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Preliminary observations on cherry (*Prunus avium* L.) infested by the cherry fruit fly (*Rhagoletis cerasi* L.) in protected and unprotected stands

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Abstract: *Preliminary observations on cherry* (Prunus avium *L.*) *infested by the cherry fruit fly* (Rhagoletis cerasi *L.*) *in protected and unprotected stands*. The European cherry fruit fly has great economic importance in Poland and all over the world. The infestation of sweet cherry fruits by its larvae can reach 100%. The aim of the study was to compare the levels of infestation of a wild cherry, as well as the sweet cherry cultivars 'Vanda', 'Summit' and 'Alex' growing in a chemically controlled orchard, and the cultivar 'Regina' in an ecological orchard. The abandonment of chemical control in part of the orchard led to a high level of fruit infestation on unprotected trees. Fruit damage on the late ripening cultivar 'Alex' was 45%, while on the 'Regina', from the ecological orchard, it was 52%. The percentage of infested fruits was similar regardless of the part of the tree crown (bottom, middle or upper) from which the fruits were sampled. The fruit ripening time of the cultivars had a significant impact on the levels of fruit infestation. Late ripening cultivars were infested to a higher degree than mid-ripening cultivars, which is in accordance with the previous observations of other authors. No parasitoids of *Rhagoletis cerasi* larvae were found in either stand. This also refers to the stand of wild cherry, in which the occurrence of the parasitoid *Psytallia carinata* had been noted in the previous year. This research should be continued in more stands, including also the parasitic wasps of *R. cerasi* pupae.

Key words: *Rhagoletis cerasi*, pest management, cherry cultivar, parasitoid

INTRODUCTION

Fruit flies (Tephretidae) are a group of insects of great economic importance all over the world (Smit et al. 2013). This family includes the European cherry fruit fly, *Rhagoletis cerasi*, the most dangerous pest of sweet cherries in Poland and Europe (Maciesiak and Olszak 2009, Daniel and Grunder 2012). It is a univoltine, stenophagic species that infests the fruit of trees and shrubs of the genus *Prunus* sp. and *Lonicera* sp. (White and Elson-Harris 1992).

The biology of the European cherry fruit fly is closely correlated with the host plant's phenology (Zwölfer 1983). The fly overwinters as a pupa in the soil where it undergoes an obligatory diapause, which can be extended to up to 2–3 years (Boller and Prokopy 1976, Vallo et al. 1976). The end of the *R. cerasi* diapause coincides with the end of winter; however, the pupa remains in the postdiapausal stage in the soil until the temperature exceeds 5°C (Moraiti et al. 2014). The emergence of flies takes place after the cherry blossoms, usually just before the ripening of the fruit. After copulation, the females of *R. cerasi* lay single eggs into the fruits and the hatched larvae feed on their pulp, which leads to the rotting and decaying of the fruits. After the period of feeding, the larvae emerge from the fruits and fall into the soil, where they become pupae (Boller 1966b, Daniel and Grunder 2012).

In Poland, there is a constant increase in the *R. cerasi* population in practically all areas of the sweet and sour cherry production. Without proper protection of crops, fruit damage by this pest can reach up to 100% (Fimiani 1983). The European cherry fruit fly poses a severe challenge for fruit growers, especially because market standards regulating the number of infested fruits in a yield are very low. In Poland, this threshold is up to 2%, in other European countries 4%, and in Turkey, for exported fruits this standard is 0% (Kepenekci et al. 2015).

Since 2014, in Poland and other EU countries, all professional users of plant protection products are obliged to follow the principles of integrated pest management (Act of 8 March 2013 on plant protection products). According to this, it is recommended that pesticide treatments are limited, in favour of non-chemical methods. At the same time, the demand for ecological fruit, including sweet cherry fruit from organic orchards, where conventional chemical pest control is not applied, is increasing year by year (Tamm et al. 2004, Rozpara et al. 2010).

