PHOTOSYNTHESIS AND CARBOHYDRATE METABOLISM OF HEALTHY AND LEAFROLL DISEASED POTATO PLANTS

(MET EEN SAMENVATTING IN HET NEDERLANDS)

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Promotor: Professor Dr. E. C. Wassink

aan de nagedachtenis van mijn ouders

CURRICULUM VITAE

Teneinde te voldoen aan de wens van de Faculteit der Wiskunde en Natuurwetenschappen, volgt hier een overzicht van mijn studie.

In 1943 behaalde ik aan de R.H.B.S. te Goes het einddiploma. Van 1945-1948 was ik ingeschreven als student aan de Landbouwhogeschool te Wageningen.

In 1948 werd mijn studie voortgezet aan de Rijksuniversiteit te Leiden, alwaar ik in 1952 het candidaatsexamen aflegde en in 1956 het doctoraalexamen biologie, met als hoofdvak plantkunde en als bijvakken dierkunde en plantenziektekunde.

Tijdens mijn studie voor het doctoraalexamen werden de volgende onderwerpen bewerkt. De 'apparent free space' in aardappelschijfjes, onder leiding van Prof. Dr. T. H. VAN DEN HONERT. De betrekking tussen de kauwmusculatuur en de sculptuur van de kop bij *Cavia*, onder leiding van Prof. Dr. C. J. VAN DER KLAAUW. De embryonale ontwikkeling van de hypophyse bij de stekelbaars, onder leiding van Dr. W. VERVOORT. De adsorptie van virusdeeltjes aan kleimineralen, onder leiding van Prof. Dr. T. H. THUNG te Wageningen.

Vanaf 1956 ben ik als gastmedewerker verbonden aan het Laboratorium voor Plantenphysiologisch Onderzoek van de Landbouwhogeschool te Wageningen. Het beschreven onderzoek werd daar verricht onder leiding van de directeur, Prof. Dr. E. C. WASSINK, aanvankelijk tevens in overleg met Prof. Dr. T. H. THUNG †1960, eertijds hoogleraar in de Virologie.

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CHAPTER I

SURVEY OF LITERATURE[1]) AND SCOPE OF THE INVESTIGATION

'Virus diseases', according to BAWDEN and PIRIE (1952), 'are most plausibly regarded as metabolic disturbances of the host,...'. A similar definition of virus diseases seems to leave ample room for a physiological study.

In § 1 of this introduction, some of the physiological aspects of the leafroll disease are discussed. A parallel is often drawn between potato leafroll and beet yellows, because these diseases have many characteristics in common so that sometimes both were studied by the same investigators. In the Netherlands especially, much research has been devoted to the study of the above diseases, which was greatly stimulated by the pioneer work of QUANJER.

In § 2, related aspects of the physiology in healthy plants are surveyed; in § 3, the scope of the present investigation is presented.

§ 1 PHYSIOLOGICAL ASPECTS OF THE LEAFROLL DISEASE IN POTATO PLANTS

a Accumulation of carbohydrate and phloem conductivity

One of the most remarkable symptoms in potato leafroll and sugar beet yellows is the accumulation of large quantities of carbohydrate in the leaves of infected plants. These diseases thus give rise to an increase in the C/N ratio of the leaf; they contrast in this respect with the diseases of the mosaic type which lead to a decrease in this ratio (*cf.* MARTIN, 1954; BAWDEN, 1959).

Potato leafroll is one of the long-known virus diseases, and was first studied by QUANJER (1913, 1916, 1919), who later, in partial collaboration with ROLAND, was also the first to study beet yellows (QUANJER, 1934; ROLAND, 1936) For both diseases, QUANJER suggested that mechanical disturbances in the phloem (described as phloem necrosis and phloem gummosis, for leafroll and beet yellows respectively) would inhibit translocation, and lead to accumulation of carbohydrate[2]).

Accumulation of starch in leaves of leafroll diseased plants was first demonstrated by QUANJER (1919), and in the same year also by other investigators (NEGER, 1919 and ESMARCH, 1919). The latter authors did not assume that the leafroll disease was caused by virus infection, as suggested by QUANJER (1916) from grafting experiments.

NEGER suggested that the accumulation of starch resulted directly from inhibition of the diastase activity in leaves of diseased plants. Similar views were proposed by OORTWIJN BOTJES (1920), who observed accumulation of carbohydrate before any visible signs of phloem necrosis. MURPHY (1923) confirmed

[1]) An exhaustive review of literature on potato leafroll has recently been published by BODE (1962).

[2]) For further anatomical description of the diseased phloem, *see* ESAU (1938, 1960).

this observation. THUNG (1928) examined enzyme activities in healthy and leafroll diseased plants in a comparative study of the various processes in which carbohydrates are involved. He found no differences between healthy and diseased plants; during respiration, *e.g.*, which appeared increased in diseased leaves, starch disappeared as readily from diseased leaves as from healthy ones. From further observations on loss of carbohydrate during a dark period, and from other evidence, THUNG supported QUANJER's conception that accumulation occurred as the result of inhibited translocation, initiated by phloem necrosis.

Increased respiration rates in leaves of yellows diseased sugar beets have been demonstrated by VAN RIEMSDIJK (1935). In analogy to similar observations in potato leafroll (*see* above), KLINKENBERG (1945) noticed accumulation of carbohydrate in beet yellows before visible signs of phloem gummosis. These results further illustrate the similarity between potato leafroll and beet yellows.

It may well be assumed that leafroll is essentially a disease of the phloem. The virus which until now never has been isolated, presumably, is restricted to this tissue as well. This might be derived from the similarity in physiological respects between leafroll and beet yellows. The beet yellows virus, according to ESAU (1960), indeed is limited to the phloem, at least in the earlier stages of infection; later on it is found also in the other tissues of the plant (*cf.* also ESAU *et al.*, 1961).

Carbohydrates presumably accumulate because of inhibited translocation. The exact reason for this inhibition as yet appears undecided. Recent investigators suggest that first physiological disturbances in the phloem become effective, which are followed up by mechanical disturbances. For sugar beet yellows, *e.g.*, it has been proposed that a decrease in the phosphatase activity in the phloem might inhibit translocation, and lead to carbohydrate accumulation (VAN DUUREN, 1955; HENKE, 1957; SOMMER, 1957). This suggestion is based on modern conceptions on translocation which are discussed in more detail in § 2*b*.

A quite different possibility for accumulation was proposed by COÏC (1945), who suggests that the growth meristems in leafroll diseased plants are 'indifferent' towards unlimited supply of nutrient substances. The author referred to the work of JAHNEL (1940), who demonstrated shortage of growth substances in the various organs of leafroll diseased plants (*cf.* also BAUMEISTER, 1951). The indifference of the growth meristems, according to COÏC, would lead to reduced plant growth and subsequent accumulation of carbohydrate in leaves.

Growth reduction in diseased plants indeed might account for part of the accumulation. Certainly, reduction of leaf surface is one of the causes for the low tuber yield in diseased plants (*cf.* BALD and HUTTON, 1950).

b Rate of photosynthesis

Photosynthesis in leaves of leafroll diseased plants is reduced, as has been demonstrated by several investigators (MÜLLER, 1932; VON WITSCH and POMMER, 1954). Several possible causes have been proposed.

MÜLLER (1932) suggested that the main reason for the decreased rate of photosynthesis would be partial closure of stomata in diseased leaves; gas exchange, consequently, would be affected (*see* also FRIEDRICH, 1938). Especially under field conditions, this may have a large effect on photosynthesis (*cf.* § 2*a*).

VON WITSCH and POMMER (1954) proposed that the stomatal effect was only one of the reasons for the reduced rate of photosynthesis. Another would be the high sugar concentration of diseased leaves. MÜLLER, earlier, had rejected this possibility because he did not find any improvement of photosynthesis in plants which had been kept in the dark, and lost part of the accumulated carbohydrate.

The decrease in chlorophyll content in diseased leaves also has been held responsible for the low rate of photosynthesis. BODE (1951) compared tuber yields in different potato varieties and observed a close correlation between reduced tuber yield and reduction in clorophyll content.

Decrease in the rate of photosynthesis in yellows diseased beets has been reported by SCHULTZ (1958). The same factors as mentioned above might be responsible.

c Nitrogen metabolism

Nitrogen metabolism in potato leafroll appears to be relatively unaffected. Total N in leaves of diseased plants seems lower than that in leaves of healthy plants, mainly because of a relative decrease in protein (HENKE, 1956). The glutamine content of diseased leaves is reported to be increased, probably because of its relation with sugar (REINDEL and BIENENFELD, 1956). Analogous data were obtained for sugar beet yellows (HENKE, 1954; VAN DUUREN, 1955).

The increase in C/N ratio in leaves of diseased plants, thus, seems to be entirely on account of the increase in carbohydrate content.

d Influence of environmental conditions on symptoms

Data on the effect of environmental conditions on the expression of leafroll symptoms in diseased plants are relatively scarce. Most reports are on the effect of fertilisers. It is generally accepted that application of nitrogen masks external symptoms in potato leafroll (FELTON, 1948; ARENZ, 1949; WATSON and WILSON, 1956; WENZL and REICHARD, 1961). In this respect, it is interesting to note that translocation, apparently, is not improved. This may be derived from the experiments of WATSON and WILSON, who observe the same degree of phloem necrosis (which may well be considered as a measure for inhibition of translocation) in + nitrogen and control plants. FELTON remarks: 'Although healthy and leafroll plants could not be distinguished with certainty in the + nitrogen series, the total yield of the leafroll plants was still less than one-half that of the healthy plants'.

With regard to the effect of weather conditions, low light intensities seem to suppress development of symptoms in potato leafroll (WILSON, 1955; WATSON and WILSON, 1956), as well as in sugar beet yellows (WATSON, 1955).

Several investigators have drawn attention to the similarity of leafroll symp-

toms and those of the deficiency diseases of the potato. HOVELAND *et al.* (1954), *e.g.*, compare leafroll symptoms to those induced by phosphorus deficiency. They actually observe a decreased phosphorus content in leaves of diseased plants which, however, we were unable to confirm in our experiments (*cf.* Chapter IV, § 2).

§ 2 RELATED ASPECTS IN HEALTHY PLANTS

a Photosynthesis and carbohydrate formation in leaves

Photosynthesis in potato plants has been studied by LUNDEGÅRDH (1922), CHAPMAN (1951), CHAPMAN and LOOMIS (1953) and WINKLER (1961).

LUNDGÅRDH and also CHAPMAN and LOOMIS studied the effect of different CO_2-concentrations on the rate of photosynthesis, and found a linear increase with CO_2-concentrations up to 0.15% (higher concentrations were not applied). GAASTRA (1959), studying the CO_2-effect on photosynthesis in several crop plants, observed CO_2-saturation at concentrations of about 0.10%. He assumes that the effect of still higher concentrations on photosynthesis, reported by the above authors, may have been due to insufficient control of leaf temperatures, which may have risen above those actually recorded.

This effect of CO_2-concentration on photosynthesis indicates that under field conditions photosynthesis generally is limited by the CO_2-diffusion rate, which is partly conditioned by stomatal opening. When this is applied to potato leafroll, it seems likely that the partial closure of stomata is at least one of the reasons responsible for the reduced rate of photosynthesis in diseased plants.

Another factor which may influence photosynthesis is the carbohydrate content of the leaf. This factor may be discussed in some more detail here, since there is a large increase in the carbohydrate content in leaves of leafroll diseased plants.

Although it is often suggested that accumulation of carbohydrate in leaves reduces the rate of photosynthesis, it appears that this is not necessarily so. KURSANOV (1933) observed, when the bark of apple trees was girdled, a decrease in the rate of photosynthesis. MOSS (1962) noticed the same effect after the removal of fruit in corn and tomatoes. Both authors assume that the decrease in rate of photosynthesis is connected with an increase in the sugar content of leaves. On the other hand, several other investigations show no such effect of carbohydrates on photosynthesis. In *Elodea densa*, as reported by STÅLFELT (1945), photosynthesis proceeds at a constant rate for at least 5 hours, while it must be assumed that meanwhile sugars accumulate. HUMPHRIES (1963) observes the same net assimilation rate in detached *Phaseolus* leaves under different daylight conditions, so that the leaves presumably were unequal in carbohydrate content.

From this, it appears that different plants react to sugar accumulation in different ways. There might well be a relation with the storage capacity for carbohydrates in various plant species. When this is large, and carbohydrates

are stored away from the actual reaction sites, no effect on photosynthesis can be expected.

The effects of several other internal leaf factors on photosynthesis are discussed in Chapter IV, when our measurements on photosynthesis in healthy and leafroll diseased plants are presented.

Already long ago it was tried to obtain information on the first sugar of photosynthesis by studying the diurnal fluctuations of carbohydrate in leaves. Two publications from that period still are often referred to. BROWN and MORRIS (1893) observed that sucrose, at least quantitatively, was the most important sugar in leaves of *Tropaeolum*. DAVIS, DAISH and SAWYER (1919) studied carbohydrate fluctuations in mangold leaves and arrived at the same conclusion. A similar, though less thorough, study on carbohydrate fluctuations in potato leaves was published by DAVIS and SAWYER (1916).

The present opinion is that sucrose is the first free sugar in photosynthesis, which in *Chlorella pyrenoidosa* was demonstrated by CALVIN and BENSON (1949), making use of modern tracer techniques. Hexoses are considered to arise from inversion of sucrose (PORTER *et al.*, 1959; KURSANOV *et al.*, 1960; GLASZIOU, 1960, 1961; PAVLINOVA and TURKINA, 1961).

The study of the synthesis of di- and oligo-saccharides has been much promoted by the discovery of the uridine phosphates, which appear to play a key-rôle in sugar interconversions (LELOIR and CARDINI, 1953; CARDINI *et al.*, 1955; LELOIR and CARDINI, 1955). Also synthesis of glycogen and starch at present are considered to involve transfer of glucosyl units from UDP-glucose (LELOIR *et al.*, 1961), or from ADP-glucose (FRYDMAN, 1963).

Free sugars are rarely involved in the various carbohydrate interconversions. They first have to be phosphorylated by the action of ATP. Phosphorylated carbon compounds are also the first products of photosynthesis, from which later free sugars are derived (*cf.* CALVIN, 1956).

b Carbohydrate translocation and phloem transport mechanism

It seems quite certain now that sucrose is the form in which carbon it transported through plants. This was suggested already by MASON and coworkers in their study of translocation in cotton (*cf.* MASON and PHILLIS, 1937). Later investigators, with more suitable techniques, identified sucrose as the only sugar in the phloem sap (WANNER, 1952, 1953; ZIEGLER, 1956; ZIEGLER and MITTLER, 1959). More evidence is supplied by experiments in which $^{14}CO_2$ is assimilated, and radioactive sucrose very soon appears in the conducting tissues (*cf.* KURSANOV *et al.*, 1958, 1960; JONES *et al.*, 1959).

In some cases also sugars belonging to the raffinose family have been found in phloem sap (ZIMMERMANN, 1957; PEEL and WEATHERLEY, 1959; PRISTUPA, 1959); in several instances in even greater concentration than sucrose (ZIMMERMANN, 1957).

The mechanism of conduction of organic substances in the phloem remains a fascinating problem in plant physiology. Most investigators now visualise translocation as a mass flow of solutes. Interesting experiments have been re-

ported by WEATHERLEY *et al.* (1959), and by PEEL and WEATHERLEY (1962, 1963), the results of which are also interpreted in favour of the mass flow theory. In these experiments, sap from phloem cells is obtained by means of severed stylets of aphids (*cf.* KENNEDY and MITTLER, 1953).

Study of the anatomy of the phloem seems indispensible for a better understanding of its function. Several reviews have appeared (*cf.* ESAU, CURRIER and CHEADLE, 1957). Opinions still vary with regard to the vitality of the functioning sieve cells. Of special interest seems the occurrence of mitochondria in the phloem, principally in connection with the comparatively high respiration rate of vascular bundles (*see* below). Mitochondria have been demonstrated in mature sieve elements of several plants (KOLLMANN, 1960; ESAU and CHEADLE, 1962); however, they appear considerably modified which is interpreted by the supporters of the diffusion theory as another example of the highly specialized structure of sieve cells (*cf.* KOLLMANN).

Respiration of the vascular bundles is increased as compared with that of the surrounding tissues (KURSANOV and TURKINA, 1952; WILLENBRINK, 1957). ZIEGLER (1958) separated phloem from xylem in *Heracleum mantegazzianum*, and found that both tissues contributed to the increased respiration. KURSANOV *et al.* (1959) noticed the presence of several enzymes of the glycolytic system in the vascular bundles of the sugar beet. The high respiration rate of vascular bundles, probably, is not connected with actual phloem conduction. Rather, it is related to the introduction of organic substances into the sieve cells, and their subsequent release (ZIEGLER, 1958).

The companion cells of the phloem, especially, have long been known to show high metabolic activity. Already PHILLIS and MASON (1933) suggested that these cells and those of the phloem border parenchyma have a secretory or accumulatory function. Transformation of sugars would take place here, creating sucrose concentration gradients between leaf parenchyma and phloem (MASON and MASKELL, 1928[a], 1928[b]). YIN (1945) noticed an exceptionally high phosphatase activity in companion cells, and suggested a connection with the ideas of PHILLIS and MASON.

Increase in phosphatase activity in companion cells was demonstrated also by other investigators (WANNER, 1952, 1953; FREY, 1954). WANNER suggests that in leaf parenchyma diffusion of sugar phosphates takes place into the direction of the vascular bundles. In phloem parenchyma and companion cells, the sugar phosphates are hydrolysed, while at the same time sucrose is synthesized and introduced into sieve tubes. As already briefly mentioned in § 1*a*, this hypothesis has been applied to explain the accumulation of carbohydrate in sugar beet yellows (VAN DUUREN, 1955; HENKE, 1957; SOMMER, 1957). Decrease in phosphatase activity in the vascular bundles might inhibit parenchyma transport and lead to accumulation of carbohydrate in leaves. The results obtained by HENKE indicate that the phosphatase activity in the main leaf veins in diseased sugar beets indeed is reduced, whereas that in the remainder of the lamina is increased.

c Carbohydrate accumulation as the result of disturbed physiology

Boron deficiency in plants generally leads to accumulation of carbohydrate in leaves. There seems to be strong anatomical evidence that boron deficiency interferes with cambial activity (WARINGTON, 1926; JOHNSTON and DORE, 1929; VAN SCHREVEN, 1934, 1935; REED, 1947; PALSER and MCILRATH, 1956). The resulting vascular derangement might be the reason that sugars are not removed from leaves. Not all investigators, however, accept that the necrotic condition of the phloem is the primary cause for carbohydrate accumulation in leaves of boron deficient plants. GAUCH and DUGGER JR. (1953), and SISLER *et al.* (1956), *e.g.*, propose that boron is needed for complex formation with sugars which would facilitate translocation through protoplasmic membranes.

REED (1947) observes in boron deficient sunflowers a comparatively large inorganic phosphate content, which he connects with, apparently, increased phosphatase activity in the vascular bundles. This observation is of interest in connection with WANNER's hypothesis on translocation (*see* under *b*). The generally observed accumulation of carbohydrate in leaves of boron deficient plants, then, cannot be explained on the basis of this hypothesis which postulates, conversely, decreased activity in the vascular bundles.

Treatment of plants with maleic hydrazide also leads to accumulation of carbohydrate. In potato plants, *e.g.*, it may cause the formation of aerial tubers in leaf axils (DENISEN, 1953). Maleic hydrazide is known to inhibit haulm growth, which may well be the direct reason for accumulation. A parallel may be drawn with potato leafroll, where inhibited haulm growth, presumably, is an additional reason for carbohydrate accumulation.

§ 3 SCOPE OF THE INVESTIGATION

Though it seems well established now that the rate of photosynthesis in leafroll diseased plants is reduced, this has been investigated once more in the present study. Several internal leaf factors influencing photosynthesis appear considerably modified by the leafroll disease. Special attention has been paid to the effect of carbohydrate accumulation (Chapter IV).

A perusal of the relevant literature shows that it may be of considerable interest to compare several aspects of carbohydrate metabolism in healthy and diseased plants.

As such, a comparison of the removal of photosynthates from healthy and diseased leaves was considered important, together with a general estimation of the carbohydrate content in petiole and lamina (Chapter V).

Secondly, a more detailed study of the carbohydrate content at different times during the day and night has been (made) (Chapter V).

In the third place, it has been asked whether the composition of total carbohydrate shows marked deviations in diseased plants as compared with healthy ones (Chapter VI).

Since indications were obtained that the carbohydrate composition is primarily related to total carbohydrate content, and the latter is mostly higher in

diseased plants, it was attempted, moreover, to obtain diseased plants with low carbohydrate contents in experiments in which artificial shading was applied (Chapter VI).

CHAPTER II

MATERIAL AND METHODS

§ 1 Material

The potato variety 'Alpha' was used throughout our investigation. This variety reacts to leafroll infection with moderately-severe symptoms. Reduction of growth, *e.g.*, is quite evident but enough leaf material remains for the selection of samples.

In our experiments, secondarily infected plants were used; the tubers were obtained from the Laboratory of Phytopathology[1]), and were infected with a moderately virulent strain of the virus. While healthy tubers were newly obtained each year, our stock of diseased tubers was renewed once in two or three years.

The plants were grown in the experimental field of the laboratory; healthy and diseased plants were cultivated in the same plot. Plants suspected of being infected with any of the other virus diseases of the potato were immediately removed. The plots, if required, were sprayed with 'Gesarol', a DDT-product, for aphid control. The probably more effective organic phosphorus containing insecticides generally could not be used because sometimes leaf phosphates were estimated.

Before the tubers were planted, all sprouts except two were removed. Another one was removed after emergence so that in the end only one stem per tuber remained. Afterwards, all lateral shoots were removed. This practice was followed in order to keep conditions for the expansion of various leaves the same as nearly as possible.

It is well known that the expression of leafroll symptoms may vary widely from one year to another. During our experiments, the disease symptoms were severe in 1958 and 1961, while they were much milder in 1957 and 1960. The reason for this variation is not definitely known; weather conditions may at least partly be responsible (Chapter III, § 2). Also in single plots, the individual plants sometimes vary in symptom expression. Recently, HOYMAN (1960, 1962) reported that plants grown from sprouts near the bud-end of infected tubers develop more distinct symptoms than those grown from sprouts near the stem-end. This may be one of the reasons for the variation in single plots.

Leaves collected for analysis usually were first washed to remove adhesive soil particles. The mesophyll then was separated from petiole and larger veins; both portions were subsequently dried in a well ventilated oven for about 12 hours at 70°C, followed by 30 minutes at 105°C. The dried samples were preserved in closed glass vessels, and carbohydrate estimations usually made in the winter following the summer sampling.

[1]) We wish to thank ir. ROZENDAAL and mr. VAN BINSBERGEN for their kind help.

