ACTA
PROTOZOOLOGICA

Acta Protozool. (2012) 51: 169–177
http://www.eko.uj.edu.pl/ap
doi: 10.4467/16890027AP.12.013.0517

The Effects of *Nosema pyrausta* Infection on European Corn Borer Populations from Five European Countries

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S**ummary.** *Ostrinia nubilalis* populations from Slovakia, Romania, Austria, Serbia and Germany were collected in the autumn when the insects were in the larval stage. These insects were then established as laboratory populations. The number of pupae and adults that developed was always higher in the *Nosema pyrausta* non-inoculated (uninfected) populations than in the populations treated by the pathogen *N. pyrausta* (infected). Significant differences were also found among the populations from different countries. Infected females laid significantly fewer eggs compared to uninfected females. The average time for pupal eclosion or adult emergence was not significantly different between the uninfected and infected populations of *O. nubilalis*. However, it was found that the infected females laid their eggs significantly sooner as compared to the uninfected females (37.383 days compared to 40.089 days). Under the same conditions, populations from colder regions developed faster than those from warmer regions. The place of origin of the population did not significantly influence larval weight, larval length or pupal weight. However, larvae infected with *N. pyrausta* spores had significantly lower weight (average 0.0797 g) than uninfected larvae (0.0901 g). With regard to pupal weight, the difference between the infected and uninfected individuals was not significant. It was confirmed that *N. pyrausta* from one European country can infect and influence host larvae originating in other countries. Although there have been several statistically significant interactions with regard to the country of origin and *N. pyrausta* infection, it was not believed that *N. pyrausta* from one country would have specific effects on the mortality, developmental rate and larval or pupal weight of *O. nubilalis* populations from different countries.

Key words: *Nosema pyrausta*, *Ostrinia nubilalis*, Microsporidia.

INTRODUCTION

Nosema pyrausta Paillot (Microsporida: Nosematidae) is an obligate, intracellular parasite (Lewis *et al*. 2009) and a highly prevalent microsporidian pathogen of the European corn borer, *Ostrinia nubilalis* Hübner, 1796 (Lepidoptera, Crambidae) (Lewis and Lynch 1976; Andreadis 1984, 1986; Maddox 1987). It may potentially be used in integrated pest management for crop protection in European maise-based cropping systems (Vasileiadis *et al.* 2011).

N. pyrausta is an important biological mortality factor of *O. nubilalis* (Kramer 1959a, b). It reduces egg hatch, developmental rate, fecundity and life span of the host (Zimmack and Brindley 1957, Windels *et al*. 1976, Bruck *et al.* 2011). It also has a significant nega-

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tive effect on flight performance (Dorhout *et al*. 2011). *N. pyrausta* plays an important role in the regulation of natural populations of *O. nubilalis* in Europe and North America (Lewis *et al.* 2009), and it is maintained in populations of *O. nubilalis* by both horizontal and vertical transmission (Zimmack and Brindley 1957; Lewis 1978; Hill and Gary 1979; Andreadis 1984, 1986, 1987; Lewis and Cossentine 1986; Solter *et al*. 2005). Horizontal spread occurs through the ingestion of spores, and vertical transmission occurs from the mother to her offspring (Lewis *et al*. 2009). An important factor influencing the result of infection, especially in natural pest conditions, is the mechanism by which the infection is spread. Lewis *et al.* (2009) identified both vertical and horizontal transmission mechanisms for the spread of infection. The success of vertical transmission depends on the infection intensity, and the success of horizontal transmission depends on the larval stage (Abdel Rahman *et al*. 2010, Lopez *et al*. 2010).

