In contrast to fiber cells, the cells in the lens epithelium fail to show immunofluorescence for γ-crystallins in all stages of regeneration studied (Fig. 4). The same applies to the cells in the external wall of lens vesicle at stages V to IX (Figs. 2 and 3). It should be pointed out that those cells negative in immunofluorescence are dividing in contrast to fiber cells which are nondividing. According to our preliminary data, α- and β-crystallins are present in the lens epithelial cells as well as in the fiber cells. Thus production of γ-crystallins is characteristic for lens fiber differentiation. This conclusion is in good agreement with biochemical data obtained on the calf lens (*8*). If lens fiber differentiation is the essential event of lens differentiation, the synthesis of γ-crystallins should be one of the crucial molecular processes in lens differentiation.

Comparison of our data with that obtained by H^3-thymidine autoradiography shows that the prospective lens fiber cells begin to synthesize γ-crystallins at a definite time interval after the last DNA-synthesizing phase. Electron microscopic data on ribosomes (*9*) and autoradiographic data on intracellular distribution of RNA in the present system imply that synthesis of γ-crystallins starts after the formation of a new ribosomal population is completed.

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Plant Parasitic Nematodes: A New Mechanism for Injury of Hosts

Abstract. *Pathological effects of* Ditylenchus dipsaci *and* Meloidogyne hapla *are related to the disturbance of the auxin balance in the host by the nematode. The parasites produce an auxin inactivator, apparently enzymatic, that enables* Ditylenchus dipsaci *to stunt host stem apices and* Meloidogyne hapla *to reduce its galling potential.*

Evidence that nematodes inactivate auxins provides an explanation for some of the pathological effects of nematode infection. The mechanisms by which parasitic nematodes injure host plants have been largely a matter of speculation based upon plant symptoms of nematode injury. They have usually been described in gross terms—that is, in terms of the mechanical injury resulting from nematodes feeding or moving through the tissues of the host, or of plant tissue reactions to secretions injected by the feeding parasites, or both (*1*). That nematode pathogenicity of host plants has a sound chemical basis was demonstrated by Mountain (*2*), who showed that *Pratylenchus penetrans* in peach roots is able to hydrolyze amygdalin to release benzaldehyde and hydrogen cyanide which diffuses to neighboring cells and brings about necrosis. In research on the relation between growth regulators and pathogenesis (*3*) the role of nematodes has been virtually neglected. Although fundamental disturbances of plant growth regulatory mechanisms in pathogenicity have been suggested, there is no experimental evidence of the biochemical mechanisms involved (*4*).

The possibility that nematodes can disturb plant tissues by secreting auxins was reported recently (*5, 6*). We have now investigated the roles of auxin-inactivating systems originating from the nematode in host parasite relationships. Freshly collected nematodes such as *Ditylenchus dipsaci* and *Meloidogyne hapla* upon incubation in water release an auxin-inactivating system (*7*). A characteristic manifestation of heavy infections of *Ditylenchus dipsaci* on alfalfa is severe stunting; apparently the stem apex is unable to elongate. According to conventional auxin theory, auxin synthesized in the growing point moves downward and as part of its physiological activity plasticizes the walls of the young cells, allowing them to absorb water and enlarge and elongate. Since *D. dipsaci* invades and develops in the stem apex, stunting can be visualized as a result of inactivation of auxin synthesized by the apical meristem.

Differential growth responses, obtained by application of natural and synthetic auxins to the shoot apices of infected and healthy intact plants, were consistent with the notion that indole-3-acetic acid (IAA) was inactivated in infected plants whereas naphthalene acetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) were not inactivated. However, in view of the great variability of response of intact plant stem apexes to auxins, the difficulty of establishing equivalent concentrations of IAA, NAA, and 2,4-D for purposes of comparison, the nonuniformity in the numbers of nematodes infecting shoot apexes, and the fact that the stem apex quickly grows away from the region of influence of the sedentary nematodes, more data were required.

