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STUDIES ON THE PHYSIOLOGY OF PRUNING TEA 1—TURNOVER OF RESOURCES* IN RELATION TO PRUNING

S. Kandiah

The variations in the level of resources in the stems and roots following the pruning of tea has been investigated. Bark and wood tissues are considered separately and changes in three separate fractions of the total resources have been followed. By isolation of roots, an attempt was made to check whether there is migration of root reserves into the frame during recovery from pruning. The results indicate that root resources are largely consumed in the root system itself and that resources in the stem bark are appreciable to support bud growth on the frame. The importance of root respiration as a 'sink' for root reserves is discussed in relation to pruning practices adopted in Ceylon.

The growth of perennial plants after a severe reduction of their foliage involves the utilization of reserve food materials. Such growth occurs periodically in all deciduous temperate perennials in spring, after the autumn defoliation and winter dormancy. In the tropics too, certain tree species exhibit similar defoliation and regrowth patterns. Pruning trees is, in a sense, an artificially-imposed growth modification inducing the tree to be dependent on reserves as in the instances cited above. Physiological conditions are however probably dissimilar, in that the former is a part of the natural growth cycle of the tree while the latter is artificially induced. It is possibly because pruning is a physiologically unnatural operation that subsequent recovery is attendant with risks of poor growth, and even death. Pruning alters the natural growth rhythm of the tree. The situation is further complicated in the case of pruning tea because its cultivation itself involves its growth under unnatural conditions—the changing of a tree into a bush. Much research has been done in this field on temperate tree crops. Tropical ones, however, have received comparatively little attention.

The chemical nature of the reserves in the tree, the method of accumulation and utilization, has been studied for a long time, but knowledge in this field is still incomplete. As carbohydrates form the major component of plant tissues, they have been the object of many studies on reserve metabolism.

Most early workers considered starch as the important reserve component of carbohydrates, and variations in starch content during growth received their attention. In many perennial plants including tea, starch comprises the most readily available and osmotically inert reserve carbohydrate. Starch-sugar interconversions in response to metabolic demands have been observed in many plants. An extensive study in tea recovering from pruning was made by Tubbs (1937). Nagarajah & Pethiyagoda (1965) considered sugars and starch, defined as 'total available carbohydrates'—TAC.

* This term includes materials already available as reserves and the products of present synthesis (*eg* carbohydrates, fats and fatty acids *etc*). The terms 'resources' and 'reserves' will, therefore, be used synonymously in non-photosynthetic tissues, when there is no export of assimilates from leaves.

In most of the studies on the reserve metabolism of tea, only roots were considered. Even here the wood and bark tissues were not dealt with separately. The stem tissues of pruned frames have seldom been included in reserve studies, despite the fact that new shoots develop directly on the stem. In the present work both roots and stems were examined for reserves and their bark and wood tissues were considered separately. The purpose of this study is to extend our present knowledge by considering more categories of tissues, and more than one component of the total extractable resources in them. Variation in the level of resources during recovery of 'clean-pruned' and 'lung-pruned' bushes has been followed. By isolating at the time of pruning the root intended to be sampled, an attempt has been made to check whether root reserves are translocated in appreciable quantities to the frame during recovery from pruning.

MATERIALS AND METHODS

Plants of Clone TRI 2024 at St Coombs (1500 m amsl) were used. There were three treatments assigned to conform to a completely randomized design :

1 — Clean-pruned, by removing all leaf-bearing branches.

2 — Clean-pruned, but a root of diameter 1 to 1.5 cm at its proximal end, was cut free from the bush and allowed to remain buried in the soil until it is due for sampling. This prevents the movement of reserves between this root and the frame.

3 — Lung-pruned by retaining a sizeable leaf-bearing side branch.

Sampling procedure

The stem sample was 5 to 6 cm long and 1.5 to 2 cm in diameter, obtained from the pruned frame. A piece of root of similar length but of diameter (1 to 1.5 cm) obtained from the same bush formed the corresponding root sample.

Nine sampling occasions were spread over a period of 165 days. At each sampling occasion six bushes were sampled per treatment. The first sample was obtained on the day the treatments were imposed, and consisted of nine randomly-selected bushes. This involved 153 bushes in all.

