THE UTILIZATION OF ROOT ORGANIC COMPOUNDS DURING THE REGENERATION OF LUCERNE

By K. C. HODGKINSON*

[*Manuscript received January* 13, 1969]

Summary

Organic compounds in the roots of lucerne, labelled with ^{14}C following the assimilation of $^{14}CO_2$ by the shoots 1 day prior to complete herbage removal, were subsequently lost from the roots either through respiration or translocation into new shoots for about the first 20 days of regeneration. The majority of the compounds used in these two processes were derived from the tap-root. Autoradiography revealed that compounds labelled with ^{14}C 10 days prior to herbage removal were translocated from the roots into the new shoots.

The contribution of accumulated organic compounds to the growth of the leaves of new shoots occurred mainly during the formation and expansion of the first-formed leaves, whereas for the stems it continued for a longer period (up to 20 days). For the first 10 days of regeneration the movement of ^{14}C-labelled compounds into stems of new shoots was independent of daylight intensity whereas the movement into leaves during this period was directly related to the daylight intensity.

Export of ^{14}C-labelled photosynthates from the leaves of new shoots into the roots was measured on the sixth day and probably commenced earlier.

I. INTRODUCTION

In 1927 Graber *et al.* postulated that for lucerne "new top growths, especially in the early stages, are initiated and developed largely at the expense of previously accumulated organic reserves". This hypothesis has in the main been accepted although relatively recently the role of reserves in regeneration was queried on the grounds of insufficient and inconclusive evidence (May 1960). May concluded that a specific role for carbohydrate reserves in initiating regrowth, and in determining the rate and ultimate extent of regrowth, could not yet be considered as firmly established.

More recent evidence used to support this hypothesis has been of an indirect nature. Thus it is known that the percentage of total available carbohydrates in the tap-root declines for a period of 20–30 days after herbage removal before increasing again (Nielsen and Lysgaard 1956; Hodgkinson 1967). However, it has been argued on theoretical grounds (May and Davidson 1958; May 1960) that continued respiration in the roots following herbage removal may account for all or a major part of the reduction in the concentration of total available carbohydrates in the roots. Thus the decline in the percentage of these carbohydrates in the tap-root cannot be taken as proof that organic root reserves are directly involved in the initiation and growth of new shoots. It has also been shown that there is a relationship between root weight and the rate of shoot regeneration (Hildebrand and Harrison 1939; Leach

* Agronomy Department, University of New England, Armidale, N.S.W.; present address: Division of Plant Industry, CSIRO, Riverina Laboratory, Deniliquin, N.S.W. 2710.

1968). Furthermore, Graffis (1960) was able to show that the percentage of some carbohydrates in the tap-root was related to the rate of shoot regrowth. Again this evidence is inconclusive since the relationship may not be causal.

The first step in determining the significance of reserves in regeneration is to establish that there is movement of organic compounds from the root into the new shoots during regeneration. In this paper three ^{14}C-labelling experiments are reported which were designed to study such movement. Special reference is made to the effect of different shoot growth rates (manipulated by growing the plants in contrasting light-intensity regimes) on the pattern of utilization of root organic compounds during regeneration.

II. Materials and Methods

(a) *Treatment of Plants*

Lucerne plants (*Medicago sativa* L. cv. Hunter River) were propagated by stem tip cuttings from a single clone, inoculated with a commercial strain of *Rhizobium*, and grown in the semi-controlled environment of a glasshouse. The plants were grown singly in 9-in. pots containing river sand which had been sieved to remove particles greater than 2 mm. They were irrigated once daily with a standard Hoagland's nutrient solution and with tap water when required.

(b) *^{14}C Assimilation*

To label plant organs with ^{14}C, plants were exposed for a short period to an atmosphere of $^{14}CO_2$. Chambers made from clear Acrylite and having a capacity of 19 litres were lowered over plants and sealed to the rim of each pot with adhesive tape. The plants were placed in darkness for a period of 30 min during which time $^{14}CO_2$ was released into each chamber by dropping dilute HCl on $Ba^{14}CO_3$ contained within a small vial sealed into the top of each chamber. Small fans driven by 6-V motors and sealed inside each chamber ensured that air inside the chambers was circulated during exposure. A bank of 12 500-W Philips flood (photo) lamps provided illumination during the exposure periods. The light intensity under these lamps was approximately 1350 f.c. measured with an omnidirectional photometer at the base of the plants. The length of the exposure period and the quantity of $^{14}CO_2$ to which the plants were exposed varied for each experiment.

