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Development of *Meloidogyne chitwoodi* on Wheat[1]

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Abstract: Postinfection development of *Meloidogyne chitwoodi* from second-stage juveniles (J2) to mature females and egg deposition on 'Nugaines' winter wheat required 105, 51, 36, and 21 days at 10, 15, 20, and 25 C. At 25 C, the J2 induced cavities and hyperplasia in the cortex and apical meristem of root tips with hypertrophy of cortical and apical meristem cell nuclei, 2 and 5 days after inoculation. Giant cells induced by late J2 were observed in the stele 10 days after inoculation. Clusters of egg-laying females were common on wheat root galls 25 days after inoculation. Juveniles penetrated wheat roots at 4 C and above, but not at 2 C, when inoculum was obtained from cultures grown at 20 C, but no penetration occurred at 4 C when inoculum was stored for 12 hours at 4 C before inoculation. In northern Utah, J2 penetrated Nugaines wheat roots in the field in mid-May, about 5 months after seedling emergence. *M. chitwoodi* eggs were first observed on wheat roots in mid-July when plants were in blossom. Only 40% of overwintered *M. chitwoodi* eggs hatched at 25 C.

Key words: Columbia root-knot nematode, *Triticum aestivum,* histopathology, life cycle, postinfection development, root infection, temperature.

Potato (*Solanum tuberosum* L.) and wheat (*Triticum aestivum* L. em Thell) are two economically important crops grown in the northwestern United States damaged by the Columbia root-knot nematode, *Meloidogyne chitwoodi* Golden et al. (3,4). Recent studies have shown that the development duration of *M. chitwoodi* is greatly influenced by temperature. Duration was 20–21 days at 20 C and 82–84 days at 10 C from single cell egg to second-stage juvenile (J2) (2). J2 were also reported to invade potato roots at 10 C (4). However, information is lacking on temperature as affecting the duration of *M. chitwoodi* postinfection development and its ability to invade wheat roots. The duration of its life-cycle on wheat in the field is also unknown.

A study of *M. chitwoodi* on wheat was conducted to determine 1) influence of temperature on the duration of nematode postinfection development, 2) histological alteration of root tissues by nematode infection, 3) effect of temperature on root infection, and 4) duration of the life-cycle under field conditions.

MATERIALS AND METHODS

A *M. chitwoodi* population isolated from tomato (*Lycopersicon esculentum* Mill.) at Prosser, Washington, was used in our experiments. The nematode population was increased on wheat (*Triticum aestivum* cv. Nugaines) in a greenhouse. Infective juveniles were collected by incubating egg masses on 75-μm-pore microsieves (2).

Effect of temperature on postinfection nematode development: Three hundred sixty 2-day-old wheat seedlings were inoculated with 1,500 J2 per seedling. The J2 were poured in an aqueous suspension directly on the seedling roots during transplanting into flats containing methyl bromide-fumigated coarse sand. Seedling roots were exposed to J2 for 3 days, seedlings were then removed from the flats, and the root systems were carefully washed in tap water to remove all J2 outside the roots. Seedlings were then transplanted singly into 10-cm-d plastic pots containing 400 cm^3 fumigated coarse sand. Seedlings were divided into four groups of 90 plants each, and groups were maintained at 10, 15, 20, or 25 C in growth chambers. Four seedlings grown at 20 and 25 C were harvested at 2-day intervals, and four grown at 15 and 10 C were harvested at 5-day intervals. Roots were gently washed in tap water and stained with hot acid fuchsin in lactoglycerol (1:1:1 distilled water, glycerol, lactic acid). Nematodes were removed from root tissues with the aid of a dissecting microscope, mounted in lactoglycerol, and observed microscopically to determine their degree of development based on the presence of retained molted cuticles, morpho-

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logical conformation of the stomodaeum (foregut), and gonad development. The experiment at a given temperature was terminated when eggs were detected on wheat roots.

Histological examination of nematode infected wheat roots: Forty 2-day-old Nugaines wheat seedlings were exposed to 1,500 J2 per seedling in a flat containing coarse sand. Two days after inoculation, the seedlings were removed from the flat, washed free of sand and J2, and transplanted singly into pots containing 400 cm^3 fumigated soil (72% sand, 18% silt, 10% clay). Seedlings were grown in a growth chamber at 25 C, and roots for histological examination were collected at transplanting and at 5-day intervals thereafter. Root segments were fixed in FAA, dehydrated in a tertiary butyl alcohol series, and embedded in paraffin. Sections, 10 μm thick, were stained with safranin and fast-green, mounted in Dammar xylene, and observed under a compound microscope (1).

