THE INFLUENCE OF LEAF TO FRUIT RATIOS ON THE PHOTOSYNTHETIC ACTIVITY OF YORK AND LIVLAND APPLE LEAVES

by

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INTRODUCTION

Of the varieties of apples most commonly grown in the Great Plains area, York has presented the alternate or biennial bearing condition more than any other variety. By biennial or alternate bearing in apples the horticulturist refers not to the behavior of the individual spur itself but rather to the behavior of the tree as a whole. It is well known that the individual apple spur is characteristically biennial in its bearing habit in that the year in which it bears a fruit is followed by a year in which neither fruits nor blossoms are borne. This condition is true of the spurs of all varieties of apples but in most varieties we find that only part of the spurs produce blossoms and bear fruit in a given year. The remainder of the spurs are meanwhile producing vegetative growth only and differentiating blossom buds which will produce the crop for the following season. This is the condition favored by the fruit grower for it insures against the possibility of having crops of fruit only in alternate years.

In the alternate bearing condition as typified by York, all or nearly all of the spurs bear blossom buds in one year and, hence, since an individual spur under Kansas conditions rarely blossoms in successive years, there will be but few blossoms borne the following year.

This condition is decidedly undesirable from the standpoint of the apple grower. In years in which a crop is borne he may suffer from low financial return because of over production and in the alternating off-years he gets no return from his investment. Then, too, loss of a crop in an on-year through late frost or other reason extends the number of successive unremunerative years to two. For this reason, regularity of bearing has been an important consideration in the selection of varieties, and in consequence varieties with a marked tendency to irregular or alternate bearing have been discriminated against or are gradually being replaced by varieties exhibiting a more regular bearing tendency.

Though exhibiting almost universally the alternate bearing condition the variety York is highly regarded by practical orchardists because of other partially redeeming features such as hardiness, resistance to pests, vigor, and its good average production of marketable fruit.

If we may assume that regularity of bearing is dependent on nutritive conditions it is reasonable to assume that by proper cultural methods much could be done to remedy this undesirable habit. If it were possible the value of this variety of apples will be greatly enhanced. Before it is possible to prescribe for a condition, however, one must have some knowledge of the causes responsible for that condition.

For this reason it seemed desirable to conduct an experiment to determine if photosynthetic activity of the leaves might be one of the contributing factors in the biennial bearing habit. York, being the outstanding variety exhibiting biennial bearing, was chosen as the subject for this experiment. To afford direct comparison between the photosynthetic activity of a variety exhibiting the biennial habit and one exhibiting regular bearing it was decided to include in this study Livland which has a regular bearing tendency.

REVIEW OF LITERATURE

A popular conception regarding fruitfulness is indicated in the following statement by Brown (4): "The biennial bearing habit is apparently not an inherited trait, but when it once becomes fixed in the life of the individual there is little that can be done to change it . . . .

as the trees grow older the habit becomes fixed and it is hardly worth while to attempt to correct it." Observations of biennial bearing orchards during the previous quarter century have suggested the theory that this habit is largely due to nutritional conditions. Roberts (42) has observed five facts which suggest the view that biennial bearing is not a fixed habit of the trees.

"1. Off-year trees have been made to bear in successive years by experimental means.

"2. Removal of the blossoms before the fruit sets has resulted in successive blossoming.

"3. Removal of the leaves has stopped blossom bud formation.

"4. Orchards of annually bearing trees exist.

"5. Following a severe freeze in the late spring of 1910 the on-year changed from the even to the odd-numbered year. This occurred with all varieties."

Many suggestions have been made to explain the occurrence of biennial bearing. One of the most common theories is that the tree needs a rest after bearing a heavy crop of fruit. Roberts (43) has upset this theory by demonstrating that removal of the fruits any time after they have become as much as one-half inch in diameter seldom results in the fruit spurs fruiting in successive years. While overbearing is apparently not the direct trouble, excess blossoming may be said to be the cause, since a spur seldom produces flowers two years in succession, regardless of whether it bears a fruit or not. While excessive blossoming is associated with the off-year, it does not cause off-year bearing since excessive blossoming is itself an effect of nutrition. Roberts (44) considers that nutritional conditions which cause the formation of extreme numbers of blossom buds tend to give off-year bearing.

Overbearing having been considered a prime factor in the irregular fruiting of apples, it seems a logical conclusion that thinning the fruit would tend to result in regular bearing. Numerous experiments have repeatedly shown that commercial thinning of the fruit does not have the effect expected. The failure of thinning to induce successive bearing is apparent if one considers that the period of blossom bud differentiation occurs before commercial thinning generally is done. Roberts observed that removal of all the young fruits any time after they have "set" fails to give successive bearing.

Pickering and Bedford (35) report that the weather of England has a sort of biennial cycle which they consider to be associated with the biennial bearing of dwarf trees.

Macoun (27) has offered the same suggestion regarding alternate bearing in Canada. Whipple (54) suggested that winter killing of the blossom buds may cause off-years in Montana. Roberts (43) considers that these climatic influences do not seem to be causes of irregular bearing as it occurs in Wisconsin. He makes two other suggestions which are of more significance to the local situation than those just mentioned. One is the short growing period which is usually accompanied by rather high temperatures. The other is the relation of climate to the production of available nitrates in the soil. He mentions a third factor concerning which very little was known, namely, the relation of the short growing period to spring root development, as affecting absorptive power.

Brown (4) reports that the alternate bearing condition of apple trees is more common in fruit sections of the north and east than in those of the south or west. He finds that the biennial bearing habit does not exist in regions where climatic conditions favor the setting of a crop every year. Where frosts and rains interfere with the setting of fruit the life processes of the tree are thrown out of balance and the tree acquires the habit of overworking one year and recuperating the next. The attempt to mature a heavy crop causes a drain on the tree and no fruit buds are differentiated since the food supply of the tree is directed first to the needs of the maturing crop.

Chandler (6) observed that fruiting reduced the growth of the tree and suggested the reduced vegetative growth might cause reduced fruit bud differentiation. These results are in keeping with those of Haller and Magness (15) who found that with decreased leaf area per fruit the percentage of dry weight and total sugars in the fruits also decreased and that blossom bud formation was also inhibited. Blossom differentiation was associated with a relatively high sugar content in adjacent apples. Kraybill, Potter, Wentworth, Blood and Sullivan (24) likewise observed that the leaf area of non-fruiting spurs was invariably greater than that of fruiting spurs and that the non-bearing spurs were higher in starch, sugar, acid hydrolyzable carbohydrates, ash and dry weight than were bearing spurs. They believed that these differences in chemical constituents were at least partially responsible for the inhibition of fruit bud differentiation on bearing spurs but suggested the possibility that suppression of fruit bud differentiation may be due to a "dominance" exerted by the developing fruit through production of "growth regulators".

Magness, Overly and Luce (28) found that fruit bud formation of apples and pears did not occur on ringed branches with ten leaves per fruit but that twenty leaves per fruit provided ample fruit bud formation while abundant formation occurred with thirty or more leaves per fruit. The total sugars, dry matter and titratable acidity of fruit and spurs was always greater on branches with large leaf area per fruit than on branches of the same tree bearing fewer leaves per fruit.

Potter, Kraybill, Wentworth, Sullivan and Blood (39) observed that the leaf area of deflorated spurs from trees completely deflorated was considerably larger than that of deflorated spurs from trees on which half the spurs were allowed to blossom. The starch content of such spurs and of the bearing spurs was lower than that of deflorated spurs from completely deflorated trees.

Thies (50) studied the effect of defloration on spur leaf area in the McIntosh apple and found that the average increase in leaf area as a result of defloration to be about 35 per cent. If a tree bloomed heavily it failed to store sufficient carbohydrate reserve to insure a set of fruit buds for the next year. On the other hand, if only a small proportion of the spurs set fruit in a given year the leaf area per spur was increased and a greater carbohydrate reserve was built up. The work of Bailey (2) indicates that it is necessary to remove about 90 per cent of the apple blossoms of a heavy bloom to insure development of fruit buds for the following year.

Vyvyan and Evans (53) noted that bearing apple spurs have smaller leaves than non-bearing spurs and that non-bearing spurs on heavily loaded trees have smaller leaves than non-bearing spurs on only slightly loaded trees. They conclude that the presence of a crop may dwarf the leaves and even vegetative growth. Their observations coincide with those of Swarbrick (49).

Wiggans (55) found that non-bearing spurs on account of their greater leaf area are able to lay up a greater amount of reserves than spurs which are maturing fruits. When compared with the total reserves this excess is small but he considers that it may be significant in fruit bud differentiation.

Aldrich and Work (1) in studying the effects of the degree of fruitfulness in pears on fruit bud differentiation found that thinning to give 100 leaves per fruit and defoliating to leave ten leaves per fruit increased and decreased, respectively, the amount of fruit bud formation as compared with normal limbs. The amount of increase or decrease of bloom depended on the time the thinning or defoliation was done.

According to Mack (26) the smaller branches of alternate bearing varieties are exhausted of their reserve materials in the early part of the on-year and continue to have a low carbohydrate-nitrogen ratio until after growth has stopped. This is indicated by the fact that only very long growths or those making secondary growth, form blossom buds for the off-year. The larger branches and possibly the trunks of mature trees retained sufficient storage materials after the setting of the on-year crop to differentiate blossom buds on the short spurs near these larger branohes.

In making a study of the self sterility problem in apples, Kraus (22) found that fruit bud differentiation is dependent on nutritional conditions and that if these conditions are not favorable a high percentage of deficient or abnormal fruit buds develop. His work is substantiated by the findings of Howlett (18) in a study of the carbohydrate and nitrogen composition of developing flowers and young fruits. At the period of opening the buds were low in reducing substances, total sugars and acid hydrolyzable fraction. Flowers exhibiting tendencies to drop showed decreases in carbohydrate and nitrogen contents while those which set showed great increases in nitrogen and all types of carbohydrate material and especially in the soluble monosaccharoses.

The far famed work of Kraus and Kraybill (23) with reference to the influence of the carbohydrate-nitrogen ratio need not be discussed here save mentioning the fact that fruitfulness was found to be dependent on the proper balance of reserve food materials. Gurjar (14) also noted that changes in the carbohydrate-nitrogen ratio are accompanied by marked changes in the metabolic responses of the plant and he concluded that fruitfulness in our economic plants is dependent on the proper carbohydrate-nitrogen ratio. On the other hand, Work (56) states that there is no apparent relation between nitrogen content and carbohydrate content except that in starved plants the nitrogen content will be low and the carbohydrate content high. No indication was found that either high or low carbohydrate content inhibited either vegetative or reproductive activities of the plants in these experiments. He concluded that the concentration of carbohydrates in a plant is the result of the balance between the processes of manufacture and the many processes of use. So long as the rate of manufacture is sufficient to meet current needs the amount present does not condition the processes of vegetation and fruition. Finch (11) found the unfruitful growths of biennially bearing trees to represent an extreme of carbohydrate-nitrogen composition. Fruitful growths represent an intermediate condition of the proper balance. Potter and Phillips (40) found the spur constituent most consistently associated with blossom formation to be insoluble nitrogen. Large accumulations were followed by fruit bud formation while the accumulation of carbohydrates, such as starch, prior to July was not an indication of conditions favorable to blossom differentiation.

