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# *THE EFFECT OF ARTIFICIAL WILTING ON THE METABOLISM OF PHOSPHATE COMPOUNDS IN TEA SHOOTS AND STRAWBERRY LEAVES

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The concentrations of phosphate esters, nucleotides and sugars were measured in tea shoots and in strawberry leaves stored either under conditions in which no loss of water occurred or under severe wilting conditions. In both a reserve material other than sugars was used as a substrate for respiration. In the case of strawberry leaves, under severe wilting conditions, sucrose increased in amount. The changes in the phosphate compounds were slight when the leaves were stored without loss of water, except for the 3-phosphoglyceric acid. Under severe wilting conditions the phosphate compounds decreased in concentration but the ratio of the concentrations of the various sugar phosphates known to be enzymically linked did not change appreciably. The ratio ATP-ADP decreased, suggesting loss of respirable substrate or damage to the oxidation mechanism particularly in tea shoots.

## INTRODUCTION

The metabolic effects of artificial wilting are of great importance in the manufacture of black tea by conventional methods. During wilting, the plucked shoots (comprising the bud, the first two leaves and the included stem of the tea plant) are spread thinly on porous material (nylon tats) and allowed to wilt undisturbed for 18-20 hr until the moisture content has been reduced by about 35 per cent. This operation is referred to in the trade as 'withering'. 'Withering' is accompanied by loss of turgor and an increase in the permeability of the cell membranes.[1,2] Though phosphate compounds are intimately involved in most metabolic processes, little information is available as to the effect of wilting on the concentrations present.

In the present study the changes in the various phosphate esters during 'withering' were measured. Strawberry leaves as well as tea shoots were used as plant material, the strawberry leaves being treated as though they were tea shoots and subjected to the standard procedure used for 'withering'. Strawberry leaves were chosen because detailed information about the constituent phosphate esters, nucleotides and sugars was already available.[3,4]

## RESULTS

Table 1 shows the concentrations of phosphate esters, nucleotides and sugars in the fresh, stored and 'withered' strawberry leaves and in 'fresh' and 'withered' tea leaves.

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TABLE 1 — *Concentration of Phosphate esters, nucleotides and sugars in strawberry leaves and tea shoots after storage under different conditions*

| Compound | Strawberry leaves | | | Tea shoots | | |
|---|---|---|---|---|---|---|
| | Fresh | Stored 20 hr no wilting | 'Withered' 20 hr | Fresh (var. Assimica) | Stored 20 hr no wilting (var. TRI 777) | 'Withered' 20 hr (var. TRI 777) |
| Glucose-6-phosphate* | 19.9 | 19.8 | 5.3 | 11.6 | 9.6 | 6.6 |
| Fructose-6-phosphate* | 4.6 | 4.4 | 1.3 | 2.2 | 1.4 | 0.8 |
| Glucose-1-phosphate* | 2.6 | 2.8 | 1.1 | 1.2 | 0.6 | 0.5 |
| Fructose-1,6-diphosphate* | 0.39 | 0.36 | 0.36 | — | — | — |
| Glucose-1,6-diphosphate* | 0.7 | 0.74 | 1.41 | 0.2 | 0.06 | 0.01 |
| Sucrose-6-phosphate* | 0.36 | 0.39 | 0.68 | 0.05 | 0.01 | 0.01 |
| 3-phosphoglycerate* | 22.2 | 13.3 | 7.8 | — | — | — |
| 3-UMP | 1.3 | 1.9 | 2.0 | 1.5 | 1.0 | 2.7 |
| 5-UMP | 0.8 | — | — | 1.1 | 2.0 | 3.5 |
| UDP-sugars | 14.8 | 18.3 | 11.3 | 12.1 | 22.5 | 15.7 |
| UDP-glucose* | 10.6 | 9.8 | 4.4 | 5.0 | 7.1 | 3.4 |
| UDP | 2.7 | 2.9 | 3.9 | 1.5 | 3.8 | 4.0 |
| CTP | 2.1 | 2.3 | 1.8 | 0.9 | 5.6 | 0.1 |
| UTP | 3.8 | 4.6 | 2.9 | 3.2 | 7.0 | 0.58 |
| AMP* | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| ADP | 5.5 | 4.9 | 6.4 | 4.1 | 7.3 | 7.0 |
| ADP* | 5.9 | 4.6 | 6.5 | 5.1 | 9.2 | 9.7 |
| ATP | 11.2 | 8.9 | 8.7 | 10.0 | 18.1 | 0.6 |
| ATP* | 9.2 | 7.0 | 5.9 | 8.0 | 15.0 | 0.1 |
| Glucose* | 2130 | 2390 | 1150 | — | 32 | — |
| Fructose* | 1718 | 1820 | 1275 | — | — | — |
| Sucrose* | 6472 | 6125 | 7720 | — | 175 | — |

