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Changes in starch distribution within an embryonic shoot of Norway spruce [*Picea abies* (L.) Karst.] before resumption of mitotic activity

Abstract: The above results supplemented earlier research on the structure and development of Norway spruce embryonic shoots and focuses on changes in starch distribution in winter and early spring. Starch accumulation and mobilization is characteristic for cells that play an important role in morphogenesis. The observed starch distribution within an embryonic shoot suggests that starch indicates the places of future mitotic and morphogenetic activities of the developing shoot. Changes of the rate of starch accumulation in a bud are affected by temperature. This study showed that especially important are the course of temperature fluctuations.

Additional key words: temperature, PAS and Feulgen reaction

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Introduction

Starch is the main storage compound in higher plants. Changes in its content are observed during such processes as photosynthesis, accumulation of storage compounds, generative reproduction and seed ripening. Changes in starch content have been recorded at different time intervals, from hourly and daily in leaves to seasonal changes in other organs (Chaumont et al. 1994). Seasonal changes in starch content are also observed in perennial plants, such as trees. Essiamah and Eschrich (1985) distinguished four phases in the annual starch cycle: accumulation in autumn, dissolution during the dormancy, resynthesis at the end of dormancy, and dissolution during bud break. Starch reserves are related to changes in the activity of organs or tissues (Smith et al. 1992).

This study focused on changes in starch distribution in the embryonic shoot of Norway spruce bud in winter and early spring.

Materials and methods

Material originated from grafts of *Picea abies* clone 04–118 (Serwy) grown on a second generation seed orchard (established in 1981) in the 'Zwierzyniec' Experimental Forest near Kórnik (52°15'N, 17°04'E). Buds were collected every week from January till May in 1997 and in 1998, from the middle part of the graft crown. Embryonic shoots, isolated from the buds, were fixed in a chromium-acetate solution (CrAF) and embedded in paraffin. Sections, 9 μm thick made by a rotary microtome, were treated with Schift's reagent for PAS reaction (Berlyn and Miksche 1976, modified by Hejnowicz 1982), starch grains stained red, or for Feulgen reaction (Gerlach 1972, modified by Hejnowicz 1982), for observations of mitoses. The observations were made under a light microscope.

Results

Sesonal changes of starch distribution within the shoot apex and leaf primordia were studied. Preliminary observations carried out in November and December 1997 showed that starch was abundant in the autumnal embryonic shoot. In late December it was absent. Again it was detected in buds fixed on February the 3rd, in basal parts of bud scales located at the base of the embryonic shoot and in the pith. In the shoot apical meristem and in needle primordia starch was still undetectable (Fig. 1). About two weeks later, starch was detectable in all parts of the embryonic shoot, including procambium and the apex (Fig. 2).

