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Meloidogyne javanica-Rhizoctonia solani disease complex of peanut

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Summary – The interaction of *Meloidogyne javanica* and *Rhizoctonia solani* was studied on peanut in greenhouse and field microplot experiments. The effects of *R. solani* on reproduction of *M. javanica* was variable, with nematode *Pi* having a greater effect on nematode reproduction than did the presence of *R. solani*. In microplot tests with a factorial design with four nematode *Pi* and two levels (1995) or three (1996) levels of *R. solani*, peanut pod rot and root colonization by *R. solani* were increased by the presence of *M. javanica* ($P \leq 0.05$), and the total amounts of pod rot and root colonization were positively related ($r^2 > 0.85$) to *Pi* of *M. javanica*. Pod yield was more suppressed by both pathogens than by either pathogen alone, and was negatively related ($r^2 > 0.86$) to *Pi* of *M. javanica* in both microplot experiments. The data confirm the interaction of *M. javanica* and *R. solani* on peanut. © Orstom/Elsevier, Paris

Résumé – *Le complexe pathogène de l'arachide* Meloidogyne javanica - Rhizoctonia solani – L'interaction entre *Meloidogyne javanica* et *Rhizoctonia solani* a été étudiée sur arachide en serre et en microparcelles au champ. L'action de *R. solani* sur la reproduction de *M. javanica* est variable, la *Pi* du nématode ayant une plus grande influence sur sa reproduction que la présence de *R. solani*. Lors d'expériences en microparcelles ayant une disposition factorielle et comportant quatre niveaux de *Pi* du nématode et deux (1995) ou trois (1996) niveaux de *R. solani*, la pourriture des gousses d'arachide et la colonisation des racines par *R. solani* sont accrues en présence de *M. javanica* ($P \leq 0,05$) ; les valeurs totales de la pourriture des gousses et de la colonisation des racines sont corrélées positivement ($r^2 > 0,85$) à la *Pi* de *M. javanica*. La récolte en gousses est plus affectée en présence des deux agents pathogènes que si un seul d'entre eux est présent, et cette récolte est corrélée négativement ($r^2 > 0,86$) aux *Pi* de *M. javanica* dans l'une et l'autre expériences en microparcelle. Ces données confirment donc l'interaction entre *M. javanica* et *R. solani* sur l'arachide. © Orstom/Elsevier, Paris

Keywords: *Arachis hypogaea*, disease complex, *Meloidogyne javanica*, peanut, *Rhizoctonia solani*, root-knot nematode.

Nematode-fungal disease complexes, especially those involving *Meloidogyne* spp., are common on many crops (Golden & Van Gundy, 1975; Diomandé *et al.*, 1981; Abawi & Barker, 1984; Starr *et al.*, 1989). The association of nematodes and fungi on plants may cause synergistic, additive, or antagonistic effects with respect to disease development and yield suppression. Synergistic associations are generally attributed to the enhancement of fungal infections due to the physiological effects on the plant of nematode parasitism (Golden & Van Gundy, 1975; Starr & Aist, 1977). In the synergistic association of *Meloidogyne incognita* and *Rhizoctonia solani* on okra and tomato, roots of both crops were colonized to a greater extent by *R. solani* in the presence of *M. incognita* compared to colonization of plants exposed to *R. solani* alone (Golden & Van Gundy, 1975). Siddiqui and Husain (1992) reported a similar effect of *M. incognita* on the colonization of chickpea roots by *Macrophomina phaseolina*. Infection of cotton by *M. incognita* increased the susceptibility of wilt-susceptible and wilt-resistant cotton genotypes to *F. oxysporum* f. sp. *vasinfectum* (Starr *et al.*, 1989; Jeffers & Roberts, 1993).

Not all associations of nematodes with soilborne fungal pathogens result in synergistic effects. Starr *et al.* (1996) reported that the effects of *Sclerotium rolfsii* and *M. arenaria* on yield of peanut and incidence of southern blight were additive over a range of different inoculum levels of both pathogens. Jorgenson (1970) reported antagonistic effects for the association of *Heterodera schachtii* and *F. oxysporum* with respect to growth of sugar beets.

