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Effect of Aphid Foraging on the Intensity of Photosynthesis and Transpiration of Selected Crop Plants in Its Early Stages of Growing

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Abstract: Plant stress caused by aphid foraging can cause a number of disturbances in its vital functions. The degree of disturbance depends on the duration of the stress factor and its intensity. The aim of this study has been to evaluate the impact of aphids (*Acyrthosiphon pisum* Harris, *Rhopalosiphum padi* L., *Myzus persicae* Sulzer) foraging on plants and the intensity of photosynthesis and transpiration in several crops (*Pisum sativum* L., *Triticum aestivum* L., *Brassica napus* L.). The laboratory experiment conducted for this purpose revealed that aphids feeding on plants affect the course of photosynthesis and transpiration in crops. Statistically significant correlations were determined between the efficiency of these processes and the number of aphids as well as the duration of their foraging. The average values of the intensity of photosynthesis and transpiration in the test crops were found to increase with the increasing number of aphids in a short feeding period (day 5). A longer foraging time (10, 15 days) coincided with a decrease in the intensity of photosynthesis and transpiration. A detailed analysis of the three species of crops demonstrated that the effect of the biotic stressors on the course of the analysed processes could vary, depending on the host plant.

Keywords: Aphidoidea; photosynthesis; transpiration; *Brassica napus* L.; *Pisum sativum* L.; *Triticum* L.; aphids feeding

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1. Introduction

Aphids (Aphidoidea) are a group of sucking phytophages of the order Hemiptera [1–4]. The widespread occurrence of aphids and the significant impact of their foraging on the development of the host plants make aphids one of the most economically dangerous pests in agricultural, horticultural and vegetable crops [5,6]. The harmfulness of aphids consists of the direct, mechanical damage to the plant tissues and ingestion of the primary plant metabolites necessary for their proper development; another problem is that aphids can transfer many viral diseases to plants [7–10]. Moreover, these phytophages affect the photosynthesis of host plants by causing morphological (e.g., a decrease in the assimilation surface due to the deformation of leaves), metabolic (loss of the photosynthetic pigments chlorophyll a and b) and mechanical changes (clogging of stomata with honeydew) [11–14].

The unquestionable role of photosynthesis and its effect on increasing yields have been the basis for numerous studies [15–18]. Araus et al. [18], however, draw attention to the fact that the improvement of photosynthesis in order to raise the yields of crops has stimulated many extensive studies in the past decade, but none of them have produced practical and conclusive results. Owing to scientific progress, it is now possible to fully understand this process and translate this knowledge into greater food security, but only with a multidisciplinary scientific approach [18,19]. Among the multitude of factors that influence photosynthesis, the effect on the process effected by the foraging of phytophages has not been explored completely [20]. Frier et al. [11], based on the observed variability regarding the factors that shape photosynthesis, concluded that foraging by insects tended to have a negative impact on this process, although there were also cases of positive and neutral effects caused by phytophages (i.e., tolerance). The main reason for such discrepancies is identified as the variation of the plant-insect interaction, the intensity of the foraging by insects, sites where insects are foraging on plants, the type of plant tissues damaged, and the way the plants are damaged. The varied effect of *Aphis pomi* on the metabolic processes in *Malus domestica* was demonstrated in a study by Pincebourde and Ngao [21], who also showed a strong correlation between the age of leaves and the impact of the insects on the biochemical processes in the host plant. The negative effect of *Aphis glycines* on photosynthesis in the soybean (*Glycine max*) and of *Rhopalosiphum padi* on wheat (*Triticum aestivum*) has been demonstrated by Macedo et al. [22,23] and Cabrera et al. [24], who analysed the effect of *Schizophis graminum* foraging on *Hordeum vulgare.* Other research shows an increase in the intensity of photosynthesis as a result of the compensatory response of plants to the damage caused to infested tissues [25], for example due to the foraging of aphids (*Tuberolachnus salignus*, *Pterocomma salicis*) on willow (*Salix viminalis*) or by cotton aphid (*Aphis gossypii*) on cotton plants (*Gossypium hirsutum*) [26,27].

The examples given above attest to the high variation in the correlation between phytophages and photosynthesis, largely dependent on the plant species and insect species. Analysis of the influence of phytophages feeding on the most important agricultural crops can aid in the evaluation of the tolerance of crop species to foraging by aphids. Publications dealing with the protection of pea, winter wheat and winter oilseed rape include information on the economic thresholds of the harmfulness of aphids to these plant species. However, these thresholds refer to most plants in later developmental stages, whereas aphids can settle on crops already in the early stages of plant development and may affect their growth and development in a significant manner. Hence, in this experiment we attempted to make an evaluation of the abundance of aphids growing on plants from the 4- to 6-leaf stage in order to determine how the number of these insects adversely affected the processes of transpiration and photosynthesis in plants, and which would finally cause a decrease in their yields.

Identifying the number of aphids which adversely affects plant transpiration and photosynthesis may be a contribution to further research on the impact of these insects on the yield of the crops studied. The experiment was conducted on cultivars of the crops that are most commonly grown in Poland [28]. Wheat (*Triticum* L.) belongs to the principal crops typically of the moderate climate zone, and in Poland it is cultivated on around 20% of all arable land, which makes it one of the most common crops in this country [29]. The most popular oil crop is oilseed rape (*Brassica napus* L.). Poland is one of the biggest producers of this crop in Europe [30]. Among grain legumes, pea (*Pisum sativum* L.) is the plant that is cultivated over the largest area of arable land in Poland [31].

