Plant Physiol. (1971) 48, 792–794

Effect of Wilting on Carbohydrates during Incubation of Excised Bean Leaves in the Dark

Received for publication July 1, 1971

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ABSTRACT

Excised bean (*Phaseolus vulgaris*) leaves were used to compare the changes in various carbohydrates during dark incubation in a wilted and turgid condition. After a rapid (less than 1 hour) wilt, leaves were incubated at a constant water content (75% of original fresh weight).

The starch content of the wilted leaves decreased faster than it did in the turgid leaves. This accelerated starch loss was accompanied by an increase in free (alcohol-soluble) sugars, while no similar increase was observed in the turgid leaves. The increase in free sugars in the wilted leaves during accelerated starch loss was sufficient to account for the decreased starch content in wilted leaves compared to turgid leaves. Thus, total carbohydrate decreased at the same rate in both wilted and turgid leaves during dark incubation.

The free sugar which accumulated in the wilted leaves was mainly sucrose. There was no increase in sucrose content of turgid leaves during incubation, although there was a rapid loss of starch. Glucose and fructose also increased during incubation, but there was no difference between the wilted and turgid leaves. These results suggest that wilting of detached leaves caused a conversion of starch to sucrose in the dark.

It is well known that wilted leaves contain less starch than turgid leaves (5, 6, 8, and references therein). In spite of the numerous studies on the composition of various carbohydrates in plants during moisture stress, little is known about the time course of the changes. In leaves of intact plants, a moisture stress usually requires several hours, perhaps days, to develop, depending on the relative rates of water absorption and transpiration. However, excised leaves can be quickly wilted, since water absorption is prevented by excision; after excised leaves are wilted, they can be maintained at a reduced but constant water content by preventing transpiration. This technique in previous studies (8, 9) showed an accumulation of free sugars during a period of accelerated starch breakdown in wilted leaves. The present study was undertaken to determine what free sugars accumulate during this period and to compare the quantity accumulated with the quantity of starch lost.

MATERIALS AND METHODS

Bean plants (*Phaseolus vulgaris*, L. var. Tendergreen) were grown in soil in flats in the greenhouse. Fully expanded primary leaves were excised from 2-week-old plants that had been exposed to a light intensity of 2500 ft-c (fluorescent and incandescent) for at least 12 hr in a growth chamber. A sample of five leaves, each from a different plant (about 5 g), was used for each incubation time. The midribs were removed, and half of each leaf was weighed and allowed to wilt by exposure of the leaves to the conditions of the growth chamber. The other half was weighed and kept turgid by floating on water in the growth chamber. After the wilted leaves had lost 25% of their original fresh weight, both samples were weighed and placed in closed containers containing humid air. Separate pairs of samples were incubated in the dark for 3, 6, 9, 12, 18, 24, 36, 48, and 72 hr at room temperature (25 C). The closed chambers consisted of 3-lb coffee cans containing pure water. The leaves were in contact with the water only through the vapor phase.

After incubation, the samples were weighed and immersed in 95% ethanol and thoroughly extracted with 80% (v/v) ethanol. The extracts were evaporated to dryness, re-evaporated, and then taken up in H_2O after removal of lipids with chloroform. The water-soluble material was passed successively through columns of Dowex-50-H^+ and Dowex-1-formate.

Reducing sugars were determined on the deionized solution by reaction with 3,5-dinitrosalicylic acid (2) and comparison with glucose as a standard. Sucrose was determined by difference in reducing sugar before and after a 2-hr treatment of aliquots of the samples with invertase (2.5 units). Glucose was determined by the glucose oxidase method (Glucostat, Worthington Biochemical Corp.). The free sugar content was obtained from the amount of reducing sugars after invertase treatment.

Starch was extracted from the ethanol-insoluble residue by the method of McCready *et al.* (7) and determined by a total sugars method analogous to their anthrone method, in which α-naphthol is used in place of anthrone (9). Standards were prepared from potato starch in the same manner.

