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# RESPIRATORY METABOLISM IN DETACHED SHOOT TIPS OF THE TEA PLANT

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The output of carbon dioxide from detached tea shoots under different conditions of storage was measured using Pettenkoffer tubes. The possible effects of stomatal changes on carbon dioxide output by tea shoots kept illuminated for long periods of time and also in the dark were investigated. The results do not appear to support the view that the physical state of the stomata influences carbon dioxide output. The results also showed that carbon dioxide production is not affected by the presence or absence of light. The respiratory quotient of tea shoots measured manometrically was almost unity for both fresh and withered shoot tips. From the respiration-time curve an estimate of the quantity of carbon dioxide evolved during a 24 hr withering period was obtained. The sugar equivalent of this figure was in close agreement with the loss in dry weight of the tea shoots withered for the same time.

The utilization of reserves as respiratory substrates is an important factor controlling the respiration intensity. The carbohydrate and nitrogenous fractions were, therefore, examined. No simple quantitative relationship between any nitrogenous or carbohydrate fractions and carbon dioxide output was detected.

The first step in orthodox tea manufacture is a withering process during which the plucked tea shoots are thinly spread on tats for a period of about 18 hr during which time the leaf loses about 35% of its moisture. Withering is accompanied by loss of turgor and an increase in the permeability of the cell membranes (Sanderson 1968). Several chemical changes are known to occur during this period (Sanderson 1964a). Some of the significant changes are :

1 — An increase in total free amino acids due to protein breakdown (Bhatia & Deb 1965; Roberts & Sanderson 1966),

2 — An increase in the caffeine content—most probably due to the enzymic synthesis of the alkaloid from amino acids,

3 — Changes in the levels of organic acids, notably a decrease in the levels of malic and succinic acids (Sanderson & Selvendran 1965),

4 — An increase in total carotenoids (Tirimanne & Wickremasinghe 1965),

5 — Changes in the levels of keto acids—notably an increase in the $\alpha$ - keto-isocaproic acid content (Wickremasinghe 1967),

6 — The high-energy phosphates ATP and UTP and the respiratory intermediates glucose-6-phosphate and fructose-6-phosphate decrease appreciably (Selvendran & Isherwood 1971),

7 — The activity of polyphenol oxidase reaches an indefinite maximum when withered from 10 to 18 hr (Sanderson 1964b),

8 — A slight decrease in the content of chlorophylls, these being partially transformed to phaeophytins (Wickremasinghe, Kirtisinghe, Perera & Perera 1965),

9 — The level of carbohydrates undergoes marked changes during withering (Sanderson & Perera 1965). These workers have shown that there is a large rapid decrease in soluble carbohydrates during the first few hours following plucking which is followed by a steady increase in these substances during the withering period; the final level was slightly higher than at the time of plucking.

A general feature during storage of detached leaves is the predominance of catabolic reactions in which carbohydrates and proteins are believed to be broken down to soluble constituents; these products tend to accumulate in detached leaves. Respiration in plant tissues, results in the biological oxidation of substrates particularly the sugars. In the detached leaf, when the initial level of primary substrates is exhausted, other materials may be mobilized for utilization as respiratory substrates (Yemm 1965). Such diversions to meet the respiratory demands of tissues would be expected to produce changes or adjustments in the catabolic products accumulating in detached leaves. It is therefore, possible that conditions of storage and their effects on respiration may be closely related to the enzymatic changes occuring in tissues as well as the catabolic products accumulating in the detached leaf. These products are believed to have an important bearing on the quality of black tea (Roberts 1962). A preliminary study of the respiratory metabolism of detached tea shoots, therefore, seemed warranted.

The respiratory behaviour of detached tea shoots under different conditions of storage was studied by measuring the output of carbon dioxide. In most plants carbohydrates are the main source of respiratory substrates. Other substrates that may be concerned with respiration are fats, proteins and organic acids (Yemm 1965), but there are hardly any known fatty reserves in leaves and organic acids being intermediate oxidation products are not normally consumed for meeting energy requirements of the tissue. In the present study, the soluble carbohydrate fractions and the different nitrogenous fractions of the fresh and the withered tea shoots were examined.

