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Investigating the ability of *Pseudomonas fluorescens* UW4 to reduce cadmium stress in *Lactuca sativa* via an intervention in the ethylene biosynthetic pathway

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Abstract

A typical plant response to any biotic or abiotic stress, including cadmium (Cd), involves increased ethylene synthesis, which causes senescence of the affected plant part. Stressed plants can experience reduced ethylene and improved growth if they are inoculated with bacteria that have the enzyme ACC deaminase, which metabolizes the ethylene precursor ACC (1-aminocyclopropane-1-carboxylate). We investigated whether one such bacterium, *Pseudomonas fluorescens* UW4, reduces the production of ethylene and improves the growth of lettuce (*Lactuca sativa*) sown in Cd-contaminated potting material (PRO-MIX® BX). Plants were inoculated with the wild-type *P. fluorescens* UW4 or a mutant strain that cannot produce ACC deaminase. Cadmium-treated plants contained up to 50 times more Cd than did control plants. In non-inoculated plants, Cd induced a 5-fold increase in ethylene concentration. The wild-type bacterium prevented Cd-induced reductions in root biomass but there was no relationship between Cd treatment and ethylene production in inoculated plants. In contrast, when the concentration of ethylene was plotted against the extent of bacterial colonization of the roots, increased colonization with wild-type *P. fluorescens* UW4 was associated with 20% less ethylene production. Ours is the first study to show that the protective effect of this bacterium is proportional to the quantity of bacteria on the root surface.

Keywords

Cadmium, ethylene, plant growth-promoting bacteria, *Lactuca sativa*.

44 **Note**

45 Ethylene is an important signalling molecule for plants; it regulates many developmental
46 processes and a variety of biotic and abiotic stress responses (Glick and Stearns 2011). Under
47 mild stress, a small spike in ethylene production initiates the plant protective response to
48 environmental stressors (Chmielowska-Bąk et al. 2013). However, if the stress continues or
49 becomes greater, concentrations of ethylene increase, which induces stress symptoms including
50 leaf chlorosis and abscission, and premature senescence (Glick and Stearns 2011). One of many
51 tolerance mechanisms that can mitigate the impact of stress is the ability of plants to form
52 symbiotic relationships with plant growth-promoting bacteria (PGPB).

53 Glick et al. (1998) hypothesized that, when a plant is experiencing stress, certain PGPB
54 could promote plant growth in one of two ways: stimulation of root growth by providing the
55 plant with indole-3-acetic acid (IAA, a plant growth hormone) or via an intervention in the
56 ethylene biosynthetic pathway, whereby the plant exports some ACC (1-aminocyclopropane-1-
57 carboxylate; the precursor to ethylene) to bacteria that have colonized the plant roots. Certain
58 species of PGPB produce the enzyme ACC deaminase, which metabolizes ACC, and have the
59 potential to minimize the plant stress response by maintaining helpful, low ethylene levels (Glick
60 et al. 1998). In this study, we will test this aspect of Glick et al.'s (1998) hypothesis using the
61 PGPB *Pseudomonas fluorescens* UW4 (Glick et al. 1995), which contains a gene encoding ACC
62 deaminase (*acdS*; Cheng et al. 2010). The strain *P. fluorescens* UW4 has undergone a number of
63 designations. It was initially placed in the genus *Pseudomonas* but was classified as *Enterobacter*
64 *cloacae* UW4 in 1998, then renamed *Pseudomonas putida* UW4 in 2005 (as explained in Cheng
65 et al. 2007 and Duan et al. 2013). However, comparison of its whole genome sequence to those

of other sequenced *Pseudomonads*, as well as phylogenies of four ‘housekeeping’ genes, led to its current designation (Duan et al. 2013).

