Tannin vacuoles and starch in the development of Scots pine (*Pinus silvestris*) vegetative buds

ALINA HEJNOWICZ

Institute of Dendrology, Polish Academy of Sciences, Kórnik near Poznań

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Abstract

Tannin cells occur throughout the bud except the distal and peripheral meristem zones of the apical meristem, and the youngest cataphyll primordia. Starch is absent in winter buds. The earliest structural manifestation of spring awakening in the bud are fragmentation of tannin vacuoles and synthesis of starch in the green cells of the bud.

The tannins occurring in the vacuoles are hydrolysable giving a positive reaction for sugars (PAS). During their spring hydrolysis glucose is released. It is probably one of the sources of sugars for the synthesis of starch.

During extension growth of the bud there occurs a degradation of tannin cells in the pith, which consist in the precipitation of tannins to a condensed form.

INTRODUCTION

During several years of studies on the development of vegetative buds of Scots pine (*Pinus silvestris* L.), the results of which will be presented in a separate publication, special note was taken of the part of the bud lying directly below the apical meristem and consisting of the stem part of the bud, sterile cataphylls and subtending scales as well as primordia of axillary buds. In these tissues there exist files of cells rich in tannin substances (Fig. 1). The nature of these cells changes during the vegetative season. It was necessary to establish what these changes depend on and what could be their association with other processes taking place in the bud.

Tannins are polyhydroxyphenolic compounds. They are usually divided into hydrolysable and condensed or non-hydrolysable tannins. After hydrolysis of the first group of tannins there form carbohydrates

(usually glucose) and phenolic acids. Condensed tannins do not undergo hydrolysis and they are made up of amorphous polymers (phlobaphenes — Haslam, 1966).

In plant cells tannins perform primarily protective functions. They are usually considered to be reserve substances, associated in an ill-defined manner with the metabolism and transport of sugars (Sperlich, 1939; Paech, 1950; Durzan, Chafe and Lopushanski, 1973). It is generally believed that accumulation of tannins in a plant cell is a manifestation of its maturation, and that larger quantities of tannins are toxic for a cell. The protective barrier is the cytoplasmic membrane (tonoplast) which isolates the tannin substances occurring in the vacuole from the live cytoplasm (Baur and Walkinshaw, 1974; Hall and Walkinshaw, 1972). Tannins are also believed to play the role of growth regulators in the sense that they inhibit the action of enzymes (Swain, 1965).

The presence of tannin cells in pine buds has been observed by all authors studying the structure of buds from all representatives of the genus *Pinus*, however information is lacking about their possible role or about the changes taking place during ontogenesis. Camelfort (1956), Sacher (1955) and Hanawa (1967) believe that the occurrence of tannins in a cell at the apex of a pine shoot is a manifestation of its differentiation and thus, even though the authors do not say so explicitly, such cells could no longer be considered as meristematic. Kupila-Ahvenniemi (1966) mentions dividing cells containing unidentified ergastic substances observed in young scales covering the apex of a pine short-shoot.

That accumulation of tannins in a cell does not deprive it of mitotic capacity is known from the works of Bloch and Ball (after Hall et al., 1972). Bloch observed dividing tannin cells in the intercalary rib meristem in *Ricinus* and Ball in tissue cultures of *Sequoia sempervirens* embryos.

MATERIAL AND METHODS

The studies were conducted in the years 1969—1971 and 1974—1976 on material collected every 2—14 days from young Scots pine (*Pinus silvestris* L.) trees aged 1—15 years, and being of various origin, which grow in the nurseries and experimental fields of the Institute of Dendrology of the Polish Academy of Sciences. The buds were subjected to the commonly used microtechniques, fixed in Crai or FAA, embedded in paraffine and cut on a rotating microtome. Sections were stained with Ehrlich's hematoxylin by the progressive or regressive

method, or subjected to histochemical reactions for DNA (Feulgen reagent), carbohydrates (PAS) and tannins (FeCl_3). More detailed data on methods will be presented in the paper on the development and structure of pine buds.

RESULTS

In a pine bud tannin cells occur in the stem part in the cortex and pith and in the epidermis and mesophyll of scales. Tannins are one of the components of vacuoles. Within the apical meristem tannin cells reach to the distal zone, occurring singly in the rib meristem (Fig. 2). However, they do not occur in the remaining zones of the apex, that is in the peripheral zone and in the distal zone which include according to American terminology the zone of apical initials and the central mother cells zone.

During the winter tannin vacuoles occur in the form of single vesicles completely filling the inside of the cell and reducing the protoplast to a thin layer along the cell wall. In the young pith tannin cells are usually elongated parallelly, to the axis of the bud and the nucleus is shifted to the acropetal pole of the cell (Fig. 3). The tannin vacuoles give a positive reaction for tannins with FeCl_3 and for carbohydrates with PAS exhibiting an intensive red pigmentation following the latter test.

