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SEASONAL CHANGES IN THE AMOUNT OF CARBOHYDRATES AND PHOTOSYNTHETIC ACTIVITY OF *PINUS PUMILA* REGEL ON ALPINE IN CENTRAL JAPAN

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Abstract: Branches and needles of *Pinus pumila* REGEL were collected from a site in the Yatsugatake mountain range in central Japan They were divided into current, 1-year-old, 2-year-old and 3-year-old parts Then, soluble carbohydrate (total sugars and starch) included in each of them was analyzed by SOMOGYI-NELSON method Photosynthetic rate of needles was measured by an infrared CO_2 gas analyzer Total sugar contents of branches and needles showed high levels in the winter season, but starch content levels were low In the younger part, total sugar content showed a higher level than in the older part Net photosynthetic rate of needles was the highest in summer, and was slightly negative in winter

1. Introduction

In central Japan, the greater part of the alpine region above the forest limit is dominated by *Pinus pumila* scrub except for some volcanoes. This dwarf pine is an evergreen conifer whose branches elongate one node every year (OKITSU, 1988). We can distinguish the age of branches on account of traces of their elongation.

In woody species, there are no specific organs such as in perennial herbaceous species for storing soluble carbohydrates produced during the growing season It is suggested that in woody species, the trunk, branch, root and needle play a role of a storage organ (KIMURA, 1969). It can be supposed that recent branches and needles of *Pinus* play this role of storage organ. The mean longevity of coniferous needles has been reported for *Abies veitchii*, *A mariesii* (KIMURA, 1963; KIMURA *et al* , 1968) and *Tsuga diversifolia* (KIMURA, 1963) According to these reports, the mean longevity was 4.4 years in *A veitchii* and 6 1 years in *A mariesii* The longevity of needles of *Pinus pumila* has also been reported (KAJIMOTO, 1989) The mean value ranged from 3 to 5 years It seemed that this difference among these needles and branches (3 to 5 years) was related to their function as a storage organ

With regard to net photosynthesis in coniferous species, some studies have been conducted One is about deciduous needles, *Larix decidua* (BENECKE *et al* , 1981), and others about evergreen needles, *Pinus radiata* (BENECKE, 1980), *Pinus montana* (HÄSLER, 1982) and *Pinus pumila* (KAJIMOTO, 1990). It is suggested that evergreen needles commence photosynthetic activity earlier and cease it later than deciduous needles, and net photosynthesis of evergreen needles is lower than that of deciduous needles. This lower rate of net photosynthesis is compensated for by a longer photosynthetic period than in deciduous needles (TRANQUILLINI, 1979). It can be supposed that evergreen needles of *Pinus pumila* commence photosynthetic activity soon after the thaw. However, there are few reports on the photosynthesis of evergreen needles at the timberline all through the year. Thus, more study of *Pinus pumila* in relation to its photosynthetic activity is required to understand the existence of a *Pinus pumila* belt at the timberline.

The aim of this study is to clarify the role of branches and needles as storage organ, and to clarify the advantage in evergreen needles under extreme environment by measurement of photosynthetic activity through out the year.

2. Materials and Methods

2.1. Sample collection

The study site is located on the Yatsugatake mountain range in central Japan. This mountain range consists of composite volcano, and is mainly composed of two ranges, north and south ranges. The study site, at about 2400 m a. s. l., is set on the north range. Dwarf woody species such as *Pinus pumila* and *Rhododendron brachycarpum* dominate there, and below this zone, *Abies veitchii, A. mariesii, Tsuga diversifolia* dominate.

Samples were collected a total of eight times over a 14 month period from May 1989 to June 1990. The samples consisted of branches and needles, which were divided into four age groups: current year, 1-year-old, 2-year-old, and 3-year-old. For the measurement of photosynthesis, samples were taken from branches both below and above the snows in the winter. After collection at the study site, the samples were taken the same day to the laboratory at Shizuoka University for analysis.

2.2. Measurement of net photosynthetic rate

In the morning after collection, the photosynthetic rates of the needles were measured with an open gas-exchange system using an infrared CO_2 gas analyzer (Fuji Electric Co., Ltd.). Current to 3-year-old needles were used as samples. Before measurement they were treated in a growth box which was kept at 15°C and about 20 klx. The open air was fed into a temperature and relative humidity control unit. Then conditioned air (15°C, 80% humidity) was transported to an assimilation chamber (Koito Industries Ltd.). The samples were set in the chamber and their cut ends immersed in a small plastic box filled with water. Metal halide lamps (Mitsubishi Electric Co., Ltd.) were used for illumination and light intensity was measured in lux with a selenium photometer (Toshiba).