The use of insecticides is so far the most effective way to control the European cherry fruit fly (Stamenković et al. 2011). Neonicotinoid insecticides are widely recommended due to their high efficacy in controlling this pest (Olszak and Masiesiak 2004, Stamenković et al. 2012). At the same time, investigations are being conducted on various non-chemical methods, including the use of natural enemies such as entomopathogenic fungi, nematodes, or parasitoids that could reduce the occurrence of *R. cerasi* (Kovanci and Kovanci 2006, Daniel 2009, Daniel and Grunder 2012). There are many species of parasitoids that could be used to control the *R. cerasi* population. So far, more than 20 species of parasitoids of the European cherry fruit fly larvae and pupae have been described (Hoffmeister 1993). The most important species are parasitic wasps *Opius magnus* Fisher (Braconidae), which cause 10–30% pupae mortality (Monako 1984) and *Psytallia carinata* Thoms. (synonyms: *P. rhagoleticola*, *Opius rhagoleticola* and *O. carinata*) (Braconidae), which cause 22–32% mortality of larvae (Łęski 1963, Lux et al. 2016). Both types of parasitoids may occur in commercial orchards as well as on wild cherries or other host plants of *R. cerasi* (Łęski 1963).

The aim of our study was to compare the degree of fruit infestation by *R. cerasi* of wild cherry and of four sweet cherry cultivars in ecological and chemically protected orchards. Simultaneously, the level of fruit infestation was estimated in an orchard in which several tree rows were excluded from chemical protection. The presence of parasitoids of *R. cerasi* larvae in the investigated wild and sweet cherry stands and the parasitization level of the pest were observed.

MATERIAL AND METHODS

Cherry trees and their stands

The observations of fruit infestation by *R. cerasi* were conducted in 2014 on wild cherry and four cultivars of sweet cherry: the mid early ripening 'Vanda' and 'Summit' and the late ripening 'Alex' and 'Regina' (Table 1). The trees grew in three different stands, in which separate observations were made. In all stands, the cherry fruits were collected in the period of their harvest maturity.

TABLE 1. Cherry cultivar characteristics, stands and date of fruit harvest

Cherry tree	Fruit ripening	Stand	Chemical control	Date of harvest
'Vanda'	mid early	Dąbrowice	partial*	7th July
'Summit'	mid early	Dąbrowice	partial*	7th July
'Alex'	late	Dąbrowice	partial*	14th July
'Regina'	late	Nowy Dwór-Parcela	non	14th July
Wild cherry	undertermined	skarpa ursynowska	non	10th July

*The trees grew both in the protected and unprotected part of the orchard.

1. Dąbrowice (near Skierniewice), the Experimental Orchard of the Institute of Horticulture in Skierniewice (Fig.1A.). In this orchard, apart from apple, grapevine, pear, peach and sour cherry trees, several dozens of rows of sweet cherry trees (ca 40 trees per row) of various cultivars were grown. Around the crop, at a distance of about 150–200 m from the sweet cherry trees, some wild cherry trees also grew. This orchard was controlled chemically, but during the year of our observations, ten rows of sweet cherry trees were left unprotected. In the protected part of the orchard two treatments against *R. cerasi* were made, using the neonicotinoid insecticide thiacloprid (Calypso 480 SC) on 27th May, and acetamiprid (Mospilan 20 SP) on 11th June. We compared fruit infestation by *R. cerasi* on the trees of the 'Vanda', 'Summit' and 'Alex' cultivars. They were situated in both protected and unprotected parts of the orchard. In the protected part we examined two, and in the unprotected part, three trees of each cultivar. The examined trees of each cultivar were situated in separate rows except the cultivar 'Vanda' and 'Summit' (protected part of the orchard), which trees grew in the same row, each cultivar on the opposite end of the row (Fig.1A).

2. Nowy Dwór-Parcela (near Skierniewice), the Ecological Experimental Orchard of the Institute in Skierniewice (Fig. 1B). In this orchard, there was no chemical control. The observations were conducted on three trees of 'Regina', situated one by one in one row. Near these trees sour cherries, peaches, apple and pear trees also grew. In the vicinity of the orchard there were also a few trees of wild cherry.

