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EFFECT OF AMMONIUM ON ROOT-KNOT NEMATODE, MELOIDOGYNE INCOGNITA, IN EXCISED TOMATO ROOTS

by

S u d i r m a n

Ir. (Agric.), the University of Mataram, Indonesia, 1984

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
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ABSTRACT

Experiments were done to determine the effect of ammonium on hatching, penetration, and development of *Meloidogyne incognita* on excised tomato roots grown on Skoog, Tsui, and White medium. The medium in five treatments contained either 1.5, 9, 54, 324 ppm ammonium or 1116 ppm nitrate. To evaluate the effect on hatching, dispersed eggs and egg masses were used in separate experiments. Results showed that with both types of inocula and with or without the presence of excised roots, the numbers of hatched juveniles decreased with increasing ammonium concentrations during 15 days.

Egg masses and second stage juveniles were used as inoculum in separate experiments to evaluate the effect of ammonium on penetration. In both experiments, increasing ammonium levels decreased the percentage of juveniles which penetrated the roots over time when compared with the control. Under similar conditions, following incubation of nematode-infected roots in water, a greater number of juveniles migrated from roots grown with high ammonium than from those in the control.

Single egg masses were used as the inoculum for development experiments. High ammonium concentrations were found to suppress nematode development. Four weeks after inoculation, most nematodes developed into adults at low ammonium concentrations with more females than males, but

only a few nematodes developed into adults at the high ammonium concentrations. At low ammonium, there were more galls, and dry weight of the roots was higher compared with that at high ammonium levels.

The effect of ammonium on nematode development was reversible. Experiments in which the ammonium concentration was increased or decreased after inoculation showed that nematode development could be altered. Increasing ammonium concentration after infection suppressed nematode development. In roots transferred to high ammonium, fewer nematodes matured three weeks after transfer and most of those that did were males. There also were fewer galls and root dry weights were lower than with constant low ammonium.

Decreased ammonium concentrations after infection enhanced nematode development. Three weeks after transfer to lower ammonium, more nematodes matured (mostly into females), and there were more galls and root dry weights were higher than in constant high ammonium. There was no effect of different ammonium concentrations on the dry weight of uninoculated roots.

To

my wife and my son

Zubaidah and Arzia P. Rahman

with patience and love

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I. INTRODUCTION

Root-knot nematodes (*Meloidogyne* spp.) are pathogens of a wide range of economically important plants (92), which are distributed worldwide, and generally recognized as causing more economic damage to food crops than any other single group of plant parasitic nematodes (2). In a worldwide survey involving 75 countries and 371 nematologists (93), *Meloidogyne* was the genus ranked first among ten nematode genera that are considered the most important pathogens (92). Indeed, root-knot nematodes are a serious threat to the world's food supply and thus to the welfare of mankind (92).

Meloidogyne populations inhabit farm soils, and their distribution is influenced by the species of host plants. If a field is planted to a susceptible crop, the *Meloidogyne* distribution in the soil is about the same as that of the crop plant roots. Most of the population is found 5 to 30 cm beneath the soil surface, and decreases to a depth of one meter in soil planted with annual plants. In soil used for perennial plants, these nematodes may be found at extreme depths of 5 m or more (102).

On a worldwide basis, the species that inhabits a region is greatly affected by the climate in the region. In cool climates where the average temperature of the coldest month of the year is near or below 0°C and the average temperature of the warmest month is about 15°C or above, the

most common species of *Meloidogyne* is *M. hapla*. Current evidence indicates that *M. hapla* has long been adapted to the northern United States and southern Canada, and to northern Europe and northern Asia. In South America, *M. hapla* is found south of about 40°S latitude and in the mountainous regions of the western part of the continent. In Africa, it is adapted to exist at altitudes above 1500 m. In Australia, it is common in the coolest states of Victoria and Tasmania (55, 102).

In the tropics, the most common *Meloidogyne* spp. are *M. incognita* and *M. javanica*. In North and South America, *M. javanica* is seldom found above 30°N and 35°S latitudes and becomes more common near the equator. In many parts of tropical Africa, Australia and southern Asia, *M. javanica* is probably the most common *Meloidogyne* spp., but *M. incognita* and *M. arenaria* are common and widespread in the same regions (56, 102).

Although *M. incognita* is a warm climate parasite, it is also an important pathogen in cold climates where high cash value crops are grown under glass (56). In glasshouses in northern climates, tomatoes, cucumbers, lettuce and many ornamental plants are often heavily attacked by *M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla* (33). These four species are the most widespread and common *Meloidogyne* spp. in the world and very probably cause more damage to farm crops than all of the other *Meloidogyne* spp. combined (91).

The relative proportion of *Meloidogyne* spp. in agricultural soils of the world is *M. incognita* 52%; *M. javanica* 31%; *M. arenaria* 8%; *M. hapla* 7%; and other species about 2% (38). Although the species are distributed in different parts of the world, they have the same basic life cycle.

The eggs of *Meloidogyne* spp. are deposited by the female within a gelatinous matrix which holds them in "egg masses" or "egg sacs" which are visible on the surface of galled roots, although on certain hosts they may occur also within the galls (42). More than 1000 eggs have been recorded within one egg mass, which may be larger in size than the female's body.

Egg development begins within a few hours of deposition, and continues until a juvenile is formed, complete with a visible stylet, coiled within the egg membrane. This is the first juvenile stage (J1), which can move in the egg but is not very active. Soon after the J1 is formed, the first molt takes place within the egg, and the juvenile becomes a second-stage juvenile (J2) (42). Shortly after the first molt, the J2 hatches, emerging through a hole made in the end of the flexible egg shell by repeated thrusting with the stylet (101, 102).

The hatched J2 may or may not leave the egg mass immediately. Usually there are some hatched J2 in the egg mass together with eggs at various stages of development. The J2 are the only juveniles of *Meloidogyne* that migrate in

the soil (109). Once the J2 leave the egg masses, they infect nearby, galled roots or locate new roots of susceptible plants to continue their life cycles (42). Research shows that J2 of *Meloidogyne* do not find roots through random movement, but are attracted to plants in response to stimuli emanating from roots (37, 84). These J2 possess well-developed, innervated cephalic sensory organs that are thought to be chemoreceptive and presumably able to locate roots (19).

Although the J2 usually enter roots directly in the region behind the root cap, they are also attracted to apical meristems, points where lateral roots emerge, penetration sites of other juveniles, and wound surfaces of roots (14, 15, 42). It was reported that the apical 2 mm of an excised tomato root actually repelled the J2 of *M. hapla*, but that from 3 mm to 8 mm back from the tip, the root tissue increased in attractiveness (42). Beyond 8 mm from the tip, the tissue was neutral or somewhat repellent to juveniles (12).

Many organic and inorganic compounds secreted by roots and microorganisms develop gradients from the root surface into the soil which may influence nematode attraction over short distances (0.5 - 1.0 cm). Klingler (52) regards certain amino acids and CO_2 as the most important root excretions for attracting plant-parasitic nematodes. It is known that some of these compounds attract *Meloidogyne* juveniles *in vitro* (10, 12).

After penetration, J2 migrate intercellularly in the cortex to the region of cell differentiation (23, 42, 60). Endo and Wergin (27) reported that cells along the path of migration become distended and compressed by migrating J2, but show no sign of nematode feeding. However, Linford (60) observed feeding by the migrating J2 (42). The J2 finally come to rest with their heads within the developing stele, near the region of cell elongation, and with their bodies in the cortex. Cell walls are pierced with stylets, and secretions from the esophageal glands are injected into the cells. These secretions cause enlargement of cells in the vascular cylinder and increase rates of cell division in the pericycle. This disruption leads to the development of elaborate permanent feeding sites, called giant cells (13, 26) which are formed by repeated mitosis without cytokinesis (47). At the same time, there is intense cell enlargement (hypertrophy) around the juvenile's head. These changes are usually, but not invariably, accompanied by enlargement of the root to form distinct galls (42, 102).

Galls usually develop one or two days after juvenile penetration, and are often the first symptoms observed on an infected plant. On a susceptible plant, gall size is often related to the number of nematodes in the tissue (22). However, gall size differs among plant species in response to nematode infection (42).

As the giant cells and galls are formed, the width of the juveniles increases, and there is considerable

enlargement of the esophageal glands. The cells of the genital primordia enlarge, forming two pronged ovaries in the female, and a single elongated testis in the male. As the J2 continue feeding, body size increases, they become flask-shaped, and the gonads lengthen (102). Several body systems, the body wall, nervous, digestive, and excretory systems, are coordinated to allow these tiny parasites to develop to maturity. The reproductive system remains small in the preparasitic juvenile but begins to develop shortly after juvenile feeding is initiated (81).

After the establishment of a host-parasite relationship and an initial feeding period of 3-8 weeks, depending on temperature, the J2 molts three times in quick succession and develops into an adult female or male (34). After the second and third molts are completed, as shown by the two loose cuticles, the stylet and median esophageal bulb disappear. Shortly after the fourth molt, the stylet and median bulb are regenerated, the uterus and vagina are formed in the female, and a perineal pattern is visible. Further development of the two female gonads is difficult to see as they elongate and become folded in the nearly globular or slightly elongated female body with a neck which may be shorter and stout or nearly as long as the body (25, 102).

Just before the second molt, the male gonad is near the posterior end of the body and the rectum is visible. After the second and third molts, no stylet is visible, the median

esophageal bulb has degenerated, and the gonad has enlarged. Then there is a rather rapid metamorphosis as the elongate, vermiform body develops within the J2 cuticle, complete with stylet, esophagus with median bulb, spicules, and sperm in the testis (25, 102).

Males are vermiform and motile and do not feed. Shortly after the fourth molt, they leave the root and move freely through the soil. Even though males are not necessary for reproduction in the four most common species, many *Meloidogyne* spp. reproduce by cross fertilization or facultative meiotic parthenogenesis (106). In populations which reproduce by amphimixis, the ability of the male to find a suitable mate is critical for survival of the species. The production of males in mitotically parthenogenetic populations is a survival mechanism for the species. In plants that are under stress, many more juveniles develop into males which do not feed and require less nutrition than females, hence lessening stress on the plant host and enabling the survival of both plant and parasite (25, 105).

Where susceptible host plants are present, one of the most important factors for nematode development is soil temperature, which is largely determined by climate. Climate depends on latitude, elevation above sea level, geographic location, and seasonal variation (102).

It has been reported that temperature is the main driving factor in *Meloidogyne* spp. for embryogenesis,

hatching, mobility, invasion of plant roots, growth, reproduction and survival (97). In general, *M. hapla* and other cool climate species have lower minimum, optimum, and maximum temperature requirements for hatching, movement, penetration, growth and reproduction than do *M. incognita*, *M. javanica*, and *M. arenaria* which occur in warmer climates. Optimum temperatures range from 15°C to 25°C for *M. hapla* and related species, and 25°C to 30°C for *M. javanica* and related species. There is very little activity of any *Meloidogyne* spp. above about 40°C or below 5°C (97, 102).

The second most important factor influencing *Meloidogyne* spp. is soil moisture, which depends on rainfall or irrigation. In agricultural soils, sufficient soil moisture for nematode activity is generally present if there is sufficient moisture for crop growth (102). As the soil dries at the beginning of the dry season, *Meloidogyne* eggs are subjected to osmotic stress. Hatching ceases, but development in the egg continues, so that all living eggs soon contain the J2. If the eggs become too dry, the J2 die; but if the eggs survive until the beginning of the rainy season, they hatch and infect plants (24, 119).

Under favorable conditions of temperature and soil moisture together with susceptible plant hosts, the population of *Meloidogyne* builds up very fast, and soon accounts for significant losses in yields of food and fibre.

Damage caused by the nematode is determined by relating pre-plant nematode densities (initial population) to growth

and yield of annual crops (7). The minimal density (damage threshold density) (6) that causes a measurable reduction in plant growth or yield varies with nematode species, host plants, cultivar and environment (7). Some damage threshold densities of *M. incognita* are as follows: 3,000-81,000 eggs/plant for soybean (71); 0.005-0.02 eggs/g of soil for tomato; 0.005-0.03 eggs/g of soil for potato (31).

It is estimated that crop losses due to *Meloidogyne* and other nematodes on a worldwide basis are, on average, about 12.3% (92). This would not be of great significance if this were evenly distributed, but it is not. The greater part of the loss is borne by those growers least able to afford it, namely the small farmers in undeveloped countries. Their losses may be as much as 25% to 50% of the available farm land of the country (92, 102).

In cool, temperate climates, damage and crop losses due to *Meloidogyne* spp. are less significant. In economic terms, however, the losses are still very real to the farmers (92). Such instances occur in conditions that favour the nematode, such as in sandy soils or in glasshouses. The losses may be expressed as decreased yields or reduced quality, which in turn lead to low returns or unmarketable produce (33, 83).

Yield losses of about 50% for tomato attacked by *M. incognita*, under the climatic conditions of Mediterranean summer, have been demonstrated, based on field experiments done in southern Italy (56). Moreover, field experiments done in North Carolina showed that *M. incognita* suppressed

yields of tomato up to 85% on the coastal plains and 20-30% in mountain areas (8). In other crops, *M. incognita* caused yield losses of 30-60% in eggplant and 50% in cantaloupe and watermelon (56). Yield decreases caused by *M. incognita* are well known in okra, sweet potato, and celery (56).

In North America, Europe or Africa, *M. incognita* is a major pest of industrial crops such as tobacco. Yield losses in tobacco in non-irrigated fields range from 30-75%, and reductions of yield by 60% and 80% were reported in sugarbeet attacked by *M. incognita* in 1976 and 1977, respectively (56).

Vegetables such as tomato, eggplant, beans, squash, watermelon, and sometimes okra, carrot, celery and yam are affected most by *M. javanica*. This species also attacks industrial and row crops, such as potato, sugarbeet, sugarcane, rice, and tobacco. In ornamentals it is a serious pest of carnation (56).

Meloidogyne arenaria is mainly a problem on vegetables such as tomato, pepper, watermelon, celery, beet, carrot and onion. The crops most susceptible to attack, however, are peanut, tobacco, and sugarbeet, especially in the southern United States (56).

Meloidogyne hapla decreased yield of tomato in North Carolina by 50% (7) and in Canada by 40% (75). Considerable losses were reported on alfalfa (36%), peanut (70%), carrot (50%), sugarbeet (20%), potato (46%), and onion (64%) (64).

Estimated percent loss due to *Meloidogyne* spp. for typical crops in the tropics are; tomato (29%), eggplant (23%), okra (22%), beans (28%), pepper (15%), cabbage (26%), soybean (26%), papaya (20%), potato (24%), coffee (19%), banana (5%), and rice (25%) (91).

In response to losses caused by *Meloidogyne* spp., many attempts have been made to control these nematodes, through management of populations. Realizing that nematodes cannot be eliminated, the overall goal is to keep the population density as low as possible, usually below an economic threshold (92).

To accomplish this, nematicides have been one of the most important and reliable means for control of a wide variety of nematodes (45, 121). These chemicals are particularly useful in high-value horticultural crops and more importantly, to enable certain crops to be produced profitably in fields previously abandoned or classified as "tired" (39, 92). Although only a few hundred ha were treated each year prior to 1950, the number of ha treated annually has grown rapidly (92). In 1985, in the United States, approximately $ 200 m was spent to treat about 800,000 ha for nematode control, usually for tobacco, cotton, peanuts, vegetables, fruit, soybean and sugarbeet (45, 121).

Since they were first introduced, various types of nematicides have been developed and reported to control *Meloidogyne* spp. Carbon disulfide, dibromochloropropane

(DBCP), Metham, VC-13, Fensulfothion, and Thionazin have been reported to be effective (6, 57, 90). Ethoprop was reported to be effective in controlling *M. incognita* in tobacco (80) and in tomato (16). Oxamyl, a systemic nematicide, effectively controlled this nematode in several host plants when applied to foliage (86).

In tomato fields infested with *M. incognita* in Southern Italy, soil fumigation with 150 L/ha of Telone II (93% 1,3-dichloropropene) increased yield by 51% (40) and at the rate of 200 L/ha by 121% (55) compared with untreated plots in a dry summer (56). In glasshouse experiments, dichloropropene-dichloropropane (DD) applied at 200 L/ha increased the production of marketable tomatoes by 126% compared with untreated plants which were heavily infested with *M. incognita* (56). Aldicarb applied at 8 kg a.i./ha before planting gave yield increases of about 200% compared with untreated controls in a tobacco field infested with *M. incognita* (41, 55).

Success in controlling nematodes by using nematicides relies on the ability of these chemicals to disrupt nematode activities such as hatching, invasion, development and reproduction. Motsinger (69) reported that Thionazin at 200 ppm suppressed the hatching of *M. incognita*. Aldicarb was reported to reduce hatching of *M. hapla* (88) and *M. incognita* (72), *M. arenaria* (67) and *M. javanica* (40). McLeod and Khair (67) reported that the organophosphates

Fenamiphos and Thionazin effectively reduced hatching of *M. javanica*, *M. incognita* and *M. hapla*.

The nematicide Oxamyl (83) has been shown to prevent both invasion and development of juveniles of *M. incognita* in cucumber seedlings when applied to the roots but affected only invasion when applied to the foliage (60). McLeod and Khair (67) found that aldicarb reduced migration of *M. incognita*, *M. hapla*, and *M. javanica*, and fenamiphos impaired the ability of juveniles of *M. arenaria* to penetrate roots (46).

McLeod and Khair (67) found that aldicarb and thionazin at 2 ppm completely prevented development of *M. javanica* juveniles in tomato seedlings, while Methomyl, Ethoprophos or fenamiphos at 4 ppm reduced development by 60% and at 8 ppm of Ethoprophos or fenamiphos or 16 ppm Methomyl, development was stopped. Nematicide concentrations which suppressed development also prevented the normal orientation of juveniles in the roots and reduced or prevented giant cell formation (72). Oxamyl also has been shown to significantly inhibit development of juveniles of *M. incognita* in cucumber roots at 6 ppm (67). However, when cucumber seedlings containing nematodes were removed from Oxamyl-treated sand 10 days after the nematicide was applied and then placed in clean sand, a significant proportion of the juveniles had developed further after 18 days (122). This suggested that the effect of Oxamyl in nematode development was reversible.

Atilano and VanGundy (3) found that treatment with Oxamyl could significantly reduce the size of adult females of *M. javanica* in tomato roots. Cotton treated with 10 ppm diflubenzuron had fewer egg masses/root system caused by *M. incognita* than did untreated controls (111). These effects are likely to be due to inhibition of normal feeding activity (29).

Unfortunately, most of the effective and widely used nematicides are toxic not only to the nematodes but also to other living organisms, particularly when nematicides are applied improperly. Although not considered a general biocide, studies in the United States and Europe have shown that DD or Vidden D could affect a number of soil organisms, such as nitrifying bacteria, which causes accumulation of phytotoxic levels of ammonia (104). Carbamates and organophosphates may be hazardous to humans and animals during and after application if proper safety precautions are not used (46). The finding that DBCP depressed the sperm counts of workers in manufacturing plants led to the cancellation of this nematicide for all registered uses despite its effective and economical value (46).

It became apparent during the 1980's that a major disadvantage of all nematicides is their inherent toxicity coupled with their potential to be environmentally hazardous (39). In attempts to obtain high levels of control, farmers have poisoned the environment by using higher-than-necessary rates of some nematicides (83). This is particularly serious

if nematicide residues or breakdown products reach unacceptable levels in either products or in groundwater (39).

For the foreseeable future, commercial agriculture will continue to depend heavily on chemical control methods because in general they are the most reliable and economical methods available, particularly since the demands for food and fibre increase from year to year (46, 121).

Research on environmentally safe chemicals, other than nematicides, to control plant parasitic nematodes has been done intensively. In tests on hatching, for instance, Lehman (59) tested the response of *Heterodera glycines* to $CaCl_2$ and NH_4NO_3 over a range of pH. He found that the cation NH_4^+ was more inhibitory than Ca^{2+}, and the influence of pH was secondary to that of inorganic ion present at pH values greater than 4.0. In addition, diffusates from host roots increased the hatching of *H. glycine* in one study (103) while those from non-hosts had no effect (74). Hatching activity was not influenced in another study of root diffusates from soybean plants (98). However, diffusates from the roots of plants such as potato stimulated hatching of *Globodera pallida* eggs, whereas diffusates from mustard roots, a non-host, inhibited hatching (32).

Once hatched, J2 must find and penetrate host roots. The infectivity of juveniles may be affected by the nutritional status of the host (66). Root-knot larvae are capable of feeding on the surface of the roots, and it is

possible that the nutrients taken in during that feeding period are important to the larvae in the subsequent process of penetration (65). In addition to the effects of host nutrients, the infectivity of juveniles is probably affected by prior chemical treatment. Hussey and Barker (43) reported that there were more juveniles of *M. incognita* penetrating roots when egg masses were used as inoculum than when dispersed eggs extracted by sodium hypochlorite were used as inoculum.

The development and reproduction of root-knot nematodes, depend on successful formation of feeding sites (coenocytes) in susceptible plants. Attempts to prevent coenocyte formation, and thus hamper nematode development, by using plant growth hormones (53, 78) or plant growth retardants (21, 35, 77) have failed. In most cases they did not inhibit coenocyte formation and if they did, they also severely damaged host plants. The induction of natural root-knot resistance reaction by artificial means has been examined in many studies. Overman and Woltz (85) proposed the use of amino acid analogs as antimetabolites for inhibiting development of *Meloidogyne* spp. in tomato roots.

The use of several fertilizers to suppress nematode development and to increase crop yields has also been reported. Fademi (30) reported that, in greenhouse tests, ammonium sulphate at 45 kg N/ha increased the vigor of rice seedlings infected by *M. incognita*, reduced the nematodes' recovery and reduced galling, when compared with

unfertilized controls. An increase in root length and yields, and a reduction in egg masses were reported on brinjal infected by *M. incognita* and fertilized by an N:P:K ratio of 12:8:6 (96). Furthermore, Vozna *et al.* (115) showed that ammonium nitrate reduced the *M. incognita* population in Begonia plants.

Rodriguez-Kabana and King (89) noted a reduction in galling of squash roots by adding urea to the soil. Glazer and Orion (36) demonstrated that hydroxyurea (HU) at rather low concentrations suppressed development of *M. javanica* in excised roots in culture. This report of HU influencing the interaction between the host and the nematode is similar to the description of the hypersensitive reaction that occurs naturally in root-knot nematode resistant plants (35, 112). This suggests that HU induces resistance in susceptible tomato roots. Kochba and Samish (53) indicated that thiourea inhibited development of *M. javanica* in peach roots.

In a study on inhibition of giant cell formation on two different media, Orion *et al.* (79) found that a high concentration (360 ppm) of ammonium nitrate in the medium inhibited coenocyte and gall formation in cultured tomato roots infected with *M. incognita*. Spiegel *et al.* (99) showed that application of ammonium chloride reduced nematode populations in roots and induced a high percentage of males in the population.

The use of ammonium to control *M. incognita* appears very promising since this substance could be applied as, or

along with, fertilizer. The objective of this reasearch was to determine the effect of ammonium on hatching, penetration, and development of *M. incognita* in excised tomato roots.

II. GENERAL MATERIALS AND METHODS

1. Plant Material

Seeds of the susceptible tomato cultivar, *Lycopersicon esculentum* Mill. UC 82, were obtained from A.L. Castle Inc., Morgan Hill, CA.

The seeds were surface sterilized in a 1:1 mixture of 95% ethanol and commercial chlorine bleach (Javex, 6% NaOCl) for 8 min and rinsed five times with sterile distilled water (54). The seeds then were sown in Petri dishes containing a 3-4 mm thick layer of 1% water agar and incubated in the dark at 25°C.

2. Growth Media and Root Cultures

The STW nutrient medium formulated by Skoog, Tsui, and White (58) was used for all experiments and modified as required. To obtain different concentrations of ammonium, the standard concentration of ammonium nitrate in the STW medium was modified in different treatments as follows: ammonium deficient, 6.67 mg/L (1.5 ppm NH_4^+); normal, 40 mg/L (9 ppm NH_4^+); 6X strength, 240 mg/L (54 ppm NH_4^+); and 36X strength, 1440 mg/L (324 ppm NH_4^+). In order to demonstrate whether the effect of the higher ammonium nitrate concentrations were due to the ammonium or the nitrate ion, the concentration of potassium nitrate in the medium was increased to 1897 mg KNO_3/L (1116 ppm NO_3^-) in one treatment, while retaining the normal concentration of

ammonium nitrate. This concentration of nitrate was equivalent to the high concentration of ammonium nitrate without the associated increase in ammonium. Thus, there were four different ammonium concentrations and one high nitrate concentration. A stock solution at each concentration was prepared as shown in Table 1.

Stock solutions were made as follows (58); stocks A, B, C, and D were diluted with distilled water into 1000 mL, 100 mL, 100 mL, and 100 mL, respectively. Stocks A, B, and C were stored at 5°C. Stock D was sterilized by filtration (Micron Separations Inc.), distributed at 1 mL per sterile tube and stored at -20°C.

To make 1 L of nutrient agar medium, 100 mL of stock A was combined with 5 mL of stock B, 10 mL of stock C, 20 g of sucrose, and 12.5 g of Difco Noble Agar. This solution was brought up to 1000 mL with distilled water. The pH was adjusted to 6.0 with 0.1 N NaOH and the mixture was then autoclaved at 15 psi for 15 min. After autoclaving, the nutrient medium was allowed to cool to about 60°C. Then, 1 mL of stock D was added to the nutrient medium and mixed thoroughly. The medium was then aseptically poured into Petri dishes (100x15 mm) at about 20-25 mL per Petri dish and allowed to solidify.

When the tomato seedlings had germinated, 1 cm-long primary root tips were cut at the hypocotyl and the excised roots were aseptically transferred to Petri dishes containing STW growth medium with different ammonium

Table 1. Composition (mg per liter) of Skoog, Tsui, and White medium modified with different ammonium and nitrate concentration(s) (based on Lauritis, *et al.*, 1982).

	Ammonium (NH_4)				KNO_3
	1.5ppm (deficient)	9ppm normal (Control)	54ppm 6X	324ppm 36X	1116ppm
Macronutrient Salts (Stock A)					
$Ca(NO_3)2$		144			
KNO_3	**80**	**80**	**80**	**80**	**1897**
KCl		65			
KH_2PO_4		38			
NH_4NO_3	**6.7**	**40**	**240**	**1440**	**40**
$MgSO_4.7H_2O$		72			
Iron (Stock B)					
$FeSO_4.7H_2O$		28			
Na_2EDTA		37			
Micronutrient Salts (Stock C)					
$ZnSO_4.7H_2O$		2.7			
$MnSO_4.H_2O$		4.9			
H_3BO_3		1.6			
KI		0.75			
Vitamins and Amino acids (Stock D)					
Nicotinic Acid		0.5			
Pyridoxine HCl		0.75			
Thiamine HCl		0.1			
Sodium glycinate		2.62			

concentrations. The root cultures were incubated in the dark at 25°C for 3 days prior to inoculation with nematodes (65).

3. Nematodes

Source and maintenance

Meloidogyne incognita (Kofoid and White, 1919) Chitwood, 1949, race 2, obtained from North Carolina State University (NC # 632), was maintained in greenhouse-grown tomato plants. Two-week-old seedlings, grown in 20x25 cm plastic pots filled with autoclaved growing medium (80% sand and 20% loam), were inoculated with one egg mass each. The plants were maintained in a greenhouse at 24-28°C, watered every 2 days and treated when necessary with 15:15:10 fertilizer. Egg masses were produced and collected after 2-3 months for use in experiments.

Dispersed eggs

The infected tomato roots, taken from *M. incognita* inoculated plants in plastic pots in the greenhouse, were carefully washed free of soil, then cut into 1 to 2 cm long segments, and comminuted in a Waring blender with 200 mL water at high speed for 15 sec. The mixture was vigorously shaken for 2 min in a 0.525% sodium hypochlorite solution, made by adding 25 mL chlorine bleach (Javex, 6% NaOCl) to the mixture (5). The solution containing the eggs was rinsed

through a series of 60, 100, 200, and 500 mesh sieves and the eggs were collected on the 500 mesh sieve (51). The masses were washed off the sieve with water and poured into centrifuge tubes and centrifuged at low speed to remove clumps of eggs and soil particles. The supernatant was decanted into sterile water and concentrated by centrifugation. The eggs then were sterilized with 0.525% sodium hypochlorite for 2 min and rinsed four times with sterile distilled water. Inocula were prepared by dilution of a suspension to give about 200 eggs per 5 μL of water.

Egg masses

Egg masses were taken from monoxenic cultures that were prepared as follows (5): egg masses were taken manually from stock cultures in the greenhouse and surface sterilized with 0.525% sodium hypochlorite for about 3 min (54). The egg masses were blotted dry on sterile filter paper and aseptically inoculated onto three-day-old excised root cultures in Petri plates. The plates were sealed with Parafilm and incubated in the dark at 25°C. About 55 to 65 days later, egg masses produced in these cultures were used as inocula. The egg masses were manually picked up and sorted for uniformity in size and color (114).

Second stage infective juveniles

Second-stage juveniles of *M. incognita* were obtained by incubating the egg masses taken from monoxenic cultures. The

egg masses were placed in a layer of sterile water (about 1-2 mm deep) in Petri dishes in the dark at 25°C. The juveniles emerging after one day were used in experiments (76, 118). Inocula were prepared by diluting a suspension to give about 150 juveniles per 5 μL aliquot.

4. Inoculation and measurement of roots

The roots in Petri plates were inoculated with *M. incognita* by placing dispersed eggs or egg masses or second-stage juveniles about 1 cm from the tips of three-day-old excised roots. Root growth was measured as fresh or dry weights.

