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RELATIONSHIP BETWEEN APHID INFESTATION AND CHLOROPHYLL CONTENT IN FABACEAE SPECIES

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We determined the chlorophyll *a* and *b* levels (SPAD readings) in uninfested leaves and in leaves after 7 and 17 days of aphid infestation in four Fabaceae species (*Pisum sativum* L., *Vicia faba* L., *Trifolium pretense* L, *Medicago sativa* L.). Feeding by pea aphids *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae) caused significant loss of chlorophyll *a* and *b* in the infested plants. Uninfested leaves on both short- and long-infestation plants had significantly higher chlorophyll *a* and *b* than infested leaves.

Key words: chlorophyll *a*+*b*, Fabaceae, *Acyrthosiphon pisum*, SPAD values.

INTRODUCTION

One of the most serious pests of commercial Fabaceae crops is the pea aphid *Acyrthosiphon pisum* Harris (Homoptera: Ahididae) (Farag et al., 2007). The pea aphid damages crops directly and is a vector of more than thirty viral diseases, including bean yellow mosaic virus, red clover vein mosaic virus and pea streak virus (Barnett and Diachun, 1986; Jones and Proudlove, 1991). All viral diseases reduce yield of Fabaceae (Cuperus et al., 1982; Garlinge and Robartson, 1998).

Chlorophyll content is one of the most important parameters in the relationships between plants and herbivores. Chlorophyll levels change during plant development (Costa et al., 2001), and can alter in response to a wide variety of stresses (Fanizza et al., 1991; Samdur et al., 2000; Lawson et al., 2001). Chlorophyll catabolism is equal to global chlorophyll synthesis, and can be reduced by insect feeding, nutritional deficiencies and pathogen infections (Ni et al., 2002). Chlorophyll loss caused by herbivore feeding is not fully understood, although herbivory-caused chlorophyll loss has been described (Carbera et al., 1994; Ni et al., 2002; Heng-Moss et al., 2003). Two well-known aphid species causing chlorophyll loss are the Russian wheat aphid *Diuraphis noxia* (Mordvilko) (Hemiptera: Aphididae) on wheat *Triticum aestivum* L. (Burd and Elliott,1996) and the greenbug *Schizaphis graminum* (Rondani) on sorghum *Sorghum bicolor* (L.) Moench (Girma et al., 1998) and wheat. Leaf feeding by sap-feeding insects causes chlorosis and necrosis, leading to significant crop loss worldwide (Ni et al., 2001). Herbivore-caused leaf chlorosis in growing plants should be studied in detail, as chlorophyll fluorescence might prove useful as an indicator of plant responses to stressors including insect damage (Haile et al., 1999).

In this work we determined the concentrations of photosynthetic pigment (chlorophyll *a* and *b*) in uninfested and aphid-infested legumes. No similar studies have been conducted to assess the effect of feeding by this species on chlorophyll levels in such a wide range of hosts; this research represents an initial effort to characterize the effect *A. pisum* feeding has on chlorophyll *a*+*b* loss in legumes.

MATERIALS AND METHODS

PLANT MATERIAL

The experiments used four legume species: pea *P. sativum* L. var. Tulipan, vetch *V. faba* L. var. Jaga, clover *T. pratense* L. var. Bona, and alfalfa *M. sativa* L. var. Radius. Seed samples of alfalfa were obtained from the Plant Breeding and Acclimatization Institute (IHAR) in Radzików/Błonie (near Warsaw, Poland),

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TABLE 1. Total chlorophyll concentration (SPAD units) (means ±SD) in uninfested (control) and infested legumes 7 days after pea aphid infestation

Plant species	Total chlorophyll (SPAD units)				
	Uninfested plants	Infested plants	t	d.f.	*P*
Pisum sativum	39.82±2.39	36.57±6.96	3.81	98	<0.001
Vicia faba	38.55±6.97	31.10±7.14	5.27	98	<0.001
Trifolium pratense	38.84±4.67	28.88±6.17	9.10	98	<0.001
Medicago sativa	30.89±5.15	26.10±6.09	3.57	73	<0.001

Student's *t*-test, comparing means between SPAD units in the legume plants.

and the others were bought from Horticultural Plant Breeding, Seed Production and Nursery in Ożarów Mazowiecki (Warsaw, Poland). Seed samples were germinated in a climate chamber and kept at 21±1°C and 70% relative humidity (RH) under a 16 h photoperiod. The plants were grown in plastic pots (7×7×9 cm) with fine garden soil commonly used for greenhouse experiments, one plant per pot. The plants were watered regularly and not additionally fertilized.

