

Article

## Flavanols and Flavonols in the Nuclei of Conifer Genotypes with Different Growth

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**Abstract:** Flavanols and flavonols of mitotic and post-mitotic nuclei in needles of *Taxus baccata* L., *Tsuga canadensis* L., and slow growing dwarf genotypes of both genera are investigated histochemically. The flavanols of nuclear chromatin and in the vacuoles stain blue with the *p*-dimethylamino-cinnamaldehyde (DMACA) reagent. Flavonols do not react with the reagent but owing to their UV absorbance they can be seen as bright yellow pigments. The nuclei in the photomicrographs obtained by microscopy were measured for flavanols at 640 nm. The vigorously sprouting *Taxus baccata* L. displays the most rapid cell cycling of the needles and the nuclei reveal clear blue and white mosaic structures. The flavanol component of *Taxus baccata* nuclei remains relatively stable most of the growing season. The dwarf genotypes also display fairly blue stained meristematic nuclei during the intense spring flush. However, after the spring flush and towards mid-summer the nuclear flavanols slowly decrease in parallel with a gradual increase in yellow staining nuclear flavonols. A mixture of blue stained flavanols and yellow flavonols results in greenish coloration of the nuclei. The greenish tint becomes more pronounced when the parenchyma cells mature and age. At the same time, the cytoplasm of the dwarf genotypes also begins to attain a more yellow tint. This trend continues towards mid-summer and autumn, particularly in the nana genotypes. It would appear that the yellow staining flavonols are linked to restricted growth conditions. In the present study, it becomes evident that the species-typical

endogenous growth potential is related to both flavanol and flavonol allocation into the nuclei. The vigorously growing species of *Taxus* and *Tsuga* have a higher capacity for recruitment of flavanols into the nuclei than the very slow growing dwarf species.

**Keywords:** flavanols; flavonols; DMACA; *Taxus baccata* L.; *Tsuga canadensis* L.; slow growing dwarf genotypes; cell nucleus

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## 1. Introduction

Flavonoids are ubiquitous in plants [1,2]. These so-called secondary products have many functions mainly in protecting against biotic and abiotic aggressors [3]. The flavanols as a subgroup of the flavonoids were selectively detected in nuclei by their blue histochemical stain in fresh tissue. This finding was also confirmed by a highly selective physical method [4]. The flavanols appear to be bound to histones in nuclei of several coniferous tree species and their intranuclear distribution is highly influenced by developmental and environmental signaling networks [5]. In this context, it is of special importance that catechin, the major flavanol, was found to protect DNA and proteins from oxidative degradation by reactive oxygen species [6].

Many plants respond to increasing toxic oxygen species (ROS) by up-regulation of various polyphenols [7]. For instance, the commonly occurring quercetins limit damaging ROS in stressed tissue [8]. However, it should be noted that these oxygen radicals do not only have negative functions. It is well known that they are important signals in mitogen-activated protein kinase cascades, which trigger the expression of specific sets of genes [8].

The present study deals with coniferous species that have different growth patterns and focuses on their nuclear flavonoids. Both flavanols and flavonols are detected in conifer nuclei [9]. A previous paper reported that growth depression during short periods of drought and heat was characterized by a loss of nuclear flavanols and an appearance of nuclear flavonols [10]. Therefore, the goal of our study was to investigate, in addition to the flavanols, the role of nuclear flavonols in the complex interplay during different growth conditions. Up until now, the role of phenols in nuclei appears to be highly complex and actual knowledge is poor and still fraught with many questions and uncertainties.

## 2. Materials and Methods

### 2.1. Materials

Adult coniferous trees range from vigorous to weak growth in the following species order: *Taxus baccata* L., *T. baccata aurea*, *T. baccata nana* (Green diamond group, a morphological subgroup of the nana types). This study compared the vigorously growing *Tsuga canadensis* L. with the dwarf *Tsuga nana* L. The trees were investigated in 2010, 2011, and 2012. All the trees were between 12 and 15 years old and grow in a mixed species plantation at the Botanical Garden of the University of Munich in Freising-Weihenstephan. About 3000 nuclei from needles (current year growth) were investigated from each of *Taxus baccata* and *Tsuga canadensis*. From each of the weakly growing genotypes, about

800 nuclei were studied. In all trees, the most intense sampling was carried out just after bud break during high rates of cell cycling.

*Tsuga canadensis* samples were collected at three dates, (a) in early April 1-year old mature needles before bud break, (b) in early May sprouting buds with still elongating needles, and (c) in mid-July mature needles. In addition, about 200 seed wings from *Tsuga canadensis* were also sampled and analyzed.

