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Volume changes in the pollen grain of *Corylus avellana* L. (Corylaceae) during development

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Pollen development in 8-year-old hazel plants (*Corylus avellana* L.) from Central Italy was monitored in order to investigate the chilling completion date of the catkins. Meiotic division occurred in September and pollen development continued until mid-October when the first haploid mitosis divisions were observed. The catkins elongated gradually during the summer until mid-October when their growth slowed. In mid-December the catkins resumed rapid growth and released pollen. From the end of October the pollen grain volume decreased by 12% on average, reaching its minimum value on 6th November. From this time onwards volume increased again to reach its maximum value at the beginning of December, just before pollination. In mid-October the daily mean temperature dropped below 12.4°C and remained at this low level during the succeeding period; a relatively high mean temperature, above 4.5°C, was recorded in the second week of December. The beginning of dormancy could be correlated with the slowing down of catkin growth and the decrease in the volume of the pollen grains after the first haploid mitosis. When the chilling requirement is fulfilled, at about 640 chill units, the pollen grain volume reaches its maximum value.

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The flower buds of many deciduous trees are formed during the previous vegetative growing season and the differentiation of the floral parts begins before the winter dormancy period (Felker et al. 1983).

In hazel, which flowers in winter, the male floral parts are differentiated at an early stage in the growing season and the male catkins are well developed when the leaf abscission occurs in autumn and they enter dormancy. In hazel the chilling requirement necessary for the resumption of growth ranges from 100 to 860 hours for the male catkin and from 290 to 1550 hours for the female flower, depending on the cultivar (Germain 1994). These values are generally calculated using greenhouse forcing of the fruiting shoots, but generally the dormancy stage is difficult to distinguish because there is no reliable physiological marker to identify this period (Faust 1989). Sometimes the meiotic stage of the mother cells has been used to mark the end of the rest period, as in the peach (Young & Houser 1980), but this cannot be used in all plants (Felker et al. 1983).

In an earlier paper (Frenguelli et al. 1992) focusing on spontaneous hazel trees, we determined the chilling requirement to break dormancy using the method suggested by Ashcroft et al. (1977). This requirement was statistically calculated using the pollination starting date and the temperature for a period of 7 years. However, the results in successive years were not as good as we had foreseen.

In this study we have tried to identify the beginning and the end of the dormancy period of hazel catkins by monitoring the development of the pollen grains.

MATERIALS AND METHODS

8-year-old hazel plants (*Corylus avellana* L., Corylaceae) were used in this investigation. The plants grow in an orchard along the Tiber river valley (Central Italy), 170 m above sea level.

At the beginning of the summer, 8 plants were chosen at random, on each plant 4 branches were marked, and on each branch the male inflorescences, with the young budding catkins, were identified. The length and diameter of the marked catkins was measured every week; parts of catkins with similar development were collected from the plant and the anthers were embedded in resin for observation under the microscope. Some anthers were squashed in a drop of Alexander's stain (Alexander 1969) and, after being warmed, examined under the microscope.

Some anthers from the middle of a catkin were opened and shaken onto a microscope slide prepared with immersion oil. The equatorial and polar axes of 100 developing pollen grains, chosen at random from each anther, were measured at 400× magnification, using a Leitz Dialux microscope equipped with an ocular micrometer. Their volume was calculated using the formula $\frac{4}{3}\pi a^2b$ where a is the equatorial axis/2 and b is the polar axis/2 given that the hazel pollen resembles an ellipsoid in projection (Pacini 1990). The pollen viability was tested by the fluorochromatic reaction test (Heslop-Harrison & Heslop-Harrison 1970).

Pollination was followed by means of a 7-day volumetric Hirst-type pollen trap (Lanzoni, Bologna, Italy) positioned at ground level at about 50 m from the plants. A description of the trap and the methods used are explained in an earlier paper (Frenguelli et al. 1981).

Meteorological data were collected by an automatic station which provided hourly temperature records. For chilling requirements we have used the model proposed by Richardson et al. (1974) which is based on the accumulation of chill-units (CU), where 1 chill-unit equals 1 hour exposure between 2.5 and 9.1°C, 0.5 chill-unit equals 1 hour exposure between 1.5 and 2.4°C or 9.2 and 12.4°C. The heat

accumulation to induce flowering after dormancy is expressed in growth degree hours (GDH), obtained by the summation of hourly temperatures above 4.5°C which represents the base temperature (Faust 1989).

