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ORIGINAL PAPER

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THE PARASITISATION OF THE ROSE TORTRIX [(*Archips rosana* (L.)] POPULATION IN THE APPLE ORCHARD IN THE WIELKOPOLSKA REGION, POLAND

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ABSTRACT

The rose tortrix is a polyphagous species, which is dominant in the leafroller complex (Tortricidae) in orchards, including apple orchards and fruit-bearing shrubs in western and southeastern Poland. Rose tortrixes massively feed on leaf rosettes, developing leaves, buds, flowers and fruit buds in orchards. The population of this phytophage is regulated by environmental factors such as its natural enemies, e.g. parasitoids. The aim of the study was to determine the degree of parasitisation of rose tortrix pupae and to determine the role of individual families of parasitoids in reducing their population. The study showed that parasitoids from the Ichneumonidae, Chalcididae, Tachinidae, Euliphidae and Pteromalidae families reduced the rose tortrix population. The parasitoids of the Ichneumonidae family were the most effective entomophages. The degree of total parasitisation of rose tortrixes in individual years of the research was similar. The effectiveness of these entomophagous parasitoids is influenced by environmental factors – above all by weather conditions, the number of crop protection treatments and the ecological infrastructure of orchards.

Key words: parasitisation, *Archips rosana*, Hymenoptera, Diptera, apple orchard

INTRODUCTION

The rose tortrix [*Archips rosana* (L.)] is a polyphagous species, which is dominant in the leafroller complex (Lepidoptera, Tortricidae) in orchards, including apple orchards and fruit-bearing shrubs in western and southeastern Poland [Kot and Jaśkiewicz 2007, Tian and Piekarska-Boniecka 1998]. It is also a phytophage that frequently feeds on ornamental trees and shrubs growing in urban agglomerations [Polat and Tozlu 2010]. Rose tortrixes massively feed on leaf rosettes, developing leaves, buds, flowers and fruit buds in orchards. This reduces the yield of crops, which results in economic loss. The biting of leaves of ornamental plants reduces their aesthetic value, which is so important for urban greenery. Rose tortrix caterpillars hatch in late April, when apple-trees are in the green bud phase. They feed until late May and early June. Then they pupate at their feeding site, i.e. in rolled leaves. Moths appear in the second half of June and fly until the end of July [Płuciennik and Tworkowska 2004]. It is necessary to annually apply chemical crop protection products to reduce the population of rose tortrixes in orchards. Nevertheless, chemical treatments may be difficult or strictly forbidden in urban agglomerations due to the presence of people and animals [Tomalak 2003].

The population of this phytophage is regulated by environmental factors such as its natural enemies, e.g. parasitoids. Representatives of the following

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families of rose tortrix parasitoids are predominant: Ichneumonidae, Braconidae, Chalcididae (Hymenoptera) and Tachinidae (Diptera). To date more than 100 species of parasitoids limiting the rose tortrix population have been found [Yu et al. 2012, Aydoğdu 2014]. More than 30 species of parasitoids of the Ichneumonidae family, reducing the number of rose tortrixes feeding in orchards, have been found in western Poland [Piekarska-Boniecka and Trzciński 2013, Piekarska-Boniecka et. al. 2019]. Parasitoids appear in the eggs, larvae and pupae of rose tortrixes [Piekarska-Boniecka and Trzciński 2013, Aydoğdu 2014, Ercan et al. 2015].

Earlier studies on limiting the rose tortrix population by parasitoids in apple orchards in the Wielkopolska region showed considerable diversification in the degree of parasitisation. It ranged from 15.5 to 40.1%. The research was conducted only in one growing season in individual orchards [Piekarska-Boniecka et al. 2019]. Therefore, it was the research was continued in the orchard where the highest parasitisation of this species was observed.

The aim of the study was to determine the degree of parasitisation of rose tortrix pupae and to determine the role of individual families of parasitoids in reducing their population.

MATERIAL AND METHODS

Locality

The research was conducted the apple orchard in the village of Jarogniewice (52°09'14.9"N 16°41'08.7"E) in the Wielkopolska region between 2014 and 2016. The orchard occupied an area of 120 ha. The research was conducted in a 5-ha plot with 18-year-old apple-trees of the 'Idared' and 'Jonagold' cultivars. There was grassland between the rows of trees in the orchard, and there was herbicide fallow in the rows. The experimental site was adjacent to a shrub belt with a length of 300 m and a width of 7–8 m. The vegetation in the shrub belt mainly consisted of the blackthorn plum tree (*Prunus spinosa* L.), the common pear tree (*Pinus communis* L.), the common elm (*Ulmus campestris* L.), the European spindle tree (*Evonymus europaea* L.), the hawthorn (*Crataegus monogyna* Jacq.), the whitethorn (*C. oxyacantha* L.), the elder (*Sambucus nigra* L.), the dewberry (*Rubus caesius* L.), the blackberry (*Rubus suberectus* Ander. ex Sm.), the briar rose (*Rosa canina* L.), the common carrot (*Daucus carota* L.), the common yarrow (*Achillea millefolium* L.), the common nettle (*Urtica dioica* L.) and the couch-grass (*Agropyron repens* L.).

