Leptine Glycoalkaloids Reduce Feeding by Colorado Potato Beetle in Diploid Solanum sp. Hybrids

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Abstract. Leptine glycoalkaloids in leaves of the weedy diploid potato, Solanum chacoense Bitt., have been shown to reduce feeding by Colorado potato beetle (CPB; Leptinotarsa decemlineata Say.). Development of cultivated potatoes with natural resistance to CPB has the potential to reduce costs and environmental impacts of production by reducing pesticide use. Through efforts to move the genes controlling leptine biosynthesis into cultivated potato, a series of hybrids was generated between the high leptine producing S. chacoense and a cultivated type, S. phureja Juz. and Buk. These hybrids were evaluated for solanine (+chaconine), leptinins, leptines, and total steroidal glycoalkaloid content. All hybrids contained leptines, but at different levels (ranging from 117 to 802 mg·g−1 dry weight of leptine aglycon). Some hybrids appeared to convert solanine (+chaconine) to leptine and leptine efficiently and had no detectable solanine in sampled leaves. To verify the biological significance of these glycoalkaloids, leaf tissue was subjected to feeding assays with second instar CPB. CPB feeding rate ranged from 38 to 87 mm2·d−1 and was most closely correlated with leptine concentration. A minimum leptine level of 300 mg/100 g fresh leaves suppressed feeding by 50%, and levels below this had no effect on CPB feeding.

Development of potatoes (Solanum L. sp.) with natural resistance to the Colorado potato beetle (CPB) (Leptinotarsa decemlineata) may reduce costs and environmental impacts of production by reducing pesticide use and enhancing insect resistance management strategies. The introduction of cultivars expressing the Bacillus thuringiensis protein toxin is one example of this approach (Perlak et al., 1993). Steroidal glycoalkaloids (SGAs) occur naturally in potato and other crops and confer resistance to CPB and other potato pests (Sanford and Ladd, 1992; Tingey, 1984). SGAs have been described as nonspecific antifeedants (Sturckow and Low, 1961) or toxins (Smith 1984). Both the concentration of SGA and the specific SGA influence antifeedant activity (Tingey 1984). However, expression of these SGAs must be confined to leaves and not affect tuber quality. The potato cultivar ‘Lenape’ was removed from the market due to excessive SGA levels in tubers (human heath risk limit is 100 mg·kg−1 fresh weight (FW) (Zitnak and Johnston, 1970).

Specific steroidal glycoalkaloids known as leptines are common in certain accessions of the noncultivated diploid potato [Solanum chacoense (chu)] and are known to reduce feeding by CPB (Carter, 1987; Sinden et al., 1980; Sinden et al., 1986; Sturckow and Low, 1961). Leptines have anticholinesterase-type activity, effectively deterring feeding by CPB when present at 1 mM concentrations (Sturckow and Low, 1961; Tingey 1984). The SGAs solanine and chaconine also deter CPB feeding, but must be present at concentrations >6 mM. More importantly, leptines are found only in the leaf tissue, so are absent from tubers, unlike solanine and chaconine (Kuhn and Low, 1961; Lawson et al., 1992; Sanford et al., 1995; Sinden et al., 1986; Veilleux and Miller, 1998).

There have been ongoing efforts to introgress the genes controlling leptine production into cultivated tetraploid potato [Solanum tuberosum (tbr)] (Sanford et al., 1996; Veilleux et al., 1992). The enzyme systems regulating the conversion of solanidine (SD, the aglycon of solanine and chaconine) to solanine, chaconine and leptines, however, remain poorly understood (Lawson et al., 1993). Different clones of leptine-producing potato may vary, however, in the efficiency of conversion from solanine to leptines (Sinden et al., 1980). A chc clone (PI 458310) has been used as a parent in hybrid crosses in several studies, because 90% of its SGAs are leptines (Sinden et al., 1986; Veilleux and Miller, 1998). Hybrids between chc and tbr must be horticulturally acceptable and possess foliar levels of leptines sufficient to reduce CPB feeding. It is important to note that foliar leptine levels necessary to impart significant feeding deterrence are not well defined.