In spring, about 7 days prior to initiation of mitotic activity in the buds, there occur changes in tannin cells the first signal of which is the appearance on the surface of the vacuoles of slight invaginations and protuberances (Fig. 4), which gradually increase, as a result of which the initially single vacuole becomes fragmented into several smaller ones. At the same time the nucleus migrates to the centre of the cell and only after that mitosis takes place (Fig. 5).

At the time when in the apical meristem mitotic activity is resumed, there forms, between the upper part of the old bud that formed towards the end of the last vegetative period and the new part that formed already in the current season, a transitory zone which is best seen in the young pith. It is characterized by a loose arrangement of cells with numerous intercellular spaces. It is also designated by tannin cells the nature of which on both sides of the boundary is different. In the new bud the tannin cells have much fragmented and very numerous vacuoles while in the old one they are single (Fig. 6). These differences are particularly obvious after Feulgen and PAS reactions, after which the tannin vacuoles stain yellowish-red in the new bud and have a gray pigmentation in the old one. Tannin cells

in the zone belonging to the old bud gradually lose their meristematic ability while in the new bud they divide quite intensively.

Changes in the nature of tannin vacuoles in the spring begin first in the scales and in the stem of the bud in the zone of the oldest, short-shoots located lowest along the axis of the bud and over several days they spread over other parts of it. However, in the cells of the rib meristem tannin vacuoles undergo fragmentation about two weeks later than at the base of the bud.

The process of vacuole fragmentation advances as the mitotic activity increases and internodes elongate. In that period, among the numerous cell families in the young pith there occur many consisting of tannin cells (Fig. 7).

In the process of winter bud formation, the early phases of which take place still within the old bud, there form new tannin cells. At first they occur in the protoderm of scale primordia and in the rib meristem (Fig. 8). At this stage of new bud development and during June and July tannin vacuoles after PAS take on a yellow colour. A bright red colour is attained only in August and this primarily in the cells of the pith at the level of the youngest short shoots zone. In the zone of sterile cataphylls and in the zone of long-shoots they preserve a yellow colour even in the winter bud.

Towards the end of June, when in the axils of newly formed scales axillary bud primordia (short-shoots) form small vacuoles begin to coalesce. The process of vacuole fusion progresses successively during the summer and autumn so that before winter in all the tannin cells within the whole bud there are only single large vacuoles. Only in the basal parts of the subtending scales for axillary short- and long-shoots in some cells the vacuoles remain fragmented even over the winter.

Besides the tannin cells of meristematic nature, in all phases of bud development there occur in the bud axis outside the primary vascular bundle tannin cells which exceed all adjacent cells in size (Fig. 9). They become distinct more or less simultaneously with the first sieve cells and they grow as the adjacent cells divide. During internode elongation in May their protoplasts undergo degradation. Sacher (1954) refers to these cells as secretory.

In the winter bud it is also possible to find single cells or groups of them with granular inclusions (Fig. 10) which also give a positive reaction for tannins, but a negative one for sugars. They accumulate primarily in the zone of sterile cataphylls (terminal), above the long-shoots zone. They do not have a meristematic nature and they seem to be in the process of dying as could be judged by their further fate.

Fig. 1. Bud in longitudinal section (mid-March). In the pith files of tannin cells are visible. Stained with Ehrlich's hematoxylin by the regressive method. Notes: a) Between the apex and the long-shoots zone there is sterile cataphyll zone undescribed on the photograph, which in the following vegetative season will become the lower sterile bracts zone. b) "The sterile bracts zone" consists of two parts, the lower one formed towards the end of the season (n-2), corresponding in the winter bud to the sterile cataphyll zone and the upper formed at the beginning of the season n-1

Fig. 2. Apical meristem in longitudinal section (mid-June). Tannin cells in the rib meristem and young pith. Feulgen reaction counterstained with Fast-Green. Abbreviations: DZ — distal zone, PZ — peripheral zone, RM — rib meristem zone, CP — cataphyl primordium, C — young cataphyll

Fig. 3. Cells of young pith in longitudinal section. In the tannin cells nuclei are located polarly, while in cells without tannins centrally (January). Stained with Ehrlich's hematoxylin by the progressive method

Fig. 4. Tannin vacuoles in cells of young pith in longitudinal section at the onset of fragmentation (mid-March). Stained with Ehrlich's hematoxylin, by the progressive method

Fig. 5. Dividing cells of pith in longitudinal section. Tannin vacuoles are fragmented (mid-April). Stained with Ehrlich's hematoxylin by the regressive method

Fig. 6. Transitory zone between the old and new bud in longitudinal section (mid-May). Stained with Ehrlich's hematoxylin by the progressive method

