Plant Soil (2006) 288:309–318
DOI 10.1007/s11104-006-9119-y

# The use of transgenic canola (*Brassica napus*) and plant growth-promoting bacteria to enhance plant biomass at a nickel-contaminated field site

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Received: 3 May 2006 / Accepted: 29 August 2006 / Published online: 6 October 2006


**Abstract** The applicability of transgenic plants and plant growth-promoting bacteria to improve plant biomass accumulation as a phytoremediation strategy at a nickel (Ni)-contaminated field site was examined. Two crops of 4-day old non-transformed and transgenic canola (*Brassica napus*) seedlings in the presence and absence of *Pseudomonas putida* strain UW4 (crop #1) or *P. putida* strain HS-2 (crop #1 and 2) were transplanted at a Ni-contaminated field site in 2005. Overall, transgenic canola had increased growth but decreased shoot Ni concentrations compared to non-transformed canola, resulting in similar total Ni per plant. Under optimal growth conditions (crop #2), the addition of *P. putida* HS-2 significantly enhanced growth for non-transformed canola. Canola with *P. putida* HS-2 had trends of higher total Ni per plant than canola without *P. putida* HS-2, indicating the potential usefulness of this bacterium in phytoremediation strategies. Modifications to the planting methods may be required to increase plant Ni uptake.

**Keywords** Transgenic canola · Plant growth-promoting bacteria · Nickel-contaminated soil · Phytoremediation

**Abbreviations**

| | |
|---|---|
| ACC | 1-Aminocyclopropane-1-carboxylate |
| C | Control or non-transformed canola |
| T | Transgenic canola |

## Introduction

Metal phytoremediation, involving the harvest of plants grown in metal-contaminated soil and the combustion of this plant material to recover metals, is considered to be one of the potentially more effective methods for reducing metal concentrations in contaminated soils. The selection of plants for metal phytoremediation is based on one of two differing approaches; use either plants that are capable of accumulating elevated levels of metals in shoot tissue or plants that are capable of a high level of shoot biomass. Numerous plant species identified as metal hyperaccumulators are capable

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of accumulating 1–3 orders of magnitude greater metal concentrations than non-hyperaccumulators (Lasat 2002; McGrath et al. 2001). Often plants are natural metal hyperaccumulators, for example species of *Alyssum*, *Thlaspi* and *Berkheya* are Ni hyperaccumulators (McGrath et al. 2001), or plants may be engineered with transgenes to enhance hyperaccumulation (Clemens et al. 2002). The disadvantage of using hyperaccumulators as part of a phytoremediation strategy is that these plants generally have a slow growth rate and low amounts of above-ground biomass for harvesting.

The second approach to metal phytoremediation is to use a plant species that is capable of accumulating high amounts of above-ground biomass. The disadvantage of this approach is that these plants have lower and more variable levels of metal accumulation in shoot tissue compared to hyperaccumulators, however these plants generally grow faster so there is the potential for multiple harvests within a growing season. In order to facilitate maximum metal accumulation and above-ground biomass despite the plant stress induced by exposure to metal-contaminated soil, some studies have examined the use of transgenic plants manufactured to alter metal detoxification or accumulation (Krämer and Chardonnens 2001; Clemens et al. 2002). Another approach to reducing metal-related plant stress is to manufacture transgenic plants that produce enzymes, which reduce metal stress-induced ethylene production (Stearns and Glick 2003). There are several transgene products that can alter ethylene production, however the focus of this study examines the production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase required to breakdown plant ACC, the immediate precursor to ethylene. Plants that are stressed produce elevated levels of ACC, which is then converted to ethylene by ACC oxidase. Elevated levels of ethylene in plants are associated with reduced chlorophyll content, shoot height and biomass.

Trangenic plants that produce the enzyme ACC deaminase to breakdown the ethylene precursor (ACC), especially in their roots, or are associated with plant growth-promoting bacteria that produce ACC deaminase grow better than plants in the absence of ACC deaminase when exposed to environmental stressors. In laboratory studies, transgenic plants expressing the gene for the enzyme ACC deaminase are more resistant to growth inhibition in metal-contaminated soil (Stearns et al. 2005; Grichko et al. 2000; Nie et al. 2002) as well as inhibition by other stressors such as flooding (Grichko and Glick 2001), plant pathogens (Robison et al. 2001) and salt (Sergeeva et al. 2006). Similarly, in laboratory studies, the presence of plant growth-promoting bacteria that produce ACC deaminase and indoleacetic acid, increases plant resistance to organic and inorganic contaminants (Reed and Glick 2005; Reed et al. 2005; Belimov et al. 2005; Burd et al. 1998; Mayak et al. 2004a), plant pathogens (Wang et al. 2000) as well as flood and drought conditions (Grichko and Glick 2001; Mayak et al. 2004b).

