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# Plant-Parasitic Nematodes Attacking Chickpea and Their In Planta Interactions with Rhizobia and Phytopathogenic Fungi

Chickpea (*Cicer arietinum*) is a cool-season food legume second in importance as a pulse crop in the world after beans (*Phaseolus vulgaris*). It is an important protein source in many regions of the semi-arid tropics (30). Chickpea is grown in 47 countries and is a significant component of subsistence cropping systems for farmers in the Indian Subcontinent, West Asia, the Mediterranean Basin, and certain areas of East and North Africa. More than 90% of the chickpea crops are grown in eight countries, including India, Pakistan, Iran, Turkey, Ethiopia, Mexico, Australia, and Canada, in decreasing order.

Many species of plant-parasitic nematodes have been reported in the roots and rhizosphere of chickpea in the major growing regions in the world (Table 1). However, only certain nematode species are considered constraints to chickpea production, causing an estimated 14% in annual yield losses (61,68). The symptoms and signs of nematode parasitism on chickpea differ depending upon the nematode's feeding habit. Moreover, nematode attacks can make plants more sensitive to other biotic and abiotic stresses, and overall result in stunting and poor yield (70). Symptoms caused by nematode attack are nonspecific and nondescript, and they are largely a consequence of disruption of normal processes of plant growth, and absorption and translocation of water and nutrients. Consequently, damage by nematodes to chickpea often goes unnoticed and is attributed to other causes, such as lack

of fertility or deficient soil moisture. For example, even 50% reductions in chickpea yield caused by the chickpea cyst nematode, *Heterodera ciceri*, cannot be predicted by inspection of the upper part of the plant (35). For these reasons, plant-parasitic nematodes are often referred to as the "unseen enemies" of crops.

The most important nematode pathogens of chickpea include root-knot nematodes (*Meloidogyne* spp.), root-lesion nematodes (*Pratylenchus* spp.), cyst-forming nematodes (*Heterodera* spp.), and the reniform nematode (*Rotylenchulus reniformis*). In this paper, we will focus on the geographical distribution and pathogenicity of these nematodes, along with the symptoms, epidemiology, and management of the diseases they cause on chickpea. In addition, we will describe some of the most common and efficient diagnostic methods that are available for nematodes. Finally, we will address the complex interactions that occur between some nematodes and other microorganisms that coinfect chickpea, including rhizobia and the fungal pathogen *Fusarium oxysporum* f. sp. *ciceris*, which causes Fusarium wilt.

## Root-Knot Nematodes

Sedentary endoparasitic root-knot nematodes of the genus *Meloidogyne* are among nature's most successful plant parasites. *Meloidogyne* species infect thousands of different hosts, including herbaceous and woody monocotyledonous and dicotyledonous plants, and cause serious losses to numerous agricultural crops worldwide (28,57,61). The root-knot nematode species known to damage chickpea are *M. arenaria*, *M. incognita*, and *M. javanica*, warm climate species that attack chickpea in the Indian Subcontinent, and *M. artiellia*, a species well adapted to cool and wet conditions, that is widely distributed in the Mediterranean Basin (3,9,70).

Root galling is the typical symptom of parasitism by *Meloidogyne* spp. in susceptible crops. However, compared with large galls induced by *M. arenaria*, *M. incognita*, and *M. javanica* on chickpea, *M. artiellia* produces small galls or no galls (85). The most evident sign in roots infected by this latter species are the egg masses produced by the nematode, which may contain 200 to 600 eggs (Fig. 1) (39). The limited hyperplasia in the vascular cylinder and the cortical cells that surround the feeding site of *M. artiellia* seems to determine the small gall size in this species (85). Also, the frequently observed protrusion of bodies of adult *M. artiellia* females from infected roots, resembling the parasitic habit of adult cyst-nematode females, seems to be congruent with the lack of root galling (Fig. 1).

Parasitism by root-knot nematodes in chickpea involves the establishment of permanent feeding sites called giant cells in the root cortex, endodermis, pericycle, and vascular parenchyma (85). Feeding sites are sinks for plant photosynthate. In addition, deformation and blockage of vascular tissues at feeding sites limit translocation of water and nutrients, and further suppress plant growth and reduce seed yield (57,85). In chickpea, the number of giant cells in the feeding site (averaging four to six) and the area of individual giant cells are not influenced by root-knot nematode species or isolates within species (85). However, the number of nuclei per giant cell is significantly lower for *M. artiellia* than for *M. arenaria*, *M. incognita*, or *M. javanica* (Fig. 2). Similarly, maximum diameters of nuclei and nucleoli are significantly greater in giant cells induced by *M. artiellia* isolates compared with those induced by *M. arenaria*, *M. incognita*, or *M. javanica* (85) (Fig. 2).

Uneven patches of chickpea plant growth are commonly associated with soils

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infested with root-knot nematodes. Heavily infected plants show stunted growth, less branching, and leaves with pale green to yellow color (Fig. 3A). The extent of yield loss caused by root-knot nematodes is determined by a number of factors, including the nematode species, the population density, pattern of nematode distribution in soil, the soil cropping history, chickpea cultivar, climate, and soil type. Although a variety of mathematical functions have been proposed to describe the relationships between the initial nematode population density in soil and crop damage, the most widely accepted one is the Seinhorst model [ $y = m + (1 - m)z^{P-T}$  when  $P \geq T$ , and  $y = 1$  when  $P < T$ ] (63). In this model,  $y$  = relative value of the plant growth parameter being measured;  $m$  = the minimum value of  $y$  (y at a very large ini-

tial nematode population density);  $P$  = the initial nematode population density;  $T$  = the tolerance limit (initial population at which plant growth is not impaired; and  $z$  = a constant  $< 1$  reflecting nematode damage, with  $z^{-T} = 1.05$  (Fig. 4). Threshold levels for *M. incognita* and *M. javanica* on chickpea grown under greenhouse conditions were established at 0.2 to 2.0 second-stage juveniles (J2) per gram of soil (52,58,64); however, tolerance to *M. javanica* was identified in some chickpea cultivars (65). Comparatively, the tolerance limits of chickpeas to infection by *M. artiellia* under field conditions in micro-plot experiments were much lower, i.e., 0.14 and  $< 0.02$  eggs and J2 per  $\text{cm}^3$  of soil for winter- and spring-sown crops (Fig. 4), respectively (20). Chickpea yield losses of up to 60% were caused by severe *M. in-*

*cognita* infections in India (3); whereas in southern Italy, initial *M. artiellia* populations of 2 eggs and J2 per  $\text{cm}^3$  of soil caused a yield reduction of 50% in winter sowings, and about 80% in spring sowings (20).

*Meloidogyne* spp. attacking chickpea have a wide host range including many weeds, which constitute natural, alternative hosts for the nematodes and enable their survival and population increase (Fig. 3B) (13,69). Moisture, texture, and temperature are major soil physical factors affecting distribution and reproduction of those nematodes. *M. artiellia* completes one generation per growing season (21), mostly because of a combination of low, insufficient rainfall and high temperature that prevail in spring in the Mediterranean Basin. However, *Meloidogyne* spp. infect-