Excised roots were carefully removed from the Petri dish culture and cleaned of medium adhering to the root surface. The root was placed on filter paper, and immediately the fresh weight was obtained on an analytical balance.

To obtain dry weight, the clean roots were placed in sacs made from paper towels, and dried in a 70°C oven for 48 h. The dry weight was then obtained on an analytical balance. The dry weight of the roots was calculated by subtracting the dry weight of sacs alone from the dry weight of sacs with roots.

5. Root staining

The roots were stained with acid fuchsin as described by Ayoub (4). The acid fuchsin solution was prepared by

adding one part 0.1% acid fuchsin to 19 parts lactophenol (= 1 part phenol, 1 part lactic acid, 2 parts glycerol, 1 part water). About 100 mL of the solution or enough to cover the roots, was poured into a beaker and heated to boiling point. The roots were placed into the boiling staining solution for about 1 min. The stained roots were then transferred to a second beaker and rinsed with cold water for about 2 min to remove excess stain and to stop further heating, and then transferred to another beaker and covered with clear lactophenol solution for destaining. The clear roots with stained nematodes inside were observed under a stereomicroscope after 6 h of destaining.

6. Statistical Analysis

Each experiment was designed as a completely randomized block with five treatments, each replicated five times. The data were subjected to analysis of variance (100) and each treatment mean was compared with the control by the test of least significant difference (LSD), at 0.05 or 0.01 level significance, to determine the significant effects of ammonium concentrations. Where necessary, the data were analyzed after transformation of percentages to angles (angle = arc sin $\sqrt{x}$)

III. EXPERIMENTS AND RESULTS

1. Hatching

Experiment

These experiments were to investigate the effects of different ammonium concentrations on hatching. Two types of inocula were used: (i) dispersed eggs were used to obtain individuals at a similar stage of egg development (fully differentiated eggs) and (ii) egg masses.

An experiment was conducted with dispersed eggs, using 25 Petri dishes, which were divided into five groups. Four groups contained STW medium, each with a different ammonium concentration, namely deficient, normal (control), 6X-strength and 36X-strength. One group contained STW medium with a high potassium nitrate concentration.

The dispersed eggs were inoculated into 3-day-old excised roots, by placing 5 μL aliquots containing about 200 eggs each, into each of a treatment series of five Petri dishes. Every treatment was replicated five times. Most of the excess water covering the eggs was evaporated by keeping the open Petri dishes in a laminar air flow for about 40 sec, then the dishes were sealed with Parafilm and incubated in the dark at 25°C. The hatched juveniles were counted and taken out of the dish daily, for up to 15 days using a fine, mounted hair. The percent of hatched juveniles was determined after the eggs remaining in the dishes had been counted (48).

Similar experiments were done with egg masses as inocula. Since only one three-day-old excised root was used as a host, one egg mass was the unit of inoculum. One egg mass of *M. incognita* was inoculated onto an excised root per dish containing STW medium in the treatment series. The juveniles which hatched from each egg mass were counted and removed from the dish daily for 15 days. The percent of hatched juveniles from an egg mass was determined after the eggs remaining in the egg mass had been separated by the sodium hypochlorite treatment (48) and counted. To release the remaining eggs in the egg masses, each egg mass was put in a 1-ml plastic vial half-filled with 5% NaOCl, shaken vigorously for 15 sec and kept for about 60 min in an incubator at 25°C (49). The eggs liberated from the egg masses were counted under a stereomicroscope. The percentage of hatched eggs was determined by dividing the number of hatched eggs with total hatched eggs and unhatched eggs.

Using both types of inoculum, the experiments were conducted both with and without excised roots.

Results

With increasing ammonium concentration, there was an inhibition of hatching of the nematodes in all experiments (Figs 1 and 2). The cumulative percentages of hatched juveniles in the experiments with dispersed eggs are shown in Fig. 1. The reduction in the cumulative percentage of hatched juveniles due to increasing ammonium concentrations

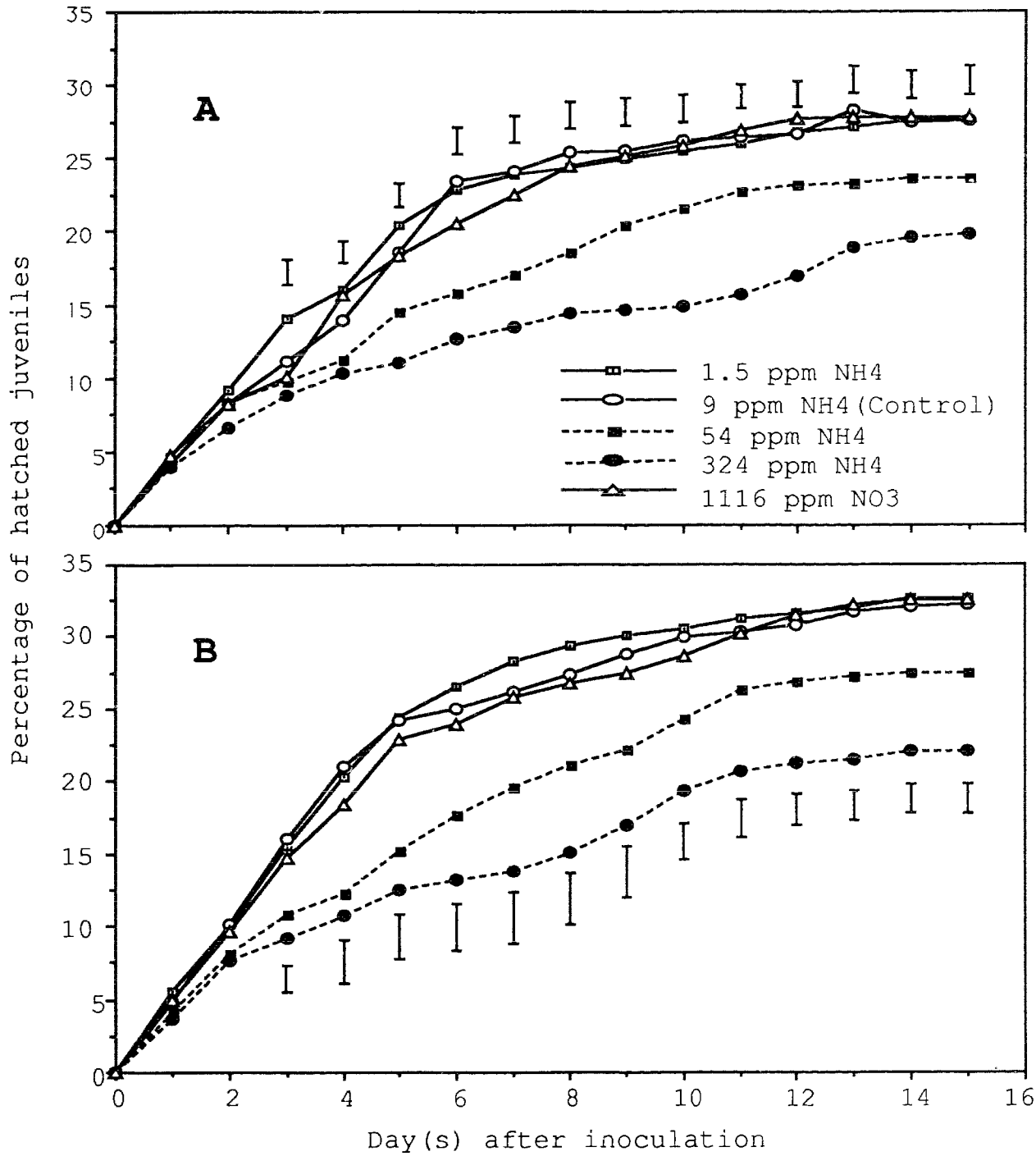

Figure 1. Cumulative percentage of hatched *Meloidogyne incognita* juveniles from dispersed eggs exposed to different ammonium or high nitrate concentration(s), A) without and B) with the presence of excised tomato roots. Vertical bars represent LSD (P = 0.05).

started from the 3rd-day of the experiment with dispersed eggs, either in the presence or in the absence of roots. From the 3rd-day until the end of the experiments, the cumulative percentage from treatments with 54 ppm and 324 ppm was significantly lower than that in the control. The percentage hatch was significantly lower with the higher ammonium concentrations. However, there were no significant differences between treatments with deficient ammonium or with high nitrate and the control.

The cumulative percentages of hatched juveniles in the experiments with egg masses are summarized in Fig. 2. The reduction in hatching started from the 2nd- and the 3rd-days, with and without the presence of the roots, respectively. From the 3rd- and 4th-days both with and without the presence of the roots, the cumulative percentages from treatment with 54 ppm and 324 ppm were significantly lower than that in the control. The percentage was lower with the higher ammonium concentrations. There were no significant differences between treatments with deficient ammonium or high nitrate and the control.

In both types of inocula, the rates of hatching in treatments with 54 and 324 ppm were slower than those in the control. During the first 7 days with dispersed eggs and 5 days with egg masses, the rates of hatching in the control were high, whereas in treatments with 54 and 324 ppm they were consistently slow until the end of the experiments. The

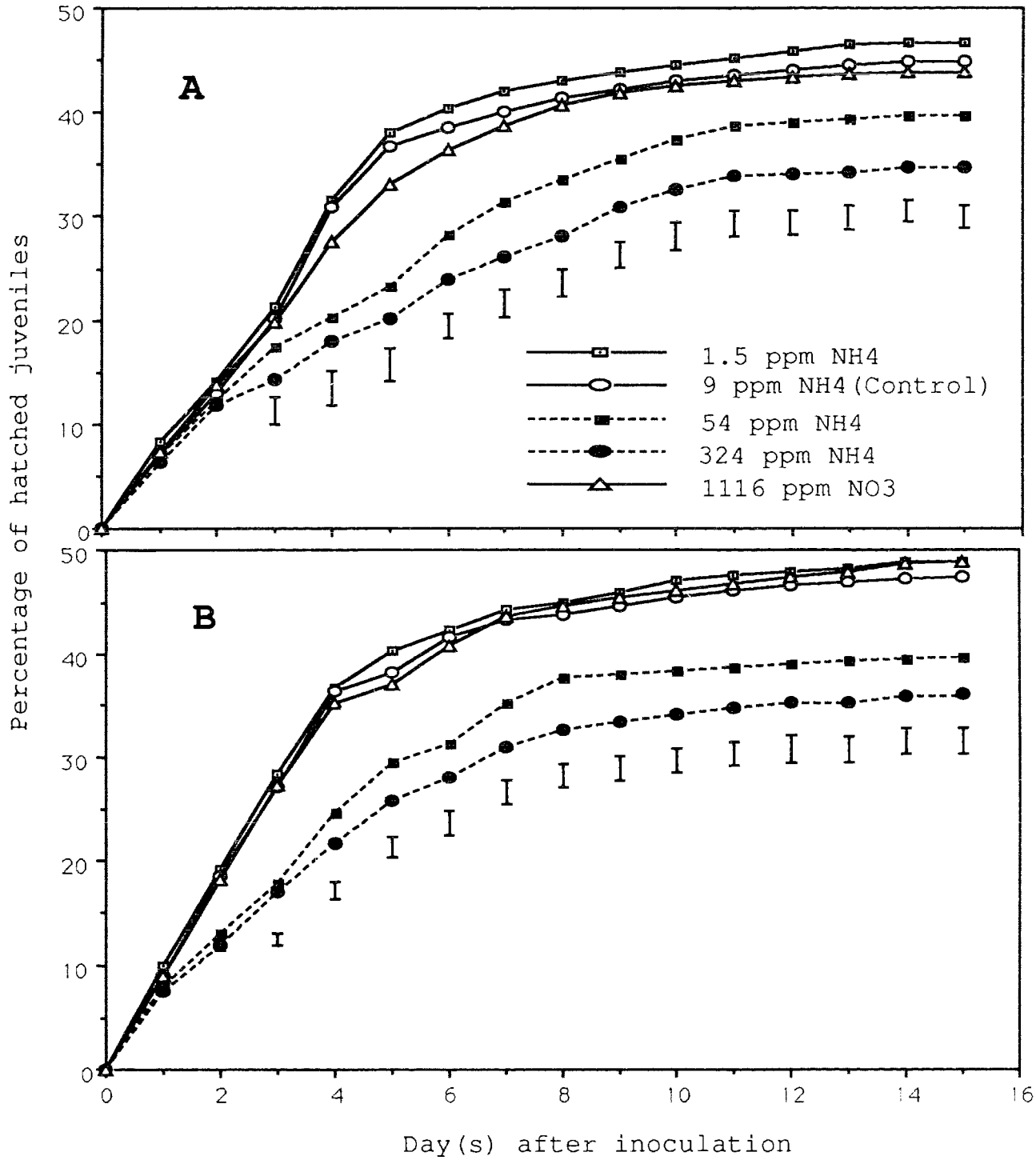

Figure 2. Cumulative percentage of hatched *Meloidogyne incognita* juveniles from a single egg sac exposed to different ammonium or high nitrate concentration(s), A) without and B) with the presence of excised tomato roots. Vertical bars represent LSD (P = 0.05).

rates of hatching in treatments with deficient ammonium and high nitrate were similar to those in the control.

There was a tendency for increased hatching in the presence of roots both with dispersed eggs (Fig. 1) and with egg masses (Fig. 2). The significant effect of roots was clearly shown by the control or treatment with deficient ammonium on all days of observation. Such effects were observed after the 5th- and the 8th-day for the treatments with 54 and 324 ppm, respectively, using dispersed egg inoculum (Fig. 1). In the experiments with egg masses, however, the roots had no effect on hatching at high ammonium concentrations.

The type of inoculum significantly affected hatching. Figs 1 and 2 demonstrate that the cumulative percentage of hatched juveniles from egg masses was higher than that from dispersed eggs.

2. Invasion

Experiment

This experiment investigated the effects of different ammonium concentrations on the invasion of nematodes into excised roots. Two types of inocula were used; egg masses and second-stage juveniles.

The experiment was conducted using 225 Petri dishes containing STW medium. The dishes were divided into five groups of 45. Each of four groups contained STW medium with one of the ammonium test series, and the fifth group had the

high nitrate concentration. Excised roots were grown in each dish, as described.

One egg mass was inoculated into each dish, which was sealed and incubated as described. Five replicate roots of each treatment were carefully harvested at 2, 3, 4, 5, 6, 7, 9, 11, and 14 days following inoculation. Each root was gently washed in a dish to remove surface nematodes, which then were counted. The roots were stained and the number of juveniles within the roots was counted.

A similar experiment was done by using about 150 second-stage juveniles in 5 μL aliquots as inocula. The same observations were made.

Results

The numbers of juveniles from a single egg mass that penetrated the roots are presented in Appendix I. Proportionally, there was a significant reduction in penetration by juveniles as the ammonium concentration was increased. Such a reduction was not observed in the treatment with high nitrate. The reduction was noted at all time intervals. As shown in Fig. 3, there were significantly lower percentages of juveniles in the roots that had the 54 ppm and 324 ppm treatments than those in the control. However, there were no significant differences between 1.5 ppm ammonium or high nitrate and the control. With regard to the time of exposure, at normal and deficient ammonium, the percentages of juveniles that penetrated the roots continued

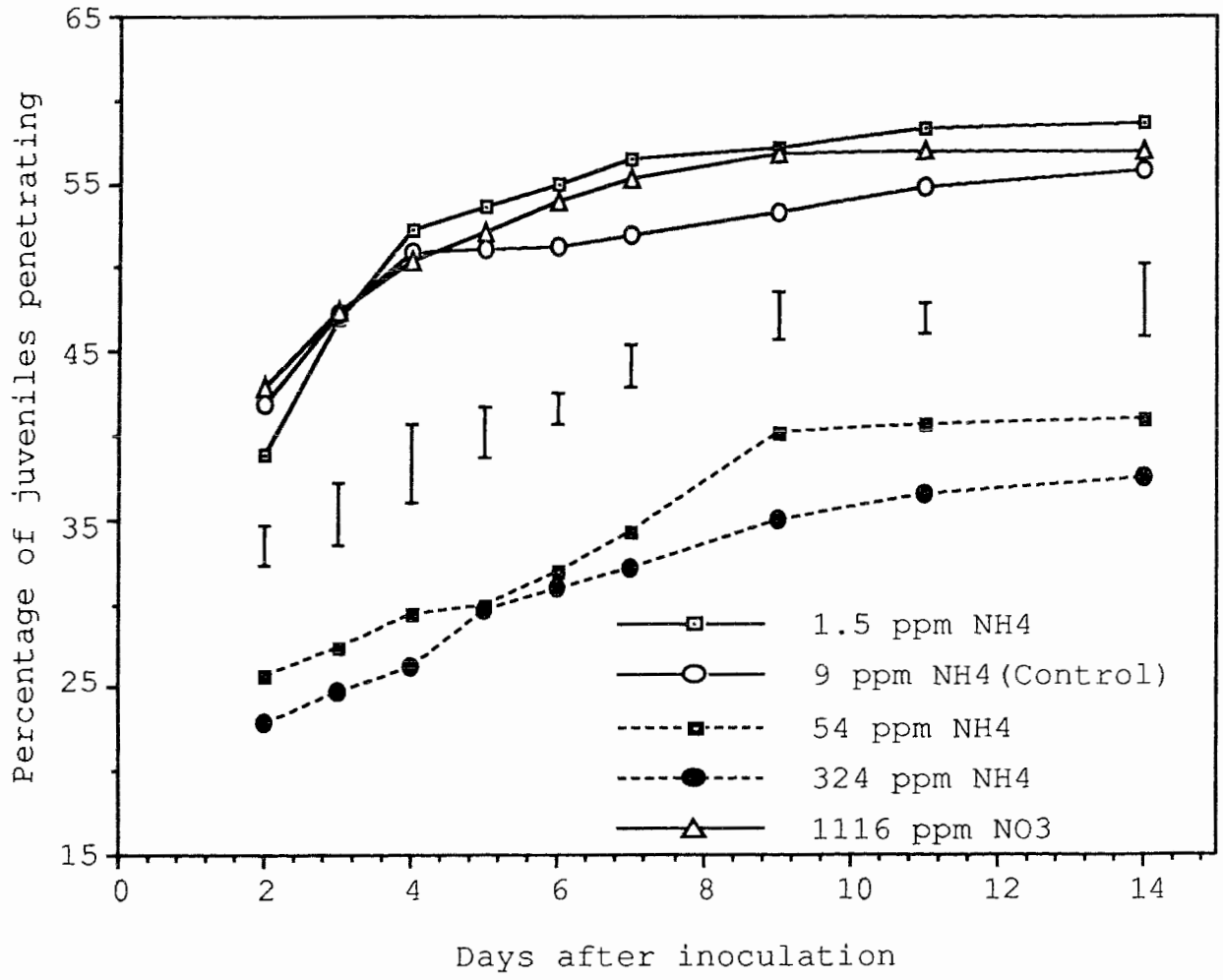

Figure 3. Percentage of *Meloidogyne incognita* juveniles, emerging from a single egg mass, that penetrated excised tomato roots grown on STW medium containing different ammonium or high nitrate concentration(s). Vertical bars represent LSD (P = 0.05)

to increase rapidly during the first 4 days, then slowly until the 14th-day. The percentages of juveniles that penetrated the roots in treatments with 54 ppm and 324 ppm increased slowly from the 2nd-day until the end of the experiments.

In the second experiment, the numbers of juveniles that penetrated the roots are summarized in Appendix II. The percentages of juveniles that penetrated the roots inoculated with second-stage juveniles decreased with increasing ammonium concentrations (Fig. 4). A reduction in the percentage of juveniles that penetrated the roots was observed at all time intervals. There were significantly lower percentages of juveniles in the roots that had the 54 ppm and 324 ppm treatments than those in the control. There were no significant differences in the percentages of juveniles penetrating the roots between treatments with deficient ammonium and control up to the 5th-day. From the 6th- until the 14th-day, however, the percentages of juveniles penetrating the roots were significantly different.

At all time intervals, the percentages of juveniles that penetrated the roots inoculated with egg masses were lower than those in excised roots inoculated with second-stage juveniles.

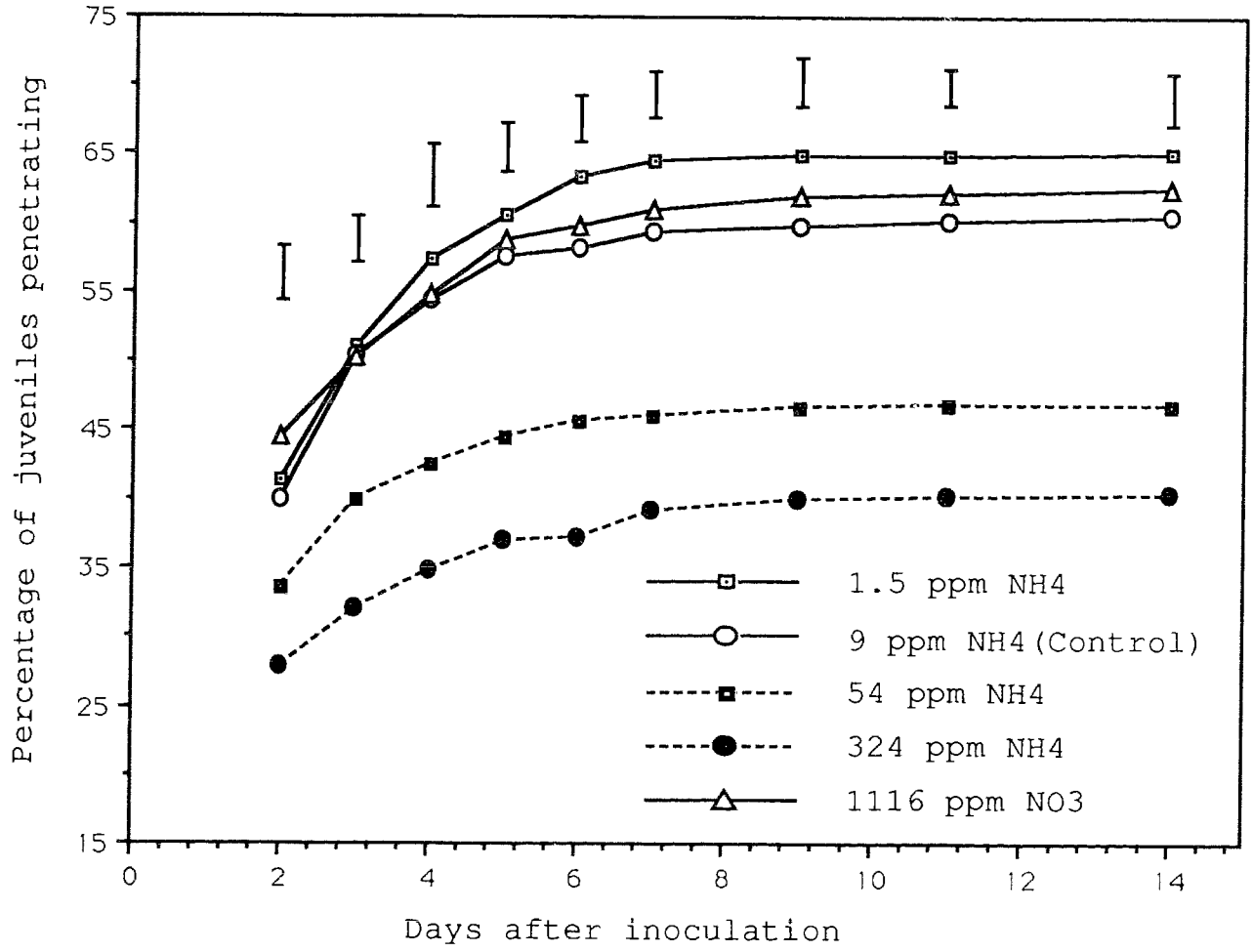

Figure 4. Percentage of inoculated *Meloidogyne incognita* juveniles penetrating excised tomato roots on STW medium containing different ammonium or high nitrate concentration(s). Vertical bars represent LSD (P = 0.05).

3. Migration

Experiment

This experiment was designed to show the effects of different ammonium and a high nitrate concentration(s) on nematode-host compatibility. It was conducted with 175 Petri dishes which were divided into five groups of 35. Each of four groups contained STW medium with one of the ammonium test series, and the fifth group had the high nitrate concentration. Excised roots were grown in each dish, as described previously. About 150 2nd-stage juveniles in 5 μL of water per dish were inoculated into excised roots grown on the STW medium.

Five replicate excised roots of each treatment were removed at 2, 3, 4, 5, 6, 7, and 14 days after inoculation. Each root was washed to remove nematodes from the surface of the roots, then placed in a Petri dish filled with a 3-4 mm layer of sterile distilled water and incubated in the dark at 25°C. After 24 h, all of the juveniles that emerged from the roots were counted and the nematodes inside the roots were counted after staining. The percentage of juveniles migrating from the roots was determined. The juveniles that had migrated from the roots grown in high ammonium were reinoculated into roots grown in normal ammonium (control) to determine their infectivity.

Results

The comparisons of the numbers and the percentages of juveniles that migrated from the roots are summarized in Appendix III and Fig. 5, respectively. At all time intervals, with an increase in ammonium concentration, the percentage of juveniles that migrated from the roots increased. As shown in Fig. 5, significantly more juveniles left the roots in treatments with 54 ppm and 324 ppm than that in the control. The proportion of juveniles that left the roots in deficient ammonium or high nitrate was similar to that in the control.

In the control, deficient ammonium and high nitrate treatments, there was a small increase in the proportion of juveniles that left the roots up to the 4th-day, but at other time intervals, the proportion of juveniles leaving the roots was relatively constant. In high ammonium concentrations, however, a large proportion of juveniles leaving the roots was noted after the 3rd-day, and remained relatively constant for the rest of the time intervals.

Although a few juveniles migrated from excised roots grown in the control (normal ammonium), low ammonium or high nitrate concentration treatments, the percentage was small compared with that from the 54 and 324 ppm treatments. The juveniles that had migrated from the roots grown in high ammonium and were inoculated into the roots grown in normal ammonium (control), except 14 days after inoculation, penetrated the roots and induced galls.

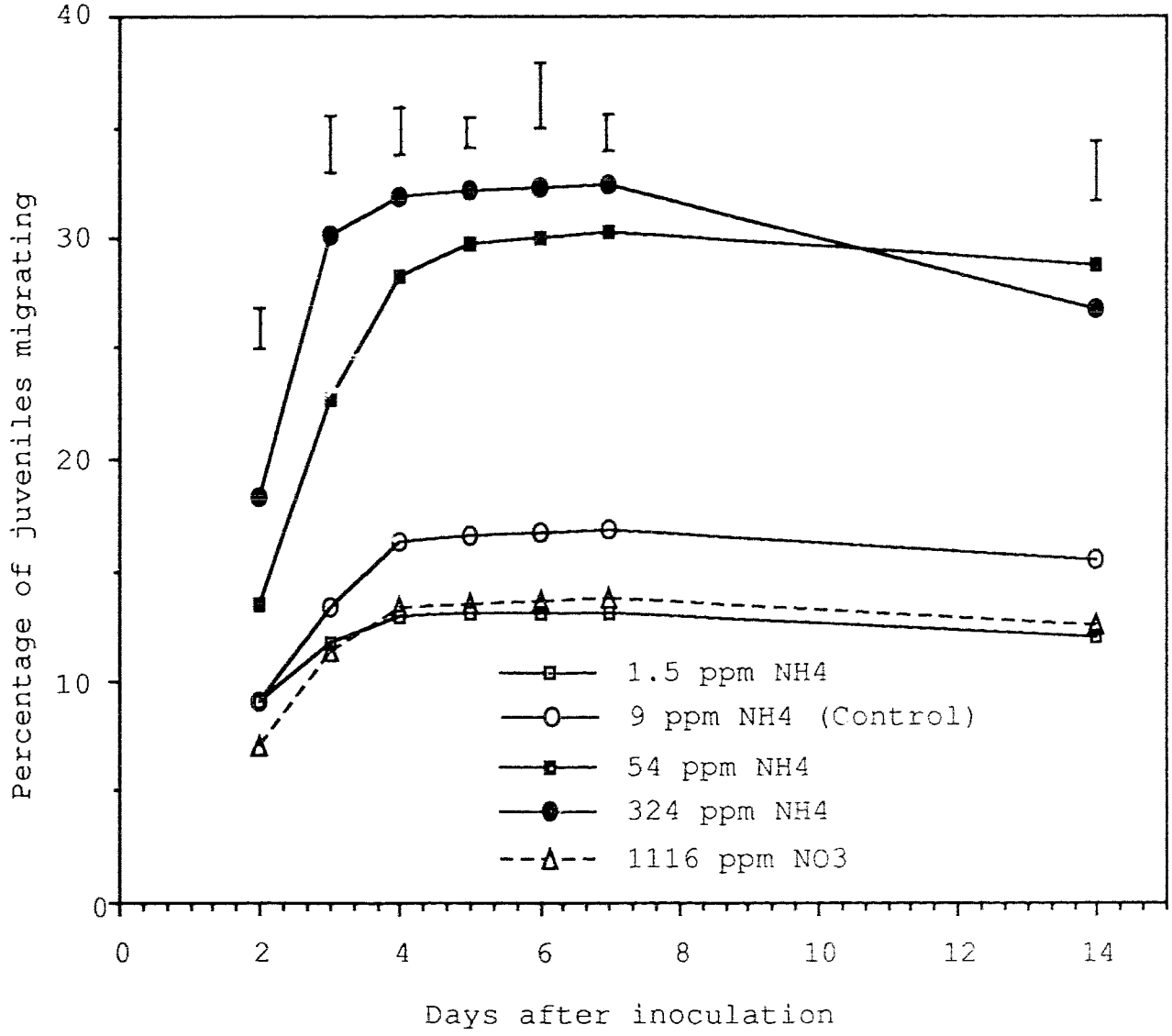

Figure 5. Percentage of *Meloidogyne incognita* juveniles migrating, after 24 h incubation, from infected excised tomato roots grown on STW medium containing different ammonium or high nitrate concentration(s). Vertical bars represent LSD (P = 0.05).

