

Development of the False Root-knot Nematode, *Nacobbus aberrans*, on Sugarbeet¹

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Abstract: The duration of the embryogenic development of *Nacobbus aberrans* (= *N. batatiformis*) took 9–10 days at 25 C and 51 days at 15 C. The J₁ molted in the egg; hence the J₂ emerged from the egg. The effect of distilled water and root leachates of kochia and sugarbeet was investigated at 5, 10, 15, 20, and 25 C. Root leachates did not significantly affect the percent of cumulative hatch of eggs, but temperature did significantly affect emergence of juveniles ($P = 0.05$). Less than 1, 5, and 20% of eggs hatched at 5, 10, and 15 C, respectively. The percent of cumulative hatch at 20 C was four times greater than at 15 C, while the highest percentage of juveniles emerged at 25 C. The duration of postembryogenic development from J₂ inoculation until the appearance of mature females with egg masses took 38 days, and the life cycle from egg to egg was completed in 48 days at 25 C. All immature stages, young females and males were migratory endoparasites. Young females were able to leave the root swellings, where they developed from juvenile stages, and re-enter the root, where they formed a true gall and became sedentary. Thirty days after inoculation with J₂ nematodes, specimens were detected in root tissues at 10, 15, 20, 25, and 30 C, but not at 5 C. Five days after inoculation at 23 C (± 2 C), juveniles had penetrated the roots and caused slight swellings of the tip and axis of sugarbeet feeder roots. Large cavities extended from the cortical parenchyma to the periphery of the stelar area, and 50 % of the central cylinder was destroyed 25 days after inoculation at 23 C. No syncytia formation were detected in the sugarbeet root swellings infected with juveniles. Syncytia were associated only with adult females; hyperplasia, abnormal proliferation of lateral roots, and asymmetry of root structure were additional anatomical changes induced by adult females. Only very smooth annules but no cuticular ornamentations were noted by SEM on the perineal area of adult females. **Key words:** life cycle, embryogenesis, postembryogenesis, egg hatch, root leachates, histopathology, morphology.

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The false root-knot nematode *Nacobbus aberrans* Thorne and Allen, a nearctic species distributed in North and South America, is reported to damage sugarbeet (*Beta vulgaris* L.) and solanaceous crops such as potato (*Solanum tuberosum* L.) and tomato (*Lycopersicon esculentum* Mill.) (3,8,12,13). Information on the biology of *N. aberrans* and anatomical alterations caused by its penetration of sugarbeet and tomato roots has been reported (5,6,7,8,9,10). Schuster et

al. (10) reported that juveniles of *N. aberrans* (= *N. batatiformis* Thorne and Schuster) (11) did not molt in the egg. Clark (1), however, stated that *N. aberrans* (= *N. serendipiticus* Franklin) (11) emerged as J₂ (second-stage juvenile). Other aspects of the life cycle and host relations of the nematode species are unclear. The purpose of this paper was to study (i) the embryogenic development of *N. aberrans* (= *batatiformis*) at different temperatures, (ii) the effect of root leachates and temperature on egg hatch, (iii) parasitism on sugarbeet, and (iv) detailed morphology of the mature female.

MATERIALS AND METHODS

A Nebraska *N. aberrans* (= *N. batati-*

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formis) population from kochia (*Kochia scoparia* [L.] Schrad.) was used in our experiments. To obtain inocula for experimentation, nematodes were reared on sugarbeet cv. AH 14 plants in a greenhouse.

Embryogenic development: To determine the duration of embryogenic development, five single-cell eggs were enclosed in a cavity slide filled with distilled water. The slide was contained in a petri dish (5-cm-d) lined with moist filter paper to retard evaporation. The petri dish was stored in a growth chamber at 15 and 25 C (± 1 C) and embryogenic phases recorded on a daily basis. There were four replications.

