

Presence of glycoalkaloids in phloem of *Solanum* plants

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Recibido: 02-11-10 Aceptado: 11-07-11

Abstract

Solanum glycoalkaloids have been reported to play an important role in chemical defense against insects. However, their defensive role against phloem-feeding insects like aphids has been controversial. This study demonstrates the presence of α -solanine and α -chaconine –the two major potato glycoalkaloids– in the phloem of potato (*Solanum tuberosum*) and a wild relative (*S. commersonii*). These glycoalkaloids are also present in *M. euphorbiae* aphids reared on potato plants, demonstrating that these phloem-feeding insects do ingest these metabolites when reared on their host plants. These compounds are known to be toxic against many insect species, including aphids. The presence of glycoalkaloids in the phloem of *Solanum* plants and their ingestion by *M. euphorbiae* from their host plant phloem indicated the tolerance of this aphid specie to these metabolites and one or more detoxification mechanism may be involved.

Keywords: *Solanum tuberosum*, Solanaceae, aphids, glycoalkaloids, phloem.

Presencia de glicoalcaloides en el floema de plantas del género *Solanum*

Resumen

Los glicoalcaloides presentes en el género *Solanum* han sido reportados por desempeñar un rol de defensa química contra insectos. Sin embargo, su rol en defensa contra insectos que se alimentan del floema, como los áfidos, es motivo de controversia. En este estudio se demuestra la presencia de α -solanina y α -chaconina, los dos principales glicoalcaloides presentes en *S. tuberosum*, tanto en el floema de esta especie como en el floema de *S. commersonii*, una especie silvestre del mismo género. De igual manera, los mismos fueron determinados en la especie de áfidos *M. euphorbiae* cuando fueron alimentados de sus plantas hospederas, *S. tuberosum*. Estos compuestos son conocidos por ser tóxicos contra muchas especies de insectos, como los áfidos, por lo que la presencia de glicoalcaloides en el floema de las plantas de *Solanum* y su ingestión a través de este por *M. euphorbiae*, indica la tolerancia de esta especie de áfidos a dichos metabolitos. Esto hecho estaría indicando que uno o más mecanismos de desintoxicación se ven involucrados en el proceso.

Palabras clave: *Solanum tuberosum*, Solanaceae, áfidos, glicoalcaloides, floema.

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Introduction

Some of *Solanum* glycoalkaloids appear to play a role in mediating the adaptation of insects to their hosts, and several investigations have pointed out the importance of glycoalkaloids in the resistance of *Solanum* plants against aphids (1- 3).

The presence in the phloem sap of many secondary compounds is known however no report on the presence of the glycoalkaloids in phloem sap of *Solanum* plants has ever been published, despite its importance in assessing the biological significance of several observed effects of phloem sap on aphids (1, 3). In this study the presence of glycoalkaloids in the phloem of *S. tuberosum* and of a wild relative, *S. commersonii*, as well as in the tissues of aphids (*Macrosiphum euphorbiae*) feeding on potato are reported, and the possible implications are discussed.

Materials and methods

Insect and plant materials

Individuals of *M. euphorbiae* were collected in potato fields near Montevideo. Aphids were kept on potato plants (cv. Kennebeck) in a Phytotron at $24 \pm 2^\circ\text{C}$ with a photoperiod of 18L: 6D and 15,000 lux.

S. commersonii Dun. ex Poir plants were collected at Los Cilindros near Montevideo. *S. tuberosum* tubers (potato cultivar 386480.9 CIP) were kindly provided by Dr. F. Vilaró. Plants were cultivated from these tubers, and when the plants reached a height of 40 cm they were translocated to a Phytotron, in the conditions indicated above.

Phloem sap collection

Phloem sap collection was carried out as described by Groussol et al. (4). Twenty leaves were collected from completely developed plants by cutting through the base of the petioles, and distributed to vials (five leaves per vial) containing collection solution (20 mM EDTA, 0.05 M phosphate

buffer, pH 7.4). The ends were recut, rinsed with the same solution, and transferred to a new vial also containing collection solution (1 mL). Exudation was allowed for 24 h in the dark and the solutions were then stored at -20°C until further processing. The phloem sap solution was lyophilized, redissolved in AcOH 5% and then passed through a preconditioned Sep-Pak C18 cartridge to purify the glycoalkaloids fraction following the procedure suggested by Ferreira et al. (5). The glycoalkaloids were eluted with MeOH and the solvent removed under vacuum.

