Observations on the Feeding and Symptomatology of *Xiphinema* and *Longidorus* on Selected Host Roots[1]

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Abstract: *In vitro* feeding of *Xiphinema brevicolle, X. index* and *Longidorus africanus* on roots of host seedlings is described. Both *Xiphinema* spp. fed mainly along roots rather than at tips and up to several days at a single site. Feeding of *L. africanus* was confined to root tips and lasted up to 15 min. No visible short term reaction of roots parasitized by the *Xiphinema* spp. could be discerned, but both swelling and cessation of growth of root tips were observed within 20 hr after feeding by *L. africanus*. Long-term (12-month) symptoms on roots of several host plants caused by cultured populations of *X. brevicolle, X. index, X. italiae, L. africanus* and *L. brevicaudatus* are described. All the *Xiphinema* spp. caused a thinning and distinct darkening of root systems and, at some sites, a breakdown of the cortex. Both species of *Longidorus* caused stubby and swollen root tips. Root symptom severity was in proportion to nematode population levels. *Key Words*: Feeding, Symptoms, *Xiphinema brevicolle, X. index, Longidorus africanus.*

Information on the feeding habits of *Xiphinema* and *Longidorus* and the short-term symptom reaction of parasitized roots is an important aid in understanding their host-parasite relationships and capacity to transmit viruses. Only a few detailed feeding studies have so far been carried out with *Xiphinema* (4, 5, 8), and are entirely lacking with *Longidorus*. In the present paper observations on the feeding of three species of *Xiphinema* and two species of *Longidorus* and the pathological symptoms they cause on host plant roots are reported.

MATERIALS AND METHODS

In vitro feeding studies were carried out with *X. brevicolle, X. index,* and *L. africanus* on grape seedlings (*Vitis vinifera* L.), dwarf nettle (*Urtica urens* L.), and bur marigold (*Bidens tripartita* L.). These hosts were selected since they were found in earlier studies to support nematode reproduction. Stock cultures of the three nematode species were maintained on grape roots in polyethylene bottles (2, 3). The nematodes were extracted from the soil and washed in distilled water before inoculation. Surface-sterilized seeds of the three host plants were placed on 0.75% water agar in petri dishes. After germination, single drop suspensions containing 20–100 nematodes (females + larvae) were placed on the agar near the roots. The cultures were kept in the dark at room temperature (20–25 C) and nematode movements and feeding, as well as root reactions, were intermittently observed and, where possible, photographed, for periods of up to four weeks. No attempt was made to attain aseptic conditions. Feeding of *X. index* was also observed on nonsterilized, sectioned pieces of grape roots on 0.75% water agar in petri dishes.

The appearance of roots of plants inoculated with nematode cultures was compared with those of healthy noninoculated check plants. Hand-picked populations of *X. brevicolle, X. index, X. italiae, L. africanus* and *L. brevicaudatus* were reared on rooted grapevine cuttings; in addition, *X. brevicolle* was cultured on rooted rose cuttings (*Rosa indica* L.) and sour orange seedlings (*Citrus aurantium* L.) and the two *Longidorus* spp. were also cultured on bur marigold seedlings. The cultures were raised on plants growing

