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Effect of Carbohydrate Demand on the Remobilization of Starch in Stolons and Roots of White Clover (*Trifolium repens* L.) after Defoliation

B. BAUR-HÖCH, F. MACHLER and J. NÖSBERGER[1]

Institut für Pflanzenwissenschaften, Eidgenossische Technische Hochschule, Universitatstrasse 2, CH-8092 Zurich, Switzerland

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ABSTRACT

White clover plants were grown from stolon tips in growth cabinets and then defoliated. Thereafter, changes in the contents of non-structural carbohydrates such as starch, sucrose, glucose, fructose, maltose, and pinitol in stolons and roots were monitored. Initial contents of carbohydrate reserves, photosynthetic supply of new carbohydrates and carbohydrate demand after defoliation were varied by growing the plants at various CO_2 partial pressures, by varying the extent of defoliation and by removing either roots or stolon tips at the time of defoliation.

Remobilization of carbohydrate reserves in stolons increased proportionally to their initial contents and was greater when plants had been severely defoliated, suggesting that carbohydrates were remobilized according to availability and demand. Starch was the predominant reserve carbohydrate. Starch degradation was associated with decreased contents of sucrose, glucose and fructose in young stolon parts and roots but not in old stolon parts suggesting that starch degradation was not strictly controlled by the contents of these sugars. A decrease in the demand for carbohydrates by removal of roots did not decrease starch degradation but increased the contents of sucrose, glucose, and fructose. Removal of stolon tips decreased starch degradation and contents of sucrose, glucose, and fructose. The results suggest that starch degradation was controlled by a factor other than sucrose, glucose, and fructose which was exported from stolon tips, e.g. gibberellin.

Key words: White clover, storage carbohydrates, remobilization, regrowth.

INTRODUCTION

The growth of herbaceous plants after defoliation depends on the presence of carbohydrate reserves in stubbles and roots. A relationship between the content of non-structural carbohydrates in defoliated tissues and the regrowth of leaves has been shown for *Dactylis glomerata* (Davidson and Milthorpe, 1966) and *Phleum pratense* (Smith, 1974). Regrowth of white clover after defoliation has been investigated by King, Lamb, and McGregor (1978) and by Ryle, Powell, and Gordon (1985). Gordon, Ryle, Mitchell, Lowry, and Powell (1986) showed that regrowth of defoliated white clover was associated with a decrease in starch and other carbohydrates in stolons and roots.

In this study, the effect of assimilate demand after defoliation on the remobilization of carbohydrates in stolons and roots was investigated. Carbohydrate supply and carbohydrate demand varied due to the following conditions. Stolons with different initial carbohydrate contents were studied, the extent of defoliation was varied, and sink organs such as stolon tips and roots were removed. Non-structural carbohydrates in stolons and roots were analysed for starch, which is the main storage carbohydrate in white clover leaves (Scheidegger and Nösberger, 1984), for pinitol, which is the main sugar in leaves, petioles, and nodules of white clover (Davis and Nordin, 1983), and for sucrose, glucose, fructose, and maltose. The results show that starch degradation increases with the demand for carbohydrates, but that it is not strictly associated with low levels of sucrose, glucose, and fructose.

[1] To whom correspondence should be addressed.

MATERIALS AND METHODS

Plant material and growth conditions

Plants of white clover (*Trifolium repens* L. cv. Milkanova) were grown from clonal stolon tips (six per box) in growth chambers (Therma). Each box (40 × 17·5 × 12·5 cm) contained silica sand (0·8–1·2 mm) and received 100 cm^3 nutrient solution (Hammer, Tibbits, Langhans, and McFarlane, 1978) twice a day. Light was provided by fluorescent lamps (75 per cent) and incandescent bulbs (25 per cent by total wattage). Photon irradiance was increased weekly beginning with 100 μmol quanta m^{-2} s^{-1} at plant height and was 500 μmol quanta m^{-2} s^{-1} from the beginning of the fourth week until the end of the experiment. The photoperiod was 16 h. Day/night temperature was 18/13 °C and relative humidity was 75/80%.

