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Effect of Uneven Distribution and Different Levels of Nematode Invasion on Symptom Development and Nematode Reproduction on Mung Bean

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ABSTRACT

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The uneven distribution of nematodes was created by inoculating 400 second-stage juveniles of *Meloidogyne incognita* in the aliquots of 1, 2, 4, and 8 ml of water to the root system of mung bean seedlings grown in culture pouches. The number of nematodes within a gall varied from 1 to 36 nematodes per gall. The highest density was 36, 24, 17, and 8 nematodes within a gall in the 1, 2, 4, and 8 ml treatments, respectively. The distribution of nematodes is conceivably uneven in a field situation. The uneven distribution of nematodes affected the number of nematodes within the galls and masked the severity of nematode invasion when the disease was assessed by the number of galls or galling index. Another experiment was conducted by inoculating 250, 500, 900, and 1800 nematodes in 4 ml of water to the mung bean seedlings grown in culture pouches. The numbers of galls and egg masses per root system increased as the inoculum level increased from 250 nematodes to 900 nematodes. Increasing the inoculum level to 1800 nematodes did not significantly increase the number of galls but decreased the number of egg masses significantly. The results showed that the optimum level of inoculum for maximum reproduction of nematodes was different from that for symptom development. In addition to the understanding of host-parasite relationship between *M. incognita* and mung bean, the results of this study may contribute to the improvement of the precision of researches involving disease severity and nematode reproduction by the awareness of the effect of uneven distribution and different levels of nematode invasion.

Keywords: *Meloidogyne incognita*, mung bean, uneven distribution, reproduction

Mung bean, *Vigna radiata* (L.) Wilczek, is an important pulse crop in many Asian countries. Mung bean sprouts are popular in Taiwan as vegetables. They are important traditional food in Korea [8]. The root-knot nematode *Meloidogyne incognita* (Kofoid & White) Chitwood is an important parasite of legumes [1, 19]. Contrary to the many researches on soybean [1, 7, 9, 11, 12, 14], very little information is available on mung bean in relation to *M. incognita*. Researches on host-parasite relationship provide basic understanding of diseases and may be helpful in formulating nematode disease management strategies. The number of nematodes invaded a plant is related to

the demand and supply of nutrients between the parasites and the host. The distribution of nematodes is conceivably uneven in a field situation. The soil around the roots of a host plant is bound to have more nematodes than the soil in the inter space. Even after tillage, the nematode population may remain unevenly distributed and will have differential effect on the individual plants in the next crop. The purpose of this study was to investigate the effect of uneven distribution and different levels of nematode invasion on symptom development and nematode reproduction on mung bean.

The root-knot nematode *M. incognita* was originally collected from the experimental station of the National Taiwan University and maintained on mung bean seedlings. Egg masses were hand picked and hatched. Second-stage juveniles were collected every day and kept at 15℃. They were used within three days. Mung bean seeds were surface sterilized and sown in zip-lock plastic bags with paper from grocery bags as supporting substrate [21]. They were kept at 28℃ in a growth chamber (Fig. 1A) for five days. Extra seedlings were grown so that seedlings of similar growth vigor could be selected for different treatments.

In the first experiment, the uneven distribution of nematodes was created by adding different volumes of water containing the same amount of nematodes to the culture pouches of the same size in which the mung bean seedlings were grown. The aliquots of 1, 2, 4, and 8 ml of nematode suspension containing 400 nematodes each were inoculated onto the roots of mung bean seedlings. The pouches were placed horizontally in a plastic tray and kept at 28℃ in a growth chamber for one day [21]. Then the pouches were hung up in the 28℃ growth chamber for 14 days. The roots of the seedlings were then stained with acid-fuchsin [2]. The number of galls and the number of nematodes penetrated the roots within each gall were counted under a dissecting microscope. There were four replicates for each treatment. The experiment was repeated twice.

