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RESEARCH

Induced Senescence Promotes the Feeding Activities and Nymph Development of *Myzus persicae* (Hemiptera: Aphididae) on Potato Plants

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ABSTRACT. The effect of dark-induced senescence on *Solanum tuberosum* L. (Solanales: Solanaceae) plants was assessed on the feeding behavior and performance of the green peach aphid, *Myzus persicae* Sulzer (Hemiptera: Aphididae). Senescence was induced by covering the basal part of the plant with a black cloth for 5 d, avoiding the light passage, but keeping the apical buds uncovered. The basal part of control plants was covered with a white nonwoven cloth. The degree of senescence was determined by measuring the chlorophyll content of the covered leaves. The performance and feeding behavior of *M. persicae* were studied on the uncovered nonsenescent apical leaves. The aphid's performance was evaluated by measuring nymphal mortality and prereproductive time. Aphid feeding behavior was monitored by the electrical penetration graph technique. In plants with dark-induced senescence, the aphids showed a reduction in their prereproductive time. Aphids also spent more time ingesting sap from the phloem than in control plants and performed more test probes after the first sustained ingestion of phloem sap. These data suggest that *M. persicae*'s phloem activities and nymph development benefit from the nutritional enrichment of phloem sap, derived from dark-induced senescence on potato plants. The induced senescence improved plant acceptance by *M. persicae* through an increase in sap ingestion that likely resulted in a reduction in developmental time.

Key Words: *Solanum tuberosum*, green peach aphid, electrical penetration graph

The green peach aphid, *Myzus persicae* Sulzer (Hemiptera: Aphididae), is a piercing–sucking insect that ingests plant phloem fluid through modified mouthparts (stylets). To select the host plant, aphids penetrate plant tissue by inserting their stylets between the cell walls of the epidermis and the mesophyll (Tjallingii and Hogen Esch 1993). On the way to the phloem, the cells of the mesophyll may be punctured by the stylets. When aphids reach the sieve tube, they test the cellular content and, if it is preferred, they begin to ingest phloem sap. The process of host acceptance takes several hours, during which the aphid and the plant interact closely (Pollard 1973, Tjallingii and Hogen Esch 1993, Miles 1999, Cherqui and Tjallingii 2000, Tjallingii 2006, Will and van Bel 2006). By using the electrical penetration graph (EPG) method, it has been possible to thoroughly study plant penetration by the aphid's stylets (McLean and Kinsey 1964; Tjallingii 1978, 1985, 1988). EPG signals have been correlated with aphid's activities as well as with tissue locations of the stylet's tips (Tjallingii and Hogen Esch 1993), and thus constitute a valuable tool to study plant and aphid inter- and intracellular interactions at the plant tissue level. For example, EPGs have been used to study induction of resistance or susceptibility of the plant by aphids (Prado and Tjallingii 2007), differences in behaviors among the different species and morphs of aphids (Boquel et al. 2011), mechanisms of virus transmission by aphids (Martin et al. 1997, Fereres and Moreno 2009, Tjallingii et al. 2010), and effects of different host plants on behavior of different aphid species (Alvarez et al. 2006, Le Roux et al. 2010).

M. persicae has a broad host range and attacks many economically important plants. It is the primary aphid species infesting potato crops (Kuroli and Lantos 2006), but some examples of plant resistance have been reported. Alvarez et al. (2006, 2014) found that potato cultivar 'Kardal' (*Solanum tuberosum* L., Solanales: Solanaceae) presents

resistance against *M. persicae*, but the resistance diminishes with the age of the leaf and susceptibility is related to the induction of foliar senescence. Kardal has resistance to *M. persicae* at the phloem level, and *M. persicae* is not able to colonize the young leaves but can survive and reproduce on mature to senescent leaves.

Foliar senescence is a developmentally programmed degeneration process that constitutes the final step of leaf development (Buchanan-Wollaston 1997, Lim et al. 2007). It is an active process and involves a highly regulated decrease in photosynthesis, chloroplast degradation, macromolecule (proteins, nucleic acid and lipids) degradation, loss of chlorophyll, and nutrient mobilization to different destinations of the plant (Buchanan-Wollaston 1997, Page et al. 2001, Liu et al. 2008). These physiological changes are evident in differential expression of genes related to various functional categories and activation of signaling pathways (He et al. 2002; Buchanan-Wollaston et al. 2003, 2005; Gepstein et al. 2003; Breeze et al. 2008). As a result of protein degradation (i.e., Rubisco), amino acids are mobilized and enrich the phloem sap. In this sense, leaf senescence could favor aphids, as a higher content of amino acids in the sap could stimulate ingestion and improve nutrition.