The treatments were imposed on November 2, 1970, on which date the first sample was obtained. The second, third and fourth samples were obtained at approximately ten-day intervals. The sampling dates for the remaining samples were decided on the basis of the growth stage of the new shoots on the frame. At the time of the fourth, fifth, sixth and seventh samples, the average size of the new shoots on the frame was as shown in Fig. 1 left to right. The lung shoots on the bushes of treatment 3 were removed at the time of the seventh sampling, 84 days after pruning. The new shoots were tipped (cut 10 to 15 cm above the frame) at the time of the eighth sample, 125 days after pruning. The ninth sample was obtained 40 days after tipping.

The pieces of stem and root sampled in the field were immediately brought to the laboratory, washed, separated into bark (all tissues outside the cambium) and wood; cut into small pieces and placed in an oven at 80°C for overnight drying. Samples of about 1.5 to 2 g of wood, and 0.5 to 1 g of bark were enclosed in 3 x 6 cm muslin bags, left in the oven for a further period of not less than six hours, cooled in a desiccator and weighed.

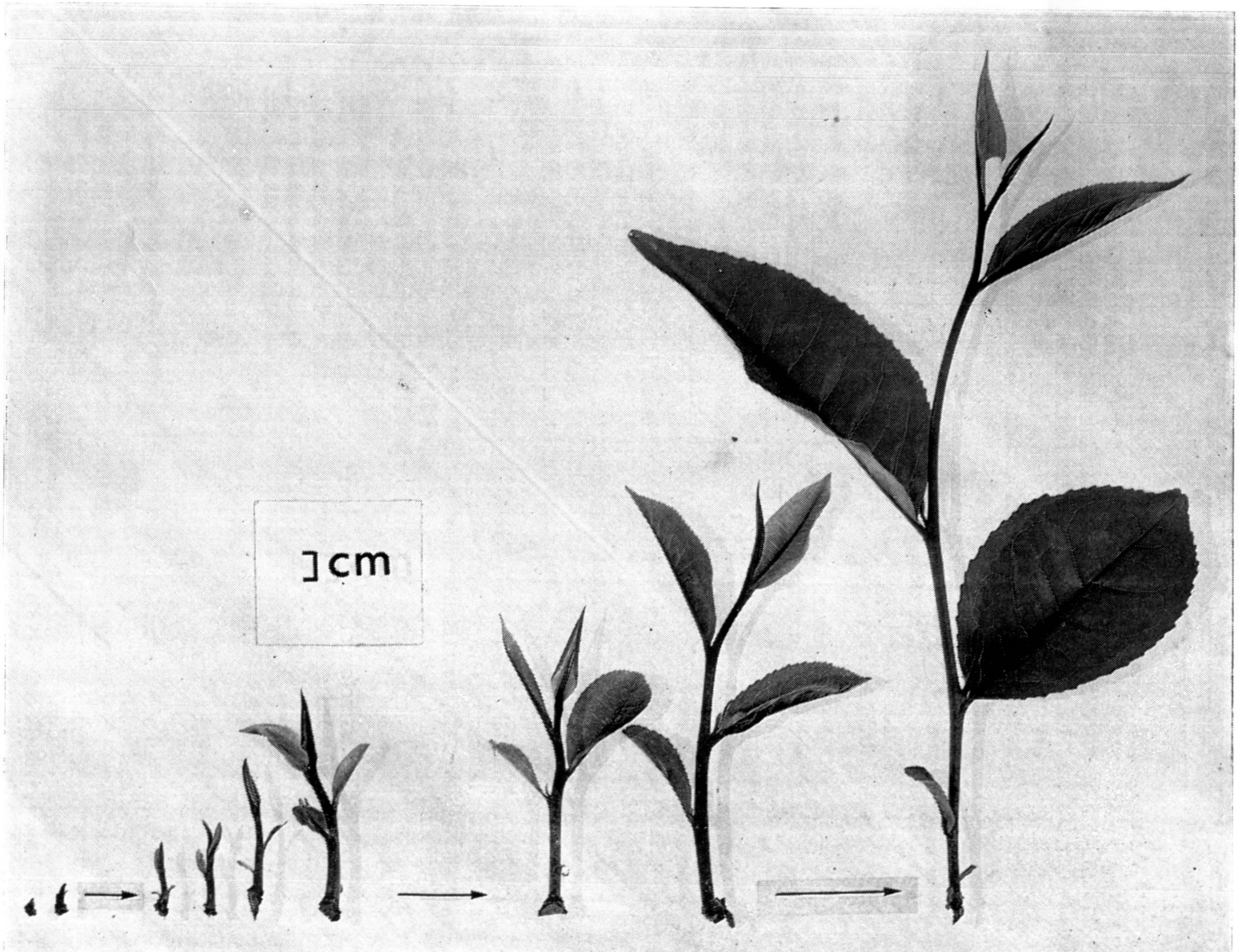

FIG. 1—*Samples of typical shoot size—left to right, 1st batch 32 days, 2nd batch 49 days, 3rd batch 66 days, 4th batch 84 days after pruning*

Analytical procedure

A modification of a method in regular use for extraction of carbohydrate resources in apple trees (Priestley 1962) was employed. In Priestley's method the carbohydrate resources are extracted in three fractions, (a) soluble sugars and glucosides, (b) starch and (c) hemicelluloses.

A disadvantage in this method is the long time taken for estimating the carbohydrates in the three fractions after conversion to sugars. To avoid this it was decided to estimate the resources in each of these fractions in terms of extractable material, by simple measurement of weight changes after extraction. This involved determining the initial dry weight of the samples in the muslin bags and then determining the loss in weight after extraction of each fraction. The extraction procedure was as follows :

1 — The sample-bags were placed in Soxhlet extractors and extracted for 10 hr with 70 to 80% aqueous methanol. The dry weight of the residue was determined. The loss in dry weight gives the weight of methanol-soluble material.

2 — The residue was similarly extracted for 1 hr with 1% HCl in 96% aqueous methanol, followed by extraction with 95% aqueous methanol for a further hour. This results in the solubilization of starch. The samples were then dried and extracted with water for 10 hr. The dry weight of the residue was determined, and the loss in dry weight of the residue from 1 gives the weight of hot-water soluble material in the residue.