Several studies regarding protozoan interactions with local populations of the host species showed that there were differences in these interactions depending on the locality, such as with *Nosema apis* in Poland (Topolska *et al.* 2008), *Nosema ceranae* in Europe (Higes *et al.* 2010) and *Nosema meligethi* in Europe (Lipa and Hokkanen 1992). Additionally, more *Bombus* species were attacked with only one microsporidian species: *Nosema bombi* (Larsson 2007). *N. pyrausta* is a parasite of *O. nubilalis* and is found in many countries worldwide; however, it was found only in some populations of *O. nubilalis* in Slovakia and the Czech Republic (Cagáň *et al.* 2006). Therefore, the question remains: are some populations of *O. nubilalis* susceptible to infection by the parasite? In this study, our objective was to confirm that parasite infection of *O. nubilalis* by *N. pyrausta* does not depend on the place of origin of *O. nubilalis* larvae. The main aim of this study was to evaluate the effects of *N. pyrausta* strain collected in one country (Slovakia) to mortality, developmental rate and larval or pupal weight of *O. nubilalis* populations from different European countries (Slovakia, Romania, Austria, Serbia and Germany).

MATERIALS AND METHODS

Five *O. nubilalis* populations in the larval stage were collected in the autumn. Romanian larvae were collected from the vicinity of Bucharest (N44°30′, E26°32′), Serbian larvae were collected from the region situated north west from Novi Sad (N45°20′, E19°45′), Austrian larvae were collected near the city of Klagenfurt (N46°45′, E14°34′), German larvae were collected from the Upper Rhine region (N47°48′, E7°36′) and Slovakian larvae were collected from maize fields near the town of Nitra (N48°19′, E18°09′).

Laboratory populations were established from the collected larvae and maintained at 25 ± 1°C with a 16:8 (L:D) photoperiod. Larvae hatched from the eggs were reared in 250 ml glass containers (60 mm diameter) containing 125 ml of standard, semi-artificial diet described by Nagy (1970). Four egg clusters (20 eggs per egg cluster) were placed in each container. After eclosion, adults were released into cages (200 × 300 × 600 mm) and allowed to oviposit on paper strips (150 × 100 mm). Adults were fed 2% (w/v) sucrose water supplied in small plastic trays lined with cotton. Egg masses deposited on the paper strips were collected daily and placed in the glass containers with fresh food to establish a continuous population.

The Malpighian tubules of 10 fifth-instar larvae of the first laboratory generation were microscopically examined for the presence of microsporidian spores (Canning and Vavra 2000). The same examination of 10 randomly chosen fifth-instar larvae was performed in each new laboratory generation, until the larvae were used in the experiments. During the laboratory rearing, no microsporidian infection was detected in the population. This is an important examination to perform in any laboratory study with *O. nubilalis* larvae (Andow *et al.* 1999). After the development of more than three generations in the laboratory, the populations were used for the experiment.

The *N. pyrausta* spores used in these experiments were isolated from the Malpighian tubules of *O. nubilalis* larvae collected near Dechtice, Slovakia (N48°33′, E17°35′). The microsporidian infection was determined by dissecting the larvae and examining their Malpighian tubules in wet mount preparations. Fresh spores of *N. pyrausta* were obtained as follows: Malpighian tubules dissected from 25 infected fifth-instar larvae were homogenised in a glass tissue grinder, and then the homogenate was diluted in 25 ml of distilled water and centrifuged at 2000 rpm (672 × g) for 15 min. (Windels *et al*. 1976, Siegel *et al*. 1986). The supernatant was discarded, and the pellet containing the spores was resuspended in 25 ml of distilled water. The centrifugation step was repeated three times. After the spore concentration of the final suspension was determined using a Neubauer chamber, the suspension was adjusted to a concentration of 1×10^6 spores/ml. The suspension was stored at 4°C until use in the experiments during the next 2–3 days.

In the first experiment, the impact of *N. pyrausta* on *O. nubilalis* larvae mortality and development rate was tested. Fifty-five larvae that were just emerging from their eggs were placed in vials (500 ml) with the artificial diet. Experiments were performed in 4 replicates for the control variant and in 4 replicates for the variants with *N. pyrausta* spores. In the vials containing *N. pyrausta*, the spore suspension of *N. pyrausta* was added to the surface of the fresh artificial food in the vial (500 spores per mm^2) when the larvae were one or two days old. Larvae developing on either the inoculated or non-inoculated diet were checked for infection after ten days. Five larvae from each vial were microscopically checked for the presence of spores to confirm that the infection was acquired and that the control populations were infection free. If a minimum of four out of five larvae showed infection with *N. pyrausta,* the vial was considered to be "infected." The remaining 50 larvae in the vial, with the microsporidian spores still on the surface of the diet substrate, were used in the experiment. The mortality of the larvae was

