AN ABSTRACT OF THE THESIS OF

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Title Physiological Changes Induced In Peach Foliage By The Western X Disease Virus

Abstract approved

The irregular yellow to red leaf symptoms occurring in peach foliage invaded by the Western X disease virus suggested that the normal foliar metabolism was affected. Physiological disturbances have been reported in the leaves of several yellows and mosaic type virus diseases including peach yellows, little peach, potato leaf-roll, and tobacco mosaic. Changes in the respiration rate, starch content, and nitrogen content have been frequently found. In the yellows group respiration rate and starch content were reported to increase and nitrogen content to decrease. In mosaic type viruses, respiration generally increased, starch decreased slightly, and nitrogen accumulated. Apparently no one has made similar studies with peaches infected with Western X disease virus. Therefore, a study was undertaken of respiration rates, starch content, and nitrogen content occurring in peach leaves infected with this virus.

The rate of respiration of healthy leaves was found to vary greatly in preliminary comparisons of diseased and healthy leaves. Therefore, before studying diseased leaves further, the factors affecting respiration of healthy leaves were determined. This was done in order to be able to more critically select healthy checks for comparison with diseased leaves. Respiration rates of healthy leaves varied according to the maturity of the foliage both at the terminal and along the current season growth. The highest rates occurred at the terminal nodes of actively growing limbs prior to terminal bud differentiation. Respiration rates at such terminals were about four times greater than the rates at basal leaves on the same limbs. After terminal bud differentiation had occurred, a variation of only twice the respiration rate was found between terminal and base. In all cases respiration decreased from the terminal to the base of current season growth. Time of day was another factor which affected respiration with the same leaf respiring up to 36 percent more in the afternoon than in the morning.

Respiration rates of diseased and healthy leaves were next compared in relation to both leaf and limb maturity. In three experiments conducted prior to, during, and after terminal bud differentiation, respiration rates of the larger half leaf samples were compared

on corresponding nodes of diseased limbs and healthy limbs. When the respiration of all the leaves on the healthy and diseased current season growth was averaged separately the rates of the diseased leaves exceeded the healthy in all three experiments. In the first experiment before terminal bud differentiation the diseased leaves exceeded the respiration of the healthy leaves by 74 percent. In the second experiment, in which terminal growth was becoming less active, the average respiration of diseased leaves exceeded the average respiration of healthy leaves by 168 percent, while in the final experiment in which both diseased and healthy terminal buds were fully matured the respiration rate in diseased tissue exceeded the healthy by 275 percent. The data for these experiments showed that the average respiration rates of healthy leaves occurring after terminal bud maturity, had decreased to one-fourth the rate occurring prior to maturity. However, the rates of diseased tissue compared before and after terminal maturity had only decreased to about one-half their earlier rates. Thus the greater variation amounting to 275 percent increase in respiration rate of diseased leaves occurring after terminal bud maturity, appears to be a result of two factors, a stimulation in respiration rate due to the presence of the virus and a slower decrease in respiration rate in the diseased leaves as maturity advances.

In another series of experiments a relationship between symptom severity and respiration was established. A wide range of symptoms was selected using leaves of only one stage of maturity. By careful and uniform selection of leaves chosen to represent the various stages and by selecting small respiration samples from only the most typical portions of the leaves within the stages, a relationship between symptom severity and respiration rate was demonstrated. All diseased leaves respired at a greater rate than comparable healthy leaves and as symptoms became more severe the respiration rates became greater. This increase was consistent whether determined by respiration per gram fresh weight of tissue or respiration per milligram of nitrogen.

Leaf starch content and leaf nitrogen content were determined at each degree of symptom severity. As symptoms became more severe additional starch accumulated. Nitrogen decreased slightly in the diseased tissue at all stages of symptom severity. The ratio of starch to nitrogen in the healthy leaves was taken as 100/100 and deviations in the diseased leaves were calculated in percent. The increased starch content and decreased nitrogen content resulted in greater starch nitrogen ratios than in the case of healthy leaf tissue. Due to the continued accumulation of starch as symptom severity advanced, the starch nitrogen ratios were correspondingly greater at more advanced stages of the disease. The physiological changes reported here in Western X disease in peach coincide with those generally reported to occur in the yellows group of virus diseases. The data obtained in this study on respiration, starch accumulation and decrease in foliar nitrogen all add to the evidence that Western X disease virus must be classified with the yellows group of viruses.

PHYSIOLOGICAL CHANGES INDUCED IN PEACH FOLIAGE BY THE WESTERN X DISEASE VIRUS

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PHYSIOLOGICAL CHANGES INDUCED IN PEACH FOLIAGE BY THE WESTERN X DISEASE VIRUS

INTRODUCTION

The Western X disease virus produces abnormal growth responses in its various host plants, all of which suggest a disturbed metabolism. The disease is characterized in the sweet cherry by a retardation of fruit maturity, lack of normal fruit color development, and loss of typical cherry flavor. In the peach, a yellows type symptom occurs, followed by a leaf necrosis becoming increasingly severe as the disease progresses. In the wild chokecherry (Prunus demissa), premature autumn leaf colorations appear during the summer months occasionally developing into a necrotic breakdown.

At the present time little information exists as to the nature of the physiological disturbances which the Western X disease virus brings about in its host plants. In this study, the peach was selected as the experimental plant in an attempt to learn whether abnormalities accompanying the development of the disease are discernible by physiological methods. The peach was chosen because of the clear-cut foliar symptom expression, extending over several months of each growing season. This afforded a long period of time each year for experimental study.

The symptoms produced by the Western X disease virus suggested that it belonged to the yellows group of viruses. These viruses tend to increase the rate of respiration and cause excessive accumulation of carbohydrate in the leaves. The result is a marked change in carbohydrate nitrogen ratios. These factors were selected as the

basis of the research for this thesis problem.

Preliminary studies indicated that in order to evaluate and obtain consistent results with diseased materials a comprehensive study of normal peach foliage would be necessary. Therefore an examination was made of the following factors to determine their effect on the selection of leaves for respiration studies: maturity of the foliage up and down current season growth, length and number of nodes developed on current season growth, and the effect of time of day.

REVIEW OF LITERATURE

The Western X little cherry disease was first reported on peach during 1936 by Blodgett (6, pp.89-95) in Idaho. While he failed to demonstrate its virus nature at this time, he did note its similarity to the X disease occurring in the Eastern States. Richards and Hutchins (31, p.19) working in Utah during 1939 and 1940 appear to be the first to have demonstrated the virus nature of the disease. Zeller and Evans (47, pp.452-453) reported the earliest appearance in Oregon of the disease and transmitted the virus from peach to peach. They had also noted its occurrence in eastern Oregon previously during 1939. The disease has now been reported in Washington (30, pp.116-119), California (28, pp.916-925), Colorado (7, pp.474-475), and British Columbia (19, pp.260-262). In Oregon, Zeller and Milbrath (48, p.920), found that the Western X virus also causes the red leaf chokecherry disease and the little cherry disease in sweet and sour cherry orchards of Wasco County, Oregon. Thus this one virus has been shown to be responsible for these three diseases.

Throughout Oregon, Washington, Idaho, Utah, and British Columbia, Western X little cherry disease is considered to be the most important virus disease occurring in peaches (41, p.45). In eastern Oregon in Baker, Malheur, Wasco, and Umatilla counties, it is commonly found in peach and also in cherries in Wasco county. The severity of the disease in Utah is demonstrated by a reported 83 percent infection found during 1943 in a survey of orchards of three peach growing

counties (41, p.46). In Utah the greatest incidence of diseased cherry trees coincided with the occurrence of the disease in both peach and chokecherry (41, p.46).

No information could be found concerning respiration rates of healthy peach foliage in relation to maturity, either of the leaf or of the limb upon which the leaf was produced. Reports of starch content of healthy peach foliage were also lacking. However, Dunlap (14, pp.354-357) has studied a similar problem by showing that where peach was infected with peach yellows virus, the total carbohydrate increased and total nitrogen decreased, thus showing an increased carbohydrate nitrogen ratio. Smith (35, p.44) also referred to yellows type viruses including little peach and peach yellows as being associated with the accumulation of leaf starch in diseased leaves.

The effect of leaf and limb maturity was shown to be of such importance in healthy respiration rates of other plants by Kidd, West, and Briggs (18, p.376) and Hover and Gustafson (17, pp.33-39) that this factor should be considered in studying the peach. Likewise as the leaf symptoms of Western X disease are of yellows type involving early leaf chlorosis followed by a general yellowing and necrosis, the increased starch accumulation and the changes in carbohydrate nitrogen ratios accompanying other yellows type diseases seemed worthy of investigation in the case of Western X disease. The knowledge that other yellows type viruses such as potato leaf-roll, little peach, and peach yellows have affected starch accumulation, led to an

examination of starch content in Western X diseased peach leaves. In the case of potato leaf-roll, respiration rates have been reported to increase in the presence of the virus. Since respiration rates were to be studied in this problem, the pertinent literature relating to those processes was reviewed.

The rates of respiration in many healthy plants taken on a fresh or dry weight basis are known to decrease with age. This may be shown by comparing rates of leaf respiration at varying stages of leaf maturity. Hover and Gustafson (17, pp.33-39) found that as the leaves of corn, sorghum, wheat, and oats increased in age their respiration rates decreased. They also noted as the leaves became still older there was a tendency for respiration to again increase. Nicolas (25, p.209) demonstrated that the rate of leaf respiration is dependent on age, but he neglected to distinguish between the changes in respiration due to differences in the age of the leaf and those due to differences in age of the plant.

Kidd, West, and Briggs (18, pp.376-379) have studied rate of respiration in relation to age of entire plants and plant parts. They found in Helianthus annuus that the respiration of the entire plant decreased continuously from the time of germination to maturity. At maturity their plants respired at one tenth the rate of the germinating seedling. Mature lower leaves located on the main stem were also found to respire at about one tenth the rate of the top cluster of leaves when tested 30 days after germination. In discussing this decrease in respiration rate with age, these workers suggested the effective

amount of respiring cell matter per gram dry weight becomes less as the age of the plant increases but they were uncertain as to whether this was due to a decreasing amount of protoplasm or respiring enzymes per gram dry weight. They felt that a knowledge of the protoplasmic nitrogen content, particularly of the young leaves, might throw light on the causes underlying the reason for the fall of respiration with age. Hover and Gustafson (17, pp.33-39) noted that as a cell increases in size and age, the total amount of protoplasm probably remained the same while the dry material increased. Then assuming that if respiration were connected with protoplasm and the amount of respiration of an old leaf was calculated on the basis of total dry weight, the rate per gram of dry weight would be less than in the young leaves though the rate per gram of protoplasm might be the same. These workers suggested that a respirational method taking into account only the amount of protoplasm would be more accurate, and respiration might be calculated on the basis of amino nitrogen or total nitrogen.

