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

Cristina R. Machado-Assefh,1,2 Alejandro F. Lucatti,2 and Adriana E. Alvarez2,3

1Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), CCT-Salta, Av. Bolivia 5150, 4400 Salta, Argentina
2Cátedra de Química Biológica, Facultad de Ciencias Naturales, Universidad Nacional de Salta (UNSa). Avda. Bolivia 5150. CP 4400. Salta, Argentina
3Corresponding author, e-mail: alvarez@natura.unsa.edu.ar

J. Insect Sci. 14(155): 2014; DOI: 10.1093/jisesa/ieu017

**ABSTRACT.** The effect of dark-induced senescence on *Solanum tuberosum* L. (*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. 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
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 expressor of PR genes5 (cpr 5) hypersenescent51 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 ± 3 ºC, 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 ± 2 ºC, 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 apterous 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 Lichtenhaler (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 ± 2 ºC, 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-GΩ input resistance and 50× 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 ± 2 ºC, 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, stylopathy 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).
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 v2011.0p 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 2).

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 sustained 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 senescence-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 senescence-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.

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) | P value |
|----------------------|--------------------|-----------------|---------|
| Prereproductive time  | 6 ± 0.3            | 7 ± 0.5         | 0.0027* |
| Nymph mortality      | 31                 | 43              | 0.440p  |

* Mann–Whitney test significant differences at P ≤ 0.05.

p Fisher’s exact test significant differences at P ≤ 0.05.
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 dark-
ness, 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.
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
foliar sap for feeding (Buchanan-Wollaston et al. 2005). Although
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).

Table 2. Mean (± 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 ± 0.58 | 2.14 ± 0.52 | 0.5087 |
| | 2 | Duration of first probe per insect | min | 4.94 ± 3.03 | 3.74 ± 2.42 | 0.2342 |
| | 3 | Number of probes per insect | # | 45 ± 24 | 31 ± 3 | 0.0961 |
| | 4 | Number of probes (shorter than 3 min) after first sustained E2 | # | 1 ± 1 | 9 ± 2 | 0.0032* |
| | 5 | Time from the start of first probe to first sustained E2 | min | 231.6 ± 30.38 | 182.04 ± 27.03 | 0.3356 |
| Pathway + probing | 6 | Number of pathway events per insect | # | 54 ± 24 | 41 ± 3 | 0.082 |
| | 7 | Duration of pathway (excluding E1a, F, and G) per event | min | 4.27 ± 0.43 | 3.98 ± 0.32 | 0.2494 |
| Phloem contact phase | 8 | Number of single E1 events (without subsequent E2) | # | 6 ± 1 | 7 ± 1 | 0.5689 |
| | 9 | Number of E1 events (both single E1 events + those followed by E2) | # | 10 ± 2 | 13 ± 5 | 0.0914 |
| | 10 | Number of E12 events (combined E1 and E2) | # | 2 ± 0 | 3 ± 1 | 0.0466* |
| | 11 | Duration single E1 per event | min | 2.8 ± 0.37 | 3.07 ± 0.36 | 0.8355 |
| | 12 | Duration of E1 per event | min | 3.34 ± 0.53 | 3.12 ± 0.44 | 0.8949 |
| | 13 | Duration of E2 per event | min | 19.35 ± 12.58 | 19.73 ± 3.74 | 0.0699 |
| | 14 | Duration of E12 per event | min | 36.96 ± 15.27 | 20.15 ± 3.76 | 0.6501 |
| | 15 | Maximum duration (for each insect) of single E1 per event | min | 5.46 ± 0.75 | 7.36 ± 1.31 | 0.584 |
| | 16 | Maximum duration (for each insect) of E1 per event | min | 13.30 ± 4.16 | 10.53 ± 2.07 | 0.8355 |
| | 17 | Maximum duration (for each insect) of E12 (combined E1 and E2) | min | 44.39 ± 16.50 | 50.46 ± 11.29 | 0.2899 |
| | 18 | Total duration of E1 by insect | min | 34.30 ± 7.67 | 36.57 ± 5.27 | 0.4168 |
| | 19 | Duration single E1 by insect | min | 18.92 ± 4.38 | 20.79 ± 3.67 | 0.8061 |
| | 20 | Total duration of E2 by insect | min | 34.70 ± 16.40 | 76.04 ± 13.80 | 0.0285* |
| | 21 | Total duration of E12 by insect | min | 50.08 ± 16.40 | 91.82 ± 13.80 | 0.0031* |
| Cell puncture | 22 | Total number of potential drops per insect | # | 141 ± 13 | 159 ± 13 | 0.385 |
| | 23 | Duration potential drop per event per insect | s | 4.64 ± 0.17 | 4.36 ± 0.12 | 0.0642 |
| | 24 | Total duration of potential drop by insect | min | 10.68 ± 0.99 | 11.56 ± 0.97 | 0.5087 |
| Host acceptance | 25 | Number and percentage of aphids that performed sustained E2 | # (%) | 9 (60%) | 14 (82%) | 0.2433* |

