Leaf Chlorosis and Twig Discoloration in 'Delicious' Apple Trees Having Mn-Induced Internal Bark Necrosis[1]

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Abstract. **'Red Prince Delicious' apples/'M 7' rootstocks, growing in sand culture and receiving ½, 5 or 50 ppm Mn, developed internal bark necrosis (IBN), and an Fe-deficiency chlorosis in its severest expression, at the highest level of Mn supply. Fe was as high in leaves of chlorotic as in those of non-chlorotic plants, but the chlorosis was cleared up by increasing the Fe supply.**

Ground twigs showed a brownish coloration which had a max reflectance at 700 mμ. Differences in coloration were correlated with the Mn supply, the severity of IBN symptoms and the Mn/Fe ratio in the leaves and bark, but not with the Mn/Fe ratio in the ground twigs.

Several investigators, in studying IBN of the 'Delicious' apple, have reported abnormalities of young leaves of trees supplied with high amounts of Mn. Berg and Clulo (2) characterized the symptoms as Mn toxicity, but did not describe them. They stated that the *"intensity varied with the concn [of Mn] used."* Shannon (11) referred to the symptoms as an *"interveinal chlorosis characteristic of iron deficiency."* The authors, in a previous report (12), noted the same phenomenon at all levels of Mn supply, but increasing in frequency of occurrence and severity with increasing Mn concn in the nutrient supply. They regarded it as being a typical iron deficiency symptom. However, no one seems to have examined Fe levels in detail, or Mn-Fe relationships in apple plant parts, in relation to Mn level of supply and the occurrence of IBN.

This paper is a report of the Fe level and Fe/Mn ratio in plant parts of 'Red Prince Delicious' apple trees used in studying ^{54}Mn distribution in relation to IBN. A detailed description of the manner of conducting the experiment was presented in the earlier paper (12).

In brief, 'Red Prince Delicious'/'M 7' trees were grown in sand culture and supplied with nutrient solutions containing ½, 5 or 50 ppm Mn. Fe was supplied as NaFe DTPA to provide 6 ppm Fe. At monthly intervals the sand was flushed with tap water and fresh nutrient solution placed in the reservoir for each tree.

Periodically, laterals, or tips, were removed to limit growth of the trees to that suitable for the greenhouse area available for the experiment. Before growth resumed the 2nd and 3rd seasons (1968 and 1969), the previous season's growth was cut back to 38 cm.

At irregular intervals bark patches were removed from the trees of a different one of the 12 replicates for Mn and Fe analyses. Leaves, shoots and twigs were also sampled 8 months after the initiation of the experiment.

Twigs were ground in a CRC Micro Mill. Color differences observed between ground twig samples were characterized at a wavelength of 700 mμ with a reflectance attachment for a "Spectronic 20" colorimeter.

All of the leaf, shoot and twig samples for chemical analyses were ashed in a muffle furnace at 550°C., dissolved in concd HNO_3, and diluted to volume. Mn and Fe were determined on a Perkin-Elmer 303 Atomic Absorption Spectrophotometer.

The chlorosis of young leaves mentioned earlier occurred in 17, 58 and 91%, respectively, of trees grown at the ½, 5 and 50 ppm levels of Mn supply. Severity of the chlorosis was not estimated for individual trees but was less severe with respect to loss of green color and no. of leaves affected at the lower levels of Mn supply. Chlorotic, tip leaves sampled in Nov. 1968, had an Fe concn of 62 ppm No comparable non-chlorotic tip leaves were obtained, but analysis of non-chlorotic mid-shoot leaves obtained at the same time showed the same concn of iron. Several investigators (5, 6, 7, 10, 13) have found young, or tip leaves, of several tree fruits to contain a lower concn of Fe than mid-shoot leaves. Since, in this case, tip leaves, though chlorotic, contained the same concn of Fe as mid-shoot leaves, they were not quantitatively deficient in Fe.

Further, analyses of a composite of all leaves, chlorotic and non-chlorotic, on shoots at the time of cutting back prior to the 3rd season of growth (1969) showed concn of Fe to be virtually the same in samples which contained chlorotic leaves and those which did not (Table 1). Both were somewhat below the range considered normal for apple leaves, but above a deficiency range (15). The Mn concn and the Mn/Fe ratio were considerably higher in the former sample.

It is not uncommon to find the Fe level to be the same, or unrelated to plant condition, in leaves of various plants considered normal compared with those showing Fe deficiency symptoms (1, 3, 4, 8, 9, 14).

That the chlorosis is a functional iron deficiency in this case, however, is shown by the occurrence of greening when the Fe supply was increased 10-fold without altering the Mn supply. However, it is not known whether this was the minimum required to effect the correction. Hence, it seems more meaningful to delineate the situation in terms of a Mn/Fe ratio rather than Mn level alone.

Fe concn was influenced by the level of Mn supply only in the twigs, being higher at the intermediate (5 ppm) level of supply. These data are not shown in tabular form. The Mn/Fe ratio was higher in all tissues at the highest (50 ppm) level of Mn supply (Table 2), reflecting the dominating effect of the level of Mn in the tissue in determining the ratio.

In bark patches the Mn/Fe ratio varied with time (Fig. 1) as well as with the level of Mn supply. The sharp rise from the 8th to the 10th month (50 ppm treatment) coincides with the initiation of a new flush of growth following the cutback of shoots early in 1969. It was subsequent to the appearance of morphological symptoms of IBN and considerably subsequent to the appearance of "islands" of Mn concn in the tissue. The decline in the Mn/Fe ratio after the 10th month suggests a degree of regulation by the uncontrolled environment, or the plant, over the ratio, since the Mn, Fe and moisture supplies were kept relatively constant.

