Inhibition of Syncytia Formation and Root-knot Nematode Development on Cultures of Excised Tomato Roots

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Abstract: Two different defined growth media were used to culture aseptically the root-knot nematode, *Meloidogyne incognita,* on excised roots of tomato, *Lycopersicon esculentum* cv 'Marglobe.' One of these media, STW, was a formulation by Skoog, Tsui, and White and the other, MS, a formulation by Murashige and Skoog. From 1 through 4 weeks, inoculated tissues were fractured to observe root infection, giant-cell formation, and nematode development with the scanning electron microscope (SEM). Four weeks after inoculation, the fresh weights of roots and developmental stages of nematodes were recorded. SEM observations indicated that roots cultured on the STW medium had normal growth and infection sites with galls that supported the development of mature females by 4 weeks. Roots cultured on the MS medium were less vigorous and had infection sites with galls containing only one to four syncytialike cells that did not support the development of mature females. Eighty percent of the larvae infecting roots cultured on the MS medium failed to develop into mature females. To determine which factor(s) affected root growth and nematode development, inoculated and uninoculated roots were grown on media consisting of different combinations of the organic and inorganic fractions of the STW and MS formulations. These experiments indicated that the organic fraction of STW was essential for normal root growth; however, the inorganic fraction of MS inhibited normal gall formation and nematode development. Further testing of the inorganic fractions revealed that the high concentration of ammonium nitrate in the MS medium was a factor that inhibited giant-cell formation and nematode development. *Key Words:* ammonium inhibition, *Meloidogyne incognita.*

For development and reproduction, root-knot nematodes depend on successful formation of feeding sites, referred to as syncytia, giant cells, or giant transfer cells, in susceptible plants. These syncytia usually fail to develop in nonhost plants and resistant cultivars (3,6,7). Attempts to prevent the formation of syncytia by using plant growth hormones (10,18,23), plant growth retardants (5,11,17,19), antibiotics (16), and herbicides (14) have either failed to inhibit syncytial formation or severely damaged the host plant. Control of nematodes through disruption of syncytial development thus remains elusive.

Preliminary attempts in our laboratory to culture the root-knot nematode on excised roots of tomato (20) indicated that syncytial formation and nematode development were impaired in a medium formulated by Murashige and Skoog. An understanding of the factor(s) in the medium that might affect nematode development could lead to new control measures. Therefore, a more comprehensive study was undertaken to identify the factor(s) further.

MATERIALS AND METHODS

Seed of tomato, *Lycopersicon esculentum* cv Marglobe, were surface sterilized for 15 min in 1% sodium hypochlorite, rinsed three times with sterile distilled water, and placed in petri dishes containing 1% water agar. After the seeds germinated, 1-cm lengths of the primary root tips were excised and transferred to petri dishes containing one of two chemically defined media (Table 1). A formulation by Skoog, Tsui, and White (24), herein designated STW, consisted of inorganic salts and an organic fraction of glycine, thiamine, pyridoxine, and nicotinic acid. A formulation by Murashige and Skoog (15), herein designated MS, had a much higher inorganic salt concentration and an organic fraction containing only thiamine and inositol. Six modifications of these media were prepared by combining the salt and the organic fractions as follows: 1) the organics of MS + the complete STW formulation; 2) the organics of MS + the salts of STW; 3) the organics of STW + the complete MS formulation; 4) the organics

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TABLE 1. The composition of STW and MS media (mg/L).

	Skoog, Tsui, & White (STW)	Murashige & Skoog (MS)
Inorganic fraction		
$Ca(NO_3)_2 \cdot 4H_2O$	144	—
KNO_3	80	1900
KCl	65	—
KH_2PO_4	38	170
NH_4NO_3	40	1650
$CaCl_2$	—	440
$MgSO_4 \cdot 7H_2O$	72	370
$FeSO_4 \cdot 7H_2O$	23	27.85
Na_2EDTA	37.25	37.25
$ZnSO_4 \cdot 7H_2O$	2.7	8.6
$MnSO_4 \cdot 4H_2O$	4.9	16.9
H_3BO_3	1.6	6.2
KI	0.75	0.83
$NaMoO_4 \cdot 2H_2O$	—	0.25
$CuSO_4 \cdot 5H_2O$	—	0.025
$CoCl_2 \cdot 6H_2O$	—	0.025
Organic fraction		
Nicotinic acid	0.5	—
Pyridoxine HCl	0.75	—
Thiamine HCl	0.1	0.4
Glycine	2.0	—
Inositol	—	100
Sucrose	20.000	20.000
Agar	15.000	15.000

of STW + the salts of MS; 5) STW + 1900 ppm KNO_3, designated STWK; and 6) STW + 1650 ppm NH_4NO_3, designated STWN.

