Seasonal Dynamics and Entomophthoralean Infection of the Pea Aphid, *Acyrthosiphon pisum* Harris

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Abstract


The seasonal dynamics of the pea aphid, *Acyrthosiphon pisum* Harris, and entomopathogenic fungi from the order Entomophthorales attacking the aphid were analysed in alfalfa and field pea crops during the growing seasons of 1998 and 1999 at Nitra-Malanta. In both years, pea aphid populations on pea showed a pattern with one peak, culminated at flowering and pod formation. The infestation level on alfalfa was low in both years. Entomopathogenic fungi attacking the pea aphid were identified as *Erynia neoaphidis* Remaudière and Hennebert and *Conidiobolus obscurus* (Hall and Dunn) Remaudière and Keller. Both pathogens infected the aphid on pea, but only *E. neoaphidis* was found in the alfalfa plots. Infected aphids were not found on alfalfa during 1999. The maximum levels of infected aphids on pea were 10.30% and 48.39% in 1998 and 1999, respectively. During both years alate aphids were more frequently attacked than apterous ones. Correlation coefficients indicated a positive relationship between the number of infected aphids and precipitation, but this relationship was weak or moderately strong. A strong correlation was found between the number of dead aphids and number of alate aphids counted 5 to 10 days earlier.

Key words: Erynia neoaphidis; Conidiobolus obscurus; Acyrthosiphon pisum; aphid pathogens

The pea aphid, *Acyrthosiphon pisum* Harris (Homoptera: Aphididae), is a cosmopolitan pest of annual and perennial legumes. This aphid, under conditions of central Europe, is holocyclic monocious and lives nearly exclusively on plants from the family Fabaceae (HOZÁK 1968). It is known to transmit a large number of plant viruses and may cause serious losses in yield of pea, alfalfa and other leguminous crops (HARPER & KALDY 1982; MAITEKI & LAMB 1985; HINZ 1991; SOROKA & MACKAY 1990b). Both annual and perennial crops have been investigated to determine a pattern of pea aphid abundance. This and the seasonal dynamics of the aphid have been studied in alfalfa and field pea crops in Europe and North America (DUNN & WRIGHT 1955; HOZÁK 1968; ŽRALOVIĆ 1970; STARY 1974; SOROKA & MACKAY 1990a; BOMMARCO & EBOM 1996).

Under natural conditions, pea aphid populations may often be reduced by mycoses, commonly caused by two fungal species belonging to the order Entomophthorales. They are *Conidiobolus obscurus* (Hall & Dunn) Remaudière & Keller and *Erynia neoaphidis* Remaudière & Hennebert. These species were recorded from *A. pism* in Britain (BROBYN & WILDING 1977; WILDING 1975), Poland (BALAZY et al. 1990; BAYTO 1966), Russia (RONINA 1971), U.S.A. (PICKERING et al. 1989; PICKERING & GUTIERREZ 1991; FENG et al. 1992b), Canada (MACLEOD 1955) and Australia (CAMERON & MILLER 1981; MILLER 1982). Many of these authors found epizootics in pea aphid populations, so that pathogens are considered to be important biological agents for aphid control (HALL & DUUN 1958; KREZIOVÁ 1972; HALL & NARIK 1982; LATGE et al. 1982).


Of the entomophthoralean pathogens, *Entomophthora aphidis* Hoffm. (synonym of *Erynia neoaphidis*) was recorded during an outbreak of pea aphid in Slovakia in 1921 (VIENWERTH 1921 in STARY 1974), as well as in Moravia and Slovakia in 1926 (DRASTICH & ROZYSYPAL 1927 in STARY 1974).

The aim of this study was to determine the seasonal dynamics of the aphid in pea and alfalfa plots, to identify entomophthoralean fungi infecting the aphids, and to evaluate the impact of pathogens on pea aphid populations under conditions of south-western Slovakia.
MATERIAL AND METHODS

In 1998 and 1999, the seasonal dynamics and entomopathogens of the pea aphid were studied in field plots of pea (variety Olivín, 0.5 ha) and alfalfa (variety Páďava, 0.5 ha, second year growth used in both years), situated at Nitra-Malanta (48°19'N, 18°09'E). No insecticides or fungicides were applied to the experimental plots.

