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Title: NATURE OF RESISTANCE TO VERTICILLIUM DAHLIAE KLEB. IN STRAINS OF PEPPERMINT (MENTHA PIPERITA L.) DEVELOPED BY IRRADIATION

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Verticillium dahliae Kleb. causes a wilt disease of peppermint. Attempts to control the disease by soil fumigation, crop rotation, flaming of stubble, and deep plowing have met with only limited success. Development of resistant varieties through conventional breeding procedures is difficult because commercial peppermint is male-sterile. Several strains of commercial peppermint that show field tolerance to Verticillium wilt have been developed by irradiating mint stolons, planting them under wilt conditions in the field, and selecting for resistance.

The purposes of this study were (1) to test these irradiated strains of mint for their reaction to Verticillium wilt in Oregon (2) to compare these strains with commercial peppermint for their reaction to root penetration and subsequent infection by V. dahliae (3) to determine sites and mechanisms of resistance that might function in the

resistant strains.

Field experiments showed that the irradiated peppermint strains had a significantly lower incidence of wilt than did the non-irradiated commercial 'Mitcham'. Disease severity in individual resistant plants was sometimes as great as in susceptible plants. Therefore, disease incidence is more important than disease severity in selecting resistant strains.

Dead stems of resistant strains had fewer microsclerotia of the fungus than did susceptible Mitcham. This shows that irradiated strains were less extensively invaded and would return less inoculum to the soil.

Oil yield data from field plots showed that yield of the irradiated strains was not depressed in relation to Mitcham.

Growth of Verticillium on stem pieces and sap-extract media of the resistant strains and on Mitcham control suggested that nutritional differences were not related to resistance and that inhibitory substances, possibly phenolic compounds, were present.

The polyphenoloxidase (PPO) level of healthy, field grown plants fluctuated throughout the growing season and dropped to a low point at the time of flowering. The level of this enzyme did not seem to be associated directly with resistance but may be related to the types of phenolic compounds in the plants.

The low level of PPO at the time of flowering suggested that

plants with flowers might be more susceptible to wilt than non-flowering cuttings. Inoculation experiments showed, however, that differential resistance was maintained after plants had flowered. Flowering had no influence on wilt susceptibility and selection of a resistant variety could be made without regard to flowering.

Resistant, moderately resistant, and susceptible strains of mint were invaded by conidia of Verticillium within 30 minutes after inoculation. Thus, resistance is not related to ability of the fungus to initially invade plants.

Experiments using a cotton and mint isolate of V. dahliae showed that the cotton isolate is only weakly virulent to mint. The cotton isolate is, therefore, a different physiological strain of the fungus from the mint isolate.

Roots of resistant, moderately resistant, and susceptible mint plants were penetrated nearly equally by V. dahliae, suggesting that resistance is not wholly dependent on resistance to root penetration.

Resistance to Verticillium is present in stems as well as roots of mint plants. When the root system was bypassed by direct stem inoculation or by inoculation of cut shoots, resistance was maintained.

Cross-protection was demonstrated when mint strains were inoculated with a cotton isolate of Verticillium and challenged one week later by a mint isolate. This suggests that active resistance mechanisms in peppermint are present and can be initiated by an avirulent strain of the fungus.

Nature of Resistance to *Verticillium dahliae* Kleb. in Strains of Peppermint (*Mentha piperita* L.) Developed by Irradiation

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NATURE OF RESISTANCE TO *VERTICILLIUM DAHLIAE* KLEB. IN STRAINS OF PEPPERMINT (*MENTHA PIPERITA* L.) DEVELOPED BY IRRADIATION

INTRODUCTION

Verticillium dahliae Kleb. (= *V. albo-atrum* var. *menthae* Nelson) causes a wilt disease of peppermint (*Mentha piperita* L.). This disease caused a drastic reduction of commercial peppermint production in the Midwest. As a result, a rapid expansion of mint production occurred in Oregon and Washington in the 1940's and is continuing presently. The Willamette Valley of Oregon is especially suitable for production of high quality peppermint oil but *Verticillium* wilt has gradually become more widespread there, threatening the future of the crop.

Various methods have been tried to effect control of this disease. Among these are soil fumigation, crop rotation, and various cultural practices such as flaming of stubble and deep plowing (Berry and Thomas, 1961; Horner and Dooley, 1965; Green, 1958).

Soil fumigation provides a measure of control but is not economically feasible for large acreages. Crop rotation is practiced by growers, but because the fungus is known to exist for many years in the absence of a host or on non-host crops (Nelson, 1950, p. 165; Horner, 1962, p. 11; