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CHLOROPHYLL CONTENT OF APHID-INFESTED SEEDLING LEAVES OF FIFTEEN MAIZE GENOTYPES

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We measured the total chlorophyll (Chl *a*+*b*) content in seedling leaves of fifteen maize cultivars infested by two studied aphid species (oligophagous *Rhopalosiphum padi* L., monophagous *Sitobion avenae* F.) 7 and 14 days after the beginning of infestation, using a SPAD-502 chlorophyll meter. Chlorophyll loss was more severe in *R. padi*-infested than in *S. avenae*-infested plants. Chlorophyll depletion was greater after long-term (14 days) than after short-term aphid infestation in the investigated host systems. Seedlings of Złota Karłowa and Tasty Sweet were more damaged by aphid feeding; Ambrozja and Płomyk plants were less damaged by aphid feeding.

Key words: Total chlorophyll (Chl *a*+*b*) content, SPAD units, maize, *Rhopalosiphum padi*, *Sitobion avenae*.

INTRODUCTION

Maize is one of the most important crop species, widely cultivated throughout the world. *Zea mays* is increasingly used in bioethanol and biogas production and in the pulp and paper industry. Maize can grow under different climatic conditions, but high temperature and humidity can accelerate the population growth of many phytophagous insects that infest this host (Fornalé et al., 2012; Herrmann, 2013; Reddy et al., 2013; Semenčenko et al., 2013). Aphids (Hemiptera, Aphidoidea) represent the major group of arthropods heavily infesting *Z. mays* cultivars (Dahmardeh et al., 2010; Khatoon et al., 2010, Lewis et al., 2010). In Poland, maize plants are colonized by four aphid species: *Metopolophium dirhodum* Walk., *Rhopalosiphum maidis* F., *Rhopalosiphum padi* L. and *Sitobion avenae* F. (Pieńkosz et al., 2005; Strażyński, 2008). This group of highly specialized phloem-feeding hemipterans has coevolved multi-level adaptations facilitating exploitation of the resources provided by their host plants (Czerniewicz et al., 2011a; Sprawka et al., 2011; Chrzanowski et al., 2012; Sprawka et al., 2012; 2013a). These insects cause a wide spectrum of detrimental effects in attacked organs, including mechanical disruption of penetrated tissues, depletion of photoassimilates, and intensification of many intracellular processes (Sempruch et al., 2010; Sytykiewicz et al., 2011a; Goławska et al., 2012). Aphid salivary secretions contain a variety of hydrolytic enzymes and biologically active substances that modulate metabolic reactions within the host. In some cases, aphid infestation contributes to the activation of premature senescing process and programmed cell death in plant organs (Carolan et al., 2009; Anstead et al., 2010; Sytykiewicz et al., 2011b; Sprawka et al., 2013b). Colonization of the different host systems may lead to transmission of a wide range of pathogenic plant viruses, such as barley yellow dwarf virus (BYDV), bean leafroll virus (BLRV), beet western yellows virus (BWYV), chickpea chlorotic stunt virus (CpCSV), faba bean necrotic yellows virus (FBNYV), maize dwarf mosaic virus (MDMV), soybean dwarf virus (SbDV) and sugarcane mosaic virus (SCMV) (Makkouk and Kumari, 2009; Higashi and Bressan, 2012; Stewart et al., 2012; Zielińska et al., 2012; Ferriol et al., 2013).

Changes in the total chlorophyll (Chl *a*+*b*) content of foliar tissues is an important indicator of disturbed chloroplast development and impaired photosynthetic capacity in plants exposed to a broad spectrum of biotic and abiotic stressors. Many studies have shown that aphid infestation can trigger

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severe Chl breakdown within the host. An imbalance between biosynthesis and catabolic turnover of green pigments in plant tissues indicates profound inhibition of photosynthesis process (Heng-Moss et al., 2003; Smith et al., 2005; Botha et al., 2006; Diaz-Montano et al., 2007; Goławska et al., 2010). Portable leaf-clip-type chlorophyll meters perform rapid, repetitive and nondestructive estimates of Chl concentrations in living plants (Hawkins et al., 2009; Jinwen et al., 2009; Coste et al., 2010; Gholizadeh et al., 2011; Shrestha et al., 2012; Ghosh et al., 2013). The use of these reliable and advanced instruments for measurement of green pigment content is not restricted to controlled laboratory bioassays but can be extended to experiments in the plants' natural environment (Uddling et al., 2007; Ruiz-Espinoza et al., 2010; Neto et al., 2011; Cerovic et al., 2012). SPAD-502 chlorophyll meters have been used to obtain accurate measurements in arbitrary SPAD units reflecting absolute foliar Chl concentrations (Arunyanark et al., 2008; Marenco et al., 2009; Mielke et al., 2010; Ling et al., 2011; Shrestha et al., 2012). A significant linear relationship between SPAD readings and leaf nitrogen status in tissues of monocotyledonous species has been documented (Rostami et al., 2008; Swain and Sandip, 2010; Liu et al., 2012; Ghosh et al., 2013). To the best of our knowledge there are no published data from studies using a SPAD-502 meter to assess aphid-affected changes in the Chl content of tissues of maize plants representing a variety of genotypes. A wide range of maize cultivars presumably should reflect different levels of susceptibility to damage from aphid colonization and consequently different degrees of Chl decrement in infested leaves. In this study we compared the total Chl ($a+b$) content of seedling leaves of fifteen *Z. mays* genotypes infested by two aphid species, oligophagous *R. padi* and monophagous *S. avenae*. We also monitored the increase in size of the aphid populations on *Z. mays* plants. The relationship between the total Chl content of seedling leaves (nondestructive SPAD readings) and Chl $a+b$ concentrations measured spectrophotometrically in leaf extracts of uninfested (control) maize plants is estimated in this study.

MATERIALS AND METHODS

PLANT MATERIAL

Seeds of the fifteen *Z. mays* genotypes used in this study (Ambrozja, Anawa, Delitop, Dobosz, Eleganza, Gucio, Makila, Nana, Narew, Płomyk, Rataj, Tasty Sweet, Touran, Waza, Złota Karłowa) were obtained from Syngenta (Warsaw, Poland), Hodowla Roślin Smolice, Grupa IHAR (Smolice, Poland), KWS Polska (Poznań, Poland), Hodowla Roślin Snowidza (Snowidza, Poland) and local garden shops. The seeds were germinated and seedlings were grown in a climate chamber at 22±2°C/16±2°C (day/night), 65±5% relative humidity, 100 μM m^{-2} s^{-1} light intensity and a long-day photoperiod (L16:D8). Maize seedlings were individually planted in round plastic pots (10 cm diam., 9 cm high) filled with general-purpose horticultural substrate widely used for greenhouse experiments, with no supplementary fertilization.

APHIDS

Wingless parthenogenetic females (apterae) of the bird cherry-oat aphid (*Rhopalosiphum padi* L.) and the grain aphid (*Sitobion avenae* F.) were collected from infested cereal plants growing in Siedlce district. The aphid colonies were maintained for a year on seedlings of winter wheat cv. Tonacja in the Department of Biochemistry and Molecular Biology, University of Natural Sciences and Humanities in Siedlce. The aphids were reared in an environmental chamber under the same conditions as described for seedling cultivation. Apterous 1- or 2-day-old adults of the two aphid species were used for the bioassays.

INFESTATION EXPERIMENT

Leaves of 14-day-old seedlings of each maize cultivar were infested with adult apterous females of *R. padi* or *S. avenae* – 10 (I-10) or 40 (I-40) individuals per plant. Uninfested seedlings were the control. Aphid-stressed and control maize plants were isolated in gauze-covered plastic cylinders (20×50 cm). The density of the insect populations developing on the *Z. mays* plants was monitored 7 and 14 days post infestation (dpi).

NONDESTRUCTIVE CHLOROPHYLL MEASUREMENT

The total chlorophyll (Chl $a+b$) content of seedling leaves of the fifteen maize genotypes was measured in situ with a SPAD-502 meter (Minolta Corp., Ramsey, NJ). This instrument measures SPAD units, defined as the ratio of light transmittance at two peak wavelengths (650 and 940 nm) (Goławska et al., 2010; Mielke et al., 2010; Filipović et al., 2013). The total Chl content values given here are means of 25 SPAD readings from different locations of both leaf sides. In making the measurements we avoided the vein areas of the leaf blades. The relative concentration of green pigments in foliar tissues of aphid-damaged and control maize plants was estimated at 7 and 14 dpi.

TABLE 1. Total chlorophyll content (SPAD units, mean ±SD) in leaves of *R. padi*-stressed maize seedlings

Maize genotype	7 dpi			14 dpi		
	U	I–10	I–40	U	I–10	I–40
Ambrozja	27.6±2.1	27.5±2.0	26.8±1.5	30.1±2.9	29.2±2.3	27.5±1.8
Anawa	26.8±1.8	26.7±1.5	26.1±1.2	29.3±2.7	29.0±2.0	28.2±2.3
Delitop	28.4±3.5	27.4±2.2	25.6±1.8	35.5±3.8	33.7±2.9	30.4±2.7*
Dobosz	25.7±1.3	24.3±1.4	22.8±0.9*	27.3±1.8	24.6±1.2*	22.3±0.9*
Eleganza	24.6±1.1	23.6±1.1	22.5±0.8	28.7±2.3	27.2±1.6	25.3±1.4*
Gucio	27.5±2.3	26.0±1.7	23.2±1.3*	33.5±3.1	31.4±2.8	27.9±1.9*
Makila	28.9±3.1	26.9±1.9	25.3±1.0*	32.8±2.7	30.3±2.6	27.5±1.6*
Nana	27.2±2.5	25.7±1.4	23.2±1.4*	30.5±2.9	28.4±1.4	25.9±1.0*
Narew	22.1±0.9	22.1±0.8	22.1±0.8	25.6±1.3	25.6±1.3	25.6±1.3
Płomyk	26.2±1.7	26.0±1.8	25.1±1.4	29.4±2.5	28.8±1.7	27.5±1.2
Rataj	25.3±1.4	25.3±1.2	25.3±1.6	28.9±2.2	28.9±1.9	28.9±2.4
Tasty sweet	28.4±3.5	25.5±1.0*	22.5±1.2*	30.1±2.8	23.3±1.0*	20.5±0.9*
Touran	27.5±2.6	25.8±1.7	24.0±1.1*	32.7±3.0	30.1±2.5	28.1±2.1*
Waza	26.0±1.5	25.4±1.5	24.2±1.5	29.8±2.1	28.5±1.5	27.4±1.4
Złota Karłowa	27.9±2.8	24.6±1.1*	20.3±0.7*	28.4±2.4	23.9±1.2*	19.1±0.8*

*$P \leq 0.01$ (Student's t-test) – significant difference in leaf total Chl between aphid-stressed and uninfested seedlings; dpi – days post initial infestation; U – uninfested plants; I–10 – seedlings initially infested with 10 aphids; I–40 – seedlings initially infested with 40 aphids.

TISSUE-DESTRUCTIVE CHLOROPHYLL QUANTIFICATION

Freshly collected control samples (10 g) of seedling leaves of five maize genotypes (Ambrozja, Płomyk, Tasty Sweet, Waza, Złota Karłowa) were homogenized at 4°C for 5 min in 40 cm^3 ice-cold 80% (*v*/*v*) acetone. The extracts were filtered through two layers of gauze and then centrifuged at 5000 × g for 10 min. The pellet was discarded and the supernatants were analyzed. Chlorophyll *a* and *b* content was determined by measurement of absorbance at 644, 662 and 750 nm using a Hewlett-Packard UV-Vis spectrophotometer (model 8453). The levels of Chl *a* and *b* in the tested samples were estimated by the following empirical formulas (Ihl et al., 2000):

$$\text{Chl } a \text{ (nmol cm}^{-3}) = 11.30 \times (A_{662} - A_{750}) - 1.11 \times (A_{644} - A_{750})$$

$$\text{Chl } b \text{ (nmol cm}^{-3}) = 18.07 \times (A_{644} - A_{750}) - 2.87 \times (A_{662} - A_{750})$$

Total Chl (*a*+*b*) content in the seedling leaves is expressed in μg g^{-1} fresh weight (FW).

