RESISTANCE OF TOMATO PLANTS TO VERTICILLIUM ALBO-ATRUM

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by

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ABSTRACT

Comparative studies have been made with tomato varieties resistant and susceptible to V. albo-atrum.

When inoculated through roots, both varieties became infected. In susceptible plants the amount of mycelium in the stem, and the damage to the leaves, increased rapidly for some time after inoculation. The increase became less with time, and finally stopped. In resistant plants, a limited invasion of the lower stem was accompanied by extensive tylosis. However, the fungus soon disappeared from the stem, and the plant recovered from the mild symptoms which developed initially. The growth of susceptible plants was markedly reduced by infection, but in resistant plants a slight to moderate stimulation was often obtained. The cuttings, and the detached leaves, when inoculated, responded in the same way as the root-inoculated plants. The fungus gradually disappeared from resistant cuttings. The age of a plant did not have much influence on its ultimate response to infection. Root damage at the time of inoculation invariably caused the symptoms to develop sooner, but a similar treatment a few days earlier caused significant reduction in the development of symptoms.

Indole-3-acetic acid, at low concentrations, stimulated the symptoms, but reduced them at higher concentrations. The different treatments designed to increase slightly, or reduce the level of auxins in inoculated susceptible plants stimulated and reduced the symptoms respectively. But synthetic auxins, applied at low to moderate concentrations before inoculation, always reduced disease. The anti-auxins did not always reduce the symptoms at higher concentrations in the way that cycocel, a growth inhibitor did. The effect of a growth regulating substance on disease development in susceptible plants could not be correlated, in most cases, with its effect on mycelial growth in vitro. But those substances which induced resistance in susceptible plants also usually inhibited their growth.

Tissue extracts, tracheal saps and diffusates from healthy resistant and susceptible plants did not show any differential effect on the growth of mycelium in vitro, or on spore germination. Treatments with glucose, casamino acids and ethanol, for a few days after inoculation, always induced increased symptoms in inoculated resistant cuttings compared with untreated ones. But these cuttings always recovered when the treatment was stopped. Similarly, no appreciable reduction in resistance

was obtained by treatment with various metabolic inhibitors. But some of the treatments enabled the fungus to survive longer in resistant cuttings than usual. From experiments using tissue segments, tracheal saps and plant-diffusates, evidence was obtained that some antifungal substance might have been produced in resistant tissue in response to infection. This was probably responsible for the resistant reaction.

CONTENTS

I. INTRODUCTION

Verticillium albo-atrum Reinke and Berthold is the cause of wilt in a wide variety of herbaceous and woody plants. Some of these diseases are of considerable economic importance. Tomato is one of the more important hosts of this fungus in the Great Britain. The wilt of tomato, commonly referred to as " Sleepy disease ", has been ascribed to two fungi, V. albo-atrum and Fusarium oxysporum f. lycopersici (Bewley, 1922), the former being more common in the Great Britain.

The problem on which this work is based is one of fundamental importance in plant pathology. Most of the fungal pathogens responsible for wilt diseases are limited in their host range. Even within a single host species, a pathogen may successfully parasitize one variety, but not another closely related one. The question naturally arises - what makes one plant susceptible and another resistant to the same pathogen ? In spite of a considerable amount of work done during the last two decades, the nature of resistance in higher plants to fungal attack is very little understood, except in a few host-parasite combinations. This is particularly true for tomato wilt caused by V. albo-atrum , in which very little is known regarding the basis of resistance. The work reported in this thesis has been based on a study of the disease reaction of two varieties of tomato, genetically related to each other, but showing marked difference in their response to the pathogen. One is highly susceptible to V. albo-atrum , but the other is highly resistant. It was thought that a comparative study with two such closely related varieties would give

a better understanding of the problem.

In this work, no attempt has been made to separate the problems of parasitism and resistance. These are parallel problems. It was thought, therefore, that any contribution to the problem of parasitism would also help to understand the mechanism of resistance.

The first part of this work is mainly concerned with a study of disease development in the two varieties of tomato. In this work, attempts were made to analyze in detail the different effects of the pathogen on two varieties, and to locate the site of resistance. The second part deals with attempts to modify the normal responses of the two varieties to the disease by treatment with various growth regulating substances. The last part of the work deals with studies on the mechanism of resistance. In this an attempt was made to determine if any antifungal substance was produced in resistant plants as a result of infection.

II THE DISEASE

An account of the disease, incorporating observations made in the course of this work, and by other workers is given below.

It is known that five species of Verticillium can induce disease symtpoms in tomato plants (Isaac, 1953). Of these, V. albo-atrum and V. dahliae are the more virulent pathogens, and V. albo-atrum is the species mostly responsible for Verticillium wilt of tomato.

Verticillium spp. are soil-borne pathogens. V. albo-atrum is largely restricted to the upper 24 inches of soil (Wilhelm, 1950a). Verticillium spp. do not normally survive in the soil for long periods in the absence of a suitable host. But on occasions they have been found to persist for at least 3-4 years (McKay, 1926; Zeller, 1936; Luck, 1953), and in one instance for more than 9 years (Wilhelm, 1955). Survival in soil depends on the continued viability of microsclerotia (Luck, 1953; Schreiber and Green, 1962). Evidences from various investigations suggest V. albo-atrum to be a root-inhabiting type of pathogen (Isaac, 1953), or a soil-invader (Wilhelm, 1951; Wilson and Porter, 1958; Van den Ende, 1958), but

not a soil-inhabiting type. In sterilized soil V. albo-atrum grows rapidly, but in non-sterile soil it rarely spreads by independent saprophytic growth (Wilhelm, 1951; Sewell, 1959) unless susceptible hosts are present. V. albo-atrum is a poor competitor in the soil, and does not survive there for a long time. This may be due to the presence, in the soil microflora, of many microorganisms that are strongly antagonistic to it (Wilson and Porter, 1958; Lockwood, 1959; Yin et al., 1955; Kerr, 1961). There are reports also of good control of wilt by addition to the soil of organic amendments that might stimulate the development of many antagonists at the expense of parasites, such as V. albo-atrum (Grassi, 1957; Yin et al., 1955; Tolmsoff and Young, 1959).

Wilhelm (1951) did not find V. albo-atrum in the rhizosphere of tomato or on its root surface which is known to carry many antagonistic organisms (Kerr, 1961). But according to Wilhelm (1951), tomato can cause a rapid increase of V. albo-atrum in the soil. This may depend on partial or complete neutralization of the fungistatic or mycolytic principles present in the soil by the root-exudate from a host plant (Schreiber and Green, 1963).

Verticillium wilt occurs under a wide variety of soil conditions, but it is often particularly severe in soils rich in organic matter (Bewley, 1922; Presley, 1950). Although the disease is generally recognized as one favoured by alkaline soils (Garrett, 1938) its occurrence and severity are not greatly affected by soil reaction within the range in which susceptible hosts are commonly grown (Wilhelm, 1950b).

Infection normally takes place through the root. Evidence from work with pure cultures indicate that V. albo-atrum can infect undamaged roots of tomato plants and subsequently reach the stele (Bewley, 1922; Derbyshire, 1956). Similar observations have also been made for other hosts, such as potato and cucumber (Van der Meer, 1925), Sainofin (Isaac, 1946) and pea (Dufrenoy, 1927). Van den Ende (1958) found that V. albo-atrum entered tomato plants through root hairs or directly through the cortex. Selman and Buckley (1959) studied invasion of seedling tomato roots in sterile water culture and in non-sterile soil. They found that undamaged roots were penetrated in both cases, but in water culture only when the level of sucrose was very high did the fungus reach the stele. Roots were

invaded not through the root hairs, but through the cells that produced them. The fungus spread through the cortex mainly intracellularly, then penetrated the endodermis and pericycle and eventually reached the stele. They obtained ample evidence to show that infection occurred much more readily through injured roots than through uninjured ones. Selman and Buckley also showed that a systemic invasion of the host plant was possible only when the roots were damaged.

The fungus, having reached xylem vessels, for the most part is confined to them until the host is moribund or dead. In severely infected plants, the fungus may be found in few tracheids and xylem parenchyma cells. It grows through the vessels in both directions, and often reaches the top of the plant. The hyphae spread laterally from one vessel to another through pits in the cell wall.

The syndrome, as observed under greenhouse conditions, is as follows. The earliest symptom is often an epinasty of lower petioles, but this does not always occur. Some 7-10 days after inoculation, yellow blotches appear on the lower leaves starting mostly at the tips and gradually extending downwards.

Later, other leaves are affected in the same way as the disease spreads upwards mostly in an acropetal sequence. Wilting normally follows yellowing of the leaflet, but in severe infections it may develop earlier and if the conditions are favourable, most of the leaves may wilt badly while they are still green. Initially, the leaves show transient wilting. But with time, wilting becomes permanent. Wilting is generally followed by desiccation. In less severely infected plants desiccation may follow yellowing with no intervening wilting phase. In severely infected plants, wilted leaves are usually shed before they become desiccated leaving a crown of small, dark green leaves at the top. The infected plants show various degrees of stunting ranging from slight to severe. Sometimes the symptoms appear initially on one side of the plant only, but they ultimately spread to the other side. Adventitious roots, in excess of those which appear on healthy plants, are initiated from the base of stem gradually spreading upwards, but they rarely grow further. Browning of xylem elements, mostly vessels, extending through the root and some distance up the stem, is another characteristic symptom of this disease.

Under greenhouse conditions infected plants

are rarely killed. If the plants are not severely infected they often recover partially during the summer, and continue to grow at the top. One or two axillary shoots may develop from the lower leaves.

Numerous observations show that the nutrition of host may have considerable effect on the development of disease. Increased incidence and/or severity of disease with increase in nitrogen supply has been reported for tomato (Roberts, 1943), Cotton (Presley, 1950; Ranney, 1962) and antirrhinum (Isaac, 1957b). Roberts reported also that a deficiency of potash tended to increase the incidence of disease. Presley (1950) found that an application of potash reduced Verticillium wilt of cotton. Isaac (1957a) found that wilt of lucerne was very severe in soils rich in superphosphate. Roberts (1944) showed that tomato plants became more resistant to Verticillium infection if the leaf-shoot ratio was reduced. This was thought to be due to a reduction in the carbohydrate content of the host. Selman and Buckley (1959) found a correlation between the level of sucrose in the medium and inoculum potential of V. albo-atrum.

The most important environmental factor influencing this disease appears to be temperature.

Bewley (1922) found that the disease developed well at temperatures from 15°-24°C., being most rapid at 21°-23°C., and at 25°C. and above it was progressively inhibited.

III SURVEY OF THE LITERATURE

In vascular diseases symptom expression is variable. However, the most prominent symptom is usually a wilting of the leaves which may affect the whole plant. Various views have been expressed from time to time to explain the mechanism of wilting in different hosts and the relevant literature has been reviewed a number of times (Kamal, 1954; Dimond,1955; Threlfall, 1957; Blackhurst, 1961; Sadasivan, 1961).

A brief summary of the various mechanisms suggested to be responsible for wilting and associated symptoms are given below. This will be followed by a review of the works so far done on the nature of resistance to wilt diseases.

Wilting of leaves is generally associated with an imbalance in water economy of the host leading to an increasing water deficit. Considerable disagreement exists, however, regarding the mechanism by which such a deficit is induced in an infected plant.

According to one view, toxic metabolites produced in the host as a result of infection, act directly on leaf cells altering their permeability and upsetting the normal course of metabolism (Gäumann, 1951; Subramanian and Saraswathi Devi, 1959). This

leads to excessive water loss from the leaves in which water retaining capacity has been seriously impaired.

Gottlieb (1943a) detected, in the tracheal sap from Fusarium infected tomato plants, a toxin which caused wilting when applied to cut shoots. This toxin was not characterized. Toxins have been found in the cell-free culture filtrates of many wilt pathogens and a role has been ascribed to them in the respective diseases. Culture filtrates from different species of Verticillium have been shown to produce symptoms in various hosts, such as tomato (Bewley, 1922; Green, 1954; Threlfall; 1957; Blackhurst, 1963), cotton (Kamal, 1954), hop (Talboys, 1957), and mint (Nelson, 1950). However, the fact that a pathogen produces a toxin in vitro may have no significance in explaining its pathogenicity. Dimond and Waggoner (1953a) distinguished between toxin produced in vitro and a "vivotoxin" which is produced in the diseased plant as a result of host-pathogen interaction and which functions in the production of symptoms. Of the many wilt toxins recorded from in vitro studies, only fusaric acid has so far been detected from diseased plants (Lakshminarayanan and Subramanian, 1955; Page, 1959). However, there are differences between the symptoms caused by infection and by

fusaric acid alone. It is also not known if the concentration of fusaric acid in diseased plant is sufficient to induce symptoms. Dimond and Waggoner (1953c) found more ethylene in Fusarium infected tomato plants than in healthy ones. Epinasty and initiation of adventitious roots are symptoms normally associated with plants infected with Fusarium and Verticillium. Similar symptoms were produced in healthy plants when they were treated with ethylene.

Water imbalance in a diseased plant may also result from reduced water supply to the leaves. A considerable decrease in transpiration rate per unit area of leaf surface has been noted for tomato plants infected with Fusarium oxysporum f. lycopersici (Scheffer and Walker, 1953) and V. albo-atrum (Scheffer et al., 1956; Threlfall, 1957). In both cases, the reduced rate of ranspiration was associated with an increase in resistance of stems to water flow. In many cases the correlation between wilting and reduced water movement in the stem was good (Melhus, et al. 1924; Harris, 1940; Dimond and Waggoner, 1953b; Beckman et al., 1953). A reduction in the movement of water through infected stem may be caused by occlusion of the vessels by mycelium, or by substances and structures produced by host-pathogen interaction, such as gels and

tyloses. In many plants infected with Fusarium, or Verticillium, mycelium has been found to be widely distributed in the vascular tissue, often causing complete occlusion of the vessels. But only in a few cases has the effect of mycelial plugging on the transport potential of the host been assessed (Ludwig, 1952; Waggoner & Dimond, 1954; Threlfall, 1957). There, it has been found that the presence of mycelium alone cannot account fully for the reduction in water flow in infected stem.

Tylosis and gummosis have been observed in Verticillium wilts of potato (Pethybridge, 1916; Van der Meer, 1926), hop (Talboys, 1958b), Fusarium wilts of banana (Wardlaw, 1930) and water melon (Wilson, 1936), and many other diseases. In diseases, such as Fusarium wilt of melon (Sleeth, 1933) oak wilt due to Ceratocystis fagacearum (Struckmeyer et al., 1954) and Dutch elm disease (Zentmeyer, 1942) tylosis was thought to be extensive enough to cause wilting.

Plugs of hyaline materials have been recorded for many vascular wilts, including Verticillium infected tomato (Bewley, 1922), and Fusarium infected tomato (Ludwig, 1952), and Pierson et al. (1955) Ludwig (1952) suggested that these plugs in Fusarium infected tomato plants were composed of pectic substances. According

to Gothoskar et al. (1953) wilting in Fusarium infected tomato plants was caused when vessels were plugged by pectic substances liberated from the host cell walls by the action of pectic enzymes secreted by the pathogen. Chambers and Corden (1963) re-examining this problem, found plugs only rarely in diseased stems, and did not think them to be responsible for wilting.

The existence of cell wall degrading enzymes in the culture filtrate has been shown for many wilt inducing pathogens, particularly Verticillium and Fusarium. Such enzymes have also been detected from stem tissues of plants infected with Verticillium and Fusarium. Leal and Villanueva (1962) working with forty strains of Verticillium spp. found that pectic enzymes were produced only by the virulent strains. Similarly Paquin and Coulombe (1962) showed that a virulent strain of Fusarium oxysporum f. lycopersici produced more pectic enzymes in vitro than did an avirulent strain. These observations and other evidence strongly suggest that cell wall degrading enzymes may be responsible for some of the symptoms in wilt diseases, particularly wilting (Gothoskar et al., 1953; Scheffer et al., 1956; Wood, 1961). On the other hand, McDonnel (1962) and Mann (1962), both working with mutants of F. oxysporum f. lycopersici of varying

pathogenicity, found no correlation between pathogenicity of a strain and its ability to produce pectic enzymes.

In various wilt diseases certain symptoms, such as epinasty, initiation of adventitious roots, proliferation of xylem parenchyma and formation of tyloses appear to be growth responses rather than effects of toxins. Many of the synthetic growth regulators induce similar responses when applied to the plant (Zimmerman and Wilcoxon, 1935; Zimmerman and Hitchcock, 1941). Pegg and Selman (1959) demonstrated significant increases in the level of indole-3-acetic acid (IAA) in young tomato plants infected with V. albo-atrum. Since the fungus was found to produce IAA in vitro, it was suggested that accumulation of IAA in diseased plant might be responsible for many of the symptoms. This view finds support from the recent work of Sequeira and Kelman (1962) who showed similar increases in the level of IAA in tobacco and banana plants infected with Pseudomonas solanacearum. Beckman et al. (1953) suggested that in oak wilt caused by Ceratocystis fagacearum auxin produced by the pathogen altered the plasticity of cell wall thus allowing formation of tyloses which ultimately blocked the vessels and caused wilting. However, the evidence

so far known is not sufficient to make any proper assessment of the role of IAA in the wilt syndrome.

In Pseudomonas wilt of banana, no evidence of toxic effects to the photosynthetic mechanism or, to guard cell function has been noticed even until the final stages of disease (Beckman et al., 1962) The evidence indicated that a continuous decrease in the water supply to the leaves was the primary factor responsible for wilt and other symptoms.

Nature of resistance to wilt diseases

The reaction of a plant to a particular pathogen is a character inherent to it. This reaction may vary between the extremes of complete resistance or immunity, and complete susceptibility. The resistance of a plant may be controlled by specific single genes (monogenic), or by more than one gene (polygenic). Varieties showing the first type of resistance are usually highly resistant to particular races of the pathogen, but may be susceptible to some other races. Varieties in the second group show somewhat lower resistance and their response to pathogen may be modified by a change in the environmental conditions. Both Loran Blood and Gem resistant varieties of tomato have monogenic resistance to V. albo-atrum.

The resistance of the host may be expressed through (1) exclusion of the potential pathogen, (2) restriction in the establishment of infection and (3) inactivation of the mechanism responsible for the production of symptoms. The importance of a study of defence reactions in the plant is widely accepted, but very few studies have been made, until comparatively recently, on this problem. The literature on the different aspects of disease resistance in plants has been reviewed in several recent works (Walker and Stahman, 1955; Allen, 1959; Akai, 1959; Farkas and Kiraly, 1962; Tomiyama, 1963; Cruickshank, 1963).

Resistance of plants to vascular pathogens appears to be particularly complex, because not only is the xylem of all the plant organs invaded, but also the parenchymatous tissue in the root and the vascular bundles. The mechanism of such resistance may operate, therefore, in different parts of the plants at different stages of infection. Blackhurst (1961) has reviewed the literature relating to disease resistance of the plants to vascular pathogens.

The substances exuding out of the roots into the soil become a part of the chemical environment of the root, and may exert considerable influence on the microorganisms in the rhzosphere. This effect

may vary with genera and species (Starkey, 1938; Rovira, 1956). Timonin (1940) working with flax wilt caused by F. oxysporum f. lini, found that Trichoderma viride which is highly antagonistic to different species of Fusarium grew better in the root exudates from a resistant variety than in that from a susceptible one. Timonin (1941) showed later that the root exudate from resistant variety inhibited spore germination and that this inhibition was correlated with its high hydrocyanic acid content. The exudate from susceptible variety had no such inhibitory effect and did not contain any detectable hydrocyanic acid. Trione (1960b) did not find, however, any good correlation, between the hydrocyanic acid content of flax varieties and their resistance to the pathogen. Agnihotrudu (1955) found that F. udum, the cause off wilt in pigeon pea, survived for shorter periods in the rhizosphere of resistant varieties than in that of susceptible varieties. He isolated, from the rhizosphere of resistant varieties, many actinomycetes antagonistic to F. udum. These organisms caused maximum inhibition on a medium containing root extracts from resistant variety, but on a similar medium with extracts from susceptible variety inhibition

was slight. Walker (1935) produced evidences to suggest that resistance of Alaska pea varieties to F. oxysporum f. pisi may be associated with the presence, in their roots or root exudates, of some substances inhibitory to the pathogen. Buxton (1957a) worked with three pathogenic races of F. oxysporum f. pisi sharply defined on 3 varieties of pea. He found a positive correlation between resistance to a given race and the capacity of root exudates to inhibit germination. But mycelial growth was not differentially affected by these exudates. Similar host-specific effects were also obtained with rhizosphere extracts of the three varieties (Buxton, 1957b). Buxton (1962) extended his observations to Panama wilt of banana caused by F. oxysporum f. cubense. Here also, he found a close correlation between resistance to a particular race, and the inhibitory effect of root exudates on spore germination. According to Buxton, part of the resistance mechanism in pea and banana plants to Fusarium infection might operate in rhizosphere through the effect of root exudates on spore germination.

Conidia and microsclerotia of V. albo-atrum germinate poorly in all natural soils. Schreiber and Green (1963) found that the root exudates from a

host plant (tomato) overcame the fungistatic effect of soil to a much greater extent than that from a non-host (wheat). No study on similar lines has yet been made with the exudates from resistant tomato varieties. Lacy and Horner (1962) working with Verticillium wilt of peppermint, found a lower population of the fungus in the rhizosphere of resistant varieties than in that of susceptible varieties only for the first 4 weeks, and not thereafter. They did not find any correlation between resistance of a variety to infection and invasion of its root cortex by the pathogen. From the evidence it does not appear that the root exudates play a decisive role in the defence mechanism of plants. But it is possible that such exudates, by restricting infection to some extent through their inhibitory action on spore germination, may allow time for other mechanisms to become operative.

In wilt diseases there are only a few instances where resistance has been found to operate, at least partly, through some structural changes in the root tissue in response to infection. In Panama wilt of banana, F. oxysporum f. cubense is checked after moderate to considerable initial growth in the cortex of the roots as well as of sucker by the development

of suberized cambiform tissue (Wardlaw, 1930). The new tissue is formed in the ground parenchyma generally at right angles to the line of invasion. This reaction was found to be same in both susceptible and resistant varieties.

Infection of the roots of tolerant and sensitive varieties of hop with mild and virulent strains of V. albo-atrum was studied by Talboys (1958a). He found that initial invasion of the host tissue can be checked to varying extents by lignification of cell walls and occlusion of penetrating hyphae by sheaths of cellulosic material in cell walls of epidermis and cortex (lignitubers). The cortical defence mechanism restricted the progress of a mild pathogen more effectively than that of a virulent one. In a tolerant variety, the fungus is excluded from the stele by suberization of the cell walls of the endodermis, but in a sensitive variety a similar response to infection was not common. In water melon wilt caused by F. niveum, the fungus cannot penetrate through suberized endodermis, but can enter the stele by invading the root tip region (Butler and Jones, 1949). Lignitubers have been observed also in Verticillium infected roots of potato and cucumber (Van der Meer, 1925), and tomato (Derbyshire, 1956; Selman and Buckley, 1959).

However, the importance of these structures in the resistance of a plant is yet to be assessed.

Nothing is known about the anatomical responses of the root tissue in resistant varieties of tomato to Verticillium infection. It appears from the works of Threlfall (1957) and Blackhurst and Wood (1963) that the fungus may probably penetrate through the root cortex of resistant Moran Blood plants to reach stele and proliferate there.

Once a pathogen reaches the tissue of a host it may encounter chemical conditions not favourable for its continued growth. Endeavours have often been made to find a parallelism between disease resistance of a plant and the presence, in its tissue, of some substances toxic to the pathogen. Phenols, and many of their oxidation and addition products have often been implicated in this type of resistance. But only in a few cases has the presence of some inhibitory substances in the healthy tissue been found to be well correlated with resistance.

Lee and Tourneau (1958) found a good correlation between resistance of certain varieties of potato to V. albo-atrum and a high level of chlorogenic acid in their roots. It was shown later that the

young plants of both varieties had very high, inhibitory levels of chlorogenic acid in the root tips, the sprout tips and vascular tissue (McLean et al., 1961). Concentration of phenols decreased as the plants matured, and this decrease was much more pronounced in susceptible varieties than in resistant varieties. Patil et al. (1962) also found some correlation between the pre-infection level of chlorogenic acid in potato plants and their resistance to Verticillium infection. A similar correlation has been found between the presence of certain phenols in potato varieties and their resistance to common scab disease (Johnson and Schaal, 1952). Valle (1957) found an inhibitory level of chlorogenic acid in the leaves of potato varieties resistant to Phytopthora, but in the leaves from susceptible plants concentration was much lower. Differential fungitoxicity of host tissues to different formae of F. oxysporum has been studied by Moore and Chupp (1952), and more recently by Davis (1964). Only in cabbage yellows did the evidence favour any participation of selective fungitoxicity of host tissue in resistance mechanism (Davis, 1964).

In wilt diseases, once the hyphae reach xylem

vessels they may proliferate there and subsequently traverse long distances unimpeded by transverse partitions. In such cases the resistance of a plant may depend on the absence in the xylem sap, of nutrients essential for the continued growth of the pathogen, the presence in the sap of some fungitoxic substances, post-infection production of such substances, structures and substances produced in the vessels in response to infection and limiting physically the spread of the pathogen, inhibition of the production of fungal toxins, neutralization of such toxins, or immunity to them.

Garber (1956) proposed a nutrition-inhibition hypothesis according to which a plant would be resistant if it did not contain particular substances which the pathogen may require for its continued growth. By irradiation, Buxton (1956) produced mutants of F. oxysporum f. pisi. Some of these mutants having requirements for a certain amino acid, showed reduced virulence to pea plant than the autotrophic and virulent p rent strain. Virulence was restored to these mutants when they were supplied with nutrients for which they were deficient. Similar results had been obtained earlier with mutants of Venturia inaequalis (Kline et al., 1957) and Erwinia carotovorum (Garber and Shaeffer,

1957).

Gottlieb (1943b) found that mycelial growth of F. oxysporum f. lycopersici on sap expressed from resistant tomato plants was consistently lower than that on sap from susceptible plants. The fungistatic constituent of the sap from resistant plants, although not identified, was found to be fairly stable. Wood (1961) analyzed tracheal saps from tomato varieties, resistant and susceptible to V. albo-atrum, and did not find any qualitative difference between them. The sap from susceptible plants supported slightly better growth of the fungus and had more of nitrogenous substances and minerals than that from resistant plants. Blackhurst (1961) did not find any consistent difference in the composition of saps from Gem varieties of tomato resistant and susceptible to V. albo-atrum. The fungus grew equally well in cultures containing extracts, or insoluble plant material from the two varieties.

A post-infectional increase in phenols and related compounds to fungitoxic concentrations has been suggested to be responsible for resistance to many diseases. However, conclusive evidence is lacking in most cases.

Rubin et al. (1947) observed a marked increase in the level of phenols in resistant potato varieties

infected with Phytophthora infestans. It was shown later that infection with an incompatible race only caused such an increase (Tomiyama et al., 1956). Rubin and Perevyazkina (1951) noted considerable increase in polyphenol and tannin contents of resistant cotton varieties infected with V. albo-atrum. This increase was most remarkable in the roots. There was no such increase in susceptible varieties in which tannin remained evenly distributed. On the other hand, Gubanov (1958), and Krasnoschekova and Runov (1962) suggested that phenol and tannins might have a negative effect on resistance of cotton varieties to Fusarium and Verticillium infection. A post-infectional increase of phenol concentration and polyphenol oxidase activity was claimed for tomato varieties resistant to F. oxysporum f. lycopersici (Menon and Schachinger, 1957). Susceptible varieties, when infected, did not show any significant increase.

Activation of oxidases is as widespread in diseased tissues as the post-infectional rise in phenols. The infected resistant tissue shows, in many cases, a higher oxidase activity than the infected susceptible tissue, or the healthy one. A positive correlation between peroxidase activity of infected potato varieties and their resistance to Phytophthora has been recorded

by Umaerus (1959). Wilding (1960) observed considerable increases in polyphenol oxidase and peroxidase activity in the stem tissue of resistant pea variety after inoculation with F. oxysporum f. pisi. A similar increase in the oxidative capacity of resistant tomato varieties after inoculation with V. albo-atrum, has been recently observed by Deese and Stahman (1962a). No such increase was recorded for infected susceptible plants. Quinone test was rapid and very strong in cultures from resistant tissue inoculated with the fungus. Deese and Stahman also studied the oxidase activity in Fusarium infected banana (1962b) and tomato (1962c) plants. In banana plants, there were increased oxidase activity, as well as higher level of phenols in infected resistant variety than in infected susceptible variety. In the case of tomato plants, a decrease of reducing substances was noted in stem juice from infected tomato plants. Deese and Stahman suggested that phenol-oxidation products such as quinones might inactivate important hydrolyzing enzymes secreted by the pathogen and consequently suppress its growth.

Thus it would appear that the resistance of a plant to wilt diseases depends not so much on the nature of xylem fluid as on the post-infectional metabolic activity of host cells. This becomes further evident when it is found that the resistance of a plant can be

broken, or at least reduced by treating it with certain substances well known for their role as inhibitors of metabolic activity. Gothoskar et al. (1955) were the first to report such a breakdown of resistance in tomato varieties highly resistant to F. oxysporum f. lycopersici. Inoculated resistant cuttings, when treated with metabolic inhibitors such as 2,4-dinitrophenol, sodium fluoride, thiourea and sodium diethyldithiocarbamate, developed considerable disease symptoms. These substances did not have much effect on the growth of pathogen in vitro. Gothoskar and his coworkers suggested, therefore, that the breakdown of resistance was due to the effect of these inhibitors on host metabolism, particularly on respiration. Studying wilt of flax due to F. oxysporum f. lini, Nair (1958) was able to increase disease incidence in resistant plants by treating them with 2, 4-dinitrophenol, thiourea, and maleic hydrazide which is known to inhibit respiration of higher plants. Considerable increase in disease symptoms was also recorded in tobacco plants resistant to Pseudomonas solanacearum when they were treated with the reducing agents, glutathione, ascorbic acid and sodium sulphite 2 days before inoculation (Maine and Kelman, 1961). Both glutathione and ascorbic acid are known to inhibit polyphenoloxidase activity (Henze, 1956). Similarly,

Noveroske et al. (1962) obtained a reduction of resistance in Malus atrosanguinea to Venturia inaequalis when the plants were treated with 4-chlororesorcinol, an effective inhibitor of polyphenol oxidase. Christiansen and Weniger (1955) showed that resistance of potato tubers to Phytophthora could be reduced by treating them with substances inhibitory to polyphenol oxidase. On the other hand, the growth of the fungus on susceptible tubers was suppressed when treated with substrates for polyphenol oxidase.

Narcotics are known to inhibit enzymes at relatively high concentration (James, 1953) and have some effect on the metabolism of plants. Gothoskar et al. (1955) recorded considerable disease symptoms in Fusarium resistant tomato cuttings when they were treated with ethanol after inoculation. But urethane, a strong narcotic, did not have any effect on resistance.

Resistance may also express itself in the production of tyloses and gum barriers in the vessels of infected plants thus blocking the spread of pathogen. In Panama disease of banana the hyphae were sometimes found embedded in gum completely filling the vessel. It is not known if the hyphae could grow through such a barrier. Many plants which normally do not produce

tyloses do so when infected with a pathogen, particularly those causing vascular disease. According to Chattaway (1930) tylosis and gummosis are both formed by ray parenchyma cells in response to a stimulus. Whether gum, tyloses or both would be formed depends on the size of pit aperture. According to Powers (1954) tyloses are produced not because of contact with air as suggested earlier by Klein (1923), but primarily in response to the decomposition products of invaded cells. This agrees partly with the suggestion made early by Haberlandt (1923). In infected plants tyloses are generally produced in advance of the pathogen. According to Beckman (1956) tylosis may be initiated in elm plants infected with Ceratostomella ulmi by a shift in the cell metabolism involving an increase of carbohydrases and production of cell wall degrading enzymes. From his studies on Fusarium wilt of sweet potato, McClure (1950) concluded that tylosis was stimulated when the level of toxic products in the vascular system of infected plants was low. Very few tyloses developed near the pathogen where the concentration of toxic substances was very high. As concentration of such substances decreased with distance from the pathogen, tyloses were more and more produced. Occlusion of many vessels with tyloses

increased gradually the concentration of toxic substances in other vessels until a concentration inhibitory to tylose formation was attained. Talboys (1958b) also made similar suggestion to explain the formation of tyloses in Verticillium infected hop plants.

Tyloses have often been implicated in the production of wilting in leaves. But in other cases it has been shown that tyloses by blocking the vessels may help in confining the pathogen to a restricted area (Wardlaw, 1930; McClure, 1950; Talboys, 1958b). Hyphae are not commonly found in same vessel as tyloses. Penetration of the tylose blockage, or invasion of the tyloses by the pathogen has rarely been observed (Talboys, 1958b). In Fusarium wilts of banana (Wardlaw, 1930) and radish (Peterson and Pound, 1960) tyloses were equally distributed in resistant and susceptible varieties. Talboys (1958b) found an inverse relationship between tylosis and mycelial growth in Verticillium wilt of hop. There were very few tyloses in plants showing acute symptoms. But in a mild syndrome resulting from an infection of sensitive and tolerant varieties respectively with a mild and virulent strain of the pathogen, tylosis was extensive. According to Talboys, the slight invasion of the vascular system of

root determined by a strong extra-vascular defence mechanism in a mild syndrome, would stimulate tylosis. The fungus would not be able to spread further and this would induce further tylosis. If the extra vascular defence mechanism fails, as in the acute syndrome, then extensive invasion of the root vascular system would suppress tylosis. This would lead to further colonization. Talboys (1958b) postulated that the extra-vascular host-pathogen interaction, which constitutes the determinative phase of the disease regulated the amount of mycelium in the vascular elements of the root. This situation appeared to be self-perpetuating and determined the subsequent course of host-pathogen interactions sometimes leading to the development of symptoms that constitute the expressive phase of the disease. These ideas have been elaborated in much more detail in a recent paper (Talboys, 1964). In tolerant varieties extensive tylosis was accompanied by a compensatory development of additional xylem elements. Threlfall (1957) studying Verticillium wilt of tomato, found very many tyloses in resistant Loran Blood variety, but only very few in susceptible Ailsa Craig variety. Hyphae were restricted to the base of stem. Using the same varieties of tomato as Threlfall, Blackhurst and Wood

(1963) did not notice much difference in tylosis and spread of the pathogen between them. The importance of tylosis in localizing infection receives support from a different type of work. Smalley (1962) obtained complete control of Dutch elm disease by injecting 2, 3, 6-trichlorophenoxyacetic acid into the plants before they were inoculated. In the susceptible plants thus made resistant, tyloses were widespread in large vessels.

