

## TRANSPIRATION INFLUENCES CADMIUM UPTAKE BY BABY LEAF SPINACH (*SPINACIA OLERACEA* L.)

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### ABSTRACT

Cadmium (Cd) is a toxic heavy metal whose concentration in soils is rising. This study investigated the effect of transpiration on Cd uptake by plants using soil conditions akin to those experienced by field crops. Two experiments were performed using baby leaf spinach (*Spinacia oleracea* L.) grown in soil containing Chloride (Cl) at a typical concentration of 70 mg kg<sup>-1</sup>. Experiment 1 explored plant response to Cd over the range ~0.04 to 10 mg kg<sup>-1</sup> so that a non-toxic concentration could be selected for Experiment 2. Experiment 2 tested the hypothesis that transpiration and Cd uptake were related using soil amended to 70 mg Cl kg<sup>-1</sup> and 0.24 mg Cd kg. Differences in transpiration were achieved by manipulating atmospheric CO<sub>2</sub> (~400 and ~640 μL CO<sub>2</sub> L<sup>-1</sup>) and air temperature (22/14, 26/18 and 30/22°C day/night). In Experiment 1, Cd in the foliage varied from ~2 to ~100 mg kg<sup>-1</sup> DW, yet there was no evidence of Cd toxicity. In Experiment 2, temperature and [CO<sub>2</sub>] caused large differences in plant growth. [Cd] was lower in chambers with increased temperature and higher in those with elevated CO<sub>2</sub>. Despite differences in growth, transpiration and foliar Cd uptake were positively and strongly correlated, even when both parameters were expressed on a DW basis. The data are consistent with Cd transport by mass flow towards the roots being a substantial contributor to Cd uptake. Higher [Cd] at elevated CO<sub>2</sub> raises concerns about future chronic dietary exposure to Cd. The findings challenge the interpretation of earlier studies on the effects of CO<sub>2</sub> and temperature on Cd uptake and may partly explain the inter-seasonal variation in Cd uptake by field crops.

**Keywords:** Transpiration, Chloride, Elevated CO<sub>2</sub>, Temperature, Canopy Area

### 1. INTRODUCTION

Plants contribute a large proportion of our dietary Cadmium (Cd), which is perhaps the heavy metal of most concern for human health (Clemens *et al.*, 2013). Discharges of Cd into the environment have increased due to increases in industrial activity and to Cd-contamination of phosphorus fertilizers (Williams and David, 1976). Phosphorus fertilizers have been used in

many countries to increase the productivity of horticulture and agriculture (Chen *et al.*, 2011) and are a major source of Cd in farmed soils worldwide; Australian soils are no exception (Andrews *et al.*, 1996; Jinadasa *et al.*, 1997). As a result, chronic Cd toxicity in humans, through continued low-level dietary exposure, is a global health concern and the selection of crop plants with low Cd uptake has become a focus of activity (Clemens *et al.*, 2013). However, gaps remain

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in our understanding of the effects of edaphic and environmental factors on Cd uptake by plants.

Transpiration causes water and solutes to flow towards the roots of plants (Salt *et al.*, 1995); consequently, influences on transpiration have the potential to affect Cd uptake. Were such influences to also affect plant Dry Weight (DW), the association between transpiration and Cd uptake might be stronger than that between Cd concentration and transpiration/unit mass; however, evidence for this appears conflicted. For example, the amount of Cd in the tops of bush beans (*Phaseolis vulgaris* L.) grown in a liquid culture was proportional to the amount of water transpired (Hardiman and Jacoby, 1984), as was the uptake of Cd from soil by radish (*Raphanussativus* L.) (Lorenz *et al.*, 1994; Kashem and Singh, 2002). In contrast, Florijn and van Beusichem (1993) found no relationship between shoot Cd and transpiration in pot experiments with high and low Cd-accumulating lines of maize (*Zea mays* L.) and similar results were obtained for radish and soybean (Lagerwerff and Biersdorf, 1972; Cunningham *et al.*, 1977). Likewise Greger *et al.* (2003) (unpublished results, cited by (Ekvall and Greger, 2003) found that Cd translocation to the shoot of willow (*Salix viminalis* L.) was not affected by transpiration rate. However, the results of some of these studies are possibly complicated by the effects of Cd toxicity on water relations (Poschenrieder *et al.*, 1989; Perfus-Barbeoch *et al.*, 2002); moreover, the role of transpiration rate in Cd transport in the xylem is contested (Salt *et al.*, 1995; Hart *et al.*, 1998).