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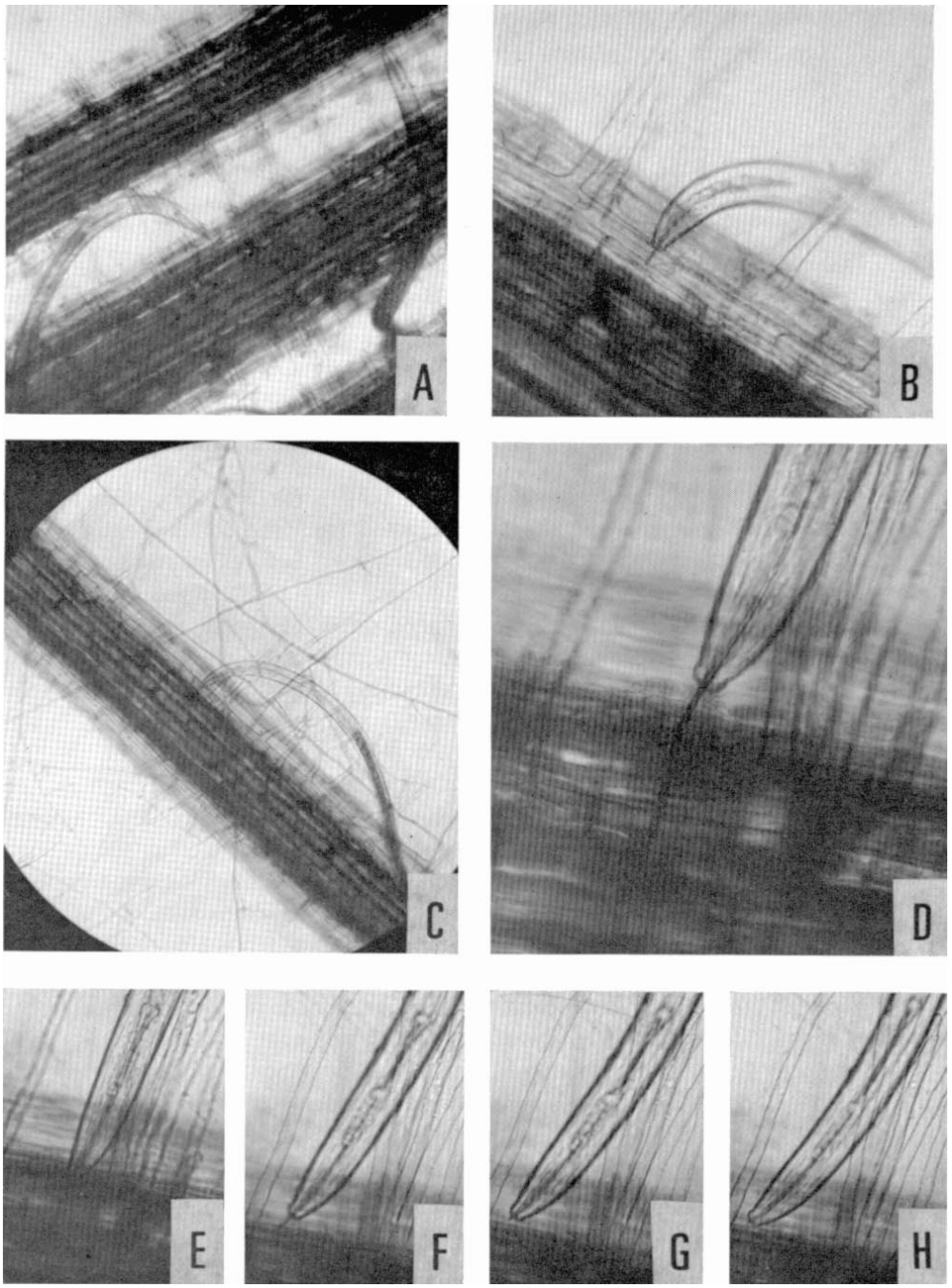

FIG. 1. Feeding of *X. brevicolle* and *X. index* on roots of dwarf nettle: **A**. Two females of *X. brevicolle* feeding; **B**. Larva of *X. brevicolle* inserting onchiostyle into root; **C**. Female of *X. index* feeding; **D**. Female of *X. index* with slightly bent onchiostyle deeply inserted into root; **E–H**. Stages in simultaneous withdrawal and retraction of onchiostyle by female of *X. index*.

in heat-sterilized soil in 500-cc polyethylene bottles, as described earlier (2, 3), and maintained at 23–25 C in a growth chamber. Inocula consisted of fifty nematodes (females + larvae) per bottle. Plants were removed periodically for root observations during the 12 months after inoculation; the soil was screened through 200 and 350 mesh screens, washings were placed on extraction funnels for 24 hr at room temperature, and the nematodes were counted.

RESULTS

IN VITRO FEEDING OBSERVATIONS: Both larvae and females of *X. brevicolle, X. index* and *L. africanus* were observed to feed *in vitro*. Following inoculation, *X. brevicolle* moved along the roots without feeding and many appeared dead after 24 hr: by 48 hr, however, 20 nematodes were either feeding or probing the root surface with quick jabs of their stylets. Feeding occurred at various root locations but rarely near the root tips (Fig. 1, A & B). Feeding nematodes pressed their lip region against the epidermis and gradually (average 8 min) inserted the stylet to various depths in the root tissue, depending on root diameter, often reaching the stele. Curvature of the stylet within the root was common as has been observed by others for *X. diversicaudatum* (8) and *X. index* (5). Feeding periods lasted several hr and even up to three days. The nematodes were rather still during feeding except for occasional motion of their posterior, and positions were not uniform, some were outstretched, others arched and some inclined at an angle. Rapid (up to 90 per min) contractions of the esophageal bulb were intermittently observed, but passage of material into the gut was not visible. After feeding, nematodes invariably withdrew their onchiostyles without retracting them, and with the onchiostyle extended began to move along the root in search of new feeding sites. No conspicuous changes in appearance or shape of the parasitized roots, at the feeding site, or within the feed cell could be seen during feeding or shortly after feeding had stopped.