The aim of this study was to determine the effect of the abundance of and the duration of foraging by pea aphids *Acyrthosiphon pisum* Harris, bird cherry aphids *Rhopalosiphum padi* L. and peach aphid *Myzus persicae* Sulzer on the processes of photosynthesis and transpiration of plants in early developmental stages. The test plants were: peas (*Pisum sativum* L.), winter wheat (*Triticum aestivum* L.) and winter rapeseed (*Brassica napus* L.).

2. Materials and Methods

2.1. Plants

The experiment included three plant species:

- pea cv. Milwa—a cultivar registered in Poland, in 2005 (Plant Breeding Smolice zp. z o. o., IHAR group). This is a cultivar which matures early and uniformly, suitable for cultivation throughout Poland. There are no data on its vulnerability to foraging by aphids;
- winter what cv. Opal—a cultivar registered in Germany, in 2021 (Syngenta Seeds). This is a cultivar with high baking quality, tolerant to septoria leaf blotch, DDt, fusarium

head blight, and septoria ear blotch. It is suitable for early and late sowing. There are no data regarding its tolerance to aphid foraging;
- winter oilseed rape cv. SY Florida—a hybrid cultivar (Syngenta Crop Protection AG; registered in Poland in 2015). This is a cultivar with high yield potential, suitable for cultivation throughout Poland. It is characterised by high autumn vigour. The yields are 15–22% higher than those of the reference cultivar (post-registration cultivar experiment 2014–2016, 2019). This cultivar is resistant to blackleg of Brassica and stalk break. There are no data regarding its tolerance to aphid foraging.

The plant species and cultivars were selected for their suitability as host plants for the analysed aphid species. Seeds for the plants were provided by the University of Warmia and Mazury in Olsztyn, from the Bałcyny Plant Production and Experimental Station The plants were grown in a SANYO MLR 35O-H air-conditioned chamber under constant thermal (18 °C) and humidity conditions (relative air humidity 70%), with an L14: D10 photoperiod.

2.2. Aphids

Aphids came from mass cultures, maintained in SANYO MLR 351-H phytotron chambers (temperature 20 °C, relative air humidity 70%, photoperiod L16: D8). Pea aphids (*Acyrthosiphon pisum* Harris) were grown in the laboratory of the Chair of Entomology, Phytopathology and Molecular Diagnostics at the University of Warmia and Mazury in Olsztyn. Peach aphids (*Myzus persicae* Sulzer) and bird cherry-oat aphids (*Rhopalosiphum padi* L.) were grown in the laboratory of the Chair of Botany and Ecology at the University of Zielona Góra. *A. pisum* was cultured as a multi-clone colony on the *Pisum sativum* L., Hubal; *R. padi* was cultured as a multi-clone colony on the *Triticum aestivum* L., Bamberka; *M. persicae* was cultured as a multi-clone colony on the *Brassica rapa* subsp. Pekinensis (Lour.) Hanelt.

Aphids have been cultured since 2008. In order to maintain the vitality of colonies, wingless aphids were transferred to non-infected plants every other week. The transfer of aphids to new plants consisted of cutting of aphid-infested leaves and placing them on new plants, in order to enable aphids to move to fresh plants.

2.3. Measurements of the Intensity of Photosynthesis and Transpiration

The entomological material (*A. pisum*- AP, *R. padi*- RP, *M. persicae*- MP) was placed on the plants at the 4–6 leaf stage (4 weeks after germination). Observations of each plant (pea, wheat and oilseed rape) were carried out in four variants in 10 replications each:

- control: without aphids (AP_K; RP_K; MP_K);
- 5 wingless females (AP_5; RP_5; MP_5);
- 10 wingless females (AP_10; RP_10; MP_10);
- 15 wingless females (AP_15; RP_15; RP_15).

The number of aphids throughout the experiment was always the same. In each variant, the number of aphids was monitored daily, and newly hatched aphids were removed. Each plant was isolated in a plastic cylinder with a fine mesh on top.

The plants were placed in a SANYO MLR 35O-H phytotron chamber with constant settings (temperature 20 °C, relative humidity of air 70%, photoperiod: L14: D10). Measurements of the intensity of photosynthesis and transpiration were made at equal time intervals. The first observation took place 5 days after placing the aphids on the plants, and it was followed by subsequent observations after 10 and 15 days.

Measurements of the intensity of photosynthesis and transpirations were made with a LI-COR 6400 (DMP AG SA LTD) portable gas analyser. The parameters were measured at a constant CO_2 concentration of 400 ppm, and light 1000 μmol m^{-2} s^{-1}. The source of photons consisted of a LED Light Source lamp, emitting light with a spectrum of the main peak concentrated in the 670 mm band and the minor peak of 465 nm. The measurements were made on the youngest, fully developed, randomly selected leaves, repeating each measurement 5 times. The results of the research show the average values of the intensity of photosynthesis and transpiration for each combination.