Values are —μmoles/100 g fr. wt.
*These were determined enzymically.

## DISCUSSION

*Strawberry leaves.* The small changes in the concentrations of the sugars either during storage with no loss of water, or under 'withering' conditions, as compared to the loss of respirable substrate calculated from the $CO_2$ produced, clearly showed that reserve substances were being metabolized. The average respiration rate of leaves of temperate plants in summer is about 0.44 mg $CO_2$/hr/ g fr. wt.[5] equivalent to 10 μ moles $CO_2$/hr/g fr. wt. In 20 hr this would be equivalent to 3333 μ moles hexose/100 g leaves. However, the combined loss of glucose, fructose and sucrose in the experiment when the leaves were stored with no loss of water was 332 μ moles/100 g leaves (a loss of less than 5% of the total hexose) which meant that reserve materials were being rapidly degraded and used for respiration. The same was true when the leaves were wilted. The nature of these reserve materials is not definitely known and it is possible that many compounds may contribute to different extents to the formation of $CO_2$ depending on the exact physiological state of the leaves. When starch and sugars are present in appreciable amounts it is usually assumed that they are responsible for a large part of the respired $CO_2$. In the present experiments it was known that both sugars and starch were definitely present. In addition, in experiments on strawberry leaves the level of sucrose actually increased during wilting which meant that a reserve polusaccharide was converted into sucrose. Similar observations have frequently been recorded in experiments on the leaves of whole plants subjected to drought conditions (for references prior to 1953, see Ref 6). A study of the content of starch and sugars of lucerne,[7] showed that during the first 24 hr drought conditions, the level of sucrose rose to a maximum while the reducing sugars remained almost constant and the starch declined.

The storage of leaves in punched polythene bags caused very little change in the concentrations of the phosphate esters except for 3-phosphoglyceric acid. This in contrast to the others, decreased markedly. It is possible that it was converted into other compounds rather than used as a substrate for respiration; serine increases more than two-fold during the 'withering' of tea leaves.[8] The 3-phosphoglycerate would be converted to serine via phosphohydroxypyruvate and phosphoserine. Most of the enzymes catalysing the formation of serine from 3-phosphoglycerate have been found in plant and animal tissues.[9,10] The changes in the nucleotides were relatively small and showed no clear pattern.

The changes in the concentrations of the phosphate compounds during 'withering' were more striking than those when water loss is prevented, the most noticeable feature being the large fall in the amount of sugar monophosphate present. Calculation of the ratios of the concentrations of those phosphate compounds which are known to be closely linked enzymically indicated that the ratios changed very little even though the absolute concentration of some of the phosphate esters in withered leaves fell to 25 per cent of the values in stored leaves. This indicated that most of the enzymic complexes were still intact in the'withered' leaves. Values for various pairs of phosphate compounds are given in Table 2.

The main exceptions were the ratios ATP/ADP and UTP/UDP which fell during 'withering'. This fall may reflect the loss of respirable substrate from the cytoplasm or damage to the oxidation mechanisms. The amount of both sucrose and sucrose-6-phosphate was higher in the 'withered' leaves than in those stored under conditions in which no loss of water occurred.

