III

RESPIRATORY DRIFTS

MUCH of the pioneer work on plant respiration depended on measurements of CO_2-output, and a favourite method of working was to sweep the carbon dioxide out of the respiration chamber into a Pettenkofer absorption tube by means of a continuous air-stream. About the turn of the century, F. F. Blackman devised a switch which automatically diverted the gas-stream through a series of Pettenkofer tubes at fixed intervals. He applied it to the investigation of CO_2-outputs of tissues, prolonged over intervals of weeks or months, and so initiated what has proved to be one of the major approaches to the study of plant respiration and the basis of modern fruit storage.

DETACHED LEAVES

The first respiration drifts to be examined in detail were those of detached leaves of cherry-laurel (*Prunus laurocerasus*). The choice was dictated by experimental convenience, and the leaf was chosen as one that would not deteriorate too rapidly when isolated. The records were followed through until obvious necrosis set in, and the characteristic spontaneous drift thus made known became the starting-point for further manipulation. The normal conditions of experiment confined mature cut leaves in a dark chamber with their petioles dipping into water and a continuous current of air passing through at a temperature around 20° C. The complete time curve of CO_2-emission is represented in Fig. 7[1] for leaves that were maintained at 16·5° C. Most subsequent experiments have been run a little warmer. Blackman analysed this curve into six phases—characterized as follows:

Phase	*Duration in days*	*Slope*	*Colour of leaves*
1	1	high level	green
2	6	rapid fall	green
3	6	low level	green → pale green
4	c. 20	steady rise	pale green → yellow
5	c. 20	steady fall	yellow → brown
6	..	sharp rise	brown

[1] This figure has not been previously published. It is a reduction of a wall-diagram designed by the late Dr. F. F. Blackman to illustrate his results and used by him in lectures to the Cambridge Botany School to whom I am indebted for permission to use it here. Much of the work on cherry-laurel has not been published in any journal and the remarks above are based on Dr. Blackman's lectures and on personal communications.

from Plant Respiration

~~Respiratory~~

F.F. Blackman. 1958.

Before attempting any analysis of the metabolic processes underlying these changes, it will be well to compare them with corresponding results for other species. Blackman chose *Tropaeolum majus* leaves as a contrasting type, and their time curve of CO_2-emission is also represented in Fig. 7. The form of the curve is very similar to that for cherry-laurel; but the drift is much faster, and, to make the successive phases correspond, the time axis for *Tropaeolum* has been expanded twofold.

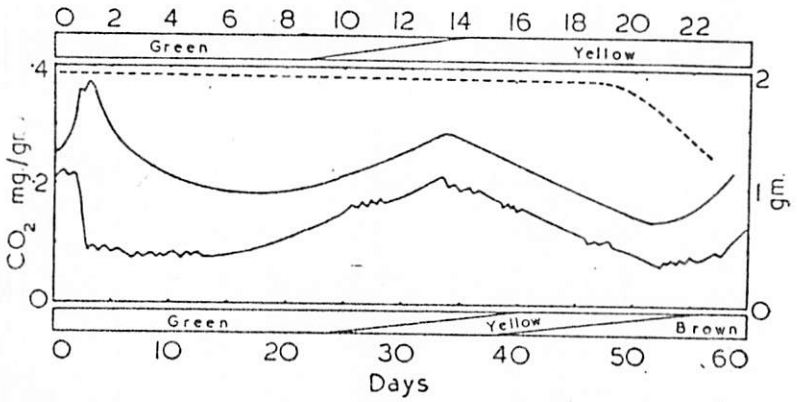

FIG. 7. Respiration drifts of detached cherry-laurel and *Tropaeolum* leaves. Bottom curve and time scale refer to cherry-laurel; upper solid curve and time scale to *Tropaeolum*. The broken line gives the leaf weight of *Tropaeolum* with ordinates on the right.

The drift curves of barley leaves have also been studied in detail. Individual curves show much variation; but also constant features that afford interesting comparisons with cherry-laurel. In Fig. 8, a generalized barley curve, based on numerous experiments in the Oxford laboratory, is plotted with one for cherry-laurel at an approximate temperature of 20° C. The time scales are very different. The drift that may occupy over a month in cherry-laurel is completed in about six days by barley. Conversely, the initial rate of cherry-laurel is about one-fifth that of barley and, in spite of fluctuations, a wide difference of rate is maintained. Nevertheless, by suitably adjusting the time scales, certain similarities of form, and also some important differences, are easy to see. Phases 1 and 2 occur in both; but the phase 2 of barley is interrupted at a relatively high level and no low, steady phase 3 is observable. It is, in fact, not always evident with cherry-laurel either at the higher temperature. Phases 4 and 5 are represented in the barley curve by a high undulating plateau on which a rise (phase 4) and fall (phase 5) round a single peak are not clearly defined. There is usually a small rise after 4–5 days (phase 6) followed by a sudden plunge towards extinction. Curves for wheat published by Krotkov (1939) show phases

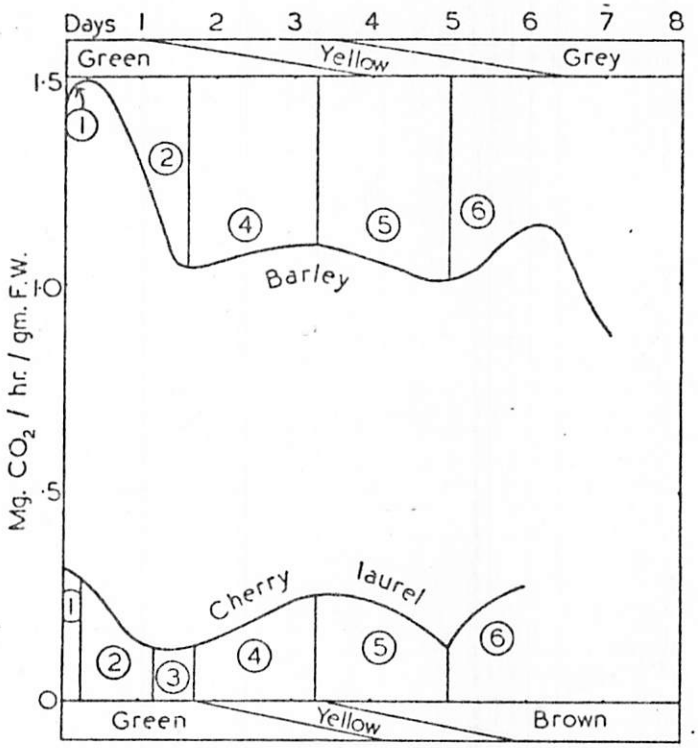

FIG. 8. Comparison of detached barley and cherry-laurel leaf drift-curves.

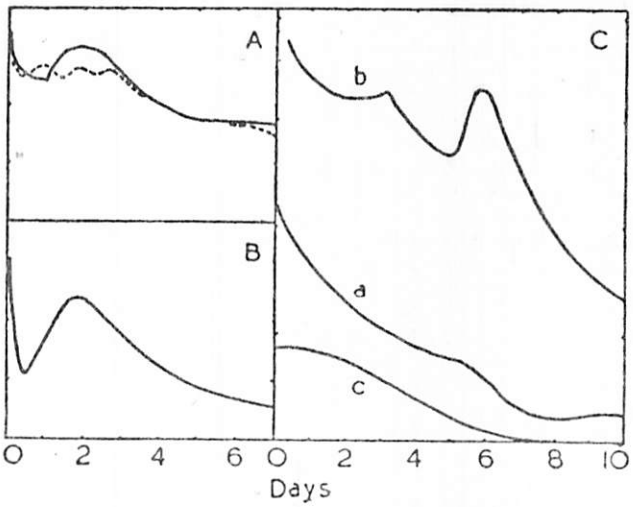

FIG. 9. CO_2-drift curves. A, Sudan grass (two curves) and B, Kikuyu grass (Wood, Cruickshank, and Kuchel, 1943). C, barley leaves from different positions of insertion on a mature stem. *a*, top leaf still unrolling; *b*, second leaf, lamina fully spread; *c*, old leaf towards base of stem, losing colour. Author's data. CO_2-scales omitted for clarity.