(c) *Harvesting*

At the predetermined harvest times (experiments 1 and 3) roots were washed free of sand. The plants were dissected into organs, quickly frozen, and subsequently freeze-dried. Freeze-dried samples were weighed to obtain dry weights and then ground in a microhammer mill to pass through a 1·0-mm sieve.

In experiment 2 one shoot from each plant was removed at ground level and prepared for autoradiography.

(d) *^{14}C Analyses*

Rapid and accurate measurement of ^{14}C in the ground samples was obtained by combustion of the samples to CO_2, absorption of the CO_2 into ethanolamine, and counting of the absorbent using the liquid scintillation method. Details of this procedure have been previously described (Hodgkinson and Veale 1966).

(e) *Autoradiography*

In experiment 2, shoots were cut half-way along their length and placed up against Ilford X-ray film (Ilford Ilfex safety base). A thin plastic sheet was placed between the plant material and the X-ray film. The plant material and the X-ray film were then clamped together and placed in a deep-freeze unit at $-15°C$ for 14 days. After exposure the films were developed and photographic prints taken from them.

(*f*) *Description of Experiments*

(i) *Experiment 1: Effect of Light Intensity on Utilization of Accumulated Organic Compounds during Regeneration*

Plants selected for this experiment were 4 months old and had reached the one-tenth flowering stage (i.e. when one-tenth of the stems on each plant had open flowers). One day prior to complete herbage removal two out of the four replicate plants in each treatment were labelled with ^{14}C by exposing the leaves for a period of 15 min to an atmosphere of $^{14}CO_2$ containing 100 μCi of ^{14}C and an initial concentration at the start of the light period of 0·1% CO_2. The exposures were carried out in the mid-afternoon. Following exposure the plants were kept in darkness and 24 hr later the herbage was completely removed from each plant (cut down to a level of 2·5 cm above the sand surface). The plants were then placed in one of three light regimes: daylight, 20% daylight, and darkness. These were obtained respectively as follows:

(1) Plants were left uncovered.
(2) Plants were placed under a canopy of shade cloth which reduced the normal light intensity by 80% (measured by an omnidirectional photometer).
(3) Plants were placed in a light-proof box.

The mean daily maximum and minimum air temperatures within the three light regimes during the experimental period were 28·2 and 13·9°C respectively in daylight, 26·7 and 13·9°C respectively in 20% daylight, and 27·5 and 15·4°C respectively in darkness. The differences in temperature between light regimes would have slightly influenced growth and respiration rates. However, such variation was considered to be insignificant in relation to the large variation resulting from differences in light intensity.

Four shoots on each plant were permitted to grow. At 0, 10, 20, and 30 days after complete herbage removal four plants from each light treatment were harvested. Two of these four plants were analysed for ^{14}C content.

(ii) *Experiment 2: Determination by Autoradiography of the Distribution within New Shoots of Organic Compounds Translocated from Residual Organs during Regeneration*

This experiment was conducted on 11-month-old plants which had already been defoliated once earlier. Shoots were exposed to an atmosphere of $^{14}CO_2$ containing 393 μCi of ^{14}C and an initial concentration of 0·29% CO_2 for a period of 3 hr, either 10 days or 1 day prior to a second herbage removal 30 days after the first. Then four shoots on each plant were permitted to grow.

To prevent the photosynthesis of $^{14}CO_2$ respired by the roots, a layer of silicone rubber was poured on to the surface of the sand around each plant and allowed to solidify. A small hole was formed on one edge of the silicone rubber layer to permit the application of nutrient solution to the sand medium. As an additional precaution, the pots in the glasshouse were placed in a row at right angles to the stream of air from an Aquacool air-cooling unit, which operated constantly in the glasshouse compartment. The watering holes in the silicone rubber layer in each pot were placed away from the direction of the air flow.

On the 20th day after herbage removal one new shoot from each plant was removed and autoradiographed.

(iii) *Experiment 3: Exportation of ^{14}C-labelled Photosynthate from New Leaves to Roots during Regeneration*

Experiment 3 was conducted on plants 2 months of age. At the one-tenth flowering stage the herbage was completely removed from the plants. One shoot on each plant was permitted to grow. Four plants were harvested at each of the following times: 6, 10, 14, 20, 30, and 40 days after complete herbage removal. On the predetermined harvest day, the selected plants were exposed for 10 min to an atmosphere of $^{14}CO_2$ containing 31 μCi of ^{14}C, the initial CO_2 concentration being 0·06%. These exposures were given at about midday. The plants were kept in darkness for a period of 3 hr after exposure, then harvested, and the ^{14}C content of each organ determined. In this paper the distribution of ^{14}C-labelled photosynthates to the roots is considered.

