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Penetration, Post-penetration Development, and Reproduction of *Meloidogyne incognita* on *Cucumis melo* var. *texanus*

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Abstract: Cucumis melo var. *texanus,* a wild melon commonly found in the southern United States and two accessions, Burleson Co. and MX 1230, expressed resistance to *Meloidogyne incognita* in preliminary experiments. To characterize the mechanism of resistance, we evaluated root penetration, post-penetration development, reproduction, and emigration of *M. incognita* on these two accessions of *C. melo* var. *texanus*. Additionally, we evaluated 22 accessions of *C. melo* var. *texanus* for their reaction against *M. incognita* in a greenhouse experiment. Fewer ($P \leq 0.05$) J2 penetrated the root system of *C. melo* var. *texanus* accessions (Burleson Co. and MX 1230) and *C. metuliferus* (PI 482452) (resistant control), 7 days after inoculation (DAI) than in *C. melo* 'Hales Best Jumbo' (susceptible control). A delayed ($P \leq 0.05$) rate of nematode development was observed at 7, 14, and 21 DAI that contributed to lower ($P \leq 0.05$) egg production on both accessions and *C. metuliferus* compared with *C. melo*. Though J2 emigration was observed on all *Cucumis* genotypes a higher ($P \leq 0.05$) rate of J2 emigration was observed from 3 to 6 DAI on accession Burleson Co. and *C. metuliferus* than on *C. melo*. The 22 accessions of *C. melo* var. *texanus* varied relative to their reaction to *M. incognita* with eight supporting similar levels of nematode reproduction to that of *C. metuliferus*. *Cucumis melo* var. *texanus* may be a useful source of resistance against root-knot nematode in melon.

Key words: Cucumis melo, *C. melo* var. *texanus*, *C. metuliferus,* emigration, melon, *Meloidogyne incognita,* post-penetration development, resistance, and root-knot nematode.

The root-knot nematode, *Meloidogyne incognita*, is an important plant-parasitic nematode on melon, *Cucumis melo*, in the United States (Sasser, 1979; Lamberti, 1979; Heald et al., 1988; Ploeg and Phillips, 2001; Sikora and Fernandez, 2005). Few management options are available to growers to manage *M. incongita* on melon. The use of fumigant nematicides has been successful (Hamill and Dickson, 2005); however, the use of fumigants or other nematicides are becoming limited or banned by legislation because of potential hazards to environmental or public health (Ristaino and Thomas, 1997; Nyczepir and Thomas, 2009). The development of root-knot nematode resistant varieties have been successful in cotton, peanut, pepper, tomato, and tobacco (Ogallo et al., 1997; Simpson and Starr, 2001; Starr et al., 2002; Thies et al., 2003; Starr and Mercer, 2009). Currently, no commercially available melon varieties are resistant to *M. incognita*. Sources of resistance in *C. melo* are lacking though many closely related species and botanical varieties have been evaluated for resistance to *M. incognita* (Thomason and McKinney, 1959; Fassuliotis and Rau, 1963; Fassuliotis, 1967; Nugent and Dukes, 1997). A high level of resistance to *M. incognita* was reported in *C. metuliferus* (Fassuliotis, 1970; Wehner et al., 1991; Walters et al., 2006), but numerous attempts to incorporate this resistance into *C. melo* have been unsuccessful due to cross-incompatibility (Fassuliotis, 1977; Norton and Granberry, 1980; Chen and Adelberg, 2000). Thus, identifying resistance to *M. incognita* within *C. melo* subspecies or botanical varieties would be beneficial for plant breeders and plant pathologist to introgress resistance into melon varieties.

Cucumis melo is a diverse species composed of tropical and subtropical botanical varieties that are potential sources of resistance to melon diseases. A wild botanical variety, *C. melo* var. *agrestis* has been reported to have resistance to a closterovirus that causes melon yellowing disease (Sorie et al., 1996) and var. *texanus* has been reported to be a putative source of resistance to *M. incognita* in preliminary greenhouse trials (Faske, 2010). *Cucumis melo* var. *texanus* is commonly found in agricultural fields in the southern United States and Mexico. Though widely distributed in North America, little is known about this source of resistance to *M. incognita*. Thus, characterizing the mechanism of resistance in *C. melo* var. *texanus* to *M. incognita* and evaluating the reaction of several accessions of *C. melo* var. *texanus* against *M. incognita* would further our understanding of this potential source of resistance to root-knot nematodes.

