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Interactions between *Fusarium oxysporum* f. sp. *tracheiphilum* and *Meloidogyne* spp. in *Vigna unguiculata*. 1. Effects of different inoculum densities on Fusarium wilt

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The effects of chlamydospores and conidia of *Fusarium oxysporum* f. sp. *tracheiphilum* at different initial spore concentrations were compared in the wilt-susceptible cowpea cultivar California Blackeye No. 5 (CB5). In glasshouse experiments with one inoculum density of either *Meloidogyne incognita* or *M. javanica*, chlamydospores resulted in greater incidence and severity of Fusarium wilt than conidia at the same inoculum densities. Wilt symptoms also increased on wilt-resistant cultivar CB3 as inoculum densities of *M. javanica* were increased. When three cultivars were infested with moderate or high densities of both *F. o. tracheiphilum* and *M. javanica*, only CB5 developed severe wilt at either inoculum density. The wilt-tolerant cultivar Grant had mild wilt symptoms in most plants at moderate inoculum densities, and a tenfold increase in inoculum did not increase wilt ratings. CB3, however, had higher incidence and severity of Fusarium wilt symptoms at high inoculum densities, although 60% of the plants survived for 9 weeks.

INTRODUCTION

Interactions between *Meloidogyne* spp. and *Fusarium oxysporum* have been reported in many crops, and the literature on this subject was reviewed recently (Mai & Abawi, 1987). Most papers on these interactions reported the use of inoculum at either unspecified concentrations or densities that were unnaturally high. Roots commonly were wounded, either by cutting, transplanting, or making inoculation holes in the soil. Further, conidia generally were used as fungal inoculum, although chlamydospores are the natural survival structures and primary inoculum of *F. oxysporum* (Nelson, 1981). Studies of the effects of different inoculum densities have demonstrated an increase in susceptibility to Fusarium wilt at moderate or high levels of *Meloidogyne* spp. and moderate levels of *F. oxysporum* in watermelon (Sumner & Johnson, 1973) and cotton (Garber *et al.*, 1979; Starr *et al.*, 1989). Levels of inocula of *M. incognita* up to 10^3 juveniles per plant had no effect on Fusarium wilt symptoms or on infection of tomato plants by *F. oxysporum* (Jones *et al.*, 1976; Sidhu & Webster, 1981). Higher nematode inoculum levels increased chlorosis and propagule count of *Fusarium* in two tomato cultivars resistant to *F. oxysporum* (Sidhu & Webster, 1981), but did not reduce the monogenic resistance in four other cultivars (Abawi & Barker, 1984).

M. javanica has twice been reported to enhance Fusarium wilt symptoms in cowpeas (*Vigna unguiculata*). Thomason *et al.* (1959) reported that the presence of *M. javanica* increased xylem necrosis in stems of the wilt-tolerant cultivar Grant and the wilt-susceptible cultivar Chino 3 when grown in pots containing loam infested with *F. oxysporum* f. sp. *tracheiphilum*, but they did not quantify their inocula. Swanson (1984) found that the addition of *M. javanica* to sand infested with conidia of *F. o. tracheiphilum* increased vascular discoloration at the primary node in both the wilt-resistant cultivar California Blackeye No. 3 (CB3) and the wilt-susceptible cultivar California Blackeye No. 5 (CB5). In CB3, vascular discoloration increased with increasing inoculum densities of both eggs of *M. javanica* and conidia of *F. o. tracheiphilum*. However, vascular discoloration was slight except at high inoculum concentrations (5×10^5 conidia and over $2{\cdot}5 \times 10^3$ nematode eggs per plant). Swanson concluded that a predisposing interaction is possible in CB3 in the field. Further, Thomason *et al.* (1959) demonstrated that soil fumigation in cowpea

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fields reduced root-knot and Fusarium wilt in the *Fusarium*-tolerant cultivar Grant.

Before future studies could be conducted on the nature of reported interactions between *F. o. tracheiphilum* and *Meloidogyne* spp. on cowpeas, it was necessary to determine realistic inoculum densities and inoculation methods that would give moderate levels of disease with low variability. The objectives of these investigations were: to compare conidia and chlamydospores of *F. o. tracheiphilum* as inoculum; to develop techniques for inoculating cowpea with *Meloidogyne* spp. and chlamydospores of *Fusarium* without wounding roots; to determine whether *Meloidogyne* spp. can increase susceptibility to *F. o. tracheiphilum* at realistic inoculum densities of both the nematode and fungus; and to determine the identity and density of *F. o. tracheiphilum* and *Meloidogyne* spp. in a field of Fusarium wilt-resistant cowpea that showed Fusarium wilt symptoms. Part of this research has been reported briefly (Harris & Ferris, 1988a).

MATERIALS AND METHODS

All experiments were conducted in a glasshouse (24 ± 5 C) or laboratory at the University of California, Davis, from 1986 to 1989. The following materials and methods were common to all glasshouse experiments. Cowpea seeds were surface-disinfested in 0·5% NaOCl for 10 min, then rinsed by soaking in several changes of sterile distilled water for 10 min. Seeds were germinated by placing in a humid chamber for 1–2 days, then seedlings of similar size were selected and one was sown in aerated-steam-treated sand in each pot. The sand was maintained at 27 ± 5 C by keeping pots in either constant-temperature water baths or environmental growth chambers. Each plant was fertilized with half-strength Hoagland's solution (Hoagland & Arnon, 1950), generally on alternate days.