3. Skarpa ursynowska, Warsaw, the campus of the Warsaw University of Life Sciences – SGGW (Fig.1C). In this stand, three trees of wild cherry grew on the slope of the escarpment. The crowns of these trees started from about 150 cm above the ground, while the trees themselves were about 12 m high. During our

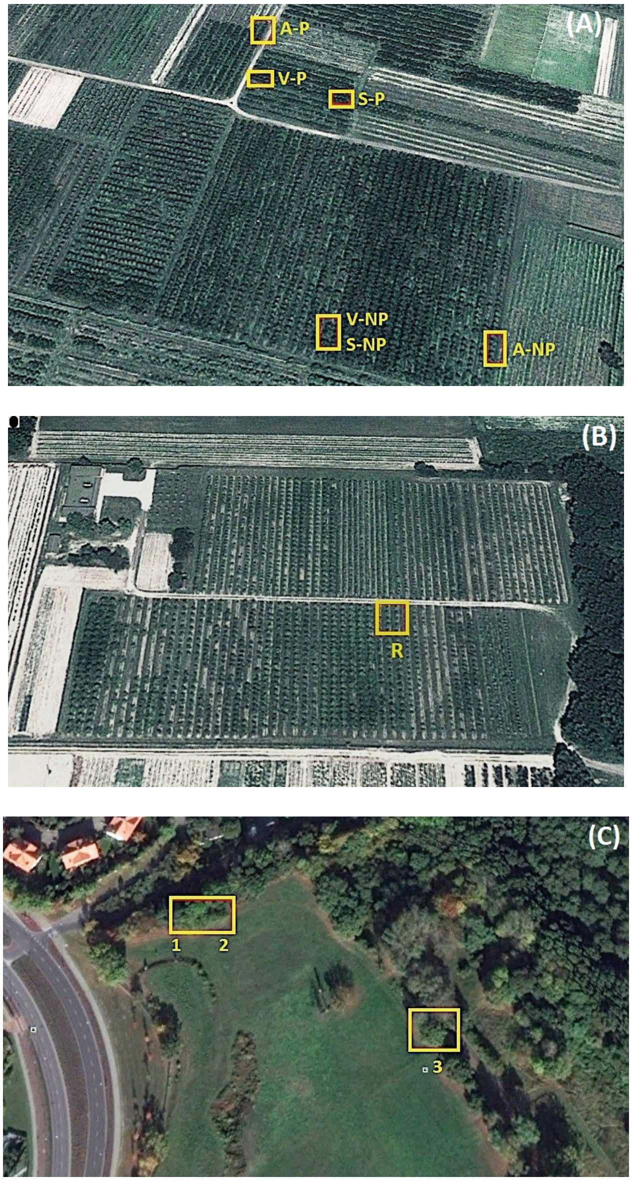

FIGURE 1. Stands of cherry trees, in which the fruit infestation by *Rhagoletis cerasi* was observed. (A) Dąbrowice, (B) Nowy Dwór-Parcela, (C) skarpa ursynowska, Warsaw: A – 'Alex', V – 'Vanda', S – 'Summit'; R – 'Regina'; NP – non-protected stand, P – protected stand; 1, 2, 3 – the number of the wild cherry trees (on the basis of google map)

study, the fruit of these wild cherry trees were ripening at the beginning of July.

Fruit sampling and laboratory tests

From each tree, 150 fruits were collected. They were sampled randomly from three parts of the tree crown – the bottom (starting from the first branch fork), middle and upper parts. From each part of the crown (each ca 1.5 m high), 50 fruits were sampled. The height of the cultivated trees did not exceed 5 m. However, wild cherry trees reach a height of 20 m or more. The fruits from these trees were sampled only from the lower part of the crown, to the height of 5 m from the ground, which corresponded to the height of the cultivated trees of sweet cherry. The samples, 50 fruits each, were packed into separate plastic bags, labelled and transported to the laboratory.

Laboratory tests were conducted in the Department of Applied Entomology at WULS-SGGW, where the fruits were transported. To assess the level of fruit infestation, we followed the method described by Moraiti et al. (2012). Each sample was placed in a Styrofoam box (24.5×14.5×7 cm) filled with a sterile sand layer of ca 1 cm thick (Fig. 2). In the box lid, holes of 5 mm in diameter, 3 mm apart from each other were drilled. The bottom edges of the box lid leaned against the upper edges of the bottom of the box and were additionally glued together with adhesive tape. Fruits were placed onto the box lid and during the following days the larvae came out from the fruits, fell down through the lid holes to the sand and formed pupae.

