Jurnal Perlindungan Tanaman Indonesia, Vol. 24, No. 2, 2020: 133–138
DOI: 10.22146/jpti.23978
Available online at http://jurnal.ugm.ac.id/jpti
ISSN 1410-1637 (print), ISSN 2548-4788 (online)

Research Article

Responses of *Capsicum annuum* Varieties toward Root Knot Nematode *Meloidogyne incognita* Infection

Resty Islamiati Putri[1)], Siwi Indarti[1)]*, & Ani Widiastuti[1)]

[1)]Department of Plant Protection, Faculty of Agriculture, Universitas Gadjah Mada
Jln. Flora No. 1, Bulaksumur, Sleman, Yogyakarta 55281 Indonesia

**Corresponding author. E-mail: siwi.indarti@ugm.ac.id*

Received April 17, 2017; revised July 30, 2017; accepted November 4, 2020

ABSTRACT

Chili Pepper (*Capsicum annuum*) is one of the major vegetable commodities in Indonesia. One of the challenges in chili pepper cultivation is the infection of *Meloidogyne incognita* which causes gall formation in root systems. The purposes of this research were to determine 1. the response of four varieties of chili pepper against *M. incognita* infections; 2. damaged intensity caused by this pest in the root system, and; 3. development of *M. incognita* populations in root systems (rhizosphere). This research was conducted in Plant Pest Science Laboratory and Greenhouse of the Department of Crop Protection, Faculty of Agriculture, University of Gadjah Mada. This research included cultivation of three chili pepper varieties (*Cabai Merah Besar*, Pilar F1; *Cabai Merah Keriting*, Kastilo F1; and Paprika, Red Star 2060), rearing of *M. incognita* , inoculation, and observation. Chili pepper respond to *M. incognita* infections were evaluated based on agronomic aspects of the plants, such as root histopathology observation, disease intensity, and nematode populations. Results showed that each variety of chili pepper expressed various responses to infections. All varieties had different root weight and length, but had similar sensitivity based on the appearance of root damage and egg mass scoring of *M. incognita*. Although nematode population could develop on roots, observation from root tissues showed lignification after infection of J2 *M. incognita.* Chili pepper var. Red Star 2060 was more susceptible against *M. incognita* than chili pepper var. Pilar F1 and chili pepper var. Kastilo F1.

Keywords: chili pepper; host plant response; *M. incognita*

INTRODUCTION

Chili pepper is a major vegetable commodity in Indonesia, which is both consumed locally and exported to other countries. Chili pepper production averagely reaches 1,915,120 million ton each year (Sekretariat Jenderal Kementerian Pertanian, 2016).

Most chili pepper variety are susceptible to the root-gall nematode, *Meloidogyne incognita* (Oka *et al*., 2004). Chili pepper yield lost due to the infection of *M. incognita* is estimated to reach 12–90%, depending on population levels, and the biotic and abiotic factor of its surrounding environment (Sasser & Freckman, 1987). Oka *et al*. (2000) stated that economic loss due to the infection of root-gall nematodes may reach US $100 million each year globally.

M. incognita are well-known for its ability to reproduce rapidly at temperatures above 18°C and Secondary infection by plant pathogen, such like a fungus. In the the process of penetration and feeding, Infection of *M. incognita* on plant root become subject to infection by fungal pathogens. Its effects to plant host may even spread wider in tropic climates due to environmental conditions that affect nematodes reproduction, survival and dispersion (Luc *et al*., 2005). *M. incognita* have not been considered to be a serious pest in San Joaquin Valley on bell peppers, another chili variety. However, the nematode is a serious pest on bell peppers in Coachella Valley due to its desert condition (Aguiar *et al.,* 2014). Environmental differences may indirectly affect *M. incognita* by alternating temperature, soil pore sizes, and water availability. Temperature may affect metabolic changes, movement, and activity. *M. incognita* in general requires higher temperature (such as in tropical climates) between 25–30°C while suitable soil type for nematode movement are soil types that are light and sandy (Mulyadi, 2009).

Infected host roots show galls and swollen root tissue, which contain nematodes. In addition, root veins of infected plants were plugged by sap-like substances. This inhibits water and nutrition trans-location from roots to other organs (Vovlasa *et al*., 2005).

