Effects of Insect Damage on Glycoalkaloid Content in Potatoes (*Solanum tuberosum*)

Jason J. Hlywka,*,† Gerald R. Stephenson,† Mark K. Sears,† and Rickey Y. Yada‡

Departments of Environmental Biology and Food Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Potato plants (*Solanum tuberosum*), cultivar Superior, were subjected to insect damage by Colorado potato beetles (*Leptinotarsa decemlineata*) and potato leafhoppers (*Empoasca fabae*) to assess the influence of pest-related stresses on glycoalkaloid content in tubers. Detection and quantification of the glycoalkaloids, solanine and chaconine, were achieved using a C_{18} reversed phase HPLC computer integrated system equipped with a UV photodiode array detector at 208 nm. Field and growth room studies indicated that the tuber glycoalkaloid concentrations of potatoes subjected to defoliation damage by Colorado potato beetles were consistently greater than those concentrations found in tubers from undamaged plants. Damage to plants by potato leafhoppers did not have any apparent effect on tuber glycoalkaloid content. These results indicate that a food crop not protected from common pests may produce elevated levels of natural toxins, possibly affecting the degree of food safety.

Keywords: *Glycoalkaloids; solanine; chaconine; Colorado potato beetle; pest damage; Solanum tuberosum*

INTRODUCTION

Much concern in the area of food safety is focused on synthetic, otherwise known as man-made, chemicals and their occurrence in or on food commodities. Comparatively less attention is given to the residues of natural toxins or pesticides commonly found in food plants. It has been estimated that greater than 99% of all toxins consumed by an individual are naturally occurring (Ames *et al.*, 1990a). In sheer quantity, this could amount to 1.5 g/day per person, which is approximately 10 000 times the estimated daily intake of synthetic pesticides (Ames *et al.*, 1990a). The existence of naturally produced pesticides within plants is not a new concept but rather falls under the broad theory of coevolution of plants and their phytophagous predators (Ryan and Byrne, 1988). All plants possess a means of defense against pest predators, be it morphological, physiological, chemical, or some combination of the three.

An alternative method of pest control includes the development of pest-resistant varieties through genetic manipulation. The level of resistance conferred to a domesticated food crop is often achieved through crosses with "wild" species (Flanders *et al.*, 1992). These species are often capable of producing a wider array of chemical defense compounds at higher concentrations. The recent introduction of pest-resistant varieties of celery and potato, containing levels of natural toxins detrimental to humans, reiterates the fact that natural does not equate to safe (Ames *et al.*, 1990b). As with synthetic chemicals, efforts must be made to quantify the production of natural toxins and assess the toxicity posed to consumers prior to the release of such pest-resistant varieties.

* Address correspondence to this author at the Department of Food Science and Technology, University of Nebraska, Lincoln, NE 68583-0919 (e-mail jhlywka@unlinfo.unl.edu).

† Department of Environmental Biology.

‡ Department of Food Science.

Chemicals produced by a plant as a defense mechanism are often considered to be secondary or stress metabolites. The toxin content of damaged plant tissue is often more likely to be higher than that in undamaged tissue, having been induced by the pest stress (Young, 1991). For example, root damage to *Brassica* species by insect pests will cause increased concentrations of toxic glucosinolates within the foliage (Birch *et al.*, 1992). Khan and Harborne (1991) observed increased foliar concentrations of alkaloids in *Atropa acuminata* Royle ex Lindl., a Solanaceous plant native to Asia and closely related to *Atropa belladonna* L., following mechanical damage and feeding by insects.

