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Susceptibility to Root-Knot Nematodes in Cotton Lines Resistant to the Fusarium Wilt/Root-Knot Complex[1]

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ABSTRACT

The cotton (*Gossypium hirsutum* L.) breeding lines CABCS'-1-81, CAHUS-2-81, and LEBOCAS'-3-81, developed by the multiple adversity resistance (MAR) technique, have been reported to be resistant to the fusarium wilt [*Fusarium oxysporum* Schlecht f. sp. *vasinfectum* (Atk.) Synd. and Hans.]/root-knot nematode [*Meloidogyne incognita* (Kofoid and White) Chit.] complex. Host wilting was the sole criterion upon which resistance to the complex was based. Resistance to the nematode, usually predicted upon the inability of the nematode to reproduce on the host, was never directly measured. Our objective, therefore, was to measure the resistance of these breeding lines to the nematode alone. When nematode inoculated plants were grown in 15 cm pots in replicated greenhouse tests, the cotton breeding lines supported levels of *M. incognita* egg production that did not differ from that produced on the nematode susceptible 'TAMCOT SP37'. All lines supported greater ($P = 0.01$) egg production than did the root-knot nematode resistant 'Auburn 634'. In replicated microplot tests, seed cotton yields of the breeding lines were not different from that of the susceptible TAMCOT SP37 at several nematode inoculum levels. Similarly, reproduction of *M. incognita* on susceptible and putatively nematode-resistant cotton lines did not differ in microplot tests. Thus, while the cotton lines CABCS'-1-81, CAHUS-2-81, and LEBOCAS-3-81 may be more resistant under field conditions to fusarium wilt than is TAMCOT SP37, our study indicates they are not more resistant to *M. incognita*. Therefore, the indirect selection for resistance to root-knot nematodes based on host wilting, even in the presence of both pathogens, was found to be an inadequate test of resistance to *M. incognita*.

Additional index words: Host resistance, Screen for resistance, *Meloidogyne incognita* (Kofoid and White) Chit., *Fusarium oxysporum* Schlecht f. sp. *vasinfectum* (Atk.) Synd. and Hans., *Gossypium hirsutum* L.

THE root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chit., is an economically important pathogen of cotton (*Gossypium hirsutum* L.) throughout the USA (10). Losses in western Texas are estimated at 175 000 bales of lint annually (13). Additionally, root-knot nematodes frequently form disease complexes with other soilborne pathogens resulting in increased crop damage. *Meloidogyne incognita* forms disease complexes on cotton with *Pythium debaryanum* Hesse (6), *Rhizoctonia solani* Kuhn (6,8), and *Fusarium oxysporum* Schlecht f. sp. *vasinfectum* (Atk.) Synd. and Hans. (9). The development of disease complexes involving *M. incognita* often results in a reduction in the plant's resistance to soilborne fungal pathogens (15). However, infection by the nematode has no effect on resistance to fusarium wilt in selected cultivars of tomato (*Lycopersicon esculentum* Mill.) or squash (*Cucurbita* spp.) (1,7).

Because of the common interaction of root-knot nematodes and the fusarium wilt pathogen on cotton, Kappelman and Bird (12) measured resistance to the fusarium wilt/root-knot nematode complex in fields infested with both pathogens. Usually resistance to ne-

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matodes is measured in terms of the plant's inability to support nematode reproduction or the plant's growth response to nematode infection (17). In Kappelman and Bird's procedure, however, plants were rated for nematode resistance only on the basis of wilt symptom development; there was no direct measurement of the plant's reaction to *M. incognita.* With many agronomic crops, high nematode resistance precludes wilt by the fungus (15). Thus, an assumption that a relative decrease in wilt is indicative of a relative increase in resistance to the nematode was integral to their study.

We have observed that the cotton cultivar 'TAMCOT SP21', which was released as a fusarium wilt/root-knot complex resistant cultivar (3), is susceptible to *M. incognita.* In a field infested with *M. incognita,* yields of TAMCOT SP21 were nearly doubled in response to soil treatment with the fumigant nematicide ethylene dibromide (Starr, Veech, and Orr, unpublished data). In this paper we report the response of cotton lines assumed to be resistant to infection by *M. incognita* based on the work of Kappelman and Bird (12), and comment on the utility of the indirect method (measuring wilt symptoms) of assessing resistance to root-knot nematodes.

