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Nutrient Uptake among Subspecies of *Cucurbita pepo* L. Is Related to Exudation of Citric Acid

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Abstract. **Exudation of organic acids by roots has been implicated in uptake of minerals from soil. Three cultivars within each of two subspecies of summer squash (*Cucurbita pepo* ssp. *ovifera* D. S. Decker var. *ovifera* and *C. pepo* ssp. *pepo* var. *pepo*) were grown in the field. Plants of ssp. *pepo* had higher concentrations of K, P, and Zn than those of ssp. *ovifera*. These same cultivars were grown under P sufficient and depleted conditions in hydroponics, to measure exudation of organic acids from roots. When grown in hydroponics, tissues of ssp. *ovifera* had similar or higher concentrations of nutrients than ssp. *pepo*. Therefore, differences in tissue composition of field-grown plants are likely due to differences in nutrient uptake ability, not inherent differences in tissue composition between subspecies. Phosphorus nutrition played a significant role in exudation of organic acids into the hydroponics solution. For both subspecies, P depletion resulted in exudation of more citric and succinic acid, and less oxalic and tartaric acid. Under P depletion, ssp. *pepo* exuded more citric acid than ssp. *ovifera*. When soil was eluted with solution containing root exudates, the exudates from ssp. *pepo* eluted more K, Mg, Fe, and Zn than did those from ssp. *ovifera*. Among subspecies of *C. pepo*, exudation of organic acids, particularly exudation of citric acid in response to P depletion, is associated with the plant's ability to accumulate more inorganic nutrients when grown in the field.**

Among domesticated *Cucurbita pepo*, the subspecies *pepo* (*C. pepo* ssp. *pepo* var. *pepo*) includes the "true" zucchini and many pumpkins, and ssp. *ovifera* (*C. pepo* ssp. *ovifera* var. *ovifera*) includes summer squash known as straight necks, crooknecks, patty pans, and winter squash (Decker, 1988; Paris, 2001). In field studies on the phyto-extraction of organic contaminants from soil, shoots of ssp. *pepo* accumulated a substantial amount of 2,2-bis(p-chlorophenyl)-1,1-dichloroethylene (DDE), a breakdown product of DDT, but ssp. *ovifera* did not (White et al., 2003a). This uptake of DDE was correlated with differences among the two subspecies in uptake of various nutrients and heavy metals available in trace quantities in the same soil (White et al., 2003a). These studies suggested there is genetic variation among *C. pepo* in their ability to forage for and take up nutrients from soil. An understanding of this variation may help to predict growth and yield on different soil types, and to refine fertilizer use in squash production.

Plant roots exude metabolites and/or proteins that can chelate or dissolve cations bound to soil particles and increase their availability for plant uptake from the soil solution. This chelating activity disrupts the soil structure on a microscopic scale, and releases molecules that are tightly bound within soil aggregates. For example, adding solutions of organic acids or other chelates to soil released organic carbon and organo-chlorine contaminants bound to mineral particles in soil (Yang et al., 2001). When organic acids were added to DDE-contaminated soil, plants growing in the soil took up greater quantities of DDE than plants in soil without added organic acids (White and Kottler, 2002).

Citric acid has often been suggested as the particular root exudate that disrupts soil structure under nutrient deficient conditions. Several experiments have shown that citric acid exudation is promoted by P depletion (Dinkelaker et al., 1989; Gardner et al., 1982; Gerke et al., 1994; Lipton et al., 1987; Ohwaki and Hirata, 1992), or iron depletion (Gerke et al., 1994). Specific environmental perturbations, such as aluminum toxicity, also induce organic acid exudation (Gerke et al., 1994; Ishikawa et al., 2000; Luo et al., 1999). Plant species differ in the organic acids exuded in response to environmental perturbation (Bhattacharyya et al., 2003; Ishikawa et al., 2000; Luo et al., 1999; Ohwaki and Hirata, 1992; Rengel, 1997). Differences in exudation between genotypes of *Lupinus albus* L. were only seen when plants were P deficient (Egle et al., 2003; Lucus-Garcia et al., 2001). Although differences in organic acid exudation have been noted among genotypes within other species (Gahoonia et al., 2000; Ishikawa et al., 2000; Keller and Römer, 2001), there are few reports linking within-species variation in organic acid exudation to nutrient uptake. In a comparison of two *Spinacea oleracea* L. cultivars, differences in organic acid exudation were related to differences in tissue composition of heavy metals (Römer and Keller, 2002).

Our previous studies of field-grown *C. pepo* found a within-species variation in uptake of nutrients (White et al., 2003a, 2005). The objectives of this study were to determine 1) if the differences in nutrient concentrations are related to nutrient uptake ability, 2) how the subspecies differ in exudation of organic acids from the roots and how P availability alters organic acid exudation, and 3) whether nutrient uptake ability is related to the rate of exudation of organic acids from the roots. Three cultivars were selected from each of the *C. pepo* subspecies *ovifera* and *pepo*. These were grown in hydroponics under P-sufficient or P-depleted conditions to measure organic acid exudation, and solutions of exudates were tested for their ability to extract nutrients from soil.