The roots were inoculated by placing egg masses of *M. incognita,* obtained from monoxenic culture of the nematode, 1 to 2 cm from the root cultures after lateral roots had emerged. Each treatment was done in six or eight replicates. All cultures containing roots and/or nematodes were incubated in the dark at 25 C.

Root samples were removed from the cultures 1, 2, 3, and 4 weeks after inoculation and prepared for observation with the scanning electron microscope (SEM). To prepare the tissue, root segments containing galls were quickly removed from the culture and placed in vials containing 3% glutaraldehyde in 0.05M phosphate buffer, pH 6.8, at 22 C. Chemical fixation for 2 to 24 h was followed by dehydration in an ethanol series. The galled tissues were transferred from 100% ethanol to liquid nitrogen and fractured with the edge of a razor blade. The fractured segments of the gall were then thawed in 100% ethanol and critical point dried from liquid carbon dioxide. Next, the gall segments were placed on stubs and coated with 20–30 nm of gold-palladium in a Technics Hummer (Alexandria, Virginia) sputtering device. The coated specimens were viewed and photographed with a Hitachi (Mountain View, California) HHS-2R SEM operating at 10 or 15 kV.

Root growth and nematode development were examined after 4 weeks. Root growth was determined by weighing the fresh root. The developmental stages were determined by sampling 50 ± 5 nematodes per culture from galled roots that were stained for 5 min in boiling acid fuchsin-lactophenol and cleared with lactophenol.

RESULTS

Fig. 1 summarizes comparisons of the fresh weight of roots grown on the STW and MS media. Thirty days after inoculation, the average fresh weight of a culture grown on the MS medium was only 30% of that on the STW medium. Similarly, the MS medium severely limited the development of the larvae (Fig. 2). Only 12.5% of the population isolated from the MS cultures developed into mature females, whereas 90% of the nematode population

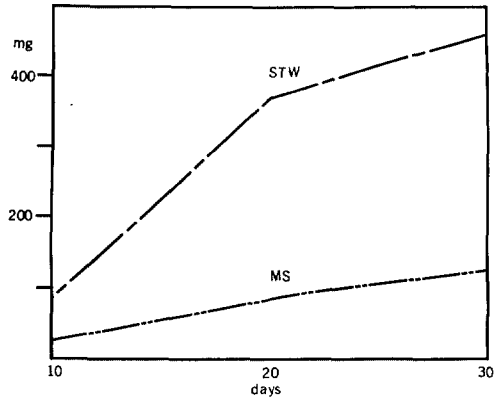


FIG. 1. Fresh weight of tomato roots 10, 20, and 30 days after inoculation with egg masses from the root-knot nematode, *Meloidogyne incognita.* Infected tissues were cultured in media formulated by Skoog, Tsui, and White (STW) or by Murashige and Skoog (MS). At 30 days, fresh weight differences, i.e., 467.5 ± 49.8 verses 141.0 ± 17.5, are significant ($P = 0.05$) according to Student's t-test.

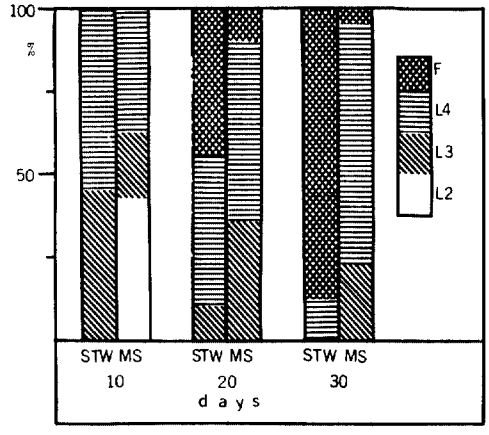


FIG. 2. Effects of STW and MS media on the development of *Meloidogyne incognita* 10, 20, and 30 days after inoculation. L2, L3, L4, and F, respectively, represent second-stage larvae, third-stage larvae, fourth-stage larvae, and females.

that was cultured on roots grown with the STW medium attained the same stage.

