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Effects of Genotype and Transpiration Rate on the Uptake and Accumulation of Perchlorate (ClO_4^-) in Lettuce

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Although evidence of perchlorate accumulation in plants exists, there is a scarcity of information concerning the key factors and mechanisms involved. To ascertain whether genotypic variation in perchlorate accumulation occurs within lettuce, hydroponic plant uptake experiments were conducted with five types of lettuce (*Lactuca sativa* L.), which were grown to market size at three perchlorate (ClO_4^-) concentrations (1, 5, or 10 μg/L). Perchlorate accumulated in the leafy tissues to varying amounts, ranging from 4 to 192 μg/kg fresh weight (FW), and the ranking of perchlorate accumulation was crisphead > butter head > romaine > red leaf > green leaf. The effect of transpiration rate on perchlorate accumulation was further examined using crisphead, butter head, and green leaf lettuce. By growing lettuce in controlled-environment chambers with two climatic regimes, "cloudy, humid, cool" (80% RH, 18/15 °C, 250 μmol/m^{2}s photosynthetic photon flux density (PPFD)) and "sunny, dry, warm" (∼50% RH, 28/18 °C, 500 μmol/m^{2}s PPFD), up to 2.7-fold differences in transpiration rates were achieved. Across all three genotypes, the plants that transpired more water accumulated more perchlorate on a whole-head basis; however, the effect of transpiration rate on perchlorate accumulation was not as great as expected. Despite 2.0−2.7-fold differences in transpiration rate, there were only 1.2−2.0-fold differences in perchlorate accumulation. In addition to whole-head analysis, plants were sectioned into inner, middle, and outer leaves and processed separately. Overall, the ranking of perchlorate accumulation was outer leaves > middle leaves > inner leaves. Transpiration rate has a clear effect on perchlorate accumulation in lettuce, but other factors are influential and deserve exploration.

Introduction

Perchlorate (ClO_4^-) emerged as a drinking-water contaminant in the late 1990s, and has since been detected in various water supplies throughout the United States, where it can potentially have adverse effects on human health. Perchlorate interferes with normal iodide uptake by the human thyroid (*1*, *2*), and reduced iodide uptake can disrupt normal thyroid function. This is especially important for sensitive subpopulations such as developing fetuses and breast-feeding infants. In 2005, the United States Environmental Protection Agency adopted a reference dose of 0.0007 mg/kg·d based on the National Academies of Science review concerning the health effects of perchlorate ingestion (*3*). For a 70-kg adult drinking 2 liters of water per day, this reference dose equates to a drinking-water equivalent standard of 24.5 μg/L perchlorate. In March 2004, California adopted a public health goal of 6 μg/L in drinking water (*4*). This value was determined using a reference dose of 0.00037 mg/kg·d and the assumption that 60% of perchlorate ingestion is from water and the remainder from food; however, no dietary data were available to validate their assumption. It remains unknown to what extent humans are exposed to perchlorate from eating, for example, contaminated vegetables.

Recently, perchlorate has been detected in an increasing number of dietary sources including milk products, fresh produce and, most recently, in wine and beer (*5*−*9*). Perchlorate was detected in leafy vegetables from across the United States at concentrations ranging from below detection to 628 μg/kg fresh weight (FW) (*7*). One obvious source of dietary perchlorate exposure is from produce grown in the lower Colorado River valley. The Colorado River is contaminated with low concentrations (5−9 μg/L) of perchlorate (*6*), and the water is used to irrigate vast acreages in southeastern California and southwestern Arizona, where winter lettuce (*Lactuca sativa* L.) is a major crop. Recently, there has been concern among farmers, consumers, and regulators as to whether these crops are safe to eat.

The Environmental Working Group (EWG) in conjunction with Texas Tech University conducted a market survey in which 22 lettuce samples were purchased from retail grocers in California and four out of 22 samples contained detectable perchlorate, between 30−100 μg/kg FW (*10*). Perchlorate was also found in iceberg lettuce sampled from agricultural fields in the lower Colorado River region ranging from below detection to 142 μg/kg FW (*6*). While helpful in understanding perchlorate accumulation in vegetables, these surveys do not reveal whether differences in perchlorate accumulation among lettuce varieties exist. Moreover, to understand the key factors of perchlorate uptake in lettuce, it is necessary to grow plants under carefully controlled conditions where climatic factors, nutrient availability, and perchlorate concentration can be closely monitored.