If auxins are inactivated in infected plants, it would be reasonable to expect less auxin in infected shoot tips than in healthy ones. We found this to be the case (Fig. 1). When infected and noninfected shoot tips were lyophilized, extracted for auxin in the conventional manner, and tested by the avena "first internode test" (*8*), less IAA was found,

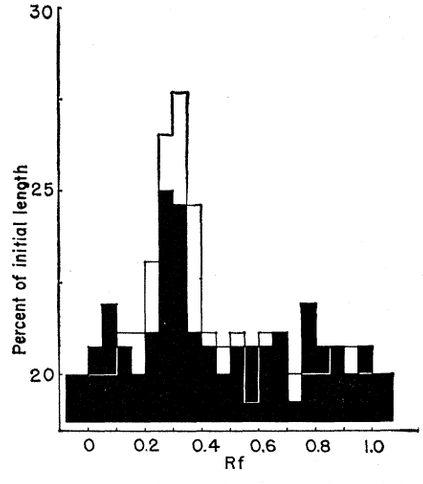

Fig. 1. Growth regulator activity of infected (with *Ditylenchus dipsaci*) and noninfected alfalfa shoot tips. Histograms indicate the distribution of plant growth regulator activity (indicated as percentage increase in growth of the first internode of *Avena*) with increasing R_F values in chromatograms of auxin extracts of infected (shaded) and noninfected (unshaded) alfalfa shoot tips. Tissue extracts were developed with a mixture of water, methanol, and isobutanol.

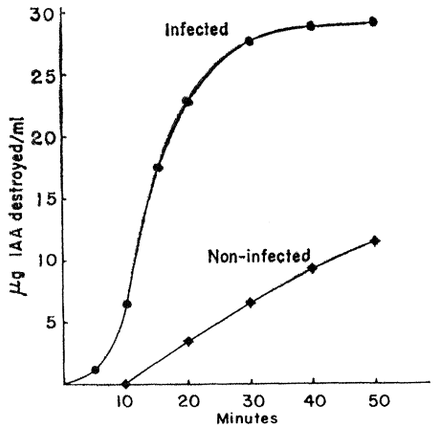

Fig. 2. Kinetics of oxidation of indole acetic acid (IAA) in alfalfa shoot tips. The reaction mixtures contained $10^{-4}M$ dichlorophenol and $MnCl_2$, $8 \times 10^{-4}M$ IAA, 1 ml enzyme solution, and 1.5 ml phosphate buffer; final *p*H, 6.1. Enzyme solutions were prepared by grinding shoot tips with fine sand, centrifuging the suspension, and collecting the supernatant.

on a dry weight basis, in infected tissue.

If a stunted stem apex is prevented from growing by inactivation of the auxin synthesized by the growing point, then such a tip could be expected to possess greater inactivating potential than a normal point. We found that when water extracts of ground tissue were incubated with IAA after the method of Galston and Dalberg (*9*), the infected shoot tips possessed a greater capacity for destroying IAA than healthy tips (Fig. 2). Not only was the capacity greater but the rate curves were entirely different, thereby suggesting the presence of a characteristic system in infected tissue. The destruction of IAA in healthy tissue can be explained in terms of peroxidase and other oxidases, normally found in healthy cells, which possess the ability to destroy IAA. That the disappearance of the IAA substrate in these tests is due to destruction of IAA was indicated by assays with Salkowski reagent which is specific for the indole nucleus under these conditions (*10*).

To establish that the intimate presence of the nematode in the growing point was unnecessary for stunting it was mandatory to demonstrate reduced growth with the auxin inactivating system released by the nematode in vitro. This was accomplished by infiltrating germinating seedlings under reduced pressure with solutions obtained from *D. dipsaci,* just prior to the period of rapid elongation, and by measuring differential growth. The results of such tests with seedlings of alfalfa, oats, wheat, and cucumber are shown in Table 1. Reduced growth, significant at least at the 5-percent level, was demonstrated with all the seedlings. The fact that neither IAA inactivation in vitro nor reduced growth of seedlings could be obtained with boiled solutions obtained from nematodes suggests a reaction of an enzymatic type. Although absorption of enzymes by roots (*11*) and tissue cultures (*12*) has been reported, to our knowledge no such report exists of absorption by growing shoots. With infiltration under reduced pressure, a reasonable replacement of the gases in the intercellular spaces with enzyme solution can be expected. The intimate contact of enzyme and auxin for the inactivation reaction may be explained in two ways—either the enzyme enters the cell or the auxin comes out. In the meristematic region of the growing point one can conceive of enzyme uptake by the process of pinocytosis (*13*). Since it is well known that auxin diffuses through cell walls (*14*), it is possible that the inactivation reaction takes place in the intercellular spaces. In such a system the auxin in the intracellular spaces would normally

