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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 leptinine 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 mm^2·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* (*chc*)] 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 (4*x*) of *chc* (2*x*) were crossed to *tbr* (4*x*). Leptine and total glycoalkaloid concentrations in foliage varied among hybrids. In hybrid populations and through F_4 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

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

Clone[z]	Parents	Year tested 1994	1995
RPC-1	*phu* (PP5) x *chc* (8380-1)	X	
RPC-2	*phu* (AD2-4) x *chc* (55-1)	X	
RPC-3	*phu*(AD3-4) x *chc* (55-1)		X
RPC-4	*phu* (AD29-1) x *chc* (55-1)	X	X
RPC-5	*phu* (PP5) x *chc* (8380-1)	X	X
RPC-7	*phu* (PP5) x *chc* (8380-1)		X
RPC-8	*phu* (AD4-1) x *chc* (8380-1)		X
CP-1	*chc* (8380-1) x *phu* (BARD1-3)	X	X
CP-2	*chc* (8380-1) x *phu* (BARD1-3)	X	X
CP-4	*chc* (8380-1) x *phu* (BARD1-3)	X	X

[z]P or RP or *phu* = *S. phureja*, or C *chc* = *S. chacoense.*

leptine concentrations in cultivated potato. Clones of *chc* were crossed with the cultivated diploid (2*x*) *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 4*x* x 2*x* hybrids would consist of only one-fourth unadapted *chc* germplasm compared to one-half for *tbr* x 4*x 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 promising hybrids between *chc* and *phu* (Veilleux and Miller, 1998). The simpler genetic system of monoploids may facilitate our understanding of inheritance of glycoalkaloids.

For work reported herein, we studied a population of 2*x* hybrids between *phu* and *chc* generated by Veilleux and Miller (1998). Our primary goal was to assess whether the concentrations of leptines found in these hybrids suppressed CPB feeding. The minimum and effective dose to reduce feeding by 50% (ED_{50}) levels of total and individual SGAs that affected CPB feeding were also defined. Actual amounts of leaf tissue consumed were measured to deduce biologically active concentrations of SGAs. 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 ºC, 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 SGAs, solanine (and chaconine, for hybrids), leptinine and leptine, were extracted and hydrolyzed to their corresponding aglycons, solanidine (SD), leptinidine (LD), and acetylleptinidine (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} HCl in methanol. The headspace was purged with N_2 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 NH_4OH added to increase pH > 10. Vials were then centrifuged at 1800 g_n for 10 min. Supernatants were partitioned against 3 mL of benzene. Duplicate 1 mL aliquots of the benzene phase were placed in amber vials and evaporated to dryness at 50 °C under N_2 gas. The residue containing SGA aglycons was redissolved in 500 µL chloroform for injection onto 15 m × 0.53 mm i.d. × 0.25 µm RT_x1 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 mm^2 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 × 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 ºC 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 mm^2 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

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.

	Aglycon concn ($\mu g \cdot g^{-1}$ DW)[z]				Ratio of aglycons		Leaf area
Clone[y]	SD	LD	ALD	Total	ALD/Total	ALD + LD/Total	consumed (mm^2)
chc	1823 b[x]	5265 d	8932 d	16020 de	0.56 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

[z]SD = solanidine, LD = leptinidine, ALD = acetylleptinidine, total = total aglycons.
[y]P or RP = *S. phureja*, *chc* or C = *S. chacoense*, *tbr* = *S. tuberosum* 'Russet Burbank'.
[x]Values in columns represent means from three replications, each with 10 samples. Mean separation within columns by Fisher's protected LSD, $P < 0.05$.

aglycons to the total (nontransformed) in the hybrids, *tbr* and *chc* from each of the replicates, were used in statistical analyses. Within each year of testing, three full replicates of feeding trials and SGA analyses were conducted. Analysis of variance was conducted using SAS's general linear model procedures (SAS Institute Inc., Cary, N.C.). Multiple regression analysis was used to identify alkaloids having the most significant effect on CPB feeding.

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 (Tables 2 and 3). Among the hybrids, RPC-6 and CP-1 accumulated the highest total SGAs (Tables 2 and 3). In 1994, RPC-1 and RPC-2 contained the lowest levels of total SGAs of tested materials (including *tbr* but not significantly different from *tbr*), and these two hybrids did not differ significantly from each other for quantities of individual aglycons (Table 2). In 1995, RPC-3 and RPC-7 both had lower total aglycons than *tbr*, though again not significantly different from *tbr* (Table 3).