Previous plant uptake experiments under controlled conditions have been conducted using rather high perchlorate concentrations (*11*, *12*), usually in the context of phytoremediation (*13*−*17*). This was partially due to limits in analytical instrumentation; however, recent methods have been developed that provide low μg/kg detection limits (*18*, *19*), so these challenges no longer exist. While the previous perchlorate uptake studies are informative, they are not conclusive and leave many questions unanswered. One essential question is to what extent do low levels of perchlorate lead to potentially harmful levels of accumulation within edible plant tissues?

While it is clear that evidence of perchlorate uptake by plants exists, the full scope of plant uptake is not understood. To date, there have been no controlled plant uptake experiments at relevant (i.e., low μg/L) concentrations coupled with accurate quantification of the resulting low levels of perchlorate in plants. In addition, there is a scarcity of information available concerning the fundamental mechanisms and key controlling factors surrounding perchlorate accumulation in higher plants. The main objectives of this research were (1) to evaluate any genotypic differences in perchlorate accumulation in lettuce when exposed to environmentally relevant levels and (2) to understand the role

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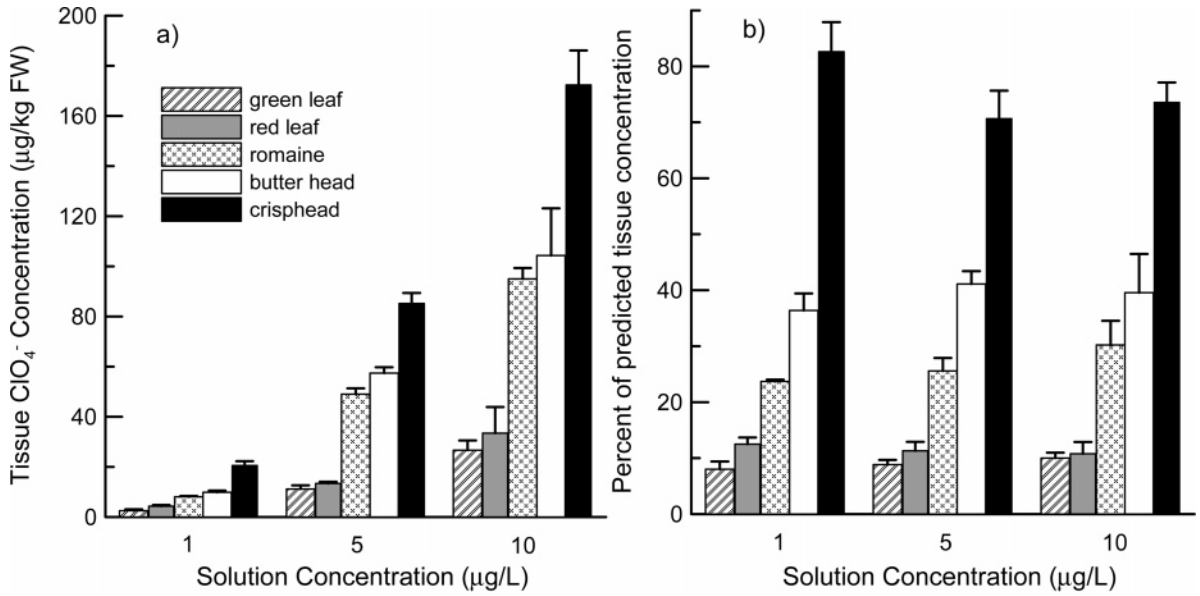

FIGURE 1. (a) Final tissue perchlorate concentrations (whole-head analysis) and (b) perchlorate tissue concentration expressed as a percentage of the value predicted based on transpirational water flux (see eq 1) for five types of lettuce hydroponically grown at 1, 5, or 10 μg/L perchlorate. Error bars represent standard error of the mean where $n = 3$ with the exception of the 1 and 5 μg/L red leaf treatments where $n = 2$.

of transpiration rate in plant uptake and storage of perchlorate within edible tissues. To our knowledge, this is the first mechanistic study of perchlorate accumulation in lettuce at environmentally relevant concentrations.

Experimental Section

Reagents. All solutions were prepared using 18 M′Ω·cm water or better, and all salts used for nutrient solutions were ACS certified 99% purity or higher. Perchlorate standards ranging from 0.250−20 μg/L were prepared by dilution of a liquid 1000 mg/L perchlorate standard solution (SPEX CertiPrep, Metuchen, NJ). Perchlorate treatments were administered using the same standard stock solution.

Lettuce Seeds. Five types of clay-coated lettuce seeds were donated from commercial seed distributors in southeastern California. Redleaf ("Redtide"), romaine ("Conquistador"), and butter head ("Baja") were obtained from Champion Seed Company (Coachella, CA) while crisphead ("Bubba") and green leaf ("Shining Star") were obtained from Keithly Williams Seed Company (Holtville, CA).