4. Development

Experiment

This experiment was to determine the effect of different ammonium concentrations on nematode development and root growth. The experiment was conducted with 300 Petri dishes which were divided into five groups of 60. Each of four groups contained STW medium with one of the ammonium test series, and the fifth group had the high nitrate concentration. Excised roots were grown in each dish, as described previously. Egg masses were used for inocula. One egg mass was inoculated onto a three-day-old root in each dish. There were parallel control (non-inoculated) treatments and each treatment was replicated five times. The dishes were sealed and incubated as described. Since the root growth was based on both fresh and dry weights, the inoculated treatments were made in two sets of experiments. One set was examined with a dissecting microscope for nematode development and the other set was used for dry weight. Roots from each treatment were harvested at weekly intervals for 4 weeks.

For nematode development and root growth based on fresh weight, the galls were counted and the fresh weight of the excised roots was determined. Then the population and the stages of nematode development, based on Taylor and Sasser (102), were assessed after staining the roots and examining them under a microscope. The stages recorded were (Fig. 6 and Fig. 7): vermiform, middle second-stage, late second-

Figure 6. Development of *Meloidogyne incognita*. A: Vermiform. B: Middle second-stage. C: Late second-stage. D: Third/fourth stage. E: Young adult female. F: Young adult male. (Magnification: 120X).

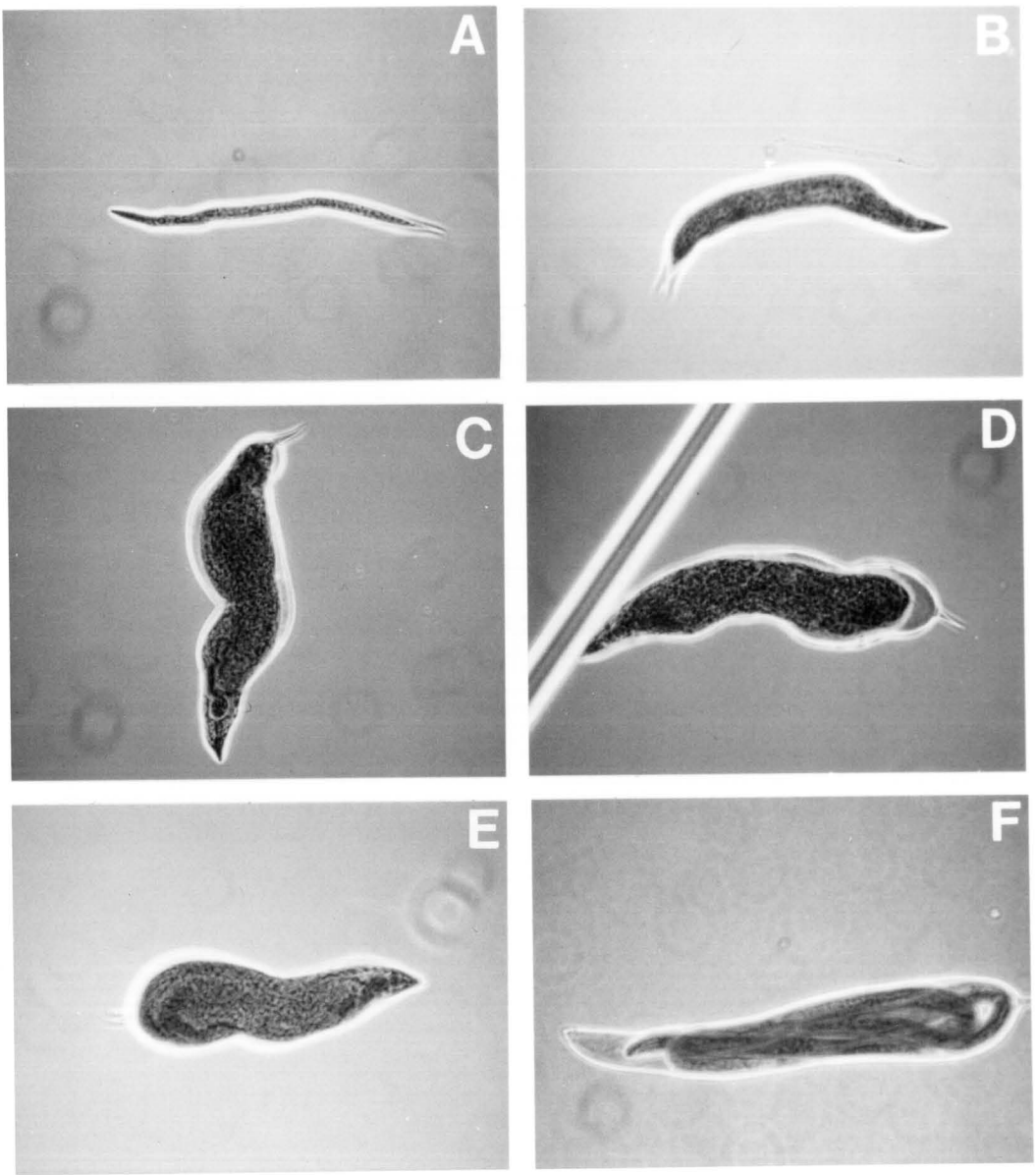
A
B
C
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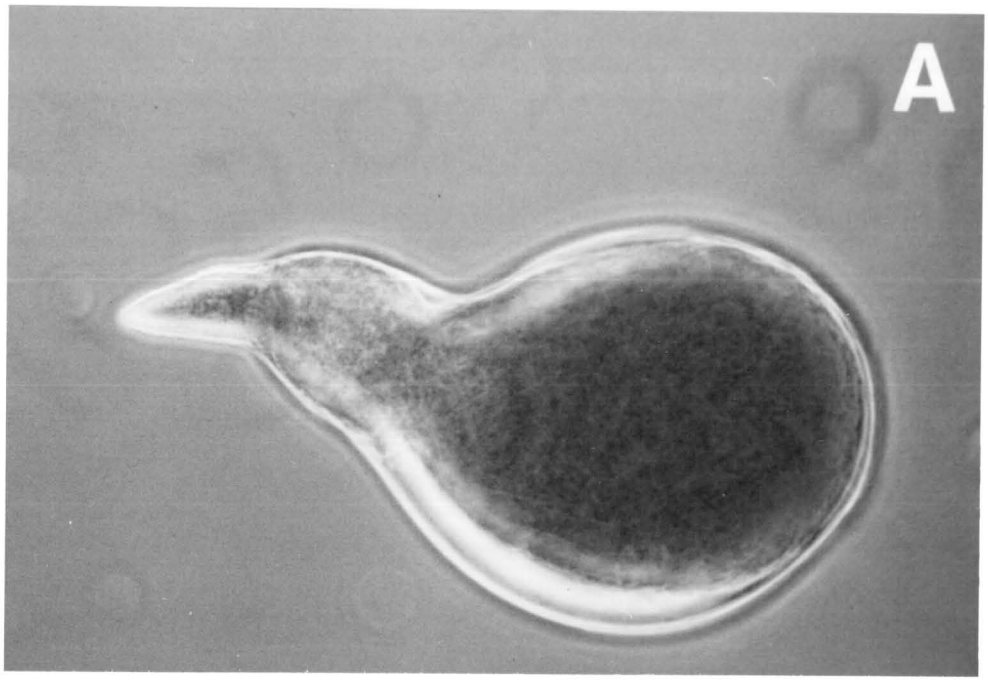
Figure 7. Adult of *Meloidogyne incognita*.

A: Female B: Male (Magnification: 120X)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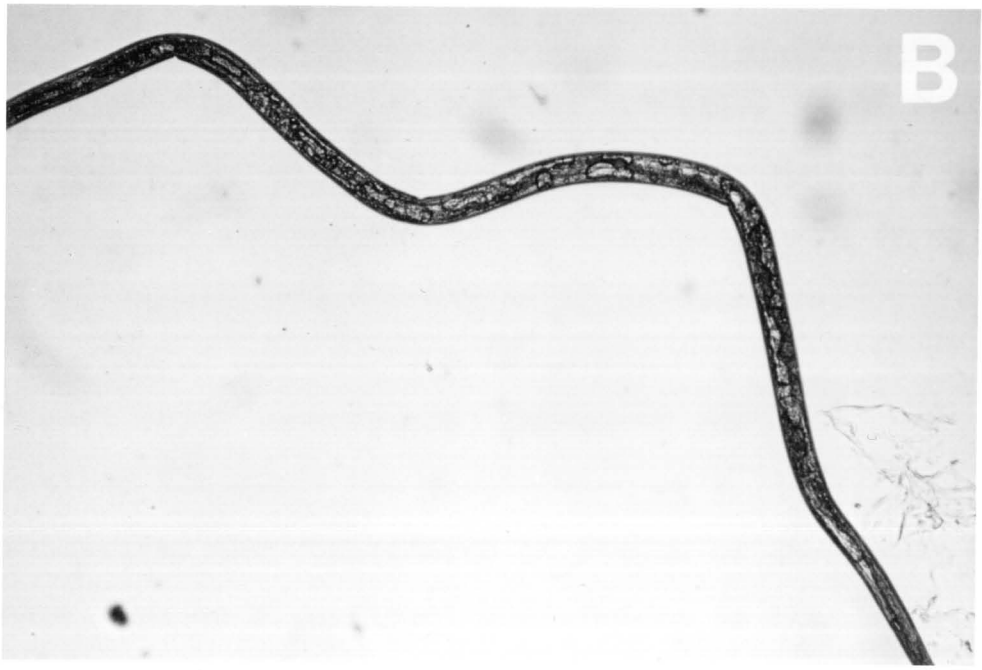
B

stage, third- and fourth-stages combined because of the difficulties in differentiating between them, adult female and adult male. At the same weekly intervals, root growth based on the dry weight was determined after drying the inoculated roots, using the other set of experimental but uninoculated roots.

Results

The fresh and dry weights of uninoculated, and the fresh weight of inoculated roots are summarized in Appendices IV and V, respectively. Ammonium or high nitrate concentration did not significantly affect the dry weights of uninoculated roots at any time of observation.

The infectivity recorded was based on the galls induced by the nematodes. For treatments with deficient ammonium, the control and high nitrate, most of the galls were first observed on the 3rd-day following inoculation. Most of galls in treatments with 54 ppm and 324 ppm ammonium were first observed on the 4th- and 5th-day after inoculation.

One week after inoculation, increased ammonium concentrations inhibited the development of *M. incognita*. Fig. 8 summarizes the comparisons of stages of development. While there were no significant differences in the percentage of middle 2nd-stage, there were significant differences between treatments with 54 ppm and 324 ppm ammonium and control in the percentage of late 2nd-stage.

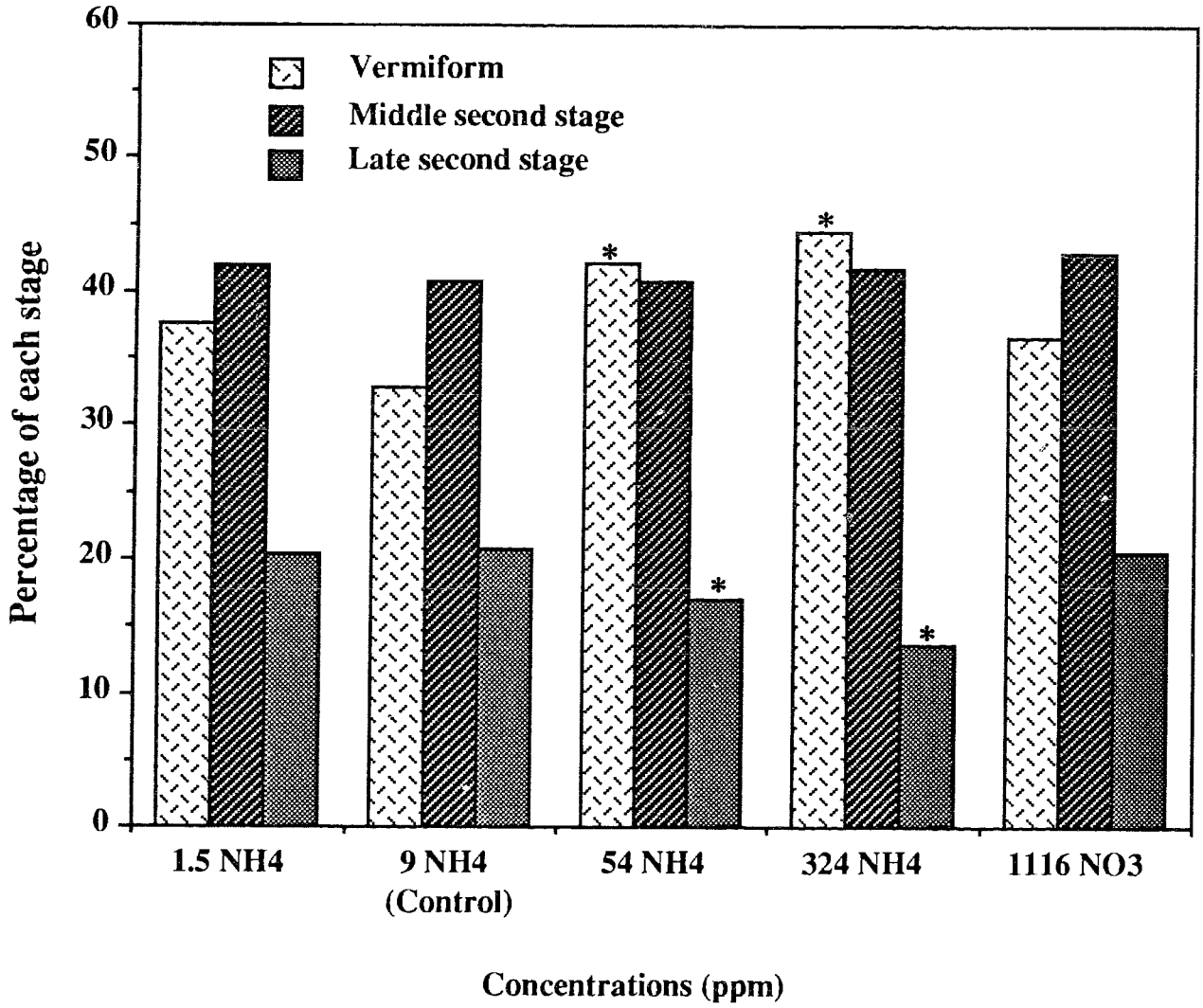

Figure 8. Percentage of stages of development one week after inoculation with *Meloidogyne incognita* egg masses into excised tomato roots grown on STW medium containing different ammonium or high nitrate concentration(s).Bars with * are significantly different from control (P = 0.05).

Only a few late 2nd-stage juveniles were observed in treatments with 54 ppm and 324 ppm ammonium. Compared with the control, there were reductions in the numbers of developed juveniles of about 20% and 35% in treatments with 54 ppm and 324 ppm ammonium, respectively. The percentage observed in treatment with 54 ppm was significantly higher than that in 324 ppm. The percentages of late 2nd-stage juveniles in treatments with deficient ammonium or high nitrate were not significantly different from those in the control.

For the number of galls induced by the nematodes and the dry weights of inoculated roots, there were significant differences between treatments and the control. As shown in Fig. 9, the number of galls in the treatment with 324 ppm was significantly lower than that in the control. In addition, a few of the galls in treatment with 54 ppm and many with 324 ppm ammonium were necrotic; these were first observed on the 6th-day following inoculation. There were no significant differences, however, among treatments with deficient, 54 ppm ammonium, high nitrate and the control.

In Fig. 10 dry weights are compared. Although there were no differences between high ammonium and the control, the dry weights in treatment with deficient ammonium were heavier than those in treatments with high ammonium. The dry weight in treatment with high nitrate was not significantly different from those in all treatments and the control.

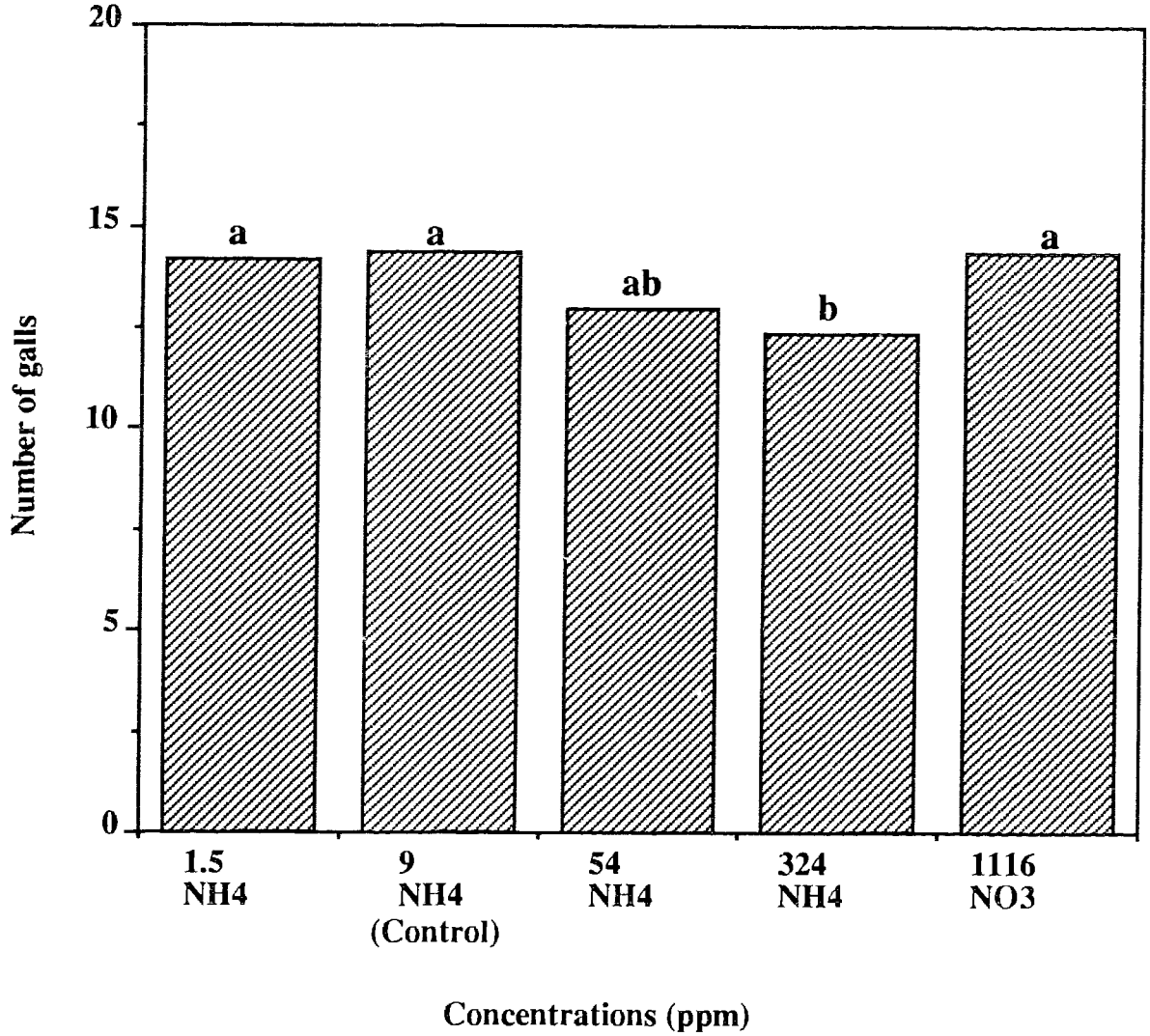

Figure 9. Numbers of galls induced by *Meloidogyne incognita*, one week after inoculation with egg masses, on excised tomato roots grown on STW medium containing different ammonium or high nitrate concentration(s). Bars with the same letter are not significantly different ($P = 0.05$).

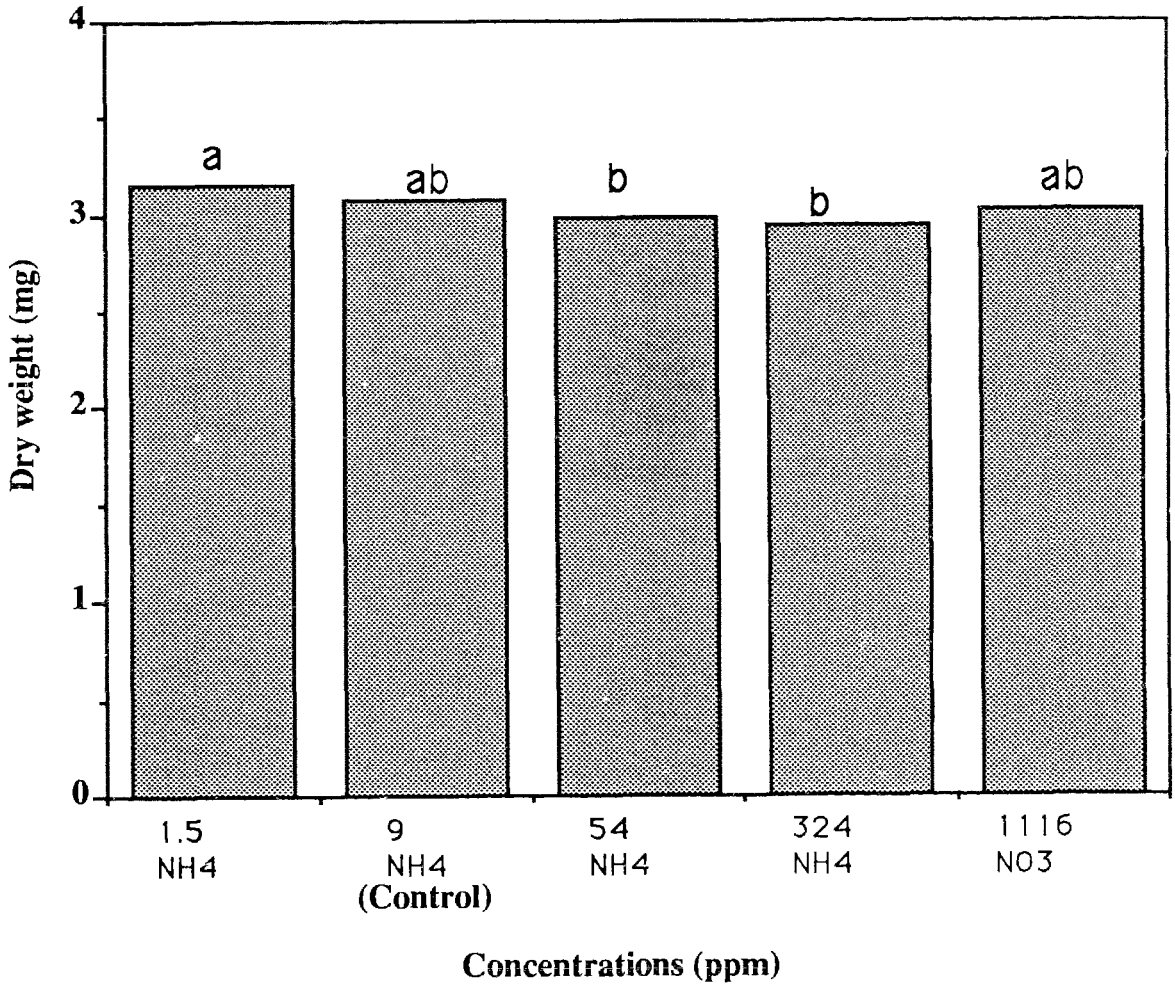

Figure 10. Dry weights of *Meloidogyne incognita* infected excised tomato roots, one week after inoculation with egg masses, grown on STW medium containing different ammonium or high nitrate concentration(s). Bars with the same letter are not significantly different ($P = 0.05$).

The percentages of juveniles that developed further, *two weeks after inoculation*, are summarized in Fig. 11. Here, the stages of development are emphasized in the 3rd- and 4th-stages. There were significantly fewer nematodes that attained these stages in treatments with high ammonium. Compared with the percentage in the control, there were reductions of the 3rd- and 4th-stages of at least 30% and 75% in treatments with 54 ppm and 324 ppm ammonium, respectively. Between high ammonium concentrations, significantly fewer developed juveniles as concentrations increased. The percentages of the 3rd- and 4th-stages in treatments with deficient ammonium and high nitrate were not significantly different from those in the control.

Ammonium concentrations significantly affected the number of galls. As shown in Fig. 12, there were significantly fewer galls in treatments with 54 ppm and 324 ppm ammonium compared with that in the control. In addition, the number of necrotic galls increased in high ammonium levels. There were no differences in the number of galls among treatments with deficient ammonium, high nitrate and the control.

Ammonium concentrations significantly affected the dry weights of inoculated roots. Fig. 13 summarizes the comparisons of the dry weights. The dry weights in treatments with 54 ppm and 324 ppm were lower than those in the control. The dry weights in treatments with deficient

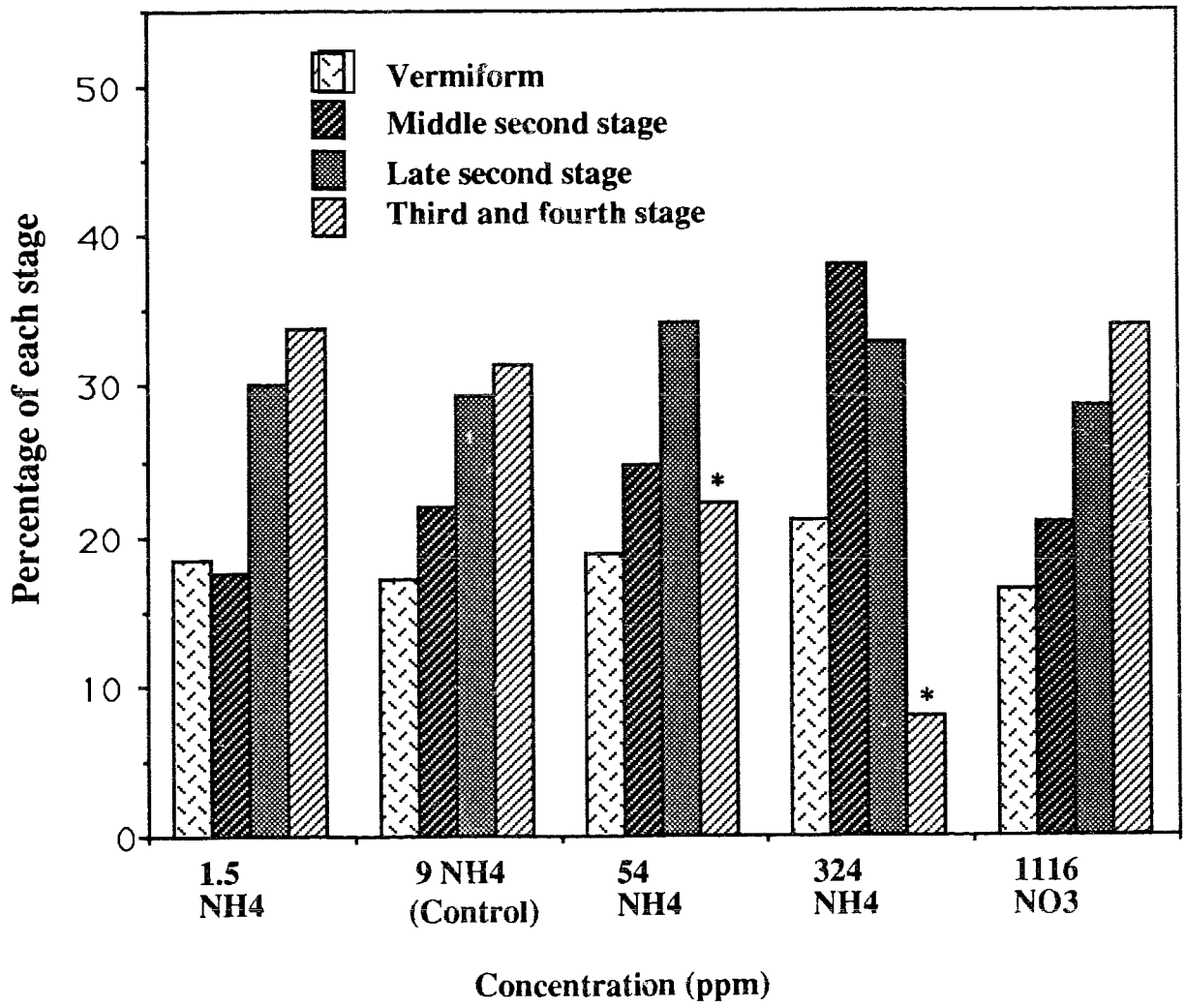

Figure 11. Percentage of stages of development two weeks after inoculation of *Meloidogyne incognita* with egg masses into excised tomato roots grown on STW medium containing different ammonium or high nitrate concentration(s). Bars with * are significantly different from control ($P = 0.05$)

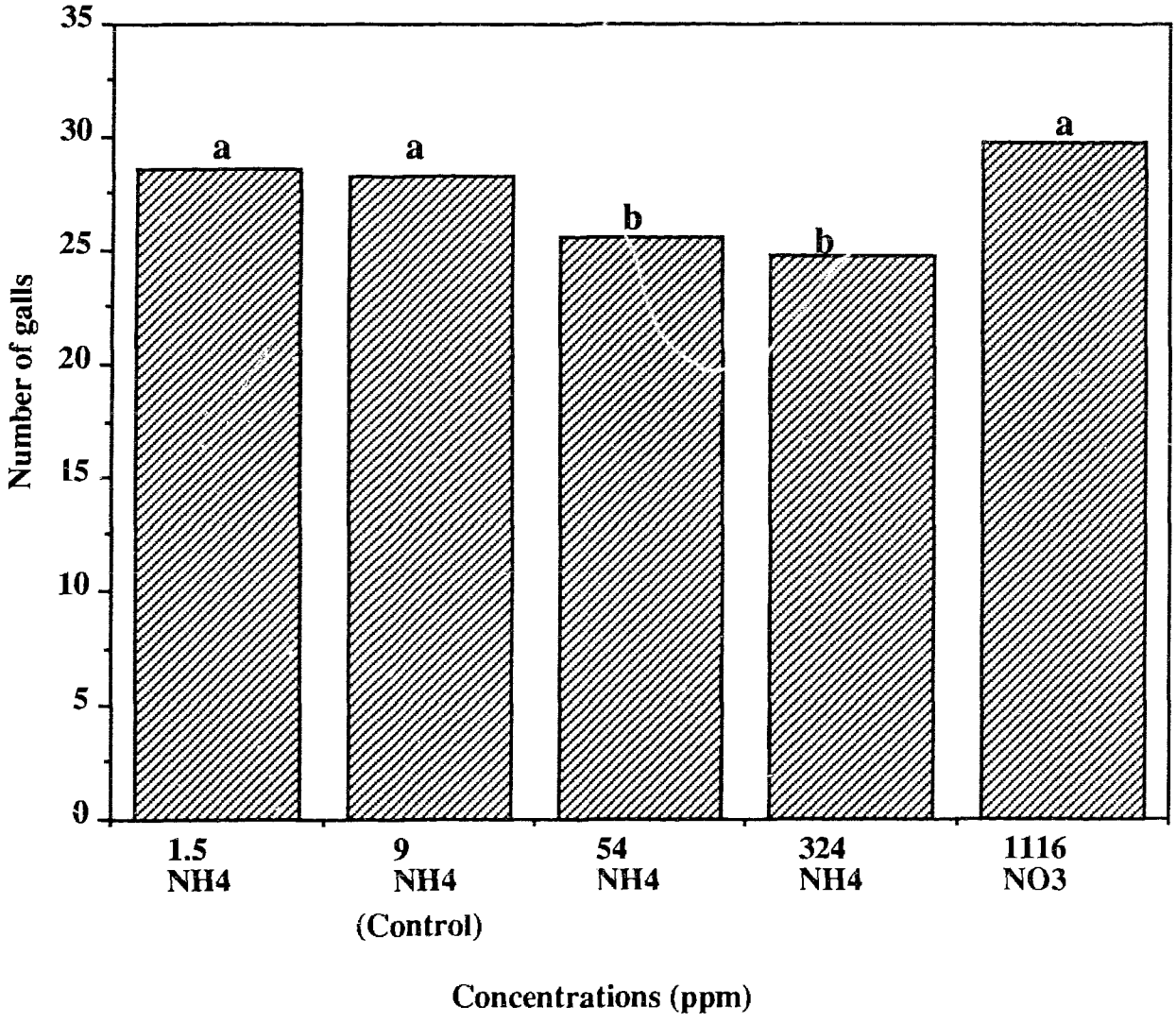

Figure 12. Numbers of galls induced by *Meloidogyne incognita*, two weeks after inoculation with egg masses, on excised tomato roots grown on STW medium containing different ammonium or high nitrate concentration(s). Bars with the same letter are not significantly different (P = 0.05).

