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TOMATO ISOLINES BY THE NON PATHOGEN, CHAETOMIUM.

The Ohio State University, Ph.D., 1972
Agriculture, plant pathology

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INDUCED RESISTANCE TO FUSARIUM WILT IN SUSCEPTIBLE TOMATO
ISOLINES BY THE NON PATHOGEN, CHAETOMIUM

DISSertation

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By

Pritam Singh Verma, B.Sc. (Ag), M.Sc. (Ag. & A. H.)

* * * * * *

The Ohio State University

1972

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PUBLICATIONS


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Major Field: Plant Pathology

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INTRODUCTION

Fusarium wilt of tomato, incited by *Fusarium oxysporum* f. sp. *lycopersici*, is one of the classical vascular wilt diseases of plants. Because of its importance in the principal tomato-producing areas of the world, efforts to develop resistant cultivars have continued since 1910. At first resistance was of the polygenic type (horizontal), but since 1939-40 emphasis has shifted to the monogenic type (vertical). The success of the breeding program has been complicated by the field appearance of race 2 in 1960, but resistant cultivars have been developed. Control of Fusarium wilt depends mostly on the use of resistant cultivars and cultural practices.

Since the first report (44) in 1957 that a *Cephalosporium* sp. inhibited symptom development of Fusarium wilt of tomato, continued research at The Ohio State University with this *Cephalosporium* has resulted in an accumulation of evidence on the modification of susceptibility of tomato (1, 4, 11, 39, 44), cowpeas (27), and cotton (42) to their respective Fusarium wilt pathogens.

*Chaetomium* spp. frequently have been found in soil and in the rhizosphere of tomato plants. Members of this genus also were isolated from apparently healthy Bonny Best plants. A hypothesis was proposed that *Chaetomium* spp. were internal residents of tomato plants and might modify the susceptibility of Fusarium wilt susceptible cultivars. Several isolates of *Chaetomium* were selected for investigation.
Continued research has demonstrated that certain isolates were effective in retarding and inhibiting symptom development of Fusarium wilt of Bonny Best tomato. Using the host plant in a selective capacity, isolates of Chaetomium were selected that varied in their capacity to retard and inhibit symptom development of Fusarium wilt. A preliminary report on possible modification of susceptibility (induction of resistance) of tomato to Fusarium wilt by a Chaetomium sp. has been published (46).

Many workers have emphasized the desirability of using isolines of the host and pathogen for comparative studies of host-pathogen interaction in disease susceptible and resistant cultivars. Isolines of host and pathogen, genetically characterized for factors that control disease reaction and virulence, are highly desirable components of any study of the nature of disease resistance. The approach selected in this study was to employ two susceptible isolines of tomato, Lycopersicon esculentum Mill., Improved Pearson (IP), and Pearson VF 11 (VF) and race 2 of Fusarium oxysporum f. sp. lycopersici (Sacc.) Snyd. & Hans. (hereafter referred to as Fusarium). The use of isolines will help in studying detectible differences which are related to susceptibility to Fusarium and the differences which occurred as a result of induction of resistance in both tomato isolines.

The objectives of this study were to determine: (i) extent of colonization of susceptible tomato isolines by race 2 of Fusarium; (ii) extent of colonization of susceptible tomato isolines by the non pathogen Chaetomium; (iii) effect of Chaetomium on Fusarium wilt symptom development in susceptible tomato isolines; (iv) degree of
resistance (modification of susceptibility) induced in susceptible
tomato isolines by Chaetomium to race 2 of Fusarium; (v) interaction
of race 2 of Fusarium and Chaetomium in vitro; and (vi) the effect of
total phenols in the host on the induction of resistance.
REVIEW OF LITERATURE

There has recently been renewed interest in biological control. Resistance to fungus plant pathogens induced by earlier or simultaneous inoculation with a strain of the same or another organism is commonly described as cross protection, acquired immunity, or induced resistance (modification of susceptibility). The phenomenon has been demonstrated in various host plant species against several plant pathogens.

A number of key research reports concerning the induced resistance to fungus plant pathogens are summarized below. These reports relate to the present study in two main ways: first, they provided insights leading to the objectives, and second, they provided information making possible the development of techniques.

Resistance Induced by Non-Pathogenic Formae Speciales of Fusarium oxysporum.

It has long been known that, while the pathogenic formae speciales of *Fusarium oxysporum* are restricted in their pathogenicity to a narrow range of hosts, root penetration does occur on non suscepts as well as in highly resistant cultivars of the host. Shroeder and Walker (43) demonstrated that when pea plants were exposed to *F. oxysporum f. sp. conglutinans*, the latter was isolated from the lower stems, although no disease symptoms were noted except occasional vascular browning. Winstead and Walker (50) showed that the pectolytic enzyme extract from cultures of several formae speciales caused vascular browning in non suscepts as
well as in suscepts, presumably due to the breakdown of wall materials which in some way released phenols which were converted to melanins by host polyphenol oxidase.

Probably one of the first studies to indicate protection of plants by a strain of the same fungus was that of Borlaug (1945). His field studies were designed to determine the effect of races 6 and 11 of *F. oxysporum* f. sp. *lino*, singly and in combination, on yield of four flax varieties. Much less disease occurred in the flax varieties inoculated with mixtures of the two races than when inoculated with either race alone. In laboratory tests there was no apparent antagonism between the two races in agar cultures. Suppression of disease with mixtures of the races as inoculum also was demonstrated in steam sterilized soil in the greenhouse (5).

Davis (13, 14, 15) studied the reaction of suscepts and non suscepts to various formae speciales of *F. oxysporum* when aseptically produced seedlings were transferred to agar and exposed to a given formae speciales. The roots were excised 10 mm below the crown before transfer. Invasion of non suscepts was the general rule. Gums and tyloses were found less frequently and to a lesser degree in the parasitized vessels of suscepts. While continuous mycelial strands were common in the xylem of suscepts, they were always discontinuous in the vessels of non suscepts. The parasite never proliferated into the cortex of non suscepts beyond the immediate area of root wounding. He questioned whether the higher concentrations of occluding materials found in non suscepts were responsible for limiting the distribution of the fungus, or whether they were merely by-products of a more basic modification of susceptibility. In further experiments (16) he
inoculated a number of plants, including tomato, with non-pathogenic forms, and then reinoculated with the respective pathogenic form at intervals of 1 to 15 days. The length of intervals did not alter the results. The disease was regularly suppressed in those plants previously exposed to a non-pathogenic form. Seedlings were less resistant if the roots were injured at the time of the addition of the pathogen. He regarded it improbable that phytoalexins, antibiotics, or antibodies were responsible for resistance. He suggested that the resistance barrier was due to auxin imbalance, respiratory dysfunction, or membrane disruption which might result in a hypersensitive response.

Bega (3) and McClure (33) described a reduction in development of Fusarium wilt symptoms of sweet potato when fresh cuttings of sweet potato were inoculated with *Fusarium solani*, causal agent of the foot rot disease, and then with *F. oxysporum f. sp. batatas*. Severe wilt symptoms developed on the control sprouts which were not inoculated with *F. solani*. Effectiveness of the wilt-reducing treatment was dependent on the time interval between the two inoculations.

Davis (17) tried the effect of non-pathogenic formae speciales on control of tomato Fusarium wilt in greenhouse experiments. Tomato plants growing in treated compost were inoculated at the five to seven leaf stage with *f. sp. batatas*, *f. sp. vasinfectum*, *f. sp. cubense*, and *f. sp. trachaeiphilum*, respectively. This was done by uprooting and dipping in a microconidial suspension and replanting, or by spraying the root ball with a spore suspension. Four to seven days later the tomato wilt fungus was added to the soil. The disease was retarded equally by all four non pathogens, although the effect was only a delay.
This inhibition occurred only when the wilt fungus inoculum was poured on the soil without disturbing the roots. These experiments were all with the very susceptible Bonny Best cultivar. While these facts are of interest, they obviously offer no practical means of control.

Matta (30) found that wilt symptoms were reduced after preinoculation of tomato roots with *Verticillium dahliae* Kleb. and *Fusarium oxysporum* f. sp. *dianthi* and f. sp. *callistephi*. A similar effect was observed when tomato cuttings were preinoculated with f. sp. *dianthi*. Inhibition was greatest with a two-day interval between preinoculation with *F. oxysporum* f. sp. *dianthi* and inoculation with the wilt fungus.

Langton (25) inoculated susceptible tomato cuttings by standing them in suspension of mixtures of "bud cells" of *F. oxysporum* f. sp. *lycopersici* (FL) and f. sp. *pisi* (FP). There was marked reduction in symptom development compared with suspension of FL alone. The reduction in symptoms was related to the ratio of FP to a fixed level of inoculum of FL, the reduction being moderate at 1:3 and complete at 4.9:1. When heat killed cells of FP were substituted for live inoculum no reduction in symptoms followed. Mitigation appeared to be related to reduction in extent of invasion by FL.

Mas (28) has also shown that resistance of melon plant toward its respective pathogenic formae of *F. oxysporum* can be increased by previous inoculation with non-pathogenic formae or races of the same species.

Buxton and Perry (7) reported that the severity of pea wilt caused by *F. oxysporum* f. sp. *pisi* on cultivar Onward was decreased by simultaneous inoculation with *F. solani* f. *pisi*. The decrease in wilt
symptoms was statistically significant and was greater when the amount of *F. solani f. pisi* in the mixed inoculum was increased. Inoculating plants with *F. solani* before *F. oxysporum* resulted in less wilt than inoculating plants with both fungi simultaneously. Buxton (6) also reported a decrease of pea wilt by inoculating pea plants with non-pathogenic cultures of *F. solani* plus *F. oxysporum f. sp. pisi* race 1.

**Resistance Induced by Non Pathogens of Tomato to Fusarium Wilt.**

Smith (44), in 1957, reported the isolation of a species of *Microomonospora* (later determined to be a species of *Cephalosporium* [11]) from one tomato plant remaining healthy in a group of seedlings inoculated with *F. oxysporum f. sp. lycopersici*. This *Cephalosporium* (C) isolate retarded growth of the wilt fungus on agar. When roots of tomato plants were placed in a liquid culture of C before planting in Fusarium-infested soil, the wilt symptoms were considerably reduced. When roots were immersed in Knop's solution containing the sterile filtrate from a C culture for four days prior to inoculation with the wilt fungus, the disease rating was 1-4 compared to 6-9 for untreated controls on a scale of 0 = no symptoms to 10 = severely diseased or dead. Although *Cephalosporium* alone induced no macroscopic symptoms on inoculated tomato plants, it was isolated from about 20% of such plants.

