



Frontispiece

Fusarium-resistant (Pearson VF-11) tomato plants treated as follows (left to right):

non-inoculated control;

inoculated with Fusarium oxysporum f. sp. lycopersici alone;

inoculated with Meloidogyne javanica and F. oxysporum f. sp. lycopersici;

inoculated with M. javanica alone.

THE REDUCTION OF RESISTANCE OF TOMATO TO
FUSARIUM WILT BY MELOIDOGYNE JAVANICA (NEMATODA)

by

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B.Sc. Hons., University of East Anglia, 1970

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THE REQUIREMENTS FOR THE DEGREE OF
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Wilt by Meloidogyne Javanica (Nematoda)

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ABSTRACT

"Pearson VF-11" (VF) and "Pearson A-1 Improved" (IP) tomato (Lycopersicon esculentum) plants are moderately resistant and susceptible, respectively, to the vascular wilt fungus, Fusarium oxysporum f. sp. lycopersici, race 1. This study examined the responses of each variety to combined infections of Fusarium and the root-knot nematode, Meloidogyne javanica, under various controlled experimental conditions. Nematode infections reduced the resistance of VF, and increased the susceptibility of IP plants to Fusarium. The extent of these disease interactions was measured by comparing disease symptoms (expressed as indices of leaf chlorosis) and fungal colonisation (expressed as numbers of Fusarium propagules recovered from homogenised tissues) of plants infected with both M. javanica and Fusarium with those of plants infected with Fusarium alone. The occurrence of more extensive Fusarium colonisation of nematode-infected than of nematode-free roots suggests that galled root tissue forms a particularly favourable environment for fungal growth. There was no appreciable fungal colonisation of stems of Fusarium-resistant plants either in the presence or absence of M. javanica, while stems of Fusarium-susceptible plants were readily colonised in the absence, and more so in the presence of the nematode.

In Fusarium-resistant plants, greater disease interactions occurred a) in 4-week than in 8-week old plants, b) with increasing inoculum levels (up to 10,000 larvae per plant) of M. javanica, and c) with increasing time intervals (up to 35 days)

between inoculation with M. javanica and subsequent inoculation with Fusarium. There was a positive disease interaction in Fusarium-resistant plants when each pathogen was inoculated on a separate root system of the same plant, though a greater interaction occurred when both pathogens were inoculated on the same root system. This study has provided further support for the hypothesis that changes in a plant's physiology due to nematode infection influence its subsequent response to fungal invasion.

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INTRODUCTION

The predisposition of plants to fungal attack by nematode infections has been the subject of numerous investigations (Powell, 1971; Bergeson, 1972). Although the observed disease interactions include a wide range of pathogenic fungi and many species of both ecto- and endoparasitic nematodes, particular attention has been given to those interactions involving Fusarium vascular-wilt fungi and the sedentary, endoparasitic root-knot nematodes (Meloidogyne spp.).

Young (1939), on the basis of field trials, was the first to suggest that root-knot nematode infections can reduce the Fusarium resistance of tomatoes. Jenkins and Coursen (1957) found that the incidence of Fusarium wilt in highly-resistant "Chesapeake" tomatoes was as high as 100% in the presence of Meloidogyne incognita acrita, and 60% in the presence of M. hapla. Similar nematode infections also broke the intermediate Fusarium-resistance of "Rutgers" tomatoes. However, Binder and Hutchinson (1959), in repeating the work of Jenkins and Coursen, were unable to detect any change in the Fusarium-resistance of "Chesapeake" tomatoes by M. incognita acrita infections.

A number of subsequent studies on Meloidogyne/Fusarium interactions in tomato, some involving Fusarium-resistant and others Fusarium-susceptible varieties, have resulted in further contradictions as to whether the response to Fusarium is altered by the nematode infection (Table I). There were considerable differences in some of the experimental conditions used in these studies (Table I), and these may have been partly

responsible for the contradictory results. Binder and Hutchinson (1959), in attempting to explain the differences between their results and those of Jenkins and Coursen (1957), suggested that the size of the nematode inoculum may be an important consideration. This possibility has not been investigated further. The influence of the time interval between nematode and fungal inoculation was reported by Melendez and Powell (1967), and Porter and Powell (1967) in their work on Meloidogyne/Fusarium interactions in tobacco. They showed that plants were maximally predisposed to Fusarium infection when nematode inoculation preceded fungal inoculation by 3 or 4 weeks, and suggested that some change in host physiology, associated with the mature nematode infections, was necessary before the fungus could become optimally established. Melendez and Powell (1967) also showed that the galled tissues of nematode-infected tobacco roots were more readily colonised by Fusarium than were nematode-free root tissues.

Another indication that alterations in host physiology by nematode infections may be involved in Meloidogyne/Fusarium disease interactions was given by Bowman and Bloom (1966). They observed a reduction in the Fusarium-resistance of tomato by M. incognita even when the infection sites of the nematode and the fungus were separated on a split-root system.

The purpose of this study was to answer the following questions: --

a) Can Meloidogyne infection reduce the Fusarium-resistance of --

tomato?

- b) If so, what are some of the conditions that determine the extent of the resistance reduction?
- c) What might be the mechanism of such a disease interaction?

The study used two varieties of tomato, Lycopersicon esculentum Mill. (one resistant and one susceptible to Fusarium); the root-knot nematode, Meloidogyne javanica (Treb.) Chitwood; and the tomato vascular wilt fungus, Fusarium oxysporum f. sp. lycopersici (Sacc.) Snyder & Hans., race 1. Experiments 1 to 4 investigated the effects of 1) nematode inoculum level, 2) host plant age, 3) time interval between inoculation with each pathogen, and 4) separate infection sites for each pathogen on the extent of the disease interaction in Fusarium-resistant plants. Experiment 5 examined the disease interaction in Fusarium-susceptible plants.

Table I. A summary of the experimental conditions used in, and the results of recent studies on Meloidogyne/Fusarium interactions in tomato.

Authors	Occurrence of a positive disease interaction ^a	Nematode inoculum level (no./plant)	Age of host when nematodes inoculated	Time between inoculation with nematode and with fungus
Jenkins and Coursen, 1957	No (susceptible) Yes (resistant)	50,000 ^b	15 cm. seedlings	Simultaneous
Binder and Hutchinson, 1959	No (resistant)	1,000	> 6 weeks	14 days
Cohn and Minz, 1960	No (susceptible) Yes (resistant)	?	?	?
Bowman and Bloom, 1966	Yes (resistant)	9,000	6 weeks	14 days
Kawamura and Hirano, 1967	Yes (susceptible) No (resistant)	?	Seedlings	Simultaneous and 16 days
Goode and McGuire, 1967	Yes (resistant)	?	?	?
Bergeson et. al., 1970	Yes (susceptible)	10,000 and 16,500	2 weeks	7 and 14 days

^a Some experiments involved Fusarium-resistant and others Fusarium-susceptible tomato varieties.

^b Inoculum level quoted by Binder and Hutchinson, 1959.

GENERAL MATERIALS AND METHODS

1. SOURCES AND MAINTENANCE OF MATERIALS

A. TOMATO PLANTS

Two isolines of tomato were used in this study: "Pearson A-1, Improved" (IP), which is susceptible to Fusarium oxysporum f. sp. lycopersici; and "Pearson VF-11" (VF), which contains single-dominant-gene resistance to race 1 of Fusarium. These two varieties were chosen firstly because of their genetic similarity and secondly because their physiological responses to Fusarium have been well-documented (Mace and Veech, 1971; Beckman et al., 1972). Seeds were obtained from two sources: Peto Seed Co., Inc., Saticoy, California, U. S. A.; and Dr. J. Veech, National Cotton Pathology Research Laboratory, College Station, Texas, U. S. A. Although the specific interest of this study was the reduction of the Fusarium resistance of VF plants by M. javanica, the response of IP plants to Fusarium alone was also recorded in each experiment and compared with the extent of resistance reduction.

All plants were raised, and experiments conducted in a glasshouse at Simon Fraser University, Burnaby, B. C. Plants were grown in 10 cm. plastic pots containing a pasteurised peat/loam/perlite mixture (obtained from Burnaby Lake Greenhouses Ltd., Burnaby, B. C.). Seeds were sown directly into the pots of soil to obviate subsequent transplanting of seedlings. Every 2 weeks the soil was fertilised with a 5-10-10 commercial mixture ("Vigoro", Swift Canadian Co., Ltd., Toronto), and plants were watered daily with tap water.

Supplementary lighting from cool-white fluorescent tubes was used to maintain a 15-hour photoperiod throughout the year. The glasshouse temperature was maintained at 26° C (\pm 5° C in extreme weather conditions) with the aid of a natural gas heater and extractor fans.

B. MELOIDOGYNE JAVANICA

Lima bean plants (Phaseolus lunatus, var. L-136) served as hosts for maintaining populations of M. javanica. This variety of lima bean is particularly tolerant of M. javanica in that it supports large populations without significant galling. The initial nematode population and the bean seeds were obtained from Dr. S. D. Van Gundy, Dept. of Nematology, University of California, Riverside, U. S. A.

A stock culture was maintained through the course of the study by periodically transferring between ten and twenty egg sacs of M. javanica from the roots of old, infected to the roots of young, healthy bean plants. In order to avoid the plants becoming pot-bound, with resulting rotting of the galls and loss of nematode stock, roots were trimmed and plants repotted at 8-week intervals. This process ensured the continuation of root growth and, therefore, new sites for re-infection by the nematode larvae which were constantly hatching from eggs produced in the mature galls.

C. FUSARIUM OXYSPORUM F. SP. LYCOPERSICI

Cultures of F. oxysporum f. sp. lycopersici, race 1, were obtained from Dr. W. C. Snyder, University of California,

Berkeley, U. S. A. Virulent colonies of the fungus were maintained on test tube slopes of potato dextrose agar. Freshly-inoculated slopes were incubated initially at $21 \pm 1^{\circ}$ C for 7 days to obtain sporulating colonies, then subsequently at 5° C to minimise further growth. At approximately 6-week intervals Fusarium propagules were transferred to fresh slopes.

2. INOCULATION PROCEDURES

In all experiments, with the exception of Experiment 5 which was concerned solely with IP plants, the basic types of inoculation treatments for the two tomato varieties were as follows: --

- a) VF plants; four types of inoculation: 1) M. javanica alone, 2) Fusarium alone, 3) M. javanica plus Fusarium, 4) non-inoculated controls.
- b) IP plants; two types of inoculation: 1) Fusarium alone, 2) non-inoculated controls.

However, specific details of the treatments were not constant throughout the study, so consequently additional descriptions are given at the beginning of each experiment. There were seven replicate plants per treatment in all experiments except Experiment 5 in which there were eight.

A. M. JAVANICA INOCULATION

It was considered preferable to inoculate experimental plants with second-stage larvae, rather than egg sacs of M. javanica since, with larvae, infection was possible immediately

upon inoculation, whereas with egg sacs infection would have followed the staggered hatching period of many days. The method used, therefore, allowed a more exact estimation of the time of nematode infection. This was of particular importance in Experiment 3.

M. javanica larvae were obtained from eggs using the following procedures. With the aid of a dissecting microscope and fine forceps large numbers of mature egg sacs were removed from the galled roots of stock plants. The egg sacs, stored at room temperature in a beaker of aerated tap water, hatched over a period of 1 to 2 weeks. Twenty-four hours before inoculation the larvae and egg sacs were transferred to hatching baskets (Fig. 1) which enabled the separation of larvae from egg sacs and organic debris. The baskets, made from a fine stainless steel mesh, were lined with filters of 3-ply porous paper and suspended in crystallising dishes containing aerated tap water. Active larvae moved through the filters and collected in the dishes. After 24 hours the numbers of larvae in 1 ml. samples of a clean suspension were determined using a Doncaster counting dish, and the average count of five samples was used as an estimate of larval concentration.

Volumes of a suspension containing the desired numbers of larvae (usually 10,000 per plant) were made up to 30 ml. with tap water. Using a Pasteur pipette, each 30 ml. volume was divided into 15 x 2 ml. aliquots which were individually injected at 4 cm. depths into the soil around one plant. This method

gave fairly uniform infections of the root systems. Control plants were treated similarly but with 15 x 2 ml. aliquots of nematode-free tap water.

B. FUSARIUM INOCULATION

Fusarium inocula consisted of suspensions of conidia (mostly microconidia), large numbers of which were obtained by growing the fungus in Tochinai broth. Two-hundred ml. volumes of autoclaved broth in 500 ml. Ehrlenmeyer flasks were inoculated with Fusarium and incubated on a rotary shaker at room temperature. Seventy-two hours of fungal growth resulted in dense mixtures containing conidia and mycelia. Clean suspensions of conidia were obtained by filtering these mixtures through four layers of cheese cloth (to remove mycelia), centrifuging the filtrates at 2250 rpm for 10 minutes, then resuspending the conidia in distilled water. The process was repeated and the concentrations of conidia were adjusted, with the aid of a haemocytometer, to 5×10^6 conidia per ml., thus giving the actual inocula.

Fungal inoculations were done at specified time intervals (which varied between experiments) after the nematode inoculations. Half of the nematode-infected and half of the nematode-free plants were inoculated with Fusarium. Plants to be inoculated were removed from their pots; the roots were washed carefully in tap water to remove most of the soil without unduly damaging the root systems, then dipped into conidial suspensions for 2 minutes. Control plants were treated similarly

but the roots were dipped in Fusarium-free tap water. Immediately after treatment the plants were repotted with loosely-packed soil and watered thoroughly.

3. ESTIMATION OF DISEASE SEVERITY

A. DISEASE INDEX METHOD

The method was designed to place a quantitative estimate on the extent of visible disease symptoms. For this purpose leaf chlorosis, being the most readily-recognisable symptom of plants infected either with M. javanica and Fusarium or with Fusarium alone, was used as a disease indicator. Chlorosis was first visible in the lower leaves, usually between 8 and 12 but sometimes as long as 20 days after Fusarium inoculation, and then spread acropetally. On the first day of symptom occurrence, then subsequently every 4 - 7 days for the duration of the experiment, each fully-expanded leaf of each plant was rated for degree of chlorosis on a 0 - 4 scale (Fig. 2), according to the following criteria: --

- 0 - green healthy leaf
- 1 - slightly chlorotic leaf
- 2 - moderately chlorotic leaf
- 3 - severely chlorotic leaf
- 4 - dry, dead leaf.

For each plant, leaf ratings were totalled and divided by the number of leaves rated to obtain the disease index, which, therefore, ranged from 0, for a completely healthy plant, to 4, for a plant with all its leaves dead.

Low disease indices were frequently recorded for M. javanica - infected and control plants since some of their leaves usually showed slight chlorosis. The "disease index", therefore, although primarily intended as a quantitative estimate of symptoms of Fusarium-infection, is not disease-specific.

B. FUSARIUM PROPAGULE COUNT METHOD

This method, which was designed to place a quantitative estimate on the extent of Fusarium colonisation, measured the number of Fusarium propagules recovered from root and shoot tissues at the completion of an experiment. Plants were processed for propagule counts after the final disease indices had been recorded, which was between 25 and 41 days after Fusarium inoculation.