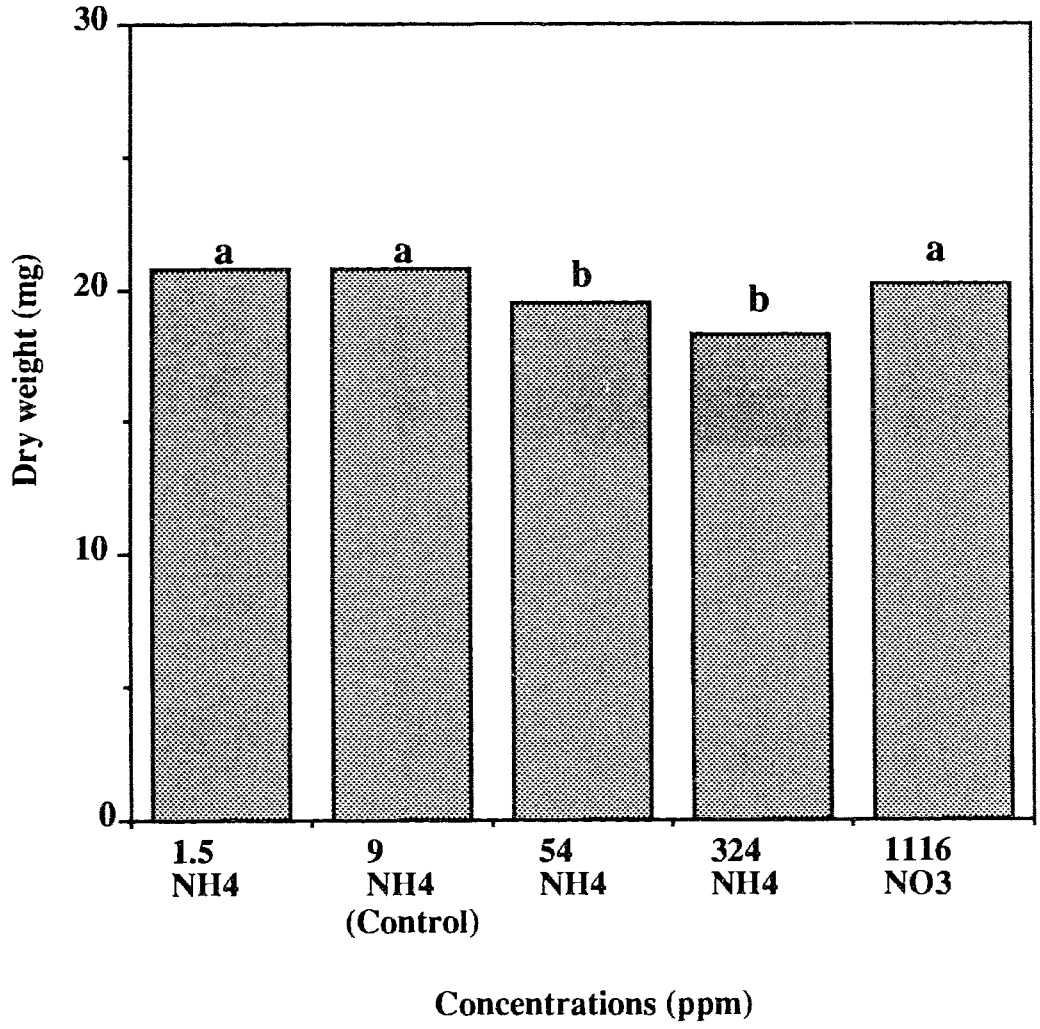

Figure 13. Dry weights of *Meloidogyne incognita* infected excised tomato roots, two weeks after inoculation with egg masses, grown on STW medium containing different ammonium or high nitrate concentration(s). Bars with the same letter are not significantly different ($P = 0.05$).

ammonium and high nitrate were not significantly different from those in the control.

Further nematode development was severely inhibited by increasing ammonium concentrations *three weeks after inoculation*. Fig. 14 summarizes the comparisons of the stages of development. There were significantly more nematodes in the vermiform, middle and late second stages and fewer in the 3rd- and 4th-stages in treatments with 54 ppm and 324 ppm than there were in the control. The percentages of the first three stages and the later two stages in treatment with 324 ppm were significantly higher and lower, respectively, than they were in 54 ppm. There were no significant differences among treatments with deficient ammonium, high nitrate and the control.

In development into adults, high ammonium concentrations significantly inhibited development into females but enhanced development into males. The percentages of adult females in treatments with 54 ppm and 324 ppm ammonium were significantly lower than those in the control. There were no significant differences between treatments with 54 ppm and 324 ppm, or among treatments with deficient ammonium, high nitrate or the control. Adult males comprised less than 15% of the adult population in the control, but they comprised more than 60% and 80% in treatments with 54 ppm and 324 ppm ammonium, respectively. The population of adult males in treatments with deficient ammonium and high

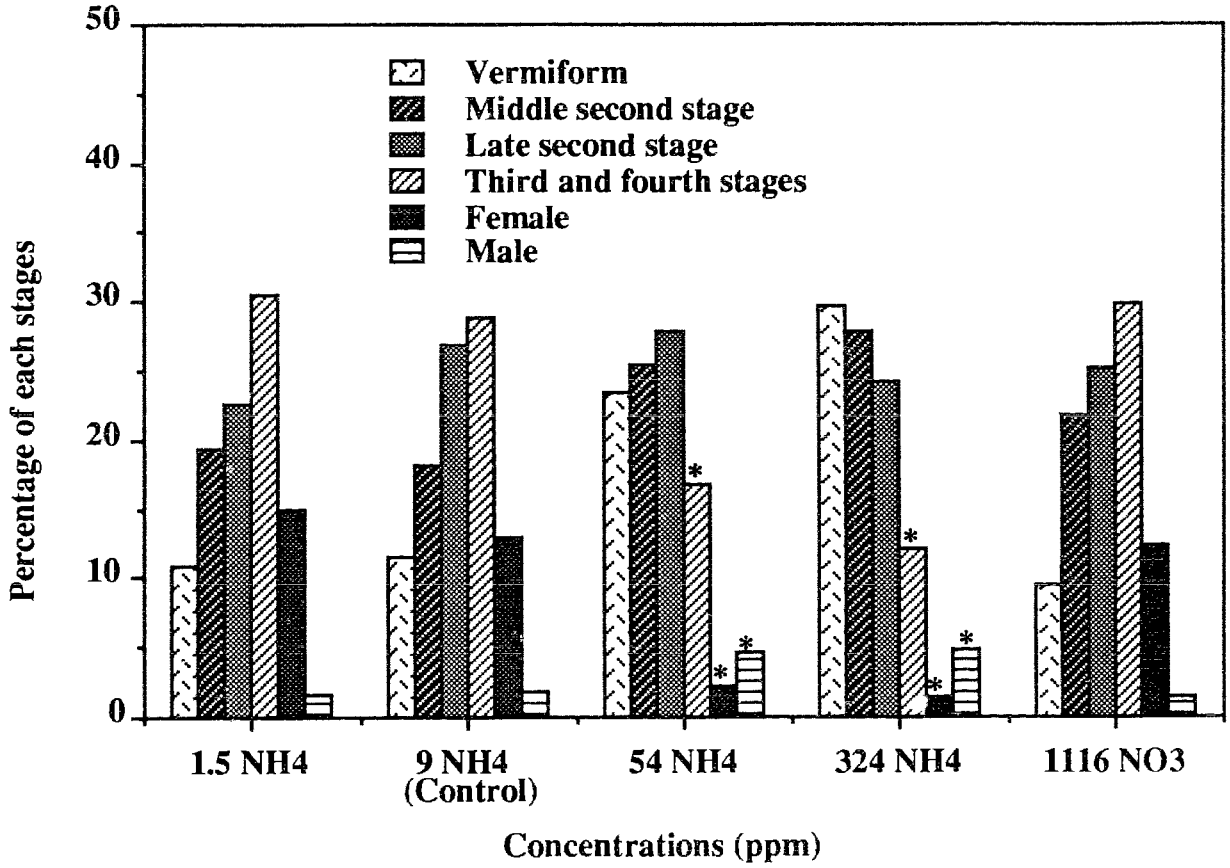

Figure 14. Percentage of stages of development three weeks after inoculation of *Meloidogyne incognita* egg masses into excised tomato roots grown on STW medium containing different ammonium or high nitrate concentration(s). Bars with * are significantly different from control ($P = 0.05$).

nitrate were not significantly different from that in the control.

The suppression of nematode development can also be observed in the numbers of galls. High ammonium concentrations significantly reduced the number of galls. As shown in Fig. 15, there were fewer galls at the higher concentrations of ammonium, but there were no significant differences among treatments with deficient ammonium or high nitrate and the control.

The effects of different ammonium concentrations on the numbers of galls paralleled the effect on dry weights of inoculated roots. As shown in Fig. 16, the roots in the higher ammonium treatments were significantly less than in the control. However, there were no significant differences among treatments with deficient ammonium, high nitrate or the control.

Four weeks after inoculation, the inhibition of nematode development was even more pronounced. Fig. 17 summarizes the comparisons of the stages of development. Most of the nematodes in treatments with high ammonium were inhibited from developing beyond the 2nd-stage. There were no significant differences in the proportion of the 3rd- and 4th-stages. High ammonium concentrations, however, significantly suppressed development into adult females. Compared with the proportion of adult females in the control, there were reductions of at least 85% and 95% in

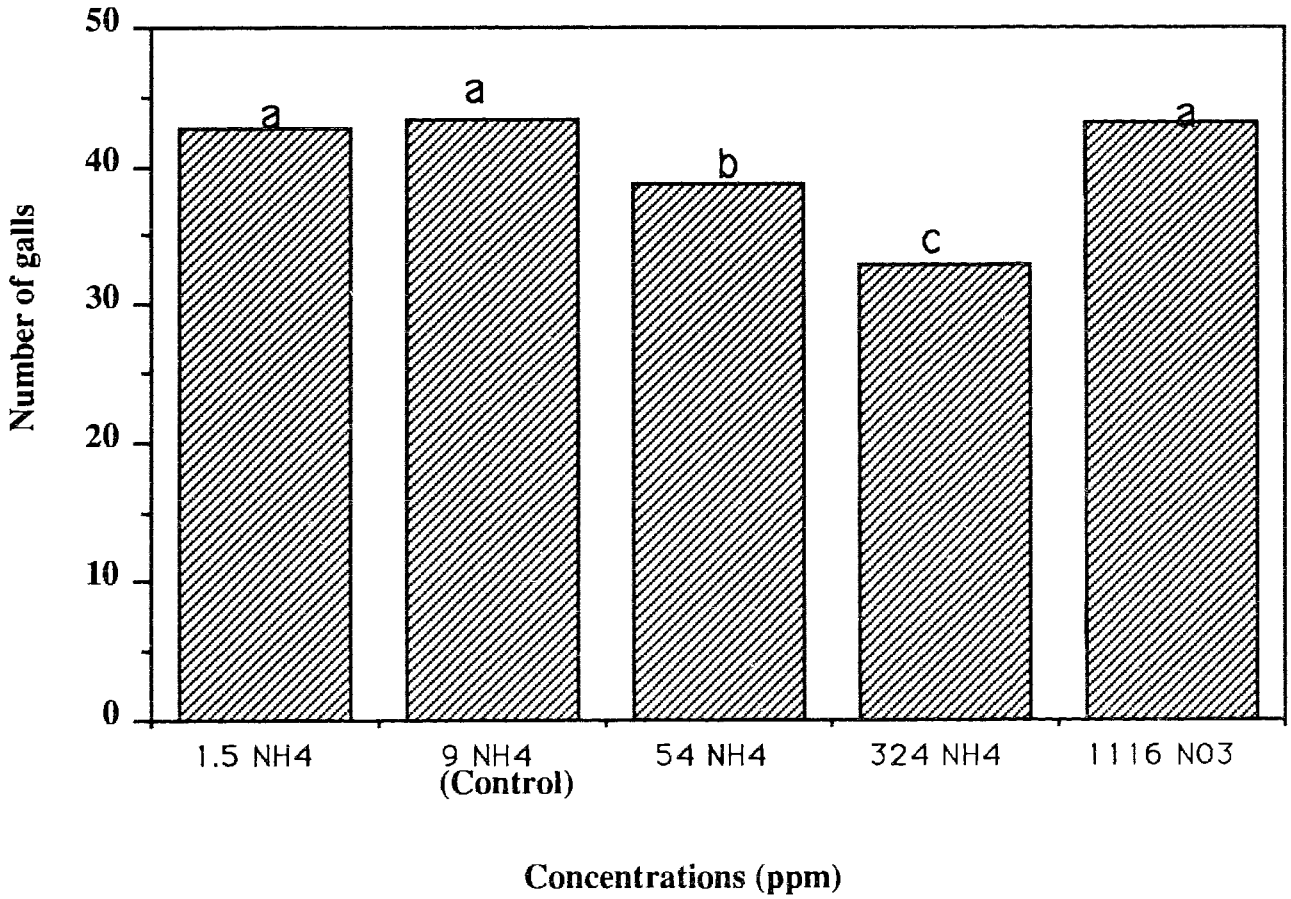

Figure 15. Number of galls induced by *Meloidogyne incognita*, three weeks after inoculation with egg masses, on excised tomato roots grown on STW medium containing different ammonium or high nitrate concentration(s). Bars with the same letter are not significantly different (P = 0.05).

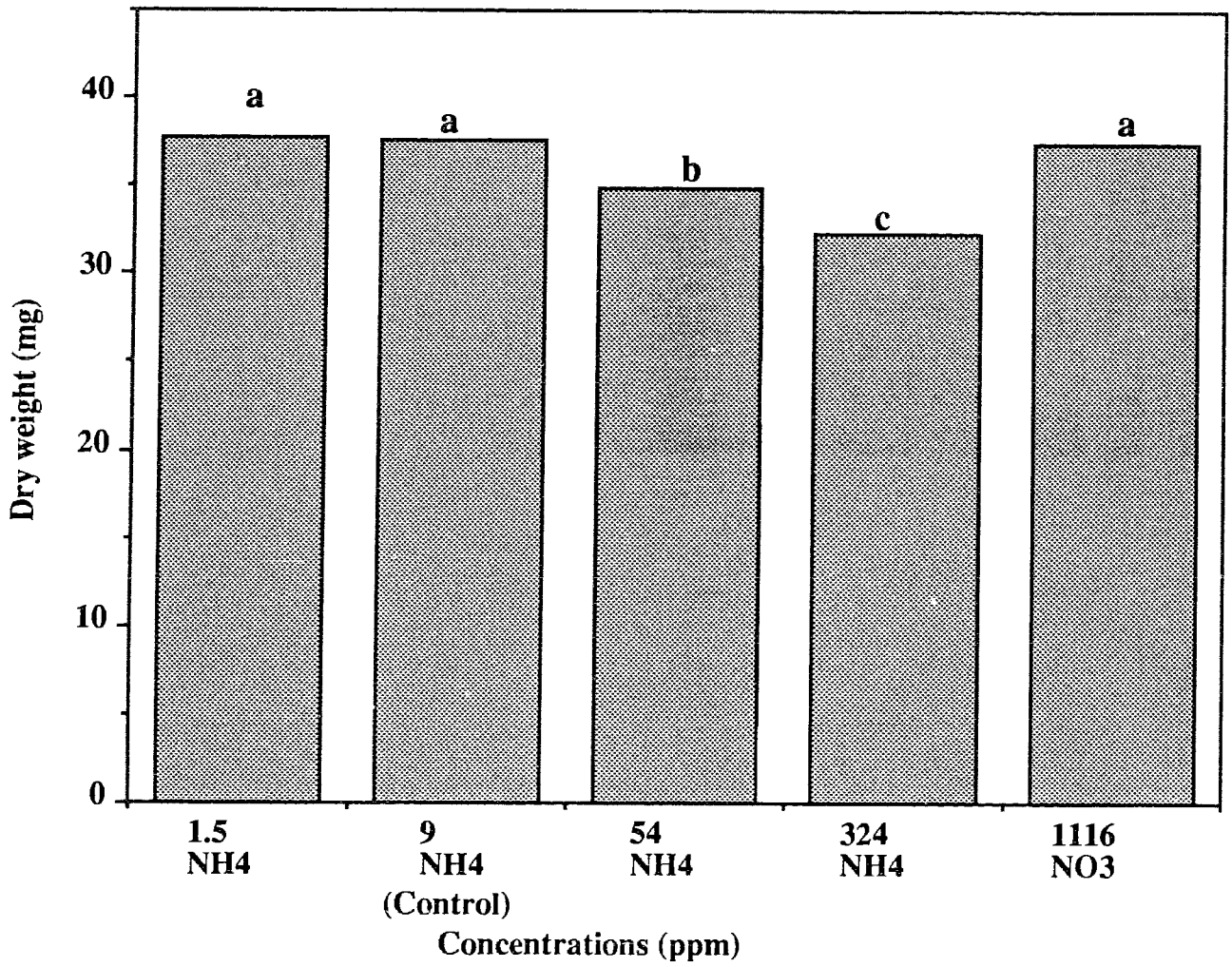

Figure 16. Dry weights of infected excised tomato roots, three weeks after inoculation with *Meloidogyne incognita* egg masses, grown on STW medium containing different ammonium or high nitrate concentration(s). Bars with the same letter are not significantly different (P = 0.05).

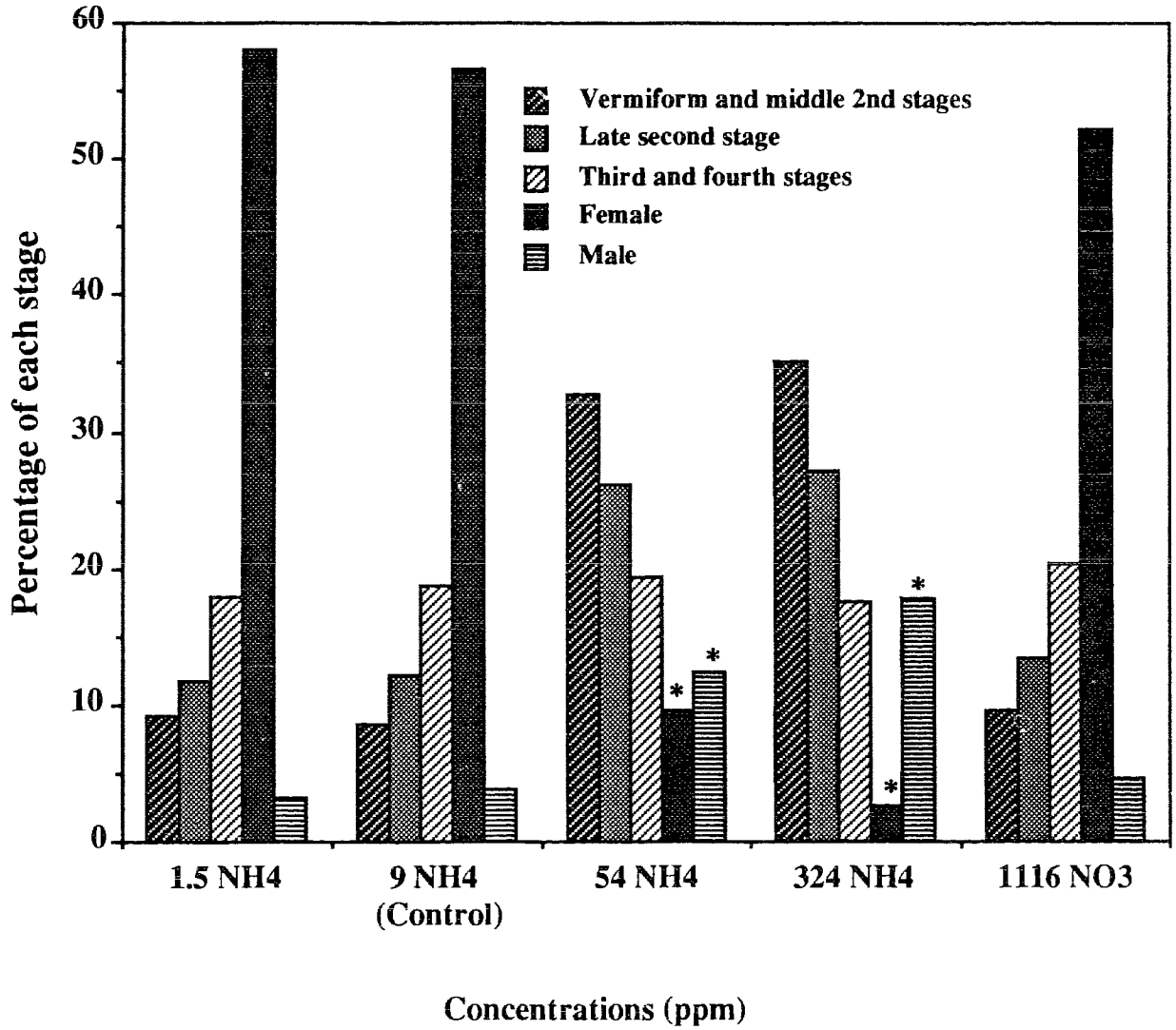

Figure 17. Percentage of stages of development four weeks after inoculation with *Meloidogyne incognita* egg masses into excised tomato roots grown on STW medium containing different ammonium or high nitrate concentration(s). Bars with * are significantly different from control (P = 0.05).

treatments with 54 ppm and 324 ppm, respectively. The proportion of adult females in the treatment with 54 ppm was significantly greater than with 324 ppm. The adult females with deficient ammonium or high nitrate showed no significant differences from the control.

In contrast to the effect on adult females, high ammonium enhanced the development of adult males. Adult males comprised less than 7% of the adult population in the control, but they comprised more than 50% and 75% in treatments with 54 ppm and 324 ppm, respectively. The percentage of males in the last two treatments were significantly different. For the same stage of development, there were no significant differences among treatments with deficient ammonium, high nitrate and the control.

High ammonium concentrations reduced the numbers of galls. As shown in Fig. 18, there were fewer galls in the high ammonium treatments compared with the control, and the numbers of galls in treatment with 324 ppm was significantly lower than with 54 ppm. There were no significant differences observed among treatments with deficient ammonium, high nitrate and the control.

As a result of the reduction in the number of galls, the dry weights of inoculated roots also were significantly lower at high ammonium (Fig. 19). Between high ammonium concentrations, the dry weights of the roots decreased significantly as the concentration increased. Although the dry weight in the treatment with deficient ammonium was

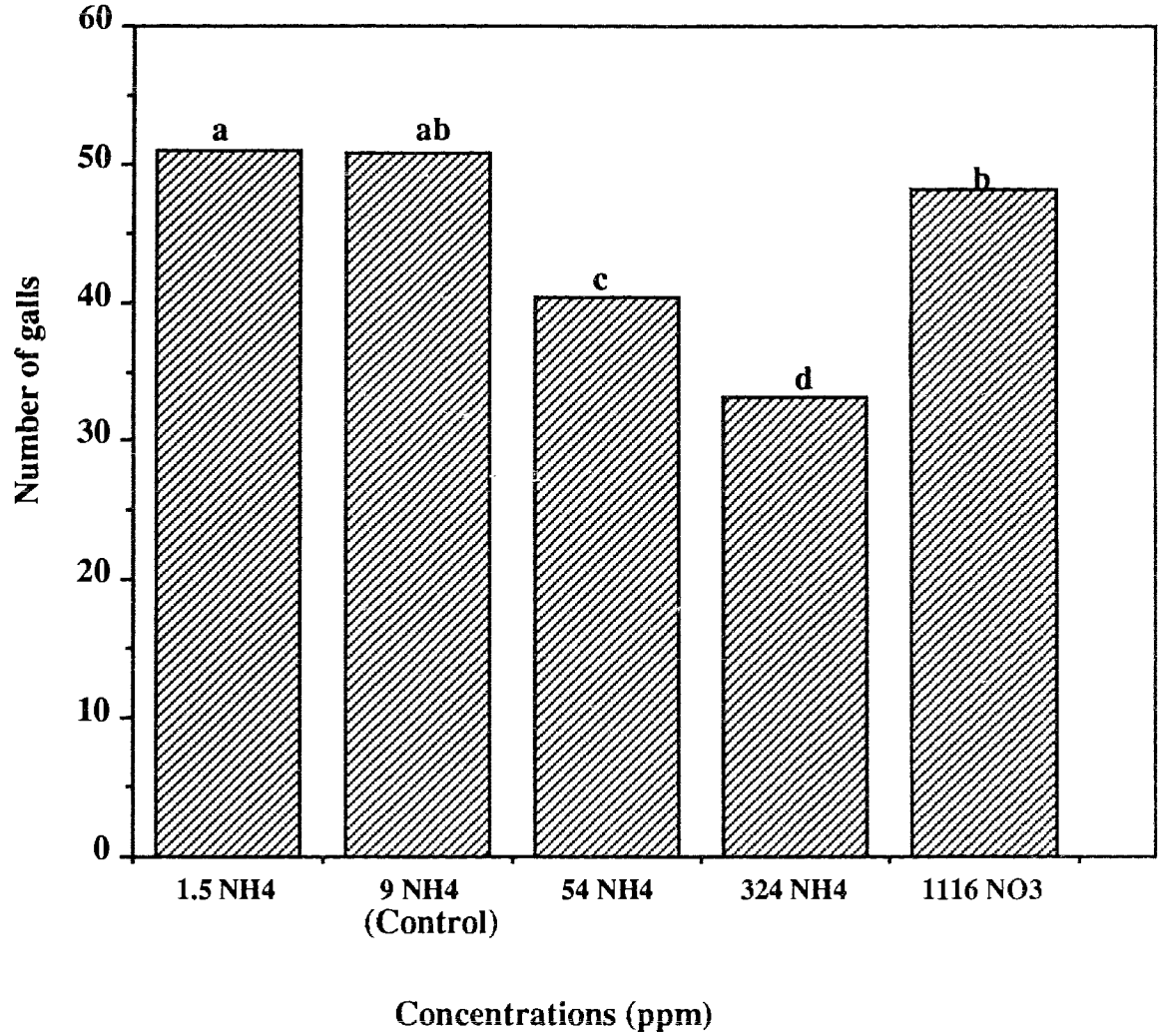

Figure 18. Numbers of galls induced by *Meloidogyne incognita*, four weeks after inoculation with egg masses, on excised tomato roots on STW medium containing different ammonium or high nitrate concentration(s). Bars with the same letter are not significantly different (P = 0.05).