Seed Germination and Seedling Transplantation. Lettuce seeds were rinsed of their clay-coat and germinated by rolling the seeds into the top edge of a moistened germination paper, the bottom of which was placed in a beaker of chelater-buffered nutrient solution for approximately 10 d before transplanting. Water was added daily to compensate for evaporation of the nutrient solution. Twenty seedlings were transplanted to each nursery of 22-L of chelator-buffered nutrient solution that was continuously aerated. Each seedling fit into a drilled hole on the nursery lid, supported by a modified Horticube foam collar (Smithers-Oasis, Kent, OH). Basal nutrients were supplied as (μM) NO_3^-, 4800; NH_4^+, 200; P, 80; K, 1080; Ca, 1900; Mg, 500; S, 500; Fe, 20; Mn, 0.6; Zn, 8; Cu, 2; B, 10; Mo, 0.1; Ni, 0.1; and Cl, 21.4 (*20*). For controlling trace metal availability, the solution contained 57.7 μM HEDTA, which is a 25 μm excess above the sum of the Fe, Mn, Cu, Zn, and Ni concentrations. To buffer the nutrient solution at pH 6, the solution contained 1 mM MES and 0.5 mM NaOH.

After 7 d in the nursery, plants of similar size were selected as replicates and each was transplanted to a 4-L, opaque, HDPE bucket of continuously aerated nutrient solution (as previously described) to which a prescribed level of perchlorate was added. Opaque buckets and lids were used to inhibit light penetration and thus to minimize algal growth. In addition, microbial growth was minimized by changing nutrient solutions every 3−7 d during the course of each experiment. A subsample of the exhausted solution was obtained and the buckets were replaced with clean, acid-washed buckets containing fresh nutrient solution. During the course of the experiments, pH, water level, and phosphate concentrations were regularly checked and adjusted to appropriate levels (pH = 6.0 ± 0.05; phosphate = 80 μM). In addition, the transpirational water loss was measured and replaced daily using 18 M′Ω·cm water. Transpirational water loss was easily measured using the experimental units described above. Because the collars and lids are watertight, any water loss is due only to transpiration and can be measured gravimetrically.

Genotypic Uptake Experiment. Uptake experiments were conducted using controlled-environment growth chambers within which factors such as photosynthetic photon flux density (PPFD), temperature, and relative humidity could be closely monitored and controlled. Five types of lettuce were grown with prescribed perchlorate concentrations of 1, 5, or 10 μg/L, with three replicates. Plants were grown to approximate market size under the following "standard" conditions: 65% relative humidity, 25 °C during 16 h of light and 18 °C during 8 h of dark. During light hours, PPFD was ramped up to 500 μmol/m^{2}s and held there for 6 h before symmetrically ramping back down to zero. After the plants reached approximately marketable size, shoots were separated from roots and the whole-head (i.e., all of the leafy tissue) was processed according to the procedure described later in this section.

Transpiration Rate Experiment. Based on the results of the first study, crisphead, butter head, and green leaf were chosen for the transpiration experiment because they represented extremes in perchlorate accumulation. These three genotypes were grown in two different environments to obtain different rates of transpiration. There was a "cloudy, humid, cool" environment in which plants were exposed to the following conditions: 80% relative humidity, 18 °C during 16 h of light and 15 °C during 8 h of dark. During light hours,