§ 2 METHODS

a Measurements of photosynthesis

Photosynthesis was measured with the aid of the WARBURG technique. This method, applied to measurements on higher plants, has first been described by WASSINK (1946); it enables several simultaneous measurements of different leaves and thus seems to be especially suited for purposes of comparison.

The apparatus used in our experiments is of the normal type with single manometers. A shaking frame, supporting the manometers, is mounted in front of a water thermostate. The water is circulated by means of a small pump to ensure even distribution of temperature. Lamps are mounted under the bath, with two fans for heat removal.

The vessels (with a volume of 40 ml, each) consist of a bottom part and a lid so that the leaf material can easily be introduced. Small protuberances at the inner side of the bottom part support a perforated plexiglass plate on which leaf discs are arranged. The leaf material is thus prevented from contact with the buffer solution at the bottom of the vessel.

Light is provided by high pressure mercury lamps (Philips HO, 450 W; type 57103 G). For obtaining lower light intensities, the vessels were wrapped in one or two layers of cheese cloth. An envelope of black cloth excluding all light was used in the determination of respiration rates which were measured simultaneously with rates of photosynthesis. The light intensity (erg/cm^2.sec) was determined with a calibrated MOLL's microsurface thermopile, adapted for measurements under water. Correction was made for the infrared radiation which was taken apart by application of the SCHOTT RG 8 filter in a separate measurement.

The CO_2-concentration in the vessels was maintained during the measurements at 1.26%. This was achieved by the application of the 0.5 M WARBURG buffer No. 9 (*cf.* WARBURG, 1928), a mixture of carbonate and bicarbonate in the ratio 15:85 (v/v).

Ten small leaf discs (with an area of 0.28 cm^2, each), usually taken from different leaves, were arranged on the plexiglass plates in the vessels (*see* above); the upper surface of the discs was turned towards the light source. Care was taken that the composition of the material was approximately the same in each vessel. It was taken into account, *e.g.*, that the rate of photosynthesis might be different in upper and lower leaflets of a single leaf.

In a single measurement, diseased leaves mostly were compared with healthy ones. Two of the ten available manometers were then used for thermo-barometer control. Two sets of four remained, one for measurements with healthy leaves and the other for measurements with diseased leaves. One of the manometers of each set was used for measuring respiration, the other three for measuring photosynthesis at three different levels of light intensity. Measurements which were first carried out in the morning usually were repeated in the afternoon using fresh material, which was thus distributed that those vessels which first contained discs from healthy leaves, now held discs from diseased

leaves, and *vice versa*. Inaccuracies that may arise from slight differences in light intensity and vessel constant thus may be eliminated. Usually four readings were made with intervals of 15 minutes; during the whole of this time, the rate of photosynthesis proceeded at a steady rate.

The WARBURG method, as stated before, seems to be especially suited for comparative measurements. Also the fact that discs from different leaves can be taken together in a single vessel seems to be an advantage. The measured rate of photosynthesis then may be considered to represent that of an average leaf.

In most measurements, it was noticed that the leaf discs held at higher light intensities tended to wilt. Wilting apparently results from evaporation upon absorption of radiant energy. It draws attention to the fact that leaves under high light intensities assume a higher temperature than those under low intensities. Such differences in temperature may be quite appreciable (*cf.* WAGGONER and SHAW, 1952; ANSARI and LOOMIS, 1959).

Small leaf discs as used in our measurements usually show lower rates of photosynthesis than larger discs taken from the same leaf. In comparative measurements, it was found that the rate of photosynthesis per unit leaf area in larger discs (1.58 cm^2 as compared with 0.28 cm^2, as generally used) was about 20% higher.

b Estimation of carbohydrates

The dried material to be analysed was first ground in a mortar before extractions were made. When a sufficient amount of material was available, a constant portion (500 mg) was used for extraction of sugars with ethanol, another portion (200 mg) for extraction of starch with $HClO_4$.

α Soluble sugars. Sugars were extracted with 80% ethanol. To ensure complete recovery of sugar, usually two successive extractions were made; the extracts were centrifuged, filtered through paper, and combined. After parts of the extracts were treated with active charcoal (trade mark 'Norit') for clarification and removal of glycosides, the total sugar content was determined with anthrone.

The removal of glycosides from sugar extracts, evidently, is necessary when the sugar content is determined with the anthrone reagent. Glycosides, if not removed, are hydrolysed in the reaction and determined along with sugar. Active charcoal, in this respect, proved effective; preliminary experiments had shown that sugars in ethanol extracts are not adsorbed.

In several of the experiments discussed in Chapter V, the glycoside content of the material was determined as the difference in total sugar content between non-clarified and clarified extracts. It appeared that there were no marked quantitative differences between healthy and diseased plants; the glycoside content of the lamina was in the order of 200–300 mg/10 g res. dry wt, that of the petiole in the order of 100 mg/10 g res. dry wt. It was further observed that the glycoside content of leaves in the potato remained approximately constant throughout the day.

Sugar extracts were chromatographed, using Whatman No. 1 filter paper and *n*-butanol:ethanol:water (4:1.1:1.9; v/v) as a solvent. The sheets were cut in strips as recommended by MATTHIAS (1954). Usually, descending chromatography was employed.

Sugar spots were localized by spraying with aniline hydrogen phthalate and naphthoresorcinol, for the development of hexoses and ketose-sugars respectively.

After localisation, the spots were cut from the paper, the sugars washed into test tubes and determined with anthrone. Since an arbitrary volume of the extracts had been used, the results of the chromatographic separation were obtained in percentages; absolute amounts were calculated using the data on total sugar which had been obtained before.

The anthrone reaction was first applied by DREYWOOD (1946) as a qualitative test for carbohydrates. In our determinations, we used the quantitative method, originally developed by TREVELYAN and HARRISON (1952), and modified by YEMM and WILLIS (1954). It appeared in the course of the investigation that ethanol had some influence on the colour development during the reaction, though it is not listed among the substances which may show this effect (*cf.* SCOTT and MELVIN, 1953). Therefore, when dilution of the original sugar extraxts is desired, this should be done with 80% ethanol; calibration curves should also be made with sugar solutions in 80% ethanol.

β Starch. Starch was extracted with $HClO_4$, and specifically isolated, according to the method of PUCHER *et al.* (1948). After being dissolved in water, it was determined with the anthrone reagent.

Usually, a sufficient amount of material was collected so that starch and sugar could be extracted each from a separate fraction of the material. In 1960, when only relatively small quantities of material were collected, starch was determined in the residue after sugar extraction. The first method, however, has to be preferred because of the fact that during sugar extraction, especially in decanting supernatants, some of the material may easily be lost.

γ Reference basis for carbohydrates. In the experimental results, carbohydrates are referred to residual dry matter (dry matter minus total carbohydrate). This, according to MASON and MASKELL (1928[a]), provides an approximately constant basis of reference. While the carbohydrate content of the lamina is referred to 10 gram residual dry weight, that of the petiole is mostly referred to 'corresponding' residual dry weight, *i.e.* the weight corresponding to 10 gram lamina (to be calculated for each leaf separately). The latter reference basis makes allowance for the surface of adjoined lamina, and seems especially suited for the detection of carbohydrate accumulation in petioles of diseased leaves.

c Estimation of phosphates and chlorophyll

In some of the photosynthesis experiments, the phosphate and chlorophyll contents of leaves were estimated.

Phosphates were extracted from freshly gathered material by adding a certain volume of 20% TCA, resulting in a final concentration of 5%. Separately,

the inorganic, the soluble organic and the insoluble phosphate fractions were determined, using the method described by LINDEMAN (1958).

Chlorophyll was estimated comparatively in healthy and diseased leaves by determining the extinction values of extracts in the colorimeter at 665 mμ. Equal surface areas of healthy and diseased leaves were extracted with a fixed volume of ethanol; the material had been killed before by immersion in boiling water.

CHAPTER III

SYMPTOM EXPRESSION AND EFFECT OF WEATHER CONDITIONS

§ 1 Symptoms

The first leafroll symptoms usually appear about two or three weeks after emergence of the plants. In 1957, *e.g.*, at the time of the first experiment of June 17–20, three weeks after emergence, diseased plants could already be clearly distinguished from healthy ones. The plants showed the usual erect appearence, the lower leaves were cupped rather than rolled, and a faint yellow discoloration of the foliage could be observed. Healthy plants at that time had just started the formation of tubers; diseased plants not yet while these latter had fewer and much shorter stolons. Subsequent analysis of the samples that were taken showed that carbohydrates did not yet accumulate in the leaves of diseased plants, so that external disease symptoms seem to be present before accumulation can be demonstrated. The latter, however, cannot be expected to have been large as early as this; some accumulation may have been present in the lower leaves but not detected in the analysis because higher and lower leaves were analysed together.

At later growth stages, stunted growth of diseased plants and leaf area reduction generally become more apparent. These symptoms and the unnatural stiffness confer a quite specific habitus to diseased plants so that they 'can be seen through', which is certainly not so in healthy plants. In these latter, the leaves at the lower half of the plant may overshadow each other even to the extent that yellow patches develop.

It has been reported that virus-laden tubers often remain hard and non-putrefied in the soil (*cf.* Coïc, 1945). In a few cases, undecayed mother tubers indeed were found at the time of the harvest, but not more frequently in diseased plants than in healthy ones. Though the stems of the diseased plants were shorter than those of the healthy ones, about the same number of leaves was present in both. Flowering also remained unaffected.

Several of the above observations now may be illustrated with data collected in the first two years of the experiments (*cf.* Tables I and II; Fig. 1).

Stem length (*cf.* Table I) appears considerably reduced in the diseased plants of 1958, much more than in 1957 when symptoms were milder. It will be noticed that also large differences in stem length may occur between healthy plants grown in different years.

In 1958, leaves were sampled at various times during the growing season. Since mostly the lower leaves and those higher at the stem were collected separately, a fairly complete picture can be given for that year with regard to leaf surface reduction in diseased plants. This is illustrated in Fig. 1.

Direct measurements of leaf area were not always obtained, the various leaves in Fig. 1, therefore, have been represented by the calculated residual dry weight (dry weight less total carbo-

hydrate); leaf area reduction in diseased plants may then be estimated (*see* percentages to the right). The petiole weight in Fig. 1 has been given as 'corresponding' weight, which is the residual dry weight, corresponding to 10 g residual lamina. The respective figures can be found in Tables 6, 8, 10, 12 of the Appendix.

TABLE I. Stem length, fresh weight, and number of leaves per stem in healthy and diseased plants, grown in 1957 and 1958.

Time of harvest	Stem length (cm)		Fresh weight (g)		Number of leaves	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
June 17–20, 1957 (average of 7 stems)	13	11	12.3	10.2	–	–
July31–August 3, 1957 (average of 7 stems)	80	72	102	91	23	21
July 25–26, 1958 (two separate stems)	55(49)	35(38)	66(61)	40(46)	22(23)	21(22)

Leaf area reduction in diseased plants is most apparent in the higher leaves. In 1958, this was about 40% in the 15th leaf as compared with an average of 25% in the 5th leaf.

Fig. 1 shows clearly that the 10th leaves, sampled in the experiments of July 3–4 and July 7–9, at that time were not yet fully developed. Especially the weight of the diseased leaves is markedly less than that of the full-grown leaves sampled in the experiments of July 25–26 and August 11–13 (*cf.* also Table VI, Chapter V).

The average corresponding petiole weight is about the same in the different diseased leaves. In healthy leaves, a clear decrease is observed when going from lower to higher leaves. From this again, it may be derived that the reduction in leaf area is most evident in the higher leaves, which seems in agreement with

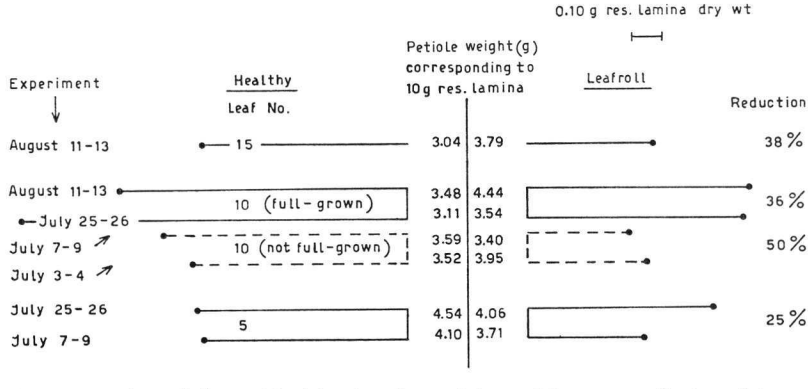


FIG. 1. Presentation of the residual lamina dry weight and 'corresponding' petiole weight of various leaves along the stem, and reduction in leaf area in diseased plants (experiments of 1958).

the general observation that the disease is revealed more clearly in relation to the development of the plant.

More detailed data on tuber yield of diseased plants are presented in § 2 of this chapter (*cf.* Table III). Some comments on the number and size of the tubers may be given here (*cf.* Table II).

TABLE II. Number and size of tubers, and total yield as observed at two different harvests in 1957.

	August 1–10, 1957 (7 plants)		August 10–20, 1957 (18 plants)	
	Healthy	Diseased	Healthy	Diseased
Total number	104	47	223	122
Large	21(20%)	14(30%)	30(13%)	23(19%)
Medium	–	–	71(32%)	43(35%)
Small	83(80%)	33(70%)	122(55%)	56(46%)
Total yield	3170 g	1950 g	8060 g	4430 g

It has been remarked before that there are relatively few stolons in diseased plants, which, moreover, are extremely short. The number of tubers appears accordingly small.

The data on tuber size in Table II were obtained at two different harvests in 1957. In the first harvest, only large and small tubers were distinguished, whereas in the second harvest medium-size tubers were grouped separately. Although more data would be needed to draw definite conclusions, it would appear that in diseased plants proportionally more large tubers are produced than in healthy plants.

§ 2 EFFECTS OF WEATHER CONDITIONS ON SYMPTOMS

The expression of superficial symptoms in leafroll diseased plants varied widely in the different years in which experiments were made. Whereas the disease symptoms in 1957 and 1960, especially towards the end of the growing season, were mild, they were severe in 1958 and 1961, and remained so throughout the season[1]).

Weather conditions and mineral nutrition both have an effect on symptoms in diseased plants (for references, *see* Chapter I, § 1*d*). Since the experimental fields in which our plants were grown are equally well dressed with fertiliser

[1]) In 1959, no experiments comparable with those of the other years were performed. Most of the time had to be used for analysis of the extensive series of samples collected in 1958, so that only preliminary observations along some other lines could be made.

each year, only correlations with the prevailing weather conditions could be expected.

In table III, data are presented on superficial symptoms, carbohydrate content of the leaves, and tuber yield as observed in four different years. Weather conditions are summarized in Table IV.

TABLE III. Superficial symptoms, carbohydrate content of leaves, and tuber yield in four different years. Carbohydrate content in mg/10 g res. dry wt; tuber yield in g per plant (one shoot only, lateral branches were removed).

		1957	1958	1960	1961
Date of planting		May 13	May 23	June 3	May 19
Superficial symptoms	(June 10–20)	moderate	severe	mild	severe
Carbohydrate content of full-grown leaves (Healthy-Diseased)	(July 20–31)	1480–1920	680–1680	2600–2380	1070–1800
Tuber yield (Healthy-Diseased)	(August 10–20)	450–250			
	(September 1–10)			290–150	
	(September 20–30)		600–200		no data

The features presented in Table III and their possible relation with the weather conditions during growth may now successively be discussed.

a Superficial symptoms

Disease symptoms in Table III have been characterized as mild, moderate and severe. The denotation was mainly on the examination of superficial characteristics such as stunted growth, yellowing of the foliage and marginal curling of the lower leaves.

The mild superficial symptoms as observed in 1960 might well be related to the exceptional weather in that year. During the months June, July and August only about one-third of the sunshine in the other years was measured; rainfall was accordingly high. Especially the low light intensities may have influenced symptom expression in 1960. Notably these have been reported to suppress development of the external symptoms in leafroll diseased plants (WILSON, 1955; WATSON and WILSON, 1956).

Further pertinent connections between symptoms and weather conditions could not be found, in 1957, *e.g.*, symptoms were milder than in 1958 and 1961, but weather conditions did not appear much different.

b Carbohydrate content of leaves

An unusually large carbohydrate content was observed in 1960, in the leaves of both healthy and diseased plants. This again might be related to the low

TABLE IV. Weather conditions during the growing season in four different years[1]).

	Rainfall (mm per day)				Temperature (°C)				Sunshine (min per day)			
	1957	1958	1960	1961	1957	1958	1960	1961	1957	1958	1960	1961
June	1.5	1.4	1.9	1.5	Max. 23.0 Min. 11.4	20.3 10.7	22.4 12.2	21.6 11.5	440	280	130	390
July	2.6	3.6	4.1	3.1	Max. 23.3 Min. 14.0	22.2 13.3	20.3 12.1	20.5 12.2	300	300	90	240
August	4.5	2.8	5.2	3.2	Max. 20.1 Min. 12.6	22.5 14.2	21.1 12.6	21.2 12.7	280	260	70	300
Average	2.9	2.6	3.7	2.6	Max. 22.1 Min. 12.7	21.7 12.7	21.3 12.3	21.1 12.1	340	280	100	310

[1]) The data were obtained by courtesy of the Laboratory for Physics and Meteorology of the Agricultural University at Wageningen.

light intensities during growth, as following observations suggest.

POHJAKALLIO (1951) and BODLAENDER (1963) report that low light intensities promote haulm growth in the potato, which is accompanied by a sharp decrease in tuber yield. It would seem that in plants growing under these conditions the translocation stream, which at the later growth stages normally is in the direction of the tubers, is now largely reversed. Though relevant data, as far as we know, are not available, a possible explanation for this might be that low light intensities cause a delay in the induction of tuber formation. Carbohydrates which are not consumed in stimulated haulm growth might then accumulate in the leaves.

It remains difficult to see why, in 1960, in diseased leaves no surplus accumulation over that in healthy leaves occurred since the tuber yield of diseased plants was about one-half that of healthy ones. Presumably, decreased rate of photosynthesis and leaf area reduction in diseased plants should each be taken into consideration.

The carbohydrate content of the healthy leaves in 1957 also seems to be somewhat higher than that in 1958 and in 1961. The general weather conditions in 1957, however, were not much different from those in 1958 and 1961, so that there are no indications for any correlation.

c Tuber yield

The tuber yield of diseased plants usually was about half or less than half the yield of healthy plants.

Nearly this reduction was observed also in 1960 when, especially towards the end of the growing season, diseased plants superficially could be hardly recognized from healthy ones. A similar observation was made by FELTON (1948), who studied the effect of nitrogen application in potato leafroll. It appeared that in the + nitrogen series, diseased plants could not be distinguished with certainty from healthy ones, whereas there was a large difference in tuber yield. It would thus seem that superficial symptoms alone cannot serve as a reliable measure for the severity of the disease.

In 1960, the yield of both healthy and diseased plants was small as compared with that in the other years. There seems to be a marked relation with the carbohydrate content of the leaves, which, as discussed in section *b*, was exceptionally high in that year.

CHAPTER IV

MEASUREMENTS OF PHOTOSYNTHESIS

§ 1 Photosynthesis in leaves of diseased plants

The measurements of photosynthesis described in this chapter were made in 1958, when leafroll symptoms were severe. Rates of photosynthesis were determined in both younger and older leaves, several times during the season.

In Fig. 2, the results of a typical measurement are presented; rates of photosynthesis in the 5th and 10th leaves of healthy and diseased plants are compared at different light intensities (leaves were numbered starting near the base of the stem; imperfectly developed leaves low at the stem were disregarded).

The rate of photosynthesis in the younger (10th) diseased leaf is the same as that in the comparable healthy leaf; that in the older (5th) diseased leaf appears considerably reduced, both at high and low light intensities. Rates of photosynthesis recorded in Fig. 2 have been corrected for respiration which is usually higher in diseased leaves than in healthy ones. Measurements of respiration, however, were not very accurate because of the relatively small difference between readings.

The above results are in agreement with those obtained elsewhere (for referen-

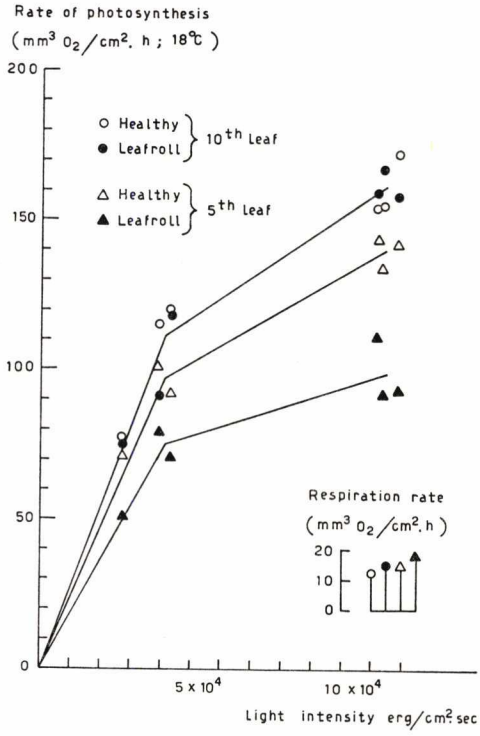


FIG. 2. Rate of photosynthesis at relatively high CO_2-concentration (1.26%) in the 5th and 10th leaves of healthy and diseased plants. Plants are about two months old.

ces, *see* Chapter I, § 1*b*). Most of these earlier publications deal with photosynthesis under field conditions, and generally large differences between healthy and diseased leaves are observed[1]).

In the measurements presented in Fig. 2, the rate of photosynthesis in healthy leaves at the highest light intensity applied was about 160 mm^3 $O_2/cm^2.h$ (18 °C). This intensity of 11×10^4 $erg/cm^2.sec$, evidently, is not sufficiently high to attain full light saturation of photosynthesis.

It may be assumed that a further increase in light intensity would have resulted in a higher rate of photosynthesis, as in fact was observed in later measurements. In leaves of healthy plants, growing in culture solutions, rates of photosynthesis in the order of 250–300 mm^3 $O_2/cm^2.h$ (20 °C) were measured at light intensities of 15×10^4 $erg/cm^2.sec$. In the later measurements, however, larger leaf discs were used (1.58 cm^2 as compared with 0.28 cm^2), and higher CO_2-concentrations applied (3.88 % as compared with 1.26 %).

In literature, several measurements on photosynthesis in potato plants are reported. Rates of photosynthesis of about 250 ml $CO_2/cm^2.h$ were found by LUNDEGÅRDH (1922), and by CHAPMAN and LOOMIS (1953). Much lower rates, in the order of 110–120 mm^3 $CO_2/cm^2.h$, were found by VON WITSCH and POMMER (1954), and by WINKLER (1961). The obvious difference in photosynthetic rate is probably due to differences in experimental conditions. The higher rates were measured at relatively high CO_2-concentrations (0.15 %), the lower rates in normal air (0.03 % CO_2).

