COLOR VARIATION IN STROBILI OF DOUGLAS-FIR

Kim K. Ching, Harvey Aft, and Terry Highley[1]

Introduction

Differences in color of male and female strobili of Douglas-fir often are observed in nature by those working in various phases of tree improvement. Questions have been raised on the mode of inheritance of these visible characteristics and their relation with other factors associated with growth of trees, such as thermo-sensitivity in controlling time of flowering.

A perusal of literature on color of the reproductive organs, the strobili or "flowers" of Douglas-fir, discloses much controversial and incomplete information. Morgenthal (1950) described the female flowers of Douglas-fir as being green or purple, but female flowers tinged with red and green also were reported by the Forestry Branch, Canada (1956). The most recent publication by Franklin (1964) described the female conelets of Douglas-fir as green or red, and the green form as more common. In fact, the inflorescence ranges from green to deep red, with intermediate gradation of pink-red to yellowish green; therefore, describing color in strobili of Douglas-fir by visual observation is inadequate.

Information gathered through personal communications with O. Sziklai suggests that the ratio of flower color of Douglas-fir varies according to locations. For example, a 10-year phenological observation on 128 trees at the University Research Forest, Haney, British Columbia, revealed 28 percent of them had deep red flowers, but only 6 percent of a group of 84 Douglas-fir trees on the campus at Oregon State University exhibited such a color characteristic. Evidently, the particular trait of flower color in Douglas -fir may be nonclinal as was found by Pyror (1963) in _Eucalyptus sideroxylon_ in Australia and by Rubner (1938) _Picea_ abies in Europe. Furthermore, color appears not to have

---

[1]Forest Geneticist, Research Chemist, and Assistant in Forest Products, Forest Research Laboratory and School of Forestry, Oregon State University, Corvallis, Oregon.

a simple Mendelian inheritance but to be more in line with a pattern of multiple gene inheritance.

Flower color and pigments

Generally speaking, color of a plant can be produced either by the refraction of light by physical structures and surfaces or by the absorption of definite wavelengths by pigments present in the plant body. In the study of flower color, of course the latter phenomenon is found. The flower pigments can be divided easily into two groups, the fat-soluble carotenoids and the water-soluble flavonoids. The former group usually is located in the plastid bodies of the plant cell, and is responsible for the showing of red, orange, and yellow color. Clevenger (1964) reported that the latter group produces red, blue, and yellow colors and can be found in the cell vacuole.

The actual colors displayed by flowers are generally not pure pigments either of the flavonoid or carotenoid type. Paech (1955) stated color depends first upon the pigments present and, second, upon other conditions such as concentration of pigments in the cell sap, acidity of the cell sap, and presence and kinds of inorganic ions in the vacuole.

Moreover, genetic phenomena such as mutation, dominance, and epistacy are important in the expression of flower color. Apart from genes that are responsible for production of pigments, there are many other genes that control localization and pattern of pigments and regulate acidity of the sap. Environmental elements such as temperature and light intensity also have been known to affect the pattern of flower color and degree of manifestation.

Methods

At the Forest Research Laboratory, Oregon State University, we have adopted two different approaches in studying flower color in Douglas-fir. One is concerned with describing the character differences that develop when making crosses on trees with various flower colors, to study certain basic patterns of inheritance with these genetic varieties. The other approach is the biochemical-genetic method that involves new analytic techniques such as ultraviolet absorption spectrophotometry and paper or thin-layer chromatography to separate and identify the various pigments present in the materials studied.

Results and discussion

Red, yellow, and green'female strobili, as well as red and green male strobili, were collected from Douglas-fir trees growing at Corvallis, Oregon, for the study of pigment compounds (Figure 1). Flavonoid compounds were separated from these strobili by extraction with methanol and one percent methanolic hydrochloric acid. Paper chromatographic and ultraviolet spectral techniques were used for isolation and identification.

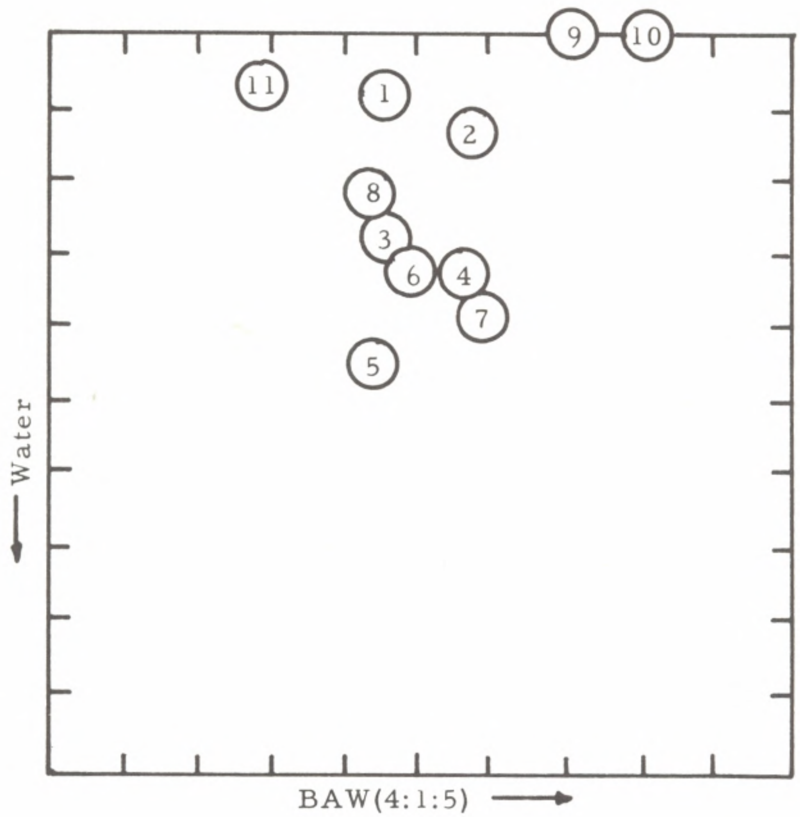
Eleven flavonoid compounds were found and identified in all the flower phenotypes. One particular compound, cyanidin-3-monoglucoside, was found only in the red flowers. Highley (1964) found no differences in flavonoids in male and female flowers of the same phenotype (Figure 2).