Materials and Methods

Six cultivars of summer squash were used in these experiments. 'Early Prolific', 'Hybrid Crescent', and 'Zephyr' belong to the ssp. *ovifera*, while 'Black Beauty', 'Gold Rush', and 'Raven' belong

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to the ssp. *pepo*. Seeds sources were Johnny's Selected Seeds, Albion, Maine ('Gold Rush', 'Raven', and 'Zephyr'); Seedway, Hall, N.Y. ('Black Beauty'); and Gurney's Seed Co., Yankton, S. Dak. ('Early Prolific' and 'Hybrid Crescent').

Field plot. These cultivars were grown in 2002 and 2003 in a field plot at Lockwood Farm in Hamden, Conn. (lat. 42N, long. 73W, altitude 50 m above sea level). The soil was a fertile fine sandy loam with pH 6.7 composed of 56% sand, 36% silt, 8% clay, and 1.4% organic carbon. Analysis after the first growing season indicated the soil contained the following concentrations of nutrients in $mg \cdot kg^{-1}$ dry soil: K = 1032, Ca = 1144, Mg = 2718, P = 755, Fe = 9585, Mn = 312, Cu = 47, and Cu = 10. No fertilizer was added to the soil in the 2 years of this experiment. The plot was covered with black polyethylene mulch. Duplicate hills for each cultivar were placed randomly in the field plot. Seeds were germinated for 3 d in the laboratory before hills of several seedlings were planted in the plot at 2 × 2-m spacing. Seedlings were planted in early June. Hills were later thinned to three plants. The plot was weeded and watered as necessary. Plants were examined daily, and fruit were picked when they exceeded 200 g fresh weight. These harvests began in early July, and continued into August. Plants were then destructively harvested in early August to obtain tissue samples from vegetation, as described in White et al. (2003a).

Analysis of tissue. The fresh weight of fruit was summed over all harvests. The fresh weights of roots, stems, and leaves were measured for each hill at the destructive harvest. Subsamples of each tissue; roots, stems, leaves, and fruit, were saved for chemical analysis. Vegetation was thoroughly rinsed with tap water to remove soil particles, finely chopped, and stored in a freezer. Inorganic elements were quantified in tissue samples that were dried at 95 °C for 24 h. Subsamples of 0.5 g were digested in 10 mL concentrated HNO_3 on a hot plate for 30 min, and then diluted to 100 mL in water. Concentrations of all elements were determined by inductively coupled plasma emission spectroscopy (ICP) (Atom Scan 16; Thermo-Jarrell Ash, Franklin, Mass.). The whole-plant tissue concentrations were calculated from the concentrations in individual parts, [ ], and the dry weight of each part (DW) according to the equation:

$$[plant] = ([root] \times DW_{root} + [stem] \times DW_{stem} + [leaf] \times DW_{leaf} + [fruit] \times DW_{fruit}) / DW_{plant} \quad [1]$$

Hydroponics studies. Four trials were conducted in hydroponics to compare root exudates of these six cultivars. Seeds were germinated on filter paper, then transferred to a static nutrient solution with adequate P and grown until the seedlings had three true leaves. Then they were transferred to troughs with a once-through flow of nutrient solution supplied at a rate of 0.5 $mL \cdot min^{-1}$/plant. To deplete P in the plants, they were supplied a complete nutrient solution without P for 7 to 10 d. All other nutrients were maintained at an adequate concentration. After the plants ceased growing due to phosphorous depletion, P was re-supplied to some plants for 3 to 5 d. This depletion and re-supply procedure minimized differences in size and vigor of plants at the time of collection of root exudates. All experiments commenced in a growth room maintained at 25 °C with a 12-h photoperiod of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux density. For trials conducted in July, August and February, root exudates were collected under this condition. For a trial in June, the plants were transferred to a greenhouse and grown under ambient light during the collection of exudates. Exudates were collected when plants were ≈4 weeks old, weighed ≈15 g fresh weight, and had about six true leaves.

Stock solutions contained (per L): 27.2 g KH_2PO_4, 101.1 g KNO_3, 189 g $Ca(NO_3)_2 \cdot 4H2O$, 98.6 g $MgSO_4 \cdot 7H_2O$, 2.0 g Fe EDTA (sequestrene), 1.24 g H_3BO_3, 0.676 g $MnSO_4 \cdot H_2O$, 0.115 g $ZnSO_4 \cdot 7H2O$, 0.100 g $CuSO_4 \cdot 5H_2O$, and 0.0484 g $Na_2MoO_4 \cdot 5H_2O$. The standard nutrient solution was a 2.5 mL aliquot of each stock solution diluted to 1 L in de-ionized water. KCl replaced KH_2PO_4 to deplete P in the plants. After the pretreatment to deplete P, plant roots were transferred to 125-mL foil-wrapped Erlenmeyer flasks containing 100 mL of solution. There was one plant per flask, held in place with a foam plug. Three plants of each cultivar were fed nutrient solution containing KH_2PO_4, and three were fed a solution containing KCl. These solutions were exchanged once a day. Air was bubbled into the solutions through glass micropipettes.