3 — The sample bags were then transferred into a litre flask and covered with N H_2SO_4 and refluxed for 1 hr. The acid was then drained off and the samples washed in running tap water for 1 hr. The dry weight of the residue was determined. The loss in dry weight of the residue from 2 gives the weight of fraction corresponding to 'hemicelluloses' in the sample.

The loss in weight of the empty bags after these extractions was found to be negligible. The samples need not, therefore, be taken out of the bags for dry weight determinations once the initial weights of the bags are known. The coefficient of variability of the estimates of weight losses after 1, 2 and 3 for six replicates of bark and wood samples from the same source was found to be less than 4%.

The Soxhlet extractors of 100 ml siphoning capacity that were used could each take six bags of wood sample or 12 bags of bark sample of the size used in this experiment. A heating unit for six flasks could, therefore, be used to extract 36 wood samples or 72 bark samples at a time. This is advantageous in experiments involving large number of samples.

Expression of results

The estimates of resources in the three fractions were expressed as a percentage of the residue left after final extraction (g/100g residue dry weight). The residue after extraction is considered to represent the structural cell material within which resources are stored. Expression of results on this basis, for tissues that do not grow appreciably during the period of study, overcomes certain limitations of using initial dry weight (Priestley 1962).

The three fractions are distinguished as (a) the methanol fraction, (b) the hot water fraction and (c) the sulphuric acid fraction; and are abbreviated into MF, HF and SF respectively. The term 'total resources' is used to denote all three together.

RESULTS

Total resources

The variation in the total extractable resources in the stem and root tissues of the bushes receiving the three treatments are presented in Fig. 2 (A to D). The following features are evident from the graphs :

1 — There are more resources available per unit of residual material in the bark than in the wood. (Note that the curves for bark and wood are drawn to different scales.)

2 — While the levels of resources in the bark of stem and root do not differ markedly, those in the wood tissues do—the wood in the root has much more resources per unit residual matter than the stem wood.

3 — The change in the level of resources in the bark following pruning is different from that in the wood.

Bark (Fig. 2 A & B)

In the stem, bark resources are depleted following pruning (Fig. 2A). Although there appear to be intermittent phases of replenishment, these do not become marked until the time of the seventh sampling—84 days after pruning by which time the shoots would have begun to export photosynthates. Tipping, at sample 8, seems to have checked this process of replenishment, resulting in the resources in the last sample collected 40 days after tipping, being still less than that at the time of pruning. Differences between treatments are not pronounced.

