Amer. J. Bot. 71(5): 666–675. 1984.

FORMATION AND MORPHOLOGY OF AN IRON PLAQUE ON THE ROOTS OF TYPHA LATIFOLIA L. GROWN IN SOLUTION CULTURE[1]

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ABSTRACT

Roots of *Typha latifolia* L. exposed to Fe^{2+} under reduced conditions in solution culture developed visible coatings (plaques) of an oxidized Fe compound that extended as much as 15–17 μm into the rhizosphere. Iron concentrations were significantly less and discoloration was not apparent on the surface of roots exposed to Fe-$(BPDS)_3$, Fe^{3+}, Fe-EDDHA, and Fe-EDTA. The extent of plaque formation increased with the concentration of Fe^{2+} in solution and with pH of the solution in the range of 3.0 to 4.6. Above pH 4.6, oxidation of Fe^{2+} in the culture solution may have reduced precipitation of Fe on the root surface. Plaque development was most extensive approximately 1.0 cm from the root tip, but all root surfaces showed some Fe staining. Scanning electron micrographs of plaqued roots, grown both in solution culture and in the field, provided support for a model of cast formation by oxidation and precipitation of Fe on external cell surfaces.

IN NATURAL WETLANDS, including contaminated wetlands in the Sudbury region, we have commonly observed coatings of an Fe compound on the roots of *T. latifolia.* Such coatings, henceforth plaques, have been observed previously on roots of *Phragmites communis* (Taylor and Crowder, 1983c), *Oryza sativa* (Boone, Bristow and VanLoon, 1983; Chen, Dixon and Turner, 1980a, b; Bacha and Hossner, 1977; Green and Etherington, 1977; Bartlett, 1961), *Spartina alterniflora* (Mendelssohn and Postek, 1982), and a number of other bog and wetland plants (Armstrong and Boatman, 1967; Jones and Etherington, 1970). Bartlett (1961) also found plaques on the roots of some upland plants exposed to waterlogged conditions. Chen et al. (1980a) and Bacha and Hossner (1977) identified alpha-FeOOH and gamma-FeOOH as the primary constituents of such plaques on the roots of *Oryza sativa.*

Howeler (1973) suggested that formation of an Fe plaque may diminish the root's capacity to absorb essential nutrients, and a number of authors have suggested that oxidation of the rhizosphere may reduce the toxicity of reduced substances such as Fe^{2+}, Mn^{2+}, and S^{2-} (Mendelssohn and Postek, 1982; Chen et al., 1980a, b; Armstrong, 1978, 1972, 1967, 1964; Bacha and Hossner, 1977; Howeler, 1973; Jones and Etherington, 1972). The results of Taylor and Crowder (1983a, b) demonstrated the tolerance of *Typha latifolia* L. to Cu and Ni contamination, but implicated different tolerance mechanisms. Plants growing in a contaminated marsh near Sudbury, Ontario, effectively excluded Cu and Ni from aerial tissues (Taylor and Crowder, 1983a), while plants grown in solution culture accumulated considerable concentrations of Cu and Ni in aerial tissues (Taylor and Crowder, 1983b). Taylor (1983) and Taylor and Crowder (1983b) suggested that an Fe plaque could be involved in the immobilization of Cu and Ni at the rhizosphere of *T. latifolia,* thus accounting for apparent exclusion of these metals under contaminated field conditions.

In order to test the latter hypothesis at a later date, we have developed a technique to generate plaques on the roots of *T. latifolia* grown in solution culture. This paper reports on this technique, investigates some of the chemical parameters affecting plaque formation, and describes the morphology of the resulting Fe plaques.

METHODS AND MATERIALS—Dormant overwintering rootstocks of *T. latifolia* were collected from an uncontaminated marsh on Hill Island, Lansdowne, Ontario. Previous results

[1] Received for publication 12 July 1983; revision accepted 7 December 1983.

This study was supported by funds from the National Research Council of Canada (grant no. A0330 and contract no. OSU82-00224) and the Queen's University Advisory Research Committee. We would also like to thank Henry Versteppen of the Department of Anatomy for technical assistance with scanning electron microscope techniques, Sheila Macfie for assistance in collection of field specimens, and Dr. Bill Newcomb for assistance in preparation of photographic plates.

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indicated that plants collected from the Hill Island site and a contaminated site near Sudbury, Ontario, showed similar metal uptake patterns and were equally tolerant of Cu and Ni (Taylor, 1983; Taylor and Crowder, in press). Plants from both sites were equally capable of plaque formation. Rootstocks transferred to flats in a growth chamber, gave rise to new vegetative shoots which were severed from the parent rootstocks and mounted on covers of black buckets of 5-l capacity (5 plants per bucket). Plants were mounted with Terostat type VII putty (Teroson GmbH, Heidelberg) with the roots extending down into the nutrient solution below. Nutrients were supplied as full strength Hoagland's solution (pH 5.5) (Hoagland and Arnon, 1938), with iron supplied as 5.0 μg g^{-1} Fe-EDTA (Ferric ethylenediaminetetraacetic acid) (Jacobson, 1951). Plants were maintained in a controlled-environment chamber at 23 C for a 16-hr light period and at 15 C during darkness. Light intensity was 175 μEm^{-2} s^{-1} at the leaf bases, level, increasing to 1,000 μEm^{-2} s^{-1} as leaves grew towards the light source. Plants maintained in this fashion continued to generate new vegetative offshoots which, when severed from the parent plant and allowed six weeks recovery and growth, were suitable for experimental purposes.

