J Chem Ecol (2014) 40:599–608
DOI 10.1007/s10886-014-0447-8

Glycoalkaloids of Wild and Cultivated *Solanum*: Effects on Specialist and Generalist Insect Herbivores

Paula Altesor · Álvaro García · Elizabeth Font · Alejandra Rodríguez-Haralambides · Francisco Vilaró · Martín Oesterheld · Roxina Soler · Andrés González

Received: 25 March 2014 / Revised: 5 May 2014 / Accepted: 9 May 2014 / Published online: 27 May 2014
© Springer Science+Business Media New York 2014

Abstract Plant domestication by selective breeding may reduce plant chemical defense in favor of growth. However, few studies have simultaneously studied the defensive chemistry of cultivated plants and their wild congeners in connection to herbivore susceptibility. We compared the constitutive glycoalkaloids (GAs) of cultivated potato, *Solanum tuberosum*, and a wild congener, *S. commersonii*, by liquid chromatography coupled to mass spectrometry. We also determined the major herbivores present on the two species in field plots, and tested their preference for the plants and their isolated GAs in two-choice bioassays. *Solanum commersonii* had a different GA profile and higher concentrations than *S. tuberosum*. In the field, *S. tuberosum* was mostly attacked by the generalist aphids *Myzus persicae* and *Macrosiphum euphorbiae*, and by the specialist flea beetle *Epitrix argentinensis*. In contrast, the most common herbivore on *S. commersonii* was the specialist sawfly *Tequus* sp. Defoliation levels were higher on the wild species, probably due to the chewing feeding behavior of *Tequus* sp. As seen in the field, *M. persicae* and *E. argentinensis* preferred leaf disks of the cultivated plant, while *Tequus* sp. preferred those of the wild one. Congruently, GAs from *S. commersonii* were avoided by *M. persicae* and preferred by *Tequus* sp. The potato aphid performed well on both species and was not deterred by *S. commersonii* GAs. These observations suggest that different GA profiles explain the feeding preferences of the different herbivores, and that domestication has altered the defensive capacity of *S. tuberosum*. However, the wild relative is still subject to severe defoliation by a specialist herbivore that may cue on the GAs.

Keywords Plant defense · *Solanum* · Glycoalkaloids · Herbivore specialization · Plant domestication

P. Altesor · Á. García · E. Font · A. Rodríguez-Haralambides · A. González (✉)
Facultad de Química, Universidad de la República, Montevideo, Uruguay
e-mail: agonzal@fq.edu.uy

F. Vilaró
Instituto Nacional de Investigación Agropecuaria (INIA)-Las Brujas, Canelones, Uruguay

M. Oesterheld
Ifeva, Facultad de Agronomía, Universidad de Buenos Aires/ Conicet, Buenos Aires, Argentina

R. Soler
Department of Terrestrial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), Wageningen, The Netherlands

Introduction

Plant domestication for human consumption has involved centuries of selective breeding, in which certain traits have been favored. As a result, herbivore resistance in cultivated plants may have been debilitated, since human-selected genotypes allocate more resources to the growth of edible parts and photosynthetic tissue, and less to defensive traits (Bazzaz et al. 2009; Herms and Mattson 1992; Rosenthal and Dirzo 1997). In particular, certain chemical defenses have been selected against by humans to avoid intoxication (Bautista et al. 2012; Gepts 2004), and chemical protection has been applied externally, at least during the past century, in the form of pesticides. It may hence be expected that cultivated plants should be more susceptible to herbivory than their wild

relatives, and this has been shown for a few species (Bellota et al. 2013; Cole 1997; Dávila-Flores et al. 2013; Gols et al. 2008a,b; Massei and Hartley 2000; Poelman et al. 2008; Rosenthal and Dirzo 1997; Small 1996; Wise et al. 2001). Wild species related to cultivated crops often have been studied by plant breeders searching for favorable traits, usually linked to resistance against biotic or environmental factors. However, few studies have compared simultaneously the defensive chemistry of cultivated plants and their wild congeners, their herbivore susceptibility, and the causal link between these factors (Cole 1997; Gols et al. 2008a,b; Poelman et al. 2008). Besides furthering our fundamental knowledge about the actual role and relative importance of plant defensive chemicals against insect herbivores, these comparative studies also provide valuable insight that can be used by crop scientists and agro-ecologists to design crop-management practices that enhance the efficacy of pest control.

Insect herbivores have evolved strategies for counteracting plant chemical defenses, and diet breadth is a major part of those strategies (Schoonhoven et al. 2005). Generalist (polyphagous) species feed on plants from different families, while specialists utilize one or a few plant species, usually within a single genus (monophagous) or a single family (oligophagous) (Schoonhoven et al. 2005). Specialist herbivores usually tolerate the defensive metabolites of their host plants, or even benefit from them for their own development or as anti-predatory defenses (Ali and Agrawal 2012; Schowalter 2006). Specialization is the norm among herbivorous insects, with generalists only accounting for about 10 % of the species (Bernays and Graham 1988). Specialization implies for a plant a higher risk that at least some insect species will be adapted to its chemical defense. Chemically defended species, therefore, while protected against generalists, are still attacked by a narrow spectrum of specialist herbivores. Domesticated relatives, in turn, may be at greater risk of attack by a broader spectrum of herbivores, if they produce a smaller amount or less diverse defensive chemicals. Therefore, a comparison of wild and cultivated plant species regarding their chemical defensive capacities should incorporate, as a relevant factor, the degree of dietary specialization of the herbivores involved.

The genus *Solanum* (Solanaceae) is characterized by the production of steroidal glycoalkaloids (GAs) (Eich 2008). Chemically, these metabolites possess interesting structural and electronic features as a result of combining a rigid steroidal skeleton, a basic heterocyclic nitrogen, and a polar sugar moiety. GAs are indeed bioactive, and usually have been associated with plant defense against herbivorous insects (Flanders et al. 1992; Fragoyiannis et al. 1998; Friedman 2002; Jansky et al. 2009; Kowalski et al. 1999; Lorenzen et al. 2001; Mulatu et al. 2006; Nenaah 2011a; Nenaah 2011b; Rangarajan et al. 2000; Tingey 1984; Yencho et al. 2000). In the cultivated potato, *Solanum tuberosum*, the GAs have been selectively reduced by breeding, since they have a bitter taste and are potentially toxic to humans in concentrations higher than 200 mg/kg of fresh tuber (Gregory et al. 1981; van Gelder et al. 1988). The most common GAs in *S. tuberosum* are α-solanine and α-chaconine, which usually make up for more than 95 % of the GA mixture (Friedman et al. 1997). These are glycosides of the same steroidal aglycone solanidine, varying in their trisaccharide sugar moiety. GAs from several wild *Solanum* species that occur in South America have been studied. Among these, *S. commersonii*, a wild and native species that grows as a weed in Southern South America, has been shown to produce mostly tretrasaccharide GAs, including tomatine, commersonine, demissine, dehydrocommersonine, and dehydro-demissine (Vázquez et al. 1997). Different populations of *S. commersonii* show great variability in their GA contents, both qualitatively and quantitatively (from 30 to 40000 mg/kg of fresh leaf biomass) (Pianzzola et al. 2005). While *S. commersonii* and its hybrids with *S. tuberosum* have been studied for pathogen resistance, there have been no reports on natural insect herbivory on *S. commersonni*, its connection to their GA contents, or its comparison with the cultivated congenere.

