ONTOGENY OF *DAUCUS CAROTA* IN RELATION TO *MELOIDOGYNE HAPLA* WITH A PRELIMINARY ENDOMYCORRHIZAL STUDY

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ABSTRACT

ONTOGENY OF *DAUCUS CAROTA* IN RELATION TO *MELOIDOGYNE HAPLA* WITH A PRELIMINARY ENDOMYCORRHIZAL STUDY

By

Lucille A. Slinger

The growth and development of *Meloidogyne hapla* (northern root-knot nematode) infected carrots (cv. Spartan Premium) was significantly ($P = 0.05$) retarded 32 to 88 days after planting. Increased root galling indicated a *M. hapla* life cycle duration of 16 days. Tap root development was initiated approximately 36 days after planting. Three distinct growth phases were observed for Spartan Premium carrots grown in muck soil. During the first four days seed reserves appeared to be incorporated into basic structural components. Between day four and day sixteen there was a rapid increase in growth, followed by a relatively steady gradual increase as the tap root developed. Spartan Premium carrots grown in a nematode-free environment were marketable by 76-80 days after planting. The maturity of the *M. hapla* infected carrots was delayed, reaching marketable weights by 96 days after planting. Gold Pak was a slower maturing cultivar. It had a reduced total plant efficiency, greater degree of galling and supported a larger population density of

M. hapla than Spartan Premium. A greenhouse experiment for testing carrot cultivar and parent line susceptibility to *M. hapla* indicated a positive correlation with field test results for only three cultivars: Spartan Classic, M 3489 and Danvers. No positive correlation was observed for cultivar host potential and susceptibility. Gold Pak carrots were mycorrhizal 30 days after planting. The degree of endomycorrhizal infection by *Glomus* spp. increased with time.

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DEDICATION

To those who have shown me
the immense value of life
especially:
Ann
Mom
Dad

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INTRODUCTION

Michigan was second in the nation in fresh market and processing carrot (Daucus carota L.) production in 1975. The Michigan carrot industry represents about 10 per cent of the nation's carrot production and is a ten to twelve million dollar industry (233). An estimated crop loss of 20 per cent is attributed annually to damage caused by plant-parasitic nematodes (206). The northern root-knot nematode (Meloidogyne hapla Chitwood, 1949) is indigenous to Michigan and is the major nematode pathogen of the United States carrot industry (27,233). Michigan carrots are produced in muck soils, predominately in Arenac, Clinton, Lapeer, Newaygo and St. Clair counties (150). In Michigan, carrots are produced in rotation with celery, onions or mint. All of these plants are good hosts for M. hapla from a reproductive potential standpoint and are subject to economic losses caused by this nematode (27,97). Continuous cropping of carrots for three years in the presence of M. hapla resulted in losses of up to 89% (250).

The objectives of this investigation were to: (i) define the basic growth and development of a Michigan carrot cultivar in noninfested and root-knot nematode-infested muck

soil; (ii) define the growth and development of the carrot plant in relation to specific population densities of *M. hapla*; (iii) analyze a greenhouse method of evaluating parent lines and trial cultivars of *D. carota* for *M. hapla* resistance and host potential; and (iv) determine if an endotrophic mycorrhizal association exists between carrots and fungi of the Endogonaceae. The study will be used as a base to establish a predictive pest management model for the economic losses of carrots caused by the northern root-knot nematode, contribute to a better understanding of this host-parasite relationship and to assist in the development of improved control methods.

LITERATURE REVIEW

Carrot

Carrots are a popular raw or cooked vegetable and rank high in food efficiency (146). They are high in vitamin A, average in food energy, iron and protein, and low in ascorbic acid, niacine, riboflavin and thiamine (236). Their composition, particularly that of vitamin A, varies with cultivar and maturity (241).

Carrots belong to the Umbelliferae family (66), and little is known about the systematics of this biennial species. Investigations on taxonomy of polymorphic species are complicated by numerous semi-cultivated and cultivated forms as well as weedy populations (241). Babb and others list over 389 names for the orange-fleshed varieties (7).

The origin and development of western cultivars of carrots has been documented by several workers (10,109,138, 140,234). They suggested that the original Afghanistan domesticated purple root and a yellow variant spread simultaneously into the Mediterranean region about 1300 A.D. The white and orange root cultivars are mutants of this yellow variant. As far as is known, all cultivated forms were derived from the sub-species D. carota sativus L. (197).

The carrot is a cool-season vegetable requiring vernalization for completion of the life cycle (197). The first major contribution on carrot production, storage and use in the United States was published by Gregory in 1882 (82). Numerous others have since addressed themselves to this subject (5,24,122,126,153,160,197,217,241). The majority of the crop in the Midwest is produced in muck soil. Carrots grown in muck soil generally have a smoother root than those produced in mineral soil and are preferred for fresh market (241). Ninety-nine per cent of the carrots in Michigan are for fresh market (149).

The National Carrot Improvement Program (USDA) and seed companies seek to develop carrot breeding stocks which are disease and nematode resistant, emerge earlier, are higher in carotene, uniformily high in seed set, even-colored, non-bitter and of a suitable size for particular uses in fresh market, freezing or canning (197). Much basic information on carrot physiology and ontogeny is needed to optimize the implication of these goals. Detailed studies of the anatomical changes and two major growth phases of the carrot were reported by Esau (92) and Havis (62). The four main regions in the seedling carrot during primary growth are the meristematic stele, meristematic cortex, meristematic epidermis and the root cap (63). Primary growth is completed with the development of the centrifugal xylem at about eleven days

after germination (63). Secondary growth is derived primarily from periclinal vascular cambium division, first in the xylem and phloem and then in the parenchyma tissue. The hypocotal and the primary root form the storage organ produced by this excessive secondary growth. The carotene or orange appearance is present about 37 days after germination (63).

No extensive study of the growth and development of carrots in muck soil has been published. Carrots respond differentially to soil type, irrigation and fertilization (11,26,82). In 1936, Barnes (11) did an extensive study of carrots grown in mineral soil, but reported information from only two harvest dates during the later stages of growth. He reported that environmental factors such as soil moisture, nutrients, temperature and length of day all affect development, particularly carotene content. The optimal growth range is 10.5 to 20.1 C (126,241), and the shape of the carrot is largely determined by the average temperature during growth. Stress caused by low moisture or nutrient deficiency results in the shape response to temperature being enhanced. Low soil moisture potential results in the production of smaller and more tapered carrots. In a more detailed study of carrots growing in mineral soil, Werner (240) reported the composition of dry matter, sugar and carotene content throughout growth and storage.

Phan and Hau (175) presented a general descriptive analysis of the morphological and chemical changes that agree with morphological changes observed by Esau. Leaves are the first organs to grow actively, reaching 13-18 cm within two weeks. At this time, lateral roots become numerous. In the sixth week of growth, primary root extension occurs. Thirty days later, the primary root begins to enlarge as a storage organ. At this time, root growth is faster than leaf growth. With the onset of root enlargement, a second active extension of leaves occurs, reaching a maximum length of 26-29 cm. The rate of growth reaches a maximum at biological maturity, that being defined as the time when the sugar content remains constant while the carrot remains chemically active in a slow growth process.

Chemical analysis of carrot growth has been studied by a number of workers (37,40,175,190,240). Carotene content, dry matter, and reducing sugars vary with stage of root growth, time in storage, soil type, cultivar and temperature (25,37,57). Muck grown roots are generally higher in ascorbic acid and lower in phenols, reducing sugars, carotene and percentage dry matter than mineral grown carrots, and maintain a brighter orange color with little browning (37, 151). Carotene content is not usually reported to vary with soil type (81,177,241); however, Chipman and Forsyth (37) found that muck-grown carrots have less carotene.

The implications of morphological and chemical properties on carrot breeding were analyzed by Dooker (57). He integrated information on the relative magnitude of genetic, macroenvironmental and genotype-environmental interactions on characteristics of economic importance in carrot production.

Northern Root-knot Nematode

History

The genus Meloidogyne was described by Goeldi in 1887 as the second plant-parasitic nematode known to man. Berkely (17) reported root-knot infection of cucumbers in England in 1855. May (145) observed symptoms of this infection on violets, for the first time in the United States, in 1888. Other early reports of this genus in the United States were made by Neal (157), Atkinson (6), Stone and Smith (214), and Bessey (18,19).

Prior to 1949, root-knot and cyst (Heterodera spp.) nematodes, both which are sedentary endoparasites, were placed in the genus Anguillulina by Goodey (77), later in the genus Ditylenchus by Filipjev, Caconema by Cobb (43), Heterodera marioni, Heterodera radicicola, Tylenchus and Anguillula (116). In 1949, Chitwood (38), after making a morphological study of the root-knot nematodes, removed them from the genus Heterodera and reassigned five species and

one subspecies to the genus Meloidogyne. At this time, he described the northern root-knot nematode, Meloidogyne hapla and stated the morphological characteristics which differentiate the genera. The root-knot nematode causes gall formation, has a soft body as an adult female and deposits the eggs externally in a gelatinous matrix. Cyst nematodes, Heterodera spp., do not induce galls and the female body forms a cyst around the retained eggs. Because of the taxonomic discrepancies prior to 1949, it is difficult to interpret early studies on M. hapla.

Taxonomy and Morphology

As of June 1975, there were 39 described species of Meloidogyne (64). The northern root-knot nematode (M. hapla Chitwood 1949) is a member of the following taxa: Tylenchida, Tylenchina, Heteroderoidea, Meloidogynidae, Meloidogyne Goeldi 1887 (46). Species can be differentiated by electrophoric patterns of body content (103,229), as well as morphological and pathological characteristics (155,242). Triantaphyllou reported that there are two races of M. hapla, Type A and Type B. Type A has 15 to 17 chromosomes and reproduces by amphimixis and meiotic parthenogenesis. Type B has 45 chromosomes and reproduces by mitotic parthenogenesis (227,228). Others have reported the existence of physiological races based on ability to feed and reproduce on a particular host (58,80,163,164,180,192,193,230).

Genetic variability of populations has also been associated with climatic adaptation and temperature tolerance ranges (54).

Detailed morphological characteristics of M. hapla were compiled by Whitehead in 1968 (46,242). The perineal pattern of M. hapla was first described by Taylor et al. (218). The histology of M. hapla has been studied by Elsea (62) and ultra structure details of the female body wall by Ibrahim et al. (106), larval cuticle by Ibrahim and Hollis (107), as well as intestinal ultrastructure by Ibrahim (105).

Life Cycle

The life cycle of M. hapla involves the egg, four larval stages and the mature female and male. Eggs are deposited outside the body in a protective gelatinous matrix, frequently embedded in the host roots (94). They undergo embryogenesis forming the first-stage larva (227). A molt occurs and the second-stage vermiform larva emerges as the infective stage (250). Second-stage larvae migrate in the soil to roots. They penetrate the subapical meristematic region of the root and become embedded in the vascular parenchyma of the stele. Penetration is acquired by repeated thrusts of the stylet, lip suction and chemical interaction on the cell walls. A larva feeds on the cells it penetrates by intercellular and intracellular movement (59,116,134).

This stage initiates lysogenous growth in the plant and second stage larvae become saccate. Two more molts occur, forming increasingly saccate third and fourth-stage larvae. With the final molt, the reproductive systems are mature, forming the didelphic swollen female or the monorchic vermiform male (70). Sex reversal, induced by environmental or chemical stress, can occur in the prefemale up to two-thirds of completion of second-stage development. A diarchic vermiform male results from this sexual reversal (226).

Distribution and Hosts

The northern root-knot nematode has a worldwide distribution and is indigenous to Michigan and other temperate climatic regions (27,46,99,155). It has a cosmopolitan distribution (208,237), including many hosts of major economic importance: nearly all vegetables, clover, lucerne, groundnuts, soybean, pyrethrum, coffee, cotton, maize and watermelon (27,46,78,239). Many weeds are also hosts of *M. hapla* (171,225). Plants that are resistant to the northern root-knot nematode include many grasses and cereals (16, 71). There has also been considerable breeding of resistant cultivars of host plants, although with limited economic success (1,36,47,79,85,100,102,104,152,165,171,210,216).

Symptomatology

There are both internal and external symptoms of *M. hapla* infection. External primary symptoms include the

presence of medium size galls, with or without proliferation of the nearby roots. Secondary external symptoms include reduced yields, wilting, stunted plants, yellowing of the foliage, premature death, delayed maturity, and poor bloom (14,27,191,205). The internal symptoms include lysogenous growth initiated by second-stage larvae, hypertrophy and hyperplasia.

M. hapla-induced lysogenous growth has been described in detail by several workers (55,173,205,221,223). It is reported that upon embedding in the pro-vascular parenchyma region, hypertrophy is initiated near the nematode head. This results in the formation of 1-4 transfer (giant) cells characterized by dense cytoplasm, multinucleation, and thickened cell walls. Host tissue reaction to penetration, feeding and subsequent synctial development is well documented (21,37,60,98,99,173). Although the nematode invasion takes place, development of the nematode to maturity does not occur unless transfer cells are formed. Electron microscopic studies report the breakdown of these transfer cells soon after the female ceases egg production (173).

Host hyperplasia responses occur near the posterior region of the nematode body (173). The chemical stimulus reaches several hundred cells and results in the formation of galls which are the primary symptom of the northern root-knot infection (173). The exact chemical mechanism of

initiation of hypertrophy and hyperplasia has not been defined. It is reported that galls are detectable prior to synctia formation and are the result of cortical hyperplasia (60,173).

There are two theories about syncytia formation. One maintains that initially a dissolution of a few provascular or cortical cells occurs near the nematode head followed by a coalescence of cytoplasms and organelles and the deposition of a single, thick secondary cell wall (21,60,173). The second is that syncytia arise from single cells through hypertrophy, and repeated karyokinesis without cytokinesis (98,99,173).

Ecology

Penetration by the infective second-stage larvae is variable. Bird and Wallace (22) found that only 2.9 per cent of a high population (60,000) entered tomato roots within 48 hours. Kinlock and Allen (125) checked tomato roots after 10 days and reported entry of 65.3 per cent of an introduced population of 125, and 47.3 per cent of an introduced population of 1,000. The time for penetration of carrots and onions has been reported as 24 hours (27,205). Smith and Mai (205) also stated that more than one larva may enter the root at the same site. The associated root galls are detectable in 1-9 days after root entry (205).

Duration of the life cycle is dependent on environmental conditions as well as host association. The shortest reported life cycle was 19 days on tomato (232). The longest was 72 days on onions under Michigan field conditions (27). Tyler (232) and Wong and Mai (251) reported temperature influences on life cycle duration. Wong and Mai stated for lettuce at 21°C, the life cycle was completed in 54 days, while at 32°C it only took 20 days. Brody (27) reported a life cycle of 45 days for carrots, 72 days for onions and 56 days for celery, all under greenhouse conditions.

The reproductive potential of M. hapla has been studied by several workers under various conditions. Host association and environmental factors influence the rate of reproduction (22,85,222,250,251). Hendricks et al. (94) reported an average of 467 eggs per mass. Tyler observed that one female deposited 2,882 eggs "without becoming exhausted" (232).

The cold temperature tolerance of M. hapla has been studied widely. Berfeson (16) reported that eggs and juveniles survive better at 0°C than those of other Meloidogyne spp. Daulton and Nesbaum (54) found that eggs were viable after 250 days of field temperatures which reached 0°C. Sayer (194) reported that juveniles survived freezing to -7°C in salt solutions better than M. incognita. He suggested that it was due to dessication of the nematode with the resultant suppression of ice crystal formation point

within the nematode, influencing the survival in temperate climates. He also reported that in Ontario, Canada, winter conditions tend to reduce the field population by 75 per cent. Other studies suggest even greater cold tolerance (27,53). In Michigan and other temperate climates, the overwintering form is the egg (27,99,194,223). Elsewhere the second-stage juvenile is also an overwintering form (46,223).

M. hapla has a lower tolerance to high temperatures than other Meloidogyne spp. (22,54,221). Optimum temperatures for M. hapla are reported as: hatching, 25°C; mobility, 20°C; invasion, 15-20°C; growth, 20-25°C; and galling, 25-30°C (22,88,110,160). In muck soil, Wong and Mai (251) reported optimum day-night temperatures for movement and invasion as 21.1 and 26.7°C.

Disease Complexes

M. hapla is a predisposition agent for bacteria, fungi and other nematode disease agents. In bacterial associations, increased severity and increased incident of disease are reported as well as weakening and breaking of host resistance (84,87,101,117,133,176,213). Similar results are reported for M. hapla and fungal associations (28,44,115, 118,137,179,215,219). The results of interaction studies between M. hapla and other plant-parasitic nematodes are varied. Johnson and Mausbaum reported that M. hapla suppressed reproduction of Pratylenchus brachyurus (114).

In sugar beets, Heterodera schachtii and M. hapla develop independently, producing their own characteristic pathological changes of tissue (119). Griffin reported that Ditylenchus dipsaci was a predisposition agent for M. hapla on lucerne (85). Kinlock and Allen (125) observed that in mixed populations of M. hapla and M. javanica on tomatoes, M. javanica dominated.

Economic Losses

Economic losses attributed to known population densities of M. hapla have been reported for some crops and specific cultivars. Olthof and Potter (167) from Ontario, Canada, reported the following crop losses at a population density of 18,000 M. hapla per kilogram of soil: cabbage, 9%; cauliflower, 24% with delayed maturity; lettuce, 46%; potatoes, 46%; and onions 46%. Norton (162) reported a 36% loss to lucerne. Copper (48) reported a 70% loss in groundnuts. Sugar beet losses of 20% were attributed to M. hapla infection by Grunjuic and Paunovic (89). The amount of damage varies from case to case, but with severe infection, nearly total crop loss can result.

Control

Control methods for nematodes generally require a multiphase program including exclusion, population reduction, use of resistant varieties and protection practices. Protection

practices involve the establishment and enforcement of quarantines, as well as the use of certified stock and clean cultural practices. In undisturbed field soil conditions, nematode movement is limited to about 100 cm per year (211). Exclusion practices include the use of hot water or chemical root dips especially for ornamentals (51,52,91,93,142,143, 235). Chemical soil fumigants are reported effective for field conditions (67,120,132,158,182,185,188,244,245). Chemical fumigation is most effective when incorporated with crop rotation since no fumigation process eliminates an entire nematode population (27,48,95). More effective control will probably be obtained by breeding resistant crop cultivars (46). Few resistant cultivars are developed at present. Biological control possibilities include increasing populations of trapping fungi, parasitic bacteria and protozoans, and predaceous nematodes in the soil (108,156).

Northern Root-knot Nematode and Carrot

Northern root-knot nematode infection of carrots is economically significant and of worldwide distribution. The economic loss is defined in terms of direct yield reduction as well as increased production costs attributed to control methods. Ritter (184) reported a five per cent annual loss in Southern Europe and in the Mediterranean region. He included the losses in commercial exchange due to quarantine

and inspection measures, as well as research funding on the parasite physiology and resistant cultivar breeding. Other European countries have also reported infection of carrots. The pathogenesis and distribution of this nematode has been extensively studied in Poland (31,94,194,209). Grujicic and Paunovic (89) estimated a 20 per cent loss in Yugaslavia. Linhardt and Bagger (135) observed severe attacks in Denmark. Anderson (2) reported that M. hapla is the only root-knot species naturally associated with carrots in Sweden. Hahn (90) noted a 10 per cent galling of carrots on light soil at Maize, Germany. In Rhineland, Germany, M. hapla infection of carrots was reported in 1972 (76). Several different carrot production areas of Russia are also infected. Tulaganov and Aheptal (231) found infected carrots on five collective farms in Samarkand, Uzbekistan, and Karemova (123) reported infestation in the Tashkent region. Other regions infested include the Alma Ata region (187) and the Turkmenia region (203). Brown (29) reported that infections cause complete crop failures in Great Britain. Similar situations have been found in Israel (45), Japan (166), New South Wales (3,4) and New Zealand (83).

In the Western Hemisphere, severe infestations were reported in Brazil by Petenucci (204). The Ontario and Montreal vegetable growing areas of Canada are also infested (167,168,169,170,204,222,225). Townshend (225) stated that

M. hapla is the most important nematode associated with carrots in Ontario. In the United States, nematodes cause an annual estimated 20 per cent loss to the carrot industry (206). Brody (27) reported that even at low population densities M. hapla is a major economic problem of Michigan grown carrots. Wilson (248) found a 50 per cent loss with M. hapla infection increase from 5 per cent to 93 per cent in Ohio muck fields. This nematode is also significant in Arizona (181) and New York (199), and it can be assumed that M. hapla is the most important nematode problem of carrots in the United States (241).

The symptoms of M. hapla infection of carrots are well documented. Primary symptoms include the formation of galls on both the primary and secondary roots. This is associated with proliferation of nearby roots and branching or malformation of the primary root (4,14,15,30,49,246,248). Secondary symptoms most often observed are yield reduction, hairy root (246) and death under heavy infestation (14). There are conflicting reports on the infection of the hypocotyl storage area and tap root (4,14,15,30,49). Wilson (248) reported that the degree of infection has no influence on top growth, but wide variability in the amount of root injury. Brody (27) observed galling symptoms are most frequently followed by a split tap root and then the hairy root condition. The secondary internal modifications are assumed to

be the same as observed in other host plants of M. hapla.

The life cycle of M. hapla is similar to its development on other hosts. Brody (27) reported that it is a 45 day life cycle on mineral soil grown carrots, and Okada (166) found that three generations of M. hapla occur from May to October with soil temperatures ranging from 15°C to 29°C. Only one generation developed from October to May with a temperature range of 0°C to 15°C. He also reported that no larvae hatched at temperatures below 9.5°C. Hendrick et al. (94) noted that in Poland two life cycles are completed annually, the first in 9 to 13 weeks. He also found that egg masses had an average of 467 (range 25 to 1,337).

Brody (27) reported that most of the invading second-stage larvae penetrated carrot roots within 24 hours; however, for unknown reasons, only 0-4 per cent of the introduced population entered the root. The penetration occurred adjacent to the root cap and, after migration to the provascular region, the second-stage larvae oriented themselves with their anterior ends toward the distal terminus of the root.

Stein (212) studied the spread of M. hapla associated with carrots grown under field conditions. He found that during the first year horizontal movement of the nematode population was 5 to 6 cm. In the second year, this increased

to 10 to 15 cms. When M. hapla was associated with lettuce, horizontal movement was greater than 100 cm in two years.

The influence of the soil environment on M. hapla associated with carrots was studied by Wilson (248). He found that at a pH of 5.3, 25.3 per cent of the carrots were infected while at a pH of 4.5, 26.6 per cent were infected. This represented a 23 per cent differential in the yield. He concluded that little or no correlation existed between high and low levels of soil nutrients and the per cent of nematode infection on different crops. Shubina (200,201, 202) reported that the use of mineral fertilizers increased the number of some nematodes in carrot fields; however, none of these were phytopathogenic species.

Several workers have investigated chemical variation in infected and healthy carrots (41,127,129). They all refer to their investigations as studies on the defense mechanism of the carrot against M. hapla. Knypl et al. (127) showed an accumulation of IAA-oxidase inhibiting compounds in infected roots. Peroxidase activity in the storage root and the fibrous side roots also increased in response to M. hapla infection. There was a greater concentration of phenols and chlorogenic acid in the galled side compared to the healthy side of the roots. They suggest that local increases of auxin concentration following inhibition of IAA-oxidase by chlorogenic acid may be a factor

responsible for induction of root tissue growth and gall formation around the penetration and feeding sites of M. hapla.

Chylinska et al. (41) suggested that stunted growth and subsequent branching of the storage hypocotyl root may be caused by the inhibition of protein synthesis in the terminal part of the primary root. They also found that the overall response of the plant to M. hapla is increased protein and RNA content. There is no effect, however, on protein or RNA synthesis in the hypocotyl storage root of carrot, although the total content of RNA is increased.

Knypl and Janas (129) investigated a tolerant and a susceptible variety, finding that in comparison to healthy carrots both cultivars had: (i) RNA and protein concentrations highest in galled secondary roots, (ii) the ratio of ^{14}C-uracil incorporation into RNA was highest in galled roots and (iii) radioactive protein was lower in galled roots than in all the other tissues. In the tolerant cultivar, galled secondary roots had: (i) a doubling of the RNA synthesis rate with a constant RNA concentration, (ii) a 70 per cent increase in protein content and specific radioactivity was lowered by 60 per cent, and (iii) RNase activity per mg protein decreased by 80 per cent. In the sensitive cultivar, galled side roots compared with healthy roots had: (i) RNA and protein contents increased by 20 and

30 per cent respectively, (ii) RNA synthesis was stimulated; whereas, specific radioactivity (ct/min/mg fresh wt) of protein was not modified, and (iii) the specific activity of RNase was halved. In the secondary vascular tissue of the storage root of infected carrots, the tolerent cultivar had RNA synthesis inhibition. Protein synthesis was stimulated and RNase activity decreased, compared with healthy plants. In the sensitive cultivar, corresponding tissue had no change in specific activity of RNA and protein with an increase in RNase specific activity. Infection, resulting in gall formation, also resulted in accumulation of RNA in infected tissue. The RNA increase is attributed to increased synthesis, as well as decreased RNA breakdown and protein accumulation.

Control of M. hapla infection of carrots has been investigated by more researchers than any other phase of the pathogenic association. One control method is the use of tolerant or resistant cultivars. Investigations of tolerance of various cultivars have been conducted. Safrygiva (187) stated that "lwlnyaya lyribimitsa" is a resistant variety while "Shantene 2461" had a 67 per cent infection of roots. Berbec (13) stated that "Namtejska(Nantes)" was least affected by M. hapla while a forage type, "St. Valery", was most affected in Poland. Brzeski (34), another Polish investigator, worked with 13 cultivars in field and

greenhouse studies giving the mean per cent branched roots for various cultivars including: Danvers half long, 20%; Slenders, 18%; and Nantes, 27%. He also noted that nematode population density increase in pots of the various cultivars was not significantly different.

In the United States, Wilson (246) screened 35 cultivars in Ohio muck soil under field conditions. He concluded that very little cultivar resistance to nematode infection occurred. He noted little correlation between the growth shape of the cultivar (long or short) and the per cent of malformation. He concluded that early or forced cultivars were more susceptible to M. hapla. Clark (42) screened 222 carrot cultivars, for multiple disease and M. hapla resistance, finding three numbered lines with multiple disease resistance potential.

Brzeski (34) stated that differences in cultivars can be attributed to different degrees of nematode attack and development, or to a specific reaction of different cultivars. He found no correlation between penetration and population development of nematodes with the degree of branching of carrots, suggesting that the degree of branching is related to physiological difference among the cultivars, as supported by Knypl (129). He further speculated that the degree of branching of carrot roots is an inherent characteristic.

Other methods of control include crop rotation and fumigation. Most nematicide tests have been conducted in the Western world. Wilson (247) studied the effect of 20 different nematicides in muck soil. The most effective control was attained using ethylene dibromide at 9 and 12 gal/acre, methyl bromide, chloropicrin, and 1,3-D-(1,3-dichloropropene,1,2-dichloropropene) at 30 and 45 gal/acre. Cohn et al. (45) supported this work by reporting that EDB (ethylene dibromide) and DBCP (1,2-dibromo-3-chloropropene) increased yields up to 77.6 per cent and marketable produce up to 142.6 per cent in Israel. Sherf and Stone (199) found good control on muck in New York with 1,3-D at 40 gal/acre and EDB at 6 gal/acre. They reported poor control with N-methyl dithiocarbamate dihydrate at 25 gal/acre. Renolds (224) found carrot yields increased three and four fold in sandy loam and coarse textured soils of Arizona by using EDB at 4.5 and 6 gal/acre, respectively. Lear et al. (131) tested 17 nematicides for yield reduction and tainting. They noted reductions with 100 lbs applied zinc trichlorophosphate 32 per cent (Dow 9B), D-D mixtures, Dowfume N and dichlorobutene. Taint occurred with D-D and Dowfume N after one and two years application.

Workers outside the United States report similar findings. Petenucci (174) noted good control in Brazil using DBCP. Yields were depressed at high concentrations of DBCP.

Weisher (238) and Hahn (90) reported on nematicide tests in Germany. Weischer stated that 1,3-D was not effective in high humus soils due to the persistent nature effecting subsequent crops. Hahn stated that in light soil, Vapam at 100 cc per sq m was more effective than 90 cc 1,3-D, although Vapam, at that rate, resulted in a yield depression.

It is notable that bromide fumigants may be phytotoxic to some poor host crops of M. hapla used in rotation with carrots such as onions (156). Petenucci (174) and Olthof and Potter (169) both suggest that, although fumigation is an expensive process, economic returns exceed cost.

The most economical means of control is by crop rotation. Continuous growing of carrots results in a build-up of the population of M. hapla (3,27,112,238,249). Other crops frequently reported used in sequence with carrots which increase the nematode population density include celery, parsnip, potato, mint and chickory (27,30,97,146,212,248). Crops reported that reduce population density and subsequent carrot infection rate include radishes, onions, grasses, sweet corn, turnip, rape, summer barley, rye and other small grains (4,27,30,33,212,248,249).

In summary, it was noted by Jacob (112) that population densities of larvae from soil do not give a complete picture of effects of preceding crops because many nematodes are removed with host root crops such as carrots and chickory.

Weischer (238) suggests that individual agriculture practices alone will not give optimal reduction of crop damage; however, proper rotation can prevent further build-up of new populations. He suggests the need for an integrated pest management program for nematode control.

Vesicular-Arbuscular Mycorrhizae

In 1885, Frank (69) coined the term mycorrhizae for the "fungus-root" structure he observed. Marx (144) defined this as a symbiotic-parasitic association between specific fungi (symbionts) and roots, rhizomes, or thalli of plant hosts in which both associates normally benefit from the relationship.

Mychorrizae are classified into three types: ectomychorrizae, endomychorrizae and ectoendomychorrizae (74,144). Ectomychorrizae are characterized by a root-fungus association in which hyphae form a mantle around the root and by the harteg net which is a network of hyphae encircling the root cortical cells intercellularly. Endomychorrizae are characterized by hyphae of the fungal symbiont being found intracellularly in the plant cortical tissue. In the 1975 publication of the proceedings of a symposium on mycorrhizae there is a detailed review of information available on endomycorrhizae covering evolution, classification, culturing,

physiology, fine structure, effects on growth, ecology and biological interactions (189). Ectoendomycorrhizae are characterized as having features of both the ecto- and endo-type; a fungal mantle is present as well as intracellular hyphal penetration in root cortical cells. Very little is known about this third form (144).