Sanford et al. (1994, 1995, 1996, 1997) evaluated hybrid and backcross populations between chc and tbr for leaf leptine content, tuber size, and tuber SGA content. Chromosome doubled derivatives (4x) of chc (2x) were crossed to tbr (4x). Leptine and total glycoalkaloid concentrations in foliage varied among hybrids. In hybrid populations and through F2 generations, tuber size was reduced and tuber SGA content remained higher than the level accepted for human consumption. Backcrossing to the tbr parent reduced tuber SGA levels to acceptable levels. No correlation was found between leaf and tuber SGA content (Sanford et al., 1995).

Veilleux et al. (1992) employed a different strategy to enhance...
leptine concentrations in cultivated potato. Clones of chc were crossed with the cultivated diploid (2x) S. phureja (phu), to serve as a bridge cross to tbr (Carter, 1987). This approach has several advantages. Solanum phureja tubers have good horticultural qualities and are consumed in many countries (Haynes, 1972). In addition, phu produces unreduced gametes naturally, which facilitates crossing with tbr. The resulting 4x x 2x hybrids would consist of only one-fourth unadapated chc germplasm compared to one-half for tbr x 4x chc hybrids. By doubling chc for crossing to tbr, horticultural quality also may be compromised due to inbreeding when using chromosome doubled chc. (Sanford et al., 1995). Third, phu is responsive to anther culture, allowing derivation of monoploids from progeny between chc and phu (Veilleux and Miller, 1998). The simpler genetic system of monoploids may facilitate our understanding of inheritance of glycoalcaloids.

For work reported herein, we studied a population of 2x hybrids between phu and chc generated by Veilleux and Miller (1998). Our primary goal was to assess whether the concentrations of leptins found in these hybrids suppressed CPB feeding. The minimum and effective dose to reduce feeding by 50% (EDso levels of total and individual SGA that affected CPB feeding were also defined. Actual amounts of leaf tissue consumed were measured to deduce biologically active concentrations of SGA. The objectives were to 1) characterize differences in SGA concentration among phu x chc hybrids, 2) identify differences among hybrids for feeding rates by CPB, 3) determine the relationship between feeding rates and SGA concentration, and 4) identify hybrids for breeding efforts to move SGA genes into tbr.

**Materials and Methods**

**Plant material.** Hybrids were generated between phu and chc by Veilleux (Veilleux and Miller, 1998). Hybrids with the prefix ‘CP’ were from crosses with chc as the female parent and ‘RPC’ were from crosses with phu as female parent. The hybrids were generated by crosses between phu clones BARD 1-3, PP5 or anther-derived homozygous doubled monoploid derivatives of PP5, and chc clones 8380-1 and 55-1 (derived from PI 458310) (Table 1). The selections listed in Table 1 represent plants that were sufficiently vigorous to complete a life cycle and were used in the CPB feeding studies.

The tbr cultivar ‘Russet Burbank’ and a chc parent (PI 458310) were included in all feeding trials as controls. Preliminary experiments comparing phu and tbr indicated no significant difference in SGA content or insect feeding rates (data not presented). Therefore, tbr was selected as a control to compare feeding results on hybrids to a commercial cultivar. Tubers were sprouted in the greenhouse, and plants were maintained under cool-white fluorescent supplemental lighting (14 h photoperiod), days/nights of 29/18 °C, and twice weekly fertilization with 200 ppm N from Peters General Purpose 20–20–20 water soluble fertilizer (Scotts, Marysville, Ohio). The sixth leaf (fully expanded) was harvested for the CPB feeding assays. Two experiments were conducted (1994 and 1995), each having three replicate feeding trials and SGA analyses. In 1995, some additional hybrids were tested along with five of the clones from 1994 (Table 1).

