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CEREAL AND LEGUMINOUS HOST RESPONSE TO *LONGIDORUS BELLOI* FEEDING

by

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Summary. The pathological and histological effects of *Longidorus belloi* Andres et Arias feeding on cereal and leguminous plants were investigated under controlled conditions. Feeding behaviour was similar to that of other *Longidorus* species. The root-tip was penetrated by the odontostyle through several layers of cells causing the formation of a terminal gall. Most galls were formed on wheat and fewest on vetch among the hosts studied. The cell response in the hosts differed: hypertrophy of the meristematic tissues was detected in vetch, wheat and barley roots, together with lysis of the cell contents and walls in barley. This is considered to be a hypersensitive response, as the tissues that were devoid of inclusions quickly collapsed. In rye grass and lentil the occurrence of a systematic hyperplasia reaction indicated that nematode feeding had induced cellular modifications at a distance from the cells penetrated by the odontostyle. This may be related to their being poor hosts.

The new species *Longidorus belloi* Andres et Arias (1988) was found associated with cereal crops in Spain (Andres and Arias, 1985). Experiments were done under controlled conditions to obtain information on the reaction of some cereals and leguminous crops to the nematode.

Cohn (1975) states that the ability of a nematode to feed on a particular plant does not in itself indicate that the plant is a host in the sense of supporting reproduction. Nevertheless, the histological reaction of the plant in response to the nematode's feeding can be indicative of its susceptibility or resistance to the parasite and hence indirectly, at least, of its host status.

Materials and Methods

Seedlings of barley (*Hordeum vulgare* L.), wheat (*Triticum aestivum* L.), rye grass (*Lolium rigidum* G.), vetch (*Vicia sativa* L.) and lentil (*Lens culinaris* Medic.) were transplanted singly into 5 cm diameter clay pots containing 10 ml sterilized sand with 20 replicates for each cultivar. Each pot was inoculated with 20 adult females of *L. belloi* extracted from naturally infested soil. The pots were kept in a growth chamber at 22°C, 60% RH and 3000 lux. Three weeks after nematode inoculation, the plants were gently removed from the sand to obtain intact root-tips.

The number of nematode induced galls on each root system were counted using a stereoscopic microscope. A few plants of each host were removed from the pots at one to two weeks after nematode inoculation and the galls excised

from the roots. These were then fixed in 3% glutaraldehyde in 0.05 M sodium cacodylate buffer pH 7.2 for 4 h, rinsed in the same buffer and post-fixed in 2% osmium tetroxide for 4 h at 4°C, followed by staining in 0.5% aqueous uranyl acetate, dehydration in an ascending series to absolute ethanol and embedding in Spurr's medium. Sections, 2 µm thick, were cut with an LKB ultratome III, stained with toluidine blue and observed under a Zeiss photomicroscope.

Results

L. belloi fed on all the plants tested and produced galls at the root-tips. However, the number of galls per root differed significantly between hosts (Table I). Based on the

TABLE I - Number of galls on root tips induced by *Longidorus belloi* in different hosts.

Host	N. of galls/root system
Wheat	44 ± 1.2 (a)
Barley	23 ± 1 (bd)
Rye grass	30 ± 0.7 (c)
Lentil	22 ± 2.1 (d)
Vetch	15 ± 3.8 (e)

N.B.: Statistical significance was determined according to Student's t-test. Means followed by different letters in columns are significantly different. (P = 0.05).