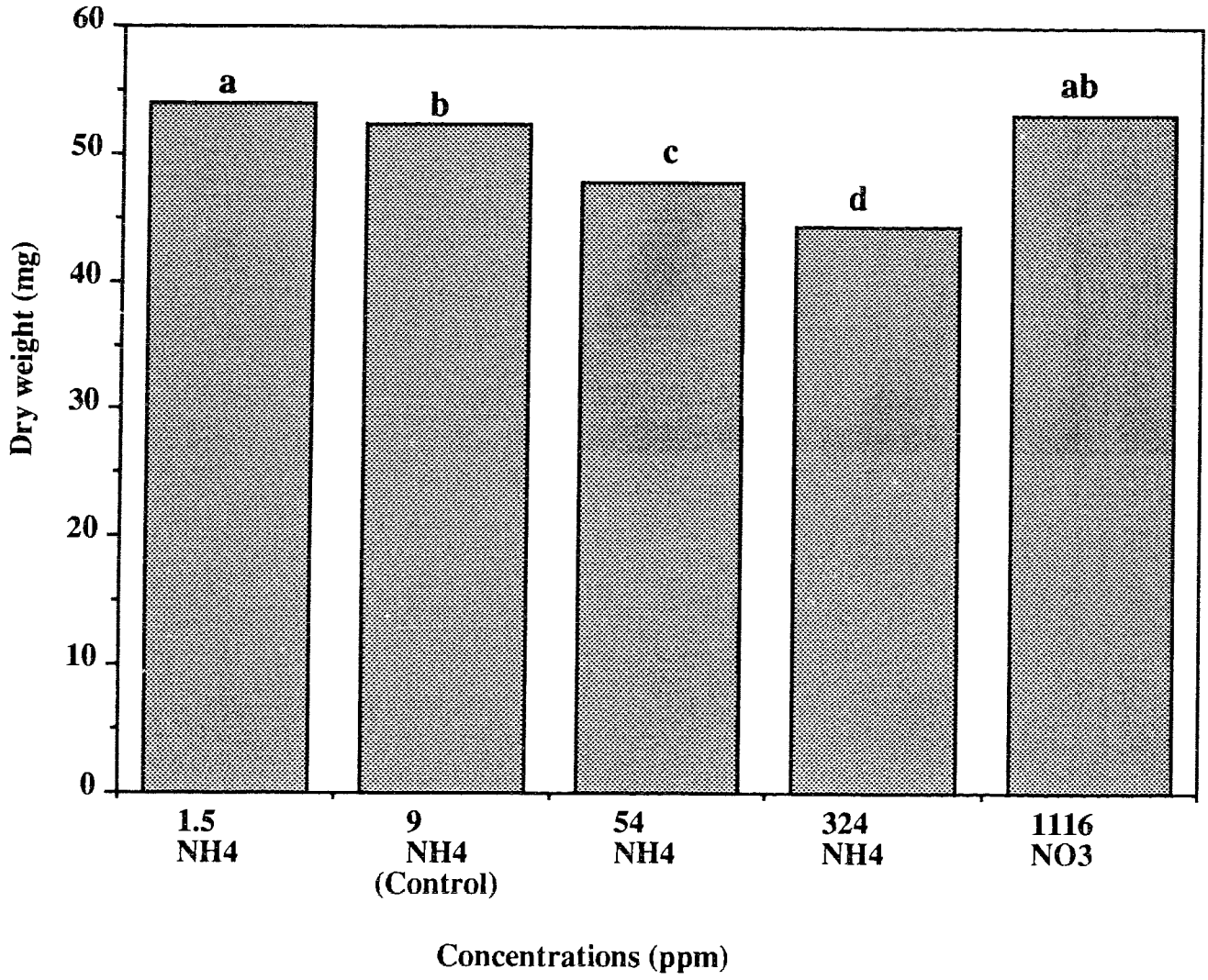

Figure 19. Dry weights of infected excised tomato roots, four weeks after inoculation with *Meloidogyne incognita* egg masses, grown on STW medium containing different ammonium or high nitrate concentration(s). Bars with the same letter are not significantly different ($P = 0.05$).

significantly greater than in the control, that with high nitrate was not significantly different.

5. Development with increasing ammonium

Experiment

This experiment was designed to examine the effect on nematode development of increasing ammonium concentrations after nematodes infected the roots. The experiment used 240 Petri dishes each containing an excised root and STW medium with deficient ammonium concentration (1.5 ppm NH_4^+). Three day-old seedlings were excised and the roots were aseptically transferred into Petri dishes containing STW medium with 1.5 ppm ammonium and with 2% agar. On the medium with the higher agar concentration, the lateral roots grew on only the surface of the medium so that the excised roots could easily be removed without damage. Three days after excision, each of 160 excised roots was inoculated with a single egg mass; the remaining 80 dishes with roots were not inoculated. The experimental dishes were sealed and incubated as described.

One week after inoculation, ten infected roots were carefully removed from the dishes (leaving 150 with nematodes). The numbers of galls, fresh weight and nematode development were recorded from five infected roots. The other five infected roots were dried at 70°C after the number of galls had been recorded. On the same day, five uninoculated roots were also carefully removed from the

dishes (leaving 75 without nematodes) to record the fresh and dry weights. The rest of the roots were aseptically transferred into Petri dishes containing standard 1.25% agar and STW medium that included ammonium, either deficient, normal, 6X-strength, and 36X-strength, or a high nitrate concentration, which were then labelled as I-I (constant low ammonium = CLA), I-II, I-III, I-IV, and I-V, respectively. Each treatment was replicated five times. The dishes were resealed and incubated as described. On the new growth media, samples of roots were harvested weekly for 3 weeks. The numbers of galls were counted, and from five replicates, the fresh and dry weights of the roots were measured. At the same intervals, the population and the stages of nematode development were assessed after staining the roots and examining under a dissecting microscope.

Results

The stages of development, *one week after inoculation*, are summarized in Fig. 20. The average number of galls induced by nematodes was 14.6/excised root with dry weight of 3.06 mg.

The stages of nematode development *One week after increasing ammonium* are summarized in Fig. 21. Increasing the ammonium concentrations from deficient after infection significantly inhibited nematode development. Compared with treatment I-I (CLA), the percentage of nematodes that developed into the 3rd- and 4th-stages in treatments I-II,

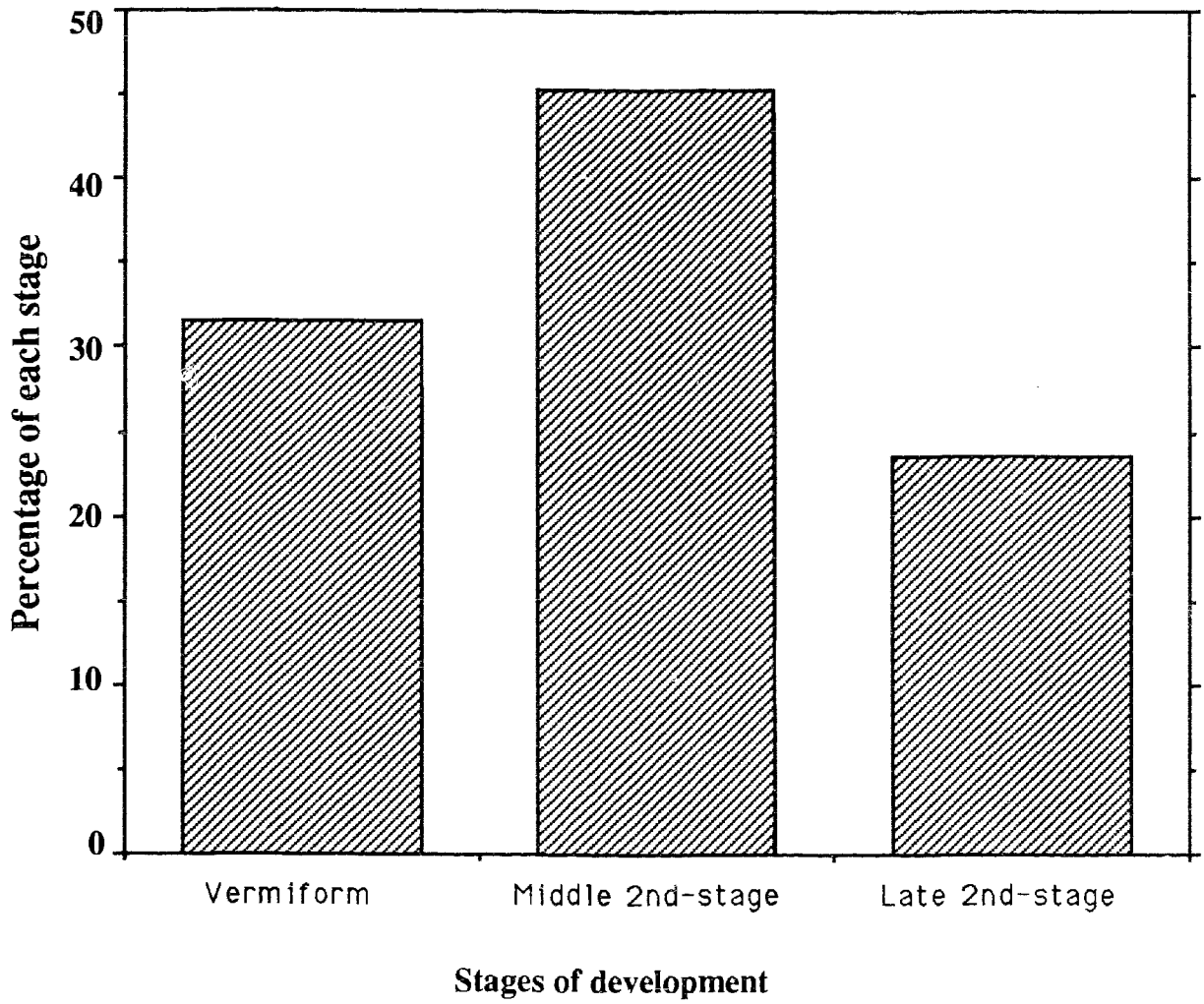

Figure 20. The percentage of stages of development one week after inoculation with a single egg mass of *Meloidogyne incognita* in excised tomato roots grown on STW medium containing 1.5 ppm ammonium.

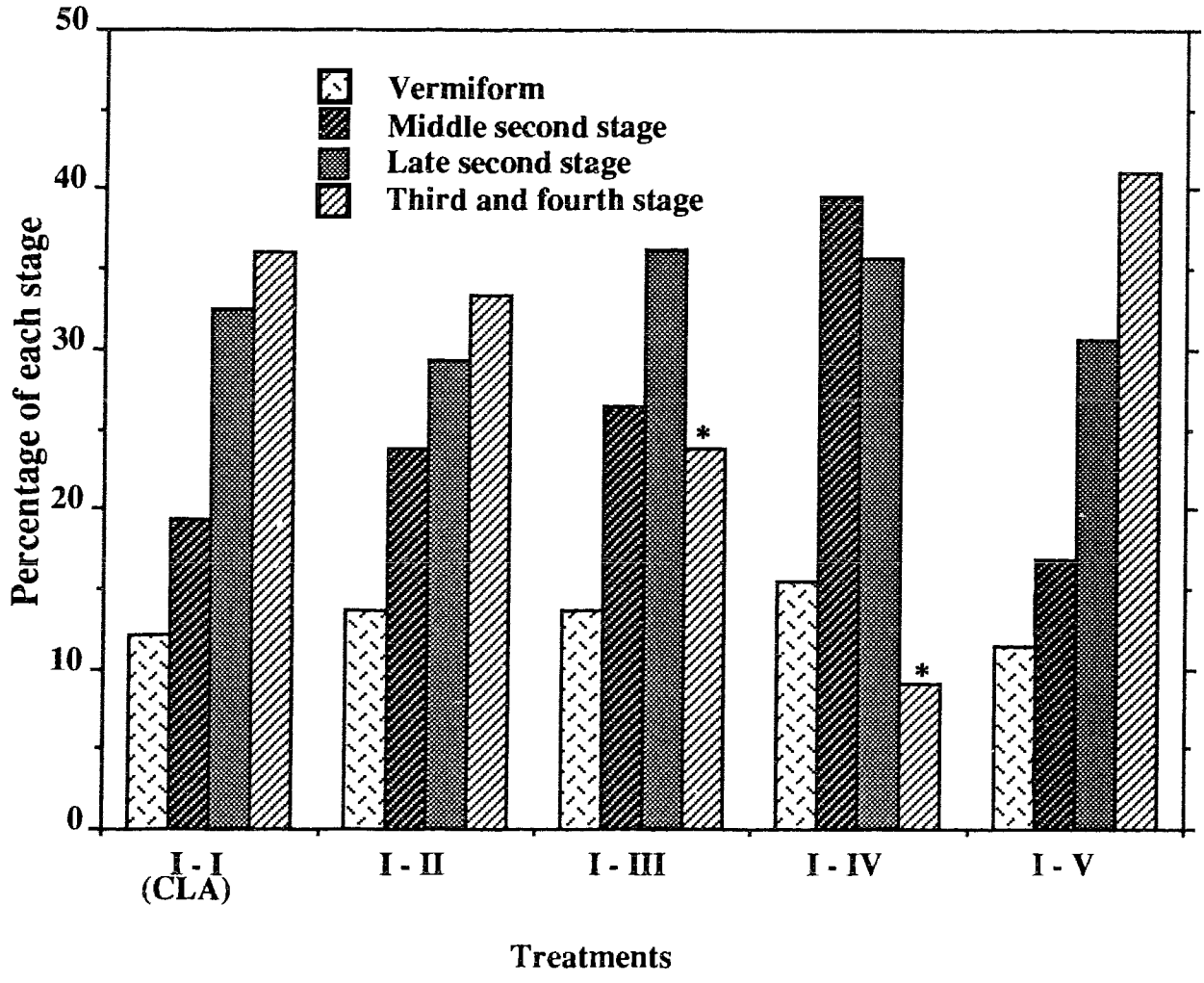

Figure 21. Percentage of stages of development of *Meloidogyne incognita* in excised tomato roots grown on STW medium one week after increasing ammonium or high nitrate concentration(s). Bars with * are significantly different from constant low ammonium (CLA) (P = 0.05). Axis labels I-I to I-V are described on p 61.

I-III, and I-IV, were reduced by about 10%, 30% and 80%, respectively, and these reductions were significantly different from each other. The percentage in treatment I-V was significantly higher than that in treatment I-I (CLA).

The development of the nematodes in treatments I-II, I-III, and I-IV was mostly disrupted at the middle and late 2nd-stages. There were significantly more nematodes in these stages in treatments I-II, I-III, and I-IV than that in treatment I-I (CLA). The percentages of nematodes in these early stages in treatment I-V, however, were significantly lower than those in treatment I-I (CLA), but this is because significantly more had developed to the third stage.

The suppression of nematode development with increases in ammonium concentrations resulted in a reduction in the number of galls and the dry weights of roots. As shown in Fig. 22, the increased ammonium concentrations significantly reduced the numbers of galls. There was about a 10% reduction in treatments I-III and I-IV, compared with treatment I-I (CLA). There were no differences between treatments I-II and I-I (CLA), or between I-II and I-III, between I-III and I-IV, and between I-V and treatment I-I (CLA).

The significant reduction in the number of galls resulted in significant differences in the dry weights of the roots (Fig. 23). Similar to the effects on the numbers of galls, the dry weights in treatments I-III and I-IV were about 10% lower than in treatment I-I (CLA). The dry weight

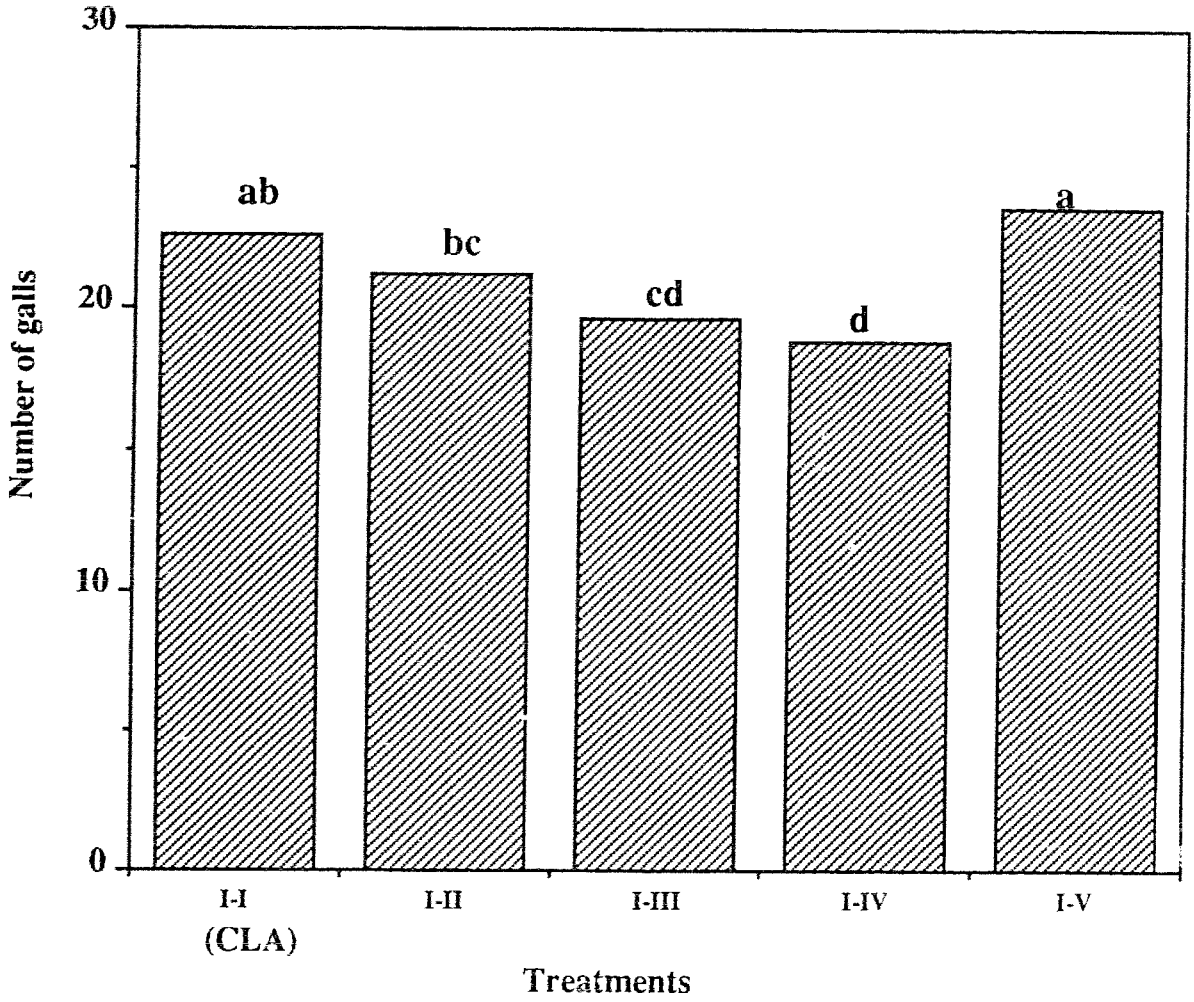

Figure 22. Number of galls induced by *Meloidogyne incognita* on excised tomato roots one week after increasing ammonium or high nitrate concentration(s) on STW medium. Bars with the same letter are not significantly different (P = 0.05). CLA = constant low ammonium. Axis labels I-I to I-V are described on p 61.

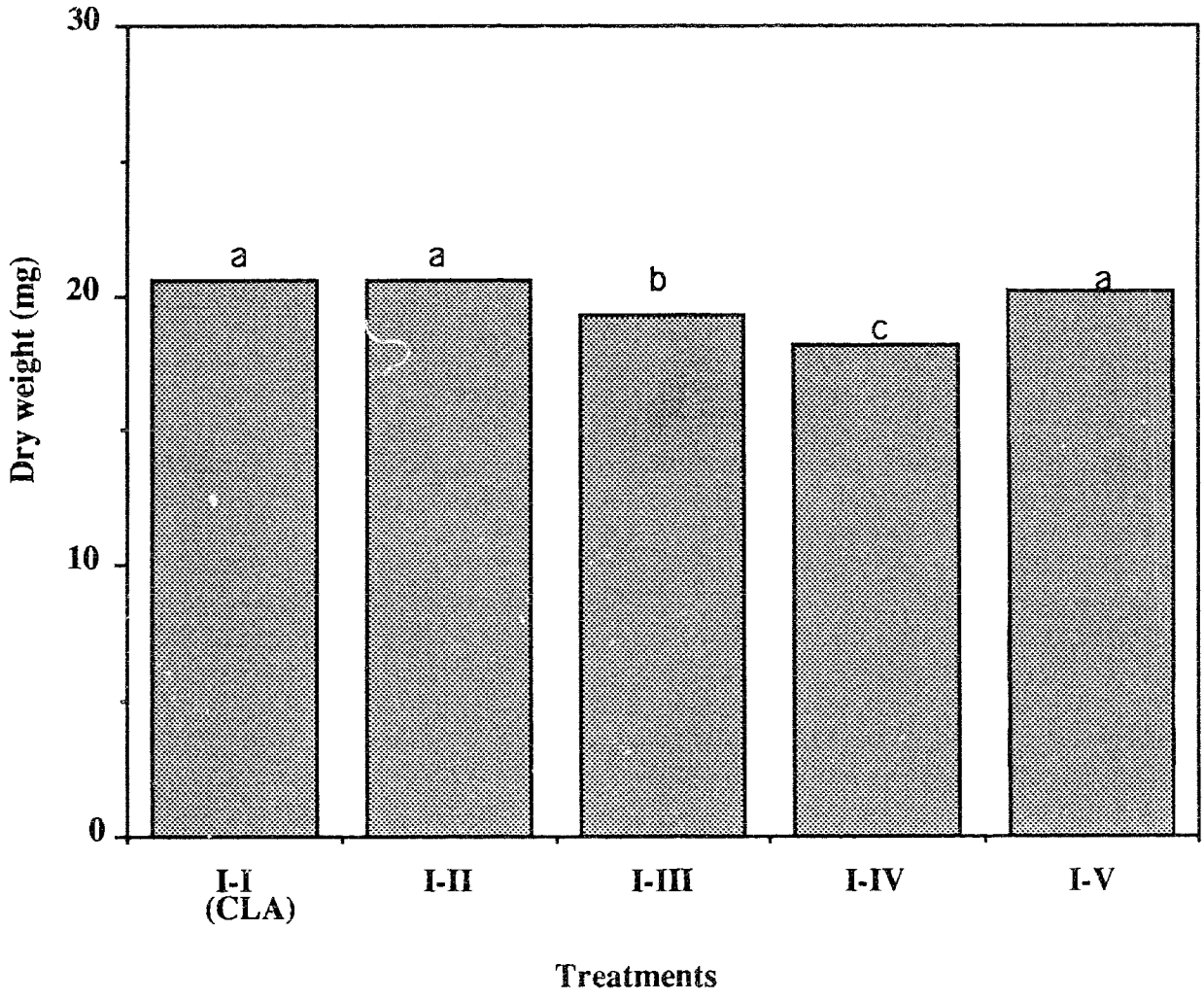

Figure 23. Dry weights of *Meloidogyne incognita* infected excised tomato roots one week after increasing ammonium or high nitrate concentration(s). Bars with the same letter are not significantly different (P = 0.05). CLA = constant low ammonium. Axis labels I-I to I-V are described on p 61.

in I-III was significantly greater than in I-IV. There were no significant differences, however, among treatments I-II, I-V and treatment I-I (CLA).

Two weeks after increasing ammonium, the development of nematodes was severely inhibited. Most of the nematodes in treatments I-III and I-IV were still in the 2nd stage. As shown in Fig. 24, the percentages of the 3rd- and 4th-stages in treatments I-III and I-IV were about 30% and 50%, respectively, lower than that in treatment I-I (CLA). The percentage in treatment I-IV was about 25% lower than that in I-III. The percentage in treatment I-V was not significantly different from that in treatment I-I (CLA).

The suppression of development became more pronounced as fewer nematodes developed into adult females in treatments with increased ammonium. The percentages in treatments I-III and I-IV were at least 75% and 80%, lower, respectively, than in treatment I-I (CLA). Adult females in treatments I-II or I-V were not significantly different from that in treatment I-I (CLA). In contrast, increasing ammonium concentrations after infection favored the development of adult males. Although adult males comprised less than 10% of the adult population in treatment I-I (CLA), I-II or I-V, they comprised more than 65% in treatments I-III or I-IV. The percentage of males in treatment I-III or I-IV was significantly higher than in treatment I-I (CLA).

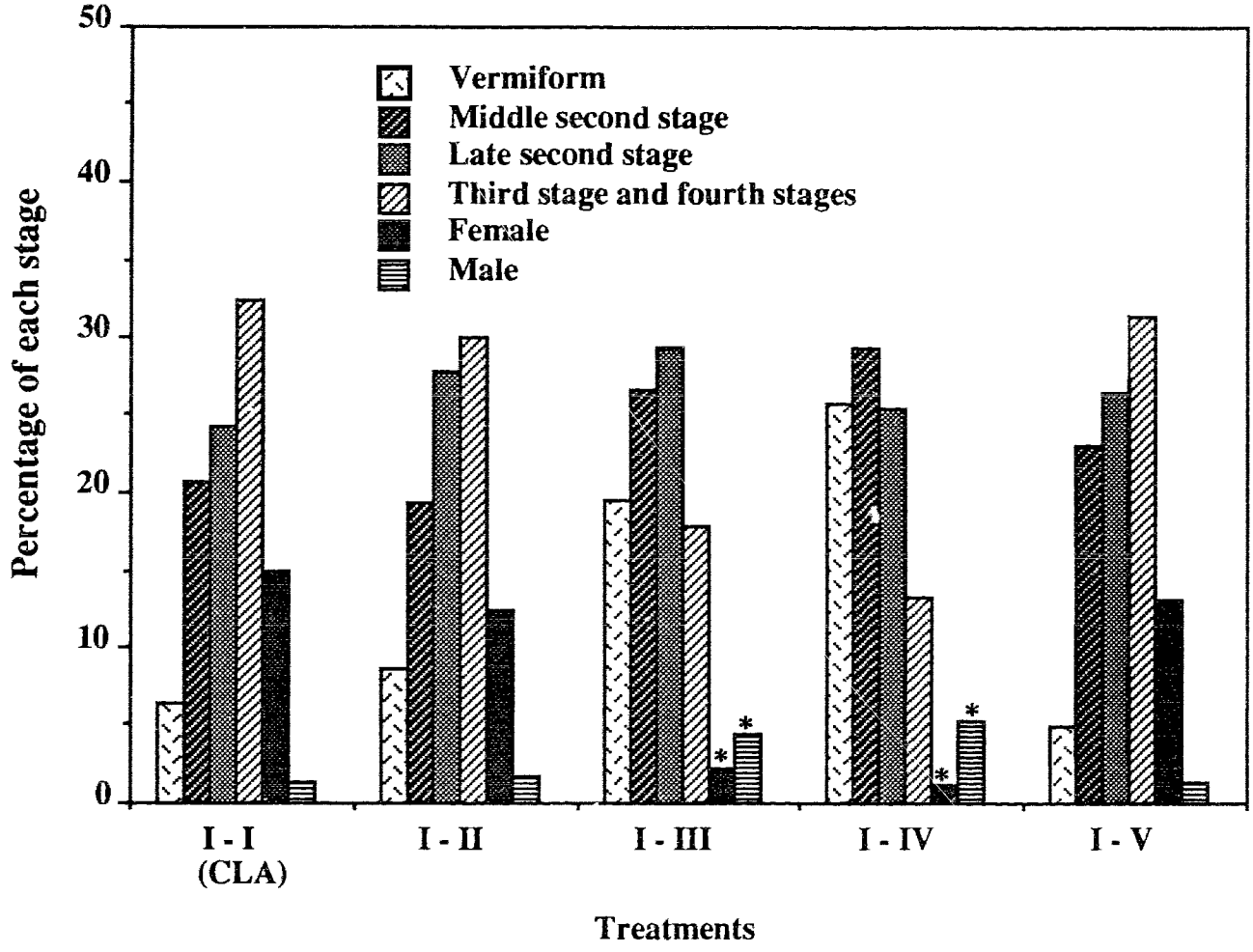

Figure 24. Percentage of stage of development of *Meloidogyne incognita* in excised tomato roots in STW medium two weeks after increasing ammonium or high nitrate concentration(s). Bars with * are significantly different from constant low ammonium (CLA) (P = 0.05).Axis labels I-I to I-V are described on p 61.

Fig. 25 shows that in treatments I-III and I-IV, there were at least 15% and 25% fewer galls, respectively, than in treatment I-I (CLA). No significant differences were observed between treatments I-V and I-I (CLA).

The significant differences in the number of galls accounted for the differences in the dry weights of roots (Fig. 26). Compared with roots in treatment I-I (CLA), treatments I-III and I-IV were lower in weight by about 5% and 15%, respectively. Although dry weights in treatments I-II, I-V, or treatment I-I (CLA) were slightly different, these differences were not significant.