2.4. Statistical Analysis

In order to determine the relationship between the intensity of transpiration and photosynthesis versus the number of foraging specimens of the analysed aphid species (in the set time intervals), Pearson's linear correlation *r* coefficient was calculated. The foraging of the three aphid species on the selected host plants was analysed by evaluating the intensity of photosynthesis and transpiration. The average values of these parameters obtained in this study were submitted to an assessment of the distribution of data using the W Shapiro-Wilk test. Differences between the means characterised by unimodal distribution were evaluated using the Generalized Linear Model GLM, including the Gamma type of data distribution. Data with normal distribution were assessed using the single-factorial ANOVA test. Groups of means describing the intensity of photosynthesis and transpiration that did not differ statistically significantly were labelled with the same letter index: a, b or c (Tukey's HSD test). Statistical calculations and their visual presentation were performed in Statistica 13.1 (Dell Inc., Tulusa, OK, USA).

3. Results

In order to determine the relationship between the intensity of photosynthesis and transpiration in plants on which different numbers of individuals of the analysed aphid species were foraging, the Pearson linear correlation *r* was computed (Table 1). In the case of *A. pisum*, a significant positive correlation ($r = 0.92$) was found between the increase in the number of feeding aphids and the intensity of photosynthesis on the 5th day of the experiment (Table 1). After 10 and 15 days, it was found that the increase in the number of foraging individuals of this species was correlated with a significant decrease in photosynthesis (10 days, $r = -0.55$; 15 days, $r = -0.85$). The same relationship was observed for *A. pisum* in the case of transpiration intensity. The increase in the number of foraging *R. padi* individuals after 5 and 10 days was significantly, negatively correlated ($r = -0.88$; $r = -0.88$, respectively) with the intensity of photosynthesis. On the 15th day of the experiment, this relationship was positive ($r = 0.32$) but was not statistically significant (Table 1). In the case of the transpiration process, an increase in the number of feeding aphids caused a significant decrease in this process only on the 5th day of the experiment ($r = -0.66$). As the foraging time of *R. padi* increased, the transpiration coefficient was positive (10 days, $r = -0.76$; 15 days $r = 0.29$). The increasing number of foraging *M. persicae* individuals was correlated with an increase in the intensity of photosynthesis, but significant *r* values were recorded only on the 5th and 15th day of the experiment ($r = 0.86$, $r = 0.59$, respectively). In the case of transpiration, a negative correlation was found between the increase in the number of feeding aphids on the 5th and 10th day of the experiment and the intensity of this process. After 15 days, this relationship became positive ($r = 0.61$) (Table 1).

The effects of aphid foraging on the intensity of photosynthesis and transpiration were analysed on peas (*A. pisum*), winter wheat (*R. padi*) and oilseed rape (*M. persicae*). Data showing the values of the analysed parameters determined for the three plant species showed different types of distribution (normal and unimodal). Depending on the model of distribution, the significance of differences between means from the different combinations was tested with the generalised linear model (GLM) or with the single-factor ANOVA (Table 2).

The results of our analyses indicate that differences in the intensity of photosynthesis and transpiration of the test plants, at different time intervals (from 5th to 15th day of observations) were statistically significant (Table 2).

Analysis of the impact of the tested variables (time of foraging, number of aphids) on the ongoing metabolic processes in peas demonstrated an increase in the intensity of photosynthesis in the first series of observations (day 5) as the number of foraging insects increased (Figure 1). The intensity of photosynthesis in plants with 5 foraging

A. pisum individuals was higher (6.93 µmol CO_2 m^{-2} s^{-1}) than in the control plants (6.81 µmol CO_2 m^{-2} s^{-1}). Higher values of this parameter were also noted in plants with 10 individuals (10.23 µmol CO_2 m^{-2} s^{-1}) and 15 individuals (10.74 µmol CO_2 m^{-2} s^{-1}) (Figure 1).

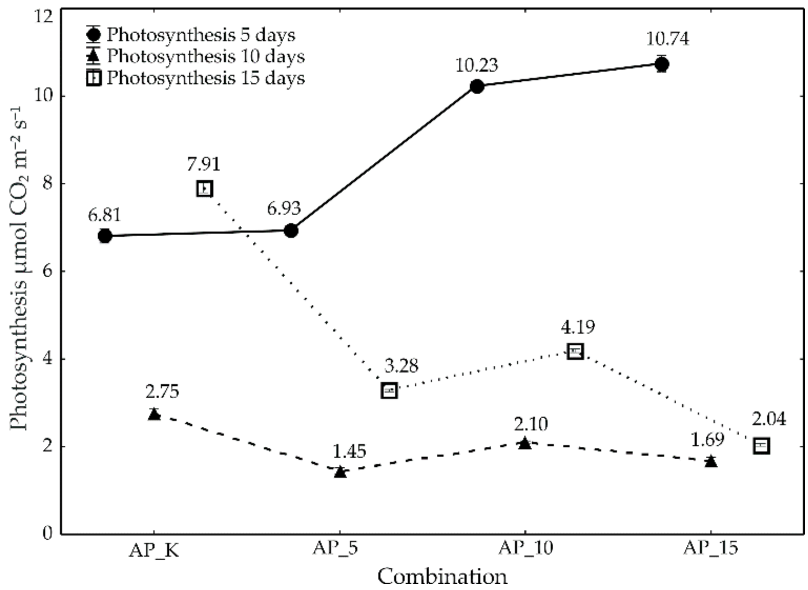

Figure 1. Intensity of the course of photosynthesis in the set time intervals in peas with different numbers of foraging aphids of *A. pisum*. Vertical lines at mean values indicate SE.

Table 1. Values of the Pearson linear correlation coefficient *r* for the intensity of photosynthesis and transpiration and for the number of foraging aphids in the set time intervals (N = 20).

