Spring Shoot Growth in Douglas-Fir May Be Initiated by Gibberellins Exported from the Roots

Abstract. *Trials conducted under controlled environments demonstrated that the delay of bud activity of Douglas-fir (*Pseudotsuga menziesii*) seedlings occasioned by low temperature of the soil could be eliminated by application of gibberellic acid. Analyses of field-grown plants showed a parallel increase in bud activity, level of gibberellin-like compounds in xylem sap, and soil temperature during February and March.*

A population of potted Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] seedlings of Oregon provenance, which had been maintained out of doors all winter, was placed, in late February, in a controlled environment chamber programmed for a daily 9-hour photoperiod and a constant air temperature of 20°C. The soil temperature for one half of the plants was maintained at 5°C, while that for the second half was 20°C. The average bud break of the seedlings growing in the cold soil was about 2 weeks later than that of plants in the 20°C soil.

A second population of seedlings, divided into two groups as before, was placed in the above growth regimes. Half of the plants of each group were sprayed to drip point with a solution of gibberellic acid [100 parts per million (ppm)] at the end of each daily photoperiod. The remaining plants were sprayed similarly with distilled water. No effect of the gibberellic acid treatment was noted for plants grown in warm soil, these data being in agreement with those reported for *Pseudotsuga macrocarpa* by Lockhart and Bonner (*1*). However, the plants which were treated with gibberellic acid and grown in 5°C soil broke their buds as rapidly as the plants in the 20°C soil. Two further trials confirmed these results and demonstrated that the mean bud burst of plants treated with gibberellic acid was more rapid than that of a control population grown in 5°C soil. A Student's *t*-test of the data showed the difference to be significant at the 1 percent level. Even more marked were the differences in terminal bud activity of the treated and control plants; 71 percent of the former initiated terminal shoot growth during the experimental period, as opposed to only 24 percent of the control seedlings.

Earlier work (*2*) demonstrated that the reduction in shoot growth associated with low soil temperatures was not occasioned by reduced water or mineral uptake. An alternative explanation is that, when roots are grown in cold soils, shoot growth processes are slowed by reduced export, from the roots, of some plant growth regulatory substance or substances. The validity of this hypothesis has been demonstrated for *Zea mays* (*3*).

Crozier *et al.* (*4*) report gibberellin-like substances in Douglas-fir shoots, and current work at Oregon State University (*5*) has demonstrated that gibberellin-like compounds may be synthesized in the roots.

Figure 1 illustrates a relation between soil temperature and the levels of gibberellin-like compounds in xylem sap expressed from Douglas-fir seedling roots with a pressure chamber apparatus (*6*). These data are compatible with the hypothesis that soil temperature influences the levels of gibberellin-like substances moving from the root to the shoot. No comparable relation has been established for the cytokinins.

Plant species that have a requirement for an annual chilling period normally have this need fully satisfied by spring. Wareing (*7*) notes that in such cases the bud activity in spring is independent of photoperiod and is probably related to temperature. However, so far all reports, in which control of growth initiation in spring has been discussed, including the one by Lavender and Hermann (*8*) for Douglas-fir, have reported shoot rather than root temperatures. Campbell (*9*) notes that relative timing of bud burst between individuals or between provenances of Douglas-fir is very consistent from year to year and is unaffected by aging. A genetic response keyed to the relatively consistent increase of soil temperature with time in the spring could give such a response pattern.

Finally, the suggestion that soil temperature may play a major role in governing the initiation of bud activity of Douglas-fir in spring is supported by the authors' field observation and fits well with existing knowledge of the precision of control of Douglas-fir phenology by the climate in which it has evolved (*9, 10*).

The above data have important implications for the design of future studies of the growth physiology of Douglas-fir.