Meloidogyne javanica (Tomaszewski *et al.*, 1994) and *R. solani* (El-Wakil *et al.*, 1984) are important pathogens of peanut (*Arachis hypogaea*) in Egypt and have been reported to be involved in disease complexes either together or with other organisms on several crops (Batten & Powell, 1971; Golden & Van Gundy, 1975; Sankarialingman & McGawley, 1994; Walker, 1994), but their association on peanut has not been investigated previously. The objective of this study was to quantify the effect of the association of these two pathogens on peanut.

Materials and methods

Rhizoctonia solani was isolated from peanut roots exhibiting symptoms of root rot and root galling and Koch's postulates fulfilled (data not shown). The isolate of *R. solani* used for all experiments was identified

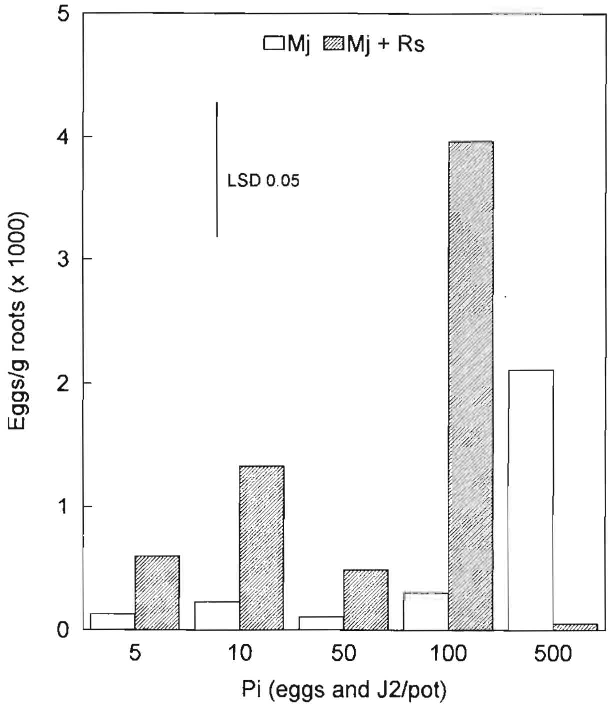

Fig. 1. *Effect of root infection by* Rhizoctonia solani *on reproduction of* Meloidogyne javanica *on peanut in a greenhouse experiment.*

as belonging to anastomosis group (AG)4 by comparison with isolates of known AG. *Meloidogyne javanica* was obtained from potato in Texas, and was parasitic on peanut (Abdel-Momen & Starr, 1997).

Meloidogyne javanica was reared on tomato (*Lycopersicon esculentum*) cv. Rutgers and eggs for inoculum were extracted from infected roots with 0.5 % NaOCl (Hussey & Barker, 1973). *Rhizoctonia solani* was maintained on potato dextrose agar. Inoculum of *R. solani* was prepared by growing the fungus on autoclaved oat grains for 15 days at 27 °C. Colonized oat grains were air-dried and then ground in a blender for 15 s prior to use as inoculum.

The effect of *R. solani* on reproduction of *M. javanica* was determined in a greenhouse experiment using a two × six factorial design. Two inoculum densities of *R. solani* (0 and 0.1 g/pot) and six initial population densities (*Pi*) of *M. javanica* (0, 5, 10, 100, and 500 eggs/pot) were tested. Thirty-cm-diam. pots were filled with a loamy sand soil, infested with each pathogen alone or combined. Seeds of peanut cv. Florunner were germinated in moist paper towels and one seedling was transplanted to each pot. To estimate nematode reproduction, as number of eggs per gram roots, eggs were extracted from infected roots with 0.5 % NaOCl (Hussey & Barker, 1973) at 8 weeks after planting.

The combined effects of *M. javanica* and *R. solani* on nematode reproduction, root colonization and pod rot by *R. solani*, and peanut yield was tested in 1995 and 1996 in field microplots. Microplots were plastic cylinders (55 cm diam × 45 cm deep) filled with and buried in loamy sand soil (85 % sand, 7 % silt, 8 % clay, pH 7.5). Microplots were fumigated before planting with metham sodium (380 L/ha) to eliminate existing pathogen populations. For these microplot experiments, nematode inoculum was prepared by chopping infected tomato roots into small fragments and mixing these fragments with the infested soil from which the roots were obtained. This mixture of infested soil and infected root fragments was then mixed with pasteurized sand (1:2 v/v). The number of second-stage juveniles (J2) and eggs in the inoculum mix was estimated from 500 cm^3 samples following elutriation (Byrd *et al.*, 1976) and centrifugation (Jenkins, 1964). Eggs were extracted with 0.5% NaOCl (Hussey & Barker, 1973) from root fragments collected during elutriation. Different amounts of this highly infested soil was mixed into the upper 25 cm of microplot soil to obtain the desired nematode *Pi* in each microplot. Ground oat grain inoculum of *R. solani* was also incorporated into the upper 25 cm of soil in the appropriate microplots. Eight seeds of Florunner peanut were planted in each microplot immediately after infesting the soil, microplots were thinned to three plants/microplot following emergence.