*EPG replicates.
#Fisher’s exact test.
* Mann–Whitney test. Significant differences, P ≤ 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, especi-
ally 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
aphid, but further research of the effect of these changes on aphid fit-
ness is needed. Another example is the black pecan aphid,
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
aphid, but further research of the effect of these changes on aphid fit-
ness is needed.
Tjallingii, W. F. 1990b. Stylet penetration parameters from aphids in relation to host-plant resistance. Symp. Biol. Hung. 39: 411–419.

Tjallingii, W. F. 1995. Aphid-plant interactions: what goes on in the depth of the tissues? Proc. Exp. Appl. Entomol. 6: 163–169.

Tjallingii, W. F. 2006. Salivary secretions by aphids interacting with proteins of phloem wound responses. J. Exp. Bot. 57: 739–745.

Tjallingii, W. F., and T. Hogen Esch. 1993. Fine structure of aphid stylet routes in plant tissues in correlation with EPG signals. Physiol. Entomol. 18: 189–200.

Tjallingii, W. F., E. Garzo, and A. Fereres. 2010. New structure in cell puncture activities by aphid stylets: a dual-mode EPG study. Entomol. Exp. Appl. 135: 193–207.

van Doorn, W. G. 2005. Plant programmed cell death and the point of no return. Trends Plant Sci. 10: 478–483.

Walling, L. L. 2000. The myriad plant responses to herbivores. J. Plant Growth Regul. 19: 195–216.

Walling, L. L. 2008. Avoiding effective defenses: strategies employed by phloem-feeding insects. Plant Physiol. 146: 859–866.

Walling, L. L. 2009. Adaptive defense responses to pathogens and insects. Plant Innate Immun. 51: 551–612.

Watanabe, A., N. Takagi, H. Hayashi, M. Chino, and A. Watanabe. 1997. Internal Glu/Glu ratio as a potential regulatory parameter for the expression of a cytosolic glutamine synthetase gene of radish in cultured cells. Plant Cell Physiol. 38: 1000–1026.

Weaver, L., S. Gan, B. Quirino, and R. Amasino. 1998. A comparison of the expression patterns of several senescence-associated genes in response to stress and hormone treatment. Plant Mol. Biol. 37: 455–469.

Weaver, L. M., and R. M. Amasino. 2001. Senescence is induced individually darkened Arabidopsis leaves, but inhibited in whole darkened plants. Plant Physiol. 127: 876–886.

Will, T., and A. J. E. van Bel. 2006. Physical and chemical interactions between aphids and plants. J. Exp. Bot. 57: 729–737.

Wittenbach, V. A. 1977. Induced senescence of intact wheat seedlings and its reversibility. Plant Physiol. 59: 1039–1042.

Zhu-Salzman, K., R. A. Salzman, J. E. Ahn, and H. Koiwa. 2004. Transcriptional regulation of sorghum defense determinants against a phloem-feeding aphid. Plant Physiol. 134: 420–431.

Received 30 November 2012; accepted 26 April 2013.