MATERIALS AND METHODS

The population of *M. incognita* used for all tests was reared on 'Rutgers' tomato in the greenhouse. The following cotton lines were examined for their reaction to *M. incognita* in greenhouse or microplot tests: 'TAMCOT SP37' (susceptible to *M. incognita*) (3); CABCS'-1-81, CAHUS-2-81, and LEBOCAS'-3-81 (all putatively resistant to *M. incognita* (4,5); and 'Auburn 634' (resistant to *M. incognita*) (19).

Cotton seeds were germinated and grown on paper towel rag dolls at 30°C, and after 7 days growth, uniform seedlings were transplanted singly into 15 cm diam pots filled with a loamy sand soil (85% sand, 7% silt, and 8% clay, pH 7.5) and maintained on a greenhouse bench. Each seedling was inoculated with root-knot nematodes by pouring a suspension of 10^4 *M. incognita* eggs, collected from tomato roots using the NaOCl technique (11), over the roots at transplanting. Plants were harvested 6 weeks after inoculation and the roots washed gently under running water to remove adhering soil. Nematode eggs, extracted from a 5 g fresh-weight sample of each root system by the NaOCl technique (11), were counted under ×40 magnification using a stereomicroscope, and the number was expressed as eggs/g root fresh weight. Eight single plant replications of each cotton line were tested.

Reproduction of *M. incognita* and seed cotton yield in response to nematode infection were examined in field microplot tests. Microplots were plastic cylinders (55 cm diam by 45 cm deep) filled with and buried in a loamy sand soil (85% sand, 7% silt, 8% clay, pH 7.5). All plots were fumigated with methylbromide (1 kg/10 m^2) to eliminate existing pathogen populations. Plots were infested with *M. incognita* immediately prior to planting by mixing highly infested soil from greenhouse cultures with the noninfested soil of the microplots. Sufficient infested soil was added to the microplot soil to establish nematode population treatments of 0 (no soil added), 17, or 170 eggs and infective juveniles per 100 cm^3 soil. In 1983, six acid-delinted cotton seed (TAMCOT SP37, CABCAS'-1-81, CAHUS-2-81, or LEBOCAS'-3-81) were planted into each plot. Plots were irrigated as needed and fertilized according to soil test recommendations. Plots were hand harvested at maturity to obtain seed cotton yields per plot. Composite soil samples (eight cores 2.5 cm diam by 25 cm deep per plot) were collected from each microplot at harvest and nematode populations estimated following elutriation of soil samples (20). The reproduction index (RI = final population density/ initial population density) of *M. incognita* was determined for each cotton line. There were four microplot replications of each treatment; the experiment was a randomized complete block design.

The microplot test was repeated in 1984 using five nematode inoculum levels (0, 0.3, 3, 17, and 33 eggs and juveniles/100 cm^3) and five replications of each treatment. For this test, seed cotton yields of each cotton line (TAMCOT SP37, CABCAS'-1-81, and LEBOCAS-3-81) were regressed against the log (X + 1) transformation of the initial nematode population density (2,16) and slopes of the regression lines compared using a *t* test (14).

Table 1. Reproduction of *Meloidogyne incognita* in a greenhouse test on three cotton lines putatively resistant (PR), resistant (R), or susceptible (S) to *M. incognita.*

Cotton line	*M. incognita* eggs per g root fresh wt†
	no.
TAMCOT SP37 (S)	2750
CABCS'-1-81 (PR)	3040
CAHUS-2-81 (PR)	3190
LEBOCAS-3-81 (PR)	3110
Auburn 634 (R)	50
LSD (0.05)	850

† Values are means of eight replications.

