

## Effect of Soil Salinity on Internal Browning of Potato Tuber Tissue in Two Soil Types

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### ABSTRACT

A study was carried out with potato (*Solanum tuberosum*; cv. Atlantic) during 2001 and 2002 to determine the effect of soil salinity on internal tuber browning. The effect of varying levels of soil salinity on proline content, polyphenol oxidase enzyme activity, and chlorogenic acid content in potato leaves and tubers was examined. NaCl treatments (2.1, 4.25, 6.38, 8.5 g NaCl L<sup>-1</sup>) were applied to the pots, the first 46 days after planting, and four additional treatments were applied, each about 7 days apart. Increasing NaCl concentrations resulted in an increase in browning of tuber tissue and proline content in the tubers. Chlorogenic acid content in the leaves increased up to 6.4 g NaCl L<sup>-1</sup>, but then decreased at 8.5 g NaCl L<sup>-1</sup> and in tubers tended to be maximal at the highest saline concentration tested (8.5 g NaCl L<sup>-1</sup>). Increasing NaCl concentration resulted in a reduction in yield per plant and average tuber weight, and also increased tuber number. There were major differences in the impact of salinity over the 2-year period, which was probably due to the impact of the growing media; a low organic matter (about 1% OM) silty loam soil and a high organic content (about 90% OM) Muck soil were used in 2001 and 2002, respectively. Tuber browning increased linearly with salinity in 2002, but only markedly increased at 8.5 g NaCl L<sup>-1</sup> in 2001. Sodium and chloride ion concentration was always greater (about two times) at equivalent application rates in the 2002 trial. The high organic matter content soil retained sodium and chloride ions more effectively than the silty loam soil and enhanced the impact of increased salinity

concentration on physiological properties of potato plants and particularly on tuber tissue browning and proline accumulation.

### RESUMEN

Con el objeto de determinar el efecto de la salinidad del suelo sobre el oscurecimiento interno del tubérculo de papa (*Solanum tuberosum*; cv. Atlantic), se hizo un estudio durante los años 2001 y 2002. Se examinó el efecto de la variación de niveles de salinidad del suelo sobre el contenido de prolina, actividad de la enzima polifenol oxidasa y contenido de ácido clorogénico en las hojas y tubérculos de papa. Los primeros 46 días después de la siembra se aplicó a las macetas los tratamientos de ClNa (2.1, 4.25, 6.38, 8.5g de ClNa L<sup>-1</sup>) y 4 tratamientos adicionales cada 7 días. El incremento de concentración de ClNa dio como resultado un aumento del oscurecimiento del tejido del tubérculo. El contenido de ácido clorogénico en las hojas aumentó hasta los 6.4g de ClNa L<sup>-1</sup> pero luego disminuyó a los 8.5g y los tubérculos propendieron a tener mayor tamaño a las más altas concentraciones salinas probadas (8.5g ClNa L<sup>-1</sup>). El incremento de la concentración de ClNa dio como resultado una reducción del rendimiento por planta y del peso promedio del tubérculo, pero aumentó el número de tubérculos. Hubo diferencias importantes en el impacto de la salinidad sobre el período de los dos años, lo cual probablemente fue debido al impacto de los medios de cultivo, uno con baja cantidad de materia orgánica (alrededor de 1%) suelo fangoso con arcilla y un suelo con alto contenido de materia orgánica (alrededor de 90%) con estiércol húmedo, suelos que se usaron en 2001 y 2002 respectivamente. El oscurecimiento del tubérculo se incrementó linealmente con la salinidad en el 2002, pero solo aumentó marcadamente con 8.5g ClNa

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**L<sup>-1</sup> en el 2001. La concentración de los iones cloro y sodio fue siempre mayor (aproximadamente dos veces) a índices de aplicación equivalentes en la prueba del 2002. El suelo con alto contenido de materia orgánica retuvo los iones de cloro y sodio con mayor efectividad que el suelo fangoso arcilloso e intensificó el impacto de la concentración de salinidad sobre las propiedades fisiológicas de las plantas de papa y particularmente en el oscurecimiento del tejido y acumulación de prolina.**

## INTRODUCTION

Potatoes are grown globally under many different cultural conditions, including arid and semiarid regions where soils are often highly saline from soluble salts in irrigation water and fertilizers. The use of saline water for irrigation increases the salt concentration in the soil and thereby affects plant development by reducing plant growth, yield, and quality (Akilan et al. 1997). Some irrigation waters contain NaCl, which contributes to the direct effects of excess salt on plant growth that may be described as (1) an osmotic stress, reducing water availability; (2) ion imbalance stress, caused by antagonistic effects on nutrient elements; and (3) specific toxicity of Cl<sup>-</sup>, Na<sup>+</sup>, or both (Villora et al. 2000).

In potatoes several factors such as injury during mechanical harvesting, infection by pathogens, heat necrosis, and physiological stress are thought to cause internal disorders of tuber tissue (Friedman 1997). These symptoms range from localized to extensive gray to brown discoloration. Internal discoloration causes severe losses in both table and process stock (Bachem et al. 1994). Excessive browning during frying can result from enzymatic-based internal tuber discoloration (Rodríguez-Saona et al. 1997).