We chose to study cadmium (Cd) as stressor because it is a common contaminant in agricultural soils due to a number of industrial and agronomic activities (Adriano 2001) and it is readily taken up by *Lactuca sativa* (lettuce; Baldantoni et al. 2016). Previous studies involving PGPB with the *acdS* gene either involved assessing the effect of bacteria on plant growth or on ethylene production (Glick 2012 and references therein), but very few have measured both response variables in the same experiment. One example is Madhaiyan et al. (2007); in their study the effects of two PGPB (*Methylobacterium oryzae* and *Burkholderia* sp.) on plant growth were assessed using potted *Lycopersicon esculentum* (tomato) plants and ethylene production was assessed using tomato grown in water-filled pouches. Therefore, the stress-reducing mechanism involving ACC deaminase has not previously been tested directly.

In this study, *Lactuca sativa* L. var Grand Rapids was grown in jars such that ethylene could be collected from the headspace. Prior to sowing, dry potting material, PRO-MIX® BX (Premier Tech Horticulture, Rivière-du-Loup, Canada), was autoclaved for 15 min at 121°C then 50 g (400 mL) was placed into each of 30 autoclaved 1.4 L Mason jars. The potting material was wetted with autoclaved reverse osmosis (RO) water, and left to sit until fully moistened. One half of the jars were set aside as controls; 50 mL of a stock cadmium chloride (CdCl_2) solution were added to each of the other jars, resulting in a final concentration of 40 mg of Cd/kg of potting material. Jars were sealed and undisturbed for two weeks to allow the Cd to diffuse through the potting material and to establish an equilibrium between the growth medium and the ‘soil’ solution.

To determine the specific effect of ACC deaminase on Cd-induced ethylene, two bacterial strains were used: a mutant strain of *P. fluorescens* UW4 that is unable to produce ACC deaminase due to a gene knockout but secretes IAA at the same concentrations as the wild-type (Li et al. 2000) and wild-type *P. fluorescens* UW4, which produces ACC deaminase and IAA. Additionally, seeds were inoculated with either a low or a high concentration of each bacterial strain to determine whether higher bacterial concentrations would result in a further reduction of ethylene concentrations. Based on Madhaiyan et al. (2007) and Hall et al. (1996) respectively, the target concentrations of bacteria in liquid culture were measured by absorbance at a wavelength of 600 nm (OD₆₀₀): 0.2 (low) and 0.4 (high). Thus, a total of 5 treatments were applied: no bacteria and low and high concentrations for each of two bacterial strains, with three replicates for each treatment. A standard curve for each bacterial strain was created to determine the bacterial cell counts (using a haemocytometer) over a range of OD₆₀₀ readings (data not shown) and the cell density of the two strains was not identical at a given OD₆₀₀. Accordingly, the estimated number of bacterial cells/mL for each treatment will be reported rather than the OD₆₀₀ of the culture.

Bacterial stock cultures were maintained in glycerol at -80°C. Subcultures were grown in tryptic soy broth (TSB), then in DF minimal medium, then in 0.3 M MgSO₄ following the protocol of Penrose and Glick (2003). Once the bacteria were in MgSO₄, *L. sativa* seeds were surface-sterilized in 70% ethanol for 1 min, in 1.2% sodium hypochlorite for 3 min, and then rinsed for 1 min three times with autoclaved RO water. The seeds were soaked for 1 h in one of the four bacterial cultures or 0.3 M MgSO₄ (control) in sterilized Petri dishes, following the protocol of Penrose and Glick (2003). The seeds were then transferred to new sterilized Petri dishes containing autoclaved filter paper saturated with autoclaved RO water until the radicles

emerged (24 h), before being placed in their respective jars (one seed per jar). Each jar was covered with a Bio-Shield® Sterilization Wrap, which permitted gas exchange but prevented microbial contamination.

The plants were cultivated for 3 weeks in a growth chamber set to 22°C, 60% relative humidity, and a 16 h photoperiod ($124 \pm 3 \mu\text{mol}/\text{m}^2/\text{s}$). Due to the low nutrient content in potting material, 5 mL of half-strength Hoagland's nutrient solution was added to each jar at the end of the first and second weeks of the growth period.