The respirational rates of virus diseased plants and the factors affecting respiration have been studied by several workers. Thung (38, p.70) considered that the increased respiration rate of potato leaves infected with potato leaf-roll was due primarily to the increased carbohydrate content of the diseased leaves, as the increased carbohydrate provided a greater concentration of respirable substrate. Whitehead (44, p.967) noted that the rate of respiration of potato leaves infected with potato leaf-roll virus increased before

any leaf rolling or excess starch accumulation occurred in the leaves but not necessarily before sugars begin to accumulate. He also found that as the disease progressed carbohydrate accumulation occurred in the leaves at the expense of the tubers. This produced profound changes in the normal metabolism as evidenced in the increased rate of respiration. Whitehead (45, p.73) attributed the increased respiration rate to the additional available substrate rather than to the presence of the potato leaf-roll virus in the plant.

Dunlap (14, pp.354-357) compared the total nitrogen and carbohydrate contents of a number of virus infected plants in relation to their respiration rates. He noted that in plants infected with a yellows type virus, particularly yellows of peach, plum, asters, ragweed, or leaf-curl of raspberry, that the total carbohydrate increased and the total nitrogen decreased so that in this group the carbohydrate nitrogen ratio was increased. Conversely plants infected with mosaic type virus diseases such as mosaic of tobacco, tomato, pokeweed (Phytolacca decandra), cucumber, and raspberry, all indicated an increase in total nitrogen and a decrease in total carbohydrate, with a resultant decrease in carbohydrate nitrogen ratio. Brewer et al. (9, p.850) found a similar lowering of the carbohydrate nitrogen ratio in tobacco plants infected with mosaic, which they associated with a decrease in total carbohydrate but not in nitrogen. Dunlap (14, pp.354-357), unlike Whitehead (44, p.967) and Thung (38, p.70), did not find where carbohydrate had increased that respiration also increased. He showed that in both virus groups the respiration rates

of younger diseased leaves was greater than in older diseased leaves, but the older diseased leaves respired slower than older healthy leaves. Campbell (11, p.430), in comparing healthy and leaf-roll infected potato plants showed that the infected plants had a higher percentage of total nitrogen when considered on a fresh weight basis but they were about the same nitrogen content on a dry weight basis.

Caldwell (10, p.223) in studying respiration of tomato leaves infected with yellow tobacco mosaic reported that the carbon dioxide given off in respiration by infected plants was always greater than that from healthy plants. This was true regardless whether the determinations were made on initial dry weight, residual dry weight, or residual nitrogen, and whether carbon dioxide output was measured in oxygen or nitrogen. As the carbohydrate content of these yellow mosaic infected leaves was less than normal, he concluded that increased oxidase activity was responsible for the increased respiration. Glasstone (15, pp.275-276), using mosaic infected tobacco plants in nutrient solution under carefully controlled conditions, found that the respirational rates of both healthy and diseased plants had corresponding levels until the disease occurred systemically. As the clearing of the veins became apparent the respiration rates increased in the diseased plants to later recede to about that of the healthy plants at which time the mosaic mottling could be seen. At the peak of respiration the increase of the diseased plants was about fifty percent more than that of the healthy. According to Smith (35, pp.44-52) this rapid increase in respiration took place as the

virus became systemic in the plant occurring as the veins start to clear.

Bawden and Pirie (5, p.181) have classified virus infected plants into two groups based upon changes in carbohydrate nitrogen ratios. In well illuminated plants infected with yellows type viruses the ratio was found to increase whereas in plants infected with the mosaic type the ratio was reduced. Smith (35, p.44) also noted that in yellows type virus infected plants there were alterations in the carbohydrate leaf content accompanying changes in the carbohydrate nitrogen ratio. He mentioned that carbohydrate accumulation occurs in such diseases as potato leaf-roll, "spike" of sandal, spinach blight, peach yellows, and little peach. However, the increased carbohydrate nitrogen ratio found in yellows type virus infected plants was not definite evidence of an increase in carbohydrate assimilation according to Barton-Wright and McBain (1, p.340), as they found photosynthesis to be below that of healthy plants. Quanjer (27, pp.75-77), and also Thung (38, p.70), reported a necrotic breakdown in phloem of potato leaf-roll plants resulting in an abnormal carbohydrate accumulation in leaves. Murphy (24, pp.170-175) on the other hand, found carbohydrate to accumulate prior to any detectable phloem necrosis and he suggested that the carbohydrate accumulation is associated with an enzyme disturbance with the phloem necrosis occurring as a secondary effect.

Bawden and Pirie (5, p.181) noted that carbohydrate accumulation has been attributed to the restricting effect of a necrotic phloem,

because diseased plants when kept in the dark stained strongly with iodine indicating starch, whereas similarly treated healthy plants did not. However, these authors further stated that the necrotic phloem theory could only be held by neglecting the fact that mosaic viruses which caused no phloem necrosis also reduced the rate at which starch was lost from infected cells in darkness.

Cook (12), working with the mosaic disease of sugar cane, found that while healthy leaves and healthy green appearing areas of diseased plants seemingly performed phytosynthesis in the normal manner that diseased areas showed reduced starch accumulation in the afternoon. He concluded that the starch forming power of the mosaic infected cane was reduced in proportion to the severity of infection but the ability to translocate the carbohydrate synthesis was unimpaired. This he felt was the reverse of the situation in peach yellows, little peach, and potato leaf-roll.

Bawden and Pirie (5, p.181) reported that mosaic diseases reduced the rate at which starch is synthesized in daylight and such diseases retarded its being broken down for translocation in darkness. They also felt there was no critical comparative information on the way in which mosaic and yellows type viruses affect photosynthesis but that their different effects on carbohydrate nitrogen ratios may lie here rather than in effects on the phloem. Watson and Watson (43, pp.286-287) presented evidence that phloem necrosis in one yellows type virus disease, sugar beet yellows, was not responsible for starch retention in leaves. In their work diseased leaves placed in

darkness lost as much starch as healthy leaves. They suggest that the increased carbohydrate content in the leaf was caused by changes in the leaf cells affecting the enzyme systems controlling inter-conversion of the different forms of carbohydrate and that it was not merely a passive consequence of a shift in the balance between carbohydrate production in the leaves and its distribution to and in utilization in other parts of the plant.

Schweizer (33, pp.557-560) working with potato leaf-roll had another view on carbohydrate accumulation and phloem necrosis, attributing both to a deranged protein metabolism. He considered that nitrogen passed into the young potato shoots faster in infected tubers, stopping diastatic activity so that much starch was left unhydrolysed with the result that the infected tubers remained harder for a longer period than the healthy tubers. Still other factors have been suggested as being associated with starch accumulation. Bolas and Bewley (8, p.471) working with tomato yellow mosaic found starch accumulated around the loci of leaf infection while the remaining symptomless leaf parts of diseased leaves lost starch faster than the healthy leaves. Here they felt the presence of the virus was associated with this starch accumulation in the parenchyma cells. The delay in translocation of carbohydrate from the infected parenchyma cells could have been due to the effect of the virus on carbohydrate activity. Wynd (46, pp.457-458) has also advanced the idea that changes in cell permeability due to presence of the virus could be associated with such carbohydrate accumulation.

In the presence of one mosaic type disease, paracrinkle in potato, carbohydrate is accumulated according to Barton-Wright and McBain (2, pp.545-546). They associated this accumulation with interference in the channel of transport out of the leaf, finding that sucrose could not travel in the diseased petiole with the same ease as in the healthy. However, no actual deterioration of the phloem tissues was mentioned. Bawden (3, p.286) considered this carbohydrate accumulation to be an exception to the situation in other mosaic type viruses. Spike disease virus in sandal (36, pp.27-29), and curly top virus in tomato (34, p.106), were reported to cause an accumulation of both carbohydrate and nitrogen. However, Rosa (32, pp.168-169) working with tomatoes infected with curly top virus found that carbohydrates increased in all parts of the blighted plants and total nitrogen also increased in all parts other than the leaves where it decreased. In spinach infected with spinach blight the carbohydrate content of the diseased tops was markedly greater than that of healthy plants, while in the roots both diseased and healthy plants had similar amounts of sugars and starch (39, p.384). Read (29, pp.45-48) reported that both total and reducing sugars increased in tomato leaves infected with yellow mosaic, doubling in content twelve days after inoculation.

Early workers differed on the matter of total nitrogen content in curly top infected tomato plants. Rosa (32, pp.168-169) reported a decrease of total nitrogen in such plants, whereas Shapovalov (34, p.106) working with the same plant and virus found an increase

in total nitrogen.

In the case of protein content in virus infected plants, Stanley (37, p.1159) found large increases over healthy plants in both tobacco mosaic and yellow mosaic plants with increases of two to three times more protein nitrogen. This increase in protein nitrogen was found to be due to the production in diseased plants of large amounts of high molecular weight virus protein. Martin, Ball, and McKinney (20, pp.329-330), and also Holden and Tracey (16, p.156) found no increase in total nitrogen in tomato infected with tobacco mosaic. Tobacco mosaic infected tomato plants according to Bawden and Pirie (4) contained about one-third of the nitrogen in the form of virus varying in this respect from ten to sixty percent. Where so much of the plant nitrogen was in the form of virus but without an apparent increase in protein production, Martin, Ball, and McKinney (20, pp.329-330) suggested that the virus might be produced at the expense of normal protein.

In summary, healthy leaf respiration rates appear to be related to maturity of both the leaf and the plant. Plants infected with viruses of the yellows type often show an increase in rate of respiration which may later recede as the plant approaches senescence. In the presence of yellows type viruses the foliar carbohydrate content is also found to increase and the nitrogen content to decrease resulting in an increased carbohydrate nitrogen ratio. Where a mosaic type virus is present the carbohydrate content may often decrease and the nitrogen content increase. This results in a reduced carbohydrate

nitrogen ratio.

MATERIALS AND METHODS

Two to four year old trees of the varieties J. H. Hale, Elberta, or Improved Elberta were used for these studies. The trees were spaced four to ten feet apart in an experimental nursery at The Dalles, Oregon. Where indicated some experiments were conducted using mature orchard trees. In all cases leaves chosen for experimental purposes were selected from various points on current season limb growth. Where more than one leaf was produced at a node, the first or oldest leaf was used.

In some of the limbs used, new growth was still developing at the terminals, indicating active stem elongation (See Figure 1, B), while others were used after the terminal growth had stopped and the dormant terminal bud matured (See Figure 1, A).

All of the diseased nursery trees had been inoculated with Western X disease virus and had expressed symptoms of this disease. In a few cases inoculated or naturally infected mature trees were used. Definite disease symptoms were always observed before samples were taken.