The mechanism of resistance to root-knot nematodes in *C. metuliferus* has been characterized in a few studies (Fassuliotis, 1970; Haynes and Jones, 1976; Walters et al., 2006). Fassuliotis (1970) reported that the penetration rate of *M. incognita* was similar between *C. metuliferus* and *C. melo,* 'Hales Best Jumbo', and a similar observation was reported between *C. metuliferus* (PI 482452) and *C. sativus*, 'Sumter' (Walters et al., 2006). Post-penetration development of *M. incognita* was delayed because of abnormal development of the feeding site for optimum nematode development, thus contributing to lower nematode reproduction on *C. metuliferus* as compared with the susceptible control. Further, no hypersensitivity or necrosis was associated with the infection of *M. incognita* in the root system of *C. metuliferus* (Fassuliotis, 1970; Walters et al., 2006. Though a few studies have investigated resistance related to the biology of *M. incognita* in *C. metuliferus*, it has yet to be determined if J2 emigration contributes to the mechanism of resistance.

The objectives for this study was (i) to evaluate the penetration rates, post-penetration development, and fecundity of *M. incognita* on *C. melo* var. *texanus*; (ii) evaluate the behavior of *M. incognita* J2 following root

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penetration on *C. metuliferus* and *C. melo* var. *texanus*; and (iii) compare reproduction of *M. incognita* on several North American accessions of *C. melo* var. *texanus*.

Materials and Methods

Nematode culture and inoculum: Meloidogyne incognita was isolated from cotton (*Gossypium hirsutum*) and maintained in the greenhouse on *Solanum lycopersicum* L. 'Rutgers'. Eggs were collected from infected tomato roots with 0.5% NaOCl (Hussey and Barker, 1973) and J2 were collected in a hatching chamber (Vrain, 1977). Only 24-hr-old J2 were used in this study.

Nematode biological response experiments: Three separate experiments were conducted to evaluate penetration and post-penetration development, emigration of J2, and fecundity of *M. incognita* on four *Cucumis* genotypes. Two accessions (Burleson Co. and MX 1230) of *C. melo* var. *texanus* had lower nematode reproduction in preliminary trials; therefore, they were used in these nematode biological response experiments. The resistant control, *C. metuliferus* (PI 482452), and susceptible control, *C. melo* 'Hales Best Jumbo', were used throughout this study.

A time course study was used to evaluate nematode penetration and post-penetration development. Germinated seed from each *Cucumis* genotype was planted into 85-cm^3-celled planter flats containing pasteurized sand to peat (6:1 v/v) soil mix. Seedlings were inoculated at the first true leaf stage, 2 to 3 wk after seeding, with approximately 165 J2 evenly distributed among three 2-cm-deep cavities around the seedling. This experiment was arranged in a randomized complete block design (RCBD) and repeated once. In each experiment, treatments were replicated four times per sample date. Root systems were harvested at 7, 14, and 21 d after inoculation (DAI) and washed free of soil. Nematodes were stained with acid fuchsin (Byrd et al., 1983) and classified into four stages of development; vermiform J2, sausage-shaped juveniles, female without eggs, and female with eggs.

Emigration of J2 was evaluated in a hydroponic system. Germinated seeds were planted into 85-cm^3-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 2,000 J2, evenly distributed among three 2-cm-deep cavities around the seedling. Each *Cucumis* genotype was replicated five times in a RCBD and the experiment was conducted once. Roots systems were collected 2 DAI and washed free of soil. Seedlings were transferred into individual 230-cm^3 plastic containers filled with distilled water and fitted with a plastic tube attached to a small airpump. Air was pumped through the water to provide enough oxygen to keep roots healthy. Second-stage juveniles that emigrated from the roots were collected daily from 3 to 6 DAI and enumerated.

To evaluate fecundity, freshly collected eggs were inoculated onto seedlings at the second true leaf stage, 3 to 4 wk after seeding, growing in 1025-cm^3 standard pots containing pasteurized sand to peat (6:1 v/v) soil mix. Inoculum concentration of 4,000 eggs was distributed among three 2-cm-deep cavities around each seedling. Each experiment was maintained in a greenhouse where ambient temperature ranged from 22 to 32°C. Each *Cucumis* genotype was replicated four times in a RCBD and the experiment was repeated once. Roots were harvested 7 wk after inoculation and washed free of soil. Roots were blotted dry with paper towels, rated for galling based on a six-point scale with 0 = no galls and 5 = severe galling. Single egg masses (largest) were collected from each root system and treated with 1.0% NaOCl to extract eggs from each egg mass. Root systems were weighted, cut into 1-cm pieces and treated with 1.0% NaOCl to extract eggs present. Eggs were enumerated with a stereoscope.

