INVESTIGATIONS OF INTERNAL BARK NECROSIS IN DELICIOUS APPLE TREES

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ABSTRACT

INVESTIGATIONS OF INTERNAL BARK NECROSIS
IN DELICIOUS APPLE TREES

By

Timothy Eugene Crocker

Internal bark necrosis (IBN) often referred to as "measles" was first described in detail by Hewitt and Truax in 1912. This disorder occurs predominantly on trees of the Delicious cultivar. The disease may result in a considerable reduction in growth and/or death of newly planted trees.

Many causative agents have been suggested as the cause of IBN: (1) a toxicity of the elements Mn, Fe, Cu, Co, Al, Zn, or Ni, (2) a toxic combination of two or more of these metals or (3) a deficiency of B.

An experiment was designed in 1967 to induce IBN on Delicious trees. The trees were grown in sand culture with two levels (normal and low) of Ca in combination with two levels (100 and 200 ppm) of Mn, Fe, Cu, Al, a mix of 50 ppm of each metal, and a minus B treatment. Only trees receiving the Mn or minus B treatments developed IBN. Trees treated with other treatments had a greater than

normal leaf accumulation of each respective metal, but there were no symptoms of IBN.

An experiment was conducted in 1968 to determine the concentration of Mn associated with a severity rating of IBN. Trees were grown in sand culture with normal and low levels of Ca in combination with five levels (0, 25, 50 75, and 100 ppm) of Mn. The severity of IBN increased with increasing leaf Mn content. A leaf Mn value of 500 ppm was established from results of this experiment as the value of Mn above which IBN may become severe.

An experiment was conducted in 1969 to determine if the severity of IBN occurred to a greater degree on spur-type than on Standard Delicious trees. Trees of both growth characteristics were grown in sand culture and received treatments of 50,100, and 150 ppm Mn. Neither severity of IBN nor leaf Mn were found to differ significantly for tree type. Terminal growth was significantly greater for the standard type trees.

The use of a corrective application of lime for IBN was studied in 1968. Five concentrations 0, 5, 10, 30, and 50 pounds of dolomitic hydrated lime per 100 gallons of water at three rates, 1, 2 and 3 gallons were applied at the base of the trees. Leaf Mg, soil Mg, soil Ca and soil pH were significantly increased for treatments. No significant differences in IBN or available

soil Mn occurred with lime treatments. However, IBN was not severe in any of the planlings studied.

NH_4NO_3, $(NH_4)_2SO_4$ and $NaNO_3$ applied at .5 and 1 lb. actual N per tree were compared to no N on the occurrence of IBN on Delicious trees. NH_4NO_3 and $(NH_4)_2SO_4$ increased the soil acidity and soil Mn significantly from the control (no N). No significant differences were observed for leaf N or IBN rating.

The microprobe X-ray analyzer was used to analyze thin sections of bark tissue affected with IBN. Mn and Ca were found in larger quantities in the necrotic areas than non-necrotic areas of tissue from trees treated with excess Mn. Minus B induced IBN lesions had a larger Ca concentration. K and P were found in smaller quantities within the necrotic lesions than non-necrotic areas for both Mn and minus B induced IBN.

Observations and analysis of tissue representing symptoms called IBN in areas outside of Michigan, indicated that the symptoms were unlike those induced in this study and, apparently were related to some factor not identified rather than excess Mn or deficiency B.

INVESTIGATIONS OF INTERNAL BARK NECROSIS IN DELICIOUS APPLE TREES

By

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INTRODUCTION

The popularity of the Delicious apple variety and its sports has been increasing during the last decade in all major apple growing areas of the United States and in many foreign countries. In Michigan, Delicious comprises 30 per cent of the present production, and accounts for 35 per cent of the trees in non-bearing orchards. The production of apples in North Carolina is now 51 per cent Delicious. The same is true for apple production in Washington and other apple-producing areas.

Delicious is susceptible to a disorder known as internal bark necrosis (IBN) or "measles". Hewitt and Truax (22) in 1912 were the first researchers to describe the disorder in detail, and they applied the name "apple measles". The disorder is worse on Delicious than on other major apple cultivars. Internal bark necrosis may result in the death of newly planted orchards, but more often results in reduced growth and an economical loss due to delayed production because of reduced tree growth.

Recent reports on internal bark necrosis have indicated various discrepancy opinions as to its cause. Many investigators believe it is associated with a manganese excess or boron deficiency. Still others surmise that it could be excessive amounts of other metals such as Al,

Fe, Cu, Co, Zn or Ni. Other researchers suggest an accumulation of all the heavy metals, of spray oils or of the expressed symptoms of a latent virus.

To resolve the confusion, experiments were conducted to determine if it was a heavy metal, a combination of metals, or boron deficiency. Control recommendations were tested to determine the best control practices. Studies were undertaken, also, to determine if there was an accumulation of nutrients in the necrotic areas of the bark tissue.

REVIEW OF LITERATURE

Internal bark necrosis (IBN) or "measles" have been reported on Golden Delicious, Grimes, Jonathan, McIntosh, Rome, Northwestern Greening, Stayman, York, and King David (8,30), and from personal observation on R. I. Greening. Nagai, et. al. (26) has reported IBN in Delicious and Roll trees in Japan, and Atkinson and Roberts (3) have reported the symptoms in New Zealand on Delicious.

IBN symptoms usually develop a year or two after planting, occurring on the lower branches and trunks of the trees. In very severe cases, the symptoms can appear on current seasons growth, causing severe stunting and eventual death of the tree. Many trees affected with IBN recover and grow normally after a few years. But some trees remain weak and stunted, never resuming normal growth.

Berg, et. al. (8) in West Virginia has observed three types of stem lesions characteristic of IBN--the pimply, the oedematous, and the minute superficial lesions. The pimply lesions were the most common and found in association with the other two types when they appear.

The pimply lesions developed from small necrotic areas which originated deep in the cortex or periderm. Later these areas became encysted within the bark by development of an enclosing layer of suberized cells. The first externally visible indication of this condition was raised points that appeared on the epidermal surface. These areas increased until the bark took on a characteristic rough surface with numerous cracks and splits.

Oedematous lesions occurred most commonly at the base of the trees. The bark of the oedematous lesions became swollen and had a water-soaked appearance. The outer periderm over these swellings split parallel to the axis of the stem and became loose appearing as a series of tan papery sheets covering the unruptured swollen portions.

Minute superficial lesions appeared as darkened points on the bark, which were perceptible to the touch, and were confined to the terminal portion of the current season's growth.

Berg, et. al. (7,8) and Shannon (32) reported a yellowing of the leaves sometimes as intervenal chlorosis and sometimes as large or small yellow areas. Affected leaves often abscised prematurely.

Many investigators associate an excess of manganese with the disorder (1, 2, 6, 7, 8, 13, 14, 15, 20, 21,

24, 26, 28, 31, 32, 33, 39) while some do not agree that excess manganese is the cause or only cause of IBN (10, 20, 39). Nagai, et. el. (26) has reported that the manganese content of trees grown in sand culture varied with rootstocks, and that two to three-year wood contained a higher level of manganese than did the current season's wood. Berg, et. al. and Orton, et. al. (8,28) disclosed that the manganese content of bark was less than found in the leaf; but Nagai, et. al. (26) found bark higher in manganese content than that found in the leaves.

Rogers, et. al. and Berg, et. al. (31,8) have shown IBN to be greater when ammonium nitrate or other acidifying sources of nitrogen were applied; on the other hand, Cahoon, et. al. and Hildebrand (10,23) have shown little response to sources of nitrogen.

Boron deficiency has similarly been implicated as a cause of IBN (9,24,32, 35, 37, 39), but Berg and Clulo (6) were unable to induce the symptoms with a nutrient solution absent of boron. However, some investigators have shown a response from applied boron (17,24) in correcting the disorder.

Still other investigators support the evidence that IBN may be a manganese toxicity or a boron deficiency (4, 32, 39).

Shannon (32) has induced the symptom with iron. Berg, et. al. (8) also suggested that iron may be

associated with the disorder. Zeider and Kink (39) found that high iron content seemed to delay but not prevent the symptoms caused by manganese excess. He found that a 50 ppm iron treatment gave a terminal rosetting and stunting of the trees. Forshey (20), in New York, induced the symptoms with high levels of manganese and also induced the symptoms by injections of iron, aluminum, copper, zinc, cobalt and nickel. Cobalt was as effective as manganese in inducing the symptoms, but the other elements gave a lesser response. Wave, et. al. (36), in Washington, stated that copper deficiency symptoms are very similar to those of the early stages of measles. Still other investigators (10, 20) feel that the condition may be aluminum toxicity.

Shannon (32), in a histological study, showed that deficiency symptoms of boron and excess symptoms of iron and manganese exhibited the same basic manifestation. His manganese symptoms for IBN agreed with the symptoms for IBN given by Berg, et. al. (8) and Clulo (4) to be manganese toxicity.

Wave and Stiles (36) were able to produce a measles-like symptom using superior oils, but with this condition the pimples or raised areas are associated with the lenticels rather than those occurring in smooth areas of the bark as generally observed for IBN. Zwick, et. al. (40) supported this theory and observed a definite proliferation

or corkiness of lenticels in the epidermis of the bark of young pear trees. These conditions have been observed on young Delicious trees by Downing (16).

Cheney et. al. (11) reported the symptoms of "pustule-canker", a graft transmissible bark disease of "Red Delicious" apple, as closely resembling those of measles and blister bark. But he stated that pustule-canker differed from IBN in that the former did show terminal die-back. He concluded that pustule-canker is distinct from IBN. Hickey at V.P.I. (personal correspondence) has no evidence that IBN is related to a latent virus.