1. Selection of Experimental Material

For comparative purposes, four arbitrary degrees of severity of symptom expression were chosen, no visible symptoms, early, moderate, and severe. Leaf selection for these degrees of severity was based upon a detailed description of the symptoms and a clear visual impression of their appearance in the field. In selecting leaves

Figure 1. Terminals from peach limbs selected to show the two stages of maturity considered in this study. (A) Terminal bud differentiated and limb elongation terminated for the season. (B) Terminal growth active and terminal bud not yet developed.

for experiments considerable care and time was devoted to choosing each individual leaf.

No visible symptoms. Leaves with no visible symptoms were selected from terminals on diseased trees in which no visible evidence of virus infection has as yet developed.

Early symptoms. Leaves were considered in the early symptom group when they first showed discernible evidence of infection. The only symptom considered here was a mild irregular chlorotic spotting. This spotting occurred more often on the upper leaf surface but occasionally on the lower surface. The chlorotic areas varied in shape and size. On the upper surface about five to twenty percent of the leaf area showed these faint symptoms. On the lower surfaces, symptoms were of a similar type but less clearly defined. Occasionally a reddish tinge in the main vein on the lower side, together with a puckering of the leaf tissue adjoining the midrib was observed although these were never considered as definite symptoms. Lower and upper surfaces of typical leaves with early symptoms of virus infection are illustrated in Figures 2A, 3A, 4C, and 5C.

Moderate symptoms. Leaves considered to be at the moderate stage were selected when about one-third of the upper surface leaf area showed a reddish yellow caste. No visible necrosis or falling away of tissue had as yet occurred at this stage. Symptoms were less clearly defined on the lower sides, and those that were evident did not always coincide in position with those on the upper leaf surfaces. At this stage the lower side of the leaves occasionally possessed

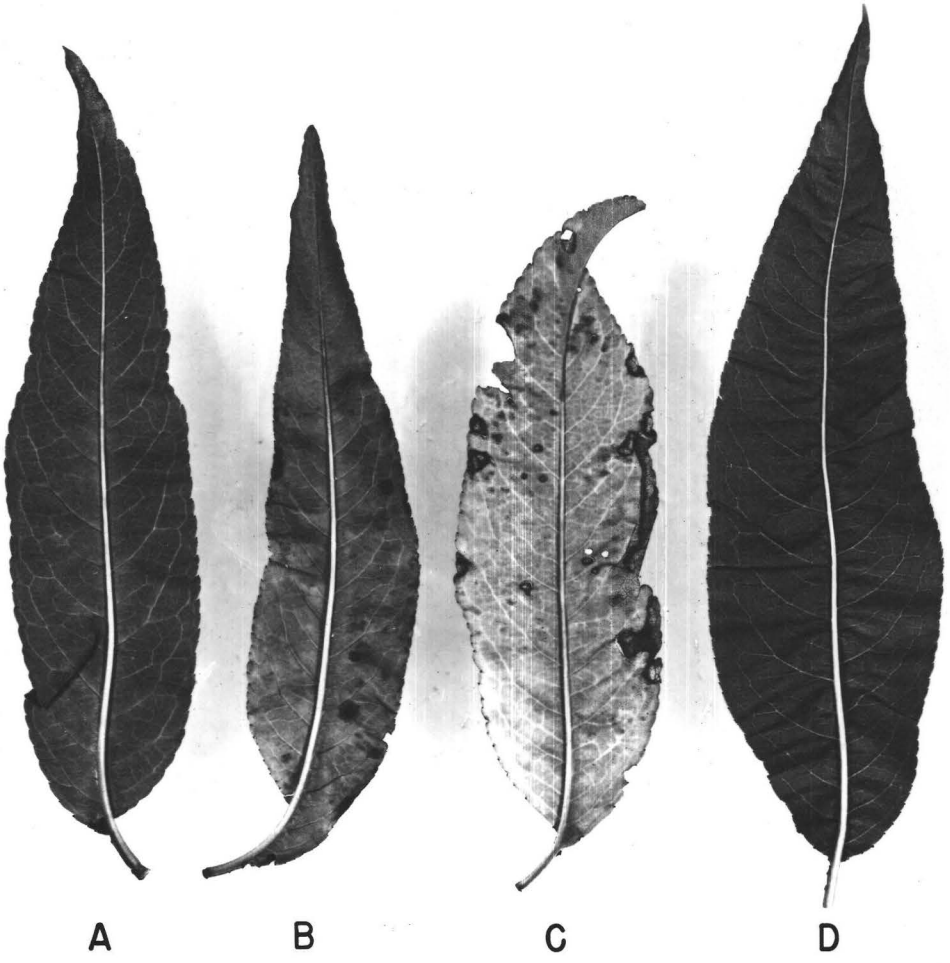


Figure 2. Lower surface of peach leaves selected to show the various stages of symptom severity used in these studies.

(A) Early stage (B) Moderate stage (C) Severe Stage (D) Healthy check

A B C D

Figure 4. Lower surface of peach leaves selected to show the various stages of symptom severity used in these studies:
(A) Severe stage (B) Moderate stage
(C) Early stage (D) Healthy check

A B C D

Figure 5. Upper surface of peach leaves selected to show the various stages of symptom severity used in these studies:
(A) Severe stage (B) Moderate stage
(C) Early stage (D) Healthy check

small irregular areas having a mild purple tinge. This symptom was rarely if ever visible on the upper surface. Leaves at the moderate stage may also show occasional mild chlorotic spotting typical of the early stage. Figures 2B, 3B, 4B, and 5B show typical leaves for this symptom group.

Severe symptoms. Severe symptoms were considered to exist when the reddish yellow discolored areas of the moderate stage had become necrotic and taken on a brownish yellow burned appearance. Although much of the necrotic tissue still remained in place, the advanced areas were about to break away from the less visibly injured portions of the leaf. These advanced stages represented about twenty to thirty percent of the total leaf area. Symptoms typical of the moderate stage occupied another twenty to twenty-five percent while a third twenty to twenty-five percent showed mild symptoms again more clearly defined on the upper leaf surface. See Figures 2C, 3C, 4A, and 5A.

2. Leaf Sampling for Respiration, Starch, and Nitrogen Studies

In the choice of experimental leaves having these four degrees of symptom severity, care was exercised that all leaves actually fell within the four stages described. Much time was spent in the actual selecting of desirable types from more than two hundred available diseased trees. The gross terminal growing point was considered as node one. The first node showing a clearly visible space below the terminal node or whorl was taken as node two. In Figure 1A and 1B the upper leaves at the right with the tendency to droop are

considered to arise from node two. The nodes were counted down the limbs to the basal or earliest node developed on current season growth. The counting of the nodes was done to determine the relative age of each leaf, always choosing leaves as closely as possible to the same age. For comparative studies, due to the immaturity and fragility of the small leaves making up node one at the terminal growing point, most sampling was done starting just below at node two. In a few early experiments leaves from node one were used. In choosing leaves for comparative studies two factors were considered. All leaves had to be of the same relative age, that is, leaves from basal nodes were chosen to compare with leaves from other basal nodes, and leaves from half way up current season growth from similar positions in comparative trees and so on. Secondly all experimental limbs had to be at about the same stage of growth and maturity at the growing point or terminal. In no case were limbs compared on an equal basis where one limb was still actively elongating at the tip and the other had ceased elongation and developed the terminal bud for the season.

3. Preparation of Leaf Tissue for Respiration Determinations

In preparation of the leaf tissue used for respiration determinations, eight millimeter discs were removed with a cork borer, taking care to avoid the main veins. The areas of the leaf chosen for removal of leaf discs, represented tissue most typical of that stage being selected. In a few cases half leaves were used cutting the

leaf longitudinally without the main vein. Samples were either chosen directly in the field, or the leaves were removed, placed in portable moist chambers to retain turgor and taken to the laboratory where the 8 mm. discs were selected. Where the small discs of tissue were taken directly in the field as in experiments requiring a later series of samples from the same leaves, such leaf discs were dropped into stoppered test tubes containing a wad of moist paper. The test tubes acted as small moist chambers and maintained turgidity in the discs until they could be used in the actual respiration determinations. In the laboratory, respiration samples usually of ten discs per sample were weighed, transferred to a Warburg flask and the respiration determinations commenced. All respiration determinations were made in a Warburg respirometer at a temperature of 30°C. Unless otherwise stated respiration determinations were made over a two hour period and were conducted and calculated according to the direct method as described by Umbriet et al (40, pp.1-16). The laboratory procedure used was as follows:

1. To dry Warburg flasks equipped with a center well, add leaf materials to the main compartment of flask.
2. Add 0.2 ml. alkali (10% KOH) to the center wells.
3. Grease attachment joints on manometers and grease and insert plugs for sidearms. Grease tops of alkali cups.
4. Add filter paper strips to alkali in center cups (for better absorption of carbon dioxide).
5. Attach flasks to manometers.

6. Place in constant temperature bath.
7. Place one flask containing alkali and filter paper only, attached to manometer in bath as thermobarometer.
8. Adjust and tighten flasks after about 5 minutes shaking in bath.
9. Allow to equilibrate, with shaking, for 20 minutes.
10. Adjust manometer fluid to zero point on closed side of monometers with stopcocks open.
11. Close stopcocks.
12. Begin readings.
13. Calculate results according to the method of Umbreit et al (40, pp.1-16) and express as microliters of oxygen taken up per gram of material per unit of time.

4. Starch Sampling and Starch Determinations

Leaves having had discs removed for determination of respiration rates were in some cases held for later determinations of starch content. Fresh weights were recorded here and the samples allowed to dry. For starch determinations the leaves were brought to constant dry weights and the starch content determined. The method used involved the use of anthrone 3-sulphuric acid followed by spectrographic analysis. This procedure was taken from several sources (21, 22, 13, 23, 42) and modified by Dr. R. O. Belkengren, Oregon State College, Corvallis, Oregon. The steps used in the method for starch determination are:

1. Weigh out one gram sample of ground plant tissue.
2. Place sample in centrifuge tube and add 80 percent ethyl alcohol and heat for one hour at 80°C or hold for four hours at room temperature. Shake occasionally. Centrifuge at 4000 revolutions per minute for a ten minute period and save supernatant liquid for determination of soluble carbohydrates or discard if starch only is wanted.*
3. Extract the residue in the centrifuge tubes once more with 80 percent ethyl alcohol. Centrifuge, pour off the supernatant liquid, and allow residue to dry in tube. Supernatant liquid again may be saved and added to that removed earlier if soluble carbohydrates are to be determined.*
4. To the residue in the centrifuge tube add 5 ml. distilled water and 6.5 ml. perchloric acid reagent (52 percent). Stir well for 5 minutes or shake, and then let stand for 15 minutes with occasional stirring.*
5. Centrifuge, pour off supernatant liquid into 100 ml. volumetric flask. Wash residue with 5 to 10 ml. distilled water. Centrifuge again, adding supernatant liquid to the volumetric flask.
6. Add 5 ml. distilled water and 6.5 ml. 52 percent perchloric acid and extract again as in steps 4 and 5.
7. Again pour contents from the centrifuge tube into the volumetric flask.
8. Make volumetric flasks up to volume (100 ml.).*

9. Filter if necessary into small dry flask.*

10. Proceed with the determination of carbohydrates by the anthrone procedure on a suitable aliquot.

11. Make up standard solutions of anhydrous glucose containing 0, 5, 10, 15, 20, 50, 75, 100, 125, micrograms of glucose per ml.