The objective of this study was to assess the potential use of transgenic canola and plant growth-promoting bacteria, *Pseudomonas putida* strain UW4 and *P. putida* strain HS-2, all expressing ACC deaminase, as potential phytoremediation strategies for Ni-contaminated soil, in situ and to validate observations of enhanced plant growth in laboratory-based studies using Ni-spiked or -contaminated soil (Stearns et al. 2005; Burd et al. 1998; Rodriguez et al. 2006). Previous field studies showed that transgenic canola in the presence and absence of *P. putida* UW4 had significantly higher shoot biomass compared to non-transformed canola in Ni-contaminated soil, however, this study was influenced by the added stress of flooding (Farwell et al. 2006). In the current study, 4-day old seedlings of non-transformed and transgenic canola in the presence and absence of either *P. putida* strain UW4 or *P. putida* strain HS-2 were transplanted at a Ni-contaminated field site and later assessed for plant growth and shoot Ni concentrations.

## Materials and methods

### Bacterial strain

Two rhizobacterial strains were used: *P. putida* UW4, a well characterized plant growth-promoting bacterium (Glick et al. 1995; Shah et al. 1997; Hontzeas et al. 2005) and *P. putida* HS-2, a new

isolate from the Ni-contaminated field site (Port Colborne, ON, Canada) used in this study (Rodriguez et al. 2006). Both strains were isolated based on the ability to utilize ACC as the only source of nitrogen (Glick et al. 1995). *P. putid*a HS-2 was also selected based on its high tolerance to Ni (13 mM in vitro) (Rodriguez et al. 2006). *P. putida* UW4 and *P. putida* HS-2 were characterized based on results using a Biolog Kit (MicroLog System, Release 4.0) and by sequence determination of the 16s rDNA.

Each strain was grown in tryptic soybean broth, centrifuged, rinsed twice with 30 mM $MgSO_4$, resuspended in 30 mM $MgSO_4$ and adjusted to an $OD_{600} = 0.5$. Two ml of the bacterial suspension was applied to the seed hole at the time of planting, where applicable.

## Plants

Non-transformed canola (*B. napus* L., cv. Westar) and a double copy transgenic canola were used in this study (Stearns et al. 2005). Transgenic canola has the gene for ACC deaminase (from *P. putida* UW4, Shah et al. 1998) under the control of the *rolD* promoter from *Agrobacterium rhizogenes* that is expressed specifically in roots (Elmayan and Tepfer 1995).

Seeds were placed in plug trays (Jack Van Klaveren Co., St. Catherines, ON, Canada) with 152 seeds per tray, containing PRO-MIX ''BX'' (general purpose growing medium) (Premier Horticulture Inc., Rivière-du-Loup, QC, Canada), and where applicable, treated with the bacterial suspension previously described. The flats were held in growth chambers (Conviron, Winnipeg, MB, Canada) at 25°C with a 16 h light (photosynthetically active radiation, 200 μmol/$m^2$/s): 8 h dark cycle. On day four, the plug trays were transported to the field site and the seedlings transplanted in the field plot described below. The transplant method was used as a preferred method for bacterial inoculation versus direct inoculation of the seeds in the field.

## Description of the field plot

The field site is located in Port Colborne, ON, Canada (17 T 0644700, UTM 4749197; 17 T 0644674, UTM 4749178) in an area contaminated with Ni and Cu resulting from the historical aerial deposition from a local nickel refinery (Frank et al. 1982; McIlveen and Negusanti 1994). Detailed soil chemistry is reported in Farwell et al. (submitted for publication). In brief, total Ni (3.0 mg/g soil) and Cu (0.39 mg/g soil) levels are elevated as well as major ion levels, specifically plant available $Ca^{2+}$ (6.6 g/l) and $Mg^{2+}$ (0.7 g/l), in this high organic matter content (50–52%), acid-neutral (pH > 6.0) soil.

## Preparation and planting

An area of 625 $m^2$ was fenced to establish boundaries for this field plot. Within the fenced area, 1.5% Round-up® (Monsanto Canada, Winnipeg, MB, Canada) was applied in the early spring of 2005 and let stand for approximately 10 days prior to ploughing and raking. The plot (13 m × 20 m) was divided into 32 subplots (1 m × 4 m) and each subplot was covered with landscape fabric around the perimeter (60 cm wide) to reduce weed growth. Within each subplot there were three rows of 25 plants with 15 cm between seedlings and 20 cm between rows.

