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BLACK BEAN APHID POPULATIONS AND CHLOROPHYLL COMPOSITION CHANGES AS RESPONSES OF GUELDER ROSE TO APHID INFESTATION STRESS CONDITIONS

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ABSTRACT

Aphis fabae Scop. is phloem-feeding insect that cause substantial damage to horticulare and agriculture worldwide due to feeding – related damage and the transmission of economically important plant viruses. These aphids cause a detrimental effects in attacked organs, like depletion of photoassimilates. Insect feeding can among others reduced chlorophyll catabolism. In the present investigations we determined the chlorophyll a and b levels (SPAD readings) in uninfested leaves and in *Aphis fabae*-infested leaves of *Viburnum opulus* shrubs, wild plants and garden variety, which were grown in green areas around Siedlce, east central Poland. Feeding by *A. fabae* affected chlorophyll a + b level. The insect feeding reduces the concentration of photosynthetic pigment in the infested shrubs. The level of chlorophyll in plants occupied by aphids was clearly lower than the level in plants where aphids were not found. We also found that chlorophyll levels increased with the progress of growing season, the level of chlorophyll differed between date of survey, being higher for the latest survey (mid-June) and that the place where the plant is located is important, wild *V. opulus* have slightly more level of chlorophyll compared to garden ones. Chlorophyll level might be useful as an indicator of plant responses to aphid damage.

Key words: *Aphis fabae*, chlorophyll a + b, garden variety, wild plants, SPAD values, *Viburnum opulus*

INTRODUCTION

The black bean aphid *Aphis fabae* Scop. belongs to a small group of polyphages within the *Aphidiaceae* family. Aphids are phloem-feeding insects that cause substantial damage to horticulare and agriculture worldwide due to feeding – related damage and the transmission of economically important plant viruses. Aphids cause a detrimental effects in attacked organs, like mechanical disruption of penetrated tissues, depletion of photoassimilates, and intensification of many intracellular processes [Sytykiewicz et al. 2011a, Goławska et al. 2012]. Aphid salivary secretions contain biologically active substances that modulate metabolic reactions within the host, activation of premature senescing process and programmed cell death in plant organs [Sytykiewicz et al. 2011a, Sprawka et al. 2013]. The black bean aphid belongs to the most important pests of broad bean in Poland. This aphid belongs to polyphagous species, which show host alternation involving seasonal movements between primary and secondary host plant. In the case of *A. fabae* the primary hosts are *Euonymus europaeus* L., *Viburnum opulus* L., *Philadelphus coronarius* L. [Kafel et al. 2010]. *Aphis fabae* has many secondary hosts. These include, among others: *Vicia faba* L., *Chenopodium album* L., *Lamium*

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purpureum L., *Papaver dubium* L., *Beta vulgaris* L., *Capsella bursa-pastoris* L., *Veronica hederifolia* L., *Amaranthus retroflexus* L. and *Capsicum annuum* L. [Webster et al. 2010, Fajinmi et al. 2011]. The choice of host species is influenced by factors such as the host genotype, the presence of other aphids and environmental conditions [Zytynska et al. 2016]. This aphid focuses attention on many features of the potential host, among others it focus on the color, smell, shape and texture of the host's surface [Schröder et al. 2017], uses mechanical and chemical receptors located on trumpets and keels, olfactory organs on antennas for their analyzes [Abbot et al. 2018]. *Aphis fabae* is causing severe crops, beetroot, broad bean, field beans, poppy, beets and other crops, losses either through direct feeding or as a virus vector. Infestation with *A. fabae* resulted in malformations of younger leaves, shoots and flowers and as a vector numerous viruses. *Aphis fabae* can cause also considerable indirect damages. The most known viruses transmitted by *A. fabae* include: alfalfa mosaic virus (AMV), cucumber mosaic virus (CMV), lettuce mosaic virus (LMV), pepper veinal mottle virus (PVMV), plum pox virus (PPV) [Nebreda et al. 2004, Fajinmi et al. 2011]. Environmental harmfulness *A. fabae* is also associated primarily with the use of insecticides to kill, killing both it and other insects valuable for the environment, e.g. predatory species, pollinators [Beketov et al. 2013]. Pesticides mainly harm through soil and water pollution, poisoning plants and animals significantly reducing the biodiversity [Beketov et al. 2013].