The atmospheric Carbon Dioxide (CO<sub>2</sub>) concentration is expected to exceed 600 µL L<sup>-1</sup> during the 21st century, leading to a global mean surface temperature warming of 1.9-4.4°C (Solomon *et al.*, 2007) and CO<sub>2</sub> concentration and temperature are among the factors that drive transpiration. Elevating the atmospheric concentration of CO<sub>2</sub> increases the rate of photosynthesis of C<sub>3</sub> plants by enhancing CO<sub>2</sub> fixation and suppressing photorespiration (Drake *et al.*, 1997). Under most conditions, elevated CO<sub>2</sub> also reduces stomatal conductance and, hence, leaf transpiration rates (Ainsworth and Rogers, 2007; Drake, 2014). In addition, elevated CO<sub>2</sub> can cause morphological changes that impact on transpiration, e.g., specific leaf weight and stomatal index (Poorter and Navas, 2003). Temperature directly affects leaf transpiration because stomatal conductance exhibits a thermal optimum and indirectly because the leaf-to-air vapour pressure deficit increases exponentially with increasing temperature, causing changes in leaf transpiration that often overwhelm any direct effects on stomata (Farquhar and Sharkey, 1982). Photosynthesis and plant growth have temperature optima and increases in temperature beyond the thermal optimum have negative

effects on both leaf and plant productivity (Berry and Björkman, 1980; Kubien and Sage, 2008). Consequently, varying the atmospheric concentration of CO<sub>2</sub> and the temperature provide a convenient means of manipulating transpiration. However, elevated CO<sub>2</sub> *per se* may influence the mobility of Cd in the rhizosphere (Li *et al.*, 2014).

The potentially variable effects of atmospheric CO<sub>2</sub> concentrations on transpiration noted above may be the source of much of the difference between studies in the effect of CO<sub>2</sub> on Cd uptake. For example, elevated CO<sub>2</sub> decreased the concentration of Cd in the roots and shoots of *Lolium* spp. and ameliorated Cd toxicity but did not affect transpiration (Jia *et al.*, 2010). However, CO<sub>2</sub> effects on transpiration are not generally reported and differences in transpiration may lie behind the reported differences in Cd uptake between cultivars within studies (Li *et al.*, 2014) and between studies (Guo *et al.*, 2011). An alternative explanation for such contrasting results may be that elevated CO<sub>2</sub> influences either root uptake of Cd (Li *et al.*, 2014) or its internal (re) distribution. In contrast, temperature appears to affect plant Cd more consistently. For example, increasing temperature increased Cd uptake by the excised roots of barley (*Hordeum vulgare* L.) (Cutler and Rains, 1974). Similarly, increased temperature increased Cd uptake by soybeans (*Glycine max* L.) (Haghiri, 1974) and ryegrass (*Lolium multiflorum* Lam.) (Almas and Singh, 2001) grown in soil in the greenhouse; however, in these studies the effect attributed to temperature would have included any concomitant effect on transpiration.

The greater the transpiration rate, the greater the potential delivery of Cd to the roots by mass flow. Chloride (Cl) is predicted to facilitate this process by increasing the concentration and mobility of Cd in the soil solution due to the formation of chlorocomplexes (Boekhold *et al.*, 1993; Kamewada and Nakayama, 2011). Moreover, Cl occurs ubiquitously in soils (Ure and Berrow, 1982) and the predicted enhancement of Cd uptake is well established (Bingham *et al.*, 1983; McLaughlin *et al.*, 1997; Wu *et al.*, 2002). The chloro complexes dissociate rapidly and complexes with this characteristic may also increase Cd uptake by decreasing the effective thickness of the diffusion boundary at the root (Degryse *et al.*, 2012). Lastly, variation of Cd uptake with transpiration could underlie the seasonal variation observed in Cd uptake/concentration by crops (Andersson and Binge, 1985); although transpiration may play a secondary role in the accumulation of Cd in grain (Hart *et al.*, 1998).

This study tested the transpiration/uptake hypothesis in the greenhouse using soil conditions that mimic those

onlarge tracts of agricultural land, i.e., a non-phytotoxic concentration of Cd (Clemens *et al.*, 2013) and of Cl (Ure and Berrow, 1982; Jinadasa *et al.*, 1997). The plant model was the C<sub>3</sub> species, baby leaf spinach (*Spinacia Oleracea* L.) and the atmospheric CO<sub>2</sub> concentration and the temperature were varied to manipulate transpiration.