Feeding by *X. index* (Fig. 1, C & D) was similar to that of *X. brevicolle* with feeding periods lasting up to 36 hr. When the nematodes were feeding on excised pieces of grape root, probing and feeding began immediately, even on roots in generally poor condition, and feeding periods were shorter (maximum 4 hr). Unlike *X. brevicolle, X. index* stylets were retracted while the nematode was still in feeding position. One case of onchiostyle withdrawal from a nettle root was photographed (Fig. 1, E–H); the process required four minutes. As with *X. brevicolle*, feeding of *X. index* occurred at several sites along the roots of all three hosts and only occasionally near tips. Similarly, no immediate changes in root shape or appearance, during or immediately after feeding, were observed.

Feeding of *L. africanus* was first observed 3 days after inoculation, and only four individuals were seen feeding (two females on bur marigold and one female and one larva on dwarf nettle). The feeding site was close to the root tips in all cases (Fig. 2, A, B). Probing was not conspicuous, onchiostyle insertion was rapid, and feeding periods were short (maximum of 15 min). Contractive movements of the oesophagus were not seen, but with two of the nematodes the entire body moved back and forth, pressing on the surface of the root, while the onchiostyle was embedded in the tissue. As with the *Xiphinema* spp., passage of material within the nematode body was not visible. After feeding, the nematode withdrew its onchiostyle and moved away from the root. After 24 hr root elongation had apparently ceased, and a slight swelling was visible at the feeding site just behind the root cap (Fig. 2, C). The swelling enlarged for three days following feeding (Fig. 2, D).

