

CHANGES IN THE CARBOHYDRATE CONCENTRATION OF PINE SEEDLINGS AFTER COOL STORAGE

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ABSTRACT

Seedlings of *Pinus mugo* and *Pinus radiata* were cool stored for 0, 6, 12 and 18 weeks and analysed for carbohydrate (as soluble sugars and starch) on removal from storage and after six weeks subsequent growth in controlled environment.

Concentrations of carbohydrate were highest at lifting (10-14% of seedling dry weight) and declined steadily through 6, 12 and 18 weeks' cool storage in both species. *Pinus mugo* seedlings retained higher concentrations in stem and root than *P. radiata* during cool storage and these were depleted during subsequent growth. *Pinus radiata* showed a similar pattern of depletion during growth following 0 and 6 weeks' cool storage but results suggests that alternative resources were utilised following 12 and 18 weeks' storage, resulting in a decline in vigour. A pre-treatment of root wrenching had no significant effect on carbohydrate concentrations in either species.

INTRODUCTION

In New Zealand cool storage of forest tree seedlings is becoming an accepted and necessary technique for holding seedlings between lifting and planting. The scale of lowland production forest planting of *Pinus radiata* D. Don has led to the development of containerised handling systems of which the cool store is an integral part. The low temperature of storage minimises planting stock deterioration, reducing respiration and the consequent build up of heat within the container. Short duration storage, 1-3 weeks, presents few problems and allows nursery production to be smoothed to planting site demand (Hocking, 1972).

For higher altitude protection forest planting seedlings need to be stored for much longer periods, 4-5 months. The seedlings are raised in low altitude nurseries for two reasons. Firstly, the longer growing season reduces the nursery period for the seedlings and secondly, management is simplified through better access to labour. However, high altitude planting sites are not accessible or clear of snow until early summer (October, November) and planting stock must be held until this time from winter lifting (June, July). Left in the seedbed for this period, seedlings begin the spring growth flush, and inevitably new tissue is lost with lifting and planting and subsequent mortalities are high. Pit storage, even at high altitude, subjects seedlings to widely fluctuating ambient temperatures and does not always prevent flushing before planting sites are clear.

Critically low water potentials can also develop in pit storage and cause permanent damage if sustained for long periods.

Cool storage with careful packaging reduces seedling deterioration to a minimum by keeping respiratory and moisture losses low and preventing flushing (Hocking and Nyland, 1971). However long-duration exposure to darkness and low temperatures could cause considerable changes to seedling physiology and seriously affect subsequent survival and vigour (Lavender and Waring, 1972).

For both *Pinus mugo* Turra and *P. radiata* it is not known what changes occur in the concentration or nature of carbohydrates during cool storage or in the period immediately following planting. In *P. ponderosa*, Hellmers (1962) associated the disappearance of starch from the stem during cool storage with a marked decrease in field survival. Ronco (1973) also found a marked depletion of soluble carbohydrate occurred during four months' storage of *Picea englemannii* seedlings, but was prevented by unseasonal frosts from associating this depletion with subsequent survival or vigour. Winjum (1963) found there was no significant change in the concentrations of starch and sugars in *Abies procera* and *Pseudotsuga menziesii* during four weeks' cool storage. Peak survivals did correlate with peak non-reducing sugar concentrations in mid-winter and these were unaffected by cool storage.

This report examines the changes in carbohydrate concentration in foliage, stem and root of *Pinus mugo* and *P. radiata* seedlings during cool storage, and after a subsequent period of growth.

METHODS

Seedlings of *Pinus mugo* and *P. radiata* were raised to planting size as 2/0 and 1/0 stock respectively. Root wrenching was applied to half the numbers of each species in the nursery bed using a reciprocating blade wrencher and maintained at weekly intervals for the latter half of the growing season.

Forty seedlings from each treatment were lifted simultaneously in July and a sample of 5 seedlings harvested. A further 5 seedlings from each treatment were potted in 1-litre pots of river sand and placed in a controlled environment growth chamber. The rest were placed in polythene-lined kraft paper bags and stored in a cool store at $1.0^{\circ}\text{C} \pm 1^{\circ}\text{C}$ with relative humidity above 95% in the bags. At intervals of 6, 12 and 18 weeks, 10 seedlings from each treatment were taken from storage, 5 were harvested and 5 potted and placed in the growth chamber. After 6 weeks in the growth chamber these 5 seedlings were taken for harvest. The growth chamber was set for a 25°C , 14 h day at $315 \mu\text{Ein.m}^{-2}\text{s}^{-1}$ (from a mixture of fluorescent and incandescent lamps) and a 15°C night. Relative humidity was not controlled and varied from 60 to 45%.