The isolate of *F. o. tracheiphilum* (160 s128) used in all experiments was race 3, identified using race differential cowpea cultivars (Harris, 1989). This isolate was collected from a cowpea field in Tulare County in 1985. Chlamydospores were produced by adding a suspension from cultures on potato-dextrose agar (PDA) to 80 g of chopped, sterile, cowpea straw plus 500 ml of nutrient solution (5 mM KNO_3 + 0·5% glucose) in 2·8-l flasks. After 2 weeks, infested straw was transferred aseptically into sterile paper bags and air-dried for 2 weeks. The dry straw was milled (Wiley mill, 40 mesh [0·42-mm] screen) and used as inoculum by mixing with dry aerated-steam-treated sand in a sterilized concrete mixer. Inoculum was quantified by dilution plating the straw on yeast-dextrose agar medium (YDA) (Nelson *et al.*, 1986) in petri dishes and counting resultant colonies.

Plants were assessed for Fusarium wilt incidence and severity with two rating systems. For disease rating, wilt symptoms were scored on a scale of 0–4, where 0 = healthy; 1 = stunted; 2 = stunted, swollen basal stem, primary leaf chlorosis, one primary leaf may have fallen, and vascular discoloration; 3 = stunted, swollen basal stem, both primary leaves fallen, and vascular discoloration; 4 = dead. This disease rating method was modified from Rigert & Foster (1987). Vascular discoloration in cross-sections of the primary node (first unifoliolate node) was scored on a scale pre-transformed to arcsines, where 0 = 0–9, 1 = 10–34, 2 = 35–64, 3 = 65–89, 4 = 90–99 and 5 = 100% of vessels discoloured (modified from Swanson, 1984). Seedlings with disease ratings of 1 do not develop other disease symptoms and usually recover from stunting (Rigert & Foster, 1987). Disease ratings of 0 or 1, or vascular discoloration scores of 0–2, indicated resistance; higher ratings indicated susceptibility. A few seedlings died or failed to develop due to genetic abnormalities, so the number of plants surviving or wilted by the end of each experiment was counted.

Effect of inoculum type and density of *F. o. tracheiphilum* and of inoculation method

Cowpea cultivar CB5 was inoculated with *F. o. tracheiphilum*, to which it is susceptible, in two experiments. Conidial inocula were prepared by placing small pieces of dried fungus, from single-spore cultures stored at 4 C, onto PDA in petri dishes. Wild-type colonies were subcultured onto PDA slants. Wild-type cultures were selected again, washed with sterile distilled water, and filtered through cheese cloth. Conidial concentrations were adjusted with the aid of a haemacytometer.

In the first experiment, conidial suspensions or dried chlamydospore-infested straw were mixed with sand in a sterilized concrete mixer. Calculated initial spore concentrations and control treatments are listed in Table 1. Infested sand for each treatment was placed in 500-cm^3 (10-cm-diameter) plastic pots approximately 375 cm^3 of sand per pot), which then were immersed in the same type of sand (uninfested) in plastic tubs. Each tub contained three pots of the same

Table 1. Recovery of *Fusarium oxysporum* f. sp. *tracheiphilum* from sterilized, air-dry sand in pots at 27 C, 19 days after inoculation, and stained *Meloidogyne incognita* in cowpea cultivar CB5 roots 5 weeks after inoculation with 10^3 eggs per plant

Type of inoculum and number of spores per pot	Initial concentration of *F. o. tracheiphilum* (spores/g of air-dry sand)	$\text{Log}_{10}(X+1)$ (initial concentration of spores/g of air-dry sand)	c.f.u./g of dry sand 19 days after inoculation	Number of mature *M. incognita* per plant
Control (no *F. o. tracheiphilum*)	0	0·000	0	53
Conidia:				
10^4	13	1·322	1	21
10^5	130	2·303	0	27
10^6	1310	3·301	15	31
10^7	13070	4·301	100	39
Chlamydospores:				
10^4	26	1·613	110	23
10^5	260	2·603	250	23
10^6	2610	3·602	4050	31
No M. incognita:				
Conidia, 10^6	1310	3·301		0
Chlamydospores, 10^6	2610	3·602		0

treatment and had no drainage holes. The tubs were immersed in constant-temperature water baths. Each tub (experimental unit) of the 10 treatments was replicated five times in a completely randomized design. Pots were left without fertilizing or watering for 2 weeks before planting, to allow fungus populations to stabilize and form resting structures. To assess survival of spores, sand was sampled from each pot 19 days after infestation with *F. o. tracheiphilum*. Samples were collected at a depth of 4–5 cm with an 11-mm-diameter cork borer. Sand from the three pots in each tub was bulked and air-dried in paper bags. A dilution series was made of each bulked sample of sand in sterile 0·1% agar water, and 1 ml of each dilution was pipetted onto petri dishes of PCNB agar (Nash & Snyder, 1962). Each dilution was replicated five times. Colonies were counted after 3 days' incubation at room temperature under fluorescent lights. Two weeks after infestation with *F. o. tracheiphilum*, 10^3 eggs of race 1 of *M. incognita* (isolate I 265) were pipetted into a hole (12 mm diameter, 20 mm deep) made in the sand in each pot. Nematode eggs were extracted with 0·5% NaOCl (Hussey & Barker, 1973) from a single-egg-mass culture maintained on tomato (*Lycopersicon esculentum* 'Rutgers') in a glasshouse. CB5 is moderately susceptible to some isolates of *M. incognita* (Swanson, 1984). Effects of *M. incognita* on Fusarium wilt were measured by including conidial and chlamydospore treatments (10^6 spores per pot) without *M. incognita*. A germinated seed of cowpea cultivar CB5 was planted immediately in each hole (one seed per pot). Four weeks after planting, each seedling was assessed for Fusarium wilt incidence and severity. The method of Byrd *et al.* (1983) was used to stain roots with acid fuchsin, except that lactic acid was substituted for HCl, and mature *M. incognita* were counted. Data on effects of inoculum concentration were analysed separately for each type of spore with the general linear models (GLM) procedure in SAS (Statistical Analysis System; SAS Institute Inc., 1985), and data on effects of *M. incognita* were subjected to analysis of variance.