## 2.2. Histochemistry and Light Microscopy

All studies were performed in fresh tissue without dehydration and embedding. The freshly cut needle sections (transverse and longitudinal) were about 20 to 25  $\mu\text{m}$  thick. They were immediately stained for 20 min with the DMACA reagent (1% *p*-dimethylamino-cinnamaldehyde dissolved in 1.5 N methanolic sulfuric acid). The DMACA reagent is specific for flavanols as well as other phenols and generates a special bright blue color. The natural state of the nuclei, commonly 7–8  $\mu\text{m}$  in diameter, was, thus, observed directly after sampling. Photomicrographs were obtained with a Zeiss Axioscop and a Zeiss UV photomicroscope. Absorption units (AU) of the micrographs were determined with the Cool Scan Digi Eye Apparatus (Nikon, Tokyo) [11]. Standard deviations (SD) are based on many observations. DAPI (diamidino-phenylindole dihydrochloride, Serva, Heidelberg, Germany) was used for visualizing DNA and yielded bright yellow to white fluorescence spots using the Zeiss fluorescence apparatus III RS. Yellow flavonols could be observed in the cells also after DAPI staining under the microscope. This yellow color was more intense after staining with the aqueous diphenylboric acid- $\beta$ -aminoethylester (DPBA, Naturstoff-reagent) and the colors were even more brilliant under the UV-microscope (Zeiss, Oberkochen, Germany) equipped with exciter-barrier filter sets G 436, FT 510, and LP 520.

Needle tissue of *Tsuga nana* was incubated for 48 h in watery solutions of BA (kinetin) at 0.2 mg/L (0.8  $\mu\text{m}$ ).

## 2.3. HPLC Analysis of Flavonoids in Needles from *Tsuga canadensis*

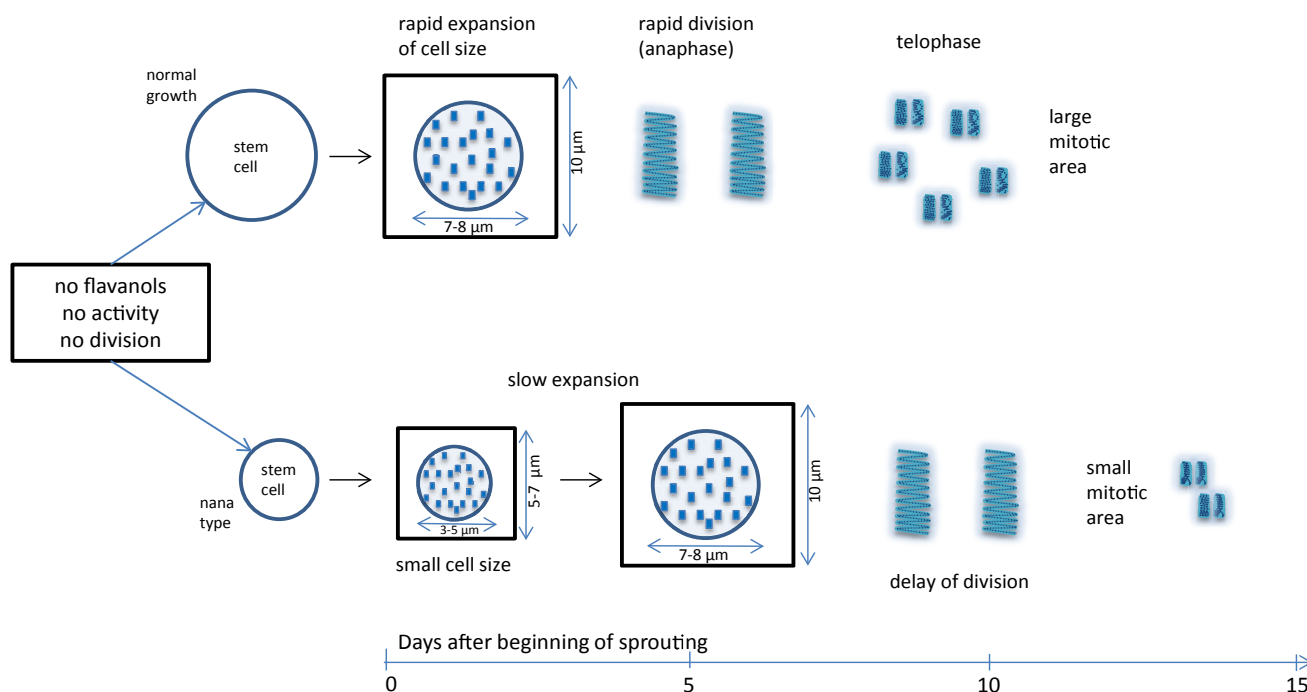
Phenolic compounds were extracted with methanol using 10 mg DW (needles) and 5 mg DW (seed wings). Determination of the phenolic compounds was performed by HPLC. They were separated on a column (250  $\times$  4 mm I.D) packed with Hypersil ODS (3  $\mu\text{m}$ ). The HPLC system consisted of an autosampler (Gilson-Abimed Model 231), two pumps (Kontron model 422), and a diode array detector (Bio Tek Kontron 540). For post column derivatization, an HPLC pump (Gynkotek Model 300C) and a VIS detector (Kontron 432) were used. Further details of the solvent gradients and blue staining of flavanols with DMACA solution are described by Feucht *et al.* [9]. Hydroxycinnamic acids, flavanols, flavones, and flavonols were identified by their UV-spectra and by comparison with commercial standards (Roth, Karlsruhe, Germany). Furthermore, acid hydrolysis of flavonol-glycosides revealed only kaempferol as aglycone. Thus, all flavonols were derivatives of kaempferol. Catechin, epicatechin, and epigallocatechin concentrations were quantified after reaction with DMACA [9] by comparison with their respective standards. The hydroxycinnamic acids and flavones were detected at 320 nm and quantified as chlorogenic acid or apigenin equivalents, respectively. Kaempferol-glycosides at 350 nm were quantified as kaempferol-3-glucoside equivalents.