Collection and analysis of root exudates. Collection of root exudates started in the middle of the photoperiod 3 to 5 d after P was resupplied to half the plants, and after disinfection of the roots. Roots were immersed for 1 h in 0.5 mM $CaCl_2$ solution with 50 $\mu g \cdot L^{-1}$ streptomycin and 25 $\mu g \cdot L^{-1}$ chloramphenicol, and then immersed for 1 h in 0.5 mM $CaCl_2$ without antibiotic. The flasks containing the plant roots were filled with fresh 0.5 mM $CaCl_2$. This solution was poured off and replaced after 24 h. The solution containing exudates was poured into a 250-mL plastic screw-cap centrifuge tube and agitated with two 5.0 × 0.8-cm pieces of anion exchange membrane in the $NaHCO_3$ form (Excellion I-200; Electropure Corp., Laguna Hills, Calif.) in order to sequester the organic acids. A second batch of solution containing exudates was agitated with the anion exchange membranes after another 24-h collection period. The anion exchange membranes were transferred to small screw-cap vials to which 3 mL of 2 M hydrochloric acid was added. This was agitated for 2 h to release the organic acids. This method of concentration and extraction of organic acids was tested with known concentrations of organic acids standards to determine the efficiency of retention on the anion exchange membranes and any background due to the membranes.

Organic acids were detected after liquid chromatography (LC) on an Agilent model 1100 LC (Agilent Corp., Palo Alto, Calif.). A 10 to 50-μL subsample was injected onto an anion exclusion column (7.4 × 300 mm, Supelcogel 610H; Supelco, Belefonte Pa.) operated at 40 °C and eluted with 0.1% v/v H_3PO_4 at 1.0 $mL \cdot min^{-1}$. Organic acids were detected by UV absorption at 210 nm and quantified by comparison to standards prepared from the pure substances (Sigma-Aldrich, St. Louis).

Analysis of tissue. Plants were harvested immediately after the collection of root exudates. They were separated into leaf, stem + petiole, and root parts, weighed, and freeze dried. Concentrations of nutrients were determined in freeze dried tissue of each plant part, as described above for field grown plants. The plant tissue concentrations were calculated using Eq. [1]. Organic acids were extracted from root tissues. A 50 mg subsample was homogenized with 5 mL of water at 4 °C. This was diluted 2:1 into a solution of 0.1% sodium azide and filtered through a 0.45-μm filter. Organic acids in this solution were determined by LC analysis as described above.

Elution of soil with solutions of root exudates. Root exudates from the February trial were used as a treatment to extract nutrients from soil. In this trial, roots were immersed in 0.25 mM $CaCl_2$ solution with or without P to collect exudates. The solution containing exudates was collected for 2 d and solutions

from four plants of each cultivar and treatment were combined. A fraction of this solution was used to assay the concentration of organic acids. The majority was used for batch elution of nutrients from soil. A 10-g subsample of a sandy loam soil (from the plot used for field studies) was agitated with 60 mL of exudates solution for 24 h at room temperature. The control consisted of soil samples eluted with 0.25 mM $CaCl_2$. The solution was centrifuged and then filtered through a 0.45-μm filter. A subsample of the eluent was acidified and nutrients were assayed by ICP spectroscopy. Three replicate soil samples were tested for each cultivar and treatment.

Experimental design and statistical analysis. There were duplicate hills for each cultivar placed randomly in the field plot. A different randomization pattern was used in 2002 and 2003. Tissue nutrients in field-grown plants were measured in two replicate subsamples of a single combined tissue sample for each cultivar. Only year and subspecies were used as main effects in analysis of variance (ANOVA). Within subspecies variation due to cultivar was pooled with variation due to experimental error.

There were three or four replicate plants of each cultivar and treatment within each hydroponics trial. The position of the cultivars was randomized in a split-plot with P treatment as the main plot. The four trials were combined for ANOVA. Trial, treatment, and subspecies were the main factors, and all interactions between these effects were included in ANOVA. Variance due to cultivar was analyzed only within subspecies. The cultivar factor was nested within subspecies in the general linear model ANOVA procedure in SYSTAT. $LSD_{0.05}$ was calculated from mean square error reported from ANOVA, and the degrees of freedom within and among comparisons.

There were three replicate soil samples eluted with solutions of root exudates for each cultivar and P treatment. Only subspecies and treatment were used as main effects in ANOVA, as root exudates from the four plants of each cultivar were combined before elution. Within subspecies variation due to cultivar was pooled with variation due to experimental error. A pair-wise correlation analysis was also done, comparing the concentration of a specific organic acid in the exudates solutions from a particular cultivar to the elution of a specific element. The variation among cultivars and replicate elutions was the source of error in this analysis. All analyses were done using SYSTAT (version 10.2; SPSS, Chicago).

Results

Inorganic elements in field-grown plants. On a whole-plant basis, plants of the ssp. *pepo* had both higher concentrations and contents of K, P, and Zn than plants of the ssp. *ovifera* (Table 1). Biomass, which included dry weight of the picked fruit, was also greater for plants of ssp. *pepo* than for ssp. *ovifera*. Thus, content of all nutrients, except Cu, was greater in plants of ssp. *pepo* than in ssp. *ovifera*. Concentrations of K, Ca, Mg, Fe, and Mn differed between 2002 and 2003, but the only interactions between subspecies and years were for Ca. Plants of ssp. *pepo* accumulated 30% to 130% more nutrients than plants of ssp. *ovifera* in 2002, and 90% to 190% more nutrients in 2003.

