Effect of Temperature and Interrelationships of the Host *Pleuroptya ruralis*, Its Primary and Secondary Parasitoids in Lithuania

Galina BARTKEVIČIENĖ¹ Rasa BERNOTIENĖ² Vytautas JONAITIS³

¹Laboratory of Avian Ecology, Nature Research Centre, Akademijos 2, Vilnius LT-08412, LITHUANIA, e-mail: galina@ekoi.lt ²Laboratory of Entomology, Nature Research Centre, Akademijos 2, Vilnius LT-08412, LITHUANIA, e-mail: rasab@ekoi.lt ³Laboratory of Entomology, Institute of Ecology, Akademijos 2, Vilnius LT-08412, LITHUANIA

ABSTRACT

Research results on the long-term dynamics (1982-2004) of the temporal dispersion of the host *Pleuroptya ruralis*, its parasitoid the *Macrocentrus cingulum* as well as its second parasitoids *Encrateola laevigata*, *Gelis ruficornis* and *Pteromalus dispar* on common nettle in Lithuania and the relationships between the air temperatures and development of these species are presented. Multiple regression analysis was carried out to determine the most important relations between dependent (appearance date, emergence peak, adult emergence duration, density) and some independent variables (air temperature, life cycle parameters of related species). The most important factors for the life cycle parameters of investigated species were determined.

The appearance dates and the emergence peaks of secondary parasitoids *Encrateola laevigata*, *Gelis ruficornis* and *Pteromalus dispar* are related with air temperature in June and the appearance date of their host *M. cingulum*. The appearance date as well as the emergence peak of *P. ruralis* are the most important factors for the appearance date and the emergence peak of *M. cingulum*. The most important factors for the emergence durations of secondary parasitoids are their densities. The emergence duration of *P. dispar* is related with air temperature in July and the emergence duration of *M. cingulum*, which is related with the emergence duration of its host. Densities of the secondary parasitoids were related with density of primary parasitoid, which was influenced by the density of the host, *P. ruralis*.

Key words: Density, emergence, Encrateola laevigata, Gelis ruficornis, Macrocentrus cingulum, Pteromalus dispar.

INTRODUCTION

Recent climate changes have influenced many living organisms, including the phenology, population size, and distribution patterns of insects (Tryjanowski *et al.*, 2010). One of the climate indicators, the temperature, has a direct impact on the annual physiologic cycle of insect development (Odum, 1971; Stange and Ayres, 2010).

Seasonality of insect development is hereditary basis, characteristic of the species and populations, but is also under the control of external environmental factors (Sokolov, 2012). Temperature is changing and it is expected to be changing in the future (IPCC, 2013), so it is of great importance to understand the response of different animal

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species to regional changes in thermal regimes. It is thought that this temperature change has influenced the phenology and distribution of organisms (Menzel et al., 2006; Parmesan, 2007), but the magnitude of these ecological changes may be relatively minor compared with those in future. The adaptation of insects to regularly changing environmental conditions may be secured by coordinating some parameters of biological systems with air temperature. The most important environmental factors affecting the beginning of insect development are the day length and air temperature. These two parameters are perceived by insect's receptors as signaling information about the environment, which allows to synchronize their development with periodic changes in weather (Sokolov, 2012). However, insect responses to climatic changes in isolation can differ considerably from their responses to the interaction of multiple components of climate change (Owain and Keith, 2008). Climate change affects the abundance, distribution and activity of natural enemies that are important for suppressing herbivore crop pests. Moreover, higher mean temperatures and increased frequency of climatic extremes are expected to induce different responses across trophic levels, potentially disrupting predator-prey interactions (Romo and Tylianakis, 2013).

We investigated *Pleuroptya ruralis* (Scopoli, 1763) (Lepidoptera: Crambidae), which is known as a serious pest of soybeans. The overwintering generation and summer generation use the nettles (Urticales, Urticaceae) and soybeans as host plants, respectively (Katayama *et al.*, 2012). *Macrocentrus cingulum* Brischke, 1982 (Hymenoptera: Braconidae), the parasitoid of *P. ruralis,* attacks caterpillars of the European corn borer also, exerting low to moderate levels of parasitism in the field (Onstad *et al.*, 1991). The second parasitoids of *P. ruralis* (*Encrateola laevigata* (Ratzeburg, 1848)), *Gelis ruficornis* Thunberg, 1827 (Hymenoptera: Ichneumonidae) and *Pteromalus dispar* (Curtis, 1827) (Hymenoptera: Pteromalidae) were also investigated.