## 2. MATERIALS AND METHODS

### 2.1. Plant Culture and Growth Conditions

A sandy-loam soil was collected from Menangle, NSW, Australia, air-dried and sieved (5 mm). The soil was of low fertility: pH in water (6.0), organic matter (0.1%), total-N (0.06%), Olsen-P (2 mg kg<sup>-1</sup>) and exchangeable cations (5.6 cmol<sub>c</sub> kg<sup>-1</sup>), Cl (15 mg kg<sup>-1</sup>). This soil was used in two experiments and, for both, 5,000±5 g was weighed into a series of plastic bags. The required volume of a solution of calcium chloride dihydrate (15 g L<sup>-1</sup>) was added to increase the background Cl concentration to ~70 mg kg<sup>-1</sup> of air-dry soil. This concentration is close to, but below, the 'world median' of ~100 mg kg<sup>-1</sup> (Ure and Berrow, 1982) and likewise is below the median of 90 mg kg<sup>-1</sup> for soils from 29 farms in Greater Sydney, Australia (Jinadasa *et al.*, 1997). The natural Cd concentration was low (~0.04 mg kg<sup>-1</sup>) and was augmented by adding aliquots of a solution of Cd chloride (100 mg Cd L<sup>-1</sup>) to the soil. The contents of the bag were mixed and the bag was placed in a pot. Seeds of two cultivars of baby leaf spinach, Ragoon and Donkey (Rijk Zwaan Australia), were sown into separate pots (15seeds per pot). Baby leaf spinach was used as the plant model as it is a known Cd accumulator (Liang *et al.*, 2013), is a small plant with rapid vegetative growth and the leaves and petioles are eaten in salads (Rogers *et al.*, 2008). The numbers of pots and amounts of Cd added are described under Experiments 1 and 2 in the following paragraphs. Both experiments were conducted in greenhouses located at Richmond, NSW, Australia (150° 45' E, 33° 36' S).

Experiment 1 defined the range of soil Cd concentrations across which baby leaf spinach cvv. Ragoon and Donkey did not exhibit symptoms of Cd toxicity. The toxicity indices used were the fresh and dry weight of the foliage (Kastori *et al.*, 1992; Perfus-Barbeoch *et al.*, 2002). Forty four pots were randomly allocated among the two cultivars and 11 different Cd concentrations (background ~0.04 mg kg<sup>-1</sup> soil and background plus 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4 and 10 mg Cd kg<sup>-1</sup> soil), i.e., there were two pots per treatment. The day/night temperature regime was 26/18°C and the

light was natural, with an average photon flux density at midday of ~1500 μM m<sup>-2</sup> s<sup>-1</sup> and a photoperiod of~13 h. The relative humidity was ~50% and the CO<sub>2</sub> partial pressure was ambient (~400 μL L<sup>-1</sup>).

Experiment 2 tested the transpiration/uptake hypothesis using the same concentration of Cl as in Experiment 1 (~70 mg kg<sup>-1</sup> soil) and a Cd concentration of 0.24 mg kg<sup>-1</sup> soil. This soil Cd concentration was non-toxic in Experiment 1. Nonetheless, similar concentrations of Cd in soil can cause concentrations of Cd in some leafy vegetables that are deemed unacceptable for human consumption (Jinadasa *et al.*, 1997; FSANZ, 2005; McLaughlin *et al.*, 2006). Sixty pots, 30 of each cultivar of baby leaf spinach, were randomly allocated among six combinations of three temperature and two atmospheric CO<sub>2</sub> treatments and each of the six combinations was allocated to a separate greenhouse chamber. That is, there were five pots per treatment. The three temperature regimes were 22/14°C, 26/18 and 30/22°C (day/night)-the highest being above the temperature optimum for growth (Rogers *et al.*, 2008; Koike *et al.*, 2011)-and the two CO<sub>2</sub> concentrations, *ambient* and *elevated*, were 400 and 640 μL L<sup>-1</sup>, respectively. The CO<sub>2</sub> was supplied from high pressure cylinders (Food grade, Air Liquide, Australia). The gas was scrubbed to eliminate any ethylene contamination (Chemisorbant Media Product 5050 CHMBX, Purafil Australia) before being injected into the chambers through solenoid valves controlled by CO<sub>2</sub> monitors (Lambda T, ADC Bio Scientific Ltd., Hoddeston, UK). The CO<sub>2</sub> concentrations were independently monitored (Model DL2e, Delta-T Devices Ltd, Cambridge, UK). All the monitors were calibrated using pure N<sub>2</sub> and two certified CO<sub>2</sub> calibration gases (406±12 and 714±16 μL L<sup>-1</sup>; AirLiquide, Australia).