checked daily, and each dead larva was examined for infection by microsporidia. During each day of the experiment, pupal eclosion and the emergence of adults were monitored. The number of pupae and adults that developed from the 50 individuals of the first instar larvae and the average time of pupal eclosion or adult emergence expressed by days were then calculated. Adults from each vial were placed into separate and isolated cages (for adults from infected and uninfected vials from each country). Dead moths were removed and frozen until *N. pyrausta* determinations could be performed. Adult infections were diagnosed from cross sections of the abdomen. At least 4 of the 5 adults from each vial had to be infected for a positive diagnosis of infection.

Egg clusters deposited on the paper strips in each cage were collected daily. The number of eggs in each cluster and the time at laying were recorded. The average number of eggs in an egg cluster was 18.25. Differences among variants were not significant; therefore, "egg cluster" was used as the method of egg laying quantification. The number of eggs counted in each of the egg clusters was counted each day. Cumulative number was then divided by 18.25.

In the second experiment, the impact of *N. pyrausta* on *O. nubilalis* larval weight and length and the impact of *N. pyrausta* on *O. nubilalis* pupal weight were tested. The experiment was performed similarly to the first experiment, with one difference. Six egg clusters with emerging first instar larvae were place in each vial to achieve a minimum level of 100 young larvae in each vial, which ensured that more than 20 larvae would develop in each vial. Corrugated paper covered by transparent folia was placed inside the vials to enhance larval entrance. Larvae that entered the shelter and did not leave during the next two days were calculated as 5th instar larvae. The larvae in the paper "traps" were weighed and placed back in the "traps" for pupation. The pupae were weighed after their eclosion.

Two-way ANOVA was used for the statistical analysis of data, using "treatment" and "population origin" as factors (Tukey's HSD test, $p > 0.05$). The interaction between both factors was also calculated. Programme "Statistica" was used for the calculations (Tables 1–3).

RESULTS

Table 1 shows the influence of the origin of the population of *N. pyrausta* on the infection of *O. nubilalis.* It shows the average number of surviving *O. nubilalis* pupae, the adults and the number of egg clusters laid by females developed from vials inoculated by protozoan (infected) and from vials without protozoan inoculation (uninfected).

The number of pupae that developed was significantly higher in uninfected treatment groups than in treatment groups infected by the pathogen, *N. pyrausta*. The number of pupae in infected treatment groups was generally lower by 34.38% as compared to that in non-infected treatment groups. The average number of animals in the pupal stage that survived was 28.65 for infected treatment groups and 18.80 for uninfected treatment groups (50 larvae were used in the beginning of the experiment). The population originally from Slovakia had the highest level of mortality (from 50, 13.38 animals survived), followed by populations from Germany (19.75), Serbia (23.50), Austria (27.50) and Romania (34.50). There was a significant interaction between population origin and infection ($P = 0.0242$). It was found that only the Romanian and Serbian populations were significantly influenced by microsporidian infection, as determined by the mortality of the larvae until they achieved the pupal stage.

A similar result was obtained after the number of *O. nubilalis* adults from infected and uninfected vials were compared. The differences among countries (P-value = 0.0001) and between treatments (P-value = 0.0001) were significant. However, calculations showed that only the Romanian and Austrian populations had a significant effect on the mortality of adults caused by the microsporidian infection.

Treatment groups that developed from vials uninfected by *N. pyrausta* had nearly twice as many egg clusters as compared to the treatment groups that developed from vials infected by the protozoan (significant difference). Moreover, the number of egg clusters significantly depended on the place of origin of the population. However, there was no country-specific population reaction to the microsporidian infection.

Although there were some differences between uninfected and infected treatment groups, the average time for pupal eclosion and the average time for adult emergence were not significantly different between the uninfected and infected treatment groups of *O. nubilalis* (Table 2). However, infected females were found to lay their eggs significantly sooner as compared to the uninfected females (37.383 days compared to 40.089 days).