Uptake of nutrients varied between different plant tissues, and tissue differences varied between subspecies. This data has been presented elsewhere (White et al., 2003a, 2005). In general, stems were a smaller proportion of total plant dry weight for ssp. *pepo* than for ssp. *ovifera*, but concentrations of all elements were greater in stems of ssp. *pepo* than in ssp. *ovifera*. Fruits were a greater fraction of total plant dry weight for ssp. *pepo* than for ssp. *ovifera*, and the concentrations of most elements in fruit tissue were higher for ssp. *pepo* than for ssp. *ovifera*. In contrast, the subspecies did not differ in leaf nutrient concentrations. In large part, ssp. *pepo* has a greater whole plant nutrient content because it produced more biomass in fruit combined with a higher concentration of nutrients in fruit tissues.

Inorganic elements in plants grown in hydroponics. Averaged over all trials in hydroponics, the dry weight of plants of ssp. *pepo* was higher than that for plants of ssp. *ovifera*, 1.97 and 1.54 g, respectively ($P < 0.002$). Phosphorus re-supply had no effect on plant weights, perhaps because of the short 3- to 5-d period of recovery from P depletion. There was no difference in root to shoot ratio between subspecies (0.20 averaged across treatments). Phosphorus depletion increased leaf dry matter content from 0.10 to 0.12 $g \cdot g^{-1}$ fresh weight ($P < 0.001$), but had no effect on other parts.

When grown in hydroponics, plants of ssp. *ovifera* had higher concentrations of Mg, P, and Mn than plants of ssp. *pepo*, but similar concentrations of other elements (Table 2). Plants of ssp. *ovifera* accumulated a higher concentration of P than those of ssp. *pepo* when it was re-supplied to the plants. The other nutrients were unaffected by P depletion or re-supply, except for a slight change in Cu.

Exudation in hydroponics. Acetic, citric, formic, lactic, malic, oxalic, pyruvic, succinic, and tartaric acids were detected in the root exudates from plants grown in hydroponics. The amount of pyruvic acid was an order of magnitude less than that for the other acids. Only the results for di- and tri-carboxylic acids are presented. Plants of ssp. *pepo* exuded more citric and oxalic acid than those of ssp. *ovifera* (Table 3). The cultivar Gold Rush was distinctive within ssp. *pepo*, in that it exuded more citric acid than 'Black Beauty' and 'Raven'.

Phosphorus depletion increased the exudation of citric and succinic acids, while that of oxalic and tartaric acid declined (Table 3). The exudation of citric acid from plants of ssp. *pepo* was significantly greater than that from ssp. *ovifera* under the P-depleted condition, 8.5 compared to 5.3 $nmol \cdot d^{-1} \cdot g^{-1}$, respectively. This difference in exudation was smaller when P was re-supplied to the plants. Thus, plants of ssp. *pepo* appeared to have a greater ability than those of ssp. *ovifera* to exude citric acid from roots under P deficiency.

The effect of P-depletion on exudation of organic acids varied among the four trials in hydroponics. Whereas tartaric acid was a major fraction of organic acids exuded from plants re-supplied with P in June, none was exuded in July, and less was exuded in August and February (data not shown). The variation from trial to trial likely indicated a variable recovery from P depletion. In previous experiments, we established that tartaric acid was only exuded from plants with no P deficiency (unpublished results). The apparent difference between subspecies in the effect of P-treatment on exudation of oxalic acid is misleading (Table 3). Due to interference with a chloride peak in the chromatograms for the July and August trials, oxalic acid was not detected in exudates from ssp. *ovifera* given the minus-P treatment. In June and February trials, when oxalic acid was detected for both treatments and subspecies, the effect of P-treatment on exudation of oxalic acid did not differ between subspecies.

Organic acids in root tissues. Although the concentrations of most organic acids within root tissue varied among the cultivars within each subspecies, the subspecies only differed in malic and succinic acids (Table 4). Oxalic and citric acid in root tissue did not

Table 1. Biomass, concentration, and uptake of nutrients per plant by subspecies of *Cucurbita pepo* when grown in field soil in 2002 and 2003. Values were calculated from the weight-averaged concentrations in each tissue; fruit, leaf, stem, and root.

Year	Sub-species	Bio mass	Elements in whole plant tissue							
			K	Ca	Mg	P	Fe	Mn	Zn	Cu
Concentration						*(mg·g⁻¹ dry wt)*				
2002	*ovifera*		42.5	20.5	4.1	3.7	0.107	0.170	0.039	0.011
	pepo		56.6	20.2	4.0	4.7	0.097	0.152	0.061	0.010
2003	*ovifera*		29.4	9.7	4.9	3.6	0.123	0.107	0.054	0.014
	pepo		37.7	15.5	4.8	4.5	0.130	0.116	0.075	0.009
$LSD_{0.05}$[z]			10.0	4.4	0.4	0.6	0.026	0.046	0.023	0.009
Significance										
Year (Y)			0.001	0.001	0.001	NS	0.010	0.004	NS	NS
Subspecies			0.003	NS	NS	0.001	NS	NS	0.015	NS
Y × subspecies			NS	0.047	NS	NS	NS	NS	NS	NS
Uptake per plant						*(g/plant)*				
2002	*ovifera*	531	24.2	10.8	2.2	2.0	0.059	0.090	0.021	0.006
	pepo	829	46.8	16.8	3.4	3.7	0.078	0.123	0.048	0.008
2003	*ovifera*	545	17.6	5.9	2.6	1.9	0.066	0.059	0.034	0.008
	pepo	1047	40.6	17.0	5.0	4.7	0.135	0.131	0.084	0.009
$LSD_{0.05}$		407	19.6	7.8	1.8	1.7	0.054	0.068	0.038	0.009
Significance										
Year (Y)		NS[y]	NS	NS	NS	NS	NS	NS	NS	NS
Subspecies		0.007	0.002	0.003	0.008	0.001	0.023	0.032	0.006	NS
Y × subspecies		NS	NS	NS	NS	NS	NS	NS	NS	NS