Plants are able to recognize, react, and activate resistant response when they are infested by parasites or pathogens (Kosuge, 1969 *cit.* Fitriyanti *et al*., 2009). Susceptible plants that are infested by root-gall nematodes will have enlarged cells followed by cell division that are not accompanied by cytokinesis. In contrast, resistant plants may respond by producing hypersensitive reaction when infested by root-gall nematodes by producing localize necrotic cells around feeding sites, which have darker colors compared to its surrounding cells (Kaplan & Keen, 1980). "Carolina Wonder" and "Charleston Belle" are example of chili varieties that are resistant against *M. incognita* based on the existence of the homogeneous gen-N (Thies & Fery, 1998 *cit.* Aguiar *et al.*, 2014). In order to manage *M. incognita*, research on the responses of four *Capsicum* spp., varieties when infested by nematodes is required. Selecting resistant varieties is essential in crop rotation to suppress nematode populations.

MATERIALS AND METHODS

M. incognita Rearing

Tomato seeds were planted is sterile growing medium placed in polybags to obtain 14-day-old plants. Each 14-day-old plant were then planted in a polybag with 45 cm in diameter. Seven days after being moved to larger polybags, tomato plants were infested with egg masses of *M. incognita.* After 60 days since infestation, tomato plants were taken and eggs of *M. incognita* were then harvested and hatched into L2 as an inoculum.

Preparation of Plant for Test

Growing medium was a mixture of sterilized soil and manure (ratio 1:1). Sterile growing medium were then placed into 45 cm diameter polybags, and filled up to ¾ of the polybag volume. Chili seeds were placed in small polybags with diameter of ± 6 cm. After 3 weeks, test plants were transplanted to larger polybags with diameter of 30 cm. Chili pepper varieties used in this study were *Cabai Merah Besar* (Pilar F1), *Cabai Merah Keriting* (Kastilo F1), and *Paprika* (Red Star 2060). Inoculation treatments in this study were: (1) inoculated with *M. incognita* and (2) untreated, with 10 replications for each treatment.

Inoculation of M. incognita on Plants

M. incognita eggs were extracted from roots of tomatoes by using 1% NaOCl solution according to methods by Hussey and Baker (1973) to clean debris. Eggs of *M. incognita* were then extracted and left at room temperature for 3–4 days until they hatched to L2. The suspension consisting of 1000 L2 M. incognita was inoculated on the 30 day old test plants using a syringe to inject the suspension into the soil around the test plants.

Observation and Data Analysis

Observation were done on histopathology of test plant roots, damage intensity, which followed procedures from Zeck (1971), and nematode populations from root tissues and soil surrounding test plants were extracted using Whitehead Tray Technique with modification (Southey, 1986). Data were then compared using an ANOVA. Significant differences were then test using a DMRT post-hoc test at α=0.05.

RESULTS AND DISCUSSION

Root Damage Intensity

Result of root damage intensity scoring based on Zeck (1971) was shown in Figure 1 and Table 1. Response between chili pepper variety were not significantly different. However, data numerically showed that *Paprika* were more susceptible due to the many galls found on its root (Figure 1A and B) and the highest gall scoring 7.7. Gall scorings were then followed by *Cabai keriting* (7.14) and *Cabai besar* (6.85). These scores indicated that all varieties tested had similar root sensitivity against *M. incognita*. This is supported by the same egg mass scores that all chili pepper varieties had (c.a. 5) (Table 2). Root damage scores from tested varieties indicate nematode population in root system that may directly impact growth, especially their leaves. Lower leaf growth may result in lower production. These results were consistent with the findings of Anwar *et al.* (2013) regarding to crop suitability as nematode host based on galls found on roots. Host crops considered as susceptible against *M. incognita* had root gall score of 6–8.2.

Scores of Root Damage by M. incognita

All three tested varieties were grouped based on the number egg masses were formed on roots based on Quesenberry *et al.* (1989). Scoring showed that all three tested varieties had similar scores implying that all plant varieties were susceptible to *M. incognita*.