Examination of fractured gall tissue from the STW medium with the SEM revealed four to six syncytia clustered near a nematode feeding site (Fig. 3.A). The giant cells were surrounded by proliferated xylem tissue (Fig. 3.B). Galls from the MS cultures had poorly developed syncytia with only one to four giant cells per nematode (Fig. 4.A). These cells were usually devoid of cytoplasm and had no obvious cell-wall invaginations. However, proliferated xylem tissue was often found associated with the cells.

Fresh-weight analysis of uninoculated roots, which were cultured on six different combinations of the organic and inorganic fractions from the STW and MS formulations, indicated that the organic fractions from either the STW or MS media supported optimum growth (Fig. 5). Growth was minimal when the organic fraction of STW was excluded from the medium. Growth responses were similar in the inoculated cultures, where root weights were 1.6 to 5 times greater than those of the uninoculated controls.

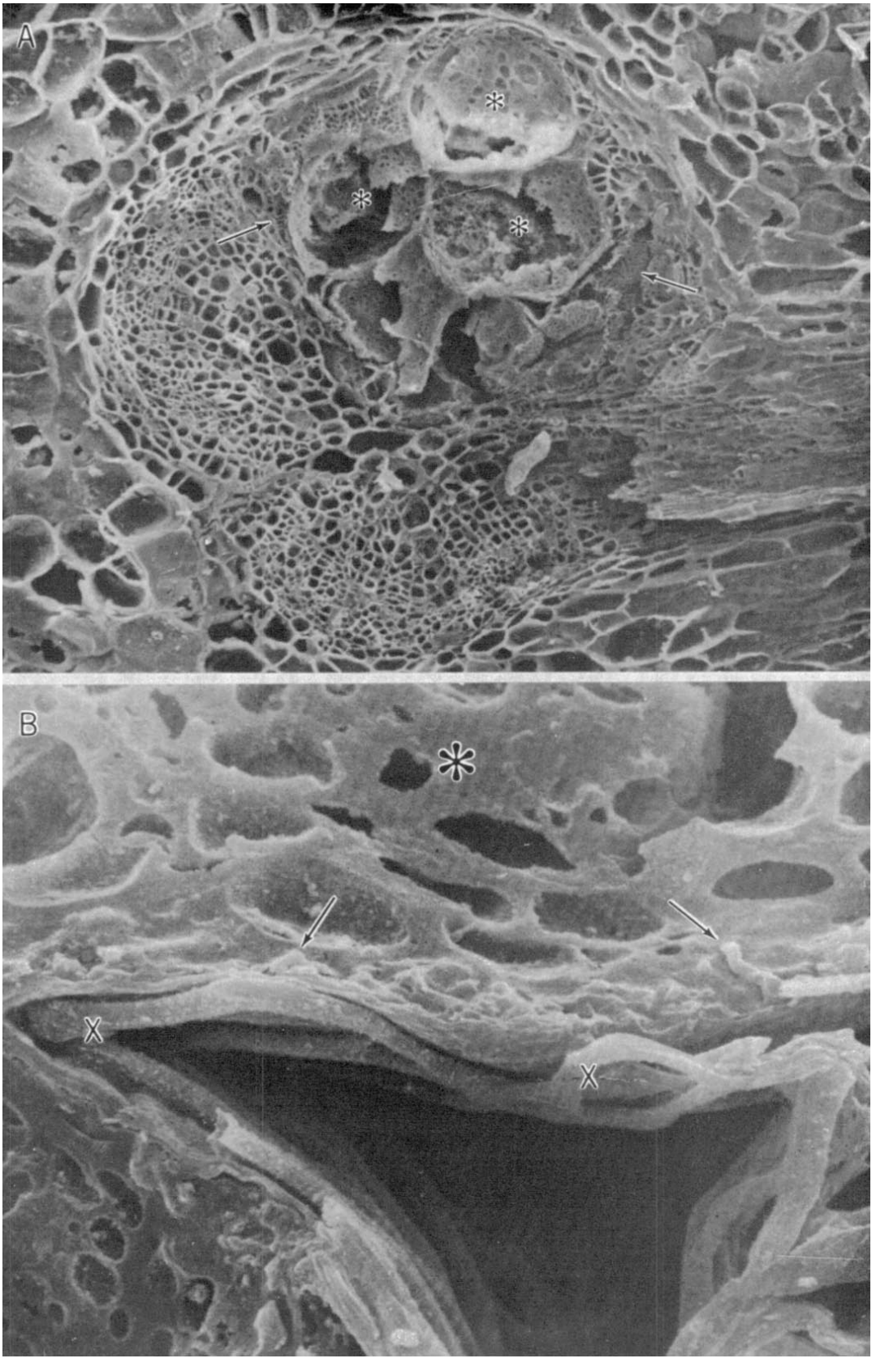
The organic fraction affected root growth, but the inorganic fraction was more important to nematode development (Fig. 6). Whenever the medium contained the inorganic fraction from STW, more than 50% of the nematode population were females. Alternatively, when the medium contained the inorganic fraction from MS, less than 25% of the population were females.