In summing up the results of the above experiments one can say that in general fruit bud differentiation and the resulting fruitfulness are determined primarily by the accumulation of reserve plant nutrients in excess of the plant's daily requirements. In the words of Murneek (33) "the apple fruit, being primarily an organ high in carbohydrate exerts a dominating metabolic influence and draws heavily on the products of photosynthesis especially during the latter part of its development. If the leaf area exceeds the requirements of the developing fruit, then a surplus of carbohydrates exists and the vegetative parts of the tree are benefited accordingly. There will be a tendency for greater vegetative development, carbohydrate storage and increased fruit bud formation."

In further studies with the tomato, Murneek (30) found that a plant under a given condition of nutrition would set a corresponding number of fruits and when that number is reached no additional flowers could be fertilized, though daily pollination was practiced. The flower clusters produced after that time became smaller, yellow in color and dropped quickly. He explained this situation by stating that the developing fruits have a dominating metabolic influence. In answering the question as to how the fruit is able to monopolize practically all of the incoming food supply he sets up two possibilities: (a) It is a case of localized nutrition brought about by gametic union and consequent rejuvenescence and the initiation of a metabolic gradient, or (b) It may be due to hormonic control of the physiological substrate, the indispensable chemical constituents of the plant.

He finds a marked increase in metabolism at the growing points immediately following fertilization. This stimulation, due to fertilization, extends to an appreciable degree beyond the tissues immediately adjoining the developing embryos. Murneek (31, 34) considers these features to parallel the situation in animal physiology wherein recent investigations have shown that not only those organs closely associated with the embryo but the entire body of the mother becomes more efficient during the prenatal period, resulting in increased storage of nitrogen, phosphorus and other mineral substances. With the animal there is no question as to the part played by hormones and in Murneek's opinion the same theory explains this condition in plants.

From the foregoing evidence, one could logically draw the assumption that the alternate bearing condition of the York apple is due to its inability to accumulate sufficient food reserves to permit fruit bud differentiation at the same time that it is maturing a crop of fruit. The results obtained by Fisher (12) in studying the comparative efficiency of a given area of leaf surface in production of fruit give weight to the above theory. In every case he found that 100 square centimeters of leaf area of annual bearing varieties produced a greater weight of fruit than an equal leaf area of biennial bearing varieties and suggested that the results might be due to differences in the photosynthetic activity of the leaves of the different varieties.

Crocker (7) after studying periodicity in tropical trees stated that while the fundamental causes of such phenomena are as yet unknown, a careful study of the internal conditions of the plant, anatomical, chemical, and microchemical, as well as the application (by injection or otherwise) of the various salts and carbohydrates and products manufactured from them is necessary to give definite assurance of the effective agents causing such periodicity. Heinicke and Hoffman (16) in studying the rate of photosynthesis of apple leaves found that the internal conditions of the leaf and of the tree as a whole will have a profound influence on the efficiency of the foliage of apple shoots. Dark green leaves are associated with a high rate of photosynthetic activity. Succulent leaves, which are subject to incipient wilting on hot days, show great reduction in their activity at such times. They state that a more complete knowledge of the assimilation rate may be expected to throw some light on other important considerations in fruit growing such as the alternate bearing condition of some varieties of apples.

Pickett (36, 37, 38) in making a study of the internal structure of apple leaves as related to photosynthetic activity found that of the seven varieties studied, viz.: Livland, Wealthy, Jonathan, Delicious, Winesap, Gano and York, York belonged in a class by itself because of its low apparent photosynthetic rate. He observed that York leaves have: (a) a lower percentage of ash, (b) usually a lower water content, (c) a lower total dry weight per unit of leaf area, (d) a more compact mesophyll. He suggested that the distinct biennial bearing habit of York may be due in part to the fact that its foliage is relatively inefficient photosynthetically, which condition apparently is due to the above mentioned characters.

Lundegårdh (25) found greater photosynthetic activity in leaves with greater area of surface in the intercellular spaces and concluded that difference in internal structure was correlated with difference in photosynthetic activity. Using Sach's iodine test Dastur (8) has shown that in ageing leaves of Abutilon asiatica, Ricinus communis, Carica papaya and a number of other species a loss of photosynthetic activity occurs first in the mesophyll cells of the margins and intravascular regions of the leaf, that is in the cells most remote from the vascular bundles and therefore from the water supply. Later work of Dastur's which was not published, shows according to Stiles (48) that there is a direct correlation between water content and rate of photosynthesis, the latter declining as the water content diminishes as the leaves approach their stage of senesence.

Consideration of the above mentioned studies leads to the hypothesis that the biennial bearing condition of York is due to its low apparent photosynthetic rate and, if such is the case, should be reflected in the photosynthetic behavior of York leaves borne on branches which exhibit varying degrees of fruitfulness.

For the present study, it was decided to adjust the leaf-fruit ratios on large scaffold branches to three widely varying degrees of productivity and to determine the effects of such varying degrees of fruitfulness on the respective photosynthetic rates. Kim (19) in studying the relation of the leaf-fruit ratio to the size and color of apple fruits found that for York at least 40 leaves per fruit were necessary to produce fruit of commercially satisfactory size and color. Ten leaves per fruit resulted in undersized, poorly colored fruit due to insufficient food supply. For this reason, it was decided to use branches with the degrees of fruitfulness represented by the following leaf-fruit ratios: 10 leaves per fruit, 50 leaves per fruit and the fruitless condition.

MATERIALS AND METHODS

Two varieties of apples were used in this study. Livland is an early or mid-summer ripening variety while York is a late season or winter variety. Livland was chosen because it is an annual bearer and according to Pickett (38) possesses the highest photosynthetic rate per unit of leaf area of any of the varieties studied. Livland is a rather dwarfed tree as compared with the tree of York which is above average in vigor probably because York has far greater leaf area per tree. Trees growing in the orchard of the Kansas Agricultural Experiment Station were used for the study. An attempt was made to select trees of average vigor and size for the varieties, growing on soil of nearly the same fertility. The York trees selected were trees 4 and 5 of row 11 and the Livland trees 12 and 14 of row 31.

Representative scaffold branches on the south one-third of the trees were selected for the study. An effort was made to choose branches approximating the desired leaf-fruit ratio but considerable adjustment was necessary to obtain the desired ratios. All injured or defective leaves were removed as were all of the small leaves found on spurs thus leaving only leaves which were about average in size for the variety. All defective fruits were likewise removed and then the desired fruit-leaf ratio obtained by either removing leaves or thinning fruits. Approximately 4,000 leaves of average size for the variety remained on each scaffold branch with the proper number of fruits for each ratio. The fruit-leaf adjustment was completed on June 4, 1935.

For the sake of brevity the following substitution of terms will be used hereafter in the manuscript:

heavy crop York = York ten leaves per fruit
medium crop York = York fifty leaves per fruit
fruitless York = York fruitless
heavy crop Livland = Livland ten leaves per fruit
medium crop Livland = Livland fifty leaves per fruit
fruitless Livland = Livland fruitless

The photosynthetic activity was determined in two ways: (a) By the weight of the total dry matter accumulated per unit of leaf area, and (b) By the saccharification method.

The Dry Weight Method

This method was developed by Sachs (45). He removed one-half of a leaf blade by cutting along the midrib at the beginning of an experiment and determined its area and dry weight. The other half of the blade was left attached to the midrib and at the end of the experiment it too was removed by cutting along the midrib and its area and dry weight determined. The gain in dry weight per unit area of leaf surface was considered as due to the carbohydrate formed in the meantime by photosynthesis. Sachs recognized that translocation and respiration would be occurring at the same time as photosynthesis and attempted to correct the results obtained by the dry weight method by adding the loss in dry weight per unit of leaf area at night to the gain in dry weight per unit of leaf area by day. Another method of correction was attempted by using a detached leaf in water as a control with the idea that translocation of carbohydrate material would be impossible under such circumstances but later investigation has shown that such detached leaves do not assimilate at the same rate as leaves attached to the plant.

Sachs' method has been severely criticized by other investigators. Brown and Escombe (5) compared the results obtained by measuring photosynthesis by the continuous gas stream method and the dry weight method. Working with catalpa bignonioides they obtained photosynthetic values two or three gimes greater for the dry weight method than those obtained by the continuous gas stream method. They concluded that all errors in the dry weight method became accumulative in the final result.

Ganong (13) believed that the dry weight method as devised by Sachs possessed possibilities if certain errors could be eliminated. He designed a punch which could be used to cut from leaves circular discs 1.128 centimeters in diameter, or 1 square centimeter in area. He found that use of the punch eliminated the error due to differences in area of the half leaves as used by Sachs. He concluded that removal of the discs did not seriously interfere with the functioning of the leaves. Kostytchew (21) reports decreased photosynthetic activity in wounded leaves of Betula pubescens and Lamium album as compared with unwounded leaves. Along with Engelmann (9) and Ewart (10) he thinks that the general cytoplasm plays no part in the binding and reduction of carbon dioxide but the process takes place exclusively in the chloroplasts. The question is still in doubt, however, for he appears to have taken no account of respiration and of the influence of wounding on this process. Kny (20) also reports that there is no direct parallelism between injury to the cytoplasm and photosynthetic inhibition.

Thoday (51, 52) did not consider the dry weight method sufficiently refined to give accurate determinations of photosynthetic activity. He was of the opinion that the calculation of the equivalent intake of carbon dioxide from the increase in dry weight to be only approximate and states that it is probably advisable to determine the increase in ash content and to deduct that increase from the increase in dry weight. The tendency of this method to give results which are too high was explained by: (a) Shrinkage in area of leaves during the experiment which may amount to as much as five per cent from morning to midday as a result of loss of turgor during insolation, (b) The lack of symmetry in respect to dry weight per unit area. He admits that the latter objection can be diminished by avoiding large veins and by using a large number of leaves for each experiment. He admits that the real increase in dry weight during a period of five hours or more can be determined with most leaves correct to the nearest milligram per square decimeter per hour but, along with Spoehr (47) and Stiles (48), considers that it cannot compare with the gas stream method for accuracy and that its virtues lie chiefly in its simplicity in principle and application.