Effect of temperature on egg hatch: To determine the effect of root leachates on egg hatch, seven egg masses (about 1,500 eggs) from sugarbeet roots were placed on a 75- μ m microsieve (20-mm-d) and enclosed in petri dishes partially filled with distilled water and root leachates of kochia or sugarbeet AH 14 sufficient to cover the egg masses. Dishes with eggs were maintained at 5, 10, 15, 20, and 25 C (± 1 C) in growth chambers, and each treatment was replicated four times. Root leachates were obtained from 1-month-old container grown plants by drenching the soil with 2 liters of distilled water and collecting the leachates during a 24-h period. The leached root diffusates were centrifuged at $935 \times g$ for 8 min and stored at 4 C during the experiment. Fresh changes of diluents and emerged juvenile counts were made daily. When egg hatch ceased, the egg masses were ground in a glass tube and numbers of unhatched eggs counted. Hatched eggs expressed as percent of total initial egg population were analyzed statistically using a split-plot in time analysis of variance.

Postembryogenic development: To study the postembryogenic development, 2-wk-old sugarbeet cv. AH 14 seedlings were inoculated with 400 J₂ and maintained at 20 and 25 C. Seedlings were harvested at 5-day intervals, and juveniles were removed from root swellings, mounted in distilled water, and observed microscopically to ascertain their development on the basis of body size and gonad development.

Nematode root infection: The effect of temperature on root infection by juveniles was investigated by inoculating 2-week-old

sugarbeet cv. AH 14 seedlings with 400 J₂. Seedlings grown at temperature of 5, 10, 15, 20, 25, and 30 C (± 1 C) were harvested after 30 and 35 days. Nematode infection was determined by staining roots with hot acid fuchsin in lactophenol and examining them with the aid of stereomicroscope.

Histological study of nematode infected sugarbeet roots: Histological root examination of inoculated sugarbeet seedlings grown at 23 C (± 2 C) were made at 5-day intervals. To observe any anatomical changes, root segments were fixed in FAA (23% formalin, 3% acetic-acid, 30% alcohol, 44% distilled water), dehydrated in TBA (tertiary butyl alcohol) series, and embedded in paraffin. Sections 10–15 μ m thick were stained with safranin and fast-green, mounted in Dummar xylene, and observed with a compound microscope (4).

Morphological study of mature females: Mature females were observed by scanning electron microscope (SEM) to study morphological structure in detail. The specimens removed from sugarbeet root tissue were killed and fixed in hot aqueous solution of 4% formaldehyde and 1% propionic acid, transferred to 1% osmium tetroxide for 12 h, and infiltrated with Spurr's resins (2); ethanol, instead of acetone, was used for dehydration. Specimens were mounted on SEM stubs, coated with gold, and examined.

RESULTS AND DISCUSSION

Embryogenic development: *N. aberrans* (= *N. batatiformis*) laid single-cell eggs that measured 82.5 μ m (70–90) \times 38.6 μ m (36–40) ($n = 16$). Egg segmentation to the four-cell stage required 12 h at 25 C and 45–48 h at 15 C. The third segmentation occurred soon after the second at 25 C, but required 14–17 h at 15 C. After the third division, the gastrula stage was attained in 87 h at 25 C and 157–181 h at 15 C. The J₁ molt (Fig. 1) was initiated 2 days and 6–7 days after the gastrula stage at 25 and 15 C, respectively. The emergence of J₂ occurred 3–4 days and 20 days after molt at 25 and 15 C, respectively. The length of embryogenic development from egg deposition until J₂ emergence was 9–10 days at 25 C and about 51 days at 15 C. At 15 C, only about 10% of the eggs hatched; the



Fig. 1. Molt of J_1 *Nacobbus aberrans* (= *N. batatiformis*) in the egg. Note molted cuticle (arrow). Scale bar = 15 μ m.

