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Short Communication

A Diffusible Auxin from *Pinus radiata* Pollen and Its Possible Role in Stimulating Ovule Development

G. B. Sweet and Philippa N. Lewis
Forest Research Institute, Rotorua, New Zealand

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Summary. Germinated pollen of *Pinus radiata* contains an auxin which is active in the *Avena* coleoptile test. It differs from all other hormones detected in pine pollen in that it is readily able to diffuse out from the pollen into an agar medium. It is suggested that, following pollination in vivo, the auxin may diffuse from the germinated pollen-tube onto the nucellus, thereby triggering the processes which allow ovule and gametophyte development to proceed. The auxin is water-soluble and may be an indole derivative.

In *Pinus* there is a time lag of appproximately 13 months between pollen germination on the nucellus and fertilisation (Ferguson, 1904); during this time, there is a steady increase in dry weight of the pollinated strobili accompanied by considerable differentiation and growth of their ovules. If ovules are not pollinated, however, they begin to degenerate immediately after the first division of the megaspore mother cell, that is, some 3 weeks after receptivity (Sarvas, 1962). It can thus be postulated that, at pollination, the pollen must provide a substance to the female strobilus which prevents ovule degeneration and also actively "triggers" the processes of differentiation that lead to gametophyte development. Such a substance clearly must be capable of diffusing out from the pollen grain onto the nucellus and, on the basis of work with other species (see, e.g. Crane, 1964), could be expected to be either a hormone or a hormone precursor. In this paper we describe the search for a hormone able to diffuse out of the germinating pollen of *P. radiata*. We show that one such hormone could be detected, and report something of its properties.

Preliminary tests showed that a number of established bioassays for auxins, gibberellins and cytokinins could detect, with sensitivity, growth substances present in agar. As pollen of *P. radiata* germinates and grows readily in vitro in this medium, it was chosen for the experiments. The pollen, after several washings

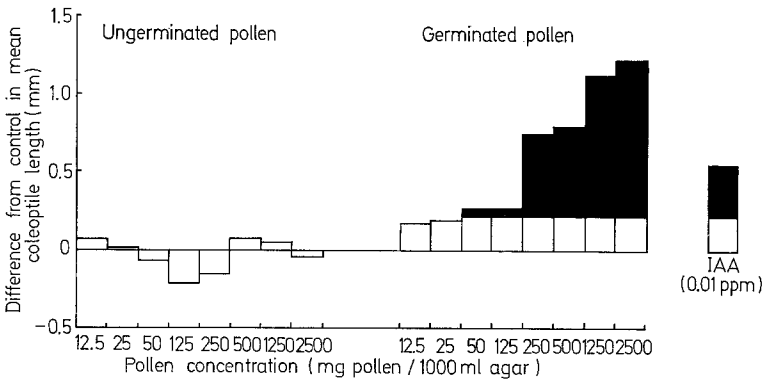

Fig. 1. Growth of *Avena* coleoptiles, placed on the surface of agar in which *Pinus radiata* pollen was incorporated. The base line (drawn through zero on the y axis) represents coleoptile growth on agar without pollen (controls). Areas shaded differ significantly from controls at the 1% level, using confidence limits as the basis for significance

in double-distilled water to remove bacterial and fungal contaminants, was incorporated into molten agar (1% in deionised water), without additives, in 9-cm Petri dishes at 40°. Subsequent germination at 26° was largely complete after 72 hr, and typical germination percentages at that time were 90% with mean pollen-tube lengths of 120 μ. Provided that the operation was carried out carefully, under sterile conditions, bacterial contamination was negligible.

A series of bioassays for gibberellins and cytokinins was made on the surface of agar containing germinated pollen but, regardless of pollen concentration, it was not possible to detect the presence of any of these hormones in the medium. Auxin straight-growth bioassays (Nitsch and Nitsch, 1956; Sirois, 1966), on the other hand, did show the presence of a substance which actively promoted *Avena* coleoptile growth. The results of a representative auxin bioassay are shown in Fig. 1. At all concentrations tested there was a positive response in coleoptile growth to the presence, in the agar, of germinated pollen, and the response was statistically significant at concentrations of 50 mg pollen per litre of agar and above. The diffusate from 1 mg pollen was equivalent, in its effect in the bioassay, to 0.05 μg indole-3-acetic acid (IAA). There was no comparable response with ungerminated pollen.

Following these assays, attempts were made to extract and partially purify the active auxin.

