EFFECTS OF SEEDLING AGE AND ROOT WOUNDING ON THE CHARCOAL ROT/ROOT-KNOT DISEASE COMPLEX ON GREEN BEAN

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ABSTRACT

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The effects of seedling age at inoculation and the mechanical wounding of roots on the development and severity of the disease complex caused by the interaction between *Macrophomina phaseolina* and *Meloidogyne javanica* on green bean were examined in two different greenhouse pot experiments. The results showed that disease severity decreased with increasing seedling age at inoculation from 4 to 6 to 8 weeks, and nematode reproduction was suppressed. However, mechanical wounding of the roots during inoculation with both pathogens increased the root-rot index, root galling severity, fungal recovery from roots, and the number of eggs per gram roots produced by the nematodes, consequently decreasing plant growth.

Key words: Macrophomina phaseolina, *Meloidogyne javanica*, *Phaseolus vulgaris*.

RESUMEN

Al-Hazmi, A. S., A. A. M. Dawabah, S. N. Al-Nadhari, F. A. Al-Yahya, y H. A. Lafi. 2016. Efectos de la edad del plantón y de lesiones en las raíces sobre la enfermedad compleja de la podredumbre/nódulos de las raíces en las judías verdes. Nematropica 46:229-234.

Los efectos de la edad del plantón en el momento de la inoculación y de las heridas producidas por manipulación mecánica en las raíces sobre el desarrollo y severidad de la enfermedad compleja causada por la interacción entre *Macrophomina phaseolina* y *Meloidogyne javanica* en judía verde fueron examinados en dos experimentos diferentes en macetas en invernadero. Los resultados mostraron que la severidad de la enfermedad y la reproducción del nematodo se redujeron al incrementar la edad del plantón de 4 a 6 y a 8 semanas. Sin embargo, el daño mecánico causado por la manipulación de las raíces durante la inoculación con ambos patógenos incrementó el índice de podredumbre, la severidad del agallamiento, la recuperación fúngica de las raíces y el número de huevos del nematodo por gramo de raíz, y consecuentemente el crecimiento de la planta se redujo.

Palavras chave: Macrophomina phaseolina, *Meloidogyne javanica*, *Phaseolus vulgaris.*

INTRODUCTION

The green bean, *Phaseolus vulgaris* L., is an important summer crop in Saudi Arabia. During recent surveys and field studies, we sometimes found concomitant infection by the fungus *Macrophomina phaseolina* (Tassi) Goldi and either *Meloidogyne javanica* (Trueb) Chitwood or *M. incognita* (Kofoid & White) Chitwood, which causes a root-rot/root-knot disease complex (Al-Hazmi, 1985). Infected plants are usually stunted, and their root systems often display heavy galling and symptoms of charcoal root-rot.

Certain biotic and abiotic factors can affect the development and severity of this disease complex on green bean and other crops (Back *et al*., 2002). These factors may include the population density of both pathogens in the soil, soil moisture and texture, age

of the plants at initial infection, plant cultivar, root wounds caused by other factors, and other biological and physical factors (Al-Hazmi, 1985; Shahzad and Ghaffar, 1992; Port, 1993; Abawi and Chen, 1998; Back *et al*., 2002).

Very few reports have investigated the effects of plant age on the development and severity of the charcoal rot/root-knot disease complex (Tu and Cheng, 1971; Antoon *et al*., 2009). Tu and Cheng (1971) reported that kenaf seedlings (*Hibiscus cannabinus* L.) inoculated with both *M. phaseolina* and *M. javanica* at 5, 10, and 15 days of age showed decreasing percentages of root rot lesions (70.3, 44.5, and 21.8%, respectively), as well as low numbers of nematode second-stage juvenile penetration (49.0, 36.7, and 12.3%, respectively). Similarly, Antoon *et al*. (2009) reported that as the age of tobacco seedlings increased from 3 to 7 wk, the severity of the disease complex caused by *M. phaseolina* and *M. javanica* decreased.

