THE BIOLOGY AND ECOLOGY OF FUSARIUM OXYSPORUM f. MELONIS IN SOIL AND THE ROOT ZONES OF HOST AND NONHOST PLANTS

Thesis for the Degree of Ph. D.

MICHIGAN STATE UNIVERSITY
ZIAEDDIN BANIHASHEMI

1968

THESIS

This is to certify that the

thesis entitled

THE BIOLOGY AND ECOLOGY OF FUSARIUM OXYSPORUM

f. MELONIS IN SOIL AND THE ROOT ZONES OF

HOST AND NONHOST PLANTS

presented by

Ziaeddin Banihashemi

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Plant Pathology

Ronald J. de Zeeuw
Major professor

Date January 4, 1968

O-169

ABSTRACT

THE BIOLOGY AND ECOLOGY OF *FUSARIUM OXYSPORUM* f. *MELONIS* IN SOIL AND THE ROOT ZONES OF HOST AND NONHOST PLANTS

by Ziaeddin Banihashemi

Fusarium oxysporum f. *melonis* (Leach and Currence) Snyder and Hansen, a soil-borne plant pathogen, is a specialized wilt pathogen of muskmelon.

The activity of f. *melonis* was studied in soil, rhizosphere, and root surface of host and nonhost plants under field conditions and controlled greenhouse conditions.

Most of the washed conidia of f. *melonis* introduced into either autoclaved or naturally infested soils disappeared after 30 days incubation at temperatures between 5 and 30°C, but more rapid decline in population took place at the higher temperatures and in naturally infested soil. The population of f. *melonis* after 14 months incubation was greatest at 15°C and lowest at 5°C in both autoclaved and natural soils. The survival units were chlamydospores associated with the previously invaded organic particles. This association is considered to be an important factor in the organisms's survival in soil.

Both rhizosphere population and number of root segments colonized by f. *melonis* increased with time in the

presence of host but not with nonhost plants. The increase in rhizosphere population of the pathogen was caused mainly by macroconidia borne in sporodochia on infected roots. Wilted host plants supported greater rhizosphere populations of both f. melonis and other fungi than healthy plants. Lower soil moisture (30-40% whc) favored both infection and increased populations in the vicinity of roots. Lower soil temperatures (15-20°C) favored disease severity, parasitic and saprophytic colonization and also survival in the soil.

The population of f. melonis in soil increased in the presence of a living host and decreased in its absence. Although the soil near host plants always contained greater numbers of propagules of f. melonis than did soil between the rows, the greatest increases in population were at the site of actually wilted plants. Population of f. melonis was also greatest on the soil surface but appreciable numbers of propagules of the pathogen were obtained below the 6 inch depth. Sporulation on infected vines and the sloughing away of loosened infected tissues accounted for the increase in population on the soil surface and in the root zone at lower levels. Infected vines were an important source of inoculum for overwintering the pathogen. In Michigan the population of f. melonis was not greatly affected by steady low winter temperatures but declined sharply in spring with the advent of milder weather. The

drop in population was assumed to be due to progressive decomposition of infected plant residues.

The pathogen was able to colonize roots of many nonhost plants under field conditions, but there was no accompanying increase of f. melonis in soil. Rhizosphere, and soil populations actually decreased with the nonhost plants. Corn decreased the pathogen more effectively than soybean in this respect, and the living soybean roots were a better substrate for f. melonis than those of corn or other grasses.

The pathogen colonized detached or sterilized tissues of either host or nonhost plants in naturally infested soil irrespective of depth buried, and under favorable conditions the pathogen can survive in colonized plant materials for several months. Colonization did not occur at 5°C but the pathogen was a more active colonizer at 15°C than at higher temperatures. The pathogen was found to be rather competitive with other soil microorganisms in colonizing plant materials saprophytically.

Two distinct physiological races of F. oxysporum f. melonis have been identified among isolates used in this research, and were compared as to their parasitic and saprophytic activities. The Iranian isolates of f. melonis infected the muskmelon variety "Persian small type" when root dipped in a spore concentration of 10^4/ml, whereas the Michigan isolates failed to infect this variety at 10^8 conidia/ml. One of these isolates representative of the

common Michigan forms, is a new fourth physiological race according to the classification of Risser and Mas. Several North American isolates of f. melonis gave similar reactions and all are considered to be of the same physiological race as Michigan isolates. The inoculum concentration proved to be important in relation to disease expression for both races on a series of muskmelon varieties.

THE BIOLOGY AND ECOLOGY OF *FUSARIUM OXYSPORUM* f. *MELONIS* IN SOIL AND THE ROOT ZONES OF HOST AND NONHOST PLANTS

By

Ziaeddin Banihashemi

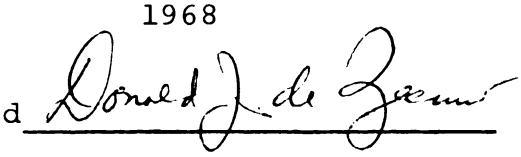
A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Botany and Plant Pathology

1968

Approved 

649202
5 20-68

ACKNOWLEDGMENTS

I wish to extend my sincerest thanks and appreciation to Dr. Donald J. deZeeuw, committee chairman and advisor, for guidance and encouragement throughout the course of these investigations, and also for reviewing and correcting the manuscript.

Sincere appreciation is also extended to Dr. John L. Lockwood for certain valuable suggestions relative to this work.

The author is also grateful to the other members of the committee; Drs. William B. Drew, William J. Hooker, Harry H. Murakishi, and Charles L. San Clemente.

The technical assistance of Mr. Richard A. Crum is greatly appreciated.

The funds and materials supplied by the following companies have been valuable aids in this research:

Chemagro Corporation
Chevron Chemical Company
Diamond Alkali Company
Dow Chemical Company
E. I. duPont de Nemours and Company
Ferry-Morse Seed Company
Morton Chemical Company
Olin-Mathieson Chemical Corporation
Rohm & Haas Company
Union Carbide Chemical Company
Upjohn Company
U.S. Rubber Company (Naugatuck Chemical Division)

TABLE OF CONTENTS

LIST OF TABLES

Table Page

LIST OF ILLUSTRATIONS

Figure | Page

INTRODUCTION

Of all plant pathogens perhaps the soil-borne forms are the most difficult to control. Among these no single species is more important than the wilt Fusarium (Fusarium oxysporum) with its approximately 66 specific forms (18). In spite of the importance and ubiquity of the species, there is comparatively little information on its behavior in the soil and in the root zone of susceptible and non-susceptible plants. Information needed for combating the soil-borne plant pathogens includes basic knowledge on mode of survival and behavior in the presence and absence of the host plants.

Fusarium oxysporum f. melonis the causal agent of melon wilt, was selected to serve as a model for studying some aspects of the biology and ecology of a wilt pathogen in soil.

Field studies and more closely controlled greenhouse and laboratory studies on the behavior of the pathogen under various environmental conditions were made in the presence and absence of the host. Parasitic invasion, saprophytic colonization and survival and competitive saprophytic ability of the pathogen were studies under various

environmental conditions. Isolates of melon wilt Fusaria from North America and Iran (Persia) were compared in many aspects of parasitism and saprophytism.

REVIEW OF LITERATURE

Host and the Pathogen.--Cucumis melo L., cantaloupe or muskmelon is the host of the pathogen under investigation in this report. The origin of muskmelon is probably tropical Africa and the secondary centers are India, Persia, Southern Russia and China (107,108).

Fusarium wilt of muskmelon was first reported by Sturgis in 1898 from United States (82). The pathogenicity of the concerning organism was first proved by Leach in 1933 (29), and the identification of the causal agent as Fusarium bulbigenium var niveum f.2. and description of the disease was completed in 1938 report by Leach and Currence (30). Revision of the genus Fusarium by Snyder and Hansen (68), changed the name of the fungus to Fusarium oxysporum f. melonis (Leach and Currence) Snyder and Hansen, the currently accepted name.

There have been conflicting reports on races of F. oxysporum f. melonis. Reid (54) compared some 290 isolates of f. melonis obtained from diseased muskmelon plants. To prevent cultural degradation all stock isolates were immediately transferred as hyphal masses to sterile soil tubes.

The various isolates were compared on Czapek's medium. He described 10 macroscopically different cultural races which also differed in relative degree of pathogenicity. Race 1 was the same as the standard culture or Miller's wild type (39). This race was predominant in nature. Because of the method he employed, he postulated that mutation was not responsible for the appearance of cultural races.

McKeen and Wensley (35) working with the same fungus and probably in the same area, stated that F. oxysporum f. melonis produces mutants in sterile soil similar to those found on conventional media and these mutants were morphologically indistinguishable from the original wild type. They also stated that many common cultural characteristics were "variable in expression and frequently impossible to evaluate objectively and meaningfully." The concept of a wild type was supported. Reid (54) indicated that variations in wilt from field to field and from year to year could best be explained by the presence of different races or different proportions of the various races. Wensley and McKeen (105) did not support this explanation and thought instead that the fungus population density might be related to the wilt incidence.

Welty (102) used 17 isolates of F. oxysporum f. melonis differing in degree of virulence. He concluded that although the races clearly differed in virulence, there was no evidence for race-specific pathogenic specialization.

Recently Risser and Mas (57) reported the occurrence of three distinct races of F. oxysporum f. melonis on the basis of specific virulence toward differential host varieties. Since the inoculum potential was not specified and is considered to be an important factor in determination of resistance (13,58) it is still not clear whether these races depend on relative or absolute resistance.

Epidemiology

Inoculum Potential.--Inoculum potential was first used and defined by Horsfall (23) as the number of infective particles present in the host environment, and severity of disease is dependent on the numbers of infective particles present. Garrett (15,16) analyzed and discussed the term inoculum potential for soil fungi and defined it as "the energy of growth of a parasite available for infection of a host at the surface of the host organ to be infected."

Miller (40) pointed out that even a low level of inoculum is sufficient to cause severe wilt by f. melonis. This was considered to be an important factor in disease epidemiology. Soil containing fewer than 15 units per gram still could cause appreciable wilt in muskmelons (34).

The degree of resistance apparent in muskmelon varieties has been found to be also dependent on the inoculum potential of F. oxysporum f. melonis (13,58). Wensley and

and McKeen (105,106) found that the population of melon wilt Fusarium increased at the site of muskmelon plants. A decline of inoculum was noticed shortly after harvest. In the greenhouse, only susceptible varieties of melon caused a site increase in inoculum, whereas the population remained constant or even decreased slightly in the case of resistant varieties. The increase in inoculum probably resulted from spores in infected root and hypocotyl tissue.

Inoculum Dispersal.--Outbreaks of soil-borne plant pathogens in newly planted fields are not uncommon, but there has been little experimental evidence to indicate how the spread of wilt Fusarium takes place.

Wensley and McKeen (105) observed large quantities of spores mostly macroconidia produced in lesions that developed on wilted muskmelon plants. Leach and Currence (30) indicated that wind-borne spores should not be overlooked as a means of dissemination.

Chlamydospores of F. oxysporum f. melonis are produced in infected plant debris and also in epidermal tissue of the hypocotyl of infected muskmelon plants (105). In confirmation of previous reports, infected muskmelon vines, containing tremendous numbers of resting structures of the wilt organisms have been observed repeatedly in Michigan (personal observation). This kind of plant residue lying

on the surface of soil disintegrate and release small pieces of infected cortical tissue. These fragments carrying inoculum may be wind-borne for considerable distances although specific evidence is lacking in the case of muskmelon Fusarium. Due to the important role of chlamydospores in the survival of many Fusaria (33,42), the significance of such wind-blown plant particles is readily understood.

Many Fusaria appear in newly planted soil under circumstances not clearly defined. Dissociation of saprophytic forms of Fusarium to the pathogenic type has only been postulated (22), but there is no evidence for this in respect to the muskmelon wilt Fusarium.

Another possibility is the introduction of the pathogen by infected seeds (30). Baker (2) recently reviewed this aspect of transmission of plant pathogens in general and states that "establishment of pathogens in many isolated areas is undoubtedly due to seed transmission."

Resistance.--McNew (36) stated that any disease outbreak depends on the inherent susceptibility of the host, the inoculum potential of the parasite, and effect of environment on parasitism and pathogenesis.

Reid (54) investigated the mode of penetration of f. melonis in resistant and susceptible varieties of muskmelons histologically. He found that the pathogen was

equally aggressive in both varieties. Soil temperature greatly affected the expression of resistance. Although both resistant and susceptible plants died at 20 or 25°C and both were resistant at 35°C, only the susceptible ones were killed at 30°C. Temperature apparently acted on pathogen aggressiveness rather than on host susceptibility. Leach and Currence (30) reported that the higher temperature only delayed disease symptoms, since no significant differences were obtained in the final amount of wilt at 30 and 35°C. At higher temperatures periderm is formed in roots and around the vascular bundles of the hypocotyl quickly enough to prevent entry of the pathogen. Grigoryan (19) reported that F. oxysporum f.f. melonis, cucumerinum, niveum and F. moniliforme (G. fujikuroi) were identical in their modes of penetration and spread in the plant tissue. Hyphae passed from cortex into pith and xylem through the medullary rays, and later into the vessels. Spread was mainly through parenchyma.

The grafting experiment performed by Cox (9) revealed that no wilt occurred when either melon or cucumber were grafted on cucumber root stock, but when melon or cucumber scions were grafted on melon root stock, and inoculated with F. oxysporum f. melonis, both plants died as a result of infection. The scion did not affect susceptibility of the root stock to infection, and relative

susceptibility of the scion was not altered by the root stock.

Expression of resistance to F. oxysporum f. melonis on muskmelons has been reported to be a function of inoculum potential (13,58) and also related to root penetrability (58). Methods of testing resistance or susceptibility of melon varieties have been reported to affect the results. Dolan (12) found that resistant and susceptible muskmelons could be differentiated best by injecting a spore suspension of F. oxysporum f. melonis between the cotyledons of seedlings. By this treatment few susceptible plants survived and most resistant varieties remained healthy. Rodriguez (58) found that when stems of very resistant plants are inoculated by the injection method, the fungus fails to spread or dies completely. Thus resistance was attributed to the pathogen's failure to spread in the host plant.

Due to discrepencies in the expression of resistance in greenhouse and field, Mortenson (41) suggested that all greenhouse studies should be applied at 30°C soil temperature. In his studies, Fusarium resistance in muskmelon appeared to be controlled by one principal dominant gene (R), plus two complementary dominant genes (A and B). Messian et al. (38) reported that resistance is a monogenic dominant.

The Soil Environment

Soil Environment and Disease Development

Temperature.--Soil temperature as a factor influencing the development of Fusarium wilt diseases has been investigated exclusively (24,97). With some exceptions the disease-temperature curve follows very closely the pathogen-temperature curve. The optimum temperature of the pathogens in culture was about 27°C. The optimum temperature for Fusarium wilt development in low optimum temperature plants such as pea, cabbage, and also in high optimum temperature plants such as tomato, watermelon, and cotton was about 27°C as well. In muskmelon wilt (30) the disease optimum is lower although the optimum temperature for the pathogen in culture is about 27°C. Muskmelons grow best at 35°C (30) and the reduction of muskmelon wilt disease at higher temperatures has been confirmed (11,40,41,54,58). The severity of disease at lower temperatures has been considered to be either a matter of host predisposition (40) or aggressiveness of the pathogen rather than a change of basic host susceptibility (54).

Moisture.--The effect of soil moisture on Fusarium wilt disease development is not as clear and definite as soil temperature since it is not as easily controlled. There is some disagreement on soil moisture influence in these cases. Walker (97) stated that the vascular fusarial

diseases, which are markedly influenced by soil temperature, are not greatly affected by soil moisture. Others reported that wilt Fusaria may be favored by either high soil moisture (8,10,63,74,109) or by low soil moisture (11, 40,87,93).

As to muskmelon Fusarium wilt, however, there is general agreement that dry weather and low soil moisture favor incidence of the disease (11,40). Reid (54) using root dip inoculation, found no significant differences in disease incidence at 25 and 50% soil water holding capacity. Crozier (11) concluded that the major factor in the appearance of "sudden wilt" (Fusarium) of muskmelon was due to drought in many of the melon growing areas of New York. Miller (40) studied the effects of soil moisture on muskmelon wilt Fusarium in rather more detail. He found that lower soil moisture increased wilt development and decreased plant growth rate. Higher soil moisture favoring host growth reduced incidence of the disease. Differences in wilting between low and high soil moisture were more distinct with 2-week than with 3-week old plants. He suggested that a predisposition factor may be operating at lower soil moisture. Plants grown under conditions of low soil moisture and moderately high soil temperature (27°C) became susceptible to the disease.

Other Factors.--Soil fertility, type, pH, aeration, etc., reportedly affect soil-borne plant pathogens. Stover

(79) stated that Panama disease caused by Fusarium oxysporum f. cubense is favored more by acid sandy loam than by alkaline silt or clay loam soils. Wensley and McKeen (105) recently found that the greater wilt potential in Fox sandy loam than in Colwood loam soils was not solely due to inoculum density of F. oxysporum f. melonis, and other factors were involved.

Stotsky, et al. (77) reported that the spread of F. oxysporum f. cubense is low in soils containing montmorillonite. Commercial banana producing soils are called short, intermediate, or long life in reference to wilt. In long life soils the disease spread was slow, thus allowed longer time in banana production and this had a direct correlation with available potassium. Addition of K to existing short life soil however, did not affect the wilt potential. Soil life, it was concluded, could be predicted from its clay minerology, but the exact effect of soil type on wilt is not understood. Montmorillonite increases bacterial respiration mainly due to its buffering action on maintaining the soil pH (78). A possible relationship between clay minerals and activity, ecology, and population dynamics of microorganisms in natural soil is suggested.

Soil Environment and the Pathogen

Detection.--Estimation of plant pathogen population in soil is essential for measuring inoculum potential. Various qualitative and quantitative methods have been employed

for microorganisms in the soil. The major problem is a lack of reliable methods for assaying the pathogen population directly (37). At present no single method of assay applies for all soil microbiological studies.

The soil dilution method is widely used for estimating soil microorganism population. One criticism of this method is that it gives an advantage to freely sporulating forms.

Selective media are still not available for most soil-borne plant pathogens. Various isolation media have been reported for estimating soil fungus propagules in general and for Fusarium species in particular (43,45,49, 67,75,103). Wensley and McKeen (103) using a modified Martin's peptone agar, estimated populations of the species F. oxysporum satisfactorily, but F. oxysporum f. melonis could be estimated only by an auxiliary pathogenicity test (104) because of similar morphology in nonpathogenic and pathogenic forms of F. oxysporum. They indicated that since F. oxysporum f. melonis occurs in soil as chlamydospores, use of a soil dilution method is reliable. Papavizas recently (45) compared 18 previously recommended media for the isolation of Fusarium species from soil. Peptone-PCNB agar was preferred for quantitative studies. Park (49) and Singh and Nene (67) claimed to have developed selective media for the isolation of soil Fusaria but Papavizas (45) found that they were not satisfactory due to low Fusarium recovery and poor restriction of associated microorganisms.

Activity in the Soil.--Waksman studying soil fungi from different regions, classified soil fungi into two groups; "soil inhabitants" and "soil invaders" (96). Garrett (16) redefined Waksman's classification of soil fungi. Soil inhabitants were characterized by "ability to survive indefinitely as soil saprophytes." The term soil invaders was changed to "root inhabiting fungi" and characterized by "an expanding parasitic phase on the living host, and by a declining saprophytic phase after its death."