**SGA analysis.** Leaflets from one side of a leaf were sampled and pooled for SGA analysis. The predominant SGA, solanine (and chaconine, for hybrids), leptine and leptine, were extracted and hydrolyzed to their corresponding aglycons, solanidine (SD), leptinidine (LD), and acetyl-leptinidine (ALD) and quantified using capillary gas chromatography (Lawson et al., 1992). About 20 mg of freeze-dried leaf tissue was placed in a 10 mL screw top vial, along with 200 µg of tomatine as an internal standard (Sigma-Aldrich Chem. Co., St. Louis, Mo.) and 6 mL of 1 mol-L⁻¹ HC1 in methanol. The headspace was purged with N2 gas, the vial sealed, and placed in a shaking water bath, 70 °C, for 4 h. At the end of the extraction–hydrolysis period, the vials were allowed to cool to 25 °C and 4 mL concentrated NH4OH added to increase pH > 10. Vials were then centrifuged at 1800 g_for 10 min. Supernatants were partitioned against 3 mL of benzene. Duplicate 1 mL aliquots of the benzene phase were plated in amber vials and evaporated to dryness at 50 °C under N2 gas. The residue containing SGA aglycons was redissolved in 500 µL chloroform for injection onto 15 m x 0.25 mm i.d. x 0.25 µm RT, 1 fused silica column (Restek Corp, Bellefonte, Pa), installed in a Hewlett-Packard 5890A gas chromatograph (Hewlett-Packard, Avondale, Pa.) using helium carrier gas at a linear flow rate of 45 cm-s⁻¹, injector temperature of 270 °C, column temperature program at 210 °C, increasing 2 °C-min⁻¹ to 270 °C, and flame ionization detector at 280 °C. For each sample, the mean aglycon concentration was based upon two GC injections. Aglycons represent ~40% of the total SGA mass (Lawson et al., 1997).

**Insect feeding assay.** Adult CPB were gathered from field plots, in Wooster, Ohio, and maintained in the greenhouse, in cages containing tbr ‘Russet Burbank’. Egg masses were harvested daily from cages, and larvae were reared on leaves of tbr until the second instar larval stage. Ten leaf disks (113 mm² including center vein) were cut with a cork borer, from leaflets opposite those collected for SGA analysis. The individual leaf disks were placed in 60 x 15 mm petri dishes containing moist filter paper and one second instar CPB larva was allowed to feed on the disk for 24 h. During feeding assays, these dishes were held in a controlled environment at 22 °C and with a 16-h photoperiod of 130 µmol-m⁻²-s⁻¹ provided by cool-white fluorescent lamps. After this period, the remaining disk area was measured (0.1 mm² accuracy) using a leaf area meter (Delta T Devices, Cambridge, U.K.) coupled to a dissecting microscope (model SZ-6045; Olympus, Lake Success, N.Y.). Ten individual insect assays were conducted for each hybrid, chc and tbr, using new groups of CPB larva for each replication.

**Statistical analyses.** Average leaf area consumed was calculated from the results of 10 feeding assays on each clone. These averages represented one replication for statistical analysis. Total and individual aglycon concentrations and the ratio of individual

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Table 1. Diploid (2n = 2x = 24) hybrids of Solanum phureja x S. chacoense used in feeding studies with Colorado potato beetle in 1994 and 1995.

| Clone | Parents | Year tested |
|-------|---------|-------------|
| RPC-1 | phu (PP5) x chc (8380-1) | x | 1994 |
| RPC-2 | phu (AD2-4) x chc (55-1) | X | 1995 |
| RPC-3 | phu(AD3-4) x chc (55-1) | X | 1995 |
| RPC-4 | phu (AD29-1) x chc (55-1) | X | 1994 |
| RPC-5 | phu (PP5) x chc (8380-1) | X | 1995 |
| RPC-6 | phu (PP5) x chc (8380-1) | X | 1995 |
| RPC-7 | phu (AD4-1) x chc (8380-1) | X | 1995 |
| CP-1 | chc (8380-1) x phu (BARD1-3) | X | 1994 |
| CP-2 | chc (8380-1) x phu (BARD1-3) | X | 1995 |
| CP-3 | chc (8380-1) x phu (BARD1-3) | X | 1995 |
| CP-4 | chc (8380-1) x phu (BARD1-3) | X | 1995 |

