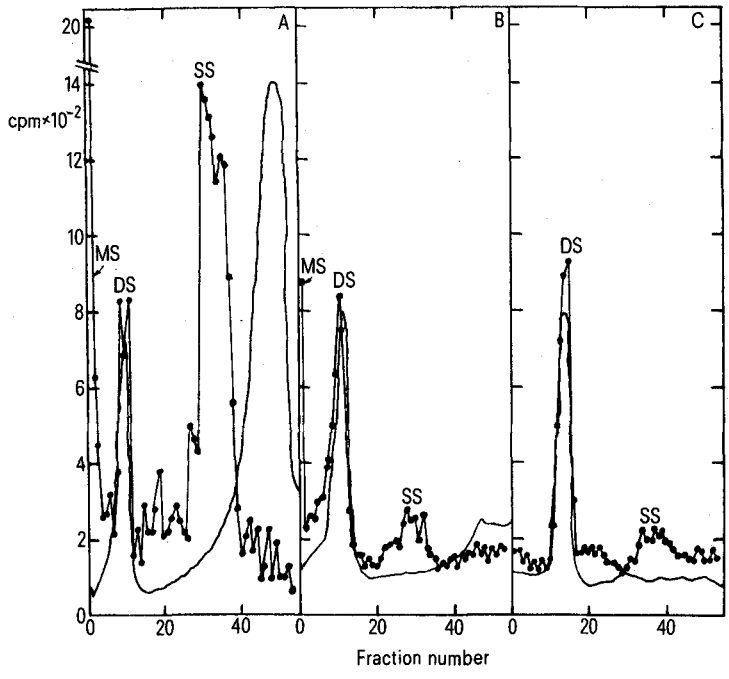

Electrophoretic analysis of rhinovirus type 2 RNA treated with RNAse before phenol extraction. *A* Control, *B* and *C* treated with 0.1 and 10μg/ml of RNAse respectively. The continuous line is the OD_{260} trace indicating the position of the marker DNA (which runs together with the DS RNA) and the ribosomal RNA which is present only in the control figure. Direction of electrophoresis from left to right.

concentrations of RNAse whereas cellular DNA was insensitive.

These results show that the DS RNA detected in cells infected with rhinovirus type 2 is present before nucleic acids are extracted with phenol. This is important in view of recent experiments establishing a crucial role for DS RNA in the mechanism of interferon's antiviral action, although the possibility that hydrogen bonding formation occurs before the extraction with the phenol has not been ruled out.

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Endogenous indolyl-3-acetic acid and pathogen-induced plant growth disorders: distinction between hyperplasia and neoplastic development

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Summary. Quantitative analysis of the auxin indolyl-3-acetic acid during the development of neoplastic (crown gall) and hyperplastic (club root) plant growth disorders revealed different physiological mechanisms.

The growth disorders induced in plants by the crown gall agent *Agrobacterium tumefaciens* have many of the characteristics of animal neoplasias[2]. In particular, cells from crown gall tumors continue to proliferate in tissue culture when freed from *A. tumefaciens* and have no requirement for external auxins or cytokinins[3]. The parasitic fungus *Plasmodiaphora brassicae* induces hyperplasias ('club root') in susceptible cruciferous plants which, however, require the continued presence of *P. brassicae* plasmodia for growth on basal media[4]. Since elevated auxin levels have been suggested as causative factors in the development of both plant diseases[5,6], endogenous levels of the major auxin indolyl-3-acetic acid (IAA) were monitored during the early stages of the diseases to clarify the physiological mechanisms involved.

Material and methods. Crown galls were induced on wounded sunflower (*Helianthus annuus* L. var. Russian Giant) with *Agrobacterium tumefaciens* (Smith and Townsend) Conn. strain B6 and seedlings of swede (*Brassica napus* var. Danestone) were infected with *Plasmodiaphora*

brassicae Woron. strain S as described previously[7,8]. Segments of sunflower stem, 4 cm in length, were excised so as to include the entire wound site; such stem segments were analyzed individually. With swede seedlings 2-cm sections containing the hypocotyl and the top of the main root were taken; 18–35 such sections were combined for analysis. Tissue samples were frozen in liquid nitrogen, ground to a powder and extracted with 80% aqueous methanol (10 ml per g). Acidic ether fractions were prepared from tissue extracts as described previously[8,9]; losses of IAA during extract purification were monitored using ^{14}C-labelled IAA[9]. IAA determination was with the pyrone fluorimetric method[9,10]; scanning of the emission spectrum (λ_{max} 482 nm) of the reaction product from purified extracts and authentic IAA standards was used in quantitative evaluation. To investigate the presence of acidic auxins other than IAA purified extracts were fractionated by paper or cellulose TLC and the zones corresponding to different R_f-values tested using a *Triticum* coleoptile auxin bioassay[11].