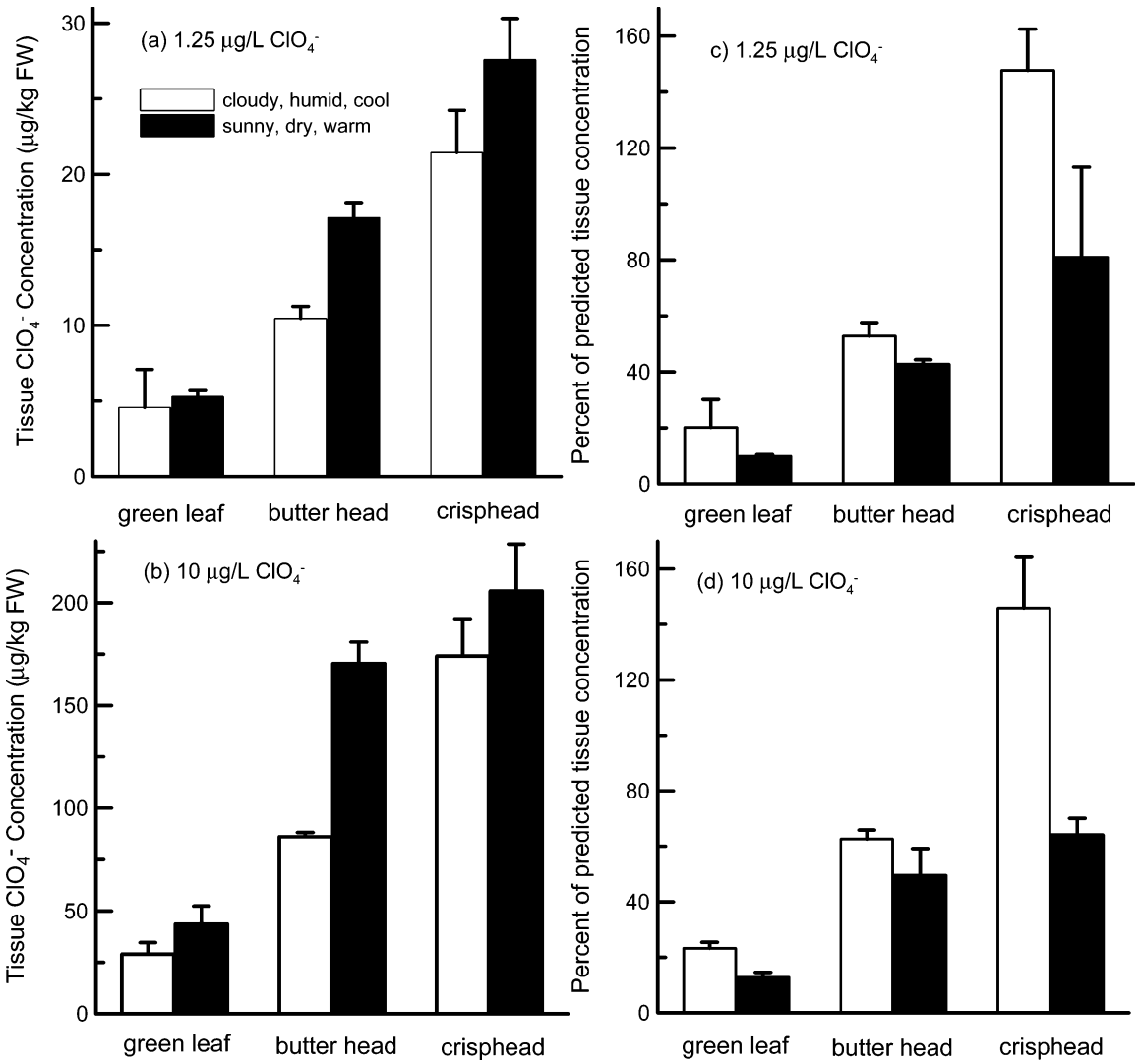

FIGURE 2. Whole-head tissue accumulation of perchlorate (a, b) in green leaf, butter head, and crisphead lettuces with the corresponding tissue concentration expressed as a percentage of the predicted value (c, d) (see eq 1) grown at 1.25 μg/L or 10 μg/L perchlorate in either a "cloudy, humid, cool" or a "sunny, dry, warm" environment in plant growth chambers. Error bars represent standard errors of the mean where $n = 3$ with the exception of the 1.25 and 10 μg/L "cloudy, humid, cool" butter head treatments and the 1.25 μg/L "cloudy, humid, cool" crisphead treatment where $n = 2$.

PPFD was ramped up to 250 μmol/m^{2}s and held there for 6 h before ramping back down to zero. There was also a "sunny, dry, warm" environment in which plants were exposed to the following conditions: ~50% relative humidity (ambient but somewhat variable), 28 °C during 16 h of light and 18 °C during 8 h of dark. During light hours, PPFD was similarly ramped, but the maximum was 500 μmol/m^{2}s.

Each genotype tested was grown with prescribed perchlorate concentrations of 1.25 and 10 μg/L in both environments with three replicates. Plants were again grown to approximately marketable size and maintained as described previously for the genotypic uptake experiment. At harvest, the roots were separated from the shoots and the head was bisected longitudinally. One-half was analyzed for whole-head perchlorate concentration, and the other half was further sectioned into outer, middle, and inner leaves and processed separately according to the procedure described next.

Tissue Processing and Analysis. Lettuce leaves were processed using a recently developed extraction procedure for perchlorate in plant matrices and analyzed by ion chromatography-electrospray ionization-mass spectrometry (IC-ESI-MS) (*18*). Briefly, leaves were sealed in a plastic bag and placed in a −22 °C freezer for at least 2 days to help rupture cell membranes. Frozen leaves were weighed, diluted at least 3:1 (w/w) with water, and macerated in a blender. Extracts were then centrifuged, filtered, and the aqueous extracts were rendered water-clear using a one-step solid-phase extraction (SPE) method. Total time for extraction and sample cleanup was 6 h.