Macrocentrus cingulum was introduced into the US from France and Korea earlier this century to control the European corn borer (Owain and Keith, 2008). It has been shown to transmit the protozoan *Nosema pyrausta* (York, 1961) between individuals of its host (Siegel *et al.*, 1986). This protozoan causes disease in corn borer. Consequently, *M. cingulum* could provide a service by increasing the spread of this pathogen, however, this parasite can also decrease the lifespan of the adult wasp and depress reproduction (Andreadis, 1980; Siegel *et al.*, 1986).

The new empirical and theoretical information about the diversity and regularities of the long-term dynamics of the phenological situation of simple and complicated biological systems and their temporal dispersion is always useful. Therefore we generalized the research results about the long-term dynamics of the temporal dispersion of the parasitoid of *P. ruralis.* the *M. cingulum* as well as its second parasitoids *E. laevigata*, *G. ruficornis* and *P. dispar* on common nettle in Lithuania. The aim of this work is to extend the earlier data and to provide basic information on the relationship between the development and the temperature of most abundant species of secondary parasitoids.

MATERIALS AND METHODS

Study site

The material was collected in five study sites in Vilnius: Pilaitė (54°41'35"N, 25°11'37"E); Karoliniškės (54°41'37"N, 25°13'48"E); Jeruzalė (54°44'44''N, 25°16'46"E); Visoriai (54°45'11"N, 25°25'23"E), Verkiai (54°44'54"N, 25°17'11"E) and in three localities in Vilnius district: Dūkštos (54°50'12"N, 24°57'38"E), Gudeliai, (54°49'42"N, 25°10'44"E) and Kiemeliai (54°52'08"N, 24°58'08"E). The material collection took place in 1982-2004.

Material collection

The materials were collected as described by Bartkevičienė *et al.* (2010). Breafly; nettle leaves with a moth larvae or pupae were collected every week throughout the adult emergence period from the beginning of June till the middle August. Each sample was kept separately in a glass test-tube with a hole stuffed by cotton wool. Tubes were kept outside at natural temperature. They were observed daily till the emergence of the moth or its primary and secondary parasitoid adults. The appearance date, the adult emergence peaks and the emergence duration and density of the host, primary and secondary parasitoids were observed and investigated. The appearance date and adult emergence peaks were expressed in days from the first of January. The emergence duration was expressed in days. The insect density was expressed as the average number of specimens per 10 m².

Air temperature

Data of the mean monthly air temperatures were obtained from the archive of the Lithuanian Hydrometeorological Service to the Vilnius Meteorological Station situated outside the city (54°37′35″N; 25°5′41″E). We used values of monthly spring and summer air temperatures (Sparks *et al.*, 2006; Bartkevičienė *et al.*, 2010), but relationships only between May, June, and July air temperatures and investigated parameters were found what is why temperatures of these months (T_{MAY} , T_{JUN} , and T_{IIII} °C) were used in the further analysis.

Statistical analysis

Multiple regression (Beta±SE, i. e. regression coefficient ± Standard Error) analysis was carried out to determine the most important relations between dependent and some independent variables. The determination coefficient (R^2), Student test (t), F-test (F), and p-level were calculated in order to estimate the statistical reliability of regression.

The appearance dates of primary and secondary parasitoids were analyzed looking for associations with air temperature, and appearance dates of their hosts. The emergence peaks of primary and secondary parasitoids were analyzed looking for associations with air temperature, appearance date, and emergence peaks of their hosts. The duration of *P. ruralis* adult emergence was analyzed looking for associations with air temperature, density, and appearance date of *P. ruralis*. The duration of adult emergence of primary and secondary parasitoids was analyzed looking for associations with air temperature, durations of adult emergence of their hosts, and their appearance dates and densities.

The density of *P. ruralis* was analyzed looking for associations with air temperature. The densities of primary and secondary parasitoids were analyzed looking for associations with air temperature and densities of their hosts.

Prior to multiple regression analysis, a correlation matrix of all investigated parameters was calculated to check for the multicollinearity. If correlation was detected between independent variables or results of multiple regression were not reliable the simple regression model was used in this case. The insect density and duration of *E. laevigata* and *P. dispar* were log-transformed to meet the normality assumption. All statistical calculations were made using the STATISTICA 6 package (Statsoft) for Microsoft Windows 2007.