Botanists generally have accepted the idea that flowering plants have sprung from gymnosperms; that is, on the evolutionary scale, the coniferous species is more primitive. Data from Highley's experiments (1964) have shown that primrose has 15 anthocyanins, and the potato has 10 anthocyanins, but only one anthocyanin is found in Douglas-fir. Such evidence already is useful in supporting the classification of plants phylogenetically, according to Swain (1963). Further experiments are required to verify the hypothesis that the mechanism that is producing the flavonoid compound in Douglas-fir is the result of simpler metabolic processes.

At the age of 2 weeks, seedlings from crosses made in 1964 were examined for color of hypocotyl. Only in the first mating type listed in Table 1 (green x green) did half the progeny have green hypocotyls. Red pigments seemed prevalent in all other crosses. Whether or not the red pigment in these seedlings is the same as was found in the flower is an interesting point for investigation. Plans are being laid for future studies.

Evidence also has been presented that the flavonoid compounds may be involved in the physiology of the reproductive process. Kuhn and Low (1949) showed that the failure of two varieties of Forsythia to cross pollinate was due to the presence or absence of a certain flavonoid in

Figure 1. Female strobili of Douglas-fir. Outside rows were deep red: the center row was green.



1) Quercetin-3-Monoglucoside
2) Kampferol-3-Monoglucoside
3) 1-Epicatechin
4) d-Catechin
5) Dihydroquercetin-3'-glucoside
6) Eriodictyol-7-glucoside
7) Naringenin-7-glucoside
8) Kampferol-3-diglucoside
9) Quercetin
10) Kampferol
11) Cyanidin-3-Monoglucoside (Found only in acid extract of red flowers).

Figure 2. Schematic chromatogram of red, green and yellow flower extracts of male and female flowers, according to Highley (1964).

Table 1. Colored Stems of Two-Week-Old Seedlings.

Mating type		Stem color		
		Red	Pink	Green
♀	♂	Percent[1]	Percent[1]	Percent[1]
Green	Green	18	32	50
Green	Red	48	48	5
Green	Wind	58	13	29
Red	Red	80	19	0
Red	Green	79	19	3
Red	Wind	79	12	9

[1]Based on all seedlings produced from the particular cross.

the pollen. Ecochard (1963) reports that, in working with Triticum, resistance to rust fungus, Puccinia graminis, was related physiologically to the ability of the plant to synthesize anthocyanin.

Seeds from controlled pollination of various combinations of floral phenotypes in 1965 have been collected and extracted. The immediate plan for this study is to test their germinative capacity and rate of germination, since Rubner (1938) reported in his studies on Norway spruce that although the varieties with red and with green cones occupy the same habitat, they have different adaptation and morphological features; seeds from the red-cone strain germinated more quickly and consequently may have a competitive advantage in the early stage of development. Intensive study of any of these previous findings may shed some light on the significance of flower color in the growth of Douglas-fir .

Literature cited

Clevenger, S. 1964. Flower Pigments. Sci. Am. 210(6) 84-92.

Ecochard, R. 1963. Genetical Characteristics of Some Ethiopian Triticum Resistant to Cercosporella herpotrichoides and Puccinia graminis. Ann. Amelior. Plantes 13(1) 5-25.

Forestry Branch Canada, 1956. Native Trees of Canada. Page 58. Dept. of Resources and Development, Canada.

Franklin, J. 1964. Color of Immature Cones of Several Pacific Northwest Conifers. For. Sci. 10(1): 103-104.

Highley, T. L. 1964. The Flavonoid Compounds of Three Floral Phenotypes of Douglas-fir. M.S. Thesis, Oregon State University, Corvallis.

Kuhn, R. and I. Low . 1949. Morphologie and Chemie der Staubgefasse von Forsythia. Chemische Berichte 82: 474-479.

Morgenthal, J. 1950. Die Wildwachsenden and Angebauten Nadelgeh8lze Deutschlands. Page 23. Gustav Fischer Co., Germany.

Paech, K. 1955. Colour Development in Flowers. Ann. Res. Plant Physiol. 6: 273-298.

Pyror, L. D. 1963. Provenance in Tree Improvement with Particular References to Eucalyptus. Proc. of World Consultation on Forest Genetics and Tree Improvement. Section 2/3. Stockholm, Sweden.

Rubner, K. 1938. Keimung von Samen Grun-und Rotzapfiger Fichten. Tharandter Forstliches Jahrbuch 89: 247-251.

Swain, T., Editor. 1963. Chemical Plant Taxonomy. Academic Press, New York, N. Y.