Twenty-four hours before sampling/harvest, the Bio-Shield® covers were replaced with Mason jar lids that were fitted with rubber septa. On the day of harvest, a dual-sided sampling needle was used to draw gases in the headspace into a sealed, sterile 5 mL vial. Later, a 1 mL sub-sample was removed from each vial using a syringe and analyzed for ethylene content using gas chromatography with a flame ionization detector (GC-FID) following the protocol of Madhaiyan et al. (2007). The aboveground biomass was placed in a paper bag and dried to constant weight at 60°C. The particles of potting material were manually removed from the plant roots, which were then rinsed twice with RO water then soaked for 5 min in 10 mM calcium chloride (CaCl_2) to desorb Cd from the root surface and remaining traces of potting material. The roots were rinsed in RO water, placed in paper bags and dried to constant weight at 60°C. The concentrations of Cd in roots and shoots were determined by acid digestion followed by inductively coupled plasma optical emission spectroscopy (ICP-OES), following the protocol of Akhter and Macfie (2012).

The effects of bacterial strain and level of inoculation on dry mass, Cd concentration and ethylene concentration were analyzed using 2-way ANOVA, after verifying normality and homogeneity of variance. In cases where ANOVA detected significant main effects, a post-hoc

test (Holm-Sidak Method) was used to determine significant differences among treatments. Regression analysis was done to compare the uptake of Cd by *L. sativa* to ethylene production.

The average Cd concentrations in the roots and shoots of all Cd-treated plants were within the range of 31 to 61 μg of Cd/g of plant tissue, and were one (roots) or two (shoots) orders of magnitude greater than those of untreated plants, which all had less than 2 μg of Cd/g of plant tissue (Fig 1). Mehmood et al. (2013) studied the Cd tolerance of *L. sativa* grown for 4 weeks in potted agricultural soil. Although the Cd concentration in the soil used by Mehmood et al. (2013) was 25% less than the concentration used in our study, the Cd content in their shoots was 50% greater. The plants in our study took up only 0.014% to 0.053% of the Cd that was in each jar, suggesting that Cd adsorbs more readily to PRO-MIX® BX than to soil, as described by Eriksson (1988). Bacterial treatments had no effect on the uptake of Cd by the plants (Fig. 1), which was also the case in similar studies conducted by Burd et al. (1998), Wu et al. (2006) and Dell'Amico et al. (2008).

Shoot dry mass was unaffected by either Cd or bacterial treatments, with all masses falling within a range of 0.005 to 0.020 g; however, standard errors were large (Fig. 2 top). Promotion of shoot growth in response to bacterial treatment is not typically seen in short-term experiments (reviewed in Glick 2012). For non-inoculated plants, Cd treatment resulted in an 80% reduction in root dry mass (Fig. 2 bottom). *L. sativa* is expected to exhibit symptoms of stress at low concentrations of Cd (Benzarti et al. 2008). In all but one case, inoculation resulted in the dry mass of Cd-treated roots being the same as the corresponding control roots, indicating that the bacteria helped to maintain root growth in the presence of Cd. The exception was for inoculation with the higher dose of the mutant bacterium. It is possible that the inability of the mutant to divert ACC might shift the bacterial/plant relationship from being beneficial to being

competitive; the higher density of bacteria in this treatment could be competing with the plant for nutrients.

The concentrations of ethylene measured from the headspace of each jar did not differ among any of the treatments (data not shown) probably due to large standard errors, which in turn were likely due to the variability seen in shoot size (Fig. 2 top). The amount of ethylene produced per gram of plant tissue was, therefore, calculated for each plant. When this ethylene concentration was plotted against the concentration of Cd in the entire plant (shoot plus root), non-inoculated plants produced up to 5 times more ethylene as the concentration of Cd increased (Fig. 3 star symbols) but there was no relationship between ethylene produced and Cd concentration for plants inoculated with either bacterial strain (Fig. 3). In addition, it is interesting to note that inoculation of the plants in the absence of Cd-treatment triggered the production of ethylene; these concentrations were as high as many of those induced by Cd.