Combination	**Number of Aphids**	
	r	***p* ***
	Pea/*A. pisum*	
Photosynthesis 5 days	0.92	0.0
Photosynthesis 10 days	−0.55	0.0
Photosynthesis 15 days	−0.85	0.0
Transpiration 5 days	0.77	0.0
Transpiration 10 days	−0.99	0.0
Transpiration 15 days	−0.65	0.0
	Cereal/*R. padi*	
Photosynthesis 5 days	−0.88	0.0
Photosynthesis 10 days	−0.88	0.0
Photosynthesis 15 days	0.32	n.s. **
Transpiration 5 days	−0.66	0.0
Transpiration 10 days	0.76	0.0
Transpiration 15 days	0.29	n.s.
	Rape/*M. persicae*	
Photosynthesis 5 days	0.86	0.0
Photosynthesis 10 days	0.03	n.s.
Photosynthesis 15 days	0.59	0.0
Transpiration 5 days	−0.72	0.0
Transpiration 10 days	−0.36	n.s.
Transpiration 15 days	0.61	0.0

* The value of the test probability *p* ** n.s. (not statistically significant; $p > 0.05$).

Table 2. Results of statistical tests (GLM, ANOVA) for the intensity of photosynthesis and transpiration in peas (*A. pisum*), winter wheat (*R. padi*) and winter oilseed rape (*M. persicae*).

Combination	df	Wald's Statistic	ANOVA F Value	*p*
		Pea—*A. pisum*		
Photosynthesis 5 days	3	761.54	-	0.00
Photosynthesis 10 days	3	-	59.22	0.00
Photosynthesis 15 days	3	7985.11	-	0.00
Transpiration 5 days	3	938,143.00	-	0.00
Transpiration 10 days	3	157,622.00	-	0.00
Transpiration 15 days	3	1,202,718.00	-	0.00
		Winter wheat—*R. padi*		
Photosynthesis 5 days	3	331.46	-	0.00
Photosynthesis 10 days	3	-	29.48	0.00
Photosynthesis 15 days	3	-	49.10	0.00
Transpiration 5 days	3	20,362.77	-	0.00
Transpiration 10 days	3	28,182.23	-	0.00
Transpiration 15 days	3	21,630.20	-	0.00
		Winter oilseed rape—*M. persicae*		
Photosynthesis 5 days	3	57.68	-	0.00
Photosynthesis 10 days	3	93.70	-	0.00
Photosynthesis 15 days	3	11.62	-	0.00
Transpiration 5 days	3	-	602.6	0.00
Transpiration 10 days	3	2637.92	-	0.00
Transpiration 15 days	3	-	784.38	0.00

On day 10 and 15 of observations, a decrease in the intensity of photosynthesis occurred relative to the control plants. After 10 days, the intensity relative to the control sample (2.75 μmol CO_2 m^{-2} s^{-1}) decreased in each variant of the number of aphids placed on the plants although these values were similar (5 aphid individuals: 1.45 μmol CO_2 m^{-2} s^{-1}, 10 aphids: 2.10 μmol CO_2 m^{-2} s^{-1}, 15 aphids 1.69 μmol CO_2 m^{-2} s^{-1}) (Figure 1). The foraging by aphids had a distinctly negative effect on the level of photosynthesis 15 days after the experiment was launched. In the last series of observations, the intensity of this process decreased (compared to the control: 7.91 μmol CO_2 m^{-2} s^{-1}) when 5 aphids (3.28 μmol CO_2 m^{-2} s^{-1}), and 10 aphids were feeding on the plants (4.19 μmol CO_2 m^{-2} s^{-1}), and the lowest intensity of photosynthesis was recorded in plants on which 15 aphids were feeding (2.04 μmol CO_2 m^{-2} s^{-1}). During the last observations (day 15), the photosynthesis intensity indicator was higher than achieved after 10 days of the foraging of pea aphids on pea, but lower than obtained during the first series of observations (day 5). The lowest value of photosynthesis in peas was observed in the variant with 5 individuals of *A. pisum* foraging for 10 days (1.45 μmol CO_2 m^{-2} s^{-1}), while the highest value was obtained in the variant with 15 aphids feeding for five days (10.74 μmol CO_2 m^{-2} s^{-1}) (Figure 1). The Tukey HSD test was run to group the means which identified the intensity of photosynthesis at different time intervals with different numbers of foraging aphids and did not differ significantly (Table 3). The level of photosynthesis of the pea plants infested with aphids differed from the control in all the variants except the first series of observations (after 5 days of foraging) and the smallest number of aphids (5 individuals).

Evaluation of the influence of the population variables (5, 10, 15 individuals of *R. padi*) on the course of photosynthesis in winter wheat cv. Opal revealed a tendency towards the decreasing efficiency of photosynthesis with the increasing number of foraging aphids. The lower rate of photosynthesis was observed at all the set time intervals except the last observations (day 15), where 15 individuals of *R. padi* were foraging on wheat plants. The foraging of the bird cherry-oat aphid most severely limited the intensity of photosynthesis after 5 days (Figure 2).

Table 3. Mean values of the parameters of photosynthesis and transpiration in the test plants during the foraging by aphids.