Aphis fabae colonized European cranberry bush *Viburnum opulus* L., commonly known as guelder rose or highbush cranberry, which is one of the most widespread shrub species. Guelder rose grows under different climatic conditions, in eastern, northeastern, western, and central Europe and in western and eastern Siberia, wildly in moist, well-drained soils and is widely cultivated in gardens in many countries in Europe and Asia [Kollmann and Grubb 2002]. The genus *Viburnum* comprises more than 230 species, many of which are used for ornamental purposes, but *V. opulus* L. is known for its bitter, edible fruits as well [Česonienė et al. 2010]. Their fruits are rich with biologically active substances known for their antioxidative properties [Li et al. 2009] and were used in traditional and folk medicine [Yilmaz et al. 2008]. Phytochemical studies on this species have shown the presence of different natural compounds including iridoids, iridoid glucosides, lantanoside, flavonoids, saponins, tannins, arbutin, ursolic acid, flavones and anthocyanins [Tomassini et al. 1997, Deineka et al. 2005]. The study shown also some dangerous chemicals like coumarin that can be dangerous to human health [Adebayo et al. 2017].

One of the most important parameters in the relationships between plants and herbivores is chlorophyll content. It amount change during plant development and can alter in response to a broad spectrum of biotic and abiotic stressors [Fanizza et al. 1991, Lawson et al. 2001]. Chlorophyll catabolism can be reduced among others by insect feeding [Goławska et al. 2010, Sytykiewicz et al. 2013] but it loss isn't understood. Leaf feeding by insects causes chlorosis and necrosis, leading to crop loss worldwide [Ni et al. 2001], so insect-caused leaf chlorosis in plants should be studied exactly, as chlorophyll level might be useful as an indicator of plant responses to insect damage [Haile et al. 1999].

In the present experiments, measurement of leaf photosynthetic pigment (chlorophyll a and b) content was used for study the effect of the aphid infestation on chlorophyll concentration in *V. opulus* plants. There are no published data from studies to assess aphid-affected changes in the chlorophyll content of tissues of guilder rose shrubs, on wild and garden plants. This research represents an initial effort to characterize the effect *A. fabae* feeding has on chlorophyll a and b loss in European cranberry bush.

MATERIAL AND METHODS

Plant material and study area. The research was carried out in 2016 and 2017 years, on *Viburnum opulus* L. shrubs growing wild and garden variety Roseum. The plants were grown in green areas around Siedlce, Poland (52°12′N, 22°7′E). The climate of the area is characterized by an annual mean temperature of 8.7°C, annual mean relative air humidity of 79%, and a total rainfall of 526 mm (https//en.tutiempo.net). During the experimental period, the weather was typical for the spring in eastern Poland. Fully expanded 1-year side shoots of the *V. opulus* L. shrubs, were used in all experiments. The measurements were conducted on leaves of two shrubs for garden variety and two shrubs for wild plants. Simultaneously, systematic entomological observations were conducted from end of April/early May to mid-June, every 10 days. Plant material was analyzed from the moment the first aphids appeared until they were gone. Plant material for chlorophyll analyses included naturally infested leaves by the black bean aphid. Control leaves were collected from the uninfested shoots of the *Vibornum opulus* L.

Entomological observations. Entomological observations were done on *V. opulus* shrubs under natural conditions. Studied morphs of the black bean aphid (*Aphis fabae* L.) were: fundatrix (the first morph, hatched from the winter eggs, always apterous), fundatrigeniae (apterous instars of fundatrix) and alatae (winged morphs). Samples of aphids were counted randomly. The number of *A. fabae* was estimated using the technique of direct counting of aphid individuals infesting 30 randomly selected, fully expanded 1-year side shoots of *V. opulus* (approx. 45 cm long).