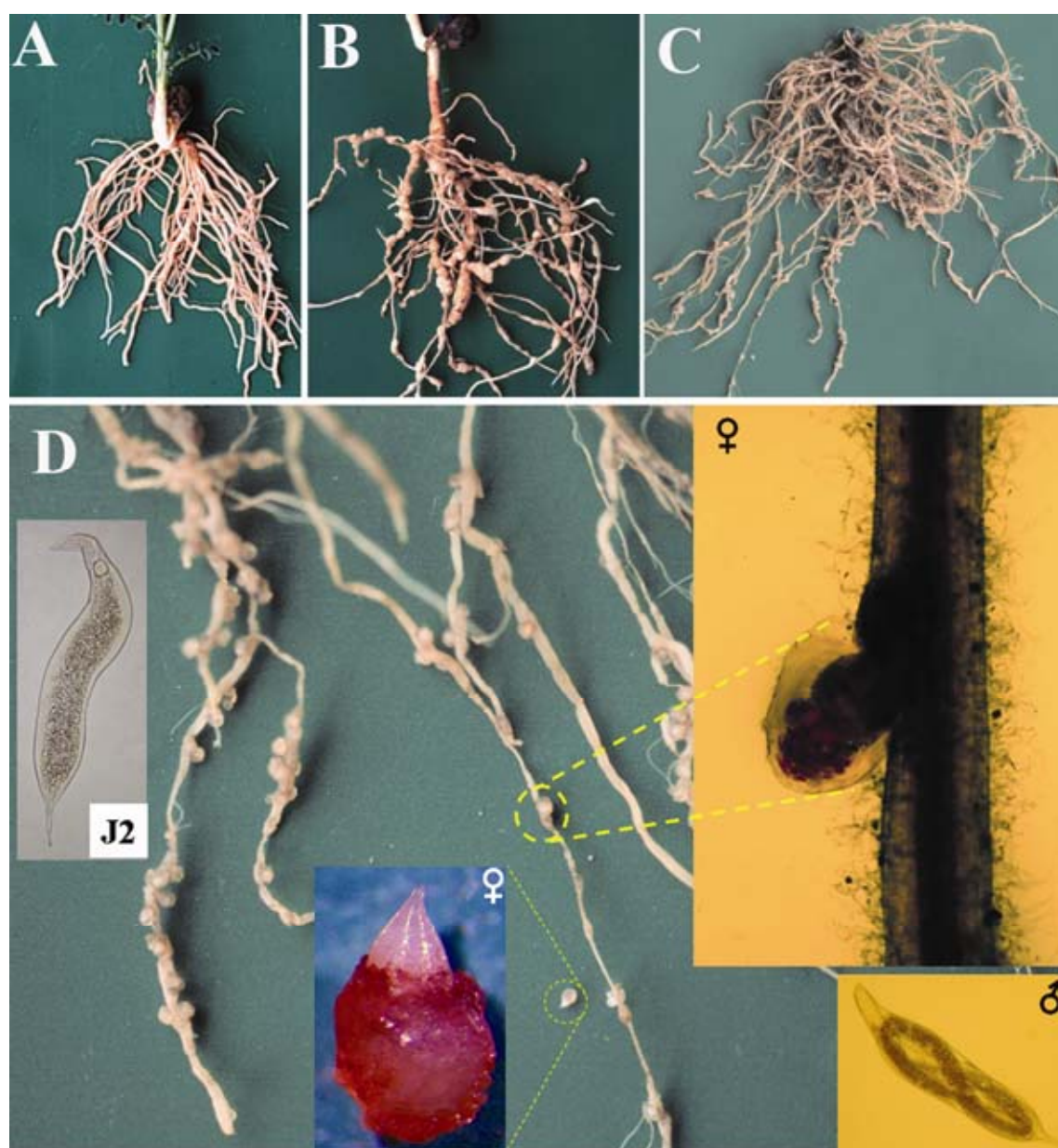


Fig. 1. Chickpea root systems. A, healthy; B to D, severely infected with root-knot nematodes. Galled roots caused by B, *Meloidogyne incognita*, and C to D, *M. artiellia*. Insets show details of *M. artiellia*: a postparasitic second-stage juvenile (J2); two closer views of mature females (♀), one attached to a root and one dissected from the root and stained with phloxine B; and an immature male (♂).

ing chickpea in the Indian Subcontinent can complete several generations within a growing season, leading to a very high postharvest nematode population density in soils (3). In any case, all these *Meloidogyne* species can persist in infested soils for 2 to 3 years protected within the infected root tissues, in the egg masses, or as J2 in anhydrobiosis (21,70).

Cultural practices play a vital role in the management of nematode-induced diseases in subsistence farming because crops of low value rarely receive any major input of fertilizer or pesticides. Although soil treatments with nematicides or solarization have been effective in the management of root-knot nematodes in chickpea, their use is prohibitively expensive (70). Fallowing is practiced commonly in Turkey and Syria to reduce nematode soil population densities by 35 to 50% per year (70). In the Indian Subcontinent, crop rotation is difficult because of the wide host range of warm-climate *Meloidogyne* species. Nevertheless, crops such as groundnut and winter cereals are not suitable hosts for *M. arenaria*, *M. incognita*, and *M. javanica* (70) and could therefore be included in rotation with chickpea. Conversely, *M. artiellia* in chickpea could be effectively managed in the Mediterranean Basin by rotation with nonhosts such as cotton, sugar beet, potato, maize, or lentil. Unfortunately, the most common crop rotation for legumes is with winter cereals, which is not useful for management of *M. artiellia* because the nematode is well adapted to winter cereals (25,39). Finally, although chickpea germplasm with resistance to *M. incognita* and *M. javanica* has been identified in India (65), poor agronomic characteristics have limited its use by farmers. Resistance to *M. artiellia* is not available, although resistance has been found in some accessions of wild *Cicer* species, including *C. bijugum*, *C. chorassanicum*, *C. judaicum*, *C. pinnatifidum*, and *C. reticulatum* (26).

## Root-Lesion Nematodes

Root-lesion nematodes are major constraints to chickpea production and rank second after the root-knot nematodes in terms of their global economic impact on crops (15,61). This is due to their wide host ranges as well as to their occurrence in almost any climate. Root-lesion nematodes are migratory endoparasites that damage chickpea roots by feeding in the cortical parenchyma and causing extensive necrosis of epidermal, cortical, and endodermal cells (15,16). Both penetration of the root and migration within root tissues are facilitated by a combination of stylet thrusting and enzymatic softening of the host cell walls (15). Under field conditions, root-lesion nematode damage to chickpea occurs in patches, with affected plants showing stunting, leaf chlorosis, root necrosis, and reduction of root growth

and shoot weight as a result of destruction of cortical parenchyma (Fig. 5).

Although species from three genera of root-lesion nematodes have been reported parasitizing chickpea, i.e., *Pratylenchoides*, *Pratylenchus*, and *Zygotylenchus* (Table 1), those of *Pratylenchus* are found most commonly in chickpea growing areas. The two major species most frequently diagnosed as causing damage to chickpea worldwide are the cereal and legume root-lesion nematode, *Pratylenchus thornei* (Fig. 6), and *P. penetrans* (9,15,22,23,70). In general, *Pratylenchus* species complete several generations per growing season, with each generation requiring about 1 month. Although large numbers of root-lesion nematodes may occur in infected roots early in the growing season, nematode populations in soil are rather low, especially in the absence of a host crop

(15). Some weed species are good hosts for *Pratylenchus* species, thus suggesting the need for implementing efficient weed management practices to reduce the nematode population in the soil and subsequent damage to crops (43,83).

*P. thornei* reproduces by mitotic parthenogenesis (31). Females of *P. thornei* lay eggs in the cortex, and the nematode completes its life cycle in about 4 weeks under suitable environmental conditions. *P. thornei* has a wide host range, including highly susceptible crops such as grain legumes (e.g., chickpea, faba bean, lentil, pea, and vetch) and small grain cereals; and moderately susceptible crops such as cauliflower, lettuce, and potato. Spinach and sugar beet are resistant to *P. thornei* (36). Under field conditions in Syria, the tolerance limit of chickpea to *P. thornei* was as low as 0.031 nematodes per cm<sup>3</sup> of

**Table 1.** Plant-parasitic nematodes associated with chickpea<sup>a</sup>

Nematode group and species	Threshold level (nematodes per cm <sup>3</sup> or g soil)	Country/region
<b>Root-knot nematodes</b>		
<i>Meloidogyne</i> spp.		
<i>M. arenaria</i>	—	Ghana, India, North Africa, Syria
<i>M. artiellia</i>	0.02-0.14	North Africa, Syria
<i>M. incognita</i>	1.0-2.0	Brazil, Egypt, India, Pakistan
<i>M. javanica</i>	0.1-4.28	Brazil, Egypt, Ethiopia, India, Pakistan
<b>Root-lesion nematodes</b>		
<i>Pratylenchoides</i> spp.		
<i>P. alkani</i>	—	Syria
<i>P. erzurumensis</i>	—	Turkey
<i>P. leiocauda</i>	—	Turkey
<i>P. ritteri</i>	—	Algeria, Tunisia
<i>Pratylenchus</i> spp.		
<i>P. alleni</i>	—	Turkey
<i>P. brachyurus</i>	—	Brazil, Turkey
<i>P. coffeae</i>	—	India
<i>P. mediterraneus</i>	—	North Africa, Syria, Turkey
<i>P. mulchandi</i>	—	India
<i>P. neglectus</i>	—	Australia, North Africa, Spain, USA
<i>P. penetrans</i>	—	North Africa, Spain, Turkey
<i>P. thornei</i>	0.031	Australia, India, Mexico, North Africa, Spain
<i>P. zeae</i>	—	Turkey
<i>Zygotylenchus guevarai</i>	—	North Africa, Spain, Turkey
<b>Cyst nematodes</b>		
<i>Heterodera</i> spp.		
<i>H. cajani</i>	—	India
<i>H. ciceri</i>	0.4-1.4	Syria, Turkey
<i>H. goettingiana</i>	—	North Africa
<i>H. swarupi</i>	—	India
<b>Reniform nematodes</b>		
<i>Rotylenchulus</i> spp.		
<i>R. reniformis</i>	0.5-1.0	India
<i>R. macrosoma</i>	—	Syria
<b>Stem and bulb nematodes</b>		
<i>Ditylenchus dipsaci</i>	—	Australia
<b>Stunt and spiral nematodes</b>		
<i>Amplimerlinius magnistylus</i>	—	Spain
<i>Aorolaimus perscitus</i>	—	Spain
<b>Basilaraimus spp.</b>		
<i>B. columbus</i>	—	India
<i>B. dimorphicus</i>	—	India
<i>B. indicus</i>	—	India, Pakistan
<i>B. seinhorsti</i>	—	India

(continued on next page)

<sup>a</sup> Data from several sources (3,4,6,9,18,22,23,27,31,32,48,52,56,59,67,68,70,77).



soil (Fig. 4), with chickpea yield being reduced to about 40% with a nematode population at planting of 2 nematodes per cm<sup>3</sup> of soil (24). Experiments in growth chambers demonstrated that root weight was significantly reduced by *P. thornei* infections, which reached high populations in all tested chickpea genotypes except for cv. JG 62, which showed tolerance to *P. thornei* (16). Results also demonstrated that nematode reproduction was significantly influenced by chickpea genotype, with cvs. JG 62 and UC 27 being the best and poorest hosts, respectively, independent of the geographical origin of the nematode isolate (16).

