Plant and Environment Interactions

Biogeochemistry of Fluoride in a Plant–Solution System

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ABSTRACT

Fluoride (F^-) pollutants can harm plants and the animals feeding on them. However, it is largely unknown how complexing and chelating agents affect F bioavailability. Two studies were conducted that measured F^- bioavailability and uptake by rice (*Oryza sativa* L.). In the first study, rice was grown in solution culture (pH 5.0) with 0, 2, or 4 mM F^- as KF to compare the interaction of F^- with humic acid (HA) and with a conventional chelating agent, *N*-hydroxyethylenthylenediaminetriacetic acid (HEDTA). In the second study, F was supplied at 0, 0.5, 1.0, and 2.0 mM KF with an additional 2 mM F^- treatment containing solution Ca at 2× (2 mM Ca) the level used in the first study, to test the effect added Ca had on F^- availability and uptake. Total biomass was greatest with HEDTA and $F^- < 1$ mM. Leaf and stem F concentrations increased exponentially as solution F^- increased linearly, with nearly no F partitioning into the seed. Results suggest that F was taken up as HF^0 while F^- uptake was likely restricted. Additionally, F^- competed with HA for Ca, thus preventing the formation of Ca–HA flocculents. The addition of soluble Ca resulted in the precipitation of CaF_2 solids on the root surface, as determined by tissue analysis and energy dispersive X-ray spectroscopy.

T HE NONESSENTIAL HUMAN ELEMENT FLUORINE (F) has long been added at approximately 0.04 mM (0.7 mg L^{-1}) to 0.06 mM (1.2 mg L^{-1}) F^- to municipal drinking water (Public Health Service, 1991). Intake ranging from 1.5 to 4.0 mg d^{-1} is considered safe and adequate for the prevention of dental caries (National Research Council, 1989). Public concern about municipal fluoridation of drinking water, soil F contamination from industry, and the application of some F-laced fertilizers have renewed interest in F effects on plants and animals. For example, hydrogen fluoride deposition from some Al smelters, coal burning power plants, and P fertilizer processing facilities can harm the foliage of plants growing in deposition zones (Arnesen, 1997; Sun and Su, 1985). Some phosphorus fertilizers and fluorosilicate suspension fertilizer additives may provide an additional source of soil F and increase the bioavailability of Al, which in either case may hinder plant growth, particularly in acidic soils (Sikora et al., 1992; Singh et al., 1979; Stevens et al., 2000). The irrigation of high-F soils located in parts of India has resulted in F-contaminated ground water (Datta et al., 1996). Fluorine may enter the animal food chain through ingested plants, where daily intake of forage >100 mg kg^{-1} F can lead to fluorosis (Shupe and Olson, 1983; Puls, 1994). Additionally, water intake at concentrations >5 to 20 mg L^{-1} F^- may be dangerous to livestock (Puls, 1994).

The National Aeronautics and Space Agency (NASA) expects to include fluoridation in the bioregenerative life support habitats being developed for long-duration planetary stays at destinations such as the moon or Mars (Barta and Henninger, 1994; Pitts and Drysdale, 1998; Tri, 1999). The space station crew will receive F^- at a level ranging from 1.5 to 4.0 mg d^{-1} supplied through dental products (Lane and Rice, 1992). Controlling bioavailable F levels in the terrestrial environment or recycled wastes from NASA space habitats requires characterization of F biogeochemical processes. Two factors controlling F bioavailability in both terrestrial and space systems are: (i) the formation of solid phases with inorganic elements; and (ii) sorption by organic matter. In nature, F bioavailability is governed by sorption reactions that include precipitation–dissolution and adsorption–desorption processes occurring at aqueous–solid interfaces. Fluorite (CaF_2) may be the most important mineral controlling F^- activity in soils (Lindsay et al., 1989). Soil organic matter may also interact with F. For example, an increase in soil pH and formation of organic-AlF complexes occurred when F^- was added to soils (Elrashidi and Lindsay, 1987). Humic acid (HA) functional group counterions may also attract F^- (Farrah et al., 1985).

Plants do not require F, and tissue concentrations from uncontaminated soils rarely exceed 30 mg kg^{-1} dry mass (Kabata-Pendias, 2001). Plant F uptake is dependent on solution F activity, pH, substrate composition, and plant species. Critical solution levels of 1.5 to 4 mM F may lead to shoot dry mass concentrations of 70 to >300 mg kg^{-1} F for several plant species (Hara et al., 1977; Bar-Yosef and Rosenberg, 1988; Stevens et al., 1998a). Thermodynamically, increased soil acidity results in greater F bioavailability and hence, greater plant uptake (Horner and Bell, 1995), while increasing bioavailable Ca results in lower leaf F content but increased root F content (Brennan et al., 1950). It is conjectured that high root F is associated with the formation of CaF_2 either outside or inside the root (Ramagopal et al., 1969). The floriculture industry has evaluated Ca as a water additive to improve the shelf life of cut flowers

Abbreviations: DAT, days after transplanting; DOC, dissolved organic carbon; EDX, energy dispersive X-ray; HA, humic acid; HEDTA, *N*-hydroxyethylenthylenediaminetriacetic acid; NC, no chelate.

stored in fluoridated water. Cut roses pulsed with $Ca(NO_3)_2$ for 72 h before being placed in fluoridated water were less susceptible to F leaf injury (Pearson-Mims and Lohr, 1990).