STATISTICAL ANALYSIS

The experiments were completely randomized and performed in three independent replicates. Each bioassay variant (aphid infestation level – exposure time) and control (nonstressed plants) comprised 10 maize seedlings of similar height. All data given are means ±SD. The significance of differences in total Chl content (SPAD units) between aphid-stressed and uninfested seedling leaves was analyzed with Student's t-test. A linear regression model was used to test the correlation between relative SPAD values and spectrophotometrically determined Chl *a*+*b* concentrations (μg g^{-1} FW) in acetone extracts of seedling leaves of uninfested maize plants. The significance of differences in the number of aphids on the maize cultivars was checked by one-way ANOVA followed by Duncan's multiple range test (DMRT). Significance was deemed at $p \leq 0.01$. The statistical calculations were done with STATISTICA 9.0 (StatSoft Poland).

RESULTS

The results on total Chl content (SPAD units) in seedling leaves of fifteen tested maize cultivars colonized by two aphid species (*Rhopalosiphum padi*, *Sitobion avenae*) are presented in Tables 1 and 2. Long-term aphid infestation (14 dpi) and higher initial number of insects (40 per plant, I–40) resulted in significantly greater diminution of chlorophyll than short-term (7-day) infestation and lower initial number of insects (10 per plant, I–10). This was true for both aphid species, but the extent of chlorophyll depletion was markedly higher in *R. padi*-stressed than in *S. avenae*-stressed plants: 14-day *R. padi* feeding caused more than 10% reduction of foliar Chl levels in seedlings of nine cultivars (Delitop, Dobosz, Eleganza, Gucio, Makila, Nana, Tasty Sweet, Touran, Złota Karłowa), whereas *S. avenae*

TABLE 2. Total chlorophyll amount (SPAD units, mean ±SD) in leaves of *S. avenae*-infested maize seedlings

Maize genotype	7 dpi			14 dpi		
	U	I–10	I–40	U	I–10	I–40
Ambrozja	27.6±3.5	27.1±2.0	25.9±1.1	30.1±2.7	29.4±1.7	28.0±1.5
Anawa	26.8±2.6	26.8±1.9	26.6±1.9	29.3±2.4	29.2±1.5	28.6±1.9
Delitop	28.4±4.2	27.2±2.5	26.3±1.5	35.5±3.2	33.9±2.8	32.4±2.8
Dobosz	25.7±3.6	24.1±1.2	22.7±0.8*	27.3±1.5	25.2±0.7	23.7±1.1*
Eleganza	24.6±2.2	24.0±0.9	23.1±0.9	28.7±1.8	27.9±0.9	26.5±1.5
Gucio	27.5±3.9	25.9±1.3	24.2±0.9*	33.5±3.0	31.2±2.5	28.2±1.6*
Makila	28.9±4.7	27.7±2.2	26.3±1.2	32.8±2.9	30.9±2.0	28.5±2.1*
Nana	27.2±2.3	26.4±1.7	25.7±1.0	30.5±2.8	29.2±1.8	27.5±1.4
Narew	22.1±1.4	22.1±0.8	22.1±0.7	25.6±1.2	25.6±1.2	25.6±1.3
Płomyk	26.2±1.7	26.0±1.5	25.2±1.2	29.4±2.7	29.0±1.4	28.2±1.6
Rataj	25.3±3.8	25.3±1.2	25.3±1.3	28.9±2.6	28.9±1.1	28.9±1.9
Tasty sweet	28.4±4.3	26.2±1.6	23.8±0.9*	30.1±2.8	27.3±0.9*	21.5±0.8*
Touran	27.5±4.1	26.1±1.3	25.0±1.0	32.7±3.0	30.4±1.8	27.9±1.4*
Waza	26.0±3.2	25.1±1.0	24.2±0.9	29.8±2.7	28.5±1.2	27.3±1.0
Złota Karłowa	27.9±2.9	25.4±1.1	23.1±0.8*	28.4±2.3	24.9±0.6*	19.4±0.6*

*$P \leq 0.01$ (Student's t-test) – significant difference in leaf total Chl between aphid-stressed and uninfested seedlings; dpi – days post initial infestation; U – uninfested plants; I–10 – seedlings initially infested with 10 aphids; I–40 – seedlings initially infested with 40 aphids.