[z]$LSD_{0.05}$ = least significant difference between subspecies across years, $P < 0.05$.
[y]Probability of no effect, NS = nonsignificant, $P > 0.05$.

Table 2. Concentration of nutrients in seedlings of *Cucurbita pepo* when grown in hydroponics in August. Values were calculated from weight-averaged concentrations in each tissue; leaf, stem, and root.

Treatment	Sub-species	Whole plant tissue concn (mg·g⁻¹ dry wt)							
		K	Ca	Mg	P	Fe	Mn	Zn	Cu
P depleted	*ovifera*	51.2	19.0	4.3	2.7	0.063	0.118	0.061	0.010
	pepo	47.2	17.9	3.6	1.6	0.063	0.075	0.061	0.006
P resupplied	*ovifera*	53.4	19.0	4.4	9.7	0.065	0.101	0.056	0.008
	pepo	50.5	20.8	4.0	6.0	0.066	0.085	0.064	0.006
$LSD_{0.05}$[z]		6.0	2.3	0.5	1.3	0.009	0.020	0.006	0.003
Significance									
Subspecies		NS[y]	NS	0.002	0.001	NS	0.001	NS	0.001
Cultivar[x]		NS	NS	0.009	NS	NS	NS	0.002	NS
P treatment		NS	NS	NS	0.001	NS	NS	NS	NS
P × subspecies		NS	NS	NS	0.001	NS	NS	NS	0.043

[z]$LSD_{0.05}$ = least significant difference between subspecies within P treatment, $P < 0.05$.
[y]Probability of no effect, NS = nonsignificant, $P > 0.05$.
[x]Effect of cultivar was restricted to variance within subspecies.

differ between the two subspecies, and re-supplying P resulted in higher concentrations of oxalic and citric acids than in P-depleted plants. We have never detected tartaric acid in root tissues, even though it was exuded from plants resupplied with P.

Elution of nutrients from soil by root exudate solutions. The solutions of root exudates from plants of ssp. *pepo* released more K, Mg, Fe, and Zn from soil samples than did those from ssp. *ovifera* (Table 5). Root exudates from P-depleted plants released more K and Mg, but less Fe and Zn. The P-resupply treatment resulted in a higher concentration of P in the solution of exudates before it was used to treat the soil. Release of Ca could not be measured because the solution used to collect root exudates contained 0.25 mM $CaCl_2$. Because P-resupply resulted in greater plant biomass than P-depletion in this trial, 2.6 and 1.5 g dry weight, respectively, there was a greater concentration of most organic acids, except citrate, in the exudates solution from plants resupplied with P.

In general, there was a poor pair-wise correlation when the release of specific cations from soil by root exudates solutions of each cultivar was correlated to the concentrations of specific organic acids in the exudates solution for that cultivar (data not shown). Release of Zn was positively correlated with concentrations of all organic acids except succinic acid ($P < 0.05$). There were significant positive correlations ($P < 0.05$) for release of Mn and concentration of citric acid, release of Fe and concentration of tartaric acid, and release of Cu and concentration of succinic acid. All other correlations were nonsignificant or negative. These comparisons did not prove that any particular organic acid was responsible for release of the cations from the soil.

Table 3. Exudation of organic acids into hydroponics solution by *Cucurbita pepo* as affected by cultivar and P treatment. Values are averages over four trials.

Treatment		Exudation rate (nmol·d^{-1}·g^{-1} plant dry wt)				
subspecies	Cultivar	Oxalic	Citric	Tartaric	Malic	Succinic
P depleted						
ssp. *ovifera*	Early Prolific	7.5	7.1	0.2	2.7	8.1
	Hybrid Crescent	4.3	4.4	0.2	0.0	5.4
	Zephyr	5.5	4.6	0.2	3.1	10.5
ssp. *ovifera*	average	5.8	5.3	0.2	1.9	8.0
ssp. *pepo*	Black Beauty	9.3	7.9	0.4	2.4	9.0
	Gold Rush	5.4	11.7	0.1	3.3	9.9
	Raven	13.0	5.9	0.2	1.7	9.9
ssp. *pepo*	average	8.9	8.5	0.3	2.4	9.6
P resupplied						
ssp. *ovifera*	Early Prolific	9.9	4.4	2.6	1.9	5.5
	Hybrid Crescent	9.3	3.7	3.5	2.1	6.8
	Zephyr	7.3	4.8	1.6	2.4	8.8
ssp. *ovifera*	average	9.0	4.3	2.6	2.1	7.0
ssp. *pepo*	Black Beauty	10.7	4.3	3.0	1.3	3.5
	Gold Rush	10.9	6.8	3.8	3.5	3.0
	Raven	9.7	4.1	5.1	2.4	7.8
ssp. *pepo*	average	10.5	5.1	3.9	2.4	4.7
$LSD_{0.05}$[z]		2.2	1.6	1.1	2.9	4.1
Significance						
Subspecies (ssp.)		0.029[y]	0.002	NS	NS	NS
Cultivar[x]		NS	0.001	NS	NS	NS
P Treatment		0.003	0.001	0.001	NS	0.009
P × ssp.		NA[w]	0.034	NS	NS	NS
Trial (T)		0.001	0.001	0.001	0.049	0.001
T × ssp.		NA	NS	NS	NS	NS
P × T		NA	0.001	0.001	NS	0.026
P × T × ssp.		NA	NS	NS	NS	NS