We used these domesticated and wild *Solanum* species as a model for a comparative study of the role of plant chemical defenses on herbivore resistance, and their differential effects on generalist and specialist herbivores. Our objective was to correlate plant domestication with herbivore diversity and abundance, defensive chemistry, and chemically-mediated host plant preference. Specifically, we assessed field herbivory levels in the cultivated *S. tuberosum* and its wild congener *S. commersonii*, we characterized their constitutive GA chemistry and quantified their GA concentration, and we evaluated the preference of the main generalist and specialist insect herbivores for both, the plant leaves and their isolated GAs.

Methods and Materials

Plants and Insects Cloned accessions of *S. tuberosum* (var. Iporá) and *S. commersonii* (accession 05.02–6) were obtained from a *Solanum* germoplasm bank located at the National Institute of Agricultural Research (INIA, Las Brujas field station, Canelones, Uruguay). Plants were propagated under laminar flow conditions, from internodal stem cuts with a single bud. Cut stems were first cultured *in vitro* for 15 days (3000 lux; 21±2 °C; 16:8 h L:D regime), on enriched agar medium containing salts, vitamins, and sucrose (Murashige and Skoog 1962; Staba 1969). Once the plants reached four nodes, they were transferred to seedbeds and placed in a greenhouse for promoting vegetative growth. After 2 weeks, plants were either transplanted in the field for the field-plot experiment, or transferred to plastic pots and allowed to grow

for 15 additional days before taking them to the laboratory for experimentation, GA extraction, or maintenance of insect cultures. Plants hence were used during the vegetative phase.

Aphids (Hemiptera: Aphididae) were cultured in an environmental chamber (19±1 °C, 60±10 % RH, 14:10 h L:D regime). *Myzus persicae* were reared on pepper plants (*Capsicum annuum*) grown from seeds, while *Macrosiphum euphorbiae* were maintained either on *S. tuberosum* or *S. commersonii* plants obtained as described above. Larvae of *Tequus* sp. (Hymenoptera: Pergidae) and adults of *Epitrix argentinensis* (Coleoptera: Chrysomelidae) were used within a few days after field collection on *S. commersonii* or tomato, respectively. They were maintained in the laboratory under controlled conditions (5000 lux, 23±2 °C, 50±10 % RH, 14:10 h L:D), on these same plants (tomato grown from seeds), until experimental use.

Field-plot Experiment The experiment was conducted in an organic (pesticide-free) research farm located at the National Institute of Agricultural Research (INIA, Las Brujas field station, Canelones, Uruguay), from February to June of 2011. To compare the diversity and abundance of herbivores in *S. tuberosum* and *S. commersonii*, cloned plants were transferred to alternate plots (3 × 3 m) separated 3 m from each other. The experiment consisted of 7 plots per *Solanum* species, and 48 plants distributed evenly in each plot. Oat seeds (*Avena sativa*) were sown in between plots to prevent the growth of weeds. Insects were sampled weekly by thorough inspection of the aerial parts of three randomly-chosen plants in each plot. Plants were sampled without repetition, and the experiment was conducted for 6 wk, hence sampling a total of 126 plants per species, and covering the entire plant cycle (vegetative growth, flowering and tuberization).

When the experiment ended, 15 plants of each species were harvested and taken to the laboratory to estimate leaf area consumption. Plants were selected from all plots, at random within the plot, without including those sampled for insects. For each plant, alternate leaves were clipped off from the entire plant, and their remaining area was measured. Measurements were done by setting each leaf flat on a paper sheet, along with a flat reference object of known area. Digital images of the leaves were then analyzed using the software ImageJ (v1.43, http://rsb.info.nih.gov/ij/). The complete leaf area (expected with no herbivory) was then estimated by digitally reconstructing the leaf margins and measuring the enclosed area. Thus, the missing leaf area, presumably lost to herbivory, was calculated and expressed as a percentage. All leaves (either measured or not) were then dried at 50 °C until constant leaf weight. Therefore, the leaves actually measured and weighted allowed for a calculation of a factor (leaf area/dry biomass) for each plant, which was used to estimate the total leaf area of the plant. These calculations indicated that 44±2 % and 43±3 % of the leaf area was actually measured for *S. tuberosum* and *S. commersonii*, respectively.

Preference Bioassays Two-choice preference bioassays were performed to compare herbivore resistance in both *Solanum* species, and the defensive role of GAs. The bioassays were conducted with the main herbivore species found in the field-plot experiment: the aphids *M. persicae* and *M. euphorbiae*, *E. argentinensis* adult beetles, and hymenopteran larvae *Tequus* sp. Leaf disks (1.2 cm diam.) were cut from healthy plants, and set flat over a layer of 2 % agar, equidistant from the center and the margins of a Petri dish (6.1 cm diam). Two experiments were performed with each herbivore, one in which the insects were offered a choice between leaf disks of both *Solanum* species, and one to evaluate the effect of GAs. For the latter, leaf disks of *S. tuberosum* were treated with topical addition of GAs from *S. commersonni*, using solvent-treated disks as controls. The extract (0.8 μg/μl) was added in 10 μl MeOH to obtain a dosage of 8 μg, which corresponds to the average amount of GAs extracted from a mass equivalent to one 1.2-cm leaf disk of *S. commersonii* (22 ±1 mg, N=10; GAs in plant: 0.36±0.09 mg/g fr.wt., N=5).

Aphid preference was evaluated as the percentage of aphids settled on each leaf disk after 24 h. Fifteen *M. persicae* or ten *M. euphorbiae* (adult and late instar nymphs) were placed in the center of the Petri dish using a soft paint brush, and the dish was left upside down for 24 h. Replicates were considered valid if more than 50 % of the aphids were alive after 24 h, and if at least half of these had settled on a leaf disk. An additional experiment was done for *M. persicae* to test if the preference for *S. tuberosum* was due to the lower amount of GAs present in this plant, as opposed to the type of GAs. To increase the amount of GAs, an extract of GAs from *S. tuberosum* was added to *S. tuberosum* leaf disks (10 μl; 1 μg/μl MeOH), and tested against control *S. tuberosum* disks (10 μl MeOH). The dosage of GAs applied corresponded to a 1.7-fold increase, given the average amount of GAs extracted from a mass equivalent to one 1.2-cm leaf disk of *S. tuberosum* (21±1 mg, N=10; GAs in plant: 0.28±0.04 mg/g fr.wt., N=5). The preference of *E. argentinensis* adults and *Tequus* sp. larvae (2nd – 5th instar) was evaluated as the percentage of leaf disk consumed (assessed visually and categorized as 0, 25, 50, 75, or 100 %). One *E. argentinensis* adult or larva of *Tequus* sp. was placed in the center of the Petri dish, and the percentage of area consumed was recorded visually every 30 min for 3 h, or until 100 % of one disk had been consumed. An additional measurement was done for *E. argentinensis* at 24 h since they fed at a slower pace. Insects that had not begun feeding in the first two observations were replaced.

Glycoalkaloid Analysis All leaves of individual plants were extracted to analyze their GA contents. Leaves were cut, weighed, and dried at 50 °C until no difference in weight was observed (0.42±0.01 g, 0.45±0.06 g for *S. tuberosum* and *S. commersonii*, respectively, N=5 plants/species). Dried leaves were grounded in a mortar with 5 ml of 1 % acetic acid, and the solution and plant tissue then were transferred to a

glass centrifuge tube for sonication (5 min) and subsequent centrifugation (5 min). The mortar was washed with 1 ml of 1 % acetic acid, and both solutions were joined before sonication and centrifugation. The supernatant solution was separated, and the plant tissue was re-extracted with 5 ml of 1 % acetic acid. The joined extracts were filtered under vacuum, and purified by solid phase extraction using a Sep-Pak C18 cartidge (1000 mg). The cartridge was conditioned with 3 × 3 ml MeOH, 3 x 3 ml water and 3 ×3 ml 1 % acetic acid; the extract then was applied to the cartridge, washed with 40 % MeOH (10 ml), and the GAs were eluted with MeOH (10 ml). The methanolic GA extract was evaporated to dryness under vacuum, weighed, and stored at −20 °C for chemical analysis or bioassays (Ferreira et al. 1993).