Greenhouse pot experiments: Twenty-two accessions for *C. melo* var. *texanus* were compared for their reaction against *M. incognita* in two separate experiments (Table 1). In each experiment, freshly collected eggs were inoculated onto seedlings at the second true leaf stage, 3 to 4 wk after seeding, growing in 1025-cm^3 standard pots containing pasteurized sand to peat (6:1 v/v) soil mix. Inoculum concentration of 4,000 eggs was distributed among three 2-cm-deep cavities around each seedling. Each experiment was maintained in a greenhouse where ambient temperature ranged from 22 to 32°C. Accessions were replicated four times in a RCBD and each experiment was completed once. The resistant

Table 1. Geographic data of *Cucumis melo* var. *texanus* accessions tested for ability to support reproduction of *Meloidogyne incognita*.

Accession or PI no.	Geographic origin	Experiment no.	Experiment ID
	Burleson, Texas	2	Burleson Co.
1230	Baja California, Mexico	2	MX 1230
1298	Saint John the Baptist, Louisiana	1	LA 1298
1301	East Baton Rouge, Louisiana	1	LA 1301
1303	Pointe Coupee, Louisiana	2	LA 1303
1307	Saint Landry, Louisiana	2	LA 1307
1314	Bossier, Louisiana	1	LA 1314
1319	Issaquena, Mississippi	2	MS 1319
1325	Tensas, Louisiana	2	LA 1325
1328	Oktibbeha, Mississippi	2	MS 1328
1329	Newton, Mississippi	1	MS 1329
1338	Hidalgo, Texas	2	TX 1338
1342	Cameron, Texas	1	TX 1342
1344	San Patricio, Texas	1	TX 1344
1347	Calhoun, Texas	2	TX 1347
1351	Jackson, Texas	1	TX 1351
1357	Burleson, Texas	1	TX 1357
1359	Palm Beach, Florida	2	FL 1359
1362	Brevard, Florida	2	FL 1362
1363	Brevard, Florida	1	FL 1363
PI 442178	Tamualipas, Mexico	1	PI 442178

control, *C. metuliferus* (PI 482452), and susceptible control, *C. melo* 'Hales Best Jumbo', was used in this study.

Experiments were harvested 7 wk after inoculation and soil was washed from the root system. Roots were blotted dry with paper towels, rated for galling, weighted, cut into 1-cm pieces and treated with 1.0% NaOCl to extract eggs present. Root galling was based on a six-point scale with 0 = no galls and 5 = severe galling. The eggs were enumerated with a stereoscope.

Statistical analysis: Data from nematode reproduction, fecundity, penetration, and emigration experiments were transformed ($\ln + 1$) to normalize and nontransformed data are reported. Pearson's correlation coefficient was calculated between root-galling and egg counts. Data were subjected to analysis of variance and mean separations by Tukey's honestly significant difference (HSD) test, whereas data from post-penetration development experiment were subject to chi square analysis using SPSS 19.0 (SPSS, Inc., Chicago).

Results

Penetration rates of *M. incognita* per root system were similar between experiments so data were combined (Fig. 1). Fewer ($P \leq 0.05$) J2 were observed at 7 and 21 DAI in both accessions of C. *melo* var. *texanus* and *C. metuliferus* than *C. melo*, the susceptible control. The average count of *M. incognita* for all three sample dates was 20.7, 30.6, and 23.1 on the accessions of *C. melo* var. *texanus*, Burleson Co., MX 1230, and *C. metuliferus*, respectively, which was lower ($P \leq 0.05$) than 63.1 on *C. melo*.