The percentage of total SGAs present as either ALD or combined LD plus ALD were calculated (Tables 2 and 3). The concentration of ALD in the hybrids ranged from 24% to 100% of the total aglycons extracted, over the 2 years of study. The percentage of total SGA aglycons present as ALD plus LD in the hybrids ranged from 33% to 100%. RPC-6 contained the highest concentration of ALD in both years (Tables 2 and 3), which represented 40% and 46%, respectively, of the total aglycons in this clone. Three of the hybrids evaluated (RPC-1, RPC-2, and RPC-7) contained no SD. Therefore, 100% of aglycons extracted from RPC-7 were ALD, while 100% of the SGAs in RPC-1 and RPC-2 were ALD plus LD.

INSECT FEEDING BIOASSAY. The leaf areas consumed in 24 h by second instar CPB ranged from 7 to 88 mm^2 in 1994 and 8 to 71 mm^2 in 1995 (Tables 2 and 3). CPB exhibited significantly lower feeding rates on *chc* in both experiments. Feeding rates on *tbr* were similar to several hybrids, averaging 72 $mm^2 \cdot d^{-1}$ in 1994 (Table 2) and 52 $mm^2 \cdot d^{-1}$ in 1995 (Table 3). For hybrids, leaf area

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.

	Aglycon concn ($\mu g \cdot g^{-1}$ DW)[z]				Ratio of aglycons		Leaf area
Clone[y]	SD	LD	ALD	Total	ALD/Total	ALD + LD/Total	consumed (mm^2)
chc	1211 ab[x]	6266 c	11126 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 bc	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 ab	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

[z]SD = solanidine, LD = leptinidine, ALD = acetylleptinidine, total = total aglycons.
[y]P or RP = *S. phureja*, *chc* or C = *S. chacoense*, *tbr* = *S. tuberosum* 'Russet Burbank'.
[x]Values in columns represent means from three replications, each with 10 samples. Mean separation within columns by Fisher's protected LSD, $P < 0.05$.

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.

		ANOVA[z]		
Source	df	Mean square	F	$P > F$
Acetylleptinidine	1	12209.1	49.05	<0.0001
Leptinidine	1	7.6	0.03	0.8623
Solanidine	1	35.2	0.14	0.7048
Error	47	11697.9		
		Parameter estimates		
Variable		Parameter	SE	Pr > |t|
Intercept		86.07	4.3	<0.0001
Acetylleptinidine		–0.0068	0.001	<0.0001
Leptinidine		0.0003	0.0016	0.8623
Solanidine		0.0003	0.0008	0.7048

[z]Average feeding rate observed was 57 $mm^2 \cdot d^{-1}$. The model had an $R^2 = 0.694$.

consumed by CPB ranged from 38 to 88 $mm^2 \cdot d^{-1}$ over the 2 years. The hybrid RPC-4 supported the highest feeding rate in both experiments (88 $mm^2 \cdot d^{-1}$ in 1994 and 71 $mm^2 \cdot d^{-1}$ 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 $\mu g \cdot g^{-1}$ DW leaf tissue did not appear to deter feeding. Furthermore, levels of ALD greater than 8200 $\mu g \cdot g^{-1}$ DW were calculated to decrease feeding by 50% (ED_{50}) relative to *tbr*.

Discussion

This study supports previous research that identified leptines as more effective than solanine, chaconine and leptinines 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 $\mu g \cdot g^{-1}$ 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 ED_{50} 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 fall 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 2*x* 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 leptinine (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 $\mu g \cdot g^{-1}$ 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

feeding levels may have been partially related to differences in this physical trait and not to alkaloids. Five leaflets from midage 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/cm^2, respectively. The *phu* clone averaged 112 (±16 SE) trichomes/cm^2. Trichome density in RPC4 averaged 56 (±5 SE) and CP2 had 94 (±9 SE) trichomes/cm^2. 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 F_1 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 F_1 hybrids expressed some LD and ALD, the *chc* must have been homozygous at the loci controlling this expression. Otherwise, the F_1 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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