### 2.2. Management and Observations

In both experiments, pots were randomly allocated positions on the greenhouse bench and were re-randomised weekly. To minimise radiant heating of the soil, the sides of the pots were shielded from direct sunlight and the bench tops were wire mesh. Two weeks after sowing, the number of seedlings was reduced to five per pot. Water was applied on demand to 60% field capacity and a dilute nutrient solution was added weekly. (Nutrient sufficiency was confirmed by tissue analysis as described below.) Six weeks after sowing, the aerial biomass was harvested from each pot and the leaf blades were separated from the stems and petioles. These components were weighed at harvest (FW) and reweighed after Drying at 60°C overnight (DW). Within each pot, dry leaf blades were composited, ground and

analysed for a complete suite of nutrient elements and for Cl and Cd. Additional observations during the second experiment included: Conductance of the three, youngest, fully-expanded leaves per pot measured under growth conditions using a steady state diffusion porometer (Model SC-1, Decagon Devices, Washington State, USA); and leaf canopy area, measured using a portable leaf area meter (Model LI-3100A, LI-COR, Lincoln, NE, USA). Canopy transpiration rate was calculated from these values. Lastly, the roots were washed out and their FW and DW recorded. The conductance data for cv. Raccoon at ambient CO<sub>2</sub> and the highest temperature regime (30/22°C day/night) were not repeatable and were consequently rejected.

Dried foliage from every pot in both experiments was ground and a subsample digested in nitric acid and hydrogen peroxide for mineral analysis using Inductively Coupled Plasma (ICP) optical emission spectroscopy (Wheal *et al.*, 2011). The elements determined were: B, Ca, Cd, Cu, Fe, K, Mg, Mn, Na, P, S, Ti and Zn. Separate subsamples were analysed using a dedicated analysers: For N by combustion and gas analysis (Leco, Michigan, USA) and for Cl by titration with coulometrically generated silver ions (Sherwood Scientific, Cambridge, UK). Soil digests in HNO<sub>3</sub> and HCl were analysed for Cd using ICP mass spectrometry. Data quality was assured by parallel analysis of reference samples and the use of titanium as an indicator of soil contamination of plants (Robinson *et al.*, 1984; Cook *et al.*, 2009); on these criteria, the Cd data for 8 of the 104 plant samples were excluded from further consideration.

### 2.3. Statistics

Cd uptake from the first experiment was analysed using ANOVA in Statistica (Version 9, Stat Soft Inc.), with effects of Cd concentration (12 levels) and cultivar (and their interaction). A square root function was used to describe the relationship between Cd uptake and Cd concentration.

For experiment 2, since there is no replication of environmental conditions (CO<sub>2</sub> x T), mean (+/-SE) dry weights, canopy area and Cd concentrations are presented for each of the six chambers and no formal statistical testing of the effects of CO<sub>2</sub> or temperature was performed. The relationship between mean Cd uptake and mean transpiration rate per chamber and cultivar was examined, both on a total plant basis and on a mass basis. Each model comprised effects of (linear) transpiration rate, cultivar and its interaction.

## 3. RESULTS

### 3.1. Plant Management

In both experiments, the foliar nutrient concentrations were: 35-50 mg kg<sup>-1</sup> B, 0.65-0.8% Ca, 12-22 mg kg<sup>-1</sup> Cu, 250-600 mg kg<sup>-1</sup> Fe, 5.4-7% K, 1.7-2.2% Mg, 320-410 mg kg<sup>-1</sup> Mn, 4.7-5.1 N, 0.6-0.8 Na, 0.35-0.45 P, 0.25-0.33% S and 30-50 mg kg<sup>-1</sup> Zn.

### 3.2. Experiment 1

For both cultivars, increasing the concentration of Cd from about 0.04 to 10 mg kg<sup>-1</sup> of soil increased the foliar Cd concentration from ~1 to ~100 mg kg<sup>-1</sup> DW (**Fig. 1**). Even at the maximum concentration of Cd, no foliar symptoms of toxicity were expressed. The mean FW per plant of both Raccoon and Donkey was similar ( $F_{(1,23)} = 0.0003$ ;  $P = 0.99$ ), as was the mean DW ( $F_{(1,23)} = 0.078$ ;  $P = 0.78$ ). The large range of soil Cd concentrations (0.04-10 mg kg<sup>-1</sup>) did not affect either FW or DW ( $F_{(11,23)} = 0.89$  and  $0.74$ ;  $P = 0.56$  and  $0.68$ ); consequently, the value of FW/DW remained constant at ~12:1 throughout.

