Nutritional Composition of Potato Foliage

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The foliage of the potato *Solanum tuberosum* (L.)was analyzed to gain understanding of the within-plant distribution of the Colorado potato beetle, *Leptinotarsa decemlineata* Say, and to develop a synthetic diet useful for rearing of this insect for experimental purposes. Foliage from three age classes was analyzed for protein, amino acid composition of protein, free amino acids, sugar, starch, nonstarch polysaccharides, dietary fiber, and trace elements. Concentrations of the amino acids glutamine, serine, asparagine, glutamic acid, proline, histidine, and arginine were higher in the youngest foliage than in the two older categories. Protein content was highest in the youngest foliage. With the exceptions of copper and phosphorus, the oldest foliage had the highest mineral levels. Concentrations of sugars were highest in the oldest foliage. The concentrations of nonstarch polysaccharides tended to be lowest in the youngest foliage.

Keywords: *Solanum tuberosum; Colorado potato beetle; protein; amino acids; carbohydrates; minerals; Leptinotarsa decemlineata*

INTRODUCTION

Foliage of the cultivated potato, *Solanum tuberosum* (L.), is a major food crop source for the Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say), an important pest in most temperate zone areas of the world where the cultivated potato is grown. Research into diverse biorational methods of CPB control, such as the use of predators and parasitoids, microbials, glycoalkaloids and protease inhibitors, and others, and basic research in insect nutrition could progress without interruption if all life stages of the CPB were available continuously. Currently, most year-round CPB cultures are maintained on greenhouse-grown potato plant foliage, an expensive and labor-intensive effort. The use of foliage in rearing CPB has other disadvantages also. The levels of foliar nutrients and feeding stimulants have been shown to vary seasonally in field-grown plants (Hare, 1983). Greenhouse conditions may compensate somewhat for the flux in natural conditions in producing plants of uniform nutrient content. However, nutrients and feeding stimulants of greenhouse-grown plants may also vary significantly between plants and by leaf age within plants. The use of host plant foliage may thus be an uncontrolled source of experimental error that could confound the effects of test substances coated on leaf tissue and fed to CPBs. Characterizing and quantifying the nutritional content of potato foliage were done as a starting point in the development of a synthetic diet suitable for year-round rearing of the CPB and to determine if our analyses uncovered any nutrition-related reasons for the observed feeding preference CPB appears to have for feeding on foliage located on the middle and upper portions of the potato plant.

MATERIALS AND METHODS

Plants. Potato *S. tuberosum* cv. Kennebec (commonly grown in the eastern United States) plants were grown in a greenhouse from 6 month old tubers (dormant for 4 months) planted in 15 cm diameter pots filled with a soiless potting mix (peat moss and vermiculite, Jiffy-Mix, Jiffy Products, Batavia, IL). Plants were watered daily and fertilized (1:100 dilution) weekly (N–P–K of 20–20–20, Peters general purpose, Grace-Sierra, Milpitas, CA). Supplemental light (500 ft.-candles at 1 m) was provided by sodium vapor lamps to maintain a 16:8 h daily light/dark regime. Eighteen plants were grown at approximately 23 °C and were 35–45 cm tall after 3 weeks. The foliage was separated into three age classes of ≤3, 5–7, and 15–21 days old. A small number of the oldest, senescent leaves present, yellow in color and wilted, were not sampled with the 21 day age class. Foliage was cut, placed in plastic bags, and frozen at −20 °C. After stems were cut from frozen leaves, two 100 g samples were weighed, freeze-dried, and reweighed to estimate water content. All remaining foliage (350–400 g of fresh weight per age class) was lyophilized, ground in a blender to a fine powder, sieved through a no. 50 (300 μm) standard testing sieve, and stored in plastic bags at −20 °C until further use.

