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Mitotic Index and Influence of Environmental Conditions in the Megagametophyte of *Picea abies*

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S u m m a r y

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From May 15 to June 25, 1995 megagametophytes (= female gametophyte, endosperm tissue in development) of spruce trees were collected. 10 preparations of each day were analyzed semi-automatically with regard to their mitotic figures using digital image analysis. All environmental data (temperature, humidity, radiation, atmospheric pressure, rainfall) were measured during this period with a weather station. The data were collected every 5 seconds. From these data the averages of a half-hour were calculated.

Number of nuclei of one megagametophyte increased from an average of 5000 at the beginning in May to 60,000 at the end of the experiment in June. Correlations were found between temperature, atmospheric pressure and mitotic index. The other parameters showed no connection to the mitotic index. The mitotic index of the megagametophytes were also examined with regard to their location in the cone (top – middle – base). All regions of the cone develop simultaneously – number of nuclei in division showed no significant differences with regard to the position of the cone from which they where collected.

Introduction

The megagametophyte of spruce trees is a very important material for cytogenetic studies (see e.g. SAX & SAX 1933, PEDERICK 1967, 1970, BORZAN 1977, 1981). Although literature on embryology in conifers is comprehensive and there are very detailed morphological descriptions of the development of the seed

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from some of the species (MAHESHWARI & SINGH 1967, SINGH 1978, HAKMAN & al. 1986), we did not find any information concerning the effect of environmental factors on cone development. Since only one study was done regarding that problem (KÖHLER & al. 1995), we considered it important to investigate female gametophytes of Norway spruce (*Picea abies* (L.) Karst.) with particular attention directed to the relation between several environmental conditions and the maturation process of the megagametophytes.

Materials and Methods

From May 11, 1995 to June 29, 1995 a weather station (Kroneis Inc.) recorded meteorological data at the Botanical Garden of the University of Graz. Meteorological data were recorded automatically every 5 sec, means of 30 min were stored.

A spruce (*Picea abies* (L.) Karst.) of 10 m in height, growing by the roadside of a rarely used street about 1 km off the weather station was chosen for this purpose. From May 15 to May 23, 1995 female cones of this tree were collected every two days, from May 24 to June 25, 1995 one cone was gathered daily at 9.00 a.m.. Collected cones were immediately dissected.

Ten megagametophytes were extracted daily from the gathered cone. In order to detect if there are differences in the stage of development megagametophytes from the proximal, the distal and the intermediate region of the cone were distinguished. In the course of 37 experimental days 370 megagametophytes were prepared for light microscope observation to follow the development of the female gametophyte.

Slides were fixed and prepared by Feulgen squash method according to DARLINGTON & LA COUR 1962, BORZAN 1981 and MÜLLER & al. 1991 with some modifications. At the earlier collection dates whole ovules had to be fixed, from 29 May intact megagametophytes could be separated from the surrounding integument before fixation.

Subsequently the total number of nuclei was determined using digital image analysis and horizontal scanning technique. Means were calculated from the numbers of prophases, metaphases, anaphases, telophases, mitotic figures and mitotic indices each experimental day and connected with meteorological data.

Results and Discussion

From May 15 till May 28 the gametophytes were very small and therefore the preparation was very difficult. Also the staining was very poor. From May 21 to June 21 the size of the gametophytes increased and the specimen were easy to prepare and showed a good staining. After June 21 we got worse material: the gametophytes had grubs, they were often flabby and brownish. Therefore we stopped the investigation June 23.

During the whole period the number of nuclei was increasing ($R = 0.77$, $P < 0.0001$). But the ascend was not exponential - as expected - it was linear. Fig. 1 shows the reason why: After May 28 a cold period began and the dividing of cells was stopped. Around June 8, the temperature increased and the dividing of cells started again. We think that first the light induced readiness for cell-dividing must exist. After this, a temperature regulated dividing of the cells occurs.

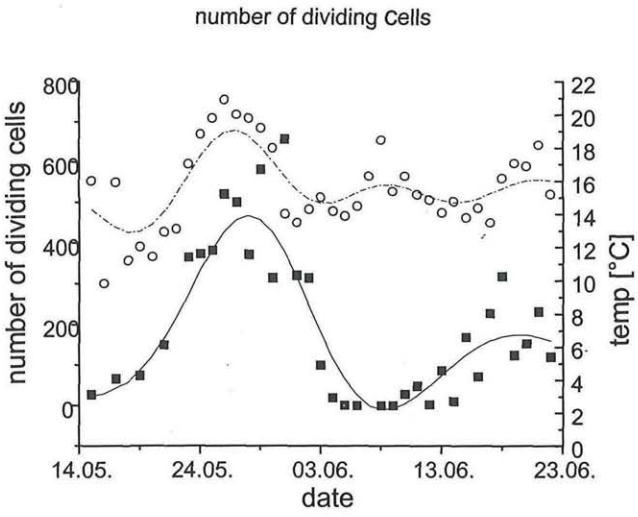

Fig. 1. Number of dividing cells versus temperature. Filled squares and line: number of dividing cells; open circels and dashed line: temperature. Fast Fourier Transform (FFT) smoothing (5 points considered for smoothing).

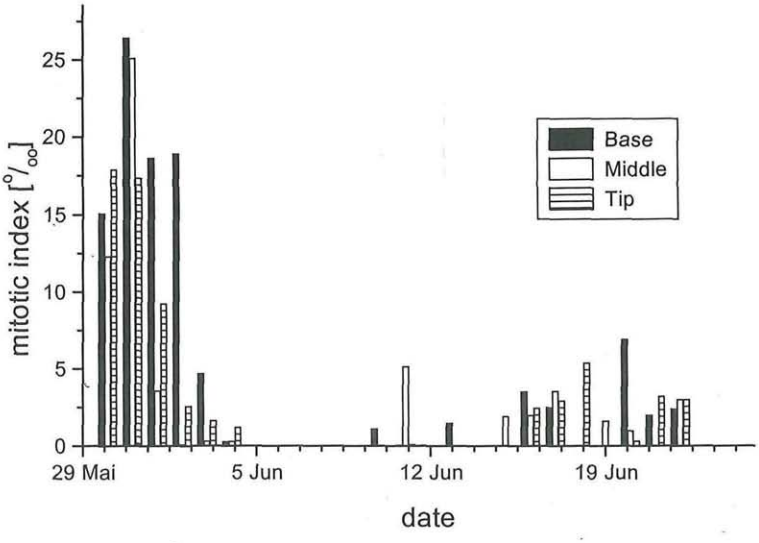

Fig. 2. Mitotic indices [‰] of the macrogametophyte within one cone; differences of preparations between base, middle tip.

The mitotic indices of gametophytes of different positions within one cone (top, middle, base) showed no correlation (see Fig. 2). The differences were not significant and less as the differences between different cones. The number of dividing cells in the megagametophyte within one cone is more similar than between those of different cones.

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