[z]$LSD_{0.05}$ = least significant difference between subspecies within P treatment, $P < 0.05$.
[y]Probability of no effect, NS = nonsignificant, $P > 0.05$.
[x]Effect of cultivar was restricted to variance within subspecies.
[w]NA = not applicable due to missing values in some trials.

Table 4. Organic acid concentrations in root tissue of *Cucurbita pepo* when grown in hydroponics in August.

Treatment		Root tissue concn (μmol·g^{-1} plant dry wt)				
subspecies	Cultivar	Oxalic	Citric	Tartaric	Malic	Succinic
ssp. *ovifera*	Early Prolific	7.2	24.1	ND[z]	51.2	111.8
	Hybrid Crescent	4.1	13.3	ND	43.1	68.0
	Zephyr	5.7	33.3	ND	74.1	88.8
ssp. *pepo*	Black Beauty	8.0	22.0	ND	38.2	188.6
	Gold Rush	4.7	30.2	ND	46.2	139.7
	Raven	5.2	28.9	ND	49.3	86.0
P depleted		5.2	21.8	ND	46.6	112.9
P resupplied		6.4	28.8	ND	54.1	114.8
$LSD_{0.05}$[y]		1.1	6.4		8.6	35.7
Significance						
Subspecies		NS[x]	NS		0.008	0.007
Cultivar[w]		0.001	0.007		0.001	0.016
P treatment		0.020	0.028		NS	NS
P × subspecies		NS	NS		NS	NS

[z]ND = not detected.
[y]$LSD_{0.05}$ = least significant difference between P treatment or subspecies means, $P < 0.05$.
[x]Probability of no effect, NS not significant, $P > 0.05$.
[w]Effect of cultivar was restricted to variance within subspecies.

Table 5. Concentration of nutrients eluted from soil samples by solutions of root exudates collected in February.

	Concns eluted from soil ($mg \cdot L^{-1}$)						
Factor	K	Mg	P	Fe	Mn	Zn	Cu
P depleted	6.7	5.5	0.54	2.00	0.16	0.05	0.03
P resupplied	5.0	5.3	0.66	2.59	0.19	0.06	0.02
ssp. *ovifera*	5.5	5.2	0.61	2.20	0.17	0.05	0.02
ssp. *pepo*	6.1	5.5	0.59	2.44	0.19	0.07	0.03
Control[z]	4.3	5.9	0.44	1.30	0.11	0.07	0.02
$LSD_{0.05}$[y]	0.5	0.2	0.05	0.18	0.03	0.01	0.02
Significance							
Subspecies	0.024[x]	0.011	NS	0.020	NS	0.001	NS
P treatment	0.001	0.018	0.001	0.001	NS	0.002	NS
P × subspecies	NS	NS	NS	NS	NS	NS	NS

[z]Control, soil eluted with 0.25 mM $CaCl_2$ without exudates.
[y]$LSD_{0.05}$ = least significant difference between P treatment or subspecies means, $P < 0.05$.
[x]Probability of no effect, NS = nonsignificant, $P > 0.05$.

Discussion

Our results show plants of *C. pepo* ssp. *pepo* accumulated a greater amount of most nutrients when grown in the field, roots of P-depleted plants exuded more citric acid when grown in hydroponics, and solutions of their root exudates released higher concentrations of K, Mg, Fe, and Zn during batch elution of soil samples, than did plants of ssp. *ovifera*. These results support the hypothesis that uptake of nutrients from soil by *C. pepo* is facilitated by exudation of organic acids from roots. However, pair-wise correlations at a cultivar level did not show that any one particular organic acid was responsible for release of nutrients. Citric acid is often implicated in nutrient uptake from soil because it has a greater ability than di-carboxylic acids to bind cations, particularly Fe^{+3} and Al^{+3} (Ryan et al., 2001). Typically, release of nutrients from soil by solutions of pure organic acids can only be shown at organic acid concentrations at or above of 10^{-2} M (Yang et al., 2001) or 10^{-3} M (White et al., 2003b). Due to restricted diffusion in soil, concentrations of organic acid exudates in the soil solution at the root surface are predicted to be on the order of 10^{-3} M (Ryan et al., 2001). However, our method of collecting organic acids exudates in hydroponics resulted in very low concentrations, on the order of 10^{-7} M. Thus, it is not surprising that our method did not show an unambiguous relation between exudation of a particular organic acid and release of a particular nutrient from soil samples.