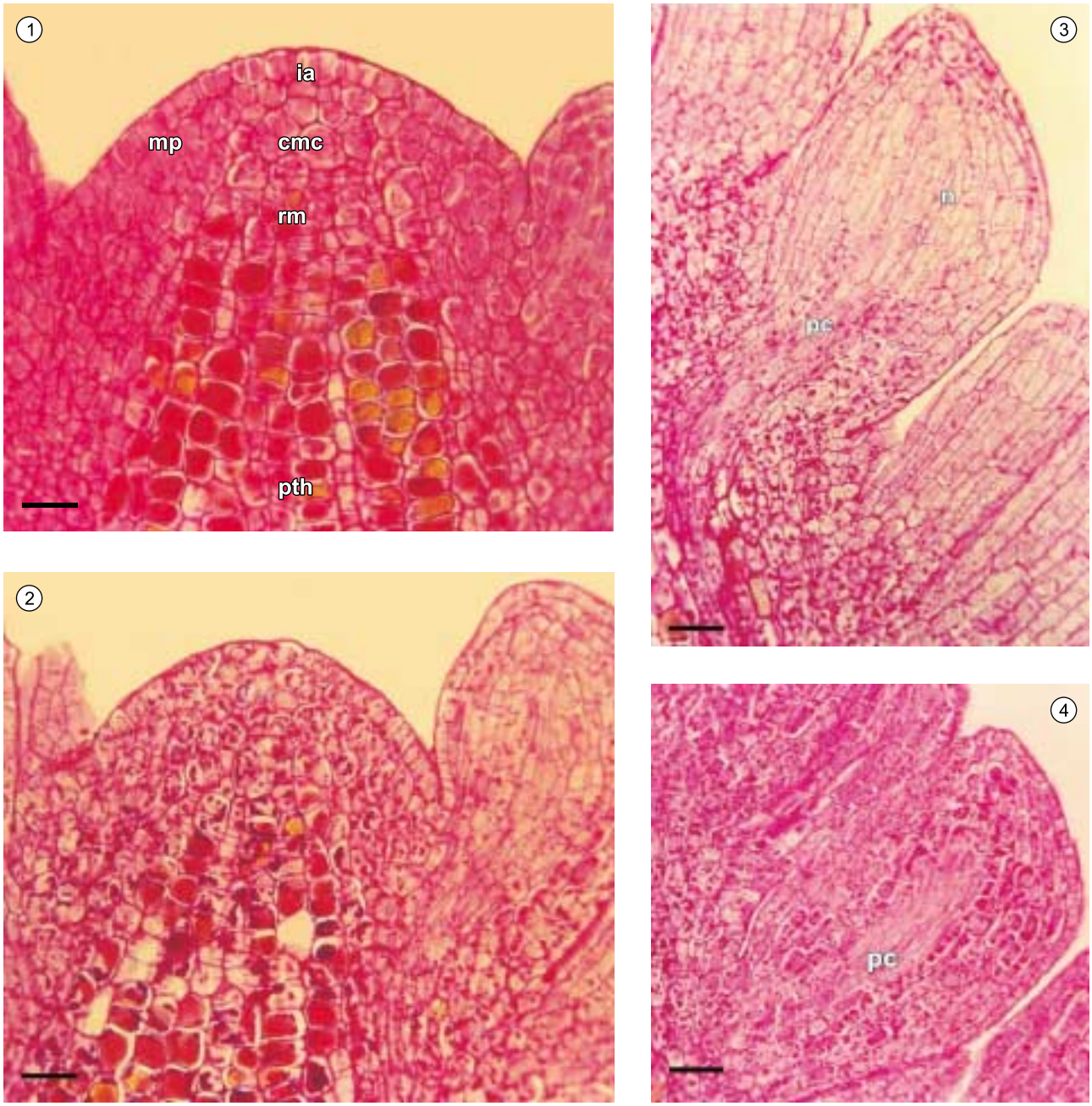
