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Host Status of Lisianthus 'Mariachi Lime Green' for Three Species of Root-knot Nematodes

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Abstract. **Lisianthus [*Eustoma grandiflorum* (Raf.) Shinn.] plants were grown in soil infested with increasing densities of *Meloidogyne hapla* Chitwood, *M. incognita* (Kofoid & White) Chitwood, or *M. javanica* (Treub) Chitwood, root-knot nematodes. Compared to tomato plants grown in soil with the same nematode numbers and species, lisianthus had less severe root symptoms, suffered less damage, and resulted in lower nematode multiplication rates. Lisianthus was a better host for *M. javanica* than for *M. incognita*, and a poor host for *M. hapla*. Lisianthus shoot weights were significantly reduced after inoculation with *M. javanica* or *M. hapla*, but not after *M. incognita* inoculation. The number of flowers produced per lisianthus plant was reduced by all three nematode species. The results show that the root-knot nematode species that are most common in California may cause significant damage in the cut-flower production of lisianthus.**

Lisianthus (*Eustoma grandiflorum,* Gentianaceae) was introduced as a cut flower into the United States from Japan in the early 1980s. The plant is native to the moist prairies of the mid- and southwestern states and is also known as Texas Blue Bell and Prairie Gentian (Halevy and Kofranek, 1984). Breeding, done mainly in Japan, has yielded a wide range of new varieties with excellent flower colors (white, pink, and blue), double or single flowers and long vase life (Cho et al., 2001). This, combined with strong consumer demand, lower prices for traditional cut flowers, and a favorable price, has resulted in a rapid increase in the production of this flower (Univ. of California, 1999). California sales in 2001 were $9.4 million, representing an increase of ≈50% compared to sales in 2000 (U.S. Dept. of Agriculture, 2002).

Due to the small seed size and slow germination and initial seedling growth, propagation is usually done by specialized companies, who then sell 3-month-old transplants for greenhouse cut flower production (Armitage et al., 2003). The time from transplanting to flowering depends on the variety, temperature, and daylength, and usually ranges between 10 and 13 weeks (Gill et al., 2000).

Lisianthus is susceptible to a wide range of fungal and viral diseases, including *Pythium* sp. , *Rhizoctonia solani*, *Fusarium solani*, *F. avenacaeum*, impatiens necrotic spot virus, cucumber mosaic virus, and bean yellow mosaic virus (Daughtrey, 2000; Dreistadt, 2001; Gill et al., 2000). In addition, insects such as aphids, whiteflies, and thrips can cause major pest problems (Dreistadt, 2001; Gill et al., 2000).

The cultivation of lisianthus, grown in greenhouses on well-draining sandy soils, at relatively warm temperatures, with a relatively long crop cycle, would favor the establishment and rapid development of some major nematode pests, such as certain species of root-knot and lesion nematodes. In spite of this, information on the susceptibility of lisianthus to nematodes is not available. Recently however, we were made aware of stunted growth of lisianthus 'Mariachi Lime Green' in a commercial greenhouse in southern California. Roots of stunted plants exhibited severe root-galling resembling root-knot nematode damage. Numerous second-stage root-knot nematode juveniles, later identified as *Meloidogyne javanica*, were extracted from these roots, as well as from soil collected around these roots. This root-knot nematode species is common in warm sandy soils in southern and central California (Siddiqui et al., 1973), and an economically important pest of numerous vegetable and fruit crops (McKenry and Roberts, 1985). Other important root-knot nematode species in California include *M. incognita* and *M. hapla* (McKenry and Roberts, 1985). The latter species is generally more predominant in the cooler climate of northern California (Siddiqui et al., 1973). The objective of this study was to determine the susceptibility and host status of lisianthus for these three commonly occurring species of root-knot nematodes.

