SHOOT APICES IN TWO HYBRID JUNIPERS

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Introduction

The apex of shoots of the Cupressaceae, to which the genus *Juniperus* belongs, is characterized by four recognizable major zones (Fig. 1): Zone I, consisting of the apical initial cells and their anticlinal derivatives; Zone II, the subapical mother cells; Zone III, the peripheral meristem; and Zone IV, the pith mother cells. Apical initials in this family and in the Taxodiaceae are reported to be only four or less per shoot; in these cells periclinal divisions occur (Fig. 4). However, the peripheral surface layer appears as a discrete protoderm. Complete elimination of periclinal divisions has been attained in the permanent shoots of *Taxodium*, and in *Araucaria* (Araucariaceae); these two genera have, therefore, been regarded as possessing a tunica and of having reached the angiosperm level of organization. The expression "potential tunica" is sometimes used for the peripheral surface layer in the Cupressaceae (Al-Sherifi, 1952).

The subapical mother cells of the Cupressaceae have characteristics common to most of the conifers in being few in number, irregularly arranged and poorly defined. This family and the Taxodiaceae (*Sequoia* excepted) have the smallest Zone II of any of the Gymnospermae. Zone III develops as lateral derivatives of cells of Zone II. In the Cupressaceae Zone III is stratified but exceedingly narrow and not clearly separable from Zone II. In contrast, the pith is easily discernible. Zone IV is made up of one to three pith mother cells in this family. However, pith is often observed very close to the apex (Figs. 2 and 3), and no pith rib meristem is formed. The pith zone is also characteristically narrow, a condition considered to be related, possibly, to the highly ramified type of branching of the shoot.

In the present study an analysis has been made of the shoot apices of terminal and first lateral branches in winter and spring periods of growth from two trees selected from a genetically mixed "swarm hybrid population" (Hall, 1952) of *J. virginiana* L. (eastern red cedar) X *J. Ashei* Buchh. (Ozark white cedar). One of the trees shows the characteristically longer terminal branches

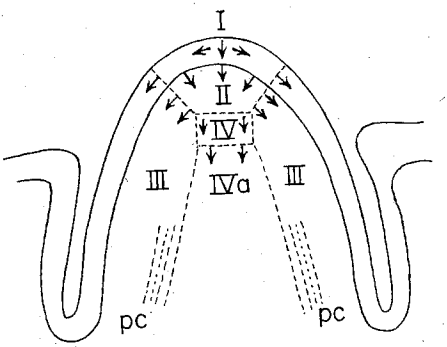

Fig. 1.—Four major zones of shoot apex of Cupressaceae (after Al-Sherifi). I: apical initials and their anticlinal derivatives; II: subapical mother cells; III: peripheral meristem; IV: pith mother cells. IVa and pc are regions of maturing pith and procambium, both situated below actual apical meristem.

