Investigations of Internal Bark Necrosis in ‘Delicious’ Apple Trees

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Abstract. In 4 experiments conducted to study internal bark necrosis (IBN) in apple, ‘Delicious’ trees were treated with Mn, Fe, Cu, and Al (100 and 200 ppm in nutrient solution), Mn, Fe, Cu, plus Al (50 ppm each) and a minus B treatment. Only trees receiving Mn and minus B developed IBN symptoms. Trees grown under normal and low levels of Ca and receiving variable concentrations of Mn (0, 25, 50, 75, and 100 ppm) developed IBN in proportion to Mn concentration. Spur-type and standard ‘Delicious’ trees did not differ in IBN severity. Bark samples with IBN symptoms, when analyzed on the electron microprobe x-ray analyzer, had greater Mn and Ca concentrations in necrotic tissue areas than in non-necrotic areas. IBN lesions induced with minus B had a higher Ca concentration in necrotic areas than in healthy tissue.

‘Delicious’ apple trees are susceptible to a disorder known as internal bark necrosis (IBN). The disorder appears as small necrotic areas in the phloem tissue and is commonly known as “measles.” In Michigan IBN is usually associated with low soil pH and high Mn in leaves. IBN has been reported on other cultivars (4, 10) but is most prevalent on ‘Delicious’.

IBN has been reported to be caused by excess Mn (1, 2, 5, 8, 10, 11, 14, 16, 17, 18 and 22), B deficiency (4, 7, 12, 17, 19 and 21), or either B deficiency or Mn excess (3, 17 and 22). Other investigators have suggested that several metals, in excess, could be the cause (10). Wave and Stiles (20) produced a measles-like symptom using superior oils.

Therefore, because of the many conflicting explanations of the disorder, experiments to investigate the causes of IBN were designed to elucidate the nutritional factors related to the disorder.

Materials and Methods

These investigations were carried out in 4 experiments, enumerated and discussed as experiments I, II, III and IV.

Experiment I: Two-year-old ‘Miller Sturdy Spur Delicious’ trees budded on M 7 were planted in 3 gal plastic containers in washed quartz sand. One of a 1/2 strength Hoagland (13) solution with varying levels of Ca and B were applied to the trees on alternate days using the Chapin ring system3. Calcium and B levels were: 1) normal (Control) Ca - 1/2X Hoagland solution; 2) low Ca - 1/6X Ca in 1/2X Hoagland solution; 3) minus B - 1/2X Hoagland solution minus B. Trees receiving the 2 Ca levels were subdivided and used for solution treatments, applied on alternate days, containing supplemental Mn, Fe, Cu, or Al at 0, 100 and 200 ppm. An additional treatment combined Mn, Fe, Cu, and Al at 50 ppm each. This resulted in a total of 21 treatments arranged in a randomized block with 2 single tree replicates.

The trees were grown outdoors on a concrete apron during the summer of 1967. In October, they were placed in cold storage at 2°C to complete the dormancy requirements. In February, 1968, the trees were removed from storage and the summer of 1967. In October, they were placed in cold storage at 2°C to complete the dormancy requirements. In February, 1968, the trees were removed from storage and placed in a greenhouse, and treatments resumed.

Experiment II: ‘Miller Sturdy Spur Delicious’ trees on M 7 were used with potting medium and basic nutrient solutions the same as in Experiment I. Supplemental solutions of Mn (100, 75, 50, 25 and 0 ppm) were applied at the rate of one per tree every other day, alternating with basic nutrient solution. A randomized block design was used. The trees were grown outside on a concrete apron during the summer of 1968 in the fall, they were moved into cold storage at 2°C and then were placed in a greenhouse in February 1969 when treatments were resumed.

Experiment III: Trees of ‘Miller Sturdy Spur Delicious’ on MM 106 and ‘Red Prince Delicious’ on MM 106 were used. Trees were placed on an automatic watering system with 1/2 strength Hoagland’s nutrient solution with Ca reduced to 1/6 strength and applied every other day. Manganese concn of 50, 100, and 150 ppm, at pH 4.5, were applied on alternate days. Plot design was a randomized block with 8 single-tree replicates.

Measurements (Experiments I, II and III) - Leaf samples from the middle of the terminal growth were taken 2-4 weeks after terminal bud formation for all experiments. These samples were analyzed spectrographically for Ca, Mg, Mn, Fe, Cu, B, and Al.

The severity rating of IBN was made at the end of the growing season in accordance with the rating in Table I. Visual rating for IBN was not made for Experiment I.

Table 1. Rating scheme for determining the severity of IBN on apple trees.