In many cases where the pathogen is well distributed in both the varieties, resistance may depend on the inhibition of the production of toxin, its inactivation or immunity to its presence. Gäumman (1957) suggested that fusaric acid was not produced in the resistant plants because of inhibition. Trione (1960a) found that fusaric acid could be synthesised in vitro by F. oxysporum f. lini only when grown on tissue from susceptible flax variety. Deese and Stahman have recently shown for Fusarium wilts of tomato (1962c), and banana (1962b) and Verticillium wilt of tomato (1962a) that considerably lower amount of cell wall degrading enzymes are produced in infected resistant tissue than in infected susceptible tissue. In the last two cases oxidase activity and the level of phenolic compounds were very high. On this basis,

Deese and Stahman suggested that resistance of banana to Fusarium, and tomato to Verticillium was due to a suppression of the formation or inactivation of pectic enzymes. Confirmatory evidence to this has been produced by Grössman (1962). He obtained inhibition of wilt symptoms when Fusarium infected tomato plants were treated with rufianic acid, a phenolic substance known to inhibit pectic enzymes.

If auxins have any role to play in wilt diseases, then their relation with phenols, and their oxidation products may be important in disease resistance. Oxidases have been known to be connected with the disappearance of auxins from the tissue. The capacity of polyphenol oxidase to inactivate IAA in vitro in the presence of oxidizable phenols have been proved (Leopold and Plummer, 1961). Higher levels of phenols and oxidases in many infected resistant plants may not allow accumulation of IAA in the tissue and thus inhibit production of symptoms. This view is disproved, however, by the findings of Sequeira and Kelman (1962). They showed that both resistant and susceptible varieties of tobacco recorded almost similar increase in IAA level when inoculated with Pseudomonas solanacearum.

Toxins produced by vascular pathogens,

including fusaric acid, are known to affect susceptible and resistant varieties in the same way. The basis for specific pathogenicity of a parasite for certain hosts has yet to be determined. A significant lead in this direction was given by Winstead and Walker (1954) working with strains of F. oxysporum f. conglutinans that are pathogenic to radish, cabbage or cotton varieties. Toxins produced in vitro was shown to have the same specificity for species and varieties as the fungus itself. Helminthosporium victoriae produces, both in vivo and in vitro, Victorin a toxin that affects only the susceptible varieties of oats (Meehan and Murphy, 1947; Pringle and Braun, 1958). Romanko (1959) later showed that Victorin, when fed to the cuttings of oat varieties, could be recovered only from extracts of susceptible plants. Culture filtrates of Periconia circinata, the cause of milo disease of sorghum, were found to be highly toxic to susceptible varieties, but did not damage resistant varieties of sorghum (Scheffer and Pringle, 1961). This suggests the presence, in the resistant tissue, of a mechanism for inactivation of toxin.

Susceptible tomato plants can be made resistant to Fusarium infection by treating them with one of a variety of growth regulators (Davis and Dimond, 1953;

Gorden and Dimond, 1959). Corden and Dimond (1959) suggested that the induced resistance in tomato plants was due to the modification of cell wall pectic substances in a way so as to make them immune to the action of pectic enzymes.

There has been considerable divergence of opinion regarding the site of resistance mechanism in plants infected with vascular pathogens. Heinze and Andrus (1945) studied disease development in cross-grafted resistant and susceptible tomato plants infected with F. oxysporum f. lycopersici. They confirmed the finding of May (1930) that resistant character cannot be transferred through grafting. All the scions on susceptible stock were severely diseased and invaded with the pathogen, but others on resistant stock showed mild symptoms only. Heinze and Andrus suggested, therefore, that the defence mechanism of a plant was located in its root system. This view was supported by Keyworth (1953) and Threlfall (1957) working with Verticillium wilts of hop and tomato respectively. Keyworth suggested that the stems of both varieties had equal and high resistance to infection. According to him the stems of susceptible variety were invaded, because heavy growth of the pathogen in its root increased the inoculum potential and lowered the

resistance of stem.

Completely different kind of results have been obtained by many workers. Snyder et al. (1946) using resistant and susceptible tomato plants inoculated with spores of F. oxysporum f. lycopersici through cut roots, found differences in fungal growth in the stem to be correlated with differences in wilting. Scheffer and Walker (1954) working with inoculated cuttings, recorded considerably more symptoms in susceptible variety than in resistant one. Scheffer (1957) later gave convincing demonstrations of differential resistance in the stems of resistant and susceptible varieties of tomato to Fusarium infection. His observations have since then been confirmed by Keyworth (1963).

Blackhurst (1961) repeated Threlfall's work on Verticillium wilt of tomato using the same varieties, but arrived at different conclusion. Observations from this and other works led her to conclude that resistance factor was present in stem also. This agrees well with the findings of Horner (1954) and Berry and Thomas (1961) both working on Verticillium wilt of peppermint.

Observations made by Provvidenti and Schroeder

(1959) studying leaf infection in tomato, indicate that even the leaves of different varieties may have differential resistance towards the pathogen.

Evidences suggest that resistance may conceivably be distributed throughout the plant and may express itself in different organs at different stages of infection and in different ways.

IV. MATERIALS AND METHODS

A. Growing of plants

The tomato plants used in this study were resistant and susceptible "Gem" varieties originally supplied by Mr. Woolliams, Summerland, British Columbia, Canada. They were selected because of their high susceptibility and resistance to wilt caused by Verticillium albo-atrum. "Gem" resistant (GR) variety was developed as a cross between "Gem" susceptible (GS) variety and Loran Blood, an American variety showing a high degree of resistance to Verticillium wilt. Its resistance is of the most stable form so far known which is that controlled by a single gene (monogenic resistance).

Plants were grown in a roof greenhouse at Imperial College, London. Seedlings were raised in seed boxes, removed from them at 4-leaf stage and transplanted to 3 in. clay pots in which they grew until they were inoculated. After inoculation the plants were repotted into 5 in. clay pots. For growing plants John Innes potting compost was used throughout. To this John Innes base fertilizer (2 parts hoof-and-horn, 2 parts superphosphate and 1 part sulphate of potash), and ground chalk were added at the rate

of 5 lb and 1 lb per cubic yard respectively. Supplementary light was provided by banks of 80W. fluorescent tubes 5 inches apart and suspended 1 ft above the tops of the plants. The greenhouse was heated in winter so that the temperature rarely went below 60°F (15.5°C). The plants grew well except during the winter months when light was probably limiting.

For some experiments plants were grown in nutrient solutions. For experiments requiring undamaged root systems, the seedlings were raised under sterile conditions. The seeds were surface sterilized by immersion in sodium hypochlorite solution (3% active chlorine) for 10 minutes followed by several washings with sterile water. Sodium hypochlorite solution contained 0.1% of Tween 20, a wetting agent. The seeds were germinated in petri-dishes on filter paper moistened with water in aseptic conditions in a constant-temperature-room at 25°C. About one week after germination, the seedlings were removed to bottles (60 ml capacity) containing full-strength nutrient solution. For most other experiments, however, the seedlings were taken out of seed-boxes at 4-leaf stage, their roots washed carefully in running water and transferred directly to the 60 ml bottles. These bottles were covered with black

polythene to cut off light. The plants were raised in greenhouse under the conditions of light and temperature already given. The Long Ashton nutrient solution as described by Hewitt (1952), minus aluminium, gallium, cobalt and nickel salts, was used throughout this work. For 100 litres of nutrient 33.6 g of KNO_3 and 54.7 g of $Ca(NO_3)_2$ were used during the winter months, but for the rest of the year these amounts were increased to 50.5 g and 82.0 g. respectively. Iron was originally supplied as ferric citrate, but owing to its instability on standing, this salt was later replaced by an iron-ethylene-diamine-tetraacetic acid complex (Fe-EDTA) supplying iron at the rate 0.15 milliequivalent per litre of nutrient solution. No symptom of iron deficiency occurred in these plants.

B. Culture of fungus

1. Origin of culture

A virulent isolate of Verticillium albo-atrum Reinke and Berthold was used in this work. This strain, originally isolated in 1955 from infected material supplied by Cheshunt Research Station, was used by Threlfall (1957) and Blackhurst (1961) in their studies on tomato wilt. It is an atypical specimen

of V. albo-atrum because it did not produce dark mycelium characteristic of this species. The fungus was re-isolated from an artificially infected GS plant and a culture derived from a single spore was used throughout this work.

2. Media

All the chemicals used in the preparation of media were of analytical reagent grade, unless otherwise stated. The pH of media was not adjusted except when assaying the in vitro fungitoxicity of certain compounds. The media used were as follows:-

(a) Sucrose-sodium nitrate (SSN)

Sucrose	-	15.0 g
KH_2PO_4	-	1.0 g
$MgSO_4, 7H_2O$	-	0.5 g
$NaNO_3$	-	2.0 g
KCl	-	0.5 g
Trace element soln.	-	1 ml.
Water	-	to 1 litre

(b) Sucrose-casamino acids (SCA)

Sucrose - 15.0 g

KH_2PO_4 - 1.0 g

$MgSO_4, 7H_2O$ - 0.5 g

Casamino acids (Difco) - 4.6 g

Trace element soln. - 1 ml

Water - to 1 litre

The trace element solution used in the preparation of media (a) and (b), was made up as follows:-

Trace element	Salt used	Amount in mg./100 ml.	p.p.m. trace element in stock soln.	p.p.m. trace element in final soln.
Iron	$FeSO_4, 7H_2O$	249	501	0.5
Copper	$CuSO_4, 5H_2O$	40	100	0.1
Zinc	$ZnSO_4, 7H_2O$	44	100	0.1
Manganeese	$MnSO_4, 4H_2O$	41	101	0.1
Molybdenum	$Na_2MaO_4, 2H_2O$	51	202	0.2
Water		100 ml.		

One ml. of this stock solution was added to a litre of medium.

Agar was added at the rate of 20 g per litre when solid medium was required and steamed for 1 hour.

(c) V8 vegetable juice agar (V8)

V8 is the trade name of a mixture of juices from tomato, celery, parsley, spinach, carrot, beet root, lettuce and watercress mildly seasoned with salt and monosodium glutamate. This is a product of Campbell's Soups Ltd., Norfolk, England. One part by volume of this juice was mixed with 5 parts of water. Agar was added to this at the rate of 20 g per litre and steamed for 1 hour.

(d) Macerates of plant tissue

Well-washed pieces of stem or root from healthy or infected plants were macerated in a Waring type Blendor with 9 times its own weight of water for 20 seconds at maximum speed. The homogenate was filtered through several layers of muslin. The filtrate was centrifuged at 3,000 r.p.m. for 15 minutes to remove solid particles and the supernatant was made up to a volume equivalent to 10 times the fresh weight of the tissue so that the macerate contained 10 per cent of the tissue by fresh weight. The macerate was used as such or added to SSN medium in certain proportions.

3. Culture vessels

Cultures were maintained on SCA-agar slopes mostly, but sometimes on V8-agar. Cultures for

obtaining inoculum (spores or mycelium) were grown in 10 oz. medicine bottles containing 50 ml. of liquid, or solid medium and incubated on the flat side. Unless otherwise stated, Erlenmeyer flasks of 150 ml. capacity and containing 25 ml. of liquid SSN medium were used for assaying the effects of different compounds on mycelial growth. Similar flasks, but containing 20 g. of small glass beads in medium were used for preparing inoculum in the form of mycelial fragments.

4. Sterilization

All media were sterilized in autoclave for 15 minutes at 120°C.

5. Inoculation and incubation of cultures

Slopes were inoculated by transferring a small bit of mycelium. Solid media in medicine bottles were inoculated by drawing a small bit of sporulating mycelium over the surface of the agar. Liquid SCA medium in medicine bottles and SSN medium in Erlenmeyer flasks were inoculated by adding 1 ml. of spore suspension. Spore suspensions were made by shaking a 10-14 day old culture on solid SCA medium with 12 ml. of sterile water. This medium was replaced by SSN medium when spore suspensions were

needed for *in vitro* fungitoxicity tests. While testing the effects of tracheal saps on mycelial growth, a suspension of mycelial fragments was used as the inoculum. To obtain this, a 2-week-old culture growing on 25 ml liquid SSN medium with glass beads was broken into small fragments by shaking with hand.

All the cultures were incubated at 25°C± 0.5°C.

6. Dry weight of mycelium

Mycelial mats were collected on muslin, washed in running water and transferred to small aluminium foil cups of known weight. Mycelia thus collected were dried in an oven at 70°C for 48 hours, cooled over $CaCl_2$ in a desiccator and weighed.

C. Preparation of inoculum

For root inoculation of soil-grown plants inocula were prepared from 10-14 day old cultures growing on SCA liquid medium. Mycelial mats were collected on muslin, washed in running water to remove traces of medium and homogenized in a Waring type Blendor with tap water (50 ml for each bottle = original volume of medium) for 15 seconds at maximum speed. This suspension of mycelial fragments was

used for inoculating plants in soil.

The plants growing in nutrient solution, cuttings and detached leaves were inoculated with spore suspension. Spore suspension was obtained by shaking 10-14 day old cultures on SCA agar medium with about 10 ml of water. The spores in suspension, collected by filtration through several layers of muslin, were washed by repeated centrifugation and resuspension in water. The concentration of spores in the final suspension was measured with a haemacytometer slide and then adjusted suitably.

D. Inoculation of plants

1. Root inoculation of plants growing in soil

Plants were inoculated by pouring 25 ml of mycelial suspension around the root system while transferring plants from 3 in. pots to 5 in. pots. During transplanting many lateral roots were deliberately damaged to ensure rapid infection. The controls were also similarly treated, but 25 ml of tap water replaced the mycelial suspension.

In a few experiments, an alternative method was used. Plants were inoculated by dipping washed roots in a mycelial suspension for a few seconds and then replanting into soil in 5 in. pots. In the

controls, the roots were dipped in tap water only.

2. Root inoculation of plants grown in nutrient solution

Before inoculation, about one-fifth of the lateral roots of each plant were trimmed with a pair of scissors. Then 1 ml of dense spore suspension (5,000,000/ml) was poured into the nutrient solution in each bottle in which the plant was growing. The plants to be kept as control had also their roots trimmed in the same way. After 24 hours the plants were transferred to larger bottles.

3. Inoculation of cuttings and detached leaves

A cut-shoot spore-inoculation technique, adapted from that of Keyworth (1950), was used. Stems of 7-11 week old plants were cut at ground level with a wet razor blade, the lower 5 mm portion trimmed under running water and the cuttings were placed in spore suspension containing 500,000 spores per ml. For the controls tap water replaced the spore suspension. The cuttings were kept for 4 hours in the greenhouse under conditions favouring moderate transpiration. Afterwards, the cuttings were removed from the spore suspension. Their lower ends were washed in running water to remove spores adhering to

the surface and the cut ends were trimmed by 5 mm before being placed in half-strength nutrient solution in bottles of 160 ml capacity. The bottles were coated on the outside with aluminium paint. The cuttings were kept in cabinets, provided with four 80W fluorescent tubes giving a light intensity of about 250 f.c., and maintained at 18°C ± 1°C, for about 2 weeks before their removal to the greenhouse. By end of the second week, most of the cuttings had produced many roots.

Healthy leaves, cut off under water near the base of the petioles, were inoculated in the same way as the cuttings. Afterwards, they were transferred to half-strength nutrient solution in 60 ml bottles covered with black polythene to cut off light. Throughout the experimental period the leaves were kept in the illuminated cabinet as mentioned before.

E. Examination of plants

1. Evaluation of morphological symptoms

The severity of disease of intact plants and cuttings was assessed by determining a disease index, or a wilting index based on the following arbitrary scales:

Disease index

0 = healthy leaf.

1 = slight wilting and/or yellowing up to 25% of the leaf.

2 = moderate wilting and/or yellowing extending from 26% to 75% of the leaf.

3 = nearly complete wilting and/or yellowing extending almost over the whole leaf.

4 = leaf dead, or shed.

Wilting index

0 = healthy leaf.

1 = slight wilting of 1 or 2 leaflets.

2 = pronounced wilting of the terminal leaflets or moderate wilting extending over the whole leaf.

3 = complete and severe wilting of the leaf.

4 = leaf dead, or shed.

With young plants, both wilting and yellowing of leaves were considered for an assessment of disease as expressed by disease index. But in other experiments, involving older and larger plants, an assessment on the basis of the disease index was not found to be very satisfactory. In these experiments yellowing was not uncommon in the lower leaves of many uninoculated plants. It was also very difficult to

distinguish between yellowing of leaf due to infection, and that due to senescence. For such plants disease was assessed on the basis of wilting symptoms only. For determination of an index the first leaf above the cotyledons was numbered 1, followed by 2 and so on. All the leaves more than 1 cm. in length were rated individually for the severity of symptoms on one of the scales mentioned above and the mean indices were computed for plants and leaves. The maximum possible value for a leaf was 4.0.

In assessing the symptoms in detached leaves similar scales, but ranging from 0 to 3 only, were used. If yellowing was evaluated separately as a symptom, then the following scale was used:

0 = healthy leaf.

1 = yellowing up to 25% of the leaf

2 = yellowing extending from 26% up to 75% of the leaf.

3 = nearly complete to complete yellowing of the leaf.

2. Anatomical studies

Plant materials such as pieces of stem, root or petiole, if not used immediately, were fixed and preserved in Formalin-Aceto-Alcohol - FAA (formalin 5 parts, glacial acetic acid 5 parts and 70%

alcohol - 90 parts by volume). For free-hand sectioning, the preserved materials were washed successively in 63% alcohol, and in water. The free-hand sections were stained in safranin-picro aniline blue following the procedure recommended by Cartwright (1929). The stained sections were mounted in Euparal. For studying tylosis only, sections were stained in picro aniline blue and mounted in 50% glycerine after the excess stain had been removed by washing.

3. Dry weight of plant organs

The materials were cut into small pieces and dried by heating in an electric oven at 100°C for 24 hours.

F. Collection of tracheal sap

The lower portion of stem was washed by spraying with water. The stem was cut 3 centimetres above ground level with a sterile scalpel. One end of a sterile rubber tubing, about 5 centimetres in length was fitted to the cut end. Into the other

end a sterile 10 ml pipette was introduced. Both the joints were made airtight by tying with very thin copper wire. The pipette was kept vertically. The top of the pipette was covered loosely with a small piece of aluminium foil to prevent contamination. The whole set was kept in the greenhouse for 24 hours, after which tracheal sap was collected and stored at -20°C for future use.

G. Studies on spore germination

Germination studies were made on clean and grease-free slides. They were placed inside plastic boxes (16.5 cm x 11.5 cm x 4 cm) on narrow glass rods resting on blotting paper moistened with water. Spore suspensions were obtained in the usual way. The spores were washed by three successive centrifugations at 3,000 r.p.m. and resuspended in sterile water. After the last centrifugation the concentration of spores in the suspension was determined and adjusted suitably. A known volume of this suspension was mixed with a known volume of tracheal saps, plant diffusates or sterile water. Thus a series of spore suspensions were produced in different germination media, but all having the same concentration of spores. One

drop of such a suspension (0.05 ml in volume) was placed at two spots on the slide at a distance of 2.5 cm from each other. The boxes were closed with covers and made airtight with vaseline. Each box contained one slide from each treatment. They were covered with black polythene and kept at room temperature (20°C - 22°C) for 24 hours. Afterwards, one small drop of cotton blue in lactophenol was added to each spot which was then covered with a cover slip for microscopic observation. All spores showing discernible germ tube growth were counted as having germinated. Germ tube growth was measured with an eyepiece micrometer. About 100 spores were counted from each slide i.e., 50 spores from each spot. Each result is based on a count of 400 spores.

H. Treatments with growth regulating substances

The following chemicals were used in connexion with different experiments.

(1) Indole-3-acetic acid (IAA), (2) indole, (3) α-napthaleneacetamide (NAM), (4) maleic hydrazide (MH), (5) 2, 4, 6-trichlorophenoxyacetic acid (2,4,6-T), (6) 2, 4-dichlorophenoxyacetic acid (2,4-D), (7) 2,4-dichloroanisole, (8), 2, 3, 5-triiodobenzoic acid (TIBA),

(9) gibberellic acid (GA) and (10) (2-chloroethyl)-trimethylammonium chloride. The chemicals 1 - 5 were from L. Light & Co., 6 from the British Drug Houses Ltd., 7 and 8 from Eastman Kodak Co., 9 from Plant Protection Ltd., and 10 from American Cyanamid Company under the trade name "Cycocel" containing 50% of the active ingredient in an inert medium.

Maleic hydrazide was dissolved in water with gentle warming over a flame. "Cycocel" was also dissolved in water. All other substances, being insoluble or only slightly soluble in water, were first dissolved in a minimum volume of ethanol before adding water to make up the required concentration. These solutions were either applied to the root system by pouring 50 ml portions around the base of the stem or sprayed on to the foliage until it was thoroughly wet. The solutions to be sprayed were always supplemented with Tween 20 (Polyoxyethylene Sorbitan monolaurate), a wetting agent at the rate of one drop (0.05ml) for each 100 ml. Sprays were applied with an atomizer. When IAA, 2,4-D or MH sprays were used, care was always taken to protect the growing point from coming in direct contact with

spray mixture. IAA, when used as such, is very unstable in solution. When sprayed on the leaf, it is rapidly absorbed so that there is very little decomposition on the leaf surface. However, in some of the experiments the plants had to be grown, or the cuttings had to be kept for sometime in solutions of IAA. So in order to circumvent the difficulty arising out of decomposition, the ammonium salt which is soluble and much more stable in solution was used instead. To prepare this salt, IAA was dissolved in minimum volume of concentrated ammonia solution (specific gravity = 0.88). The solution was then evaporated to dryness over a low flame till there was no smell of ammonia.

I. Tests on fungitoxicity

These tests were carried out in 150 ml flasks with SSN as the basal medium. The pH of the medium was adjusted to 6.0 by addition of N/10 NaOH, and N/10 HCl. Twenty-five ml portions of the medium were distributed into flasks and sterilized. Stock solutions of different compounds were prepared at strengths 27 times that of the final concentrations to be used in the experiments. Required amounts of MH and streptomycin sulphate

and required volumes of ethanol were dissolved directly in sterile water to prepare the stock solutions. All other compounds were initially dissolved in certain minimum volume of ethanol, this volume being constant for all the concentrations of a particular compound. So all the stock solutions of any compound, though of different strengths, always contained the same proportion of ethanol. For each experiment, a stock solution of ethanol was also prepared in the same way so that an ethanol control series could be included. It had the same concentration of ethanol as other stock solutions. To the sterilized basal medium, one ml of stock solution was added. For the control series, one ml of sterile water was added to each flask. Inoculation was done by adding one ml. of spore suspension to each flask. The cultures were incubated for 14 days unless otherwise stated. Afterwards, mycelial mats were collected separately from each flask and dry weights determined.

V. EXPERIMENTAL WORK

A. Morphological and anatomical studies

1. Relation of infection to the production of external and internal symptoms, to the presence of hyphae, and to certain growth responses in resistant and susceptible plants.

Threlfall (1957) working with Loran Blood and Ailsa Craig tomato plants, resistant and susceptible respectively to Verticillium albo-atrum, found that only mild symptoms developed in resistant plants. This was associated with the fact that although the roots of resistant plants became invaded the parasite did not spread into the stem. In susceptible plants, however, invasion of the stem followed the entry of the fungus into the root. He found a close correlation between leaf symptoms, vascular browning and the presence of hyphae in the petiole, the last two being very closely associated. He recorded tyloses in many vessels of infected resistant plants, but seldom found them in infected susceptible plants. Threlfall also claimed that the severity of leaf symptoms was directly related to vessel blockage.

Blackhurst and Wood (1963) made comparative

studies with root-inoculated Loran Blood and Ailsa Craig plants. They reported almost equal spread of pathogen up the stems of both varieties although there appeared only slight symptoms in resistant plants. They showed, however, that there was less fungus in the petioles of resistant plants than in those of susceptible plants. They also reported, in agreement with Threlfall (1957), that there were more tyloses in inoculated resistant plants than in inoculated susceptible plants, although the latter showed extensive tylosis as early as 15 days after inoculation. Blackhurst and Wood also made comparative studies with shoot-inoculated Loran Blood and Ailsa Craig, and Gem resistant and Gem susceptible plants. The fungus grew well inside all four varieties, but resistant plants did not produce any symptoms. They suggested that Loran Blood and possibly Gem resistant also, are resistant to V. albo-atrum in the sense that their growth was not much affected by the infection and extensive spread of the pathogen inside them.

An experiment was designed, therefore, to study the various effects of infection on the resistant and susceptible varieties used in this work.

Attempts were also made to determine how far the expression of symptoms can be correlated with the amount and the distribution of the fungus within the plant.

Eighteen, 6-week-old GR and GS plants were root-inoculated in the usual way by pouring 25 ml. of mycelial suspension around the damaged root system. There were eight controls from each variety for which water was used in place of the suspension.

External symptoms, such as yellowing and wilting of leaves, and growth responses like epinasty, height of the plant and the total number of leaves were studied. For this purpose, five GS control (GSC), five GS inoculated (GSI), five GR control (GRC) and five GR inoculated (GRI) plants were kept separate. These plants were examined 10, 14, 17, 21, 24 and 28 days after inoculation. For epinasty, adaxial angles of leaves 3-10 were measured. For other purposes, all leaves more than 1.5 cm. in length were taken into consideration. Heights of the plants were measured at the conclusion of the experiment. Dry weights were recorded twice - 14 and 28 days after inoculation, and for this purpose only three replicates, taken at random from

each treatment, were used.

For anatomical studies, on each date of sampling, two infected plants from each of the two varieties were cut off at the region of cotyledonary node, and pieces of stem including the lower portion of attached petioles were fixed and preserved in FAA. Free-hand sections were made from these stem pieces at intervals of 2.5 cm., and at the base of petioles. The sections were microscopically observed to appraise quantitatively as well as qualitatively the effect of infection on the vascular tissues of resistant and susceptible plants.

The data are presented in Tables 1-8 and Figure 3.

In this experiment, the external symptoms, including the initiation of adventitious roots, were quite prominent in some replicates when sampling started 10 days after inoculation. In fact, symptoms started appearing from the 7th day onwards.

The difference in disease intensity as expressed by disease index, between inoculated GR and GS plants was apparent within 10 days after inoculation (Table 1). At this time, inoculated GR plants showed slight wilting and a little yellowing

Table 1 Comparative reactions of resistant and susceptible plants to infection by V. albo-atrum as expressed by mean disease index per leaf

Plant	Treatment	Days after inoculation					
		10	14	17	21	24	28
GR	Control	0.8	0.8	0.8	0.8	0.8	0.9
	Inoculated	0.9	1.1	1.1	1.1*	1.1*	1.3**
GS	Control	0.7	0.7	0.6	0.6	0.7	0.8
	Inoculated	1.2	1.8*	2.2**	2.3**	2.4**	2.4**

Average of 5 replicates

* Significant difference: P = 0.05

** Significant difference: P = 0.01

of the lower leaves. Thereafter, disease development was very slow and the differences between control and inoculated GR plants became significant only at the end of the third week. The mean wilting indices for the leaves of inoculated GR plants were 0.2, 0.2, 0.3, 0.3, 0.4 and 0.4 respectively 10, 14, 17, 21 and 28 days after inoculation. In inoculated GS plants both wilting and yellowing developed very rapidly for 17-18 days after inoculation. Later on, symptoms developed more slowly

and after 4 weeks the progress of disease appeared to have been checked. The differences in disease index between control and inoculated GS plants were always considerable and very significant except on the first date of sampling. None of the control plants showed any wilting at any stage during this experiment, but some of the lower leaves turned yellow. This explains the disease index values recorded for them.

Table 2 Effect of infection on height of resistant and susceptible plants determined after 28 days

Plant	Treatment	Height in cm. Replicate No. 1	2	3	4	5	Average	Percentage increase or reduction in height
GR	Control	40.5	40.5	41.2	41.2	46.7	42.0	
	Inoculated	41.2	41.9	41.9	43.2	43.7	42.4	+ 0.95
GS	Control	38.7	41.2	41.2	43.0	43.5	41.5	
	Inoculated	17.7	25.5	28.0	30.2	32.5	26.8**	-35.42

** Significant difference: P = 0.01

In GS plants infection always resulted in a check to growth, and at the end of the experiment the percentage reduction in stem height was 35.4. The degree of stunting in diseased plants ranged from moderate to severe (Table 2). Infection did not have any appreciable effect on stem height of GR plants. The slight increase in mean height shown by the inoculated plants over the controls was not significant. But in this connexion it is of some interest that Blackhurst and Wood (1963) made similar observations for inoculated Loran Blood plants. No reason can be offered for this slight stimulation in growth resulting from infection.

Petiolar epinasty was evident in inoculated plants of both varieties within 10 days of inoculation (Table 3). Increase in adaxial angles also occurred in control plants, but there it took the form of a gradual increase with age in contrast with the sudden increase characteristic of inoculated plants. In inoculated GS plants the increases in the adaxial angles compared with the controls were very prominent in leaves 3 - 7 in the earlier stages of infection. But with time, the differences became much less marked. The upper leaves (8 - 10) of the control plants maintained, almost throughout the

Table 3 Mean adaxial angles of the leaves of control and inoculated plants recorded at regular intervals after inoculation

D	Treat-ment	Gem susceptible								Gem resistant							
		Leaf number								Leaf number							
		3	4	5	6	7	8	9	10	3	4	5	6	7	8	9	10
10	C	66	65	57	49	36	26			68	74	60	50	31	22		
	I	79	79	78	68	57	37			66	64	64	58	46	22		
		+13	+14	+21	+19	+21	+11			-2	-10	+4	+8	+15	0		
14	C	78	78	72	63	50	42	34		73	75	68	53	43	35	30	
	I	87	98	90	81	65	40	25		71	76	72	68	57	41	30	
		+9	+20	+18	+18	+15	-2	-9		-2	+1	+4	+15	+14	+6	0	
17	C	78	84	73	67	61	57	50	30	80	85	80	63	59	52	49	22
	I	87	92	90	85	72	50	36	28	73	77	77	72	66	60	56	38
		+9	+8	+17	+18	+11	-7	-14	-2	-7	-8	-3	+9	+7	+8	+7	+16
21	C	85	86	77	71	71	63	53	38	83	92	84	66	61	54	50	34
	I	-	-	92	87	74	65	53	40	78	88	86	80	70	69	61	45
		-	-	+15	+16	+3	+2	0	+2	-5	-4	+2	+14	+9	+15	+11	+11
24	C	87	88	83	79	74	75	79	67	87	92	85	74	69	67	65	58
	I	-	-	92	90	77	69	60	53	82	90	89	87	78	78	81	77
		-	-	+9	+11	+3	-6	-19	-14	-5	-2	+4	+13	+9	+11	+16	+19
28	C	91	96	89	85	79	83	81	82	91	94	89	77	80	75	83	84
	I	-	-	-	93	79	74	71	63	-	95	92	89	81	82	84	82
		-	-	-	+8	0	-9	-10	-19	-	+1	+3	+12	+1	+7	+1	-2

Average of 5 replicates
D = days after inoculation
C = Control
I = Inoculated

experiment, wider angles than those of the inoculated plants. In inoculated GR plants the adaxial angles of the lower leaves (3 - 5) rarely showed any appreciable increase over those of the controls at any stage of this experiment. For the upper leaves (6 - 10), however, the angles in inoculated plants were consistently greater than those in the controls. The difference between the lower and the upper leaves of each variety in their post-inoculation behaviour is difficult to explain. Epinasty, although pronounced in this experiment, can not, however, be regarded as a reliable symptom. It was often absent in subsequent experiments when other symptoms were highly developed.

Table 4 Mean number of leaves in healthy and inoculated plants recorded at regular intervals after inoculation

Plant	Treatment	Days after inoculation					
		10	14	17	21	24	28
GR	Control	8.2	9.6	10.4	11.4	12.0	13.6
	Inoculated	8.4	10.6	11.4	12.2	12.6	14.4
GS	Control	8.2	10.2	10.6	11.6	12.2	13.8
	Inoculated	8.8	9.8	10.0	11.2	11.8	13.2

Average of 5 replicates

Of the growth characteristics studied, the total number of leaves carried by a plant was the least affected by infection (Table 4). In the resistant variety, inoculated plants always had a slightly higher average of leaves than the controls, but the reverse was true for the susceptible variety. These results agree with those of Selman and Pegg (1957) and Threlfall (1957) who showed that in tomato plants infection reduced the total leaf area and that this was due not to a reduction in the number of leaves, but to their failure to expand.

Table 5 Effect of infection on dry weights of resistant and susceptible plants as a whole, shoot and root determined after 14 and 28 days.

