Segregation of Leptine Glycoalkaloids and Resistance to Colorado Potato Beetle (*Leptinotarsa decemlineata* (Say)) in F2 *Solanum tuberosum* (4x) x *S. chacoense* (4x) Potato Progenies

G. Craig Yencho[1]*, Stanley P. Kowalski[2]*, George G. Kennedy[3], and Lind L. Sanford[2]

[1]Department of Horticultural Science, North Carolina State University, Vernon James Research and Extension Center, 207 Research Station Road, Plymouth, NC 27962.
[2]USDA/ARS/Plant Science Institute, Vegetable Laboratory, Beltsville, MD 20705.
[3]Department of Entomology, North Carolina State University, Box 7630, Raleigh , NC 27965.
***Correspondence:** Dr. Craig Yencho, Dept. of Horticultural Science, NCSU-VJREC, 207 Research Station Road, Plymouth, NC 27962, P252.793.4428 x 147, F252.793.5142, Craig_Yencho@NCSU.edu.
*Current Address: ISAAA AmeriCenter, 260 Emerson Hall, Cornell University, Ithaca, NY 14853.

ABSTRACT

***Solanum chacoense* Bitter is resistant to the Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say). Resistance has been associated with the presence of a rare class of glycoalkaloids, the leptines. In this study, seven tetraploid, F2 *S. tuberosum* x *S. chacoense* families were evaluated for foliar production of leptines I and II, leptinines I and II, and α-solanine and α-chaconine; and screened for resistance to CPB in the laboratory and field. Resistance was correlated with the concentrations of glycoalkaloids on a family and an individual basis. Leptine concentrations ranged from undetectable to a high of 18.0 mg/g dry weight. All of the progeny produced solanine and chaconine. Family 9623 had the highest mean leptine concentration and the lowest mean leaf disk feeding and CPB defoliation levels. Family 9616 had the lowest mean glycoalkaloid concentration and ranked as one of the most susceptible families. Regression analyses of solanine + chaconine, leptine I and II, and leptinine I and II foliar concentrations versus leaf disk consumption and field defoliation revealed that only increased foliar levels of leptines resulted in decreased CPB feeding. The regression models for leptines versus leaf disk consumption and field defoliation were highly significant, accounting for 17% and 26% of the variation in consumption and defoliation, respectively. To the best of our knowledge, this is the first work reporting the impact of leptine and leptinine concentrations on CPB feeding in tetraploid, *S. tuberosum* x *S. chacoense* potato hybrids. Results are discussed within the context of breeding for resistance to CPB.**

RESUMEN

***Solanum chacoense* Bitter es resistente al escarabajo de la papa (CPB), *Leptinotarsa decemlineata* (Say). La resistencia ha sido asociada con la presencia de una clase rara de glicoalcaloides, los leptinos. En este estudio, siete familias tetraploides, F2 *S. tuberosum x S. chacoense* fueron evaluadas por la producción foliar de leptinos I y II, leptininos I y II, y α-solanina y α-chaconina, y revisadas por su resistencia al escarabajo de la papa en el laboratorio y el campo. La resistencia fue correlacionada con las concentraciones de glicoalcaloides sobre una base familiar e individual. Concentraciones de leptinos se extendieron de no percibidas hasta un máximo de 18.0 mg/g peso seco. Toda la progenie producía solanina y chaconina. La familia 9623 tenía el promedio de concentración más alto de leptinos, y el promedio más bajo tanto para el consumo de discos de hoja, como en los niveles de defoliación por el escarabajo de la papa. La familia 9616 demostró el promedio de concentración más bajo de glicoalcaloides, y se mostró como una de las familias más susceptibles. Los análisis de regresión de solanina + chaconina, y las concentraciones foliares de leptino I**

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y II, y leptinino I y II contra el consumo de discos de hoja y la defoliación en campo, revelaron que sólo los niveles foliares aumentados de leptinos resultaron en una alimentación reducida de parte del escarabajo de la papa. Los modelos de regresión para leptinos contra el consumo de discos de hoja, y la defoliación en el campo, fueron sumamente signifcantes, y explicaron el 17% y el 26% de la variación en el consumo y la defoliación, respectivamente. Que sepamos, este es el primer trabajo que informa del impacto de concentraciones de leptinos y leptininos sobre el consumo que realiza el escarabajo de la papa de los híbridos de papa tetraploides *S. tuberosum x S. chacoense*. Los resultados se evalúan dentro del contexto de la crianza para la resistencia al escarabajo de la papa.

INTRODUCTION

The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say), is a major limiting factor to potato production in the US, Canada, eastern Europe, and the former Soviet Union (Ferro 1985; Weber and Ferro 1994). Until recently, efforts to control CPB have relied almost exclusively on the use of insecticides. During the last decade, however, significant progress has been made developing potatoes resistant to CPB using conventional and transgenic breeding techniques (Perlak *et al.* 1993; Tingey and Yencho 1994).

Here, we report studies designed to increase our understanding of the genetic and mechanistic basis of CPB resistance derived from *Solanum chacoense* Bitter in tetraploid *S. chacoense* (*chc*) x *S. tuberosum* (*tbr*) hybrids. Previous work has demonstrated that *chc* is highly resistant to CPB (Stürckow and Löw 1961; Sinden *et al.* 1986b; Sinden *et al.* 1991). Resistance in *chc* has been associated with the presence of a rare class of glycoalkaloids, the leptines, which are powerful CPB feeding deterrents (Kuhn and Löw 1961). To date, *chc* is the only species reported to produce leptines (Sinden *et al.* 1991). Leptines I and II are acetylated analogs of α-chaconine and α-solanine, respectively, the commonly occurring glycoalkaloids present in all *tbr* cultivars (Osman *et al.* 1978; Sinden *et al.* 1991). A unique characteristic of the leptines is that, unlike other *Solanum* glycoalkaloids, they are synthesized only in the foliage, not the tubers (Sinden *et al.* 1986a). This is advantageous for a breeding program because glycoalkaloids present in high concentrations in tubers are toxic to humans and livestock, and high tuber glycoalkaloid levels are selected against by potato breeders (Maga 1994).