SPAD meter readings. The chlorophyll content in tissues of single leaves of the *V. opulus* plants (infested, and unifested as control) was determined with a SPAD-502 meter (Minolta Corp., Ramsey, NJ). This instrument has a self-contained light source for uniform lighting over the sampled leaf surface, and two detectors, one sensitive to red light (645 nm) and the other sensitive to infrared radiation (790 nm). The sensors convert the light into electrical currents for calculation of the SPAD value: SPAD = $A(\log(I_{or}/I_r) - \log(I_{of}/I_f) + B$, where A and B are constant, and I_r and If are respectively the currents from red and infrared detectors with sample in place with no sample in place (I_{or} and I_{of}) [Fanizza et al. 1991]. Three SPAD readings were averaged for each leaf to represent one observation. The results represent average measurements of chlorosis for 30 leaves on two plants of each, wild and garden *V. opulus* shrubs.

Statistical procedure. Before analysis, the data sets were assessed for normality of distribution and homogeneity of variance (Shapiro-Wilk and Lavene's tests were applied). A General Linear Mixed Model (GLMM) with normal distribution and identity link functions was used to investigate the factors affecting the level of chlorophyll in *V. opulus*. The models included plant chlorophyll level as the response variable and shrub (garden variety/wild plants), aphid presence (yes/no), and survey number (five surveys per each season from end of April to mid of June) as fixed factors. Individual identity of each shrubs of viburnum (four shrubs) was included as a random effect to avoid pseudoreplication. The year of the study was also included in the analysis as a random effect. The same method of analysis (GLMM with normal distribution and identity link functions) was used to investigate the factors affecting the number of aphids in *V. opulus*. The model included number of aphids as the response variable, shrub (garden variety/wild plants) and survey number (five surveys per each season) as fixed factors. Individual identity of each shrubs of *V. opulus* (four shrubs) and year of the study were included as a random effects. Parametric Pearson correlation was calculated between SPAD values and abundance of black bean aphid on guelder rose plants. Statistical analyses were performed in SPSS v. 21.0 [IBM Corp. 2012], $p < 0.05$ was considered statistically significant.

RESULTS

The level of chlorophyll differed between date of survey, being higher for the latest survey, which was in mid-June (Tab. 1). Chlorophyll levels clearly increased with the progress of growing season (Fig. 1). At the first measurement it was 27.08 and at the last one 1.5 times more. The level of chlorophyll in plants occupied by aphids was clearly lower than the level in plants where aphids were not found (Tab. 1, Fig. 2). For unifested plants it was 36. Aphid infestation significantly reduced the level of chlorophyll to 30.5. We also found that wild *V. opulus* have slightly more level of chlorophyll (34.2) compared to garden ones (32.1) (Tab. 1, Fig. 3).

In both years of the study (2016 and 2017), dynamics of *A. fabae* abundance on the studied hosts, was characterized by an initial rapid increase to reach maximum, followed by a decrease in the number of aphids until to the total disappearance of the population.

Observations of the population growth of *A. fabae* on the shoots of the guelder rose showed differences in the number of aphids. Number of aphids differed between date of survey, and the biggest number was recorded during third survey, which took place at the end of May (Tab. 2, Fig. 4). During the two surveys carried out in June, the number of aphids was much lower. The number of aphids was not determined by the variety of *V. opulus* (Tab. 2).

