Interactions of Virulent *Meloidogyne incognita* and Fusarium Wilt on Resistant Cowpea Genotypes

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Research funded in part by the University of California Statewide Integrated Pest Management Project and the California Blackeye Bean Council.

We thank A. Hall, D. Helms, W. Isom, and S. Temple for provision of seed and/or technical help and cooperation and C. Adams for advice on statistics.

Accepted for publication 17 July 1995.

ABSTRACT

Roberts, P. A., Frate, C. A., Matthews, W. C., and Osterli, P. P. 1995. Interactions of virulent *Meloidogyne incognita* and Fusarium wilt on resistant cowpea genotypes. Phytopathology 85:1288-1295.

Two isolates of *Meloidogyne incognita* from separate field sites in central California were virulent to five cowpea (*Vigna unguiculata*) genotypes carrying resistance gene *Rk* and three other isolates were *Rk* avirulent in findings based on egg production in greenhouse experiments. Egg production and root galling at the two field sites with virulent populations and at one field site with an avirulent population confirmed the greenhouse results. The effects of nematodes on wilt disease caused by *Fusarium oxysporum* f. sp. *tracheiphilum* and on cowpea yield were examined over 3 years at the two sites infested with virulent nematodes. Main plots in the split-plot experiments were treated with a nematicide or were not treated. Cowpea genotypes planted in subplots had combined nematode (gene *Rk*) and wilt resistance (genotypes CB46, 7964, and 8517), nematode resistance only (CB5), wilt resistance only (CB3), or no resistance (8679). Infection by *M. incognita* did not predispose wilt-resistant genotypes to wilt disease on the basis of cumulative incidence of plants with visible symptoms or midseason and late-season vascular discoloration ratings. On wilt-susceptible genotypes, wilt disease occurred in nematicide-treated plots and was exacerbated by nematode infection in nontreated plots regardless of the presence of *Rk*. The yield of wilt-resistant genotypes was suppressed an average of 17% as a result of nematode infection in nontreated plots compared with plots treated effectively with a nematicide. Wilt-susceptible genotypes had significantly lower yield than wilt-resistant genotypes in treated plots, and in nontreated plots yield of wilt-susceptible genotypes was suppressed an average of 37 to 65% because of the combined effects of nematode and wilt infections.

Additional keywords: 1,3-dichloropropene, root-knot nematode, soil fumigation.

Several cowpea (*Vigna unguiculata* (L.) Walp.) cultivars possessing gene *Rk* for resistance to *Meloidogyne incognita* (Kofoid & White) Chitwood have been developed for the blackeye dry bean industry in California. These cultivars, including California Blackeye 5 (CB5) and particularly California Blackeye 46 (CB46), are grown widely in central California and represent the majority of the blackeye plantings; another cultivar, California Blackeye 3 (CB3), which lacks gene *Rk*, is grown on a limited basis. Many other cowpea cultivars possessing gene *Rk* have been developed for other regions of the United States and in other countries. In all these areas, the growing of cultivars with root-knot nematode resistance based on resistance gene *Rk* provides the primary nematode management tactic (1,2,4,5,9,10,14,20–22).

The single dominant gene *Rk* was reported to confer resistance to *M. hapla* Chitwood and *M. javanica* (Treub) Chitwood in addition to *M. incognita* (5–7) in studies made with nematode isolates from the southeastern United States. However, earlier studies on California isolates of *M. javanica* revealed virulence or aggressiveness of these isolates on cowpeas with gene *Rk*, such as CB5 and CB7 (21,22). Thus, evidence existed for variability in the ability of *M. javanica* to parasitize and injure cowpea cultivars with the *Rk* gene. Fields in which significant root-knot nematode infection of resistant cowpeas was observed were generally assumed to be infested with *M. javanica*, which is widely distributed in cowpea production areas (9,20–22). However, in two cowpea fields approximately 200 km apart, populations of *M. incognita* were observed to cause extensive root galling and plant injury to cowpea cultivars possessing gene *Rk* (17). Subsequently, we have identified a third field infested with resistance-breaking *M. incognita*, and a survey of cowpea production sites is required to determine both the extent and pattern of distribution of these *M. incognita* infestations. The terms "virulent" and "avirulent" are used herein to describe nematode populations that are able to reproduce significantly (virulent) on host plants that prevent or suppress reproduction of other populations (avirulent) of the same nematode parasite species.

The fields also contained the cowpea Fusarium wilt organism, *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *tracheiphilum* (E. F. Sm.) W. C. Snyder & H. N. Hans. Differences in Fusarium wilt incidence and severity were observed in both fields and were associated with the planting of cowpea cultivars susceptible or resistant to wilt in the presence of the nematode. Breakdown of Fusarium resistance by predisposition in the presence of *M. javanica* has been reported in both greenhouse and field studies (8,10,21). *M. incognita* isolates, whether virulent or avirulent against gene *Rk*, apparently did not predispose wilt-resistant genotypes to disease in earlier studies (9,17).