12. Dilute the unknowns so that the carbohydrate content is within the range of the standards. A 10 to 1 dilution is satisfactory for leaf material.

13. Pipette 2 ml. portions of the standard and the diluted unknown solutions into test tubes and add 5 ml. of freshly mixed anthrone reagent (0.2 grams of anthrone to 100 c.c. of 95 percent sulphuric acid) to each. Mix the contents of the tubes and stopper lightly with a marble or cork.

14. Heat these in a boiling water bath for 7.5 minutes. Cool them rapidly by immersion into water at 25°C (room temperature).

15. Fill spectrophotometer cuvettes with the knowns and establish the standard curve on the spectrophotometer. A wave length of 600 m.u. and a slit width of 0.3 is usually satisfactory.

16. After establishing standard curve, proceed reading unknowns on spectrophotometer. By comparing the unknown readings with the standard curve it is possible to determine actual starch. To do this multiply glucose by 0.90 and report as starch.

* Steps in procedure in which overnight delays may occur without impairing accuracy.

One place that must be watched carefully is the addition of the anthrone reagent to the 2 mo. of unknown and standard. Thorough mixing is essential and the mixing must be done immediately as the heat liberated when water is mixed with 95 percent sulphuric acid is considerable. Careful stirring with a glass rod or bubbling of a small stream of air through the solution serves to mix it well.

5. Determination of Leaf Nitrogen Content

In some experiments leaf tissue used for respiration determinations was preserved, brought to dry weight, and nitrogen content determined by a micro-kjeldahl method (26). The following procedure was employed:

1. Bring leaf sample to constant dry weight using about 15 m.g. for nitrogen determination.
2. Place in digestion flask with 650 m.g. potassium sulphate, 16 m.g. mercurous oxide and 1 ml. of sulphuric acid.
3. Digest sample for two hours.
4. Release ammonia with 40 percent sodium hydroxide and steam distill into 5 ml. of 0.02 normal hydrochloric acid. The excess hydrochloric acid is back titrated with 0.02 normal sodium hydroxide.

EXPERIMENTAL RESULTS

1. Respiration of Healthy Peach Foliage

The respirational rates of healthy peach leaves occurring on current season growth were examined to determine what growing conditions or other factors might affect respiration. The following were considered:

a. Leaf maturity

b. Length of current season growth and number of nodes

c. Time of day

a. Leaf maturity.

Experiments involving single limbs. In three experiments each involving a single three year old J. H. Hale tree, respiration determinations were made on leaves at intervals starting at the terminals down to the base of current season growth. In each experiment one limb only was selected per tree. In the first experiment, Number 1, leaf samples were taken at node 1, the terminal, and at nodes 3, 5, 8, 13, 22, and 35, the base. Sampling was done by using a cork borer cutting out ten discs of leaf tissue, each 8 mm. in diameter, from each half of each leaf. This allowed for duplicate respiration samples at each node. At the terminal, node 1, the leaves were too small to obtain duplicate samples from one leaf hence it was necessary to use two leaves.

In the other two experiments, Number 2 and Number 3, single respirations were made at each node, not in duplicate as in

Experiment 1. In Experiment 2, due to the immature leaf size, two leaves were again needed at the terminal to obtain a total of 10 leaf punches for respiration determination. In Experiment 3, node 2 was selected as the uppermost node as was done in most later experiments, because of greater ease in sampling. The leaf tissue samples used in Experiment 3, instead of being 8 mm. leaf tissue discs were made up of half leaves cut longitudinally but excluding the main veins. A comparison of the respiration rates in the three experiments is summarized in Table 1 which shows total respirational rates over a two hour period, expressed in microliters of oxygen taken up per gram fresh weight of leaf tissue.

TABLE 1

RESPIRATION OF LEAVES FROM INDIVIDUAL J. H. HALE LIMBS[1]

Experiment 1[2]		Experiment 2[2]		Experiment 3[3]	
Node	Respiration[4]	Node	Respiration[4]	Node	Respiration[4]
1(terminal)	3460	1	3540	2	1440
3	2600	4	1990	5	897
5	2275	6	2140	7	815
8	1860	13	1260	10	650
13	1490	15	1200	15	524
22	1170	18	925	19	561
35(basal)	882	20	1060	24	511
		21	905	28	402

(1) Leaf samples taken from trees at 9:45 a.m.

(2) Terminals growing actively

(3) Terminals starting to mature

(4) Microliters of oxygen taken up in two hours per gram fresh weight

The data presented in Table 1 indicate a consistent and rapid decrease in leaf respiration rate from the terminal nodes to the mature basal nodes. In Experiment 1, the leaf at the basal node, 35, respired at the rate of 882 microliters of oxygen taken up per gram of fresh weight over a two hour period. This is less than one fourth the rate of the terminal node which took up 3460 microliters for a similar period. Likewise in Experiment 2, the decrease in rate from tip to base was rapid. In Experiment 3, in which the terminals were starting to mature and were growing less vigorously, the respiration rate was lowest, 1440 microliters at the terminal, and it continued to decrease toward the base. Comparisons of corresponding nodes in Experiment 3 with those in Experiment 1 suggest that in Experiment 3 respiration was less than half as rapid. The trend showing a decreased rate from the terminal to the mature basal leaves occurred in all three experiments. See Table 1.

Respiration experiments involving several limbs. In five more experiments studies were made of healthy respiration rates in relation to leaf maturity, using composite samples of leaves from corresponding nodes on limbs of several trees. This was done to get a better average estimate of respirations up and down current season growth. Limbs were chosen of comparable maturity and size. In Experiment 4, ten healthy Hale trees of two years of age growing in a nursery were used, while in Experiment 5, five similar two year old Hale trees were used. The terminal growth of trees in both experiments was soft and succulent with the young leaves at node 1 being very small and tender. In Experiment 4, one 8 mm. disc of leaf tissue

was removed per tree at corresponding nodes for comparison of respiration rates. Thus the ten discs in the respiration sample representing node 1, were taken one from each of the first nodes in the ten experimental trees. In Experiment 5, two leaf discs per node were used from each of the five trees, otherwise the sampling was similar to that in Experiment 4. Table 2 compares the respiration rates from the terminal node 1, down to the lowest node tested, node 20.

TABLE 2

AVERAGE RESPIRATION OF LEAVES FROM SEVERAL J. H. HALE LIMBS (1)

Experiment 4		Experiment 5	
Node	Respiration(2)	Node	Respiration(2)
1(terminal)	4842	1	6125
2	5270	3	5455
3	5005	5	4720
4	4856	7	4005
5	4140	9	3209
6	4236	11	2389
7	3502	13	2877
8	2988	15	2250
16	2376	20	2128
20(basal)	1732		

(1) Terminals growing actively. Leaf samples taken from trees at 11:00 a.m.

(2) Microliters of oxygen taken up in two hours per gram fresh weight.

The average foliar respiration rates for the ten terminals tested in Experiment 4 were about the same in the four upper nodes with nodes 2, 3, and 4, exceeding the rate of the leaves at node 1.

See Table 2. The rate of respiration of the lower nodes in both Experiment 4 and 5 decreased steadily down the stem with the leaf at the lowest node tested (node 20) respiring in each experiment at about one third the rate of the terminal leaf.

Maturity of leaves at the terminal. In Experiments 6, 7, and 8, Table 3, involving mature Improved Elberta trees, composite leaf respiration determinations were made on leaves from the terminal towards the base of current season growth. The objective was to learn whether the degree of maturity of the terminal node could be associated with rate of respiration. In Table 3, a comparison is made between a limb in which the terminal node has matured and two limbs in which terminal nodes are just beginning to mature. Leaf samples in Experiments 6 and 7 in Table 3 were taken from a total of ten trees, using one limb per tree and two discs from each leaf. In Experiment 8, only five trees were used but otherwise leaf sampling was the same.

TABLE 3

AVERAGE RESPIRATION OF LEAVES FROM SEVERAL IMPROVED ELBERTA LIMBS

Experiment 6(1)		Experiment 7(2)		Experiment 8(1)	
Node	Respiration(3)	Node	Respiration(3)	Node	Respiration(3)
1(terminal)	4979	1	2496	1	3254
2	4252	3	1930	3	2768
3	3592	5	1900	5	2759
4	3295	7	1835	7	2708
5	3215	9	1700	9	3456
6	3091	11	1617	11	2839
7	3564	13	1583	13	1991
8	3175	15	1504	15	2309
12	2630	17	1460	20	2123
16	1938				
20(basal)	1621				

(1) Terminal buds beginning to mature. Leaf samples taken from trees at 10:15 a.m.

(2) Terminal bud matured. Leaf samples taken from trees at 1:30 p.m.

(3) Microliters of oxygen taken up in two hours per gram fresh weight.

The data in Tables 2 and 3 indicate that leaf respiration is highest where the terminals are growing actively, and as the terminal bud matures, the rate declines. A respiration rate of 6125 microliters of oxygen taken up over a two hour period per gram fresh weight occurred at the actively growing terminal node in Experiment 5, Table 2.

Where the terminals had begun to mature respiration rates as measured by oxygen uptake decreased to 4979 and 3254 microliters. (Table 3, Experiments 6 and 8). After the terminal bud had fully matured, the oxygen uptake was further reduced to 2496 microliters. See Table 3, Experiment 7. In all cases leaves at lower nodes respired at a consistently lower rate than leaves at the terminals. While all respirations were made on current season growth, the age of the test trees may have also been a factor affecting respiration rate. The highest rates occurred on actively growing limbs from two year old J. H. Hale trees. (Table 2, Experiment 5). On current season growth of mature Improved Elberta trees the rates were lower. (Table 3).

b. Length of current season growth and number of nodes. Respiration rates were compared between leaves situated at corresponding nodes on long and short current season limbs to determine whether variation in the length of the limb had a bearing on respiration. Employing healthy three year old J. H. Hale trees, two trees were selected and from each tree a long and a short limb were removed. All terminals were in an actively growing vegetative state and the

trees were growing under similar conditions. Table 4 summarizes the lengths of the limbs and total number of nodes developed during the season.