2 and 4 well developed and the early stages of phase 5. Curves for Kikuyu (*Pennisetum clandestinum* Hockst.) and Sudan grass (*Andropogon sudanensis* L. and B.), also members of the same family, have been published by Wood *et al.*[1] They show obvious resemblances with those for barley, and occupy about the same time, 6 or 7 days. Phase 1 does not appear, a sharp fall (phase 2) starting immediately. This is interrupted, usually at a high level, after about one day, by a rise (phase 4), followed by a fall (phase 5). In some experiments with Sudan grass the regular rise and fall is replaced by an undulating plateau, as in barley (Fig. 9A, dotted curve). There is usually no sharp rise (phase 6) due to saprophytes. Perhaps these investigators were more successful than others in maintaining aseptic conditions. The analytical results provided also suggest that little respirable material was left in the leaves at the breakdown stage. Diametrically opposed results were shown by broad-bean leaves (Yemm, 1934) in which a prolonged and more or less continuous fall was interrupted on the fifth day by a massive rise associated with the development of saprophytes. This effect was even more pronounced in leaves suffering potassium starvation (Fig. 10). Succulent leaves of *Kleinia articulata* (Thoday and Richards, 1944) show a CO_2-drift very reminiscent of cherry-laurel and extending over 11 days. In well nourished leaves phases 1 and 2 are represented by two days showing a regular rhythm superimposed on the gradual fall. Phases 4 and 5 are represented by a well-developed single hump, and there is a later sharp rise (phase 6) associated with cell breakdown.

When these various curves are compared, the initial choice of cherry-laurel is seen to have been singularly fortunate because its stately progression of events allows scope for the full development of each successive stage. Species such as *Tropaeolum* and *Kleinia* may show the same stages with almost equal clarity; others, like barley and Sudan grass, may be referred to the same sequence supposing some stages to overlap and others to be telescoped.

Colour changes

Visible alterations accompany the CO_2-drift curve in fairly close correlation. During phases 1 and 2 little is noticeable; but by the beginning of the third phase there is a definite loss of pigment. The green colour becomes pale and begins to be replaced by yellow—except, apparently, in *Kleinia*. After the crest is passed, cherry-laurel leaves begin to turn brown, due to cellular oxidations of polyphenols. Barley and similar species without polyphenolases do not show this coloration; but the leaves become translucent and grey owing to the sap escaping from collapsing cells into the intercellular spaces. At the same

[1] Wood, Cruickshank, and Kuchel, 1943; Wood, Mercer, and Pedlow, 1944.

time a marked loss of fresh weight (Fig. 7) is caused by exudation from the surface.

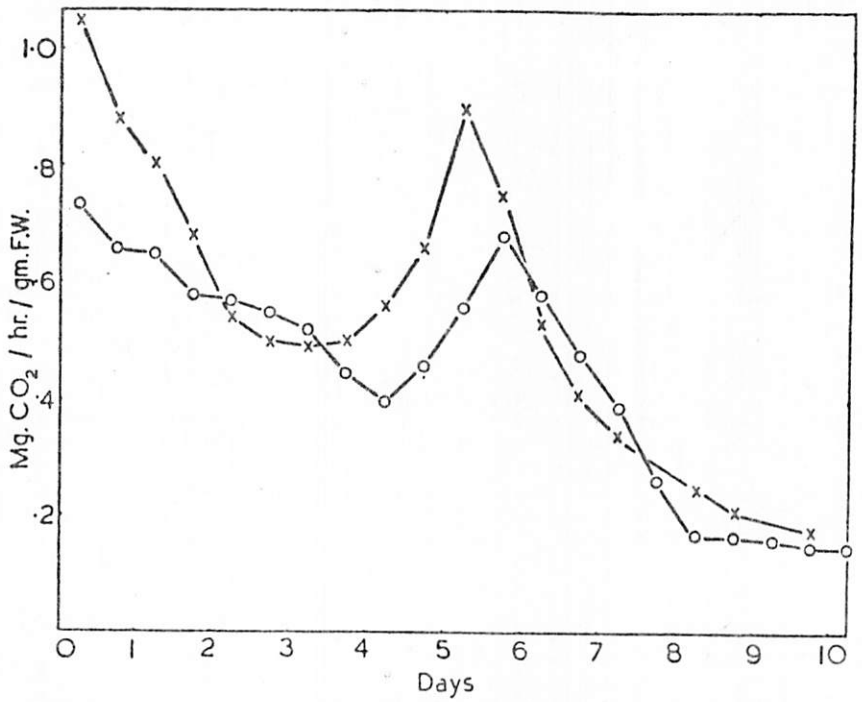

FIG. 10. CO_2-emission of broad-bean leaves (third from top) from plants raised in water culture; ○ with full mineral nutrient solution; × deficient in potassium. After Yemm, 1934.

Age

So far, description has been limited to completely expanded leaves still in full vigour and well nourished; picked, that is to say, after a period of active photosynthesis. The course of the curve depends greatly on developmental stage. In young leaves the respiratory hump or high plateau is much reduced and in very young ones may be altogether lacking. Fig. 9c represents the behaviour of leaves plucked from barley stems at three levels. Young leaves still unrolling (*a*) showed only a continuous fall to a slow rate. Leaves with their laminae recently spread (*b*) showed an interrupted fall followed by a saprophytic rise on the sixth day, and senescent leaves, already losing colour when plucked, showed a slow initial respiration that steadily declined (*c*). Young cherry-laurel leaves also showed a sharp decline to a low value with a late and much reduced rise associated with yellowing.[1]

[1] Godwin and Bishop, 1927.

Nature of the successive phases

Phase 1, immediately after detachment, corresponds more or less with the normal respiration of the leaf *in situ*. It is liable to disturbances caused by change of temperature, nearly always a rise, in passing from the open air to the experimental chamber; and by stimulation due to handling.[1] There is usually an excess of carbohydrate in the leaf, mainly starch, for example, in cherry-laurel, sucrose in barley and Sudan grass, and mixed sugars in Kikuyu. The respiration quotient (R.Q.) keeps close to unity,[2] suggesting a predominant consumption of carbohydrates. Barley leaves with a low content of available carbohydrate when cut showed an initial R.Q. = 0·88 and neither phase 1 nor phase 2 was visible in the CO_2-drift curve (Fig. 11).

Direct analysis of total carbohydrate loss presents many technical difficulties; but has been achieved with several species. The classic investigation is that of Deleano (1912) with vine leaves which at 19° C. have a long-drawn-out drift of about 20 days. He found (Fig. 12) that for the first hundred odd hours the CO_2-output could be accounted for by loss of carbohydrate, mostly starch; proteins showed no breakdown during this period (Fig. 12). More recently, with improved methods, Yemm has analysed the available carbohydrates in broad bean and barley leaves. Available carbohydrates are taken to mean the sugars and those polysaccharides for whose hydrolysis enzymes are available. They here include starch, fructosans, sucrose, glucose, and fructose, but

TABLE 11

CO_2-output and CO_2-equivalent of carbohydrate loss in mg./hr./gm. fresh weight

Data of Yemm, 1934

Leaf	*Hours*	*CO_2-output*	*Carbohydrate loss*	*CO_2 unaccounted for*
Broad bean	12	0·61	0·62	..
Barley	12	1·50	1·57	..
,, ...	12	1·45	1·10	0·35
,, ...	12	1·26	0·89	0·37
,, ...	6	1·39	1·50	..
,, mean	..	1·40	1·27	0·13
,, senescent leaves . .	12	1·01	0·44	0·57

not cellulose. Table 11 marshalls Yemm's data for the first 6–12 hours. In beans the carbohydrate loss fully accounted for the CO_2 produced,