Knowledge of both the direct effects of virulent *M. incognita* on yield potential of cowpea genotypes with the *Rk* gene and of

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any interactive effects on expression of Fusarium wilt disease in these genotypes is important in making nematode- and disease-management decisions. Currently, control of both root-knot nematodes and Fusarium wilt is based on breeding cultivars with resistance to both organisms (14). In certain crop rotation sequences, effective *M. incognita* resistance benefits succeeding crops by suppressing residual population densities. However, the existence of virulent nematode infestations makes the rational deployment of cultivars with resistance gene *Rk* more difficult and potentially much less effective. Cowpea breeding programs also may be required to account for populations of *M. incognita* with virulence to gene *Rk* and to identify and incorporate additional sources of root-knot nematode resistance (15).

The objectives of this study were i) to evaluate *M. incognita* field populations for parasitic ability and virulence on cowpea genotypes possessing resistance gene *Rk;* ii) to determine the damage potential of the virulent populations under field conditions on cowpea genotypes with and without gene *Rk;* and iii) to assess the interactive effects of virulent *M. incognita* populations on Fusarium wilt disease expression in field-grown wilt-resistant and wilt-susceptible cowpea genotypes.

MATERIALS AND METHODS

Greenhouse experiments. Isolates of *M. incognita* were collected from the three field sites described; from a cowpea field in Pixley, Tulare County, California; and from an unknown source at the University of California, Riverside (UCR). Isolates were cultured from several egg masses from greenhouse-grown plants of the susceptible tomato cultivar Tropic. Identification of isolates was made to species by female esterase and malate dehydrogenase profiles in polyacrylamide gels and by female perineal patterns (3,11) and to host race and species by the differential host test (11).

Cowpea seedlings showing vigorous growth were used in all tests. Plants were grown singly in 600-cm^3 pots (Western Pulp Products, Corvallis, OR) containing steam-sterilized loamy sand. Plants were irrigated periodically with Hoagland's solution. Inocula were prepared (12), and a suspension of 5,000 eggs in 5 ml of water was pipetted into the root zone via three holes made around each plant at 20 days after planting.

Treatments (cowpea × isolate) were arranged in a randomized complete block design and replicated six times on benches with temperature-regulated bases that maintained pot soil at 26 to 27°C. Two months after inoculation, roots were washed free of soil, damp dried, weighed, and fixed in 2% formalin. Eggs were recovered by maceration of the total root system in NaOCl solution (12).

Field experiments. Experiments were conducted over 3 years on three sites infested with *M. incognita* in the San Joaquin Valley, California. Site I was in Denair, Stanislaus County; site II was in Poplar, Tulare County; and site III was in Parlier, Fresno County. The soil classification types were site I: Hanford sandy loam (76% sand, 11% silt, 13% clay); site II: Foster fine sandy loam (72% sand, 16% silt, 12% clay); and site III: Hanford sandy loam (61% sand, 24% silt, 15% clay).

Cowpea cultivars and lines were obtained from the University of California breeding programs at Davis and Riverside. Cowpea entries were chosen on the basis of resistance to *M. incognita* (the presence of gene *Rk*) and to *F. oxysporum* f. sp. *tracheiphilum* as follows: 8679, susceptible to *M. incognita* and *F. oxysporum* f. sp. *tracheiphilum;* CB5, resistant to *M. incognita* and susceptible to *F. oxysporum* f. sp. *tracheiphilum;* CB3, susceptible to *M. incognita* and resistant to *F. oxysporum* f. sp. *tracheiphilum;* and CB46, 7964, and 8517, resistant to both *M. incognita* and *F. oxysporum* f. sp. *tracheiphilum.*

A split-plot design was used in all experiments, except year 1 at site III, which was a randomized complete block design without fumigation treatment. Cowpea entries were grown in randomized subplots within nematicide-treated and nontreated main plots. Each subplot consisted of four rows 9.5 m long spaced 0.8 m apart (sites I and III) or 1.0 m apart (site II). About 1 month after emergence, 0.9 m of each row was removed from both ends of all subplots. Each experiment was replicated four times, except year 1 at site III, which had five replications. The fumigant nematicide 1,3-dichloropropene (1,3-D) at a concentration of 0.6 liters per 100 m of row (equivalent to 78.5 liters per hectare at sites I and III and 62.0 liters per hectare at site II) was applied at least 21 days before planting through one shank per row and 30 cm deep. All plots were machine seeded with 13 seeds per meter of row. Cowpeas were grown according to standard practices for irrigated production of cowpea dry beans.

Plants in the center two rows of each subplot were hand cut near ground level 3 weeks before threshing and allowed to wind dry. Plants were passed through a small-plot thresher to collect seed, and the seed was passed through a mechanical cleaner to remove chaff. The cleaned seed per plot was weighed, and a 100-g subsample was oven dried to calculate a standardized seed dry weight with 10% moisture content. The yield index for each genotype was calculated by expressing the seed yield in the nontreated plot as a percentage of that in the treated plot within the same main plot replicate. Data are presented as the mean index from four replicates per genotype and are not based on the difference between the treatment mean yield values per genotype. Meaningful yield data were not obtained at site III because of severe insect (*Lygus* spp.) injury.

Initial nematode population densities in the soil at planting (Pi) and final population densities at harvest (Pf) were estimated from two soil samples per main plot (Pi) or per subplot (Pf), each composited from 12 cores, 2.5 × 40 cm. Samples for Pf assays were collected from the two center rows in each subplot. Second-stage juveniles (J2) and eggs were extracted by sieving a 250-cm^3 subsample through a 250-µm-pore sieve and two 45-µm-pore sieves; all screenings were extracted for 3 days in a modified Baermann funnel-mist chamber.

