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SCLEREID DISTRIBUTION IN THE LEAVES OF PSEUDOTSUGA UNDER NATURAL AND EXPERIMENTAL CONDITIONS[1]

KHALIL H. AL-TALIB AND JOHN G. TORREY

ABSTRACT

AL-TALIB, KHALIL H., and JOHN G. TORREY. (U. California, Berkeley.) Sclereid distribution in the leaves of Pseudotsuga under natural and experimental conditions. Amer. Jour. Bot. 48(1): 71–79. Illus. 1961.—A study of the distribution of sclereids in cleared leaves taken from 1-, 2-, and 4-year-old shoots of an adult tree of *Pseudotsuga menziesii* (Mirb.) Franco showed a repeated pattern of sclereid distribution along the shoot axis with many sclereids in the basal leaves grading into few or no sclereids in the terminal leaves of each year's growth. Attempts were made to influence sclereid distribution by bud defoliation of attached branches with and without auxin treatment and by testing the effects of growth-regulating substances on sclereid formation in leaves of excised buds of *Pseudotsuga* cultured in vitro. Whereas removal of the basal ¾ of the leaves at the time of bud unfolding had no effect on bud, leaf or sclereid development, removal of the leaves of the upper half or complete defoliation led to premature expansion of next year's terminal bud with leaves developing in part from presumptive bud-scale primordia. Indoleacetic acid at 0.5% in lanolin paste applied to the defoliated region prevented this premature bud expansion. Defoliation of the basal half did not affect sclereid formation in the terminal leaves. Sclereid development in leaves of prematurely expanded buds on defoliated branches was normal except in the few cases where bud expansion occurred in the presence of low-auxin concentrations. Then, sclereid development was inhibited. Sclereid formation in leaves of excised buds grown in nutrient culture was generally much less frequent than in intact branches, and auxin treatment still further reduced the frequency of sclereids. It was concluded that sclereid initiation and differentiation in the intact plant may well be under the control of hormonal factors in the plant, one of which may be auxin.

IN recent years, sclerenchymatous idioblasts or sclereids have been the subject of much investigation by plant anatomists. Their occurrence has been noted in leaves, stems, roots, fruits and seeds of angiosperms. Further, their morphology and ontogeny have been studied and their taxonomic importance evaluated (Foster, 1944, 1945, 1946, 1947, 1955a, 1955b, 1956; Bloch, 1946; Bailey and Nast, 1948; Rao, 1951).

In gymnosperms, sclereids have been less intensively studied; they have been shown to occur, however, in both the vegetative and reproductive tissues of various members of the Coniferales. Allen (1945) noted the occurrence of sclereids in the leaves of *Pseudotsuga taxifolia*. Sterling (1947) studied the ontogeny and morphology of the sclereids in the shoot of *P. taxifolia* and observed differences in the number of sclereids between individual branches. He also observed that sclereids in the cortex were less abundant but were larger than those in the pith. Griffith (1950) reported the presence of numerous crystalliferous idioblastic sclereids in the leaves of certain species of *Araucaria*. Sacher (1954) found that sclereids occur in the cortex of shoots of *Pinus ponderosa*, extending up to within 2 mm. of the shoot apex. Kitamura (1956) studied the distribution of foliar sclereids of *Sciadopitys verticillata* and found that various zones of the leaf differ in the number of sclereids