*P. thornei* appears to be adapted to suppress root growth more severely under warm, dry soils than in cool, moist soils, typical of areas with in-season rainfall or with irrigation (10). Under controlled conditions, chickpea root growth was signifi-

cantly reduced by *P. thornei* in plants grown under water stress and fluctuating temperatures in the greenhouse, but root growth was not affected if soil was maintained at water-holding capacity in a shadehouse with moderate air temperatures (10). In this context, Castillo et al. (9,14) determined that the reproductive rate for *P. thornei*, as well as hatching and penetration of chickpea roots, increase as soil temperature rises from 15 to 20°C.

In the absence of a host crop, *Pratylenchus* species survive by means of several strategies, but particularly on roots of weeds or in the soil in a quiescent state (anhydrobiosis). The ability of *Pratylenchus* species to undergo anhydrobiosis is the main reason that these nematodes are difficult to eradicate from infested fields (15). Anhydrobiosis of *P. thornei* was observed in Israel and southern Spain, where nematodes survived the dry fallow season

in soil or within root debris in an anhydrobiotic state until reactivated by fall rains (33,76).

## Cyst-Forming Nematodes

Cyst-forming nematodes, mainly *Heterodera* and *Globodera* spp., are more prone than other plant-parasitic nematodes to dispersion over time and space because eggs within cysts can tolerate long periods of desiccation and persist in soil for several years in the absence of a host plant. Dispersion of these nematodes is favored by agricultural operations and is rapid and widespread compared with that of other nematode species (57). Although three species of *Heterodera* have been reported to infect chickpea (Table 1), the chickpea cyst nematode, *H. ciceri*, is considered the most important, particularly in Syria and Turkey, where it causes considerable yield loss (23,35). *Heterodera cajani* and *H. swarupi* were reported infecting chickpea in several localities of India (3,4,67), and *H. goettingiana* in North Africa (22); however, significant damage to chickpea was not observed.

Soil infestations by cyst-forming nematodes are first limited to small circular areas that may further extend to include the entire field. Symptoms on infected chickpeas are more apparent at the flowering stage. Infected plants are stunted, with pale green leaves at an early stage of growth which later turn chlorotic (Fig. 7A). A reduction in the number of flowers and pods is observed in infected plants, which contain small or no seeds if heavily infected (35). Infected roots are poorly developed, lacking nitrogen-fixation nodules and showing cysts of adult nematode females as the sole diagnostic signs (35,37).

Second-stage juveniles (J2) emerge from eggs inside cysts, migrate through soil, penetrate chickpea roots, and migrate intracellularly through the cortex until they settle permanently and establish their feeding sites (syncytia), where they become sedentary and swollen and remain to fulfill their life cycle (Fig. 7B and C). A syncytium expands into the root stele by subsequent fusions of several pericycle and stellar cells (Fig. 7D to F). All syncytia cells are hypertrophic with dense cytoplasm and large nuclei. Swollen females rupture root tissues with the posterior end of their bodies, which then protrude from the root surface (Fig. 7D and E). *H. ciceri* eggs do not undergo dormancy as described for other cyst nematodes, but egg hatching is favored by diffusates from roots of host crops (such as chickpea and pea), suitable soil moisture, and temperature of at least 10°C (34,37,42). At 20°C, development of the nematode from penetration of roots by J2 to appearance of cysts is completed within 36 days; however, penetration of roots by *H. ciceri* is suppressed at 30°C (42). Insufficient soil

**Table 1.** (continued from previous page)

Nematode group and species	Threshold level (nematodes per cm <sup>3</sup> or g soil)	Country/region
<b>Stunt and spiral nematodes (continued)</b>		
<i>Bitylenchus</i> spp.		
<i>B. brevilineatus</i>	—	India
<i>B. dubius</i>	—	The Netherlands
<i>B. huesingi</i>	—	Spain
<i>B. vulgaris</i>	—	India
<i>Helicotylenchus</i> spp.		
<i>H. abunaamai</i>	—	India
<i>H. digonicus</i>	—	Pakistan
<i>H. dihystra</i>	—	India
<i>H. indicus</i>	—	India
<i>H. retusus</i>	—	India
<i>H. sharafati</i>	—	India
<i>H. tunisiensis</i>	—	Spain
<i>Merlinius brevidens</i>	—	India, Spain
<i>M. microdorus</i>	—	Spain
<i>Paratrophurus loofi</i>	—	Spain
<i>Scutellonema brachyurum</i>	—	India
<i>Trophurus imperialis</i>	—	Spain
<i>Tylenchorhynchus</i> spp.		
<i>T. annulatus</i>	—	India
<i>T. brassicae</i>	—	India
<i>T. clarus</i>	—	Spain
<i>T. elegans</i>	—	India
<i>T. mashhoodi</i>	—	India
<i>T. nudus</i>	—	India
<b>Ring and pin nematodes</b>		
<i>Hemicriconemoides</i> spp.		
<i>H. cocophilus</i>	—	India
<i>H. mangiferae</i>	—	India
<i>Mesocriconema</i> spp.		
<i>M. curvatum</i>	—	Spain
<i>M. ornata</i>	—	India
<i>M. spaherocephalum</i>	—	Spain
<i>Paratylenchus</i> spp.		
<i>P. microdorus</i>	—	Spain
<i>P. sheri</i>	—	Spain
<b>Dagger, needle and stubby-root nematodes</b>		
<i>Xiphinema</i> spp.		
<i>X. basiri</i>	—	India
<i>X. index</i>	—	Turkey
<i>X. italiae</i>	—	Turkey
<i>X. pachtaicum</i>	—	India, Turkey
<i>Longidorus elongatus</i>	—	India
<i>Trichodorus mirzai</i>	—	India

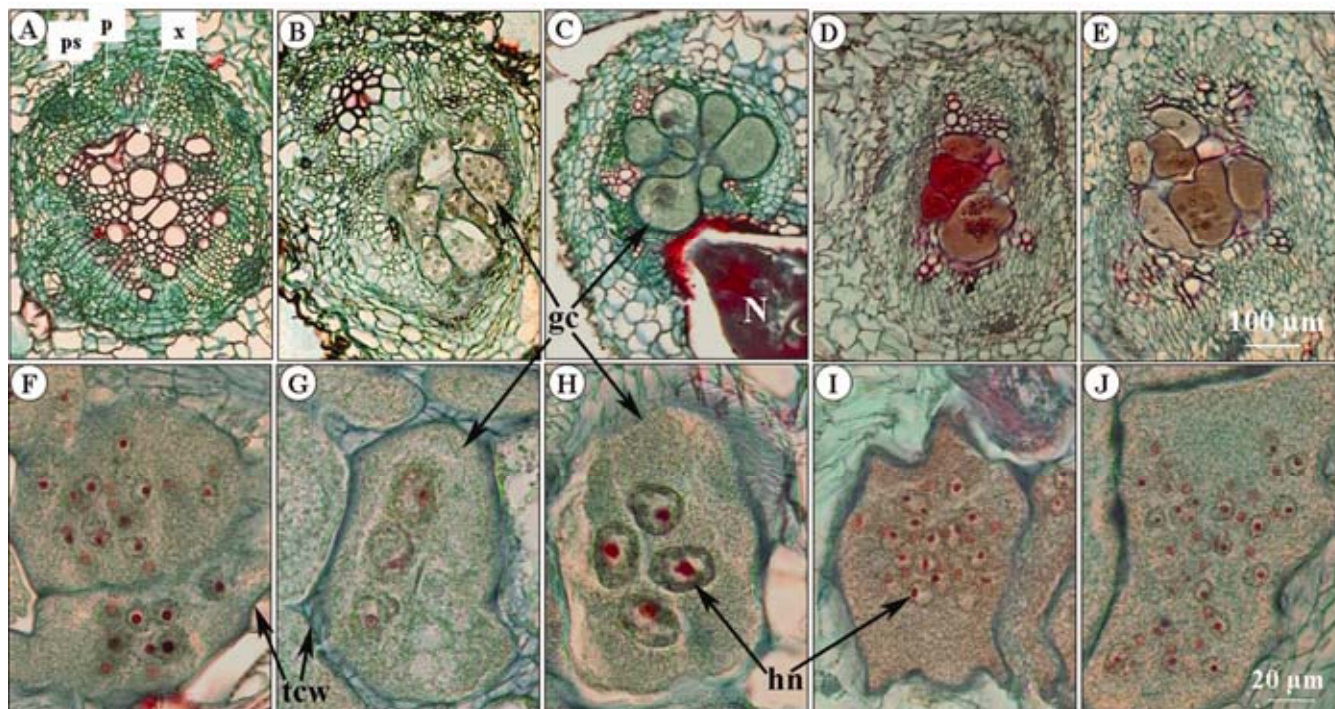


Fig. 2. Transverse sections of chickpea cultivar UC 27 stained with safranin and fast green: A, noninfected root; B, F, roots infected with *Meloidogyne arenaria*; C, G, H, *M. artiellia*; D, I, *M. incognita*; E, J, *M. javanica*, showing B-E, the anatomical changes (hypertrophy and hyperplasia) induced by nematode feeding; and F-J, details of induced giant cells. Abbreviations: gc = giant cells; hn = hypertrophied nuclei; N = nematode female; p = phloem; ps = protoxylem elements; tcw = thickened cell walls; x = xylem.