Half of the root system of each plant was removed and washed thoroughly in tap water, particular attention being paid to the removal of organic debris which may have harboured saprophytically-growing fungal mycelia. Replicate root samples from all of the plants comprising each treatment were combined, and total fresh weights determined. Roots were then cut into 1 cm. pieces and macerated in distilled water using a Virtis 45 homogeniser or a Waring Blendor. Stem tissue macerations, which consisted usually of the lower 25 cm. portions, but in Experiment 1 of the lower, middle and upper 7.5 cm. portions of the stems, were prepared similarly. Petioles were not included in these preparations. Microscopic examination of macerated

Fusarium-infected plant tissues revealed fungal conidia and mycelium fragments which presumably constituted the propagules.

Volumes of the homogenised suspensions containing 1 g fresh weight samples of root or stem tissue were diluted with sterile distilled water to give 1:10³ and 1:10⁴ dilutions. Five 1 ml. samples of each dilution were pipetted onto separate plates of a selective peptone-pentachloronitrobenzene (PCNB) agar (Nash and Snyder, 1962, modified by Bergeson et al., 1970). The plates were then incubated at 21 ± 1° C, and after 5 days fungal colonies were counted with the aid of a "Gallenkamp" colony-counter. Colonies of F. oxysporum f. sp. lycopersici were identified morphologically by their smooth, flat, off-white or sometimes dirty yellow appearance (Fig. 3). The identification was verified firstly by microscopic examination, and secondly by testing the responses of Fusarium-susceptible tomato plants to infection by several isolates of the colonies. Since the method of identification did not guarantee 100% accuracy, low background counts (presumably representing morphologically-similar Fusarium colonies) were usually recorded for the non-Fusarium-infected treatments. Assuming that each colony represented one fungal propagule, the results are expressed as numbers of propagules per gram fresh weight of root or shoot tissue.

"Student's" t-test was used for the statistical analysis of all results.

Figure 1. Hatching basket used to separate active Meloidogyne javanica larvae from egg sacs and organic debris.

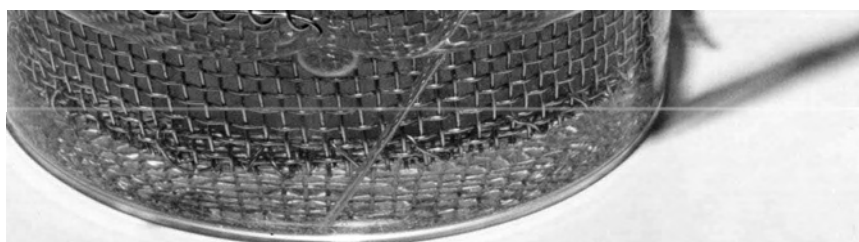


Figure 2. Degrees of chlorosis of tomato leaves rated by the disease index method according to the following criteria: --

- 0 - green, healthy
- 1 - slightly chlorotic
- 2 - moderately chlorotic
- 3 - severely chlorotic
- 4 - dry, dead.

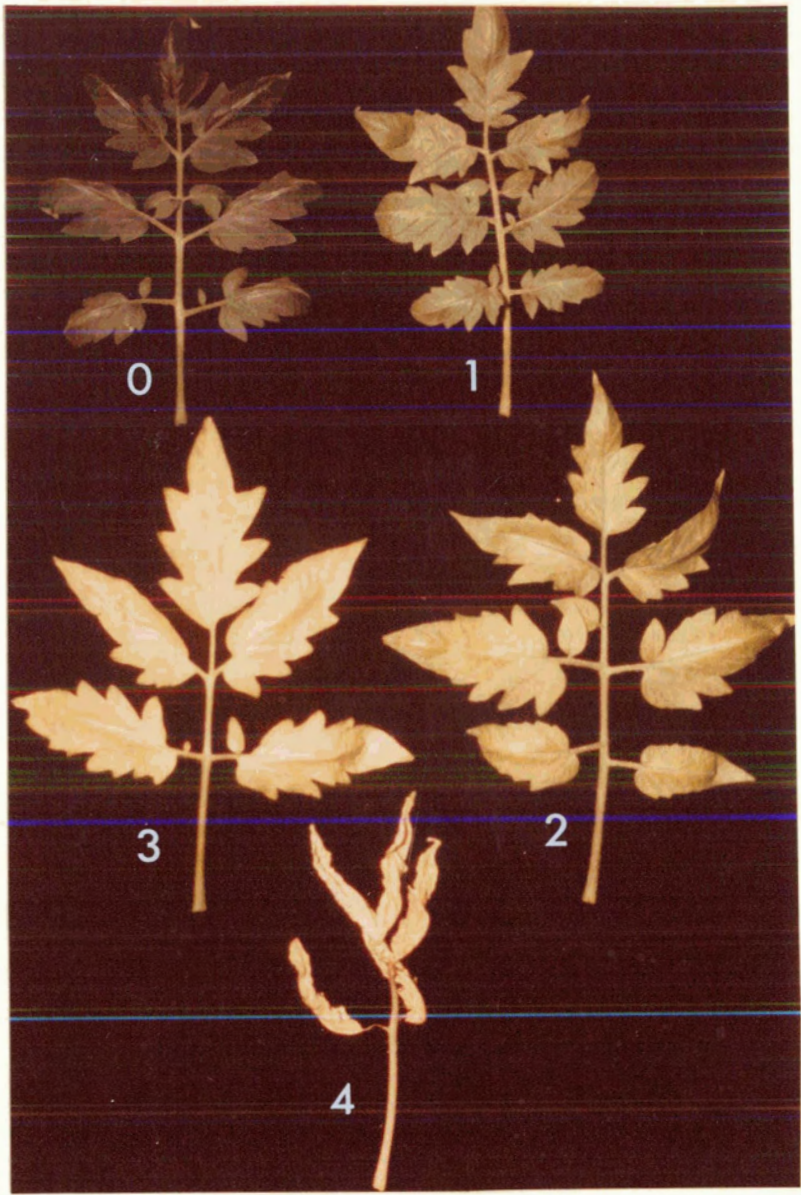
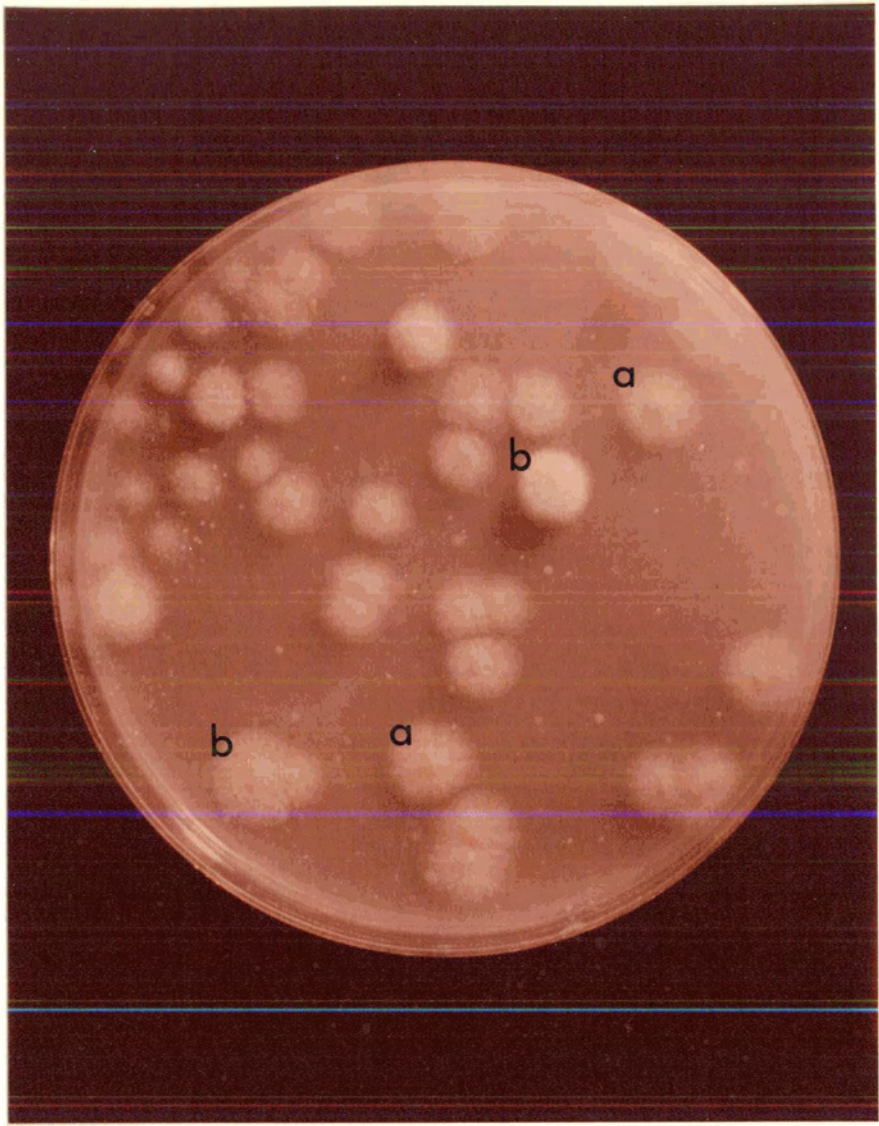


Figure 3. PCNB agar plate 5 days after inoculation with 1 ml. of dilute homogenised tomato root tissue infected with Fusarium oxysporum f. sp. lycopersici. Colonies of F. oxysporum f. sp. lycopersici (a); colonies of other Fusarium species (b).

17a.



EXPERIMENTS AND RESULTS

SECTION I. THE EFFECT OF VARIOUS FACTORS ON THE
REDUCTION OF FUSARIUM-RESISTANCE OF
TOMATO PLANTS BY MELOIDOGYNE JAVANICA

EXPERIMENT 1

INOCULUM LEVEL OF MELOIDOGYNE JAVANICA

MATERIALS AND METHODS

Using the methods described previously, 4-week old Fusarium-resistant (VF) tomato plants were treated with one of three inoculum levels of M. javanica: 1,000, 5,000 or 10,000 larvae per plant, which represented light, moderate and heavy infections, respectively. Fusarium was inoculated 22 days later. The plant treatments and their respective codes are shown in Table II.

Disease indices were taken 8, 14, 21, and 28 days after Fusarium inoculation and plants were processed for Fusarium propagule counts 7 days after the final index readings. Unfortunately, at the time of this experiment the morphological appearance of F. oxysporum f. sp. lycopersici colonies on PCNB agar had not been definitely characterised, so the propagule counts were of colonies of all species of Fusarium which grew on the inoculated plates. However, this problem may be partially overcome by comparing the propagule counts of Fusarium-infected with those of Fusarium-free plants.

The experiment was conducted between mid-March and early-June of 1973.

RESULTS

Disease symptoms, in the form of leaf chlorosis, were first seen in Fusarium-infected plants 8 days after inoculation.

Thereafter, symptoms increased steadily in all infected plants, but particularly abruptly in Fusarium-susceptible plants (treatment IP.F). Means of the disease indices recorded on each day were plotted (Fig. 4) and tabulated with their standard errors (Table III).

At all three inoculum levels, Fusarium-resistant (VF) plants infected with both M. javanica and Fusarium tended to show greater disease symptoms than did those infected with Fusarium alone. However, this trend, which began 14 days after Fusarium inoculation, was statistically significant ($P < 0.05$) only for the highest inoculum level (VF. M10000F), 21 and 28 days after Fusarium inoculation, when the disease indices were 71 and 48%, respectively, greater than those for VF plants inoculated with Fusarium alone (VF.F). Differences in disease indices between the VF.M1000F, VF.M5000F and VF.M10000F treatments were not statistically significant, though VF.M10000F plants appeared to show the greatest symptoms. However, even these symptoms never approached the severity of those of the Fusarium-susceptible response (IP.F).

Disease indices for Fusarium-infected plants were not significantly ($P < 0.05$) greater than those for the Fusarium-free controls until 14 days after fungal inoculation. The final disease index recorded for Fusarium-resistant plants infected with Fusarium alone was as high as 32% of that for the Fusarium-susceptible response. This indicates that the resistance of VF plants is not complete and explains why I

describe this variety as "moderately" resistant. The disease indices for plants inoculated with 5,000 larvae of M. javanica alone (VF.M5000) rose unexpectedly sharply, particularly after the 21-day reading, due to fairly extensive leaf chlorosis in four of the plants. Apart from the VF.M5000 treatment, disease indices of nematode-infected plants were not significantly different from those of non-infected controls.

The numbers of propagules of Fusarium species recovered from roots at the end of the experiment are shown in Fig. 5. Since colonies of all species of Fusarium were counted, readings for the non-Fusarium-infected plants were relatively high and, presumably, represent part of the rhizosphere population of various Fusarium species. Nevertheless, significantly ($P < 0.01$) more propagules were found in the roots of Fusarium-inoculated than in those of non-Fusarium-inoculated plants.

Roots of plants infected with both M. javanica and Fusarium contained significantly ($P < 0.01$) more propagules than did those of plants infected with Fusarium alone, and the number recovered increased with increasing inoculum levels of M. javanica. Fusarium colonisation of roots in the VF.M1000F, VF.M5000F and VF.M10000F treatments was 56, 113 and 148% greater, respectively, than that in the VF.F treatment. Although the root propagule count for the VF.M10000F treatment was significantly ($P < 0.01$) greater than that for the VF.M1000F treatment, the count for the VF.M5000F treatment was not significantly different from either of these. Fungal

colonisation of infected Fusarium-susceptible roots (IP.F) was considerably greater than that found in any treatment of Fusarium-resistant plants.

There was very little fungal colonisation of the stems of Fusarium-resistant plants whose roots had been inoculated with either Fusarium alone or both Fusarium and M. javanica (Table IV). Colonisation of stems of Fusarium-infected susceptible plants, however, was extensive. Surprisingly high propagule counts were recorded for stems of the IP.C and VF.C treatments, and these cannot be accounted for but presumably resulted from contamination.

Table II. Treatment codes for Experiment 1. Tomato plants treated with or without Fusarium oxysporum f. sp. lycopersici in the presence or absence of Meloidogyne javanica.

Plant variety	<u>M. javanica</u> inoculated	Inoculum level (larvae/plant)	Treatment code	
			<u>Fusarium</u> inoculated ^a	<u>Fusarium</u> not inoculated
IP	No	-----	IP.F	IP.C
VF	No	-----	VF.F	VF.C
VF	Yes	1,000	VF.M1000F	VF.M1000
VF	Yes	5,000	VF.M5000F	VF.M5000
VF	Yes	10,000	VF.M10000F	VF.M10000

^a22 days after M. javanica inoculations.

Figure 4. The effect of inoculum level of Meloidogyne javanica on the disease indices^a recorded for tomato plants at various times after subsequent inoculation with Fusarium oxysporum f. sp. lycopersici.

^aBased on degrees of leaf chlorosis (Fig. 2).