Effect of temperature on root infection by J2: Seventy-two 2-day-old Nugaines wheat seedlings in each of two experiments were inoculated with 2,000 J2 per seedling. A J2 suspension was poured onto seedling roots after transplanting into 2-cm-d plastic pots containing 90 cm^3 methyl bromide-fumigated coarse sand and maintained in growth chambers at 2, 4, 6, 8, 10, and 12 C. In the first experiment, wheat seedlings were inoculated with J2 stored at 20 C before inoculation. In the second experiment, J2 inoculum and the seedlings were maintained at 4 C for 12 hours before inoculation. After inoculation, plants in both experiments were grown in growth chambers for 13 days at the above temperatures. Harvested roots were stained, and nematodes in the roots were counted.

Nematode life-cycle under field conditions: Twenty 25-cm-d plastic pots containing sandy loam soil (72% sand, 18% silt, 10% clay) infested with *M. chitwoodi* egg masses with a mean of 50 eggs/cm^3 soil were placed about 30 cm deep in a trench at Logan, Utah, in November 1983. The soil around the pot walls was compacted to avoid air cushion formation between the soil and pot walls. One Nugaines wheat seed was sown in each pot in the last week of November 1983. Snow covered the pots from late November until April 1984. Wheat seeds germinated under the snow and seedlings were present in the pots in April. From April until July, wheat plants from four pots were harvested monthly and the root systems were observed for nematode infection. No nematode infection was detected in April. Twelve egg masses (2,160 eggs) were recovered from the soil in the pots and incubated on 75-μm-pore microsieves in distilled water at 25 C to ascertain the viability of the over-wintered eggs. The eggs were incubated until egg hatch ceased.

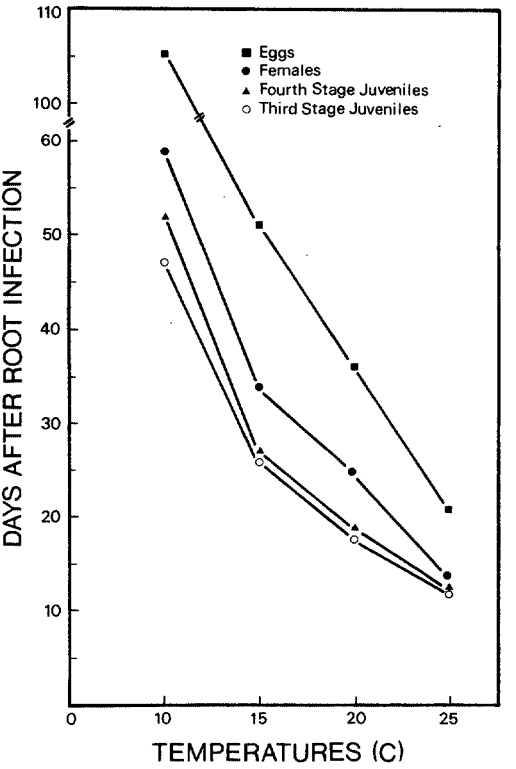

FIG. 1. First appearance of several developmental life-stages of *Meloidogyne chitwoodi* in roots of wheat grown at different temperatures.

RESULTS AND DISCUSSION

Effect of temperature on postinfection nematode development: At 10, 15, 20, and 25 C, postinfection nematode development required 105, 51, 36, and 21 days, respectively (Fig. 1).

Second-stage juveniles required more time to develop and molt than did J3 and J4, and J3 developed more rapidly than did J4 at all temperatures (Fig. 1). Development of J3 was completed after 5, 1, and 1 days at 10, 15, and 20 C, respectively,