In the same laboratory Bhelwa, Phillips and Allison (4) inoculated tomato seeds with a suspension of conidia and hyphae of an isolate of *Cephalosporium* and the resultant 30-day-old seedlings were inoculated with the wilt fungus. Three weeks later all but a few seedlings had severe Fusarium wilt. Two of these seedlings had no internal discoloration and the remainder showed only varying amounts of such discoloration.
They proposed that a non-pathogen in or on the seed may become associated with seedlings in such a way as to mitigate symptom development of wilt. Also in the same laboratory, Chisler et al. (11) reported that agitation of tomato seedling roots in a suspension of *Cephalosporium* for several hours resulted in great retardation of Fusarium wilt symptoms.

Allison and Phillips (1) used Bonny Best seedlings in a selective capacity and demonstrated biotypes of *Cephalosporium* that varied in their capacity to retard and inhibit symptom development of Fusarium wilt of tomato. This was actually the use of selective pressures to obtain more effective biotypes in control of Fusarium symptoms. Mitigation of symptoms increased with the increase in the interval between inoculations with the two fungi.

In the same laboratory the modification of susceptibility has also been demonstrated in cotton (42) and cowpeas (27) against Fusarium wilt by prior inoculation with a *Cephalosporium* sp. In 1970 Verma and Allison (46) reported that a *Chaetomium* sp. was an internal resident of tomato plants and was highly effective in delaying and retarding Fusarium wilt symptoms in the Bonny Best cultivar.

Youssef (53) reported that symptoms of tomato wilt were inhibited in highly susceptible tomato plants treated with *Penicillium chrysogenum* cultures or with filtrates of such cultures. Panteleev and Shklyar (36) found that when tomato seedling roots were dipped in culture filtrates of a *Pseudomonas* sp. before planting in Fusarium-infested soil, the percentage of infected plants was halved and yield was increased 12-16%.
This phenomenon of induced resistance is known also to occur in sweet potato against *Ceratocystis fimbriata* Ell. & Halst. (48), in potato (35), in soybean (9, 37) against pathogenic species of *Phytophthora*, in tobacco against *Peronospora tabacina* Adam. (12) and *Thielaviopsis basicola* (Berk. & Br.) Ferr. (22), and with the rust fungi in beans (49, 51), wheat (24) and sunflower (52). A resistant response was also induced in etiolated hypocotyls in *Phaseolus vulgaris* L. by non pathogens of beans (*Helminthosporium carbonum* Ullstrup, race 1, or *Alternaria* sp.) or a race of *Colletotrichum lindemuthianum* (Sacc. & Magn.) Scribner to which the bean variety was resistant (40).

**Mechanisms of Induced Resistance in Fusarium Wilt Diseases.**

Fawcett (20) emphasized the need for studying multiple invasions of plants, and several diseases resulting from two or more pathogens have been described. They may result from simultaneous invasion by different pathogens or from successive invasions occurring soon after one another. Two pathogens entering a host may act synergistically or one may lessen the damage caused by the other.

The examples of induced resistance cited earlier may be broadly divided into three types, depending on the apparent mechanisms involved, with more than one mechanism suggested in many cases. The mechanisms include structural modifications of the host tissue (vascular wilt pathogens), inhibitors of germination (rust), and the induction of phytoalexins (strongly indicated in cross-protection of soybean and potato, and not ruled out in any of the examples).

According to McClure (33), the mechanism of protection of sweet-potato sprouts against wilt by prior inoculation with the foot rot
Fusarium may exist in the xylem of the sprouts. As the foot rot Fusarium causes but a limited rot, it may be that the tissues of an invaded sprout develop delimiting barriers which, once formed, are effective against the wilt Fusarium. Histological examinations of diseased sprouts revealed the presence of numerous tyloses in the vessels in advance of the fungus. Tyloses are also commonly found in the vessels of plants inoculated with the wilt Fusarium, and the method by which that pathogen by-passes tylosis-clogged vessels, by growing into unclogged vessels, is described (33). In the case of sprouts with the wilt Fusarium, it may be that the foot rot pathogen promotes the production of tyloses in all vessels, thus leaving no unclogged vessel by which the wilt pathogen could by-pass the tylosis-clogged vessels.

Perry (38) studied the mechanism underlying the decrease caused by F. solani f. pisi in the severity of pea wilt due to F. oxysporum f. pisi by examining sites of possible interaction in soil and within the host. Fusarium solani produced no substance in vitro that inhibited spore germination or growth of F. oxysporum, and there was no evidence that the two fungi competed to the detriment of F. oxysporum either in the pea rhizosphere or in the soil. Some water extracts of foot rot lesions contained a substance inhibitory to the germination of F. oxysporum spores, but it could not always be demonstrated. No inhibition was caused by exudates or extracts of pea roots with F. solani. However, F. solani colonized the epidermis and outer cortex of pea roots more rapidly and more extensively than did F. oxysporum. Three days after inoculation with F. solani, pea roots respired 25% more than uninoculated. The physical nature and metabolic activity of the cortex
was altered through infection by *F. solani*, so he concluded that it was these changes alone that delayed the progress of *F. oxysporum* to the stele, resulting in a decrease in wilt.

The mechanism of Fusarium wilt symptom suppression by *Cephalosporium* sp. in tomato has been investigated and several theories have been proposed. Smith (44) postulated that an antibiotic substance produced by *Cephalosporium* sp. (at that time tentatively identified as *Micromonospora* sp.) might inhibit the growth of *F. oxysporum* f. *lycopersici* in *vivo*. Chisler (10) demonstrated *in vitro* inhibition of *Cephalosporium* sp. of toxin production by *F. oxysporum* f. *lycopersici* but offered no direct evidence that this occurred *in vivo*. Rodebaugh (41) reported that when susceptible Bonny Best plants were inoculated with *Cephalosporium* before inoculation with the wilt fungus, less pectin methylesterase and polyphenoloxidase activity was noted 18 days after wilt pathogen inoculation. *Cephalosporium* had little or no effect on enzyme production in monogenic-resistant plants. He suggested that inhibition is due either to reduction of pectic enzyme production and limitation of the amount of available carbohydrates or to direct inhibition of *Fusarium* growth. Phillips, Leben and Allison (39) demonstrated that *Cephalosporium* sp. invaded vessels in the stem and produced mycelium and tylosis obstructions in tomato seedlings. These were confined to primary vessels already formed at the time of treatment, and *Cephalosporium* sp. did not spread extensively within the stem. In contrast, the plants inoculated with *Fusarium* mycelia were found in all vessels including secondary vessels formed after inoculation, and *F. oxysporum* f. *lycopersici* spread extensively through the stem. In
plants treated with Cephalosporium sp. and inoculated with *F. oxysporum* f. *lycopersici*, obstructions were rarely observed in secondary vessels and extensive spread of the pathogen did not occur. They suggested that one mechanism by which Cephalosporium sp. reduced symptoms is by vessel obstructions induced by the Cephalosporium treatment that reduced entry or prevented extensive spread of *F. oxysporum* f. *lycopersici* in the plants.

Davis (16) working on cross-protection in Fusarium wilt diseases, did not find evidence for accumulation of gum and tyloses, formation of phytoalexin, antibiotics of fungus origin, or production of antibodies. However, he hypothesized an alteration in host metabolism. The initial inoculation could induce auxin imbalance, respiratory dysfunction, membrane disruption, etc., which would in turn result in hypersensitive response. The products of this reaction could act as barriers to the advance of the pathogen.

Langton (25) studied the interactions of the tomato with two formae speciales of *Fusarium oxysporum*. He postulated three main mechanisms to account for the dual inoculation mitigation effect: (i) a purely fungal interaction resulting in the suppression of the disease potential of *F. oxysporum* f. *lycopersici*, (ii) antibiotic production by the tomato in response to the fungi, and (iii) a physical localization reaction such as that postulated by Beckman (2) to explain resistance to Fusarium wilt in certain varieties of tomato and host plants of other species following root inoculation.

All non-pathogenic forms of *F. oxysporum* tested on tomatoes initially induced greater increase of phenols in leaves and stems than
those induced by the pathogenic forms (31). The correlation suggests that the induced resistance of tomato plants after inoculation with non-pathogenic forms is reflected by, and perhaps depends upon, the increase of phenolic substances which might act by inhibiting the pathogen, directly or by means of their oxidation products, and/or by inciting more complex metabolic changes such as accumulation of growth substances (29) leading to formation of defense barriers in the xylem vessels.

Mechanism of induced resistance in Phaseolus vulgaris to bean anthracnose by non-pathogens of beans (Helminthosporium carbonum Ullstrup, race 1, or Alternaria sp.) or by a race of Colletotrichum lindemuthianum (Sacc. & Magn.) Scribner to which a bean cultivar is resistant (varietal non-pathogenic race) suggested that phaseollin and the other phenolic substances associated with visible cell collapse are not the primary sources of protection (40). The times of appearance of induced resistance were the same following inoculation with H. carbonum, Alternaria sp., or a varietal non-pathogenic race of C. lindemuthianum, suggesting that metabolic alterations occurring 0-24 hr after inoculation reflect the primary protective response of bean hypocotyls to these fungi.

Effect of Chaetomium sp. on Tomato.

Chaetomium sp. has frequently been found in the rhizosphere of tomato plants and as an internal non-pathogen of tomato (21). In one experiment, young tomato plant roots were artificially inoculated with Chaetomium sp. and planted in sterilized soil in pots. Chaetomium sp. became established in soil, on the root surface and in root tissue and
was also isolated from stems. The roots of tomato five months after
inoculation with Chaetomium became corky (21).

Under gnotobiotic conditions (26) Chaetomium sp. has been reported
to grow on the roots of dwarf tomato and caused an increase in the
height of plants as compared to control. It was concluded that this
fungus growing in the rhizosphere has growth-regulative effects on
tomatoes resulting in increased height.
MATERIALS AND METHODS

HOST MATERIAL:

A major consideration in the selection of a host–pathogen complex was the availability of genetically similar susceptible plants. Two cultivars of tomato, *Lycopersicon esculentum* Mill., Improved Pearson (IP), and Pearson VF11 (VF), whose pedigrees were known and related were selected. Seeds of both cultivars were donated by Peto Seed Co., Inc., Saticoy, Calif., USA. The two cultivars have comparable genetic backgrounds and may be considered isolines. The cultivars were developed by O. S. Cannon (8). IP is an inbred Fusarium wilt susceptible line, VF is an isolate of IP carrying single gene resistance to Fusarium wilt incited by race 1 of *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyd. & Hans. However, both cultivars are susceptible to race 2 of *F. oxysporum* f. sp. *lycopersici*.