III. Results

(*a*) *Dry Weight Changes of Organs during Regeneration* (*Experiment 1*)

During the 30-day period after cutting the dry weights of the stubble and lateral roots changed little and were not significantly influenced by the three light regimes (Fig. 1). However, the dry weight of the tap-root steadily declined during the first 20 days at a similar rate for all the light regimes. This decline continued during the period of 20–30 days for the plants placed in the 20% daylight and darkness regimes whereas for the plants in the daylight regime the tap-root ceased decreasing in weight between the 20th and 30th day.

Fig. 1

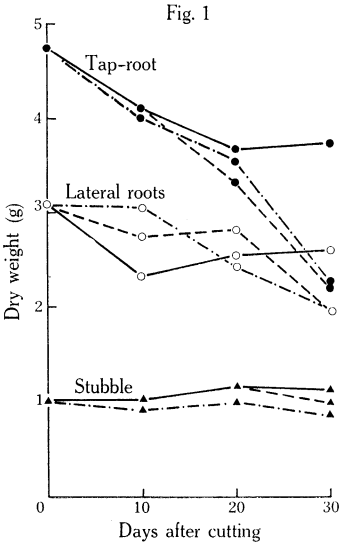


Fig. 2

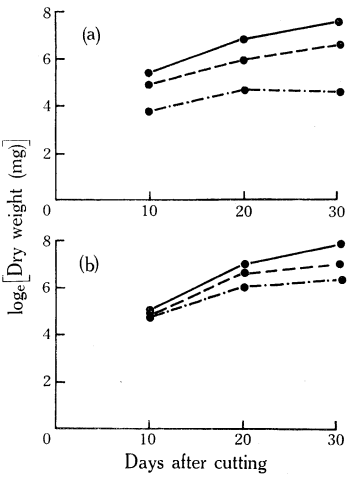


Fig. 1.—Changes in the dry weight of the tap-root, lateral roots, and stubble during regeneration of lucerne following complete herbage removal. Plants regrew in one of three light regimes: daylight (——), 20% daylight (— —), and darkness (—·—·—).

Fig. 2.—Changes in the dry weight (log$_e$) of leaves (*a*) and stems (*b*) during regeneration of lucerne following complete herbage removal. Light regimes as for Figure 1.

The growth of the new leaves and stems is shown in Figures 2(*a*) and 2(*b*). During the period up to the 10th day there was little effect of light on the growth rate of the new stems, but in contrast the growth rate of the new leaves was influenced by the light regimes prior to the 10th day [Fig. 2(*a*)]. After the 10th day the growth rate of both the stems and leaves was significantly depressed by the 20% daylight and darkness regimes.

(*b*) *Utilization of Accumulated Organic Compounds during Regeneration* (*Experiment 1*)

Organic compounds labelled with ^{14}C 1 day prior to complete herbage removal were translocated from the residual organs into the new shoots during regeneration as shown by the increase in the ^{14}C content of the regenerating shoots [Figs. 3(*a*), 3(*b*), and 3(*c*)]. The period during which most of this utilization occurred was the first 20 days after cutting for plants regrowing in the daylight and 20% daylight

treatments. When plants regrew in darkness, translocation of ^{14}C-labelled organic compounds from the residual organs into the new shoots continued for at least 30 days [Fig. 3(*c*)].

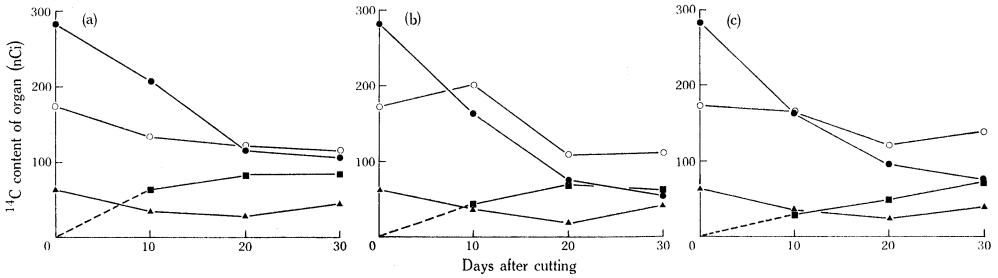


Fig. 3.—Changes in the ^{14}C content of each plant organ with time during the 30-day period after complete herbage removal. Plants regrew in either daylight (*a*), 20% daylight (*b*), or darkness (*c*). ○ Lateral roots. ● Tap-root. ■ New shoots. ▲ Stubble.