Seven days after placement of the fruit samples into the boxes, we counted the number of *R. cerasi* pupae in the

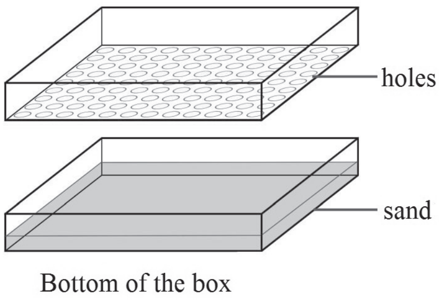

FIGURE 2. Scheme of the box used for the assessment of fruit infestation by *Rhagoletis cerasi*

sand. As some larvae could have still remained inside the fruits, each fruit was additionally cut with a knife to search for the presence of larva inside. During our fruit survey no larvae were found. Therefore, it could be concluded that the number of pupae we collected from the sand reflected the actual level of cherry fruit infestation. The level of fruit infestation was determined as a percentage of the number of pupae that were obtained from the sample of 50 fruits, assuming that only one larva of *R. cerasi* inhabited one fruit.

In order to assess the degree of parasitization of *R. cerasi* larvae by the potential parasitic wasps, the collected pupae were left for the obligatory diapause for the next seven months. They were kept at a temperature of 23°C for 14 days and then transferred to a plant growth chamber (Panasonic MLR-352) set at 4°C and 65% ±5 RH. To initiate the post-diapause development and enclosion, seven months later the boxes were transferred to the plant growth chamber (Panasonic MLR-352) at a temperature of 23°C, and relative humidity of 65% ±5 RH and L16/D8 photoperiod. The emergence of flies and possible parasitoids was examined every morning for 10 days. The enclosed individuals were systematically removed from the boxes.

Statistical analysis

Statistical analysis was performed using the generalized linear models GLM. For the number of infected fruits, Poisson distribution was assumed while for the percentage of fly emergences, the binomial distribution was applied. To compare multiple means, Tukey correction was used. The analysis was performed using the GENMOD function in the SAS 9.4 package. Means are given as ±1 *SE*.

RESULTS

The effect of chemical control on fruit infestation by *R. cerasi*. In the Dąbrowice stand, the percentage of fruit infestation was significantly influenced by chemical control ($\chi2 = 297.81$, $df = 1$, $P < 0.0001$), cultivar ($\chi2 = 95.53$, $df = 2$, $P < 0.0001$) and the interaction between chemical control and the cultivar ($\chi2 = 99.1$, $df = 2$, $P < 0.0001$). Interestingly, there was no effect of the part of the tree crown (bottom, middle or upper) on fruit infestation by *R. cerasi* ($\chi2 = 2.84$, $df = 2$, $P = 0.076$). Similarly, the effects of chemical control × crown part interaction ($\chi2 = 4.01$, $df = 2$, $P = 0.2298$), cultivar × crown part interaction ($\chi2 = 2.09$, $df = 4$, $P = 0.1096$) and chemical control × cultivar × crown part interaction ($\chi2 = 1.65$, $df = 4$, $P = 0.1897$) were all non-significant.

In contrast to the unprotected trees, the protected ones had a very low percentage (0.3%) or no infested fruits ('Alex') – Figure 3. Interestingly, on the protected trees fruit infestation was noted only in the upper part of the tree crown. Only two pupae were obtained

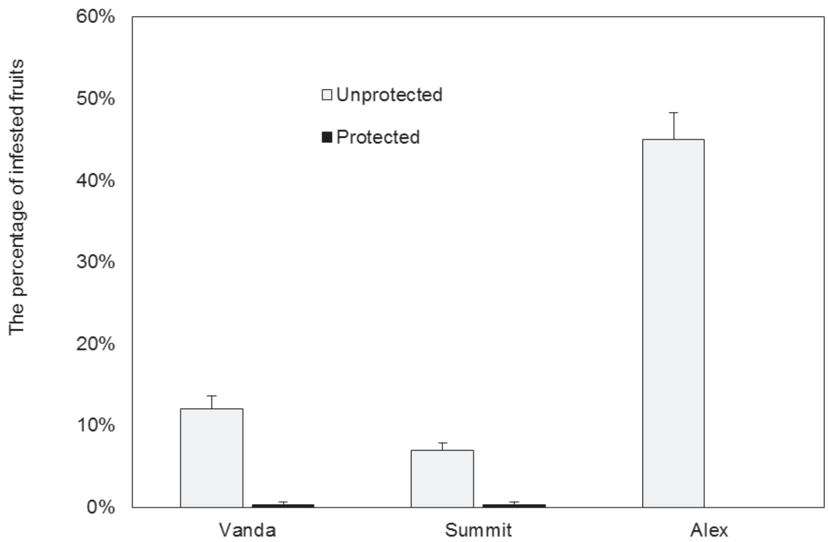

FIGURE 3. The impact of chemical control on the percentage of fruit infestation by *Rhagoletis cerasi* in the experimental orchard in Dąbrowice in 2014

from these trees. After the period of diapause, one deformed individual and one fully developed and vital fly emerged.