In the early 1960s, pioneering research into the properties of the leptines was conducted in Europe. Sinden *et al.* (1991) described the early work conducted with this species in Europe and Lawson *et al.* (1993) published a detailed review on the biochemistry and genetics of the steroid alkaloids present in *chc*. Stürckow and Löw (1961) and Kuhn and Löw (1961) were the first to report that leptines derived from *chc* were potent feeding deterrents to CPB. Sinden *et al.* (1986a) screened more than 60 *chc* accessions for the presence of leptines in an attempt to describe the occurrence of leptine production in *chc* and found that only a small number of accessions produce these compounds. Further, relatively few sibs within these accessions synthesized leptines, which led Sinden *et al.* (1986a) to hypothesize that leptine synthesis is coded by a single or a few genes. Recent studies conducted by Ronning *et al.* (1999) in segregating F1, inter F1, and backcross families of *chc* support this hypothesis.

Introgressing the genes for leptine accumulation into a more adapted *tbr* background has been slow. One reason for this is the fact that *chc*, a diploid, does not produce sufficient numbers of unreduced gametes and most 4x/2x, *tbr* x *chc* crosses fail. Kobayashi *et al.* (1994) cultured internode explants of the *chc* clone 8380-1 (2n=2x=24) from PI 458310, which produces high levels of leptines in high proportions (ca. 90%) *in vitro* in a regeneration medium and recovered 4x regenerants that crossed readily with 4x *tbr* (Sanford *et al.* 1997).

In the current study, seven 4x, F2 *S. tuberosum* x *S. chacoense* families derived from these materials were evaluated (1) to study segregation of glycoalkaloids; (2) to obtain estimates of the genetic basis of their inheritance; and (3) to correlate resistance to CPB with foliar glycoalkaloid concentrations.

MATERIALS AND METHODS

Genetic Materials

Seven F2 families of 33 siblings each were developed by LLS at the USDA/ARS Beltsville Agricultural Research Center, Beltsville, MD. The F2 families were derived from selfed F1 clones of interspecific crosses between a synthetic tetraploid *chc* clone (2n=4x=48), derived from the diploid *chc* clone 8380-1 (2n=2x=24), as pollen donor (Sanford *et al.* 1996). The following 4x *tbr* parents were used to generate the F1s: B0175-21 (family 9603); B3692-4 (family 9611); B9335-17 (family 9613); Superior (family 9619); B3692-4 (family 9623); B0180-24 (family 9625); and Atlantic (family 9627).

Decisions for selecting the F1's for selfing were made on the basis of leptine content (high and low leptine content), plant vigor and relative fertility. All of the 4x *tbr* parents were half-sibs from crosses with 4x 8380-1. Seedlings were planted in the greenhouse for tuber production and the tubers were sent to GCY at the North Carolina State University, Vernon James Research and Extension Center (NCSU VJREC), Plymouth, NC.

Laboratory Resistance Assay

Plants—Tubers of each clone were planted in 25-cm-diameter x 19-cm-deep plastic pots filled with Cornell peat-lite soil mix A (Boodley and Sheldrake 1982) and placed in a greenhouse with a 16-hr photoperiod and mean daily temperature of ca. 22.5 C. Natural lighting of the greenhouse was supplemented by metal halide multivapor growth lights. All plants were fertilized weekly with a solution of 100 ppm 20-10-20 liquid fertilizer (Peters Peat-Lite Professional, W. R. Grace & Co., Fogelsville, PA).

No-choice Leaf Disk Bioassay—One or two fully expanded leaves from plants at the pre-bloom to initial flowering stage were excised three to five nodes below the plant terminal. Five 16-mm-diameter leaf disks were cut from the leaflets using a cork borer, avoiding the mid-vein when possible. The total fresh-weight of the 5 disks was then taken and the five leaf disks were placed singly in 55-mm petri dishes containing filter paper moistened with three drops of distilled water from a Pasteur pipette. Seven first instar CPB larvae that had not fed previously on foliage were then placed on the leaf disk, the dish sealed with parafilm and placed in a room maintained at 27 C. The larvae were allowed to feed for 24 hrs, after which they were counted and leaf disk consumption was measured using a digital image analysis system (Color Image Analysis System, CID Inc., Vancouver, WA) incorporating leaf disk areas before and after consumption. Larval mortality was determined from the mean of number of surviving larvae in each of the five petri dishes.

Field Resistance Trial

Plot Layout—The field trial was planted on March 18, 1997, at the NCSU VJREC, Plymouth, NC. The soil type was a Portsmouth fine sandy loam, and fertilization and herbicide applications were in accordance with commercial recommendations. The experimental design used was a randomized complete block with two replications. Each replication consisted of six 61-m rows spaced 0.97 m apart. Experimental plots (family sibs) were randomly assigned within each replication. Each plot consisted of two hills (one tuber per hill) spaced 30.5 cm apart. Plots were spaced 91.4 cm apart. To determine uniformity of CPB feeding damage, one susceptible (cv. Atlantic) plot was randomly assigned to each row, while a single plot of 8380-1 was present in each replication. Both replications were bordered on the east and west by two fallow rows, then a solid four-row planting of the cv. Atlantic, and on north and south they were bordered by 9.1 m of fallow area and a solid planting of cv. Atlantic.