Effect of Fe source—After preparation as described above, five experimental plants were allocated to each of six treatments. Treatments included Fe^{2+} as $FeSO_4$, Fe-$(BPDS)_3$ (ferrous-4,7-di(4-phenylsulfonate) 1,10-phenanthroline or bathophenanthroline disulfonic acid), Fe^{3+} as $FeCl_3$, Fe-EDDHA (ferric ethylenediamine-di(o-hydroxyphenylacetic acid)), Fe-EDTA, and a control treatment with no added Fe. The Fe^{2+} chelate was prepared with $FeSO_4$ and the Na salt of BPDS, and the Fe^{3+} chelates were prepared with $FeCl_3$ and EDDHA or Na_2EDTA. All solutions, except the control, contained 20 μg g^{-1} Fe, and were renewed once every 3 days of the 12-day exposure. The pH of the growth solution was adjusted to 5.0 twice daily with KOH or HCl. Solutions were maintained in a deoxygenated condition by bubbling with N_2.

Effect of Fe^{2+} concentration—Four experimental plants (new vegetative offshoots) were allocated to each of six treatments. Fe^{2+} was supplied at concentrations of 5, 10, 20, 50, 75, or 100 μg g^{-1} as $FeSO_4$. Nutrient solutions were renewed daily and the pH adjusted twice daily with KOH or HCl to pH 4.2. Solutions were maintained in a deoxygenated state by bubbling with N_2 for the duration of the 7-day experiment.

Effect of pH—Four experimental plants (new vegetative offshoots) were allocated to each of 6 treatments and one control. Plants were supplied with 50 μg g^{-1} Fe^{2+} as $FeSO_4$ at pH values ranging from 3.0 to 5.0 in 0.4 unit increments. The control treatment was supplied with deionized water adjusted to a pH of 4.2. Nutrient solutions were renewed daily and the pH was adjusted twice daily with KOH or HCl. Solutions were maintained in a deoxygenated state by bubbling with N_2 for the duration of the 7-day experiment.

Analysis of plant tissues—At the completion of each experiment, plants were harvested, washed twice with distilled water, and divided into leaf and root material. Only the top 25 cm of the four tallest leaves, some of the oldest leaf material, were used for metal analysis. The leaf material was dried to constant weight at 60 C, and 0.3 to 0.5 g were digested for 12 hr at 120 C with 2 ml HNO_3 and 1 ml of H_2O_2 in Teflon pressure chambers. The chambers were then washed twice with distilled water and the resulting solution was made to volume (25 ml) for determination of Fe. Iron from the root surface was extracted using the DCB (dithionite-citrate-bicarbonate) technique of Jackson (1958) as modified by Taylor and Crowder (1983c). Approximately 0.3 to 0.5 g (estimated dry wt) of fresh root material was placed in a solution of 40 ml of 0.3 M sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$) and 5 ml of 1.0 M sodium bicarbonate ($NaHCO_3$) at room temperature (21 C). Three grams of sodium dithionite ($Na_2S_2O_4$) was added and the mixture was agitated continuously for 3 hr. After the 3-hr extraction period, the wash was collected and the roots were washed three times with 15 ml of deionized water. The resulting solution was made to volume (100 ml) with deionized water. To determine the amount of Fe remaining in the roots after washing, the root material was then dried to constant weight at 60 C and prepared for determination of Fe as previously described for leaf material.

Iron concentrations were determined by atomic absorption spectrophotometry following the methods described by Taylor and Crowder (1983c). The DCB solutions produced considerable interference with the absorption of the Fe wavelength, so the spectrophotometer was calibrated with Fe standards prepared with 40 ml of 0.3 M $Na_3C_6H_5O_7 \cdot 2H_2O$, 5 ml of 1.0 M $NaHCO_3$, and 2.172 g of

TABLE 1. *Concentrations of Fe in roots and leaves of* T. latifolia *exposed to various Fe sources. Values are* $\bar{x} \pm SE$ *of DCB-extractable Fe, concentration of Fe remaining in washed roots, percent of total root Fe remaining in washed roots, and concentration of Fe in leaves, of plants exposed to* Fe^{2+}, *Fe-(BPDS)*$_3$, Fe^{3+}, *Fe-EDDHA, Fe-EDTA, and a deionized water control*[a]

Variable	Fe Source					
	Control	Fe^{2+}	Fe-(BPDS)$_3$	Fe^{3+}	Fe-EDDHA	Fe-EDTA
DCB-extractable Fe (% by wt)	ND	6.15 ± 0.30 a	0.47 ± 0.06 bc	1.33 ± 0.13 d	0.65 ± 0.05 c	0.16 ± 0.04 b
Fe in washed roots ($\mu g\ g^{-1}$)	55 ± 6 a	1,073 ± 50 b	291 ± 17 d	161 ± 25 c	288 ± 14 d	75 ± 6 a
% of total Fe in washed roots		1.7 ± 0.1 a	6.1 ± 0.5 b	1.2 ± 0.1 a	4.3 ± 0.6 c	5.6 ± 0.5 b
Leaf Fe ($\mu g\ g^{-1}$)	86 ± 8 a	622 ± 45 b	181 ± 17 c	122 ± 4 ac	268 ± 72 d	130 ± 8 ac

[a] Note: For each variable, any two values which share the same letter (to the right of each value) do not differ at the $P < 0.05$ probability level; ND = not determined.

