

## MECHANISM OF RESISTANCE TO *MELOIDOGYNE INCOGNITA* IN RESISTANT COTTON GENOTYPES

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### ABSTRACT

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*Meloidogyne incognita* is an economically important plant-parasitic nematode on cotton (*Gossypium hirsutum*) across the U.S. Cotton Belt. Few studies have characterized the mechanism of resistance in cotton based on the biology of *M. incognita*. Penetration, post-penetration development, and reproduction of *M. incognita* were determined on eight cotton genotypes. Resistant genotypes included Clewewilt, Wild Mexican Jack Jones (WMJJ), five primitive accessions; TX-1174, TX-1440, TX-2076, TX-2079, and TX-2107 and one susceptible control, cv. Deltapine (DP) 90. Root penetration by *M. incognita* on developing radicles was similar among resistant genotypes and susceptible control. The mechanism of resistance for all resistant genotypes was based on delayed ( $P \leq 0.05$ ) maturity of *M. incognita*. This delayed maturity contributed to lower eggs per gram of root, egg masses per root system, and Pf/Pi values; however, eggs per egg mass were similar among all genotypes. Thus, resistance to *M. incognita* had a greater influence on nematode development than penetration or fecundity. The rate of delayed nematode development varied among resistant genotypes, thus suggesting diversity in resistant genes among these cotton genotypes. Further, these data confirm resistance in the primitive accessions from Mexico and suggest that they may be useful new sources of resistance to *M. incognita*.

*Key words:* Cotton, *Gossypium hirsutum*, *Meloidogyne incognita*, penetration, post-penetration development, resistance, root-knot nematode.

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### RESUMEN

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*Meloidogyne incognita* es un nematodo de importancia económica en algodón (*Gossypium hirsutum*) en todo el cinturón algodonero de los Estados Unidos. Pocos estudios han caracterizado los mecanismos de resistencia en algodón con base en la biología de *M. incognita*. Se determinó la penetración, desarrollo post-penetración y reproducción de *M. incognita* en ocho genotipos de algodón. Los genotipos resistentes incluyeron Clewewilt, Wild Mexican Jack Jones (WMJJ), y cinco accesiones primitivas (TX-1174, TX-1440, TX-2076, TX-2079, y TX-2107). También se incluyó el cultivar Deltapine (DP) 90 como control susceptible. La penetración de *M. incognita* en radículas en desarrollo fue similar entre los genotipos resistentes y el control susceptible. El mecanismo de resistencia para todos los genotipos resistentes estuvo basado en un retraso en la madurez de *M. incognita* ( $P \leq 0.05$ ). Este retraso en la madurez contribuyó a reducir el número de huevos por gramos de raíz, masas de huevos por sistema radical y los valores Pf/Pi. Sin embargo, el número de huevos por masa fue similar entre todos los genotipos. Por tanto, la resistencia a *M. incognita* tuvo una mayor influencia en el desarrollo del nematodo que en la penetración o fecundidad. La tasa de reducción en el desarrollo del nematodo varió entre los genotipos resistentes, sugiriendo diversidad en los genes de resistencia entre estos genotipos de algodón. Adicionalmente, estos datos confirman la resistencia de las accesiones primitivas de

México y sugieren que estas accesiones pueden ser útiles como nuevas fuentes de resistencia a *M. incognita*.

*Palabras clave:* algodón, *Gossypium hirsutum*, *Meloidogyne incognita*, penetración, desarrollo post-penetración, resistencia, nematodo agallador.

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## INTRODUCTION

The root-knot nematode, *Meloidogyne incognita*, is the most widely distributed and economically important plant-parasitic nematode affecting U.S. cotton (*Gossypium hirsutum*) production (Koenning *et al.*, 2004; Thomas and Kirkpatrick, 2001). In 2004, root-knot nematodes accounted for yield losses of 547,728 bales or, approximately 2.2% of total U.S. cotton production (Blasingame, 2005).

Genetic resistance is an environmentally safe and economical method to manage root-knot nematodes on cotton. Though there is limited availability of resistance to *M. incognita* in cotton cultivars with high yield potential, valuable sources of resistance are available among *Gossypium* spp. germplasm lines (Robinson *et al.*, 2001). Most studies of genetic resistance to *M. incognita* in cotton have focused on relatively few sources of resistance, mainly the Auburn and M-series sources of resistance (Shepherd *et al.*, 1996; Shepherd, 1974), Clevevilt 6, and a few Acala-type cottons (Oakley, 1995; Ogallo *et al.*, 1997). Numerous other sources of resistance to *M. incognita* in the cotton germplasm collection have been reported (Robinson and Percival, 1997; Shepherd, 1983) but have not been examined to further characterize that resistance.