CPB Counts and Defoliation Ratings—A CPB population for the field study was established by collecting adult beetles from a grower's field (Bell Farms, Roper, NC, Washington County) on May 11, 1997. The adults were held, unfed, in plastic containers overnight and released in the experiment on May 12 (55 DAP, pre-bloom stage) at ca. 10 AM. To determine if antixenosis-type resistance was present in the genetic materials, adults were released between the rows every 91 cm at the rate of ca. four beetles per plot (two adults/plant) and allowed to freely orient to the plants for ca. 24 hrs, Counts of the number of adults observed per plot were made the following day from 10 AM to 2 PM.

After the adult counts were completed, a second release at the rate of two beetles per plot was made on May 22 to augment the beetles present in the experiment. On May 23, weekly counts of the number of adult beetles, small larvae (first and second instar larvae), large larvae (third and fourth instar larvae), and egg masses per plot were initiated. During each count, plots were also assessed for defoliation using a pretransformed, 13-point visual defoliation rating scale (Little and Hills 1978). On June 20 only adults were counted, and on June 23 and 27 only defoliation scores were taken.

Glycoalkaloid Quantification

Tissue collection—Three fully expanded leaves from 26 sibs from each family were excised in the greenhouse and brought to the lab. Each was rinsed in distilled water and blotted dry with tissue paper. Leaflets were removed, weighed, packaged in WhirlPak Bags™, and stored at 5 C. for 2 to 4 hrs, then transferred to -80 C. Packages in groups of 10 to 15 were opened and freeze dried for 24 hrs. Packages were resealed and stored at room temperature in plastic bags containing a desiccant.

Quantification—Tissue samples were sent to the USDA/ARS BARC for glycoalkaloid quantification. Glycoalkaloids were extracted from 0.25 g dry weight (DW) of leaves in boiling 100% ethanol containing 5% acetic acid using the methods of Sanford *et al.* (1996). Concentrations of the gly-

coalkaloids of α-solanine, α-chaconine, leptines I and II, and leptinines I and II were quantified as mg/g DW by analytical HPLC using methods described by Sinden *et al.* (1986a) and Sanford *et al.* (1996). The glycoalkaloids α-solanine, α-chaconine (Sigma), and leptines and leptinines from 8380-1 (Sinden *et al.* 1986a) were used as the chromatographic standards for identification and quantification of glycoalkaloids.

Statistical Analyses—Area under the defoliation curve (AUDC) for the CPB field defoliation study was calculated using methods described by Ruppel (1983). Pearson product-moment linear correlation coefficients and GLM ANOVA computations were calculated using the computer software package SuperAnova v1.11 (Abacus Concepts, Berkeley, CA). Frequency histograms and regression analyses were computed using the computer software package DataDesk 4.1 (Data Description, Inc., Ithaca, NY).

RESULTS

Glycoalkaloid Analyses

Table 1 presents the mean, minimum and maximum values observed, and family variance estimates associated with each glycoalkaloid for each of the seven families. To determine total glycoalkaloids (TGA) present in each sample, indi-

TABLE 1.—*Family-wise range, mean and variance estimates of leptine I + II, and solanine + chaconine foliar concentrations and their proportions expressed as % of TGA. Note: each trait is sorted by family mean in ascending order.*

Family	Trait	n[1]	Min	Max	Mean[2]	LSD	Variance
9619	Leptines I & II	26	0.3	5.6	1.9	a	1.9
9627	(mg/g DW)	26	0.3	8.9	3.5	a	5.6
9613		26	0.5	12.1	3.5	a	7.7
9611		26	0.1	12.6	5.7	b	9.7
9625		26	0.0	14.8	6.5	bc	17.2
9603		26	0.8	18.0	7.6	cd	11.1
9623		26	0.0	15.6	8.4	d	22.8
9619	Solanine + Chaconine	26	0.7	24.8	5.3	a	30.5
9627	(mg/g DW)	26	1.8	16.9	6.1	a	15.3
9603		26	1.0	15.3	6.7	ab	14.0
9613		26	1.6	16.5	7.6	ab	17.9
9611		26	2.2	17.3	7.7	ab	14.4
9625		26	1.1	25.7	9.5	b	31.9
9623		26	1.4	52.6	13.0	c	94.1
9619	TGA	26	1.8	27.8	8.5	a	36.8
9627	(mg/g DW)	26	5.3	19.9	10.4	ab	18.9
9613		26	5.9	24.7	13.1	bc	26.2
9603		26	5.0	27.0	15.2	cd	27.7
9611		26	8.3	23.6	15.4	cd	15.4
9625		26	5.3	37.0	17.4	d	55.8
9623		26	3.7	52.6	23.4	e	80.0
9619	% Leptines I & II	26	3.1	56.6	26.0	a	210.6
9613		26	5.4	64.3	27.0	a	277.2
9627		26	1.6	68.0	34.2	ab	289.3
9611		26	0.5	64.6	37.1	b	338.3
9623		26	0.0	65.2	38.3	b	363.6
9625		26	0.0	70.4	38.4	b	330.0
9603		26	14.9	79.9	50.5	c	282.5
9603	% Solanine + Chaconine	26	13.0	70.9	43.0	a	301.9
9611		26	19.4	97.2	49.7	ab	404.3
9623		26	26.2	100.0	52.1	ab	349.7
9625		26	19.7	100.0	53.3	ab	453.1
9627		26	22.0	98.2	56.2	b	431.8
9619		26	13.6	96.4	57.5	b	560.3
9613		26	20.9	89.8	57.5	b	557.5

[1]n= number of genotypes.

[2]Family means within a family with same letter are not different (Fisher's Protected LSD $p<0.05$).

vidual glycoalkaloids were summed. Correlation coefficients (data not shown) of the concentrations of solanine and chaconine, which share the aglycone solanidine, indicated that the concentrations of these compounds were highly correlated (r=0.92). Likewise, foliar leptine I and II levels, which share the aglycone acetyl-solanidine, were also highly correlated (r=0.95). Because of these high correlations, and the fact that solanine and chaconine affected CPB performance similarly as did leptine I and leptine II (see Figure 4 for comparison of leptine I and II), solanine and chaconine, and leptines I and II were combined for all analyses, except those pertaining to the effect of leptines on CPB resistance.