The common cultivated potato, *Solanum tuberosum* L., proves an excellent food crop for studying the occurrence of natural toxins. As a member of the Solanaceae family, potatoes also readily synthesize natural pesticidal toxins known as glycoalkaloids. Glycoalkaloids are nitrogen-containing steroidal glycosides synthesized throughout the plant. Like many secondary metabolites, glycoalkaloids are thought to function in the chemical defense system of the plant, acting as general, nonspecific protectants or repellents against potential pest predators (Roddick, 1979; Osman, 1980). The inhibitory effects of glycoalkaloids on both fungal and insect pests of the potato indicate that their evolutionary significance is most likely to be as natural pesticides (Jadhav *et al.*, 1981). The two most common glycoalkaloids found in potatoes, solanine and chaconine (Figure 1), are fetal toxic in mammals (Keeler *et al.*, 1978; Renwick *et al.*, 1984), teratogenic in chickens (Mun *et al.*, 1974), and teratogenic and embryotoxic to frogs (Friedman *et al.*, 1991). Glycoalkaloids have been described, on a quantitative basis, as the most highly consumed natural toxin in the North American diet (Hall, 1992), because the consumption of potatoes averages almost 70 kg/year per person. Glycoalkaloids are of concern to human health because they inhibit cholinesterase activity (Bushway *et al.*, 1987; Roddick, 1989) and are membrane disruptive (Roddick *et al.*, 1986, 1988). The current maximum residue limit for

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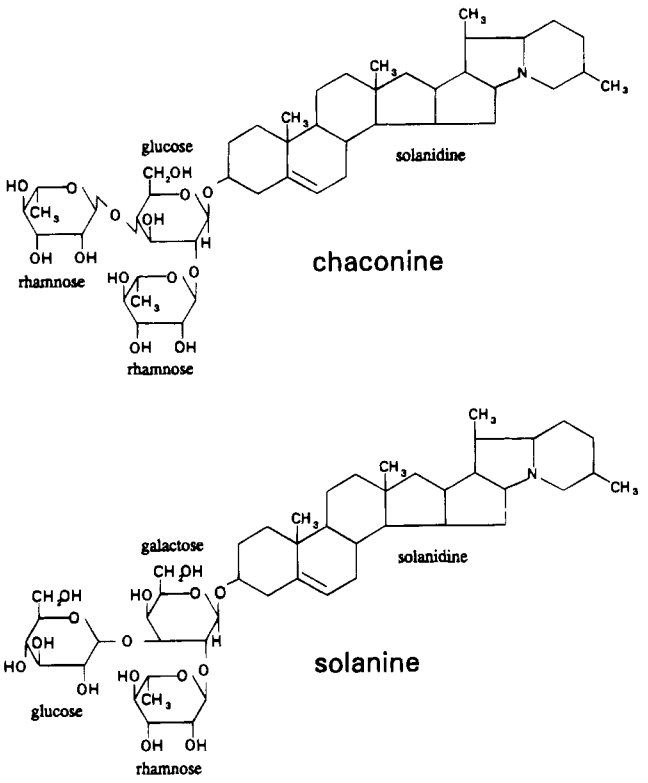

Figure 1. Chemical structures of chaconine and solanine glycoalkaloids.

glycoalkaloids in fresh potatoes intended for human consumption is 20 mg/100 g of fresh weight, or 1000 mg/kg of dry weight, assuming 20% dry matter. The glycoalkaloid content of potato chips has been investigated (Sizer *et al.*, 1980); however, recent concern in the potato chip industry regarding high glycoalkaloid content in certain potato cultivars appears to have prompted an industry-driven selection for cultivars with even lower glycoalkaloid concentrations (Yada, personal communication).

Most research directed at glycoalkaloids has concentrated on the effects of environmental and physical changes incurred during the growth, harvesting, storage, and processing of potatoes [see reviews of Jadhav *et al.* (1981, 1992) and Maga (1981)]. Very few studies have specifically examined the effects of pest-related biological stress on glycoalkaloid residues (Locci and Kuć, 1967; Deahl *et al.*, 1973; Frank *et al.*, 1975; Sanford *et al.*, 1992). The objective of this research was to determine the tuber glycoalkaloid concentrations of a common potato cultivar, Superior, following insect damage by Colorado potato beetles (*Leptinotarsa decemlineata* Say) and potato leafhoppers (*Empoasca fabae* Harris) during growth. Colorado potato beetles and potato leafhoppers are two primary insect pests of potatoes, contributing to serious losses in tuber yields (Tolman *et al.*, 1986; Zehnder and Evanylo, 1989). Colorado potato beetles are considered to be a major pest threat in Ontario and have developed resistance to many synthetic insecticides (OMAF, 1990).