Shelton, et. al. (33), in North Carolina, working with ^{54}Mn, found that it did accumulate in the necrotic areas of the bark tissues. Eggert and Hayden (18) at Purdue have shown, with a modified histochemical technique that manganese does have a relationship to IBN of apples and was accumulated in the necrotic areas of the bark tissue.

Cahoon and Banta (10), at Wooster, Ohio, stated that more than one factor may be involved with IBN. They believed that the disorder was associated with the total accumulation of heavy metals and believed aluminum to be directly involved. Forshey (20) stated that it was an interference with the normal nitrogen metabolism of the plant with an accumulation of toxic nitrogenous

intermediates that was responsible for the characteristic damage to the tissue. Eggert et. al. (19) believed that the accumulation of toxic amounts of manganese in the tissue could be responsible for the death of the tissue.

Fucik's (21) research with apple trees grown in water culture with varying levels of manganese and calcium, indicated that calcium was an important regulator in the absorption of manganese.

Many investigators have linked the occurrence of IBN with low soil pH and soils high in manganese (8, 3, 23, 31). Hildebrande (24) found that one ton of high calcium lime raised the pH from 4.0 to 5.0 and with this the leaves of the affected trees regained normal leaf characteristics. Clulo (3) found that calcium hydroxide, calcium carbonate, magnesium carbonate, magnesium oxide, and sodium carbonate all reduced the development of the disorder. Other researchers (8,20) report the use of lime as the common correction for IBN.

EXPERIMENTS

Experiment I

In the summer of 1967, an experiment was conducted to determine whether heavy metals and/or deficient boron would induce the disorder of internal bark necrosis (IBN) in young Delicious apple trees.

Two-year-old nursery trees of the variety Miller Sturdy Spur Delicious budded on E.M. VII were planted in three-gallon plastic pails in clean washed quartz sand. Approximately one-third of the roots and tops of the trees was removed from the trees before planting in the small container.

The trees were placed on a concrete apron at the Horticultural Research Center. The containers were fitted for automatic watering using the Chapin ring system.[1] Pumps were used to deliver a measured amount of solution from storage drums. The trees were watered each day using nutrient solutions. The amount of solution applied at each watering was approximately 1,000 ml. This amount of solution proved sufficient to leach the containers and retard salt accumulation.

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[1] Chapin Watermatic Company. New York.

The experimental design was a randomized block with two single-tree replicates.

Normal Ca trees were watered on alternate days with a complete one-half Hoagland[2] nutrient solution. Trees receiving the minus B treatment were watered on alternate days with a one-half Hoagland solution minus B. The trees receiving the low Ca treatment were watered on alternate days with a one-half Hoagland solution containing one-sixth of the normal concentration of Ca. Nutrient solutions were adjusted to a pH of 4.5.

Separate solutions with pH adjusted to 4.5 were made and applied manually to the trees on alternate days. These solutions provided the following treatments: 100 ppm Mn, 200 ppm Mn, 100 ppm Fe, 200 ppm Fe, 100 ppm Cu, 200 ppm Cu, 100 ppm Al, and a solution of 50 ppm each Mn, Fe, Cu and Al. Thus the final array of treatments was for minus boron trees plus the applied treatment with two single tree replicates as follows:

	100 ppm				200 ppm				
Low Ca (53.3 ppm)	Mn	Fe	Cu	Al	Mn	Fe	Cu	Al	MEM*
Normal Ca (160 ppm)	Mn	Fe	Cu	Al	Mn	Fe	Cu	Al	MEM

*MEM - minor element mix containing 50 ppm each of Mn, Fe, Cu and Al.

In (October) 1967, the trees were placed in cold storage at 35° F and were stored for five months to

[2]D. R. Hoagland and D. I. Arnon. 1950. The water-culture method for growing plants without soil. Calif. Agr. Exp. Cir. 347.

satisfy the rest requirement of the trees. On February 23, 1968, the trees were removed from storage and placed on greenhouse benches in the Plant Science Greenhouses. All fertilization treatments were then continued.

Leaf samples, from the middle of the terminal growth, were taken in August of 1967 and again in May of 1968. Bark samples were taken from the old (1967) and new bark (1968) in May of 1968. These samples were analyzed for Ca, Mg, Mn, Fe, Ca, B, Al nutrient composition in the plant Analysis Laboratory by spectrographic analysis.

Results

From visual observation made in 1967, only the 100 and 200 ppm Mn and minus B treatments developed symptoms of IBN. All but two of the trees treated with 100 and 200 ppm Mn developed IBN symptoms. Of the four trees that received minus B, three of them developed IBN symptoms. After the trees had received treatments for three months in the greenhouse, the incidence of IBN was recorded. It was found that all trees receiving 100 and 200 ppm Mn and minus B had symptoms of IBN. The Mn treatments (Figure 1C) developed more severe symptoms of IBN as denoted by the numerous raised necrotic areas (pimples) on the bark, than the minus B (Figure 1A) which had a sparse distribution of pimples.

The results of spectrographic analysis of leaf samples taken in 1967 and 1968, and the results of bark samples of old (1967) and new (1968) bark, Table 1, showed the Mn content of the leaf and bark tissues increased significantly over the controls for the 100 and 200 ppm Mn treatments. Leaf Mn was significantly higher in 1968 than in 1967, and the low Ca level, an average for two years,(leaf 881 and bark 589 ppm), was significantly higher in Mn than the normal Ca level, (leaf 677 and bark 476 ppm). With the leaf Mn values, there was a significant years x Ca level and years x treatment interaction. The year x Ca level interaction was denoted by the small change in Mn content for 1967; average for low Ca level 437 ppm, average for normal Ca level 380 ppm; compared to the large change in Mn with Ca level for 1968; average for low Ca level 1,325 ppm; average for normal Ca level 964 ppm. The years x treatment interaction, Table 1, showed that there was a greater increase of Mn with treatment for 1968 than there was for 1967.

The results, Table 2, showed the leaf and bark tissues of the minus B treatment to be significantly lower in B than the control tissues. Little difference was found in B content within treatments with regard to bark age.

A very pronounced rossette condition and stunting was observed with the 200 ppm Fe treatment (Figure 1B).

TABLE 1.--Mn content of leaf and bark tissue as affected by varying concentrations of Mn and Ca.

Treatment	Low Ca (53.3 ppm) 1967	Low Ca (53.3 ppm) 1968	Normal Ca (160 ppm) 1967	Normal Ca (160 ppm) 1968	Average 1967	Average 1968
			Leaf Manganese ppm			
Control	95	141	73	82	84	112
100 ppm Mn	719**	2161**	539*	1445**	629**	1803**
200 ppm Mn	585**	2065**	599**	1673**	592**	1869**
50 ppm Mn[1]	350	933**	351	658*	350	795**
Average	437	1325	390	964	414a	1145
			Bark Manganese ppm			
Control	28	60	28	43	28	51
100 ppm Mn	1010**	1024**	590**	1235**	800**	869**
200 ppm Mn	1013**	1096**	750**	714**	881**	1165**
50 ppm Mn[1]	288	196	209	244	248	220
Average	559	595	394	585	489	576

* Means significantly different from control at 5% level.
** Means significantly different from control at 1% level.
a 1967 Mn means significantly different from 1968 at 1% level.
1 Mix containing 50 ppm Mn, Fe, Cu and Al.

TABLE 2.--B content of leaf and bark tissue as affected by an absence of B from the nutrient solution.

Treatment	Leaf B ppm 1967	1968	Average
Control	23.7	43.1	33.4
Minus Boron	5.4**	7.1**	6.7**

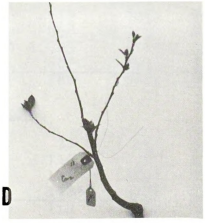
** Means significantly different from control at 1% level.

Figure 1 Delicious trees in second year of heavy metal and minus B treatments.

A. A tree from minus B treatment. IBN severe.
B. A tree from high (200 ppm) Fe. No IBN, pronounced rosetting.
C. A tree from high (200 ppm) Mn. IBN severe.
D. A tree from high (200 ppm) Cu. No IBN, very short terminal growth.
E. A tree from high (200 ppm) Al. No IBN.
F. A tree from the mix (50 ppm each of Mn, Cu, Fe and Al). No IBN.


A


B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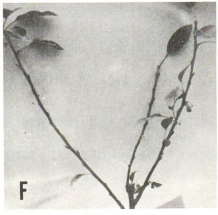
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These symptoms were very similar to those described by Shannon (32) to be Fe toxicity.

The leaf Fe values (Table 3) increased significantly for 1968 over 1967. There was no significant difference between Ca levels for Fe, low Ca 893 ppm; normal Ca 562 ppm. The average Fe value showed a significant increase for the 200 ppm treatment over the control.

TABLE 3.--Fe content of leaf and bark tissue as affected by varying concentrations of Fe and Ca.

	Low Ca (53.3ppm)		Normal Ca (160 ppm)		Average	
Treatment	1967	1968	1967	1968	1967	1968
			Leaf Iron ppm			
Control	274	333	168	579	221	456
100 ppm Fe	825*	1,020*	601	858	713	939
200 ppm Fe	914*	1,568**	722*	1,025	818*	1,296*
50 ppm Fe[1]	604	1,259**	386	687	495	873
Average	654	1,000	469	787	562a	893
			Bark Iron ppm			
Control	53	56	58	71	55	63
100 ppm Fe	94	160**	103	112	98	136*
200 ppm Fe	136**	183**	106	121	121*	152**
50 ppm Fe[1]	136**	230**	91	172**	113*	201**
Average	105	157	89	119	97a	138

* Means significantly different from control at 5% level.
** Means significantly different from control at 1% level.
a 1967 Fe means significantly different from 1968 at 1% level.
1) Mix containing 50 ppm Mn, Fe, Ca and Al.