Fig. 3. A, *Meloidogyne artiellia*-infected chickpea plants showing stunted growth and leaves with pale green to yellow color. B, Black nightshade (*Solanum nigrum*) root system severely infected with *Meloidogyne incognita*.

moisture during the chickpea growing season, such as that occurring in Syria and Turkey, suppresses nematode infection and therefore allows the nematode to complete only one generation per cropping season (42).

Chickpea is highly susceptible to damage by *H. ciceri*. The tolerance limit of the crop ranges from 0.4 to 1.4 eggs and J2 per  $\text{cm}^3$  of soil (Fig. 4). Nematode population densities of 8 and 16 eggs + J2 per  $\text{cm}^3$  of soil can cause yield losses of 20 and 50%, respectively. A complete crop failure can occur in fields infested with as few as 60 eggs + J2 per  $\text{cm}^3$  of soil (35). *H. ciceri* has a rather narrow host range among food legumes, including chickpea, lentil, and pea, but it can reproduce, although poorly, on alfalfa, bean, lupine, and *Vicia* spp. As a result, this nematode can be effectively controlled by crop rotations. In Syria, yield

of chickpea increased by factors of 2.1, 4.5, and 7.9 in fields where suitable host crops were not sown for 1, 2, or 3 years, respectively, compared with yield produced under continuous chickpea (62). Fallowing is a common practice in Turkey and Syria, and it reduces the soil population densities of the nematode by 35 to 50% per year (35).

Potential sources of resistance to *H. ciceri* in genotypes of *Cicer* spp. that could be transferred to commercial chickpea cultivars were investigated. Unfortunately, none of 10,000 lines of *C. arietinum* screened so far (26,77) were found to be resistant to *H. ciceri*; however, resistance was identified in some accessions of *C. bijugum*, *C. pinnatifidum*, and *C. reticulatum* (49,71). Among these wild *Cicer* species, only *C. reticulatum* is readily crossed with *C. arietinum*, the only cultivated spe-

cies in the genus (49). The resistant reaction of *C. bijugum* to the cyst nematode is evidenced by the absence of syncytia and the death of several root cells surrounding the lip region of infecting J2 (Fig. 8).

### Reniform and Other Nematodes

Reniform nematodes (*Rotylenchulus* spp.) are semi-endoparasites of roots and occur commonly in tropical and subtropical regions. The term "reniform" refers to the kidney-shape body form of the sedentary mature female protruding from infected roots (Fig. 9). *Rotylenchulus reniformis* is the most economically important species of reniform nematodes, and is also an important pathogen of chickpea in Egypt, Ghana, and India (Table 1) (3,27,56,59). *Rotylenchulus macrosoma* has been reported in chickpea fields in Syria, but it has never been documented infecting chickpea roots (70). Infections of chickpea by *R. reniformis* have been associated with chickpea decline in India and Egypt. In India, high soil infestations with *R. reniformis* occur in irrigated chickpea fields, whereas population densities of this nematode are lower in rain fed areas (3). Infected plants are distributed in patches and show pale green leaves, stunted growth, and early senescence. The damage threshold for *R. reniformis* in greenhouse-grown chickpeas was estimated at 0.5 to 1.0 nematodes per g of soil (18,48), with up to 80% yield reduction occurring at 10 nematodes per g of soil (48).

The life cycle of *R. reniformis* on chickpea has not been assessed, but studies in



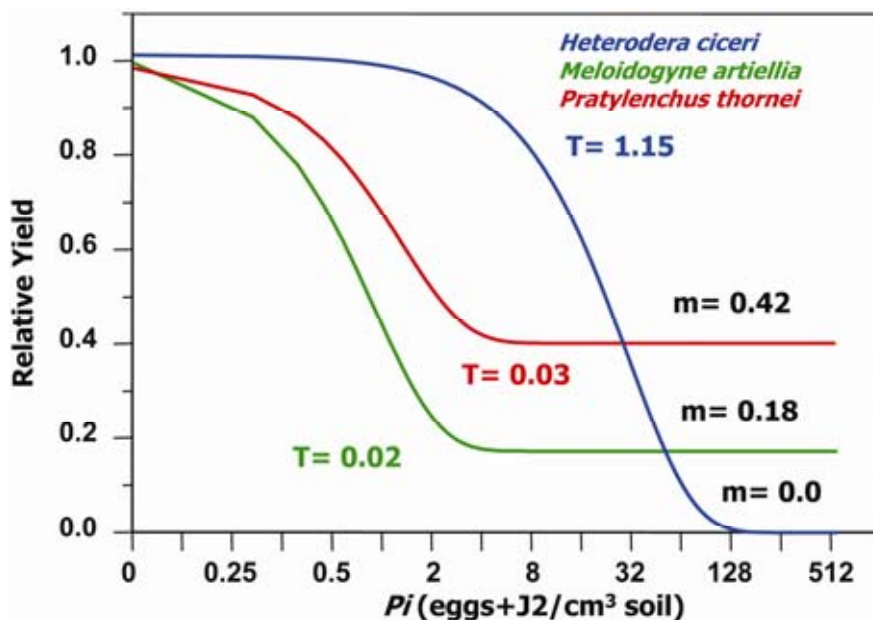


Fig. 4. Relationships between relative yield and initial population densities ( $P_i$ ) of different species of plant-parasitic nematodes attacking chickpea. Abbreviations:  $m$  = minimum value of relative yield (relative yield at a very large initial nematode population density);  $T$  = tolerance limit.

other susceptible hosts indicate that it takes about 25 to 30 days, depending upon soil temperature. The thermal limits for reproduction of this nematode are between 15 and 36°C, with an optimum of 30°C. The J2 and subsequent juvenile stages are not plant-parasitic. The molt of J4 juveniles gives rise to approximately equal numbers of vermiform parasitic females and non-parasitic males. Vermiform females penetrate the epidermis and cortical parenchyma of roots and usually stop to establish a permanent feeding site on a single endodermal cell (Fig. 9A). The anterior part of the nematode body remains embedded within the root, whereas the posterior end protrudes from the root surface and swells during maturation of the reproductive system to assume a kidney-like shape (Fig. 9A). In the stele, infective females induce the formation of a feeding site called a syncytium (Fig. 9B). This syncytium originates from an endodermal cell and enlarges subsequently by incorporating cells from the pericycle, vascular parenchyma, and sometimes phloem, by means of partial dissolution of common