Variation in the extent of defoliation

Plants at 37-d-old were either slightly defoliated (all unfolded leaves removed including developing leaves with stage 0·9 after Carlson, 1966) or severely defoliated (all unfolded leaves removed including developing leaves with stage 0·4 to 0·9 after Carlson, 1966). The reduction in leaf dry matter was 81·3% and 98·5% for slightly and severely defoliated plants, respectively. Control plants were not defoliated.

Variation in initial carbohydrate contents of stolons

Some plants were grown for 3 weeks in ambient CO_2 concentration and then transferred to growth chambers with either 20 or 100 Pa CO_2 and kept there for 4 weeks. The plants were then defoliated severely and regrown in ambient CO_2 concentration.

Variation in sink activity

Plants at 37-d-old were severely defoliated. The growing tips (0·5 cm) or the roots were also removed from some plants. Those without roots were placed on the surface of nutrient solution in germination dishes.

Sampling of plant parts

Plants were sampled at intervals after defoliation and dissected into leaves, stolons, and roots. Some stolons were dissected into main and lateral shoots. The main shoot was dissected into parts according to age (young, medium, old), all of the same length. Plant parts were freeze-dried and dry weight was determined.

Extraction and analysis of carbohydrates

Freeze-dried, homogenized plant parts (80–100 mg) were extracted twice in 80% (v/v) ethanol (5·0 cm^3 each time) by stirring the mixture for 30 min at 60 °C. Extracts were combined, evaporated and dissolved in 1·5 cm^3 water. Proteins were precipitated after addition of 0·1 cm^3 lead acetate (10% w/v). Extracts were filtered (0·45 μm pore size), fractionated by HPLC (HP 1090 Liquid Chromatograph, Hewlett Packard, Palo Alto, California, USA) on an ion exchange column (HPX87P, Bio Rad, Richmond, California, USA) with water as the mobile phase (flow rate: 0·4 cm^3 min^{-1}); carbohydrate fractions were detected by refractometry (HP 1037A Refractive Index Detector) using sucrose, glucose, fructose, maltose, and pinitol as reference substances. Pinitol was kindly provided by Dr Baumgartner, Institut für Lebensmittelwissenschaft, ETH Zürich, Switzerland. The residue of the ethanol extract was resuspended and extracted three times in 0·5 N NaOH (5·0 cm^3 each time) by stirring the mixture for 30 min at 60 °C (after Wagner, Keller, and Wiemken, 1983). Two cm^3 of diluted extracts were mixed with anthrone reagent (4·0 cm^3), boiled for 15 min and absorbance was measured at 620 nm with glucose as a standard. Starch was completely extracted with this method. No additional starch was extracted when, after NaOH-extraction, residues were treated with amyloglucosidase (Boehringer, Mannheim, FRG). Only few cell wall components were included in the extracts. This was checked by comparing extraction in the presence of NaOH as described above with extraction in the presence of α-amylase after Smith (1969). Carbohydrate contents per 100 mg residual dry weight were only 1·0 mg ($\pm$1·17 mg, confidence interval, $P \leq 0{\cdot}05$) higher in NaOH- extracts than in α-amylase-extracts.

RESULTS

The decrease in non-structural carbohydrates in stolons after defoliation

The content of non-structural carbohydrates in stolons decreased after defoliation (Fig. 1A). The decrease was greater after severe defoliation than after slight defoliation and appeared to respond to the demand for reserve carbohydrates by the growing leaves, which we supposed would be greater following severe rather than slight defoliation.

The content of non-structural carbohydrates in stolons was higher when plants had been kept at 100 Pa CO_2 before defoliation than when they had been kept at 20 Pa CO_2 (Fig. 1B). After defoliation, the higher contents decreased to a greater extent than did the lower contents but the stolons with the lower contents exhausted their supplies sooner than the stolons with the higher contents. The results suggest that the extent of remobilization of reserve carbohydrates in stolons depended upon their availability.