MATERIALS AND METHODS

Field Studies. *Location.* Field studies were conducted from April to August, 1991, and from May to August, 1992, at the Ontario Ministry of Agriculture and Food Horticultural Research Station in Simcoe, ON (42° 52′ N 80° 18′ W).

Design and Treatments. Certified seed tubers of the early-maturing potato cultivar Superior were planted on April 25, 1991, and May 13, 1992. Seed tubers were planted by hand, but plots were mechanically furrowed and hilled. Black plastic mulch was installed, where appropriate, following planting. Field plots were treated with the herbicide metribuzin, prior to plant emergence, on May 8, 1991, and May 26, 1992. The black plastic mulch was removed or slit open, depending on the treatment intended, as the potato plants emerged. The plants emerged on approximately May 16, 1991, and June 5, 1992.

Field plots contained four treatments involving individually caged plants. Nylon mesh cages (Lumite, Atlanta, GA), with approximate dimensions 1 m × 0.5 m × 1 m, were installed on metal frames over plants representing the four treatments on May 9, 1991, and June 5, 1992. Caged treatments were as follows: hand-weeded controls without black plastic mulch (HW), controls with black plastic mulch (BPM), Colorado potato beetles with black plastic mulch (CPB), and potato leafhoppers with black plastic mulch (PLH).

Colorado potato beetles, from a rearing colony at the University of Guelph (M. K. Sears), were introduced to treatment cages as two adult male and female pairs on June 5, 1991. In 1992, Colorado potato beetles were applied to treatment plants as two to three egg masses per plant on June 25. The egg masses were collected from field plants and consisted of approximately 35 eggs per mass. The egg masses were placed at petiole and stem junctions on the plant, such that they were sheltered from wind and rain.

Potato leafhoppers, from a rearing colony at the University of Guelph (M. K. Sears), were introduced to treatment cages as 15 adult leafhoppers per plant on June 5, 1991, and June 30, 1992. Leafhoppers were transported to field plots in disposable plastic Ependorff pipet tips sealed with parafilm and cotton and kept on ice.

Treatments were arranged in a randomized complete block design (Snedecor and Cochran, 1989), with 5 and 10 replications of each treatment in 1991 and 1992, respectively. Field plot dimensions were approximately 12 m × 60 m, such that within a row treatment plants were planted on 2 m centers, separated by at least 6 m containing 10 untreated plants. Rows were separated by a spacing of 1 m. Treatment plants were harvested on August 5, 1991, and August 14, 1992. Analyses of variance were carried out on yield characteristics and glycoalkaloid concentrations of tubers. When necessary, data were transformed via a $\log_e$ transformation to satisfy the assumption of normality required by analysis of variance. Significant ($p \leq 0.05$) treatment effects were determined using pairwise contrasts of means (Snedecor and Cochran, 1989).

Tuber Sampling. Tubers from each treatment plant were harvested by hand and immediately placed into labeled brown paper bags. Tubers were kept at 8 °C and were sampled within 24 h. Tubers from each treatment plant were rinsed under water and divided by size into groups of greater than and less than 5 cm in diameter. A French-fry slicer (Starfrit, Atlantic Promotions Inc., Mississauga, ON) was used to obtain a median longitudinal (apical-basal) strip, approximately 1 cm wide, from each tuber. Tuber tissues exhibiting chlorophyll greening, mechanical damage, or disease were not sampled. The strips from each treatment plant were pooled to create subsamples of the two size groups. The fresh weight of tuber subsamples was recorded. Tuber samples were immediately placed in self-sealing freezer bags and frozen at −24 °C. Samples were lyophilized (Stokes Model 902001-8, The Pennwalt Corp., Philadelphia, PA; Labconco Model 77500, Labconco Corp., Kansas City, MO) for a minimum of 96 h. Dried tuber samples were weighed and machine ground (Wiley cutting mill, mesh size 40, Thomas Scientific, Swedesboro, NJ). Ground tissue was mixed well and stored in glass vials at −24 °C until extraction and analysis.