Bark analysis (Table 3) for the Fe treatments showed a significant increase in Fe content in 1968 over 1967 and

and low Ca, an average for two years 131 ppm, over normal Ca, 104 ppm. With the average Fe values for bark, all but the 100 ppm treatment in 1967 were significantly different from the control.

A symptom of Cu toxicity is shown by the 200 ppm Cu treatment (Figure 1D). The symptom was expressed as short terminal growth and stunting of the foliage of the tree. Some trees showed terminal die-back.

Leaf Cu values (Table 4) in 1968 were significantly greater than in 1967. No significant difference was observed for Ca level, average value for two years, low Ca 62.3 ppm and normal Ca 53.5 ppm. For the average treatment values of Cu in 1967 and 1968, all but the 1967 100 ppm treatment were significantly different from the control. There was a significant year x treatment interaction because of the small change in Cu for the control and 100 ppm treatment partly due to the control receiving a smaller amount of Cu, versus the larger accumulation for the 50 and 200 ppm treatment.

Bark analysis for Cu (Table 4) showed a significant increase in Cu for 1968 over 1967 and no significant difference between Ca levels, an average for two years, low Ca 65.8 ppm and normal Ca 58.5 ppm. With the average treatment Cu values for 1967 and 1968, all but the 100 ppm Cu treatment in 1967 showed a significant increase over the control.

TABLE 4.--Cu content of leaf and bark tissue as affected by varying concentrations of Cu and Ca.

Treatment	Low Ca (53.3 ppm) 1967	Low Ca (53.3 ppm) 1968	Normal Ca (160 ppm) 1967	Normal Ca (160 ppm) 1968	Average 1967	Average 1968
			Leaf Copper ppm			
Control	11.4	19.1	10.7	19.0	11.1	19.1
100 ppm Cu	32.9	59.1*	23.1	56.1*	28.0	57.6*
200 ppm Cu	32.2	106.2**	58.0*	104.8**	45.1*	105.5**
50 ppm Cu 1/	47.0*	120.1**	110.0**	117.0**	78.6**	118.0**
Average	30.9	76.1	50.5	74.2	40.7a	75.1
			Bark Copper ppm			
Control	22.1	30.5	23.5	30.4	22.8	30.5
100 ppm Cu	55.4*	74.2**	38.0	58.8*	46.7	66.5**
200 ppm Cu	58.1**	74.4**	58.2**	74.3**	58.2**	74.3**
50 ppm Cu 1/	64.0**	147.4**	88.7**	96.0**	76.4**	121.7**
Average	52.4	81.6	49.9	64.9	51.0a	73.3

* Means significantly different from control at 5% level.
** Means significantly different from control at 1% level.
a 1967 Cu means significantly different from 1968 at 1% level.
1/ Mix containing 50 ppm Mn, Fe, Ca and Al.

There was a significant year x Ca level x treatment interaction. The treatment x Ca level interaction was the result of the Cu content of 200 ppm Cu being higher than 100 ppm for normal Ca, but not for low Ca. The interaction of years x treatments resulted from a greater increase in Cu for the treatments over the controls for 1968; and the interaction with Ca levels x years seen by the average value of Cu for all treatments for low and normal Ca being much closer in 1967, low Ca 52.4 ppm and normal Ca 49.9 ppm, than in 1968, low Ca 81.6 ppm and normal Ca 64.9 ppm.

The 200 ppm Al treatment (Figure 1E) showed a delayed foliation of the tree. The terminal bud would break first, and then the lateral buds would leaf-out one to three days later. The symptom looked very similar to that of a tree that had not received adequate cold to satisfy the rest requirement.

The leaf Al values (Table 5) showed a significant increase in Al for 1968 over 1967. There was no significant difference between Ca levels, average values for two years, low Ca 422.4 ppm and normal Ca 377.7 ppm. The 200 ppm treatment was significantly different from the control in all cases; and the 100 ppm treatment was significantly different from the control in 1968.

The bark analysis (Table 5) for Al was not significantly influenced by treatment.

The mix treatment, which was a mixture of 50 ppm each of Mn, Fe, Cu and Al, had symptoms very similar to those of the copper treatment (Figure 1F). These symptoms would be expected after observing the Cu values for the mix treatment in Table 4.

Discussion

In the experiment where heavy metals and minus B were used to try to induce IBN, IBN occurred with only 100 and 200 ppm Mn and minus B treatments.

That IBN was associated with high leaf value of 500 to 2,000 ppm manganese agreed with the research

reported by Zeiders et. al. (39), Berg et. al. (8) and others (1, 2, 6, 7, 13, 14, 15, 19, 20, 21, 24, 26, 28, 31, 33).

TABLE 5.--Al content of leaf and bark tissue as affected by varying concentrations of Al and Ca.

	Low Ca (53.3 ppm)		Normal Ca (160 ppm)		Average	
Treatment	1967	1968	1967	1968	1967	1968
			Leaf Al ppm			
Control	177	325	151	328	164	327
100 ppm Al	277	666**	322	549*	299	608**
200 ppm Al	486*	840**	360*	685**	423**	762**
50 ppm Al[1]	322	284	233	391	278	338
Average	316	529	267	489	291.3a	508.9
			Bark Al ppm			
Control	51	63	51	50	51	56
100 ppm Al	89	64	88	74	89	69
200 ppm Al	63	70	64	76	64	73
50 ppm Al[1]	51	64	63	53	57	58
Average	64	65	66	64	65	64
	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

N.S. No significant difference between means.
* Means significantly different from control at 5% level.
** Means sginificantly different from control at 1% level.
a 1967 Al mean significantly different from 1968 at 1% level.
1) Mix containing 50 ppm Mn, Fe, Ca and Al.

Low B values (5 to 7 ppm B) were associated with IBN agreed with the research of Zeiders et. al. (39), Young et. al. (37), Shannon (32) and Hildebrand (23), but Berg et. al. (8) and other researchers (6, 31, 34) were

not able to find an association between low B and IBN. This relationship may not exist if the trees in their studies had not depleted the residual boron supply in various tissues of the tree.

Berg et. al. (8) showed Mn values to be higher in leaf than in bark tissue, which agreed with the results of this experiment. Nagai et. al. (26) reported Mn content to be higher in two to three-year old bark than in current season's bark. However, in this experiment current season growth was found to be higher in Mn than the older bark.

The high 100 and 200 ppm Fe treatments gave typical rosetting and stunting of the tree as reported by Shannon (32) and Zeiders et. al. (39). But no IBN was observed on the trees as reported by Shannon (32). The root symptoms of dark roots for the 100 and 200 ppm Fe treatments were the same as reported by Zeiders (39), and this may be a result of the sequestrene form of Fe used.

The mix treatment showed near toxic concentrations of Mn, Fe and Cu but there were no visible or external symptoms of IBN. This evidence did not support the work of Cahoon et. al. (10) or Forshey (20) who reported that the disorder could be caused by an accumulation of heavy metals. Zeiders et. al. (39), stated that high levels of Fe would delay, but not inhibit the expression of the symptoms of IBN. This, in part, was seen by the mix not

showing IBN where a high Mn value (Table 1; 50 ppm treatment low Ca 1968) and high Fe value (Table 3; 50 ppm treatment low Ca 1968) were observed.

With leaf values, only Mn was found to be increased with the low level of Ca. Fucik (21) has reported that Ca did have an effect on the uptake of Mn. With bark values, the low Ca level had higher values for Mn and Fe; and Cu and Al values were not significantly affected by Ca level.

With regard to bark, Mn, Fe and Cu were found in greater concentrations in the new bark (1968). Al, unlike the other elements, was not found to accumulate in bark tissue even though significantly different accumulations of Al with amount applied were observed in the leaf. This suggests that Al was not held in the bark tissue and thus precluded the possibility of Al excess causing IBN.

From this experiment it was concluded that IBN could be the result of excess Mn or deficient B, and that it was not associated with excess amounts of Fe, Cu or Al.

Experiment II

In the summer of 1968, an experiment was designed to determine the minimum level of Mn that would induce IBN.

Miller Sturdy Spur Delicious trees on E.M. VII rootstock were used with potting, nutrient solutions (to supply low and normal Ca), and plot design being the same as for Experiment I. The solutions of Mn (100, 75, 50, 25, and 0 ppm) were applied at the rate of 1,000 ml per tree every other day, alternating with the Ca solutions.

The trees were grown during the summer, 1968, at the Horticulture Research Center and in the fall, 1968, moved into cold storage. The trees were moved to the greenhouse in February, 1969, and the treatments were then resumed.

Leaf samples from the center of current season's growth were taken in August of 1968 and again in May of 1969. Nutrient analysis was determined in the Plant Analysis Laboratory by spectographic analysis. The severity of IBN was rated for each tree in September, 1968, and again in May, 1969. The rating is presented in Table 6.

TABLE 6.--IBN rating scheme for determining the severity of IBN on apple trees.

Rating	Number of pimples Present in 3 sq. cm.	Age of bark
1	None	2 Year
2	1 to 5	2 "
3	More than 5	2 "
4	1 to 5	1 "
5	More than 5	1 "

Results

The IBN rating (Table 7) was significantly greater for low Ca than normal Ca in 1968. There was no significant difference with Ca levels in 1969 but there was a significant interaction for Mn treatment x Ca level. This was a result of the high ratings in 1969, of the 25 and 50 ppm Mn for the low Ca level. In 1968 only the 75 and 100 ppm Mn were significantly different from the control for the normal Ca level; while with the low Ca level, the 50, 75 and 100 ppm Mn were significantly different from the control. In 1969, only the 25 ppm Mn level with normal Ca level was not significantly different from the control.