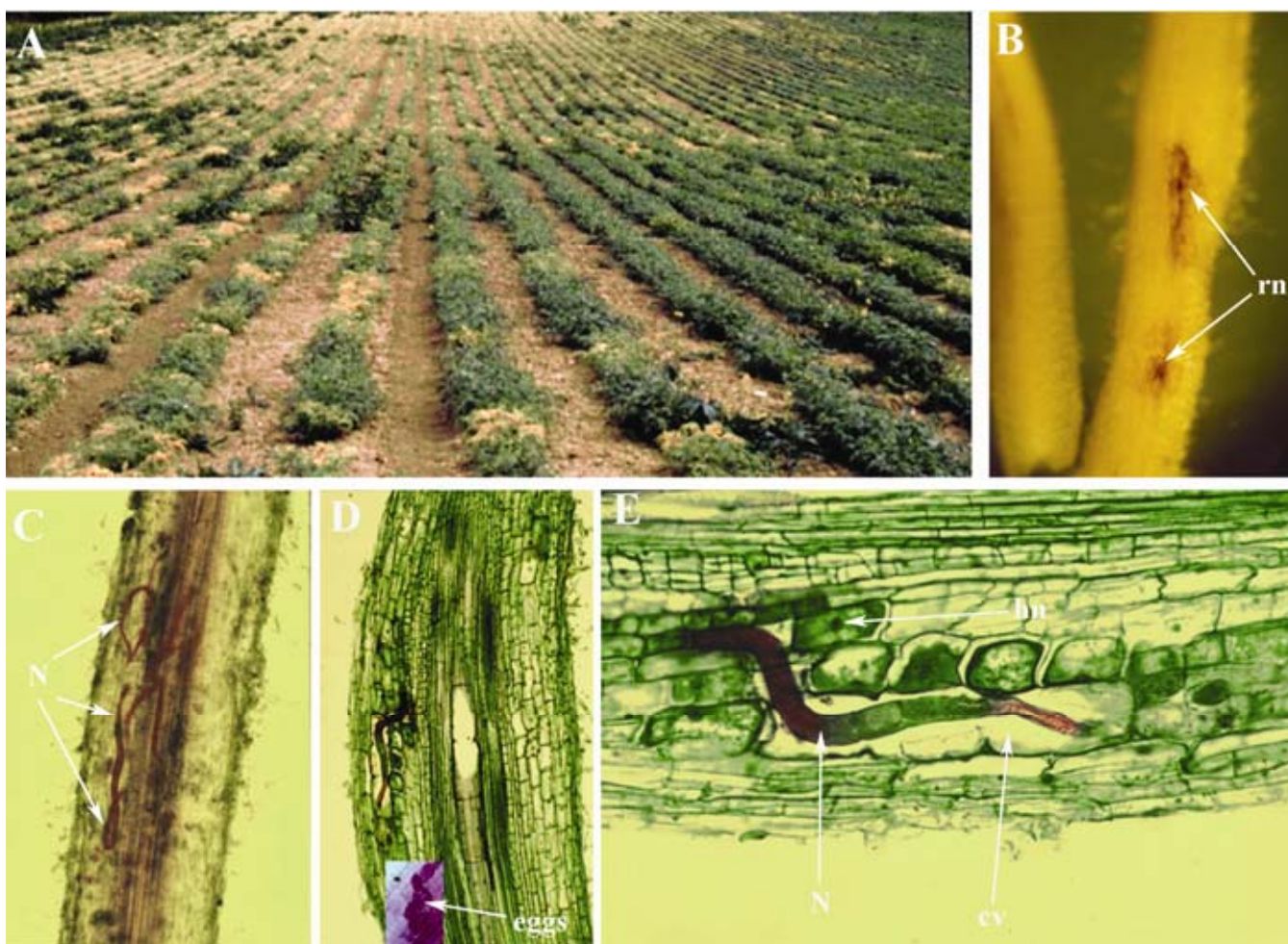


Fig. 5. A, Stunted and chlorotic chickpea plants, and B, root necrosis (rn) on roots caused by infections with *Pratylenchus thornei* on chickpea; C-E, Histopathological changes induced by the feeding and migration of the nematode (N) showing cavities (cv), hypertrophied nuclei (hn), and granular cytoplasm of cells adjacent to nematode pathway, and eggs deposited inside the root.



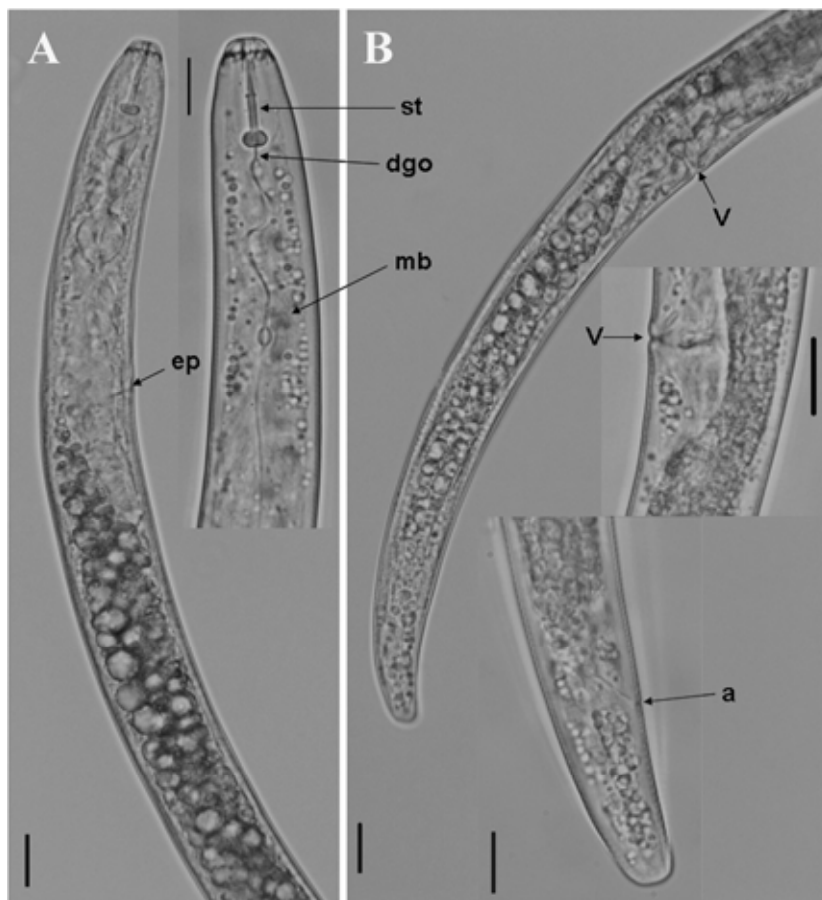


Fig. 6. Micrographs of the cereal and legume root-lesion nematode *Pratylenchus thornei*. A, Female anterior region showing stylet (st), dorsal pharyngeal gland orifice (dgo), median pharyngeal bulb (mb), and excretory pore (ep). B, Female posterior region showing vulva (v), tail and anus (a). Scale bars = 10 µm.

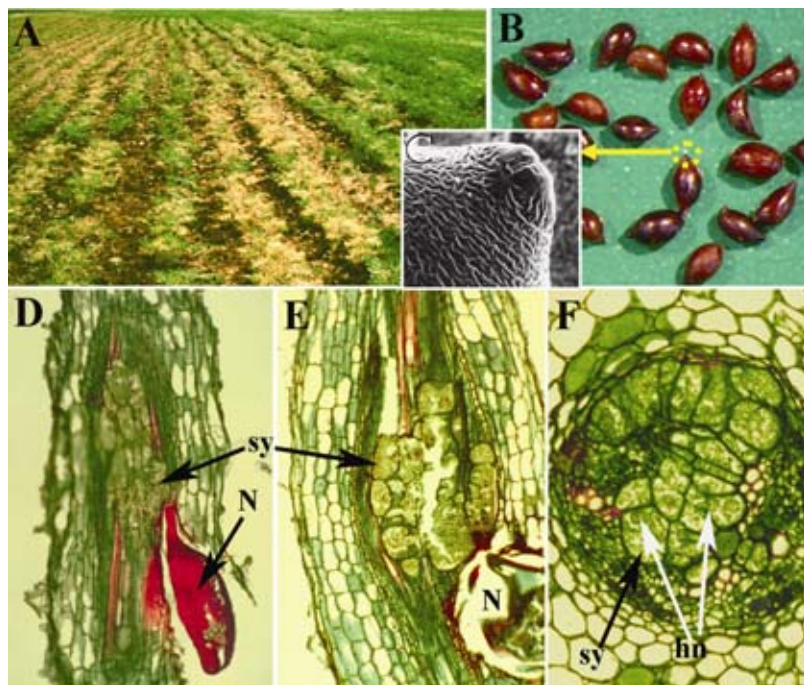


Fig. 7. A, Field symptoms caused by infections of chickpea with *Heterodera ciceri*. B, Brown mature cysts of *H. ciceri*; C, a detail of the vulval cone under scanning electron microscopy showing fenestra. D-F, Histopathological changes induced by *H. ciceri* showing the nematode (N), the permanent feeding site inducing syncytium (sy) with hypertrophic cells with dense cytoplasm and hypertrophied nuclei (hn); and stained with safranin and fast-green.

cell walls. Syncytial cells have densely staining granular cytoplasm and enlarged nuclei, and are slightly hypertrophied (Fig. 9B). Mature females deposit between 60 and 200 eggs into a gelatinous matrix which flows out from the vulva (72).

*R. reniformis* has a wide host range, including legumes (common bean, black gram, chickpea, pigeonpea, and soybean), cereals (maize, rice, sorghum, and wheat), vegetables (cucumber, eggplant, potato, and tomato), fruits (banana, citrus, grapevine, and tropical fruits), and oilseed crops (castor, groundnut, and sunflower). Consequently, management of *R. reniformis* by crop rotation is very difficult to practice, and alternative nonhost crops must be tested before they can be recommended. Other practices such as altering planting dates have had some success; for example, advancing of planting date of paddy rice (*Oryza sativa*) in India significantly reduced soil population densities of *R. reniformis* (6,38). The use of soil solarization was effective in reducing the population density of *R. reniformis*, but its use is very expensive and is not feasible for a pulse crop (66).

Other nematode parasites reported to infect chickpea comprise a long list of migratory ectoparasites, including dagger, needle, pin, ring, spiral, stunt, and stubby-root nematode species (Table 1). However, pathogenicity to chickpea of all these nematodes has not been demonstrated, except for *Helicotylenchus indicus*, *Tylenchorhynchus brassicae*, and *Tylenchorhynchus vulgaris* (32,60,78). Finally, the oat race of the stem and bulb nematode, *Ditylenchus dipsaci*, was reported in South Australia to cause severe yield losses in young chickpea plants, whereas adult plants were resistant to the nematode (77).

## Diagnosis of Nematodes Pathogenic to Chickpea

The accurate identification of plant-parasitic nematodes infecting crops is a prerequisite for designing effective management strategies. This is particularly relevant for chickpea, where several species of *Meloidogyne*, *Heterodera*, and *Pratylenchus* have been reported to be pathogenic to chickpea in several growing areas. Identification of these species is mostly based on morphology and morphometric traits of adult females or cysts (57), but such a task is hindered by the intra-specific variability of morphological traits and the need for experienced diagnosticians. Diagnostic characteristics differentiating *Meloidogyne* spp. include the tail shape and length of J2 juveniles (Fig. 10E to H) and the characteristic perineal pattern of adult females (Fig. 10A to D). The perineal pattern of *M. artiellia* is typical, showing a very distinct inner area which contains the vulva and anus that is surrounded by a few coarse lines arranged

in a figure-eight with a large base and a small top (Fig. 10B). Similarly, the tail shape and length of the J2 in *M. artiellia* is typically rounded and short compared with those of other species infecting chickpea (Fig. 10E to H).