other unhatched eggs retained the J_2 in a quiescent state. Six percent of these eggs hatched when they were incubated at 25 C. At 25 C, the embryogenic development of *N. aberrans* (= *N. batatiformis*) was shorter than *N. aberrans* (= *N. serendipiticus*) at 17 C (9–10 days vs. 12–17 days) but was longer at 15 C (51 days vs. 12–17 days) (1). In our study, the J_1 molt occurred in the egg (Fig. 1) and the J_2 emerged from the egg. This differs from the findings of Schuster et al. (10) who examined only embryonated eggs of *N. batatiformis* which did not reveal any larval molt prior to hatching. Our observations with *N. aberrans* (= *N. batatiformis*) were similar to Clark's (1) detailed studies on the embryogenic development of *N. serendipiticus* which also found that the first molt was in the egg. No studies on the effect of temperature on the duration of the embryogenic development of *N. aberrans* have been reported, so it is not known if the eggs of other populations of this species retain quiescent J_2 at 15 C or lower temperatures

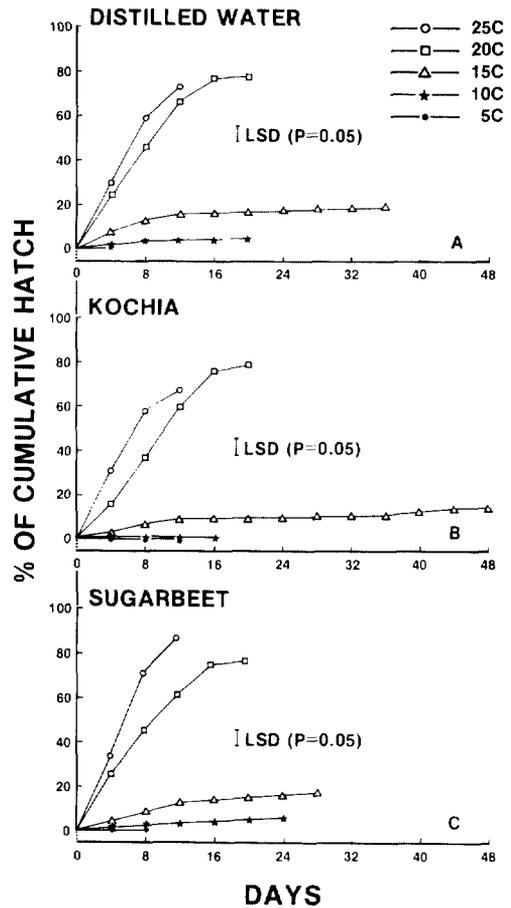


Fig. 2. Influence of temperatures 5, 10, 15, 20, and 25 C (± 1 C) on percent cumulative hatch of *Nacobbus aberrans* (= *N. batatiformis*) in distilled water, kochia, and sugarbeet root leachates.

as do those of *N. aberrans* (= *N. batatiformis*).

Effect of temperature on egg hatch: The total egg hatch at 5 C was less than 1% of the initial number of eggs and similar to cumulative egg hatch at 10 C, but less ($P = 0.05$) than at other temperatures (Fig. 2 A, B, C).

At 10 C, cumulative egg hatch was less ($P = 0.05$) than at 15 C between 4 and 20, 8 and 16, and 8 and 24 days in distilled water, kochia, and sugarbeet leachates, respectively, and was significantly less ($P = 0.05$) than at 20 and 25 C in all media used at all time intervals. Hatch of *N. aberrans* eggs was not significantly different between any of the media used (Fig. 2 A, B, C).