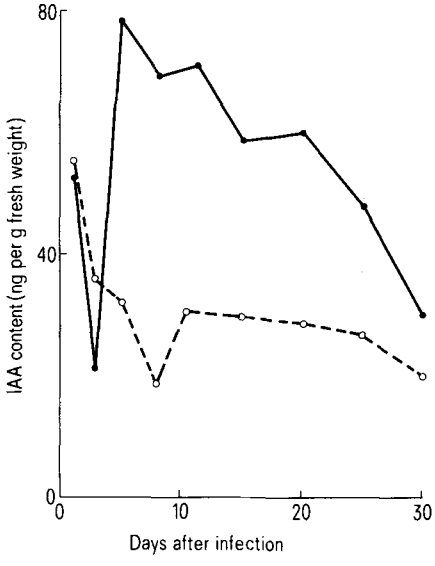

Figure 1. Endogenous IAA levels in control (○) and *Agrobacterium tumefaciens*-infected (●) sunflower stem. Tumorization was first visible 5 days after infection.

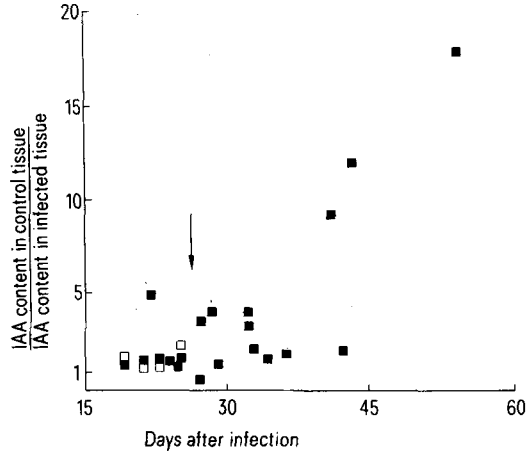

Figure 2. Endogenous IAA content of control and *Plasmodiaphora brassicae*-infected root and hypocotyl of swede seedlings. Arrow marks onset of visible club root symptoms. (■): fresh tissue; (□): lyophilized tissue. Samples were taken from 5 initial sowings (4 or 6 sampling dates per experiment); club root development occurred in 72–95% of emergent infected seedlings.

Results and discussion. Endogenous IAA levels rose within 5 days of *A. tumefaciens* infection and remained elevated during the succeeding 20 days (fig. 1). Visible tumors appeared 5 days after infection; by 20 days large tumors (10 g fresh weight) had developed. Endogenous IAA levels in *P. brassicae*-infected swede root and hypocotyl were, in contrast, lower than in corresponding control plants both before and after club root symptoms developed (fig. 2); this was so when both fresh and lyophilized tissue was analyzed (IAA levels in uninfected swede root and hypocotyl were 15–104 ng per g fresh weight and 314–1409 ng per g dry weight). When acidic ether fractions from the sunflower and swede tissues were separated by paper or cellulose TLC and analyzed by a *Triticum* coleoptile straight-growth test over 90% of the auxin activity co-chromatographed with IAA.

The kinetics of change of endogenous IAA levels in *A. tumefaciens*-infected tissue are consistent with a role of localized increases in auxin content in the induction of crown gall tumors. In *P. brassicae*-infected root and hypocotyl such hyperauxiny is not apparent. Paradoxically, auxins induce root swellings in turnip seedlings[12], but it is unclear whether this involves the same cellular changes as does the club root disease.

The distinction which can be made between crown gall and club root in the induced changes in auxin content reflects the subsequent tissue culture behaviour. The 'genetic engineering' carried out by *A. tumefaciens* in the host plant[13] results in the insertion or expression of genetic information for regulating IAA synthesis or metabolism in the transformed host cells. This may occur in the first 30 h following infection as has been demonstrated for the synthesis of crown gall-specific amino acid derivatives[14]. These genetic changes are stable and are manifested in the auxin-autotrophy of crown gall tissue cultures. The club root pathogen, however, utilizes the host plant in part of its life cycle while inducing no apparent genetic changes. The hyperplasia in *P. brassicae* infected plants may be induced by the synthesis of cytokinins or other cell-division factors by the pathogen itself[15]. Consequently, club root resistance is unlikely to involve hormonal mechanisms.

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