§ 2 POSSIBLE REASONS FOR REDUCED PHOTOSYNTHESIS IN LEAVES OF DISEASED PLANTS

Several internal factors influencing photosynthesis are modified by the leafroll disease. At one time or another, most of these have been held responsible for the reduced rate of photosynthesis in diseased plants. In Table V, some data on such factors are assembled; they have been collected in two parallel series of experiments, in which photosynthesis was measured at 18 °C and 15 °C respectively.

For each series, seven plants were selected, on each of which three successive leaves at the lower part of the stem were marked so that they could easily be recognized. At three different times in the growing season, one of these leaves was collected. The rate of photosynthesis was then measured, while simultaneously the other determinations were made.

The following remarks may be made on possible correlations.

1. The chlorophyll content of diseased leaves is reduced, especially in older plants (Column II). In the potato variety 'Alpha', infected with a moderately virulent strain of the leafroll virus, the reduction may amount to as much as

[1]) Increased respiration rates in leaves of diseased plants are also found by CLINCH and co-workers (University of Dublin). They observe that respiration in diseased plants increases with leaf age, whereas respiration in healthy plants normally is greater in younger than in older leaves (private communication).

TABLE V. Rate of photosynthesis in leaves of healthy and diseased plants, in relation to several internal leaf factors (*see* text). Column I: Rate of photosynthesis (mm^3 $0_2/cm^2.h$; 80,000 $erg/cm^2.sec$); II: Chlorophyll content per unit leaf area (colorimeter readings); III: Percentage dry matter in lamina; IV: Water content of lamina (mg/100 discs $\sim$ 28 cm^2); V and VI: Carbohydrate content of lamina (mg/100 discs $\sim$ 28 cm^2; starch and sugar respectively); VII, VIII and IX: Phosphate content of lamina (μg/20 discs $\sim$ 5.6 cm^2; inorganic, TCA-soluble organic and TCA-insoluble P respectively). Plants emerged in the first week of June.

	I	II	III	IV	V	VI	VII	VIII	IX
Series I (18 °C)									
Healthy leaf									
June 26	164	0.49	8.9	1170	0.75	4.95	18.5	19.5	58.2
July 10	148	0.54	11.0	1130	1.70	9.00	17.4	19.6	36.4
July 31	124	0.43	12.4	1000	2.15	7.50	14.3	19.8	34.1
Diseased leaf									
June 26	161	0.43	12.1	910	3.15	9.30	25.8	21.3	79.5
July 10	136	0.46	13.6	990	5.70	17.40	16.2	21.8	39.8
July 31	95	0.26	16.6	810	15.10	21.00	19.5	22.3	31.6
Series II (15 °C)									
Healthy leaf									
June 27	146	0.49	9.3	1150	0.35	4.50	18.1	19.6	50.0
July 11	136	0.55	10.1	1230	2.95	10.50	14.6	20.5	36.5
July 28	113	0.41	11.1	1080	0.65	6.60	12.7	19.1	34.4
Diseased leaf									
June 27	132	0.47	12.4	970	8.80	13.20	18.4	19.8	66.1
July 11	113	0.41	13.7	980	6.15	18.30	14.9	22.9	40.7
July 28	83	0.30	16.2	800	11.60	20.10	14.5	19.4	34.5

30%. The reason for the decrease in chlorophyll content is not known; the diffuse yellowing of leaves, characteristic for the 'yellows' type of virus disease, probably is due to a cause different from that producing the spotted pattern in leaves of plants affected by the mosaic type of disease. Repeatedly, it has been assumed that the decrease in chlorophyll content of diseased leaves is one of the main causes for the reduced rate of photosynthesis (BODE, 1951).

A study on the effect of different chlorophyll concentrations on photosynthesis in various plant species has been published by GABRIELSEN (1948). The chlorophyll factor in photosynthesis, according to this author, is only noticeable at low light intensities as rarely found in nature; it is therefore of little importance under ecological conditions. When this conclusion is applied to the leafroll disease, it might follow that the decrease in photosynthesis, observed in that part of the curve in which O_2 production is proportional to light intensity, is partly due to the reduction in chlorophyll content. At the higher light intensities, the effect of chlorophyll would be concealed by the effect of other factors, especially those influencing the dark processes in photosynthesis.

2. The accumulation of carbohydrate in the lamina is one of the most conspic-

uous symptoms of the leafroll disease. As indicated by the data in columns V and VI, there is a large increase with time in both starch and sugar content of diseased leaves. The presence of large quantities of carbohydrate in the leaves of diseased plants often has also been considered as a possible cause for the reduced rate of photosynthesis (VON WITSCH and POMMER, 1954). MÜLLER (1932), however, found no improvement of photosynthesis when diseased plants were kept in the dark for a short time, and lost part of the accumulated carbohydrate.

Theoretically, an effect of carbohydrates on the rate of photosynthesis can be expected only when they accumulate in the actual reaction chain. This, in most cases, is at least partly prevented by the deposition of carbohydrate as starch. Indeed, the greater proportion of the carbohydrate which is accumulated in diseased leaves occurs as starch. Sugars, however, accumulate as well, but in our later discussion (*cf.* Chapter VI) it is suggested that at least part of these sugars is stored at intra-cellular sites not directly involved in normal carbohydrate interconversions. Accumulation of carbohydrate in the lamina, thus, not necessarily leads to reduction in the rate of photosynthesis. In some instances, however, a correlation between the sugar content of leaves and photosynthesis has been demonstrated (MOSS, 1962).

3. The water content per unit leaf area, which has been determined as the difference between fresh and dry weights of samples, is decreased in diseased leaves as compared with healthy ones (Column IV). Together with the increased carbohydrate content, it accounts for the relatively high percentage of dry matter (Column III).

Any effect of the water content of leaves on photosynthesis, presumably, is mediated by changes in hydration of the protoplasm which may influence its permeability for carbon dioxide (SIMONIS, 1952). As discussed under 4, there are indications that there is relatively more protoplasm per unit leaf area in diseased leaves; when this is added to the decreased water content, it might be suggested that dehydration of protoplasm is at least one of the reasons for the reduced rate of photosynthesis.

4. The phosphate content of diseased leaves generally is increased as compared with that of healthy leaves, especially the organic phosphate content (Columns VII, VIII and IX).

The effect of inorganic P on photosynthesis is discussed by LINDEMAN (1952), and by PIRSON *et al.* (1952). Both observe a decrease in rate of photosynthesis, in *Lemna* and *Ankistrodesmus* respectively, with a decrease in inorganic P-content. The inorganic phosphate content in diseased leaves, however, is much the same as that in healthy leaves (Column VII) so that no effect on photosynthesis in diseased plants can be expected.

The TCA-soluble, organic phosphate fraction in diseased leaves appears to increase consistently with leaf age (Column VIII). This observation as such, however, does not contribute to the understanding of the decrease in photosynthesis. More information might be expected from a further separation of this fraction; work along this line is in progress.

The relative increase in the TCA-insoluble phosphate fraction in diseased leaves (Column IX) might indicate relatively more protoplasm per unit leaf area, as already referred to under 3.

5. Apart from the factors discussed above, some other particulars of the diseased plant may affect its rate of photosynthesis, especially under field conditions. NEGER (1919) and MÜLLER (1932) found that the stomata of diseased leaves were less open than those of healthy leaves, which increases stomatal diffusion resistance. Since diffusion of carbon dioxide towards chloroplasts usually is the rate limiting factor in photosynthesis, stomatal opening in diseased leaves certainly is one of the factors responsible for the low rate of photosynthesis, at least under field conditions.

Partial opening of stomata cannot account for the decreased rate of photosynthesis in diseased leaves as observed in our own measurements. Relatively high CO_2-concentrations as applied in our measurements (1.26%) probably overcome stomatal diffusion resistance (GAASTRA, 1959).

The rate of photosynthesis under field conditions may further be limited by the unnatural, stiff appearance of the diseased plants, and by the marginal curling of the leaves.

Several internal leaf factors influencing the rate of photosynthesis appear considerably modified by the leafroll disease. It is not easy to decide which of these are predominant in the reduction of the rate of photosynthesis in diseased plants. Decreased chlorophyll content, and increased carbohydrate content probably are of small significance. Dehydration of protoplasm may be more important because it may indicate both increased internal diffusion resistance for CO_2 and quantitative reduction of the ultimate reducing agent.

CHAPTER V

TRANSLOCATION AND DAILY FLUCTUATIONS OF CARBOHYDRATE IN LEAVES

§ 1 Introduction and qualitative sugar composition

The presence of relatively large carbohydrate contents in leaves of diseased plants can be explained best when it is visualized that during translocation less carbohydrate is removed from these leaves than from those of healthy plants. In experiments, carried out in several years, it was tried to demonstrate the assumed difference in translocation between healthy and diseased leaves by comparing carbohydrate contents of evening and morning samples. The experiments were carried out at different times during the growing season, so that it proved possible to obtain at the same time information on the progress of the accumulation of carbohydrate in diseased leaves with time.

Daily fluctuations of carbohydrate were studied mainly to see whether these fluctuations in diseased leaves, though presumably at a high level of accumulated carbohydrate, show the same general pattern as in healthy leaves. These experiments were also carried out at different times during the growing season.

In both types of experiments, lamina and petiole were analysed separately, with a view to the localization of the assumed disturbance in the phloem. Part of the carbohydrate removed from the lamina, *e.g.*, might be retained in the petiole, and not be translocated further up or down the stem.

As pointed out in Chapter I, it has been suggested by several investigators that carbohydrate interconversions might be affected by the leafroll disease. Especially for that reason, a complete carbohydrate analysis was carried out in most experiments so that the carbohydrate interconversions in the leaf could be studied in greater detail.

By chromatography of ethanolic extracts from potato leaves, the presence of sucrose, glucose and fructose in all parts of the plant is easily demonstrated. The presence of some more sugars (probably oligosaccharides) may be demonstrated as well, but these occur in only relatively small quantities; for this reason, they have not been included in our discussion. The same sugars which are found in healthy plants also occur in diseased ones, so that qualitative differences in sugar composition do not exist.

§ 2 Brief description of experiments

a General survey

In 1957, only experiments on translocation were carried out. At two different times in the growing season, leaves were sampled in the evening and in the morning on several successive days (experiments of June 17–20 and July 31–August 3). In this year, disease symptoms were moderately severe and differences in translocation between healthy and diseased leaves were small.

Most of the experiments described in this chapter were carried out in 1958, when both nocturnal translocation (experiments of July 7–9 and August 11–13) and daily carbohydrate fluctuations (experiments of July 3–4 and July 25–26) were studied. In 1958, disease symptoms were severe; differences in translocation between healthy and diseased leaves were generally large, and daily fluctuations in diseased leaves occurred at a much higher level of carbohydrate than those in healthy leaves.

In 1960, only daily fluctuations were studied (experiments of July 29–30 and August 4). Disease symptoms in this year were mild, and daily fluctuations occurred at the same high level in leaves of both healthy and diseased plants, presumably owing to accumulation of carbohydrate in healthy leaves as well.

The last experiment in this chapter is that of July 24–25, 1961, when nocturnal translocation from different leaflet pairs of the compound potato leaf was studied. In 1961, leafroll symptoms were severe, and differences in translocation between healthy and diseased leaves again were demonstrated.

b Sampling techniques

Leaves for analysis in most experiments were collected according to a similar scheme (experiments of 1957; experiments of July 3–4 and July 25–26, 1958). The number of plants selected was the same as that of the samples. Per sample, one leaf was collected from each plant, taking care that the material especially with respect to leaf age was evenly distributed.

In 1957, as well as in the experiment of July 3–4, 1958, both young and older leaves were collected in the same samples. In the experiment of July 25–26, 1958, the '5th' and '10th' leaves were sampled separately, *viz.* (since 6 successive samples were taken) one of 6 leaves round the 5th, and one round the 10th.

In order to concentrate more clearly on sampling leaves with distinct numbers, fewer samples were taken, and more plants used, in the experiments of July 7–9 and August 11–13, 1958. In both experiments, carbohydrate contents of evening and morning samples were compared in two successive nights; in each night, four different plants were used. In the experiment of July 7–9, the 5th (or 6th) and 10th (or 11th) leaves were collected at sunset, and the 6th (or 5th) and 11th (or 10th) leaves at sunrise, the following day. The same method was followed in the experiment of August 11–13, when the 10th and 15th leaves were sampled[1]). Although in these latter experiments, the distinction between the different leaves was comparatively exact, it cannot be claimed that the 10th leaf in the experiment of July 7–9 was precisely the same as that with the same number in the experiment of August 11–13: in the meantime, some of the lower leaves may have decayed.

In the above method, making use of entire leaves, usually satisfactory results were obtained in case of healthy plants. In the experiments on daily fluctuations, *e.g.*, normal curves were obtained, with differences in carbohydrate content between the successive samples, corresponding fairly well to the time of the

[1]) The numbering of leaves was according to sequence of emergence. The first few leaves low at the stem which are usually imperfectly developed were not included.

day. The results obtained for diseased plants appeared less reliable; irregular curves were often obtained, showing large and inconsistent differences in carbohydrate content between successive samples. The reason for this may be a rather large variation in carbohydrate content, either between corresponding leaves on different plants, or between different leaves on the same plant. The relatively small number of leaves collected per sample, then, might not yield reliable average carbohydrate contents, representative for the time of the day at which the samples were taken.

Since the selection of a greater number of plants was disadvantageous in other respects, it was tried, in later experiments carried out in 1960, to reduce the variability of the material by collecting leaflets instead of entire leaves. In the experiment of July 29–30, and in that of August 4, seven leaves on different plants were selected from which the leaflets were collected in such a way that they constituted complete, diversely composed 'leaves' in each of the samples (*cf.* Fig. 3). The method entailed that petiole material could not be collected.

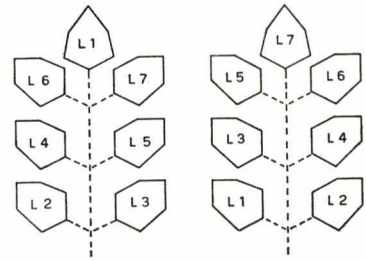


FIG. 3. Example of sampling of leaflets from seven different leaves (L1-L7), at two harvests (*cf.* text).

It turned out, however, that the new method did not improve the results. Apparently, the variation in carbohydrate content between leaflets, now introduced, was equally large as that between entire leaves.

Left and right leaflet halves were collected in the experiment of July 24–25, 1961, when translocation was compared in the 1st and 3rd leaflet pairs of a single leaf. Each of the samples contained six half leaflets, one from each of three successive leaves on two different plants. The remaining opposite leaflets of each pair were used for obtaining additional information on the increase of carbohydrate during the day.

§ 3 PRESENTATION OF RESULTS, AND DISCUSSION OF SOME FEATURES NOT DIRECTLY RELATED TO THE DISEASE

The data on carbohydrate composition, obtained in the experiments of 1957, 1958, 1960 and 1961, have been presented in chronological order in Tables 1–16 of the Appendix. Some of the experiments (data on starch and total sugar only) have been presented in Figs. 4, 5, 6, 7 and 9, illustrating the text in this and the next section.

Most of the experiments were carried out with fair weather conditions. Fluc-

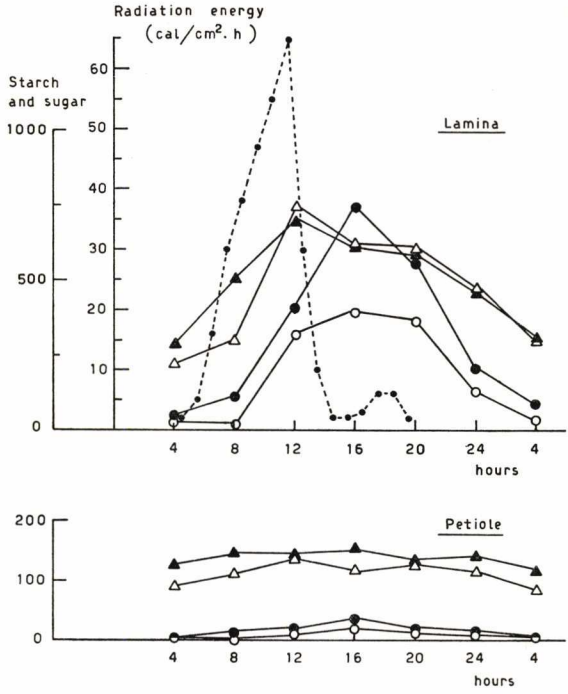


FIG. 4. Fluctuations of starch and sugar (mg) in 10 g res. dry lamina and corresponding petiole (young leaves; experiment of July 3-4, 1958). Healthy leaves: o starch, △ sugar; diseased leaves: ● starch, ▲ sugar.

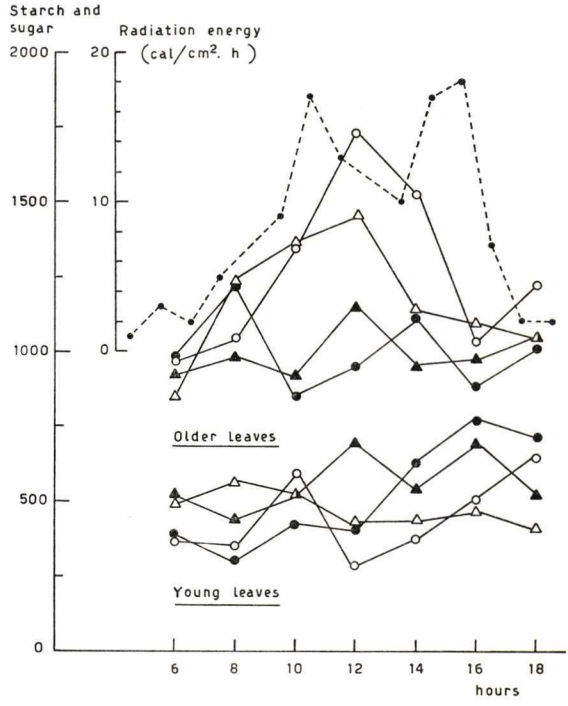


FIG. 5. Fluctuations of starch and sugar (mg) in 10 g res. dry lamina (young and older leaves; part of the experiment of July 29-30, 1960). Healthy leaves: o starch, △ sugar; diseased leaves: ● starch, ▲ sugar.

tuations in carbohydrate content strongly depend on light intensity, as is illustrated in Figs. 4 and 5.

In the experiment of July 3–4, 1958 (Fig. 4), the radiation energy fell off sharply between 12 and 13 h because of the onset of a thunderstorm. The rise in carbohydrate content of the leaves during the first part of the day, then, came to a sudden stop. During the daylight hours of the experiment of July 29–30, 1960 (Fig. 5), there was a heavy drizzle contrary to weather predictions. The radiation energy was correspondingly small, and, in 3 out of the 4 leaves analysed, the carbohydrate content did not rise above the level at the beginning of the day.

Whereas, in most years, a comparatively low carbohydrate content was found in healthy leaves, there was an unusually high carbohydrate content in 1960. A comparison of Figs. 4 and 5 will show the difference. The carbohydrate content of the younger (not yet full-grown) leaves in 1960 is already relatively high, but in the older (mature) leaves it reaches a level which is 3 or 4 times that in the leaves analysed in 1958.

In the experiments of 1958, when disease symptoms were severe, it was not taken into account that growth retardation in diseased plants may lead to differences in leaf development, showing up especially clearly when healthy and diseased leaves with the same sequential number are compared. This, *e.g.*, complicates the interpretation of the results obtained on the carbohydrate composition in young leaves. Anticipating this discussion (*cf.* Chapter VI), it may be remarked here that there is a lower relative starch content in the lamina of diseased leaves than in that of healthy ones, which is clearly related to accumulation. It now appears that in the young, non-accumulating diseased leaves analysed in the experiment of July 3–4, as well as in the 10th diseased leaf analysed in the experiment of July 7–9, there is, conversely, a higher relative starch content than in the corresponding healthy leaves (*cf.* Tables 5 and 7 of the Appendix, and Figs. 4 and 6). In view of the above, this is difficult to explain; it suggests that the observed difference in carbohydrate composition between young healthy and diseased leaves is connected with a difference in leaf development, arising from growth retardation in diseased plants.

A difference in development between young healthy and diseased leaves indeed existed in the experiment of July 7–9, 1958, as may be seen from the comparison, made in Table VI, of leaf areas measured then and later (experiment of August 11–13) in the same year.

The numbering of leaves according to the sequence of their formation may not have quite the same significance in these experiments, so that direct comparison of leaf areas may not always be appropriate. Therefore, leaf areas in diseased plants have also been given in percentages of those in healthy plants. It appears that during the interval between both experiments the 10th diseased leaf has increased considerably in size, whereas the corresponding healthy leaf has not, which implies that in the experiment of July 7–9 a large difference in leaf development existed.

It is true that similar differences in leaf development exist both when young

TABLE VI. Comparison of leaf areas, determined in the experiments of July 7–9 and August 11–13, 1958. Figures indicate total area of 4 leaves in cm².

	July 7–9			August 11–13	
	Healthy	Leafroll		Healthy	Leafroll
Leaf No. 5					
July 7, 20 h	770	360(47%)			
July 8, 4 h	850	390(46%)			
July 8, 20 h	520	360(69%)			
July 9, 4 h	480	300(62%)			
Average	660	350(53%)			
Leaf No. 10			*Leaf No. 10*		
July 7, 20 h	900	370(41%)	August 11, 19 h	1080	740(69%)
July 8, 4 h	970	390(40%)	August 12, 5 h	1040	780(75%)
July 8, 20 h	880	410(47%)	August 12, 19 h	840	750(89%)
July 9, 4 h	920	480(52%)	August 13, 5 h	750	580(77%)
Average	920	410(45%)	Average	930	710(76%)
			Leaf No. 15		
			August 11, 19 h	680	290(43%)
			August 12, 5 h	880	490(56%)
			August 12, 19 h	780	390(50%)
			August 13, 5 h	670	380(57%)
			Average	750	390(52%)

and older leaves are compared. Connected differences in physiology, however, may show up especially clearly in younger leaves because in that case mature or fully developed healthy leaves are compared with immature and still expanding diseased ones.

The above suggestion that differences in leaf development may be connected with difference in carbohydrate composition seems supported by the results of the experiment of July 29–30, 1960. Then, immature leaves of both healthy and diseased plants were selected according to external appearance, and differences in carbohydrate composition were not observed (*cf.* Table 13 of the Appendix). However, disease symptoms were mild in that year and, moreover, healthy plants accumulated carbohydrate to the same degree as diseased plants.

§ 4 DISCUSSION OF RESULTS

a Accumulation of carbohydrate in lamina, petiole and stem, in relation to translocation

The data obtained in the experiments of July 7–9 and August 11–13, 1958, on nocturnal carbohydrate translocation from different leaves, are illustrative for the accumulation pattern in diseased plants; they are reproduced in Figs. 6 and 7.