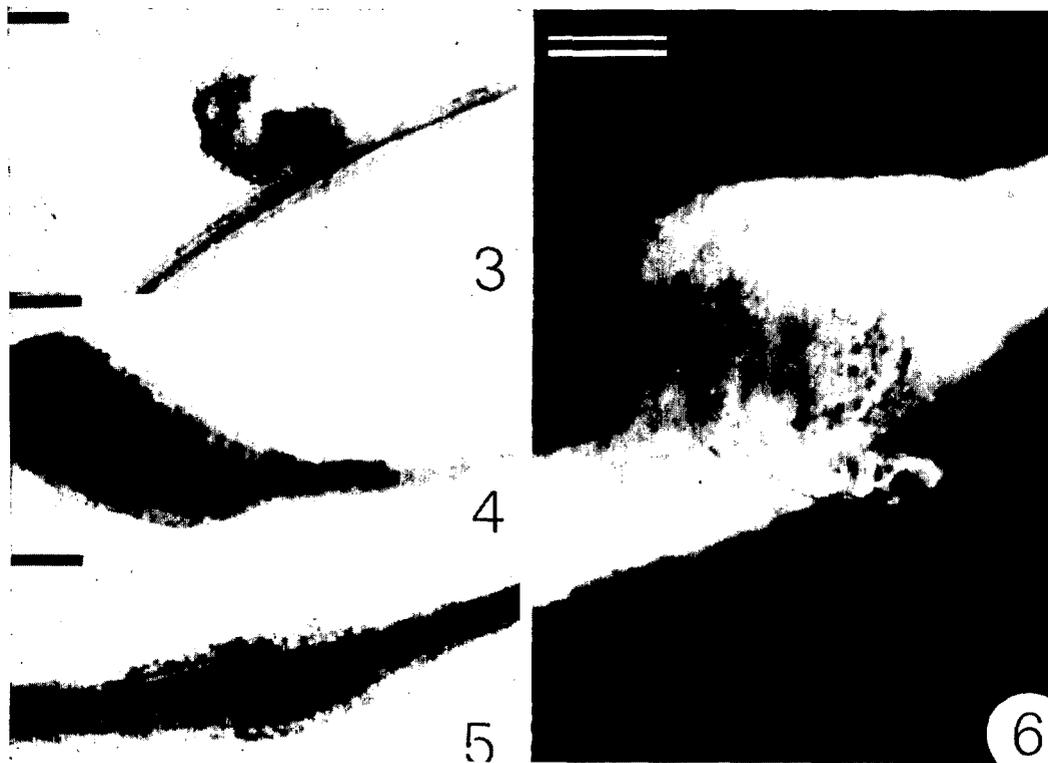
The duration of juvenile emergence was shorter than that of embryogenic develop-

ment in all media at 15 C, indicating the eggs were in advanced stage of development at the beginning of the experiment. Hatch of *N. aberrans* eggs in distilled water and root leachates at 15 C was less ($P = 0.05$) than at 20 and 25 C at all time intervals. There were no significant differences between the numbers of juveniles emerged in all media at 15 C (Fig. 2 A, B, C).

At 20 C, the duration of emergence was 20 days in distilled water and root leachates, and the cumulative hatch was about four times that at 15 C but less ($P = 0.05$) than that at 25 C between 8 and 12, 4 and 12, and 4 and 20 days in distilled water, kochia, and sugarbeet root leachates, respectively. Between 16 and 20 days, the cumulative hatch of *N. aberrans* eggs was similar to that at 25 C in distilled water but more ($P = 0.05$) than that at 25 C in kochia root leachate. Cumulative hatch of eggs was less ($P = 0.05$) in sugarbeet leachate than in distilled water and kochia leachate between 16 and 20 days at 20 C (Fig. 2A, B, C).

At 25 C, hatch of eggs in distilled water and kochia root leachate was less ($P = 0.05$) than that in sugarbeet root leachate between 8 and 12 days (Fig. 2 A, B, C).