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Materials and Methods

Nematode inocula. Populations of three *Meloidogyne* sp. were used: *M. incognita* race 3 from cotton (San Joaquin Valley, Calif.), *M. javanica* from cowpea (Chino, Calif.), and *M. hapla* from alfalfa (San Bernardino, Calif.). Species and races were identified with isoenzyme electrophoresis and on differential host tests (Eisenback and Triantaphyllou, 1991). Populations of *M. incognita* and *M. javanica* were increased and maintained on tomato 'UC82', and *M. hapla* on tomato 'Hypeel 45' grown in steam-sterilized sandy soil (93% sand, 4% silt, 3% clay) in a greenhouse (25 to 32 °C, natural light). Inoculum was obtained from nematode-infested tomato roots ≈10 weeks after inoculation by washing the root system free of sand and shaking each root system in a 3-L container for 2 min in 400 mL, 1% NaOCl using a commercial paint shaker (Radewald et al., 2003). Eggs released from the roots were collected on a 25-μm pore-size sieve and washed into a container. The eggs in the resulting suspensions were counted at 40× magnification and the suspensions were diluted with tap water to contain 100, 1000, or 10,000 eggs per 15 mL. For inoculation, 15 mL of nematode egg suspension were thoroughly mixed into 3.5 L steam-sterilized coarse sand. This was then used to fill a 3.5-L plastic pot. Twelve pots were prepared for each combination of the three inoculum densities and the three *Meloidogyne* species (12 × 3 × 3 = 108 pots). In addition, 12 pots were prepared without nematodes (no nematode control). The complete experiment was done twice, in Spring 2001 (Expt. 1), and Fall 2002 (Expt. 2).

Plants. Three-month-old transplants of lisianthus 'Mariachi Lime Green' (Sakata Seed America Inc., Morgan Hill, Calif.) were planted into sixty 3.5-L plastic pots with each combination of inoculum density and *Meloidogyne* species replicated six times. On the same day, one 5-week-old seedling of tomato 'UC82' was transplanted into each of the 60 remaining pots. Pots with lisianthus and tomato were placed on two separate but adjacent benches in a greenhouse and then completely randomized over the bench. One week after transplanting, all pots were fertilized with 10 g of a slow-release fertilizer (17N–6P–10K). Plants were watered through an automated drip system and grown for 13 weeks.

Collection and analysis of data. At harvest, all plants were carefully removed from the pots. Fresh weights of tops and roots were determined. The tomato and lisianthus roots were indexed for galling on a scale from 0 to 10 (0 = no galls; 10 = 100% galled; Bridge and Page, 1980). For each lisianthus plant, the number of flowers that had fully opened at time of harvest was counted. Eggs were extracted from each of the root systems as described previously for inoculum preparation and counted at 40× magnification.