In both years, the pea plots were surveyed from early May on, soon after the crop emerged, until the beginning of July when the crops matured and were harvested. Pea aphid counts began when the first aphids appeared and continued at approximately 3-day intervals. Observations in alfalfa started in the first half of April in either year. In 1998, the alfalfa plot was monitored just until the pea harvest, but in 1999 for the whole growing season. 25 randomly selected plants were investigated in three replications at each sampling date: one pea branch and one alfalfa stem of a plant were taken for the analyses. The numbers of living aphids and fungus-killed aphids per plant were recorded. The categories of alate and apterous forms were counted separately. With peas, each single branch was carefully examined and the categories of aphids were counted. With alfalfa, the aphids were shaken off the stem onto a sheet of white paper and counted. After shaking, the stem was examined and mummies of dead aphids were collected. Aphids which still remained on analysed stems (mostly aphids of younger instars) were also taken into account. After the aphids were sampled, notes were taken on the stage of plant development.

All dead aphids with external symptoms of entomopatholaean infection (a conspicuous position on the leaves, colour changes, adhesion of the cadavers to the plant surface by rhizoids etc.) were removed from the substrate and placed into plastic vials. In the laboratory, the cadavers were investigated under a microscope to find characteristic fungal structures and to confirm the infection.

Identification of a fungus was based on external symptoms of the diseases and morphology of sporulating structures. Dimensions of primary spores, conidiophores and nuclei were measured. To obtain a pure sample of spores for exact measurement, freshly killed animals were individually transferred from the vials to a piece of wet filter paper at a bottom of a small Petri dish (diameter 50 mm). A microscope slide was put directly over the dead aphid at a distance of 3-4 mm and the dish was closed with its lid. In this simple humid chamber, primary conidia shooting out of conidiophores were projected onto the slide.

Weather data (daily precipitation and mean daily temperature) were taken from the Agrometeorological Station of the Slovak Agricultural University in Nitra situated at Nitra-Malanta.

RESULTS

Figures 1 and 2 show the pattern of seasonal dynamics of the pea aphid in pea plots. In 1998, the first aphids were observed on May 11. Aphid density remained relatively low till May 25, when the number of aphids per branch was higher than 10. Then the population increased to a peak of approximately 70 aphids per branch at the beginning of June. Aphid density peaked during flowering and beginning of pod formation. After reaching the peak, aphid density dropped sharply and did not recover prior to pea harvest. In 1999, the pattern of abundance was similar to that of 1998. Aphid density reached a peak in the first week of June, when the population surpassed 18 aphids per branch. Similarly to 1998, aphid density decreased as the season progressed and the crop became senescent. No aphids were observed after 6 July when all plants were yellow and dry. In both years a maximum number of alatae was recorded at the growth stage of development of pods in the second half of June. The highest abundance of alatae was 1.07 and 0.64 aphids per branch in 1998 and 1999, respectively.
Analysis of variance revealed a significant difference ($P = 0.05$) between the peak aphid abundance on pea in the two years.

Infestation levels of pea aphid in alfalfa plots are shown in Figs 3 and 4. In 1998, aphid density gradually increased from the beginning of May up to 12 June when, on average, 2 aphids per stem were recorded. Then the population declined until sampling finished at a routine cutting on 1 July. Next year, the population reached a peak of more than 3 aphids per stem on 31 May when the population growth was interrupted by cutting. However, the aphids re-appeared in the cut plots and within a week reached a level of 2.6 aphids per stem. In the third week of June, the population decreased to a very low level and did not recover. The density of alate aphids remained at low to negligible levels through the whole sampling season in 1998. The highest count for a sampling date was 7 alateae on 75 stems. In 1999, alateae had a similarly low abundance just in mid-June.

Fig. 2. Seasonal dynamics of pea aphids (number of aphids per pea branch) and number of infected aphids per branch analysed in pea crop at Nitra-Malanta (1999)

Analysis of variance revealed a significant difference ($P = 0.05$) between the peak aphid abundance on alfalfa in the two years.

Two fungal pathogens from the order Entomophthorales were found in the aphid populations on pea. They were identified as Erynia neoaphidis and Conidiobolus obscurus. Comparisons between counts of aphids killed by each pathogen species gave no significant differences neither in 1998 nor in 1999 ($P = 0.05$).

In 1998, fungal infection in the pea plot started on 3 June, i.e. 24 d after colonisation of the crop by aphids (Fig. 1). The level of infestation by fungi was extremely low up to 15 June (< 1% of dead aphids), after which the number of recorded cadavers suddenly increased. The highest density of aphid cadavers occurred on 19 June (10.30% of all aphids), two weeks after the peak of aphid population density.