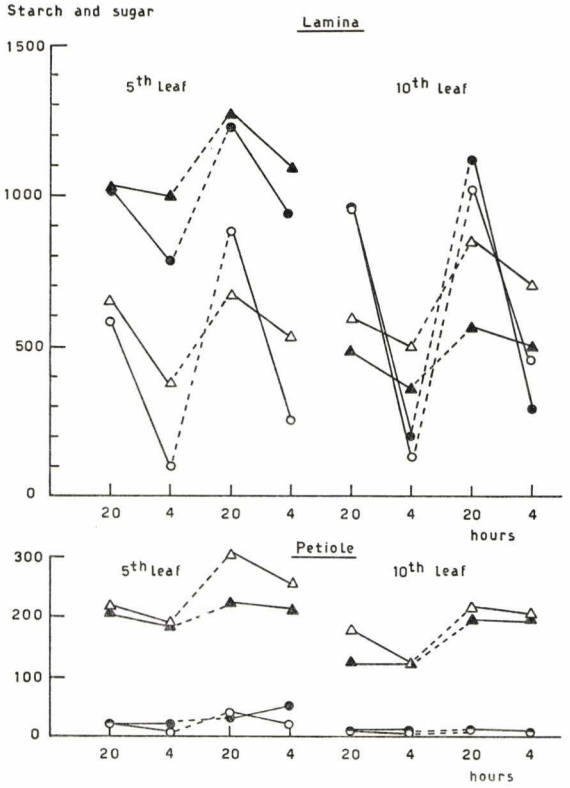


FIG. 6. Translocation of carbohydrate (mg) from 10 g res. dry lamina and corresponding petiole (5th and 10th leaves; experiment of July 7-9, 1958). Healthy leaves: o starch, △ sugar; diseased leaves: ● starch, ▲ sugar.

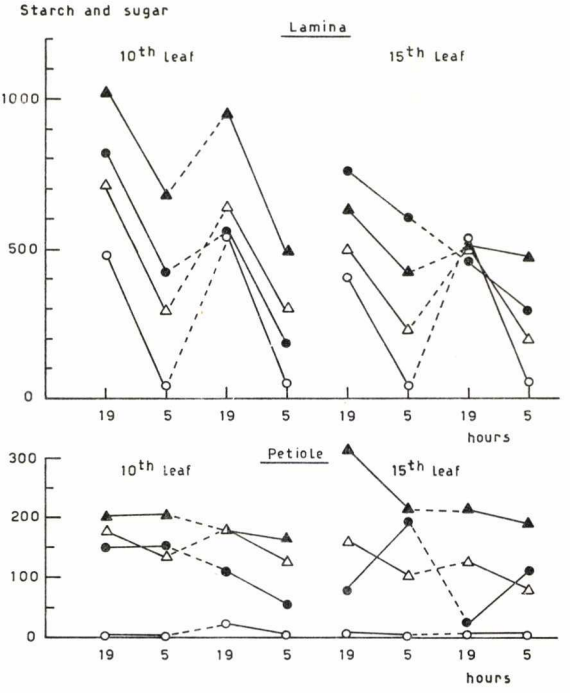


FIG. 7. Translocation of carbohydrate (mg) from 10 g res. dry lamina and corresponding petiole (10th and 15th leaves; experiment of August 11-13, 1958). Healthy leaves: o starch, △ sugar; diseased leaves: ● starch, ▲ sugar.

It appears that leaves of diseased plants accumulate carbohydrate as soon as they reach maturity. Only in the 10th diseased leaf, analysed in the experiment of July 7–9, and immature at that time, no accumulation is observed.

The largest accumulation in the lamina is usually found in leaves at the lower part of the stem, *e.g.*, in the 5th leaf analysed in the experiment of July 7–9. The lamina accumulation never reaches the same high values in leaves at the higher part of the stem. The largest accumulation in the petiole, conversely, is found in the higher leaves, *e.g.*, in the 15th leaf analysed in the experiment of August 11–13.

The above difference in accumulation pattern between leaves in different positions on the stem is also observed in the experiment of July 25–26, 1958, discussed later in this chapter (*see* under *b*). The data obtained in this latter experiment, together with those illustrated above, are reproduced in Fig. 8, in which the accumulation in lamina and corresponding petiole has been calculated as the difference in carbohydrate content between diseased and healthy leaves.

Fig. 8 clearly shows that when the accumulation in the lamina is large (5th

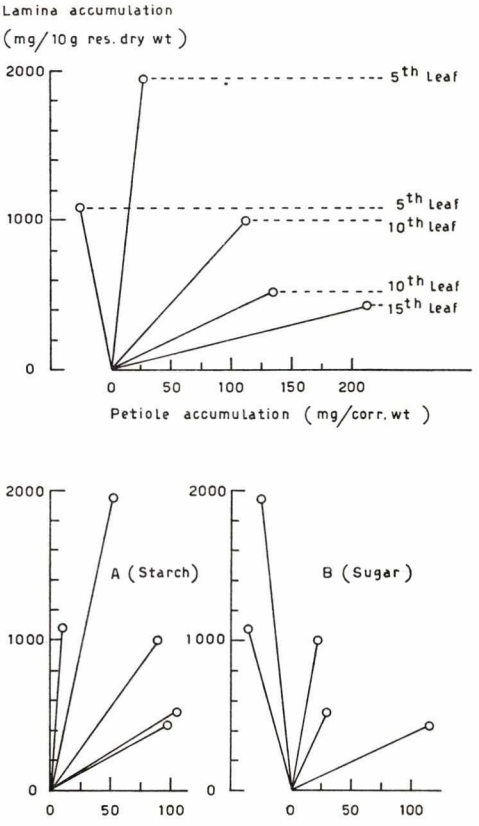


FIG. 8. Accumulation of carbohydrate in lamina and petiole of leaves in different positions on the stem. Average values; experiments of July 7-9 (5th leaf), July 25-26 (5th and 10th leaves) and August 11-13 (10th and 15th leaves).

leaf), there is only a small accumulation in the petiole, and *vice versa* (10th and 15th leaves). It further appears (*cf.* Figs. A and B), that the accumulation in the petiole is mainly on account of starch; sugar accumulates only in petioles of higher leaves.

Accumulation of carbohydrate in the lamina and accumulation in the petiole, thus, are conversely related. It may, moreover, be concluded that, apart from the general effect of leaf age on accumulation, there is another effect which is related to the position of the leaf on the stem.

The data on accumulation in lamina and petiole obtained in the experiments of July 7–9 and August 11–13, may now be compared with the data on translocation determined in the same experiments as the difference in total carbohydrate content between evening and morning samples (*cf.* Table VII).

Translocation of carbohydrate from both lamina and petiole of the different healthy leaves is remarkably constant. No significant difference is observed, *e.g.*, between the experiment of July 7–9 and that of August 11–13.

Translocation from the lamina of diseased leaves mostly, but not always, appears reduced. A large relative decrease in translocation is observed in the 5th leaf (July 7–9), and in the 15th leaf (August 11–13). The absence of a decrease in translocation from the 10th leaf analysed in the experiment of July 7–9, probably, is connected with the absence of accumulation in the lamina of this leaf. In the 10th leaf analysed in the later experiment of August 11–13, however, there is a distinct accumulation in the lamina, while translocation seems uninhibited.

Translocation from the petiole of diseased leaves appears reduced in all leaves. The data obtained in the 1st and the 2nd night of the experiments are not always consistent, but in general a good correlation with the data on accumulation is obtained. It may well be assumed that part of the carbohydrate which is removed from the lamina is subsequently retained in the petiole. In several instances, this leads to an increase in the carbohydrate content of the petiole overnight, as in the 15th leaf analysed in the experiment of August 11–13.

Experiments on translocation have also been carried out in 1957 and 1961. In the experiments of 1957, leaves along the whole length of the stem were collected for analyses, and, presumably partly because of this and partly in connection with the absence of severe leafroll symptoms, no clear indications for inhibited translocation from diseased leaves were obtained (*cf.* Tables 1–4 of the Appendix). In the experiment of July 24–25, carried out in 1961 when leafroll symptoms were much more severe than in 1957, a relative decrease in translocation from both the 1st and 3rd leaflet pairs of a single diseased leaf again could be demonstrated (*cf.* Table 16 of the Appendix).

It thus appears that in diseased leaves, generally, a relative decrease in the amount of carbohydrate lost during the night from lamina and petiole can be demonstrated. A clear correlation with accumulation, however, is not always evident. Part of the decrease during the night is on account of respiration, and introduces an uncertainty. Furthermore, it appears difficult to obtain exact figures on the carbohydrate loss from diseased leaves. Relatively large differences, *e.g.*, are observed in different nights, especially in petioles. The most

TABLE VII. Translocation of carbohydrate (mg) from 10 gram residual dry lamina and corresponding petiole (experiments of July 7–9 and August 11–13, 1958).

Experiment of July 7–9					Experiment of August 11–13				
	Total carbohydrate (Lamina)		Total carbohydrate (Petiole)			Total carbohydrate (Lamina)		Total carbohydrate (Petiole)	
Healthy leaf, No. 5					Healthy leaf, No. 10				
20 h	1230		239		19 h	1190		183	
4 h	480	—750	200	—39	5 h	330	—860	147	—36
20 h	1550		342		19 h	1180		204	
4 h	780	—770	271	—71	5 h	350	—830	128	—76
Diseased leaf, No. 5					Diseased leaf, No. 10				
20 h	2060		226		19 h	1840		346	
4 h	1780	—280	205	—21	5 h	1100	—740	351	+ 5
20 h	2500		257		19 h	1510		287	
4 h	2020	—480	263	+ 6	5 h	670	—840	217	—70
Healthy leaf, No. 10					Healthy leaf, No. 15				
20 h	1540		185		19 h	900		167	
4 h	630	—910	123	—62	5 h	270	—630	105	—62
20 h	1870		225		19 h	1030		132	
4 h	1150	—720	211	—14	5 h	250	—780	80	—52
Diseased leaf, No. 10					Diseased leaf, No. 15				
20 h	1450		131		19 h	1390		394	
4 h	550	—900	126	— 5	5 h	1020	—370	405	+11
20 h	1680		207		19 h	970		235	
4 h	790	—890	199	— 8	5 h	760	—210	302	+67

probable reason for this is the variation in carbohydrate content between samples (*cf.* Sampling techniques, § 2*b*).

In the foregoing discussion on accumulation, only the total carbohydrate content of the leaf was considered. In the lamina, both starch and sugar accumulate; the accumulation in the petiole, however, is mainly on account of starch (experiment of August 11–13, 1958; Table 8 of the Appendix). Also in petioles in which no clear accumulation can be demonstrated, a relative increase in the percentage of starch in total carbohydrate can be observed (experiment of July 7–9, 1958; Table 12 of the Appendix). The latter was also noticed in the experiment of July 31–August 3, 1957 (*cf.* Table 4 of the Appendix).

Stem material was analysed at the end of the experiment of July 31–August 3, 1957, but the carbohydrate content appeared the same for stems of healthy and diseased plants. Stems were also collected at the end of the experiment of July 25–26, 1958. The stems of one of the healthy plants and one of the diseased ones, used in the experiment, were divided into four parts, which were analysed separately. The results are presented in Table VIII, in which also the fresh weight and the length of the different stem parts have been indicated.

Again, no accumulation of carbohydrate in the diseased stem is demonstrated As in petioles, a relative increase in starch percentage, however, is evident. It thus seems that leafroll infection in the potato variety 'Alpha' does not lead to increase in the carbohydrate content of the stem, though large quantities of carbohydrate accumulate in lamina and petiole.

b Daily fluctuations of carbohydrate in leaves

Two experiments on daily fluctuations were already presented in § 3, to show the influence of weather conditions, and to illustrate the difference in carbohydrate content between plants grown in 1958 and 1960 (*cf.* Figs. 4 and 5).

In the experiment of July 3–4, 1958 (Fig. 4), with young leaves, daily fluctuations occurred at the same level in healthy and diseased plants. In the healthy plants, the same amount of sugar was observed as in the diseased plants; in the latter, however, relatively more starch was found which was related to differences in leaf development resulting from growth retardation in diseased plants. The curves for sugar were of nearly identical shape in healthy and diseased leaves, indicating that the sampling technique was adequate for non-accumulating leaves. In the petioles of diseased leaves, a clear relative increase in starch was observed, which seems to be a conspicuous symptom of the disease, the more so since it occurs already before there is a significant accumulation of carbohydrate (*see* also under *a*).

Differences in carbohydrate level between healthy and diseased plants neither were observed in the experiment of July 29–30, 1960 (Fig. 5), this time because the healthy plants evidently accumulated carbohydrate as well as the diseased ones. Daily fluctuations were irregular in both, so that it was concluded that the sampling technique, making use either of entire leaves or of individual leaflets, yielded unreliable data for accumulating plants (*cf.* § 2*b*).

Another experiment on daily fluctuations, not presented before, was carried

TABLE VIII. Carbohydrate content (mg/10 g dry wt) in different parts of the stem (experiment of July 25–26, 1958).

	Length (cm)	Fresh wt (g)	Dry wt (g)	Total carb.	Starch	%	Sucrose	Glucose	Fructose
Healthy plants									
(Top)	15	8.66	0.63	640	8	1	300	225	105
	13	16.68	1.58	1020	18	2	450	350	200
	12	18.31	1.96	1240	16	1	610	390	220
(Bottom)	9	17.26	2.03	1210	16	1	560	355	275
	49	60.91	6.20						
Diseased plants									
(Top)	12	5.12	0.43	700	20	3	200	270	210
	10	10.36	1.07	1060	76	7	440	390	150
	8	14.99	1.61	1110	133	12	440	350	190
(Bottom)	8	16.01	1.74	860	96	11	280	395	85
	38	46.48	4.85						

out July 25–26, 1958, when the 5th and 10th leaves were analysed separately; the data are illustrated in Fig. 9, and may be discussed in some more detail.

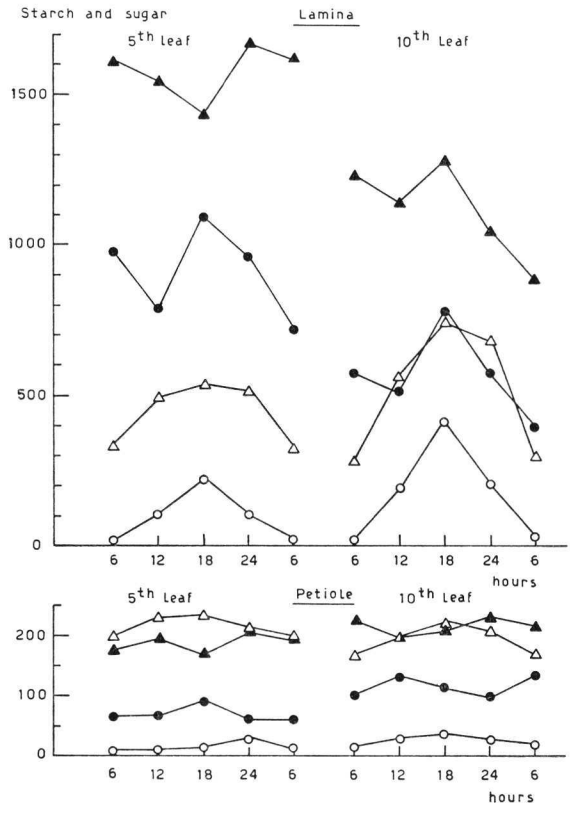


FIG. 9. Fluctuations of starch and sugar (mg) in 10 g res. dry lamina and corresponding petiole (5th and 10th leaves; experiment of July 25-26, 1958). Healthy leaves: o starch, △ sugar; diseased leaves: ● starch, ▲ sugar.

Daily fluctuations in the healthy 5th and 10th leaves occur at the same relatively low level of carbohydrate as observed in all other experiments carried out in 1958. In the early morning hours, there is hardly any starch left in the leaves. The fluctuations in both lamina and petiole show the normal diurnal pattern, and the relatively large increase in carbohydrate content in the lamina of the 10th leaf during the first part of the day probably reflects comparatively high photosynthetic activity in the younger leaf.

Daily fluctuations in the diseased 5th and 10th leaves occur at a relatively high level of accumulated carbohydrate. This applies to both lamina and petiole.

The difference in accumulation pattern between leaves in different positions on the stem (*cf.* Fig. 8) is again clearly illustrated. In the 5th (the lower) leaf, there is more carbohydrate accumulated in the lamina, and less in the petiole, than in the 10th (the higher) leaf. In the lamina, starch and sugar are accumulated; the accumulation in the petiole is restricted to starch, the sugar content of the petiole of the 5th diseased leaf being even smaller than that of the petiole of the corresponding healthy leaf.

The fluctuations in both lamina and petiole of diseased plants appear much less regular than those observed in healthy plants. It is questionable, however, whether the data obtained for diseased plants reflect real diurnal variation. As indicated by the present data, and also by the data obtained in the experiments discussed under *a*, there are relatively large differences in carbohydrate content between different leaves on the same plant. Similar differences occur between corresponding leaves on different plants. This makes adequate sampling of leaf material from diseased plants difficult, and a variation in carbohydrate content between successive samples may easily result, interfering with normal daily fluctuations.

When it is, *e.g.*, assumed that the 10th leaf collected in the sample of 6 h, July 25 (*cf.* Fig. 9) was in a somewhat lower position on the stem than the leaves collected in the other samples, both the relatively high carbohydrate content of the lamina and the relatively low starch content of the corresponding petiole might be accounted for. Similarly, the relatively low carbohydrate content of the lamina of the 10th leaf collected in the sample of 6 h, July 26, and the relatively high starch content of the corresponding petiole, might be explained assuming that this particular leaf was in a somewhat higher position on the stem than the leaves collected in the other samples.

The results on daily fluctuations in lamina and petiole in diseased leaves, thus, are hard to evaluate. The irregular course, as actually observed, may often be explained by assuming variation in leaf material between successive samples (*cf.* small print, above). We should like to suggest that the daily fluctuations in the older diseased leaves are essentially unaffected, as in the younger leaves. They are, however, shifted to a relatively high level of accumulated or residual carbohydrate.

c Survey and conclusions

In the experiments, carried out in 1958 when leafroll symptoms were severe, a distinct accumulation of carbohydrate in leaves of diseased plants could be demonstrated. Whereas the accumulation in the lamina appeared largest in the lower leaves of the plants, the accumulation in the petiole appeared largest in the higher leaves. It was found that, in general, a reasonably good correlation between accumulation in lamina and petiole and inhibited translocation exists, so that it may well be accepted that the accumulation of carbohydrate in diseased plants results from inhibition of translocation. The reason for this inhibition is left out of discussion here; however, it may be safely assumed that it is associated with the presence of the leafroll virus in the phloem.

In order to explain the above accumulation pattern in lamina and petiole, we whould like to suggest that the virus, carried by the mother tuber, proceeds rapidly into the first formed (lower) leaves of the developing sprout, invading even the minor veins in the lamina. The associated phloem disturbance largely prevents translocation so that the bulk of the assimilates is retained *in situ*, and only relatively small amounts enter the petiole. During further development of the plant, the virus obviously proceeds more slowly into the newly developing (higher) leaves, keeping confined to the major phloem bundles of the petiole.

A comparatively large part of the assimilates, thus, can be translocated from the lamina, but is subsequently retained in the petiole in which it accumulates as starch.

The suggestion may be extended to include the progress of the disease into the individual leaflets of a single leaf. Generally, more carbohydrate is accumulated in the top leaflets than in those below, which would indicate that the virus invades the top leaflets first, and the lower leaflets afterwards.

The above suggestion agrees well with the data obtained on the carbohydrate content of the petiole of the 15th diseased leaf, analysed in the experiment of August 11–13. It was observed that in the evening samples a comparatively large sugar content was present, which was replaced by a comparatively large starch content in the morning samples; a decrease during the night in total carbohydrate content could not be demonstrated. It might be derived that, during the day, sugar was translocated from the lamina, but subsequently retained in the petiole. This sugar was retardedly converted into starch during the night.

The results on daily carbohydrate fluctuations in lamina and petiole of accumulating diseased leaves appear complex. Examination of the data obtained in the individual samples suggests that the irregular course of the fluctuations may often be attributed to variation in material between successive samples. It was suggested that in the older leaves, daily fluctuations were essentially unaffected by the leafroll disease; this indeed was found in the younger leaves.

CHAPTER VI

CARBOHYDRATE COMPOSITION

§ 1 Carbohydrate composition in the lamina

The discussion of the carbohydrate composition in the lamina, in section *a*, is based on the data obtained in the experiments of 1958, when leafroll symptoms were severe. The experiments, as described in Chapter V, deal with the carbohydrate involved in diurnal variations; they offer only indirect information on the accumulated carbohydrate in diseased leaves. At the end of this section, the results are compared with those obtained in the experiments of 1957 and 1960, when leafroll symptoms were much less severe, and healthy plants apparently accumulated carbohydrate as well.

In a special experiment, carried out in 1961, several selected leaves of diseased plants were artificially shaded with the result that the accumulated carbohydrate gradually disappeared from these leaves. The analytical data are discussed in section *b*, and apply more specifically to the carbohydrate, retained in diseased leaves during the accumulation process.

a Carbohydrate composition in young and older leaves

α Relation between starch and total carbohydrate. The relation between starch and total carbohydrate during daily fluctuations in young and older leaves is presented in Figs. 10 and 11, respectively.

During daily fluctuations in healthy plants, the same relation between starch and total carbohydrate appears to exist in young and older leaves. The relation is non-linear below 700 mg total carbohydrate, and indicates that from an initial rise in the carbohydrate content of the leaf, an increasingly important proportion is on account of starch. Above the level of 700 mg, the relation is linear so that the proportion of starch in a further increase in the carbohydrate content of the leaf remains constant. When the carbohydrate content of the leaf decreases, the same relations as indicated above are manifest in the reversed order.

In young diseased leaves, the relation between starch and total carbohydrate seems to differ from that in healthy leaves. The data, assembled in Fig. 10, indicate that in the diseased leaves a larger proportion of a rise or fall in total carbohydrate is on account of starch than in the healthy leaves. In Chapter V, § 3, it has been pointed out that the young healthy and diseased leaves, collected in the experiments of 1958, are not strictly comparable since a difference in development could be demonstrated (*cf.* Table VI). It seems possible that the above difference in the relation between starch and total carbohydrate is connected with this difference in leaf development. This suggestion is supported by the data, obtained in the experiment of July 29–30, 1960. In this experiment, leaves of equal development were collected, and a difference in relative starch content between young healthy and diseased leaves was not observed (*cf.* Table 13 of the Appendix).