Foliar TGA levels in the F2 families ranged from a low of 1.8 mg/g DW in clone 9616-8 to 52.6 mg/g DW for clone 9623-13. Leptine concentrations ranged from undetectable in clones 9623-13 and 9625-33, to <1.0 mg/g DW for 18 clones spread across all seven families, to a high of 18.0 mg/g DW for clone 9603-9. All of the individuals in this study (182 total) produced solanine and chaconine. However, only two clones, 9619-23 and 9619-30 produced less than 1.0 mg/g DW solanine+chaconine. In contrast, clone 9623-13 had the highest foliar levels of solanine + chaconine at 52.6 mg/g DW.

Approximately 36% of the TGAs present in these families were synthesized as leptines, while 53% were present as either solanine or chaconine, and the remainder were present as leptinines (11%). Aside from clones 9623-13 and 9625-33, which did not produce any detectable levels of leptines, only nine clones out of the 182 studied produced <5% of their TGA as leptines. At the upper end of the distribution, only two clones, 9603-31 and 9603-33, produced >75% of their TGA as leptines. In contrast, percent solanine + chaconine levels ranged from 13% to 100%.

The frequency distributions of the seven families studied are shown in Figure 1. Family 9623 had the highest mean foliar concentrations of TGA, solanine + chaconine, and leptines, while 9616 had the lowest concentrations of these compounds. Compared to the other families, the frequency distribution of 9616 was more highly skewed toward lower levels of glycoalkaloids, while the distribution of 9623 was more evenly distributed. Family 9623, which had the highest mean TGA, solanine + chaconine, and leptines concentrations, had the lowest mean leaf disk feeding and area under the defoliation curve levels (AUDC) (Table 2, Figures 2b and c). In contrast, family 9616, which was ranked as the lowest glycoalkaloid producer, ranked as one of the most susceptible families.

CPB Resistance Screens

Adults—Highly significant differences ($p<0.001$) in adult abundance were observed between the families 24 hrs after the initial release of adults into the plots (Figure 2a). An average of less than one adult per plant settled on the progeny of families 9623 and 9625, compared with greater than two adults per plant settling on families 9603 and 9613. Families 9623 and 9625 had the highest and second highest glycoalkaloid family means, and the highest and third highest leptine means, respectively (Table 1). However, no significant relationships ($p=0.05$) were observed when family means of TGA, solanine + chaconine, or leptine concentration versus adult settling were subjected to regression analysis (data not shown).

No-choice Leaf Disk Consumption and Choice Field Defoliation Feeding Studies—Significant differences in feeding were observed between families for both the free-choice, field defoliation and the no-choice, laboratory feeding studies (Table 2, Figures 2b and c). Generally, there was good agreement between the rankings of the families for each trait. For example, as measured by adult abundance, leaf disk consumption and AUDC, families 9623 and 9625 were ranked amongst the most resistant families, while 9613 ranked the most susceptible.

The family means for leaf disk consumption and AUDC were regressed to determine if the mean level of resistance of a given family observed in the field, as measured by AUDC, could be predicted by leaf disk consumption. The regression model was highly significant ($p=<0.007$) with leaf disk consumption accounting for ca. 76% of the variation in the mean AUDC by family. However, this relationship was not as clear when leaf disk consumption and AUDC were regressed by clone. The regression model was highly significant ($p < 0.0001$), but an $r^2=0.11$ indicated that a given clone's performance in the field as measured by AUDC was poorly predicted by knowledge of leaf disk consumption.

Relation Between Glycoalkaloids and Resistance to CPB—Individual regression analyses of foliar concentrations of TGA, solanine + chaconine, leptine I and II, and leptinine I and II versus AUDC and leaf disk consumption, respectively, revealed that in these families foliar levels of solanine + chaconine had no measurable impact on CPB feeding (Figure 3). In contrast, increased foliar levels of leptines resulted in decreased CPB feeding in both the choice field defoliation studies and no-choice leaf disk consumption. The linear regression model fit to each dataset was highly significant ($p<0.0001$) accounting for 26% and 17% of the variation in