Specific enzymes are produced in the potato plant in response to damage and stress (Hammerschmidt 2001). Increase of proline in the leaves and tubers of potatoes corresponds with a rise in osmotic potentials of the organs, indicating a possible role for proline in adaptation to salinity (Heuer 1999). Proline content increased with an increase in NaCl concentration as well as with an increase in duration of stress in *Phaseolus mungo* L. seedlings (Dash and Panda 2001). Proline concentration is considered to be an important parameter for measuring the stress tolerance capacity in plants (Delauney and Verma 1993; Yoshida et al. 1995).

Internal tuber browning is a complex symptom that may occur in potatoes produced in fields irrigated with saline water. In this study, it is hypothesized that an increase in NaCl concentration in the soil could be responsible for escalating internal tuber browning disorder in potato tubers. The objectives of this research were (1) to verify the effect of increasing NaCl concentration in soil on internal tuber browning, (2) to evaluate the effect of increasing NaCl concentration in soil on the biochemical responses of potato plants related to internal tuber browning, and (3) to measure the impact of increasing NaCl concentration in soil on tuber yield, size and weight.

## MATERIALS AND METHODS

### *Plant Growth Conditions*

Potato tubers cv Atlantic of about 200 g and visibly free from disease were selected for the experiments. Tubers were surface-disinfested in 10% sodium hypochlorite for 20 min, rinsed with sterilized distilled water, and air-dried at 15 C for 2 days prior to planting. The seed tubers were planted whole in pots (57-L capacity) containing a low organic matter (about 1% OM) silty loam soil in 2001 and a high organic content (about 90% OM) Muck soil in 2002. The experiments consisted of randomized blocks in a completely randomized plot design, with 10 plants per replicate per treatment and four replicates per treatment, giving a total of 40 experimental units per treatment. The main plot effects were different concentrations of NaCl.

Five NaCl treatments were applied in both 2001 and 2002: 0, 2.1, 4.25, 6.38, and 8.5 g NaCl L<sup>-1</sup>, ranging from mild saline conditions (2.1 g NaCl L<sup>-1</sup>) to concentration comparable to saline water (8.5 g NaCl L<sup>-1</sup>). The first application of the five NaCl treatments was applied to the pots 46 days after planting (DAP, when the plants were in the stage of tuber initiation) with four additional applications, each about 7 days apart.

To verify the amount of Na<sup>+</sup> and Cl<sup>-</sup> that was effectively present in the system, soil samples (5 g) from each pot were collected at harvest, and sodium and chloride content was analyzed, and cation exchange capacity (CEC) was determined (Rhoades 1982).

### *Biochemical Analyses*

In both years, biochemical analyses were carried out on each of 10 samples of compound leaves collected 90 DAP, when plants had reached early senescence. The fourth leaf

below the inflorescence on the main stem of each replicate plant considered median in terms of height and diameter was selected. These leaf samples were weighed, frozen in liquid nitrogen, and stored at -20 C for further biochemical analyses. Tubers were harvested from each pot, and the number and weight of tubers per plant were recorded. Tuber browning was evaluated (described below) in four tubers selected at random from each replicate of every treatment (40 tubers per treatment). The remaining tubers were ground and stored at -20 C for biochemical analyses.

Proline concentration was measured according to Moore and Stein (1948) following extraction with methanol:chloroform:water (12:5:3 v/v), (Bielski and Turner 1966). Polyphenol oxidase activity was assayed using 0.5 g of ground leaf and tuber samples suspended in 1.5 mL of phosphate buffered saline at pH 7.3 (Ray and Hammerschmidt 1998), followed by centrifugation at 20,000  $g_n$  for 10 min. This enzyme was assayed at 480 nm with L- $\beta$ -3,4-dihydroxyphenylalanine (DOPA) as substrate (Constabel et al. 1995). Chlorogenic acid analysis was performed according to the method described by Griffiths et al. (1992), where 0.2 g of each sample was suspended in a 2 mL aqueous solution containing urea (0.17 M), acetic acid (0.10 M), and a further 1 mL of water was added. After the addition of 1 mL of sodium nitrate (0.14 M), 1 mL of sodium hydroxide (0.5 M) was added. The resulting suspension was centrifuged at 2,250  $g_n$  for 10 min, and an aliquot (1 mL) of the supernatant was then transferred to a cuvette where its absorbance was determined at 510 nm. Chlorogenic acid content was plotted against a standard curve varying from 25 to 400 mg L<sup>-1</sup> from commercially available 5-caffeoylquinic acid (Sigma).