For carbohydrate analysis foliage samples were harvested from mature foliage, stem samples from the central main stem and root samples from the taproot and its primary branches. New tissue in the form of extending shoots or white unsuberised roots was removed for separate drying. Samples were immediately freeze-dried or held in liquid nitrogen for up to 48 h until they were freeze-dried. Dried samples were stored in plastic bags at -16°C until analysis. For analysis all samples were ground to pass through a 0.5 mm sieve and further freeze-dried for 24 h. Analyses were made on 100 mg samples. All individuals from the unstored treatments were analysed separately for an estimate

of variability. Individuals from all other treatments were bulked by weight and sub-sampled for analysis.

Soluble sugars were extracted and concentrations determined using the anthrone test against fresh glucose as described by Ebell (1969). Starch was extracted with perchloric acid according to the method of McCready *et al.* (1950), and concentration determined with the anthrone test against fresh glucose standards containing an identical concentration of perchloric acid. These determinations were corrected to 0.9 of their value to give starch concentration.

RESULTS

Changes during Storage

The results were analysed using a three-way ANOVA (wrenching *v.* storage *v.* tissue) separately for soluble sugars, starch and total carbohydrate. In both species wrenching had no significant effect on the concentration of any carbohydrate. However the effects of both storage period and tissue were highly significant (Table 1). With the almost identical results from unwrenched and wrenched treatments, separate discussion is not warranted and the results have been combined. Detailed results for wrenched and unwrenched seedlings are given in the summary table, Table 2.

At lifting, seedlings of both species contained 10-14% of their dry weight as soluble sugars and starch. In the foliage and stem soluble sugars formed the principle resource but the roots had an equally high or higher concentration of starch.

After six weeks in cool storage clear differences in the extent of carbohydrate depletion emerged between the two species (Fig. 1). Total carbohydrate in the stem fell 4% in *P. mugo* and 30% in *P. radiata*. In the roots total carbohydrate fell 22% in *P. mugo* and 38% in *P. radiata* with much of this difference arising from starch depletion, 28% in *P. mugo* against 66% in *P. radiata*. The fall in total carbohydrate in the foliage was very similar in both species following all storage periods.

After 12 and 18 weeks' storage further depletion of both soluble sugar and starch in *P. radiata* was clearly evident in all tissues. Starch was very low and total carbohydrate concentrations were reduced to 25% in the foliage, 42% in the stem and 38% in the root on those at lifting. *P. mugo* after 12 and 18 weeks' storage also showed depletion but not to the same extent. Foliage concentration was reduced to 34% of that at lifting, stem to 61% and root to 73%. In both relative and absolute terms *P. mugo* maintained a greater carbohydrate resource throughout cool storage, especially in the root system.

Changes during the Six Weeks following Storage

Both species showed a marked depletion of the carbohydrate resource in seedlings during the period in the growth chamber following 0 and 6 weeks' cool storage (Fig. 2). After 12 and 18 weeks' storage, however, a clear difference in the nature of the depletion became evident between the two species. In *P. mugo* a marked decrease in concentration occurred during the growth period following all cool storage treatments. A feature of this decrease was the fall in concentration of carbohydrate of the root system where it had been maintained throughout storage. Stem sugar concentration also fell markedly but foliar sugar concentrations, with the exception of those from the unstored seedlings,

TABLE 1—Analysis of variance table for sugars, starch and total carbohydrate following cool storage

