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Changes in the photosynthetic performance of infested leaves of two barley cultivars by aphids

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Abstract

The influence of the aphids on leaf gas-exchange, chlorophyll fluorescence and photosynthetic pigments content of two barley cultivars – Obzor and Aheloy 2 – was studied in the Institute of agriculture – Karnobat and Agricultural University – Plovdiv during 2008–2009. It was established that the photosynthetic performance of the aphids-infested leaves was negatively influenced, and that effect was stronger expressed in the cv. Aheloy 2. Net photosynthetic rate of the infested leaves from both cultivars was lower than the healthy leaves due to the lower pigments content, the less efficient use of the captured light energy and probably the higher stomata limitation.

Key words: aphids, barley, chlorophyll fluorescence

Introduction

Direct damage from aphids results in reduction of yield and quality of crop production due to torsion, warping and yellowing of leaves and - weak growth and development of attacked plants. According to Goszczynski and Cichocka (1998) and Vassilev and Lecheva (2003), the factors listed are only external signs of damage leading to low yields, due to the pathophysiological changes occurring in the leaves of the host by stimulating the respiratory stress, lowering the rate of foliar gas exchange, content of chlorophyll, sugars and proteins. The extent of damage to plants depends on the density of aphids, aphids species and varietal tolerance (Lecheva et al., 2001) of the host. According to Haile et al. (1999) the mechanisms of physiological tolerance of plants to pests are not fully understood, but there is a view that some of them are related to the photosynthetic process. Therefore, research interest focuses on photosynthetic characteristics of leaves infested by aphids, which is currently focused on two aspects:one - clarification of sensitive units, limiting integral photosynthetic process (Goszczynski and Cichocka, 1998; Shannag et al., 1998) and two - using of photosynthetic parameters for assessing the physiologic tolerance of plant genotypes to different pests (Blanco et al., 1992; Riedell and Blackmer, 1999; Haile et al., 1999). According to Czerwinski (1978), the main reason for the reduction of photosynthetic rate in leaves attacked by insects is reduced content of chlorophyll. Goszczynski and Cichocka (1998) admitted that the somewhat negative effect is due to the postponement stomata limiting because of the so-called "honey dew". To assess the physiological tolerance of plant genotypes to aphids used parameters of foliar gas exchange and chlorophyll fluorescence, and information about the spectrum of light reflected from the leaves. Blanco et al. (1992) considered that the parameters of fast chlorophyll fluorescence kinetics may serve as early indicators of damage by aphids in barley, alfalfa, peas and others cultures. Haile et al. (1999) established significant genotypic specificity in the degree of inhibition and restoration of foliar gas exchange and chlorophyll fluorescence parameters in different genotypes of winter wheat infested by russian wheat aphid. Riedell and Blackmer (1999) indicate an opportunity to evaluate the tolerance of different wheat genotypes by the same pest spectrum of reflected light from the leaves.

Materials and methods

Studies were conducted at the Institute of Agriculture - Karnobat and Agricultural University – Plovdiv during the period 2008 - 2009 year. Under study were healthy and infested by aphids leaves of two cultivars of barley – Aheloy 2 and Obzor. We studied two cultivars of barley due to their different sensitivity. Plants were grown in pots under laboratory conditions, some of them were colonized and multiplied aphids, others without aphids served as controls. Average density - 12 aphids per leaf from attacked plants were selected. Marked the leaves were used for analysis of foliar gas exchange, chlorophyll fluorescence, contents of photosynthetic Pigments and anatomy - morphological characteristics. Leaf gas exchange (net photosynthesis rate - A, the intensity of transpiration - E and stomatal conductivity - gs) was determined by photosynthetic portable LCA-4 system (ADC ltd, England) in not pike off and carefully cleaned from aphids leaves under the following conditions: illumination (PHARE) - 1000 - 1200 μmol m-2 s-1, temperature 30 - 32^0C and relative humidity - 60 - 65%. The main parameters of chlorophyll fluorescence: Fv / Fm - potential activity photosystem 2 (FS2), Y - quantum yield or actual functional activity of FS2, qP - photochemical quenching and qN – not photochemical quenching in the same leaves was determined with Chlorophyll Fluorometers MINI-PAM (H. Walz, Germany) procedure described earlier (Bozhinova and all, 1999). Photosynthetic pigments were extracted with 85% acetone, their extintions are set at Spekol 11, and their contents evaluated by McKinney (1941). Anatomy - morphological measurements of healthy and infested leaves are made at the Department of Botany and Agrometeorology, using Cytology technique (Nikolov and Daskalov, 1966) and Convenient Method for studying grass leaf epidermis (Hilu and Randall, 1984). Leaves were examined by two cultivars of barley attacked by aphids and attacked sample (control), and fixed in 75% ethyl alcohol. Cuttings are made from the middle part of fully developed leaf lamina, as they are made permanent microscopic preparations. The thickness of mesophile and number of stomata with eyepiece was measured (10X micrometer). 30 measurements of length and width of stomata are made (100X with eyepiece micrometer). It is estimated the number of mm ². Data are processed by the methods of variation statistics.