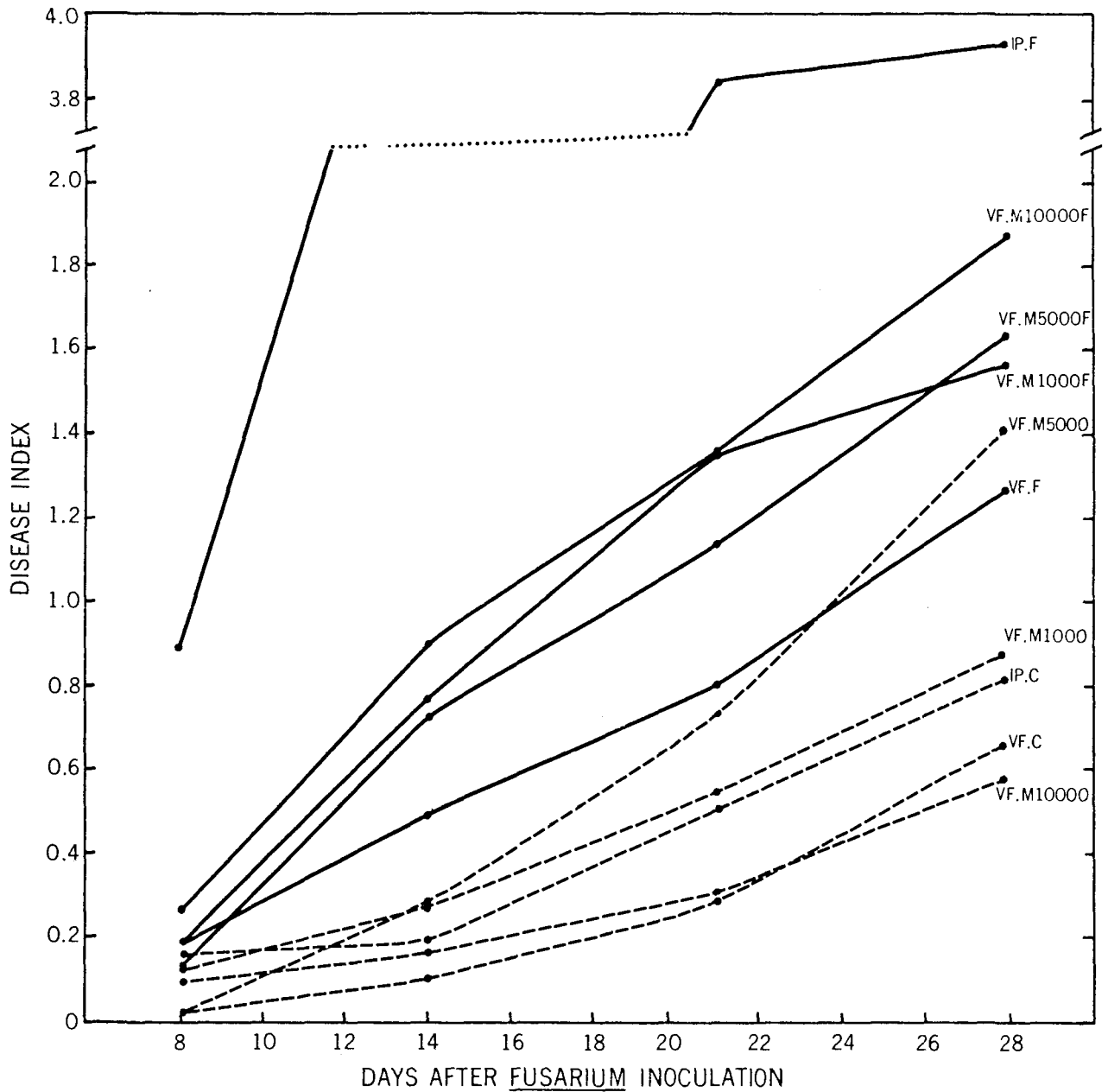


Table III. The effect of inoculum level of Meloidogyne javanica on the disease indices^a (means[±]S.E.) recorded for tomato plants at various times after subsequent inoculation with Fusarium oxysporum f. sp. lycopersici.

Plant Treatment	Disease indices at various times after <u>Fusarium</u> inoculation			
	8 Days	14 Days	21 Days	28 Days
IP.C	0.16 [±] 0.05	0.19 [±] 0.06	0.51 [±] 0.09	0.83 [±] 0.10
VF.C	0.02 0.02	0.10 0.04	0.28 0.11	0.66 0.20
VF.M1000	0.12 0.03	0.27 0.07	0.54 0.13	0.89 0.22
VF.M5000	0.02 0.02	0.29 0.12	0.72 0.23	1.42 0.41
VF.M10000	0.09 0.03	0.17 0.03	0.31 0.05	0.58 0.08
IP.F	0.88 0.09	2.84 0.18	3.84 0.03	3.93 0.03
VF.F	0.18 0.08	0.50 0.10	0.80 0.06	1.27 0.05
VF.M1000F	0.16 0.06	0.78 0.15	1.36 0.26	1.57 0.24
VF.M5000F	0.12 0.03	0.73 0.14	1.13 0.21	1.65 0.22
VF.M10000F	0.27 0.08	0.91 0.18	1.37 0.23	1.88 0.22

^aBased on degrees of leaf chlorosis (Fig. 2).

Figure 5. The effect of inoculum level of Meloidogyne javanica on the number (means \pm S.E.) of propagules of Fusarium species per gram fresh weight of tomato root tissue following inoculation with Fusarium oxysporum f. sp. lycopersici.

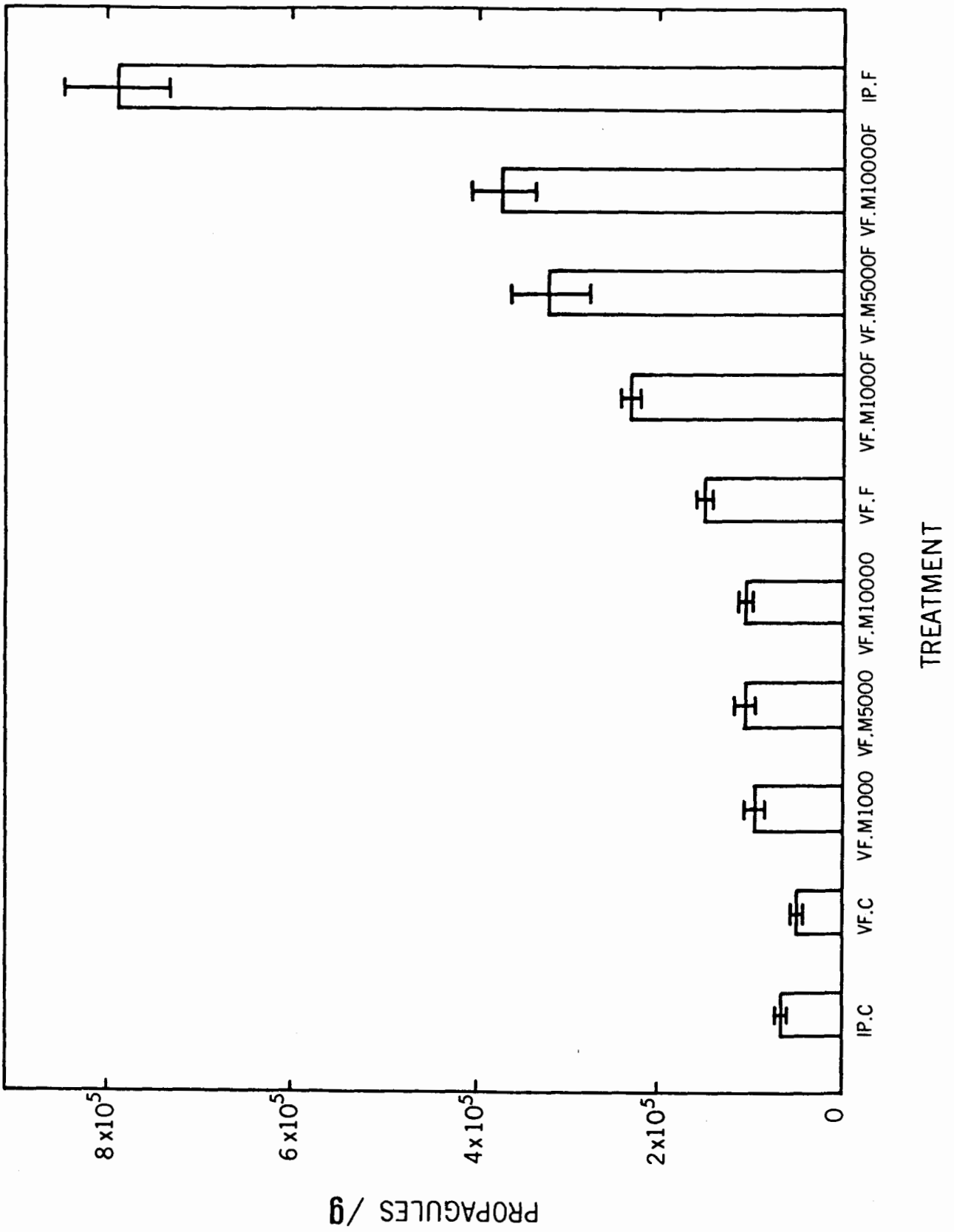


Table IV. The effect of inoculum level of Meloidogyne javanica on the number of propagules of Fusarium species per gram fresh weight of tomato stem tissue following inoculation with Fusarium oxysporum f. sp. lycopersici.

Plant Treatment	Number of Propagules/g
IP.C	$19^{\pm}2 \times 10^3$
VF.C	$107^{\pm}7 \times 10^3$
VF.M1000	0
VF.M5000	0
VF.M10000	0
IP.F	$166^{\pm}8 \times 10^3$
VF.F	$3^{\pm}1 \times 10^3$
VF.M1000F	$2^{\pm}1 \times 10^3$
VF.M5000F	$3^{\pm}1 \times 10^3$
VF.M10000F	$2^{\pm}1 \times 10^3$

EXPERIMENT 2

AGE OF THE TOMATO HOST

MATERIALS AND METHODS

Fusarium-resistant (VF) tomato plants of two different ages, 4-weeks ("young") and 8-weeks ("old"), were inoculated with 10,000 larvae of M. javanica per plant. After weighing the root systems of ten equivalent "young" and "old" plants, the relative inoculum sizes were estimated as 26,000 and 9,000 M. javanica larvae, respectively, per gram fresh weight of root. At the time of nematode inoculation "young" plants had four and "old" plants eight true leaves. Fusarium was inoculated 22 days later. The plant treatments and their respective codes are shown in Table V.

Disease indices were taken 12, 16, 20 and 26 days after Fusarium inoculation and plants were processed for Fusarium propagule counts immediately after the final index readings.

The experiment was conducted between mid-April and late-July of 1973.

RESULTS

The disease indices recorded for treatments of Fusarium-resistant plants with both M. javanica and Fusarium (VF.Y.MF and VF.O.MF) were significantly ($P < 0.05$) greater than those for treatments with Fusarium alone (VF.Y.F and VF.O.F), (Fig. 6 and Table VI). This trend was more apparent in "young" than in "old" plants. Also, the disease symptoms of "young" plants which had

been inoculated with both pathogens tended to be more extensive than those of similarly-treated "old" plants, though this difference was not statistically significant.

"Young" Fusarium-infected susceptible plants (IP.Y.F) expressed greater disease symptoms than did the "old" plants (IP.O.F). Twenty days after inoculation, plants of the IP.Y.F treatment were almost dead, giving a disease index of 3.52 which was significantly ($P < 0.01$) greater than the index of 1.90 for plants of the IP.O.F treatment. There were no apparent differences, however, between disease indices of "young" and "old" VF plants when infected with either Fusarium or M. javanica alone.

VF plants of both ages exhibited a high degree of Fusarium resistance, in terms of symptom development, when inoculated with Fusarium alone, since the maximum disease indices obtained for these treatments were less than 6% of those of the Fusarium-susceptible responses. Indices for both "young" and "old" Fusarium-infected susceptible plants were significantly ($P < 0.01$) greater than those for resistant plants infected with both pathogens, from 20 days after Fusarium inoculation onwards. In "young" VF plants significantly ($P < 0.001$) greater disease indices were recorded for nematode-infected than for control plants on the 20- and 26-day readings, but this did not occur in "old" VF plants.

For both age groups of plants, significantly ($P < 0.001$) more Fusarium propagules were recovered from the roots of

resistant plants infected with both M. javanica and Fusarium (VF.Y.MF and VF.O.MF) than from those infected with Fusarium alone (VF.Y.F and VF.O.F), (Fig. 7). Roots of "young" VF and IP plants which had been inoculated with Fusarium alone harboured significantly ($P < 0.001$) more (69 and 300%, respectively) Fusarium propagules than did those of the similarly-inoculated "old" plants. Also, significantly ($P < 0.001$) more propagules were recovered from the roots of "young" than from those of "old" VF plants infected with both pathogens. None of the treatments of resistant plants resulted in such extensive Fusarium colonisation of roots as that found in the Fusarium-infected susceptible plants (IP.Y.F and IP.O.F).

Very few Fusarium propagules were recovered from stems of VF plants whose roots had been inoculated with Fusarium, whether or not M. javanica also was present. However, large numbers of propagules occurred in the stems of both "young" ($492 \pm 40 \times 10^3$ /g fresh weight) and "old" ($432 \pm 27 \times 10^3$ /g fresh weight) Fusarium-infected susceptible plants.

Table V. Treatment codes for Experiment 2. Tomato plants treated with or without Fusarium oxysporum f. sp. lycopersici in the presence or absence of Meloidogyne javanica.