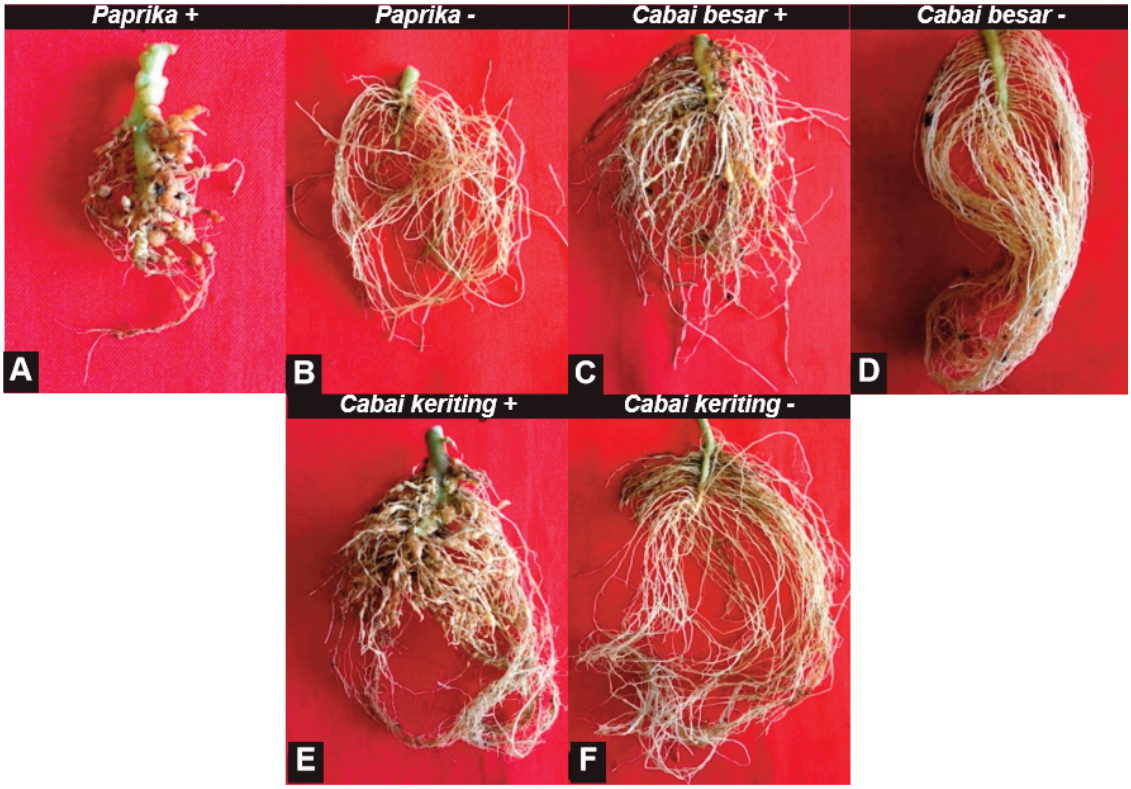

Figure 1. Roots of test plants 60 days after inoculated (DAI); (A) Paprika roots inoculated with *Meloidogyne incognita* and (B) not inoculated; (C) *Cabai besar* roots inoculated with *Meloidogyne incognita* and (D) not inoculated; (E) *Cabai keriting* roots inoculated with *Meloidogyne incognita* and (F) not inoculated

Table 1. Root damage of four *Capsicum annuum* varieties inoculated with *Meloidogyne incognita*

C. annuum variety	Damage scores
Cabai Keriting +	7.14 a
Cabai Merah Besar +	6.85 a
Paprika +	7.71 a
Control	0.00 b

Note: Numbers followed by different numbers were significantly different based on a DMRT post-hoc test (α = 0.05); var. (+) indicated that plants were inoculated with nematodes.

Table 2. Scoring of *Meloidogyne incognita* egg masses from test plant 60 days after inoculated (DAI)

Treatment	Egg mass scores
Cabai Keriting +	5.00 a
Cabai Merah Besar +	5.00 a
Paprika +	5.00 a
Control	0.00 b

Note: Numbers followed by different numbers were significantly different based on a DMRT post-hoc test (α = 0.05); var. (+) indicated that plants were inoculated with nematodes.

Root Histopathology

Histopathology observation showed that there was absorption of safranin on inoculated chili pepper varieties. This observation used safranin (red) and fast green (green). Coloring using safranin was used to color lignin and lignified cells, while fast green was used to show cytoplasm and cellulose (Johansen, 1940; Sass, 1971).

Samples from 6-day-old test plants showed a variation of resistant response to nematodes (Figure 2). *Paprika* roots (Figure 2A) showed accumulation of red in transport veins. This indicates that lignification process by plants. This process results in cell becoming impermeable to water and nutrition from outside causing cells to die (Slusarenko *et al.,* 2000). Response may occur at different times due the different sensitivity levels of plants to *M. incognita* infection (Thomas *et al,* 1995). Lignification that is considered as a resistant response of *Paprika* roots was not able to counter *M. incognita* numbers causing *Paprika* to possess the highest root damage scores. Populations from *Paprika* were higher compared to other varieties.