<table>
<thead>
<tr>
<th>Rating</th>
<th>Number of pimples present in 3 sq. cm.</th>
<th>Age of bark</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>2 year</td>
</tr>
<tr>
<td>2</td>
<td>1 to 5</td>
<td>2 year</td>
</tr>
<tr>
<td>3</td>
<td>More than 5</td>
<td>1 year</td>
</tr>
<tr>
<td>4</td>
<td>1 to 5</td>
<td>1 year</td>
</tr>
<tr>
<td>5</td>
<td>More than 5</td>
<td>1 year</td>
</tr>
</tbody>
</table>

Experiment IV: The localization and approximate amount of certain elements within necrotic areas of diseased tissues were studied. Bark samples were taken from trees showing IBN and from control trees. Sample preparation for microprobe analysis differed from that of Rasmussen et al. (15) in that sections were cut 15 to 20μm and then coated with a thin layer (approximately 15 Å) of carbon for electrical conductivity. Elemental analysis were made on an ARL4 electron microprobe x-ray analyzer Model EMX-SM using 25 KV acceleration voltage and 0.125 microamp sample current on subsequent samples (6).

The location and relative amount of each element in question were determined from a line scan across the sample where the x-rays (Kα radiations) having a characteristic wave length for each element were plotted on a X, Y recorder, and x-ray photomicrograph from radiations detection. Secondary electron photomicrographs were made of the area being studied, and x-ray photomicrographs indicated the relative concen and location of the element. By use of line scans, x-ray and secondary electron photomicrographs precise location and

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Results and Discussion

Experiment I: Only trees treated with 100 and 200 ppm Mn or minus B developed IBN. The Mn treated trees developed more severe IBN than the minus B trees. Manganese values in leaves (Table 2) were higher with added Mn than the control. Trees with low Ca nutrient solution had a higher Mn value than normal Ca trees. Those trees with minus B nutrient solution had significantly less B than control trees (Table 2).

Trees treated with 100 and 200 ppm Fe had a pronounced rosette condition and were stunted in growth as described by Shannon (17). Correspondingly there was more iron present than in the control (Table 2) especially with low Ca. These treatments did not induce IBN.

Trees treated with 100 and 200 ppm Cu had short terminal growth and stunted foliage but no IBN. The 200 and 500 ppm Cu treatments resulted in a significant increase in Cu content of the leaves over controls (Table 2). The combination of elements gave a larger accumulation of Cu in the leaves and bark, than the 100 ppm Cu.

A delay in foliation after cold storage was observed with the 100 and 200 ppm AI treatments. However, IBN symptoms did not develop. The Al concn (Table 2) of leaves increased significantly in the 200 ppm Al treatment, but there was no significant increase in bark Al.

Leaf and bark values for the element combination under low Ca (Table 2) resulted in an increase of the various elements except Al, but no IBN was observed. This suggests that IBN is not caused by combined excesses of these elements although leaf Mn was as high as 933 ppm, rather high levels of Fe (Table 2) or other elements may delay or suppress the expression of IBN symptoms.

Experiment II: Results of Experiment I indicated that excess Mn induced IBN. Efforts were then made to determine the minimum level of Mn that would induce IBN.

Table 4. Rating of IBN and Leaf Mn on 'Delicious' trees receiving varying levels of Ca and Mn after 1 growing season.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rating IBN</th>
<th>Mn (ppm)</th>
<th>Rating IBN</th>
<th>Mn (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.00</td>
<td>120.8</td>
<td>1.00</td>
<td>80.8</td>
</tr>
<tr>
<td>25 ppm Mn</td>
<td>1.50</td>
<td>500.1**</td>
<td>1.25</td>
<td>191.0</td>
</tr>
<tr>
<td>50 ppm Mn</td>
<td>2.50*</td>
<td>703.0**</td>
<td>1.25</td>
<td>329.0*</td>
</tr>
<tr>
<td>75 ppm Mn</td>
<td>2.75*</td>
<td>996.1**</td>
<td>2.25</td>
<td>462.3**</td>
</tr>
<tr>
<td>100 ppm Mn</td>
<td>3.75*</td>
<td>1,168.0**</td>
<td>2.50*</td>
<td>629.3**</td>
</tr>
</tbody>
</table>

***Means significantly different from control at 5% and 1% levels.

The IBN rating (Table 4) showed a significant effect of Ca level on response to different Mn concn. The rating for the low Ca solution was significantly higher than the control for the 50, 75 and 100 ppm Mn treatments; however, the rating of IBN for the normal Ca solution was only significantly higher than the control with 100 ppm Mn treatment. All Mn leaf values showed increases with an increase in Mn concn applied.

Low Ca treatment resulted in a greater quantity of Mn being absorbed in the same period of time than the normal Ca level. The 50 ppm Mn treatment with low Ca solution (Table 4) showed an IBN rating of 2.5 and a leaf Mn value of 703 ppm. The normal Ca solution required 100 ppm Mn for a comparable IBN rating and leaf Mn level.

A given severity rating had a wide range of leaf Mn concn (150 to 350 ppm) correlated with it. A leaf composition of approximately 500 ppm Mn caused eventual tree death. Many trees exceeded this Mn value, but were essentially dead at the time of sampling.

The rating system for IBN was thought to be adequate when devised. However, a rating system employing a wider range of values would not have grouped in one category trees severely affected the first year. The top IBN rating value of 5 was too low to permit evaluation of further IBN development.