2.3. Measurement of content of carbohydrate

Branches and needles were dried in a convection oven (Tabai Espec Corp.) at 80°C for 48 hours. Five samples per each age were used for this measurement. Each dried sample was ground to fine powder with a mill (Yoshida seisakusho). The powder was boiled in 80% ethanol for 3 hours. This solution was filtered through Millipore filter, and the extract was evaporated. The water-soluble fraction of this was hydrolyzed for 3 hours with 0.2% hot HCl and was used for the determination of total sugar. The filtered residue was extracted with hot water for 3 hours, then filtered again. And the extract was hydrolyzed again. This hydrolyzed extract was used for the determination of starch Then, content of soluble carbohydrate (total sugar, starch) was measured by modified SOMOGYI-NELSON method (MASUZAWA, 1977).

3. Results and Discussion

3.1. Photosynthesis

The rate of net photosynthesis of the needles on 3 March is shown in Table 1. From late November to May, the site was covered with much snow. *Pinus pumila* scrub was almost covered with snow. In the early winter, snow accumulates on the bush As the depth of snow increases, the accumulated snow becomes compact and crusty as a result of wind action (OKITSU and ITO, 1984). In the needles attached to the branches above the snow, net photosynthesis showed negative values. Similarly, the rate of net photosynthesis for needles collected below the snow was also negative. However, under the high light condition (10 to 30 klx) the net photosynthetic rate of needles below the snow showed a slightly higher level than that of needles above the snow. In other evergreen woody species, winter dormancy of photosynthesis has been reported (TRANQUILLINI, 1979). This dormancy is not solely induced by cold temperature. In this measurement, similar dormancy was recognized

Table 2 shows the net photosynthesis of needles in early spring season (22 May). The snow around the study site thawed in late May of 1989. After the thaw, branches of *P. pumila* were exposed again. The photosynthetic rate of needles above the snow

Table 1 Net photosynthesis of needles (current to 3-year-old) above and below snow measured on 3 March.

Illuminance (klx)	Net photosynthesis ($mgCO_2\ g^{-1}$ d wt h^{-1})	
	Needles above snow	Needles below snow
0	−0 98	−1 13
4	−0 80	−0 93
11	−0 64	−0 06
30	−0 38	−0 09

Table 2 Net photosynthesis of needles (current to 3-year-old) above and below snow measured on 22 May

Illuminance (klx)	Net photosynthesis ($mgCO_2\ g^{-1}$ d wt $\cdot h^{-1}$)	
	Needles above snow	Neddles below snow
0	−0 36	−0 54
1 6	−0 24	−0 08
12	+0 33	+0 42
22	+0 79	+0 60
38	+1 45	+1 22

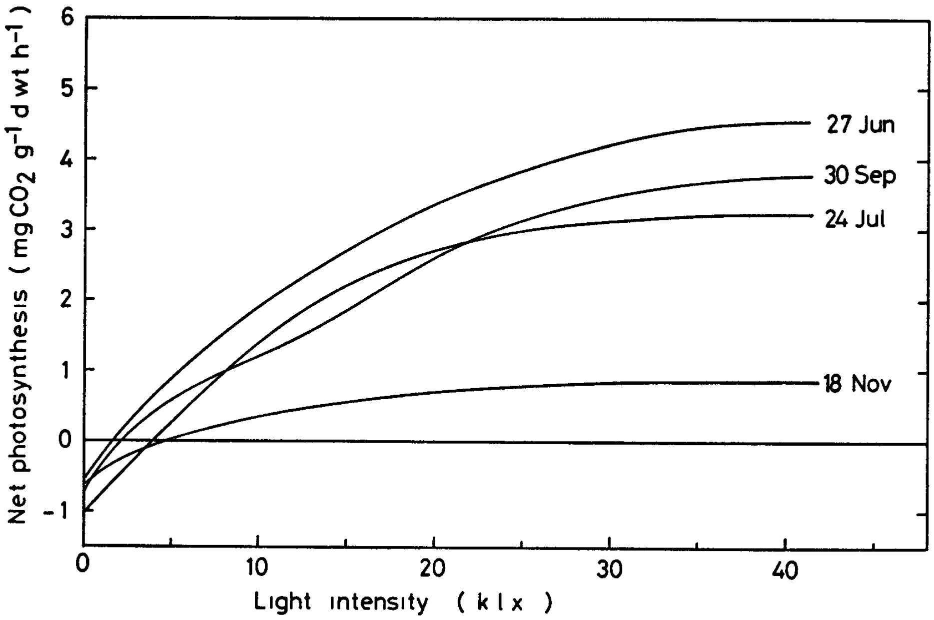

Fig. 1. Net photosynthesis of needles (current to 3-year-old) of Pinus pumila at 15°C.