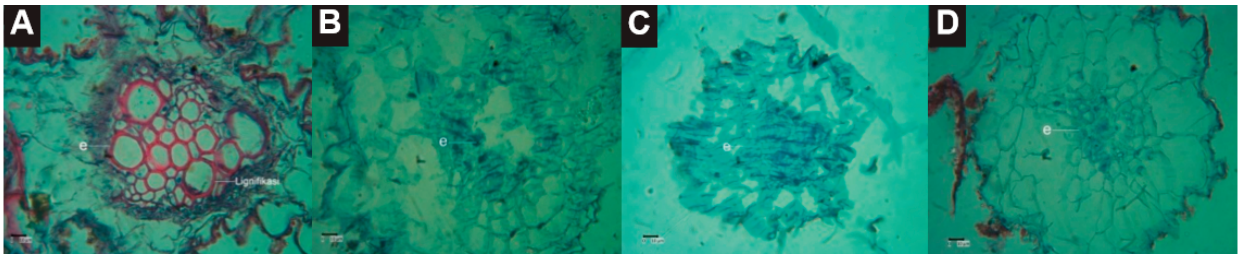

Figure 2. Chili pepper roots 6 days after inoculated (DAI) with *Meloidogyne incognita* (bar size: 10 µm); (A) *Paprik*a inoculated with *M. incognita*, (B) *Cabai besar* inoculated with *M. incognita*, (C) *Cabai keriting* inoculated with *M. incognita,* (D) control, (e) veins

Besides that, *Paprika* had higher sensitivity compared to other varieties.

Population Analysis of M. incognita

The population of L2 *M. incognita* inoculated on test plants were able to develop on all chili varieties. Analysis of *M. incognita* population at 60 days after inoculation showed that nematodes were found in both soil and root tissues (Table 3, 4, and 5).

Root-gall nematode populations found in the soil around roots were L2 (Figure 2). The finding of nematodes at this stage indicates nematodes infected and develop on test plants (Table 3). These findings were like the research by Anwar *et al.* (2013). Although prior inoculations of *M. incognita* to soil were very high, only 3 nematodes per 100 ml of soil were recovered. This may be caused by the speed of L2 *M. incognita* to find host and their ability to penetrate root systems (Mulyadi, 2009). Data showed that average *M. incognita* populations collected from all 3 varieties were not significantly different.

L2 M. incognita Populations in Root Tissue

Root damage intensity correlated to *M. incognita* populations in root tissue (Anwar *et al*., 2013) and indicated that plants were susceptible to nematodes. Populations from inoculated treatments were significantly different compared to the control (Table 4). Average numbers of L2 *M. incognita* per 0.2 g of *Cabai keriting* roots were higher compared to other varieties. This may be due to L2 *M. incognita* to not be quick enough to form feeding sites on host plants. The longer it took for nematodes to develop between stage, the longer it will took them to complete their life cycle. The toughness of root tissues also affected nematode ability to penetrate root tissue (Mulyadi, 2009). Suitability of a plant to be a host of *M. incognita* does not solely depend on the L2 population (Figure 3), but also population of other stages, such as L3, L4 or adult females (Figure 4), number of galls, number of egg masses produced and reproduction levels.

Table 3. Average of L2 *Meloidogyne incognita* population per 100 ml of soil

Treatment	Average L2 *M. incognita* population per 100 ml of soil
Cabai Keriting +	1.86 a
Cabai Merah Besar +	1.90 a
Paprika +	2.04 a
Control	0.00 b

Note: Numbers followed by different numbers were significantly different based on a DMRT post-hoc test (α = 0.05); var. (+) indicated that plants were inoculated with nematodes.

Table 4. Population L2 *Meloidogyne incognita* from root tissue

Treatment	Average L2 *M. incognita* population/0.2 g of root tissue
Cabai Keriting +	471.14 a
Cabai Merah Besar +	190.43 b
Paprika +	311.71 b
Control	0.00 c

Note: Numbers followed by different numbers were significantly different based on a DMRT post-hoc test (α = 0.05); var. (+) indicated that plants were inoculated with nematodes.