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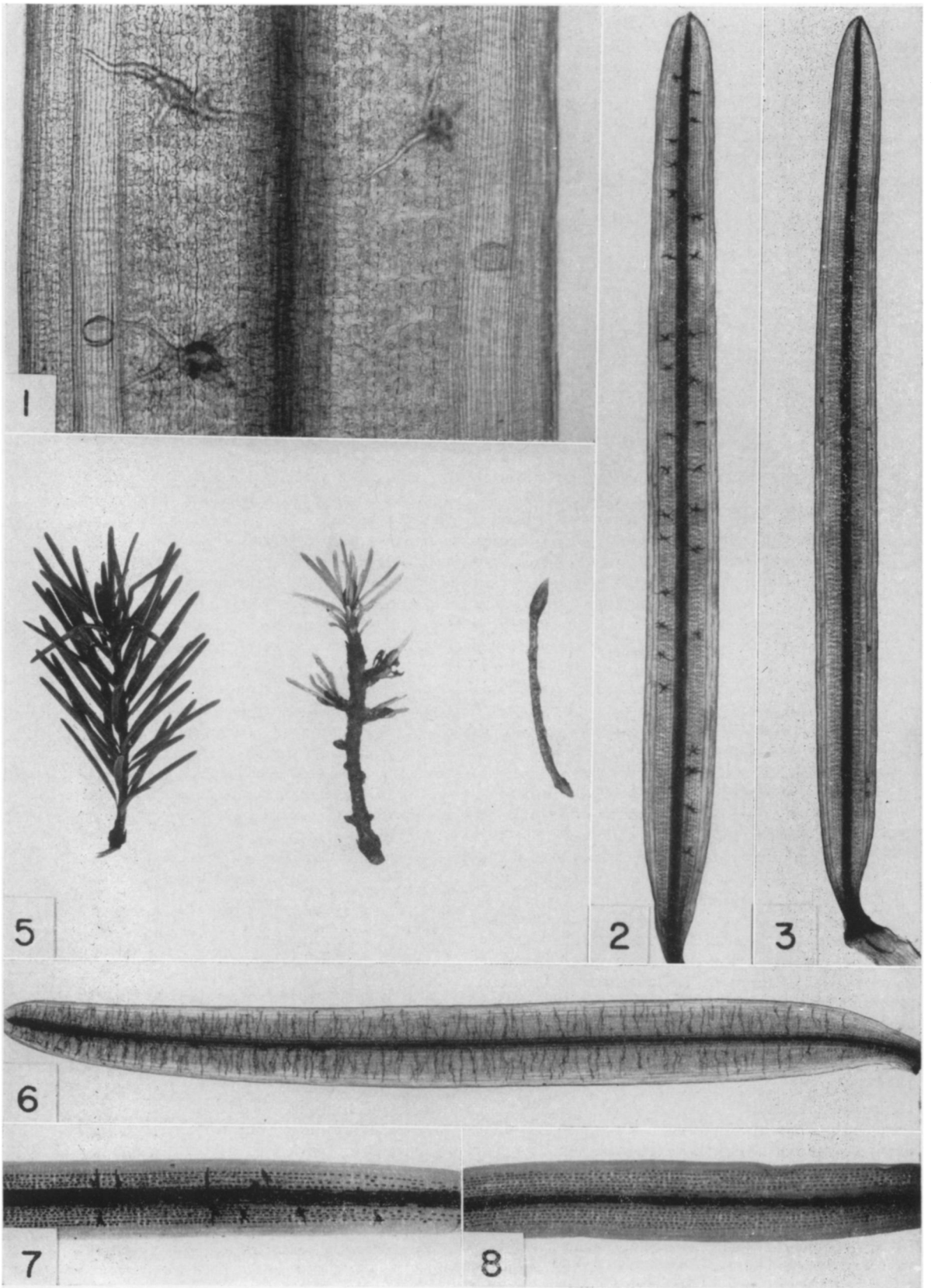
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TABLE 1. *Sclereid distribution in leaves along the shoot axis of 1- and 2-year-old branches of Pseudotsuga menziesii*

A. *1-year-old branch (1954)*

Segment number (15 leaves per segment)	Number of sclereids per segment	Average number of sclereids per leaf and S.E.
1 (Terminal)	32	2.13 ± 0.41
2	128	8.53 ± 0.72
3	228	15.20 ± 0.82
4	338	22.53 ± 0.79
5 (Basal)	286	19.07 ± 2.95

B. *2-year-old branch*

Segment number	Number of leaves in segment	Total number of sclereids	Average number of sclereids per leaf and S.E.
	1954		
1 (Terminal)	20	31	1.55 ± 0.27
2	19	158	8.33 ± 0.62
3	15	245	16.33 ± 0.65
4	16	428	26.75 ± 0.95
5 (Basal)	18	450	25.0 ± 2.28
	1953		
1 (Terminal)	15	34	2.27 ± 0.35
2	17	99	5.82 ± 0.38
3	18	228	12.67 ± 0.72
4	19	356	18.74 ± 0.59
5	15	258	17.20 ± 1.87

present with the highest number of sclereids occurring toward the tip of the leaf. With the exception of the study by Kitamura, there appears to be no quantitative study of sclereid distribution in plants.

During a preliminary study of the occurrence of sclereids in the leaves of *Pseudotsuga menziesii* (Mirb.) Franco[2] (Douglas fir), there was noted a rather striking and distinctive pattern of sclereid distribution in the leaves along the shoot axis. The regularity of the pattern suggested that sclereid formation in leaves of *P. menziesii* was rigidly controlled by internal physiological factors and that experimental methods might be used to upset the regularity of sclereid formation in an attempt to discover the physiological factors affecting sclereid distribution. In this paper are presented the data concerning sclereid formation in leaves of *P. menziesii* in nature and an account of certain experiments designed to affect the regular pattern of sclereid formation.