Regardless of what may be said against this method, the fact remains that it does give comparable results and as yet no method has been devised which is accepted as absolutely accurate. A true measure of photosynthetic activity will be obtained only when a method is devised capable of making a determination of the total energy changes taking place in the plant. Precautions were taken to diminish as many sources of error as possible, among these were: (a) Fifty leaves were selected for sampling each individual branch for each experiment; (b) Dust, spray residue and similar deposits were washed from the leaves twenty-four hours prior to collection of the first samples; (c) In the removal of each disc all possible care was taken to avoid cutting the large veins; (d) Only fully mature, comparable leaves were used; (e) Leaves showing injury caused by fungi, insects or wind were avoided though as the season advanced it became impossible to secure entirely normal leaves because of severe leaf miner and red spider infestation; (f) All leaves used were located on the south one-third of the outer periphery of the tree and at heights ranging from three to six feet from the surface of the ground; (g) The spacing of the trees was such that at no time from 5:00 a. m. to 2:00 p. m. were the leaves used shaded by foliage of other trees; (h) The trees used were growing under conditions of soil and soil management which were as nearly identical as possible.

Pickett (38) found that apple leaves growing in the orchard attained their greatest weight per unit of leaf area at about 2:00 p. m. and their least weight per unit of leaf area at about 5:00 a. m. From 2:00 p. m. to 5:00 a . m. the processes of respiration and translocation proceed at a faster pace than does the apparent photosynthesis as determined by changes in dry weight. Accordingly at 5:00 a. m. the first set of punches was collected, the first punch from each leaf being taken from the right side of the leaf and near the tip. The punches were collected in tightly stoppered, previously weighed, clean vials which were then taken to the laboratory and the green weight quickly determined. The open vials were then placed in the electric evaporating oven at a temperature of 95 degrees C. for a period of 24 hours at which time the dry weight was determined. At 2:30 p. m. a second set of punches was collected in a similar fashion from the left side of the leaf. At 5:00 a. m. the following day another set was collected from the right side of the leaf just below the punch taken the previous morning. At 2:30 p. m. another set was collected from the left side of the leaf. Four sets of punches so collected gave data as to increase in weight on two days with the decrease in weight over one night. Temperatures and relative humidity figures were recorded at the time of each collection. All samples were collected in the following order: heavy crop York, medium crop York, fruitless York, heavy crop Livland, medium crop Livland and fruitless Livland.

On the day before a set of samples was to be taken 50 leaves, average in every respect, were selected and labeled with numbered cardboard tags to aid in identifying the leaves. At the same time the leaves were carefully washed with water to remove dust and spray residue. Climatic conditions had been favorable to fungous attacks necessitating heavy applications of Bordeaux mixture shortly after the first collection of samples was made.

Since water was not effective in removing the Bordeaux spray residue, thereafter a one-fourth per cent solution of glacial acetic acid was used in washing the leaves followed by a rinsing with water to remove the acid solution. This method was satisfactory in removing the spray residue and apparently had no injurious effects on the foliage though its effect on photosynthesis is not known. However, even after careful washing traces of the spray residue could be detected on the pubescent lower surface of the leaves and in the depressions adjacent to the midrib and veins on the upper surface.

Collections of samples were made on the following dates: June 6 and 7, June 19 and 20, June 27 and 28, July 3 and 4, and July 22 and 23, 1935 giving a total of 10 days of collection and observation.

The Saccharification Method

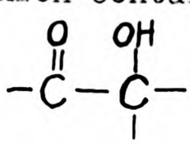
At the same time that the leaf punches were collected 40 to 50 leaves were collected from each branch for use in the saccharification method of determining photosynthetic activity. The leaf blades only were collected and were quickly taken to the laboratory where they were washed in dilute (one-fourth per cent) glacial acetic acid solution and then rinsed in water to remove the acid solution. After hurried wiping to remove the excess water the leaf blades were placed in hardware cloth baskets and placed in the electric oven to be dried at a temperature of 70 degrees C. During the first hour the oven door was left slightly ajar to hasten the removal of the excess moisture. After drying over night the leaves were ground with a mortar and pestle to a powder which would pass through a forty mesh sieve and the powder stored in labeled bottles until such time as chemical analyses could be made.

By this method the entire carbohydrate content is hydrolyzed to hexoses and the products thus formed are calculated as glucose by the reduction of Fehling's solution. The procedure as modified by Shafer and Hartman (46) follows: One and one-half gram samples of the dry leaf powder were weighed into 500 cc. Erlenmeyer flasks, 150 cc. of two and one-half per cent hydrochloric acid added and the mixture boiled in a water bath under reflux condensers for two hours. The preparation was then cooled under the tap and brought to near neutrality with a sodium hydroxide solution and then filtered. The general procedure in this method then is to clarify the filtrate with a saturated solution of neutral lead acetate, filter and then to precipitate the lead with sodium oxalate solution and refilter and then test the filtrate for the presence of lead with solid sodium oxalate and precipitate the remainder of the lead with sodium oxalate solution if necessary. The final filtrate is then brought up to volume in 250 cc. volumetric flasks with distilled water. MacGillivray (29) has reported that clarification with lead and its accompanying deleading does not affect the reducing power and consequently can be dispensed with hydrolyzed extracts of many plant tissues. Only whenever tannins or similar reducing impurities are present in the extract did he consider clearing necessary.

Preliminary experiments here showed that clarification of the first filtrate did not alter the results appreciably but gave slightly lower carbohydrate contents as compared with unclafified filtrates. Therefore in all cases the filtrate obtained after bringing the preparation to neutrality was brought to volume directly with distilled water in 250 cc. volumetric flasks.

Fehling's solution is prepared in the following manner. Part A is made by dissolving 69.28 gms. of copper sulphate in 1,000 cc. of distilled water. Part B is made by dissolving 346 gms. of sodium potassium tartarate (Rochelle salts) in 500 cc. of distilled water and 100 gms. of sodium hydroxide in another 500 cc. of distilled water. Both parts of solution B are then mixed and kept until needed.

Twenty-five cc. of each of solutions A and B are then transferred to a 300 cc. Erlenmeyer flask into which exactly 50 cc. aliquots of the hydrolyzed sample is pipetted. This solution is then heated in a hot water bath at 80 degrees C. for 30 minutes following the method developed by Quisumbing and Thomas (41). The flask is then removed and cooled rapidly. Twenty-five cc. of potassium iodide-iodate solution is then added. This is prepared by dissolving 60.0 gms. potassium iodide and 5.4 gms. potassium iodate in 1,000 cc. of water and adding a few drops of concentrated sodium hydroxide solution to prevent the liberation of free iodine from the solution. Then approximately 16 cc. of five normal sulphuric acid are added quickly, the flask being shaken while the acid is being added to prevent the formation of hydriodic acid while the cupric oxide is being dissolved. Then 20 cc. of a saturated solution of potassium oxalate are added and the mixture titrated with a standard one-tenth normal solution of sodium thiosulphate. In titrating, the preparation is brought near the end point at which time a few cubic centimeters of soluble starch solution are added and then the titration completed, the addition of the starch causing the end point to become more distinct. The number of cc. of the sodium thiosulphate solution required to oxidize the excess iodine is then subtracted from the amount required when 50 cc. of distilled water is used in place of the hydrolyzed sugar solution. The net titration value thus obtained is the amount of sodium thiosulphate which is necessary to oxidize the iodine which has been used to oxidize the cuprous oxide precipitated through reduction of cupric hydroxide by the reducing sugars in the hydrolyzed leaf extract. Multiplying this net titration value by 6.36 gives the number of milligrams of metallic copper reduced by the sugar. Quisumbing and Thomas tables (41) were used for calculating dextrose from the copper values obtained.

The chemistry of the above procedure is as follows: The hydrolyzed leaf extract contains more or less hexose sugar which contains a potentially active "ose" or sugar group () in its structural formula. Fehling's solution is essentially a solution of cupric hydroxide held in alkaline solution by the presence of the Rochelle salts. When heated with a solution of reducing sugar oxygen is withdrawn by the "ose" group from the cupric hydroxide which is thus transformed into cuprous oxide which is insoluble in the solution and separates from it as a reddish precipitate.

The sulphuric acid is added as directed above to release free iodine from the iodide-iodate solution which then oxodizes the cuprous oxide to cupric copper. The potassium oxalate is added to unite with all the divalent copper and the sodium thiosulphate oxidizes the excess iodine.

Standardization of the sodium thiosulphate solution is necessary. and was done as follows: A one-tenth normal solution of potassium biiodate was prepared. Exactly 50 cc. of this solution were pipetted into a 300 cc. Erlenmeyer flask and to this was added a solution of three grams of potassium iodide dissolved in 25 cc. of water. Then 10 cc. of five normal sulphuric acid were added and the mixture titrated with one-tenth normal sodium thiosulphate solution. The number of cc. of biiodate used should be equal to the number of cc. of thiosulphate used.

If the number of cubic centimeters of the thiosulphate solution divided by the number of cubic centimeters of biiodate does not equal one the dividend is a factor by which all tritration values must be multiplied as a corrective factor.

Analysis Of Spurs For Carbohydrate Content

It was considered advisable to determine the effect of the various fruit leaf ratios on the carbohydrate content of the spurs since it is now generally conceded that individual spur composition is closely associated with individual spur behavior according to Murneek (32).

Spur samples were collected from each branch on July 1. Representative non-bearing spurs were collected with about 75 spurs constituting a sample in the case of York and about 50 in the case of Livland. The samples were taken in the early forenoon following a day of bright sunshine as recommended by Bradford (3) and Hooker (17). The spurs were taken to the laboratory at once and their green weight determined. The material was then dried to constant weight at 75 degrees C. in a ventilated electric oven and prepared for chemical analysis by grinding in an iron mortar to a powder which would pass through a forty mesh sieve. Dry weights were taken of the unground spurs.

A similar collection of spurs was made August 13 at which time difficulty was experienced in making the collection due to the branches showing severe defoliation on account of dry weather and red spider attack.

The carbohydrate content of the spurs was determined by the same method used in analysis of the leaf tissue.

PRESENTATION OF DATA

Tables I to V show the total dry weight, acid hydrolyzable carbohydrate and water contents of York and Livland apple leaves on June 6, 7, 19, 20, 27 and 28 and July 3, 4, 22 and 23. These tables also show the daily variation in the total dry weight, acid hydrolyzable carbohydrate and water content of the leaves on each of the above dates.

Table VI shows the average dry weight, acid hydrolyzable carbohydrate and water content of the leaves throughout the experiment.

Table VII shows the average daily changes in total dry weight, acid hydrolyzable carbohydrate and water contents of the leaves throughout the experiment.

Table VIII exhibits a comparison of the rate of gain in total dry weight by the leaves on the first day of each collection of samples with the gain on the second day.

Table IX exhibits a comparison of the water content of the leaves on the first day of each collection of samples compared with that of the second day.

Table X gives the mean relative humidity, mean daily temperature, soil moisture content and the mean daily gain of total dry matter per square meter of leaf area.

Table XI compares the water and total acid hydrolyzable carbohydrate contents of spurs on July 1, and August 13.

Figure A presents graphically the variation in water content of York and Livland apple leaves throughout the experiment.