Aqueous lettuce extracts were then analyzed using IC-ESI-MS (Dionex, Sunnyvale, CA) with a minimum reporting limit of 0.8 μg/kg FW (*18*). Chromatographic separation was achieved using an IonPac AS16 (2 × 250 mm) analytical column equipped with an IonPac AG16 (2 × 50 mm) guard column with 45 mM NaOH eluent at a flow rate of 0.3 mL/min. Flow from the IC was directed into a Finnigan Surveyor MSQ Plus single quadropole MS (Thermo Electron Corporation, Waltham, MA). To correct for potential ion suppression, $Cl^{18}O_4^-$ was used as an internal standard at a concentration of 1 μg/L in all standards and samples, and was added just prior to analysis.

The MS was equipped with an AXP-MS auxiliary pump, pumping 50/50 acetonitrile/water at a flow rate of 0.3 mL/

TABLE 1. Summary Table of the 3-way ANOVA for the Log-transformed Data from the Transpiration Rate Experiment Showing the Relative Contributions of Error and the Level of Statistical Significance for Each Tested Factor and Their Interactions

source	df	mean square	F	sig
corrected model	11	0.984	48.061	<0.0001
intercept	1	72.467	3537.875	<0.0001
genotype	2	1.654	80.728	<0.0001
perchlorate concentration	1	6.781	331.051	<0.0001
climate	1	0.244	11.923	0.002
genotype × concentration	2	2.974×10^{-3}	0.145	0.866
genotype × climate	2	1.653×10^{-2}	0.807	0.460
dose × climate	1	4.356×10^{-5}	0.002	0.964
genotype × concentration × climate	2	2.726×10^{-3}	0.133	0.876
error	21	2.048×10^{-2}		
total	33			
corrected total	32			

TABLE 2. Relative Differences in Mean Values for Tissue Perchlorate Concentration and Transpiration Ratio for Plants Grown in the "Sunny, Dry, Warm" Environment versus Those Grown in the "Cloudy, Humid, Cool" Environment (Three Types of Lettuce Were Grown at Either 1.25 or 10 μg/L Perchlorate)

	tissue concentration	transpiration ratio
1.25 μg/L		
green leaf	1.3	2.5
butter head	1.6	2.0
crisphead	1.3	2.6
10 μg/L		
green leaf	1.4	2.5
butter head	2.0	2.5
crisphead	1.2	2.7

min. Matrix diversion was used to divert all of the IC flow to waste for the first 9 min of each run, and then the IC flow joined the acetonitrile/water so that a total flow rate of 0.6 mL/min was provided to the MS for each analysis. The retention time of perchlorate was ~13 min under these conditions. Negative ion monitoring of SIM99 = m/z 99-(±0.5), SIM 101 = m/z 101 (±0.5), and SIM 107 = m/z 107 (±0.5) (corresponding to $^{35}Cl^{16}O_4^-$, $^{37}Cl^{16}O_4^-$, and $^{35}Cl^{18}O_4^-$, respectively) was utilized with a dwell time of 0.30 s per ion. SIM 99 was used exclusively for the quantification of perchlorate in all standards and samples. Identification of perchlorate in the unknowns was confirmed by retention times as well as the SIM 99 to SIM 101 ratio of 3:1, reflecting the natural isotopic abundance of ^{35}Cl to ^{37}Cl. SIM 107 was used to quantify the $Cl^{18}O_4^-$ internal standard and thus to correct for ion suppression (*18*). Chromeleon Version 6.6 (Dionex, Sunnyvale, CA) was used to control the instrumentation and to quantify perchlorate.

Statistics. Simple inspection of the whole-head perchlorate concentrations from both experiments revealed that the standard deviations were roughly proportional to the means for each treatment; this heterogeneity of variance was confirmed using Bartlett's test (*21*). Moreover, the tissue concentration data in both cases did not conform to a linear additive model, and the data were, therefore, log-transformed. This transformation corrected the data to meet the criteria for normality, homogeneity of variance, and additivity so that analyses of variance (ANOVAs) could be properly performed (*21*). The ANOVAs were conducted using SPSS version 11.0 for Mac (SPSS, Inc., Chicago, IL).