### 3. Results

#### 3.1. Approximate Growth Parameters of the Species

The climatic conditions varied considerably in 2010, 2011, and 2012, especially in temperature and water supply. These tree species have different crown architectures and a rough estimate of tree heights was 12 m for the pyramid-shaped *Tsuga canadensis* and 3 m for the bushy growing *Taxus baccata*. The small subspecies *Taxus baccata aurea* is 1.5 m tall and the extremely dwarfed nana types of *Taxus* and *Tsuga* both measure 0.5 m. Histological changes in development during the initial phase of cell cycling are presented in Figure 1. Stem cells of shoot tips showed no special activity towards any differentiation. In this state there is no synthesis of specialized metabolites, such as phenols or nuclear flavanols. Outside the stem cells, the peripheral flanks of the apex form lateral primordia, which are active in cell division and flavanol synthesis.

**Figure 1.** The beginning of sprouting in the normal and the *Tsuga canadensis nana* types; the dwarf type has smaller stem cells and a major group of the descendant cells, including the nuclei, is reduced in size; both cell expansion and cell division are delayed; in the end, mitotic areas are smaller. Stem cell nuclei have no flavanols.

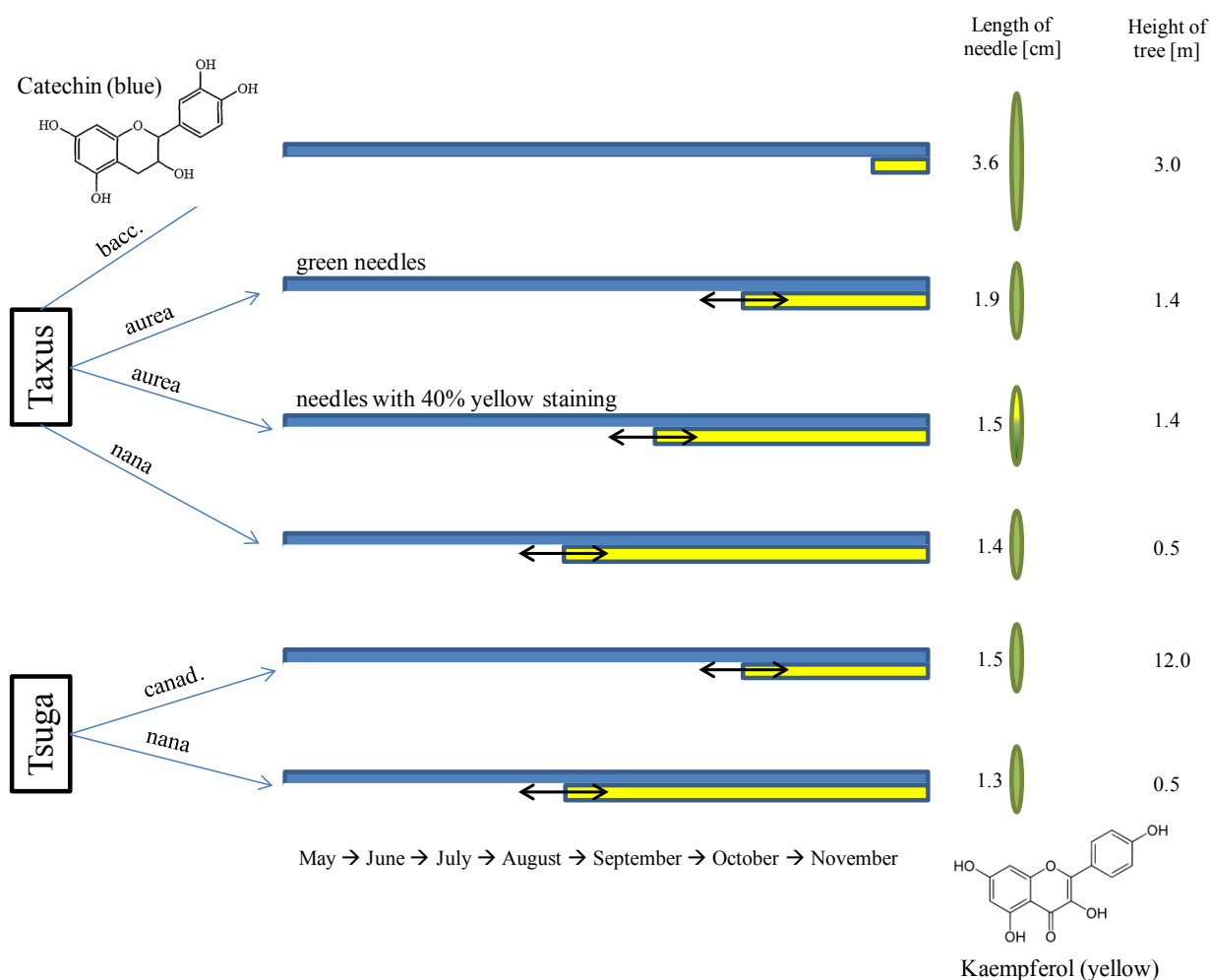


At the onset of needle growth the sizes of both the cells and nuclei of the nana type of *Tsuga* were slightly smaller than those usually found in needles of the normally growing *Tsuga canadensis* (Figure 1). Presumably, the small cells suffered from a lack of expansion proteins and also the nuclei (3–5 μm in diameter) were more compacted than those of the vigorous tree (7–8 μm in diameter). Such tightly condensed nuclei had fewer mobile domains because of a lack of interchromosomal spacing. In the end, mitosis was restricted and delayed (Figure 1).