Population of L3, L4, and Adult M. incognita in Root Tissue

Coloring was done to precisely show *M. incognita* due to difficulties of microschophically differ between nematodes and root tissue. Results showed significantly different L3, L4, and adult populations between inoculated and controls (Table 5). *Paprika* was relatively susceptible against *M. incognita*. High populations collected from *Paprika* root system is caused by its soft root tissues compared to *Cabai besar* and *Cabai keriting*, which caused nematodes

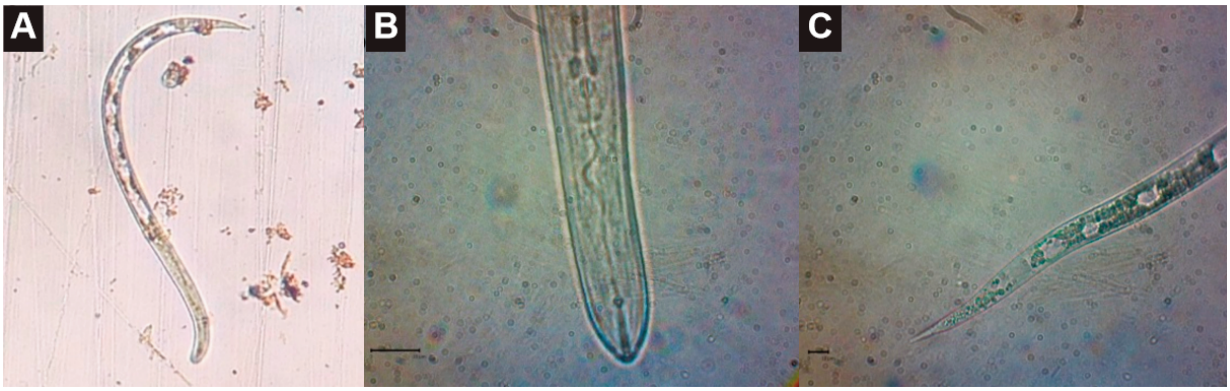

Figure 3. (A) L2 *Meloidogyne incognita*; (B) anterior *M. incognita* (bar size: 10 µm); (C) posterior *M. incognita* (bar size: 10 µm)

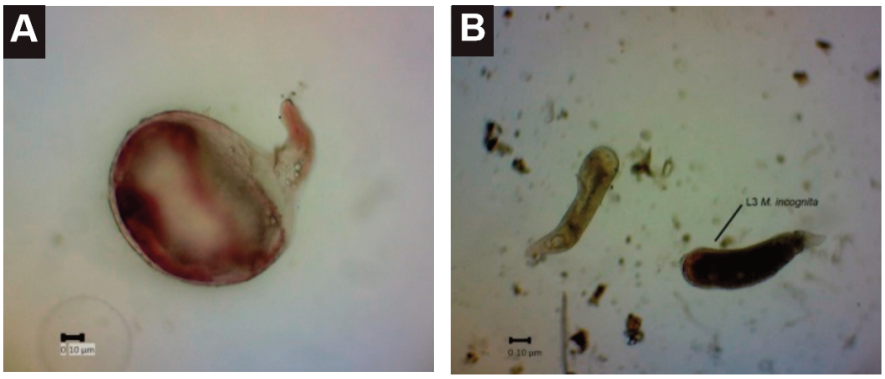

Figure 4. *Meloidogyne incognita*; (A) Female *M. incognita* (bar size: 10 µm) and (B) L3 *M. incognita* (bar size: 10 µm)

Table 5. Average population of L3, L4, and adult female *Meloidogyne incognita* from root tissue

Treatment	Average population of L3, L4, and adult females/0.2 g root tissue
Cabai Keriting +	70.28 c
Cabai Merah Besar +	94.71 b
Paprika +	123.0 a
Control	0.00 d

Note: Numbers followed by different numbers were significantly different based on a DMRT post-hoc test (α = 0.05); var. (+) indicated that plants were inoculated with nematodes.

to easily locate feeding sites and develop to further stages. Root galls indicated *M. incognita* ability to form feeding sites. The higher the ability of nematodes to form feeding sites, the more severe the galls that are formed.

CONCLUSION

Histopathology observation showed that *Paprika* variety was more sensitive to *M. incognita* infection compared to *Cabai keriting* and *Cabai besar*. *Cabai keriting, Cabai besar,* and *Paprika* root systems were similarly susceptible to *M. incognita* infection based on their scores on root damage and egg masses. *M. incognita* developed well on *Paprika* variety based on the populations of nematodes in 100 ml of soil surrounding plant root and populations of L3, L4, and adult nematodes in root tissue. *Paprika* was a susceptible chili pepper variety against *M. incognita.*

ACKNOWLEDGEMENT

We would like to thank Istikhana, the laboratory technician of the Nematology Laboratory, who has assisted in providing materials and *M. incognita* population and Ratna Widowati, S.P. for suggestions regarding to nematode inoculation techniques.