The numbers of eggs and J2 in roots at harvest were estimated by macerating a 10-g subsample of chopped fresh roots from 10 root systems per subplot in 1% NaOCl. These root systems were indexed for galling symptoms at harvest on a scale of 0 to 4, where 0 = no galls, 1 = 1 to 25%, 2 = 26 to 50%, 3 = 51 to 75%, and 4 = 76 to 100% of the root system galled.

Ten plants removed from the outer two rows of each subplot in midseason and the 10 plants used for nematode assays at harvest were rated for vascular discoloration in stems sliced longitudinally through and above the root-stem transition zone. At sites I and II in year 1, the number of plants per subplot with visible foliar wilt symptoms (stunting, chlorosis, epinasty, or death) was counted weekly for 7 weeks starting in the seventh week after planting when symptoms became visible. The percentage of disease incidence was calculated from the total number of plants in each subplot. *F. oxysporum* f. sp. *tracheiphilum* was not present at site III, and no Fusarium wilt disease was found.

Statistical analyses were performed with the SAS general linear models procedure for split-plot analysis of variance (field experiments) or randomized complete block analysis of variance (field experiment in year 1 at site III and greenhouse experiments) (18). All nematode population density data were transformed ($\log_{10}[n + 1]$) before analysis.

RESULTS

Nematode infection and reproduction. The greenhouse experiment was performed twice with similar results, except that infection levels were higher in the second experiment. Only results of that experiment are presented (Table 1). Egg production levels of the five *M. incognita* isolates provided a strong criterion for differentiating the presence (in genotypes Mississippi Silver,

CB5, CB46, 7964, and 8517) and absence (in genotypes 8679 and CB3) of resistance gene *Rk* and for differentiating avirulence (isolates from UCR, site III, and Pixley) and virulence (isolates from sites I and II) to gene *Rk* in nematode isolates. The virulent isolates from sites I and II produced as many or more eggs on genotypes with gene *Rk* as they did on the susceptible genotypes 8679 and CB3, indicating that gene *Rk* was unable to suppress reproduction significantly (Table 1). In contrast, the egg production levels of avirulent isolates from UCR, site III, and Pixley were significantly suppressed on resistant genotypes compared with egg production on the two susceptible genotypes. Significant differences were apparent between virulent and avirulent isolates and between resistant and susceptible cowpea genotypes on the basis of comparison of overall isolate means and overall genotype means, respectively (Table 1). The *M. incognita* isolates from site I and UCR were identified by host differential reaction (11) as race 1, and isolates from site II, site III, and Pixley were identified as race 3. Thus, both *Rk*-avirulent and *Rk*-virulent populations occurred within each of these host races.

In the field experiments, the preplant (posttreatment) mean initial *M. incognita* population densities (J2 and eggs per 250 cm^3 of soil, with 20% extraction efficiency) for treated and nontreated plots differed significantly except in year 2 at site I (Table 2). Fumigation suppressed the numbers of eggs recovered from roots in all years at site I and in year 2 but not in year 1 at site II (Tables 3 and 4). Egg production by nematode populations virulent on gene *Rk* was high on all genotypes at sites I and II in all years (Table 3 and 4). Egg production did not differ on genotypes at sites I and II, except on CB46 in year 2 at site I (Table 3). Significant fumigation treatment × genotype interactions occurred in year 3 at site I because high levels of nematode infection occurred in nonfumigated plots compared with fumigated plots. Severe Fusarium wilt injury to plants of wilt-susceptible CB5 restricted nematode reproduction and contributed to the interaction (Table 4).

Egg production and root-galling indexes of the *M. incognita* population at site III (population avirulent to resistance gene *Rk*) assessed in nontreated plots during 2 years showed consistent, significant separation of genotypes (Table 5). High levels of egg production and root galling occurred on susceptible genotypes 8679 and CB3 compared with significantly lower levels on resistant genotypes CB5, CB46, 7964, and 8517. These results show the effects of resistance gene *Rk* in restricting infection and reproduction by the avirulent *M. incognita* population at this site. However, this population was able to reproduce on resistant genotypes to some extent (Table 5), as found in the controlled greenhouse experiment (Table 1).

Fusarium wilt symptoms. The percentage of plants with vascular discoloration at harvest provided a good assay of Fusarium wilt infection in all years at sites I and II (Table 6). Midseason assessments were equally useful and allowed a similar discrimination of treatment and genotype effects (data not shown). Differences in overall levels of Fusarium wilt disease occurred between years and sites; but consistent genotype, and to some extent fumigation treatment, effects were found. Low incidence of vascular discoloration occurred on the wilt-resistant genotypes CB3, CB46, 7964, and 8517 in all experiments, and fumigation treatment did not affect ratings on these wilt-resistant cowpeas (Table 6). The means of all experiments indicated that 0 to 9.4% of resistant plants had vascular discoloration (Table 6). Vascular discoloration ratings were greater in all experiments on the wilt-susceptible genotypes 8679 and CB5; means of plants with vascular discoloration ranged from 38.0 to 89.7% (Table 6). Incidence of vascular discoloration was greater in nontreated plots than in fumigant-treated plots on wilt-susceptible genotypes, with significant differences in four of 10 comparisons (Table 6).