TABLE 4

LENGTH OF CURRENT SEASON GROWTH AND NUMBER OF NODES ON TWO LONG AND TWO SHORT J. H. HALE LIMBS

Limb	Length of Limb cm.	Number of Nodes
Short	24.5	26
Short	24.0	27
Long	67.5	38
Long	88.0	39

Respiration rates were determined on the leaves situated at nodes 2, 7, 14, and 21 taken from the two short and two long limbs. Table 5 presents the average respiration rates obtained.

TABLE 5

RESPIRATION OF LEAVES AT FOUR NODES ON LONG AND SHORT CURRENT SEASON J. H. HALE LIMBS(1)

Limb	Node Number	Average Respiration(2)	Difference in Oxygen Uptake of Long Over Short Limb(3)
Long	2(terminal)	2535	85
Short	2	2450	
Long	6(7 missing)	1530	195
Short	7	1335	
Long	14	1210	182
Short	14	1028	
Long	21	888	147
Short	21(basal)	741	

(1) Terminals growing actively. Leaf samples taken from trees at 11:00 a.m.

(2) Microliters of oxygen taken up in two hours per gram fresh weight.

(3) Average difference in oxygen uptake of long over short limbs 152 microliters.

The average respirations of leaves from the long limbs (as expressed in oxygen uptake) was always higher than that of leaves from comparable short limbs. (Table 5). In comparing node 14 in the short and long limbs the average oxygen uptake is 1210 microliters as compared with 1028 microliters. This increased rate of respiration of the long over the short limbs was fairly consistent throughout the experiment. The long limbs in this experiment were more than twice the length of the short limbs and had developed about twelve more nodes (Table 4). As both long and short limbs constituted current season growth the question might be raised as to the justification of comparing any particular node, as for example node 7, on a limb having only 26 nodes with node 7 on a limb having 38 nodes. In comparing the relative maturity, node for node, of the two lengths it is possible that node 7 of the longer limb could be younger, having appeared later in the season than node 7 of the short limb. The respiration rates shown in Table 5 seem to suggest this, as in every case the longer limbs possessed higher rates of respiration at corresponding nodes. In a further experiment (Table 7), comparative respirations were made of long and short limbs comparing basal leaves, leaves at the midpoints or central nodes, and leaves at the terminals. The objective was to compare leaves of the same apparent age rather than at the same numerical node as counted back from the terminals. Thus a leaf in the midpoint of a short limb might be expected to be of about the same age or maturity as the leaf at the midpoint of a long limb. Table 6 summarizes the length and number of nodes in the

three long and three short limbs used in this type of experiment. Three J. H. Hale trees, three years of age, were used and from each, one long and one short limb was selected.

TABLE 6

LENGTH OF CURRENT SEASON GROWTH AND NUMBER OF NODES DEVELOPED IN THREE LONG AND THREE SHORT J. H. HALE LIMBS

Limb	Length of Limb cm.	Average Length cm.	Number of Nodes	Average Number of Nodes
Long	80.5		39	
Long	57.0		36	
Long	69.0	69	38	38
Short	28.5		30	
Short	29.0		27	
Short	28.5	29	35	31

The long limbs shown in Table 6 averaged 7 more nodes and over twice the length as compared with the short limbs. Using these limbs, comparative respirations were made of leaves selected at the terminals, at the base, and at central nodes midway between the terminals and the base. Table 7 presents the average respiration rates obtained.

TABLE 7

RESPIRATION OF LEAVES AT TERMINAL, CENTRAL, AND BASAL NODES, ON LONG AND SHORT CURRENT SEASON J. H. HALE LIMBS(1)

Limb	Node	Respiration(2)	Difference in Oxygen Uptake of Short Over Long Limbs(3)
Long	Terminal	2060	
Short	Terminal	2140	80
Long	Central	1085	
Short	Central	1089	4
Long	Basal	586	
Short	Basal(4)	713	127

(1) Terminals growing actively. Leaf samples taken from trees at 9:30 a.m.

(2) Microliters of oxygen taken up in two hours per gram fresh weight.

(3) Average difference in oxygen uptake of short over long limbs 105 microliters of oxygen.

(4) Lowest leaves remaining used. In one case lowest leaf was two nodes above original basal leaf and in another three nodes above.

The respiration rates of leaves at similarly located nodes on long and short limbs were compared by two methods. One method involved counting nodes back from the terminals on both long and short limbs and comparing leaf respiration at nodes 2, 7, 14, and 21, (Table 5). In the other method, respiration rates were compared in leaves at terminal, central, and basal nodes regardless of the number of nodes occurring on the limb, (Table 7). In this second method closer comparative respiration rates occurred between the nodes compared than in the first method. While the experiments summarized in Tables 5 and 7 show a trend only, this information was kept in mind in further sampling. Limbs of about the same number of nodes were looked for. If they were not available comparisons between nodes were made with some consideration given to the overall amount of seasonal growth.

c. Time of day.

In two experiments duplicate samples of leaf discs were taken from the same leaves, one sample in the morning before exposure to sunlight and a second in the late afternoon. The objective was to determine whether leaf respiration rates varied from morning to afternoon.

In the first experiment, the early morning sampling preceded the afternoon sampling. In the second experiment the procedure was reversed with the afternoon sampling being done first, and followed by the second sampling the next morning. The order of sampling was reversed in an attempt to equalize any effect of leaf injury on

respiration rate as caused by the cutting out of the discs of leaf tissue.

The first experiment involved five J. H. Hale trees three years of age all growing actively at the terminals. One limb of similar size and number of nodes was chosen from each of the trees and covered in the evening at 7:00 p.m. with two layers of black cloth. Such covers were applied in order to be able to obtain morning leaf respiration samples unexposed to sunlight. The next morning at 7:30 a.m. the first of the leaf respiration samples was taken, working as much as possible under the black shades on the limbs.

Respiration samples on the five covered limbs were selected from leaves at five locations ranging from the terminal to the base. Duplicate samples, each of two discs of leaf tissue were first cut out from the basal leaf on each of the five limbs. Such a sample contained a total of ten leaf discs and represented an average of the five basal leaves. The next pair of samples from the five limbs was selected at a point between the base and the central nodes, thirdly at the central nodes then halfway between the central node and the terminals and finally at the terminals. As in other experiments, because of the immaturity of the terminal, the node just below was used. After removing the leaf discs from the leaves, they were dropped into stoppered test tubes containing a wad of moist paper. The test tubes were held in the dark while sampling the trees and the samples exposed to a minimum of light in weighing and preparation for the respirational determinations. The Warburg apparatus

was darkened with a black cloth during the respiration determinations in order to reduce light exposure to the leaf discs and any resultant error in respiration introduced by the discs doing photosynthesis. On completion of the early morning sampling, the five limbs were uncovered and exposed to sunlight. Later in the day at 3:00 p.m. identical samples were taken from the same leaves on the same limbs. Respiration determinations were again made as before and the morning rates compared with the afternoon rates. Respirations made at both times of day again showed a rapid decrease in rate from the actively growing terminals down to the base of current season growth. Average afternoon respiration rates exceeded those in the morning by 36 percent. At both times of the day the terminal leaves were respiring over four times the rate of the basal leaves. See Table 8 and Figure 6.

TABLE 8

MORNING AND AFTERNOON RESPIRATION RATES OF J. H. HALE LEAVES PRIOR TO MATURING OF TERMINAL BUDS

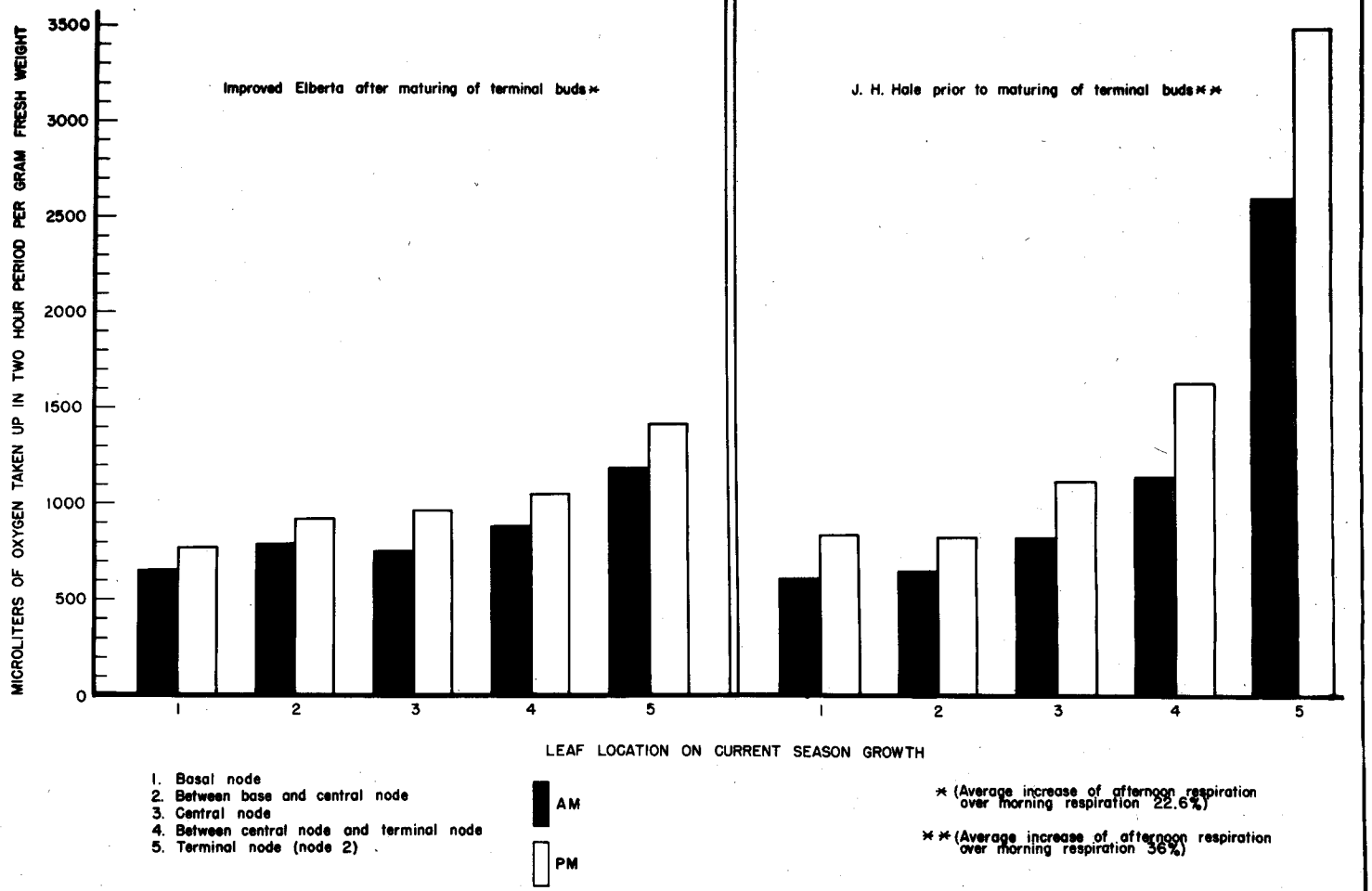
Leaf Location	Morning Respirations(1)	Afternoon Respirations(1)	Percent Increase
Terminal (node 2)	2580	3460	34
Between central and terminal node	1135	1625	43
Central node	823	1120	36
Between basal and central node	638	828	30
Basal node	606	835	38

Average increase of afternoon respiration over morning respiration 36%.