A factorial experimental design for microplot tests was used to determine the effects of each pathogen. In 1995, two inoculum densities of *R. solani* (0 and 0.01 g/500 cm^3) and four *Pi* of *M. javanica* (0, 5, 10, and 50 eggs and J2/500 cm^3 soil) were tested. In 1996, three inoculum densities of *R. solani* (0, 0.01, and 0.02 g/500 cm^3) and four *Pi* of *M. javanica* (0, 10, 50, and 100 eggs and J2/500 cm^3) were tested. There were five replications of each treatment each year.

Microplots were sampled twice, once at 8 weeks after planting and again at crop maturity (140 days after planting) to estimate nematode population densities. A composite soil sample of eight cores, each 2.5 cm diam. × 25 cm deep, was collected from each microplot. J2 were extracted from 500 cm^3 of soil by elutriation (Byrd *et al.*, 1976) and centrifugation (Jenkins, 1964). Eggs were extracted from root fragments collected during elutriation with 0.5 % NaOCl (Hussey & Barker, 1973). Reproductive factors (*Rf*) were calculated as midseason (*Pm*) or final (*Pf*) nematode population densities divided by *Pi*.

Peanuts were dug at 140 days after planting, air-dried, and the pods were harvested by hand. To estimate the percentage of rotted pods, 100 pods were selected randomly from the yield of each microplot and examined for symptoms of pod rot. The percentage root colonization by *R. solani* for each treatment was determined in 1996. One hundred root segments (each 1 to 2 cm long) were taken arbitrarily from the roots of each microplot, rinsed with tap water, surface sterilized with 0.5 % NaOCl for 1.5 min, rinsed with sterilized water, placed in 10 cm diam. Petri dishes containing tannic acid-benomyl-agar medium, a selective medium for *R. solani* (Flowers, 1976), and incubated at 27 °C. Percentage of peanut roots colonized by *R. solani* was estimated based on the number of root segments from which colonies of *R. solani* developed.

Data from different experiments were subjected to analysis of variance using the general linear model procedures of SAS (SAS Institute, Cary, NC 27511, USA) to determine the effects of treatments on nematode population densities, pod rot, root colonization by *R. solani*, and pod yield. Mean separations were made using Fisher's LSD.

Results

Rhizoctonia solani did not affect the reproduction of *M. javanica* at nematode *Pi* of 5, 10, or 50 eggs/pot, but at a *Pi* of 100 eggs/pot in greenhouse tests, the final number of nematode eggs per gram roots was greater ($P \leq 0.05$) in the presence of *R. solani* compared to that in the absence of *R. solani* (Fig. 1). At a *Pi* of 500 *M. javanica* eggs/pot, the final number of eggs per gram roots was reduced ($P \leq 0.01$) by 98 % in the presence of *R. solani* compared to that produced in the absence of *R. solani*.

In microplot experiments, the midseason Rf values of *M. javanica* were not affected ($P \leq 0.05$) by the presence of *R. solani* (data not shown). At crop maturity, there was a general trend for *Rf* values to decline with increasing *Pi* (Fig. 2). The effect of *R. solani* was to increase *Rf*, but this trend was significant only in 1996 ($P \leq 0.05$).

No symptoms of pod rot were observed in plots not infested with *R. solani*. The percentage of pods with symptoms of rot caused by *R. solani* was greater ($P \leq 0.05$) in plots also infested with *M. javanica* than in plots infested only with *R. solani* (Fig. 3), and was positively related to the *M. javanica Pi* in both years ($r^2 = 0.86$ for 1995 and $r^2 = 0.89$ for 1996; $P \leq 0.01$). An increase in the concentration of inoculum of *R. solani* from 0.01 to 0.02 g/500 cm^3 did not affect the percentage of rotted pods in 1996 ($P \leq 0.05$).