The percentage of plants showing visible symptoms of Fusarium wilt disease was scored weekly from the onset of symptoms for several weeks in all experiments at sites I and II. Similar trends in symptom progression were found across experiments; representative data are presented for the experiments in year 1 at sites I (Fig. 1A) and II (Fig. 1B). The wilt-resistant genotypes showed trace or no visible wilt symptoms in both fumigant-treated and nontreated plots. There were no measurable effects of high levels of *M. incognita* infection on expression of wilt resistance by the wilt-resistant genotypes CB3, CB46, 7964, and 8517. The susceptible genotypes differed in their expression of wilt disease. Wilt incidence on genotype 8679 was intermediate between the resistant genotypes and the highly susceptible CB5 (Fig. 1). The higher incidence of wilt in nontreated compared with treated plots was not significant for genotype 8679 but was significant on most sampling dates for genotype CB5 (Fig. 1). These results conform closely to the incidence of vascular discoloration later in the season (Table 6), except that the progression and expression of visible symptoms was more discriminating than vascular discoloration ratings in assessing treatment and nematode effects and for indicating resistance to wilt disease.

Yield. Seed yield (kilograms per hectare) was determined in all experiments at sites I and II. The response of yield to fumigation treatment was used to calculate a yield index to interpret the influence of nematode and wilt infection among cowpea genotypes. At site I, a significant interaction between treatment and

TABLE 1. Egg production by five isolates of *Meloidogyne incognita* on seven cowpea genotypes in a greenhouse experiment[v]

	Mean $\log_{10}$ (eggs + 1) per gram of fresh root					
	Race 1 isolates		Race 3 isolates			
Genotype	Site I	UCR[w]	Site II	Site III	Pixley	Overall genotype means
8679	3.73 a[x]	4.32 a	4.10 a	4.09 a	3.98 a	4.04 a[y]
CB3	3.09 b	4.23 a	4.25 a	3.81 a	4.36 a	3.95 a
Mississippi Silver	3.58 a	3.20 b	4.46 a	2.87 b	3.28 b	3.48 b
CB5	3.79 a	2.23 d	4.39 a	2.63 bc	3.26 b	3.26 c
CB46	4.05 a	2.85 bc	4.42 a	2.28 c	2.42 c	3.21 c
7964	3.81 a	2.53 cd	4.13 a	2.47 bc	2.89 bc	3.17 c
8517	3.59 a	2.82 bc	3.56 b	2.36 c	3.10 b	3.09 c
Overall isolate means	3.66 B[z]	3.17 C	4.19 A	2.93 D	3.33 C	

[v] Analyses of variance were performed on $\log_{10}(n+1)$ transformed egg count data.
[w] University of California, Riverside.
[x] Values in columns followed by same lowercase letter are not significantly different at $P \leq 0.05$ based on LSD t test for genotype × isolate interaction (LSD for $P = 0.05$ is 0.47 and for $P = 0.01$ is 0.62).
[y] Values for overall effects among genotypes followed by same lowercase letter are not significantly different at $P \leq 0.05$ based on LSD t test (LSD for $P = 0.05$ is 0.21).
[z] Values for overall effects among isolates followed by same uppercase letter are not significantly different at $P \leq 0.05$ based on LSD t test (LSD for $P = 0.05$ is 0.18).

genotype affected yield in years 1 and 3; only treatment and genotype main effects were significant in year 2 (Table 7). Treatment effects were greatest on the wilt-susceptible genotypes 8679 and CB5, as shown by the yield indexes of 35 to 63 for these genotypes. Yield of these genotypes was lower than that of resistant genotypes in treated plots because of wilt disease (Table 7). Wilt-resistant genotypes CB3, CB46, 7964, and 8517 had an average yield index of 83, with a range of 67 to 100. These responses to treatment can be attributed primarily to the impact of virulent nematode infection on wilt-resistant cowpeas.

TABLE 2. Initial population densities of *Meloidogyne incognita* eggs and second stage juveniles (J2) in soil samples collected from field plots after nematicide treatment at planting time[y]

Experiment	Eggs and J2 per 250 cm^3 of soil	
	Treated	Nontreated
Site I		
Year 1	0.2	22.4
Year 2	0.5	2.4
Year 3	<0.1	3.6
Site II		
Year 1	0	18.1
Year 2	<0.1	12.2
Site III		
Year 1	NA[z]	71.2
Year 2	NA	18.8

[y] Values in nematicide-treated and nontreated plots for the same site and year are significantly different ($P \leq 0.05$) according to LSD t test, except year 2 at site I.
[z] Not assayed.

TABLE 3. Egg production by *Meloidogyne incognita* on six cowpea genotypes over 2 years at sites I and II grown in nematicide-treated and nontreated field plots infested with *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *tracheiphilum*

Year Genotype	Eggs per gram of root[w] (genotype mean)	
	Site I	Site II
Year 1		
8679	2.410	2.227
CB3	2.835	2.322
CB5	1.945	2.379
CB46	2.244	2.415
7964	1.935	2.390
8517	2.588	2.292
Treatment mean		
Treated	1.678	1.718
Nontreated	3.065	2.933
LSD ($P = 0.05$)		
Treatment[x]	0.285	NS[z]
Genotype[y]	NS	NS
Year 2		
8679	2.251 b	2.779
CB3	2.628 b	2.971
CB5	2.156 b	3.184
CB46	3.240 a	3.556
7964	2.294 b	2.351
8517	2.345 b	3.108
Treatment mean		
Treated	1.727	2.023
Nontreated	3.244	3.896
LSD ($P = 0.05$)		
Treatment	1.004	0.583
Genotype	0.525	NS

[w] Data are means of four replications, analyzed and presented following transformation to $\log_{10}(n + 1)$ eggs per gram of root.
[x] Indicates differences between nematicide treatment means (treated and nontreated) averaged over genotypes (applied only in the absence of treatment × genotype interaction).
[y] Indicates differences among genotype means averaged over nematicide treatments (applied only in absence of treatment × genotype interaction). Significantly different genotype means are not followed by the same letter.
[z] Not significant.