The endomycorrhizae are subdivided into two groups, on being septate or nonseptate fungi (75). Vesicular-arbuscular (VA) mycorrhizae are the nonseptate type (74) found almost ubiquitously on plant roots (74). Gerdemenn (74) reported that VA mycorrhizae colonize most plants important to agriculture. They function primarily to enhance water and nutrient uptake by the plant.

Fungi Endogonaceae produce VA mycorrhizae. The hyphae may be found on the root surface, but not in sufficient quantity to produce a mantle (124). Nicolson (159) described the hyphae in the soil as dimorphic and composed of thick-walled nonseptate hyphae with small thin-walled lateral branches. Vesicles and large thick-walled spores are borne in the soil (74). Variable sized hyphae are present within the plant cortex, but the stele is not infected (113). Multibranching of hyphae within the cells is defined as arbuscules. These are suggested to be functional as micro-haustoria. Vesicles are formed intercellularly and intracellularly serving as food storage organs (73).

VA mycorrhizae colonization does not significantly modify the external part of the root (72).

Much work has been done indicating that VA mycorrhizae increase plant growth (50,111,183,186,189). Several workers indicated that VA mycorrhizae may be functional in pathogen determent (68,183,186). No work has been reported for mycorrhizae colonization of carrots or the possible interaction of M. hapla and mycorrhizae. Several studies indicate that VA mycorrhizae and plant-parasitic nematodes are both ubiquitous to crop plants (74,141,183,189).

MATERIALS AND METHODS

Ontogeny of Spartan Premium Carrot

Growth Conditions

Spartan Premium, a new hybrid carrot cultivar almost ready for commercial introduction in Michigan, was selected for this investigation. In field trials, this cultivar appears to have resistance or tolerance to M. hapla (8). Ten seeds (1974 source 74W278) were planted in each of 250 containers of muck soil. Four different sizes of plant containers were used to minimize greenhouse space and maximize the volume of soil available for root development. Fifty six-inch clay pots were used for plants to be harvested during the first 28 days after seeding. Plants to be harvested from day 28 to 48 were grown in eight-inch pots, while plants for day 48 to 68 were grown in ten-inch pots. Plants harvested during the last 32 days were grown in drainage tiles (14.8 cm in diameter X 32.2 cm in depth) filled with approximately 4.5 liters of soil. All pots were cleaned and steam sterilized for 2 hours prior to use.

The muck soil used in this investigation was obtained from a Grant, Michigan, carrot field infested with M. hapla.

The initial population density of M. hapla was 5 second-stage larvae per 100 cm^3 soil, as determined by the centrifuge floatation technique (207). Half of the containers of each size were filled with steam sterilized (3.5 hrs) muck and the other half were filled with field soil. The soil analysis for sterilized and infested muck were the same (pH 6.8, 66-73 ppm insoluble salts, 54-67 ppm nitrates, 1.3-1.7 ppm magnesium). The per cent total salts were: 11.7-13.2 nitrates, 0.9-2.0 potassium, 17.2-17.3 calcium and 4.5-4.9 magnesium. The soil was characterized as a coarse aggregate of organic material.

To assure nematode infection, 5 ml water suspensions of 100 second-stage larvae of M. hapla per container were added to the non-sterilized soil at the time of planting. It was added directly onto the seeds. The M. hapla innoculum was obtained by using a standard shaker technique for nematode extraction (207), using celery from culture boxes of M. hapla maintained by the Michigan State University Nematology Laboratory.

The carrots were maintained under 18 hours of light and watered daily. Greenhouse air temperatures ranged from 12 to 33°C with an estimated mean of 21°C. Humidity ranged from 0 to 40 with an estimated mean of 30. No additional fertilizer or pest control chemicals were applied prior to planting or during the 100 days of growth. The plants were

thinned to three plants per pot on day 14 and to one plant per pot 21 days after seeding. Nematode loss due to thinning was assumed negligible.

Harvesting Procedures

Four replicates grown in the sterilized muck and four from the infested muck were randomly selected from the specified container size group every 96 hours. The roots were washed and prepared for analysis as outlined by Schuurman and Goedewaagon (196). All of the soil and root systems were removed from the pots intact, and individually soaked for several hours in cool water. Adhering soil was then carefully washed from roots, beginning with the lower portion of the roots, using a gentle stream of cool tap water. The remaining debris was removed from the roots using forceps. The plants were blotted, wrapped in moist paper towelling and stored at 5-7°C in a closed plastic bag. Plant analyses were made within 96 hours after washing. Carrots harvested on days 80 and 84 were maintained with adequate moisture at 5-7°C for 8 and 4 days, respectively, prior to harvesting.

Evaluation Procedure

Each carrot shoot system was evaluated for number of leaves, height, fresh weight, dry weight and area. Each root system was evaluated for root area, number, order, and length, and galling, fresh weight, dry weight and economic value index.

Leaf and root area measurements were made with a Li-Cor Model Li-3000 Portable area meter with the Li-3050A transparent belt conveyor accessory. The secondary roots were spread as thin as possible on the belt, and an average of three readings was recorded. Each shoot system was divided into individual leaflets and stems and evaluated for area. These readings should be close approximations of the actual plant surface areas. Beginning with day 36, area of the enlarged storage root was determined by using the area formula of a right cylinder or a frustrum of a cone.

Root order, number, and length were obtained using two different methods. During the first 36 days, the entire root systems of all replicates were evaluated. Because of the extensiveness of the root system and the time required for evaluation, after day 36, only an "average carrot" selected from the replicates was analyzed. The tap root was divided into tenths and the second order root closest to the division was removed for evaluation. The total number of roots of each order and their respective lengths were then calculated, based on the analysis of those second order roots in relation to the total number of second order roots on the plant. Root evaluation was made using a tray with an attached grid (Figure 1.1). The roots were floated in a thin film of water. This prevented desiccation and facilitated separation and analysis of the roots.

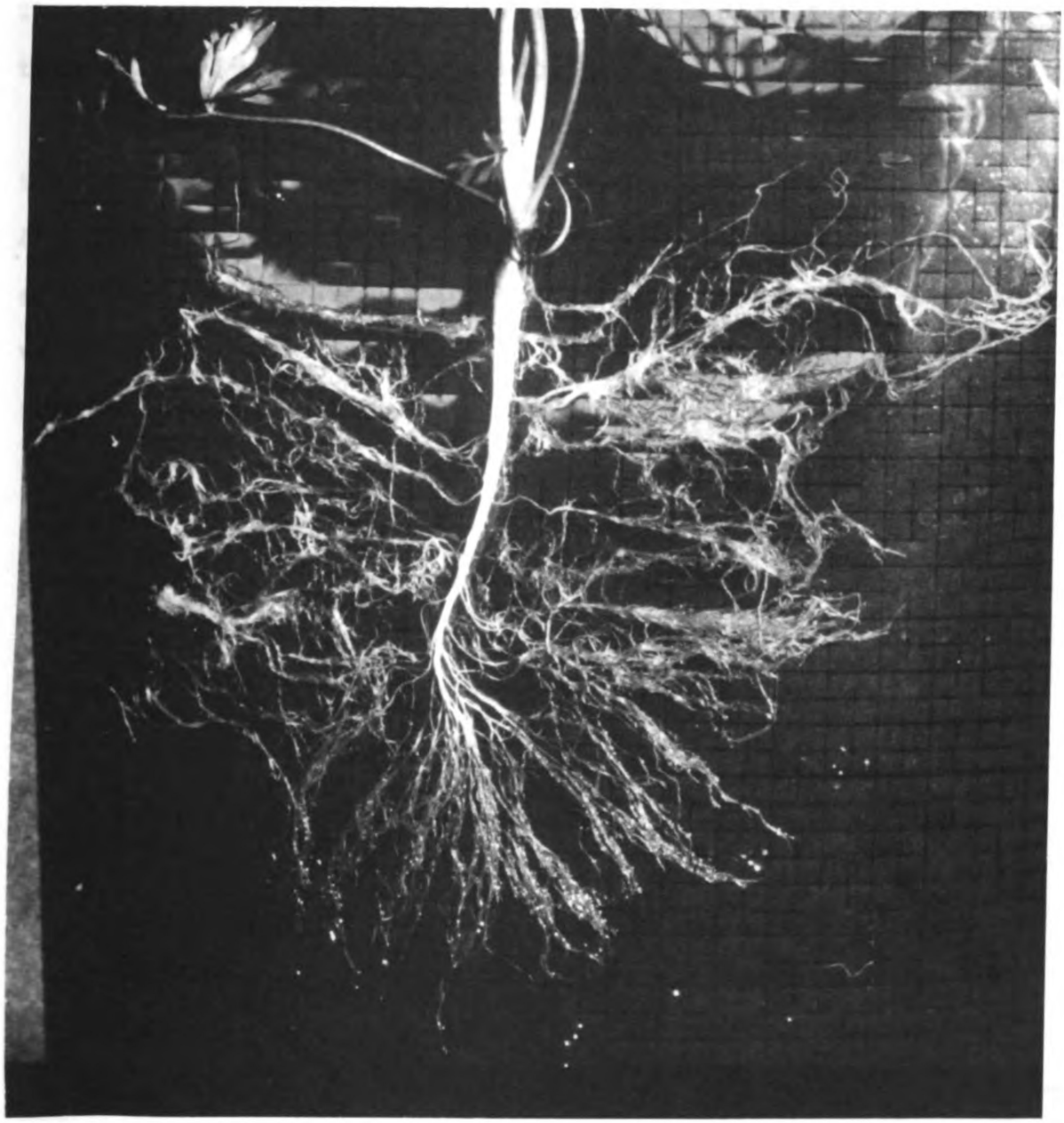

Figure 1.1. Forty-eight day old Spartan Premium Carrot root system on centimeter measuring grid.

All root systems were evaluated for nematode galls. This was done by floating the roots on a dark surface in a thin film of water and counting the galls.

Fresh weights of the root and shoot systems were obtained by direct weight in a preweighed and dried crucible. A four-place Mettler balance was used. Dry weights of the shoot and root systems were obtained by drying to a constant weight (± 0.1 mg for the first 36 days and 1.0 mg for the remaining harvest days) at 105°C.

Storage roots were graded for economic value, beginning 52 days after seeding. The economic index is based on the per cent of carrots deformed beyond fresh market use. Roots prior to day 52 were analyzed only for the location and number of galls on the primary root.

Pathology, Distribution and Population Density of Meloidogyne hapla

Growth Conditions

Gold Pak, an open pollinated cultivar, that is highly susceptible to M. hapla infection, and Spartan Premium, a new experimental hybrid cultivar which appears to have tolerance to M. hapla, were selected for this investigation. They were subjected to initial population densities of 0, 10, 100, and 1000 second-stage larvae of M. hapla.

One hundred and twenty-eight tile pots (14.8 cm in diameter X 32.2 cm depth) were filled with steam-sterilized muck soil, as described for the ontogeny study. Ten seeds (Gold Pak 1971 source 411027 or Spartan Premium 1974 source 74W278) were planted in each container. The M. hapla inoculum of 10, 100, or 1000 per container was introduced at planting, directly around the seeds, in 5 ml, 5 ml and 20 ml water suspensions, respectively. The containers were maintained in groups of 16 for each population density of each cultivar.

M. hapla inoculum was obtained by the shaker extraction technique (207), from celery grown in culture boxes of M. hapla maintained by the Michigan State University Nematology Laboratory. A perineal pattern of one nematode was used to confirm the species identification.

The plants were maintained under greenhouse conditions for 120 days. The air temperature ranged from 12.2 to 44.5°C with an estimated mean of 21.6°C, and the humidity ranged from 0 to 60 with an estimated average of 20. The carrots were grown under 18 hours of light and watered daily. No preplant or seasonal growth fertilizer was used. Pest control for red mites, aphids and white flies was achieved using nicotine, Pyrellin, Sevin, Plectron, Plant Fume 103, and Malathion (Appendix A). Plants showed symptoms of tip burn after fumigation with Plant Fume 103.

All of the containers were thinned to three plants on day 14 and again to one plant on day 21. Nematode loss in thinning was assumed negligible.

Harvesting and Evaluation Procedure

Four replicate plants grown under each initial nematode density for each cultivar were harvested every 30 days. The entire plant and all of the soil were removed from the containers. Rhizosphere soil was defined as that soil immediately around the root system. The total plant and rhizosphere soil were weighed. The rhizosphere soil was then washed from the roots into a 12 liter pail with 8 liters of cool water. The roots were blotted with paper towelling and reweighed. The galls present on the root systems were evaluated by spreading roots on a dark surface in a thin film of water and counting. The nematodes were extracted from the roots using the standard shaker technique for 72 hours at 125 rpm (207). A 400-mesh screen was used for collection of the nematodes.

Nematode counts were made for the rhizosphere soil and for a 100 g sample of the remaining pot soil. The need to study the distribution of M. hapla in the carrot environment is based on a study by Hogger and Bird (96) of the distribution of M. incognita in Cypres, and Pratylenchus brachyurus in Glycine max and Sorghum halepense. They emphasize the

importance of separate root and rhizosphere soil analysis for nematodes. As in their study, all nematode population densities were evaluated for root, rhizosphere and soil populations. The centrifuge floatation technique was used for the soil analysis. A 400 mesh screen was used for collection of M. hapla. Samples were stored at 5-7°C until microscopic quantitative population estimates were made.

The shoot system of each carrot was evaluated for fresh weight and height. All weighings were made on a one-place Mettler balance with a ± 0.1 g.

Cultivar and Parent Line Susceptibility to Meloidogyne hapla

Growth Conditions

Fifteen carrot cultivars and parent lines with known field test ratings for M. hapla resistance were selected for greenhouse evaluation for susceptibility to M. hapla (Table 1.1). Ten seeds (1975) of each of the cultivars and parent lines were planted in four replicate ten-inch pots of steam sterilized (4 hours) muck soil. A 5 ml water suspension of 100 second-stage larvae of M. hapla was added to each pot directly around the seeds at the time of seeding. Greenhouse growth conditions, watering, fertilization and pest control were the same as outlined for the population dynamics study (p. 29).

Table 1.1. Cultivars and parent lines evaluated for *M. hapla* susceptibility.[1]

Cultivar/Parent line	Source	Field Rating[2]
M 5986	411008	T-R, with some S
M 5988	72W161	T-R, most are R
M 5987	GR 66/67	S, most are S
M 3489	C923/13238	S-R, most are S
Gold Pak	411027	S, typical susceptible to *M. hapla*
Danvers	410007	S, typical susceptible to *M. hapla*
Spartan Bonus	40119L	R-T, some are S
Spartan Fancy	43129	S-T, most are S
Spartan Delite	43123	T-R, a few are S
Spartan Classic	411056	R-T, some S but most T-R
Spartan Premium	74W278	R-t, most are T-R
Spartan Winner	74W271	S, most are S
Spartan Delux	73W38	T, segs S to R
(1304M/872)-1-S-CM	Ca416/16657	seg. more R than S
(1304M/872)-1-M-CM	C418/16656	seg. more S few R

[1]S = susceptible; T = tolerant; R = resistant; seg. = segregating population.

[2]Based on reference (8).

After 60 days of growth, the soil was washed from the plant roots and each root system was indexed for galling caused by M. hapla and economically indexed based on the per cent of tap root deformed beyond fresh market use. The following gall index was used:

Gall index	Fraction of root system galled
1	0
2	>0 - 1/10
3	>1/10 - 3/10
4	>3/10 - 7/10
5	>7/10 - 9/10
6	>9/10 - 10/10

M. hapla were extracted from the roots using the shaker technique (206) at 125 rpm for 72 hours. The nematodes were collected using a 400 mesh screen, and stored at 5-7°C until quantified by microscopic observation.

Mycorrhizae Investigation

Ten seeds of Gold Pak (1971 source 411027) carrots were planted in each of forty ten-inch pots of steam-sterilized muck soil. One hundred ml of Glomus macrocarpus var. geosporus-infected sand was added to half of the pots at planting. A centrifuge floatation extraction of spores, using heavy sugar, indicated the inoculum level was approximately 12,000 spores of Glomus sp. per 100 ml of sand. The carrots were grown as outlined for the population dynamics study (p. 29).

Five replicate plants of each the infected and non-infected muck-grown carrots were harvested 30, 60, 90, and 120 days after planting. The root systems were washed and stained using a modification of the method outlined by Bird, Rich and Glover (23). The modification consisted of five minutes of staining and five minutes for destaining. Microscopic observations were made to evaluate the mycorrhizal infection of the roots.

RESULTS AND DISCUSSION

Ontogeny of Spartan Premium Carrots

Results

Shoot system.--Shoot emergence occurred by the eighth day after seeding. Meloidogyne hapla infection retarded the average number of leaves and average shoot height of plants. The noninfected mature carrots had 10 leaves while the infected had an average of 8.4 leaves, although this was not a significant (P=0.05) growth retardation (Figure 2.1). M. hapla infection, however, significantly (P=0.05) retarded shoot height from 12 to 52 days after planting (Figure 2.2). The maximum average height of mature Spartan Premium carrots grown in the absence of M. hapla was 47.1 cm by the 84th day of growth. In the presence of M. hapla, the maximum height was 44.8 cm by the 96th day of growth.

The shoot surface area was 41 per cent less in the M. hapla infected carrots than those grown in the noninfested soil. This significant (P=0.05) retardation was observed 12 days after seeding and continued through the 88th day of growth (Figure 2.3). Maximum average shoot surface areas for nematode-free and M. hapla infected carrots were 1306 cm^2

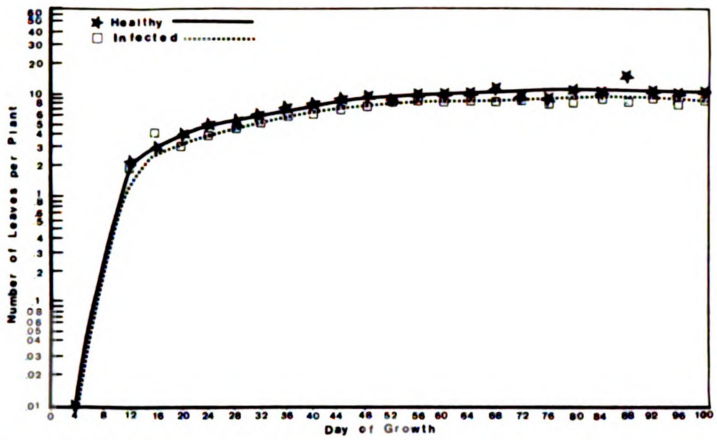

Figure 2.1. Number of leaves per Spartan Premium carrot infected with M. hapla.

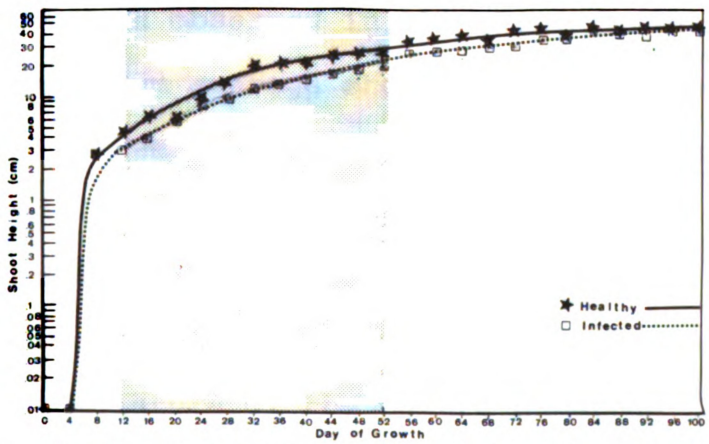

Figure 2.2. Shoot height of M. hapla infected carrots was significantly (P=0.05) less than noninfected Spartan Premium carrots days 12 through 52.

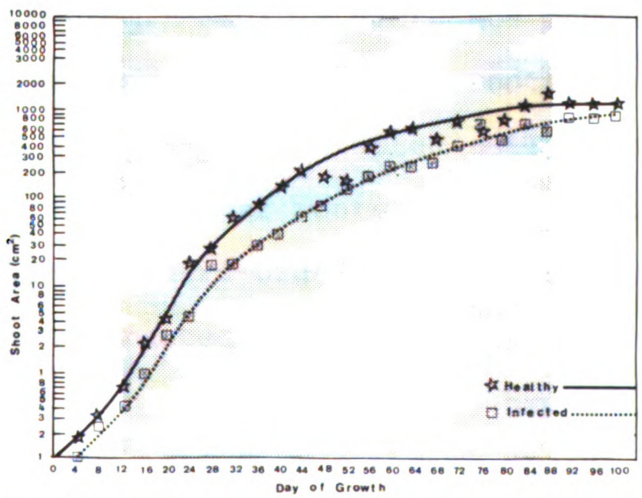

Figure 2.3. Shoot area of M. hapla infected Spartan Premium carrots was significantly (P=0.05) less than noninfected carrots days 12 through 88 after planting.

by the 88th day, and 769 cm^2 by the 100th day of growth, respectively.

M. hapla infection of Spartan Premium carrots resulted in a significant (P=0.05) 48 per cent retardation in shoot dry weight (Figure 2.4). This difference was evident from day 16 to 88. The reduction is reflected in maximum weight differences of 4.69 g and 2.45 g at 100 days after planting. The same trend is observed in the shoot fresh weight (Figure 2.5). The maximum average shoot fresh weights were 31 g on day 84 for the nematode-free plants and 23 g on day 96 for the M. hapla infected carrots. This was a 36 per cent reduction in growth.

Using the fresh and dry weights of the shoot system, the per cent moisture of the mature nematode-free carrots was 84.9 per cent. The infected carrots' shoot system had 89.4 per cent moisture.

Root system.--Radicle emergence occurred by the fourth day after seeding. Second-order roots were observed by the 8th day in the M. hapla infested soil, while noninfected plants had second-order roots by the 4th day after seeding (Figure 2.6A). Third-order roots were observed on day 16 for both the infected and noninfected carrots (Figure 2.6B). The fourth-order roots were first observed 28 days after seeding for the noninfected and on day 32 for the M. hapla infected muck-grown carrots (Figure 2.6C). Fifth-order

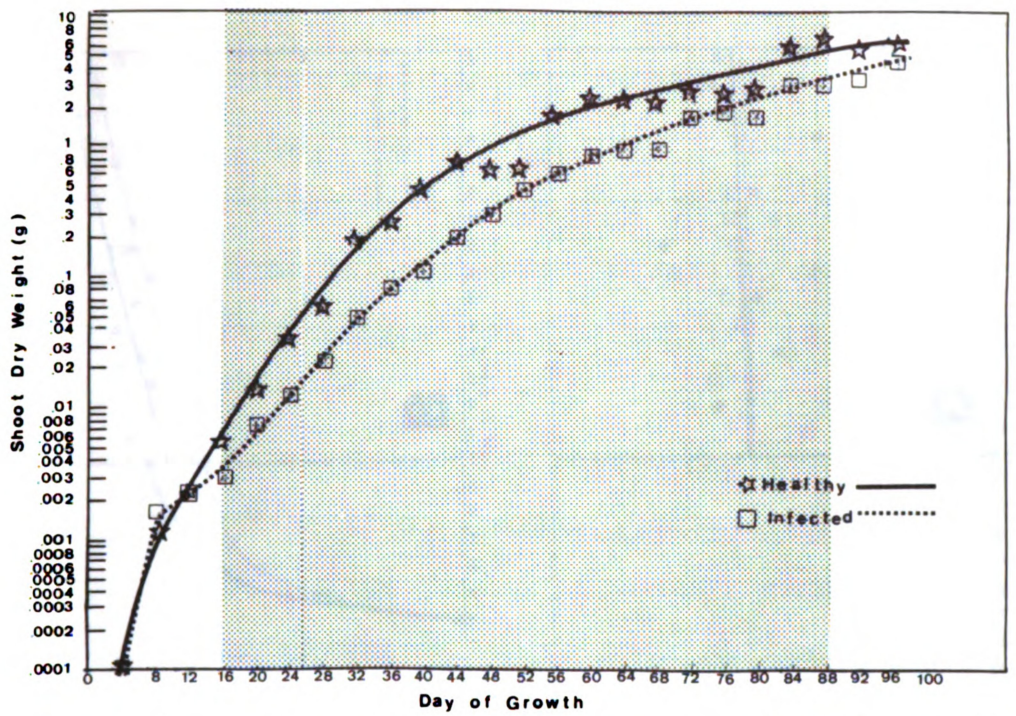

Figure 2.4. Dry weight of Spartan Premium Shoot significantly less (P = 0.05) for infected carrots days 16 through 88.

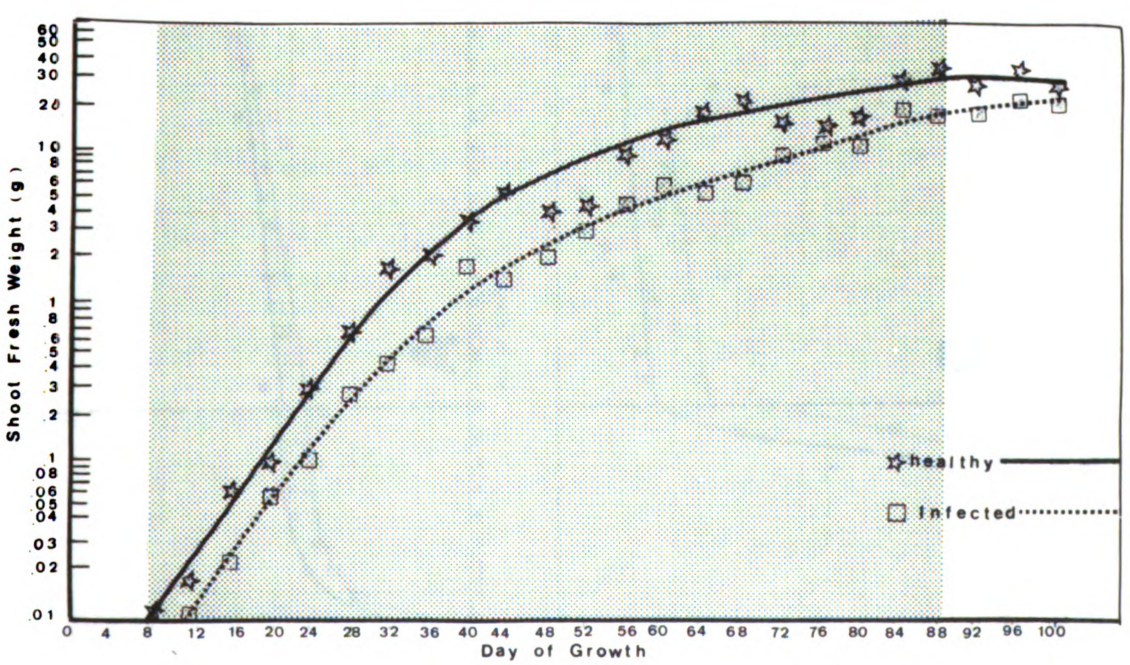

Figure 2.5. Spartan Premium carrot shoot fresh weight significantly less (P = 0.05) for infected days 8 through 88.

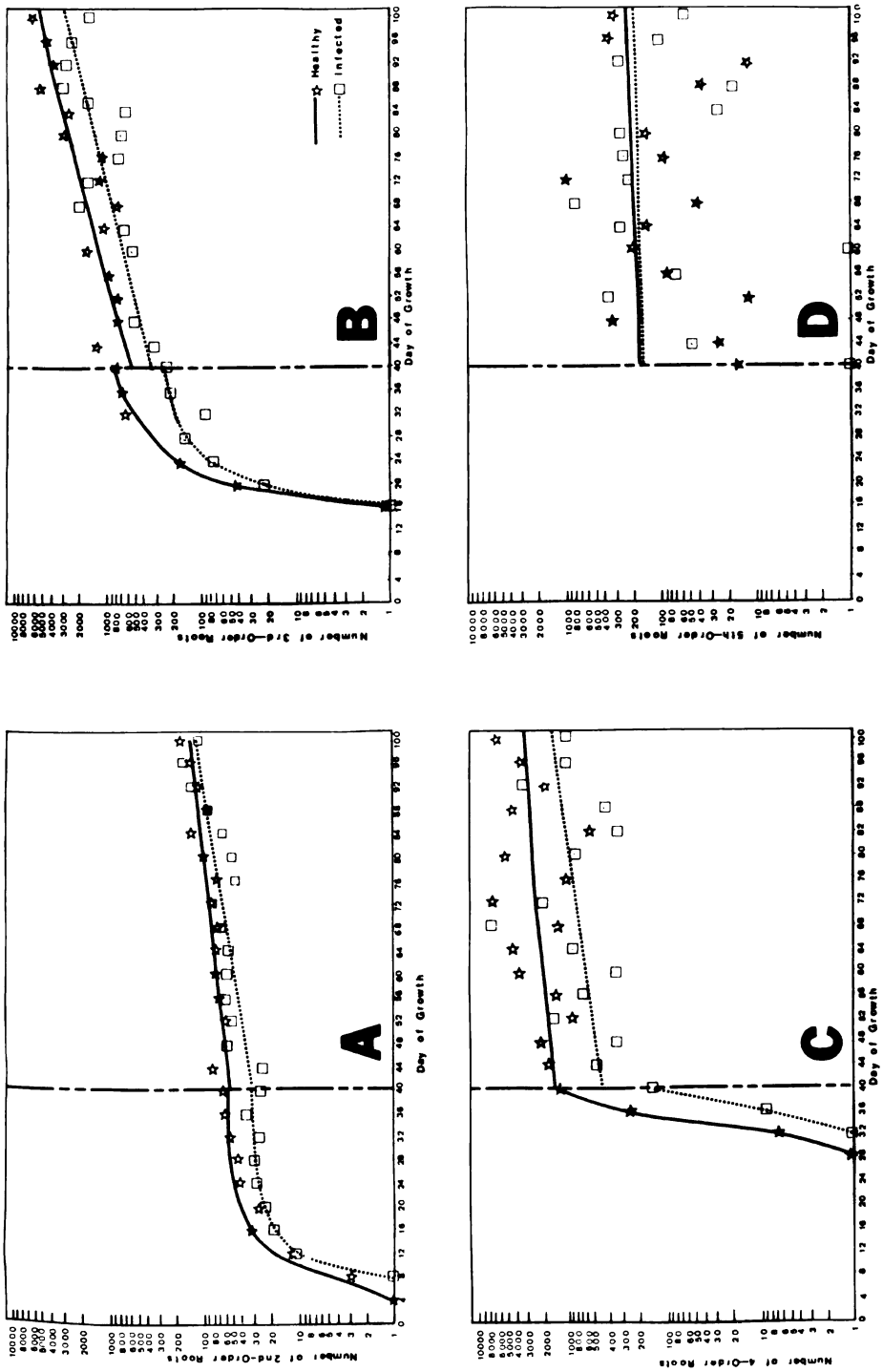

Figure 2.6. Number of roots of each order for Spartan Premium carrots. A = 2nd-order, B = 3rd-order, C = 4th-order and D = 5th-order.

roots were first noted on day 40 and 44 for the noninfected and infected carrots, respectively (Figure 2.6D). Sixth-order roots were observed on noninfected plants only on the roots of 48 day old carrots; whereas, M. hapla-infected carrots had sixth-order roots after 64, 68, 76 and 80 days of growth.