RESULTS

The secondary parasitoid *E. laevigata* was common species of *M. cingulum* parasitoids on the nettle. In 1982-2004 it was responsible for 0-17.8% of mortality of *M. cingulum*. Higher degree of infestation of *M. cingulum* with *E. laevigata* was recorded in 1984 (13.3%), 1995 (17.8%) and 2002 (15.2%). It has been established that the *E. laevigata* was absent in 1994, 1999-2001. The secondary parasitoid *G. ruficornis* was responsible for 0-16.4% of the mortality of *M. cingulum*. Higher degree of infestation of *M. cingulum* with *G. ruficornis* was recorded in 1995 (15.1%) and 1996 (16.4%). It was absent in 2001-2002. The secondary parasitoid *P. dispar* was one of the most common species of *M. cingulum* parasitoids on the nettle. It was responsible for 0-79.8% of the mortality of *M. cingulum*. Higher degree of *M. cingulum* with *P. dispar* was recorded in 1990 (53.5%), 1999 (71.0%) and 2000 (79.8%). It was absent on the *M. cingulum* in 1993, 1995 and 2001-2002.

The appearance date and emergence peak

Results of multiple regression showed (R² = 0.51, $F_{2.20}$ = 10.45, p < 0.001) that *P. ruralis* appearance date was related with T_{MAY} (Beta = -0.57±0.16 SE, t = -3.60, p < 0.002) and a less related with T_{JUN} (Beta = -0.51±0.16, t = -3.22, p < 0.004). The emergence peak of *P. ruralis* (R² = 0.52, $F_{2.20}$ = 10.86, p < 0.001) was more related with T_{JUN} (Beta = -0.60±0.16, t = -3.86, p < 0.001) and less related with T_{MAY} (Beta = -0.47±0.16, t = -3.04, p < 0.01).

The *M. cingulum* appearance date was not related with air temperature, but related with *P. ruralis* appearance date (Simple regression: Beta = 0.75 ± 0.15 , R² = 0.57, F_{1.20} = 26.45, *t* = 5.14, *p* < 0.0001). The most important factor for the emergence peak of *M. cingulum* was the emergence peak of *P. ruralis*. The emergence peak of *M. cingulum*, like the emergence peak of *P. ruralis*, was also related with T_{UN} (Table 1).

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Table 1. Simple regression of *M. cingulum* emergence peak and *M. cingulum* appearance date, *P. ruralis* emergence peak, T_{JUN} (June air temperature).

Dependent	Independent	Beta±SE	R ²	F _{1.20}	t	<i>p</i> -value
<i>M. cingulum</i> emergence peak	M. cingulum appearance date	0.65±0.17	0.43	14.79	3.85	0.001
	P. ruralis emergence peak	0.75±0.15	0.58	27.25	5.22	0.000
	T _{JUN}	-0.66±0.17	0.42	15.06	-3.88	0.001

Secondary parasitoid *E. laevigata* started to emerge in 1-5 of July, *G. ruficornis* and *P. dispar* started to emerge in 6-10 of July: 198.3 ± 8.7 , 200.8 ± 8.9 , 199.8 ± 7.6 d (days) respectively. The emergence peaks were detected in 202.7 ± 8.3 , 205.0 ± 8.4 , 204.8 ± 7.5 d respectively. Insects of all species could be found until the middle August.

The earliest appearance date of *E. laevigata* was determined in 1996 (183 d) and the latest appearance date was detected in 1987 (212 d). The emergence peak during these years was detected from 187 d (1989) to 218 d (1984). The earliest appearance date of *G. ruficornis* was detected in 1984 and 2004 (213 d). The emergence peak during these years was detected from 192 d (1989, 1998, and 1999) to 222 d (1987). The earliest appearance date of *P. dispar* was determined in 1983, 1989, and 1999 (187 d) and the latest appearance date of *P. dispar* was determined in 1983, 1989, and 1999 (187 d) and the latest appearance date of *P. dispar* was detected in 1984, 1996, and 2004 (208 d). The emergence peak during these years was detected from 192 d (1983, 1989, and 1999) to 218 d (1984).

Results of multiple regression revealed that the appearance dates and the emergence peaks of secondary parasitoids were related with T_{JUN} and the appearance date of *M. cingulum*. Emergence peak of *M. cingulum* was excluded from the analysis to avoid multicolinearity. The higher the temperature in June and the earlier the appearance date of *M. cingulum*, the earlier the appearance date and emergence peak of the secondary parasitoids (Table 2).

