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MEASUREMENTS OF CHLOROPHYLL FLUORESCENCE AS AN AUXILIARY METHOD IN ESTIMATING SUSCEPTIBILITY OF CULTIVATED HAZEL (*Corylus* L.) FOR FILBERT APHID (*Myzocallis coryli* Goetze)

Magdalena Gantner[1], Władysław Michałek[2]

[1]Department of Entomology, University of Life Sciences,
Leszczyńskiego 7, 20-069 Lublin, Poland, e-mail: magda.gantner@up.lublin.pl
[2]Department of Plant Physiology, Akademicka 15, 20-950 Lublin, Poland

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Abstract

The influence of aphid feeding on chlorophyll *a* fluorescence in the leaves of four cultivated hazel cultivars, with different levels of resistance to filbert aphid (*Myzocallis coryli* Goetze), was studied. The maximum effect of photosystem reaction measured on dark-adapted hazel leaves (Fv/Fm parameter) and maximum efficiency of photon energy PAR conversion to chemical energy in light conditions (Y parameter) were estimated twice, in the leaves of four hazel cultivars with different levels of resistance to filbert aphid, using a fluorometer PAM-2000 by Walz GmbH – Germany. The analysis of changes of these parameters showed that aphid feeding caused a reaction in all tested cultivars. The most visible reduction of the Fv/Fm and Y values as a result of aphid feeding was observed in the cultivars 'Cud z Bollwiller' and 'Olbrzymi z Halle', numerously colonized by aphids. A smaller number of aphids found on the leaves of more resistant cultivars – 'Katalońki' and 'Lamberta Biały', caused a weaker response of plants and a smaller decline in the value of this parameter. 'Cud z Bollwiller' cultivar showed higher tolerance than other tested cultivars to stress caused by the feeding of sucking insects. The Fv/Fm and Y parameters can be regarded as reliable indexes useful in diagnosing susceptibility of hazel cultivars to aphids, helpful in determining, for example, harmfulness thresholds.

Key words: hazelnut, cultivars, filbert aphid, chlorophyll fluorescence, photosystem II

INTRODUCTION

Unfavourable environmental factors, which include, among other things, cold, drought, excess light, mechanical damages or gaseous pollutants, can disturb physiological processes in plants growing in natural conditions, limiting their growth and yield. In particular, most of them affect adversely the process of photosynthesis (Brüggemann et al. 1995; Kacperska, 2002; Murkowski, 2004). Biotic stress factors for plants also include insects which, depending on the mouth organs, chewing or sucking, cause injuries to assimilation tissues. Changes in photosynthetic indexes in connection with the measurements of chlorophyll content in control plants and those colonized by arthropods belong to the most frequently used direct tests that estimate the effect of pests on plants (Leszczyński, 2001).

One of the completely non-invasive and at the same time sensitive methods that allows to study photosynthesis *in vivo* is the analysis of the parameters of chlorophyll *a* fluorescence. This method is especially useful in studies on the effect of various environmental stresses on plants (Bolh r-Nordenkampf and Öquist, 1993; He et al. 1993; Skórska, 2002). The aim of this study was to compare the same photosynthetic parameters in the leaves of four hazel cultivars with different levels of resistance to filbert aphid (*Myzocallis coryli* Goetze) and to determine the tolerance of these cultivars to the feeding of this species.

MATERIALS AND METHODS

The studies were conducted in the years 2004-2006 on a 2.5-hectare productive plantation in Końskowola in the Lublin region. Four cultivars with different levels of resistance to *M. coryli* were chosen to be studied. These were as follows: 'Cud z Bollwiller' ('Minnas') and 'Olbrzymi z Halle' ('Giant from Halle') – with a high level of acceptance by aphids, as well as 'Kataloński' ('Luizen Zellernuss') and 'Lamberta Biały' ('White Fillbert'), whose level of acceptance was 2-3 times lower. The level of hazel acceptance by *M. coryli* was expressed in percent, as a quotient of the populations of larva and adult individuals inhabiting the leaves of a given cultivar and the total population of individuals found on all the cultivars. Aphids (larvae and adults) were counted on 100 leaves, from 5 shrubs of each cultivar during the whole vegetation period, in 10-day intervals. At the same time, field measurements of the chlorophyll fluorescence parameters were performed twice, on the same shrubs, using a fluorometer PAM-2000 by Walz GmbH – Germany (sign by vectors on Fig. 2). The first measurement was performed at the end of May, when aphids occur singly, and the other one at the turn of June and July, in the period of maximum abundance of filbert aphids. The measurements were performed on the leaves free of aphids (control) and on the leaves that were colonized by them. The selected leaves, both the control ones and those colonized by aphids, had a similar location on the plant and were in a similar physiological condition. In order to maintain the same study conditions, the control leaves were not subjected to earlier treatments designed to remove dirt. The measurements were made in triplicate on the designated five shrubs of each cultivar. The following parameters were estimated:

Fv/Fm – the maximum effect of photosystem reaction measured on dark-adapted hazel leaves (Bolh r-Nordenkampf and Öquist, 1993)
Y – maximum efficiency of photon energy PAR conversion to chemical energy in light conditions (Schreiber, 1997)

The results for the respective parameters were processed by means of two-way analysis of variance and the Tukey test was used to separate the groups of means ($p \leq 0.05$). The means marked with the same letters do not significantly differ.

RESULTS AND DISSCUSION

The data included in Figure 1 show that the cultivars ‘Cud z Bollwiller’ and ‘Olbrzymi z Halle’ were conventionally considered to be highly accepted by aphids as compared to the cultivars ‘Kataloński’ and ‘Lamberta Biały’, characterized by poor acceptance by the pest. The former two cultivars were colonized, respectively, by 36 and 37% of all individuals, while the two latter ones were invaded by 17 and 10%, respectively (Fig. 1). The average number of aphids on 100 hazel leaves, in the group of cultivars of low acceptance, ranged, depending on the date, from 30.2 to 93.9, while in the cultivars regarded as highly accepted it ranged from 77.9 to 229.9 individuals (Fig. 2). In the course of observations, clear seasonal differences in the number of *M. coryli* individuals could be seen in the tested cultivars. The greatest number of aphids colonized hazel leaves in 2005, while in 2004 their number was 6 times lower. In the last year of the study, pest abundance was high, as in 2005, especially in the cultivar ‘Olbrzymi z Halle’. Despite the seasonal differences, the population of aphids reached their maximum number at the turn of June and July every year, which was maintained until the end of July in the year of aphids gradation (2005) (Fig. 2).

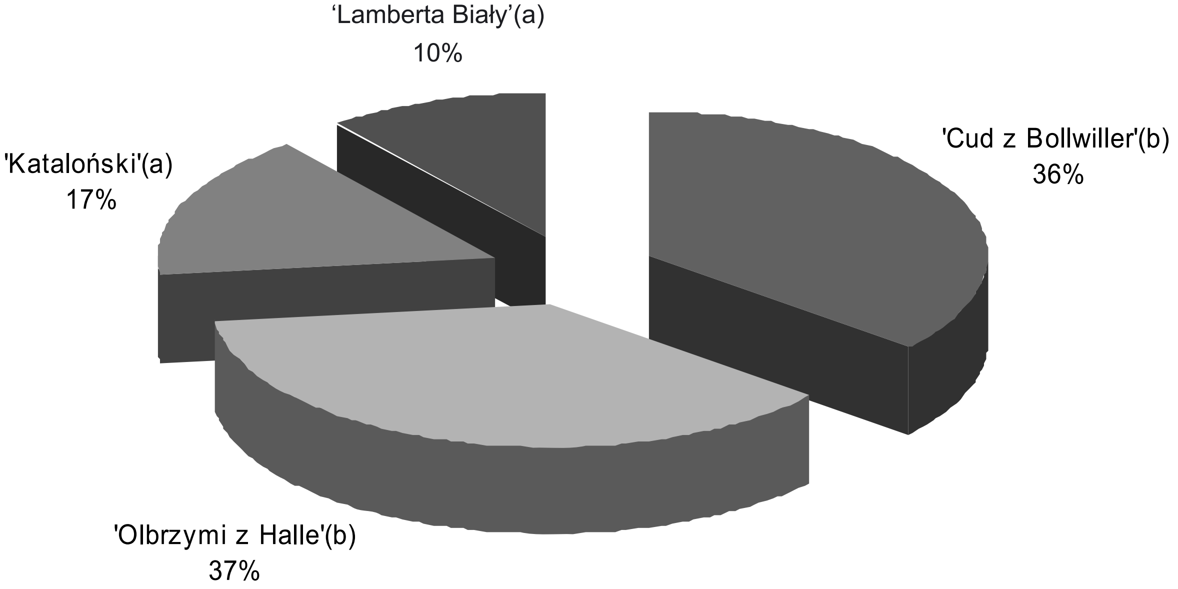

Fig. 1. Level of acceptance of four hazelnut cultivars by *M. coryli* in 2004-2006 (^{abc} – Means followed by the same letters do not differ significantly at $p \leq 0.05$)