Chemical Analyses. Lithium 2.75 eluent (1.2 mL) (Pickering Labs) was added to a 70 mg sample of potato leaf powder. Amino acids were extracted by picking up and dispensing the mixture for 3 min with a pipet. The mixture was then centrifuged for 3 min at 5000*g* with a Beckman microfuge to remove particulates. The supernatant (600 μL) was mixed with 600 μL of lithium diluent (lower ionic concentration than lithium eluent) and centrifuged for 3 min. Supernatant (20 μL) was injected by autosampler (0.583 mg of leaf powder/20 μL injection). High-performance liquid chromatography (HPLC) was performed on a Dionex BioLC system equipped with a Dionex AI-450 automation controller. The amino acid separations were carried out on a Dionex Li-cation exchange column, 4.6 × 150 mm, custom packed with a 5 μm 10% cross-linked soft gel. The gradient flow rate was 0.3 mL/min with ternary elution. The three eluents were Lithium eluents 2.75, 7.5, and regenerant (to wash salts off column) (Pickering Labs). The sample was derivatized by postcolumn reaction with ninhydrin (Trione, Pickering Labs), and absorbance was monitored at 520 nm. Standards consisted of acidic, basic, and neutral amino acids (Sigma, A-9906). L-Asparagine and L-glutamine standards were prepared separately. The mean and standard deviation were calculated in nanomoles per milligram for each amino acid detected, on the basis of triplicate injections of one extraction from a composite sample in each age class.

Protein was extracted from 10 replicated samples of a lyophilized powder composite from each age class with 0.1 N NaOH following the procedures of Jones et al. (1989) to estimate the protein content of potato foliage. The Bradford Coomassie dye reagent (Bradford, 1976) obtained from Pierce

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Laboratories, Rockford, IL (Pierce Coomassie plus reagent), was used to assay for extracted protein. The absorbances of a set of bovine serum albumin standards at 595 nm were used to construct a regression line, and the protein concentrations of the samples were estimated from the equation for the fitted line.

To extract protein for amino acid analysis, 15 g of leaf powder from ≤3 day old foliage was homogenized on ice in 175 mL of ice-cold buffer, with an IKA Ultra-Turrax (IKA, Cincinnati, OH) for five cycles of 30 s mix and 2 min cool. The buffer consisted of 50 mM NaCl, 50 mM Tris (pH 7.4), 20 mM $MgCl_2$, 0.1 mM EDTA, 10% glycerol, 2 mM PMSF, 2 μg/mL leupeptin, and 5 mM β-mercaptoethanol (Mehta et al., 1992). The homogenate was filtered through two layers of cheesecloth, and the filtrate was spun at 5000*g* for 30 min at 2 °C. The supernatant was spun at 100000*g* for 1 h at 2 °C. The supernatant was applied to a 4.5 × 60 cm low-pressure glass column containing Sephadex G-25 gel filtration media. A Tris–EDTA–NaCl–mercaptoethanol buffer was used to equilibrate the column and to separate the sample into a 320 mL protein band. The protein-containing solution was precipitated with $(NH_4)_2SO_4$, 70% saturation, and spun at 5000*g* for 30 min at 2 °C. The precipitate was placed in dialysis tubing and dialyzed against buffer for 30 h. The protein redissolved and was applied to a column packed with Sephadex G-25 equilibrated with 0.050 M $(NH_4)_2SO_4$. The protein band was frozen and lyophilized.

Amino acid content analysis of protein extracted from ≤3 day old foliage was performed by vapor phase hydrolysis of duplicate samples for 1.5 h at 155 °C with 100 μL of 6 N HCl–phenol (100:1 v/v) (Wagner et al., 1993). After hydrolysis, the samples were dried and diluted with borate buffer prior to analysis using the aminoQuant OPA-FMOC system (Hewlett-Packard, Palo Alto, CA). The proteins casein and egg albumin were subjected to the same analysis, since these are two commonly available, inexpensive substitutes for foliar protein in insect diets.

The trace metal composition of potato leaves was analyzed using a wet ash (HNO_3/H_2O_2) digestion of samples (Miller-Ihli, 1988). After digestion, deionized distilled water was added and heated to dissolve the sample. Samples were cooled, diluted to volume, and filtered. Three subsamples were prepared from each age-class sample, and each digest was analyzed in triplicate by inductively coupled plasma atomic emission spectrometry (ICP-PS3000, Leeman Laboratories, Lowell, MA) to obtain data for Ca, Co, Cu, Cr, Fe, Mg, Mn, Ni, P, V, and Zn. The sodium and potassium contents of similarly treated samples were determined by flame atomic emission spectroscopy (Model PS3000, Perkin-Elmer Corp., Norwalk, CT). NIST standard reference materials were run as controls to verify analytical accuracy.