Duration of adult emergence

Results of multiple regression revealed that duration of *P. ruralis* adult emergence was negatively related with appearance date of *P. ruralis*: the earlier was the appearance date the longer was the duration of adult emergence. More important factor influencing the emergence duration of *P. ruralis* was T_{JUL} . Increased T_{JUL} affected the shorter *P. ruralis* emergence duration (Table 3).

Duration of *M. cingulum* emergence was also related with T_{JUL} like in the case of *P. ruralis*. In our case, the relationship between emergence duration of *M. cingulum* and emergence duration of its host *P. ruralis* (Beta = 0.81±0.13, R² = 0.66, F_{1.21} = 40.26, t = 6.34, p < 0.000) was tighter comparing to the relationship between emergence duration of *M. cingulum* and T_{JUL} (Beta = -0.62±0.17, R² = 0.39, F_{1.21} = 13.21, t = -3.63, p < 0.002). So, we excluded T_{JUL} and used only the emergence duration of *P. ruralis* in the model (Table 4). Results of multiple regression showed that emergence duration of *M. cingulum* was related with *M. cingulum* appearance date and with the

emergence duration of *P. ruralis* (Table 4). The earlier *M. cingulum* started to emerge the longer was the duration of adult emergence like in the case of *P. ruralis*. The most important factor influencing the emergence duration of *M. cingulum* was the emergence duration of *P. ruralis*.

Table 2. Multiple regression analysis of the appearance dates and the emergence peaks of secondary parasitoids and some independent ($T_{,IUN}$, *M. cingulum* appearance date) parameters.

Dependent	Independent	Beta±SE t		<i>p</i> -value		
	T _{JUN}	-0.43±0.16	-2.65	0.016		
E. laevigata appearance date	M. cingulum appearance date	0.58±0.16	3.63	0.002		
Regression model: R ² = 0.61, F _{2.17} = 12.57, <i>p</i> < 0.001						
<i>E. laevigata</i> emergence peak	T _{JUN}	-0.55±0.15	-0.55±0.15 -3.78			
	M. cingulum appearance date	0.50±0.15	3.44	0.003		
	Regression model: R ² = 0.67, F _{2.17} = 16.14	, <i>p</i> < 0.0002		^		
	T _{JUN}	-0.58±0.12	-4.88	0.000		
G. ruficornis appearance date	M. cingulum appearance date	0.50±0.12	4.20	0.001		
Regression model: R ² = 0.78, F _{2.17} = 30.35, <i>p</i> < 0.000						
	T _{JUN}	-0.47±0.15	-3.13	0.006		
G. ruficornis emergence peak	M. cingulum appearance date	0.52±0.15	3.48	0.003		
	Regression model: $R^2 = 0.65$, $F_{2.17} = 16.10$	0, <i>p</i> < 0.000				
D diener opportunge date	T _{JUN}	-0.52±0.12	-4.2	0.001		
P. dispar appearance date	M. cingulum appearance date	0.56±0.12	4.51	0.000		
	Regression model: $R^2 = 0.77$, $F_{2.17} = 27.9$	6, <i>p</i> < 0.000				
P. dispar emergence peak	T _{JUN}	-0.57±0.11	-5.25	0.000		
	M. cingulum appearance date	0.54±0.11	4.50	0.000		
	Regression model: R ² = 0.82, F _{2.17} = 38.55	9, <i>p</i> < 0.000		۰		

The emergence duration of *E. laevigata* was 14.2 ± 8.2 d and varied from 5 (1987, 2004) to 40 (1996) d. Results of simple regression showed that the most important factor for the emergence duration of *E. laevigata* was the density of *E. laevigata* (Table 5). The emergence duration of *E. laevigata* was also statistically related with the appearance date of *E. laevigata*. The earlier *E. laevigata* started to emerge the longer was the emergence duration. No relationships between the emergence duration of *E. laevigata* and air temperature or emergence duration of *M. cingulum* were determined.

The emergence duration of *G. ruficornis* was 14.8 ± 5.0 d and varied from 10 (1989, 1991, 1994, 1997-1999, 2003-2004) to 25 (1988, 1990) d. Results of simple regression showed that the only important factor for the emergence duration of *G. ruficornis* was the density of *G. ruficornis* (Table 6). No relationships between the

emergence duration of *G. ruficornis* and the air temperature, emergence duration of *M. cingulum* or appearance date of *G. ruficornis* were detected.