Integrated protection from pests and diseases was provided to the apple-trees in the orchard. Both in 2014 and 2015 five treatments were applied to protect the crops from pests. The following crop protection products were used: Karate Zeon 050 CS (Syngenta, GB), Calypso 480 SC (Bayer, D), Steward 30 WG (DuPont, CH), Pirimor 500 WG) (Syngenta, GB) and Cyren 480 EC (Cheminova, DK). In 2016 there were three crop protection treatments with the following products: Karate Zeon 050 CS (Syngenta, GB), Calypso 480 SC (Bayer, D) and Pirimor 500 WG (Syngenta, GB).

Methods

Each year between May and June the feeding grounds of rose tortrix larvae were examined and pupae in rolled leaves were selected in a designated area of the orchard. The pupae were collected from the orchard twice a week throughout the study period. All the pupae were collected from randomly selected trees. We tried to collect 1 000 pupae per orchard each year. The pupae were placed individually in glass tubes and capped with cotton wool. They were then transferred to wooden boxes and reared in the orchard under a roof. They were checked daily and the moths and parasitoids which appeared were recorded. The pupae which did not develop into insects were observed under a microscope in order to define their parasitoids.

Parasitoid families of the Hymenoptera order were identified according to the key developed by Goulet and Huber [Goulet and Huber 1993], whereas species of the Ichneumonidae family were identified according to the key developed by Kasparyan [Kasparyan 1981].

The data on the weather conditions during the research were based on the average monthly temperatures measured at the Research Station in Turew, Agricultural and Forest Environ-ment Institute, Polish Academy of Sciences, Poznań, Poland. The weather conditions were similar in individual years of the research, especially in 2014 and 2015 (Fig. 1). 2014 and 2015 were warmer than 2016. In July 2014 and in August 2015 the highest air temperatures were recorded.

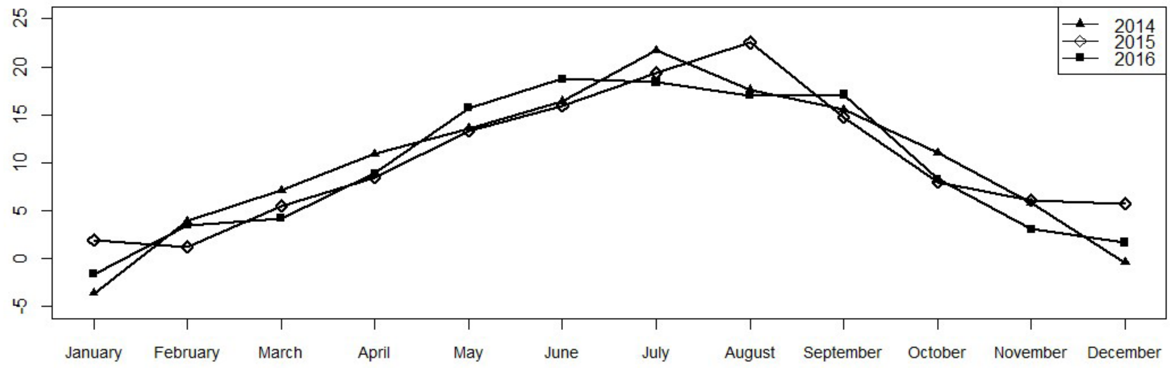

Fig. 1. Average monthly air temperatures in particular study year

In each year of the research the spring and summer months were warm. During this period air temperatures were similar, but in May and June 2016 they were higher than in the other years.