Ph or RP or phu = S. phureja, or C chc = S. chacoense.
Table 2. Leaf concentrations of principle aglycons of *Solanum* sp. and hybrids and leaf area consumed by second instar Colorado potato beetle. Plants were grown in the greenhouse in 1994.

| Clone  | SD    | LD       | ALD     | Total   | ALD/Total | ALD + LD/Total |
|--------|-------|----------|---------|---------|-----------|---------------|
| chc    | 1823 b | 5265 d   | 8932 d  | 16020 de| 0.36 f    | 0.89 d 7 a    |
| CP-1   | 2380 b | 4122 cd  | 6355 cd | 12857 cd| 0.49 ef   | 0.81 c 59 bc |
| CP-2   | 1994 b | 2720 bc  | 3910 bc | 8624 bc | 0.45 de   | 0.77 c 78 c  |
| CP-4   | 1453 ab| 2680 bc  | 2366 ab | 6498 ab | 0.36 bc   | 0.77 c 84 c  |
| RPC-1  | 0 a   | 1663 b   | 1165 ab | 2828 a  | 0.41 cde  | 1.00 e 88 c  |
| RPC-2  | 0 a   | 1664 b   | 2047 ab | 3711 ab | 0.55 f    | 1.00 e 65 c  |
| RPC-4  | 2905 b | 2342 b   | 2554 cd | 7802 abc| 0.33 b    | 0.63 b 86 c  |
| RPC-6  | 7031 d | 4437 d   | 8021 d  | 19490 e | 0.40 cd   | 0.63 b 38 ab |
| tbr    | 5175 c | 0 a      | 0 a     | 5175 ab | 0.00 a    | 0.00 a 72 c  |

Values in columns represent means from three replications, each with 10 samples. Mean separation within columns by Fisher’s protected LSD, *P* < 0.05.

Aglycon concn (µg g⁻¹ DW) * Ratio of aglycons * Leaf area consumed (mm²)

Table 3. Leaf concentrations of principle aglycons of *Solanum* sp. and hybrids and leaf area consumed by second instar Colorado potato beetle. Plants were grown in the greenhouse in 1995.

| Clone  | SD    | LD       | ALD     | Total   | ALD/Total | ALD + LD/Total |
|--------|-------|----------|---------|---------|-----------|---------------|
| chc    | 1211 ab| 6266 c   | 1126 d  | 18603 f | 0.61 cd   | 0.94 e 8 a    |
| CP-1   | 9571 e | 1239 ab  | 3467 b  | 14284 ef| 0.24 ab   | 0.33 b 69 b   |
| CP-2   | 3270 abc| 423 a   | 2917 b  | 6610 abc| 0.64 d    | 0.68 d 66 b   |
| CP-4   | 7135 de| 729 a    | 3148 b  | 11012 cde| 0.30 b    | 0.36 b 67 b   |
| RPC-3  | 1356 ab| 142 a    | 1815 ab | 3314 ab | 0.58 cd   | 0.62 cd 60 b  |
| RPC-4  | 2907 abc| 148 a   | 2298 b  | 5385 ab | 0.44 bcd  | 0.47 bcd 71 b |
| RPC-6  | 3845 bcd| 2170 b  | 5914 c  | 11929 de| 0.46 bcd  | 0.63 d 45 b   |
| RPC-7  | 0 a    | 0 a      | 1601 a  | 1601 a  | 1.00 e    | 1.00 e 70 b   |
| RPC-8  | 4301 bcd| 379 a   | 2756 b  | 7436 bcd| 0.37 bc   | 0.43 bcd 64 b |
| tbr    | 5346 cd| 0 a      | 0 a     | 5346 ab | 0.00 a    | 0.00 a 52 b   |

*SD* = solanidine, *LD* = leptinidine, *ALD* = acetylleptinidine, *total* = total aglycons.

Values in columns represent means from three replications, each with 10 samples. Mean separation within columns by Fisher’s protected LSD, *P* < 0.05.