In 1999, infection began on 4 June, i.e. 29 d after the start of pea crop infestation (Fig. 2), but no infection was
detected at the next two observations. Similarly to 1998, the highest number of killed aphids were found on 19 June (90 individuals = 25.21%). However, in relative expression, the highest infection was recorded on 28 June when 48.39% (15 individuals) of the aphids were killed by the pathogens. Fungal infection in the population continued till 6 July. After this date no living or dead aphids were found.

Table 1 shows, for the pea crops, the proportion of aphids killed by *E. neaphidids* and that killed by *C. obscurus* in categories of alate and apterial separately. Infection by *C. obscurus* of alate aphids was 12 times (1998) or 10 times (1999) more frequent than of apterial ones; similarly, alatee were 4.4 times (1998) or 2 times (1999) more frequently infected by *E. neaphidids* than apterae. However, when the proportions of individual pathogens on alate and apterae were compared by analysis of variance, significant differences (*P* = 0.05) were only detected for infection by *C. obscurus* in both years.

In 1998, aphids in alfalfa were killed only by *E. neaphidids*, and altogether 21 infected aphids were found among 1030 pea aphids analysed during the sampling season. This infection appeared in the crop in the third decade of June. In 1999, surprisingly, aphids on alfalfa were not attacked by entomopathogens throughout the growing season.

Results of correlation analysis are shown in Table 2. Correlation coefficients indicated a positive relationship between the number of infected aphids and precipitation, but this relationship was weak or moderately strong. A strong correlation was found between the number of dead aphids and the number of alate aphids counted 5 to 10 days earlier.

**DISCUSSION**

In previous reports, seasonal patterns of abundance of pea aphids varied considerably between annual and perennial crops. On field peas the population pattern generally exhibits a typical growth curve with one peak. The aphids usually appeared soon after emergence of the crop (MAITEKI et al. 1986), populations were relatively low.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Aphid form</th>
<th>Number of dead aphids per 75 plants</th>
<th>Number of all aphids per 75 plants</th>
<th>Proportion of dead aphids (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Erynia neaphidids</em></td>
<td>alate aphids</td>
<td>14</td>
<td>512</td>
<td>2.73a</td>
</tr>
<tr>
<td></td>
<td>apterial aphids</td>
<td>151</td>
<td>24 206</td>
<td>0.62a</td>
</tr>
<tr>
<td></td>
<td>alate aphids</td>
<td>14</td>
<td>138</td>
<td>10.14a</td>
</tr>
<tr>
<td></td>
<td>apterial aphids</td>
<td>149</td>
<td>3 011</td>
<td>4.95a</td>
</tr>
<tr>
<td><em>Conidobolus obscurus</em></td>
<td>alate aphids</td>
<td>32</td>
<td>512</td>
<td>6.25a</td>
</tr>
<tr>
<td></td>
<td>apterial aphids</td>
<td>126</td>
<td>24 206</td>
<td>0.52b</td>
</tr>
<tr>
<td></td>
<td>alate aphids</td>
<td>26</td>
<td>138</td>
<td>18.84a</td>
</tr>
<tr>
<td></td>
<td>apterial aphids</td>
<td>57</td>
<td>3 011</td>
<td>1.89b</td>
</tr>
</tbody>
</table>

Means within the same section followed by the same letter are not significantly different (Tukey's test, *P* = 0.05)
Table 2. Correlation coefficients between the number of infected aphids and precipitation, or numbers of alate aphids

<table>
<thead>
<tr>
<th></th>
<th>P&lt;</th>
<th>Pdays</th>
<th>ALATE5-10</th>
<th>ALATE6</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEADA</td>
<td>0.4084*</td>
<td>0.1519*</td>
<td>0.7122**</td>
<td>0.3727*</td>
</tr>
<tr>
<td>DEADA</td>
<td>0.0038*</td>
<td>0.2787*</td>
<td>0.7775**</td>
<td>0.5609*</td>
</tr>
</tbody>
</table>

P< 0.01, P > 0.05

DEADA – number of dead aphids per 75 pea branches in populations per analysed day

P< cumulative precipitation counted from the day when the first aphids appeared

Pdays – number of days with measurable precipitation counted from the day when the first aphids appeared

ALATE5-10 – number of living alate aphids per 75 branches recorded five to ten days before

ALATE6 – average number of living aphids per 75 branches for six preceding days

until flowering, then they rose rapidly during pod formation and fell abruptly as pods matured and dried (BOM-MARCO & EKBOB 1996; MAITEKI et al. 1986; SOROKA & MACKAY 1990a). A similar trend of infestation of pea crops was observed in our experiments. The highest abundance was observed at the flowering stage and beginning of pod formation. Moreover, the 1998 infestation was significantly heavier than that in 1999 (P = 0.05). The heavy infestation in 1998 is attributed to favourable weather conditions. Dry and relatively warm conditions through May and June resulted in the outbreak. In 1999, warm and dry weather in late spring was also associated with an increase in aphid numbers from a low level in early May to a maximum on June 7. However, heavy rain on June 8 caused a decrease of population. The next increase on June 11 was again depressed by heavy rain. Further rainy days at the end of June suppressed the population once more, and the change in plant quality associated with maturing aided this suppression.