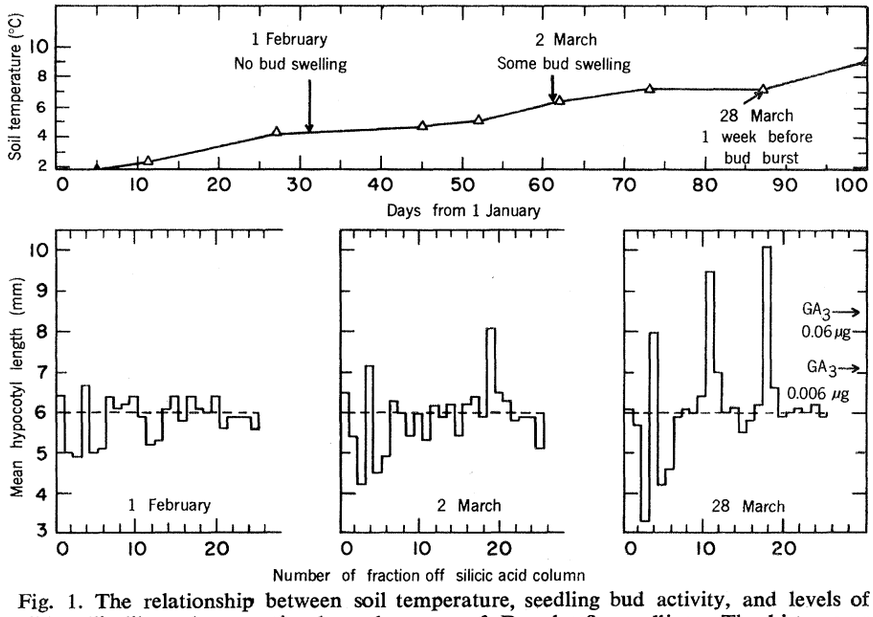

Fig. 1. The relationship between soil temperature, seedling bud activity, and levels of gibberellin-like substances in the xylem sap of Douglas-fir seedlings. The histograms illustrate the relative levels of gibberellin-like substances, as detected by the lettuce hypocotyl bioassay of Brian *et al.* (*11*). Authentic gibberellic acids (*GA*) were found in the following fractions: GA_3, the 11th fraction; GA_4, first fraction; GA_5, sixth fraction; GA_7, fifth fraction; GA_9, second fraction; and GA_{13}, eighth fraction.

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References and Notes

1. J. A. Lockhart and J. Bonner, *Plant Physiol.* **32**, 492 (1957).
2. D. P. Lavender and W. Scott Overton, *Oreg. State Univ. For. Res. Lab. Res. Pap. 13* (1972).
3. R. K. Atkin, G. E. Barton, D. K. Robinson, *J. Exp. Bot.* **24**, 475 (1973).
4. A. Crozier, H. Aoki, R. P. Pharis, R. C. Durley, *Phytochemistry* **5**, 5137 (1970).
5. G. B. Sweet, J. B. Zaerr, in *Proceedings of the International Plant Physiology Symposium on Mechanisms of Regulation of Plant Growth* (Palmston North, New Zealand, in press).
6. P. F. Scholander, H. T. Hammel, E. D. Bradstreet, E. A. Hemmingsen, *Science* **148**, 339 (1965); R. B. Vasey, in *Abstracts, First North American Forest Biology Workshop* (Michigan State University, 1970).
7. P. F. Wareing, *Annu. Rev. Plant Physiol.* **7**, 191 (1965).
8. D. P. Lavender and R. K. Hermann, *New Phytol.* **69**, 675 (1970).
9. R. K. Campbell, *J. Appl. Ecol.*, in press.
10. G. B. Sweet, *Silvae Genet.* **14**, 46 (1965).
11. P. W. Brian, H. G. Hemming, D. Lowe, *Ann. Bot. (Lond.)* **28**, 369 (1964).

12. Supported by NSF grant GB31304 to D.P.L. and Fulbright-Hays grant to G.B.S.

5 July 1973

Membrane Continuities Involving Chloroplasts and Other Organelles in Plant Cells

Abstract. *Plant cell membranes were examined in thin sections by electron microscopy. Numerous membrane continuities appeared between chloroplasts and other organelles of the fern* Pteris vittata *L., and occasionally in the leaves of the tomato* Lycopersicon esculentum *Mill. A number of these continuities had not been reported earlier or had been seen only infrequently.*

Although many continuities between different organelles and membrane systems have been reported, some possibilities, especially those involving the chloroplast, have not yet been documented (*1*). In this report we describe at least 14 different combinations of continuities between organelles and membrane systems in the fern gametophyte of *Pteris vittata* L., and describe several that involve the outer membrane of the chloroplast. We also present evidence from higher plants which suggests that such continuities may be more common than is generally recognized.

The observations on *Pteris* were made on the cells of two different prothallia (6- and 12-cell specimens), which were grown as reported (*2*), fixed in 3 percent glutaraldehyde in cacodylate buffer, postfixed in osmium tetroxide, dehydrated in acetone, and embedded in an epoxy plastic. Leaves of tomato (*Lycopersicon esculentum* Mill.) were similarly processed.