TABLE 7.--Average IBN rating for Delicious trees grown with varying levels of Mn and Ca for two seasons.

Treatment	1968		1969	
	Normal Ca (160 ppm)	Low Ca (53.3 ppm)	Normal Ca (160 ppm)	Low Ca (53.3 ppm)
	IBN Rating			
Control	1.00	1.00	1.00	1.00
25 ppm Mn	1.25	1.50	1.50	4.50**
50 ppm Mn	1.25	2.50*	2.75*	5.00**
75 ppm Mn	2.25*	2.75**	5.00**	5.00**
100 ppm Mn	2.50*	3.75**	5.00**	5.00**

* Means significantly different from control at 5% level.
** Means significantly different from control at 1% level.

The rating of IBN (Table 8) showed a significant effect of Ca level on the response to different Mn

concentrations. The rating for IBN for the low Ca level was significantly higher than the control for the 50, 75 and 100 ppm treatments; however, the rating of IBN for the normal Ca level was only significantly different from the control with the 100 ppm Mn. All leaf Mn values, except the 25 ppm Mn treatment at the normal Ca level, were significantly different from the control.

TABLE 8.--Rating of IBN on Delicious trees receiving varying levels of Ca and Mn after one growing season.

	Normal Ca (160 ppm)		Low Ca (53.3 ppm)	
Treatment	Rating IBN	Mn ppm	Rating IBN	Mn ppm
Control	1.00	80.8	1.00	120.8
25 ppm Mn	1.25	191.0	1.50	500.1**
50 ppm Mn	1.25	329.0*	2.50*	703.0**
75 ppm Mn	2.25	462.3**	2.75*	996.1**
100 ppm Mn	2.50*	629.3**	3.75**	1,168.0**

* Means significantly different from control at 5% level.
** Means significantly different from control at 1% level.

The 50 ppm Mn treatment for the low Ca level showed an IBN rating of 2.5 and a leaf Mn value of 703 ppm. The normal Ca level required the 100 ppm Mn treatment to give a comparable IBN rating of 2.5 and a Mn leaf value of 629.3 ppm.

The rating of IBN for the low Ca level was significantly greater than the normal Ca level in 1968 (Table 9). In 1969, the low Ca level did not significantly increase the rating of IBN above the normal Ca level. The leaf

concentration of Ca followed the same pattern as the rating of IBN with the low Ca level being significantly less than the normal Ca level in 1968, but it was not significantly different in 1969. The leaf concentration of Mn was significantly greater with the low Ca level in both 1968 and 1969.

TABLE 9.--Leaf concentration of Ca, Mn and rating of IBN as affected by normal and low Ca levels.

Calcium level	IBN Average Rating	Ca %	Mn ppm
		1968	
Normal (160 ppm)	1.55	1.05	337
Low (53.3 ppm)	2.25*	.83*	549**
		1969	
Normal (160 ppm)	3.05	1.13	559
Low (53.3 ppm)	4.10	1.39	673**

* Means significantly different at 5% level.
** Means significantly different at 1% level.

Figure 2 shows the IBN rating had a positive linear relationship with leaf Mn. The correlation coefficient was 0.5791 and was significant at the .0005 level. The standard error of estimate was ±177.5 ppm Mn.

Figure 3 shows that both low and normal Ca were significantly greater than the control in severity of IBN at all dates. The IBN rating for low Ca increased significantly from October to March while the normal Ca increased only slightly for this period. The rating of IBN

Figure 2. Leaf Mn content of Delicious trees correlated with IBN rating observed after one year of Mn treatments.

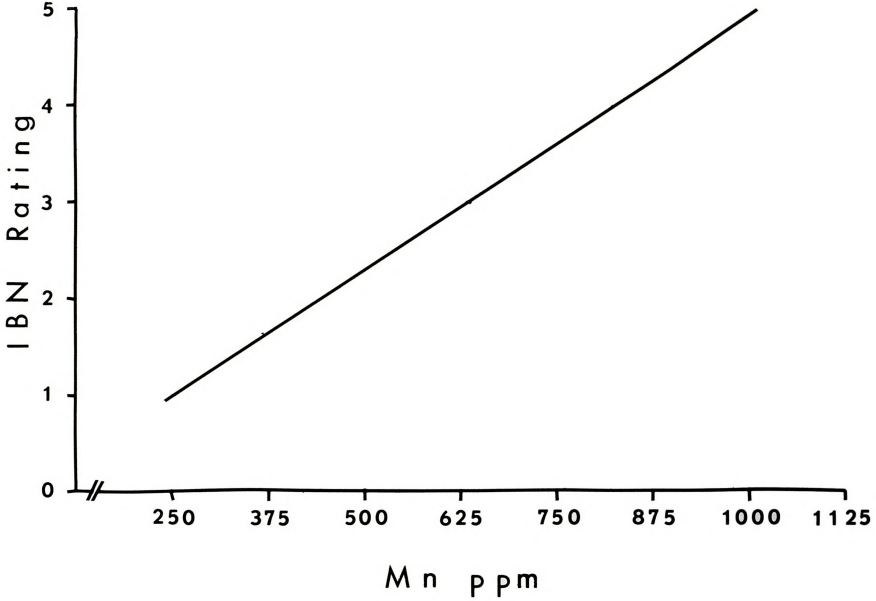
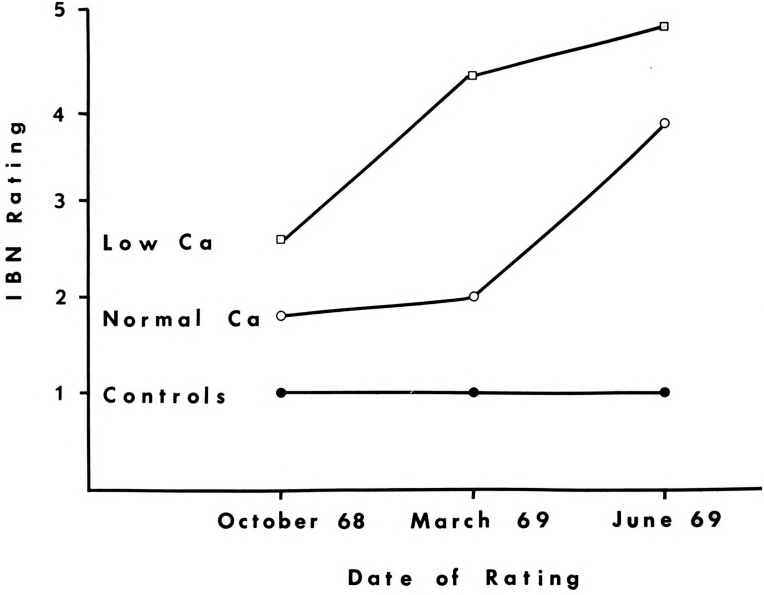

IBN Rating
0
1
2
3
4
5
250
375
500
625
750
875
1000
1125
Mn ppm

Figure 3. Average rating of IBN for low Ca, normal Ca and control observed in October, March and June.


5
4
3
2
1
IBN Rating
Low Ca
Normal Ca
Controls
October 68
March 69
June 69
Date of Rating

for normal Ca increased significantly from March to June, but the low Ca increased only slightly and this may have been due to the trees receiving low Ca having a maximum rating for IBN in March. The controls were rated one for all rating dates.

Discussion

The results of the different Mn concentrations showed that the severity of IBN increased with increasing levels of Mn. However, a given severity rating could have a wide range (150 to 350 ppm) of Mn correlated with it.

The low Ca level resulted in a much greater quantity of Mn being absorbed in a shorter period of time than the normal Ca level. Fucik (21) showed this relationship. This increased uptake of Mn was evident by the more severe IBN rating given to trees grown with the low Ca solution. Lower levels of Mn applied induced a more severe IBN on trees receiving the low Ca solution.

IBN increased while the trees were in storage. This may explain why the growers have observed a severe condition of IBN in the spring after the first growing season for young trees. These results suggest that the substance that caused tissue death or necrosis was also active during the storage period.

A leaf composition value of approximately 500 ppm Mn was necessary before young trees would develop severe

IBN and need corrective measures. Tree death occurred at Mn concentrations between 800 and 1,000 ppm as reported by other researchers (8,3). Many trees greatly exceeded this Mn value, but these trees were, in reality, dead at the time of sampling. The rating of 5 for IBN indicated that the trees would probably die during the next growing season or during the dormant period.

The rating system for IBN was thought to be adequate when it was devised. However, a rating employing a wider range would not have grouped in one category trees severely affected the first year. The highest IBN rating value was too low to permit evaluation of further IBN development. Because the low Ca level and normal Ca level were closer together in terms of IBN rating for the higher Mn treatments the second year than the first year (Table 7), this suggests a need for an expanded rating system.

Experiment III

In the summer of 1969, an experiment was designed to determine if spur-type Delicious trees were more susceptible to IBN than standard Delicious trees. The plot design was randomized block with eight single-tree replicates.

Trees of Miller Sturdy Spur Delicious on MM 106 and Red Prince Delicious MM 106 rootstock were root and top pruned and potted in washed quartz sand in three-

gallon plastic pails. All trees were placed on the automatic watering system with the nutrient solution being one-half strength Hoagland solution with Ca reduced to one-sixth strength applied every other day.