According to some authors, the seasonal dynamics of the pea aphid on perennial crops is characterised by two peaks, one in spring and the other in autumn (DUNN & WRIGHT 1955; HOZÁK 1968). However, in former Czechoslovakia, STARÝ (1974) identified in alfalfa three (four) peaks of abundance throughout the year: a spring peak was caused by high numbers of fundatrix; a peak in spring-summer (summer) was the result of an outbreak of virgines; a summer peak, if it occurred, was in fact a postponed spring-summer peak owing to weather conditions; at last an autumn peak was the increase of sexuales. The seasonal dynamics of aphids on alfalfa in our experiments were very similar in both years. There was only one apparent maximum (spring-summer). In 1998, the population was analysed only until the end of June. Therefore, we could not confirm an eventual autumn peak. In 1999, cutting weakened the spring-summer peak, and the following decrease was probably caused by wet weather in June when the sum of precipitation was 54% higher than the long-term average for this month (Table 3). A similar weather pattern was also observed in July.

The level of infestation by the alate form of aphids was low for both crops and years. In general, the population trend of alatae was similar to apterae. But the method used for aphid sampling in the crops might have caused some errant numbers of alatae because they may have flown away just before or during counting. Though a sweeping method would have been better, we used the method of counting per branch/stem because it allowed us to analyse also the proportion of dead aphids in a population. According to HOZÁK (1968), results with the aforementioned sampling methods approximately reflect the population curve of the pea aphid.

Fungal diseases more or less regularly reduce aphid populations, but levels of infection vary among years, localities and populations. Many researchers have recorded aphid mortality to be higher than 50% (WILDING 1975; PICKERING et al. 1989; KISH et al. 1994).

Humid conditions are emphasised to be necessary for fungal infection in aphid populations. Over 30% of pea aphids were infected only when the relative humidity remained above 90% for at least 8 h/d during 7 to 12 d before sampling (WILDING 1975). Epizootics by entomophthoralean fungi followed a period during which relative humidity exceeded 90% for at least 8 h/d, rain fell for

Table 3. Weather data (mean month temperature and sum of precipitation) measured at Nitra

<table>
<thead>
<tr>
<th>Month</th>
<th>Long-term average*</th>
<th>1998</th>
<th>1999</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>temperature (°C)</td>
<td>rainfall (mm)</td>
<td>temperature (°C)</td>
</tr>
<tr>
<td>May</td>
<td>14.8</td>
<td>55.0</td>
<td>15.1</td>
</tr>
<tr>
<td>June</td>
<td>18.3</td>
<td>70.0</td>
<td>19.7</td>
</tr>
<tr>
<td>July</td>
<td>19.7</td>
<td>64.0</td>
<td>21.0</td>
</tr>
<tr>
<td>August</td>
<td>19.3</td>
<td>58.0</td>
<td>-</td>
</tr>
<tr>
<td>September</td>
<td>15.4</td>
<td>37.0</td>
<td>-</td>
</tr>
<tr>
<td>October</td>
<td>9.1</td>
<td>41.0</td>
<td>-</td>
</tr>
</tbody>
</table>