Growth Room Studies. *Plant Material and Growth Room Conditions.* Certified seed tubers of the cultivar Superior were cut into pieces containing no less than three sprout eyes. Seed pieces were planted in a peat-based growing medium (Pro-Mix, Premier Brands Inc., Stamford, CT) in 9 L, 25 cm diameter plastic pots (Nursery Supplies Ltd., Fairless Hills, PA). Plants were fertilized biweekly with 20N/20K/20P fertilizer (Plant Products, Bramalea, ON) at a rate of 3 g/L. Growth

room conditions were as follows: day and night temperatures of 25 and 15 °C, respectively, 16 h of daylight, 58% relative humidity, and a photosynthetic photon flux density of 463 μmol^{-1} m^{-2}.

Colorado Potato Beetle Defoliation Experiment. Fifteen potato plants were enclosed in individual nylon mesh cages (Lumite) approximately 2 weeks following plant emergence. At this time, Colorado potato beetles were collected from a field plot, and five beetles were placed in each treatment cage. There were five treatments examined (0, 25, 50, 75, and 100%), all relating to the percent defoliation of the plant by Colorado potato beetles. Treatment cages with 0% defoliation were controls and did not contain beetles. Beetles were removed from treatment cages once the desired percent defoliation was achieved. A defoliation level of 25% took approximately 23 days, whereas plants at 100% defoliation were exposed to beetles for the 7 week duration of the experiment. Potato plants were arranged in a randomized complete block design on the growth room bench. The five treatments were replicated three times. Tubers were harvested 10 weeks postplanting and 7 weeks post-treatment.

Manual Defoliation Experiment. Potato plants at the four to five leaf stage were mechanically defoliated using a hand hole-puncher. A total of 10 leaves per plant were clipped, approximately 10 holes per leaf, to simulate Colorado potato beetle damage. Manual clipping of the leaves was carried out twice weekly for a period of 5 weeks, achieving approximately 70% defoliation of the plant. Potato plants not mechanically damaged served as control treatments. Potato plants were arranged in a completely randomized design on the growth room bench with each treatment replicated four times. Plants were harvested 10 weeks post-treatment, 12 weeks postplanting.

Tuber Sampling. Tubers from growth room experiments were rinsed under water and enumerated, and the total fresh weight was recorded. Tubers less than 2 cm in diameter were discarded. A single tuber was selected from each plant within an experiment for glycoalkaloid analysis. Tubers were selected for similar shape, size, and weight whenever possible. Tubers exhibiting chlorophyll greening were not sampled. Sample tubers were sliced to facilitate freeze-drying and were immediately placed in a −24 °C freezer. Sample tubers were lyophilized (Labconco Model 77500) for a minimum of 96 h. Dried tuber samples were ground and stored as previously described.

Extraction of Solanine and Chaconine. The extraction of solanine and chaconine glycoalkaloids followed a modified procedure of Carmen *et al.* (1986). Five grams of lyophilized tuber tissue were homogenized in a Waring blender (Fisher Scientific, Toronto, ON) for 5 min at high speed with 80 mL of extracting solution [2% (v/v) acetic acid in methanol] and 20 mL of water. The resultant homogenate was filtered (Whatman Paper No. 1 5.5 cm, Whatman International Ltd., Maidstone, England) under vacuum. The blender container and cover were rinsed with extracting solution and passed through the filter. The filtrate volume was brought to 150 mL with extracting solution. A 60 mL aliquot of filtrate was transferred to a 250 mL round-bottom flask and the methanol removed on a rotary evaporator at 50 °C (Büchi Rotavapor, Brinkmann Instruments, Rexdale, ON). Five milliliters of ion-pairing reagent [20 mM 1-heptanesulfonic acid, 1% acetic acid (v/v) in water] was added to the flask and the flask agitated vigorously on a mechanical mixer for 30 s. The contents of the flask were transferred to a 25 mL graduated cylinder. Agitation with ion-pairing reagent was repeated twice more and the volume brought to 25 mL. The sample was transferred to a polycarbonate centrifuge tube and centrifuged for 5 min at 7500*g* (Sorvall Model RC-5B, DuPont, Wilmington, DE). A single-use C_{18} solid phase extraction cartridge column (Waters Sep-Pak, Millipore Corp., Westboro, MA) was attached to a 10 mL plastic syringe and conditioned with approximately 5 mL of methanol followed by 5 mL of ion-pairing reagent. A 10 mL aliquot of the sample supernatant was loaded into the syringe and passed through the column at a rate of 1−2 drops/s. This was followed by 5 mL of an acetonitrile/water 20:80 (v/v) wash solution, again at a rate of 1−2 drops/s. Any remaining solvent in the cartridge column was removed by empty syringe plunges of forced air. All eluate to this point was discarded as waste. Solanine and chaconine glycoalkaloids were eluted from the column with 2 mL of an acetonitrile/water 50:50 (v/v) solution and collected in a small (12 × 75 mm) test tube. The sample was mechanically mixed and filtered through a 0.22 μm nylon filter (Micron Separations Inc., Westboro, MA) prior to injection into a high-performance liquid chromatograph. All solvents and reagents used were of Optima or HPLC grade quality (Fisher Scientific, Fair Lawn, NJ).