The number of roots of each order increased rapidly during the first 36-40 days after seeding. This was followed by the observance of 5th and 6th-order roots and a slow increase in the number of second, third, and fourth order roots through the 100th day after seeding (Figure 2.6). The total number of roots for each order was estimated from growth information of a portion of the root systems for days 40 to 100. This was determined by the best fit line (P=0.05) for the estimated values. The number of roots on a mature plant at 100 days after seeding was greatest for 4th-order roots and least for 6th order roots (Table 2.1). They ranged from 4,200 to 10 per order for the nematode-free plants and from 2,200 to 39 per order for the M. hapla infected plants.

The total length of roots for each order followed the same trend as the number of roots for each order (Figure 2.7). M. hapla infected carrots had retarded root length for each order except for the 5th and 6th-orders. The estimated lengths of roots for 100 day old root systems of noninfected

Table 2.1. Estimated number and length of each root order on a Spartan Premium Carrot 100 days after seeding for nematode-free and M. hapla infected plants.

Root System	Order of Roots 2nd	3rd	4th	5th	6th	Total on Plant
Noninfected, Number/Plant	140	3,100	4,200	220	10[1]	7,500
Infected, Number/Plant	110	2,200	1,600	190	39[2]	4,200
Noninfected, Root Length (cm)/Plant	1,900	7,500	2,100	130	3.1[1]	13,000
Infected, Root Length (cm)/Plant	1,300	3,800	1,100	140	20.8[2]	6,500

[1]Found only on root system 48 days after seeding.

[2]Found on root systems from day 64, 68, 76 and 80.

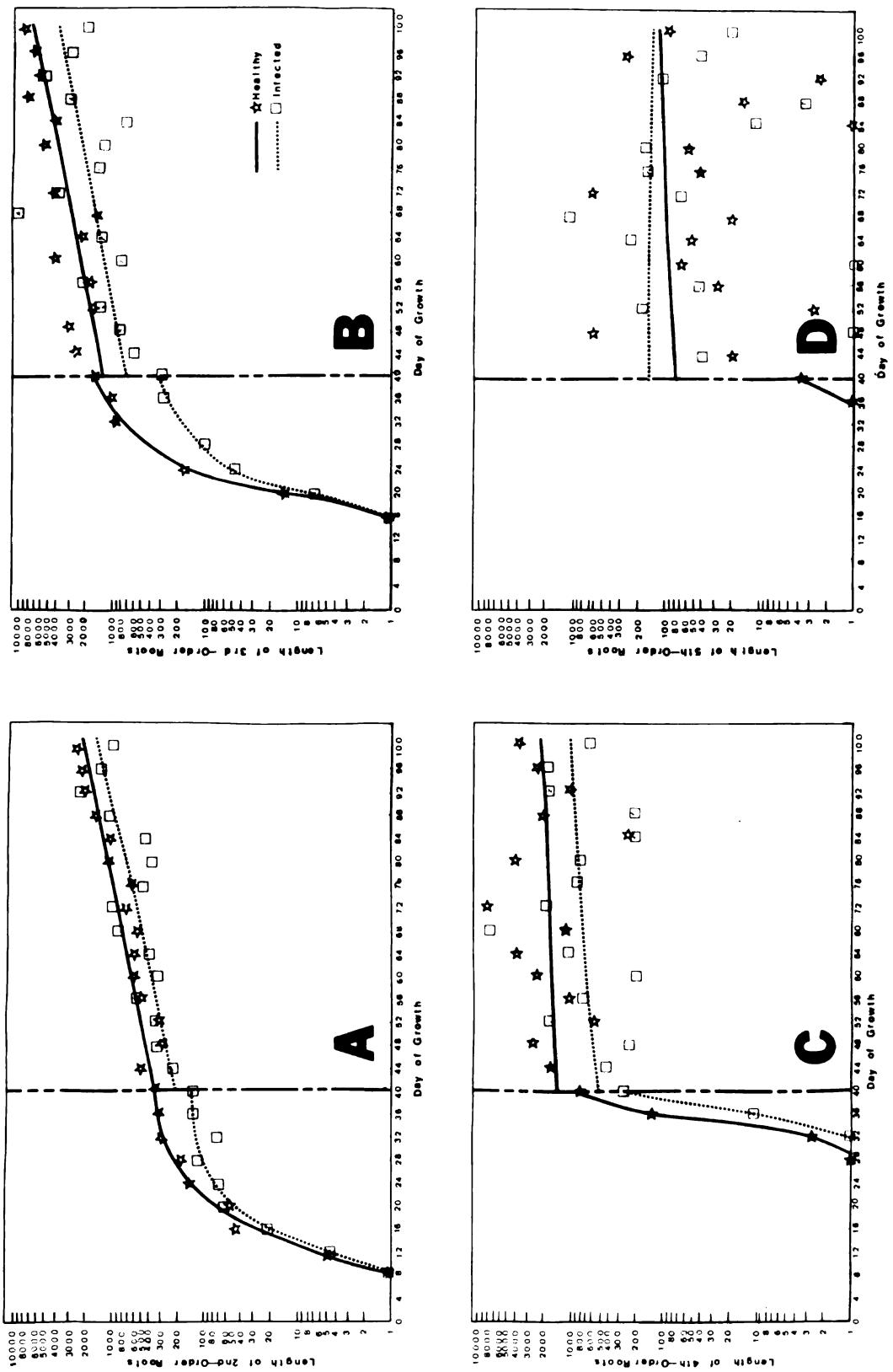

Figure 2.7. Spartan Premium carrot root length for each order.
A = 2nd-order, B = 3rd-order, C = 4th-order and D = 5th-order.

carrots ranged from 7,500 cm to a total length of 3.1 cm of 6th-order roots (Table 2.1). The M. hapla infected carrots had a retarded length of roots ranging from 3,800 cm to a total length of 20.8 cm of 6th-order roots found on day 64, 68, 76 and 80.

Fresh weight of secondary roots was determined for days 36 through 100 (Figure 2.8). There was an initial rapid increase in growth from day 36 to 44, followed by a gradual increase. The estimated secondary fresh root weight for 100-day-old nematode-free carrots was 3 grams. M. hapla infected carrots had only 1.8 g of secondary roots.

Spartan Premium carrots grown in the absence of M. hapla were marketable (approximately 70 g) by 76-80 days after seeding. M. hapla infected carrots did not reach this weight until 96 days after seeding (Figure 2.9). Carotene (orange appearance) was present by day 36 for the nematode-free carrots, but not until day 44 for the nematode infected carrots. There was a steady increase in tap root fresh weight from day 36 to 88. This gain was prolonged to day 96 for the M. hapla infected carrots (Figure 2.9). The infected carrots were also branched and occasionally exhibited hairy root symptoms.

The economic index was an estimated 57.5 average for carrots harvested in the first 52 days studied. For days 52 through 100 the economic index was an average of 41. The average index of infected carrots was 48.2. Noninfected

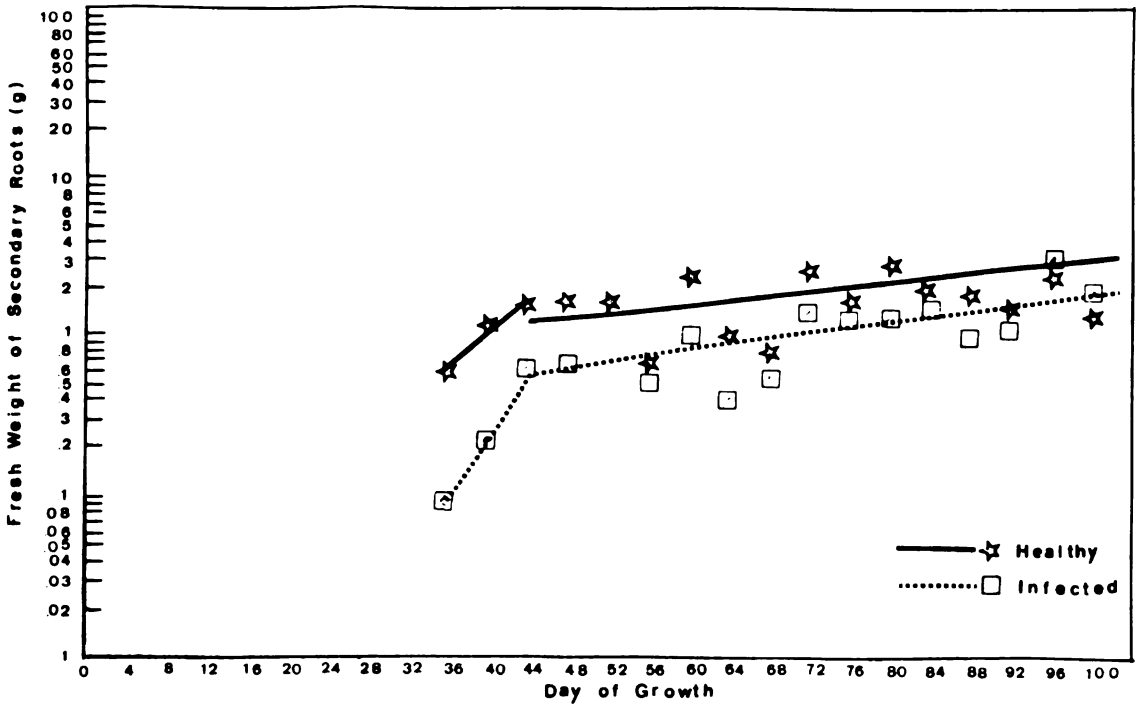

Figure 2.8. Spartan Premium carrot secondary root weight for days 36 through 100.

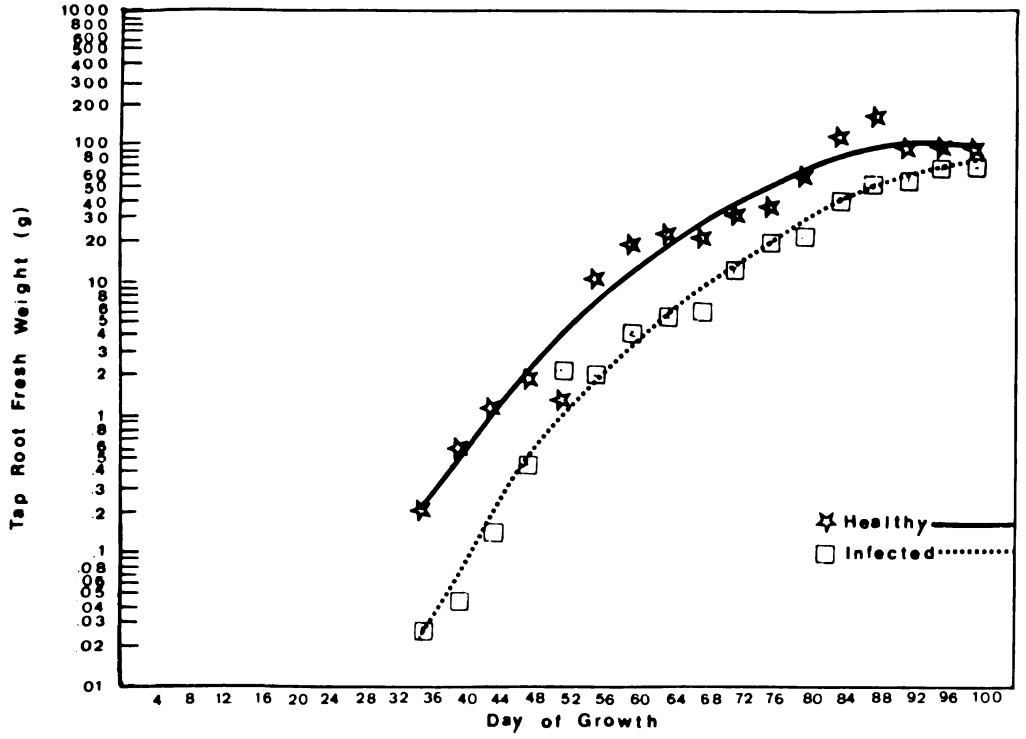

Figure 2.9. Spartan Premium carrot tap root fresh weight for growth days 36 through 100.

carrots had an economic index average of 3 for the entire 100 days observed.

The number of M. hapla induced galls present on the root system of infected carrots increased throughout the 100 days of growth, reaching a maximum of 52 galls per plant (Figure 2.10). There were five levels of gall densities observed: first, 4.75 galls per plant during days 20 to 32; second, 17 galls per plant on days 36 to 48; third, 30 galls per plant on days 52 to 64; fourth, 45 galls per plant on days 72 to 84; and fifth, 52 galls per plant on days 92 to 100.

Root area increased for the first 88 days of growth of the noninfected plants. M. hapla significantly (P=0.05) retarded root area from day 16 to 96 (Figure 2.11). Maximum area estimated for a nematode-free carrot was 328 cm^2, while M. hapla infection retarded this by approximately 100 cm^2 for the 100 day old carrots.

The total number of roots rapidly increased for the first 40 days after seeding (Figure 2.12). This was followed by a gradual increase. The maximum number of roots found on nematode-free carrots was estimated at 7,500. M. hapla infected carrots had an estimated 4,200 roots per plant (Table 2.1). The length of roots reflected this same trend (Figure 2.13). The estimated lengths of roots in a system were 13,000 cm for the nematode-free and 6,500 cm for the infected carrot plants.

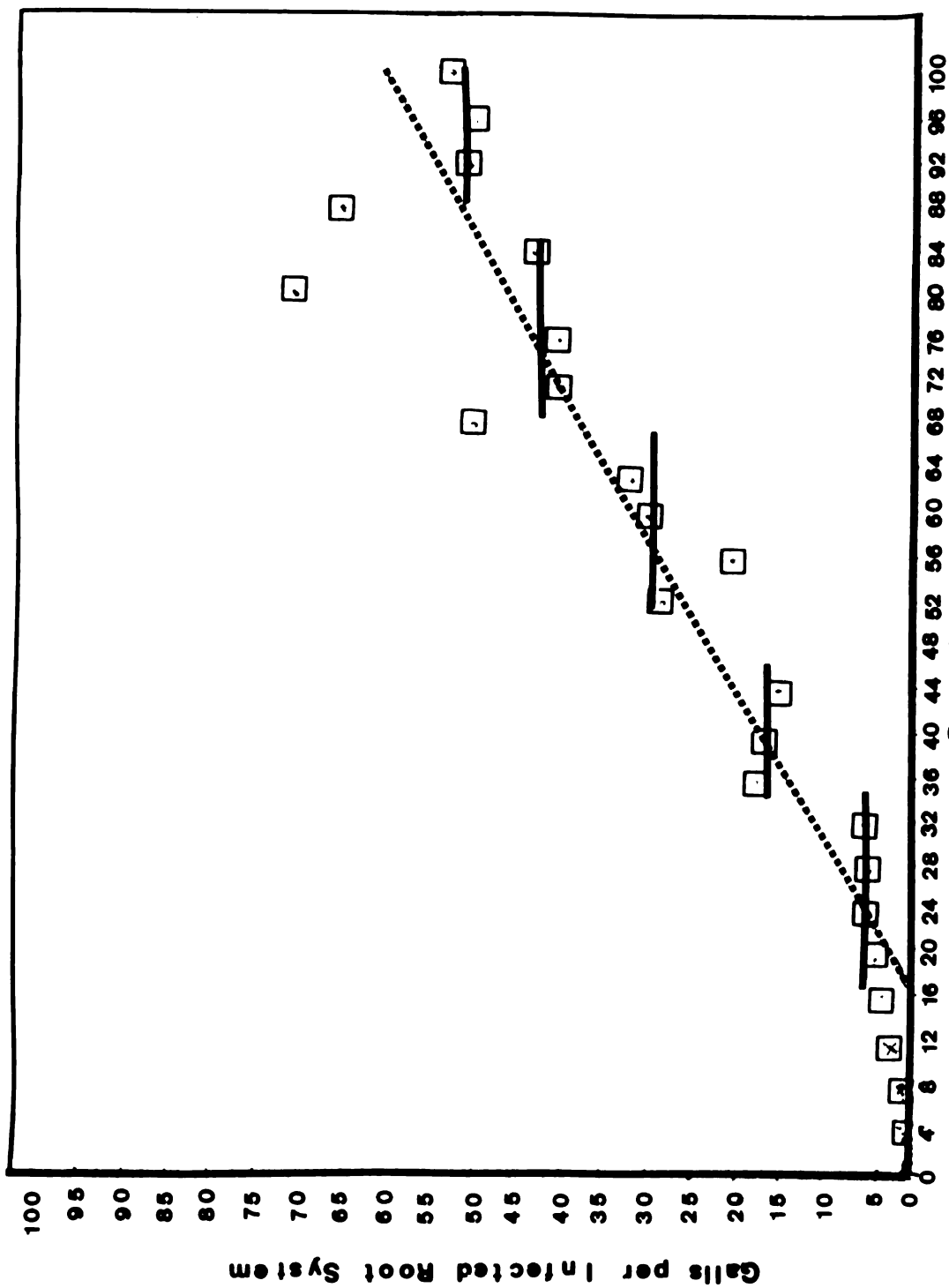

Figure 2.10. Gall found on Spartan Premium carrot root system.

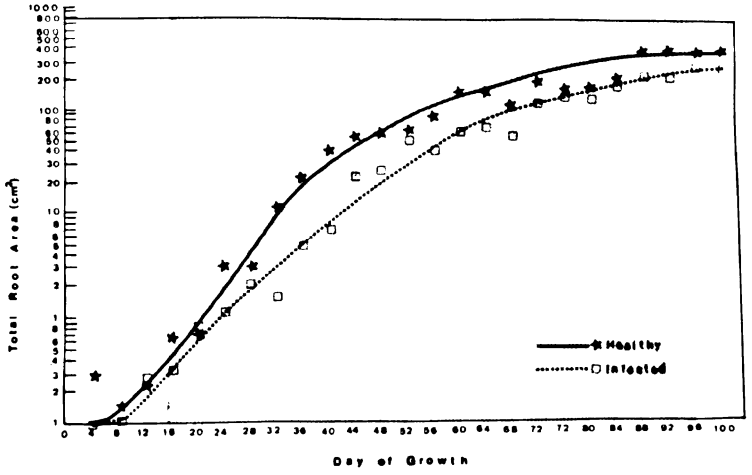

Figure 2.11. Root surface area of M. hapla infected Spartan Premium carrots significantly (P=0.05) less days 16 through 96.

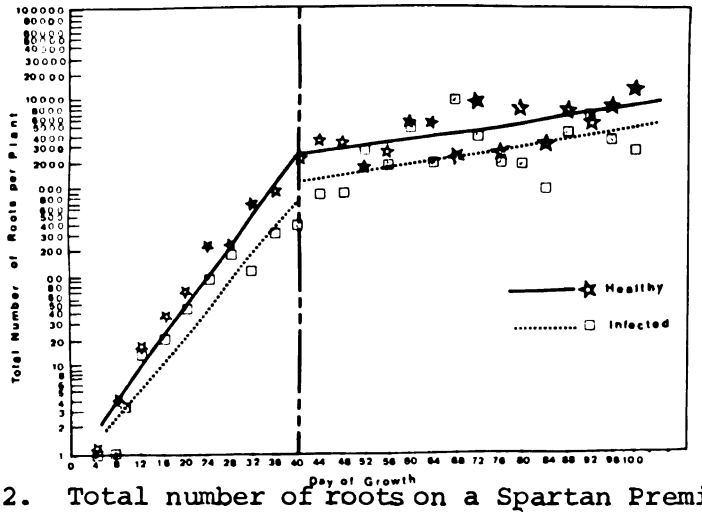

Figure 2.12. Total number of roots on a Spartan Premium carrot infected and noninfected by M. hapla. Days 0 through 40 are average of four replicates. Day 44 through 100 based on one plant analysis.

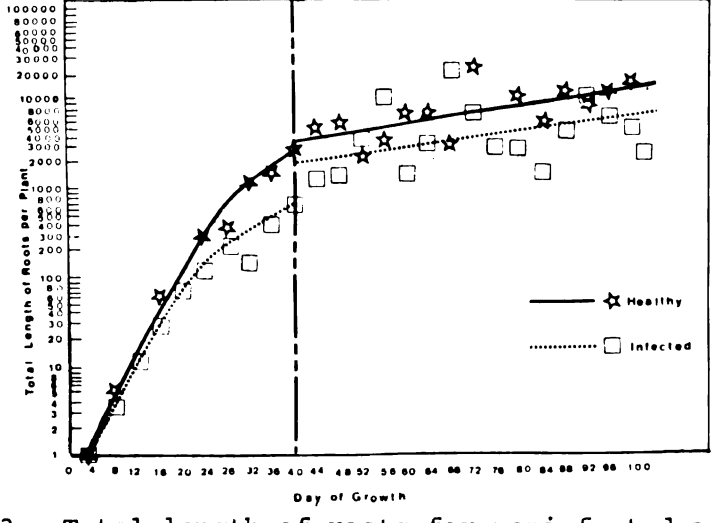

Figure 2.13. Total length of roots for noninfected and M. hapla infected Spartan Premium carrots. Day 0 through 40 is an average of four replicates. Day 44 through 100 is based on one representative plant root system.

M. hapla significantly (P=0.05) retarded root fresh weight for days 24 through 96 of growth (Figure 2.14). The steady increase in fresh weight was observed through day 84 for the noninfected carrots and through day 96 for the M. hapla infected carrots. The maximum weight of the non-infected carrots was 107 g of roots, while the infected carrots reached a maximum of 72.2 g of roots.

The dry weight of the roots reflected a different trend in plant growth (Figure 2.15). In the first 12 days, the infected carrots had significantly (P=0.05) higher dry weights. On day 16 of growth, the infected and noninfected carrots were approximately equal in root dry weight. This was followed by days 32 through 100, for which root dry weight was significantly (P=0.05) less than the noninfected carrots. The maximum average dry weights of roots were 11.12 g for the noninfected and 7.12 g for the M. hapla infected carrots.

Total plant.--The trends of growth observed for the root and shoot systems were used in the total plant results. The area of the total plant (Figure 2.16) indicated that infection by M. hapla significantly (P=0.05) retarded plant total surface area for growth from day 4 through 88. The maximum area of the nematode-free carrots was 1400 cm^2 while the infected carrots had a maximum area of 980 cm^2.

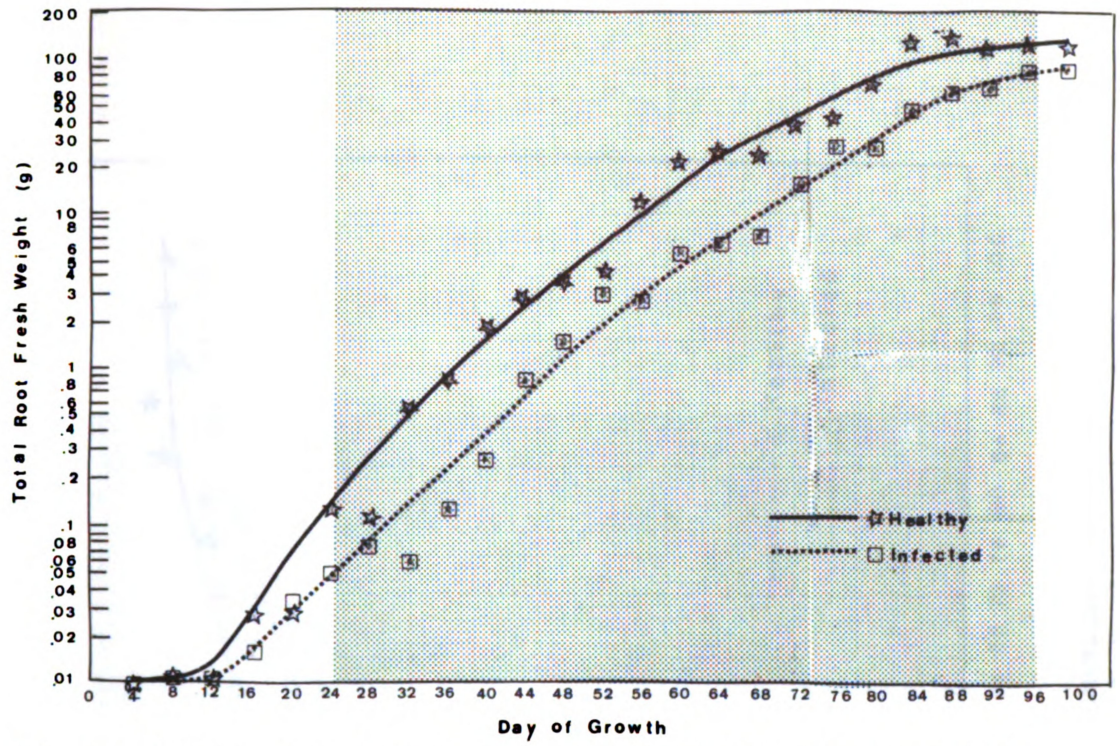

Figure 2.14. Fresh weight of Spartan Premium carrots M. hapla infected and noninfected. Significantly (P=0.05) different days 24 through 96.

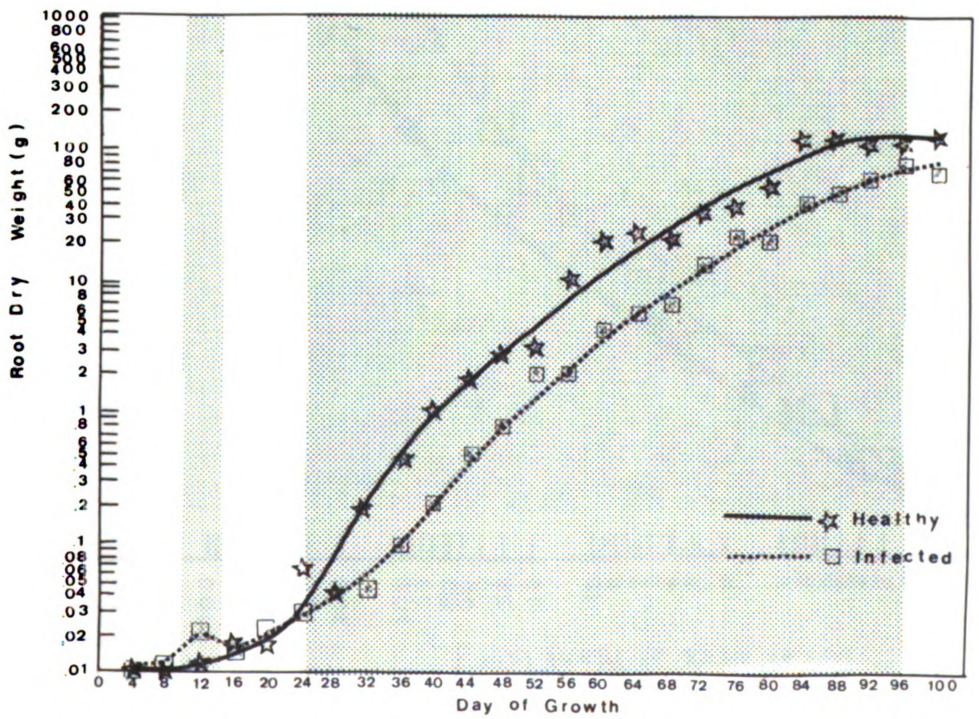

Figure 2.15. Root dry weight of Spartan Premium carrots significantly less (P=0.05) for infected plants day 24 through 96. Significantly more for infected day 12.

