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THE EFFECT OF *PRATYLENCHUS FALLAX* ON WHEAT, BARLEY AND SUGAR BEET ROOTS

BY

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Pratylenchus fallax Seinhorst penetrated root tips, the region of root hair development and the junction of main and lateral roots of wheat, barley and sugar beet. Many tended to penetrate together at one site. In microbiologically sterile cultures, roots of sugar beet rapidly became necrotic: barley and wheat roots were damaged less and more slowly. Transverse sections of wheat roots showed that cells were severely damaged and collapsed before symptoms showed externally. The endodermis became thickened and discoloured in response to heavy attack. *P. fallax* reproduced better on roots of wheat and barley than on those of sugar beet.

Pratylenchus fallax occurs in barley fields in England (Corbett, 1970) and in orchards and meadows in the Netherlands (Seinhorst, 1968) but nothing is known of its biology. As it was associated with patchy growth of barley in sandy soils in England its effects on roots of wheat, barley and sugar beet in the absence of other microorganisms were studied.

MATERIALS AND METHODS

Roots of two varieties of barley (*Hordeum vulgare* L.) and one of sugar beet (*Beta vulgaris* L.) were grown on Carew & Schwarting's (1958) basal medium, and two varieties of wheat (*Triticum aestivum* L.) on White's medium (Mountain, 1955). Nematode behaviour was the same on one variety of wheat as on the other, and on one variety of barley as the other. To provide the roots, seeds were sterilised in 0.1 % mercuric chloride, washed in sterile water and placed on the agar. Roots more than 1 cm long were excised and placed individually on the appropriate agar medium in Petri dishes. Two cultures of *P. fallax* derived from single females were grown on lucerne callus on Krusberg & Blickenstaff's medium (1964). From these, batches of 130 to 140 sterile, live adults and larvae were extracted (Webb, 1971) and pipetted in a little sterile water into separate Petri dishes near an excised root. The Petri dishes were incubated at 20-25 °C. In the tests, three roots of each host were inoculated with each nematode isolate and three not. Two wheat-root cultures became contaminated with micro-organisms and were discarded: all other inoculated dishes remained uncontaminated. All three hosts were observed periodically, and transverse sections of infected wheat cv. Jufy were cut 10μ thick at weekly intervals for four weeks

after inoculation. Sections were fixed first in FAA, then in osmic acid and stained of the slides with toluidine blue.

At the end of the experiment *P. fallax* were extracted from the roots and agar on the wheat, barley, and sugar beet respectively using Stemerding's (1963) method.

OBSERVATIONS ON ROOT CULTURES

Nematode behaviour. The day after inoculation, more *P. fallax* were attracted to barley and wheat roots than to sugar beet roots. On all three hosts, nematodes clustered about root tips, the region of root-hair development and the junction of laterals with the main root. Many penetrated together at these points. Four days after inoculation most of the nematodes were at these sites, and within a week many were inside the roots near them. Twelve days after inoculation nematodes were still feeding on and through the root cap, at the junction of lateral and main roots, at the tips of emerging lateral roots and within the root hair zone. Most of the nematodes fed inside the roots, but a few were only partially embedded. In sugar-beet roots, nematodes fed for relatively long periods apparently without moving; one fed at the same site for a day and another for 2 days. Many nematodes and eggs were in barley and sugar-beet roots and on the agar alongside; many eggs but few nematodes were in the agar alongside wheat roots. There were many tracks in the agar along roots and at root tips. Seven weeks after inoculation many nematodes and eggs were in barley and wheat roots but few in the older parts of sugar-beet roots. Nematodes began to leave the older heavily infested parts of barley and wheat roots especially at main and lateral root junctions, and re-invaded healthy parts of roots towards the tip. In sugar-beet nematodes continued to leave parts of the root system where the brown discolouration was severe and to re-invade healthy roots towards the tip. This migration persisted in all three hosts until the experiment ended 10 weeks after inoculation.