High-Performance Liquid Chromatography. Solanine and chaconine glycoalkaloids were separated and quantified using a Shimadzu Model LC-6A high-performance liquid chromatograph (HPLC). A 100 μL aliquot of sample was separated on a 250 × 4.6 mm Ultracarb 5 μm ODS(30) reversed-phase C_{18} column (Phenomenex, Torrence, CA). The two glycoalkaloids were detected by a Shimadzu SPD-M6A photodiode array UV−vis detector at 208 nm and quantified on a Shimadzu SPD-M6A computer program (version 2.12) integrated to the detector. The HPLC conditions used to separate solanine and chaconine were as follows: an isocratic mobile phase of acetonitrile/ammonium phosphate-buffered water 50:50 (v/v) at a flow rate of 0.7 mL/min and a temperature of 22 °C.

RESULTS AND DISCUSSION

Potato plants were visibly stressed by foliar damage from both insect treatments. Colorado potato beetles caused extensive defoliation, while potato leafhoppers caused the characteristic symptoms of "hopperburn", with numerous curled leaves with brown tips. Cages proved very successful at protecting treatment plants from indigenous field pest populations. It was also observed that the growth of "control" plants was extremely vigorous within cages. The use of black plastic mulch as a weed deterrent did not have a significant effect on tuber glycoalkaloid content compared with hand-weeded treatments.

The effect of Colorado potato beetles on the productivity of Superior potato plants was severe. In 1991, some plants did not produce any tubers >5 cm in diameter. Colorado potato beetles caused significant ($p \leq 0.05$) decreases in the number of tubers per plant, the total tuber dry weight, and the average dry weight of each tuber. These results agree with the observations of Zehnder and Evanylo (1989). In addition to effects on foliage and yield of tubers, there was a significantly ($p \leq 0.05$) higher mean solanine concentration observed in >5 cm diameter tubers from CPB-treated plants harvested in 1991 compared with tubers from BPM plants (Figure 2). This difference reflected an approximate 30% increase in solanine concentration of tubers from CPB-damaged plants over that observed in tubers of undamaged plants.

Within the growth room, percent defoliation by CPB did not have a significant effect on the number of tubers produced per plant; however, 50, 75, and 100% defoliation resulted in a significant ($p \leq 0.05$) reduction in the total tuber dry weight per plant. Severe defoliation also significantly ($p \leq 0.05$) reduced average tuber dry weight. Defoliation damage of plants by CPB resulted in the production of tubers with significantly higher glycoalkaloid concentrations (Figure 3). The total glycoalkaloid content of tubers from plants exposed to beetles for the duration of the experiment was more than 50% higher than the mean concentration in tubers from undamaged plants.

Unlike the results of defoliation by Colorado potato beetles, tuber characteristics from plants manually defoliated were not significantly different from those of