In some preliminary studies to check the analytical methods, sugars were chromatographed on Whatman No. 1 paper using descending chromatography with ethyl acetate:pyridine:water (10:4:3) as the solvent. Standards were spotted and chromatographed as markers and the sugars were located by applying *p*-anisidine (1) to the marker strips. Individual sugars were eluted from sections of the chromatograms and determined quantitatively by the α-naphthol total sugar method (9).

RESULTS

Preliminary Studies. Figure 1 shows the fresh weight of an excised leaf (wilted and turgid) when treated and incubated as described in "Materials and Methods." These results show that the turgid leaves were maintained at a constant turgid condition during incubation which was the same as the initial water content. The wilted leaves were maintained at a

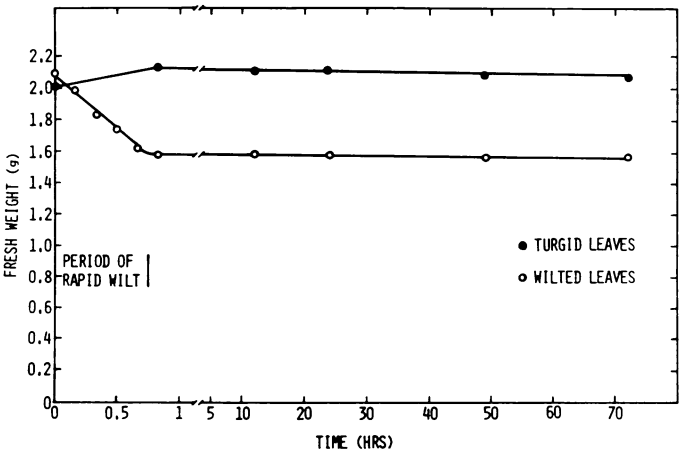

FIG. 1. The fresh weight of turgid and wilted excised leaves during incubation in the dark in a humid chamber.

constant but reduced water content during the incubation. The time course of carbohydrate changes reported in this paper occurred during incubation at constant water content.

When sugars extracted from bean leaves were chromatographed and sprayed with *p*-anisidine, three spots were observed. The R_F values of these spots corresponded to sucrose, glucose, and fructose. When the sugars were eluted from these chromatograms and quantitative determinations were made, the amount of sucrose and glucose in tissue extracts was the same as determined enzymatically as described in "Materials and Methods." However, this correspondence was true only after the extracts had been purified by passing through Dowex 50-H^+ and Dowex 1-formate. If the Glucostat procedure was used prior to this treatment, it markedly underestimated the amount of glucose. The amount of glucose plus fructose determined from chromatography followed by determination of eluted sugars was the same as the amount of reducing sugars determined. If reducing sugar determinations were made without purification with Dowex resins, the determination markedly overestimated the amount of glucose plus fructose. Thus, it appears that there are compounds other than glucose, fructose, and sucrose in tissue extracts that react with the 3,5-dinitrosalicylic acid reducing sugar reagent; these are removed by ion exchange resins. There was a quantitative recovery of glucose, fructose, and sucrose when solutions of these sugars were passed successively through Dowex 50-H^+ and Dowex 1-formate.

Carbohydrate Changes in Wilted and Turgid Leaves. Figure 2A shows the time course of the loss of starch from turgid and wilted excised leaves incubated in the dark. The starch content decreased faster in the wilted leaves than in the turgid leaves and was essentially complete in the wilted leaves after 12 hr and in the turgid leaves after 24 hr. The starch content did not reach zero but leveled off at an amount which was 25% of the initial content. Figure 2B shows the time course of the content of free sugars (reducing sugars of extract after treatment with invertase) in these same leaves. During the time of accelerated starch loss due to wilting there was an increase in the amount of free sugars. The free sugars did not accumulate appreciably in the turgid leaves even during the time of most rapid starch breakdown.

Figure 3 shows the time course of the total carbohydrate content (the sum of the values in Fig. 2, A and B) during the incubation. It appears from these data that there was no difference between wilted and turgid leaves in the rate of carbohydrate loss. In other words, at any given time during accelerated starch breakdown in wilted leaves, the additional amount of carbohydrate lost from starch was recovered in the free sugars. These free sugars were subsequently metabolized at the same rate as the sugars which were released from starch in the turgid leaves, resulting in no difference in the rate of net loss of carbohydrates between wilted and turgid leaves.