Mechanical wounding may predispose plant roots to infection by facilitating the entry of root pathogens (Back *et al*., 2002). A number of reports have clearly illustrated that the root damage caused by some endo- and ecto-parasitic nematodes aided in the establishment and development of diseases that were caused by some soil-borne pathogens (Storey and Evans, 1987; Evans and Haydock, 1993; Orion *et al.*, 1999). For example, Inagaki and Powell (1969) reported that when tobacco plants were subjected to an artificial (mechanical) root wounding treatment, symptoms of black shank were significantly more severe than those observed in plants inoculated with *Phytophthora parasitica* Dastur var. *nicotianae* (Breda de Haan) Tucker and the lesion nematode, *Pratylenchus brachyurus* (Godfrey) Filipjev & Sch. Stekh, without root-wounding. In another study, mechanical wounding of the small roots of peanuts increased the severity of Cylindrocladium black rot (CBR) caused by *Cylindrocladium crotalariae* (Loos) and *M. hapla* compared with non-wounded plants (Diomande and Beute, 1981).

This study aimed to evaluate the role of seedling age at inoculation and the mechanical wounding of roots on the development and severity of the charcoal rot/root-knot disease complex on green bean under greenhouse conditions.

MATERIALS AND METHODS

Macrophomina phaseolina

A pure culture of the fungus, *M. phaseolina*, was obtained from the fungal plant diseases unit, Plant Protection Department, King Saud University (the late Dr. S. El-Hussaini). The fungus was originally isolated from green bean plants that were infected with charcoal root-rot near Riyadh, Saudi Arabia. This indigenous isolate was then cultured on potato dextrose agar media (PDA) in Petri dishes at 24 to 26°C for one week. Inoculum was prepared as follows, 500-ml conical flasks were each filled with 150 g of barley grains and bran (3:1 v/v), and soaked with water overnight, then autoclaved for 30 min over two consecutive days. After cooling, each flask was inoculated with a small disc (8 mm diameter) obtained from the periphery of the 7-day-old cultures. The flasks were then incubated at 25 ± 1°C for 2 wk (Bugbee, 1974; Khanzada *et al*., 2012). During incubation, the flasks were shaken twice a day to ensure a uniform distribution and mycelial growth. To obtain the sclerotia, which were used as the fungal inoculum, the cultures were thoroughly mixed, and the mixture was processed in an electric blender in batches of 100 g in 250 ml sterilized distilled water. The cultures were blended five times for 30 s each. The contents were then passed through several layers of muslin cloth to obtain the sclerotia (Khanzada *et al.*, 2012). The sclerotia were counted, and the suspension was adjusted to the required concentration. The fungal inoculum consisted of eight sclerotia per gram soil per pot (Al-Hazmi, 1985).

Meloidogyne javanica

Inoculum of *M. javanica* consisted of eggs that were extracted in 0.5% sodium hypochlorite (Hussey and Barker, 1973) from a greenhouse culture on tomato cv. 'Rutgers'. The extracted eggs were immediately washed several times with running tap water, and the egg suspension was adjusted to contain 1,200 eggs per ml. Each plant was inoculated with 12,000 eggs (= 8 eggs/g soil).

Test plants

Seeds of the green bean cv. 'Contender', which is susceptible to local Saudi isolates of *M. phaseolina* and *M. javanica* (Al-Nadhari, 2007), were sown in a clean plastic tray containing autoclaved loamy sand soil (85.12% sand, 6% loam, 8.88% clay). One week after germination, the young seedlings were transferred individually into small (14 cm diameter) plastic pots containing 1,500 g of the same autoclaved loamy sand soil, and maintained in a greenhouse at 24 to 26°C until the end of the experiments.

Inoculation of plants

At the time of inoculation with the nematode and (or) the fungus, 4-wk-old green bean seedlings

were carefully re-potted, and the soil of each pot was thoroughly mixed in a plastic bag with the nematode and (or) with the fungal inoculum as appropriate. Most of the seedlings exhibited five trifoliate leaves, which is suitable for inoculation with both *M. phaseolina* and/or *M. javanica* (Al-Hazmi, 1985). The infested soil was then returned to its designated pot according to the test treatments, and the designated seedling was replanted. Each treatment in both tests was replicated five times.

Pots of both tests were arranged on a bench in the greenhouse (24 to 26°C) in a randomized complete block design (RCDB). All seedlings were irrigated as needed and fertilized (1.5-18-18, NPK), (Vauxhall®, Greenhaven Garden Centre, Alberta, Canada). Each pot received 60 ml of a solution containing 10 ml Vauxhall® per l000 ml water every 2 wk.