The same experiment was carried out using collection solution in the absence of EDTA as control experiment.

Extraction and preliminary clean up of glycoalkaloids from aphids

Insects (fresh weight: 100-300 mg) were collected from the upper leaves of *S. tuberosum* plants into culture tubes (13 × 100 mm) and immediately sonicated in AcOH 5% for 24 hr, and centrifuged at 4,000 rpm for 10 min. The supernatant was applied to a preconditioned Sep-Pak C18 cartridge, washed with water, and the glycoalkaloids were eluted with MeOH (5 mL). The solvent was removed under vacuum to give the glycoalkaloid extracts.

Analysis of glycoalkaloid

Each phloem extract and solution obtained as control experiment were analyzed by TLC according to Ferreira et al. (5) and them were analyzed by HPLC-MS.

The LC system consisted of an HP 1100 pump and Jasco AS-950-10 autosampler. The flow-rate was 0.2 mL/min. The LC column used was a Symmetry C-8 (3 μm , 150 × 2.1 mm I.D.). The mobile phase was a binary mixture of 0.02 M aqueous ammonium formate buffer, pH 3.8 (solvent A), and 0.02 M of the same buffer in MeOH (solvent B). The gradient was initially A: B 70:30, then it was linearly brought to 100% B over 25 min. It

was brought back to A: B 70:30 and kept at this ratio for 10 min before the next injection.

MS was performed on an ion trap instrument (Finnigan LCQ Classic, San Jose, CA, USA), using an ESI interface in positive mode. The heated capillary temperature was 225 °C, and nitrogen was used as sheath and auxiliary gas at flow rates of 60 and 2 (arbitrary units), respectively. The spray voltage was set to 4.5 kV yielding a typical spray current of 5 mA. Mass calibration for the ion trap instrument was performed using the standard mixture of caffeine, Met-Arg-Phe-Ala tetrapeptide and Ultramark 1620. Alternating full scans in the range of m/z 840-1200 and data dependent MS/MS scans were used. In MS/MS experiments, an isolation width of 1.3 Da was used and the relative collision energy was 35 % (arbitrary units). The mass spectra data were processed by averaging and background subtraction.

Results

When *S. tuberosum* and *S. commersonii* phloem saps were analyzed by HPLC both showed a double peak with the same retention times as the two main glycoalkaloids in potato, α -solanine (compound **1**; ret. time, 12.16 min) and α -chaconine (compound **2**; ret. time, 12.24 min) (figure 1).

The mass spectra (electrospray ionization) of the corresponding glycoalkaloids were characterized by the ions produced through three major fragmentation processes and their pseudomolecular ion $[M + H]^+$, according to the nomenclature system proposed by Domon and Costello (6).

The first eluting compound (**1**) showed an $M+H^+$ ion at m/z 868, indicating a molecular weight of 867, identical to that of α -solanine. The MS/MS spectrum of this ion is the same that corresponding a solanidine-containing glycoside. The ions at m/z 526 and 558 were assigned to a solatriose resi-

due linked to a solanidine aglycone, confirming the identification of compound **1** as α -solanine (7-9). The compound co-eluting with α -chaconine (compound **2**) showed a pseudomolecular ion $M+H^+$ at m/z 852, indicating a molecular weight of 851, equal to the molecular weight of α -chaconine. The MS/MS spectrum of the pseudomolecular ion at m/z 852 indicating the presence of the aglycone solanidine, and the fragments at m/z 750 and 706 confirmed the identity of compound **2** as α -chaconine (7-9).

The HPLC chromatogram of the aphid extract showed, among others, two peaks co-eluting with α -solanine (compound **1**; ret. time, 12.16 min) and α -chaconine (compound **2**; ret. time, 12.24 min) (figure 2). The analysis of the pseudomolecular ions $M+H^+$ for each compound and their MS/MS spectra, allowed their identification as α -solanine and α -chaconine (table 1).

Discussion

It is well known that *S. tuberosum* produces α -solanine and α -chaconine as the main glycoalkaloids. *S. commersonii* on the other hand, has different chemotypes which differ in the amount and types of glycoalkaloids produced (10), including α -solanine and α -chaconine.