In the second experiment, the same materials and methods were used, except as detailed below. Because of the low infection rate of conidia in the previous experiment, the lowest inoculum concentration of conidia (10^4 per pot) was omitted. Further, to increase the chances of infection by conidia, seedling roots were cut with a knife *in situ* 2 weeks after sowing, immediately before the conidial and nematode suspensions were poured into the knife slit. Roots were wounded only in the conidial treatments to confirm that conidia added to soil can infect cowpeas. On the day of

inoculation with conidia, germinated seeds of cowpea cultivar CB5 were planted in sand infested with chlamydospores at four different rates. One seed was planted in each 460-cm^3 (14-oz) polystyrene cup, immediately followed by inoculation with 10^3 eggs of *M. javanica* (isolate J255, a subculture of isolate J-7c-54 described later). *M. javanica* was used in this experiment to determine whether a putative virulent nematode isolate (Harris & Ferris, 1991a) could increase incidence or severity of Fusarium wilt in a susceptible cowpea cultivar, because *M. incognita* had no effect on wilt in the previous experiment. Treatments were conidia at 2×10^2, $\times 10^3$, and $\times 10^4$ per cm^3 of sand, chlamydospores at 2×10^1, $\times 10^2$, $\times 10^3$, and $\times 10^4$ per cm^3 of sand (all with *M. javanica*), control (*M. javanica* but no *F. o. tracheiphilum*) and 2×10^3 conidia or chlamydospores without *M. javanica*. Plants were grown for 5 weeks after infestation of sand. Roots were stained as described for the previous experiment, weighed, and the number of females, egg masses and galls counted. Regression coefficients and intercepts of the disease models for conidia and chlamydospores were compared by *t*-test.

Inoculum density of *M. javanica*

Germinated seeds of wilt-resistant cowpea cultivar CB3 were planted in sand in 500-cm^3 plastic pots (one seed per pot). Juveniles of *M. javanica* were pipetted immediately into each planting hole at rates of 0, 10, 10^2, 10^3 or 10^4 nematodes per pot. Nematodes were obtained from single-egg-mass cultures of isolate J-7c-54 of *M. javanica* on tomato cultivar UC82 by placing infected roots in a Seinhorst mist apparatus (Seinhorst, 1950). Juveniles were collected at 8-h intervals and stored for up to 3 days at 10–15 C with aeration, until enough were obtained for the inoculations. This putative virulent nematode isolate was collected in 1954 by I. J. Thomason from cowpeas in southern California, and was used in the interaction experiments of Thomason *et al.* (1959) and Swanson (1984), and in our subsequent experiments (Harris & Ferris, 1991a, b). Each treatment was replicated 12 times in a randomized complete block design with split plots, with environmental growth chambers as blocks and position within the chambers (front, middle or back) as main plots. Treatments were completely randomized within main plots. After 4 weeks, seedlings were transplanted carefully, to minimize root damage, into 1320-cm^3 (13·5-cm-diameter) plastic pots. Each pot contained approximately 900 cm^3 of sand infested with chlamydospores to give a density of 2×10^4 colony-forming units (c.f.u.) per cm^3 of sand. Plants were scored for disease rating, vascular discoloration at the primary node, and root galling (Daulton & Nusbaum, 1961) 8 weeks after inoculation with *F. o. tracheiphilum* (i.e. when the cowpeas were 12 weeks old) (Table 2). Galling index values were: 0 = free from galls; 1 = trace, less than 5 galls; 5 = very slight, trace to 25 galls; 10 = slight, 26 to 100 galls; 25 = moderate, galls numerous, mostly discrete; 50 = moderately heavy, galls numerous, many coalesced; 75 = heavy, galls very numerous, mostly coalesced, root growth slightly retarded; 90 = very heavy, mass invasion, slight root growth; 100 = extremely heavy, mass invasion, no root development. Data for wilt ratings were analysed with the GLM procedure in SAS.

Table 2. Effect of initial inoculum density of juveniles of *Meloidogyne javanica* on the incidence of Fusarium wilt, and on final galling index values, of wilt-resistant cowpea cultivar CB3 inoculated with *Fusarium oxysporum* f. sp. *tracheiphilum* at 2×10^4 c.f.u. per cm^3 of sand

Initial number of *M. javanica*	Proportion of plants with Fusarium wilt	Galling index value (mean ± S.D.)
0	0·09	0 ± 0
10	0·40	36 ± 16
100	0·67	39 ± 27
1,000	0·75	72 ± 16
10,000	1·00	88 ± 6

One stem section was sampled from each block for each treatment, surface-disinfested in 0·5% NaOCl, and placed in petri dishes of YDA medium (Nelson *et al.*, 1986). Emergent colonies were examined microscopically, and the presence of *F. oxysporum* was confirmed in the stems of diseased plants. Root samples also were collected from each block for each treatment, adult female nematodes were extracted from galls, and perineal patterns were cut (Hartman & Sasser, 1985) and examined to confirm that galling was caused by *M. javanica*.