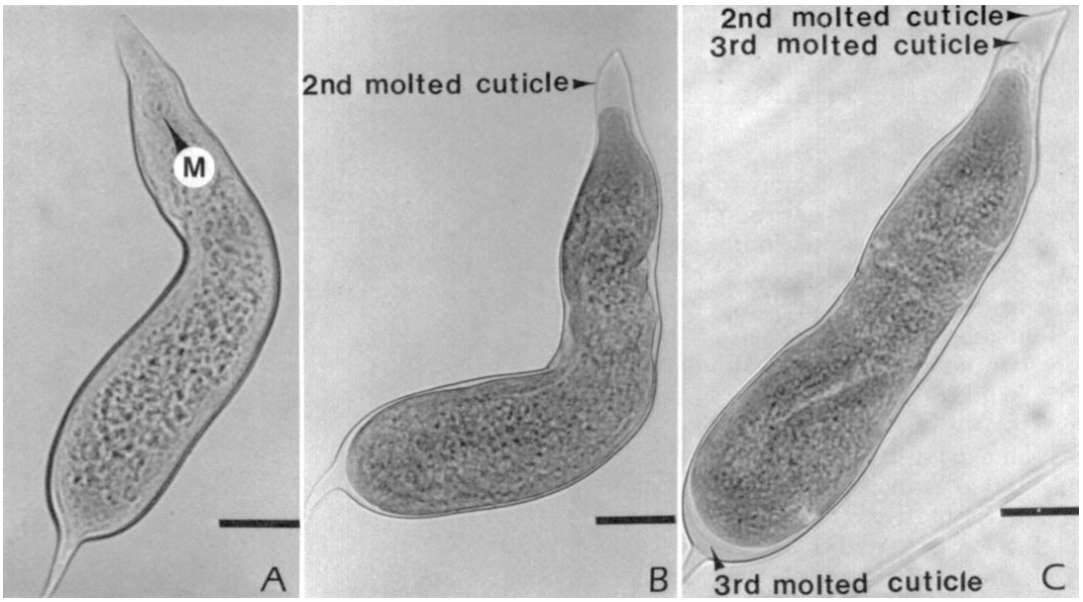

FIG. 2. Life stage juveniles of *Meloidogyne chitwoodi*. A) Second-stage juvenile, M = metacorpus. B) Third-stage juvenile. C) Fourth-stage juvenile. Scale bars = 52 μm.

and after 12 hours at 25 C (Fig. 1). Third- and fourth-stage juveniles were inactive, lacked stylets, and contained partially developed stomodaea (Fig. 2B, C). These later stages molted much faster than did the J2, an active stage with a well-developed stomodaeum (Fig. 2A).

Males were not found, suggesting that this *M. chitwoodi* population did not reproduce by cross-fertilization.

Considering that the pre-infection development of *M. chitwoodi* was 82–84 days at 10 C and 20–21 days at 20 C (2), the length of nematode life-cycle from egg to egg was 187–189 days at 10 C and 56–57 days at 20 C.

Histological examination of nematode-infected wheat roots: M. chitwoodi J2 invaded root tips and (or) axial roots. Root tips usually became swollen soon after penetration. Cavities were observed in the cortex in infected root tips 2 days after inoculation (Fig. 3B). Apical meristem and cortical cells divided, giving rise to hyperplastic cortex containing cells with enlarged nuclei and prominent nucleoli 5 days after inoculation (Fig. 3C, D). Giant cells induced by J2 were observed in the stele 10 days after inoculation (Fig. 3E). Giant cells enlarged when females were first observed, and they occupied up to a third of the stele 15 days after inoculation (Fig. 3F). Partial obliteration of the stele was caused by giant cells 20 days after inoculation when females were close to oviposition (Fig. 3G). Clusters of egg-laying females were observed in single galls at 25 days after inoculation (Figs. 3H, 4). During egg deposition, root galls with many females became completely covered by egg masses (Fig. 4).

Temperature effect on root infection by J2: M. chitwoodi J2 penetrated Nugaines wheat roots at 12, 10, 8, and 6 C, when inoculum was stored at either 20 or 4 C (Table 1). Slight nematode infection occurred on wheat seedlings maintained at 4 C and inoculated with J2 stored at 20 C, but no infection was observed at 4 C when the inoculum was also stored at 4 C. No infection occurred at 2 C. More J2 were present in root galls after 13 days when the inoculum was stored at 20 C than when it was stored 4 C. *M. chitwoodi* J2 infected wheat roots at temperatures lower than those reported in similar experiments where the lowest temperature used was 10 C (4).