Liquid chromatography-mass spectrometry (including tandem MS-MS) was performed using a HPLC instrument (Agilent 1200, Agilent Technologies, Palo Alto, CA, USA) coupled to an ion trap mass spectrometer (Esquire 6000, Bruker Daltonics GmbH, Bremen, Germany). Samples were analyzed using a reversed-phase Luna C18 analytical column with 100 mm length, 3 mm internal diam and 3 μm particle size (Phenomenex, Torrance, CA, USA), maintained at 40 °C. Extracts (1 mg/ml) were injected in MeOH (5 μl) and the GAs were separated using a linear gradient of 20 % to 30 % of acetonitrile in formic acid (10 mM) in 15 min. The flow rate was 0.35 ml/min and the split ratio before introduction to the mass spectrometer was 1:1. Mass spectrometry was performed in positive-ion mode, using the following conditions: electrospray source endplate offset voltage=−500 V; capillary voltage=−4000 V; nebulizer=40 psi; dry gas flow=9.0 L/min; dry gas temperature=365 °C. Nitrogen was used as the drying and nebulizing gas. Acquisition and data analysis were performed with Bruker Compass Data Analysis 4.0 SP 1 (version 1.2 SR1, BukerDaltonik GmbH). The relative and absolute quantification of GAs in the extracts was based on the peak areas of the $[M + H]^+$ ions. Absolute quantification was done by extrapolation from a calibration curve obtained from standard solutions of α-solanine (Sigma-Aldrich; 10, 100, 1000 ppm; $r^2>0.999$). Hence, the net quantitative values are expressed as α-solanine equivalents.

Statistical Analysis Insect abundance in *S. tuberosum* and *S. commersonii* was compared by a *repeated-measures ANOVA*, using sampling weeks as within-subjects factor [insect counts were transformed to $\sqrt{(x+1)}$]. The percentages of missing leaf area (herbivory) in the field-plot experiment, and the effects of plant rearing in the choice bioassays with *M. euphorbiae*, were analyzed by the *Mann–Whitney U test*. Insect preference in choice bioassays was analyzed by the *Wilcoxon signed-rank test* for paired samples. Differences were considered significant at $P<0.05$. Data are expressed as mean ± SEM throughout.

Results

Field-plot Experiment The main herbivorous insect species found throughout the experiment were the aphids *M. persicae* and *M. euphorbiae*, adult leaf beetles of *E. argentinensis*, and sawfly larvae *Tequus* sp. In addition, a few adults of *Diabrotica speciosa* (Coleoptera: Chrysomelidae) were sporadically observed in both plant species, and will not be considered any further. The aphids and *E. argentinensis*, both regarded as potato pests, were more abundant on *S. tuberosum* (Figs. 1a, b), whereas larvae of *Tequus* sp. were found almost exclusively on *S. commersonii* (Fig. 1c) (see figure legend for statistical details). The wild *S. commersonii* showed lower total leaf area but a significantly higher relative missing leaf area than the cultivated potato *S. tuberosum* (Table 1). The lower leaf area of *S. commersonii* was accounted for by its lower specific leaf area, since foliage biomass, either fresh or dry, was not significantly different between species (Table 1).

Preference Bioassays In agreement with its behavior in the field, *M. persicae* preferred to settle on leaf disks of *S. tuberosum* when tested against *S. commersonii* ($P=0.002$) (Fig. 2a1). This preference was partially explained by the GAs present in *S. commersonii*, since *S. tuberosum* leaf disks treated with GAs from the wild species were less preferred than disks treated with solvent ($P=0.02$) (Fig. 2a). This differential behavior was a result of the particular GA composition of *S. commersonii* because the addition of extra GAs extracted from *S. tuberosum* to *S. tuberosum* leaf disks had no effect on the preference of *M. persicae*, with 48.8±2.3 % and 51.2±2.3 % of the aphids settled on control or GA-supplemented leaf disks, respectively (*Wilcoxon signed-rank test*, $P=0.61$, $N=41$). In disagreement with its behavior in the field, *M. euphorbiae* showed no preference for settling on leaf disks of either species (*Wilcoxon signed-rank test*, $P=0.18$ and $P>0.99$, for aphids grown on *S. tuberosum or S. commersonii*, respectively) (Fig. 2b1). As expected from these results, the addition of GAs extracted from *S. commersonii* had no effect on the preference of *M. euphorbiae* for *S. tuberosum* leaf disks (*Wilcoxon signed-rank test*, $P=0.07$ and $P=0.30$, for aphids grown on *S. tuberosum or S. commersonii*, respectively) (Fig. 2 b2).

Food choice bioassays for the chewing herbivores agreed with the behavior in the field. *E. argentinensis* beetles preferred to feed on *S. tuberosum* ($P<0.05$) (Fig. 2c1), although this choice was not explained by a significant rejection to GAs from *S. commersonii* ($P=0.37$) (Fig. 2c2). The larvae of *Tequus* sp., which were found in the field almost exclusively on *S. commersonii*, showed a clear preference for this species ($P<0.001$) (Fig. 2d1), and this preference was due, at least in part, by a preference for *S. commersonii* GAs, as indicated for the higher preference for *S. tuberosum* leaf disks treated with *S. commersonii* GAs ($P<0.001$) (Fig. 2d2).