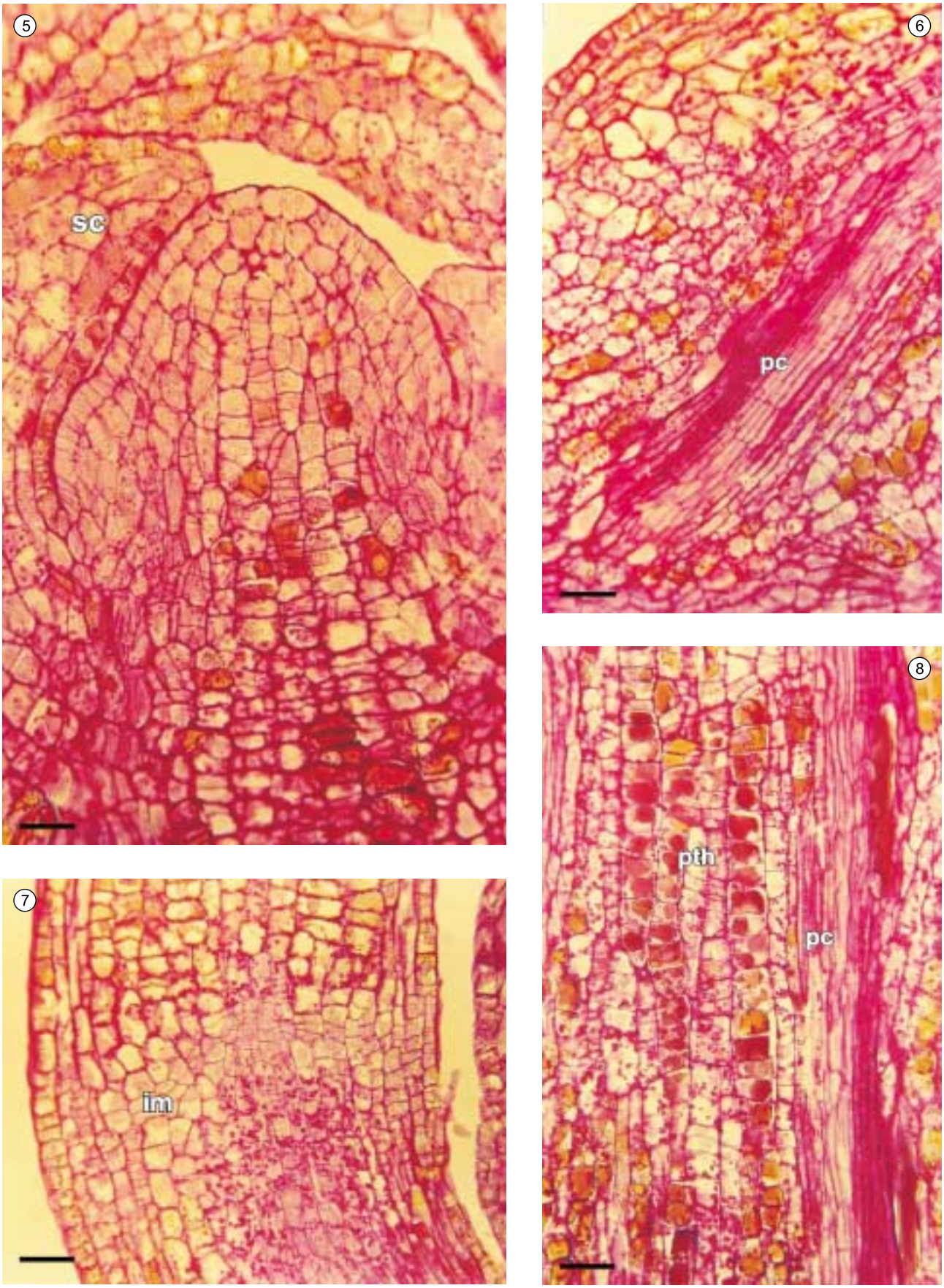