(1) Microliters of oxygen taken up in two hours per gram fresh weight.

FIGURE 6

COMPARISON OF MORNING AND AFTERNOON RESPIRATION OF HEALTHY PEACH LEAF TISSUE



The second experiment comparing morning and afternoon respirations involved five Improved Elberta trees, three years of age in which the terminal buds had matured for the season.

In this experiment the sampling technique was identical with that of the previous experiment, excepting that the order of sampling was reversed. Here the afternoon leaf samples were taken at 3:00 p.m. and the morning samples were taken the next day at 7:30 a.m. As in the other experiment, dark cloth covers were placed over the experimental limbs at 7:00 p.m. to prevent exposure to early morning sunlight.

In this second experiment using limbs in which the terminal buds had matured, the average afternoon respiration exceeded the morning respiration by 22.6 percent. At the terminals, respiration took place at about twice the rate occurring at basal nodes. In the earlier experiment where the terminal buds had not matured, afternoon respiration exceeded morning respiration by 36 percent and the terminal leaves respired four times more rapidly than leaves at basal nodes. See Table 9 and Figure 6.

TABLE 9

MORNING AND AFTERNOON RESPIRATION RATES OF IMPROVED ELBERTA LEAVES AFTER MATURING OF TERMINAL BUDS

Leaf Location	Morning Respirations(1)	Afternoon Respirations(1)	Percent Increase
Terminal (node 2)	1180	1415	28
Between central and terminal node	876	1059	19
Central node	742	957	29
Between basal and central node	773	917	19
Basal node	649	768	18

Average increase of afternoon respiration over morning respiration 22.6%.

(1) Microliters of oxygen taken up in two hours per gram fresh weight.

2. Respiration of Diseased Peach Foliage

a. Respiration rates on individual diseased and healthy limbs.

Diseased and healthy limbs were selected for respiration comparisons, and samples were taken from each starting at the terminal node and proceeding to the oldest or basal node. Diseased leaves from a three year old J. H. Hale tree were chosen from a limb having 26 nodes of current season growth. A similar healthy limb from a tree of corresponding age and size was used for comparison. In both limbs the terminals were beginning to mature but the terminal bud had not yet visibly differentiated. In preparing the infected leaf tissue for respiration samples, the leaves were cut longitudinally, excluding the main vein. That half of the leaf having the greater severity of symptoms was chosen. This method of sampling gave an overall estimate of the respiration rate of the diseased leaf tissue. The method is not critical in relation to variation of the severity of symptoms within the leaf. The longitudinally cut half leaves usually contained leaf tissue in several varying stages of symptom expression together with areas showing no visible symptoms. Respirations of such diseased material might be expected to indicate average rates of respirations.

The diseased leaves were found to have a much greater rate of respiration than comparable healthy leaves, but there was little indication that any one stage of severity of symptoms varied from another in respiration rate. See Table 10. Such lack of any trend might be attributed to the method of sampling. In the diseased

leaves the range of severity of symptoms varied both in degree and in proportion with some leaves showing more or less of certain symptoms than others. Only at the terminal, node 2, did the healthy check exceed the diseased in respiration rate.

TABLE 10

RESPIRATION OF DISEASED AND HEALTHY J. H. HALE LEAVES
FROM LIMBS WITH TERMINAL BUDS BEGINNING TO MATURE

Node	Healthy Respiration(1)	Diseased Respiration(1)	Percent Increase Over Healthy	Severity of Disease Symptoms
2(terminal)	1660	1330	-20	No visible symptoms
3	1250	1430	14	Very early
4	1180	1730	47	Early
5	865	1220	41	Very early
6	1010	1550	53	Moderate
7	620	1300	109	Early
9	535	995	86	Moderate
10	535	1350	152	Very early
12	518	1180	128	Early to moderate
13	647	2010	211	Very early
14	527	840	59	No visible symptoms
19	509	938	84	Very early
20	591	845	43	Very early
21	568	745	31	Very early
24(basal)	483	850	76	No visible symptoms

Average percent increase of diseased respiration over healthy respiration 74%.

(1) Microliters of oxygen taken up in two hours per gram fresh weight.

Two other similar experiments were carried out. In one of these the J. H. Hale variety was again used. See Table 11. The terminals of the diseased and healthy limbs in this second J. H. Hale experiment were maturing but the terminal buds had not yet fully developed. These limbs were more mature than those shown in Table 10. Rates of respiration of the diseased leaves in both experiments were higher than those of the healthy. This increased rate in the diseased leaves was again borne out in the final experiment of this group, using Improved Elberta in which the terminals had fully matured for the season. See Table 12.

TABLE 11

RESPIRATION OF DISEASED AND HEALTHY J. H. HALE LEAVES FROM LIMBS WITH TERMINAL BUDS MATURING BUT NOT FULLY DEVELOPED

Node	Healthy Respiration(1)	Diseased Respiration(1)	Percent Increase Over Healthy	Severity of Disease Symptoms
2(terminal)	560	1123	101	Early
4	415	911	120	Early
5	488	941	93	Early
7	393	847	116	Severe
8	347	970	180	Moderate
9	312	970	211	Moderate to severe
15	368	1075	192	Early to moderate
18	348	859	147	No visible symptoms
19	331	889	169	Early to moderate
21	377	960	155	No visible symptoms
25	275	909	231	Early
29(basal)	216	871	303	Early

Average percent increase of diseased respiration over healthy respiration 168%.

(1) Microliters of oxygen taken up in two hours per gram fresh weight.

TABLE 12

RESPIRATION OF DISEASED AND HEALTHY IMPROVED ELBERTA LEAVES FROM LIMBS WITH TERMINAL BUDS MATURED

Node	Healthy Respiration(1)	Diseased Respiration(1)	Percent Increase Over Healthy	Severity of Disease Symptoms
2(terminal)	332	880	165	No visible symptoms
4	293	892	204	Very early
5	332	825	148	Severe
6	270	815	202	Severe
17	194	870	348	Moderate
19	173	900	420	Very early
21	166	970	484	Moderate
23	209	895	328	Moderate
26(basal)	186	517	178	Very early

Average percent increase of diseased respiration over healthy 275%.

(1) Microliters of oxygen taken up in two hours per gram fresh weight.

In the three experiments shown in Tables 10, 11, and 12, the average percent increase of respiration of diseased leaves ranged from 74 percent in the first of the three experiments to 168 percent in the second and finally to 275 percent in the third. Examination of the data suggests that this increased average may be partially associated with limb maturity. The respiration rates of healthy leaves dropped greatly as the terminals matured and the terminal bud differentiated. This coincides with the earlier observations of decreasing respiration rates in healthy foliage as the terminals mature. Tables 2 and 3. On the other hand, while the diseased leaves also decreased with maturity the rate of decrease was much less than in the healthy leaves. Accordingly, the average percent increase in respiration rates of the diseased over the healthy showed a rise from 74 percent to 275 percent as maturity progressed.

b. Severity of symptom expression and respiration.

The experiments summarized in Tables 10, 11, and 12, all showed a respirational increase of diseased over healthy tissue. The increase became greater later in the season as the leaves and limbs matured. In these experiments no clear-cut relationship was demonstrated between severity of symptoms and rate of respiration other than that all diseased leaves indicated increased respiration.

Four additional experiments were carried out to determine whether a relationship could be found between respiration rate and severity of symptoms. In order to properly segregate the various stages of disease severity, extreme care was exercised in leaf

sampling. Leaves selected for Experiments 16 and 17 were taken from the lower third of the current season growth while leaves in Experiments 18 and 19 were chosen from as close to the central node as possible. See Tables 13 and 14. This slight change in sampling location was necessary because more diseased leaves remained attached in the vicinity of the central nodes than on the lower portions of current season growth. All limbs possessed about the same number of nodes and were of similar maturity with terminals growing actively at the time of leaf sampling. Experiments 16 and 18 involved three year old J. H. Hale trees and Experiments 17 and 19 Improved Elberta trees of a similar age. In Experiment 16, the leaves were removed from the trees in the afternoon at 3:00 p.m. and in the other three experiments in the morning about 10:00 a.m. A large number of diseased trees was employed for selecting leaves of varying degrees of symptom severity. Whenever possible not more than one symptom type was taken from each tree. In Experiment 16, samples of three leaves were used to represent each of the stages, early, moderate, severe, no visible symptoms, and healthy. In the other three experiments five leaves of each stage were used to make up a sample. Final selection of the actual leaves in each stage of symptom severity was made in the laboratory under a uniform source of transmitted light. As all leaves showed a range of symptom expression, the choice of location of the samples within the leaf was a critical step in the procedure. After careful examination of each leaf, discs were removed from those portions of the leaf which were most typical of

TABLE 13

SYMPTOM SEVERITY AND LEAF RESPIRATION IN J. H. HALE, EXPRESSED PER GRAM FRESH WEIGHT AND PER MILLIGRAM OF TOTAL NITROGEN(1)

Experiment	Symptom Severity	Resp./gm. Fresh Weight(2)	Percent of Healthy	Resp./mg. Total Nitrogen(3)	Percent of Healthy
16					
	Healthy	1091	100	846	100
	No visible symptoms	1110	102	1148	136
	Early	1340	123	1402	166
	Moderate	1705	156	1655	196
	Severe	1550	142	1607	190
Experiment 18					
	Healthy	1625	100	799	100
	No visible symptoms	1610	99	754	94
	Early	1980	121	1294	162
	Moderate	1935	119	1490	186
	Severe	2385	147	1456	182

(1) Terminals growing actively. Leaf samples taken from trees in Experiment 16 at 3:00 p.m. and in Experiment 18 at 10:00 a.m.