Fig. 7. Groups of tannin cells in the pith in longitudinal section (mid-April). Stained with Ehrlich's hematoxylin by the progressive method

Fig. 8. Tannin cells in the rib meristem 0.08 mm from the apex in cross section (mid-June). PAS reaction

Fig. 9. Secretory tannin cells external to the primary vascular bundle, in longitudinal section (early April). PAS reaction

Fig. 10. Degenerating tannin cells in the young pith from the sterile cataphylls zone in longitudinal section (mid-December). PAS reaction

Fig. 11. Apical meristem of the shoot in longitudinal section. Tannin vacuoles and starch grains in the rib meristem, young pith and sterile cataphylls (early April). PAS reaction

Fig. 12. Cells of the young pith in cross section. Starch grains and disintegrating tannin vacuoles are visible (late April). PAS reaction

Fig. 13. Fragment of a new winter bud in longitudinal section in semi-polarized light. Starch grains shine (mid-May). Stained with Ehrlich's hematoxylin by the regressive method

Fig. 14. Cells of the distal zone in the apical meristem in cross section. Small starch grains are visible (mid-June). PAS reaction

A characteristic symptom of spring bud awakening in pine is the synthesis of starch, which is absent in the winter bud. It appears only in the phase preceding by a few days the first cell divisions, together with changes taking place on the surfaces of the tannin vacuoles. Starch appears first in the scales subtending axillary buds, in sterile cataphylls and in the pith (Fig. 11) that is in tissues that during the winter and during the whole development of the bud are rich in chlorophyll. The spring maximum of starch content in the bud coincides with the period when the tannin vacuoles are most fragmented (Fig. 12).

Early in the spring apical meristems and needle primordia are devoid of starch. When the needle primordia begin to elongate starch appears in their terminal parts, but during the stage of intensive development (during April and May) it disappears completely. Within the apical meristem starch is present only in the rib meristem (Fig. 13) but not in the distal zone, or peripheral zone nor in the youngest scale primordia.

In the newly formed bud the synthesis of starch in June and July is very intensive. Temporarily it appears also in the distal zone of the apex (Fig. 14), however, in the peripheral meristem and in the youngest scale primordia it can not be found yet. Large quantities of starch are synthesized in the rib meristem, in the young pith and in the basal parts of scales subtending short-shoots.

In autumn the amount of starch in the bud falls. Small quantities of it are still to be found in buds fixed in the first half of December, and single grains can still be found in the winter, but only in the basal parts of subtending scales. From about mid-December till mid-March, starch is absent in the bud.

DISCUSSION

When attempting to explain the role of tannin vacuoles in the process of spring activation of buds, it is necessary to consider two possibilities, that either it is an active or a passive role. In the latter case tannins would only represent an indication of changes in the volume of vacuoles resulting from the water relations between the vacuole and the cytoplasm.

The process of fragmentation of the central vacuole in the cell into several small ones and then their coalescence has long been known and was described for the cells of the stalk of the *Drosera* tentacle as early as in 1885 by de Vries (after Dangeard, 1956). It is a reaction of cells to sudden external stimuli as a result of which changes occur in the content of water in the vacuole-cytoplasm relations

(Dangeard, 1956). Changes in water relations between a vacuole and the cytoplasm are also the direct cause of coalescence of several vacuoles in a cell, a phenomenon occurring in the process of cell differentiation, and also of the reverse phenomenon depending on the disintegration of a single vacuole into several small ones, which are observable in cells that regain a meristematic nature (Dangeard, 1956).

From various data in the literature it is known that there exists a strict, metabolic relation between tannins and sugars. Direct evidence for such a relation was provided by Zaprometova and Bukhlayeva (1970). These authors established that in young shoots of Scots pine radioactive CO_2 is incorporated already after 30 minutes (in 6.17%) into phenolic compounds.

Data exists that aromatic secondary substances (among which tannins are also included) may be also involved in the basic metabolism of plants. Runeckles (1963) who applied universally labelled phenolic substances to intact plants found subsequently that several phenylpropane derivatives were partially converted into soluble sugars (glucose, fructose and saccharose).

On the basis of indirect evidence, Sperlich (1939) and Paech (1950) mention also a strict metabolic connection between tannins and sugars.

From experiments conducted in recent years in an electron microscope it appears that the synthesis of tannins and their transport take place through the endoplasmatic reticulum and the Golgi apparatus (Chafe and Durzan, 1973; Durzan, Chafe and Lopushanski, 1973). Clowes and Juniper (1968) represent the opinion that in certain specialized cells plastids are responsible for the synthesis or accumulation of phenolic substances.