Effect of seedling age and root wounding

Two trials were conducted. One test evaluated the effect of seedling age at inoculation on the severity of the charcoal rot/root-knot disease complex. In this test, six treatments were established: 1) control, healthy 4-wk-old seedlings, 2) 4-wk-old seedlings inoculated with nematode alone (N), 3) 4-wk-old seedlings inoculated with the fungus alone (F), 4) 4-wk-old seedlings inoculated with both pathogens (N + F), 5) 6-wk-old seedlings inoculated with both pathogens, and 6) 8-wk-old seedlings inoculated with both pathogens (Table 1).

The second trial evaluated the effect of the mechanical wounding of roots at inoculation on the severity of the root-rot/root-knot disease complex. In this test, 4-wk-old seedlings were transplanted into the pots and five treatments were established as follows: 1) control, healthy seedlings, 2) inoculation with nematode alone, 3) inoculation with fungus alone, 4) inoculation with both pathogens without wounding, and 5) inoculation with both pathogens after root wounding. Wounding of roots was accomplished by making three punctures, 3 mm behind the root cap with a fine needle (0.4 mm diameter) (Diomande and Beute, 1981; Carter, 1981).

Termination of the tests

The plants were uprooted 60 days after inoculation with the nematode and/or the fungus in both trials. The roots were washed with running tap water, and the total fresh weight of each plant was recorded. Nematode infection was determined based on the number of root galls, and nematode reproduction was determined by the number of eggs produced. The gall index (0-5) (Taylor and Sasser, 1978) and nematode reproduction factor (Rf) (Oostenbrink, 1966) were calculated for each plant. The charcoal rot index for each root system was determined according to a 0-10 scale (Iqbal *et al*., 2010).

All data were analyzed with SAS, 2013, and the treatment mean values were separated using Fisher's protected least significant differences (FPLSD).

RESULTS

Effect of seedling age

As the age of the seedlings at inoculation increased from 4 to 8 wk, the severity of the disease complex caused by the interaction between *M. phaseolina* and *M. javanica* decreased (Table 1). Compared with inoculation of seedlings at 4 or 6 wk of age, inoculation of 8-wk-old seedlings resulted in significantly ($P \leq 0.05$) increased plant growth, less root galling, a lower root-rot index and lower

Table 1. The effects of seedling age at inoculation with *Meloidogyne javanica* (N) and *Macrophomina phaseolina* (F) on the severity of the disease complex on green beans cv. 'Contender', 60 days after inoculation.

Treatment	TFW[x](g)	No. galls/g root	Root-rot Index[z] (0-10)	% Reisolation of the fungus
Control	33.3 a[y]	-	-	-
Nematode (N)	27.6 b	18.1 c	-	-
Fungus (F)	26.4 b	-	4.6 b	40.0 b
N+F (at 4 weeks old)	17.0 e	48.7 a	6.6 a	65.0 a
N+F (at 6 weeks old)	19.7 d	23.6 b	4.2 b	35.0 b
N+F (at 8 weeks old)	23.8 c	11.3 d	3.0 c	25.0 b

[x]TFW= total fresh weight of shoots and roots.
[y]Values are means of five replicates. Means followed by the same letter(s) in a column are not significantly different at $P \leq 0.05$.
[z]On a scale of 0-10 where, 0 = 0.00%, 1 = up to 10%, 2 = 11-20%, 3 = 21-30%, 4 = 31-40%, 5 = 41-50%, 6 = 51-60%, 7 = 61-70%, 8 = 71-80%, 9 = 81-90%, and 10 = 91-100% of the root area is infected (Iqbal *et al.*, 2010).

percentage of fungal re-isolation (Table 1). Similarly, nematode reproduction was suppressed ($P \leq 0.05$) with increasing age of the seedlings (Table 2).

Effect of root wounding

Mechanical wounding of the roots at inoculation significantly ($P \leq 0.05$) increased the root-rot index, the number of root galls, and fungal re-isolation (%), compared with the non-wounded treatment, but wounding also decreased plant growth (Table 3). Root wounding increased the number of nematode eggs per gram root that were recovered but did not increase the number of egg masses that were found per root system or the nematode reproduction factor (Table 4).

Table 2. The effects of seedling age at inoculation with *Meloidogyne javanica* (N) and *Macrophomina phaseolina* (F) on the nematode reproduction on green beans cv. 'Contender', 60 days after inoculation.