The production of leaf dry matter after defoliation

The production of leaf dry matter was strongly affected by the extent of defoliation (Fig. 2A). Slightly defoliated plants continued with the production of new leaves at the same rate as undefoliated plants. In contrast, leaf production of severely defoliated plants was considerably retarded due to the absence of growing leaves at an advanced developmental stage.

Leaf dry matter production after defoliation was affected by the partial pressure of CO_2 in the atmosphere before defoliation and thus by the initial carbohydrate content of the stolons (Fig. 2B). Plants showed a higher production rate of leaf dry matter when they had been kept at 100 Pa CO_2 before defoliation than when they had been kept at 20 Pa CO_2 suggesting that leaf dry matter production increased with the initial carbohydrate content in the stolons.

The content of the main carbohydrate fractions in young, medium, and old stolon parts and in roots

Starch was the predominant non-structural carbohydrate in stolons and roots (Table 1). The content on a residual dry weight basis, was highest in old stolon parts, lower in young stolon parts and lowest in roots. Lateral

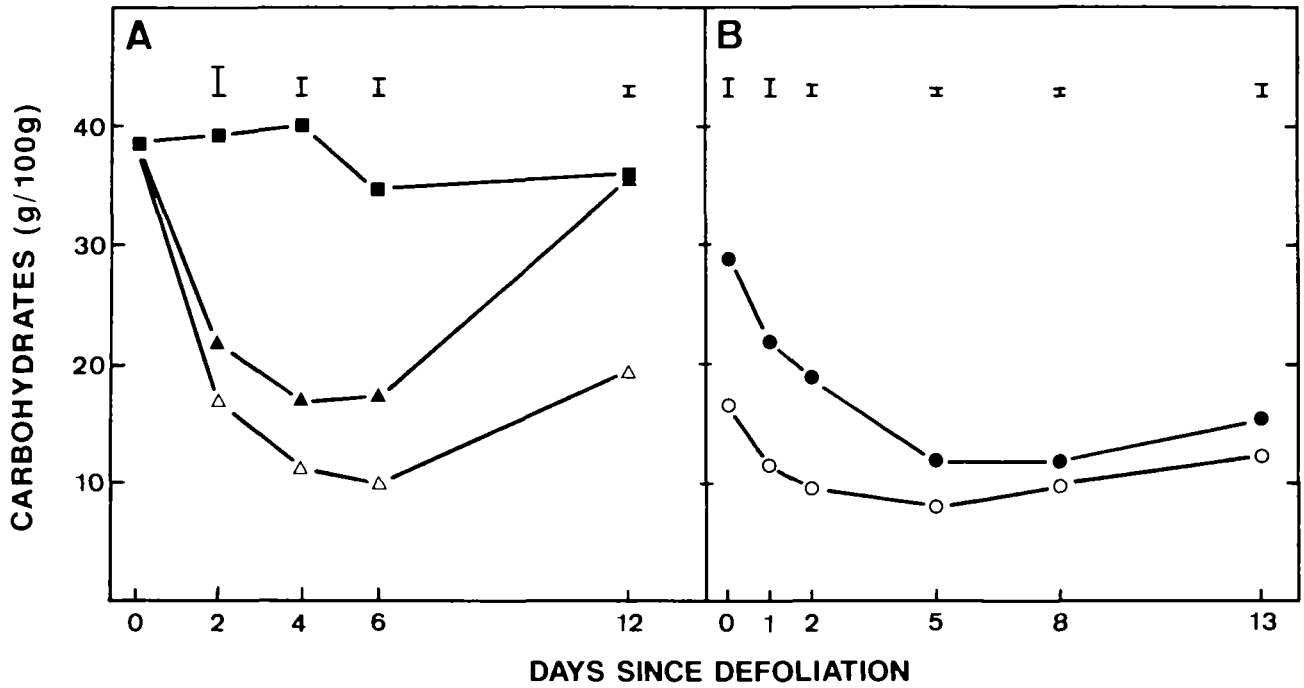


FIG. 1. Decrease in content of non-structural carbohydrates on a residual dry weight basis in stolons after defoliation. (A) Effect of the extent of defoliation: Slight defoliation (▲), severe defoliation (△), control (■). (B) Effect of initial content of carbohydrates in stolons after growth at 100 Pa CO_2 (●) and 20 Pa CO_2 (○) and after severe defoliation. Means of six replications are indicated. Vertical bars are s.e.

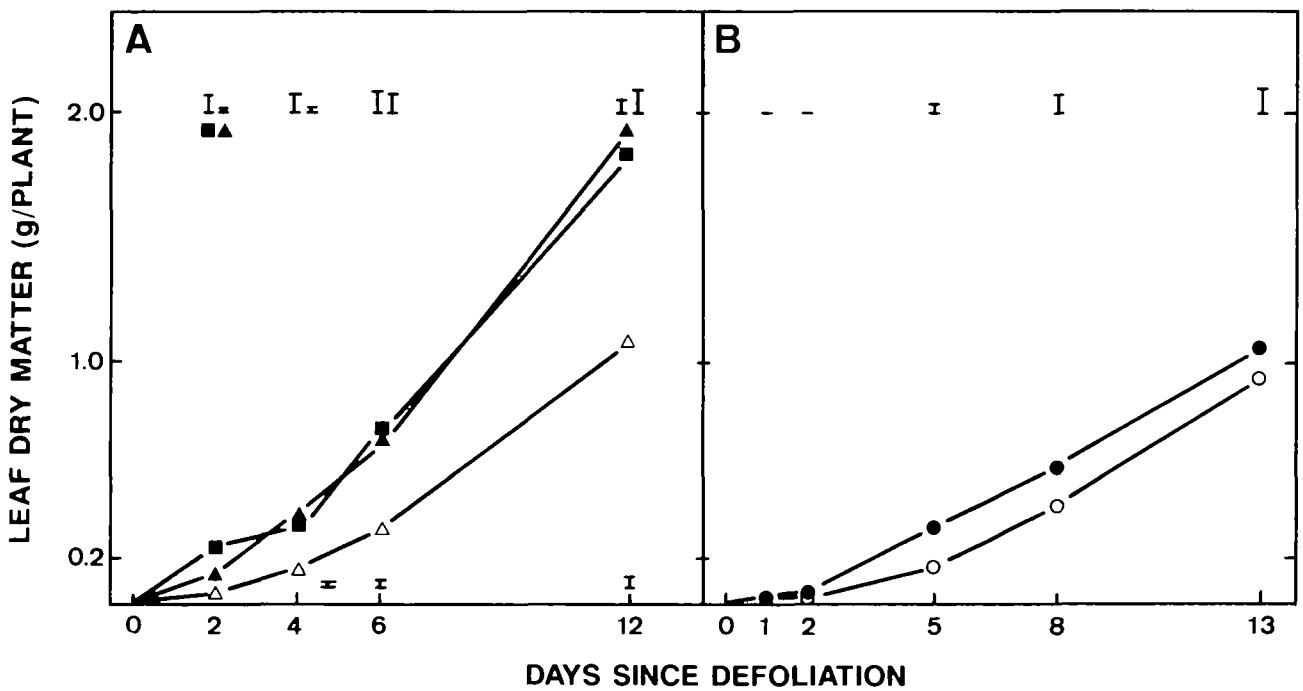


FIG. 2. Production of leaf dry matter per plant after defoliation. (A) Effect of the extent of defoliation: Slight defoliation (▲), severe defoliation (△), control (■). (B) Effect of initial content of carbohydrates in stolons after growth at 100 Pa CO_2 (●) and 20 Pa CO_2 (○) and after severe defoliation Means of six replications are indicated. Vertical bars are s.e.