Most studies of resistance in cotton have focused on inheritance (Bezawada *et al.*, 2003; McPherson *et al.*, 2004; Ynturi *et al.*, 2006; Zhang *et al.*, 2007; Zhou *et al.*, 1999), whereas a few studies have investigated resistance related to the biology of

*M. incognita* (Jenkins *et al.*, 1995; McClure *et al.*, 1974; Minton, 1962). Resistance to *M. incognita* was related to post-penetration factors within the root that delayed nematode development on Clevevilt 6 and Auburn 623 RNR sources of resistance (Jenkins *et al.*, 1995; McClure *et al.*, 1974; Minton, 1962). Auburn 623 RNR was derived from a transgressive segregation from a cross between Clevevilt 6 and Wild Mexican Jack Jones (Shepherd, 1974). Thus, previously evaluated *M. incognita* biology on cotton was on a few closely related sources of resistance. Characterization of *M. incognita* biology on other resistant genotypes would further our understanding of nematode resistance in cotton.

The cotton genotypes chosen for this study were five primitive accession originally collected from the Yucatan region of Mexico (Robinson and Percival, 1997). However, four of the five putatively resistant accessions supported lower reproduction of *M. incognita* only in one of the two tests conducted by Robinson and Percival (1997). Therefore, the objectives of this study were to confirm the resistance of each of these five cotton accessions and to characterize the mechanism of resistance in these putatively resistant cotton genotypes.

## MATERIALS AND METHODS

*Meloidogyne incognita* was isolated from cotton (*G. hirsutum*) and maintained in the greenhouse on *Solanum lycopersicum* L. cv. 'Rutgers'. Second-stage juveniles (J2) were

collected in a hatching chamber (Vrain, 1977) and only 24-hr-old J2 were used in this study.

The cotton genotypes selected for study included five primitive accessions from Mexico; TX-1174, TX-1440, TX-2076, TX-2079, and TX-2107. Cotton genotypes, Wild Mexican Jack Jones (WMJJ, TX-2516) and Clevevilt (SA-245) were the resistant controls and cv. Deltapine (DP) 90 was the susceptible control.

The mechanism of resistance to *M. incognita* was characterized based on root penetration, post-penetration development, and reproduction. Penetration was determined on developing cotton radicals. The root tip of seedlings with a radical length of 5-cm were inserted individually in a plastic cylinder (0.5 cm-diam  $\times$  5.5 cm-length) then covered with pasteurized sand (<710  $\mu$ m diam). Approximately, 100 J2 in 100  $\mu$ l of water were inoculated into the sand 1-cm below the root tip. Inoculated roots were incubated at 28°C for 2 d inside a moisture chamber. Nematodes were stained with acid fuchsin (Byrd *et al.*, 1983) to aid in visualizing J2 within the root tissue. Genotypes were replicated four times and the experiment repeated once.

A time course study was utilized to compare nematode post-penetration development among cotton genotypes. Germinated cotton seeds were planted into 85 cm<sup>3</sup> celled planter flats containing pasteurized sand to peat (6:1 v/v) soil mix. Seedlings were inoculated at the first true leaf stage with approximately 1,000 J2/500 cm<sup>3</sup> of soil, evenly distributed among three 2-cm deep cavities around the seedling. Cotton seedlings were incubated in a growth chamber at 30°C with 12 hr darkness in a 24 hr period. The root systems from these plants were harvested at 7, 14, and 21, d after inoculation (DAI) and washed free of soil. Nematodes in the roots were stained with acid fuchsin (Byrd *et al.*,

1983) and were classified into four stages of development; vermiform J2, sausage shaped juveniles, female without eggs, and female with eggs. The experiment was arranged in a randomized complete block design (RCBD) and each genotype was replicated four times per harvest date.

Nematode reproduction and fecundity were assayed in a greenhouse trial. Germinated cotton seeds were planted into 656 cm<sup>3</sup> Deepots (Stuewe and sons, Inc, Corvallis, OR) containing pasteurized sand to peat (6:1 v/v) soil mix. Seedlings were inoculated, as described above, at the first true leaf stage with 1,000 J2/500 cm<sup>3</sup> of soil. The root systems from these plants were harvested at 42 and 56 DAI and washed free of soil. The experiment was arranged in a RCBD and each genotype was replicated four times per harvest date. Egg masses were stained with phyloxine B (Daykin and Hussey, 1985) and eggs were extracted from root systems with NaOCl (Hussey and Barker, 1973). Final population (Pf) values were eggs extracted per root system. Reproduction and fecundity were determined by counting eggs per root system, egg masses per root system, and eggs per gram of root, then calculating eggs per egg mass and Pf/Pi values.