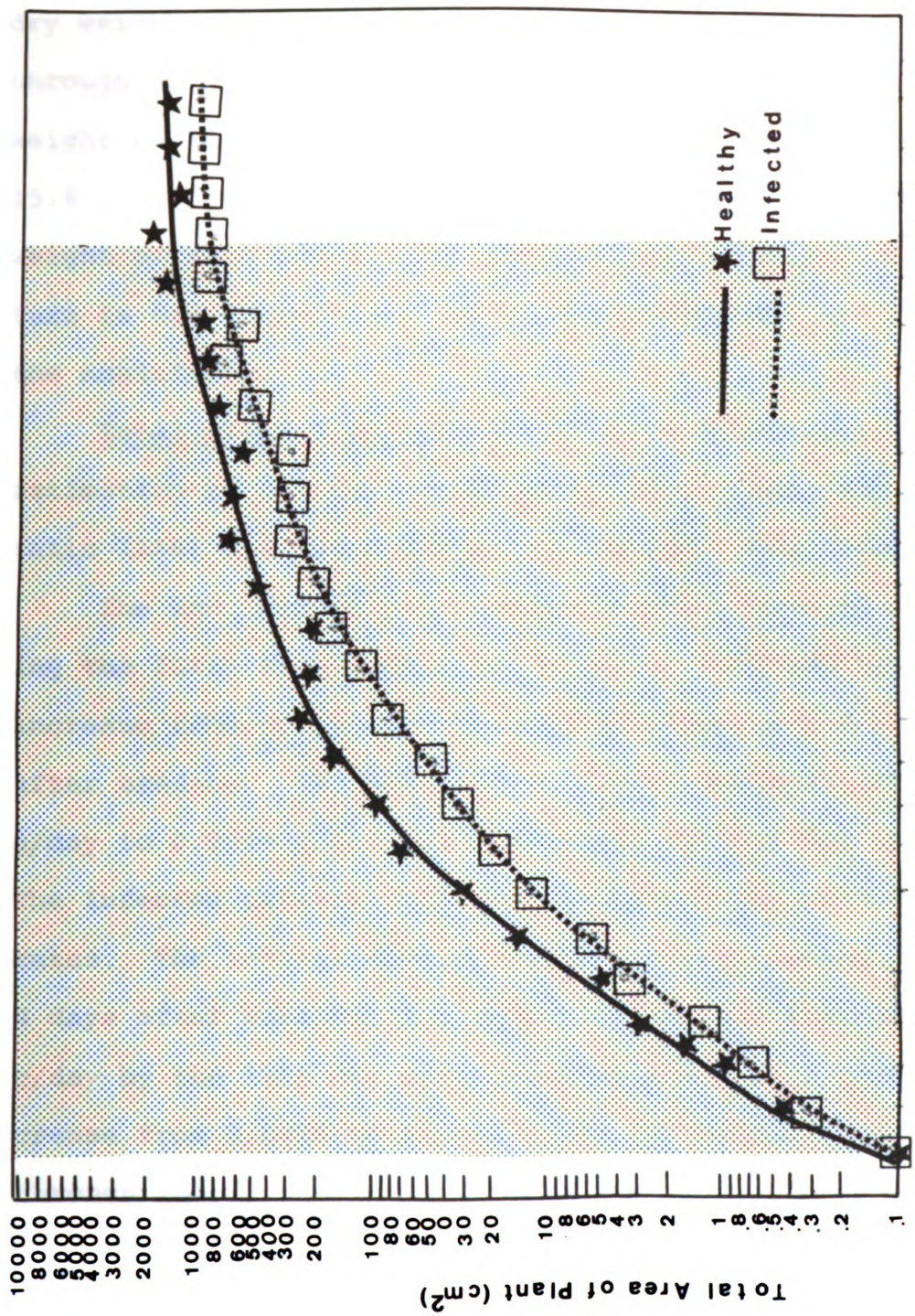

Figure 2.16. Total surface area of Spartan Premium carrot significantly less (P=0.05) for M. hapla infected plants days 4 through 88 of growth.

M. hapla infection significantly (P=0.05) retarded the dry weight and fresh weight of the total plant for days 32 through 96 of growth (Figures 2.17 and 2.18). Maximum fresh weight of the noninfected plants was 138 g fresh weight and 15.8 g dry weight. The infected plants had a maximum fresh weight of 95.2 and 9.8 g of dry weight. The per cent moisture in the carrots was 89.7 for the infected and 88.6 for the noninfected.

Total plant summary.--Total plant analysis included the estimation of the net assimulation rate (NAR), the leaf area ratio (LAR) and the relative growth rate (RGR) (Appendix B).

The NAR (Figure 2.19) showed a rapid drop in weight during the first four days after seeding, followed by a rapid increase from -0.250 g/day to 0.003 g/day by the 12th day after seeding. The NAR then gradually declined from 0.0007 g/day to 0.0004 g/day for the growth between day 16 and 100. The infected carrots followed the same trend with modified rates. The initial drop was only to -0.01 during the first 8 days after seeding, followed by the rapid increase to 0.018 g/day by the 12th day after seeding and then a gradual decrease from 0.0025 to 0.0015 g/day for growth from day 16 through 100.

The RGR (Figure 2.20) was similar to the NAR. The M. hapla infected carrots had an earlier minimum peak of -0.15 g/day on day 4 after seeding, followed by the maximum

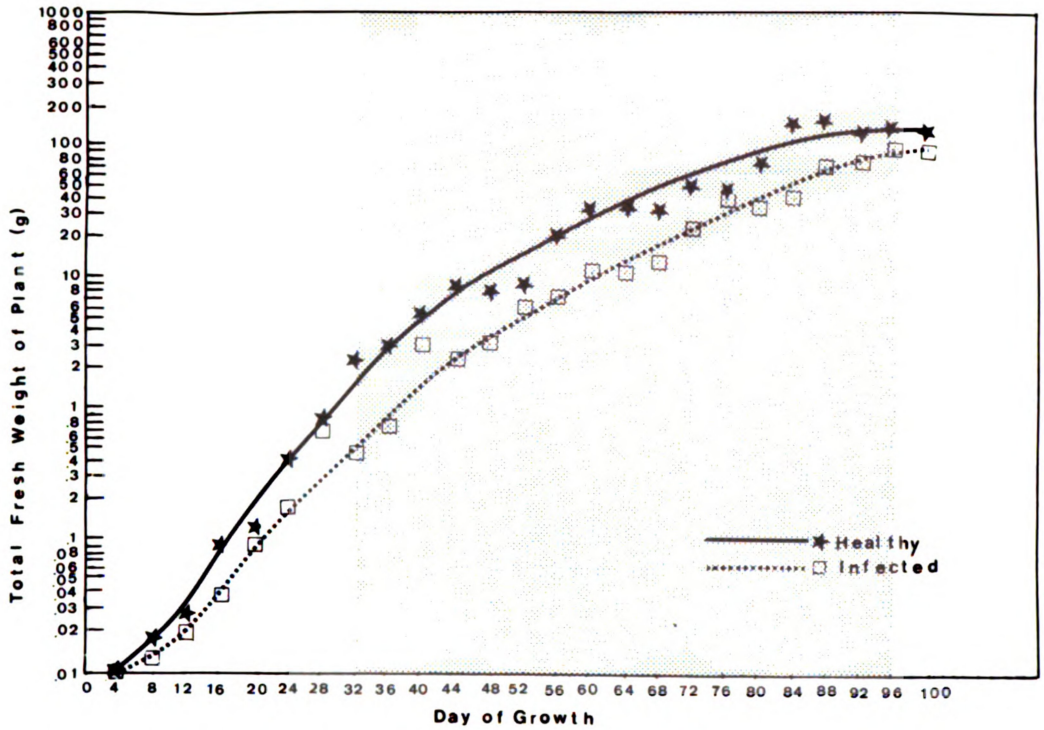

Figure 2.17. Fresh weight of M. hapla infected carrots significantly less (P=0.05) than noninfected carrots days 32 through 96.

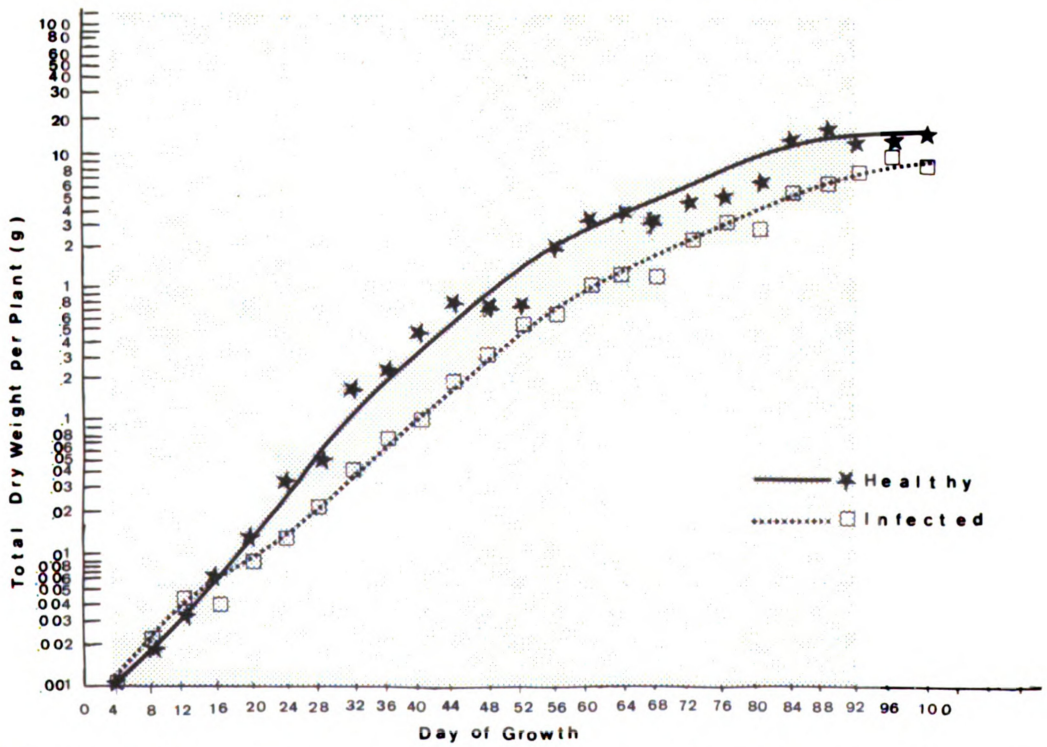

Figure 2.18. Dry weight of Spartan Premium carrots significantly greater (P=0.05) than noninfected carrots days 4 through 16, significantly less than noninfected days 20 through 92.

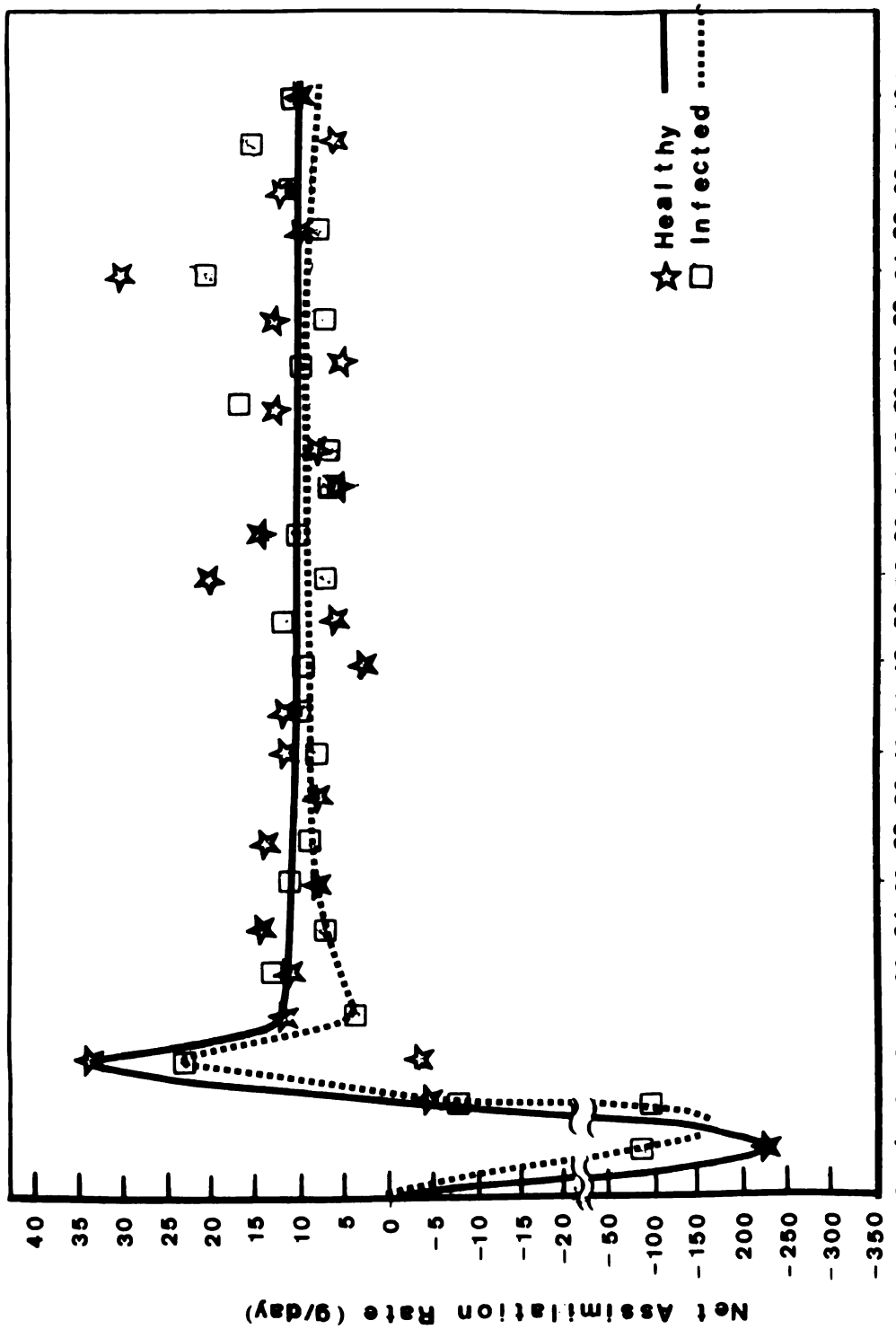

Figure 2.19. Net assimulation rate of Spartan Premium carrots and M. hapla infected Spartan Premium carrots.

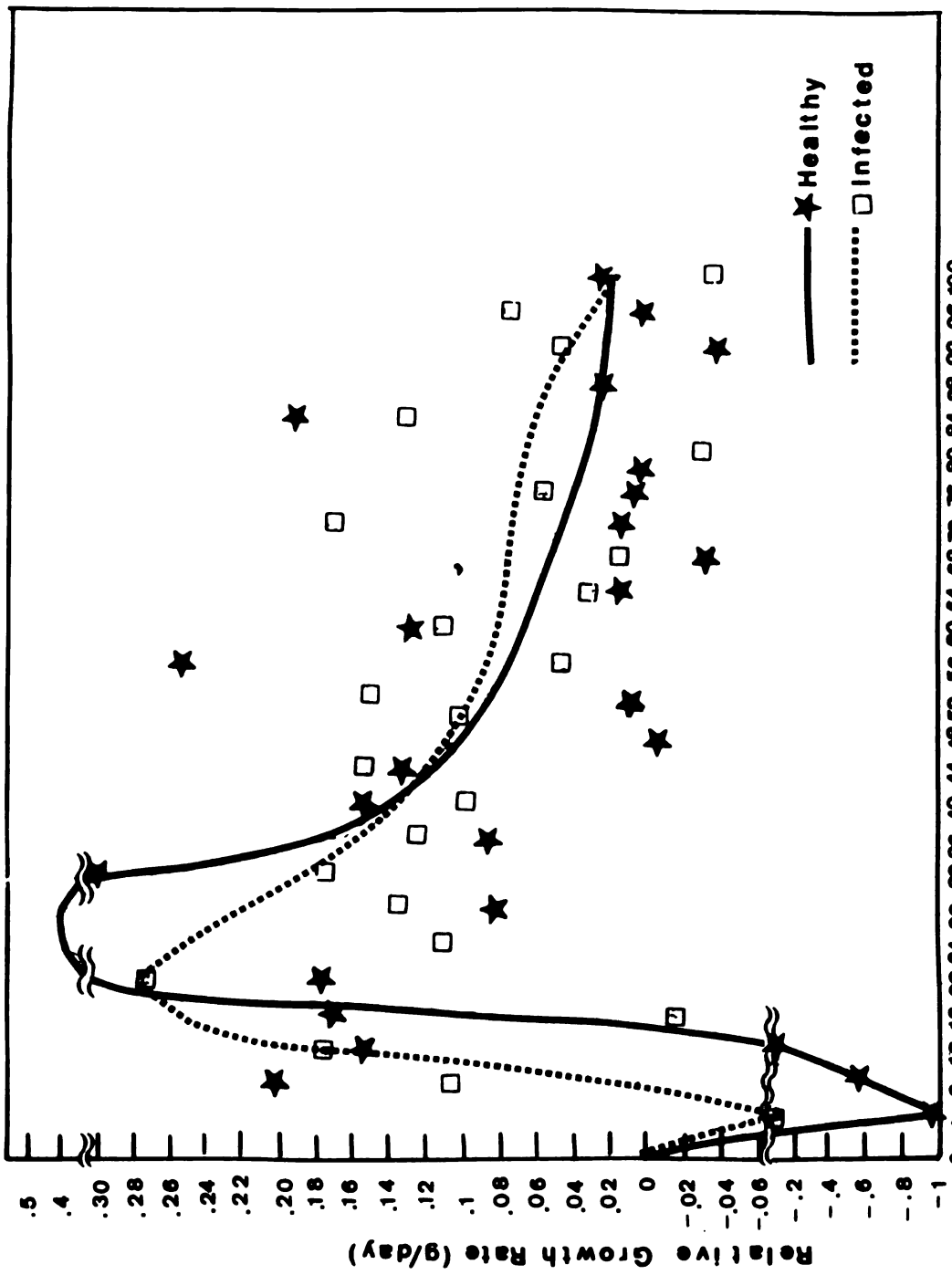

Figure 2.20. Relative growth rate of Spartan Premium carrots infected and noninfected by M. hapla.

peak of 0.272 g/day on the 12th day after seeding and then followed by a gradual decline through day 100. The non-infected carrots had peaks of -0.9 g/day on the 4th day after seeding, 0.42 on the 26th day and was followed by a more rapid decline through the 100th day of growth than those of the infected carrots.

LAR (Figure 2.21) had a rapid initial increase through day 28 for the noninfected carrots and day 30 for the infected carrots. This was followed by a gradual decline as the carrot matured. M. hapla retarded the initial rate of LAR increase, but the maximum value for the infected and non-infected carrots were approximately the same 380 cm^2/g. The gradual decline of the noninfected carrots was more rapid, reaching a steady state by day 88 after seeding at approximately 50 cm^2/g. No steady state was reached for the infected carrots.

Discussion

Spartan Premium carrot cultivar is a rapidly maturing hybrid or early variety. In this study, the carrots were of marketable size by 76-80 days after seeding. The noninfected carrots appeared to begin senescence after 92 days. Carrots infected by M. hapla exhibited delayed maturity and a slower growth rate, as well as the symptoms of branched tap roots and galling of roots, with or without proliferation of the adjacent roots (Figure 2.22). Delay in maturity

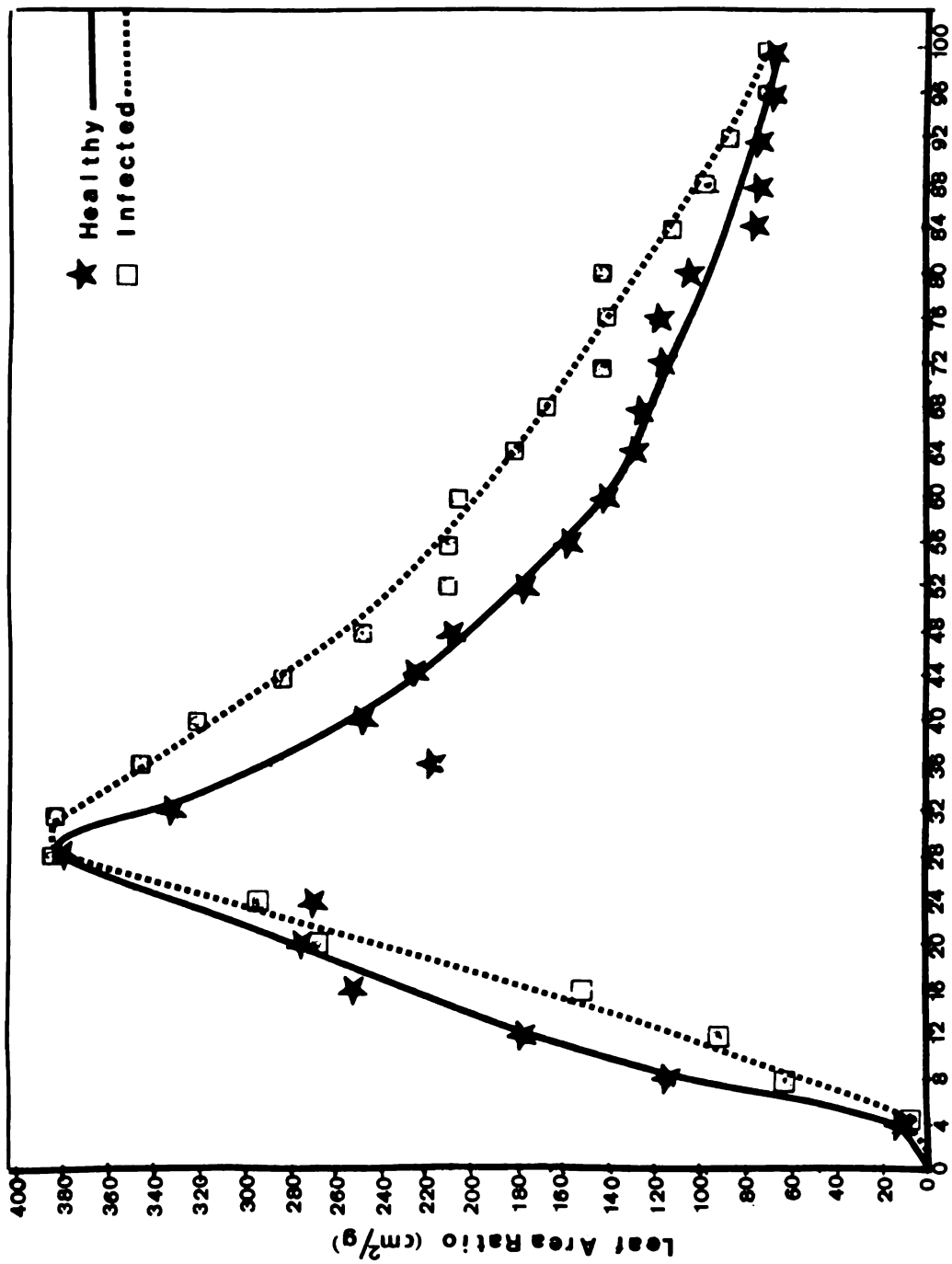

Figure 2.21. Leaf area ratio of Spartan Premium carrots noninfected and infected by M. hapla.

Figure 2.22. M. hapla infected carrot tap roots expressing delayed maturity and branching. Noninfected carrot tap root in center of photo.

was the result of an overall retardation of plant surface area for nutrient and light absorption. There was, as a result, a significant (P=0.05) retardation in the growth rate, based on fresh weight and dry weight, in relation to time. Gall formation on the root systems also was an important factor in this growth retardation. The disruption of the vascular system caused by these galls reduced the plant growth potential.

Delayed maturity represents a significant economic loss due to the loss in marketable tons of carrots per acre, as well as the loss of early season market price margins.

The degree of error in this study increased with maturation of the carrots. The fresh weight represents a degree of error increase as the amount of water retained on the root surface increases proportionally with the extensiveness of the root system. The other error accountable in weight values was the extremely low values for the initial days of growth. This may account for the discrepancies found between the early day fresh and dry weights, particularly of the roots. The error accountable in relation to surface area was the increasing difficulty to attain the total surface area of the leaflets and secondary roots as the carrots matured. Thus, a proportional error was seen in these values. The surface area, however, should be a close approximation. These errors were minimized by the genetic variability

within the four replicate plants used for each days evaluation.

In looking at the total secondary root development there was an initial period of rapid increase, and then a steady but slower rate of increase for the remaining days studied. The analysis involved the establishment of the best fit line for days 0 to 40 and for days 40 to 100. The R^2 values of the first days analyzed were always nearly 1.0. This was as expected, since the whole root systems of each of the replicates were analyzed. For days 40 to 100, however, much differentiation was seen in the R^2 values of the lines. The method of analyzing only a portion of the root systems and relating this to the whole is probably the major error. There was considerable variation among the four replicates, particularly in the infected carrots. This increased the difficulty of selecting the "representative carrot" from the replicates to be evaluated. This selection highly influenced the root number, order and length values.

The growth analysis of the whole plant in response to the environment and to the effects of Meloidogyne hapla infection is expressed in terms of the net assimulation rate (NAR), leaf area ratio (LAR) and the relative growth rate (RGR). The NAR is the dry weight increase of the plant in relation to the unit leaf area in relation to time. The LAR is the ratio of the leaf area to dry weight of the leaves.

The RGR is the rate of charge of the log of fractional change of the plant weight over a given unit of time (Appendix B).

In this study, the R^2 values for the LAR curve was nearly 1.0. This was fitting the points to a second degree polynomial function formula. The LAR indicated that the infection of carrots delayed maturity and reduced growth potential. The infected carrots had a greater LAR which, related to the photosynthetic ability, would indicate a reduced rate in relation to the noninfected carrots. The initially more rapid increase in LAR of the noninfected carrots probably reflects the rapid change in weight of the plants.

In this study, the NAR pointed out the initial utilization of seed reserves (days 0 to 4), followed by a rapid increase in the NAR as the basic plant structures were formed. This rapidly increasing NAR continued through the time of radical emergence, shoot emergence, secondary root initiation, and cotyledon and first true leaf appearance. As the carrot matured, an almost steady state was seen between the increase in plant weight and the shoot area. After maturity, there was a drop in shoot area as the plant gradually begins to lose leaves. Infected carrots followed the same trends with lower NAR values.

The RGR reflected the same plant development trends as the NAR. The gradual decline was much more evident, however,

in the RGR. This continuous decline with the development of the tap root was expected, that is, more gradual for the infected plants with the delayed maturity and reduced growth rate than for noninfected plants.

It is crucial to observe the root order, number and length in a plant's growth particularly, in relation to nematode problems. The subapical meristematic region is the area of entry for the second-stage larvae of M. hapla. The carrot system is very complex with up to six orders of roots. The sixth order of roots observed in this investigation probably are the result of the proliferation of roots near the galls on the infected root systems. The greater number of 5th-order roots in a mature infected carrot root system also was probably due to the galls and root proliferation caused by M. hapla infection. The number and arrangement of the length of total roots of each order followed a logical pattern in the infected and the noninfected carrots. The second-order root system was limited. There were more 3rd- and 4th-order roots than any other. The extensiveness of the root systems was adequate to provide multiple sites for infection for high densities of the northern root-knot nematode.

In this study, the gall count was used as an indicator for the degree of infection in relation to time. It may also reflect the rate of reproduction of the nematode on

this particular carrot cultivar. There are many influential factors involved in this reading. First, a generally increasing trend in the number of galls on a root system was seen. The R^2 value was 0.87 for a straight line (P=0.05). Along this time span, there were five respective levels of galling noted. The first one, at about 5 galls per plant, indicated the initially introduced population of 100 second-stage larvae, plus the 5 larvae per 100 g of infested field soil. The plateau at 20 to 32 days of growth may represent the second generation of nematodes or infection by larvae which hatched from egg masses present in the soil. If the lifecycle is completed in approximately 16 days, the third level could represent the third generation of nematodes, or if the lifecycle is completed in 32-34 days, it may be the second generation of the initial population density of second-stage larvae. The fourth and fifth levels were not as distinct as the first three. They may represent the fourth and fifth generations of the initial population, respectively, or they may be the second generation of the second level population from egg masses and the third generation of the initially introduced population (level 1). From this study it would appear that the life cycle of M. hapla under these greenhouse conditions in muck soil on carrots is less than the 45 days indicated by Brody (27). It may also be a weakness of this study if two life cycles of M. hapla were occurring in the soil during this study.

The overall growth and carrot plant development seen in this investigation support that which was described by Phan and Hsu (175). Cultivar differences are seen as the shoot height increases more slowly in Spartan Premium carrots, but at a greater total height (15-20 cm more) than the cultivar they studied. The root development coincides with their observations. The phases of growth observed by Esau (63) and Haves (92) are also distinctly visible in the RGR and NAR growth analysis. Although not anatomically differentiable, it also appeared that a third type of growth could be expressed as the tap root enlargement occurs. This would give the Spartan Premium carrot three distinct phases of growth. Days 0 to 4, days 4 to 16, and days 16 to marketable size about 76-80 days after seeding. The appearance of the carotene, or orange color, coincided with Haves' (92) reported observation of 37 days after seeding.

The per cent moisture in the Spartan Premium carrots correlated closely with Watt and Merill's (236) estimation of 88.2 per cent. The 88.6 and 89.7 moisture values for the noninfected and infected carrots, respectively, are within experimental error.

The overall development of M. hapla infected and non-infected muck-grown carrots indicated a significant (P=0.05) growth differential for days 32 to 88 of growth. This reflected secondary symptoms typical of the infection caused by the northern root-knot nematode, M. hapla.

Pathology, Distribution and Population Density of Meloidogyne hapla

Results

Pathology.--In this study, carrots with introduced population densities of 0, 10, 100 and 1,000 second-stage larvae of M. hapla are referred to as noninfected, low, medium and high soil infestation levels, infected carrots or population densities, respectively. These ratings are not intended to be relevant to naturally occurring field population densities of M. hapla.

The primary pathological symptom, root galling, increased with time for all four of the introduced population densities on both the Gold Pak and Spartan Premium cultivars (Figure 3.1). For Gold Pak carrots with low and medium population densities, similar trends for increased root galling were observed. The low population density galls per carrot plant increased from 0.75 to 88, which was a 117-fold increase for growth from days 30 to 120. The medium population density on Gold Pak carrots increased root galling from 1.63 to 273.25, which was a 168-fold increase from days 30 to 120. The high population density on carrots increased from 5.75 to 849.5, which was a 149-fold increase in galls per plant for growth from day 30 to 120. The degree of root galling decreased with the increase in the high population densities.