The ^{14}C content of the three residual organs (stubble, tap-root, and lateral roots) is also shown in Figures 3(*a*), 3(*b*), and 3(*c*). It can be seen that during the 30-day period after herbage removal, one-half to two-thirds of the ^{14}C initially present in the tap-root disappeared. This decline was highly significant ($P<0\cdot01$). There was a slight decline in the ^{14}C content of the lateral roots during the 30-day period of regeneration, but this was not statistically significant. The ^{14}C content

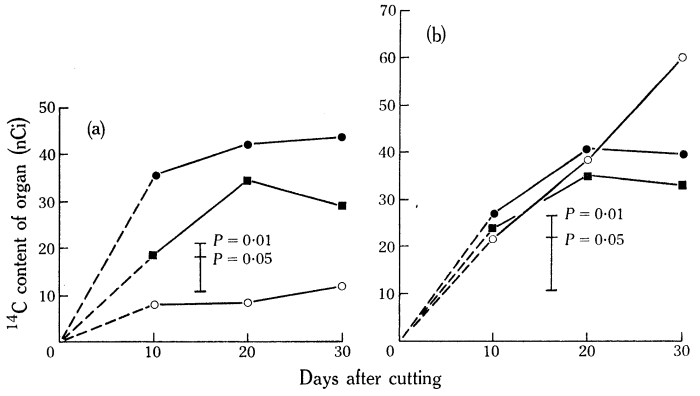


Fig. 4.—Changes with time in the ^{14}C content of leaves (*a*) and stems (*b*) during the 30-day period after complete herbage removal as influenced by daylight (●), 20% daylight (■), and darkness regimes (○).

of the stubble declined during the initial 20-day period after herbage removal and then increased slightly. Differences in light intensity during regeneration had no significant effect on the ^{14}C content of the stubble or lateral roots but did have a highly significant ($P<0\cdot01$) effect on the rate of decline of the ^{14}C content of the tap-root. From these results it is concluded that all or a major portion of the organic compounds translocated into the regenerating shoots were mobilized from the tap-root.

There were differences in the ^{14}C content of the new leaves and stems due to the light treatments [Figs. 4(*a*) and 4(*b*)]. Overall, the ^{14}C content of the leaves [Fig. 4(*a*)] was significantly greater for the daylight than for the 20% daylight treated plants and that of the latter was in turn significantly greater than that of the plants in darkness. By contrast, the ^{14}C content of the stems [Fig. 4(*b*)] was unaffected by the three light treatments during the first 20 days. Between the 20th and 30th day the ^{14}C content of the stems of plants in darkness increased significantly above that of the stems of plants in the daylight and 20% daylight treatments.

The movement of ^{14}C-labelled compounds into the new leaves was mainly completed by the 10th day (or earlier), whereas movement into the new stems continued for longer.

The overall decline in the ^{14}C content of the tap-root after complete herbage removal may be attributed to respiration and movement of organic compounds from the residual organs into rapidly growing shoot tissues. The quantities of ^{14}C used in these two processes are compared in Table 1. Loss of ^{14}C from the plant

TABLE 1

LOSS OF ^{14}C FROM THE PLANT BY RESPIRATION AND THE INCORPORATION OF ^{14}C INTO NEW SHOOT TISSUE FOLLOWING COMPLETE HERBAGE REMOVAL

Treatment	Growth Period	^{14}C Content (nCi) of Residual Organs used for:		Total ^{14}C Content (nCi)	Percentage of Total ^{14}C used for:	
		Respiration	New Shoot Tissue		Respiration	New Shoot Tissue
Daylight	Days 0–10	81	62	143	56	44
	Days 10–20	89	21	110	81	19
20% daylight	Days 0–10	74	42	116	64	36
	Days 10–20	172	28	200	86	14
Darkness	Days 0–10	125	30	155	81	19
	Days 10–20	107	17	124	86	14

by respiration has been calculated by the decline in the total ^{14}C content of the plants during regeneration. It was assumed that the ^{14}C-labelled metabolizable compounds within the tap-root were available equally as respiratory substrates and for translocation into new shoots during regeneration.

During the first 10 days after herbage removal, 44% of the ^{14}C lost from the residual organs was incorporated into new shoot tissue when plants regrew in normal daylight. As the light intensity declined, a decreasing proportion of the ^{14}C lost from the residual organs was incorporated into new shoot tissue. For all light regimes, the proportion incorporated into new shoot tissue was smaller (14–19%) and the proportion used for respiration correspondingly larger during the period 10–20 days after herbage removal than during the first 10 days. There was little difference between the three light regimes in the relative proportions used for respiration and incorporation into new shoot tissues during the period 10–20 days after herbage removal.