The ameliorating effect of Ca may be related to its ability to bind with F forming insoluble solid phases before entering the root or perhaps within the xylem in the case of cut flowers. Once F has entered the apoplasm, it may proceed through the plasmalemma or react with various cations within the apoplasm, thus affecting plant nutrition. There have been many reports on the interference of F with membrane function, enzyme activity, and other metabolic processes (Yang and Miller, 1963; Shupe and Olson, 1983; Murphy and Hoover, 1992; Miller, 1993). These effects may not be offset by Ca nutrition alone, which might explain why Ca was unable to completely inhibit F toxicity in the aforementioned studies.

The objectives of this study were to characterize F uptake and partitioning in 'Super Dwarf' rice, characterize the effects of organic complexing agents, i.e., humic acid (HA) and N-hydroxyethylenthylenediaminetriacetic acid (HEDTA) used to enhance iron (Fe) solubility in solution cultures has on F uptake, and determine the ameliorating role Ca has on F uptake.

MATERIALS AND METHODS

Plant Culture

Two studies were conducted with 'USU–Super dwarf' rice. Due to system volume constraints, highly productive dwarf crop species are being considered for use in NASA's Advanced Life Support program (Bugbee, 2000). The focus of Study 1 was to characterize the complexing agents HA and HEDTA with regard to solution F solubility and to measure F uptake and partitioning within the plant. The focus of Study 2 was to determine the ameliorating role Ca has on F uptake.

Rice seed used in both studies were germinated in a 40-mm layer of moist inert medium (Isolite, Sumitomo Corp., Denver, CO) and a week after emergence the seedlings were transplanted (0 d after transplanting [DAT]) to a deep solution hydroponic system comprised of large polyethylene tubs (10 plants per tub), each containing 50 L nutrient starter solution (Tables 1, 2, and 3). Each polystyrene tub cover had 10- to 2.5-cm holes drilled and each hole contained a foam plug to support a single plant above the nutrient solution. The nutrient solution liquid level was checked daily with a portable glass manometer bearing a mark showing the liquid level setting. A refill solution (Tables 1 and 4) was added whenever the solution level dropped below the manometer mark to replace nutrients and water that were removed through plant uptake. The nutrient solution in each tub was vigorously mixed and aerated using polyvinyl chloride manifolds fed from an in-house air supply. Nutrient solution electrical conductivity (0.6 ± 0.07 dS m^{-1} beginning and 1.5 ± 0.09 dS m^{-1} end of study) was measured daily and pH (5.0 ± 0.5 units) was measured daily and adjusted, via acid/base. It should be noted that F treatments were added only once and F was not replaced in refill solutions. Greenhouse air temperature (28°C/22°C, light/dark), and daily canopy photosynthetic photon flux (29 mol m^{-2} d^{-1}) were measured periodically during the studies.

Table 1. Solution inorganic components for Study 1. The starter solution was used to fill the system and the refill solution was used to replace transpired water. The complexing agents for the starter solution are listed in Table 2.

Salt	Starter solution	Refill solution A†	Refill solution B
	m*M*		
KF	0, 2, or 4	0.0	0.0
KCl‡	4.0	0.0	0.0
KNO$_3$	1.0	0.0	1.0
NH$_4$Cl	0.0	2.0	0.5
Ca(NO$_3$)$_2$	1.0	1.0	0.5
KH$_2$PO$_4$	0.5	0.5	0.5
MgSO$_4$	0.5	0.5	0.25
K$_2$SiO$_3$	0.1	0.0	0.0
	μ*M*		
Fe§	50.0	7.5	7.5
ZnSO$_4$	4.0	2.0	2.0
MnCl$_2$	3.0	3.0	1.8
H$_3$BO$_3$	2.0	1.0	0.4
CuCl$_2$	1.0	1.0	1.0
Na$_2$MoO$_4$	9.0×10^{-2}	3.0×10^{-2}	3.0×10^{-2}

† The 0 m*M* F treatments received 4 m*M* KCl to normalize K with the 4 m*M* KF treatments. This solution was used daily to replace the first 25 L of transpired water in 2 m*M* KF treatments and the first 50 L of transpired water in the 0 and 4 m*M* KF treatments, after which refill solution B was used.

‡ Only the 0 m*M* F treatments received 4 m*M* KCl to normalize K with the 4 m*M* KF treatments.

§ Total Fe concentration in starter and refill solutions were equivalent among treatments. All the Fe in the HEDTA treatment starter solution was supplied as Fe-HEDTA and all the Fe in the HA and NC treatments was supplied as FeCl$_3$. The refill solution contained Fe as FeCl$_3$ for all treatments. Total Fe in each system was theoretically ample to supply plant Fe requirements.