Comparison of moderate and high inoculum densities of both *F. o. tracheiphilum* and *M. javanica* on three cultivars

Three cowpea cultivars were tested, and their reported reactions to *F. o. tracheiphilum* are CB3, resistant; CB5, susceptible; and Grant, tolerant

(Thomason *et al.*, 1959). Grant is a selection of Chino 3 and is the cultivar that first was reported to be more susceptible to Fusarium wilt in the presence of *M. javanica* (Thomason *et al.*, 1959). All three cultivars are susceptible to *M. javanica*. Each cultivar was inoculated at a high, moderate, or zero level of *F. o. tracheiphilum* and *M. javanica*. The high level (2×10^5 c.f.u. of *F. o. tracheiphilum* per cm^3 of sand plus 10^5 nematode eggs per plant) approximated those that Swanson (1984) reported reduced resistance to *F. o. tracheiphilum* in CB3. The moderate level (2×10^4 c.f.u. per cm^3 of sand plus 10^3 nematode eggs per plant) was considered optimal from the above experiments, based on disease level, variability and densities occurring naturally in the field. The density of *F. oxysporum* was higher than that occurring naturally in the field (Rigert, 1985), but the nematode density approximated densities that could be found in soil of severely infested fields (Harris, 1984).

Polystyrene cups (460 cm^3) were filled with chlamydospore-infested sand (as described earlier), then one germinated seed was planted in each cup. Eggs of *M. javanica* (isolate J-7c-54) were obtained as described above, and 5 ml of egg suspension was pipetted into each planting hole. Sterilized straw and distilled water were added to the sand in control treatments (zero level). Eggs of *M. javanica* were used as inoculum because they are surface-sterilized during bleach extraction, and are easier to extract. Further, eggs presumably have the potential to infect over a longer period than second-stage juveniles, and asynchronous infection undoubtedly occurs in the field. To determine nematode viability, a sample of eggs was placed on a 500-mesh (0·026-mm) sieve in tap water, and hatched juveniles were collected and counted every 1–3 days. Over 38 days, 9·1% of the eggs hatched. Cups with the same treatment were nested in groups (tubs) of three, and each group was replicated five times, as described earlier. Tubs were randomized within water baths (blocks) in a complete factorial design. Foliar Fusarium wilt symptoms were recorded daily, and as plants wilted and died, they were assessed for disease rating, vascular discoloration, root galling, stem length, and numbers of pods and seeds. Nine weeks after inoculation, all surviving plants were assessed. Results for each cultivar were analysed by one-way analysis of variance in the SAS GLM procedure, and weighted least-squares techniques were used for disease rating and vascular discoloration to minimize the residual sum of squares. This conservative adjustment was necessary to compensate for unequal variances in wilt ratings. Stem sections from plants with doubtful symptoms were plated onto PCNB agar, and resultant colonies were subcultured onto YDA slants to confirm infection by *F. oxysporum*. Root samples also were collected from each treatment, stained as described earlier, and root-knot nematodes counted.

Identity and density of *F. o. tracheiphilum* and *Meloidogyne* spp. in a cowpea field

One field of cowpea cultivar CB3 that showed Fusarium wilt symptoms was found in Tulare County, California in 1988. This field was sampled to determine the identities and natural densities of *F. o. tracheiphilum* and *Meloidogyne* spp. in field soil. Small sections were cut from the lower and middle stem of plants that showed vascular discoloration. The sections were surface-sterilized in 1·0% NaOCl for 1 min, then placed on PCNB agar. Fungi were subcultured onto PDA and carnation leaf-piece agar (Fisher *et al.*, 1982), and identified by colony appearance, microscopic morphology, and differential host test (Nelson *et al.*, 1986). Root-knot nematodes also were isolated from root galls and single-egg-mass cultures were established on tomato cultivar UC82. The nematodes were identified by perineal-pattern morphology and differential host test (Hartman & Sasser, 1985). Two to 3 months after harvest, four composite soil samples were collected from areas where Fusarium wilt symptoms had been severe. Each sample was composited from 20–25 cores, 5–30 cm deep. The soil was a sandy loam, classed as Eutric Fluvisol with a medium textured surface layer (FAO–Unesco, 1975). Ectoparasitic and juvenile nematodes were extracted from soil with a modified semiautomatic elutriator and sugar flotation technique (Byrd *et al.*, 1976). Endoparasitic nematodes and newly hatched juveniles were extracted by placing the organic matter collected from the elutriator onto Baermann funnels in a Seinhorst mist apparatus (Seinhorst, 1950) for 7 days. Nematodes were counted separately for each extraction technique with a dissecting microscope, and the total number of nematodes of each genus was calculated for each sample. *F. oxysporum* also was isolated from each composite soil sample, by dilution plating 5 g of air-dry soil onto PCNB agar by the methods of Nash & Snyder (1962). Colonies that appeared to be *F. oxysporum* were subcultured onto PDA in petri dishes, incu-