Root damage on barley and wheat

First week. Five days after inoculation a brown diffuse patch was seen on the stele of a barley root where a lateral root emerged, but there was no visible reaction in wheat roots. Inoculated and uninoculated wheat had about 8 lateral roots; lateral root development in barley was very sparse. Transverse sections of inoculated wheat roots showed epidermal and cortical cells with broken walls (Fig. 1) and sometimes neighbouring cells had collapsed. The many nematodes that invaded the roots created cavities containing the remnants of cell walls in the cortex (Fig. 3). Nematodes were found in the large cells of the main root surrounding the base of lateral roots that they were penetrating.

Second week. Some roots of barley and wheat were discoloured slightly; one barley root tip had a microscopic lesion and some were irregularly swollen for about 5 mm behind the tip. The "collar" of epidermal cells of main roots sur-

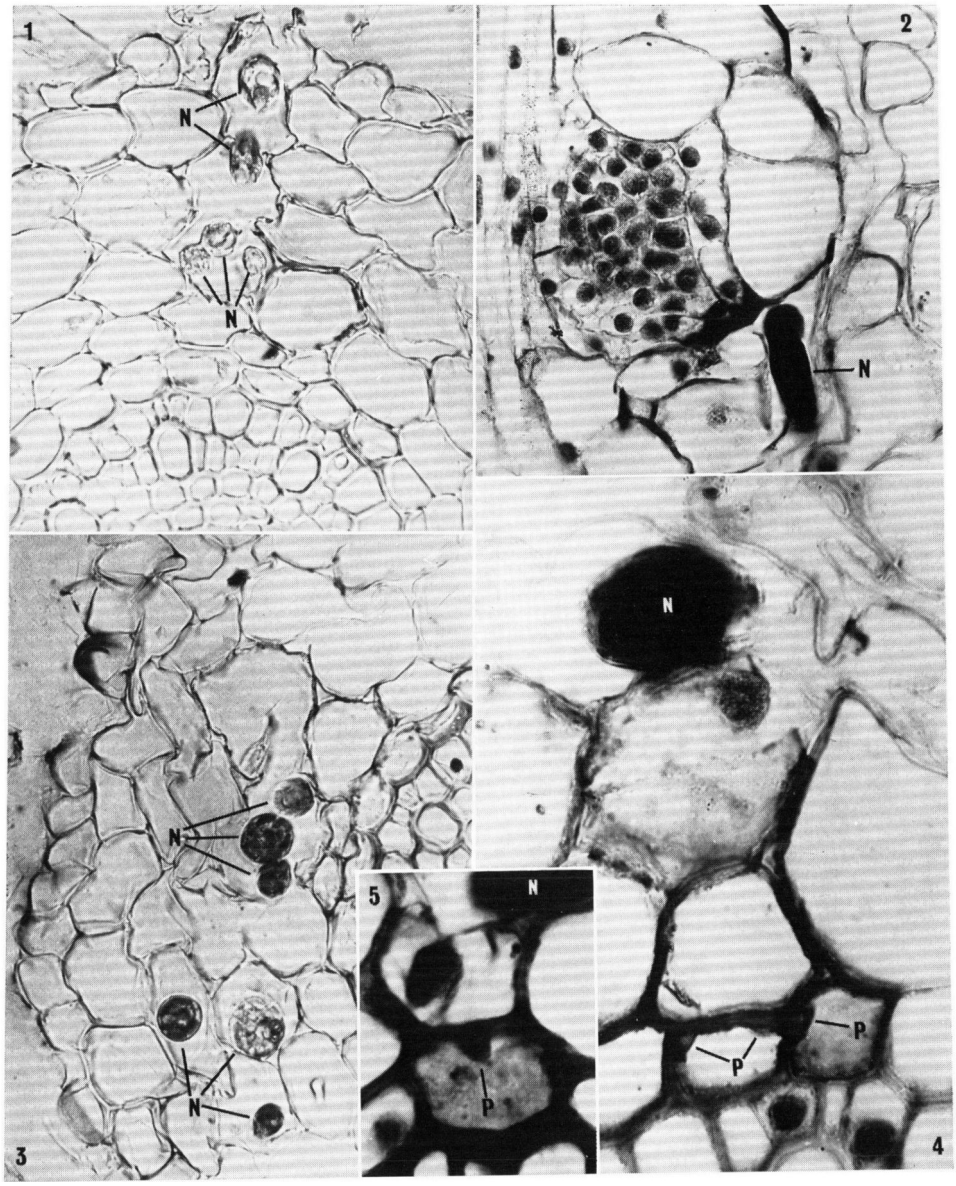

Fig. 1. *P. fallax* (N) in the cortex of wheat roots one week after inoculation, having ruptured epidermal and cortical cell walls.