Data from the nematode penetration and reproduction assays were subject to Analysis of Variance and mean separations by Tukey's Honestly Significant Difference (HSD), whereas data from nematode development assay were subject to Chi Square Analysis using SPSS 16.0 (SPSS Inc., Chicago, Ill). Data from nematode reproduction trials were transformed ( $\ln + 1$ ) and non-transformed data are presented in graphs.

## RESULTS

Root penetration by *M. incognita* at 2 DAI was similar among susceptible and

resistant genotypes with a mean of 10.7 J2 in seedling radicals. Further, the total number of nematodes in developing root systems at 7 DAI from the time course study was similar among genotypes (Fig. 1). The number of nematodes per root system in DP 90 increased ( $P \leq 0.05$ ) from 34 to 98 individuals from 7 to 21 DAI, respectively, whereas numbers in resistant genotypes, averaged 24 to 26 individuals from 7 to 21 DAI, respectively. Total *M. incognita* per root system at 14 DAI was lower ( $P \leq 0.05$ ) in all resistant genotypes (except TX-2079) than in DP 90 (Fig. 1). The number of nematodes per root system in TX-2079 decreased ( $P \leq 0.05$ ) from 14 to 21 DAI, whereas no change was observed in other resistant genotypes. Total *M. incognita* per root system at 21 DAI was lower ( $P \leq 0.05$ ) in all resistant genotypes than DP 90 (Fig. 1). No difference in total *M.*

*incognita* per root system at 21 DAI was observed among resistant genotypes.

Development of *M. incognita* at all sample dates was delayed ( $P \leq 0.05$ ) in resistant genotypes relative to nematode development in DP 90 (Fig. 2). The proportion of the population present as females at 7 DAI and egg-laying females at 14 and 21 DAI was lower ( $P \leq 0.05$ ) on all resistant genotypes than on DP 90. Female *M. incognita* without eggs on DP 90 comprised 0.23, and females with eggs comprised 0.19 and 0.82 of total *M. incognita* per root system at 7, 14, and 21 DAI, respectively. The proportion of females with or without eggs at each sample date was similar among resistant genotypes (Fig. 2).

The rate of delayed development of *M. incognita* at all sample dates in Clevevilt differed ( $P \leq 0.05$ ) from WMJJ, TX-1440, TX-2076, and TX-2079 whereas WMJJ differed

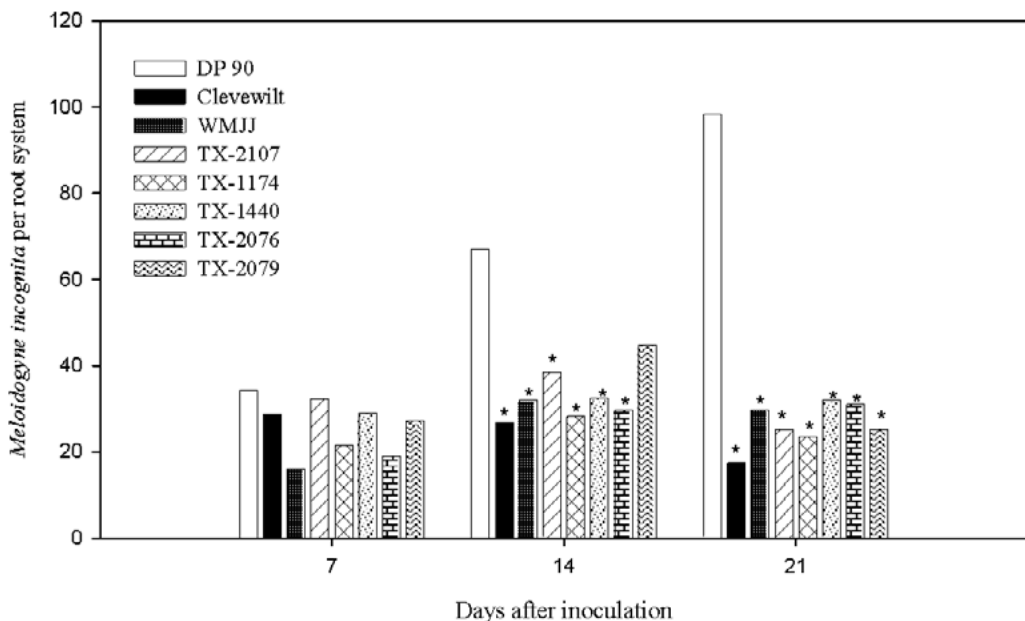


Fig. 1. Root penetration by *Meloidogyne incognita* on eight cotton genotypes at different days after inoculation. Initial population density of *M. incognita* was 1,000 J2/500 cm<sup>3</sup> soil. Bars with an asterisk (\*) within specified days are significantly different from the susceptible control (DP 90) according to Tukey's HSD  $\alpha = 0.05$ .

