Oegoconia novimundi* (Busck, 1915)**									×
Depressariidae									
***Depressaria chaerophylli* Zeller, 1839**									
Geometridae									
Ligdia adustata (Denis & Schiffermüller, 1775)									×
Philereme transversata (Hufnagel, 1767)									×
Erebidae									
Hypena proboscidalis (Linnaeus, 1758)									×
***Hypena rostralis* (Linnaeus, 1758)**									
Noctuidae									
Cirrhia icteritia (Hufnagel, 1766)									×
Mesapamea secalis (Linnaeus, 1758)									×
Mesapamea secalella Remm, 1983									×
Euxoa nigricans (Linnaeus, 1761)			×						
Amphipoea fucosa (Freyer, 1830)		×							
***Agrotis segetum* (Denis & Schiffermüller, 1775)**		×							
***Oligia latruncula* (Denis & Schiffermüller, 1775)**									
***Xestia xanthographa* (Denis & Schiffermüller, 1775)**									
Pterophoridae									
Pterophorus pentadactyla (Linnaeus, 1758)									×
Pyralidae									
Hypochalcia ahenella (Denis & Schiffermüller, 1775)									×
Hypsopygia costalis (Fabricius, 1775)		×							
Crambidae									
***Cydalima perspectalis* (Walker, 1859)**									
Yponomeutidae									
Yponomeuta evonymella (Linnaeus, 1758)		×							
***Yponomeuta malinellus* Zeller, 1838**									

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CONCLUSION

Pheromone lures from different manufacturers have different levels of attraction and require independent setting of harmfulness thresholds.

Transparent traps are slightly less attractive than green ones, which have little effect on the number of males captured and, thus, the monitoring results.

A fundamental problem in monitoring is the high proportion of *G. funebrana* and other non-target species, which makes it difficult to decide on possible control interventions.

In addition to the composition of the pheromone, the proportion of *G. funebrana* in a trap with the pheromone lure for *G. molesta* is fundamentally influenced by the character of the surroundings (fruit tree species).

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