Data analysis. Firstly, the normality of distributions of the observed traits was tested with the Shapiro-Wilk test of normality [Royston 1995]. The analysis of the number of parasitoids limiting the rose tortrix population was based on the χ^2 test. This test assessed the dependency between the research years and the total number of all parasitoid families parasitising rose tortrixes, and the dependency between the research years and the number of individuals of the Ichneumonidae family only. Differences in parasitisation between consecutive years were assessed with the Kruskal-Wallis rank sum test [Kruskal and Wallis 1952]. The parasitisation diversity profiles were analysed to characterise the degree of parasitisation in individual years of the study. Parasitisation diversity profiles enable the analysis of parasitisation by assessing their functional traits [Patil and Taillie 1979, 1982]. Diversity profiles are functions that depend on the parameter that indicates the presence of rare and abundant species. This method is very sensitive to slight differences in the abundance of species and indicates the presence of dominant species in the environment. The profile method was used to analyse the parasitisation of rose tortrixes by all parasitoid families and so-called β-profiles were determined [Patil and Taillie 1979, 1982]. The diversity of parasitisation by parasitoids of the Ichneumonidae family was presented by means of the diversity profiles determined on the basis of the first derivative. The areas under the β-diversity profiles for individual parasitoid families were also analysed. They enable assessment of differences in the size of these families. The R software (version 3.6.2) by R Core Team [2019] was used for statistical calculations.

RESULTS

Between 2014 and 2016 3,000 rose tortrix pupae were collected in the apple orchard in the Wielkopolska region (Tab. 1). 2,520 (84%) rose tortrix moths developed from the pupae, 404 (13.5%) of the pupae were parasitised and 76 (2.5%) of the pupae dried. The rose tortrix population was limited by five parasitoid families, i.e. Chalcididae, Eulophidae, Ichneumonidae and Pteromalidae of the Hymenoptera order and Tachinidae of the Diptera order. The parasitoids of the Ichneumonidae family were the most effective entomophages because they reduced the rose tortrix population to the greatest extent, i.e. by 9.9% (294 pupae). The other parasitoid families parasitised the rose tortrixes to a small extent, i.e. by 0.1–2.1%.

The relation between the numbers of individual parasitoid families and the years of the study was analysed by means of the χ^2 independence test. The test showed that the numbers of individual parasitoid families and thus the degree of parasitisation changed in individual years (χ^2 test = 50.734, p-value = 0.0011).

The degree of total parasitisation of rose tortrixes in individual years of the research was similar, especially in 2014 and 2015, when it amounted to 13 and 11.1%, respectively. In 2016 the degree of parasitisation was slightly higher, i.e. 16.3% (Tab. 1).

Table 1. The parasitisation of *Archips rosana* pupae in orchards in Jarogniewice, 2014–2016

	Years			
	2014	2015	2016	Total 2014–2016
Total pupae	1000	1000	1000	3000
Parasitised by Chalcididae	14 (1.4%)	16 (1.6%)	33 (3.3%)	63 (2.1%)
Parasitised by Eulophidae	2 (0.2%)	–	–	2 (0.1%)
Parasitised by Ichneumonidae	98 (9.8%)	80 (8%)	116 (11.6%)	294 (9.9%)
Parasitised by Pteromalidae	2 (0.2%)	1 (0.1%)	1 (0.1%)	4 (0.1%)
Parasitised by Tachinidae	14 (1.4%)	14 (1.4%)	13 (1.3%)	41 (1.3%)
Total parasitisation	130 (13%)	111 (11.1%)	163 (16.3%)	404 (13.5%)

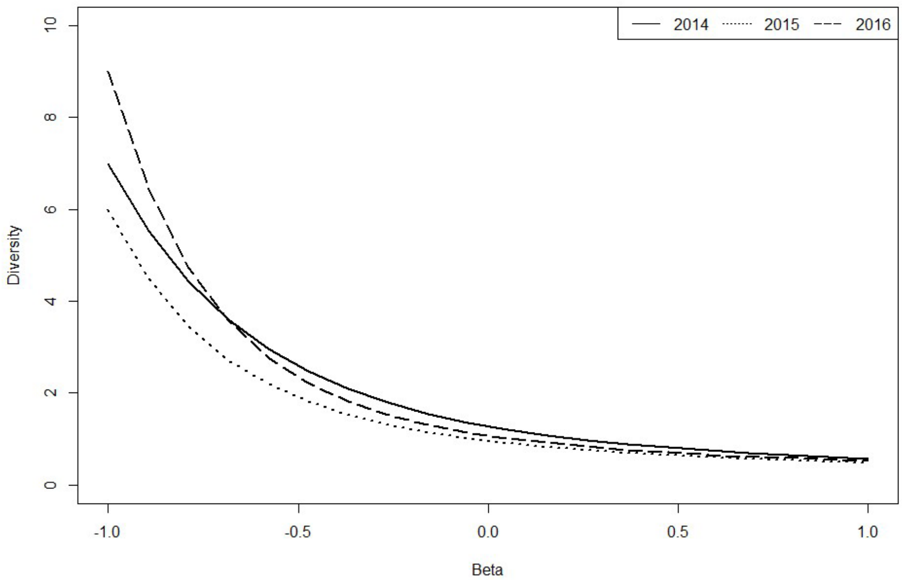

Fig. 2. Variation in the parasitisation of *Archips rosana* pupae by all parasitoid families between 2014 and 2016, expressed by means of β-diversity profiles