Experiment III: There was no significant difference in the incidence of IBN between standard-type and spur-type trees. Terminal growth was significantly greater on standard trees than on spur-type trees (Table 5). Higher leaf Mn as a result of high Mn rates resulted in more severe IBN (Table 6).

Table 5. Standard versus spur-type 'Delicious' apple trees receiving varying levels of (150, 100, and 50 ppm) on the occurrence of IBN.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Leaf Mn ppm</th>
<th>IBN Average rating</th>
<th>Terminal Growth in inches</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>876</td>
<td>3.67</td>
<td>14.0</td>
</tr>
<tr>
<td>Spur-type</td>
<td>889</td>
<td>3.71</td>
<td>**</td>
</tr>
<tr>
<td>NS</td>
<td>**</td>
<td>NS</td>
<td>**</td>
</tr>
</tbody>
</table>

**Means significantly different at the 1% level. NS Means not significantly different.

Mn rates resulted in more severe IBN (Table 6).

Table 6. IBN rating and Mn leaf concn of standard and spur-type 'Delicious' apple trees as affected by 3 concn of Mn (150, 100, and 50 ppm).

<table>
<thead>
<tr>
<th>Level of Mn ppm</th>
<th>Leaf Mn ppm</th>
<th>IBN average rating</th>
<th>Terminal growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>503</td>
<td>1.67</td>
<td>11.75</td>
</tr>
<tr>
<td>100</td>
<td>1,004**</td>
<td>4.37**</td>
<td>11.55</td>
</tr>
<tr>
<td>150</td>
<td>1,123**</td>
<td>4.81**</td>
<td>11.30</td>
</tr>
<tr>
<td>**Means significantly different from 50 ppm at 1% level. NS Means not significantly different from each other.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Experiment IV: Line profile analysis of normal apple bark tissue (Fig. 1), showed Mn and B distribution in the tissues to be uniform. The average background count for Mn was 34 count/sec and the average Mn count was 44 count/sec. No unusually large quantities of Mn were found in any areas of the normal bark tissue. The Mn x-ray photomicrograph for normal bark tissue showed a uniform distribution of Mn for a large area of normal tissue.

A corresponding x-ray photomicrograph of Mn and a line profile analysis of IBN lesion (Fig. 2) showed Mn to accumulate in necrotic lesions caused by the Mn treatment. The line scan showed that Mn content increased in the necrotic area and was highest in the center of the necrotic area. The background count, line B, was uniform throughout the sample.

Secondary electron micrographs showed the cellular detail of the tissues (Fig. 3A and 4A). A triangle designates the center of the necrotic lesion of a high Mn treatment when viewed as a secondary electron image (Fig. 3A).

The Ca photomicrograph (Fig. 3D) of the same area as the high Mn photomicrograph, showed an increased concn of Ca within the necrotic lesion. Corresponding photomicrographs for K, (Fig. 3C) and P (Fig. 3B) showed decreased concn of these elements within the lesion.
photomicrograph (Fig. 4D) showed an increase of Ca within the necrotic lesion. Potassium and P were shown to decrease within the necrotic lesion as shown by the photomicrographs for K and P (Fig. 4C and 4B).

Line profile analysis of minus B apple bark tissue (Fig. 1A, line scan C) shows Mn content to be uniformly distributed within the tissue. Manganese content was low as shown by Mn count, averaging approximately 19 counts/sec; and the average background (line scan D), was 8 count/sec.

Quantitative determination of Mn was accomplished by comparing the unknown Mn value to the count/sec of K α radiation that would be given by exciting the pure element Mn under the same instrument conditions used in the experiment. The background count of the sample was then subtracted from the actual count of K α radiation recorded when the Mn within the sample was excited. This adjusted count was then divided by the count expected for the pure element, thus giving the percentage of Mn present. By this method, it was determined that the amount of Mn present in the necrotic lesion was approximately 6,000 ppm.

Eggert et al. (9) have shown by histochemical techniques that Mn does accumulate in necrotic lesions. They further stated that the amount of Mn found (1,000 ppm) were large enough to cause death of the tissue. Shelton et al. (18), using 54Mn in nutrient solutions, showed slight accumulations of the metal in necrotic lesions of 'Delicious' apple trees.

The lesions of Mn and minus B-induced IBN were similar in some respects. Both showed an accumulation of Ca within necrotic lesions. Both were similar in regard to K and P content found in each respective lesion, but Mn lesions and minus B lesions were different in Mn and B content. Manganese induced lesions were shown to have a high Mn content, and minus B induced lesions were shown to have a low B content. These results might suggest that excess Mn and deficient B could result in the accumulation of a similar metabolic substance that would cause death of the tissue. Forshey (10) stated that an accumulation of nitrates or nitrogenous compounds, as a result of high concn of metals, in the tissue could result in death of the tissue.

Since it has been shown that the condition can be induced with excess Mn or low B and that Ca accumulates in both types of lesions, it seems possible that tissue death may result from a similar metabolic substance. The accumulation of Ca may result from Ca migration to the lesion area and neutralizing the unidentified toxic substance. However, it is unlikely that excess Mn or low B per se is the cause of tissue death.

Literature Cited