Na_2SO_3 (sodium sulfite) made to volume (100 ml) with deionized water. This solution substitutes Na_2SO_3 for $Na_2S_2O_4$ in equimolar concentrations with respect to Na. Standards prepared in this fashion mimicked the interference of the DCB solutions, but were stable over long periods of time (Taylor and Crowder, 1983c). Sample dilution decreased, but did not eliminate, interference. Hence when sample dilution was required for the DCB analysis, standards with equal dilution ratios were also prepared. Concentrations of Fe were expressed as percent Fe by weight or as $\mu g\ g^{-1}$. For calculation of DCB-extractable Fe, dry root weights were corrected for the removal of FeOOH by the extraction technique (corrected wt = dry wt ± 1.591 wt of Fe extracted; 1.591 = MW FeOOH/MW Fe).

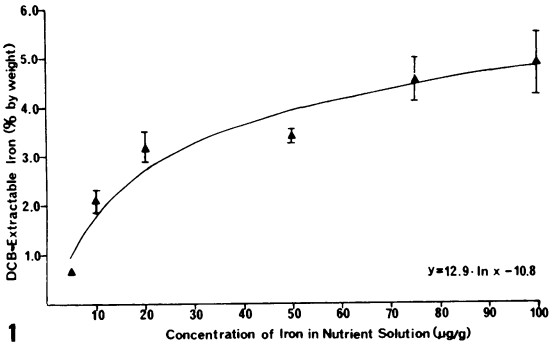
Results were analyzed using Duncan's Multiple Range test available on the SPSS statistical package subprogram oneway, and by simple regression using the SPSS statistical package subprogram scattergram (Nie et al., 1975).

Scanning electron microscopy—Heavily plaqued and lightly plaqued roots prepared in 50 $\mu g\ g^{-1}$ Fe^{2+} at pH 5.0, roots collected in the field, and roots of control plants of *T. latifolia* were frozen in liquid nitrogen and freeze dried. Specimens were mounted on aluminum mounting stubs, coated with gold-palladium with a Polaron ES-100 coating unit, and observed with an Hitachi S-450 scanning electron microscope at an acceleration voltage of 20 kev.

RESULTS—Roots of plants supplied with 20 $\mu g\ g^{-1}$ Fe^{2+} at pH 5.0 developed visible staining of root surfaces with a reddish-brown coloration, typical of oxidized iron compounds. Roots of plants supplied with Fe^{3+} showed some coloration, but this was not intimately associated with the root surface and was largely lost during the deionized water rinse. Roots of plants supplied with Fe-(BPDS)$_3$, Fe-EDDHA, and Fe-EDTA showed no coloration. These observations were verified by the results of the DCB extractions. Roots of plants exposed to 20 $\mu g\ g^{-1}$ Fe^{2+} showed significantly higher ($P < 0.05$) DCB-extractable Fe than roots exposed to any other Fe source (Table 1). Roots exposed to Fe^{3+} also showed significantly higher ($P < 0.05$) DCB-extractible Fe than roots exposed to the Fe chelates, all of which showed similar concentrations, although DCB-extractable Fe was higher with Fe-EDDHA than with Fe-EDTA (Table 1).

The proportion of total root Fe extracted by the DCB solution was high, more than 94% for all the Fe sources. A significantly higher proportion ($P < 0.05$) of the total root Fe was extracted from roots exposed to the Fe^{2+} and Fe^{3+} treatments as compared to the three Fe chelate treatments, however, the concentration of Fe remaining in washed roots was also highest in the Fe^{2+} treatment (Table 1). There were also significant differences ($P < 0.05$) in the concentrations of Fe in leaf material. Leaves from the Fe^{2+} treatment had significantly higher concentrations than all other treatments. The remaining treatments showed some significant differences ($P < 0.05$) (Table 1). When compared to the Fe^{2+} treatment, BPDS inhibited uptake and translocation of Fe to the leaves. When compared to the Fe^{3+} treatment, EDDHA increased, and EDTA did not affect, uptake and translocation to the leaves (Table 1).