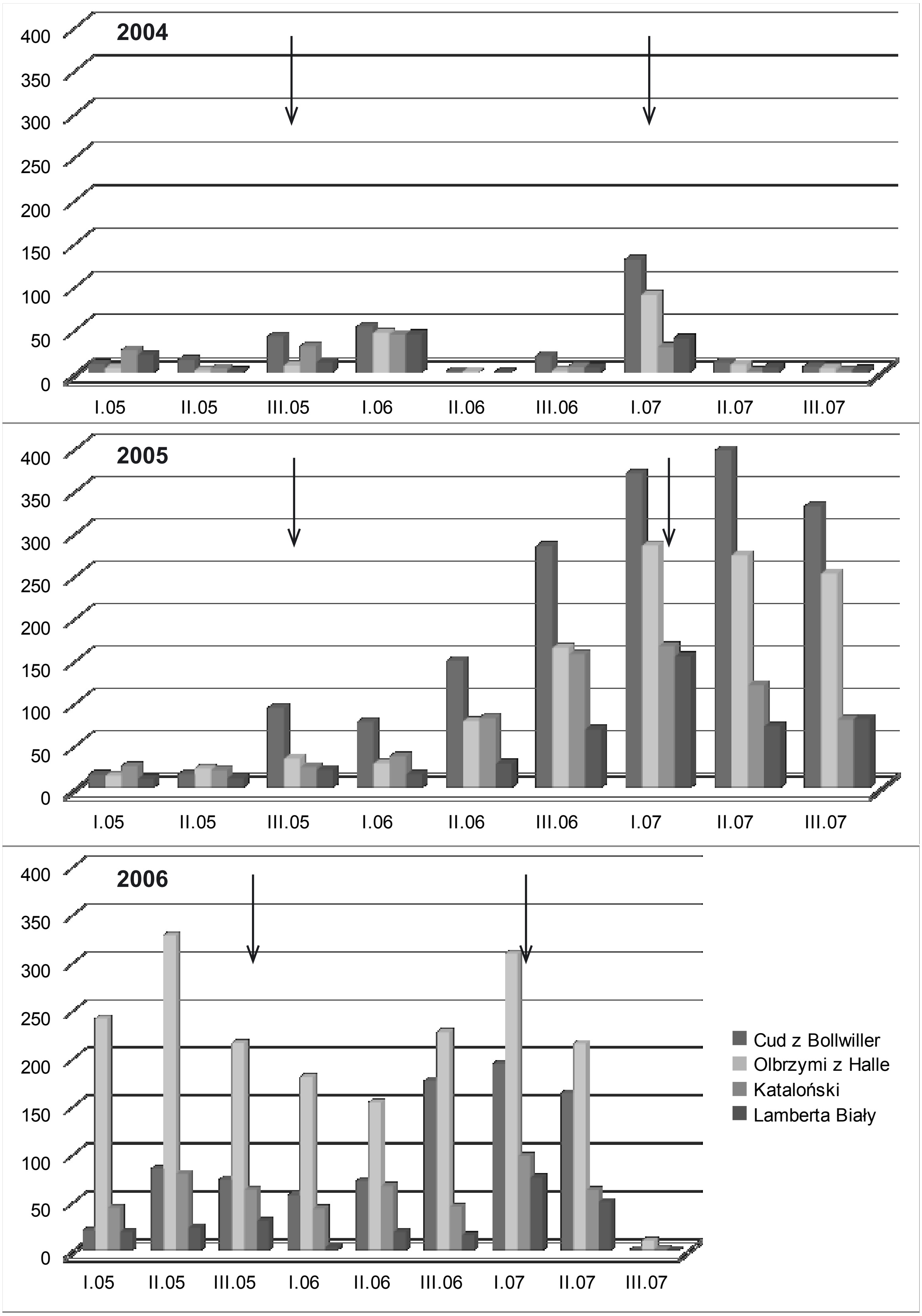

Fig. 2. Quantitative changes of *M. coryli* on hazel in 2004-2006

Table 1 presents the changes in the chlorophyll *a* fluorescence parameters in the control treatment (uninfested leaves) and after stress caused by aphid feeding, with the measurements made in the period 2004-2006 under field conditions in the leaves of four cultivated hazel cultivars.