PATHOGEN MATERIAL:

A culture of race 2 of *F. oxysporum* f. sp. *lycopersici* (hereafter called *Fusarium*) was obtained from Dr. J. A. Veech, USDA, ARS, Beltsville, Md. Genetic stability of the pathogen from one experiment to the next was insured by lyophilizing stock cultures. The isolate was increased in a shake culture on a pH 5.0 medium-B (18) amended with 0.1% yeast extract.
Preparation of Lyophilized Stock Cultures of Pathogen.

After growing one week in a shake culture, the culture was removed from the shaker and left undisturbed for two hr. This allowed the "bud cells," hereafter called conidia, similar in size and shape to microconidia, to settle to the bottom of the flask. The medium was decanted and an equal volume of 10% Difco bacto skim milk (w/v) added. After resuspending the conidia in the milk, the flask remained undisturbed for two hr. The skim milk was decanted leaving the conidia on the bottom of the flask. Additional skim milk was added to the flask. The amount of milk added is only important as it affects the final concentration of conidia in lyophilization tubes. The skim milk carrier was sterilized in the autoclave for 15 min at 15 lb pressure (121 C). The conidia were resuspended in the skim milk and aliquots of the suspension were aseptically transferred to 10 x 108 mm Wheaton gold band vacules. The suspensions were lyophilized with freeze dryer-vacuum equipment.

Preparation of Pathogen Inoculum:

The lyophilized content of one vacule was used to seed 100 ml of a shake culture medium (18). Seeded shake cultures were incubated for 5-7 days at 25 C. The conidia were collected by repeated centrifugation and washing with sterile distilled water. The final washing was filtered through glass wool and the filtrate was centrifuged and the supernatent discarded. The conidia were washed several times with sterile water and brought to the desired concentration using a hemacytometer. To prevent germination during preparation of the inoculum, conidial suspensions were kept in an ice bath at 4 C and used as inoculum the same day.
18

NON PATHOGEN MATERIAL:

Source.

During 1969 isolations of *Chaetomium* were made from Bonny Best plants which had previously been inoculated with *Chaetomium* and later with *F. oxysporum f. sp. lycopersici*. The plants chosen for reisolation of *Chaetomium* were those that were free of symptoms of Fusarium wilt or with only slight symptoms. *Chaetomium* was re-isolated from roots, stems, and petioles. Isolates of *Chaetomium* showing the same degree of inhibition of Fusarium wilt symptoms and that looked alike in culture were classified in group A. Isolate B of *Chaetomium* was from apparently healthy tomato plants from a field at OARDC, Wooster, Ohio, during the summer of 1971.

Preparation of Chaetomium (Non Pathogen) Inoculum.

To decrease variability, isolates of the non pathogen were stored in a soil medium as described by McKeen and Wensley (34). Ascospore inoculum was produced by culturing on oatmeal agar in Petri plates. The cultures were incubated at room temperature for 7-10 days. Ascospores were harvested by flooding plates with 10-15 ml of sterile double-distilled water and then scraping the agar surface with a spatula. Usually, several plates of each isolate were used for each batch of ascospores by bulking in a 250 ml Erlenmeyer flask containing sterile double-distilled water. The suspension was filtered through a double layer of cheesecloth to remove small bits of agar or other debris and then adjusted to a desired concentration of ascospores with a hemacytometer.
TOMATO SEEDLING PRODUCTION:

To obtain uniform tomato seedlings, seeds were sown in a sterilized soil mixture in wooden flats. The soil consisted of one part horticultural perlite, one part Canadian peat moss and two parts loam soil. The same composition of soil was used in other greenhouse experiments unless otherwise specified. Seedlings were watered as needed and no additional fertilizer was applied. Plant age was measured from the time of seeding. The seedlings grew in the greenhouse for various intervals at 25-27 °C with supplementary light (GE cool white) providing a 16 hr photoperiod. For all the experiments, visually uniform seedlings were selected.

GREENHOUSE EXPERIMENTS:

Soil in 4-in sterilized plastic pots was generally used. Plants were watered as needed. A commercial fertilizer starter, 9-45-15 of NPK, was applied after transplanting at the rate of 1/2 oz per gallon of water and the soil was saturated. The greenhouse temperatures ranged between 24 to 29 °C with occasional daytime periods of 30 °C. Supplementary light was provided.

FUSARIUM WILT SUSCEPTIBILITY OF TOMATO ISOLINES:

Host Colonization.

1. Effect of inoculum concentration:- Fifty test and 10 control plants (25 days old) of each cultivar were divided into six equal groups for studying the effects of inoculum concentration. The roots were washed in tap water, and the main axis of plants was transversely severed at the junction of root and hypocotyl. The cut ends of plants
of five groups of each cultivar were placed for one hour in concentrations of 5 x 10^6, 5 x 10^5, 5 x 10^4, 5 x 10^3, or 5 x 10^2 conidia per ml; the sixth group (control) was treated with water. After inoculation the cuttings were stuck in sterile soil and held in a mist chamber for three days. They were then placed on a greenhouse bench until examined. This experiment was repeated twice.

a) **Killing and stunting** — Thirty days after inoculation, the number of plants of each cultivar that died as a result of inoculation at the various inoculum concentrations was determined. From the plants that survived, the number showing distinct stunting (less than 80% of the size of the controls) was determined.

b) **Extent of vascular discoloration** — This experiment was designed to determine how far from the point of inoculation (using the five inoculum concentrations given earlier) vascular discoloration could be detected. Plants were sampled 20 days after inoculation. Sections were cut at 1 cm intervals along the main axis. The sections were examined microscopically for the presence of discolored vessels, and the sections containing discolored vessels farthest removed from the site of inoculation were recorded.

c) **Percentage of vascular bundles invaded** — Twenty-five days after inoculation the main axis of five plants of each inoculum concentration was washed in tap water and surface sterilized in 25% Clorox (NaOCl 5.25% v/v) solution, for 2 min. Sections of 2 mm thickness were cut every 1 cm along the axis, placed on Vogel's "N" medium (47) containing 2% sucrose and incubated at 25 C for
3 days. The numbers of major vascular bundles with fungal growth and the total number of bundles were counted for each axis and the results expressed as "fungal invasion percentage," i.e., the percentage of invaded bundles in an axis.

2. Effect of the site of inoculation: Thirty plants (25 days old) of each cultivar were prepared as described previously for studying the effect of the site of inoculation. The 30 plants were divided into groups of 10. The roots of one group were cut transversely 2 cm below the junction of hypocotyl and root. The second group was transversely cut through the junction of hypocotyl and root. The third group was transversely cut through the cotyledonary node. The plants were then inoculated by placing them in a suspension of $5 \times 10^5$ conidia/ml for one hour. The cuttings were then stuck in sterile soil and held in a mist chamber for three days and then placed on a greenhouse bench. Controls consisted of 10 plants of each cultivar similarly treated but placed in sterile water instead of a conidial suspension.

a) Disease index -- After transplanting, the plants were rated on the scale of 0-5 (0 = healthy, 5 = dead) at the appropriate time. Details of the disease index are given later. Plants were examined every day for 30 days but data were taken at intervals of 10 days.

b) Percentage of vascular bundles invaded -- Five plants of each cultivar were sampled for each inoculation site. Percentages of invaded vascular bundles were determined at five day intervals until 30 days after inoculation, according to the procedure described earlier.
c) **Colony count per gram fresh weight of tissue** -- An attempt was made to estimate the amount of fungus present in diseased tissue because the fungus is restricted to xylem vessels and propagules could not be removed by homogenization from old plants. Ten days after inoculation the main axis of the stem was washed in tap water, weighed and surface sterilized in 25% Clorox for two minutes. The segments of each stem were homogenized in 100 ml sterile double-distilled water using an Omnimixer at speed 8 for one minute. Four 0.5 ml samples of serial tenfold dilutions were plated on Vogel's "N" medium containing 2% sucrose and 30 ppm of Rose Bengal. The entire procedure was aseptically accomplished and colonies were counted 3-4 days after inoculation at 25 C.

**COLONIZATION OF TOMATO ISOLINES BY THE NON PATHOGEN (CHAETOMIUM):**

1. **Distance of ascospore transport determined microscopically:**— Twenty-five-day-old plants were removed from the soil, washed in tap water, and the main axis was transversely severed at the junction of root and hypocotyl. The cuttings were inoculated by immediately placing the cut ends in a suspension of **Chaetomium**, isolate A (5 x 10^7 ascospores/ml). The cuttings were held in the ascospore suspension for four hours. The ascospores were kept suspended by intermittent stirring. Upon removal from the ascospore suspension, the leaves were removed, the stem was washed with water to remove surface ascospores, and the main axis was transversely sectioned at 1 cm intervals. The sections were examined microscopically for the presence of ascospores. The experiment was repeated three times with 10 plants of each cultivar. The distance
from the end of the cutting to the farthest section containing asco-
spores was recorded.

2. **Distance of ascospore transport determined by tissue plating:**

Ten 25-day-old plants of each cultivar were surface sterilized after
washing with tap water by immersing in 25% Clorox for one minute. They
were then rinsed with sterile distilled water and the main axis was
transversely severed at the junction of root and hypocotyl. The cut-
tings were placed in an ascospore suspension of the same concentration
used in the previous experiment or in sterile water for the controls.
The inoculation period was four hours. Upon removal from the ascospore
suspension, the leaves were removed and the main axis was again
surface-sterilized and rinsed. Transverse sections were cut at measured
intervals along the axis. The sections were transferred onto acidified
(pH 4.0) potato-dextrose agar containing 20 ppm streptomycin. The
plates were incubated for five days at room temperature and examined for
the presence of the non pathogen. The experiment was repeated three
times.