Fig. 1. Apex of an embryonic shoot (material collected 23.01.97), PAS reaction – in January starch was still undetectable. Bar = 0.03 mm; mp – peripheral meristem, ia – apical initials, cmc – central mother cells, rm – rib meristem, pth – young pith

Fig. 2. Apex of an embryonic shoot (10.02), PAS reaction – in mid-February starch was detectable in all parts of the embryonic shoot, including the apex. Bar = 0.04 mm

Fig. 3. Young needles (10.02), PAS reaction – in the needles starch distribution spread in the acropetal direction; starch was visible in the basal part of needles. Bar = 0.04 mm; n – young needle, pc – procambium

Fig. 4. Young needle (3.03), PAS reaction – starch was still present in needles but the procambium contained less starch. Bar = 0.045 mm; pc – procambium



Figs. 5–8. PAS reaction – in April starch was not visible in the apex (fig. 5), in the procambium of needle (pc – fig. 6) and the procambium of axial part of embryonic shoot (pc – fig. 8) and in the cells of intercalary meristem (im – fig. 7). Starch was abundant in the adjacent cells.
pth – young pith, sc – scale; Bar = 0.03 mm – figs. 5, 6, 8; = 0.04 mm – fig. 7

In the needles starch distribution spread in the acropetal direction, i.e. from the base (Fig. 3) to the apical part. In March starch amount decreased and it was less abundant than in late February. It was still present in the apical meristem and in basal parts of the needle primordia, while the procambium contained less starch (Fig. 4). In mid-March (11.03) starch was undetectable in the peripheral meristem of the apex. In April it was not visible in the procambium (Figs. 6 and 8) and in the shoot apex (Fig. 5), but it still was seen in the pith cells and in the basal and apical parts of young needles. In the cells of intercalary meristem, which could be distinguished in that period at the needle base, starch was absent, but it was abundant in the adjacent cells (Fig. 7).

In 1998 changes in starch distribution in the embryonic shoot were similar. Starch appeared on February the 13th, 10 days later than in 1997 and was visible in basal parts of bud scales, in the pith and in needle primordia. It was not detected in the apical meristem. In late February starch was visible in all parts of the embryonic shoot. In early March, as in 1997, starch decreased, and in mid-March it was not detectable in the procambium and in the peripheral meristem. In April starch was present only in pith cells and in young needles. It was not observed in the apical meristem and procambium of the embryonic shoot, nor in the intercalary meristem of needles.

The first divisions occurred in the youngest leaf primordia. In 1997 they were observed on March the 3rd. A week later mitoses were observed in all young needles, in the procambium of the axial part of the embryonic shoot, and in the peripheral meristem of the apex. In 1998 the first cell divisions were recorded on March the 13th, and a week later in young needles. In the whole embryonic shoot mitoses were noticed as late as on 26th March.

Discussion

The above presented results supplemented earlier research on the structure and development of Norway spruce buds (Hejnowicz and Obarska 1995) and focused on changes in starch distribution in the embryonic shoot in winter and early spring. In January the PAS reaction did not detect starch in the spruce embryonic shoot. This is consistent with results of earlier studies on pine (Hejnowicz 1982) and spruce buds (Hejnowicz and Obarska 1995). In these works, changes in starch distribution within shoot primordia in the period preceding the resumption of mitotic activity were not analysed, and the presence of starch was regarded as a symptom of the onset of bud development. However, observations under a transmission electron microscope revealed that in January in some cells of the embryonic shoot small starch grains were present in the plastids, even in the cells of the apical meristem (Guzicka and Woźny, in press).

It seems that starch accumulation and mobilization is characteristic of cells that play an important role in morphogenesis. Starch may perform the function of an 'energy bank' in the energy-consuming processes of development (for example Stamp 1987). The important role of starch during organogenesis has been confirmed by *in vitro* studies. For example, callus of *Nicotiana tabacum* does not produce shoot primordia in media containing gibberellin, which prevents the accumulation of large amounts of starch (Thopor et al. 1986). Research on *Humulus lupulus* has shown that explants cultured on media containing auxin and cytokinin intensively accumulate starch and produce shoot primordia. In the control without growth regulators starch also accumulated but at a lower concentration and no shoot primordia have been developed (Fortes and Pais 2000). The authors suggest that processes associated with mitotic activity are preceded by a period of high starch accumulation. High amounts of starch have also been observed during somatic embryogenesis in cassava (Stamp 1987). In the embryonic shoot of Norway spruce starch accumulation preceded spring bud development and cell divisions. At first starch appeared in the parts of the embryonic shoot with future mitotic activity; procambium and peripheral meristem. After the mitotic activity was started, starch in that part disappeared, while it was abundant in adjacent cells. In spruce buds starch was detected about a month before the first mitotic divisions were observed, two months before the size of the embryonic shoot started to increase, and three months before bud break takes pleace in late April. Lack of starch detectable under a light microscope in December and January seems to indicate, as suggested earlier (Guzicka 1999), that the period of deep dormancy of the bud is relatively short, lasting only several weeks. Changes of the rate of starch accumulation in a bud are certainly affected by ambient temperature. Sarvas (1967) suggests that bud growth resumed when the required "heat sum" is achieved. Cannell (1985) concluded that most important is the number of chilly days during winter and spring, or the number of days with a mean air temperature >5°C (Hejnowicz 1982). A comparison of data on air temperature in 1997 and 1998 (Fig. 9) showed that mean temperatures are less important than the course of their fluctuations. Although January 1998 was generally much warmer (accumulated temperature: 58.9°C) than January 1997 (accumulated temperature: –3.8°C), in 1997 starch accumulation started earlier than in 1998. The slower starch accumulation in 1998 could have been caused by a deep decrease in temperature in first day of February (daily –9.2°C; daily minimum –13.2°C). In 1997 the first