During the first test period (I), when the number of aphids was small, a decrease in the parameter Fv/Fm was observed, on average by 13% compared to the control, which indicates the photoinhibition changes in photosystem II in the leaves infested by aphids (Kacperska, 2002; Skórska, 2002; Murkowski, 2002). The highest decrease, by 22%, was in 2006, and the lowest (5%) in 2005. In 2004 the decrease of this parameter in the leaves after aphid feeding was reduced by an average of 12% in comparison with the control. The results show that, after aphid feeding, photoinhibition changes were observed in photosystem II in all the hazel cultivars, except the cultivar 'Lamberta Biały'. The maximum effect of photosystem II measured on dark-adapted hazel leaves was significantly lower in the infested leaves of the cultivar 'Kataloński' in 2004, in the cultivars 'Cud z Bollwiller' and 'Olbrzymi z Halle' in 2005, and in 2006 in all the cultivars in question (Gorkom, 1986).

The value of the second test parameter (Y), determined on the first measurement date, was reduced more strongly after aphid feeding in comparison with the parameter Fv/Fm. On the contrary, like in the case of the parameter Fv/Fm, the highest decline of 23% was recorded in 2005, and significantly lower (15%) in 2006 as compared to the control. In 2004 the decrease in this parameter after aphid feeding was relatively high compared to the control (more than 20%). There were no significant differences between cultivars in the initial value of this parameter measured in the leaves which were not colonized by aphids. The clearest reduction of the coefficient Y as a result of aphid feeding was observed in all cultivars in the year 2005, while in 2004 only in the cultivars 'Kataloński' and 'Lamberta Biały'. In the last year of the study, no significant changes caused by aphid feeding were found, except for the cultivar 'Kataloński' (Table 1).

As a result of aphid feeding, at the end of June, when the hazel aphid population reaches its maximum number on hazel leaves, a decrease in Fv/Fm was observed, on average by 28% compared to the control, which indicates the photoinhibition changes in photosystem II of the leaves. In 2004 the decrease in this parameter was the highest (38%), while in the years 2005 and 2006 it was at a similar level of 23%. There were no significant differences between cultivars in the initial value of this parameter measured in control leaves. In each year of observation, the highest decrease of the parameter Fv/Fm was noted in the leaves of the cultivars 'Cud z Bollwiller' and 'Olbrzymi z Halle' (respectively, 48 and 46% in 2004, 23 and 21% in 2005, 21 and 38% in 2006)

On the second measurement date, the parameter Y was reduced on average by 27% after aphid feeding in comparison with the control. The highest decrease was recorded in 2005, while almost twice lower in 2004. As a result of aphid feeding, the ability to convert PAR photon energy to chemical energy in light conditions decreased in the leaves of all cultivars. The highest reduction was recorded in 2005 in the leaves of the cultivar 'Cud z Bollwiller' (51%) and the lowest in 2004 in the leaves of the cultivar 'Lamberta Biały' (14%). There were no significant changes in the value of this parameter in the leaves of the cultivar 'Kataloński' in 2004. In contrast to the parameter Fv/Fm, the cultivars showed significant differences in the initial value of the parameter Y in the control leaves (Table 1).

As a result of using the fluorescence method, a decrease in the efficiency of photosynthesis after stress caused by aphids feeding was found in the leaves of all the tested hazel cultivars (Maxwell and Johnson, 2000; Murkowski and Skórska, 1997; Schreiber et al., 2000). During the first test period, the value of the chlorophyll fluorescence parameters differed both in the control leaves and in those infested by aphids. As determined during the second test period, the initial values in the control leaves of the tested cultivars were similar, suggesting their comparable photosynthetic activity (Murkowski, 2004). Under the influence of stress caused by sucking insects, a decrease in the potential effectiveness of the photochemical reaction in PS II, compared to the control, was observed, particularly in the cultivars 'Cud z Bollwiller' and 'Olbrzymi z Halle', the most intensely infested by aphids. A smaller number of aphids found on the leaves of the cultivars 'Kataloński' and 'Lamberta Biały' caused a much weaker reaction of plants, revealing a smaller decline in the value of the parameters.