MATERIALS AND METHODS.—Fresh leaves and buds were obtained from a single old tree of *P. menziesii*, approximately 50 ft. tall and 1 ft. in diameter at the base, growing in the Botanical Garden, University of California, Berkeley. Preserved specimens of *P. menziesii*, *P. macrocarpa*, *P. glauca* and *P. sinensis* were obtained from the Herbarium of the University of California.

The fresh material was collected at monthly intervals from October 1953 to June 1957, was killed and fixed in formalin–acetic acid–alcohol (FAA) with aspiration and was then stored in fresh FAA until needed. For the study of sclereid distribution, large numbers of leaves from branches at various levels on the tree were collected, marked according to their location along a branch and then cleared as whole leaves in sodium hydroxide.

For clearing, the leaves were first boiled in 70% ethyl alcohol to remove pigments (several hours for old leaves from the intact plant; 30–60 min. for leaves from cultured buds). After washing in water, the leaves were placed in sodium hydroxide solution (5% for old leaves; 2.5% for young leaves from cultured buds) and placed in an oven at 35–40°C. Several changes of sodium hydroxide solution were needed to complete clearing in some cases. After washing with water again, the leaves were dehydrated through an ethyl alcohol series to 70%, at which stage they were stained with safranin. Dehydration was then completed through to absolute ethyl alcohol, followed by 2 washes of absolute ethyl alcohol–xylene (1:1) and finally with pure xylene, then mounting in piccolyte. In cleared preparations of this type, cellular detail is quite evident and the large ramifying sclereids are readily discernible under relatively low magnification (fig. 1).

Defoliation experiments on the intact tree were performed in order to determine the effect, if any, of leaf removal on the distribution of sclereids in the remaining leaves. Complete or partial defoliation of the newly formed branches was made slightly before or at the time of expansion of the bud, which occurs between February and April at Berkeley. The unopened buds were descaled with forceps and then the leaves removed individually with scissors and sharp-pointed forceps. Lanolin paste was applied to the defoliated areas of the branches to

[2] Recent nomenclatural changes require the use of this name in place of the more familiar one, *P. taxifolia* (Lamb.) Britt.

Fig. 1–3; 5–8.—Fig. 1–3. Cleared leaves of *Pseudotsuga menziesii*.—Fig. 1. Portion of cleared leaf of *P. menziesii*, showing mature ramifying sclereids in spongy parenchyma on either side of the midrib. The elliptical structures are tylosoids in the resin canal cavity. ×45.—Fig. 2. Cleared whole leaf of *P. menziesii* taken from the basal part of a 1-yr.-old branch. Note the abundance of stellate-shaped sclereids. ×5.—Fig. 3. Cleared whole leaf of *P. menziesii* taken from the uppermost part of a 1-yr.-old branch. Note the complete lack of sclereids. × 5.—Fig. 5. One-yr.-old branches of *P. menziesii:* left, untreated intact branch with next year's unexpanded terminal bud; center, completely defoliated branch treated with lanolin paste, showing next year's prematurely expanded terminal bud and lateral buds; right, completely defoliated branch treated with 0.5% IAA in lanolin, showing next year's unexpanded terminal bud. ×1.—Fig. 6. Cleared whole leaf of *P. sinensis*, showing numerous filiform sclereids. ×7.5.—Fig. 7–8. Cleared leaves of *P. menziesii* taken from excised buds cultured in vitro for 2 mo. ×10.—Fig. 7. Medium contained 10^{-6} M indolebutyric acid.—Fig. 8. Medium contained 10^{-4} M indolebutyric acid. Note complete absence of sclereids.

TABLE 2. *Parallel samples of leaves of P. menziesii with and without lateral buds in their axils*[a]

Number of leaves in sample	Total number of sclereids in sample	
	Bud in axil	No bud in axil
1	19	18
1	10	11
1	14	14
1	23	22
4	91 (22.7)	90 (22.5)
4	147 (36.8)	149 (37.2)
6	158 (26.3)	139 (23.1)
23	329 (14.3)	336 (14.5)

[a] The average number of sclereids per leaf is given in parentheses.

prevent desiccation. In certain experiments, growth-regulating compounds were added to the lanolin paste to determine their effect on sclereid formation on the premise that the leaves when attached might influence bud expansion by virtue of their production of auxin. Plain lanolin was applied to control branches. All applications were renewed every 2 days.