Because the concentration of nitrogen differed by 60-fold between the MS and STW formulations, additional media were tested for the effects of nitrate and ammonium on the enhancement or reduction of root growth and nematode development. Adding 1900 ppm of potassium nitrate or 1650 ppm of ammonium nitrate to the STW medium did not significantly affect the fresh weight of the uninoculated root cultures (Fig. 7); nor did potassium nitrate affect nematode development. However, ammonium nitrate reduced nematode development by 75% (Fig. 8). SEM observations of fractured galls grown on STW medium with supplemental ammonium nitrate showed poorly developed syncytia with only a few giant cells (Fig. 4.B). These syncytia resembled those found in inoculated roots cultured on MS medium.

DISCUSSION

The lack of normal syncytia formation and nematode development in the MS medium supports the concept that the maturation of larvae depends on the formation of syncytia (3,6,7). Although root growth was inhibited in the MS formulation also, factors affecting root growth appear to be independent from those affecting syncytia formation and nematode development. This observation is supported by results showing that root growth was enhanced primarily by the organic fractions in both media, but nematode development was severely ham-

FIG. 3. A) Portion of a fractured root illustrating the feeding site of the root-knot nematode 2 weeks after inoculation in the STW medium. Small cells normally found in the vascular cylinder are replaced by several large giant cells (*) whose cytoplasm is dense but slightly vacuolated. The giant cells, which form the syncytium, are surrounded by proliferated xylem tissue (arrows). ×100. B) Portion of a cell wall from a giant cell (*) that developed on inoculated roots grown in the STW medium. Apposed to the outer wall of the giant cell are the thickened walls from the adjacent xylem (X) cell. ×4400.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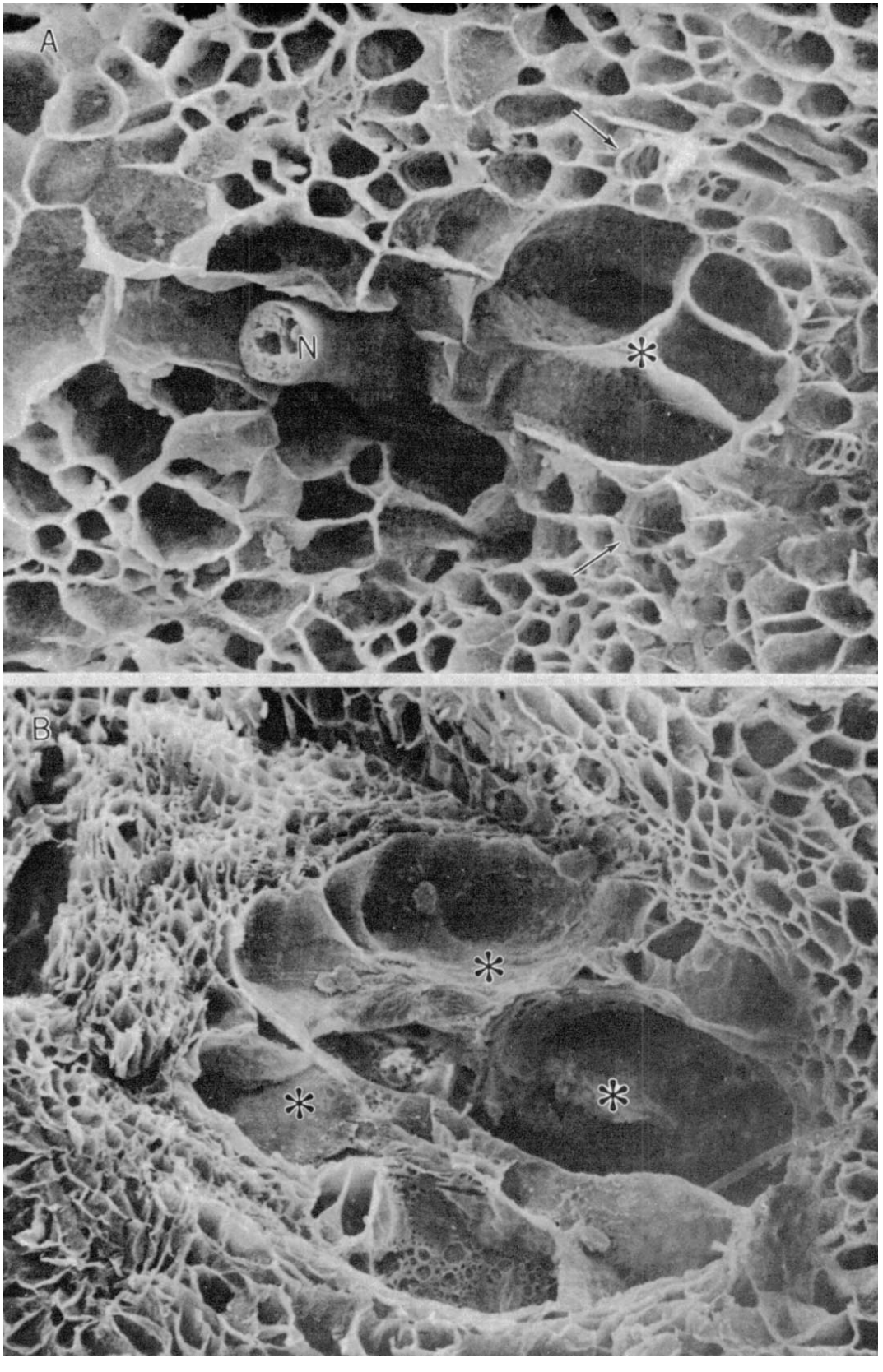
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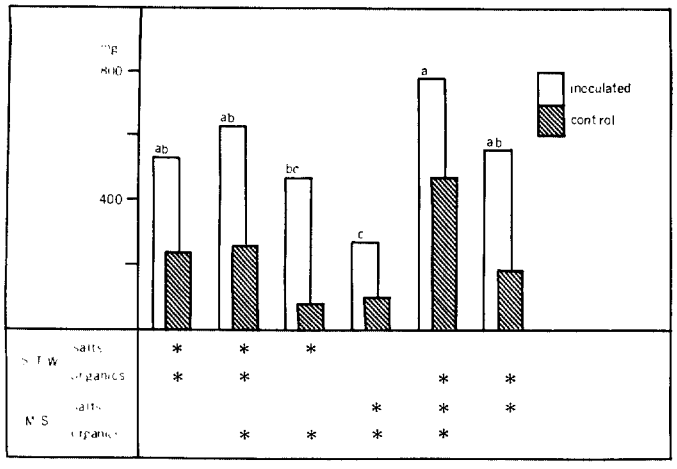

FIG. 5. Fresh weights of inoculated and control root cultures after 30 days. Roots were grown in media consisting of six combinations of the salt and organic fractions that are normally present in either the STW or MS formulation. Weights labeled with the same letter do not differ (P = 0.05) according to Duncan's multiple-range test.

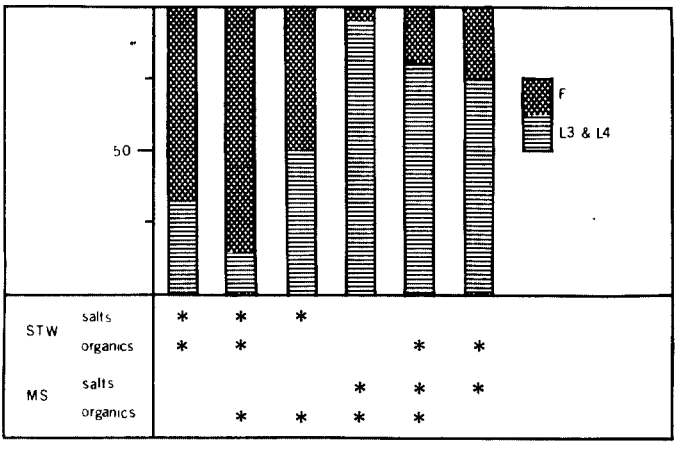

FIG. 6. Effects of STW and MS components on the development of *Meloidogyne incognita* 30 days after inoculation. L3, L4, and F, respectively, represent third-stage larvae, fourth-stage larvae, and females.

pered by the inorganic fraction of the MS medium.