A study by Cichocka et al. (1992) showed a slower rate of photosynthesis after a few weeks of preying by aphids *Pemphigus bursarius*, *P. spyrothecae*, *Cavariella aegopodi*, *Macrosiphoniella sanborni*, *Aphis fabae*, *Myzus persicae* and *Acyrthosiphon pisum* on all the studied host plants. A strong reaction of the photosystem to preying aphids was especially well visible in different species of trees and shrubs. For example, photosynthesis in hawthorn leaves injured by *Dysaphis crataegi* decreased 4-5 times as compared to the control (Cichocka and Goszczyński, 1986). In certain conditions, however, plants – due to the induced defence – tolerate the presence of pests. The authors explain this phenomenon with growth stimulation, the increase of photosynthetic intensity, the change in the distribution of assimilative substances or fast necrotization of tissues. In this way, they are capable of compensating the losses, which balances the negative effect of phytophagous species, thanks to which plants remain in a good condition and they yield well (Thomson et al. 2003). Retuerto et al. (2004) showed a distinct increase of efficiency of the photosystem in the leaves of holly (*Ilex aquifolium*) attacked by scale insects (*Coccus* sp.). As a comparison, the increase of photosynthetic intensity in cucumber plants (*Cucumis sativum*) balanced the loss of assimilation area in almost 80%. That loss appeared as a result of the preying of snails *Helix aspersa* (Thomson et al., 2003). Poor infection of bean, strawberry, chrysanthemum and cucumber plants by red spiders caused increased intensity of photosynthesis, which contributed to increased metabolism and induction of defensive reaction, leading as a consequence to losses and even growth stimulation and improved yielding (Tomczyk, 2001).

Table 1.

Chlorophyll fluorescence parameters in leaves of four hazelnut cultivars in 2004-2006

Cultivar	First measurement date				Second measurement date			
	Fv/Fm		Y		Fv/Fm		Y	
	control	infested	control	infested	control	infested	control	infested
				2004				
Cud z Bollwiller	0.75^{c}	0.69^{bc}	0.63^{c}	0.57^{bc}	0.80^{d}	0.42^{a}	0.64^{c}	0.44^{a}
Olbrzymi z Halle	0.70^{bc}	0.64^{b}	0.59^{bc}	0.54^{b}	0.79^{d}	0.43^{a}	0.59^{bc}	0.46^{a}
Kataloński	0.64^{b}	0.44^{a}	0.60^{c}	0.38^{a}	0.79^{d}	0.62^{b}	0.59^{bc}	0.57^{b}
Lamberta Biały	0.73^{c}	0.72^{c}	0.58^{bc}	0.43^{a}	0.80^{d}	0.51^{c}	0.55^{b}	0.47^{a}
Mean	0.71	**0.62**	**0.60**	**0.48**	**0.80**	**0.49**	**0.59**	**0.49**
Effect significance:								
Cultivar (c)	***		***		***		***	
Infested (i)	***		***		***		***	
c x i	***		***		***		***	
				2005				
Cud z Bollwiller	0.78^{c}	0.73^{b}	0.53^{c}	0.43^{b}	0.80^{c}	0.61^{b}	0.59^{d}	0.29^{a}
Olbrzymi z Halle	0.70^{b}	0.64^{a}	0.53^{c}	0.36^{a}	0.79^{c}	0.62^{b}	0.57^{d}	0.39^{b}
Kataloński	0.66^{a}	0.63^{a}	0.59^{d}	0.41^{b}	0.77^{c}	0.62^{b}	0.52^{c}	0.37^{b}
Lamberta Biały	0.80^{c}	0.80^{c}	0.60^{d}	0.52^{c}	0.79^{c}	0.57^{a}	0.52^{c}	0.31^{a}
Mean	**0.73**	**0.70**	**0.56**	**0.43**	**0.79**	**0.61**	**0.52**	0.34
Effect significance:								
Cultivar (c)	***		***		**		***	
Infested (i)	***		***		***		***	
c x i	**		***		**		***	
				2006				
Cud z Bollwiller	0.78^{d}	0.55^{ab}	0.53^{bc}	0.45^{a}	0.81^{c}	0.64^{b}	0.63^{c}	0.44^{a}
Olbrzymi z Halle	0.69^{c}	0.50^{a}	0.57^{bc}	0.45^{a}	0.79^{c}	0.50^{a}	0.58^{bc}	0.40^{a}
Kataloński	0.60^{b}	0.49^{a}	0.59^{c}	0.54^{bc}	0.79^{c}	0.63^{b}	0.53^{b}	0.41^{a}
Lamberta Biały	0.61^{b}	0.55^{ab}	0.59^{c}	0.49^{ab}	0.79^{c}	0.69^{b}	0.54^{b}	0.44^{a}
Mean	**0.67**	**0.52**	**0.57**	**0.48**	**0.79**	**0.61**	**0.57**	**0.424**
Effect significance:								
Cultivar (c)	**		**		***		**	
Infested (i)	***		***		***		**	
c x i	***		ns		***		*	