### Data Collection and Analyses

Tuber tissue discoloration was measured after a 30 day post-harvest incubation period (24 h dark at 15 C). Tubers were cut into three sections, 25%, 50%, and 75% of the distance from the apical end of the sample tubers. A sharp knife was used to ensure a smooth cut face. Fresh-cut tuber sections were placed cut surface down on a glass plates, 40 × 30 cm and 2 mm thick. A digital image analysis technique was used to assess tuber tissue discoloration (Kirk et al. 2001). The glass plates were used to prevent surface contamination of the scanner glass and permitted multiple samples to be prepared and moved to the scanner for image production. The glass plate was transferred to a flatbed scanner (Epson Perfection 4870 Photo Pro; Epson America, Inc., Long Beach, CA,

USA) with optical resolution of 4800 dpi, (hardware resolution 4800 × 9600 dpi [maximum]) controlled by PC. Scanner control software (Epson Scan) generated an image of the cut tuber surfaces against a black background. The image was formed from light reflected from the cut tuber surfaces. The brightness value of the image controlled the light intensity of every pixel in the image. The contrast value controlled the differences between light and dark regions of the image. The scanner control software was able to automatically adjust the brightness and contrast of the image by comparing the relative size of the pale tuber surfaces against the black background. A photograph-quality image was taken and stored for analysis. A typical image in joint photographic experts group format (JPEG) occupies 0.6 megabyte. The image files created with the scanner software were loaded into the image analysis software (Sigma Scan Pro ver. 5.0, Jandel Scientific, San Rafael, CA, USA). The black background has 0 light intensity units (LIU), while pure white has 255 LIU. The image of the cut tuber surface was selected for analysis and isolated from the adjacent regions of the image. The surface of tuber sections area was selected with the “fill” tool, which encompassed all pixels within a given area brighter than the cut-off threshold. The area selection cut-off threshold was set to 10 LIU, effectively allowing the software to exclude all parts of the image darker than 10 LIU, e.g., the black background. The average reflective intensity (ARI) of all the pixels within the image gave a measurement of browning of the tuber tissue of each sample. The ARI was measured in three sections from the apical end of the daughter tuber, described above. The degree of darkened tissue per tuber was expressed as a single value (mean ARI) calculated as the average ARI of the three sections evaluated from eight seedpieces (sampling unit) 30 days after inoculation. Tuber tissue browning was expressed relative to the ARI of the control treatments. The relative average reflective intensity of a treatment was calculated as follows:

$$\% \text{ RARI} = \left[ 1 - \frac{\text{MeanARI}_{\text{saline treatment}}}{\text{MeanARI}_{\text{control}}} \right] * 100$$

% RARI has a minimum value of 0 (no measurable discoloration) and maximum value of 100 when the ARI value  $\leq 10$  LIU (tuber surface is completely blackened).

Linear and non-linear regression analyses were used to describe the responses of the measured variables to increase in NaCl concentration (JMP version 5.01, SAS software).

## RESULTS

The applications of salt to the soil effectively increased Na<sup>+</sup> and Cl<sup>-</sup> content in the in both years (Table 1). The soil used in the experiment conducted in 2002 had greater cation exchange capacity (CEC) than in 2001 (Table 1). This characteristic was indicated by the higher content of Na<sup>+</sup> and Cl<sup>-</sup> ions recovered from soil samples at the end of each experiment. Sodium and chloride ion concentration was always greater (about two times) at equivalent application rates in the 2002 trial. Na<sup>+</sup> and Cl<sup>-</sup> ionic content of the soil increased with NaCl concentration applied into the soil in both years (Table 1).

TABLE 1—Cation exchange capacity (CEC) and sodium and chloride ions content in the soil samples collected from plots of potato cv Atlantic at different concentrations of soil NaCl during 2001 and 2002.

Salinity concentration (g NaCl L <sup>-1</sup> )	CEC (me/100 g)		Sodium (mg/kg)		Chloride (me/100g)	
	2001	2002	2001	2002	2001	2002
0	12.8	27.8	32	70	150	270
2.1	14.4	26.9	94	208	158	244
4.25	12.9	28.0	176	386	187	296
6.38	21.9	28.9	296	501	203	415
8.5	10.7	29.1	413	691	215	380

TABLE 2—Linear and non-linear relations of % RARI, leaf and tuber proline content, chlorogenic acid content and yield components with increasing salinity concentration (g NaCl L<sup>-1</sup>)