Nutrient Solution Composition

Study 1

The starter solution was generally the same for all treatments, except for the F concentration and complexing agent. The three F treatments were 0, 2, and 4 m*M* F, which were added as KF at 14 DAT (Table 1). The three organic complexing agent treatments were: (i) 50 μ*M* synthetic chelate HEDTA supplied as FeCl$_3$–HEDTA; (ii) HA at 1 m*M* C and 50 μ*M* Fe supplied as FeCl$_3$; and (iii) no complexing agent (NC) and 50 μ*M* Fe supplied as FeCl$_3$ (Table 2). The HA (Aldrich Chemical Co., Milwaukee, WI) used in both studies was dissolved with KOH and desalted by placing it in 15 000 molcular weight cutoff dialysis tubing and soaking it in distilled–deionized water until the solution electrical conductivity (EC) was <0.10 dS m^{-1} (approximately 3 d). Non-F treatments received an additional 4 m*M* KCl at 14 DAT to match the K concentration of the 4 m*M* F treatments. This resulted in a total of nine treatments with three replicates (50-L tubs) per treatment.

All the tubs received a daily refill solution to replace water and nutrients lost via plant uptake. However, K was left out of the refill solutions until either 25 L (2 m*M* KF treatment) or 50 L (0 and 4 m*M* KF treatments) of refill were used, respectively. This allowed for the nutrient solution K in all treatments to decrease with plant uptake to a level (~3 m*M*)

Table 2. Complexing agent treatments for Study 1. The starter solution was used to fill the system. The complexing agents were not replaced by refill solution. The inorganic components for the starter solutions are listed in Table 1.

Treatment (symbol)	Starter solution
No chelate (NC)	–†
HEDTA + FeCl$_3$ (HEDTA)	0.05 m*M* Fe-HEDTA
Humic acid (HA) + FeCl$_3$	1.00 m*M* HA (as C)

† There was no complexing agent used in the NC treatments.

Table 3. Beginning nutrient solution treatment compositions for Study 2. The starter solution was used to fill the system. The refill solution, used to replace transpired water, is listed in Table 4.

Salt†	0 mM F	0.5 mM F	1 mM F	2 mM F	2 mM F + 2× Ca
			mM		
KF	0.0	0.5	1.0	2.0	2.0
KCl	1.0	0.5	0.0	0.0	0.0
NaNO$_3$	0.0	0.0	0.0	1.0	0.0
KNO$_3$	1.0	1.0	1.0	0.0	0.0
Ca(NO$_3$)$_2$	1.0	1.0	1.0	1.0	1.5
CaCl$_2$	0.0	0.0	0.0	0.0	0.5
KH$_2$PO$_4$	0.5	0.5	0.5	0.5	0.5
MgSO$_4$	0.5	0.5	0.5	0.5	0.5
K$_2$SiO$_3$	0.2	0.2	0.2	0.2	0.2
			μM		
Fe-HEDTA	50.0	50.0	50.0	50.0	50.0
ZnSO$_4$	4.0	4.0	4.0	4.0	4.0
MnCl$_2$	3.0	3.0	3.0	3.0	3.0
H$_3$BO$_3$	2.0	2.0	2.0	2.0	2.0
CuCl$_2$	2.0	2.0	2.0	2.0	2.0
Na$_2$MoO$_4$	0.09	0.09	0.09	0.09	0.09

† Each treatment contained 3, 0.5, 2.7, and 0.5 mM of N, P, K, and Mg, respectively. The 0, 0.5, 1, and 2 mM F treatments contained 1 mM Ca and the 2× Ca treatment contained 2 mM Ca.

typically found in our standard nutrient solution (Table 1, column 2 without KCl). When the nutrient solution EC rose above 1.3 dS m^{-1}, (~50 DAT), DI water containing 7.5 μM FeCl$_3$ was used as the refill solution to prevent further increases in EC, while maintaining adequate Fe nutrition.

Study 2

All treatments contained the same starter solution until 14 DAT when F treatments were added at 0, 0.5, 1, and 2 mM F and 2 mM F + 2mM Ca (Table 3). Initial solution K was equivalent (2.9 mM) across all treatments. The study had a total of five treatments with three replicates (50-L tubs) per treatment. All the treatments received the same refill nutrient solution to replace nutrients and water loss via plant uptake. The refill solution was modified over time to maintain balanced nutrition (Table 4).

The chemical equilibrium model GEOCHEM-PC (Parker et al., 1995) was used to predict metal ion activities among nutrient solution treatments. The accompanying literature states that many of the equilibrium constants have been extracted from older work and some of the equilibrium constants have not been verified. GEOCHEM-PC does not contain data for HA, so equilibrium constants were taken from the literature (Van Dijk, 1971; Takahashi et al., 1997; Hering and Morel, 1988) and entered into the model. Likewise, we replaced the $-\log K_{sp}$ values for solid phases CaF$_2$ and MgF$_2$ in the

Table 4. Refill solution compositions for Study 2. The refill solutions were used to replace transpired water.