TABLE 3. Abundance of *R. padi* populations developing on the investigated maize cultivars

Maize genotype	7 dpi		14 dpi	
	I–10	I–40	I–10	I–40
Złota Karłowa	31 ± 2.20^{a}	84 ± 4.56^{a}	122 ± 8.54^{a}	217 ± 11.52^{a}
Tasty sweet	25 ± 1.37^{b}	67 ± 3.78^{b}	98 ± 7.29^{b}	164 ± 9.38^{b}
Dobosz	14 ± 0.85^{c}	52 ± 3.15^{c}	61 ± 5.2^{c}	115 ± 6.53^{c}
Gucio	13 ± 0.62^{c}	46 ± 2.94^{d}	55 ± 4.9^{d}	98 ± 5.9^{d}
Makila	10 ± 0.38^{cd}	35 ± 2.72^{e}	33 ± 2.68^{e}	90 ± 4.74^{de}
Touran	8 ± 0.21^{d}	28 ± 2.16^{f}	30 ± 2.10^{e}	76 ± 3.92^{f}
Nana	8 ± 0.14^{d}	23 ± 1.50^{fg}	27 ± 1.19^{f}	69 ± 3.61^{g}
Eleganza	7 ± 0.19^{de}	21 ± 1.32^{g}	23 ± 1.05^{g}	52 ± 2.30^{h}
Delitop	6 ± 0.09^{e}	24 ± 1.13^{fg}	20 ± 0.94^{g}	45 ± 2.14^{hi}
Płomyk	6 ± 0.08^{e}	19 ± 0.32^{g}	2 ± 0.19^{i}	14 ± 1.27^{k}
Waza	5 ± 0.06^{e}	22 ± 0.94^{g}	16 ± 4.70^{gh}	31 ± 1.95^{j}
Ambrozja	5 ± 0.07^{e}	13 ± 0.28^{h}	3 ± 0.24^{i}	9 ± 0.86^{l}
Anawa	2 ± 0.04^{f}	8 ± 0.17^{i}	–	–
Narew	–	–	–	–
Rataj	–	–	–	–

Values are means ±SD. Means bearing the same letter within column do not differ significantly by Duncan's MRT test at $P \leq 0.01$, dpi – days post initial infestation; dash indicates the absence of aphids; I–10 – seedlings initially infested with 10 aphids; I–40 – seedlings initially infested with 40 aphids.

infestation led to such a Chl decline in tissues of six cultivars (Dobosz, Gucio, Makila, Tasty Sweet, Touran, Złota Karłowa). The decrement in green pigment levels was highest in insect-stressed seedlings of Złota Karłowa and Tasty Sweet (~30% decrease at 14 dpi, I–40). The Chl content of the Rataj and Narew cultivars was unaffected by aphid infestation. The other investigated cultivars varied in the extent of chloro-

TABLE 4. Abundance of *S. avenae* populations developing on the investigated maize cultivars

Maize genotype	7 dpi		14 dpi	
	I–10	I–40	I–10	I–40
Złota Karłowa	17±1.38 a	38±2.19 a	86±5.17 a	109±7.25 a
Tasty sweet	14±0.65 b	33±1.57 b	57±4.39 b	96±6.47 b
Dobosz	9±0.58 c	29±1.35 c	44±3.12 c	85±6.12 c
Gucio	7±0.52 d	24±1.18 d	30±2.85 d	52±4.83 d
Makila	7±0.49 d	20±0.83 e	23±2.67 e	34±2.71 e
Touran	6±0.42 de	16±0.62 g	19±1.34 ef	30±2.38 e
Nana	5±0.36 e	19±0.71 ef	17±0.92 f	25±1.94 f
Eleganza	4±0.30 f	13±0.57 h	11±0.76 g	22±1.73 fg
Delitop	4±0.21 f	11±0.48 i	15±0.80 f	19±1.24 g
Waza	4±0.27 f	6±0.23 jk	10±0.64 g	17±0.96 g
Płomyk	3±0.18 g	8±0.35 j	1±0.03 h	6±0.49 h
Ambrozja	2±0.15 h	5±0.19 k	1±0.08 h	1±0.09 i
Anawa	1±0.07 i	3±0.18 l	–	–
Narew	–	–	–	–
Rataj	–	–	–	–

Values are means ±SD. Means bearing the same letter within column do not differ significantly by Duncan's MRT test at $P \leq 0.01$. dpi – days post initial infestation; dash indicates the absence of aphids; I–10 – seedlings initially infested with 10 aphids; I–40 – seedlings initially infested with 40 aphids.

phyll loss in response to aphid feeding. Long-term *R. padi* colonization (14 dpi, I–40) caused Chl reduction ranging from 3.7% (Anawa) to 18.3% (Dobosz); the decline in *S. avenae*-attacked seedlings ranged from 2.4% (Anawa) to 13.2% (Dobosz).