Figure B shows the variation of the total dry weight content of the leaves during the experiment.

Figure C shows the variation of the acid hydrolyzable carbohydrate content of the leaves during the experiment.

Figure D presents the data for mean relative humidity, mean daily temperature, soil moisture content and the average daily gain in total dry weight per square meter of leaf area.

Table I. Daily variation in total dry weight, total acid hydrolyzable carbohydrate and water contents of York and Livland apple leaves at Manhattan, Kansas June 7 and 8, 1935.

Variety	Leaves per fruit	Time	Dry weight per square meter leaf area - gms. Total	Change	Total gain*	Acid hydrolyzable carbohydrates per square meter leaf area gms. Total	Change	Total gain*	Water per cent of total weight Total	Change
York	10	a. m.	63.22			13.06			69.48	
		p. m.	68.44	+5.22	6.88	15.47	+2.41	6.25	57.98	-11.50
		a. m.	66.78	-1.66		11.63	-3.84		65.10	
		p. m.	69.12	+2.34	4.00	12.69	+1.06	4.90	50.47	-14.63
York	50	a. m.	57.60			10.54			66.87	
		p. m.	62.24	+4.64	5.66	12.53	+1.99	2.39	58.55	-8.32
		a. m.	61.22	-1.02		12.13	- .40		63.08	
		p. m.	63.58	+2.36	3.38	13.45	+1.32	1.72	62.50	- .58
York	fruit-less	a. m.	61.98			11.50			66.21	
		p. m.	68.48	+6.50	8.62	15.25	+3.75	6.21	57.31	-8.90
		a. m.	66.36	-2.12		12.79	-2.46		62.47	
		p. m.	68.18	+1.82	3.94	14.61	+1.82	4.28	59.00	-3.47
Livland	10	a. m.	59.18			13.05			68.93	
		p. m.	65.32	+6.14	9.14	15.21	+2.16	3.36	61.88	-7.05
		a. m.	62.32	-3.00		14.01	-1.20		69.59	
		p. m.	63.30	+ .98	3.98	13.90	- .11	1.09	62.25	-7.34
Livland	50	a. m.	58.46			12.82			68.14	
		p. m.	60.86	+2.40	3.72	13.08	+ .26	1.02	62.37	-5.77
		a. m.	59.54	-1.32		12.32	- .76		68.75	
		p. m.	62.00	+2.46	3.78	13.23	+ .91	1.67	62.34	-6.41
Livland	fruit-less	a. m.	50.92			10.19			69.24	
		p. m.	56.64	+5.72	8.66	12.66	+2.47	4.86	63.44	-5.80
		a. m.	53.70	-2.94		10.27	-2.39		69.05	
		p. m.	55.60	+1.90	4.84	12.21	+1.94	4.33	63.40	-5.65

* Total gain is the sum of the gain by day and the loss by night.

Table II. Daily variations in dry weight, total acid hydrolyzable carbohydrate and water content of York and Livland leaves at Manhattan, Kansas June 19 and 20, 1935.

Variety	Leaves per fruit	Time	Dry weight per square meter leaf area - gms. Total	Change	Total gain*	Acid hydrolyzable carbohydrates per square meter leaf area gms. Total	Change	Total gain*	Water per cent of total weight Total	Change
York	10	a. m.	66.12			10.47			66.76	
		p. m.	69.28	+3.16	4.64	13.03	+2.56	+4.31	57.02	-9.74
		a. m.	67.80	-1.48		11.28	-1.75		60.68	
		p. m.	71.78	+3.98	5.46	11.34	+ .06	+1.81	58.62	-2.06
York	50	a. m.	62.10			11.48			62.27	
		p. m.	64.70	+2.60	3.54	11.45	- .03	.28	57.05	-5.22
		a. m.	63.76	- .94		11.14	- .31		60.40	
		p. m.	65.58	+1.82	2.76	9.26	-1.88	-1.57	58.17	-2.23
York	fruit-less	a. m.	62.24			11.37			63.64	
		p. m.	64.68	+2.44	3.74	11.78	+ .41	+ .85	56.61	-7.03
		a. m.	63.38	-1.30		11.34	- .44		60.19	
		p. m.	67.04	+3.66	4.96	9.85	-1.49	-1.05	57.27	-3.92
Livland	10	a. m.	67.94			11.71			69.21	
		p. m.	71.78	+3.84	10.28	12.06	+ .35	+ .53	59.81	-9.40
		a. m.	65.34	-6.44		11.88	- .18		62.40	
		p. m.	71.34	+6.00	12.44	11.91	+ .03	.21	61.53	- .87
Livland	50	a. m.	69.42			11.54			70.26	
		p. m.	72.70	+3.28	5.52	12.46	+ .92	+1.48	60.87	-9.39
		a. m.	70.46	-2.24		11.90	- .56		63.26	
		p. m.	72.70	+1.76	4.00	11.24	- .66	- .10	61.83	-1.43
Livland	fruit-less	a. m.	72.72			12.13			68.61	
		p. m.	76.56	+3.84	8.56	12.91	+ .78	+1.71	70.04	+1.43
		a. m.	71.86	-4.72		11.98	- .93		64.49	
		p. m.	74.80	+2.94	7.66	12.92	+ .94	+1.87	62.04	-2.45

* Total gain is the sum of the gain by day plus the loss by night.

Table III. Daily variation in total dry weight, total acid hydrolyzable carbohydrate and water contents of York and Livland leaves at Manhattan, Kansas June 27 and 28, 1935.

Variety	Leaves per fruit	Time	Dry weight per square meter leaf area - gms. Total	Change	Total gain*	Acid hydrolyzable carbohydrates per square meter leaf area gms. Total	Change	Total gain*	Water per cent of total weight Total	Change
York	10	a. m.	70.36			11.19			66.16	
		p. m.	75.62	+5.26	9.54	13.11	+1.92	3.62	56.56	-9.60
		a. m.	71.34	-4.28		11.41	-1.70		**	
		p. m.	76.06	+4.72	9.00	12.62	+1.48	3.18	57.15	
York	50	a. m.	57.16			9.05			66.62	
		p. m.	63.60	+6.46	9.86	10.24	+1.19	1.63	57.62	-9.00
		a. m.	60.20	-3.40		9.80	- .44		**	
		p. m.	64.50	+4.30	7.70	10.76	+ .96	1.40	57.26	
York	fruit-less	a. m.	67.64			11.45			64.96	
		p. m.	74.96	+7.32	10.42	12.43	+ .98	1.07	54.47	-10.49
		a. m.	71.86	-3.10		12.34	.09		**	
		p. m.	76.98	+5.12	8.22	13.92	+1.58	1.67	58.09	
Livland	10	a. m.	62.62			11.69			69.03	
		p. m.	69.03	+6.44	12.40	11.34	- .35	- .15	60.35	-8.68
		a. m.	63.10	-5.96		11.14	- .20		**	
		p. m.	67.34	+4.24	10.20	12.57	+1.43	1.63	60.05	
Livland	50	a. m.	69.22			11.97			68.06	
		p. m.	75.38	+6.16	11.34	13.38	+1.41	2.47	58.30	-9.76
		a. m.	70.20	-5.18		12.31	-1.07		**	
		p. m.	76.52	+6.32	11.50	14.11	+1.80	2.87	58.09	
Livland	fruit-less	a. m.	62.64			10.74			72.70	
		p. m.	69.98	+7.34	13.92	11.22	+ .48	.81	65.66	-7.04
		a. m.	63.40	-6.58		10.89	- .33		**	
		p. m.	69.56	+6.16	12.74	12.65	+1.76	2.09	61.23	

* Total gain is the sum of the gain by day and the loss by night.
** Leaves wet - no green weight taken.

Table IV. Daily variation in total dry weight and total acid hydrolyzable carbohydrate and water contents of York and Livland leaves at Manhattan, Kansas July 3 and 4, 1935.

Variety	Leaves per fruit	Time	Dry weight per square meter leaf area - gms.			Acid hydrolyzable carbohydrates per square meter leaf area gms.			Water per cent of total weight	
			Total	Change	Total gain*	Total	Change	Total gain*	Total	Change
York	10	a. m.	60.10			10.00			61.96	
		p. m.	65.30	+5.20	6.82	10.43	+ .43	+ .45	56.53	-5.43
		a. m.	63.68	-1.62		10.41	- .02		59.44	
		p. m.	66.70	+3.02	4.64	9.98	- .43	- .41	57.47	-1.97
York	50	a. m.	73.12			12.62			59.14	
		p. m.	77.90	+4.78	8.10	11.85	- .77	+ .23	54.59	-4.55
		a. m.	74.58	-3.32		12.85	+1.00		58.58	
		p. m.	79.06	+4.48	7.80	11.19	-1.76	- .76	54.59	-3.99
York	fruitless	a. m.	71.42			11.85			58.57	
		p. m.	76.06	+4.64	6.90	12.03	+ .18	+ .58	54.08	-4.49
		a. m.	73.80	-2.26		11.63	- .40		58.19	
		p. m.	76.68	+2.88	4.54	12.09	+ .46	+ .86	54.94	-3.25
Livland	10	a. m.	67.52			10.86			64.10	
		p. m.	72.64	+5.12	8.94	11.55	+ .69	+1.10	60.31	-3.79
		a. m.	68.82	-3.82		11.14	- .41		63.15	
		p. m.	71.98	+3.16	6.98	10.58	- .56	- .15	60.11	-3.04
Livland	50	a. m.	80.28			13.76			62.89	
		p. m.	85.50	+5.22	9.38	15.25	+1.49	+1.85	57.43	-5.46
		a. m.	81.34	-4.16		14.89	- .36		61.17	
		p. m.	85.52	+4.18	8.34	13.51	-1.38	-1.02	58.46	-2.71
Livland	fruitless	a. m.	67.96			11.27			65.48	
		p. m.	72.58	+4.62	8.12	11.05	- .22	+ .30	59.50	-5.98
		a. m.	69.08	-3.50		11.57	+ .52		62.79	
		p. m.	73.48	+4.40	7.90	10.70	- .87	- .25	60.03	-2.76

* Total gain is the sum of the gain by day and the loss by night.

Table V. Daily variation in total dry weight, total acid hydrolyzable carbohydrate and water contents of York and Livland leaves at Manhattan, Kansas July 22 and 23, 1935.