RESULTS

From June until mid-October, the young catkins grew at about 0.15 mm/day, reaching a length of around 22 mm in mid-October (Fig. 1). From November to the beginning of December only slow catkin growth was recorded. In the second week of December the catkins resumed growth, initially very slowly, but after a few days the growth rate increased progressively until in January it was about 1 mm/day. The diameter of the catkin did not undergo any significant variation from October to December, after which it grew progressively until January (Fig. 1).

In August the pollen mother cells were vacuolated and wrapped in a thick callose layer. The first meiotic divisions occurred in mid-September and the tetrad stage, with the microspores enveloped in a thickened callosic wall, lasted throughout September when the callose was completely dissolved with the formation of the primary exine. The development of grains continued until mid-October when the first haploid mitosis occurred (Fig. 2).

Some of the pollen grains were viable, bigger, spherical, swollen and with evident pores and very large onci, while others, were non viable, smaller, sub-triangular and deflated. The volume of viable pollen was, on average, 2480 µm³ at the meiosis cleavage, and decreased by about 10% immediately after this stage, then increased progressively to reach 5100 µm³ when the pollen was binucleate (Fig. 2).

From mid-October the cytoplasmic material of the grains

gradually became more dense and the volume decreased, by 12% on average, reaching a mean value of 4500 µm³ on 6th November. From mid-November the volume increased again, slowly at first and more quickly at the beginning of December, reaching a maximum value of 6180 µm³ on 8th December and decreasing again by about 9% at anther opening. The first pollen grains were released into the atmosphere on 16th December.

The average volume of dead pollen grains remained unaltered until pollination, and never exceeded 3100 µm³. We estimated that 25% of all the pollen grains inside the anthers were dead.

The lengths of the equatorial and polar axes changed in different ways during pollen development. At first the polar axis grew more than the equatorial axis, so that the P/E ratio changed from 0.73 to 0.78 at the binucleate stage (Fig. 3). After this the polar axis decreased markedly and the P/E ratio changed from 0.78 to 0.67 at the beginning of November. When the volume increased again, the polar axis increased more than the equatorial axis, in this way the shape of the grain changed from oblate to oblate-spheroidal, reaching the value P/E=0.88 just before pollination (Fig. 3).

The mean temperature stayed at the same high level until the end of August, and then decreased. In mid-October the average temperature was below 12.4°C. A relatively high mean temperature, above 4.5°C, was recorded in the second week of December (Fig. 4). The chill-units, calculated from mid-October to the first week of December, reached 640 and the heat accumulated during the week before pollination was 350 GDH.

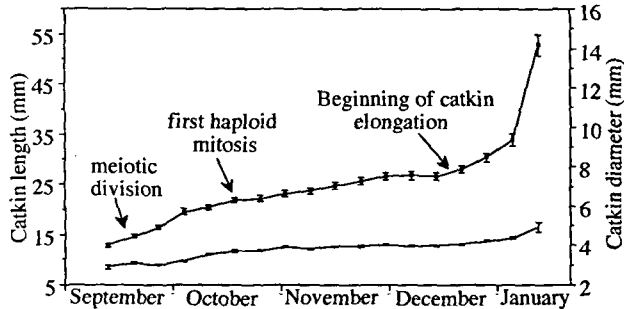


Fig. 1. Average catkin length (■) and diameter (solid line) during pollen development. Bars represent ± standard error.

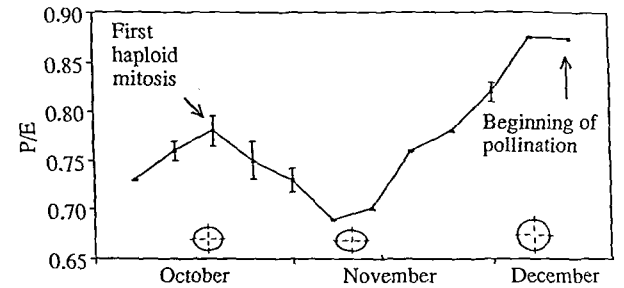


Fig. 3. Changes in the P/E ratio and the shape of the pollen grain in the course of development. Bars represent ± standard error.

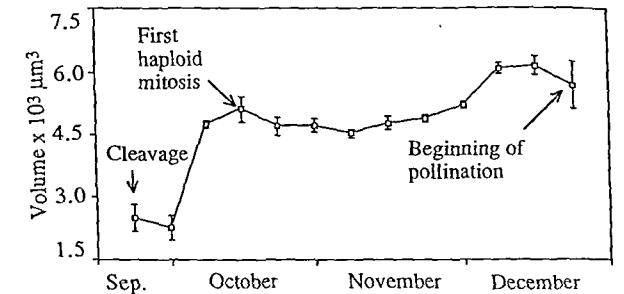


Fig. 2. Changes in microspore/pollen grain volume in the course of development. Bars represent ± standard error.