(2) Microliters of oxygen taken up in two hours per gram fresh weight. Average of two determinations.

(3) Microliters of oxygen taken up in two hours per milligram of total nitrogen. Average of two determinations.

TABLE 14

SYMPTOM SEVERITY AND LEAF RESPIRATION IN IMPROVED ELBERTA, EXPRESSED PER GRAM FRESH WEIGHT AND PER MILLIGRAM OF TOTAL NITROGEN(1)

Experiment	Symptom Severity	Resp./gm. Fresh Weight(2)	Percent of Healthy	Resp./mg. Total Nitrogen(3)	Percent of Healthy
17					
	Healthy	994	100	723	100
	No visible symptoms	1220	123	663	92
	Early	1530	154	1174	162
	Moderate	1630	164	1162	161
	Severe	1680	169	1017	141
Experiment 19					
	Healthy	1280	100	833	100
	No visible symptoms	1440	112	839	101
	Early	1645	129	1037	124
	Moderate	2055	160	1688	203
	Severe	2280	178	1554	187

(1) Terminals growing actively. Leaf samples taken from trees at 10:00 a.m.

(2) Microliters of oxygen taken up in two hours per gram fresh weight. Average of two determinations.

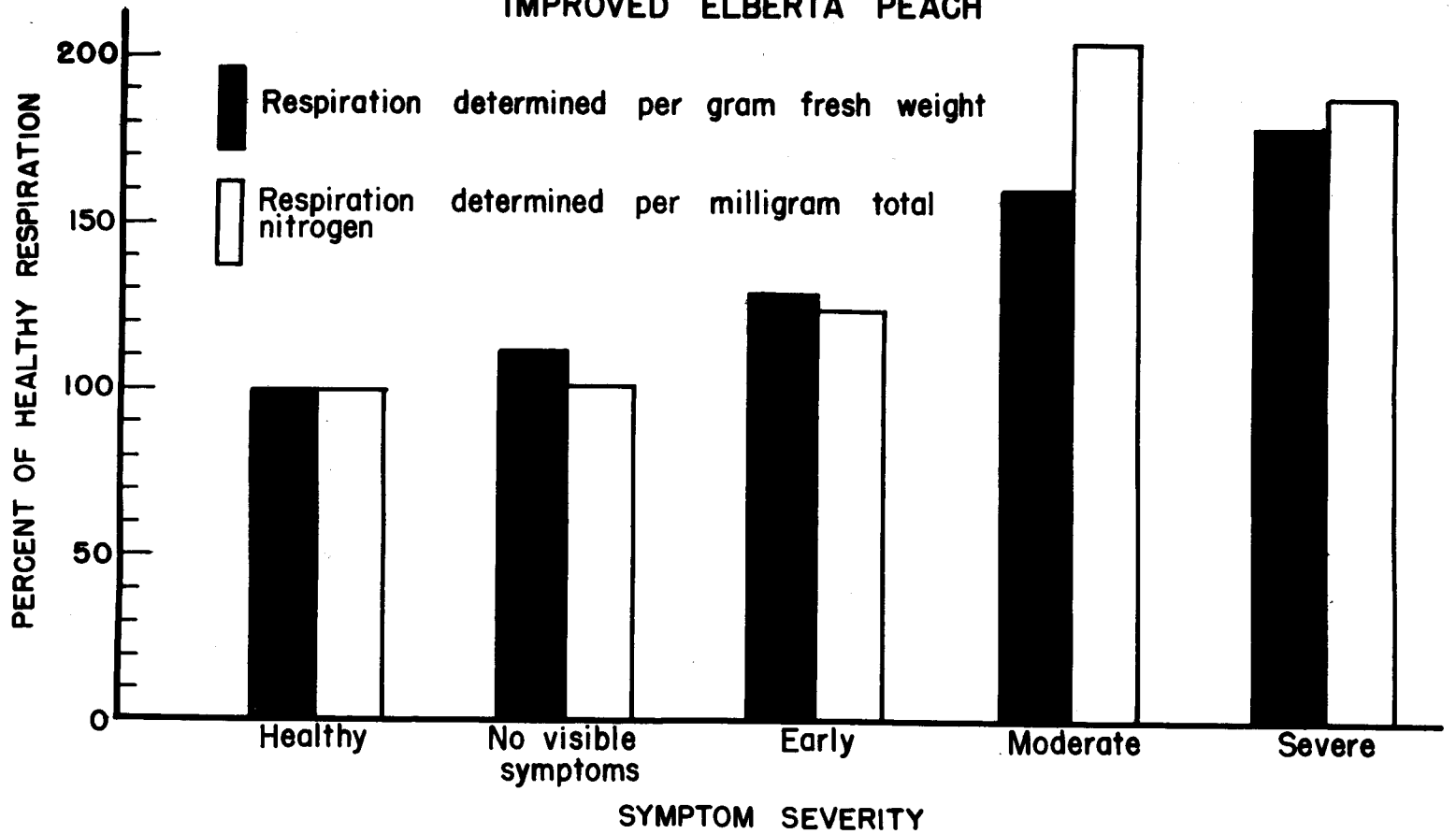
(3) Microliters of oxygen taken up in two hours per milligram of total nitrogen. Average of two determinations.

the stage of maturity being selected. Respiration determinations were made at once, using three, eight mm. leaf discs from each leaf. All the discs were retained and total nitrogen content determined. The remaining portions of the leaf samples after discs had been removed were dried and saved for starch analyses. Duplicate samples from the same leaves were employed for determinations of respiration rate, starch content, and total nitrogen content.

Previous respirations of healthy and diseased foliage had been made on a basis of respiration per gram fresh weight of leaf tissue. In this series of four experiments, a second method was introduced based upon respiration per milligram of total leaf nitrogen. Respiration was found to increase in the diseased leaves as symptoms became more severe. This increase took place when respiration was measured per gram fresh weight and also when measured per milligram total nitrogen. In the J. H. Hale Experiments 16 and 18, and in one Improved Elberta, Experiment 19, respiration based on nitrogen content showed a greater increase in rate in diseased leaves than when determined on a fresh weight basis. The effect of leaf maturity on diseased and healthy respiration rate calculated by nitrogen content was not determined, as there was only a small range in the leaf maturity in these experiments. All data indicate that respiration rates tend to increase with each degree of symptom severity whether respiration is determined on a fresh weight or total nitrogen basis. See Tables 13 and 14 and Figure 7.

FIGURE 7

COMPARISON OF SYMPTOM SEVERITY AND LEAF RESPIRATION
IN
IMPROVED ELBERTA PEACH



3. Starch and Nitrogen Content in Diseased and Healthy Foliage

The leaves used in the four previous experiments, 16, 17, 18, and 19, had been dried and analyzed for starch and the leaf discs analyzed for total nitrogen. Using this information a comparison could be made of the starch nitrogen ratios occurring in healthy and diseased leaves. The ratios of starch to nitrogen in the healthy leaves was taken as 100/100 and deviations in the diseased leaves were calculated in percent. The starch and nitrogen content for each variety was averaged and the starch nitrogen ratio compared for the healthy and for the varying degrees of disease severity. These data are summarized in Tables 15 and 16, and illustrated by bar graph in Figure 8.

The data in these four experiments show an increased accumulation of starch to occur in the diseased leaves as symptom severity becomes more advanced. At the severe symptom stage starch accumulation was 54 percent greater than the healthy in the J. H. Hale variety and 111 percent greater in Improved Elberta. Total nitrogen did not vary to any great extent with symptom severity, however, it was lower than in healthy leaves. In the J. H. Hale variety nitrogen decreased more than in the Improved Elberta while the increase in starch was greater in the latter variety. The increase in starch and decrease in nitrogen resulted in greater starch nitrogen ratios when compared with healthy leaves. In both varieties these ratios became progressively greater as symptoms increased in severity with the Improved Elberta showing slightly greater ratios than the J. H. Hale.

TABLE 15

SYMPTOM SEVERITY AND STARCH NITROGEN RATIOS
IN J. H. HALE LEAVES

Symptom Severity	Starch Content(1)	Nitrogen Content(2)	Starch Nitrogen Ratio	Average Ratio Increase Over Healthy
Healthy	14.2	15.8	100/100	--
No visible symptoms	13.6	13.9	96/88	8
Early	20.7	10.9	146/69	77
Moderate	21.5	10.6	151/67	84
Severe	21.8	10.8	154/68	86

(1) Milligrams starch per gram fresh weight of leaf tissue. Average of two experiments.

(2) Milligrams total nitrogen per gram fresh weight of leaf tissue. Average of two experiments.