Another experiment was conducted by inoculating 250, 500, 900, and 1800 nematodes in 4 ml of nematode suspension to the seedlings. The culture pouches were placed horizontally in a plastic tray and kept at 28℃ in a growth chamber for one day after inoculation. Then the pouches were hung up in the 28℃ growth chamber [21]. At 2, 7, and 28 DAI (days after inoculation), the numbers of galls on each root system were counted directly under a dissecting microscope without pulling the seedlings off the culture pouches because the zip-lock plastic bags were transparent (Fig. 1B). The numbers of necrotic galls were counted at 28 DAI. At 31 DAI, the number of egg masses was counted under a dissecting microscope. The plastic bags were cut open to facilitate the counting of egg masses on the underside of some galls. There were four replicates for each treatment. The experiment was repeated twice.

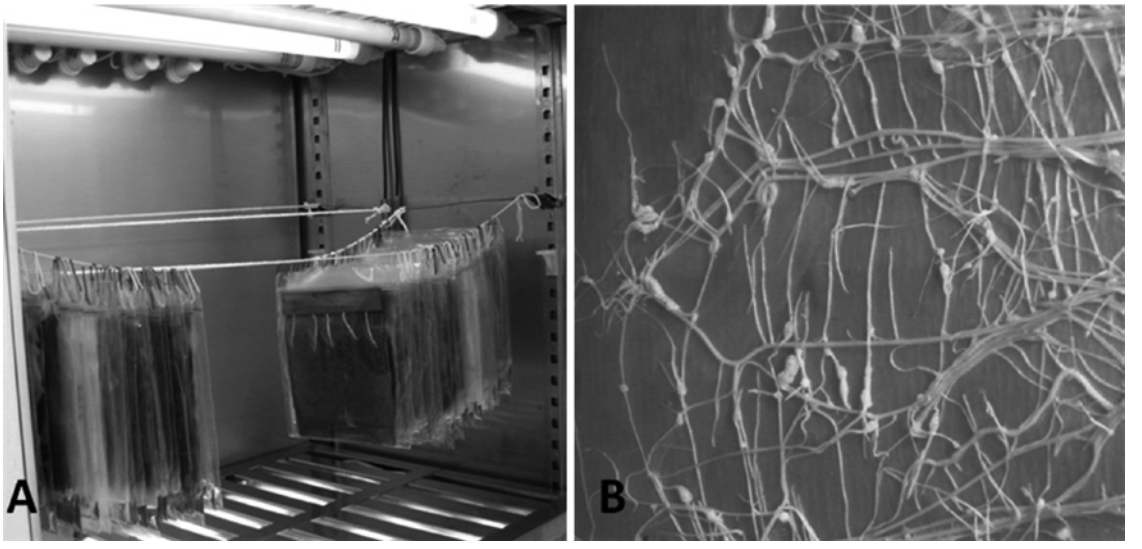

Fig. 1. Culture pouches. A. Germinating mung bean seeds in culture pouches made of zip-lock plastic bags with paper from grocery bags as supporting substrate in a growth chamber. B. Transparent zip-lock plastic bag allowing direct observation of galls on the root system under a dissecting microscope without pulling the seedling off the culture pouch.

In the repeated experiment, the data were combined with the first one because same trend was observed in the two experiments.

In the first experiment, the number of nematodes within a gall varied from 1 to 36 nematodes per gall (Table 1). The clustering of nematodes was most severe when the inoculum was in 1 ml suspension. There were 2.3 galls with 21-36 nematodes per gall in the 1 ml treatment while none in the 4 and 8 ml treatments. The highest density was 36, 24, 17, and 8 nematodes within a gall in the1, 2, 4, and 8 ml treatments, respectively. The volume of the inoculum affected the even distribution of the nematodes in each treatment.