APHIDS

The pea aphids *Acyrthosiphon pisum* Harris used in the experiments came from stock culture kept at the University of Natural Sciences and Humanities in Siedlce, Poland. The aphids were reared on pea seedlings *P. sativum* L. var. Tulipan (Fabaceae) in an environmental chamber (21±1°C, 16 h photoperiod, 70% RH). They were transferred to the studied legumes for one generation (Apablaza and Robinson, 1967). Then the adult apterous females were used in the experiments.

ENTOMOLOGICAL OBSERVATIONS

The entomological observations were made on isolated plants in plastic cylinders (50×50×50 cm) in an environmental chamber (21±1°C, 16 h photoperiod, 70% RH). The experiment was initiated when the legume plants were 3 days old. Then 25 adult apterous females were placed on each plant of each legume species. At two intervals of infestation (7 and 17 days) the pea aphids (adult apterae, larvae and adult alatae) were counted on 10 plants of each legume.

SPAD METER READINGS

The chlorophyll content in tissues of single leaves of the legume plants (infested, and uninfested 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 I_f are respectively the currents from red and infrared detectors with sample in place and with no sample in place (I_{or} and I_{of}) (Fanizza et al., 1991). Five SPAD readings were averaged for each leaf to represent one observation. The results represent average measurements of chlorosis for five leaves on ten plants of each legume.

STATISTICAL ANALYSIS

Comparisons of total chlorophyll concentration (SPAD units) between infested and control legume plants were subjected to two-tailed and unpaired Student's *t*-test. One-way ANOVA was carried out for number of aphids on the studied plants, followed by Duncan's test. Correlations between chlorophyll *a* and *b* content and number of aphids were calculated. All statistical analyses used Statistica for Windows v. 6.0 (StatSoft, 2003).

RESULTS

Average values of SPAD readings decreased under the stress of *A. pisum* feeding. For uninfested plants at the first measurement they ranged from 30.89 for *M. sativa* to 39.82 for *P. sativum*, and at the second measurement from 29.65 for *M. sativa* to 36.80 for *V. faba* (Tabs. 1, 2). Aphid infestation significantly reduced the level of chlorophyll *a* and *b* irrespective of the duration of infestation. At 7 days of infestation, *A. pisum* caused significant loss of chlorophyll *a* and *b* versus the values for uninfested plants of all studied legumes (Tab. 1). The decrease during the shorter period of infestation was greatest in vetch and clover (Tab. 1). At 17 days of infestation, *P. sativum* plants still showed a slight, nonsignificant decrease of chlorophyll *a*+*b*; at that interval the differences in chlorophyll *a*+*b* content between control and infested plants were significant for *V. faba*, *T. pratense* and *M. sativa* (Tab. 2). At 17 days the prolonged infestation produced stronger stress reactions in tissues of vetch and clover (Tab. 2).

TABLE 2. Total chlorophyll concentration (SPAD units) (means ±SD) in uninfested (control) and infested legumes 17 days after pea aphid infestation

Plant species	Total chlorophyll (SPAD units)		t	d.f.	*P*
	Uninfested plants	Infested plants			
Pisum sativum	34.87±5.64	33.64±3.70	0.66	98	NS
Vicia faba	36.80±4.69	30.68±7.29	4.99	98	<0.001
Trifolium pratense	35.39±3.94	27.19±3.33	11.25	98	<0.001
Medicago sativa	29.65±3.88	23.80±2.70	6.75	73	<0.001

Student's *t*-test, comparing means between SPAD units in the legume plants.