The effect of the cherry cultivar on fruit infestation by *R. cerasi.* The comparison of percentages of infested fruits in all unprotected stands revealed that the cherry cultivar has a significant impact on the level of fruit infestation by *R. cerasi* ($\chi2 = 62.76$, $df = 4$, $P < 0.0001$). The highest percentage of fruit infestation (52.4%) was noted for the late ripening cultivars, 'Regina', and (45.1%) 'Alex', while the lowest percentage (6.7%) was noted on the mid early ripening 'Summit' (Fig. 4). There were no significant differences in the degree of fruit infestation between the mid-early ripening cultivars 'Summit' and 'Vanda' ($\chi2 = 1.46$, $P = 0.155$), late ripening cultivars 'Alex' and 'Regina' ($\chi2 = 2.01$; $P = 0.144$) or wild cherry and the mid-early cultivar 'Vanda' ($\chi2 = 1.95$, $P = 0.061$). The remaining comparisons between cherry cultivars were statistically significant.

Parasitization of *R. cerasi* larvae

In this study, no pupae of *R. cerasi* were found to contain parasitoids (Table 2). This was also the case for fruit samples taken from wild cherry trees, where in the last season the parasitic wasp, *P. carinata*, which parasitizes *R. cerasi* larvae, had been noted. In contrast to the parasitic wasps, we recorded the frequent enclosions of *R. cerasi* flies, ranging from 72 to 87% of adults.

Statistical analysis revealed the significant impact of the cultivar on the percentage of fly emergences ($\chi2 = 10.592$, $df = 4$, $P = 0.032$). Post hoc tests have detected a significant difference between the mean percentage of fly emergences from the late ripening cultivar 'Regina' and the mid-early cultivar 'Vanda'.

DISCUSSION

As our study shows, abandonment of chemical control against *R. cerasi* in part

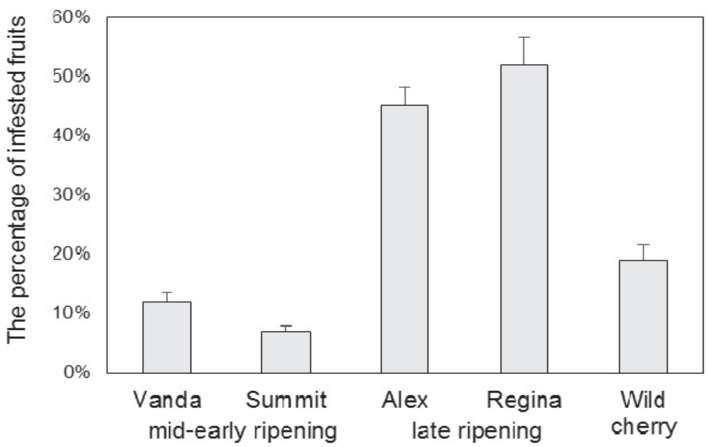

FIGURE 4. The effect of cherry cultivar on the degree of fruit infestation by *Rhagoletis cerasi* in the unprotected stands. 'Vanda', 'Summit' and 'Alex' were located in the unprotected part of the orchard in Dąbrowice. 'Regina' grew in the ecological orchard in Nowy Dwór-Parcela, and wild cherry on skarpa ursynowska in Warsaw

TABLE 2. Mean (±*SE*) percentage of fly and parasitoid emergences from pupae that were obtained from cherry fruits infested by *Rhagoletis cerasi* in 2014

Cultivar	No of pupae collected from all fruit samples	Fly emergence (%)	Parasitoid emergence (%)
'Vanda'	54	87.03 ±0.046 a	0
'Summit'	30	86.67 ±0.062 ab	0
'Alex'	203	80.79 ±0.028 ab	0
Wild cherry	86	76.74 ±0.046 ab	0
'Regina'	236	71.61 ±0.029 b	0