Fig. 2. *P. fallax* (N) feeding at a lateral root initial within the cortex of the main root.

Fig. 3. *P. fallax* (N) in a cavity caused by breaking cells in the cortex of wheat roots, one week after inoculation.

Figs. 4 & 5. Peg-like outgrowths and thickenings (P) in the outer tangential cell walls of the endodermis of wheat roots four weeks after inoculation with *P. fallax* (N).

rounding the base of a lateral root of wheat started to turn brown. Transverse sections of wheat roots showed that cortical cells near nematodes were darker and had thicker walls than unaffected cells. One root contained a nematode in an endodermal cell. Nematodes also fed on lateral-root initials within the cortex, and discoloured the tips (Fig. 2).

Third week. In inoculated barley roots, discolouration at a lateral and main root junction became visible to the naked eye. Transverse sections of inoculated wheat roots showed the outer walls of endodermal cells stained darker and were thicker where nematodes were present. Nematodes also fed in and under the root cap, at the growing point, and stopped the root tip from growing.

Fourth week. The endodermis of one barley root turned brown for much of its length. Wheat roots had cortical cells with thick, dark staining walls, and endodermal cells had granular, dark staining contents. The outer tangential walls of endodermal cells near nematodes were irregularly thickened on their inner surfaces by deposited material that produced irregular, inward growing, "pegs" of tissue (Fig. 4 and 5).

Seventh week. Barley roots were swollen behind the tip, with the surface rough and brown for a few millimetres, but wheat roots were more irregularly thickened over greater lengths, giving a "lumpy" or beaded appearance. The stele of wheat roots was brown in places but in barley was brown along its whole length. Root tips of wheat, and the junctions of main and lateral roots, had brown lesions.

Eighth week. The cortex of barley roots was discoloured, and root necrosis general. The stele of wheat roots was necrotic, but not the cortex, though heavily attacked roots had rough surfaces and had lost outside cells.

Tenth week. The stele and cortex of barley roots were brown and necrotic, and the root irregularly swollen for about 5 mm behind the tip. The cortex of one root disintegrated completely about 3 mm behind the tip, exposing the stele and releasing nematodes into the agar. Cortical cells of wheat died. In both species, lateral roots were stubby and necrotic.

Root damage on sugar beet

Four days after inoculation brown lesions were visible at the junction of the main and a lateral root, where nematodes that had penetrated during the first day after inoculation were feeding. A week after inoculation, more lateral roots developed in inoculated than uninoculated sugar beet (mean 13.5 secondary roots compared with 6.0). The second week after inoculation, many roots had discrete brown lesions and others were generally discoloured. Heavily attacked root tips swelled and became club-shaped, and there were often distinct lesions at main and lateral roots. In one culture severe necrosis was accompanied by a dark-brown substance diffusing into the agar.

Seven weeks after inoculation, the stele was extensively brown, the cortex associated with dark-brown lesions on the stele became necrotic, and several root tips swelled and became dark brown and rough surfaced. The condition

of attacked roots worsened until, at the end of the experiment 10 weeks after inoculation, both stele and cortex were all dead or dead in discrete lesions, with the stele the darker brown. Some of the lesions were associated with localised swellings. Some of the root systems had short, stubby, dark-brown laterals swollen at the tip and with rough surfaces. In many roots, cells fell off the outside of attacked areas, and a dark-brown substance diffused into the agar.