The degree of total parasitisation of rose tortrixes in individual years of the study was analysed by assessing the functional traits of β-diversity profiles (Fig. 2). The analysis confirmed that the degree of parasitisation in 2016 was higher than in 2014 and 2015. It also confirmed that in 2014 and 2015 the degree of parasitisation was similar.

In individual years of the study the rose tortrix population was limited by 4 or 5 families of parasitoids. In each year of the study the tortrixes were parasitised by representatives of the Chalcididae, Ichneumonidae, Pteromalidae and Tachinidae families. In 2016 the Chalcididae family had the highest share (3.3%) in reducing the rose tortrix population, whereas in 2014 and 2015 the shares were lower and very similar to each other. The Ichneumonidae family parasitised the rose tortrix population to the greatest extent. In 2016 it was the highest, i.e. 11.6%. In 2014 and 2015 the Ichneumonidae family reduced the rose tortrix population to a lesser extent, which was similar in both years. The share of the Pteromalidae family in the regulation of the rose tortrix population was small – it ranged from 0.1 to 0.2%. The parasitoids of the Tachinidae family parasitised rose tortrixes to a slightly higher extent, i.e. 1.3–1.4% – these values were similar in individual years of the study. The parasitoids of the Eulophidae family reduced the rose tortrix population only in 2014. Their share in parasitisation was negligible.

The analysis of the rate of parasitisation of rose tortrix pupae by individual parasitoid families in the subsequent years of the study showed that there were no statistically significant differences between the years (Kruskal-Wallis chi-squared = 0.14177, p-value = 0.9316).

The area under the β-profile of parasitic diversity was analysed to show the numbers of individual parasitoid families. The analysis showed that in 2014 and 2015 the numbers of parasitoid families were the most diversified, as evidenced by the values of the area under the β diversity profile, i.e. 3.9136 and 3.8871, respectively. In 2016 the diversity was smaller and the area under the β diversity profile amounted to 3.0497.

In 2014 parasitoids started flying out of the tortrix pupae in mid-June and this period ended in late July (Fig. 3). First, parasitoids of the Ichneumonidae and Tachinidae families appeared. In early July representatives of the Chalcididae family began to fly out. Single individuals of the Eulophidae and Pteromalidae families appeared in mid-July. The largest number of parasitoids began to fly in late June – these were represen-