The state in which a fungus occurs in soil is important in understanding its activities there. Direct microscopic observation is often useful. Fusaria are most consistantly associated with organic particles and much less as free spores or hyphal fragments in soil (98,99). Park (46) concluded that the chlamydospore is a survival structure in soil fungi, and strains possessing the ability to form them will have a greater chance of being classified as members of the soil microflora. When mycelium or conidial inoculum of Fusarium oxysporum was added to the soil, the survival structures were found to be chlamydospores. Plant materials placed on or beneath the surface of inoculated soil were colonized by F. oxysporum with abundant production of conidia on the colonized substrate. These were then released into soil and dispersed by water (48). No continuous growth was maintained in soil. He also noted that Fusarium colonization of plant materials previously colonized by

other organisms required a high inoculum potential. Park (48) considered F. oxysporum a pioneer fungus in respect to substrate colonization. Growth of the fungus in soil was limited and it shortly produced chlamydospores. Addition of fresh organic materials induces germination of chlamydospores and colonization of the added substrate. Warcup (100) found that very few viable colonies of F. oxysporum could be obtained by isolating hyphal fragments from the soil.

Garrett (16,17) distinguished between saprophytic survival and saprophytic colonization. He remarked that

> By saprophytic survival is meant the survival of a parasite in dead host tissue that it originally invaded and occupied whilst they were still alive, i.e. during its parasitic phase. By saprophytic colonization is to be understood the invasion of dead host tissue in competition with purely saprophytic fungi and other soil microorganisms.

True saprophytic survival occurs through a continued slow mycelial development of the parasite within the dead host tissue and this must be distinguished from dormant survival in the form of resting spores or sclerotia (17).

Garrett (16) indicated that saprophytic ability like pathogencity is a general character of the organism. In order for a fungus to colonize a substrate it must have ability to compete with other soil microorganisms. Three factors were considered to be essential in saprophytic colonization of a substrate: (1) competitive saprophytic ability, (2) inoculum potential, and (3) proper environmental

conditions including the population of competing fungi and other soil microorganisms. The competitive saprophytic ability was defined as "the summation of physiological characteristics that make for success in competitive colonization of dead organic substrates." As in pathogenicity, the term "saprophytic ability" or "competitive saprophytic ability" will make sense only if it is used in strict reference to a particular type of substrate (17).

Four characters have been used by Garrett (16) to indicate a high competitive saprophytic ability: (1) high growth rate of hyphae, and rapid germination of spores, (2) good enzyme-producing equipment, (3) production of antibiotic toxins, and (4) tolerance of antibiotics produced by other microorganisms. Butler (5), using the Cambridge Method, concluded that high rate of growth and antibiotic production was not related to the saprophytic ability of fungus. Tolerance to antibiotic was related to competitive saprophytic ability. Wastie (101), using the agar plate method, concluded that the rate of linear growth rather than tolerance to antibiotic was a factor for success in saprophytic colonization.

Information on the saprophytic status of vascular Fusaria is fragmentary and scanty. Subramanian and Zachariah (84,110) found that F. oxysporum f. vasinfectum, the cotton wilt pathogen, is able to colonize up to 100% of the detached or autoclaved pieces of cotton root or stem buried

in unsterilized soil. Park (47) reported that vascular wilt of oil palm caused by F. oxysporum could competitively colonize sterilized plant materials placed on the surface of inoculated alien soils. The fungus was considered a soil inhabitant. Rao (51), using the agar plate method, found that F. oxysporum f. cubense and F. oxysporum f. vasinfectum were vigorous soil saprophytes whereas Verticillium dahliae and Rhizoctonia solani were poor saprophytes. The validity of this method for measuring saprophytic ability of a fungus can be questioned, since R. solani is considered a good soil saprophyte. Stover (81) studied the competitive saprophytic ability of F. oxysporum f. cubense by the Cambridge Method. He found that the banana wilt pathogen at high levels of inoculum could colonize dead tissue but was a poor competitor compared with Fusarium solani and F. roseum with much lower indigenous populations. His method of identifying the pathogenic form of Fusarium oxysporum was based on cultural interactions rather than the pathogenicity test. He concluded that evidence for saprophytic growth of the banana wilt pathogen in soil is now well established but its ability to compete with soil inhabiting fungi in substrate colonization is not great.

Activity in the Root Zone

The Root Zone and Its Nature.

--The term "root zone" is here used to include both rhizosphere and the root surface.

The term "Rhizosphere" was originally introduced and defined by Hiltner (21) in 1904 as "that region of the soil which is under immediate influence of the plant roots." He found that the soil near roots harbored a heavier microbial population than soil distant from the roots.

No attention was given to Hiltner's discovery for a quarter of a century until the classical works of Starkey in the period of 1929-38 (69-73) again focused attention on the microbiology of the rhizosphere. Starkey noticed that type, age, and condition of any given plant, and proximity of roots influenced neighboring microbial activity in soil, and he also emphasized the possible concomitant importance of rhizosphere flora to the growing plant. Bacteria were more affected in root zones than actinomycetes and fungi and were found in greater numbers in legume than in nonlegume rhizospheres. Size of the plant and root system does not determine stimulation, but rather its physiology through the quality and quantity of root excretion (70). He stated that the greatest effect of the plant appears on the root surface and in the soil in contact with the root, but effects may extend for several millimeters beyond the root surface. The important findings of Hiltner and Starkey have been thoroughly investigated and confirmed throughout the world.

The rhizosphere soil is generally a thin layer adhering to the root after the loose soil and clumps have

been removed by shaking (27). Since this amount varies depending on type of root and amount of moisture, Clark (7) suggested that the "Rhizoplane" might be a more sensitive index area for root zone investigations pertaining to qualitative effects of roots on soil microorganisms. He defined rhizoplane as "the external surface of plant roots together with any closely adhering particles of soil or debris" or "the plant root surface." Timonin (91) preferred to use rhizosphere, rhizoplane and histosphere (root tissue), to include all regions affected by the root system. Rovira recently (61) attempted to define the rhizosphere and concluded that the rhizosphere is not a uniform, well defined volume of soil. Many workers thus attempted to use terms such as "outer rhizosphere," "inner rhizosphere," "root surface" and "rhizoplane" for each zone of the soil. The heterogenous nature of soil, however, makes it impossible to define precisely where each of these zones begins and ends. According to Rovira (61) the most satisfactory method of many described for obtaining the rhizosphere flora is by washing roots gently in sterile water, and the rhizoplane microflora by subsequent vigorous washing of the same roots. One foresees difficulties in separating rhizosphere from the rhizoplane organisms, however, where a heavily sporulating fungus may be growing directly on the root surface, removing the rhizosphere soil even with a gentle washing method would release "rhizoplane" spores or even hyphal fragments as well.

Since Hiltner's time, the rhizosphere effect has been considered to be a result of root excretions or root exudates. Even before the nature of materials present in the root exudates was definitely known, Lochhead and co-workers (32) discovered that amino acid-requiring bacteria were stimulated most in the rhizosphere. Similar results were obtained with fungi (86). Lochhead stated that an amino acid supply at or near the root may arise by liberation from roots, systhesis by other microorganisms, and decomposition of cellular debris.

Many organic compounds have been identified in root exudates of various types of plants (62). Simple sugars and amino acids in root exudates influence the spore germination of many soil-borne plant pathogens and soil saprophytes as well (65). Root exudates of various plants either stimulate or inhibit the germination of fungal propagules and mycelial growth and also effect the attraction of zoospores or nematodes and the emergence of eelworm cysts (62).

Exudations have been reported to be greatest during the first two weeks of growth (50). Exudation is the most important factor contributing to the supply of nutrients in the rhizosphere of young seedlings but as the plant develops exudation declines and decomposition of moribund root hairs, epidermal cells, and cortex contributes the main portion of the nutrients to rhizosphere microorganisms. The site of exudation has been considered to be mainly in

the region of elongation and the root-hair zone (62). Factors influencing the growing plant's physiology affect the quality and the quantity of root exudates. A decrease in soil moisture (25,26), increase in soil temperature (66), increase in light intensity (59), injury to roots, and infection with plant pathogens (65) cause increased root exudation. Various foliar treatments changed rhizosphere microflora by changing the exudation (52,94).

The Root Zone in Relation to Susceptibility, Resistance and Immunity

Although it has been more than 60 years since Hiltner discovered the rhizosphere phenomenon, its importance in respect to plant pathogens has not been appreciated until recently. Studies on several soil-borne plant pathogens combined with resistant, susceptible, and nonhost plants have disclosed some important aspects of pathogenesis in the root zone. Timonin (88) studied the rhizosphere effect of flax and tobacco plants resistant and susceptible to wilt and root rot respectively. Rhizosphere population was always greater with the susceptible varieties. Higher counts of microorganisms in the rhizosphere of susceptible plants have been observed by others as well (6,31,83,85,88,89,90,92). Schroth and Hildebrand (65) indicated that there is no convincing evidence that a toxic component of the exudate significantly influenced the activity of a fungus pathogen

and played an important role in disease resistance. Resistance to infection has also been attributed to the root zone microflora (14,111).

Root exudates have both direct and indirect effects on inoculum potential (65). The direct effect is by supplying nutrients in the rhizosphere for multiplication of and infection by the pathogen. The indirect effect results from associative changes in other rhizosphere microorganisms. Papavizas and Davey (44) and Bateman (3) found that Thielaviopsis basicola increased markedly in the rhizosphere of beans (a host plant), but only after the appearance of root rot symptoms. Nonhost plants (wheat and corn) did not influence population of the pathogen. He concluded that growth and reproduction of T. basicola in soil were associated with its parasitic activity. Papavizas and Davey (44) found that T. basicola was more abundant in the rhizosphere of beans infected with moderately to strongly pathogenic isolates than from the rhizosphere of beans infected with mildly pathogenic isolates.

Reyes (55) studied the population changes of Fusarium solani f. pisi, Fusarium oxysporum f. lycopersici, and Fusarium solani in soil and in the rhizosphere of various plants under various environmental conditions. Fusarium was stimulated in the rhizosphere of susceptible plants but not in that of nonhosts. He could not detect directly what kind of propagules were being stimulated (spores or hyphae).

Stimulation of F. solani f. pisi was evident up to two millimeters distance from the root and the effect was more intense around the younger portions of the roots. The stimulating effect was observable 4 days after planting and reached its maximum on the eighth day, but lasted even beyond 64 days.

Lacy recently (28) found that the population of Verticillium dahliae was greater in the rhizosphere of host and nonhost plants than in nonrhizosphere control soil. While susceptible and resistant mint species (hosts) and tomato a symptomless host supported similar population of the mint strain of V. dahliae, the nonhost plants such as wheat, corn and beans tended to support fewer numbers of Verticillium propagules. There were no significant differences in number of root penetrations between resistant and susceptible mint species. Symptomless host and nonhost plants had fewer penetration sites on roots than either resistant or susceptible mint species.

Armstrong and Armstrong (1) found that pathogenic isolates of Fusarium oxysporum could be isolated from stems and roots of nonsusceptible plants. Waite and Dunlop (97) isolated banana wilt Fusarium from roots of several species of grasses. Nonsusceptible plants were found to be important on the survival of bean root rot Fusarium (66).

The influence of host susceptibility on root zone populations of plant pathogens is summarized by Schroth and

Hildebrand (65): "It appears probable therefore, that, although the population of root-infecting fungi may decline in the absence of susceptible plants, the ability of certain of them to colonize tissues of non-suscepts, and others to produce limited mycelial growth in their rhizospheres, may enable a number of pathogens to produce and maintain an effective inoculum for establishing diseases when a susceptible crop is again planted."

MATERIALS AND METHODS

Culture Media.--Various culture media recommended for the isolation of soil fungi in general and Fusarium spp. in particular were tested (43,45,49,67,75,103). PDA+NPX (75, 76) was found to be a satisfactory isolating medium. When this medium was used at pH 4 colonies of Fusarium oxysporum f. melonis appeared typically pink to salmon color. TMN (Trimethylnonanol), a nonionic surfactant, at 500 ppm concentration was later used to replace the NPX. The TMN-PDA medium gave all the advantages of NPX-PDA and in addition tended to repress the colony diameter of all fungi and enhance the pinkish color of F. oxysporum viewed from beneath.

Modified potato dextrose Agar (TMN-PDA) standardized for this work consisted of: Fresh potato extract--500 ml (200 gms. of peeled potatoes/500 ml distilled water), Agar--17 gms. (Difco Bacto Agar), dextrose 20 gms. and distilled water 500 ml. The TMN (0.5 ml) was added prior to autoclaving. Before pouring plates, the TMN-PDA (at 40°C) was acidified to pH 4.0-4.2 with 50% lactic acid. Poured plates were generally used the same day and were not used if they had been stored for more than 2 days since old plates produced fewer colonies.

Experimental Soils.--Sandy loam top soil collected from two locations was used in all experiments. All soil was screened (4-mesh) to remove stones and trash. Soils heavily infested with the muskmelon wilt Fusarium was collected in Wayne County (Howard Gerst Farm) and stored at 14% moisture in closed cans until used. The control soil used for artificial infestation studies was obtained from the M.S.U. horticulture farm. This soil was autoclaved and stored in a closed metal container for at least one week before use. The saturation point (56) was found to be 35-40% by weight of soil--100% of its water holding capacity. The soil pH was between 6.5-7.0.

Inoculum Preparation.--Mycelium from a 4-day old single spore, sporodochial culture of Fusarium oxysporum f. melonis was transferred into a 500 ml flask containing 100 ml of potato dextrose broth (extract fresh potato 200 gms./ 500 ml and dextrose 10 gms.) at pH 6.5. Cultures were incubated at room temperature on a reciprocal shaker (140 shakes per minute) for 3-4 days. The conidia were centrifuged down at low speed and washed three times with sterile distilled water. The conidia were resuspended in sterile distilled water to give the desired concentration of conidia in the soil to be infested. Soil and inoculum were mixed and stored in a covered plastic-lined metal container in a 65-76°F greenhouse for 2-3 months before use. Soil moisture was about 12%. Population of the pathogen was determined by

agar plate assay as needed and appropriate dilution made with autoclaved or unsterilized soil.

Environmental Control.--For root zone studies plants were grown in one-liter plastic containers in temperature tanks at the desired temperature (fluctuation ±1°C). Aliquots of soil (1100 gms. oven-dry basis) were apportioned to each container. The units were irrigated with clear plastic (Naglon) tubes about 6" long. The lower end of each tube was closed and ten holes 2 mm in diameter were made along the length. Tubes were placed in the soil diagonally from bottom to top. To reduce evaporation, the containers were usually covered with 3-hole plastic tops. Two (2 cm diameter) for the plant stems and one for the irrigation tube. Pots were weighed daily and deionized water added as required. A day length of 14 hours was maintained during the fall, winter and spring months using supplemental illumination at 400 f.c. from cool white fluorescent bulbs. Greenhouse temperature was controlled during these months between 65-72°F. In summer the temperature at times exceeded 90°F for several hours.

Soil Sampling and Soil Assay.--Soil samples for assay were collected from melon-wilt infested fields throughout the year with a Hoffer soil tube and placed in individual plastic bags. Assay plating was usually done within a day. A standardized soil dilution plate method was employed throughout this work. Individual soil samples were mixed

and all visible organic particles removed. One gram (dry weight equivalent) was placed in 100 ml of sterile distilled water and mixed for 10 minutes. Further dilutions were made with 0.1% water agar. Each diluted soil suspension to be assayed was shaken vigorously for 30-60 sec. and 1/2 ml pipeted into each of 5-10 petri plates containing TMN-PDA. After the first plate, the suspension was again mixed and the process repeated. The seeded plates were incubated at room temperature for 4-6 days and examined daily. Numbers of Fusarium oxysporum and other fungi were determined separately for each sample and calculated per gram of oven-dry soil.

Rhizosphere Assays.--The evening before a rhizosphere assay was to be made, soil moisture was brought to desired percent whc. The entire soil mass was knocked from the plastic container and test plant roots carefully removed and shaken to remove most soil particles. Plants of each sample with their roots intact were brought to the laboratory for root zone study. Samples of root and adhering soil weighing 1-2 grams were placed in sterile 500 ml flasks containing 100 ml of sterile distilled water. The mouth of each flask was covered with aluminum foil to prevent loss by splashing and then they were shaken for 30 minutes on a reciprocal shaker (280 shakes/min). The contents of each flask were next filtered through double layers of cheese cloth into a 250 ml flask. This filterate with plant particles eliminated;

was the "rhizosphere soil suspension." Samples of the rhizosphere soil suspension were further diluted with 0.1% water agar and 3-6 plates seeded per assay. The amount of rhizosphere soil in a given suspension was determined back by oven drying a 20 ml sample at 105°C for 12 hours. After the dry rhizosphere soil in a sample was determined, fresh weight of root in the gross sample could be calculated. Assays of fungal propagules in the rhizosphere were expressed as numbers per gram fresh weight of root and/or numbers per gram oven-dry rhizosphere soil.

Root Surface Studies.--Harley and Waid's methods (20) were used with some modifications. The same root sample used for rhizosphere assay was used to assay for root colonization. Roots that had been collected on the filter cloth were placed in clean 500 ml flasks containing 100 ml distilled water and shaken (280/min) for 10 minutes. The wash water was decanted through filter cloth for each successive washing. Nine washings were found safe and sufficient for the removal of all spores and most hyphae clinging to the root surface. One experiment was conducted to compare the root washing efficiency by the shaker and running tap water method (720 l/hr) (Figure 1). The shaker method removed soil saprophytes more efficiently and retained more f. melonis than the running tap water method. This experiment was repeated several times with the same results. Roots to be plated for fungus colonization were cut

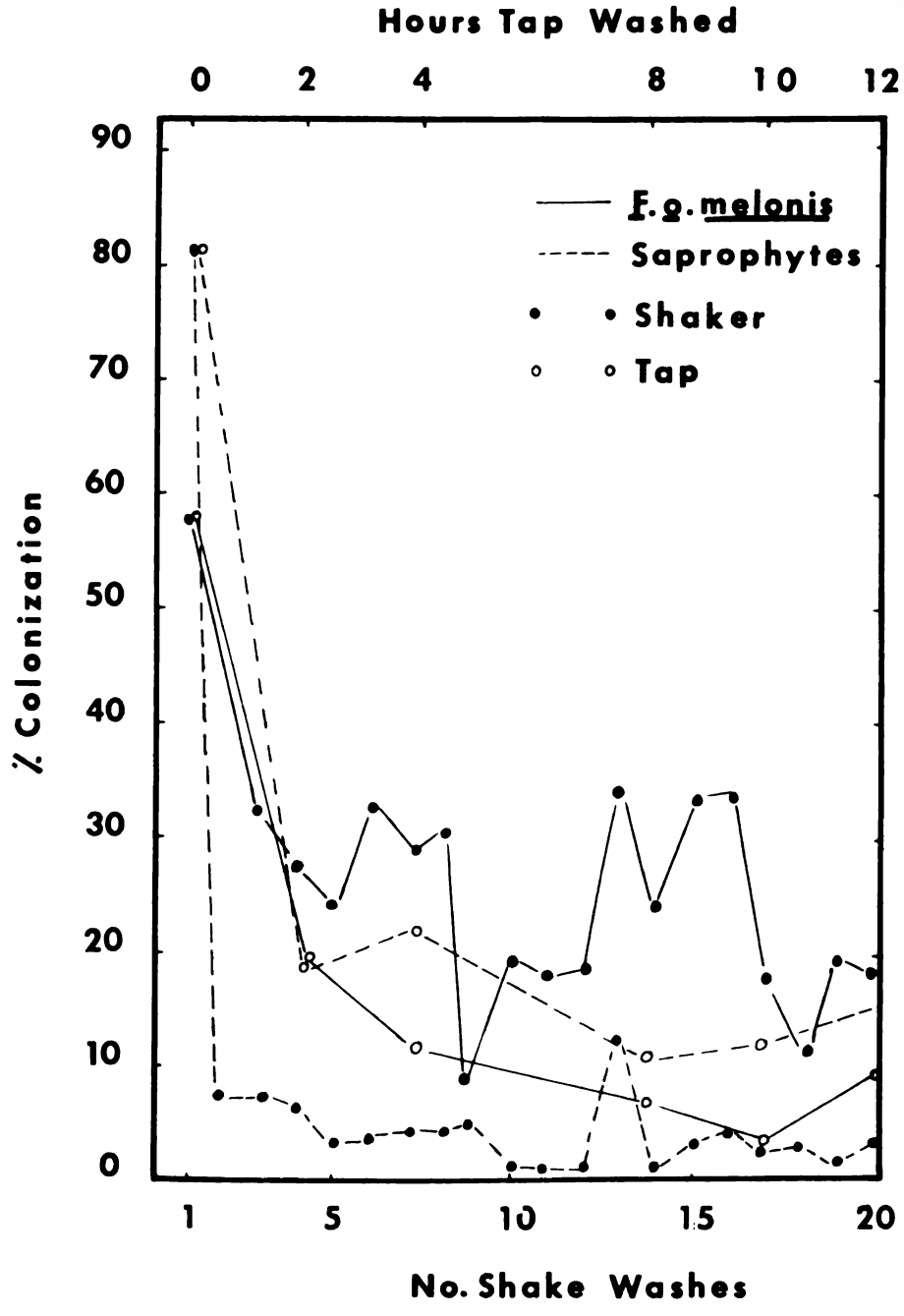


Figure 1. Comparative efficiency of two methods of root washing on recovery of root surface fungi (Fusarium oxysporum f. melonis and soil saprophytes). Harosoy soybean planted in autoclaved soil artificially infested with f. melonis (M-4).

aseptically into 2-3 mm segments. Each sample consisting of about 300 segments from randomly selected roots, was plated in rows on TMN-PDA and incubated for 4-6 days at room temperature. Separate counts were made for root segments in each sample colonized by Fusarium oxysporum and by other fungi, and these expressed as percent colonization of the total segments plated. Several forms of Fusarium oxysporum were present as in unsterilized field soil, colonization by f. melonis was estimated by index pathogenicity tests.