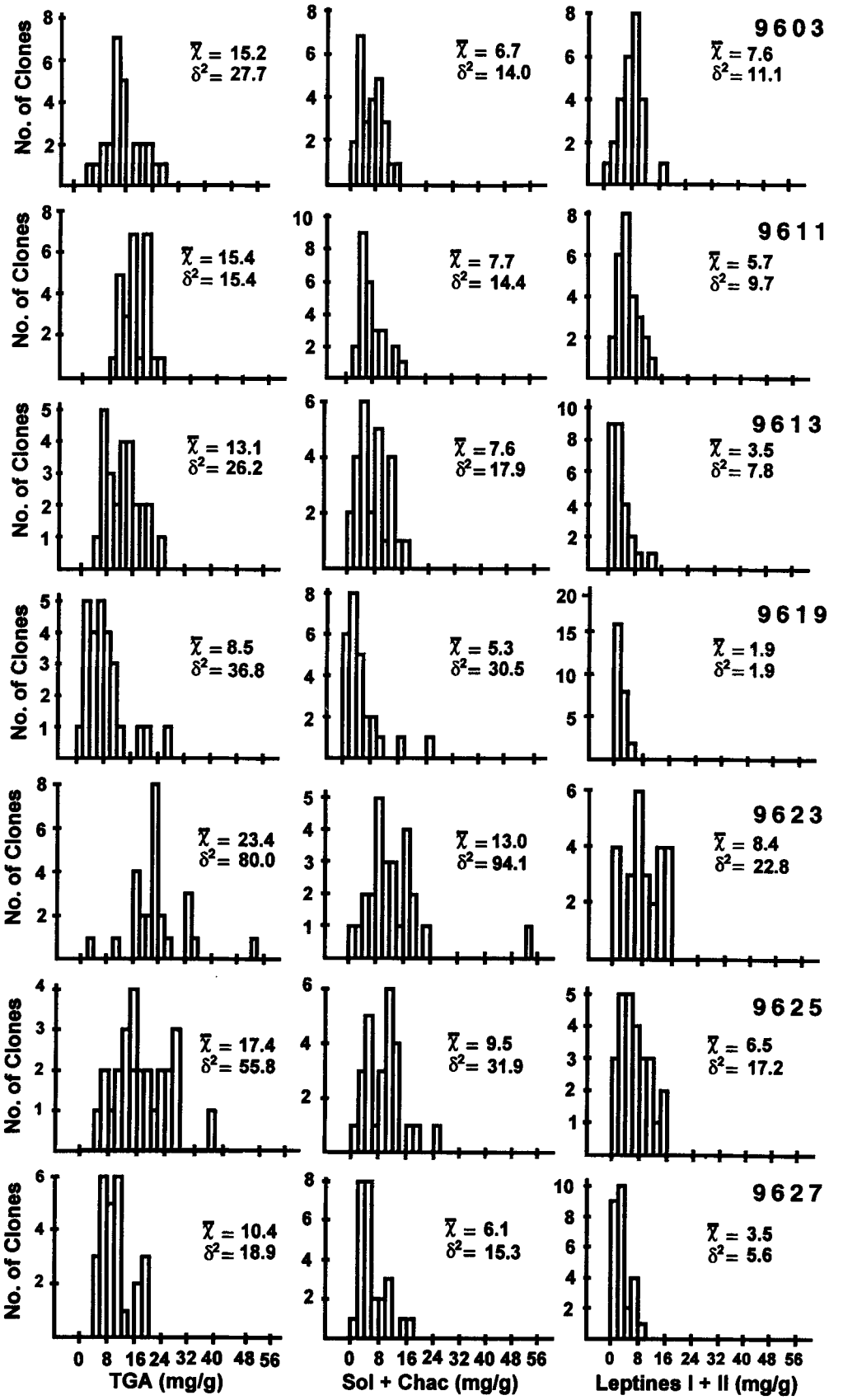

FIGURE 1.
Frequency distributions of total glycoalkaloids (TGA), solanine + chaconine (Sol + Chac.), and leptines I+ II of the seven F2 *Solanum tuberosum* (4x) x *S. chacoense* (4x) potato families.

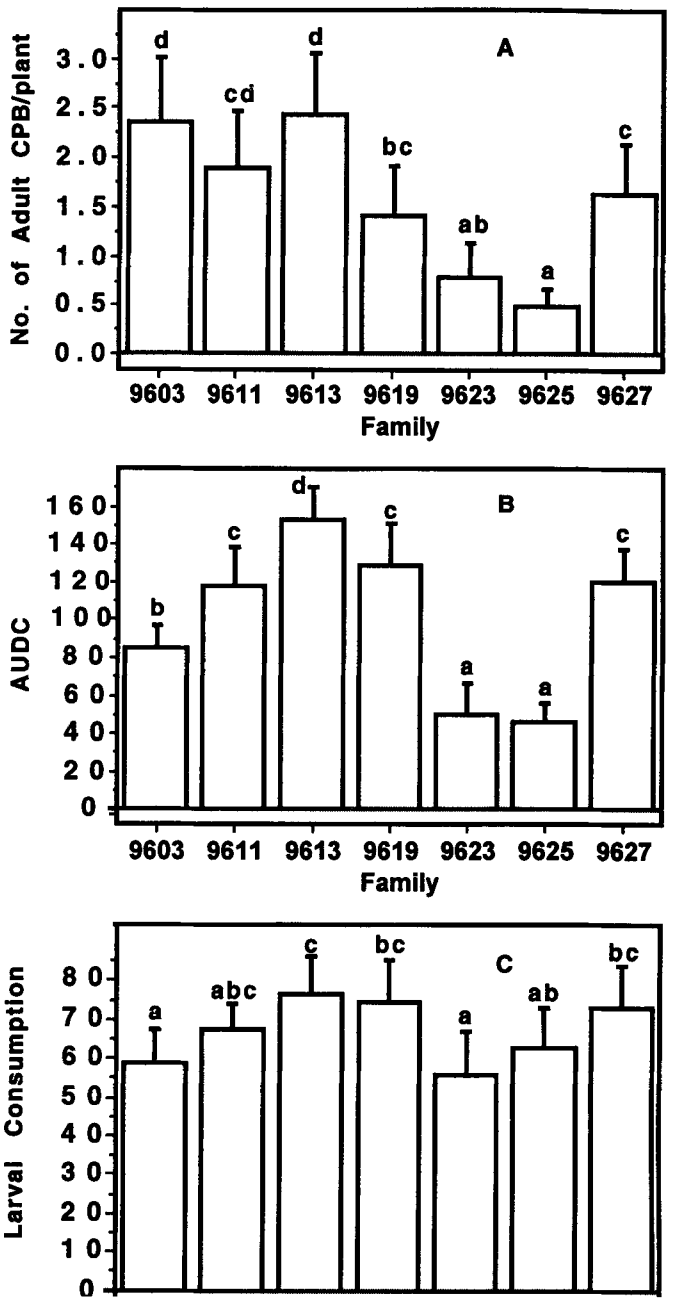

FIGURE 2.
Family means of number of adult CPB/plant (A) and area under the CPB defoliation curve (AUDC) (B) in a field experiment, and larval consumption (mm^2) in a laboratory experiment (C). Differences in means were tested using Fisher's Protected LSD (p=0.05) and 95% confidence interval error bars are provided for each family.