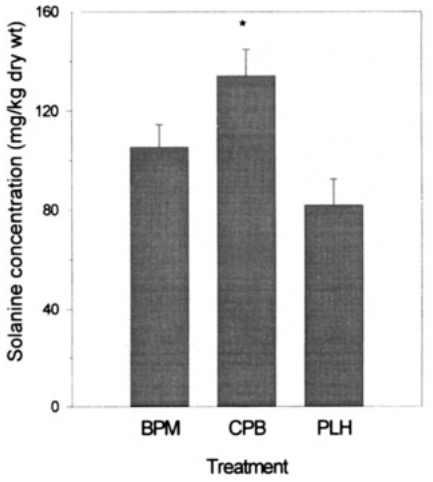

Figure 2. Solanine concentration of large Superior tubers (>5 cm in diameter) harvested at Simcoe, ON, 1991, following exposure to insect pests: BPM, black plastic mulch; CPB, Colorado potato beetle + BPM; PLH, potato leafhopper + BPM; *, significantly different from black plastic mulch (BPM) at $p \leq 0.05$. Data are the mean of five replicates. (±) standard errors are indicated by capped vertical bars when larger than symbols at each point.

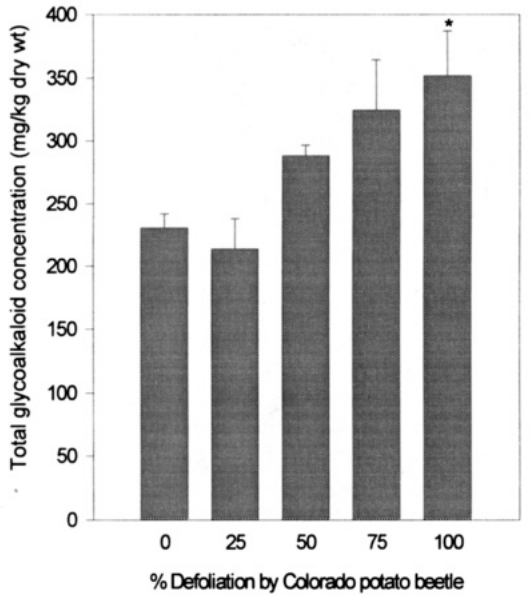

Figure 3. Total glycoalkaloid concentration of growth room grown Superior potato tubers following 0, 25, 50, 75, and 100% defoliation by Colorado potato beetles (CPB): *, significantly different from 0% defoliation at $p \leq 0.05$. Data are the mean of three replicates. (±) standard errors are indicated by capped vertical bars when larger than symbols at each point.

undamaged plants. Manual defoliation with hand clippers mimicked the aggressive chewing damage by beetles. The extent of defoliation sustained by clipped plants was approximately 70%, and the mean glycoalkaloid content of 330 mg/kg of dry weight was similar to the concentration of 324 mg/kg of dry weight (dw) found in plants 75% defoliated by CPB. However, the glycoalkaloid concentration of tubers from undamaged plants was higher in the manual defoliation study (286 mg/kg of dw) compared with the CPB defoliation study (230 mg/kg of dw). These results suggest that the interaction of Colorado potato beetles and host potato plant results in a higher tuber glycoalkaloid concentration than would be implied by just the percent defoliation or, more likely, that the manual defoliation technique employed was not totally representative of feeding

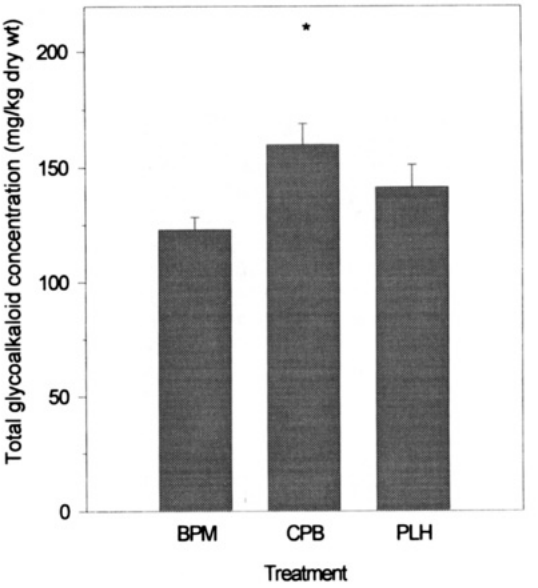

Figure 4. Total glycoalkaloid concentration of large Superior tubers (>5 cm in diameter) harvested at Simcoe, ON, 1992, following exposure to insect pests: BPM, black plastic mulch; CPB, Colorado potato beetle + BPM; PLH, potato leafhopper + BPM; *, significantly different from BPM at $p \leq 0.05$. Data are the mean of 10 replicates. (±) standard errors are indicated by capped vertical bars when larger than symbols at each point.

behavior by beetles. Although beetles will defoliate an entire plant and even attack the stem, they exhibit a preference for the tender young tissue, which ironically contains higher concentrations of glycoalkaloids (Kozukue *et al.*, 1987; Friedman and Dao, 1992).