D	Plant	Mean dry wt. (g.)			Percentage increase or reduction in dry wt.		
		Plant	Shoot	Root	Plant	Shoot	Root
14	GRC	4.63	3.60	1.03			
	GRI	4.75	3.80	0.95	+2.5	+5.5	-7.7
	GSC	3.65	2.85	0.80			
	GSI	2.60**	2,31*	0.29**	-28.8	-18.9	-63.5
28	GRC	10.13	7.73	2.40			
	GRI	11.13	8.10	3.03	+9.8	+4.6	+26.2
	GSC	10.60	8.55	2.05			
	GSI	1.88**	1.53**	0.35**	-82.2	-84.4	-82.9

Average of 3 replicates
* Significant difference: P = 0.05
** Significant difference: P = 0.05
D = Days after inoculation

That infection has considerable effect on dry-matter production in GR and GS plants, is evident from Table 5. The mean dry weight of inoculated GR plants was slightly greater than that of the controls on both occasions when samples were taken, but the differences were not statistically significant. In GS plants, on the other hand, infection caused a highly significant reduction in dry weight as soon as 14 days after inoculation. Two weeks later, the percentage reductions in dry weight for whole plant, shoot and root were much higher. Root growth was adversely affected in GR plants in the earlier stages of infection. At the end of the experiment, however, inoculated plants showed a much higher dry weight of root than the controls. In GS plants also, in the early stages of infection, the root was more affected in its growth than the shoot, but in the later stages both appeared to have suffered equally.

Table 6 Mean number of vessels containing hyphae, or tyloses and showing browning of wall at selected heights of stem and at different stages after inoculation

	D	Height of section above cotyledonary node (cm.)													
		Gem susceptible								Gem resistant					
		2.5	5.0	7.5	10.0	12.5	15.0	17.5	20.0	2.5	5.0	7.5	10.0	12.5	15.0	17.5
Hyphae	10	72	58	29	7	0				6	5	3	2	0	0	
	14	59	53	20	21	6	1	0		0	0	0	0	0	0	0
	17	89	78	44	26	11	4	0		2	0	0	0	0	0	0
	21	82	49	49	39	17	5	0		0	0	0	0	0	0	0
	24	43	22	21	12	15	3	0		0	0	0	0	0	0	0
	28	38	32	27	23	23	14	5	0	0	1	2	2	0	0	0
Vascular browning	10	12	9	9	5	0				1	0	0	0	0	0	
	14	6	5	5	4	1	0	0		1	1	0	0	0	0	0
	17	8	12	10	9	10	0	0		3	1	0	0	0	0	0
	21	11	6	6	5	5	2	1		0	0	0	0	0	0	0
	24	7	7	6	6	5	4	1		0	0	0	0	0	0	0
	28	6	4	5	2	4	4	0	0	0	1	2	2	0	0	0
Tyloses	10	1	1	2	2	0				36	28	30	24	25	23	
	14	3	3	4	5	5	10	1		20	12	10	6	5	7	6
	17	3	4	5	7	6	5	0		60	38	25	19	10	11	9
	21	15	15	23	19	18	13	15		49	32	26	20	16	14	15
	24	29	34	32	30	26	30	23		68	90	90	63	31	32	25
	28	52	55	56	53	54	53	58	47	88	119	107	68	35	34	29

D = days after inoculation

Average of 2 replicates

Table 7 Mean percentage of vessels containing hyphae, or tyloses and showing browning of wall at selected heights of stem and at different stages after inoculation.

	D	Height of section above cotyledonary node (cm.)													
		Gem susceptible								Gem resistant					
		2.5	5.0	7.5	10.0	12.5	15.0	17.5	20.0	2.5	5.0	7.5	10.0	12.5	15.0	17.5
Hyphae	10	42	36	20	7	0				2	2	1	1	0	0	
	14	24	22	10	12	4	1	0		0	0	0	0	0	0	0
	17	31	29	17	12	6	4	0		1	0	0	0	0	0	0
	21	23	16	16	13	7	4	0		0	0	0	0	0	0	0
	24	11	6	8	5	6	1	0		0	0	0	0	0	0	0
	28	10	8	7	7	7	5	2	0	0	0	1	1	0	0	0
Vascular browning	10	7	6	6	4	0				1	0	0	0	0	0	0
	14	4	4	4	5	2	0	0		0	0	0	0	0	0	0
	17	5	5	5	6	6	0	0		1	0	0	0	0	0	0
	21	5	3	3	3	3	1	1		0	0	0	0	0	0	0
	24	2	2	2	3	2	2	1		0	0	0	0	0	0	0
	28	2	1	2	1	2	2	0	0	0	0	0	0	0	0	0
Tyloses	10	1	1	1	2	1				16	13	16	14	19	17	
	14	1	1	2	3	3	12	4		11	8	7	5	7	8	7
	17	1	1	2	3	3	5	3		19	12	10	8	7	8	7
	21	4	5	7	6	7	7	11		16	12	11	11	9	9	9
	24	7	9	9	11	10	13	13		14	19	22	17	11	11	9
	28	13	14	16	16	16	18	22	19	18	23	25	16	9	9	8

Average of 2 replicates

D = days after inoculation

When observation started 10 days after inoculation, fungal hyphae were extensively distributed in inoculated GS plants (Tables 6 and 7). At this stage they were mainly confined to vessels of the primary protoxylem and metaxylem (Fig. 1). Hyphae were found to pass freely through pits from one vessel to another, but lateral growth was not extensive enough to infect more than a few vessels in the secondary xylem. Even at this early stage of infection, about 40 per cent of the total number of vessels with the hyphae were nearly completely occluded by the fungus. With time, the fungus spread upwards and hyphae were observed also in some tracheids and in a few xylem parenchyma cells adjacent to heavily infected vessels. But hyphae were never found to proliferate in xylem parenchyma cells or spread beyond them. This agrees with the findings of Blackhurst and Wood (1963). While the fungus was gradually spreading in the upper parts of the stems, it started disappearing from the lower parts. The beginning of a progressive reduction in the number as well as the percentage of vessels containing hyphae was apparent in the lower part of the stem as soon as 21 days after inoculation (Tables 6 and 7). The disappearance of hyphae from vessels

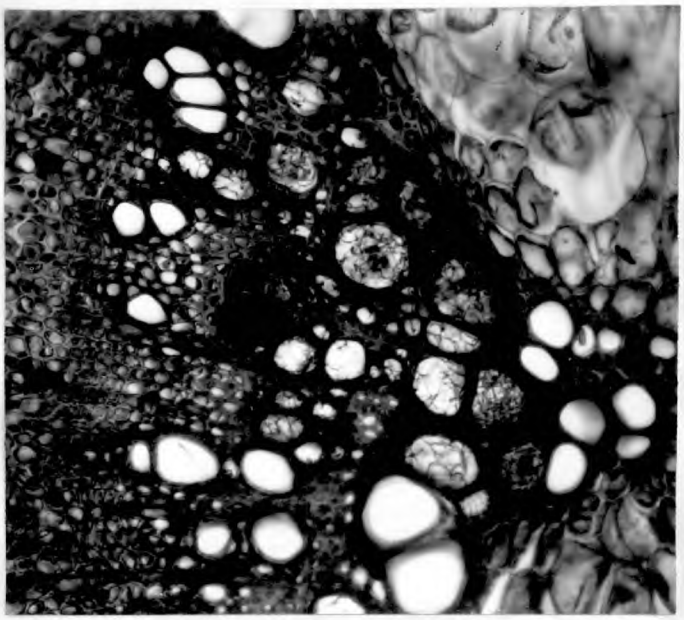


Fig. 1. Transverse section at the base of stem from root-inoculated Gem susceptible plant showing mycelium in vessels and tracheids. (x 160)

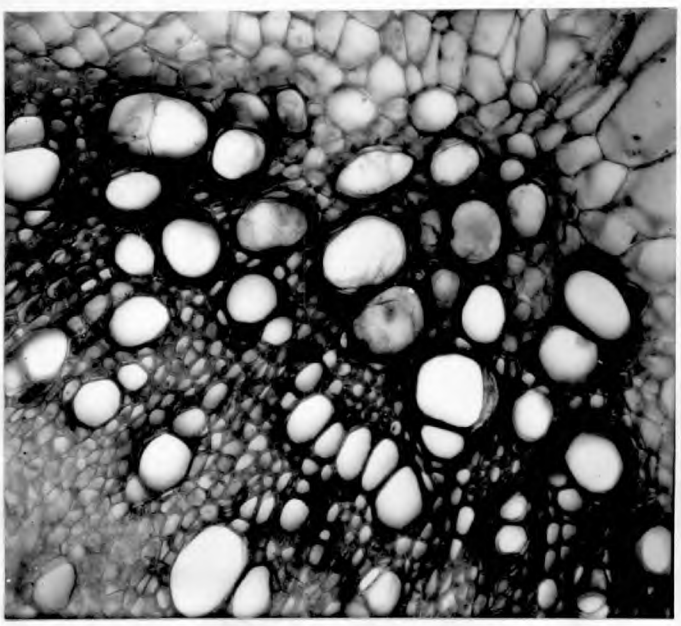


Fig. 2. Transverse section at the base of stem from root-inoculated Gem resistant plant showing tyloses in vessels. (x 160)

occurred also in the middle portion of the stem, but somewhat later. When the observations ended 28 days after inoculation, the fungus was still spreading upwards. It is evident from the results that hyphae began to die as early as 21 days after inoculation. A rapid increase in the amount of the mycelium followed by its gradual disappearance later has also been reported by Matta and Dimond (1963) in wilt of susceptible tomato plants caused by Fusarium oxysporum f. lycopersici. The first signs of the disappearance of the fungus from the plant occurred at the same time as the check in the progress of foliar symptoms i.e., about 21 days after inoculation (Fig. 3). Identical observations were made by Matta and Dimond. Such coincidence probably emphasizes the relation between the amount of mycelium in the stem and foliar symptoms.

Hyphae were found in many vessels of petioles of inoculated GS plants (Table 8). Although the results for petioles were less evident, here too there was a suggestion that hyphae were present in fewer vessels in the older petioles of infected plants.

Fig. 3. RELATION OF FOLIAR SYMPTOMS IN SUSCEPTIBLE PLANTS TO DISTRIBUTION OF HYPHAE AND TYLOSES AT THE BASE OF STEM — DATA EXPRESSED AS PERCENTAGE OF MAXIMUM POSSIBLE VALUE.

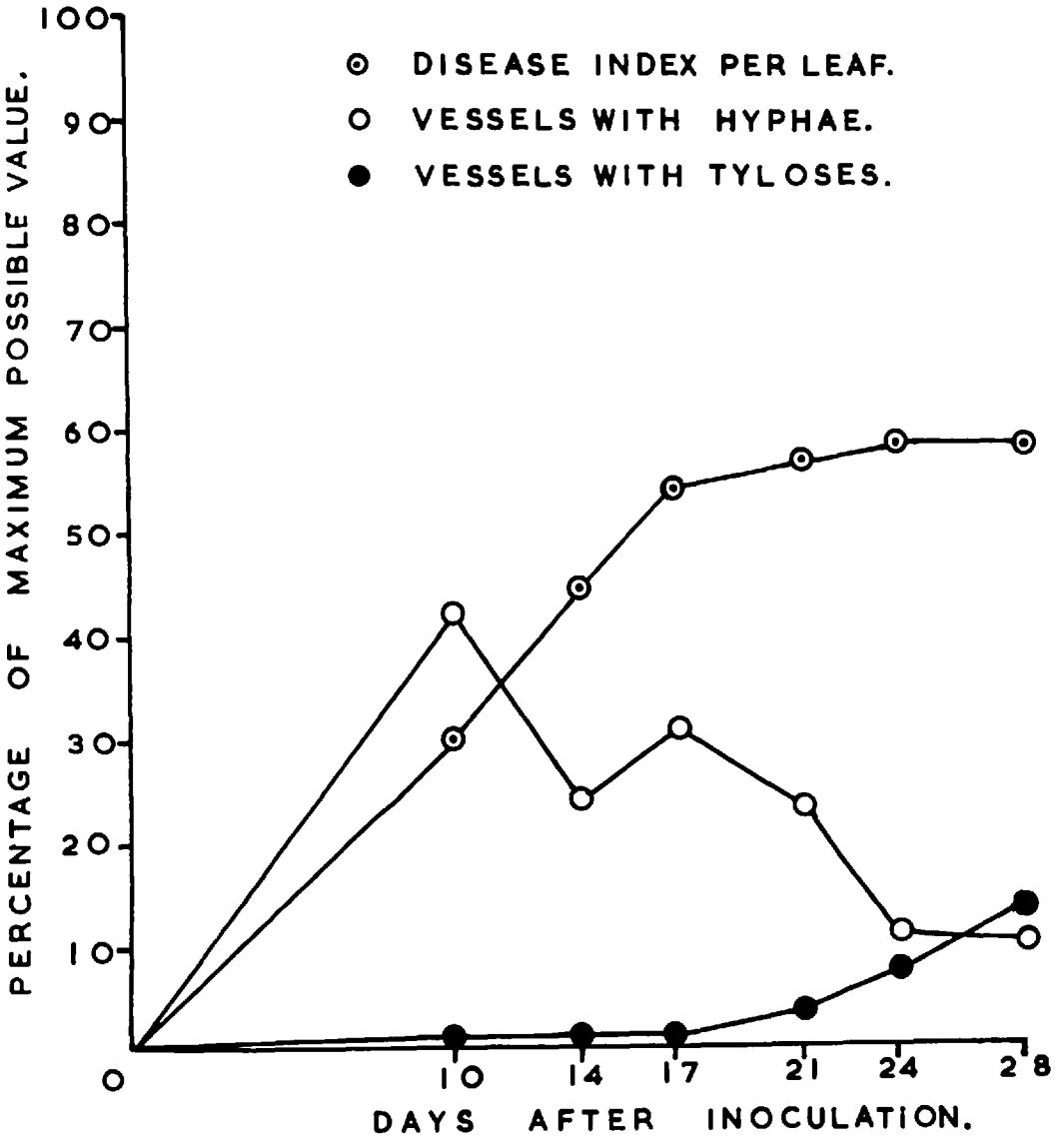


Table 8 Mean percentage of vessels at the base of petiole containing hyphae, or tyloses and showing browning of wall at different stages of infection

	D	Leaf number														
		Gem susceptible								Gem resistant						
		2	3	4	5	6	7	8	9	1	2	3	4	5	6	7
Hyphae	10	-	22	25	16	12				0	0	2	1	0	0	0
	14	18	29	8	5	3				0	0	0	0	0	0	0
	17	5	-	28	8	4	2			0	0	0	0	0	0	0
	21	4	-	-	6	7	8	0	0	-	-	0	0	0	0	0
	24	-	-	13	7	8	0	0		0	-	0	0	0	0	0
	28	-	-	11	19	14	15	4	1	-	-	0	0	0	0	0
Vascular browning	10	-	5	6	5	3				0	0	0	0	0	0	0
	14	6	3	2	2	0				0	0	0	0	0	0	0
	17	5	-	4	3	2	1			0	0	0	0	0	0	0
	21	9	-	-	2	2	2	0	0	-	-	0	0	0	0	0
	24	-	-	4	4	2	3	1		0	-	0	0	0	0	0
	28	-	-	3	4	3	2	1	0	-	-	0	0	0	0	0
Tyloses	10	-	0	0	1	0				13	0	22	24	12	9	13
	14	6	9	7	9	3				7	7	19	9	12	8	8
	17	4	-	6	8	8	5			9	9	6	7	9	4	9
	21	7	-	-	6	6	10	7	1	-	-	5	6	8	5	7
	24	-	-	10	14	17	15	11		14	-	12	14	9	11	13
	28	-	-	2	15	9	15	13	16	-	-	41	21	11	15	17

Average of 2 replicates

D = Days after inoculation

In inoculated GR plants the hyphae were never observed more than 10 centimetres above the cotyledonary node (Table 6). Hyphae were initially observed in a few vessels of the primary xylem. Later, hyphae were generally absent except at occasional points where 1-3 vessels contained 1 or 2 strands of hyphae. A few vessels of petioles of inoculated GR plants contained hyphae at the first sampling, but not thereafter (Table 8). This also suggests that hyphae die sometime after they have become established in resistant tissue. This is in line with earlier observations made for Fusarium wilt (Scheffer and Walker, 1954) and Verticillium wilt (Blackhurst and Wood, 1963) of tomato. The few isolated strands of hyphae which were observed as late as 28 days after inoculation may have been dead and in the process of dissolution. These results agree with those of Threlfall (1957), but differ from those of Blackhurst and Wood (1963) in showing that growth of the fungus in the stem of resistant plants was much more restricted than in the stem of susceptible plants.

Vessel collapse was observed only in the stems of inoculated susceptible plants. A collapsed

vessel is not hexagonal, or oval in cross section, but is instead quite irregular in shape. Often the opposite walls come into contact thus narrowing the lumen of the vessel. In the lower internodes, vessel collapse was observed only in a few metaxylem vessels of medium size. In the upper internodes, however, a high percentage of the vessels in the smaller vascular bundles were collapsed, and generally there was proliferation of xylem parenchyma around these bundles. In petioles vessel collapse was seen only occasionally. Hyphae were absent from most of the collapsed vessels. Chambers and Corden (1963) made similar observations for Fusarium wilt of tomato. This suggests that some fungal, or host metabolites may be responsible for vessel collapse. Wardlaw (1930) observed collapsed vessels in the roots of banana plants infected with Fusarium oxysporum f. cubense. He attributed it to pressure exerted on weakened vessel walls by expansion and proliferation of surrounding xylem parenchyma cells. The possibility that these changes may result from high levels of auxin in diseased tissue is supported by the work of Chambers and Corden (1963). They showed that vessel collapse

could be induced in the stem and petiole of tomato cuttings within 48 hr. by keeping them in dilute indole-3-acetic acid (IAA) solution, or raw culture filtrate of Fusarium oxysproum f. lycopersici. This fungus is known to produce IAA in vitro (Gruen, 1959). The same is also true for V. albo-atrum (Pegg and Selman, 1959).

Vascular browning was never very prominent in the inoculated plants of this experiment (Table 6). In the stems of inoculated GS plants, it was found generally in those vascular bundles where there was extensive mycelial development, and was restricted to the vessels of primary xylem. Occasionally browning was observed in some vessels without hyphae being present. It appears from Table 6 that vascular browning generally followed the upward spread of the fungus, but there were instances when it appeared in advance of the growing hyphae. Vascular browning regularly occurred in petioles of inoculated GS plants, but only in few vessels. In inoculated GR plants vascular browning was only occasionally observed in the stems. It was never recorded from the petioles.

In some later experiments, vascular browning was found to be quite extensive in the stem of severely diseased susceptible plants, particularly in

the upper internodes.

Tyloses are not common in uninoculated and undamaged plants of the two varieties used in this work. Sometimes they have been found at the base of mature stems, but even then in a few vessels only. In this experiment, two control plants from each of the two varieties, when sectioned at the base of the stem 28 days after inoculation, showed tyloses in less than 2 per cent of the vessels.

Inoculation with V. albo-atrum generally, though not invariably, stimulated production of tyloses in both GR and GS plants. The extent of such stimulation was, however, different for these two varieties.

In inoculated GR plants many vessels had tyloses as soon as 10 days after inoculation (Table 6). In the lower part of the stems tyloses were distributed over the primary xylem, but higher up they occurred mostly in metaxylem vessels. In the course of the next 18 days tyloses developed in very many new vessels. They were observed in the secondary xylem and sometimes in tracheids also. The considerable increase in the distribution of tyloses does not appear to be very striking in Table 7. This is due to the fact that the total number of vessels in

a cross-section also increased considerably during the last 18 days thus balancing, to a certain extent, any increase in the number of vessels with tyloses when this is expressed on a percentage basis. It will appear from Table 6 that tyloses were much more common in the lower 4 inches of the stem than in the upper parts. In the lower part of the stem tyloses were sometimes so numerous in a vessel that the lumen was completely occluded (Fig. 2).

There was also extensive tylosis in petioles of inoculated GR plants (Table 8) and this was evident within 10 days of inoculation.

In inoculated GS plants tyloses were seen initially in very few vessels (Table 6). However, they gradually developed in many new vessels and by the time of last sampling were common in the vascular bundles. Nevertheless, it is clear that there were many fewer vessels with tyloses in inoculated GS plants than in inoculated GR plants at each stage of infection. In inoculated GS plants tylosis was more extensive in the upper parts of the stem than in the lower. This may be particularly significant because the fungus was very restricted in its spread in the upper part of the stem. However, tyloses were rarely observed together with hyphae in the same vessel.

Tyloses were practically absent from the petioles of inoculated GS plants when sampling commenced 10 days after inoculation. Within the next 4 days they were observed in a few vessels, and the number of such vessels gradually increased with time (Table 8).

The results show that tylosis was stimulated in both resistant and susceptible plants as a result of infection, and that tyloses were initiated in advance of the growing hyphae in both varieties. However, the two varieties show clear differences in the speed of their response to infection. In resistant plants the response is rapid and persistent, but in susceptible plants the response comes later and develops gradually. Tyloses appear to be connected, in some way or other, with the resistant reaction. The fact that the late stimulation of tylosis in inoculated GS plants almost coincides with the beginning of the disappearance of the fungus from the stem (Fig. 3) may be significant in this connexion.

Often deposits of brownish materials were visible in the intercellular spaces bordering infected vessels in primary xylem. These deposits may break the connexions between xylem cells by plugging the pits (Chambers and Corden, 1963). Occasionally, in

free-hand sections, a vessel was found to be filled with dark brown material, but this was too infrequent to be of any importance.

In inoculated GS plants showing pronounced symptoms, proliferation of xylem parenchyma was observed in some of the infected vascular bundles. In the lower part of the stem, it was not common in the larger bundles, but it sometimes occurred around the smaller bundles. In the upper part of the stem, however, many small vascular bundles showed proliferation of xylem parenchyma with characteristic transverse walls. Many of these bundles had also a few collapsed vessels and deposits of brownish materials in the intercellular spaces.

The observations made in course of this experiment, show how differently GR and GS plants respond to infection by V. albo-atrum. The association between foliar symptoms, such as wilting and the distribution of mycelium in the stem and petioles is apparent, but to what extent wilting depends on the amount of mycelium in vessels is not clear. In inoculated susceptible plants, as many as 42 per cent of vessels were variously plugged with hyphae as soon as 10 days after inoculation. But in view of the findings of Ludwig (1952), Waggoner and Dimond

(1954) and Threlfall (1957) it appears to be unlikely that vessel occlusion by hyphae was distributed widely enough to be solely responsible for wilting. The frequent occurrence of hyphae in the petioles of susceptible plants may be significant in this connexion. Dimond and Edgington (1960) have shown for Fusarium-infected tomato plants that to maintain an undiminished flow through a vascular bundle in which effective radius has been reduced by 50 per cent due to occlusion by hyphae, the increase of pressure required in a petiole is more than 500 times of that required in a stem. From their findings it appears that occlusion of vessels in a petiole is much more important for wilting in a leaf than similar occlusion in the stem. The slight initial wilting shown by inoculated resistant plants was possibly due to occlusion of vessels by tyloses which developed in large numbers in both stems and petioles soon after inoculation.

Because the disease was well advanced when sampling began, it was not known if the fungus was well distributed in the stems of susceptible plants before symptoms appeared. It is also not clear if the fungus spread considerably in the stems of resistant plants and then rapidly disappeared, or

if it was restricted to a few vessels from the beginning. Indirect evidence, such as lack of vessel collapse, or the occurence of vascular browning in very few vessels suggests the second explanation. An inverse relationship between the extent of tylosis and the amount of mycelium in the vessels of infected plants is indicated for both resistant and susceptible varieties. This was also reported by Talboys (1958b) in hop plants infected with V. albo-atrum. The anatomical studies further suggest that growth regulating substances may be implicated in the syndrome.

2. Further studies on the distribution of the fungus in resistant and susceptible plants, and relationship between foliar symptoms and the presence of hyphae, vascular browning and tyloses in the vessels

An experiment was designed to clarify a few points raised in connexion with the previous one.

Twenty, 7-week-old GR and GS plants were root-inoculated. There were adequate controls from each variety. After 4, 7, 9 and 12 days of inoculation, two GR and two GS plants were taken at random for anatomical studies. The wilting index was determined for each plant. Free-hand sections were made

from fresh materials at the cotyledonary node, 2.5 centimetres below and 5.0, and 10.0 centimetres above that point. These sections were observed microscopically to determine the percentages of vessels with hyphae, or tyloses. Sections were also made from two control plants of each variety at the cotyledonary node 12 days after inoculation. Tyloses were found in 1.2 per cent and 1.0 per cent of vessels in control GR and GS plants respectively. The results in respect of inoculated plants are shown in Table 9.

It is evident from these results that although the fungus infects the roots of resistant plants and reaches the lower parts of the stem, it does not grow much further. The fungus remained restricted to a few vessels in resistant plants at a time when it was well spread in susceptible plants. Further, signs of disappearance of the fungus from resistant plants were evident as soon as 12 days after inoculation. An early stimulation of tylosis appears to be intimately connected with infection in resistant plants, but whether this is the cause or an effect of a resistance mechanism in such plants is not clear. The observations made in the course of this experiment also showed that the fungus was

Table 9 Percentage of vessels with hyphae, or tyloses in resistant and susceptible plants at early stages of infection

D	Plant	Hyphae				Tyloses			
		CN-2.5 cm.	CN	CN+5 cm.	CN+10 cm.	CN-2.5 cm.	CN	CN+5 cm.	CN+10 cm.
4	GR	0	0	0	0	2.9	1.7	1.0	0
	GS	0	0	0	0	0.9	1.0	0.6	0
7	GR	0.6	0	0	0	4.5	4.4	0.9	0
	GS	3.6	2.9	3.2	0.9	1.3	0.9	0.4	0
9	GR	2.7	1.7	0.6	0	22.0	18.3	13.2	12.3
	GS	4.8	5.7	3.3	1.7	1.5	0.9	1.0	1.0
12	GR	0.4	0	0	0	17.9	14.6	11.6	11.7
	GS	23.5	24.5	17.0	11.8	0.9	1.0	1.4	1.9

Average of 2 replicates

D = Days after inoculation

CN = Cotyledonary node

well distributed inside the stem before the leaves wilted. This is in agreement with the findings of Kamal (1954) for wilt of cotton caused by Verticillium dahliae Kleb.

Two weeks after inoculation, six GR and six GS plants were taken at random for a study of vessel occlusion. Wilting index per leaf was recorded for each plant. Free-hand sections were prepared from all plants at the cotyledonary node. These were observed microscopically for a visual assessment of occlusion in the vessels. Each vessel in a section was rated for the extent of occlusion in it, caused either by hyphae or tyloses, on a scale as follows: 0 = no occlusion; 1 = about $\frac{1}{4}$ of lumen occluded; 0.5 = about $\frac{1}{2}$ of lumen occluded; 0.75 = about $\frac{3}{4}$ of lumen occluded and 1.0 = complete occlusion. The number of vessels in each class was multiplied by the factor representing the degree of occlusion. The sum total of all such values, divided by the total number of vessels in the section and multiplied by 100, gave a rough estimate of the degree of dysfunction in vessels due to occlusion. The results, shown in Table 10, express the degree of dysfunction in vessels as a percentage of the total capacity for conduction.

Table 10 Relationship between wilting and vessel occlusion in resistant and susceptible plants 14 days after inoculation.

Plant	Replicate No.	Wilting index	Percentage of vessels with		Degree of dysfunction in vessels as percentage of total conducting capacity		
			Hyphae	Tyloses	Due to hyphae	Due to tyloses	Total
GR	1	0	0	8.2	0	5.3	5.3
	2	0	0	11.5	0	6.2	6.2
	3	0	0	14.6	0	7 2	7 2
	4	0	0	18.6	0	8.9	8.9
	5	0.3	0	45.0	0	28.1	28.1
	6	0.4	3.2	49.3	C.5	26.3	26.8
GS	1	0.5	12.8	9.7	6.6	4.7	11.3
	2	0.9	18.2	16.9	8.9	10.0	18.9
	3	1.0	23.6	21.2	8.6	15.4	24.0
	4	1.5	29.8	3.5	14.7	2.6	17.3
	5	2.1	30.3	1.8	16.5	1.2	17.7
	6	2.2	36.2	0.7	18.3	0.7	19.0

The results suggest some relation between the amount of wilting in a plant and the degree of dysfunction in its vessels due to occlusion. Occlusion of vessels caused by hyphae was found to be more important as a factor in wilting than that caused by tyloses. Wilting in inoculated susceptible plants increased with an increase in vessel occlusion due to hyphae. In inoculated resistant plants, hyphae were mostly absent at the base of the stem. Tyloses were common to such plants, but only mild wilting developed when considerable vessel occlusion occurred due to extensive tylosis.

Three apparently healthy and twenty-six leaves showing symptoms were collected from the lower parts of diseased GS plants between 9 and 16 days after inoculation. The severity of wilting and yellowing were assessed for each leaf on the basis of scales mentioned previously. The values 0, 1, 2 and 3 in that scale stand for no, slight, moderate and complete symptoms respectively as recorded in Table 11. Sections were made at the base of petiole and the percentages of vessels with hyphae, browning or tyloses were recorded. Results are given in Table 11 which does not include two of the three apparently healthy leaves because they showed neither

Table 11 Relationship of foliar symptoms to the presence of hyphae, vascular browning and tyloses in petioles from infected susceptible plants.

Serial No. of leaf	Degree of symptoms produced		Percentage of vessels with		
	Wilting	Yellowing	Hyphae	Browning	Tyloses
1	nil	nil	8.6	10.0	2.8
2	"	slight	0	0	1.8
3	"	"	4.3	2.8	7.1
4	"	"	0.5	2.1	6.3
5	"	moderate	0	0	5.7
6	"	"	0	0	20.5
7	"	"	0	0	0
8	slight	nil	13.8	2.1	5.3
9	"	slight	8.3	13.8	0
10	"	moderate	6.6	8.7	10.8
11	"	complete	2.9	0	14.2
12	moderate	nil	8.8	4.4	4.4
13	"	"	17.9	15.3	0
14	"	"	5.2	0	4.2
15	"	slight	15.5	10.7	3.6
16	"	"	14.6	9.3	1.3
17	"	"	21.6	10.8	2.7
18	"	"	8.8	1.5	2.9
19	"	"	3.0	7.0	2.9
20	"	moderate	8.7	17.4	5.2
21	"	"	30.0	24.3	2.8
22	"	complete	13.3	30.0	3.3
23	"	"	20.3	25.4	3.3
24	complete	slight	3.4	5.2	1.7
25	"	moderate	3.4	1.7	5.2
26	"	"	6.9	10.6	7.0
27	"	"	26.7	9.0	16.7

hyphae, nor browning nor tylosis in the vessels.

It appears that yellowing does not have much relation to the presence of hyphae in petioles. Slight to moderate yellowing occurred in some leaves even when the petioles were free of hyphae. The degree of wilting, however, can be correlated with the presence of hyphae, although the correlation is by no means a very close one. There were hyphae in all twenty wilted leaves and also in three out of nine leaves not showing any wilting. An increase in wilting of leaves was usually accompanied by a wider distribution of hyphae, but in three out of four completely wilted leaves hyphae were observed in many fewer vessels than was expected. Vascular browning was closely associated with the presence of hyphae. No correlation was found between foliar symptoms and tylosis in petioles.

3. Various effects of infection on the plants growing in nutrient solution

To confirm some of the observations made earlier, and to test the possibility of using plants growing in nutrient solution for studying different aspects of disease, the following experiment was done.

Three, 3-week-old GR and GS plants grown in

nutrient solution, were root-inoculated. In each plant, 4 lateral roots were trimmed with a pair of scissors before inoculation to facilitate infection. Three controls from each of the two varieties also had their roots trimmed in the same way.

Within 10 days of inoculation, root growth was definitely checked in inoculated GS plants. There was no sign of stunting in the shoots at that time. In the course of the next 2 days yellowing developed in the lower leaves of all 3 inoculated GS plants in which stunting was also evident. Inoculated GR plants did not show any shoot symptoms within 15 days after inoculation. By this time, all inoculated GS plants showed a smaller root system consisting of many fewer and somewhat shorter lateral roots than control plants. In GR plants no such effect of infection was evident. Disease and wilting indices for leaves of inoculated GS plants were 0.55 and 0.35 respectively 15 days after inoculation. Leaf area was measured for all plants. This was done by placing individual leaves on a graph paper, drawing the outline of leaf blade on it and then determining the area of leaf. Dry weights were recorded for root, stem and leaf in milligrams. Results, being the averages of 3 replicates, are given in Table 12.

Table 12 Effect of infection on leaf area and dry weights of plant organs determined 15 days after inoculation.

Plant	Leaf area (Sq.cm.)	Dry wt. (mg.)				Percentage increase or decrease due to infection				
		Plant	Root	Stem	Leaf	Leaf area	Dry weight Plant	Root	Stem	Leaf
GRC	42.9	194	41	41	112					
GR1	53.6**	230**	44	48**	138**	+24.9	+18.5	+7.3	+17.0	+23.2
GSC	63.6	268	56	58	154					
GS1	34.2**	177**	40**	41**	96**	-46.0	-33.9	-28.5	-29.3	-37.7

* Significant difference: P = 0.05

** Significant difference: P = 0.01

Results support the observations made earlier that infection stimulates growth in GR plants and checks that of GS plants. Inoculated GR plants recorded highly significant increases in total leaf area and dry weights of the plant as a whole, stem and leaf over the controls. Increase in dry weight of root was not statistically significant. Considerable reduction in leaf area in GS plants resulting from infection agrees with the results of Selman and Pegg (1957) and Threlfall (1957). It is also clear that plants growing in nutrient solution can be usefully employed in different experiments involving the production of normal disease symptoms.

4. Disease development in inoculated cuttings

The experiment described below was designed primarily to determine whether cut shoots inoculated through their cut ends developed typical disease symptoms similar to those that develop in root-inoculated plants. This technique of inoculation eliminates root as a factor in infection. It was hoped that the results obtained by using this technique would give some basic information regarding the site and nature of resistance in a plant.

Stem cuttings about 12 centimetres in height, and taken from 8-week-old plants, were inoculated by the method described earlier. There were five inoculated and five control plants from each of the two varieties. They were kept in an illuminated, constant temperature cabinet ($18^{\circ}\pm1^{\circ}C$) for 3 weeks before the transfer to greenhouse.