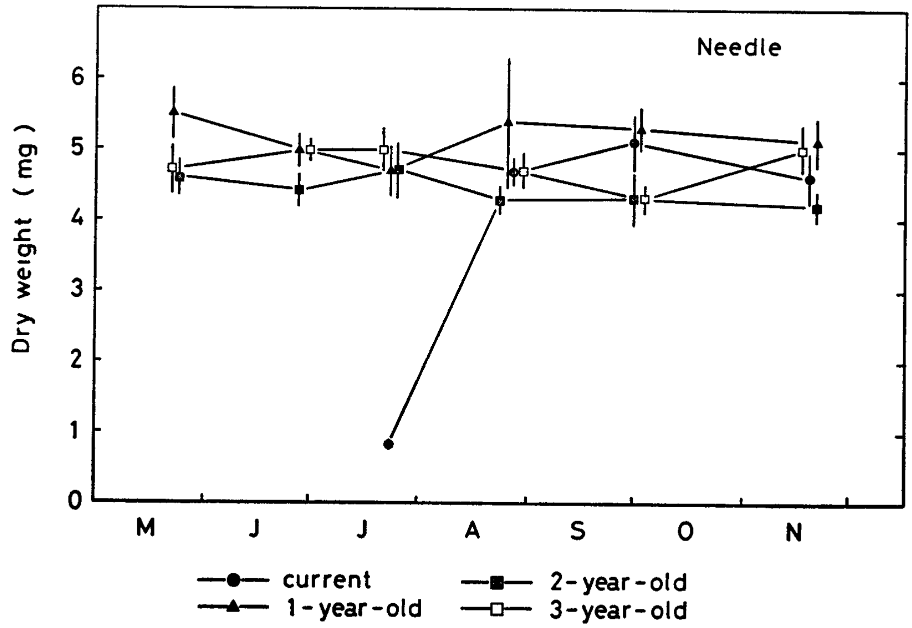

Fig 2 Seasonal change in dry weight of a single needle leaf. Vertical line represents one standard error of the mean ($n \geq 17$).

shows a lower level under low light intensity (0 to 1.6 klx). On the other hand, that of needles below the snow shows a relatively high level. Under high light intensity (12 to 38 klx) the rate of needles above the snow shows a higher level, whereas that of needles below the snow shows a lower level.

Figure 1 shows the seasonal change in net photosynthesis of the needles. The rate attained its highest level at a light saturation point of about 4.5 $mgCO_2 \cdot g^{-1}$ d.

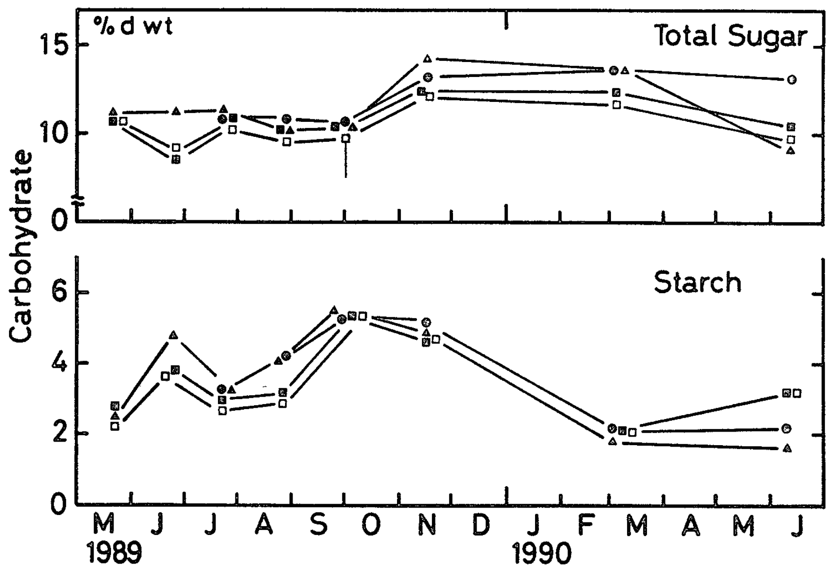

Fig 3 Seasonal change in the amount of carbohydrate (as % d wt.) of branches. Symbols are the same as Fig 2. Vertical line represents one standard error of the mean (n=3)