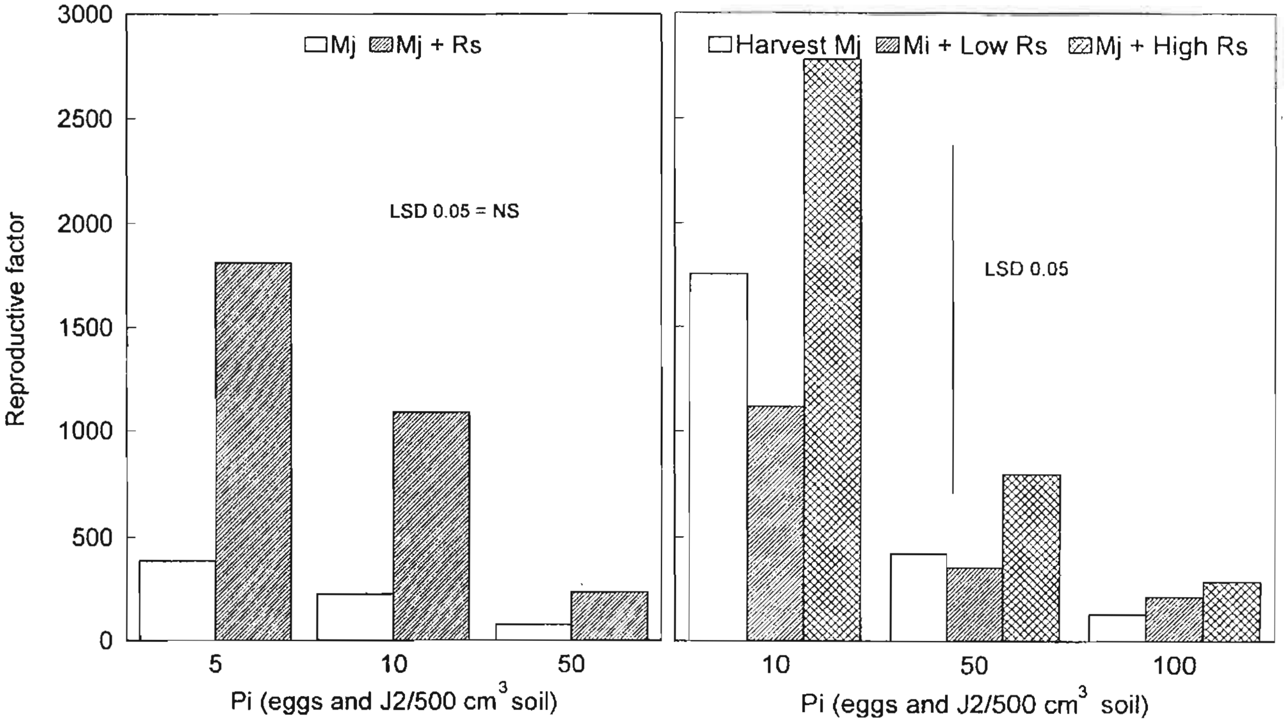

***Fig. 2.** Effect of root and pod rot caused by* Rhizoctonia solani *(Rs) on the reproductive factor (Rf) of* Meloidogyne javanica *on peanut in field microplots (Left: 1995. Right: 1996. Low Rs and High Rs = 0.01 g and 0.02 g of inoculum per 500 cm^3 soil, respectively).*

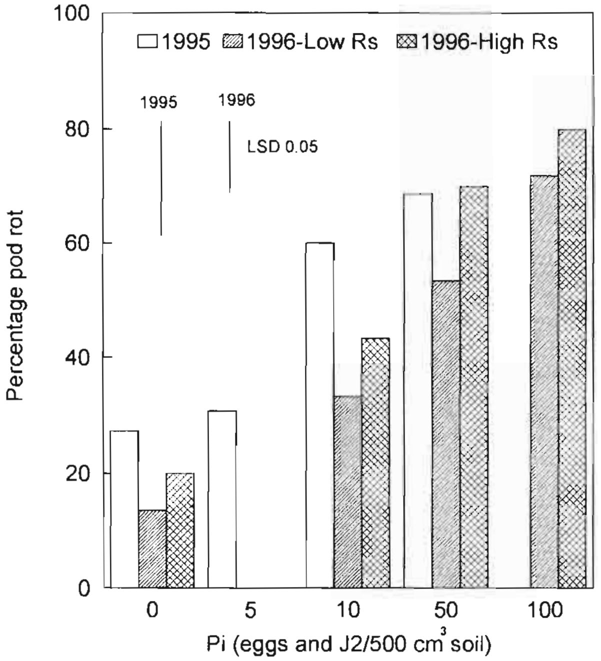

Fig. 3. *Effect of initial population densities* (Pi) *of* Meloidogyne javanica *on percentage pod rot of peanut by* Rhizoctonia solani *(Rs) in field microplots (Low Rs and High Rs = 0.01 g and 0.02 g of inoculum per 500 cm^3 soil, respectively).*