In the 1992 field study, yield characteristics of harvested Superior tubers were different from those observed in 1991 or in the growth room. In 1992, no treatment was observed to affect the number of tubers per plant, the average tuber dry weight, or the percent dry matter of tubers. Despite the apparent absence of treatment effects on yield characteristics, there were significant differences in glycoalkaloid concentration observed between treatments (Figure 4). Once again, plants exposed to insect defoliation via CPB produced tubers with significantly higher ($p \leq 0.05$) total glycoalkaloid concentrations than plants not exposed to beetles. The significant difference between treatments observed in total glycoalkaloid concentration was reflected in the individual solanine and chaconine concentrations of tubers, which were approximately 30% higher than the concentrations of tubers from undamaged BPM plants. In a concurrent 2 year field study with the late-maturing cultivar Kennebec, increased tuber glycoalkaloids were not observed with either of the insect treatments (Hlywka, 1993).

Within all experiments, there existed an apparent inverse relationship between tuber size and glycoalkaloid content. In fact, the glycoalkaloid concentration of smaller diameter tubers was consistently greater than concentrations found in larger tubers and, in some cases, exceeded the maximum residue limit. The greater concentration of glycoalkaloids in the dermal, surface tissue of tubers (Maga, 1980; Bushway *et al.*, 1983; Kozukue *et al.*, 1987) and the greater surface area to volume ratio of smaller tubers are likely explanations for this observation.

In addition to the effects of pest-related stresses on glycoalkaloids, it was also observed that year to year differences in climate also had an effect on the basal

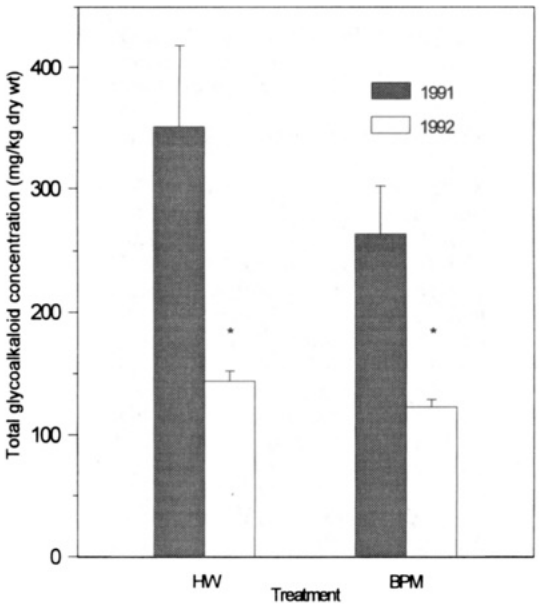

Figure 5. Comparison of 1991 and 1992 total glycoalkaloid concentrations of large, HW (hand weeded) and BPM (black plastic mulch) Superior tubers (>5 cm in diameter) harvested at Simcoe, ON: *, significantly different between years at $p \leq 0.05$. Data are the mean of 5 and 10 replicates in 1991 and 1992, respectively. (±) standard errors are indicated by capped vertical bars when larger than symbols at each point.

level of glycoalkaloids found in potato tubers. Higher glycoalkaloids were observed during the summer of 1991, which was warm and sunny, than were observed in 1992, which was quite cloudy, wet, and cool (Figure 5). Ponnampalam and Mondy (1986) also observed seasonal variation in tuber glycoalkaloid concentrations but did not elucidate the actual cause. Sinden and Webb (1972) reported that potatoes grown in geographic locations characterized by a short, cool growing season produced tubers with higher glycoalkaloid concentrations.