To assess the use of transgenic canola and plant growth-promoting bacteria for phytoremediation strategies, two separate crops were planted in the spring (referred to as Crop #1) and summer (referred to as Crop #2) of 2005. Crop #1 consisted of 16 subplots with one row of each non-transformed canola treatment [non-transformed canola, *B. napus* L., cv. Westar (referred to as the control, C); C inoculated with *P. putida* UW4 (C + UW4); C inoculated with *P. putida* HS-2 (C + HS-2)] and 16 subplots with one row of each transgenic canola treatment [transgenic canola (T); T inoculated with *P. putida* UW4 (T + UW4); T inoculated with *P. putida* HS-2 (T + HS-2)]. Crop #1 was transplanted into the field on May 30 and 31, 2005 and had a total of 400 canola seedlings for each of the six treatments (C, C + UW4, C + HS-2, T, T + UW4, T + HS-2).

The same plot layout was used for crop #2; however, in this case there were four treatments; 16 subplots with one row of each non-transformed canola treatment (C and C + HS-2) and 16 subplots with one row of each transgenic

canola treatment (T and T + HS-2). Crop #2, transplanted into the field August 8 and 9, 2005 following raking and weeding, had a total of 400 canola seedlings for C + HS-2, T, and T + HS-2, and only 275 for C.

## Plot maintenance and harvesting methods

For each crop, the field plot was examined weekly to assess plant survival, and to maintain the crop (watering and weeding) as required. The plants were harvested prior to flowering (at the first signs of bud formation) to meet the requirements of the authorization for field testing these transgenic plants (Confined Research Field Trial Application 05-UOW1-272-CAN; Plant Products Directorate, Canadian Food Inspection Agency, Ottawa, ON, Canada). The period the canola plants were exposed to Ni-contaminated soil for crop #1 was 23 days (from May 30 or 31 to June 23 or 24, 2005) and during this time daily temperatures ranged from 9.0 to 31.0°C (average of 20.4°C) and total precipitation was 24.4 mm (Environment Canada 2005a, b). Crop #2, transplanted August 8 or 9, and harvested September 7 or 8, was exposed to the Ni-contaminated soil for 30 days. The daily temperatures ranged from 12.0 to 30.0°C (average of 21.8°C) and total precipitation was 62.7 mm (Environment Canada 2005c, d).

On the day(s) of harvest, canola plants were carefully removed from the soil, measured for shoot length, assessed for insect damage, placed in labelled plastic bags, stored on ice and transported to the University of Waterloo. For the assessment of insect damage, plants were given a score from zero to four, where plants with a score of zero had no damage and plants with scores of one (0–5%), two (>5–15%), three (>15–50%) or four (>50%) had damage affecting a percentage of the total surface area. Insect damage was reported as percent occurrence. At the laboratory, shoots were immediately cleaned with tap water and rinsed with deionized water, dried at 80°C for two days and weighed. Subsamples with minimal insect damage (score of ≤2) were cleaned, dried, ground and stored in 15 ml plastic vials for metal analysis.

## Chemical analyses

Soil samples were collected prior to transplanting the seedlings in each subplot (three per subplot) at a depth of 3–5 cm, and stored in labelled plastic bags at 4°C prior to Ni analyses at the University of Guelph Laboratory Services Division (Guelph, ON, Canada). Soil samples and the reference soil (National Institute of Standards and Technology, NIST 2711 soil) were digested with concentrated nitric acid and hydrochloric acid, and measured on a Varian Vista Pro (radial) inductively coupled plasma-optical emission spectrometer (ICP-OES) (Varian Canada Inc., Mississauga, ON, Canada). A subset of samples were also analyzed following diethylenetriaminepentaacetic acid (DTPA) extraction (modified from Lindsay and Norvell 1978) to estimate the concentration of bioavailable Ni.

For plant Ni analyses, cleaned, dried shoot tissue was finely ground with a mortar and pestle and 30 mg aliquots transferred to each of two 1.5 ml microcentrifuge tubes (Progene, St-Laurent, QC, Canada) per plant. The tissue was digested in 0.75 ml of trace metal grade 1 N $HNO_3$ (Fisher Scientific, Nepean, ON, Canada), incubated at 65°C for 3 h, and then cooled and centrifuged at 20,000×*g* for 10 min. A volume of 100 µl of supernatant was transferred to 900 µl of Milli-Q water in 1.5 ml microcentrifuge tubes and stored at 4°C for a minimum of 7 days prior to analysis. Samples were run on an atomic absorption (AA) spectrophotometer (Varian SpectrAA 880), with a GTA 100 graphite furnace (Varian Canada Inc., Mississauga, ON, Canada) (standard curve was between 1 and 5 µM Ni).