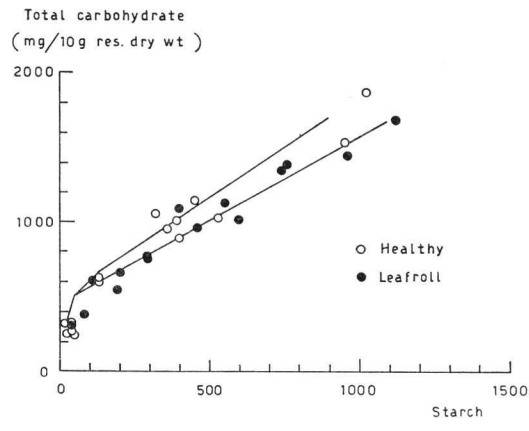


FIG. 10. Relation between starch and total carbohydrate (mg/10 g res. dry wt) in young leaves (experiments of 1958). Healthy: o; diseased: ● (young leaves, July 3-4; 10th leaf, July 7-9; 15th leaf, August 11-13).

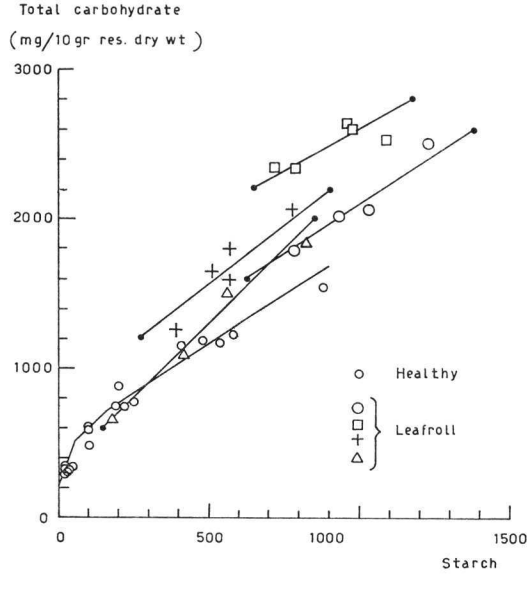


FIG. 11. Relation between starch and total carbohydrate (mg/10 g res. dry wt) in older leaves (experiments of 1958). Healthy: o; diseased: ○ (5th leaf, July 7-9); □ and + (5th and 10th leaves, respectively, July 25-26); △ (10th leaf, August 11-13).

In older diseased leaves, the relation between starch and total carbohydrate has been presented as a set of straight lines, one for each analysed leaf. The presentation in Fig. 11 suggests that the relation between starch and total carbohydrate during daily fluctuations is approximately the same in full-grown healthy and diseased leaves, but shifted in the latter to a high level of accumulated carbohydrate.

In the determination of the □ and + lines, indicating daily fluctuations, the sequence of carbohydrate determinations has been taken into account, so that the slope of the presented lines is indeed the most probable with respect to that of the lines which might be drawn by connecting the carbohydrate contents of the successive samples (*cf.* Table 9 of the Appendix). The ○ and △ lines, indicating translocation during the night, have been constructed by connecting

average carbohydrate values as determined in the evening- and morning samples (*cf.* Tables 7 and 11 of the Appendix).

The slope of three of the presented straight lines appears not much different from the slope of the straight part of the curve, representing the relation between starch and total carbohydrate in healthy leaves. No explanation can be offered for the markedly different slope of the △ line, referring to the 10th leaf in the experiment of August 11–13. It might have been expected that in this leaf, with relatively small accumulation, the relation between starch and total carbohydrate would appear to be about the same as that in healthy leaves.

In Chapter V, it has been demonstrated that part of the carbohydrate formed during the day is not removed from diseased leaves during subsequent darkness. It may be visualized that at least part of the carbohydrate thus retained is withdrawn from daily fluctuations, and stored as residual sugar (which is no longer in equilibrium with starch). As a result, the relation between starch and total carbohydrate in daily fluctuations is shifted to a higher residual sugar content than before. Another part of the non-removed carbohydrate, as may be further suggested, is retained in the leaf in normal starch $\rightleftarrows$ sugar equilibria, and is manifest in a gradual increase in the starch content of the leaf.

The above explanation of the accumulation process is illustrated in the scheme of Fig. 12.

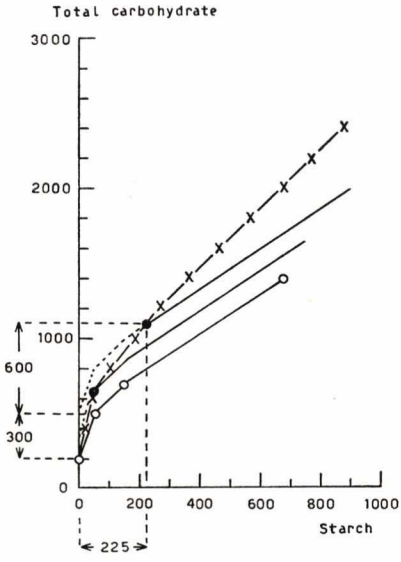


FIG. 12. Schematic presentation of the relative decrease in starch content in leaves of diseased plants (explanation in text).

The relation between starch and total carbohydrate in healthy leaves has been presented by the o—o curve, which is the same as that in Figs. 10 and 11.

Two further curves have been presented to indicate the relation between starch and total carbohydrate as observed in accumulating leaves. The assumed residual sugar content of these leaves is raised by 150 and 300 mg, respectively.

A comparison with the set of straight lines in Fig. 11 indicates that the daily fluctuations, as determined in the experiments, run along the full-drawn part of the curves in the scheme. The dotted part represents the non-removed carbohydrate which is not stored as residual sugar, but is maintained in normal starch $\rightleftarrows$ sugar equilibria. Daily fluctuations, thus, are superimposed on the x-x-x line, indicating the gradual accumulation of starch in diseased leaves.

It is difficult to decide from the available data which part of the non-removed carbohydrate is 'accumulated' as residual sugar, and which part is maintained in normal starch $\rightleftarrows$ sugar equilibria. In the above scheme, it has been assumed that the ratio is 1:2, so that when a total amount of 900 mg carbohydrate is retained, 300 mg is 'accumulated' as residual sugar, while 600 mg (of which 225 mg is starch) remains in normal starch $\rightleftarrows$ sugar equilibria, as illustrated by the example in Fig. 12. It will be clear that during the first stages of accumulation, a much smaller proportion of the accumulated carbohydrate occurs as starch as later on, when (according to the x-x-x line in the scheme) equal amounts of starch and sugar are accumulated.

β Sugar interrelations. The sugar composition during daily fluctuations in young and older leaves is presented in Figs. 13 and 14, respectively.

The sugar composition in young and older leaves of healthy plants appears to be approximately the same. In Figs. 13 and 14, it has been represented by identical straight lines, which indicate that, during daily fluctuations, hexose and sucrose increase or decrease linearly with increase or decrease of the total sugar content of the leaf. A large proportion of the sugar is on account of sucrose, while the proportion of hexose is relatively small.

In young diseased leaves, the sugar interrelations differ slightly from those in healthy leaves. The data, assembled in Fig. 13, indicate that in the diseased leaves, a smaller proportion of total sugar during daily fluctuations is on account of sucrose, and a correspondingly larger proportion on account of hexose than in the healthy leaves. It seems possible that this difference is connected with the difference in development between young healthy and diseased leaves, as

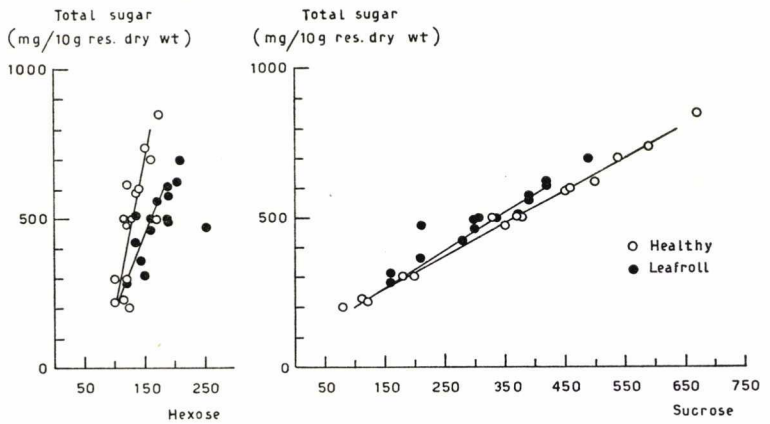


FIG. 13. Relation between total sugar (mg/10 g res. dry wt) and hexose and sucrose, respectively, in young leaves (experiments of 1958). Healthy: o; diseased ● (young leaves, July 3-4; 10th leaf, July 7-9; 15th leaf, August 11-13).

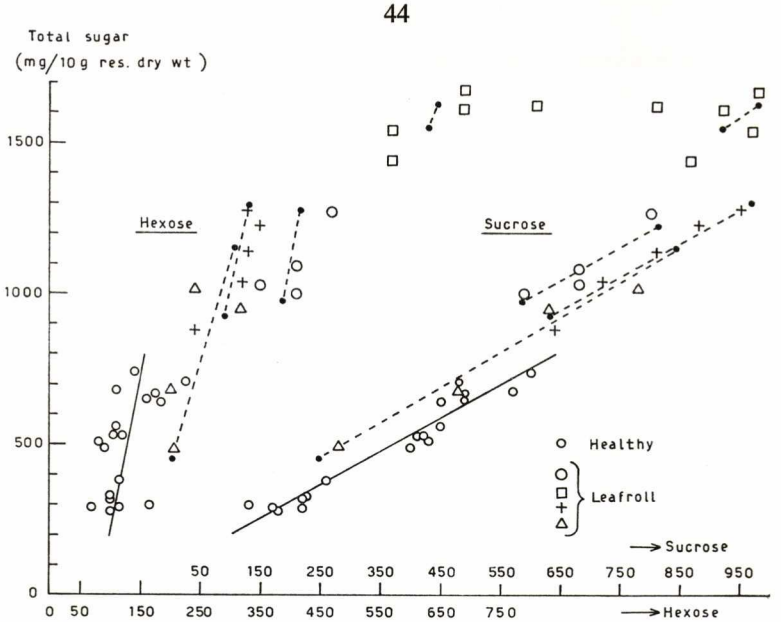


FIG. 14. Relation between total sugar (mg/10 g res. dry wt) and hexose and sucrose, respectively, in older leaves (experiments of 1958). Healthy: o; diseased: ○ (5th leaf, July 7-9); □ and + (5th and 10th leaves, respectively, July 25-26); △ (10th leaf, August 11-13). □ : left group of 5: hexose, right group: sucrose.

previously suggested with regard to the difference in relative starch content between these leaves. The results obtained in the experiment of July 29–30, 1960, referred to earlier, support this suggestion (*cf.* under α).

In the older diseased leaves, the sugar interrelations have been presented as a set of straight lines, one for each of the analysed leaves (*cf.* Fig. 14).

In the determination of the positions of the different lines, the same difficulties are encountered as in the presentation of the relation between starch and total carbohydrate in accumulating leaves, illustrated in Fig. 11. The relative position of the △ line now agrees well with the position of the other lines.

The different sugar levels have been chosen so that they correspond with the starch levels in Fig. 11. The excessively long stretch of the △ line, thus, is due to the deviating slope of the △ line in Fig. 11, indicating large quantitative variation of sugar in the diurnal pattern. The short stretch of the □ line, for the same reason, is connected with the relatively strong inclination of the starch line in Fig. 11.

The most remarkable feature in Fig. 14 is the strong increase in the relative hexose content of diseased leaves. This increase is mainly due to fructose, as indicated by the data on hexose analysis in Tables 7, 9 and 11 of the Appendix. The presentation suggests further that the sugar composition during daily fluctuations is essentially the same in full-grown healthy and diseased leaves (the lines are fairly parallel). In the latter, however, they are shifted towards an increasingly high level of residual sugar, especially hexose.

In the previous discussion of the relation between starch and total carbohydrate, it has been suggested that part of the non-removed carbohydrate in diseased leaves is stored as residual sugar. This residual sugar is withdrawn from

daily fluctuations so that its composition may well be different, which would explain the shift in position of the normal sugar equilibria. The other part of the non-removed carbohydrate, contributing to accumulation as well, is probably maintained in normal equilibria. The above explanation is illustrated in the scheme of Fig. 15.

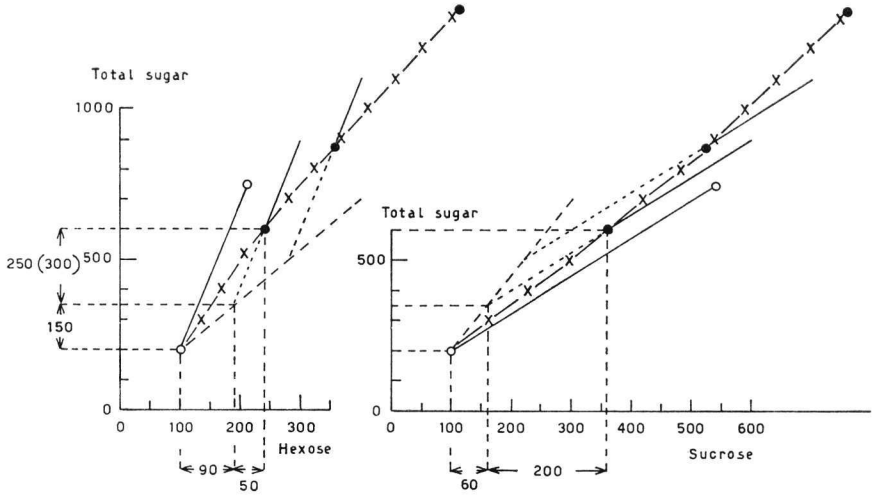


FIG. 15. Schematic presentation of the sugar accumulation in leaves of diseased plants (explanation in text).

The sugar composition in healthy leaves has been presented by the o—o lines. They indicate that the residual sugar level of the leaves consists of equal parts of hexose and sucrose, while the ratio of these sugars during daily fluctuations is 20:80 (which is approximately the ratio as observed in the experiments). Two further pairs of lines are presented (each partly dotted, and partly full-drawn), corresponding in total carbohydrate contents with the starch lines in Fig. 11. They represent the sugar composition in accumulating leaves, in which the residual sugar content is raised by 150 and 300 mg, respectively. These lines are superimposed on the --- line, representing the composition of this residual sugar. The --- line indicates 'specific' accumulation since the residual sugar is withdrawn from normal daily fluctuations, and is stored in a specific composition pattern. The dotted part of the individual composition lines represents the composition of the other part of the accumulated carbohydrate which is maintained in normal equilibria. Daily fluctuations as observed during the experiments, finally, run along the full-drawn part of the composition lines, and are superimposed on the x-x-x line, indicating 'general' accumulation.

In the construction of the 'specific' accumulation line, it has been assumed that the ratio between hexose and sucrose in the specifically accumulated sugar is 60:40. This is only an approximation, since from the available data the exact ratio cannot be determined. It is clear, however, that the proportion of hexose in this residual sugar (especially that of fructose) is considerably larger than the hexose proportion in the sugar involved in daily fluctuations (20%).

In the scheme, the earlier assumed ratio of 1:2 between the specifically accumulated carbohydrate and the carbohydrate retained in normal equilibria has been maintained (*cf.* small print, page 43). Thus, when 150 mg carbohydrate is accumulated as residual sugar, another fraction of 300 mg carbohydrate is retained in normal starch $\rightleftarrows$ sugar equilibria (50 mg starch $\backsim$ 250 mg sugar). Each of these accumulated fractions show a specific sugar composition, as illustrated by the example in Fig. 15.

In the experiments of 1958, discussed above, the carbohydrate content of healthy leaves was generally low, while there was a distinct accumulation in diseased leaves. However, healthy plants may accumulate carbohydrate as well, as is shown by the data obtained in 1957, and still more clearly by those obtained in 1960. Differences in carbohydrate content between healthy and diseased leaves were small in 1957 and even absent in 1960, which may be partly related to the mild external symptoms in those years.

While, thus, the carbohydrate content was approximately the same in healthy and diseased leaves, the carbohydrate composition did not show conspicuous differences either. It seems possible that the reason for the absence of differences in composition is connected with the absence of clear leafroll symptoms. The 'specific' accumulation, then, might be restricted to diseased plants. It is also possible, however, that the 'specific' accumulation may occur in healthy plants as well and may become manifest only when the carbohydrate content of the leaves exceeds normal values. Information concerning this question may be obtained by comparing the carbohydrate composition of accumulating and non-accumulating leaves on the same plants.

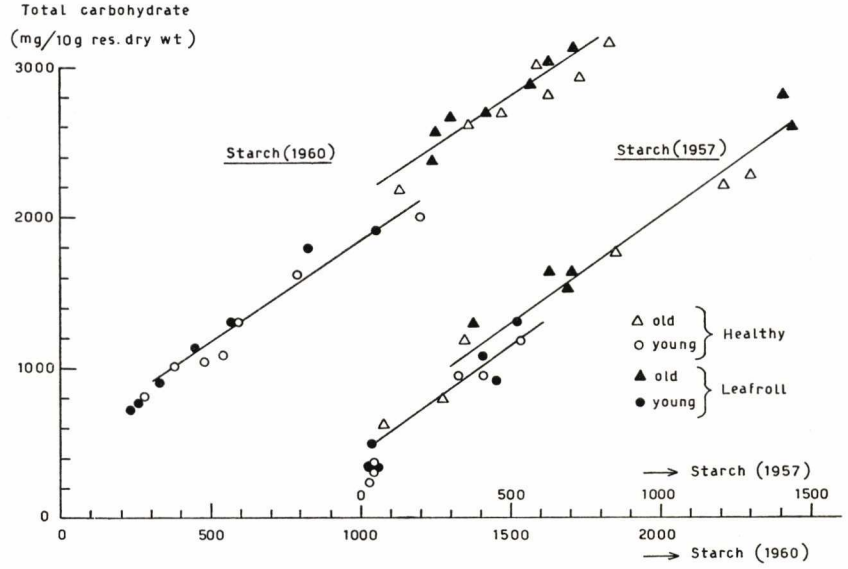


FIG. 16. Relation between starch and total carbohydrate (mg/10 g res. dry wt) in young and older leaves in 1957 (experiments of June 17-20 and July 31-August 3), and in 1960 (experiment of July 29-30; 20-8 h). Healthy leaves: ○ young, △ old; diseased leaves: ● young, ▲ old.

In Fig. 16, the relation between starch and total carbohydrate in young and older leaves has been presented, as observed in the experiments of 1957 and 1960.

In the determination of the relation in the young leaves of 1957, it has been taken into account that the relation is non-linear at low total carbohydrate levels (*cf.* Figs. 10 and 11). The reason that the relation between starch and total carbohydrate has been chosen, and not the sugar interrelations, is that the data on sugar composition showed larger variations.

The presentation in Fig. 16 indicates that the relation between starch and total carbohydrate in the older, accumulating leaves is shifted to a higher level of residual sugar than in the young, non-accumulating leaves. This means that, in 1957 and 1960, the same specific accumulation of carbohydrate occurred in both healthy and diseased plants. The withdrawal of sugar from normal daily fluctuations, and subsequent storage in specific composition, thus appears a general phenomenon, and becomes manifest when the carbohydrate content of the leaf exceeds normal values.

b Carbohydrate composition in shaded leaves

In a special experiment, carried out in 1961, it was tried to remove the accumulated carbohydrate from diseased plants by enveloping some selected leaves in a single layer of cheese-cloth, reducing light intensities by about 60%. The analytical data obtained in this experiment are discussed in the present section, and apply specifically to the accumulated carbohydrate, which is generally inactive in diurnal variations. A short survey of the experimental conditions and general results may first follow.

Experiment of July 17–23, 1961

α Experimental conditions. On four healthy and four diseased plants, three successive, full-grown leaves were shaded. Unshaded leaves on other plants served as controls. The plants were divided into two comparable sets from which, parallel to each other, leaf samples were collected. The samples contained six leaflet halves, at each harvest one leaflet being taken from each of the selected leaves on two plants. Since the samples were restricted to the first leaflet pair counted from the top of the leaves, only four could be collected. They were taken in the evening and in the morning of the 2nd and the 6th night, following shading.

The weather conditions during the experiment, together with those of the two preceding days are surveyed in Table IX.

In order to see whether the relatively long shading period might affect the physiological condition of the leaves, leaf samples in which the chlorophyll content was estimated were collected before and after the shading period. Although at the end of the shading period small yellow patches were noticed in some of the leaflets, chlorophyll destruction on the whole proved small, as indicated by the data in Table X.

β General results. The observed changes in total carbohydrate content of shaded and control leaves are illustrated in Fig. 17. Detailed data on the carbohydrate composition during the shading period are recorded in Table 17 of the Appendix.

Fig. 17 shows that there is a large difference in carbohydrate content between healthy and diseased leaves which agrees with the severity of leafroll symptoms in 1961. It further appears that the accumulated carbohydrate in diseased leaves is nearly completely removed after five days of partial shading.

TABLE IX. Weather conditions during the experiment of July 17–23, 1961

	Rainfall (mm)			Temperature (°C)		Sunshine (min)
	8h	14h	19h	Max.	Min.	
July 15	–.–	0.1	1.1	19.4	11.7	100
16	1.4	5.8	0.1	16.9	11.6	137
July 17	–.–	–.–	–.–	20.7	9.7	256
18	1.9	8.7	0.6	18.8	13.2	102
19	0.3	1.9	2.6	17.6	12.5	57
20	4.9	0.3	0.4	16.8	12.6	6
21	–.–	0.2	–.–	16.0	13.4	19
22	–.–	0.2	–.–	16.0	12.8	51
23	0.2	–.–	–.–	17.0	12.4	222

TABLE X. Relative extinction values, measured at 665 mμ in ethanol extracts of equal leaf areas (experiment of July 17–23, 1961).

	Healthy leaves		Diseased leaves	
	Control	Shaded	Control	Shaded
1st plant set	0.42–0.43	0.40–0.37	0.26–0.24	0.26–0.24
2nd plant set	0.41–0.46	0.38–0.37	0.25–0.24	0.28–0.29

The loss of carbohydrate from the leaves of healthy plants estimated in the 2nd night following shading is about the same as that in the 6th night. Less carbohydrate is lost from the shaded leaves than that from the unshaded ones, which probably may be attributed to the difference in initial carbohydrate content. The difference in translocation from the shaded leaves of diseased plants, as observed between the 2nd and the 6th night following shading, also might be attributed to the difference in initial carbohydrate content. This, however, cannot explain a similar difference in translocation from the unshaded leaves. Especially, the relatively large loss of carbohydrate in the 6th night seems unaccountable, the more so since in the experiments described in Chapter V, in general, a relative decrease in translocation from diseased leaves was demonstrated. It has been remarked before that the relatively large variation in carbohydrate content of the diseased material may well be the reason that in the analysis often irreproducible results are obtained (*cf.* Chapter V, § 2*b*).

α Relation between starch and total carbohydrate. The relation between starch and total carbohydrate in shaded and nonshaded leaves, observed in the experiment of July 17–23, 1961, is presented in Fig. 18.