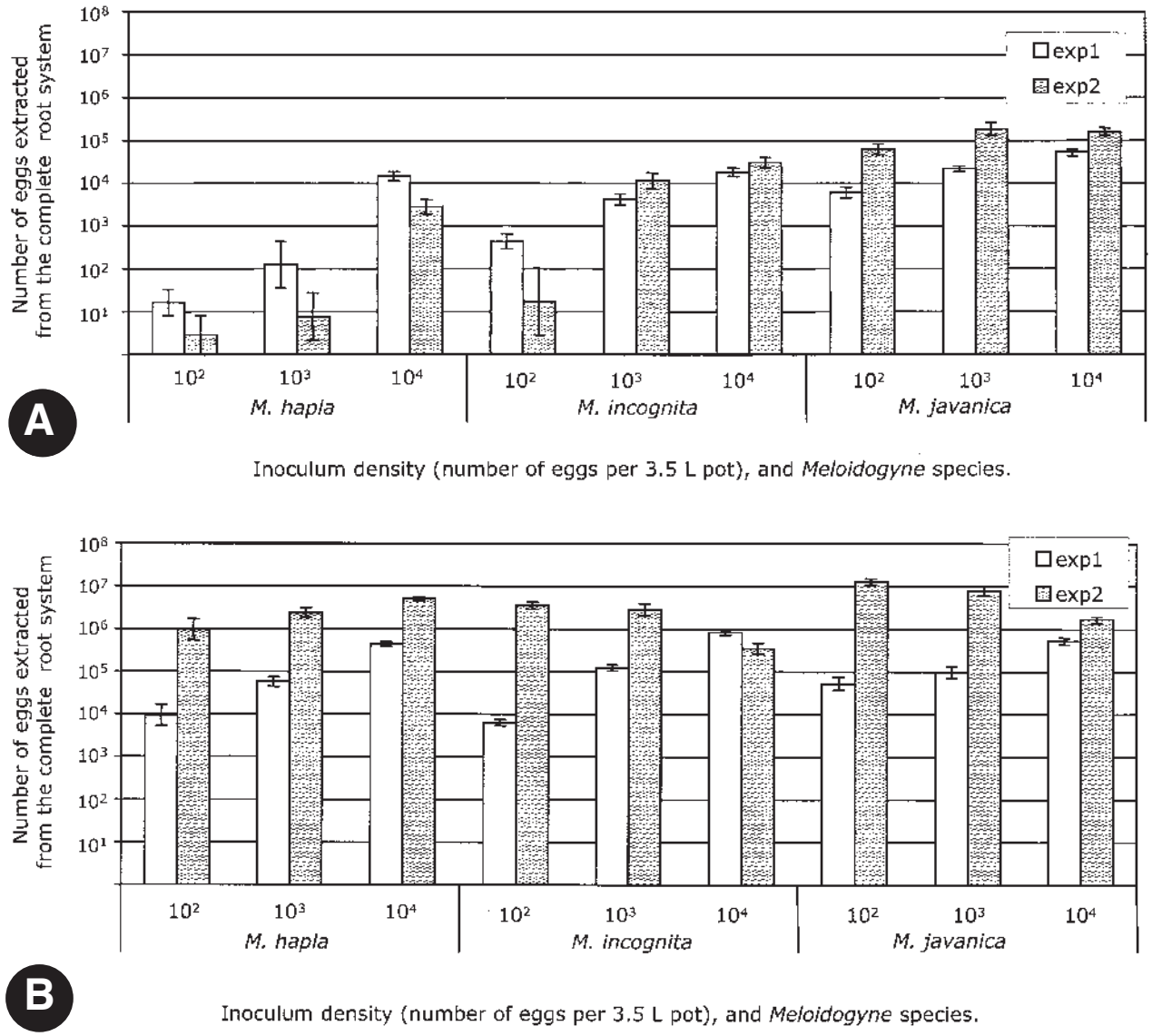

Fig. 1. Effect of inoculum density of three *Meloidogyne* species on the numbers of eggs recovered from roots of (**A**) lisianthus or (**B**) tomato in two replicated experiments. Error bars represent 2× standard error.

Effects of *Meloidogyne* species and inoculum density on shoot and root fresh weights, lisianthus flower numbers, root galling, and egg production [log (n + 1) transformed] were analyzed in an ANOVA procedure, and means were separated with Duncan's range test ($P = 0.05$) using SAS statistical software (SAS Institute, Cary, N.C.).

Results

Nematode reproduction and symptoms. With one exception (*M. hapla* on lisianthus), the numbers of extracted nematode eggs were much greater in Expt. 2 than in Expt. 1 (Fig. 1 A and B). With the same *Meloidogyne* species, tomato roots always yielded more eggs than lisianthus roots (Fig. 1 A and B). However, *M. javanica* produced about equal (Expt. 2) or higher (Expt. 1) numbers of eggs per gram of root on lisianthus compared to tomato (data not shown). In Expt. 1, there was a positive correlation between the inoculum density and final egg density on both plants. In Expt. 2, however, tomato plants that were inoculated with lower densities of *M. incognita* or *M. javanica* yielded the highest final egg populations (Fig. 1B). Averaged over the two experiments, *M. javanica* had the highest multiplication rate, on both tomato and lisianthus. On the latter plant, *M. incognita* had a higher reproduction than *M. hapla*. On tomato, the reproduction was not significantly different between *M. incognita* and *M. hapla*. Root galling was more severe on tomato (average galling index of 5.3 on inoculated plants) than on lisianthus (average galling index of 2.3). The average galling index on tomato was higher after inoculation with *M. hapla* (5.7) or *M. javanica* (5.5) than after inoculation with *M. incognita* (4.8). Galling on lisianthus roots inoculated with *M. javanica* was higher (4.3) than on roots inoculated with *M. incognita* (2.9) or *M. hapla* (2.4).

Plant damage. The effects of increasing *M. hapla* inoculum densities on shoot growth were similar for tomato and lisianthus (Fig. 2 A and B). Significant reductions in fresh shoot weights occurred at inocula of 1000 (Expt. 1) or 10,000 (Expt. 2) *M. hapla* eggs per pot. In contrast to tomato, which suffered significant damage even after inoculation with only 100 *M. incognita* eggs, shoot growth of lisianthus was not significantly affected at any of the *M. incognita* inoculum densities (Fig. 2A). *Meloidogyne javanica* started to reduce top growth of both tomato and lisianthus at inoculum densities of 100 or 1000 eggs per pot (Fig. 2 A and B). Overall, *M. incognita* reduced the top weight of tomato by 59%, and was more damaging than *M. javanica* (29%) or *M. hapla* (18%). For lisianthus, however, the situation was reversed as *M. javanica* and *M. hapla* were more damaging than *M. incognita*, reducing top weights by 33%, 22% and 7%, respectively.