Results and Discussion

To ensure that the perchlorate concentration remained relatively constant within each bucket, sub-samples of the exhausted solutions at each solution change were collected. We placed an emphasis on analyzing perchlorate in the samples from the solution changes in the later portion of the experiment, when the plants were large. Representative samples from the last solution change for all genotypes in the first uptake experiment were analyzed at each exposure level (1, 5, and 10 μg/L), as well as one replicate from each genotype for the 5 μg/L level at every solution change. In addition, the last three solution changes for crisphead, butter head, and green leaf were analyzed for the 1 and 10 μg/L level. For most of the solutions analyzed, the concentration of perchlorate remaining in solution was 79−118% of the initial concentration, with the exception of three samples (71, 75, and 188%, in the final solution changes for the 1 μg/L butter head, the 5 μg/L butter head the 1 μg/L romaine treatments, respectively). The depletion of perchlorate in solution was also estimated for each plant based on the mass of perchlorate stored in the tissues at the termination of the experiment, along with the mass of perchlorate available to the plant over the course of the entire experiment. These calculated depletions for the genotypic uptake experiment ranged from 0.7 to 22% of the initial concentration.

For the transpiration rate experiment, representative samples from the last solution change at each concentration-climate-genotype combination were analyzed, as well as samples from the last three solution changes for crisphead at each concentration-climate combination. The concentration of perchlorate remaining in solution ranged from 76 to 119% of the initial concentration for most samples, with the exception of just five samples. Three out of five of these samples (27, 46, and 64% of the initial concentration) were obtained from the crisphead 1.25 μg/L level for the "sunny, dry, warm" environment. The remaining two samples were also from crisphead, but were from solutions in the "cloudy, humid, cool" environments; one for the 1.25 μg/L level (69% of initial) and one for the 10 μg/L level (50% of initial). Crisphead was later shown to take up the most perchlorate; therefore, when transpiration rates were increased, perchlorate was depleted more rapidly from these solutions. The estimated overall perchlorate depletion in each bucket over the course of the transpiration rate experiment ranged from 1.5 to 10% of the initial concentration. Thus, the perchlorate concentrations were generally within ±20% of the initial values across both experiments.

Genotypic variability in perchlorate accumulation was demonstrated for different lettuce types grown under the same experimental conditions (Figure 1a). All lettuce types exhibited a linear increase in tissue accumulation with increasing perchlorate in solution. Perchlorate accumulated in the leafy tissues to varying amounts, ranging from 4 to 192 μg/kg FW and the ranking of perchlorate accumulation was crisphead > butter head > romaine > red leaf > green leaf. This ranking is similar to that found previously for mean perchlorate accumulation in a survey of lettuce in North America (*7*) with the exception of crisphead, which contained the least amount of perchlorate in that study. This is likely