Measured variable and figure location	Year	Equation	Co-efficient	Standard error	T value	P value	Adjusted r <sup>2</sup>
% RARI Fig. 1	2001	$RARI_{p2001} = 6.25 + 0.68e^{0.013c}$	6.25	0.512	16.411	<0.0001	0.82
			0.68	0.221	-5.730	<0.0001	
	2002	$RARI_{p2002} = 4.86 + 0.78c$	0.013	0.020	8.711	<0.0001	0.77
			4.86	0.642	7.569	<0.0001	
Proline (leaf) Fig. 2A	2001	$Pt_{2001} = 73.0e^{0.09c}$	0.78	0.110	7.018	<0.0001	0.69
			73.0	6.899	10.581	<0.0001	
	2002	$Pt_{2002} = 38.0 - 0.53c$	0.09	0.015	6.074	<0.0001	0.09
			38.0	2.055	18.494	<0.0001	
Proline (tuber) Fig. 2B	2001	$Pt_{2001} = \frac{158.8}{1 + e^{\frac{(c+0.31)}{3.85}}}$	0.53	0.394	-1.338	0.1975	0.81
			158.8	20.571	7.715	<0.0001	
	2002	$Pt_{2002} = 79.6e^{0.02c}$	3.85	1.6011	2.401	0.0280	0.56
			-0.31	0.876	-0.349	0.7309	
Chlorogenic Acid Fig. 4A	2001	$CAI_{2001} = 310.5 + 19.6c$	79.5937	1.596	49.887	<0.0001	0.84
			1.4782	0.306	4.821	0.0001	
	2002	$CAI_{2002} = 296.2 + 2.2c$	310.5	8.920	34.809	<0.0001	0.41
			19.6	2.244	8.711	<0.0001	
Yield Fig. 5A	2001	$Y_{2001} = 0.72 - 0.02c$	296.2	2.813	105.457	<0.0001	0.64
			2.22	0.708	3.122	0.0075	
	2002	$Y_{2002} = 2.04 - 0.08c$	0.72	-0.021	32.820	<0.0001	0.44
			-0.02	0.004	-5.626	<0.0001	
Tuber number Fig. 5B	2001	$Tn_{2001} = 7.60 + 0.01c$	2.04	0.109	18.580	<0.0001	0.06
			-0.08	0.021	-3.784	0.0014	
	2002	$Tn_{2002} = 23.0 + 0.36c$	7.6	0.508	15.031	<0.0001	0.15
			0.01	0.097	1.066	0.3002	
Tuber weight Fig. 5C	2001	$Tw_{2001} = 95.4e^{-0.06c}$	23.0	1.021	22.508	<0.0001	0.81
			0.36	0.196	1.809	0.0871	
	2002	$Tw_{2002} = 89.5e^{-0.06c}$	95.4	2.644	36.072	<0.0001	0.69
			0.06	0.006	8.911	<0.0001	
	2002	$Tw_{2002} = 89.5e^{-0.06c}$	89.5	3.886	23.014	<0.0001	0.69
			0.06	0.010	6.218	<0.0001	

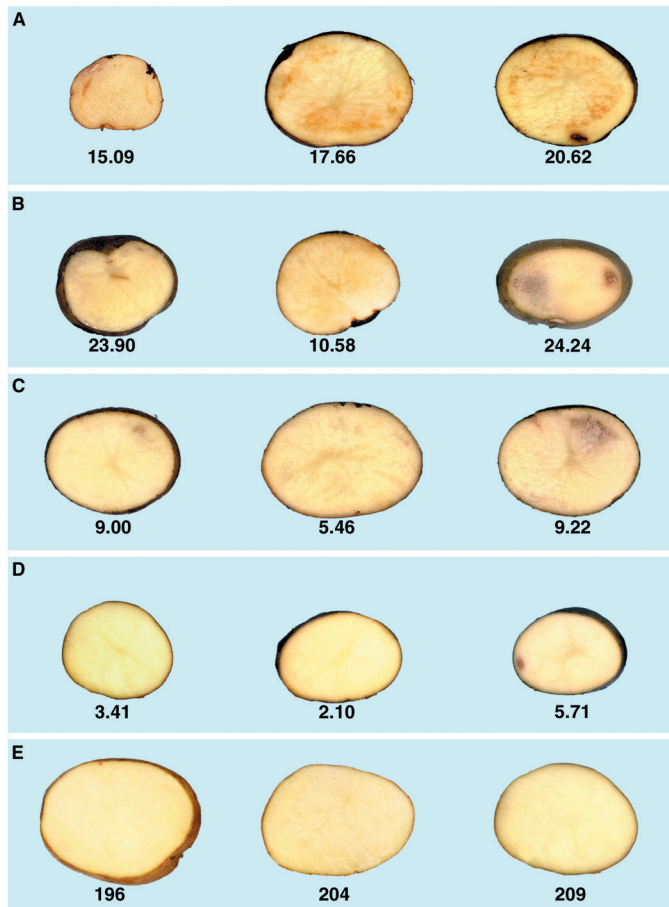
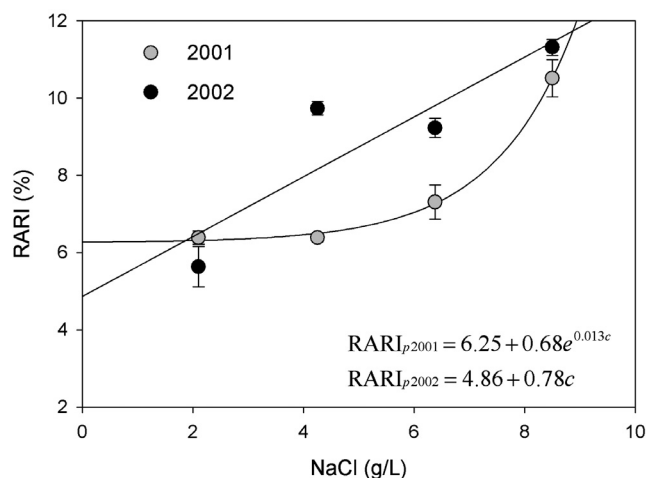


FIGURE 1.