3. **Distribution of Chaetomium in previously inoculated tomato
cuttings:**

Forty 25-day-old plants of each cultivar were removed from
the soil and washed in tap water and the main axis of the plants was
transversely severed at the junction of the root and hypocotyl. The
cuttings were inoculated by placing the cut ends in a suspension of
$5 \times 10^7$ ascospores/ml of Chaetomium, isolate A, and left for 24 hr in a
growth room. Control plants were given the same treatment except they
were placed in sterile water instead of ascospore suspension. The cut-
tings were transplanted in sterile soil and placed on a greenhouse bench.
Isolations were made to determine the pattern of *Chaetomium* spread in inoculated cuttings of each cultivar. Five rooted cuttings were taken from each cultivar at 7, 14, 22 and 30 days after inoculation. The uppermost expanded leaf of each cutting had been tagged at the time of inoculation. Reisolations of *Chaetomium* were attempted from stem sections taken from the base of each cutting, from immediately below the tagged leaf (top), and from midway between these points (middle). Isolations were also attempted from new stem growth two inches above the tagged leaf and from roots. Portions of the cuttings were surface sterilized in 25% Clorox for two minutes. Transverse segments were cut at measured intervals along the axis and plated on potato-dextrose agar. The plates were incubated at room temperature for 6-9 days and examined for the presence of *Chaetomium*. At the same intervals additional plants were removed and examined microscopically for the presence of discolored vessels and other microscopic changes.

**STUDIES ON INDUCTION OF RESISTANCE IN TOMATO TO FUSARIUM WILT:**

**General Procedure of Inoculations.**

Twenty-five-day-old plants of IP and VF were removed from the soil and washed in tap water and the main axis of the plants was transversely severed at the junction of root and hypocotyl. The cuttings of each cultivar were inoculated with *Chaetomium*, isolates A and B (inducer). The cuttings were placed in a wide mouth 250 ml Erlenmeyer flask containing 100 ml of an ascospore (5 x 10^7/ml) suspension in sterile double distilled water and left for 24 hr in a growth room at 26 C with supplementary lighting (GE cool white) providing a 14 hr photoperiod.
The ascospore suspension of the non pathogen (inducer) was then replaced with Hoagland's solution (23). After an additional 24 hr the roots were injured by cutting off the lower portions, about 0.5 cm. Control cuttings were treated the same except they were placed in 100 ml of sterile double distilled water without ascospores. These cuttings were then inoculated with Fusarium \( (5 \times 10^5 \text{ conidia/ml}) \) for a 2 hr period (challenge inoculation). After the challenge inoculation the cuttings were transplanted into steamed soil in pots, held in a mist chamber for three days and then placed on the greenhouse bench.

**Induction of Resistance under Controlled Environmental Conditions.**

Twenty-five-day-old plants of VF and IP were prepared as described above. Isolate A of Chaetomium was used as it was found to be more effective in the previous experiment. The procedure and concentration of inoculum for the induced and for the challenged inoculations were the same as described in the previous experiment. After inoculations the plants were potted in vermiculite, and Hoagland's solution plus micro-nutrients was used as a part of the watering regime. All the plants were placed in an environmental chamber with 12 hr of light \( (26 \text{ C}) \) and 12 hr of darkness \( (23 \text{ C}) \) with relative humidity of 60 ± 5%. A comparable control treatment of plants of both cultivars was run side by side.

**Effect of Killed Ascospores of Chaetomium on Development of Fusarium Wilt Symptoms.**

In order to find out if killed ascospores of Chaetomium, isolate A were effective in reducing the symptoms of Fusarium wilt, 25-day-old
plants of IP and VF were used as described earlier. The cuttings were placed in a killed ascospore suspension for 48 hr. The ascospores were killed by heating at 37°C for 12 hr in a 1% Formalin solution (19). Formalin was removed under sterile conditions by alternate washings and centrifugation. After 48 hr the cuttings were challenged by Fusarium for two hr. The cuttings were transplanted in steamed soil and the pots were placed on a greenhouse bench.

Wilt Development in Tomato Inoculated with Combination of Fusarium and Chaetomium, Isolate A.

An effort was made to find out if mixed inocula of Fusarium and Chaetomium could induce protection to Fusarium wilt in tomato. Both cultivars, IP and VF, were employed. Twenty-five-day-old plants of both were prepared and used for inoculation as described previously. The cuttings were placed in a combination of inoculum (Fusarium and Chaetomium, isolate A) for two hr. The mixture was in a 1:1 ratio of Fusarium and Chaetomium, isolate A, consisting of $10^6$ spores per ml. The plants were transplanted into steamed soil and the pots were placed on a greenhouse bench. Observations were recorded every 10 days for 30 days.

Isolation from Induced Resistant (protected) Plants of IP and VF.

An effort was made to isolate from protected plants which might indicate the extent of colonization by Fusarium and Chaetomium. The plants of both cultivars were prepared and used for inoculations as described earlier. The inducer (Chaetomium, isolate A) and challenger (Fusarium) inoculations were as described. Isolations were made at
10-day intervals until the end of the experiment. The stems of the plants were washed in tap water and surface sterilized in 25% Clorox solution for one minute. Sections of 5 mm thickness were cut along individual plant stems at 1 cm intervals and placed on acidified PDA in Petri dishes. Isolations were also made from petioles of green leaves. The control plants in this experiment were those inoculated with Fusarium only.

Quantitative Estimate of Chaetomium (inducer) and Fusarium (challenger) in Protected Plants of VF and IP.

Twenty-five-day-old plants of each cultivar were prepared and used. Induced inoculation (Chaetomium, isolate A) was followed by challenger (Fusarium). After inoculation the plants were potted in vermiculite. Hoagland's solution containing micronutrients was applied as part of the watering regime. The experiment was under controlled environmental conditions. The control plants were non induced but challenged. A quantitative estimation (colonies/g fresh weight of tissue) was made according to the procedure described earlier. Colony counts were made at 3 day intervals up to 21 days.

ESTIMATION OF DISEASE INDEX:

Shortly after the first appearance of wilt symptoms and at regular intervals thereafter, plants were examined and evaluated for Fusarium wilt symptoms. A rating scale of 0-5 was used in the experiments.

0 = no disease symptoms,

1 = slight downward curvature of petioles and slight stunting,

2 = 0-30% of the leaves yellowed,
3 = 31-80% of the leaves yellowed,
4 = 81-100% of the leaves yellowed or abscised,
5 = apex wilted or plant dead.

At the end of the experiment the plants were harvested and examined for vascular discoloration, which gave additional support to the value of the disease rating scale.

Calculation of Degree of Resistance Induced.

\[
\text{Degree of resistance induced} = 100 - \frac{\text{disease index of induced and challenged plants} \times 100}{\text{disease index of non induced (control) but challenged plants}}
\]

QUANTITATIVE DETERMINATION OF TOTAL PHENOLS:

The procedure of plant tissue extraction for quantitative determination of total phenols is presented in detail in Figure 1. The plant material used for extraction was washed in sterile double-distilled water. The fresh weight of stems and leaves was determined separately, as well as the dry weight of their residues following extraction.

Characterization of the Ethanol Extract.

The total phenols in the ethanol extract were determined by a modified Folin-Denis method, as described by Swain and Hillis (45). For this test 0.5 ml aliquots of ethanol extract were used and compared to the same volume of standard ethanolic solutions of chlorogenic acid. The optical density (OD) at 725 nm of the processed solutions was measured using a Spectrophotometer 240. These values were related to a chlorogenic acid standard curve and the results were referred to as equivalent-milligrams of chlorogenic acid per unit dry weight of
Fresh tissue

Maceration in 70% ETOH (1:4 w/v)

Filtered through tared Whatman No. 1 filter paper and washed with 20 ml 70% ETOH

Residue

Filtrate - crude extract

Dried at 100°C overnight

Dry weight

Centrifugation (10,000 g for 10 min)

Added water (1:1 v/v)

Evaporated ETOH under vacuum at 40°C

Fractionated twice with ethyl acetate (1:2 v/v)

Aqueous fraction with suspended brown material discarded

Ethyl acetate fraction dried under vacuum at 40°C

Redissolved in 95% ETOH

Ethanol extract

Figure 1. Procedure for extraction of phenols from plant tissues.
extracted tissue or percent increase over the controls. The extracts were diluted or concentrated as necessary in order to obtain adequate values for OD which could be related to the chlorogenic acid standard curve.
RESULTS

FUSARIUM WILT OF SUSCEPTIBLE TOMATO ISOLINES: HOST COLONIZATION:

Effect of Inoculum Concentration.

Killed and stunted plants:– The effect of five inoculum concentrations (5 \times 10^6, 5 \times 10^5, 5 \times 10^4, 5 \times 10^3, and 5 \times 10^2 conidia/ml) is recorded as the percent of plants killed and is presented in Figure 2. Disease symptoms appeared simultaneously on all parts of the cutting, reflecting the method of inoculation. The plants of both cultivars were more susceptible as the inoculum concentrations increased. The percentage of killed plants was less in cultivar VF as compared to IP at all inoculum concentrations. Cultivar VF was significantly different from IP at the 5% level of significance. Cultivars IP and VF inoculated with 5 \times 10^2 conidia/ml had 95% and 96% survival, respectively. If observation of survival had been made beyond 30 days after inoculation, it is probable that some additional killing of plants would have occurred with the inoculum concentration of 5 \times 10^2 spores/ml.

The percentages of VF and IP plants that were stunted as a result of inoculation at the various inoculum concentrations are given in Figure 2. Plants less than 80% of the size of the controls were considered stunted. An analysis of variance indicated that there was no significant difference between the two cultivars. Increased inoculum levels did increase stunting of both cultivars.
Extent of vascular discoloration: The average distance that discoloration of the vascular bundles extended from the point of inoculation in both VF and IP plants inoculated at the various inoculum concentrations is presented in Figure 3. In both cultivars there was a direct relationship between the extent of discoloration and the inoculum concentration. An analysis of variance did not demonstrate any significant difference between the two cultivars.

Percentage of vascular bundles invaded: Average estimates of the percentage of invaded vascular bundles of plants inoculated with the various inoculum concentrations are presented in Figure 4. All the vascular bundles were considered invaded in plants which died as a result of inoculation. The percentages of vascular bundles invaded were higher as the inoculum concentration increased. At lower levels both cultivars were similar in percentage of vascular bundles invaded. At higher inoculum concentrations the cultivars differed considerably in this respect. Mycelium was frequently observed in IP at 12 cm above the inoculation site, although it was usually not abundant at that level in the stem. In the plants of VF, mycelium was observed at 10 cm above the inoculation site in the stem.

Effect of the Site of Inoculation.