Based on data from three years (Figure 2), the mean length of the needles ranged from 35 mm in *Taxus baccata* to 15 mm in *Tsuga canadensis*. The needles of the longest leader shoots of the top crown

are excluded from the calculations. *Taxus baccata aurea* is a small bush that develops a yellow color in the distal area of the needles of the upper light-exposed canopy. The yellow pigmentation is mainly due to the thick cell walls of the upper and lower epidermal tissue. Some faint yellow staining was also seen in the cytoplasm. The partially yellow needles of *Taxus baccata aurea* reached 15 mm in length whereas the green needles were 19 mm long. Both extremely stunted nana types had much smaller needles, namely 13 mm and 14 mm (SD values were about 1 mm for all needle groups). The overview presented in Figure 2 shows that blue staining flavanols were dominant in the *Taxus baccata* nuclei, which had the longest needles. The yellow flavonols of the nuclei increased in color towards the end of the growing season and this phenomenon was particularly evident in the dwarf genotypes.

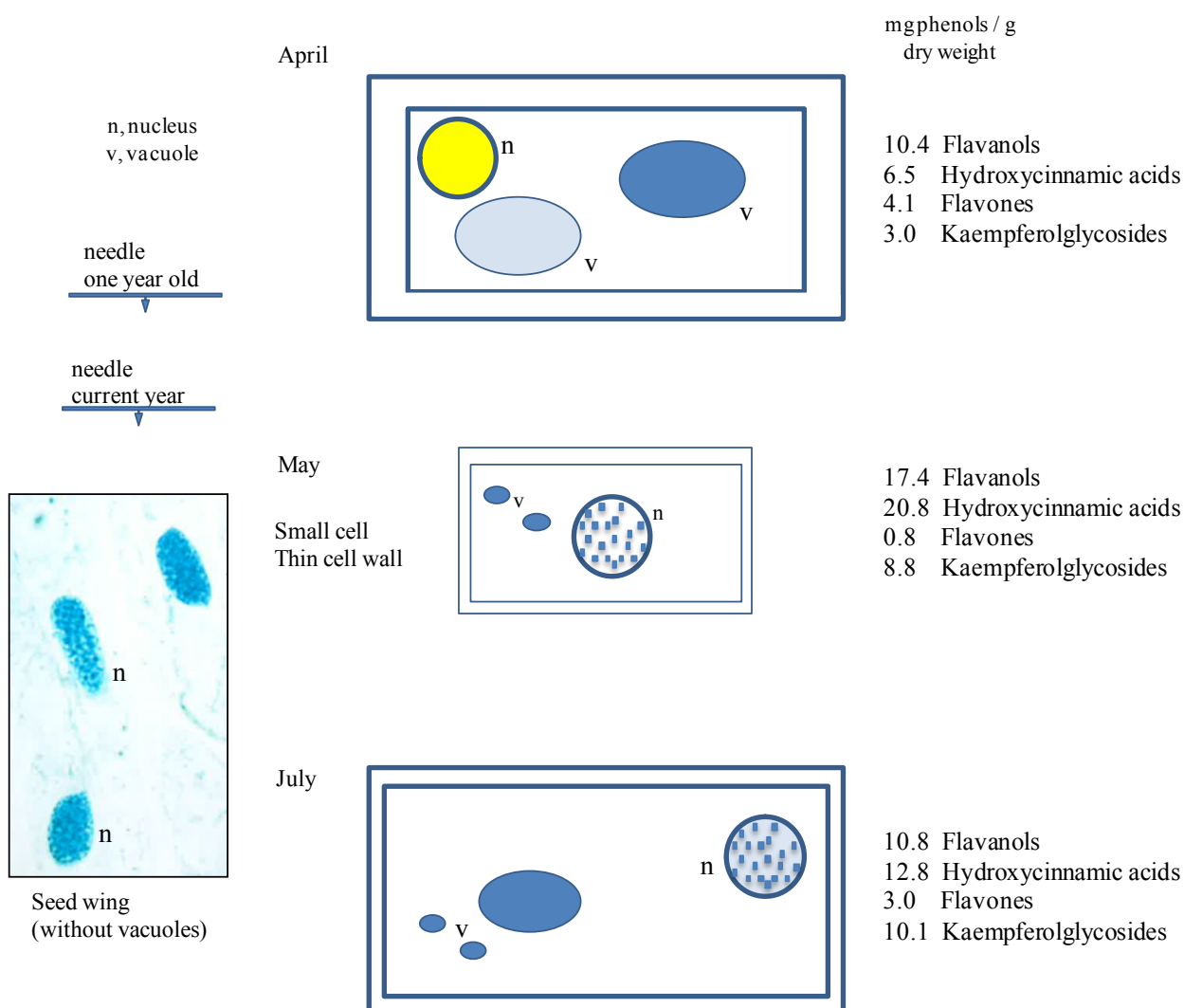
**Figure 2.** Appearance of yellow flavonols in the blue staining nuclei during the annual growth period of the conifers; as a result, the blue nuclei take on a greenish color; an early change to greenish nuclei is correlated with reduced needle size and tree height.