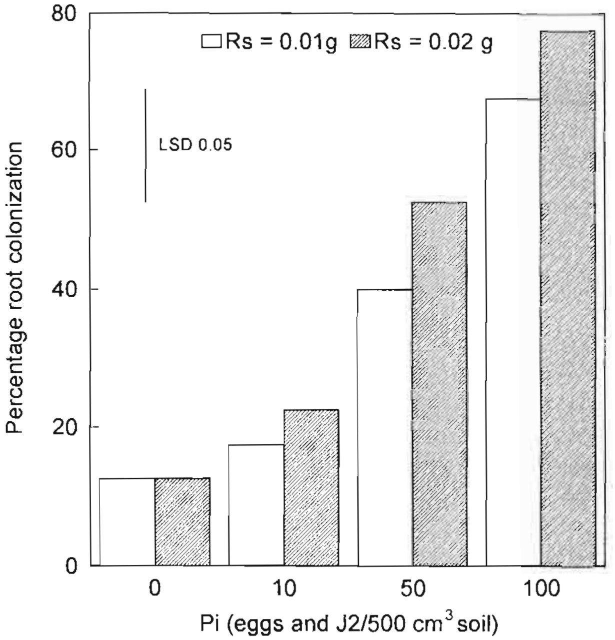

Fig. 4. *Effect of initial population densities* (Pi) *of* Meloidogyne javanica *on percentage colonization of peanut roots by* Rhizoctonia solani *(Rs) in field microplots in 1996.*

Colonization of peanut roots by *R. solani* also was positively related to *M. javanica Pi* (r^2 = 0.83; $P \leq 0.01$; Fig. 4), and visual examination of root segments indicated that the fungal growth was concentrated on and around root galls. The increase in *R. solani* inoculum from 0.01 to 0.02 g/500 cm^3 soil did not affect the percentage of root colonized by *R. solani* ($P \leq 0.05$).

Both *R. solani* and *M. javanica* suppressed yield of peanut ($P \leq 0.05$) in 1995 and 1996 (Fig. 5), and the interaction of the two pathogens was significant ($P \leq 0.05$) in both years. A negative relationship between *M. javanica Pi* and pod yield was observed in both years (r^2 = 0.88 in 1995 and r^2 = 0.86 in 1996; $P \leq 0.01$). In 1996, *R. solani* alone at 0.02 g/500 cm^3 soil caused greater ($P \leq 0.01$) yield suppression than that caused by 0.01 g/500 cm^3 soil.

Discussion

The pathogenicity of *R. solani* on peanut was confirmed by fulfilling Koch's postulates. This is consistent with previous reports of other isolates of *R. solani* AG-4 causing limb (Barnes *et al.*, 1989) and pod rot of peanut (Baird *et al.*, 1993). Parasitism of peanut by *M. javanica* (Tomaszewski *et al.*, 1994; Abdel-Momen & Starr, 1997), which is rare on peanuts in the United States, also was confirmed.

As reported with other crops (Batten & Powell, 1971; Golden & Van Gundy, 1975; Sankarialingman & McGawley, 1994; Walker, 1994), the combined effects of *M. javanica* and *R. solani* on yield of peanut were consistently greater than the effects of either pathogen alone. The synergistic nature of this association was most evident at the lower nematode *Pi* levels. At the highest population densities of *M. javanica* and *R. solani*, the damage by each pathogen alone was sufficiently large that no synergistic effect was observed. *Meloidogyne javanica* also increased the peanut pod rot and root colonization by *R. solani*. Colonies of *R. solani* were most frequently observed to develop from the gall tissue of peanut roots in the present study. This observation was consistent with the hypothesis of Golden and Van Gundy (1975) that *R. solani* is more attracted to the gall tissue compared with nongalled root tissues.