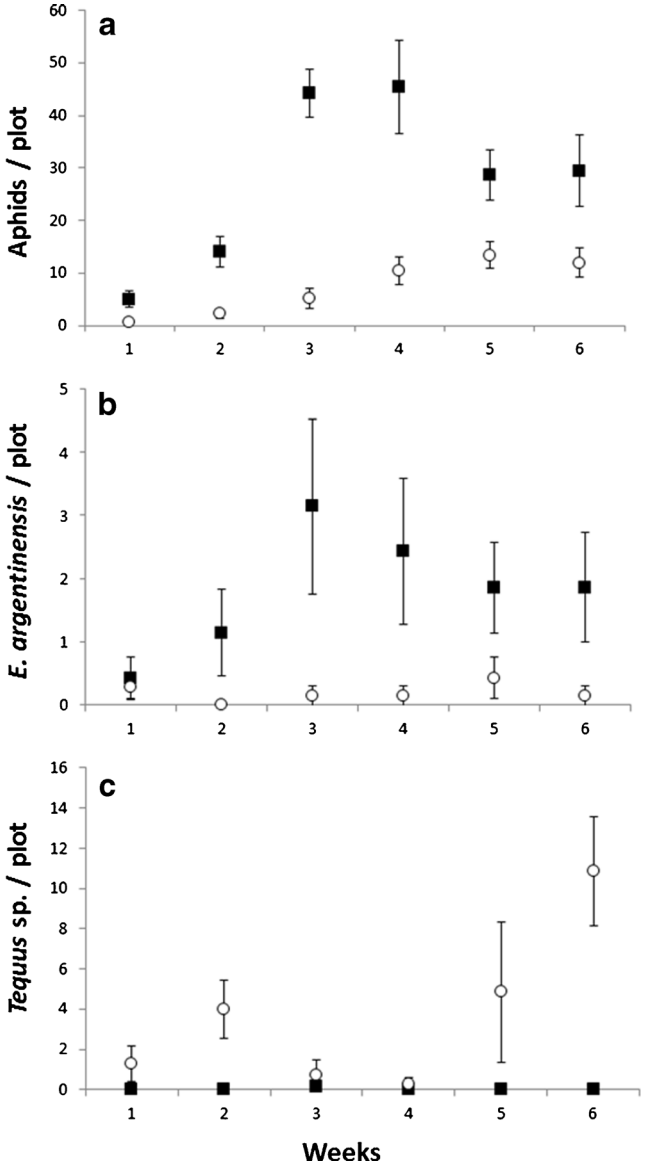

Fig. 1 Abundance of herbivorous insects in the cultivated potato *Solanum tuberosum* (black squares) and the wild congener *S. commersonii* (open circles). **a** Aphids (*Myzus persicae* and *Macrosiphum euphorbiae*); **b** adults of *Epitrix argentinensis*; **c** larvae of *Tequus* sp. Data points represent the number of insects sampled in 3 plants of a single plot. *Repeated measures ANOVA*: Aphids: $F_{(1,12)}$=38.30, P<0.001; *E. argentinensis*: $F_{(1,12)}$=9.48, P<0.01; *Tequus* sp.: $F_{(1,12)}$=36.52, P<0.001

Glycoalkaloid Analysis LC-MS analysis of GA extracts of the accession of *S. commersonii* used in this study showed 7 chromatographic peaks (Fig. 3a, Table 2). The major compound (**5**), representing 80 % of the total GA mixture, showed a $[M + H]^+$ ion of *m/z* 1016.5, which suggested a GA with a tetrasaccharide sugar moiety (Distl and Wink 2009). In further MS-MS analysis of the $[M + H]^+$ ion, its fragment ions indicated the loss of a terminal pentose (132 mass units, *m/z* 884) and three hexoses (each 162 mass units). The loss of this sugar moiety (lycotetraose) resulted in a fragment of *m/z* 398, which corresponds to the common aglycone solanidine (Fig. 3 b, Table 2) (Distl and Wink 2009). This information, along with previous reports of GAs from *S. commersonii* (Vázquez et al. 1997), allowed for the identification of dehydro-demissine as the major GA. The next more abundant GA in *S. commersonii* (**6**) showed two additional mass units in its $[M + H]^+$ ion (Table 2), and the MS-MS fragments showed the same fragment pattern of the sugar moiety lycotetraose, with an aglycone of *m/z* 400 that corresponds to demissidine. Therefore, GA **6** was identified as demissine, which has also been reported as a major GA in *S. commersonii* (Vázquez et al. 1997). Two minor GAs, **3** and **4**, were identified tentatively as dehydro-tomatine and dehydro-commersonine by comparison of their mass spectra with those previously reported GAs (Distl and Wink 2009). Dehydro-tomatine showed a $[M + H]^+$ of *m/z* 1032, and MS-MS fragment ions that matched the loss of a lycotetraose sugar moiety, resulting in a fragment of *m/z* 414 that corresponds to the aglycone tomatidenol. Dehydro-commersonine showed a $[M+H]^+$ of *m/z* 1046, and MS-MS fragment ions that matched the loss of four hexose moieties (648 mass units, commertetraose), with the aglycone solanidine (*m/z* 398). Hence, four of the seven GAs from *S. commersonii* were identified, which combined represent roughly 91 % of the GA mixture. The remaining peaks (1, 2 and 7) also correspond to GAs given their mass spectra, and remain to be identified.

The glycoalkaloid extracts from *S. tuberosum* were similarly analyzed, showing 6 peaks in their LC-MS chromatograms (Table 2). The two major peaks, **8** and **9**, representing more than 95 % of the GA mixture, were identified as the common GAs α-solanine and α-chaconine, respectively. The identification was based on the eluting order, the $[M + H]^+$ ions of *m/z* 868 and 852, the MS-MS fragment ions corresponding to the trisaccharide sugar moieties solatriose and chacotriose, and the fragment of *m/z* 398 that corresponds to the aglycone solanidine (Distl and Wink 2009). The unidentified peaks 10–13 are also regarded as GAs from their mass spectra. The net concentration of GAs in the plant tissue

Table 1 Fresh and dry plant foliage biomass, foliar area and consumed leaf-area of *Solanum tuberosum* and *S. commersonii* after the field-plot experiment

	S. tuberosum	*S. commersonii*	Mann–Whitney U
Fresh biomass (g) (N=10 plants)	185±33	104±13	W=128; P=0.08
Dry biomass (g) (N=10 plants)	11.4±2.1	6.9±0.9	W=124.5; P=0.14
Foliar area (cm^2) (N=15 plants)	2943±415	1262±170	W=307; P=0.002
Consumed leaf-area (%) (N=15 plants)	1.23±0.14	2.95±0.36	W=141; P<0.001