Kochia and sugarbeet are good hosts of *N. aberrans* (= *N. batatiformis*). The root leachates obtained by the method used in this experiment showed no difference in effect on egg hatch; its influence was erratic and changed at different temperatures. Cumulative hatch of eggs was similar at 5, 10, and 15 C in all media used. Hatch in sugarbeet root leachate was less ($P = 0.05$) at 20 C and greater at 25 C than hatch in distilled water. There was no difference in egg hatch number in distilled water and kochia root leachate at all temperatures. This indicates no persistent and stable action of the root leachates on the hatch of *N. aberrans* eggs. The hatch of *N. aberrans* eggs was greatly influenced by temperature. Less than 20% of nematode eggs hatched at 5, 10, and 15 C, and 70% or more hatched at 20 and 25 C, indicating that 20 and 25 C are more favorable for



Figs. 3-6. Symptoms induced by *Nacobbus aberrans* (= *N. batatiformis*) in sugarbeet roots. Scale bars = 400 μ m. 3) Two juvenile stages in a root tip. 4-5) Light swellings caused by juveniles stages on the root tip and along the root axis. 6) Mature female in semiendoparasitic position on a large gall.

N. aberrans egg hatch than 5, 10, and 15 C.

Postembryogenic development: Juveniles are migratory endoparasites (Fig. 3), and the J₂ invade plant root tips and axis where they induce slight swellings (Figs. 4, 5). The swellings may extend over a large portion of the root axis. Schuster et al. (10) reported root tip swellings but not on the root axis of sugarbeet infected with *N. aberrans* juveniles. Five days after inoculation, only J₂ were observed in root swellings at 20 and 25 C. Juveniles appear to be gregarious, as large colonies were often seen in the same root swelling. At 20 and 25 C, J₃ occurred in 14 and 11 days, J₄ in 22 and 16 days, and immature females were observed in the swellings 27 and 23 days after inoculation, respectively. The first true galls containing young females occurred in 59 and 30 days, and mature females with egg masses were noted 75 and 38 days after inoculation at 20 and 25 C, respectively. The duration of postembryogenic development of *N. aberrans* (= *N. batatiformis*) on sugarbeet at 20 C until the appearance of immature females was shorter than *N. aberrans* (= *N. serendipiticus*) on tomato (27 days vs. 35 days); egg masses of *N. serendipiticus* were used as the inoculum source rather than J₂ (6). The appearance of mature females with egg masses required 75 days compared with 43 days for *N. serendipiticus*. For *N. aberrans* (= *N. batatiformis*) at 25 C, the length of postembryogenic development and the appearance of immature females and mature females with egg masses was only slightly longer than *N. aberrans* (= *N. serendipiticus*) on tomato (23 days vs. 20 days and 38 days vs. 36 days) (6). Males were observed in the root swellings later than immature females (59 and 30 days after inoculation at 20 and 25 C, respectively). Prasad and Webster (7) reported that males *N. aberrans* (= *N. serendipiticus*) develop