Salt†	0–21 DAT†	21–37 DAT	37–63 DAT
		mM	
NH$_4$NO$_3$	2.0	0.0	0.0
NH$_4$Cl	0.0	2.0	1.0
KNO$_3$	0.0	2.0	3.0
Ca(NO$_3$)$_2$	1.0	1.0	1.0
KH$_2$PO$_4$	0.5	0.5	0.5
MgSO$_4$	0.5	0.5	0.5
		μM	
FeCl3	7.5	7.5	7.5
ZnSO$_4$	2.0	2.0	2.0
MnCl$_2$	3.0	3.0	3.0
H$_3$BO$_3$	1.0	1.0	1.0
CuCl$_2$	1.0	1.0	1.0
Na$_2$MoO$_4$	0.03	0.03	0.03

† Days after transplant. Refill solutions were adjusted periodically to moderate pH changes with NH$_4^+$ and to meet K demand.

GEOCHEM-PC model with the respective constants 9.1 and 10.1, obtained from Elrashidi and Lindsay (1985).

Sampling and Analyses

During Study 1, nutrient solution samples (20 mL) were taken weekly and analyzed for dissolved organic carbon (DOC) using a Phoenix 8000 carbon analyzer (Tekmar-Dohrmann, Cincinnati, OH). Another set of 10 mL samples was taken for F analysis. They were mixed with 10 mL of total ionic strength adjustment buffer (TISB) and then analyzed for F$^-$ using an Orion combination ion selective electrode (Orion Research, Cambridge, MA). Additional 100 mL samples were taken from Study 1 at the time of plant sampling (28, 65, and 100 DAT), and sent to NASA's Kennedy Space Center for nutrient elemental analysis using inductively coupled plasma atomic emission spectrometry (ICP-AES), but only the Ca values are presented.

For Study 1, six plants per tub were harvested at 28 and 65 DAT and the remaining plants (four per tub) were left until the final harvest (100 DAT). For Study 2, five plants were harvested at 35 and 63 DAT (only 63 DAT data presented). During each harvest, tissue was separated into seeds, chaff, leaves, stems, and roots for Study 1 or shoot (leaves + stem) and roots for Study 2. Roots were not rinsed to preserve existing surface coatings; however, excess nutrient solution was manually expressed from the roots before drying. All tissue was oven-dried (80°C for 3 d) before weighing. Tissue samples were ground (2-mm sieve), digested with HNO$_3$ and H$_2$O$_2$, and inorganic elemental content determined by ICP-AES. Additional samples were processed for F$^-$ detection using the alkali fusion–selective ion electrode technique described by McQuaker and Gurney (1977). Briefly, plants (250 mg) were fusion digested with 16 M KOH in Ni crucibles in a muffle oven at 600°C for 30 min. Following cooling, the samples were resolublized with 10 mL water and pH adjusted to 8 to 9 with HCl. They were then diluted to 100 mL and filtered through Whatman no. 42 paper. Equal volume of sample and TISB were combined and analyzed for F$^-$ with an ion selective electrode.

In Study 2, samples of 63 DAT oven-dried roots were mounted on stubs, coated with C using an ion beam sputterer, and examined with a Hitachi S-4000 scanning electron microscope (SEM). Energy dispersive X-ray (EDX) digital dot mapping for F and Ca was performed with the Link EXL 1000 EDX system (Oxford Instruments, Oak Ridge TN). Roots for

EDX dot mapping were oven-dried instead of using the typical tissue preparation process, i.e., solution fixation, which washes away root coatings. Additional oven-dried ground shoot tissue from the 0 mM, 2 mM, and 2 mM F + 2x Ca treatments were put through a sequential extraction procedure to determine the different binding forms of Ca and F in the shoots (Fink, 1991). Briefly, soluble Ca and F was determined by mixing oven-dried ground tissue (200 mg) with 6 mL ultra pure water in 20-mL glass scintillation vials for 12 h at 23°C. This was followed by a 6 mL 2 M acetic acid (CH_3COOH) extraction representing pectates/phosphate Ca and F, and finally a 6 mL 2 M HCl extraction representing cell wall and oxalate Ca and F. Samples were passed through a 0.2 µM glass filter following each extraction. Oven-dried material may skew Ca values toward less soluble fractions (Bradfield, 1977). Because our interest was in relative fraction changes among treatments, the use of oven-dried material was adequate for our purposes. The extracts were analyzed for Ca by ICP-AES and F by ion selective electrode, using the methods previously described.

Statistical Analysis

A randomized 3 × 4 factorial design with three complexing agents, four levels of F, and three replicates were adopted for Study 1 and a randomized design with three replicates was adopted for Study 2. Data were analyzed using analysis of variance (ANOVA) and the level of significance was calculated from the F values of the ANOVA (Prism 3.0, Graphpad Software, San Diego, CA). Means separations were determined by using least significant differences (LSD).