Three weeks after increasing ammonium, nematode development was severely inhibited at the higher concentrations, treatments I-III or I-IV where most of the nematodes did not develop further than 2nd stage (Fig. 27). There were no significant differences in the 3rd- and 4th-stage, but compared to those in treatment I-I (CLA), there were significant reductions in the percentage of adult females by at least 85% and 95% in treatments I-III and I-IV, respectively. The proportions of adult females in treatments I-II or I-V were not significantly different from those in treatment I-I (CLA). The development of adult males increased with increasing ammonium concentration. While the adult males were less than 5% of the adult population in treatment I-I (CLA), they were more than 50% and 80% in treatments I-III and I-IV, respectively. The percentages of

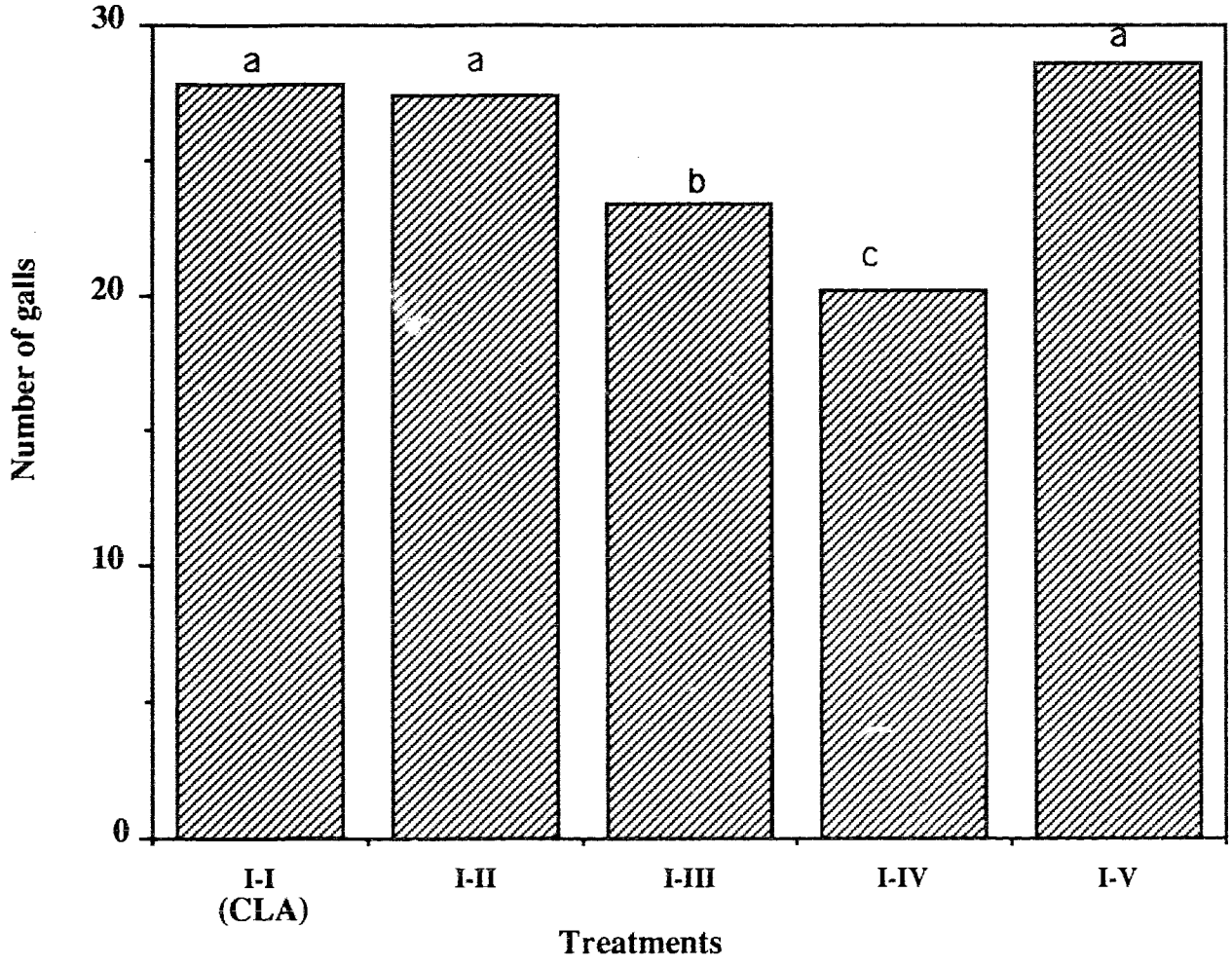

Figure 25. Numbers of galls induced by *Meloidogyne incognita* on excised tomato roots two weeks after increasing ammonium or high nitrate concentration(s) on STW medium. Bars with the same letter are not significantly different ($P = 0.05$). CLA = constant low ammonium. Axis labels I-I to I-V are described on p 61.

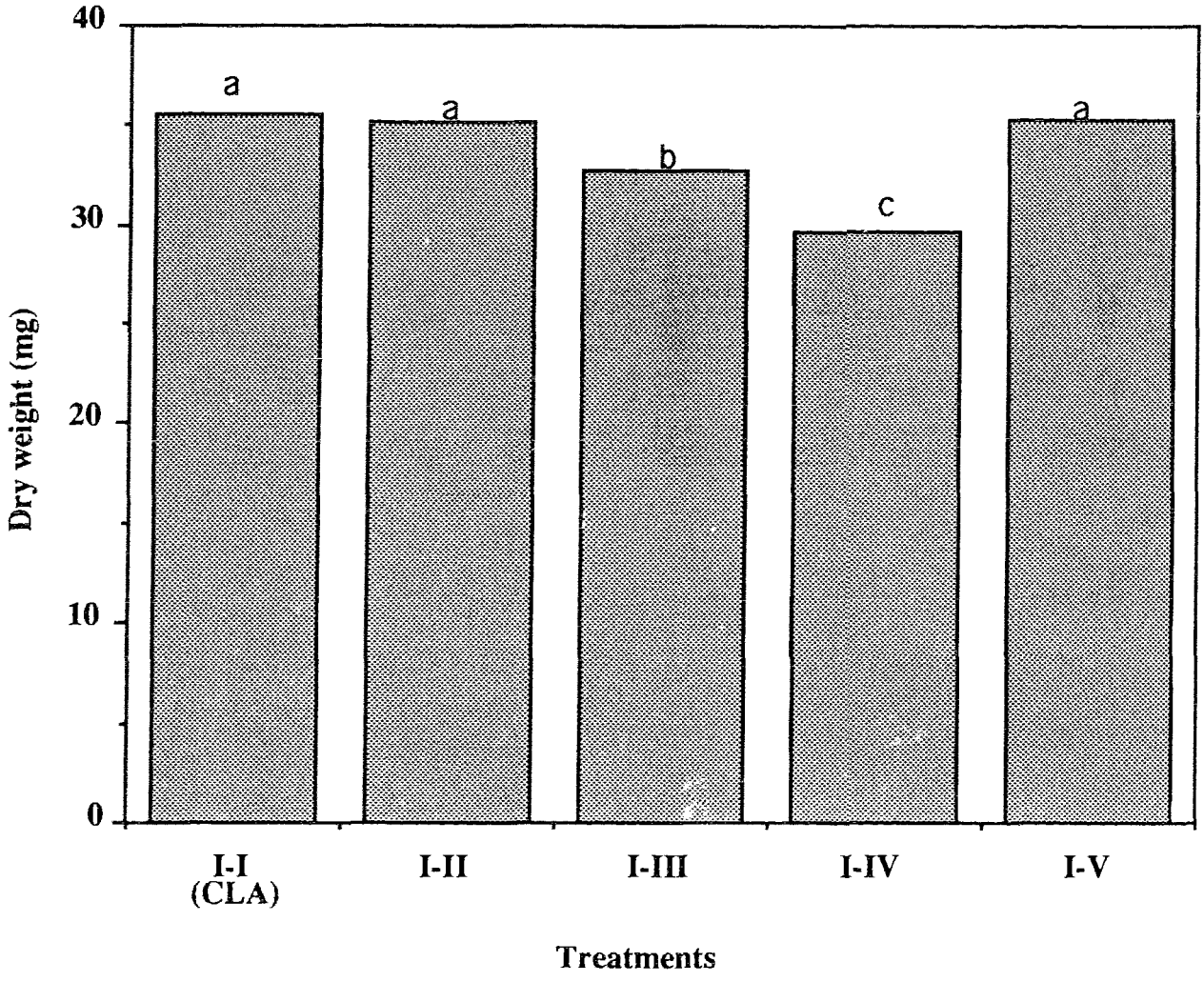

Figure 26. Dry weights of *Meloidogyne incognita* infected excised tomato roots two weeks after increasing ammonium or high nitrate concentration(s). Bars with the same letter are not significantly different (P = 0.05). CLA = constant low ammonium. Axis labels I-I to I-V are described on p 61.

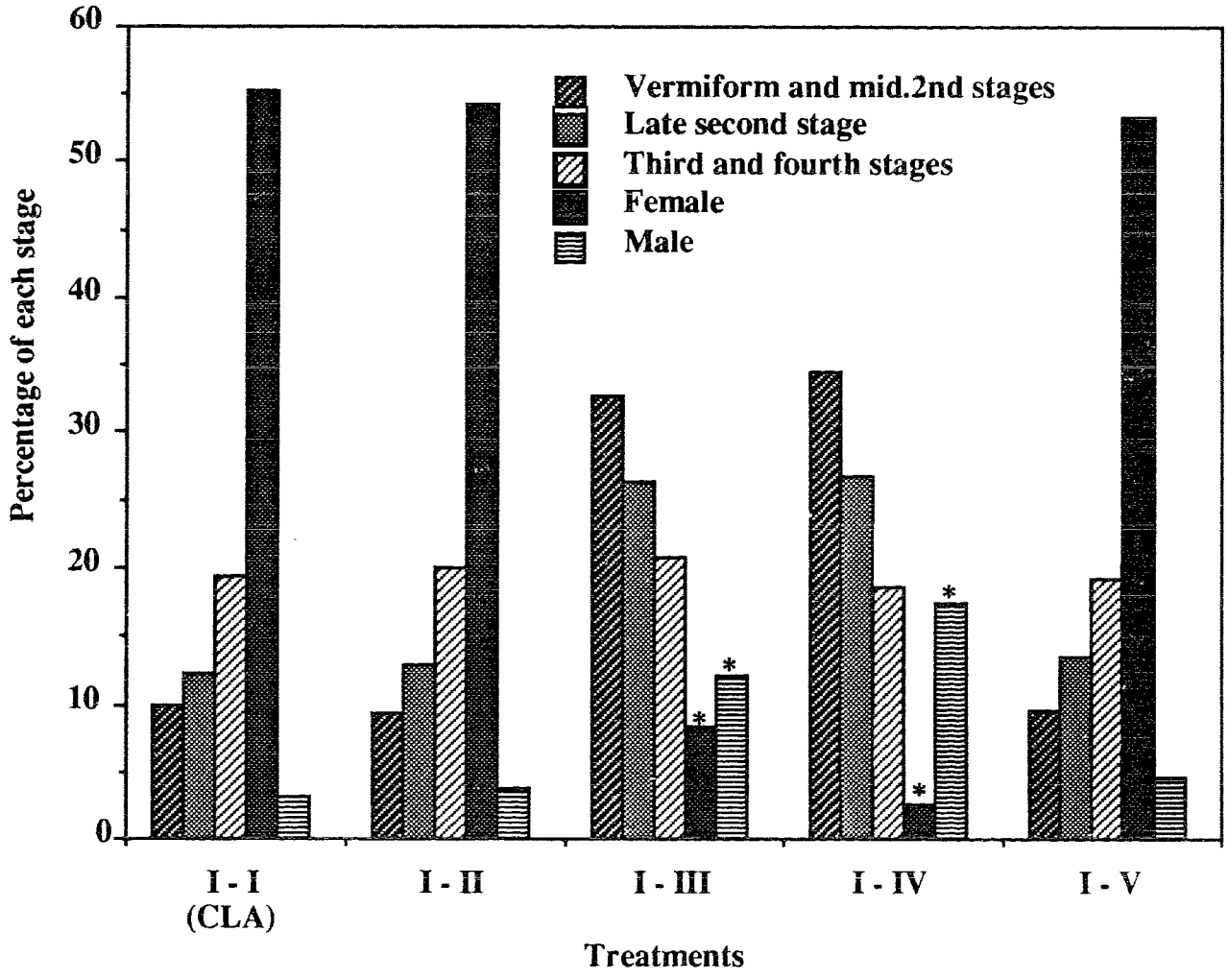

Figure 27. Percentage of stage of development of *Meloidogyne incognita* in excised tomato roots three weeks after increasing ammonium or high nitrate concentration(s). Bars with * are significantly different from constant low ammonium (CLA) (P = 0.05).Axis labels I-I to I-V are described on p 61.

adult males in treatments I-II or I-V were not significantly different from those in treatment I-I (CLA).

Significant differences were observed in the number of galls. Fig. 28 shows that in treatments I-III and I-IV, there were about 15% and 25%, respectively, fewer galls than those in treatment I-I (CLA). No significant differences were observed between treatments I-II and I-V and treatment I-I (CLA).

As a consequence of the decreased numbers of galls, the roots were significant lower in dry weight. As presented in Fig. 29, compared with the dry weights in treatment I-I (CLA), treatments I-III and I-IV were about 8% and 15% lower, respectively. The dry weights in I-II and I-V were not significantly different from those in treatment I-I (CLA).

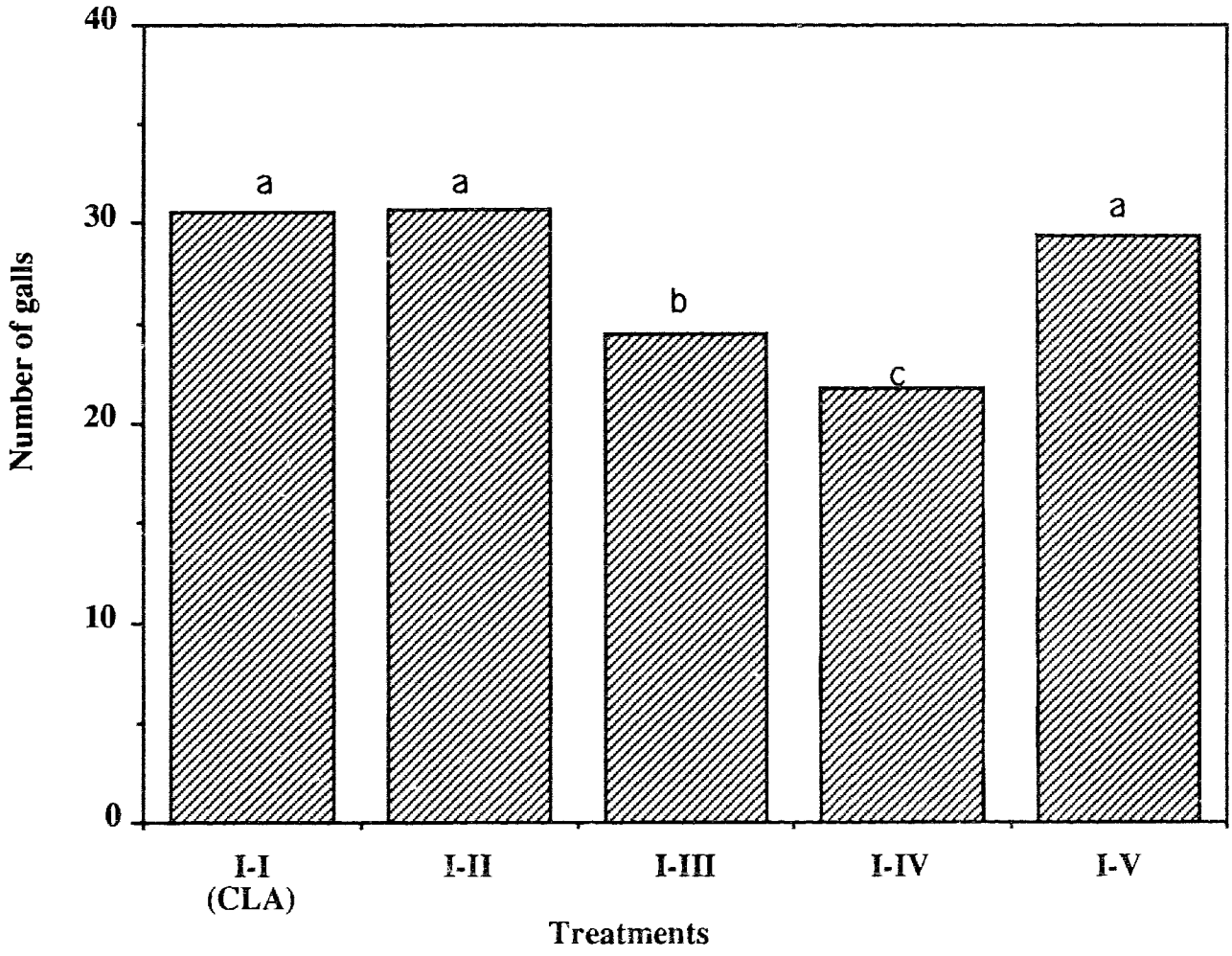

Figure 28. Numbers of galls induced by *Meloidogyne incognita* on excised tomato roots three weeks after increasing ammonium or high nitrate concentration(s) on STW medium. Bars with the same letter are not significantly different ($P = 0.05$). CLA = constant low ammonium. Axis labels I-I to I-V are described on p 61.

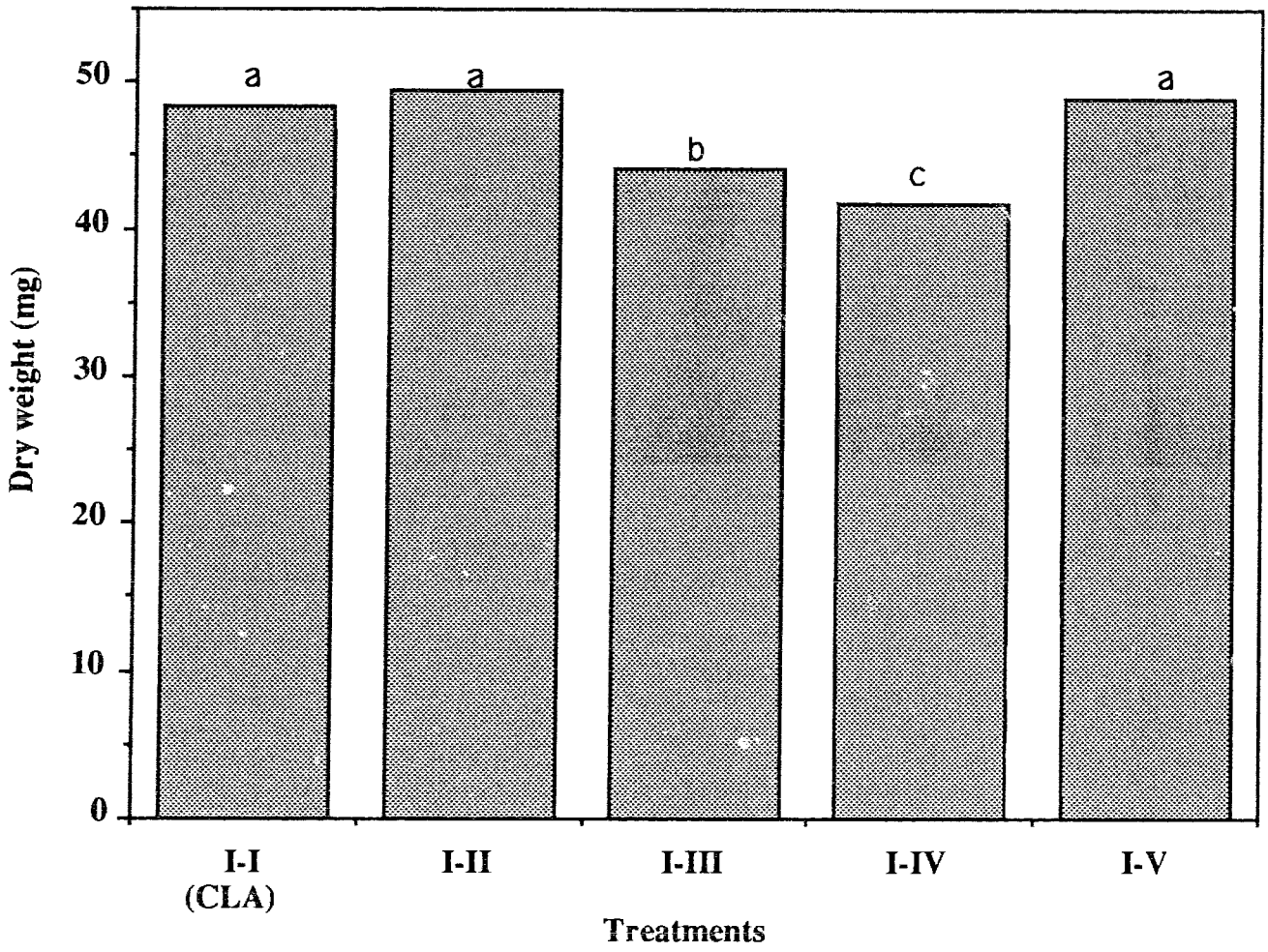

Figure 29. Dry weights of *Meloidogyne incognita* infected excised tomato roots three weeks after increasing ammonium or high nitrate concentration(s). Bars with the same letter are not significantly different (P = 0.05). CLA = constant low ammonium. Axis labels I-I to I-V are described on p 61.

6. Development with decreasing ammonium

Experiment

This experiment was designed to examine the effect on nematode development of decreasing ammonium concentrations after infection. To achieve this purpose, an experiment was done similarly to that described above, but instead of being grown on deficient ammonium (1.5 ppm), the excised roots were grown on 36X-strength ammonium (324 ppm). Three-day-old seedlings were excised and the roots were aseptically transferred into 240 Petri dishes containing STW medium with 36X-strength ammonium and with 2% agar. On the medium with the higher agar concentration, the lateral roots grew on only the surface of the medium so that the excised roots could easily be removed without damage. Three days after excision, each of 160 roots was inoculated with a single egg mass, but the remaining 80 dishes were not inoculated. The dishes were sealed and incubated as described.

One week after inoculation, 10 infected roots were carefully removed from the dishes. The number of galls, the fresh weight and nematode development were recorded from five infected roots. The other five roots were dried in a 70°C oven after the galls had been counted. On the same day, five uninoculated roots were carefully removed from their dishes for observation of fresh and dry weights. The rest of the roots were aseptically transferred into Petri dishes of STW medium containing either deficient, normal, 6X-strength, 36X-strength ammonium, or high nitrate concentration, which

were then labelled IV-I, IV-II, IV-III, IV-IV (constant high ammonium = CHA), and IV-V, respectively. Each treatment was replicated five times. The dishes were resealed and incubated as described. While in the new growth media, samples of the roots, five replicates for each treatment, were harvested weekly for 3 weeks. The galls were counted, and the fresh and dry weights of the excised roots were determined. At the same intervals, the population and the stages of nematode development were assessed after staining the roots and examining them under a dissecting microscope.

Results

One week after inoculation, the stages of development of the nematodes were summarized (Fig. 30). The nematodes in these stages of development induced galls on the average of 11.2 per excised root, the average dry weight of which was 2.94 mg. At this time, several galls were necrotic.

One week after decreasing ammonium, the decreased ammonium concentrations significantly enhanced nematode development. The stages of development were summarized (Fig. 31). There were significantly more nematodes that developed into later stages in treatments IV-I, IV-II, IV-III and IV-V than in treatment IV-IV (CHA). The percentage of late 2nd-

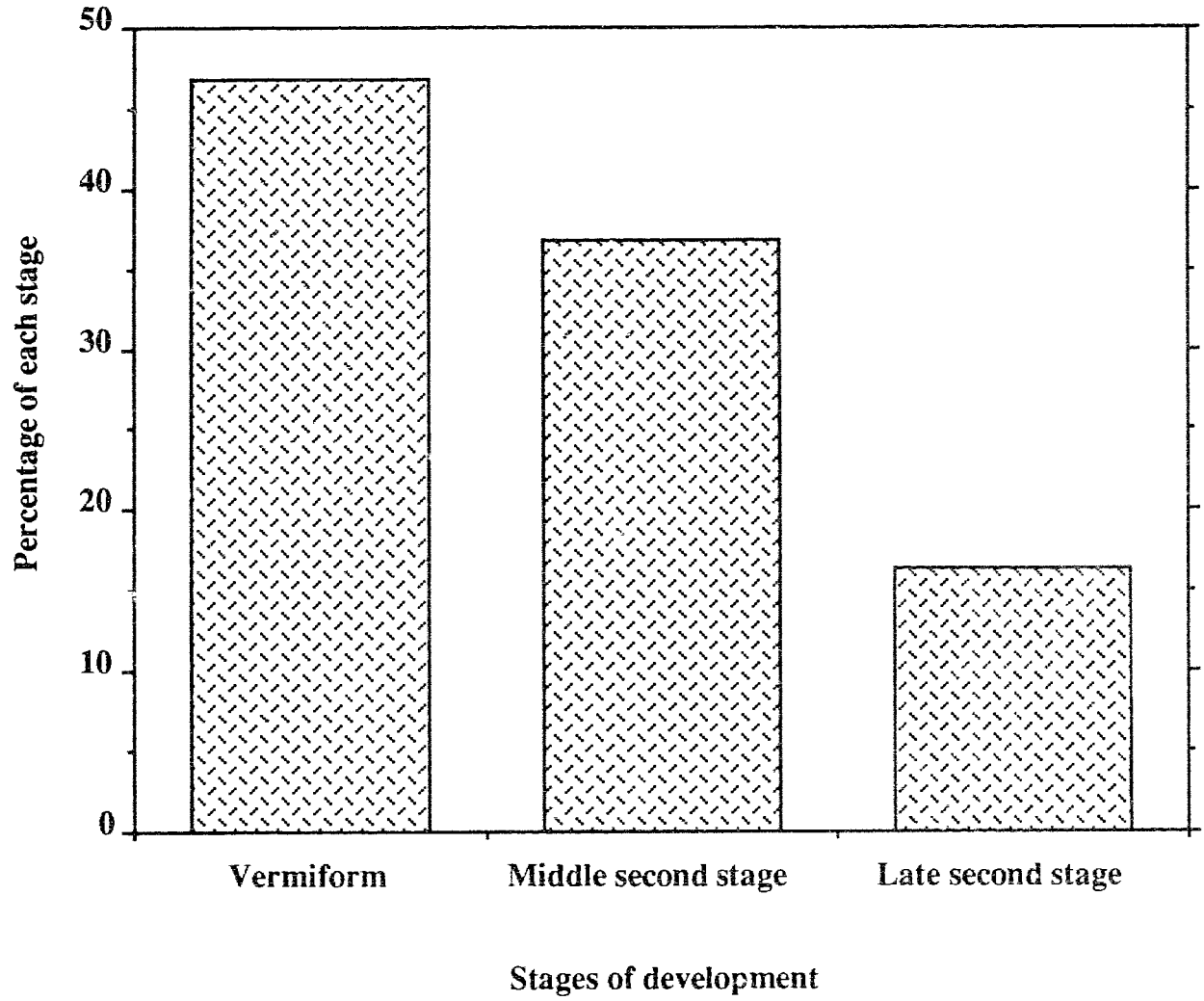

Figure 30. The percentage of stage of development one week after inoculation of *Meloidogyne incognita* with a single egg mass, in excised tomato roots on STW medium containing 324 ppm ammonium.

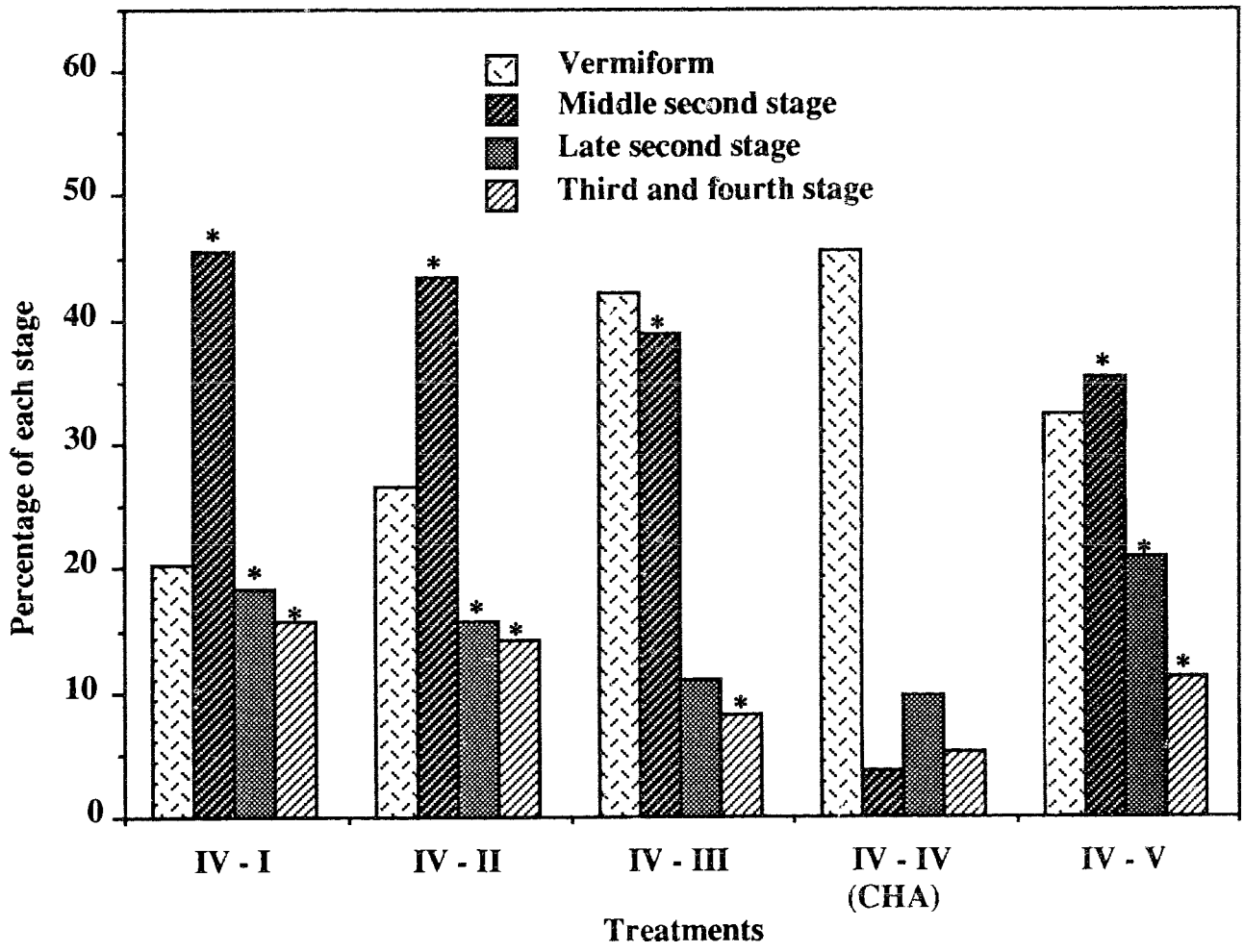

Figure 31. Percentage of stages of development one week after decreasing ammonium or high nitrate concentration(s) of *Meloidogyne incognita* in excised tomato roots on STW medium. Bars with * are significantly different from constant high ammonium (CHA) (P = 0.05). Axis labels IV-I to IV-V are described on p 76.

stage in treatment IV-IV (CHA) was significantly lower than in treatments IV-I, IV-II, or IV-V, but not significantly different from that in IV-III. Significant differences were also observed in the numbers of nematodes that developed into the 3rd- and 4th-stages. The percentage in treatment IV-IV (CHA) was significantly lower than in treatments IV-I, IV-II, and IV-V, but not significantly different from that in IV-III. Compared with the percentages in treatments IV-I or IV-II, treatment IV-V were significantly higher in vermiform and lower in the middle 2nd, late 2nd and 3rd stages.

