

significant slowing of growth. In fact, the transpiration ratio (the ratio of total transpiration (in g) to total dry matter production (in g) during a given interval of time) decreased in the period of the experiment. This ratio indicates the degree of inhibition of growth by an applied substance<sup>6</sup>. The results reaffirm that the primary effect of the esters is on the stomata, and that the efficiency of mesophyll photosynthesis is not appreciably altered. No other toxic effects on the plants have been found.

The suppression of stomatal opening is slightly more persistent with the esters than with ABA. This may be a result of greater penetration into the plant, or of breakdown over a period of time. A compound which releases ABA by slow hydrolysis might be more effective than the same dose of the active acid applied at one time, which could then be subject to rapid inactivation<sup>7</sup>.

The methyl and phenyl esters of abscisic acid thus seem to function effectively as antitranspirants, and because of their prolonged action may be suitable for protecting field crops during droughts.

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## Auxin Production by Phylloplane Fungi

DURING a study of the distribution of fungi on living leaves of sycamore (*Acer pseudoplatanus*) a succession of filamentous forms has been found, with *Aureobasidium pullulans* colonizing the expanding leaves, followed by *Cladosporium herbarum* and finally by *Epicoccum nigrum*. These three species have commonly been found on the surfaces of green leaves of a wide range of plants which have been studied by many workers. The veinal distribution of these fungi on sycamore led to a search for them in surface-sterilized leaves, and all three species have been isolated<sup>1</sup>. *Aureobasidium*, however, has also been isolated from surface sterilized bud scales, shoots and even from the roots of seedlings, and it has been described as an endophyte in sycamore and other trees<sup>2</sup>. The relationship between *Aureobasidium* and the higher plant could be in the form of symptomless parasitism, or, conversely, there could be some benefit to, or other influence on, the higher plant. Aspects of the physiology of the three common filamentous phylloplane fungi have been investigated to see if growth promoting substances can be produced by them, for Jump<sup>3</sup> suggested that a phytohormone produced by *A. pullulans* (*Dematiella pullulans*) may have been responsible for forking in red pine.

The three fungi were grown at different temperatures and for various times on a reciprocal shaker, with 100 ml. of Czapek's Dox medium in 1 l. flasks to ensure adequate aeration. At the end of the growing period the medium was checked to ensure that there was no contamination. The mycelium was separated by centrifugation, and the culture filtrate was used in a dilution series at 25° C with *Avena* coleoptiles for evidence of growth-promoting substances. When tryptophane was used as the N

source there was a marked effect of the culture filtrates on the coleoptiles compared with controls which were incubated in distilled water or in uninoculated culture medium. Maximum elongation was obtained with filtrates from cultures of *Aureobasidium* and *Epicoccum* which had been grown at 15° C rather than at 5° C or at 25° C. Cultures of *Aureobasidium* which were collected after 7 days caused a 62% elongation of the coleoptiles compared with the controls. *Epicoccum* cultures harvested after 15 days of incubation produced an elongation of 55% over the controls. *Cladosporium* was incubated at 5° C and at 25° C, and the filtrates caused elongations of 41% and 35% after 21 days and 7 days of incubation respectively.

*Aureobasidium* and *Epicoccum* were subsequently grown for 7 days at 25° C in diffuse light on a reciprocal shaker, and the mycelium separated by centrifugation. The filtrate was extracted at 2° C for 16 h with peroxide-free ether. The extract was evaporated to dryness in a rotary evaporator at 30° C, and taken up into a small volume of ether for chromatography, when isopropanol/NH<sub>3</sub> (specific gravity 0.880)/H<sub>2</sub>O (10:1:1 v/v/v) was found to be the most satisfactory solvent. The three sprays used were Ehrlich's and Salkowski's reagents, and Prochazka's reagent was used for fluorescence in ultraviolet light.

The chromatograms of the total ethereal extract from the *Aureobasidium* culture filtrate showed five well-defined spots, but three of the five spots from the *Epicoccum* extract were not as clearly separated. One of the five spots from both fungi corresponded closely in colour reaction and *R<sub>F</sub>* value to indole-3-acetic acid (IAA), and elution of unsprayed chromatograms indicated 0.15 µg/ml. and 0.05 µg/ml. of IAA for *Aureobasidium* and *Epicoccum* respectively. A second spot from *Aureobasidium* and the three poorly separated spots from *Epicoccum* overlapped the indole-3-aceto nitrile (IAN) spot. The *R<sub>F</sub>* values were similar, and colour reactions approximated to those for IAN. Elution of unsprayed chromatograms indicated 0.065 µg/ml. and 0.0125 µg/ml. from *Aureobasidium* and *Epicoccum* respectively. Valadon and Lodge<sup>4</sup> have shown that both IAA and IAN can be produced by *Cladosporium* at 0.015 µg/ml. and 0.02 µg/ml. respectively.

The production of these and possibly other auxins *in vitro* is of potential interest to all who work with leaves, and to those who work on factors which influence bud and seed dormancy. Tukey<sup>5</sup> has shown that auxins and their possible precursors can be found in plant leachates, and that leachates can be absorbed by both leaves and roots. Some, at least, of these auxins may have been produced by fungi living on leaf surfaces or even within the tissues of the higher plants. The production of auxins by phylloplane fungi *in vivo* would suggest that these fungi may play a much fuller role in nature, for example in growth regulation within the ecosystem, than has previously been suspected. Further studies on these interrelationships are being carried out.

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