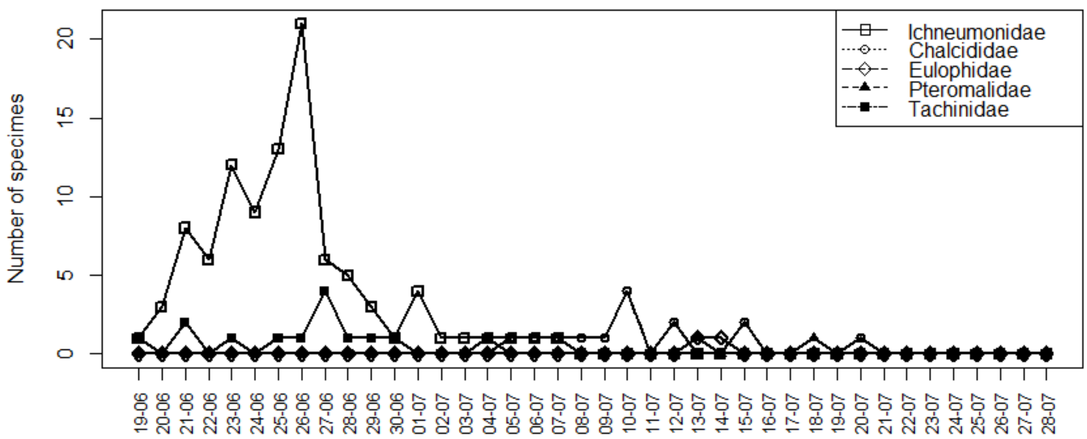

Fig. 3. The emergence of parasitoids from *Archips rosana* pupae in 2014

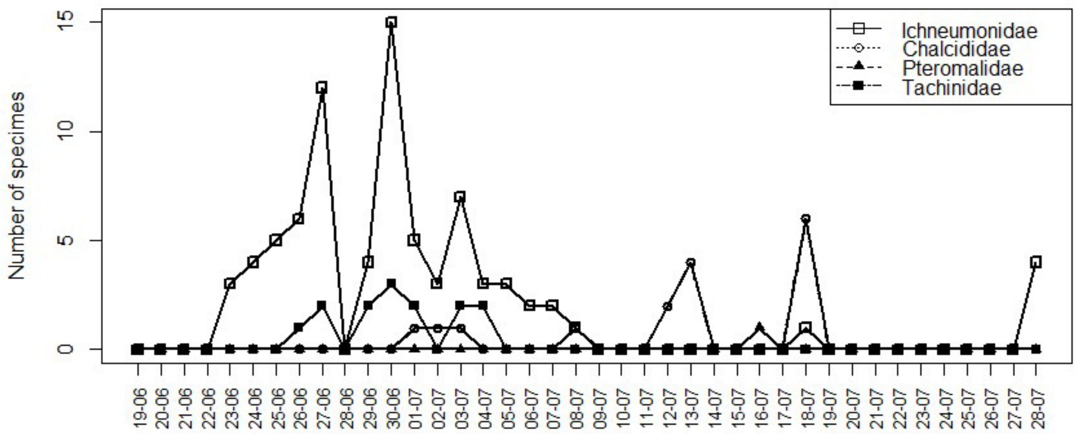

Fig. 4. The emergence of parasitoids from *Archips rosana* pupae in 2015

Table 2. The parasitisation of *Archips rosana* pupae by parasitoids of the Ichneumonidae family in orchards in Jarogniewice, 2014–2016

Species	Years			
	2014	2015	2016	Total 2014–2016
Anomaloninae				
Trichomma enecator (Rossi, 1790)	3 (0.3%)		1 (0.1%)	4 (0.1%)
Ichneumoninae				
Phaeogenes semivulpinus (Gravenhorst, 1829)	9 (0.9%)			9 (0.3%)
Metopiinae				
Exochus mitratus (Gravenhorst, 1829)		1 (0.1%)	1 (0.1%)	2 (0.1%)
Pimplinae				
Apechthis quadridentata (Thomson, 1877)		1 (0.1%)	1 (0.1%)	2 (0.1%)
Apechthis rufata (Gmelin, 1790)		1 (0.1%)	4 (0.4%)	5 (0.2%)
Itoplectis alternans (Gravenhorst, 1829)			1 (0.1%)	1 (0.03%)
Itoplectis maculator (Fabricius, 1775)	82 (8.2%)	77 (7.7%)	107 (10.7%)	266 (8.9%)
Pimpla rufipes (Miller, 1759)			1 (0.1%)	1 (0.03%)
Pimpla turionellae (Linnaeus, 1758)	4 (0.4%)			4 (0.1%)
Total parasitisation	98 (9.8%)	80 (8%)	116 (11.6%)	294 (9.9%)