RESULTS AND DISCUSSION

Growth and Partitioning

In Study 1, plants treated with 4 mM F or plants grown without a complexing agent (NC) had the lowest relative growth rate (RGR) values (gram dry mass of growth per gram dry mass of biomass per day) (Fig. 1). Plants grown with HEDTA had greater RGR than plants grown with HA at 0 mM F, but if 2 mM F or 4 mM F was added then HEDTA and HA treatment effects were indistinguishable. The 2 mM F treatment resulted in plant F toxicity while NC plants suffered from Fe deficiency (Fig. 1). In Study 2, a linear response was found between F$^-$ measured in solution and re-
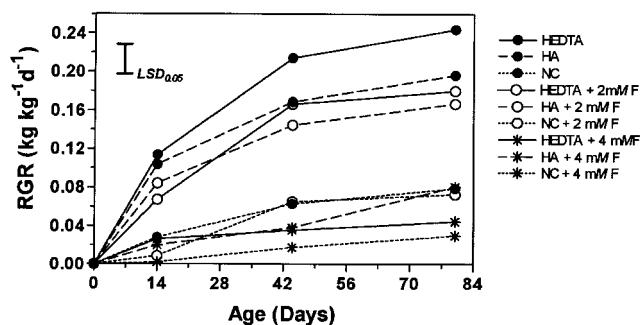
sulting biomass, where solution F$^-$ above 0.5 mM inhibited total plant biomass (Fig. 2).

In Study 1, HA had no influence on F partitioning in the plant, so the data were pooled to study F effects. The 2 mM F treatment resulted in the following tissue F values: root (3436 ± 1743 mg kg^{-1}) = stem (1436 ± 604 mg kg^{-1}) = leaves (1315 ± 408 mg kg^{-1}) > chaff (35 ± 16 mg kg^{-1}) > seed (1.4 ± 1.8 mg kg^{-1}). Even with a solution value of 4 mM F, seed F remained <3 mg kg^{-1}. The relatively low seed content is a typical response to elements that are transported via the xylem or restricted by a barrier that prevents F transport into the seed. Laszlo (1994) has found that the soybean [*Glycine max* (L.) Merr.] seed coat greatly limits Ca transport from the phloem and apoplasm into the seed. Seeds from Vernal sandwort [*Minuartia verna* (L.) Hiern] and broadleaf dock (*Rumex obtusifolius* L.) grown in F-contaminated soils contained approximately 35 mg kg^{-1} F in decoated seeds, but values increased by more than 1000-fold if the seed coats were included in the analysis (Cooke et al., 1976). Although there are few reports on F partitioning into the seed portion of grain crops, relatively low seed F was found for soybean (Ghiasseddin et al., 1981) and rice (Singh et al., 1979; Sun and Su, 1985), thereby supporting our results.

Fluoride Bioavailability

Plant water uptake and tissue F data were used to determine if F uptake was an active, passive, or restricted process in our studies. If shoot F content were greater than the total amount supplied via transpiration (i.e., nutrient solution uptake), F uptake was likely active. If shoot F content approximated transpiration-supplied F, then uptake was likely passive, and if shoot content was less than the transpiration-supplied F, then uptake may have been restricted. Estimates of nutrient solution Ca and F speciation were calculated using the chemical equilibrium model GEOCHEM-PC (Parker et al., 1995) (Table 5). By knowing the total amount of F taken up by the plant relative to water uptake via transpiration, the F activity required to support passive F uptake could be calculated (Table 5, Column 7).

The F$^-$ nutrient solution activities among treatments were orders of magnitude greater than HF^0 activities, suggesting that if F$^-$ uptake were predominantly pas-

Fig. 1. Complexing agent and F effects on rice relative growth rate (RGR) in Study 1. Least significant difference (LSD$_{0.05}$) was used to compare treatment means. HA, humic acid; HEDTA, *N*-hydroxyethylenthylenediaminetriacetic acid; NC, no complexing agent.
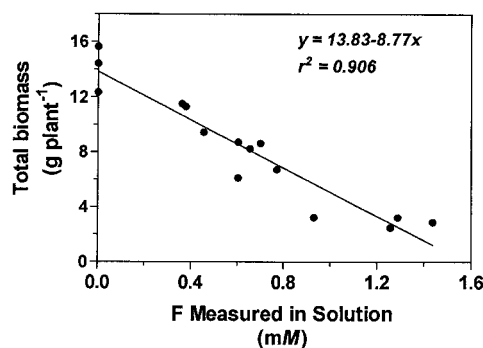

Fig. 2. In Study 2 a linear relationship exists between total plant biomass and solution F, as measured with an ion selective electrode. Each data point represents one observation.

Table 5. Nutrient solution activities calculated with GEOCHEM-PC.

Treatment	Complexing agent	Ca^{2+}	F^-	HF^o	CaF_2 solid	Passive F uptake†
				— mM —		
				Study 1		
0 mM F	HEDTA	0.626	–‡	–	–	–
	Humic acid	0.216	–	–	–	–
2 mM F	HEDTA	0.179	0.472	7.54×10^{-3}	0.734	1.2×10^{-2}
	Humic acid	0.042	0.975	1.55×10^{-2}	0.461	4.1×10^{-2}
4 mM F	HEDTA	0.012	1.803	2.27×10^{-2}	0.981	9.3×10^{-3}
	Humic acid	0.007	2.366	2.37×10^{-2}	0.690	7.3×10^{-3}
				Study 2		
0 mM F	HEDTA	0.661	–	–	–	–
0.5 mM F	HEDTA	0.595	0.258	4.07×10^{-3}	0.103	1.5×10^{-3}
1.0 mM F	HEDTA	0.450	0.297	4.76×10^{-3}	0.332	2.7×10^{-3}
2.0 mM F	HEDTA	0.179	0.472	6.00×10^{-3}	0.734	4.2×10^{-3}
2.0 mM F + 2× Ca	HEDTA	0.743	0.231	4.63×10^{-3}	0.867	6.0×10^{-3}