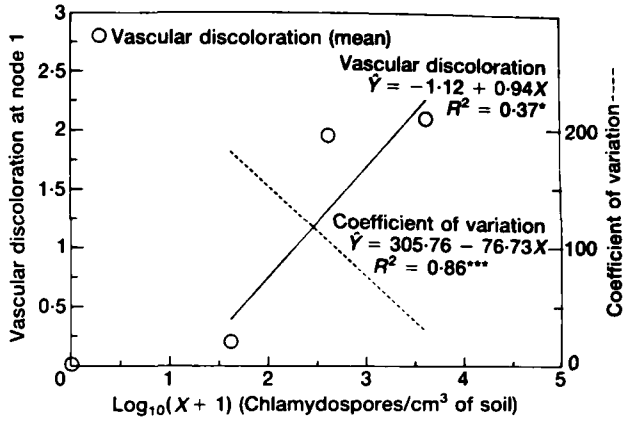

Fig. 1. Effect of inoculum density of chlamydospores of *Fusarium oxysporum* f. sp. *tracheiphilum* on vascular discoloration at the primary node of wilt-susceptible cowpea cultivar CB5 infected with *Meloidogyne incognita* (first experiment). Each data point is the mean of five replications, but regressions were not performed on means. (*), (***) Indicate significant coefficients of determination at $P = 0{\cdot}05$ and 0·001, respectively.

bated at 24–25 C in the dark for 3 days, and then colony diameter was measured. Colonies with diameter greater than 2 cm were macerated in 5 ml of sterile, distilled water, and each isolate was tested for pathogenicity by inoculating two to five seedlings of cowpea cultivars CB3 and CB5, as follows. Germinated seeds were sown in sand in trays. After 11 days, seedling roots were cut, and the seedlings were soaked in the fungal suspension for at least 2 min, before they were transplanted into aerated-steam-treated U. C. soil mix (Baker, 1957) in 190-cm^3 (6 oz) polystyrene cups. The plants were maintained in a glasshouse (approximately 27 C), and Fusarium wilt symptoms were recorded weekly after 3 weeks.

RESULTS

Effect of inoculum type and density of *F. o. tracheiphilum* and of inoculation method

When conidia were added to sand in the first experiment, the disease ratings and vascular discoloration ratings for all treatments were low ($< 0{\cdot}54$), and were not different from controls (no *F. o. tracheiphilum*) ($P < 0{\cdot}05$). Dilution plating of the sand collected from pots just after planting seedlings indicated that survival of conidia was low (Table 1). The concentration of chlamydospores, however, had a significant effect on vascular discoloration, and a linear model best described the relationship between inoculated treatments on a $\log_{10}(X + 1)$ scale ($P < 0{\cdot}01$) (Fig. 1). The coefficient of variation was inversely proportional to the $\log_{10}(X + 1)$ of the chlamydospore inoculum density. When disease rating was used as a measure of Fusarium wilt, there was a difference ($P < 0{\cdot}01$) between the control (no *F. o. tracheiphilum*) and all *Fusarium*-infested treatments. The linear model comparing infested treatments was significant at $P = 0{\cdot}053$. Linear contrasts indicated that controls (no *F. o. tracheiphilum*) had more *M. incognita* ($P < 0{\cdot}05$) than treatments infested with either conidia or chlamydospores (Table 1). *M. incognita* did not affect the severity of Fusarium wilt at one inoculum level (10^6 conidia or chlamydospores per pot), even though some nematodes infected roots and matured.

In the second experiment, the inoculum densities of conidia and chlamydospores affected both disease rating (Fig. 2) and vascular discoloration (Fig. 3) ($P < 0{\cdot}01$). Treatments infested with chlamydospores had greater disease ratings and vascular discoloration ratings than conidial treatments with root slicing at each inoculum density. The number of plants showing Fusarium wilt symptoms also increased with *Fusarium* inoculum density. For both disease rating and vascular discoloration, the intercept for chlamydospores was higher than that for conidia ($P < 0{\cdot}001$), but the regression coefficients did not differ ($P < 0{\cdot}05$). The coefficients of variation were inversely proportional to the $\log_{10}(X + 1)$ of spore concentrations, as for the previous experiment. The equations for the linear models, and their coefficients of determination, for Fusarium wilt ratings and coefficients of variation are given in the legends for Figs 2 and 3. Addition of *M. javanica* had no effect (at $P = 0{\cdot}05$) on mean

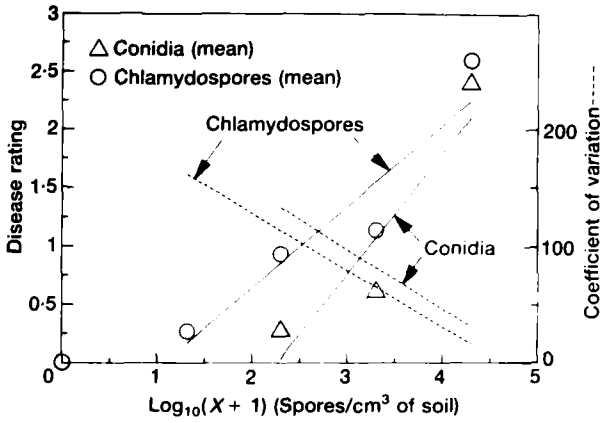

Fig. 2. Effect of inoculum density of either conidia or chlamydospores of *Fusarium oxysporum* f. sp. *tracheiphilum* on disease rating of wilt-susceptible cowpea cultivar CB5 infected with *Meloidogyne javanica* (second experiment). Each data point is the mean of five replications, but regressions were not performed on means.
For chlamydospores:
disease rating $\hat{Y} = -0{\cdot}76 + 0{\cdot}70X$ ($R^2 = 0{\cdot}52$, $P < 0{\cdot}001$);
coefficient of variation $\hat{Y} = 225{\cdot}20 - 48{\cdot}70X$ ($R^2 = 0{\cdot}61$, $P < 0{\cdot}001$).
For conidia:
disease rating $\hat{Y} = -2{\cdot}36 + 1{\cdot}04X$ ($R^2 = 0{\cdot}66$, $P < 0{\cdot}001$);
coefficient of variation $\hat{Y} = 250{\cdot}22 - 51{\cdot}09X$ ($R^2 = 0{\cdot}90$, $P < 0{\cdot}001$).