The emergence duration of *P. dispar* was 16.3 ± 4.6 d and varied from 10 (1989, 1999, 2002 and 2004) to 25 (1985, 1986) d. Results of simple regression showed that the most important factor for the emergence duration of *P. dispar* was density of this species (Table 7). The emergence duration of *M. cingulum* and T_{JUL} were also important for the duration of *P. dispar*. No relationship between the emergence duration of *P. dispar* and the appearance date of this species was detected.

Density

Densities of all investigated insect species were not related with air temperature. The density of *M. cingulum* was related with the density of *P. ruralis* (Beta = 0.47±0.19, $R^2 = 0.22$, $F_{1.20} = 5.72$, t = 2.39, p < 0.027). Results of simple regression showed that densities of the secondary parasitoids were related with density of their host *M. cingulum*. The density of *M. cingulum* was the most important for the *E. laevigata* density (Beta = 0.73±0.16, $R^2 = 0.54$, $F_{1.17} = 19.53$, t = 4.42, p < 0.000), the less important for densities of *G. ruficornis* (Beta = 0.69±0.17; $R^2 = 0.48$, $F_{1.18} = 16.37$, t = 4.05, p < 0.001) and *P. dispar* (Beta = 0.52±0.21, $R^2 = 0.28$, $F_{1.17} = 6.46$, t = 2.54, p < 0.021).

Table 3. Multiple regression analysis of <i>P. ruralis</i> adult emergence duration and some independent (T ₁₁₁ ,	,
P. ruralis appearance date) parameters.	

Dependent	Independent	Beta±SE	t	<i>p</i> -value		
P. ruralis emergence duration	T _{JUL}	-0.73±0.14 -5.19		0.000		
	P. ruralis appearance date -0.36±0.14 -2.54 0.019					
Regression model: $R^2 = 0.61$, $F_{2.20} = 15.62$, $p < 0.000$						

Table 4. Multiple regression of *M. cingulum* emergence duration and some independent (*M. cingulum* appearance date, *P. ruralis* emergence duration) parameters.

Dependent	Independent	Beta±SE	t	<i>p</i> -value			
M. cingulum emergence duration	M. cingulum appearance date	-0.37±0.13	-2.85	0.010			
	P. ruralis emergence duration 0.65±0.13 4.93 0.00						
Regression model: R ² = 0.70, F _{2.19} = 22.15, <i>p</i> < 0.000							

Table 5. Simple regression of E. laevigata emergence duration and its density, appearance date, M.
<i>cingulum</i> emergence duration and $T_{\mu\mu}$.

Dependent	Independent	Beta±SE	R ²	F _{1.17}	t	p-value
<i>E. laevigata</i> emergence duration	E. laevigata density	0.59±0.19	0.34	9.29	2.81	0.009
	E. laevigata appearance date	-0.57±0.20	0.32	8.05	-2.83	0.011
	M. cingulum duration	0.30±0.23	0.09	1.71	1.31	0.209
	T _{JUL}	-0.26±0.23	0.07	1.23	-1.11	0.283

Table 6. Simple regression of <i>G. ruficornis</i> emergence duration and its density, appearance date, <i>I</i>	М.
<i>cingulum</i> emergence duration and T _{JUL} .	

Dependent	Independent	Beta±SE	R ²	F _{1.18}	t	p-value
<i>G. ruficornis</i> emergence duration	G. ruficornis density	0.54±0.20	0.30	7.55	2.75	0.013
	G. ruficornis appearance date	-0.08±0.23	0.01	0.13	-0.36	0.372
	M. cingulum duration	0.17±0.23	0.03	0.52	0.72	0.480
	T _{JUL}	-0.41±0.22	0.17	3.78	-1.94	0.068

Table 7. Simple regression of *Pteromalus dispar* emergence duration and its density, appearance date, *M. cingulum* emergence duration and T_{utu}.