Significant differences were found in the development of populations from different countries. The average time of pupal eclosion, average time of adult emergence and average time of egg laying of populations from Slovakia or Germany were shorter than those from Austria, Romania and Serbia. Table 2 shows that the average time of pupal eclosion of the population from Slovakia was 26.038 days and that of the population from Serbia was 30.983 days. A similar result was found for the average time of adult emergence or average time of egg laying. Generally, under the same conditions, populations from colder regions developed faster than those from warmer regions. Statistical analysis did not show any interactions between country of

Table 1. Average number of *O. nubilalis* pupae, adults and egg clusters developed from 50 first instar larvae. Data were calculated as an average from 4 replicates. Means in succeeding rows followed by the same letter are not significantly different (ANOVA, Tukey's HSD test, $p > 0.05$). If the differences were not significant, letters were not used. Population origin is the country where *O. nubilalis* individuals were collected. NP spores shows the differences among vials uninoculated (NP-No) and inoculated (NP-Yes) by *N. pyrausta* spores (the infection was confirmed – for details see Material and Methods). *x* – average; *s* – standard deviation.

	Number of pupae	Number of adults	Number of egg clusters
Population origin	*x* ± *s*	*x* ± *s*	*x* ± *s*
Germany	19.75 ± 3.03ab	18.50 ± 3.39b	41.63 ± 13.53c
Slovakia	13.38 ± 4.55a	10.88 ± 3.62a	8.88 ± 6.53a
Romania	34.50 ± 9.67d	28.75 ± 9.54c	20.63 ± 12.36ab
Austria	27.50 ± 7.71c	21.00 ± 5.81b	23.25 ± 10.29b
Serbia	23.50 ± 7.43bc	16.25 ± 5.26ab	12.38 ± 6.40ab
F-ratio	23.36	23.80	17.79
P-value	0.0001	0.0001	0.0001
NP spores	*x* ± *s*	*x* ± *s*	*x* ± *s*
NP-No	28.65 ± 10.21b	23.30 ± 8.68b	28.00 ± 16.03b
NP-Yes	18.80 ± 6.62a	14.85 ± 5.37a	14.70 ± 11.19a
F-ratio	44.73	49.16	24.16
P-value	0.0001	0.0001	0.0001
Interactions	*x* ± *s*	*x* ± *s*	*x* ± *s*
Germany *x* NP–	20.25 ± 2.95abc	19.25 ± 3.63bc	51.00 ± 11.68
Germany *x* NP+	19.25 ± 3.03abc	17.75 ± 2.95bc	32.25 ± 7.33
Slovakia *x* NP–	17.25 ± 1.79ab	13.50 ± 2.50ab	12.75 ± 6.42
Slovakia *x* NP+	9.50 ± 2.87a	8.25 ± 2.49a	5.00 ± 3.74
Romania *x* NP–	43.00 ± 4.47e	37.25 ± 4.32d	30.25 ± 10.43
Romania *x* NP+	26.00 ± 4.74bcd	20.25 ± 4.32bc	11.00 ± 3.39
Austria *x* NP–	33.00 ± 7.21de	25.75 ± 3.56c	29.25 ± 9.36
Austria *x* NP+	22.00 ± 2.55bcd	16.25 ± 3.11ab	17.25 ± 7.22
Serbia *x* NP–	29.75 ± 3.27cd	20.75 ± 2.59bc	16.75 ± 4.02
Serbia *x* NP+	17.25 ± 4.66ab	11.75 ± 2.86ab	8.00 ± 5.24
F-ratio	3.28	4.58	0.81
P-value	0.0242	0.0052	0.5300

origin and microsporidian infection with regard to the time of development.

The place of origin of the population did not significantly influence larval weight, larval length or pupal weight (Table 3). However, larvae infected with *N. pyrausta* spores had significantly lower weight (average 0.0797 g) than those uninfected by the protozoan (0.0901 g). Similar results were observed for larvae length (18.61 mm versus 17.59 mm). For pupal weight, the difference between infected and uninfected individuals was not significant (Table 3). Differences based on the country of origin were not significant. The specific

Table 2. Average time of pupal eclosion, adult emergence and egg laying of *O. nubilalis*. Animals developed from 50 first instar larvae in 4 replicates were used in the experiment. Means in succeeding rows followed by the same letter are not significantly different (ANOVA, Tukey's HSD test, $p > 0.05$). If the differences were not significant, letters were not used. Population origin is the country where *O. nubilalis* individuals were collected. NP spores shows the differences among vials uninoculated (NP-No) and inoculated (NP-Yes) by *N. pyrausta* spores (the infection was confirmed – for details see Material and Methods). *x* – average; *s* – standard deviation.