Plant variety	<u>M. javanica</u> <u>inoculated</u>	Plant age	Treatment code	
			<u>Fusarium</u> <u>inoculated</u> ^a	<u>Fusarium not</u> <u>inoculated</u>
IP	No	Young ^b	IP.Y.F	IP.Y.C
IP	No	Old ^c	IP.O.F	IP.O.C
VF	No	Young	VF.Y.F	VF.Y.C
VF	No	Old	VF.O.F	VF.O.C
VF	Yes	Young	VF.Y.MF	VF.Y.M
VF	Yes	Old	VF.O.MF	VF.O.M

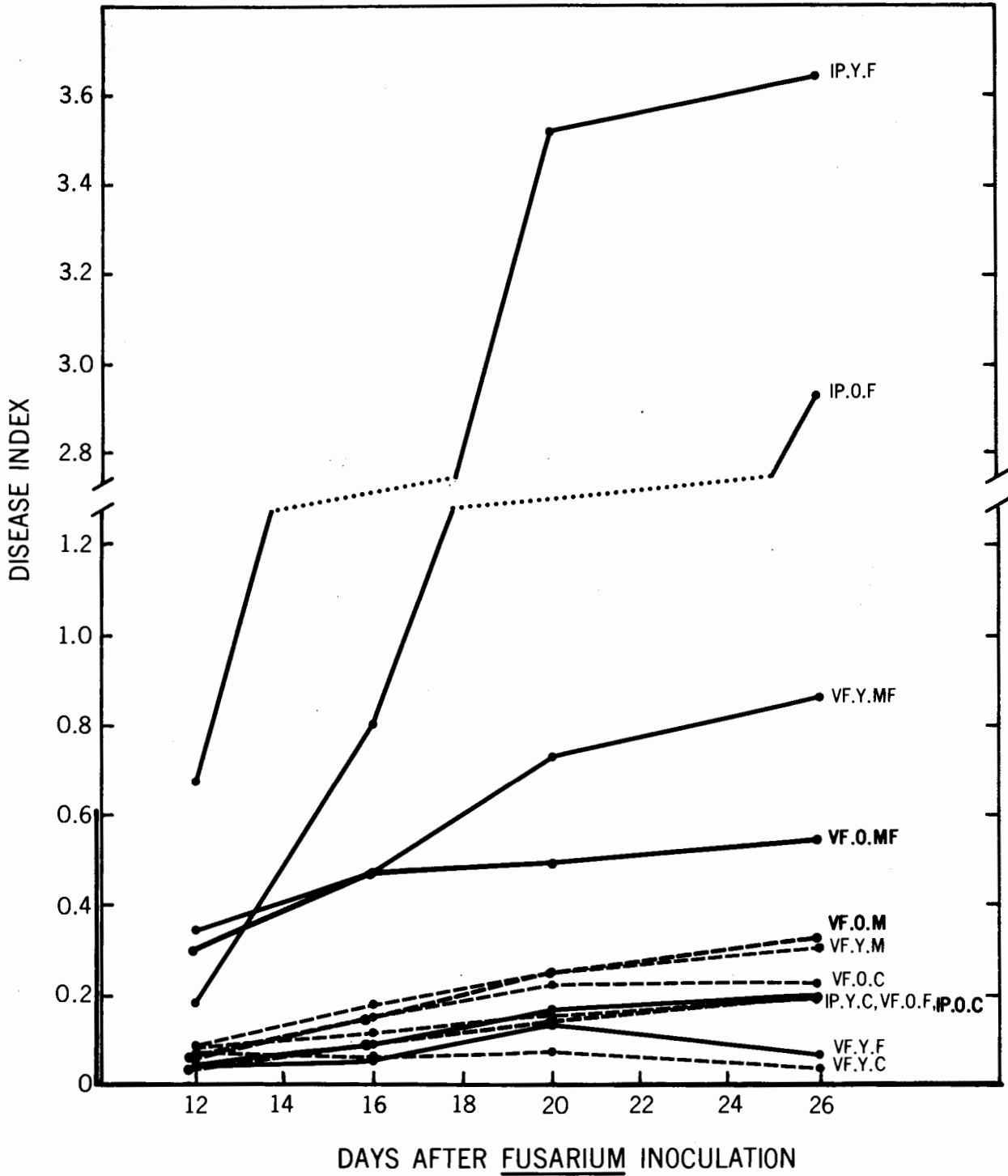
^a22 days after M. javanica inoculations.

^bPlants 4 weeks old at the time of M. javanica inoculations.

^cPlants 8 weeks old at the time of M. javanica inoculations.

Figure 6. The effect of age of tomato plants on the disease indices^a recorded at various times after inoculation with Fusarium oxysporum f. sp. lycopersici in the presence or absence of Meloidogyne javanica.

^aBased on degrees of leaf chlorosis (Fig. 2).



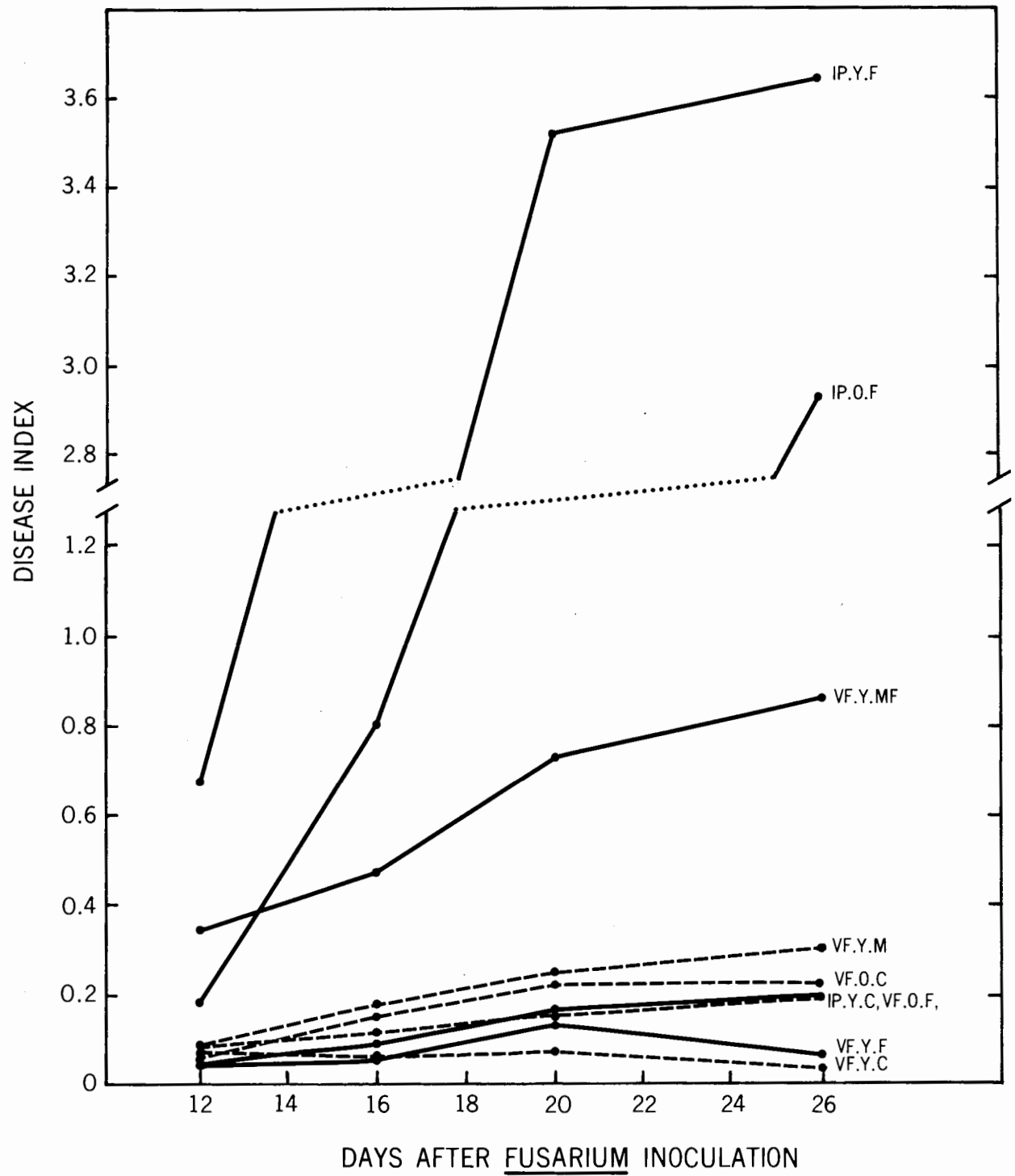
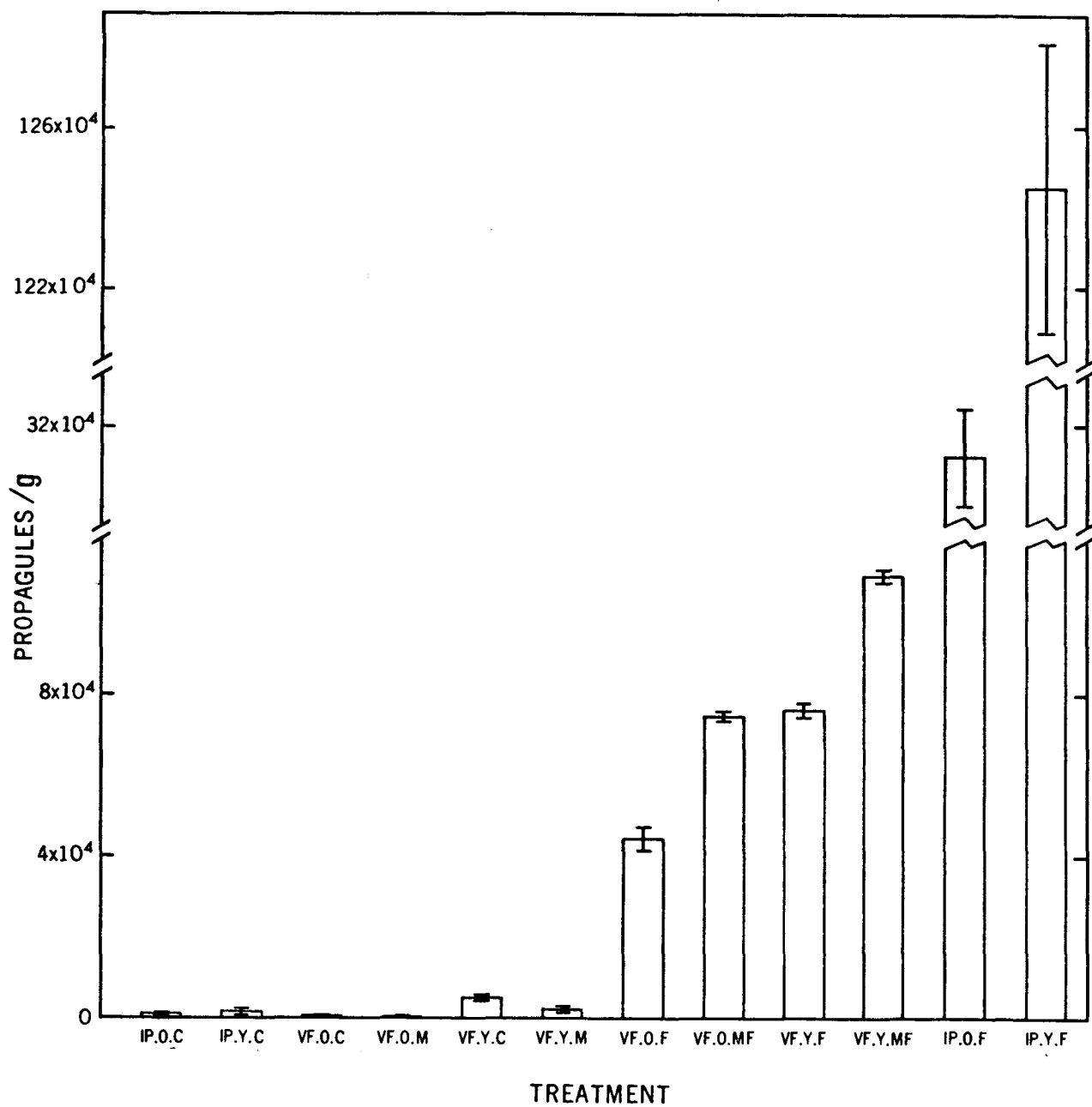


Table VI. The effect of age of tomato plants on the disease indices^a (means \pm S.E.) recorded at various times after inoculation with Fusarium oxysporum f. sp. lycopersici in the presence or absence of Meloidogyne javanica.

Plant Treatment	Disease indices at various times after <u>Fusarium</u> inoculation			
	12 Days	16 Days	20 Days	26 Days
IP.O.C	0.03 [±] 0.02	0.09 [±] 0.02	0.14 [±] 0.03	0.19 [±] 0.03
IP.Y.C	0.08 0.04	0.11 0.04	0.15 0.02	0.19 0.08
VF.O.C	0.06 0.03	0.15 0.03	0.22 0.06	0.22 0.05
VF.Y.C	0.07 0.05	0.06 0.04	0.07 0.02	0.03 0.02
VF.O.M	0.05 0.02	0.15 0.04	0.25 0.05	0.33 0.08
VF.Y.M	0.08 0.05	0.18 0.06	0.25 0.03	0.30 0.06
IP.O.F	0.18 0.07	0.80 0.26	1.90 0.37	2.93 0.28
IP.Y.F	0.67 0.13	2.09 0.33	3.52 0.24	3.64 0.27
VF.O.F	0.04 0.03	0.09 0.03	0.16 0.04	0.19 0.05
VF.Y.F	0.04 0.04	0.05 0.04	0.13 0.05	0.06 0.05
VF.O.MF	0.29 0.07	0.47 0.09	0.49 0.12	0.54 0.13
VF.Y.MF	0.34 0.13	0.47 0.13	0.73 0.12	0.86 0.12

^aBased on degrees of leaf chlorosis (Fig. 2).

Figure 7. The effect of age of tomato plants on the number (means \pm S.E.) of Fusarium propagules per gram fresh weight of root tissue following inoculation with Fusarium oxysporum f. sp. lycopersici in the presence or absence of Meloidogyne javanica.



EXPERIMENT 3

TIME INTERVAL BETWEEN INOCULATION WITH MELOIDOGYNE JAVANICA
AND WITH FUSARIUM OXYSPORUM F. SP. LYCOPERSICI

MATERIALS AND METHODS

Four groups of Fusarium-resistant (VF) tomato plants were inoculated with 10,000 M. javanica larvae per plant, 35, 25, 15, and 5 days before subsequently inoculating with Fusarium. The times of nematode inoculation were staggered so that the plants could be inoculated with Fusarium on the same day, at which time all plants were 9 weeks old. The four time intervals were chosen so that the infecting nematodes would have developed to the following stages by the time of Fusarium inoculation: --

- 5 days - second-stage larvae feeding on the induced giant cells;
- 15 days - developing nematodes in the process of moulting and, therefore, not feeding;
- 25 days - adult females feeding and growing rapidly;
- 35 days - adult females feeding and extruding eggs.

The plant treatments and their respective codes are shown in Table VII.

Disease indices were taken 10, 14, 20 and 25 days after Fusarium inoculation and plants were processed for Fusarium propagule counts 1 day after the final index readings.

The experiment was conducted between mid-April and mid-July of 1973.

RESULTS

Greater disease symptoms occurred in VF plants which had been inoculated with both M. javanica and Fusarium than in those which had been inoculated with Fusarium alone (VF.F), and the longer the time interval between inoculation with each pathogen the higher were the disease indices recorded (Fig. 8 and Table VIII). When the interval was 5 or 15 days (VF.M5F and VF.M15F, respectively) the increases in disease indices over the VF.F treatment were small and not statistically significant. The increases were significant ($P < 0.01$), however, with intervals of 25 and 35 days (VF.M25F and VF.M35F). The disease indices for these treatments, taken 25 days after Fusarium inoculation, were 45 and 82%, respectively, greater than the corresponding index for the VF.F treatment. For treatments involving inoculation with both pathogens, the only significant ($P < 0.05$) differences in disease indices were between a) the VF.M35F and VF.M5F, and b) the VF.M35F and VF.M15F treatments.

Ten days after Fusarium inoculation there was no significant difference in disease indices between plants of the VF.M35F treatment and Fusarium-infected susceptible plants (IP.F). Subsequently, however, indices for the susceptible response increased more rapidly and, 15 days later, were 100% greater than those of the VF.M35F treatment. Disease indices recorded for non-Fusarium-infected plants were all quite low (0.02 - 0.42) except for those of the VF.M25 treatment, though

the higher indices resulting from this treatment were not significantly different from those of the M. javanica-free controls.

Significantly ($P < 0.05$) more Fusarium propagules were recovered from roots of VF plants infected with both M. javanica and Fusarium than from those of plants infected with Fusarium alone (VF.F) with the exception of the 15-day inoculation interval treatment (VF.M15F) in which 21% fewer propagules were recovered (Fig. 9). The increases over the VF.F treatment in numbers of propagules recovered from roots were 14, 46, and 39% for the VF.M5F, VF.M25F and VF.M35F treatments, respectively. There was no significant difference in root propagule counts between the 25- and the 35-day interval treatments. The highest propagule count for the roots of resistant plants infected with both pathogens (155×10^3 propagules/g in the VF.M25F treatment) was only 31% of that for the roots of Fusarium-infected susceptible plants (IP.F).