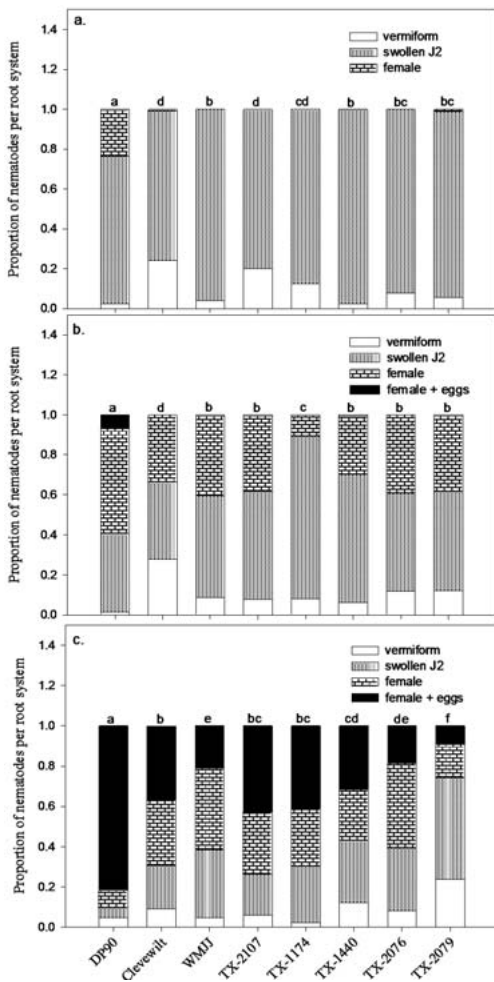


Fig. 2. Post-penetration developmental stages of *Meloidogyne incognita* on eight cotton genotypes at (a.) 7, (b.) 14, and (c.) 21 days after inoculation. Initial population density was 1,000 J2/500 cm<sup>3</sup> soil. The susceptible control was DP 90. Different letters over bars indicate significant difference at  $P \leq 0.05$  according to chi-square analysis applied in pairs of genotypes.

( $P \leq 0.05$ ) from TX-1174 (Fig. 2). Alternately, the rate of delayed nematode development at all sample dates was similar between TX-2076 and WMJJ and between TX-2076 and TX-1440. In addition to the delayed nematode development at 7 DAI, an augmented delay ( $P \leq 0.05$ ) was

observed in Clevevilt and TX-1174 at 14 DAI and in TX-2079 at 21 DAI relative to nematode development in other resistant genotypes (Fig. 2).

Reproduction by *M. incognita* on cotton root systems was similar between sample dates, so these data were combined. Fewer ( $P \leq 0.05$ ) eggs per gram of root were observed on TX-1174, TX-2076, and TX-2079 than on DP 90 (Fig. 3). All resistant genotypes except Clevevilt and WMJJ had fewer ( $P \leq 0.05$ ) egg masses per root system and lower ( $P \leq 0.05$ ) Pf/Pi values than DP 90. Cotton genotypes TX-1174, TX-2076, and TX-2079 had fewer ( $P \leq 0.05$ ) egg masses per root system than Clevevilt or WMJJ. No difference was observed for eggs per egg mass among genotypes with an average of 720 for all genotypes. Only, TX-1174 had fewer ( $P \leq 0.05$ ) egg masses per root system, eggs per gram of root, and lower ( $P \leq 0.05$ ) Pf/Pi value than Clevevilt or WMJJ.

## DISCUSSION

These data herein confirms the resistance to *M. incognita* in the five primitive cotton accessions from the Yucatan region of Mexico as originally reported by Robinson and Percival (1997). Further these data suggest that the resistance of TX-1440, TX-2076, and TX-2107 is similar in magnitude to that of Clevevilt and WMJJ but that the resistance of TX-1174 and TX-2079 is of greater magnitude than that of Clevevilt or WMJJ.