Only genotype influenced seed yield in both years at site II (Table 8). Because fumigation treatment was less effective, nematodes suppressed yields of all genotypes in treated as well as nontreated plots, and the yield indexes did not significantly separate genotypes. Yields averaged over treatments were lower for the wilt-susceptible genotypes 8679 and particularly CB5 in most comparisons with wilt-resistant genotypes (Table 8).

DISCUSSION

The results from greenhouse and field experiments clearly demonstrate the existence of *M. incognita* populations (and the isolates cultured from them) that express virulence to root-knot resistance gene *Rk* in cowpea. Egg production levels on several cowpea genotypes possessing gene *Rk* were high and typical of a compatible or susceptible host response. Resistance conferred by gene *Rk* has been reported previously to be ineffective against some populations of *M. javanica* (8–10,20–22), but until recently there was no indication that virulence occurred within *M. incognita* (17). Because the two field sites (I and II) with populations of *M. incognita* virulent on *Rk* are geographically separated, these populations probably arose independently at each site. The sites

TABLE 4. Egg production by *Meloidogyne incognita* on six cowpea genotypes in year 3 at site I grown in nematicide-treated and nontreated field plots infested with *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *tracheiphilum*

Genotype (G)	Eggs per gram of root[x]	
	Treated	Nontreated
8679	2.270*	3.600 bcd
CB3	1.987*	3.539 cd
CB5	2.013*	2.917 d
CB46	1.799*	4.290 ab
7964	1.822*	3.954 abc
8517	1.664*	4.403 a
LSD ($P = 0.05$)		
Treatment × G_1[y]	0.726	
Treatment × G_2[z]	0.850	

[x] Data are means of four replications, analyzed and presented following transformation to $\log_{10}(n + 1)$ eggs per gram of root.
[y] Indicates differences among genotype means for the same nematicide treatment. Significantly different means in a column (treated or nontreated) are not followed by the same letter.
[z] Indicates differences between means of the same genotype with different nematicide treatments (* = significant difference) or means of different genotypes with different nematicide treatments.

TABLE 5. Egg production and root-galling indexes of *Meloidogyne incognita* on six cowpea genotypes at site III over 2 years grown in nontreated field plots infested with *Meloidogyne incognita* [w]

Genotype	Year 1		Year 2	
	Eggs per gram of root[x]	Gall index[y]	Eggs per gram of root	Gall index
8679	4.009 a	1.69 a	2.950 a	0.79 a
CB3	4.311 a	1.91 a	3.215 a	0.89 a
CB5	2.824 b	0.66 b	1.600 bc	0.11 b
CB46	1.646 d	0.29 bc	2.533 ab	0.10 b
7964	1.990 cd	0.46 bc	1.339 c	0.14 b
8517	2.366 bc	0.23 c	1.731 bc	0.03 b
LSD ($P = 0.05$)[z]	0.544	0.35	0.949	0.21

[w] Data are means of four (year 2) or five (year 1) replications. Data (year 2) are from nontreated plots only of a split-plot experiment that included nematicide-treated and nontreated main plots. Treatment × genotype interaction was significant ($P = 0.05$) for all variables. Year 1 was a randomized complete block design on nontreated plots.
[x] Egg data transformed to $\log_{10}(n + 1)$ per gram of root.
[y] Index scale: 0 = no galls, 1 = 1 to 25%, 2 = 26 to 50%, 3 = 51 to 75%, and 4 = 76 to 100% of the root system galled.
[z] LSD indicates differences among genotype means. Significantly different means in a column are not followed by the same letter.

have had different cropping systems, but cowpea cultivars with gene *Rk* have been grown frequently. It seems likely that selection for virulence has occurred on *Rk*-cowpea cultivars in each case. Recently, a third site with *Rk*-virulent *M. incognita* has been found on a different farm in the same county as site II.

The comparisons of egg production levels on resistant genotypes of avirulent and virulent nematode isolates provide a measure of the effectiveness of gene *Rk* in suppressing avirulent *M. incognita*. Some egg production occurs with all three avirulent isolates (site III, Pixley, and UCR), but at low levels. This confirms other reports that gene *Rk* resistance does not confer immunity but suppresses infection adequately for plant protection (1,2,5,20,22). The resistance conferred by gene *Rk* does not appear to be influenced significantly by the several genotype backgrounds compared in this study.