† Solution F concentration required to support the passive F uptake theory. Values calculated from shoot F concentration, shoot dry mass, and water uptake per plant through 65 d after transplant (DAT) for Study 1 and 63 DAT for Study 2.
‡ Treatment does not contain F.

sive, then shoot F concentrations should have been higher. Therefore, it may be concluded that F^- uptake was restricted. However, it is possible that the more membrane-permeable HF^0 species was the primary form absorbed by the plants. Stevens et al. (2000) dismissed the possibility of HF^0 being a major player in F uptake in their study because its activity at pH 5.5 was 10^3 times lower than F^- activity. However, HF^0 activity increases as solution pH decreases (log $K_a = -2.24$, Elrashidi and Lindsay, 1985) and tomato (*Lycopersicon esculentum* Mill.), oat (*Avena sativa* L.), ryegrass (*Lolium multiflorum* Lam.), wheat (*Triticum aestivum* L.), and *Chlorella* F toxicities also worsened with decreasing pH (Horner and Bell, 1995; Rai et al., 1998; Stevens et al., 1998b). The permeability coefficient for HF^0 passing through a lipid bilayer membrane is approximately 1.4×10^{-4} cm s^{-1}, whereas F^- is 4.9×10^{-11} cm s^{-1} (Gutknecht and Walter, 1981). It is conceivable that HF^0 uptake might exceed F^- uptake, even at pH 5.5. If HF^0 were the major species taken up by rice, then its activity should match the predicted passive F uptake value. Indeed, this is the case (Table 5). Plants grown in neutral to alkaline pH soils would likely tolerate higher soil F because available F would be present mostly as F^-, which possibly has restricted uptake (Horner and Bell, 1995; Stevens et al., 2000). Additionally, other elements

found in calcareous soils, namely Ca, Mg, and P, form insoluble solids with F.

Chemical equilibrium modeling predicted significant F × HA × Ca solution interactions in Study 1, where increasing total solution F or HA resulted in less free Ca^{2+} activity (Table 5, Study 1). This prediction was supported by the nutrient solution data, where lower Ca^{2+} concentrations were measured in solutions containing HA or increasing F concentrations (Fig. 3). The model also predicted a decrease in HA–Ca complexation with increasing solution F, where concentrations from the 0, 2, and 4 mM F treatments would result in 66, 48, and 30% of HA complexing with Ca, respectively. By monitoring nutrient solution DOC, we observed the behavior predicted by the model (Fig. 4). Lower solution F likely resulted in increased HA flocculation with Ca and subsequent precipitation. Others have reported similar findings (Farrah et al., 1985; Kremlenkova and Gaponyuk, 1984). In contrast, solution F did not affect HEDTA × Ca interactions (Fig. 4), which is also supported by the model.

Calcium × Fluoride Interactions

In Study 2, root Ca levels increased significantly with increasing solution F, and the 2× Ca treatment resulted
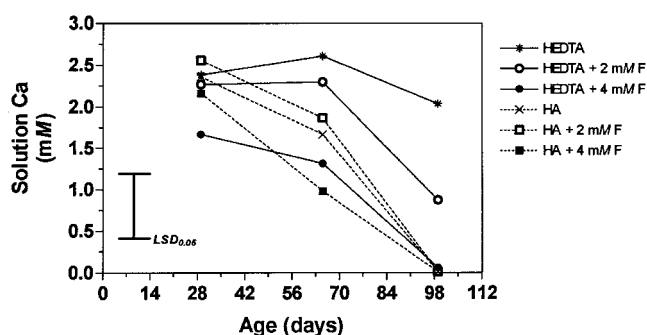
Fig. 3. Solution F and complexing agent effects on nutrient solution Ca concentration over time in Study 1. HA, humic acid; HEDTA, *N*-hydroxyethylenthylenediaminetriacetic acid. Least significant difference (LSD$_{0.05}$) was used to compare treatments. Measurable Ca tended to decline with increasing solution F or if HA was included in the solution.
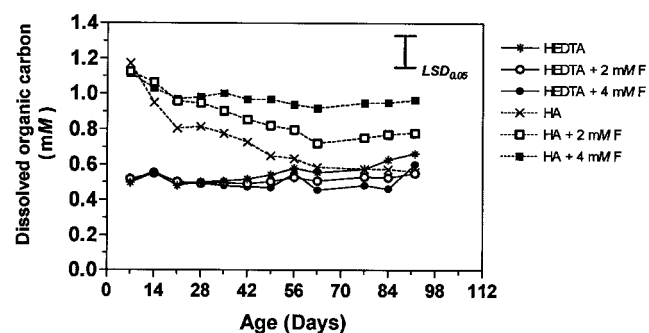
Fig. 4. Solution F and complexing agent effects on nutrient solution dissolved organic carbon (DOC) over time in Study 1. HA, humic acid; HEDTA, *N*-hydroxyethylenthylenediaminetriacetic acid. Least significant difference (LSD$_{0.05}$) was used to compare treatment means. With increasing solution F, more HA remained in solution, likely due to a decrease in HA–Ca flocculation. Carbon in the HEDTA treatment came from the chelate.