[1] See Audus, 1935; Godwin, 1935; Barker, 1935.
[2] Yemm, 1934; McKee, 1937*a*.

and in barley the mean discrepancy was less than 10 per cent. Senescent leaves showing no phase 1 (cf. Fig. 9cc) had a large excess of CO_2, not of carbohydrate origin, from the start. Corresponding results for wheat by Krotkov indicated that loss of carbohydrates, especially sucrose,

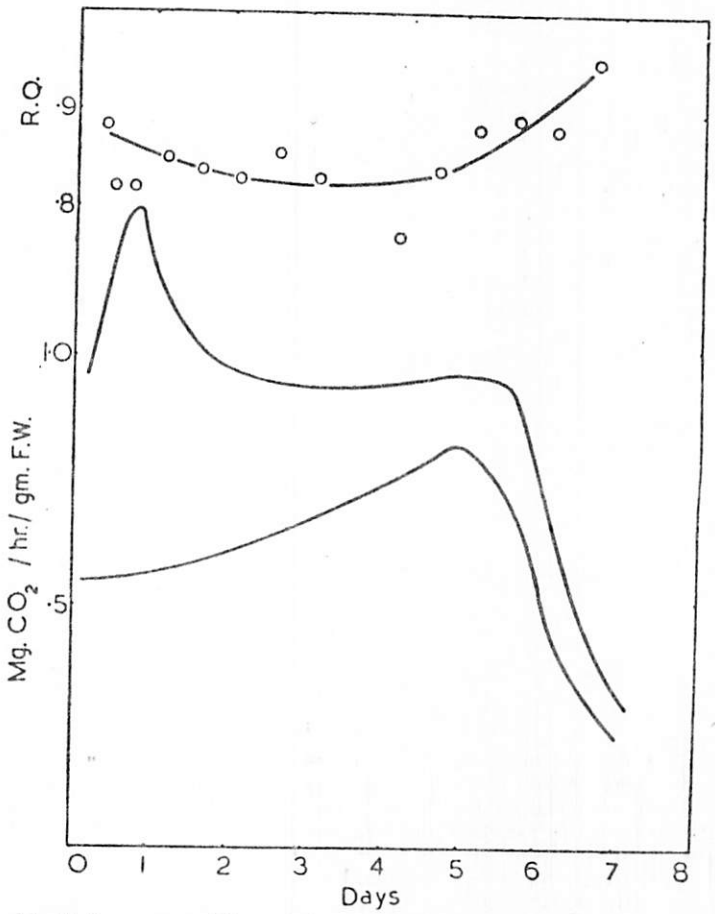

FIG. 11. Bottom curve, CO_2-emission of barley leaves cut after a period of darkening on the plant. Middle curve, CO_2-emission of leaves cut simultaneously but after a period of illumination. Top curve, R.Q. of leaves darkened before cutting. After Yemm, 1934.

accounted for the CO_2-output of the first 24 hours, a period which appeared to cover both phase 1 and phase 2. Krotkov's results are summarized in Table 12. The grasses examined by Wood *et al.* showed an excess of CO_2-production over carbohydrate loss even on the first day. The authors remark that their carbohydrate fractions were not exhaustive. Further, the CO_2-drift curves show no phase 1, and shorter periods, such as those employed by Yemm with barley, might

have told a different tale. The same remark may also be applied to the rhubarb-leaf data of Vickery and Pucher (1939).

Phase 1 and its transition to the early part of phase 2 indicate what goes on in the leaf while still attached to the plant. It is a phase in which available carbohydrates provide the respirable material, but

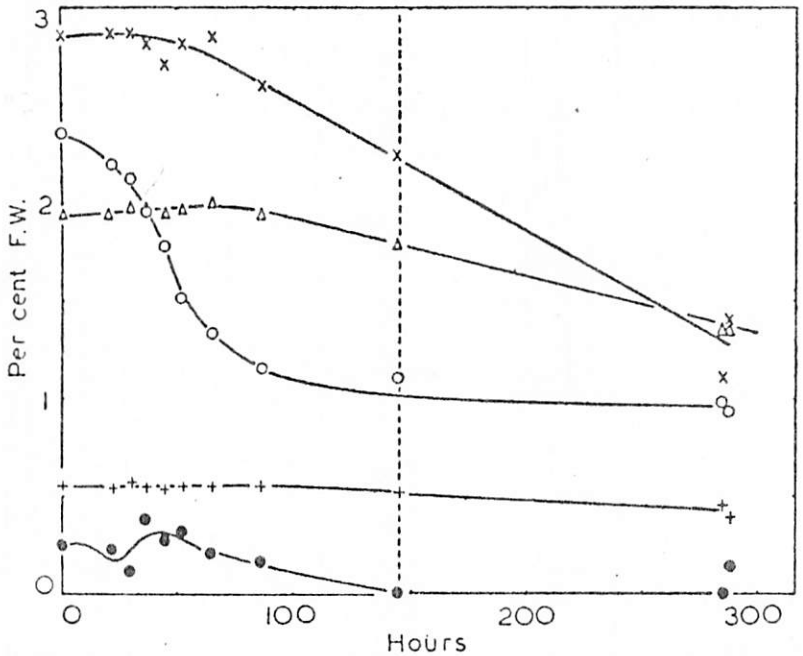

Fig. 12. Analyses of halved vine leaves, kept in the dark. ○, starch; ●, sucrose; ×, hexose; △, total acid calculated as tartaric; +, coagulable protein. The persistent 'starch' fraction after 144 hours gave no iodine colour reaction. Up to this time (indicated by a broken vertical line) total carbohydrate loss was equivalent to CO_2-output.
Data of Deleano, 1912, adjusted to allow for variations in control half-leaves.

TABLE 12

CO_2-output and CO_2-equivalent of carbohydrate loss by wheat leaves in mg./gm. F.W.

Data of Krotkov, 1939

Day	1	2	3	4	5
Phase	1–2	3	3	4	4
CO_2-output	6·89	7·26	10·09	10·08	5·09
CO_2-equivalent of carbohydrate loss	7·05	2·79	1·91	1·61	0·95
$100 \times \frac{\text{carbohydrate } CO_2}{CO_2\text{-output}}$	102	38	19	16	19

this is not the same thing as saying that carbohydrates are always and only the substrate of normal plant respiration. Not all leaves show

phase 1, which is typical of juvenile to mature leaves in a high state of carbohydrate nutrition. Very young leaves, senescent leaves, leaves of plants forced in greenhouses, or picked with a low carbohydrate content after darkening, show an immediate and usually rapid decline of CO_2-emission.

Phase 2 is shown to a greater or lesser extent by almost all leaves. Its rapid decline is associated with a progressive exhaustion of the

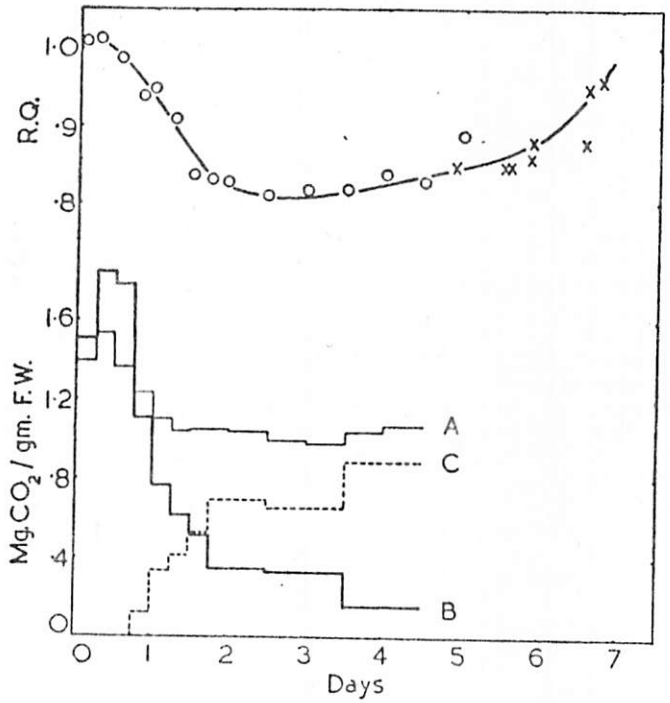

FIG. 13. Cut barley leaves. Curve A, CO_2-emission; curve B, CO_2-equivalent of carbohydrate loss; curve C, CO_2 unaccounted for. Top curve, respiratory quotient. The continuation of the R.Q. curve is added from a second experiment. After Yemm, 1936.