Reliable diagnostic methods based on protein or nucleic acid analyses are now available and have significantly improved the routine identification of *Meloidogyne* species. Analysis of isozyme electrophoretic patterns proved valuable in separating *Meloidogyne* spp. damaging chickpea (17,29) (Fig. 11). The esterase pattern of *M. artiellia* revealed a characteristic two-band phenotype, M2, (Fig. 11) which can be clearly differentiated from phenotypes of other root-knot nematodes infecting chickpea, viz. *M. arenaria* (A1, A2, or A3 phenotypes), *M. incognita* (I1 phenotype), and *M. javanica* (J3 phenotype) (Fig. 11). Similarly, the malate dehydrogenase phenotype of *M. artiellia* revealed a weak band with higher relative mobility than those phenotypes of other root-knot nematodes infecting chickpea (Fig. 11). In addition, several DNA-based analyses have been successfully used for the diagnosis of *Meloidogyne* spp. damaging chickpea, including polymerase chain reaction (PCR) assays using primers derived from sequence characterized amplified regions (SCAR), restriction fragment length polymorphisms (RFLP) of the internal transcribed spacer (ITS), and phylogenetic studies of the 18S, ITS1–5.8S-ITS2, and D2–D3 region of 28S of ribosomal DNA (rDNA) sequences (17,86).

Identification of root-lesion nematodes, *Pratylenchus* spp., is based on morphology and morphometric traits of adult females and males, of which the number of lip annuli, presence of males, stylet length, shape of spermatheca, and position of vulva and post-vulval uterine sac are particularly important diagnostic characters (15,46). Although morphology continues to be the basis for the identification of *Pratylenchus* spp., new technologies based on biochemical and molecular analyses are also becoming increasingly important for practical identifications and phylogenetic studies (1,2,5,8,19). Andrés et al. (5) found that species-specific isozyme phenotypes obtained by isoelectrofocusing successfully differentiated some *Pratylenchus* species infecting chickpeas, such as *P. brachyurus*, *P. neglectus*, *P. penetrans*, and *P. thornei*. Similarly, Carrasco-Ballesteros et al. (8) developed a pair of SCAR primers specific to *P. thornei* and identified repetitive sequences (satDNA) in *P. thornei*, which can easily be amplified from DNA extracts obtained from different life stages of the nematode. Similarly, although the identification of cyst nematodes attacking chickpea is primarily based on the morphology of cuticle surrounding the vulva in the mature cyst (fenestral patterns), molecular identification of ITS1

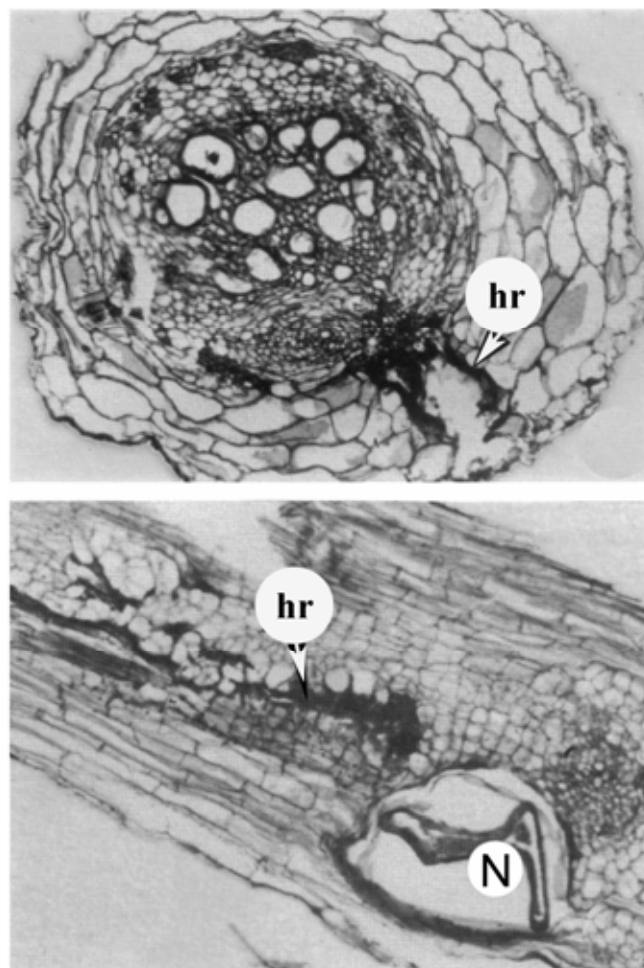
and ITS2 sequences, as well as RFLP profiles generated by different restriction enzymes, may help with the identification of *H. ciceri* (47,74,75). Finally, identification of *Rotylenchulus* species is based mainly on morphological characters of vermiform females, but sequences from the 18S, ITS1–5.8S-ITS2, and D2–D3 of 28S rDNA from *R. reniformis* are also available in the GenBank database and may help with the specific identification of this nematode.

### Interactions with Rhizobia and Phytopathogenic Fungi

Plant-parasitic nematodes attacking chickpea may develop complex interrelationships with soilborne microorganisms occupying a similar ecological niche in the chickpea rhizosphere, including beneficial (symbiotic) and deleterious (pathogenic) plant-associated microorganisms. These nematode interactions are important biological and phytopathological phenomena of great significance for chickpea production.

Free nitrogen fixation by chickpea roots through effective symbiotic association

with *Mesorhizobium* and *Sinorhizobium* rhizobia can enable cultivation of the crop in many nitrogen-poor soils (55). Yield potential of chickpea depends on the associated rhizobia and plant genotype that together influence the symbiotic performance and the symbiosis response to biotic and abiotic stresses (73). Nematode parasitism may influence symbiotic nodulation on legumes roots by associated rhizobia and thus nitrogen fixation (40). Studies in greenhouse conditions showed that *M. incognita* infected more than 25% of nodules induced by *Mesorhizobium* in chickpea cv. UC 648 (84). Moreover, histological observations of the nematode-infected nodules showed that J2 had established permanent feeding sites in the vascular bundles of the nodules and induced the formation of giant cells (Fig. 12). Disruption of xylem elements was associated with enlargement of the nematode body and expansion of giant cells. However, the integrity of bacteroids inside the nodule was not affected by the nematode infection. Although the development of nodules was apparently not suppressed by nematode infection, histological examination of



**Fig. 8.** Histopathological observations showing the reaction of a resistant wild chickpea accession (*Cicer reticulatum*) to *Heterodera ciceri* showing the absence of syncytia formation and hypersensitive reaction (hr) with necrosis of several root cells surrounding the nematode juvenile (N).



infected tissues suggested premature senescence (84). Other studies have shown that rhizobial nodulation can be impaired by parasitism by root-knot nematodes; for example, *M. incognita* and *M. javanica* significantly decreased the number of functional nodules per root system on several chickpea cultivars (50,54,82). Also, infections of chickpea by *P. thornei* or *R. reniformis* induced a reduction in the number of nodules formed on infected roots compared with that formed in uninfected ones (18,79).

Nematode parasitism plays a significant role in the development of other chickpea diseases caused by soilborne pathogens. Interactions between plant-parasitic nematodes and fungi can vary considerably over plant genotypes, cultivars, and lines, as indicated in studies of disease complexes

on multiple chickpea genotypes (7,11,12,81). In fact, a number of comprehensive studies have been carried out concerning interactions between plant-parasitic nematodes, including *Pratylenchus* spp. and *Meloidogyne* spp., and pathogenic fungi, mainly *F. oxysporum* f. sp. *ciceris*, the causal agent of Fusarium wilt of chickpea (7,11,12,81).

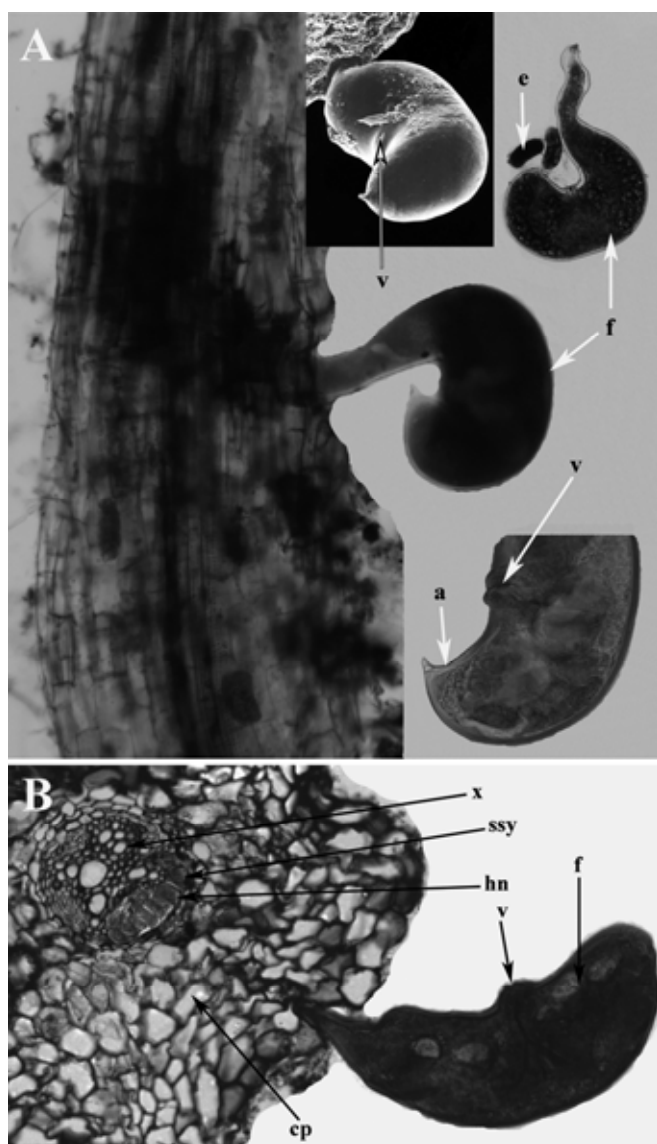
Management of Fusarium wilt in chickpea is mainly by the use of race-specific resistance, which is widely recognized as the safest, most economical, and most effective control method for the disease (41,45,53). Studies in India have shown that interactions of *F. oxysporum* f. sp. *ciceris* and *M. incognita* or *M. javanica* in chickpea can lead to a breakdown in the resistance in chickpea plants against an unidentified race of the Fusarium wilt