shoots were young and compared well with young parts of the main stolons. A relatively high proportion of total plant dry matter consisted of lateral shoots and roots. Therefore, starch in lateral shoots and roots contributed considerably to the total starch content per plant. On a residual dry weight basis the content of sucrose was lowest in roots and showed little variation within the stolon system. The contents of glucose, fructose, maltose, and

TABLE 1. *Content of the main carbohydrate fractions before defoliation in young, medium, and old stolon parts and in roots on a residual dry weight basis (A) and as total weights per plant (B)*

Means of three (young part of main shoot), six (medium part of main shoot), and nine (old part of main shoot, lateral shoots, roots) determinations ± s.e. are shown.

	Starch	Sucrose	Glucose	Fructose	Maltose	Pinitol
A (g per 100 g)						
Main stolon						
Young part	9·9 ± 1·4	2·8 ± 0·2	5·7 ± 0·70	4·1 ± 0·53	1·2 ± 0·10	2·8 ± 0·07
Medium part	24·4 ± 1·3	4·5 ± 0·3	0·5 ± 0·10	0·3 ± 0·03	0·7 ± 0·06	0·8 ± 0·05
Old part	44·1 ± 2·4	4·0 ± 0·2	0·3 ± 0·04	0·2 ± 0·05	0·4 ± 0·02	1·1 ± 0·03
Lateral stolons	11·1 ± 0·6	2·9 ± 0·1	4·1 ± 0·15	2·4 ± 0·14	1·0 ± 0·02	2·4 ± 0·09
Roots	4·3 ± 0·1	2·0 ± 0·1	1·3 ± 0·12	0·9 ± 0·08	*	0·5 ± 0·04
B (mg)						
Main stolon						
Young part	4·2 ± 0·5	1·2 ± 0·1	2·4 ± 0·30	1·7 ± 0·20	0·5 ± 0·06	1·2 ± 0·04
Medium part	25·1 ± 1·4	4·6 ± 0·3	0·5 ± 0·06	0·4 ± 0·03	0·7 ± 0·06	0·9 ± 0·06
Old part	66·9 ± 1·8	6·0 ± 0·2	0·5 ± 0·06	0·3 ± 0·07	0·6 ± 0·04	1·6 ± 0·10
Lateral stolons	43·0 ± 2·8	11·3 ± 0·6	15·9 ± 1·00	9·5 ± 0·70	3·8 ± 0·20	9·3 ± 0·40
Roots	40·8 ± 2·4	18·7 ± 1·2	12·7 ± 1·10	8·4 ± 0·70	*	5·2 ± 0·50

* Below the limits of the method.

pinitol were highest in young stolon parts (including lateral shoots) and lowest in old stolon parts and roots. However, due to the great proportion of root dry matter per plant, the low contents of these carbohydrates in roots contributed considerably to the total content per plant.

Contents of the various carbohydrate fractions in the different plant parts after defoliation as affected by the removal of roots and stolon tips.

(1) Defoliation without removal of roots and shoot tips (Fig. 3, circles). The starch content decreased in all plant parts following defoliation. The proportion of remobilized starch was similar in the various stolon parts and was lower in roots. In young stolon parts, the decrease in starch was associated with a decrease in sucrose, glucose, and fructose during the early phase suggesting that remobilization of starch would be induced by a decrease in sugars. However, in old stolon parts only an initial decrease in sucrose, glucose, and fructose was found. Thereafter, the sugars appeared to increase in these plant parts and it is suggested that this was the result of the remobilization of starch.

(2) Defoliation with removal of roots (Fig. 3, squares). The removal of roots did not affect the decrease in starch content of the stolons to any significant extent. However, removal of roots increased the contents of sucrose, glucose, and fructose, first in old stolon parts and later in young stolon parts. The results suggest that sugar contents in stolons increased due to a decreased sink activity in the absence of roots. However, this obviously did not slow down the remobilization of starch.