## Statistics

Plant growth measurements and Ni concentrations were expressed as the mean ± standard error (SE) for each treatment or group. Significant differences between treatments or groups for each crop were determined using analysis of variance followed by a Tukey test. All statistical analyses were conducted at $\alpha = 0.05$ using SYSTAT 10 (Statistical Package for the Social Sciences, 2000).

## Results

The majority of the transplanted canola seedlings survived for crop #1 (≥93%). However, for crop #2, survival was reduced for both non-transformed (63–71%) and transgenic (75–79%) treatments due to high temperatures and very dry soil conditions at the time of transplanting. More than half of the mortality occurred within the first 6 days following the transplantation to Ni-contaminated soil. Insect damage, affecting ≥88% of the plants in crop #2, may also have contributed to the increased mortality of crop #2.

There were significant differences in growth between the two crops and between non-transformed and transgenic canola treatments. Crop #1 with a shorter growing/exposure period had average shoot length and biomass measurements of 15–18 cm and 0.6–1.3 g, respectively for all treatments (Fig. 1a, b). Crop #2 had average shoot lengths that were twice as large (32–41 cm) and an average shoot biomass that was four-times as great (3.2–6.4 g) as crop #1. There was significantly greater shoot length ($P \leq 0.014$) and biomass ($P < 0.001$) for all transgenic treatments compared to the corresponding non-transformed treatments (C vs. T; C + UW4 vs. T + UW4; C + HS-2 vs. T + HS-2) for both crops. For crop #1 there were no significant differences in shoot length or biomass for canola in the presence or absence of UW4 or HS-2 with the exception of reduced growth of T + UW4 compared to T ($P < 0.001$). However, for crop #2, which had a longer growing/exposure period, there were significant increases in shoot length ($P \leq 0.02$) and biomass ($P \leq 0.003$) for both non-transformed and transgenic canola inoculated with HS-2 with the exception of shoot biomass for transgenic canola.

Total Ni concentrations in the soil samples were in the range of 1.9–3.3 mg total Ni/g soil [mean ± SE ($n$); 2.3 mg Ni/g soil ± 0.149 (9)] with bioavailable Ni concentrations between 23 and 31% of total Ni. Analyses of Ni content in canola shoots indicated differences in Ni accumulation between non-transformed and transgenic canola treatments planted in Ni-contaminated soil. Transgenic canola had lower shoot Ni concentrations than non-transformed canola and for crop #2, there were trends toward a decrease in biomass with an increase in shoot Ni concentration (Fig. 2a, b). At similar shoot Ni concentrations (13–25.99 μg Ni/g dry weight), there was a significant increase in biomass for transgenic canola compared to non-transformed canola in the presence and absence of *P. putida* HS-2 ($P \leq 0.041$) (Fig. 3).

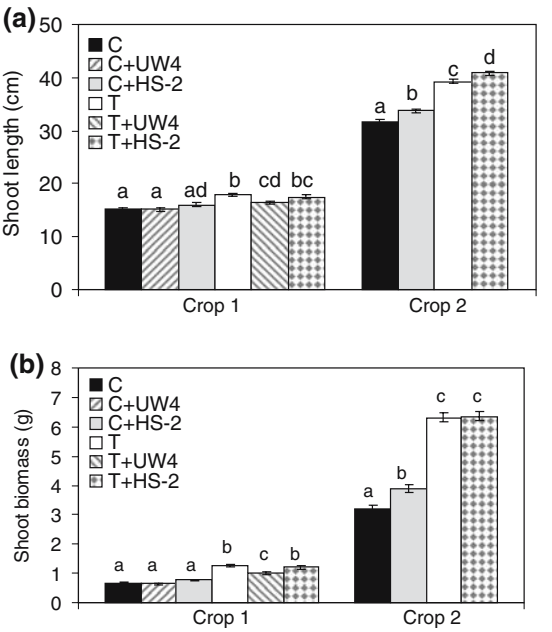


**Fig. 1** Mean ± SE shoot length (cm) (**a**) and shoot biomass (g dry weight) (**b**) for two crops of canola transplanted in 2005. Treatments included non-transformed canola *B. napus* L., cv. Westar (C); transgenic canola (T); C inoculated with *P. putida* UW4 (C + UW4); C inoculated with *P. putida* HS-2 (C + HS-2); T inoculated with *P. putida* UW4 (T + UW4); T inoculated with *P. putida* HS-2 (T + HS-2). Sample numbers ($n$) ranged from 160 plants per treatment for crop #1 to 173–244 plants per treatment for crop #2 for both shoot length and biomass. Different letters represent statistical differences between treatments for each crop ($P < 0.05$)