Results and discussion

External signs of direct damage of aphids resulting in reduction of yield and quality of crop production was studied by several authors, but research into pathophysiological aspect in our coutry is limited and incomplete information in particular on changes in photosynthesis of barley plant attacked by aphids motivated conduct the survey.

Data on foliar gas exchange in healthy and infested leaves of both barley cultivars Aheloy2 and Obzor are shown in Figure 1. We consider that the values of net photosynthesis speed (A)in the healthy leaves are in the range of 3 to 6 μmol CO2 m-2 s-1. The values of cultivar Aheloy 2 are higher than cultivar Obzor, but - overall, the invasion of aphids reduced the intensity of foliar gas exchange and the intensity of Transpiration (E). Of the three tested parameters - rate of net photosynthesis (A), intensity of Transpiration (E) and stomatal conductance (gs) - at - high degree of speed is suppressed net photosynthesis (A), in which a cultivar Aheloy 2 is - clear expressed. The results obtained for the negative effect of aphids on the rate of net photosynthesis (A) in two cultivars of barley broadly correspond with the known literature data for other aphids, such as the degree of inhibition of A depends on the number of aphids, plant species and genotype and the duration of exposure (Shannag et al., 1998; Haile et al., 1999; Goszczynski and Cichocka, 1998).

The leaves of the studied, barley cultivars was consisted of lamina and sheath, and leaf blade on the bottom side was smooth with a clear and prominent median nerve in the upper surface of striated veined. Major epidermal cells were of two types-short and elongated, arranged in parallel rows. Cells were extended with straight sides.The leaves of the both cultivars were **amphistomous type.**

Net rate of photosynthesis depends on many factors, one of which was stomatal limiting. The stomata were located in parallel rows on the surface and the upper and lower epidermis, a slight overstated of their number in a cultivar Obzor. Closing cells were dumbbell form. Tend to increase in the number of stomata greater attacked plants compared to control (Table 1) and reducing their width and length (Table 1), probably due to reaction of the plant to attack by aphids. Motor cells were located fan-shaped on the surface of the upper epidermis and formes motor stripes on both sides of the main vein. Mesophile was composed of homogeneous cells arranged in rows between two evenly epidermis. There was increasing of its thickness, which was probably the plant's response to attack by aphids (Table 1). Vascular bundles were closed collateral with parenchymatous collar one-line cells.

Reducing the width and length of stomata was probably the main reason for their strong inhibition of Transpiration (E), this gives us grounds to support the opinion of Goszczynski and Cichocka (1998) that some of the factors limiting the A could be associated with limited access of CO_2.

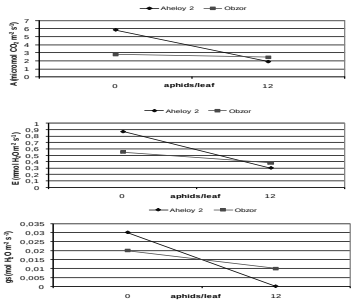

Fig. 1. Influence of leaf aphids on photosynthetic rate (A), transpiration rate (E) and stomatal conductance (gs) in the leaves of two barley cultivars –Aheloy 2 and Obzor.

Table 1. Anatomy – morphological indicators of healthy and infested leaves of barley

Ad surface (AdS) and ab surface (AbS) indicators: number of stomata (NS in mm^2), lenght of stomata (LS μm) width of stomata (WS μm) and mespphyle thickness (MT μm)

	Aheloy 2 barley cultivar						Obzor barley cultivar					
	Control (0 aphids)			12 aphids on leaf			Control (0 aphids)			12 aphids on leaf		
	X	S%	M/m	X	S%	M/m	X	S%	M/m	X	S%	M/m
adS NS	134.63	8.39	1.42	174.63	6.99	1.32	196.01	7.42	1.25	209.71	6.06	1.32
adS LS	47.46	3.97	1.13	46.0	5.29	1.21	37.3	10.81	1.5	38.7	9.33	1.53
adS WS	21.8	7.84	1.35	16.26	7.73	1.35	17.76	7.63	1.33	17.79	12.05	1.57
abS NS	122.11	12.55	1.48	190.36	6.09	1.28	150.28	7.04	1.32	146.88	6.5	1.35
abS LS	47.46	3.97	1.13	46.0	5.29	1.21	47.7	8.07	1.47	42.7	7.17	1.36
abS WS	19.4	5.67	1.22	16.26	7.73	1.35	22.23	10.0	1.66	16.1	8.83	1.46
MT	79.66	9.6	1.5	88	7.54	1.42	53.66	14.25	2.33	77.0	6.94	1.28