pathogen (44,51,80,81). Similarly, in Spain, results from growth chamber experiments with moderate and high fungal inoculum densities clearly demonstrated that in chickpea genotypes with partial resistance to Fusarium wilt, the severity of the disease was significantly increased by coinfection with *F. oxysporum* f. sp. *ciceris* race 5 and *M. artiellia* (Fig. 13) (12). This effect occurred regardless of the fungal inoculum density and the geographic origin of *M. artiellia* (Italy or Syria), except for cultivar CPS 1, in which a significant increase in Fusarium wilt severity occurred only with the highest fungal inoculum density (12). Moreover, results from those experiments suggested that coinfection by the two pathogens can overcome complete resistance to *F. oxysporum* f. sp. *ciceris* race 5 in some chickpea genotypes (e.g., lines CA 334.20.4, CA 336.14.3.0, and UC 27), but not in others (line ICC 14216 K), and that the breakdown of such resistance occurs irrespective of the initial inoculum density of the fungus and the geographic origin of the nematode (e.g., lines CA 334.20.4, and CA 336.14.3.0), or require a high inoculum density of *F. oxysporum* f. sp. *ciceris* race 5 (cv. UC 27) (Fig. 13) (12). Interestingly, this genotype-specific resistance breakdown by *M. artiellia* infection did not occur for other pathogenic races of *F. oxysporum* f. sp. *ciceris* such as races 0, 1A, and 2. Thus, stability of Fusarium wilt resistance in genotypes with complete resistance (e.g., lines BG-212, CA 334.20.4, CA 336.14.3.0, ICC 1416 K, PV 61, UC 27, and WR-315) or complete susceptibility (line P 2245) to these races of *F. oxysporum* f. sp. *ciceris* was not compromised by coinfection of the plant with both pathogens (P. Castillo, unpublished results).

Conversely, simultaneous or sequential coinfection of chickpea by *P. thornei* and *F. oxysporum* f. sp. *ciceris* race 5 did not modify severity of Fusarium wilt in chickpea genotypes susceptible to wilt (11). However, coinfection of roots following inoculations with 5 nematodes per cm<sup>3</sup> and 5,000 chlamydo-spores per g of soil resulted in significantly higher numbers of propagules of the fungus in wilt-susceptible cv. CPS 1 but not in wilt-resistant cv. UC 27. Such a difference in responses of chickpea genotypes was reduced by increasing the nematode inoculum to 10 nematodes per cm<sup>3</sup> of soil, which resulted in a significant increase of root infection by *F. oxysporum* f. sp. *ciceris* race 5 in both cultivars irrespective of fungal inoculum densities (250 or 2,000 chlamydo-spores per g of soil) (11).

## Concluding Remarks

Attacks by root-knot, root-lesion, and cyst-forming nematodes can significantly limit chickpea production worldwide, particularly in subsistence agriculture. Although the extent of crop yield loss is



**Fig. 9. A,** Mature females (f) of the reniform nematode *Rotylenchulus reniformis* infecting chickpea roots showing details of vulva (v), anus (a), and deposited egg (e). **B,** Histopathological observations of the permanent feeding site induced by *R. reniformis* showing enlarged endodermal cells forming the stelar syncytium (ssy) with hypertrophied nuclei (hn), distorting the xylem tissue (x), and showing the anterior part of the body embedded in the cortical parenchyma (cp).

mainly determined by the nematode population density in soil, other factors in the agro-ecosystem (e.g., climate, soil conditions, cultivar susceptibility, etc.) may also contribute to significant yield reductions.

Accurate and quick diagnoses of the nematode species and pathogenic variants are a prerequisite for the proper management of nematodes in chickpea. While conventional identification of nematodes based on

their morphological features can be hindered by insufficient expertise of diagnosticians, the use of newly developed diagnosis technologies based on biochemical and DNA analyses can be of help. Man-

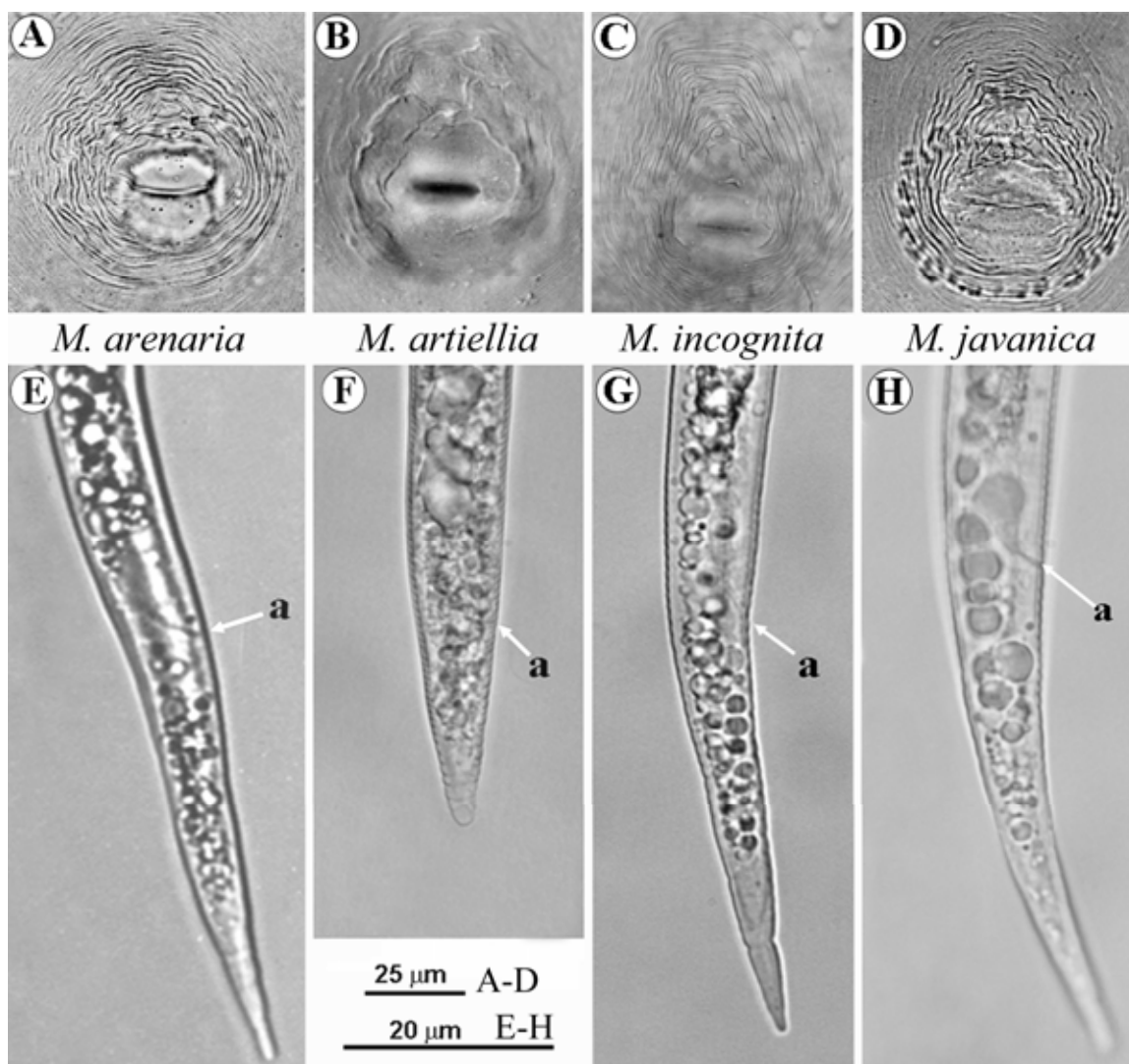


Fig. 10. A-D, Micrographs showing differences among the female perineal patterns, and E-H, second-stage juvenile tails of root-knot nematodes attacking chickpea. A, E, *Meloidogyne arenaria*; B, F, *M. artiellia*; C, G, *M. incognita*; D, H, *M. javanica*. Abbreviation: a = anus.