Pathogenicity Tests.--Distinguishing Fusarium oxysporum f. melonis from other forms in the species was possible only by using pathogenicity tests. A representative group of isolates, usually between 10-40 were tested in various experiments where needed. If the sample being assayed was from a known high disease situation a small index test sufficed. Four to five day old cultures of F. oxysporum from soil or plant material assays were transferred to medicine bottle slants and incubated 7 days at room temperature. An incubation at 27°C under continuous white, cool fluorescent light gave the best conidial production with a particularly striking increase in macroconidia. Inoculum was made by adding 30 ml of deionized water to each culture bottle and shaking for 30-60 sec. to suspend the spores. This inoculum suspension was then used in either of two procedures: (A) The shaker method (104) in which bare rooted melon seedlings

(10-14 day Hale's Best Jumbo grown in vermiculite) were used. Plants were in vials of inoculum on a shaker (60 shakes/min) and agitated continuously for about two weeks. (B) The root dip method in which the bare-rooted test seedlings were dipped in the inoculum and transplanted to 3" pots of steamed soil. Both methods were fast and reliable, but the shaker method required more attention and more care in watering. The shaker method required less greenhouse space. Three seedlings were tested for each isolate being indexed whichever method was followed. All inoculations were begun in late afternoon or evening to reduce transpiration shock and plants being transplanted to soil were protected for 2 days with newspaper.

Yellowed, wilted and dead plants were recorded daily up to 15 days for the shaker method and 3-4 weeks for the root-dip method. More than 3500 isolates of F. oxysporum were index tested for pathogenicity to muskmelon during the course of this research.

EXPERIMENTAL RESULTS

Physiological Races of Fusarium oxysporum f. melonis

Attempts were made to identify physiological races of F. oxysporum f. melonis among isolates used in the course of research. Many isolates obtained from various sources were compared for pathogenicity on muskmelon varieties. One domestic variety of muskmelon (Persian) proved to be resistant or nearly immune to a highly pathogenic isolate (M-4). One hundred highly pathogenic cultures isolated from soil or plants in a single field in southern Michigan were tested and failed to produce disease symptoms on Persian melon. In very rare cases f. melonis could be isolated but only from basal parts. Subsequent inoculation with reisolated cultures showed that there was no change in virulence on Persian melon.

Several attempts were made to find an American isolate capable of wilting Persian melon. Resistant plants were grown in naturally infested soil for six months. None of the Persian melons wilted but susceptible controls wilted within a short time. Pathogenic isolates of F. oxysporum f. melonis obtained from several parts of Michigan, Wisconsin, Minnesota, and Ontario likewise failed to cause any disease

symptoms in Persian melon. All of the above isolates were pathogenic to domestic varieties such as Iroquois, Harvest Queen, Spartan Rock, Hearts of Gold, Delicious 51, and Honey Rock.

A rhizosphere soil sample of a single wilted melon plant obtained from Iran gave very distinctive colonies of a Fusarium oxysporum. The Iranian isolates were highly pathogenic not only to Persian melon but also all other available melon varieties from Iran or the United States. Iranian melon varieties however were highly resistant to all the North American isolates of f. melonis (Table 1). Heavy infestation, as can be achieved in autoclaved soil may cause a damping-off reaction in some otherwise resistant melon seedlings. The Persian melon however was resistant to damping-off by the Michigan isolate M-4 at 25°C soil temperature, but occasionally susceptible at 15°C. In order to determine whether a longer contact time would break down resistance, 40 Persian melon seedlings were inoculated with isolate M-4 and transplanted into steamed soil. None produced disease symptoms after 5 months.

Comparison of Iranian and Michigan isolates:

Growth rate in culture.--It was of interest to know whether Iranian and Michigan strains of f. melonis obtained from two distinct geographical regions had the same temperature requirement in culture. Agar discs cut with a

TABLE 1. Comparison of pathogenicity of North American and Iranian isolates of F. oxysporum f. melonis on differential muskmelon varieties.

Melon vars.	Sources of Isolates				
	Michigan	Wisconsin[a]	Minnesota[b]	Canada[a]	Iran
Persian	R	R	R	R	S
6 Iranian melon vars. (P-1 to P-6)	R	R	R	R	S
Hales Best Jumbo	S	S	S	S	S
Iroquois	S	S	S	S	S

[a]Supplied by Dr. G. M. Armstrong.

[b]Supplied by Dr. C. J. Eide.

R: Resistant to wilt; S: Susceptible to wilt.

cork borer from the edge of six-day cultures of Iranian isolate I-5 and Michigan isolate M-4 were transferred to PDA plates. Three replicate plates were prepared for each isolate at each temperature. Radial growth at 12, 16, 20, 24, 28, 32, 34, 36, and 40°C was measured daily for six days. Optimum temperature for growth in culture was 28°C for both isolates but M-4 grew better at lower temperatures (Figure 2). Only I-5 was able to grow slightly at 36°C and both failed to grow at 40°C. Interaction of the temperature and culture factor appeared to be consistent but a

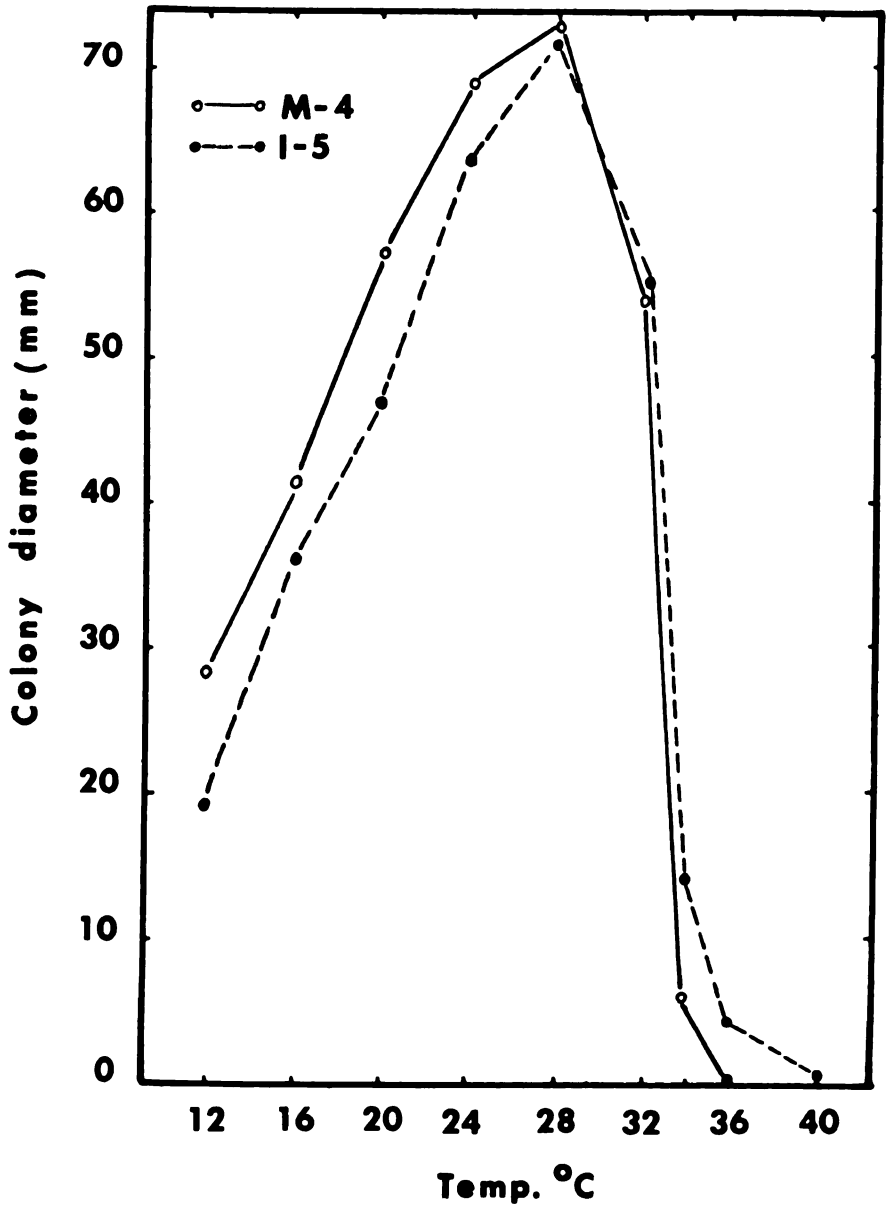


Figure 2. Radial growth of two strains of F. oxysporum f. melonis isolates I-5 (Iran) and M-4 (Michigan) grown at temperatures between 12 and 40°C for 6 days.

statistical analysis showed the differences were below the level required for significance.

Pigmentation.--Both the Iranian and Michigan isolates produced pigments on acidified PDA. On nonacidified PDA, Michigan isolates produced a very faint pinkisk color and only at 24 and 28°C. The color characteristic proved to be a reliable identification of Iranian isolates because a pinkish-purple pigment developed both in the culture medium and in aerial mycelium at all temperatures 12-36°C. Color differences between I-5 and M-4 were especially clear at 32°C and this temperature was used to identify and separate them in subsequent expeiments. On acidified TMN-PDA all cultures of Fusarium oxysporum that were isolated produced distinctly salmon colored colonies without reference directly to pathogenic capability. Iranian isolates of f. melonis were distincly more highly colored than Michigan isolates on both acidified and nonacidified PDA. Pathogenicity testing could be reduced considerably in some experiments because of this characteristic since 90% or more of the colonies identified by strong color were pathogenic to Persian melons.

Differential hosts.--Although various cultures of F. oxysporum f. melonis differed in degree of pathogenicity, no clear cut separation of them by reaction of differential varities had been known. Three highly pathogenic cultures, M-4, M-21 (Michigan isolates), and I-17 (Iranian isolate)

were tested on 20 melon varieties in a search for differential hosts. Two week old plants grown in sterile vermiculite were root-dipped in inoculum at 10^6 condia/ml and grown for 30 days in pots of sterilized soil. Roots of the controls were similar treated by dipping in deionized water. Trasplanted seedlings received normal greenhouse care (July temperature 25-30°C). Plants were examined daily and numbers of yellowed, wilted and dead plants were recorded up to 30 days after inoculation.

All varieties were susceptible to the Iranian isolate I-17 (Figure 3A). All of the Iranian melon varieties (P-1, P-2, P-3, P-5, P-6), and two of the American varieties (Persian small type and Smith's Perfect) were resistant to the Michigan isolates M-4 and M-21 and remained symptomless. Several other American varieties were partially resistant to Michigan isolates and a few Iranian melons were partially resistant to the Iranian isolate. Although all plants of the variety Spartan Rock wilted when inoculated with Michigan isolates, only 10-20% died.

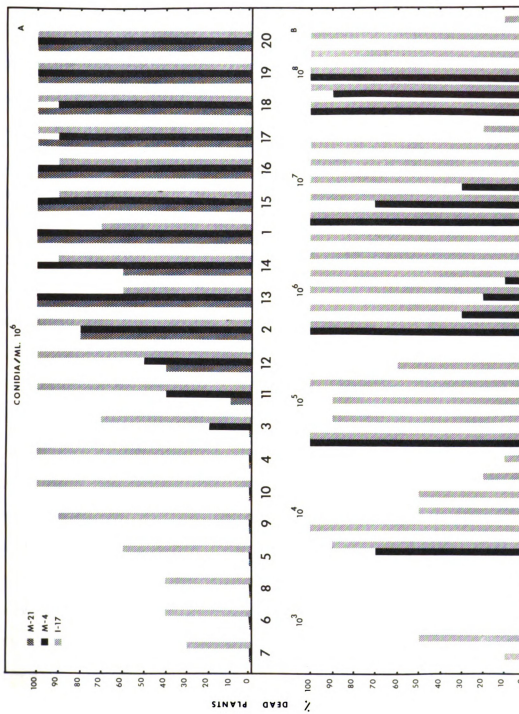
The incubation period was different in Michigan and Iranian isolates. When varieties of melon equally susceptible to both Iranian and Michigan isolates were inoculated with the same inoculum concentration, the disease symptoms appeared 2-3 days earlier with Michigan isolates.

FIGURE 3.

A. Comparison of susceptibility of 20 muskmelon varieties inoculated with Iranian (I-17), and Michigan (M-4 and M-21) strains of F. oxysporum f. melonis. Data were taken 30 days after inoculation by root dipping in conidial suspension (1,000,000/ml).

B. Reactions of six differential muskmelon varieties toward various concentrations of inoculum of Iranian (I-17), and Michigan (M-4) strains of F. oxysporum f. melonis. Data were taken 30 days after inoculation by root dipping.

Var. No.	Muskmelon Variety	Source
1.	Rocky Ford	U.S.A.
2.	Harvest Queen	U.S.A.
3.	Honey Dew	U.S.A.
4.	Smith's Perfect	U.S.A.
5.	Persian small type	U.S.A.
6.	P-1 (Kadkhoda Hosseinie sabz)	Iran
7.	P-3 (Baba-kharman Darreh-gaz)	Iran
8.	P-6 (Schakhteh Mashhad)	Iran
9.	P-5 (Kadkhoda Hosseinie zard)	Iran
10.	P-2 (Langrood)	Iran
11.	Spartan Rock	U.S.A.
12.	Hales Best Jumbo	U.S.A.
13.	Burpee Hybrid	U.S.A.
14.	Pride of Wisconsin	U.S.A.
15.	Schoon's Hard Shell	U.S.A.
16.	Delicious 51	U.S.A.
17.	Casaba	U.S.A.
18.	Hearts of Gold	U.S.A.
19.	Milwaukee Market	U.S.A.
20.	Honey Rock	U.S.A.


M-21
M-4
I-17
CONIDIA/ML. 10^6
A
DEAD PLANTS %
10^3
10^4
10^5
10^6
10^7
10^8
B
VARIETY NO.

Varietal response to concentration of inoculum.--
The melon varieties differed in resistance under uniform concentration of inoculum. An experiment was designed to determine whether higher or lower inoculum concentrations would affect the expression of resistance in selected varieties. Michigan (M-4) and Iranian (I-17) isolates of F. oxysporum f. melonis at six levels, 10^3 - 10^8 conidia/ml, were inoculated to 6 of the varieties used previously. Two-week old seedlings were root dip inoculated and then incubated at 25°C for 30 days. In treatments where plants were remained symptomless up to 30 days, 5 of the plants were used for confirming isolations.

Final counts expressed as percentages of dead plants are illustrated in Figure 3B. The Iranian isolate (I-17) caused appreciable disease in 2 varieties at an inoculum concentration of 10^3/ml, but the Michigan isolate (M-4) did not. At a concentration of 10^4, all varieties became diseased with the Iranian isolate but the Michigan isolate killed only 70% of the comparatively susceptible Rocky Ford melon. A concentration of at least 10^6 conidia/ml was required for less susceptible varieties such as Harvest Queen and Honey Dew to be seriously affected with the Michigan isolate. Muskmelon varieties Persian, P-1 (an Iranian variety of melon) and Smith's Perfect were mainly resistant to the Michigan isolate up to 10^8 conidia/ml. Only one inoculated plant of Smith's Perfect variety died at the

10^6 concentration and otherwise all remained alive and symptomless up to the end of the experiment. At and above 10^7/ml there was slight stunting in some resistant plants. A temporary wilting in resistant varieties occurred 3 days after inoculation with 10^7 conidia/ml of the Michigan isolate but not with the Iranian isolate. Smith's Perfect variety received the most severe shock from the Michigan isolate and remained stunted up to the end of experiment. Higher inoculum concentration of both isolates produced disease symptoms earlier. The Iranian isolate could not be recovered from stems of symptomless inoculated plants. The Michigan isolate on the other hand could be isolated from stems of some symptomless plants inoculated with spore concentrations of 10^5/ml or above but not from Persian melon inoculated with any concentration.

The experiment indicated that the Iranian and Michigan isolates belong to two distinct physiological races of F. oxysporum f. melonis on the basis of host specificity.

After this work had been completed, a paper by French workers (57) came to the attention of the author. They described three physiological races of F. oxysporum f. melonis based on differential host specificity. Seeds of their differential muskmelon varieties were generously supplied by Dr. Georgette Risser. These were sown and the plants inoculated by root-dip with M-4 and I-17 isolates of F. oxysporum f. melonis. Two of the varieties,

"Ogon No. 9" and "C.M. 17187" were extremely susceptible to the Michigan isolate and resistant to the Iranian isolate (Table 2). On the other hand, one muskmelon variety (Doublon) was resistant to the Michigan isolate and susceptible to the Iranian isolate.

TABLE 2. Comparative pathogenicity of Iranian, Michigan and French isolates of F. oxysporum f. melonis on differential muskmelon varieties.

Melon varieties	Race 1 (after Risser and Mas)	Race 2	Race 3	Michigan isolate (M-4)[a]	Iranian isolate (I-17)[a]
Charentais T.	S	S	S	S	S
Doublon	R	S	S	R	S
Ogon No. 9	R	R	S	S	R
C.M. 17187	R	R	S	S	R
Persian	-	-	-	R	S
Smith's Perfect	-	-	-	R	S
Rocky Ford	-	-	-	S	S

[a] 2×10^6 washed conidia/ml inoculum used in a greenhouse trial. Five to 10 plants were inoculated with each isolate.

If the race classification of F. oxysporum f. melonis is to the 4 varieties used by Risser and Mas (57) then the Iranian isolate could be considered as race 2 and the Michigan isolate based on the reaction of Doublon would

be a new race or race 4. Reactions of the 3 races described by Risser and Mas on my differential varieties are not known. If the Iranian isolate is race 2, then there are at least 4 races of F. oxysporum f. melonis. Probably all North American isolates of F. oxysporum f. melonis used by the author could be grouped in the tentative new race 4.