available carbohydrates, particularly sucrose and the polysaccharides. Relations between individual carbohydrates are complex and are gone into more fully on p. 102. All appear to be readily inter-convertible, and for present purposes it is enough to regard the total drain upon the pool. By the end of phase 2 the carbohydrates have been much reduced, but are not exhausted. As it proceeds, there is a progressive increase of carbon dioxide not accounted for by carbohydrate loss; and at the same time a progressive decline of the respiratory quotient from 1·0 to 0·8, a further indication that some other class of substrates is being called upon. Detailed results have been provided for barley and broad beans by Yemm (Figs. 13, 35, and 36).

The end of phase 2 is reached when the CO_2-emission curve no longer falls, and this may occur at either low or high level. If the rate at the end of phase 2 (r_2) is related to the initial rate (r_1) the values of r_2/r_1 obtained with different species fall into a more or less continuous series. Leaves with long drifts have low r_2/r_1 values and short-drift leaves have high ones (Table 13).

TABLE 13

Characteristics of CO_2-drift curves of leaves of various species

	Temp. °C.	CO_2-*output* (*mg.* CO_2/*gm. F.W./hr.*)		r_2/r_1	*Duration of drift in days*
		r_1	r_2		
Cherry-laurel (Blackman)	16·5	0·21	0·07	0·33	54
„ „ (Godwin and Bishop)	20·8	0·30	0·13	0·43	20
Kleinia articulata	25·0	0·09	0·04	0·45	9
Tropaeolum majus	16·5	0·37	0·19	0·51	21
Kikuyu grass	24·5	0·27	0·15	0·56	5
Sudan grass	24·5	0·28	0·17	0·61	?
Wheat	20·0	0·31	0·20	0·65	5
Barley (Yemm)	25·0	1·5	1·00	0·67	6
„ (McKee)	21·0	1·2	0·9	0·75	6
Broad bean	25·0	0·65	0·59	0·91	4

This might be taken to indicate that long-drift leaves reduced their carbohydrate content to a lower level than short-drift leaves before utilization of secondary substrates became considerable. Direct analytical data do not bear this out; the ratio of available carbohydrates at the end of phase 2 to the initial is about 1/2 in the most diverse leaves; and the concentration relative to fresh weight is lower in short-lived leaves than in long (Table 14).

TABLE 14

Available carbohydrate in long- and short-drift leaves at the end of phase 2

	Average drift in days	*Available carbohydrate**		
		Per cent. F.W.	*Per cent. initial*	
Cherry-laurel	20	2·47	49·1	Onslow, unpublished
Vine	20	4·28	59·2	Deleano, 1912
Barley	6	1·79	49·7†	Yemm, 1934
Wheat	5	0·8–0·15	61·5–26·7	Krotkov, 1939

* Calculated as hexose equivalent. † Mean of 4.

Phase 3, the period of slow CO_2-evolution at a steady level, is not often revealed, because the rise of phase 4 anticipates its occurrence. It

probably has analogues in the ground respiration of partially starved wheat roots[1] and the endogenous respiration of unicellular algae.[2] Both the last are highly resistant to poisoning by cyanide, unlike the floating respiration induced by addition of sugars. The effect of cyanide on phase 3 respiration of cherry-laurel does not seem to have been examined; but these examples agree in having a slow rate associated with a low content of available carbohydrate.

Phase 4. Whatever the duration of the drift, the carbohydrates now make a progressively smaller contribution to the CO_2-output. The first attempt to identify a secondary substrate appears to have been that of Godwin and Bishop (1927), using cherry-laurel. They observed that the cyanogenetic glycoside (prulaurasin) was stable for about 4 days, i.e. until towards the end of phase 2; but that rapid loss of HCN occurred during the early stages of phase 4. The glucose released by the hydrolysis of the glycoside was naturally regarded as available for respiration and as contributing to the secondary rise of respiration rate. A temporary increase of hexose concentration occurred at this point, while sucrose and starch continued to diminish steadily.[3] In young leaves with delayed phase 4, glycoside hydrolysis was delayed correspondingly.

This obviously represents a rather special case, and the CO_2-equivalent of the glycoside lost was only a small fraction of the additional CO_2-emission (Fig. 14). A significant observation later made by Yemm (1934) and McKee (1937*a*) with barley was the lowering of the R.Q. which sank to about 0·82 when phase 4 was well advanced (Fig. 13). The only possible cause for such a fall appeared to be the massive breakdown of proteins. Organic acids would tend to raise the R.Q. if respired, and were not believed to be present in sufficient quantities to contribute much carbon dioxide. This was later verified by Somers.[4]

The behaviour of proteins and their products in detached leaves has been extensively studied.[5] The time at which protein breakdown becomes detectable at 20–25° C. varies from less than a day in the grasses to about 5 days for vine. It anticipates visible yellowing, breakdown of the chlorophyll, and lowering of the R.Q. It appears to occur, at least in some leaves, while the carbohydrate loss is still adequate to account for total CO_2-production.

[1] Lundegårdh, 1949.

[2] Emerson, 1927.

[3] Analyses carried out for the authors by Mrs. Onslow.

[4] Somers, unpublished.

[5] In vine, Deleano, 1912; barley, McKee, 1937*a*, 1950, Yemm, 1937; tobacco, Mothes, 1931, Vickery, Pucher, Wakeman, and Leavenworth, 1937; *Tropaeolum*, Michael, 1935; broad bean, Mothes, 1925; runner bean, Mothes, 1925; Sudan grass, Wood, Cruickshank, Kuchel, 1943; Kikuyu grass, ibid.; oat, Cruickshank and Wood, 1945; belladonna, James, 1950*b*.

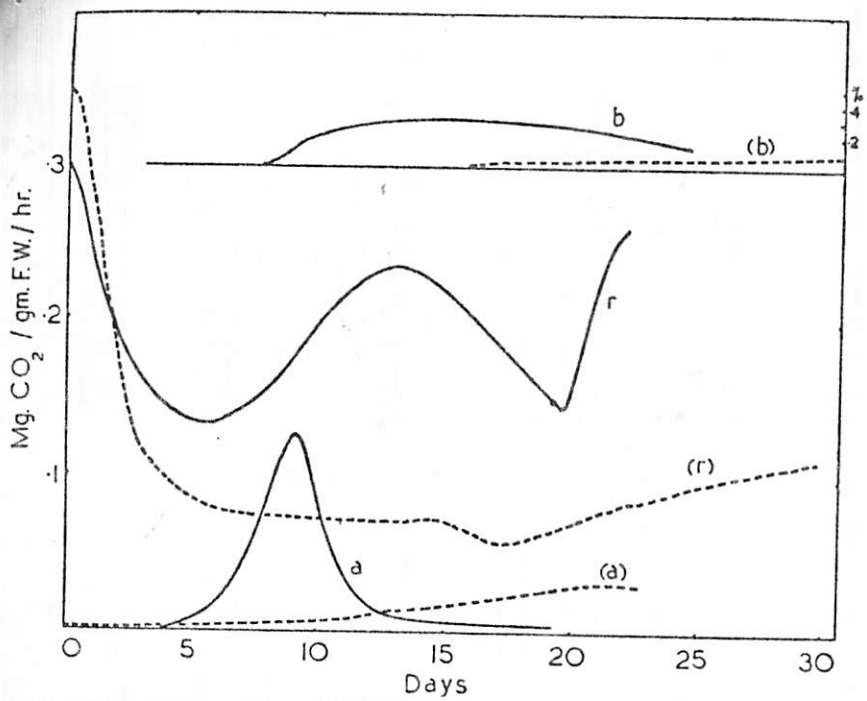

FIG. 14. CO_2-emission and loss of HCN-glycosides from cherry-laurel leaves. *a* and (*a*) loss of glycoside as CO_2-equivalent; *b* and (*b*) rate of yellowing as percentage of leaf surface per hour; *r* and (*r*) rate of CO_2-emission. Continuous lines—leaves 21 months old; broken lines—leaves 2½ months old. After Godwin and Bishop, 1927.