Variety	Leaves per fruit	Time	Dry weight per square meter leaf area - gms. Total	Change	Total gain*	Acid hydrolyzable carbohydrates per square meter leaf area gms. Total	Change	Total gain*	Water per cent of total weight Total	Change
York	10	a. m.	59.88			10.81			57.17	
		p. m.	61.08	+1.20		10.67	- .14		54.93	-2.24
		a. m.	62.32	+1.24		10.77	+ .10		57.17	
		p. m.	64.22	+1.90		9.90	- .87		56.77	- .40
York	50	a. m.	71.52			12.47			55.59	
		p. m.	70.48	-1.04		11.24	-1.23		53.89	-1.70
		a. m.	73.08	+2.60		12.45	+1.21		55.56	
		p. m.	74.64	+1.56		12.34	- .11		52.80	-2.76
York	fruit-less	a. m.	66.64			11.96			54.71	
		p. m.	67.58	+ .94		11.89	- .07		52.00	-2.71
		a. m.	68.88	+1.30		11.50	- .39		54.52	
		p. m.	72.22	+3.34		11.42	- .08		51.36	-3.16
Livland	fruit-less	a. m.	51.64			8.21			64.53	
		p. m.	51.12	- .52		8.64	+ .43		63.05	-1.48
		a. m.	52.40	+1.28		7.90	- .74		64.32	
		p. m.	53.58	+1.18		8.73	+ .83		62.10	-2.22
Livland	50	a. m.		Fruit harvested and no samples taken						
		p. m.								
		a. m.								
		p. m.								
Livland	10	a. m.		Fruit harvested - therefore no samples taken						
		p. m.								
		a. m.								
		p. m.								

* Total gain not possible because of variation in data.

Table VI. Average dry weight, acid hydrolyzable carbohydrate and water contents of York and Livland leaves at Manhattan, Kansas, 1935.

Variety	Time	York	York	York	Livland	Livland	Livland
Leaves per fruit		10	50	fruitless	10	50	fruitless
Total dry weight per square meter leaf area gms.	a. m.	65.16	65.43	67.42	64.60	69.89	61.63
	p. m.	68.76	68.92	70.98	69.09	73.83	65.39
	average	66.96	67.17	69.20	66.84	71.86	63.51
D-glucose per square meter leaf area gms.	a. m.	11.10	11.45	11.77	11.93	12.69	10.51
	p. m.	11.92	11.43	12.52	12.39	13.38	11.36
	average	11.51	11.44	12.14	12.16	12.78	10.93
Water content per cent of total weight	a. m.	62.66	58.70	60.38	66.63	66.07	66.80
	p. m.	56.35	60.91	55.51	60.78	59.96	63.04
	average	59.33	58.70	57.82	63.51	62.81	64.82

Table VII. Average daily changes in total dry weight, acid hydrolyzable carbohydrate and water contents of York and Livland leaves at Manhattan, Kansas, 1935.

Variety	Time	York	York	York	Livland	Livland	Livland
Leaves per fruit		10	50	fruitless	10	50	fruitless
Total dry weight per square meter leaf area gms.	a. m. to p. m.	4.11	3.93	4.30	4.49	3.97	4.61
	p. m. to a. m.	-2.26	-2.17	-2.19	-4.80	-3.20	-4.43
	a. m. to a. m.	6.37	6.10	6.49	9.29	7.17	9.04
D-glucose per square meter leaf area gms.	a. m. to p. m.	1.18	.14	.96	.47	.59	.91
	p. m. to a. m.	-1.83	- .01	- .85	- .48	- .68	-1.10
	a. m. to a. m.	3.01	.15	1.81	.95	1.27	2.01
Water content per cent of total weight	a. m. to p. m.	-6.31	-4.21	-4.87	-5.85	-6.11	-3.76

Table VIII. Showing decreased gain in total dry weight on second day of each two day collection of samples.

Variety / Leaves per fruit	York 10	York 50	York fruitless	Livland 10	Livland 50	Livland fruitless	Total	Average daily gain
June 6	6.88	5.66	8.62	9.14	3.72	8.66	42.68	7.11
June 7	4.00	3.38	3.94	3.98	3.78	4.84	27.92	4.65
June 19	4.64	3.54	3.74	10.28	5.52	8.56	36.28	6.05
June 20	5.46	2.76	4.96	12.44	4.00	7.66	37.28	6.21
June 27	9.54	9.86	10.42	12.40	11.34	13.92	67.48	11.25
June 28	9.00	7.70	8.22	10.20	11.50	12.74	59.36	7.89
July 3	6.82	8.10	6.90	8.94	9.38	8.12	48.26	8.04
July 4	4.64	7.80	4.54	6.98	8.34	7.90	40.20	6.70
Total	27.88 23.10	27.12 21.64	29.68 21.66	40.76 31.60	29.96 27.62	39.26 33.14		
Average	6.97 5.77	6.78 5.41	7.42 5.41	10.14 7.90	7.39 6.90	9.81 7.90		

Table IX. Showing decreased water content of leaves on second day of each two day collection. All data given in per cent of green weight.

Variety	York		York		York		Livland		Livland		Livland			
Leaves per fruit:	10		50		fruitless		10		50		fruitless		Total	Average
June 6	63.73		62.71		61.76		65.40		65.25		66.34		385.14	64.19
June 7		57.93		62.79		60.73		65.92		65.54		66.22	379.13	63.19
June 19	61.89		59.66		60.12		64.51		65.56		69.32		381.04	63.51
June 20		59.65		59.28		58.73		61.96		62.54		63.26	365.42	60.90
June 27	61.36		62.11		59.71		64.69		63.18		69.18		380.23	63.67
June 28		*		*		*		*		*		*		
July 3	59.24		56.86		56.32		62.20		60.16		62.49		357.27	59.65
July 4		58.45		56.58		56.56		61.63		59.81		61.40	354.43	59.07
Totals	246.22	176.03	241.34	178.45	237.91	176.02	256.80	189.51	254.15	187.89	267.33	190.88		
Average	61.55	58.67	60.34	59.48	59.48	58.67	64.20	63.16	63.54	62.63	66.83	63.62		

* Leaves were wet and no green weights were taken.

Table X. Showing variation in mean relative humidity, mean daily temperature, soil moisture content and mean daily gain of York and Livland apple leaves at Manhattan, Kansas June 6 to July 23, 1935.

	June 6	June 7	June 19	June 20	June 27	June 28	July 3	July 4	July 22	July 23
Mean relative humidity	68 %	69.5	61.5	70.5	69	81	64.5	63.5	47	51
Mean daily temperature	54 °	58.25	66.25	71.75	72.25	72.0	83.5	83.5	90.5	90.5
Soil moisture			18.6	18.6	21.3	21.3	19.3	19.3	15.2	15.2
Mean daily gain total dry weight per square meter leaf area	7.11	4.65	6.05	6.21	11.25	9.89	8.04	6.70	*	*

* Mean daily gain of total dry weight per square meter of leaf area not calculated because of extreme fluctuation.

Table XI. Acid hydrolyzable carbohydrate contents (expressed as d-glucose) and water content of spurs of York and Livland apples at Manhattan, Kansas, 1935.

Variety	York		York		York		Livland		Liv-land		Livland	
Leaves per fruit	10		50		fruitless		10		50		fruitless	
	D-glucose per cent dry weight	Water per cent green weight	D-glucose per cent dry weight	Water per cent green weight	D-glucose per cent dry weight	Water per cent green weight	D-glucose per cent dry weight	Water per cent green weight	D-glucose per cent dry weight	Water per cent green weight	D-glucose per cent dry weight	Water per cent green weight
July 1	17.70	51.99	18.86	50.24	19.28	52.06	18.13	54.19	17.84	53.31	18.18	54.74
August 13	16.64	49.73	16.36	45.35	16.24	47.92	17.84	51.87	18.60	54.02	17.26	52.27
Change	-1.06	-2.26	-2.50	-4.89	-3.04	-4.14	- .29	-2.32	+ .76	+ .81	- .92	-2.47

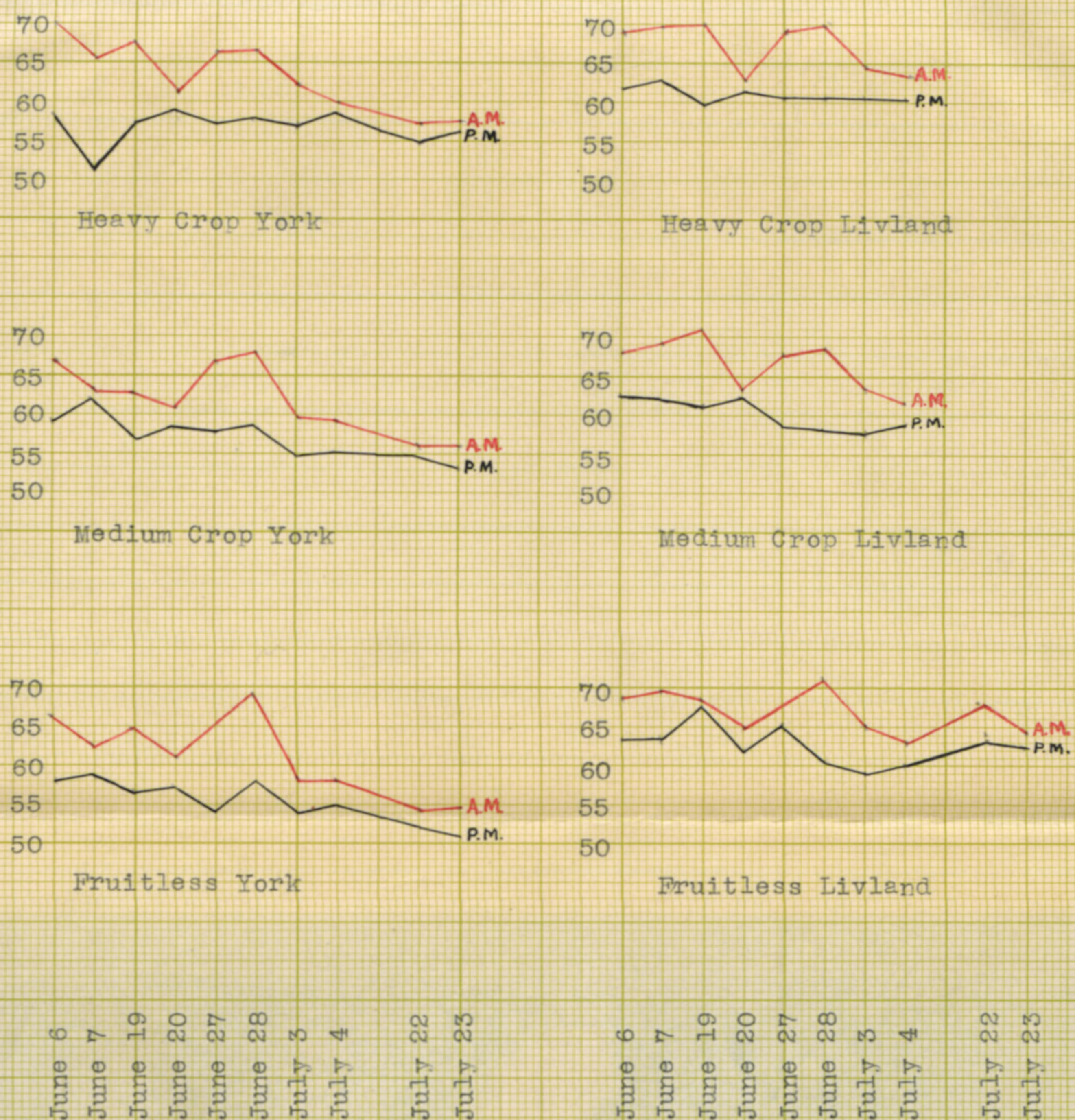


Figure A. Seasonal variation of the moisture content of York and Livland apple leaves in percent of green weight.