Survival of the Organism at Different Soil Temperatures

An experiment was designed to study the population changes of F. oxysporum f. melonis in autoclaved and non-autoclaved soils at different soil temperatures for a period of 420 days. The inoculum suspension of isolate M-4 consisted mainly of microconidia with a few mycelial fragments and hyaline chlamydospores. Sandy loam top soil was collected from within melon rows in a field heavily infested with F. oxysporum f. melonis in January 1966 and screened to remove debris. Part of the soil was sterilized and then left on the greenhouse bench for 10-15 days for recolonization by air-borne saprophytes. Species of Trichoderma and Penicillium predominated.

Inoculum suspension was mixed with lots of autoclaved and nonautoclaved soils and each soil lot divided into 5 one-liter plastic top containers with tight fitting tops (about 1200g/container). Non-treated samples were saved for control assays. Soil moisture was maintained at about 12% on oven dry basis throughout the experiment. One container

of each soil lot was incubated at each of the temperatures--5, 15, 20, 25, and 30°C. Soil samples were obtained by coring to the container bottom with a sterile glass tube. Three cores of soil were removed, mixed, and re-divided into 2-4 sub-samples per assay. Five acidified TMN-PDA plates were used per sub-sample. The high level of inoculum added to the soil together with high soil dilution made it possible to exclude the effects of most other fungi including the F. oxysporum naturally present.

Soil populations immediately after infesting with the pathogen were considered to be 100% for subsequent assays. Autoclaved soil contained 317×10^5 propagules/g oven-dry soil of F. oxysporum including f. melonis and the nonautoclaved soil 65×10^5 when assayed. The controls had none and 0.03×10^5 respectively in autoclaved and nonautoclaved soil.

The greatest decline in population occurred within the first 30 days regardless of temperature or soil (Figure 4). The rate of decline was faster at the higher temperatures and in nonautoclaved soil than at lower temperatures or in autoclaved soil. At the higher temperatures, especially in nonautoclaved soil, f. melonis reached a rather stable level sooner than was the case at lower temperatures or in autoclaved soil. In autoclaved soil the changes in population of f. melonis at lower temperatures were rather gradual, whereas at 25, and 30°C there was a considerable

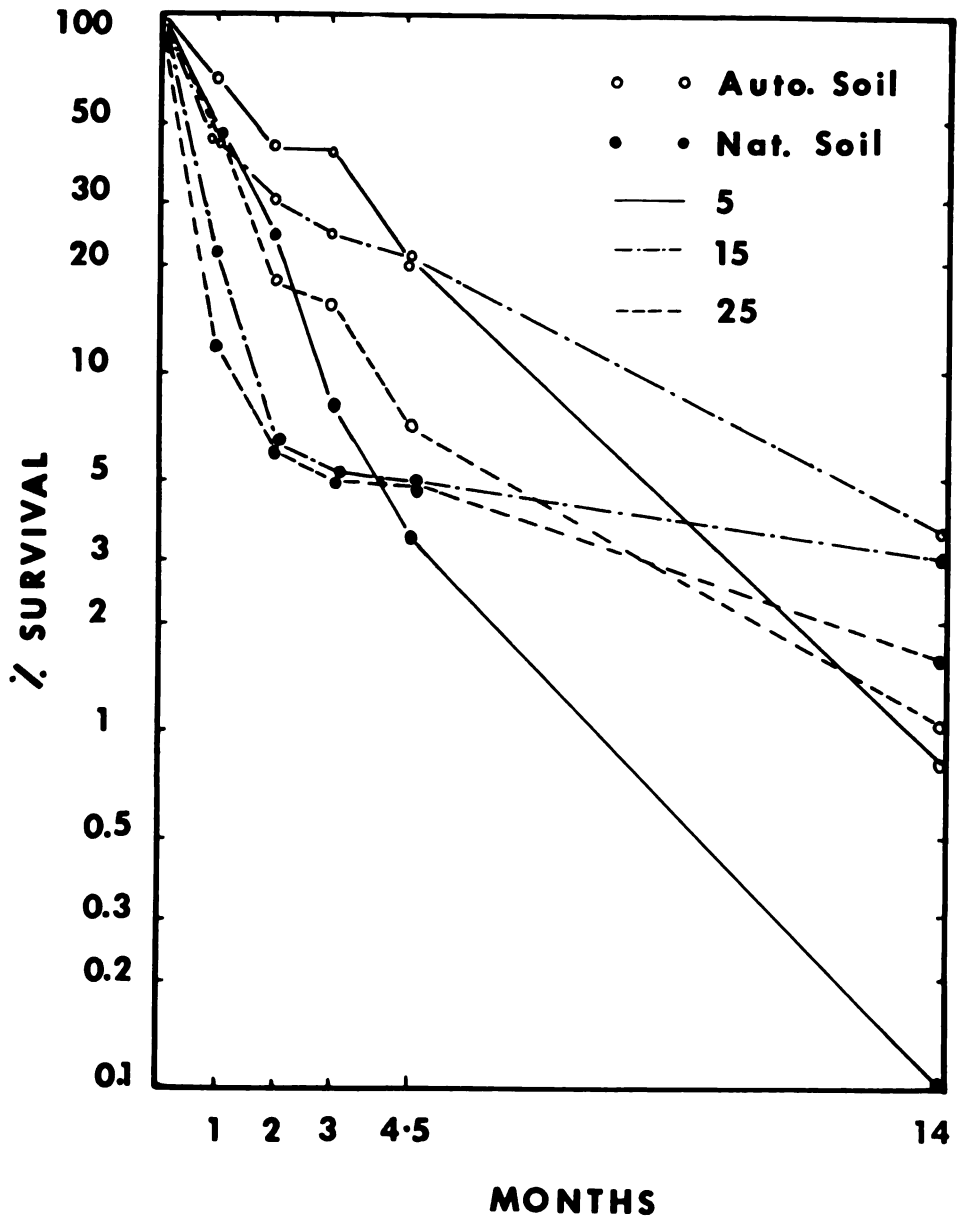


FIGURE 4. Survival of F. oxysporum f. melonis (M-4) added to nonautoclaved and autoclaved soil stored at various temperatures. (Initial population of M-4 in autoclave and nonautoclaved soils: 317 × 10^5 and 65 × 10^5 per gram oven-dry soil respectively.)

decrease 90 days after incubation. The most interesting results were obtained at 420 days (Figure 4). The lowest populations were at 5 and highest at 15°C in autoclaved and nonautoclaved soils.

Another experiment was performed to determine the fate of conidia introduced into nonautoclaved soil. Macroconidia of isolates M-4 and I-17 grown under continuous flourescent light at 27°C, were washed three times with distilled water, mixed with nonsterile soil and incubated at 5, 15, and 25°C. Five days later they were examined microscopically by Nash and Snyder's method (42) using 0.1% phenolic rose bengal. Very few germinated conidia of either isolate could be detected at any temperature. At 15 or 25°C some of the macroconidia appear to have changed to chlamydospores without germinating. At 5°C no germination or conversion into chlamydospores could be detected. When soil samples were examined 40 days after incubation at 15 and 25°C, no macroconidia could be observed. A few spherical thickwalled chlamydospores were detected.

The experiment indicated that a few conidia are converted into chlamydospores at 15°C or more and that conversion probably takes place without germination. Perhaps this is due to lack of available nutrients.

The Organism as Affected by Host and Nonhost Plants

Population changes in the root zone of wilt susceptible muskmelons in naturally infested soil.--Population changes taking place in the root zone of a typical susceptible host were studied. Among the considerations were rhizosphere populations and root colonization at various growth stages; effects of soil moisture on disease development and root zone microflora; and the effect of infection on populations of F. oxysporum f. melonis and other soil fungi in the vicinity of roots.

Naturally infested soil supplemented with 300 ppm 12-12-12 fertilizer was planted with wilt susceptible Hale's Best Jumbo. Nonplanted soil was used as control. After 3 days most seeds had germinated and soil moisture was adjusted to the experimental levels--40% and 70% whc (16% and 28% total moisture). The units were incubated at 25°C in temperature tanks during the experiment.

Root and soil samples were taken from 3 of the pots of each moisture level 2, 4, 6, and 8 weeks after planting and assayed for infection and root zone populations.

Root colonization and numbers of propagules of f. melonsi in the rhizosphere increased progressively with time at both moisture levels but higher populations occurred at the lower moisture (Figure 5). No rhizosphere effect was noticed at 2 weeks and root colonization was detectable

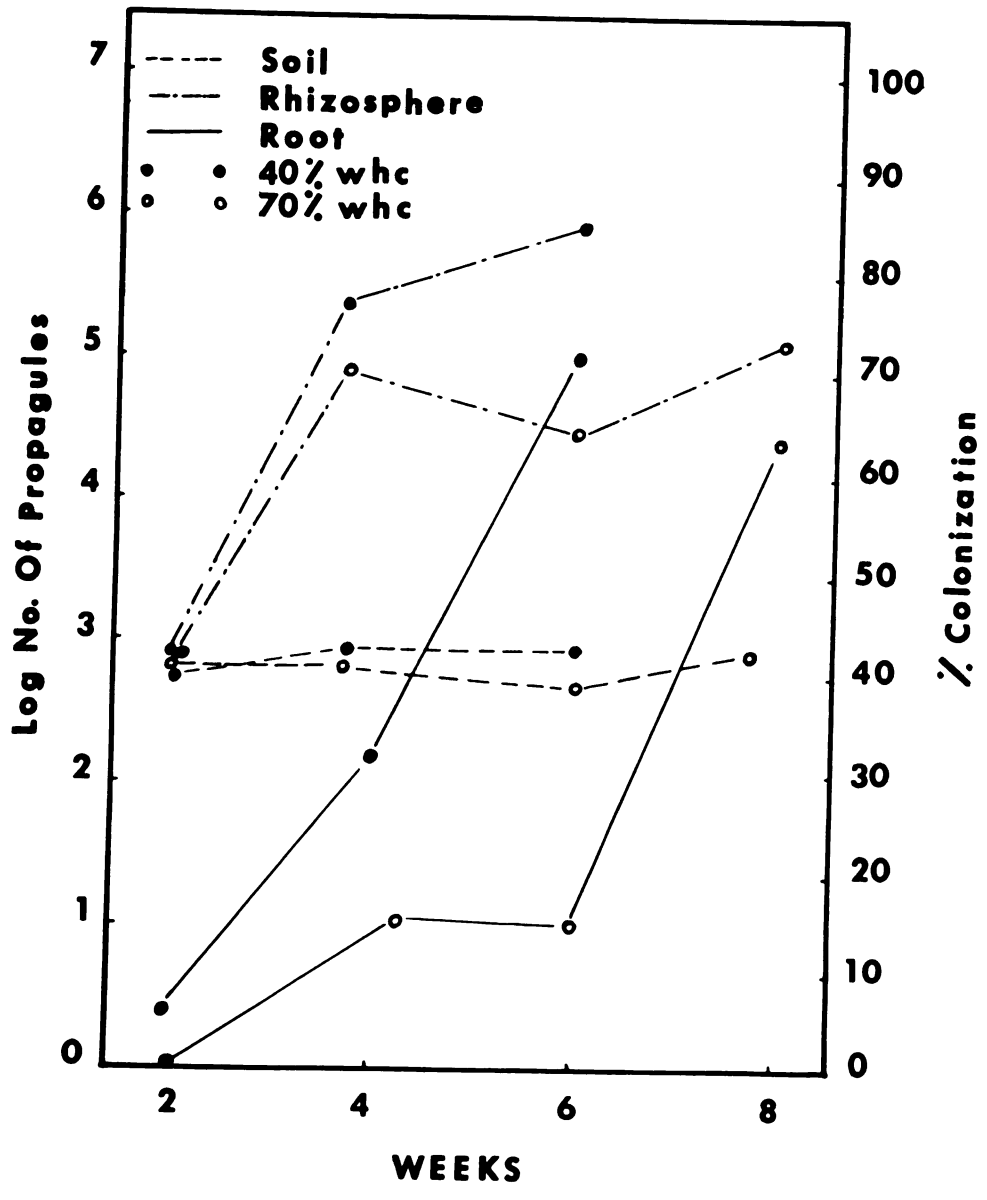


FIGURE 5. The effect of two soil moisture levels in naturally infested soil on rhizosphere population of F. oxysporum f. melonis and its root colonization of wilt susceptible melon var. Hale's Best Jumbo at 2, 4, 6, and 8 weeks after planting.

only at the lower soil moisture. Disease symptoms had not appeared at this time but the pathogen could be isolated from stems of some symptomless plants growing at the lower soil moisture.

Rhizosphere influence on F. oxysporum f. melonis was appreciable at 4 weeks at both moisture levels and the pathogen could be isolated from aerial parts of plants. There was a slight decline in both rhizosphere population and root colonization by F. oxysporum f. melonis at 70% whc in the 6 week assay. Wilt symptoms were present at 3-4 weeks in the lower soil moisture, but delayed to 6-8 weeks in the higher soil moisture. Only two moribund plants were left in the 40% whc soil at 6 weeks and this casued a considerable increase in F. oxysporum f. melonis propagules in the rhizosphere (Table 3). At 70% whc the maximum population of the pathogen occurred 8 weeks after planting. F. oxysporum f. melonis remained relatively constant in the control soils during the experiment.

(a) Comparison between rhizospheres of healthy and infected plants.--Rhizosphere population and root colonization of systemically infected plants (wilted or apparently healthy but not decayed) were compared with those in non-infected plants (Table 4). Infected plants harbored greater numbers of f. melonis and other fungi in their rhizosphere than the healthy plants, but there was not a good correlation between infection and root colonization.

TABLE 3. Rhizosphere population of F. oxysporum f. melonis and root colonization of wilt susceptible melon var. Hale's Best Jumbo grown for various times in naturally infested soil at 25°C and either 40 or 70% whc.[a]

% whc	Propagules per: g fresh wt of root				Propagules per: g rhizo. soil				Colonization of root segments			
	Weeks				Weeks				Weeks			
	2	4	6	8	2	4	6	8	2	4	6	8
	No. × 10^3								%	%	%	%
40	0.15	33	79.0	-[b]	0.95	278	853	-	8.8	31.6	72.5	-
70	0.15	14	1.6	25.6	0.77	79	34	158	0.0[c]	15.6	14.5	64

[a]Population of F. oxysporum f. melonis in naturally infested soil ranged from 9-11 × 100 and 7-10 × 100 in 40 and 70% whc respectively per g oven-dry soil.

[b]All plants were dead.

[c]Based on 10 isolates tested for pathogenicity.

TABLE 4. Comparison of rhizosphere population of F. oxysporum f. melonis and root colonization of healthy and systemically infected living muskmelon plants at 70% whc.

Weeks after planting	Prop./g rhiz. soil		Colonization of root segments	
	Healthy	Infected	Healthy	Infected
	No. × 10^3		%	%
2	0.0	3.0	0	0
4	3.0	235.0	44	4
6	80.0	100.0	14	7
8	31.0	280.0	51	87

Changes in population of other soil fungi in the root zone during growth were similar to those of F. oxysporum f. melonis (Figure 6). A Penicillium predominated over all other fungi except the pathogen. There were also some changes in the species present during growth and disease development.

Direct microscopic observations of rhizosphere soils and of infected roots revealed that the increased F. oxysporum f. melonis in the rhizosphere was mainly due to sporulation of the pathogen on infected roots. Scattered or grouped sporodochia bearing conidia and conidiophores were observed. Macroconidia were the predominant forms. Few mycelial branches were present on the infected roots. The situation was similar for either naturally or artificially

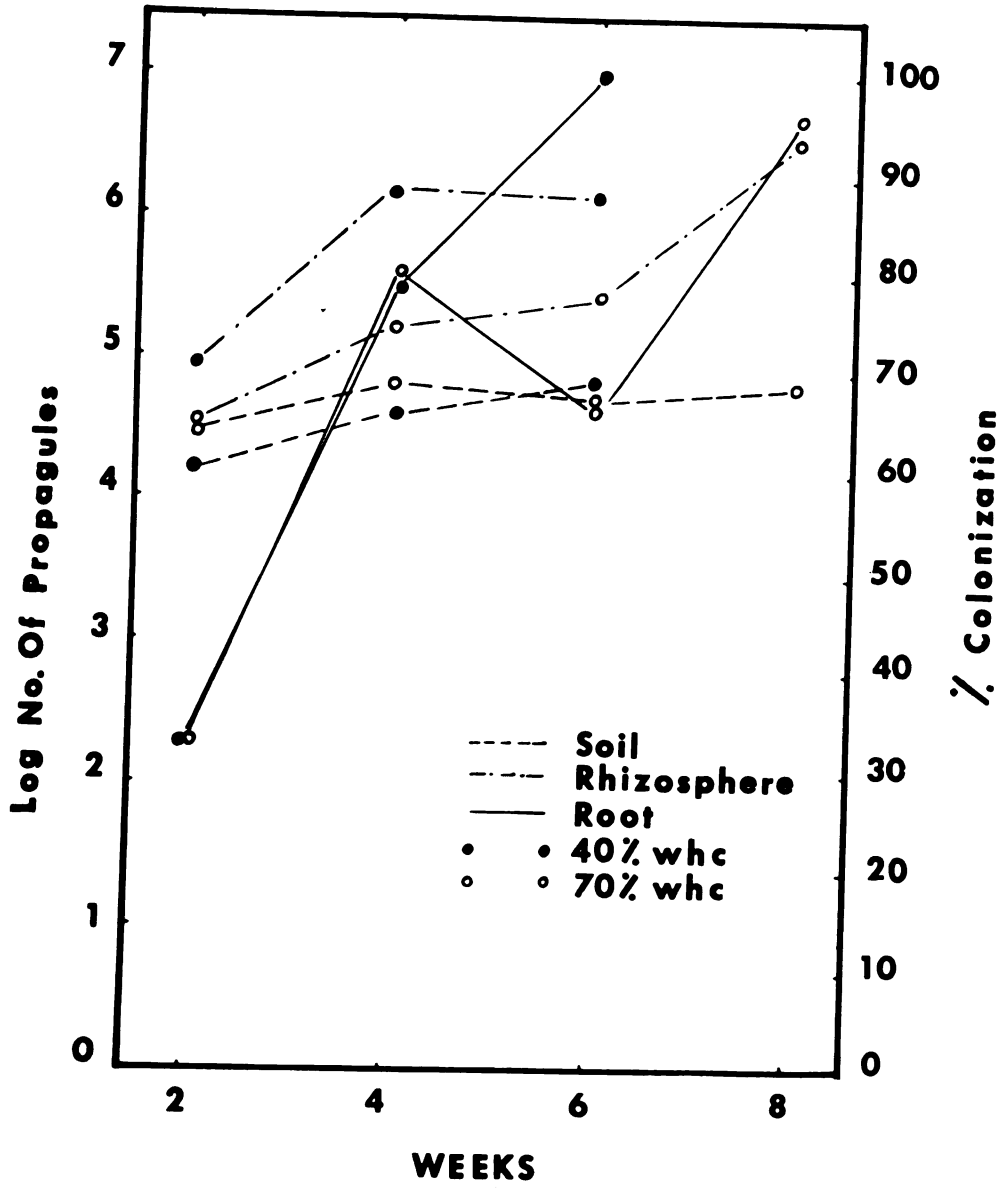


FIGURE 6. The effect of two soil moisture levels on rhizosphere population and root colonization of a wilt susceptible melon var. Hale's Best Jumbo by saprophytic fungi in naturally infested soil. Assays at 2, 4, 6, and 8 weeks after planting.

infested soils. When wilt susceptible melons were grown in naturally infested soil in root observation boxes, large numbers of absorptive roots were killed prior to the appearance of wilt symptoms.

The differences in root zone populations at 40 and 70% whc were probably due to several factors. Growth of muskmelon plants is profoundly affected by soil moisture after the first two weeks. At 30 days, plants growing at 40% whc remained much smaller than those at 70% whc. Miller (40) reported that lower soil moisture predisposed the melon to Fusarium wilt. This is confirmed in the present investigation. Higher soil moisture delayed the disease but did not prevent it. Increase of other micro-organisms in the rhizosphere of plants growing at lower soil moisture has been attributed to increase in root exudation and root hair formation (50).