The general shape of the curve, representing the relation between starch and total carbohydrate in the non-accumulating healthy leaves, is much the same as that previously observed in the experiments of 1958 (*cf.* Figs. 10 and 11). The

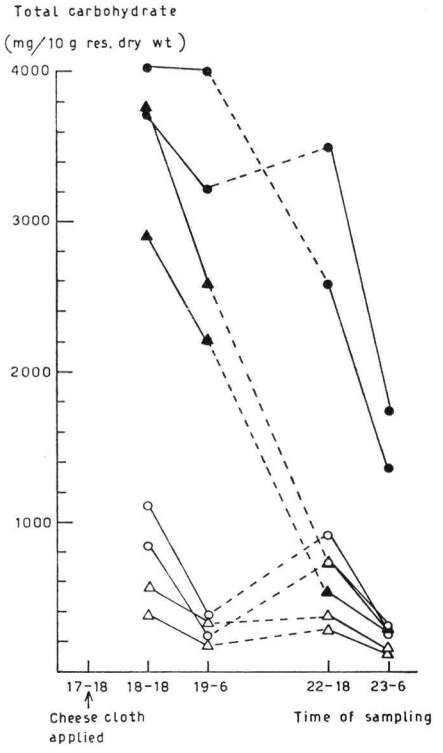


FIG. 17. Changes in total carbohydrate content (mg/10 g res. dry wt) in healthy and diseased leaves as the result of partial shading (experiment of July 17-23, 1961) Healthy leaves: o control, △ shaded; diseased leaves: ● control, ▲ shaded.

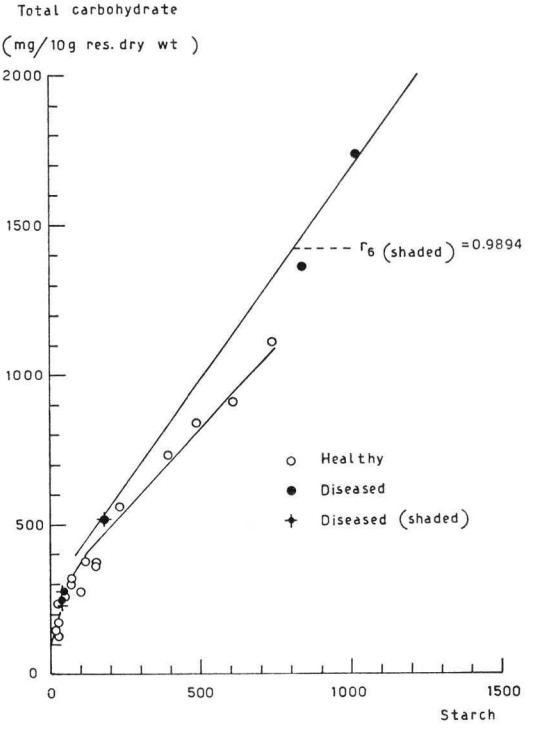


FIG. 18. Relation between starch and total carbohydrate (mg/10 g res. dry wt) in shaded leaves (experiment of July 17-23, 1961). Healthy: o; diseased (control): ●, diseased (shaded): +. Total carbohydrate contents above 2000 mg have not been presented; they can be found in Table 17 of the Appendix.

relation is linear above a certain level of total carbohydrate (400 mg, in the present experiment).

The relation between starch and total carbohydrate in the accumulating diseased leaves is represented by a single straight line ($r_6 = 0.9894$) which has been calculated from the data obtained in the shaded leaves only, including carbohydrate contents above 2000 mg which have not been presented in Fig. 18.

The relative starch content of the non-shaded leaves is not significantly different, but the data have not been included in calculating the correlation, mainly to underline that in the present section the discussion is restricted to the non-active or accumulated carbohydrate. In the non-shaded leaves, a definite (though relatively small) part of the estimated carbohydrate may be actively involved in diurnal variations which are not limited by low light intensities as in the shaded leaves.

In the experiments of 1958, discussed in section *a*, no direct information on the composition of the accumulated carbohydrate was obtained. It proved possible, however, to arrive at a preliminary conception. The x-x-x line in the scheme of Fig. 12, *e.g.*, was suggested to represent the relative starch content in the accumulated carbohydrate. It was constructed, assuming that in the accumulation process part of the non-removed carbohydrate in diseased leaves is stored as inert sugar, while the remaining part is retained in normal starch $\rightleftarrows$ sugar equilibria.

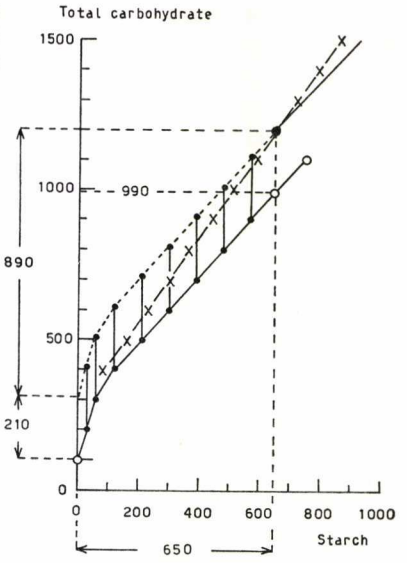
The present line appears identical with the x-x-x line in the scheme of Fig. 12. It may be concluded that the inert sugar, stored in the accumulation process, is gradually remobilized during the degradation of the accumulated carbohydrate in shaded leaves; it is removed, simultaneously with the carbohydrate originally accumulated in normal starch $\rightleftarrows$ sugar equilibria.

The absolute value of the starch content of diseased leaves found in the experiment of 1961 is much higher than that found in the experiments of 1958. This suggests that in 1961 a relatively small proportion of the accumulated carbohydrate is stored as inert sugar, while a large proportion is retained in normal starch $\rightleftarrows$ sugar equilibria. In the discussion of the results obtained in 1958, it was assumed that for each 100 mg inert sugar, 200 mg carbohydrate was retained in normal starch $\rightleftarrows$ sugar equilibria. The present ratio appears to be roughly 1:4, as calculated in the example below, and illustrated in Fig. 19.

The o-o-o line in Fig. 19 represents the relation between starch and total carbohydrate in healthy leaves, and is identical with the line representing the same relation in Fig. 18. The x-x-x line represents the relation between starch and total carbohydrate in shaded diseased leaves, and is identical with the line ($r_6 = 0.9894$) representing this relation in Fig. 18.

The amount of starch in a total accumulation of 1100 mg (*cf.* Fig. 19) is observed to be 650 mg, corresponding to a total carbohydrate content of 1200 mg of which 100 mg is the normal residual sugar content of the leaf. The 650 mg of accumulated starch is in normal equilibrium with sugar (as observed in healthy leaves), making a total of 890 mg (990 — 100) carbohydrate which in retained in normal starch $\rightleftarrows$ sugar equilibria. The remaining 210 mg (1100 — 890) represents the inert sugar fraction of the accumulated carbohydrate. The

FIG. 19. Schematic presentation of the relative decrease in starch content in shaded leaves of diseased plants (explanation in text).



ratio between the inert fraction and the carbohydrate accumulated in normal equilibria, thus, is 210:890 (or roughly 1:4).

β Sugar interrelations. The sugar composition in shaded and non-shaded leaves, observed in the experiment of July 17–23, 1961, is presented in Fig. 20.

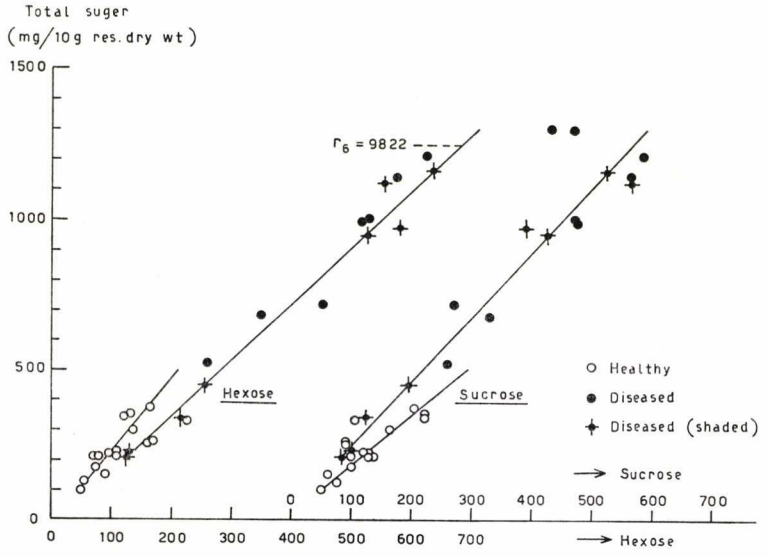


FIG. 20. Relation between total sugar (mg/10 g res. dry wt) and hexose and sucrose, respectively, in shaded leaves (experiment of July 17-23, 1961). Healthy: o; diseased (control): ●, diseased (shaded): +.

The sugar composition in the non-accumulating healthy leaves, as observed in the present experiment, differs only in minor aspects from that observed in the experiments of 1958 (*cf.* Fig. 13 and 14). The residual sugar content of the leaves (100 mg) is lower than that observed before, and the ratio between hexose and sucrose in changes in total sugar content appears to be 40:60, compared with 20:80 in the earlier experiments.

The sugar composition in the accumulating diseased leaves also is much the same as that observed earlier. It has been indicated by single straight lines, calculated from the data obtained in shaded leaves only (*cf.* under α). The obvious increase in relative hexose content is mainly due to fructose, as indicated by the data in Table 17 of the Appendix. It appears that the sugar composition in the non-shaded leaves is approximately the same as that in the shaded leaves.

In the preliminary conception of the accumulation process, illustrated in Fig. 15, it was assumed that part of the accumulated sugar is stored as inert sugar with a considerably higher hexose/sucrose ratio than that observed in healthy leaves (60:40 as compared with 20:80). The relative position of the x-x-x lines in Fig. 15, constructed on this assumption, agrees well with the relative position of the lines, indicating the sugar composition in the shaded diseased leaves of the present experiment. The hexose/sucrose ratio of the inert sugar fraction now appears to be roughly 80:20, as calculated in the example below, and illustrated in Fig. 21.

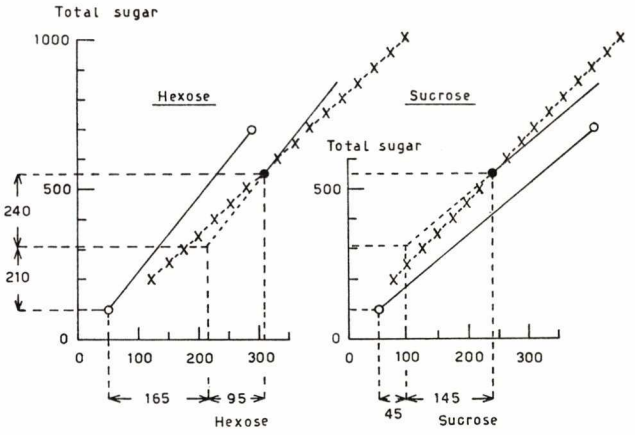


FIG. 21. Schematic presentation of the sugar accumulation in shaded leaves of diseased plants (explanation in text).

The o-o-o and x-x-x lines in Fig. 21 representing the sugar composition in healthy leaves and shaded diseased leaves respectively are identical with those representing the sugar composition in healthy leaves and shaded diseased leaves in Fig. 20.

The amount of sugar in a total accumulation of 1100 mg carbohydrate is 450 mg (the previous example under α is further developed), and corresponds to a total sugar content of 550 mg of which 100 mg is the normal residual sugar content of the leaf. The 450 mg of accumulated sugar is composed of 260 mg

hexose and 190 mg sucrose (*cf.* Fig. 21). Previously, it was calculated that in a total accumulation of 1100 mg, 210 mg occurred as inert sugar, leaving 240 mg (450 — 210) sugar in normal equilibria (95 mg hexose and 145 mg sucrose). The 210 mg of inert sugar, thus, consists of 165 mg (260 — 95) hexose and 45 mg (190 — 145) sucrose, roughly corresponding to the ratio of 80:20.

Summarizing the discussion in sections *a* and *b*, it appears that in diseased leaves a decrease in the relative starch content, and an increase in the relative hexose (mainly fructose) content, can be clearly noticed. Leafroll diseased plants, thus, are characterized especially by large hexose (fructose) contents. These results are best explained when it is visualized that in the accumulation process an inert sugar fraction is built up, in which the hexose/sucrose ratio is considerably higher than normally observed in non-accumulating healthy leaves.

The outline of the above conception was presented in section *a*, in which the diurnal variations in healthy and diseased leaves were studied. The carbohydrate equilibria during diurnal variations appeared approximately the same in both healthy and diseased leaves. The preliminary conception of the accumulation process could be confirmed in section *b*, when the data were studied which were obtained in an experiment carried out to enable the study of the accumulated carbohydrate exclusively. Diurnal variations in this experiment were limited by the application of artificial shading, which, moreover, resulted in a gradual degradation of the accumulated carbohydrate. It was concluded that the inert sugar fraction was gradually broken down during degradation, and removed from shaded leaves simultaneously with the carbohydrate accumulated in normal equilibria.

From additional data, discussed at the end of section *a*, it appeared that in healthy leaves sometimes a decrease in the relative starch content could be observed as well, as in 1960 when the carbohydrate content of healthy plants was exceptionally high. The above described accumulation process, it may therefore be suggested, is of general occurrence, and becomes manifest when the carbohydrate content of the leaf exceeds normal values.

§ 2 Carbohydrate composition in the petiole

The discussion on the carbohydrate composition in the petiole is based mainly on the data obtained in 1958. Petioles were analysed in 1957 as well, but the leafroll symptoms in that year were much less severe than those in 1958. In 1960 and 1961, petiole material was not collected because of the newly introduced technique of sampling individual leaflets instead of entire leaves, as before.

In most samples, starch and total sugar were determined. A complete sugar analysis was carried out in the experiment of July 25–26, 1958 (*cf.* Table 10 of the Appendix). While the sucrose content in the lamina is mostly much higher than the hexose content, they appear approximately equal in the petiole, mainly because of the higher glucose content.

The relation between starch and total carbohydrate in the petiole, as observed in the different experiments of 1958, is presented in Fig. 22.

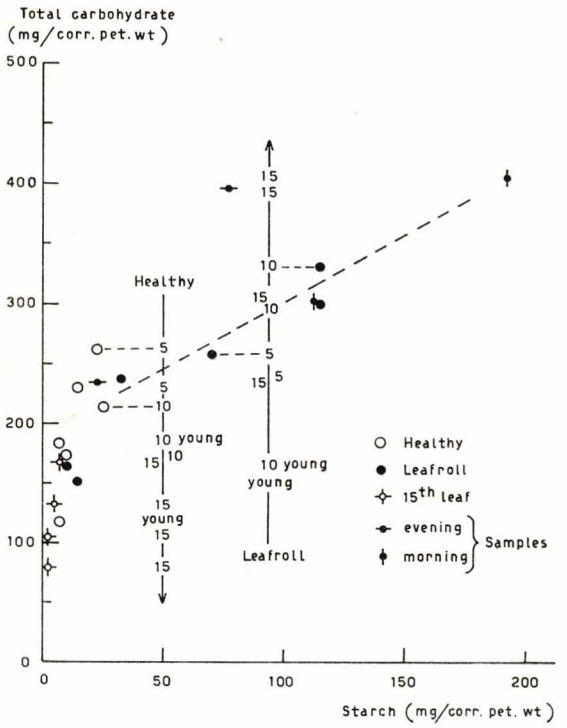


FIG. 22. Relation between starch and total carbohydrate (mg/corr. wt) in the petiole (experiments of 1958; Tables 6, 8, 10 and 12 of the Appendix). Except for the 15th leaf (+ symbols), average values have been plotted; further explanation in text.

The carbohydrate content of the petiole in healthy plants is generally larger in the lower leaves than in those higher at the stem, as indicated by the left column of leaf numbers in Fig. 22 (leaf numbers correspond to dots at the same height). There is no direct correlation with the carbohydrate content of the lamina, but the difference in carbohydrate content between petioles of lower and higher leaves can at least partly be explained by taking into account the comparatively large corresponding petiole weight of the lower leaves, and the comparatively small corresponding petiole weight of the higher leaves (*cf.* Fig. 1, Chapter III). The starch content of the petiole in healthy plants, on the whole, is small, especially when compared with the starch content of the lamina.

The carbohydrate content of the petiole in diseased plants is generally higher in the higher leaves than in those below, as indicated by the right column of leaf numbers in Fig. 22. The situation in diseased plants, thus, is opposite to that in healthy plants. In diseased plants, there is a less clear trend in corresponding petiole weight than in healthy plants (*cf.* Fig. 1, Chapter III), and the comparatively large carbohydrate contents of the petioles of the higher leaves indicate real petiole accumulation.

The accumulation of carbohydrate in the petiole appears conversely related

to the accumulation of carbohydrate in the lamina, as pointed out in Chapter V (*cf.* Fig. 8). It was already remarked that the accumulation in the petiole, in most diseased leaves, is nearly entirely on account of starch, the sugar content of the petiole in diseased leaves being about equal to that in healthy leaves. This again is clearly shown by the presentation in Fig. 22. The --- line, averaging the data on the relative starch content of the petiole in accumulating diseased leaves, indicates that from a total amount of 100 mg carbohydrate, 90 mg is accumulated as starch. Only in the petioles of leaves higher at the stem, sugar is sometimes accumulated as well, especially towards the end of the day. This, *e.g.*, was found in the evening samples taken from the 15th leaf, analysed in the experiment of August 11–13, 1958. The large sugar content of the evening samples (— symbols) appears replaced by an equally large starch content of the morning samples (| symbols), suggesting conversion of sugar into starch during the night. The relative starch content of the morning samples agrees well with the --- line in Fig. 22, while the relative starch content of the evening samples does not.

In Chapter V, § 4*c*, it was suggested that the phloem disturbance in the higher leaves is still largely limited to the petiole. Sugar, thus, is translocated from the lamina but subsequently retained in the petiole, and finally accumulated as starch. This may well explain the temporary sugar accumulation in the petiole.

In the experiment with older leaves, carried out in 1957 (*cf.* Table 4 of the Appendix), a comparatively large starch content of the petiole in diseased leaves was observed as well. Indications for accumulation in the petiole, however, were not obtained, at least not when referring carbohydrate contents to 10 g res. dry wt (corresponding petiole weights could not be calculated).

CHAPTER VII

GENERAL DISCUSSION

The results obtained in Chapters IV, V and VI may be briefly surveyed first.

The rate of photosynthesis in leafroll diseased plants, especially that in older leaves, appears reduced as compared with that in corresponding leaves of healthy plants (Chapter IV).

Generally, large amounts of carbohydrate are found to accumulate in diseased leaves. Accumulation is found in both lamina and petiole, but not in stems. Accumulation in the lamina is comparatively large in leaves at the lower part of the stem; accumulation in the petiole, conversely, is comparatively large in leaves at the higher part of the stem (Chapter V).

The accumulation of carbohydrate, generally, can be correlated with a relative reduction in transport from leaves of diseased plants (Chapter V).

Whereas absolute amounts of starch are generally much larger in diseased leaves than in healthy ones, it appears that the relative starch content (the amount of starch in relation to total carbohydrate) is decreased in diseased leaves. Differences in sugar composition between healthy and diseased leaves are observed as well (Chapter VI).

a Photosynthesis

The rate of photosynthesis in full-grown leaves of diseased plants is reduced. The precise reason for this remains obscure; there are several possible explanations (*cf.* discussion, Chapter IV). In literature, it has often been suggested that the accumulation of carbohydrate in leaves of infected plants might be of special importance (for references, *see* Chapter I). Evidently, both accumulation of carbohydrate and reduced rate of photosynthesis are related to the intensity of leafroll symptoms; this, however, does not necessarily imply that the mentioned processes are also causally related.

Sink size and rate of photosynthesis in plants usually are correlated (KURSANOV, 1933; MOSS, 1962; HUMPHRIES, 1963). MOSS showed that removal of fruit in corn and tomatoes (reduction in sink size) reduced assimilation; he suggested a direct relation with the increased sugar content of the plants. However, in considering the possible effect of increased sugar content on photosynthesis, not only 'active' sinks (meristematic regions) but also 'passive' sinks (such as the storage capacity of the cell) should be taken into account. Theoretically, an effect of carbohydrate on photosynthesis can be expected only when sugars accumulate at the site of their production, thus disturbing equilibrium concentrations in the reaction chain. When they are stored away from the reaction sites, no effect can be expected. Plants such as the potato, in which a large proportion of the carbohydrate formed during photosynthesis is deposited as starch, then, might not show any clear effect of carbohydrate accumula-

tion on photosynthesis. Our results discussed under *d*, moreover, indicate that during accumulation in the potato part of the sugar is withdrawn from active metabolism, thus further increasing the storage capacity of the plant.

b Accumulation of carbohydrate and inhibited translocation

Accumulation of carbohydrate in leaves of infected plants was demonstrated soon after the first description of the leafroll disease (ESMARCH, 1919; NEGER, 1919; QUANJER, 1919). QUANJER illustrates the distribution of starch in diseased plants in several schematical figures, and the results obtained in our own investigation (Chapter V) agree well with his description:

'Les feuilles inférieures que l'on reconnaît malades à leur enroulement, prennent par la réaction à l'iode une teinte tout aussi sombre que la soirée précédente. Il est clair que ces feuilles malades perdent très hâtivement leur pouvoir d'assimiler; et qu'elles cèdent peu de chose à la tige puisque celle-ci ne contient presque pas d'amidon. Seulement le sommet de la tige contient un peu d'amidon provenant des feuilles supérieures d'apparence encore saine, où la circulation n'est entravée que dans les pétioles' (QUANJER, 1919).

This particular distribution of starch in secondarily infected plants, according to QUANJER, agrees well with the extension of phloem necrosis. The same would hold for primarily infected plants, in which both accumulation of starch and phloem necrosis occur in leaves towards the top of the plants.

THUNG (1928) extended QUANJER's observations, and studied the dissolution of starch in leaves when plants were kept in the dark. He visualized the compound potato leaf as a simple one, and observed that in secondarily infected plants starch was retained longest near the top and leaf margin. In primarily infected plants, starch was retained longest near the base and in the central part of the leaf. THUNG was successful in obtaining corresponding patterns in leaves of healthy plants by incision of the smaller veins in the lamina and incision of the common petiole, respectively.

The above certainly leads to the suggestion that the leafroll disease is primarily localized in the phloem, while the accumulation of carbohydrate in the leaf is caused by inhibited translocation. In our experiments, described in Chapter V, a correlation between decreased translocation and carbohydrate accumulation usually could be demonstrated. However, accurate determinations of transport appeared difficult because of comparatively large variation in carbohydrate content between samples.

Differences in transport need not necessarily be large to account for an accumulation which gradually comes about in the course of the vegetation period. It therefore seems unjustified to conclude that the direct cause for accumulation should not be located in the phloem if, as sometimes occurs, no conspicuous differences in translocation between healthy and diseased leaves are found (WATSON and WATSON, 1951; WATSON, 1955, experimenting with sugar beet yellows).