Table 1. Comparison of the concn of Fe and Mn and the Mn/Fe ratio of chlorotic[z] vs. green leaves. Composites were made without regard to Mn supply. Samples taken at beginning of 3rd season of growth (1969).

Fe deficiency symptoms	Mn (ppm)	Fe (ppm)	Mn/Fe ratio[y]
No	128	77	2.32
Yes	460	72	19.79

[z]Because of a paucity of leaves, "chlorotic" samples contained some green leaves.
[y]Mn/Fe ratios are averages of ratios for individual samples and are not calculated from Mn and Fe averages.

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Table 2. Mn/Fe ratio in leaves, twigs and bark of 'Red Prince Delicious' apple trees supplied with ½, 5 or 50 ppm Mn in the nutrient solution.

Mn concn (ppm)	Mn/Fe ratio		
	Leaves	Twigs	Bark patches
½	2.0	4.4	0.4
5	2.8	9.6	0.6
50	29.6	77.7	4.6
LSD 5%	16.7	52.3	0.9

Rusty discolorations were often observed on the surface of the wood of trees supplied with 50 ppm Mn when bark patches were removed. Figure 2 shows one such longitudinally-oriented streak.

When twigs taken at the cutback preceding the 2nd season's growth were ground, differences in color varying from off-white to "toast" brown were observed. These color differences were related to the level of supply of Mn as determined by reflectance values at 700 mμ. Samples from trees receiving 50 ppm Mn were darker than samples from trees at the 2 lower levels of supply (Table 3).

A highly significant correlation coefficient (-0.783) between the severity of IBN and the reflectance of light at 700 mμ from ground tissue suggests the possibility that the color of ground 'Red Delicious' twigs might be developed as an index to predict the likelihood that trees in the field would develop external visual symptoms of IBN. It has not been determined, however, that the color change precedes the occurrence of external IBN symptoms.

The ground twig color was not associated with the twig concn of Mn, Fe, or the Mn/Fe ratio.

Highly significant correlation coefficients of -0.583 and -0.601, respectively, were calculated between ground twig color and bark Mn concn and twig color and bark Mn/Fe ratio. This indicates that the color quality of ground twig tissue was a good indicator of both Mn concn and Mn/Fe ratio in the bark. This is also true for leaf Mn and Mn/Fe ratio. The correlation coefficients were -0.614 and -0.433, respectively; the former was highly significant. That these correlation coefficients are larger than those between the color and Mn concn, or Mn/Fe ratio, in the twigs themselves may have been due to the high % of wood in the ground twig sample. Earlier, we have shown (16) that the wood contains a lower concn of Mn than the bark, and that they do not invariably parallel each other in changes in concn, or in the relationship of their concn at any sampling time.

Our data and observations indicate that a terminal leaf chlorosis typical of

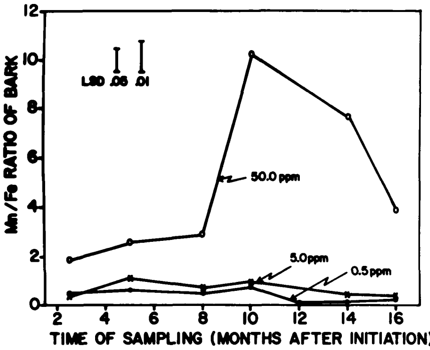

Fig. 1. Mn/Fe ratio of bark patches removed at indicated time, from 'Red Prince Delicious'/'M 7' apple trees, growing in sand culture and supplied with nutrient solutions containing ½, 5 or 50 ppm Mn.

Fe-deficiency accompanies the occurrence of IBN in 'Red Prince Delicious' resulting from an increasing supply of Mn. The chlorosis was cleared up by increasing the Fe supply without increasing the supply of Mn.

The chlorosis is not associated with a lower level of leaf Fe, but with a higher level of Mn and so a larger Mn/Fe ratio. Although the phenomenon can be explained in terms of Mn alone, expressing the relationship as the Mn/Fe ratio emphasizes what appears to be a quantitative Mn/qualitative Fe situation.

Differences in color of ground twig tissue are associated with the level of supply of Mn and with the concn of Mn in bark and leaves, but not with the concn in the twigs themselves. The differences are similarly correlated with Mn/Fe ratios.

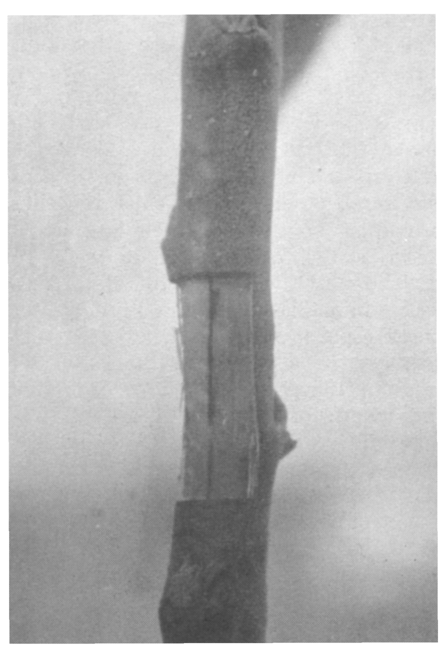

Fig. 2. Exposed wood showing rusty discolored streak associated with 50 ppm Mn in nutrient solution supplied to 'Red Prince Delicious' apple trees growing in sand culture.

Table 3. Reflectance of light[z] at 700 mμ from ground twigs taken at the cutback prior to t the second season's growth of 'Red Prince Delicious' trees.

Mn (ppm) in nutrient solution	Reflectance (%)
½	71.3
5	72.3
50	64.5
LSD 1%	2.9

[z]A low "% reflectance" is associated with a dark color of ground twig tissue and vice versa.

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