The lack of a relationship between concentrations of ethylene and Cd in the plant may have been a result of low sample size but it led us to question whether the seeds were evenly coated by the bacteria during inoculation and/or if the roots were unequally colonized by the bacteria. Therefore, a second set of plants was grown to evaluate the relationship between the production of Cd-induced ethylene and the extent of root colonization. The methods were the same as for the experiment described above with two modifications: (1) The Cd concentration was increased to 50 mg Cd/kg of potting material to ensure that the plants were experiencing Cd-stress. (2) Plants were grown for only two weeks before harvesting, because a preliminary experiment determined that two-week-old plants were sufficiently large to produce measureable concentrations of ethylene (data not shown).

On the day of harvest, ethylene was sampled and measured as described above. Excess potting material was removed from the roots, which were processed immediately. It was not possible to remove every particle of potting material without also dislodging bacteria from the root surface. Roots were finely chopped and weighed into a sterile test tube to which 2 mL of a saline water-agar solution (0.85% NaCl in 0.1% bacteriological-grade agar) was added (Dr. George Lazarovits, A&L Biologicals, pers. comm., 2015). Each tube was vortexed for 30 sec, and 100 μ L of the resultant liquid sample was spread onto an agar plate containing TSB growth medium. All plates were then sealed using ParaFilm® and incubated at 30°C for 24 h. The colonies of wild-type or mutant *P. fluorescens* UW4 present on the plate after incubation were counted in an area measuring 2.5 cm $\times$ 2.5 cm, and these counts were used to calculate the number of colonies per gram of each sample. Regression analysis was done to compare the root colonization by *P. fluorescens* UW4 to ethylene production by *L. sativa*.

When ethylene concentration was plotted against the number of colony-forming units (CFU) present per gram of below-ground plant tissue, the expected relationships were found despite low sample sizes. The concentration of ethylene produced by plants inoculated with mutant *P. fluorescens* UW4 did not vary with the number of colonies formed (Fig. 4a) and there was a negative correlation between ethylene production and CFU on roots inoculated with wild-type *P. fluorescens* UW4 (Fig. 4b).

For the mutant *P. fluorescens* UW4, no correlation between root colonization and ethylene production was expected because the gene encoding the ACC deaminase enzyme has been disrupted (Li et al. 2000). Therefore, it cannot hydrolyze any ACC that may be exported by the plant, and should not have an effect on the levels of ethylene produced by *L. sativa*. For wild-type *P. fluorescens* UW4, an increase in root colonization leading to a decrease in the ethylene

concentration in the headspace of the jar was expected, as both the presence of ACC deaminase and sufficient association of the bacterium with the plant roots are required to have a reduction in ethylene production (Hontzeas et al. 2004).

Unexpectedly, initial inoculation with a low or a high bacterial concentration had no effect on the extent of root colonization by either mutant or wild-type *P. fluorescens* UW4, as the CFU counted were highly variable and unrelated to inoculum concentration (Fig. 4). It is possible that the efficiency of colonization was greater for seeds that germinated more quickly (throughout the study, it was common for germination to be asynchronous), or that variation in seed coat texture influenced adhesion of bacteria to the seeds as they were sown. The variability in colonization could explain why the results for ethylene production within each treatment were also highly variable. High variability could be due to the seed inoculation method used; therefore, other methods, such as the root dip method (Bressan and Borges 2004) or direct inoculation of the substrate (Gagné et al. 1993), may elicit more efficient colonization.

Despite the variability in colonization, the results of our study support Glick et al.'s (1998) hypothesis and confirm the ability of the wild-type *P. fluorescens* UW4 to reduce ethylene levels in *L. sativa* and maintain root growth under Cd-stress.