Glycoalkaloids are not translocated within plants (Roddick, 1982); thus, any increased concentrations observed in the tubers would result from synthesis within the tubers. Furthermore, if the increased synthesis of glycoalkaloids in the tubers is resultant from direct pest interaction with the foliage, there must exist some signal–response mechanism. The mechanism for induction of glycoalkaloid synthesis has yet to be determined (Bergenstråhle *et al.*, 1992a); however, the response is assumed to be multigenic (Sinden *et al.*, 1984). The application of various phytohormones to plant foliage and directly on tubers may affect glycoalkaloid concentrations of tubers (Jadhav *et al.*, 1973; Mondy *et al.*, 1977; Ponnampalam and Mondy, 1986; Bergenstråhle *et al.*, 1992b); however, no one specific plant chemical has been implicated in glycoalkaloid synthesis. The occurrence of action potentials within plant tissues upon wounding and other stresses (Davies, 1987a,b; Wayne, 1993) also merits consideration in the increased synthesis of glycoalkaloids, especially in the case of long-distance signal transduction from foliage to tubers. Higher glycoalkaloid concentrations were consistently observed in the tubers of CPB-damaged Superior potato plants, whereas no change in glycoalkaloid concentration was observed in tubers of plants exposed to the stress of PLH. This could possibly be related to the type of stress imparted upon feeding on the plant by each of these insects. Colorado potato beetles are very destructive and aggressive chewing insect pests, whereas potato leafhoppers are phloem feeders. Foliar wounding of potato plants, similar to CPB damage, can result in the systemic expression of a proteinase inhibitor gene via a phytohormone signal involving abscisic acid (Peña-Cortés *et al.*, 1989). Bergenstråhle *et al.* (1992a) found the addition of abscisic acid to tuber disks to be ineffective at altering glycoalkaloid levels, although, as the authors observed, abscisic acid may be released from endogenous pools and become involved in the wound accumulation response.

Glycoalkaloids are natural pesticidal toxins found in all plant parts, including tubers, of the common cultivated potato, *S. tuberosum*, as well as in other plant species in the Solanaceae family. The glycoalkaloids are constitutively expressed compounds synthesized throughout the life cycle of the plant. However, synthesis may be induced or suppressed under certain conditions. The biosynthetic pathway of glycoalkaloid synthesis has yet to be elucidated, as are the mechanisms of induction. The maximum residue limit for glycoalkaloids in tubers approved for human consumption is 20 mg/100 g of fresh weight, or 1000 mg/kg of dry weight, assuming 20% dry matter. In this study, the exposure of potato plants, cultivar Superior, to severe defoliation by Colorado potato beetles resulted in significantly higher glycoalkaloid concentrations within tubers. The significant increase in total glycoalkaloid concentration was reflected by increases in both glycoalkaloids measured, solanine and chaconine. In the 1991 field study and growth room experiments, increases in glycoalkaloid content were accompanied by decreases in the dry weight of tuber yields. It is apparent from the 1992 field study, however, that significant differences in glycoalkaloid content can occur in tubers from pest-stressed plants without an associated effect on tuber yield or size characteristics. Although both Colorado potato beetles and potato leafhoppers caused visible plant stress, only Colorado potato beetle damage resulted in tubers with higher glycoalkaloid concentrations. This could be based upon the dissimilar feeding behaviors of these two insects, which produce different wound responses in the host plant. Significantly higher tuber glycoalkaloid concentrations were not observed in growth room plants that were mechanically defoliated to simulate defoliation by Colorado potato beetles.

Ames *et al.* (1990a) postulated that plants which are stressed or damaged, as through attacks by or in competition with pest organisms, may produce higher levels of natural pesticides than unstressed plants. The judicious use of synthetic pesticides to alleviate a pest stress, for which there have been no documented cases of harm (Hall, 1992), may in fact result in a higher degree of food safety, as the production of natural toxins to combat the pest stress may be reduced. Our results indicate that potato plants stressed by Colorado potato beetle damage have the potential to produce tubers with higher glycoalkaloid concentrations than unstressed plants. Thus, plant produce damaged by pests or from plants stressed by pests may not be as safe to consume as produce from plants protected by synthetic pesticides, even though there may not be any visual signs of pest damage. Avoiding the use of synthetic pesticides is an option that some people may prefer, but pest-related stresses must be prevented by alternative methods if the safest possible produce is the goal.

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