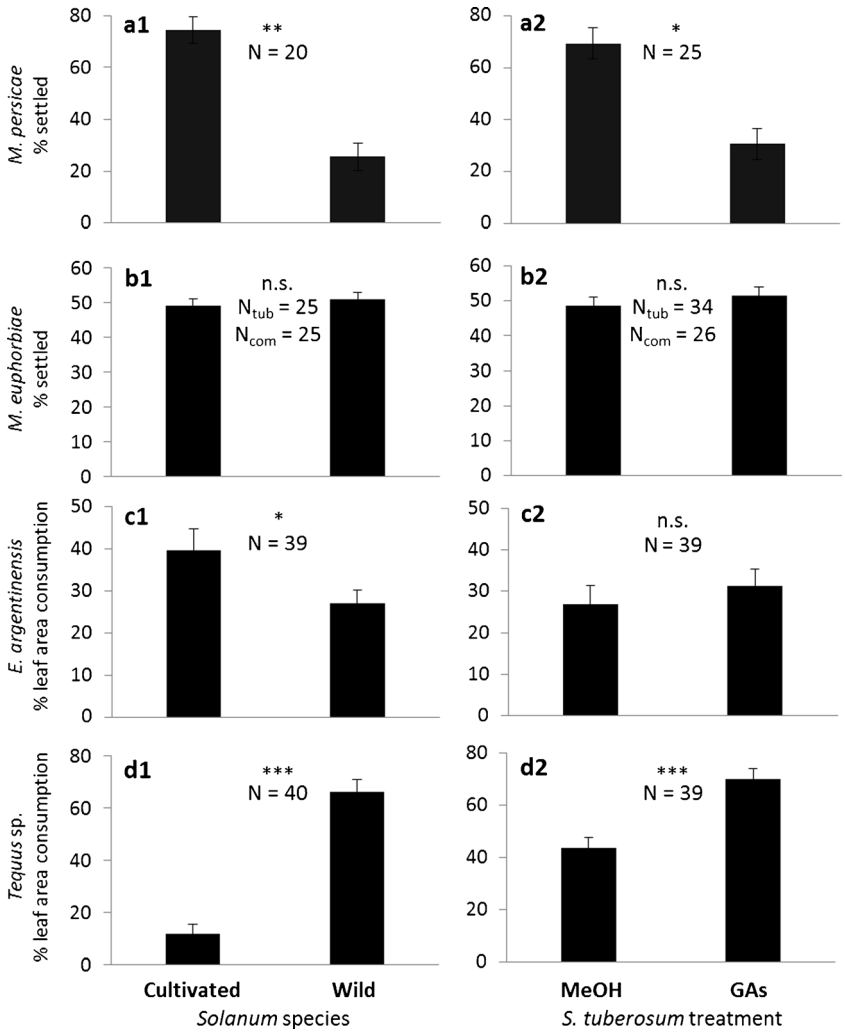

Fig. 2 Preference of the aphids *Myzus persicae* **(a)** and *Microsiphum euphorbiae* **(b)**, *Epitrix argentinensis* beetles **(c)**, and *Tequus* sp. larvae **(d)**, between leaf disks of the cultivated *S. tuberosum* or the wild *S. commersonii* (left), and between leaf disks of *S. tuberosum* treated either with solvent (control) or with Glycoalkaloids (GAs) extracted from *S. commersonii* (right). Preference is shown as % settling for the aphids, and as % leaf consumed for the chewing insects. Asterisks show significant differences according to the *Wilcoxon signed-rank test* (*=P<0.05, **=P<0.01, ***=P<0.001). Error bars indicate SEM; n.s. means not significant; N is the number of replicates. Because the two populations of *M. euphorbiae*, raised on both *Solanum* species, showed no differences in the bioassays (*Mann-Withney U test*, W=596.5, P=0.43 and W=920, P=0.06, for b1 and b2, respectively), and were combined for the figure (N_{tub} and N_{com} refers to the number of replicates for *M. euphorbiae* raised on *S. tuberosum* or *S. commersonii*)

(leaves) was higher in the wild species *S. commersonii*, which contained 1.46±0.14 mg/g of leaf biomass (dry weight) (N=5), more than twice the GA concentration of *S. tuberosum* (0.65±0.19 mg/g of dry leaf biomass) (N=5) (*Mann–Whitney U test*: W=10, P=0.03).

Discussion

Our field results showed that the two *Solanum* species differ in the diversity and abundance of insect herbivores. While aphids and the chrysomelid beetle *E. argentinensis* were more abundant on *S. tuberosum*, the sawfly larvae *Tequus* sp. were almost exclusively on the wild species *S. commersonii*. The chewing feeding habit of *Tequus* sp. most likely explains that *S. commersonii* lost more leaf area than *S. tuberosum*, which was in turn mostly attacked by phloem-sucking aphids. The observed host-plant associations may be, at least in part, influenced by differences in the defensive chemistry of the plants, as shown by the chemical analyses of GA extracts from healthy plants. Not only the concentration of GAs was significantly higher in the wild species *S. commersonii*, but also the GAs differed in their chemistry, particularly in the glycosidic part of the molecule. The main GA in *S. commersonii* was dehydrodemissine, a tetraglycoside of solanidine, the same aglycone of the triglicosydes α-solanine and α-chaconine, the main GAs in *S. tubersoum*.

The importance of the glycosidic portion of GAs in modulating their biological activity was not unexpected (Güntner et al. 1997; Roddick et al. 1992), and the results of our choice bioassays are consistent with this notion. In fact, GAs significantly affected host plant selection, and they did so differently for two of the herbivores included in our study. The aphid *M. persicae*, which preferred to feed on the cultivated plant, showed a negative response toward the GAs from *S. commersonii* when these were applied on *S. tuberosum* leaf disks. Such response was not due to an increase in the total GA concentration, since the aphids showed no avoidance

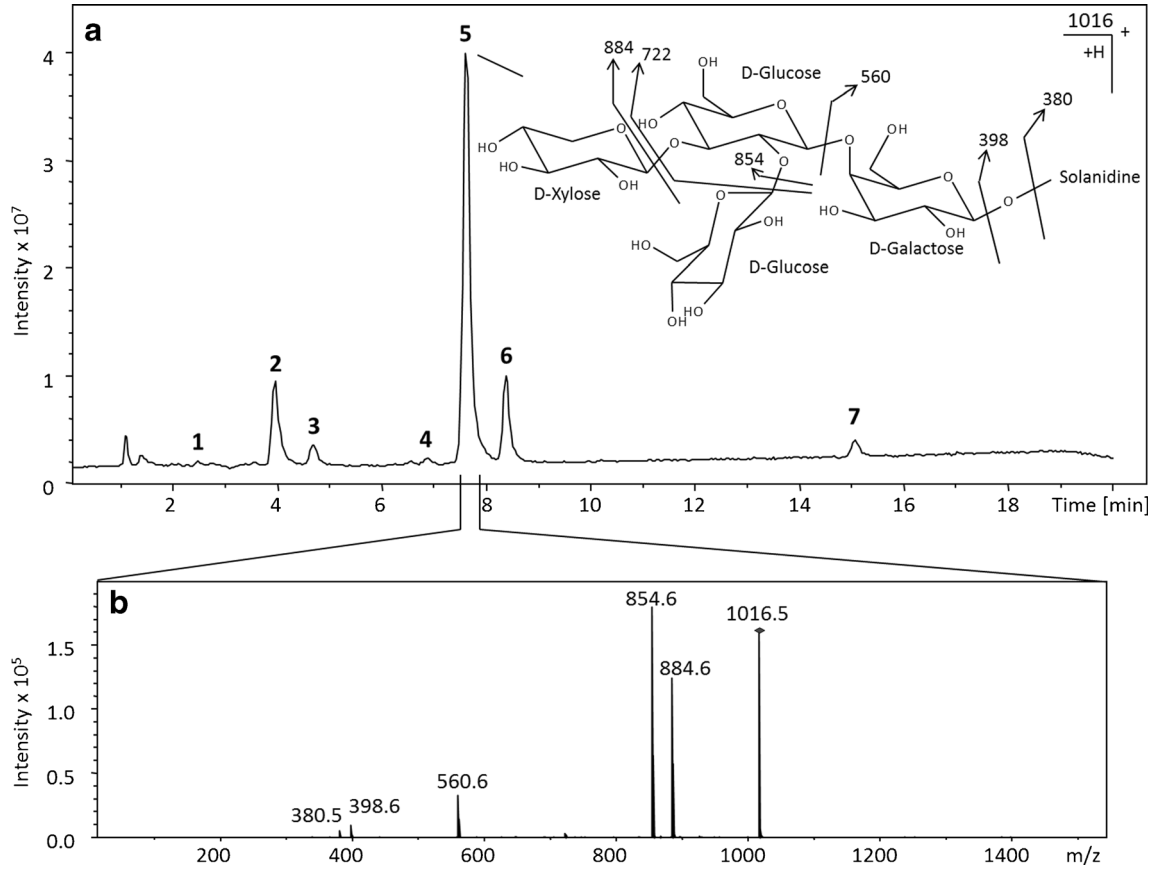

Fig. 3 **a** Typical total ion chromatogram (TIC) of glycoalkaloid (GA) extracts from *Solanum commersonii*, including the structure and fragmentation scheme of the main GA, dehydro-demissine. **b** MS-MS fragmentation of the $[M+H]^+$ ion for the identification of dehydro-demissine. Daughter ions correspond to different losses of sugar moieties and to the aglycone solanidine (*m/z* 398.6)

toward leaf disks with an increased amount of *S. tuberosum* GAs. In the case of *Tequus* sp. larvae, which preferred to feed in *S. commersonii*, the GAs from the wild species were a phagostimulant when added to *S. tuberosum* leaf disks, likely explaining the clear host plant preference shown by *Tequus* sp. larvae both in the field and in the bioassays. Therefore, our results show that producing more defensive GAs, while conferring protection against generalist herbivores, does not necessarily result in less herbivory damage, due to specialized insects such as *Tequus* sp., which may be cued on these compounds. Interestingly, the sawfly genus *Tequus* is restricted to the Neotropics and reported to feed exclusively on *Solanum* species (Schmidt and Smith 2006), sharing therefore evolutionary history with GA-producing plants. Further, host plant specialization and sequestration of chemical defenses is not unusual in sawflies (Barker et al. 2002; Bowers et al. 1993; Crockett and Boevé 2011; Eisner et al. 1974; Müller et al. 2001; Opitz et al. 2010, 2012; Prieto et al. 2007; Schaffner et al. 1994; Schaffner and Boevé 1996), including members of the *Tequus* family Pergidae (Carne 1962; Tait 1962; Morrow et al. 1976; Schmidt et al. 2000, 2010). It thus is conceivable that *Tequus* sawflies are cueing on *S. commersonii* GAs to locate their host plant, or even to obtain chemical defenses for their own protection against predators, hypotheses that deserve further investigation.