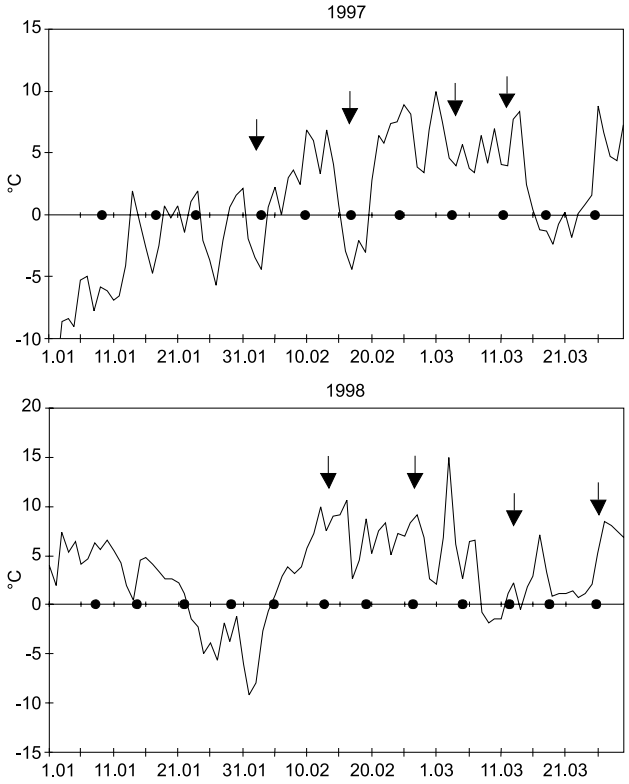


Fig. 9. Mean temperature of January–March 1997 and 1998:
• – time of bud collection;
↓ – starch appeared, maximal starch accumulation, first mitoses, mitoses in the whole embryonic shoot, respectively

mitoses were observed earlier than in 1998. A week after the first mitotic divisions, they were observed in all parts of the embryonic shoot. In 1998 mitoses were observed in all parts of the embryonic shoot two weeks after the first mitoses. The slower spread of mitotic activity in 1998 could have been caused by low temperature between the 9th and 16th of March (Fig. 9). The observed changes in starch distribution within the embryonic shoot during the winter and early spring (Fig. 10) suggest that starch indicates the places of future high mitotic and morphogenetic activities, of the developping shoot.

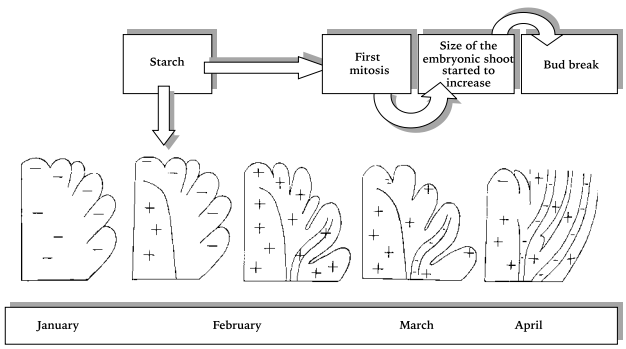


Fig. 10. Sequence of facts in spruce bud during winter and spring: [+] starch was visible; [–] starch was not detected

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