OBSERVATIONS AND EXPERIMENTAL RESULTS.—*The distribution of foliar sclereids in intact shoots.*— Sclereids are found as scattered idioblasts in the spongy parenchyma of the leaf of *Pseudotsuga menziesii* and are absent from the palisade parenchyma. They are distributed in approximately equal numbers on both flanks of the midrib (fig. 2), but are never found in the spongy parenchyma adaxial to the vein. The prevailing diffuse distribution of sclereids throughout the spongy mesophyll of *P. menziesii* contrasts strikingly with some of the species of *Monstera* where they are restricted to a specific series of mother cells (Bloch, 1946) or as in *Mouriria* and *Boronia* where they are associated with vein endings (Foster, 1947, 1955b).

A wide range of variation in the number of sclereids in the leaves of an individual branch was noted. The difference in sclereid number in the basal leaves of a 1-yr.-old branchlet (fig. 2) and in the terminal leaves of the same branchlet (fig. 3) was very striking. A detailed analysis was made of the average number of sclereids in the leaves in relation to the distance of the leaves from the terminal bud. A 1-yr.-old branch was divided along the axis into segments of 15 leaves each and the total number of sclereids in the leaves of each segment was counted (table 1A).

It was at first supposed that the observed variation in sclereid number was associated with the age of the leaves, with the younger leaves containing fewer sclereids than the older leaves. A similar analysis was made of 2-yr.-old and 4-yr.-old branches. From table 1B it becomes apparent that the pattern of sclereid distribution is repeated each year in the 2-yr.-old branch and the sclereid formation is not related to age of leaf per se but to position of the leaf along the shoot axis for each year of shoot development. In fig. 4 is shown the sclereid distribution in successive years of shoot growth in a 4-yr.-old branch expressed as average number of sclereids per 20 leaves. For each year's new shoot growth, regardless of the age of the branch, the average number of sclereids per leaf is at a minimum in the apical leaves, rises to a maximum in the leaves toward the base until at the extreme base the average number decreases. There is variation in the different maxima; this variation is evident in table 1B as well as in fig. 4. The consistent decrease in number of sclereids at the base was related to the fact that several of the most basal leaves have few or no sclereids, so that the average number per segment is reduced.

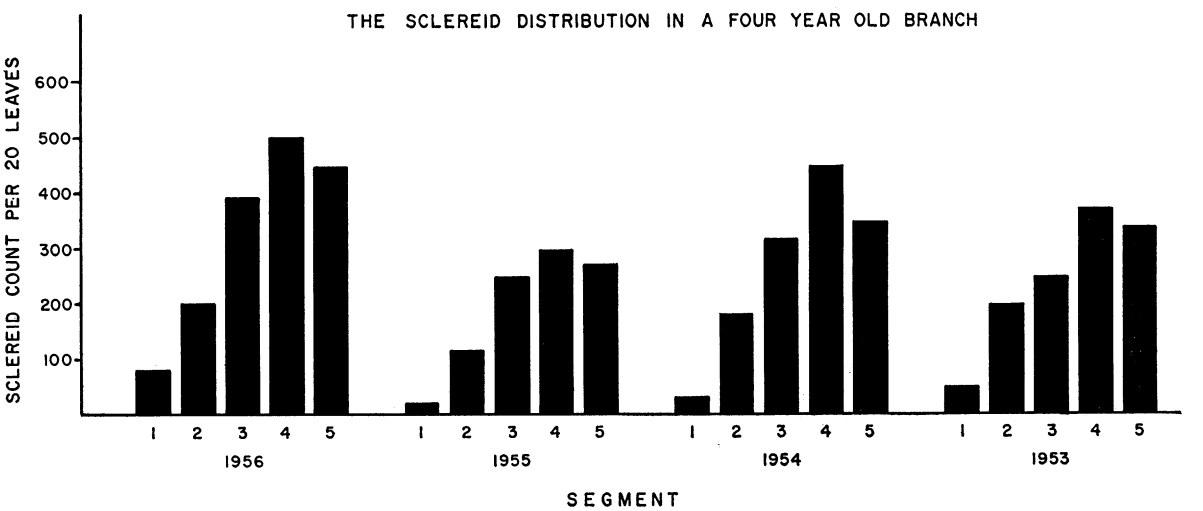

Fig. 4. Histogram showing the pattern of sclereid distribution along the axis of a 4-yr.-old branch of *P. menziesii*. Each segment contained approximately 20 leaves.

TABLE 3. *The distribution of sclereids in the terminal and basal samples of leaves of preserved shoot specimens of Pseudotsuga*[a]

	Number of sclereids per sample	
	Terminal	Basal
P. glauca	542 (108)	828 (165)
P. macrocarpa	204 (41)	533 (106)
P. sinensis	1006 (201)	1713 (342)
P. menziesii	15 (3)	93 (18)

[a] 5 leaves per sample. (The average number of sclereids per leaf is given in parentheses.)

