CELLULASE IN PHYTOPARASITIC NEMATODES

BY

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An enzyme which produces reducing substances from carboxymethylcellulose (CMC) is present in homogenates of phytoparasitic and some myceliophagous nematodes but is absent from Panagrellus sp., Neodiplogaster sp. and from Trichinella spiralis. Temperature inactivation time, optimum ionic strength, pH optima, and substrate specificity were determined for Meloidogyne sp. and Ditylenchus myceliophagus preparations. Characteristics of cellulase differed in the two nematodes. Highest quantities of CMC-ase were present in preparations from root-knot and cyst nematode larvae.

Enzymes which attack plant cell walls must play a prominent role in the life of phytoparasitic nematodes. The intact surface of a root offers a formidable barrier to an infective larva. Linford (1942) observed that a root-knot nematode repeatedly thrusts its stylet against a small area of a single cell and continues this behavior for many minutes before it punctures the wall. Once inside the root, a larva may feed on cell contents immediately or may migrate to its final location. Its path of migration sometimes leaves a trail of destruction, as in the Heteroderas, or the path may be intercellular, with little evidence of damage to the host. Cell wall degradation is a prominent feature in formation of the characteristic syncytia about the head of developing root-knot and cyst nematodes and also occurs in the formation of the extensive cavities found in plants infected with Ditylenchus dipsaci. Cell wall destruction is not confined to larval forms, but is caused by such adult nematodes as Pratylenchus and Radopholus. From the morphology of the stylet and the associated esophageal glands, it seems clear that cell wall destroying enzymes are extruded from the tip of the stylet during penetration of roots, intracellular migration, and formation of syncytia or cavities associated with certain phytoparasitic nematodes.

The chemistry of the plant cell wall is incompletely known. Northcote (1958) listed the following constituents:

- **cellulose** — polymers of glucose with traces of other monosaccharides.
- **hemicellulose** — polymers of glucose, galactose, mannose, arabinose, xylose, rhamnose, and uronic acids.
- **pectic substances** — polymers of galacturonic acid, galactose, and arabinose.
- **lignin** — derivatives of p-hydroxyphenyl propane.

A recent analysis (Jensen & Ashton, 1960) showed that primary wall of onion
root tip cells consists of a complex mixture of cellulose, pectic substances, soluble non-cellulosic polysaccharides, hemicellulose, and insoluble non-cellulosic polysaccharides. In addition to carbohydrates, non-woody plant cell walls also contain up to 10\% protein (Northcote, op. cit.). The nematode must have an array of enzymes to degrade the complex, orderly structure of the plant cell wall.

Several workers reported cellulase in phytoparasitic nematodes but did not characterize the enzyme or enzymes. Tracey (1958) found 4.7 times as much cellulase in homogenates of *D. dipsaci* as in *D. myceliophagus*. He measured activity by following production of reducing sugars from a cellulose suspension at pH 5. The enzyme was absent from *Turbatrix aceti*. Krusberg (1960) measured cellulase by viscometry of solutions of carboxymethylcellulose (CMC). He found CMC-ase in homogenates of *D. trifurcatus*, *D. dipsaci*, and *P. zeae* with a suggestion that it was more concentrated in *P. zeae* than in *D. dipsaci*. Morgan & McAllan (1963) also demonstrated cellulase by viscometry on a solution of methyl cellulose. They calculated that *P. penetrans* had seven times as much enzyme as *Heterodera trifoli*ii. The absence of this enzyme from *Turbatrix aceti* was confirmed.

This work was designed to study the types and characteristics of cell-wall degrading enzymes in phytoparasitic nematodes as a basis for investigations of the nature of resistance.

**MATERIALS AND METHODS**

Root-knot nematodes were harvested from tomato grown in 15-cm pots of sand in the greenhouse and from tomato grown in fumigated field plots. When female nematodes of the second generation had large egg masses, the plants were held without water for 7-10 days. After the roots were washed and cut into small pieces with a hand slicer, they were placed in trays lined with nylon mesh in a mist chamber. The trays drained into buckets from which the larvae were recovered by sieving and centrifugation, after which they migrated through paper tissue into aerated water. After further centrifugation, larvae were stored at —20° C. *Ditylenchus myceliophagus* nematodes were collected under mist from commercial mushroom spawn infected with pure populations. In this process, nematodes settled to the bottom of a 12-liter bucket through which 300 cc of tap water flowed per hour. At each centrifugation the nematodes were concentrated and the supernatant fluid discarded. The final product was a viscous suspension of several million nematodes per cc of distilled water, with no microscopic evidence of fungi or bacteria. *Tylenchulus semipenetrans* was furnished by S. D. Van Gundy of Riverside, California, and *Heterodera schachtii* by D. Viglierchio of Davis, California. Small quantities of other nematodes were grown on alfalfa callus in sterile agar cultures by the method of Krusberg (1960) or on fungus cultures in agar, furnished by D. Taylor of Urbana, Illinois, and R. Adams of Morgantown, West Virginia.

Homogenates were prepared by exposing suspensions of larvae in water for 6 minutes to sonic oscillations in a 60-watt Measuring and Scientific Equipment ultrasonic disintegrator through the courtesy of J. A. Alford. After centrifugation