Disease index: The effect of three sites of inoculation on the disease index is presented in Figure 5. The disease index recorded for cultivar VF was always less than for cultivar IP regardless of inoculation site. In the early stage of disease development at inoculation sites A and C, both cultivars had a similar response to the pathogen. However, 30 days after inoculation cultivar IP was always more susceptible.
Figure 2. Effect of five inoculum concentrations of Fusarium on killing and stunting of plants of two tomato cultivars 30 days after inoculation.
Figure 3. Effect of five inoculum concentrations of *Fusarium* on the extent of vascular discoloration measured 20 days after inoculation.
Figure 4. Effect of five inoculum concentrations of *Fusarium* on the fungal invasion percentage of IP and VF plants 25 days after inoculation.
According to the data (Fig. 5) the plants of both cultivars inoculated at sites A and B had higher disease indices than the plants inoculated at the other inoculum site.

**Percentage of vascular bundles invaded:** The percentage of vascular bundles invaded of the plants of both cultivars, inoculated at three inoculation sites, are given in Figures 6 and 7. This parameter measures the extent of colonization of the plant rather than the amount of fungus present. The extent of colonization of invaded vascular bundles in both cultivars increased with time after inoculation. These data also agree with that of disease index (Fig. 5), indicating the extent of colonization at inoculation site C was much less than that of the other inoculation sites. The extent of colonization in both cultivars was similar when plants were inoculated at sites A and B. The maximum extent of colonization in both cultivars occurred in plants inoculated at site B. A rapid increase in the extent of colonization was noted beginning 15 days after inoculation.

**Homogenization and colony count:** This experiment was an attempt to get an estimate of the amount of mycelium present in plants inoculated at three different inoculation sites. Homogenization and plating out suggested a variability in the amount of fungus within plants of a cultivar. This is not surprising in view of the variation in the nature and size of the fungus propagules produced by homogenization, particularly as the fungus is restricted to xylem vessels which are difficult to homogenize.

The fewest colonies were recovered from plants which had been inoculated at the cotyledonary node (Table 1). This supports the data
of the previous two experiments. The number of colonies recovered was always less from cultivar VF than from IP regardless of inoculation site. Plants inoculated at the junction of root and hypocotyl appeared to have the maximum amount of propagules per gram fresh weight of tissue.
Time in days after inoculation

Figure 5. Effect of site of inoculation on disease index. IP and VF cultivars of tomato were inoculated with Fusarium \(5 \times 10^5\) conidia/ml. Inoculation sites were at transverse cuts (A) 2 cm below the junction of root and hypocotyl, (B) junction of root and hypocotyl, (C) cotyledonary node.
Figure 6. Effect of site of inoculation on fungal invasion. Cultivar IP inoculated with Fusarium \(5 \times 10^5\) conidia/ml). Inoculations were at transverse cuts (A) 2 cm below the junction of root and hypocotyl, (B) junction of root and hypocotyl, (C) cotyledonary node. Standard of error of mean \(\pm 5\%\).
Figure 7. Effect of site of inoculation on fungal invasion. Cultivar VF inoculated with Fusarium (5 x 10^5 conidia/ml): Inoculation sites were at transverse cuts (A) 2 cm below the junction of root and hypocotyl, (B) junction of root and hypocotyl, (C) cotyledonary node. Standard error of mean ± 8%.
Table 1. Effect of inoculation site on amount of Fusarium from homogenized samples of stem 10 days after inoculation.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Average number of Fusarium colonies/g fresh wt of stem</th>
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<tr>
<td></td>
<td>SITE OF INOCULATION&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>IP</td>
<td>$3.3 \times 10^4$ (± 11%)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>VF</td>
<td>$3.0 \times 10^4$ (± 16%)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Three sites of inoculation: A = inoculated 2 cm below the junction of root and hypocotyl; B = inoculated at the junction of root and hypocotyl; C = inoculated at cotyledonary node.

<sup>b</sup> Standard error of mean expressed as a percentage of the mean.

EFFECT OF CHAETOMIUM ON TOMATO:

Ascospore transport determined microscopically:— The rate of ascospore transport in the two cultivars was 5.8 cm per four hr period. Ascospore transport distance was determined for cuttings held in a sterile distilled water pretreatment for 45 min prior to the addition of ascospore suspensions. The transport distance observed in plants inoculated immediately after cutting was compared with the transport distance observed in the water pretreated plants. The experiment, repeated three times with 10 plants of each cultivar, indicated that only a slight decrease in the rate of ascospore transport occurred in the plants pretreated in water before inoculation. Thus, cutting the main axis does not seem to induce a delayed shock that could reduce the transpirational pull and thereby reduce the rate of ascospore transport.
The ascospore assay appears to afford a better index of the sites where ascospores are retarded rather than their true transport distance. A transverse section of stem of cultivar VF shows an accumulation of ascospores at a vessel end wall (Fig. 8). The ascospores appeared to gradually leak past this barrier and were rapidly transported to the next end wall. As additional ascospores accumulated, a blockage occurred and fewer ascospores passed through the barrier. This mechanism appears to account for the progressive decrease in ascospore transport.

**Ascospore transport distance determined by tissue plating:**— Ascospore transport distance using a tissue plating revealed that ascospore transport distribution was systemic. The average transport distance for both cultivars, as determined by tissue plating of 30-day-old plants, inoculated for four hours was 7.0 cm. The apparent increase in the rate of transport is not surprising when the greater sensitivity of the tissue plating is considered. Tissue plating data agreed with the data of the previous experiment in that the transport distances were the same for the two cultivars.

**Distribution of Chaetomium, Isolate A, in Previously Inoculated Tomato Cuttings.**

No macroscopic symptoms were observed on the plants of either cultivar previously inoculated with *Chaetomium*. The plants appeared to be as healthy and vigorous as controls treated with water only. A slight yellowing of leaves was observed within the first week after transplanting. Vascular browning was observed 1 cm above the cut ends of cuttings before transplanting. The results of this experiment are detailed in Table 2. It was demonstrated in previous experiments that this
Figure 8. Transverse section of living tomato stem showing accumulation of ascospores at a xylem vessel end wall.
Table 2. Distribution of Chaetomium, isolate A, in previously inoculated tomato cuttings.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Position on Plant</th>
<th>Days after Inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>IP</td>
<td>New stem</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Top</td>
<td>2/10</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>4/10</td>
</tr>
<tr>
<td></td>
<td>Base</td>
<td>9/10</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>2/10</td>
</tr>
<tr>
<td>VF</td>
<td>New stem</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Top</td>
<td>2/10</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>5/10</td>
</tr>
<tr>
<td></td>
<td>Base</td>
<td>8/10</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>3/10</td>
</tr>
</tbody>
</table>

Numerator: Number of segments positive for Chaetomium.
Denominator: Total number of segments.

inoculation method systemically introduced ascospores into the tomato plant, as evidenced from isolations 4 hr after inoculation. It appeared that Chaetomium rapidly spread throughout the plant and could be isolated from all parts after seven days. Both cultivars had a similar distribution of Chaetomium as an internal resident in all the plant parts. The fungus became well established in roots and in the base of the stem. The fungus could be isolated from leaf petioles as well.
Figure 9. Isolates A (above) and B (below) of Chaetomium growing from sections of tomato stems plated on PDA.
INDUCTION OF RESISTANCE IN TWO FUSARIUM WILT SUSCEPTIBLE ISOLINES BY CHAETOMIUM UNDER GREENHOUSE CONDITIONS.

An attempt was made to induce resistance in cultivars IP and VF to Fusarium wilt using two isolates (A and B) of Chaetomium (inducer). The design was a split plot 2 x 2 x 4 with four replications. There were 10 plants in each replication. The four treatments were as follows: (i) inducer (Chaetomium) alone, (ii) inducer followed 48 hr later by a challenged inoculation of Fusarium, (iii) sterile double-distilled water treatment (non-induced), (iv) sterile double-distilled water followed 48 hr later by a challenge inoculation of Fusarium (non-induced but challenged inoculation). Disease indices were recorded starting after transplanting and were taken at five day intervals for 30 days. The degree of resistance induced was calculated according to the formula stated earlier.

The average disease index of four replications was determined for each treatment (Table 3, Figs. 10, 11, 12). The plants inoculated with the two isolates of Chaetomium (non pathogen) remained healthy and vigorous comparable to the control plants which were treated with sterile double-distilled water only.

In cultivar IP inoculation with Chaetomium prior to challenge inoculation of Fusarium significantly reduced the disease index for all intervals at which data were taken (Fig. 10). Both isolates of Chaetomium were effective in reducing wilt symptoms as measured by disease index, however, isolate A was more effective than B (Fig. 10). By the end of the experiment the disease index of those plants induced by isolate A of Chaetomium and then challenged with Fusarium was 50%.
Table 3. Effect of *Chaetomium*, isolates A and B, in inducing resistance to *Fusarium* wilt in tomato.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Treatment</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>IP</td>
<td>Induced (isolate A) and challenged</td>
<td>0.4</td>
<td>0.57</td>
<td>1.25</td>
<td>1.55</td>
<td>1.90</td>
<td>2.25</td>
</tr>
<tr>
<td></td>
<td>Induced (isolate B) and challenged</td>
<td>0.5</td>
<td>0.8</td>
<td>1.30</td>
<td>1.75</td>
<td>2.25</td>
<td>2.65</td>
</tr>
<tr>
<td></td>
<td>Non Induced but challenged</td>
<td>1.05</td>
<td>1.90</td>
<td>2.75</td>
<td>3.2</td>
<td>3.75</td>
<td>4.25</td>
</tr>
<tr>
<td>VF</td>
<td>Induced (isolate A) and challenged</td>
<td>0.28</td>
<td>0.5</td>
<td>1.10</td>
<td>1.30</td>
<td>1.85</td>
<td>2.10</td>
</tr>
<tr>
<td></td>
<td>Induced (isolate B) and challenged</td>
<td>0.47</td>
<td>0.75</td>
<td>1.21</td>
<td>1.58</td>
<td>2.16</td>
<td>2.25</td>
</tr>
<tr>
<td></td>
<td>Non Induced but challenged</td>
<td>0.95</td>
<td>1.80</td>
<td>2.80</td>
<td>3.05</td>
<td>3.45</td>
<td>4.05</td>
</tr>
</tbody>
</table>

* a The values are average of 4 replicates, each replicate consisted of 10 plants

less than in the plants not induced but challenged. The plants induced with *Chaetomium* prior to the challenge inoculation of *Fusarium* continued to maintain resistance after 30 days.