Overall, non-transformed canola treatments had 34–54% more Ni per g shoot tissue than the corresponding transgenic canola treatments (Table 1). The addition of plant growth-promoting bacteria, strain UW4 or HS-2, had no significant effect on shoot Ni concentration ($P > 0.05$). Shoot biomass and the corresponding shoot Ni concentrations were used to calculate total Ni per plant (Table 1). The addition of *P. putida* HS-2 resulted in a 10–12% increase in Ni per plant for

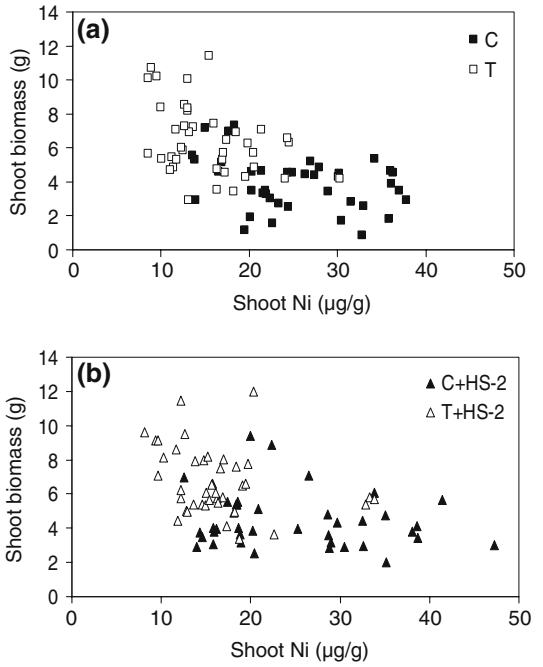


**Fig. 2** Shoot biomass (g dry weight) versus shoot Ni concentration (µg Ni/g dry weight) for non-transformed canola (*B. napus* L., cv. Westar) (C) and transgenic canola (T) (**a**) and canola treated with *P. putida* HS-2 (C + HS-2; T + HS-2) (**b**) for crop #2

non-transformed and transgenic canola (although not statistically significant) but only for the crop with the longer growing season (crop #2). Although there was higher biomass for the transgenic canola, the lower Ni concentrations

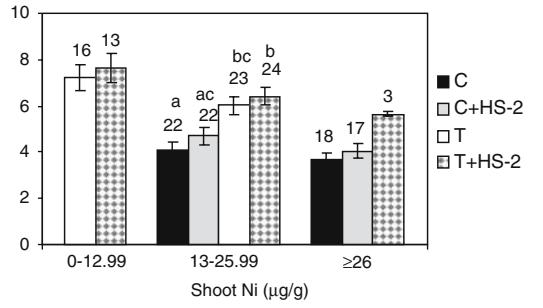


**Fig. 3** Mean ± SE shoot biomass (g dry weight) for ranges of shoot Ni concentrations (µg Ni/g dry weight) for non-transformed canola (*B. napus* L., cv. Westar) (C), transgenic canola (T) and canola treated with *P. putida* HS-2 (C + HS-2; T + HS-2) for crop #2. The number above the bar represents the sample number for that group. Different letters represent statistical differences between treatments for each shoot Ni concentration range ($P < 0.05$)

resulted in total Ni per plant that was not significantly different between the non-transformed and transgenic canola (Table 1). However, when total Ni per plant was calculated for plants with similar shoot Ni concentrations (13–25.99 µg Ni/g dry weight), there was a 20–26% increase in Ni per plant for transgenic canola compared to non-transformed canola for crop #2 (Table 2). Total Ni per plant was statistically greater for transgenic canola relative to non-transformed canola for crop #2, but only for plants in the absence of *P. putida* HS-2.

## Discussion

The increase in growth of transgenic compared to non-transformed canola is likely a function of both increased ACC deaminase activity and lower Ni concentrations in the shoots. The significantly higher level of Ni per g tissue in non-transformed canola may be attributed to differences in root growth between plants as a function of the method of planting (i.e., use of transplants). Preliminary studies at this site found little difference between the shoot Ni concentration in non-transformed canola [mean ± SE (*n*); 29.25 µg Ni/g ± 1.72 (96)] and transgenic canola [mean ± SE (*n*); 27.60 µg Ni/g ± 1.67 (96)] when the seeds were planted directly in Ni-contaminated soil. This trend is consistent with later studies, using canola seeds, that examined the impact of flooding stress at the same Ni-contaminated site (Farwell et al. 2006). Similarly, no significant difference was found in shoot Ni concentrations between non-transformed and *rolD* transgenic canola grown in a growth chamber from seed in Ni-spiked soil (Stearns et al. 2005) or Ni-contaminated soil from the study site (Rodriquez et al. 2006). Since transgenic plants, with the root specific *rolD* promoter, have greater root length (>3 cm) than non-transformed canola when planted in uncontaminated soil (Stearns et al. 2005), the roots of the transplanted transgenic canola plants may have grown into deeper, less contaminated soil resulting in lower Ni uptake. The Ni concentration in subsurface soil samples (2.9 Ni mg/g soil) was lower compared to surface soil samples (6.3 Ni mg/g soil). Thus, root elongation of transgenic canola, which was first