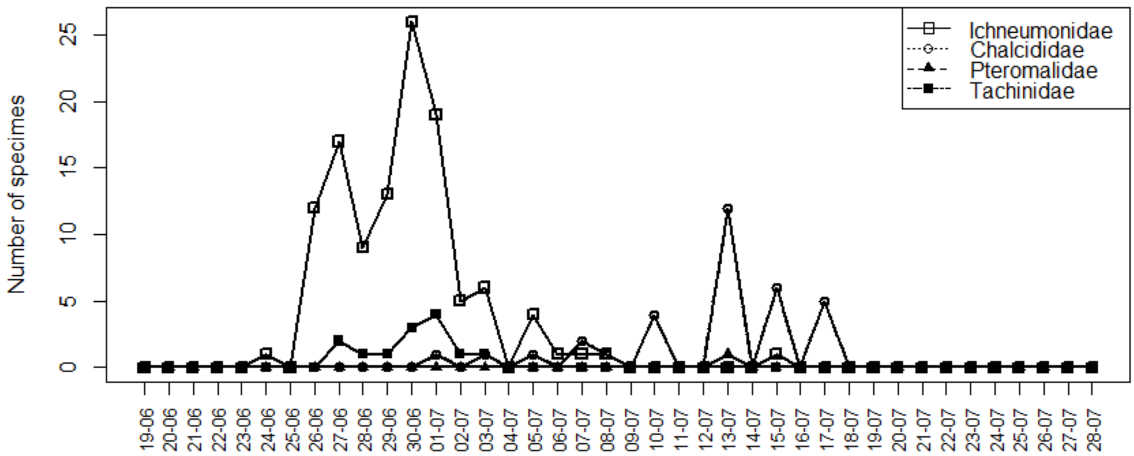

Fig. 5. The emergence of parasitoids from *Archips rosana* pupae in 2016

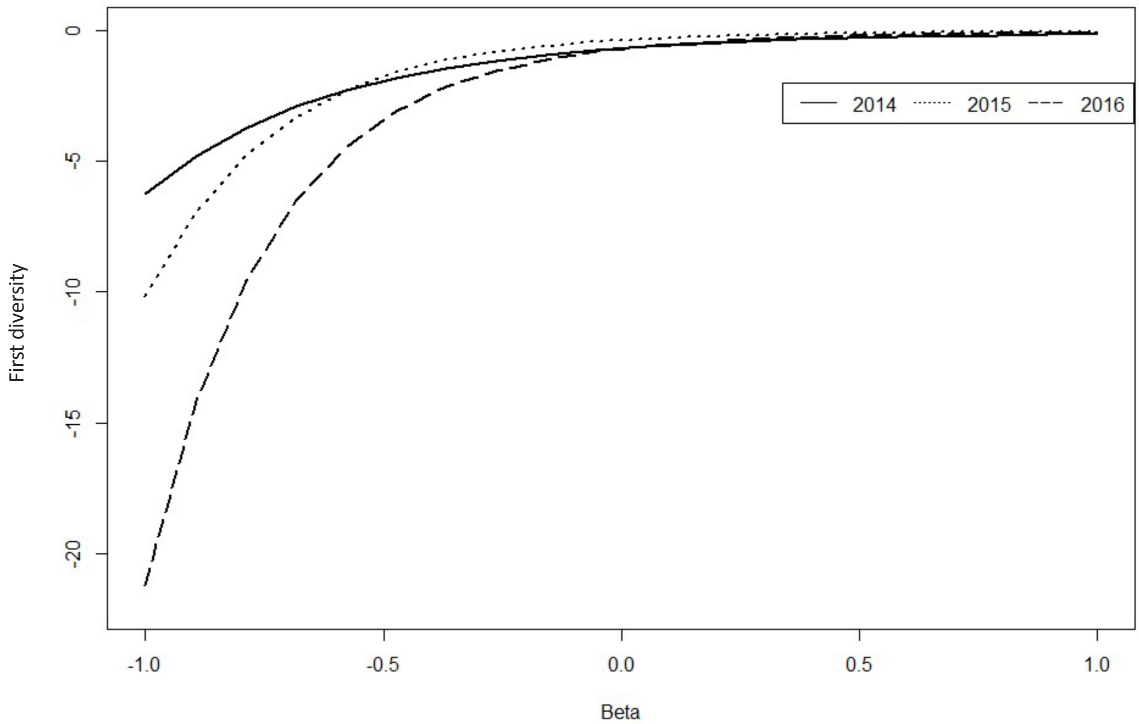

Fig. 6. The profiles of diversity in the parasitisation of *Archips rosana* pupae by the Ichneumonidae between 2014 and 2016, determined by means of the first derivative

tatives of the Ichneumonidae and Tachinidae families.

In 2015 the flights of parasitoids continued with intervals from late June to late July (Fig. 4). In late July parasitoids were observed only at the end of the month. The Ichneumonidae appeared first. Later the Tachinidae and Chalcididae began to fly. There was one specimen of the Pteromalidae family observed in mid-July. The most parasitoids flew in late June – these were representatives of the Ichneumonidae, Tachinidae and Chalcididae families.

In 2016 the flights of parasitoids lasted shorter than in the previous years. They started in late June and ended in mid-July (Fig. 5). First, the Ichneumonidae appeared. They were followed by the Tachinidae. In early July representatives of the Chalcididae family appeared. There was one specimen of the Pteromalidae family observed in mid-July. The most parasitoids flew in late June – these were representatives of the Ichneumonidae, Tachinidae and Chalcididae families again.

The relation between the numbers of individual Ichneumonidae species and the years of the study was analysed by means of the χ^2 independence test. It showed that the species abundance, and thus the degree of parasitisation changed in individual years, (χ^2 test = 39.343, p-value < 0.001).

The parasitoids of the Ichneumonidae family reduced the rose tortrix population to a similar extent in individual years of the study. In 2014 and 2015 the degree of parasitisation amounted to 9.8 and 8%, respectively (Tab. 2). In 2016 it was slightly higher, i.e. 11.6%.