With the exception of a few in the VF.M5F treatment, no Fusarium propagules were found in the stem tissues of Fusarium-infected resistant plants, whether or not M. javanica also was present. Nevertheless, large numbers ($476 \pm 35 \times 10^3$ /g) of propagules were recovered from the stems of Fusarium-infected susceptible plants. Comparatively low propagule counts ($10 \pm 1 \times 10^3$ /g) recorded for stems in the VF.M5F and IP.C treatments may have been due to contamination or to false colony identification.

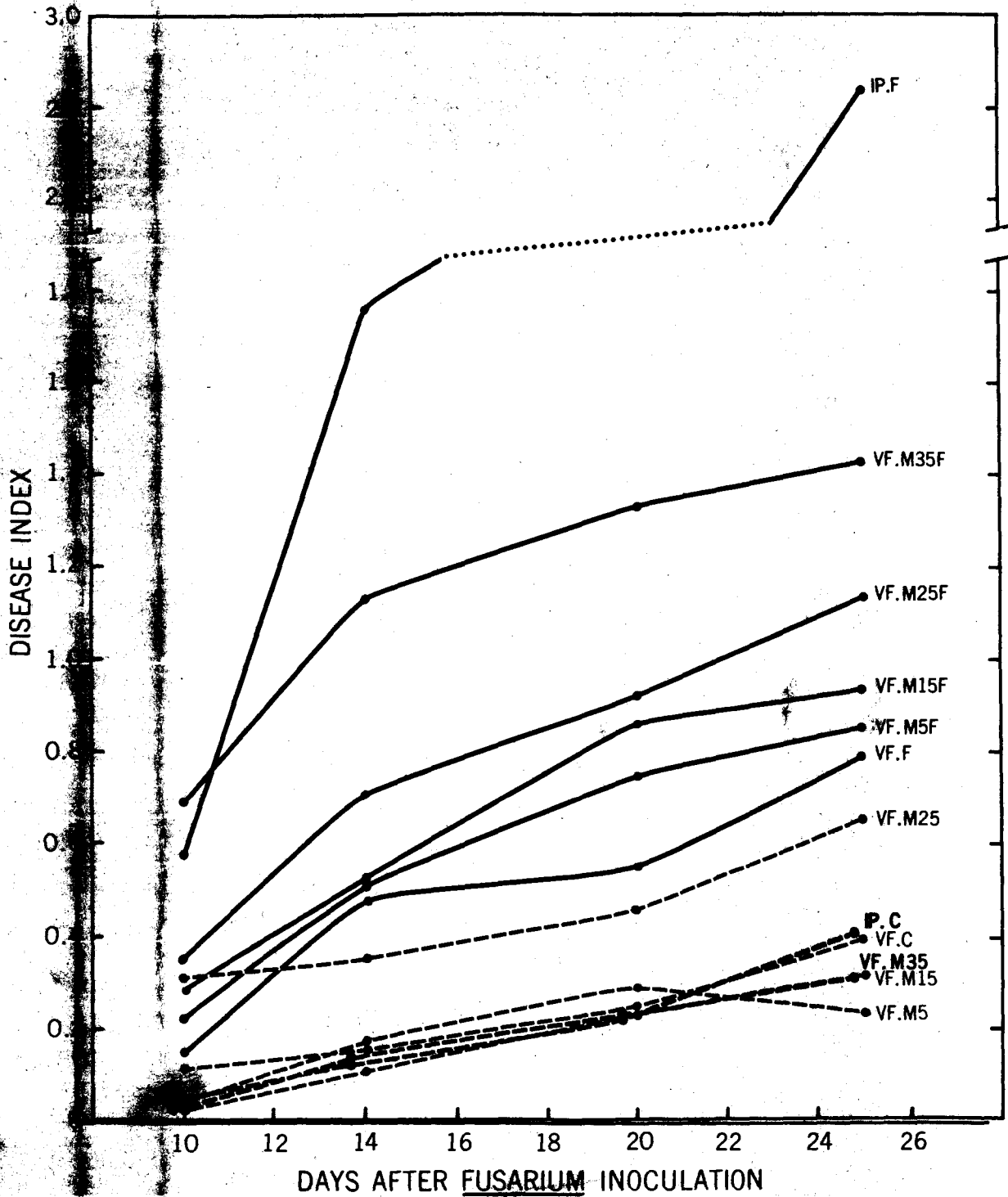
Table VII. Treatment codes for Experiment 3. Tomato plants treated with or without Fusarium oxysporum f. sp. lycopersici in the presence or absence of Meloidogyne javanica.

Plant variety	<u>M. javanica</u> inoculated	Time interval ^a (days)	Treatment code	
			<u>Fusarium</u> inoculated	<u>Fusarium</u> not inoculated
IP	No	--	IP.F	IP.C
VF	No	--	VF.F	VF.C
VF	Yes	5	VF.M5F	VF.M5
VF	Yes	15	VF.M15F	VF.M15
VF	Yes	25	VF.M25F	VF.M25
VF	Yes	35	VF.M35F	VF.M35

^aBetween inoculation with M. javanica and with Fusarium.

Figure 8. The effect of time interval between inoculation with Meloidogyne javanica and with Fusarium oxysporum f. sp. lycopersici on the disease indices^a recorded for tomato plants at various times after Fusarium inoculation.

^aBased on degrees of leaf chlorosis (Fig. 2).



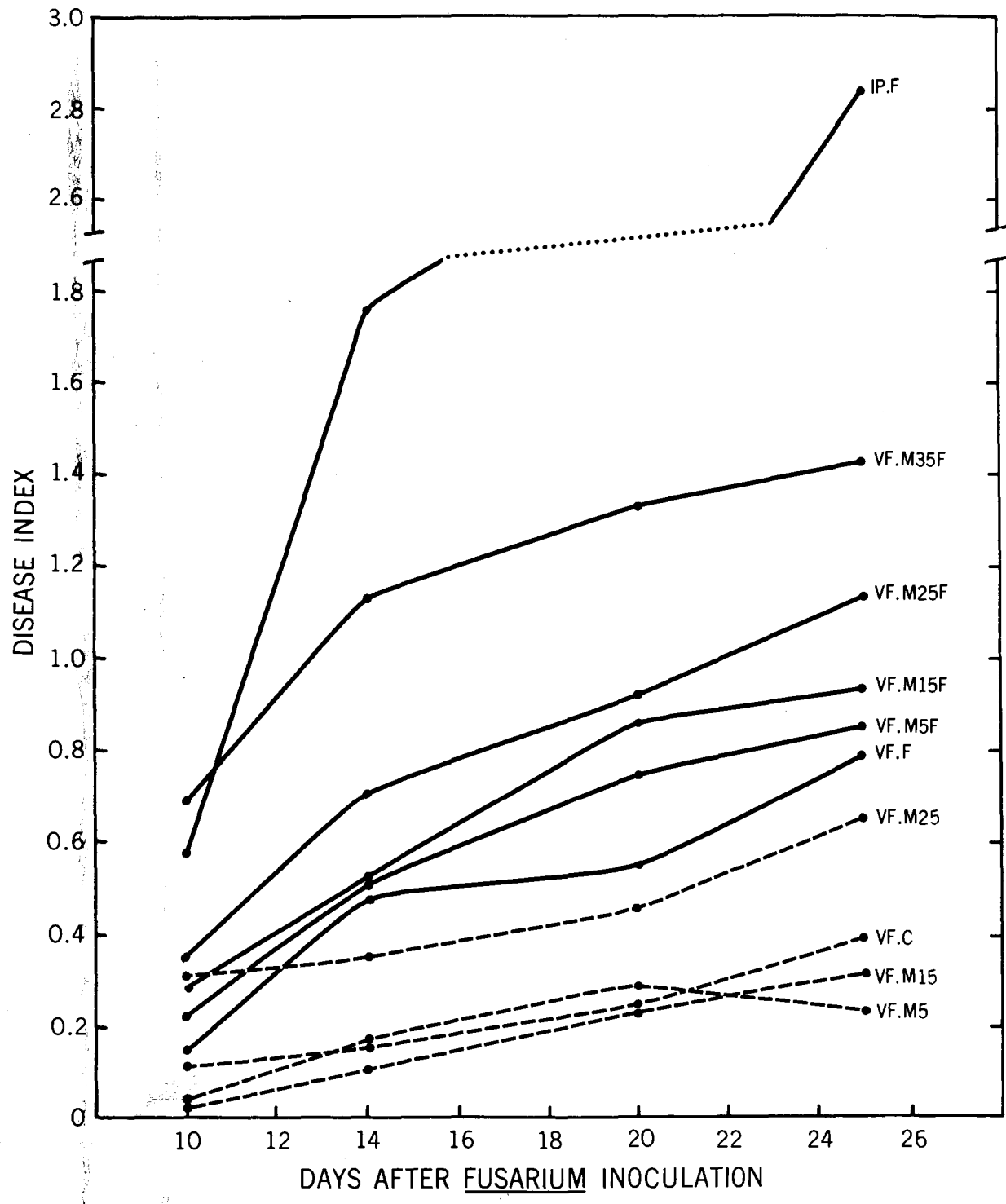
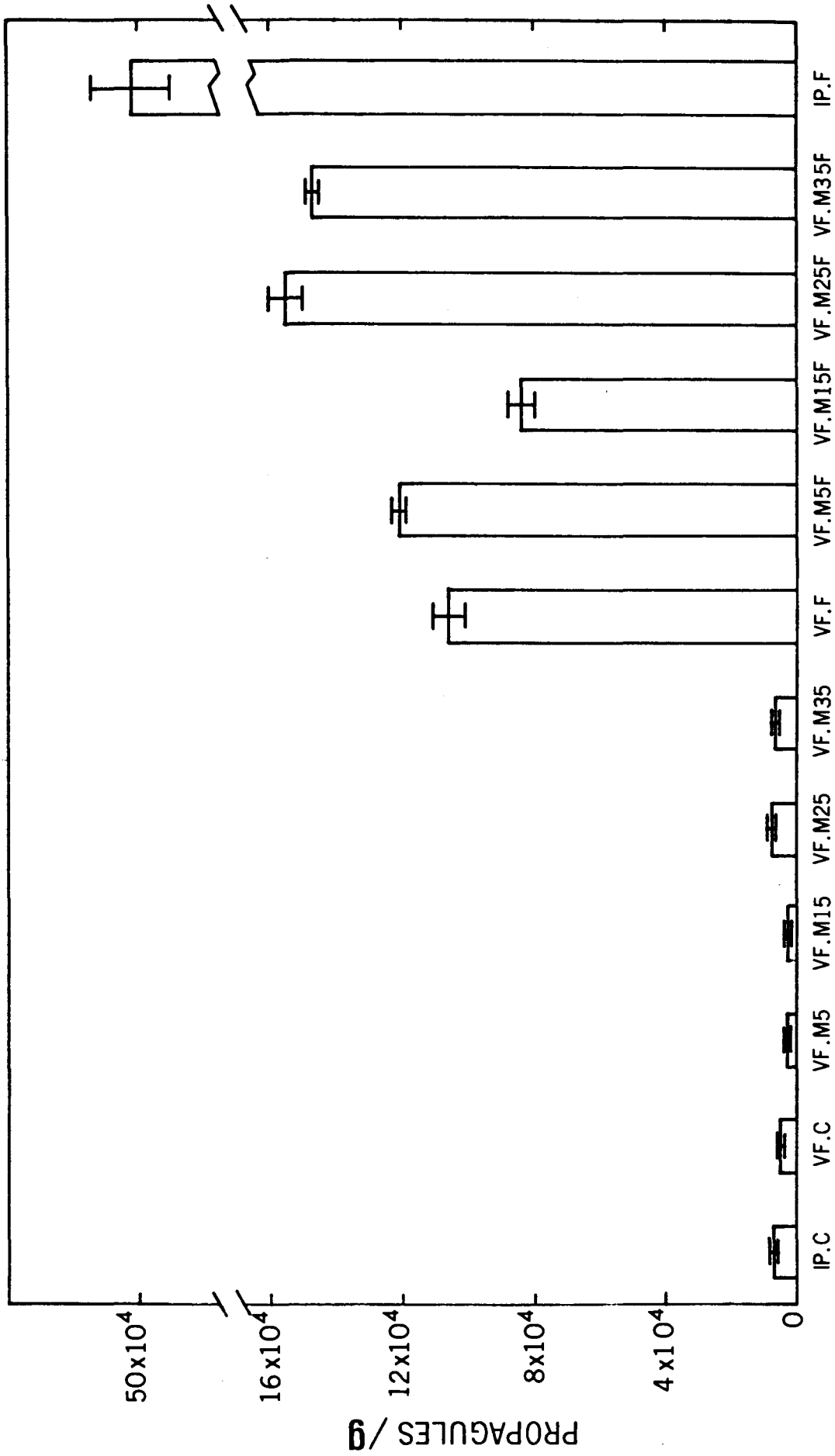


Table VIII. The effect of time interval between inoculation with Meloidogyne javanica and with Fusarium oxysporum f. sp. lycopersici on the disease indices^a (means \pm S.E.) recorded for tomato plants at various times after Fusarium inoculation.

Plant Treatment	Disease indices at various times after <u>Fusarium</u> inoculation			
	10 Days	14 Days	20 Days	25 Days
IP.C	0.04 \pm 0.04	0.13 \pm 0.05	0.23 \pm 0.06	0.42 \pm 0.10
VF.C	0.11 0.06	0.15 0.06	0.24 0.07	0.39 0.12
VF.M5	0.04 0.02	0.17 0.08	0.29 0.08	0.23 0.05
VF.M15	0.02 0.02	0.10 0.03	0.23 0.04	0.31 0.03
VF.M25	0.31 0.08	0.35 0.09	0.45 0.09	0.65 0.13
VF.M35	0.03 0.02	0.14 0.06	0.24 0.06	0.32 0.12
IP.F	0.57 0.07	1.76 0.12	2.12 0.28	2.84 0.37
VF.F	0.15 0.03	0.48 0.08	0.54 0.03	0.78 0.07
VF.M5F	0.22 0.04	0.51 0.06	0.75 0.17	0.85 0.21
VF.M15F	0.28 0.06	0.52 0.12	0.86 0.16	0.93 0.13
VF.M25F	0.35 0.13	0.71 0.09	0.92 0.12	1.13 0.06
VF.M35F	0.68 0.09	1.13 0.13	1.33 0.14	1.42 0.11

^aBased on degrees of leaf chlorosis (Fig. 2).

Figure 9. The effect of time interval between inoculation with Meloidogyne javanica and with Fusarium oxysporum f. sp. lycopersici on the number (means \pm S.E.) of Fusarium propagules per gram fresh weight of tomato root tissue.