Development of *M. incognita* was delayed ($P \leq 0.05$) at all sample dates in both accessions of C. *melo* var. *texanus* and *C. metuliferus* as compared with *C. melo* (Fig. 2). The nematode population present as females at 14 DAI and egg-laying females at 21 DAI was lower ($P \leq 0.05$) on both accessions of C. *melo* var. *texanus* and *C. metuliferus* than *C. melo*. The proportion of females and egg-laying females at 14 and 21 DAI on *C. melo* was 0.40 and 0.58, respectively. No egg-laying females were observed on *C. metuliferus* and empty galls were observed on both accessions of C. *melo* var. *texanus* and *C. metuliferus*.

Second-stage juveniles emigrated at all sample dates from the root system of all *Cucumis* genotypes. More ($P \leq 0.05$) J2 emigrated 3 DAI from the root system of accession Burleson Co. and *C. metuliferus* than *C. melo* (Fig. 3). The average count of J2 for all four sample dates was 102.6 and 100.6 from accession Burleson Co. and *C. metuliferus*, respectively, which was more ($P \leq 0.05$) than 36.8 from *C. melo*.

Nematode reproduction on *Cucumis* root systems was similar between experiments so data were combined (Fig. 4). Fewer ($P \leq 0.05$) eggs were produced by *M. incognita* on both accessions of C. *melo* var. *texanus* and *C. metuliferus* than on *C. melo* (Fig. 4). A lower ($P \leq 0.05$) root-gall rating was observed on accession MX 1230 and *C. metuliferus* as compared with *C. melo*. Fecundity of mature females remaining in the root system was similar among *Cucumis* genotypes with an average 432 eggs/egg mass for all genotypes.

In the two greenhouse experiments, a lower ($P \leq 0.05$) nematode reproduction index and root-gall rating was observed on the resistant control, *C. metuliferus* (PI 482452), than on the susceptible control, C. *melo* 'Hales Best Jumbo' (Fig. 5). In both experiments, galling was positively correlated ($r = 0.41$ and $r = 0.49$) with eggs produced by *M. incognita*. A few accessions of *C. melo* var. *texanus*, MX 1230, LA 1302, LA 1303, LA 1307, LA

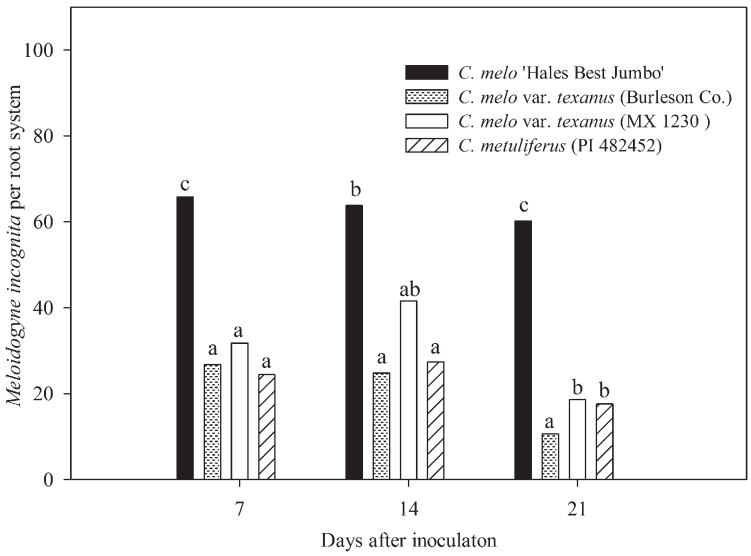

Fig. 1. Number of *Meloidogyne incognita* at 7, 14, and 21 d after inoculation in four *Cucumis* genotypes. Genotypes consisted of a resistant control, *C. metuliferus* (PI 482452); susceptible control, *C. melo* 'Hales Best Jumbo'; and two accessions of *C. melo* var. *texanus* (Burleson Co. and MX 1230). Initial population density of *M. incognita* was 200 J2/100-cm^3 soil. Different letters over bars indicate significant differences at $\alpha = 0.05$ according to Tukey's HSD test.