Combination	Number of Aphids							
	Control		5 Aphids		10 Aphids		15 Aphids	
			Pea—*A. pisum*					
Photosynthesis 5	6.81	a *	6.93	a	10.23	b	10.74	b
Photosynthesis 10	2.75	c	1.45	a	2.10	b	1.69	a
Photosynthesis 15	7.91	d	3.28	b	4.19	c	2.04	a
Transpiration 5	3.71	b	3.46	a	3.69	b	7.98	c
Transpiration 10	3.42	d	2.79	c	2.42	b	1.65	a
Transpiration 15	7.69	d	1.76	a	2.92	c	2.86	b
			Winter wheat—*R. padi*					
Photosynthesis 5	3.32	c	1.32	b	1.12	b	0.77	a
Photosynthesis 10	3.17	b	2.62	b	1.59	a	1.54	a
Photosynthesis 15	1.94	b	1.30	a	1.45	a	2.28	c
Transpiration 5	0.72	d	0.31	b	0.54	c	0.29	a
Transpiration 10	0.77	b	0.52	a	0.85	c	1.31	d
Transpiration 15	3.79	b	2.47	a	4.01	c	3.81	b
			Oilseed rape—*M. persicae*					
Photosynthesis 5	0.25	a	0.39	ab	0.52	b	1.02	c
Photosynthesis 10	0.24	a	0.97	c	0.54	b	0.40	b
Photosynthesis 15	0.29	a	0.41	ab	0.47	b	0.45	b
Transpiration 5	3.10	c	2.43	b	2.32	a	2.48	b
Transpiration 10	2.81	d	1.71	a	2.02	b	2.27	c
Transpiration 15	1.43	b	1.02	a	1.55	c	1.72	d

* Means in rows followed by the same letter do not differ (Tukey's HSD test).

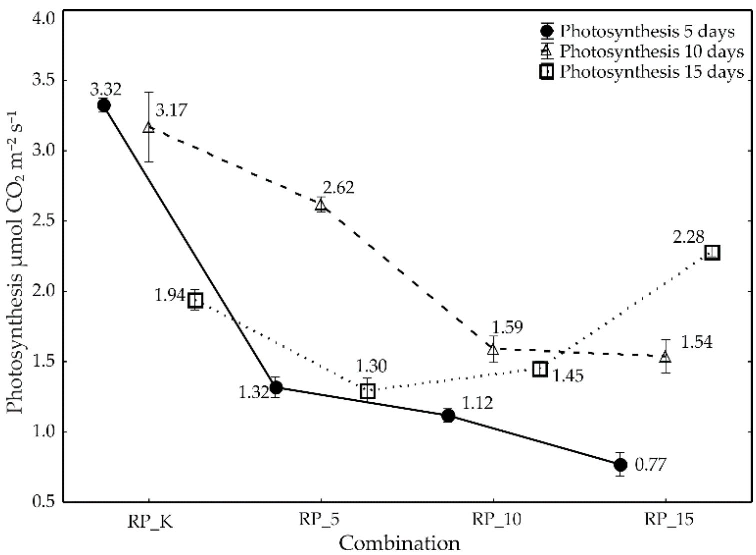

Figure 2. Intensity of photosynthesis at the set time intervals in winter wheat with different numbers of foraging aphids of *R. padi*. Vertical lines at mean values indicate SE.

The photosynthesis of plants on which aphids were feeding was reduced in comparison with the control plants (3.32 µmol CO_2 m^{-2} s^{-1}) when 5 (1.32 µmol CO_2 m^{-2} s^{-1}), 10 (1.12 µmol CO_2 m^{-2} s^{-1}) and 15 individuals (0.77 µmol CO_2 m^{-2} s^{-1}) of *R. padi* were feeding. On the fifth day of the experiment, both the highest (control: 3.32 µmol CO_2 m^{-2} s^{-1}) and the lowest photosynthesis rate (during the foraging of 5 individuals of *R. padi*: 0.77 µmol CO_2 m^{-2} s^{-1}) were observed (Figure 2). Moreover, the post-hoc Tukey test revealed homogenous clusters relative to the intensity of photosynthesis between the control and the variant with 5 aphids on the 10th day of the experiment (Table 3).

The results of the measurements of photosynthesis in winter oilseed rape damaged by *M. persicae* and the control demonstrated a reverse tendency to the one induced by the foraging of bird cherry-oat aphids. The research results revealed the stimulation of the intensity of photosynthesis as increasing numbers of aphids were placed on the plants. Photosynthesis on the fifth day of observations increased successively (control: 0.25 µmol CO_2 m^{-2} s^{-1}, 5 aphids: 0.39 µmol CO_2 m^{-2} s^{-1}, 10 aphids: 0.52 µmol CO_2 m^{-2} s^{-1}, 15 aphids: 1.02 µmol CO_2 m^{-2} s^{-1}). A similar tendency was observed on the 15th day of the experiment, except for a small decrease when 15 aphids were feeding on the plants (control: 0.29 µmol CO_2 m^{-2} s^{-1}, 5 aphids: 0.41 µmol CO_2 m^{-2} s^{-1}, 10 aphids: 0.47 µmol CO_2 m^{-2} s^{-1}, 15 aphids: 0.45 µmol CO_2 m^{-2} s^{-1}). The intensity of photosynthesis in the control group remained similar throughout the entire experiment (0.25, 0.24, 0.29 µmol CO_2 m^{-2} s^{-1} on day 5, 10 and 15, respectively). The intensity of photosynthesis in the control samples during each observation (day 5, 10 and 15) was lower than the corresponding values obtained from plants on which 5, 10 and 15 individuals of peach aphid were feeding (Figure 3), although the Tukey HSD test showed a statistically significant similarity on day 5 and 15 of the experiment between the control and the plants with 5 foraging individuals of *M. persicae* (Table 3).