Population changes in soil as affected by host and nonhost plants.--Changes in amount of the pathogen in field soil is influenced by the crop grown. The first of 2 experiments to study the influence of host vs. nonhost plants was performed on the M.S.U. Botany farm. Soil samples were taken from around plants or between melon rows and at various depths. Table 5 shows how wilted and nonwilted plants or the absence of plants in control soil affect the population of f. melonis. Soil samples were collected in October 1966 a week after harvest. At this time tissue isolations

were only successful from stems of wilted plants. Soil around wilted plants had 20 times as much f. melonis as around healthy plants and about 500 times as much as non-planted soil. Both diseased and healthy plants increased the pathogen considerably in comparison to control soils. Of the Fusarium oxysporum colonies obtained around diseased plants, 97% were pathogenic to melons and from control soils only 1% was pathogenic. The highest populations were obtained from surface soil.

TABLE 5. Effect of wilted melons, nonwilted melons and of melon-free soil on the population of F. oxysporum f. melonis.

	No. of propagules/g soil						
	F.ox.f.melonis			Other F.oxysporum			Other Fungi
Sample Depth:	0-2"	2-6"	6-12"	0-2"	2-6"	6-12"	0-12"
Sample	No. × 100						
Wilted melons	313.0	37.0	16.0	0.0	9.0	4.0	2440.0
Healthy melons[a]	11.0	8.0	0.0	9.0	2.0	3.0	440.0
Control soil[b]	0.0	0.6	0.0	28.0	19.4	8.0	395.0

[a]No recovery of F. oxysporum f. melonis from vines.

[b]Between rows.

The experiment was continued until May 1967 (Table 6). Cropping with a susceptible melon increased the pathogen considerably, whereas a sharp decline in population occurred during fallowing, especially on the soil surface. There were

TABLE 6. Effect of susceptible melon and melon-free period on population changes of F. oxysporum f. melonis.

	No. of propagules/g soil								
	F.ox.f.melonis			Other F.oxysporum			Other Fungi		
Sample Depth:	0-2"	2-6"	6-12"	0-2"	2-6"	6-12"	0-2"	2-6"	6-12"
Sample Date	Within Rows:			No. × 100					
Oct. 1966	112	17.0	5.3	5.0	7.0	3.7	1760	1570	135
May 1967	2	1.7	0.4	6.0	8.3	3.6	524	170	66
	Between Rows:[a]								
Oct. 1966		0.22			18.98			395	
May 1967		0.20			39.80			196	
	Within rows:								
Jan. 1967:									
0-1"		24.0			24.0			950	
1-4"		2.7			2.6			640	

[a]Average of 3 depths.

proportionately smaller declines in deeper soil. F. oxysporum f. melonis was not obatined from surface soil in the control areas (between rows) but only from the 2-6" depth. It would appear that inter-row sites were not readily contaminated from plants in the rows. Low recovery of the pathogen from between rows also points out that the pathogen had not grown well as a saprophyte in the inorganic soil. Samples obtained in January 1967 contained more

wilt Fusarium than those obtained in May. This indicates that freezing conditions during winter were less detrimental to the pathogen than the relatively mild temperatures of late April and May. Much sloughed-off tissue was found around both infected and noninfected melon vines. This decomposition and destruction of the tissue structures in the early spring may account for the reduction of pathogen population in May.

The second experiment was performed on the Gerst farm, which was heavily infested with F. oxysporum f. melonis. After the melon crop had been harvested, the field was left unploughed until the next spring. Soil samples were obtained in melon rows at different depths during March 1966 (Table 7). Populations of F. oxysporum f. melonis, other Fusarium oxysporum and other soil fungi decreased with depth. Soil moisture, PH, and % organic matter were nearly alike at all depths. Although more than 60% of the F. oxysporum cultures obtained from the surface were pathogenic to melon, the proportion was lower at other levels. At 3-8" the proportion was higher than at 1-3" but the total number was not. This probably indicates the influence of infected root system at the lower levels. It is also evident that most of the inoculum of F. oxysporum f. melonis dervied from sporulation on infected plants and the release of infected cells

on the soil surface. The field was otherwise quite uniformly populated with the pathogen as nearly equal numbers of propagules were found on samples from different areas.

TABLE 7. Vertical distribution of F. oxysporum f. melonis and other soil fungi in an infested melon field-March 23, 1966.

Sample Depth	Soil H2O	Organic Matter	Soil PH	No. of propagules/g soil[a] F. oxysporum f. melonis	Other F. oxysp.	Other Fungi
in.	%	%			No. × 10^3	
0-1	15.4	3.2	6.2	6.1	3.4	150
1-3	14.5	3.1	5.8	1.2	3.3	110
3-8	15.5	3.0	5.8	1.8	2.2	80

[a]Average of 5 replicates.

Effect of susceptible host, nonhost, and of a crop-free period on propagule populations.--Soil samples were obtained from the top 4-inch soil layer at several times between August 1965 and April 1967. Table 8 summarizes the results. The presence of a susceptible melon host increased the population of F. oxysporum f. melonis. Maximum numbers of propagules were reached in August when most melon plants were badly infected with Fusarium. Although the pathogen decreased about 30% from its August high population by January 1966, it subsequently remained unchanged until early spring.

TABLE 8. Population changes of F. oxysporum f. melonis and other soil fungi at intervals in a single field during 20 month period.[a]

Sample Date	Crop or Residue	Propagules/g soil		
		F. oxy. f melonis	Other F. oxysp.	Other Fungi
		No. × 10^3		
Aug. 18, 1965	melon plant[b]	9.1	11.9	142
Jan. 22, 1966	melon residue (under snow)	3.3	2.2	75
Mar. 23, 1966	melon residue (thawing)	3.0	3.0	110
June 18, 1966	soybean plant (seedling)	0.2	1.7	40
July 21, 1966	soybean plant[d] (flowering)	1.6	3.6	70
Oct. 15, 1966	soybean residue	0.2	1.8	120
April 1, 1967	soybean residue	0.2	4.0	140

[a]Howard Gerst farm, Belleville, Michigan.

[b]Soils were collected from within plant rows. Before soil dilution was made plant materials were removed.

[c]Population of all forms of F. oxysporum between rows: 3 × 10^3 pro./g.

[d]Soil was completely covered with soybean roots.

This again indicated that a freezing period maintained the level of population of F. oxysporum f. melonis almost unchanged. Between April and May 1966 the field was ploughed. In early June half of the field was planted with soybeans and the other half with corn. By June 1966 populations of

F. oxysporum f. melonis in samples of soil from the ploughed field had dropped considerably and then remained relatively constant to the end of the experiment (April 1967). The ratio of pathogenic to nonpathogenic F. oxysporum decreased after melon residues had been decomposed. In the earlier period (August 1965-March 1966) more than 50% of the F. oxysporum isolates were pathogenic to melons, but later (June 1966-April 1967), populations contained only about 12% of the melonis form. The sharp drop was thought to be due to decomposition of most of the infected melon residues during early June.

Effect of soybean and corn cropping and nonplanted soil on propagules populations.--Soil samples were collected within and between rows of soybeans and corn in April 1967 and populations compared with the soil samples which had been examined in June 1966 (Cf. Table 8). The June 1966 samples were collected from rows of soybean and corn in the seedling stage and served to represent approximately the initial population of the whole field as root influences were considered to be negligible (Table 9).

Populations of F. oxysporum f. melonis both within the soybean rows and between rows (nonplanted control soil) were similar and remained unchanged throughout the experiment. Of 40 isolates of F. oxysporum obtained from within corn rows in April 1967, none was pathogenic to susceptible melons. These results agree with the results in another

rhizosphere population study (Table 12). There was also reduction of other Fusarium oxysporum within corn rows as compared to between corn rows or within soybean rows.

TABLE 9. Effect of soybean and corn cropping and of non-planted soil on the soil population of F. oxysporum f. melonis and other soil fungi.[a]

Sample Date	Location of Soil Samples	Propagules/g soil		
		F. oxy. f melonis	Other F. oxysp.	Other Fungi
			No. × 1000	
June 18, 1966	Soybean & Corn rows	0.24	1.7	40
April 1, 1967	Soybean rows	0.21	4.0	140
April 1, 1967	Corn rows	0.00[b]	2.5	100
April 1, 1967	Between rows of corn & soybean	0.25	3.1	100

[a]Howard Gerst farm, Belleville, Michigan.

[b]Based on 40 isolates used for pathogenicity test.

Results of the experiments indicated that the pathogen is capable of colonizing roots of many nonhost plants without accompanying increase of f. melonis in soil. The population of f. melonis in soil increased in the presence of a host and decreased in its absence. The greatest increase in population of the pathogen was at the site of infected plants and on the soil surface. A considerable amount of f. melonis could be detected below 6 inches deep. Under Michigan conditions population of the pathogen was

not greatly affected by steady low temperatures but declined sharply in Spring. The drop in population was assumed to be due to progressive decomposition of infected melon residue which is an important source of inoculum in the field.

Population changes in root zones of host vs. nonhost plants-Greenhouse.--Several experiments were designed to investigate the activity of f. melonis in the root zones of several host and nonhost plants in the greenhouse.

In the first experiment naturally infested soil was infested with 9 month old soil inoculum of the Iranian isolate of f. melonis (I-5). Three levels of inoculum were used to obtain low (100), intermediate (1000), and high (10,000 propagules/g). Control soil was natural soil without I-5 inoculum (having about 240 propagules/g soil of Michigan forms of f. melonis).

Seeds of soybean, corn (nonhosts), Persian and P-1 melons (respectively susceptible and semiresistant to the Iranian isolate but both highly resistant to Michigan isolates) were planted and incubated at 25°C. Soil moisture was maintained at 40% whc. Root zone and soil analyses were made 4 weeks after planting (Table 10).

Persian melon is susceptible to I-5 and had the highest colonization of roots by I-5. Corn had the lowest. The frequency of root colonization was dependent on the inoculum density. Identification of the inoculum organism

TABLE 10. *F. oxysporum* f. *melonis* (I-5), other *F. oxysporum*, and other fungi in the rhizosphere and as root colonizers of host and nonhost plants grown in field soil artificially infested with different levels of inoculum of the pathogen (I-5).

	Rhizosphere Population Assayed[a]											
	Inoculum isolate (I-5)				Other *F. oxysporum*				Other Fungi			
Initial Inoculum Level[b] (I-5)	1	2	3	4	1	2	3	4	1	2	3	4
Plant	No. × 10^3								No. × 10^5			
Persian melon (S)	0.0	0.0	2.5	4.7	72	3.1	2.5	4.7	29	10.0	9.2	2.8
P-1 melon (R)	0.0	0.0	4.7	0.0	5	1.8	4.7	0.0	30	27.0	1.7	8.4
Soybean (NH)	0.0	0.0	0.0	0.0	243	5.0	0.0	0.0	14	10.0	7.2	6.9
Corn (NH)	0.0	0.0	0.0	0.0	168	1.9	7.6	2.2	3	2.0	3.5	2.1
Control soil	0.0	0.05	0.5	3.5	4.2	4.8	4.5	5.0	1	1.0	1.1	1.1
Inoculum (I-5) Level	Root Colonization											
	1	2	3	4	1	2	3	4	1	2	3	4
Plant	%	%	%	%	%	%	%	%	%	%	%	%
Persian melon (S)	0.0	2.0	12	49	60	41	57	47	22	27	25	12
P-1 melon (R)	0.0	0.0	1	32	49	55	26	41	33	38	54	19
Soybean (NH)	0.0	0.0	2	24	79	70	62	59	8	10	18	14
Corn (NH)	0.0	0.0	0	1	57	43	37	40	46	45	50	40

S: Susceptible, R: resistant, NH: nonhost

[a]No. propagules/g oven-dry soil.

[b]Level 1:0, 2:100, 3:1000, 4:10,000 propagules of I-5/g oven-dry soil.

among other forms of Fusarium oxysporum was relatively easy at the highest inoculum level. At the lowest level of inoculum no I-5 colonization could be detected in the resistant host (P-1 melon) or in either of the nonhost plants.

Rhizosphere populations of I-5 were comparatively low at all inoculum levels and on all plants. This failure was partly due to the presence of great numbers of other soil fungi, particularly several species of Penicillium. Many propagules of the pathogen may have been lost because of the high dilution of rhizosphere samples and low numbers of plates used. None of the susceptible or resistant hosts or nonhost plants showed disease symptoms either at low (100 p/g) or intermediate (1000 p/g) levels of inoculum. Most of the susceptible and a few of the resistant melons died at the highest (10,000 p/g) level while the nonhost plants remained apparently healthy at all levels of inoculum. Infection in some host plants, especially the resistant melon, took the form of crown decay.

Root colonization by other forms of native F. oxysporum was high in all plants, particularly in soybeans. These forms decreased in the rhizosphere of all plants where infestation by the Iranian isolate was added and were highest in the absence of I-5. Root colonization by other soil fungi (excluding F. oxysporum types) was highest on corn and lowest on soybean in all levels of added I-5 inoculum. Rhizosphere populations of other fungi increased

considerably with growth of all the plants except corn relative to the nonplanted control soils. These other fungi also decreased in the rhizosphere of all plants as the level of I-5 inoculum was increased.

Population changes of the Iranian isolate I-5 were compared for previously planted and nonplanted soils. After roots had been removed, soils from each chosen treatment were individually mixed and assayed in the same way as nonplanted soils. Planted soils were assayed only at inoculum level 1 and 4, but nonplanted soils at all levels (Table 11). The highest recovery of isolate I-5 was less than the starting level e.g. 8500 vs. 10,000 in soil planted with Persian melon.

It is probable that there was some loss of propagules in naturally infested soil during the experimental period but also there may have been dilution errors when the soil was infested intitially.

Cropping with the susceptible (Persian) and semi-resistant (P-1) melons maintained the greatest numbers of the pathogen whereas with the nonhosts, especially corn, the pathogen tended to decrease to lower population than in the control soils.

There appear to be no clear relationship between the root/soil ratio and survival or increase of I-5. The amount of roots varied greatly for the different varieties, being maximum for soybean and corn and minimum for Persian melon.

TABLE 11. Population changes of F. oxysporum f. melonis (I-5) in nonplanted and planted sandy loam field soil artificially infested with different levels of the Iranian strain (I-5) of f. melonis.

Initial level of inoculum in field soil (prop./g)	Control	Persian	P-1	Soybean	Corn
	No. of propagules/g soil × 100				
0	0.0	0	0	0	0
100	0.5	-	-	-	-
1000	5.0	-	-	-	-
10,000	35.0	85	55	30	20

[a]Dash indicates assay not made.

Activity of the organism in the root zone of nonhost plants-Field.--Several investigations on the multiplication and survival of f. melonis in the presence and in the absence of host and nonhost plants were carried out in two fields with histories of muskmelon wilt. The Howard Gerst farm field had been heavily infested with melon wilt in the summer of 1965. The cropping history of this field was as follows:

Year	Crop	Note
1961	Melon	few infected plants with Fusarium
1962	?	
1963	Tomato	
1964	Wheat	
1965	Melon (wilt suscept)	50% loss due to F. oxysporum f. melonis
1966	Soybean and corn	

The other field was at East Lansing, Michigan, and was a loam soil naturally infested with f. melonis.

Results obtained from assays on soybeans and corn on the Gerst farm are summarized in Table 12. Root samples were taken between June 1966 and April 1967. The percentage of root segments colonized by F. oxysporum f. melonis remained almost constant during the experiment, except during early stages soybean growth. Actively growing corn gave very low recovery of melon wilt Fusarium. Overwintered roots of soybean and corn which had been left undisturbed were alike as to recovery of F. oxysporus f. melonis. Colonization of corn roots by F. oxysporum f. melonis tended to increase as the plant aged but soybean root colonization was highest at the seedling stage. Recovery of F. oxysporum f. melonis in partially disintegrated

TABLE 12. Occurrence of F. oxysporum f. melonis and other soil fungi in the root zone of field grown soybean and corn at various stages of growth.[a]

Sample Date	Growth stages and Weeks after planting	F.ox.f.melonis		Other F. oxysporum		Other Fungi	
		Soybean	Corn	Soybean	Corn	Soybean	Corn
		Root Colonization					
		%	%	%	%	%	%
June 66	Seedling (2)	21	0	53	1.3	94	48.7
July 66	Flowering (6)	8	3	19	27.0	74	77.0
Sept.66	Fruit Set (11)	6	2	39	47.5	92	100.0
Oct. 66	Post-harvest (16)	6	-[b]	62	-	84	-
Apr. 66	Pl. Residues (42)	9	7	41	11.0	92	93.0
		Propagules/g rhizosphere soil					
		No. × 10^3					
June 66	Seedling (2)	40	0.0	40	0.0	800	460
July 66	Flowering (6)	0	0.0	0	0.4	17	80
Sept.66	Fruit Set (11)	-	0.8	16	5.8	250	340
Oct. 66	Post-harvest (16)	-	-	-	-	-	-
Apr. 66	Pl. Residues (42)	38	0.1	200	39.9	7500	1600

[a]Howard Gerst farm, Belleville, Michigan.

[b]Dash indicates no assays were made.

Note: For population of fungi in field soil see Table 33.

overwintering roots of nonhost plants demonstrates how the pathogen can survive for long periods and provide inoculum for succeeding years.

Rhizosphere population trends were similar to root colonization in both soybeans and corn. Soybean at the seedling stage had considerably higher numbers of propagules of F. oxysporum f. melonis than control soil. Soybean roots used in this trial had been stored in a plastic bag for two weeks at 5°C before assay. Probably the cortical tissue softened causing easy removal of superficial cells in the washing process and an increased recovery of the pathogen. Actively growing corn plants had little F. oxysporum in the rhizosphere, but some increase was noticed in eleven-week old plants. Soybean roots collected in April 1967 gave relatively high rhizosphere numbers of the wilt organism. This again was probably due to the release of loose cortical cells into washing medium. Numbers of F. oxysporum f. melonis in rhizosphere of corn decreased after overwintering.

Species of grass growing among noncultivated plants in most fields may contribute to and influence the biology of soil-borne plant pathogens uniquely. Different species of grass were collected from different fields presently or recently cropped to melon and submitted to assay. Grasses (e.g. crabgrass) supported the pathogen to some extent (Table 13). Usually grasses in early and active growth stages were not good hosts for any F. oxysporum.

TABLE 13. Root colonization of grass species by F. oxysporum f. melonis in the field.

Sample Date	Current Cropping	Root Colonization		
		F. ox. f. melonis	Other F. oxysporum	Other Fungi
		%	%	%
June 1966	Soybean[a]	0.00	0.00	84.00
July 1966	Soybean	7.10	2.90	54.00
July 1966	Melon	1.00	9.00	87.00
Sept. 1966	Melon	7.50	19.50	87.00
Jan. 1967	Melon	1.50	28.50	50.00

[a]The soybean field had been previously cropped with melon.

The results indicate that under field conditions the pathogen can colonize roots of many nonhost plants, thus maintaining its existence in the absence of susceptible host plants. Nonhost plant types influences the amount of colonization on roots by the pathogen. The pathogen also survives in colonized tissues between growing seasons in Michigan.

Comparison of Michigan and Iranian strains in the root zone.--The following experiments were performed in autoclaved soil artificially infested with either Iranian (I-5) or Michigan (M-4) isolates of f. melonis. Root zone

activities of these isolates were compared in several ways such as host vs. nonhost effects and soil temperature or soil moisture effects.