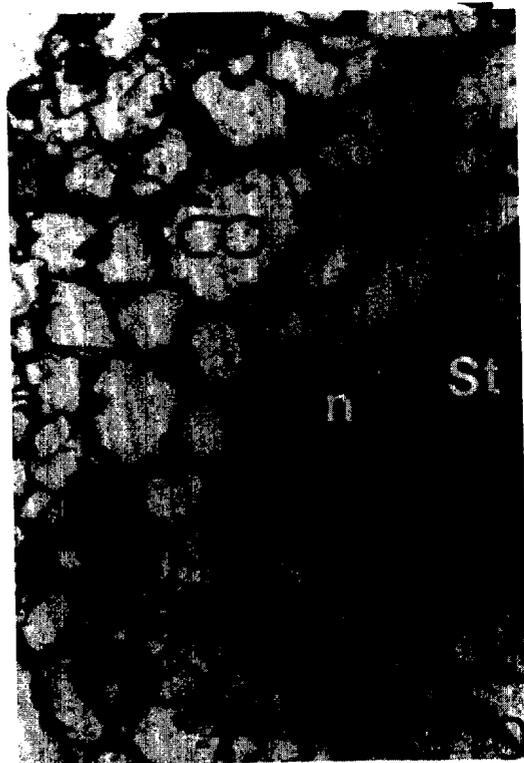
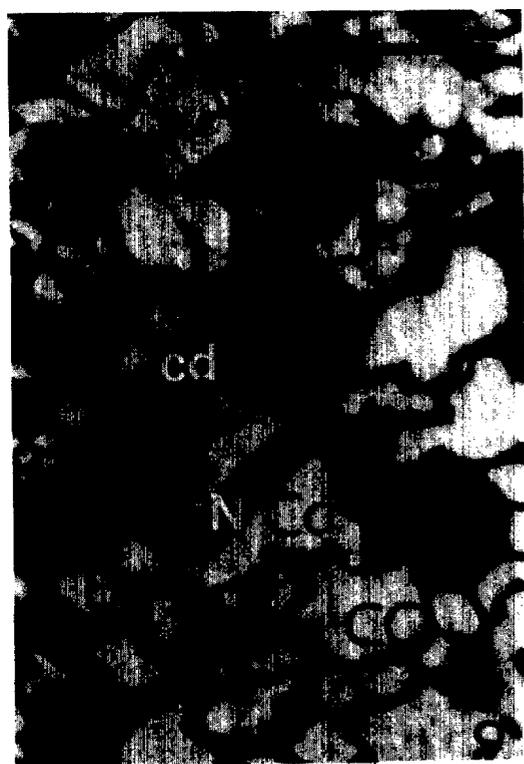
faster than females on tomato, but this did not occur in our experiments.

Males leave the root swellings in search of the sedentary mature females in the true galls. During our observations, after the artificial removal of a gelatinous matrix containing males from a true gall, a new gelatinous matrix was deposited and males were found in the new matrix one week later, indicating that fertilization may occur after the females become sedentary. Mature females are sedentary endoparasites; however, they were observed in a semiendoparasitic feeding position with swollen posterior portions, of the body protruding from the gall surface (Fig. 6). We hypothesize that young females are able to leave the root swellings, where they developed from juvenile stages, and re-enter the root where they form a gall and become sedentary. This hypothesis is supported by the semiendoparasitic habit of some adult females (Fig. 6) and the findings of Quimi (8) who reported gall formation after tomato root inoculation with immature females. The root tissues where the females develop are so heavily damaged by the juveniles that it is necessary for immature females to find new healthy roots and new feeding sites for syncytium development and reproduction.

Considering that embryogenic development was 10 days, the life cycle from egg to egg was about 48 days at 25 C, somewhat longer than for an English and South American population of *N. aberrans* which was 35 and 28 days at the same temperature (8). The differences in the life cycle from egg to egg, reported at similar temperatures (7,8) and also observed during our experiments, may be explained by the different time intervals spent by immature females after leaving the root swellings and before re-entering the root to form the gall and complete its life cycle.



Figs. 7-10. Anatomical changes induced by *Nacobbus aberrans* (= *N. batatiformis*) juvenile stages in sugarbeet roots compared to an uninfected root. Scale bars = 50 μ m. 7) Uninfected root. CO = cortex, En = endodermis. 8) Longitudinal section of a root tip with a large cavity (CA) extending from the cortex (CO) into 50% or more of the stelar area (St), N = nematode, 25 days after inoculation. 9) Primary root cross section showing a nematode (N) in a cavity (CA) close to parenchymal (cd) and endodermal (Ed) cells with dense cytoplasm, CO = cortex, 5 days after inoculation. 10) Secondary root cross section showing a cavity (Ca) in the cortex (CO) extending into the periphery of stelar area (St), 5 days after inoculation. Note the hypertrophic nucleus (nu) in a cell of cortex. n = nematode.



During our observations, gall formation was induced only by adult females and the root swellings were always associated with juveniles. This is in agreement with findings by Clark (1) and Quimi (8) for *N. aberrans* (= *N. serendipiticus*) and a South American population of *N. aberrans* on tomato but differs from those of Prasad and Webster (7) who stated that gall formation was associated with the third-stage females of *N. aberrans* (= *N. serendipiticus*) on tomato.