(c) *Distribution within New Shoots of Organic Compounds Translocated from Residual Organs during Regeneration (Experiment 2)*

Autoradiographs of shoots which had regrown for 20 days after complete herbage removal are shown in Figures 5–8. The prominent feature is that ^{14}C-labelled organic compounds were uniformly distributed within each leaf and region of the stems.

The most heavily labelled portions of the shoots were the basal regions of the main stem, the lower older leaves, stipules, and petioles. These portions were the first-formed leaf and stem tissue. Leaf and stem tissue formed later were not so heavily labelled with ^{14}C.

Another feature of these autoradiographs is that some of the ^{14}C which was assimilated 10 days prior to herbage removal, and subsequently incorporated into organic compounds in the root system, was translocated into new shoots during regeneration.

The pattern of labelling of the new shoots was similar for the plants in which root organic compounds were labelled with ^{14}C 10 days or 1 day prior to herbage removal (Figs. 5 and 6 cf. Figs. 7 and 8).

(d) *Distribution of Photosynthate to the Roots during Regeneration (Experiment 3)*

When the shoots were exposed to $^{14}CO_2$ for 10 min at different stages of regeneration and the plants harvested 3 hr later it was found that a part of the ^{14}C-labelled photosynthate had been translocated to the roots, as the following tabulation shows:

Days after herbage removal	6	10	14	20	30	40
Percentage of ^{14}C in the roots	29·2	19·9	13·1	13·9	17·3	12·7

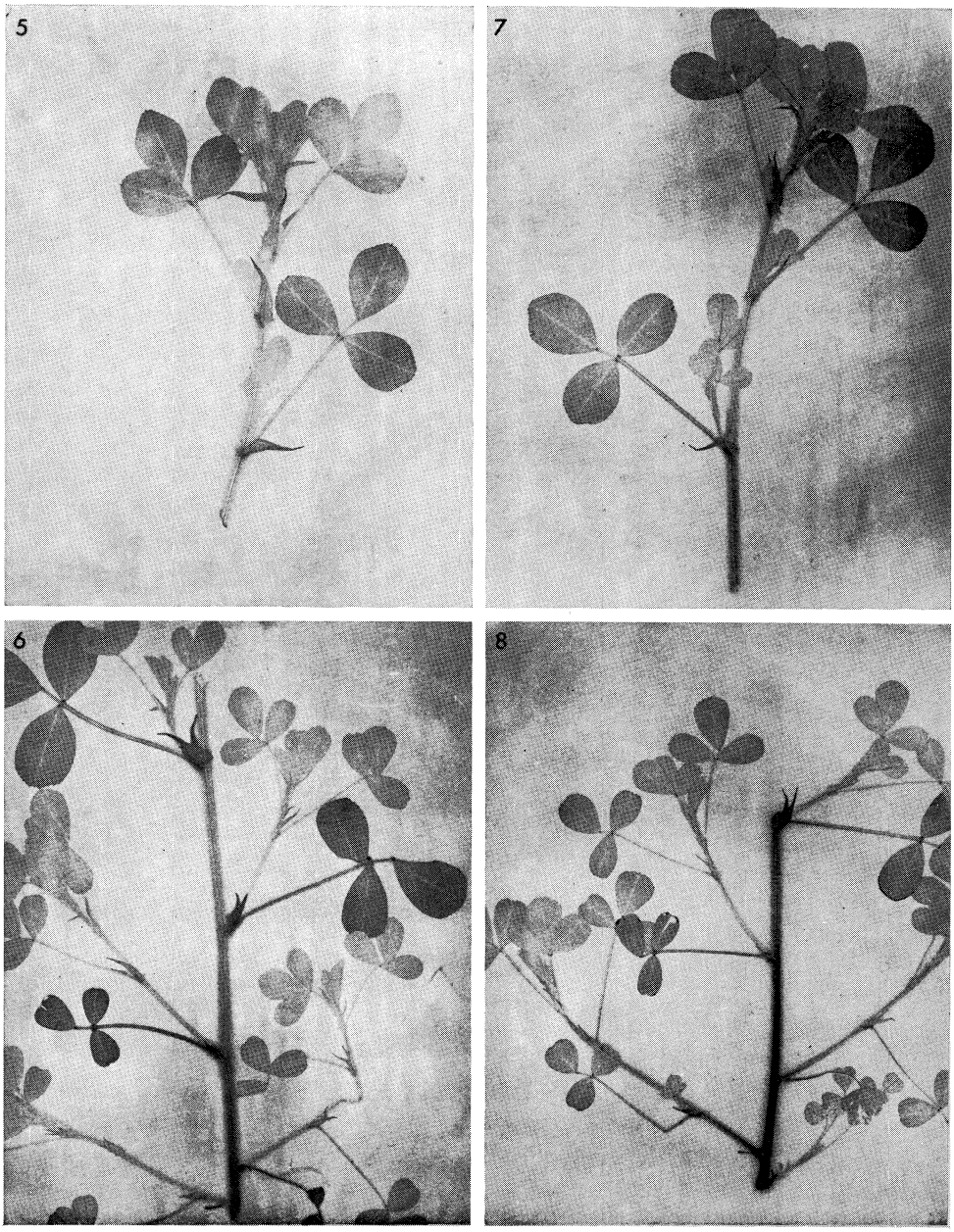
The necessary difference for significance between percentages is 3·8 ($P = 0·05$) and 5·0 ($P = 0·01$). During the early phase of regeneration (sixth day) a large portion of the photosynthate was translocated into the roots. By the 14th day the proportion declined to 13% and thereafter (14th–40th day) changed little.