The number of flowers produced by lisianthus plants was reduced the most by *M. javanica* at the highest inoculum density of 10,000 eggs per pot (Table 1). *Meloidogyne*

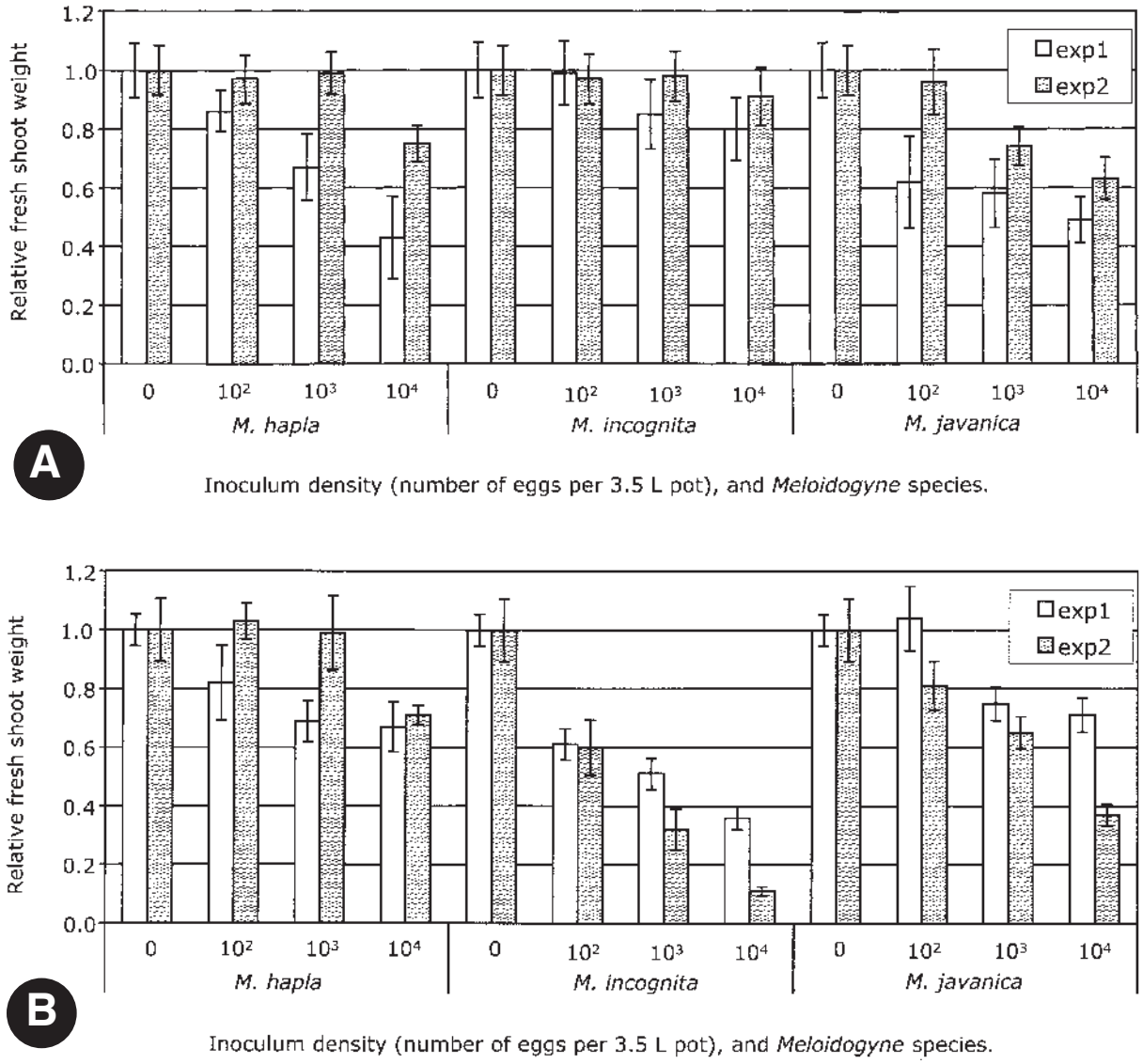

Fig. 2. Effect of inoculum density of three *Meloidogyne* species on the fresh shoot weight of (**A**) lisianthus or (**B**) tomato relative to non-inoculated controls in two replicated experiments. Error bars represent 2× standard error.

hapla also reduced the number of flowers, but surprisingly, the reduction was greatest at intermediate inoculum densities. The least damaging species based on the number of flowers produced was *M. incognita*, resulting in significantly fewer flowers only in one treatment (Expt. 2, inoculum 10,000).