	Average time (days) of pupal eclosion	Average time (days) of adult emergence	Average time (days) of egg laying
Population origin	*x* ± *s*	*x* ± *s*	*x* ± *s*
Germany	27.925 ± 2.36ab	31.768 ± 2.47ab	37.145 ± 2.44ab
Slovakia	26.038 ± 1.65a	29.656 ± 1.24a	34.288 ± 2.39a
Romania	30.901 ± 1.61bc	34.144 ± 1.59bc	38.588 ± 1.94bc
Austria	30.676 ± 2.00bc	34.954 ± 1.94c	41.788 ± 3.30c
Serbia	30.983 ± 1.96c	36.201 ± 1.80c	41.871 ± 2.97c
F-ratio	8.93	13.27	12.36
P-value	0.0001	0.0001	0.0001
NP spores	*x* ± *s*	*x* ± *s*	*x* ± *s*
NP-No	29.491 ± 3.17	33.508 ± 3.12	40.089 ± 3.67a
NP-Yes	29.119 ± 2.30	33.181 ± 2.84	37.383 ± 3.68b
F-ratio	0.31	0.26	10.9
P-value	0.5808	0.6154	0.0025
Interactions	*x* ± *s*	*x* ± *s*	*x* ± *s*
Germany *x* NP–	28.050 ± 2.01	32.515 ± 1.31	38.640 ± 1.83
Germany *x* NP+	27.800 ± 2.67	31.020 ± 3.06	35.650 ± 2.03
Slovakia *x* NP–	25.135 ± 0.97	28.813 ± 0.60	36.000 ± 2.13
Slovakia *x* NP+	26.940 ± 1.70	30.500 ± 1.14	32.575 ± 1.00
Romania *x* NP–	31.428 ± 1.71	34.579 ± 1.95	39.775 ± 2.08
Romania *x* NP+	30.375 ± 1.30	33.710 ± 0.93	37.400 ± 0.59
Austria *x* NP–	30.870 ± 2.25	35.043 ± 1.82	43.578 ± 3.10
Austria *x* NP+	30.483 ± 1.69	34.866 ± 2.06	40.165 ± 2.52
Serbia *x* NP–	31.970 ± 2.12	36.591 ± 1.87	42.450 ± 2.98
Serbia *x* NP+	30.000 ± 1.13	35.810 ± 1.63	41.125 ± 2.81
F-ratio	0.88	0.72	0.23
P-value	0.4893	0.5866	0.9183

effect of microsporidia infection was confirmed only in the Romanian population. The infected larvae from this population were significantly smaller than the uninfected larvae.

DISCUSSION

O. nubilalis larvae were infected by *N. pyrausta* spores soon after they hatched from eggs. This was likely due to significantly higher mortality of animals infected by *N. pyrausta*. Our result is similar to those of Siegel *et al.* (1986), who found an average mortality

Table 3. Average weight of *O. nubilalis* larvae and pupae, and average length of larvae calculated from 20 individuals (average from 4 replicates). Means in succeeding rows followed by the same letter are not significantly different (ANOVA, Tukey's HSD test, $p > 0.05$). If the differences were not significant, letters were not used. Population origin is the country where *O. nubilalis* individuals were collected. NP spores shows the differences among vials uninoculated (NP-No) and inoculated (NP-Yes) by *N. pyrausta* spores (the infection was confirmed – for details see Material and Methods). *x* – average; *s* – standard deviation.