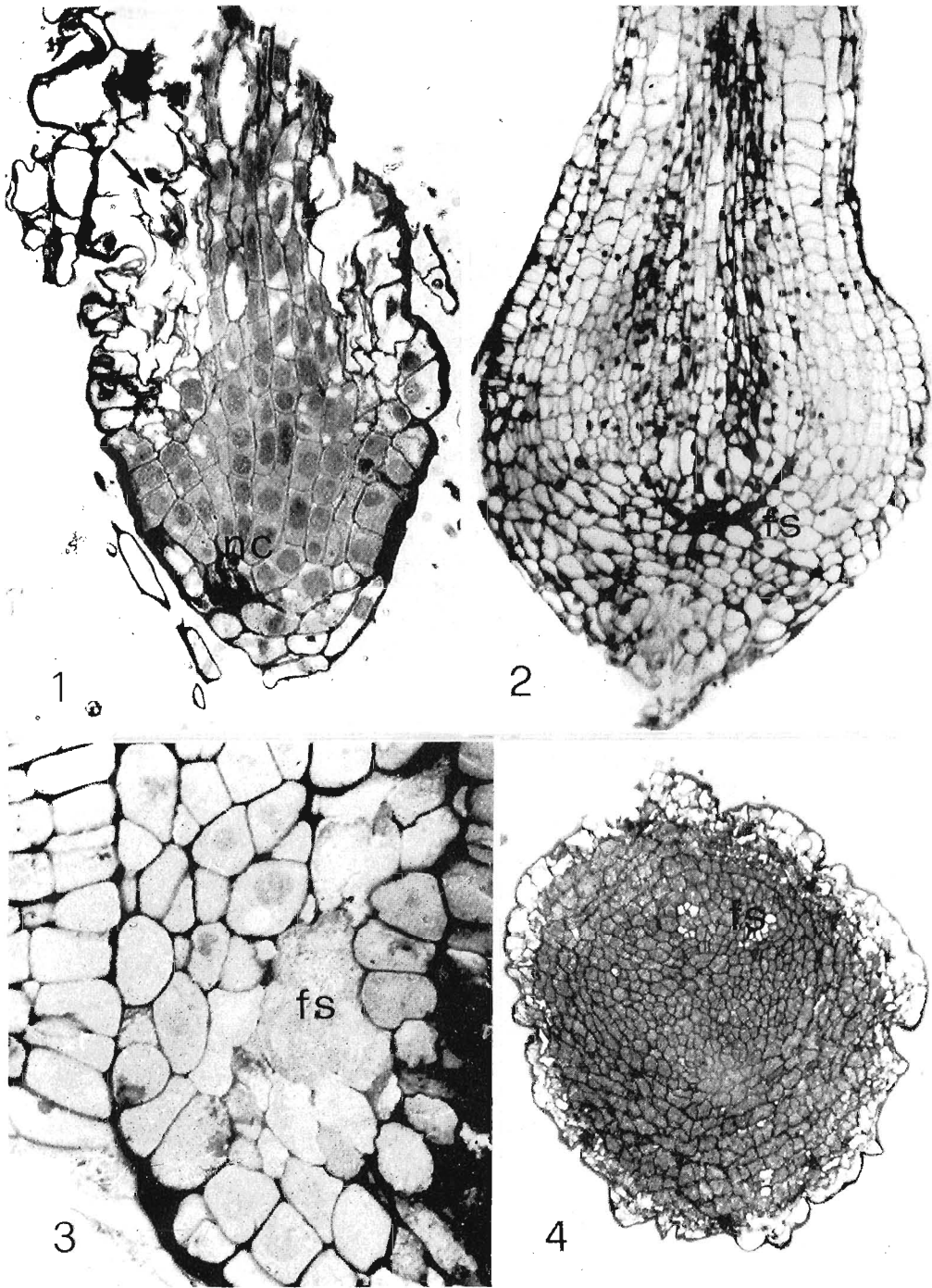


Fig. 1 - Longitudinal section of wheat root with dark cells, representing one site of *Longidorus belloii* odontostyle penetration. The cortical cells around the feeding site show hypertrophy. Another previous feeding site (arrow) has induced early maturation of the cells, resulting in their becoming highly vacuolated. (x420).

Fig. 2 - Longitudinal section of root tip gall on barley induced by *L. belloii*. The feeding area is localized at the initial cells. Swelling of the root tip appeared to be due to a slight hypertrophy of cortical and procambial cells. Vessels are detectable very close to the injured meristematic cells. (x350).

Fig. 3 - Details of the feeding site of *L. belloii* in a barley root. A lysigenous cavity results from the feeding action. Cells delimiting the lysed ones have a hypertrophic appearance. (x1200).

Fig. 4 - Transverse section of infested rye grass. Necrotic empty cells represent the feeding site (fs). As a consequence all the meristematic, cortical and procambial cells show a prominent hyperplasia with abundant small cells. (x270).

number of galls, wheat would be regarded as the best host for *L. belloii* among the plants tested.