Source of variance	d.f.	S.S.	M.S.	F ratio	Signif.
Pinus mugo					
Sugar					
Storage	3	17249.4	5749.8	25.4	P < 0.001
Wrenching	1	8.3	8.3	0.1	
Tissue	2	4637.9	2318.9	10.2	P < 0.001
Interactions	17	16025.8	942.7	4.2	P < 0.001
Within	20	4536.7	226.8		
Total	43	63382.8			
Starch					
Storage	3	692.5	230.8	7.9	P < 0.001
Wrenching	1	34.2	34.2	1.2	
Tissue	2	3060.1	1530.1	52.5	P < 0.001
Interactions	17	420.8	24.7	0.8	
Within	20	583.2	29.2		
Total	43	6728.7			
Total Carbohydrate					
Storage	3	24742.9	8247.6	33.5	P < 0.001
Wrenching	1	46.5	46.5	0.2	
Tissue	2	9400.2	4700.1	19.1	P < 0.001
Interactions	17	16197.8	952.8	3.9	P < 0.001
Within	20	4913.6	245.6		
Total	43	55672.7			
Pinus radiata					
Sugar					
Storage	3	28181.2	9395.7	54.1	P < 0.001
Wrenching	1	569.1	284.6	1.6	
Tissue	2	9944.5	4972.2	28.6	P < 0.001
Interactions	17	19849.6	708.9	4.1	P < 0.001
Within	20	4900.4	173.5		
Total	43	96762.9			
Starch					
Storage	3	7568.8	2522.9	47.4	P < 0.001
Wrenching	1	86.7	43.4	0.8	
Tissue	2	1971.2	985.6	18.5	P < 0.001
Interactions	17	6143.9	219.4	4.1	P < 0.001
Within	20	1809.1	53.2		
Total	43	25747.1			
Total Carbohydrate					
Storage	3	63840.1	21280.0	78.9	P < 0.001
Wrenching	1	341.3	170.7	0.6	
Tissue	2	3133.1	1566.6	5.8	P < 0.005
Interactions	17	10382.5	370.8	1.4	P < 0.050
Within	20	9171.0	269.7		
Total	43	96309.8			

TABLE 2—The concentration of total soluble sugars and starch in the foliage, stem and root of *Pinus mugo* and *Pinus radiata* seedlings following both cool storage and a subsequent six week period of growth

Storage Period	Tissue	Unwrenched				Weekly Wrenched			
		After Sug	Storage St	After Sug	Growth St	After Sug	Storage St	After Sug	Growth St
(mg/g)									
<i>Pinus mugo</i>									
0	Fol	134	9	47	7	154	13	41	6
	Stem	68	18	33	10	80	21	25	9
	Root	55	43	26	7	56	35	32	10
6	Fol	52	9	52	7	61	5	66	6
	Stem	92	17	48	16	62	9	41	12
	Root	54	36	23	7	37	20	37	9
12	Fol	53	6	44	6	61	4	56	11
	Stem	58	9	36	10	57	10	44	12
	Root	39	26	19	7	48	28	30	9
18	Fol	51	8	49	7	41	6	30	5
	Stem	50	14	27	9	39	11	31	13
	Root	39	28	9	11	38	33	12	8
<i>Pinus radiata</i>									
0	Fol	128	20	44	13	120	16	45	7
	Stem	73	36	43	22	120	10	39	12
	Root	43	62	48	28	48	63	35	22
6	Fol	60	5	50	2	73	3	57	2
	Stem	59	12	40	5	59	17	36	6
	Root	41	18	42	5	51	25	42	11
12	Fol	38	4	42	8	61	5	51	14
	Stem	44	14	45	19	51	13	44	20
	Root	32	8	39	16	33	10	29	10
18	Fol	25	7	25	5	33	7	28	4
	Stem	39	12	48	21	40	10	33	17
	Root	32	12	24	25	28	11	22	20

fell only slightly. Growth of new tissue was quite pronounced in *P. mugo* (Fig. 3) and tended to increase with longer storage periods.

Pinus radiata seedlings showed a similar pattern of decrease in carbohydrate concentration during the growth periods following 0 and 6 weeks' storage. However, during the growth periods following 12 and 18 weeks, both shoot and root tissues showed a slight increase in concentration of both sugars and starch. Foliar concentrations showed little change during the growth period following storage for 6, 12 and 18 weeks. Growth following storage (Fig. 3) was much less than that in *P. mugo* but more evenly distributed between shoot and root.