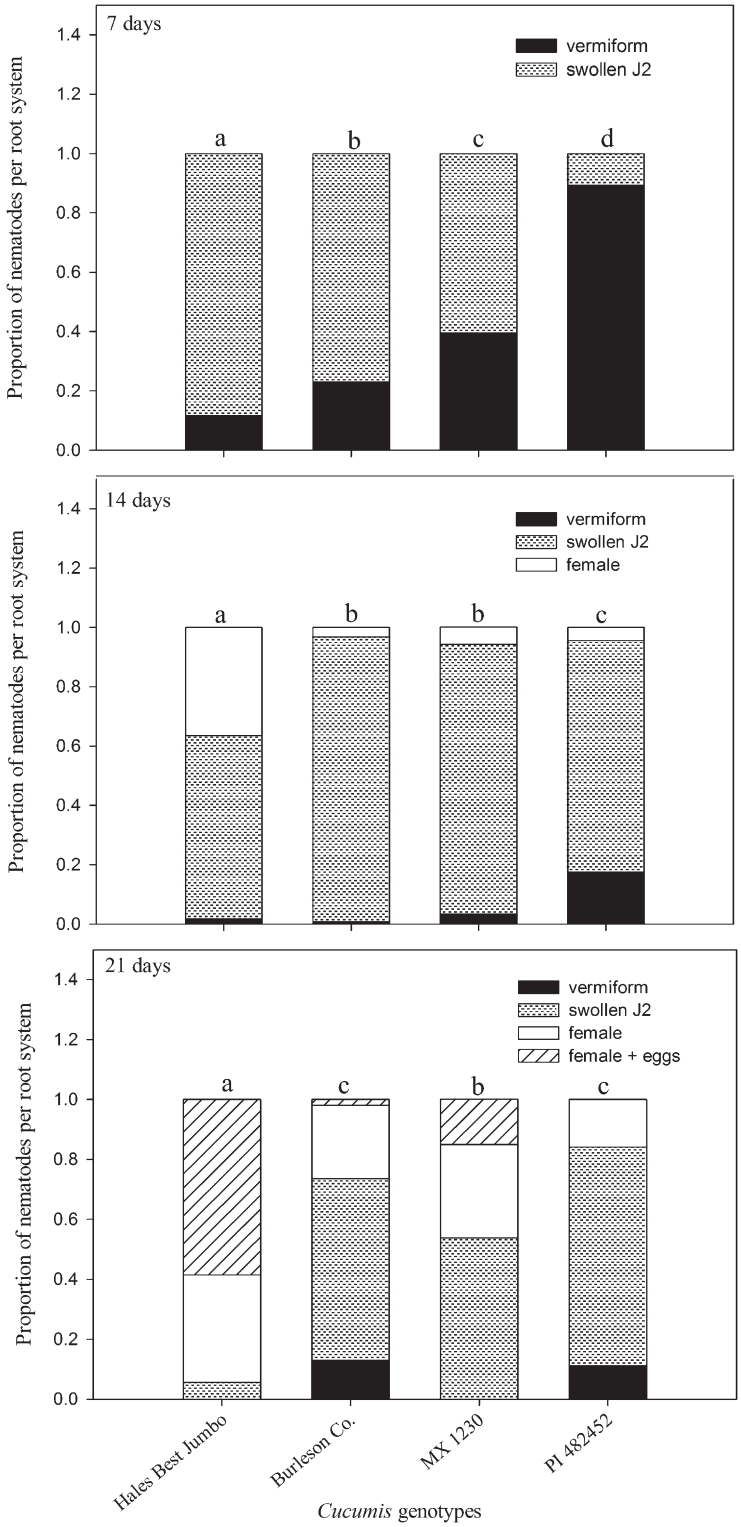

FIG. 2. Number of *Meloidogyne incognita* development stages at 7, 14, and 21 d after inoculation in four *Cucumis* genotypes. Genotypes consisted of a resistant control, *C. metuliferus* (PI 482452); susceptible control, *C. melo* 'Hales Best Jumbo'; and two accessions of *C. melo* var. *texanus* (Burleson Co. and MX 1230). Initial population density was 200 J2/100-cm^3 soil. Different letters over bars indicates a significant difference at $P \leq 0.05$ according to chi-square analysis applied in pairs of genotypes.