Leaf sugar and starch contents were analyzed by extracting duplicate 500 mg samples in Teflon tubes with 10 mL of hexane, followed by 12 mL of 80% methanol (Li et al., 1988). Aliquots (0.5 mL) of the methanol extract were dried and the trimethylsilylated oxime derivatives were prepared as follows: Pyridine reagent containing hydroxylamine hydrochloride and β-phenyl D-glucopyranoside (internal standard) was added to the dried extract. After heating for 30 min at 75 °C and cooling, 0.5 mL of hexamethyldisilazane and 4 drops of trifluoroacetic acid were added. After centrifugation, the supernatant was analyzed on a Hewlett-Packard 5840A gas–liquid chromatograph (GLC) equipped with a flame ionization detector. The column was a HP-1 (Hewlett-Packard, Rockville, MD) 10 m wide-bore fused silica cross-linked methyl silicone capillary column with 0.53 mm i.d. and 2.6 μm film thickness. Sugars were identified by comparison with retention times of standards and quantitated by the equation

$$\text{sugars (as \% of dry matter)} = A_s R_s W_{is} / A_{is} R_{is} W_s \times 100$$

where A_s and A_{is} are peak areas, W_s and W_{is} are dry weights of sample and internal standard, and R_s and R_{is} are amount/area for sugars and internal standards, respectively.

Table 1. Free Amino Acid Content of Lyophilized Potato Foliage Sampled from Three Leaf-Age Classes, Expressed in Nanomoles per Milligram of Dry Foliage[a]

amino acid	≤3 days	5–7 days	15–21 days
aspartic	7.32	6.99	6.59
serine	13.27	7.83	8.23
asparagine	25.36	2.24	*b*
glutamic	20.94	13.63	13.82
glutamine	18.93	4.79	3.6
proline	2.16	1.22	0.7
glycine	1.61	2.05	2.15
alanine	11.46	12.46	7.08
citrulline	0.14	0.22	0.21
valine	0.91	0.48	0.75
methionine	0.15	0.08	0.12
isoleucine	0.57	0.36	0.57
leucine	0.83	0.35	0.91
tyrosine	0.90	0.80	0.88
phenylalanine	1.82	1.05	1.12
β-alanine	0.27	0.08	0.03
GABA	7.92	5.50	5.65
tryptophan	2.84	2.21	2.46
ethanolamine	2.58	1.28	1.50
ornithine	0.10	0.04	0.12
lysine	0.99	0.75	0.89
histidine	0.83	0.33	0.28
arginine	2.95	0.42	0.17
threonine	1.75	1.75	*b*

[a] One extraction of a composite sample taken from 15 plants was analyzed per age class. [b] Not found.

Starch content was determined on the methanol insoluble residue, which was suspended in 10 mL of H_2O and heated to 130 °C for 1 h. After cooling to 55 °C, 2 mL of a mixture of amyloglucosidase (2 mg/mL buffer) and acetate buffer (4 M, pH 4.8) was added. The solution was incubated at 55 °C for 2 h. Aliquots of hydrolysate were dried, derivatized, and analyzed for glucose with GLC as described above. To estimate total dietary fiber, 48 mL of 95% ethanol was added to the remaining enzyme-treated mixture, followed by filtration after 1 h and washing with 10 mL of 78% ethanol and 10 mL of 95% ethanol. A final 10 mL acetone wash was performed, and residues were dried overnight at 105 °C. Crude protein and ash were determined and subtracted from dry residue weights, with the balance attributed to total dietary fiber (Li and Andrews, 1988).

Where applicable, the statistical analysis procedures (*SAS/Stat User's Guide*, 1988) were used to determine significant differences in mean values reported in the tables.