TABLE 16

SYMPTOM SEVERITY AND STARCH NITROGEN RATIOS
IN IMPROVED ELBERTA LEAVES

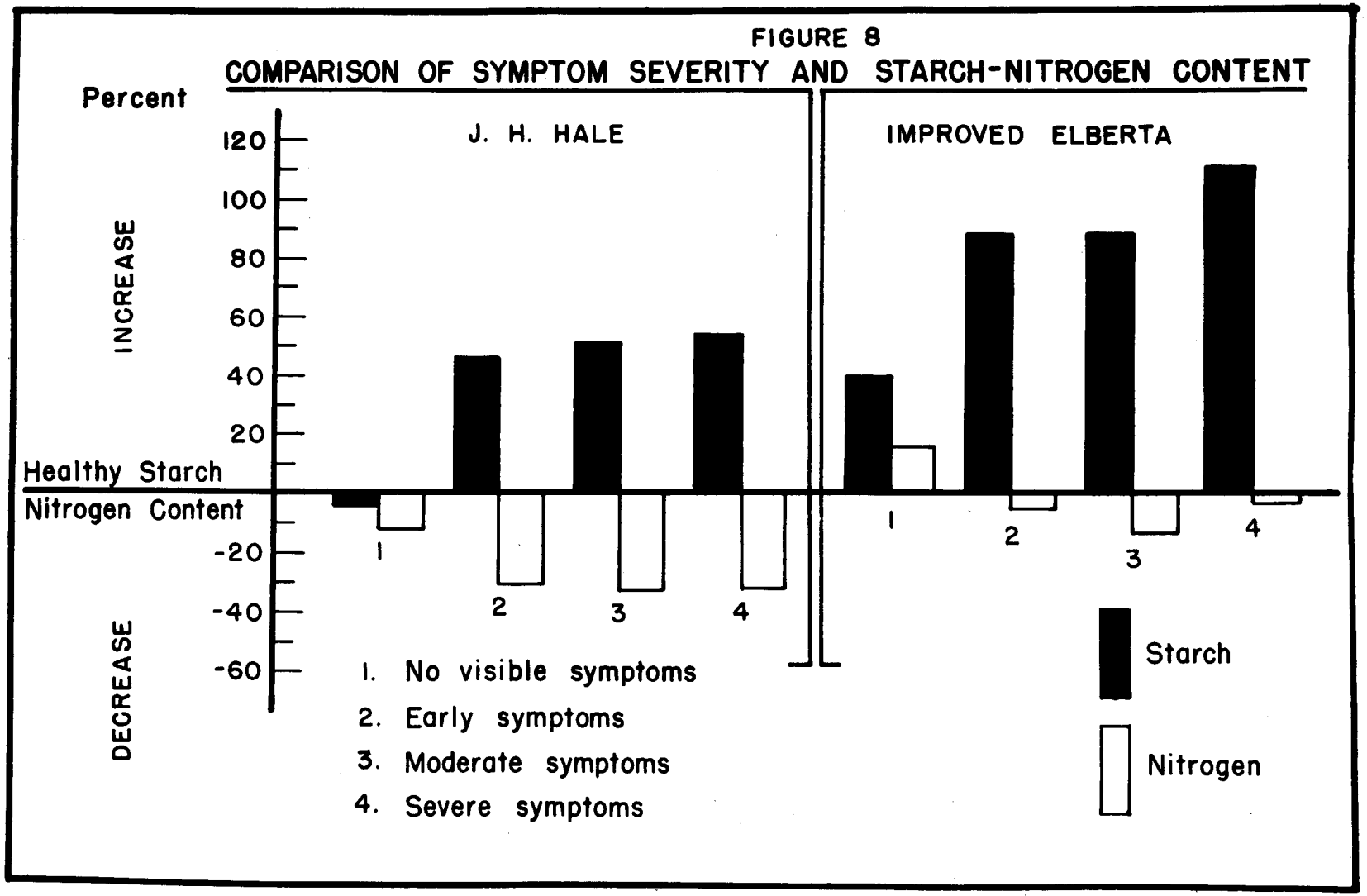
Symptom Severity	Starch Content(1)	Nitrogen Content(2)	Starch Nitrogen Ratio	Average Ratio Increase Over Healthy
Healthy	11.9	11.6	100/100	—
No visible symptoms	16.2	13.4	140/116	24
Early	21.2	11.1	188/95	93
Moderate	22.3	10.1	188/87	101
Severe	25.0	11.4	211/98	113

(1) Milligrams starch per gram fresh weight of leaf tissue. Average of two experiments.

(2) Milligrams total nitrogen per gram fresh weight of leaf tissue. Average of two experiments.

FIGURE 8

COMPARISON OF SYMPTOM SEVERITY AND STARCH-NITROGEN CONTENT



DISCUSSION

In general the symptoms of Western X disease on the various host plants resemble yellows type virus diseases. However, infected peach leaves are not entirely typical of the yellows group, as irregular chlorotic and yellow spots develop which later become necrotic and drop out to form a lace-like leaf pattern. The symptoms produced by the Western X disease little cherry complex on its various hosts suggest a disturbed metabolism and several phases of the problem are being investigated by the Oregon Agricultural Experiment Station in order to reach a better understanding of this disease. Changes in leaf respiration, starch accumulation, and nitrogen content have been found to occur by other workers in both yellows and mosaic type diseases, therefore, these factors were selected for study of Western X disease. The peach was chosen as an experimental host because it expresses foliage symptoms for several months of the growing season.

In preliminary experiments in which the respiration of diseased and healthy leaf tissue was compared, little consideration was given to the method of choosing healthy check leaves. Such leaves were selected at random from what appeared to be material comparable to the diseased. In general the leaves in the early stages of the disease respired more rapidly than healthy leaves and the respiration rate in the diseased leaves became greater with increasing symptom severity. However, results were erratic, especially in the severe stages where respiration rates occasionally varied widely. In

preliminary experiments of this kind wide variations also occurred in the respiration rate of healthy leaves, occasionally exceeding respiration rates in diseased leaves. Study of the problem led to the observation that no average respiration rate of healthy tissue existed, but the rate of respiration of each individual leaf varied according to its state of maturity and position on the limb. Healthy leaves at the terminal consistently respired more rapidly than the older leaves. A detailed study of the factors affecting respiration rates of healthy foliage seemed essential before further comparisons could be made with diseased peach leaf tissue.

Respiration rates in healthy peach leaves were found to decrease with age. Actively growing leaves at terminal nodes respired about four times the rate of leaves at basal nodes, however, where the terminal bud had matured for the season, terminal respiration averaged only about twice that of the basal leaves. This decrease in respiration in more mature foliage agrees with the findings of other workers including Kidd et al (18, pp.376-379) who studied respiration and maturity in Helianthus annuus L. and with Hover and Gustafson (17, pp.33-39) who studied the problem in cereal crops.

While there is agreement in the literature that respiration decreases with age both in the leaves and in other plant tissues, very little information was found specifically relating to respiration of peach leaf tissue. A number of possible factors which might affect respiration rates of normal tissue were studied prior to investigating diseased foliage. Length of current season growth, time of day in

selecting samples, entire leaf samples compared to discs of leaf tissue, and state of maturity of the limb based on terminal bud formation were found as the most important factors to be considered.

The effect of time of day on respiration was studied by selecting leaf samples in the early morning and afternoon from the same leaves. In the first experiment of this type morning sampling preceded afternoon sampling while in the second experiment, the sampling order was reversed. This was done in an attempt to offset any stimulus in respiration resulting from leaf injury accompanying the removal of the first samples. Time of day proved to have a considerable effect on respiration rate with afternoon respiration exceeding morning respiration by as much as 36 percent.

In comparing respiration of leaves on individual diseased and healthy limbs the diseased leaves showed a consistently higher respiration rate. Where larger leaf samples (half leaves) were used no clear-cut relationship could be shown between respiration and severity of symptom expression. The same diseased leaf usually expressed varying degrees of the symptoms considered to be severe, moderate, early, and healthy appearing. Therefore, the disc method of sampling was found in later experiments to give more reliable comparisons as more exact samples of the symptom types could be selected.

While no relationship could be determined between respiration rate and symptom severity using the larger half leaf samples, the effect of leaf and limb maturity on diseased and healthy respiration

rate could be compared. In three experiments conducted prior to, during, and after terminal bud differentiation, respiration rates of the half leaf samples were compared on corresponding nodes of diseased limbs and healthy limbs. When the respiration of all the leaves on the healthy and diseased current season growth was averaged separately, the rates of the diseased leaves were greater than the healthy in all three experiments. In the first experiment before terminal bud differentiation, the diseased leaves exceeded the respiration of the healthy leaves by 74 percent. In the second experiment, in which terminal growth was becoming less active, respiration of diseased foliage was higher than that of healthy foliage by 168 percent, while in the final experiment in which both diseased and healthy terminal buds were fully matured, the diseased leaves respired at a rate of 275 percent above healthy leaves. The data for these experiments showed that the average respiration in healthy leaves occurring after terminal bud maturity had decreased to one-fourth the rate occurring prior to maturity. However, in diseased tissue compared before and after terminal maturity the respiration rate had only decreased by about one-half. Thus the greater variation amounting to 275 percent increase in respiration of diseased tissue occurring after terminal bud maturity, appears to be a result of two factors, a stimulation in respiration rate due to the presence of the virus and a slower decrease in respiration rate in the diseased leaves as maturity advances.

By careful leaf sampling, using leaf discs only of those

portions of diseased leaves most typical of the various stages of symptom expression, leaf respiration was found to increase as symptom expression became more severe. This increase in respiration rate occurred consistently whether determined by respiration per gram fresh weight of tissue or respiration per milligram of nitrogen.

The importance of consistently and carefully selecting the varying degrees of symptom severity was demonstrated in a series of preliminary experiments. They showed that respiration rates for the various stages differed greatly according to the choice of leaves. This was especially true if leaves chosen to represent the severe symptom stage were approaching senescence. In these very advanced stages of the disease, necrotic and almost dead tissue predominated and respiration dropped below that of healthy tissue. Therefore, leaves selected for the severe symptom stage were taken before senescence had occurred, and with this type of tissue respiration was consistently higher than at any other stage of symptom expression.

The increased starch nitrogen ratios found occurring in the leaves of both J. H. Hale and Improved Elberta infected with Western X little cherry disease suggest that this yellows appearing virus disease coincides physiologically with other diseases in the yellows group. Starch accumulation was found to occur in direct proportion to the degree of symptom severity. In the Improved Elberta variety at the severe stage of the disease, the increase of starch was over 100 percent. The decrease in nitrogen content occurring in the diseased leaves also corresponded to reported decreases in other

yellows type virus diseases. Following Bawden and Pirie's classification (5, p.181) Western X disease would be classified as a yellows type virus disease. According to their classification the yellows group tends to have an increased carbohydrate content and a decreased nitrogen content, while the mosaic group has decreased carbohydrate and increased nitrogen content.

The data obtained in this study on respiration, starch accumulation, and loss of nitrogen all add to the evidence that the Western X disease virus must be classified with the yellows group of viruses.

SUMMARY

1. In studying certain physiological disturbances of the foliage accompanying Western X little cherry disease in peach, respiration rates, starch content, and nitrogen content of diseased and healthy leaves were determined.
2. A separate study of the factors affecting respiration of healthy leaves was undertaken in order to more properly evaluate the respiration of diseased leaves.
3. Respiration rates of healthy peach leaf tissue determined on a fresh weight basis were greatest at the terminal of elongating limbs and decreased in rate as the terminal buds matured.
4. Leaves on all limbs decreased in respiration rate from the terminal to the base of current season growth regardless of stage of maturity of the terminal.
5. In order to compare leaves from separate limbs, comparable stages of maturity of the terminal bud and similar stages of leaf maturity had to be considered.
6. Respiration rates of healthy leaf tissue determined in the afternoon exceeded those determined in the morning.
7. Diseased leaves respired more rapidly than healthy leaves. As the limbs matured, the respirational rates of diseased leaves decreased at a slower rate than comparable leaves from healthy limbs.

8. Respiration rates increased in diseased leaves as the disease symptoms became more severe. This increase with severity occurred when respiration was determined on a fresh weight basis and also when determined on a total nitrogen basis.
9. An accumulation of starch occurred in all diseased leaves becoming greater with disease severity.
10. Total nitrogen content in all the diseased leaves was lower than in corresponding healthy leaves but did not vary greatly with symptom severity.
11. The increase in starch and decrease in nitrogen resulted in a greater starch nitrogen ratio than in healthy leaf tissue. The starch nitrogen ratios were correspondingly greater at more advanced stages of the disease due to the continued accumulation of starch as symptom severity advanced.
12. The data obtained in this study on respiration, starch accumulation, and loss of nitrogen all add to the evidence that the Western X disease virus must be classified with the yellows group of viruses.

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