This study serves as a vital intermediate step towards determining if the commercial application of *P. fluorescens* UW4 in a large-scale agricultural situation will have sufficient benefits to crop growth, health, and yield. The determination of this agricultural impact with respect to soil contamination by Cd is important because fertilizers are one of the primary anthropogenic sources of Cd deposition (Micó et al. 2006), and they are particularly problematic in Ontario, where application of fertilizers to agricultural fields has been shown to double the surface soil Cd levels when compared with Cd levels in the subsoil, thereby increasing the

amount of Cd that is available for uptake by plants (Sheppard et al. 2009). However, there are still further questions that must be addressed before any potential large-scale benefit of PGPB can be determined. For example, this line of research should be conducted in an agricultural field to more accurately determine the effect of *P. fluorescens* UW4 on Cd-stressed crop species.

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References

Adriano, D.C. 2001. Trace elements in terrestrial environments. Biogeochemistry, bioavailability, and risks of metals. Springer-Verlag, New York. N.Y.

Akhter, M.F., and Macfie, S.M. 2012. Species-specific relationship between transpiration and cadmium translocation in lettuce, barley, and radish. J. Plant Stud. **1**(1): 2-13. doi:10.5539/jps.v1n1p2.

Baldantoni, D., Morra, L., Zaccardelli, M., and Alfani, A. 2016. Cadmium accumulation in leaves of leafy vegetables. Ecotox. Environ. Saf. **123**(1): 89-94. doi:10.1016/j.econenv.2015.05.019.

Benzarti, S., Mohri, S., and Ono, Y. 2008. Plant response to heavy metal toxicity: comparative study between the hyperaccumulator *Thaspi caerulescens* (ecotype Ganges) and

- 248 nonaccumulator plants: lettuce, radish, and alfalfa. *Environ. Toxicol.* **23**(5): 607-616.
249 doi:10.1002/tox.20405.
- 250 Bressan, W., and Borges, M.T. 2004. Delivery methods for introducing endophytic bacteria into
251 maize. *BioControl* **49**(3): 315-322. doi:10.1023/B:BICO.0000025372.51658.93.
- 252 Burd, G.I., Dixon, D.G., and Glick, B.R. 1998. A plant growth-promoting bacterium that
253 decreases nickel toxicity in seedlings. *Appl. Environ. Microbiol.* **64**(10): 3663-3668.
254 PMID:9758782.
- 255 Cheng, Z., McConkey, B.J., and Glick, B.R. 2010. Proteomic studies of plant-bacterial
256 interactions. *Soil Biol. Biochem.* **42**(10): 1673-1684. doi:10.1016/j.soilbio.2010.05.033.
- 257 Cheng, Z., Park, E., and Glick, B.R. 2007. 1-aminocyclopropane-1-carboxylate deaminase from
258 *Pseudomonas putida* UW4 facilitates the growth of canola in the presence of salt. *Can. J.*
259 *Microbiol.* **53**(7): 912-918. doi:10.1139/w07-050.
- 260 Chmielowska-Bąk, J., Lefèvre, I., Lutts, S., and Deckert, J. 2013. Short term signaling responses
261 in roots of young soybean seedlings exposed to cadmium stress. *J. Plant Physiol.* **170**(18):
262 1585-1594. doi:10.1016/j.jplph.2013.06.019.
- 263 Dell'Amico, E., Cavalca, L., and Andreoni, V. 2008. Improvement of *Brassica napus* growth
264 under cadmium stress by cadmium-resistant rhizobacteria. *Soil Biol. Biochem.* **40**(1): 74-
265 84. doi:10.1016/j.soilbio.2007.06.024.
- 266 Duan, J., Jiang, W., Cheng, Z., Heikkilä, J.J., and Glick, B.R. 2013. The complete genome
267 sequence of the plant growth-promoting bacterium *Pseudomonas* sp. UW4. *PLoS ONE*
268 **8**(3): e58640. doi:10.1371/journal.pone.0058640