In 2016, the first individuals of *A. fabae* aphids on the *V. opulus* shoots was observed on May 5. After this date, a gradual increase in the size of the analyzed population was recorded, and maximum density was observed on May 24. In the following days, a reverse tendency was observed, i.e. a decrease in the number of aphids, and on July 3, *A. fabae* was not found on the host. In 2017, the first individuals of aphids were observed on the *V. opulus* on April 30. From May 9, an increase in the number of aphids was observed. Maximum level of *A. fabae* population was observed at the turn of May and June, and later a decrease in the number of the studied aphid population was noted, leading to its disappearance at the beginning of July. The number of aphids on the studied guelder rose plants, in fact, did not correlate with SPAD readings (Pearson correlation, $r = 0.07$, $p < 0.05$).

Table 1. Results of General Linear Mixed Model on level of chlorophyll in *Viburnum opulus* (normal error distribution), random effects: ID of bushes $p = 0.342$, year $p = 0.480$

Fixed effects	Estimate	Standard error	*t*-test	*P*-value
Intercept	37.142	2.238	16.59	<0.001
Aphids (absent)	5.697	0.171	33.40	<0.001
Variety (garden)	–2.050	0.765	–2.67	0.007
Survey no 1	–11.614	0.270	–43.04	<0.001
Survey no 2	–8.343	0.309	–26.94	<0.001
Survey no 3	–5.773	0.249	–23.21	<0.001
Survey no 4	–3.092	0.326	–9.47	<0.001

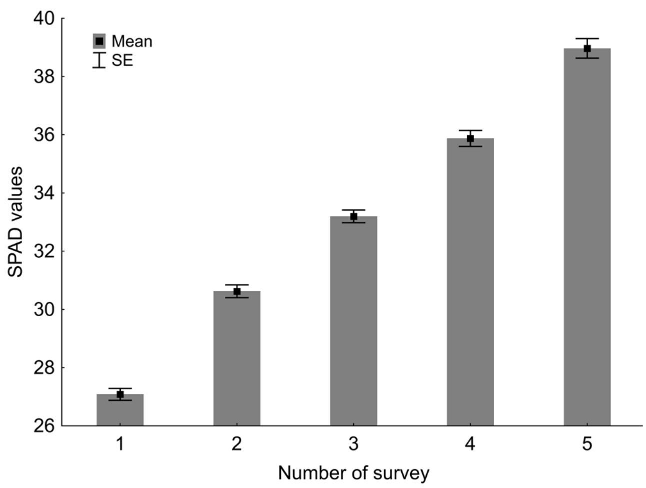

Fig. 1. Comparison of level of chlorophyll in *Viburnum opulus* depending on the date of material collection (survey no 1 – end of April/early May, survey no 5 – mid of June, $n = 120$ leaves for each dataset). Mean ±standard error (SE) are indicated. The level of chlorophyll differed statistically between each of the five surveys, pairwise comparison ($p < 0.05$)

Table 2. Results of General Linear Mixed Model on number of aphids in *Viburnum opulus* (normal error distribution), random effects: ID of shrubs $p = 0.354$, year $p = 0.544$

Fixed effects	Estimate	Standard error	*t*-test	*P*-value
Intercept	11.333	1.910	5.93	<0.001
Variety (garden)	–1.898	2.311	–0.821	0.412
Survey no 1	–7.746	0.988	–6.65	<0.001
Survey no 2	5.712	1.165	5.78	<0.001
Survey no 3	9.337	0.974	9.58	<0.001
Survey no 4	7.733	1.212	6.38	<0.001

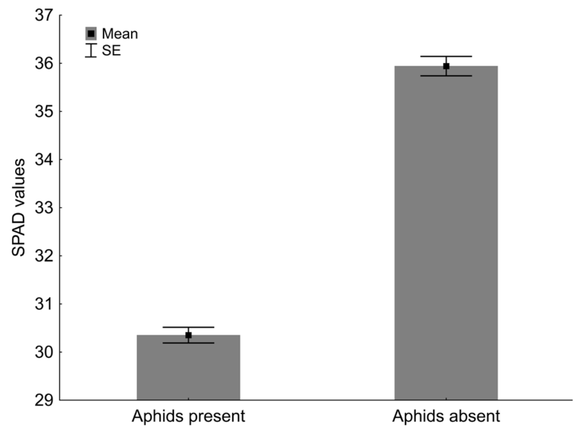

Fig. 2. Comparison of level of chlorophyll in *Viburnum opulus* depending on the presence/absence of aphids ($n = 120$)