While no distinction was made between aphid species during field monitoring, only two common generalist species, *M. persicae* and *M. euphorbiae*, were later identified. Interestingly, these species showed different responses in their host plant preferences in choice laboratory bioassays. As already discussed, *M. persicae* preferred to settle on the cultivated *S. tuberosum*, and was deterred by GAs from the unpreferred plant *S. commersonii*. On the other hand, *M. euphorbiae* showed no preference, and it was not deterred by the defensive chemicals. The absence of preference was independent from previous experience, since *M. euphorbiae* raised on *S. tuberosum* or *S. commersonii* showed equal responses. These results show that herbivores with similar feeding habits and broad diet preferences may respond quite differently to specific plant defensive metabolites. While a general mechanism may be expected regarding the tolerance of highly polyphagous herbivores to a given type of chemical defense, our results clearly show that some generalists are significantly more capable than others for withstanding moderate amounts of plant toxins, as shown here by *M. euphorbiae*.

Table 2 Chromatographic, spectroscopic and relative abundance data for the main glycoalkaloids (GAs) from *Solanum commersonii* and *S. tuberosum*

Species	GA	Rt (min)	Compound	Molecular formula	$[M+H]^+$	Main fragment ions[a] MS-MS of $[M+H]^+$	% Area $[M+H]^+$ mean ± SEM[b]
S. commersonii	1	2.5	n.i.[c]		1033	1014.5/900.4/882.3/870.5/ 853.5/558.3/576.1/379.6	0.44±0.12
	2	3.9	n.i.		1031	898.5/850.3/736.4/718.2/574.3/ 556.5/433.3/412.4/394.6	7.3±1.8
	3	4.7	Dehydro-tomatine	$C_{50}H_{81}NO_{21}$	1032.5	900.3/870.3/576.4/567.3/**414.4**/396.4	1.29±0.22
	4	6.9	Dehydro-commersonine	$C_{51}H_{83}NO_{21}$	1046.6	1038.5/1016.4/884.4/560.5/**398.4**/390.5	1.33±0.08
	5	7.6	Dehydro-demissine	$C_{50}H_{81}NO_{20}$	1016.5	884.6/854.6/722.6/560.6/**398.6**/380.5	80.4±1.9
	6	8.4	Demissine	$C_{50}H_{83}NO_{20}$	1018.6	886.6/856.6/724.7/562.5/**400.6**/382.6	7.84±0.63
	7	15	n.i.		1195.6	1063.6/1033.5/1016.6/739.2/709.3/ 577.3/433.2/415.4/397.4	1.43±0.12
S. tuberosum	8	7.9	α-Solanine	$C_{45}H_{73}NO_{15}$	868.7	722.6/706.7/560.6/**398.7**	21.38±0.52
	9	8.2	α Chaconine	$C_{45}H_{73}NO_{14}$	852.7	834/706.4/560.5/**398.4**/380.5	75.53±0.39
	10	12.9	n.i.		1085.6	1069.3/1066.3/939.4/923.5/777.3/ 615.3/598.5/592.9/576.4/511.1/ 493.2/431.6/365.1/347.3	0.15±0.04
	11	13.9	n.i.		1069.6	965.3/923.4/777.3/597.4/432.4	0.36±0.10
	12	15.8	n.i.		1047.6	723.5/577.3/415.4/397.4/309.1	0.61±0.15
	13	16.3	n.i.		1031.7	996.3/869.5/723.4/577.4/561.4/543.4/ 415.5/397.5/379.5	1.98±0.39

[a] Fragment ions in bold correspond to the aglycones. [b] Average and error based on 5 independent extracts. [c] Not identified

Previous studies have looked into the performance of *M. persicae* and *M. euphorbiae* on other wild *Solanum* species, with variable results with respect to plant resistance (Flanders et al. 1997; Fréchette et al. 2010; Le Roux et al. 2007; Pelletier et al. 2010; Pompon et al. 2010), and no clear relationship between aphid performance and GA contents in the plant (Flanders et al. 1992). Regarding the evaluation of specific GAs, the tetraglycoside tomatine has been shown to moderately decrease the performance of *M. euphorbiae*, but the two major GAs from *S. tuberosum*, α-solanine and α-chaconine, caused no effect (Güntner et al. 1997). In another study, α-solanine and α-chaconine were effective against *M. persicae*, although the experiments were conducted with higher dosages in relation to the dosages tested against *M. euphorbiae* (Fragoyiannis et al. 1998). In our study, when tested at the dosages that naturally occur in the plant, and even increasing this dosage by addition of supplementary GAs from *S. tuberosum*, we found no effects on the preference of *M. persicae*, indicating that it is the type of GAs, and not their amount, what caused the preference of *M. persicae* for *S. tuberosum*.

In the case of *E. argentinensis*, an oligophagous chrysomelid beetle that feeds on several solanaceous plants, both the field experiment and the choice bioassays showed that the beetles preferred to feed on *S. tuberosum*. However, they were not deterred by *S. commersonii* GAs. Some tolerance to these metabolites may be expected in a species that specializes at the family level on the metabolite-rich Solanaceae. Similarly, previous studies on *E. cucumeris* found no clear trend between GAs and plant resistance to this beetle (Flanders et al. 1992; Tingey and Sinden 1982). On the other hand, these studies showed an effect of simple and glandular trichomes on *E. cucumeris* performance (Flanders et al. 1992), which may explain its preference for *S. tuberosum* in our field study, given that *S. commersonii* shows higher density of both types of trichomes (P. Altesor, unpublished).

In summary, our study provides a comparison of patterns of herbivory and constitutive chemical defenses of two closely related *Solanum* species; a cultivated one that has undergone decades of plant breeding for optimizing productivity, and a wild species that grows as a weed and that is therefore likely to be under strong natural selection. We have shown that herbivory differs both qualitatively and quantitatively between these plant species, with generalist and oligophagous insects preferring the cultivated *S. tuberosum*, and a specialist insect showing a strong preference for the wild *S. commersonii*. We also have shown that the wild plant produces more and structurally different GAs, the typical defensive metabolites of the genus *Solanum*, and established a clear relationship between these plant chemicals and the host plant preference, either positive or negative, of one specialist and one generalist insect. Therefore, plant domestication has seemingly weakened the chemical defensive capacity of the cultivated potato, but the better defended wild relative may still suffer severe damage as a

result of specialization. Finally, we have shown that the defensive role of GAs cannot be assumed equally effective against all generalist herbivores. This supports the current view regarding the improbable generalization of the impact of plant defenses on generalist and specialist herbivores (Ali and Agrawal 2012). Comparative system-approaches in plant-insect research can provide novel insights for the design of sustainable plant production systems, and an excellent study system to investigate some general but unanswered questions in ecology regarding the functioning of terrestrial communities.