TABLE 15

	Time proteins stable (days)	*Length of drift (days)*
Vine	5	20·5
Belladonna . . .	2	7
Barley	< 1	6
Oats	< 1	4
Sudan grass . . .	< 1	?
Kikuyu grass . . .	< 1	5

The changes resulting from protein breakdown are numerous and complex and have been most closely related to the respiratory sequence in barley.[1] In mature leaves, protein breakdown is already apparent after 6 hours isolation, and may even be happening in attached leaves as part of a translocation mechanism. Such hydrolyses do not necessarily lead on to a complete respiratory breakdown of the nitrogenous materials. During the first 24 hours, the CO_2 produced by barley leaves

[1] Yemm, 1937.

appears to come wholly, or almost wholly, from the available carbohydrates. During this period the loss of protein is accounted for by accumulation of amino-acids, glutamine, and other soluble organic nitrogen compounds such as peptides. There is no accumulation or release of ammonia. The glutamine, however, accumulates in excess of the amount that could have been preformed in the lost protein; accounting for about 4 times its whole loss of amide-N.[1] It is therefore possible that some protein is fully decomposed to provide ammonia for the additional amide groups, and that a small CO_2-contribution might result.

Much more definite changes were observed after 48 hours, i.e. when the leaves had passed from phase 2 into phase 4. The accumulation of glutamine continued, asparagine was formed rapidly, implying a corresponding formation and fixation of ammonia, and there was even a release of some free ammonia (Fig. 15). At the same time, amino-N began to diminish,[2] and the conclusion seems inescapable that complete protein degradation was occurring with the formation of the two final products, ammonia and carbon dioxide.

The breakdown of protein was much slower in young leaves, and the glutamine formed could have arisen directly, in agreement with the absence or postponement of phase 4 previously noted (p. 50) for young leaves.

TABLE 16

Barley leaves. Results given in mg. N *per gm. fresh weight*

Data of Yemm[1]

Period of isolation (hr.)	*Loss of protein* N		*Amide* N *in lost protein*		*Increase of glutamine* N	
	Young	*Mature*	*Young*	*Mature*	*Young*	*Mature*
24	1·17	0·94	0·060	0·09	0·060	0·42
48	2·26	2·34	0·115	0·23	0·125	0·84
72	..	2·60	0·250	0·26	0·250	0·95

Similar results are available for mature leaves of oats, Sudan and Kikuyu grasses.[3] All show early loss of protein with accumulation of amino-nitrogen. Glutamine formation is much less than in barley, but asparagine accumulates rapidly after 48 hours; i.e. with the beginning of phase 4. Free ammonia is released later. At the same time, i.e. towards the end of phase 4, amino-N begins to be reduced. The individual amino-acids contributing to it have been to some extent

[1] Data of Yemm in Chibnall, 1939, p. 219.

[2] Amino-N exclusive of that in amides (glutamine and asparagine); probably mainly in amino-acids.

[3] Wood and collaborators, loc. cit.

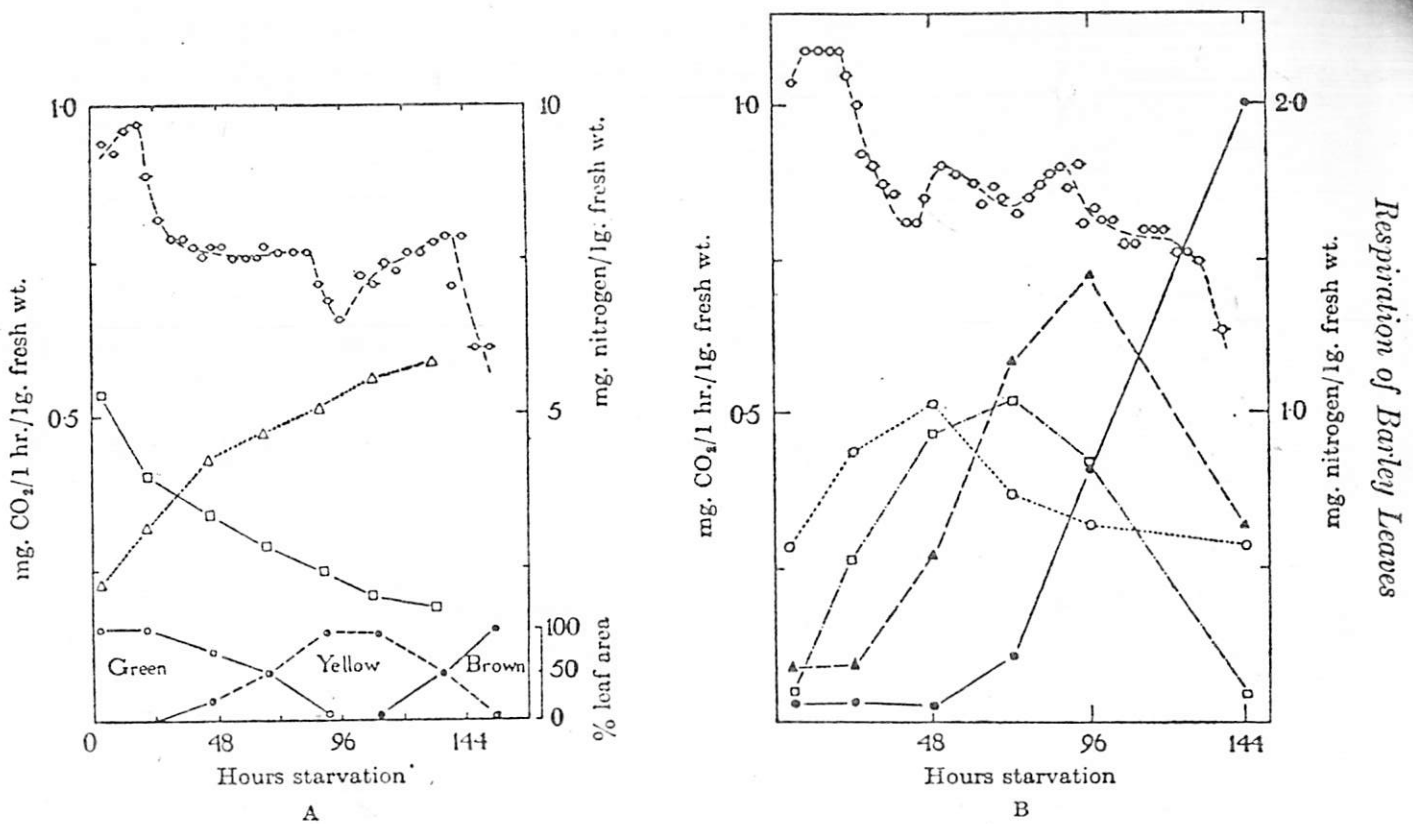

FIG. 15. Changes of soluble nitrogen fractions in detached barley leaves. A. –○– CO_2-emission; –□– total insoluble-N; ···△··· total soluble-N. Percentage of area green, &c., shown below. B. ···○··· amino-N; –·–□–·– glutamine-N; – – ▲ – – asparagine-N; —●— ammonia-N. The CO_2-emission curve –○– is given above. From Yemm, 1937.

particularized; glutamic acid and cystine appear to break down relatively fast, tyrosine and tryptophan more slowly.[1]