1325, MS 1328, TX 1338, and Burleson Co. expressed a lower ($P \leq 0.05$) root-gall rating and supported lower ($P \leq 0.05$) levels of egg production than the susceptible control (Fig. 5). Further, egg production was numerically lower on these eight accessions compared with the resistant control. Numerically, the lowest gall rating and egg production was observed on MX 1230, LA 1303, and LA 1325 among these eight accessions of

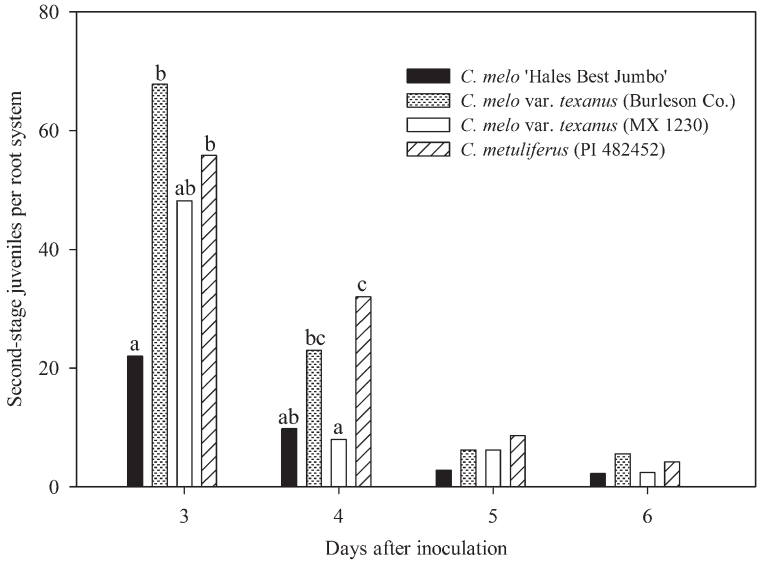

FIG. 3. Number of *Meloidogyne incognita* second-stage juveniles emigrating from roots of four *Cucumis* genotypes. Genotypes consisted of a resistant control *C. metuliferus* (PI 482452); susceptible control, *C. melo* 'Hales Best Jumbo'; and two accessions of *C. melo* var. *texanus* (Burleson Co. and MX 1230). Initial population density of *M. incognita* was 2400 J2/100-cm^3 soil. Different letters over bars indicate significant differences at $\alpha = 0.05$ according to Tukey's HSD test.

C. melo var. *texanus*. The average egg production on these three accessions was less than 5% of the nematode reproduction of the susceptible control. Alternately, nematode reproduction was higher on accession LA 1314, MS 1319, PI 442178, TX 1344, and TX 1351 than the susceptible control. Finally, there was no interaction between nematode reproduction and geographical origin of accessions.

DISCUSSION

Cucumis melo var. *texanus* is a wild melon commonly found in agricultural fields in the southern United States. There is limited data on this melon as a potential source of resistance to *M. incognita*. These data herein suggest that *C. melo* var. *texanus* is resistant to *M. incognita* and the mechanism of resistance is similar to that of *C. metuliferus*. The mechanism of resistance observed in *C. melo* var. *texanus* was related to three different effects on *M. incognita*. The first effect of resistance was a reduction in root penetration in *C. melo* var. *texanus* and *C. metuliferus* relative to the susceptible control. These results differ from those reported for *M. incognita* penetration rates in *C. metuliferus* (Fassuliotis, 1970; Walters et al., 2006) where the number of J2 did not differ between *C. metuliferus* and the susceptible

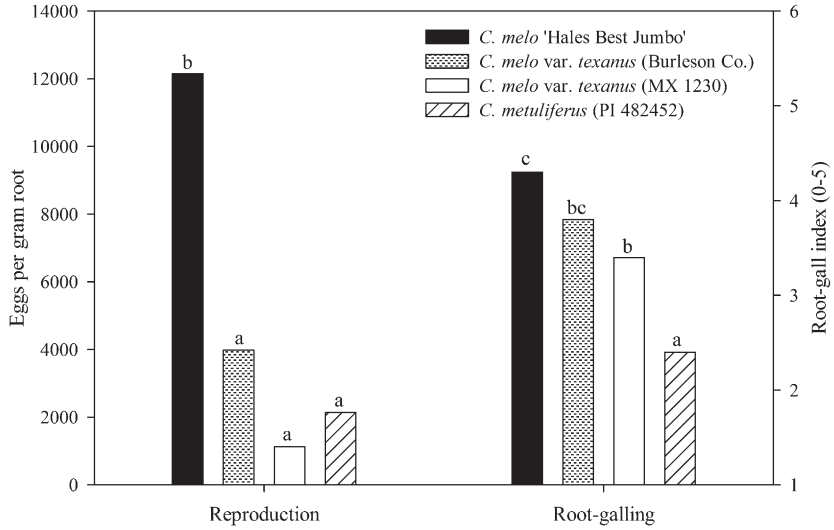

FIG. 4. Root-gall rating and reproduction of *Meloidogyne incognita* on four *Cucumis* genotypes. Genotypes consisted of a resistant control, *C. metuliferus* (PI 482452); susceptible control *C. melo* 'Hales Best Jumbo'; and two accessions of *C. melo* var. *texanus* (Burleson Co. and MX 1230). Plants were harvested 7 wk after inoculation of 400 eggs/100-cm^3 soil. Different letters over bars indicate significant differences at $\alpha = 0.05$ according to Tukey's HSD test.