Numbers in columns marked with different bold printed letters are significantly different.

of an orchard can lead to a high level of fruit infestation in unprotected trees. Fruit damage on the late ripening cultivar 'Alex' was over 45% of infested fruits, which was comparable to the level of infestation of another late ripening cultivar, 'Regina' (52%), from the ecological orchard, where no insecticides had been applied for years. Therefore, one may wonder what the potential source of the European cherry fruit fly may be, in an orchard that has been intensively protected against this pest for years. Obviously, some flies could have avoided contact with the insecticide, or the quantity of the sprayed insecticide might have been insufficient to kill the insects. Such a situation might occur when spraying of tree crowns is inaccurate. In our study, fruit infestation on the protected trees was recorded only in the upper parts of the crowns, which during spraying indeed could not have been fully reached by the insecticide. Admittedly, only one vital fly emerged from a total sample of 900 fruits. However, if the fly turned out to be fertile, it could potentially lay eggs into up to 200–300 cherry fruits. According to various authors, under field conditions the fertility of the European fruit fly lies between 30–100 eggs per female, while in the laboratory, it can reach 400 or more eggs laid by one female during her lifetime (Łęski 1963, Boller 1966a). Wild cherry trees growing in the close vicinity of the protected orchard could also have been a source of flies that could infest fruits of unprotected sweet cherry trees. Such a possibility is supported by a previous report by Maciesiak and Olszak (2009). The authors indicate that chemical treatments against *R. cerasi* should be carried out very precisely, and the potential vicinity of unprotected orchards or natural stands of wild cherry and other host plants of *R. cerasi* should also be avoided.

In our study, regardless of the part of the tree crown (bottom, middle or upper) in which fruits were growing, the level of their infestation by *R. cerasi* was similar. This is in accordance with observations by Łęski (1963), who also found that the fly infests cherry fruits uniformly within the tree crown. Our research also confirmed the significant impact of the cherry cultivar on fruit damage by the European cherry fruit fly. Late ripening varieties ('Alex' and 'Regina') were much more heavily infested than the mid-

-early ripening cultivars ('Vanda' and 'Summit'). A similar pattern of infestation of sweet cherry cultivars that differed in their times of fruit ripening has also been found by other authors (Łęski 1963, Banzo et al. 2012). In our study, the level of fruit damage in wild cherry and mid-early cultivars was similar. This is consistent with the phenology and ripening period of these cherries (Łęski 1963).

Our study did not reveal the presence of parasitoids of *R. cerasi* larvae, either in the chemically protected or ecological orchard, or in the stand of wild cherry. Some authors emphasize (e.g. Daniel 2009) that the effectiveness of the parasitoids of *R. cerasi* larvae depends on the size of the cherry fruit. In the case of cultivars with large fruits, the ovipositor of parasitoids may be too short to reach the larva in the fruit and insert an egg into its body. This may also explain why we did not find parasitoids of this type in the fruits of cultivated sweet cherries. This does not exclude, however, the presence of parasitoids of *R. cerasi* that parasitize pupae in the soil, especially in stands with no chemical pest control. As shown by numerous studies, the use of neonicotinoid insecticides generally has a negative effect on the survival of parasitoids (Prabhaker et al. 2011, Gentz et al. 2010). Therefore, the possibility of the occurrence of parasitic wasps in Dąbrowice, both in the protected and unprotected part of the orchard, was rather low.

As opposed to the commercial cultivars of sweet cherry, wild cherry has much smaller fruits, and therefore, parasitoids can parasitize larvae in such fruits (Łęski 1963, Daniel 2009). The lack of parasitoids in wild cherry samples from skarpa ursynowska remains unclear. A year ago, the parasitoid of *R. cerasi* larvae, *P. carinata*, was noted in this stand and caused a fly parasitization of above 30% (Lux et al. 2016). Perhaps we took too small a fruit sample from this stand and/or fruits were collected too late, when the majority of the parasitized larvae of *R. cerasi* had already fallen down into the soil. However, taking into account the results of the laboratory tests on *P. carinata* overwintering (Łęski 1963), it is rather unlikely that the lack of parasitoids was connected with the reduction of their population caused by low temperatures during the winter preceding our study.