Discussion

This is the first detailed study on the host status and susceptibility of lisianthus for the economically important root-knot nematodes *M. hapla*, *M. incognita*, and *M. javanica*. In absolute terms, tomato was a better host to all three nematode species than lisianthus. However, in relative terms (eggs per gram root), lisianthus was as good a host for *M. javanica* as tomato. Lisianthus was a poor host for *M. hapla*, with an overall average Pf/Pi (final population/initial population) value of 0.7, but it was a good host for *M. incognita* (average Pf/Pi: 8.4) and an even better host for *M. javanica* (average Pf/Pi: 187.8). That egg production on both tomato and lisianthus was generally higher in Expt. 2 than in Expt. 1 is probably because the average soil temperatures were slightly lower in the first experiment (24.6 °C in Expt. 1 vs. 26.3 °C in Expt. 2). A very high nematode population increase occurred on tomato in Expt. 2, particularly on plants inoculated with only 100 *M. javanica* eggs (average Pf/Pi: 136,944). The inverse relationship between inoculum density and final populations of *M. incognita* and *M. javanica* on tomato in Expt. 2 indicates a rapid multiplication of nematodes on tomato in Expt. 2, resulting in substantial root damage, loss of available feeding sites, and a lower multiplication rate (Seinhorst, 1967).

The effects of the nematodes on fresh shoot weights were most dramatic in tomato with *M. incognita*, and in lisianthus with *M. javanica*. The average effects on lisianthus flower production were similar for all three nematode species: compared to non-inoculated pots, the percentages of flowers produced in

Table 1. Effect of *Meloidogyne* on the average number of open flowers produced per lisianthus plant (N = 6).

	Inoculum			
Meloidogyne sp.	0	100	1000	10,000
M. hapla				
Expt. 1	2.7 a[z]	1.7 ab	1.2 b	2.5 a
Expt. 2	3.0 a	1.0 bc	0.5 c	2.0 ab
M. incognita				
Expt. 1	2.7 a	1.7 a	1.3 a	1.3 a
Expt. 2	3.0 a	2.7 ab	1.7 ab	1.2 b
M. javanica				
Expt. 1	2.7 a	1.8 ab	2.0 a	0.7 b
Expt. 2	3.0 a	2.5 a	2.3 a	0.5 b

[z]Different letters represent significant differences (at $P = 0.05$) within a row.

nematode inoculated pots were 53%, 58%, and 58% for *M. hapla*, *M. incognita*, and *M. javanica,* respectively. Therefore, the differences in the host status of lisianthus for the three *Meloidogyne* species did not correspond with the damage caused by these nematodes. For example, there was a substantial reduction in fresh shoot weight and in the number of flowers in lisianthus that had been inoculated with *M. hapla*, although lisianthus was a poor host for this nematode. Similar results where plants suffer considerable damage, but are relatively poor hosts, have been obtained with several other plant–nematode associations, e.g., carrots and *Longidorus africanus* (Kolodge, 1980), onion and *M. hapla*, and narcissus and *Pratylenchus penetrans* (Stemerding and Kuiper, 1968).

In both experiments with lisianthus and *M. hapla*, the fresh shoot weights decreased as nematode numbers increased, but this was not reflected in the flower production. Significantly fewer flowers were produced at the inoculum density of 1000 eggs, than at the highest density of 10,000 eggs. Given sufficient time, lower shoot weights would generally result in fewer flowers (Halevy, 1985).

In conclusion, our results show that although there are significant differences in the host status of lisianthus for the three *Meloidogyne* species tested, the presence of any of these three root-knot nematode species in soil where lisianthus is to be grown can lead to a substantial yield reduction. Furthermore, the damage observed in our experiments, where plants were grown in steam-sterilized sand, may underestimate the potential damage under field conditions. Lisianthus plants are highly susceptible to soil-borne fungi, such as *Pythium, Fusarium*, and *Rhizoctonia* sp., and in numerous studies the synergistic effect between *Meloidogyne* and such fungal pathogens on plant damage has been demonstrated (Bergeson, 1972; Taylor, 1990). In California, lisianthus for cut-flower production is generally grown in greenhouses on well-draining soils. In combination with the presence of a host, these conditions would favor a rapid build-up of all three *Meloidogyne* sp. included in this study. Preventing the introduction of nematodes with planting stock, irrigation water, or tools and machinery is probably the most important nematode management strategy, because none of the "traditional" chemical fumigant and non-fumigant nematicides are currently registered for greenhouse-grown cut flowers in California. Nematode analysis of soil samples is a useful means to detect and monitor root-knot nematodes. Few options are available to control existing *Meloidogyne* infestations, but soil-steaming has been shown to provide good levels of preplant control (Lamberti, 1979). Bio-rational products, such as DiTera®, and Neem-based products may provide a future option for nematode management, but their efficacy and allowed use in an ornamental greenhouse situation remains to be evaluated. There is no information on the availability of sources of resistance in lisianthus.

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