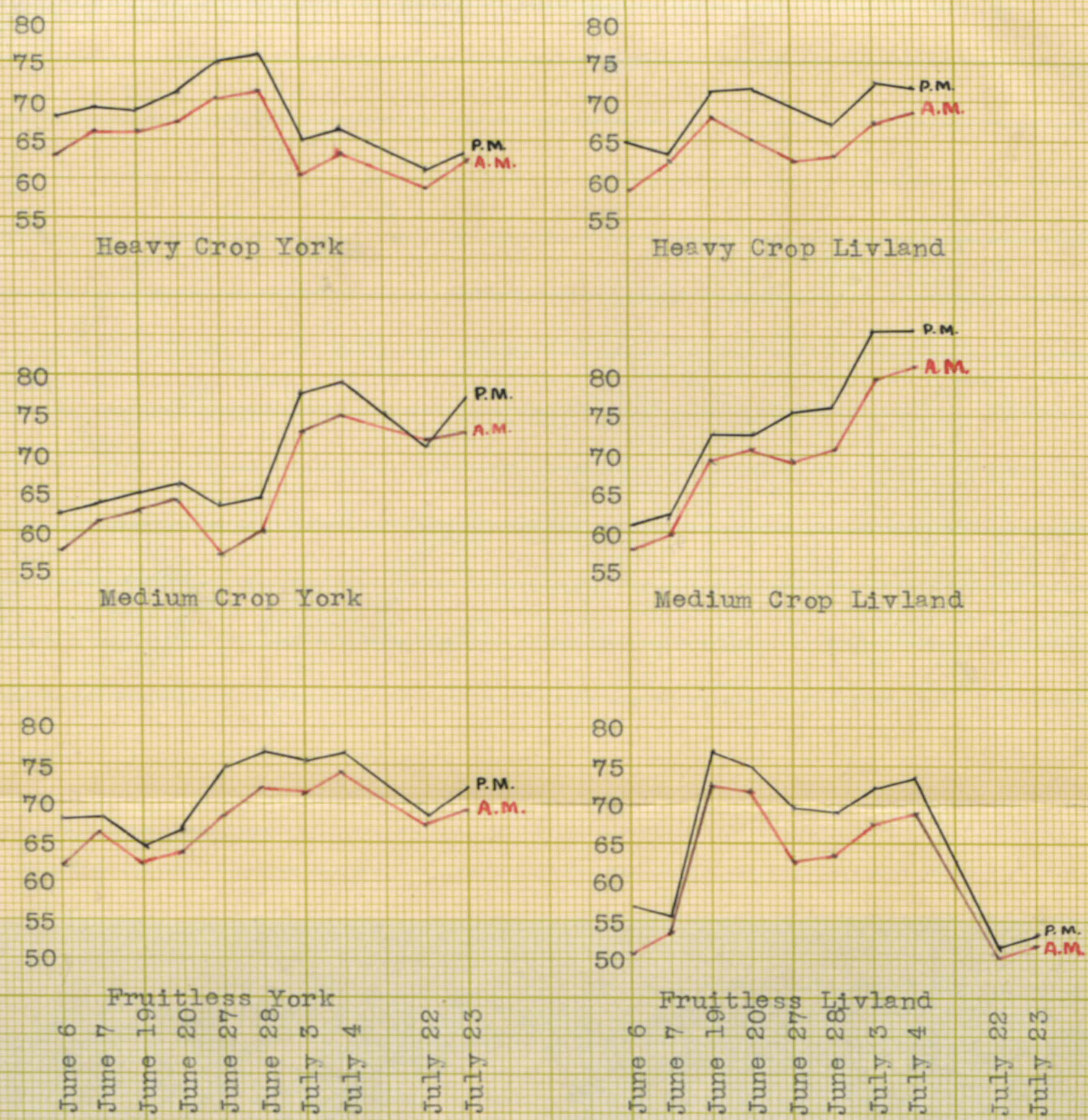


Figure B. Seasonal variation of total dry weight of York and Livland apple leaves in grams per square meter of leaf area.

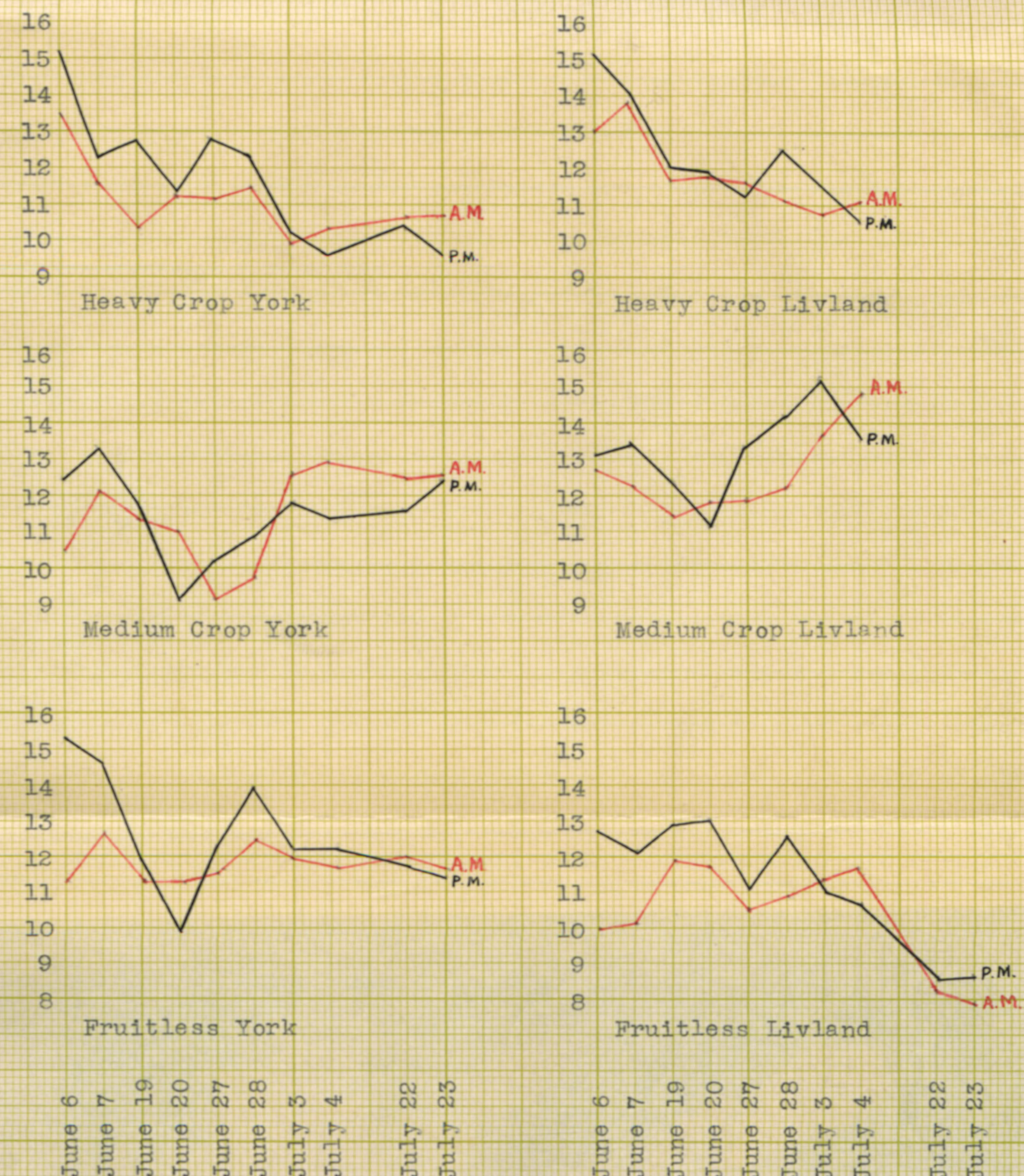


Figure C. Seasonal variation of acid hydrolyzable carbohydrate content (expressed as grams of d-glucose per square meter of leaf area) of York and Livland apple leaves.