TREATMENT

EXPERIMENT 4

SEPARATE INFECTION SITES FOR MELOIDOGYNE JAVANICA AND
FUSARIUM OXYSPORUM F. SP. LYCOPERSICI

MATERIALS AND METHODS

All of the tomato plants in this experiment were induced to form adventitious root systems (II) in pots adjacent to those containing the primary root systems (I) (Fig. 10). This was achieved by laying the stems of 8-week old plants on their sides to touch the soil in the adjacent pots, securing the stems with bent-over plant ties at the cotyledonary nodes and then banking soil against the stems. Within 24 hours the terminal parts of the stems, having responded phototropically, were vertically oriented, and within a week the adventitious root systems were visible in the region of the cotyledonary nodes.

Root systems I of 12-week old Fusarium-resistant (VF) plants were inoculated with M. javanica. As adequate numbers of hatched larvae were not available for this experiment the inoculum consisted of approximately 3,000 larvae and 7,000 unhatched eggs (in egg sacs) per plant and presumably, therefore, M. javanica infection occurred over several days owing to staggered egg hatching. Forty-two days after nematode inoculation Fusarium was inoculated on root systems II in all Fusarium treatments except VF.M_IF_I in which root systems I, which were already infected with M. javanica, were inoculated. All of the plant treatments are summarised in Table IX.

Disease indices were taken 20, 27, 34, and 41 days after Fusarium inoculation. Root systems I and II were processed separately for Fusarium propagule counts 2 days after the final index readings. Stems were not analysed for Fusarium colonisation in this experiment.

The experiment was conducted between early-September and mid-February of 1973-74.

RESULTS

Disease symptoms were not readily apparent in Fusarium-infected plants until about 20 days after inoculation (Fig. 11 and Table X). VF plants which had been inoculated with M. javanica and Fusarium, whether both on the same root system (VF.M_IF_I) or each on separate root systems (VF.M_IF_{II}) showed greater disease symptoms than did those which had been inoculated with Fusarium alone (VF.F_{II}), though this trend was statistically significant ($P < 0.05$) only in the case of the VF.M_IF_I treatment. Neither of these two treatments of VF plants with both pathogens produced disease symptoms as extensive as those of the Fusarium-infected susceptible plants (IP.F_{II}).

Significantly ($P < 0.05$) greater disease indices were recorded for those plants which had been inoculated with both pathogens on the same root system than for those which had been inoculated with each on separate root systems, between 20 and 34 days after Fusarium inoculation. After this time disease

symptoms in plants of the VF.M_IF_I treatment did not increase further whereas those in plants of the VF.M_IF_{II} treatment continued to increase.

From the 27-day index reading onwards, VF plants infected with M. javanica alone (VF.M_I) showed significantly ($P < 0.05$) greater symptoms than did non-infected control plants (VF.C). However, symptoms of VF plants infected with Fusarium alone were not significantly greater than those of the controls. In this experiment, therefore, VF plants, in terms of symptom expression, exhibited high resistance to Fusarium in the absence of M. javanica.

Significantly ($P < 0.001$) more Fusarium propagules were recovered from the Fusarium-inoculated root systems of M. javanica-infected plants, whether the inoculation sites of each pathogen had been on the same (VF.M_IF_I) or on separate root systems (VF.M_IF_{II}), than from those of non-M. javanica-infected plants (VF.F_{II}), (Fig. 12). However, significantly ($P < 0.05$) more propagules were found in the Fusarium-inoculated root systems in the VF.M_IF_I than in the VF.M_IF_{II} treatment.

No Fusarium propagules were found in either root system of non-Fusarium-infected plants except 1×10^3 propagules/g in root system I of control IP plants (IP.C), and very few or no propagules were recovered from the non-Fusarium-inoculated root systems of any Fusarium-treated VF plants. However, large numbers of propagules were present in both root systems

of Fusarium-infected susceptible plants (IP.F_{II}), though there were significantly ($\underline{P} < 0.001$) more in root system II than in root system I.

Figure 10. The production of a double root system on a single tomato plant. Root system I -- primary; root system II -- adventitious.

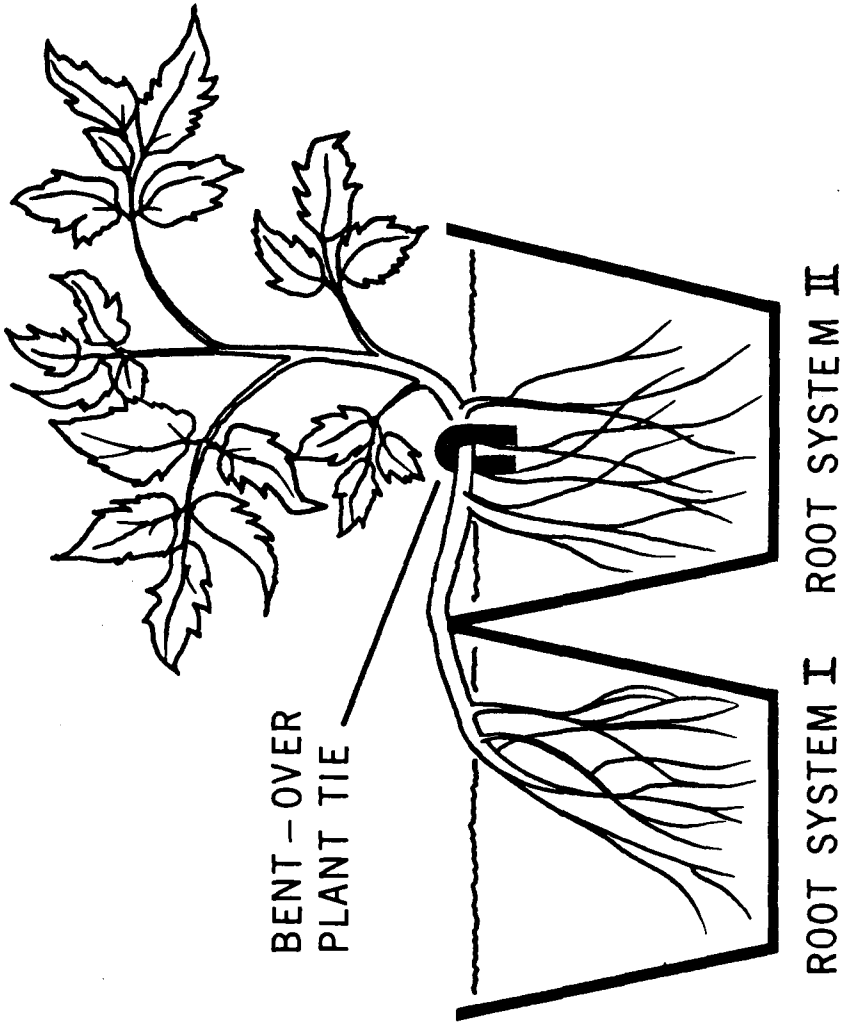


Table IX. Treatment codes for Experiment 4. Tomato plants treated with or without Fusarium oxysporum f. sp. lycopersici in the presence or absence of Meloidogyne javanica.

Plant variety	<u>M. javanica</u> inoculated on root system I	Treatment code		
		<u>Fusarium</u> inoculated ^a	Root system inoculated	<u>Fusarium</u> not inoculated
IP	No	IP.F _{II}	II	IP.C
VF	No	VF.F _{II}	II	VF.C
VF	Yes	VF.M _I F _I	I	VF.M _I
VF	Yes	VF.M _I F _{II}	II	--

^a42 days after M. javanica inoculations.

Figure 11. The effect of separate infection sites for Meloidogyne javanica and Fusarium oxysporum f. sp. lycopersici on the disease indices^a recorded for tomato plants at various times after Fusarium inoculation.

^aBased on degrees of leaf chlorosis (Fig. 2).

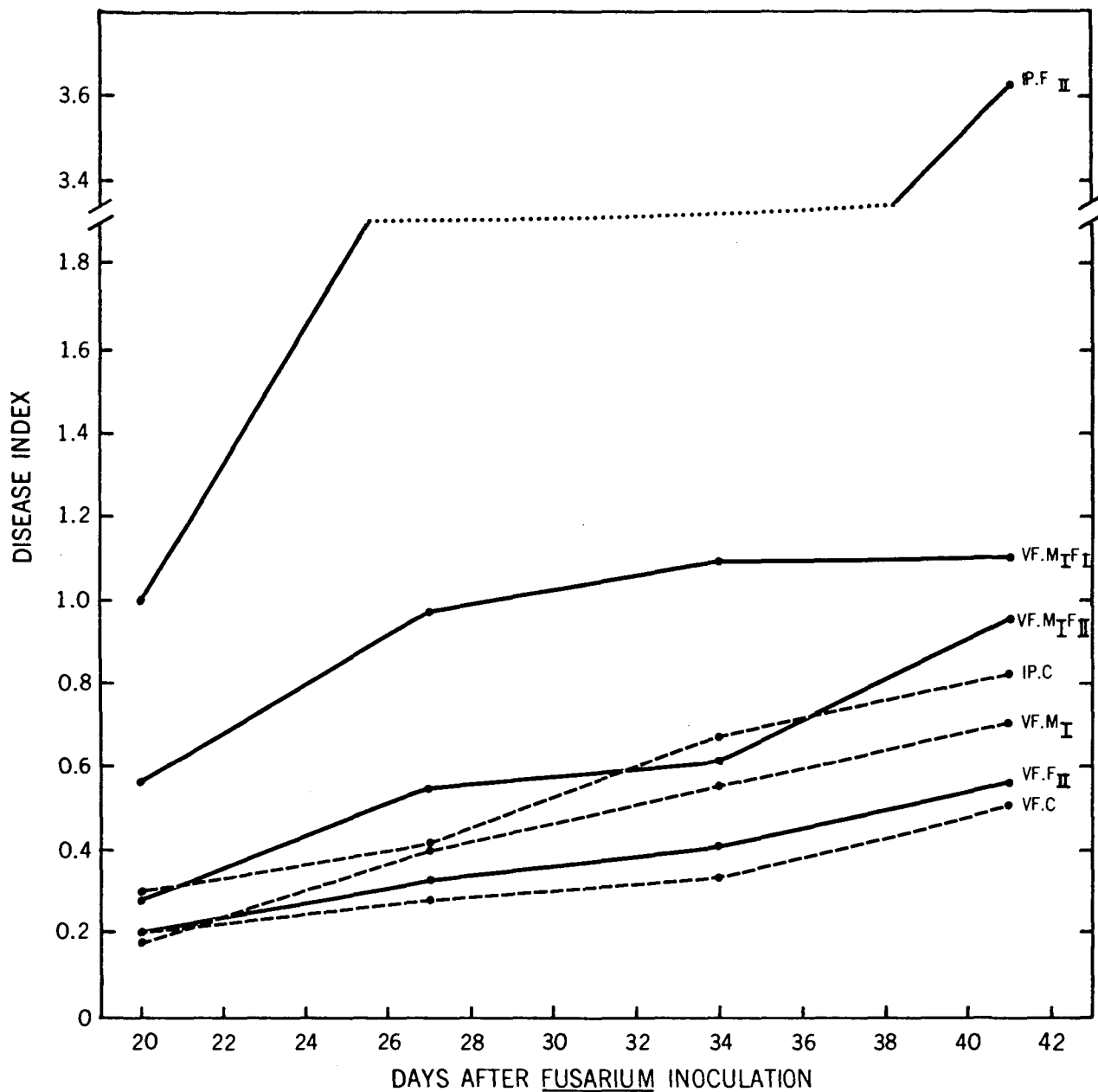
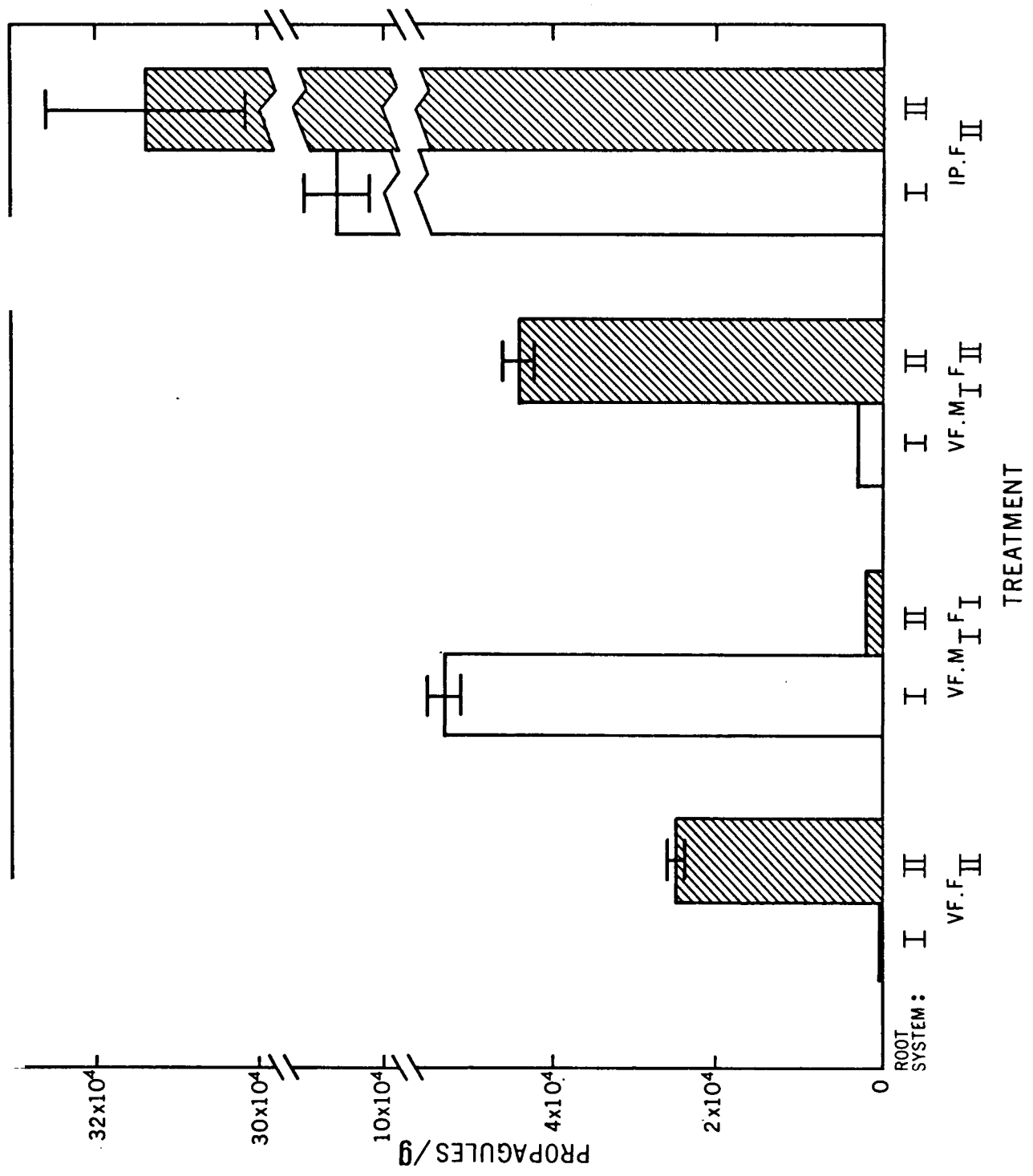


Table X. The effect of separate infection sites for Meloidogyne javanica and Fusarium oxysporum f. sp. lycopersici on the disease indices^a (means \pm S.E.) recorded for tomato plants at various times after Fusarium inoculation.

