Ascorbic acid modulates aphid success on potato: a role for reactive oxygen species?

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Introduction

One of the proposed mechanisms of plant defence against insect herbivory is the production of pro-oxidant allelochemicals[1], the toxicity of which are maximised by appropriate regulation of their redox state[2]. In the current work, the capacity of phloem feeding aphids to grow and develop on potato leaves with altered redox status was investigated. Aphids reared on leaves with a high ascorbic acid (AsA) concentration were able to develop and reproduce more rapidly than those reared on low AsA leaves. This presentation outlines initial investigations into the mechanism of aphid success on high AsA leaves.

Materials and Methods

Myzus persicae genotype G was cultured on excised leaves of potato (*Solanum tuberosum* cv. Desiree) in cages as shown in Fig. 1. Cages were maintained at 18°C with a 16h light, 8h dark photoperiod.

AsA was determined by HPLC according to Tedone *et al.*[3] following extraction of plant or insect tissues in 5% metaphosphoric acid containing 5 mM tris(2-carboxyethyl)phosphine hydrochloride.

Figure 1 – Aphid rearing cages used in this study

Superoxide dismutase, catalase, monodehydroascorbate reductase, dehydroascorbate reductase and glutathione reductase activities were extracted from plant and insect tissues in 50 mM potassium phosphate buffer pH 7.4, 100 mM NaCl, 2 mM EDTA, 1 mM $MgCl_2$. Ascorbate peroxidase activity was extracted in the same buffer containing 2 mM AsA. All activities were measured spectrophotometrically using standard assay procedures. Peroxidase activities were extracted and measured according to Perez *et al.*[4] using *o*-phenylenediamine as substrate.

Results

1. L-Galactono-1,4-lactone Enhances Leaf AsA and Aphid Fecundity

In order to manipulate the redox balance of potato leaves, different concentrations of GalL were supplied in the buffer reservoir of aphid cages. A single one day old nymph was inoculated into each cage and culture continued for 15 days. At the end of the culture period, the number of aphids present was recorded and the AsA concentration of leaves, aphids and aphid honeydew determined.

- GalL treatment increased leaf AsA concentration
- Increased leaf AsA resulted in increased honeydew AsA
  → GalL enhanced phloem AsA
- Increased leaf AsA weakly enhanced aphid AsA
- Increased leaf AsA resulted in enhanced aphid fecundity

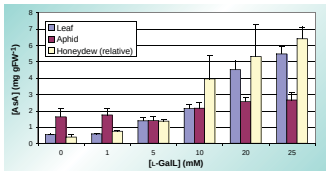


Figure 2 – Effect of GalL on AsA concentration in leaf and aphid tissues

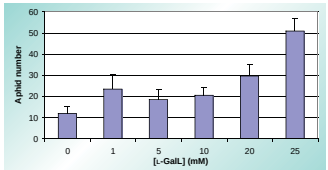


Figure 3 – Effect of GalL on growth and reproduction of *M. persicae* on potato leaves.

2. Aphids Cultured on High AsA Leaves Feed more Consistently

Previous studies suggest *M. persicae* require dietary AsA[5], therefore increased aphid fecundity may have been the result of increased availability of dietary AsA. Conversely, the large amounts of AsA excreted in honeydew suggested excess AsA was available. To determine whether changes in phloem AsA concentration affected aphid feeding behaviour, aphids were observed over 48 h by time lapse photography.

- Aphids feeding on 25 mM L-GalL treated leaves spent more time feeding and less time exploring
  → Consistent with greater palatability of high-AsA phloem
  → Mechanism for faster growth and reproduction rates?

Figure 4 - Still from time-lapse video of aphids feeding on untreated or L-GalL treated potato leaves

3. Aphids Contain AsA-Glutathione Cycle Enzymes but Lack Ascorbate Peroxidase

High AsA phloem may be more palatable to aphids due to decreased concentrations of oxidised defence compounds. Reduction of such compounds may occur either passively through spontaneous chemical reaction or actively by enzyme-catalysed reactions. Aphid extracts were therefore tested for antioxidant enzyme activities.

- Aphids contain enzyme activities for the reduction of DHA via the AsA-glutathione cycle
- Aphids lack ascorbate peroxidase for the detoxification of H_2O_2
- Reduction of oxidised defence compounds using AsA?

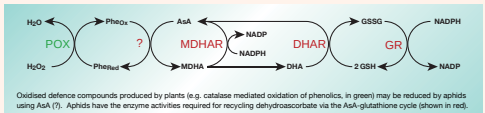
Enzyme[a]	Specific Activity (μmol min⁻¹ mg protein⁻¹) Potato Leaf	Aphid
SOD[b]	4.49 ± 0.08	10.05 ± 0.54
CAT	30.79 ± 4.72	21.56 ± 1.89
POX	5.81 ± 0.70	1.54 ± 0.25
APX	0.20 ± 0.03	ND[c]
MDHAR	0.18 ± 0.01	0.27 ± 0.02
DHAR	0.19 ± 0.03	0.02 ± 0.00
GR	0.05 ± 0.01	0.01 ± 0.00

[a] SOD, superoxide dismutase; CAT, catalase; POX, peroxidase; APX, ascorbate peroxidase; MDHAR, monodehydroascorbate reductase; DHAR, dehydroascorbate reductase; GR, glutathione reductase
[b] μg protein required for 50% inhibition of NADH oxidation rate in a superoxide generating assay
[c] ND = not detected

Table 1 – Antioxidant enzyme activities in potato leaves and aphids

Conclusions

In this preliminary study we have shown that aphids feeding on leaves with enhanced AsA concentrations have increased growth and reproduction rates. Aphids feeding on high AsA leaves had slightly higher concentrations of AsA however; concentrations of AsA excreted in honeydew correlated more closely with leaf AsA concentrations suggesting excess AsA was excreted. Aphids feeding on high AsA leaves spent a greater proportion of time feeding and less time exploring the leaf surface allowing for greater nutrient uptake. These data are consistent with reduced prevalence of oxidised defence compounds (e.g. phenolics). Determination of aphid enzyme activities suggests a possible role for AsA in detoxification of oxidised defence compounds by aphids (Fig. 5).



Oxidised defence compounds produced by plants (e.g. catalase mediated oxidation of phenolics, in green) may be reduced by aphids using AsA (?). Aphids have the enzyme activities required for recycling dehydroascorbate via the AsA-glutathione cycle (shown in red).

Figure 5 – Potential pathway for the detoxification of oxidised defence compounds by aphids

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