There is evidence that aphid infestation alters expression of plant genes that are potentially involved in the conversion of the feeding site into metabolic sinks (Moran and Thompson 2001, Moran et al. 2002, Alvarez et al. 2013). This suggests that sap intake by aphids may be hydraulically equivalent to plant sinks, such as fruits or roots. However, the interaction established between the plant and the aphid is more complex than a natural source–sink interaction of plant tissues (Douglas 2003).

In wheat and oat plants infested with *Schizaphis graminum* (Rondani), the concentration of amino acids, particularly glutamine,

increases locally and systemically. The amino acid glutamine is the main form of transport of nitrogen from senescing leaves to sink organs (Kamachi et al. 1992, Watanabe et al. 1997, Buchanan-Wollaston et al. 2003).

It has been demonstrated that a large number of cellular metabolism genes change their expression both in senescence and under aphid herbivory. Many of these differently expressed genes influence changes in the physiological state of the plants, from source to sink, which suggests that aphids manipulate the host plant response for their own benefit. It has also been suggested that these changes are necessary to prepare the feeding site and may mediate the aphid's ability to establish a colony on a particular host plant genotype, thus promoting a plant susceptibility to aphid feeding (Moran and Thompson 2001; Moran et al. 2002; De Vos et al. 2005, 2007; De Vos and Jander 2009; Alvarez et al. 2013). On the other hand, it has also been proposed that plants may use senescence as a resistance mechanism. In *Arabidopsis*, the hypersenescent phenotype related to *constitutive expresser of PR genes5 (cpr 5)* *hypersenescent1* mutant seems to be associated with the increase on plant resistance on this mutant, as aphids reproduction and growth rate were reduced (Pegadaraju et al. 2005, 2007).

Here, senescence was induced in the basal part of plants to evaluate the effect that it has on aphid's performance and feeding behavior on the apical uncovered leaves. The hypothesis is that the turnover and remobilization of nutrients from the senescent basal part of the plant improve performance and feeding behavior of *M. persicae* on *S. tuberosum* plants, promoting plant acceptance.

Materials and Methods

Plants and Aphids. Potato plant 'PO 97.11.9' was used for the experiments because of its susceptibility to *M. persicae*. This cultivar was provided by the germplasm bank of INTA Balcarce, Buenos Aires, Argentina. The propagation of plants was performed in vitro on Murashige and Skoog medium (pH 5.8) including vitamins and 3% sucrose. After 2 wk, the rooted plantlets were transferred to soil in a growth chamber at $22 \pm 3^\circ\text{C}$, about 70% relative humidity (RH), and a photoperiod of 16:8 (L:D) h. All plants were evaluated in the preflowering stage, and no tuberization processes were evident or ever noticed for the *Solanum* genotype used.

M. persicae colonies were reared on radish (*Raphanus sativus* L.). All aphids used in the experiments came from a colony maintained at the Natural Sciences Department, National University of Salta, Argentina. This colony was initiated from a single virginoparous apterous aphid collected in the field in 2009. Colonies were reared in a climate chamber at $22 \pm 2^\circ\text{C}$, 30–40% RH, and a photoperiod of 16:8 (L:D) h to induce parthenogenesis. A new colony was started every week, and newly molted adult apterae aphids were used for the experiments.

Senescence Induction and Chlorophyll Determination. Senescence was induced by covering the basal part of 4-wk-old *S. tuberosum* plants for 5 d with black cloth bags that block the passage of light but allow gas exchange. The control plants were covered with bags of nonwoven porous white cloth that allow the passage of light and gas exchange. Both in the test plants and control plants, the apical buds remained uncovered until the end of the evaluation. The grade of senescence was determined in the covered leaves by measuring chlorophyll a, chlorophyll b, and total chlorophyll content by spectrophotometry. Chlorophylls were extracted from potato leaves with buffered aqueous acetone (80% v/v containing 2.5 mM buffer phosphate, pH 7.8). Leaf discs were cut (16 mm), weighed, and then ground to complete dissolution in a mortar with 2 ml of buffered acetone. The homogenate was collected and washed three times (each washing with 1.5 ml of the same buffered acetone). After centrifugation, the supernatant was taken to a final volume of 8 ml and absorbance was measured at 663 and 646 nm with a spectrophotometer (MetroLab 325 BD). Chlorophyll content was then calculated and expressed as microgram of chlorophyll per milliliter of solution per gram of fresh foliar tissue as described by Lichtenthaler

(1987) and Porra et al. (1989). Chlorophyll content from one randomly chosen leaf of the covered portion of the plant was determined from four randomly selected plants from both the dark-induced and control treatment.