Most of the typical symptoms including wilting, yellowing and initiation of adventitious roots developed in inoculated cuttings, but epinasty did not appear prominently. Wilting was evident in plants of both varieties within 7 days of inoculation, although there was considerable variation

among the replicates in each set. Thereafter, differences became apparent in the two varieties. The differences in mean wilting indices between the control and the inoculated plants were never significant statistically for the resistant variety, but always highly significant for the susceptible variety, except when first sampled 7 days after inoculation (Table 13).

Table 13 Wilting indices per leaf for cuttings of resistant and susceptible plants recorded at regular intervals

Plant	Treatment	Days after inoculation				
		7	14	21	28	35
GR	Control	0.45	0.60	0.55	0.60	0.50
	Inoculated	0.43	0.80	0.82	0.85	0.75
GS	Control	0.22	0.40	0.34	0.32	0.28
	Inoculated	0.60*	1.07**	1.39**	1.49**	1.45**

Average of 5 replicates

* Significant difference : P = 0.05

** Significant difference : P = 0.01

The slight initial wilting shown by all the plants was probably attributable to the absence of roots. Wilting caused by the parasite became apparent in later stages as was evident from the differences between the controls and the inoculated plants.

The heights of plants were measured at the end of experiment. Infection was found to have reduced the average heights of GR and GS plants by 1.9 per cent and 46.6 per cent respectively.

At the end of the experiment, the inoculated cuttings were sectioned at certain points. The results of microscopical observation are summarized in Table 14.

The similar extent of proliferation of xylem parenchyma in the cuttings of GR and GS plants suggests that the fungus was initially well spread in both varieties.

The lower incidence of the fungus in GR cuttings also suggests that it has already started disappearing from the stem. A similar behaviour of resistant cuttings was previously reported by Scheffer and Walker (1954) for tomato wilt due to

Table 14. Presence of hyphae, and nature of tylosis and proliferation of xylem parenchyma in the inoculated cuttings from resistant and susceptible plants

Plant	Height of section from base (cm.)	Percentage of vessels with hyphae	Tylosis	Proliferation of xylem parenchyma
GR	5.0	4.3	Extensive	Extensive
	10.0	2.1	Extensive	Moderate
	12.5	0	Slight	Nil
GS	5.0	13.8	Moderate	Extensive
	10.0	3.6	Slight	Moderate
	12.5	0	Slight	Nil

Average of 2 replicates

Fusarium oxysporum f. lycopersici. It is evident from these facts that resistance is not localized in the roots as often suggested, but is also present in the stem.

The cuttings were found to be useful as test material, because they responded to inoculation in the same way as root-inoculated plants.

5. Distribution and survival of the fungus in inoculated cuttings from resistant and susceptible plants.

The results from previous experiments have shown that both in root-, and shoot-inoculated plants the fungus starts disappearing a certain time after it has become established in the xylem. The timing of this disappearance is, however, different for resistant and susceptible plants, occurring much earlier in the former than in the latter. Resistant plants, inoculated through root, or shoot, may show slight symptoms of disease initially, but they always soon recover. As inoculum is present and well distributed in shoot-inoculated plants, this recovery means that an infected resistant plant frees itself of the fungus and does not merely become tolerant to the parasite and its effects in the vascular system. It was, therefore, of some interest to make a detailed study of the distribution of the fungus in cuttings from GR and GS plants.

Stem cuttings, about 17.5 centimetres long and taken from 11-week-old plants, were used in this experiment. Eighteen cuttings from each variety were inoculated following the usual method. There were four control plants from each variety.

Wilting was assessed with four inoculated and four control plants from each variety 14 and 21 days after inoculation. None of the controls showed any wilt at any time. The wilting indices per leaf for GR and GS cuttings were respectively0.06 and 0.73 after 14 days and 0.06 and 0.85 after 21 days. Every third day, for 21 days, two inoculated cuttings from each variety were removed for anatomical studies. Free-hand sections were made from the stem at intervals of 2.5 centimetres and examined microscopically. The results of these observations are summarized in Table 15.

It appears that the inoculum became well distributed in the conducting channel of both GR and GS cuttings within a few days of inoculation. The rate of spore germination was somewhat lower in GR cuttings than in GS cuttings. In the infected vessels mostly germ tubes were observed, sometimes in a bunch, originating from the vessel wall at a particular point, but rarely from all the sides. Active fungal growth in the form of proliferation of hyphae, was not observed in GR cuttings. It was observed in GS cuttings only from the 18th day onwards. When sampling concluded 21 days after inoculation, the fungus was found to be still spreading

Table 15 Percentages of vessels with hyphae or tyloses in inoculated cuttings of resistant and susceptible plants

D	Plant	Height of section from cut end in centimetres								
		Hyphae								
		2.5	5	7.5	10	12.5	15	17.5	20	22.5
3	GR	16	1	2	0					
	GS	9	3	2	0					
6	GR	11	6	1	2	1	0			
	GS	22	11	5	6	4	3			
9	GR	12	9	7	7	5	1	1	0	
	GS	15	10	9	10	6	4	2	1	
12	GR	16	10	8	8	8	3	1	0	
	GS	33	25	16	13	12	7	4	0	
15	GR	16	7	4	4	9	1	0	0	
	GS	23	19	10	15	19	12	1	0	
18	GR	22	12	11	9	8	3	0	0	0
	GS	28	24	11	17	13	11	18	1	0
21	GR	18	13	4	2	1	0	0	0	0
	GS	32	26	33	21	23	16	18	14	7

D	Plant	Height of section from cut end in centimetres								
		Tyloses								
		2.5	5	7.5	10	12.5	15	17.5	20	22.5
3	GR	4	10	5	6					
	GS	1	5	6	3					
6	GR	10	13	24	19	1	0			
	GS	4	5	6	7	5	5			
9	GR	10	18	20	14	11	7	4	0	
	GS	6	8	9	12	8	5	5	2	
12	GR	10	14	12	11	12	7	5	0	
	GS	4	5	8	6	7	4	4	0	
15	GR	6	9	16	17	12	12	1	0	
	GS	5	8	15	14	13	7	5	0	
18	GR	13	9	27	16	14	14	15	4	0
	GS	5	9	10	8	9	10	7	8	0
21	GR	6	15	24	32	30	12	11	6	3
	GS	10	11	15	15	14	8	7	7	7

Average of 2 replicates

D = Days after inoculation

through GS cuttings by infecting more and more vessels and extending upwards. In GR cuttings, on the other hand, the distribution of hyphae never became extensive. In them hyphae were never observed higher than 17.5 centimetres from the base i.e., height of a cutting at the time of inoculation. Further, there was also clear evidence that hyphae gradually disappeared from the resistant stem. This process was first evident near the top of GR cuttings as soon as 15 days after inoculation and gradually affected the lower parts. It is possible that if the experiment had been continued for a longer period, the fungus would have been found near the base only. The results confirm the earlier conclusions about the inverse relationship between the distribution of hyphae and tyloses in infected plants and also the earlier stimulation of tylosis in inoculated resistant as compared with inoculated susceptible plants.

6. Studies with detached leaves

Provvidenti and Schroeder (1959) found that the leaves of tomato plants could be infected by spraying them with a spore suspension of V. albo-atrum. In susceptible varieties such infection caused enlarging lesions, followed by premature leaf fall and it sometimes led to systemic invasion of the plant. In a resistant variety, however, the intensity of symptoms on infected leaves gradually decreased. Griffiths and Isaac (1963) have recently shown that when sprayed on to plants as a spore suspension, V. dahliae from Brussels sprouts, invariably failed to infect the leaves of tomato plants. Similarly V. tricorpus, isolated originally from tomato, was found to infect tomato leaves only in the presence of an adequate source of carbohydrate, but even under these conditions it never produced spreading lesions. Griffiths and Isaac also found, in the sap expressed from healthy leaves of tomato, a water-soluble, thermolabile substance which although not lethal to conidia suspended in distilled water, inhibited their germination.

In the previous experiments some correlation was found between wilting in a leaf and the

presence of hyphae in its petiole. In resistant plants the leaves rarely wilted and the hyphae were seldom present in the petioles. But in susceptible plants wilting was common and the affected leaves always had hyphae in their petioles. It was, therefore, of some interest to determine how differently the detached leaves from GR and GS plants would behave when inoculated with a spore suspension through their cut ends.

Detached leaves were inoculated in the same way as were cuttings. In preliminary experiments healthy, fully developed leaves were used. Inoculated GS leaves generally showed somewhat higher wilting indices than inoculated GR leaves. But the variations within each set were too great to permit any general conclusion. Further complication resulted from occasional wilting of control leaves and the appearance of yellowing in most of the leaves within 3 - 4 days. As many of the leaves, including those of the controls, showed almost complete yellowing within a week, the experiments had to be discontinued at that stage. Benzimidazole when added to the dilute nutrient solution at 40 p.p.m., did not maintain the greenness

of leaves. It was found, however, that if somewhat younger, and still developing leaves were used better results were obtained. These leaves were less affected by yellowing and could be maintained for observation for a longer period. Results of two experiments in which such leaves were used are given in Table 16.

Table 16 Development of wilting and yellowing in detached leaves

Expt. no.	No. of replicates	Plant	Days after inoculation							
			Wilting				Yellowing			
			4	7	11	14	4	7	11	14
1	3	GRC	0	0	-	0	0.5	1.0	-	2.5
		GRI	0	0	-	0	0	0.7	-	2.0
		GSC	0	0	-	1.0	0	0.5	-	2.0
		GSI	0	1.7	-	2.3	0.3	1.0	-	2.3
2	4	GRC	0	0	0	0.3	1.0	1.5	1.5	2.3
		GRI	0.3	0.5	0.5	0.8	1.0	1.5	2.3	2.5
		GSC	0	0	0	0	1.0	1.5	2.0	2.3
		GSI	1.0	1.5	1.5	1.5	1.0	1.8	1.8	2.5

Average of 3 replicates D = Days after inoculation

Although there was some variation from one experiment to another, the pattern was the same in both. Inoculated GS leaves always gave higher wilting indices than inoculated GR leaves. Yellowing was equally extensive in all the leaves.

In the second experiment, at the end of sampling, two inoculated leaves of each variety were sectioned at the middle of the petioles and the sections observed microscopically. The mean percentages of vessels with hyphae, browning and tyloses were respectively 44.4, 4.0 and 3.0 for GR leaves and 53.0, 8.5 and 0.5 for GS leaves. The differences between the two varieties were relatively small. But the trends were the same as those shown by root- and shoot-inoculated plants. Thus it appears that the qualitative differences existing between GR and GS plants are also reflected in the behaviour of their leaves when detached and inoculated.

7. Effect of infection on the production and growth of lateral roots

The results from the previous experiments and the findings of Selman and Pegg (1957) leave no doubt as to the inhibitory effect of Verticillium infection on the root growth of susceptible tomato plants. This is not unexpected, because the roots are the first to be affected by the fungus. The growth of roots has sometimes been found to be checked even before foliar symptoms were apparent. Reduced root growth in the soil is characteristic of most of the wilt diseases. Peterson and Pound (1960) studied root growth in radish plants infected with Fusarium oxysporum f. conglutinans. In infected susceptible plants, secondary root development was increasingly reduced with time until it stopped completely in severely diseased plants. The initiation of adventitious roots from the lower part of the stem is a very characteristic response of susceptible tomato plants to Verticillium infection. But such roots never develop any further. An attempt was made, therefore, to determine the nature of effects of Verticillium infection on root growth in susceptible tomato plants.

Six, 3-week-old GS plants, grown in nutrient

solution, were used in this experiment. Before inoculation, four lateral roots of each plant were trimmed with a pair of scissors. Three of these plants were inoculated, and the rest were kept as controls.

Symptoms started appearing in the inoculated plants from the 9th day onwards. By the end of the second week not only were the symptoms well developed, but the plants also showed signs of growth inhibition in both the root and the stem. Sixteen days after inoculation, two small, recently initiated secondary roots were selected in each plant for styding their rate of growth. Fine threads of different colours were tied round each one of them so that the roots could be easily recognized. The length of each root from its point of origin to the tip was measured. Similar measurements were taken after 72 hours and again after 120 hours. The mean growth of six roots from healthy and infected plants were respectively 21 mm. and 25 mm. for the 3-day-period, 16 to 19 days after inoculation, and 13.4 mm. and 8.6 mm. for the subsequent 2-day-period 19 to 21 days after inoculation. Differences in the rate of growth between the roots of control and infected plants were not significant statistically ($P = 0.05$) on either occasion. Further, the mean total growth in length of the six roots from

control and infected plants over the 5-day-period for which measurements were recorded, were almost identical. This suggests that infection probably did not affect the growth of individual roots to any appreciable extent.

After the measurements for root growth had been taken, the lateral roots from each plant were collected by removing them at their points of origin. Each root was placed into either one of the two classes viz., (1) secondary, or (2) tertiary and others according to its origin, and its length was measured. For each plant, the number of roots in each class and their total length were determined. Afterwards, the lateral roots from each plant were dried separately in an oven for dry weight determination. Results are summarized in Table 17.

It appears that neither the number, nor growth in length of the secondary roots were much affected by infection. But the mean number of tertiary and other lateral roots was significantly less in infected plants. The mean total length of such roots seemed to be reduced as a result of infection, but the reduction in this case just failed to be significant. If all the lateral roots are concidered, then it also appears that the reduction due to infection was significant for

Table 17 Growth of lateral roots in control and inoculated susceptible plants recorded 21 days after inoculation

Treatment	Lateral roots									Dry wt. (mg.)
	Secondary			Tertiary and others			All			
	Number	Total length (cm.)	Average length (cm.)	Number	Total length (cm.)	Average length (cm.)	Number	Total length (cm.)	Average length (cm.)	
Control	55	462	8.40	386	997	2.58	441	1459	3.31	122
Inoculated	49	446	9.06	222	611	2.75	271	1057	3.90	46
L.S.D. (P = 0.05)	NS	NS		102.5	407.1		127.4	310.2		53.4

Average of 3 replicates

NS = Non significant interaction

both total number and total length as well as dry weight of such roots. But the mean length of the lateral roots, irrespective of their origin, was not reduced by infection.

The results indicate that the reduced root systems in infected susceptible plants is due to a reduction in the development of new laterals, particularly those of tertiary origin and others of still higher order. The mean length of the lateral roots and their rate of growth were little affected by infection.

8. Effect of plant age on infection

Selman and Pegg (1957) observed that the young tomato plants used in their work showed much less severe symptoms than the older plants when infected with V. albo-atrum. Some relation between the physiologic maturity of a plant and the development of Verticillium wilt has been reported (McLean, 1955; McLeod and Thompson, 1959; and Nelson, 1950). In general more severe symptoms have been found to be associated with the maturity of a plant which, of course, is related to its age.

To test the resistance of GR and GS plants of varying ages to infection, two experiments were done. In the first experiment three lots of seeds

were sown at intervals of 2 weeks. The plants were root-inoculated in the usual way when the youngest ones became 2 weeks old. For each age-group, there were three inoculated and three control plants. Inoculated GR plants did not show any symptoms. The severity of disease in inoculated GS plants was assessed at regular intervals on the basis of wilting. The results are shown in Table 18.

Table 18. Wilting indices for leaves of inoculated susceptible plants of different age-groups

Age at the time of inoculation (weeks)	Days after inoculation		
	14	21	28
2	0.1	0.4	1.0
4	0.1	0.5	1.0
6	1.0	1.3	1.4

Average of 3 replicates.

There was practically no difference in disease development between 2- and 4-week-old GS plants. But in 6-week-old GS plants wilting appeared much earlier and was also more pronounced than in the other two groups at the beginning, but after 4 weeks this difference became less marked.

Dry weights of the plants as a whole, the shoots and the roots were recorded at the conclusion of the experiment. The results are shown in Table 19.

Table 19 Mean dry weights of healthy and inoculated plants of different age groups recorded 28 days after inoculation

Age (weeks)	Plant	Dry weight (g.)			Percentage increase or reduction due to infection		
		Plant	Shoot	Root	Plant	Shoot	Root
2	GRC	5.45	4.60	0.85			
	GRI	4.30	3.65	0.65	-21.1	-20.7	-23.6
4	GRC	7.75	6.60	1.15			
	GRI	7.60	5.90	1.70	-1.9	-10.6	+47.8
6	GRC	11.15	8.90	2.25			
	GRI	13.65	10.05	3.65	+22.4	+12.9	+60.0
2	GSC	5.30	4.65	0.65			
	GSI	1.45	1.25	0.20	-72.7	-73.2	-69.3
4	GSC	9.20	7.65	1.55			
	GSI	3.75	3.20	0.55	-59.3	-58.2	-64.5
6	GSC	15.15	12.0	3.15			
	GSI	8.45	6.65	1.80	-44.3	-44.6	-42.9

Average of 3 replicates

Infection always caused a reduction in dry weight of susceptible plants, the shoot and the root being affected almost equally. The younger plants in which wilting was much delayed and less prominent were affected more by infection than the older plants in which wilting appeared earlier and developed rapidly. The inhibitory effect of infection on the growth of susceptible plants appears to be inversely related to their age within certain limits. Infection also had some effect on the dry matter production in resistant plants. This effect varied considerably with the age of the plant. In 2-week-old plants considerable reduction in dry weight was evident. For 4-week-old plants the effect was very slight, but with 6-week-old plants considerable increase in dry weight was recorded. Further, the shoot and the root were affected differently in their growth by infection, the growth of the latter being promoted considerably in 4- and 6-week-old plants. A similar trend has been noticed in an earlier experiment also, but there the promotion of root growth was not as marked as in this experiment.

The delay in the onset of wilting in young susceptible plants might have been due to the method of inoculation used. In this method a much smaller

proportion of roots would be damaged in younger plants than in the older plants in which the root system is very large. In the second experiment, therefore, a different method of inoculation was used.

Resistant and susceptible plants, 2, 7, 10 and 14 weeks old, were inoculated by dipping their washed roots in mycelial suspension. The onset of wilting was recorded for each plant. Two weeks after inoculation, they were assessed for the severity of wilting. The results are set out in Table 20.

The 2-week-old GS plants showed just the beginning of symptoms when sampling was done. They were kept under observation for a further period of 9 weeks to study the course of disease development in such young plants. The mean wilting indices per leaf 3, 4, 7, 9 and 11 weeks after inoculation were 0.9, 1.8, 1.7, 2.1 and 2.1 respectively.

The results indicate that the ultimate amount of infection in a susceptible plant is little affected by its age at the time of inoculation. Nevertheless, some relation between the age of a plant and the course of symptom expression is apparent. Wilting appears earlier in the older plants and develops rapidly, while in the younger plants it is late to appear and develops slowly. A young susceptible plant, when

Table 20 Effect of the age at the time of inoculation on the onset and the severity of wilting in susceptible plants recorded after 15 days.

Age of the plant (weeks)	Onset of wilting in 3 replicates in days after inoculation	*Mean wilting index per leaf
2	12, 15, 17	0.4
7	8, 9, 11	2.0
10	9, 10, 11	1.7
14	7, 7, 7	2.3

* Average of 3 replicates

infected, may not soon develop very pronounced symptoms. But its growth is affected to a much greater extent than that of an older plant. It cannot be said, therefore, that the young susceptible plants show more resistance to infection than the older plants.

In the resistant variety, a high degree of resistance to infection was expressed uniformly by all plants irrespective of their age at the time of inoculation. Even then the younger plants could not escape completely the inhibitory effect of infection. On the other hand, infection stimulated growth of the older plants.

9. Root injury as a factor in infection

Penetration of undamaged tomato roots by V. albo-atrum has been reported (Bewley, 1922; Derbyshire, 1956; Selman and Buckley, 1959). However, Selman and Buckley (1959) has shown that root injury facilitates infection and that the systemic invasion of a plant never occurs unless the roots are damaged. But Selman and Pegg (1957), using young tomato plants in their work, did not find any great effect of root damage either on the period of incubation or on infection.

In different experiments so far described, some roots have always been deliberately damaged in the expectation that this would encourage disease development in inoculated plants. However, in view of the disagreement among previous workers as regards the importance of root damage to infection, the problem was re-examined.

Plants of susceptible variety were always grown in nutrient solution for this series of experiments. It was assumed that the root systems of plants grown in this way were undamaged.

In the first experiment, six 3-week-old plants were inoculated by adding 1 ml. of concentrated spore suspension to the nutrient solution in which they

were growing. In half of them, four secondary roots had been trimmed just before inoculation with a pair of scissors. Uninoculated plants with undamaged, or damaged roots were maintained as control. Wilting was recorded at regular intervals to assess the severity of disease.

In the inoculated plants with damaged roots wilting started to appear between 9 and 12 days after inoculation, and then developed gradually. The mean wilting indices per leaf for these plants were 0.1, 0.4, 0.8 and 1.1 after 9, 14, 21 and 28 days respectively. In the inoculated plants with undamaged roots, wilting was not apparent within 21 days of inoculation (Fig. 4). Thereafter, it developed slowly, the mean index per leaf being 0.3 after 28 days. The observations trongly suggest that in young tomato plants at least, damage to even a few roots may be sufficient to aid invasion and hasten the appearance of symptoms. The same experiment was repeated with 3-week-old plants, but five lateral roots were trimmed this time instead of four. The experiment was continued for 28 days after inoculation. The results, shown in Table 21, confirm those of the first experiment.

The effect of root damage on infection was investigated further. Seven-week-old plants were given

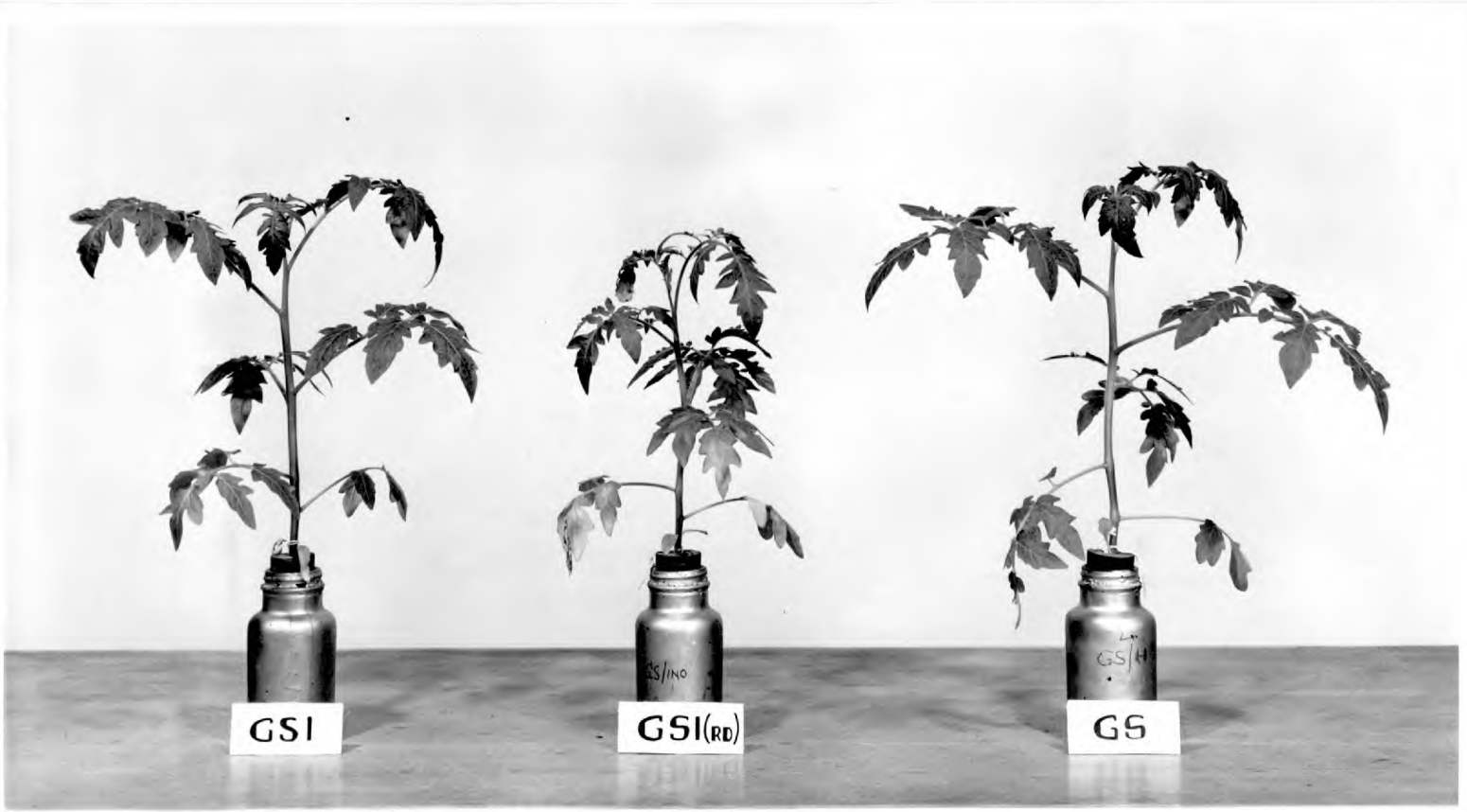


Fig. 4. Healthy (GS), inoculated (GSI) and inoculated, root damaged (GSI/RD) Gem susceptible plants 21 days after inoculation

Table 21 Mean wilting indices per leaf of plants with undamaged or damaged root system.

Treatment	Days after inoculation			
	7	14	21	28
Undamaged	0	0	0.58	0.75
Damaged	0.27	0.46	0.66	0.79

Average of 3 replicates

four different treatments by trimming 1, 3, 5 or 8 secondary roots at the time of inoculation. The treated plants as well as others with undamaged roots were inoculated in the standard way. There were uninoculated controls for each treatment. None of them, however, showed any wilting. The results in respect of inoculated plants are reported in Table 22.

Table 22 Mean wilting indices per leaf recorded at regular intervals after inoculation following treatments varying in the severity of root damage

Number of roots trimmed	Days after inoculation					
	10	14	17	21	24	28
Nil	0	0.02	0.17	0.35	0.53	0.63
1	0.13	0.19	0.46	0.66	0.70	0.80
3	0.03	0.05	0.43	0.64	0.67	0.70
5	0.03	0.05	0.37	0.80	0.88	0.92
8	0.02	0.10	0.42	0.77	0.91	0.88

Average of 4 replicates

The association between damage to the root and an early expression of symptoms was evident again. All inoculated plants with damaged roots showed more wilting than those without such damage. It also appeared that wilting developed slightly more in plants with 5, or 8 roots damaged than in those with 1, or 3 damaged roots. However, the differences between different root damage treatments were always small.

A reduction in disease symptom was noted for Fusarium wilts of pea (Wade, 1929) and banana (Rishbeth, 1955) when the roots were damaged before inoculation. Studying Fusarium wilt of tomato, Keyworth and Dimond (1952) found that severe root pruning a few days before inoculation reduced wilting considerably, but similar damage at the time of inoculation caused increased symptoms. Reduced infection also resulted when the roots were damaged by treatments with hot water, or a number of chemicals.

To test if a pre-inoculation damage to the roots would reduce the symptoms in Verticillium wilt of tomato, a preliminary experiment was done. Six, 6-week-old plants were inoculated in the standard way. In three of them, five secondary roots had been trimmed 5 days before inoculation. In others same number of roots were trimmed just before inoculation.

Difference in the mean wilting indices for leaves between the two treatments was not pronounced at first, but became significant ($P = 0.05$) after 4 weeks. In the plants in which roots were damaged at the time of inoculation wilting developed gradually with time. But in other plants in which the roots were damaged 5 days before inoculation, the progress of wilting was very slow.

In a further experiment, 6-week-old plants were given five different treatments varying in the severity and timing of root damage. These plants along with those having undamaged roots were inoculated in the standard way. For each treatment, there were uninoculated controls to see if the damage to roots had any effect on the growth and development of the plants. As the plants grew too tall the experiment had to be discontinued after 3 weeks. The treatments and the mean wilting indices for leaves as recorded 14 and 21 days after inoculation, are recorded in Table 23.

All inoculated plants in which the roots were damaged, irrespective of the timing of such a treatment, showed more wilting than those with undamaged roots. There was not much difference between the treatments involving the trimming of five or ten

Table 23 Effects of different treatments involving root damage before, or at the time of inoculation on the development of wilting in susceptible plants

Number of roots trimmed	Time of treatment in relation to the date of inoculation	Wilting index per leaf Days after inoculation	
		14	21
Nil		0.08	0.15
5	0	0.27	0.56
10	0	0.19	0.60
5	-5*	0.27	0.57
10	-5	0.11	0.34
10	-10	0.08	0.33

* A minus sign followed by a number indicates that the treatment was given so many days before inoculation.

roots on the day of inoculation, and five roots 5 days before inoculation. But in those plants in which ten roots had been trimmed either 5, or 10 days before inoculation, wilt development was perceptibly slower than it was in other plants with damaged roots, but more than that in plants with undamaged roots. None of the controls showed any abnormality in its growth behaviour.

B. Studies with growth regulating substances

1. Effects of indole-3-acetic acid on healthy and inoculated plants

In the Verticillium wilt of tomato it has been suggested that a post-infectional increase in IAA may be responsible for some of the disease symptoms which appear to be growth responses rather than the effect of toxins (Pegg and Selman, 1959). Assays of infected stems and leaves showed considerable increase in growth promoting activity over the healthy controls. Similar assays showed that filtrates from cultures of the parasite also had considerable growth promoting activity. In the anatomical studies with infected plants, proliferation of xylem parenchyma and vessel collapse were found in addition to tylosis. These changes may be dependent on an alteration in the auxin levels of infected plants. A series of experiments were designed, therefore, to study the effects of externally applied IAA on healthy as well as on infected plants.

Six-week-old plants of both varieties were cut at ground level under tap water. The cuttings, with 6-7 leaves, were transferred to full strength nutrient solutions containing 0, 1, 3, 10 and 30 p.p.m. IAA.

Cuttings were kept in the greenhouse avoiding direct sunlight. The solutions were changed once, 4 days after inoculation. Data recorded 4 and 7 days after inoculation in respect of wilting, epinasty (mean of four lower leaves) and formation of adventitious roots are summarized in Table 24.

The results show a graded effect of different concentrations of IAA on wilting and epinasty of the petioles. These effects were apparent even at the lowest concentration used in this experiment. Adventitious root production was stimulated at low concentrations, but retarded at higher concentrations. The effects of IAA were more pronounced on resistant than on susceptible cuttings.

Table 24 Effects of IAA treatments on healthy cuttings from resistant and susceptible plants

Concentration (p.p.m.)	Gem resistant					Gem susceptible				
	W.I.		Epinasty		A.R.	W.I.		Epinasty		A.R.
	D		D		D	D		D		D
	3	7	3	7	7	3	7	3	7	7
Nil	0.2	0.8	84	91	20	0.3	1.1	77	92	4
1	0.3	1.1	95	113	65	0.4	0.9	90	113	60
3	0.6	1.8	103	128	60	0.4	1.4	89	118	45
10	1.3	2.5	113	128	40	1.4	2.0	116	131	30
30	1.8	2.6	116	124	0	1.4	2.3	103	110	5

Average of 3 replicates
W.I. = Mean wilting index per leaf
A.R. = Mean number of adventitious roots
D. = Days after inoculation

This experiment was repeated with slight modifications. Six-week-old plants, grown in nutrient solution, were used instead of cuttings. The treatment with IAA continued for 3 weeks. The plants were kept in the greenhouse for this period. Nutrient solutions were changed twice a week. Wilting and epinasty (mean of 4 lower leaves) were recorded at regular intervals. Production of adventitious root protuberances from the base of stem were recorded at the end of the experiment. Yellowing developed to almost the same extent in all the treatments. It has not been included in Table 25 which shows the other results.

Table 25 Effects of different concentrations of IAA on resistant and susceptible plants

Plant	Concentration (p.p.m.)	Wilting index per leaf			Epinasty			A.R.
		D			D			D
		10	14	21	10	14	21	21
GS	Nil	0.4	0.6	0.8	67	65	73	Few
	1	0.8	0.9	1.0	88	109	118	Moderate
	3	0.6	0.8	0.9	92	110	104	Few
	10	0.4	0.7	0.7	88	97	107	Few
	30	0.2	0.6	0.6	95	98	112	Very few
GR	Nil	0.5	0.6	0.9	67	66	73	Very few
	1	0.8	0.9	1.0	100	112	125	Moderate
	3	0.9	0.9	0.9	85	100	96	Moderate
	10	0.3	0.6	0.7	88	94	107	Few
	30	0.3	0.7	0.8	85	86	107	Very few

Average of 3 replicates
A.R. = Adventiticus root protuberance
D. = Days after inoculation

All the plants used in this experiment showed slight wilting. In both varieties, there was a slight increase in wilting with the two lower concentrations of IAA, i.e., 1 and 3 p.p.m. With 10 and 30 p.p.m. IAA, on the other hand, there was evidence of slight reduction in wilting. Treatment with IAA always stimulated epinasty, but it was most effective when used at 1 p.p.m. Maximum initiation of adventitious roots was also obtained with 1 p.p.m. IAA. At higher concentrations root initiation was retarded.

It appears from the results that IAA, at lower concentrations, may stimulate the production of symptoms while at higher concentrations its effect would probably be inhibitory. It was decided, therefore, to test the effect of various concentrations of IAA on symptom expression in infected plants.

Six-week-old GR and GS plants were treated with different concentrations of IAA viz., 0, 1, 3 and 10 p.p.m. by spraying solutions on to the leaves 4 days before, and 4 days after inoculation. Plants were inoculated by pouring 25 ml. of a mycelial suspension around the root system at the time of transfer from 3 inch to 5 inch pots. Uninoculated controls were kept for each variety for the different treatments.

All the IAA-treated plants showed certain growth responses soon after spraying, but the plants gradually became normal in appearance, the time taken to recover being proportional to the concentrations of IAA applied. Inoculated resistant plants did not wilt. The results in respect of inoculated susceptible plants are shown in Table 26.

Table 26 Mean wilting index for leaves of inoculated susceptible plants treated with different concentrations of IAA.