X = average; S = ; M/m = maximum : minimum

Another limiting parameter (A) can be quantity and the relations of photosynthetic pigments. Although between the absorption of light energy from the leaves and quantity of pigments (Terry, 1980), there was no linear relation but their reduction may be somewhat limited A. The data indicated in Fig. 2 show that the feeding of aphids reliably reduces the amount of chlorophyll and carotenoids in both cultivars. Amount of chlorophyll in attacked leaves variants (12 number per leaf aphids) in a cultivar Aheloy 2 decreased by 20.98% and that of carotenoids by 14.29%. In the corresponding version of the quantity of cultivar Obzor photosynthetic pigments decreased even more - a significant 26.96% in chlorophyll, and 27.61% for carotenoids. In a cultivar Aheloy 2 ratio chlorophyll a / chlorophyll b increased and the ratio chlorophylls / carotenoids decreased, while the cultivar Obzor chlorophyll ratio a / chlorophyll b significantly reduced and the ratio chlorophylls / carotenoids steadily rising. Given the substantial and significant reduction of the pigments in barley attacked leaves may accept the opinion of Czerwinski (1978), that's one of the main reasons for reducing their photosynthetic rate. Reduction of photosynthetic pigments in attacked leaves may be explained mainly by the fact that they are an important source of nitrogen for aphids.

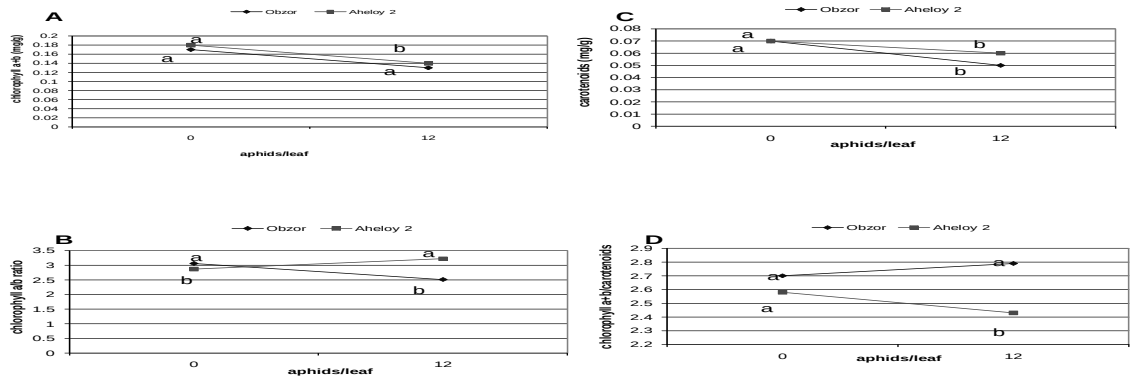

Figura 2. Influence of leaf aphids on both content (A, C) of and ratios between (B, D) photosynthetic pigments in the leaves of two barley cultivars – Aheloy 2 and Obzor. In the figure, different letters (a, b) express significant differences between treatments (P = 0.1%), with "a" representing the highest value.

Inhibition of net photosynthetic rate (A) in barley leaves attacked by insects is due to many factors directly related to breaches in photosynthetic electronic transport and carbon assimilation and indirectly - in other physiological processes. To a certain extent the nature of these disorders can be judged by the parameters of chlorophyll fluorescence. It is known that in conditions of stress reaction fluorescence emission in plants growing and changing its kinetics. This was in the biotic stress caused by the attack of aphids in alfalfa and wheat (Blanco et al., 1992; Haile et al., 1999).

Data reflecting changes in some fluorescent parameters in leaves infested by aphids (12 number per leaf) in both cultivars are shown in Fig. 3. We see that in this density of the insects are not reported significant changes in the ratio Fv / Fm, which is an indicator of the functional potential of FS2. While the values of quantum yield (Y) and fighting photochemical (qP) of fluorescence decreased, while those of nefotohimichnoto fighting (qN) increased, which most - generally reflects the nature of the negative impact of aphids on photosynthetic function. Data show that inhibition of Y and qP in sensitive cultivar Aheloy 2 (Fig. 3 A) is - significantly compared with that for a relatively tolerant cultivar Obzor (Fig. 3 B), which corresponds with data on net photosynthetic rate (Fig. 1) and the amount of photosynthetic pigments (Fig. 2) in leaves infested by aphids in two cultivars.