Previous work indicated that potassium and ammonium might be involved in nematode suppression (1,8,21,22). Our preliminary data supported that concept. The inorganic fraction of the MS formulation contained all the nutrients found in STW. Therefore, the lack of essential growth elements did not appear to be a factor inhibiting the formation of syncytia. Alternatively, the MS medium contained higher levels of potassium and ammonium nitrate than did the STW.

Other studies (9,13) have indicated that the concentration of potassium nitrate does not affect syncytial formation or nematode development. Although our results support that conclusion, potassium nitrate caused inoculated plants to have a significantly greater fresh weight of root tissue than did the control plants. This weight difference could be attributed solely to the formation of galled tissue stimulated by nematodes. Alternatively, the statistically significant interaction obtained in this study could result also from the root growth stimulation that occurred in the presence of the nema-

FIG. 4. A) Fractured tomato root after inoculation with the root-knot nematode (N) in the MS medium. After 4 weeks, one to four giant cells, usually void of cytoplasm, are normally present; but the syncytia are considerably smaller than those that develop on roots grown in the STW medium. Xylem tissue (arrows) is associated with the giant cells but generally does not surround the entire syncytium (*). ×600. B) Fractured root from tissue grown in STW medium supplemented with NH_4NO_3. The syncytia that develop on this medium normally consist of giant (*) cells, which are highly vacuolated or void of contents. Only occasionally are they associated with xylem tissue. As a result, these syncytia more closely resemble those grown on MS than those that develop on the normal STW formulation. ×300.

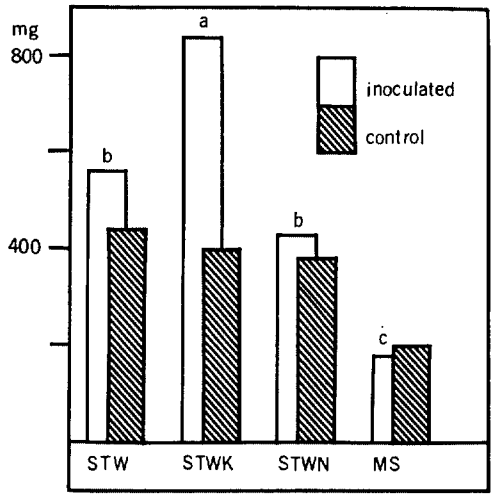

FIG. 7. Thirty-day fresh weight of inoculated and control cultures of excised tomato roots grown on the following media: STW; STW + 1900 ppm KNO_3 (STWK); STW + 1650 ppm NH_3NO_3 (STWN); and MS. Fresh weights accompanied by different letters differ (P = 0.05) according to Duncan's multiple-range test.

tode and with high concentrations of potassium nitrate.

In most studies in which syncytia development was depressed by growth retardants, such as maleic hydrazide (5) and morphactin (19), or by deficiencies in organic (12) and inorganic nutrients (2,4) plant growth was also inhibited. However, in our study the high concentration of ammonium did not depress root growth, but it did inhibit

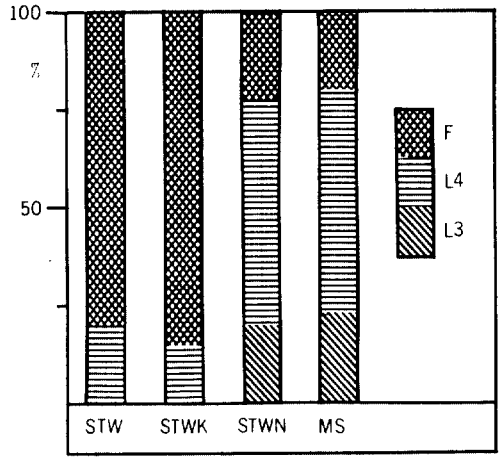

FIG. 8. Effect of the four media described in Fig. 7 on the development of *Meloidogyne incognita* 30 days after inoculation. L3, L4, and F respectively, represent third-stage larvae, fourth-stage larvae, and females.

syncytia formation and nematode development. Possibly the ammonium salt either affects plant metabolic processes related to the formation of syncytia or has a direct adverse affect on the nematode. This precise mode of action of ammonium, which cannot be determined at this time, is of interest because of its possible practical application.

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