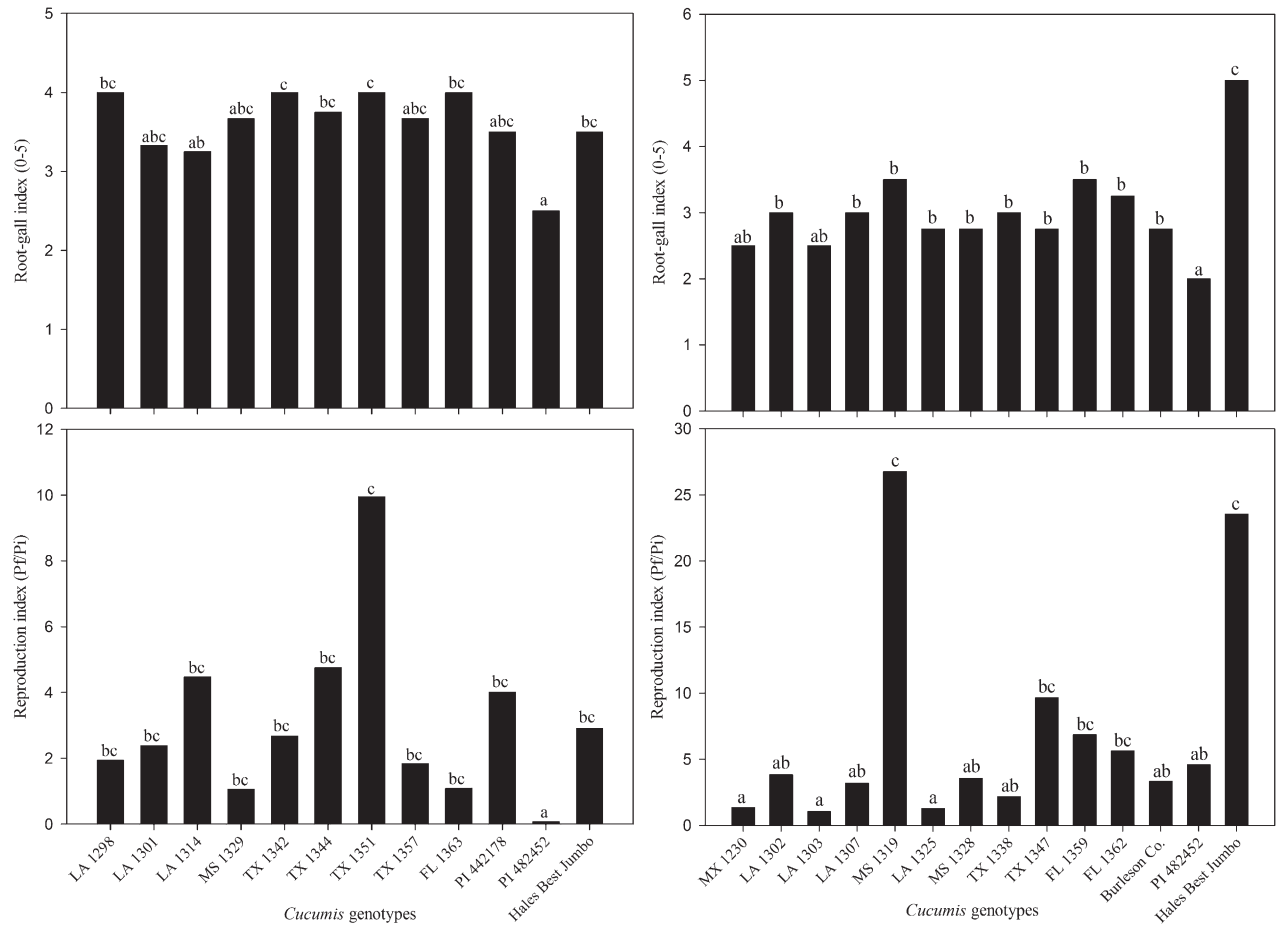

FIG. 5. Root-gall rating and reproduction of *Meloidogyne incognita* on 22 accessions of *Cucumis melo* var. *texanus* from various locations across North America. Resistant control was *C. metuliferus* (PI 482452) and susceptible control was *C. melo* 'Hales Best Jumbo'. Plants were harvested 7 wk after inoculation of 400 eggs/100-cm^3 soil. Different letters over bars indicate significant differences at $\alpha = 0.05$ according to Tukey's HSD test.