It is not so easy to calculate the amount of CO_2 derivable from the protein losses as from the carbohydrate, on account of the more numerous and dubious changes occurring. The Kikuyu results are relatively simple in that the sum of protein+amino+amide+ammonia nitrogens

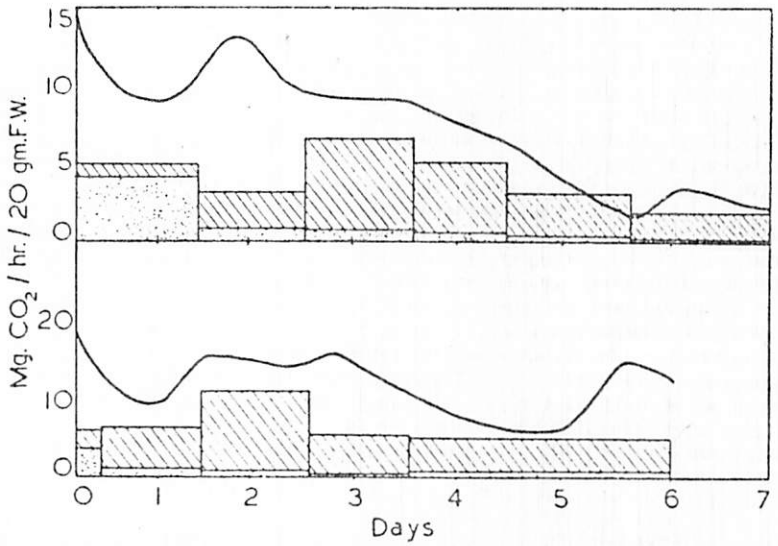

FIG. 16. Kikuyu grass leaves. CO_2-emission is shown by the continuous curves. The CO_2-equivalent of carbohydrate lost is shown by dot shading and the CO_2-equivalent of protein loss by line shading. Two separate experiments are represented. From Wood, Mercer, and Pedlow, 1943.

remained approximately constant over the whole drift period. It may therefore be assumed that the changes among them are self-contained, and that no carbon passes between them and other substances except CO_2. There is no measurable formation of glutamine or unidentified N-compounds. Wood, Mercer, and Pedlow propose the following conventions: Protein-C and amino-acid-C = 3×protein-N and 3×amino-N respectively; asparagine-C = 3·43×amide-N. The amount of protein-C disappearing and not accounted for by asparagine formation is given by the algebraic sum of the C-changes of protein, amino-acids, and asparagine. It is presumed that this is all oxidized to CO_2. Results of two experiments calculated on these assumptions are given in Fig. 16. The degradation of protein is seen to give rise to a progressively larger proportion of the total CO_2-output, and to account for most of it towards the end of the drift.

The estimation is rather more complex for barley on account of the

[1] Wood and Cruickshank, 1944.

accumulation of glutamine and unidentified N-compounds to a considerable amount. To make the calculation, Yemm (1950) uses the arbitrary assumption that the carbon of the unidentified N-compounds weighed twice as much as the nitrogen; and further that glutamine-C = 2·14 × glutamine-N (= 4·28 × unstable amide-N), asparagine-C = 1·71 × asparagine-N (3·42 × stable amide-N), protein-C = 3·1 × protein-N, and amino-C = 2·8 × amino-N. On this basis, he found that, by the end of the drift in eight experiments, the C-loss from protein sources had accounted for between 20 per cent. to 40 per cent. of the total CO_2 emitted. In three experiments in which the carbohydrates were determined, their total loss of carbon was equivalent to 35, 47, and 29 per cent. of the total CO_2-emission respectively. In one experiment in which the leaves started with an exceptionally high carbohydrate content, the proportion rose to 71 per cent. Apart from this one experiment there appears to be a substantial amount—some 20–40 per cent.—of the CO_2 whose origin is not accounted for in either carbohydrate or protein loss. A similar discrepancy is noticeable in Fig. 16. According to available data this cannot come from organic acids such as malic and citric.[1]

Visible yellowing of the leaves is also a characteristic of phase 4. It begins usually in the early stages and is more than half completed by the end (Figs. 7 and 8). It is due to breakdown of the chlorophylls which, according to Michael, is measurable before yellowing can be seen. The breakdown of protein may begin earlier than either. In mature barley leaves, proteins begin to disappear within the first 6 hours, chlorophyll losses become detectable after about 24 hours, and visible yellowing after about 48 hours. In *Tropaeolum* leaves[2] protein loss is more rapid than chlorophyll loss for 2 or 3 days, and thereafter runs a more or less parallel course. Yellowing is first visible after 5 days. If protein breakdown is delayed, chlorophyll breakdown is delayed also.[3]

Extracted chlorophylls are unstable in light and air and probably owe their stability *in vivo* to a chlorophyll-protein linkage. Phyllochlorin, defined by Mestre to include 'the green pigments of the plastid', is likely to consist of a chromoprotein in which the porphyrin of chlorophyll is weakly linked to protein through the magnesium atom. Although a pure chlorophyll-protein has not yet been prepared, there is considerable evidence for the existence of such substances in leaves.[4] The breakdown of the proteins within the grana of the chloroplasts may therefore unmask the chlorophyll, and make it available to respiratory breakdown. Attempts have been made to discover whether the chloroplast

[1] Somers, unpublished; Wood, Cruickshank, and Kuchel, 1943.
[2] Michael, 1935.
[3] Wood, Cruickshank, and Kuchel, 1943.
[4] Lubimenko, 1921, 1927; Noack, 1927. See Rabinowitch, 1945, for discussion.

proteins are more or less stable than the cytoplasmic. Chibnall and Grover (1926) found that the ratio of chloroplast protein-N/cytoplast protein-N had not changed in *Phaseolus multiflorus* leaves after 4 days isolation. Chloroplast and cytoplasmic proteins were also found to break down at similar rates in Kikuyu grass (Wood *et al.*, loc cit.); but in Sudan grass leaves chloroplast proteins were lost the faster.

Phase 5. The transition from phase 4 to phase 5 is well defined in leaves such as cherry-laurel with a sharply peaked respiration hump (Fig. 7). In others, where the peak is replaced by a high plateau (Figs. 8 and 9), no sharp division shows in the CO_2-drift curve. Nevertheless, the end of the plateau corresponds with a fundamentally different condition from that of its beginning, as is indicated by the drifts of the various N-fractions (Fig. 15). These come to successive maxima in the order amino-acids, glutamine, asparagine, representing so many successive stages in the consumption of the proteins. As each in turn begins to disappear, a further step has been taken towards exhaustion. In the type of curve with a single peak, the secondary substrates may be supposed to consist mainly of one, or to become available more or less in unison; when it is replaced by a rolling plateau, the substrates perhaps become available in successive waves.

The proteins are not completely removed when phase 5 comes to its end, and usually they then appear to be approaching a steady value at a fairly high level (Fig. 15A). One possible reason might be that the tissues contain a proportion of non-digestible, or at least highly resistant, protein; another is mentioned on the next page in connexion with phase 6.

During phase 5 the R.Q., which has dropped to about 0·8 in phase 4, begins to rise again (Fig. 13). This would happen if there were a considerable consumption of organic acids at this late stage, stated by Moyse to occur in leaves of *Rumex acetosa* which have a relatively high content of oxalic acid. A more usual reason for the rise lies in the changed nature of the protein breakdown. While the formation of amides, associated with incomplete oxidation, leads to R.Q.s in the neighbourhood of 0·7, the further oxidation of amides and amino-acids to carbon dioxide and ammonia gives R.Q.s in the range of 1·0 to 1·3. It is this latter change that predominates in phase 5 (Fig. 15 and Table 21). Similar results were obtained by Moyse with wheat and *Polygonum fagopyrum*.