Nematode populations with virulence to *Rk* at sites I and II effectively removed the influence of nematode resistance from the treatment and genotype comparisons, because all genotypes had a susceptible host response. The Fusarium wilt-resistant genotypes (CB3, CB46, 7964, and 8517) did not exhibit evidence that wilt resistance was broken or weakened by *M. incognita* infection. Two of these genotypes, CB3 and 7964, were reported to possess different race 3 wilt-resistance genes (16), but they performed similarly in our experiments. A separate greenhouse study with isolates of the same *M. incognita* populations also demonstrated that *Rk*-virulent *M. incognita* does not break wilt resistance (9). Virulence to gene *Rk* is not functionally related (at least directly) to the factor or factors that predispose wilt-resistant cowpeas to become wilt susceptible when infected with *M. incognita*. *M. javanica* is able to overcome *Rk* gene resistance and to predispose wilt-resistant cowpeas to wilt disease, but it is not clear whether the two processes are related in that pathosystem (8–10,20,21).

On wilt-susceptible genotypes CB5 and 8679, the incidence of wilt disease and its impact on plant growth and yield were exacerbated by *M. incognita* infection. Disease progress on CB5 was significantly greater in nontreated plots in which nematode

TABLE 6. Percentage of plants with vascular discoloration symptoms at harvest of six cowpea genotypes over 3 years at sites I and II grown in nematicide-treated and nontreated field plots infested with *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *tracheiphilum*[t]

Year	Site I			Site II		
Genotype (G)	Treated	Nontreated	Mean (G)	Treated	Nontreated	Mean(G)
Year 1						
CB5	85.0 a	90.0 a	87.5	14.5	45.8	30.2 a
8679	71.3 b*	88.8 a	80.0	33.3	47.9	40.6 a
CB3	1.3 c	3.8 b	2.5	4.2	14.6	9.4 b
CB46	0.0 c	1.3 b	0.6	2.1	4.2	3.1 b
7964	0.0 c	1.3 b	0.6	2.1	14.6	8.3 b
8517	0.0 c	0.0 b	0.0	10.4	4.2	7.3 b
Treatment (T) mean	26.3	30.8		11.1	21.9	
LSD ($P = 0.05$)						
T[u]		NA[v]			NS[w]	
G[x]		NA			20.2	
$T \times G_1$[y]		7.1			NS	
$T \times G_2$[z]		7.7			NS	
Year 2						
CB5	90.7	85.6	88.5 a	46.0 a*	67.9 a	56.9
8679	89.6	89.9	89.7 a	26.7 b*	49.4 b	38.0
CB3	5.8	4.4	5.1 b	3.9 c	2.5 c	3.2
CB46	3.2	5.8	4.5 b	1.3 c	1.3 c	1.3
7964	2.9	3.5	3.2 b	0.0 c	3.2 c	1.6
8517	1.6	8.6	4.6 b	0.0 c	1.3 c	0.6
Mean (T)	32.3	31.7		13.0	20.9	
LSD ($P = 0.05$)						
T		NS			NA	
G		5.8			NA	
$T \times G_1$		NS			12.8	
$T \times G_2$		NS			15.7	
Year 3						
CB5	55.3 b*	83.8 a	69.5			
8679	78.2 a	90.4 a	84.3			
CB3	5.0 c	2.5 b	3.8			
CB46	0.0 c	6.3 b	3.1			
7964	0.0 c	0.0 b	0.0			
8517	4.9 c	2.5 b	3.7			
Mean (T)	23.9	30.9				
LSD ($P = 0.05$)						
T		NA				
G		NA				
$T \times G_1$		11.8				
$T \times G_2$		16.9				

[t] Data are means of four replications.
[u] Indicates differences between nematicide treatment means (treated and nontreated) averaged over genotypes (applied only in absence of T × G interaction).
[v] Not applicable because T × G interaction is significant.
[w] Not significant.
[x] Indicates differences among genotype means averaged over nematicide treatments (applied only in absence of T × G interaction). Significantly different means (G) are not followed by the same letter.
[y] Indicates differences among genotype means for the same nematicide treatment. Significantly different means in a column (treated or nontreated) are not followed by the same letter.
[z] Indicates differences between means of the same genotype with different nematicide treatments (* = significant difference) or means of different genotypes with different nematicide treatments.

infection was not suppressed compared with plots treated with a fumigant that selectively controlled nematode infection. Although susceptible genotype 8679 does not carry the primary wilt-resistance genes that the wilt-resistant genotypes possess and it had high vascular discoloration ratings, the slower disease progress compared with CB5 (Fig. 1) indicates that 8679 has partial or moderate resistance to infection by *F. oxysporum* f. sp. *tracheiphilum*.

Seed yields and the calculated yield indexes further discriminated between the relative impacts of nematode and wilt infections. Results from site I provided the best basis for interpretation because of the highly effective fumigant treatment in each year that reduced nematode initial population densities. Yield indexes of the wilt-resistant cowpea genotypes provided a measure of the impact of virulent *M. incognita* on yield because the wilt resistance eliminated any significant effects of wilt disease. Nematode infection suppressed seed yield by an average of 17% (index of 83), although the index range was from 67 to 100 at site I. In the absence of wilt disease, populations of *M. incognita* virulent on nematode resistance gene *Rk* can suppress cowpea yield, regardless of whether the gene is present (as in CB46, 7964, and 8517) or absent (as in CB3). Insect damage at pod set precluded collection of yield data at site III, but the nematode-resistant genotypes grew vigorously and showed no signs of nematode injury. The healthy root systems of resistant plants in these plots had little galling or nematode reproduction, which agreed with previous reports that *Rk* resistance was effective against avirulent populations of *M. incognita* (1,2,4).