**Table 1** Mean ± SE (*n*) for canola shoot biomass (g dry weight), shoot Ni concentration (μg Ni/g dry weight) and total Ni per plant (μg Ni) for two separate crops of canola treatments (non-transformed canola *B. napus* L., cv. Westar (C); transgenic canola (T); C inoculated with *P. putida* UW4 (C + UW4); C inoculated with *P. putida* HS-2 (C + HS-2); T inoculated with *P. putida* UW4 (T + UW4); T inoculated with *P. putida* HS-2 (T + HS-2) grown in Ni-contaminated soil

| Crop# | Treatment | Shoot biomass (g dry weight) | Shoot Ni (μg Ni/g dry weight) | Total Ni per plant (μg Ni) |
|---|---|---|---|---|
| 1 | C | 0.79 ± 0.08 (32)$^{a}$ | 29.06 ± 2.86 (32)$^{a}$ | 19.25 ± 1.89 (32)$^{a}$ |
| | C + UW4 | 0.75 ± 0.08 (32)$^{a}$ | 31.03 ± 3.81 (32)$^{a}$ | 19.61 ± 2.47 (32)$^{a}$ |
| | C + HS-2 | 0.80 ± 0.07 (32)$^{a}$ | 27.81 ± 3.18 (32)$^{a}$ | 18.70 ± 1.93 (32)$^{a}$ |
| | T | 1.21 ± 0.67 (32)$^{b}$ | 15.15 ± 1.28 (32)$^{b}$ | 19.22 ± 2.65 (32)$^{a}$ |
| | T + UW4 | 1.09 ± 0.08 (32)$^{a}$ | 14.28 ± 1.12 (32)$^{b}$ | 15.52 ± 1.80 (32)$^{a}$ |
| | T + HS-2 | 1.34 ± 0.14 (32)$^{b}$ | 14.95 ± 1.55 (32)$^{b}$ | 18.44 ± 2.43 (32)$^{a}$ |
| 2 | C | 3.89 ± 0.25 (40)$^{a}$ | 25.32 ± 1.14 (40)$^{a}$ | 95.16 ± 6.25 (40)$^{b}$ |
| | C + HS-2 | 4.48 ± 0.26 (40)$^{a}$ | 24.65 ± 1.43 (40)$^{a}$ | 107.61 ± 7.64 (40)$^{b}$ |
| | T | 6.46 ± 0.33 (40)$^{b}$ | 15.40 ± 0.80 (40)$^{b}$ | 95.45 ± 5.13 (40)$^{b}$ |
| | T + HS-2 | 6.75 ± 0.31 (40)$^{b}$ | 16.28 ± 0.94 (40)$^{b}$ | 106.43 ± 6.51 (40)$^{b}$ |

Different letters represent statistical differences between treatments for each crop ($P < 0.05$)

**Table 2** Mean ± SE (*n*) for canola shoot biomass (g dry weight), shoot Ni concentration (μg Ni/g dry weight) and total Ni per plant (μg Ni) for plants with a similar range of shoot Ni concentrations (13 to 25.99 μg Ni/g dry weight)