In searching further for morphological relationships which might influence sclereid distribution, leaves which bore axillary buds were sampled. A number of leaves with lateral buds in their axils were taken from various branches, together with leaves at the same level containing no axillary buds. In table 2 the data presented show that the presence of a lateral bud does not affect the number of sclereids formed in the leaf subtending it. Although the number of sclereids formed in each leaf varies from one level of the axis to another, the data further indicate that the distribution of sclereids in the leaves is very similar on all sides of the axis at a given level.

The distribution of sclereids in the leaves of preserved specimens of *Pseudotsuga glauca*, *P. macrocarpa*, *P. sinensis* and *P. menziesii* was also studied. These specimens had been collected from geographical regions outside the San Francisco Bay area. As is seen in the data in table 3, striking variations in the average number of sclereids from species to species was observed. In *P. sinensis*, the sclereids were long and filiform (fig. 6) in contrast to the stellate shape in the other species studied. In general, however, the pattern of sclereid formation in leaves along the shoot axis was consistent, with the basal leaves containing more sclereids than the upper or terminal leaves within any one year's growth. Because of the regularity of pattern of sclereid formation, *Pseudotsuga* seemed to be particularly suited for studies of the physiological mechanisms controlling the process. Experimental procedures were devised which might be expected to upset or modify normal sclereid occurrence. Of particular interest were experiments with defoliated shoot branches growing on the intact tree and experiments with isolated buds of *P. menziesii* cultured in vitro.

The effects of defoliation and auxin treatment on leaf development. — Within the dormant bud of *Pseudotsuga* immediately prior to bud expansion there are found the fully formed, unexpanded leaves of the current year's growth. These are surrounded and enclosed by thin, brown, outer bud scales and inner transparent bud scales which loosen and unfold as the bud expands. Within the bud at the shoot apex itself, the bud scale and leaf primordia for next year's bud are already beginning to form. In the fully formed but unexpanded leaves, sclereid initiation has already been completed, while sclereid initiation in primordial leaves has not yet occurred. Defoliation of the unexpanded buds at the time of bud expansion was performed in order to study the effect of leaf removal on the development of the remaining leaves and to ascertain the effect of defoliation on sclereid development.

Removal of up to ¾ of the lower leaves of the branchlet did not affect the normal development of the remaining terminal leaves. It was found, however, that complete defoliation or removal of the leaves of the upper half of the elongating branch caused a premature expansion of the next year's terminal bud. Since at the time of defoliation these leaves were primordial and sclereid initiation had not yet been determined, it was of special interest to study the effect of defoliation on leaf development and sclereid formation in these prematurely expanding structures.

Following removal of the leaves along the upper half of the bud or all of the leaves of the bud, there occurred within a period of 4–8 wk. a premature expansion of next year's bud (fig. 5). The leaves of this newly expanded bud, as well as the shoot, are considerably smaller than normal. These leaves develop from the oldest basal primordia which, under ordinary conditions, would have formed bud scales. Thus the defoliation alters the

TABLE 4. *The effect of defoliation and of auxin treatment on the premature expansion of terminal buds in P. menziesii*

Concentration of indoleacetic acid in lanolin (%)	Number of branches tested	Number of dead buds	Number of unexpanded buds	Number of expanded buds
	Lower half defoliated			
0.0	10	0	10	0
0.1	10	0	10	0
0.5	10	0	10	0
1.0	10	4	6	0
	Lower three-fourths defoliated			
0.0	25	0	25	0
0.1	25	0	25	0
0.5	25	0	25	0
1.0	25	16	9	0
	Upper half defoliated			
0.0	10	1	2	7
0.1	10	0	7	3
0.5	10	1	8	1
1.0	10	9	1	0
	Completely defoliated			
0.0	20	1	2	17
0.1	20	0	15	5
0.5	20	2	16	2
1.0	18	16	2	0

normal developmental pattern of the foliar primordia, causing certain primordia to develop into leaves which otherwise were destined to become bud scales.

The time of defoliation is critical, there being a limited period only during which defoliation will produce premature bud expansion. In Berkeley, only during April and May, when the meristem is forming primordia which normally form scales, was it possible to cause premature bud expansion through defoliation.

Experiments were set up to determine the relative effectiveness of partial defoliation of the upper and lower portions of the branch and complete defoliation with and without auxin treatment on bud expansion. The results of these experiments are summarized in table 4.