Example images of tuber tissue browning from potato plants (cv Atlantic) grown in a range of soil NaCl concentrations, (A) 8.5, (B) 6.38, (C) 4.25, (D) 2.1, and (E) 0 g NaCl L<sup>-1</sup>. The numbers in (A-D) are percentage RARI (relative average reflective intensity) values. The numbers in (E) are ARI values in light intensity units (LIU).



### Tuber Tissue Browning

Examples of images of tuber tissue browning from potato plants (cv Atlantic) grown in a range of soil NaCl concentrations are shown in Figure 1. The higher percentage RARI values indicate more intense tissue browning. The numbers annotating the 0 g NaCl L<sup>-1</sup> are ARI values in light intensity units and ranged from 210 to 214.

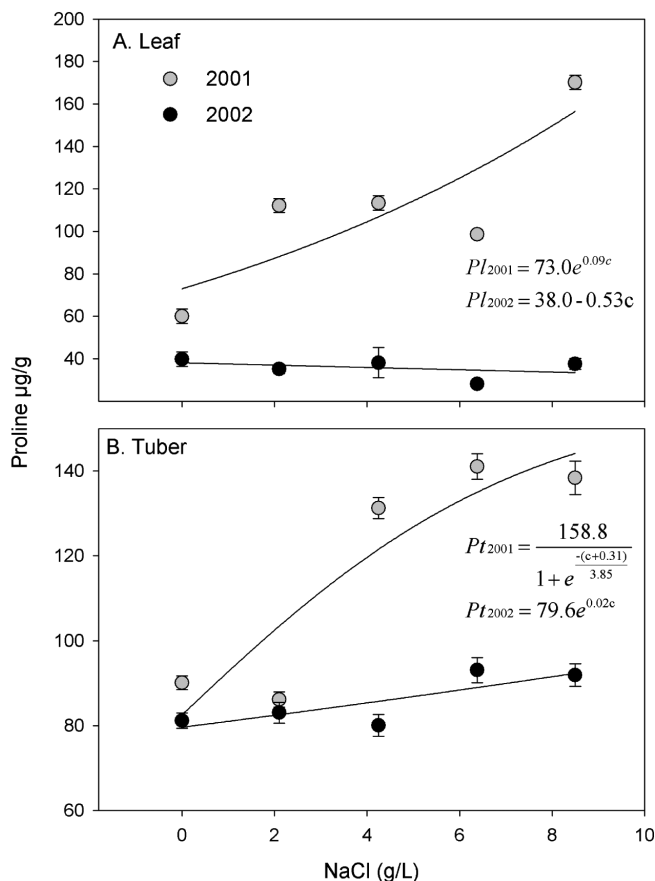
Tuber tissue browning increased exponentially in 2001 and linearly in 2002 with increasing saline concentration (Figure 2). Tuber browning increased linearly with salinity in 2002 but only markedly increased at 8.5 g NaCl L<sup>-1</sup> in 2001. Values of percentage RARI ranged from 6.39 to 10.5 and 5.64 to 11.3 in tubers grown in saline conditions in 2001 and 2002, respectively (Figure 2). The percentage RARI was maximal at the highest concentration of salinity (8.5 g NaCl L<sup>-1</sup>) in 2001 and 2002 (Figure 2). The slopes of the lines derived from non-linear regression of percentage RARI on salinity concentration were significantly different from 0 in 2001 and 2002, respectively (Table 2).

### Biochemical Analyses

In 2001, proline accumulation in the leaves increased exponentially with the concentration of NaCl (Figure 3A). Proline accumulation in leaf tissue was greatest (about 170 µg/g) at 8.5 g NaCl L<sup>-1</sup> (Figure 3A). In 2002, proline accumulation in the leaves did not increase with saline concentration (Figure 3A). The proline content in leaf tissue was lower in 2002 at all comparative salinity concentrations than in 2001. Proline accumulation in tubers increased logarithmically with the concentration of NaCl in 2001, but in 2002 the rate of increase was linear but at a lower rate (Figure 3B). The slopes of the lines derived from non-linear and linear regression of proline content in leaves and tubers on salinity concentration were significantly different from 0 in 2001 and 2002, respectively (Table 2). The correlations between percentage RARI and proline content of tubers were 0.40 and 0.49 in 2001 and 2002, respectively (data not shown).