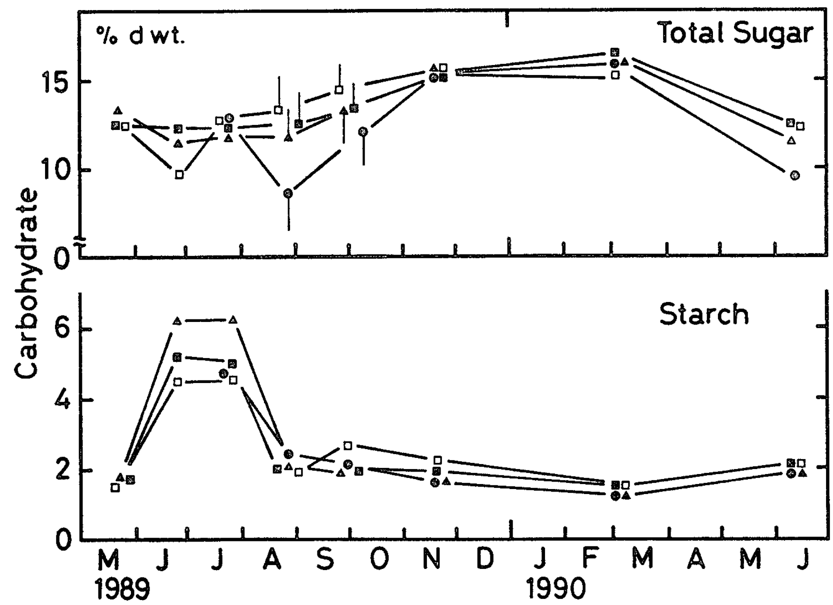

Fig 4. Seasonal change in the amount of carbohydrate (as % d wt) of needles. Symbols are the same as Fig 2. Vertical line represents one standard error of the mean (n=3).

wt.·h^{-1} on 27 June. From early summer to early fall, the photosynthetic rate at the light saturation point maintained 3 to 4.5 mg. In late fall (Nov.), *P. pumila* scrub was exposed to cold wind, so the photosynthetic rate showed a very low level less than 1 mg. It can be supposed that needles of *P. pumila* at this study site begin photosynthesis as soon as the light condition becomes good in the spring, and maintain a high level of photosynthetic activity till fall. However, in winter, under a low light condition due to packing of snow, the activity of snowpacked needles lowers till next spring, whereas the activity of exposed needles was at a lower level because of exposure to the wind.

3.2. *Carbohydrate content*

Figure 2 shows the seasonal change in the dry weight of each age of needle. Current needles elongated in one month from late July to late August, and attained to 5 mg in September. Except for current needles, the weight of a single needle was nearly constant (about 5 mg) all through the growing season with slight fluctuation.

Figure 3 shows seasonal changes in the amount of carbohydrates in the branches. Total sugar content was lower in summer than in winter. The highest level of 14.3% d. wt. was found in 1-year-old branches in November. The lowest level was 8.8% d. wt. of 2-year-old branches in June. Starch content of branches maintained low levels in the spring, then increased temporarily toward June, and then increased toward September. In fall, the content showed the highest level of 5.4% d. wt., then the rate decreased toward mid-winter.

Figure 4 shows the seasonal change in the amount of carbohydrates in the needles. Total sugar content was low in summer and then increased toward winter. The highest level was 16.5% d. wt. in March. The rate decreased toward early spring. In early growing season, it reached almost the same level as in the year before. Starch content of needles was at a high level during summer (from June to August). Except the summer season, the level was nearly constant, about 2%.

In the summer, the starch content showed high levels, and the total sugar content was low (Figs. 3 and 4). The peak of starch content was from June to July in the needles, and was from September to November in the branches. On the contrary, in the winter season, the total sugar content maintained a high level, and starch content declined till the next spring (Figs. 3 and 4). It can be supposed that carbohydrate gained as starch by photosynthesis in summer was transported from needles to branches till fall, and transformed to sugar in the late growing season, and restored in branches and needles as sugar in the winter season. These results suggest the role of branches and needles as storage organs. The high level of total sugar in winter may be related to cold tolerance.

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