Mn concentrations of 50, 100 and 150 ppm were used. Applications were of 1,000 ml of the Mn solutions with the pH adjusted to 4.5 applied to the trees every other day alternating with the nutrient solution.

Leaf samples were taken in August, 1969, and the nutrient composition values were determined as before. The trees were rated in September, 1969, as to the severity of IBN in accordance with the previous rating scheme.

Results

The results (Table 10) showed no significant difference between the two types of trees on the occurrence of IBN. Terminal growth was significantly greater on the standard trees than on the spur-type trees.

TABLE 10.--Standard versus spur-type Delicious apple trees receiving varying levels of Mn (150, 100 and 50 ppm) on the occurrence of IBN.

Type Tree	IBN Average Rating	Leaf Mn ppm	Terminal Growth in inches
Standard	3.67	876	14.0
Spur-type	3.71	889	9.0
	N.S.	N.S.	**

** Difference between means significant at the 1% level.
N.S. No significant difference between means.

The results showed (Table 11) the leaf Mn concentration and IBN rating significantly increased with the increasing concentrations of the Mn treatments. Both 100 and 200 ppm Mn were significantly different from the 50 ppm treatment for leaf Mn and IBN rating. There was no significant tree type x Mn interaction, and no significant difference in terminal growth was observed with Mn treatment.

TABLE 11.--IBN rating and Mn leaf concentration of standard and spur-type Delicious apple trees as affected by three concentrations of Mn (150, 100 and 50 ppm).

Level of Mn ppm	Leaf Mn ppm	IBN Average Rating	Terminal Growth
50	503	1.87	11.75
100	1,004**	4.37**	11.55
150	1,123**	4.81**	11.30
			N.S.

N.S. Means not significantly different from each other.
** Means significantly different from 50 ppm at 1% level.

Discussion

The results showed no difference between standard and spur-type Delicious trees treated with varying Mn levels in regard to the severity of IBN. This differs from field observations made by growers and extension agents, who have reported that the disorder (IBN) occurred to a greater degree on the spur-type Delicious trees.

The leaf concentration and severity rating of IBN were found to increase with increasing concentrations of Mn as found in previous experiments. The terminal growth of the standard Delicious trees was significantly greater than the growth of the spur-type Delicious trees.

Experiment IV

In the spring of 1967 and 1968, new Delicious plantings in Michigan were surveyed to determine the possibility of IBN developing. Observations from the previous summer showed that it was almost impossible to determine in older orchards whether the trees were just beginning to develop the measled condition, were in the measled condition, or had grown out of the disorder with only the symptoms and not the condition remaining. The only positive way to determine the current stage of IBN was by determining the nutrient content of leaves from the trees.

Letters were mailed to different nuseryman in Michigan asking for the name and address of growers in Michigan who had purchased 100 or more Delicious trees for planting in 1967 or 1968. The growers were then contacted and leaf samples taken from each of the newly planted orchards. Leaf samples were obtained from many nursery blocks of young Delicious trees. Nutrient analysis was conducted for each sample in the Plant Analysis Laboratory.

Manganese and boron compostion values were used to determine if the young trees would develop IBN. From these analyses three orchards were selected in southwest Michigan as being suitable for study of corrective measures for IBN.

In the two orchards where the existing trees were used, one orchard was of the variety Miller Sturdy Spur on seedling rootstock, and the other orchard was Miller Sturdy Spur on E.M. VII rootstock. Existing trees were not used in the third orchard because the trees were four to five years old. Therefore, young Delicious trees on seedling rootstocks were planted for this experiment.

In the orchards where existing trees were used, the solutions of 50, 30, 10 and 5 pounds of hydrated dolomitic lime (composition Mg, 20.5% and Ca, 37.5%) per 100 gallons of water were applied at the rate of 1, 2 or 3 gallons per tree. The solution was applied at the base of the trees where a shallow basin, one and one-half feet in diameter and three to four inches deep, was constructed. The 0 concentration (control) did not receive any solution.

The experimental design was randomized block with three two-tree replicates.

Also included in these two orchards was an experiment designed to determine if the source or amount of nitrogen applied to the trees would enhance the occurrence of IBN. The nitrogen sources were ammonium nitrate,

sodium nitrate and ammonium sulfate applied at rates of one pound and one-half pound of actual nitrogen per tree. The control trees received no nitrogen. The plot design was a randomized block with three two-tree replicates.

In the third orchard, where high concentrations of manganese were found in the leaves of the older trees, young Delicious trees on seedling rootstock were planted at a spacing of five feet between trees with the row of young trees planted between two existing tree rows. After planting, the lime solutions, as stated before, were applied into the planting holes after filling. The experimental design was randomized block with three one-tree replicates.

Soil samples were taken six inches from the base of the tree and to a depth of six inches at all locations in the fall of 1969. Complete soil analysis, including available Mn, was determined by the Soil Testing Laboratory at Michigan State University.

Leaf samples were taken from the test plots in August of 1969 and complete nutrient analysis determined. The severity of IBN was observed in the fall of 1969 in accordance with the previous rating system.

With each of three orchards, the grower was asked to carry out his normal spray program and other cultural practices.

Results

The survey data showed that most of the young plantings sampled had a leaf Mn content between 0 and 200 ppm (Table 12). Only 2 of the orchards sampled had manganese values above 500 ppm.

TABLE 12.--Mn content of leaves from newly-planted young Delicious trees (samples the year of planting).

ppm Mn in leaves	Number of Orchards 1967	1968	Total	% of Total
0- 99	11	5	16	21.05
100-199	18	27	45	59.21
200-299	4	4	8	10.53
300-399	3	0	3	3.94
400-499	1	1	2	2.63
500-599	1	0	1	1.32
600 or more	1	0	1	1.32
Total	39	37	76	100.00

Table 13 shows the manganese content of leaves taken from young Delicious trees budded and growing in Michigan nusery rows. Most of the trees had a manganese content in the range of 100-199 ppm. This has been reported as the normal manganese concentration range for Delicious apple trees (12).

No significant location x treatment interactions were observed for the two sites where existing trees were used, therefore, the leaf and soil analysis results are presented as an average of the two sites.

TABLE 13.--Mn leaf content of young Delicious trees in nursery row.

Mn ppm in leaves	Rows Samples	% of Total
100-199	21	87.50
200-299	2	8.33
300-399	1	4.17
Total	24	100.00

The leaf composition values for Ca, Mg and Mn (Table 14) showed no significant difference from the control with increasing lime concentration. The leaf Mn values were seen to decline with increasing lime concentration; however, the leaf Mn value was much lower for the control than the 350 to 524 ppm found when the trees were first sampled in 1967. No significant difference was found between treatments on the severity of IBN, which was considerably less than in 1967.

TABLE 14.--Leaf analysis values and IBN rating of Delicious trees treated with lime slurry solutions. Existing orchards.

Lb. of hydrated dolomitic lime/100 gal. H_2O	1969a			
	Ca%	Mg%	Mn ppm	IBN
0	1.60	.30	174	1.17
5	1.62	.31	173	1.02
10	1.53	.29	154	1.33
30	1.59	.30	149	1.34
50	1.53	.30	154	1.34
	N.S.	N.S.	N.S.	N.S.

N.S. Means not significantly different from 0 level.
a Average of two sites.

Soil Mg and pH were found to be significantly increased for the 50 lb. lime treatment over the control. The other treatments showed no significant increase (Table 15). There was no significant difference between treatments for soil Ca and soil Mn.

TABLE 15.--Soil analysis results from Delicious trees treated with lime slurry solutions. Existing orchards.

Lb. of hydrated dolomitic lime/ 100 gal. H_2O	1969a			
	Soil Ca lb./acre	Soil Mg lb./acre	Soil Mn ppm	Soil pH
0	1,226	335	44.0	5.55
5	1,436	345	48.1	5.68
10	1,522	405	66.6	5.86
30	1,528	540	51.9	6.36
50	1,429	669*	50.0	6.72*

a Average of two sites.

The rate of application of the lime slurry solution (Table 16) significantly increased the soil pH value for the three-gallon rate. There was no significant increase for any of the other parameters with increasing rates of the lime slurry solution; however, leaf Ca, soil Ca, and soil Mg showed a slight increase with increasing rate of application.

The leaf composition values of the planted Delicious trees treated with the lime slurry solutions (Table 17) showed the Ca value not to be significantly

increased with increased amounts of lime. The leaf Mg values did increase with increasing concentration of lime, and the highest concentration (50 lbs) was significantly higher than the control. The leaf concentrations of Mn were not significantly different from the control, but there was a slight decline in Mn content for the 5, 10 and 30 lb. treatments.

TABLE 16.--Soil analysis results from Delicious trees treated with the lime slurry solutions. Existing orchards.

Gal. of slurry per tree	Ca leaf %	Mg leaf %	1969a Ca soil lb./acre	1969a Mg soil lb./acre	Soil pH
1	1.43	.37	1,355	417	5.80
2	1.44	.37	1,301	427	6.10
3	1.49	.37	1,356	450	6.22*

* Means significantly different from one-gallon treatment at 5% level.
a Average of two sites.

TABLE 17.--Leaf analysis values and IBN rating for planted Delicious trees treated with the lime slurry solutions. Newly planted trees.

Lb. of dolomitic hydrated lime/ 100 gal. H_2O	Ca %	Mg %	1969 Mn ppm	IBN
0	1.33	.23	488	3.33
5	1.55	.24	447	2.22
10	1.63	.35	458	2.00
30	1.44	.38	443	2.33
50	1.35	.42*	481	2.44

* Means significantly different from 0 level at 5% level.