3.2. Seasonal Changes of Phenolic Compounds from Entire Needles of *Tsuga Canadensis* and the Importance of Cell Structures

The phenolic pattern of the entire needles is presented in Figure 3 and gives an overview of the proportions of diverse phenol groups in relation to flavanols. The relatively inactive needle group, namely 1-year old needles, was sampled in April before bud break and compared with just sprouting needles from early May and also with needles from mid-July which had down-regulated cell cycling.

**Figure 3.** Total content of phenols at different stages of *Tsuga canadensis* needle development; the different cell sizes and thickness of cell walls together with different amounts of vacuolar flavanols made it impossible to quantify nuclear flavanols; however, it was possible to sample seed wings in developing cones which have no vacuolar flavanols.



The flavanols of all three-needle groups are located in the nuclei and vacuoles of both mesophyll cells and the epidermis. During the winter rest period up to April, the nuclei have no flavanols. However, in May the nuclei of young developing cells contain flavanols in relatively high concentrations; but the high value of 17.4 mg flavanols per mg DW needs to be explained. Some tiny vacuoles with flavanols are repeatedly found in cells outside the meristem clusters. Furthermore, the relatively high amount of flavanols is partly due to the small cell size and therefore a relatively high number of cells and nuclei per mg DW is the result. Also, the relative thin cell walls in May contribute to a higher proportion of cytoplasm per unit DW. Cell wall thickness was greater in April and July.

In April, the hydroxycinnamic acids accounted for only a third or half the amount compared to May and July. The flavone group was lower during rapid cell cycling (May) compared with the two other time periods. The kaempferol-glycoside concentrations were slightly higher in May and July compared with the resting needles from April. The flavanols were mainly located in vacuoles. The results clearly demonstrate that the diverse phenolic compounds changed constantly and that it was not possible to measure only the nuclear flavanols.

Based on the above results, it is clear that only highly specialized tissue is suitable for determining nuclear flavanols. Fortunately, most seed wings of *Tsuga canadensis* have no vacuoles (inset in Figure 3) although it is advisable to check them before analysis. It is advantageous that the seed wings of entire cones of the same branch behave synchronously. This means that at any one sampling date seed wings are either with or without vacuoles. Thus far, the main flavanol compounds of the nuclei, as determined by HPLC, were catechin and epicatechin. In addition, small quantities of B1 and B type proanthocyanidins were detected. The striking result of this study is the verification of the presence of flavanols in the nuclei.

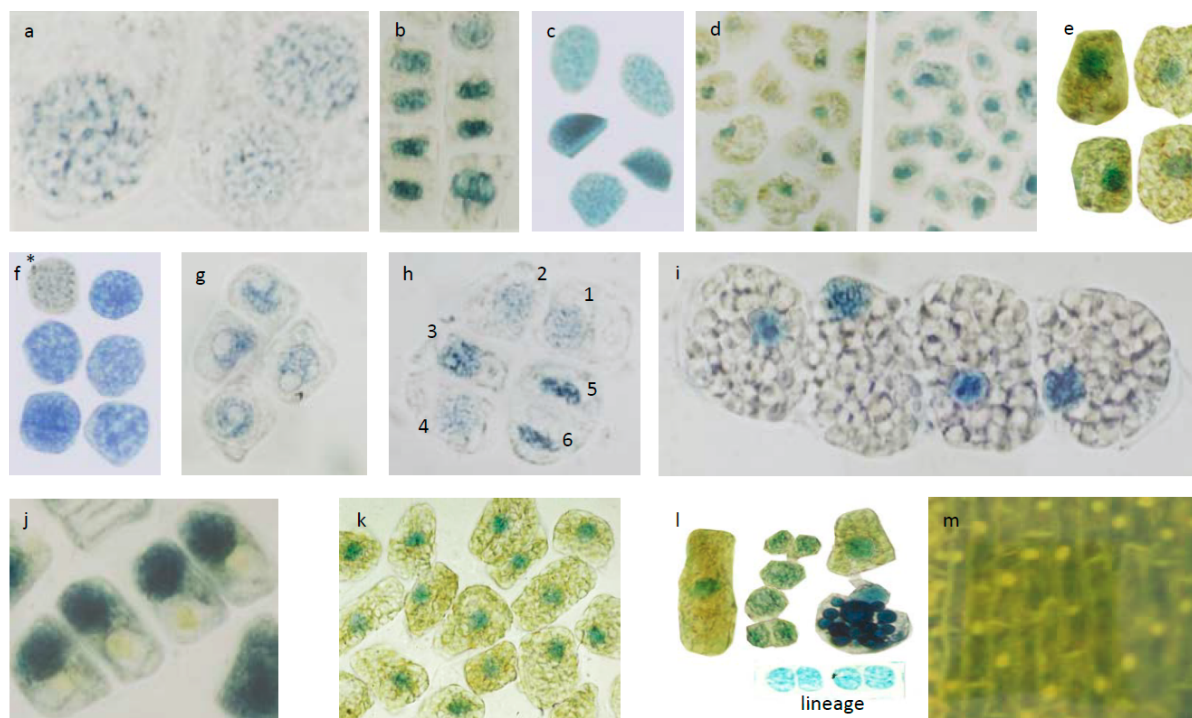
### 3.3. Micrometric Histology of Nuclear Flavanols from Needles of the *Tsuga* Genotypes