Although the sites of biosynthesis and accumulation of glycoalkaloids are reasonably well understood, the mobility of glycoalkaloids within the plant has long been the subject of debate (11). Since glycoalkaloids are thought to be part of the plant's defensive mechanisms against pests and pathogens, and are more concentrated in peripheral tissues, it is reasonable to surmise that they may also be present in parts of the transport conduits that are susceptible to predation, especially the phloem.

Previous reports tend not to support the idea of large-scale transport of glycoalkaloids in potato and tomato (11), but they do not exclude the possibility that glycoal-

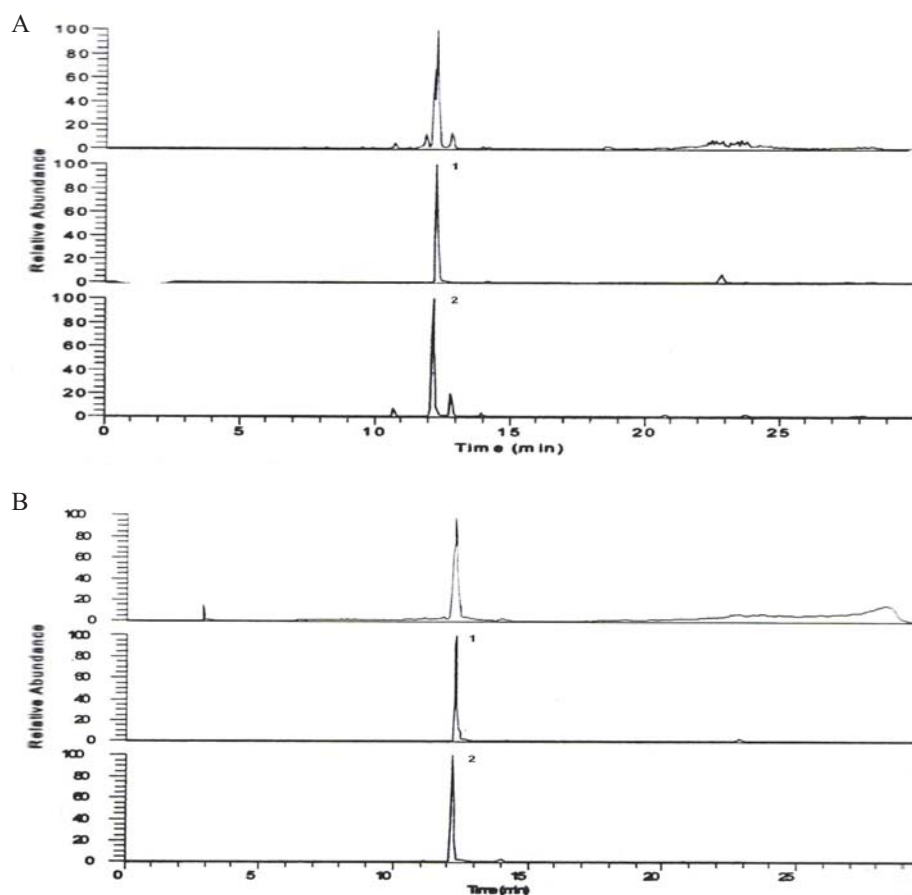


Figure 1. HPLC-MS chromatograms of *S. tuberosum* extract (A) and *S. commersonii* extract (B). Total Ion Current chromatogram; (1) Selective Ion Monitoring for m/z 868; and (2) Selective Ion Monitoring for m/z 852.

Table 1
Diagnostic ions of the glycoalkaloids from *S. tuberosum*, *S. commersonii* and *M. euphorbiae* by ESI-HPLC-MS

Glycoalkaloids	[M+H] ⁺	Diagnostic ions (% abundance)
α -solanine (1)	868	850 (18), 750 (4), 722 (54), 706 (100), 558 (6), 526 (8), 398 (18), 380 (4).
α -chaconine (2)	852	750 (15), 706 (100), 398 (91), 380 (15).

kaloids in these and other *Solanum* species might be present in sieve tubes.