The *R. padi* populations on the *Z. mays* seedlings reached higher abundance than the *S. avenae* populations (Tabs. 3, 4). Adult apterous females of both aphid species produced no offspring on Narew and Rataj plants, and there were no aphids on seedlings of cv. Anawa at 14 dpi, indicating this genotype's high resistance to these aphids. Aphid density per plant at 14 dpi was low on Ambrozja (9 *R. padi*, 1 *S. avenae*) and Płomyk (14 *R. padi*, 6 *S. avenae*). The other tested cultivars differed greatly in aphid abundance. For both aphid species at 14 dpi it was highest on Złota Karłowa (217 *R. padi*, 109 *S. avenae*) and Tasty Sweet (164 *R. padi*, 96 *S. avenae*).

We used the uninfested controls to check the accuracy of the SPAD readings versus spectrophotometric measurements of total Chl content in tissues of all tested *Z. mays* varieties. Table 5 presents the correlations between mean SPAD values and chlorophyll *a*+*b* amounts ($\mu g \cdot g^{-1}$ FW) in uninfested seedling leaves. Statistical analyses showed a strong positive correlation between the noninvasive SPAD readings and spectrophotometrically determined chlorophyll concentrations in acetone extracts prepared from seedlings of the studied *Z. mays* cultivars ($p \leq 0.01$).

TABLE 5. Correlation between total chlorophyll content (SPAD units) and chlorophyll *a*+*b* amount ($\mu g \cdot g^{-1}$ FW) in uninfested (control) seedling leaves of selected maize cultivars

Maize genotype	R^2
	SPAD–Chl abs
Ambrozja	0.98
Anawa	0.93
Delitop	0.95
Dobosz	0.91
Eleganza	0.94
Gucio	0.92
Makila	0.95
Nana	0.93
Narew	0.96
Płomyk	0.95
Rataj	0.96
Tasty sweet	0.94
Touran	0.92
Waza	0.95
Złota Karłowa	0.90

SPAD-Chl abs represents the relationship between SPAD values and spectrophotometrically determined chlorophyll *a*+*b* concentrations in acetone extracts of seedling leaves of uninfested maize plants; FW – fresh weight; R^2 – coefficient of determination (linear regression model).

DISCUSSION

There are many recent reports on the destructive effects of aphid infestation on the functioning of various mono- and dicotyledonous plant systems (e.g., Czerniewicz et al., 2011b; Sempruch et al., 2011; Chen et al., 2012; Goławska et al., 2012; Łukasik et al., 2012; Sempruch et al., 2012; Bak et al., 2013; Riedell et al., 2013). One such effect is chlorosis (Heng-Moss et al., 2003; Smith et al., 2005; Botha et al., 2006; Diaz-Montano et al., 2007; Goławska et al., 2010). Highly bioactive effector molecules in aphid salivary gland secretions may cause severe damage to infested plants (Cooper et al., 2010; Cooper et al., 2011; Nicholson et al., 2012; Rao et al., 2013). Although *Z. mays* is one of the most important model plants and a valuable crop, there has been no published assessment of aphid-evoked changes in the total chlorophyll concentration of seedlings representing a wide range of genotypes. In this study we assessed the tolerance of a diverse range of maize varieties to infestation by aphids (*Sitobion avenae*, *Rhopalosiphum padi*). *S. avenae* is a monophagous and monoecious insect infesting a large array of plants of the family Poaceae (Gao and Liu, 2013; Li et al., 2013; Svobodová et al., 2013), whereas the life cycle of oligophagous *R. padi* involves seasonal migration between two groups of taxonomically distant host plants. The primary hosts of *R. padi* are a few members of the genus *Prunus*, and the secondary hosts include a broad range of cereals and grasses (Stoetzel and Miller, 2001; Aslan and Uygun, 2005; Łukasik 2009; Coulette et al., 2013).