Tannin cells in a pine bud occur in these zones which throughout the period of bud development are rich in chlorophyll (scales, cortex, pith). The positive PAS reaction that vacuoles give indicates that the tannins present here belong to the group of hydrolysable tannins, and, therefore, have within their structure the glucose group (free sugars if they are present in the bud can be washed out of tissues by micro-techniques). In spring, when tannin vacuoles undergo fragmentation starch appears in the cells. The intensity of PAS reaction weakens. These changes indicate that the tannins present in the vacuoles undergo hydrolysis and the released glucose groups are transported to the cytoplasm, becoming one, though probably not the only source of sugars, which in the plastids undergo condensation to starch. A source of precursors for starch synthesis, besides the products of tannin hydrolysis, may also be the products of lipid hydrolysis as happens in germinating seeds. In rape the first starch grains in the root cap of

the embryo appear already a few hours after imbibition (even after 3 hrs) and they are the first morphological symptom of metabolic activation in cells observable in the light microscope (Kuraś and Teleżyński, unpublished).

In the early period of new bud formation (May-July) the binding of glucose by tannic acids is limited. This is indicated by the weak PAS reaction tannin vacuoles give in the rib meristem, in the young pith and in the scales subtending short-shoots. At the same time, in these zones of the bud, intensive synthesis of starch takes place. During August and September synthesis of starch drops while the intensity of PAS reactions in the tannin vacuoles increases.

Pith cells which contain granular deposits that do not give a positive reaction for sugars, are probably in the condition which eventually all tannin cells of the bud attain after complete hydrolysis of the tannins present in the vacuoles. Such cells with degraded protoplasts and destroyed plasmatic membranes occur commonly in young, no longer elongating shoots. Presumably the tannins present there belong to the condensed, non-hydrolysable group which occur in mature tissues of pine as mentioned by Hegnauer (1962).

It is also pertinent to mention here the results of interesting observations of Riding and Gifford (1973) conducted on the apices of embryo and developing seedlings of *Pinus radiata*. In embryos isolated from dry seeds there was no starch. It appeared only 24 hours after imbibition, and then in all zones of the stem apex. In later phases of apex development starch was present only in the rib meristem. Tannins appeared only after germination of the seeds. On the other hand, proteins and lipids are reserve substances in the embryo and during the early phases of seedling development (Riding and Gifford 1973).

CONCLUSIONS

It was established that tannin cells of pine buds, occurring in the ground tissues and in the rib meristem are capable of division. Results of cytochemical observations suggest that vacuoles in these cells contain hydrolysable tannins, which liberate glucose in the spring. After hydrolysis the tannins undergo sedimentation and are visible as granular deposits in dying cells of pith in the young shoot.

On the basis of the data presented in this study it may be that there exists a causal relationship between the spring changes in the nature of tannin vacuoles and the metabolism of starch. The onset of tannin vacuole fragmentation and the simultaneous appearance of starch in spring, both in the tannin cells and the parenchyma cells

devoid of tannins are the first structural indications that spring awakening has begun, and this takes place about a month before external manifestations of dormancy breaking (bud swelling, scale separation) observable about 2 weeks after mitotic activity are resumed.

A detailed description of the relationships indicated by this study would be only possible after cytological and biochemical studies based on specific techniques of tissue fixing and cytochemical analyses. These methods were not required in the present study the main purpose of which was to observe the seasonal changes of the structures within a pine bud.

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Author's address

Dr. Alina Hejnowicz

Institute of Dendrology

Polish Academy of Sciences,

63-120 Kórnik near Poznań; Poland

Wakuole taninowe i skrobia w rozwoju wegetatywnych pąków sosny zwyczajnej (Pinus silvestris L.)

Streszczenie

Praca obejmuje fragment kilkuletnich badań anatomicznych nad rozwojem pąków *Pinus silvestris* L. Stwierdzono, że w zimowych pąkach nie ma skrobi oraz że w prawie wszystkich częściach pąka występują komórki taninowe, w których taniny stanowią jeden ze składników wakuoli. Nie ma ich tylko w strefach merystemu dystalnego i merystemu peryferycznego w obrębie wierzchołka, a także w najmłodszych zawiązkach łusek i igieł.

Najwcześniejszymi, strukturalnymi przejawami wiosennego przebudzenia pąków jest rozpad pojedynczych wakuoli taninowych i synteza skrobi. Prawdopodobnie oba te zjawiska są ze sobą przyczynowo związane.

Występujące w wakuolach taniny należą do grupy hydrolizujących, dających pozytywną reakcję na cukry (PAS). W czasie wiosennej ich hydrolizy następuje uwalnianie glukozy, która jest prawdopodobnie jednym ze źródeł cukrów dla syntezy skrobi.

W okresie wzrostu wydłużeniowego pąka następuje degradacja komórek taninowych, polegająca na strąceniu tanin, przechodzących stopniowo w formę skondensowaną.