Nematode life-cycle under field conditions: Snow covered the ground from late November 1983 until late March in 1984 at Logan, Utah. During the winter months, the soil temperature remained around 1 C. On 15 April, no nematodes were detected in seedling roots that had emerged under snow cover. Forty percent of *M. chitwoodi*

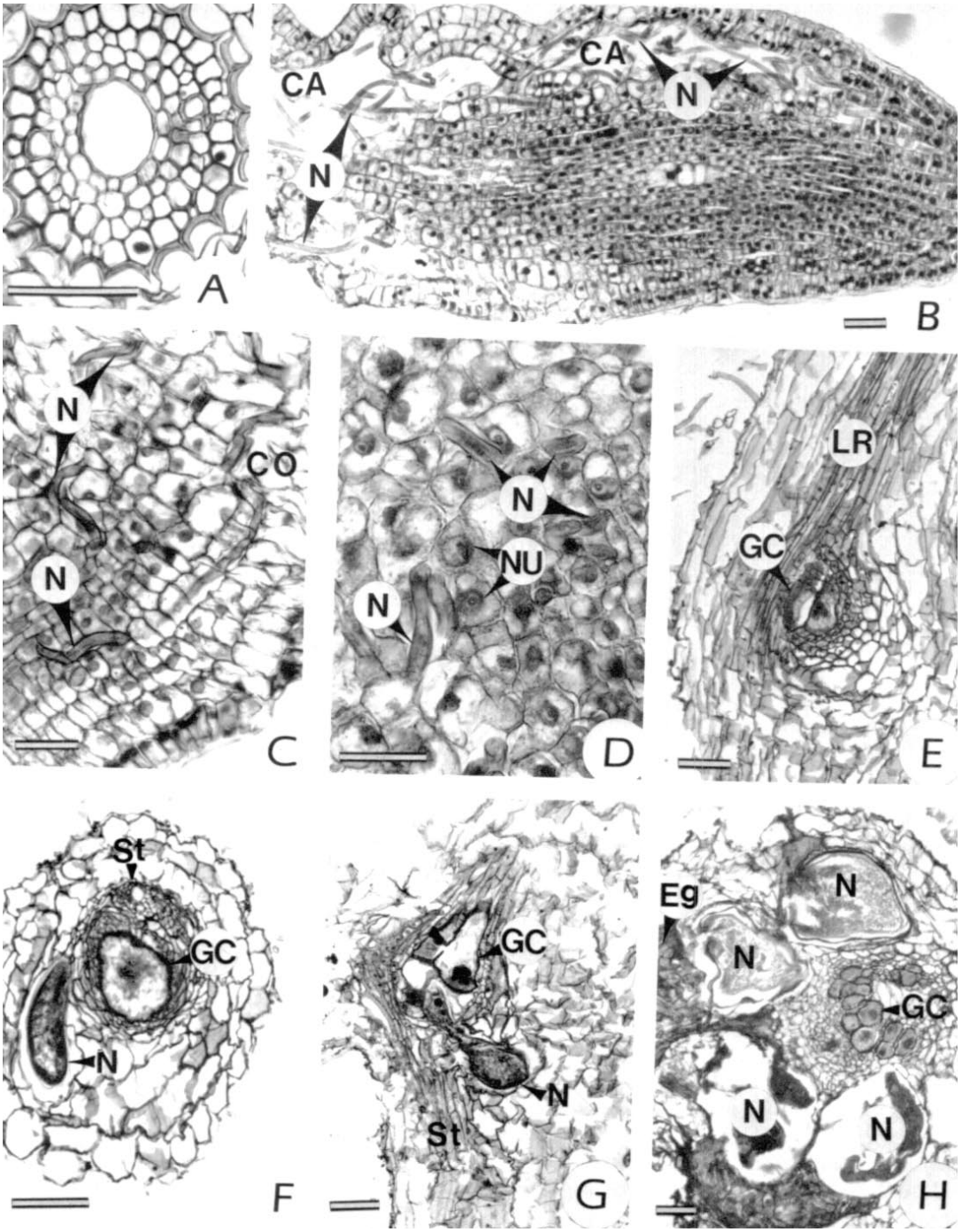

FIG. 3. Anatomical changes induced by *Meloidogyne chitwoodi* on 'Nugaines' wheat roots compared with noninfected root. A) Noninfected root. B) Longitudinal section of a root apex showing cavities (CA) induced by second-stage juveniles (N) in the cortex (Co), 2 days after inoculation. C) Cross section of a root apex showing second-stage juveniles (N) in the cortex (Co), 5 days after inoculation. D) Cross section of a root apex showing second-stage juveniles (N) adjacent to cortical cells with hypertrophic nuclei (NU) and prominent nucleoli, 5 days after inoculation. E) Root cross section showing a giant cell (GC) induced by a late second-stage juvenile in the stele (St) adjacent to a lateral root (LR), 10 days after inoculation. F) Root cross section showing a large giant cell (GC) occupying one-third of the stele (St) and induced by a young female (N), 15 days after inoculation. G) Longitudinal section of root showing a young female (N) feeding on giant cells (GC) that have partially obliterated the stele (St). H) Root cross section showing clusters of egg-laying females (N) around giant cells (GC) in the stele (St). Eg = eggs. Scale bars = 100 μm.

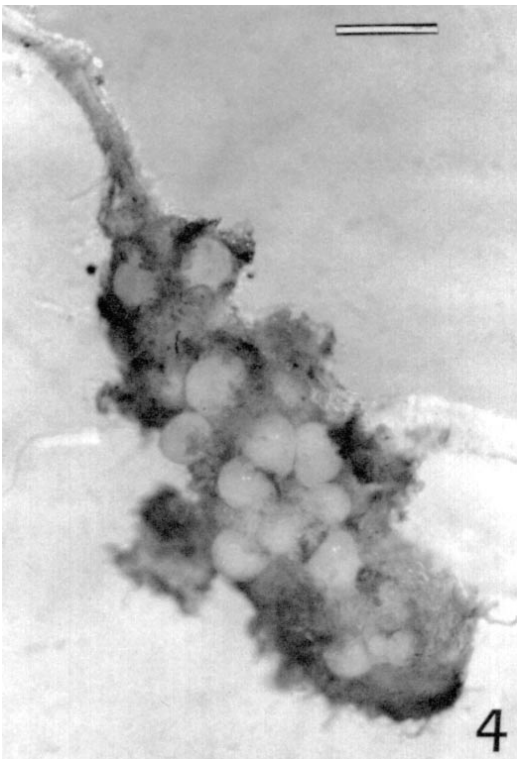

FIG. 4. 'Nugaines' wheat root gall infected with a colony of *Meloidogyne chitwoodi* egg-laying females. Scale bar = 0.71 mm.