Treatment	No. egg masses/ root system	No. eggs/g root (× 1000)	Reproduction factor (Rf)[z]
Control	-	-	-
Nematode (N)	260.8 b[y]	4.4 b	5.6 a
Fungus (F)	-	-	-
N+F (at 4 week old)	307.6 a	6.2 a	4.3 b
N+F (at 6 week old)	163.6 c	2.4 c	2.1 c
N+F (at 8 week old)	82.8 d	1.0 d	1.1 d

[y]Values are means of five replicates. Means followed by the same letter(s) in a column are not significantly different at $P \leq 0.05$.
[z]Reproduction factor (Rf) = Pf (final egg count/root system)/Pi (initial egg inoculum/root system).

Table 3. The effects of mechanical wounding of seedling roots at inoculation with *Meloidogyne javanica* (N) and *Macrophomina phaseolina* (F) on the severity of the disease complex on green beans cv. 'Contender', 60 days after inoculation.

Treatment	TFW[x](g)	No. galls/g root	Root-rot Index[z] (0-10)	% Reisolation of the fungus
Control	31.5 a[y]	-	-	-
Nematode (N)	27.2 b	18.6 c	-	-
Fungus (F)	25.2 c	-	4.8 b	35.0 c
N+F (+ wounding)	14.5 e	53.8 a	7.4 a	80.0 a
N+F (no wounding)	17.9 d	38.9 b	6.8 a	60.0 b

[x]TFW= total fresh weight of shoots and roots.
[y]Values are means of five replicates. Means followed by the same letter(s) in a column are not significantly different at $P \leq 0.05$.
[z]On a scale of 0-10 where, 0 = 0.00%, 1 = up to 10%, 2 = 11-20%, 3 = 21-30%, 4 = 31-40%, 5 = 41-50%, 6 = 51-60%, 7 = 61-70%, 8 = 71-80%, 9 = 81-90% and 10 = 91-100% of the root area is infected (Iqbal *et al*., 2010).

Table 4. The effects of mechanical wounding of seedling roots at inoculation with *Meloidogyne javanica* (N) and *Macrophomina phaseolina* (F) on nematode reproduction on green beans cv. 'Contender', 60 days after inoculation.

Treatment	No. egg masses/ root system	No. eggs/g root (× 1000)	Reproduction factor (Rf)[z]
Control	-	-	-
Nematode (N)	227.8 a[y]	4.3 c	5.4 a
Fungus (F)	-	-	-
N+F (+ wounding)	242.2 a	7.4 a	4.6 b
N+F (+ wounding)	238.2 a	5.9 b	4.6 b

[y]Values are means of five replicates. Means followed by the same letter(s) in a column are not significantly different at $P \leq 0.05$.
[z]Reproduction factor (Rf) = Pf (final egg count/root system)/Pi (initial egg inoculum/root system).

DISCUSSION

Both the age of the green bean seedlings and root wounding at inoculation had a strong effect on the synergistic outcome of the interaction between the two pathogens. Older seedlings were relatively more resistant to the effects of the disease complex than younger ones. Our results support previous reports that as the age of kenaf (Tu and Cheng, 1971) or tobacco (Antoon *et al.*, 2009) seedlings increased, the severity of disease due to the interaction between *M. phaseolina* and *M. javanica* decreased. It appears that as seedlings age, they become more tolerant or resistant to both pathogens. Compared with younger seedlings, older plants possessed more developed connective tissues and thicker cell walls, which might hinder plant penetration by many pathogens (Tu and Chen, 1971).

Root wounding at the point of inoculation increased the severity of the disease complex and enhanced plant damage. Some reports suggest that root wounding, whether made by nematodes or by any artificial (mechanical) root-wounding method, produces more severe disease symptoms compared with non-wounded plants (Carter, 1981; Owens and Specht, 1966; Inagaki and Powell, 1969; Diomande and Beute, 1981; Evans and Haydock, 1993; Orion *et al*., 1999). *Meloidogyne javanica* and root wounding might have an additive effect, leading to a more severe disease. The wounding could assist root penetration by *M. phaseolina* and predispose plants to infection. Root damage might also increase the synergistic interaction between the two pathogens. It has been reported that the mechanical wounding of peanut roots increases the severity of the Cylindrocladium black rot (CBR) caused by *C. crotalariae* and *M. hapla*, compared with non-wounded plants (Diomande and Beute, 1981), regardless of the exact mechanism.

The age of plants and root wounding by pathogens or parasites, may play important roles in the outcome of interactions between plant-parasitic nematodes and some soil-borne pathogens.

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