There was no consistent trend with regard to effects of *R. solani* on the reproduction of *M. javanica*. Overall, the *Pi* had a greater influence on final nematode population densities than did the presence or absence of *R. solani*. Premature senescence of peanut due to combined infection by *M. javanica* and *R. solani*, however, would be expected to limit total reproduction by *R. solani*, as was observed in the *Fusarium*

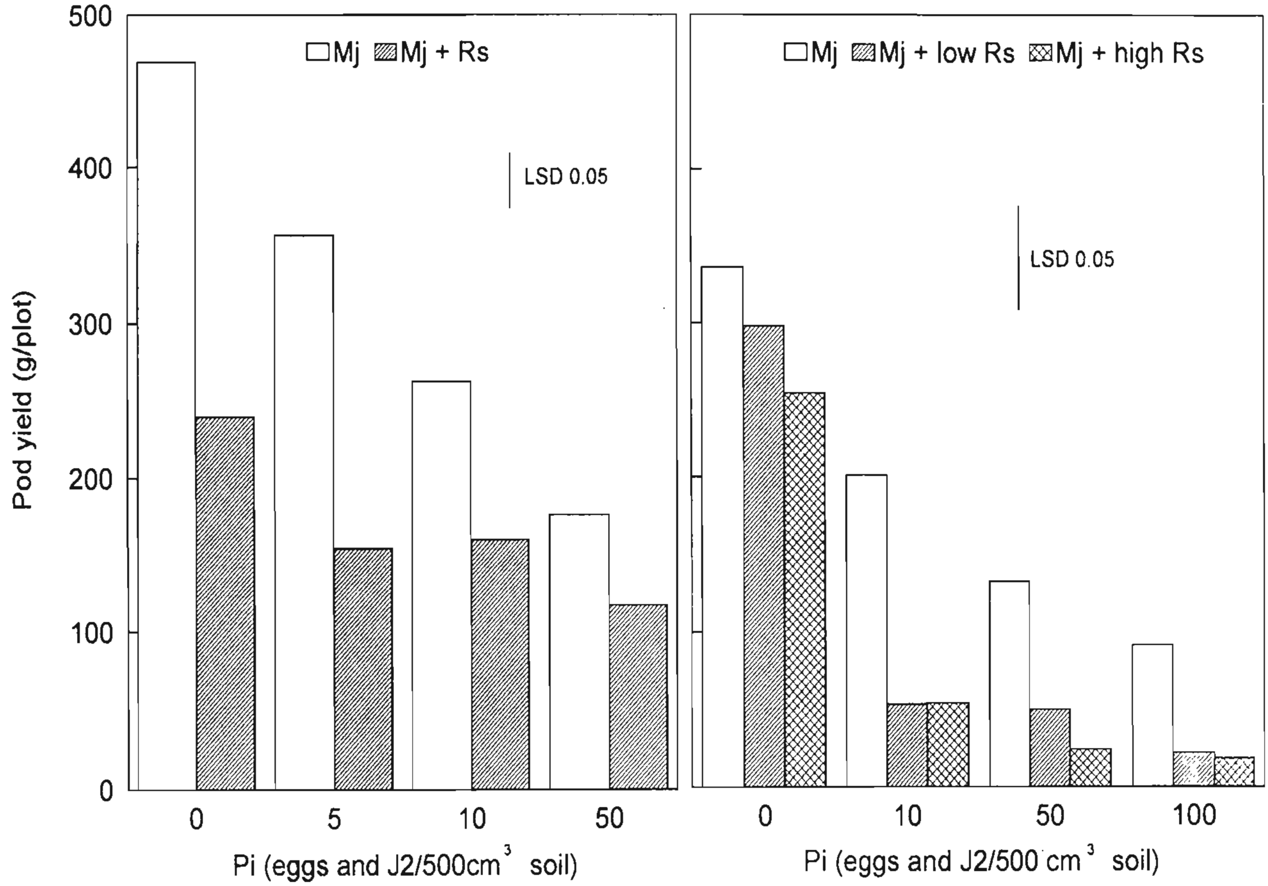

Fig. 5. *Combined effects of* Meloidogyne javanica *(Mj) and* Rhizoctonia solani *(Rs) on pod yield of peanut in field microplots (Left: 1995. Right: 1996. Low Rs and High Rs = 0.01 g and 0.02 g of inoculum per 500 cm^3 soil, respectively).*

wilt/root-knot nematode complex of cotton (Starr *et al.*, 1989).

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