The IBN rating was less for all levels of the lime concentrations, but none of the means was significantly different.

Soil analysis results from the planted Delicious trees (Table 18) showed the soil Ca value increased with higher concentrations of lime.

TABLE 18.--Soil analysis results for planted Delicious trees treated with the lime slurry solutions. Newly planted trees.

Lb. of dolomitic hydrated lime/ 100 gal. H_2O	1969 Ca Soil lb./acre	Mg Soil lb./acre	Mn Soil ppm	pH
0	555	129	24.7	6.26
5	692	147	26.7	6.50
10	732	199	24.3	6.95
30	693	368	26.6	7.26*
50	850*	510**	33.1	7.76**

* Means significantly different from 0 level at 5% level.
** Means significantly different from 0 level at 1% level.

Soil Mg increased with all amounts of liming material. There was no significant difference between treatments with regard to available soil manganese. The soil pH value was raised with increasing concentrations of liming material. Only the 50 lb. concentration was significantly different from the control (0 treatment) in all cases except soil manganese.

The leaf composition values for trees that were treated with three sources of nitrogen and a control are

given in Table 19. Leaf value for N, Mn, and the severity of IBN were not significantly different from the control for any source of nitrogen. The Mn value, however, tended to increase for all nitrogen sources. NH_4NO_3 and $(NH_4)_2SO_4$ had slightly higher values for IBN than the control and the $NaNO_3$ had the lowest rating of any source.

TABLE 19.--Leaf composition and IBN rating of trees receiving different sources of N. Existing orchards.

Source	N %	1969a Mn ppm	IBN rating
NH_4NO_3	2.38	208	1.54
$(NH_4)_2SO_4$	2.38	231	1.67
$NaNO_3$	2.35	217	1.33
Control	2.41	165	1.50
	N.S.	N.S.	N.S.

N.S. Means not significantly different from the control.
a Average of two sites.

Soil analysis for the trees treated with the nitrogen sources (Table 20) showed NH_4NO_3 and $(NH_4)_2SO_4$ to be significantly higher in soil Mn than the control. Soil acidity was found to be significantly higher where the ammonium nitrate and sulfate were used. The $NaNO_3$ was found to be slightly lower than the control with regard to soil acidity.

No significant differences were found with regard to the one pound and one-half pound of actual N rates applied.

TABLE 20.--Soil analysis results of trees receiving difference sources of N.

Source	1969a Soil Mn ppm	pH
NH_4NO_3	97.2*	4.66**
$(NH_4)_2SO_4$	78.8*	4.73**
$NaNO_3$	52.6	5.46
Control	45.2	5.37

* Means significantly different from control at 5% level.
** Means significantly different from control at 1% level.
a Average of two sites.

Discussion

Growers and extension personnel had indicated that IBN was a large problem in Michigan. However, leaf analysis survey data of young Delicious plantings did not suggest IBN to be present in most Michigan plantings. The results showed two orchards out of 76 sampled would probably need corrective lime application for IBN, since a leaf Mn value above 500 ppm was found.

The lesser amount of IBN observed could result because the symptoms of IBN remain visible on the bark of the tree after the tree has grown out of the IBN condition. Therefore, orchards may have been reported as having the disorder when, in fact, the orchard had outgrown the condition even though the bark symptoms were still visible.

Sampling in the nursery showed young trees were not accumulating toxic amounts of Mn in the nursery.

Thus the trees that did develop IBN were accumulating the Mn after being planted in the growers' orchards.

Leaf analysis data showed leaf Ca not to be significantly different from the controls in the liming experiment. With regard to leaf Mg the existing trees did show a significant difference between treatment and the 0 level of lime (control). The leaf Mn value did not increase significantly from the 0 level of lime, but in the nitrogen experiment the leaf Mn value increased slightly with all forms of nigrogen, with $(NH_4)_2SO_4$ having the highest Mn value. Kenworthy (25) showed the uptake of Mn by cherry trees was greater when NH_4NO_3 was applied than when no N was used.

IBN rating was not significantly affected by treatment; however, the 0 level of lime for the planted Delicious tree lime treatments and the NH_4NO_3 and $(NH_4)_2SO_4$ forms of N had the higher rating for IBN.

The soil Mn in the lime experiment was observed to increase slightly with increasing liming material and with decreasing soil acidity. This does not agree with Pailoor's work (27) where he found soil Mn to diminish with decreasing acidity. Also this discrepancy was shown by the fact that the soil Mn values were not in agreement with the leaf Mn content where a slight decrease was seen in leaf Mn with 5, 10 and 30 lb. concentrations of liming solution. One explanation for the higher soil Mn values

could be the extracting method used in the soil tests. The method employed was a 0.1 N HCl solution. This solution could possibly extract the easily reducible Mn that was not considered available for uptake by the plant, thereby giving a higher apparent available soil Mn value.

The soil Mn and pH were significantly increased for the NH_4NO_3 and $(NH_4)_2SO_4$ forms of N over the control. Forshey (20) has reported that the ammonium forms of N will increase soil acidity; this increased available Mn. The $NaNO_3$ resulted in a higher soil pH value than the other sources of N. This was in agreement with what has been reported (8) about the failure of $NaNO_3$ to increase soil acidity to the same degree as the ammonium nitrate and sulfate.

The two older orchards where lime slurry solutions and nitrogen treatments were applied probably would have grown out of the IBN condition without any corrective measures. Both of these orchards, one showing 524 ppm Mn and the other 350 ppm Mn, were above or just below the critical diagnostic value of 500 ppm Mn. This value had been established from the results of the varying Mn levels experiment and from previous work by Kenworthy to be the value for Mn above which IBN would develop. Also, when no lime or nitrogen was applied, the leaf Mn value decreased to 165-174 ppm in 1969, showing that without treatment of lime, the Mn value decreased below the level that would cause symptoms.

Experiment V

Investigations were made to determine the location and approximate amounts of elements within the necrotic areas of the diseased tissues. Bark samples were taken from trees treated with excess manganese and low boron that were showing IBN, and a sample was taken from the control trees for comparison. Sample preparation for microprobe analysis differed from that described by Rasmussen (29) in that the sections were cut 15 to 20 microns thick, and then coated in a Varian vacuum evaporator with a thin layer of carbon for conductivity.

The instrument used was the ARL[3] electron microprobe X-ray analyzer Model EMX-SM. The instrument conditions were 25 KV acceleration voltage and 0.05 microamp sample current with the first samples run, and 15 KV acceleration voltage and 0.125 microamp sample current on subsequent samples.

The location and amount of the element in question was recorded in two ways. The first way being a line scan across the sample where the X-rays (Kα radiations), that have a characteristic wave length for each element, were recorded on a X,Y recorder. The second way was oscillograms (photographs) prepared from images displayed on the cathode ray tube by either secondary electrons or

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[3]Applied Research Laboratory, Inc., Sunland, Calif.

Kα radiations detection. The secondary electron oscillograms showed the area being studied, and the X-ray oscillograms showed the concentration and location of the element. By use of the line scans and oscillograms, the precise location and semi-quantitative amount of a given element could be determined.

Results

Line profile analysis of normal (control) apple bark tissue, Figure 4, showed the Mn and B distribution in the tissues to be uniform. The average background count was 34 count/sec, and the average Mn count was 44 count/sec. No unusually large quantitites of Mn were found in any areas of the normal bark tissue. The Mn X-ray oscillogram for the normal bark tissue showed a uniform distribution of Mn for a large area of the normal tissue.

Corresponding X-ray oscillogram of Mn and a line profile analysis, Figure 5, showed Mn to accumulate in the necrotic lesion. B, the line scan analyzing for Mn content increased when the necrotic area was analyzed, and the Mn content was found the greatest in the middle of the necrotic area. The background count, line scan B, was uniform through the sample.

With use of cryostat sections of bark tissue, the cellular detail of the tissues could be determined, as shown in Figures 6B and 7B.

Figure 4A. Line profile analysis of B deficient bark demonstrating the distribution of Mn. One large square on the graph equals 50μ. Full graph scale was 100 count/sec. D designated the average background counts.

Figure 4B. Line profile analysis and X-ray oscillogram of normal bark demonstrating the distribution of Mn. One 20μ square on the graph equals a similar and corresponding square on the oscillogram. The line scan progressed from point A to B as indicated on the inserted X-ray oscillogram.