As in other plant species, many of the organic acids exuded from roots of *C. pepo* varied with P depletion. Phosphorus depletion increased exudation of citric and succinic acid, as seen in various *Lupinus* L. species [*L. albus*, *L. angustifolius* L., and *L. luteus* L.] (Egle et al., 2003), *Hordeum vulgare* L. (Gahoonia et al., 2000), *Spinacea oleracea* (Keller and Römer, 2001), *Medicago sativa* L. (Lipton et al., 1987), and other leguminous crops (Ohwaki and Hirata, 1992). In *C. pepo*, the increase in exudation of citric acid from roots under P deficiency was greater for plants of ssp. *pepo* than for ssp. *ovifera*. It is interesting that *C. pepo* 'Gold Rush' exuded more citric acid than any other cultivar under P deficiency. In field experiments, 'Gold Rush' was most effective at extracting weathered DDE from the soil (White et al., 2003a). However, this particular cultivar did not take up more nutrients than other cultivars in ssp. *pepo* when grown in the field (White et al., 2003a, 2005).

The subspecies *pepo* and *ovifera* may differ both in their ability to forage in soil, and in their nutrient requirements for growth and fruit production, or the tendency to accumulate higher concentrations in tissues. When seedlings were grown in hydroponics, there was no inherent tendency for plants of ssp. *ovifera* to have lower tissue concentrations of nutrients than ssp. *pepo* (Table 2). At least during vegetative growth, differences in uptake by field-grown plants were likely due to differences in nutrient uptake ability. The two subspecies of *C. pepo* may differ in their ability to forage in soil, and in their degree of P deficiency when grown in soil. Tissue P in field-grown plants of both subspecies was less than that in plants resupplied with P, but more than that of P-deficient plants grown in hydroponics (Tables 1 and 2). From this comparison, we suspect the field grown plants were partially P deficient and that their citric acid exudation systems were likely induced. Under this condition, the greater ability of plants of ssp. *pepo* to exude citric acid likely played a role in facilitating their greater uptake of nutrients from soil. Because plants of ssp. *ovifera* were less able to extract P and other nutrients from soil, they are likely to require higher soil fertility for good yields than would plants of ssp. *pepo*. A high efficiency for uptake of nutrients may be a liability in soil contaminated with heavy metals. *Cucurbita maxima* Duch. fertilized with municipal solid waste had 10 times more cadmium in fruit than control plants, while no such increase in cadmium was observed in fruit of tomato (Ozores-Hampton et al., 1994).

The relation between plant P status and citric acid exudation has been studied most thoroughly in *Lupinus* species. Various *Lupinus* species differ in tolerance for P-depleted soils, and also in exudation of organic acids in response to P (Egle et al., 2003). The formation of proteoid roots that release a large amount of citric acid is a well-characterized response of *L. albus* to P deficiency (Gerke et al., 1994). Whereas *L. albus* roots exude far more citric acid than any other organic acid under P deficiency, *C. pepo* exuded similar amounts of several organic acids. Iron deficiency has also been shown to affect organic acid exudation in various species (Gerke et al., 1994; Liang and Li, 2003; Waters and Blevins, 2000). However in *L. albus*, Fe deficiency results in a different pattern of organic acid exudation than that of P deficiency (Gerke et al., 1994; Liang and Li, 2003). Although Waters and Blevins (2000) observed *C. pepo* formed proteoid roots in hydroponics as a result of Fe deficiency, we did not see formation of proteoid roots due to P deficiency.

Others have hypothesized that the amount of organic acid exuded from roots is directly related to the concentration in root tissues (Ohwaki and Hirata, 1992). However, comparing our data for organic acid exudation to that for organic acid concentration in roots of *C. pepo* suggests that there is no simple relation between the two. Phosphorous depletion increased the rate of exudation of citric acid, but decreased the concentration of citric acid in root tissues. Phosphorus treatment did not affect the tissue concentration of succinic acid, even though it affected exudation. Thus, the rate of exudation of organic acids by roots was not simply related to the concentration in root tissues, particularly when comparisons were made across treatments. It is likely that specific exudation mechanisms exist for each organic acid, under the control of nutrient status or some other aspect of plant metabolism.

Literature Cited

Bhattacharyya, P., S.C. Datta, and P. Dureja. 2003. Interrelationship of pH, organic acids, and phosphorus concentration in soil solution of rhizosphere and non-rhizosphere of wheat and rice crops. Comm. Soil Sci. Plant Anal. 34:231–245.

Decker, D.S. 1988. Origins, evolution, and systematics of *Cucurbita pepo* (Cucurbitaceae). Econ. Bot. 42:4–15.

Dinkelaker, W., V. Romheld, and H. Marchner. 1989. Citric acid secretion and precipitation of calcium citrate in the rhizosphere of white lupin (*Lupinus alba*). Plant Cell Environ. 12:285–292.

Egle, K., W. Römer, and H. Keller. 2003. Exudation of low molecular weight organic acids by *Lupinus albus*, *Lupinus angustifolius* and *Lupinus luteus* as affected by phosphorus supply. Agronomie 23:511–518.