AUDC and leaf disk consumption, respectively (Figure 3). Further dissection of the effects of specific leptine fractions (i.e., leptine I and leptine II) and leptinine I and leptinine II indicated that leptine I and II deterred CPB feeding, while leptinines I and II did not appear to affect CPB feeding (Figure 4).

DISCUSSION

To the best of our knowledge, this is the first report to investigate the effect of leptine and leptinine concentrations on CPB feeding in 4x, *S. tuberosum* x *S. chacoense* potato hybrids. Introgression of leptines into cultivated potato has been difficult (Sanford *et al.* 1996). However, the recent development of a tetraploid *chc* clone by Kobayashi *et al.* (1994) has mitigated these difficulties and successful crosses of these clones with several 4x, *tbr* breeding lines have been made (Sanford *et al.* 1996). The F2 families used in the current studies were derived from these genetic materials (Sanford *et al.* 1996). The F2 progenies in this study are moderately to highly fertile, and they are segregating for a wide variety of traits including leptine levels, resistance to CPB, plant vigor, tuberization, tuber yield, and tuber dormancy.

Studies by McCollum and Sinden (1979), Sinden *et al.* (1986a) and Ronning *et al.* (1998) have demonstrated in diploid progenies that leptines are moderately to highly heritable and that leptine production is probably coded by a few genes having both additive and dominance effects. Sinden *et al.* (1986a) hypothesized that, at least in diploid progenies, recessive genes affect the proportion of leptines in the total pool of glycoalkaloids present in the foliage, and epistatic genes appear to modify the quantity of leptines produced. Ronning *et al.* (1998) characterized F1 *chc* x *chc* progeny as high or low based on percent leptine content and made backcrosses and crosses among F1's using high x high, low x low, high x low and low x high individuals. They found that the low x high, and high x high families produced results consistent with a single recessive gene. In contrast, five of eight high x low and four of six low x low families produced results contrary to a single gene model suggesting the presence of additional genes regulating the inheritance of leptine glycoalkaloids in *chc*.

The inheritance of leptines is undoubtedly more complicated in the tetraploid progenies surveyed in this study. While our work does not permit precise estimations of the number of genes involved in the synthesis of leptines, the frequency distributions observed for leptines I and II were often moderately to highly skewed toward reduced glycoalkaloid production. This supports the hypothesis that their inheritance is probably coded by a few genes, with at least one