In the root bark the trends are similar but more marked and uniform (Fig. 2 B). Besides, in the lung-pruned bush the phase of depletion is steadier and quicker than in the clean-pruned bush or in the isolated root; and replenishment starts much earlier. A notable feature in the root bark is that the isolated root too shows a trend comparable with that in the pruned bushes.

Wood (Fig. 2 C & D)

Unlike stem bark, stem wood shows negligible changes in levels of reserves following pruning (Fig. 2 C). In the root wood, in contrast, the changes are very marked. In all treatments there is depletion of resources following pruning (Fig. 2 D). Depletion of resources become more marked after the third sample—23 days after pruning. In the clean-pruned bushes depletion continues up to the time of tipping. In the lung-pruned bushes such depletion is checked very early. Tipping, however, seems to have depleted the level of resources, and in the final sample collected 40 days after tipping, there is not much difference between clean-pruned and lungpruned bushes.

In treatment 2, where the root was isolated, the reserves became depleted but rather unsteadily compared with that in attached roots. Even after 165 days the reserve level did not decrease to the same extent as that in the attached roots. In this respect the wood tissues in roots differed from the bark in roots.

Component resources

The variation in the level of the three component fractions of total resources in the bark and wood are presented in Figs. 3 and 4.

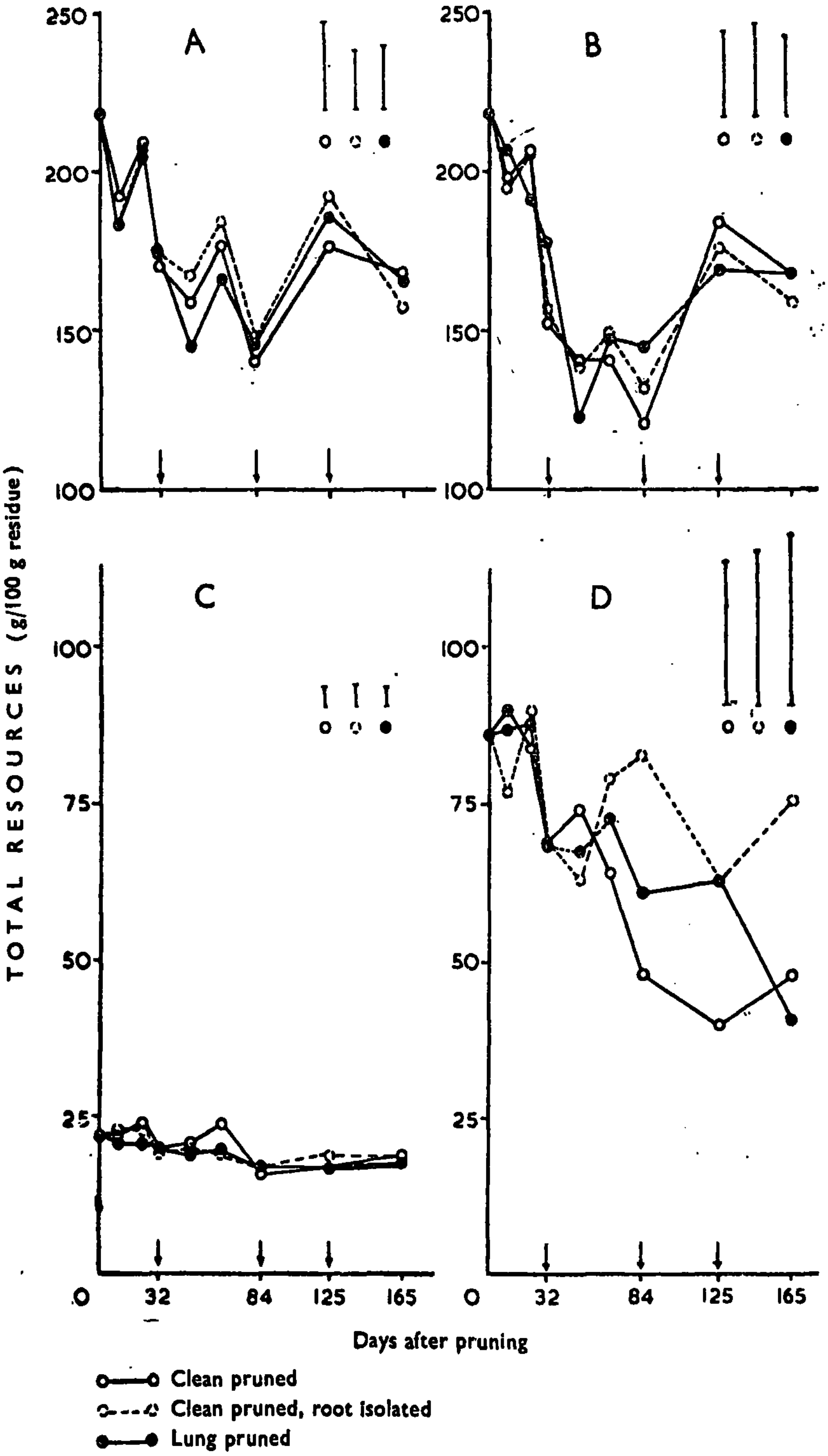

FIG. 2—*Total resources in (A) stem bark, (B) root bark, (C) stem wood and (D) root wood—Arrows indicate time of bud break, removal of lung shoots, and tipping—Bars represent LSD (P=0.05)*