^{abc} – Means followed by the same letters (for one parameter) do not differ significantly at p≤0.05

*effect significance at the level p≤0.05, **p≤0.01, ***p≤0.001; ns – not significant

To summarize, it can be concluded that only one hazel cultivar – 'Cud z Bollwiller' – showed more tolerance to the feeding of sucking insects than the other tested hazel cultivars, despite the highest number of aphids recorded on their leaves in the years 2005 and 2006. The present studies, which are of preliminary nature, are an evidence of the satisfying efficiency of the measurements of chlorophyll fluorescence in hazel using the Fv/Fm parameter – the maximum effect of photosystem measured on dark-adapted hazel leave, and the Y parameter – maximum efficiency of photon energy PAR conversion to chemical energy in light conditions as a reliable index useful in diagnosing susceptibility of hazel cultivars to aphids. Therefore, they can be regarded as a reliable index useful in diagnosing susceptibility of hazel cultivars to aphids, helpful in determining, for example, harmfulness thresholds. They also point to the need of continuing them, with a controlled stock of individuals in glasshouse conditions.

CONCLUSIONS

1. The initial values of the Fv/Fm parameter in the leaves of four tested hazel cultivars that were not colonized by aphids were similar, suggesting their comparable photosynthetic activity.
2. Under the stress caused by insect feeding, the potential efficiency of the photochemical reaction in PS II was reduced, particularly in the hazel cultivars 'Cud z Bollwiller' and 'Olbrzymi z Halle', the most numerously colonized by aphids.
3. The hazelnut cultivar 'Cud z Bollwiller' showed higher tolerance to stress caused by the feeding of sucking insects than the other tested cultivars.

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Pomiary fluorescencji chlorofilu jako metoda pomocna w ocenie podatności leszczyny uprawnej (*Corylus* L.) na zdobniczkę leszczynową (*Myzocallis coryli* Goetze)

Streszczenie

Badano wpływ żerowania mszyc z gatunku zdobniczka leszczynowa (*Myzocallis coryli* Goetze.) na fluorescencję chlorofilu *a* w liściach leszczyny uprawnej czterech odmian, różniących się podatnością na występowanie szkodnika. Pomiary wykonano przy użyciu fluorymetru typu PAM wyznaczając parametry Fv/Fm i Y w dwóch terminach różniących się liczebnością występowania mszyc. Z analizy zmian wartości parametrów wynika, że żerowanie mszyc spowodowało reakcję u wszystkich badanych odmian. Uwidoczniło się to w zdecydowanym spadku wartości parametrów Fv/Fm i Y u odmian Cud z Bollwiller i Olbrzymi z Halle, licznie zasiedlanych przez mszyce. Mniejsze ilości mszyc stwierdzone na liściach odmian bardziej odpornych – Kataloński i Lamberta Biały, spowodowały słabszą reakcję roślin i mniejszy spadek wartości tego parametru. Odmiana Cud z Bollwiller wykazała większą niż pozostałe odmiany leszczyny uprawnej tolerancję na stres wywołany żerowaniem owadów ssących. Parametry Fv/Fm i Y można uznać za miarodajny wskaźnik pomocny w diagnostyce wrażliwości odmian roślin na szkodniki, przydatny w wyznaczaniu między innymi progów szkodliwości.