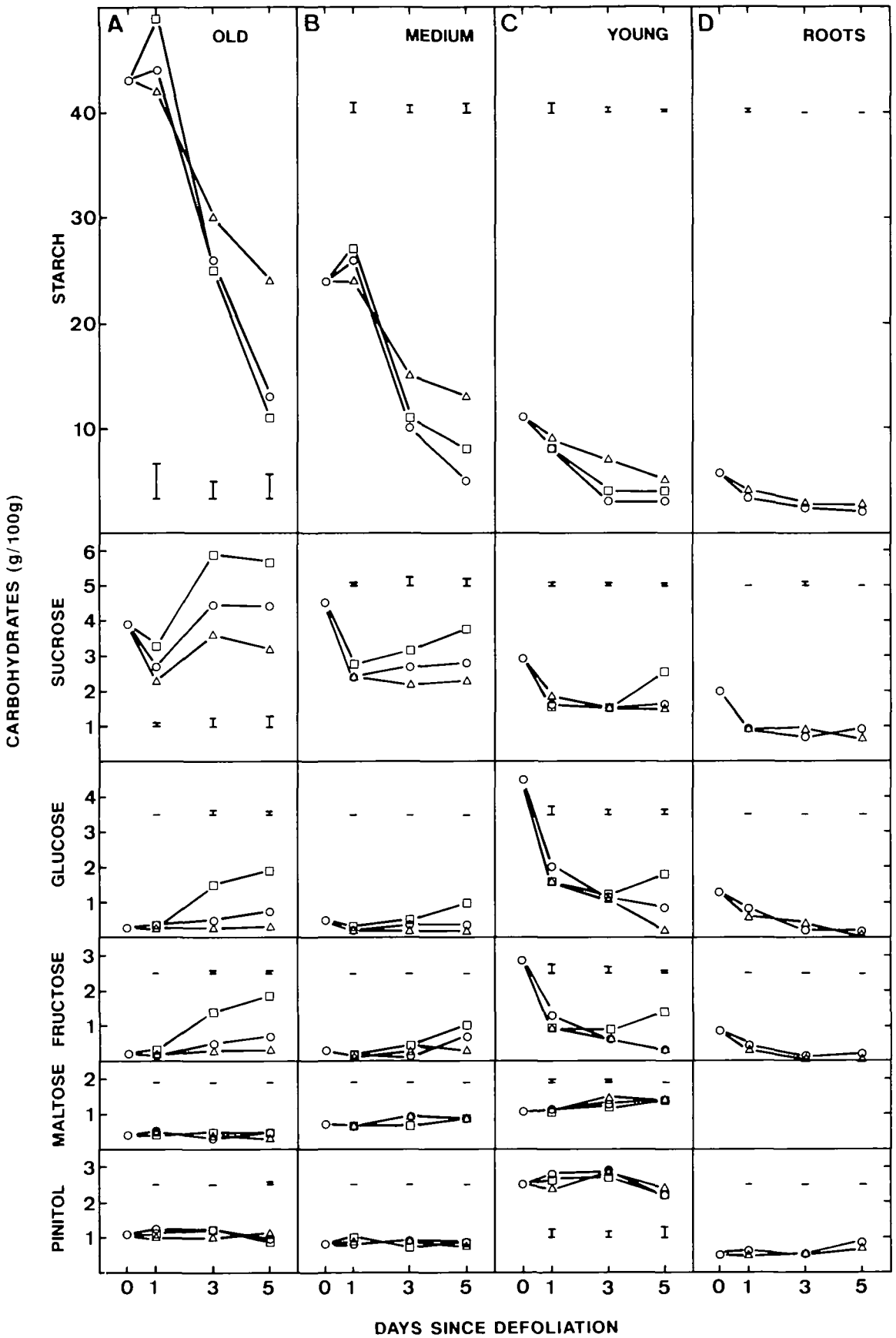
(3) Defoliation with removal of stolon tips (Fig. 3, triangles). The removal of stolon tips decreased the remobilization of starch in older stolon parts. This appeared to be meaningful considering the decreased sink activity in the absence of the stolon tips. However, the decrease in the remobilization of starch was not associated with an increase in sugar contents. On the contrary, contents of sucrose, glucose, and fructose decreased, probably as a result of decreased remobilization of starch. Starch and sugars in young stolon parts and roots were less affected by the removal of stolon tips.