Plants supplied with Fe^{2+} at concentrations ranging from 5 to 100 $\mu g\ g^{-1}$, and plants supplied with 50 $\mu g\ g^{-1}$ Fe^{2+} at pH values ranging from 3.0 to 5.0 all showed visible staining of



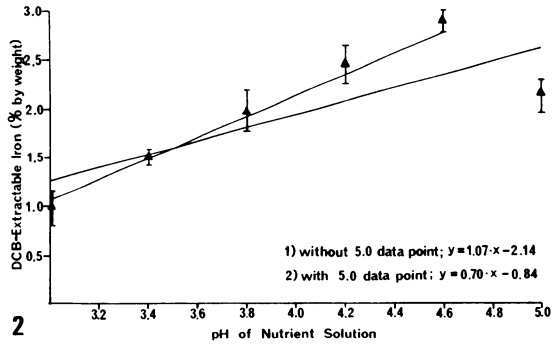
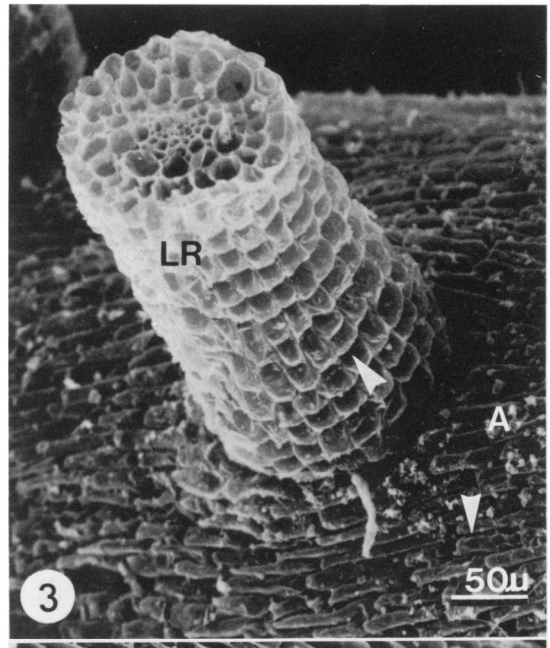


Fig. 1, 2. **1.** Relationship between DCB-extractable Fe on roots of *T. latifolia* and concentration of Fe^{2+} in the nutrient solution. The regression equation indicates a significant correlation at the $P < 0.001$ level. **2.** Relationship between DCB-extractable Fe on roots of *T. latifolia* and pH of the nutrient solution. Two regression analyses were performed, with and without the pH 5.0 values. The regression equations indicate significant correlations at the $P < 0.001$ level.



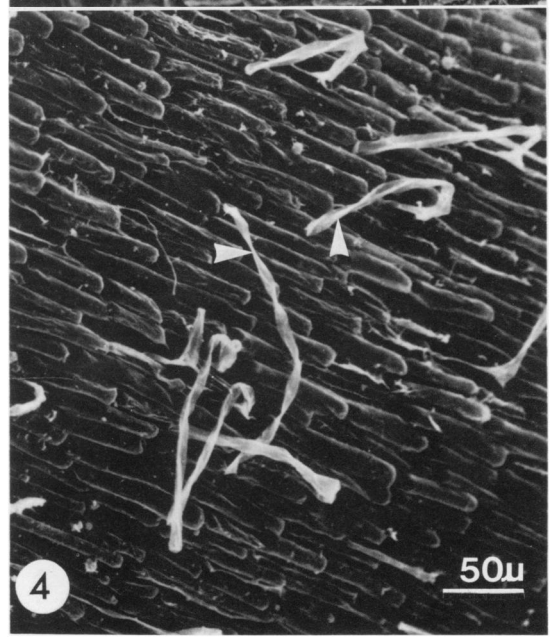


Fig. 3, 4. Scanning electron micrographs of unplaqued, control roots. **3.** Lateral root (LR) emerging from the main axis (A); most cells appear undamaged and largely free of external deposits. Note that junctions between cells (arrows) are free of deposits and thus cells are clearly discernible. **4.** Root hairs (arrows) emerging from a young root. Root hairs appear undamaged and the root surface is free of deposits. Scale bars = 50 μm.

root surfaces. The visual appearance of the staining was not equal throughout all portions of the root, but appeared to match the regions of intense O_2 evolution as described by Armstrong (1971). The most heavily stained portions of the root were approximately 1.0 cm above the root tip, extending for variable lengths up the root. Staining decreased from this most concentrated portion as one approached either the root tip or the proximal region, but was apparent on all portions of the root. The proximal regions of lateral roots showed more intense staining than nearby root surfaces. High Fe^{2+} concentrations and high pH treatments produced the strongest visible staining. Control plants showed no such staining.

Corresponding to these observations, there was a significant ($P < 0.001$) curvilinear relationship between concentration of Fe^{2+} in the nutrient solution and DCB-extractable Fe on roots (Fig. 1). Increasing the pH of the nutrient solution also resulted in a significant ($P < 0.001$) increase in DCB-extractable Fe on the roots (Fig. 2). Roots showed progressively