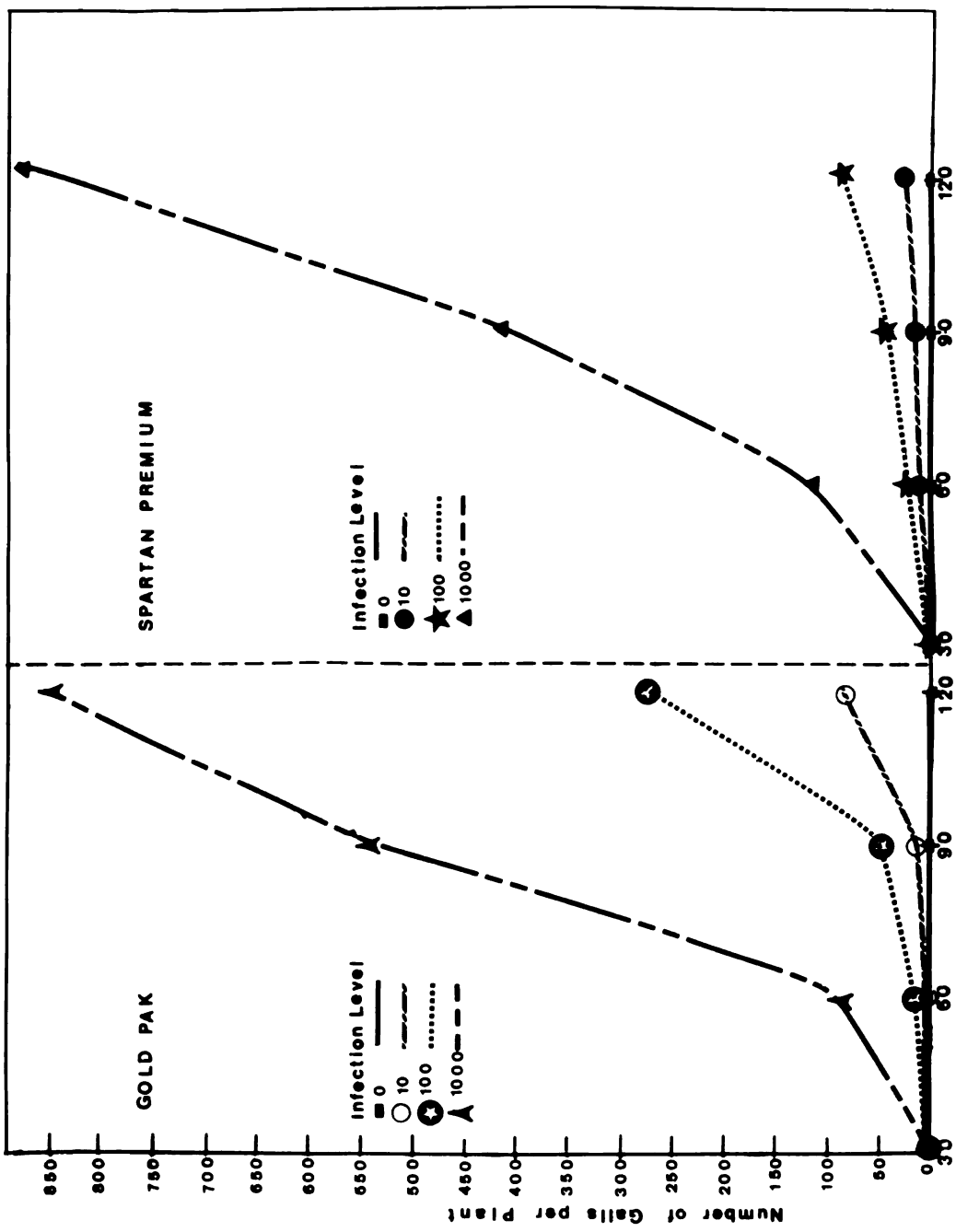

Figure 3.1. The number of galls found on carrots infected with M. hapla.

The Spartan Premium carrots with low population densities had the greatest rate of galling increase from 0.25 to 52.5 at day 120, which was a 263-fold increase. For the medium population densities, galls per plant had increased from 9.0 to 87.5 by day 120, which was a 9.8-fold increase. The high nematode population densities increased from 6.9 to 874, which was a 127-fold increase in galls per plant for days 30 to 120.

At 30 days after planting, there was a significantly (P=0.05) greater number of galls per root system for the Spartan Premium carrots than the Gold Pak carrots. There was also a significantly (P=0.05) greater number of galls per root system of the medium and high population density carrots of each cultivar than for the low and noninfected carrots of each cultivar. After day 30, there was a significantly (P=0.05) greater number of galls found on the high population density carrots than in the medium, low or noninfected density carrots of each cultivar. There was no significant difference in root galling between the cultivars.

Secondary symptoms of M. hapla infection were evaluated by root, shoot and total plant fresh weights, as well as by shoot height. The 120th day after planting, heights of Gold Pak carrots with initial populations of 0, 10, 100, and 1,000 second-stage juveniles per pot, were 35, 37, 36.25 and 40.25 cm, respectively, while the Spartan Premium carrots

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were 43.75, 34.86, 45.13 and 35.13, respectively (Figure 3.2). At 30 and 60 days after seeding, a significantly (P=0.05) greater height was observed for the Spartan Premium cultivar than the Gold Pak cultivar carrots. The noninfected Spartan Premium carrots had significantly greater height than all other carrots on day 30 after seeding. No significant difference was observed at later growth for the population densities within a cultivar or between the two cultivars.

Maximum shoot fresh weights (Figure 3.3) observed for Gold Pak carrots with initial populations of 0, 10, 100 and 1,000 second-stage juvenile M. hapla per pot were 31.7, 33.8, 37.4 and 46.9 g, respectively, while the Spartan Premium carrots had 22.1, 23.0, 30.5 and 25.5 g, respectively. Significant differences were observed for shoot fresh weight at specific times between the cultivars, as well as within each of the cultivars, for the different M. hapla population densities (Table 3.1).

Root fresh weights (Figure 3.4) at 120 days after planting for Gold Pak carrots with initial populations of 0, 10, 100 and 1,000 second-stage juveniles per pot were 92.6, 103.5, 110.7 and 84.8 g, respectively, while the Spartan Premium carrots had 183.5, 129.9, 130.5 and 134.5 g, respectively. Root fresh weights were significantly (P=0.05) different at specific times between the cultivars, as well as within each cultivar (Table 3.2).

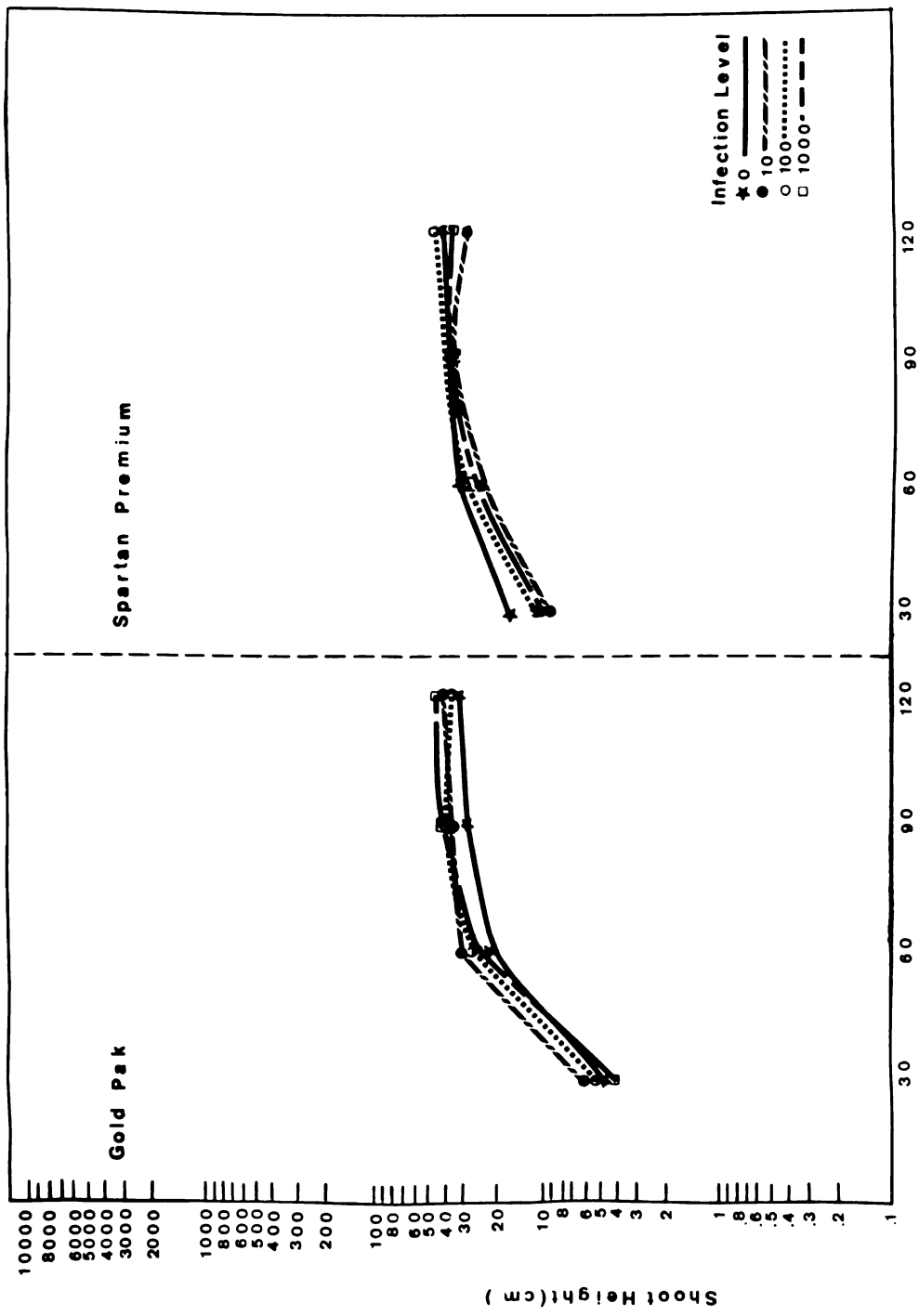

Figure 3.2. Shoot height of Spartan Premium and Gold Pak carrots infected by M. hapla.

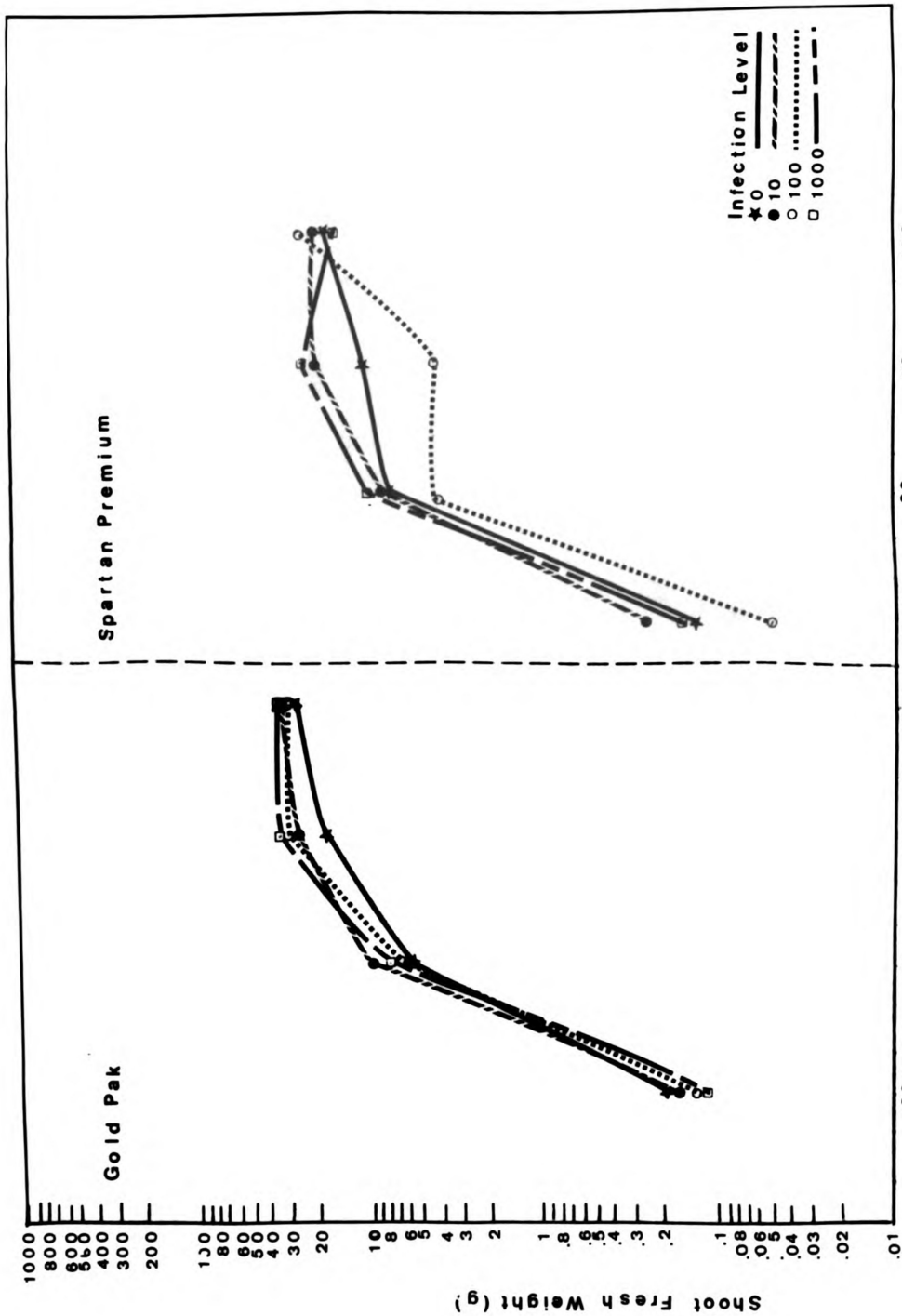

Figure 3.3. Shoot fresh weight of Spartan Premium and Gold Pak carrots infected by M. hapla.

Table 3.1. Shoot fresh weight significant differences (P=0.05) in the Spartan Premium and Gold Pak carrot cultivars infected with different population densities of *M. hapla*.[1]

	Days of Growth			
	30	60	90	120
Between cultivars[2]	None	S.P. med. < G.P. low	S.P. med. < G.P. low, med. & high S.P. noninf. < G.P. high	S.P. noninf. & low < G.P. high
Within the Spartan Premium cultivar	None	med. < high	med. < low & high	None
Within the Gold Pak cultivar	None	high > noninf., low & med.	noninf. < high	None

[1]Noninf., low, med. and high are used to designate the carrots associated with introduced *M. hapla* population densities of 0, 10, 100 and 1,000, respectively.

[2]G.P. = Gold Pak cultivar; S.P. = Spartan Premium cultivar.

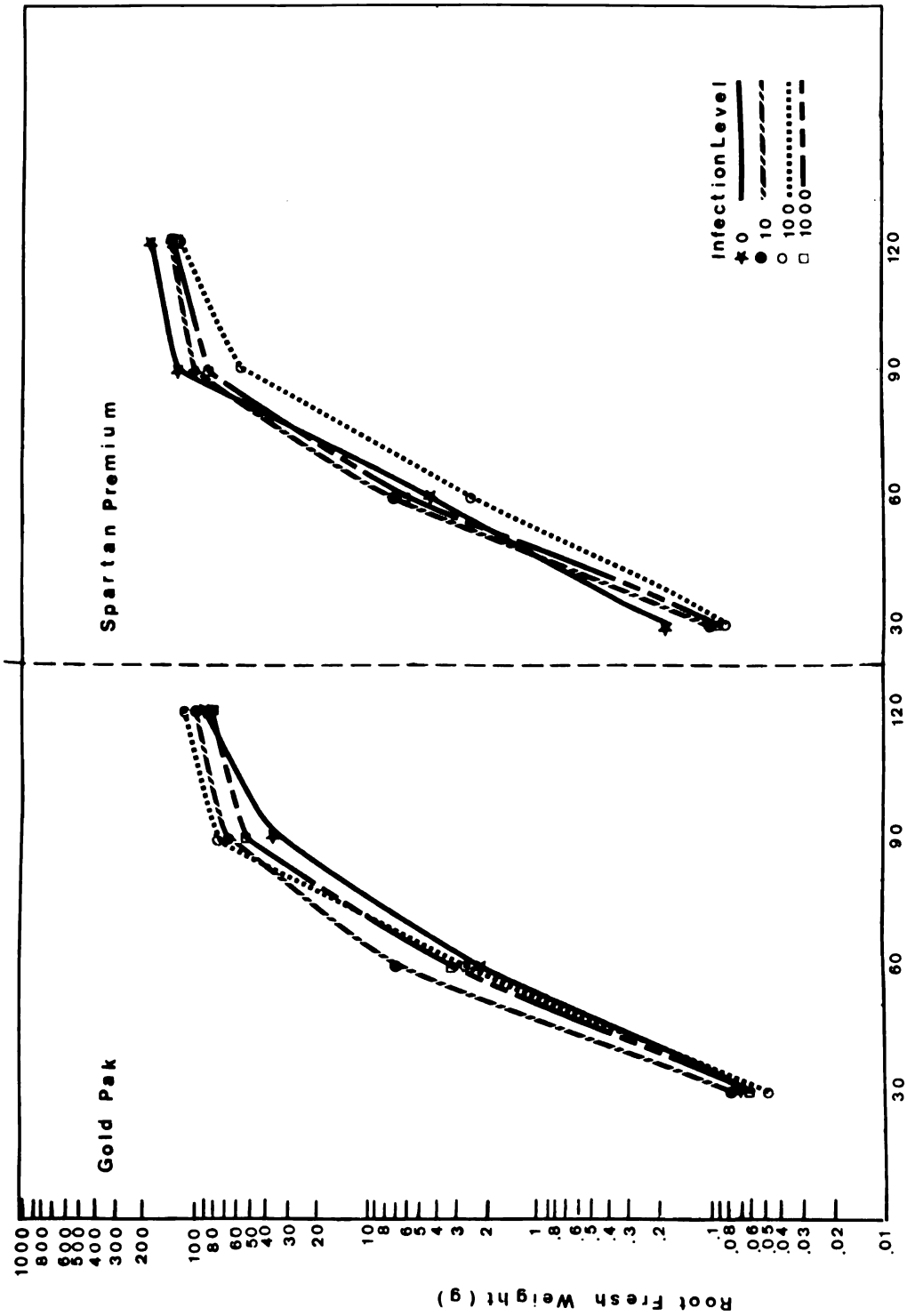

Figure 3.4. Root fresh weight of M. hapla infected Spartan Premium and Gold Pak carrots.

Table 3.2. Root fresh weight significant differences (P=0.05) in the Spartan Premium and Gold Pak carrots infected with different population densities of *M. hapla*.[1]

	Days of Growth			
	30	60	90	120
Between cultivars[2]	S.P. noninf. > G.P. (all)	None	G.P. low,high & noninf. < S.P. low, high & noninf. G.P. med. < S.P. noninf. & low	S.P. noninf.> G.P. (all)
Within the Spartan Premium cultivar	noninf. > low, med. & high	None	med. < noninf., low & high noninf. > low, med. & high	noninf. > low, med. & high
Within the Gold Pak cultivar	None	None	med. > low, high & noninf.	None

[1]Noninf., low, med. and high are used to designate the carrots associated with introduced *M. hapla* population densities of 0, 10, 100 and 1,000, respectively.

[2]G.P. = Gold Pak cultivar; S.P. = Spartan Premium cultivar.

Total plant weights (Figure 3.5) at 120 days after seeding were, for the noninfected, low, medium and highly infected Gold Pak carrots, 124.2, 137.2, 148.1 and 131.7 g, respectively. The Spartan Premium carrots had 205.6, 152.9, 161 and 160.3 g, respectively. Significant differences observed for the total plant fresh weight occurred at different times within the cultivars and between the cultivars (Table 3.3).

The economic index (see page 34) of the four replicates 120 days after planting for the noninfected, low, medium and highly infected Gold Pak carrots was 0, 25, 75 and 100, and for the Spartan Premium carrots it was 0, 50, 50 and 75, respectively.

Population Distribution.--Qualitative microscopic estimations of the M. hapla population densities extracted indicated the majority of the nematodes were present in the roots and rhizosphere soil (Figure 3.6). For all introduced population densities the number of M. hapla present in the soil increased from day 30 to 120 on both cultivars. Pot soil maximum number of nematodes was 9 per 100 g sample of soil (Figure 3.6A). A steady population density increase was observed for the low population density carrots of both cultivars. The medium and high population densities indicated a reduction in the soil nematode population density for the Gold Pak cultivar at day 90, while the Spartan Premium cultivar remained the same or slightly less than the

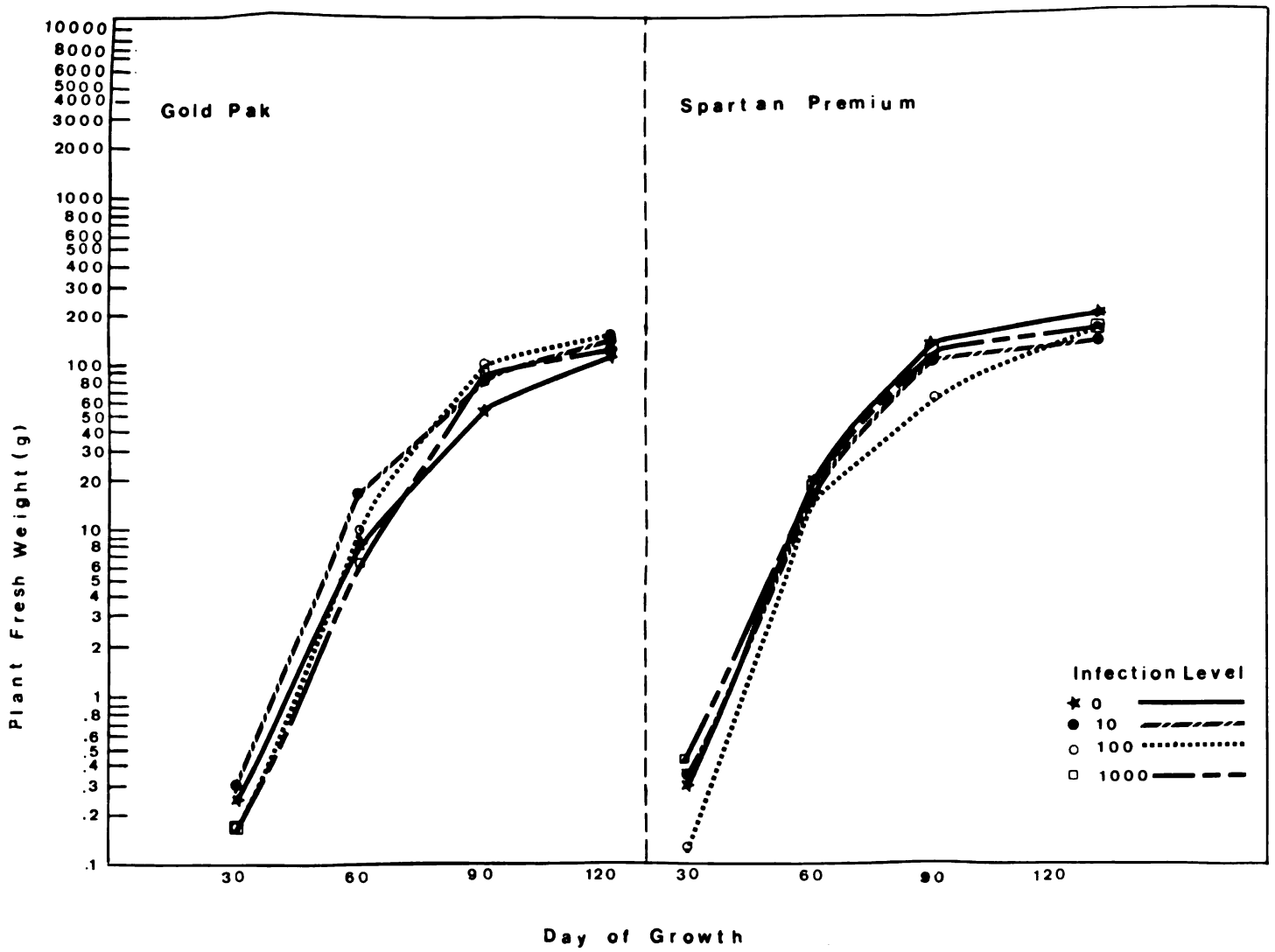

Figure 3.5. Fresh weight of M. hapla infected Spartan Premium and Gold Pak carrots.

Table 3.3. Total plant fresh weight significant differences (P=0.05) in the Spartan Premium and Gold Pak carrots infected with different population densities of M. hapla.[1]

	Days of Growth			
	30	60	90	120
Between cultivars[2]	S.P. high > G.P. med. & high	G.P. high < S.P. low, noninf. & high	S.P. med. < G.P. med. S.P. high, med. & low > G.P. noninf.	None
Within the Spartan Premium cultivar	med. < high	med. < noninf.	med. < noninf., low & high	None
Within the Gold Pak cultivar	med. & high < noninf. & low	high < low	med. > noninf., low & high	None

[1]Noninf., low, med. and high are used to designate the carrots associated with introduced populations densities of 0, 10, 100 and 1,000 second-stage larvae of M. hapla, respectively.

[2]G.P. = Gold Pak cultivar; S.P. = Spartan Premium cultivar.

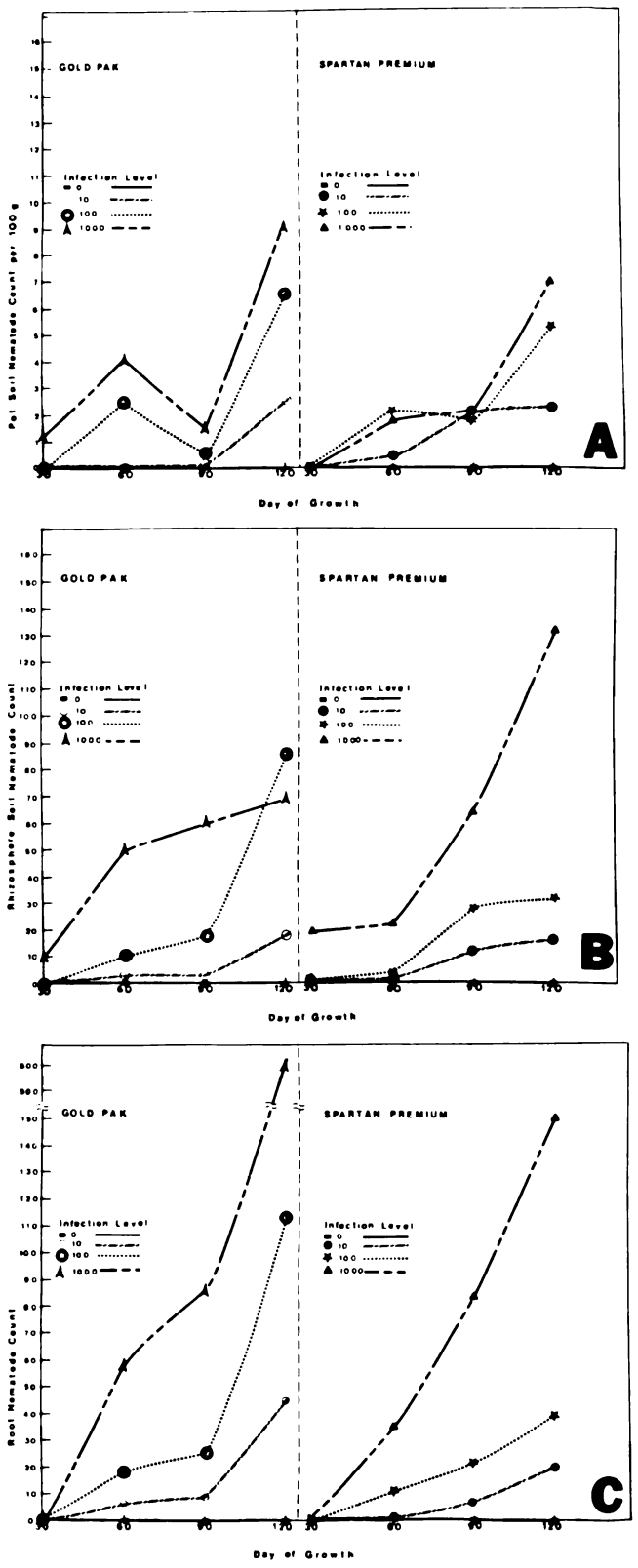

Figure 3.6. Nematode distribution: 2nd stage larvae of M. hapla found on Gold Pak and Spartan Premium carrots. A in 100 g pot soil, B in rhizosphere soil and C in root system.

60 day population density in the soil. At each population density, maximum populations in the Spartan Premium cultivar was less than those found in the soil of the Gold Pak cultivar.

Nematode population densities of the rhizosphere soil and roots followed similar trends for all densities of each cultivar, except in the case of the high population density in carrot rhizosphere soil at 120 days in the Gold Pak cultivar. This population density did not increase as rapidly in the rhizosphere soil as in the root population of M. hapla (Figures 3.6B & C). The maximum number of nematodes present in the root system of noninfected, low, medium and highly infected Gold Pak carrots 120 days after seeding were 0, 43.8, 113.3 and 596.3, respectively. The Spartan Premium carrots had 0, 19.3, 38.3 and 149.3, respectively. The maximum number present in the rhizosphere soil of the Gold Pak cultivar was 0, 16.5, 86.3 and 68, while the Spartan Premium cultivar had 0, 17.3, 32.8 and 132, respectively.

In the root populations, the highly infected Gold Pak carrots had significantly (P=0.05) more nematodes than those found in any of the other populations of either cultivar, on days 30 and 120 after seeding. On day 60, Gold Pak and Spartan Premium highly infected carrots had significantly (P=0.05) more nematodes than all other carrots, while at day 90, significantly more nematodes were present in both the medium and highly infected carrots of both cultivars.

In the rhizosphere soil, the highly infected Spartan Premium carrots had significantly (P=0.05) more nematodes than were present in any of the other carrot nematode associations. At day 60 after planting, Gold Pak highly infected carrots had significantly (P=0.05) greater numbers of nematodes than the noninfected, low and medium infected carrots of this cultivar. By 120 days, the Spartan Premium highly infected carrots had nematode population densities that were significantly (P=0.05) greater than all other population densities. The medium and highly infected Gold Pak carrots were significantly (P=0.05) greater than those of all other population densities. The medium and highly infected Gold Pak carrots were significantly (P=0.05) greater than all other noninfected or low infected carrots. In total, during the early days of growth there was a significant difference between the cultivars. There was also a significant difference in rhizosphere soil nematode population densities.