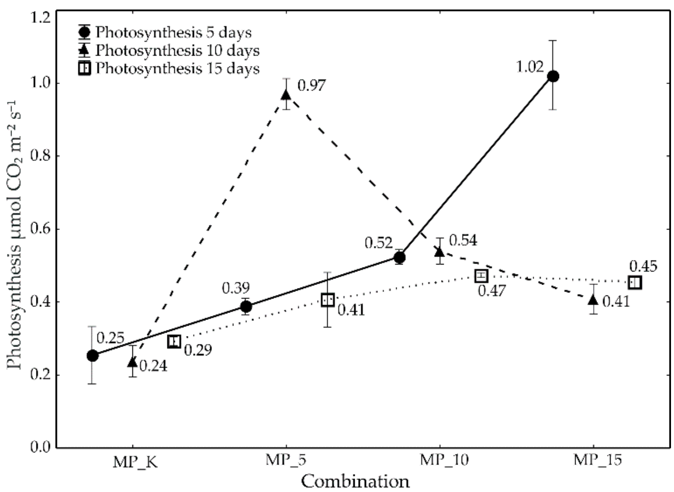

Figure 3. Intensity of the course of photosynthesis in the set time intervals in oilseed rape with different numbers of foraging aphids of *M. persicae*. Vertical lines at mean values indicate SE.

The intensity of transpiration occurring in the three plant species (pea, wheat, oilseed rape) exposed to foraging by aphids was measured throughout the whole experiment. The results of statistical tests revealed a statistically significant effect of the foraging by *A. pisum*, *R. padi* and *M. persicae* on the transpiration (Table 2). Moreover, the intensity of this process in the pea plants at the set time intervals showed (as in the case of photosynthesis) an increase on day 5 and a decrease on days 10 and 15. The only difference relative to the intensity of photosynthesis was a slight decrease on the fifth day of foraging by 5 aphids (3.46 µmol CO_2 m^{-2} s^{-1}) and by 10 aphids (3.69 µmol CO_2 m^{-2} s^{-1}) relative to the control plants (3.71 µmol CO_2 m^{-2} s^{-1}) (Figure 4). The value of transpiration on the fifth day of foraging by 15 *A. pisum* individuals (7.98 µmol CO_2 m^{-2} s^{-1}) was the highest of all the values obtained in the experiment. The lowest value of this parameter occurred on the 10th day of foraging by 15 *A. pisum* individuals (1.66 µmol CO_2 m^{-2} s^{-1}) (Figure 4). On the fifth day, the mean values of the intensity of transpiration tested using the Tukey HSD showed a lack of statistically significant differences between the control and the plant with 10 aphids (Table 3). On day fifteen, the biggest decrease in transpiration relative to the control (7.69 µmol CO_2 m^{-2} s^{-1}) was noted when 5 aphids were feeding on the plant (1.76 µmol CO_2 m^{-2} s^{-1}), and a slightly smaller decrease appeared in the plants with 10 (2.92 µmol CO_2 m^{-2} s^{-1}) and with 15 individuals of *A. pisum* (2.86 µmol CO_2 m^{-2} s^{-1}) (Figure 4).

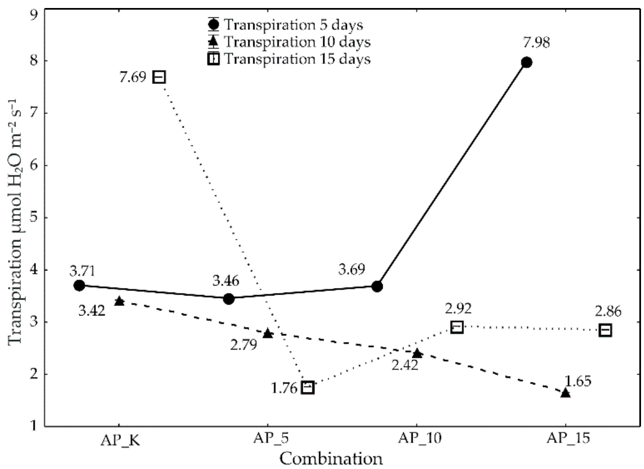

Figure 4. Intensity of transpiration in the set time intervals at pea plants with different numbers of foraging aphids of *A. pisum*. Vertical lines at mean values indicate SE.

The highest level of transpiration in wheat exposed to *R. padi*, in all the quantitative variants of aphids, was achieved on the 15th day of the experiment (Figure 5). During the last series of observations, transpiration was lower relative to the control (3.79 µmol CO_2 m^{-2} s^{-1}) due to the foraging by 5 bird cherry-oat aphids (2.47 µmol CO_2 m^{-2} s^{-1}) but was higher in plants with 10 aphids (4.01 µmol CO_2 m^{-2} s^{-1}) and with 15 individuals of *R. padi* (3.81 µmol CO_2 m^{-2} s^{-1}) (Figure 5). The multiple comparison test demonstrated lack of significant differences on that day between the control and the sample with 15 aphids (Table 3). On day 5, when 5 aphids were feeding on the plants, a decrease was observed in the intensity of transpiration (0.31 µmol CO_2 m^{-2} s^{-1}), which was higher in plants with 10 individuals of *R. padi* (0.54 µmol CO_2 m^{-2} s^{-1}), but again slightly lower due to the foraging by 15 aphids (0.29 µmol CO_2 m^{-2} s^{-1}) (Figure 5).