The severe impact of Fusarium wilt on wilt-susceptible genotypes is shown by yields of CB5 on sites I and II. Wilt disease significantly suppressed yield in treated plots in which the nematode impact was minimal, confirming that root-knot nematode infection is not required for wilt disease expression in cowpea. In contrast, disease symptoms and yield loss to Fusarium wilt are found in cotton only in the presence of *M. incognita* infections (13,19). The low yield indexes of wilt-susceptible genotype 8679 and particularly CB5 indicated that *M. incognita* infection exacerbates wilt disease on wilt-susceptible genotypes. The indexes are particularly significant because they are based on comparisons of yield in nontreated plots with the wilt-suppressed yields in treated plots. Many plants of CB5 were stunted and died prematurely in nontreated plots heavily infested with *M. incognita*, a result of the

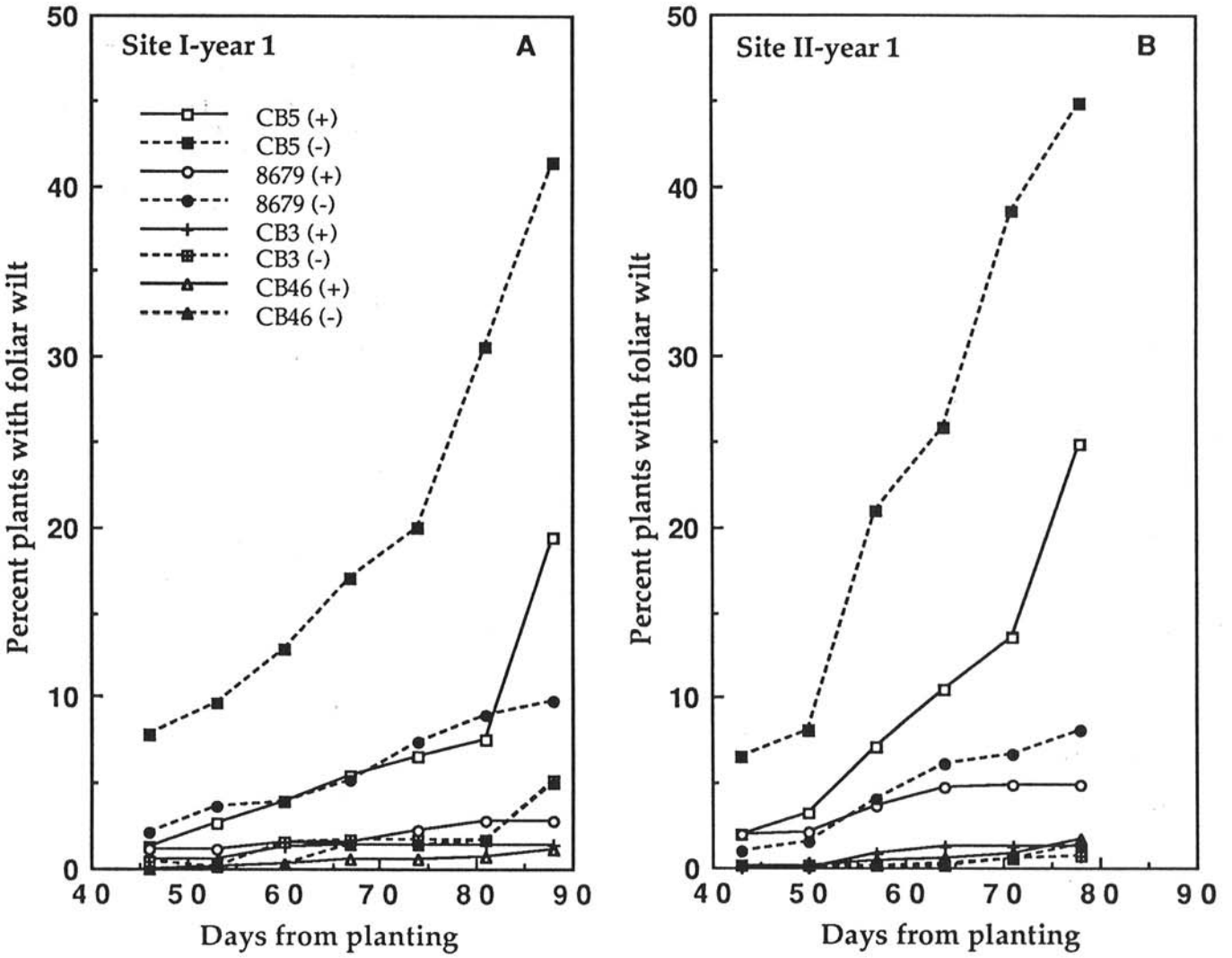

Fig. 1. Effects of cowpea genotype resistance status and soil fumigant nematicide treatment on the percentage of plants with foliar wilt symptoms from 6 weeks after planting until plant maturation in year 1 at **A**, site I and **B**, site II. On the basis of a significant treatment × genotype interaction, different ($P \leq 0.05$) means on the same day of assay were found in nontreated plots between CB5 and all other genotypes on each assay date at both sites and between 8679 and all other genotypes on days 46 and 53 at site I and on days 64 and 78 at site II. Different ($P \leq 0.05$) wilt means between treated (+) and nontreated (–) plots of the same genotype were found only for CB5 at each site. Significant ($P \leq 0.05$) mean differences for treatment × genotype interaction occurred on all assay days except days 74 and 88 at site I and days 43 and 50 at site II. On these dates, significant ($P \leq 0.05$) mean genotype effects were found between CB5 and the other genotypes. Data for CB46, 7964, and 8517, which carry the same resistance complement, were combined as CB46 because they were not different in any analysis of variance test.