- 269 Eriksson, J.E. 1988. The effects of clay, organic matter and time on adsorption and plant uptake
270 of cadmium added to the soil. *Water Air Soil Poll.* **40**(3): 359-373.
271 doi:10.1007/BF00163740.
- 272 Gagné, S., Dehbi, L., Le Quéré, D., Cayer, F., Morin, J.-L., Lemay, R., and Fournier, N. 1993.
273 Increase of greenhouse tomato fruit yields by plant growth-promoting rhizobacteria
274 (PGPR) inoculated into the peat-based growing media. *Soil Biol. Biochem.* **25**(2): 269-
275 272. doi:10.1016/0038-0717(93)90038-D.
- 276 Glick, B.R. 2012. Plant growth-promoting bacteria: mechanisms and applications. *Scientifica*
277 **2012**: 963401. doi:10.6064/2012/963401.
- 278 Glick, B.R., and Stearns, J.C. 2011. Making phytoremediation work better: maximizing a plant's
279 growth potential in the midst of adversity. *Int. J. Phytoremediat.* **13**(1): 4-16.
280 doi:10.1080/15226514.2011.568533.
- 281 Glick, B.R., Karaturovic, D.M., and Newell, P.C. 1995. A novel procedure for rapid isolation of
282 plant growth-promoting pseudomonads. *Can. J. Microbiol.* **41**(6): 533-536.
283 doi:10.1139/m95-070.
- 284 Glick, B.R., Penrose, D.M., and Li, J. 1998. A model for the lowering of plant ethylene
285 concentrations by plant growth-promoting bacteria. *J. Theor. Biol.* **190**(1): 63-68.
286 doi:10.1006/jtbi.1997.0532.
- 287 Hall, J.A., Peirson, D., Ghosh, S., and Glick, B.R. 1996. Root elongation in various agronomic
288 crops by the plant growth promoting rhizobacterium *Pseudomonas putida* GR12-2. *Isr. J.*
289 *Plant Sci.* **44**(1): 37-42. doi:10.1080/07929978.1996.10676631.

- Hontzeas, N., Saleh, S.S., and Glick, B.R. 2004. Changes in gene expression in canola roots induced by ACC-deaminase-containing plant-growth-promoting bacteria. *Mol. Plant Microbe In.* **17**(8): 865-871. doi:10.1094/MPMI.2004.17.8.865.
- Li, J., Ovakim, D.H., Charles, T.C., and Glick, B.G. 2000. An ACC deaminase minus mutant of *Enterobacter cloacae* UW4 no longer promotes root elongation. *Curr. Microbiol.* **41**(2): 101-105. doi:10.1007/s002840010101.
- Madhaiyan, M., Poonguzhali, S., and Tongmin, S. 2007. Metal tolerating methylotrophic bacteria reduces nickel and cadmium toxicity and promotes plant growth of tomato (*Lycopersicon esculentum* L.) *Chemosphere* **69**(2): 220-228. doi:10.1016/j.chemosphere.2007.04.017.
- Mehmood, F., Rashid, A., Mahmood, T., and Dawson, L. 2013. Effect of DTPA on Cd solubility in soil – Accumulation and subsequent toxicity to lettuce. *Chemosphere* **90**(6): 1805-1810. doi:10.1016/j.chemosphere.2012.08.048.
- Micó, C., Recatalá, L., Peris, M., and Sánchez, J. 2006. Assessing heavy metal sources in agricultural soils of a European Mediterranean area by multivariate analysis. *Chemosphere* **65**(5): 863-872. doi:10.1016/j.chemosphere.2006.03.016.
- Penrose, D.M., and Glick, B.R. 2003. Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. *Physiol. Plantarum* **118**(1): 10-15. doi:10.1034/j.1399-3054.2003.00086.x.
- Sheppard, S.C., Grant, C.A., and Drury, C.F. 2009. Trace elements in Ontario soils – mobility, concentration profiles, and evidence of non-point-source pollution. *Can. J. Soil Sci.* **89**(4): 489-499. doi:10.4141/cjss08033.