The total nematode counts reflected these same increasing trends for each cultivar and each population density (Figure 3.7). The Gold Pak and Spartan Premium highly infected carrots had significantly (P=0.05) more nematodes than low, medium or noninfected carrots of each cultivar. An exception was found at 120 days after seeding when the Spartan Premium highly infected carrots were not significantly different than the Gold Pak medium infected carrot

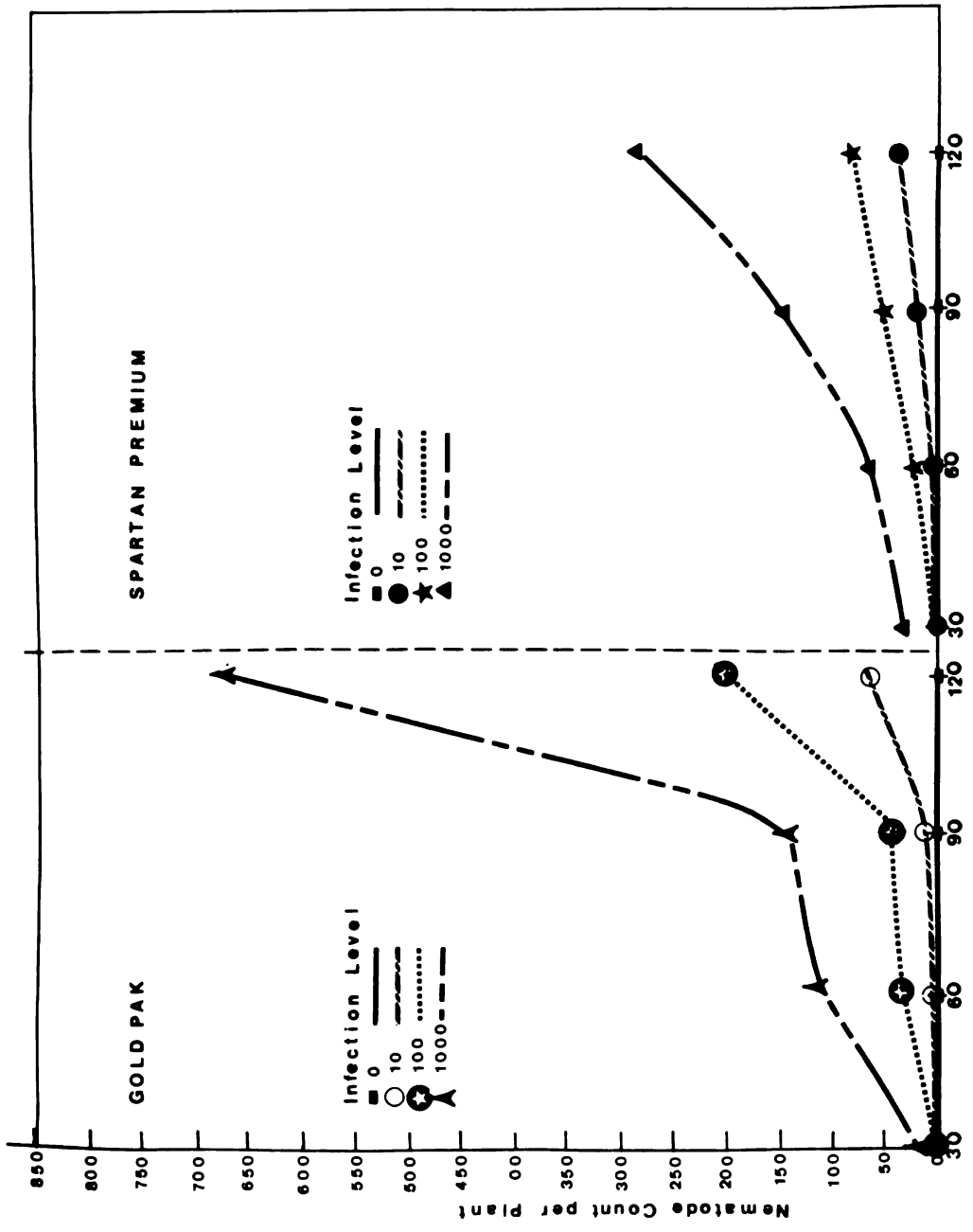

Figure 3.7. Second-stage larvae of M. hapla found per carrot plant.

nematode population density count. No significant (P=0.05) difference was seen for the low, medium and noninfected carrots of either cultivar.

Population density.--Each population of nematodes increased with time (Figure 3.8). High population densities of M. hapla on Spartan Premium carrots indicated a reduced rate of increase for higher initial population densities. This was not seen for the Gold Pak cultivar which supported greater nematode population density increases with time than did the Spartan Premium cultivar.

Discussion

Spartan Premium carrots did not express the retarded growth observed in the ontogeny study. Factors which may account for this include plant tissue injury caused by pest control chemicals, as well as the detrimental growth effects from a heavy infestation of aphids on the carrots. The use of four replicates and only four harvest days also limited the detection of any significant differences in growth between infected and noninfected carrots.

As seen in the ontogeny study, Spartan Premium carrots tend to suppress reproduction rate in high population densities of M. hapla. The Gold Pak cultivar had a greater host potential than the Spartan Premium cultivar, as shown by greater increase in galls and number of nematodes in rhizosphere soil, roots and pot soil.

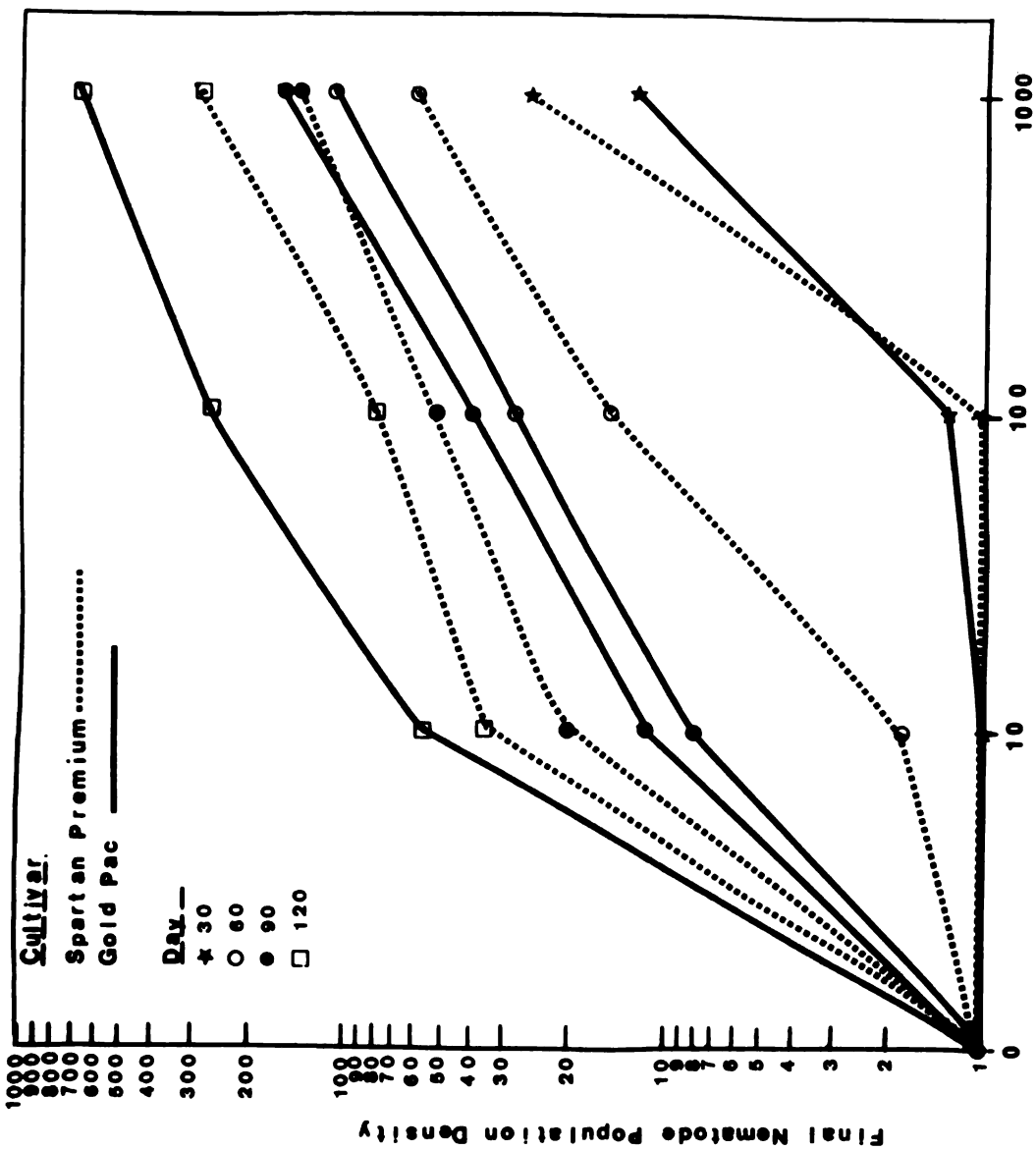

Figure 3.8. Increase in nematodes for each introduced population of M. hapla.

Error factors in this study include the method limitations for nematode extraction. The centrifuge floatation and shaker techniques gave the best nematode recovery rate, but all techniques are limited. Jones (121) reported that the number of nematodes extracted by current techniques in sandy soil is never over 70 per cent. In addition, the high organic content of muck soil increases the difficulty of nematode extraction.

The root extraction technique is highly influenced by the amount of tissue used in the extraction procedure. A proportionally greater error is present in the data in relation to time and root development. Nematodes were extracted from the entire carrot root. The optimum ratio would have been 1 g of roots per 25 ml of solution (130).

The greater number of nematodes in the roots and rhizosphere soil was expected. Stein (211) in 1965 reported that nematodes spread in field microplots of carrots no more than 5 or 6 cm per season. It was also reported by Lounsberry and Viglierchio (138,139) and Weiser (239) that tomato roots have demonstrated an attractiveness for M. hapla larvae. This same attraction phenomena has been reported for other Meloidogyne spp. on a variety of different hosts.

As was expected the number of galls increased with time, closely correlating with the increase in nematode population densities. A high initial attrition was seen for the introduced population densities. At day 30, the low

level of infection, in relation to the introduced population densities, was influenced by several factors. There were no roots available for introduced second-stage larvae of M. hapla for 4 to 7 days after planting. Another factor is the limited mobility of the larvae and the number of sub-apical meristematic penetration sites. There was also more than one plant in a pot during the first 21 days after planting. As the root matured, there was no longer a limited number of penetration sites. The number of sites available, increased per root system in response to the infection. The number of galls found should reflect the number of nematodes which have infected the root system. There was some difficulty in counting all galls due to the small size of M. hapla induced galls. However, a more accurate technique, using a staining procedure, would have curtailed nematode root extraction counts.

The trends observed in plant, root and shoot fresh weights were all about the same. There was a greater increase in weight of the infected carrots than the noninfected carrots in certain instances, probably due to the manifestation of galling and root proliferation symptoms. The highest population density with the most root galling, reflected growth rate retardation. This was probably due to the decrease in nutrient and water uptake, which is a result of hyperplastic symptoms affecting the vascular system of the plants.

Gold Pak cultivar had a greater susceptibility, as seen in the economic index as well as in the nematode and gall counts. This cultivar is a late maturing open pollinated cultivar. The Gold Pak cultivar had 33 per cent greater shoot growth with 37.7 per cent less root development than the Spartan Premium cultivar. It is probably this reduced photosynthetic efficiency which causes late maturation of the Gold Pak carrots. At 120 days after planting, there was a 25.6 per cent difference in plant fresh weight between cultivars. All carrots were significantly beyond the fresh market weight of approximately 70 g. Spartan Premium cultivar control did weigh more than all infected carrots of either cultivar. The medium infected plant weighed the least, probably because the hyperplastic response was inadequate to compensate for the retarded growth. It appears that at higher levels of infection, the hyperplastic response does compensate for the retarded plant growth. The Gold Pak carrots responded differently, as the greatest weight was seen for the medium infected carrots. This would indicate that the hyperplastic symptom expression significantly influenced total plant fresh weights.

Genetic variability, although it should be minimal for the hybrid, Spartan Premium, and experimental variations were observed for the Gold Pak and Spartan Premium cultivars. In comparison to other studies, the population dynamic study

indicated that plant growth was retarded in the Spartan Premium plant. The same muck soil used in the ontogeny study was used for this study. It appears that an insoluble salts deficiency observed in the cultivar and mycohorrizal studies may have been influential in this study. The host potential of the Spartan Premium carrots based on the number of galls was 8.6 per cent less than that found in the cultivar study. The Spartan Premium carrot appears to have a medium host potential rating. The Gold Pak carrot growth was greater than that seen for the mycohorrizal study. The host potential correlates well with the cultivar study, indicating a medium rating.

Cultivar and Parent Line Evaluation

Results

The extent of root galling caused by M. hapla was significantly (P=0.05) less for Spartan Classic (2.0 gall index) than parent line M 5988 (5.0 gall index). There were no other significant differences in root galling among the 15 cultivars and parent lines (Table 4.1). The final population densities of M. hapla were not significantly (P=0.05) influenced by the various cultivars and lines (Table 4.1). They ranged from 0.57 to 134.0 second-stage larvae per root system. Five of the cultivars and lines had an economic index of 50 or less (Table 4.1).

Table 4.1. Cultivar and parent line gall indices, economic indices and nematode count per root system.

Cultivar/Parent Line	Gall Index	Economic Index[1]	Nematode Count[2]
Gold Pak	2.75	100	19.75
Danvers	4.50	100	68.50
M 5987	3.25	50	2.0
Spartan Winner	3.25	75	2.25
Spartan Fancy	3.75	75	8.50
M 3489	4.75	75	103.75
(1304/872)-1-M-CM	3.50	75	1.75
Spartan Delux	2.50	75	14.0
Spartan Classic	2.0	50	1.0
(1304/872)-1-S-CM	3.75	50	10.0
M 5986	3.25	75	134.25
Spartan Delite	3.0	75	0.75
M 5988	5.0	75	27.75
Spartan Bonus	2.5	25	14.0
Spartan Premium	2.75	50	86.25

[1]Per cent of four replicates deformed beyond fresh market use.

[2]Second-stage larvae of M. hapla in root system 60 days after planting.

Discussion

With an initial population density of 100 second-stage larvae of M. hapla, a positive correlation between known field ratings (8) and greenhouse results existed for only the Spartan Classic, M 3489 and Danvers (Table 4.2, cultivars and parent lines arranged in order of increasing field test results for resistance to M. hapla (8)).

Root gall indices can be used to evaluate tolerance, resistance or susceptibility. The field test information (8) related to greenhouse data indicated that nonsusceptible cultivars and parent lines have a gall indices of 3.25 or less (Table 4.1 and Table 4.2). With this definition, a positive correlation for field and greenhouse ratings for susceptibility was observed for the following cultivar and parent lines: Danvers, M 3489, Spartan Fancy, (1304M/872-1-M-CM, M 5987 and Spartan Winner. Tolerant to resistant cultivars or parent lines with a positive gall index correlation were Spartan Bonus, Spartan Classic, Spartan Delux, M 5986, Spartan Delite and M 5988. Definite discrepancies were seen for M 5988, Gold Pak and (1304/872)-1-S-CM.

The demarcation of the economic indices was 50 per cent. Tolerant cultivars and lines with a positive correlation to field results were Spartan Classic, (1304/872-1-S-CM, Spartan Premium and Spartan Bonus. Susceptible cultivars and lines with an economic index which correlated positively

Table 4.2. Cultivar and parent line *M. hapla* evaluation for host potential and resistance. Listed by increasing resistance based on field ratings.

Cultivar/Parent Line	Greenhouse Rating		Field Rating (8)
	Host Potential	Resistance[1]	
Gold Pak	Medium	S	S, Typical Susc.
Danvers	High	S	S, Typical Susc.
M 5987	Low	S-T	S, Most are S
Spartan Winner	Low	S	S, Most are S
Spartan Fancy	Low	S	S-T, Most are S
M 3489	High	S	S-R, Most are S
(1304/872-1-M-CM	Low	S	Seg., Most are S Few are R
Spartan Delux	Medium	T-S	Seg. S to R
Spartan Classic	Low	T	T, Some S, Most T-R
(1304/872)-1-S-CM	Medium	T-S	Seg., More R than S
M 5986	High	S	T-R, Some S
Spartan Delite	Low	T-S	T-R, Few S
M 5988	Medium	S	T-R, Most R
Spartan Bonus	Medium	T-R	R-T, Some S
Spartan Premium	High	T	R-T, Most T-R

[1]S = Susceptible to *M. hapla*; R = Resistant to *M. hapla*; T = Tolerant to *M. hapla*.

with the field ratings were Gold Pak, Danvers, Spartan Winnter, Spartan Fancy, M 3489, and (1304/872)-1-M-CM. Discrepancies were seen for M 5987, Spartan Delux, M 5986, Spartan Delite and M 5988.

The evaluation of susceptibility based on both gall indices and economic indices indicate that field test results (8) correlated with the greenhouse test for the Danvers, Spartan Fancy, M 3489 and (1304/872)-1-M-CM cultivars and lines. The tolerance to resistant correlation existed for Spartan Classic, Spartan Bonus and Spartan Premium cultivars.

Nematode population density in the roots can be viewed as an evaluation of the host potential of the cultivar. Plant host potential may or may not correlate with the resistance or susceptibility of a particular cultivar (Table 4.2). Two susceptible carrots, Danvers and M 3489, as well as two resistant carrots, M 5986 and Spartan Premium, had high population densities of M. hapla (greater than 50 second-stage larvae extracted from 60 day old root systems by the shaker method (207) (Table 4.1). Several susceptible carrots (M 5987, Spartan Winner, Spartan Fancy and (1304/872)-1-M-CM) had low population densities of M. hapla (10 or less larvae per root system). The high population found on Spartan Premium was present on only one of the four replicates.

Greenhouse evaluation of carrot has a number of limitations. Portions of the carrot population show genetic variability for susceptibility or resistance. This is seen

by comparing the results of the population dynamics study and this investigation. The number of second-stage larvae of M. hapla extracted from 60 day old root systems of Spartan Premium may not have a high host potential rating. The Gold Pak information closely correlated with 17.5 and 19.8 root nematode counts. This would confirm the medium rating of host potential. The fact that only four replicates were evaluated is another major limitation, and to increase the accuracy, more replicates of each cultivar or parent line should be studied. The initial population of M. hapla may have been too high for a good evaluation, since there were deformed carrots even in the field tested resistant rated cultivars and lines. Other influencing factors which may have affected the results were insoluble salts deficiency in the muck soil and phytoburn caused by pest controlling chemicals (Appendix A).

Future studies should include an adequate fertilization program, a reduction in the introduced population density of M. hapla and an increase in the number of replicate plants.

Mycorrhizal Investigation

Results

Vesicular-arbuscular mycorrhizae were present in all carrot roots grown in the soil infested with Glomus spp. The degree of colonization increased with the age of the

plant (Figure 5.1). The greatest rate of increase in vessicle and arbuscule development was from day 30 to day 60 after planting. Colonization was not observed in any of the 20 root systems grown in the absence of Glomus spp. There were no significant differences (P=0.05) in the plant weight and root weights of the colonized and control carrots (Figure 5.2). The colonized plants weighed less until later growth days, at 120 days after planting they weighed 27 per cent more.

Discussion

Endomycorrhizal associations have been reported to enhance nutrient uptake for various crops (50,111,183,186,189). In carrots, this nutrient uptake increase or growth enhancement was not seen until after 90 days of growth. It would appear to be of minimal beneficial value to carrots, particularly of early cultivars such as Spartan Premium which attains a marketable size by 80 days of growth. The muck soil used in this investigation had an insoluble salts deficiency. This was severe enough to reduce the Gold Pak carrots overall growth by about 60 per cent. The mycorrhizal influence on growth should have been enhanced by this deficiency. On day 120 of growth the colonized carrot weighed 49 per cent less than the Gold Pak carrots of the population dynamic study. Further work is needed to establish any role that endomycorrhizae may have in carrots as growth enhancers,

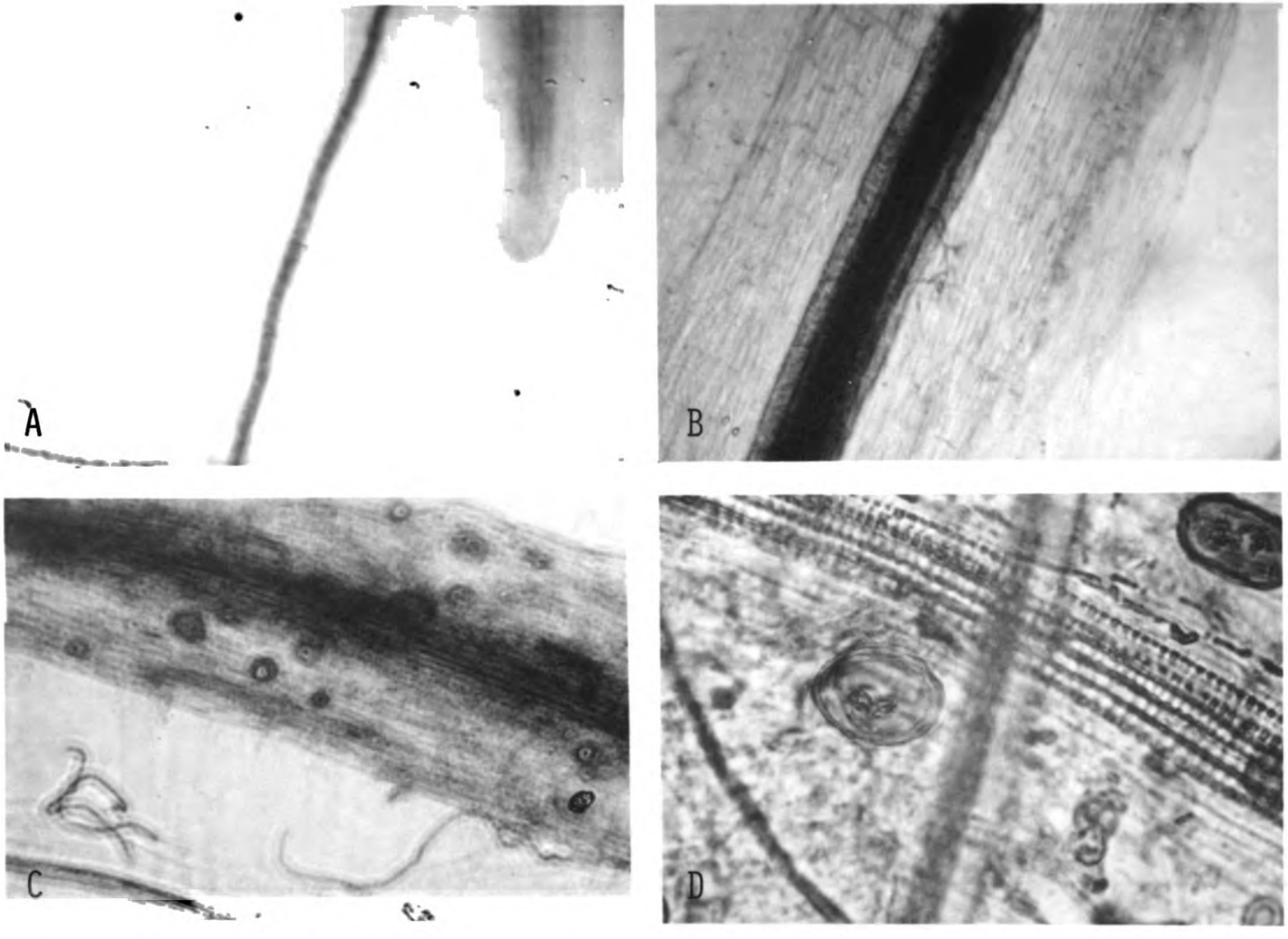

Figure 5.1. Gold Pak carrot root colonized by Glomus macrocarpus var. geosporus. A - noninfected, B - 30 days after planting, C - 60 days after planting, D - 90 and 120 days after planting.

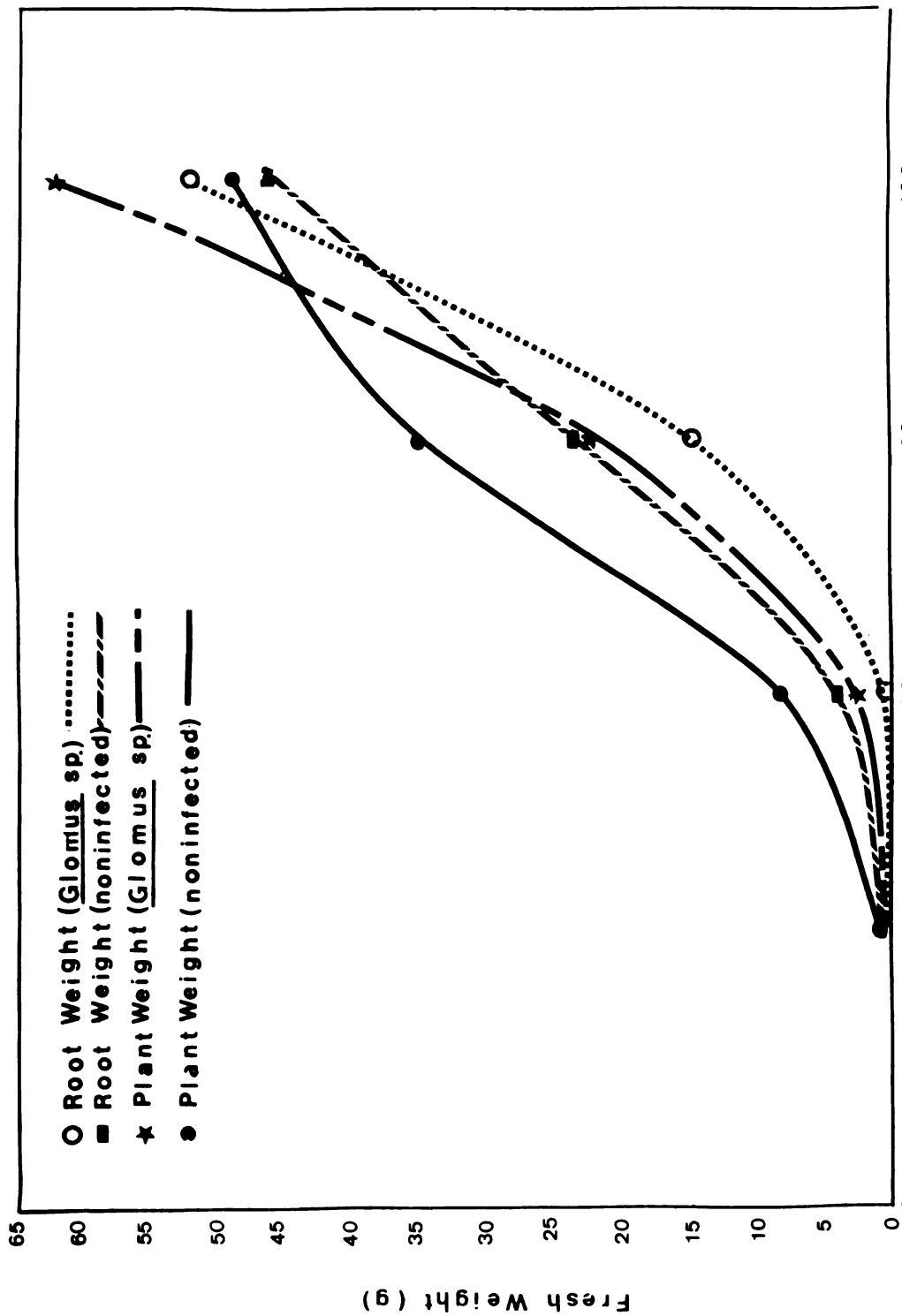

Figure 5.2. Effect of colonization of carrot root by Glomus macrocarpus var. geosporus.

plant efficiency and disease protectants, especially in relation to nematodes. The degree of colonization found under field conditions should also be assessed. The extensiveness and rate of colonization, as well as the possible growth effects, are related to the infecting Endogonacea species.

Preliminary Pest-Crop Ecosystem Model

Data from these investigations were used for development of a preliminary pest-crop ecosystem model for the growth and development of carrots (cv Spartan Premium) in relation to the economics of nematode control. The objective was to develop a simple model based on easily observable plant characteristics. Relative growth, absolute growth and net assimilation rates were not suitable for the model. The relative growth rate predicted relative changes among various components of the plant, but could not be used to predict differences between plants. The absolute growth rate had a high degree of variability with no significant observable trends. The net assimilation rate was a relative measure which did not specify the state of growth of the carrot. It also had a high variability. The only measures of plant growth with enough consistency to be of predictive value were leaf surface area and fresh weight of the tap root. Both these measures also fulfilled the criterion of easy observability.

The initial step in designing the model was to determine the leaf surface area to taproot fresh weight ratio (LATR), and its regression line ($R^2 = 0.93$).

$$\ln \text{LATR} = 7.88 - 0.068 \times \text{Time}$$

The regression $\ln W_{tf} = 0.712 + 0.0179 \times$ Time of taproot fresh weight was a good predictor of taproot fresh weight (W_{tf}) at any later time ($R^2 = 0.89$). It was noted that the regression for $\ln W_{tf}$ in relation to time has the same form for all levels of M. hapla infection tested (Table 5.1). The principal variation in the regression for the various levels of infection (0, 10, 100, 1000 introduced second-stage juveniles of M. hapla) was the Y intercepts (Table 5.1).

Regressions were used for the growth model. The fresh weight of the taproot must be estimated from leaf surface area or determined by weighing the taproots. Using the leaf surface area, the taproot weight for carrots (cv Spartan Premium) is estimated from equations:

$$\text{LATR} = L/W_{tf} = 7.88 - 0.068 \times \text{Time}$$

$$W_{tf} = L/(7.88 - 0.068 \times \text{Time})$$

where LATR = leaf surface area to taproot fresh weight ratio

W_{tf} = weight of the taproot

T = the time in days of harvest

L = leaf surface area.

Table 5.1. Regression of the natural log of the ratio of leaf surface area to taproot fresh weight of carrots (cv Spartan Premium) for 36-88 days after planting [ln (Taproot fresh weight) = A + B x Time].

Introduced Population (*M. hapla*)	A (Y intercept)	B (Slope)	R^2
0	-3.579	0.0804	0.923
10	-3.819	0.0811	0.877
100	-4.41	0.083	0.945
1000	-3.843	0.081	0.89

Estimation of taproot fresh weight increases the degree of error.

After determination of the fresh weight of the taproot for some time during the season, the Y intercept can be found ($A = \ln W_{to} - 0.081 \times T_o$). Using the intercept value the yield can be predicted by $W_{tf} = e^{(A - 0.081 \times T_0)}$.

The pest-crop ecosystem model for the growth and development of carrots (cv Spartan Premium) in relation to the economics of nematode control has three steps:

1. Circa 36 days after planting (T_0), the taproot fresh weight must be determined. Estimation can be made based on leaf surface area using the equations:

$$LATR = L_0/W_{TO} = 7.88 - 0.068 \times T_0$$

$$W_{TO} = L_0/(7.88 - 0.068 \times T_0)$$

The Y intercept of the regression line is calculated from $A = \ln W_{TO} - 0.081 \times T_0$ and predicted yield per plant is obtained using $W_{TF} = e^{(A - 0.081 \times T_0)}$.