DISCUSSION

The carbohydrates in this study were analysed in the form of soluble sugars (principally fructose, glucose, sucrose with any tri- or tetrasaccharides) and starch to include most of the primary reserves on which respiration and subsequent establishment of a

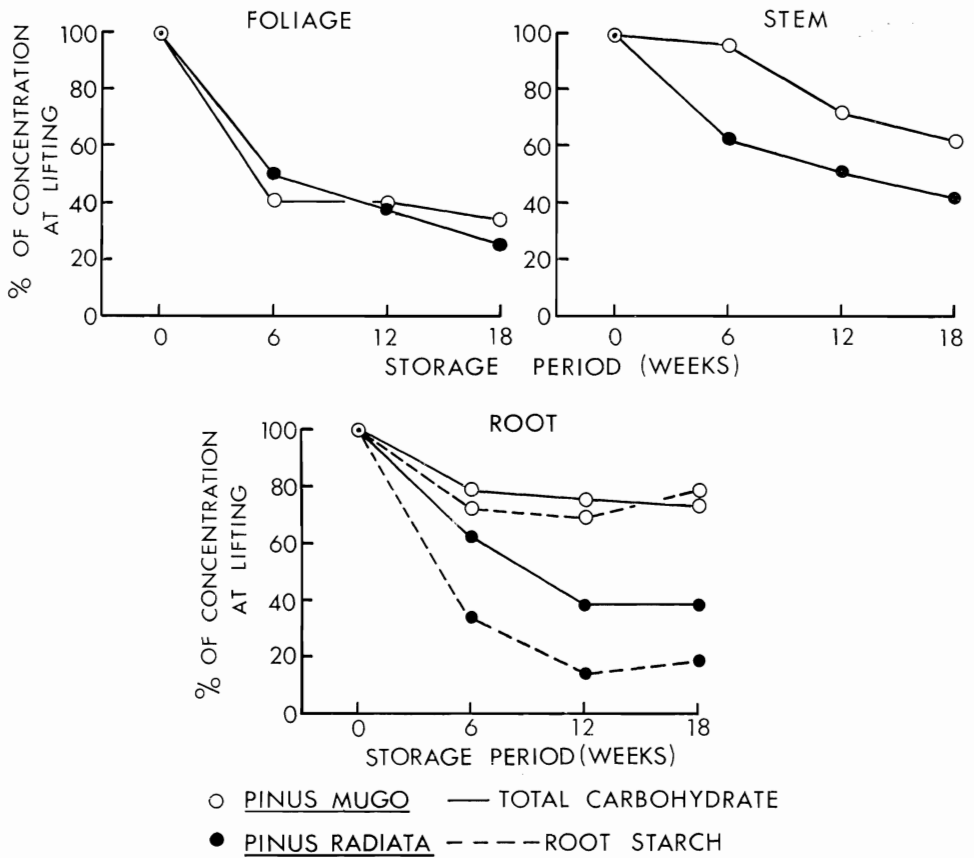


FIG. 1—Changes in the concentration of total carbohydrates and root starch in the tissues of *Pinus mugo* and *Pinus radiata* seedlings following cool storage for 0, 6, 12 and 18 weeks. Results expressed as % of the concentration at lifting.

stored seedling would depend. The remaining soluble carbohydrate fraction, the cyclitols, were not analysed and could comprise 10-20% of the available carbohydrate in seedlings of this age. With the exception of starch, reserve carbohydrates are indistinguishable from those involved in cell function and metabolism and constitute together the carbohydrate "resource" available to the seedling. Interconversions of carbohydrates are rapid and much more needs to be known about the function and fate of individual compounds before the resource can be split into fractions of metabolic "reserve" or "active" carbohydrate.

These results show that there was a marked decrease in the carbohydrate content of *P. mugo* and *P. radiata* seedlings during cool storage. A primary cause of this would have been respiratory consumption. The evidence suggests that the highest rate of consumption occurred immediately following lifting when depletion, especially in the foliage, was considerable in both cool-stored and potted seedlings. This common decrease in foliar concentrations following lifting could have occurred through stimulated respiration or translocation at the higher temperatures of the packing shed during grading