control. Nematode penetration rates were estimated later than previous studies and nematode emigration was observed 3 DAI for all *Cucumis* genotypes, which may have affected recorded penetration rates (Herman et al., 1991). A higher rate of nematode emigration was observed from the root system of both accessions of *C. melo* var. *texanus* and *C. metuliferus*. Thus, the second effect was that most of the J2 failed to establish a feeding site and emigrated from the roots. Differences in J2 emigration have been reported between resistant and susceptible genotypes of peanut and soybean (Herman et al., 1991; Bendezu and Starr, 2003; Dhandaydham et al., 2008). It is possible the J2 were responding to cucurbitacians similar to those reported in *C. sativus* (Haynes and Jones, 1976). The third effect was a delayed rate in the development of *M. incognita* to reach maturity, which is consistent with earlier studies of a reduced or delayed development of root-knot nematodes (Fassuliotis, 1970; Walters et al., 2006). This effect was observed as early as 7 DAI, which contributed to fewer egg-laying females but had little effect on fecundity. Thus, post-penetration factors that suppressed nematode development had little or no effect on individual nematode that reach maturity.

The mechanism of resistance to *M. incognita* in *C. metuliferus* has also been related to an increased stimulation of juveniles toward maleness (Fassuliotis, 1970). Empty galls were observed in the biological response experiments on both accessions of *C. melo* var. *texanus* and *C. metuliferus*, which may have contained males that left the root. Potentially, contributing to fewer eggs produced by *M. incognita* on the root system of these accessions.

These data suggest the resistance to *M. incognita* in a few accessions of *C. melo* var. *texanus* is similar in magnitude to that of *C. metuliferus*. *Cucumis metuliferus* was the most resistant *Cucumis* genotype in this study. These results are consistent with other reports of resistance to *M. incognita* in *C. metuliferus* (Wehner et al., 1991; Walters et al., 1993, 1999). A few accessions, MX 1230, LA 1302, LA 1303, LA 1307, LA 1325, MS 1328, TX 1338, and Burleson Co. were moderately resistant to *M. incognita*. Further, an average reproduction factor of 1.2 for accessions, MX 1230, LA 1303, and LA 1329 is

the lowest of all botanical varieties or subspecies of *C. melo* yet reported. Nugent and Dukes (1997) reported an average reproduction factor of 5.5 at a similar inoculum level for two accessions, PI 183311 and PI 140471, of *C. melo* subsp. *melo*. In this study, two accessions TX 1351 and MS 1319 were highly susceptible and, in comparison with the very resistant accessions (MX 1230, LA 1303, and LA 1329), would suggest a major effect in resistance. A similar effect was identified in *C. sativus* var. *hardwickii* as a single gene for resistance to *M. javanica* (Walters et al., 1997). This single resistance gene in *C. sativus* var. *hardwickii* has been reported to be effective against *M. javanica* and *M. arenaria* (Walters et al., 1996, 1999) whereas the resistance in *C. metuliferus* has been reported to be effective on these species and *M. incognita* (Wehner et al., 1991; Walters et al., 1993). Thus, this new source of resistance in *C. melo* var. *texans* may confer resistance to other species of *Meloidogyne*.

Cucumis melo var. *texanus* was not highly resistant to *M. incognita*, but it appears to be a potential source of resistance. Little progress has been made to integrate the resistance in *C. metuliferus* into melon (*C. melo*) because of cross-incompatibility (Fassuliotis, 1977; Norton and Granberry, 1980; Chen and Adelberg, 2000). *Cucumis melo* var. *texanus* is closely related to melon and could be used to develop root-knot nematode resistant varieties. Development of root-knot nematode resistant varieties would be beneficial in the management of root-knot nematode in commercial melon production.

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