	Larval weight (g)	Larval length (mm)	Pupal weight (g)
Population origin	*x* ± *s*	*x* ± *s*	*x* ± *s*
Germany	0.0881 ± 0.029	18.725 ± 2.29	0.0712 ± 0.024
Slovakia	0.0837 ± 0.022	18.075 ± 2.17	0.0675 ± 0.020
Romania	0.0798 ± 0.023	17.35 ± 3.45	0.0629 ± 0.008
Austria	0.0854 ± 0.020	17.95 ± 3.00	0.0713 ± 0.011
Serbia	0.0875 ± 0.022	18.4 ± 2.71	0.0704 ± 0.014
F-ratio	0.89	1.75	1.81
P-value	0.4725	0.1417	0.1293
NP spores	*x* ± *s*	*x* ± *s*	*x* ± *s*
NP-No	0.0901 ± 0.023a	18.61 ± 2.79a	0.0698 ± 0.018
NP-Yes	0.0797 ± 0.023b	17.59 ± 2.41b	0.0675 ± 0.016
F-ratio	10.69	8.52	1.00
P-value	0.0013	0.0039	0.3182
Interactions	*x* ± *s*	*x* ± *s*	*x* ± *s*
Germany *x* NP–	0.0958 ± 0.027b	19.05 ± 2.06b	0.0729 ± 0.019
Germany *x* NP+	0.0804 ± 0.028ab	18.4 ± 2.46b	0.0695 ± 0.028
Slovakia *x* NP–	0.0864 ± 0.025b	18.6 ± 2.37b	0.0714 ± 0.026
Slovakia *x* NP+	0.0810 ± 0.019ab	17.55 ± 1.80ab	0.0636 ± 0.011
Romania *x* NP–	0.0960 ± 0.021b	19.45 ± 3.65b	0.0636 ± 0.008
Romania *x* NP+	0.0636 ± 0.008a	15.25 ± 1.26a	0.0621 ± 0.008
Austria *x* NP–	0.0822 ± 0.020ab	18.1 ± 2.57b	0.0724 ± 0.012
Austria *x* NP+	0.0887 ± 0.020b	17.8 ± 1.81b	0.0702 ± 0.010
Serbia *x* NP–	0.0902 ± 0.018b	17.85 ± 2.69b	0.0689 ± 0.016
Serbia *x* NP+	0.0849 ± 0.026ab	18.95 ± 2.62b	0.0719 ± 0.011
F-ratio	4.18	6.25	0.52
P-value	0.0029	0.0001	0.7181

of transovarially infected larvae that was 36% higher than for uninfected larvae. Goertz *et al*. (2004) tested two *Nosema sp*. isolates on the *Lymantria dispar* larvae and observed total mortality rates that ranged from 77 to 100%. When the third larval instars were infected, a higher larval mortality of *L. dispar* larvae (86.4%) infected by microsporidium *Nosema lymantriae* was observed as compared to when the fifth larval instars were inoculated (40.9%) (Goertz and Hoch 2008). Abdel Rahman *et al.* (2010) also found that the *O. nubilalis* first larval instar was the most susceptible to *N. pyrausta* infection.

Generally, the mortality of the larvae was relatively high in both the control and infected vials. In these ex-

periments, larvae were used that had just hatched from eggs. There are many factors that influence the development of larvae to the pupal stage. In natural conditions, the mortality of young larvae is very high. For example, from 100 eggs that were laid by the previous generation, two animals survived (Benedek and Hanó 1986). In other studies, the mortality of young larvae was from 15% (Hudon and LeRoux 1986) to 82.5% (Ross and Ostlie 1990). Small disturbances in the micro-space where the larvae exist, such as the accumulation of water in a portion of the vial, may cause increased mortality of the larvae. To eliminate such factors, the experiments were repeated. Larvae used in these experiments were from regions with partial second generations of the pest. Therefore, some larvae in the laboratory went into diapause. In natural conditions, diapause occurs during the winter. If diapause continues at 25°C, the larvae die after a period of time (personal experience of authors of this paper). Under laboratory conditions, there was a higher rate of mortality of the larvae that originated from more northern regions. Populations from southern regions were most likely completely bivoltine; therefore, the development of individuals from these localities continued uninterrupted in the laboratory. For the Slovakian or German populations, the influence of diapause remains to be seen. With regard to the effect of temperature, the same effects should be observed in the Austrian population from Klagenfurt. However, Klagenfurt is more southern than the Slovakian or German localities, and daylight likely influenced the diapause potential of this *O. nubilalis* population.