Large differences (larger, in fact, than is necessary to account for accumulation), as generally found in our experiments, might indicate relatively high

respiration rates in diseased leaves. Increased respiration rates in diseased plants, indeed, have been demonstrated (THUNG, 1928; CLINCH and co-workers, private communication). They were also observed in our own investigation when, simultaneously with photosynthetic rates, respiration rates were determined (Chapter IV). Increased respiration rates in diseased leaves might also account for at least part of the estimated difference in nocturnal loss of carbohydrate between healthy and diseased leaves. Increased respiration in diseased leaves, might be – either in part or entirely – due to substrate limitation of the rate of respiration in healthy leaves.

Inhibition of translocation, presumably, also affects the activity of growth hormones, and stunted growth as observed in diseased plants might well be explained along this line. Reduced meristematic activity in diseased plants, and, in consequence, reduced utilisation of carbohydrates in growth may well give rise to an additional increase in the carbohydrate content of the leaf (*cf.* COÏC 1945).

Considering all this, it may be concluded that accumulation of carbohydrate in leaves of diseased plants chiefly arises as the result of inhibited translocation of assimilates from the leaf.

c Reasons for inhibited translocation

When QUANJER (1913) discovered necrosis as a constant symptom of the leafroll disease, he suggested that this would lead to accumulation of carbohydrate in leaves of infected plants. In one of his later publications (QUANJER, 1919), he demonstrated a close correlation between phloem necrosis and accumulation in both secondarily and primarily infected plants, each of these with their own, specific symptoms (*cf.* under *b*). Phloem necrosis still seems to be one of the best-founded possible explanations for inhibited translocation and accumulation. However, several observations are left unexplained. The most important of these seems to be the fact that in leaves of young plants carbohydrate accumulates before the first signs of necrosis in the phloem are visible (OORTWIJN BOTJES, 1920; MURPHY, 1923). The same holds for sugar beet yellows, characterised by phloem gummosis (KLINKENBERG, 1945).

It is conceivable, therefore, that the primary effect of the leafroll virus is a physiological disturbance of the phloem, followed by collapse and necrosis of sieve tubes. Presumably, similar processes are active in healthy plants towards the end of the annual vegetation period, when translocation declines, and callose is formed in sieve tubes. An interesting suggestion has been put forward by VAN DUUREN (1955), HENKE (1957) and SOMMER (1957); these authors, working with sugar beet yellows, reject the view that a mechanical disturbance of the phloem is the primary cause for accumulation; they suggest that the phosphatase activity of the vascular bundle in diseased plants is affected (for further discussion, *see* Chapter I).

In our own investigation, it appeared that carbohydrate may accumulate also in leaves of healthy plants. In 1960, *e.g.*, the carbohydrate content of healthy leaves appeared even higher than that of diseased leaves (Chapter V). Presu-

mably, such accumulation is induced by unfavourable weather conditions (Chapter III); it shows that carbohydrate may accumulate in leaves, independent of the mechanical condition of the phloem.

d Interconversion of carbohydrates

Interconversion of carbohydrates in leaves of diseased plants has been studied before in order to elucidate the accumulation process. NEGER (1919), *e.g.*, suggested inhibition of diastase activity in diseased leaves, which he assumed might explain accumulation. THUNG (1928), however, noticed that during respiration starch disappeared as readily from diseased leaves as from healthy ones. BARTON-WRIGHT and M'BAIN (1932) failed to demonstrate the presence of sucrose in petioles and stems of leafroll diseased plants, but their results seem to have been due to improper analytical methods. Recent investigations by BOSER (1958 and 1960), on glycolysis in potato plants affected by different virus diseases (among which leafroll), indicate only small differences in enzyme activity between healthy and diseased plants.

In our study, we observed – aside of a marked increase in total carbohydrate – a decrease in the relative starch content, and an increase in the relative hexose content (especially that of fructose) in accumulating leaves (Chapter VI). These observations are best explained by assuming that in the accumulation process part of the soluble sugar in diseased leaves is stored at sites intra-cellularly separated from those directly involved in the 'active' metabolism, manifest in diurnal variations. The sugar thus stored, assumedly, is no longer in equilibrium with starch, and differs in composition from the remaining 'active' sugar by a larger hexose (mainly fructose) content (Figs. 12 and 15). The 'active' metabolism in diseased plants appears essentially unaffected; the starch $\rightleftarrows$ sugar equilibria in diurnal variations are the same as those in healthy plants (Figs. 11 and 14).

The above mentioned 'specific' accumulation of sugar appears not primarily related to the leafroll disease, but is connected with accumulation in general. This may be derived from our experiments carried out in 1960, when equally large carbohydrate contents were present in both healthy and diseased plants, and conspicuous differences in carbohydrate composition were not observed (Fig. 16).

The following is an attempt to arrive at an overall picture of the carbohydrate metabolism in leafroll diseased plants inasmuch as it can be based on the facts communicated in the previous pages.

Concurrent with the superficial symptoms, the rate of photosynthesis appears decreased, both in the light saturated and in the light limited range. The photosynthetic process thus appears more or less generally inhibited; there is, so far, no direct evidence for a specific partial process to be attacked, the chloroplast mechanism appears more or less disabled in a general sense.

The second conspicuous phenomenon is the gradual increase in carbohydrate content in diseased leaves which is due to inhibited transport. This causes the

diurnal variations to take place on top of increasing levels of residual carbohydrate. Together with this, the composition of total carbohydrate shows a relative decrease in starch, and an increase in hexose, especially fructose. The normal equilibrium between the various compounds thus appears disturbed. The most likely explanation for this is that part of the accumulated carbohydrate is stored as 'inert' sugar at sites intra-cellularly separated from those involved in the 'active' carbohydrate metabolism as observed in normal diurnal variations. It is conceivable, *e.g.*, that at the assumed special sites starch synthesizing enzymes are absent, while the sucrose synthesizing enzymes are less active than those at the sites involved in diurnal variations.

The process of storage of 'inert' sugar in specific composition may be defined as 'specific' accumulation, against 'general' accumulation which includes the accumulation of carbohydrate in normal starch $\rightleftarrows$ sugar equilibria. Our data indicate that the specific type of accumulation, however, is not a specific property of the virus-infected plant, but is part of the phenomenon of overall-accumulation. In some cases we found large amounts of carbohydrates accumulated in leaves of healthy plants, accompanied by similar changes in composition as normally occurring in diseased leaves.

Moreover, the separation between specific accumulation and accumulation on the basis of the normal starch $\rightleftarrows$ sugar equilibria is not an absolute one, since the 'inert' sugar fraction, apparently, may be remobilized, as was found by artificial shading of diseased leaves, and removed simultaneously with the carbohydrate in normal composition.

The leafroll disease appears essentially a disease of the phloem; the accumulation of carbohydrate is due to inhibited transport which may account for the stunted growth of diseased plants as well. The decreased rate of photosynthesis, probably, is not directly related to the accumulation of carbohydrate, but rather due to secondary changes in the chloroplast which may, probably, in general be described as a premature 'ageing' reaction, owing to the presence of the disease.

SUMMARY

In the present investigation, the rate of photosynthesis and several aspects of the carbohydrate metabolism in healthy and leafroll diseased potato plants of the variety 'Alpha' were studied. The diseased plants were infected with a moderately virulent strain of the leafroll virus, and showed secondary symptoms.

The rate of photosynthesis was measured in leaf discs with the aid of the WARBURG technique, while relatively high CO_2-concentrations (1.26%) were applied (Chapter II, § 2*a*).

The starch and sugar content of dried material was determined with the anthrone reagent; paper chromatography was used to separate the different sugars (Chapter II, § 2*b*).

The leafroll symptoms in diseased plants varied considerably from year to year. The superficial (visible) disease symptoms, the carbohydrate content of the leaves and the tuber yield in different years were compared with the prevailing weather conditions (Chapter III, § 2). Especially in 1960, the weather conditions were abnormal; *e.g.*, the number of hours sunshine was only one third of that measured in the other years. In 1960, the superficial symptoms were mild, the carbohydrate content, also that of the healthy leaves, was abnormally high and clearly correlated with a low tuber yield (Table III).

The rate of photosynthesis of diseased leaves appeared considerably reduced as compared with that of healthy ones, both at low and at higher light intensities. The rate of respiration, however, was relatively high in diseased leaves (Chapter IV, § 1).

To account for the comparatively low rate of photosynthesis in diseased leaves, several internal factors of the leaf, influencing photosynthesis, were studied in more detail (Table V). Of special importance may be the dehydration of the protoplasm in diseased plants, which was apparent from the comparatively low water content and the comparatively high TCA-insoluble phosphate content per unit leaf area. A water deficit increases the internal diffusion resistance of the leaf, and certainly influences photosynthesis also in a more direct sense.

The aspects of the carbohydrate metabolism which were studied in more detail were the diurnal carbohydrate variation in the leaf, and the composition of the carbohydrate in healthy and diseased plants (Chapters V and VI, respectively).

Qualitative differences in carbohydrate composition between healthy and diseased plants were not observed (Chapter V, § 1).

In leaves of diseased plants, generally, a high carbohydrate content was noticed. The analysis of the distribution of the accumulated carbohydrate over the different leaves of the plant showed that the accumulation in the lamina was largest in the lower leaves, whereas the accumulation in the petiole was largest in the higher leaves (Fig. 8).

In general, a reasonably good correlation between accumulation in lamina and petiole and decreased transport could be demonstrated (Table VII).

In the stem of diseased plants, no conspicuous accumulation was observed. However, the percentage of starch appeared relatively high (Table VIII).

The daily carbohydrate fluctuations in diseased leaves appeared shifted towards a more or less high level of accumulated carbohydrate. The daily fluctuations in accumulating leaves, mostly, showed rather irregular courses. This was attributed to the variation in carbohydrate content between different leaves, which makes adequate sampling difficult (Chapter V, § 4*b*, *see* also § 2*b*).

The quantitative carbohydrate composition in the lamina appeared somewhat modified in diseased plants with respect to that in healthy plants. The relative starch content (starch relative to total carbohydrate) appeared decreased, and the relative hexose content, especially that of fructose, increased (Chapter VI, § 1*a*). This was explained by assuming that in the accumulation process part of the accumulated sugar is withdrawn from the active carbohydrate metabolism, manifest in the diurnal variations. The 'inert' sugar fraction is no longer in normal equilibrium with starch (decreased relative starch content), and furthermore differs in composition from the 'active' sugar fraction by a relatively high hexose (especially fructose) content (Figs. 12 and 15).

It thus seems possible to distinguish between 'specific' accumulation (*viz.* accumulation of sugar in specific composition) and 'general' accumulation, which includes carbohydrate remaining in normal equilibria.

It appeared, in the experiments of 1960, that similar changes in carbohydrate composition also occur in leaves of healthy plants with unusually high carbohydrate contents (Fig. 16). Apparently, the modified carbohydrate composition, as generally observed in diseased leaves, is not the direct consequence of the virus infection, but primarily related to accumulation which may occur in healthy plants as well.

In a separate experiment, several intact leaves on the plant were artificially shaded, so that their carbohydrate content gradually decreased, while, moreover, the daily fluctuations were reduced to a minimum (Chapter VI, § 1*b*). The composition of the accumulated carbohydrate during the degradation appeared largely the same as that observed during the building up of the accumulation. The previously stored 'inert' sugar fraction, apparently, is remobilized in the degradation process, and disappears from the leaf simultaneously with the carbohydrate accumulated in normal composition.

The carbohydrate composition in the petiole appeared characterized by a relatively high starch content (Chapter VI, § 2). Sometimes a temporary accumulation of sugar was observed. Apparently, a larger or smaller part of the assimilated carbohydrate is translocated from the lamina but subsequently retained in the petiole where it is finally accumulated as starch.

The leafroll disease of the potato plant appears to be essentially a disease of the phloem. The accumulation of carbohydrate in leaves of infected plants, as well as several other disease symptoms, can be explained by inhibited transport. Secondary changes, presumably, in the protoplasm are responsible for the reduced rate of photosynthesis.

SAMENVATTING

In het hiervoor beschreven onderzoek werden de fotosynthesesnelheid en enkele aspecten van het koolhydraatmetabolisme in gezonde en bladrolzieke aardappelplanten van het ras ‚Alpha' bestudeerd. De zieke planten waren geïnfecteerd met een matig virulente stam van het bladrolvirus en vertoonden secundaire symptomen.

De fotosynthesesnelheid werd gemeten in bladschijfjes met behulp van de WARBURG techniek, terwijl betrekkelijk hoge CO_2-concentraties (1,26%) werden toegepast (Hoofdstuk II, § 2*a*).

Het zetmeel- en suikergehalte van gedroogd materiaal werd bepaald met het anthron-reagens; papierchromatografie werd toegepast om de verschillende suikers van elkaar te scheiden (Hoofdstuk II, § 2*b*).

De bladrolsymptomen in zieke planten bleken van jaar tot jaar sterk te variëren. De oppervlakkige ziektesymptomen, het koolhydraatgehalte van het blad en de knolopbrengst in verschillende jaren werden vergeleken met de heersende weersomstandigheden (Hoofdstuk III, § 2). Speciaal in 1960 waren de weersomstandigheden abnormaal; het aantal uren zonneschijn b.v. was slechts 1/3 van dat in andere jaren. De oppervlakkige ziektesymptomen in 1960 bleken bijzonder mild, het koolhydraatgehalte, ook dat van het gezonde blad, was abnormaal hoog en duidelijk gecorreleerd met een lage knolopbrengst (Tabel III).

De fotosynthesesnelheid van zieke bladeren bleek aanmerkelijk lager dan die van gezonde, zowel bij lage als hogere lichtintensiteiten. De ademhalingssnelheid van zieke bladeren daarentegen was relatief hoog (Hoofdstuk IV, § 1).

Als mogelijke oorzaken van de lage fotosynthesesnelheid in zieke bladeren werden verschillende inwendige factoren van het blad, die de fotosynthesesnelheid beinvloeden, nader onderzocht (Tabel V). Van deze factoren hebben het lage chlorophylgehalte en het hoge koolhydraatgehalte van zieke bladeren waarschijnlijk slechts een geringe invloed op de fotosynthesesnelheid. Van meer belang lijkt de dehydratatie van het protoplasma in zieke planten, die werd afgeleid uit het relatief laag watergehalte en het relatief hoog TCA-onoplosbaar fosfaatgehalte per eenheid van bladoppervlak. Een waterdeficit verhoogt de interne diffusieweerstand van het blad en beinvloedt de fotosynthese zeker ook in meer directe zin.

Hoewel hierover geen nadere gegevens werden verkregen, spelen onder veldomstandigheden waarschijnlijk nog andere bijzondere kenmerken van de bladrolzieke plant, zoals de geringe openingstoestand van de huidmondjes en de habitus van de plant, een rol bij de verlaging van de fotosynthesesnelheid.

De aspecten van de koolhydraatstofwisseling, die nader werden onderzocht, waren de dagelijkse koolhydraatvariatie in het blad en de koolhydraatsamenstelling in gezonde en zieke planten (Hoofdstukken V en VI).

Qualitatieve verschillen in koolhydraatsamenstelling tussen gezonde en zieke planten werden niet opgemerkt (Hoofdstuk V, § 1).

In bladeren van zieke planten werd in het algemeen een hoog koolhydraatgehalte aangetroffen. Een analyse van de verdeling van het geaccumuleerde koolhydraat over de verschillende bladeren van de plant gaf aan dat de accumulatie in de bladschijf het grootst was in de lager aan de stengel geplaatste bladeren, terwijl de accumulatie in de bladsteel juist het grootst was in de hoger aan de stengel geplaatste bladeren (Fig. 8).

In het algemeen bleek een goede correlatie aantoonbaar tussen koolhydraatophoping in bladschijf en bladsteel en gereduceerd transport (Tabel VII).

In de stengel werd geen noemenswaardige accumulatie opgemerkt; wel bleek het zetmeelpercentage in de stengel van zieke planten relatief hoog (Tabel VIII).

De dagelijkse koolhydraatfluctuaties in zieke bladeren bleken verschoven naar een hoog koolhydraatniveau tengevolge van de accumulatie. Meestal vertoonden de dagelijkse fluctuaties in accumulerende bladeren een betrekkelijk onregelmatig verloop. Dit werd toegeschreven aan de variatie in koolhydraatgehalte tussen verschillende bladeren, die een adequate bemonstering bemoeilijkt (Hoofdstuk V, § 4*b*, *zie* ook § 2*b*).

De quantitatieve koolhydraatsamenstelling in de bladschijf bleek in zieke planten gewijzigd ten opzichte van die in gezonde planten. Het relatieve zetmeelgehalte (zetmeel betrokken op totaal koolhydraat) bleek verlaagd en het relatieve hexose gehalte, speciaal dat van fructose, verhoogd (Hoofdstuk VI, § 1*a*). Dit werd verklaard door aan te nemen dat in het accumulatieproces een deel van de geaccumuleerde suiker wordt onttrokken aan de actieve stofwisseling, die zich manifesteert in de dagelijkse koolhydraatfluctuaties. De ‚inerte' suikerfractie zou niet langer in normaal evenwicht zijn met zetmeel (verlaagd relatief zetmeelgehalte) en zou verder in samenstelling verschillen van de ‚actieve' suikerfractie door een relatief hoog hexose (speciaal fructose) gehalte (Fig. 12 en Fig. 15).

Het lijkt zodoende mogelijk om ‚specifieke' accumulatie (accumulatie van suiker in specifieke samenstelling) te onderscheiden van ‚algemene' accumulatie, waarbij inbegrepen het in normale samenstelling geaccumuleerde koolhydraat.

Nu bleek, speciaal uit de experimenten van 1960, dat gelijksoortige veranderingen in koolhydraatsamenstelling ook optreden in bladeren van gezonde planten met een abnormaal hoog koolhydraatgehalte (Fig. 16). Blijkbaar is de gewijzigde koolhydraatsamenstelling, zoals die algemeen wordt gevonden in zieke bladeren, niet een direct gevolg van de virusinfectie, doch primair gecorreleerd met het verschijnsel accumulatie dat zich ook in gezonde planten kan voordoen.

In een afzonderlijk uitgevoerd experiment werden enkele intact aan de plant gelaten bladeren kunstmatig beschaduwd, zodat het koolhydraatgehalte langzaam verminderde, terwijl bovendien de dagelijkse koolhydraatfluctuaties tot een minimum waren beperkt (Hoofdstuk VI, § 1*b*). De samenstelling van het geaccumuleerde koolhydraat tijdens de afbraak bleek in hoofdzaak gelijk aan die tijdens het totstandkomen van de accumulatie. De aanvankelijk gevormde ‚inerte' suikerfractie wordt blijkbaar geremobiliseerd in het afbraakproces en

verdwijnt vervolgens uit het blad, tegelijk met het in normale samenstelling geaccumuleerde koolhydraat.

De samenstelling van het koolhydraat in de bladsteel van zieke planten bleek gekenmerkt door een relatief hoog zetmeelgehalte (Hoofdstuk VI, § 2). In enkele gevallen werd een tijdelijk verhoogd suikergehalte waargenomen. Blijkbaar wordt een meer of minder groot deel van het geassimileerde koolhydraat uit de bladschijf getransporteerd naar de bladsteel, waar het wordt achtergehouden en tenslotte opgeslagen in de vorm van zetmeel.

De bladrolziekte van de aardappelplant lijkt essentieel een ziekte van het phloeem te zijn. De koolhydraatophoping in bladeren van geinfecteerde planten en verschillende andere ziektesymptomen kunnen worden verklaard uit een verminderd transport. Daarnaast doen zich waarschijnlijk secundaire verschijnselen voor, die wellicht mede de oorzaak zijn van de verlaagde fotosynthesesnelheid.

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APPENDIX

TABLE 1. Translocation of carbohydrate (mg/10 g res. dry wt) from the lamina in young plants (experiment of June 17–20, 1957). Glucose and fructose were determined together as hexose.

	Total carb.		Starch	%	Total sugar	Sucrose	%	Hexose	%
Healthy plants									
June 17, 20 h	1180		530	45	650	540	83	110	17
18, 8 h	360	—820	50	14	310	230	74	80	26
18, 20 h	940		320	34	620	510	83	110	17
19, 8 h	310	—630	40	13	270	190	71	80	29
19, 20 h	950		410	43	540	450	83	90	17
20, 8 h	240	—710	30	12	210	130	62	80	38
Average, 20 h	1020		420		600	500		100	
8 h	300	—720	40		260	180		80	
Diseased plants									
June 17, 20 h	1310		520	40	790	610	77	180	23
18, 8 h	490	—820	40	8	450	320	70	130	30
18, 20 h	920		450	49	470	370	80	100	20
19, 8 h	340	—580	30	10	310	210	67	100	33
19, 20 h	1080		400	37	680	500	74	180	26
20, 8 h	350	—730	60	17	290	200	68	90	32
Average, 20 h	1100		460		640	490		150	
8 h	400	—700	50		340	240		100	

TABLE 2. Translocation of carbohydrate (mg/10 g res. dry wt) from the petiole in young plants (experiment of June 17–20, 1957). Sugar extracts were not chromatographed.

	Healthy plants					Diseased plants				
	Total carb.		Starch	%	Total sugar	%	Total carb.		Starch	%	Total sugar	%
June 17, 20 h	665		87	13	580	87	840		99	12	740	88
18, 8 h	465	—200	14	3	450	97	500	—340	22	5	480	95
18, 20 h	500		68	14	430	86	545		55	10	490	90
19, 8 h	435	— 65	13	3	420	97	350	—195	12	4	340	96
19, 20 h	565		137	24	430	76	515		116	23	400	77
20, 8 h	320	—245	19	6	300	94	350	—165	21	6	330	94
Average, 20 h	575		97		580		635		90		540	
8 h	405	—170	18		390		400	—235	18		380	

TABLE 3. Translocation of carbohydrate (mg/10 g res. dry wt) from the lamina in older plants (experiment of July 31–August 3, 1957). Glucose and fructose were determined together as hexose.

	Total carb.	Starch	%	Total sugar	Sucrose	%	Hexose	%
Healthy plants								
July 31, 20 h	2210	1210	55	1000	670	67	330	33
August 1, 8 h	790 —1420	270	34	520	300	58	220	42
1, 20 h	1770	850	48	920	530	58	390	42
2, 8 h	620 —1150	80	13	540	320	59	220	41
2, 20 h	2280	1300	57	980	700	71	280	29
3, 8 h	1180 —1100	340	29	840	530	63	310	37
Average, 20 h	2090	1120		970	630		340	
8 h	860 —1230	230		630	380		250	
Diseased plants								
July 31, 20 h	2600	1440	55	1160	620	53	540	47
August 1, 8 h	1520 —1080	690	45	830	460	55	370	45
1, 20 h	1640	700	43	940	580	62	360	38
2, 8 h	1290 — 350	370	29	920	460	50	460	50
2. 20 h	2810	1410	50	1400	950	68	450	32
3, 8 h	1640 —1170	630	38	1010	750	74	260	26
Average, 20 h	2350	1190		1160	710		450	
8 h	1480 — 870	560		920	560		360	

TABLE 4. Translocation of carbohydrate (mg/10 g res. dry wt) from the petiole in older plants (experiment of July 31– August 3, 1957). Sugar extracts were not chromatographed.