## MATERIALS AND METHODS

All plant material for this study was taken from Clone TRI 2024 and consisted of young shoot-tips only. 'Withered samples' refer to the shoot-tips withered on tats for 24 hr. The respiratory quotient of the fresh and withered samples were measured manometrically and found to be almost unity in both cases. A detailed examination of the respiration rates of tea shoots during withering was carried out. It was intended to measure the rate of carbon dioxide output by tea shoots at different stages of wither. As withering proceeds it may be possible that the stomatal apertures could be subject to physical changes and these in turn may affect the rate of respiration. The basic question as to whether the physical state of stomata has any effect on carbon dioxide output had, therefore, to be first resolved. For this purpose, a set of six samples of tea shoots (20 g) were laid out to wither on tats, while another set of similar samples were placed with their stems dipping in water. They were taken at different times and their carbon dioxide output measured using Petten Koffer tubes. Light may have an effect on respiration rate of the tea shoots standing in water through its influence upon the supply of respiratory substrates resulting from photosynthesis. This experiment was therefore conducted in the dark. It was, also, repeated keeping the two sets of tea shoots in the light. A third experiment was conducted to compare the respiration rates of tea shoots undergoing withering in light and in dark.

### *Loss in dry weight on withering*

The bud, first leaf, second leaf and stem of each shoot tip was separately dissected into equal parts. About 50 shoot tips were dissected to obtain two batches of samples whose fresh weights were then recorded. One batch was immediately placed in a pre-heated oven and dried for 6 hr at 90°C. The other batch was withered for 24 hr and then oven dried under the same conditions and dry weights recorded.

*Extraction and estimation of sugars*

The sample of tea shoots (15 g) was extracted twice in boiling 80% ethanol and the combined volume reduced on a rotary evaporator. After centrifuging, the supernatant was purified by passing sequentially through columns of (i) Polyclar AT-(polyvinyl pyrrolidone), (ii) Dowex—50W cation exchange resin, and (iii) Amberlite-CG4B anion exchange resin. The effluent fraction which contained the sugars was made to volume and an aliquot hydrolysed with 0.01 N HCl. Suitably diluted aliquots of the original effluent fraction and the hydrolyzed fraction were later used for the estimation of free reducing sugars and total sugars respectively according to the method of Park & Johnson (1949). Fig. 1 shows a summary of the procedure used to separate nitrogenous fractions.

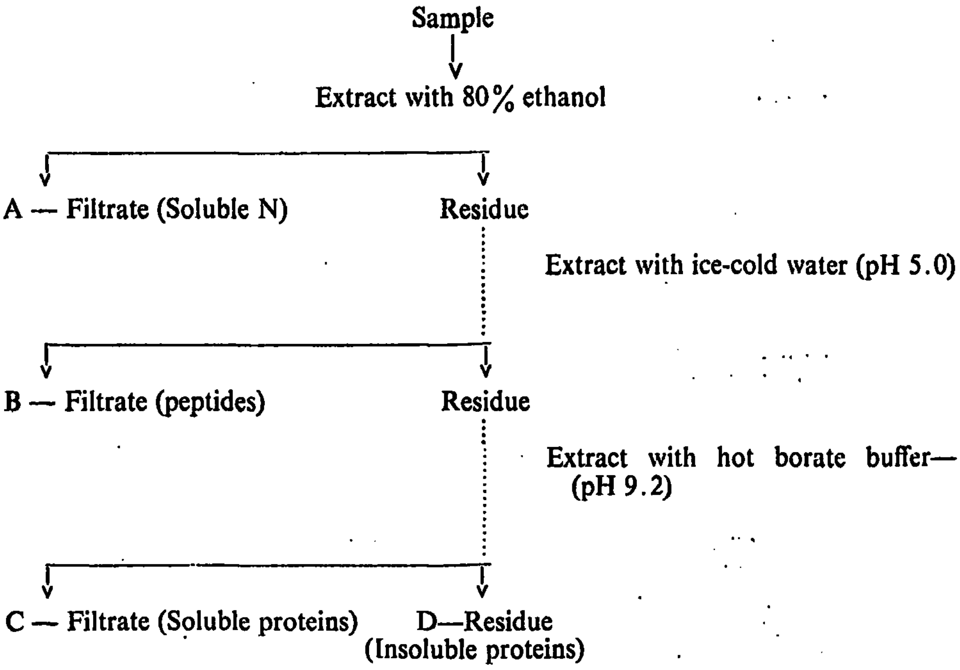

Fig. 1 — Separation of nitrogenous fractions

Suitably diluted aliquots of fraction A were taken for determination of free amino acids by the method of Yemm & Cocking (1955). Aliquots of fractions A, B, C and D were used for the determination of nitrogen by Kjdheldal digestion.