Generally, the number of pupae or the number of adults that developed was dependent on the country of origin. Additionally, the influence of the microsporidian infection on development was significant. However, the aim of this study was to find an interaction between the microsporidium and the corn borer population. Although it was found that there were some significant interactions between the microsporidium and the corn borer population in the countries of origin, it was not hypothesised that *N. pyrausta* from one country had any specific effect on the mortality of *O. nubilalis* pupae, adults or egg laying ability from another country. *N. pyrausta* had significant effects on *O. nubilalis* populations, but based on our observations, we cannot confirm that this is the case for all populations of *O. nubilalis*.

We found that healthy females laid twice as many egg clusters as compared to infected females. These data are in agreement with earlier studies on longevity and fecundity, where infected females laid an average of 48% (Zimmack *et al*. 1954), 39% (Zimmack and Brindley 1957) and 33% (Siegel *et al*. 1986) fewer eggs than uninfected females. Our results demonstrate a clear impact of *N. pyrausta* on *O. nubilalis* egg production. According to Bruck *et al.* (2001), egg production in *N. pyrausta* infected *O. nubilalis* populations was reduced to 70 and 67%, when maintained at 16 and 27°C, respectively, compared to the uninfected population.

Zimmack and Brindley (1957), Kramer (1959a), Windels *et al.* (1976), Siegel *et al*. (1986) and Sajap and Lewis (1992) note that infection of *O. nubilalis* by *N. pyrausta* directly causes abnormal larval and pupal development and mortality. Infection reduced fecundity, longevity, larval weight and the number of egg clusters that were laid. We found that the place of origin of the population was mainly responsible for the speed of pupal and adult development or day of egg laying. Protozoan infection influenced only the day of egg laying; it was significantly earlier in infested populations. We suggest that uninfected animals likely survive longer, with the consequence being that egg laying continues for a longer period of time.

There are many studies regarding the influence of protozoan infection on the weight of larvae or pupae. Windels *et al*. (1976) reported heavier male pupae when *O. nubilalis* was infected with *N. pyrausta*. Goertz *et al.* (2004) did not find any significant differences in pupal weight between infected and uninfected females and males of *L. dispar* infected with *N. lymantriae.* A slightly prolonged feeding period, higher relative growth rates and additional larval stages indicated that infected larvae could overcome nutritional constraints due to metabolic changes or competition by the parasites for nutrients (Henn and Solter 2000). On the other hand, the presence of infection significantly decreased the pupal weight of females (Sajap and Lewis 1992). Abdel-Rahman and Cagáň (2001) confirmed that infection by *N. pyrausta* had no significant effect on the total amount of food consumption by different larval instars of *O. nubilalis* but that the efficiency of conversion of ingested food to body substance was significantly affected by infection. This resulted in a significant decrease in pupal weight in infected individuals. This study confirmed that the larval weight of uninfected animals was significantly higher as compared to that of infected animals. However, the pupal weight of infected animals was not significantly lower as compared to uninfected animals. It is interesting to note that despite

the differences among different populations with regard to mortality or development, the populations showed no significant differences with regard to animal weight. Therefore, it seems that climatic conditions may influence the developmental speed of some species; however, all of the species still develop to the same size regardless of the different conditions.

With regard to larval size, the place of origin of the larvae did not have any influence on this characteristic, while the infection itself did influence this characteristic. A specific, significant effect of *N. pyrausta* infection was confirmed only in the case of the Romanian population. The interaction indicates that *N. pyrausta* affects populations in certain parameters differently depending on population origin. However, it is difficult to generalise this point because four other populations did not show this result.

Acknowledgements. This work was supported by the Excellence Centre for Agrobiodiversity Conservation and the Benefit project implemented under the Operational Programme Research and Development financed by the European Fund for Regional Development and the project of the Slovak Agency VEGA No. 1/0597/10.

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Received on 15th December, 2011; revised on 29th May, 2012; accepted on 7th June, 2012