Aphid Performance. The performance of *M. persicae* on senescent and control plants was evaluated by recording nymphal mortality and prereproductive time (from newly born nymph to adult). Two recently molted apterous adults (1–3 d old) were transferred together to the uncovered apical leaves and enclosed in 20-mm-diameter clip cages on the abaxial side of the leaves of each plant. Four or five clip cages per plant and eight plants per treatment were used. The cages were placed on the first to fifth fully expanded leaf, counting from the top of the plant, taking care not to break the stem. After 24 h, adults and nymphs were removed, leaving one newly born nymph per leaf. The condition of this individual, dead or alive, was recorded daily until the production of the first progeny. Experiments were conducted at $22 \pm 2^\circ\text{C}$, approximately 60% RH, and a photoperiod of 16:8 (L:D) h. In total, 35 insects for the senescent plants and 30 for the control plants were evaluated on eight plants for each treatment. Each plant supported four to five cages in the apical uncovered portion of the plant.

Aphid Feeding Behavior: EPG. The DC-EPG technique (Tjallingii 1985, 1988) was used to monitor probing of apterous young adult aphids. Two plants of each treatment were placed in a Faraday cage; probing behavior including ingestion of two aphids on each plant was recorded simultaneously for 6 h. Aphids were placed on the abaxial side of a leaf, which was nearly fully expanded in the uncovered bud of senescent and control plants. Before exposure to the plant, the aphid was attached to the electrode. The electrode was a 2- to 3-cm-long gold wire (20 μm in diameter), conductively glued (water-based silver glue) to the dorsum—while immobilized by a vacuum-suction device. The other end of the gold wire was attached to a 3-cm-long copper wire (0.2 mm in diameter) and connected to the input of the head stage amplifier with a 1-G Ω input resistance and 50 \times gain. The plant electrode, a 2-mm-thick, 10-cm-long copper rod, was inserted into the soil of the potted plant and connected to the plant voltage output of the EPG device (Giga-8, manufactured by Wageningen University, Wageningen, The Netherlands). In addition to the plants, the aphids and the first-stage amplifiers were set up in a Faraday cage. The recording was started immediately after aphid wiring, at $20 \pm 2^\circ\text{C}$, under constant light in the laboratory, and about 1 h after collecting the aphids from the colony. Signals of eight aphids, two per plant on each setup, were acquired and recorded. Data acquisition and waveform analysis were mediated by PROBE 3.0 software (Laboratory of Entomology, Wageningen University, The Netherlands).

EPG Waveforms, Waveform Patterns, and Parameters. The EPG signals were analyzed by distinguishing the following waveform phases, types, or subtypes: 1) waveform C, stylet pathway phase; waveform E, phloem phase, was separated into 2) waveform E1, sieve element salivation and 3) waveform E2, phloem sap ingestion with concurrent salivation; 4) waveform E1e, putative extracellular watery salivation; 5) waveform F, derailed stylet mechanics (stylet penetration difficulties); and 6) waveform G, active uptake of water from xylem elements (Tjallingii 1990a,b). Waveform events were defined as single, uninterrupted occurrences of any of the above waveform types or subtypes. Waveforms were characterized into five broad categories of EPG variables following the nomenclature of Tjallingii (<http://www.epgsystems.eu>): 1) number of times waveforms occurred per insect, 2) average duration of waveform per event or per insect, 3) maximum duration of waveform for each insect, 4) time to the first occurrence of a waveform from the start of the experiment, and 5) number or percentage of aphids performing sustained phloem ingestion (sE2: uninterrupted period of E2 longer than 10 min). These variables were calculated for each plant treatment (BAZ Excel workbook for calculation of Aphid EPG variables by Edgar Schliephake, <http://www.epgsystems.eu/downloads>).

php). The terminology used for some variables was modified according to Backus et al. (2007) and Sarria et al. (2009).

Statistical Analysis. The content of chlorophyll a, chlorophyll b, and total chlorophyll of basal covered leaves was compared between senescent and control plants using Student’s *t*-test for independent samples. Contrasts were made between the means of covered versus control plants.