It is apparent that the presence of the leaves prevents premature expansion of the terminal bud. However, it is clear that only the terminal leaves, i.e., only the upper ¼ of the leaves along the axis, contribute significantly to the suppression of bud expansion, since no bud expansion occurred when up to ¾ of the lower leaves were removed. One can also conclude from this experiment that indoleacetic acid applied to the stem surface in place of the upper leaves after defoliation effectively prevents bud expansion at relatively low concentration for this method of treatment. At high concentration, the auxin becomes toxic. It is interesting to note that the inhibitory effect must be transmitted in nonpolar fashion toward the apex. Complete defoliation differs in its effect from defoliation of the upper half in that, besides inducing a higher proportion of prematurely expanded terminal buds, it causes earlier terminal bud expansion and also expansion of lateral buds along the axis (fig. 5).

The effect of defoliation and auxin treatment on sclereid formation.—Defoliation of the lower ¾ of the branch did not affect the number of sclereids in the remaining terminal leaves of the bud. The lack of effect on sclereid distribution in these terminal leaves of the bud was not unexpected since the terminal leaves at the time of bud opening had already formed their sclereid initials. The early determination of sclereid initials in all the fully formed leaves of the expanding bud precluded the study of factors influencing sclereid initiation in these leaves. However, in the case of the premature expansion of the bud of next year's growth following defoliation, sclereid initiation actually occurs as a part of the development of the leaves from their primordial condition at the meristem. Here the effects of defoliation and auxin treatment on sclereid formation can be observed.

During the course of these studies on *Pseudotsuga*, no sclereid formation in the bud scales of vegetative buds was ever observed. On occasion, sclereids were found in the scales and bracts of the female cones. It was interesting, therefore, to observe sclereid formation in leaves of prematurely expanded buds following defoliation with and without auxin treatment.

In table 5 are presented data from counts made of leaves from prematurely expanded buds following complete defoliation. In one treatment, lanolin only was applied to the defoliated area; in other treatments lanolin containing 0.1% and 0.5% indoleacetic acid had been applied. The leaves were killed and cleared after they had completed their expansion. Since auxin treatment usually suppresses bud expansion, only in those cases where bud expansion did occur, such as at low IAA concentration, were determinations of sclereid distribution possible.

The number of sclereids in the leaves of newly expanded buds following defoliation and treatment with plain lanolin was found to vary widely, from as few as 3 or 4 to as many as 30 or 35 per leaf. In general, however, the average number of sclereids per leaf approached that of normal leaves. Thus, primordia which would normally develop into bud scales lacking sclereids have developed into essentially normal leaves with typical sclereid distribution.

Auxin treatment, which was shown to suppress leaf expansion in the terminal bud, appears also to inhibit sclereid formation in those leaves which do develop following defoliation.

Sclereid formation in leaves of isolated buds grown in vitro.—In experiments with isolated buds of *P. menziesii* grown in nutrient media (Al-Talib and Torrey, 1959), leaf development was found to be markedly influenced by the constituents of the medium. Of particular interest was the effect of auxins on leaf development and of sclereid initiation in these buds. Leaf expansion occurred in the absence of added auxin in the medium and, in the case of most auxins, when they were added at concentrations of 10^{-6} M. However, α-naphthaleneacetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) at 10^{-6} M stimulated callus develop-

TABLE 5. *Sclereid formation in leaves of prematurely expanded buds of P. menziesii following defoliation, with and without IAA treatment*

Sample number	Number of newly formed leaves	Total number of sclereids in sample	Average number of sclereids per leaf
	Lanolin only		
1	23	191	8.3
2	11	210	19.1
3	15	165	11.0
	Lanolin plus 0.1% IAA		
1	7	6	0.9
2	12	23	1.9
3	8	5	0.6
	Lanolin plus 0.5% IAA		
1	14	6	0.4

ment at the expense of leaf growth. At higher concentrations (10^{-5} and 10^{-4} M), all auxins tested retarded leaf expansion. Two reputed auxin antagonists, 2,6-dichlorophenoxyacetic acid (2,6-D) and 2,3,5-triiodobenzoic acid (TIBA) at 10^{-6} M had no effect on leaf development. At higher concentrations, they also produced a retardation of leaf expansion.

In table 6 are presented data showing the effect of various growth-regulating substances related to auxins on sclereid formation in leaves of excised buds cultured in vitro. The leaves examined in this experiment were taken at random from the basal part of 10 buds cultured for 2 mo. beginning in November under conditions described by Al-Talib and Torrey (1959). The basic medium containing 2% sucrose and added auxin was used throughout.