Concentration (p.p.m.)	Days after inoculation		
	14	21	28
Nil	1.6	2.0	2.3
1	1.4	2.1	2.5
3	1.3	2.0	2.3
10	1.3	2.6	2.9

Average of 3 replicates

The onset of wilting was delayed in the IAA-treated, inoculated plants by 2-3 days; the delaying was more prominent at 10 p.p.m. than at the other two concentrations. The progress of wilting in the IAA-treated plants was initially somewhat slower than that in the untreated plants. But later, wilting developed more rapidly in the treated plants so that at the end

of the experiment these plants had higher wilting indices than the inoculated controls. The slightly lower values obtained with 3 p.p.m. IAA cannot be explained.

Another experiment was done on similar lines, but with higher concentrations of IAA starting at 10 p.p.m. which was the maximum concentration used in the previous experiment, and up to 100p.p.m. Eight-week-old GS plants were used in this experiment. IAA, at different concentrations, were applied to some of these plants 6 days before and 6 days after inoculation. One set of plants were inoculated but not treated with IAA. There were also uninoculated controls for each treatment. Wilting was assessed at regular intervals. Thirty-five days after inoculation, all the replicates were sectioned at the cotyledonary nodes. The sections were observed microscopically to determine the percentage of vessels with hyphae or tyloses. The results are summarized in Table 27.

In this experiment treatment with IAA did not generally delay the appearance of wilting in inoculated plants as in the previous experiment. But the progress of wilting was very slow in all the treated, inoculated plants for the first 3 weeks. During the

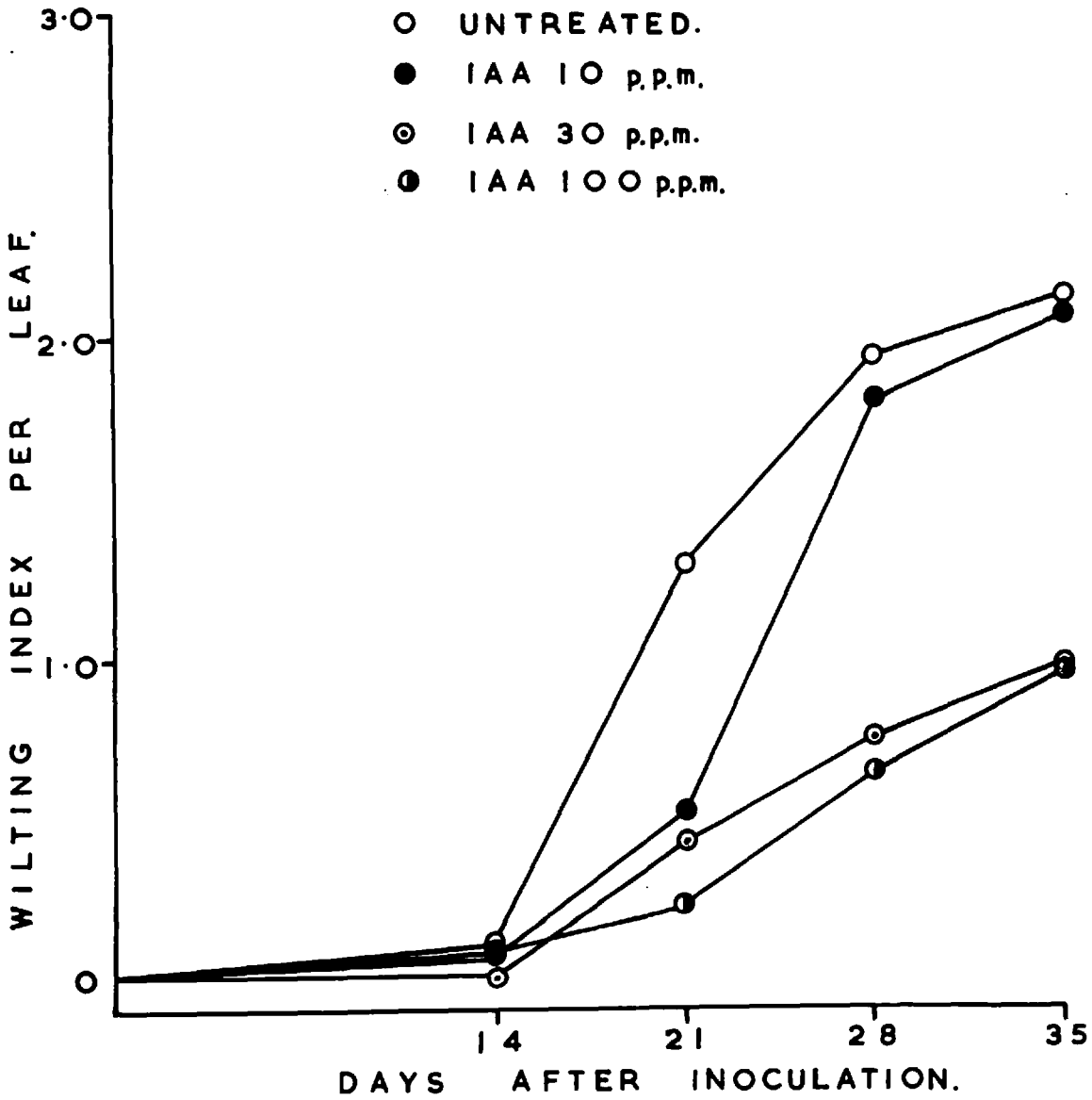
Table 27 Effects of different concentrations of IAA on wilting index per leaf and distribution of tyloses and hyphae at cotyledonary node

Concentration (p.p.m.)	Wilting index per leaf				Percentage of vessels		
	Days after inoculation				Control	Inoculated	
	14	21	28	35	Tyloses	Tyloses	Hyphae
Nil	0.1	1.3	2.0	2.1	3.4	3.8	32.1
10	0.1	0.5	1.8	2.1	5.9	4.3	27.0
30	0	0.4	0.8	1.0	6.4	15.2	10.4
100	0.1	0.2	0.6	1.0	14.5	18.7	16.5

Average of 3 replicates

next 2 weeks wilting spread very rapidly in plants treated with 10 p.p.m. IAA as is shown in Figure 5. At the end, mean wilting index for this treatment was slightly higher than that of the control series when calculated on the basis of plants, instead of leaves. In other treatments with higher concentrations of IAA, the rate of wilt development increased somewhat during the last two weeks, but even then a considerable inhibitory effect of IAA was evident at the end. Anatomical studies reveal certain interesting facts. Treatment with IAA, at all concentrations, caused

Fig. 5 EFFECT OF IAA ON DEVELOPMENT OF WILTING IN SUSCEPTIBLE PLANTS.



increased tylosis, the increase at 100 p.p.m. being very significant. The inoculated plants receiving 30 and 100 p.p.m. IAA, showed a wide distribution of tyloses - a situation somewhat comparable to what happens in inoculated resistant plants. In these plants hyphae were found in many fewer vessels than in the controls. All the treated plants showed various growth responses within 2 hours after the treatment. The plants receiving 10 and 30 p.p.m. IAA recovered within 3 - 4 days after treatment. At 100 p.p.m., however, the plants took a longer time to recover completely from the effects of IAA treatment.

The effect of still higher concentration of IAA on disease development in susceptible plants was studied in a subsequent experiment. Six-week-old plants were given only one treatment with 200p.p.m. IAA 4 days before inoculation. This treatment considerably delayed the onset of wilting. At the end of the experiment, 95 and 93 per cent reductions respectively in wilting and distribution of hyphae at cotyledonary node were recorded for this treatment in respect of the controls. The IAA-treated plants were somewhat shorter than the controls. They also showed considerable twisting of the stem even at the end of the experiment.

The observations so far made suggest that IAA when applied at lower concentrations such as 1 and 3 p.p.m. may accelerate development of wilting and some other symptoms. At the somehwat higher concentration of 10 p.p.m. an initial period of inhibition is followed by a period of rapid development of symptoms. By applying still higher concentrations of IAA the initial period during which symptoms develop only slowly is considerably prolonged so that even after long periods the plants do not exhibit pronounced symptoms of disease. On the basis of these observations it appears that work on the following lines would repay further study.

(1) the effect on pathogenesis of any treatment which is supposed to increase the level of auxins in the plant

(2) the effect on pathogenesis of any treatment that would reduce the level of auxins in the plant

(3) the effects on pathogenesis of various synthetic auxins and anti-auxins.

2. Effects of apex-removal and other treatments on symptom expression

A series of experiments were done to see how symptoms develop in inoculated plants after various treatments designed to increase or decrease their endogenous level of auxins.

Auxins are known to be produced by a plant in its growing apex. It was thought, therefore, that a removal of the apical bud followed by subsequent removals of the axillary buds would decrease the level of available IAA in the inoculated plants. Similarly, it was thought that a treatment with gibberellic acid, supposedly an auxin synergist, would cause an increase in the level of auxin.

Nine, 7-week-old GS plants, grown in soil, were root-inoculated. One set of three plants had their apical buds removed with a sterile razor blade 10 days after inoculation The axillary buds were also removed from these plants in the same way as soon as they emerged. Three plants in another set were sprayed with 100 p.p.m. gibberellic acid 10 and 15 days after inoculation. Three plants were left untreated to serve as controls. There were 3 uninoculated controls too for each set. These did not develop any symptoms. Data recorded 21 and 28 days after inoculation

are reported in Table 28.

Table 28 Effects of apex-removal and gibberellic acid treatment on the development of wilting in inoculated susceptible plants

Treatment	Wilting index per plant		Number of leaves showing wilting	
	D		D	
	21	28	21	28
Untreated	16.0	21.3	8	11
Apex removed	14.0	14.6	8	8
Gibberellic acid	25.6	28.6	11	12

Average of 3 replicates

D = Days after inoculation

The results were as expected. The removal of apical and axillary buds considerably slowed down the development of wilting. This is shown by the fact that between 21 and 28 days after inoculation three new leaves developed wilting in untreated plants, but in those from which the buds were removed wilting did not affect any new leaf. In contrast, gibberellic acid at 100 p.p.m. stimulated wilting considerably, particularly during the first week, or so after it had

been applied to plants. Sections were taken from all the inoculated plants at the cotyledonary nodes, and at regular intervals above that point. As regards the presence of hyphae and tylosis, there was not much difference between untreated plants and those without buds. But in gibberellic acid treated plants there were hyphae in many more, and tyloses in fewer vessels than in the untreated plants.

The inhibitory effect of the removal of apical and axillary buds on the development of wilting was studied further. Nine, 6-week-old GS plants, grown in nutrient solution, were inoculated by trimming five secondary roots and pouring 1 ml. of a spore suspension (5,000,000 spores/ml.) into the medium (60 ml.). In one set of three plants, apical buds were removed on the day of inoculation, and in another set, 7 days after inoculation. All the axillary buds were removed from both sets of plants as soon as they emerged. Another three plants were left untreated to act as controls. There were uninoculated controls too for each set. None of them developed any symptoms. Data recorded at regular intervals after inoculation are shown in Table 29.

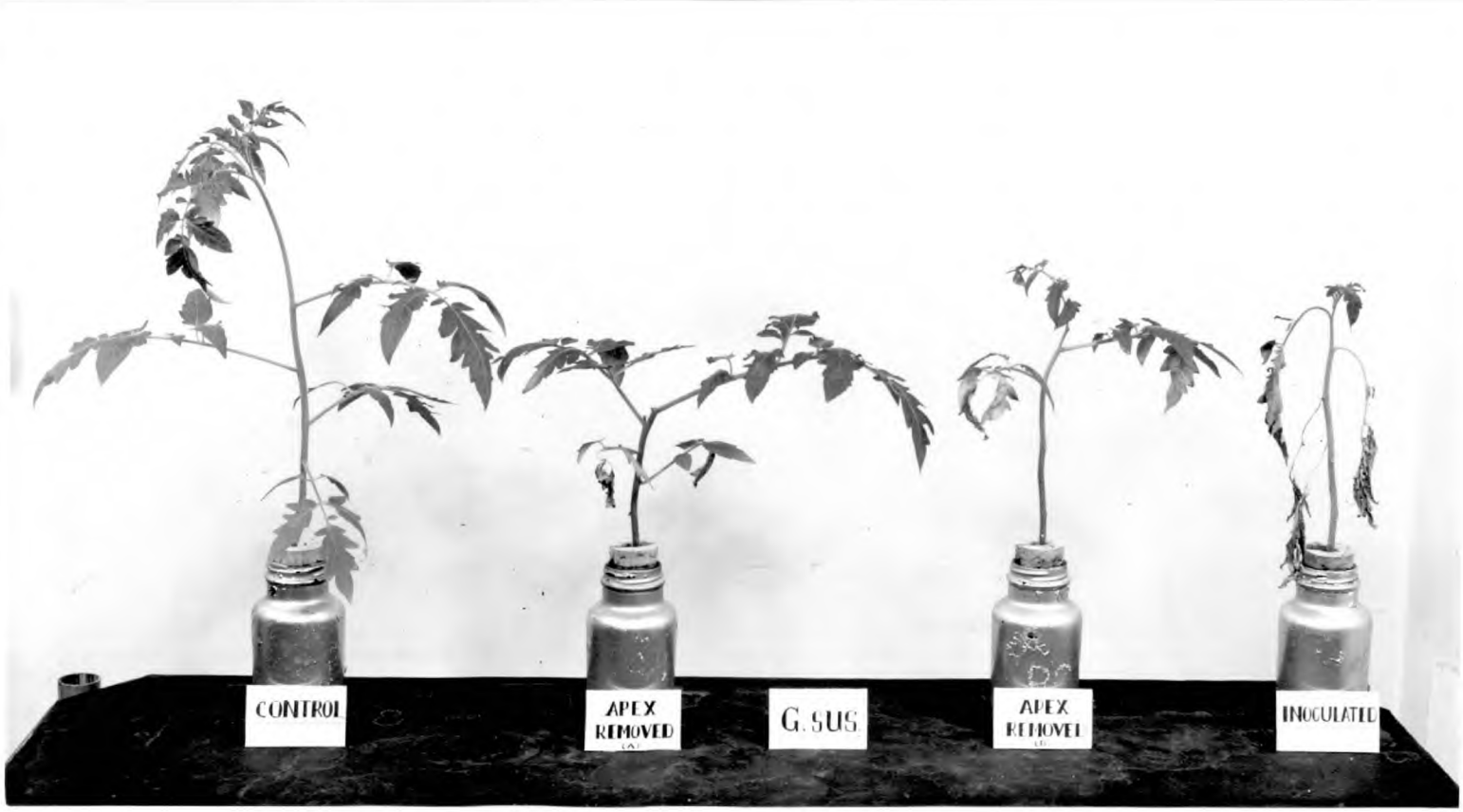
Table 29 Effect of the removal of apical and axillary buds at different times after inoculation on development of wilting in susceptible plants

Treatment	Wilting index per plant					
	Days after inoculation					
	7	10	14	17	21	28
Untreated	1.3	8.0	19.0	21.0	23.6	28.0
Apex removed on the day of inoculation	4.3	11.0	13.0	14.0	14.6	14.6
Apex removed 7 days after inoculation	3.6	7.6	10.6	14.6	17.0	18.0

Average of 3 replicates

The results confirm the earlier observation that removal of apical and axillary buds from an inoculated susceptible plant reduces considerably the development of symptoms. The inhibitory effect of removing the apical bud does not become apparent immediately, but after about 2 weeks. It was also found that an early treatment reduced symptoms more effectively than a late treatment (Fig. 6).

The reduction in disease symptoms resulting



Healthy | Inoculated (apex removed on the day of inoculation) | Inoculated (apex removed 7 days after inoculation) | Inoculated

Fig. 6. Healthy and inoculated and treated, inoculated Gem susceptible plants 28 days after inoculation

from apex-removal may be due to a lowering of the level of endogenous auxin. If that is so, then treatment with an antiauxin, like maleic hydrazide should also suppress symptoms. Again, if axillary buds are allowed to develop after the apex has been removed, then the endogenous level of auxin would not be reduced, but may even be increased above the normal level. Such a situation may cause an increase of symptoms. These possibilities were investigated in the next experiment.

Twelve, 11-week-old GS plants, grown in soil, were root-inoculated. In six plants, apical bud had been removed 7 days before inoculation. In three of them axillary buds were allowed to grow whereas in other three they were removed regularly. Three plants were sprayed with 300 p.p.m. maleic hydrzide solution 3 days after inoculation. Another three were kept as controls. There were uninoculated controls for each treatment. These plants did not develop any wilting, but uninoculated plants which had been sprayed with maleic hydrazide developed considerable yellowing in the leaves. The results in respect of inoculated plants are given in Table 30.

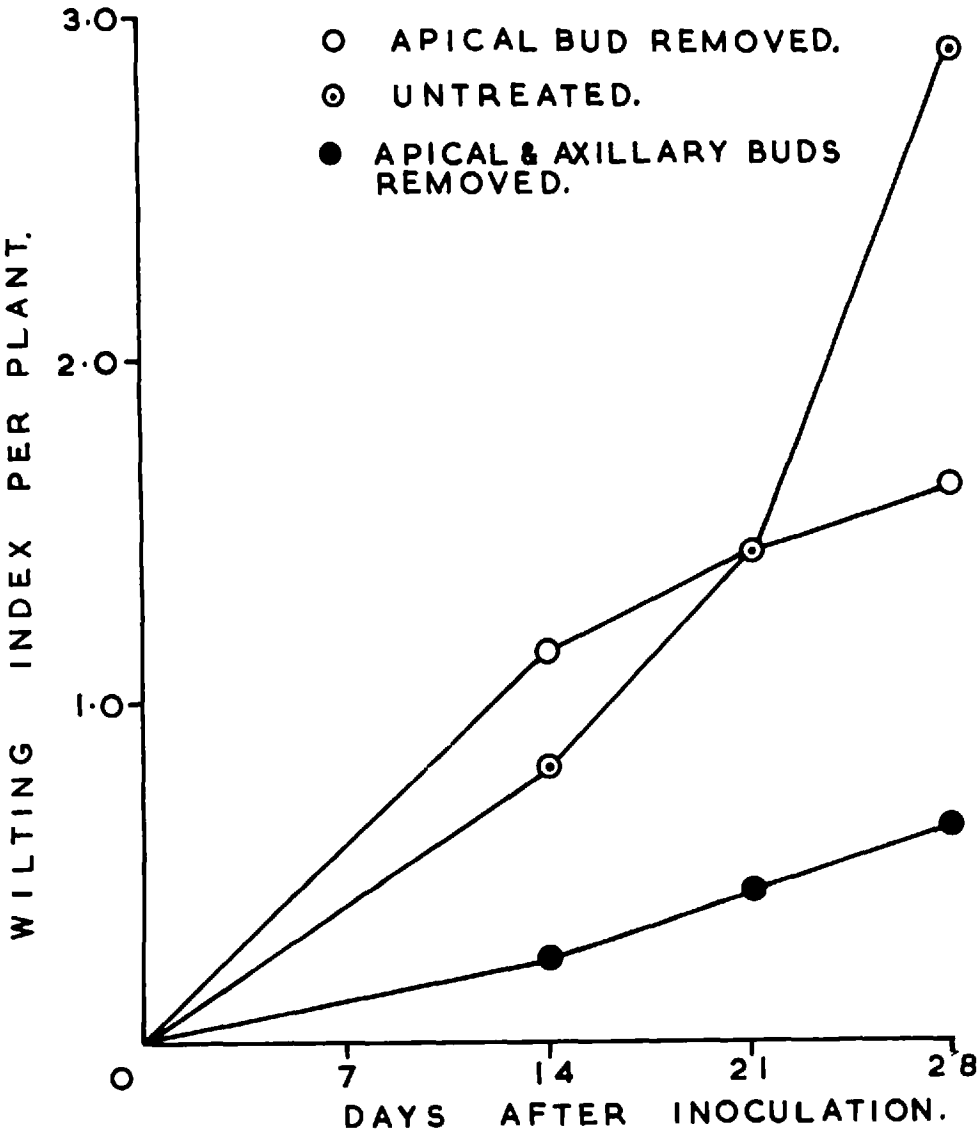
Table 30 Effects of different treatments designed to alter the level of endogenous auxin in the plant on the development of wilting in inoculated susceptible plants

Treatment	Wilting index per plant		
	Days after inoculation		
	14	21	28
Untreated	8.3	14.6	29.3
Apical and axillary buds removed	2.6	4.6	6.6
Apical bud removed	11.6	14.6	16.6
Maleic hydrazide - 300p.p.m.	6.3	27.0	31.3

Average of 3 replicates

The results show how strikingly wilt development can be inhibited if the apical bud is removed 7 days before inoculation. But this treatment is effective only if axillary buds are also removed (Fig. 7). If the axillary buds are allowed to develop, then wilting develops to an extent at least comparable to that characteristic of untreated plants. Many

Fig.7. EFFECT OF THE REMOVAL OF APICAL AND AXILLARY BUDS ON DEVELOPMENT OF WILTING IN SUSCEPTIBLE PLANTS.



leaves in these axillary shoots wilted, a condition never noticed in untreated plants even when they were severely diseased. However, these leaves were not considered for an assessment of wilting. There was a big difference in the number of leaves between untreated plants and those from which apex was removed, the mean number at the end of the experiment being 13 for the former, and 5 for the latter. This would explain the considerable difference in wilting index between untreated plants and those without apex, but with axillary shoots (Table 30). The acceleration in disease development caused by maleic hydrazide, an auxin antagonist, does not fit in with other results. It may be an effect of the high concentration employed.

In the last of this series of experiments, an attempt was made to determine the effects of various treatments on wilt development in the plants from which the apical bud has been removed. The idea was to increase the level of auxin in such plants, and to see if that would offset the inhibitory effect that normally results from bud removal.

Fifteen, 6-week-old GS plants, grown in soil, were root-inoculated in the standard way. Twelve of them had their apical buds removed at the time of

of inoculation. Apical buds were allowed to develop further in three of them, but from other nine they were removed regularly. Of these nine plants, three were sprayed with 100 p.p.m. gibberellic acid 4 days after inoculation and three similarly treated with 10 p.p.m. IAA 7 days after inoculation. Another three did not receive any treatment. Wilting indices, assessed 14, 21 and 28 days after inoculation, are shown in Table 31.

Table 31 Effects of different treatments on wilt development in inoculated susceptible plants

Treatment	Wilting index per plant			Wilting index per leaf		
	D			D		
	14	21	28	14	21	28
Untreated	1.6	6.3	9.0	0.2	0.5	0.7
AR(-AB)	0	0.5	2.6	0	0.1	0.5
" " + IAA	0	0	0	0	0	0
" " + GA	4.3	11.3	11.3	0.8	2.3	2.3
AR(+AB)	0.6	2.3	10.0	0.1	0.4	1.5

Average of 3 replicates
D = Days after inoculation
AR(-AB) = Apex removed and axillary buds removed
AR(+AB) = Apex removed, but axillary buds present

The results confirm the earlier findings that in inoculated plants from which apex has been removed, wilt development is inhibited strongly in absence of axillary buds, but, if anything, stimulated when these buds are allowed to develop. It appears that the stimulatory effect of gibberellic acid on wilt development can be obtained even when the level of auxin in a plant is subnormal. The complete absence of wilting in plants receiving IAA treatment cannot be explained.

A considerable suppression of disease symptoms in plants from which all the buds were removed has been the most interesting finding from this series of experiments. These plants had a thicker stem and showed thicker and darker green leaves than the normal plants. The leaves had a thick rachis and slightly upturned margin.

3. Effect of gibberellic acid on disease development

An acceleration of disease development in inoculated susceptible plants, when treated with gibberellic acid (GA), has already been described. A preliminary experiment was done to confirm this, and also to determine the effect of GA on inoculated resistant plants.

Six, 6-week-old plants of each variety, grown in nutrient solution, were root-inoculated. Three of the inoculated plants from each variety had been previously treated with 100 p.p.m. GA 6 days before inoculation. Wilting was recorded at regular intervals and the results are reported in Table 32.

Table 32 Mean wilting index for leaves of inoculated resistant and susceptible plants with or without gibberellic acid treatment

Plant	Treatment	Days after inoculation		
		14	21	28
Gem resistant	Untreated	0	0.12	0.18
	Gibberellic acid	0	0.07	0.60
Gem susceptible	Untreated	0.22	0.52	0.69
	Gibberellic acid	0.78	1.31	1.40

Average of 3 replicates

Gibberellic acid induced rapid development of wilting in susceptible plants even when applied a few days before inoculation. This stimulatory effect of GA was particularly evident between 10 and 21 days after inoculation. Another interesting feature was the progress of wilting in treated, inoculated resistant

plants. The amount of wilting in such plants was comparable with that in untreated, inoculated susceptible plants. Of three treated, inoculated resistant plants showing symptoms, the most severely affected one had fungus in 12 per cent of vessels at the stem base. But two other plants did not show any fungus in the same position.

Dimond and Corden (1957) studied the effect of GA on Fusarium wilt of tomato. They found an increase in symptoms when sprayed with 10, or 20 p.p.m. GA, but similar treatment with 5p.p.m. GA reduced the symptoms. According to them, spraying on the day of inoculation was more effective than later.

In the previous experiments pre-, as well as post-inoculation treatments with 100 p.p.m. GA have been found to accelerate symptom production equally. In order to confirm these results which had been obtained from two different experiments, and also to determine the effect of GA at lower concentrations, another experiment was done. Twelve, 11-week-old susceptible plants, grown in soil, were root-inoculated in the standard way. Three of these plants had already been treated with 100 p.p.m. GA 3 days before inoculation. Three plants each in two other groups

were treated with 10, and 100 p.p.m. GA 10 days after inoculation. Three remaining plants were maintained as inoculated controls. For each treatment there were three uninoculated controls too. Three weeks after inoculation, the mean wilting index for the leaves of inoculated plants was 2.44 with 100 p.p.m. GA applied 3 days before, and 1.48 and 2.78 with 10 and 100 p.p.m. GA respectively applied 10 days after inoculation. The untreated, inoculated plants had a mean index of 1.86. The uninoculated plants did not wilt. So it appears that at 10 p.p.m. GA slows down wilt development. But at 100 p.p.m. its effect is to stimulate wilting when applied 3 days before, or 10 days after inoculation.

In a further experiment, 11-week-old GR plants were inoculated by the root-dip method. Nine of them had about one-fifth of their lateral roots damaged by trimming with a pair of scissors. In nine other plants, the roots were not damaged at the time of inoculation. Within each group, three plants had been sprayed with 100 p.p.m. GA 6 days before inoculation, and another three received the same treatment 4 days after inoculation. Three plants in each group did not receive any treatment with GA. The appropriate

uninoculated controls were included in each treatment. There was no visible effect of root damage on the growth of the plants. None of the uninoculated plants showed any wilting. Data in respect of eighteen inoculated plants are shown in Table 33.

Table 33 Mean wilting index for leaves of inoculated resistant plants with intact or damaged roots and receiving pre- or post-inoculation gibberellic acid treatment

Treatment	Days after inoculation					
	Intact roots			Damaged roots		
	14	21	28	14	21	28
Untreated	0	0.06	0.04	0	0	0
Gibberellic acid (pre-inoculation)	0.24	0.28	0.38	0.27	0.35	0.39
Gibberellic acid (post-inoculation)	0	0	0	0.56	0.64	0.89

Average of 3 replicates

Untreated, inoculated plants did not practically show any wilting. Wilting was also absent from the inoculated plants with undamaged roots and receiving a post-inoculation GA treatment. The inoculated plants in all other treatments showed signs

of wilting 6 - 7 days after inoculation. Yellowing and wilting appeared simultaneously in the terminal leaflet and gradually spread into the next two leaflets. Only rarely was the whole leaf affected. Root damage did not induce more severe symptoms when the plants received a pre-inoculation treatment with GA. But when GA was applied after inoculation, root damage caused a considerable increase in the severity of the symptoms compared with any other treatment. In most of the plants treated with GA before inoculation, there was little progress of the disease during the last 2 weeks of the experiment. This might have been due to the disappearance of the effects caused by GA.

All the inoculated plants were sectioned at the cotyledonary node, and the sections were examined microscopically. In the plants with intact roots, the fungus was present in less than 1 per cent of vessels. In other plants with damaged roots, it was present in about 3 per cent of vessels. Tylosis was extensive in all the inoculated plants.

The plants receiving 100 p.p.m. GA, always showed considerable extension of the internodes. This was particularly prominent in the upper part of the stem. The leaves in such plants developed a larger

surface and were pale green in colour. The leaflets in the new leaves developing after GA treatment, very often showed an entire margin thus losing their characteristic segmented form. But these formative effects of GA treatment did not continue to appear for more than 2 - 3 weeks.

In a further experiment an attempt was made to find out if the availability of GA over a long period would induce a gradual, and continued progress of symptom development in inoculated resistant plants. Nine, 9-week-old GR plants, grown in soil, were inoculated by the root-dip method. About one-third of the lateral roots in each plants was trimmed at the time of inoculation. Six of these plants had been sprayed with 100 p.p.m. GA 6 days before inoculation. Of these plants, three received two further applications of GA 6 and 18 days after inoculation. Controls consisting of uninoculated plants with damaged roots were kept for each treatment. The results are presented in Table 34.

The GA-treated, inoculated plants always showed more symptoms than the inoculated controls. No wilting was noticed in uninoculated plants. The difference in wilting between the inoculated plants receiving 1 and 3 applications of GA was appreciable

Table 34 Mean wilting index for leaves of inoculated resistant plants receiving different gibberellic acid treatments

Treatment	Days after inoculation		
	14	21	28
Untreated	0	0.04	0.10
Gibberellic acid - 6 days before inoculation	0.14	0.23	0.25
Gibberellic acid - 6 days before, and 6 and 12 days after inoculation	0.39	0.48	0.43

Average of 3 replicates

on all the occasions when sampling was done. But these plants showed definite signs of recovery towards the end of the experiment. It appears that GA induced stimulation of symptoms is entirely dependent on the presence of fungus in the plant. Gibberellic acid cannot make the fungus overcome the resistance mechanism in the plant and survive.

4. Effect of maleic hydrazide on disease development

In the experiment described earlier it was found that maleic hydrazide (MH) treatment resulted in an increase of symptoms in inoculated susceptible

plants. All the treated plants were seriously affected in their growth and general development, and inoculation made the damage more severe. Maleic hydrazide is an anti-auxin known to have profound influence on plant development (Leopold and Klein, 1952). It has been used successfully in certain other diseases to increase susceptibility, or to reduce resistance (Waggoner and Dimond, 1952; Nair, 1958). But crown gall caused by Agrobacterium tumifaciens, and in which excess of auxin may be a factor contributing to disease, was suppressed by MH (Waggoner and Dimond, 1953). If the suggestion that excess accumulation of auxin in Verticillium infected tomato plants is responsible for some of the disease symptoms is correct, then treatment with maleic hydrazide should reduce symptoms. It was decided, therefore, to study the various effects of maleic hydrazide treatments on resistant and susceptible plants.

For each variety, there were eighteen 10-week-old plants divided into three groups of six each. The plants in one group were sprayed twice with 300 p.p.m. MH solution, 6 days before, and 7 days after inoculation. For the other group, 50 ml. of 100 p.p.m. MH solution was poured around the base of stem each day for 6 consecutive days commencing from

one week before inoculation. The remaining six plants were left untreated. In each group three plants were inoculated by root-dip method, and the other three were kept as uninoculated controls. Wilting and other symptoms were recorded until the end of the fourth week after inoculation. After that all the inoculated plants were sectioned at the cotyledonary node. The sections were examined micrscopically to determine the distribution of tyloses and hyphae in vessels. The results of the morphological and anatomical studies are summarized in Table 35.

Maleic hydrazide was more effective when added to the soil than when sprayed on the foliage. In inoculated susceptible plants MH treatments increased the symptoms considerably. In both the treatments, however, wilt development was considerably slower than in the controls in the early stages of infection. The late stimulation of wilt development in treated, inoculated plants may be caused by the activity of MH as a strong metabolic inhibitor. This view is supported by the anatomical studies. Treated GS plants showed a 2 to 5-fold increase in the amount of fungus over the control. In these plants many of the vessels with hyphae were completely blocked. All

Table 35 Effects of maleic hydrazide treatments on wilting, and distribution of hyphae and tyloses at cotyledonary node of inoculated plants

Plant	Treatment	Wilting index per leaf			Percentage of vessels with	
		Days after inoculation				
		14	21	28	H	T
GR	Untreated	0	0.04	0.10	0.8	26.5
	MH - to soil	0	0.36	0.45	3.5	2.0
	MH - spray	0	0	0	2.4	4.6
GS	Untreated	1.11	1.18	1.23	12.6	2.1
	MH - to soil	1.01	1.87	2.06	68.6	1.0
	MH - spray	0.69	1.10	1.57	27.9	1.6

Average of 3 replicates

H = Hyphae T = Tyloses

the treated plants were very much stunted and showed extensive yellowing of leaves. The root systems of these plants were considerably reduced. These inhibitory effects of MH were much more pronounced in inoculated plants. Resistant plants responded to MH treatment in the same way as the susceptible plants. More wilting developed in inoculated plants treated through the soil

than in the untreated plants, but there was not much progress in wilting. The treated, inoculated resistant plants had hyphae in a few more vessels than the untreated, inoculated plants, but in the former tyloses were found in many fewer vessels than in the latter. This suggests that tyloses can not be initiated by cells which have been damaged, or which have a low level of auxin. But the mechanism of resistance was still operative under such conditions.

In the next experiment maleic hydrazide was used at much lower concentrations that did not affect plant development to any great extent. Seven-week-old GS plants were given one treatment with 50 ml. of 1, 3 or 10 p.p.m. MH around the base of the stem 4 days before inoculation. For each treatment, three plants were inoculated and three were kept as controls. There were also controls not treated with MH. Some of these were inoculated, others were not. The development of wilting in inoculated plants was assessed at regular intervals and the results are presented in Table 36.