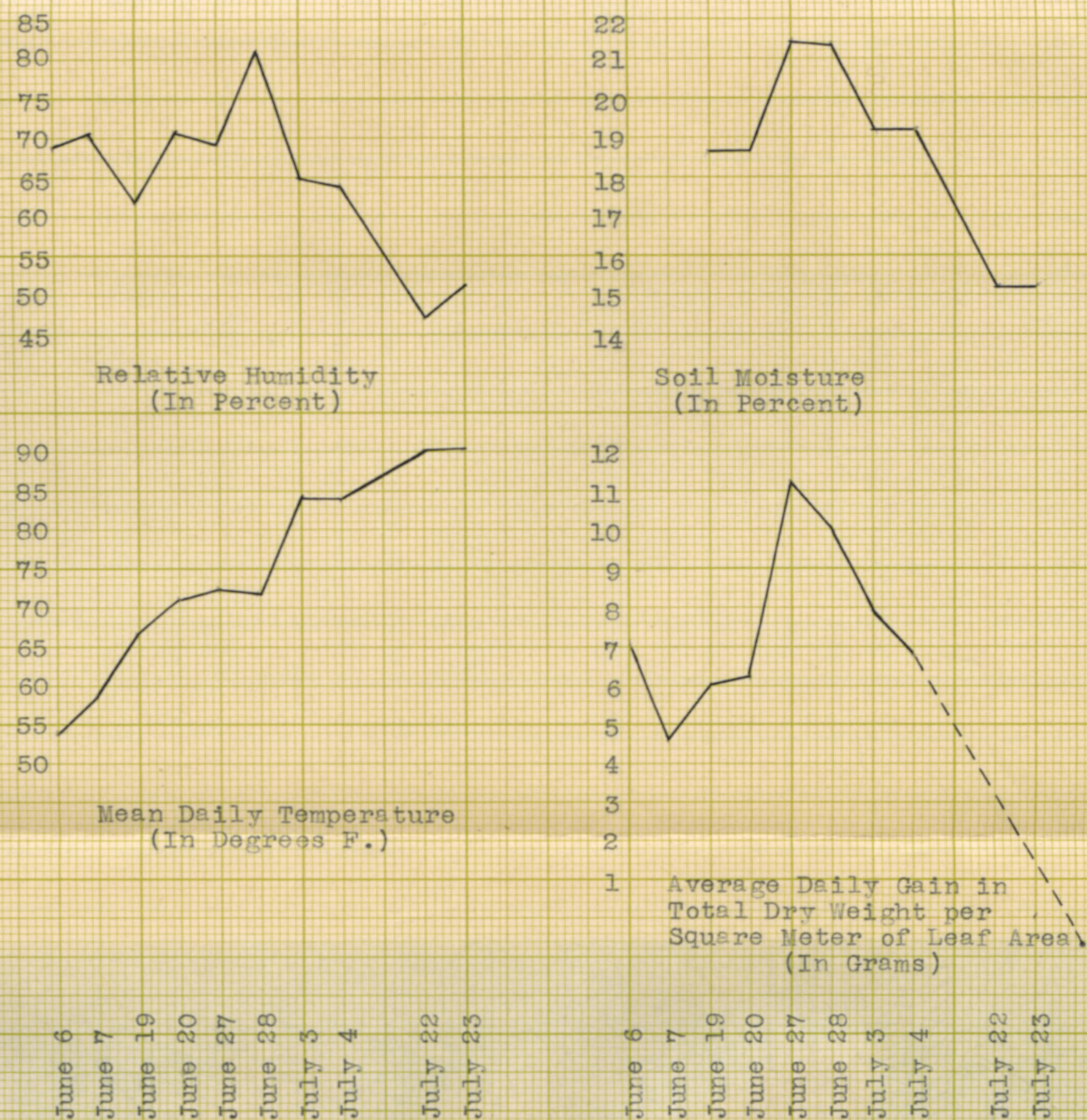


Figure D. Seasonal variation in air temperature, relative humidity, soil moisture, and average daily gain in total dry weight of York and Livland apple leaves at Manhattan, Kansas, June 6 to July 23, 1935.

Moisture Content

York leaves showed a constantly lower water content than Livland leaves (tables I-VII). The fruitless York leaves had the lowest water content and the heavy crop York leaves the highest. In the case of the Livland leaves the fruitless condition showed the highest water content and the heavy crop the lowest water content. The greatest average daily decrease in water content, expressed in percentage of green weight, was exhibited by the heavy crop York and the lowest by Livland fruitless leaves. The water loss of moderately fruitful York was lower than that of fruitless York while for Livland the greatest loss was shown in the moderately fruitful condition (table VII).

With regard to variation of water content of the leaves throughout the season it will be observed from the graphs (figure A) that all of the York samples showed a noticeable decrease in water content from June 6 to July 23 whereas the Livland leaves, while showing a decrease, did not decrease nearly as rapidly and in the case of fruitless Livland showed even a slight increase on July

22 and 23.

Total Dry Weight

The average dry weight per square meter of leaf area varied widely during the season (tables I-VI). The greatest weight was shown by medium crop Livland while the lowest was shown by fruitless Livland. The York leaves did not differ much in their average dry weight, it being 66.96 grams per square meter for heavy crop York and 69.20 grams for fruitless York.

The greatest average daily gain in dry weight was shown by the Livland leaves, York being lower in all cases (table VII). The variation in the average daily gain of total dry weight was greater for Livland leaves than for York leaves.

The weights per square meter of leaf area varied throughout the season for each of the adjusted ratios (figure B). Heavy crop York showed a gradual increase from June 6 to June 28 and thereafter a decided general decrease. Medium crop York and fruitless York showed increases to July 4 and smaller decreases followed by slight increases July 23. Heavy crop Livland showed marked increase up to June 19 and varied only slightly thereafter. Medium crop Livland showed a constant and rapid increase in weight up to July 4. Fruitless Livland started with a much lower weight per square meter of leaf area, increased rapidly until June 20 then decreased noticeably and made a partial recovery until July 4 after which the weight decreased to approximately the same figure as at the start of the experiment.

Total Acid Hydrolyzable Carbohydrate Content

This characteristic showed greater variation and less consistent results than either moisture or dry weight (tables I-VI). In general, Livland leaves showed a higher d-glucose content than the York leaves but the mean value for the fruitless Livland leaves was the lowest of all. Medium crop Livland showed the highest d-glucose content with heavy crop Livland the second highest. Fruitless York showed only slightly lower d-glucose value than heavy crop Livland to be followed by heavy crop York and medium crop York in that order.

The mean daily change in d-glucose content showed extreme fluctuation (table VII). With reference to this consideration heavy crop York showed by far the greatest gain followed by fruitless Livland, fruitless York, medium crop Livland, heavy crop Livland and medium crop York. The latter showed such wide fluctuation from day to day that its mean daily gain of d-glucose amounted to only .15 gram per square meter of leaf area.

In seasonal variation of d-glucose content heavy crop York showed a rather constant gradual decrease from June 6 to July 23 (figure C). Medium crop York showed a decrease from June 6 to June 20, an increase to July 4 and then a gradual leveling off. Fruitless York showed a similar decrease from June 6 to June 20, an increase from June 20 to July 3 followed by a slow decrease. Heavy crop Livland showed a gradual decrease from June 6 to July 4, similar to heavy crop York. Medium crop Livland showed a slight decrease from June 6 to June 20 followed by a sharp increase to July 4. Fruitless Livland showed a fairly constant d-glucose content until July 4 after which a sharp decrease occurred.

It was observed (table VIII) that the gain in total dry weight per square meter of leaf area on the second day of each two day collection of samples was less than the gain on the first day of each collection. In only one period, that of June 19 and 20, was the average daily gain in total dry weight per square meter of leaf area of all samples greater on the second day than on the first and in that case the gain was only slightly greater on the second day. In all other cases the gain on the second day was appreciably smaller than on the first day.

Moisture content of the leaves was likewise less on the second day than on the first day of each collection of samples (table IX).

The mean daily temperature showed a gradual increase from June 6 to July 23 (table X). At the same time the relative humidity showed a decrease on June 19, a great increase on June 28 and then decreases on July 3 and 4 and extreme decreases on June 22 and 23. Soil moisture figures show an increase to June 27 and 28 then a sharp gradual decrease to July 22 and 23. The average daily gain of total dry matter per square meter of leaf area for all leaves shows smaller gains on June 19 and 20 than on June 6 and 7, great increases in gain on June 27 and 28, somewhat smaller gains on July 3 and 4. On July 22 and 23, the photosynthetic activity was so irregular as to approximate a loss rather than a gain and could not be calculated (tables V and X). Conditions were so extreme that the leaves showed actual losses in dry weight by day and gains by night.

Analysis Of Spur Tissue

The samples of spur tissue were analyzed for acid hydrolyzable carbohydrates. The samples collected on July 1 showed higher water and higher acid hydrolyzable carbohydrate contents than did the samples collected on August 13 in all but one instance (table XI). The medium crop Livland spurs showed a slight increase in water content and in acid hydrolyzable carbohydrates on August 13 as compared with samples collected on July 1. York spurs showed lower water contents than Livland spurs at both periods of collection. York spurs had higher acid hydrolyzable carbohydrate contents than Livland spurs on July 1 but showed greater decreases in this constituent than the Livland spurs and a lower acid hydrolyzable carbohydrate content on August 13 than the Livland spurs. The fruitless condition in both Livland and York showed the greatest decrease in acid hydrolyzable carbohydrates.

DISCUSSION

The data of a chemical investigation with leaves of York and Livland apples, under three widely varying leaf-fruit ratios, extending through the period from June 6 to July 23 indicate that these two varieties differ in their photosynthetic response to environmental conditions and that within each variety we find varying response to varying physiological conditions which apparently arise from different leaf-fruit ratios.

In many respects the leaves and the fruits must be considered as the two most important parts of the bearing apple spur. Though persisting only during the growing season these organs nevertheless constitute the bulk of the spur system during the period of its greatest activity. Their relation to one another beyond reasonable doubt determines the future production of fruit by the plant. The developing fruit being primarily carbohydrate in content, it exerts a greater toll on the leaves which are nourishing it as it develops and the correlation between carbohydrate formation and carbohydrate consumption may be expected to be reflected in future production of fruit.

The increase in dry weight of the leaves shows some interesting features. The heavy crop York leaves showed a gradual increase in dry weight until June 28 after which a sharp decrease occurred. At this stage the young fruits were developing rapidly and it is evident that they had grown to such size that the leaves were no longer able to furnish sufficient carbohydrate material to meet the requirements of the developing fruits and as a result the leaf reserves were being utilized for fruit development. Medium crop York leaves showed a gradual increase in total dry weight to July 4 reaching a maximum weight 3.44 grams greater per square meter of leaf area than the heavy crop York leaves in spite of the fact that the medium crop York leaves had the lower initial weight per unit of leaf area. This is indicative of the fact that decreased drain on the medium crop York leaves, due to the greater number of leaves per fruit as compared with heavy crop York leaves, allowed the fruits on the medium crop York branch to grow for a longer time and reach a greater size before their demands became great enough to cause a decrease in the weight per unit of leaf area. Moreover, additional evidence of the decreased need for nourishment by the medium crop York fruits is found in the fact that the weight per square meter of leaf area of medium crop York leaves did not decrease nearly as much as did the weight of the leaves of heavy crop York. In the case of fruitless York the leaves showed a rather gradual rate of increase until June 28 after which a constant weight was maintained until after July 4 when a slight decrease occurred. The fact that the maximum weight per unit of leaf area was less than that of medium crop York indicates that the presence of developing fruit may be conducive to greater photosynthetic activity.

In considering the seasonal changes in the dry weight of Livland leaves we find a somewhat similar condition. Heavy crop Livland leaves showed a sharp increase from June 6 to June 19 after which the weight remained fairly constant until July 4 after which time the fruit was harvested. The fact that a decrease in weight did not appear as in the case of heavy crop York may be explained by the relatively greater average leaf area of Livland as compared with York. The medium crop Livland leaves showed a rapid constant increase in weight per unit of leaf area from June 6 to July 4 and reached a maximum weight per square meter of leaf area 12.88 grams greater than that of heavy crop Livland. Fruitless Livland leaves had a lower initial weight per unit of leaf area, made an extremely rapid increase to June 19, followed by a slight tendency to decrease until July 4 after which the weight decreased to less than the initial weight. Here again the lack of fruit appeared to inhibit photosynthetic activity in that the maximum weight per unit of leaf area attained was only slightly greater than that of the heavy crop Livland leaves.

With regard to average daily changes in total dry weight it is observed that both medium crop York and medium crop Livland showed the lowest gains from morning to afternoon and the greatest losses for each variety from afternoon to morning. Both fruitless York and fruitless Livland showed greater gains from morning to afternoon than heavy crop York and heavy crop Livland but heavy crop Livland and fruitless York leaves showed a slightly greater loss from afternoon to morning than fruitless Livland and fruitless York. The average daily apparent photosynthetic rate (increase by day + loss by night) shows fruitless York having a slightly greater figure than heavy crop York which is slightly higher than medium crop York. The heavy crop Livland leaves show a slightly higher photosynthetic rate than fruitless Livland but both of these show distinct advantages over medium crop Livland.