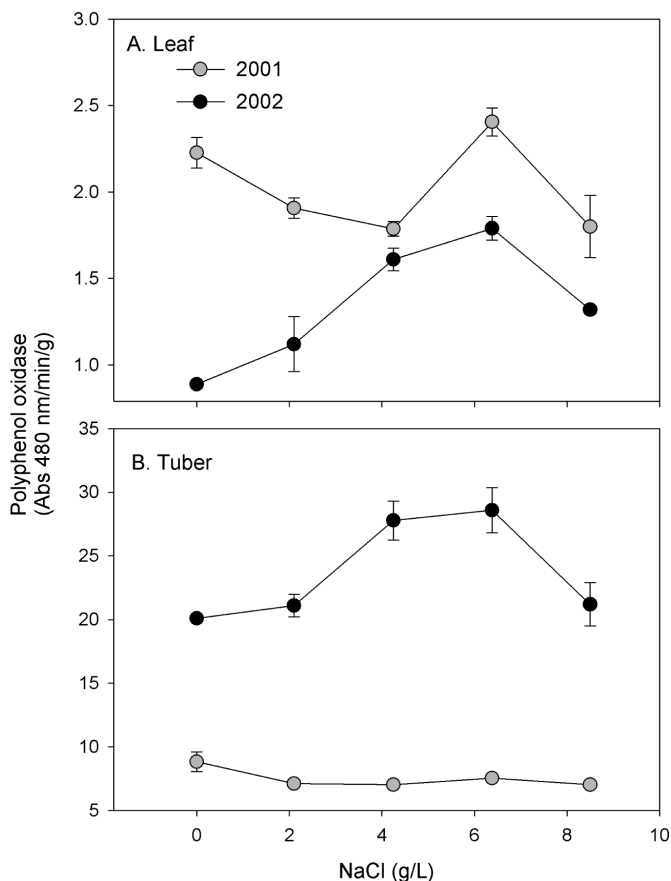
Figure 2.

Tuber tissue browning measured as percentage RARI in tubers of potatoes (cv Atlantic) harvested from plants grown in a range of soil NaCl concentrations. The formulae are the predicted (RARI<sub>p</sub>) values from 2001 and 2002 trials and *c* = salinity concentration in g NaCl L<sup>-1</sup>. The error bars are the standard deviations around mean percentage RARI values.



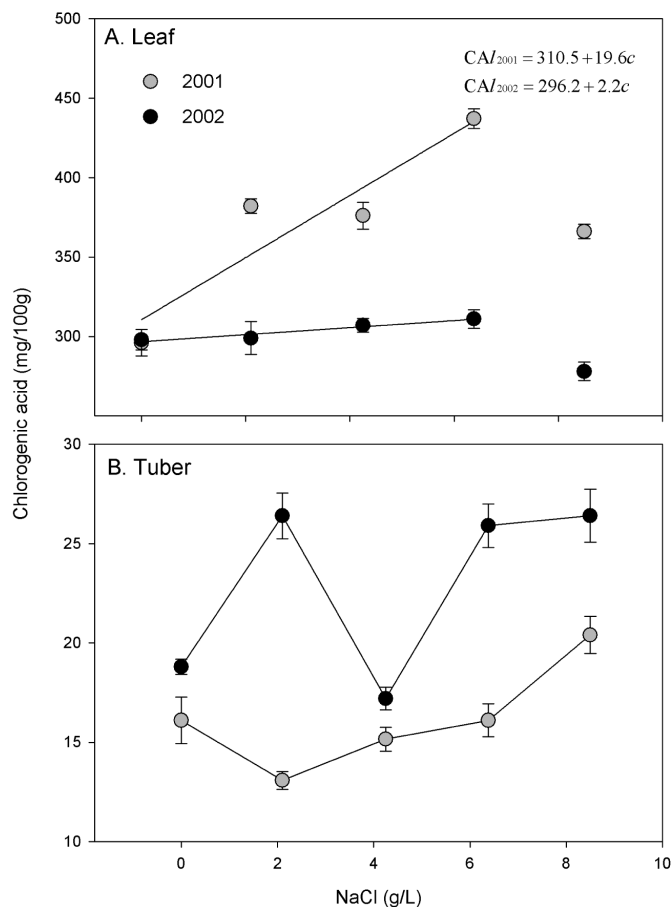
**Figure 3.** Proline content in (A) leaves and (B) tubers of potatoes (cv Atlantic) grown in a range of soil NaCl concentrations. The formulae are the predicted proline content (P) of leaves (l) and tubers (t) from 2001 and 2002 trials and  $c$  = salinity concentration in g NaCl L<sup>-1</sup>. The error bars are the standard deviations around mean proline content values.

Polyphenol oxidase (PPO) activity in leaves decreased initially with NaCl concentration then increased at 6.38 g NaCl L<sup>-1</sup> and decreased again at 8.5 g NaCl L<sup>-1</sup> in 2001 (Figure 4A). PPO activity increased with saline concentration to 6.38 g NaCl L<sup>-1</sup> then decreased at 8.5 g NaCl L<sup>-1</sup> 2002 (Figure 4A). PPO activity in leaves was lower at all equivalent salinity concentrations in 2002 in comparison to 2001 (Figure 4A). PPO activity in tubers did not change with increasing saline concentration in 2001, but increased with saline concentration to 6.38 g NaCl L<sup>-1</sup> then decreased at 8.5 g NaCl L<sup>-1</sup> in 2002 (Figure 4B). PPO activity in tubers was greater at all equivalent salinity concentrations in 2002 in comparison to 2001 (Figure 4B).



**Figure 4.** Polyphenol oxidase activity in (A) leaves and (B) tubers of potatoes (cv Atlantic) grown in a range of soil NaCl concentrations. The error bars are the standard deviations around mean polyphenol oxidase activity values.