Table 2. The reproduction index (RI) of *Meloidogyne incognita* on four cotton lines putatively resistant (PR) or susceptible (S) to *M. incognita* based on microplot tests and the yield response of cotton in nematode infected and noninfected microplots, 1983.†

Cotton line	RI	Seed cotton yield‡	
		Noninfested	Infested
		g/plot	
TAMCOT SP37 (S)	23	25	16*
CABCAS'-1-81 (PR)	42	30	10*
CAHUS-2-81 (PR)	21	30	6**
LEBOCAS-3-81 (PR)	21	28	8**
LSD (0.05)	NS	NS	NS

*,** Significantly different from the noninfested treatment at $P = 0.05$ and 0.01, respectively, based on paired *t* test.
† RI = final nematode population density/initial nematode population density.
‡ Infested plots had initial population densities of *M. incognita* of 17 eggs and infection juveniles per 100 cm^3 soil.

Table 3. The effect of initial populations (Pi) of *Meloidogyne incognita* on the reproduction index on cotton lines putatively resistant (PR) or susceptible (S) to *M. incognita* in microplot tests, 1984.†

Cotton line	Pi/100 cm^3 soil				$m \pm SE$	r^2‡
	0.3	3	17	33		
	reproduction index					
TAMCOT SP37 (S)	5280	280	70	20	3370 ± 1780	0.58*
CABCAS'-1-81 (PR)	710	50	20	10	440 ± 80	0.89**
LEBOCAS-3-81 (PR)	750	80	10	10	550 ± 100	0.86**

*,** Significant at the 0.05 and 0.01 probability levels, respectively.
† Reproduction index = final nematode population/initial nematode population.
‡ Values of the slope and standard errors of the estimate for the regression equation $y = mx + b$, where y = reproduction index and x = log (Pi + 1).

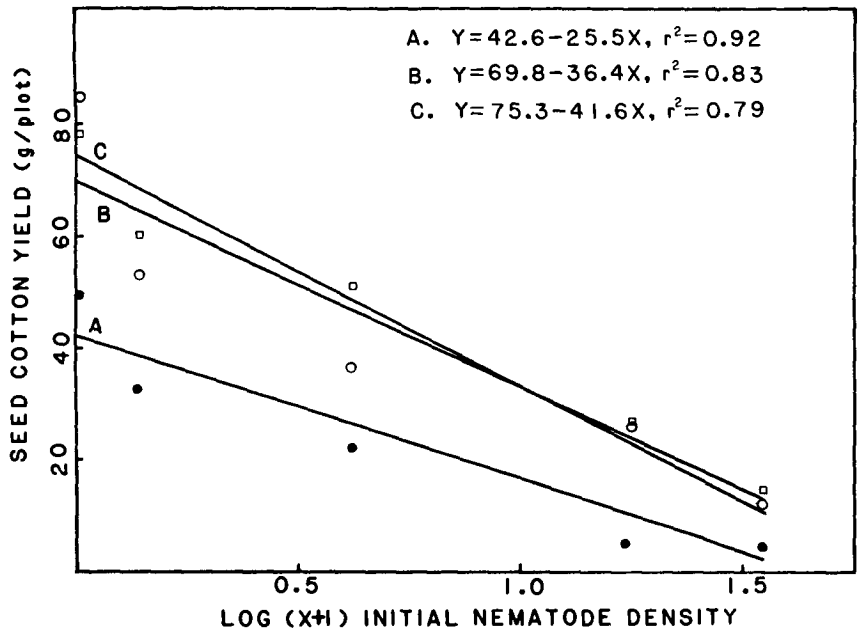

Fig. 1. Relationship of log (X + 1) transformed initial nematode population densities to seed cotton yields of A. TAMCOT SP37 (●), B. CABCS'-1-81 (○), and C. LEBOCAS-3-81 (□).

RESULTS

Meloidogyne incognita reproduced on all cotton lines tested. Reproduction was lower ($P = 0.01$) on Auburn 634 than on the other four cotton lines that were not significantly different (Table 1). Roots of all plants, except Auburn 634, were heavily galled.

In the 1983 microplot test, there was no difference among the cotton lines tested with respect to RI (nematode reproduction index) or seed cotton yields when the microplots were infested at the rate of 17 eggs and infective juveniles/100 cm^3 soil (Table 2). At 170 nematodes/100 cm^3 soil, all plants were severely damaged and most did not survive until maturity; therefore, no data were collected from these treatments.