TABLE 7. Seed yield and yield index (YI) of six cowpea genotypes over 3 years at site I grown in nematicide-treated and nontreated field plots infested with *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *tracheiphilum*[t]

Year Genotype (G)	Seed yield (kg/ha) Treated	Nontreated	Mean (G)	YI
Year 1				
8679	2,341 c*	1,013 b	1,676.8	46 bc
CB3	3,761 a*	2,522 a	3,141.7	67 ab
CB5	2,581 c*	902 b	1,741.3	35 c
CB46	3,458 ab	2,802 a	3,129.9	81 a
7964	3,416 ab	2,756 a	3,085.8	81 a
8517	3,150 b	2,820 a	2,985.3	89 a
Mean (T)	3,117.7	2,135.9		
LSD ($P = 0.05$)	NA[u]		NA[v]	25.5[w]
$T \times G_1$[x]	568.9			
$T \times G_2$[y]	687.0			
Year 2				
8679	1,881	1,056	1,468.4 c	58
CB3	2,841	2,279	2,560.2 b	81
CB5	1,992	1,229	1,610.8 c	63
CB46	3,181	2,695	2,937.8 a	86
7964	3,138	2,490	2,813.9 ab	81
8517	3,073	2,701	2,887.0 ab	89
Mean (T)	2,684.4	2,074.9		
LSD ($P = 0.05$)	228.6		372.4	NS[z]
$T \times G_1$	NS			
$T \times G_2$	NS			
Year 3				
8679	2,293 c*	1,372 c	1,832.3	60 cd
CB3	3,269 ab	2,978 a	3,123.1	92 ab
CB5	3,222 ab*	1,380 c	2,300.6	43 d
CB46	3,731 a*	2,828 ab	3,279.2	76 bc
7964	2,755 bc	2,751 ab	2,753.3	100 a
8517	3,198 ab*	2,307 b	2,752.4	71 bc
Mean (T)	3,077.9	2,269.1		
LSD ($P = 0.05$)	NA		NA	21.4
$T \times G_1$	660.9			
$T \times G_2$	619.2			

[t] Data are means of four replications.
[u] Indicates differences between nematicide treatment (T) means (treated and nontreated) averaged over genotypes (applied only in absence of T × G interaction). NA = not applicable because T × G interaction is significant.
[v] Indicates differences among genotype means averaged over nematicide treatments (applied only in absence of T × G interaction). Significantly different means (G) are not followed by the same letter.
[w] Indicates differences among genotype means. Significantly different YI means are not followed by the same letter.
[x] Indicates differences among genotype means for the same nematicide treatment. Significantly different means in a column (treated or nontreated) are not followed by the same letter.
[y] Indicates differences between means of the same genotype with different nematicide treatments (* = significant difference) or means of different genotypes with different nematicide treatments.
[z] Not significant.

combined effects of *M. incognita* virulence to gene *Rk* in the presence of wilt disease.

Avirulent and virulent populations with respect to resistance gene *Rk* of cowpea occur within *M. incognita*. Virulent populations have high reproduction potential on cowpea cultivars with the *Rk* gene and cause significant suppression of seed yield on these cultivars. Unlike virulent *M. javanica* populations, the virulent *M. incognita* populations do not incite breakdown of Fusarium wilt resistance in cowpea. Infection by *M. incognita* does exacerbate Fusarium wilt disease on wilt-susceptible genotypes, greatly suppressing growth and yield. The existence of *M. incognita* populations virulent to gene *Rk* makes the deployment of resistant cultivars difficult. Fusarium wilt resistance should be incorporated into all cowpea cultivars, whether or not nematodes are present as a disease component. Additional resistance factors effective against *M. incognita* and *M. javanica* populations that are virulent to gene *Rk* must be introduced into cowpea through selective breeding (15).

TABLE 8. Seed yield and yield index (YI) of six cowpea genotypes over 2 years at site II grown in nematicide treated and nontreated field plots infested with *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *tracheiphilum*[w]

Genotype	Year 1 Seed yield (kg/ha)[x]	YI	Year 2 Seed yield (kg/ha)	YI
8679	1,291.6 bc	68	1,718.6 c	77
CB3	1,826.7 a	103	2,462.0 a	95
CB5	923.4 c	101	1,351.9 d	82
CB46	1,290.3 bc	101	2,141.5 b	81
7964	1,285.1 bc	91	2,096.1 b	87
8517	1,337.9 b	82	1,683.9 c	93
LSD ($P = 0.05$)	332.0[y]	NS[z]	289.3	NS

[w] Data are means of four replications.
[x] Seed yield values are means for nematicide-treated and nontreated plots for each genotype.
[y] Indicates differences among genotype means averaged over nematicide treatments (applied only in absence of T × G interaction). Significantly different genotype means are not followed by the same letter.
[z] Indicates differences among genotype means. NS = not significant.

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