RESULTS AND DISCUSSION

The physiological or free amino acid content of cv. Kennebec potato leaf powder of three age classes is shown in Table 1. Serine, asparagine, glutamic acid, glutamine, proline, β-alanine, and arginine showed the most difference between the oldest and the youngest foliage, being higher in the metabolically more active young foliage. The amino acids found to be stimulatory to the Colorado potato beetle (alanine, valine, serine, GABA) (Hsiao and Fraenkel, 1968; Mitchell, 1974) were detected at levels equal to or greater than those previously reported by Hare (1983), whose data showed they varied in concentration over the growing season. Several amino acids were detected that were not reported previously (asparagine, glutamine, citrulline, β-alanine, ethanolamine, ornithine, histidine). We found only one-fourth as much lysine as listed by Hare (1983). In addition to the role of some free amino acids as feeding stimulants for the CPB, other amino acids, such as aspartic acid, arginine, asparagine, glutamic acid and glutamine, may contribute to CPB nutrition due to their relatively high concentration. Asparagine and glutamine have been shown to have important effects on growth

Table 2. Protein Levels, in Milligrams per Gram of Dry Weight, Found in Three Age Classes of Potato *S. tuberosum* Cv. Kennebec Foliage Extracted with 0.1 N NaOH, pH 11, Based on 10 Replicates from a Composite Sample for Each Age Class, ± SEM[a]

	age of foliage		
	≤3 days	5–7 days	15–21 days
protein	187.4 ± 3.5a	181.6 ± 2.0a	155.3 + 2.9b

[a] Means followed by different letters are significantly different at $p = 0.01$, least-square means, SAS Institute, Carey, NC.

Table 3. Amino Acid Composition of Protein Extracted from *S. tuberosum* Foliage, Compared with Values for Casein and Egg Albumin, Expressed in Milligrams of Amino Acid per 100 g of Protein

amino acid	potato foliage[a]	casein	egg albumin
aspartic	5.36	6.65	9.27
glutamic	5.90	10.69	12.07
serine	2.01	4.83	6.36
histidine	1.07	3.29	2.38
glycine	3.33	2.21	4.11
threonine	2.46	3.88	4.29
alanine	8.54	3.45	6.58
arginine	9.92	5.48	7.64
tyrosine	4.74	5.98	3.65
valine	8.08	5.21	6.23
methionine	5.58	3.92	4.30
phenylalanine	7.87	5.62	6.06
isoleucine	6.45	5.12	4.97
leucine	10.88	6.69	8.38
lysine	11.64	11.43	8.97
proline	6.17	15.61	4.76

[a] Composite of ≤3 day old foliage.

and amino acid composition of hemolymph in the silkworm larvae, *Bombyx mori* (Ito and Inokuchi, 1992).

Our estimate of the protein content of the youngest potato foliage was higher than any of the seasonally variable, extractable protein levels reported by Hare (1983). The relatively high levels of leaf protein reported here (Table 2) are probably related to the use of 0.1 N NaOH (pH 11) as the extractant, in contrast to use of a pH 8 phosphate buffer which yielded considerably less protein (Hare, 1983). This method has been shown to remove more ribulose 1,5-diphosphate carboxylase-oxygenase, the major protein in leaf tissue, than extraction at lower pH (Jones et al., 1989). The significance of leaf protein to phytophagous insect growth and reproduction is well documented (Mattson, 1980; Scriber and Slansky, 1981). Insects can selectively consume the more nutritious plant parts.

The amino acid composition of potato foliar protein is not very similar to either casein or egg albumin (Table 3). The most notable differences occur between potato foliar protein and casein, where potato has about half as much glutamic acid, serine, histidine, and proline as does casein. Potato protein has about twice as much alanine, arginine, and leucine as does casein. Egg albumin has about twice as much aspartic acid, glutamic acid, serine, and histidine as foliar protein. In addition to this, since the secondary and tertiary structures of these proteins also determine how efficiently they can be digested and thus utilized by the CPB for growth processes, it may be advantageous to incorporate a plant protein into a synthetic diet instead of an animal protein (Broadway and Duffey, 1988).

Table 4 lists the quantities of trace metals found in potato leaf powder. A literature search did not reveal any comparable information on the trace metal content of potato foliage. The youngest foliage generally contained the lowest levels of trace metals, with the exceptions of phosphorus and copper. This information suggests that none of the commercially available mineral mixes for insects are similar in content to that shown here by analysis of potato foliage.