The degree of rose tortrix parasitisation by individual Ichneumonidae species in consecutive years of the study was analysed on the basis their diversity profiles, which were determined by means of the first derivative (Fig. 6). The analysis confirmed that the degree of parasitisation in 2016 was higher than in 2014 and 2015 and that it was similar in the latter two years.

The rose tortrix population was limited by nine species of parasitoids of the Ichneumonidae family (Tab. 2). The highest species diversity was observed in 2016, when seven species were identified, whereas in 2014 and 2015 four Ichneumonidae species were found. *Itoplectis maculator* was the dominant species in the parasitoid complex in each year of the study. Its share in rose tortrix parasitisation ranged from 7.7 to 10.7%. The other Ichneumonidae species were less effective because they reduced the rose tortrix population by 0.1–0.9%.

The assessment of the degree of parasitisation based on the analysis of diversity profiles determined by means of the first derivative also confirmed the fact that the highest species diversity of the parasitoids of the Ichneumonidae family was observed in 2016 and that the most abundant species in the parasitoid complex was found then (Fig. 6).

The species of the *Apechthis* genus and the *Pimpla rufipes* and *Exochus mitratus* species are pupal endoparasitoids of insects of the Lepidoptera and Hymenoptera orders. The species of the *Itoplectis* and *Pimpla turionellae* genera are polyphagous pupal endoparasitoids. The *Trichomma enecator* and *Phraeogenes semivulpinus* species are larvo-pupal endoparasitoids of the Lepidoptera order [Yu et al. 2012].

DISCUSSION

The research on the parasitisation of rose tortrix pupae [*Archips rosana* (Linnaeus Linnaeus)] in the apple orchard in the Wielkopolska region showed that the population of this insect was reduced less than in an earlier study conducted in the same orchard [Piekarska-Boniecka et al. 2019]. In this study the parasitisation ranged from 11.1 to 13.5%, whereas in the previous study the rose tortrix population was reduced by 40.1%. Such high parasitisation of rose tortrixes in the previous study may have been caused by a lower number of crop protection treatments against pests in this orchard. There were only three treatments in early spring [Piekarska-Boniecka et al. 2019]. In 2015 the lowest rose tortrix parasitisation was observed. It may have been caused by the fact that this year was warmer than the others and the highest air temperatures were recorded in the summer months. The higher air temperatures may have caused the drying of the pupae and the death of parasitoid larvae in the hosts. This study showed that the rose tortrix population was the most reduced in 2016. The higher parasitisation rate in 2016 may be explained by the fact that in the spring months, i.e. May and June, the air temperatures were higher than in the other years. The higher air temperatures may have influenced the activity of parasitoids in penetrating the environment and finding hosts.

The degree of rose tortrix parasitisation in apple orchards in Poland is considerably diversified. Earlier studies conducted in western Poland showed that the rose tortrix population was reduced by 4.7–23.6% [Piekarska-Boniecka and Trzciński 2013]. The population of rose tortrix larvae and pupae in apple orchards near Lublin was parasitised by 7.2–26.4% [Kot 2007]. Płuciennik and Olszak [2010] observed that the number of larvae of this phytophage in apple orchards in different regions of Poland decreased by 2.4–32.4%. Ercan et al. [2015] found that 0.8–33.9% of rose tortrix eggs in apple, cherry, quince and apricot orchards in Anatolia, Turkey was parasitised.

Earlier studies clearly showed that the parasitisation of rose tortrix larvae and pupae in European orchards was also diversified. Mey [1987] found that the number of rose tortrix larvae and pupae in apple orchards in eastern Germany was reduced by 28.9%. Aydoğdu [2014] studied the parasitisation of rose tortrix larvae and pupae in cherry orchards in Turkey and found that parasitoids reduced their population by 19.8–20.7%. The parasitisation of the larvae and pupae of other leafrollers (Lepidoptera, Tortricidae) in orchards was also diversified. According to Balazs [1997], the degree of parasitisation of leafroller larvae in apple orchards in Hungary generally ranged from 10 to 20%. Velcheva et al. [2010] found that the decrease in the population of leafroller larvae and pupae in plum orchards near Sofia, Bulgaria ranged from 1.6 to 11.1%.

The rose tortrix population was limited by five families of parasitoids, three of which, i.e. Chalcididae, Ichneumonidae and Tachinidae, were previously recorded in this host in apple orchards in western Wielkopolska, including orchards in Jarogniewice [Piekarska-Boniecka and Trzciński 2013, Piekarska-Boniecka et al. 2019]. Aydoğdu [2014] conducted research on the parasitisation of rose tortrixes in cherry orchards in Turkey and also observed the presence of the Chalcididae, Ichneumonidae and Tachinidae families in the complex of parasitoids of this phytophage.