over-wintered eggs extracted from soil in pots on 15 April hatched in 29 days at 25 C. From mid-April until late May 1984, the soil temperature at 15 cm deep increased from about 5 C to about 18 C. The first J2 were detected in seedling roots on 15 May. Soil temperatures averaged 10–15 C during early June. The first J4 was found in roots on 15 June although J2 were still present in roots of plants with swollen unsheathed spikes. Soil temperature increased to 18–25 C in late June, and the first egg mass was found on 3 July in plants with sheathed spikes.

These field observations indicate that when winter wheat grows during cool spring weather in the Intermountain region, it escapes severe attack by *M. chitwoodi*. Plants were in advanced development when they became infected with

TABLE 1. Effect of temperature on penetration of 'Nugaines' wheat roots by *Meloidogyne chitwoodi* second-stage juveniles (J2), 13 days after inoculation with 2,000 J2 exposed to 20 or 4C for 12 hours before inoculation.

Growth temperature (C) ± 1	Number of J2 per gram fresh roots		Percentage of J2 in root gall	
	20 C	4 C	20 C	4 C
12	2,804** a	1,354 a	100** a	41 a
10	2,039** a	957 a	77** b	0.4 a
8	1,358** a	219 b	29** c	0 b
6	161** b	35 c	4** d	0 b
4	11** c	0 d	0 d	0 b
2	0 d	0 d	0 d	0 b

Values are means of six replicates. Column means followed by common letters are not different according to Duncan's multiple-range test ($P = 0.01$). ** Indicates more ($P = 0.01$) J2 in root tissues and in root galls with 20 C inoculum compared with 4 C inoculum according to the Student's *t*-test.

nematodes, and eggs were laid when plants were in blossom; such late infections have little effect on wheat production, which explains the lack of yield suppression of wheat by *M. chitwoodi* in microplot experiments (3). Parasitism by *M. chitwoodi* of spring wheat in the seedling stage, however, can seriously impact production (3). *M. chitwoodi* might also be expected to inflict greater damage on winter wheat in the warmer Pacific Northwest where nematode become active earlier in the spring. Under all environmental conditions, however, wheat maintains or increases soil populations of *M. chitwoodi*.

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