Table 4. Average Trace Metal Content of Potato *S. tuberosum* Cv. Kennebec Foliage, in Micrograms per Gram of Dry Leaf Powder, Sampled from Three Leaf-Age Classes[a]

element	≤3 days	5–7 days	15–21 days
calcium	5469 ±458a	10238 ± 523 b	12940 ± 459c
cobalt	0.191 ± 0.073a	0.319 ± 0.086a	0.418 ± 0.073a
chromium	0.745 ± 0.057a	1.042 ± 0.067a	1.106 ± 0.057b
copper	27.63 ± 5.47a	33.65 ± 6.24a	19.65 ± 5.47a
iron	165.76 ± 15.62a	196.86 ± 17.81a	209.25 ± 15.62a
potassium	23276 ± 974a	24281 ± 1110a	34411 ± 974b
magnesium	6101 ± 309a	7903 ± 352b	7373 ± 309b
manganese	79.54 ± 3.82a	120.93 ± 4.36b	143.55 ± 3.82c
sodium	80.62 ± 22.34a	135.30 ± 25.47a	472.5 ± 22.34b
nickel	1.67 ± 0.14a	1.91 ± 0.16a	2.43 ± 0.14b
phosphorus	9538 ± 283a	9859 ± 323a	8422 ± 283b
vanadium	1.41 ± 0.165a	3.05 ± 0.195b	3.92 ± 0.165c
zinc	59.47 ± 3.48a	63.44 ± 3.97a	74.15 ± 3.48b

[a] Means in a row followed by the same letter are not significantly different at p=0.05. Based on three samples of a composite per age class.

Table 5. Sugars, Starch, and Total Dietary Fiber Content of Three Age Classes of Potato *S. tuberosum* Cv. Kennebec Foliage in Grams per 100 g of Dry Leaf Powder, ± SD, $N = 2$ per Age Class[a]

carbohydrate	<3 days	5–7 days	15–21 days
sucrose	1.29 ± 0.14a	1.72 ± 0.14b	1.97 ± 0.14b
glucose	0.76 ± 0.26a	0.91 ± 0.28a	1.94 ± 0.28b
fructose	1.30 ± 0.22a	1.83 ± 0.24a	3.53 ± 0.24b
total sugars	3.35 ± 0.53a	4.46 ± 0.56a	7.44 ± 0.56b
starch	1.0	0.9	0
dietary fiber	23.9 ± 0.40a	24.1 ± 0.10a	25.4 ± 0.50a

[a] Means in a row followed by the same letter are not significantly different at $p = 0.05$.

Information on the carbohydrate content of potato leaf powder is listed in Table 5. Overall, sucrose, glucose and fructose are present at rather low concentrations, totaling at most about 5% of the dry weight of the leaf. The concentration of starch is also quite low, as might be expected in most photosynthetic tissue. The amount of total dietary fiber (TDF), which is the residue remaining after starch, fat, protein, sugars, alcohol soluble material, and ash have been accounted for, is about 25% of the dry foliage weight. About 61% of the TDF is composed of nonstarch polysaccharides (NSP). Preliminary analyses (not included in Table 5) showed that galacturonic acid, galactose, glucose, mannose, xylose, arabinose, inositol, and rhamnose were present, with glucose (mainly as cellulose) and galacturonic acid (as pectins) constituting about 85% of the total NSP.

Phytophagous insect synthetic diets frequently contain high levels of sucrose and/or glucose as carbohydrate sources. Sugars often have a phagostimulatory role for leaf-eating insects, but may also inhibit feeding above a certain level. However, the small amounts of sucrose, glucose, and fructose detected suggest that complex carbohydrate structures digestible by CPB may be more appropriate as energy sources. Although the older foliage contains the highest levels of simple sugars, the oldest foliage is eaten by CPB only if younger foliage is unavailable, thus pointing to factor(s) other than sugars as determining foliage consumed.

It is likely that many additional foliar components may function in host plant selection and suitability for

CPB growth and reproduction. However, the present study identifies and quantifies the major classes of nutrients present in potato foliage that are required to meet the basic nutritional needs of the CPB. Protein content and specific amino acids may be the most important of the nutritional factors determining within-plant distribution of the CPB.

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