Nematode root infection: Thirty and thirty-five days after inoculation with J₂, roots of sugarbeet seedling were found infected by *N. aberrans* (= *N. batatiformis*) stages at 10, 15, 20, 25, and 30 C (± 1 C) but not at 5 C. However, very few juvenile stages were observed in root seedlings at 10 C. Egg masses were found on galled sugarbeet roots 30 days after inoculation at 30 C, a shorter period than the 43 days for *N. aberrans* (= *N. serendipiticus*) on tomato (7). Only root swellings, but not true galls, were observed at temperatures lower than 25 C after 30 days.

Histological study of nematode infected sugarbeet roots: All the juvenile stages of *N. aberrans* (= *N. batatiformis*) invade the cortical parenchyma of sugarbeet roots and create cavities in the cortical cells (Fig. 8). Five days after inoculation, cavities were induced in the cortical layer adjacent to the endodermis (Fig. 9). Dense cytoplasm was observed in the endodermis and adjacent internal layer of cortical cells as a result of the nematode feeding (Fig. 9). Cells with dense cytoplasm and hypertrophic nuclei were seen in the cortex as early as 5 days after inoculation. In some cases cavities extended from the cortical parenchyma to the periphery of the central cylinder (Fig. 10). Damage in the stelar area caused by juveniles was more accentuated after 10 days, and 50% of the central cylinder was dissolved in infected roots after 25 days (Fig. 8). Previous studies (9, 10) did not report stelar damage, and differences may be due to the virulence of the population. Host differences were also reported with other *N. aberrans* populations (3,13). The South American *N. aberrans* populations are able to infect potato (3), a nonhost of *N. aberrans* (= *N. batati-*

formis) (13). Schuster et al. (10) reported that juveniles were associated with syncytium formation, but during our observations syncytia formation was never detected in root swellings infected with the juveniles. Syncytia were associated only with adult females.

The first small polynucleate syncytium induced by the adult was observed 25 days after inoculation. Accentuated asymmetry of root structure, with abnormal proliferation of secondary lateral roots due to the nematode feeding activity, was observed 30 or 35 days after inoculation (Fig. 11).

Morphological observation of mature females: The head of mature females is hemispherical with four distinct postlabial annules. The rounded and slightly raised cephalic plate shows the oval prestoma that is oriented dorsoventrally and surrounded by six pit-like labial sensilla, three on each side. The subdorsal and subventral lobes are ovoidal. Two conspicuous rectangular