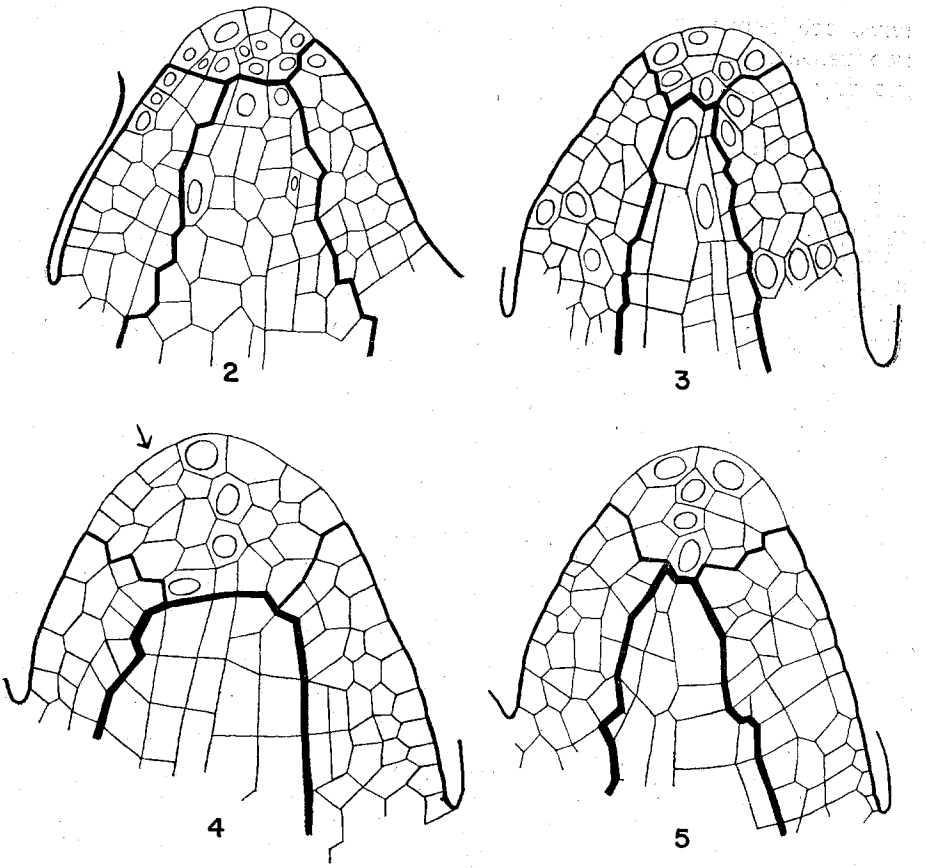

Fig. 2.—Median longisection of shoot apex of long whip. May, 1959. Heavy lines delimit apical meristem above and area of pith. Fig. 3.—Median longisection of shoot apex of lateral of long whip. May, 1959. Fig. 4.—Median longisection of shoot apex of short whip. May, 1959. Fig. 5.—Median longisection of shoot apex of lateral of short whip. May, 1959.

(long whips), as well as certain leaf characteristics of *J. virginiana,* whereas the other one possesses only short terminal branches (short whips) and whip and spur leaves of the *J. Ashei* type. The choice of these two segregates rests on the assumption that it is advantageous to have plants from a similar genetic background. Herbarium specimens are on deposit at the Herbarium of Southern Illinois University, along with typical *J. Ashei* from near Eureka Springs, Arkansas, and typical *J. virginiana* from the Nashville Basin procured by the writer. Not all of the external structural features of the two segregates selected are identical with *Ashei* or *virginiana,* but the terminal branch pat-

terns are remarkably close in the two trees selected from the naturally growing population.

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MATERIALS AND METHODS

The two trees selected are in a natural population of cedars located approximately seven and one-half miles south of Carbondale, Illinois on limestone outcroppings on a hillside near U. S. Highway 51. One of the trees shows much longer terminal branches than the other.

Shoot apices were killed and fixed in F.A.A. Infiltration followed the usual procedures with ethyl alcohol-tertiary butyl alcohol series, and the specimens were embedded in paraffin (Johansen, 1940). Serial longitudinal sections were cut at 10 μ. Very lightly stained sections using Safranin O and Fast Green FCF proved most satisfactory in delimiting apical meristem from adjacent areas. Median longisections were selected for comparisons. Twelve apices of each type were collected in February and again in May. These included long terminals and their first laterals and short terminals and their first laterals. From these four to six median longisections of each type from each collection were obtained. The number of samples analyzed was arbitrarily chosen and the samples themselves were random ones. Samples from both trees were collected on the same collecting trip of each month.

Square millimeter area determinations were made from camera lucida drawings with the use of a planimeter. Included angle readings within the shoot apices at various levels were also made from camera lucida drawings, using a protractor. By included angle is meant that angle read in degrees from apex of shoot to outer limits of level of flanking tissue, pith, or first leaf. Square millimeter areas of apical meristem included the outlines of tips of stems that were clearly differentiated in the staining process from underlying areas (Figs. 2-5). The upper level of pith, as indicated, was easily determinable, as was the level of the first leaf. The level of flanking tissue is interpreted to indicate the upper level of first detectable periclinal walls in the sub-protodermal areas (i.e., peripheral meristem of Zone III). All measurements are shown in Table 1.

RESULTS

The results of the studies are summarized in Figure 6 and Table 1. Outlines in Figure 6 are drawn to scale. Therefore, actual size differences can be easily noted. The uppermost horizontal lines indicate the level of flanking tissue, the next lower ones the level of pith. The lowermost limits of the diagrams indicate the positions of leaves. Proportionate differences are more easily read from Table 1.