2. Taproot weights can be converted to predicted yield per acre by:

$$Y_0 = W_{tf} \times D$$

where L_0 = leaf surface area at harvest time

W_{TO} = weight of taproot at harvest time

T_0 = time of taproot harvest (days)

A = Y intercept of regression line

W_{TF} = taproot weight at time desired

D = density or number of plants per acre.

3. An economic pest-crop ecosystem model must reflect the carrot production loss caused by different levels of investation, the cost of pest management strategies and predicted yield. The average cost of soil fumigation in 1975 was 167.00 (± $69.00) per acre. To determine the economics of fumigation, the cost of crop production must be evaluated.

$$NP = GP - CP$$

where NP = net profit

GP = gross profit

CP = cost of production.

If $NP - FC > El_{Mh}$ or $EL > FC$

where FC = cost of fumigation (nematode control)

EL_{Mh} = estimated losses caused by *M. hapla*

then application of an appropriate soil fumigant is economically feasible (Figures 6.1, 6.2 and 6.3).

More work must be done to substantiate the relationship between fall nematode densities and the degree of expected losses for various carrot cultivars. From this investigation it is possible to look at the number of nematodes extracted per pot in relation to introduced populations (Figure 3.8). The application of this model, however, is based on known nematode densities. A serious problem implicated is the use of the centrifuge technique for measurement of the actual nematode density in muck soil.

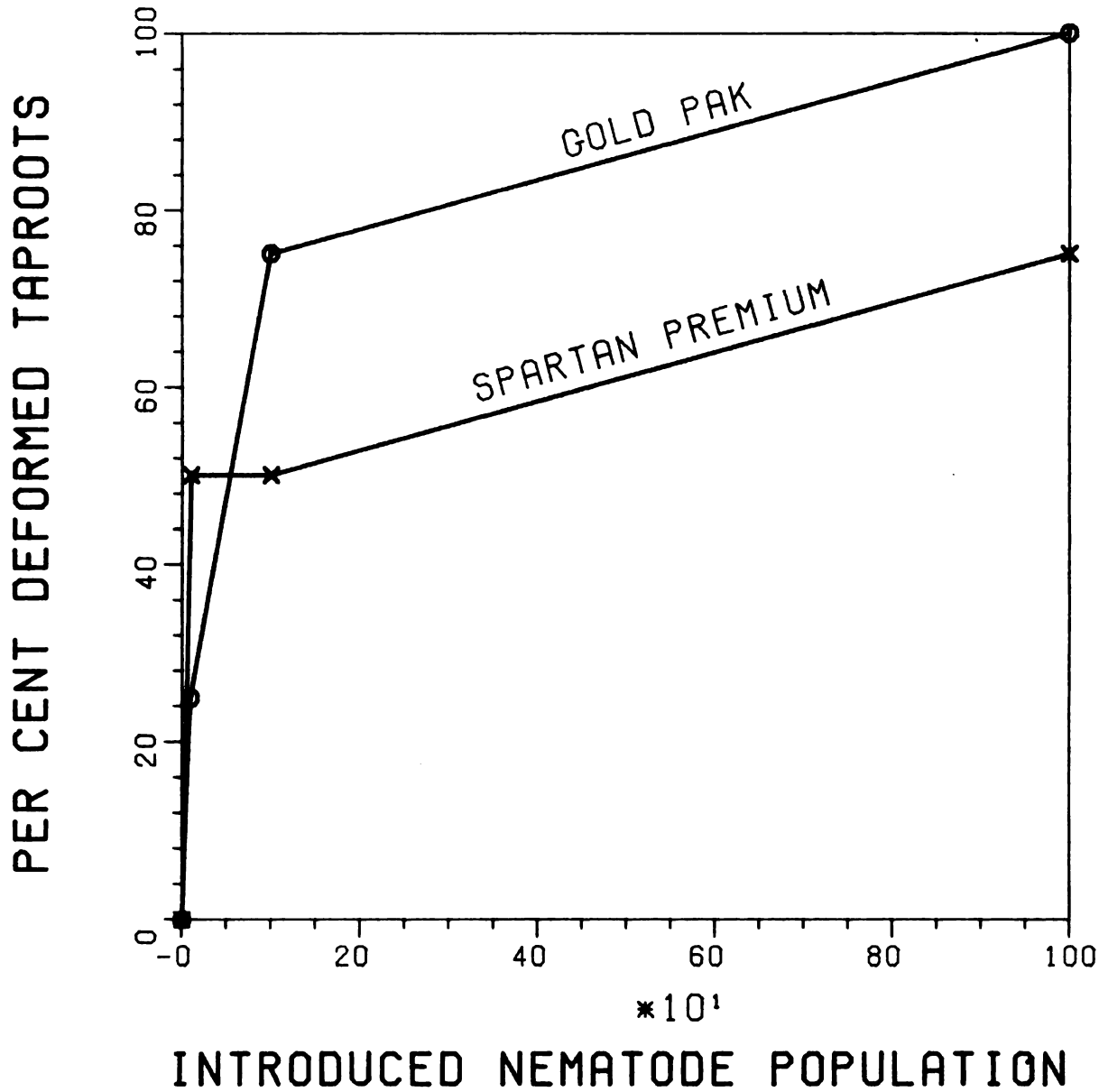

Figure 6.1. Economic index in relation to introduced nematode populations.

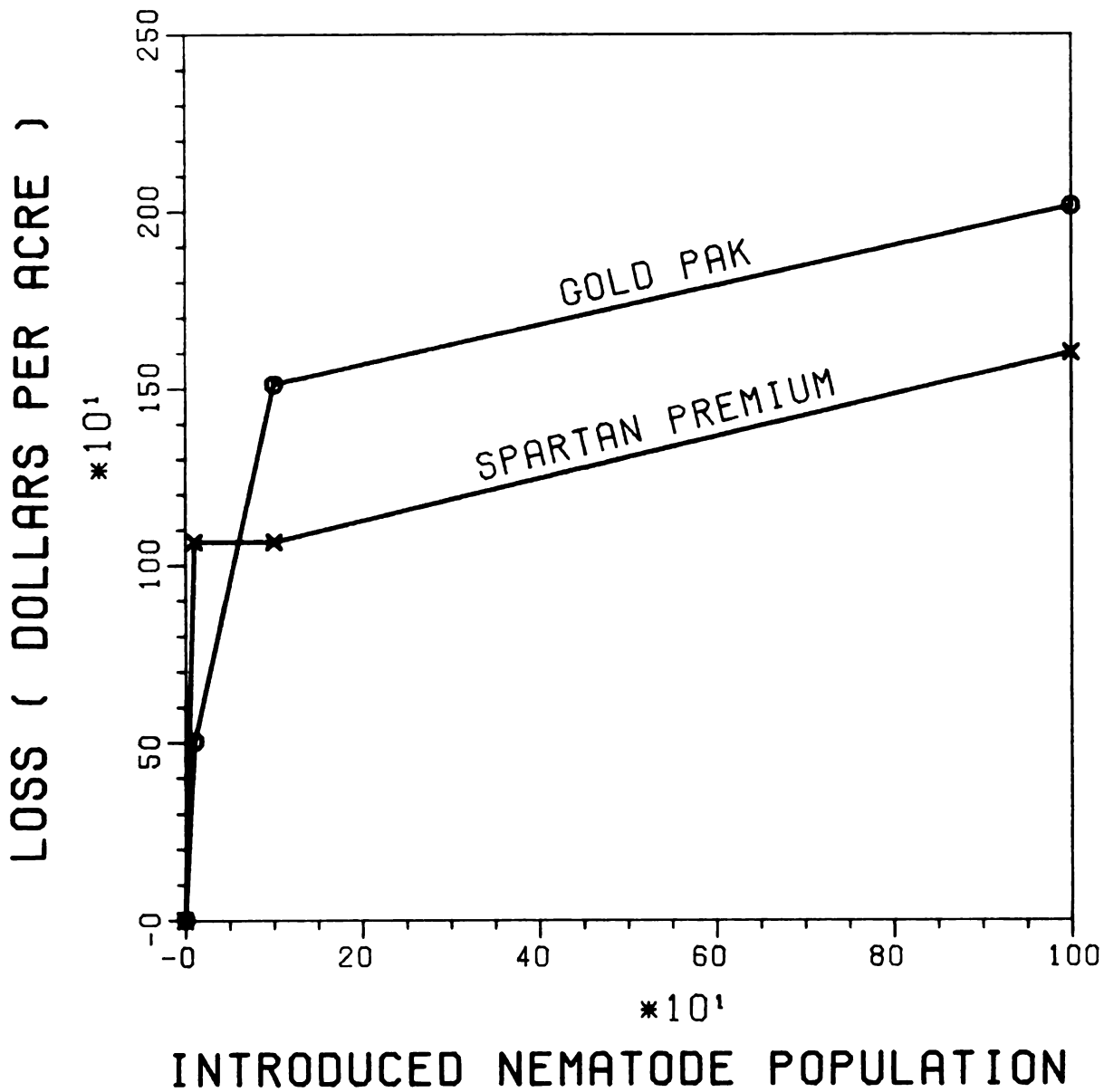

Figure 6.2. Loss of dollars per acre caused by nematode infestation.

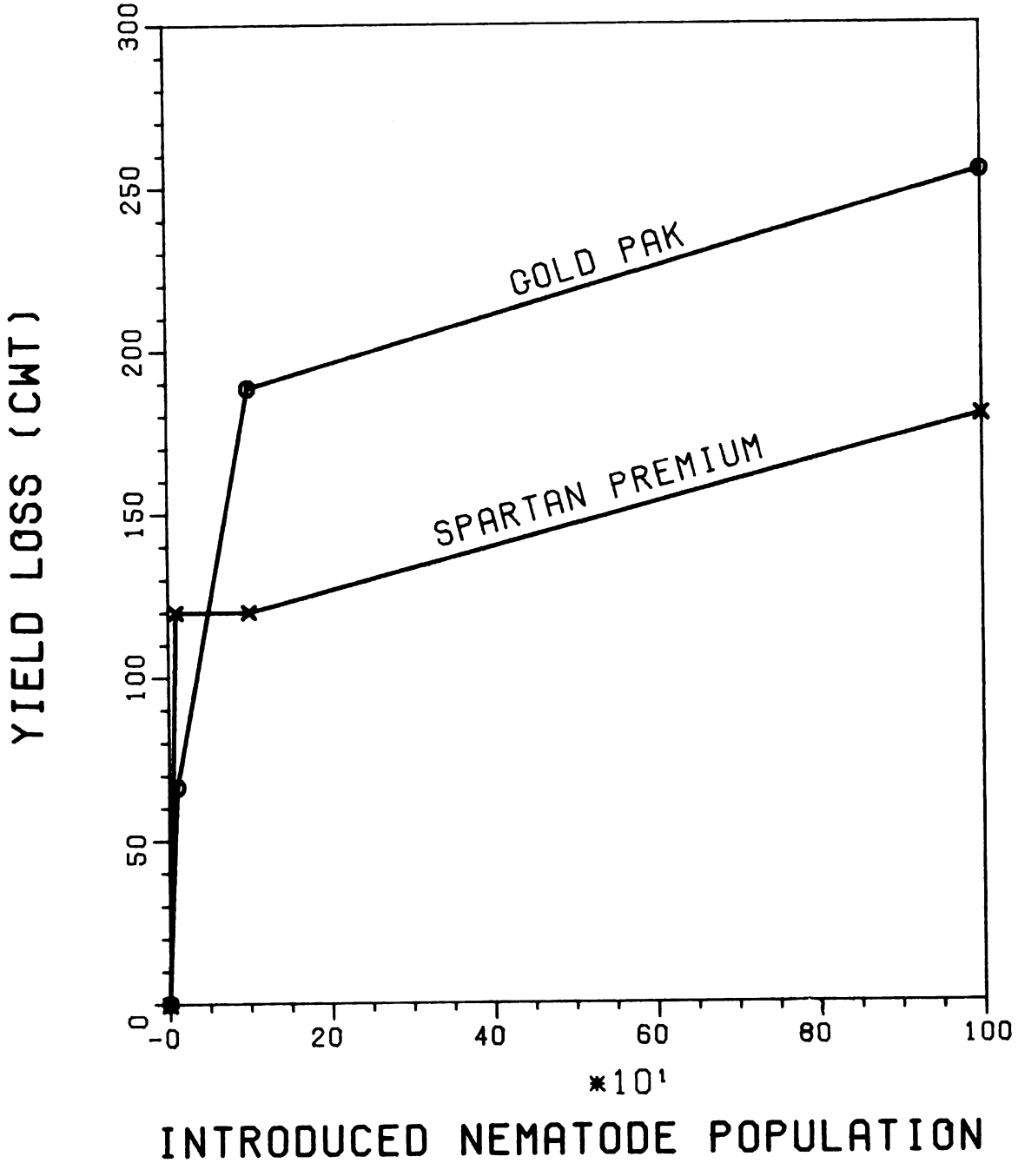

Figure 6.3. Yield loss in cwt per acre in relation to introduced nematode population.

Further work should be done to determine the degree of nematode recovery possible by this technique in field samples of muck soils. The relationship between fall populations of M. hapla and spring infection levels should also be defined.

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APPENDICES

APPENDIX A

POST CONTROL PROGRAM

EXPERIMENT				
Population Dynamics	Mycorrhizal Investigation	Cultivar Trials		
Days of Growth	Applied		Chemical/ Fumigant	Rate Used
71	38	--	Pyrellin E.C.[1]	15 ml/gal
75	42	--	Pyrellin E.C.	15 ml/gal
79	46	--	Pyrellin E.C.	15 ml/gal
87	54	--	Sevin[2]	1 tbs/gal
92	59	--	Sevin	1 tbs/gal
92	59	--	Plectron 50W[3]	1 tbs/gal
97	64	--	Plant Fume 103[4]	15,000 cu ft
98	65	--	Nicotine	20,000 cu ft
114	82	15	Nicotine	20,000 cu ft
115	83	16	Plectron 50W	1 tbs/gal
115	83	16	Malathion[5]	1 tbs/gal
--	118	51	Nicotine	10,000 cu ft

[1]Pyrellin E.C.--Pyrethrine 0.6% + Rotenone 0.5%.

[2]Sevin--Carbayl(1-naphtyl n-methylcarbamate).

[3]Plectron 50W--Tricyclohexyltin hydroside 50%

[4]Plant Fume 103--(smoke generated) 0,0,0,0-tetraethyl dithio-pyrophosphate 15%.

[5]Malathion--50% 0,0-dimethyldithiophosphate of diethyl mercaptosuccinate.

APPENDIX B

GROWTH ANALYSIS FORMULA

Net Assimulation Rate (NAR)[1]

$$NAR = \frac{W_2 - W_1}{T_2 - T_1} \times \frac{\ln L_2 - \ln L_1}{L_2 - L_1}$$

Relative Growth Rate (RGR)[1]

$$RGR = \frac{\ln W_2 - \ln W_1}{T_2 - T_1}$$

Leaf Area Ratio (LAR)[1]

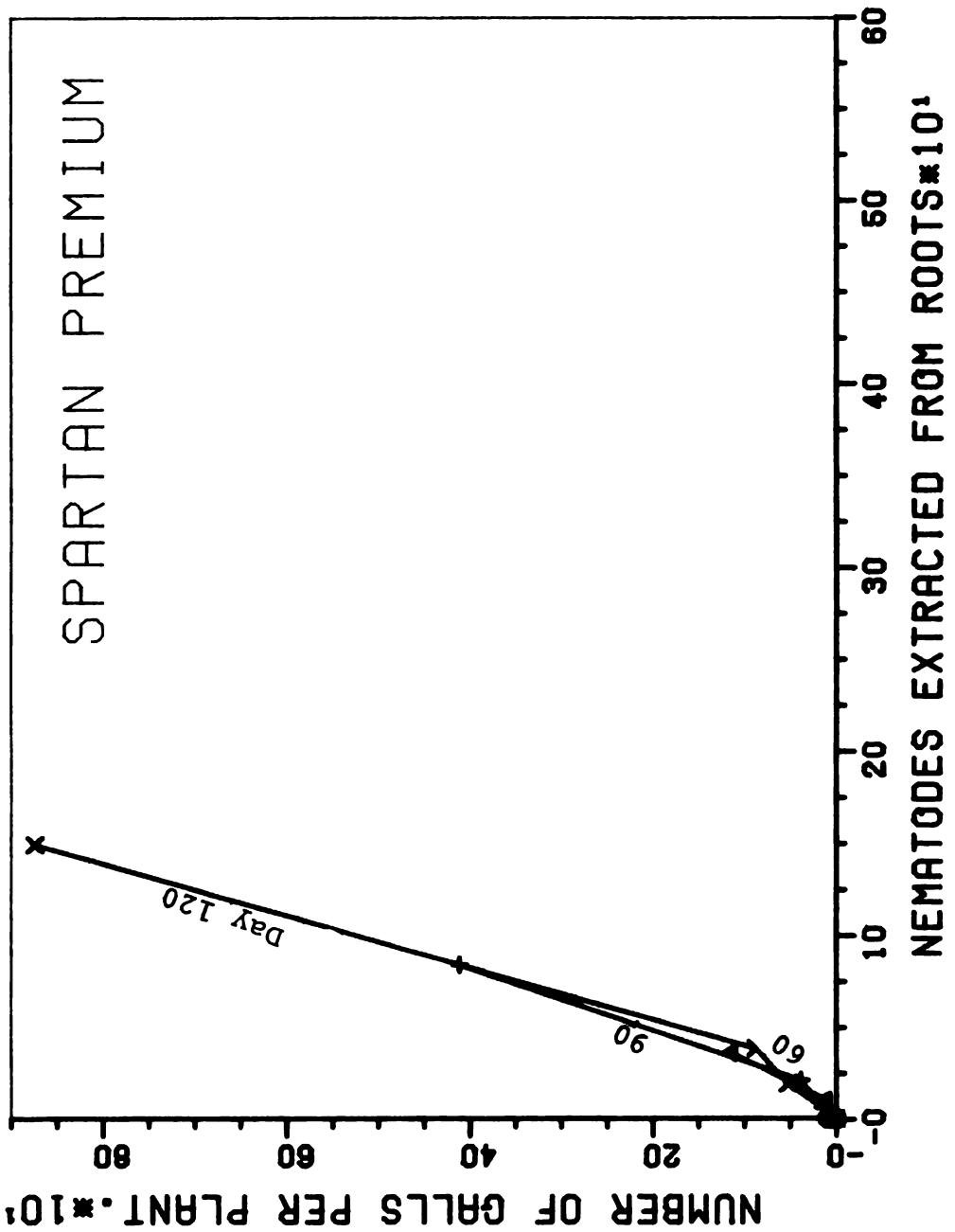
$$LAR = \frac{L_1 + L_2}{W_1 + W_2}$$

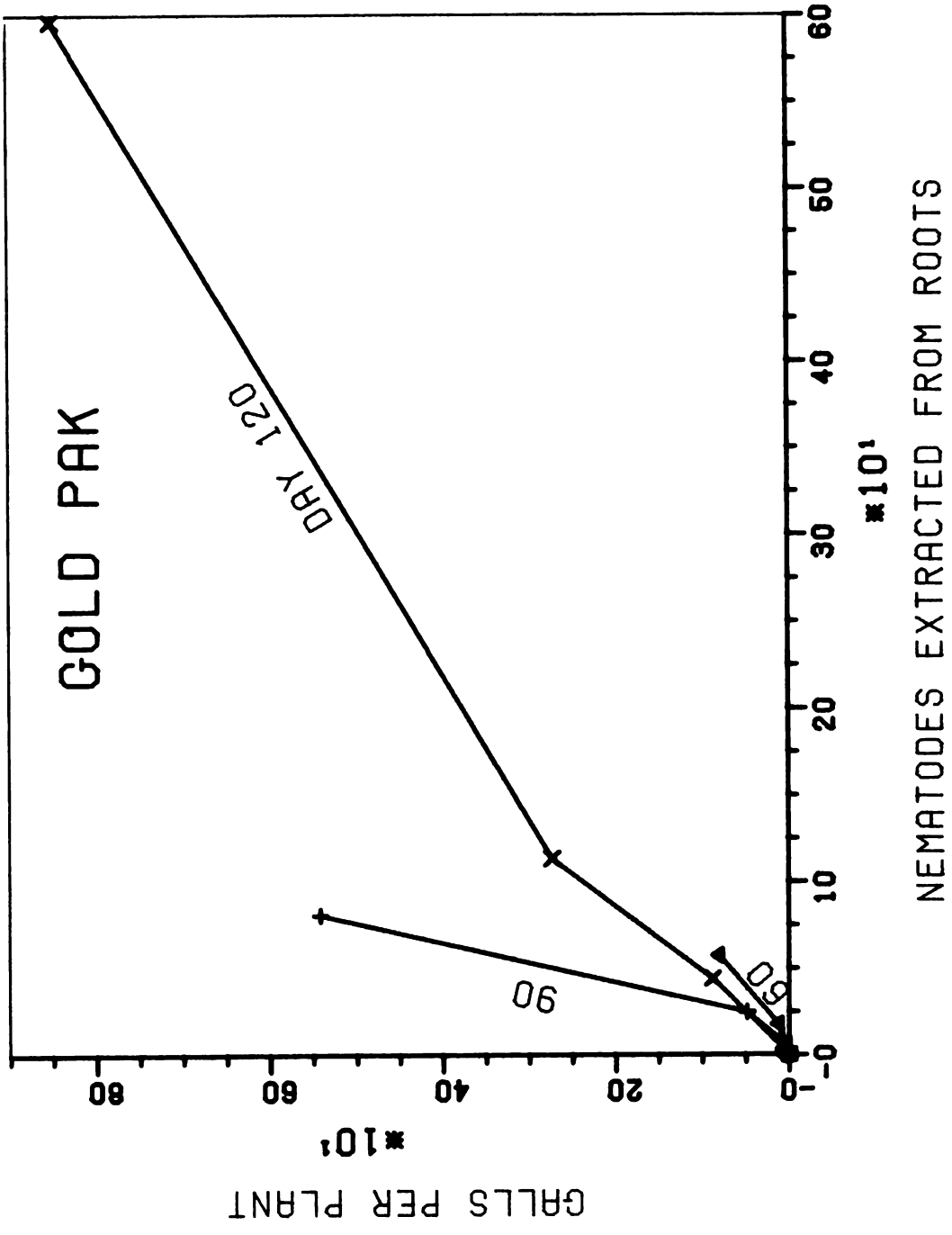
---

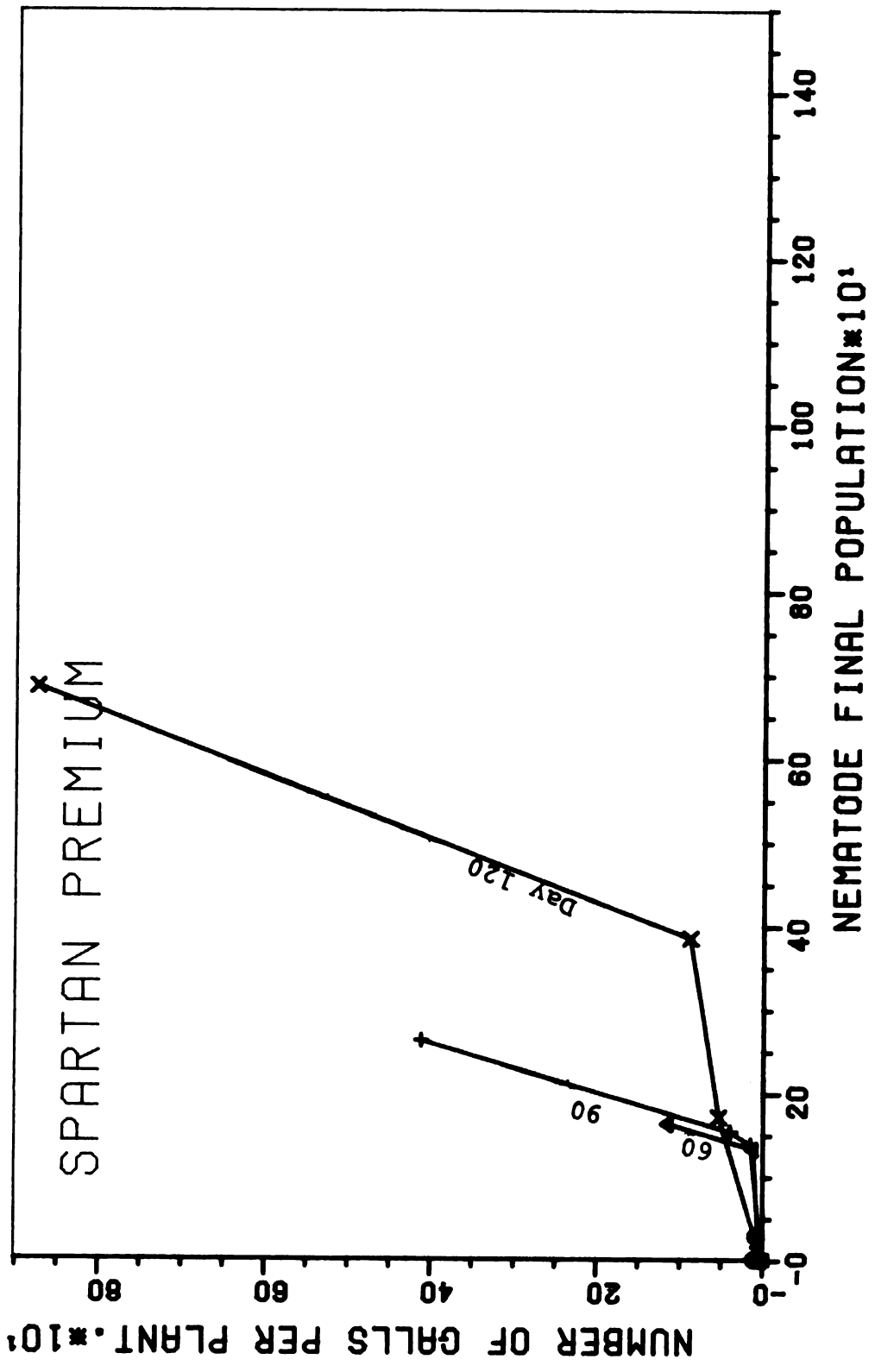
[1] L = leaf area (cm^2)
T = time (days)
W = plant dry weight (g)
ln = natural log