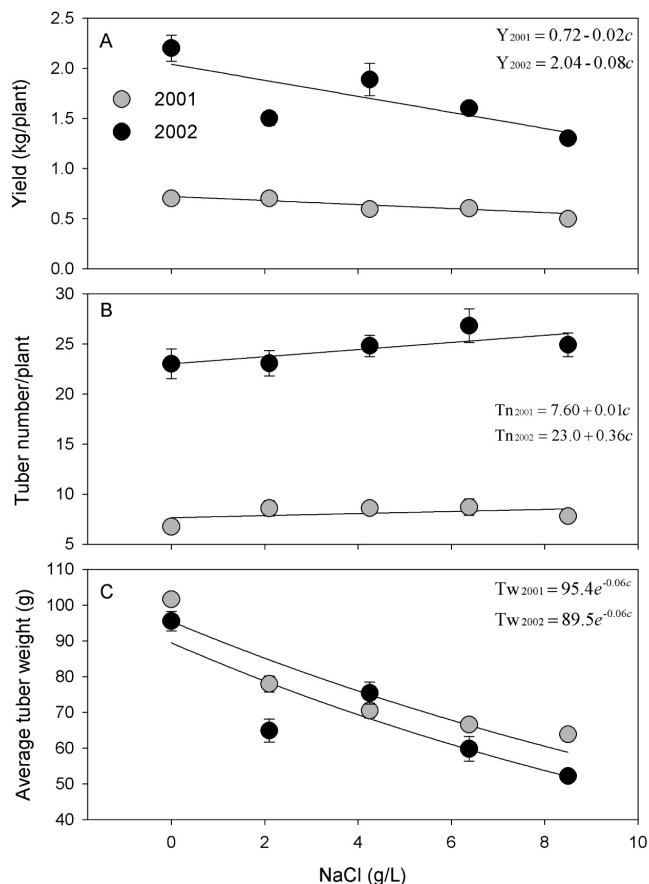
Chlorogenic acid content in the leaves increased linearly from about 300 to 440 and 300 to 310 mg/100 g (from 0 to 6.38 g NaCl L<sup>-1</sup>) in 2001 and 2002, respectively (Figure 5A). In both years, the chlorogenic acid content of leaves decreased at a saline concentration of 8.5 g NaCl L<sup>-1</sup>. Chlorogenic acid content in tubers initially decreased from about 16 to 12.5 mg/100 g at 0 to 2.1 g NaCl L<sup>-1</sup> then increased to about 20 mg/100 g at 8.5 g NaCl L<sup>-1</sup> (Figure 5B). Chlorogenic acid content in tubers was lower at all equivalent concentrations in 2001 than in 2002 (Figure 5B). The slopes of the lines derived from linear regression of chlorogenic acid content in leaves on salinity concentration were significantly different from 0 in 2001 and 2002, respectively (Table 2).



**Figure 5.** Chlorogenic acid content in (A) leaves and (B) tubers of potatoes (cv Atlantic) grown in a range of soil NaCl concentrations. The formulae are the predicted chlorogenic acid content (CA) of leaves (*l*) from 2001 and 2002 trials and *c* = salinity concentration in g NaCl L<sup>-1</sup>. The error bars are the standard deviations around mean chlorogenic acid content values.

### Yield Analysis

The average yield per plant decreased linearly at a rate of 0.02 kg per plant/g NaCl L<sup>-1</sup> in 2001 (Figure 6A). In 2002, the average yield per plant decreased linearly from about 2.25 to 1.5 kg/plant with increasing NaCl concentration at a rate of 0.08 kg per plant/g NaCl L<sup>-1</sup> (Figure 6A). The average yield per plant at equivalent saline concentrations was greater in 2002 than in 2001 (Figure 6A). The slopes of the lines derived from linear regression of yield on salinity concentration were significantly different from 0 in 2001 and 2002, respectively (Table 2).



**Figure 6.** (A) Yield per plant, (B) tuber number per plant, and (C) average tuber weight of potatoes (cv Atlantic) harvested from plants grown in a range of soil NaCl concentrations. The formulae are the predicted yield per plant (*Y*), tuber number (*Tn*), and average tuber weight (*Tw*) from 2001 and 2002 trials and *c* = salinity concentration in g NaCl L<sup>-1</sup>. The error bars are the standard deviations around mean values of yield per plant, tuber number per plant, and average tuber weight of potatoes.

The average number of tubers per plant did not increase with salinity concentration in 2001 (Figure 6B). In 2002, the average tuber number per plant increased linearly with soil salinity concentrations at a rate of 0.36 tuber number per plant/g NaCl L<sup>-1</sup> (Figure 6B). The average number of tubers per plant was greater at all soil salinity concentrations in 2002 than in 2001 (Figure 6B). The slopes of the lines derived from linear regression of tuber number per plant on salinity concentration were significantly different from 0 in 2001 but not in 2002 (Table 2).

Average tuber weight decreased exponentially with increase in salinity concentration at equivalent rates in 2001 and 2002, respectively (Figure 6C). The slopes of the lines derived from non-linear regression of average tuber weight on salinity concentration were significantly different from 0 in 2001 and 2002, respectively (Table 2).