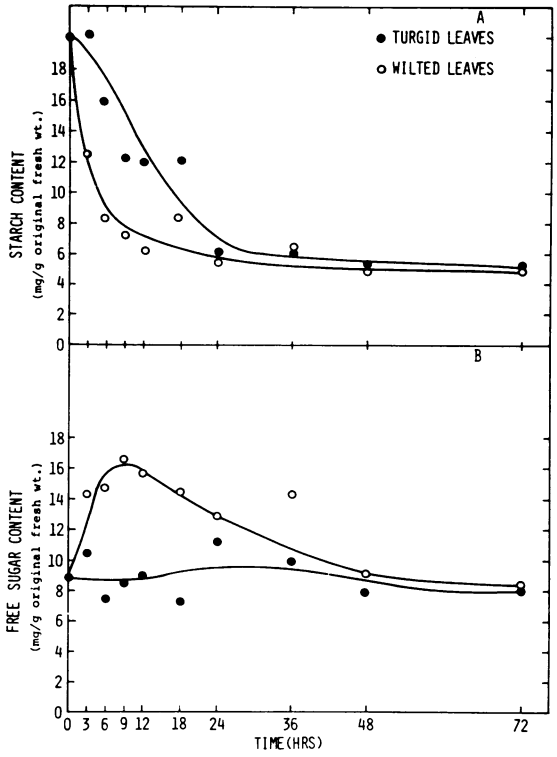

FIG. 2. Changes in starch (A) and free (alcohol soluble) sugar (B) content of turgid and wilted excised leaves during incubation in the dark in a humid chamber.

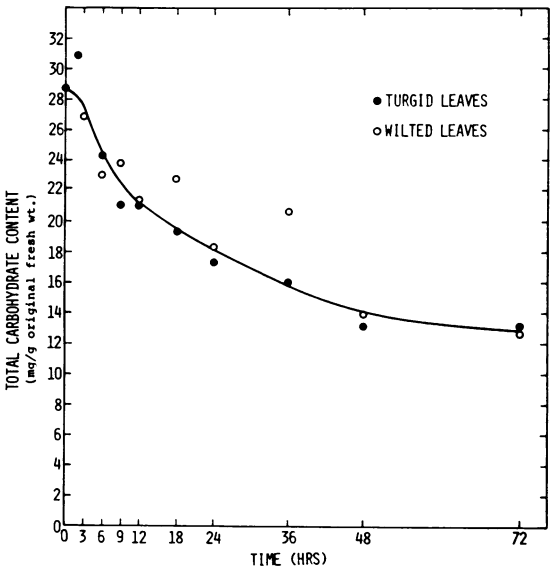

FIG. 3. Changes in the total (starch plus reducing sugar plus sucrose) carbohydrate content of turgid and wilted excised leaves during incubation in the dark in a humid chamber.

Perhaps the most striking effect of wilting in these experi-

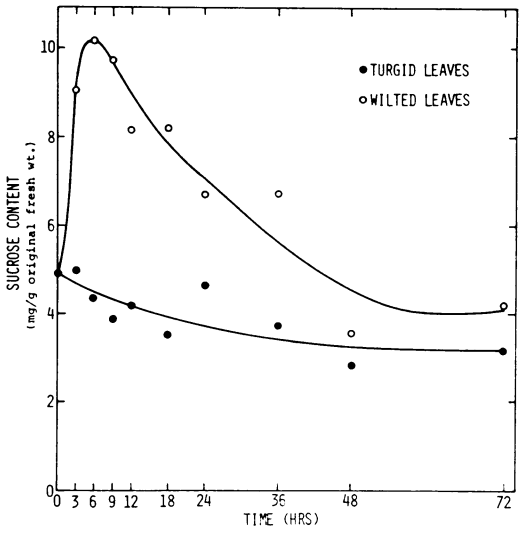

FIG. 4. Changes in the sucrose content of turgid and wilted excised leaves during incubation in the dark in a humid chamber.