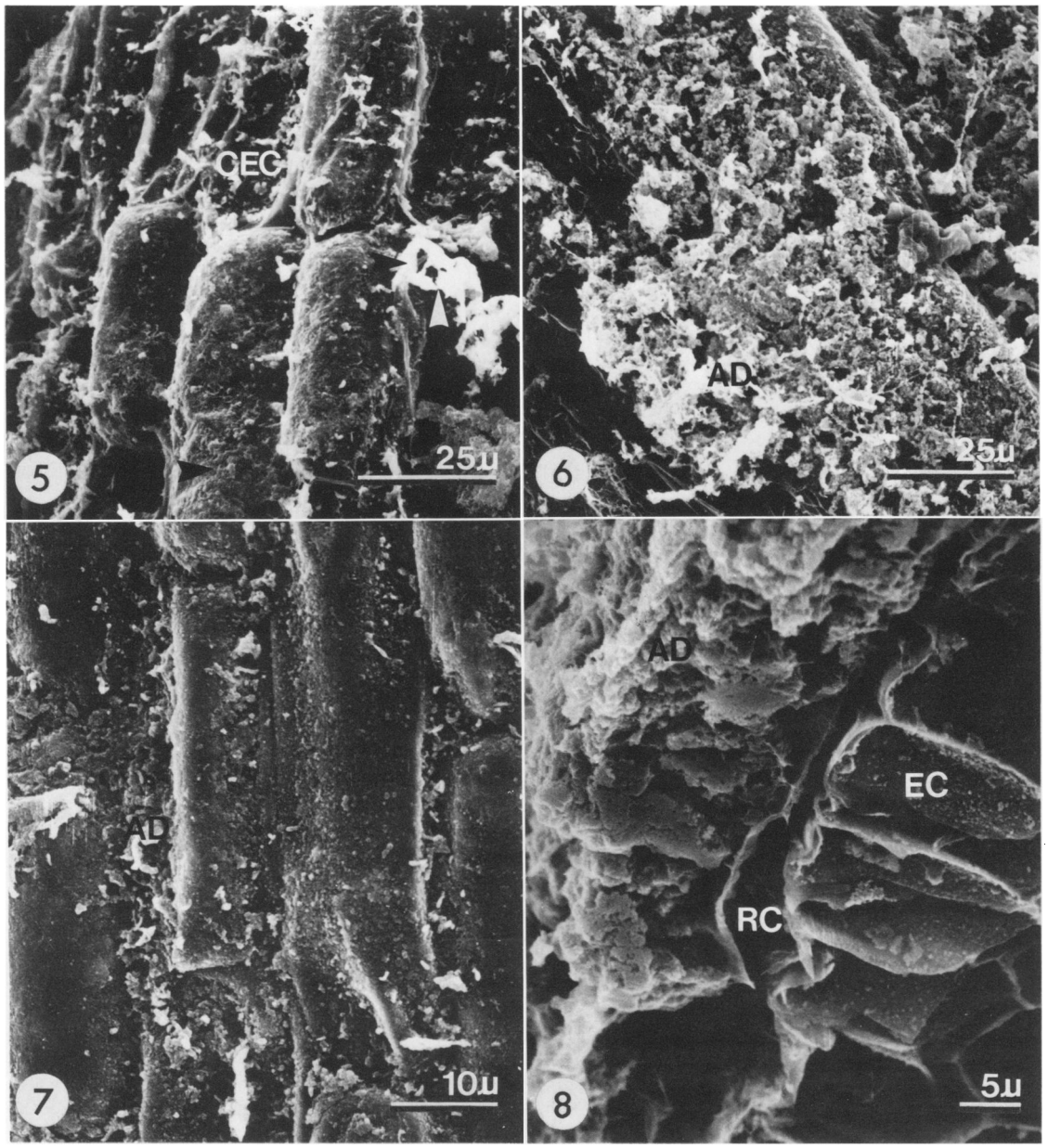


Fig. 5–8. Scanning electron micrographs of plaqued roots prepared in 50 μg g^{-1} Fe^{2+} at pH 5.0. **5.** Lightly plaqued root surface showing light deposits (arrows) on the root surface and considerable collapsing of epidermal cells (CEC). **6.** Root cap surface 0.5 mm from the root tip with considerable amorphous deposits (AD) masking the root surface proper. **7.** Amorphous deposits (AD) collecting between cells, masking cell boundaries. **8.** Cross section of heavily plaqued root near the root tip. Note thick amorphous deposit (AD), some 15–17 μm in thickness, completely masking the remnants of the root cap (RC) and epidermal cells (EC).

higher DCB-extractable Fe up to pH 5.0 when a decline was observed. Iron remaining in the roots after washing also showed a significant positive relationship with pH ($r = 0.669$, $P < 0.001$) and with the concentration of Fe^{2+} in solution ($r = 0.846$, $P < 0.001$), however, the amount of Fe remaining after washing was small, an average of 3.7 ± 0.30% of total root iron in the pH experiment, and an average of 1.5 ± 0.14% of total root Fe in the Fe^{2+} concentration experiment. Leaf concentrations were unrelated to pH or concentration of Fe^{2+} in the nutrient solution.