Nevertheless, loss of these substances did not wholly account for the CO_2-output of Kikuyu grass (Fig. 16). To fill the gap it is possible that hitherto unavailable carbohydrate might begin to break down. Support for this idea is given by Buston's analyses of hemicelluloses in detached leaves of vine, runner beans, and maize. After prolonged periods of isolation, although there was no loss of pectin and insoluble hemicelluloses,

he found considerable breakdown of hemicelluloses that could be made water-soluble by treatment with caustic soda. These consisted largely of pentosans, uronic anhydrides, and some hexans. In maize the loss amounted to 19 per cent. of their initial weight which accounted for 14·7 per cent. of the total dry matter of the leaves. The R.Q. to be expected by the complete oxidation of such substances to CO_2 would approximate unity, and so tend to raise the observed R.Q. above the earlier values around 0·8. There is no indication from Buston's results at what stage the breakdown of the less resistant hemicelluloses is fastest. It seems plausible to suppose that the incipient degradation of protein structure during phase 5 allows hydrolysing enzymes to approach the hemicelluloses previously inaccessible at their location in the cell wall.

Phase 5 is terminated by the collapse of cell organization, perhaps brought on by the rapidly increasing amounts of ammonia liberated at this stage. Feeding belladonna leaves with heavy doses of ammonium sulphate or arginine, from which ammonia is released, brings on the collapse at an earlier stage than feeding with other amino-acids.

Phase 6. So far as the individual cell is concerned, the end comes somewhat suddenly The protoplasmic organization breaks down so that semipermeability is lost and the vacuole's contents ooze into the intercellular spaces and even on to the leaf surfaces. Taking the complex organ as a whole, this end point is somewhat slurred by the idiosyncrasies of cell tempo. Owing to the progressive injection of its intercellular spaces, the leaf becomes translucent and greyish and at the same time there is a pronounced loss of weight (Figs. 7 and 8). Autolysis, i.e. unorganized enzyme catalysis, accelerates numerous reactions previously in abeyance, such as the oxidation of polyphenols in tea, and the hydrolysis of tropane alkaloids in belladonna. The irreversible oxidation of polyphenols as, for example, in leaves of cherry-laurel, belladonna, and beans, causes browning of the tissues; in other species there is no marked colour change. It is remarkable that while the general effect of autolysis is to accelerate the breakdown of the cell substances remaining, loss of proteins comes almost to a standstill (Fig. 15A). This is perhaps explained by an inactivation of proteases outside the living structure of the cell. Barley leaves, disintegrated immediately after detachment, show a slower rate of protein hydrolysis than whole leaves (Fig. 17), and at the same time the protective effect of the proteins on chlorophyll is maintained. The author found that mushes of fresh barley leaves kept under toluene were still bright green after 5 days when normal leaves are completely yellow.

It is not known how much of the rising CO_2-output of phase 6 is due to the leaf's own enzymes; the great bulk of it comes from the

respiration of saprophytic moulds and bacteria, which at this stage, if not before, gain access to the cell contents.

Causes of the drift

A drift of respiration rates happens in all tissues and those of detached leaves may be regarded to a very large degree as accelerations of the drifts that would have occurred had the leaves been left undisturbed on

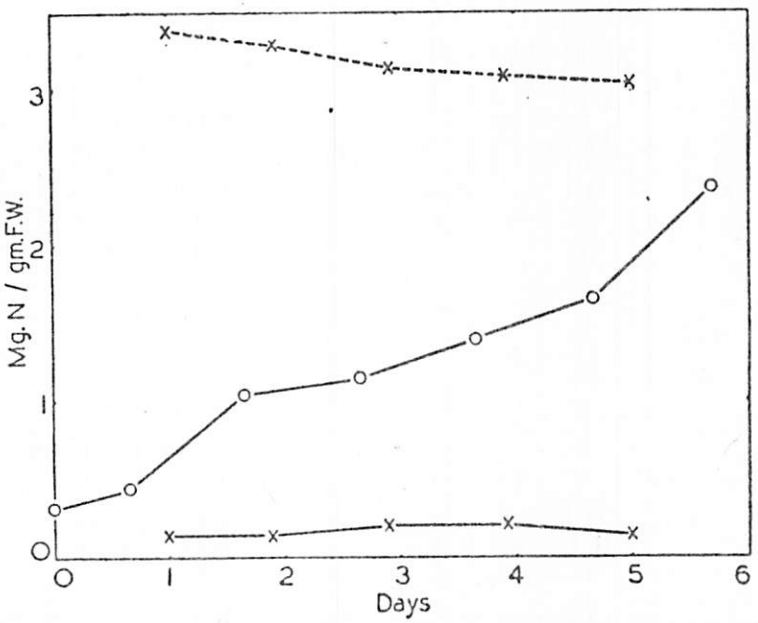

FIG. 17. Asparagine formation in cut leaves from barley seedlings about 4 weeks old. ○ asparagine-N in intact leaves; × in ground leaves under toluene. The slow rate of protein breakdown in the ground leaves, shown by the broken curve, may be compared with that of normal leaves in Fig. 15A. Data of McKee, 1937*a*.

the plant (Fig. 29). Their yellowing and exhaustion have something in common with the normal condition of leaves in autumn (cf. p. 80). For either drift, an explanation involves both depletion of substrates and changes of protoplasmic condition. It is easy to appreciate that the isolation of darkened leaves will accelerate starvation; but it commonly also leads to faster degradation of their protoplasts. The distinction between plastic and protoplasmic proteins in leaves is vague, even supposing it to exist; detachment seems to make at least a part of the structural protein more liable to early respiratory breakdown.

Stability of the proteins seems to be the key to the length of the drift (see Table 15, p. 51). Unfortunately it is not at present possible

to give any clear statement of the factors involved.[1] There seems to be fairly general agreement that in young leaves protein hydrolysis is slight, and that the tendency increases with age; conversely, the capacity for synthesis is high in young leaves and becomes less with age though it may persist in some degree, at least in detached leaves, so long as they remain green.[2] The difference between the drifts of leaves detached when young and when more mature seems to be closely related to their different degrees of protein stability.

It is not clear why this stability is reduced by cutting. Much has been written about the effect of carbohydrates in stabilizing and protecting the proteins of cut leaves; but recorded results cannot be said to bear this out. The time taken for the respiration of secondary substrates to become apparent is not lengthened by increased carbohydrate content. Wheat leaves with 1·3 per cent. total sugars showed no lengthening of phase 2 nor delay in arriving at phase 4 when compared with leaves having only 0·3 to 0·4 per cent. The change always happened after one day when the sugar-rich leaves still had some 0·8 per cent. of sugars as against 0·15 per cent. in the others. The sugar-rich leaves went on using both sugar and secondary substrates until their final collapse.[3] An extreme example is afforded by some barley leaves examined by Yemm. By a preparatory period of darkening while still attached to the plant, their available carbohydrates were reduced from 5·43 to 1·78 per cent. F.W. and their initial respiration rate from 0·95 mg. CO_2/gm. F.W./hr. to 0·54. This last value was below that of the normal inflexion point between phases 2 and 4, with the result that the CO_2-emission rate showed no fall, but a steady climb from the outset (Fig. 11, p. 46). The total difference of CO_2-emission between these and a similar batch of leaves kept in the light was 50·8 mg./gmF.W., and the difference of available carbohydrate was equivalent to 53·9 mg. From this it seems possible to infer that the breakdown of the secondary substrates was virtually identical at both levels of carbohydrate supply. Further, protein hydrolysis is at its fastest in barley leaves immediately after picking, when carbohydrates are abundant and are providing the whole of the respiratory carbon dioxide.[4] Mothes (1926) found that *Vicia faba* leaves, picked after active photosynthesis, also lost protein rapidly during the first 24 hours. Tobacco leaves, illuminated after detachment, showed massive increases of starch and sugars; but their losses of protein during the first 75 hours were as great as those of leaves kept in the dark.[5] Illuminated cherry-laurel leaves have been