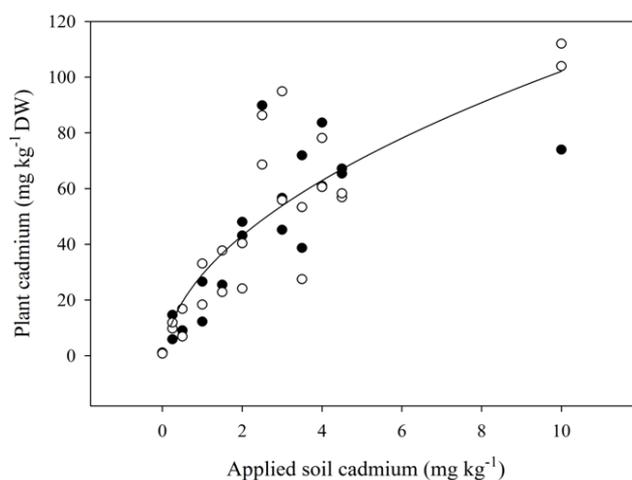
### 3.3. Experiment 2

For the DW components, there was little difference between the cultivars and the highest temperature regime depressed plant DW (**Fig. 2**). The adverse temperature effect was expected, because the highest temperature exceeded the optimum for baby leaf spinach (Rogers *et al.*, 2008; Koike *et al.*, 2011). Elevated CO<sub>2</sub> increased DW at all temperatures; however, the increase was minimal at the highest temperature (**Fig. 2**). Interestingly, the concentration of Cd increased at elevated CO<sub>2</sub> and decreased at the highest temperature and the canopy transpiration rate varied widely from 0.47-6.66 m mol s<sup>-1</sup> (**Fig. 3**). The two highest transpiration rates occurred at elevated CO<sub>2</sub> in the two lower temperature regimes (**Fig. 3**).

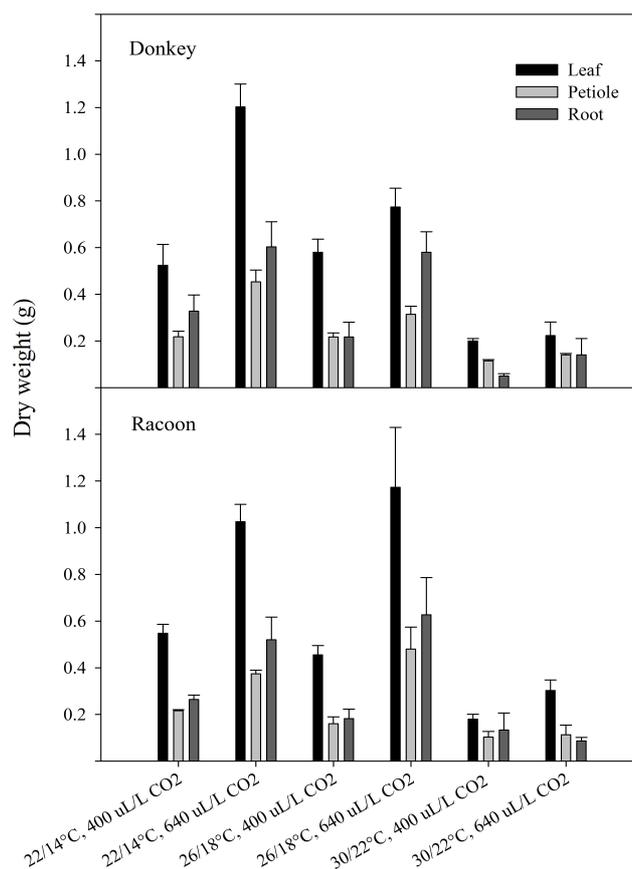
The Cd uptake by the canopy and canopy transpiration rate for the two cultivars (**Fig. 4**) can be described by a linear relationship, since there were no cultivar ( $P = 0.36$ ) or cultivar by transpiration rate ( $P = 0.61$ ) effects. The relationship ( $r^2 = 0.77$ ,  $P < 0.001$ ) is Equation 1:

$$Cd\ uptake = 1.76 (1.13) + 1.78(0.33) \times \{Transpiration\ rate\} \quad (1)$$