The data on acid hydrolyzable carbohydrates expressed as d-glucose showed greater fluctuation and variability than the dry weight.

Heavy crop York leaves had a higher initial d-glucose content per unit of leaf area than either medium crop York or fruitless York and showed a rather constantly decreasing tendency from June 6 to July 23 indicating that as the fruits developed in size the total carbohydrate content was decreased to meet the increased demand for nutrients. Medium crop York showed a sharp decrease from June 7 to June 27 followed by a sharp increase to July 3 and a constant figure to July 23. Fruitless York leaves showed a similar sharp decrease from June 6 to June 20 followed by a rapid rise to June 27 and then a slow gradual decrease to July 23.

The heavy crop Livland leaves showed a rather constant and fairly rapid decrease in d-glucose content from June 6 to July 4, the curve being similar to the heavy crop York curve. This again indicates that under a high degree of fruitfulness the carbohydrate content of leaves is decreased to meet the demands of the rapidly developing fruits. The medium crop Livland leaves exhibited a slow decrease from June 6 to June 20 after which a rapid increase to July 4 occurred, being similar to the curve for medium crop York from June 6 to July 4. The fruitless Livland leaves showed fairly constant d-glucose content from June 6 to July 4 deviating in both directions from the average but after July 4 a sharp decrease occurred sending the figure for total carbohydrates to the lowest exhibited by any set of leaves. Here again the inhibiting effect of a fruitless condition on photosynthetic activity appears to be revealed in that the acid hydrolyzable carbohydrate content does not reach the extremes shown by either the heavy crop or the medium crop condition. The sudden decrease in d-glucose content may be explained by the fact that at Manhattan Livland ripens its fruit by about July 10 and evidently thereafter the photosynthetic activity of the leaves decreases greatly.

Fruitless York leaves showed a greater total acid hydrolyzable carbohydrate content than either heavy crop York or medium crop York whereas fruitless Livland leaves were lower than not only heavy crop Livland and medium crop Livland but were also lower than any of the York leaves. Both medium crop Livland and heavy crop Livland were higher in acid hydrolyzable carbohydrates than any of the York leaves with medium crop Livland being noticeably higher than heavy crop Livland.

With reference to average daily fluctuations in the total acid hydrolyzable carbohydrate content heavy crop York showed the greatest increase from morning to afternoon of the York leaves and medium crop York the least. Of the Livland leaves the fruitless Livland showed the greatest gain from morning to afternoon and heavy crop Livland the least. The greatest decrease from afternoon to morning in total acid hydrolyzable carbohydrates in the York leaves was shown by heavy crop York and the least decrease by medium crop York in which the daily decrease amounted to only .01 gram per square meter of leaf area.

Fruitless Livland and medium crop Livland on the other hand both showed greater losses in acid hydrolyzable carbohydrates from afternoon to morning than heavy crop Livland, in the order given. The apparent daily gain in total acid hydrolyzable carbohydrates thus shows heavy crop York the highest followed by fruitless Livland, fruitless York, medium crop Livland, heavy crop Livland, and medium crop York in the order given.

In no case was the increase in acid hydrolyzable carbohydrates as great as the increase in total dry weight.

No actual measurements were made of the rate of increase in size of the fruit borne on the heavy crop branches as compared with that on the medium crop branches but it was observed that the fruit on the heavy crop York branches did not grow nearly as rapidly or reach as large size as that on the medium c rop branches. It also showed a tendency to wilt much sooner and to a greater degree than fruit on the medium c rop branches. There was less difference in the size of the heavy crop Livland fruit as compared with the medium crop Livland fruit but the advantage again belonged to the medium crop fruit. The heavy crop Livland fruit showed a more pointed or conic shape than the medium crop fruit and gave indications of not having matured properly as judged from its color, shape and flavor.

Heavy crop York leaves appeared to be smaller in size than medium crop or fruitless York leaves and exhibited a lighter color with a more limp or flaccid condition. They also showed greater wilting tendencies than medium crop or fruitless leaves.

The York leaves were noticeably lower in water content than Livland leaves which is in keeping with their lower photosynthetic rate as determined through their increase in dry weight. Heavy crop York leaves had a slightly higher water content than medium crop York which was slightly higher in this respect than fruitless York. Fruitless Livland leaves were higher in water content than heavy crop Livland leaves and both were higher than medium crop Livland. In Livland leaves increased water content was directly correlated with increased dry weight and increased total carbohydrate content per square meter of leaf area. With York leaves increased water content seemed to be inversely correlated with increased dry weight and total carbohydrate content per square meter of leaf area.

With regard to seasonal variation in water content there appeared to be a gradual decrease for all leaves as the season advanced with all leaves showing a sudden decrease June 19 and 20 and a subsequent increase June 27 and 28.

Livland leaves seemed to show some relationship between increased water content and higher apparent photosynthetic rate but York leaves showed little relationship between these two characters.

The decrease in total dry weight per square meter of leaf area on the second day of each collection of samples indicates that wounding the leaves does lower the photosynthetic activity of leaves. Since the decrease in rate of gain of total dry weight on the second day is accompanied by a decreased water content it suggests that wounding of the leaves decreases photosynthetic activity by lowering the water content of leaves.

As shown in figure D photosynthetic activity as expressed in daily gain of total dry weight per unit of leaf area is directly associated with the relative humidity of the atmosphere and soil moisture content. It is inversely correlated with air temperatures. On July 22 and 23 the air temperatures were so high and the soil moisture content and relative humidity were so low that photosynthesis was practically at a standstill.

The change in acid hydrolyzable carbohydrate content of spur tissue is associated with the amount of water loss shown by the spurs. The least decrease in York spurs was shown by the heavy crop condition. The least decrease in Livland spurs was shown by the medium crop condition which showed an actual increase in d-glucose content during the period of observation. The fruitless condition in both York and Livland showed the greatest decreases in d-glucose. York spurs showed greater losses in this constituent than did Livland spurs which is in keeping with the smaller leaf area of York.

CONCLUSIONS

Increased apparent photosynthetic activity does not appear to be directly associated with increased leaf to fruit ratios. The fact that for both York and Livland leaves the average daily increase in dry weight of the medium crop leaves was distinctly lower than for either the heavy crop leaves or the fruitless leaves would suggest that a heavy setting of fruit is necessary to stimulate the leaves to greater photosynthetic activity. Here the fact that the fruitless condition showed a slightly greater daily gain in dry weight conflicts with the previous statement. However, the experiment measured only the apparent increase in dry weight and there is no exact way of determining the amount of respiration and translocation of material which occurred under the highly fruitful condition as compared with the fruitless condition. To be sure the decrease in weight by night is used as an indication of the probable amount of decrease due to respiration and translocation but there is a possibility that these processes do not occur at the same pace by day as by night. The greater metabolic activity accompanying a high degree of fruitfulness would necessarily place a heavier drain on the leaves by day than would the fruitless condition. From this viewpoint it seems possible that the loss in weight by the heavy crop condition during the day would be even greater compared with the fruitless condition than the actual losses from afternoon to morning show. If this should be the case one may assume that the fruitless condition would reach its maximum weight early in the day and then suffer a great slowing up in the rate of weight increase because of the accumulation of the products of photosynthesis which tend to retard photosynthesis. On the other hand, a greater need for nutrient materials by the young fruits would result in a greater consumption of these materials and their accumulation would never progress to the point where their presence might inhibit further manufacture.

The seasonal distribution of increases in dry weight per unit of leaf area also adds force to the above premise. The heavy crop conditions of both York and Livland show a slow early increase in weight per unit of leaf area followed by, in the case of York, a rapid decrease and in the case of Livland by a fairly constant figure. The slightly fruitful conditions show a fairly constant increase in weight throughout the experiment indicating that the leaves are able to synthesize more dry weight than the developing fruits require.

The fact that in all cases there was a general increase in dry weight per unit of leaf area up to approximately June 28 or later would indicate that nutrient material should have been available for fruit bud differentiation which is usually considered to have taken place by June 20. The data for acid hydrolyzable carbohydrate content on the other hand by showing marked decreases from June 6 to June 28 in medium crop York and Livland leaves followed by increases after that date, whereas the heavy crop York and Livland leaves show a continued gradual decrease after June 28, may suggest that decreases in total carbohydrates from June 6 to June 28 may be the result of fruit bud differentiation. The fact that the fruitless York leaves show a similar decrease to June 20 and then an increase to June 28 may be due to fruit bud differentiation. The fruitless Livland leaves showed no such decrease in total carbohydrate material up to June 28, holding a rather constant carbohydrate content until July 4 after which a great decrease occurred reflecting the greater photosynthetic capacity of Livland leaves.

The great decrease in dry weight and acid hydrolyzable carbohydrates exhibited by fruitless Livland leaves after the period in which Livland ripens its fruit indicates that after the normal time for the removal of the need for large quantities of nutrients the leaf becomes more or less senescent.

Wounding the leaves reduces their water content and their relative photosynthetic capacity. In the case of leaves of low photosynthetic capacity, such as York, wounding by hail, wind, insects or other pests may have considerable effect on the trees' synthesis of reserve nutrients.

The rate of photosynthetic activity as determined by gain in total dry weight is influenced by soil moisture content, relative humidity and air temperatures.

The conclusion presents itself that the biennial bearing habit of York is not altogether due to its lower rate of photosynthetic activity. Other factors seem to be involved.

SUMMARY

1. York leaves are lower in water content than Livland leaves.

2. York leaves show less daily gain of total dry matter than do Livland leaves.

3. Leaves of medium crop York and Livland make smaller daily gains of total dry weight than do leaves of heavy crop and fruitless York and Livland.

4. York leaves have less weight per unit of leaf area than do Livland leaves.

5. Heavy crop York and Livland leaves did not attain as great a weight per unit of leaf area as do medium crop and fruitless York and Livland leaves. Fruitless York and Livland leaves did not attain as great weight per unit of leaf area as medium crop York and Livland leaves.

6. Heavy crop York and Livland leaves showed a gradually decreasing acid hydrolyzable carbohydrate content from June 6 to July 23.

7. Fruitless York and Livland leaves showed a more or less constant acid hydrolyzable carbohydrate content from June 6 to July 4 after which York leaves decreased

slightly and Livland leaves decreased greatly.

8. Medium crop York and Livland leaves had a decrease in acid hydrolyzable carbohydrate content from June 6 to June 20 after which constant increases occurred until after July 4.

9. Livland leaves showed great decreases in total dry weight, acid hydrolyzable carbohydrate and water content after July 4 at which time their fruit was ripening.

10. The daily increase in total dry weight was invariably greater than the daily increase in acid hydrolyzable carbohydrates.

11. Increases in daily gain of total dry weight were directly correlated with increases in relative humidity and soil moisture and inversely correlated with increasing temperature.

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