Gahoonia, T.S, F. Asmar, H. Giese, G. Gissel-Nielsen, and N.E. Nielsen. 2000. Root-released organic acids and phosphorus uptake of two barley cultivars in laboratory and field experiments. European J. Agron. 12:281–289.

Gardner, W.K, D.G. Parberry, and D.A. Barber. 1982. The acquisition of phosphorus by *Lupinus albus* L. Plant Soil 68: 33–41.

Gerke, J., W. Römer, and A. Jungk. 1994. The excretion of citric and malic acid by proteoid roots of *Lupinus albus* L.; Effects on soil solution concentrations of phosphate, iron, and aluminum in the proteoid rhizosphere in samples of an oxisol and a luvisol. Zeitschrift für Pflanzenernährung und Bodenkunde 157:289–294.

Ishikawa, S., T. Wagatsuma, R. Sasaki, and P. Ofei-Manu. 2000. Comparison of the amount of citric and malic acids in Al media of seven plant species and two cultivars each in five plant species. Soil Sci. Plant Nutr. 46:751–758.

Keller, H. and W. Römer. 2001. Cu, Zn, and Cd acquisition by two spinach cultivars depending on P nutrition and root exudation. Zeitschrift fur Pflanzenernahrung und Bodenkunde 164:335–342.

Liang, R.X. and C.J. Li. 2003. Differences in cluster-root formation and carboxylate exudation in *Lupinus albus* L under different nutrient deficiencies. Plant Soil 248:221–227.

Lipton, D.S., R.W. Blanchar, and D.G. Blevins. 1987. Citrate, malate, and succinate concentration in exudate from P-sufficient and P-stressed *Medicago sativa* L seedlings. Plant Physiol. 85:315–317.

Lucus-Garcia, J.A., C. Barbas, A. Probanza, M.L. Barrientos, and F.J.G. Manero. 2001. Low molecular weight organic acids and fatty acids exudates of two *Lupinus* cultivars at flowering and fruiting stages. Phytochem. Anal. 305:305–311.

Luo, H.M, T. Watanabe, T. Shinano, and T. Tadano. 1999. Comparison of aluminum tolerance and phosphate absorption between rape (*Brassica napus* L.) and tomato (*Lycopersicon esculentum* Mill.) in relation to organic acid exudation. Soil Sci. Plant Nutr. 45:897–907.

Ohwaki, Y. and H. Hirata. 1992. Differences in carbolic acid exudation among P-starved leguminous crops in relation to carboxylic acid contents in plant tissues and phospholipid levels in roots. Soil Sci. Plant Anal. 38:235–243.

Ozores-Hampton, M., B. Schaffer, H.H. Bryan, and E.A. Hanlon. 1994. Nutrient concentrations, growth, and yield of tomato and squash in municipal solid-waste-amended soil. HortScience 29:785–788.

Paris, H.S. 2001. History of the cultivar-groups of *Cucurbita pepo*. Hort. Rev. 25:71–170.

Rengel, Z. 1997. Root exudation and microflora populations in rhizosphere of crop genotypes differing in tolerance to micronutrient deficiency. Plant Soil 196:255–260.

Römer, W. and H. Keller. 2002. Variability of Cu, Zn and Cd content of spinach cultivars depending on P nutrition. Gartenbauwissenschaft 67:255–264.

Ryan, P.R., E. Delhaize, and D.L. Jones. 2001. Function and mechanism of organic anion exudation from plants roots. Ann. Rev. Plant Physiol. Mol. Biol. 52:527–560.

Waters, B.M. and D.G. Blevins. 2000. Ethylene production, cluster root formation, and localization of iron III reducing capacity in Fe deficient squash roots. Plant Soil 225:21–31.

White, J.C. and B.D. Kottler. 2002. Citrate mediated increase in uptake of weathered 2,2-bis(p-chlorophenyl)-1,1-dichloroethylene residues by plants. Environ. Toxicol. Chem. 21:550–556.

White, J.C., X. Wang, M.P.N. Gent, W. Iannucci-Berger, B.D. Eitzer, N.P. Schultes, M. Arienzo, and M.J. Incorvia Mattina. 2003a. Subspecies-level variation in phytoextraction of weathered p,p´-DDE by *Cucurbita pepo*. Environ. Sci. Technol. 37:4368–4373.

White, J.C., M.J.I. Mattina, W.Y. Lee, B.D. Eitzer, and W. Iannucci-Berger. 2003b. Role of organic acids in enhancing the uptake of weathered DDE by plants. Environ. Pollution 124:71–80.

White, J.C., Z.D. Parrish, M. Isleyen, M.P.N. Gent, W. Iannucci-Berger, B.D. Eitzer, and M.I. Mattina. 2005. Influence of nutrient amendments on the phytoextraction of weathered 2,2-bis(p-chlorophenyl)-1,1-dichloroethylene by cucurbits. Environ. Toxicol. Chem. 24:987–994.

Yang, Y., D. Ratte, B. Smets, J. Pignatello, and D. Grasso. 2001. Mobilization of soil organic matter by complexing agents and implications for polycyclic aromatic hydrocarbon desorbtion. Chemosphere 43:1013–1021.