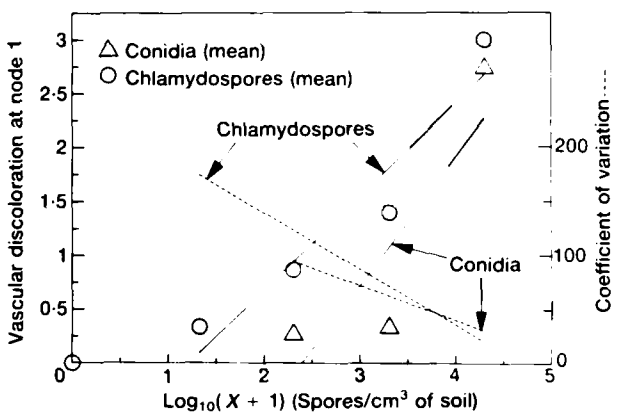

Fig. 3. Effect of inoculum density of either conidia or chlamydospores of *Fusarium oxysporum* f. sp. *tracheiphilum* on vascular discoloration at the primary node of wilt-susceptible cowpea cultivar CB5 infected with *Meloidogyne javanica* (second experiment). Each data point is the mean of five replications, but regressions were not performed on means.
For chlamydospores:
vascular discoloration $\hat{Y} = -1{\cdot}05 + 0{\cdot}87X$ ($R^2 = 0{\cdot}52$, $P < 0{\cdot}001$);
coefficient of variation $\hat{Y} = 243{\cdot}06 - 51{\cdot}87X$ ($R^2 = 0{\cdot}83$, $P < 0{\cdot}001$).
For conidia:
vascular discoloration $\hat{Y} = -2{\cdot}85 + 1{\cdot}19X$ ($R^2 = 0{\cdot}62$, $P < 0{\cdot}001$);
coefficient of variation $\hat{Y} = 169{\cdot}37 - 32{\cdot}40X$ ($R^2 = 0{\cdot}99$, $P < 0{\cdot}001$).

disease rating or vascular discoloration in the susceptible cowpea cultivar (CB5), although the number of plants showing Fusarium wilt symptoms increased from two to six in the chlamydospore treatments. Equivalent conidial treatments showed no differences in disease incidence or severity. Linear contrasts indicated that controls (*M. javanica* but no *F. o. tracheiphilum*) had more *M. javanica* per g of stained root (mean = 157) than the conidial treatments (mean = 75) ($P < 0{\cdot}001$). However, these controls had no more *M. javanica* per g (at $P = 0{\cdot}05$) than chlamydo-

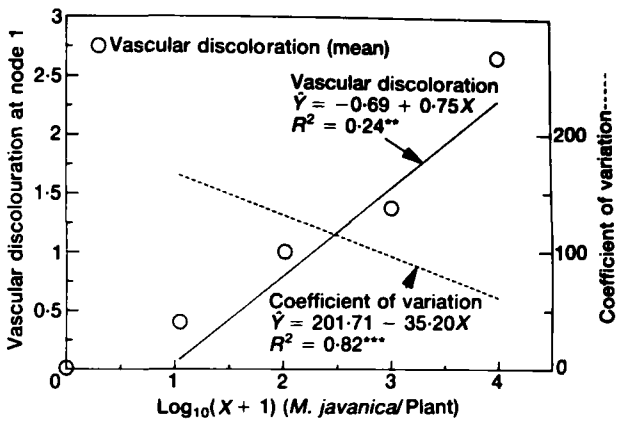

Fig. 4. Effect of inoculum density of juveniles of *Meloidogyne javanica* on vascular discoloration at the primary node of wilt-resistant cowpea cultivar CB3 inoculated with *Fusarium oxysporum* f. sp. *tracheiphilum*. Each data point is the mean of 12 replications, but regressions were not performed on means. (**), (***) Indicate significant coefficients of determination at $P = 0{\cdot}01$ and 0·001, respectively.

Table 3. Effect of *Fusarium oxysporum* f. sp. *tracheiphilum* and *Meloidogyne javanica* inoculum density on Fusarium wilt ratings, plant death, root galling, and pod and seed production in three cowpea cultivars 9 weeks after inoculation with both pathogens

Cultivar and putative resistance to wilt	Inoculum level	Disease rating	Vascular discoloration at primary node	Galling index value	Proportion of plants dead	Number of pods	Number of seeds
Grant	0	0·3 B[a]	0·2 B	0·0 B	0·00	3·2 A	14·1 A
(tolerant)	Moderate	1·5 A	2·1 A	30·4 A	0·07	4·1 A	14·1 A
	High	2·2 A	2·7 A	49·6 A	0·13	3·7 A	1·9 B
CB3	0	0·0 C	0·0 C	0·0 C	0·00	3·1 A	15·3 A
(resistant)	Moderate	1·8 B	1·7 B	28·0 B	0·13	3·3 A	11·3 A
	High	2·8 A	3·9 A	50·2 A	0·40	2·2 B	5·5 B
CB5	0	1·2 B	1·4 B	0·0 C	0·20	3·4 A	12·6 A
(susceptible)	Moderate	3·9 A	4·9 A	18·6 B	0·93	1·7 B	5·0 B
	High	3·7 A	4·7 A	28·4 A	0·93	0·9 B	2·3 B

[a] Within cultivars and columns, means not followed by a common letter differ significantly ($P < 0{\cdot}05$) according to a *t*-test.

spore treatments (mean = 135). There also was an inverse linear relationship between chlamydospore inoculum density and total number of *M. javanica* (significant at $P = 0{\cdot}05$), when controls (no *F. o. tracheiphilum*) were omitted from the analysis.