Isolated A and B of *Chaetomium* (inducer) were also effective in reducing the disease index in cultivar VF (Fig. 11). Again isolate A of *Chaetomium* was slightly more effective than isolate B in reducing the wilt symptoms.
Figure 10. Induced resistance to Fusarium wilt in cultivar IP by Chaetomium: (1) noninduced (control) but challenged, (2) induced (Chaetomium, isolate B) and challenged, (3) induced (Chaetomium, isolate A) and challenged.
Figure 11. Induced resistance to Fusarium wilt in cultivar VF by Chaetomium: (1) non induced (control) but challenged, (2) induced (Chaetomium, isolate B) and challenged, (3) induced (Chaetomium, isolate A) and challenged.
Figure 12. Degree of resistance to Fusarium wilt induced in cultivars IP and VF by isolates A and B of Chaetomium.
Figure 13. Resistance (protection) in tomato to race 2 of Fusarium wilt, induced by Chaetomium, isolate A: (1) IF, induced and challenged (protected), (2) IP, non induced but challenged, (3) VF, induced and challenged, (4) VF, non induced but challenged.
The degree of resistance induced for both cultivars was calculated from the data in Table 2 according to the formula stated earlier. The degree of resistance induced by Chaetomium isolate A was 70 and 72%, respectively, for IP and VF after 10 days of challenge inoculation (Fig. 12). This induced resistance decreased in both cultivars with time after challenge inoculation. The cultivars were not significantly different from each other with respect to degree of resistance induced by Chaetomium (isolate A and B). However, isolate A of Chaetomium was different from isolate B in inducing the resistance in both cultivars to Fusarium at all three intervals after challenge inoculation.

**INDUCTION OF RESISTANCE IN FUSARIUM SUSCEPTIBLE TOMATO ISOLINES UNDER CONTROLLED ENVIRONMENTAL CONDITIONS.**

Since the temperature in the greenhouse was variable, the induction of resistance was studied under controlled conditions in a growth room. Isolate A of Chaetomium was used as the inducer. The design of the experiment was a randomized block with four replications. Each replication consisted of five plants. The treatments were as follows: (i) inducer (Chaetomium) alone, (ii) inducer followed 48 hr later by challenged inoculation of Fusarium, (iii) treatment of sterile double-distilled water (non induced), (iv) sterile double-distilled water treatment followed 48 hr later by challenged inoculation of Fusarium (non induced but challenged inoculation). Disease indices were recorded for 30 days at five day intervals after transplanting. The degree of resistance induced was calculated as previously described.

The average disease index of four replications was calculated for each treatment (Fig. 14). Plants of both cultivars inoculated with
Chaetomium and treated with sterile double-distilled water remained healthy and vigorous and no macroscopic symptoms were observed.

Chaetomium, isolate A (inducer) was significantly effective in reducing Fusarium wilt symptoms in both cultivars as compared to the non-induced but challenged plants (Fig. 14). The reduction of Fusarium wilt symptoms by this inducer was observed at each consecutive five day interval following inoculation with Fusarium (challenger). Even 30 days after the challenge inoculation, the inducer was still effective and reduced the disease index by approximately 50%. In both cultivars induced and challenged plants continue to retain their resistance to wilt after 30 days. In non-induced but challenged plants of both cultivars the disease indices continued to increase at a faster rate. Both cultivars were similar with respect to induced resistance by Chaetomium, isolate A.

The degree of resistance induced in both cultivars (Fig. 15) was higher at early stages of disease development. However, as the time after treatment increased, the degree of resistance induced tended to decrease. Both cultivars were similar with respect to the degree of resistance induced. The degree of resistance induced by Chaetomium, isolate A after 10 days was 72 and 68%, respectively, for VF and IP cultivars and 49 and 47% respectively after 30 days.

Effect of killed ascospores of Chaetomium, isolate A, on development of Fusarium wilt in tomato cultivars IP and VF.

To study the possible effects of killed ascospores of Chaetomium in retarding Fusarium wilt symptoms, an experiment was designed in a
Figure 14. Induced resistance to Fusarium wilt under controlled environment in cultivars IP and VF by Chaetomium, isolate A: (1) IP, non induced (control) but challenged, (2) IP, induced (Chaetomium, isolate A) and challenged, (3) VF, non induced (control) and challenged, (4) VF, induced (Chaetomium, isolate A) and challenged.
Figure 15. Degree of resistance to Fusarium wilt under controlled environment induced in cultivars IP and VF by Chaetomium, isolate A.
Table 4. Effect of Chaetomium isolate A in inducing resistance to Fusarium wilt pathogen in tomato under controlled conditions.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>IP</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Challenged</td>
<td>1.0</td>
<td>1.85</td>
<td>2.60</td>
<td>3.15</td>
<td>3.50</td>
<td>4.18</td>
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<td>2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Induced and challenged</td>
<td>0.38</td>
<td>0.58</td>
<td>1.20</td>
<td>1.45</td>
<td>1.86</td>
<td>2.20</td>
</tr>
<tr>
<td>VF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Challenged</td>
<td>0.90</td>
<td>1.78</td>
<td>2.55</td>
<td>3.05</td>
<td>3.37</td>
<td>4.03</td>
</tr>
<tr>
<td>2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Induced and challenged</td>
<td>0.26</td>
<td>0.50</td>
<td>1.05</td>
<td>1.25</td>
<td>1.78</td>
<td>2.04</td>
</tr>
</tbody>
</table>

a The values of four replicates, each replicate consisted of 10 plants.
randomized block with four replications. Each replication consisted of 10 plants. The treatments were as follows: (i) killed ascospores of Chaetomium, (ii) killed ascospores of Chaetomium followed by challenge inoculation (Fusarium) 48 hr later, (iii) sterile double-distilled water, (iv) sterile double-distilled water followed by challenge inoculation (Fusarium) 48 hr later. The disease indices were recorded at six intervals up to 30 days.

The average disease indices of four replicates are presented in Figure 16. Plants of treatments (i) and (iii) were healthy and vigorous. No organism was isolated from these plants and they were free from disease symptoms. The killed ascospores of Chaetomium were ineffective in retarding the symptoms of Fusarium wilt in both cultivars (Fig. 16). Plants treated with killed ascospores and challenged (Fusarium) developed wilt symptoms comparable to those that were challenged only.

**Fusarium wilt development in IP and VF cultivars inoculated with a mixture of Fusarium and Chaetomium, isolate A.**

Mixed inocula of Fusarium and Chaetomium were studied as to the induction of protection to Fusarium wilt in cultivars IP and VF. The experiment was under greenhouse conditions and was designed in a randomized block with four replications. Each replication consisted of five plants. The treatments were as follows: (i) inoculation with a mixture of Fusarium and Chaetomium ($10^6$ spores/ml, 1:1 ratio), (ii) sterile double distilled water (non induced), (iii) challenge inoculation with Fusarium ($5 \times 10^5$ spores/ml). The disease index was recorded at three different intervals up to 30 days after inoculation.
Figure 16. Effect of killed ascospores of Chaetomium, isolate A, on development of Fusarium wilt in cultivars IP and VF: (1) IP, challenged by Fusarium, (2) IP, induced (killed ascospores of Chaetomium, isolate A) and challenged, (3) VF, challenged by Fusarium, (4) VF, induced (killed ascospores of Chaetomium, isolate A) and challenged.
Figure 17. Development of Fusarium wilt in cultivars IP and VF inoculated with mixed inocula of Fusarium and Chaetomium, isolate A: (1) IP, Fusarium, (2) IP, mixed inocula of Fusarium and Chaetomium, (3) VF, Fusarium, (4) VF, mixed inocula of Fusarium and Chaetomium.
The average disease index of four replicates was determined for each treatment (Fig. 17). Non-induced plants of both cultivars remained healthy. In both cultivars the mixture of inocula of Chaetomium isolate A and Fusarium did not induce any significant protection to Fusarium wilt. However, there was a slight decrease in the index over the non-induced but challenged treatments.

Quantitative estimation of Chaetomium, isolate A (inducer) and Fusarium (challenger) in induced resistant (protected plants of IP and VF).

The amount of Fusarium and Chaetomium, isolate A, was estimated in induced resistant (protected) plants by a plating technique. Four plants of each cultivar, which grew in a controlled environment, were used at intervals for a quantitative estimation of culturable mycelium of both organisms. The results are expressed as the average number of colonies/g of fresh stem. Controls consisted of challenged (non-protected) plants of each cultivar.

The average number of colonies of four replicates was determined for each treatment (Fig. 18, 19). The amount of Chaetomium increased within the first three days and continued to increase for 15 days after the challenge inoculation of IP. The number of Chaetomium colonies in the protected plants became constant after 15 days. According to the data, the amount of Fusarium (challenger) in protected plants increased at a steady rate after six days and continued to increase for 21 days. After the 24th day the number of Fusarium colonies in protected plants decreased. It should be emphasized that the amount of Chaetomium was greater than that of Fusarium in the stems for 15 days. Later, Fusarium
Figure 18. Quantitative estimation of Chaetomium, isolate A (inducer) and Fusarium (challenger) in induced resistant (protected) plants of IF: (1) Fusarium in non-induced (control) but challenged plants, (2) Fusarium in induced resistant (protected) plants, (3) Chaetomium, isolate A, in induced resistant (protected) plants. Standard error of mean, ± 20%.
Figure 19. Quantitative estimation of Chaetomium, isolate A (inducer) and Fusarium (challenger) in induced resistant (protected) plants of VF: (1) Fusarium in non-induced (control) but challenged plants, (2) Fusarium in induced resistant (protected) plants, (3) Chaetomium, isolate A, in induced resistant (protected) plants. Standard error of mean, ± 20%.
was more abundant than \textit{Chaetomium}. The amount of Fusarium in non-protected plants was significantly higher than in protected plants of IP.

According to data in Fig. 19, amount of \textit{Chaetomium} in protected plants of VF increased rapidly until the 12th day and then more slowly until it reached the maximum at the 18th day. The quantity of Fusarium in protected plants of VF remained significantly less than \textit{Chaetomium} for 12 days. After 12 days the amount of Fusarium increased at a more rapid rate. The quantity of Fusarium in non-protected plants of VF was significantly higher than in the protected plants.