Results

**SGA analyses.** Individual and total SGA concentrations, and SGA ratios varied among the hybrids (Tables 2 and 3). The hybrid parent *S. chacoense* contained solanidine (SD), confirming the presence of solanine (and chaconine). Detection of leptinidine (LD) and acetylleptinidine (ALD) indicated the presence of leptinines and leptines, respectively. All hybrids contained ALD, but varied for presence of SD and LD. As expected, *tbr* contained only SD (Tables 2 and 3). The total concentration of ALD in *S. chacoense* was the highest of all materials tested, in both experiments. Feeding rates on *S. chacoense* in both experiments. Feeding rates on *tbr* in both experiments. Feeding rates on *tbr* in both experiments. Feeding rates on *tbr* in both experiments.
consumed by CPB ranged from 38 to 88 mm²·d⁻¹ over the 2 years. The hybrid RPC-4 supported the highest feeding rate in both experiments (88 mm²·d⁻¹ in 1994 and 71 mm²·d⁻¹ in 1995). Feeding rate was reduced by 47% (1994) (significant) and by 13% (1995) (nonsignificant) of tbr when CPB were fed hybrid RPC-6. Lines CP-1, CP-2, CP-4, and RPC-4 supported CPB feeding levels that were 15% to 30% higher than tbr in 1994 and the same lines plus RPC-7 were 28% to 37% higher than tbr in 1995, but these rates were not significantly different from tbr (Tables 2 and 3).

**Statistical Analyses.** Statistical analysis of feeding rate and SGA concentration in all clones tested over 2 years indicated no significant differences or interactions between the 2 years (data not presented). In addition, there were no significant interactions among the SGAs and CPB feeding, in either year of study. Multiple regression analysis of the concentrations of the three aglycons versus the leaf area consumed indicated that ALD concentrations were most closely correlated with feeding rates (Table 4; P < 0.0001). ALD concentration was more important than total SGA content as well. With increasing levels of ALD in the hybrids, feeding was suppressed. The concentrations of solanidine and leptinidine had no significant effect on insect feeding. Based on the model (Table 4), ALD concentrations <6500 µg·g⁻¹ DW leaf tissue did not appear to deter feeding. Furthermore, levels of ALD greater than 8200 µg·g⁻¹ DW were calculated to decrease feeding by 50% (ED₉₀) relative to tbr.

**Discussion**

This study supports previous research that identified leptines as more effective than solanine, chaconine and lepinotinines as antifeedants to the CPB (Carter, 1987; Sinden et al., 1980, 1986; Sturckow and Low, 1961). Concentrations of ALD, the aglycon of leptines, were significantly correlated to feeding suppression by CPB (Table 4), indicating that leptines were more important than other SGAs or total SGA content in predicting feeding rates. We found a minimum ALD concentration of 8200 µg·g⁻¹ DW leaf tissue was required to suppress feeding 50%. This concentration is equivalent to 120 mg ALD per 100 g FW of hybrid leaf material (15% DW), or 300 mg leptines/100 g FW (assuming 40% of leptine mass is aglycon) (Lawson et al., 1997). Other researchers have observed that clones with levels of leptines ranging from 120 to 300 mg/100 g FW had less feeding damage than those containing lower levels (<51 mg/100 g FW) (Sinden et al., 1986). This observed ED₉₀ of ALD provides plant breeders with a target level of ALD expression, to allow rapid screening of hybrids without use of insect bioassays.

All chc x phu hybrids evaluated contained leptines, as observed previously (Veilleux and Miller, 1998). Levels of aglycons varied between years of testing, but those clones which contained the highest concentrations of ALD and total aglycons were consistent between years and comparable with levels in previous research (Veilleux and Miller, 1998). Total SGA content is under polygenic control and can be modified by photoperiod, irradiance, and quality and may vary by the stage of growth or plant part sampled (Deahl et al., 1991; Tingey, 1984). Leptine (ALD) levels were generally higher in 1994 than 1995, perhaps due to slightly higher levels of irradiance during spring versus full production (Tables 2 and 3). *Solanum chacoense* plants grown under higher irradiances had increased leptine production and reduced CPB feeding levels than plants grown under lower irradiances (Deahl et al., 1991).