In the 1984 test, there was a significant negative correlation between the transformed initial nematode population densities and seed cotton yields for the three cotton lines tested (Fig. 1). While the slopes of the regression lines for all cotton lines differed significantly ($P = 0.05$) from zero, the slopes for putatively nematode-resistant CABCS'-1-81 and LEBOCAS'-3-81 did not differ from that of the susceptible TAMCOT SP37. In this test, all cotton lines supported high levels of nematode reproduction (Table 3), with the RIs on the susceptible TAMCOT SP37 being nominally higher than the RIs on the two putatively nematode resistant cotton lines. However, there was no significant difference among the three cotton lines with respect to the slopes of the regression lines for the relationship between initial nematode populations and RI.

DISCUSSION

Our studies provide no evidence to support the concept presented previously (12), which state that cotton germplasms failing to show wilt symptoms in the presence of the fungus *F. oxysporum* f.sp. *vasinfectum* and *M. incognita* are necessarily resistant to the nematode. Resistance to plant-parasitic nematodes is generally defined as relative inability of the nematode to reproduce on the host (host suitability) and/or reduced host damage caused by nematode infection (host sensitivity) (17). In our tests, the putatively nematode resistant breeding lines (CABCS'-1-81, CAHUS-2-81, and LEBOCAS'-3-81) and the susceptible cultivar (TAMCOT SP37) supported nearly equal levels of *M. incognita* reproduction. The RI values for all cottons were high and not indicative of significant resistance to reproduction. A RI of less than 1.0 is indicative of a high level of resistance to nematode reproduction.

The putatively nematode resistant breeding lines and the moderately susceptible cultivar apparently were equally sensitive to infection by *M. incognita* when measured in terms of seed cotton yield. We recognize that relative yield levels are difficult to establish, however, the lack of a significant difference in the slopes of the regression lines (Fig. 1) indicates that all three cotton lines tested exhibited a similar decrease in seed cotton yields as initial nematode populations increased. While the putatively nematode resistant cotton lines did give greater total yields at each nematode-treatment level than did TAMCOT SP37, the proportional decreases in yield with increasing nematode populations were similar. If CABCS'-1-81 and LEBOCAS'-3-81 were less sensitive (more resistant) than TAMCOT SP37 to damage by *M. incognita*, then the slopes of their respective regression lines would have been significantly flatter than the slope of the regression line for TAMCOT SP37. Thus, while the breeding lines apparently have higher levels of fusarium wilt resistance than does TAMCOT SP37 (12), they do not exhibit a corresponding increase in resistance to root-knot nematodes.

While *M. incognita* does increase the susceptibility to fusarium wilt in many cotton lines, and resistance to wilt in the presence of both *F. oxysporum* f. sp. *vasinfectum* and *M. incognita* may indicate resistance to both pathogens, the data presented herein indicate

that cotton lines resistant to fusarium wilt are not necessarily resistant to the nematode. In such instances, resistance to fusarium wilt would be of a type that is unaffected by root-knot nematode infection. Abawi and Barker (1) reported that resistance to fusarium wilt in some cultivars of tomato was negated by *M. incognita* infection; Caperton et al. (7) reported a similar situation with squash. In other wilt resistant cultivars of tomato or squash, resistance to fusarium wilt was unaffected by the nematodes (1,7). Our data, taken with that of Kappelman and Bird (12), suggest that the effects of *M. incognita* on fusarium wilt of cotton may also be cultivar specific.

Based on our data, we suggest that an indirect screening procedure based solely on wilt symptoms may not accurately determine resistance in cotton to *M. incognita*. A direct assay for nematode resistance, however, is more time consuming and labor intensive than the indirect method. If a cost:benefit analysis dictates that the indirect method is preferred for routine screening, we would argue that at least the final selections should be assessed by a direct assay before cultivars are reported to be nematode resistant. Shepherd (18) suggested that indirect selection for resistance to fusarium wilt in cotton, based on resistance to *M. incognita*, is possible. Such a system may be more accurate than the one proposed by Kappelman and Bird (12), but again the possibility of cultivar-specific effects exists. Thus, while the use of indirect selection procedures may be helpful in a breeding program, and may result in selection of germplasm with resistance to both pathogens, it is necessary that at some point in the program a direct test be used to ascertain the plant's response to both *F. oxysporium* f. sp. *vasinfectum* and *M. incognita*.

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