\textbf{Distribution of Chaetomium, isolate A (inducer) and Fusarium (challenger) in induced resistant (protected) plants of IP and VF.}

To determine the pattern of fungal spread in induced and challenged (protected) plants under greenhouse conditions, isolations were made from plants previously induced (Chaetomium, isolate A, $5 \times 10^7$ ascospores/ml) and challenged (Fusarium $5 \times 10^5$ conidia/ml) 24 hr later, according to the procedure described earlier. Non-induced (water) and challenged plants of each cultivar served as controls. Five plants of each cultivar were brought to the laboratory and processed for isolations. Sections from predetermined parts of the plant were plated. Petri plates were maintained at room temperature for a week.

Isolations made from induced resistant (protected) plants of IP and VF after 10 days of challenge inoculation revealed that Fusarium progressed through the hypocotyls, as evidenced from the maximum number of sections yielding Fusarium (Table 5). Chaetomium could be isolated from all parts of the plant, including petioles. Fusarium could not be isolated from the top or from petioles. Fusarium grew from a few
<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Position on Plant</th>
<th>Days after Challenge Inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protected</td>
<td>Non-protected</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>C</td>
</tr>
<tr>
<td>IP</td>
<td>Petoles</td>
<td>2/10</td>
</tr>
<tr>
<td></td>
<td>Top</td>
<td>2/10</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>2/10</td>
</tr>
<tr>
<td></td>
<td>Hypocotyl</td>
<td>8/10</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>2/10</td>
</tr>
<tr>
<td>VF</td>
<td>Petoles</td>
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</tr>
<tr>
<td></td>
<td>Middle</td>
<td>3/10</td>
</tr>
<tr>
<td></td>
<td>Hypocotyl</td>
<td>7/10</td>
</tr>
</tbody>
</table>

F = number of sections showing Fusarium
C = number of sections showing Chaetomium
Numerator = number of sections positive for either organism
Denominator = total number of sections
sections of roots and the middle portions of some protected plants. It was also evident from the data that tissues from non-induced but challenged (non-protected) plants of both cultivars yielded much more Fusarium than those from tissues of protected plants during the same period of time.

Twenty days after challenge inoculation in both cultivars, Fusarium could be isolated from the middle portion of plants more frequently than Chaetomium. However, Chaetomium was still prevalent in the tops, petioles and root sections. At this stage of disease development, Fusarium in non-protected plants was significantly more abundant than in protected plants. It is clear from the data obtained from protected plants that Chaetomium effectively prevented the colonization of Fusarium in the early stages of disease development. Chaetomium could consistently be isolated from petioles and roots of the plant.

Quantitative changes in the total phenol content of stems and leaves of tomato cultivars VF inoculated with Fusarium and Chaetomium, isolate A.

Thirty-five-day old plants were prepared for inoculation as described earlier. The cuttings were inoculated with either Fusarium or Chaetomium by immersing the cut ends in inoculum for a period of four hours. The inoculum used was a suspension of $5 \times 10^5$ and $5 \times 10^7$ spores/ml of Fusarium and Chaetomium, respectively. Cuttings used as checks were prepared at the same time and placed in sterile distilled water instead of spore suspensions. After inoculation, the plants were potted in vermiculite, and Hoagland's solution plus micronutrients were
included as a part of the watering regime. All plants were placed in an environmental chamber with 12 hr of light (26 C) and alternating with 12 hr of darkness (23 C). Each treatment consisted of 15 plants divided into three groups of five plants. The groups were assayed separately. Stems and leaves from VF plants which had been previously inoculated with Fusarium or Chaetomium, isolate A, and uninoculated control plants were extracted and analyzed for their total phenol content at six different periods after inoculation (Table 6, Fig. 20).

VF plants inoculated with the non pathogen (Chaetomium) had more phenol content in stems and leaves than in those plants inoculated with the pathogen (Fusarium). The trend of variation with time of the phenol content was followed up to 19 days. At first, a much higher concentration of phenols was found in the plants inoculated with the Chaetomium, isolate A. Thereafter, the phenol content tended to decrease and to approximate that of controls after 8 and 19 days in leaves and stem, respectively (Fig. 20). While in the plants inoculated with Fusarium, the phenol content continued to increase up to 16 and 19 days in stems and leaves, respectively.

The phenol concentration of leaves was always higher than the stem in both treatments (Table 6). The percent increase over the control (Fig. 20) indicated that the trend of increase of phenol content was greater in stems than in the leaves.
Table 6. Variation of total phenol content (expressed as mg chlorogenic acid equivalents per g dry tissue) in leaves and stems of VF tomato cuttings inoculated with Fusarium only, with Chaetomium, isolate A, only, and in non-inoculated control. The data represent an average of three replications, each composed of five plants.

<table>
<thead>
<tr>
<th>Inoculated with</th>
<th>Plant part</th>
<th>3</th>
<th>5</th>
<th>8</th>
<th>12</th>
<th>16</th>
<th>19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fusarium</td>
<td>Stem</td>
<td>2.16</td>
<td>3.63</td>
<td>3.23</td>
<td>4.89</td>
<td>5.17</td>
<td>5.16</td>
</tr>
<tr>
<td></td>
<td>Leaf</td>
<td>4.35</td>
<td>5.82</td>
<td>6.39</td>
<td>7.13</td>
<td>8.31</td>
<td>12.30</td>
</tr>
<tr>
<td>Chaetomium</td>
<td>Stem</td>
<td>5.86</td>
<td>6.68</td>
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<td>2.96</td>
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<tr>
<td></td>
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<td>3.78</td>
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<td>3.75</td>
<td>3.71</td>
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<td>4.12</td>
<td>3.92</td>
<td>5.21</td>
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Figure 20. Quantitative changes in total phenol content in stems and leaves of cultivar VF induced by Fusarium and Chaetomium, isolate A: (1) induced by Fusarium in stems, (2) Fusarium in leaves, (3) induced by Chaetomium, isolate A, in stems, (4) Chaetomium, isolate A, in leaves.
DISCUSSION

Fusarium Wilt of Susceptible Tomato Isolines: Host Colonization

The objectives enumerated in the introduction can now be considered in the light of results obtained. Studies on effect of inoculum concentration on the development of Fusarium wilt in two susceptible tomato cultivars indicate that these two cultivars are affected similarly when measured by the percentage of killed and stunted plants, the extent of vascular discoloration and percentage of vascular bundles invaded. Increased levels of inoculum resulted in an increase in severity of the wilt symptoms.

The data indicate that the extent of colonization of susceptible tomato cultivars by the pathogen depends on the site of inoculation. It was demonstrated that the disease index is much lower in both cultivars inoculated at the cotyledonary node than at either of the other inoculation sites (Fig. 5). Percentage of vascular bundles invaded was determined in plants of both cultivars, which were inoculated at the cotyledonary node and other two inoculation sites (Figs. 6 and 7). Percentage of vascular bundles invaded was much lower in plants inoculated at the cotyledonary node than at either of the other sites. This is in agreement with the disease indices obtained from the plants inoculated at these three sites. The amount of culturable mycelium in plants of both cultivars inoculated at the three different sites was determined by homogenization, plating, and colony count (Table 1). Low
numbers of *Fusarium* colonies/g fresh weight of stem were culturable from plants inoculated at the cotyledonary node as compared to the plants which were inoculated at the other two sites. However, the homogenization and the colony count technique resulted in some variability in the amount of culturable mycelium within plants of a cultivar. Although both cultivars are susceptible to *Fusarium* wilt, the greater susceptibility when inoculations are made below the cotyledonary node, suggests two morphological zones, tissue above the cotyledonary node and tissue below the cotyledonary node. This should be considered in comparative studies of susceptible tomato cultivars to *Fusarium* wilt.

When plants are inoculated with *Fusarium* through a transverse cut in the plant axis, conidia become distributed throughout the primary xylem. This, along with the estimates of the degree of colonization, suggests that the conidia transported to the upper portions of the axis, although perhaps not lysed, do not germinate or grow as well as they do in the lower portions of the axis. It is proposed that some factor(s) is responsible for retarding the germination of spores or growth of mycelium in the axis tissue above the cotyledonary node. If the factor(s) is a naturally occurring compound whose effectiveness is determined by concentration, then it should be found in the highest effective concentration in the upper portion of the plant and at lower concentration in the lower portion of the plant axis. Further research on this problem could be very productive.

**Effect of Chaetomium on Tomato as an Internal Resident**

The distance of transport of *Chaetomium* ascospores was determined in both cultivars microscopically and by tissue plating. The rate of
 ascospore transport in the two cultivars was similar following a four hour inoculation period. Distribution of Chaetomium was also determined in previously inoculated cuttings of both cultivars (Table 2). Isolations made from such plants, at one week intervals for 30 days, demonstrated presence of Chaetomium in most of the plant parts, suggesting systemic distribution following this method of inoculation.

The concentration and distribution of spores within the plant are important factors in pathogenesis. In this regard, both the tissue plating method and observation of stem sections microscopically have useful applications. Tissue plating shows the distance ascospores are transported from the point of inoculation. This method, however, does not show whether spore distribution is uniform or discontinuous within the vascular system. Location of ascospores in the vascular tissue can be determined microscopically but this does not provide an accurate measurement of the maximum transport distance.

Ascospores were probably transported through a vessel at a uniform rate until they reached an end wall. Passage through these pores was slower, hence a build-up of ascospores occurred at these sites. Ascospores that pass through the pores were transported to the next end wall where lodging again occurred. This process probably continued throughout much of the stem. Regardless of the amount of lodging, some ascospores do pass through the pores in the end walls and continue up the stem in the transpiration stream. At some point along the way the ascospores are too few in number to permit detection by the sectioning technique used, but the tissue plating method of detection demonstrated that ascospores were transported even farther in the stem.
It seems, therefore, that even during very brief inoculation periods nearly systemic distribution of ascospores could occur if the inoculated plants were transpiring. The limiting factors to systemic distribution of ascospores are probably the diameter of the vessels and of the pores in vessel end walls. It is highly improbable that tyloses sufficient to inhibit the systemic distribution of ascospores could be induced during this brief time.

In assessing the injurious factors of plant disease, there is always the tacit assumption that one is able to compare these factors with "normal" controls. Healthy-appearing plants, however, are associated in nature with multitudes of other organisms. While most of these organisms are thought as saprophytes, they could incite subtle or striking effects upon plant growth of significance to the study of plant pathology.