Long Whips

The stem apex of the long whips is proportionately the largest of any

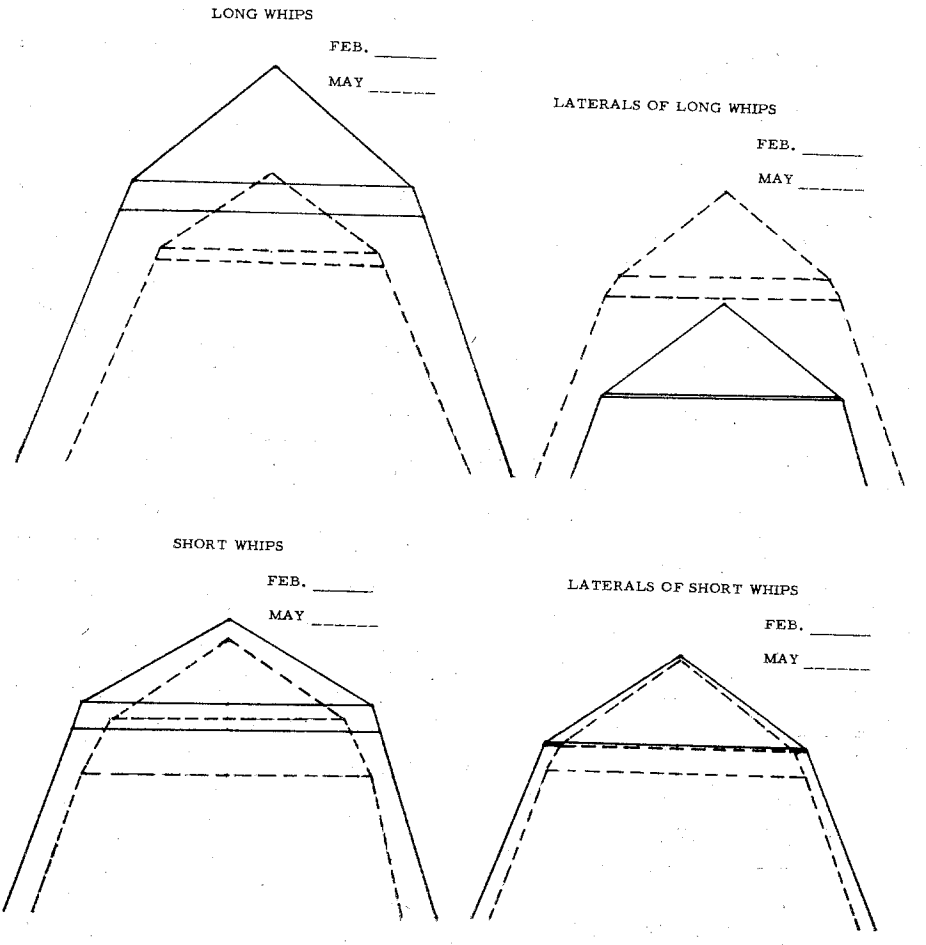

Fig. 6.—Diagram to show relative distances of upper level of flanking tissue (uppermost horizontal lines); upper level of pith (next lower horizontal lines); and upper level of first leaf (lowermost limits of outlines) from shoot apex in long and short whips and in their first lateral branches.

in the late winter when it is interpreted to include the volume from apex to the level of the first leaf. By late spring the long whip apex has become smaller but proportionately broader and lower and with its first leaf proportionately closer to the summit. Its pith is now nearest to the summit of any of the types reported. The amount of apical meristem has decreased markedly and is the smallest of any of the shoot types. The readings of included angles at the levels of first detectable flanking tissue, pith and first leaf all show an increase over

TABLE 1.

LONG WHIPS

	LEVEL OF:	TERMINALS				FIRST LATERALS			
		Included Angles of Shoot Apices	Areas of Shoot Apex within Included Angles (mm.²)	Ratios of Diameter of Shoot Apex to Height	Areas of Apical Meristem (mm.²)	Included Angles of Shoot Apices	Areas of Shoot Apex within Included Angles (mm.²)	Ratios of Diameter of Shoot Apex to Height	Areas of Apical Meristem (mm.²)
February	Flanking Tissue	100°	0.139	2.55	.....	102°	0.106	2.50	.....
	Pith	97°	0.195	2.21	(0.159)	101°	0.112	2.52	(0.119)
	First Leaf	65°	1.154	1.24	.....	79°	0.300	1.63	.....
May	Flanking Tissue	104°	0.079	2.77	.....	101°	0.080	2.46	.....
	Pith	103°	0.096	2.52	(0.097)	98°	0.132	2.20	(0.102)
	First Leaf	68°	0.664	1.35	.....	64°	0.601	1.26	.....