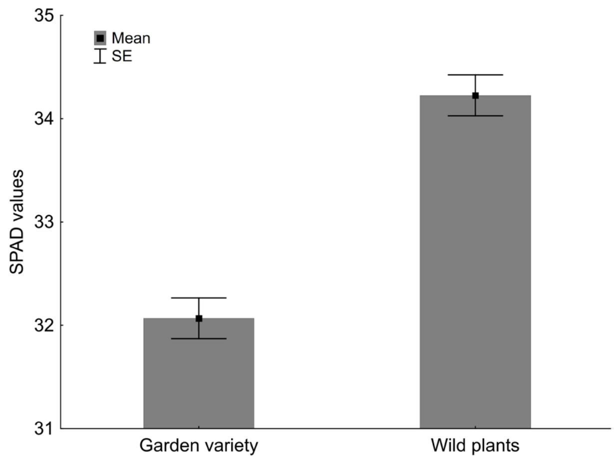

Fig. 3. Comparison of level of chlorophyll in *Viburnum opulus* depending on the garden variety/wild plants ($n = 120$)

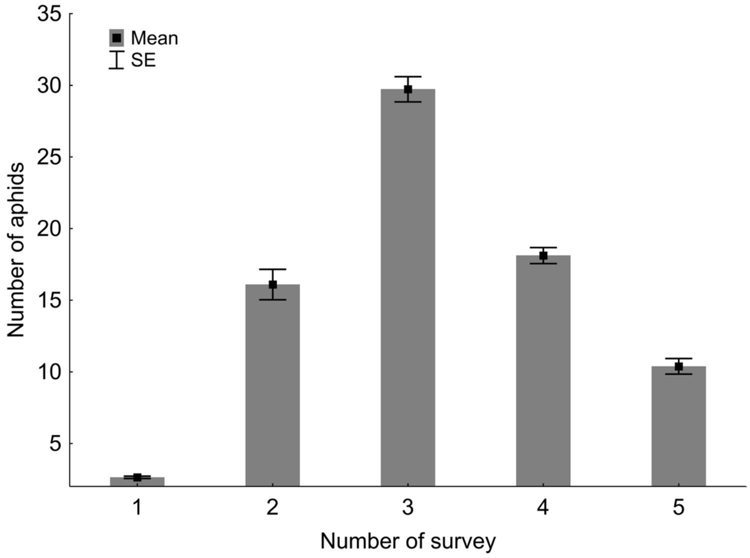

Fig. 4. Comparison of number of aphids in *Viburnum opulus* depending on the date of material collection (survey no 1 – end of April/early May, survey no 5 – mid of June, $n = 120$ shoots for each dataset). Mean ±standard error (SE) are indicated. The number of aphids differed statistically between each of the five surveys, pairwise comparison ($p < 0.05$)

DISCUSSION

Noninvasive methods of plant analysis have become increasingly popular in biology. These methods allow to characterize important physiological processes of intact plants without injurious sampling of the plant tissues. One of the most widely used group of these methods is optical measurement of leaf chlorophyll content [Samsone et al. 2007]. Chlorophyll fluorescence can be used to assess plant adaptation to an environment and to measure the stress level experienced by a plant [Oxborough 2004].