312 Wu, S.C., Cheung, K.C., Luo, Y.M., Wong, M.H. 2006. Effects of inoculation of plant growth-
313 promoting rhizobacteria on metal uptake by *Brassica juncea*. Environ. Pollut. **140**(1):
314 124-135. doi:10.1016/j.envpol.2005.06.023.
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Figure captions

Fig. 1. Concentrations of Cd in shoots (top) and roots (bottom) in *L. sativa*. Plants were grown in untreated or Cd-treated PRO-MIX® BX (40 mg Cd/kg) with one of 3 bacterial treatments (non-inoculated, mutant or wild-type *P. fluorescens* UW4); the roots were inoculated with either low ($OD_{600}=0.2$) or high ($OD_{600}=0.4$) densities of each bacterial type. Due to differences between the bacterial strains, the low and high inocula resulted in slightly different bacterial densities. All Cd-treated plants contained greater Cd concentrations than control plants in both the shoots ($F=79.364$, $p<0.001$) and the roots ($F=27.574$, $p<0.001$). No effects of bacterial treatment were found for either the shoots ($F=0.725$, $p=0.586$) or the roots ($F=0.427$, $p=0.427$) and there was no interaction between the Cd and the bacterial treatments. Error bars represent the standard error of each mean; $n=3$.

Fig. 2. Dry mass of shoots (top) and roots (bottom) of *L. sativa*. See caption of Fig. 1 for an explanation of the experimental treatments. Shoot dry mass was unaffected by Cd ($F=0.487$, $p=0.493$) or the bacterial treatments ($F=1.048$, $p=0.408$) and there was no interaction between the two types of treatments. Overall, root dry mass was unaffected by Cd ($F=0.313$, $p=0.582$) or the bacterial treatments ($F=1.039$, $p=0.412$) but there was an interaction between the Cd treatment and the bacterial treatments ($F=3.247$, $p=0.037$). A post-hoc Fisher LSD test was used to determine that two sets of Cd-treated roots were smaller than their respective controls, as indicated by *. Error bars represent the standard error of each mean; $n=3$.

Fig. 3. Correlation between Cd concentration in the entire plant and ethylene production by *L. sativa*. Plants were grown in untreated or Cd-treated PRO-MIX® BX (50 mg Cd/kg) with one of 3 bacterial treatments (non-inoculated, mutant or wild-type *P. fluorescens* UW4); the roots were inoculated with either low ($OD_{600}=0.2$) or high ($OD_{600}=0.4$) densities of each bacterial type. There was no significant relationship between Cd concentration and ethylene production when all bacterial treatments (all symbols) were combined ($r^2=0.008$, $p=0.656$), or when the mutant ($r^2=0.034$, $p=0.916$) or wild-type ($r^2=0.319$, $p=0.369$) were analyzed independently. For the non-inoculated plants (star symbol), ethylene production increased with Cd concentration ($r^2=0.82$, $p=0.035$).

Fig. 4. Correlation between ethylene production by *L. sativa* and colonization success by mutant and wild-type *P. fluorescens* UW4. Seeds were inoculated with one of two densities of bacterial culture (corresponding to an OD_{600} of 0.2 or 0.4). There was no significant correlation between the colonization of *L. sativa* roots and rhizosphere by mutant *P. fluorescens* UW4 and the production of ethylene by *L. sativa* ($r^2=0.06$, $p=0.359$). There was a significant negative correlation between the colonization of *L. sativa* roots and rhizosphere by wild-type *P. fluorescens* UW4 and the production of ethylene by *L. sativa* ($r^2=0.29$, $p<0.001$). Additionally, the colonization of *L. sativa* roots was not proportional to the initial inoculum for either strain of *P. fluorescens* UW4.

Fig. 1. Concentrations of Cd in shoots (top) and roots (bottom) in *L. sativa*.

155x210mm (150 x 150 DPI)

Fig. 2. Dry mass of shoots (top) and roots (bottom) of *L. sativa*.

156x153mm (150 x 150 DPI)

Fig. 3. Correlation between Cd concentration in the entire plant and ethylene production by *L. sativa*.

148x119mm (150 x 150 DPI)

Fig. 4. Correlation between ethylene production by *L. sativa* and colonization success by mutant and wild-type *P. fluorescens* UW4.

168x224mm (150 x 150 DPI)