Continuities were observed in *Pteris* between the outer membrane of the chloroplast and each of the following: (i) the rough endoplasmic reticulum (Figs. 1 and 4), (ii) the plasma membrane (Fig. 2), (iii) the outer membrane of the mitochondrion (Fig. 4), (iv) the tonoplast, (v) the dictyosome vesicle, and (vi) the microbody (the latter three are not shown here). If a junction between the outer membranes of the nucleus and chloroplast were seen in this organism, it would complete the possible combinations between the chloroplast and other major membranous organelles.

Continuities observed between other organelles in *Pteris* (most not illustrated here) include conjoinings between the outer mitochondrial membrane and (i) the plasma membrane and (ii) the tonoplast; between the dictyosome vesicles and (i) the plasma membrane and (ii) the tonoplast; and between the endoplasmic reticulum and (i) the outer nuclear membrane, (ii) the dictyosome cisterna, (iii) the plasma membrane, and (iv) the outer mitochondrial membrane (Fig. 4). The interconnections between the endoplasmic reticulum and these organelles have been noted in several organisms (*3*).

Several multiple conjunctions have been observed in *Pteris*. A somewhat complicated situation is shown in Fig. 4: The perichloroplast and perimitochondrial spaces are in continuity through a cisternum of the rough endoplasmic reticulum; this is in addition to the chloroplast-mitochondrion continuity at another site in the same photomicrograph. Serial sections reveal that the two mitochondrial profiles in Fig.

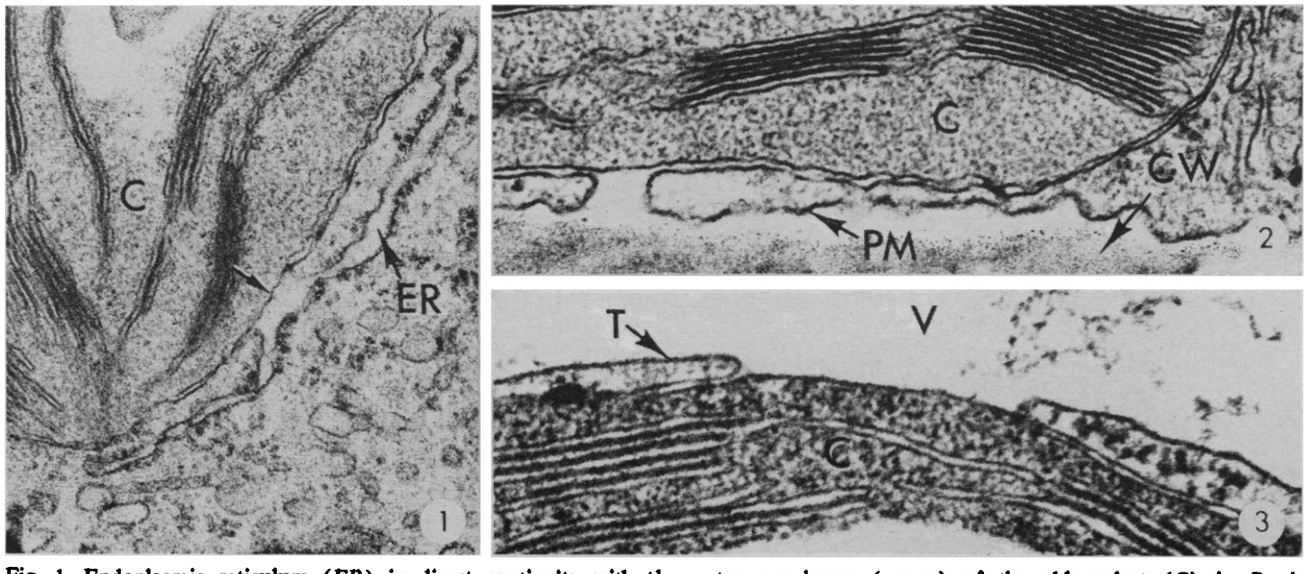

Fig. 1. Endoplasmic reticulum (*ER*) in direct continuity with the outer membrane (arrow) of the chloroplast (*C*) in *Pteris vittata* L. (× 40,000). Fig. 2. Outer membrane of the chloroplast (*C*) in continuity with the plasma membrane (*PM*) near the cell wall (*CW*) of *P. vittata* (× 62,000). Fig. 3. Outer membrane of the chloroplast (*C*) in continuity with the tonoplast (*T*) of the vacuole (*V*) in a leaf cell of tomato, *Lycopersicon esculentum* Mill. At another location, the same chloroplast outer membrane is in continuity with the plasma membrane (as in Fig. 2), so that the perichloroplast space is open to the vacuole and to the outside of the protoplast simultaneously (× 76,000).