As ammonium concentrations decreased , the numbers of galls significantly increased. As shown in Fig. 32, the number of galls in treatments IV-I, IV-II, IV-III, and IV-V was significantly greater than in treatment IV-IV (CHA). Similarly, the dry weight of roots in treatment IV-IV (CHA) was significantly lower than in treatments IV-I, IV-II, IV-III, or IV-V (Fig. 33).

The stages of development, *two weeks after decreasing ammonium*, are summarized in Fig. 34. Most of the nematodes in treatment IV-IV (CHA) were still in the vermiform, middle and late 2nd-stages, whereas in treatments IV-I, IV-II, or IV-V many had developed into adults. The percentages in the 3rd- and 4th-stages in treatments IV-I, IV-II, IV-III or IV-V were significantly higher than in treatment IV-IV (CHA). Although there were no adult females in treatment IV-IV

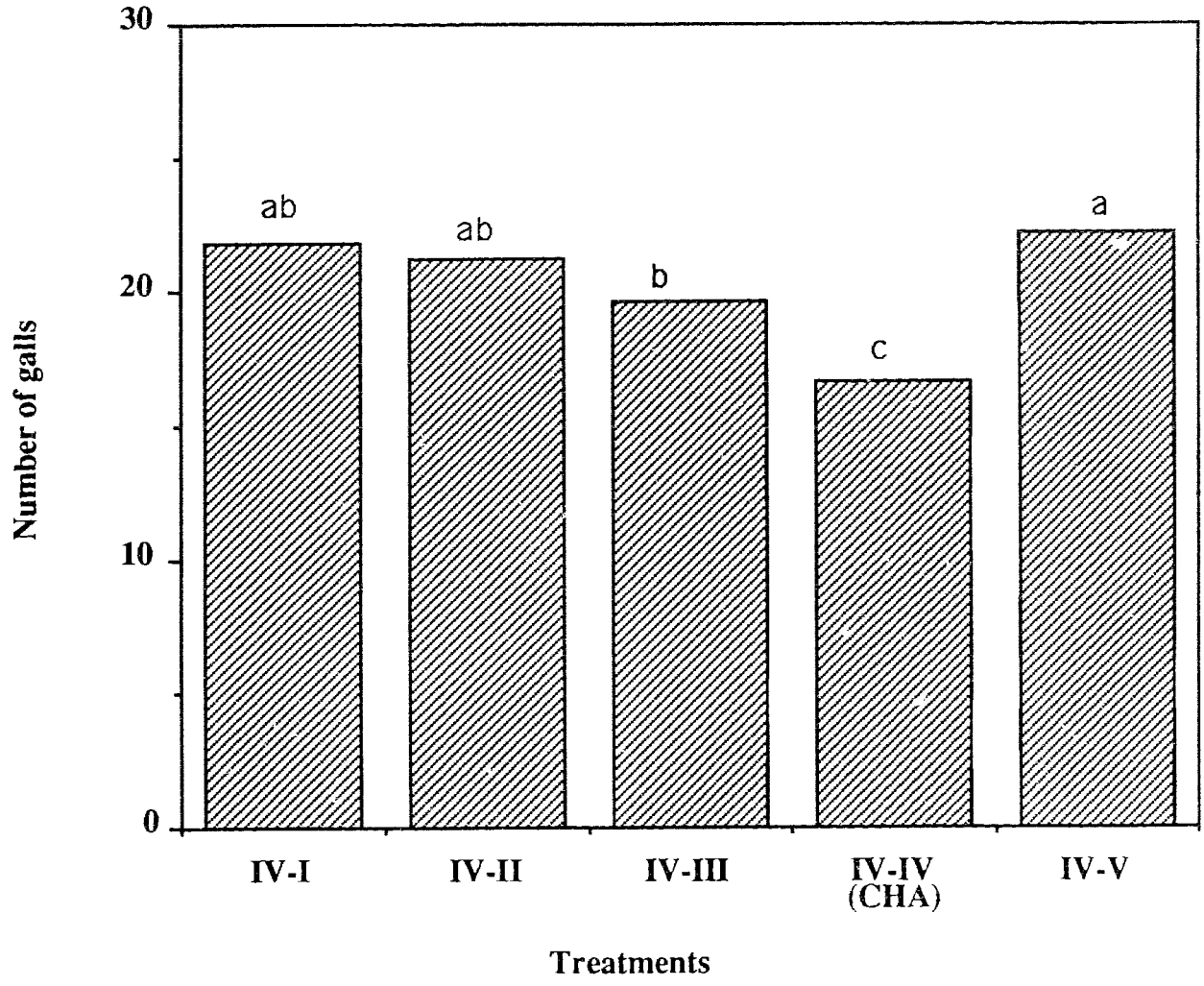

Figure 32. Numbers of galls induced by *Meloidogyne incognita* in excised tomato roots one week after decreasing ammonium or transfer into high nitrate concentration(s) of STW medium. Bars with the same letter are not significantly different ($P = 0.05$). CHA = constant high ammonium. Axis labels IV-I to IV-V are described on p 76.

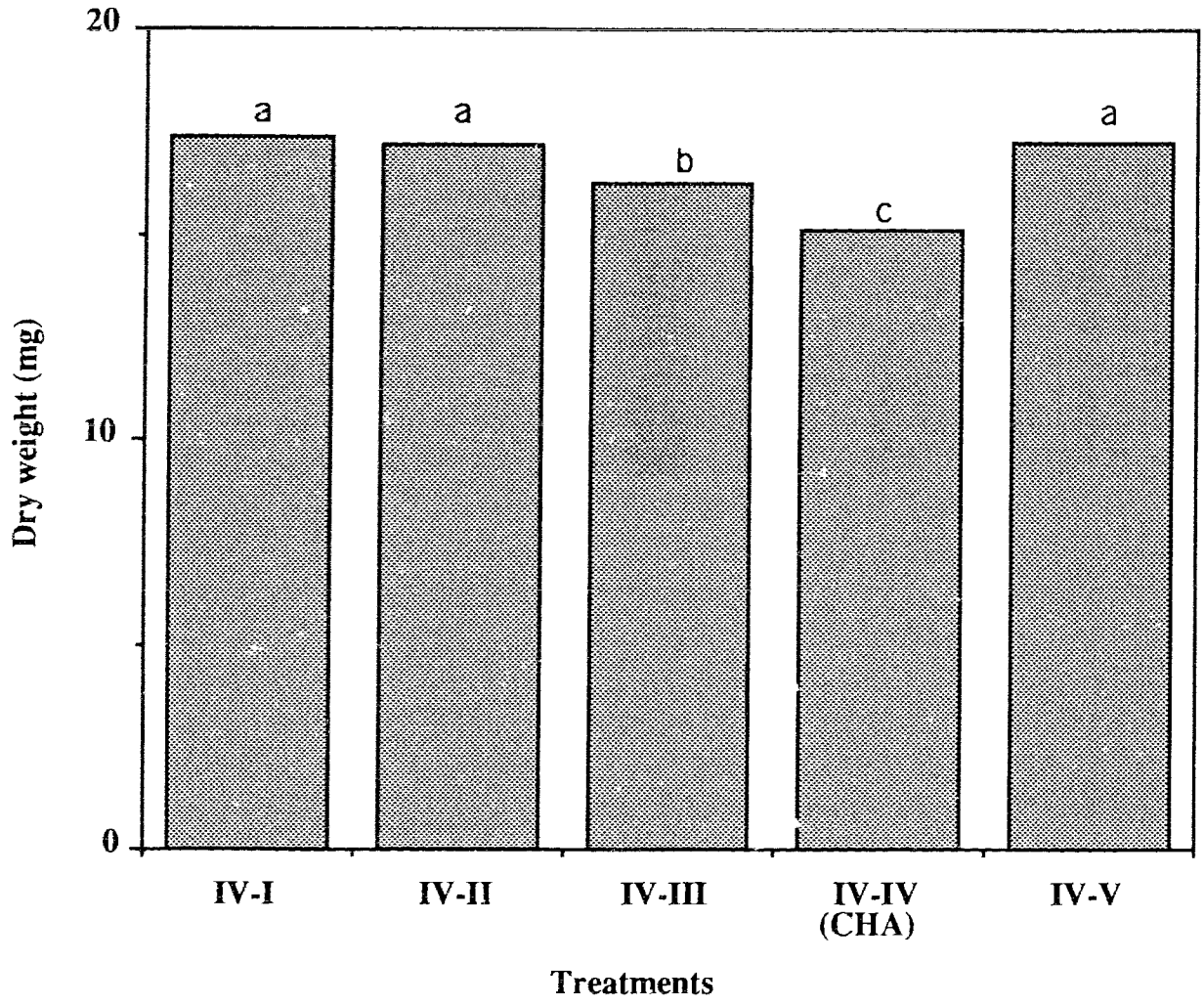

Figure 33. Dry weights of *Meloidogyne incognita* infected excised tomato roots one week after decreasing ammonium or transfer into high nitrate concentration(s) of STW medium. Bars with the same letter are not significantly different ($P = 0.05$). CHA = constant high ammonium. Axis labels IV-I to IV-V are described on p 76.

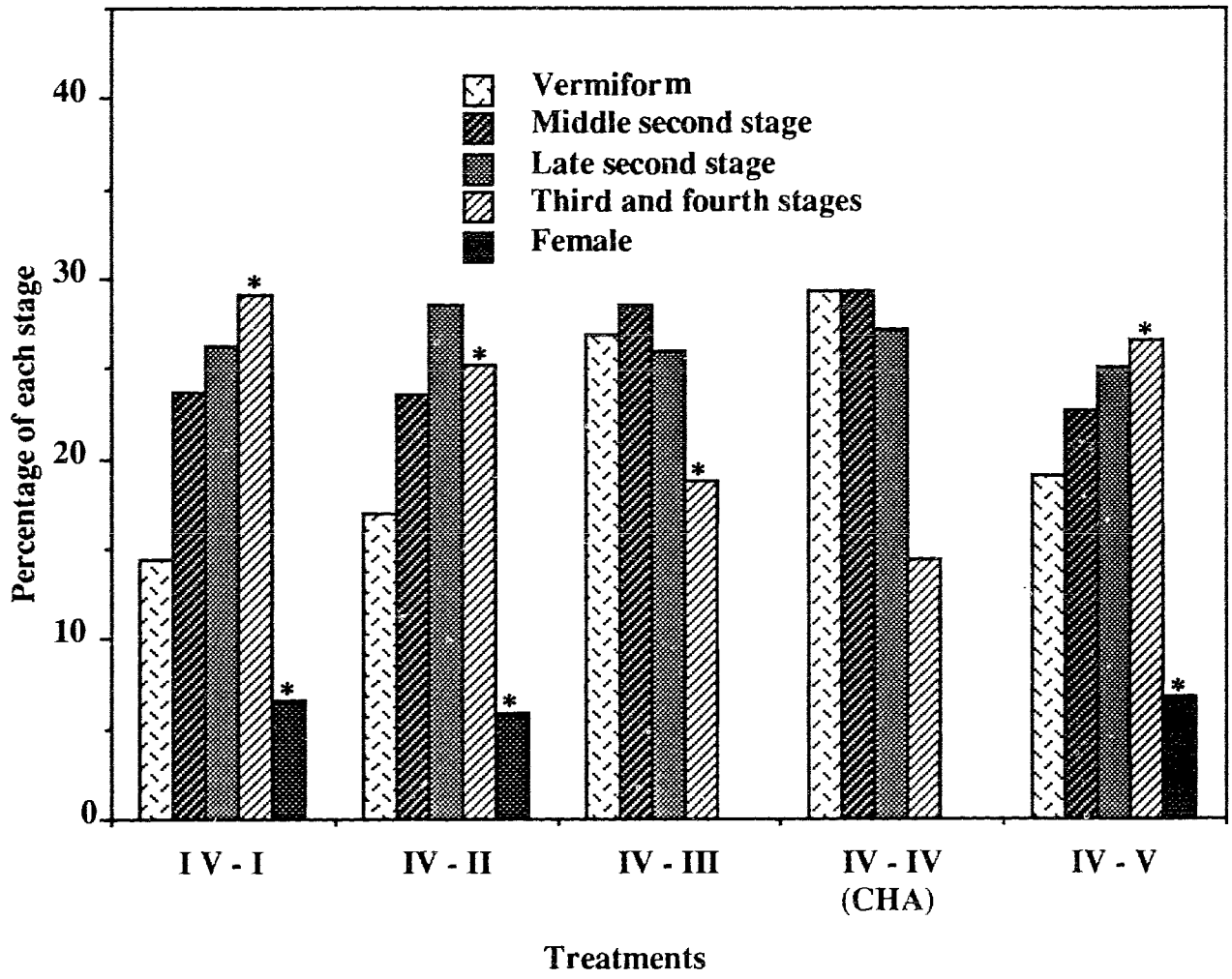

Figure 34. Percentage of stages of development two weeks after decreasing ammonium or transferring into high nitrate concentration(s) of *Meloidogyne incognita* in excised tomato roots on STW medium. Bars with * are significantly different from constant high ammonium (CHA) (P = 0.05). Axis labels IV-I to IV-V are described on p 76.

(CHA), there were 6.64%, 5.80% and 6.79% females in IV-I, IV-II, and IV-V, respectively.

The enhancement of nematode development by decreasing the ammonium concentrations resulted in an increase in the number of galls (Fig. 35) and dry weight (Fig. 36). The number of galls in treatment IV-IV (CHA) was significantly fewer than in treatments IV-I, IV-II, IV-III or IV-V. Similarly, the dry weight of roots in treatment IV-IV (CHA) was significantly lower than in IV-I, IV-II, IV-III, or IV-V.

Three weeks after decreasing ammonium, more nematodes had developed significantly further in the lower ammonium treatments. Fig. 37 summarizes the stages of development. Except in treatment IV-III and in treatment IV-IV (CHA), most nematodes had developed into later stages and in particular, more developed into adult females in the lower ammonium treatments. There were significantly more adult females in treatments IV-III, IV-II, IV-I, and IV-V than in treatment IV-IV (CHA). The percentage of adult males was not significantly different. However, the decreased ammonium concentrations clearly enabled more nematodes to develop into females in comparison with IV-IV, where more than 75% of the adults were males; the proportions of males in treatments IV-I and IV-II were less than 15%.

Decreasing ammonium concentrations significantly increased the numbers of galls. Fig. 38 shows that the

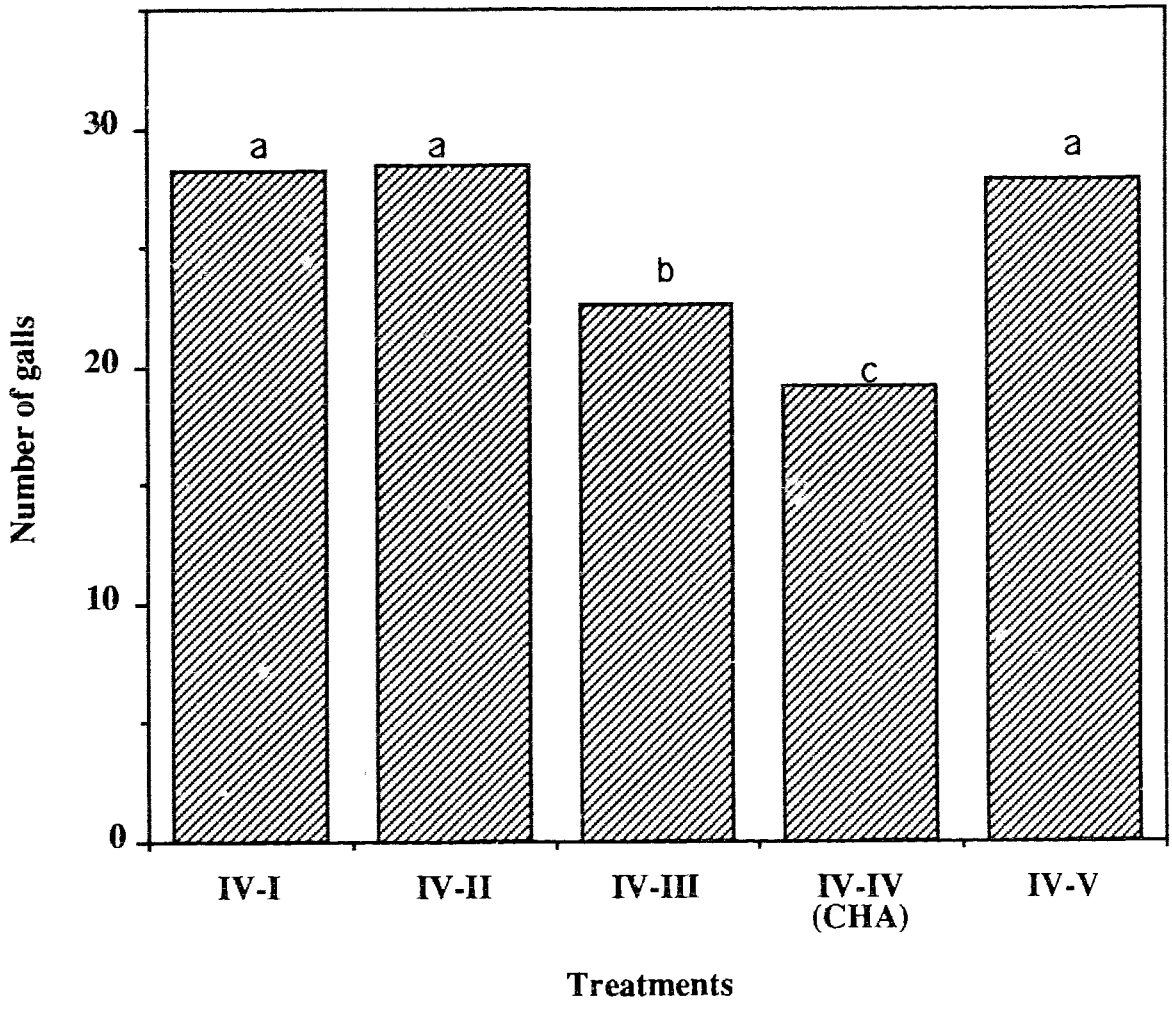

Figure 35. Numbers of galls induced by *Meloidogyne incognita* in excised tomato roots two weeks after decreasing ammonium or transfer into high nitrate concentration(s) of STW medium. Bars with the same letter are not significantly different (P = 0.05). CHA = constant high ammonium. Axis labels IV-I to IV-V are described on p 76.

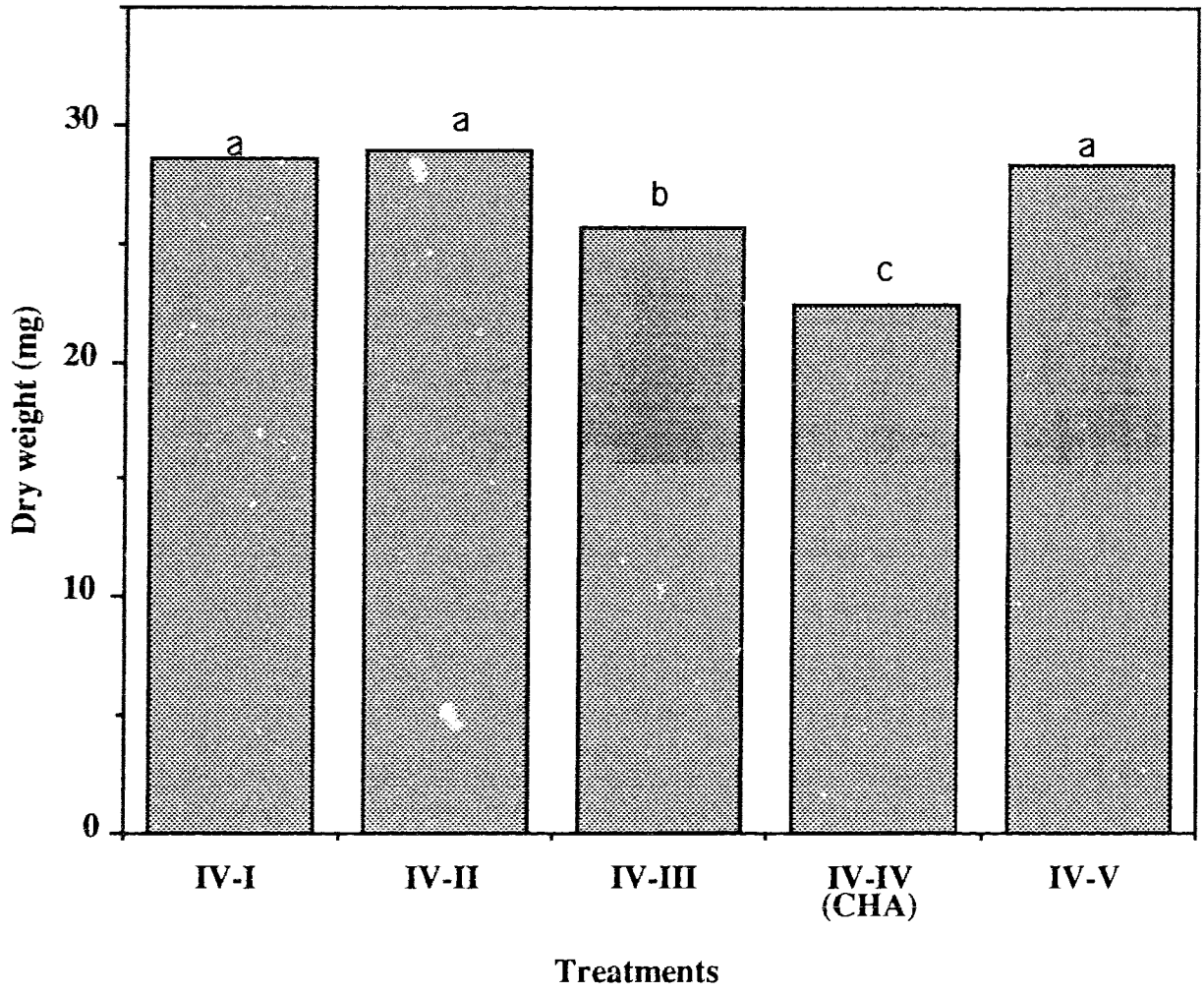

Figure 36. Dry weights of *Meloidogyne incognita* infected excised tomato roots two weeks after decreasing ammonium or transferring into high nitrate concentration(s) of STW medium. Bars with the same letter are not significantly different (P = 0.05). CHA = constant high ammonium. Axis labels IV-I to IV-V are described on p 76.

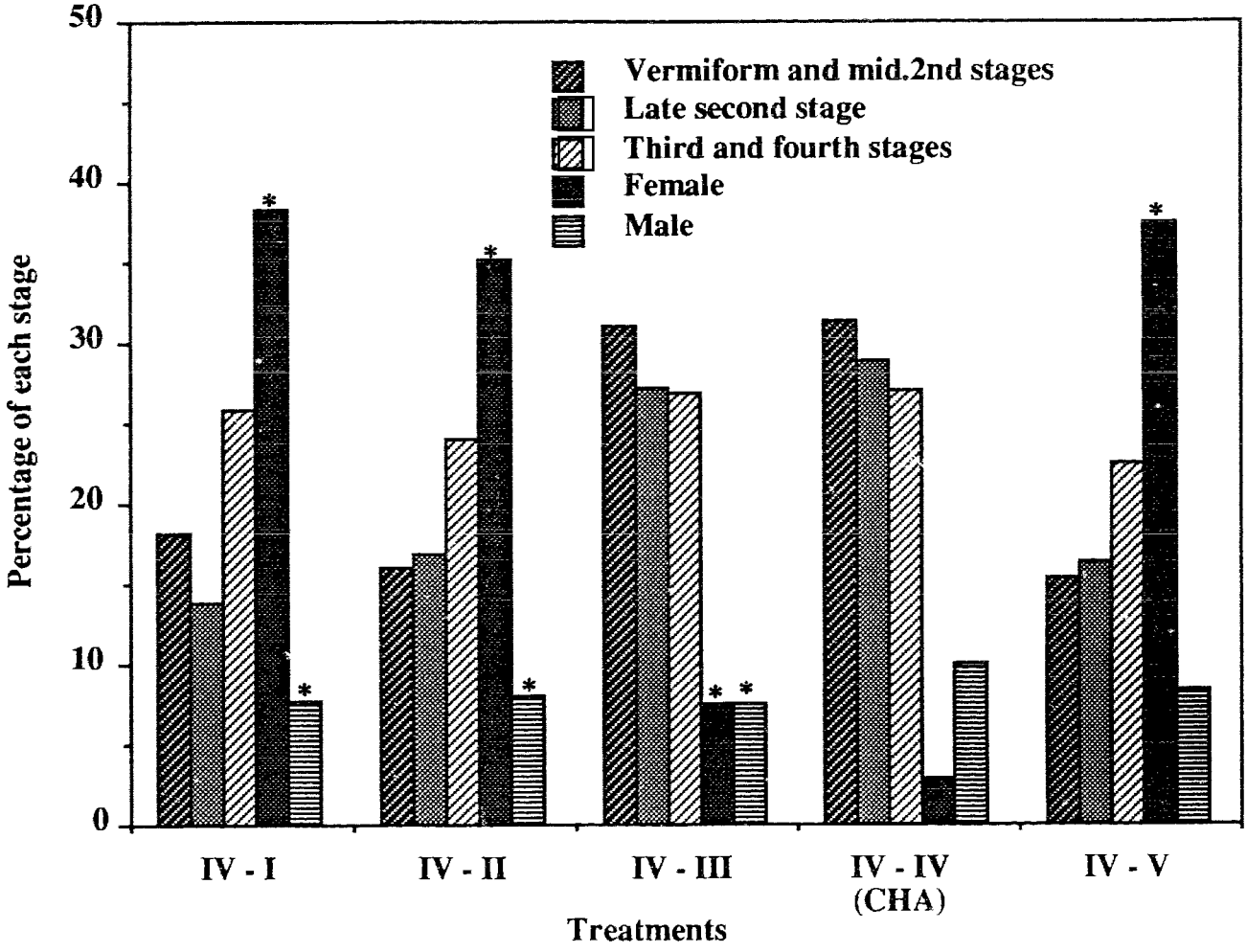

Figure 37. Percentage of stages of development three weeks after decreasing ammonium or transferring into high nitrate concentration(s) of *Meloidogyne incognita* in excised tomato roots on STW medium. Bars with * are significantly different from constant high ammonium (CHA) (P = 0.05). Axis labels IV-I to IV-V are described on p 76.