Comparison of scanning electron micro-

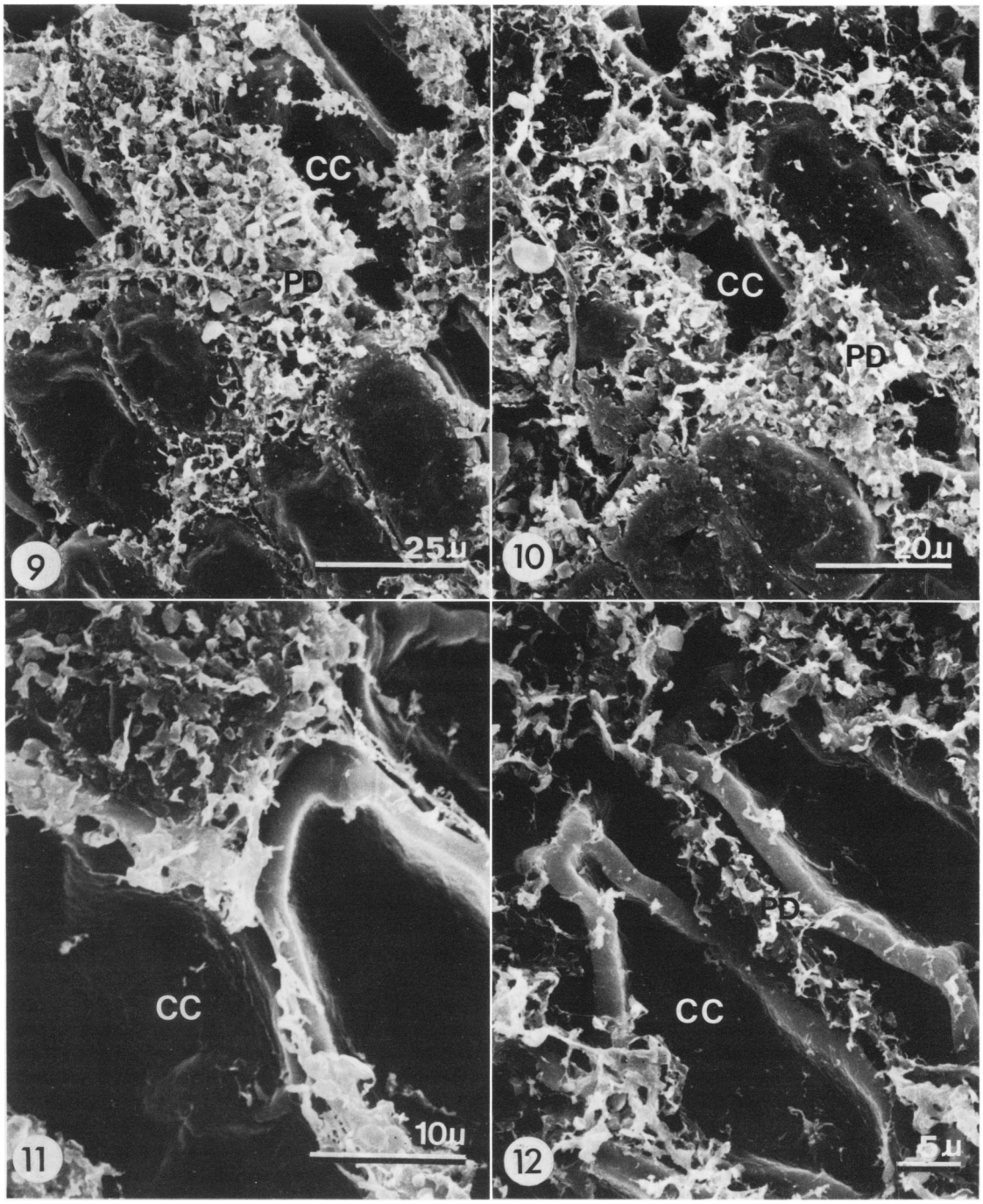


Fig. 9–12. Scanning electron micrographs of plaqued roots collected in the field. **9.** Root surface with considerable particulate deposits (PD). One cell is completely masked by the deposits, while others are clearly visible. Cell cavities (CC) show some filling with particulate deposits. **10.** Root surface with both particulate (PD) and amorphous (arrows) deposits. Open cell cavity (CC) shows considerable filling with particulate deposits. **11.** Magnified view of two open cell cavities (CC) created either by shearing off or collapse of the outer cell wall. Cavities show no apparent filling with particulate deposits. **12.** Magnified view of two open cell cavities (CC), apparently caused by shearing off of the outer cell wall. Note particulate deposits (PD) between cell casts and covering adjacent cells.

graphs of plaqued and unplaqued roots showed clearly the extent of plaque formation. Surfaces of unplaqued roots were relatively free of external deposits and each individual cell was discernible with little sign of deformation (Fig. 3, 4). On the surfaces of young roots, root hairs were visible and undamaged (Fig. 4).

In contrast, surfaces of even the lightly plaqued roots showed some deposits and, while each individual cell was discernible, considerable collapsing of the outer tangential cell walls was evident (Fig. 5). We were unable to locate root hairs in plaqued preparations. On the most heavily plaqued roots, external deposits masked the outline of individuals cells (Fig. 6–8). These deposits were particularly evident at the junctions between cells (Fig. 7), although in some cases the deposits formed an even layer over a large portion of the root surface, completely masking the root surface (Fig. 8). The plaque itself appeared amorphous in form, and extended into the rhizosphere up to a depth of 15–17 μm, a depth approximating the thickness of an epidermal cell (Fig. 8).