Table 6. Effect of F treatment on total biomass and tissue F and Ca composition from Study 2.

F treatment	Plant dry mass	Shoot F†	Root F†	Shoot Ca	Root Ca†
	g plant^{-1}		mg kg^{-1}		
0 mM F	14.12 a‡	24.9 a	48.2 a	5 163 a	2 107 a
0.5 mM F	10.77 ab	71.7 b	195.3 b	3 097 b	2 307 a
1.0 mM F	7.81 bc	148.5 bc	540.8 b	3 615 b	2 650 a
2.0 mM F	2.84 d	394.9 d	3 217 c	3 753 b	6 313 b
2.0 mM F + 2× Ca	6.05 cd	315.7 cd	21 627 d	4 068 ab	51 200 c
P value	<0.0001	<0.0001	<0.0001	0.0054	<0.0001

† One-way ANOVA with log transformed data.
‡ Mean separation by Tukey test. Means within a column followed by the same letters are not significantly different at P = 0.05.
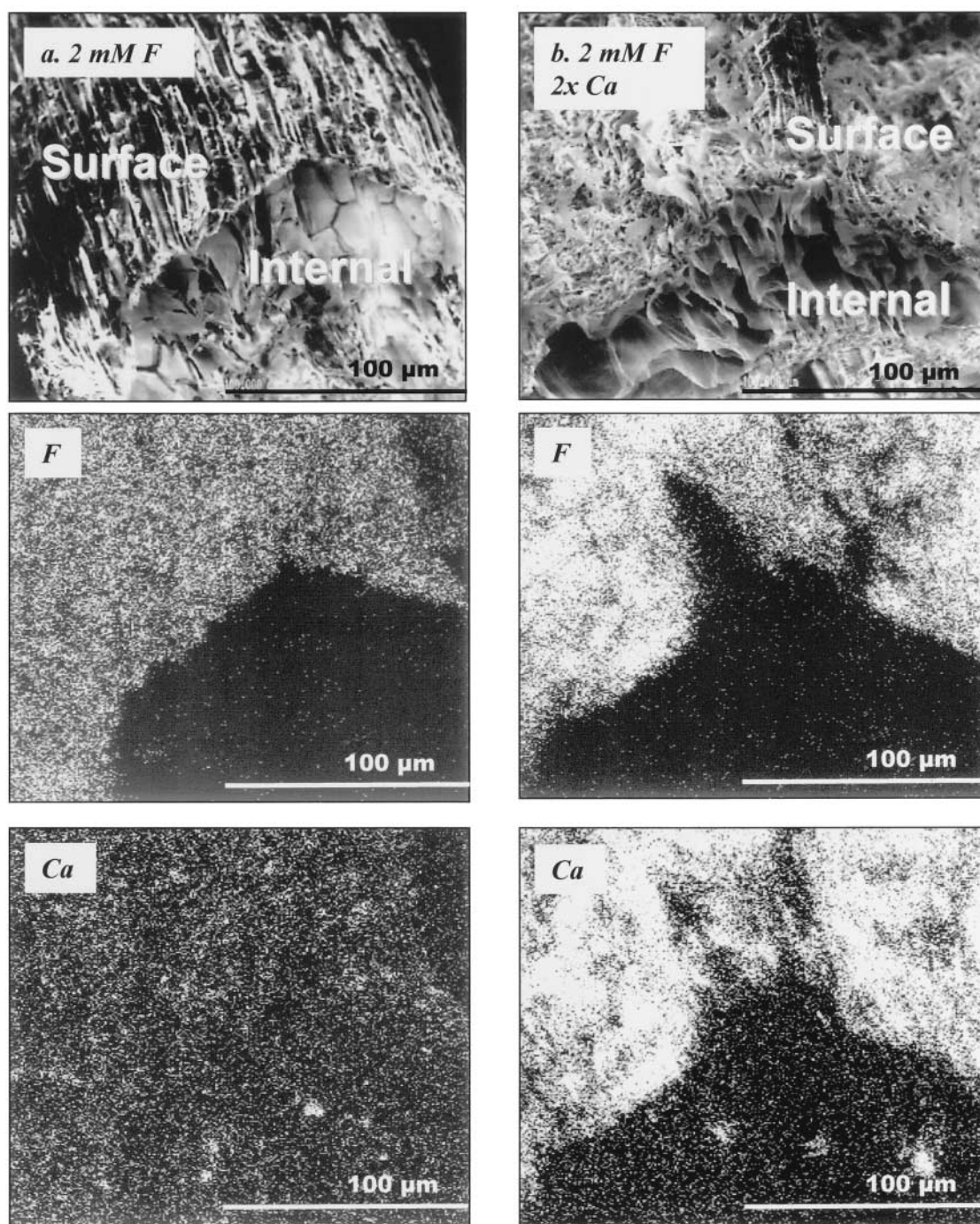