There are many reports on the destructive effects of insects infestation, including aphid, on the functioning of plant systems, e.g. chlorosis [Zvereva et al. 2010, Goławska et al. 2012, Łukasik et al. 2012]. *Aphis fabae* infestation was shown to reduce chlorophyll levels in guelder rose plants. This indicates symptoms of chlorosis in the infested shoots and leaves and adds important data for *A. fabae*, an aphid species, which has been identified as a serious pest of horticulare and agriculture plants [Kafel et al. 2010, Webster et al. 2010, Fajinmi et al. 2011]. Fanizza et al. [1991] reported a drop in chlorophyll content under a different stress treatment. Leaves of stressed plants synthesized less chlorophyll pigment. In our work the studied *V. opulus* plants differed in the effect of black bean aphid feeding on chlorophyll a + b level. Aphid feeding adversely affected the plants and directly affected chlorophyll content. The chlorophyll a + b concentration in unifested guelder rose plants was significantly higher than in aphid-infested guelder rose plants. The chlorophyll concentration in *V. opulus* plant tissues differed between date of survey and between place of occurrence the guelder rose plants. Chlorophyll levels increased with the progress of growing season and depended on the plant's species and locations. Other studies show impact aphids on concentrations of chlorophyll, but that impact may be vary. Goławska et al. [2010] showed that *A. pisum* infestation reduced chlorophyll concentration in several species of the *Fabaceae* family (*Pisum sativum*, *Vicia faba*, *Trifolium pratense*, *Medicago sativa*). Also, Sytykiewicz et al. [2013] found chlorophyll loss in plants infested by *Rhopalosiphum padi* and *Sitobion avenae* and chlorophyll loss was dependent on aphid species, time of aphid infestation and maize genotypes. Chlorophyll depletion was greater after long-term aphid infestation. Chlorophyll level was higher for the latest survey (mid-June) and higher for wild plants. Similar to our research, Burd and Eliot [1996], Riedell and Blackmer [1999] found a decline in chlorophyll concentration in infested leaf tissues of cereals, wheat and barley. More green pigment was lost from leaves infested by aphids longer period. Heng-Moss et al. [2003] reported that infested by *Diuraphis noxia* wheat isolines had higher levels of chlorophyll and carotenoids than unifested plants and prolonged this aphid feeding caused more chlorophyll loss in wheat leaves. Gutsche et al. [2009] found *D. noxia* feeding negatively impacts the photosynthetic capacity in studied cultivars of barley.