Acknowledgments The authors acknowledge technical assistance by personnel of the National Institute for Agricultural Research (INIA) for processing germplasm material, and for field and greenhouse support. Glycoalkaloid standards were kindly provided by Prof. Fernando Ferreira of Universidad de la República, Uruguay. Dr. Nora Cabrera of Universidad Nacional de la Plata, Argentina, identified *Epitrix argentinensis*. Prof. Jeremy McNeil of Western University, Canada, provided helpful suggestions for the manuscript. Mass spectrometry facilities were funded by the European Union Grant EU-URY 2003–5906. The National Agency for Research and Innovation (ANII) granted fellowships to P. Altesor and A. García. Support by the graduate program in Biological Sciences PEDECIBA is also acknowledged.

While in proof, *Tequus* sp. was identified as *Tequus schrottkyi* (Knw.), by Dr. Stefan Schmidt, Curator for Hymenoptera, Zoologische Staatssammlung, Munich, Germany.

References

Ali JG, Agrawal AA (2012) Specialist *versus* generalist insect herbivores and plant defense. Trends Plant Sci 17:293–302

Barker A, Schaffner U, Boevé J-L (2002) Host specificity and host recognition in a chemically-defended herbivore, the tenthredinid sawfly *Rhadinoceraea nodicornis*. Entomol Exp Appl 104:61–68

Bautista A, Parra F, Espinosa-García FJ (2012) Efectos de la domesticación de plantas en la diversidad fitoquímica. In: Rojas JC, Malo EA (eds) Temas selectos en ecología química de insectos. El Colegio de la Frontera Sur (ECOSUR), México, pp 253–267

Bazzaz FA, Chiariello NR, Coley PD, Pitelka LF (2009) Allocating resources to reproduction and defenses. Bioscience 37:58–67

Bellota E, Medina RF, Bernal JS (2013) Physical leaf defenses–altered by *Zea* life-history evolution, domestication, and breeding–mediate oviposition preference of a specialist leafhopper. Entomol Exp Appl 149:185–195. doi:10.1111/eea.12122

Bernays E, Graham M (1988) On the evolution of host specificity in phytophagous arthropods. Ecology 69:886–892

Bowers MD, Boockvar K, Collinge SK (1993) Iridoid glycosides of *Chelone glabra* (Scrophulariaceae) and their sequestration by larvae of a sawfly, *Tenthredo grandis* (Tenthredinidae). J Chem Ecol 19: 815–823

Carne PB (1962) The characteristics and behaviour of the saw-fly *Perga affinis affinis* (Hymenoptera). Aust J Zool 10:1–38

Cole RA (1997) The relative importance of glucosinolates and amino acids to the development of two aphid pests *Brevicoryne brassicae* and *Myzus persicae* on wild and cultivated brassica species. Entomol Exp Appl 85:121–133. doi:10.1046/j.1570-7458.1997.00242.x

Crockett SL, Boevé J-L (2011) Flavonoid glycosides and naphthodianthrones in the sawfly *Tenthredo zonula* and its host-plants, *Hypericum perforatum* and *H. hirsutum*. J Chem Ecol 37: 943–952. doi:10.1007/s10886-011-0001-x

Dávila-Flores AM, DeWitt TJ, Bernal JS (2013) Facilitated by nature and agriculture: performance of a specialist herbivore improves with host-plant life history evolution, domestication, and breeding. Oecologia 173:1425–1437. doi:10.1007/s00442-013-2728-2

Distl M, Wink M (2009) Identification and quantification of steroidal alkaloids from wild tuber-bearing *Solanum* species by HPLC and LC-ESI-MS. Potato Res 52:79–104

Eich E (2008) Solanaceae and Convolvulaceae: Secondary metabolites. Springer, Germany

Eisner T, Johnessee JS, Carrel J (1974) Defensive use by an insect of a plant resin. Science 184:996–999

Ferreira F, Moyna P, Soule S, Vázquez A (1993) Rapid determination of *Solanum* glycoalkaloids thin-layer chromatographic scanning. J Chromatogr A 653:380–384

Flanders KL, Hawkes JG, Radcliffe EB, Lauer FI (1992) Insect resistance in potatoes: sources, evolutionary relationships, morphological and chemical defenses, and ecogeographical associations. Euphytica 61: 83–111

Flanders KL, Radcliffe EB, Hawkes JG (1997) Geographic distribution of insect resistance in potatoes. Euphytica 93:201–221

Fragoyiannis DA, McKinlay RG, Mello JPFD (1998) Studies of the growth, development and reproductive performance of the aphid *Myzus persicae* on artificial diets containing potato glycoalkaloids. Entomol Exp Appl 88:59–66

Fréchette B, Bejan M, Lucas E, Giordanengo P, Vincent C (2010) Resistance of wild *Solanum* accessions to aphids and other potato pests in Quebec field conditions. J Insect Sci 10(161):1–16. doi:10.1673/031.010.14121

Friedman M (2002) Tomato glycoalkaloids: role in the plant and in the diet. J Agric Food Chem 50:5751–5780

Friedman M, McDonald GM, Filadelfi-keszi PM (1997) Potato glygcoalkaloids: chemistry, analysis, safety, and plant physiology. Crit Rev Plant Sci 16:55–132

Gepts P (2004) Crop domestication as a long-term selection experiment. Plant Breed Rev 24 (Part 2):1–44

Gols R, Bukovinszky T, van Dam NM, Dicke M, Bullock JM, Harvey JA (2008a) Performance of generalist and specialist herbivores and their endoparasitoids differs on cultivated and wild *Brassica* populations. J Chem Ecol 34:132–143. doi:10.1007/s10886-008-9429-z

Gols R, Wagenaar R, Bukovinszky T, van Dam NM, Dicke M, Bullock JM, Harvey JA (2008b) Genetic variation in defense chemistry in wild cabbages affects herbivores and their endoparasitoids. Ecology 89:1616–1626

Gregory P, Sinden SL, Osman SF, Tingey WM, Chessin DA (1981) Glycoalkaloids of wild, tuber-bearing *Solanum* species. J Agric Food Chem 29:1212–1215

Güntner C, Gonzalez A, Dos Reis R, González G, Vázquez A, Ferreira F, Moyna P (1997) Effect of *Solanum* glycoalkaloids on potato aphid, *Macrosiphum euphorbiae*. J Chem Ecol 23:1651–1659

Herms DA, Mattson WJ (1992) The dilemma of plants: to grow or defend. Q Rev Biol 67:283–283. doi:10.1086/417659

Jansky SH, Simon R, Spooner DM (2009) A test of taxonomic predictivity: resistance to the Colorado potato beetle in wild relatives of cultivated potato. J Econ Entomol 102:422–431

Kowalski SP, Domek JM, Deahl KL, Sanford LL (1999) Performance of Colorado potato beetle larvae, *Leptinotarsa decemlineata* (Say), reared on synthetic diets supplemented with *Solanum* glycoalkaloids. Amer J Potato Res 76:305–312

Le Roux V, Campan EDM, Dubois F, Vincent C, Giordanengo P (2007) Screening for resistance against *Myzus persicae* and *Macrosiphum euphorbiae* among wild *Solanum*. Ann Appl Biol 151:83–88. doi:10.1111/j.1744-7348.2007.00155.×

Lorenzen JH, Balbyshev NF, Lafta AM, Tian X, Sagredo B, Casper H (2001) Resistant potato selections contain leptine and inhibit development of the Colorado potato beetle (Coleoptera: Chrysomelidae). J Econ Entomol 94:1260–1267