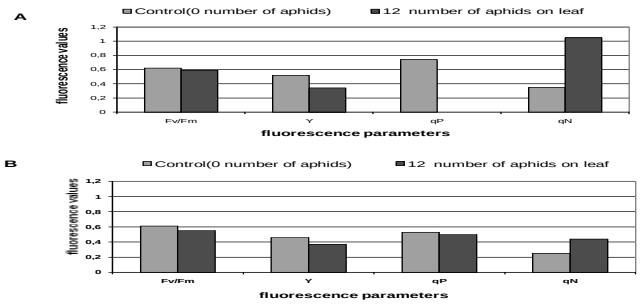

Figura 3. Influence of leaf aphids on some chlorophyll fluorescence parameters in the leaves of two barley cultivars – Aheloy 2 (A) and Obzor (B).

As with other stress effects, and in this case indicators in steady photosynthesis (Y, qP and qN) are much less sensitive than those in dark adapted leaves (Fv / Fm). According to Haile et al. (1999) inhibition of photosynthetic rate (A) in infested by Russian wheat aphid wheat genotypes is mainly due to - low efficiency of photochemical processes. Along with this possible reason, net photosynthetic rate (A) in infested by aphids barley leaves may be limited on biochemical level, as suggested for example by Tafrina deformans infected leaves of peach (Piperkova and Vassilev, 1999). In this case electron transport processes may be inhibited (indicator for which the reduced values of Y and qP) in the feedback path due to an excess of ATP and reducing equivalents, as a result of diminished use in the processes of the Calvin cycle in a significant part of the light energy is utilized disappear as heat (qN values increase).

Conclusions

Invasion of aphids inhibited net photosynthetic rate in leaves of barley cultivar Aheloy 2 and cultivar Obzor. Found a negative effect due to the complex of factors, some of which have reduced amount of photosynthetic pigments in - the low efficiency of utilization of light energy in photosynthetic processes and possibly increased stomatal limiting. The negative effect of aphids is highly developed in the sensitive cultivar Aheloy 2.

Given the high sensitivity, non-destructive nature and speed of measurement of fluorescent and gas detection parameters with modern portable systems Photosynthetic [LCA-4 (ADC), Li 6000 (Li - Cor) and the MINI-PAM (H. WALZ)], is recommends their use in screening tests for physiological tolerance of barley genotypes to aphids.

References

Blanco, L. R., Adamson H. Y., Hales D. F. (1992). Chlorophyll fluorescence kinetics as a measure of stress in plants infested with aphids. Implications for studies of resistance. Journal of the Australian Entomological Society, 31: 221 – 222.

Czerwinski, W. (1978). Fizjologia voslin, PWN, Warszawa, 605 pp.

Goszczynski, W., Cichocka E. (1998). Effects of apids on their host plants. In: Aphids in natural and managed ecosystems (Nieto Nefria, JM, Dixon, AFG., eds.) 197 – 203. Universidad de Leon, Leon, Spain, ISBN 84 – 7719 – 628 – 1.

Haile, F. J., Higley L. G., Ni X. Z., Quisenberry S. S. (1999). Physiological and growt tolerance in wheat to Russian wheat aphid (Homoptera; Aphididae) injury. Environmental Entomology, 28: (5), 787 – 794.

Hilu, K. W., Randall J. L. (1984). Convenient Method for studying grass leaf epidermis. Taxon, 33, 413 – 415.

Lecheva, I., Nikolova A., Kuncheva A. (2001). Interrelations among the host plant, the aphid Hyalopteris pruni (Geoffroy) and the predatory insect species in plum orchard. In: Aphids in a New Millennium. Sixth International Symposium on Aphids, Rennes (France), 3 – 7 September, 2001, 39 p.

McKinney, G. (1941). Absorption of light by chlorophyll solutions. J. Biol. Chemistry, 140: 315 – 322.

Riedell, W. E., Blackmer T. M. (1999). Leat reflectance spectra of cereal aphid – damaged wheat. Crop Science, 39: (6), 1835 – 1840.

Shannag, H. K., Thorvilnson H., El M. K. – Shatnawi (1998). Changes in photosynthetic and transpiration rates of cotton leaves infested with the cotton aphid Aphis gossypi: Unrestricted infestation. Annals of Applied Biology, 132: (1), 13 – 18.

Terry, N. (1980). Limiting factors in photosynthesis. I. Use of iron stress to control photochemical capacity in vivo. Plant Physiology, 65: 114 – 120.

Vassilev, A., Lecheva I. (2003). Ghanges in the photosynthetic performance of Hyalopterus pruni(Geoffr.) – infested leaves of two plum cultivars having different sensitivity. Plant science, 40, 159 – 164.