The distribution of Chaetomium (non pathogen) as an internal resident in tomato is one of the important factors in inducing resistance to Fusarium wilt. In this investigation the non pathogen established itself in the roots and base of the plants very well. No macroscopic symptoms were observed on these plants and they remained healthy and vigorous. Chaetomium is a soil inhabitant and has also been reported colonizing the roots of previously inoculated tomato plants in sterile soil (21). The fungus was also isolated from stem and other parts of old plants. Lindsey and Baker (26) studied the effect of Chaetomium on dwarf tomatoes grown under gnotobiotic conditions. They reported that plants infested with Chaetomium were taller than the germ-free plants. They suggested that single fungi growing in the rhizosphere have growth
regulative effects on tomatoes, resulting in either increased height or dry weight. In no instance was there any injurious effect incited by the fungus.

**Induction of Resistance in Tomato Cultivars Susceptible to Fusarium Wilt.**

The induction of resistance in tomato to Fusarium wilt by Chaetomium has been studied in both greenhouse and controlled environment experiments. A high level of resistance was induced in cultivars IP and VF to Fusarium wilt by Chaetomium (Figs. 11, 14 and Tables 3, 4). In previous studies, Verma and Allison (46) reported that an isolate of a Chaetomium was an internal resident of tomato plants and was highly effective in delaying and retarding Fusarium wilt symptoms of Bonny Best seedlings. Chaetomium, a non pathogen of tomato in the present research, was established as an internal resident in tomato plants when inoculated 48 hr prior to Fusarium inoculation. No visible host cell response to the non pathogen was observed in tomato plants after induced inoculation. The mycelia of the non pathogen presumably grew within a large number of host cells with no apparent damage to the host.

Chisler (10) demonstrated that as the time interval between Cephalosporium treatment and Fusarium inoculation increased, the amount of vascular discoloration decreased, resulting in less Fusarium wilt symptoms in tomato. When seedlings were inoculated with Fusarium immediately after Cephalosporium treatment, no reduction in vascular discoloration occurred. The same was true for Chaetomium in the reduction of Fusarium wilt symptoms in tomato in the present research.
When tomato cuttings were inoculated with a mixed suspension of *Chaetomium* and *Fusarium*, no mitigation of wilt symptoms was observed. When killed ascospores of *Chaetomium* were used, resistance to Fusarium wilt was not induced. These results seem to confirm that induced resistance to Fusarium wilt is not simply due to antagonistic metabolites produced by *Chaetomium*.

In this study a significant degree of resistance in tomato to Fusarium wilt was induced by inoculation with *Chaetomium* (Figs. 12 and 15). Since there have been several reports of induced resistance (modification of susceptibility) to Fusarium wilt of tomato, cotton and cowpea by using non pathogens (27, 42, 44), it is evident that the induced resistance described here is probably not a phenomenon peculiar to *Chaetomium*.

The induction of resistance in susceptible tomato cultivars suggests alteration of host tissue that protects the induced tissue against *Fusarium*. The potential for a resistance response exists in both IP and VF; if induced, the plants are protected. The potential for inducing such a response is possessed by both the A and B isolates of *Chaetomium*. Both non pathogen and host have an active role in determining the outcome of the relationship.

What is the nature of the alterations of host tissue which constitute the induced resistance to Fusarium wilt? In this study, the amount of culturable mycelium of *Fusarium* was reduced drastically in induced resistant (protected) host plants. The quantity of culturable mycelium of *Chaetomium* exceeded that of *Fusarium* in such plants for 15 days in IP and for 18 days in VF. Fusarium wilt symptom development
has been correlated with the quantity of *Fusarium* in protected plants (39). The decrease in symptom development in plants induced with *Chaetomium* prior to challenge inoculation of *Fusarium* can be attributed partly to the decreased amount of *Fusarium* in these plants.

*Cephalosporium* has been isolated from the upper parts of Bonny Best tomato plants with induced resistance to Fusarium wilt (10, 41, 44), but not in all cases. In this study, however, isolations from protected plants of IP and VF demonstrated the presence of the inducer *Chaetomium* in most of the plant parts. In addition to the upper plant parts including petioles, *Chaetomium* also could be isolated from the roots. The ability of *Chaetomium* to colonize tomato cultivars as an internal resident is an important factor in inducing resistance to Fusarium wilt. Although there does not appear to be any significant antagonism between *Fusarium* and *Chaetomium* in vitro, it is possible that there could be such in vivo.

Induced resistance in certain host plants has been reported following inoculation of the host with forms or races of *F. oxysporum* that are non-pathogenic to that host (16, 28, 30). This induced resistance has been associated in some cases with increased phenols in the host immediately following invasion. This increase has been greater than when the host plants were inoculated with a pathogenic form of the species of *Fusarium* (31). Quantitative changes in the total phenol content of cultivar VF inoculated with *Fusarium* vs. induced with *Chaetomium* then inoculated, were compared to determine whether phenolics might be involved in the mechanism of induced resistance. The total phenols in the crude extract, according to the quantitative analysis,
increased with time in both *Fusarium* inoculated and healthy control plants (Table 6). However, total phenols in the crude extract of *Chaetomium* inoculated plants increased in stem and leaf up to five and eight days after inoculation, respectively, when a maximum concentration was reached, and then decreased. There was a stronger increase of phenol content in stem and leaf of *Chaetomium* induced plants than in *Fusarium* inoculated plants up to the eighth day (Fig. 20).

The high level of resistance induced by *Chaetomium* to *Fusarium* wilt in VF plants in the first 10 days appears to be related to phenol production. The protective effect of *Chaetomium* reached a maximum at 10 days in VF, then tended to decrease slightly. The correlation suggests that inoculation with the non pathogen, *Chaetomium*, may have stimulated the host to produce greater amounts of phenolic substances, which could be responsible for resistance. The increase of phenolic substances induced by *Chaetomium* might act by inhibiting the *Fusarium* pathogen, directly or by means of oxidation products of phenol and/or by inciting more complex metabolic changes such as accumulation of growth substances (29) leading to formation of defense barriers in the xylem vessels. *Cephalosporium* has been reported to cause tyloses obstruction in xylem vessels of tomato (39) which reduced entry or prevented extensive spread of *Fusarium* in the plant, thereby reducing the symptoms of wilt.

Finally, it must be emphasized that the study of induced resistance, as well as the mechanism of induced resistance to *Fusarium* wilt, can be a useful tool in the study of the disease process, host resistance, and pathogen variability. Also, the phenomenon of induced resistance should be investigated further as a possible means of disease control.
SUMMARY

The effect of inoculum concentration and the site of inoculation on Fusarium wilt development with respect to killed and stunted plants, vascular discoloration, percentage of vascular bundles invaded, cultivable mycelium and disease index was investigated in two susceptible host isolines, IP and VF. Both cultivars were similar in susceptibility and showed progressive sensitivity as the inoculum concentration increased.

Plants of both cultivars were inoculated with *Fusarium* at three different sites (2 cm below the junction of hypocotyl and root, at the junction of root and hypocotyl, and at the cotyledonary node) and lower disease indices were recorded in plants of both IP and VF when inoculated at the cotyledonary node than either of the two inoculation sites, even though there was nearly systemic distribution of fungus conidia through the vascular system of the stem. The host plants of both cultivars inoculated at cotyledonary node also yielded low percentage of invaded vascular bundles and a lower amount of cultivable mycelium in the stem. Maximum levels of disease, percentage of invaded vascular bundles and cultivable mycelium in stem were recorded in plants of both cultivars inoculated at the junction of root and hypocotyl. It is concluded that two morphologically distinct zones, tissue above the cotyledonary node and tissue below the cotyledonary node, should be considered separately in physiological and induced resistance studies of Fusarium wilt of tomato.
The effect of Chaetomium as an internal resident in the tomato cultivars was studied by measuring ascospore transport distance and distribution in stems. Ascospores were introduced directly into the shoot vascular system through a transverse cut at the junction of root and hypocotyl. The rate of ascospore transport was similar, and the average transport distance determined for both cultivars at the end of a four hour inoculation period was 5.8 cm. A similar but more sensitive experiment, using tissue plating to measure transport distance, indicated that some ascospores were transported up to 7 cm. Isolations made from Chaetomium inoculated tomato plants at one week intervals for 30 days indicated that the fungus was present in all the plant parts. This suggested that Chaetomium became an internal resident of these two tomato cultivars without exhibiting any macroscopic disease symptoms on tomato and the plants remained healthy and vigorous.

Resistance to race 2 of Fusarium oxysporum f. sp. lycopersici was induced in two susceptible tomato cultivars, IP and VF by infiltration of vascular system of stem with a suspension of ascospores of Chaetomium, a non pathogen of tomato. A high level of resistance was induced in IP and VF in both greenhouse and controlled environment studies. The degree of resistance to Fusarium wilt induced in both cultivars by Chaetomium was approximately 70% after 10 days of challenge inoculation which, however, was reduced to 50% after 30 days. A period of 48 hr was required for Chaetomium (inducer) to become established in stem of tomato to achieve maximum degree of induced resistance to Fusarium wilt. Resistance to Fusarium wilt in tomato was not induced by killed ascospores of Chaetomium.
The amount of culturable mycelium of *Fusarium*, determined by homogenization and colony count procedure in induced resistant (protected) plants, was drastically reduced as compared to non-induced but challenged (non-protected) plants of IP and VF. Isolations from protected plants of IP and VF indicated the presence of *Chaetomium* (inducer) in all the plant parts.

The total phenol contents of stems and leaves from cultivar VF plants which had been inoculated with *Fusarium* or *Chaetomium*, isolate A, were extracted and analyzed at six different periods after inoculation. *Chaetomium*, isolate A, induced stronger increase of phenols in stems and leaves than in those plants inoculated with *Fusarium* during the first week after inoculation. Thereafter, the total phenol content in *Chaetomium* inoculated plants tended to decrease, whereas, in *Fusarium* inoculated plants they kept increasing during the time course of the experiment. This leads us to believe that the increase of phenols in tomato induced by *Chaetomium* may account partially for the resistance to *Fusarium* wilt. The phenolic substances may affect *Fusarium* or may induce metabolic changes, such as accumulation of growth substances leading to the formation of defense barriers in the xylem vessels against the pathogen.

This work has demonstrated that *Chaetomium* can be used as a tool for inducing resistance to *Fusarium* wilt of tomato. It is proposed that further research is needed to make use of this phenomenon for practical purposes.
LITERATURE CITED