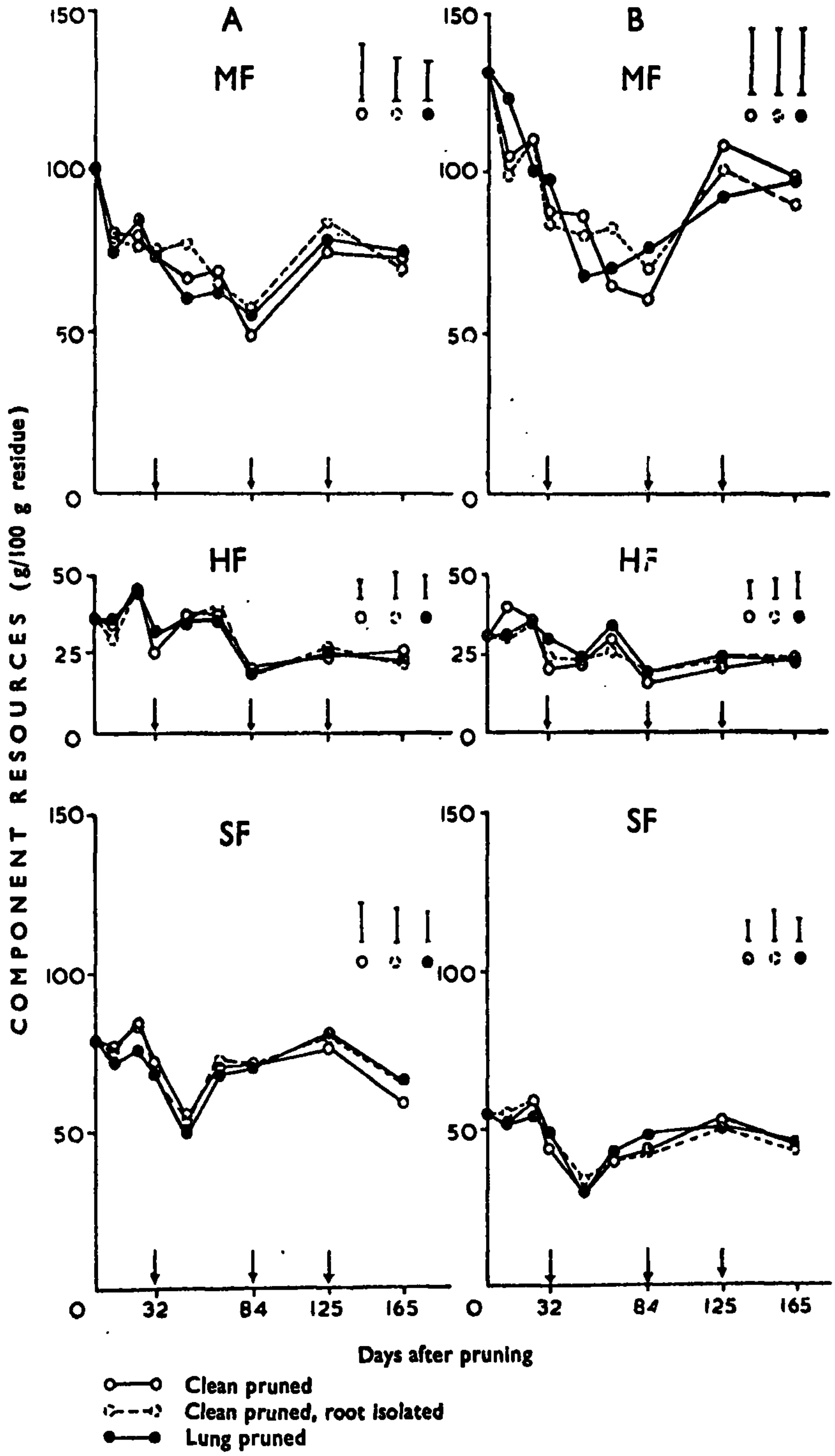

FIG. 3—*Component resources in the bark of* (A) *Stem, and* (B) *root-methanol, hot water and sulphuric acid fractions are indicated by MF, HF