LITERATURE CITED

Aguiar, J.L., O. Bachie, & A. Ploeg. 2014. Response of Resistance and Susceptible Bell Pepper

(*Capsicum annuum*) to a Southern California *Meloidogyne incognita* Population from a Commercial Bell Pepper Field. *Journal of Nematology* 46: 346–351.

Anwar, S.A., M.M Mahdi, & F.A. Chaudhry. 2013. Evaluation of Two Vegetables against *Meloidogyne incognita* Infection. *Pakistan Journal of Zoology* 45: 1285–1290.

Fitriyanti, D., Mulyadi, & C. Sumardiyono. 2009. Mekanisme Ketahanan Kentang (*Solanum tuberosum*) terhadap Nematoda Sista Kuning (*Globodera rostochiensis*). *Jurnal Hama Penyakit Tumbuhan Tropika* 9: 46–53.

Hussey R. S., & K. R. Barker. 1973. A Comparison of Methods of Collecting Inocula of *Meloidogyne* spp., Including a New Technique. *Plant Disease Reporter* 57: 1025–1028.

Johansen, D.A. 1940. *Plant Microtechnique*. McGaw-Hill Books, New York. 523 p.

Kaplan, D.T., & N.T. Keen. 1980. Mechanisms Conferring Plant Incompatibility to Nematodes. *Revue de Nématologie* 3: 123–134.

Luc, M., R.A. Sikora, & J. Bridge (eds.). 2005. *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*. CAB International, Wallingford, UK. 841 p.

Mulyadi. 2009. *Nematologi Pertanian*. Gadjah Mada Press, Yogyakarta. 339 p.

Oka,Y., Koltai, H., Bar-Eyal, M., Mor, M., Sharon, E., Chet, I. and Spiegel, Y. 2000. New Strategies for The Control of Plant Parasitic Nematodes. *Pest Management Science* 56: 983–988.

Oka, Y., R. Offenbach, & S. Pivonia. 2004. Pepper Rootstock Gaft Compatibility and Responsse to *Meloidogyne javanica* and *Meloidogyne incognita*. *Journal of Nematology* 36: 137–141.

Quesenberry, K.H., D.D. Baltensperger, R.A. Dunn, C.J. Wilcox, & S.R. Harry. 1989. Selection of Tolerance to Root-Knot Nematodes in Red Clover. *Crop Science* 29: 62–65.

Sass, J.E. 1971. *Botanical Microtechnique*. Third Edition. The Iowa State University Press, Iowa. 252 p.

Sasser, J.N. & D.W. Freckman. 1987. A World Prospective on Nematology: The Role of Society, p. 7–14. *In* J.A. Veech & D.W. Dickerson (eds.), *Vistas on Nematology: A commemoration of the Twenty-fifth Anniversary of the Society of Nematologists*. Society of Nematologists, Inc., Hyallsville.

Sekretariat Jenderal Kementerian Pertanian. 2016. *Outlook Komoditas Pertanian, Sub Sektor Hortikultura: Cabai*. Pusat Data dan Sistem Informasi Pertanian Sekretariat Jenderal Kementerian Pertanian Tahun 2016, Jakarta. 89 p.

Slusarenko, A.J., R.S.S. Fraser, & L.C. van Loon. 2000. *Mechanism of Resistance to Plant Disease*. Kluwer Academic Publishers, Dordrecht. 62 p.

Southey, J.F. 1986. *Laboratory Methods for Work with Plant and Soil Nematodes*. Her Majesty's Stationary Office. London. 202 p.

Thies, J.A. & R.L. Fery. 1998. Modified Expression of the *N* Gene for Southern Root-knot Nematode Resistance in Pepper at High Soil Temperatures. *Journal of the American Society for Horticultural Science* 123: 1012–1015.

Thomas, S.H., L.W. Murray, & M. Cardenas. 1995. Relationship of Preplant Population Densities of *Meloidogyne incognita* to Damage in Three Chile Pepper Cultivars. *Plant Disease* 79: 557–559.

Vovlas, N., D. Mifsud, B. B. Landa & P. Castillo. 2005. Pathogenicity of the Root-knot Nematode *Meloidogyne javanica* on Potato. *Plant Pathology* 54: 657–664.

Zeck, W.M. 1971. A Rating Scheme for Field Evaluation of Root–Knot Nematode Infestations. *Pflanzenschutz-Nachrichten Bayer* 24: 141–144.