DISCUSSION

In contrast to *P. thornei*, which Baxter & Blake (1967) found did not prefer any part of the wheat root system, *P. fallax* preferred to invade main roots of all three species used in these tests at points where lateral roots emerge. Possibly this is because the lateral roots are endogenous and as they emerge they separate rows of cells and make openings in the epidermis. The nematodes clustered here and around root tips. Many penetrated the roots close together. The nematodes may cluster at these points because they are attracted to them by substances that diffuse from rapidly growing cells or from those damaged or pierced. Alternatively nematodes may attract one another when penetrating cells. Some of Seinhorst's (1967, 1970) population models are based on the assumption that "nematodes do not attract or repel each other when invading roots" (Seinhorst, 1970). Clustering from whatever cause invalidates models based on the assumption that this describes adequately the relationship between different numbers of nematodes at different moments in time. Further investigation is required to see if the models even provide good approximations.

How *Pratylenchus* spp. affect roots of arable crops in microbiologically sterile conditions has been little studied. Baxter & Blake (1968) showed that *P. thornei* took 3 weeks to destroy cortical cells and form cavities in wheat roots. Lesions became visible only after 6 weeks, by which time the stele was necrotic but structurally unaltered.

My observations show that *P. fallax* caused lesions in roots of wheat, barley and sugar beet so is a direct pathogen of these crops. Damage to sugar beet roots was soon evident and 4 days after inoculation became conspicuous. Barley showed slight symptoms a week after inoculation, and wheat not until nearly two weeks after inoculation. However, sections of wheat roots showed that cells in the cortex had been damaged before the roots showed external symptoms. Browning of the endodermis produced visible lesions some time before the cortex became necrotic. Townshend (1963a; 1963b) found that the endodermis also reacted more strongly than the cortex in roots of plants attacked by *P. penetrans*, and browning of the endodermis in cabbage roots was accompanied by the accumulation of polyphenols (Acedo & Rohde, 1971). Four weeks after inoculation, when the endodermis of wheat roots in my tests showed brown lesions, it was also thickened by irregular deposits on the inner surface of the outer tangential walls opposite cortical cells damaged by the nematode. Similar thickening has not been reported in plants invaded by *P. penetrans*.

Mamiya (1970) found *P. penetrans* frequently in the endodermis and stele of *Cryptomeria japonica* seedlings, and Acedo & Rohde (1971) found it in the stele of cabbage roots 6 weeks after inoculation. *P. fallax* occurred in cells of the endodermis of wheat roots, but not in the stele. Root sections were made for only four weeks following inoculation, by when the nematode may not have had enough time to penetrate the endodermis into the stele. Acedo & Rohde (1971) found evidence that *P. penetrans* reproduced best in necrotic tissue. *P. fallax* did not: sugar beet, which produced most necrosis, yielded only 83 nematodes per dish 10 weeks after inoculating with 135 $\pm$ 5 nematodes, whereas wheat, which produced least necrosis yielded 1018; barley which was intermediate in reaction, yielded 878. The nematodes left necrotic parts of roots of all these species to invade fresh tissue. Possibly, therefore, the endodermis is unattractive to *P. fallax*, which did not penetrate into the stele because the endodermis became necrotic rather than because it resisted invasion mechanically.

I thank K. Warwick for making the root sections.

Zusammenfassung

Die Wirkung von Pratylenchus fallax *auf die Wurzeln von Weizen, Gerste und Zuckerrüben*

Pratylenchus fallax wanderte in die Wurzelspitzen, die Wurzelhaarzone und die Verbindungsstelle von Haupt- und Nebenwurzeln von Weizen, Gerste und Zuckerrüben ein. Viele neigten dazu, gemeinsam an einer Stelle einzudringen. In Sterilkulturen wurden Zuckerrübenwurzeln schnell nekrotisch. Die Wurzeln von Gerste und Weizen wurden in geringerem Maße und langsamer geschädigt. Querschnitte von Weizenwurzeln zeigten, daß die Zellen schwer geschädigt waren und zusammenfielen, bevor die Symptome äußerlich sichtbar wurden. Die Endodermis war bei schwerem Befall verdickt und verfärbt. *P. fallax* vermehrte sich in den Wurzeln von Gerste und Weizen besser als in denen von Zuckerrüben.

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