Results indicate that α -solanine and α -chaconine are present in the phloem sap of *S. tuberosum* and its wild relative, *S. com-*

mersonii. They might be loaded into the phloem acting as a systemic insecticide, protecting the plants from sap-feeding insects, and the translocation of some glycoalkaloids would then be an inevitable precondition of their presence there. It can be sup-

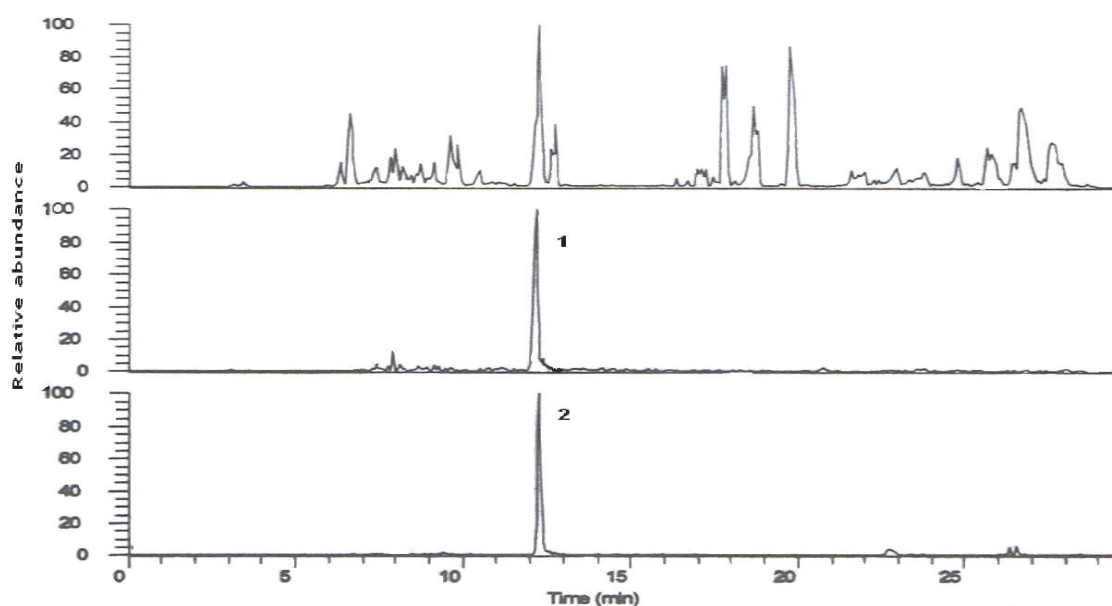


Figure 2. HPLC-MS chromatograms of *aphids* extract. Total Ion Current chromatogram; (1) Selective Ion Monitoring for m/z 868; and (2) Selective Ion Monitoring for m/z 852.

posed that the presence of glycoalkaloids in the phloem might have evolved primarily to protect the vascular tissue, with transport of the glycoalkaloids being an indirect, secondary effect (12).

The glycoalkaloids are known to possess pesticidal activity against several insects feeding on potato leaves and they are extremely toxic against the aphid *Schizaphis graminum*, which does not feed on potato (2). We have previously assayed the activity of several glycoalkaloids against *M. euphorbiae*, an important potato pest and virus vector (1, 13, 14). Although they usually produced a decrease in the reproductive rate of nymphs, none of the glycoalkaloids tested proved to be lethal at the assayed concentrations. These observations are compatible with the presence of glycoalkaloids in the phloem sap of two *Solanum* species and in aphid tissues.

Conclusions

The results suggest that *M. euphorbiae* aphids do feed and ingest glycoalkaloids

from their host plants, and perhaps possess detoxification mechanisms against these metabolites.

These findings encourage further work in this field. If this mechanism of detoxification is confirmed, the glycoalkaloids levels present in the cultivated potato may play a more complex role than previously supposed.

Acknowledgements

This research was supported by grants from the Programa de Desarrollo de las Ciencias Básicas (PEDECIBA, Uruguay), Consejo Sectorial de Investigación Científica (CSIC, UDELAR, Uruguay) and Consejo Nacional de Investigación Científica y Tecnológica (CONICYT-BID, Uruguay, grant 030). We are grateful to Prof. J.G. Roddick for valuable comments, Dr. R. Carballo for entomological identification and Dr. F. Vilaró for supplying the potato cultivar. Dr. Valerie Dee carried out linguistic revision of this paper.

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