*Long-term average according to ŠPÁNIK & TOMLAIN (1997)
at least 5 h/d for 3 or more consecutive days, and the mean maximum temperature after the rain was at least 20°C (MISONNIER et al. 1970). E. neaphidus reached epidemic proportions in aphid populations when daily rainfall averaged 0.44 inches (11.18 mm, converted by authors) with a mean relative humidity of 81.2% (MACLEOD 1955). C. obscurs and E. neaphidus require a saturated or almost saturated atmosphere for sporulation (WILDING 1969). In our experiments we found a significantly heavier aphid infection in 1999 than in 1998. The higher infection in 1999 is probably connected with the over double total precipitation in the season than in the year before (Table 3). According to VORONINA (1971), at least 55 to 65 or more rainy days per growing season are necessary for a mass infestation of pea aphid populations by entomophthorosis in Russia. But the frequency and distribution of the precipitation over the days is more important than the total amount (SHANDS et al. 1963). On the other hand, KISH & ALLEN (1978) emphasised that an excess of precipitation can be undesirable by lowering the density of airborne conidia in an environment and by washing the spores off cadavers. In our experiments, correlation analysis revealed a positive correlation ($r = 0.4048$) between the level of infection and cumulative precipitation in 1998. But this was not confirmed in 1999. A positive correlation between an entomophthoralean infection and variables associated with moisture were also found by FENG et al. (1992a), WILDING (1975) and ELKINGTON et al. (1991). In contrast, SHANDS et al. (1963) found no relationship between total precipitation during a growing season and incidence of infected potato aphids, Macrosiphum euphorbiae Thomas, in north-eastern Maine (U.S.A.).

Resting spores, overwintering propagules of the pathogens, seem to be a reservoir of the disease in nature. They may produce infectious conidia several months after remaining on the soil (COREMANS-PELSENBER 1981). However, conidia of the pathogens cause a much higher level of mortality in the insects than resting spores. This may explain why the infection of aphids appears rarely in spring months (KREIZOVÁ 1972). GUSTAVSON (1969) assumes that the low temperature of the soil, the main source of resting spores, at the start of the vegetation period is the reason why in nature it takes a long time before the first infection occurs. In England, E. neaphidus never infected pea aphids before mid-June and was most frequent in late July and early August (WILDING 1975). In our observations, the first infected aphids in pea crops occurred after 24 or 29 aphid days in 1998 or 1999, respectively. No killed aphids were found before the beginning of June. We suppose that two years research is a short time to get a consistent explanation of this phenomenon, but a certain period is probably necessary for the fungus to spread and its outbreak in populations. KRAZIČ (1970) considered that under the climatic conditions of Slovakia the fungi did not threaten the fundatrices but might be observed usually at the end of the outbreak, when the aphids were generally weakened by ecological conditions.

An efficient vector of fungal infection is essential to spread the infection among aphid populations. According to WILDING & PERRY (1980), aphid migration is an important means to distribute pathogen inoculum. Alate aphids play a principal role in dissemination of mycoses into aphid populations (RABASSE & ROBERT 1975). The aphid infection at Nita-Malanta usually increased when the numbers of alate aphids culminated. When we analysed the relationship between the number of dead aphids per day and the number of alate aphids counted 5 to 10 days earlier, we found a strong correlation ($r = 0.7122^*$ in 1998, and $r = 0.7775^*$ in 1999). This may suggest that alate forms are responsible for the transmission of infection into populations, and a period of 5 to 10 days is necessary for development of the pathogen in the vector, as well as subsequent infection of other aphids in populations. LIZEN et al. (1985) found that alate aphids of A. pism are about six times more susceptible to infection by E. neaphidus than alatum adults. We observed that alate aphids were more frequently infected by both pathogens than alatum ones, which could confirm the higher susceptibility of alate individuals to the infection. On the other hand, this fact could be responsible for the strong correlation mentioned above.

In the alfalfa crops the number of aphids killed by a fungus was low in 1998 and no infected aphids were found in 1999. A relatively low infestation of alfalfa by aphids, with never more than 3.5 pea aphids per stem, could be a reason for the low infection in the population. According to PICKERING & GUTIERREZ (1991), however, an E. neaphidus epidemic in an A. pism population was maintained at a density of less than four aphids per alfalfa stem, and the pathogen depressed this population effectively. DEDRYVER (1981) also thinks that pathogenicity of E. neaphidus seems to be density independent. We assume that a certain minimum number of insects is required for initial infection, but this density only gives the potential for production of conidia. Other circumstances, favourable weather conditions especially, are also very important to start epizootics. The alfalfa and pea crops in our experiments grew close to each other so that the aphid populations developed under the same weather conditions. In spite of that, the infection levels were notably different. There must thus be further important factors in the aphid-pathogen system. For instance, different microclimate conditions in different crops may play a certain role. It is well known that pea aphid populations are created with green and red forms and, besides, this species is also divided into races according to host plant (POSYLAJEVA 1982). Maybe the races on pea and alfalfa differ in their predisposition to fungal infection. RAMOSKA & TODD (1985) found that even feeding on different plants could influence indirectly the development of a pathogen within hosts.
References


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Súhrn


Kľúčové slová: Erynia neoaphidis; Conidiobolus obscurus; Acrithosiphon pisum; patogény vošiek

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