Nymphal mortality between senescent and control plants was compared by Fisher’s exact test, and prereproductive time was compared by Mann–Whitney *U* rank sum test.

The EPG variables were compiled individually for each aphid and then averaged across all insects in each treatment to provide means and SEM. In the case of multiple waveform events or probes, duration was averaged from the average per individual. The Mann–Whitney *U* rank sum test was used to test for differences between senescent and control data from *S. tuberosum* because EPG variables did not follow a normal distribution. The Fisher’s exact test was applied for the analysis of the number of aphids showing sustained ingestion (E2 event, longer than 10 min) between treatments. All statistical analyses were performed using InfoStat Professional v2011p software (<http://www.infostat.com.ar>; Di-Rienzo et al. 2011).

Results

Chlorophyll Determination. Leaves of plants covered with black cloth for 5 d were etiolated and showed a reduced content of chlorophyll a, chlorophyll b, and total chlorophyll when compared with leaves of control plants (Student’s *t*-test, *P* ≤ 0.05; Fig. 1).

Aphid Performance. Differences were observed on the aphid’s performance between plants with dark-induced senescence and control plants. The prereproductive time on the apical uncovered leaves of plants (with induced senescence in the basal leaves) was shorter than on apical uncovered leaves of control plants (Mann–Whitney *U*-test, *P* ≤ 0.0027). On the other hand, a small (12%), but nonsignificant, difference (Fisher’s exact test, *P* = 0.440) was found for the percentage of nymphs surviving on the plants covered with a black cloth (Table 1).

Feeding Behavior. The EPG variables were classified into five groups as described by Tjallingii (<http://www.epgsystems.eu/downloads.php>) considering its relation to the aphid’s activity or stylet location in the plant tissue (Table 2). Nonprobing and overall probing behavior during pathway periods (stylet route to the phloem, Table 2, variables 1–7) differed between senescent and control plants only in the number of test probes (stylet withdrawals within 3 min) after first sus-

tained phloem feeding (variable number 4), which was significantly higher for plants with dark-induced senescence.

Significant differences between the two treatments were found in phloem contact-phase variables. In plants with dark-induced senescence, the number of salivation followed by ingestion (E12) events per insect (Table 2, variable 10) was significantly higher than in control plants. Total time in phloem phase event per insect was significantly longer in senescent-induced plants (Table 2, variable 21), and this was due to a significantly longer time in the phloem sap ingestion phase (Table 2, variable 20). In contrast, the time in phloem salivation (E1, variable 19) did not differ between treatments. The percentage of aphids showing sustained phloem feeding (a period of E2 longer than 10 min) in plants with dark-induced senescence was higher than in the control plants (82 vs. 60%, respectively, Table 2, variable 25). However, this difference was not statistically significant.

Discussion

Senescence is a very complex process involving the expression of thousands of genes and many signaling pathways that lead to metabolic changes, such as hydrolysis of macromolecules and a massive remobilization of the hydrolyzed molecules that finally enrich the phloem sap (Buchanan-Wollaston et al. 2003, 2005; Liu et al. 2008). The contention proposed is that the physiological changes in the tissues and particularly in the composition of the phloem sap due to induced senescence have an impact in the aphid’s performance and probing behavior favoring *M. persicae*, as a higher content of amino acids in the sap could stimulate ingestion.

The induction of senescence by covering the basal part of *S. tuberosum* plants shortened the prereproductive time of *M. persicae* nymphs feeding on the apical uncovered leaves (Table 1). On the other hand, induced senescence did not affect the nymph mortality. As such, it can be inferred that the suitability of *S. tuberosum* (cultivar PO97.11.9) as a host for *M. persicae* was not affected by dark-induced senescence. Induced senescence also had an impact on *M. persicae* feeding behavior. This was evident in differences found in EPG phloem phase variables. Aphids spent more time ingesting phloem sap in the apical buds of plants with dark-induced senescence than on control plants, which suggests that induction of senescence promotes aphid ingestion. It has previously been shown that susceptibility or resistance in *Solanum* genotypes is mostly related to phloem factors (Alvarez 2007; Le Roux et al. 2008, 2010) and that foliar senescence allowed *M. persicae* to settle on senescent leaves of resistant cultivar Kardal, so it can be inferred that the increased ingestion in senescent-induced plants is related to phloem changes (Alvarez et al. 2014). Aphids performed significantly more test probes after the first sustained E2. The role of these test probes is still unknown, although it has been shown that in their way to the phloem, aphids repeatedly insert their stylets into the mesophyll cells performing brief probes, probably to prepare the tissue for feeding (Tjallingii 1985, 1988, 1995), and it is likely that they do it after sustained feeding. The plant acceptance by aphids was slightly enhanced by the induction of senescence, as the percentage of aphids that reached sustained phloem feeding in plants with dark-induced senescence was higher (82%) than the control plants (60%), although differences did not reach statistical significance. There are reports of aphid–plant interaction showing that modifications of plant physiology benefit aphids.