IV. Discussion

During the regeneration of lucerne, the decline in the dry weight of the tap-root (Fig. 1) may be accounted for by three processes. Metabolic organic compounds in the tap-root may be translocated into new shoots, respired away, or translocated into the fine lateral roots.

It has been widely assumed that the synthesis and initial growth of new shoot tissue following herbage removal is largely at the expense of organic compounds mobilized from the underground organs of lucerne (for example, Graber *et al.* 1927; Mitchell and Denne 1967). Using ^{14}C as a tracer, direct evidence of this movement of organic compounds from the tap-root into new shoots during regeneration has been obtained in experiment 1 and 2. Thus the importance of the tap-root as a source of organic compounds which are mobilized and utilized in the synthesis of new tissue during regeneration of lucerne is firmly established.

Other recent studies (Marshall and Sagar 1965; Davidson and Milthorpe 1966*a*; Ehara, Yamada, and Maeno 1966; McWilliam 1968) have shown that in a



Figs. 5–8.—Autoradiographs of the new shoots harvested on the 20th day after complete herbage removal. The root system was labelled with ^{14}C either 1 day (Figs. 5 and 6) or 10 days (Figs. 7 and 8) prior to herbage removal. Four shoots per plant were permitted to grow but only one is shown in each figure. The upper regions of the shoots are shown in Figures 5 and 7, and the lower regions in Figures 6 and 8.

number of grasses there is a source of organic compounds for new leaf growth following defoliation. This source has been shown to be the stem bases rather than the roots.

Organic compounds in the tap-root were also used as respiratory substrates during regeneration as evidenced by the decline in the total ^{14}C content of the plant. The majority of this respiratory loss may be accounted for by the decline in the ^{14}C content of the tap-root. However, respiration of smaller quantities of ^{14}C-labelled compounds would have occurred in the lateral roots and stubble, but these losses were too small to reach statistical significance. Also some of the ^{14}C-labelled compounds translocated into the new shoots may have been utilized as respiratory substrates in addition to incorporation into cell walls and cytoplasm.

A higher proportion of ^{14}C was used in respiration than for translocation into new shoots during regeneration (Table 1). This supports the hypothesis that metabolizable compounds in the tap-root are used in the main as respiratory substrates and to a lesser extent in the formation of new shoot tissue during regeneration (cf. May and Davidson 1958; May 1960; Davidson and Milthorpe 1966*b*).

There was no evidence from these present experiments that organic compounds are translocated from the tap-root into lateral roots during the early stages of regeneration. The dry weight and ^{14}C content of the lateral roots did not change significantly during the 30-day period after cutting. Since extension of lateral roots almost ceases for a period of about 15 days after complete herbage removal (Hodgkinson 1967) it is unlikely that they would be a "sink" for organic compounds mobilized from the tap-root.

It can be envisaged that the organic compounds which are either used in respiration or translocated into new shoots are derived from pools of metabolizable compounds in the tap-root (cf. Davidson and Milthorpe 1965). These organic compounds would include mono- and disaccharides and amino acids (Steward, Bidwell, and Yeman 1958; Canny 1962; Pate 1962). During the stress of defoliation some complex organic compounds such as proteins (Steward, Bidwell, and Yeman 1958) and starch (Dobrenz and Massengale 1966) are hydrolysed to simpler units which would enter pools of metabolizable compounds. The validity of using ^{14}C as a tracer of the movement of organic compounds during regeneration relies on the assumption that the compounds in the metabolic pools in the tap-root are uniformly labelled with ^{14}C. Autoradiographic studies by the author (Hodgkinson, unpublished data) of the distribution of ^{14}C in the transverse and longitudinal sections of the tap-root 24 hr after exposing the shoots to $^{14}CO_2$ indicated that labelling is uniform throughout non-conducting tissue.