Sectioned galls from roots of the different hosts showed that the nematode inserted its odontostyle into the meristematic tissue and induced a column of necrotic cells. Around the feeding site considerable histological changes occurred in the surrounding cells, but differing between the hosts.

In wheat the cells became hypertrophic. The feeding site was almost always restricted to one side of the root and involved relatively few cells whose contents stained deeply. Neighbouring cells were enlarged, indicating they had become hypertrophic. Galls exposed to nematodes for two weeks contained areas of cells that were largely devoid of their contents (Fig. 1). Hypertrophy was also evident in barley roots, where the feeding site was established close to the initial cells. The procambial tissue was subjected to an

early maturation and vascular tissue reached the apex of the root (Fig. 2). A row of necrotic cells indicated the pathway of the nematode odontostyle and the contents of neighbouring cells were lysed, with breakdown of the cell walls (Fig. 3). Galled roots of rye grass showed hyperplasia and synchronized division of the cortical and procambial cells (Fig. 4). Cells had divided to give two or four uninucleate daughter cells. The cytoplasm of the cortical cells stained deeply with toluidine blue, demonstrating that the cell contents were still intact, in contrast with the cells that had been fed on (Fig. 4). Galled roots of lentil showed a marked hyperplastic response of the cortical and procambial tissue, particularly on the inside of the root swelling, related to the feeding site. On the side of the gall opposite to the feeding site, the cortical cells were enlarged and with an irregular profile and little cytoplasm, indicating they had become differentiated cells. Procambial cells were also