In the maize cultivars we examined the extent of chlorophyll depletion in response to aphid feeding depended on the genotype. Oligophagous *R. padi* caused more Chl *a*+*b* loss than monophagous *S. avenae* in the stressed seedlings. Riedell and Blackmer (1999) reported similar results in wheat cv. Sharp infested by greenbug (*Schizaphis graminum* Rond.) and Russian wheat aphid (*Diuraphis noxia* Mordv.): Chl *a*+*b* content was lower under aphid feeding than in the nonstressed control, and the loss was greater under *S. graminum* than under *D. noxia* feeding. More green pigment was lost from mature (fully expanded) wheat leaves than from young leaves. In our work the extent of Chl loss in foliar tissues depended on the initial abundance of hemipterans on the maize seedlings and the duration of exposure to feeding. Similarly, Heng-Moss et al. (2003) found that prolonged *D. noxia* feeding on wheat leaves caused more Chl loss (~5-fold decrease after 13 days) than after short-term infestation. In their study, Russian wheat aphid feeding caused significant loss of chlorophyll content in susceptible Betta wheat but aphid-resistant Betta-*Dn1* wheat showed only slightly lower total Chl than uninfested plants. Chl levels in *D. noxia*-tolerant Betta-*Dn2* and control plants were quite similar, but 11 and 13 days after the initial infestation the aphid-infested Betta-*Dn2* leaves showed higher Chl content than the uninfested control. Haile et al. (1999) reported that *D. noxia*-tolerant wheat line PI262660 completely recovered its photosynthetic capacity 7 days after aphids were removed from the colonized plants; there was no such compensation reaction in tissues of susceptible Arapahoe wheat or introduction line PI137738, showing a high level of antibiosis. In experiments by Smith et al. (2005), *D. noxia*-stressed leaves of wheat cv. Wichita showed a ~53% decline in total Chl content (SPAD units) versus control leaves; isogenic lines PI 372129 and P243781 showed Chl loss of 53% and 21% respectively. In leaves of two *Triticum aestivum* cultivars, Tugela-DN (resistant) and Tugela (susceptible), Botha et al. (2006) found that *D. noxia* feeding caused a gradual decrement of total chlorophyll versus the uninfested controls during 16 days of colonization. The decrease was greater in the susceptible cultivar than in Tugela-DN. After 7 days of infestation by the pea aphid (*Acyrthosiphon pisum* Harr.), Chl *a*+*b* content was lower in leaves of four legume hosts: alfalfa (*Medigaco sativa* L. var. Radius), clover (*Trifolium pratense* L. var. Bona), pea (*Pisum sativum* L. var. Tulipan) and vetch (*Vicia faba* L. var. Jaga) (Goławska et al., 2010). Interestingly, in that experiment the decline was lower after 17 days than after 7 days.

Even a small population of the soybean aphid (*Aphis glycines* Mats.) significantly inhibited photosynthetic processes in attacked soybean plants (Macedo et al., 2003): there was high suppression of gas exchange in plant tissues under an intermediate level of aphid infestation (20–49 aphids per leaflet), and medium and high *A. glycines* densities (≥50 aphids per leaflet) caused an increase of F_o (nonvariable fluorescence) values, indicating damage to the PSII reaction system. In susceptible *T. aestivum* cultivars colonized by *D. noxia*, Burd and Elliott (1996) found elevated F_o levels, which may disrupt electron transfer in the PSII system and reduce D1 protein synthesis. Soybean aphid feeding altered the quenching coefficients (qP, coefficient of photochemical fluorescence quenching; qN, coefficient of non-photochemical fluorescence quenching) in tissues of infested soybean plants, according to Macedo et al. (2003), who suggested that aphid colonization may suppress the xanthophyll cycle, which participates in protecting photosystem II under stress conditions by modulating the thylakoid membrane pH gradient. Haile and co-workers (1999) reported substantial reduction of photosynthetic efficiency in wheat plants attacked by *D. noxia*. Some authors have speculated that the Chl loss in aphid-injured plants

may be connected with elevated biosynthesis of different defensive secondary metabolites such as saponins (Haile et al., 1999; Goławska et al., 2010).