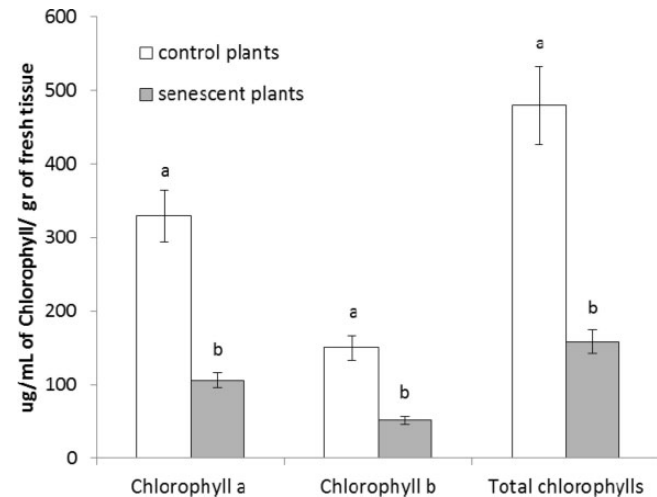


Fig. 1. Content of chlorophyll a, chlorophyll b, and total chlorophyll in control versus senescent leaves of *S. tuberosum*. Different letters indicate statistical differences (Student’s *t*-test, *P* ≤ 0.05).

Table 1. Prereproductive time of *M. persicae* (in days) and percent nymphal mortality during the prereproductive period on the apical leaves of senescent and control *S. tuberosum* plants

	Senescent (n = 35)	Control (n = 30)	<i>P</i> value
Prereproductive time	6 ± 0.3	7 ± 0.5	0.0027 ^a
Nymph mortality	31	43	0.440 ^b

^aMann–Whitney test significant differences at *P* ≤ 0.05.
^bFisher’s exact test significant differences at *P* ≤ 0.05.

Table 2. Mean (\pm SEM) EPG variables, as means per insect, during 6 h of monitoring of *M. persicae* probing behavior in the apical leaves of *S. tuberosum* plants with induced senescence and control plants

Related to	Variables N°	EPG variable	Unit	Control (n° = 15)	Senescent (n = 17)	P value
Nonprobing and overall probing	1	Time from start of the experiment to first probe per insect	min	2.34 \pm 0.58	2.14 \pm 0.52	0.5087
	2	Duration of first probe per insect	min	4.94 \pm 3.03	3.74 \pm 2.42	0.2342
	3	Number of probes per insect	#	45 \pm 24	31 \pm 3	0.0961
	4	Number of probes (shorter than 3 min) after first sustained E2	#	1 \pm 1	9 \pm 2	0.0032*
	5	Time from the start of first probe to first sustained E2	min	231.6 \pm 30.38	182.04 \pm 27.03	0.3356
Pathway + probing	6	Number of pathway events per insect	#	54 \pm 24	41 \pm 3	0.082
	7	Duration of pathway (excluding E1e, F, and G) per event	min	4.27 \pm 0.43	3.98 \pm 0.32	0.2494
Phloem contact phase	8	Number of single E1 events (without subsequent E2)	#	6 \pm 1	7 \pm 1	0.5689
	9	Number of E1 events (both single E1 events + those followed by E2)	#	10 \pm 2	13 \pm 5	0.0914
	10	Number of E12 events (combined E1 and E2)	#	2 \pm 0	3 \pm 1	0.0466*
	11	Duration single E1 per event	min	2.8 \pm 0.37	3.07 \pm 0.36	0.8355
	12	Duration of E1 per event	min	3.34 \pm 0.53	3.12 \pm 0.44	0.8949
	13	Duration of E2 per event	min	19.35 \pm 12.58	19.73 \pm 3.74	0.0699
	14	Duration of E12 per event	min	36.96 \pm 15.27	20.15 \pm 3.76	0.6501
	15	Maximum duration (for each insect) of single E1 per event	min	5.46 \pm 0.75	7.36 \pm 1.31	0.584
	16	Maximum duration (for each insect) of E1 per event	min	13.30 \pm 4.16	10.53 \pm 2.07	0.8355
	17	Maximum duration (for each insect) of E12 (combined E1 and E2)	min	44.39 \pm 16.50	50.46 \pm 11.29	0.2899
	18	Total duration of E1 by insect	min	34.30 \pm 7.67	36.57 \pm 5.27	0.4168
	19	Duration of single E1 by insect	min	18.92 \pm 4.38	20.79 \pm 3.67	0.8061
Cell puncture	20	Total duration of E2 by insect	min	34.70 \pm 16.40	76.04 \pm 13.80	0.0285*
	21	Total duration of E12 by insect	min	50.08 \pm 16.40	91.82 \pm 13.80	0.0313*
	22	Total number of potential drops per insect	#	141 \pm 13	159 \pm 13	0.385
	23	Duration potential drop per event per insect	s	4.64 \pm 0.17	4.36 \pm 0.12	0.0642
	24	Total duration of potential drop by insect	min	10.68 \pm 0.99	11.56 \pm 0.97	0.5087
Host acceptance	25	Number and percentage of aphids that performed sustained E2	# (%)	9 (60%)	14 (82%)	0.2433 ^b