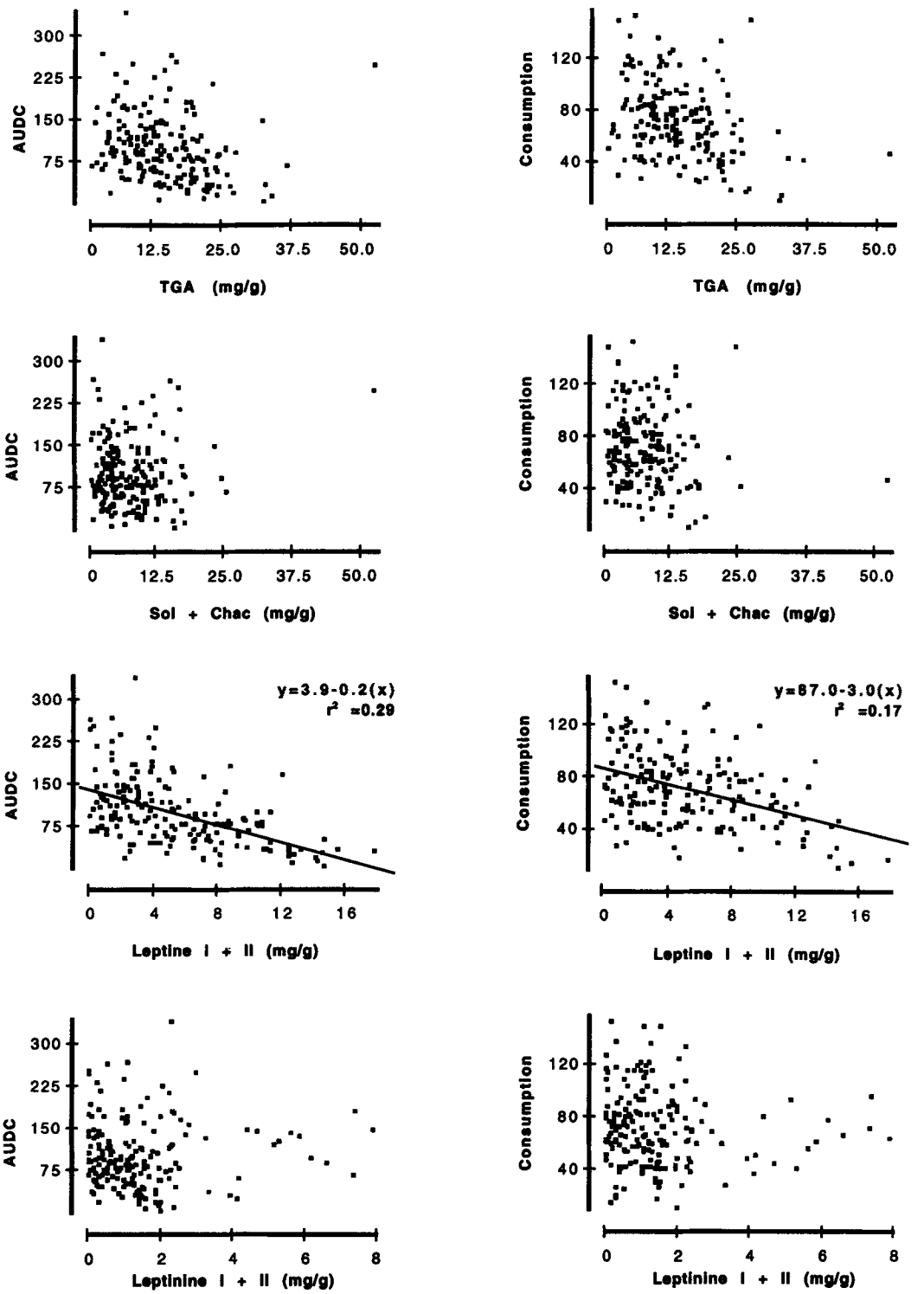

FIGURE 3.
Regression analyses of total glycoalkaloids (TGA), solanine + chaconine (Sol + Chac), and leptines I+ II, and leptinines I + II versus area under the CPB defoliation curve (AUDC) in the field and larval consumption (mm^2) in the laboratory, respectively. The regression models for leptine I + II versus AUDC and consumption were highly significant ($p<0.0001$) and are presented with coefficients of determination (r^2) levels calculated for each linear model.

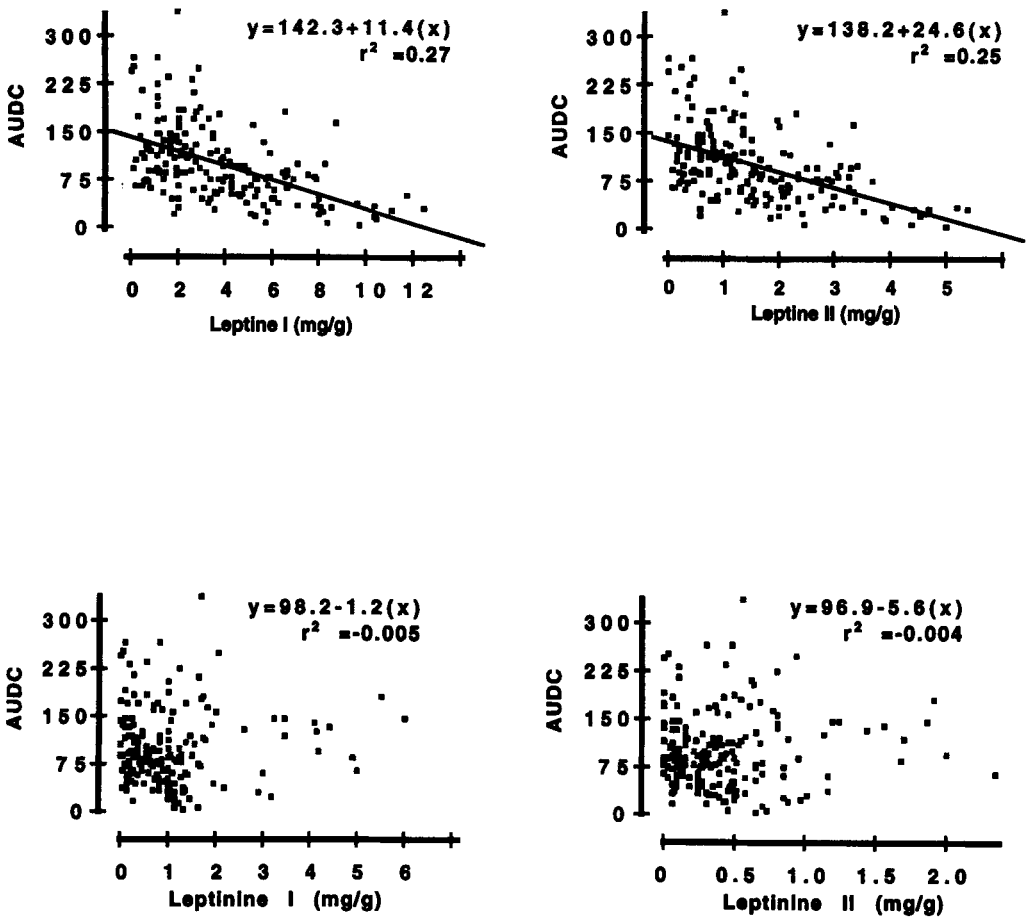

FIGURE 4.
Regression analyses of leptine I and II and leptinine I and II fractions versus area under the CPB defoliation curve (AUDC) in the field. The regression models and coefficient of determination (r^2) levels calculated for each model are presented.

having a relatively strong effect. Parent-offspring regression analyses of the concentration of leptines (mg/g DW) of the F1 clones used to generate the F2 progenies were moderately significant (p=0.06; r^2=0.44; b =0.22) further suggesting that a few genes code for leptine production (data not shown). This relationship is conservative because the selfed F1 clones used to generate the seven F2 families in this study were not grown in the same environmental conditions. More precise heritability estimates for the leptines were not calculated in this study because the F1 parents used to generate the seven F2 families were not available and model assumptions (e.g., random-matings, diploid Mendelian inheritance) were not met (Fehr 1987).

Differences in adult abundance suggest that antixenosis is probably an important component of the resistance to CPB observed in these progenies. Previous *inplanta* work conducted by Stürckow and Löw (1961) and Sinden *et al.* (1986a), and *explanta*, artificial diet work recently completed by Kowalski *et al.* (1999) support this hypothesis. In our study, the mean number of adults observed per plant for clones with foliar leptine levels of less than 1.0 mg/g DW was 2.4 (n=20). In contrast, for clones that had foliar leptine concentrations greater than 12.5 mg/g DW, we observed 0.2 adults/plant (n=13). In the leaf disk consumption assays, we did not observe significant differences in larval mortality between high and low leptine clones (data not presented). In fact, very few larvae died. This might be because the 24-hr feeding period used for this assay was not a sufficient time period in which to observe such effects. However, in the field experiment where CPB experienced chronic exposure to leptines, we did not observe any overt differences (e.g,. dead larvae at the base of a plant) in larval mortality between high and low leptine clones during the course of the study. Similarly, Kowalski *et al.* (1999) using an artificial diet did not

detect any differences in larval mortality using three concentrations of purified leptine I.