TABLE 3. Abundance (means ±SD) of pea aphids on the studied legume species

Plant species	Days after infestation*	
	7 days	17 days
Pisum sativum	134.70±15.05 a	213.60±38.84 a
Vicia faba	119.70±14.46 b	150.60±24.53 b
Trifolium pratense	10.90±2.33 c	2.60±0.70 c
Medicago sativa	6.50±1.35 c	1.70±0.95 c

*Values with different letters within columns differ significantly by Duncan's test at p<0.01. Values are means from 10 plants on the sampling date for each legume.

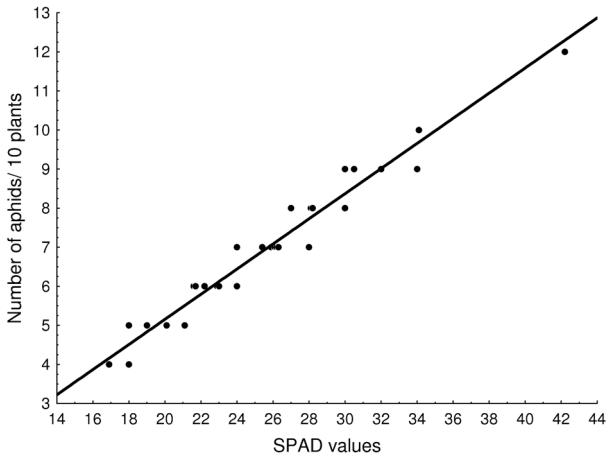

Fig. 1. Relationship between SPAD values and abundance of pea aphid on alfalfa 7 days after infestation (y = -1.282 + 0.32166 * A-SPAD, R= 0.96).

There were clear differences in pea aphid population development between the studied legume plants. The number of pea aphids was highest on pea plants and lowest on alfalfa (Tab. 3).

The number of aphids on the studied plants was significantly correlated with SPAD readings only for alfalfa after seven days of infestation (R = 0.96; p < 0.05; Pearson correlations) (Fig. 1).

DISCUSSION

In this work, *A. pisum* infestation was shown to reduce chlorophyll levels in several species of the *Fabaceae* family. This indicates symptoms of chlorosis in the infested plants and adds important new data for *A. pisum*, an aphid species whose genome has been sequenced (Ollivier et al., 2010; IAGC, 2010) and which has been identified as a serious pest of legume crops. In *Vitis vififera*, Fanizza et al. (1991) reported a drop in chlorophyll content under a different stress treatment. Leaves of stressed plants apparently synthesized less chlorophyll pigment. In our work the studied Fabaceae cultivars differed in the effect of pea aphid feeding on chlorophyll *a*+*b* concentrations. Other studies also show that responses differ. Rafi et al. (1996) found that *D. noxia* reduced chlorophyll levels in resistant cereals, while Heng-Moss et al. (2003) reported that total chlorophyll and carotenoid concentrations differed among Betta wheat isolines in response to *D. noxia* feeding: infested Betta-*Dn2* plants had higher levels of chlorophylls and carotenoids than uninfested plants, but infested Betta-*Dn1* plants had the same chlorophyll and carotenoid levels as uninfested plants. Burd and Eliot (1996) found a significant decline in chlorophyll concentration in infested leaf tissue of *D. noxia*-susceptible wheat and barley, whereas total chlorophyll concentration was not significantly affected by *D. noxia* in resistant wheat or barley. In our study the amount of chlorophyll (as SPAD units) differed between infested and uninfested plants. The chlorophyll *a*+*b* concentration in uninfested Fabaceae plants was significantly higher than in aphid-infested Fabaceae plants. Aphid feeding adversely affected the plants and directly affected chlorophyll content. Interestingly, the chlorophyll concentration in pea plant tissues at 17 days of infestation was similar to the level in the respective uninfested plants. This indicates that aphid feeding may have less effect on chlorophyll loss in this species in the long term.