Calculation of the percentage of SGAs present as leptines may provide some indication of the efficiency of conversion of solanine to leptines (Sanford et al., 1997; Veilleux and Miller, 1998). In a comparison of selected 2x crosses between chc and tbr, 80% of total SGAs were leptines in all hybrids (Sanford et al., 1997). Mean CPB larval weight was significantly reduced after feeding for 3 d on these hybrids. Although some of the clones (RPC-1, RPC-2, and RPC-7) we tested contained all of their SGAs as either leptine (LD) or leptine (ALD), suggesting that these are highly efficient converters of solanine to leptines, absolute levels of SGAs were too low to affect CPB feeding. In addition, these relative ratios among the SGAs varied between the 2 years, supporting the importance of screening these materials over multiple seasons (Tables 2 and 3).

Several of the hybrids tested, all containing some leptines, supported higher feeding levels than tbr. In many cases, these lines had lower SD and total SGA content than tbr and low concentrations of ALD (<4000 µg·g⁻¹ DW). Other research examining impact of SGAs and leaf trichomes on CPB resistance indicated trichomes may be more important than SGAs in some *Solanum sp*. (Barbour and Kennedy, 1991; Neal et al., 1989). Differences in trichome density among somatic hybrids of diploid interspecific *Solanum* clones contributed to reduced feeding rates by CPB larva (Jansky et al., 1999). Trichome density, shape and length was evaluated in several lines (chc, tbr, phu, RPC4, and CP2), in a separate set of observations, to determine if CPB

![Table 4. Analysis of variance (ANOVA) and regression parameter estimates from results of feeding rates by Colorado potato beetle on Solanum phureja x S. chacoense hybrids and parents.](image)
feeding levels may have been partially related to differences in this physical trait and not to alkaloids. Five leaflets from midridge leaves (fully expanded) on at least two plants of each clone were collected and a 1 cm by 0.5 cm tissue area was excised from each side of the midvein of each leaflet. The number of trichomes was counted on each piece and the structure evaluated. All clones had hairs in the range of 0.5 to 1 mm in length, and most were single, but a few had multiple branching. The chc parent had the same trichome density as tbr, 92 (±3 SE) vs. 123 (±23 SE) trichomes/cm², respectively. The phu clone averaged 112 (±16 SE) trichomes/cm². Trichome density in RPC4 averaged 56 (±5 SE) and CP2 had 94 (±9 SE) trichomes/cm². These observations were from different plants, grown at a different time from those used in the CPB feeding and SGA analysis experiments, so few conclusions may be drawn. However, RPC4 and CP2 supported higher feeding than tbr, but only RPC4 had a lower trichome density than tbr whereas chc supported lower feeding rates and had a similar trichome density to tbr and phu. Additional studies would be required to examine if trichome density, length or structure directly affected CPB feeding levels.

All of the hybrids evaluated in this study were F₁ hybrids, and all expressed leptines. Since the phu parent did not produce any leptines, expression of leptines in the hybrids was due to genes from the chc parent. For SD, alleles would come from both parents (phu and chc both express SD). The chc parents were derived from a cross-pollinating self-incompatible population; therefore it is unknown if these parents were homozygous for leptine expression. However, because all of our F₁ hybrids expressed some LD and ALD, the chc must have been homozygous at the loci controlling this expression. Otherwise, the F₁ hybrids would have segregated. Therefore, dominance of leptine gene(s) is indicated. Higher levels of leptine expression and greater CPB resistance may be obtained in advanced generation hybrids through homozygosity of alleles controlling leptine production, especially if additive genetic variance is important. The hybrid RPC-6, which had significantly reduced feeding rates compared to the other hybrids examined, appeared to convert much of its leaf solanine into leptines, suggesting it as a good candidate for future breeding efforts to move the genes for leptine biosynthesis into cultivated potato.

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