None of the clones in this study produced foliar leptine levels equal to that reported for their diploid *chc* 8380-1 progenitor (ca. 19 mg/g DW and 73% of TGA) (Sanford *et al.* 1996). The highest leptine producer observed in this study was clone 9603-9. This clone produced 17.9 mg/g DW leptines (12.5 mg/g DW leptine I and 5.4 mg/g DW leptine II) representing 67% of the TGA pool and had one of the lowest AUDC levels (29.3) observed in the study. This level of leptines present in 9603-9 was close to that observed in 8380-1. Based on our observations, foliar concentrations of greater than 12 mg/g DW leptines appear to provide a significant amount of protection against CPB defoliation (Figure 3). In this study, 14 clones possessed > 12 mg/g DW leptines present in their foliage. Of these 14, five produced greater than 60% of their TGA pool as leptines, while 11 of 14 produced at least half of their TGA as leptines. The ratio of leptine I to leptine II in these clones averaged 2.4 with a low of 1.8 and a high of 3.2. Based on this it would appear that leptine I is preferentially produced compared to leptine II, as is chaconine over solanine.

Regression analyses show a clear relationship between the concentration of leptines present in the foliage, and CPB leaf disk consumption and field defoliation. In contrast, neither leptinine I and II nor solanine and chaconine were implicated in resistance. Further, neither the leptine content as percentage of TGA nor the ratio of leptine I to leptine II appeared to affect CPB feeding. Only increasing foliar concentrations of leptines decreased CPB feeding. It also appears that leptine I and leptine II may not be equally efficacious at deterring CPB feeding. Comparison of slopes of the lines calculated for the leptine I and II versus CPB AUDC regression models (11.4 versus 24.6, respectively) suggests that leptine II may be more active than leptine I. However, this hypothesis needs to be confirmed.

The only difference between leptine I and chaconine, and leptine II and solanine is the acetylation of the C-23 of the steroid aglycone that these compounds share (Sinden *et al.* 1986b). Sinden *et al.* (1986b) speculated that the acetylation of C23 which markedly affected the response of both larvae and adults to the foliage of *chc*, was important for the expression of resistance to CPB. Kowalski *et al.* (in press), using artificial diets clearly demonstrated that, at biologically relevant concentrations, leptine I and chaconine had significantly different effects on CPB. This evidence suggests that the leptines are the most plausible explanation for the resistance observed in these progenies.

Based on our work, however, it appears that a significant amount of variation in the resistance in these progenies remains unexplained. Glycoalkaloid expression is affected greatly by environmental factors such as temperature and light (Maga 1994) and this along with GxE and plant/insect interactions undoubtedly contribute to some of the unaccounted variation observed in resistance. But, it is also possible that other unidentified resistance and/or susceptibility factors may be segregating in these materials. Yencho *et al.* (1996) working with *S. berthaultii*-mediated resistance to CPB uncovered this same phenomenon when they used RFLP markers to identify quantitative trait loci (QTLs) for the glandular trichomes associated with resistance to CPB resistance in diploid *S. tuberosum* x *S. berthaultii* progenies. By comparing QTLs for the trichomes and physiochemical factors associated with CPB resistance to actual phenotypic measure of resistance, they found a strong and consistent QTL that was not associated with any previously known resistance mechanism. The identity of this factor is still unknown.

Continuing advances in our ability to isolate and quantify biologically active plant metabolites and to genetically manipulate plant biosynthetic pathways through the use of DNA-based selection and plant transformation protocols promise to attenuate many of the problems associated with the development of insect-resistant crops, including potato. With respect to the glycoalkaloids, the molecular cloning of solanidine glucosyltransferase (SGT), an enzyme that catalyzes the glycosylation of the aglycone solanidine to form α-chaconine, demonstrates that potato glycoalkaloid metabolism can be manipulated (Moehns *et al.* 1997). It may be possible to down-regulate glycoalkaloid biosynthesis to reduce harmful glycoalkaloids or to up-regulate in a tissue-specific fashion those that impart insect and disease resistance (Tingey 1984; Valkonen *et al.* 1996). The quantification of the leptines present in these progenies and the determination of their effect on CPB feeding, and the recent identification of RAPD and RFLP markers linked to leptine production in diploid *chc* populations (Ronning *et al.* 1999), are valuable prerequisites to the identification of molecular markers associated with leptines, and/or the cloning of genes associated with leptine biosynthesis.

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TABLE 2.—*Family-wise range, mean and variance estimates of CPB leaf disk consumption and area under the defoliation curve (AUDC) field defoliation levels. Note: each trait is sorted by family mean in ascending order.*

Family	Trait	n[1]	Min	Max	Mean[2]	LSD	Variance
9623	Consumption	33	10.1	136.3	55.5	a	1023.3
9603	(mm^2)	33	16.1	119.6	58.9	a	608.9
9625		33	16.9	126.6	62.5	ab	859.3
9611		33	26.1	100.2	67.6	abc	306.9
9627		33	27.7	121.9	73.0	bc	870.0
9619		33	27.1	149.3	74.3	bc	916.6
9613		33	40.0	152.7	76.3	c	775.8
9625	AUDC	33	5.5	101.0	46.3	a	724.5
9623		33	2.0	247.8	49.7	a	2119.3
9603		33	29.3	168.8	84.9	b	983.3
9611		33	35.0	266.0	118.0	c	3296.4
9627		33	37.5	252.8	119.8	c	2328.7
9619		33	41.3	339.3	128.7	c	4040.2
9613		33	55.8	263.5	152.6	d	2526.7

[1]n= number of genotypes.
[2]Family means within a family with same letter are not different (Fisher's Protected LSD $p<0.05$).

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