For this type of study it is very important to compare only small clusters of nuclei, which had developed in the same microenvironment of a small tissue sector because throughout a needle there are substantial changes in physiological conditions. The 3 nuclei of *Tsuga canadensis* (Figure 4a) were located in close spatial proximity and were 6, 7, and 9  $\mu\text{m}$  in diameter. This corresponded to a volume of 31, 38, and 60  $\mu\text{m}^3$ . It is remarkable that the most activated nucleus reaches double the volume of the adjacent one. The largest nucleus is characterized by wide interchromatin spaces allowing a high directional transport of metabolites. In contrast, the two smaller nuclei indicated a more diffuse and less defined blue mosaic expression, a pattern that may be characteristic of lower activity that could be assigned to early G 1 phase. Their blue colored chromatins revealed absorbance values of 40 and 47 compared with 62 for the enlarged S phase nucleus.

The two lineages (Figure 4b) allowed fine-tuned differences to be observed in the flavanol densities of telophase, metaphase, and interphase nuclei. The lineage on the left displayed a changing density from very dark blue to moderate blue (from AU 44 to 38). The structures of the stretched chromosomes at transit from metaphase to rod-like anaphase suffered from pulling stress. This apparently caused some diffusivity of the flavanols, thereby promoting slight viscoelastic dilation as often observed in stretched molecules and often found in *Taxus* in our previous investigations (unpublished data).



**Figure 4.** Nuclear flavanols as blue and, if combined with flavonols, as greenish clusters of the *Tsuga* and *Taxus* genotypes.



As shown in Figure 4c, fully developed nuclei of *Tsuga canadensis* have a certain elasticity in their structures. Two nuclei are shown which were pressed by the cytoplasm towards the cell walls. Thus, they have a high degree of physical compaction and a high flavanol density (AU 86, SD 4.5,  $n$  15), compared to three non-pressed ones. The latter were larger in size and located in the center of the cell (AU 69, SD 4.3,  $n$  15). (For simplicity of presentation, these nuclei were removed from the original photos and combined in Figure 4c.)

As frequently observed within the same needle of *Tsuga Canadensis*, there were cell clusters with nuclei showing either blue or more greenish colors (Figure 4d). Physiological changes were apparently in play, causing differential activation of the flavonoid branches within a needle. The somewhat greenish nuclei consist of blue staining flavanols combined with yellow flavonols. The blue group of nuclei reached AU values of 65 (SD 2.0,  $n$  15).

As shown in the dwarfed *Tsuga nana* (Figure 4e), after the spring flush all mesophyll cells of a needle could be covered with a yellow tint in the cytoplasm. This is linked to the appearance of green nuclei. As the growth season progressed, the yellow color of the flavonol component intensified. To sum up, the nuclei of *Tsuga nana* give the following message: the more yellow, the less active.

Furthermore, application of cytokinin to *Tsuga nana* (Figure 4f) resulted in a fairly strong promotion of nuclear flavanols, attaining AU values up to 73 (SD 2.2,  $n$  40) compared with controls (AU 47, SD 3.5,  $n$  40). One pale control nucleus is shown in the left row (asterisk \*). Many more nuclei, up to 300, were stained in this respect (not shown). A central finding of this work is the fact that cytokinin is engaged in delivering more flavanols to the nuclei.