Contents of maltose and pinitol were neither affected by defoliation nor by the removal of stolon tips and roots suggesting that these carbohydrates were not important reserves and that maltose was not a product of starch degradation.

DISCUSSION

The breakdown of starch in stolons and roots of white clover was controlled by the plant's demand for carbohydrates. Defoliation caused a decrease in the supply of carbohydrates from organs of photosynthesis and induced starch degradation in stolons and roots. The extent of the breakdown of starch was greater after severe than after slight defoliation and depended on the initial starch content of the reserve tissues. It appears that degradation of starch could be controlled by the levels of sucrose,

FIG 3. Contents of the various carbohydrate fractions on a residual dry weight basis in old (A), medium (B), and young (C) stolon tissue and in roots (D) after defoliation (○), after defoliation and removal of roots (□), and after defoliation and removal of stolon tips (△). Means of three (old stolon part and roots), two (medium stolon part) and four (young stolon parts: 1 main shoot + 3 lateral shoots) determinations are shown. Vertical bars indicate s.e.


A
OLD
B
MEDIUM
C
YOUNG
D
ROOTS
CARBOHYDRATES (g/100g)
STARCH
SUCROSE
GLUCOSE
FRUCTOSE
MALTOSE
PINITOL
DAYS SINCE DEFOLIATION
0 1 3 5

glucose, and fructose present in the respective organs. Increased demand for carbohydrates would thus be indicated by decreased sugar levels. However, this study shows that the degradation of starch does not respond strictly to the content of these sugars. The induction of starch breakdown was associated with a decrease in sugar levels in young stolon tissues and roots only, whereas starch degradation in old stolon parts occurred in the presence of high levels of sucrose, glucose, and fructose. The removal of the roots increased the sugar contents in the stolons due to decreased carbohydrate consumption but did not decrease the degradation of starch. These results suggest that the control of starch degradation by assimilate demand does not occur by means of the levels of sucrose, glucose, and fructose although it has to be admitted that the compartmentation of the sugars within the tissues has not been investigated and that the actual concentrations in starch storing cells are unknown. The removal of the stolon tips decreased both the degradation of starch and the sugar levels in old stolons. Assuming that this decrease in sugar levels in whole tissues of old stolons indicated a decrease in sugar levels in starch storing cells it can be suggested that starch degradation was controlled by a factor other than sucrose, glucose, and fructose and that this factor was exported from the stolon tips. Starch degradation in cereal seeds is induced by gibberellins (Mundy, Hejgaard, Hansen, Hallgren, Jorgensen, and Munck, 1986; Tittle and Spencer, 1986). For legume seeds, a control via a plant growth regulator has not been unequivocally demonstrated. However, our results would be explained by a control of starch degradation in white clover stolons by gibberellins exported from stolon tips, by an increase in gibberellin concentration and starch degradation in stolons and roots after removal of the foliage and by a decrease in gibberellin concentration and starch degradation after removal of the stolon tips.

Pinitol appears to be a reserve carbohydrate in conifers (Diamantoglou, 1974). However, no such role could be found for pinitol in white clover. Smith and Phillips (1982) came to similar conclusions for pinitol in legumes in general. Thus, the role of pinitol in legumes remains unclear.

CONCLUSIONS

This study illustrates different aspects of starch remobilization in stolons and roots of white clover after defoliation. It is shown that starch degradation is controlled by the demand and supply of carbohydrates and that factors other than the levels of glucose, fructose, and sucrose appear to be the signals of this control.

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