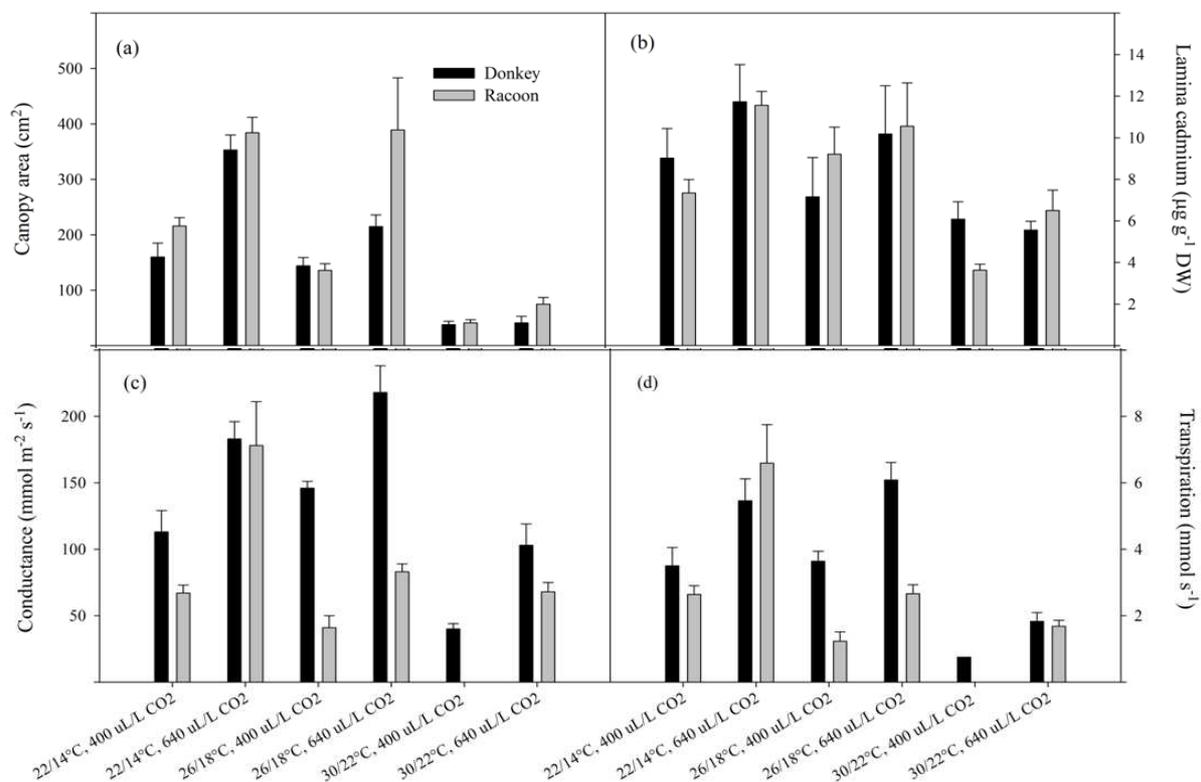
where, the units of Cd uptake are mg and of canopy transpiration rate are mmol s<sup>-1</sup> and the values in parentheses are standard errors. That is, there is a strong, positive relation between transpiration and Cd uptake that strongly supports the transpiration/uptake hypothesis.



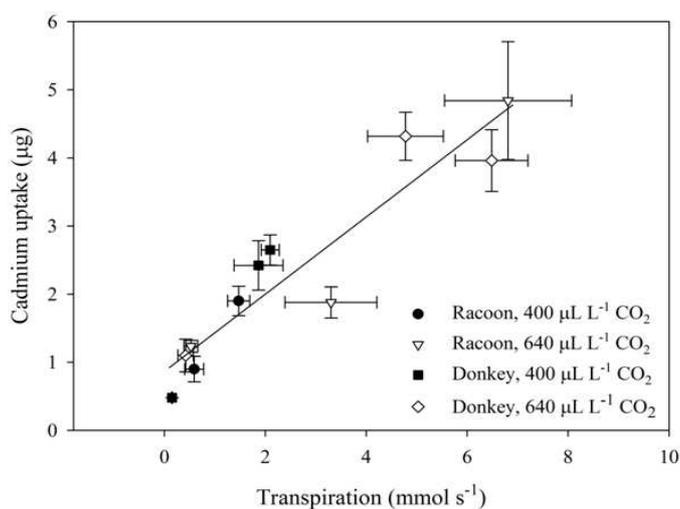
**Fig. 1.** The concentration of Cd applied to the soil and the concentration measured in the dry foliage of Racoon (closed circles) and Donkey (open circles) in Experiment 1. The trend line is a visual aid