Uneven distribution of nematodes is expected in a field situation because there will be more nematodes at the site of each host plant than areas away from the plant. The uneven distribution of nematodes could result in different densities of nematodes within galls as demonstrated in the present research. While there was better distribution of nematodes at inoculation in the 8 ml treatment as indicated by the lack of galls with more than 10 nematodes per gall, the total number of galls was significantly lower than the other treatments. Fewer galls and fewer nematodes per gall indicated that the nematode invasion was less successful. The poorer success of invasion in the 8 ml treatment could be the result of poorer backlash for nematode penetration to the roots because of too much water. Tsai and Van Gundy[22] observed that the head of the nematode *Pratylenchus scribneri* turned perpendicular to the body when feeding on plant tissue. This posture indicated that penetration of the nematode head into the plant tissue required a physical force generated in part by backlash. This is very likely also true for *M. incognita*. The excess water in the 8 ml treatment conceivably reduced the backlash and reduced the rate of successful penetration of the nematodes to the roots.

Galling index has been used by many researchers to evaluate the disease severity caused by the root-knot nematodes [3, 5, 13, 15, 23]. While it is convenient to use, it has its drawback. The results of this research showed that the number of nematodes within the galls could vary as much as 36 folds. Therefore, galling index or the number of galls can not truly reflect the severity of the nematode infection.

In the second experiment, the numbers of galls and egg masses per root system increased as the inoculum level increased from 250 nematodes to 900 nematodes (Table 2). Increasing the inoculum level to 1800 nematodes did not significantly increase the number of galls but decreased the number of egg masses significantly. The results showed that the optimum level of inoculum for maximum reproduction of nematodes was different from that for symptom development. The inoculation of 900 and 1800 nematodes per mung bean seedling produced the highest number of galls while the inoculation of 500 nematodes per mung bean seedling produced the highest number of usable egg masses. Although the number of egg masses in the 900 nematodes treatment was not significantly different from that in the 500 nematodes treatment, the egg masses of the former were contaminated by fungus from necrotic galls.

The number of galls per root system increased from 2 DAI to 28 DAI at all the inoculum levels, indicating that the nematodes continued to invade the roots for a long period of time after inoculation.

There were 54.6% and 84.1% necrotic galls at 28 DAI at the inoculum levels of 900 nematodes and 1800 nematodes, respectively. No necrotic gall was found at the lower inoculum levels (Table 2). The results indicated that when the load of nematodes at a particular section of the root exceeded certain level it caused damage to the root tissue and the gall became necrotic. No egg mass was found on the necrotic galls. It is very likely that a balance must be maintained between the demand of the nematodes and the supply of nutrients by the host plant in order to achieve the highest level of nematode reproduction. The number of egg masses produced per root system with the inoculation of 1800 nematodes was only 41.4 % of that produced with the inoculation of 900 nematodes. These findings are in conformity with those reported by Khan, *et al.* [10]. They found that the reproduction rate of *M. javanica* on balsam was the highest when inoculated with 250 nematodes and the lowest when inoculated with 8000 nematodes.

The egg masses were smaller than usual on the galls with over-crowded nematodes within the galls in this study. It is possible that the nutrition for each nematode provided by that section of host root was insufficient for the production of normal amount of eggs due to the competition of nutrients between the nematodes within a gall of higher nematode density.

In conclusion, the uneven distribution of nematodes affected the number of nematodes within the galls and masked the severity of nematode invasion when the disease

Table1. Effect of volume of *M. incognita* inoculum[1] on the density of nematodes within the galls on mung bean roots[2]

Inoculum volume (ml)	Number of galls[3]					Total number of galls[3]
	With 1-2 nematodes per gall	With 3-5 nematodes per gall	With 6-10 nematodes per gall	With 11-20 nematodes per gall	With 21-36 nematodes per gall	
1	67.5 b	12.0 a	5.0 a	2.0 a[4]	2.3 a[4]	88.8 a
2	81.3 a	5.8 b	1.8 c	1.0 a[5]	0.5 b[5]	90.3 a
4	79.5 a	6.8 b	4.0 ab	1.8 a[5]	0.0 b	92.0 a
8	58.0 c	7.3 b	3.8 b	0.0 b	0.0 b	69.0 b

[1] There were 400 nematodes in each suspension.
[2] Paper from grocery bags (pre-soaked in water for one day) were used as supporting substrates for mung bean seedlings in culture pouches.
[3] Means within each column with different letters are significantly different at $p \leqq 0.05$ according to Duncan's multiple range test.
[4] Round galls.
[5] Elongate galls.