### 3.4. Micrometric Histology of Nuclear Flavanols from Needles of the *Taxus* Genotypes

Commonly, meristematic cells of the bushy *Taxus baccata* undergo periclinal divisions to form a cell lineage. However, sometimes the plane of division changed from periclinal to anticlinal (Figure 4g). Strikingly, despite the clonal character of the 4 descendants, each one had its own “blue face”, indicating an individual spatial and temporal epigenetic modification of the flavanol pattern with AU values ranging between 55 and 65 (SD 5). Furthermore, the maximum cell cycling activity was at a distinct stage associated with one or two large whitish nucleoli each being as much as 3–4  $\mu\text{m}$  in diameter (Figure 4g). Such nucleoli reflect a highly active interphase state, taking place when ribosomal RNAs are produced.

In other cases, mitotic nuclei were in interphase (Figure 4h), evidently running through a short period of low activity (number 1, 2, and 4, with AU values of 57, 55, and 56). Mainly in early G1 the interphase nuclei generally have a lower active state. In addition, there was one quadrate, fairly blue colored nuclear area in transit from late telophase to G1 (number 3, AU 77) and also a correct telophase showing two sickle-shaped compressed “half-moon” nuclei (number 5 and 6, AU 75 and 83). The telophase conformations are about 20% smaller in area than the interphases. This means that at telophase the flavanols are triggered to accumulate up to a dark blue compaction.

Under rare specific metabolic conditions, the four cells even of a meristematic cell lineage curiously underwent a metabolic shift towards extreme deposition of starch grains (Figure 4i). Nevertheless, the nuclei retained their ability to modify the flavanols (AU 85–91,  $n$  4), thereby forming deep blue patches of heterochromatin, which points to a strict down-regulation of sizeable sets of genes. (Between the starch grains there are black shadows, which are somewhat misleading.)

In conifers, to our knowledge, the meristem cells have never been found to be vacuolated. A really unique example ever observed in a 4-celled lineage (Figure 4j) is shown by medium-sized vacuoles located adjacent to the nuclei. Hypothetically, yellow staining phenols are kept away from the nuclei. The color of the rather diffuse nuclei is extremely dark blue, obviously due to unusually over-expressed flavanol synthesis (AU 90, SD 0.6). Studies of the needles from the dwarf *Taxus aurea* (Figure 4k) revealed that the cytoplasm can attain a strong yellow color and the nuclei turn to a greenish tint.

The nuclear activities of the dwarf *Taxus baccata nana* are documented by examples of different needles (Figure 4l). During spring, the ability to form cell lineages is linked to light blue and clearly mottled blue nuclei (AU 42, SD 2.0). Within a recently formed 4-celled lineage, there is commonly, as expected, no variation in flavanol density of the nuclei and also no yellow surrounding cytoplasm. However, as the growth slowed down, 6 more greenish nuclei appeared and two cells of this lineage underwent an anticlinal cell division (Figure 4l). A greenish colored lineage is exceptional and apparently related with the early appearance of yellow flavanols in the *Taxus nana* genotype. Interestingly, a further situation is also shown in Figure 4l as a single large cell was subjected to a fairly high developmental activity in flavanol synthesis and deposition of these flavanols into about 10 small vacuoles. The nucleus responsible for such a differentiation obviously displays pale blue staining (53 AU). By contrast, two adjacent large cells with a yellow cytoplasm developed slightly greenish nuclei as a sign of relative inactivity. Indeed, the presence of notable amounts of flavanols in nuclei of *Taxus nana* is documented by yellow fluorescence as indicated clearly by DPBA (Figure 4m).

## 4. Discussion

### 4.1. Nuclear Flavanols during the Metabolically Greatly Intensified S Phase

S phase means stress phase. The metabolic stress is multiplied during the S phase because DNA and distinct proteins are doubled in quantity from 2 C to 4 C in a time period of only a few hours. Here, the proline residues of peptides [12], the lysins of basic histones [13], and the hydrophobicity of flavanols [14] play a crucial role. This requires intensified enzymatic systems, loosening of histone N-terminals, and a high degree of histone acetylation. An armada of enzymes, such as helicases and polymerases, as well as numerous micro-molecular interactions, operate at maximal levels. This results in a high potential for DNA damage. For this reason, the pluripotent stem cells of plants have a very low number of cell cycles in order to conserve high genetic stability [15].

The large interchromosomal spaces (Figure 4a) of the highly dispersed chromatin allow high mobility of messengers and macromolecules, predominantly the cycling dependent kinases (CdKs). Acetylation of histones causes the opening of chromatins to produce the hyper-activated structures of nucleosomes. Even recognition of damaged DNA and its repair depends on an acetylated status together with blobs of heterochromatins as protectors against UV micro-irradiation [16]. Taken together, the flavanol patterning of the most expanded nucleus (Figure 4a) fulfills all the properties claimed by a theoretical S phase model.

The stability of chromosomes, as well as the partitioning of genes into euchromatin and heterochromatin, is apparently supported by flavanols. In addition to the structural modification stress, chemical stress by oxygen also results in faulty synthesis of proteins and DNAs along with mitogen-activated kinases. These processes are triggered by competent ROS molecules, which, however, need strict control by radical scavenging activities [8,17]. Misregulation by the inevitable ROS molecules is also controlled by recruitment of catechin and epicatechin [18]. After all, the transit from G1 to S phase is promoted by auxin [19].