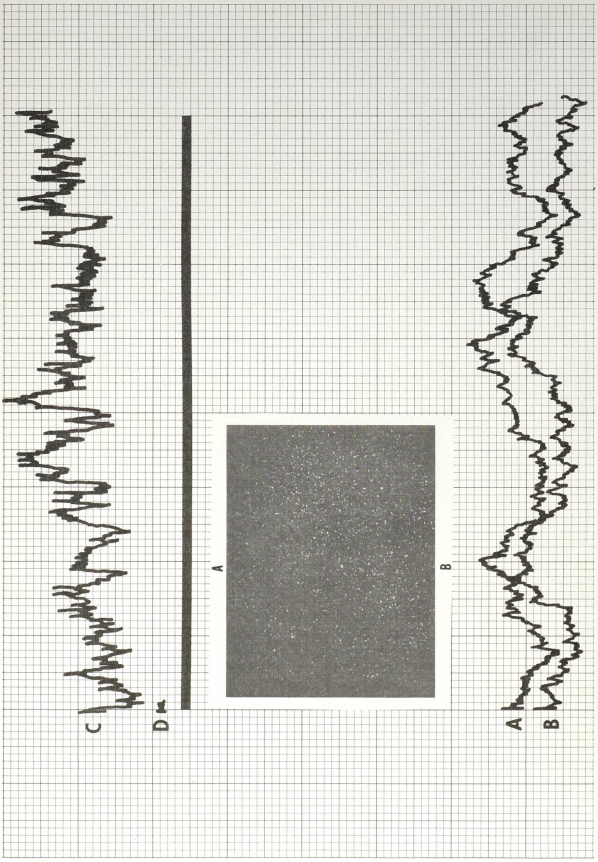
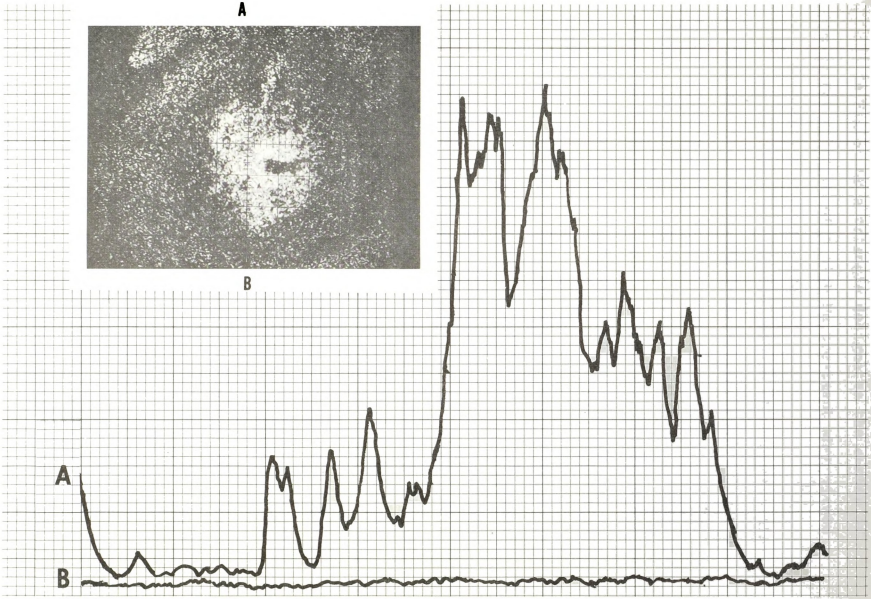

A
B
C
D
A
B

Figure 5. Line profile analysis and X-ray oscillogram of IBN bark demonstrating the distribution of Mn. One 50μ square on the graph equals a similar and corresponding square on the oscillogram. The line scan progressed from point A to B as indicated on the inserted X-ray oscillogram.


A
B
A
B

In Figure 6B a triangle designates the center of the necrotic lesion of a high Mn treatment when viewed as a secondary electron image.

The Ca X-ray oscillogram, Figure 6D, corresponding to the same area as the Mn X-ray and secondary electron oscillograms, showed an increased concentration of Ca within the necrotic lesion. Corresponding X-ray oscillograms for K, Figure 6C, and P, Figure 6A, showed decreased concentrations of these elements within the lesion.

In Figure 7B, a triangle designates the center of the necrotic lesion induced with the minus B treatment used for analysis. The corresponding Ca X-ray oscillogram, Figure 7D, showed an increase of Ca within the necrotic lesion. K and P were shown to decrease within the necrotic lesion as shown by the X-ray oscillograms for K and P, Figures 7C and 7A.

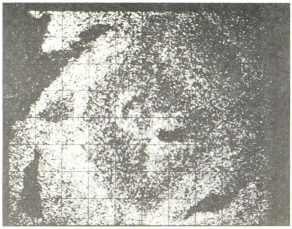
Line profile analysis of minus B apple bark tissue, Figure 4A, line scan C, showed the Mn content to be uniformly distributed within the tissue. The Mn content was low as shown by the Mn count, averaging approximately 19 count/sec. on a 100 count/sec. scale; and the average background count, line scan D, being 8 count/sec.

Discussion

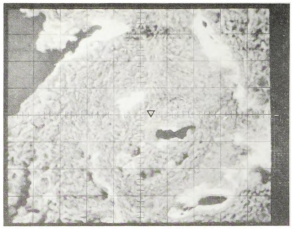
Determination of the Mn content in the necrotic lesions of trees treated with Mn was done with the use

Figure 6. Bark tissue of apple tree grown with a high (200 ppm) Mn treatment and sectioned by using the cryostat procedure. All X-ray oscillograms had exposure times of 3 minutes.

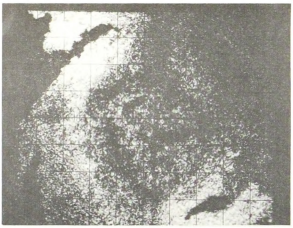
A. P X-ray oscillogram
B. Secondary electron oscillogram
C. K X-ray oscillogram
D. Ca X-ray oscillogram



A



B

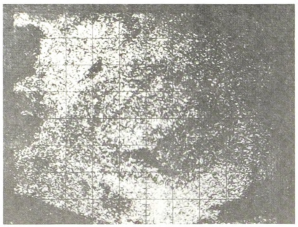


C

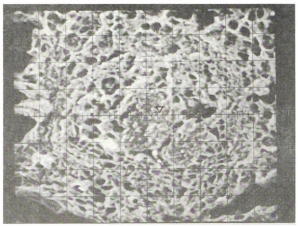
D

Figure 7. Bark tissue of apple tree grown in minus B nutrient culture and sectioned by using the cryostat procedure. All X-ray oscillograms had exposure time of three minutes.

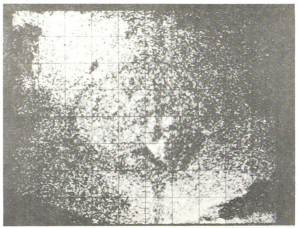
A. P X-ray oscillogram
B. Secondary electron oscillogram
C. K X-ray oscillogram
D. Ca X-ray oscillogram



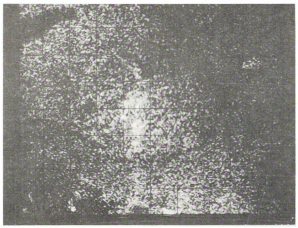
A



B



C



D

of the microprobe. The determination was accomplished by knowing the count/sec. of Kα radiation that would be given by exciting the pure element Mn with the same instrument conditions used in the experiment. The background count of the sample was then subtracted from the actual count of Kα 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 0.6% or 6,000 ppm.

Eggert, et. al. (19) has shown by histochemical techniques that Mn does accumulate in the necrotic lesions. He further stated that the concentrations of Mn found (1,000 ppm) were large enough to be causing the death of the tissue. Shelton et. al. (33) using ^{54}Mn has shown a slight accumulation of the metal in the necrotic lesions after treating Delicious apple trees with a nutrient solution containing ^{54}Mn.

The lesions of the Mn and minus B induced IBN were similar in some respects. Both showed an accumulation of Ca within the necrotic lesions, both were similar in regard to the K and P content found in each respective lesion, but the Mn lesions and minus B lesions were different in Mn and B content. The Mn lesions were shown to

have a very high Mn content, and the minus B lesions were shown to have a very low B content. These results might suggest that excess Mn and deficient B could result in the accumulation of a common material, and this material could cause death of the tissue. Forshey (20) stated that an accumulation of nitrates or nitrogenous compounds, as a result of high concentrations 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 accumulation in both types of lesions, it seems possible that tissue death may result from a common substance. The accumulation of Ca may result from Ca migration to the lesion area and neutralizing an unidentified toxic substance. However, it is unlikely that excess Mn or low B per se is the cause of tissue death.

Experiment VI

From review of the literature, personal correspondence, and observations, it appeared that many different bark symptoms were being called IBN, or "measles".

In the spring of 1969, a preliminary investigation of different symptoms of IBN was begun in several apple-growing regions. This investigation was highly exploratory and conducted for a general comparison of symptoms that were being called IBN.

Leaf samples from diseased trees in North Carolina, Georgia, and Washington were analyzed for nutrient content. Visible observations were made of symptoms of IBN in the above mentioned states, plus New York, Ohio and Ontario. Bark samples from North Carolina and Washington were analyzed for nutrient content by using the microprobe.

Results

A wide variation in symptoms was observed, as shown by the large pox-like condition of "Western measles" (Figure 8A) compared to the bark checking of "North Carolina measles" (Figure 8B). Also, there was no underlying necrotic lesions when the bark was removed from the "North Carolina measles" (Figure 8C). These bark symptoms were different from those induced with Mn or Low B (Figure 1A and 1C).

The nutrient leaf analysis, Table 21, showed a wide variation in Mn, Fe, B and Al for trees sampled, all of which were showing some visible symptom called "measles". The only nutrient values that seem to be slightly above normal were the Al value for Georgia, and the North Carolina number 2 sample and the B value for the Washington number 2 sample. The Mn value for the Washington number 1 sample was lower than normally expected.

Figure 8. Symptoms that have been called "measles".

A. Pox-like symptoms called "Western measles".
B. Bark checking symptoms called "North Carolina measles".
C. Outer bark removed from B shows no underlying necrotic lesions.

A

B

C

Figure 8

TABLE 21.--Leaf nutrient contents of trees with "measle" symptoms from different areas.

Area	Leaf Content ppm Mn	Fe	B	Al
Georgia	79	147	26.4	612
North Carolina 1	40	112	47.6	228
North Carolina 2	43	195	49.0	619
North Carolina 3	238	136	63.2	152
Washington 1	17	94	42.9	240
Washington 2	84	242	85.1	379

Discussion

Bark symptoms for "measles" in North Carolina, Georgia and Washington, were found to be very different from those in Michigan. Symptoms similar to those found in Michigan occurred in Ohio, New York and Ontario. The leaf nutrient contents were different for different areas. The North Carolina and Washington bark analyzed with the microprobe showed a difference in nutrient content, but with no large accumulation of any of the elements.