In this experiment, treated, inoculated plants showed more wilting initially than the untreated, inoculated plants (Fig. 8). But later on, wilt

Fig. 8. EFFECT OF MALEIC HYDRAZIDE (MH) ON DEVELOPMENT OF WILTING IN SUSCEPTIBLE PLANTS.

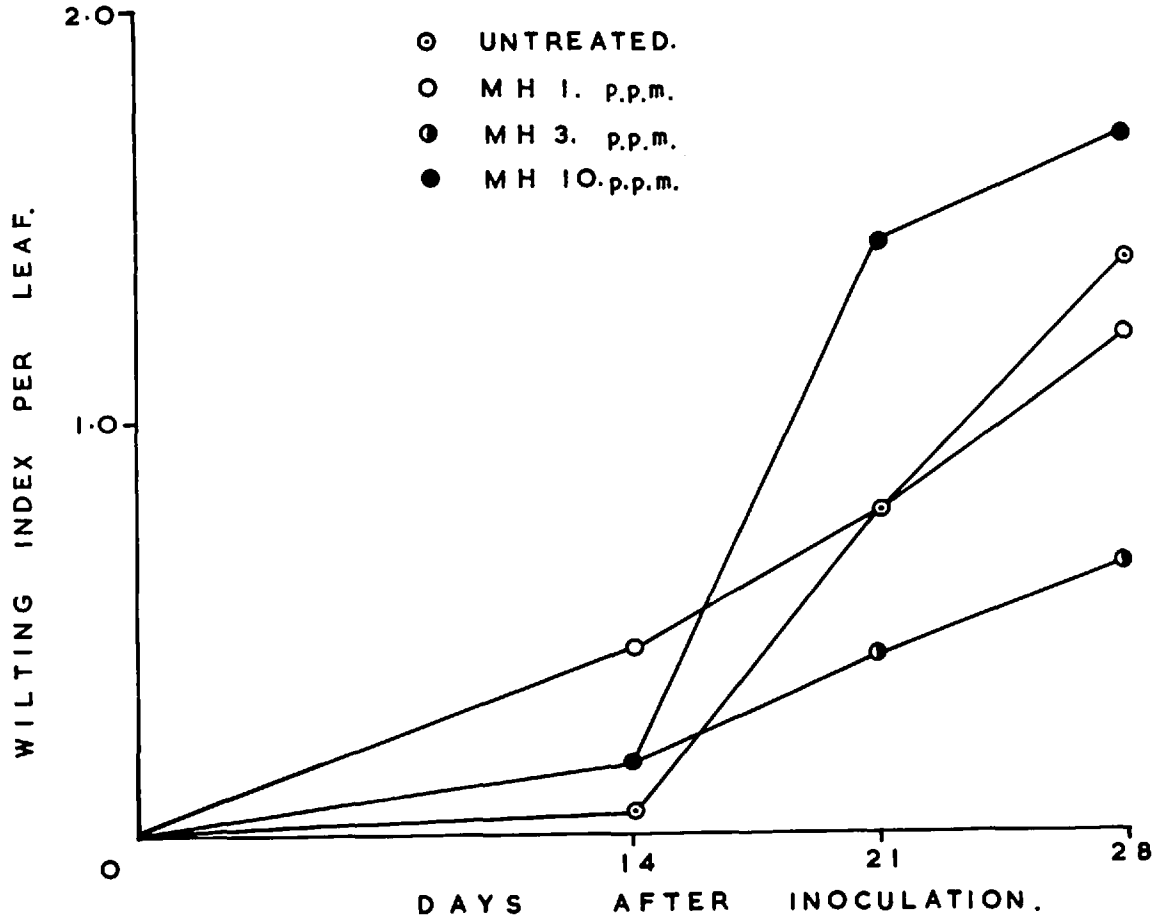


Table 36 Wilting index for leaves of inoculated susceptible plants treated with different concentrations of maleic hydrazide

Treatment	Days after inoculation		
	14	21	28
Untreated	0.05	0.79	1.39
MH - 1 p.p.m.	0.45	0.79	1.21
MH - 3 p.p.m.	0.18	0.44	1.07
MH -10 p.p.m.	0.18	1.43	1.69

Average of 3 replicates

development was somewhat retarded in plants treated with 1 and 3 p.p.m. MH so that the final values for these plants were lower than the inoculated controls. At 10 p.p.m. MH, however, the inoculated plants showed more wilting than the inoculated control. These were also the plants which showed some adverse effect of MH treatment on growth because they were somewhat stunted. It is just possible that the anti-auxin effects of MH on disease development can be obtained only at lower concentrations. At higher concentrations, these effects would be more than neutralized by the harmful effect of MH on metabolism.

5. Effects of maleic hydrazide and gibberellic acid on root and shoot growth in healthy and infected plants

Both maleic hydrazide and gibberellic acid stimulate symptom production in inoculated susceptible plants at high concentrations. In the plants treated with both GA and MH, a very rapid wilting has been found to take place within a short period. This period of rapid disease development occurs earlier for GA-treated plants than for MH-treated plants. In inoculated, MH-treated plants, the immature leaves rarely show much wilting, but they generally dry up. This also happens to the apical bud. In inoculated plants receiving GA, there is no similar desiccation. All these effects may develop from a restricted water supply.

It is known that maleic hydrazide treatment may alter the shoot-root ratio in tomato plants towards abnormally high values (Greulach, 1951). If this does happen, then the blocking of some of the larger vessels by hyphae could drastically reduce the supply of water to the shoot and severe symptoms could be produced quickly. The unusually rapid wilting in GA-treated, inoculated plants suggests also some serious interference with the availability of water to the leaves. But GA does not greatly

inhibit root growth, even at high concentrations (Brian, 1957).

In the following experiment the effects of MH and GA on root and shoot growth in tomato plants were studied. Nine, 7-week-old GS plants, grown in soil, were root-inoculated. Three of them were sprayed with 300 p.p.m. MH solution 3 days after inoculation. Three others were similarly treated with 100 p.p.m. GA. There were three untreated controls. Uninoculated controls were also kept. Wilting was assessed 14 and 21 days after inoculation. After the last sampling, the root and the shoot were collected separately for each replicate and dried to constant weights. The results are summarized in Table 37.

Both GA and MH reduced dry-matter production, but their effects were more pronounced in the root than in the shoot. Maleic hydrazide was, however, more inhibitory in its effect on growth than GA. In MH-treated plants, infection did not reduce the dry weights of root and shoot as markedly as in other treatments. It appears, therefore, that reduced root growth resulting from infection and MH treatment could not have been primarily responsible for the

Table 37 Effects of maleic hydrazide and gibberellic acid on wilt development, and dry matter production in susceptible plants

Treatment	Wilting index per leaf		Dry wt. (g.)		
	Days after inoculation		Shoot	Root	Shoot / root
	14	21			
Untreated - C	0	0	7.52	1.45	5.16
" - I	0.82	2.55	1.41	0.39	3.61
MH - C	0	0	3.63	0.28	12.53
MH - I	0.30	3.30	1.06	0.23	4.63
GA - C	0	0	6.39	0.79	8.06
GA - I	1.42	2.66	0.82	0.17	4.63

Average of 3 replicates

C = Control

I = Inoculated

severe symptoms recorded. But hyphal occlusion of many vessels in the root, or stem of a plant in which the root is much restricted in its capacity may be the deciding factor in inducing rapid wilting. The reduction of root growth following infection was greatest in GA-treated plants. Since the leaves in GA-treated

plants have larger surface area than those in untreated plants, a considerably reduced root system coupled with the presence of hyphae in vessels may easily bring about sudden wilting.

6. Effects of certain growth regulating substances on disease development in susceptible plants

The results so far reported suggest that it is possible to alter the normal disease reaction in susceptible tomato plants by giving them various treatments designed to increase or decrease the level of auxins in the plants. Treatments with different growth regulating substances have been found to induce resistance in susceptible tomato varieties to Fusarium wilt (Davis and Dimond, 1953; Corden and Dimond, 1959) and Verticillium wilt (Hilborn, 1953). Nothing in detail is known, however, about Hilborn's work. Similar treatments may also increase the susceptibility of tomato varieties to certain other diseases (Rowell, 1949; Croxall et al., 1957). It became, therefore, of some interest to study the effects of various growth regulating substances on Verticillium wilt of tomato

In the first experiment indole, 2,4-D and 2,4,6-T were tested for their effects on the disease. Indole is an auxin synergist. 2,4,6-T is known to

inhibit growth induced by simultaneously applied IAA or 2,4-D (Aberg, 1956). 2,4-D is a synthetic auxin well known for its growth promoting activity.

Twenty-four, 7-week-old plants from each variety were used in this experiment. They were arranged in four groups of six. Plants in one group were not given any treatment, those in other groups were treated with indole, 2,4-D or 2,4,6-T. Fifty ml. of 10 p.p.m. 2,4-D solution was applied to the soil in the pot, 7 days before inoculation. The plants reacted so strongly within 24 hours that no further application was made. Fifty ml. of 10 p.p.m. 2,4,6-T solution was applied to soil each day for 6 consecutive days commencing from a week before inoculation. Indole at 100 p.p.m. was sprayed twice on to the leaves, 5 and 7 days after inoculation. Three of the six plants in each treatment were inoculated in the standard way. Wilting was assessed at regular intervals up to 28 days after inoculation. At the end of sampling, the number and size of axillary shoots were recorded for each plant. Afterwards, sections were prepared from all the susceptible plants at the cotyledonary node, and observed microscopically to determine the distribution of hyphae and tyloses in vessels. Since the inoculated resistant plants

did not wilt, they were not used for anatomical studies. The results in respect of susceptible plants only have been presented in Table 38.

Table 38 Effects of different growth regulating substances on wilting and distribution of hyphae and tyloses at cotyledonary node of inoculated susceptible plants

Treatment	Wilting index per leaf			Percentage of vessels with	
	Days after inoculation				
	14	21	28	Hyphae	Tyloses
Untreated	1.38	1.66	1.92	36.5	3.5
Indole	0.21	1.60	1.98	20.0	6.3
2,4-D	0	0	0.08	1.0	18.4
2,4,6-T	0	0	0.18	3.5	10.2

Average of 3 replicates

Indole retarded the disease reaction initially to an appreciable extent, but thereafter wilting developed rapidly. At the end, indole treated plants were more wilted than the controls. In its effect on the disease reaction in susceptible plants, indole at 100 p.p.m. acted almost as did IAA at 10 p.p.m. Both 2,4-D and 2,4,6-T reduced the disease very effectively.

Anatomical studies revealed a number of interesting points. None of the treatments, except that with 2,4-D, induced tylosis in healthy plants. Even in 2,4-D treated plants tyloses were seen in only 6.3 per cent of the vessels. In inoculated plants, however, both 2,4-D and 2,4,6-T induced tylosis to an appreciable extent and this was associated with a restriction of hyphae to a few vessels. In this respect 2,4-D treated susceptible plants, when inoculated, behaved almost like inoculated resistant plants. Indole treated plants showed symptoms similar to those of the control plants, but many fewer of the vessels contained hyphae.

Of the substances tested, indole at 100 p.p.m. did not affect the growth and development of the plant. When treated with 2,4-D at 10 p.p.m., the plants showed slight stunting, and twisting and epinasty in the lower part of the stem. The leaves developed after the treatment were malformed, often looking like fern leaves with prominent venation. These effects of 2,4-D treatment continued to appear till the end of the experiment. Six applications of 2,4,6-T at 10 p.p.m. produced similar effects as 2,4-D, but caused greater modification of the leaf form.

Small outgrowths of tissue sometimes developed along the leaf veins. 2,4,6-T broke apical dominance. Most of the axillary shoots had their leaves rolled into a ball.

A second experiment was done on the same line as the first one with four other compounds known to have growth regulating properties. 2,4-dichloroanisole (DCA) is an anti-auxin (Bonner and Thurlow, 1949). 2,3,5-triiodobenzoic acid (TIBA) is well known for its anti-auxinic activity. (2-Chloroethyl)-trimethylammonium chloride (Cycocel) can cause progressive suppression of growth over a wide range of concentrations without delaying developmental processes. In tomato plants it is known to induce responses which appear to be the reverse of those caused by gibberellins (Witter and Tolbert, 1960). Napthaleneacetamide (NAM) is a synthetic auxin with strong growth regulating activity.

Six-week-old plants of both varieties were used. All the compounds were applied to the soil. TIBA and NAM, both at 50 p.p.m., were applied only once 4 days before inoculation. Cycocel at 200 p.p.m. and DCA at 10 p.p.m. were applied twice, 4 days before and after inoculation. Inoculation was done in the

standard way. In inoculated, NAM-treated plants the leaves suddenly became chlorotic and necrotic within a week after inoculation. Because of this damage, wilting was not assessed for these plants, but they were studied anatomically. Since inoculated resistant plants did not develop wilting in any treatment, they have not been included in Table 39 which shows data for susceptible plants only.

Table 39 Effects of different growth regulating substances on wilting and distribution of hyphae and tyloses at cotyledonary node of inoculated susceptible plants

Treatment	Wilting index per leaf			Percentage of vessels with	
	Days after inoculation				
	14	21	28	Tyloses	Hyphae
Untreated	0.41	0.72	0.95	12.2	18.8
DCA	0.26	0.86	0.79	8.8	22.6
Cycocel	0.05	0.05	0.10	16.2	1.5
TIBA	0.08	0.07	0.06	12.2	0.9
NAM	-	-	-	23.3	0

Average of 3 replicates

Both cycocel and TIBA gave excellent control of wilt at the concentrations employed. Both stimulated tylosis considerably even in the healthy plants in which tyloses were seen in about 10 per cent of vessels. Extensive tylosis occurred in all NAM-treated plants, although in those inoculated these were somewhat more than in healthy plants. The complete absence of hyphae from treated, inoculated plants suggest that excellent control of the disease can be obtained with NAM. The effect of DCA on wilting, tylosis or fungal distribution was not clear. But at the concentration used it showed slight anti-auxin effect by stimulating the production and growth of axillary shoots to some extent. In this respect TIBA showed a very strong effect, and cycocel a moderate effect. Their effects were slightly more pronounced in resistant than in susceptible plants. Cycocel did not induce any abnormal growth response even with two applications at 200 p.p.m. The leaves, particularly those developed after treatment, developed intense, dark green colour. They were somewhat thicker than normal leaves. The newly developed internodes did not extend much. The plants developed very stocky and compact type of growth.

(a) Effect of 2,4-dichlorophenoxyacetic acid

In one of the earlier experiments 2,4-D was found to give good control of wilt at 10 p.p.m. when applied a week before inoculation. But at this concentration, the plants showed considerable twisting and bending of stem, epinasty of the petioles, and frenching and rolling of lamina in newly developed leaves. In the following experiment, an attempt was made to see if control of wilt could be achieved with a lower concentration of 2,4-D. There was the possibility also that 2,4-D at lower concentrations might increase the severity of disease symptoms as IAA did.

Thirty six, 6-week-old GS plants were arranged in six groups of six each. Plants in one group did not receive any treatment and served as the controls. Those in other groups were treated with 1,3 or 10 p.p.m. 2,4-D before or after inoculation. Treatments were given by adding 50 ml. of the different test solutions to the soil around the stem base. Three plants in each group were inoculated in the standard way; another three served as controls. The treatments, and the mean wilting indices for the leaves of inoculated plants, recorded at regular

intervals after inoculation, are shown in Table 40.

Table 40 Wilting indices for leaves of inoculated susceptible plants treated with different concentrations of 2,4-dichlorophenoxyacetic acid

Concentration (p.p.m.)	Time of treatment*	Days after inoculation		
		14	21	28
0	-	0.61	1.04	1.31
1	- 4	1.40	1.69	1.65
3	- 4	0.86	2.27	2.23
10	- 4	0.23	0.30	0.54
3	+ 4	1.31	1.90	2.11
10	+ 4	0.86	2.37	2.47

Average of 3 replicates

* Minus or plus sign followed by a number indicates so many days before or after inoculation

At 10 p.p.m. when applied before inoculation, 2,4-D did not reduce wilt development as markedly as in the previous experiment. At lower concentrations, however, more wilt developed than in the controls. This stimulation was particularly evident during the first three weeks after inoculation. When the treatment was given 4 days after inoculation, the disease was

stimulated at both 3 and 10 p.p.m., the latter being more effective. Here also, a very rapid progress in wilting was noticed within the first 3 weeks.

Anatomical studies with sections prepared from cotyledonary nodes showed considerable tylosis in plants receiving a pre-inoculation treatment with 10 p.p.m. 2,4-D. This agrees with observations made earlier. But in all other treatments, inoculated plants showed tyloses in fewer, and hyphae in more vessels than untreated, inoculated plants. The uninoculated plants whether treated or not, did not wilt.

In two subsequent experiments, however, somewhat different results were obtained. In both the experiments, 2,4-D reduced wilting even at 1 and 3 p.p.m. when applied 4 days before inoculation. On both occasions, 3 p.p.m. gave better control of wilt than 1 p.p.m. When applied 4 days after inoculation, a slight increase in wilting was recorded at 1 p.p.m. and a more significant increase at 3 p.p.m.

It appears that the effect of 2,4-D on disease reaction depends on the timing of treatment in respect of inoculation. At lower concentrations, 2,4-D would reduce the symptoms when applied before inoculation, but stimulate them if applied after inoculation.

(b) Effect of napthalene acetamide

Napthaleneacetamide was used in one of the preliminary experiments. But its effect on wilt development could not be properly studied. However, anatomical studies with these plants revealed complete absence of hyphae and extensive tylosis in them. This suggests that NAM may be very effective in inducing resistance in susceptible plants to the disease, so the following experiment was done to investigate this possibility.

Seven week-old GS plants were treated with 1, 3, 10 or 30 p.p.m. NAM 4 days before inoculation. In each treatment, 50 ml. of test solution was poured into the soil around the stem base. Half of the plants in each treatment, and similar number of untreated plants were root-inoculated in the standard way. Uninoculated controls were maintained with both untreated and treated plants.

Symptoms were never prominent in the plants of this experiment. Even then the inhibitory effect of NAM on symptom expression was very clear (Table 41). It was effective in reducing wilt considerably even at 1 p.p.m. At 3 p.p.m. and above there was complete control of wilt.

Table 41 Wilting index for leaves of inoculated susceptible plants treated with different concentrations of napthaleneacetamide

Concentration (p.p.m.)	Days after inoculation		
	14	21	28
0	0.14	0.62	0.79
1	0.05	0.26	0.37
3	0	0	0
10	0	0	0
30	0	0	0

Average of 3 replicates

The reduction in stem height was evident at all the concentrations employed, but this effect was more prominent at higher concentrations. At 30 p.p.m., there was a slight bending of the stem, and near the base of stem a few white patches could be observed indicating proliferation of tissue. Other treated plants did not show any abnormality in their growth.

(c) Effects of 2,3,5-triiodobenzoic acid and 2,4,6-trichlorophenoxyacetic acid

Both TIBA and 2,4,6-T have been found previously to retard symptom expression in susceptible plants. Further studies were made with these two compounds, and their effects on the disease over a range of concentrations studied.

Thirty, 7-week-old GS plants, arranged in five groups of six each, were used in the first experiment. One group of plants was not given any treatment. The plants in each of the remaining four groups were treated with 1, 3, 10 or 30 p.p.m. TIBA. Treatment was given only once, 4 days before inoculation, by pouring 50 ml. of test solution to the soil. From each group three plants were inoculated in the standard way. Wilting in inoculated plants was assessed at regular intervals after inoculation and the data are presented in Table 42.

Table 42 Wilting index for leaves of inoculated susceptible plants treated with different concentrations of 2,3,5-triiodobenzoic acid

Concentration (p.p.m.)	Days after inoculation		
	14	21	28
0	0.15	0.79	1.39
1	0.19	0.46	1.31
3	0	0.58	0.69
10	0	0.15	0.28
30	0	0.76	1.71

Average of 3 replicates

Even at 1 p.p.m. TIBA reduced wilting slightly, and this effect became more and more pronounced with increase in its concentration up to 10 p.p.m. The rapid progress of wilting at 30 p.p.m. can not, however, be explained. In view of the excellent control of wilt obtained earlier with 50 p.p.m. TIBA, this result appears to be rather anomalous. To resolve the difference between the results from two different experiments, TIBA was tested again at 50 p.p.m. Treatment was given 4 days before inoculation. Mean wilting indices for leaves, 28 days after inoculation, were 0.64 and 0.30 for untreated and treated plants respectively. This suggests that TIBA, at high concentrations, may have some toxic effect on the plants. Such an effect may neutralize partly or completely the action of TIBA in reducing wilting.

When applied at 30 or 50 p.p.m., TIBA considerably modified the form of leaves which grew after the treatment. The new leaves had translucent veins and showed pronounced pubescence. The leaves also showed epinasty although it was never very pronounced. The plants were very stunted and showed odd curvatures in the lower part of the stem. With decrease in concentration, these effects became less and less pronounced until at 1 p.p.m. there was none.

The effects of different concentrations of 2,4,6-T on disease development was studied further with 7-week-old GS plants. The treatments applied in the first experiment were repeated by giving six applications of 10 p.p.m. 2,4,6-T on six consecutive days beginning from a week before inoculation. In two other treatments, 2,4,6-T was applied at 30 or 100 p.p.m. 4 days before inoculation. The treatments were applied by adding the substance to the soil as described earlier. The treated plants along with the untreated ones were inoculated in the standard way. None of the uninoculated controls which were maintained for all the treatments developed any symptoms.

Table 43 Effects of 2,4,6-trichlorophenoxyacetic acid treatments on wilt development in inoculated susceptible plants

Concentration (p.p.m.)	Number of applications	Wilting index per leaf		
		Days after inoculation		
		14	21	28
0	-	0.05	0.36	0.67
30	1	0	0.06	0.26
10	6	0.50	1.32	1.92
100	1	0	0.47	0.72

Average of 3 replicates

Six applications of 10 p.p.m. 2,4,6-T did not reduce wilting as found in the preliminary experiment, but considerably increased it. Inhibition was obtained, however, with one application at 30p.p.m., but a similar treatment with 100 p.p.m. did not show any appreciable effect. In the background of what was achieved with one application of 30 or 100 p.p.m. 2,4,6-T, the results from six applications with 10p.p.m. are anomalous.

(d) Effects of various treatments with cycocel

A considerable reduction in disease symptoms in susceptible plants when treated with a high concentration of cycocel, has already been described. Unlike most of the compounds, so far studied for their effects on disease, cycocel did not induce any abnormal growth response in the plants except that it reduced extension of the internodes. Its effect on the disease was studied in more detail in three subsequent experiments.

In the first experiment, cycocel was used at four different concentrations, 3, 10, 30 and 100 p.p.m. Only one application was made to the soil, 4 days before inoculation. The appropriate inoculated and uninoculated controls were included, but none of the latter showed any symptoms. Wilting was assessed

for the inoculated plants at different periods after inoculation. Anatomical studies were made with sections prepared from all the replicates at the region of the cotyledonary node. The results in respect of wilting in inoculated plants have been summarized in Table 44.

Table 44 Effects of different concentrations of Cycocel on wilting index and distribution of tyloses and hyphae at cotyledonary node of inoculated susceptible plants

Concentration (p.p.m.)	Wilting index per leaf			Percentage of vessels with -	
	Days after inoculation				
	14	21	28	Tyloses	Hyphae
0	1.41	1.73	1.98	3.9	27.9
3	0.79	1.27	1.58	13.3	8.6
10	0.90	1.12	1.35	14.3	6.0
30	0.93	1.04	1.21	16.2	13.2
100	0.46	0.56	0.66	19.5	10.8

Average of 3 replicates

Cycocel was effective in inhibiting wilting even at 3 p.p.m. This effect became more and more prominent at successive higher concentrations used. In most of the treatments wilting was not only less

when sampling commenced 14 days after inoculation, but its progress was also less rapid during the next 2 weeks than in the inoculated controls (Fig. 9). There was a direct correlation between increased tylosis in treated plants and the concentration of cycocel employed. This effect was found to be moderate in healthy plants, and at 100 p.p.m. tyloses were found in 5.9 per cent of vessels only. In the inoculated plants, on the other hand, tylose formation was stimulated remarkably by cycocel treatment. A marked reduction in hyphal distribution was evident in all the treatments, but there was no graded effect within the range of concentrations used.

The second experiment was done partly to confirm the results from the first one, and also to test the effectiveness of a higher concentration of cycocel on wilt, when applied only once. Cycocel at 30, 100 or 300 p.p.m. was applied 4 days before inoculation. Sampling was continued for an extended period of 6 weeks in an attempt to find out how long the effect of cycocel on the disease would last.

Considerable reduction in symptoms were obtained with all 3 concentrations used. In treated, inoculated plants, there was slight wilting at 30 p.p.m., and

Fig. 9. EFFECT OF CYCOCEL ON DEVELOPMENT OF WILTING IN SUSCEPTIBLE PLANTS.

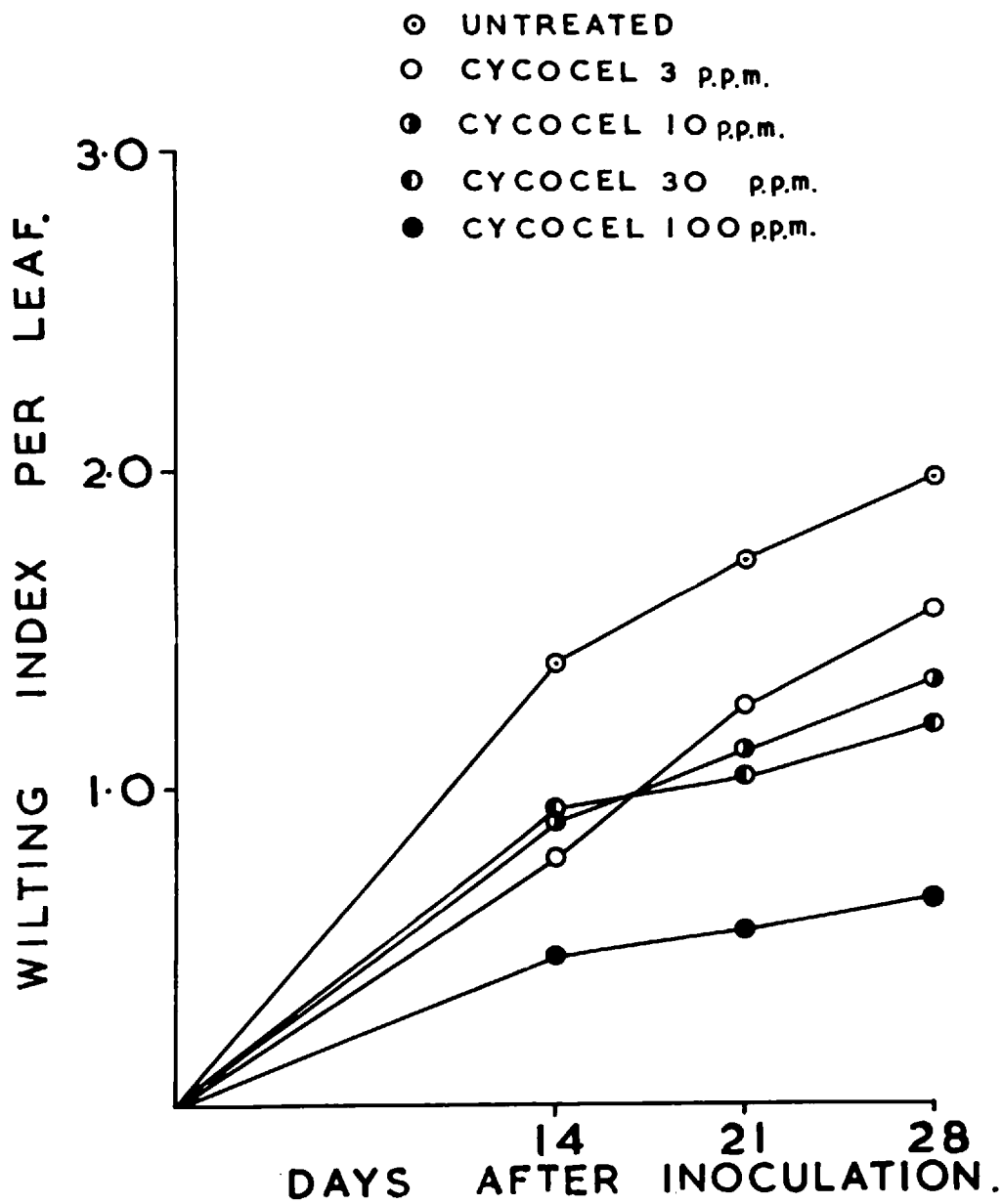


Table 45 Wilting index for leaves of inoculated susceptible plants treated with different concentrations of Cycocel.

Concentration (p.p.m.)	Weeks after inoculation				
	2	3	4	5	6
0	0.90	1.09	1.70	1.83	1.99
30	0.32	0.35	0.48	0.82	1.05
100	0	0	0.30	0.64	0.89
300	0	0	0.18	0.47	0.72

Average of 3 replicates

none at 100 and 300 p.p.m. during the first 3 weeks. Thereafter, wilting developed gradually in all the treated plants and this was associated with a corresponding reduction in the effects of the treatments on plant growth. In this experiment also, treatment with Cycocel increased considerably the percentage of vessels with tyloses and reduced the same for vessels that became infected. The inhibitory effect of Cycocel on plant growth does not persist for more than 2 - 4 weeks, depending upon the concentration used. Similarly, its effect on wilt development also disappears gradually. It seemed, therefore, to be quite likely that one or

more further treatments after inoculation might have given complete control of the disease.

In the last of this series of experiments, Cycocel was used at 30 and 100 p.p.m. only. There were three sets of 7-week-old plants for each concentration employed. Treatments were given 4 days before, and 8 and 20 days after inoculation. One set of plants received the first treatment only. The second set of plants received the first two treatments. Those in the third set received all three treatments. Inoculated, uninoculated and uninoculated, treated controls were maintained. Four weeks after inoculation, the mean wilting indices (per leaf) for the plants receiving 1, 2 and 3 treatments at 30 p.p.m. were 0.42, 0.36 and 0.34 respectively. At 100 p.p.m. the indices for 1, 2 and 3 treatments were respectively 0.44, 0.24 and 0.10. The inoculated controls had a mean index of 1.09, while the uninoculated controls had no wilt. The plants receiving two or three applications of 100 p.p.m. Cycocel showed considerable yellowing in the lower leaves. This effect was more prominent in uninoculated than in inoculated plants.

It does not appear from the results that two or three applications of Cycocel give much better

control of wilt than one such application. On the other hand, more than one application at a high concentration, such as 100 p.p.m. may be harmful for the plants and cause considerable yellowing.

7. In vitro fungitoxicity tests

It has been possible to modify the normal response of the host to the pathogen by treatment with different growth regulating substances. Some of these treatments induced considerable changes in the gross morphology of the shoot. This suggests a shift in host metabolism. But a close correlation was also found between the effect of any treatment on the disease and its effect on the fungal distribution in the host.

To check the toxicity of these compounds to V. albo-atrum, the mycelial growth was studied in SSN medium supplemented with them at concentrations ranging from 1 - 100 p.p.m. The tests were done in the way as described before. Each treatment was replicated five times. While testing Cycocel and MH, SSN medium served as the control. But for all other substances which had to be dissolved initially in ethanol, an ethanol control series was also included. The medium in this series had the same concentration of ethanol as was present in all other media containing different strengths of a particular

compound. Mycelial dry weights were determined after 14 days of incubation. The results from different experiments have been summarized in Tables 46 and 47.

Table 46 *In vitro* fungitoxicity of Cycocel and maleic hydrazide to *V. albo-atrum*

Concentration (p.p.m.)	Dry wt. of mycelium (mg.)*	
	Cycocel	Maleic hydrazide
0	50.0	50.0
1	68.0	29.1
10	68.8	24.0
100	94.3	22.3

* Average of 5 replicates

Table 47 *In vitro* fungitoxicity of various plant growth regulators to *V. albo-atrum*

Concentration (p.p.m.)	Dry wt. of mycelium (mg.)*					
	NAM	TIBA	IAA	GA	2,4-D	2,4,6-T
0	26.0	26.0	69.0	69.0	17.8	17.8
1	21.5	34.4	69.2	73.0	18.3	16.0
10	32.4	8.0	69.0	66.2	19.0	7.5
100	18.8	6.4	86.8	51.3	37.0	-

* Average of 5 replicates

With most of the compounds tested, a graded effect was obtained with increase in their concentrations. Cycocel and IAA both of which suppress symptoms effectively at 100 p.p.m., appreciably stimulated mycelial growth at that concentration. No correaltion is indicated between the effect of 2,4-D on mycelial growth and the disease. Disease development is retarded by GA at lower concentration and stimulated at higher concentration. Its effect on mycelial growth was just the opposite. The effect of NAM on mycelial growth was rather irregular. At 100 p.p.m., mycelial growth was considerably inhibited. But NAM has been previously found to reduce wilting markedly at much lower concentrations. All the three anti-auxins tested, viz., TIBA, 2,4,6-T and MH, behaved rather uniformly in their gradual inhibition of mycelial growth. Only in these cases could some correlation be found at lower concentrations between the effect of a compound on the disease and its effect on mycelial growth. Even then there was disparity between the concentrations that exert most inhibitory effect on mycelial growth and on the disease. There is evidence to suggest that these compounds stimulate symptom production at higher concentrations at which they markedly inhibited the growth of the pathogen <u>in vitro</u>.

C. Studies on the nature of *Verticillium* resistance in tomato plants

There are conflicting views on the location of *Verticillium* resistance in the tomato plant (Threlfall, 1957; Blackhurst and Wood, 1963). The observations made in course of the experiments described earlier, suggest that resistance is present both in the roots and the stems. There are indications also that the leaves of different varieties may show differential resistance to infection. There is no doubt about the fact that the fungus infects the roots in both varieties, and subsequently reaches the stem. In the susceptible variety, the fungus proliferates rapidly, and spreads into new areas of the stem. But in the resistant variety, it remains restricted, mostly to the lower part of the stem, and there too, its growth is very sparse. In the resistant plants, the fungus is unable to develop to an extent that can cause injury to the host. It appears from this that the principal resistance to *Verticillium* infection may be located within the vascular elements. This view is supported by the results of experiments with cut shoots of both varieties. A

series of experiments were done to provide further insight into the nature of this resistance.