In contrast to the parasitic wasps, we recorded frequent enclosions of *R. cerasi* flies from pupae diapausing in the laboratory. The percentage of emergent flies significantly depended on the cherry cultivar. In the case of the late ripening 'Regina', fly emergence was 15% lower than in the case of the mid early ripening 'Vanda'. Under natural conditions, some pupae usually die during the winter diapause, while other individuals (ranging from 15 to even 90% of individuals in a population), may have a prolonged diapause extended to two or more years (Łęski 1963, Moraiti et al. 2014). A similar situation could also have occurred in our study. The fruits of various cultivars may differ in nutrient abundance, which could also, to varying degrees, affect the condition of the tephretid larvae (Papadopolous et al. 2002), and consequently, the mortality of the pupae during the diapause. In insects, prolonged diapause may be beneficial under unstable climatic conditions and low food availability (Saulich 2010, Moraiti et al. 2014, Moraiti and Papadopoulos 2017). In our study, the climatic conditions in all stands, as well as the conditions during diapause, were similar for all *R. cerasi* pupae. However, cherry cultivars differed strongly in their levels of fruit infestation. 'Regina' was the most infested cultivar (52%), while 'Vanda' was infested only to a low degree (12%). It is likely that when the fruit infestation is high (and females have difficulties finding still unoccupied fruits for oviposition) it could be beneficial for the fly to prolong its emergence to the following year in order to avoid potentially high competition for food and a place for offspring development. Thus, in the ecological orchard, the population of *R. cerasi* with a greater percentage of individuals practising prolonged diapause could have evolved as a reserve to ensure survival. This is a very interesting issue, and research on the influence of the sweet cherry cultivar on the degree of prolonged diapause and the mortality of the European cherry fruit fly should be continued.

CONCLUSIONS

1. Even single seasonal abandonment of chemical protection of some trees in an orchard can cause high levels of fruit infestation in these trees by the European cherry fruit fly.
2. *Rhagoletis cerasi* infests cherry fruits similarly within the bottom, middle and upper parts of the tree crown, and the time of fruit ripening of a cherry cultivar has a significant impact on fruit infestation by this pest. The late ripening cultivars 'Regina and 'Alex' were much more heavily infested than the mid early ripening 'Summit' and 'Vanda'.
3. The parasitoids of *R. cerasi* larvae were not found in either the chemically protected orchard or the ecological orchard, or in the natural stands of wild cherry, in which they were noted last year. These studies should be continued, including more stands and also parasitoids of *R. cerasi* pupae.

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Streszczenie: *Wstępne obsewcje występowania nasionnicy trześniówki* (Rhagoletis cerasi *L.*) *na czereśni ptasiej* (Prunus avium *L.*) *w sadach chronionych chemicznie i ekologocznych*. Nasionnica trześniówka ma duże znaczenie ekonomiczne w Polsce i na świecie. Porażenie owoców czereśni przez larwy tej muchówki może sięgać nawet 100%. Celem pracy było porównanie stopnia porażenia przez nasionnicę trześniówkę owoców dziko rosnącej czereśni ptasiej, odmian towarowych czereśni 'Vanda', 'Summit' i 'Alex' w sadzie chronionym chemicznie oraz 'Regina' w sadzie ekologicznym. Pominięcie zabiegów insektycydami w części sadu spowodowało wysoki stopień porażenia owoców niechronionych drzew. Uszkodzenie owoców późno dojrzewającej odmiany 'Alex' osiągnęło wartość 45%, a 52% na odmianie 'Regina' z sadu ekologicznego. Procent porażenia owoców przez nasionnicę był podobny bez względu na to, z jakiej części korony (dolnej, środkowej czy górnej) były zbierane owoce. Wczesność odmiany czereśni miała istotny wpływ na poziom porażenia owoców. Odmiany późne były silniej porażane przez nasionnicę niż odmiany średnio wczesne, co jest zgodne z wynikami innych autorów. W żadnym ze stanowisk nie znaleziono parazytoidów larw nasionnicy trześniówki. Dotyczyło to też stanowiska dzikiej czereśni ptasiej, w którym rok wcześniej odnotowano występowanie parazytoidów *Psytallia carinata*. Badania te powinny być kontynuowane w większej liczbie stanowisk z uwzględnieniem także parazytoidów poczwarek nasionnicy trześniówki.

Słowa kluczowe: *Rhagoletis cerasi*, system ochrony roślin, odmany czereśni, parazytoid

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