The exact mechanism by which aphids affect plant metabolism is not fully understood, but Heng-Moss et al. (2003) speculated that by feeding mainly on phloem tissue the aphids change the pH either on the luminal side of the thylakoid membrane, preventing the formation of zeaxanthin, or on the stromal side where regeneration of violaxanthin takes place. Burd and Elliott (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 over-reduction in the system. One of two pathways of natural degradation of chlorophyll *a* is the oxidative bleaching pathway (Janave, 1997). Ni et al. (2001) showed that feeding by chlorosis-eliciting *D. noxia* or the non-chlorosis-eliciting bird cherry-oat aphid *R. padi* did not cause any changes in the oxidative bleaching pathway or chlorophyllase activity as compared with uninfested plants. However, Ni et al. (2002) showed that *D. noxia* feeding caused significant loss of chlorophyll *a* and *b* in the damaged regions: on two different sampling dates, undamaged regions of *D. noxia*-infested leaves showed significantly higher chlorophyll *a* and *b* concentrations than in uninfested leaves. *D. noxia*-infested wheat leaves showed significantly greater Mg-dechelatase activity than *R. padi*-infested and uninfested wheat leaves. Those assays of chlorophyll degradation enzymes indicated that *D. noxia* feeding significantly increased Mg-dechelatase activity in damaged and non-damaged leaf regions as compared with uninfested leaves, on both sampling dates. Mg-dechelatase activity in aphid-damaged regions was significantly higher than in undamaged leaf regions. This suggested that undamaged regions of *D. noxia*-infested leaves compensated for the pigment loss in the damaged regions, and that Mg-dechelatase activity changed dynamically from a localized response to a systemic response as the infestation period prolonged. Those assays clearly demonstrate the dynamic nature of plant responses to aphid feeding, which were initially localized and limited to the site of feeding, and then transformed to a whole-leaf response, indicating that *D. noxia* may elicit signaling chemical transduction between damaged and undamaged regions of infested leaves (Ni et al., 2002).

Haile et al. (1999) and Heng-Moss et al. (2003) found a significant decline of the photosynthetic rate in aphid-injured leaves and speculated that it may have resulted from increased synthesis of chemical defense compounds in response to herbivory. The decline in chlorophyll concentration found in our experiment may also be due to increased production of defensive compounds. Among the studied Fabaceae species the number of aphids was lowest on *M. sativa* plants, indicating that they are less attractive to *A. pisum*. Earlier work demonstrated that alfalfa plants are resistant to *A. pisum*. Alfalfa contains numerous secondary plant metabolites, including carotenoids (Livingstone et al., 1980) and saponins (Oleszek, 1999, 2000; Oleszek et al., 1992; Stochmal et al. 2001a, b). They have been suggested as possible chemical defensive agents of alfalfa plants against generalist herbivores (Nozzolillo et al., 1997; Oleszek, 1999; Osbourn, 2003). Goławska et al. (2008) found that saponins play an important role in alfalfa defense against the pea aphid. Szynkarczyk et al. (2001) showed that high-saponin alfalfa lines reduced pea aphid performance and phloem sap ingestion. Goławska et al. (2006) found big differences in pea aphid feeding behavior on alfalfa lines with low and high saponin content. Three alfalfa saponins (zanhic acid tridesmoside, 3-GlcA, 28AraRhaXyl medicagenic acid glycoside, 3-GlcA, 28AraRha medicagenic acid glycoside) were found to be inhibitors of *A. pisum* feeding (Goławska, 2007).

The present data on the effect of *A. pisum* feeding on chlorophyll *a*+*b* concentrations in Fabaceae tissues show changes in chlorophyll *a*+*b* concentrations in response to *A. pisum* feeding, and suggest the presence of a feeding-induced stress response in the studied legume species. SPAD measurements comprise a reliable, quick and nondestructive method useful not only for study of chlorophyll accumulation rates in different parts of the same leaf but also for determination of insect-plant interactions. Further research should investigate the mechanisms of acceptance/resistance for the Fabaceae species and explore the potential use of photosynthetic pigments as markers for identifying germplasm resistant to *A. pisum* and other chlorosis-causing insects.

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