and SF respectively—Other symbols are as in FIG. 2*

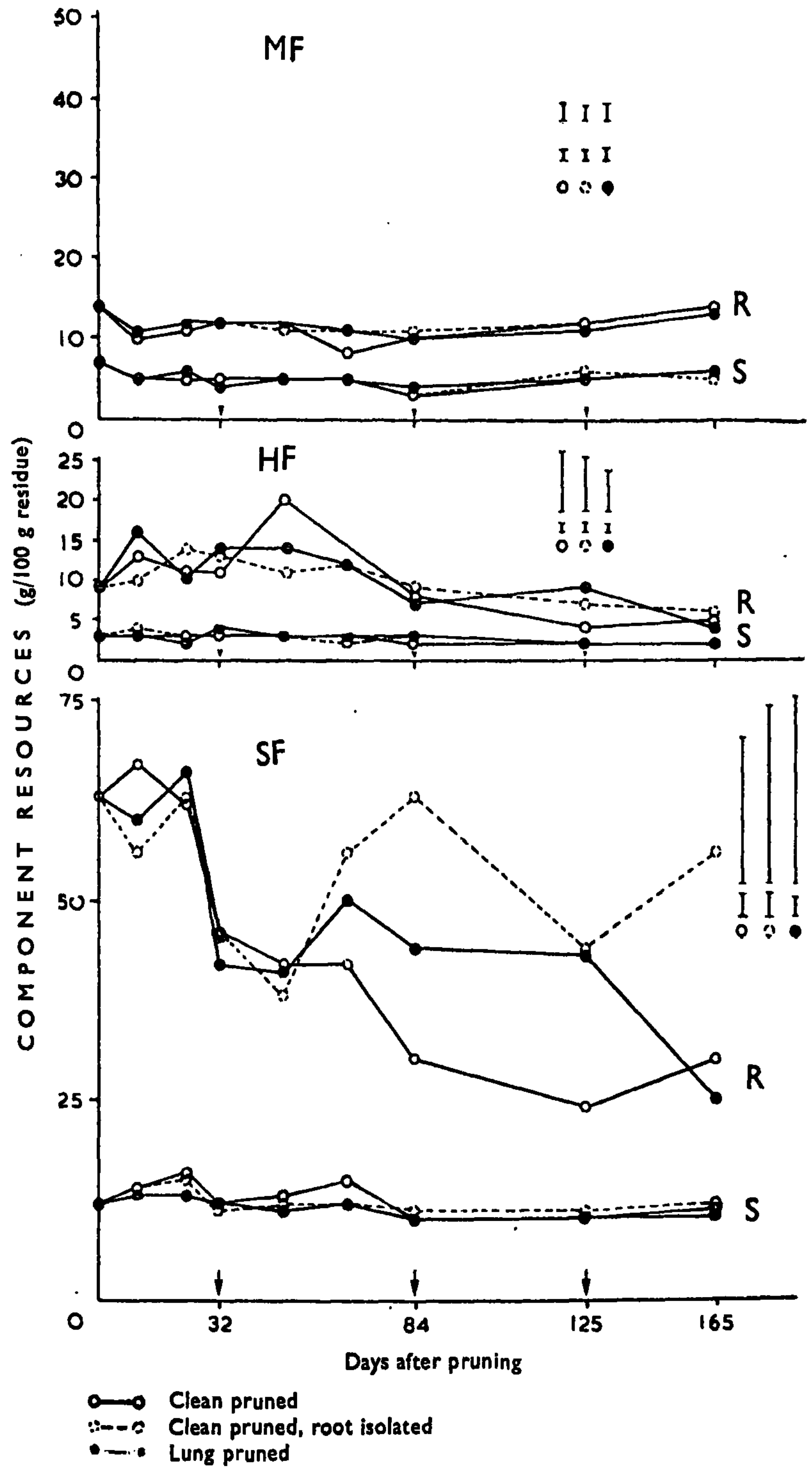

FIG. 4—Component resources in the wood of root, R, and stem, S—Other symbols as in FIG. 3