1. Cultural studies with plant extracts

Attempts were made to determine whether the extracts from resistant and susceptible varieties had any differential effect on the growth of the pathogen *in vitro*. The supernatants were collected by centrifugation from the macerates made with root and stem segments of 6-week-old resistant and susceptible plants. The supernatants were either used as such in 50 ml. portions in medicine bottles, or added, in equal volume, to 25 ml. of double strength SSN medium. Sterilization and subsequent inoculation were done in the usual way. The extracts, when used alone, supported very poor growth of the fungus. This may be due to a lack of nutrients, or may also be due to the release, into the extracts, of inhibitory substances during maceration of the tissue. In the SSN-supplemented extracts, the fungus grew very well. But neither with the root extracts, nor with the stem extracts from the two varieties was there any indication of differential effects on fungal growth. The experiment was repeated with the extracts from the roots and the stems of resistant and susceptible

plants of different age groups. Again, there was no difference between the two varieties.

The difference between the two varieties in their capacity to promote growth of the pathogen may depend on the presence or absence of one or more substances in them. But any such would most probably be masked by the effects of many other substances released from tissues into the extracts.

2. Effects of tracheal saps from healthy plants on spore germination and fungal growth in vitro.

Since the fungus grows in xylem vessels for most of its existence inside the host, the next experiment was designed to compare the effects of tracheal saps from two varieties on spore germination and fungal growth in vitro. Tracheal saps were collected from 11-week-old GR and GS plants and stored at -20°C. Before use, the frozen saps were thawed, and then sterilized by passing through a micropore filter. The sterile saps were then used as follows.

(1) Five ml. portions of the saps were transferred to sterile culture tubes. These tubes and others with 5 ml. of SCA liquid medium were inoculated with

0.25 ml. of mycelial suspension. The synthetic medium was included for the purpose of comparison. Dry weights of mycelia were determined after 12 days of incubation. The mean dry weight of four replicates were 2.9, 3.0 and 19.7 for GR sap, GS sap and SCA medium respectively.

A sterile, thoroughly washed spore suspension containing 3,000,000 spores per ml. was used in spore-germination studies. One-tenth ml. of this suspension was added to 2.9 ml. of sap, from GR or GS plants or sterile water. The three suspensions thus made had the same concentrations of 100,000 spore per ml., but in three different media. Spore-germination studies were made with these suspensions in the way already described. The results based on a count of at least 400 spores are shown in Table 48.

Table 48 Effect of tracheal saps on germination of spores of V. albo-atrum

Medium of germination	Percentage germination	Mean germ tube length (μ)
Water	7.8	7.40
GR sap	94.4	74.02
GS sap	87.7	66.31

The saps collected from resistant and susceptible plants appear to be very poor in nutrients. Further, they do not differ greatly in their effects on spore germination and germ tube growth. The results discount the possibility that the poor growth of the fungus in resistant plants may be due to the presence of certain inhibitory substances in the tracheal sap of healthy plants, at the time when they become infected.

3. Effects of certain nutrients on expression of resistance

A relation between wilt symptoms in tomato plants and their content of carbohydrate has been suggested by Roberts (1944). It has been shown by Selman and Buckley (1959) that V. albo-atrum infects tomato roots severely only in the presence of high level of carbohydrate. The importance of nitrogen in tomato wilt has also been indicated (Roberts, 1943). In view of these findings, a preliminary experiment was carried out to determine the effects of certain nutrients on Verticillium wilt resistance in tomatoes.

Resistant tomato cuttings were inoculated with a spore suspension, and then transferred to half-strength nutrient solution containing 0.1 per cent of

glucose or casamino acids. Uninoculated, treated controls, and inoculated and uninoculated controls were maintained. Treatments with glucose and casamino acids continued for 4 days after which the cuttings were transferred to half-strength nutrient solution. During the period of treatment the solutions were changed daily to keep bacterial contamination to a minimum. The cuttings were trimmed slightly (2 - 3 mm.) at the cut end every second day for the first 6 days to prevent clogging of the cut ends. Wilting was assessed at weekly intervals until the end of the fourth week. Since all the replicates showed wilting, they have been included in Table 49.

Both glucose and casamino acids stimulated wilting in inoculated plants, the effect of glucose being much more pronounced in this respect. Glucose treatment caused more wilting even in the uninoculated controls. Among the inoculated plants, only in glucose treatment did the wilting index show any increase between 14 and 28 days of inoculation. In untreated, and casamino acids-treated plants there were signs of recovery after 14 days. It was decided, therefore, to

Table 49 Effects of glucose and casamino acids on wilt development in resistant cuttings

Treatment	Wilting index per leaf			
	Days after inoculation			
	7	14	21	28
Nil - C	0.37	0.38	0.35	0.30
" - I	0.67	0.85	0.83	0.76
Glucose - C	0.57	0.46	0.52	0.56
" - I	1.02	1.39	1.35	1.55
Cas. acids - C	0.31	0.33	0.30	0.27
" " - I	0.65	1.01	1.01	0.92

Average of 3 replicates

C = Control

I = Inoculated

study further the effect of glucose on resistance. In the next experiment, the resistant cuttings were treated with 0.1 and 0.5 per cent glucose in half-strength nutrient solution for 6 days after inoculation. The appropriate controls were included. The uninoculated cuttings, whether treated with glucose or not, showed the same kind of wilting. The mean wilting indices

for the leaves of such cuttings varied between 0.45 and 0.62 on different occasions when sampling was done. Table 50 includes the results from the inoculated cuttings only.

Table 50 Effects of different concentrations of glucose on wilt development in inoculated resistant cuttings

Treatment	Wilting index per leaf			
	Days after inoculation			
	7	14	21	28
Nil	0.43	0.79	0.82	0.78
Glucose 0.1%	0.99	1.24	1.10	1.0
Glucose 0.5%	0.95	1.11	1.02	0.96

Average of 3 replicates

Glucose treatment increased wilting considerably in the inoculated cuttings during the first two weeks after inoculation. Thereafter, the glucose-treated cuttings recovered gradually. The glucose induced stimulation in wilting could have been due to a stimulation of mycelial growth in the cuttings. But there was no apparent difference in the distribution of the fungus between the cuttings receiving glucose treatment and those which did not.

4. Effect of ethanol on expression of resistance

The resistance of potato tubers to Phytophthora infestans has been found to be reduced by treatment with ethanol and other alcohols (Behr, 1949; Tomiyama et al., 1957). Varieties thus treated also became temporarily susceptible to Fusarium rot (Behr, 1949). The lowering of resistance may have been due to the reduced metabolic activity in host tissue caused by the action of ethanol as a narcotic. Scheffer and Walker (1954) studying Fusarium wilt of tomato, obtained similar results with ethanol, but not with other alcohols.

Experiments on similar lines were done to determine the effect of ethanol on the resistance of tomatoes to Verticillium wilt.

Cuttings from 8-week-old GR plants were inoculated, and then transferred to half-strength nutrient solution containing 0.2, 0.5 and 1.0 per cent ethanol. There were inoculated, uninoculated and ethanol-treated, uninoculated controls too. The cuttings had their cut ends trimmed by 2 -3 mm. every second day, when the solutions were also changed. Wilting was recorded at the end of the first and second week. The results are given in Table 51.

Table 51 Effects of different concentrations of ethanol on wilting in resistant cuttings

Treatment	Wilting index per leaf			
	Healthy		Inoculated	
	D		D	
	7	14	7	14
Nil	0.31	0.62	0.30	0.60
Ethanol 0.2%	0.50	1.31	0.77	1.56
Ethanol 0.5%	0.51	1.46	0.82	2.17
Ethanol 1.0%	0.90	1.47	0.70	2.30

Average of 3 replicates

D = Days after inoculation

A continuous treatment with ethanol for 14 days caused considerable increase in wilting over the controls in both uninoculated and inoculated cuttings. This effect was, however, more prominent in inoculated plants, particularly at two higher concentrations employed. In some of the inoculated cuttings which had been treated with 0.5 and 1.0 per cent ethanol, the fungus was found to grow out of the cut ends of the plants. All the inoculated cuttings were sectioned 7.5 centimetres above the base at the end of sampling, and the sections were examined

microscopically. Ethanol treatment, at different concentrations, was found to have increased the percentage of infected vessels 2- to 3-fold over the controls. It is possible, therefore, that ethanol caused increased wilting in resistant cuttings, because it stimulated fungal growth at the concentrations employed. So the effect of ethanol on growth of the pathogen *in vitro* was tested in SSN liquid medium. Ethanol did not cause any significant change in mycelial dry weight when it was used at concentrations ranging from 0.05 to 1.0 per cent.

In view of the considerable wilting caused even in uninoculated, treated plants it was decided to reduce the period of treatment. In the next experiment, done along the same lines as the first one, ethanol treatment was continued for 6 days only after inoculation. The trimming of cut ends stopped when ethanol treatment was discontinued. In addition to wilting, the production of adventitious roots from the cuttings were also estimated over a period of 28 days after inoculation.

The inoculated cuttings receiving different treatments with ethanol, always showed more wilting than the inoculated controls (Table 52). Nevertheless,

Table 52 Effects of different concentrations of ethanol on inoculated resistant cuttings

Treatment	Wilting index per leaf				Number of roots		
	D				D		
	6	14	21	28	6	14	21
Untreated	0.67	0.85	0.83	0.76	2	30	48
Ethanol 0.2%	0.67	1.22	1.17	1.17	0	17	40
Ethanol 0.5%	0.82	1.36	1.36	1.26	0	3	32
Ethanol 1.0%	1.25	1.52	1.53	1.36	0	0	25

Average of 3 replicates

D = Days after inoculation

all of them were showing signs of recovery during the last week of the experiment. There was also a close correlation between the action of ethanol at any concentration in inducing wilting initially, and its inhibition of root production. Ethanol treatment also inhibited root production in uninoculated plants, but there the effect was much less marked.

In another experiment, inoculated resistant cuttings in sets of three each were treated with ethanol at 0.5 per cent for 6 and 10 days respectively after inoculation. There were appropriate controls including inoculated and uninoculated cuttings as well as

uninoculated, ethanol-treated ones. In addition, cuttings from susceptible plants of the same age group were also inoculated for the purpose of comparison. All the uninoculated resistant cuttings showed a certain amount of wilting, but the data recorded in respect of inoculated cuttings only have been included in Table 53.

Table 53 Wilting index for leaves of inoculated cuttings from resistant and susceptible plants

Plant	Treatment	Days after inoculation			
		7	14	21	28
GR	Untreated	0.43	0.82	0.79	0.80
	Ethanol 0.5% 6 days	1.17	1.52	1.50	1.30
	Ethanol 0.5% 10 days	1.03	1.76	1.66	1.47
GS	Untreated	0.60	1.07	1.39	1.49

Average of 3 replicates

In ethanol-treated, inoculated resistant cuttings, the development of wilting was considerably more than in the untreated, inoculated controls. This increased wilting, resulting from ethanol treatment,

compares favourably with the wilting in inoculated susceptible cuttings. But the development of wilting did not follow the same course in susceptible and ethanol-treated resistant cuttings. In the former, wilting developed rapidly during the first 3 weeks, and then slowly in the fourth week after inoculation. In the latter, the wilt development was strikingly rapid for the first 2 weeks, but there was no further progress during the next 2 weeks. It is possible that in inoculated resistant cuttings the defence mechanism was temporarily altered under the influence of ethanol resulting in considerable wilting. When ethanol treatment was discontinued, the cuttings recovered. Anatomical studies were made at the end of the experiment with the sections prepared from the middle of the inoculated resistant cuttings. Hyphae were observed in 5.9 per cent of vessels in untreated cuttings and in 6.0 to 9.1 per cent of vessels in the cuttings receiving different ethanol treatments. The difference between untreated and ethanol-treated cuttings, in respect of the distribution of the fungus, does not appear to be large enough to explain considerable wilting in the latter.

5. Effects of metabolic inhibitors on resistance

A relation between resistance and host metabolism has often been suggested. In the experiments described earlier it has been shown that resistance can be induced in susceptible plants by treatment with certain growth regulators. For most of these compounds the correlation between their capacity to induce resistance and to cause formative changes in tomato plants was good. Many of these substances are also known to cause metabolic changes in tomato plants (Davis and Dimond, 1953). If resistance of a host is dependent on its metabolic activity, then a treatment which affects metabolism may cause some changes in resistance. This was nicely demonstrated by Gothoskar et al. (1955) who induced normal symptoms of Fusarium wilt in resistant tomato cuttings which had been treated with one of a variety of metabolic inhibitors. Deese and Stahman (1962a) recently studied some of the changes in resistant and susceptible stem tissues of tomato inoculated with V. albo-atrum. They noted, in resistant tissues, a significant increase in oxidase activity after inoculation. Such tissues also gave a strong reaction for quinones. It became, therefore,

of some interest to study the effects of metabolic inhibitors on resistance of tomato plants to V.albo-atrum.

In the first experiment, 2,4-dinitrophenol (DNP), thiourea, sodium diethyldithiocarbamate (DIECA) and sodium fluoride were tested for their effects on resistance. The role of DNP in respiration is to uncouple oxidation from phosphorylation. Sodium fluoride prevents glycolysis by inhibiting enolase. Thiourea and DIECA both inhibit polyphenol oxidase and ascorbic acid oxidase. Sodium fluoride, thiourea and DIECA were used as 10^{-3}M and 10^{-4}M solutions in water. But DNP was used as 10^{-4} and 10^{-5}M solutions. Resistant cuttings from 8-week-old plants were inoculated and kept in various inhibitor solutions for 5 days. The inoculated controls were kept in water for that period. Afterwards, they were transferred to half-strength nutrient solution. Controls were also maintained with uninoculated as well as inhibitor-treated, uninoculated cuttings. The experiment was run in the greenhouse. Wilting and yellowing were assessed 21 days after inoculation. Only two treatments, 10^{-4}M DNP and 10^{-3}M thiourea, caused any appreciable increase in symptoms in

inoculated cuttings over the controls. The mean disease indices for the leaves were 0.78, 1.01 and 1.13 for untreated and 10^{-3}M thiourea-, and 10^{-4}M DNP-treated, inoculated cuttings. The treatments with DNP caused considerable yellowing of the lower leaves. The cuttings treated with other inhibitors behaved as did untreated cuttings.

The effect of DNP at 10^{-3}M and 10^{-4}M was tested on the resistant cuttings in the same way as in the first experiment. All the cuttings treated with 10^{-3}M DNP became seriously damaged within 3 days after inoculation. This was thought to be a direct effect of DNP on the host rather than the result of reduced resistance. At 10^{-4}M DNP, the inoculated cuttings did not show any increase in wilting over inoculated, untreated controls, but there was considerable yellowing in the lower leaves.

In another experiment, salicyladoxime, 4-chlororesorcinol, DIECA and 8-hydroxyquinoline were tested for their effects on resistance. Both salicyladoxime and 4-chlororesorcinol inhibit polyphenol oxidase, and 8-hydroxyquinoline inhibits ascorbic acid oxidase. Eighteen GR cuttings and three GS cuttings were inoculated, and then transferred to half-strength nutrient solution. Controls were kept

for both varieties with similar numbers of uninoculated cuttings. After 3 days, inoculated lots of three cuttings each were transferred into a modified nutrient solution containing 10^{-3}M 4-chlororesorcinol, salicyladoxime, DIECA or 8-hydroxyquinoline. The modified nutrient solution consisted of the same amounts of $Ca(NO_3)_2$, $MgSO_4$ and KNO_3 as present in Long Ahston nutrient solution, but dissolved in M/300 phosphate buffer instead of water. For treatment with DIECA, the pH of the nutrient solution was maintained at 7.0, because this compound decomposes at a lower pH (James and Garton, 1952). For all others, a pH of 6.0 was maintained as it is very close to the pH of tomato sap which is about 5.8. The uninoculated cuttings were similarly treated. Three inoculated and three uninoculated cuttings of both the varieties were transferred into the modified nutrient solution maintained at pH 6.0. The remaining three inoculated and three uninoculated GR cuttings were also kept in same nutrient solution, but at pH 7.0 to serve as control for DIECA treatment. Treatment with inhibitors was continued for a week. During this period, nutrient solutions were changed every second day or earlier if the pH changed by more

than 0.2. After 7 days of treatment the cuttings were transferred to half-strength nutrient solution.

The experiment was run in an illuminated, constant temperature cabinet (16°C.- 18°C.). Wilting index for the leaves of inoculated plants were recorded for each treatment 21 and 28 days after inoculation. The uninoculated, treated controls did not show any appreciable wilting. At the end of the experiment, the inoculated cuttings were sectioned 7.5 and 15.0 centimetres above the cut end, and the sections were studied microscopically. The results are summarized in Table 54. None of the treatments appears to have been successful in inducing inoculated GR cuttings to develop symptoms to any significant extent. In all the treatments, except that with DIECA, more wilting developed initially than in the untreated controls. But all the cuttings showed definite signs of recovery during the last week of the experiment. The distribution of the fungus in the lower part of the cuttings treated with salicyladoxime, DIECA and 8-hydroxyquinoline was much higher than that in the inoculated controls, and compared favourably with that in susceptible cuttings. But in the upper part of all the GR cuttings, the

Table 54 Effects of various metabolic inhibitors on inoculated resistant cuttings

Plant	Treatment	Wilting index per leaf		Vessels with hyphae(%)	
		Days after inoculation		Height of section from base	
		21	28	7.5 cm.	15.0 cm.
GR	Untreated (pH 6.0)	0.23	0.19	12.5	10.9
	4-Chlororesorcinol	0.37	0.28	10.2	7.6
	Salicyladoxime	0.46	0.39	18.2	7.5
	8-hydroxyquinoline	0.35	0.23	26.0	11.3
	Untreated (pH 7.0)	0.26	0.20	11.9	9.8
GR	DIECA	0.25	0.24	24.3	11.7
GS	Untreated(pH 6.0)	1.25	1.28	20.3	24.1

Average of 3 replicates

fungus was evidently disappearing. A comparison between inhibitor-treated, inoculated GR cuttings and inoculated GS cuttings makes it clear that the treatments with various inhibitors could not modify the resistance mechanism sufficiently to induce continued development of symptoms in GR cuttings. All the treatments inhibited root production in the cuttings during the first 2 weeks. This may have been responsible for the slightly increased symptoms found in treated cuttings. The leaves were dark green and curled after treatment with 8-hydroxyquinoline and there were prominent axillary shoots from the upper leaves. In 4-chlororesorcinol-treated cuttings the root tips were often red, and there were red scars on the treated surface.

This experiment was repeated with slight modifications. Seven-week-old resistant plants, grown in nutrient solution, were used. The plants in lots of six were transferred to the modified nutrient solution containing 10^{-3}M of different inhibitors. The treatments continued for 5 days after which the plants were transferred to nutrient solution for a day before being inoculated and potted. Out of six plants in each treatment, three were

inoculated by the root-dip method and others were kept as control. The pre-inoculation stage of this experiment was carried out in the illuminated cabinet mentioned before, but after inoculation the plants were kept in the greenhouse for the next 4 weeks. During this period there was no indication, in any of the treated, inoculated plants, of a reduction in resistance.

6. Experiments with tissue segments

Spencer et al. (1957) detected an antifungal substance from stem and root tissues of broad bean plants. When short segments of the stems and the roots were placed on agar seeded with the spores of Aspergillus niger, a clear zone of inhibition was produced around the segments. But this inhibitory activity was absent from the fluid expressed from the tissue or from its extract. Indeed, such extracts stimulated growth. With segments from the petiole of tomato, however, they could not find any inhibitory effect on fungal growth. An attempt was made, by using similar technique, to determine if the segments from resistant tomato stem had any inhibitory effect on the growth of V. albo-atrum.

Petri-dishes containing SCA agar medium were

inoculated by placing one loopful of spore suspension at the centre and spreading it uniformly over a circular area about 2 cm. in diameter. After 24 hours, 0.5 cm. segments from the surface-sterilized GR and GS stems were placed, in groups of two, one centimetre beyond the margin of the colony. Each Petri dish included one pair of segments from each of GR and GS stem, but they were placed on opposite sides of the colony. In 4 - 5 days time, the advancing margin of the colony reached the segments and grew over them. In none of the six replicates there was any suggestion of the inhibition of fungal growth. It was thought to be possible that SC. agar medium which supports very good growth of the fungus might have helped it to overcome any inhibitory effect of the stem segments. The experiment was repeated, therefore, with SSN agar medium which supports only moderate growth, and water agar. The results were essentially the same. These observations suggest that in healthy resistant plants there are no antifungal compounds which could account for its resistance to this fungus. There is, however, still the possibility that such compounds may be produced in the tissue of resistant plants as a response to infection. An attempt was made, therefore, to explore

this possibility. Spencer et al. (1957) have shown that fungicides when placed on top of a segment of tomato leaf petiole placed upright on nutrient agar seeded with spores, can inhibit growth of the fungus around the base of the segment. This means that the compound moves down through the tissue of the petiole and diffuses into agar. Similarly, it is expected that any substance produced on or near the cut surface of a segment as a result of inoculation would also diffuse out of the tissue into agar. If the substance has any fungistatic effect and provided it is present in sufficient concentration, then the growth of the fungus will be checked. Keeping this possibility in mind, a series of experiments were done.

Surface-sterilized portions from GR and GS stems were cut into 0.5 cm. long segments by means of a sterile scalpel. These segments were placed on moist filter paper in sterile Petri dishes. All the segments in particular Petri dishes were inoculated at the cut surfaces by touching with an inoculating needle which had previously been dipped in a spore suspension (10,000,000 spores/ml.) Controls were kept with uninoculated segments maintained in the same

way. The segments were incubated at 20°C. After 48, or 72 hours they were removed in groups of three and placed side by side one centimetre beyond the margin of a growing 5 to 7-day-old colony on SSN agar medium. Each Petri dish had three infected segments on one side of the colony and three healthy segments on the other side. The segments from GR and GS stems were kept in separate Petri dishes. The fungus grew equally well on both GR and GS stem segments. In some segments, the fungus grew out of the base into agar, but before long these segments were enveloped by the growing colony. The GR and GS stem segments, whether inoculated or not, behaved in the same way. There was no evidence of any inhibitory effect. The experiment was repeated and essentially similar results were obtained. Tomiyama et al. (1958) have shown that resistance of potato tuber slices to Phytophtora infestans depends on their thickness and the concentration of zoospores in the inoculum. If the concentration of inoculum is relatively high for a slice of certain thickness, then its resistance mechanism may fail. A much diluted spore suspension (500,000 spores/ml.) was used, therefore, in the next experiment. The experiment was done in the same way as before. There were 5

Petri dishes for the stem segments of each variety. In most of these, the growth of the colony was not inhibited. But in 2 Petri dishes with segments from resistant stem, there was a narrow zone of inhibition between the advancing margin of the colony and inoculated stem segments. This zone of inhibition did not persist, however, for more than two days. Ultimately the colony grew over these segments. The experiment was repeated by using dilute spore suspension as the source of inoculum. There were 6 Petri-dishes for each kind of stem segments. Among the Petri dishes with resistant stem segments, there was bacterial growth around the base of inoculated segments in 3 and around the base of healthy segments in 2. Similarly, for susceptible stem segments, there was bacterial contamination around both healthy and inoculated segments in 3 Petri dishes. Bacterial growth was evident within 2 days of transferring the segments on to agar. Two days later (4 days after transfer to agar), all the Petri dishes were examined. Healthy GR or GS stem segments and inoculated GS stem segments did not inhibit the growth of the colony. If there was bacterial growth around the base of some of these segments, then very slight inhibition was

noted in some cases. Inoculated GR stem segments definitely checked the advance of the growing colony and 2.0 - 3.5 mm. wide inhibition zones were noted. When bacteria grew around the base of some of the inoculated segments, striking inhibition of fungal growth was noticed. The inhibition zone varied, in its width, between 4.0 and 8.0 mm. The observations suggest that some substance with antifungal properties may be produced within the resistant tissue when the latter is inoculated with the pathogen. This substance diffuses out of the tissue into agar and checks the growth of the colony. It is possible that this substance is produced in larger amount in presence of certain bacteria. Many such bacteria may be present in soil on the surface of, or near the roots of resistant plants in which the defence reaction initially occurs.

7. Extracts, tracheal saps and plant diffusates from healthy and inoculated plants

From the different experiments described so far, some evidence has been obtained which indicate the post-infectional formation, in the resistant tissue, of a substance with antifungal activity. The liberation of such a compound at the site of infection, particularly

in infected vascular tissue, might help to explain the natural resistance shown by GR plants to Verticillium infection. The fungus has always been found in the tracheae of inoculated plants. It almost disappears from resistant tissue very soon after inoculation. In inoculated susceptible plants a similar disappearance is not common, but it has been found occasionally at a late stage of infection. A number of experiments were done to investigate this aspect of the problem.

Comparative studies were made with tissue extracts from healthy and infected plants of both varieties. Six-week-old GR and GS plants were inoculated by root-dip method. Healthy plants were kept as controls. Three weeks after inoculation, when the symptoms were very pronounced in inoculated GS plants and only slight in similar GR plants, stem segments were collected from both control and inoculated plants for maceration. The extracts from different tissues were added, in equal volume, to 25 ml. of double strength, or normal strength SSN medium and their effects on fungal growth tested. The stem extracts from healthy and inoculated plants did not show any differential effect on fungal growth. This was true for both the varieties.

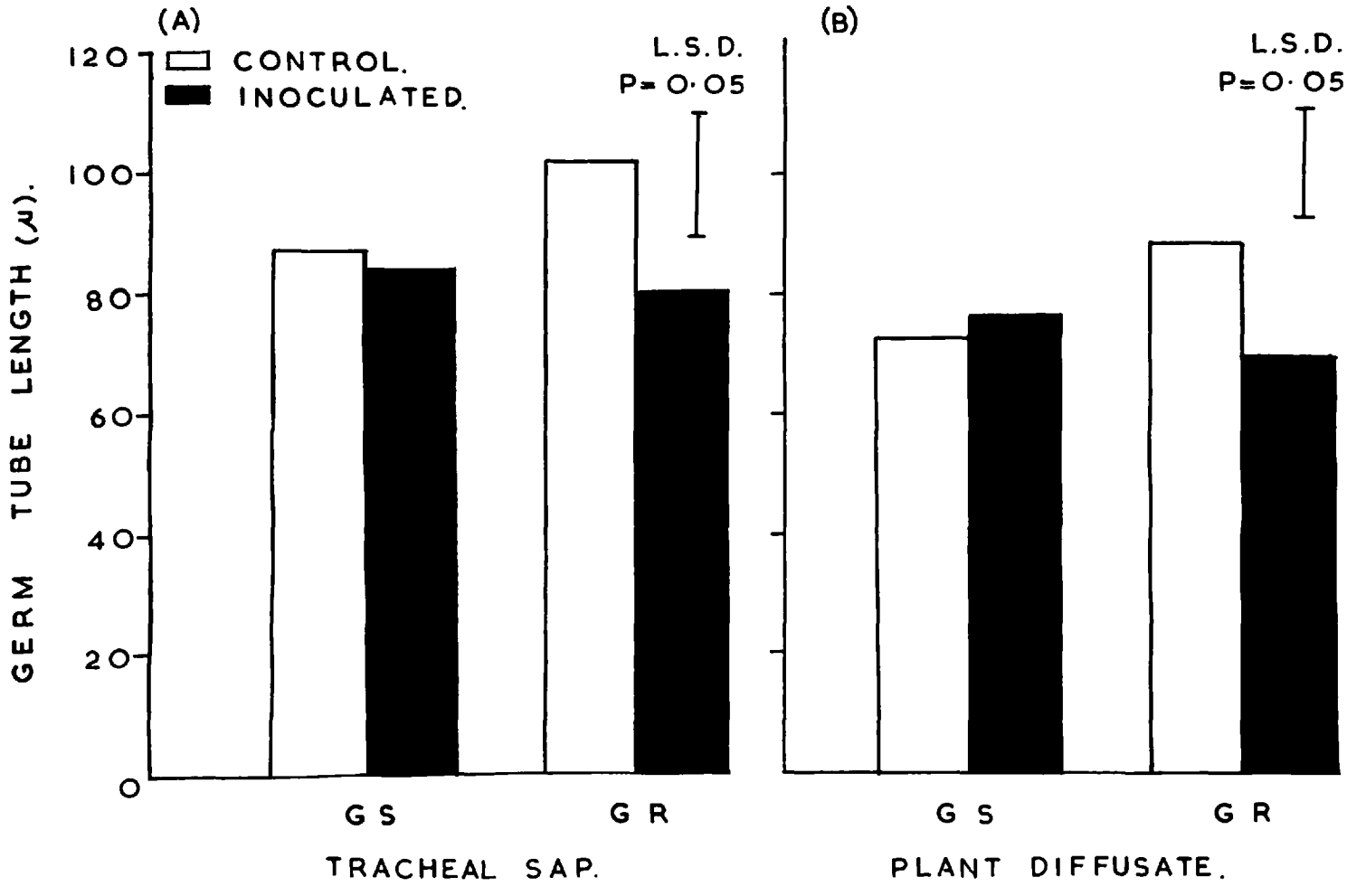
In the next experiment, tracheal saps from healthy and infected plants were tested for their effect on spore germination and mycelial growth. Collections were made from six GRC and GSC, and twelve GRI and GSI plants 10 days after inoculation. The plants were about 7 weeks old when sap was collected from them. The volume of tracheal sap collected from healthy plants was much higher than that collected from inoculated plants. These saps were stored at -20°C., thawed immediately before use and sterilized by passing through a micropore filter. Their effects on spore germination and mycelial growth were assessed in the way already described. For studies on mycelial growth, there were 3 replicates for each treatment. The mean dry weights of mycelium on the sap from GRC, GRI, GSC and GSI plants were 1.57, 1.50, 1.50 and 1.65 mg. respectively 12 days after inoculation. The differences within each variety, between two different treatments, was not significant at P = 0.05 level. The results from spore germination studies, as shown in Table 55, are based on the counts of 405-415 spores. For statistical analysis, however, only 5 spores were counted at random from each spot i.e., 10 from each slide. The results, graphically illustrated in Fig. 10(A) is based, therefore, on a count of 40 spores.

Table 55 Effects of tracheal saps from healthy and inoculated plants on spore germination

Medium of germination	Percentage germination	Mean germ tube length (μ)
Water	8.71	8.22
GRC sap	95.02	101.39
GRI sap	95.06	80.48
GSC sap	94.23	86.90
GSI sap	95.31	84.98

The different tracheal saps stimulated spore germination almost to the same extent. There was no apparent effect of the infection. The sap from GRC plants promoted germ tube growth considerably more than the sap from GSC plants. A similar observation has been made earlier. There was no appreciable difference between germ tube growth on the saps from GSC and GSI plants. The sap from GRI plants reduced germ tube growth considerably in comparison to that from GRC plants. The difference in germ tube growth between these two treatments was statistically significant. These result give some support to the suggestion that some antifungal substance might be produced in the resistant tissue as a result of infection. The concentration of such a substance

Fig. 10. EFFECT OF TRACHEAL SAPS AND PLANT DIFFUSATES ON GERM TUBE GROWTH.



in the sap from GRI plants was obviously very low. In root-inoculated GR plants, the fungus is found in very few vessels, even at the early stage of infection. The antifungal substance may be present at fungitoxic concentrations at the sites of its production and may inhibit fungal growth locally. Its concentration in the sap generally may be relatively low. In inoculated resistant cuttings, the fungus becomes well distributed immediately after infection, but after some time it starts to disappear. In such material, the hypothetical substance would probably be produced at more sites, and its concentration in tracheal sap is expected to be higher than in the root-inoculated plants. The next experiment was done, therefore, with cuttings.

The top 18 centimetre portions of 10-week-old plants were used in this experiment. Three GR and four GS cuttings were inoculated through their cut ends, and transferred to half-strength nutrient solution. Three cuttings from each variety were kept as controls. After 3 weeks, they were removed from the bottles, and the lower part of the stem from which adventitious roots developed was trimmed off in each case. The

remaining portions of the stems, cut into 6 cm. segments, were surface-sterilized in sodium hypochlorite solution after sealing their cut ends with a mixture of vaseline and wax. These segments were cut into 0.5 cm. long cylinders by means of a sterile scalpel. These cylinders were aseptically transferred to Petri dishes and stood upright on the surface of ionagar. Ionagar No. 2, a product of Oxo Ltd., London, is a highly purified agar prepared by ion-exchange process. It permits better diffusion of large molecules, and is recommended for antibiotic assay procedures.

Each Petri dish, 6 cm. in diameter, contained 10 ml. of 1.6% ionagar. All the cylinders from the cuttings from one treatment were distributed among 3 Petri dishes. After 6 hours of incubation at 25°C., the cylinders from each treatment were separately collected and transferred to oven for dry weight determination.

The layer of ionagar in 3 Petri dishes for each treatment was cut into thin slices and transferred to a 250 ml. Erlenmeyer flask containing 25 ml. of sterile water. The flasks were put on a horizontal shaker and shaken for 2 hours at the rate of one hundred movements per minute. After shaking, the diffusate was collected from each flask, centrifuged at 10,000 rp.m. for 15 minutes and the supernatant was stored at -20°C.

Before use, the frozen diffusates were thawed and then evaporated to complete dryness by concentrating under reduced pressure. .The dry substances were dissolved with 100 p.p.m. streptomycin sulphate solution added at the rate of 5 ml. per 1 g. dry weight of stem tissue. Streptomycin was added to check bacterial growth in the diffusates. It does not inhibit mycelial growth of Verticillium at concentrations up to 400 p.p.m. (Voros, 1963). This has been confirmed also in this laboratory. To 1.9 ml. of concentrated diffusate, 0.1 ml. of a spore suspension (500,000 spores/ml.) was added. The four different spore suspensions thus produced, all having the same concentration of spores as well as streptomycin sulphate, were used in spore germination studies. This was done in the same way as before. The results based on a count of approximately 400 spores are shown in Table 56. The results of a statistical analysis based on a count of 40 spores have been graphically illustrated in Fig. 10 (B).