In our study the rose tortrix population was most effectively reduced by parasitoids of the Ichneumonidae family. The highest effectiveness of these entomophages in parasitising rose tortrixes feeding in orchards was also observed in earlier studies by Piekarska-Boniecka and Trzciński [2013] and Aydoğdu [2014]. An earlier study conducted in the orchard in Jarogniewice showed that the parasitisation of the rose tortrix population by the Ichneumonidae was much higher (35.9%) than in this study (9.9%) [Piekarska-Boniecka et al. 2019]. Such considerable differences in the degree of parasitisation was caused by various environmental factors [Kasparyan 1981, Fitton et al. 1988, Kaźmierczak 2010]. The most important of them were the weather conditions, especially droughts, which prevented proper development of parasitoid larvae in the hosts, as well as the number of crop protection treatments, which also destroyed useful entomofauna, reduced the number of alternative hosts for parasitoids and the ecological infrastructure of orchards. Orchards adjacent to trees and bushes ensure favourable conditions for the development of entomophages. They provide refuge during chemical treatments in orchards and an environment for the development of alternative hosts for these entomophages [Olszak et al. 2009].

The dominance of the Ichneumonidae family in the complex of parasitoids limiting the population of leafrollers feeding in the orchard environment in Poland was also reported in studies by Kot [2007] and Płuciennik and Olszak [2010]. Maharramova [2010] studied the parasitisation of leafrollers in the orchard environment in Azerbaijan and also observed the dominance of the Ichneumonidae family. Velcheva et al. [2010] observed the dominance of the Ichneumonidae family in plum orchards near Sofia, Bulgaria.

In our study the *Itoplectis maculator* species reduced the rose tortrix population most effectively. Earlier studies conducted in apple orchards in Poland clearly indicated that this species exhibited very high effectiveness in parasitising rose tortrixes [Miczulski and Koślińska 1976, Kot 2007, Piekarska-Boniecka and Trzciński 2013, Piekarska-Boniecka et al. 2019]. The studies conducted in the fruit-growing community by Mey [1987] in Germany and by Aydoğdu [2014] in Turkey also showed the dominance of this species in the complex of rose tortrix parasitoids. *Itoplectis maculator* is closely related to the apple orchard environment and is a very stable species in this habitat. It is one of the main dominants among rose tortrix parasitoids and other leafroller species feeding in orchards [Miczulski and Koślińska 1976, Płuciennik and Olszak 2010]. Earlier studies on apple orchards in the Wielkopolska region [Piekarska-Boniecka et al. 2020] showed that *I. maculator* appeared in April and remained in this environment until October. They also showed that the period of mass appearance of the dominant parasitoid species of the Pimplinae subfamily, including *I. maculator*, in apple orchards usually occurred in the first half of July. Miliczky and Horton [2005], Debras et al. [2006], Euler et al. [2006], Dib et al. [2012] and Piekarska-Boniecka et al. [2020] also found that when there was vegetation (e.g. shrubs) growing on the edges of orchards, as was the case in this study, parasitoids migrated between these environments. Vegetation on the edges of orchards is a shelter for parasitoids during chemical treatments applied to crops. It is also the place where parasitoids can find alternative hosts. Lush vegetation on the edges of orchards increases the number of parasitoids in orchards and affects the rate of parasitisation of pests.

The results of this study, determining the dates when parasitoids exited the rose tortrix pupae, correspond with the results of an earlier study by Piekarska-Boniecka et al. (2019), in which the researchers investigated the parasitisation of rose tortrixes. The authors also observed the peak of parasitoid flights in late June and they first found parasitoids of the Ichneumonidae family. The results of this study confirmed the date when the flights of parasitoids exiting rose tortrix pupae reached their peak. They also confirmed the fact that parasitoids of the Ichneumonidae family were the first to appear. This phenomenon can be explained by the biology of parasitoids and their hosts as well as the weather conditions in Poland.

CONCLUSIONS

The abundance of the rose tortrix [*Archips rosana* (L.)], which is the dominant leafroller species in apple orchards in the Wielkopolska region, may be limited by parasitoids of the Ichneumonidae, Chalcididae and Tachinidae families. The effectiveness of these parasitoids depends primarily on weather conditions, the number of crop protection treatments and the ecological infrastructure of orchards.

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CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest.

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