Feeding by *S. graminum* biotype E on leaves of winter wheat cv. Sturdy and Largo for one hour resulted in plasmolysis and initial degeneration of cell organelles of the parenchyma; also observed was accumulation of plastoglobules and chloroplast swelling (Morgham et al., 1994). Gonzáles et al. (2002) reported ultrastructural damage to chloroplasts in leaves of *Sorghum halepense* L. infested with *Sipha flava* Forb.; light microscopy showed increased chloroplast volume, loss of grana structures and aggregation of starch granules. Rapid and uncontrolled release of high amounts of photoactive chlorophyll molecules from thylakoid membranes can unbalance the intracellular redox state, and the photodynamic action of these pigments may induce massive oxidative damage of organelles. Some researchers have shown increased chlorophyllase activity in plants subjected to aphid colonization (Ni et al., 2002; Ciepiela et al., 2005; Sytykiewicz 2007), wounding, and the action of phytopathogens (Stangarlin and Pascholati, 2000; Kariola et al., 2005). Chlorophyllase (chlorophyll chlorophyllidohydrolase, EC 3.1.1.14) is a chloroplast membrane biocatalyst responsible for hydrolytic turnover of free chlorophyll substrates into the corresponding chlorophyllide forms (Sytykiewicz et al., 2013). Plant species have evolved a variety of mechanisms participating in chlorophyll degradation and chlorophyllase regulation (Lee et al., 2010) A wide spectrum of isozymes perform different biological functions in plants. For example, isoform 1 of chlorophyllase alleviates excessive levels of reactive oxygen species generated in leaves of the model plant *Arabidopsis thaliana* stressed with the pathogenic microorganisms *Erwinia carotovora* and *Alternaria brassicicola* (Kariola et al., 2005).

The saliva produced by wingless *R. padi* females contains a variety of biocatalysts (e.g., cellulase, polygalacturonase, pectinesterase, proteolytic enzymes) participating in cleavage of chemical compounds of the cell wall and cytoplasmic membranes, leading to their disruption; *R. padi* infestation of winter wheat cv. Bezostaja led to the occurrence of large areas of hydrolysis in the foliar mesophyll (Urbańska and Niraz, 1990). Those results are in accordance with observations from Sytykiewicz (2007) indicating that *R. padi* can feed from the sieve tubes as well as parenchyma cells in bird cherry leaves. The intracellular mode of aphid stylet penetration through plant tissues can be associated with tonoplast disintegration, with subsequent release of toxic constituents from vacuoles (Urbańska et al., 2006). The salivary sheaths of *S. avenae*, on the other hand, were found to be deposited intercellularly within the epidermis and mesophyll of winter wheat cv. Sakva seedlings (Urbańska 2010). The absence of salivary diffusion into the cells surrounding the aphid stylet pathway and the presence of aphid saliva within vascular bundles confirm that *S. avenae* uses a typical phloem-feeding mode of stylet penetration. Matsiliza and Botha (2002) noted intercellular stylet penetration by *Sitobion yakini* (East.) in the epidermis and parenchyma of barley (*Hordeum vulgare* L.) var. Clipper, and found that it fed only on thin-walled sieve elements and not the thick-walled vascular bundles. Speculating from the sparse published data, we suggest that the more severe depletion of Chl from maize leaves infested with oligophagous *R. padi* than with monophagous *S. avenae* may be due to the difference in the aphids' modes of stylet penetration. The species-specific chemical composition of aphid salivary secretions should also differently affect the content of photosynthetic pigments in colonized plants.

In this work we confirmed a strong positive correlation between total Chl content (relative SPAD units) in seedling leaves and Chl $a+b$ concentrations in acetone leaf extracts of the tested maize cultivars. We also demonstrated that the SPAD-502 chlorophyll meter reliably reflects the amount of foliar Chl in *Zea mays* plants. A significant advantage of this technique is that it can quantify Chl nondestructively in plant tissues in situ. Our results provide a platform for further work to estimate a broad range of photosynthetic capacity parameters and to assess the relative expression of genes involved in chlorophyll biosynthesis and catabolic turnover in tissues of aphid-susceptible and tolerant maize plants. The detailed mechanisms underlying aphid-triggered modulation of the host's metabolism remain unclear and await more study.

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