The results of experiments 1 and 3 lead to the suggestion that bidirectional movement of organic compounds between the root and the new leaves takes place soon after the expansion of the first-formed leaves. Several explanations may be put forward for this phenomenon. Firstly, there may be true simultaneous bidirectional movement of carbohydrates within the regrowing stems once leaves begin exporting photosynthate. Evidence from translocation experiments would indicate that bidirectional movement of carbohydrates may occur in stems (Biddulph and Cory 1965; Eschrich 1967; Trip and Gorham 1968). Secondly, carbohydrates may be translocated into the buds and expanding leaf tissue, and then cease moving when the leaves reach the stage of exporting carbohydrates into the roots. Some

movement of ^{14}C-labelled compounds into new leaves beyond this stage may be accounted for by movement of ^{14}C-labelled amino acids from the roots.

The results of experiments 1 and 2 indicate that there were significant differences in the rate and amount of incorporation of ^{14}C-labelled compounds by the new leaves and stems during regeneration. Interpretation of these differences is, however, difficult because the specific activity of the organic compounds in transit from the root to the regrowing shoot would certainly vary during regeneration. Therefore quantities of ^{14}C in the new shoots may or may not be directly related to quantities of mobilized unlabelled organic compounds. However, it is possible to make a meaningful comparison between the incorporation of ^{14}C-labelled compounds into leaf and stem tissue since the specific activities of compounds entering either of these tissues would be similar.

When plants regrew in normal daylight, the growth rate of the leaves exceeded that of the stems during the first 10 days [Fig. 2(*a*) cf. 2(*b*)]. Utilization of ^{14}C-labelled compounds during this period was also greater for the leaves than for the stems. When plants regrew in a much reduced light intensity (20% daylight), where the photosynthetic rate would be low, the reduced growth of the leaves and stems was similar, as was the utilization of ^{14}C-labelled compounds from the tap-root by the leaves and stems. These results suggest that there is a direct relationship between the growth rate of the leaves and stems and the rate of utilization of organic compounds from the tap-root by the leaves and stems. The physiological mechanisms controlling the rate of utilization are not known, but there is some evidence (Booth *et al.* 1962; Seth and Wareing 1967) that growth substances would play an important role.

When plants regrew in darkness, growth of the etiolated leaves and stems was entirely dependent on the organic compounds mobilized from the tap-root. The interesting feature was that the stem continued to utilize organic compounds from the tap-root during the 30 days after cutting whereas the leaves ceased utilization about the 10th day. A similar cessation in the movement of photosynthate into leaves occurs when they are placed in darkness (Thaine, Ovenden, and Turner 1959; Williams 1964).

It has been demonstrated in these experiments that movement of organic compounds from root to shoot does take place during regeneration. However, it still remains to be shown that the quantity and availability of these compounds regulate the rate of shoot regeneration under normal light-intensity conditions.

V. Acknowledgments

Thanks are due to Mr. P. C. Bettle for technical assistance and to Drs. N. G. Smith and R. D. B. Whalley, University of New England, for helpful criticism of this paper. Financial support for this project from the Australian Meat Research Committee is gratefully acknowledged.

VI. References

Biddulph, O., and Cory, R. (1965).—Translocation of ^{14}C metabolites in the phloem of the bean plant. *Pl. Physiol., Lancaster* **40**, 119–29.

Booth, A., Moorby, J., Davies, C. R., Jones, H., and Wareing, P. F. (1962).—Effects of indolyl-3-acetic acid on the movement of nutrients within plants. *Nature, Lond.* **194**, 204–5.

CANNY, M. J. (1962).—The mechanisms of translocation. *Ann. Bot.* (N.S.) **26**, 603–17.

DAVIDSON, J. L., and MILTHORPE, F. L. (1965).—Carbohydrate reserves in the regrowth of cocksfoot (*Dactylis glomerata* L.). *J. Br. Grassld Soc.* **20**, 15–18.

DAVIDSON, J. L., and MILTHORPE, F. L. (1966*a*).—Leaf growth in *Dactylis glomerata* following defoliation. *Ann. Bot.* (N.S.) **30**, 173–84.