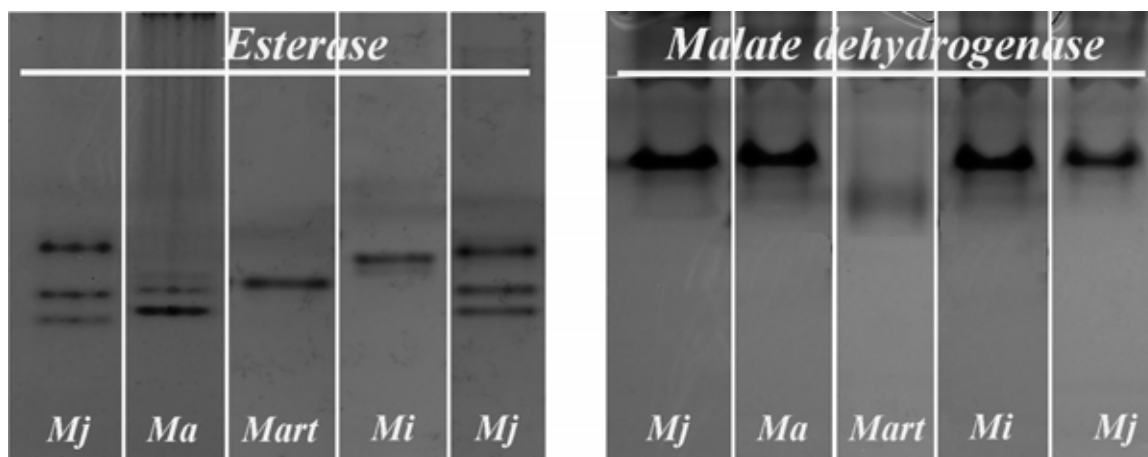


Fig. 11. Esterase and malate dehydrogenase electrophoresis patterns of protein homogenates from five young, egg-laying females of root-knot nematode species attacking chickpea: Ma = *Meloidogyne arenaria*; Mart = *M. artiellia*; Mi = *M. incognita*; and Mj = *M. javanica*.



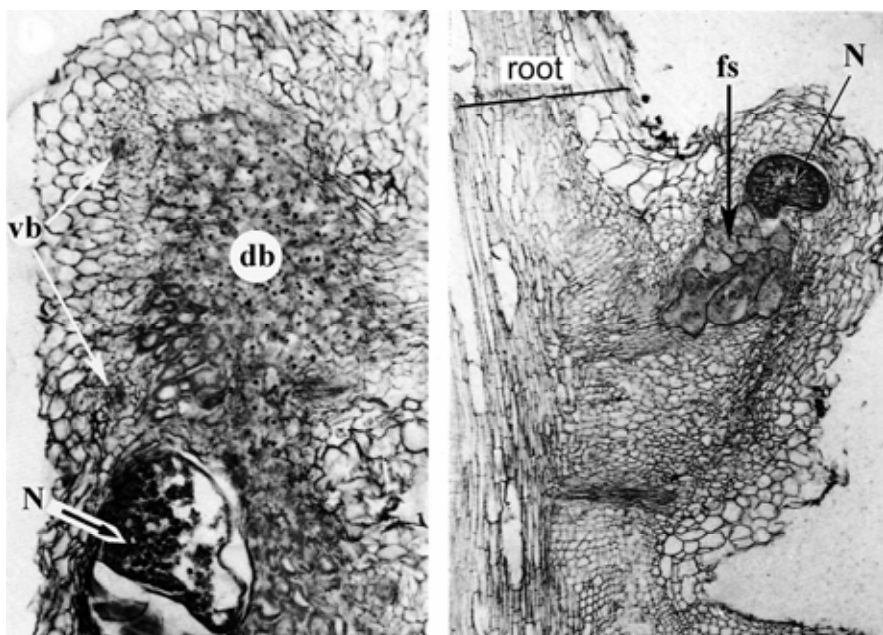


Fig. 12. Longitudinal sections of *Mesorhizobium*-chickpea (cv. UC 648) nodules infected by *Meloidogyne incognita* showing the arrangement of vascular bundles (vb), the differentiating bacteroid zone (db), and the nematode feeding site (fs) induced by the mature female (N) in the *Mesorhizobium* nodule.

agement of nematodes that attack chickpea must be based on strategies that integrate the use of available host resistance or tolerance in chickpea cultivars together with crop rotation and cultural modifications that can be practiced by farmers (e.g., time of planting). Practical and efficient integrated management of plant-parasitic nematodes in chickpea requires that high-yielding cultivars with appropriate marketable traits and resistance or tolerance to nematodes be made available to farmers. Additional studies are also needed to improve cultural management practices. Finally, further studies are needed to unravel mechanisms underlying the interactions between *Meloidogyne* species and races of *F. oxysporum* f. sp. *ciceris*. Such information would foster appropriate use of the wilt-resistant cultivars in chickpea fields infested with the two pathogens.

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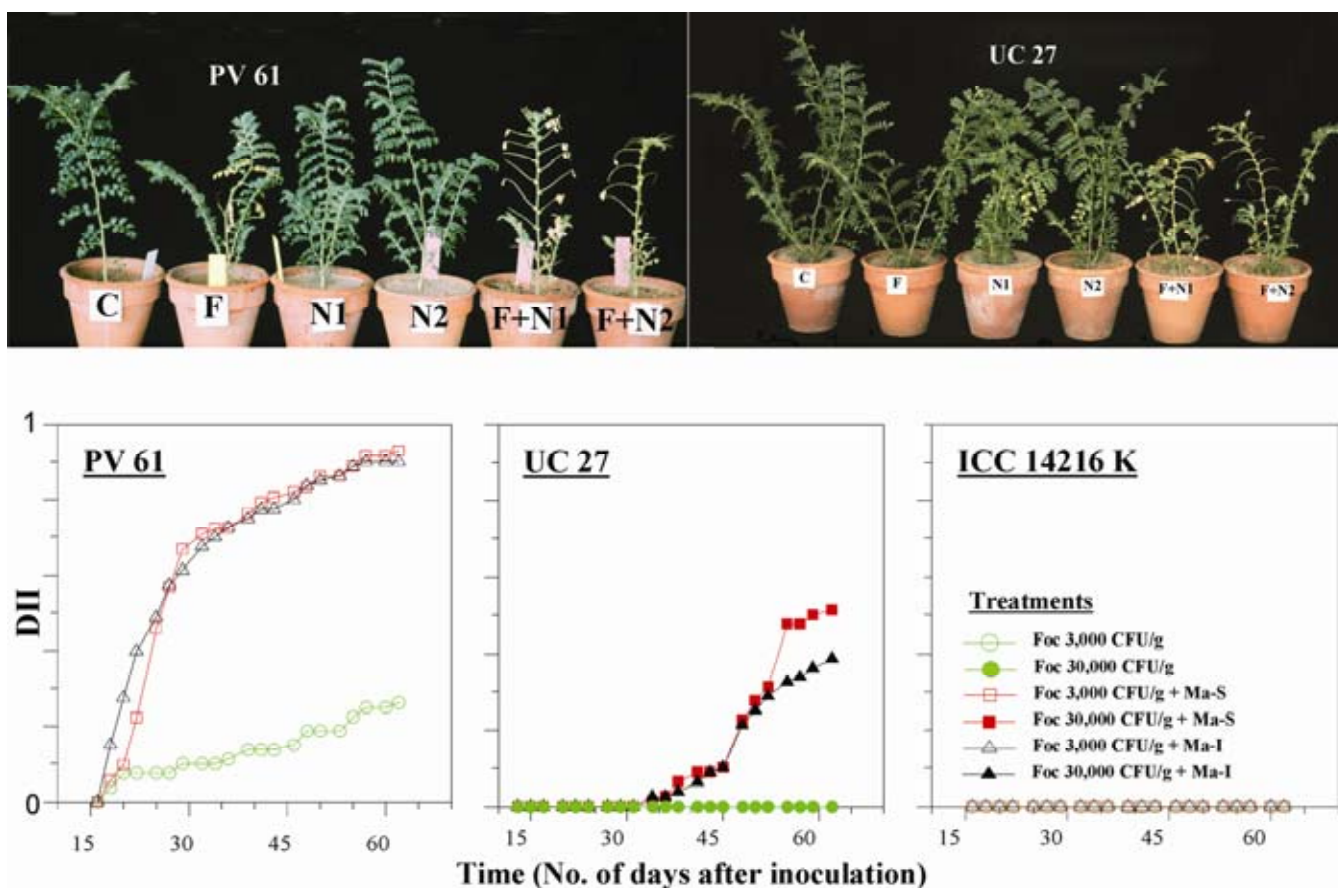


Fig. 13. Symptoms of *Fusarium* wilt in chickpea genotypes with partial (cv. PV 61) and complete (cv. UC 27 and line ICC 14216 K) resistance to *Fusarium oxysporum* f. sp. *ciceris* race 5. Progress of disease intensity index (DII) in chickpea genotypes simultaneously inoculated with 3,000 or 30,000 chlamydospores/g soil and *Meloidogyne artiellia* populations from Italy (Ma-I) and Syria (Ma-S) (20 eggs and second-stage juveniles/cm<sup>3</sup> soil). Abbreviations: C = uninoculated control plants; F = plants inoculated with the fungus only; N1 = plants inoculated with a population of *M. artiellia* from Italy only; N2 = plants inoculated with a population of *M. artiellia* from Syria only; F + N1 = plants simultaneously inoculated with the fungus and a population of *M. artiellia* from Italy; and F + N2 = plants simultaneously inoculated with the fungus and a population of *M. artiellia* from Syria.

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