Roots of plants collected in the field showed a somewhat different appearance, the texture of which more closely resembled the plaques found by Mendelssohn and Postek (1982). Although some cells were completely obscured by external deposits (Fig. 9), a number of individual cells were evident (Fig. 9–10). A large number of cells exhibited a complete loss of the outermost tangential cell wall. It was difficult to determine if the cavities were formed from shearing or collapsing of the outer cell wall (Fig. 11), although there was some evidence to indicate that the outer cell wall may have been sheared off (Fig. 12). Shearing of the outer cell wall could have occurred during root collection. However, in several instances, Fe deposits were beginning to collect in these cavities (Fig. 10), suggesting that not all the cavities were formed during collection of the roots. Much of the plaque appeared more particulate in nature than plaque material on roots prepared in the laboratory. However, an amorphous material was also observed on the surface of roots collected in the field (Fig. 10). Compared to laboratory preparations, the plaque was less evenly distributed across the surface of the root.

DISCUSSION—A number of authors have documented plaque formation under reduced conditions, presumably in the presence of Fe^{2+}. These authors have suggested that the oxidizing capacity of roots is an important factor in the process of plaque formation (Boone et al., 1983; Mendelssohn and Postek, 1982; Chen et al., 1980a, b; Bacha and Hossner, 1977; Green and Etherington, 1977; Jones and Etherington, 1970; Armstrong and Boatman, 1967). Under reduced soil conditions, Fe^{2+} is thought to be oxidized to the less soluble Fe^{3+} by oxidative agents released from the roots, thus creating a coating or plaque of an insoluble Fe^{3+} compound on the root surface.

The formation of a metal-chelate complex may be accompanied either by an increase or decrease in oxidation potential, thus stabilizing the metal in either its oxidized or reduced state. Both EDTA and EDDHA stabilize Fe in its oxidized state (Bell, 1977; Garvan, 1964), while the reverse is true for the Fe^{2+}-Fe^{3+}-BPDS couple (Bell, 1977). If plaque formation is dependent on the oxidation of Fe^{2+} to Fe^{3+}, then supplying Fe as Fe^{3+}, Fe^{3+} chelates, or Fe^{2+} chelates should effectively reduce the extent of plaque formation. This has been verified in the Fe-source experiments reported here. While DCB-extractable Fe was present on roots exposed to Fe-$(BPDS)_3$, Fe^{3+}, Fe-EDDHA, and Fe-EDTA, concentrations were significantly less than on roots exposed to Fe^{2+}.

There were also differences between the Fe-source treatments in the proportion of total root Fe extracted by the DCB solution, possibly reflecting the relative proportion of internal to external Fe. However, it was not possible to determine to what extent the DCB extraction technique removed internal Fe, or left external Fe. Furthermore, in all cases DCB-extractable Fe comprised 94% or more of the total root Fe. Thus while these differences may be statistically significant, they can not represent large differences in the site of Fe immobilization, either on the root surface or in the root proper.

Green and Etherington (1977), Bacha and Hossner (1977), and Bartlett (1961) developed plaques on the roots of plants grown in artificial substrates (agar and sand). Because Fe as $FeSO_4$ or $FeCl_3$ were the only Fe sources utilized, comparisons between these experiments and our own were not possible. Bartlett (1961) included tartaric acid in sand culture treatments, but concentrations were not reported. Boone et al. (1983) and Wallace et al. (1957) reported comparable data as part of experiments dealing with Fe nutrition. The results of Wallace et al. (1957) demonstrated that soybean plants supplied with $FeSO_4$ in sand culture fixed significantly higher concentrations of Fe on/in roots than plants supplied with Fe-EDDHA. These results agree with our own. Also consistently with the results of this study, Boone et al. (1983) found that rice plants exposed to Fe^{2+} and Fe^{3+} in solution culture ac-

cumulated more Fe on root surfaces than plants exposed to Fe-EDTA. At pH 5.3, the extent of plaque formation was greatest with Fe supplied as Fe^{2+}, but at pH 3.0 and 4.0, supply of Fe^{3+} resulted in the greatest plaque formation. We have not tested the effect of pH on the relative effectiveness of each Fe source in plaque formation. Boone et al. (1983) suggested that, while Fe^{2+} was oxidized at the root surface leading to plaque formation, the external Fe extracted from roots exposed to Fe^{3+} may have resulted from an interaction between the root surface and colloidal FeOOH present in the growth medium. Our observations concerning the nature of root coloration and its intimacy with the root surface support this view.

The results of the pH and concentration experiments were as expected. The extent of plaque formation should increase with the concentration of Fe^{2+} in solution and with increasing redox potential, until oxidation occurs spontaneously. Increased additions of $FeSO_4$ and increasing the pH of the Fe^{2+} solution both have such an effect. The decline in DCB-extractable Fe at pH 5.0 in the pH experiment may be attributed to a decline in the concentration of Fe^{2+} with oxidation of Fe^{2+} independent of the plant root. As the pH of the Fe^{2+} solutions was raised towards pH 5.0, a reddish-brown tint began to appear, indicating oxidation. At pH 5.0, despite continuous bubbling with N_2, some Fe^{3+} precipitation began to appear soon after solutions were prepared, effectively reducing the concentration of Fe^{2+}.