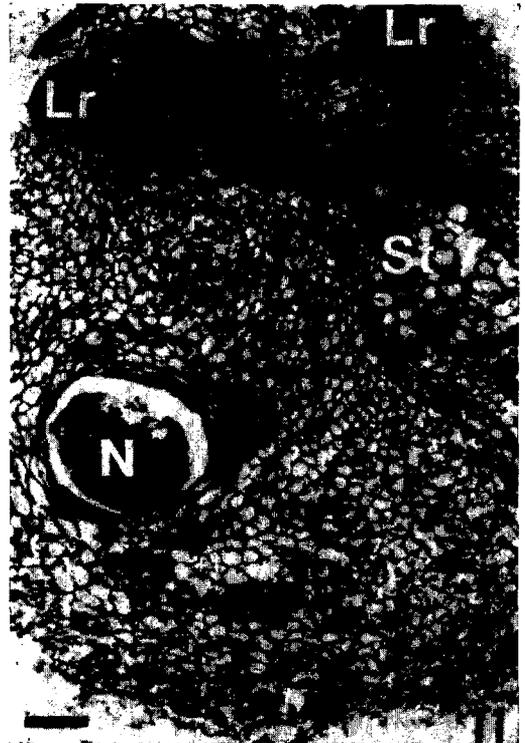
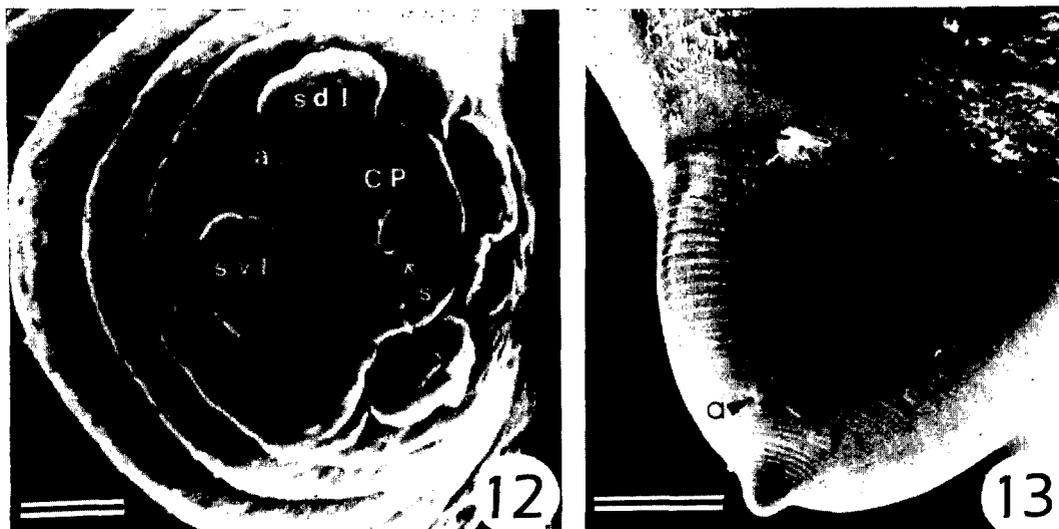


Fig. 11. Cross section of sugarbeet secondary root infected by an adult female (N) of *Nacobbus aberrans* (= *N. batatiformis*), 35 days after inoculation. Note asymmetry of the stele (St), hyperplasia or cortex (CO) and abnormal proliferation of lateral root primordia (Lr). Scale bar = 100 μ m.



Figs. 12-13. SEM micrographs of *Nacobbus aberrans* (= *N. batatiformis*). Scale bars = 2 μ m. 12) Mature female in face view. a = amphidial aperture; CP = cephalic plate; s = sensillum; sdl = subdorsal lobe; svl = subventral lobe. 13) Mature female body posterior portion. Note the smooth annules in perineal area. a = anus; lf = lateral field; v = vulva.

amphidial apertures, often covered by mucous, are present on the lateral edges of the cephalic plate, between the subdorsal and subventral lobes (Fig. 12).

The posterior portion of the nematode body is dome shaped, and the lateral field has four distinct incisures and outer aerolated bands. The transverse slit-like vulval opening is surrounded by unsculptured lips, which are slightly less than one annule thick. The anal opening is poriform and located at the 14th-15th body annules from the terminous, 23-25 annules posterior to the vulva (Fig. 13). No sculptures or special cuticular markings are present in the perineal area; that showed only smooth annules similar to the other body portion.

Our experiments have shown that the embryogenic development of *N. aberrans* (= *N. batatiformis*) was essentially the same as for other *Tylenchida* species. We have found that *N. aberrans* completes its life cycle in 48 days at 25 C. Immature females are able to leave the root swellings where they develop from endoparasitic migratory immature stages, re-enter the root, become sedentary, and form a gall with a syncytium. *Nacobbus aberrans* is similar to *Meloidogyne* species in that temperatures have a stronger effect on egg hatch than does host root leachate.

Our histological observations have

shown that *N. aberrans* severely damages the root system of sugarbeet and causes more root damage than some root-knot nematode species. In addition to hyperplasia, abnormal lateral root proliferation, asymmetry of the root structure, and syncytia formation induced by adult females, the juvenile stages cause extensive damage to cortical parenchyma and the stelar area.

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