### 4.2. The Interplay of Cytokinin, Auxin, Sucrose, and Flavanols

DNA synthesis and correct double strand compaction occurs through the action of kinetin [20]. Upon addition of cytokinin to conifer nuclei, a drastic increase in flavanols takes place as shown in the nana type of *Taxus baccata* (Figure 4f). Cytokinins, apart from scavenging free radicals, also prevent the formation of excessive free radicals and in the end are key factors in the stressful phase of DNA synthesis [21]. There may be a functional association between the well-known antioxidative effect of cytokinin [20] and the flavanols as antioxidants.

Auxin is reported to create a high cellular redox potential through reduction of ROS, thus stabilizing DNA [6]. It appears from the results presented in this paper that the abundant flavanol molecules in a nucleus produce a highly effective safeguard system by delivering enough hydrogen atoms or electrons to stabilize the overstrained molecules. In the plant kingdom, a well-known effect of cytokinins is to attract carbohydrates, overall sucrose, towards the active shoot tips [22]. In fruit trees, for example *Malus domestica*, synthesis of flavanols was enforced by providing sucrose. However, these flavanols, as determined by histology, are only deposited in vacuoles [23]. The size of the nucleoli increases with synthesis of rRNAs and this process is stimulated by auxin [24]. As auxin is engaged in transcription,

special transcription factors are involved in optimizing IAA levels [25]. As a principle, the irreplaceable auxin is possibly guided all over in the nucleus by catechins.

#### 4.3. Growth-Modifying Balance of Flavanols and Flavonols in Nuclei

*Taxus baccata* is characterized by very intense cell cycling as dominated by efficient cyclin-dependent protein kinases. The smaller growth type of *Taxus* was correlated with less nuclear flavanols and increasing flavonols (Figure 2).

The partially yellow needles of *T. aurea* exposed to full sun were smaller in size than the completely green ones. It is widely accepted that many plants accumulate flavonols as a direct response to excess sunlight and UV-radiation [3]. To present an informative example, leaves of *Phillyrea latifolia* exposed to full solar radiation were strongly reduced in size but produced ten times the amount of a quercetin-glycoside as the shaded controls [26].

Yellow fluorescing flavonols, especially quercetins, were identified in meiotic microspores and anthers of yew, yellow cypress, and juniper [9]. Flavonols, in particular, interacted with DNA as a target site [27]. Under exceptional ageing conditions under heat and water stress the blue colored flavanols of *Taxus* nuclei disappeared, whereas the yellow nuclear flavonoids became more pronounced [5,10]. In keeping with this concept, quercetin has a higher affinity to histone H3 than kaempferol and rutin [9]. Heat excess results in a very marked decrease in antioxidant enzymes, however the free radicals increase drastically [8]. In such a situation, alternative compounds such as flavonoids are of great value. As a further point, flavonols are reported to counteract the harmful effects of ROS [28] and to inhibit replicative DNA helicases during cell cycling [29]. The role of flavonols as inhibitors of the Cdk5 kinases is described accurately [30]. In this context, it is of importance that they may have the potential to inhibit both RNA and DNA synthesis [31].

Finally, it is exciting that solely flavonols can reduce tumor growth in cultivated human cancer cells [32–34]. These findings appear to indicate an evolutionarily conserved process of cell cycle inhibition in two totally different living systems, namely humans and some conifer species.

## 5. Conclusions

Previous studies indicated that the nuclei of a number of tree species, and in particular conifers, are capable of importing flavanols, which are attached to histones [5]. In addition, environmental stress factors have been shown to change transcriptionally the histone-catechin complexes [6].

The present study shows that species-typical endogenous growth potential is related to both flavanol and flavonol allocation into the nuclei. The vigorously growing species of *Taxus* and *Tsuga* have a higher capacity for recruitment of flavanols into the nuclei compared to very slow growing dwarf species. Furthermore, on considering the entire growing season, it becomes evident that the longer the flavanols reside in the nuclei the more vigorous is the growth of the species. So far, the dwarf species have shown a visible reduction of the nuclear flavanols from mid-summer to the rest period.

An important aspect of this scenario is that the blue staining flavanols compete with the yellow staining nuclear flavonols. These flavonols are linked to a reduced growth potential for the investigated trees. As a result, members of two flavonoid groups within the same nucleus control the degree of cell cycling in a balanced but adverse fashion.

It may well be that the polyphenols impose a principal impact on growth due to the property to form multi-dentate binding to nuclear proteins or DNAs. This information should be of value for the design of further experiments to determine the different binding strength of flavanols and flavonols to histones, non-histone proteins, RNAs, and DNAs of nuclei.

### Author Contributions

All authors conceived and designed the experiments; Walter Feucht and Markus Schmid performed the experiments; All authors analyzed the data; Dieter Treutter and Markus Schmid contributed reagents/materials/analysis tools; Walter Feucht wrote the paper with contribution of Markus Schmid and Dieter Treutter.

### Conflicts of Interest

The authors declare no conflict of interest.

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