## RESULTS AND DISCUSSION

*Respiratory Quotient* (RQ)

The respiratory quotients of both fresh and withered leaf were found to approach unity very closely. With most plant tissues in which sugar is the primary respiratory substrate and oxidation is complete, the RQ is unity. In most cases respiration represents the simultaneous utilization of a mixture of several substrates, so that the measured RQ represents and average for the entire complex of materials (Bonner & Galston 1952). As the RQ in the fresh sample was the same as that of the withered samples, it is assumed that there is no apparent change in the nature of the respiratory processes during withering.

### *Effect of stomatal aperture and light on carbon dioxide output*

Fig. 2B presents the respiration curves of tea shoots on withering on tats and on standing in water in the dark. The tea shoots standing in water, did not undergo any apparent physical change. Nevertheless the curves of both samples are similar. This may be taken to indicate that the $CO_2$ output was not significantly influenced by the physical state of the structure. There was a considerable drop in the rate of carbon dioxide output in the first few hours, after which the rate attained a steady value. This is a general feature in the respiratory behaviour of detached leaves (Bourne & Ranson 1965). Fig. 2A shows the respiration curves obtained when the experiment was repeated in light. The curves are similar to those obtained in the first experiment. As the tea shoots standing in water may continue to photosynthesize, this may mean that the degree of respiration was independent of the supply of photosynthetic products. Fig. 2C presents the respiration curves obtained when tea shoots were withered on tats in light and in the dark. The presence or absence of light did not appear to have had any effect on carbon dioxide output. Further, the comparatively slow decline in continuous darkness indicates that the substrate for respiration is present in excess under the conditions of the experiment.

### *Loss in dry weight due to respiration*

From the respiration curves, we could devise an average rate of respiration (assuming a linear drop). This was computed as 6 mg $CO_2$/20 g fresh weight per hr. the total carbon dioxide given off by 100 g fresh material for 24 hr is, therefore, 720 mg. Presuming that hexose sugar is the substrate and that oxidation is complete, we have

$$\underset{180}{C_6H_{12}O_6} + 6O_6 \longrightarrow \underset{264}{6\,CO_2} + 6\,H_2O$$

From this it follows that about 500 mg hexose sugar has to be respired for the production of 720 mg $CO_2$. The loss in dry weight of 12 g tea shoots during a wither period of 24 hr was 65.2 mg from which, we can compute the respiratory loss in 100 g fresh material over 24 hr as 543 mg. This loss is in close agreement with the calculated sugar equivalent respired, presuming that the loss in dry weight is carbohydrate loss.

### *Changes in sugar contents*

The content of starch present in tea shoots are reported to be extremely small (Selvendran 1970) and any contribution to the respiratory turnover can be of little account.

The contents of soluble sugars in fresh and withered shoot tips are presented in Table 1.

TABLE 1 — *Changes in sugar content of tea shoots during withering*

| | mg sugar/100 g fresh weight | |
|---|---|---|
| | Reducing sugars | Total sugars |
| Fresh shoots | 176 | 455 |
| Withered shoots | 135 | 385 |