SHORT WHIPS

	LEVEL OF:	TERMINALS				FIRST LATERALS			
		Included Angles of Shoot Apices	Areas of Shoot Apex within Included Angles (mm.²)	Ratios of Diameter of Shoot Apex to Height	Areas of Apical Meristem (mm.²)	Included Angles of Shoot Apices	Areas of Shoot Apex within Included Angles (mm.²)	Ratios of Diameter of Shoot Apex to Height	Areas of Apical Meristem (mm.²)
February	Flanking Tissue	120°	0.102	3.42	.....	112°	0.097	2.96	.....
	Pith	106°	0.195	2.77	(0.148)	111°	0.110	2.88	(0.148)
	First Leaf	73°	0.759	1.46	.....	75°	0.630	1.51	.....
May	Flanking Tissue	115°	0.076	3.00	.....	104°	0.093	2.68	.....
	Pith	98°	0.212	2.13	(0.159)	98°	0.138	2.28	(0.118)
	First Leaf	71°	0.630	1.40	.....	71°	0.555	1.39	.....

the late winter ones. The ratios of diameters to height of shoot at the same levels, comparatively, all show increases. It may be noted that within each series, as the ratios increase the included angles become greater. Conversely, as the ratios decrease the angles become less.

Short Whips

Short whip shoot apices at the three levels measured are smaller in size than long whips in the late winter season. They are proportionately broader with their first leaves and upper level of flanking tissue

closer to the apex. In the later collections the short whip apices also become smaller than in late winter. The level of pith is now farthest from the apex of any of the types reported. The actual amount of apical meristem increases somewhat instead of decreasing as the season progresses. This is the converse of the situation in the long whip apices. The readings of included angles decrease in the May collections instead of increasing as do those of the long whips. Likewise, the ratios all show a corresponding decrease in later collections, again in contrast to the changes in the long whips.

Laterals of Long Whips

The lateral shoot apices of the long whips are the smallest in late winter of any of the types reported. In contrast to the long whips their over-all dimensions increase in the later collections. However, the upper level of the pith becomes proportionately lower, whereas the upper level of the flanking tissue occurs at a higher level by May. The amount of apical meristem decreases. The included angles all decrease. The ratios of diameters to height, comparatively, also show decreases in the later collections.

Laterals of Short Whips

The lateral shoot apices of the short whips are smaller than those of the short whips at the first leaf level in the earlier collections; their dimensions decrease in the later collections, as do those of the short whips. The pith level, likewise, is lower but the upper level of flanking tissue is higher. The amount of apical meristem, the included angles and the ratios all decrease as the season progresses.

Significant structural features among the four types of shoot apices are as follows. Larger sized long whip apices occur during the late winter with a proportionately rather low level of pith and with the lowest first detectable level of flanking tissue and the second lowest included angle of shoot apex at the level of the first leaf. Smaller sized long whip apices are found in the later collections having the highest level of pith, the second highest level of flanking tissue and the third lowest included angle of shoot apex at the level of the first leaf. The first leaf appears proportionately somewhat closer to the apex, but this long whip apex is actually the highest of any of the types with its first leaf farthest from the apex. Short whip apices are lower and proportionately broader than those of the long whips, becoming narrower in the later collections and with the highest level of flanking tissue and the lowest level of pith. The first leaves are actually closer to the shoot apex than are those of the long whips.

Apices of first laterals of the long whips are proportionately broader and much lower than their terminals at the first leaf levels, but they become proportionately narrower than the terminals later in the season, although there is a marked increase in size. Earlier collections show them to be the smallest in size of any of the shoot apices. They are unlike any of the other shoot types in that they show an increase in size by the later date. Apices of the first laterals of the short whips, like those of the short whips themselves,

become smaller and proportionately narrower at the later date. Pith level also becomes relatively low. In contrast to laterals of long whips they show a decrease instead of an increase in size as the season progresses, and their included angles read at levels of flanking tissue, pith and first leaf are all greater.