(a) Effect of host vs. nonhost plant on root colonization.--Plants of melon (3 varieties), soybean, pea and crabgrass were grown for 2-4 weeks at 25°C and 40% whc in temperature tanks. Inocula of isolates M-4 and I-5 were used to infest separate soil lots. Both isolates colonized roots of resistant and susceptible melons and most nonhosts equally (Table 14). Crabgrass root was a poor substrate for both isolates. Although the Iranian melon (P-3) is resistant to M-4 and partially resistant to I-5 when inoculated by the root dip method, it was subject to damping off in soil infested with M-4. It was quite resistant to both damping off and wilt in soil infested with isolate I-5. Persian melons all remained healthy in M-4 soil but some wilted in I-5 soil. Iroquois melon was wilt-susceptible to both isolates. The root colonization of peas (Miragreen) at 2 weeks was lower with I-5 than with M-4. At 4 weeks both isolates colonized peas nearly the same.

The above experiment suggests that living nonhosts are of importance in supporting both isolates. Since the soils had been autoclaved initially and much of the natural soil microflora excluded, root colonization of the nonhost may be somewhat less important in nature.

TABLE 14. Root colonization of susceptible and resistant melons (hosts) and nonhosts (soybean, pea, and grass) by Michigan (M-4) and Iranian (I-5) strains of F. oxysporum f. melonis.[a]

Plant Variety	Root Colonization			
	F.oxys.f.melonis		Other Fungi in soil infested with:	
	M-4	I-5	M-4	I-5
	%	%	%	%
Iroquois melon[b]	46.70	52.30	16.8	20.6
Persian melon[c]	52.70	55.50	20.2	20.1
Iranian melon;[d] P-3	76.50	60.00	24.0	25.3
Harosoy soybean	48.60	41.60	15.5	13.9
Miragreen pea--2 wks	46.80	15.50	-[e]	-
--4 wks	48.30	53.40	25.4	30.7
Crabgrass --4 wks	0.74	3.10	19.2	36.4

[a]Plants were grown in autoclaved sandy loam soil artificially infested with the melon wilt Fusarium (400-600 propagules per g oven-dry soil) for 2 weeks at 25°C and 40% whc.

[b]Wilt susceptible to both M-4 and I-5.

[c]Wilt resistant to M-4 and susceptible to I-5.

[d]Wilt resistant to both races but damping-off susceptible to race M-4.

[e]Dash indicates that plates were covered with Rhizopus sp.

(b) Effect of soil temperature on root zone population.--Plants favored by warm, cold, and intermediate soil temperatures were planted in autoclaved soils infested with isolates I-5 and M-4 (4000-8000 propagules/g). Persian and Hale's Best Jumbo melon varieties (hosts) and corn (a nonhost) represented warm-termpature plants, pea (Miragreen) a cold-temperature plant and soybean (Harosoy 63) an intermediate-temperature plant. Three pots were prepared for each variety, each isolate and each incubation temperature. All pots were maintained at a soil moisture of 40% whc in this experiment. Root zone analyses were made two weeks after planting.

Both root surface colonization and rhizosphere population were generally higher at the lower temperature (Table 15). The nonhost plants did not show disease symptoms at either soil temperature or with either isolate. Most plants of the Persian and Hale's Best Jumbo melon varieties wilted and died in soil infested with the Iranian isolate, but the disease was more severe at the lower (20°C) soil temperature. On the other hand Persian melon did not wilt in M-4 soil, but approximately 50% and 20% of Hale's Best Jumbo melon wilted and died at 20 and 30°C respectively. Rhizosphere populations of I-5 were also greater than those of M-4 in both varieties of melon and at both soil temperature.

TABLE 15. Effect of soil temperatures on rhizosphere population and root colonization by Michigan (M-4) and Iranian (I-5) strains of F. oxysporum f. melonis on various host and nonhost plants.

A. Rhizosphere:

Plant Variety	Propagules/g soil				Propagules/g root			
	I-5		M-4		I-5		M-4	
	20°C	30°C	20°C	30°C	20°C	30°C	20°C	30°C
	No. × 10^4							
Persian melon	362	39.0	0.8	0.20	356.0	17.00	0.23	0.14
Hale's Best Jumbo melon	540	227.0	0.9	0.25	308.0	127.00	0.47	0.16
North Star corn	5	4.4	2.1	0.82	0.2	0.20	0.80	0.38
Harosoy 63 soybean	1	0.6	0.1	0.10	0.3	0.02	0.40	0.04
Miragreen pea	2	4.2	0.1	0.23	0.9	1.00	0.45	0.42
Control soil	0.7	0.7	0.35	0.35	-	-	-	-

B. Root Surface:

Plant Variety	Root Colonization			
	I-5		M-4	
	20°C	30°C	20°C	30°C
	%	%	%	%
Persian melon	96(S)	81(S)	72(R)	50(R)
Hale's Best Jumbo melon	86(S)	83(S)	79(S)	47(S)
North Star corn	71(NH)	60(NH)	61(NH)	49(NH)
Harosoy 63 soybean	47(NH)	60(NH)	69(NH)	69(NH)
Miragreen pea	69(NH)	67(NH)	83(NH)	61(NH)

S: susceptible; R: resistant; NH: nonhost

It is evident in this work that both isolates are rather more aggressive at lower temperatures than dependent on the "cold" or "warm temperatures" type of plant varieties used. Perhaps if somewhat lower inoculum levels and also nonautoclaved soil had been used more distinct temperature differences would have been observed.

(c) Effect of soil moisture on root zone population.-- Infested soil lots adjusted to moisture levels of 30, 40, 60, and 80% whc were planted with soybeans in order to determine the influence of the moisture on root zone populations and colonization. Isolates M-4 and I-5 were both used at 7000 propagules/g autoclaved soil. Root zone assays were made 2 weeks after planting (Table 16).

Soil moisture affected growth of soybean plants visibly. At 30% whc, plants growing in both infested and control soils were shorter, had a darker green color and more lateral roots than those at the higher mositure levels. At 80% whc, root branching was scanty and the leaves were a light green color.

There were distinct differences in root colonizations by both isolates between 30% and 40% whc. Isolate M-4 was apparently little affected by increasing the soil moisture above 40% whc, but root colonization by isolate I-5 declined as the soil moisture increased.

TABLE 16. Effect of soil moisture on rhizosphere population and root colonization of soybean var. Harosoy 63 (nonhost) by Michigan (M-4) and Iranian (I-5) strains of Fusarium oxysporum f. melonis.[a]

A. Rhizosphere:

	Propagules/g rhiz. soil				Propagules/g fresh wt. root			
% whc:	30	40	60	80	30	40	60	80
Isolate	No. × 10^2							
M-4	0.0	0.0	5.0	0.0	0.0	0.0	2.0	0.0
I-5	0.0	6.0	1110.0	40.0	0.0	2.0	440.0	40.0
Non-planted Control soil (initial)	70.0	70.0	70.0	70.0				

B. Root Surface:

	Root Colonization			
% whc:	30	40	60	80
Isolate	%	%	%	%
M-4	80	54	55	50
I-5	87	72	45	17

[a]Plants were grown for two weeks at 25°C and at 30, 40, 60, and 80% whc in autoclaved sandy loam soil artificially infested with chlamydospores of the pathogens (about 7000 propagules per g oven-dry soil).

With only one exception (I-5 at 60% whc), rhizosphere populations of both isolates decreased from that of the control soil at all levels of moisture.

Saprophytic Activities of Fusarium oxysporum f. melonis

Melon vines on the Gerst farm that had been left undisturbed during fall, winter, and early spring after harvest were collected during January and March 1966 for assay. Numbers of colonies at the first and fourth washes were calculated per ml of wash water and per g dry weight of melon vines, respectively (Table 17). Plant materials which had been washed ten times were plated and checked for the presence of various fungi.

TABLE 17. Survival of F. oxysporum f. melonis in infected melon vines.

Date of sampling	Field condition	Wash No.	Number of colonies			
			F.ox.f. melonis		Other Fungi	
			/ml wash	/g vine	/ml wash	/g vine
			$\times 10^2$	$\times 10^4$	$\times 10^2$	$\times 10^4$
Jan. 66	6" snow	1st	1.1	5.6	76.0	34.4
Mar. 66	Thawing	1st	120.0	220.0	20.0	50.0
Jan. 66	6" snow	4th	0.0	0.0	0.6	0.3
Mar. 66	Thawing	4th	14.0	27.0	4.0	8.0

Washing from the March samples gave more colonies of f. melonis and other fungi than January samples. Almost 100% of the F. oxysporum obtained from residue samples were of the pathogenic form melonis (Table 17). Colonies of F. oxysporum originated from detached pieces of tissue composed of several disintegrated host cells apparently containing chlamydospores. All of the intact vascular and corticla tissues were colonized by F. oxysporum f. melonis.

The results indicate that cortical invasion had occurred sometime after infection. The pathogen survived well in infected tissues during the winter months under extremely cold conditions. Great numbers of the wilt organism were obtained in vines beginning to decompose in early spring.

Colonization and survival in detached plant materials.--Several experiments were conducted to determine the ability of F. oxysporum f. melonis to colonize detached plant tissues in naturally infested soil. Naturally infested soil that had been stored for a year was used. The population of f. melonis in this soil was initially about 2000-3000 propagules/g soil and remained fairly constant throughout the experiment. No additional inoculum was added.

Plant segments used as colonization substrates were obtained from disease free greenhouse plants grown in steamed soil. Segments 2 inches long were usually

buried 0, 2, and 6 inches deep in plastic containers filled with the test soil. Segments located on the soil surface were lightly sprinkled with the same soil. Enough water was added to each pot to give approximately 40% whc. Units were then incubated at the desired temperatures. To reduce the moisture loss, the pots were covered and shaded to allow a natural air exchange during the experimental period. The test units were watered periodically to simulate field conditions but no precise control of moisture was attempted.

In the first experiment soil temperature effects on the saprophytic activities of f. melonis in segments of melon roots and stems were investigated. A wilt susceptible melon (var. Hale's Best Jumbo) was used at soil temperatures of 5, 15, and 25°C. After 6 weeks burial, the plant material were carefully removed and assayed. "Rhizosphere" (washing water) and colonization assays were made in the same manner as for living roots. Plant materials for colonization studies were plated after the 10th shaker wash. The "rhizosphere" sample was taken from the first wash water. Numbers of fungi were expressed either as colonies/ml wash water or percentage of cut plant segments colonized.

F. oxysporum f. melonis was present in large numbers at 15°C, less at 25°C but absent at 5°C (Table 18).

Melon stems were heavily colonized by soil saprophytes and identification of F. oxysporum was hindered. The extent of stem colonization could be more reliably estimated by plating the washing (i.e. "Rhizosphere" assay) than the tissues themselves. Plant tissues incubated in the soil at 5°C were heavily colonized by bacteria and unidentified fungi.

TABLE 18. Effect of soil temperature on saprophytic colonization of detached root and stem pieces of wilt susceptible muskmelon buried for 6 weeks in naturally infested soil by F. oxysporum f. melonis.

Plant part[a]	5°C	15°C	25°C
		Colonization	
	%	%	%
Root	0.0	3.7	1.2
		Number of colonies/ml wash water	
Stem	0.0	1300	653

[a]From Hale's Best Jumbo grown in steamed soil for 30 days. Root and stem pieces were buried in field soil without washing or other treatments.

The experiment indicated that f. melonis is capable of colonizing detached plant materials at 15°C and higher but not at 5°C. A moderately low temperature (15°C) was most favorable to saprophytic activity of F. oxysporum

f. melonis. This is a further indication that the pathogen can act as a low temperature organism.

Another experiment was conducted to study the saprophytic colonization and survival of f. melonis in resistant (90-day-old P-6) and susceptible (30-day-old Hale's Best Jumbo) melon root and stem segments. Plant materials were incubated in soil at 15°C for 28 weeks. Data were obtained at 6 and 28 weeks (Table 19).

There was no sbustrate specificity relating to wilt reactions. Colonization of the wilt resistant plant parts by f. melonis was even higher than for susceptible plants. Older tissues from P-6 melon retained more propagules than the younger tissues of susceptible melon. There appears to be no correlation between depth of burial and the extent of colonization. In nature, however depth of the substrate is an important factor in the biology of the pathogen. Cortical and vascular tissues of stem were found to be equally colonized by f. melonis. Although the population of f. melonis decreased after 28 weeks incubation, an appreciable amount still survived in disintegrating colonized tissues. This is probably one of the important factors in field survival of the pathogen. F. oxysporum f. melonis was able to survive longer in older tissues of resistant plants than in the younger tissues of susceptible melon which decomposed more rapidly.

TABLE 19. Saprophytic colonization of detached roots and stem pieces from wilt susceptible and resistant melon by F. oxysporum f. melonis and other fungi when buried at various depths in naturally infested field soil and incubated at 15°C for 6 and 28 weeks.

A. Assays of plated sections cut from buried pieces (10th wash):

Melon Var. and part	Depth buried	F. ox. melonis		Other F. oxysporum		Other Fungi	
		6 wk	28 wk	6 wk	28 wk	6 wk	28 wk
		Colonization					
	in.	%	%	%	%	%	%
H.B.J. Root	0	4.5	0.0	7.0	18.5	87.0	63.0
H.B.J. Root	2	0.0	0.0	46.6	15.0	44.0	72.0
H.B.J. Root	6	6.8	0.0	15.2	55.0	65.4	57.0
P-6 Root	0	15.0	5.5	14.0	10.5	79.6	29.0
P-6 Root	2	6.9	7.5	22.3	7.5	83.0	48.0
P-6 Root	6	14.0	7.3	4.4	7.3	78.5	88.0
H.B.J. Stem	0	3.5	2.1	9.0	39.0	100.0	63.0
H.B.J. Stem	2	1.1	1.4	6.0	24.2	100.0	92.0
H.B.J. Stem	6	1.0	3.6	4.0	31.0	100.0	97.0
P-6 Stem	0	0.0	5.5	14.0	40.5	100.0	76.5
P-6 Stem	2	4.3	0.0	9.0	43.0	100.0	89.0
P-6 Stem	6	0.8	2.8	5.2	31.0	100.0	94.5

TABLE 19. (Continued).

B. "Rhizosphere" assays from buried pieces (1st wash):

Melon Var. and part	Depth buried	F. ox. melonis		Other F. oxysporum		Other Fungi	
		6 wk	28 wk	6 wk	28 wk	6 wk	28 wk
		Number of colonies/ml wash water					
	in.						
H.B.J. Root	0	0	0	0	0	684	74
H.B.J. Root	2	0	0	4	0	1000	50
H.B.J. Root	6	0	0	0	0	1100	470
P-6 Root	0	0	0	0	0	1200	188
P-6 Root	2	0	0	0	0	500	140
P-6 Root	6	0	0	0	0	1200	600
H.B.J. Stem	0	400	0	1200	0	20000	428
H.B.J. Stem	2	0	80	4200	230	12800	1630
H.B.J. Stem	6	2200	0	2200	0	20600	272
P-6 Stem	0	450	252	1350	1012	60000	14600
P-6 Stem	2	0	146	12000	584	80000	6130
P-6 Stem	6	6600	1619	6600	4111	120000	10800

H.B.J.-Hale's Best Jumbo melon (wilt susceptible), grown 30 days in steamed soil. P-6: Iranian melon var. "Schakhteh Mashhad" (wilt resistant), grown 90 days in steamed soil.

Population changes of f. melonis and other soil fungi in soils outside the "rhizosphere" of the incubated plant materials were also compared at 28 weeks (Table 20). Control soil had more f. melonis and other F. oxysporum than soils which had been incubated with plant materials. This indicates that propagules released into the soil from disintegrated plant tissues were not important in contributing to the pathogen population. Therefore it seems that if the pathogen is introduced into a soil environment and inadequately sheltered, as in a readily decomposible tissue, the chance of its survival is poor. Germination of propagules and colonization of substrate but no return of propagules to the soil perhaps accounted for the decrease in population of f. melonis in soil containing plant materials. If this hypothesis is correct, any cultural practice that speeds complete decomposition of infected organs would lead to lower inoculum in soil. Table 19 shows that there were few colonies obtained by washing plant materials 28 weeks after incubation. Probably the low numbers resulted from the prior release of the outer, more decomposable cells into the soil. In Table 20, on the other hand, it appears that if such cells had been released, they did not contribute to population of f. melonis and F. oxysporum in the soil outside of the "rhizosphere." The numbers of other fungi were not affected materially.

TABLE 20. Effect of melon residues on the soil population of F. oxysporum f. melonis and other soil fungi in naturally infested field soil.*

Soil assayed	F. oxysporum f. melonis	Other F. oxysporum	Other Fungi
	Number of propagules/g oven-dry soil		
Control soil	3700[a]	2450	55500
H.B.J. root soil[b]	400	1200	37500
P-6 root soil[c]	2400	1600	36500
H.B.J. stem soil[b]	1320	2680	55500
P-6 stem soil[c]	1200	4200	51000

*Unsterilized, Fusarium-free melon root and stem pieces were buried in sandy loam field soil naturally infested with the melon wilt Fusarium and incubated at 15°C for 28 weeks before the soil assay.

[a]Population ranged from 1900-3700/g soil during the pre-trial storage period.

[b]Soil taken from container in which 30 day old Hale's Best Jumbo melon (wilt susceptible) root or stem pieces had been buried for 28 weeks.

[c]Soil from 90 day old P-6 melon (wilt resistant var. Schakhteh Mashhad) containers.

Saprophytic colonization of detached nonhost plant materials by F. oxysporum f. melonis was also investigated (Table 21). Plant materials were obtained from 4 week old corn and soybean seedlings growing in autoclaved soil. They were incubated at 15°C for 24 weeks in naturally infested soil. Corn roots were colonized heavily by Trichderma

viride while still growing in autoclaved soil. It is evident that f. melonis is capable of successfully colonizing detached plant tissues of some nonhosts, and can be recovered easily 24 weeks after incubation.

TABLE 21. Saprophytic colonization of detached nonhost plant materials by F. oxysporum f. melonis and other soil fungi in naturally infested field soil incubated at 15°C for 24 weeks.[a]

Plant and Part	F. oxysporum f. melonis		Other F. oxysporum		Other Fungi	
	%[b]	No.[c]	%	No.	%	No.
Corn root	0.0	0.0	0.0	0.0	100[d]	TMTC[d]
Corn leaf & stem	2.4	133.0	18.0	533.0	84	149
Soybean root	23.7	0.0	14.3	0.0	71	210

[a]Soil naturally infested with 2000-3000 propagules of f. melonis/g soil.

[b]% segments colonized.

[c]No. of colonies/ml wash water.

[d]Almost 100% Trichoderma viride.

These experiments indicate that F. oxysporum f. melonis can successfully colonize a number of detached plant materials irrepsective of origin species, part of the plant or depth buried. No colonization occurred at 5°C, but considerable colonization and survival were achieved at 15 and 25°C.

Competitive saprophytic ability.--To measure the degree of competitive saprophytic ability of the pathogen, the Cambridge method (4) was employed. Inocula of a highly pathogenic isolate of F. oxysporum f. melonis were prepared by mixing a thoroughly washed conidial suspension from a 3-day shake culture with sterile soil. The inoculum soil was incubated at room temperature for 5 to 60 days and the number of propagules of the pathogen assayed before use in each experiment. If fewer propagules were needed to prepare the experimental soil, an appropriate dilution was made with sterile soil.

Two types of natural soils were mixed with inoculum soil in this experiment: one a virgin forest soil free of f. melonis and the other a sandy loam soil naturally infested with F. oxysporum f. melonis (Michigan forms). Inoculum soil containing the desired number of propagules was diluted with the natural soil in ratios of 100/0 (inoculum control), 98/2, 90/10, 50/50, 10/90, 2/98 and 0/100 (natural soil control). Hundred-gram lots were weighed out into 100 ml sterile screw cap bottles. Twenty to 30 of 2-3 inch pieces of melon or soybean roots or stems that had been sterilized by propylene oxide were buried in the soil in each bottle. Soil moisture was adjusted to approximately 14-16% on an oven-dry basis and the experimental units incubated with the caps loosened for 2-4 weeks at 20°C.