**Fig. 2.** Mean dry weight of the components of baby leaf spinach cvv. Racoon and Donkey grown at two atmospheric concentrations of CO<sub>2</sub> in combination with three temperature regimes in Experiment 2. The error bars are SEs, showing the variation between the 3-5 plants within the chamber for each cultivar



**Fig. 3.** Mean canopy area (a), lamina Cd concentration (b), stomatal conductance (c) and canopy transpiration (d), for baby leaf spinach cv. Racoon and Donkey at two atmospheric concentrations of CO<sub>2</sub> in combination with the three temperature regimes in Experiment 2. Note that reliable conductance data could not be obtained for cv. Racoon at ambient CO<sub>2</sub> and the highest temperature regime (30/22°C day/night). The error bars are SEs, showing the variation between the 3-5 plants within the chamber for each cultivar



**Fig. 4.** Relation between Cd uptake and canopy transpiration rate in Experiment 2. Raw data are from Fig. 2 and 3. The fitted line is described by Equation 1

However, plant DW and canopy area are strongly associated in this experiment ( $r^2 = 98$ ); consequently, to avoid the potential effect of this association on the strength of hypothesis testing using the preceding relationship, we also compared the concentration of Cd in the leaves on a DW basis with the canopy transpiration rate expressed on the same basis (**Fig. 5**). As for the previous model, there were no significant effects of cultivar ( $P = 0.44$ ) or cultivar by transpiration ( $P = 0.46$ ) and the linear relation describing these data is ( $r^2 = 0.56$ ,  $P = 0.0079$ ) Equation 2:

$$Cd \text{ concentration} = 5.90(0.93) + 0.83(0.24) \times \{Transpiration \text{ rate}\} \quad (2)$$

where, the units of the Cd concentration are  $\mu\text{g g}^{-1}$  and of transpiration rate are  $\text{mmol g}^{-1}\text{s}^{-1}$ ; again, the values in parentheses are standard errors.

#### 4. DISCUSSION

None of the nutrient concentrations in either of the two experiments were growth limiting for spinach (Reuter and Robinson, 1997) and this is presumed to apply to baby leaf spinach, for which no definitive data were found. The concentration of Cl was 1.8-2.3%, which is unlikely to be toxic (Marschner, 1993) and there were no foliar symptoms of Cl toxicity. Consequently, it is unlikely that the plants suffered mineral stress. Since water was supplied on demand, the effects of the Cd challenge were not confounded by either malnutrition or drought.

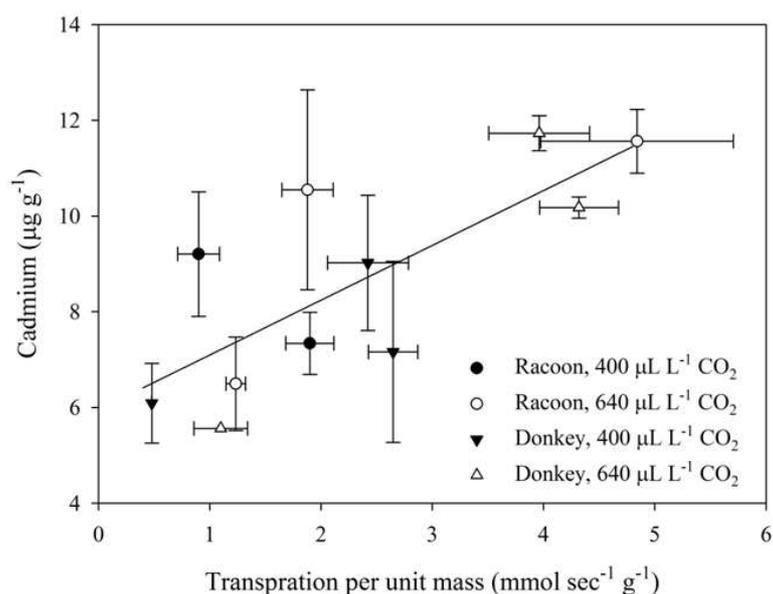
In Experiment 1 exploring plant response to Cd over the range  $\sim 0.04$  to  $10 \text{ mg kg}^{-1}$ , neither growth nor water relations showed any evidence of Cd toxicity (Poschenrieder *et al.*, 1989; Kastori *et al.*, 1992; Perfus-Barbeoch *et al.*, 2002). In all the preceding respects, the behaviour of the cultivars was indistinguishable, both being Cd tolerant and relatively strong Cd accumulators, characteristics that are well-known for spinach (Kuboi *et al.*, 1986).