300 mg pollen were germinated for 93 hr in 1% agar at a concentration of 0.3 mg pollen per ml of agar. The agar was then cut into cubes and flooded with deionised water for 5 hr. The resultant eluate was reduced in volume and shaken with peroxide-free diethyl ether, first at pH 8.5 to produce a neutral-ether

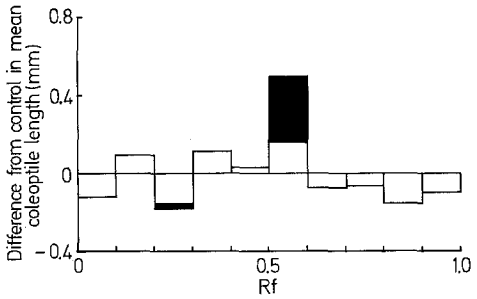

Fig. 2. Auxin bioassay of the aqueous fraction of an extract from germinated pollen of *Pinus radiata*. The chromatogram was run on Whatmans No. 3 paper in isopropanol: ammonia: water (8 : 1 : 1). Areas shaded differ significantly from controls at the 1% level, using confidence limits as the basis for significance

fraction, and then at pH 2.7 to give an acid-ether fraction and a residual aqueous fraction. The three fractions were submitted to thin layer and paper chromatography. Subsequent bioassay detected one zone in the aqueous fraction with significant promotive activity (see Fig. 2) and no activity in the other two fractions.

A more intensive extraction procedure, which broke open the pollen grains, was then applied to three further series of pollen, each of which had been germinated for a different length of time. After the appropriate germination period, the agar containing the pollen was deep-frozen to $-20°$ and then extracted with ice-cold absolute ethanol prior to reducing to a small aqueous sample and partitioning with diethyl ether as before. The aqueous fractions resulting were chromatographed and bioassayed.

The results of a representative bioassay are presented in the Table. They show that, while the auxin was not present in significant quantities until the pollen-tubes had reached a considerable length, it may well have been present, in small amount, in ungerminated and partly germinated pollen.

Table. *Effect of germination time and pollen tube length on the quantity of diffusible auxin extracted from 12 mg pollen*

Germination time (hr)	Mean pollen tube length (μ)	Bioassay response to diffusible auxin (difference in coleoptile length from control, in mm)
0	0	+0.12 NS
25	53	+0.18 NS
72	146	+0.41[a]

Difference in coleoptile length caused by IAA (0.05 μg) = 0.47mm[a]. Confidence limits = $\pm$0.22 (5%); $\pm$0.31 (1%). NS = not significant.

[a] Significant at 1% level, using confidence limits as the basis for significance

The chemical properties of the auxin showed that it was not IAA. General tests for indoles showed the presence, in small quantity, of two indole derivatives at the appropriate Rf on the chromatograms. However, we have not been able to determine whether, in fact, either of these compounds was the active auxin.

The amino acid tryptophan is known to be present in the pollen of *Pinus* (Nielsen *et al.*, 1955); and it is possible to suggest that the diffusing substance in our pollen may be tryptophan which was converted to an active auxin by bacterial action during pollen germination. We think this suggestion unlikely, however, on two grounds: (i) bacterial contamination in our germinating pollen was negligible, and (ii) the germination of pollen on an agar medium containing appropriate concentrations of added tryptophan did not result in an increased auxin response.

The evidence presented suggests the probability that, following pollen germination of *Pinus* in vivo, a water-soluble auxin diffuses out from the pollen tube into the nucellus. With the knowledge that, in *Pinus*, lack of pollination leads to ovule abortion, it is reasonable to suggest that it may be this diffusible auxin which acts to prevent ovule abortion and to allow the development of the female gametophyte to proceed.

One of the problems involved in suggesting a role in strobilus development for growth substances introduced by pollen is the very small number of pollen grains which normally reach a strobilus. In *P. sylvestris*, for example, Sarvas (1962) has shown that an average strobilus may contain about 60 ovules, and that each of these may average two or three pollen grains only, on its nucellus; the number being restricted by the size of the pollen chamber. However, there is some evidence from studies with tobacco (Muir, 1951) to show that, following pollination, the style is itself stimulated into the production of large quantities of auxin. Possibly in *Pinus* the diffusible pollen auxin may have a similar role: to stimulate the ovule into hormone production which in turn will allow its further development.

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Dr. G. B. Sweet
New Zealand Forest Service
Forest Research Institute
Private Bag
Rotorua, New Zealand