The results are similar to those from the previous experiment with tracheal saps from healthy and inoculated plants. In this experiment too, the diffusate from inoculated resistant plants supported

Table 56 Effects of plant diffusates from healthy and inoculated plants on spore germination

Medium of germination	Percentage of germination	Mean germ tube length (μ)
GRC - diffusate	98.79	97.20
GRI - diffusate	98.5	76.38
GSC - diffusate	99.50	85.83
GSI - diffusate	97.57	83.88

lesser growth of germ tube than that from healthy resistant plants. The difference in germ tube growth between the two treatments was again statistically significant ($P = 0.05$).

VI. GENERAL DISCUSSION

The present work sought to inquire into the nature of resistance of certain varieties of tomato to V. albo-atrum. As a preliminary to this, attention was directed to the various effects of infection on resistant and susceptible plants.

Infection led to a drastic check in the growth of susceptible plants which affected all their parts. The roots suffer more at the early stages of infection, but later, there is an equal reduction (proportionally) in dry weight for both the root and the shoot. This may have been due to the shedding of some of the leaves as a result of infection. The production of leaves was not affected. But the leaf area was considerably reduced, and this was because the leaves failed to expand. The increase of yellowing and wilting also further reduced the total photosynthetic area.

Infection generally slightly stimulated growth in mature resistant plants. The total leaf area was also increased as a result of infection, but there was no increase in the number of leaves. In very young plants, however, infection adversely affected the growth (Table 20).

Anatomical studies of infected plants revealed

that the fungus invaded the susceptible stems before any symptoms appeared on the leaves. Thereafter, the fungus spread very rapidly for some time both laterally and longitudinally, and then more slowly until colonization reached a maximum. Later on, the amount of mycelium gradually diminished. This was evident first at the base of the stem, but later the middle portion was also affected. In inoculated resistant plants, the fungus was observed in the roots as soon as 7 days after inoculation. It did spread into the lower part of the stem, but its growth was only sparse. Very soon afterwards, the fungus started to disappear from the stem.

The most interesting feature observed in the inoculated resistant plants was certainly the strikingly rapid formation of tyloses in the root and the lower part of the stem. Tyloses gradually developed in more and more vessels so that finally their distribution in the stem was quite extensive. From the observations it appears that the tyloses play an important role in the resistance of tomato plants by restricting the further spread of the fungus. But they may not be the primary factor responsible for resistance in such plants. The most striking evidence

for this view comes from a quite different type of experiment.

Resistant plants which had been treated with maleic hydrazide before inoculation, did not develop tyloses in more than a few vessels at the stem base (Table 35). But even in the absence of tyloses, there is no lowering of resistance.

Tylosis is often stimulated in inoculated susceptible plants, but only at a late stage of infection. This late stimulation of tylosis coincides in time with the beginning of the disappearance of the mycelium from the stem base, and also the first signs of a check to the progress of the symptoms (Fig. 3). The disappearance of the fungus from the vessels cannot have been due to an increased formation of tyloses; in fact the reverse of this is what probably happens.

The inverse relationship between the amount of mycelium and the intensity of tylosis is true for both varieties. The evidence strongly suggests that tylosis is stimulated when the amount of the mycelium in the vascular system is small, but inhibited when the mycelial growth is extensive. Similar observations were made by Talboys (1958b) and McClure (1950). Both of them postulated that some fungal metabolites

are responsible for stimulation of tylosis at lower concentration, and for its inhibition at higher concentration. In Verticillium wilt of tomato, it is doubtful, however, if the substances responsible for tylosis is of fungal origin. The cuttings of both varieties, when fed with undiluted, cell-free culture filtrate, as well as those diluted five, or ten times, do not show any appreciable tylosis within 5 days. It is possible that the concentration of metabolites in culture filtrates was too high for the initiation of tyloses. Further, extensive tylosis can be regularly induced in tomato plants by making incisions in the stem. Horizontal incisions are more effective in this respect than those made longitudinally. It seems, therefore, that tylosis is regulated by some substance probably of host origin, produced in the infected tissue as a wound response. The potentiality for such reaction exists in both resistant and susceptible plants. The inverse relationship between tylosis and the amount of mycelium in the vascular system can be explained if it is postulated that the fungal metabolites do not inhibit tylose formation at very low concentrations, but do so at higher concentration. A corrollary is

that the production of such a metabolite is related directly to the amount of mycelium present.

The evidence suggests that the fungus enters the roots of both varieties. The resistance reaction, being already in operation in the root of resistant plants, does not allow any considerable proliferation of the fungus, except for a slight spread in linear direction up into the stem. Tyloses are produced in large numbers and this checks any further upward progress of the fungus. In susceptible plants, there is no mechanism to prevent the initial growth and spread of the fungus. Also, because a high concentration of the fungal metabolite inhibits tylose formation, the fungus grows unimpeded into the stem and then spreads rapidly upwards. This continues for some time after which the older mycelium in the lower part of the stem starts dying out. Less of the fungal metabolite is now produced, and sooner or later, the normal wound response of the plant re-asserts itself.

In studying Verticillium wilt of hop, Talboys (1958a) suggested that the initial host-parasite interaction in the root was self-perpetuated in the subsequent invasion of the stem. This may also be partially true for Verticillium wilt of tomato, but

it would not explain the dying out of the fungus in susceptible stems.

It appears that the relationship between the amount of mycelium in the stem and the severity of foliar symptoms is very close. It is not known if the fungus induces wilting in plants by producing a toxin, or by physically blocking the supply of water to the leaves. Scheffer et al. (1956), and Threlfall (1957) studied the rate of transpiration in Verticillium infected tomato plants. Both found an increase in transpiration of inoculated plants over the controls early after inoculation. This was followed by a decrease in transpiration rate immediately before and during wilting. There was no evidence of altered permeability in the leaves from infected plants (Threlfall, 1957). Both Scheffer et al. and Threlfall concluded that wilting resulted from a serious interference with the supply of water to the leaves.

The observations based on a study of cross sections (Tables 7 and 10) does not probably give a valid estimate of the degree of occlusion in the vessels. A single vessel may be variously plugged at different points along its length. The actual extent

of blockage in vessels will most certainly be higher than these observations suggest. Further, the mycelium is generally found in the larger vessels which are mostly responsible for transport of water. Even then the degree of vessel occlusion observed in the stem may not explain the total wilting in the plant, in view of the findings of Ludwig (1952), Waggoner and Dimond (1954), and Threlfall (1957). But occlusion may act locally in causing wilting. Unilateral wilting is not uncommon in this disease. In a cross section of infected stem, the fungus is rarely found to be uniformly distributed among the vascular bundles, unless the plant is severely diseased. Generally, some vascular bundles have hyphae in many more vessels than the others. Further, the vascular bundles in the petioles also had hyphae in many vessels (Tables 8 and 11). Since these bundles do not have by-passes as in the stem, any occlusion here is more important for wilting than similar degree of occlusion in the stem (Dimond and Edgington, 1960). On this basis, a considerable occlusion of vessels in the petiole coupled with similar dysfunction in that part of the stem which subtends it may possibly explain wilting in infected plants.

Slight wilting sometimes developed in inoculated resistant plants, and this could have been caused by the extensive tylosis (Table 10). However, these plants had the potentiality for the development of extra xylem elements after infection. This probably compensated for any dysfunction in the early stage of infection.

The root system in an infected susceptible plant is much reduced in comparison to that of healthy plants. This was found to be due to an inhibition of the production of new roots, particularly tertiary ones. In the experiments with cuttings too, infection considerably reduced the production of adventitious roots. But infection did not reduce the growth of roots in length. The results are incompatible with the suggestion that many of the symptoms of the disease are caused because of hyperauxiny in infected plants. If this be so, then in an infected plant root elongation should be inhibited before root initiation. However, the role of auxins in root growth is not yet clearly defined, and more than one hormone, as well as an inhibitor may be involved (Audus and Shipton, 1952).

Damage to the roots considerably influences the course of infection in susceptible plants. But

this effect varies with the timing of treatment in relation to inoculation. The results showed that for susceptible plants (1) undamaged roots are not easily infected; (2) mechanical damage to even a few roots at the time of inoculation favours rapid invasion of the plant; (3) the amount of wilting is not affected to any great extent by the severity of root damage and (4) damage to some roots few days before inoculation considerably slows the development of wilt. Some of the observations are consistent with those of Selman and Buckley (1959) who found that better infection was obtained when the roots were damaged. The infection of a root by a parasite may often be limited by certain factors in the rhizosphere. So when the stele is exposed the fungus gets into the root more easily and rapid invasion follows. However, it is possible that infection occurs through some other parts of the root, because wilt also developed in plants in the absence of any apparent root injury.

A reduction in disease symptoms as a result of root injury before inoculation has also been shown to occur in Fusarium wilt of tomato (Keyworth and Dimond, 1952). In the plants with roots damaged in different ways, they detected more water soluble reducing substances than in untreated plants. Their

suggestion that the increased resistance resulting from root damage is due to a change in metabolism may be applicable to Verticillium wilt of tomato plants.

The age of the plant at inoculation has little effect on the expression of resistance, and on the ultimate amount of infection. This effect is not altered by the method of inoculation. The early onset of symptoms and their rapid development in older susceptible plants may be due to the high carbohydrate level which is normally associated with the physiological maturity of a plant.

Studies with growth regulating substances revealed a number of interesting features. At low concentrations, IAA increased the symptoms and this is consistent with the view that hyperauxiny is involved in the disease syndrome. At higher concentration, however, IAA progressively reduced symptoms.

Circumstantial evidence for the participation of auxins in the disease was obtained when it was found that by removing the apical and axillary buds from inoculated plants the symptoms can be reduced. More striking effects were obtained when the buds were

removed before inoculation. There is always an interval of about 2 weeks between the time when the apical bud is removed and the time when the inhibitory effect becomes apparent. But if the axillary buds are allowed to develop, then severe symptoms are produced. These effects, may result from a subnormal, or supranormal level of auxin the two treatments would induce. Studying Verticillium wilt of tomato, Roberts (1944) noticed only a slightly lower incidence of disease when all the buds were removed. But a significantly lower incidence of disease was obtained when all, but the young assimilatory leaves were removed. He attributed this reduction to a lowering in the rate of carbon assimilation of the host which affected the fungus. But in the present study, the distribution of the fungus at the stem base was not much lower in the plants from which buds were removed than in the untreated plants. This suggests that the inhibitory effect of bud removal on disease may not have been operated through the carbohydrate level in the host.

A post-, as well as pre-inoculation treatment with gibberellic acid (GA) invariably stimulated the development of symptoms in susceptible plants. Gibberellic acid can increase the level of free IAA

in the plants (Fang et al., 1960) and this may explain the GA-induced stimulation of the symptoms. But GA induced increased symptoms even in those plants from which the buds had been removed. It is possible that GA stimulates the disease, directly and not through IAA. The increased leaf area and reduced root growth caused by application of GA may contribute to the rapid wilting that is so characteristic of GA-treated plants. But this does not explain the inhibitory effect of GA at lower concentration.

Indole, another auxin synergist influenced the disease in the same way as did lower concentrations of IAA. Indole is known to increase the activity of IAA only to a moderate extent.

Of the four anti-auxins studied, 2,4-dichloroanisole did not show much influence on the disease at the concentration employed. The symptoms were reduced by maleic hydrazide (MH) at 1 and 3 p.p.m., but from 10 p.p.m. upwards they were stimulated. But even at the higher concentrations, MH reduced the disease initially. Later on, symptoms developed very rapidly and the plants became severely affected. It is possible that at high concentrations the harmful effect of MH as a metabolic inhibitor may completely neutralize its anti-auxin effect in reducing disease.

The distribution of the fungus was unusually extensive in MH-treated plants. Since the plants were in a very low state of metabolic activity, this increased growth of the fungus could only be explained by the death of certain elements in the vascular tissue. The serious nature of the effect of MH on root growth may be a contributory factor to this condition.

Both 2,4,6-trichlorophenoxyacetic acid (2,4,6-T) and 2,3,5-triiodobenzoic acid (TIBA) reduced the symptoms at low concentrations, but at higher concentrations a stimulation of disease was often observed. This may be due to the direct, unspecific inhibitory action on plant growth at higher concentrations which has been suggested for TIBA by Audus and Thresh (1956).

(2-Chloroethyl) trimethylammonium chloride (Cycocel) influenced the disease reaction in the way expected because of its anti-gibberellin role in growth. Unlike the anti-auxins, it did not show any abnormal effect at higher concentrations.

Both the synthetic auxins, 2,4-dichlorophenoxy-acetic acid (2,4-D) and napthaleneacetamide (NAM) induced considerable resistance to disease at low concentrations. At a concentration as low as 1 p.p.m.

NAM reduced the symptoms significantly. The disease was considerably reduced by 2,4-D at 10 p.p.m. when applied before inoculation, but was sometimes more severe at 1 and 3 p.p.m. Similar results have been obtained with IAA, also at lower concentrations. When applied after inoculation, 2,4-D increased the symptoms at both 3 and 10 p.p.m. This indicates the importance of the timing of treatment with growth regulators in relation to inoculation.

The evidence does not suggest any striking correlation between the effect of a compound on the disease, and its influence on the mycelial growth. All the three anti-auxins suppressed disease significantly at low concentrations, and this is, in fact, correlated with their effects on the mycelial growth *in vitro*. But at the higher concentrations, which reduced the mycelial growth still more, MH invariably, and both 2,4,6-T and TIBA sometimes stimulated disease development. For other compounds, the evidence points to an inverse relationship between fungitoxicity and the effect on the disease.

Whenever any treatment has induced resistance in susceptible plants to any appreciable extent, the fungus has been found to be very restricted in its

distribution inside the host. The question arises as to how the growth of the pathogen could be inhibited within the host by treatment with growth regulating substances, because a direct effect on fungal growth is ruled out for most of the compounds. The only possibility is, therefore, that they act by altering host metabolism and it is this which normally controls the growth of the fungus in vivo.

The correlation between inhibition of plant growth, and the induction of disease resistance was good for most of the compounds tested. Those which induced resistance, with the exception of NAM at lower concentration and cycocel, also showed considerable formative effects on plant growth. They also induced extensive tylosis in infected susceptible plants. There can be no doubt that these compounds induce changes in host metabolism and this somehow upset the nomral disease mechanism. The growth regulating substances, chemotherapeutically active against Fusarium wilt of tomato, were also found to alter host metabolism (Davis and Dimond, 1953).

Many of these compounds are more effective in inducing resistance when applied before inoculation than after. This means that a certain time is needed before inoculation if the treatment is to induce changes

inside the host that are unfaouvrable for the pathogen. Such changes in host metabolism may well alter the resistance to disease, because it is already known that by manipulating the balance of nutrients the disease can be considerably modified (Roberts, 1943; 1944). In many other diseases too resistance induced by growth-regulators has been found to be well correlated with the inhibition of growth in some form or other (Davis and Dimond, 1953; Beckman, 1958; Corden and Dimond, 1959). Corden and Dimond (1959) suggested that the growth regulators induce resistance by modifying pectic substances in the primary cell wall, so that they become resistant to fungal enzymes and also elongate less readily. Cell walls grow easily when adjacent carboxyl groups in the pectic substances are in methyl ester forms (Bennet-Clark, 1956). The growth regulating substances, at low concentration, may bind pectin-methylesterase (PME) to cell wall (Glasziou, 1957). Fungal PME will demethylate the highly methylated pectic substances before they are further degraded. But when the growth regulating substances are present at high concentrations, the bound PME is released from the cell wall and demethylates the esterified carboxyl groups. The carboxyl groups then get rigidly cross-linked through divalent cations,

mostly calcium. In consequence, the pectic substances in cell wall become less water soluble, too rigid for growth, and resistant to fungal enzymes. Circumstantial evidence to this has been obtained by Corden and Edgington (1960) who showed that calcium was necessary for growth regulator induced resistance to Fusarium wilt of tomato.

In the present study, symptoms were reduced by auxins and anti-auxins at low to moderate concentrations, and by cycocel over a wide range of concentrations. It seems unlikely that all these compounds, widely varying in their structure and role in plant growth, can induce similar changes in cell wall materials to make them immune to fungal enzymes. Further, the anti-auxins very often stimulate disease at higher concentrations even when plant growth is considerably inhibited. The compounds which considerably reduced the symptoms, also strikingly reduced the distribution of the fungus in the host. It seems, therefore, that resistance was induced by different growth regulating substances mainly through their indirect effect on fungal growth in vivo. The induced rigidity of the cell wall pectic substances was probably of secondary importance.

The growth of the fungus *in vivo* may have been checked in at least two ways: (1) by removal of some substances which the fungus needs for its growth, or (2) by formation and accumulation of some substances at concentration toxic to the fungus. Which of these factors is involved in *Verticillium* wilt of tomato is uncertain. The second one appears to be a possibility, since substances like IAA and 2,4-D can induce an accumulation of phenolic substances (Sequeira, 1963) which are often toxic to the fungi.

It appears that a particular level of auxin in the plants, somewhat, but not much higher than normal, is favourable for the disease. If the level in the plant is reduced below normal, or increased above the favourable level, then the symptoms are inhibited. The greater the change in auxin level from the favourable range in both directions, the greater is the induction of resistance. Most of the growth regulating substances applied undergo decomposition in the tissue. The resistance induced by them also becomes somewhat less pronounced with time in most cases. This is particularly true for a substance like IAA which decomposes very rapidly in the tissue. When applied at 10 p.p.m., IAA presumably

increases the concentration of auxin in the plant. Thus symptoms develop more slowly. But the level of auxin diminishes gradually as IAA decomposes, and after a certain time the level favourable for disease is reached. This will account for the late stimulation of symptoms. With substances like NAM and 2,4-D, there is a more permanent effect. Indole and GA, both being auxin synergist, may slightly increase the level of auxin in the plant and, therefore, cause more pronounced symptoms to develop.

The results suggest that auxins and auxin like substances may play a critical role in Verticillium wilt of tomato. The different concentrations of auxin within the tissue, resulting from various treatments, could affect the metabolism of the host either toward increased resistance or toward increased susceptibility.

Various observations indicate that resistance is not localized in any particular organ, such as the roots, but is distributed throughout the plant. The results are, therefore, different from those of Heinze and Andrus (1945), Keyworth (1953), and Threlfall (1957), but agrees with those of Blackhurst and Wood (1963), Snyder et al. (1946) and Scheffer and Walker (1954). There is an indication also that the leaves of a resistant plant are more resistant to infection than

those from a susceptible plant. In Gem resistant, the resistance was indeed very high, because the fungus never grew beyond the lower part of the stem. But even in that region its distribution was restricted to a few vessels, and from these it ultimately disappeared. Its growth in the resistant root was also very poor. The results of experiments using inoculated cuttings showed that these responded to infection in the same way as the root-inoculated plants. This is in agreement with the observations made by Blackhurst and Wood (1963), and Berry and Thomas (1961) for Verticillium wilts of tomato and mint respectively.

Histological work with inoculated cuttings gave further evidence that resistance is present in the stem. When the fungus is introduced into resistant cuttings as spores, the spores germinate well. But the fungus does not survive for long, and it gradually disappears.

The results so far discussed show that the stems, as well as the roots of resistant and susceptible plants respond differently to infection by V. albo-atrum. They agree, however, with the suggestion that in normal infection the roots are the most important sites of resistance (Talboys, 1958a; Keyworth, 1963). Any

further growth of the fungus from the root into the stem can not be successful, because the stem is also resistant. The fact remains, however, that the stems of susceptible plants may, under certain conditions, show a certain degree of resistance. Regularly in a mild syndrome, and sometimes in acute syndrome, the susceptible plants recover from the disease. This recovery is generally associated with a gradual disappearance of the fungus from the stem and with an increased formation of tyloses.

Evidence from the studies with plant extracts, tracheal saps and tissue segments show that there are no significant differences between resistant and susceptible plants in their capacity to support spore germination and mycelial growth. There is no evidence to suggest that the poor growth of the fungus in resistant plant is due to the presence of any inhibitor, in or the absence of some essential nutrients from its sap.

The importance of carbohydrates and nitrogenous compounds in securing a successful infection in tomato plants has been stressed (Roberts, 1943; 1944; Selman and Buckley, 1959). Resistant cuttings were supplied with glucose and casamino acids

for a few days after inoculation. In glucose-, and casamino-acids-treated, inoculated plants, more wilting developed during the first two weeks after inoculation than in the controls. But these plants usually recovered like the untreated plants. It is unlikely that resistance was broken by these treatments.

Many of the results which have been obtained in this study strongly indicate that resistance depends on the production of some antifungal substance in the tissue after infection. Circumstantial evidence for this is the disappearance of the fungus from root-, and shoot-inoculated plants.

The production of such a substance will be linked with the metabolism of the resistant host. Gothoskar et al. (1955) were able to break the resistance of certain tomato varieties to F. oxysporum f. lycopersici by treating the inoculated cuttings with a variety of respiratory inhibitors. They postulated that a resistant plant was continuously producing some substance which became toxic to the fungus at certain concentrations. The production of such a substance requires energy from respiration. The inhibition of respiration following different treatment also suppressed the formation of the

hypothetical substance, hence the breakdown of resistance. Increased respiration is a characteristic of infected plants. Weir (1961) showed that V. albo-atrum caused increased respiration in susceptible (Ailsa Craig), as well as resistant (Loran Blood) tomato plants. He further observed that this increase was not due to uncoupling from phosphorylation. Deese and Stahman (1962a) recorded increased oxidase activity, and a strong test for quinones in Verticillium infected resistant (V. R. Moscow and Loran Blood) tomato plants. Evidence suggested a strong metabolic activity in resistant plants after infection.

Ethanol treatment is known to reduce resistance in different diseases. In the present investigation too a treatment with ethanol induced considerably more wilting in inoculated cuttings than in the inoculated controls. But wilting did not progress in ethanol-treated plants after the first two weeks, and they always recovered in the same way as did untreated, inoculated plants. The initial stimulation of wilting in ethanol-treated plants is not because it stimulates fungal growth. It is possible that the resistance mechanism in such plants is temporarily altered by ethanol. It may also have been due to its action on the permeability of the leaf cells as suggested by Gothoskar et al. (1955). The

inhibitory action of ethanol on root growth may also have been partly responsible for the increased wilting.

The use of various metabolic inhibitors did not induce continued development of symptoms in inoculated resistant cuttings. The different treatments usually caused a slight to moderate increase in wilting in inoculated cuttings above that characteristic of the inoculated controls. This increase was evident during the first 2 - 3 weeks after inoculation, but thereafter the affected cuttings always recovered. Inoculated cuttings which had been treated with DIECA, salicyladoxime and 8-hydroxyquinoline had, in the lower region, many more infected vessels than untreated, inoculated cuttings. But in the upper region, no such difference was evident. It is possible that after these treatments, resistance was temporarily modified to allow the pathogen to grow and spread to some extent in the resistant tissue for some time. But as the normal mechanism of resistance began to function again, so did the fungus gradually disappear. The difference between the results of present study and those of Gothoskar et al. (1955) is difficult to explain because

they involve the same host. But the difference in variety and the fact that the host pathogen interactions are highly specific may be important in this connexion.

Many of the results which have been obtained in the present investigation suggest, but do not prove, the production of an antifungal substance in the tissue of resistant plants after infection. Both the tracheal saps and diffusates obtained from inoculated resistant plants caused a significant reduction in germ tube growth compared with those from healthy resistant plants.

The most likely place for the production of this hypothetical antifungal substance is the parenchyma tissue in xylem. The production of this substance may be initiated by a contact between the growing tip of a hypha and the parenchyma cell through the pit aperture in the vessel. Another possibility is, as earlier postulated by Offord (1940), that the hyphae in vessel secretes some enzymes or hormones which initiate the formation of the antifungal compound. This has been found to be true for certain host-parasite combinations (Uehara, 1959; Cruickshank and Perrin 1963). The suggested host-pathogen interaction

is not probably of the hypersensitive type, because no necrotic tissue has been noticed in the infected xylem tissue of resistant plant.

The substance may be present at fungitoxic concentrations at the sites of production and thus inhibit the growth of adjacent hyphae. Ultimately it must be released into the vascular stream where its concentration would be expected to be low at first. More and more of this substance would be released into the tracheal sap and move upwards with the transpiration stream. A fungitoxic concentration would be attained first at the top of the plant and later on in the lower regions. This may explain the gradual disappearance of the fungus from the infected stems, which always begins at the upper region of the stem, and then spreads downwards.

This substance may also be produced in infected susceptible plants. The difference between these and infected resistant plants may lie in their speed of response to the presence of the fungus. In resistant plants, because of their rapid response, the substance is produced rapidly, and soon a fungitoxic concentration is attained. In susceptible plants, the response is very slow. So the fungus spreads well inside the plant and causes symptoms. If the plant

is not severely diseased, then a fungitoxic concentration of the substance may be attained at a late stage of infection. From that point the fungus begins to disappear, and the plant starts on its way to recovery, a condition not uncommon in infected susceptible plants.

VII. SUMMARY

A. MORPHOLOGICAL AND ANATOMICAL STUDIES

1. A comparative study has been made of the effects of *Verticillium* infection on susceptible (GS) and resistant (GR) varieties of tomato.

2. Symptoms appeared in both GR and GS plants between 7 and 10 days after inoculation. GR plants soon recovered and grew normally. But GS plants developed symptoms very rapdily for a considerable time afterwards and then more slowly until the progresss of disease was stopped.

3. Infection markedly reduced both the height and dry weight of GS plants. In GR plants, on the other hand, infection stimulated growth slightly, causing increases in both height and dry weight compared with the controls. Root growth in GS plants was reduced by infection sooner than was shoot growth.

4. Infection did not affect the production of leaves significantly in either variety, but in GS plants there was a considerable reduction in leaf area.

5. Epinasty, although prominent sometimes, was not found to be an invariable symptom of this disease.

6. The fungus appeared in the infected roots of both varieties within 7 days of inoculation. It grew into the GS stem before the symptoms appeared and then spread considerably, both laterally and longitudinally. In GR plants, the fungus grew into the lower part of stem, but did not infect more than a few vessels. It soon disappeared from these vessels.

7. While the fungus was spreading into new tissues in the upper part of GS stem, it started to disappear from the lower part.

8. A close correlation was found between the occurrence and severity of foliar symptoms and the distribution of the fungus in the stem. Leaf symptoms almost ceased to develop 17 days after inoculation, when the fungus started to disappear from infected vessels.

9. A strikingly rapid tylosis was initiated in GR plants as a result of infection. Tyloses developed in advance of the growing hyphae, and were still spreading into the vessels after the disappearance of hyphae from the stem. Many tyloses also developed in inoculated GS plants, but at a much later stage of infection.

10. There was an inverse relationship between

the amount of mycelium and the intensity of tylosis in the inoculated plants of both varieties.

11. Sections from the base of petioles from GR and GS plants showed the same distribution of hyphae and tyloses as observed in the stem.

12. There was a good correlation between the degree of vessel occlusion by hyphae at the base of stem and the mean wilting index for the leaves. Vessel occlusion caused by tyloses was found to be less effective in inducing wilting than similar occlusion by hyphae.

13. Vessel collapse, occasional in its occurrence in the lower part of the stem, was not uncommon in the upper part. It may contribute, to some extent, to vascular dysfunction.

14. For the foliar symptoms, there was some correlation with the distribution of hyphae at the base of petiole, but none with yellowing of the leaf laminae.

15. Plants grown in nutrient solution, when inoculated, showed the normal disease reaction characteristic of each variety after inoculation.

16. Cuttings of GS plants, inoculated through cut ends with spore suspension, developed typical disease

symptoms. Initially, mild symptoms developed in inoculated GR cuttings, but they always recovered gradually. Generally disease intensity in inoculated cuttings was less than in root-inoculated plants. It was concluded that resistance was present in the stem as well as roots.

17. Histological studies with inoculated cuttings showed that the fungus gradually disappeared from resistant plants. The fungus disappeared first from the top, and later from the lower parts of the shoots.

18. Detached leaves from GR and GS plants were inoculated in the same way as the cuttings. Inoculated GS leaves showed more wilting than inoculated GR leaves. There were fewer vessels with hyphae and more with tyloses in GR as compared with GS leaves. The leaves of two varieties differed in their resistance to disease.

19. Infection markedly reduced root growth in GS plants. This was due to a fall in the production of new laterals, particularly those of tertiary origin. The growth of a particular root was not affected by infection.

20. The age of the plant at the time of inoculation did not affect the normal disease reaction of a variety,

or the ultimate severity of symptoms. However, in GS plants the symptoms appeared much earlier in older plants than in younger plants. The latter appeared to be more resistant to symptom expression than the former although they showed higher percentage reductions in the dry weight of plant organs.

21. Damage to a few roots at the time of inoculation always induced earlier development of symptoms in GS plants compared with undamaged plants. A similar treatment 5 to 10 days before inoculation considerably retarded the development of symptoms.

B. STUDIES WITH GROWTH REGULATING SUBSTANCES

1. Healthy GR and GS plants were fed with different concentrations of IAA in nutrient solution for 21 days. The results showed that IAA at low concentrations stimulated wilting, epinasty and initiation of adventitious roots, and reduced these symptoms at higher concentrations. The effects of different concentrations of IAA on disease development confirmed this view.

2. The removal of apical and axillary buds considerably retarded the development of symptoms in GS plants. Better results were obtained when the

treatment was given earlier in relation to the time of inoculation. If only the apical bud was removed, and if axillary buds were allowed to develop, then severe symptoms developed. When the inoculated plants from which all the buds had been removed, were treated with gibberellic acid the disease was stimulated again. But a similar treatment with IAA caused further inhibition.

3. An application of gibberellic acid markedly accelerated wilting in inoculated GS plants. Similar treatment also induced more wilting in inoculated GR plants compared with the inoculated controls. However, anatomical studies with inoculated GR plants did not show any appreciable effect of gibberellic acid on the distribution of the fungus at the stem base. Three successive applications of gibberellic acid did not induce continued development of the symptoms in resistant plants.

4. Maleic hydrazide inhibited symptom expression in inoculated GS plants at low concentrations. But at 10 p.p.m. and higher concentrations, the initial inhibition was always followed by a later stimulation. A moderate increase in wilting as a result of maleic hydrazide treatment was also found in inoculated GR plants. The maleic hydrazide-treated plants did not

show the appreciable tylosis which characterized inoculated GR plants.

5. Both gibberellic acid and maleic hydrazide reduced the dry weights of plant organs considerably. This inhibitory effect was particularly marked on the roots. Maleic hydrazide was more inhibitory than gibberellic acid.

6. None of various other growth regulating substances had much effect on disease development in GR plants. Slight stimulation of the disease in GS plants was obtained with indole inoculated GR plants. 2,4-dichloroanisole did not have much effect on GS plants, but the others did. They were investigated further for their effects on GS plants.

7. When applied before inoculation, 2,4-D reduced symptoms at 1 to 10 p.p.m. But a post-inoculation treatment always stimulated symptom development.

8. Napthalene acetamide induced resistance even at very low concentrations.

9. (2-chloroethyl) trimethylammonium chloride an anti-gibberellin, progressively inhibited the disease with increase in concentration. But even at higher concentrations, it did not cause any abnormality in

the growth and development of the plant except that it prevented extension of internodes.

10. 2,3,5-triiodobenzoic acid, and anti-auxin, inhibited the symptoms at low and moderate concentrations.

11. A reduction in the development of symptoms was also obtained with 2,4,6-T, another anti-auxin, at moderate concentrations. But at higher concentrations, its effect on disease was rather irregular.

12. No general relation was found between the effects of different growth regulating substances on disease in GS plants, and their effects on fungal growth. But most of the substances which induced resistance at certain concentrations, also caused considerable inhibition of plant growth, and also induced formative effects.

C. STUDIES ON THE NATURE OF VERTICILLIUM RESISTANCE IN TOMATO PLANTS

1. Tissue extracts from healthy plants of both varieties did not have any differential effect on the fungal growth in vitro.

2. The tracheal saps from healthy GR and GS plants supported only poor mycelial growth. But neither in this respect, nor in their effects on spore germination and germ tube growth did they behave differently.

3. Inoculated GR cuttings, when fed with low concentrations of glucose and casamino acids for 4 - 6 days after inoculation, developed more symptoms initially than the untreated, inoculated plants. However, they soon recovered afterwards.

4. A continuous treatment with ethanol for 14 days considerably reduced the resistance of GR cuttings and induced marked symptoms. But a similar treatment for shorter periods resulted in an initial increase in wilting from which the cuttings always recovered.

5. Various metabolic inhibitors were tested for their effects on inoculated resistant cuttings. Some of them induced slightly more wilting than that observed in untreated cuttings, but the treated ones always recovered. The fungus survived better in the cuttings treated with DIECA, 8-hydroxyquinoline and salicyladoxime than in untreated cuttings.

6. Segments from healthy GR and GS stems, when placed on agar, neither stimulated, nor inhibited the growth of a fungal colony. Similar segments from GR plants, when inoculated, sometimes inhibited the advancing zone of the colony. This inhibition was accentuated when some of the inoculated segments were

contaminated with bacteria. Similar contamination of uninoculated GR segments caused only a slight inhibition. Inoculated GS segments did not inhibit colony growth. The post-infection production of some antifungal substance in resistant tissue is suggested.

7. Tissue extracts from healthy and infected plants of both varieties supported mycelial growth almost to the same extent.

8. Tracheal sap from infected GR plants supported significantly less germ tube growth than similar sap from healthy GR plants. Similar results were obtained with plant diffusates. There was, however, no difference between the tracheal saps and plant diffusates from healthy and infected GS plants in this respect.

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