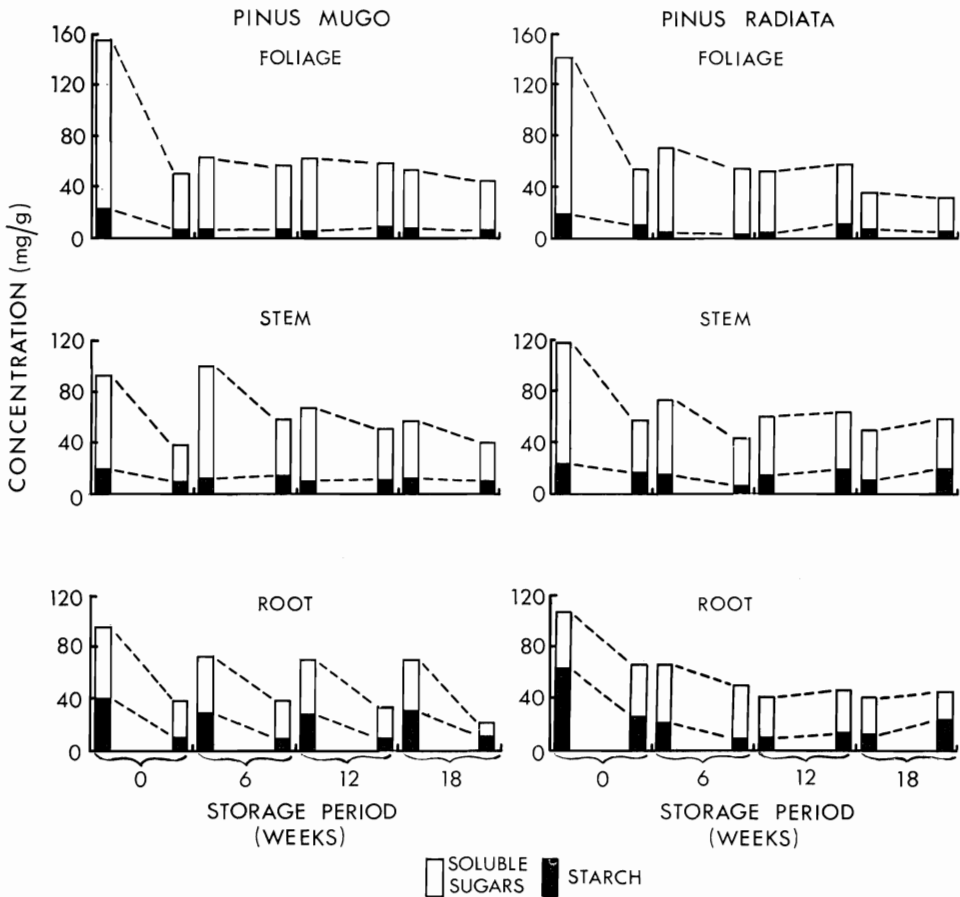


FIG. 2—Changes in the concentrations of starch and soluble sugars of *Pinus mugo* and *Pinus radiata* seedlings in the first 6 weeks following cool storage.

and packaging or potting. Also, seedlings sampled at the time of lifting were harvested directly from the nursery bed and immediately frozen in liquid nitrogen. High levels of soluble sugars were found in the foliage of these seedlings despite mid-winter temperatures. This effect has been frequently observed (Levitt, 1956, 1972; Parker, 1959, 1963; Aronsson *et al.* 1976) and associated with frost hardiness. The role of sugars and their concentration in frost hardy tissue is still imperfectly understood although it is obvious that they assist in preventing dehydration (Santarius, 1973; Aronsson *et al.* 1976). It is also possible that winter concentrations of carbohydrate in the foliage of conifer seedlings could build up through low levels of photosynthetic activity (Nielson *et al.* 1972) and the reduction of translocation at low ambient temperatures (Wardlaw, 1968).

The changes in concentration between 6 and 12, and 12 and 18 weeks' storage indicated that depletion of carbohydrate slowed with time. *Pinus mugo* was still able to retain a useful resource in the stem and root throughout storage, whereas *P. radiata* showed depletion in all tissues with very low concentrations remaining after 18 weeks' storage.