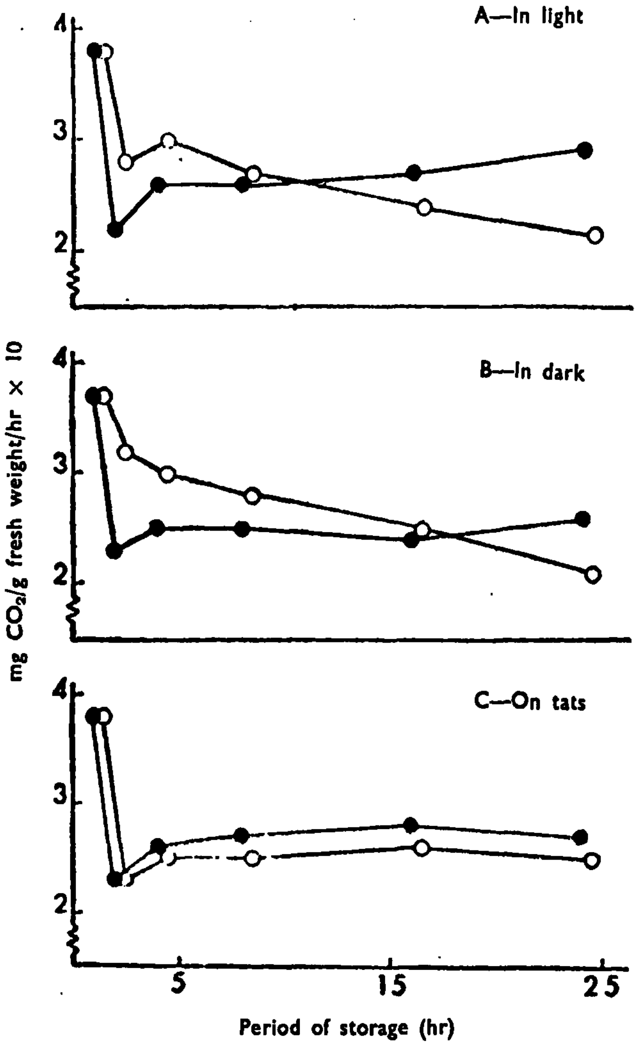

FIG. 2—*Respiration curves of detached tea shoot tips during storage. Full circles in A and B are for shoots standing in water- Hollow circles in A and B are for shoots spread on tats- Full circles in C are for shoots kept in the light Hollow circles in C are for shoots kept in the dark.*

The loss in total soluble sugars during a wither period of 24 hr was 70 mg per 100 g fresh material. This would account for less than 15% of the observed $CO_2$ output. The very low figures for free sugars obtained by Selvendran & Isherwood (1971) was probably because their samples of tea shoots had been in storage for three days during transit before analysis.

*Changes in the content of the nitrogenous fractions*

The nitrogenous fractions of fresh and withered shoot tips were examined and the results are presented in Table 2.

TABLE 2 — *Changes in nitrogenous fractions of tea shoots during withering*

| | Changes in N fractions per 100 g fresh weight | |
|---|---|---|
| | Fresh | Withered |
| Amino acids (mg Alanine) | 320 | 540 |
| Soluble—N | (mg N) | |
| | 381 | 415 |
| Peptide—N | 4 | 13 |
| Soluble Protein—N | 94 | 99 |
| Insoluble Protein—N | 524 | 516 |
| Total (by sum) | 1003 | 1043 |

The differences in the peptide fractions, the soluble protein fractions and the insoluble protein fractions were very small. There was almost a 10% increase in the alcohol-soluble nitrogen. This may be due to the enzymic breakdown of proteins and the concommitant increase in free amino acids which take place during withering of tea shoots (Roberts & Sanderson 1966). An examination of the changes in the contents of the individual amino acids during withering reveal that the consumption of these amino acids (Roberts & Sanderson 1966) would not make a major contribution to the $CO_2$ evolved. It would, therefore, appear that there is nothing here to suggest that proteins are contributory to respiratory carbon dioxide. This is in agreement with the finding of Bhatia (1965) that during the first 24 hr, there was no appreciable difference in the extent of protein breakdown, when tea shoots were withered.

Sanderson & Selvendran (1965) have shown that succinic and malic acids are markedly reduced during 18 hr withering of tea shoots of Clone TRI 2024, but all the non-volatile di and tricarboxylic acids investigated by them taken together, were not present in a quantity which would sustain the $CO_2$ output for more than a few hours. There are also no obvious fat reserves in the tea shoots.

It is unlikely, from the present work, that proteins had any influence on the carbon dioxide evolved. The analyses of carbohydrates present in the detached shoot tips suggest that for the first 24 hr, carbohydrate utilization accounted for only a part of the carbon dioxide evolved. Carbohydrate utilization appeared to stop even when there were appreciable quantities remaining in the shoot tips, and the presumption is that either this carbohyrate occurred in a form not readily accessible to the respiratory enzymes or that some other substrate became available after a time and was preferentially consumed. It may also be possible that there was a rapid turnover of some reserve polysaccharides to sugars, the latter replenishing the quantity consumed in respiration so that the nett decrease in sugar content during withering appears small. In related studies on strawberry leaves Selvendran & Isherwood (1971) surmised that reserve polysaccharides were being used for respiration *via* sucrose, especially as they found an increase in sucrose on withering. Further analyses are now required particularly for additional respiratory substrates of carbohydrate nature.

## SUMMARY

1 — About 15% of the carbon dioxide produced during a 24 hr wither could be accounted for as the loss in sugar content between fresh and withered shoot tips.

2 — There was no evidence to suggest that proteins were involved in respiration during a 24 hr period of storage.

3 — The respiratory quotients of both fresh and withered shoot tips were near unity and this may mean that the predominant substrate was carbohydrate.

4 — The physical state of the stomata does not seem to effect the rate of carbon dioxide output.

5 — The rate of carbon dioxide output appears to be independent of the supply of photosynthetic products.

6 — A major problem remaining is the nature of the endogeneous respiratory substrates.

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