Plant Treatment	Disease indices at various times after <u>Fusarium</u> inoculation			
	20 Days	27 Days	34 Days	41 Days
IP.C	0.30 [±] 0.05	0.42 [±] 0.08	0.68 [±] 0.08	0.83 [±] 0.08
VF.C	0.21 0.03	0.28 0.05	0.34 0.04	0.51 0.04
VF.M _I	0.18 0.02	0.40 0.04	0.56 0.06	0.71 0.08
IP.F _{II}	1.00 0.15	2.14 0.22	2.90 0.23	3.63 0.11
VF.F _{II}	0.20 0.03	0.33 0.06	0.41 0.07	0.57 0.08
VF.M _I F _I	0.57 0.08	0.98 0.14	1.10 0.12	1.11 0.11
VF.M _I F _{II}	0.28 0.05	0.55 0.10	0.62 0.11	0.96 0.15

^aBased on degrees of leaf chlorosis (Fig. 2).

Figure 12. The effect of separate infection sites (root systems I and II) for Meloidogyne javanica and Fusarium oxysporum f. sp. lycopersici on the number (means \pm S.E.) of Fusarium propagules per gram fresh weight of tomato root tissue.



SECTION II. THE EFFECT OF MELOIDOGYNE JAVANICA INFECTION
ON THE RESPONSE OF FUSARIUM-SUSCEPTIBLE TOMATO
PLANTS TO FUSARIUM OXYSPOURUM F. SP. LYCOPERSICI

EXPERIMENT 5

MATERIALS AND METHODS

Seven-week old Fusarium-susceptible (IP) tomato plants were inoculated with 10,000 M. javanica larvae per plant. Fusarium was inoculated 29 days later. The various plant treatments are summarised as: --

- a) M. javanica alone (IP.M);
- b) Fusarium alone (IP.F);
- c) M. javanica plus Fusarium (IP.MF);
- d) non-inoculated controls (IP.C).

Disease indices were taken 17, 22, 28, 33 and 38 days after Fusarium inoculation and plants were processed for Fusarium propagule counts 3 days after the final index readings.

The experiment was conducted between late-September and mid-January of 1973-74.

RESULTS

Disease symptoms were first apparent in Fusarium-infected plants 17 days after inoculation and thereafter increased steadily (Fig. 13 and Table XI). However, the rates of symptom development were slower than in Fusarium-infected susceptible plants in the experiments conducted during summer months (Experiments 1, 2 and 3).

Greater disease indices appeared to occur in plants infected with both M. javanica and Fusarium than in those infected with Fusarium alone, though differences between the

two were not statistically significant. Plants infected with M. javanica alone consistently gave greater disease indices than did the non-infected control plants, and these were statistically significant ($P < 0.05$) for the 33- and 38-day readings.

More Fusarium propagules were recovered from stem than from root tissues of Fusarium-inoculated plants (Fig. 14). Both the roots and the stems of plants infected with M. javanica and Fusarium contained significantly ($P < 0.001$) more propagules than did the corresponding tissues of plants infected with Fusarium alone, and this increase was considerably greater for the roots (326%) than for the stems (38%). No propagules were found in either the root or shoot tissues of non Fusarium-inoculated plants.

Figure 13. The effect of Meloidogyne javanica infection on the disease indices^a recorded for Fusarium-susceptible tomato plants at various times after inoculation with Fusarium oxysporum f. sp. lycopersici.

^aBased on degrees of leaf chlorosis (Fig. 2).

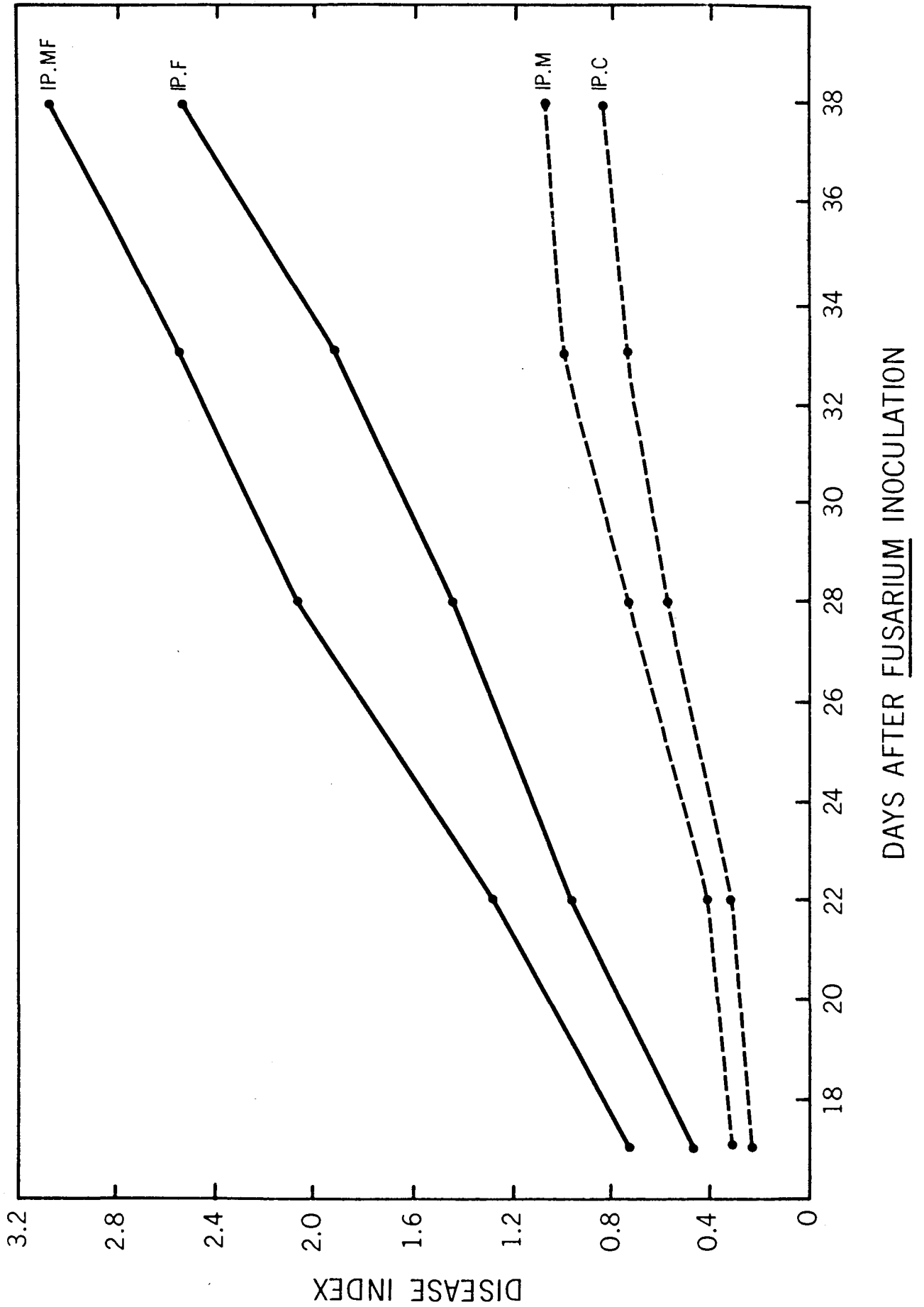
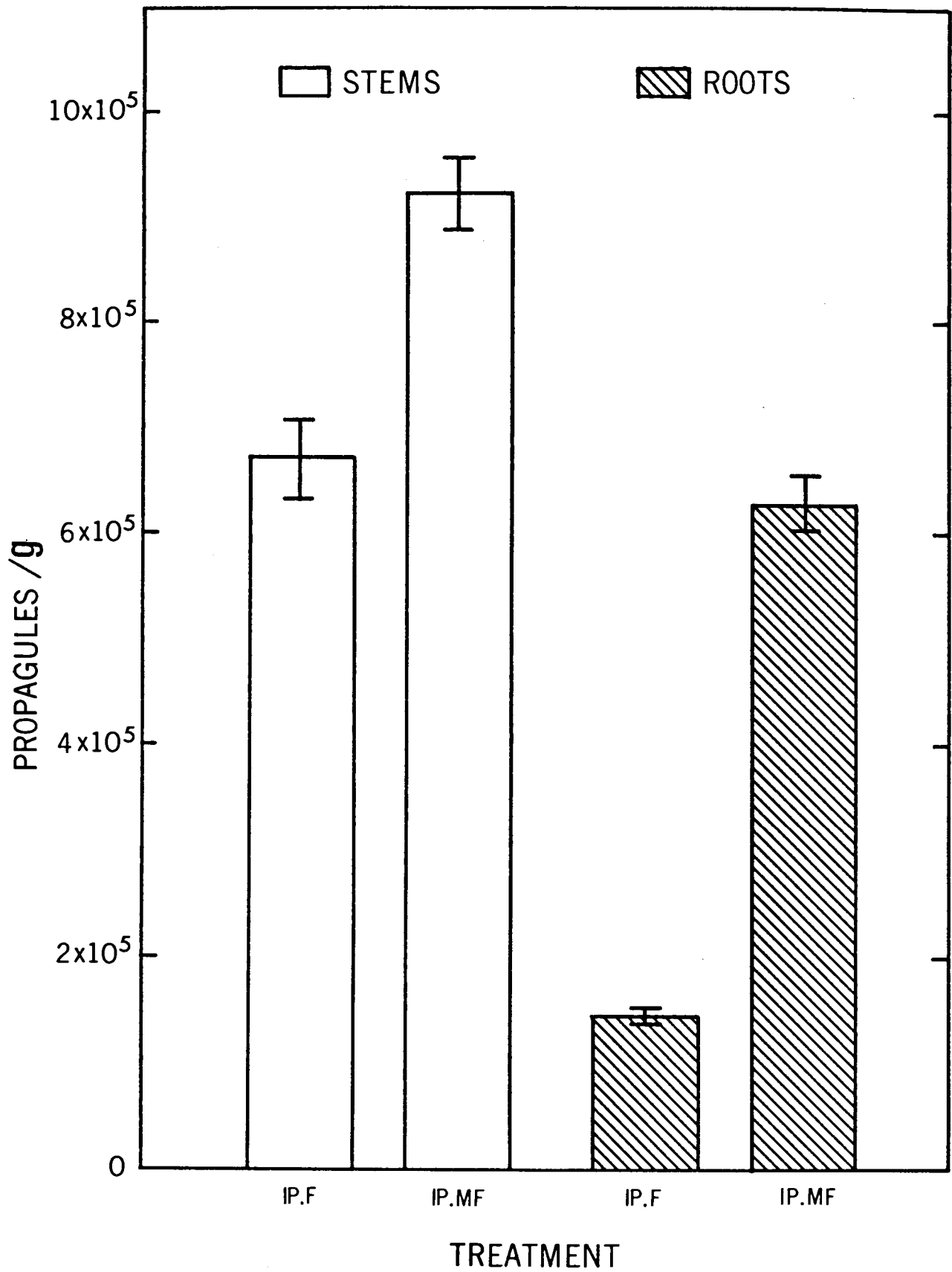


Table XI. The effect of Meloidogyne javanica infection on the disease indices^a (means \pm S.E.) recorded for Fusarium-susceptible tomato plants at various times after inoculation with Fusarium oxysporum f. sp. lycopersici.

Plant Treatment	Disease indices at various times after <u>Fusarium</u> inoculation				
	17 Days	22 Days	28 Days	33 Days	38 Days
IP.C	0.22 [±] 0.05	0.31 [±] 0.05	0.57 [±] 0.08	0.73 [±] 0.06	0.83 [±] 0.03
IP.M	0.31 0.03	0.40 0.03	0.73 0.05	1.00 0.07	1.07 0.07
IP.F	0.46 0.04	0.95 0.08	1.44 0.10	1.91 0.13	2.53 0.20
IP.MF	0.72 0.14	1.28 0.29	2.06 0.32	2.54 0.26	3.06 0.19

^aBased on degrees of leaf chlorosis (Fig. 2).

Figure 14. The effect of Meloidogyne javanica infection of Fusarium-susceptible tomato plants on the number (means \pm S.E.) of Fusarium propagules per gram fresh weight of root and stem tissue following inoculation with Fusarium oxysporum f. sp. lycopersici.



DISCUSSION

A positive disease interaction between M. javanica and F. oxysporum f. sp. lycopersici, indicated by a greater severity of disease symptoms and extent of fungal colonisation of roots of tomato plants infected with both pathogens than of plants infected with Fusarium alone, occurred in each of Experiments 1 to 5. In most instances the interaction, in terms of symptom expression, appeared to be an additive one since the disease indices of plants infected with both pathogens were no greater than the sums of the indices of plants infected with each pathogen alone. However, where the disease indices of plants infected with M. javanica alone were not significantly different from those of the non-infected controls (treatments VF.M1000 and VF.M10000 in Experiment 1; VF.0.M in Experiment 2; VF.M5, VF.M15, VF.M25 and VF.M35 in Experiment 3) there is evidence to suggest that the greater disease symptoms of plants infected with both pathogens were due not to an additive but to a synergistic effect. Synergistic interactions were most apparent where "young" plants were inoculated (Experiment 2), and where there was a 35-day interval between inoculation with each pathogen (Experiment 3). In these instances the disease indices of plants infected with both pathogens were greater than the sums of the indices of plants infected with each pathogen alone.

This study has demonstrated that M. javanica infection can reduce the resistance of VF and increase the susceptibility of IP tomato plants to Fusarium infection. Since the lowered

level of resistance in VF plants was never comparable to the susceptibility shown by IP plants when infected with Fusarium alone, the term "resistance reduction" is considered more applicable in describing the interaction in VF plants than the more commonly-used term "resistance breakage" (Bergeson, 1972). These results, therefore, are similar to those of Orion and Hoestra (1974) who reported an increased Fusarium-susceptibility of "Moneymaker" and a tendency towards resistance breakage of "Fortos" tomatoes by M. javanica and M. incognita, but not to those of Jenkins and Coursen (1957) who found that there was no increase in the susceptibility of "Red Beefsteak" but that there was a complete breakage of the resistance of "Chesapeake" tomatoes by M. incognita.

Before discussing the implications of the results, some explanation should be given of the inconsistencies in the magnitude of disease indices and Fusarium propagule counts that occurred between experiments. For example, for VF plants inoculated with Fusarium alone, maximum disease indices ranged from 0.13 ("young" plants in Experiment 2) to 1.27 (Experiment 1), and root propagule counts from 25×10^3 (Experiment 4) to 150×10^3 propagules per g fresh weight (Experiment 1). These variations were probably due to differing environmental and experimental conditions. The season during which an experiment was performed, despite the relatively constant environment of the glasshouse, is probably one of the most important considerations. The average temperature and amount of incident

light was appreciably lower in winter than in summer, and the lower temperatures may have caused the delay in appearance and development of disease symptoms in experiments conducted during winter months (Experiments 4 and 5). The nutrient levels of different batches of soil may also have varied between experiments. Such environmental factors as these are important variables affecting the severity of Fusarium wilt of tomato (Walker, 1971). Differing experimental conditions, such as the age of the plant at the time of Fusarium inoculation and the number of days after Fusarium inoculation at which plants were processed for propagule counts also might have affected the results.