Inoculum density of *M. javanica*

Fusarium wilt symptoms were slow to develop, so that by the time of assessment some plants were partially defoliated and chlorotic, due to natural senescence and damage caused by two-spotted mites. Therefore, only data for vascular discoloration at the primary node, and not for disease rating, are considered reliable measures of Fusarium wilt disease, and are presented in Fig. 4. There was a difference ($P < 0{\cdot}001$) in vascular discoloration between treatments with *M. javanica* and controls (no *M. javanica*), at this moderately high fungal density of 2×10^4 c.f.u. per cm^3 of sand. A linear model best described the

relationship between the different inoculum levels of *M. javanica* and vascular discoloration at the primary node (Fig. 4). As initial nematode inoculum density increased, the proportion of plants showing Fusarium wilt symptoms increased, as did the final galling indices (Table 2). The regression of final galling index on $\log_{10}(X+1)$ of the initial number of *M. javanica* fitted the linear model, $\hat{Y}=3{\cdot}8+21{\cdot}6X$ ($R^2=0{\cdot}76$, $P<0{\cdot}001$).

Comparison of moderate and high inoculum densities of both *F. o. tracheiphilum* and *M. javanica* on three cultivars

In the susceptible cultivar CB5, both moderate and high inoculum densities of *F. o. tracheiphilum* and *M. javanica* resulted in severe wilt, death of 14/15 plants, and fewer pods and seeds ($P<0{\cdot}01$) (Table 3). In the wilt-tolerant cultivar Grant and the wilt-resistant cultivar CB3, moderate inoculum densities caused mild wilt symptoms in 10/14 and 12/15 plants, and only one and two dead plants, respectively, and no reduction in number of pods or seeds ($P<0{\cdot}05$). A tenfold increase in inoculum density of *F. o. tracheiphilum* and *M. javanica* increased wilt ratings in CB3 ($P<0{\cdot}05$), but not in Grant. The number of pods and seeds was reduced in CB3, but only seeds were significantly fewer in Grant. Inoculum density had no effect at $P=0{\cdot}05$ on stem length. The mean galling index value for CB5 was lower ($P<0{\cdot}05$) than the means for either CB3 or Grant, which did not differ from each other. Two Grant and six CB5 control plants became infected with *F. o. tracheiphilum*, possibly due to airborne contamination, since care was taken to avoid splashing during watering.

Identity and density of *F. o. tracheiphilum* and *Meloidogyne* spp. in a cowpea field

The fungi and nematodes isolated from cowpeas sampled from the naturally infested field in Tulare County were identified, respectively, as *F. o. tracheiphilum* race 3 and *M. incognita* race 1. The four soil samples from this field had densities of *F. o. tracheiphilum* of 335, 252, 361 and 178 c.f.u. per cm^3 of air-dry soil. The same samples, respectively, had densities of 3331, 8677, 13 063 and 3074 *Meloidogyne* spp. (all stages) per 1000 cm^3 of moist soil. Three of the samples also contained low numbers of *Pratylenchus* spp., two contained *Criconemella* spp. and *Trichodorus* spp., and one contained *Hoplolaimus* spp.

DISCUSSION

In the first experiment, only 17% (8/47) of the surviving plants of cultivar CB5 infested with conidia showed Fusarium wilt symptoms, as measured by disease ratings greater than 1. This low infection of the susceptible cultivar was apparently due to the low survival of conidia when mixed with sand (Table 1). In contrast, 57% (20/35) of surviving plants infested with chlamydospores showed Fusarium wilt symptoms. The higher number of *M. incognita* in roots of control plants (no *F. o. tracheiphilum*) could have been because of larger, healthier root systems that could support more nematodes. Some of the plants infested with *F. o. tracheiphilum* were dead or dying, and roots were already decaying when sampled, so *M. incognita* may have been lost. The number of *M. incognita* per unit of root may not necessarily have differed between treatments, so roots were weighed in the second experiment. The inability of *M. incognita* to affect Fusarium wilt was confirmed in two subsequent experiments (Harris, 1989; Harris & Ferris, 1988a, 1991a, b).

In the second experiment, chlamydospores resulted in greater disease than conidia, even though the latter were added near wounded roots. Because plants infested with conidia were 2 weeks older at the time of infestation than those infested with chlamydospores, it is possible that plants in the conidial treatments were more resistant to *F. o. tracheiphilum* due to excess vascular capacity (Beckman, 1987). However, it is more likely that chlamydospores simply had greater survival and inoculum potential than conidia. Chlamydospores are the natural survival structures and primary inoculum of *F. oxysporum* (Nelson, 1981; Beckman, 1987). Therefore, it is appropriate to use chlamydospores rather than conidia as inoculum for this type of experiment. Inoculum densities of 2×10^4 spores per cm^3 of sand were required to produce Fusarium wilt symptoms in at least 75% of the plants. This inoculum density was the only level used that resulted in mean disease ratings and vascular discoloration ratings considered to reflect susceptibility. It also was the only level with coefficients of variation less than 20%. Greater variability between plants would necessitate using impractically large numbers of replicate plants. Maximum disease levels were not achieved in either this or the previous experiment, but even 2×10^4 c.f.u. per cm^3 is high compared to densities occurring naturally in the field (Rigert, 1985; Harris, 1989). On the basis of these results, it was decided to use

chlamydospores at $2 \cdot 10^4$ c.f.u. per cm^3 of soil as the standard inoculum density in subsequent experiments (Harris, 1989; Harris & Ferris, 1988a, b, 1991a, b).