**TABLE 2** — *Ratio of the concentrations of phosphate compounds known to be enzymically interconvertible in stored and 'withered' strawberry leaves*

| Compounds compared | Stored leaves | Withered leaves |
|---|---|---|
| ATP/ADP | 1.52 | 0.91 |
| UTP/UDP | 1.55 | 0.74 |
| Glucose-1-phosphate/ UDP-glucose | 0.28 | 0.24 |
| UTP/UDP-glucose | 0.47 | 0.66 |
| Fructose-6-phosphate/ glucose-6-phosphate | 0.22 | 0.24 |

The reason for the extra formation of sucrose in 'withered' leaves is not known but it may be that one of the enzymes has been affected by the changed concentrations of the intermediates due to the loss of water : a possible candidate could be the enzyme catalysing the reaction between fructose-6-phosphate and UDP-glucose to give sucrose-6-phosphate. An alternative suggestion is that it could be a physical change such as a change in viscosity of the protoplasm[11] which interferes with enzymic reactions and phosphorylation.

*Tea shoots.* These as compared with strawberry leaves are an immature tissue. This is reflected in the very low concentrations of sucrose-6-phosphate, glucose-1, 6-diphosphate and sugars present. Similar 'withering' treatments will produce more drastic changes in tea shoots than in strawberry leaves and it is probable that proteins and nucleic acids will be metabolized more extensively because the carbohydrate reserves are soon depleted. The results shown in Table 1 confirm this general picture for the concentrations of ATP, UTP and CTP after 'withering' are negligible while 3'-UMP (a possible breakdown product of nucleic acid) shows a considerable increase. The other changes are similar to those in strawberry leaves.

## EXPERIMENTAL

### *Strawberry Leaves*

Mature strawberry leaves (var. Royal Sovereign) were picked early in the morning from a field adjacent to the laboratory, washed to remove any dirt, dried between blotting paper and then sorted into samples. The leaves were picked late in the season (10 August 1966).

### *Tea Leaves*

(a) In some of the preliminary experiments, tea shoots (terminal bud with two leaves attached) collected from plants grown in a heated greenhouse in the Cambridge Botanic Gardens were used. Most of the plants were of the Assam variety. *Camellia sinensis*, var. *assamica*. They were grown in soil in large pots with a nutrient solution ; the majority was about 8-9 years old.

(b) In later experiments, shoots plucked from selected bushes at the Tea Research Institute of Ceylon, Talawakelle (1500 m amsl) were packed in polythene bags as soon as possible and flown from Ceylon to Cambridge. The samples were kept at 3-4° during transit and used in the laboratory about two days after being plucked. The term 'fresh shoots' is therefore used in a restricted sense, because of this delay ; 'chemical withering' begins immediately after the leaf is separated from the parent shoot. All experiments were carried out on shoots from Clone TRI 777.

*Storage and 'Withering' Experiments*

*Strawberry leaves.* Three samples (A, B and C) each containing 60g leaves were used. Sample A was dropped into liquid $N_2$ 1 hr after picking. Sample B was stored in a punched polythene bag for 20 hr (from time of picking) under laboratory conditions. Sufficient air was present in the bag to prevent the leaves becoming anaerobic. At the end of the storage, the leaves were dropped into liquid $N_2$. Sample C was spread uniformly on a filter paper sheet and allowed to 'wither' undisturbed for 20 hr under laboratory conditions. The moisture content of the leaves decreased by 25% during this time. At the end of this period the leaves were dropped into liquid $N_2$.

*Tea leaves.* Two samples (A and B) each containing 60 g leaves were used. Sample A was dropped into liquid $N_2$ as soon as the leaves were received from Ceylon while Sample B was 'withered' as described for strawberry leaves and then dropped into liquid $N_2$.

*Extraction of Phosphate Estes and Nucleotides, and Analyses of Constituent Compounds*

Details of the methods used have been described in earlier papers.[3,4]

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