Fig. 5. Scanning electron micrographs of (*a*) oven-dried rice root segments from the 2 mM F and (*b*) the 2 mM F + 2x Ca treatments, respectively. The energy dispersive X-ray digital dot maps represent root surface Ca and F, respectively.

in an eightfold increase in root-associated F (Table 6). The roots were not washed at harvest because any surface coatings were meant to be included in the analysis. To better discern where the F and Ca were located, SEM and EDX dot mapping of oven-dried root segments were performed. In the treatment containing moderate Ca (1 mM), the majority of root-associated F was located on the root surface and the Ca seemed to be evenly distributed among internal tissues and the root surface (Fig. 5a). However, the signal intensity for Ca and F associated with roots from the 2× Ca treatment was much stronger at the root surface (Fig. 5b). It appears that CaF_2 solids precipitated on the root surface in the 2× Ca treatment. Additionally, the 2× Ca treatment tended to lower shoot F and increase plant dry mass (Table 6), which likely was due to F becoming immobilized as CaF_2 root coatings. Although the geochemical model indicated free solution Ca^{2+} would be much higher in the 2× Ca treatments, the resulting shoot Ca values suggest the model may have over estimated free solution Ca^{2+} to some extent (Table 5, Study 2).

A three-step sequential extraction procedure was conducted on shoot tissue from Study 2 to determine Ca and F cellular distribution where: (i) the water extraction represented free Ca^{2+} and F^-; (ii) the acetic acid extraction represented Ca and F associated with the middle lamella, specifically pectates and phosphates; and (iii) the acid extraction represented cell-wall Ca and F and Ca oxalates (Bradfield, 1977). Approximately 70% of total Ca was associated with the acid-extracted fraction in plants without F. This is representative of acid extraction fractions reported from fresh rice leaf tissue (Shibata et al., 1981). However, acid-extracted Ca declined from 2850 to 1329 mg kg^{-1} with the 2 mM F treatment, and recovered somewhat to 1808 mg kg^{-1} Ca with the 2× Ca treatment (LSD = 171 mg kg^{-1}). The decrease in acid-extractable Ca from 70 to 42% with increasing solution F may reflect a decrease in Ca oxalates rather than cell wall Ca. Venkateswarlu et al. (1965) reported that >50% of leaf F is water soluble, compared with <20% of Ca, suggesting that most plant F does not bind directly with cell wall Ca. Based on our data, it appeared that F inhibited Ca oxalate formation either directly by preventing Ca from entering the plant, or indirectly by affecting oxalic acid's availability to form Ca oxalates. It is unlikely that F inhibited total oxalate formation. To the contrary, as F enters the plant predominantly as HF^0, it will dissociate to H$^+$ and F$^-$, thereby increasing the plant organic acid (i.e., oxalic acid) requirement to buffer cellular pH (Marschner, 1995). Libert and Franceschi (1987) have suggested that oxalic acid plays a pH-buffering role. Additionally, Yang and Miller (1963) reported that F-treated plants were higher in several different organic acids, although they did not report on oxalic acid specifically.

Since 51 to 55% of the extractable F was associated with pectates (acetic acid extractable), F uptake also might have interfered with Ca associated with the middle lamella. Pectate Ca increased from 880 to 1156 mg kg^{-1} (LSD = 171) with added F. Increased solution Ca had reduced F concentrations in all extracts, but it did not change the F fraction percentages. This agrees with the hypothesis that Ca physically limits the uptake of F by forming CaF_2 solids outside the plant while F interferes with plant Ca deposition and partitioning within the plant.

To more fully understand the Ca–F relationship within the plant, additional microanalytic techniques are required. Use of transmission electron microscopy (TEM) with EDAX may reveal cellular Ca and F deposition sites. A synthetic Ca pectate membrane has been used to study Ca interactions with other elements, such as Al (Blamey et al., 1993) and therefore it may be a useful tool for determining F effects on the middle lamella. Adapting X-ray absorption fine structure (XAFS) techniques to detect cell wall Ca–F solid phases or even using XAFS to analyze F effects on pectate membranes also may provide useful information at the cellular level.

SUMMARY

Pollution deposition of F on vegetation and F increases in some soils and water have increased the interest in characterizing F bioavailability and uptake in plants. We measured F uptake and partitioning in 'Super Dwarf' rice, characterized the effects of organic complexing agents on F bioavailability, and determined the ameliorating effect of Ca on F uptake. Rice growth decreased as solution KF increased from 0 to 4 mM. Roots contained the highest F concentrations, while relatively little F partitioned into the grain. In slightly to moderately acidic environments, HF^0 rather than F$^-$ may control total F uptake, but some restricted F$^-$ uptake cannot be ruled out. Although HA had no effect on F uptake, it played a role in nutrient solution dynamics, where increasing solution F greatly reduced soluble Ca and its ability to cause HA flocculation. Providing additional solution Ca greatly increased the formation of CaF_2 coatings on root surfaces and restricted F entry into plants by 20%. Based on shoot extraction data, it appeared that Ca interfered with F uptake by forming CaF_2 solid phases outside the plant, whereas, if F entered the plant then it interfered with Ca deposition and partitioning. Additional microanalytic techniques are required to better characterize how F interferes with Ca within the plant.

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