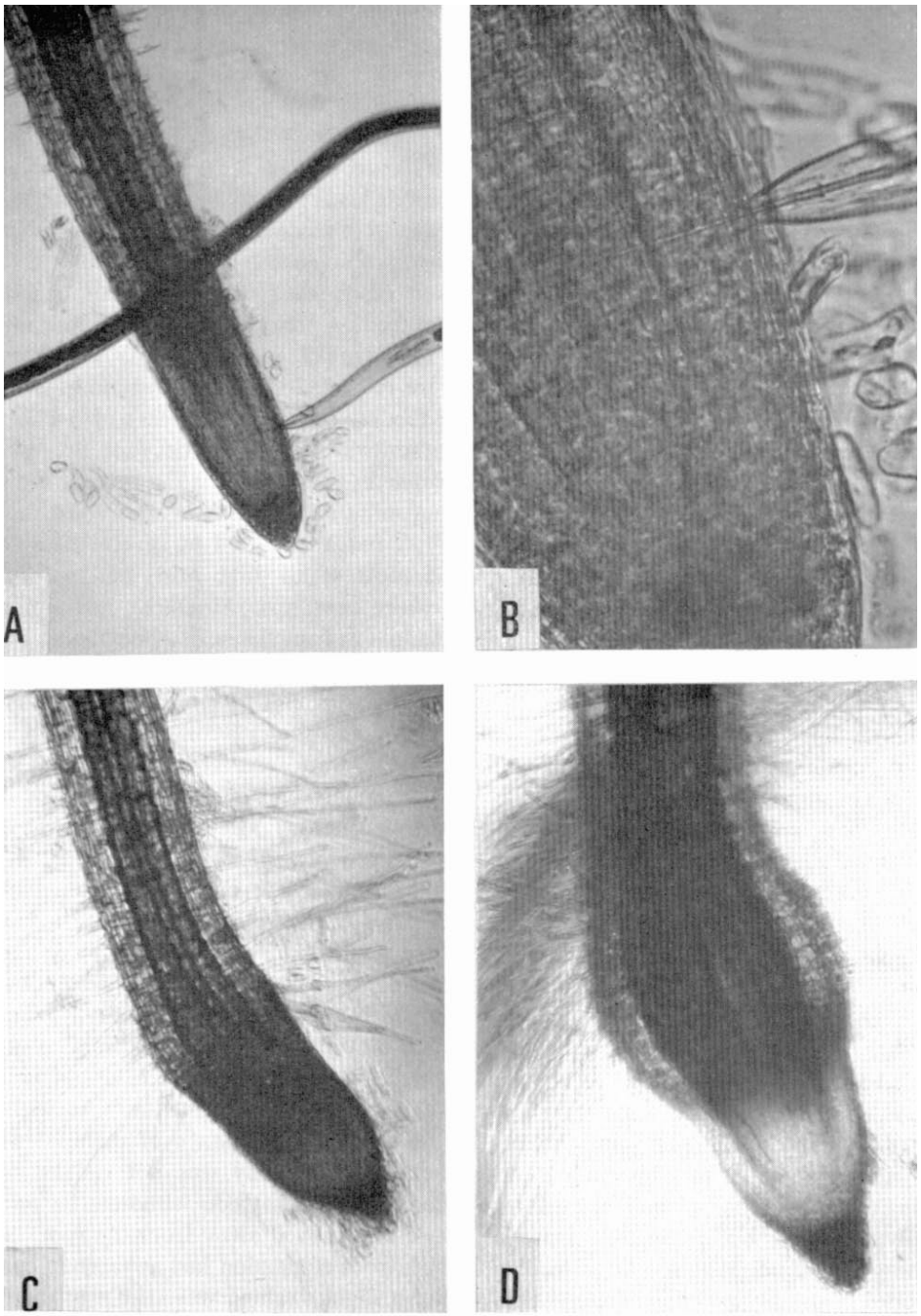

FIG. 2. Feeding of *L. africanus* on root of bur marigold: **A**. Female feeding at root tip; **B**. Penetration of onchiostyle; **C**. Swelling at root tip 20 hr after feeding; **D**. Root tip three days after feeding.