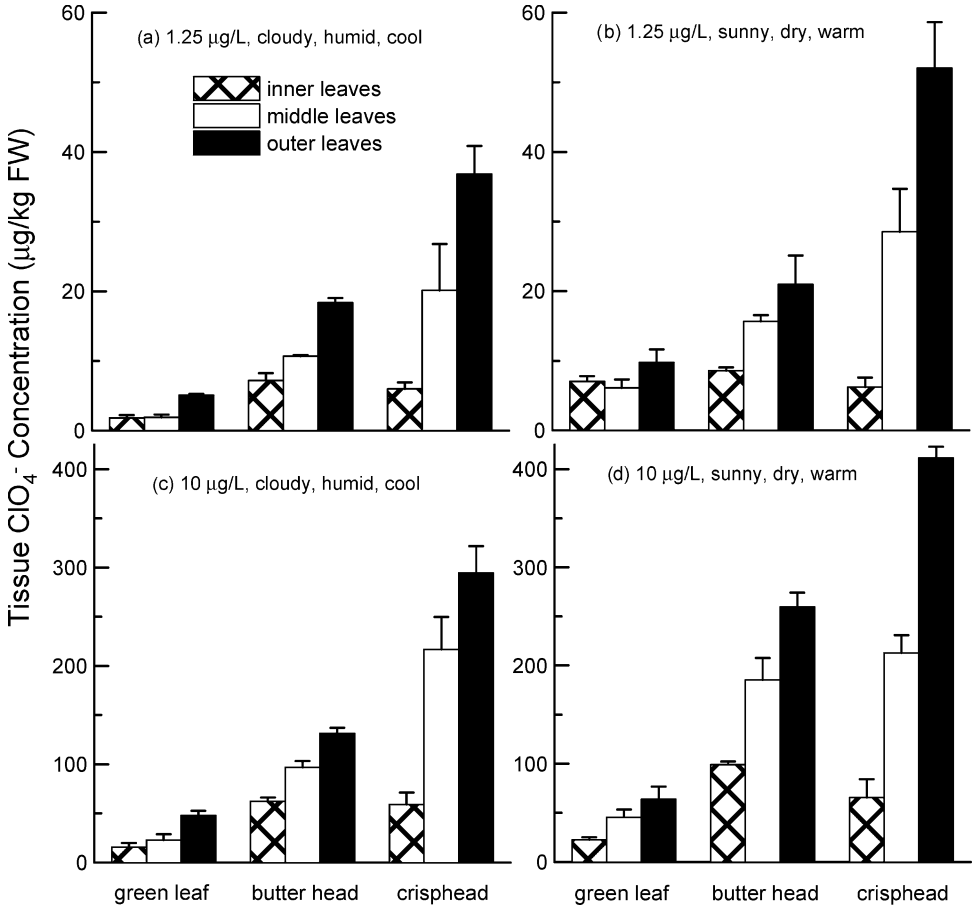

FIGURE 3. Variation in tissue perchlorate concentrations between inner, middle, and outer leaves of green leaf, butter leaf, and crisphead lettuce grown at either 1.25 μg/L (a, b) or 10 μg/L (c, d) in either a "cloudy, humid, cool" (a, c) or a "sunny, dry, warm" (b, d) environment. Error bars represent standard errors of the mean where $n = 3$ with the exception of the 1.25 and 10 μg/L "cloudy, humid, cool" butter head treatments and the 1.25 μg/L "cloudy, humid, cool" crisphead treatment where $n = 2$.

due to the removal of the outer leaves prior to analysis in the aforementioned study, but not here. When crisphead lettuce is packaged for market, the outer leaves are usually trimmed and only the edible head is packaged, which is the procedure used by Sanchez et al. (*7*) for crisphead lettuce in their study. The crisphead lettuce in our experiment was processed as whole-head with virtually no trimming prior to analysis, except where inner, middle, and outer leaves were sectioned as noted in the transpiration rate experiment.

A 2-way ANOVA between genotype and perchlorate concentration was performed on log-transformed data from the genotypic uptake experiment and the overall model was significant ($P < 0.0001$). There were significant differences with respect to tissue perchlorate concentration due to genotype ($P < 0.0001$) and to perchlorate concentration ($P < 0.0001$), but the interaction of genotype and concentration was not significant ($P = 0.385$). A post-hoc Tukey HSD test was also performed to compare across genotypes. This test indicated that of the five types, there are four distinct groups with respect to perchlorate tissue accumulation. Crisphead is in one group by itself, as it is significantly different from all of the other lettuce types ($P < 0.0001$). Butter head and romaine fall into the next group, as they are significantly different from crisphead, green leaf, and red leaf ($P < 0.0001$), but not from each other ($P = 0.429$). Green leaf and red leaf fall into the final two groups, as they are significantly different from crisphead, romaine, and butter head ($P < 0.0001$), as well as from each other ($P = 0.003$).

If perchlorate were simply taken up passively with the transpiration stream, perchlorate tissue concentration could be predicted based on the quantity of water transpired and the concentration of perchlorate in solution:

$$\text{predicted tissue concentration } (\mu\text{g/kg}) = \text{transpiration ratio (L/kg)} \times \text{solution concentration } (\mu\text{g/L}) \quad (1)$$

where transpiration ratio = total water transpired (L)/fresh weight of shoots (kg). All lettuce grown under the "standard" climatic conditions accumulates less perchlorate than predicted based on transpiration rate alone (Figure 1b). These data also further illustrate the genotypic difference in perchlorate accumulation in lettuce. Certain lettuce types (e.g., green leaf, red leaf) seem to substantially exclude perchlorate from their tissues while other types (e.g., crisphead) only minimally exclude perchlorate.

Crisphead, butter head, and green leaf lettuces were then used to assess the effect of transpiration rate on perchlorate accumulation. By growing lettuce in controlled-environment chambers with two climatic regimes, up to 2.7-fold differences in transpiration rate were achieved. Whole-head perchlorate analysis of crisphead, butter head, and green leaf again illustrated genotypic differences and, across all three genotypes, the plants that transpired more water (i.e., those grown in the "sunny, dry, warm" environment) accumulated more perchlorate (Figure 2a,b). A 3-way ANOVA between genotype, perchlorate concentration, and climate on tissue perchlorate concentration was performed on the log-transformed data and the overall model was significant ($P < 0.0001$). There were significant differences in log tissue perchlorate con-

centration due to genotype ($P < 0.0001$), perchlorate concentration ($P < 0.0001$), and climate (i.e., water transpired) ($P = 0.002$). Perchlorate concentration and genotype explained most of the variation in the data and, while significant, the effect of climate was not as great (Table 1). There were no significant interactions among genotype, perchlorate concentration, or climate.