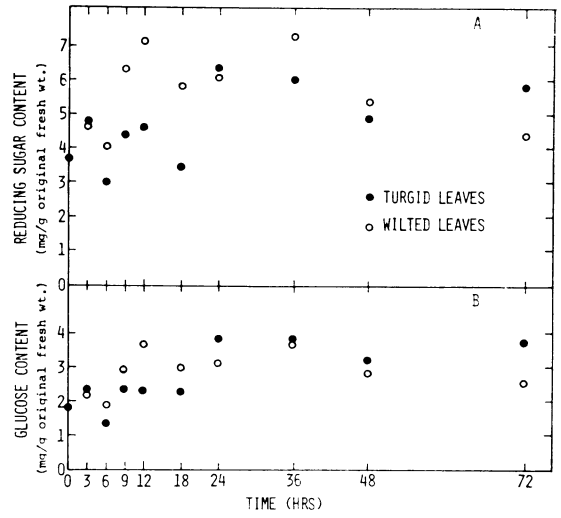

FIG. 5. Changes in the reducing sugar (A) and glucose (B) content of turgid and wilted excised leaves during incubation in the dark in a humid chamber.

ments was the effect on the sucrose content of the leaves during incubation (Fig. 4). In the wilted leaves, there was a marked increase in the sucrose content during the period of most rapid decrease in starch content. In the turgid leaves, there was no increase in sucrose accompanying the decrease in starch content. The period during which there was an accumulation in sucrose in wilted leaves corresponds to the time period during which there was an accelerated decrease in starch content due to wilting (compare Figs. 2A and 4).

The contents of reducing sugars (glucose plus fructose) and of glucose during the incubation are shown in Figure 5, A and B, respectively. As expected, the changes in glucose and reducing sugar followed essentially the same time course. There was quite a bit of variability in the reducing sugar content from sample to sample, resulting in scatter in the points in Figure 5A. The general trend, observed in both the fully turgid and wilted leaves, was an increase in reducing sugars during the period of decreasing starch content. Thus, these data do not indicate any effect of wilting on the content of reducing sugars and glucose.

DISCUSSION

The experiments reported in this paper, in which leaves are rapidly wilted and then incubated in a constant wilted condition, permit a study of the effects of wilting on changes in carbohydrates. The accelerated loss of starch due to wilting is not new, but the accompanying increase in free sugars is not well known, because many of the studies have compared well-watered plants with stressed plants and usually the stressed plants have been under stress for some time, *e.g.*, several days (5, 6). The observation that the accumulation of free sugars in wilted leaves was sufficient to account for their lower starch content during the period of accelerated starch loss has not been previously reported. This observation leads to the conclusion that in these experiments the rate of respiration was not affected by wilting, since the rate of total carbohydrate loss was the same in the wilted and turgid leaves. Measurements of oxygen uptake using standard Warburg techniques on these leaves indicate that wilting results in a decrease in respiration rate of about 10%. The measured rates of oxygen uptake agree with the rate of total carbohydrate loss during the first 12 hr calculated from Figure 3. The measurements of total carbohydrates are not precise enough for a 10% decrease in respiration to be reflected in the data in Figure 3. It is not possible to compare these measurements of the effect of wilting on respiration with other measurements (3, 4), because the water potential of the experimental tissue is not known.

The specific sugar that accumulated during accelerated starch loss due to wilting was sucrose (Figs. 4 and 5). This statement is based on the fact that the sugar which accumulates has the same R_F as sucrose in the ethyl acetate-pyridine-water solvent system and that glucose and fructose are released when the sugar is incubated with invertase. The invertase preparation which was used does not hydrolyze maltose. It is noteworthy that there was no increase in the sucrose content of turgid leaves during the time that the starch content of these leaves was decreasing. The most obvious interpretation of this result is that wilting caused starch to be converted to sucrose. It does not appear that the accelerated starch loss due to wilting was the result of a greatly accelerated loss of total carbohydrates. Thus, it appears that the starch to sucrose conversion is not a result of a marked effect of wilting on respiration rate. In should be emphasized that these experiments were with excised leaves maintained in the dark after rapid wilting. The effects of wilting on carbohydrate changes may be different in plants under natural water stress.

Acknowledgments—This work was supported by a Research Initiation Grant from Iowa State University.

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