Discussion

From the measurements of the apices of long and short branches it is apparent that the rate of development is fundamentally different in the two types. Even though pith occurs relatively near the summit in members of the Cupressaceae, when compared to those of other families of conifers, in the vigorously growing short whips it is relatively low, whereas it is extremely high in the long whips. This may be correlated with the relatively much greater amount of apical meristem tissue in the short whips at this time. The short whips at the level of their first leaves still remain proportionately somewhat broader and lower than the long whips with their first detectable periclinal divisions in the sub-protodermal region (peripheral meristem of Zone III) at an extremely high level. In general during this period the first laterals of the short whips resemble their terminals more closely than do the laterals of the long whips. However, they do share similarities as apices of laterals: decrease in included angles; decrease in their ratios; decrease in the total amount of apical meristem; and in both types the level of pith drops.

How even a very irregular mass of cells gets itself straightened out into a formed structure poses one of the most difficult problems of morphogenesis. The differences in rate of development of the shoot apices is highly suggestive of auxin differences. More important than the layering in the meristem, perhaps, are the physiologically genetic differences between the two tree types. Layering, for example, may or may not occur during certain growth phases of apices (Parke, 1959); however, the end results show quite different shapes of apices and proportions of their recognizable parts (Fig. 6). There is need for periodic collections over several seasons' growth before deciding upon the significance of layering, if any. In itself it appears to have little significance. It is possible that it is intimately connected with initiation of leaf primordia. But this, in turn, would be correlated with rate of cell divisions of the inner portions of a meristem, for we know that if the inner portions of a meristem are growing faster than the outer ones, the outer tend to be layered.

According to Hall's hypothesis the individual trees in the population with long whips reflect more "*virginiana*" genetically in this respect, whereas those with only short whips are more like "*Ashei*". The structural differences observed in the apices from the tree with long whips and from the tree with short whips only would, according to the hypothesis, reflect differences in the genetic constitution of the two individuals.

The future character of the shoot seems to be determined by the character of the shoot apex from which

it arises. Certain effects of environmental factors would be expected, and a certain amount of plasticity of apex, and therefore shoot character, probably exists. The differences between long and short shoots appear to be fundamentally comparable in character, however, to the long and dwarf shoots of *Ginkgo* (Gunckel *et al.*, 1949).

The relations between terminal and lateral apices determine the specific forms in the plant itself. The differences noted could well be the result of auxin distribution, although what determines differential distribution of auxin is not known.

Any relationship or correlation that may exist between the apices of long and short whips and ontogenetic differences in the types of leaves developing from them remains to be investigated. With the pronounced difference in appearance and behavior of these two kinds of apices it appears that the rate of leaf formation is fundamentally different. It is possible that there is a correlation with, if not a controlling influence of the rate of growth of apex to the rate of development of different portions of the leaf types formed. If this is the case, the classification of leaf types found would be referable to the growth pattern of the apex itself.

Summary

Differences in the patterns of growth in stem apices of long and short whips and their first laterals, as reflected by i) increase or decrease in volume from winter to spring; ii) changes in included angles read from apex to upper levels of flanking tissue, pith and first leaf; and iii) shifts in discernible amounts of apical meristem, are given.

1. In the spring the apex of the long whip is outstandingly different in having the smallest amount of apical meristem and the highest level of pith. It becomes proportionately somewhat broader at the first leaf level, instead of proportionately narrower, as in the case of the short whips. Its first leaf, however, remains the greatest actual distance from its shoot apex of any of the four types.
2. Unlike the other types of apices, the laterals of the long whips increase in size from the level of first leaf as the season progresses.
3. In the spring the apex of the short whips has the largest amount of apical meristem, the lowest level of pith and the highest level of flanking tissue. It is also the broadest of any apex, proportionately, at the first leaf level.
4. In the later collections the laterals of both long and short whips are alike in being smaller than their respective terminals, but the apices of the laterals of the short whips are proportionately broader than those of the long ones. In this respect they resemble more closely their own terminals.
5. According to Hall's hypothesis the differences in the types of apices would be regarded as inherent ones, reflecting differences in genetic constitution of two

individuals of a natural hybrid population of *J. virginiana* X *J. Ashei*.

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