Massei G, Hartley SE (2000) Disarmed by domestication? Induced responses to browsing in wild and cultivated olive. Oecologia 122: 225–231

Morrow PA, Bellas TE, Eisner T (1976) *Eucalyptus* oils in the defensive oral discharge of Australian sawfly larvae (Hymenoptera: Pergidae). Oecologia (Berl) 24:193–206

Mulatu B, Applebaum SW, Kerem Z, Coll M (2006) Tomato fruit size, maturity and α-tomatine content influence the performance of larvae of potato tuber moth *Phthorimaea operculella* (Lepidoptera: Gelechiidae). Bull Entomol Res 96:173–178. doi:10.1079/ber2005412

Müller C, Agerbirk N, Olsen CE, Boevé JL, Schaffner U, Brakefield PM (2001) Sequestration of host plant glucosinolates in the defensive hemolymph of the sawfly *Athalia rosae*. J Chem Ecol 27:2505–2516

Murashige T, Skoog F (1962) Revised medium for rapid growth and bioassay with tobacco tissue culture. Physiol Plant 15:473–497

Nenaah G (2011a) Individual and synergistic toxicity of solanaceous glycoalkaloids against two coleopteran stored-product insects. J Pest Sci 84:77–86. doi:10.1007/s10340-010-0329-y

Nenaah GE (2011b) Toxic and antifeedant activities of potato glycoalkaloids against *Trogoderma granarium* (Coleoptera: Dermestidae). J Stored Prod Res 47:185–190. doi:10.1016/j.jspr.2010.11.003

Opitz SEW, Boevé J-L, Nagy ZT, Sonet G, Koch F, Müller C (2012) Host shifts from Lamiales to Brassicaceae in the sawfly genus *Athalia*. PLoS One 7:e33649–e33649. doi:10.1371/journal.pone.0033649

Opitz SEW, Jensen SR, Müller C (2010) Sequestration of glucosinolates and iridoid glucosides in sawfly species of the genus *Athalia* and their role in defense against ants. J Chem Ecol 36:148–157. doi:10.1007/s10886-010-9740-3

Pelletier Y, Pompon J, Dexter P, Quiring D (2010) Biological performance of *Myzus persicae* and *Macrosiphum euphorbiae* (Homoptera: Aphididae) on seven wild *Solanum* species. Ann Appl Biol 156:329–336. doi:10.1111/j.1744-7348.2010.00389.x

Pianzzola MJ, Zarantonelli L, González G, Franco Fraguas L, Vázquez A (2005) Genetic, phytochemical and biochemical analyses as tools for biodiversity evaluation of wild accessions of *Solanum commersonii*. Biochem Syst Ecol 33:67–78

Poelman EH, Galiart RJFH, Raaijmakers CE, Van Loon JJA, Van Dam NM (2008) Performance of specialist and generalist herbivores feeding on cabbage cultivars is not explained by glucosinolate profiles. Entomol Exp Appl 127(3):218–228. doi:10.1111/j.1570-7458.2008.00700.x

Pompon J, Quiring D, Giordanengo P, Pelletier Y (2010) Characterization of *Solanum chomatophilum* resistance to 2 aphid potato pests, *Macrosiphum euphorbiae* (Thomas) and *Myzus persicae* (Sulzer). Crop Prot 29:891–897. doi:10.1016/j.cropro.2010.03.005

Prieto JM, Schaffner U, Barker A, Braca A, Siciliano T, Boevé J-L (2007) Sequestration of furostanol saponins by *Monophadnus* sawfly larvae. J Chem Ecol 33(3):513–524. doi:10.1007/s10886-006-9232-7

Rangarajan A, Miller AR, Veilleux RE (2000) Leptine glycoalkaloids reduce feeding by Colorado potato beetle in diploid *Solanum* sp. hybrids. J Am Soc Hortic Sci 125:689–693

Roddick JG, Rijnenberg AL, Weissenberg M (1992) Alterations to the permeability of liposome membranes by the solasodine-based glycoalkaloids solasonine and solamargine. Phytochemistry 31: 1951–1954. doi:10.1016/0031-9422(92)80339-g

Rosenthal JP, Dirzo R (1997) Efects of life history, domestication and agronomic selection on plant defence against insects: evidence from maizes and wild relatives. Evol Ecol 11:337–355

Schaffner U, Boevé J-L (1996) Sequestration of plant alkaloids by the sawfly *Rhadinoceraea nodicornis*: ecological relevance for different life stages and occurrence among related species. Entomol Exp Appl 80:283–285

Schaffner U, Boevé J-L, Gfeller H, Schlunegger UP (1994) Sequestration of *Veratrum* alkaloids by specialist *Rhadinoceraea nodicornis* Konow (Hymenoptera, Tenthredinidae) and its ecoethological implications. J Chem Ecol 20(12):3233–3250

Schmidt S, McKinnon AE, Moore CJ, Walter GH (2010) Chemical detoxification vs mechanical removal of host plant toxins in *Eucalyptus* feeding sawfly larvae (Hymenoptera: Pergidae). J Insect Physiol 56:1770–1776. doi:10.1016/j.jinsphys.2010.07.006

Schmidt S, Smith DR (2006) An annotated systematic world catalogue of the Pergidae (Hymenoptera). Contributions of the American Entomological Institute 34:1–207

Schmidt S, Walter GH, Moore CJ (2000) Host plant adaptations in myrtaceous-feeding pergid sawflies: essential oils and the morphology and behaviour of *Pergagrapta* larvae (Hymenoptera, Symphyta, Pergidae). Biol J Linn Soc 70:15–26. doi:10.1111/j.1095-8312.2000.tb00198.x

Schoonhoven LM, van Loon JJA, Dicke M (2005) Insect-plant biology, 2nd edn. Oxford University Press, USA

Schowalter TD (2006) Insect ecology: An ecosystem approach, 2nd edn. Press, Academic

Small E (1996) Adaptations to herbivory in alfalfa (*Medicago sativa*). Can J Bot 74:807–822. doi:10.1139/b96-102

Staba JE (1969) Plant tissue culture as a technique for the phytochemist. Recent Adv Phytochem 8:80

Tait NN (1962) The anatomy of the sawfly *Perga affinis affinis* Kirby (Hymenoptera: Symphyta). Aust J Zool 10:652–683

Tingey WM (1984) Glycoalcaloids as pest resistance factors. Am Potato J 61:157–167

Tingey WM, Sinden SL (1982) Glandular pubescence, glycoalkaloid composition, and resistance to the green peach aphid, potato leafhopper, and potato fleabeetle in *Solanum berthaultii*. Am Potato J 59:95–106

van Gelder WMJ, Vinket JH, Scheffer JJC (1988) Steroidal glycoalkaloids in tubers and leaves of *Solanum* species used in potato breeding. Euphytica 158:147–158

Vázquez A, González G, Ferreira F, Moyna P, Kenne L (1997) Glycoalkaloids of *Solanum commersonii* Dun. ex Poir. Euphytica 95:195–201

Wise IL, Lamb RJ, Smith MAH (2001) Domestication of wheats (Gramineae) and their susceptibility to herbivory by *Sitodiplosis mosellana* (Diptera: Cecidomyiidae). Can Entomol 133:255–267

Yencho GC, Kowalski SP, Kennedy GG, Sanford LL (2000) Segregation of leptine glycoalkaloids and resistance to Colorado potato beetle (*Leptinotarsa decemlineata* (Say)) in F2 *Solanum tuberosum* (4x) x *S. chacoense* (4x) potato progenies. Amer J Potato Res 77(3):167–178