	Healthy plants					Diseased plants				
	Total carb.		Starch	%	Total sugar	%	Total carb.		Starch	%	Total sugar	%
July 31, 20 h	1130		152	14	980	86	1310		520	40	790	60
August 1, 8 h	990	—140	127	13	860	87	1120	—190	450	40	670	60
1, 20 h	930		88	10	840	90	920		120	13	800	87
2, 8 h	830	—100	50	6	780	94	800	—120	130	16	670	84
2, 20 h	950		85	9	860	91	980		190	20	790	80
3, 8 h	910	— 40	100	11	810	89	1000	+ 20	230	23	770	77
Average, 20 h	1000		108		890		1070		280		790	
8 h	910	— 90	92		820		970	—100	270		700	

TABLE 5. Fluctuations in carbohydrate content (mg/10 g res. dry wt) in the lamina (young leaves; experiment of July 3–4, 1958).

	Total carb.	Starch	%	Total sugar	Sucrose	%	Hexose	%	Glucose	Fructose
Healthy plants										
July 3, 4 h	250	30	12	220	120	54	100	46	60	40
3, 8 h	320	20	6	300	180	60	120	40	–	–
3, 12 h	1060	320	30	740	590	80	150	20	–	–
3, 16 h	1010	390	39	620	500	80	120	20	–	–
3. 20 h	960	360	37	600	460	76	140	24	–	–
3, 24 h	600	130	22	470	350	74	120	26	–	–
4, 4 h	340	40	12	300	200	67	100	33	–	–
Diseased plants										
July 3, 4 h	320	40	12	280	160	57	120	43	–	–
3, 8 h	610	110	18	500	310	62	190	38	–	–
3, 12 h	1100	400	36	700	490	70	210	30	75	135
3, 16 h	1350	740	55	610	420	69	190	31	80	110
3, 20 h	1130	550	49	580	390	67	190	33	75	115
3, 24 h	660	200	30	460	300	65	160	35	–	–
4, 4 h	390	80	21	310	160	51	150	49	–	–

TABLE 6. Fluctuations in carbohydrate content (mg/corr. wt) in the petiole (young leaves; experiment of July 3–4, 1958). Sugar extracts were not chromatographed.

	Corr. pet. wt (g)	Total carb.	Starch	%	Sugar	%
Healthy plants						
July 3, 4 h	3.28	93	2	2	91	98
3, 8 h	3.44	112	1	1	111	99
3, 12 h	3.58	142	7	5	135	95
3, 16 h	3.52	133	17	13	116	87
3, 20 h	3.69	137	10	7	127	93
3, 24 h	3.60	119	6	5	113	95
4, 4 h	3.51	86	3	3	83	97
Average:	3.52	118	7	6	111	94
Diseased plants						
July 3, 4 h	4.02	129	3	2	126	98
3, 8 h	3.74	160	16	10	144	90
3, 12 h	3.33	163	19	12	144	88
3, 16 h	4.16	186	36	19	150	81
3, 20 h	4.35	157	21	13	136	87
3, 24 h	3.93	153	13	9	140	91
4, 4 h	4.14	118	4	4	114	96
Average:	3.95	152	16	11	136	89

TABLE 7. Translocation of carbohydrate (mg/10 g res. dry wt) from the lamina (5th and 10th leaves; experiment of July 7–9, 1958).

	Total carb.	Starch	%	Total sugar	Sucrose	%	Hexose	%	Glucose	Fuctose
Healthy leaf, No. 5										
July 7, 20 h	1230	580	47	650	490	75	160	25	100	60
8, 4 h	480	100	20	380	260	70	115	30	60	55
8, 20 h	1550	880	57	670	490	73	175	27	80	95
9, 4 h	780	250	32	530	420	80	105	20	60	45
Diseased leaf, No. 5										
July 7, 20 h	2060	1030	50	1030	680	66	350	34	145	205
8, 4 h	1780	780	44	1000	590	59	410	41	175	235
8, 20 h	2500	1230	49	1270	800	63	470	37	220	250
9, 4 h	2020	930	46	1090	680	63	410	37	145	265
Healthy leaf, No. 10										
July 7, 20 h	1540	950	62	590	450	77	135	23	65	70
8, 4 h	630	130	21	500	380	76	115	24	45	70
8, 20 h	1870	1020	55	850	670	79	175	21	65	110
9, 4 h	1150	450	39	700	540	77	160	23	70	90
Diseased leaf, No. 10										
July 7, 20 h	1450	960	66	490	300	61	190	39	75	115
8, 4 h	550	190	35	360	210	59	145	41	45	90
8, 20 h	1680	1120	67	560	390	69	170	31	65	105
9, 4 h	790	290	37	500	340	68	160	32	60	100

TABLE 8. Translocation of carbohydrate (mg/corr. wt) from the petiole (5th and 10th leaves; experiment of July 7–9, 1958). Sugar extracts were not chromatographed.

	Corr. pet. wt (g)	Total carb.	Starch	%	Sugar	%
Healthy leaf, No. 5						
July 7, 20 h	3.86	239	23	10	216	90
8, 4 h	4.23	200	9	5	191	95
8, 20 h	4.05	342	39	11	303	89
9, 4 h	4.24	271	19	7	252	93
Average:	4.10	263	23	8	240	92
Diseased leaf, No. 5						
July 7, 20 h	3.22	226	23	10	203	90
8, 4 h	3.80	205	23	11	182	89
8, 20 h	3.71	357	35	13	222	87
9, 4 h	4.11	263	50	19	213	81
Average:	3.71	238	33	14	205	86
Healthy leaf, No. 10						
July 7, 20 h	3.36	185	9	5	176	95
8, 4 h	3.26	123	3	2	120	98
8, 20 h	4.25	225	13	6	212	94
9, 4 h	3.47	211	7	3	204	97
Average:	3.59	186	8	4	178	96
Diseased leaf, No. 10						
July 7, 20 h	3.50	131	9	7	122	93
8, 4 h	3.25	126	7	5	119	95
8, 20 h	3.26	207	12	6	195	94
9, 4 h	3.60	199	7	4	192	96
Average:	3.40	166	9	5	157	95

TABLE 9. Fluctuations in carbohydrate content (mg/10 g res. dry wt) in the lamina (5th and 10th leaves; experiment of July 25–26, 1958).

	Total carb.	Starch	%	Total sugar	Sucrose	%	Hexose	%	Glucose	Fructose
Healthy leaf, No. 5										
July 25, 6 h	350	20	6	330	230	70	100	30	60	40
25, 12 h	590	100	17	490	400	81	90	19	40	50
25, 18 h	750	220	29	530	410	77	120	23	55	65
25, 24 h	610	100	16	510	430	85	80	15	40	40
26, 6 h	340	20	6	320	220	69	100	31	55	45
Diseased leaf, No. 5										
July 25, 6 h	2590	980	38	1610	920	57	690	43	240	450
25, 12 h	2330	790	34	1540	970	62	570	38	180	390
25, 18 h	2530	1090	43	1440	870	60	570	40	180	390
25, 24 h	2630	960	37	1670	980	58	690	42	210	480
26, 6 h	2340	720	31	1620	810	50	810	50	380	430
Healthy leaf, No. 10										
July 25, 6 h	300	20	7	280	180	63	100	37	45	55
25, 12 h	750	190	25	560	450	82	110	18	55	55
25, 18 h	1150	410	36	740	600	82	140	18	60	80
25, 24 h	880	200	23	680	570	84	110	16	55	55
26, 6 h	320	30	9	290	220	76	70	24	30	40
Diseased leaf, No. 10										
July 25, 6 h	1800	570	32	1230	880	72	350	28	130	220
25, 12 h	1650	510	31	1140	810	71	330	29	140	190
25, 18 h	2060	780	38	1280	950	74	330	26	110	220
25, 24 h	1610	570	35	1040	720	70	320	30	130	190
26, 6 h	1270	390	31	880	640	73	240	27	85	155

TABLE 10. Fluctuations in carbohydrate content (mg/corr. wt) in the petiole (5th and 10th leaves; experiment of July 25–26, 1958).

	Corr. pet. wt (g)	Total carb.	Starch	%	Sugar	%	Sucrose	Glucose	Fructose
Healthy leaf, No. 5									
July 25, 6 h	4.60	205	7	3	198	97	115	45	38
25, 12 h	4.58	238	11	5	227	95	148	45	45
25, 18 h	4.73	248	16	6	232	94	156	48	44
25, 24 h	4.05	243	30	12	213	88	158	41	44
26, 6 h	4.72	209	14	7	195	93	111	52	46
Average:	4.54	229	16	7	213	93			
Diseased leaf, No. 5									
July 25, 6 h	4.29	241	65	27	176	73	112	32	32
25, 12 h	4.03	263	67	25	196	75	110	50	36
25, 18 h	4.05	261	91	35	170	65	102	34	34
25, 24 h	3.83	268	61	23	207	77	128	43	36
26, 6 h	4.12	251	59	23	192	77	110	48	34
Average:	4.06	257	69	27	188	73			
Healthy leaf, No. 10									
July 25, 6 h	3.02	184	16	8	168	92	107	31	30
25, 12 h	3.08	225	31	14	194	86	114	47	33
25, 18 h	3.03	255	34	13	221	87	104	42	75
25, 24 h	3.03	232	27	12	205	88	118	50	37
26, 6 h	3.40	186	18	10	168	90	97	36	35
Average:	3.11	216	25	12	191	88			
Diseased leaf, No. 10									
July 25, 6 h	3.71	321	99	31	222	69	118	60	44
25, 12 h	3.53	326	131	40	195	60	113	45	37
25, 18 h	3.55	321	113	35	208	65	110	59	39
25, 24 h	3.35	323	96	30	227	70	123	63	41
26, 6 h	3.55	349	133	38	216	62	108	60	48
Average:	3.54	329	115	35	214	65			

TABLE 11. Translocation of carbohydrate (mg/10 g res. dry wt) from the lamina (10th and 15th leaves; experiment of August 11–13, 1958

	Total carb.	Starch	%	Total sugar	Sucrose	%	Hexose	%	Glucose	Fructose
Healthy leaf, No. 10										
August 11, 19 h	1190	480	41	710	480	68	225	32	90	135
12, 5 h	330	40	12	290	170	60	115	40	50	65
12, 19 h	1180	540	46	640	450	70	185	30	100	85
13, 5 h	350	50	13	300	130	45	165	55	80	85
Diseased leaf, No. 10										
August 11, 19 h	1840	820	45	1020	780	76	240	24	90	150
12, 5 h	1100	420	38	680	480	70	200	30	85	115
12, 19 h	1510	560	37	950	630	67	315	33	105	210
13, 5 h	670	180	26	490	280	58	205	42	75	130
Healthy leaf, No. 15										
August 11, 19 h	900	400	45	500	330	66	170	34	65	105
12, 5 h	270	40	16	230	110	49	115	51	50	65
12, 19 h	1030	530	52	500	370	74	130	26	55	75
13, 5 h	250	50	20	200	80	36	125	64	55	70
Diseased leaf, No. 15										
August 11, 19 h	1390	760	55	630	420	68	205	32	90	115
12, 5 h	1020	600	59	420	280	67	135	33	60	75
12, 19 h	970	460	48	510	370	73	135	27	60	75
13, 5 h	760	290	38	470	210	45	255	55	100	155

TABLE 12. Translocation of carbohydrate (mg/corr. wt) from the petiole (10th and 15th leaves; experiment of August 11–13, 1958). Sugar extracts were not chromatographed.

	Corr. pet. wt (g)	Total carb.	Starch	%	Sugar	%
Healthy leaf, No. 10						
August 11, 19 h	3.00	183	5	3	178	97
12, 5 h	3.56	147	3	2	144	98
12, 19 h	4.37	204	23	11	181	89
13, 5 h	2.98	128	3	2	125	98
Average:	3.48	166	9	5	157	95
Diseased leaf, No. 10						
August 11, 19 h	4.43	346	146	42	200	58
12, 5 h	4.73	351	149	42	202	58
12, 19 h	4.38	287	109	38	178	62
13, 5 h	4.23	217	53	25	164	75
Average:	4.44	300	114	38	186	62
Healthy leaf, No. 15						
August 11, 19 h	3.12	167	7	4	160	96
12, 5 h	3.02	105	2	2	103	98
12, 19 h	3.19	132	6	4	126	96
13, 5 h	2.84	80	2	3	78	97
Average:	3.04	121	4	3	117	97
Diseased leaf, No. 15						
August 11, 19 h	3.64	394	78	20	316	80
12, 5 h	4.44	405	193	48	212	52
12, 19 h	3.56	235	23	10	212	90
13, 5 h	3.53	302	112	37	190	63
Average:	3.79	334	102	31	232	69

TABLE 13. Fluctations in carbohydrate content (mg/10 g res. dry wt) in the lamina of immature leaves (experiment of July 29–30, 1960). Glucose and fructose were determined together as hexose.

	Total carb.	Starch	%	Total sugar	Sucrose	%	Hexose	%
Healthy leaves								
July 29, 20 h	2000	1200	60	800	420	52	380	48
29, 22 h	1620	790	49	830	560	68	270	32
29, 24 h	1310	590	45	720	420	58	300	42
30, 2 h	1020	380	37	640	380	60	260	40
30, 4 h	1090	540	50	550	310	57	240	43
30, 6 h	810	280	35	530	340	64	190	36
30, 8 h	1040	480	46	560	330	59	230	41
30, 6 h	850	360	42	490	310	63	180	37
30, 8 h	910	350	38	560	310	55	250	45
30, 10 h	1120	590	53	530	250	47	280	53
30, 12 h	710	280	39	430	190	44	240	56
30, 14 h	800	370	46	430	260	60	170	40
30, 16 h	960	500	52	460	310	67	150	33
30, 18 h	1050	640	61	410	160	40	250	60
Diseased leaves								
July 29, 20 h	1800	830	46	970	670	60	300	31
29, 22 h	1910	1050	55	860	550	64	310	36
29, 24 h	1300	580	45	720	480	67	240	33
30, 2 h	1130	450	40	680	460	67	220	33
30, 4 h	910	330	36	580	350	61	230	99
30, 6 h	760	260	34	500	220	45	280	55
30, 8 h	720	240	33	480	270	56	210	44
30, 6 h	910	390	43	520	330	64	190	36
30, 8 h	740	300	40	440	270	61	170	39
30, 10 h	940	420	45	520	250	49	270	51
30, 12 h	1090	400	37	690	380	55	310	45
30, 14 h	1170	630	54	540	340	63	200	37
30, 16 h	1460	770	53	690	450	65	240	35
30, 18 h	1240	720	58	520	250	49	270	51

TABLE 14. Fluctuations in carbohydrate content (mg/10 g res. dry wt) in the lamina of mature leaves (experiment of July 29–30, 1960). Glucose and fructose were determined together as hexose.

	Total carb.	Starch	%	Total sugar	Sucrose	%	Hexose	%
Healthy leaves								
July 29, 20 h	3160	1830	58	1330	590	44	740	56
29, 22 h	2810	1630	58	1180	630	53	550	47
29, 24 h	2920	1730	59	1190	560	47	630	53
30, 2 h	2610	1360	52	1250	640	51	610	49
30, 4 h	3010	1590	53	1420	600	42	820	58
30, 6 h	2690	1470	55	1220	610	50	610	50
30, 8 h	2180	1130	52	1050	400	38	650	62
30, 6 h	1810	960	53	850	440	52	410	48
30, 8 h	2270	1040	46	1230	630	51	600	49
30, 10 h	2700	1340	50	1360	710	52	650	48
30, 12 h	3180	1730	54	1450	640	44	810	56
30, 14 h	2660	1520	57	1140	550	48	590	52
30, 16 h	2120	1030	49	1090	560	51	530	49
30, 18 h	2260	1220	54	1040	430	41	610	59
Diseased leaves								
July 29, 20 h	3150	1700	54	1450	940	65	510	35
29, 22 h	2890	1570	54	1320	710	54	610	46
29, 24 h	2690	1420	53	1270	650	51	620	49
30, 2 h	3030	1630	54	1400	780	56	620	44
30, 4 h	2570	1250	49	1320	610	46	710	54
30, 6 h	2380	1240	52	1140	560	49	580	51
30, 8 h	2660	1300	49	1360	750	55	610	45
30, 6 h	1900	980	52	920	420	46	500	54
30, 8 h	2200	1220	55	980	550	56	430	44
30, 10 h	1770	850	48	920	610	66	310	34
30, 12 h	2100	950	45	1150	690	60	460	40
30, 14 h	2060	1110	54	950	480	50	470	50
30, 16 h	1850	880	47	970	630	65	340	35
30, 18 h	2060	1010	49	1050	680	65	370	35

TABLE 15. Fluctuations in carbohydrate content (mg/10 g res. dry wt) in the lamina of full-grown leaves (experiment of August 4, 1960). Glucose and fructose were determined together as hexose.

	Total carb.	Starch	%	Total sugar	Sucrose	%	Hexose	%
Healthy leaves								
August 4, 6 h	2610	1160	44	1450	610	42	840	58
4, 8 h	3680	1930	52	1750	730	42	1020	58
4, 10 h	3360	1810	54	1550	740	48	810	52
4, 12 h	3850	2020	52	1830	770	42	1060	58
4, 14 h	4560	2720	60	1840	700	38	1140	62
4, 16 h	4070	2470	61	1600	670	42	930	58
4, 18 h	4490	2900	65	1590	540	34	1050	66
Average:	3800	2140	57	1660	680	41	980	59
Diseased leaves								
August 4, 6 h	2600	1260	48	1340	600	45	740	55
4, 8 h	2470	1130	46	1340	540	40	800	60
4, 10 h	3240	1700	52	1540	510	33	1030	67
4, 12 h	3840	2260	59	1580	700	44	880	56
4, 14 h	4010	2480	62	1530	600	39	930	61
4, 16 h	2980	1620	54	1360	530	39	830	61
4, 18 h	3630	2170	60	1460	510	35	950	65
Average:	3240	1800	56	1440	560	39	880	61

TABLE 16. Decrease in carbohydrate content (mg/10 g res. dry wt) during the night, and increase during the day, in the 1st and 3rd leaflet pairs of a single leaf (experiment of July 24–25, 1961). Sugars were not chromatographed.

	Total carb.		Starch	Sugar		Total carb.		Starch	Sugar
Healthy (1st leaflet pair)					(3rd leaflet pair)				
July 24, 18 h	2050		1330	720		1380		900	480
25, 6 h	1130	—920	580	550		305	—1075	110	195
July 25, 6 h	860		370	490		315		75	240
25, 18 h	1670	+810	1050	620		870	+ 555	570	300
Diseased (1st leaflet pair)					(3rd leaflet pair)				
July 24, 18 h	2570		1600	970		1770		930	840
25, 6 h	2170	—400	1170	1000		1110	— 660	500	610
July 25, 6 h	1780		730	1050		800		280	520
25, 18 h	2520	+740	1480	1040		1670	+ 870	1030	640

TABLE 17. Carbohydrate contents (mg/10 g res. dry wt) in shaded and control leaves of healthy and diseased plants (experiment of July 17–23, 1961). Sugars were chromatographed, using acetic acid: *n*-butanol: water (40:160:40) as a solvent.

	Total carb.	Starch	%	Total sugar	Sucrose	%	Fructose	%	Glucose	%
Healthy plants										
Control leaves (series I)										
July 18, 18 h	840	490	58.5	350	219	62.5	65	18.5	66	19.0
19, 6 h	237	27	11.5	210	102	48.5	63	30.0	45	21.5
22, 18 h	730	390	53.5	340	221	65.0	53	15.5	66	19.5
23, 6 h	298	68	23.0	230	118	51.0	57	25.0	55	24.0
Control leaves (series II)										
July 18, 18 h	1110	740	67.0	370	203	55.0	104	28.0	63	17.0
19, 6 h	375	115	30.5	260	91	35.0	96	37.0	73	28.0
22, 18 h	910	610	67.0	300	165	55.0	51	17.0	84	28.0
23, 6 h	262	52	20.0	210	137	65.0	44	21.0	29	14.0
Shaded leaves (series I)										
July 18, 18 h	375	155	41.5	220	127	58.0	53	24.0	40	18.0
19, 6 h	173	23	13.5	150	58	39.0	52	34.5	40	26.5
22, 18 h	275	100	36.5	175	98	55.5	38	22.0	39	22.5
23, 6 h	123	24	19.4	99	46	46.0	21	21.5	32	32.5
Shaded leaves (series II)										
July 18, 18 h	560	230	41.0	330	106	32.0	165	50.0	59	18.0
19, 6 h	319	69	21.5	250	90	36.0	95	38.0	65	26.0
22, 18 h	360	150	41.5	210	134	64.0	34	16.0	42	20.0
23, 6 h	150	20	13.5	130	74	57.0	18	14.0	38	29.0

TABLE 17. Continued.

	Total carb.	Starch	%	Total sugar	Sucrose	%	Fructose	%	Glucose	%
Diseased plants										
Control leaves (series I)										
July 18, 18 h	4030	2890	72.0	1140	565	49.5	435	38.0	140	12.5
19, 6 h	4000	2700	67.5	1300	470	36.0	550	42.5	280	21.5
22, 18 h	2580	1900	74.0	680	330	48.5	230	33.5	120	18.0
23, 6 h	1360	840	62.0	520	260	50.0	203	39.0	57	11.0
Control leaves (series II)										
July 18, 18 h	3720	2720	73.0	1000	470	47.0	415	41.5	115	11.5
19, 6 h	3210	2000	62.5	1210	585	48.5	430	35.5	195	16.0
22, 18 h	3500	2510	72.0	990	475	48.0	385	39.0	130	13.0
23, 6 h	1740	1020	58.5	720	270	37.5	371	51.5	79	11.0
Shaded leaves (series I)										
July 18, 18 h	3760	2600	69.0	1160	525	45.5	500	43.0	135	11.5
19, 6 h	2580	1460	56.5	1120	565	50.5	455	40.5	100	9.0
22, 18 h	730	280	38.5	450	194	43.0	194	43.0	62	14.0
23, 6 h	277	47	17.0	230	99	43.0	67	29.0	64	28.0
Shaded leaves (series II)										
July 18, 18 h	2890	1920	66.0	970	390	40.0	450	46.5	130	13.5
19, 6 h	2210	1260	57.0	950	425	45.0	385	40.5	140	14.5
22, 18 h	520	180	34.5	340	126	37.0	160	47.0	54	16.0
23, 6 h	247	37	15.0	210	87	41.0	94	45.0	29	14.0