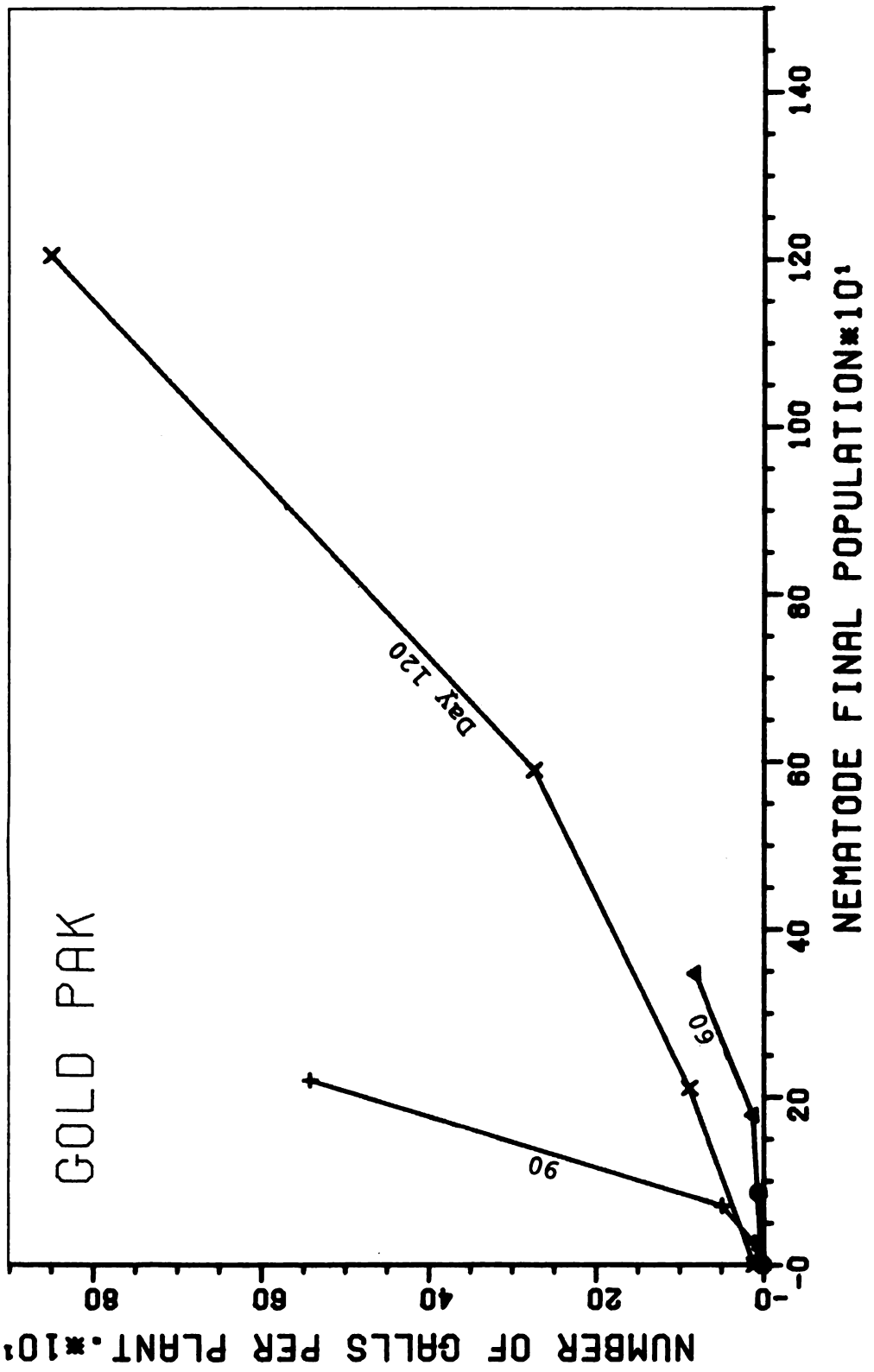
APPENDIX C

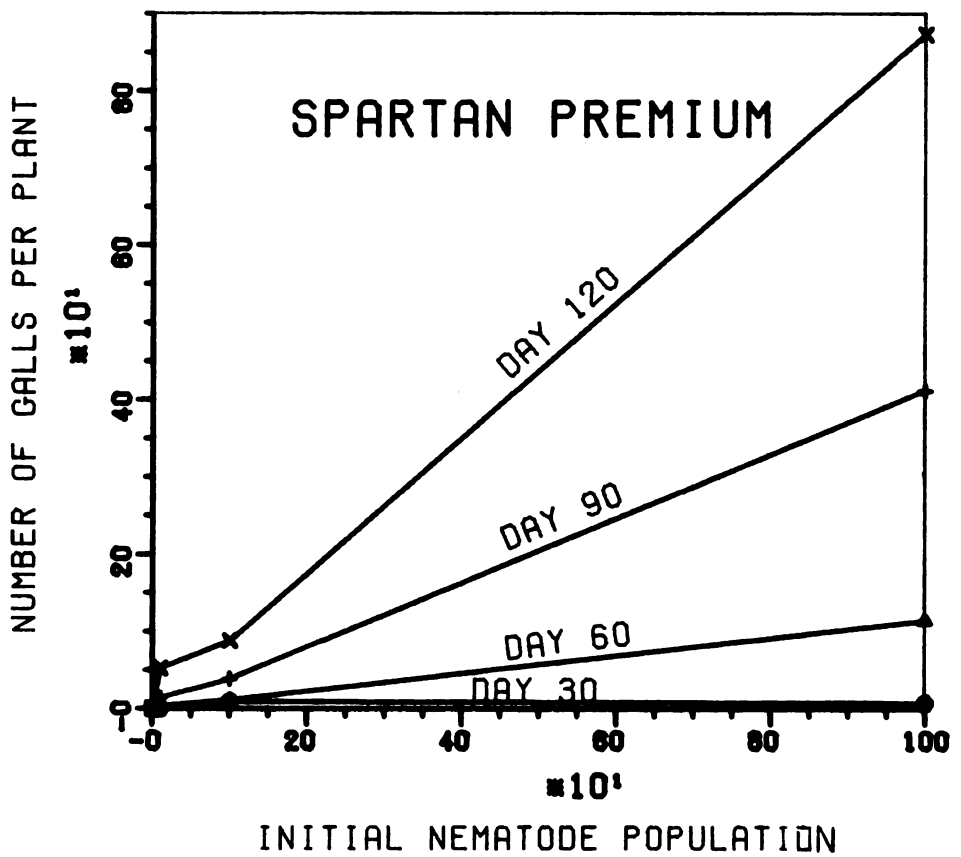
SUMMARY GRAPHS

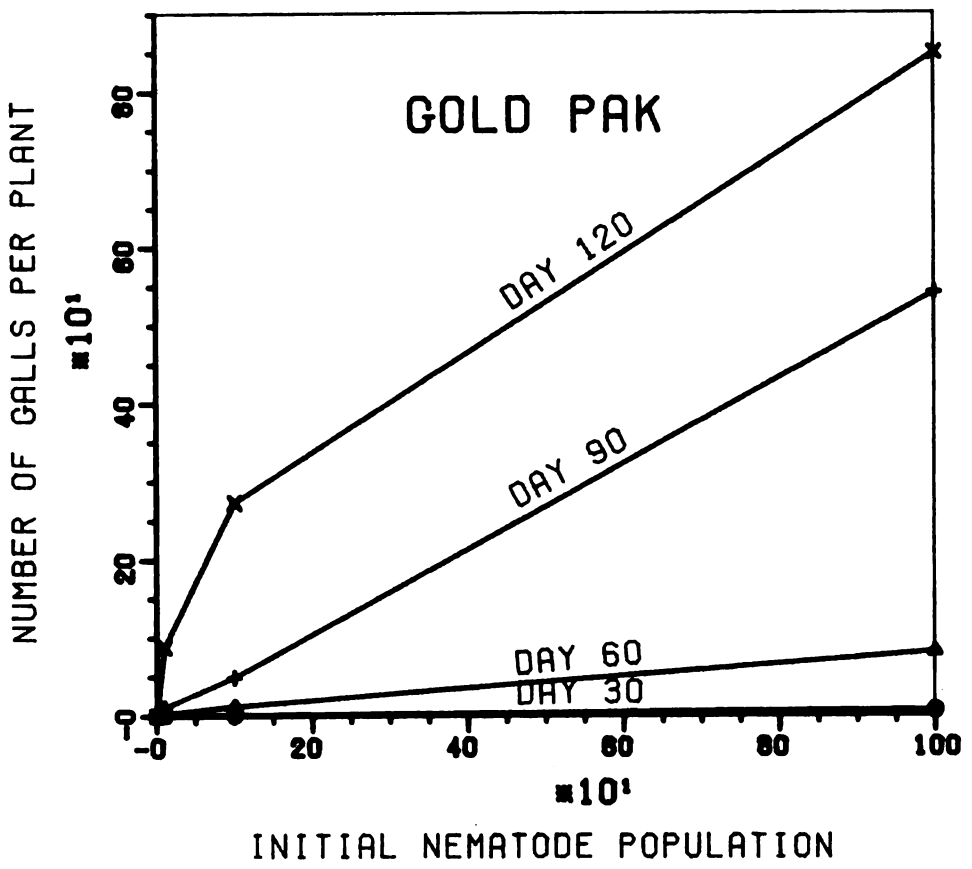

SPARTAN PREMIUM
NUMBER OF GALLS PER PLANT.*10¹
NEMATODES EXTRACTED FROM ROOTS*10¹
0
20
40
60
80
0
10
20
30
40
50
60
Day 120
90
60

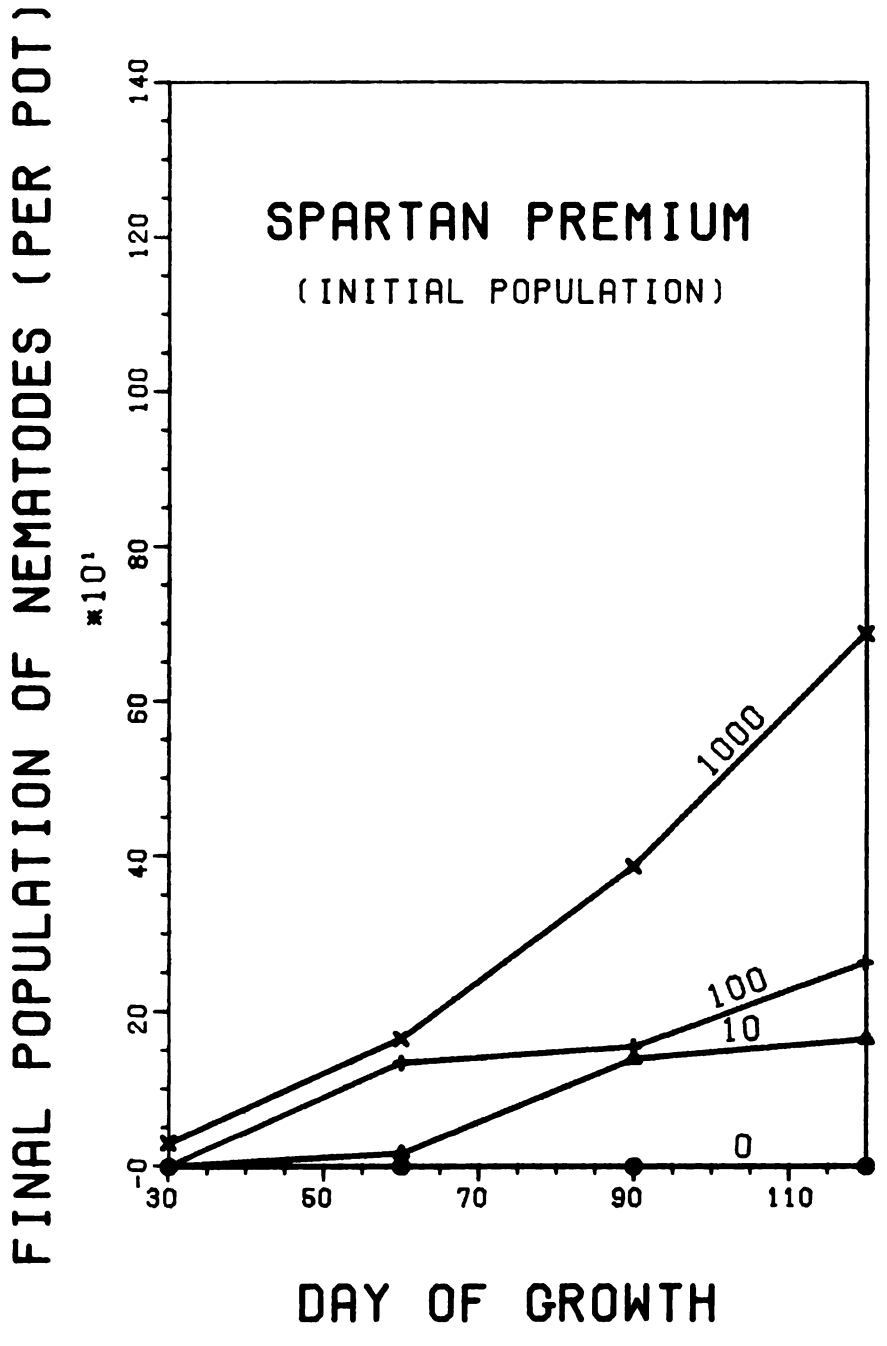

GOLD PAK
DAY 120
90
60
GALLS PER PLANT
*10²
0
20
40
60
80
NEMATODES EXTRACTED FROM ROOTS
*10¹
0
10
20
30
40
50
60

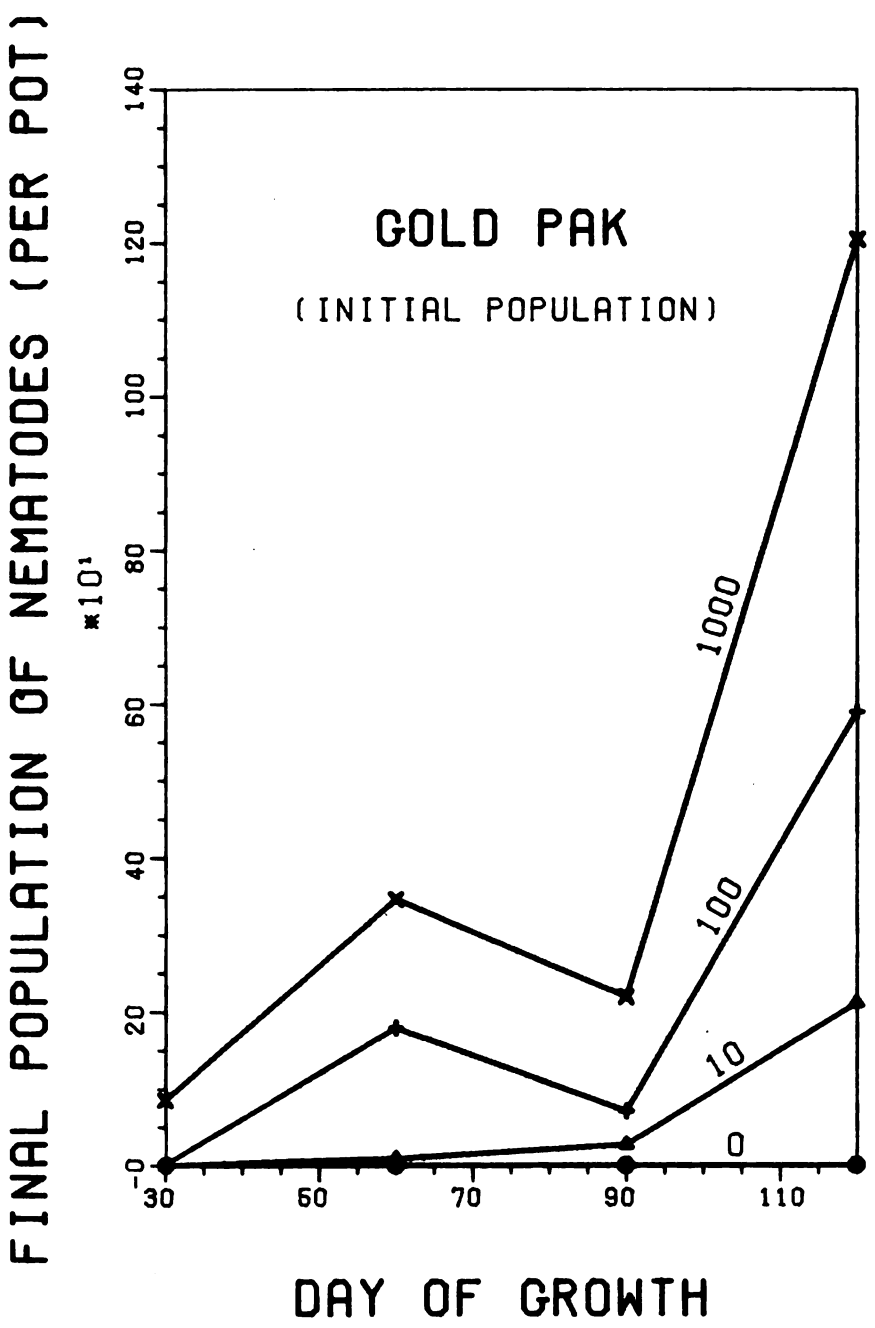

SPARTAN PREMIUM
Day 120
90
60
NUMBER OF GALLS PER PLANT.*10¹
NEMATODE FINAL POPULATION*10¹
0
20
40
60
80
100
120
140

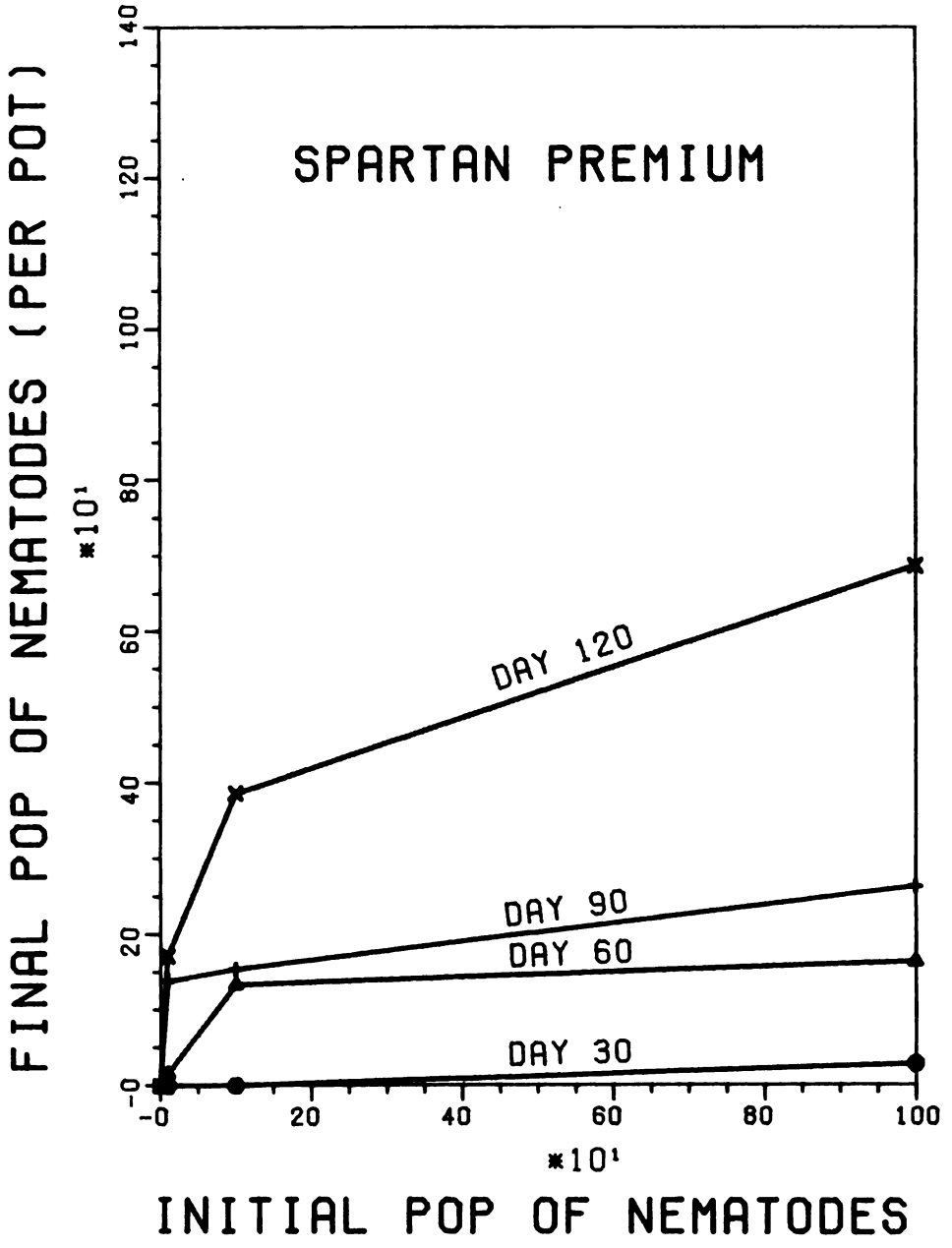

GOLD PAK
NUMBER OF GALLS PER PLANT.*10¹
NEMATODE FINAL POPULATION*10¹
0
20
40
60
80
100
120
140
Day 120
90
60

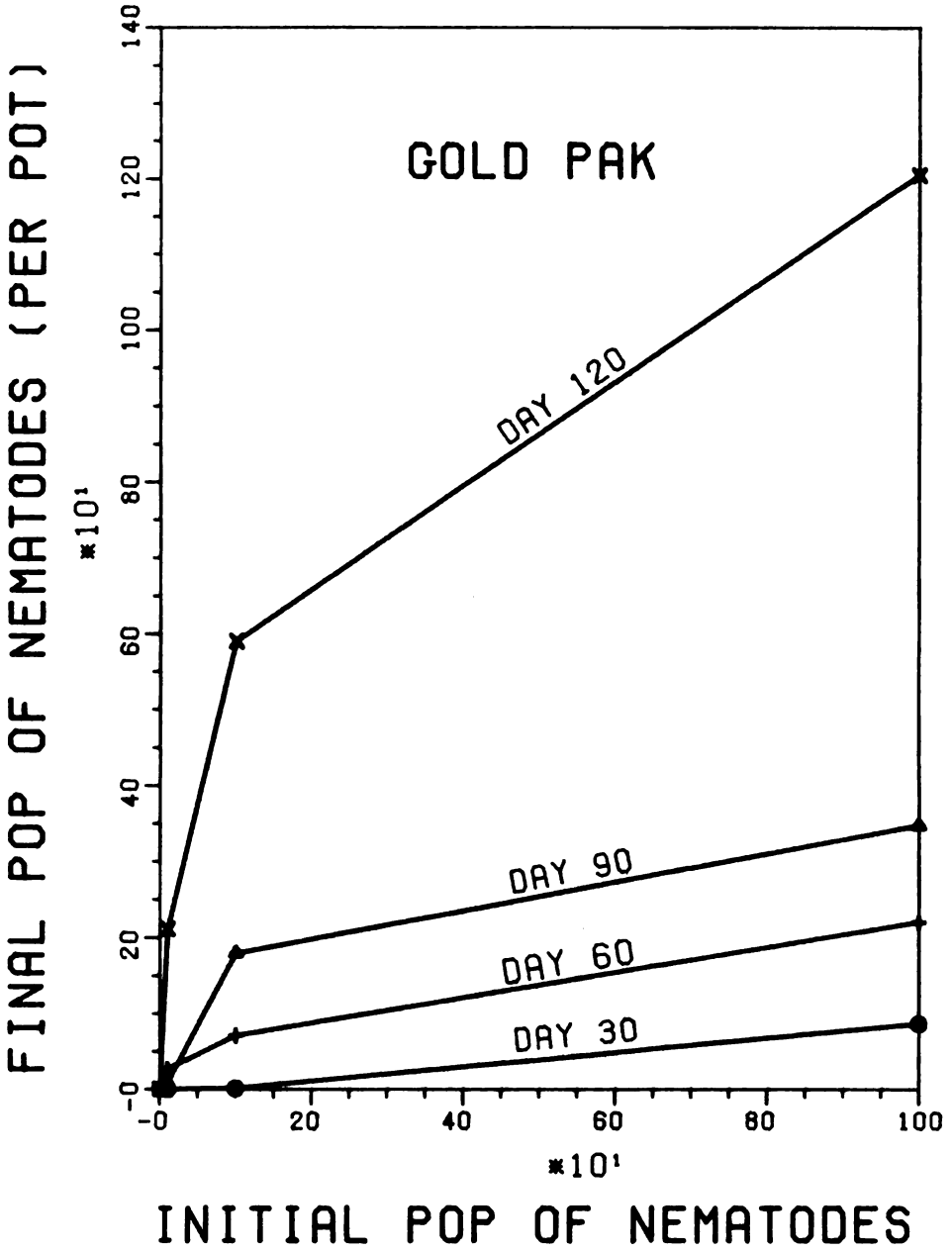

SPARTAN PREMIUM
DAY 120
DAY 90
DAY 60
DAY 30
NUMBER OF GALLS PER PLANT
x10¹
0
20
40
60
80
100
x10¹
INITIAL NEMATODE POPULATION

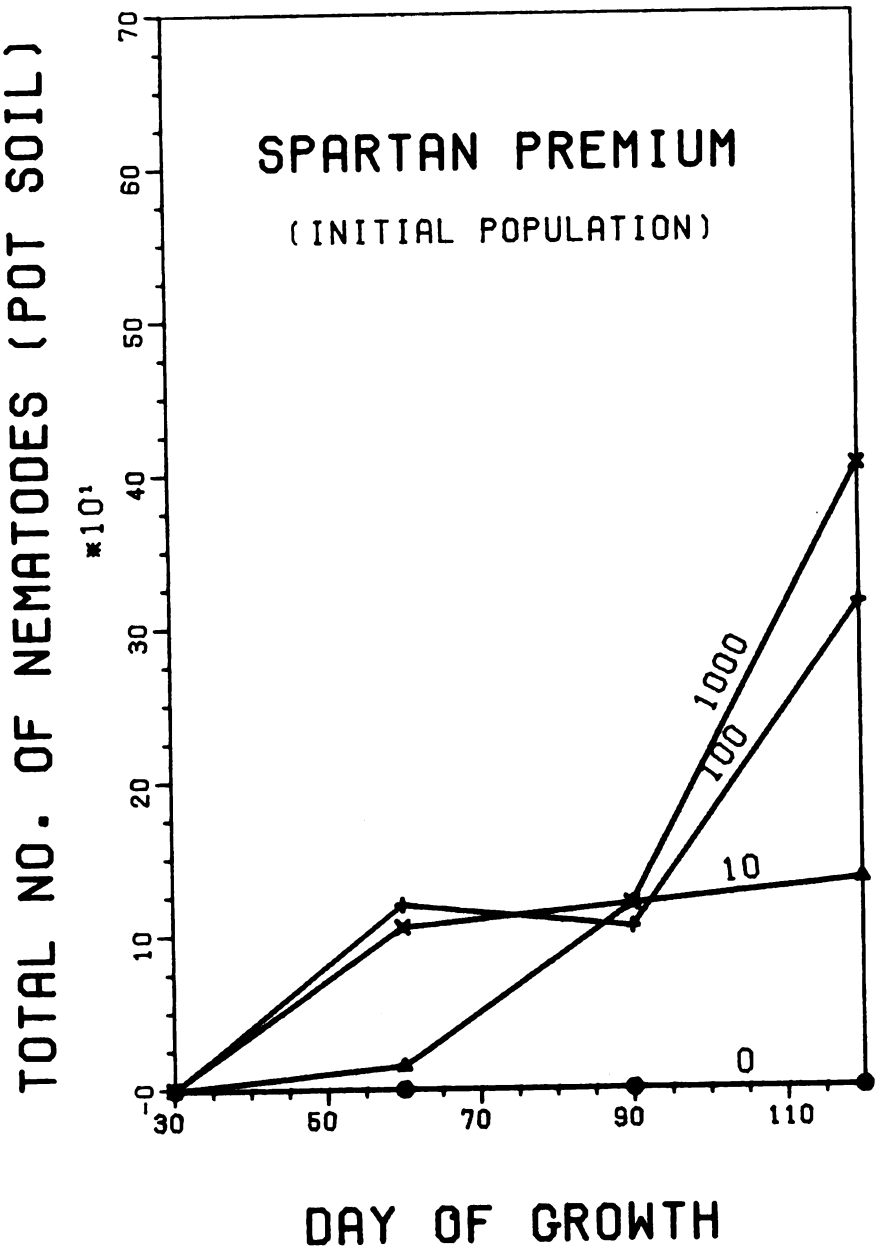

GOLD PAK
DAY 120
DAY 90
DAY 60
DAY 30
NUMBER OF GALLS PER PLANT
x10¹
0
20
40
60
80
100
x10¹
INITIAL NEMATODE POPULATION


FINAL POPULATION OF NEMATODES (PER POT)
*10¹
SPARTAN PREMIUM
(INITIAL POPULATION)
1000
100
10
0
140
120
100
80
60
40
20
-0
30
50
70
90
110
DAY OF GROWTH


FINAL POPULATION OF NEMATODES (PER POT)
*10¹
GOLD PAK
(INITIAL POPULATION)
1000
100
10
0
140
120
100
80
60
40
20
-0
30
50
70
90
110
DAY OF GROWTH


SPARTAN PREMIUM
FINAL POP OF NEMATODES (PER POT)
*10¹
INITIAL POP OF NEMATODES
*10¹
DAY 120
DAY 90
DAY 60
DAY 30
-0
20
40
60
80
100
120
140


GOLD PAK
DAY 120
DAY 90
DAY 60
DAY 30
FINAL POP OF NEMATODES (PER POT)
*10¹
INITIAL POP OF NEMATODES
*10¹
-0
20
40
60
80
100
120
140


SPARTAN PREMIUM
(INITIAL POPULATION)
TOTAL NO. OF NEMATODES (POT SOIL)
*10¹
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10
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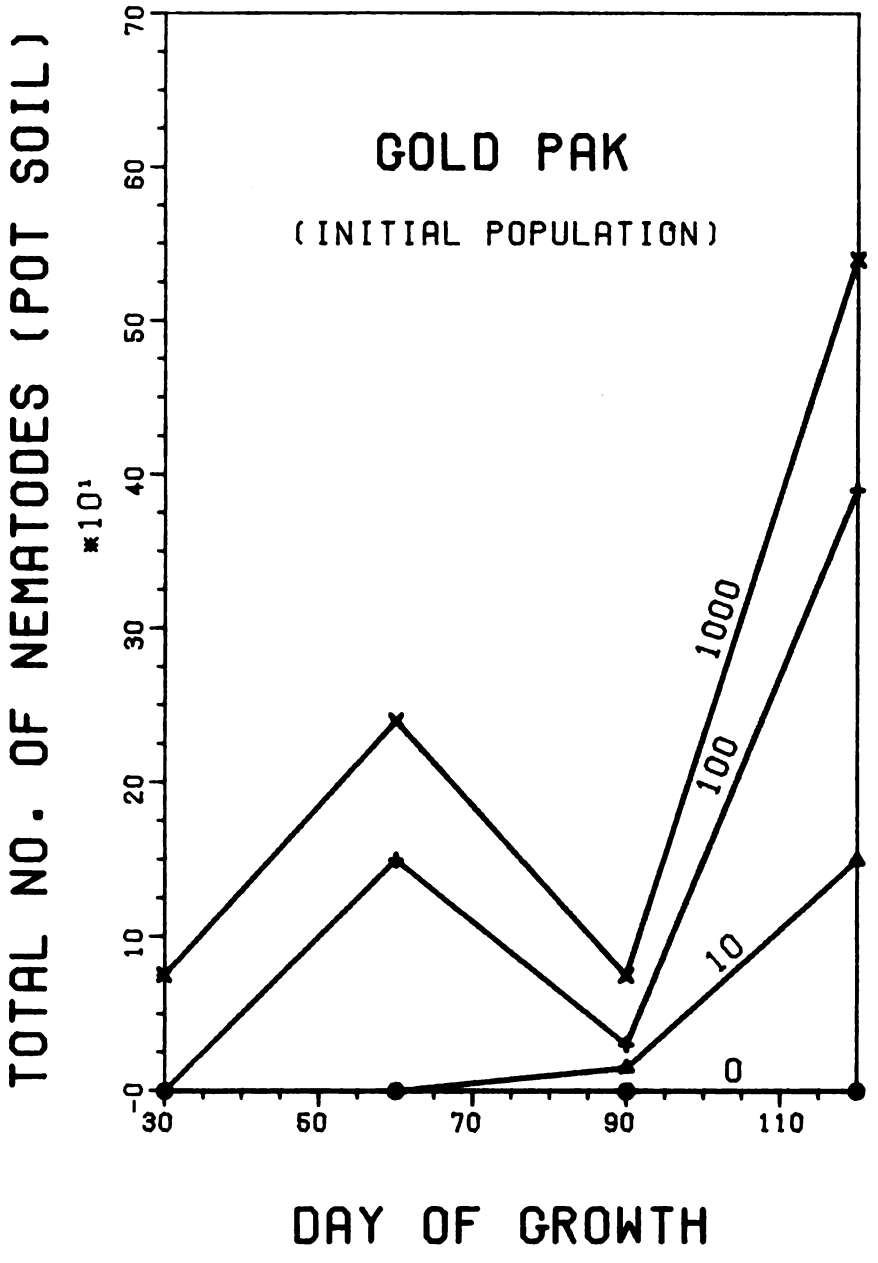
30
40
50
60
70
30
50
70
90
110
DAY OF GROWTH
1000
100
10
0


GOLD PAK
(INITIAL POPULATION)
TOTAL NO. OF NEMATODES (POT SOIL)
*10¹
0
10
20
30
40
50
60
70
30
50
70
90
110
DAY OF GROWTH
1000
100
10
0