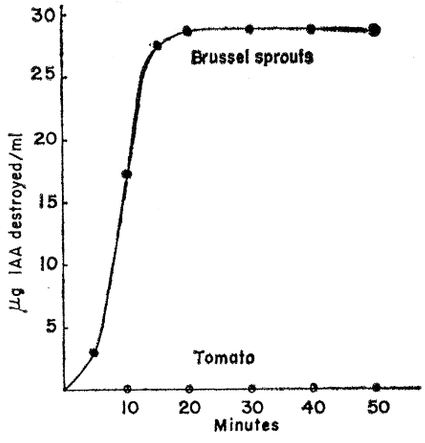

Fig. 3. Kinetics of oxidation of indole acetic acid (IAA) in uninfected brussels sprouts and tomato. The reaction mixtures contained $10^{-4}M$ dichlorophenol and $MnCl_2$, $8 \times 10^{-4}M$ IAA, 1 ml of enzyme solution and 1.5 ml of phosphate buffer; final *p*H, 6.1. Stock enzyme solutions, prepared by grinding root tissues with fine sand, centrifuging the suspension, and collecting the supernatant, were diluted fivefold before use.

be in equilibrium with that in the intercellular spaces; the destruction of the intercellular auxin would upset the equilibrium so that auxin would move out of the cell. The end result would be depletion of auxin in the tissues and reduced growth. Presumably, a similar situation would prevail in tissues infected with nematodes. We therefore believe it is reasonable to conclude that the mechanism of stunting injury effected by the stem and bulb nematode, *Ditylenchus dipsaci,* can be explained in part by the auxin produced by the plant being inactivated by an inactivating system of nematode origin.

In heavy root-knot infections of tomato and many other plants, *Meloidogyne hapla* produces a tremendous number of tiny pinhead-sized galls on a fibrous root system, whereas *M. incognita* and *M. javanica,* for example, produce massive gnarled walnut-sized galls. On the other hand, in heavy infections of brussels sprouts, *Brasica olerace* var. *gemnifera,* with three root-knot species, *M. hapla* produces galls almost invisible to the naked eye, whereas *M. incognita* and *M. javanica* produce pinhead-sized galls on a fibrous system, much like those which *M. hapla* produces on tomato. Though all three nematodes induce high concentrations of auxin in root galls (*6, 15*) only the second-stage larvae of *M. hapla* release an auxin inactivating system when incubated in water. If aque-

Table 1. Growth response of seedlings infiltrated under reduced pressure with solutions from *D. dipsaci* and with water. The seedlings were incubated for 26 or 48 hours after infiltration.

Seedlings	Time of incubation (hours)	Growth (mm)				
		Water (pressure not reduced)	Water	Solutions from nematodes		*D* at 5%*
				Boiled	Unboiled	
Alfalfa†	48		23.5	23.1	20.1	1.80
Oats‡	26		4.3	5.0	3.7	1.16
Wheat‡	26	20.6	19.8	19.7	16.8	2.39
Wheat‡	48	31.8	31.8	32.6	20.3	6.25
Cucumber†	48	28.9	27.0	27.1	22.9	2.83

* $D = QS\bar{x}$, where D (difference) is the product of $S\bar{x}$ and a factor Q; $S\bar{x}$ is a sample standard error and Q is obtained from the table of Studentized Range. See G. W. Snedecor, *Statistical Methods* (Iowa State Univ., Ames, 1959), p. 252. † Infiltrated under 600 mm-Hg for 5 minutes. ‡ Infiltrated under 300 mm-Hg for 5 minutes.

ous extracts of macerated healthy tissue of brussels sprouts and tomato are compared after the manner of Tang and Bonner (*10*) with respect to auxin inactivation (Fig. 3), it is clear that brussels sprout root tissue has a greater capacity for inactivating auxin. The response of the root tissue to these three nematodes, as manifested by root gall size, appears to be a function of auxin synthesis and auxin inactivation. In the case of tomato, for example, auxin inactivation is provided only by *M. hapla* so that *M. hapla* produces small pinhead galls, whereas *M. javanica* and *M. incognita* produce large galls. In the case of brussels sprouts the auxin inactivation system provided by *M. hapla* is supplemented by that in the host tissue so that *M. hapla* produces almost invisible galls, whereas the normally massive galls of *M. javanica* and *M. incognita* are reduced to the small pinhead-sized galls normally effected by *M. hapla* on tomato.

Our experiments thus demonstrate the ability of parasitic nematodes to disturb the growth regulatory mechanisms of plants. Consideration of the systematic or superficial manifestations of host tissue response to other parasitic nematodes indicates that interference with the plant growth regulatory mechanism is a relatively common occurrence.