| Crop # | Treatment | Shoot biomass (g dry weight) | Shoot Ni (μg Ni/g dry weight) | Total Ni per plant (μg Ni) |
|---|---|---|---|---|
| 1 | C | 0.97 ± 0.15 (11)$^{a}$ | 18.89 ± 1.08 (11)$^{a}$ | 18.06 ± 2.86 (11)$^{a}$ |
| | C + UW4 | 0.86 ± 0.09 (16)$^{a}$ | 18.52 ± 0.86 (16)$^{a}$ | 15.99 ± 2.04 (16)$^{a}$ |
| | C + HS-2 | 0.97 ± 0.11 (14)$^{a}$ | 19.17 ± 0.96 (14)$^{a}$ | 18.22 ± 1.96 (14)$^{a}$ |
| | T | 1.28 ± 0.20 (12)$^{a}$ | 15.97 ± 0.53 (12)$^{a}$ | 20.74 ± 3.33 (12)$^{a}$ |
| | T + UW4 | 1.01 ± 0.12 (14)$^{a}$ | 17.90 ± 1.08 (14)$^{a}$ | 18.10 ± 2.55 (14)$^{a}$ |
| | T + HS-2 | 1.38 ± 0.30 (12)$^{a}$ | 17.17 ± 0.94 (12)$^{a}$ | 24.14 ± 5.59 (12)$^{a}$ |
| 2 | C | 4.08 ± 0.37 (22)$^{a}$ | 19.75 ± 0.76 (22)$^{a}$ | 77.92 ± 6.22 (22)$^{a}$ |
| | C + HS-2 | 4.69 ± 0.38 (22)$^{ac}$ | 18.12 ± 0.59 (22)$^{ab}$ | 86.45 ± 8.65 (22)$^{ab}$ |
| | T | 6.02 ± 0.40 (23)$^{bc}$ | 17.83 ± 0.75 (23)$^{ab}$ | 105.95 ± 7.03 (23)$^{b}$ |
| | T + HS-2 | 6.41 ± 0.37 (24)$^{b}$ | 16.91 ± 0.46 (24)$^{b}$ | 108.37 ± 7.65 (24)$^{b}$ |

Canola treatments include non-transformed canola *B. napus* L., cv. Westar (C); transgenic canola (T); C inoculated with *P. putida* UW4 (C + UW4); C inoculated with *P. putida* HS-2 (C + HS-2); T inoculated with *P. putida* UW4 (T + UW4); T inoculated with *P. putida* HS-2 (T + HS-2). Different letters represent statistical differences between treatments for each crop ($P < 0.05$)

grown in uncontaminated soil and then transplanted in Ni-contaminated soil may account for the reduced Ni concentrations, particularly at this site where Ni contamination is due to aerial deposition.

Due to the differences in shoot Ni concentration, it is difficult to assess the full potential of transgenic canola as a phytoremediation method in this study since Ni concentration alone could account for the lower growth in non-transformed versus transgenic canola. On the other hand, increased biomass has been reported for ACC deaminase-containing transgenic tomato plants exposed to single metals such as Cd, Co, Cu, Pb, Zn or Ni (Grichko et al. 2000). In laboratory studies, growth of *rolD* transgenic canola was consistently higher than non-transformed canola both in uncontaminated and Ni-spiked soil, independent of shoot Ni concentrations which were similar among the two groups (Stearns et al. 2005). In the present study, at concentrations in the range of 13–25.99 μg Ni/g shoot, there were significant increases in biomass for transgenic canola compared to non-transformed canola for crop #2 indicating that ACC deaminase-containing transgenic canola have enhanced growth, independent of Ni concentration. Unfortunately, at lower and higher shoot Ni concentrations, there are few comparisons between non-transformed and transgenic canola. In the preliminary field study, seeds planted directly in the Ni-contaminated soil resulted in higher Ni concentrations and in this

case, trends of increased biomass of transgenic canola (although not significant) were evident at shoot Ni concentrations >40 μg Ni/g shoot dry weight. Based on the available data in the current study, ACC deaminase-containing transgenic canola contributed to enhanced plant growth, which becomes more evident the longer the plants were exposed to Ni-contaminated soil.

The potential for accumulation of Ni in canola plants is likely underestimated at this field site due to the soil conditions, specifically pH altered by $CaCO_3$ and $MgCO_3$ additions and high organic matter content. Kukier and Chaney (2004) had shoot Ni concentrations as high as ~130 μg Ni/g shoot (barley) to ~617 μg Ni/g shoot (oat) for 42 day pot experiments using similar (2.9 mg Ni/g soil) but strongly acidic (<5.24 pH), lower organic matter content (17%) Ni-contaminated soil. The addition of $CaCO_3$ and $MgCO_3$ to a pH of approximately 6, similar to field conditions, reduced plant available Ni and Ni concentrations in plant shoots (150 μg Ni/g shoot of oat) (Kukier and Chaney 2004). Other studies have also shown that additions as low as 2.5 g $CaCO_3$/kg soil decreased the solubility of Ni in soil as well as plant uptake of Ni (Robinson et al. 1999). Similar trends of reduced plant available Ni were also observed following additions of $CaCO_3$ and $MgCO_3$ in high organic matter content soil (72%) (Kukier and Chaney 2001). At the field site used in the present study, the soil has high organic matter content (50–52% dry weight), which influences the adsorptive properties of the soil. The addition of organic matter has been found to decrease Ni solubility (Kashem and Singh 2001a) and reduce Ni uptake in rice plants (Kashem and Singh 2001b). Differences in the soil conditions and soil preparation are significant factors that will affect Ni shoot concentrations and Ni phytotoxicity. Therefore, other type of soils with more plant available Ni should be assessed to further evaluate Ni shoot concentrations and plant responses related to the use of ACC deaminase-containing transgenic canola and plant growth-promoting bacteria.