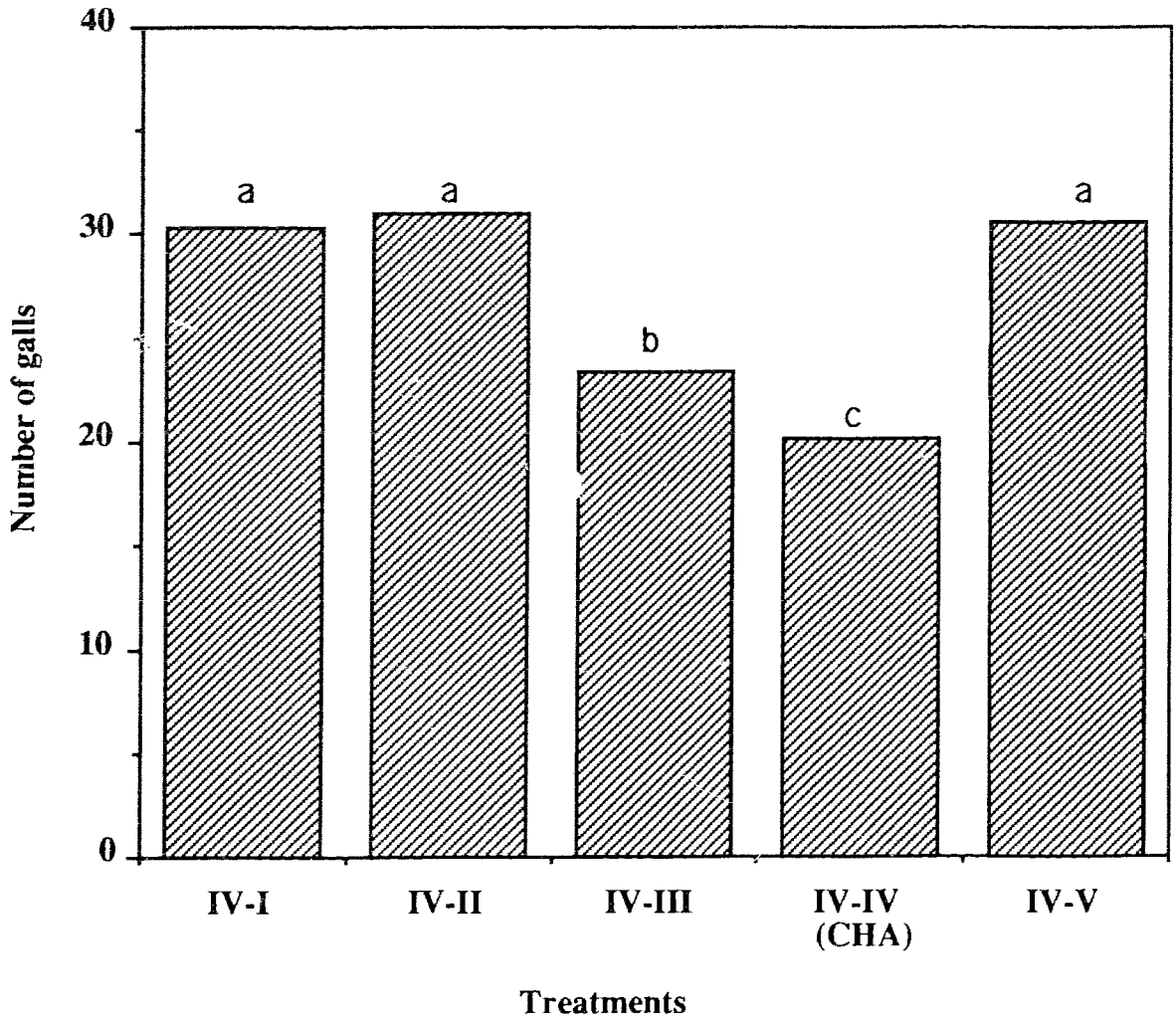

Figure 38. Numbers of galls induced by *Meloidogyne incognita* in excised tomato roots three weeks after decreasing ammonium or transfer into high nitrate concentration(s) of STW medium. Bars with the same letter are not significantly different (P = 0.05). CHA = constant high ammonium. Axis labels IV-I to IV-V are described on p 76.

numbers of galls in treatments IV-I, IV-II, IV-III, and IV-V were significantly greater than those in treatment IV-IV (CHA). Similarly, the dry weights of roots in treatments IV-I, IV-II, IV-III and IV-V were significantly greater than in treatment IV-IV (CHA) (Fig. 39).

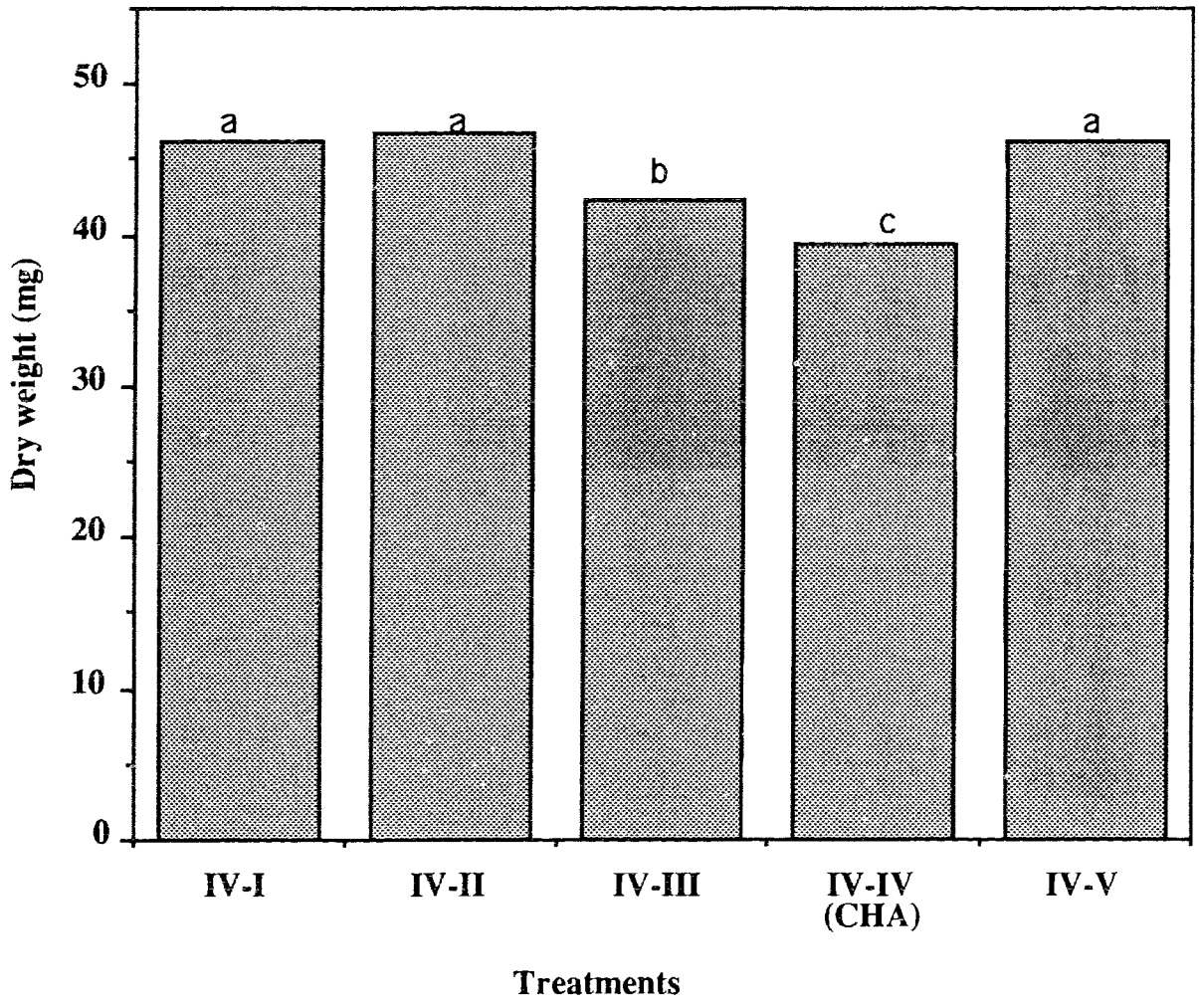

Figure 39. Dry weights of *Meloidogyne incognita* infected excised tomato roots three weeks after decreasing ammonium or transfer into high nitrate concentration(s) of STW medium. Bars with the same letter are not significantly different (P = 0.05). CHA = constant high ammonium. Axis labels IV-I to IV-V are described on p 76.

IV. DISCUSSION

Overall, the results from this research show a significant detrimental effect of ammonium on the behavior and development of *M. incognita* and this effect increases with increased concentration of ammonium in the medium. Higher than normal ammonium concentrations significantly reduced egg hatching. The responses, demonstrated by two types of inocula, were not only detectable but significant within 48 h of inoculating onto the medium (Figs 1 and 2).

In treatments with low ammonium, during the first 7 days, the rates of hatching were initially fast, then became slow. With the two types of inocula, the hatching curves diverged indicating a suppressed rate of hatching in the high ammonium concentrations. Not only were the numbers that hatched over time reduced, but also a smaller final number hatched. With high ammonium, hatching was very slow throughout the experiments. Since the rates of hatching were very low after 10 days in treatments with high ammonium and after 7 days with low ammonium, the hatching rates approached zero concurrently. It was unlikely that prolongation of the experiments would have resulted in equal final numbers hatched and so the experiments were terminated at 15 days.

Beevers and Hageman (9) reported that ammonium inhibited the activity of malate dehydrogenase in plants. Wallace (118) hypothesized that ions penetrate the eggs of

nematodes and may influence the hatch of juveniles. Therefore, it is probable that ammonium ions that penetrated egg shells and the juvenile cuticle inhibited the activity of malate dehydrogenase in the nematode (113), resulting in blockage of the intermediary fumarate reductase pathway of carbohydrate metabolism (94). This precludes the generation of energy (ATP) necessary for the subsequent vital activities of hatching, penetration, and development (112). It has been demonstrated that high salt concentrations created sufficient osmotic pressure (24, 110) to inhibit hatching of *Heterodera* spp. and *Meloidogyne* spp. and to inhibit the movement of juveniles within the eggs (24).

Considerable information has accumulated on the effects of various chemical substances, including those detected in root exudates, on the hatching of *Heterodera* spp. (59, 116, 117, 120). However, less is known about the influence of root exudates on the hatching of *Meloidogyne* spp. Early investigations (1, 18, 50, 103, 114) into the relationship of root exudates from plant seedlings and hatching of *Meloidogyne* spp. showed that root exudates of host plants were stimulatory and those of non-hosts were inhibitory. These observations agree with the results from the present study. With two types of inocula, the hatching curves showed an increased rate of hatching in the presence of excised roots. With excised roots, not only were the numbers hatched per unit time increased, but also greater final numbers hatched than those in without excised roots(Figs 1 and 2).

The fact that the hatching curves with and without the roots became non-linear after the 7th- and the 12th-days in low and high ammonium concentrations, respectively, show that the rate of hatching eventually slowed virtually to zero. Therefore, it was likely that prolongation of the tests on both with and without excised roots would not result in equal final hatching. It could be concluded that the roots provide a factor or factors whereby juveniles are able to hatch that would not do so otherwise.

The inoculum type affected hatching so that approximately 50% more juveniles emerged from egg masses than from dispersed eggs. This indicates that probably the sodium hypochlorite used during the process of egg extraction had an adverse effect on the nematodes. This result supports previous investigation (28, 43) where 0.53-1.05% (43) sodium hypochlorite treatment of *Meloidogyne* eggs decreased hatching. It was suggested that sodium hypochlorite penetrated the egg shell and caused deformities in the juveniles resulting from its strong oxidizing effect that breaks sulphide bounds and alters the orientation of tissues.

Not only did high ammonium concentrations affect the hatching of *M. incognita*, but it also severely reduced the number of juveniles penetrating into roots. This was apparently related to the direct effect of ammonium on nematodes, namely the inhibition of production of energy

required for penetration as described above (9, 94, 112, 113).

The host nutrition levels can alter the degree of host attraction, the number of available root-sites and the nematode's surface feeding activities (65). As the attraction of nematodes to their host plants is thought to be an integral part of the process of interaction with and penetration of the host, it is reasonable to expect that factors that affect the attractiveness of roots will also influence infection. It has been stated (62, 63) that the attractiveness of roots to juveniles is related to the growth of the roots. Conceivably then, the attractiveness of roots to nematodes (and thereby penetration) may be adversely affected by treatments which reduce root growth. Since the different ammonium concentrations did not significantly affect root growth but did affect nematode penetration, such a relationship was not apparent. It would seem, therefore, that if infection is dependent upon the attractiveness of roots to nematodes, such attractiveness is not necessarily dependent upon root growth as measured by dry weight. Ammonium concentrations may influence root attraction by means other than their effect on root growth. Scott and Martin (95) have shown that treatment with different ions can significantly affect the electrical potential around root tips. This mechanism may apply to the treatment here with different ammonium concentrations.

Since different ammonium concentrations significantly affected hatching, inoculation with a single egg mass resulted in different inoculum levels among the ammonium concentration treatments. Therefore, the penetration of juveniles was based on the proportion of juveniles inside the roots to the total numbers of emerged juveniles. Of interest is the fact that an increase in the number of developing juveniles per gall correlated with increase in inoculum levels, since there were more eggs hatched in normal and deficient than in high ammonium. The increase in number of juveniles per gall might seem to indicate a limitation in the number of root sites suitable for infection. Peacock (82) observed that juveniles of *M. incognita* invade the plant through an entrance made by the first juvenile. Bird (14) found galls to be equally as attractive as root tips to juveniles of *M. incognita* and with an increase in inoculum level, the proportion of juveniles that entered an excised gall also increased. He suggested that it was possible that the increase in the number of juveniles per gall resulted from a further leakage of attractive exudates from the galls. It is likely, therefore, that increased penetration of roots with low ammonium treatments was caused by the high numbers of infective juveniles. Nevertheless, the results of the experiment with roughly equal numbers of infecting juveniles showed that high ammonium concentrations had a severe detrimental effect on penetration.

Loewenberg *et al.*(61) reported that *in vitro* survival of *M. incognita* was dependent upon mineral balance in the medium. He suggested that the mineral balance may promote infection of the host. Later, Myers and Krusberg (70) found that *in vitro* 2nd-stage juveniles of *M. incognita* were capable of taking up and metabolizing uniformly labelled glucose-^{14}C. Thus it seems likely that ammonium may directly influence the fate of the juveniles. This assumption agrees with reports stating that *Meloidogyne* juveniles are capable of surface feeding, while thrusting their stylets into the surface of the roots (62), and it is possible that the nutrients taken in during that feeding period are important to the juveniles in the subsequent process of penetration (65).

The effect of high ammonium concentrations on the nematodes' activity through the host plants, other than root growth, became clear when, after 24 h incubation in water, there were significantly more juveniles leaving roots in high than in low ammonium. This indicated that the nematodes' development was halted after penetrating the roots. This phenomenon may be due to the ability of high ammonium to alter the physiology of roots, resulting in some form of incompatibility between the nematodes and roots. Incompatibility reactions occur also when *Meloidogyne*-resistant plant cultivars are exposed to *Meloidogyne* juveniles (47, 73, 88). The results from this study agree with findings reported earlier. Migration of juveniles from

roots after infection may be related to the failure of nematodes to induce feeding sites. Juveniles of *Pratylenchus scribneri* migrated out of roots of lima bean (47), and *M. hapla* and *M. incognita acrita* were observed to leave the roots of incompatible alfalfa cultivars (87). The actual mechanism(s) responsible for the movement of juveniles out of roots is still unknown. It may reflect an active host response which creates internal conditions adverse to nematodes (47).

In most studies, the 2nd-stage juveniles of *Meloidogyne* spp. were reported to readily penetrate the roots of susceptible and resistant plants. Niblack *et al.* (73) reported no differences among resistant and susceptible cultivars in penetration of soybean roots by *M. incognita* 7 days after inoculation. However, there were fewer nematodes in the roots of resistant than in the roots of susceptible cultivars 14 days after inoculation, indicating that juveniles had left the roots of resistant soybeans. Khan and Khan (49) recently reported that the number *of* M. *incognita* juveniles inside roots of cauliflower was much higher in susceptible than in resistant cultivars. In the present study, the juveniles did not leave the roots so long as the roots remained in the medium. However, when the nematode infected roots were incubated in water, significantly more juveniles left the roots that had been treated with high than with low ammonium. This was probably because after incubation of roots, the water taken by the roots lowered

the concentration of ammonium and lessened the effect of ammonium on nematodes, which then caused the nematodes, most of which were still in second-stage juveniles, to become active. This might lead to the movement of nematodes to leave the unfavorable conditions inside the roots.

Further observations showed that ammonium nitrate at high concentrations inhibited development of *M. incognita*, apparently without affecting root development. Orion *et al.* (79) reported that root growth was enhanced primarily by the organic fraction in the medium, and not by the inorganic fraction. Since the ammonium was given as ammonium nitrate, an additional test with high nitrate was conducted. High nitrate, as potassium nitrate, did not significantly affect root growth or nematode development. This is in accordance with the results of other studies (44, 64, 80) which have indicated that the concentration of potassium nitrate does not affect giant cell formation or nematode development. Although the results of the present study support that conclusion in part, nevertheless, the supplementary potassium nitrate treatment resulted in inoculated plants having a significantly greater dry weight of root tissue than the control plants. This difference could be attributed to the formation of more galled tissue by nematodes. Or alternatively, it could have resulted from the stimulation of root growth that occurred in the presence of the nematode and in a high concentration of potassium nitrate. In all

experiments, where nematode development was favored, there were more galls with a greater dry weight of roots.

The inhibition of development of *M. incognita* by high ammonium treatments, agrees with the report of Turligina (108) who stated that ammonium nitrate reduces the number of females developing from juveniles and therefore the number of galls forming on roots. Later, Orion *et al.* (79), reported that a high concentration of ammonium nitrate applied to *M. incognita* cultures on excised tomato roots suppressed giant cell development and thus indirectly hampered nematode development. In the present study, however, the concentrations of ammonium applied were much lower than that in Orion's experiment. Although this study did not include the histology of giant cells, the mechanism of suppressing rematode development could be similar to that observed by Orion, in which the suppression of giant cells and the inhibition of nematode development caused by high ammonium were similar to the effects caused by hydroxyurea and thiourea (36).

The weekly observations made in this study showed that high ammonium significantly reduced the rate of nematode development in culture. At low ammonium (normal and deficient) the nematodes developed fast and there were high percentages of females, which indicates that low ammonium creates favorable conditions for nematodes in excised roots. This was supported by the increased numbers of galls and dry weights of the roots. Davide and Triantaphyllou (21) have

stated that when environmental conditions are favorable for nematode development, sexual differentiation in the juveniles proceeds normally toward females. In contrast, with high ammonium level, the rate of development was slow, few nematodes matured, and there were a larger proportion of males in the adult population. The larger proportion of males confirms that the nematodes developed under stress conditions. Spiegel *et al* (99) reported that the application of ammonium chloride reduced the nematode populations in roots and induced a high percentage of males to develop in the population.

Triantaphyllou (105) noted that juvenile development in *M. incognita* was retarded under conditions of crowding. However, in the present study the retardation of development did not occur in relation to the numbers of nematodes per whole root system or numbers of juveniles per gall in comparison with the control. Moreover, in low ammonium, where the roots contained more nematodes than those in high ammonium (Appendix VI), there was a higher percentage of adult females than with high ammonium and this suggests that the change in sex ratio may be due to an absence of the stress of crowding. McClure and Viglierchio (66) explained that retarded development resulting from crowding, may be caused partly by reduction in the amount of food available to the nematodes or from increased nematode interactions, such as toxic effects from secretory products or competition for sites suitable for giant cell formation. Excised roots,

however, differ from intact roots in that they are not dependent upon translocation of organic compounds from the shoot. Excised roots are in intimate contact with the medium which supplies their nutrients. It follows, therefore, that disturbances in their vascular systems are not likely to result in nutritional deficiencies. This is supported by the result in the present study where even under conditions of crowding, at normal and deficient ammonium, there were higher percentages of females than in those roots in high ammonium that contained fewer nematodes (Appendix VII). Hence, the nematode sex ratio, which has now been shown (66, 105) to be at least partly controlled by host nutrition, is not greatly affected by crowding in an excised root culture. This suggests that factors other than a simple reduction in the amount of available food affect nematode development and influence sex ratio under conditions of crowding.

Resistance to nematodes in different cultivars has been associated with increased root necrosis (68), failure of the nematodes to mature (17, 68), and failure of the host to develop nematode-induced galls and giant cells (17). In the present study, most nematodes in the controls or deficient ammonium developed into adult females, and fewer grew in roots with high ammonium. Failure of the nematodes to grow within roots, namely those with higher ammonium treatment, was usually associated with necrosis within the galls. This necrotic reaction appeared as early as 6 days after inoculation. Necrosis of galls was frequent in the roots

with high ammonium and rare in control or deficient ammonium. Since ammonium did not affect root growth, it is proposed, therefore, that ammonium had an induced resistance property which complied also with Giebel's recent definition (35) that "induced resistance could be considered when the chemicals used for this purpose do not interfere with host-plant metabolism to such a degree as to be phytotoxic."

The potential of high ammonium to induce resistance through incompatibility, besides a direct effect, became more apparent with the data obtained from the experiments of increasing or decreasing ammonium concentrations following root infection by nematodes. In deficient ammonium, nematodes induced feeding sites and grew fast. There were high percentages of females, many galls and a high dry weight of roots compared with other treatments. When the roots were transferred into high ammonium concentrations, however, development of the nematodes was suppressed, with the presence of necrotic and less developed galls and reduced dry weights of the roots. After the roots were transferred into high ammonium the juveniles appeared not to develop further, which was probably due to the physiology of the roots being altered so that the cells could not support nematode development. This was confirmed by the male:female ratio of nematodes in roots transferred into high ammonium which was 100X greater than that of the constant deficient ammonium (Appendix VII). The high proportion of males in the roots transferred into high ammonium indicates that the

nematode developed under stress conditions. It is reasonable, therefore, to assume that high ammonium created adverse environmental conditions which caused the nematodes to develop into males through sex reversal. Davide and Triantaphyllou (21) reported that if conditions become adverse immediately after the juveniles have started differentiating into females, the nematodes undergo sex reversal and usually develop into males with two testes, or male intersexes.

In contrast, decreasing ammonium concentrations through transferring infected roots from high to low ammonium favored nematode development. The data show that after being transferred into low ammonium, not only did most nematodes develop into adults but also a high percentage of females occurred, with increased number and size of root galls and greater dry weight of the roots. At high ammonium (before transfer) less than 20% of juveniles had developed into the sex differentiating stage (late 2nd-stage) and the rest were still in the vermiform or middle 2nd-stage. After being transferred into low ammonium and conditions became favorable, most of the nematodes developed into females and the male:female ratio was 15X lower than that at constant high ammonium. A high proportion of females in the roots after being transferred into low ammonium indicates that nematodes readily induced feeding sites while those in the roots with constant high ammonium were starved. These results agree with those of Triantaphyllou and Hirschmann

(107), who reported that feeding initiated development toward the female whereas, prolonged starvation triggers development toward the male.

V. CONCLUSIONS

In most studies in which giant cell development was depressed by growth retardants, such as maleic hydrazide (21) and morphactin (88), or by deficiencies in organic (66) and in inorganic nutrients (11, 20) plant growth was also inhibited. However, in the present study the high ammonium concentrations did not depress root growth, but did reduce hatching, inhibit penetration, and suppress the development of *M. incognita*. Although the nature of the incompatibility and the mechanism(s) involved cannot be determined from these experiments, the findings of this study reveal that high ammonium concentrations affected nematode behavior and development both through the nematode directly and via the excised roots. On nematodes, high ammonium concentrations probably disturb physiological processes that lead to the prevention of the production of energy required for vital activities, such as hatching, penetration and development. Through the plants, high ammonium concentrations may inhibit giant cell formation and so lead to an incompatibility condition that does allow nematode to develop into males, not into females. These effects of high ammonium concentrations on the nematode are reversible.

The results of this study provide a useful perspective on the application of ammonium fertilizers. Higher than normal ammonium fertilizer treatments could potentially be very beneficial for the farmers either in developed or

developing countries since such treatments are not so hazardous as nematicides and in moderate amounts, are beneficial to soils. However, care must be taken in managing the sustained productivity of the soils because excessive chemical applications could be just as harmful to production as the presence of pathogens such as *Meloidogyne*. The testing of ammonium salts on intact plants and field tests with ammonium fertilizers, using crops tolerant to levels of ammonium, to control *Meloidogyne* spp. should be done. The value and practicality of manipulating the fertilizer regime in a *Meloidogyne* management program needs to be explored. It is possible that improved control of this devastating root-knot disease problem and an increase in crop yield could be achieved simultaneously and economically on some soils.

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APPENDIX I. The number*) of *Meloidogyne incognita* juveniles, emerging from a single egg mass, that penetrated excised tomato roots grown in STW medium containing different ammonium or a high nitrate concentration(s).

Days after inoculation	NH4 concentrations (ppm)				1116 ppm NO3
	1.5	9(Control)	54	324	
2	29.6	36.8	14.2	13.0	27.2
3	36.4	34.6	15.8	15.4	30.8
4	49.0	49.6	21.2	21.8	42.4
5	48.0	40.8	24.8	23.2	47.4
6	50.6	46.0	29.4	23.6	51.0
7	48.8	49.8	28.2	22.0	50.8
9	73.8	78.6	22.4	18.4	56.8
11	77.6	65.4	31.0	25.6	85.2
14	82.4	68.0	27.4	23.0	71.6

*) Average from five replicates

APPENDIX II. The number*) of *Meloidogyne incognita*, inoculated as second-stage juveniles, that penetrated excised tomato roots grown in STW medium containing different ammonium or a high nitrate concentration(s).

Days after inoculation	NH4 concentrations (ppm)				1116 ppm NO3
	1.5	9(Control)	54	324	
2	57.6	54.2	45.4	38.2	61.0
3	71.0	68.6	54.0	44.6	68.2
4	78.0	74.6	57.2	47.8	76.2
5	84.0	77.0	58.8	47.8	83.0
6	86.0	77.0	58.8	47.8	83.0
7	87.4	81.4	64.2	53.2	83.6
9	88.4	85.0	64.6	55.0	85.4
11	88.4	85.4	66.0	54.2	81.0
14	88.2	81.6	63.8	54.8	85.2

*) Average from five replicates

APPENDIX III. The number*) of *Meloidogyne incognita* juveniles that migrated into water, after 24 h incubation, from excised tomato roots grown in STW medium containing different ammonium or a high nitrate concentration(s).

Days after inoculation	NH4 concentrations (ppm)				1116 ppm NO3
	1.5	9(Control)	54	324	
2	5.2	5.0	5.8	6.2	4.2
3	8.0	8.6	9.8	10.8	7.6
4	10.0	11.8	12.4	12.2	9.8
5	10.6	12.2	14.0	13.6	10.6
6	10.8	12.6	14.6	14.6	10.8
7	11.4	12.8	15.2	15.6	11.2
14	10.6	12.0	15.2	15.4	10.8

*) Average from five replicates

Appendix IV. The fresh and dry weights(mg)[1] of excised tomato roots grown on STW medium containing different ammonium or a high nitrate concentration(s).

Treatments (ppm)		Days after excision[2] 10	17	24	31
			Fresh Weight		
1.5	NH4	16.26	43.18	136.15	353.91
9	NH4	15.99	43.65	134.31	352.72
54	NH4	15.72	43.37	132.72	357.24
324	NH4	16.00	43.58	131.14	355.09
1116	NO3	15.74	42.93	133.68	357.00
			Dry Weight		
1.5	NH4	1.28	3.40	11.16	29.74
9	NH4	1.26	3.44	11.10	29.64
54	NH4	1.24	3.42	11.06	30.02
324	NH4	1.26	3.44	11.02	30.04
1116	NO3	1.24	3.38	11.14	30.00

1) Average from five replicates.

2) The days are equal to one, two, three and four week(s) after inoculation of *Meloidogyne incognita* on inoculated excised roots, which was done 3 days after excision.

Appendix V. The fresh weight(mg)[1] of excised roots, inoculated with *Meloidogyne incognita*, grown on STW medium containing different ammonium or a high nitrate concentration(s).

Treatments (ppm)	Week(s) after inoculation 1	2	3	4
1.5 NH4	36.97a[2]	242.48a	370.57a	625.70a
9 NH4	36.01ab	242.09a	368.54a	607.14b
54 NH4	34.72b	226.86b	337.79b	531.05c
324 NH4	33.81b	212.68b	295.78c	468.41d
1116 NO3	35.39ab	242.16a	367.22a	616.66ab
$LSD_{0.05}$	3.03			
$LSD_{0.01}$		14.21	10.66	9.66

1) Average from five replications.

2) The numbers followed by the same latters on the same column are not significantly different at the confidence level specified.

APPENDIX VI. The numbers[1] of *Meloidogyne incognita* at different stages of development in excised tomato roots grown in STW medium containing different ammonium or a high nitrate concentration(s) over time.

Week(s) after inoculation	NH4 concentrations (ppm)				1116 ppm NO3
	1.5	9(Control)	54	324	
One					
A[2]	38.2	39.2	27.6	25.8	37.0
B	42.6	42.0	26.6	24.2	43.6
C	20.8	21.4	10.8	8.0	20.8
Total	101.6	102.6	65.0	58.0	101.4
Two					
A	16.2	17.8	14.2	14.2	18.0
B	15.4	22.8	18.4	26.0	22.8
C	26.4	30.4	25.4	22.2	31.2
D	29.4	32.6	16.8	5.6	37.0
Total	87.4	103.6	74.8	68.0	109.0
Three					
A	11.8	11.8	17.4	22.0	10.0
B	20.8	18.6	19.0	20.4	22.8
C	24.4	27.2	20.6	17.8	26.4
D	32.8	29.6	12.4	9.0	31.4
E	16.2	13.2	1.6	1.0	13.0
F	1.8	1.8	3.4	3.6	1.4
Total	107.8	102.2	74.4	73.8	105.0
Four					
A+B	9.2	8.4	23.6	24.0	9.6
C	11.6	12.0	19.2	18.4	13.6
D	17.8	18.6	14.2	12.0	20.4
E	57.6	56.0	7.0	1.6	52.6
F	3.2	3.8	9.0	12.2	4.6
Total	99.4	98.8	73.0	68.2	100.8

1) Average from five replicates

2) A = Vermiform
B = Middle second-stage
C = Late second-stage
D = Third- and fourth-stage
E = Female
F = Male

APPENDIX VII. The numbers of females, males, and the ratio of males/females 4 weeks after inoculation with a single egg mass of *Meloidogyne incognita* in excised tomato roots grown on STW medium containing different ammonium or a high nitrate concentration(s), and subsequently increasing or decreasing the ammonium concentrations.

Treatments	Number*) of		Males/Females
	Males	Females	
A. Different ammonium			
1.5 ppm NH_4	3.2	57.6	5.517
9 ppm NH_4(Control)	3.8	56.0	6.821
54 ppm NH_4	9.0	7.0	128.643
324 ppm NH_4	12.2	1.6	757.010
1116 ppmNO_3	4.6	52.6	8.849
B. Increased ammonium			
I-I (CLA)	3.2	56.8	5.616
I-II	5.8	55.0	6.937
I-III	12.0	8.2	146.380
I-IV	17.8	2.6	680.493
I-V	4.6	53.8	8.661
C. Decreased ammonium			
IV-I	3.8	18.8	20.193
IV-II	4.2	18.4	22.785
IV-III	4.0	4.0	100.000
IV-IV (CHA)	5.0	1.4	356.202
IV-V	5.4	19.6	22.434

*) Average of five replicates