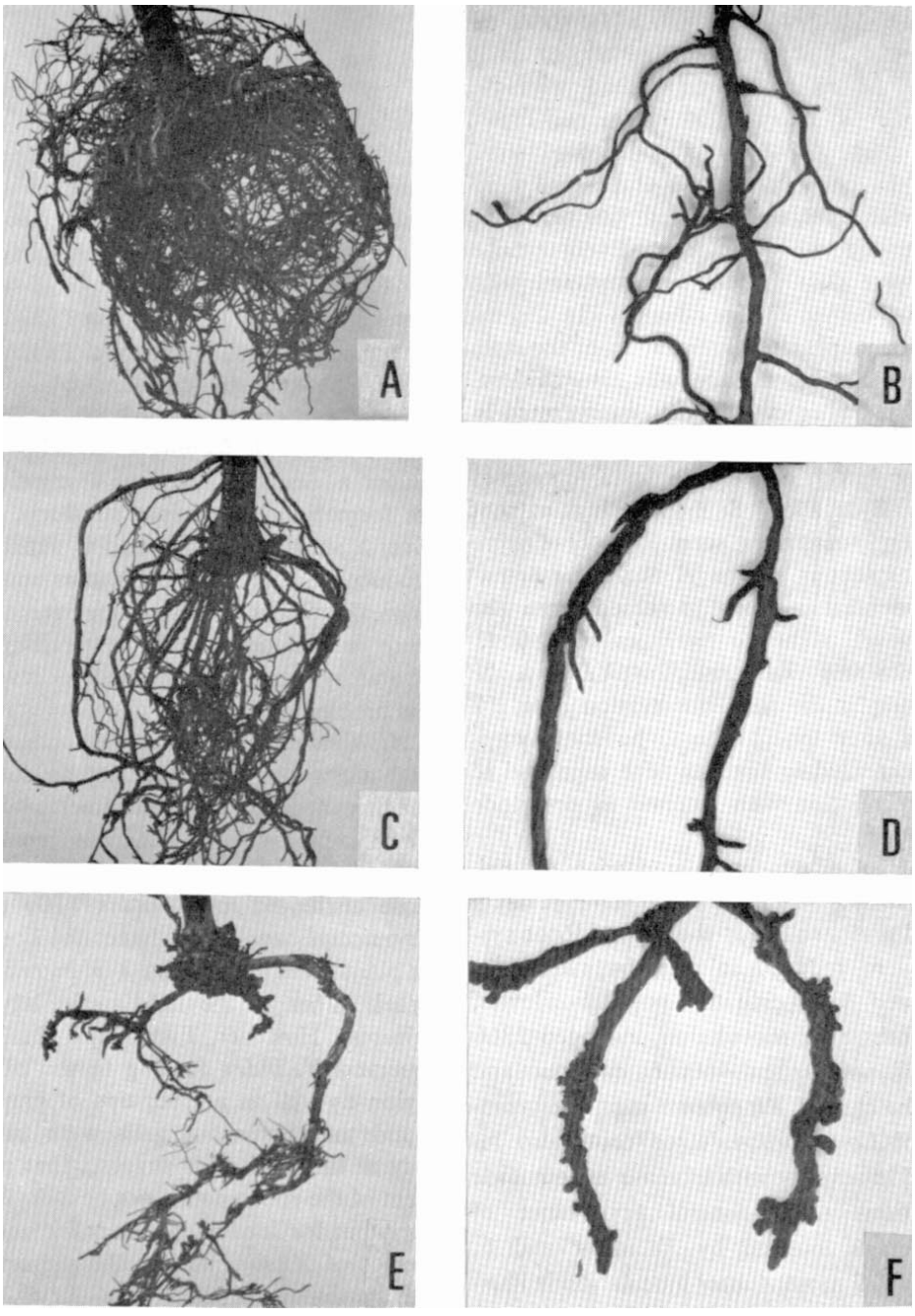

FIG. 3. Nematode damage on grape roots nine months after inoculation: **A, B.** Noninoculated controls; **C, D.** With *X. index*; **E, F.** With *L. africanus.*

OBSERVATIONS ON ROOT SYMPTOMS IN NEMATODE CULTURES: The entire root systems of all plants inoculated with *X. brevicolle, X. index* or *X. italiae* had fewer feeder roots than the controls, and were darker in color. Infected grape and rose roots were black as compared with brown in the non-infected roots; infected sour orange roots were dark brown as compared with yellowish-brown in the controls. In the infected grape plants, discoloration of the stem close to the soil surface was also evident. The cortex of infected roots disintegrated in several areas and, where nematode infestation was heavy, entire roots were sometimes black and decayed. Slight swellings at root tips were sometimes seen, but these were not conspicuous at first glance. The nematode population levels increased from the initial level of 50 to averages of 480 *X. italiae*, 2800 *X. index* and 5200 *X. brevicolle* per bottle at the final observation date, 12 months after inoculation. The root symptoms increased in severity with time and in close correlation with increase in nematode populations.

Roots of plants inoculated with *L. africanus* and *L. brevicaudatus* were severely stunted and swollen at their tips. Root systems were reduced in size, but were not discolored. Final nematode population levels, 12 months after inoculation, averaged 2300 *L. brevicaudatus* and 4600 *L. africanus* and as in the case of *Xiphinema* spp., the symptoms of *Longidorus*-infected roots also increased in severity with increase in nematode populations. The general appearance of grape roots infected by *X. index* and *L. africanus*, 9 months after inoculation is illustrated in Fig. 3.

These observations have been confirmed by examination of numerous plants serving as hosts in our nematode maintenance cultures.

DISCUSSION

From the information available to date on the pathogenicity of *Longidorus*, there is ample evidence that formation of stubby and swollen root tips is a characteristic symptom. Root symptoms similar to those described for *L. africanus* and *L. brevicaudatus* in this paper, have also been reported on lettuce for *L. africanus* (9) and on various hosts for *L. elongatus* (12, 14), *L. maximus* (13), *L. vineacola* (1), and *L. cohni* (6) and my own unpublished data. Evidently, these symptoms are a direct result of nematode feeding at root tips, which causes a cessation of root elongation and the formation of terminal swellings. Moreover, a short feeding period is sufficient to produce the effect. The stimulus and physiological process involved however, still require elucidation, and research in this laboratory has been initiated to investigate this problem.

Xiphinema individuals were observed to feed along the root and not at root tips. Feeding periods were extremely long and root symptoms were clearly different from those produced by *Longidorus*. Galls, like those attributed to *X. index* (10) and *X. diversicaudatum* (11), have not been seen on plant roots infected by *Xiphinema* spp. in Israel, either in the field or in laboratory cultures. However, Fisher and Raski (5), observed *X. index* feeding in the piliferous region as well as at root tips of grape, and found that numerous galls were produced only at the tips. This suggests that enlargement of the root tip may not be a dependable symptom for feeding by *X. index* and other species of *Xiphinema*. In the present study the symptoms produced by *X. index, X. italiae* and *X. brevicolle*—a general pattern of darkening of roots and breakdown of the cortex—are similar to those caused by *X. americanum* on roots of sugar maple, as observed by Di Sanzo and Rohde (4), and

by *X. americanum* and *X. chambersi* on strawberry roots, as reported by Perry (7).

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