Bark (Fig. 3A & B)

There are similarities in the relative proportions of the components in the bark of stem and root. In both, the methanol fraction (MF) predominates followed by the sulphuric acid (SF) and hot water (HF) fractions. As was the case for total resources, none of these components show marked differences between treatments. The depletion following pruning is seen to be contributed more by the MF and the SF than by the HF. The HF in the bark appears to be comparatively steady., Although the HF in the stem and root bark does not vary greatly, the MF is greater in the root bark, and SF in the stem bark. There are no clear indications of an inter-conversion of one fraction to another within the bark, because the graphs seem more to correspond than to alternate, as would be the case if interconversion of components had taken place.

Wood (Fig. 4)

In the stem the components in the wood seem to be little affected by pruning. In the root wood, pruning effects are most marked in the SF which forms the major component. Some fluctuation in the level of the HF is seen only in the clean pruned treatment. The MF is fairly steady.

Analysis of variance was carried out on the data. The variation due to sampling time was found to be significant ($P < 0.05$) for total resources in all treatments except root wood in the isolated root. Values for components too showed significant variation except the MF in the root wood of lung-pruned bushes, and the HF and SF in the root wood of the isolated root.

DISCUSSION

The methods previously adopted for estimating carbohydrate resources in tea are laborious and fail to differentiate between different components of the total resources. In studies where the variation of resources is of interest, a technique for estimating resources which is simple enough to allow for the study of a greater number of samples and for reasonable replication would be a great advantage., Simplicity in analysis, however, generally implies some sacrifice in clarity. The analytical procedure adopted here is a compromise between these objectives.

An attempt is made to extract resources within the samples in three steps, so that the total resources could be separated into three component fractions. Simple measurement of weight change on extraction is used to estimate the quantity of material in each fraction. This would only be a quantitative estimate of resources. However, knowledge of the compounds expected in the samples, and the extracting properties of the solvents used, serve as a guide to the nature of the compounds in each fraction. Nevertheless, a detailed analysis of each fraction would be essential before conclusions can be drawn on the functional importance of any one compound.

It is expected that the MF would contain mainly soluble sugars, amino acids and other organic acids, and in the case of bark, appreciable quantities of phenolic substances and saponins. The abundance of saponins in tea roots is noteworthy and it is very probable that they have a definite physiological role. It is likely that carbohydrate predominates in HF and SF fractions. This has been reported for apples (Kandiah 1970). HF may include largely starch and pectic substances. Polysaccharides that fail to be extracted in HF may be partly extracted in SF and this fraction could be tentatively taken to represent hemicelluloses.

Distribution of resources

The quantity of resources per unit residual matter is markedly more in the bark than in the wood. As the dry weight of wood in the tea bush is more than that of bark, the difference in content between bark and wood tissues may not vary as much as the percentage values. These results, however, indicate that an appreciable quantity of resources can be stored in the bark although this region is less bulky than the wood.

The distribution of the component fractions of the total resources was found to differ in the bark and wood. Despite the emphasis given to starch in reserve studies on tea, the hot water fraction that was expected to contain this component in the present study was found to be least responsive to pruning. In the bark MF and SF are seen to be the major components (Fig. 3) while in the wood SF predominates (Fig. 4), although HF in root wood is markedly more than in stem wood. This could either result from the incomplete extraction of starch into HF or because hemicelluloses in SF function as an important reserve component in tea. The latter is probable in view of the storage function attributed to hemicellulose in other plants (Murneek 1929 ; Priestley 1962). The apparent absence of a direct relationship between carbohydrate reserves and recovery after pruning noted in earlier studies (Pethiyagoda 1964) may be because this fraction was overlooked.