Disease indices are considered less reliable indicators of the extent of disease than propagule counts for a variety of reasons. Firstly, leaves can become chlorotic for reasons other than Fusarium or Meloidogyne infection, so sometimes high disease indices were recorded in the absence of either pathogen. Secondly, degree of leaf chlorosis is a subjective criterion and, therefore, its measurement may have been inconsistent, though the method was thorough in that it measured chlorosis of every leaf of each plant, thereby minimising such errors. However, there was usually quite a high intratreatment variance in disease indices which often resulted in a lack of statistical significance of intertreatment differences. Despite this, the method is considered useful because it provided an estimate of the overall effect of the disease inter-

action on the condition of the plants.

The extent of the disease interaction was dependent on the specific experimental conditions imposed upon the plants. There was more extensive fungal colonisation (as measured by propagule counts) of root tissue with higher nematode inoculum levels (Fig. 5). The correlation in terms of disease symptoms was less clear (Fig. 4), though the highest disease indices were consistently recorded for plants which had received the highest inoculum level (10,000 larvae) of M. javanica together with Fusarium. A similar finding by Kawamura and Hirano (1967) showed that the effect of M. incognita in increasing the Fusarium-susceptibility of "Matsudo-Ponderosa" tomatoes was intensified by increasing the number of nematodes in the inoculum.

In terms of symptom expression, there appeared to be a greater disease interaction in "young" than in "old" plants (Fig. 6). However, the fact that there was more extensive fungal colonisation of "young" than of "old" roots, whether they had been inoculated with M. javanica and Fusarium or with Fusarium alone (Fig. 7), suggests that the age of the host plant more probably determines the degree of its response to the fungus than to the combination of nematode and fungus. Support is given to this conclusion by the observation of Alon et al. (1974) that the incidence of disease in Fusarium-inoculated resistant tomatoes decreased with increasing seedling age.

In terms of symptom development, a positive disease interaction occurred with a 5- or 15-day interval between inoculation

with each pathogen, though the most extensive interactions occurred with 25- and 35-day inoculation intervals (Fig. 8). No definite explanation can be given for the apparent negative interaction, in terms of fungal colonisation of roots, with the 15-day inoculation interval (Fig. 9), though an error may have been made in calculating the dilution factor for the homogenised root sample. These results are closely similar to those of Porter and Powell (1967) and Melendez and Powell (1967), who noted that maximal predisposition of tobacco to Fusarium wilt by root-knot nematodes occurred when nematode inoculation preceded fungal inoculation by 3 or 4 weeks (i.e. 21 or 28-day inoculation intervals), though they did not relate these time intervals to developmental stages of the nematodes in the tobacco roots. Although the four inoculation time intervals used in the experiment reported here were chosen to coincide with four specific developmental stages in the life cycle of M. javanica (Materials and Methods, Experiment 3), the attempt was only partially successful. In order to determine the developmental stages of the nematodes at the time of fungal inoculation, twenty nematodes per treatment were dissected from the roots at random and their developmental stages determined. Most of the nematodes were at the expected stages but some were less well developed, possibly due to overcrowding. Also, due to the inability to eliminate sources of potential fungal infection in the soil after the time of fungal inoculation, invasion by Fusarium presumably continued to occur for

the remainder of the experimental period, when the nematodes were at more mature stages. Hence, some reservation is necessary in relating the results obtained to specific developmental stages of the nematodes at the time of fungal invasion.

The occurrence of a positive disease interaction even when the inoculation sites of M. javanica and Fusarium were on separate root systems (Experiment 4) agrees with a similar finding by Bowman and Bloom (1966) who used a split-root technique to investigate a M. incognita/Fusarium interaction in tomato. These observations suggest that translocatable or general physiological changes that occurred in the plants as a result of root-knot nematode infections affected their subsequent responses to Fusarium infection at sites distant from the nematode infection. However, in the experiment reported here, the fact that a greater disease interaction occurred when M. javanica and Fusarium were both inoculated on the same root system suggests that there may also have been a more direct interaction between these two pathogens. This possibility is inferred from these results with caution however, since the primary root system (I), which received the fungal inoculum in treatment VF.M_IF_I might have responded differently to Fusarium than the adventitious root system (II) which received the inoculum in treatment VF.M_IF_{II}. Ideally, all possible combinations of control treatments, including one with Fusarium inoculated alone on root system I, would have been included, but lack of materials and glasshouse space prohibited this.

Reviews on nematode/fungus interactions (Pitcher, 1965; Powell, 1971; Bergeson, 1972) have indicated that nematodes may aid fungal infection in a variety of ways, and it will be useful to summarise these here. a) Qualitative and/or quantitative changes in the root exudates of nematode-infected plants may provide more effective stimuli to the germination and growth of fungi in the rhizosphere. b) The wounding action of nematodes in root-penetration and, in the case of root-knot nematodes, in extrusion of egg sacs, may facilitate fungal penetration. c) Biochemical modifications of root tissues by root-knot nematodes may provide more favourable food substrates for fungi. d) Changes in the physiology of a plant may interfere with its mechanisms of resistance to fungi. The results of this study will now be discussed further with reference to the above theories.

The more extensive fungal colonisation of nematode-infected than of nematode-free roots of both VF and IP tomato plants (Figs. 5, 7, 9, 12, and 14) could have been due to a more favourable substrate provided by the galled root tissue. Histological examination of free-hand sections of VF roots which were infected with both M. javanica and Fusarium showed that fungal hyphae were present in the xylem vessels and that some of the nematode-induced giant cells were packed with hyphae (though other giant cells were not colonised). However, in VF roots infected with Fusarium alone hyphae were rarely observed in any cells other than the xylem vessels. Abundant

fungal colonisation of giant cells and nearby vessel elements has also been observed in root-knot/Fusarium-infected tobacco roots (Melendez and Powell, 1967), and tomato roots (Kawamura and Hirano, 1968). Further, Hirano (1965) showed that dry weights of mycelium of several species of Fusarium were greater when cultured in media composed of expressed juices from galled roots than when in those from healthy root tissues. The possibility therefore exists (as Bergeson (1972) indicated) that the increases in some biochemical substances, such as lipids, protein, nucleic- amino- and organic acids, in galled roots reported by Owens and Specht (1966), could form an enriched growth medium for fungi.

An additional consideration, in the case of Fusarium-resistant plants, is that the cells composing galled tissue may be incapable of a normal resistance response. Beckman et al. (1972) considered that Fusarium resistance in VF tomato plants is due to rapid tylose development in response to Fusarium infections which, as a result, become sealed-off in the xylem vessels. Tyloses result from the balloon-like expansion of xylem parenchyma or ray cells through the pits into the adjacent vessels (Talboys, 1972). In a Meloidogyne-infected root system the morphology and metabolism of some of the vascular parenchyma cells undergo striking changes in the formation of giant cells, and hypertrophy and hyperplasia of various types of root cells, sometimes including vascular parenchyma, contribute to the formation of the galls (Krusberg,

1963). It is conceivable that such modified cells, especially the giant cells, are unable to form tyloses, in which case the capacity for Fusarium resistance of a nematode-infected root system would be reduced.

There was never any appreciable Fusarium-colonisation (indicated by very low propagule counts) of the stem tissue of Fusarium-inoculated resistant plants either in the presence or absence of M. javanica (Experiments 1, 2 and 3). However, stems of Fusarium-inoculated susceptible (IP) plants were extensively colonised in the absence and even more so in the presence of nematode infections, though the actual percentage increase in colonisation was not nearly as great as that for IP roots (Fig. 14). Orion and Hoestra (1974) found that M. javanica or M. incognita increased the number of Fusarium-infected vascular bundles in the stems of Fusarium-susceptible tomatoes. Perhaps the greater fungal colonisation of Fusarium-susceptible stems was due not so much to their greater susceptibility as to an increased inoculum potential of Fusarium resulting from growth on a galled root system. This scheme could operate for the Fusarium-susceptible stems, since they support fungal growth anyway, but not for the VF stems which normally resist fungal invasion. The results reported here are contrary, in part, to those of Bergeson et al. (1970) who demonstrated more extensive growth of Fusarium in roots but not in stems of Fusarium-susceptible tomato plants when infected with M. javanica, though both sets of results emphasise that

the galled root system is the site most affected by increased fungal colonisation. This is additional support for the theory that galled roots form a more favourable environment for fungal growth.

As previously indicated, the results of Experiment 4 suggest that physiological changes that occurred in plants due to nematode infection may have been partly responsible for the disease interaction. This theory is supported further by the results of Experiment 3 which show that the greatest disease interactions occurred when the nematodes had been in the roots for 25 or 35 days before fungal inoculation, at which times they were mostly newly-moulted adult females or mature females producing and extruding eggs. Bird (1961; 1972) showed that growth of root-knot nematodes was most rapid between the completion of moulting and the commencement of egg laying, and that giant cells were largest and synthesised protein most actively during the period of rapid nematode growth and egg production. At these times, therefore, the nematodes must exert their greatest physiological stress (e.g. in terms of nutrient depletion and secretion of foreign chemical substances) on the host plant, so the results of Experiment 3 present circumstantial evidence for such a stress, or the consequences thereof, being involved in the mechanism of the disease interaction. In further support of this idea, Huang et al. (1971) found that increases in peroxidase levels of tomato plants as a result of M. incognita infection, were greatest after the final moult and

during egg production. It is therefore reasonable to assume that other biochemical changes occurring in plants due to root-knot nematode infections, including some which may be associated with the disease interaction, would be most apparent at the times of mature nematode infections. For instance, Wang (1973) found that increases in total sugar concentration in the xylem sap of M. incognita-infected tomato plants reached a maximum 4 weeks after nematode inoculation. He considered that the greater sugar content could facilitate growth and colonisation of Fusarium in the xylem. His additional finding that the sugar concentration greatly increased with higher nematode inoculum levels could explain the more extensive Fusarium colonisation of roots when infected with larger numbers of nematodes (Experiment 1). However, due consideration should also be given to the probability that larger numbers of nematodes would also a) induce greater changes in root-exudates, and b) cause more extensive root wounding, and both of these phenomena, as mentioned earlier in the discussion, have been considered as possible explanations for nematode/fungus disease interactions. However, the results of Experiment 3, which show that there was no significant difference in the extent of fungal colonisation of nematode-infected roots between the 35-day inoculation interval treatment (in which most nematodes were extruding egg sacs at the time of fungal inoculation) and the 25-day interval treatment (no egg sac extrusion at fungal inoculation), do not support the idea that root wounding, due to

egg sac extrusion, may be an important factor in facilitating fungal penetration (Bergeson, 1972).

In speculating further on how nematode-induced host physiological changes may reduce the resistance of VF plants to Fusarium, it will be useful to refer once again to the work of Beckman et al. (1972) which showed that Fusarium resistance in VF tomato plants is due to the rapid occlusion of infected xylem vessels by tylose development. This resistance mechanism was apparently less efficient even when M. javanica and Fusarium were infecting separate root systems, since this still resulted in increased fungal colonisation (Fig. 12). It is conceivable that a translocatable factor produced or induced by the nematodes in root system I interfered with the Fusarium response mechanism in root system II and reduced its efficiency. There will be no attempt here to speculate on what such a factor might have been or how it might have operated, but work by Wang (1973) has shown that root-knot nematodes can induce translocatable effects in plants. Using a split-root technique, he found that the permeability of the tonoplast and plasma membrane of non-galled tomato root cells in one half of the split-root system increased in the presence of a M. incognita infection on the other half of the root system. If nematode-produced/induced factors are transported in the xylem they could be particularly effective in influencing the Fusarium-resistance mechanism whose site of function, according to Beckman et al. (1972), is in the xylem.

Root-knot nematodes could also influence the Fusarium-resistance response by interfering with the normal metabolism of growth substances. Brueske and Bergeson (1972) found that infection of tomato with M. incognita caused a reduction in cytokinins and gibberellins in root tissue and xylem exudate, and they suggested that the decrease in cytokinins could be responsible for a plant's lower resistance to secondary infection. In addition, it has been shown that treatment of tomato plants with gibberellic acid influences the symptoms which they develop in response to infection with Fusarium (Dimond and Corden, 1957; Paquin, 1962). Orion and Hoestra (1974) found that ethrel (an ethylene-releasing compound) markedly reduced symptoms of Fusarium wilt in tomatoes, but that this therapeutic effect was counteracted by infections of M. incognita or M. javanica. From these findings, they concluded that plant growth regulators play a role in Fusarium-resistance and in the effect of the nematodes on the severity of Fusarium wilt.

The presence of leaf chlorosis in VF plants in the absence of any appreciable fungal colonisation of the stems (Experiments 1, 2 and 3) suggests the possible involvement of transported fungal toxins in the development of foliar symptoms in the disease interaction. Keyworth (1964; 1968) was able to produce symptoms of chlorosis and necrosis in the leaves of Fusarium-resistant scions when grafted onto infected Fusarium-susceptible root stocks even though the scions were not extensively invaded. Results of the experiments reported here indi-

cate an increased Fusarium-susceptibility of VF roots when infected with M. javanica, so this situation of Fusarium-resistant VF plants infected with root-knot nematodes was essentially similar to that produced by Keyworth in his resistant scion/susceptible root stock grafts. Furthermore, upon Fusarium-inoculation of the root systems, both of these conditions resulted in more extensive leaf chlorosis than was observed in controls. Keyworth considered that the effect he produced was due to a hypersensitive response of the leaves to toxic fungal metabolites produced in the infected susceptible root stock, so the same explanation may apply to the greater leaf symptoms of VF plants in the disease interaction. Bergeson (1972) suggested that fungi growing on nematode-modified tissues could undergo physiological changes causing quantitative or qualitative changes in the toxins that they produce. It is also possible that a nematode-produced/induced factor could change the activity of, or host response to, fungal toxins to the detriment of the plant. If such a factor were produced in greater amounts with more mature nematode infections then this mechanism could explain the more extensive disease symptoms which occurred with greater time intervals between inoculation with M. javanica and Fusarium (Fig. 8).

This discussion has pointed to the possible involvement of more than one mechanism in the disease interaction. It would be unwise to attempt to oversimplify the apparent complexity of this and other nematode/fungus disease interactions

which may be due to not just one, but several mechanisms linked together in a chain of complementary events. However, this study has emphasised the importance of changes in a plant's physiology due to nematode infection in influencing the effectiveness of its subsequent response to fungal invasion.

Further information leading to a better understanding of the mechanisms involved in Meloidogyne/Fusarium disease interactions could come from closer study of the biochemical processes involved in the Meloidogyne/plant relationship and in the Fusarium-resistance response.

SUMMARY

This study has shown that M. javanica infection can reduce the Fusarium-resistance of VF and increase the Fusarium-susceptibility of IP tomato plants. Resistance reduction was more apparent a) in 4-week than in 8-week old plants, b) with increasing inoculum levels (up to 10,000 larvae per plant) of M. javanica, and c) with increasing time intervals (up to 35 days) between inoculation with M. javanica and subsequent inoculation with Fusarium. A number of possible mechanisms for the disease interaction are discussed.

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