[1] There are discussions by McKee, 1937*b*, 1949; Chibnall, 1939; Petrie, 1939, 1943; Wood, 1945, who are able to come to no definite conclusions.
[2] Walkley, 1940, barley. [3] Krotkov, 1939. [4] Yemm, 1937.
[5] Vickery, Pucher, Wakeman, and Leavenworth, 1937.

observed to yellow while still glutted with starch.[1] It is true that protein synthesis may have been induced in some cut leaves by supplying them with dilute sugar solutions.[2] This appears to occur more readily in young leaves than in mature or old, and is no indication that protein breakdown is not still occurring at the usual rate. There appears to be no convincing evidence that the presence of available carbohydrates retards protein hydrolysis, though it may sometimes, but not always, assist synthesis.[3]

The drift as hitherto described requires the presence of oxygen and depends entirely on aerobic respiration; yellowing does not occur in its absence. It is readily observed that any treatment, such as blocking the stomata of *Ficus elastica* leaves with vaseline, or immersion in water, which tends to reduce access of oxygen, retards yellowing. Michael observed that yellowing and proteolysis in *Tropaeolum* leaves was accelerated in 100 per cent. oxygen and retarded in 7 per cent. The yellowing of barley leaves may be entirely prevented by an atmosphere of nitrogen or by poisoning the oxidases with cyanide. The protoplasmic structure breaks down; but the dead and translucent leaves remain bright green and have a sweet, hay-like scent instead of giving off ammonia.[4] A similar result is observed with Sudan grass under nitrogen, and it has been shown analytically that the protoplasmic structure may finally break down without appreciable loss of protein or chlorophyll.[5]

The yellowing of phase 4 is associated with the accumulation of substances which, if formed at all in an attached leaf, would probably be largely translocated away. Among them the amides are notable and it has been found that their formation is suppressed by absence of oxygen,[5] the presence of chloroform[6] or grinding up the leaves;[7] all conditions which are known to delay yellowing. The association of yellowing with oxidative respiration suggests that it may be accelerated by a substance formed as an incidental of the aerobic metabolism. A water extract of yellowed cherry-laurel leaves has been found to hasten yellowing of freshly cut leaves allowed to take it up through the petioles.[8] Similar extracts of *Tropaeolum* also accelerated the yellowing of fresh leaves laid upon them; this effect was diminished by addition of glucose.[9] The same result was also produced by a 0·4 per cent. solution of asparagine and again was diminished by addition of glucose, apparently by removal of the asparagine, at least partly, in protein synthesis.

[1] Clapham, personal communication.

[2] Mothes, 1926 (young *Phaseolus multiflorus*); Paech, 1935 (small increases in several species); Pearsall and Billimoria, 1938 (*Narcissus*). But see also p. 246.

[3] Burström, 1943 (wheat).

[4] James and Hora, 1940.

[5] Wood, Cruickshank, and Kuchel, 1943.

[6] Mothes, 1925.

[7] McKee, 1950.

[8] Godwin, personal communication.

[9] Michael, 1935.

TABLE 17

Tropaeolum leaves floating on solutions of 0·4 per cent. asparagine and 1 to 3 per cent. glucose

Data of Michael

Treatment	*Duration (days)*	*Chlorophyll (mg./10 sq. cm.)*	*Protein-N*	*Yellowing*
Distilled water . .	3	0·06	0·26	..
Glucose . . .	3	0·17	0·41	retarded
Distilled water . .	4	0·50	0·79	..
0·4 per cent. asparagine .	4	0·42	0·73	much accelerated
Analysed at start . .	0	0·46	0·83	..
Glucose + asparagine .	2	0·45	0·89	retarded

There are thus reasonable grounds for supposing that the accelerated yellowing, characteristic of detached leaves, is autocatalysed by the production of amides. The volatile autocatalysts of fruit ripening are discussed on p. 75.

DETACHED ROOTS

Roots seem to have been rarely investigated in this way; but results have been obtained for maize by Girton.[1] Roots 2–4 cm. long, obtained from young seedlings raised aseptically, were transferred to respiration

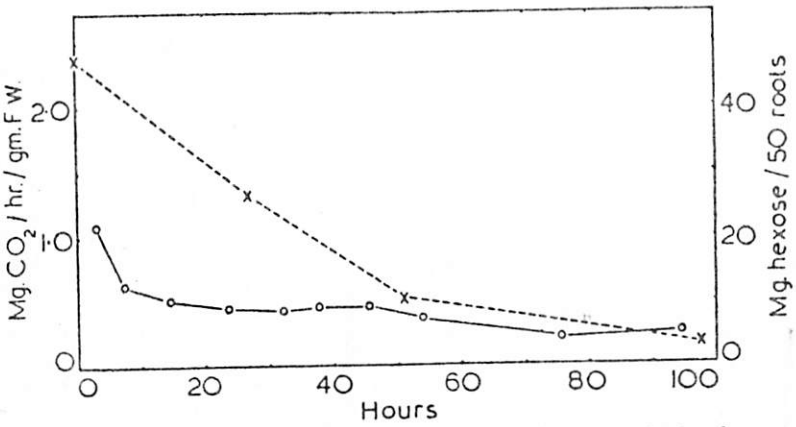

FIG. 18. CO_2-emission by maize roots, ○; and total sugar content as hexose equivalent, ×. After Girton.

chambers and CO_2-drifts in tap water followed. The course was a simple one (Fig. 18), a rapid decline during the first few hours being followed by a steady long-continued and very gradual fall. Records were carried to 7 days and normally there was no secondary rise; if bacterial intrusions occurred they were indicated by a temporary hump.

[1] Personal communication. I am greatly indebted to Prof. Girton for his permission to cite these unpublished results.

Available carbohydrates were in the form of sugars, mainly reducing sugars with a little sucrose, and showed a steady exhaustion that reduced the sugar concentration to about one-fifth during the first 2 days. In one experiment, the total CO_2-emission during this time was 54·8 mg. and the CO_2-equivalent of the simultaneous sugar loss was 52·8 mg. During a run of 172 hours the fresh weight of the samples was unaltered; but dry weight fell by 38 per cent. A very striking alteration was obtained when the roots were maintained in the presence of 2 per cent. sucrose+0·05 per cent. KNO_3. There were large increases of fresh weight, and dry weight went up by 59 per cent. in the tip region (0·5 cm.) and 52 per cent. in the young stumps, i.e. the next 2–3 cm. Such feeding also caused sustained increases of the respiration rate.

The general course of the drift curve resembles that of very young leaves (cf. Fig. 14) with its long sustained period of minimal CO_2-release. This is readily understood when the youth of the roots and their high percentage of meristematic tissue are remembered. A marked stability of the proteins appears to be indicated.

GERMINATING SEEDLINGS

The respiration rates of dormant seeds are very low, and during the early stages of germination there is always a rapid and prolonged rise. Expressed on a dry weight or similar basis this soon reaches the maximal value achieved throughout the life of the plant. The general course of events has been known a long time;[1] but not many species seem to have been examined in any detail, and, among those that have, the cereals are conspicuous. Apart from the technical importance of their germination in malting and agriculture, they present the experimental advantage that the active embryonic tissues are readily separated from their reserves.

An early study of the CO_2-drift during wheat germination was made by Rischavi (1876), who kept the germinating grains in the dark and followed the curve through to the point of exhaustion. The rate accelerated rapidly over the first 2–3 days and then more slowly until a maximum was reached after 10–11 days. After the peak a fairly rapid and continuous decline set in.[2] More detailed results are available for barley,[3] consisting of regular 3-hourly readings over 25 days. These have enabled the curve to be divided, like that for detached leaves (p. 42), into successive phases represented diagrammatically in Fig. 19.

Phase 1 lasts for about 2 days and consists of a rapid acceleration,

[1] See, for example, Sachs, 1887, p. 399; de Saussure, 1833.

[2] For other results with wheat see Godlewski, 1882; Talmas (in Doyer, 1915); Bonnier and Mangin, 1884; Gindele, 1929; Met, 1950.

[3] W. O. James and A. L. James, 1940.