In both experiments, nematodes were fewer in the presence of *F. o. tracheiphilum*, even when nematodes per g root were considered. The hypothesis that *F. o. tracheiphilum* reduces infection by *Meloidogyne* spp. needs further investigation. More *Heterodera* juveniles penetrate healthy roots of sugar beet than roots infected with *F. oxysporum* (Jorgenson, 1970). Further, several surveys showed that *Fusarium* commonly colonizes cysts and eggs of *Heterodera* spp. (Morgan-Jones & Rodriguez-Kabana, 1987).

Cowpea cultivar CB3 remained virtually resistant to *F. o. tracheiphilum* race 3 in the absence of *M. javanica* (one plant showed some leaf chlorosis and slight vascular discoloration below the primary node). As initial inoculum densities of *M. javanica* were increased, the final galling indices also increased. Final nematode populations were not counted, however, because plants were held long enough (12 weeks) for the nematodes to produce several generations. As nematode numbers and galling increased, so did the incidence and severity of Fusarium wilt, until, at 10^4 nematodes, all surviving plants were diseased. *M. javanica*, therefore, apparently reduced the resistance of cowpea cultivar CB3 to *F. o. tracheiphilum* using these methods, even when as few as 10 nematodes were added to each plant. This interaction between *M. javanica* and *F. o. tracheiphilum* in CB3 was confirmed in six subsequent experiments (Harris, 1989; Harris & Ferris, 1988a, b, 1991a, b). These results support those of Garber *et al.* (1979), Sidhu & Webster (1981) and Swanson (1984), who used different methods and inoculum type. The results suggest that a quantitative factor associated with the nematodes lowers resistance to *F. oxysporum*. Maximum Fusarium wilt severity was not attained in this experiment, but even 10^4 *M. javanica* in this volume of soil (375 cm^3) is considered a very high level in the field (even after allowing for only 20–25% infection by added juveniles). A concentration of 10^3 juveniles per plant (or pot of approximately 500 cm^3 of soil) is closer to the densities found in infested fields that show plant damage (Harris, 1984). This inoculum level (10^3 nematodes per plant) was used as the standard in other experiments (Harris, 1989; Harris & Ferris, 1988a, b, 1991a, b).

At moderate inoculum densities of *F. o. tracheiphilum* and *M. javanica* (2×10^4 c.f.u. per cm^3 of sand plus 10^3 nematode eggs per plant), almost all CB5 plants died within 8 weeks of infestation under our experimental conditions. Most plants (>70%) of Grant and CB3 showed mild Fusarium wilt symptoms and moderate root galling, but no significant ($P < 0.05$) reduction in yield in the glasshouse. The same inoculum densities produced mild to moderate Fusarium wilt symptoms in wilt-resistant CB3 in five other experiments conducted under similar conditions (Harris, 1989; Harris & Ferris, 1988a, b, 1991a, b). At high inoculum densities (2×10^5 c.f.u. per cm^3 of sand plus 10^5 nematode eggs per plant), Grant remained tolerant to Fusarium wilt, and had only moderate vascular discoloration, although seed production decreased. These results are at variance with those of Thomason *et al.* (1959), who reported that, at a similar temperature range, xylem necrosis increased greatly if *M. javanica* were added to soil in addition to spores of *F. o. tracheiphilum*. However, they did not quantify their inoculum, so comparison of results is difficult. CB3 showed higher ($P < 0.05$) incidence and severity of Fusarium wilt symptoms at high inoculum densities, and all plants had some vascular discoloration at the primary node. Six of the 15 plants died and produced no seed pods, but the surviving plants still appeared healthy after 9 weeks, and apparently remained resistant. The increase in vascular discoloration at high levels of inoculum is similar to that reported by Swanson (1984), who concluded that a predisposing interaction is possible in CB3 in the field. However, both Swanson's results and ours suggest that most plants remain tolerant of Fusarium wilt, despite infection, at realistic inoculum densities. This conclusion is supported by field observations in California in recent years. Only two fields of CB3 showing Fusarium wilt symptoms were located and reported by extension specialists from 1985 to 1987. In 1988, letters were sent to 20 extension officers, researchers and cowpea specialists throughout California, requesting information on fields with potential interactions on Fusarium wilt-resistant cowpeas. Fusarium wilt was reported in only one additional field of cowpeas. The sampling from this field of CB3, reported above, revealed high densities (Harris, 1984) of *Meloidogyne* spp. (some identified as *M. incognita* race 1) in the soil. The density of *F. oxysporum* in field soil samples (2–3 months after harvest), however, was approximately 100 times lower than the standard inoculum density used in the above experiments possibly because of clumped distribution in the field. Root-knot

nematodes isolated from other California cowpea fields also were *M. incognita*, and most isolates of *F. o. tracheiphilum* were race 3 (Harris, 1989).

Under the conditions of our experiments, infestation of soil with chlamydospores at 2×10^4 c.f.u. per cm^3 and with *M. javanica* at 10^3 nematodes per plant gave moderate levels of disease. These inoculum densities were a compromise between densities occurring naturally in field soils, and densities required to obtain maximum Fusarium wilt with minimum variability between plants. The above densities and inoculation methods which minimize root wounding were used in subsequent studies of the nature of interactions (Harris, 1989; Harris & Ferris, 1988b, 1991a, b).

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