Table2. Effect of nematode inoculum level on gall formation and production of egg masses of *M. incognita* on mung bean roots[1]

Inoculum level (No. nematodes)	Number of galls per root system[2]			% Necrotic galls at 28 DAI[2]	Number of egg masses[2]
	2 DAI[3]	7 DAI	28 DAI		
250	1.8 c	29.5 b	54.5 c	0.0 c	60.0 b
500	9.8 b	38.0 b	98.8 b	0.0 c	130.0 a
900	19.5 a	83.0 a	113.0 ab[4]	54.6 b	133.5 a[5]
1800	23.0 a	95.3 a	135.5 a[4]	84.1 a	55.3 b[5]

[1]Paper from grocery bags (pre-soaked in water for one day) were used as supporting substrates in culture pouches.
[2]Means within each column with different letters are significantly different at $p \leqq 0.05$ according to Duncan's multiple range test.
[3]DAI = Days after inoculation.
[4] Including necrotic galls.
[5] Unusable egg masses due to fungal contamination from necrotic galls.

was assessed by the number of galls or galling index. The uneven distribution of nematodes also affected the reproduction of the nematodes as indicated by the smaller egg masses. The excess number of nematodes invading the root system caused death of the galled tissues which in turn caused death of the nematodes and the reduction of the total nematode reproduction in a root system. Many researches involve nematode reproduction [4, 6, 16, 17, 18, 20]. In addition to the understanding of the host-parasite relationship between *M. incognita* and mung bean, the results of this study may contribute to the improvement of the precision of researches involving nematode reproduction by the awareness of the effect of uneven distribution and different levels of nematode invasion on nematode reproduction.

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摘　要

蔡碧雲 [1, 2]. 2010. 線蟲不均勻散佈及不同程度之侵入對綠豆苗根部病徵發展及線蟲繁殖之影響. 植病會刊 19: 243-248. ([1] 臺北市 國立臺灣大學植物病理與微生物學系；[2] 聯絡作者，電子郵件：bieyntm@ntu.edu.tw；傳眞：+886-2-2363-6490)

將 400 隻 *Meloidogyne incognita* 二齡幼蟲懸浮於 1, 2, 4 及 8 ml 之水中，再接種於培養袋中之綠豆幼苗根系上，藉以製造線蟲散佈不均勻之狀況。一個根瘤內可出現一到三十六隻線蟲。在 1, 2, 4 及 8 ml 之處理發現之最高線蟲密度分別爲一個根瘤內有 36, 24, 17 及 8 隻線蟲。田間線蟲之散佈不均勻可想而知。本試驗結果顯示線蟲之不均勻散佈影響根瘤內線蟲之數目，因此用根瘤數或根瘤指數來評估根瘤線蟲病害無法顯示眞正之嚴重性。另一個實驗將 250, 500, 900 及 1800 隻二齡幼蟲分別懸浮於 4 ml 之水中，再接種於培養袋中之綠豆幼苗根系上。當接種原由 250 隻線蟲增加到 900 隻時，每個根系之根瘤數及卵塊數隨之增加。增加接種原至 1800隻線蟲時，根瘤數未顯著增加而卵塊數則顯著減少。本試驗結果顯示最佳繁殖效果所需之接種原濃度與最佳病徵發展所需之接種原濃度不同。除了了解 *M. incognita* 與寄主綠豆之間的關係外，本試驗結果亦可對於與病害嚴重性及線蟲繁殖有關之研究工作之精確度有所貢獻。

關鍵詞：*Meloidogyne incognita*、綠豆、不均勻散佈、繁殖