After incubation, the plant materials were removed, washed ten times by the shaker method, and 150-200 of 2-3 mm segments plated for colonization assay. F. oxysporum f. melonis was identified by use of pathogenicity tests.

Virgin forest soil apparently free of Fusarium oxysporum was collected in May for colonization studies. A portion that had been stored for two weeks in the greenhouse was autoclaved and used for preparation of inoculum. Root segments of 4-month-old melon were used as a colonization substrate. The inoculum control (5-day-old) contained approximately 10^7 propagules per gram soil.

TABLE 22. Colonization of sterile melon root segments by Fusarium oxysporum f. melonis (M-4) and other soil fungi when buried in various proportions of virgin forest soil and inoculum (M-4) and incubated at 20°C for 2-4 weeks.

Inoculum in Forest Soil[a]	Segment Colonization					
	F.ox.f.melonis		Other F.oxysporum		Other Fungi	
	2 wk	4 wk	2 wk	4 wk	2 wk	4 wk
%	%	%	%	%	%	%
0	10.0[b]	0.0	9.0	37.8	76.0	85.8
2	8.0	88.0	24.0	11.0	68.0	0.9
10	7.4	80.0	45.6	20.0	35.0	0.0
50	34.0	90.0	15.0	10.0	38.0	0.0
90	40.0	100.0	51.0	0.0	5.0	0.0
98	31.0	100.0	63.0	0.0	2.0	0.0
100	82.0	100.0	0.0	0.0	0.0	0.0

[a]100% inoculum is 10^7 conidia/g (oven-dry basis) sterile forest soil. The 0% inoculum is all virgin forest soil (populations of general fungi: 10,000 propagules/g soil).

[b]Contamination error.

Recovery of f. melonis increased as incubation time increased (Table 22). Other soil fungi, however were supplanted rapidly. High inoculum level was not solely responsible for the high rate of colonization because 4 weeks after incubation more than 80% of the segments had been colonized by the pathogen at all inoculum levels.

Another experiment was designed to compare the competitive saprophytic ability of Iranian (I-17) and Michigan (M-4) strains of F. oxysporum f. melonis in sandy loam field soil (pH 6.5-7.0) naturally infested with Michigan forms of f. melonis (including M-4). Initial assays of populations in this soil were: F. oxysporum f. melonis (Michigan types) 1.1×10^3, other F. oxysporum 2.9×10^3 and other soil fungi 6.9×10^4 per g. A portion of this soil was autoclaved and used to prepare inocula with I-17 and M-4 at 10^6 propagules/g. Soil inocula were stored at room temperature for 5 days before being diluted with the natural soil in various proportions. Sterilized 4-month-old melon root segments were buried in the soil-inoculum blends and incubated at 20°C for 4 weeks.

Results (Figure 7) showed that I-17 was recovered with greater frequency than M-4--and other Michigan forms--at all inoculum levels. Native f. melonis colonized 28% of the tissues. There was an increase of colonization by the pathogen as the inoculum level was increased. Isolate M-4 appeared to be somewhat less aggressive than I-17.

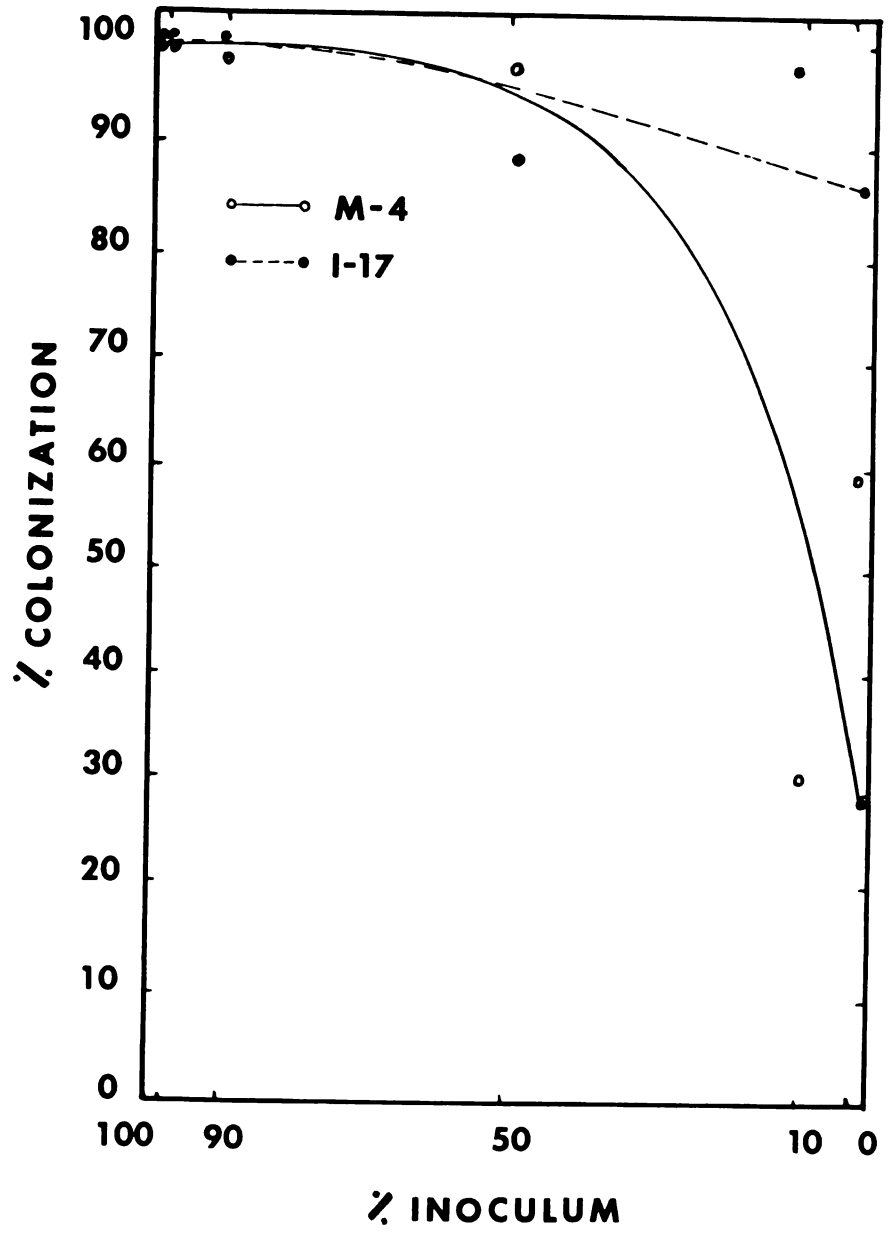


FIGURE 7. Colonization of sterile muskmelon root segments buried 4 weeks at 20°C in various proportions of field soil by F. oxysporum f. melonis (isolates M-4 and I-17).

A 20X increase of M-4 propagules (between 0 and 2% levels) increased colonization by only 35%, whereas almost the same number of propagules of I-17 added 84%. At higher inoculum levels the differences between the two isolates were not as great. Thus I-17 in a field soil containing great numbers of other fungi (70,000/g in the 98% soil, 2% inoculum) was not deterred from aggressive saprophytic colonization of a sterile substrate.

The competitive saprophytic ability of isolate I-17 was studied further using (a) soybean roots and stems as substrate, (b) a lower inoculum level than before, and (c) an older inoculum stored 2 months. Four-week-old sterile soybean roots and stems were used. The population of I-17 at 100% inoculum was about 200,000 propagules/g soil, while native soil fungi were present in 0% inoculum soil at about 73,000 propagules/g.

Isolate I-17 at 2% inoculum colonized more than 60% of the soybean roots (Table 23) and colonization was increased at higher inoculum levels. Soybean stems were a better substrate than roots. The colonization by other soil fungi decreased as inoculum concentration of the pathogen increased.

A definite discoloartion of the substrate materials occurred after invasion by soil saprophytes but not when they were occupied by I-17. Addition of as little as 2% natural soil to inoculum caused appreciable discoloration

TABLE 23. Colonization of sterile 4-week-old soybean root and stem pieces by F. oxysporum f. melonis (I-17) and other soil fungi when buried for 4 weeks at 20°C in naturally infested soil containing different levels of I-17 inoculum.

Inoculum[a] in Field Soil[b]	Substrate	Segment Colonization	
		F. oxys. f. melonis (I-17)	Other Fungi
%		%	%
0	Soybean root	0	93
2	Soybean root	63	61
10	Soybean root	62	40
50	Soybean root	74	16
90	Soybean root	100	0
98	Soybean root	100	0
100	Soybean root	100	0
0	Soybean stem	0	100
2	Soybean stem	36	73
10	Soybean stem	100	23
50	Soybean stem	100	0
90	Soybean stem	100	0
98	Soybean stem	100	0
100	Soybean stem	100	0

[a]Sterile soil inoculum containing 2×10^5 propagules/g (washed conidia of isolate I-17 had been stored in sterile soil for 2 months at room temperature before use).

[b]Field soil containing about 7×10^4 fungal propagules/g.

of the substrate pieces indicating that even at this level there was significant competition from natural flora. Microscopic examination of plant materials buried in soil at 100% and 2% levels of inoculum revealed that the pathogen had invaded most cells of the substrate and produced tremendous numbers of chlamydospores. Sporulation of the pathogen, mostly as microconidia, was also observed on the surface. This invasion of tissues and formation of resistant structures is considered to be of great importance for the pathogen's survival.

The effect of temperature on colonization was investigated concurrently with the above experiment using isolate I-17 at the 10% level of inoculum (20,000/g soil). Soils containing sterile roots segments of soybean were incubated at 5, 16, 20, and 24°C for 4 weeks. The results are shown in Table 24.

TABLE 24. The effect of soil temperature on saprophytic colonization of sterile soybean root segments by the Iranian isolate (I-17) of F. oxysporum f. melonis.

Incubation	Segments colonization	
	F. oxysporum f. melonis	Other Fungi
°C	%	%
5	0.0	16
16	79.0	23
20	62.0	48
24	30.0	71

Colonization decreased progressively at 20 and 24°C. Although there was no colonization of the substrate by the pathogen at 5°C, there was considerable colonization at 15°C. This is additional evidence to indicate that the pathogen acts as a comparatively low temperature organism.

Results of the studies on the competitive saprophytic ability of F. oxysporum f. melonis indicate that the pathogen is competitive with soil saprophytes in substrate colonization. There was no substrate specificity and the pathogen could colonize a considerable amount of substrate at 15°C or higher temperatures but not at 5°C.

DISCUSSION

The results of this investigation indicate that Fusarium oxysporum f. melonis may occur in nature as distinct physiological races of a specialized wilt pathogen, with saprophytic capability, and able to colonize roots of many nonhost plants. These saprophytic and colonization capabilities both occur under natural conditions and enables the organism to continue its existence in the absence of true host plants.

Differential reactions of muskmelon varieties toward various isolates of f. melonis have been found by various workers and used in support of race schemes. Reid (54) classified various isolates of f. melonis into so-called cultural races, mostly on the basis of colony topography. Host specialization was not detailed because resistance or susceptibility were relative rather than absolute. Relative host resistance was used as a descriptive characteristic of the cultural races. Wensley and McKeen (35,105) could not detect races of f. melonis and Welty (104) concluded that various isolates showed only degrees of virulence. This was not considered sufficient for races since all isolates could infect differential hosts to some extent.

There has been general agreement, thus, among Canadian and American workers that no clear-cut pathogenic races of Fusarium oxysporum f. melonis have been demonstrated.

As a result of present research we may conclude that many if not most of the North American isolates of f. melonis are similar in that they did not infect the muskmelon variety "Persian small type." On the other hand all of the domestic melon varieties appear to be susceptible in some degree to North American isolates. Of 100 isolates of f. melonis obtained from various parts of Michigan and other states, none produced disease symptoms on "Persian" melons. Some isolates were more virulent than others when inoculated to the susceptible American varieties of muskmelons, but all of them attempted were nonvirulent to six melons native to Iran.

Isolates of F. oxysporum f. melonis obtained from North Eastern Iran (Mashhad district) were different from the North American isolates tested. They were highly pathogenic not only to "Persian" melon but also to most of the Iranian melon varieties. More than 20 American melon varieties were highly susceptible to the Iranian isolates. The Iranian isolate typically caused severe disease symptoms at lower inoculum levels and was also more competitive saprophytically than were the Michigan isolates (Figures 3B,7). Some plants inoculated with Michigan isolates but not showing symptoms could nevertheless support Fusarium

growth as demonstrated by culturing. Iranian isolates, on the other hand, could not be reisolated from the interior of symptomless plants. The greater inherent virulence of Iranian forms thus appears to be combined with clearer expression of host symptoms than is the case with Michigan isolates. In muskmelon varieties equally susceptible to both Iranian and Michigan isolates, disease symptoms appeared 2-3 days earlier when inoculated with the Michigan form. This indicates some differences in respect to the physiology of host parasite interactions during early stages of the infection process. These differences, furthermore, are more pronounced at the lower effective levels of inoculum. Although both groups of the pathogen had somewhat similar temperature optima for radial growth in culture, the lower temperatures (12-24) generally favored Michigan isolates and higher temperatures the Iranian.

Various physiological and cultural differences between Iranian and Michigan isolates, particularly those related to host reactions indicate that the two isolate groups are distinct physiological races of Fusarium oxysporum f. melonis.

Risser and Mas (57) reported the presence of three distinct physiological races of f. melonis in France. These races are differentiated clearly by reactions on a group of host melons. Their race 3 was considered to be a new race in France. When Risser and Mas's differential varieties

were inoculated with our Michigan and Iranian isolates, the Iranian isolate gave the reaction of race 2 but the Michigan isolate was different from all 3 of their races. The Michigan isolate could infect all Risser and Mas's differential varieties except the variety "Doublon" and thus appears to be a new race (race 4) of f. melonis (Table 2).

Since the author did not have access to the isolates used by Risser and Mas, any special reaction aspects of the original 3 French races of f. melonis on the author's groups of differential hosts are not known. Whether the Iranian isolate is identical to race 2 or only similar within the published limits, remains to be investigated.

The effect of soil temperature on the survival of f. melonis in autoclaved and nonautoclaved soils indicate that most of the conidia introduced into soil will disappear within a 30-day incubation period at temperatures between 5°C and 30°C. Conversion of conidia into chlamydospores could be epidemiologically important in that the most resistant structures would be more able to survive in a competitive soil environment or in the absence of the available nutrients needed for germination. Since most f. melonis conidia disappeared 30 days after incubation at 15°C or higher and only a few chlamydospores could be detected, it is conceivable that chlamydospores are the sole survival units present at higher temperatures. The pathogen is inactive at 5°C and thus very little conversion

of conidia to chlamydospores occurs there. The slow but consistent decline of population noted at 5°C is probably due to the relatively good survival of unchanged conidia in a soil environment with low competitive microbial activity. At higher temperatures (20-25°C), in spite of the formation of resistant structures, populations decreased considerably in the 14 months of incubation (Figure 4). This signifies that even the resistant structures cannot survive indefinitely in soil. The best survival was at 15°C and possibly this demonstrates ability of the pathogen to be active in an environment less favorable for most other soil microorganisms. Perhaps also less lysis of conidia occurred before they were converted to chlamydospores. The fungus might respond either by producing resistant structures more readily at 15°C, or the resistant structures might survive longer.

All of the present studies on root zones of host and nonhost plants bring one to the conclusion that only the host plants contribute greatly to the increase of pathogen inoculum in nature. Nonhosts, however, are important in activating germination of f. melonis propagules and allowing a colonization of roots. It appears that the association of f. melonis with nonhost plants is in the form of commensalism which ensures a continuous survival in the absence of a host. Nonhost plants such as soybean and possibly other legumes are more favorable substrates

than grasses such as corn and crabgrass--at least in the early and active stage of growth.

The amount of pathogen survival in soil appeared to depend on an association with at least partially intact plant materials. Complete decomposition of invaded tissues reduces chances of the propagule's surviving if it fails to be re-established in another nearby substrate. Chlamydospores are considered to be the important survival propagulas of f. melonis, but neither can they survive long in a soil environment if not sheltered within plant tissues. Humus particles or, especially, intact tissues are therefore important resistant substrates and shelter the chlamydospores against environmental stresses for the longer term. Perhaps naked chlamydospores are exposed more intimately to the factors inducing lysis than hidden ones and thus can retain dormancy better. Warcup (98) indicated that in soil assays, Fusarium was one of the small group of fungi isolated more frequently from humus particles than as naked spores and that the spores were resistant types. Nash et al. (42) also found that Fusarium solani f. phaseoli exists in soil mainly as chlamydospores associated with or imbedded in fragments of tissue or humus particles.

Failure of propagules of f. melonis to increase in the rhizosphere of nonhosts and failure of the fungus to become established on the root surface of certain plants

may be important phenomena applicable to biological control of the pathogen under natural conditions. In this work corn and some other grasses are representative of such nonhosts. Mechanisms causing failure in increase of propagules of f. melonis in soil in the presence of these plants and also the lower recovery from root surfaces were not investigated. We might speculate that some nonhost plants are able to stimulate germination of nearby propagules and yet resist root colonization. The germinated spore would then shortly lyse in the soil environment.

Effects of soil moisture and temperature on activity of f. melonis in the root zone and on disease development were studied. Lower soil moisture levels (around 40% whc) favored disease development and rhizosphere populations in host plants. Lower moistures also increased root colonization in the case of both host and nonhost plants. These increases appeared to have resulted from physiological changes in the host brought about by reduced water. Some growth reduction was noticed in both host and nonhost plants at lower soil moisture levels. Katznelson et al. (25) found that more nutrients were released from roots at lower moisture levels. Timonin (88) indicated that formation of root hairs was more abundant in drier soil thus providing a larger surface area and a greater root excretion per gram of rhizosphere soil. Root hairs subsequently decomposed

and provide additional available nutrients in the rhizosphere. Miller (40) found that reduced moisture reduced growth of the melon. He concluded that lower moisture affected disease severity through a predisposition effect on the host.

The disease progressed faster at lower soil temperatures also. Predisposition for disease severity by lower temperatures has been claimed for muskmelon wilt (40). Reid (54), on the other hand, considered aggressiveness of the pathogen rather than host susceptibility to be the responsible factor at lower temperatures. In the present investigation lower temperatures favored parasitic and saprophytic colonization and also survival in soil by f. melonis. Root colonization was more extensive at 20 than at 30°C on both warm and cold temperature plants (Table 15). This indicates that aggressiveness of the pathogen as mentioned by Reid (54), might be the contributing factor at lower temperatures.

The effect of lower temperatures on disease severity and on the pathogen itself is an important factor in epidemiology of the disease. Southern parts of the United States are usually considered to be unfavorable for muskmelon wilt Fusarium and the disease is more important in the Northern states. Milder temperatures prevailing throughout the year in southern states probably affect survival of the pathogen in the soil and also influence progress of the disease. Faster decompsoition of organic residues in warmer climates would be detrimental to resistant structures of f. melonis

and ultimately reduce both parasitic and saprophytic activities of the pathogen.

Watermelon Fusarium (51), is prevalent in the southern states and is favored by moderately high temperatures (25-28°C). This wilt disease is better known than muskmelon wilt, probably because of its world wide distribution. Comparative studies using both watermelon and muskmelon wilt Fusaria may reveal some of the factors involved in the distribution of both wilt organisms.

Saprophytic colonization of detached or sterilized tissues of both host and nonhost plants occurred at 15°C or higher temperatures but not at 5°C. Plant materials buried at various soil depths of 0 to 6 inches were colonized to almost the same extent. Saprophytic activities of the pathogen are important for survival in the absence of living host plants. Substrate requirements are nonspecific; colonization is possible at various soil depths and temperatures between 15-25°C; and the organism persist in undecomposed colonized tissues.