Resistance did not inhibit initial root penetration by *M. incognita* in resistant genotypes. No preexisting defense structure that impedes *M. incognita* penetration has been reported on cotton (Crech *et al.*, 1995; Jenkins *et al.*, 1995; McClure *et al.*, 1974). However, the number of *M. incognita* per root system in resistant genotypes remained unchanged with increasing DAI,

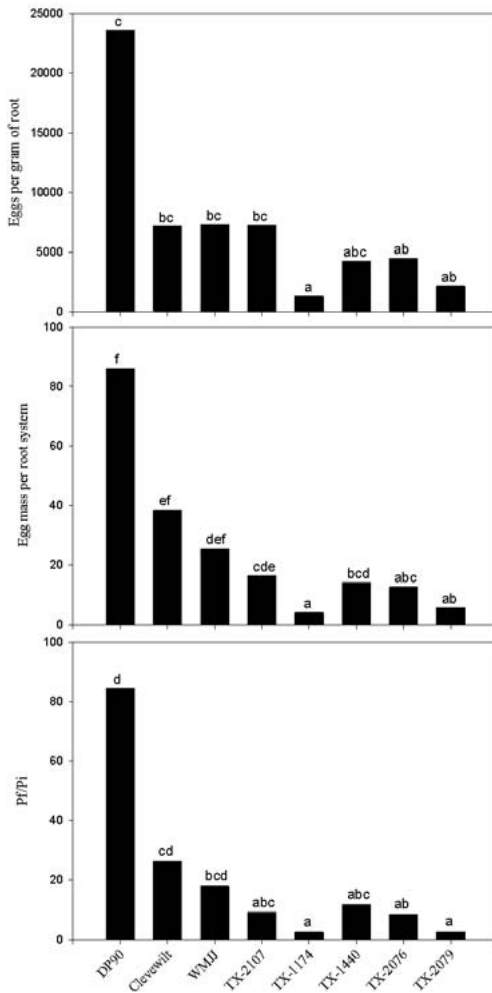


Fig. 3. Reproduction of *Meloidogyne incognita* on eight cotton genotypes measured as eggs per g roots, eggs per egg mass, and Pf/Pi. Initial population density was 1,000 J2/500 cm<sup>3</sup> soil. Final population (Pf) values were eggs extracted per root system. The susceptible control was DP 90. Different letters over bars indicate significant difference according to Tukey's HSD at  $\alpha = 0.05$ .

but increased in the susceptible control. These observations suggest that J2 may have penetrated the root system of resistant genotypes, failed to establish a feeding site, and regressed back into the soil. Increased emigration of *Meloidogyne* spp. from resistant host genotypes has been

reported previously (Bendezu and Starr, 2003; Pedrosa *et al.*, 1996).

A critical stage of nematode development occurred at 7 DAI after which *M. incognita* maturity was delayed in all resistant genotypes. This delayed maturity of *M. incognita* contributed to fewer females with eggs and lower levels of total reproduction; however, eggs per egg mass were similar among all genotypes. Thus, resistance that delayed nematode development in resistant genotypes had little influence on fecundity of individual nematodes that reached maturity. Delayed development of *M. incognita* has been reported on Clevevilt 6-3-5, M 78, and M 315, resistant cotton genotypes (Creech *et al.*, 1995; Jenkins *et al.*, 1995; McClure *et al.*, 1974). In addition to the delayed nematode development at 7 DAI, an augmented delay was observed in TX-1174 at 14 DAI and TX-2079 at 21 DAI. Thus, these two highly resistant accessions may possess an additional gene(s) for resistance to *M. incognita*. Similarly, resistance in M 315 that delayed nematode development at 8 and 24 DAI was later reported as a two-gene model for resistance to *M. incognita* (McPherson *et al.*, 2004).

Though the mechanism of resistance was similar in resistant genotypes, the rate of delayed development of *M. incognita* differed among these genotypes. Thus, suggesting diversity in resistant genes among these cotton genotypes. The rate of delayed nematode development in Clevevilt differed from three resistant accessions whereas WMJJ differed from one accession. Wild Mexican Jack Jones and the five primitive cotton accessions were collected from Mexico (Robinson, 1999; Robinson and Percival, 1997) and thus, may have developed from a similar gene pool that expresses similar resistance.

This study provides information on the mechanism of resistance, which was related to the failed ability of J2 to establish a feed-

ing site and delayed maturity of *M. incognita* in seven resistant cotton genotypes. These observations further our understanding of penetration and post-penetration development of *M. incognita* in susceptible and several resistant cotton genotypes.

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