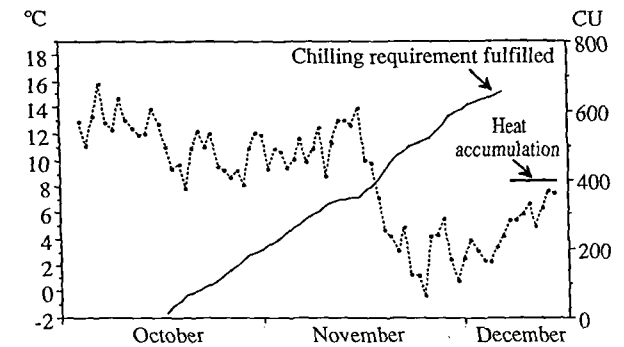


Fig. 4. Daily mean temperature (dots) during the period studied together with chilling accumulation (solid line).

DISCUSSION

The development of the male inflorescences and of the pollen grains in hazel is a very long process which begins 7–8 months before flowering, which occurs during winter after the chilling requirement to break dormancy is satisfied.

We found that catkin growth and pollen development reflects the dormancy period. Its beginning in autumn, when the mean temperature drops below 12.4°C, is characterized by the slowing down of catkin growth until a few days before pollination when the relatively warmer temperature (above 4.5°C) induces catkin axis elongation (Frenguelli et al. 1993).

A sign that the plant is entering dormancy might be a reduction in pollen size, immediately after the binucleate stage, which reaches its lowest value before mid-November. After this period pollen size increases again before undergoing a further reduction in volume just before anthesis: we can deduce that when the pollen grains reach their maximum size, the chilling requirement is fulfilled and dormancy is finished.

The relative reduction in pollen volume could be caused by dehydration, which the pollen undergoes inside the anther (Heslop-Harrison 1979). The trend of the P/E ratio suggests that the changes in volume and shape are operated by turgor changes and by an unevenly thickened intine wall. It seems that the grain is squashed at the poles where the intine is thinner, while at the equator, perhaps because of the thick onci, it shrinks. As far as the regulation of the size of pollen grains is concerned, in hazel an important role could be played by the three large onci (Heslop-Harrison et al. 1986) and by the plasma membrane which, in the viable pollen grains, is capable of regulating the flow of water coming in and out of the pollen (Bassani et al. 1994). Pollen grains, which are not viable, do not grow at all, neither do they show any significant variation in size while inside the anther. These grains seem to be the result of anomalies which occur during meiosis, which produce small, deformed and non-viable grains (Romisondo 1978).

When we examined the temperature during pollen development, our observations confirmed that from mid-October the mean temperature began to drop below the threshold of 12.4°C which, according to Richardson et al. (1974), indicates the beginning of the chilling necessary to break dormancy. At the beginning of December, when the pollen grains enlarged, 640 chill units had been reached; we assume this value indicates the chilling requirement to break dormancy.

Heat accumulation seems to have begun in the second week of December when the air temperature rose and for several days remained at a relatively high level, thus satisfying the heat requirement and initiating pollination in the second half of December.

We know that the microspores normally decrease in volume after meiotic cleavage, increase in volume during the exine and intine formation, decrease again during anther dehydration and undergo variations during dispersion in accordance with RU% (Pacini 1990). From our results it seems that a further volume variation should be taken into consideration during development including the dormancy period, at least in plants like hazel, which go through this period with their

inflorescences already developed, and pollen ready in the anther.

A reduction in pollen size during dormancy was observed in birch by Dunbar and Rowley (1984) who described the reduction of microspores diameters 13 days before dormancy. Variations in the equatorial diameter of the hazel pollen immediately after the first haploid mitosis were reported by Frenguelli et al. (1994) and confirmed in tests carried out in successive years. The same was seen in *Alnus* (alder), with time variations of some days, perhaps due to the temperature trend during development (Ferranti et al. 1996).

Therefore, variation in pollen volume in hazel could be used as a marker for determining the time of dormancy, its beginning and completion and for quantifying the chilling required to break rest. Ultrastructural investigations could give information on the role played by the different cellular components in the mechanism which determines this size variation during the chilling accumulation.

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