Chen et al. (1980a) failed to find a relationship between soil pH and DCB-extractable Fe on roots of field-grown plants. Such results may reflect a lack of suitable controls. In an experiment with rice grown in solution culture, Boone et al. (1983) found that plaque formation on roots of plants supplied with either Fe^{2+} or Fe^{3+} increased with pH of the growth medium from pH 3.0 to 5.3. Bacha and Hossner (1977) prepared plaqued roots of rice grown in sand culture, and found an increase in DCB-extractable Fe with an increase in added Fe and average solution Fe. As Bacha and Hossner utilized only four different experimental solutions, it is difficult to determine if the relationship between DCB-extractable Fe and added Fe or average Fe concentration in solution was linear or curvilinear. Their data for DCB-extractable Fe versus average concentration of Fe in solution appears to resemble our results, while the relationship between DCB-extractable Fe and added Fe appears more linear.

Based upon interpretation of scanning electron micrographs, Chen et al. (1980b) proposed two models of plaque formation. In the first model, intact cell walls provide a template for the development of polyhedral iron oxide casts through the precipitation of FeOOH on both the internal and external surfaces of the cell wall. Subsequently, the outer cell walls may decompose, permitting a junction to form between two adjacent casts. While our micrographs do not contradict such a model, neither do they provide direct support for it. We have been unable to find detached casts such as demonstrated by Chen et al. (1980b) in either our laboratory or field-grown roots. However, Chen et al. (1980b) indicated that the occurrence of such casts was a rare event. While it is possible to interpret the occurrence of a homogeneous covering of plaque material, such as observed in Fig. 6 or 8, as an example of the early stages of cast formation, other interpretations are possible.

The second model of Chen et al. (1980b) assumes that the outer tangential cell walls have decomposed prior to precipitation of FeOOH in open cell cavities. Our results provide support for this model, although there could be some argument as to whether the outer cell walls collapse or decompose. Roots collected in the field showed cell cavities, some of which appeared to be formed by shearing off of the outer cell wall. This may have resulted from physical abrasion when the roots were collected, thus accounting for the the fact that this type of cavity was only observed on field-grown roots. However, the fact that some of these cavities showed infilling with external deposits suggests that not all the cavities were formed during collection of the roots. Scanning electron micrographs of roots prepared in the laboratory provide ample evidence for the occurrence of cell cavities which have become, or are becoming, sites of FeOOH infilling.

It is generally agreed that the extent to which the plaque extends out into the rhizosphere is a function both of oxidizing capacity of the root and of the redox potential of the surrounding substrate. Most authors cite O_2 evolution as the oxidizing agent responsible for plaque formation, despite the fact that Armstrong (1967) calculated that radial O_2 loss could account for only 1/9 of the total oxidizing capacity of rice roots. Neglect of the role of enzymatic or bacterial oxidation in the formation of Fe plaques may be an error. However, hypotheses concerning the extent of plaque formation into the rhizosphere are relatively unaffected by the nature of the oxidizing agent.

Mendelssohn and Postek (1982) noted differences in the extent of plaque formation on roots of *Spartina alterniflora,* which they suggested was due to the relative differences in

substrate aeration and root O_2 loss at two sites. Where the O_2 deficit of the soil was greater and root O_2 supply was poor, roots respired anaerobically and there may not have been sufficient O_2 present to oxidize the rhizosphere. In the other site, a lower O_2 demand of the substrate, coupled with a greater O_2 supply to the root, may have resulted in more free O_2 available for oxidation of the rhizosphere. Armstrong (1967) mapped the regions of iron oxidation about the roots of *Eriophorum angustifolium,* and found the intensity of Fe oxidation reflected regions of greatest radial O_2 loss from the root.

Such an interpretation should also be applicable to the extent to which Fe^{2+} precipitates can occur in the cortex of the root. A number of authors have presented conflicting evidence in this regard. Mendelssohn and Postek (1982) found oxidized Fe to be restricted to the epidermal cell layer, while Green and Etherington (1977) found Fe precipitates extending into the cortex and along the diaphragms of the aerenchyma tissue. Iron precipitates did not penetrate the endodermis. Our preliminary results are consistent with those of Armstrong and Boatman (1967), who found Fe precipitates to extend some three cell layers into the outer cortex. As Fe^{2+} moves from the soil into the root, it must pass across a redox barrier, at which point oxidation and immobilization will occur. If roots with the greatest oxidizing potential growing in a moderately reducing soil produce plaques extending the greatest distances into the rhizosphere, then roots with the poorest oxidizing ability growing in highly reducing soils should show the greatest penetration of Fe precipitates into the root interior. We have not tested this hypothesis.

The morphology of plaqued roots prepared by the techniques described here are similar to the morphology of plaqued roots of *T. latifolia* collected in the field, and similar to the morphology of roots of other species reported in the literature. Thus the technique would appear suitable for experimenting with the effects of plaque formation on the uptake and accumulation of metals and other toxic or essential ions. Preliminary experiments in this regard have been reported by Taylor (1983).

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