^aEPG replicates.^bFisher's exact test.*Mann-Whitney test. Significant differences, $P \leq 0.05$.

Sandström et al. (2000) found that *S. graminum* and *Diuraphis noxia* (Mordvilko) induce chlorotic lesions in their host and thus alter the plant physiology, increasing the concentration of amino acids, especially glutamine. In contrast, *Rhopalosiphum padi*, which does not induce macroscopic changes in its host plants, seems to have little effect on the amino acid content of host phloem (Sandström et al. 2000). These changes are likely to be nutritionally advantageous for the aphids, but further research of the effect of these changes on aphid fitness is needed. Another example is the black pecan aphid, *Melanocallis caryaefoliae* (Davis), which feeds on mature and senescent foliage and prefers to settle on leaf discs showing chlorosis from previous feeding by the same aphid than on control discs (Cottrell et al. 2009). On the other hand, it has also been proposed that plants may use senescence as a resistance mechanism. In the *Arabidopsis* hypersenescent mutant [*PR genes5 (cpr 5) hypersenescence1*], plant resistance seems to be associated with senescence, as aphids counts were lower and the growth rate was reduced when *M. persicae* fed on this mutant. However, it is likely that this mutant is not a good host for *M. persicae* because it expresses senescence constitutively and therefore has a lower chlorophyll content. Furthermore, this mutant spontaneously undergoes cell death (Pegadaraju et al. 2005, 2007). Moreover, it is likely that it does not redistribute and recycle nutrients by manipulating the plant physiology that is necessary for aphid feeding (Sandström et al. 2000; Walling 2000, 2008, 2009; Alvarez 2007; Cottrell et al. 2009).

Covering the leaves with the black cloth proved to be an effective method to induce senescence, as the chlorophyll content was

significantly reduced (Fig. 1). There is evidence that shows chlorophyll content can be used to indicate the initiation of senescence and that the regreening ability of etiolated leaves depends on the duration of the dark phase and the plant species (reviewed by van Doorn 2005). In *Arabidopsis* and wheat, etiolation is irreversible after 2 and 4 d of darkness, respectively (Wittenbach 1977, Weaver and Amasino 2001). There have been concerns expressed about the validity of the use of dark-induced senescence as a method to mimic the senescence process because, although many of the events that occur in dark-treated leaves are known to mirror those that occurred in developmental senescence, there also many differences shown as differently expressed genes related to hormone signaling, metabolism and mobilization of nitrogen and lipids, and sugar starvation (Becker and Apel 1993, Weaver et al. 1998, Lin and Wu 2004, Buchanan-Wollaston et al. 2005). Although the limitations of the dark-induction approach should be taken into account, it still seems to be the most appropriate approach for studies on foliar senescence-insect interaction.

In summary, the findings reported here support the hypothesis that plant acceptance by aphids may be improved by foliar senescence and aphids may induce senescence for their own benefit by preparing the tissues for feeding activity, promoting an increase in the nutritional quality of the phloem sap, and coping with phloem resistance factors (Zhu-Salzman et al. 2004; Alvarez 2007; Alvarez et al. 2013). Despite this evidence, it is not clear what mechanism the aphids utilize to induce senescence, although senescence-related genes are upregulated during *M. persicae* attack on *Solanum* plants (Alvarez et al. 2013).

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