Results of studies on the competitive saprophytic ability of f. melonis indicate that even a low concentration of inoculum such as remained in soil stored for 2 years could cause colonization in more than 28% of plant segments used for substrate. The Iranian form introduced into soil at a rate of 2000 propagules/g soil colonized 64% of the substrate segments in spite of the presence of much greater

numbers of other soil microorganisms (Table 23). The organism appear to be well adapted for competition with other organisms for necessary saprophyte substrates in nature.

Stover (80) called the banana wilt Fusarium a soil inhabiting saprophyte with parasitic potentials. The muskmelon Fusarium (F. oxysporum f. melonis) according to the present findings, cannot be placed in either of Garrett's soil or root inhabitant classes of soil fungi. F. oxysporum f. melonis is not a true soil saprophyte but rather saprophytically active in or on organic fragments. It is practically incapable of saprophytic growth in the soil apart from the invaded substrate. It is therefore an intermediate type of root and soil inhabitant. It could probably be classified as an "Organic Inhabitant" or a "Tissue Inhabitant," whose chances of survival in the soil is considerably reduced by decomposition of the colonized tissue.

LITERATURE CITED

1. Armstrong, G. M., and J. K. Armstrong, 1948. Nonsusceptible hosts as carriers of wilt Fusaria. Phytopathology. 38:808-826.

2. Baker, Kenneth F. and Samuel H. Smith, 1966. Dynamics of seed transmission of plant pathogens. Ann. Rev. Phytopathology. 4:311-334.

3. Bateman, D. F., 1963. Influence of host and nonhost plants upon populations of Thielaviopsis basicola in soil. Phytopathology. 53:1174-1177.

4. Butler, F. C., 1953. Saprophytic behaviour of some cereal root-rot fungi. I. Saprophytic colonization of wheat straw. Ann. Appl. Biol. 40:284-297.

5. _____, 1953. Saprophytic behaviour of some cereal root-rot fungi. II. Factors influencing saprophytic colonization of wheat straw. Ann. Appl. Biol. 40: 298-304.

6. Buxton, E. W., 1957. Differential rhizosphere effects of three pea cultivars on physiological races of Fusarium oxysporum f. pisi. Brit. Mycol. Soc. Trans. 40:305-316.

7. Clark, F. E., 1949. Soil microorganisms and plant roots. Adv. Agron. 1:241-288.

8. Clayton, E. E., 1923. The relation of temperature to the Fusarium wilt of the tomato. Amer. Jour. Bot. 10:71-88.

9. Cox, C. E., 1944. The response of cantalope-cucumber grafts to inoculation with Fusarium bulbigenum var. niveum f. 2. Phytopathology. 34:991. (Abstr.).

10. Crawford, R. F., 1934. The etiology and control of chile wilt produced by Fusarium annuum. New Mexico Agric. Exp. Sta. Tech. Bull. 223.

11. Crozier, J. A., 1963. Some factors affecting the development of a sudden wilt disease of muskmelon in New York State. Diss. Abstr. Vol. 24:3046 (1964).

12. Dolan, D. D., 1946. Testing of segregating progenies of muskmelons for disease resistance. Ph. D. thesis, Cornell University.

13. Douglas, D. R., 1967. The effect of inoculum potential on the response of muskmelon varieties to Fusarium oxysporum f. sp. melonis. Phytopathology. 57:809 (Abstr.).

14. Eaton, F. M. and N. E. Rigler, 1946. Influence of carbohydrate levels and root surface microfloras on Phymatotrichum root rot in cotton and maiz plants. J. Agr. Research 72:137-161.

15. Garrett, S. D., 1960. Inoculum potential. Vol. 3, p. 23-58. In J. G. Horsfall and A. E. Diamond (ed.) Plant Pathology, an advanced treatise. Academic Press, New York and London.

16. _____, S. D., 1960. Biology of root infecting fungi. Cambridge University Press, 293 p.

17. _____, S. D., 1963. Soil fungi and soil fertility. Pergamon Press, Oxford and The MacMillan Company, New York, 165 p.

18. Gordon, W. L., 1965. Pathogenic strains of Fusarium oxysporum. Can. J. Botany 43:1309-1318.

19. Grigoryan, N. F., 1963. Some characteristics of pathogenic species of Fusarium on melons during wilting. Rev. Appl. Mycol. 43:160(870), 1964. (Izv. Akad. Nauk Armyan SSR) biol. Sci. 16(8):73-84(Russian).

20. Harley, J. L. and J. S. Waid, 1955. A method of studying active mycelia on living roots and other surfaces in the soil. Brit. Mycol. Soc. Trans. 38:104-118.

21. Hiltner, L., 1904. Uber neuer Erfahrungen und Probleme auf dem Gebiet der Bodenbakteriologie und unter besonderer Berucksichtigung der Grundungung und Brache. Arb. Deut. Landwirtsch. Ges. 98:59-78.

22. Hoffmaster, Donald E., 1940. A comparison of pathogenic races of Fusarium bulbigenum var. niveum. Phytopathology 30:10 (Abstr.)

23. Horsfall, J. G., 1932. Dusting tomato seed with copper sulfate monohydrate for combating damping-off. N.Y. Agr. Exp. Sta.(Cornell) Tech. Bull. 198.

24. Jones, L. R., James Johnson, and James G. Dickson., 1926. Wisconsin studies upon the relation of soil temperature to plant disease. Wisc. Agr. Exp. Sta. Res. Bull. 71.

25. Katznelson, H., J. V. Rauatt, and T. M. B. Payne, 1954. Liberation of amino acids by plant roots in relation to desiccation. Nature (London) 174:1110-1111.

26. _____, 1955. The liberation of amino acids and reducing compounds by plant roots. Plant Soil 7:35-48.

27. _____, 1965. Nature and importance of the rhizosphere. In Ecology of soil-borne plant pathogens; Prelude to biological control, K. F. Baker and W. C. Snyder (Ed.):187-209. Univ. Calif. Press, Berkeley.

28. Lacy, M. L., 1965. Influence of host and nonhost plants upon populations of verticillum dahliae in soil. Ph.D. thesis, Oregon State University, 95 p.

29. Leach, J. G., 1933. A destructive Fusarium wilt of muskmelon. 23:554-556.

30. Leach, J. G. and T. M. Currence, 1938. Fusarium wilt of muskmelon in Minnesota. Minn. Agr. Exp. Sta. Tech. Bull. 129.

31. Lochhead, A. G., M. I. Timonin, and P. M. West, 1940. The microflora of the rhizosphere in relation to resistance of plants to soil-borne pathogens. Sci. Agri. 20:414-418.

32. _____, 1959. Rhizosphere microorganisms in relation to root-diseases fungi. P. 327-338. In C. S. Holten, et al. (Ed.), Plant Pathology: Problems and Progress, 1908-1958. Univ. Wisconsin Press, Madison, Wisconsin.

33. McKeen, C. D. and R. N. Wensley, 1961. Longevity of Fusarium oxysporum in soil tube culture. Science 134:1528-1529.

34. _____, and R. N. Wensley, 1962. Population of Fusarium oxysporum f. melonis in wilt-infested soils. Phytopathology 52:21 (Abstr.)

35. _____, and _____, 1962. Cultural and pathogenic variation in the muskmelon wilt fungus, Fusarium oxysporum f. melonis. Can. J. Microbiol. 8:769-784.

36. McNew, G. L., 1960. The nature, origin, and evolution of parasitism. In Plant Pathology, an advanced treatise J. G. Horsfall and A. E. Dimond (ed.) Vol. II:19-69.

37. Menzies, J. D., 1963. The direct assay of plant pathogen populations in soil. Ann. Rev. Phytopathology 1:127-142.

38. Messian, C. M., Georgette Risser, et P. Pecant., 1962. E'tude des plantes resistantes au Fusarium exysporum f. sp. melonis dans la variete'de melon Cantaloup Charentais. Ann. Amelior. Plantes, 12:157-167.

39. Miller, J. J., 1945. Studies on the Fusarium of muskmelon wilt. I. Pathogenic and cultural studies with particular reference to the cause and nature of variations in the causal organism. Can. J. Res. (C.) 23:16-46.

40. _____, 1945. Studies on the Fusarium of muskmelon wilt. II. Infection studies concerning the host range of the organism and the effect of environment on disease. Can. J. Res. (C.) 23:166-187.

41. Mortenson, J. A., 1958. The inheritance of Fusarium resistance in muskmelons. Diss. Abstr. (19) (9):2209 (1959). Doctoral dissertation, Cornell Univ., 120 p.

42. Nash, Shirley, M. T. Christon, and W. C. Snyder, 1961. Existence of Fusarium solani f. phaseoli as chlamydospores in soil. Phytopathology. 51:308-312.

43. Nash, Shirley, and W. C. Snyder, 1962. Quantitative estimations of propagules of the bean root rot Fusarium in field soils by plate counts. Phytopathology. 52:567-572.

44. Papvizas, G. C. and C. B. Davey, 1961. Isolation of Thielaviopsis basicola from bean rhizosphere. Phytopathology. 51:92-96.

45. Papavizas, George C., 1967. Evaluation of various media and antimicrobial agents for isolation of Fusarium from soil. Phytopathology. 57:848-852.

46. Park, D., 1954. Chlamydospores and survival in soil fungi. Nature, 73:454-455.

47. _____, 1958. The saprophytic status of Fusarium oxysporum Schl. causing vascular wilt of oil palm. Ann. Bot. London, N.S. 22:19-35.

48. _____, 1959. Some aspects of the biology of Fusarium oxysporum Schl. in soil. Ann. Bot. London, N. S. 23:35-49.

49. _____, 1963. The presence of Fusarium oxysporum in soil. Brit. Mycol. Soc. Trans. 46:444-448.

50. Peterson, E. A., J. W. Rouatt, and H. Katznelson, 1965. Microorganisms in the root zone in relation to soil moisture. Can. J. Microbiol. 11:483-489.

51. Porter, D. R., 1932. Some environmental relations of watermelon wilt. Phytopathology. 22:813-825.

52. Ramachandra-Reddy, T. K., 1959. Foliar spray of urea and rhizosphere microflora of rice. Phytopathology Z. 36:286-289.

53. Rao, A. S., 1959. A comparative study of competitive saprophytic ability in twelve root-infecting fungi by the agar plate method. Brit. Mycol. Soc. Trans. 42:97-111.

54. Reid, J., 1957. Studies of the Fusaria which cause wilt in melons. Doctoral dissertation, University of Toronto.

55. Reyes, A. A. and J. E. Mitchell, 1962. Growth response of several isolates of Fusarium in rhizosphere of host and nonhost plants. Phytopathology. 52:1196-1200.

56. Riker, A. J. and Regina S. Riker, 1936. Introduction to research on plant diseases. J. S. Swift, St. Louis. 117 p.

57. Risser, Georgette, and P. Mas. 1965. Mise en evidence de plusieurs races de Fusarium oxysporum f. melonis. Ann. Amelior Plantes (Paris) 15(4):405-408. (Engl. Summ.)

58. Rodriguez, R. A., 1960. The nature of resistance in muskmelons to Fusarium wilt. Diss. Abstr. 22:1347 (1962).

59. Rouatt, J. W., and E. A. Peterson, 1960. The influence of light on the bacterial flora of roots. Nature 186:659-660.

60. Rovira, A. D., 1959. Root excretions in relation to the rhizosphere effect. IV- Influence of plant species, age of plant, light, temperature and calcuim nutrition on exudation. Plant and Soil 11: 53-64.

61. _____, 1965. Interactions between plant roots and soil microorganisms. Ann. Rev. Microbiol. 19:241-266.

62. _____, 1965. Plant root exudates and their influence upon soil microorganisms. In Ecology of soil-borne plant pathogens; Prelude to biological control, K. F. Baker, and W. C. Snyder (ed.):170-186. Univ. Calif. Press, Berkeley.

63. Ryker, T. C., 1935. Fusarium yellows of celery. Phytopathology. 25:578-600.

64. Schroth, M. N., and F. F. Hendrix, Jr., 1962. Influence of non-susceptible plants on the survival of Fusarium solani f. phaseoli in soil. Phytopathology. 52:906-909.

65. Schroth, M. N., and D. C. Hildebrand, 1964. Influence of plant exudates on root infecting fungi. Ann. Rev. Phytopathology. 2:101-132.

66. Schroth, M. N. and A. R. Weinhold, and D. S. Hayman, 1966. The effect of temperature on quantitative differences in exudates from germinating seeds of beans, peas, and cotton. Can. J. Botany. 44:1429-1432.

67. Singh, R. S. and Y. L. Nene, 1965. Some observation on the use of malachite green and captan for determination of Fusarium populations in soil. pl. Disease Reptr. 49:114-118.

68. Snyder, W. C., and H. N. Hansen., 1940. The species concept in Fusarium. Amer. J. Botany. 27:64-67.

69. Starkey, R. L., 1929. Some influences of the development of higher plants upon the microorganisms in the soil: I. Historical and Introduction. Soil Sci. 27:319-34.

70. Starkey, R. L., 1929. Some influences of the development of higher plants upon the microorganisms in the soil, II. Influence of the stage of plant growth upon abundance of organisms. Soil Sci. 27: 355-378.

71. _____, 1929. Some influences of the development of higher plants upon the microorganisms in the soil, III. Influence of the stage of plant growth upon some activities of the organisms. Soil Sci. 27:433-445.

72. _____, 1931. Some influences of higher plants upon the microorganisms in the soil, IV. Influence of proximity to roots on abundance and activity of microorganisms. Soil Sci. 32:367-393.

73. _____, 1938. Some influences of the development of higher plants upon the microorganisms in the soil, VI. Microscopic examination of the rhizosphere. Soil Sci. 45:207-250.

74. Starr, G. H., 1932. A study of diseases of canning crops (peas and corn) in Minnesota. Minn. Agr. Exp. Sta. Tech Bull. 89.

75. Steiner, G. W. and R. D. Watson, 1965. Use of surfactants in the soil dilution and plate count method. Phytopathology, 55:728-730.

76. _____, and _____, 1965. The effect of surfactants on growth of fungi. Phytopathology, 55:1009-1012.

77. Stotzky, G., J. E. Dawson, R. T. Martin, and C. H. H. Terkuile, 1961. Soil mineralogy as factor in spread of Fusarium wilt of banana. Science 133:1483-1485.

78. _____, and L. T. Rem, 1965. Influence of clay minerals on microorganisms, I. Montmorillonite and Kaolinite on bacteria. Can. J. Microbiol. 12:547-563.

79. Stover, R. H., 1956, Behaviour of Fusarium oxysporum f. cubense in different soils and soil extracts. Phytopathology. 46:27-28 (Abstr.).

80. _____, 1958. Studies on Fusarium wilt of bananas. II. Some factors influencing survival and saprophytic multiplication of F. oxysporum f. cubense in soil. Can. J. Botany 36:311-324.

81. Stover, R. H., 1962. Studies on Fusarium wilt of bananas. Competitive saprophytic ability of F. oxysporum f. cubense. Can. J. Botany 40:1473-1481.

82. Sturgis, William C., 1898. Some common diseases of melons. Conn. Agri. Exp. Sta. Ann. Rept. 22:225-235.

83. Subba-Rao, N. S., and D. L. Bailey, 1961. Rhizosphere studies in relation to varietal resistance or susceptibility of tomato to Verticillium wilt. Can. J. Botany 39:1747.

84. Subramanian, C. V., 1947. The saprophytic activity of Fusarium vasinfectum, the cotton wilt pathogen in the soil. I. Colonization of cotton roots buried in the soil. Iyengar Commem. Vol. J. Indian Bot. Soc. p. 209.

85. Thom, G., and M. Humfield, 1932. Notes on the association of microorganisms and roots. Soil Sci. 34: 29-36.

86. Thrower, L. B., 1954. The rhizosphere effect shown by some Victorian heathland plants. Australian J. Botany 2:246-67.

87. Time, E. C., 1926. The influence of soil temperature and soil moisture on the development of yellows in cabbage seedlings. J. Agr. Research 33:971-992.

88. Timonin, M. I., 1940. The interaction of higher plants and soil microorganisms. II. Study of the microbial population of the rhizosphere in relation to resistance of plants to soil-borne disease. Can. J. Res. (C.) 18:444-456.

89. _____, 1941. The interaction of higher plants and soil microorganisms. III. Effects of by-products of plant growth on activity of fungi and actinomycetes. Soil Sci. 52:395-413.

90. _____, 1947. Microflora of the rhizosphere in relation to the manganese deficiency disease of oats. Soil Sci. Soc. Amer. Proc. (1946) 11:284-292.

91. _____, 1964. Interaction of higher plants and soil microorganisms. Microbiology and soil fertility, Proceeding of the 1964 Biology Colloquim, Oregon State Univ. Press, Corvallis, Oregon, pp. 135-158.

92. Timonin, M. I., 1966. Rhizosphere effect of healthy and diseased lodge-pole pine seedlings. Can. J. Microbiol. 12:531-537.

93. Tisdale, W. B., 1923. Influence of soil temperature and soil moisture upon the Fusarium disease in cabbage seedlings. J. Agr. Research 24:55-86.

94. Venkata Ram, C. A., 1960. Foliar application of nutrients and rhizosphere microflora of Camelia sinensis. Nature (London) 187:621-622.

95. Waite, B. H. and V. C. Dunlop, 1953. Preliminary host range studies with Fusarium oxysporum f. cubense. Pl. Disease Reptr. 37:79-80.

96. Waksman, S. A., 1917. Is there any fungus flora in the soil? Soil Sci. 3:565-589.

97. Walker, J. C., 1957. Plant Pathology, 2nd Ed., McGraw-Hill, New York, 707 p.

98. Warcup, J. H., 1955. On the origin of the colonies of fungi developing on soil dilution plates. Brit. Mycol. Soc. Trans. 38:298-301.

99. _____, 1955. Isolation of fungi from hyphae present in soil. Nature (London) 175:953-54.

100. _____, 1957. Studies on the occurrence of activity of fungi in a wheat field soil. Brit: Mycol, Soc. Trans. 40:237-262.

101. Wastie, R. L., 1961. Factors affecting competitive saprophytic colonization of the agar plate by various root infecting fungi. Brit. Mycol. Soc. Trans. 44:145-59.

102. Welty, R. E., 1965. Factors influencing wilt of muskmelon caused by Fusarium oxysporum f. melonis (Leach and Currence), Snyder and Hansen. Diss. Abst. 27:1695-B(1966). Ph.D. Thesis, Univ. of Minnesota, 65 p.

103. Wensley, R. N. and C. D. McKeen, 1962. A soil suspension plating method of estimating populations of Fusarium oxysporum f. melonis in muskmelon wilt soil. Can. J. Microbiol. 8:57-64.

104. Wensley, R. N. and C. D. McKeen, 1962. Rapid test for pathogencity of soil isolates of Fusarium oxysporum f. melonis. Can. J. Microbiol. 8:818-819.

105. _____, and _____, 1963. Populations of Fusarium oxysporum f. melonis and their relations to the wilt potential of two soils. Can. J. Microbiol. 9:237-249.

106. _____, and _____, 1966. Influence of resistant and susceptible varieties of muskmelon on size of populations of the Fusarium wilt fungus and wilt in naturally infested soils. Can. J. Microbiol. 12:1115-1118.

107. Whitaker, T. W., and I. C. Jagger, 1937. Breeding and improvement of cucurbits. United States Dept. of Agri. Yearbook of Agriculture, 1937, pp. 207-232.

108. _____, and G. N. Davis, 1962. Cucurbits: Botany, Cultivation, and utilization. Leonard Hill (Books) Limited, London. Inter-science Publisher, Inc., New York. 250 p.

109. White, R. P., 1926. Tomato wilt investigations. Kans. Agr. Exp. Sta. Tech. Bull. 20.

110. Zachariah, A. T., 1949. Unpublished, cited by S. D. Garrett (1960).

111. Zak, B., 1964. Role of mycorrhizae in root disease. Ann. Rev. Phytopathology. 2:377-392.