Dependent	Independent	Beta±SE	R ²	F _{1.18}	t	<i>p</i> -value
P. dispar emergence duration	P. dispar density	0.60±0.19	0.36	10.29	3.21	0.005
	P. dispar appearance date	-0.00±0.24	0.00	0.13	-0.02	0.988
	M. cingulum duration	0.49±0.21	0.24	5.69	2.39	0.028
	T _{JUL}	-0.57±0.19	0.33	8.71	-2.95	0.009

DISCUSSION

The primary parasitoid *M. cingulum* is known to be the common species on *P. ruralis* (Bartkevičienė *et al.*, 2010). With increase in winter or early spring temperatures, the beginning of vegetation of perennial plants is starting earlier in many European countries (Romanovskaja *et al.*, 2009). The beginning of vegetation is related with the outset of phytophagous insect species. Entomologists in different countries have investigated the effects of changing temperatures on the emergence rate of different insect species (Dell *et al.*, 2005; Qiu *et al.*, 2006; Wilson *et al.*, 2007). It was determined that the appearance time of many insect species is getting earlier from the eight decade of the last century (Sokolov, 2012). There is no doubt that the appearance dates of many insect species are temperature dependent (Tryjanowski *et al.*, 2010; Sokolov, 2012).

The appearance date and adult emergence peaks of *P. ruralis* and its primary parasitoid *M. cingulum* were described by Bartkevičienė *et al.* (2010). It was noted that *P. ruralis* appearance date was related with spring temperature and emergence peak was related with temperature in June. It was also known that the adult emergence peak of *M. cingulum* had a negative correlation with June air temperature (Bartkevičienė *et al.*, 2010). Our data have shown that the adult emergence peak of primary parasitoid *M. cingulum* is related not only with T_{JUN}, but also with its appearance date and with the emergence peak of *I. ruralis* and the earlier the emergence peak of *P. ruralis* and the earlier the emergence peak of *M. cingulum*. Our results have revealed that the appearance dates and the emergence peaks of secondary parasitoids are related both with T_{JUN} and the appearance date of the host, *M. cingulum*. This study has shown that air temperature plays an important role to

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the appearance date of the host, but is not of the first importance for the primary and secondary parasitoids of the host.

Duration of *P. ruralis* emergence as well as the emergence of its primary parasitoid was related with T_{III}. It has been known that the longitudinal emergence periods of *P*. ruralis and M. grandii coincided with lower air temperature and, on the contrary, shorter emergence periods coincide with higher air temperature (Bartkeviciene et al. 2010). It has been also noted that the increased temperature is related with the shortening of the flight period duration in other insects, for example in Vespinae (Pawlikowski and Pawlikowski, 2009; Tryjanowski et al., 2010) similarly like in the case of P. ruralis and the shortening of its emergence duration. The negative impact of temperature has been also determined for longevity of parasitoids by Eliopoulos et al. (2005) and Qiu et al. (2006), but our results showed that the air temperature was not an important parameter for all investigated parasitoids as some life cycle parameters of their hosts. In our study, relationships between emergence duration of the primary parasitoid and emergence duration of its host were tighter comparing to the relationship with air temperature. Air temperature did not play a direct role to the emergence duration of some secondary parasitoids (Table 5, 6). The air temperature was the important factor only for the emergence duration of one species of the secondary parasitoid, the P. dispar.

It has been already known that a positive correlation exist between the population density of *P. ruralis* and population density of *M. cingulum* and no correlation was detected between population density and air temperature (Bartkevičienė *et al.*, 2010). According to this study, the densities of secondary parasitoids were also related only with the density of their host. No relationships between density and mean emergence date, which can be related with temperature, has been found also for butterflies (Dell *et al.*, 2005). There are some facts known about the positive influence of the temperature for some butterfly species densities (Sokolov, 2012), but our study did not reveal the relationships between densities of investigated insects and air temperatures.

CONCLUSIONS

1. The appearance dates of the secondary parasitoids are related with air temperatures in June and the appearance date of their host *M. cingulum*. The appearance date of *M. cingulum*, the parasitoid of *P. ruralis*, is not related with air temperature, but is related with *P. ruralis* appearance date.

2. The most important factor for the emergence peak of *M. cingulum* is the emergence peak of *P. ruralis.* The emergence peaks of the secondary parasitoids *E. laevigata*, *G. ruficornis* and *P. dispar* are related with air temperatures in June and the appearance date of *M. cingulum*.

3. The most important factors for the emergence durations of secondary parasitoids are their densities. The emergence duration of *P. dispar* is related with air temperature in July and the emergence duration of *M. cingulum*, which is related with the emergence duration of its host.

4. Densities of the secondary parasitoids are related with density of primary parasitoid which is related with the density of its host.

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