We estimated complexation of Cd by Cl in the soil solution (GEOCHEM-EZ, Shaff *et al.*, 2010), because the chlorocomplexes of Cd are more mobile by mass flow than Cd itself (Boekhold *et al.*, 1993; Kamewada and Nakayama, 2011). There were two assumptions: (1) The Cl concentration was fixed at  $100 \text{ mg L}^{-1}$ ; and (2) that no other ligands of significance were present. For orders of magnitude variations in the concentration of Cd in solution relevant to soil solutions ( $0.1$ - $100 \mu\text{g L}^{-1}$ ), the

proportion estimated to be present as chlorocomplexes varied relatively little (40-15%), which is informative if not unexpected (McLaughlin *et al.*, 1997; Kamewada and Nakayama, 2011). That is, these calculations suffice to show that under our conditions, an appreciable proportion of the Cd in the soil solution should have been mobile by mass flow.

The cause of the curvature in the relation between the concentration of Cd in the soil and that in the plant (**Fig. 1**) can only be speculative; however, two potential causes should not go unremarked. That is, as Cd concentrations increase, the speciation calculations reveal that the proportion of Cd present as chlorocomplexes declines as noted previously and, in addition, the root uptake mechanism may become partially saturated (Degryse *et al.*, 2012). Notwithstanding the mechanism underlying this curvature, the data show that a soil Cd concentration of  $\sim 0.3 \text{ mg kg}^{-1}$  was sufficient for the foliar concentration of Cd to be readily measured (**Fig. 1**) and this concentration was definitely not phytotoxic. Furthermore, similar concentrations of Cd in Australian soils are associated with appreciable Cd concentrations in leafy vegetables (Jinadasa *et al.*, 1997; McLaughlin *et al.*, 2006). Consequently, in Experiment 2, we used the same soil at a Cd concentration of  $\sim 0.3 \text{ mg kg}^{-1}$  and a Cl concentration of  $\sim 70 \text{ mg kg}^{-1}$  to test the transpiration/uptake hypothesis in a manner that is broadly relevant to field crops.

Experiment 2 tested the hypothesis that transpiration and Cd uptake were related and the data strongly supported this hypothesis. It is unsurprising that Cd uptake increased with increasing transpiration in our study and that on bush beans in nutrient solutions (Hardiman and Jacoby, 1984), since both studies allowed Cd to move towards the roots by mass flow (Salt *et al.*, 1995). Lorenz *et al.* (1994); Kashem and Singh, 2002) obtained similar results for Cd with radish grown in soils. Consequently, the contrasting results from other studies cited in the *Introduction* may be related to the use of conditions atypical of many agricultural soils or to interspecific differences. In addition, transpiration and the uptake of nitrate, which is mobile in the soil solution by mass flow, enjoy a strong, positive association under different atmospheric  $\text{CO}_2$  concentrations and air temperatures (McDonald *et al.*, 2002; Sherwin *et al.*, 2013). Therefore, transpiration-driven mass flow of water and dissolved solutes constitutes a plant uptake pathway that may not have been given due attention.



**Fig. 5.** Relation between leaf transpiration rate and Cd uptake on a mass basis. The bars represent standard errors. The fitted line is described by Equation 2

## 5. CONCLUSION

This study shows that Cd uptake and transpiration are strongly and positively related for baby leaf spinach under soil conditions that mimic those in many cropping soils and the findings are consistent with Cd transport by mass flow towards the roots being a substantial contributor to Cd uptake. In addition, the study also showed that elevating the atmospheric concentration of CO<sub>2</sub> tended to increase plant Cd concentrations raising concerns about future chronic dietary exposure to Cd. The findings challenge the interpretation of some earlier studies of the effects of CO<sub>2</sub> and temperature on Cd uptake and may also explain at least part of the inter-seasonal variation in Cd uptake by field crops. Consequently, there may be merit in investing more broadly in studies of the contribution of transpiration-driven mass flow of dissolved solutes as a plant uptake pathway on a wider range of crop plants under conditions relevant to the field.

## 6. ACKNOWLEDGEMENT

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### 6.1. Author's Contributions

Carlo Chehine conducted the study that was designed by Drs Holford, Ghannoum, Conroy and Milham. Data were interpreted with biometrical assistance from Dr Collins and the manuscript was prepared by Drs Holford and Milham.

### 6.2. Ethics

There are no implications of this research or its application for human or animal health. The data and figures are original and not submitted for peer review elsewhere.

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