## DISCUSSION

Internal browning of potato tubers results from oxidation of chlorogenic acid by PPO into fungitoxic quinones and further into brown pigments. The tubers from plants treated with saline solutions in this study had greater intensity of internal tissue browning, possibly due to higher concentrations of chlorogenic acid and PPO in the tubers. Although it was noted that proline, PPO and chlorogenic acid varied with NaCl stress, we cannot rule out the possibility that other compounds and enzymes could be involved in browning of internal tuber tissue. The biochemical processes involved in many potato responses are dependent on plant maturity and tuber responses may have been different had the plants been subjected to increase in soil salinity while in a different developmental stage. For example, chlorogenic acid content in leaves exposed to different salinity treatments was not affected but these treatments triggered increases in its content in the tubers.

The effects of salinity concentration are dependent on the interactions between the soil type and plant sensitivity. In this study, the soil used in 2002 had higher cation exchange capacity (CEC) thus retaining larger amounts of Na<sup>+</sup> and Cl<sup>-</sup> than the soil used in 2001. The soil used in 2002 was a Houghton Muck soil, with about 90% organic matter. Increase in organic matter leads to higher CEC (Bulluck et al. 2002). Tubers harvested from the 2002 experiment had more tissue discoloration than those from 2001 thus indicating a possible interaction between soil type, salinity and tuber tissue browning.

Proline concentration in potato tubers increased in response to increase in NaCl concentration in the soil in this study. These results correlate with other studies where it was found that free proline increased in plants exposed to increase in NaCl concentration (Guerrier 1995; Hartzendorf and Rolleschek 2001). In an experiment with cowpea, Somal and Yapa (1998) reported that there is linear relationship between

proline and NaCl concentration ( $r^2 = 0.81$ ) and that nitrogen, phosphorous or calcium deficiency increased proline levels. Wickramasinghe et al. (1987) reported accumulation of proline in the bark and leaf tissues of the rubber tree (*Hevea brasiliensis*) affected by brown bast, a disease condition that may be a physiological disorder caused by an increase in soil NaCl concentration (Paranjothy et al. 1975).

In the present study polyphenol oxidase (PPO) activity was relatively unaffected in mature leaves and tubers treated with saline solutions. PPO catalyzes two reactions: the hydroxylation of monophenols to o-diphenols, and the oxidation of o-diphenols to o-quinones. These o-quinones are highly reactive compounds that are fungitoxic and also evolve to give rise to brown pigments (Tomás-Barberán and Espín 2001). The reduction in PPO activity at a concentration of 8.5 g NaCl L<sup>-1</sup> was probably due to the excess of NaCl concentration in the tissues, which may have affected the physiology of the plants.

Treatments with NaCl had variable effects on concentrations of chlorogenic acid in mature leaves in this study. Chlorogenic acid is a phenolic compound widely distributed throughout the plant kingdom, and is the major phenolic acid found in potato tubers (Hammerschmidt 2001). Because chlorogenic acid is an o-diphenol, it is considered a much better substrate than monophenols according to the reaction mechanisms and substrate specificity of PPO (Espín et al. 2000). Oxidation of PPO into fungitoxic quinones is responsible for post-cutting darkening of tubers (Salisbury and Ross 1992), and its main function in plants appears to be to defend against pathogens (Friedman 1997). Fungal infection can initiate the production of chlorogenic acid, and oxidized chlorogenic acid has the ability to deactivate pathogen-produced pectinases, which provide support for the role of chlorogenic acid in disease resistance (Hammerschmidt 2001).

Potatoes grown under stressful conditions have a tendency to produce many small tubers. In this experiment, plants exposed to increasing salinity in the soil tended to produce many small tubers. Excessive uptake of Na<sup>+</sup> by a plant may inhibit the uptake of other nutrients such as N, causing ion imbalance stress which may result in reduced growth and yield (Cramer et al. 1991). Studies have shown that zucchini undergoing NaCl treatment (80mM) exhibited increased concentrations of Fe, Zn, Mn, and Cl in the foliage (Villora et al. 2000) while fennel treated with NaCl (2.5 g L<sup>-1</sup>) had decreased

Ca<sup>2+</sup> and K<sup>+</sup> concentration (Graifenberg et al. 1996). Considering the uptake and subsequent distribution of a given cation can inhibit or diminish both absorption and translocation of other cations toward the foliage, we suggest that Ca<sup>2+</sup> uptake may be affected by the treatments, but further investigations need to be performed.

We conclude that the highest content of Na<sup>+</sup> and Cl<sup>-</sup> in the soil (in this study, the ones caused by the treatment of 8.5 g NaCl L<sup>-1</sup>) can be a key factor on internal tuber browning. Tuber browning is a common but non-desirable characteristic that frequently affects potatoes. This deposition of brown pigments in tuber tissue of potatoes has been described as one response by tubers to infection processes that lead to disease (Friedman 1997). However, little information is available on the effect of soil salinity in tuber browning. Our results indicated that soil salinity caused tuber browning. The association between tuber browning and higher proline accumulations need to be systematically tested in tubers since no information could be found that related the two properties. Since plants respond to salinity stress through morphological, physiological and metabolic changes occurring in all plant organs, potato tubers are also likely to be affected by the nutrient imbalances imposed by excess of NaCl in the soil.

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