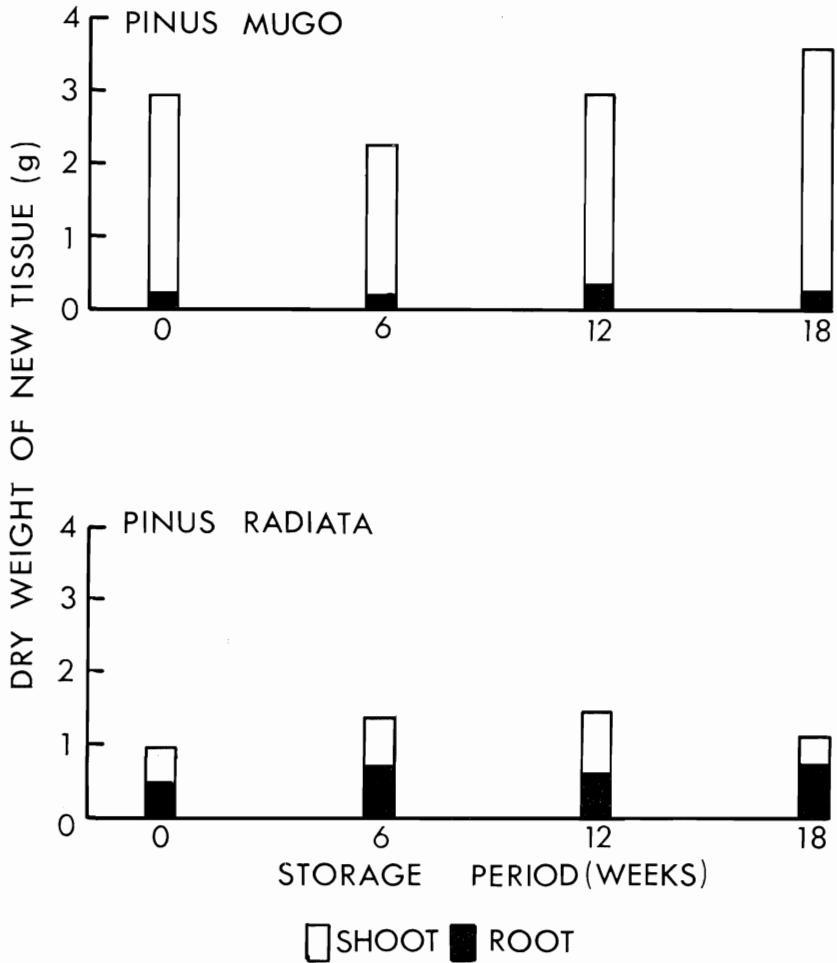


FIG. 3—The dry weight of new growth of *Pinus mugo* and *Pinus radiata* seedlings in the first 6 weeks following storage.

In the period of growth following storage the carbohydrate depletion in the stem and root of *P. mugo* occurred simultaneously with growth, especially of the shoot (Fig. 3). Photosynthetic efficiency following storage was reduced (McCracken, 1978) and it is unlikely that this would have contributed significantly to growth at this time. Clearly the carbohydrate retained by the stem and root during storage formed a vital resource for growth. The inference from Fig. 3 that growth was enhanced by cool storage arises through the earlier initiation of bud extension following longer cool storage periods allowing these treatments a longer period for shoot extension before harvest. Had time not been limiting it is probable that growth of seedlings from the longer storage periods would have been reduced through progressively lower post-storage carbohydrate concentrations.

In *P. radiata* the pattern of carbohydrate utilisation following 0 and 6 weeks' storage

was similar to *P. mugo* with growth funded largely from the stem and root resource. After 12 and 18 weeks' storage however further depletion of these resources did not occur during the growth period. Carbohydrate concentration actually increased slightly in the stem and root tissues during these periods although these increases are within the standard error estimates of the mean. Photosynthesis was very low at this time (McCracken, 1978) and probably insufficient for both growth and the increase in carbohydrate recorded. Alternative resources for respiration and growth could have been utilised, cyclitols, proteins, lipids or hemicelluloses being broken down for respiratory carbohydrate. Conversion of these resources would have been less efficient and more energy demanding for the seedling and could well have reduced subsequent vigour. In *P. radiata* this is illustrated in the fall in vigour of the 18-week-stored seedlings. *Pinus mugo* could behave similarly if storage were sufficiently prolonged.

The absence of a significant effect from wrenching is similar to that reported by Rook (1971) and demonstrates conclusively that carbohydrate concentration *per se* is not a feature of the improved survival and subsequent growth achieved by seedlings treated in this way.

These results support the general observation by Hellmers (1962) that the disappearance of starch from the main stem could mark the point of decline in post-planting vigour, especially in *P. radiata*. This species does not appear to maintain a large resource in relation to its demand and as a consequence does not store well over long periods. Maximum storage period would be 12 weeks but to allow for contingencies of bulk packaging and storage temperature a more realistic limit would be six weeks, i.e., when starch reached low levels in the stem.

In contrast *P. mugo* maintained an effective carbohydrate resource for support of new growth through 18 weeks of cool storage. This species is capable of surviving longer periods in storage than those set in this experiment although it is possible that growth in the season following storage would be reduced.

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