It is evident that growth regulators at a concentration of 10^{-4} M, where they did not cause actual death of the bud, induced a very marked reduction in the number of sclereids formed as compared to the controls without auxin (fig. 8). At lower concentrations of auxins, there was no significant difference in the leaves between the treated and the untreated buds (fig. 7). In general, the average number of sclereids formed per leaf was relatively low. In other experiments in which the isolation of the buds from the tree was made in April, late in bud development, the average number of sclereids per leaf was consistently higher. These observations suggest that early isolation of the bud in some way reduced the capacity of the leaves to initiate sclereids. Since completely normal bud development was not achieved in vitro (Al-Talib and Torrey, 1959), it is possible that nutrients essential for normal cellular differentiation were lacking in the medium used in these experiments. Auxin treatment at high concentration further suppressed the initiation process.

DISCUSSION.—The relation between auxin and the behavior of buds has been studied in the past by those interested in the problem of apical dominance and by others studying the effect of defoliation on lateral branching and leaf development. Dostál (1926) observed that the presence of young leaves retarded the growth of axillary buds. Thimann and Skoog (1934) studied the inhibitory substance in *Vicia faba* and were able to reproduce the inhibitory effect with externally supplied auxin. Avery (1935) showed that the amount of auxin in leaves tends to vary inversely with their age, an observation which has been confirmed by more recent workers (e.g., Wetmore and Jacobs, 1953). Delisle (1937) and Goodwin (1937) were both able to demonstrate that inhibitory influences from young leaves affecting associated lateral bud or leaf development could be replaced by the application of auxin in lanolin on the petiolar stumps of the excised leaves.

All of these studies suggested that in *Pseudotsuga* auxin production, especially by the terminal leaves in the expanding shoot, may act to prevent the premature expansion of the terminal bud. The experiments reported above, in which defoliated shoots were treated with auxin, tend to confirm the idea that auxin production in the young leaves is important in controlling the development of leaf primordia as well as influencing leaf expansion in the terminal bud. That foliage leaves could develop from presumptive scale primordia under experimental conditions was early observed by Goebel (1880) in experiments on *Prunus*. Such changes have been discussed from the point of view of bud-scale morphology by Foster (1928). The evidence from the present experiments clearly suggests that the determination of foliage-leaf primordia might be usefully examined with respect to the auxin relations of the structures concerned. Clearly, in the absence of determinations of the auxin production by leaves of *Pseudotsuga*, no firm conclusions concerning the role of auxin in the determination and development of foliar primordia can be made from these studies.

The determination of sclereid initials in foliar leaves of *Pseudotsuga menziesii* is clearly under some precise control of changing physiological conditions active during leaf and shoot development. Insofar as there is evidence on the matter, it would seem probable that hormonal factors, especially

TABLE 6. *The effect of various plant growth-regulators on the occurrence of sclereids in leaves of isolated buds of P. menziesii cultured in vitro for two mo.*

Growth Regulator	10^{-6} M		10^{-5} M		10^{-4} M	
	Number of leaves	Average no. of sclereids per leaf and S.E.	Number of leaves	Average no. of sclereids per leaf and S.E.	Number of leaves	Average no. of sclereids per leaf and S.E.
IAA	24	3.7 ± 0.8	18	2.8 ± 0.6	25	0.5 ± 0.1
IBA	25	2.5 ± 0.5	21	3.2 ± 0.5	29	0
NAA	19	1.7 ± 0.4	poorly developed leaves		poorly developed leaves	
2,4-D	21	2.5 ± 0.4	poorly developed leaves		poorly developed leaves	
2,6-D	24	3.4 ± 0.6	25	2.2 ± 0.5	25	0.2 ± 0.1
TIBA	20	2.5 ± 0.5	21	1.9 ± 0.4	----	----
None	24	2.3 ± 0.4				

auxins, may play a role in determining when and where sclereid initials develop. Taken together, the experiments on defoliation with and without auxin treatment and those on auxin treatment of isolated buds all suggest that auxin levels in the leaf influence the development of sclereids, with high auxin levels tending to suppress sclereid development. The observed distribution of sclereids in leaves borne along the intact shoot axis of *Pseudotsuga* fits in well with the idea that high auxin levels in the youngest terminal leaves are associated with low sclereid initiation, and low auxin levels in older, more basal leaves occur where sclereids are found more frequently. The lack of sclereids in the basalmost leaves remains unexplained.

In partially defoliated buds, removal of the basal leaves does not affect sclereid development in the remaining leaves. This result would be expected if the basal leaves in themselves contribute little auxin to the developing shoot system. In completely defoliated buds which showed premature expansion of next year's buds, sclereids do develop in the newly formed leaves derived from primordia which normally would have developed into bud scales lacking sclereids. In these buds the number of sclereids formed per leaf varied widely, some leaves containing 3 or 4 while others had 30 to 35, but, in general, the average number of sclereids per leaf approached that found in normal leaves (compare tables 5 and 1A). Unfortunately, no record was made of the gradient of sclereid distribution in leaves of plants which had been defoliated.