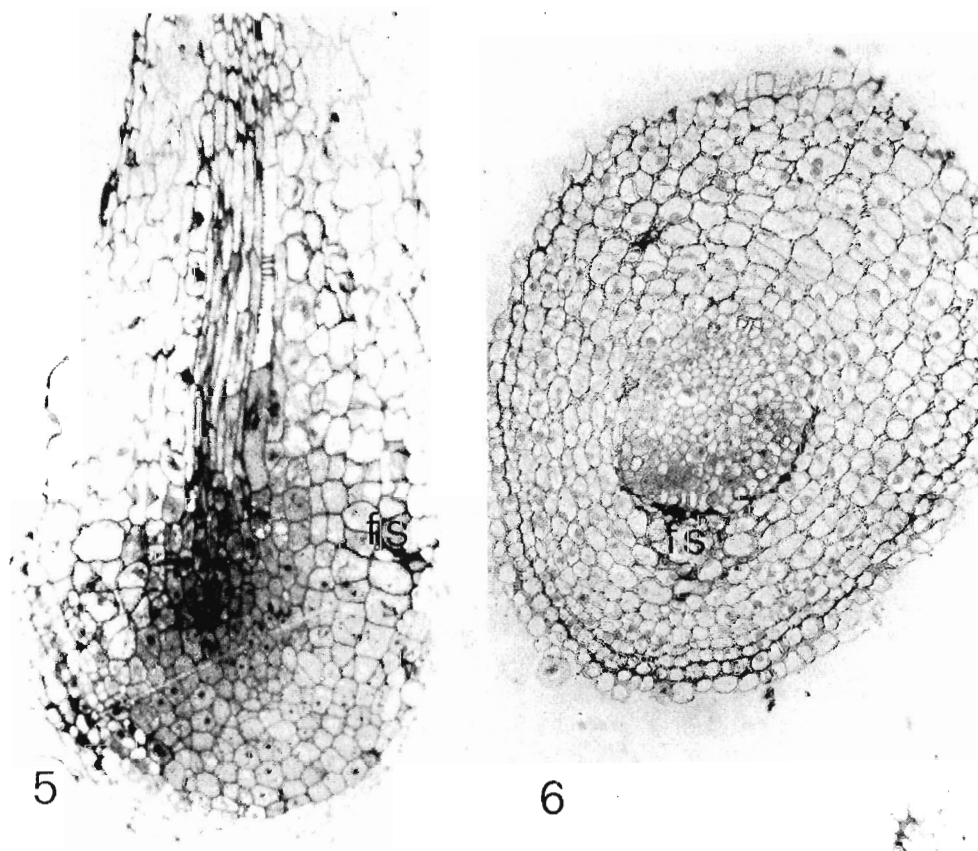


Fig. 5 - Longitudinal section of a galled lentil root. Note empty cells in the 4th layer of the cortical cells. Cortex and procambium have normal appearance with cells well stained with toluidine blue. This indicates that the cell content and the nucleus are still well preserved, but subjected to a process of hyperplasia. On the opposite side cells are highly differentiated and hypertrophied with irregular profile of the cell wall. (x380).

Fig. 6 - Transverse section of a swollen lentil root. Endodermis is involved in the stylet penetration and is darkly stained, because of necrosis. Some of the cortical cells are partially devoid of their contents because of the trophic action of the nematode. In the procambium marked hypertrophy and vessel formation are evident. Cell content is still well preserved. The cortical cells, on the side opposite to that of the feeding site are differentiated and slightly hypertrophied. (x720).

subjected to early maturation (Fig. 5). In vetch the response to nematode feeding was similar to that in lentil. Cells directly injured by the odontostyle were localized at the limit of the procambial tissue. The feeding area was widespread in the cortical cells, whose content seemed to have been digested by the enzymatic action of the nematode (Fig. 6). The procambial and cortical tissues opposite to the feeding site showed hyperplasia and a slight hypertrophy with marked vacuolation (Fig. 6), similar to the situation with rye grass.

Discussion

Galling of roots attacked by *L. belloi* are similar to those of other *Longidorus* species (Whitehead *et al.*, 1970; McElroy, 1971; Bleve-Zacheo *et al.*, 1977a, 1984; Robertson *et al.*, 1984).

Whilst the ability of a nematode to feed on a particular plant does not indicate in itself that it is a host (Cohn, 1975) there is evidence to suggest that the extent of galling is correlated with host status (Flegg *et al.*, 1970; Wyss, 1978; Griffiths and Trudgill, 1983; Bleve-Zacheo *et al.*, 1984). Thus, among the plants tested, wheat would seem to be a good host for *L. belloi* and vetch a relatively poor one.

The data presented indicate that the feeding of *L. belloi* on wheat, barley, rye grass, lentil and vetch was similar to that of other *Longidorus* species. However, the response of the different hosts to *L. belloi* feeding differed between hosts. The histological changes in the roots attacked by *L. belloi* also provide some indication of relative host status. Hypertrophy occurred in wheat and barley and cortical cells of vetch, this phenomenon frequently occurring in galls induced by *Longidorus* spp. (Bleve-Zacheo *et al.*, 1977a, b; Robertson *et al.*, 1984). However, the presence of lysigenous areas in barley indicates that wheat is a better host, as supported by the number of galls present on the roots. Lysis of the cells must be considered as a hypersensitive response, as this leads to an earlier destruction of the roots. The response to *L. belloi* feeding on barley is similar to that reported for celery infested by *L. apulus* which induced a large lysigenous cavity (Bleve-Zacheo *et al.*, 1977b). In rye grass *L. belloi* caused marked and ordered hyperplasia with the division of daughter cells, accompanied by an apparent reduction in the proportion of cytoplasm, and in the nucleus and nucleolus, whereas in celery the hyperplasia induced by *L. apulus* was disordered and led to abnormal tissue proliferation (Bleve-Zacheo *et al.*, 1977b).

The ordered development of galls induced on rye is similar to that of galls induced on various hosts by *L. elongatus* (Robertson *et al.*, 1984). There is a period during which cellular modification is initiated and feeding is limited to an abstraction of the contents of the few cells. This is followed by a period during which the contents of cells forming the feeding site are mobilized and removed. Robertson *et al.* (1984) hypothesize that *L. elongatus* has two types of feeding behaviour, one to initiate gall formation and another to remove cell contents, or alternatively that cell contents are removed only after prolonged feeding. Whatever the mechanism of feeding, it is clear from our observations and others (Towle and Doncaster, 1978) that *Longidorus* species derive food from cells far removed from the cells which contain the odontostyle tip.

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