DAVIDSON, J. L., and MILTHORPE, F. L. (1966*b*).—The effect of defoliation on the carbon balance in *Dactylis glomerata*. *Ann. Bot.* (N.S.) **30**, 185–98.

DOBRENZ, A. K., and MASSENGALE, M. A. (1966).—Change in carbohydrates in alfalfa (*Medicago sativa* L.) roots during the period of floral initiation and seed development. *Crop Sci.* **6**, 604–7.

EHARA, K., YAMADA, Y., and MAENO, N. (1966).—Physiological and ecological studies on the regrowth of herbage plants. IV. The evidence of utilisation of food reserves during the early stage of regrowth in bahiagrass (*Paspalum notatum* Flügge) with $^{14}CO_2$. *J. Jap. Soc. Grassld Sci.* **12**, 1–4.

ESCHRICH, W. (1967).—Bidirektionelle translokation in siebröhren. *Planta* **73**, 37–49.

GRABER, L. F., NELSON, N. T., LUEKEL, W. A., and ALBERT, W. B. (1927).—Organic food reserves in relation to the growth of alfalfa and other perennial herbaceous plants. Bull. Univ. Wis. agric. Exp. Stn No. 80, pp. 1–128.

GRAFFIS, D. W. (1960).—The relationship of root carbohydrates of alfalfa (*M. sativa*) at the time of harvesting to summer recovery growth. Ph.D. Thesis, University of Illinois.

HILDEBRAND, S. C., and HARRISON, C. M. (1939).—The effect of height and frequency of cutting alfalfa upon consequent top growth and root development. *J. Am. Soc. Agron.* **31**, 790–9.

HODGKINSON, K. C. (1967).—Studies on the physiology of regeneration of lucerne (*Medicago sativa* L.). Ph.D. Thesis, University of New England.

HODGKINSON, K. C., and VEALE, J. A. (1966).—The distribution of photosynthate within lucerne as influenced by illumination. *Aust. J. biol. Sci.* **19**, 15–21.

LEACH, G. J. (1968).—The growth of the lucerne plant after cutting: the effects of cutting at different stages of maturity and at different intensities. *Aust. J. agric. Res.* **19**, 517–30.

MARSHALL, C., and SAGAR, G. R. (1965).—The influence of defoliation on the distribution of assimilates in *Lolium multiflorum* L. *Ann. Bot.* (N.S.) **29**, 365–70.

MAY, L. H. (1960).—The utilisation of carbohydrate reserves in pasture plants after defoliation. *Herb. Abstr.* **30**, 239–45.

MAY, L. H., and DAVIDSON, J. L. (1958).—The role of carbohydrate reserves in regeneration of plants. I. Carbohydrate changes in subterranean clover following defoliation. *Aust. J. agric. Res.* **9**, 767–77.

MCWILLIAM, J. R. (1968).—The nature of the perennial response in mediterranean grasses. II. Senescence, summer dormancy, and survival in *Phalaris*. *Aust. J. agric. Res.* **19**, 397–409.

MITCHELL, K. J., and DENNE, M. P. (1967).—Defoliation and root development of lucerne. In "The Lucerne Crop". (Ed. R. H. M. Langer.) pp. 22–7. (A. H. & A. W. Reed: Wellington.)

NIELSEN, H. M., and LYSGAARD, C. P. (1956).—Relationship between root and top growth and organic root reserves on lucerne. *K. Vet.- og Landbohøisk. Aarsskr.* **1956**, 77–107.

PATE, J. S. (1962).—Root-exudation studies on the exchange of ^{14}C-labelled organic substances between the roots and shoot of the nodulated legume. *Pl. Soil* **17**, 333–56.

SETH, A. K., and WAREING, P. F. (1967).—Hormone directed transport of metabolites and its possible role in plant senescence. *J. exp. Bot.* **18**, 65–77.

STEWARD, F. C., BIDWELL, R. G. S., and YEMAN, E. W. (1958).—Nitrogen metabolism, respiration and growth of cultured plant tissue. *J. exp. Bot.* **9**, 11–49.

THAINE, R., OVENDEN, S. L., and TURNER, J. S. (1959).—Translocation of labelled assimilates in the soybean. *Aust. J. biol. Sci.* **12**, 349–72.

TRIP, P., and GORHAM, P. R. (1968).—Bidirectional translocation of sugars in sieve tubes of squash plants. *Pl. Physiol., Lancaster* **43**, 877–82.

WILLIAMS, R. D. (1964).—Assimilation and translocation in perennial grasses. *Ann. Bot.* (N.S.) **28**, 419–26.