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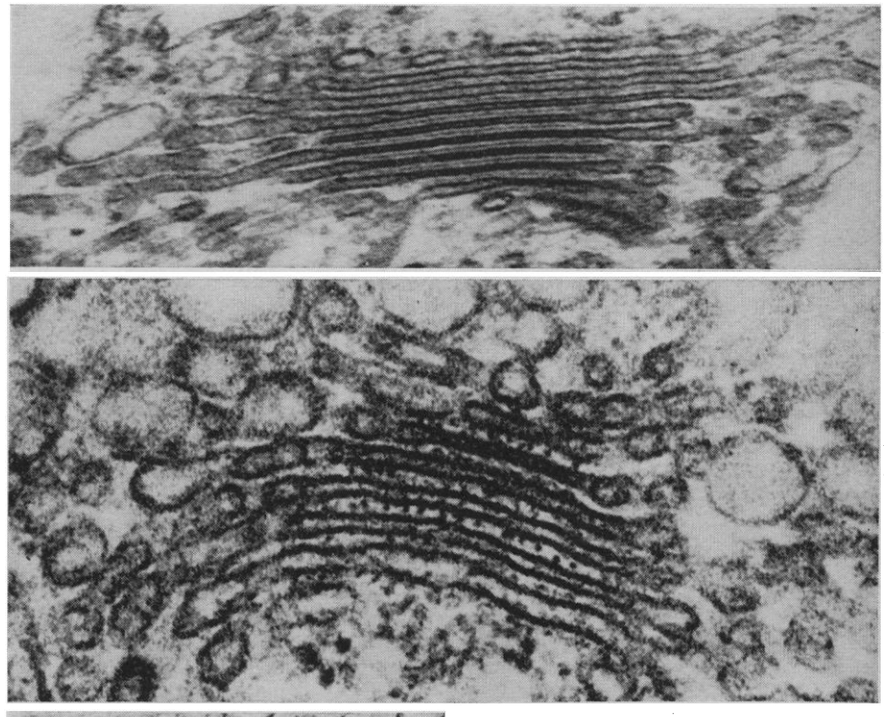
Intercisternal Elements of the Golgi Apparatus

Abstract. *In both vegetative and reproductive cells of various* Nitella *species, and in cells of other plant genera, there are fine, parallel, elongated elements lying between the cisternae of the Golgi apparatus but not in contact with the cisternal membranes.*

For some time there has been reason to believe that structural elements exist in the Golgi apparatus which have not been demonstrated in osmium tetroxide or potassium permanganate preparations. When removed from the cell the cisternae of the Golgi apparatus tend to remain associated in the typical distinct pattern of the organelle (*1*).

In both vegetative and reproductive cells of various *Nitella* spp., fixed first in cacodylate-buffered glutaraldehyde and then in osmium tetroxide, and stained first with uranyl acetate and then with lead citrate, certain views of the Golgi apparatus show an electron-dense line between some or all of the adjacent cisternae (Fig. 1). When the Golgi apparatus is viewed at what is presumed to be a 90° angle to that in which these intercisternal elements are revealed as electron-dense lines, the appearance is one of sections through individual elements (Fig. 2). This observation suggests that the elongated elements may have a definite orientation



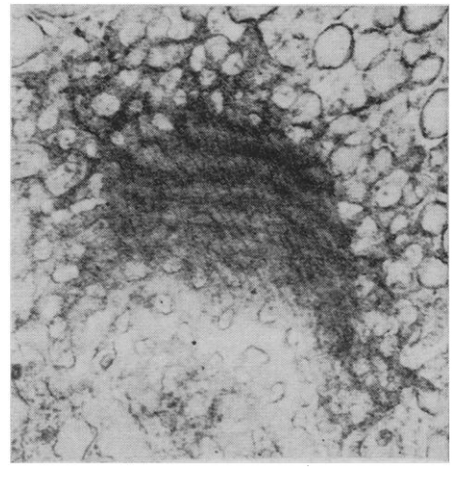


Fig. 1 (top). Electron micrograph showing electron-dense lines between cisternae of a Golgi apparatus from a vegetative cell of *Nitella* sp. (× 67,500).

Fig. 2 (center). What are presumed to be the same structures cut at an angle of approximately 90° from those in Fig. 1. From a reproductive cell of *Nitella* sp. (× 112,000).

Fig. 3 (left). An oblique cut showing the elongate, parallel character of the elements. From the same material as Fig. 1 (× 39,200).