Although Ni was the primary contaminant of interest in this study, there were also elevated levels of copper (Cu) in the soil, which can influence Ni uptake as well phytotoxic responses. In other studies, Cu has been found to compete with Ni for uptake, causing reduced accumulation of Ni in *Sinapis alba* plant roots (Fargašová and Beinrohr 1998). In the preliminary field study, average Ni and Cu concentrations of 27.23 and 8.01 μg/g shoot, respectively were found for transgenic canola planted in contaminated soil with total Ni and Cu concentrations of 2.6–6.5 and 0.34–0.74 mg/g soil, respectively. The ratio of Cu:Ni in shoot tissue is twofold higher than the ratio of Cu:Ni in the soil indicating that proportionally more Cu is being accumulated relative to Ni. Competition in metal-contaminated soil therefore may also have contributed to the lower shoot Ni concentrations in this study compared to shoot Ni concentrations in Ni-spiked soil (Stearns et al. 2005).

The addition of *P. putida* HS-2 significantly increased plant growth for non-transformed canola in Ni-contaminated soil. That trend was only evident for the longer exposure period (30 day), emphasizing the importance of the exposure duration on Ni-related plant stress responses. Although *P. putida* UW4 was only used with crop #1, similar increases in plant growth might have been expected for crop #2. In previous laboratory studies of tomato plants inoculated with *P. putida* UW4, plants were found to have lower ethylene levels and increased biomass following flooding stress (Grichko and Glick 2001). Similar trends of increased biomass were found for canola plants treated with *P. putida* UW4 when exposed to flooding conditions at the same Ni-contaminated site as the current study (Farwell et al. 2006). Various bacterial strains containing the enzyme ACC deaminase have been found to increase plant growth following exposure to Cd (Belimov et al. 2005), Cu (Reed and Glick 2005; Reed et al. 2005) and Ni (Burd et al. 1998) in soil under laboratory conditions where plant available Ni is likely higher than Ni in aged soils. The results of this study indicate that there was sufficient plant available Ni at this site to affect plant growth in non-transformed canola and that treatment with *P. putida* HS-2 increased plant tolerance to Ni exposure. Similar enhanced tolerance of non-transformed and transgenic canola to Ni-contaminated soil (from this study site) was found with the addition of

*P. putida* HS-2 in pot experiments (Rodriquez et al. 2006). The fact that *P. putida* HS-2 had no effect on plant growth for transgenic canola compared to non-transformed canola in the field is likely due to the lower Ni concentration and therefore lower Ni stress for the transgenic canola.

In conclusion, the use of ACC deaminase-containing transgenic canola increased plant growth in the field however the benefits in terms of total Ni per plant were likely underestimated due to soil conditions influencing plant available Ni and the use of transplants reducing the exposure duration of canola plants to Ni-contaminated soil and Ni accumulation in the transgenic canola. Elevated $Ca^{2+}$ levels and organic matter content as well as competition with other metals may also have contributed to the low Ni accumulation in canola shoots. The use of plant growth-promoting bacteria enhanced plant growth resulting in an approximate 10% increase in total Ni per plant. Again, the benefits of using plant growth-promoting bacteria are likely underestimated due to low Ni content in canola plants attributed to soil conditions and planting methods. Also due to the restricted growth period, as a function of the field trial authorization, the benefits in terms of enhanced biomass production may not be fully understood. Further research at field sites with higher plant available Ni would be required to assess the full potential of transgenic plants and/or plant growth-promoting bacteria in Ni-contaminated phytoremediation options. In addition, research on ACC deaminase-containing transgenic plants and/or plant growth-promoting bacteria using plants that are capable of higher Ni accumulation than canola plants would be beneficial.

**Acknowledgments** This project was funded by a Strategic Grant from the Natural Sciences and Engineering Research Council of Canada to B.R. Glick. We would like to thank Eric Azzopardi and Maria Bellantino-Perco for site co-ordination, Brian Emburgh and Co. and all the individuals from the University of Waterloo for field support. Special thanks to Rick Playle (Wilfrid Laurier University) for the use of the AA for nickel analysis. The use of transgenic canola (Confined Research Field Trial Application 05-UOW1-272-CAN), *P. putida* UW4 (Research Authorization #16578RN) and *P. putida* HS-2 (Research Authorization #16661RS) were authorized by the Plant Products Directorate, Canadian Food Inspection Agency, Ottawa, ON, Canada.

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