When completely defoliated buds were treated with auxin in lanolin, the development of foliar leaves from presumptive bud-scale primordia was markedly reduced, but in certain cases, foliage leaves did develop. In such leaves, the incidence of sclereids was very low. Here auxin clearly inhibited the development of sclereids.

From the experiments with excised buds grown in culture, it is also evident that high auxin concentrations tend to suppress sclereid development. It is uncertain whether the inhibitory effects of auxin are specific in nature or result from nonspecific inhibition of leaf development in general.

It is a striking fact that in the leaves of excised buds grown in culture, whether in the presence or absence of added auxin, the average number of sclereids per leaf is quite low, comparing very closely to the average number produced in the terminal leaves of each year's growth in the intact plant. There are at least 2 possible interpretations of this result: (1) the auxin level in the isolated buds grown in vitro is high, leading to suppression of sclereid development; or (2) isolation of the buds deprives them of some unidentified substance normally provided by the plant which is essential to sclereid differentiation. In the absence of experimental observations which bear directly on either of these interpretations, conclusions as to the controlling factors in sclereid development in these leaves must be deferred, although the possible role of auxins in determining sclereid distribution cannot be disregarded.

In recent experimental studies on sclereid formation in leaves of *Camellia*, Foard (1958) produced evidence that sclereid initials develop to maturity in detached immature leaves cultured in a liquid nutrient medium with agitation and artificial illumination. High sugar concentrations in the medium inhibited sclereid differentiation; this inhibition was attributed to an osmotic effect. In surgical experiments with leaves attached to the plant, Foard (1959) showed that sclereid formation was determined by the position along the margin of the leaf. No evidence for hormonal control was forthcoming from these experiments which do show, however, that determination of sclereid initiation in leaves is subject to experimental manipulation and thus subject to study by experimental means.

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EARLY STAGES IN THE FORMATION OF INTERNAL BARRIERS TO GENE EXCHANGE BETWEEN DIPLOID SPECIES OF SOLANUM[1]

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ABSTRACT

GRUN, PAUL. (Pennsylvania State U., University Park.) Early stages in the formation of internal barriers to gene exchange between diploid species of Solanum. Amer. Jour. Bot. 48(1): 79–89. Illus. 1961.—Internal barriers restricting development of F_1 hybrids in crosses among closely related diploid species of *Solanum* belonging to the series Tuberosa and Commersoniana were studied to determine the early stages in development of barriers to gene exchange. Both of the parental series (species complexes) are native to South America, the Tuberosa occurring along the length of the Andes, while the Commersonia occur at lower altitudes in Argentina, Paraguay, and Bolivia. They are distinguished by a fair number of morphological characters. Following crossings between the species of each series and between species of the different series comparisons were made of the number of berries formed per attempted cross, seed per berry, percentage seed germination, and F_1 vigor and pollen fertility. Although the species used were closely enough related that all could readily be hybridized, berry and seed set following crosses between species of different series were on the average lower than they were following crosses between species of the same series. These barriers are just forming and are expressed in a varying manner, so that there were significant differences between species of the same series, and even between clones of the same species in barrier expression. The barriers of some of the species were expressed only when they were tested as female or as male parent. The inter-series F_1 seeds germinated as well as did those of the parent species and the hybrids had a normal vigor and pollen fertility.

WHILE extensive information has been gained of internal barriers to crossing of plant species that are distantly related, our knowledge of early stages in the formation of barriers, those operating between closely related taxa, is somewhat less satisfactory. The object of the study reported here was to analyze barriers to exchange of genes between closely related species in the belief that such early barrier formation is a very critical part of the process of speciation. Diploid species of *Solanum* of the series (species complexes) Tuberosa and Commersoniana were chosen for intensive study because it is known that they can easily be hybridized (Choudhuri, 1944; Koopmans, 1951; Swaminathan, 1953; Hawkes, 1956b; Wangenheim, 1957a; and Magoon et al., 1958) and that, if any early barriers leading to partial isolation have evolved, they are not as yet masked by the accumulation of complex barriers leading to complete genetic isolation. Analysis of their crossing relationships was undertaken to obtain information on the first-expressed barriers, those that limit the number of fertile F_1 hybrids formed. The object of the study was to answer the following questions: (1) How does the degree of intercrossability of species that are members of the same series compare with that of species that belong

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