These data are very incomplete, but suggest an apparent discrepancy regarding the symptoms and causes of IBN. No comparisons can be made for these areas because the symptoms for each "measled" tree was different from those of the other areas.

These results show that visible symptoms cannot be relied upon for identification of IBN. In suspected cases, plant (leaf) analysis must be made to determine the presence and cause of the symptom.

SUMMARY

Experiments were conducted in 1967 and 1968 to determine if an excess of Mn, Fe, Cu, Al, a combination of these metals, or minus B would induce internal bark necrosis (IBN). The variety was Miller Sturdy Spur Delicious on EM VII Rootstock. The trees were grown in sand culture with two Ca levels in combination with 100 and 200 ppm Mn, Fe, Cu, Al, and a mix of 50 ppm of each element. Incidence of IBN was recorded and leaf and bark analyses were made.

Only excess Mn and minus B treatments induced IBN. The other treatments (Fe, Cu, Al) had high levels of the respective element in the leaves of the tree, but there was no IBN.

The low level of Ca increased the uptake of Mn, increasing the severity of IBN. The severity of IBN had a positive linear relationship with leaf Mn. A leaf Mn value of 500 ppm was established as the critical level of Mn in the leaf. Above this value, corrective measures should be taken for IBN. It was found, also, that the severity of IBN continued to increase during dormancy.

No significant differences were found between varieties, spur-type and standard trees, relative to either

severity of IBN or leaf Mn. There was a significantly larger terminal growth for the standard trees over the spur-type trees.

Electron microprobe X-ray analysis of thin sections of bark tissue with IBN showed that Mn and Ca were present in greater quantities in the necrotic areas of the tissues with IBN induced with Mn. With the minus B IBN lesion, only a high concentration of Ca was found within the necrotic area. K and P were found to be present in lesser amounts in the necrotic lesions than in non-affected tissue with both types of IBN-induced lesions.

From the results of a survey of young Delicious plantings in Michigan, only three orchards were found that might develop IBN and corrective lime applications were applied to two of these orchards. Leaf analysis results from Delicious trees sampled in the nursery showed no large accumulation of Mn or low levels of B.

Leaf Mg, soil Mg, soil Ca and the soil pH values significantly increased when dolomitic lime was applied. No significant decrease was observed for severity of IBN or available soil Mn.

Ammonium nitrate and ammonium sulfate increased the soil acidity and soil Mn over the no N treatment. Different nitrogen sources produced no significant differences in leaf Mn, leaf N or IBN.

Investigations of samples from different geographic areas showed a difference in symptoms called IBN. No single factor was related to all the varied symptoms called "measles". This suggested that there was still confusion as to the symptom and cause of IBN.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Aoki, N. and I. Okuse. 1964. Studies on 'Rough bark disease' of apple trees. V. Relationship between soil conditions and occurrence of the disorder. Japanese Soc. Hort. Sci. 33:181-194.

2. Aoki, N. 1966. Studies of 'Rough bark disease' of apple trees. IX. Effect of increasing of manganese added as $MnSO_4$ to the nutrient solution on the manganese content and symptom expression in Ralls apple grown in sand culture. Japanese Soc. Hort. Sci. 35:203-206.

3. Atkinson, J. D. and G. E. Roberts. 1960. Vigorous apple stocks fail on Otago shingle. The Orchardist of N. Z. March.

4. Benson, N. R. and C. G. Woodbridge. 1961. Apple measles. Wash. State Hort. Assoc. Proc.: 153-155.

5. Berg, Anthony and G. Clulo. 1943. Boron in relattion to IBN of apple. (abs.) Phytopathology. 33:1.

6. Berg, Anthony and G. Clulo. 1946. The relation of manganese to IBN of apple. Science, 104:265-266.

7. Berg, Anthony and G. Clulo. 1946. Manganese toxicity, a factor of IBN (apple measles). (abs.) Phytopathology. 36:395.

8. Berg, Anthony, G. Clulo (Berg) and C. R. Orton. 1958. Internal bark necrosis of apple resulting from manganese toxicity. W. Va. Univ. Agr. Exp. Bul. 414T.

9. Burrell, H. B. 1940. The boron-deficiency disease of apples. N. Y. (Cornell) Ext. Bul. 428.

10. Cahoon, G. A. and E. S. Banta. 1967. Internal bark necrosis (apple measles) of red delicious apples. Research Summary 20. Ohio Agri. Res. and Dev. Center, Wooster, Ohio. p. 47-52.

11. Cheney, Philip W., R. C. Lindner and C. L. Parish. 1970. Two graft transmissible bark diseases of apple. Plant Dis. Reptr. (in press).

12. Childers, N. F. 1966. Temperate to tropical fruit nutrition. Hort. Publ. Rutgers State Univ. pp. 815-823.

13. Clulo, Genevieve. 1949. The production of internal bark necrosis of apple in sand and soil cultures. (abs.) Phytopathology. 39:502.

14. Clulo, Genevieve. 1950. Pathological anatomy of IBN of apple. (abs.) Phytopathology. 40:5.

15. Clulo, Genevieve and A. Berg. 1949. Distribution of boron in the tissues of the apple tree. Proceedings. W. Va. Adad. Sci. 19:43-49.

16. Downing, R. S. 1967. Petroleum oils in orchard mite control. J. Entomol. Soc. British Columbia. 64:10-13.

17. Dowd, Oscar J. 1949. Observations on boron deficiency in apples in southwestern Michigan. ASHA. 53:23-25.

18. Eggert, Dean A. and R. A. Hayden. 1969. The periodate tetrabase test modified for cellular localization of manganese in plant tissue. Stain Technology. 44(3):161-162.

19. Eggert, Dean A. and R. A. Hayden. 1969. Histochemical relationship of manganese to internal bark necrosis of apple. Hort. Sci. 4(2) (2): 101.

20. Forshey, C. G. 1969. Control of measles in delicious apple trees. Proc. Mass. Fruit Growers Assoc. 75:37-38.

21. Fucik, John. 1963. Apple measle: a control may evolve from current studies of manganese and calcium interaction. Illinois Research. Spring. p. 8.

22. Hewitt, J. L. and H. E. Truax. 1912. An unknown apple tree disease. Ark. Agr. Exp. Bul. 112:481-491.

23. Hildebrand, E. M. 1939. Internal bark necrosis of delicious apple: a physiogenic 'boron-deficiency' disease. (abs.) Phytopathology. 29:10.

24. Hildebrand, E. M. 1947. IBN (measles) of delicious apples in New York in relation to pH, minor element toxicity and nutrient balance of soil. Plant Dis. Reptr. 31:99-106.

25. Kenworthy, A. L. 1954. Effect of soils, mulches and fertilizers in a cherry orchard on production, soluble solids and a leaf and soil analysis. Mich. State College Agr. Exp. Sta. Tech. Bul. 243. p. 21.

26. Nagai, K. S. Ichiki, A. Izumiya, M. Seito, S. Sakurada and C. Kamada. 1965. Studies on the nutritional disorders of apple bark. I. On "Sohibyo" caused by manganese excess. Japanese Soc. Hort. Sci. 34:265-271.

27. Pailoor, G., J. C. Shickluna and K. Lawton. 1970. Manganese availability in several Michigan soils. Mich. State Univ. Agr. Exp. Stat. Res. Report 97.

28. Orton, C. R. and G. Clulo. 1950. Manganese content of apple trees. (abs.) Phytopathology. 40:21.

29. Rasmussen, H. P., V. E. Shull and H. T. Dryer. 1968. Determination of element localization in plant tissue with the microprobe. Developments in Applied Spectroscopy. Vol. 6.

30. Rhoads, Arthur S. 1924. Apple measles with special reference to the comparative susceptibility and resistance of apple varieties to the disease in Missouri. Phytopathology. 14:289-314, Fig. 1, Pl. XVII-XXI.

31. Rogers, B. L., A. H. Thompson and L. E. Scott. 1965. Internal bark necrosis (measles) on delicious apple trees under field conditions. ASHA. 86:47.

32. Shannon, L. M. 1954. Internal bark necrosis of the delicious apple. ASHA. 64:165-174.

33. Shelton, J. E., D. C. Zeiger and W. A. Jackson. 1969. Distribution of manganese-54 in

delicious apple trees in relation to the occurrence of internal bark necrosis (IBN). Hort. Science. 4(2) (2):102.

34. Thomas, Walter, B. Mack and F. N. Fagan. 1947. Foliar diagnosis internal bark necrosis in young apple trees. Proc. ASHA. 50:1-9.

35. Vierheller, A. F. 1940. Apple measles. Va. Fruit Notes. 28:10.

36. Wave, H. E. and W. C. Stiles. 1969. Introduction of a measles-like bark necrosis in delicious apple trees by oil sprays. Proc. Mass. Fruit Growers Assoc. 75:39-41.

37. Young, H. C. and H. F. Winter. 1937. The effect of boron, manganese and zinc on the control of apple measles. Ohio Exp. Sta. bi-monthly Bul. XXII, No. 188, 147-152.

38. Young, H. C. and H. F. Winter. 1938. Apple tree measles. Phytopathology. 28:23.

39. Zeiders, K. E. and H. C. Kink. 1959. Studies on internal bark necrosis of delicious apples. Phytopathology. 49:526.

40. Zwick, R. W. and F. W. Peifer. 1968. Oils for summer control of pear psylla and their effects on pear trees. J. Econ. Entomol. 61:1075-9.