The measured perchlorate concentration expressed as a percent of that predicted based on transpirational water use illustrates the relative importance of transpiration on perchlorate accumulation. Plants grown in the "sunny, dry, warm" environment accumulated less perchlorate than predicted (Figure 2c,d), which is consistent with plants grown under "standard" conditions (Figure 1b), but more pronounced. Most plants grown in the "cloudy, humid, cool" environment also accumulated less perchlorate than predicted, but the amounts accumulated were closer to the predicted values. Moreover, crisphead grown in the "cloudy, humid, cool" environment is the only lettuce variety that actually accumulated *more* perchlorate than predicted from transpiration (Figure 2c,d). There were 2.0−2.7-fold differences in transpiration ratio between lettuce grown in the two climatic extremes, but there were only 1.2−2.0-fold differences in perchlorate accumulation (Table 2). Thus, while transpiration was shown to play a role in perchlorate accumulation in lettuce, there seems to be other factors involved that are either equally or more important than transpiration rates.

Using smartweed, Tan et al. (*17*) recently demonstrated that perchlorate is partially excluded from the transpiration stream as water is taken up, which further supports the notion that perchlorate uptake is not simply a passive process in higher plants. Perchlorate uptake in lettuce is likely a membrane-mediated process that requires the use of transport proteins, similar to those for other anions such as nitrate and chloride. Nitrate has been shown to suppress chlorate (ClO_3^-) uptake by barley roots (*22*, and references therein) and it has been suggested that these two ions share a common transport mechanism. Thus, it is possible that perchlorate and nitrate could also share a common transport mechanism in lettuce. The fact that lettuce genotypes showed different responses to uptake suggests that either the uptake mechanisms among lettuce genotypes are different, or perhaps certain types (e.g., green leaf) have more of an ability to exclude perchlorate or to metabolize perchlorate once inside the tissues. Further research is needed to fully address these possibilities.

In addition to the whole-head analyses, plants were sectioned into inner, middle, and outer leaves and processed separately in the transpiration rate experiment. In general, the ranking of perchlorate tissue accumulation was outer leaves > middle leaves > inner leaves (Figure 3 a−d). The only exception to this was the green leaf lettuce grown at 1.25 μg/L where the perchlorate concentrations in the inner leaves were similar to or slightly higher than in the middle leaves. It is noteworthy that, although crisphead accumulates more perchlorate than butter head on a whole-head basis, the inner leaves of butter head contain more perchlorate than the inner leaves of crisphead (Figure 3 a−d). Most of the perchlorate in crisphead is stored in the outer leaves, whereas in butter head and green leaf a large amount is additionally stored in the inner and middle leaves. Because most of the perchlorate in crisphead lettuce is found in the outer leaves, which are usually removed prior to human consumption, crisphead lettuce likely does not contribute much to human exposure. But, the outer leaves of green leaf and butter head *are* consumed. These lettuce types may contribute more to human exposure than previously thought not only because the outer leaves of these lettuce types are consumed, but also because the inner and middle leaves additionally contain substantial amounts of perchlorate.

The differences seen in perchlorate accumulation among inner, middle, and outer leaves of crisphead and butter head lettuce may be due to lettuce-head morphology and, in turn, the role of transpiration. The outer leaves of crisphead are quite large as compared to butter head and thus have a larger transpirational surface area. The inner leaves of crisphead are small and curl inward to form a head. Thus, the inner leaves of crisphead likely transpire much less than the outer leaves. On the other hand, the inner, middle, and outer leaves of butter head are more similar in size and in exposure to solar radiation than those of crisphead, and thus the transpiration rates of different sections are likely more similar. This observation supports the notion that the amount of perchlorate accumulation in lettuce is proportional to the amount of water transpired through the leaves.

We have established that genotypic variation in lettuce does exist with respect to perchlorate accumulation and storage. Perchlorate tends to accumulate to a greater extent in outer leaves and to a lesser extent in inner leaves across all lettuce types tested. The outer leaves tend to be older, larger, and transpire more water than the younger, smaller, inner leaves. Transpiration is clearly an important factor in perchlorate accumulation in lettuce because increasing water use increases perchlorate concentration in lettuce but, on a whole-head basis, the effect is not 100% quantitative. These results indicate that perchlorate accumulation is not simply a passive process in higher plants. First, lettuce-head concentrations are usually less than predicted based on a passive flux of perchlorate in the transpirational water stream. Second, when climate is manipulated to vary transpiration rate, the relative increase in perchlorate concentration is less than the corresponding increase in water use. Thus, while transpiration rate is important for predicting perchlorate accumulation and storage in plants, other factors (e.g., soil salinity, membrane transport, plant-mediated perchlorate reduction) are likely important as well. Elucidation of these factors will further contribute to our fundamental, mechanistic understanding of perchlorate entry into the food chain, with its consequent implications for human exposure and health.

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