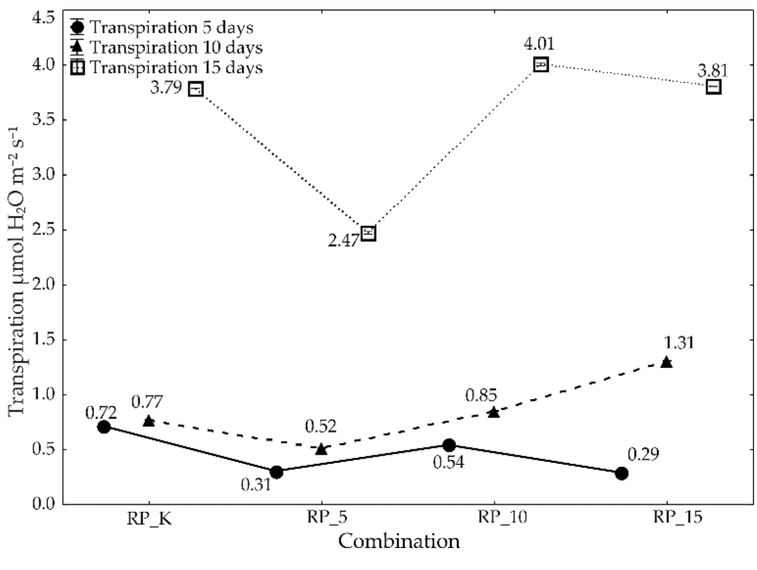

Figure 5. Intensity of transpiration at the set time intervals in winter wheat with different numbers of foraging aphids of *R. padi*. Vertical lines at mean values indicate SE.

Values for the transpiration rate on the 5th and 10th day of the study in control samples (0.72 µmol CO_2 m^{-2} s^{-1}, 0.77 µmol CO_2 m^{-2} s^{-1}) and in plants with 5 (0.31 µmol CO_2 m^{-2} s^{-1}, 0.52 µmol CO_2 m^{-2} s^{-1}) and 10 aphids (0.54 µmol CO_2 m^{-2} s^{-1}, 0.85 µmol CO_2 m^{-2} s^{-1}) were similar. The highest transpiration level was on the 15th day, in the sample with 10 aphids

(4.01 µmol CO_2 m^{-2} s^{-1}); the lowest level was on the 5th day, in plants with 15 foraging bird cherry-oat aphids (0.29 µmol CO_2 m^{-2} s^{-1}) (Figure 5).

The values for the transpiration rate in winter oilseed rape infested with peach aphids showed that during the first two series of observations (day 5 and 10) there was a decrease relative to the control (3.10 µmol CO_2 m^{-2} s^{-1}, 2.81 µmol CO_2 m^{-2} s^{-1}) in the intensity of transpiration in all experimental variants (Figure 6).

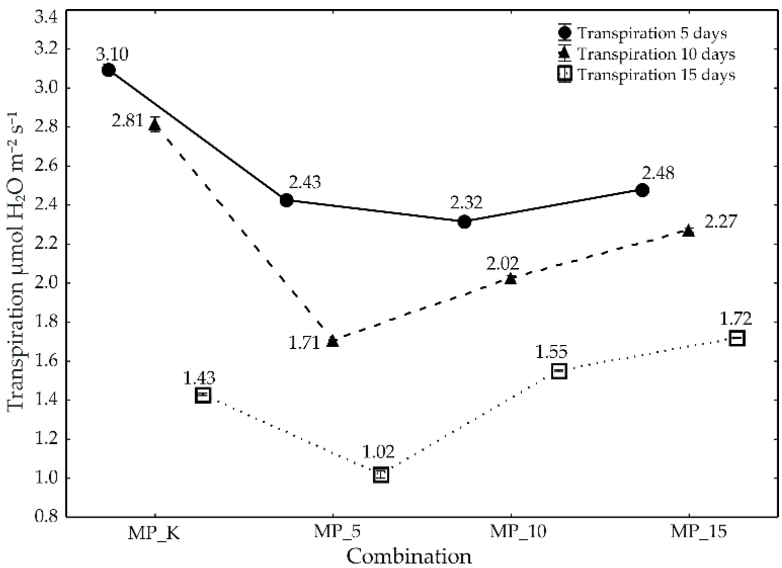

Figure 6. Intensity of transpiration in the set time intervals in oilseed rape with different numbers of foraging aphids of *M. persicae*. Vertical lines at mean values indicate SE.

After 15 days of foraging by aphids, the rate of this process decreased (compared with the control; 1.43 µmol CO_2 m^{-2} s^{-1}) in the sample with the smallest number of aphids (1.02 µmol CO_2 m^{-2} s^{-1}) but was higher in plants with 10 (1.55 µmol CO_2 m^{-2} s^{-1}) and 15 aphids (1.72 µmol CO_2 m^{-2} s^{-1}) (Figure 6). The intensity of transpiration was the lowest on day 15 in plants infested with 5 aphids (1.02 µmol CO_2 m^{-2} s^{-1}), and the highest in the control sample on day 5 (3.10 µmol CO_2 m^{-2} s^{-1}). Transpiration in pea plants occurred with different intensity to oilseed rape at the analysed time intervals. The effects of the analysed stress factors (number of aphids, time of observations) varied statistically significantly (Table 3).