Chlorophyll content of leaves is a useful indicator of photosynthetic productivity and general plant vigor, because chlorophyll is the principal photoreceptor in photosynthesis, the light-driven process in which carbon dioxide is "fixed" to yield carbohydrates and oxygen [Zarco-Tejada et al. 2002]. Changes in the amount of chlorophyll may be a part of adaptive responses. The use of non-destructive methods of chlorophyll measurement provides means of plant analysis in a wide range of biological context. Aphids – a group of specialized phloem-feeding hemipterans has coevolved multi-level adaptations facilitating exploitation of the resources provided by their host plants [Czerniewicz et al. 2011, Sprawka et al. 2011, 2012, 2013]. Insect cause a wide spectrum of detrimental effects in attacked organs e.g. mechanical disruption of penetrated tissues, depletion of photoassimilates andintensification of many intracellular processes [Goławska et al. 2012, Sytykiewicz et al. 2013]. The feeding of aphids have a profound impact on the concentration of different photosynthetic pigments. The occurrence of aphids, their feeding and honey dew excreted by aphids on leaf surface might be responsible for this decrease in the concentration of chlorophyll in areas infested by aphids. It might clog the stomata thus interfering with the gaseous exchange, which leads to increase in leaf temperature which may consequently retard chlorophyll synthesis. Infested leaf surface is responsible for reduced photosynthesis and thereby causing reduction in chlorophyll content [Botha et al. 2006, Goławska et al. 2010]. Population of aphids on plants inhibited photosynthetic processes. It was shown suppression of gas exchange in plant tissues under an aphid infestation [Macedo et al. 2003, Botha et al. 2006]. Aphid infestation evokes senescing process and cell death in plant organs [Sytykiewicz et al. 2011b, Sprawka et al. 2013]. Aphid salivary secretions contain a variety of hydrolytic enzymes and highly biologically active substances that modulate reactions in plant tissues, cleavage of the cell wall and cytoplasmic membranes, leading to their disruption, hydrolysis in the foliar mesophyll [Rao et al. 2013]. Burd and Elliot [1996] showed that aphid feeding could reduce protein synthesis, making photoinhibition irreversible as well as blocking electron transport on the acceptor site of the photosystem II reaction center, causing overreduction in the system. Oxidative bleaching pathway is one of two pathways of natural degradation of chlorophyll a [Janave 1997]. Colonization leaves by aphids may suppress the xantophyll cycle, which participates in protecting photosystem II under stress conditions by modulating the thylakoid membrane pH gradient [Macedo et al. 2003]. The depletion of chlorophyll from leaves infested with aphids may be due to the plasmolysis and initial degeneration of cell organelles of the parenchyma and at the same time accumulation of plastoglobules and chloroplast swelling was found [Morgham et al. 1994]. Ni et al. [2002] showed that *Diuraphis noxia* feeding caused loss of chlorophyll a and b in the damaged regions of infested leaves during two different sampling dates. *Diuraphis noxia*-infested wheat leaves also showed greater Mg-dechelatase activity than unifested wheat leaves. Those assays of chlorophyll degradation enzymes indicated that *D. noxia* feeding increased Mg-dechelatase activity in infested leaves. Based on the in vitro assays of chlorophyll degradation enzyme (chlorophyllase and Mg-dechelatase) activities, Wang et al. [2004] found that the chlorotic symptoms observed on *D. noxia*-infested Tugela wheat were most likely to be elicited by unbalanced chlorophyll biosynthesis and degradation. Mandal and Mukherji [2000] showed that reduction in the concentration of chlorophyll might have also been caused due to the increase in chlorophyllase enzyme, responsible for hydrolytic turnover of free chlorophyll substrates into the chlorophyllide forms, activities, which in turn affects the chlorophyll concentration in plants. It was shown increased chlorophyllase activity in infested by aphids plants [Ni et al. 2002, Sytykiewicz et al. 2013]. Gutsche et al. [2009] suggested that differences in carbon assimilation between control and infested barley plants show that *D. noxia* feeding impacts the dark reaction, specifically rubisco activity and ribulose bisphosphate (RuBP) regeneration.

Heng-Moss et al. [2003] and Goławska et al. [2010] suggested that decline of the photosynthetic rate in aphid-infested leaves may have resulted from increased synthesis of chemical defense compounds in response to herbivory. The decline in chlorophyll concentration found in our study may also be due to increased production of defensive compounds. Guelder rose contains numerous secondary plant metabolites, including carotenoids, polyphenols, flavonoids, saponins, tannins [Deineka et al. 2005, Konarska and Domaciuk 2017]. They have been suggested as possible chemical defensive agents against generalist herbivires [Osbourn 2003, Goławska et al. 2006, Goławska 2007, Goławska et al. 2008].

CONCLUSIONS

The present data on the effect of *A. fabae* feeding on chlorophyll a + b concentrations in *V. opulus* tissues show changes in chlorophyll concentrations in response to *A. fabae* feeding. It suggest the presence of a feeding-induced stress response in the studied species. Feeding by *A. fabae* caused loss of chlorophyll a and b in the infested plants. Unifested leaves of studied shrubs had higher chlorophyll a and b than infested leaves. SPAD measurements is a method useful for study of chlorophyll concentration in different parts of leaf and for determination of insect – plant interactions. Together with the other methods, chlorophyll analysis are good tool for studies in natural habitats or field experiments allowing continuous measurement of the same plant material. These methods have a special importance in biodiversity studies in native habitats and investigation of genetic resources of agricultural plants. So, further research should investigate the potential use of photosynthetic pigments as markers for identifying germplasm resistant to *A. fabae* and other chlorosis-causing insects.

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