Although it is recognized that root reserves are utilized during recovery it has not been possible to associate them with any particular aspect of recovery. As shoot development on the frame is the more evident growth manifestation in a tea bush recovering from pruning, mobilization of root reserves to maintain early shoot development has been one of the views regarding the role of root reserves. The root isolation treatment was intended to check whether there is appreciable migration of reserves from the root to the frame during recovery.

The results indicate that resources in the root bark are depleted following pruning, but it seems that such depletion is not due to consumption in new shoot growth. Depletion of resources precedes the appearance of buds on the pruned frames. Also, resource levels in the isolated roots show a decline similar to that of attached roots (Fig. 2B). It is likely that these resources are consumed in the root system itself. In the root wood the pattern is different from that in the bark (Fig. 2D). Depletion of resources in this region is most marked in clean-pruned bushes. The presence of lung shoots seems to have checked such depletion, while in the isolated root depletion was comparatively less. During the first seven weeks after pruning, however, the curves for the attached and detached roots do not vary much. If migration of reserves to support new shoot growth had occurred, it would be expected to be most marked during this period and not later, as seen here, when shoot growth on the frame is fairly advanced.

Although it is evident that in tea under cultivation the root does store more labile resources than the stem, and in this sense could be considered as a storage region, such reserves seem to be consumed mostly in the root system iteself. It is possible that the influence of root reserves in shoot growth during recovery is indirect in that they maintain root activity when demands for shoot assimilates are great.

The early reserve requirements of bud growth on a pruned frame appears to be mostly met by those in the stem bark—the stem wood being little affected during this period (Fig. 2A and C). The stem bark resources are depleted following pruning and replenishment seems to occur only after the new shoots are well established. Bud growth from regions of tea shoots isolated by ring-barking has been observed to occur (Nagarajah and Pethiyagoda 1965). The importance of stem bark as a source of reserves, at the beginning of leaf expansion in spring in temperate trees has been observed by others (Mason and Whitfield 1960 ; Tromp 1970). The reserve requirements during shoot development depends on the developmental pattern of the new shoots from dormant buds. Species that produce appreciable quantities of new tissues before new leaves begin exporting photosynthates would tend to utilize more reserves than others (Kozlowski & Keller 1966). Tea could be described as a species that establishes its assimilatory tissues fairly early in shoot development (see Fig. 1). It is possible that in tea, reserves in the stem bark are sufficient to meet initial reserve requirements of bud growth. The importance of having healthy branches on a pruned frame is a recognized fact—a healthy frame provides sufficient bark resources for bud growth. Twisted, knotty and half rotted branches are said to be poor bases for bud development (Middleton 1963 ; Scott 1963 ; Somerville 1964).

Attention has been primarily directed towards determining the importance of root reserves during recovery from pruning. A fact that is often overlooked is that in pruning tea there is a severe reduction in the shoot/root ratio. Under normal conditions the roots are totally dependent on leaves for carbon assimilates. Pruning results in the complete severence of this supply, causing the root system which has not been similarly reduced to become entirely dependent on its reserves until new shoots are well established on the frame. During this period root respiration could be an important sink for root resources. Tea in plucking has most of its feeder roots in the top layers of the soil, which become exposed after pruning, resulting in higher soil temperatures. In view of the high proportion of living cells, these roots can consume appreciable quantities of resources in respiration. This would, therefore, be more marked at lower elevations. It may be that tea after pruning could do well with less than the quantity of feeder roots normally present. Forking the soil after pruning to a certain extent achieves this and its significance to root resources is worth future investigation. Besides respiration, root growth and other physiological activities within the root could also utilize root resources at the same time. Under these conditions, dieback on the frame, if attributable to the lack of root resources, may be a secondary effect, the primary cause being the death of roots. The lung-shoots would then benefit the root more than the stem. The results indicate that replenishment of root resources starts earlier in both bark and wood in the lung-pruned bushes. This is evident in MF in the bark (Fig. 3B) and SF in the wood (Fig. 4B). In the latter although removal of lungs 84 days after pruning did not depress its level, subsequent tipping did. This could be because the marked reduction of foliage resulted in a corresponding reduction in the assimilate supply to roots. Besides, new growth on the tipped shoots too may make greater demands on assimilates at this time. This 'tipping effect' was not noticeable in clean-pruned bushes where the level of SF was in any case much lower at the time of tipping.

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