4. Discussion

The dynamics of agricultural production depends on many factors, including the important role played by phytophagous insects. It is widely acknowledged that the importance of these insects in agricultural ecosystems has been quite thoroughly investigated [32–35]. However, the damage caused by plant-eating insects is most often studied in the context of lesions in plant tissues or transmission of viruses [36,37]. The role of such insects in initiating metabolic processes in other, intact tissues is often neglected [21,38,39]. Pest foraging stress can induce plant defense responses, including changes in developmental, biochemical and physiological processes [40]. One such defense response to environmental stress is increasing the intensity of photosynthesis [41]. Moreover, the available research provides many contradictory results concerning the influence of herbivorous pest insects on the physiology of crops, and in particular such processes as photosynthesis and transpiration. The impact of phytophagous insects on gas exchange in plants has been analysed by Walter [25] and Pincebourde and Casas [38], who demonstrated that the effects mentioned were highly variable and dependent on a specific insect-plant interaction.

Aphids are among the most dangerous crop pests, able to cause large losses in crop yields, which has a direct impact on the economics of production [6,7,42]. Our study has shown a statistically significant character of the influence of foraging by aphids (*A. pisum*, *R. padi*; *M. persicae*) on photosynthesis and transpiration of the test plants: pea, winter wheat, and winter oilseed rape (Tables 1 and 2). The analysis of the impact of biotic stressors on the two processes attests to the variability of these parameters, which largely depend on the plant's individual response to the pest (Figures 1–6). Pincebourde and Ngao [21] draw attention to the fact that the influence of insects on the metabolic process is also affected by the plant's age. This study has demonstrated that the duration of foraging by aphids (5, 10 and 15 days) has a significant effect on the metabolic processes occurring in host plants (Table 1; Figures 1–6).

Trumble et al. [43] point out the ability of plants to compensate for the damage caused by foraging insects. The plant's injured photosynthetic tissues activate defence mechanisms, thereby improving the metabolic processes in order to compensate for the damage caused by phytophages [44]. Consequently, the induction of the compensation mechanism can stimulate, to a certain extent, the metabolic processes, thus increasing the intensity of photosynthesis or transpiration due to the foraging by insects [39,45–47]. The above conclusion is confirmed by the results of our experiment. A higher level of photosynthesis was observed on day 5, and the highest value was noted in plants with 15 foraging aphids. During a longer period of foraging by aphids, the efficiency of photosynthesis decreased (Figures 1–3). An exception was the photosynthesis in plants infested with bird cherry-oat aphids, where a successive decrease in the intensity was observed on day 5 and 10 as the number of foraging aphids increased. However, on day 15 of the experiment, the highest intensity of photosynthesis was achieved in cv. Opal winter wheat with 15 foraging aphids per plant (Figure 2). This may have been related to the low sensitivity of this cereal cultivar to foraging by *R. padi*. A similar tendency was noted during the observations of transpiration. The highest values of this parameter on the 5th day of the experiment were observed when the highest number of aphids (15 individuals) were foraging on the plant (Figures 1–3). However, for this process, the intensity values obtained in the plants with 5 and 10 aphids were lower than in the control, and the highest transpiration was noted in the control on day 15 of the study (Figures 1–3).

Pincebourde and Ngao [21] verified that in the early phase of the plant-insect interaction, these factors stimulate each other, as a result of which there is an increase in the metabolic efficiency of the plant and a growth in the population of the pest. A positive effect of phyophages on the metabolic processes in plants has been questioned by Zvereva et al. [48], who observed a tendency for the foraging by insects to have a negative effect on the plant's metabolic processes, which influenced the yield potential of the crops. A lowered intensity of photosynthesis and transpiration in response to the prolonged foraging by aphids has also been reported by Goszczyński [49] in apple trees, Macedo et al. [22] in soybeans, Kordan [7] in yellow lupines, Tomczyk and Wróblewski [12] in black currants and highbush blueberries. The variable nature of the interaction between phytophages and the host plant's metabolic processes has been demonstrated in studies conducted by other researchers, including Welter [25] and Pincebourde and Ngao [21]. Considering the relationships between the mean values of the two parameters, photosynthesis and transpiration, obtained in our experiment, it was demonstrated that the results were statistically significant but were of a variable character when analysing each of the three crop species (Figures 1–6; Table 2). The results showed that aphid feeding in the early stages of plant development may disturb the intensity of photosynthesis and transpiration, and the level of these changes depends on the species of the plant and the aphid.

5. Conclusions

One of the plant responses to environmental stress is to increase the intensity of photosynthesis. However, it should be emphasized that the activation of compensatory mechanisms in the plant depends on the number and feeding time of the aphids. In the face of discrepant results on the pressure of phytophagous insects on such processes as photosynthesis and transpiration, it is crucial to continue studies which will allow us to gain a better insight into these mechanisms. This study has shown that the intensity of photosynthesis and transpiration in the early phase of aphid foraging was stimulated, most probably owing to the induction of compensation mechanisms. Longer exposure of the test plants to the activity of phytophagous insects caused a decrease in the intensity of these processes. These relationships, however, were not confirmed when the foraging by *R. padi* on wheat was observed, which may indicate the variability of the plant-aphid interaction, and makes it difficult to determine a clear, unambiguous trend.

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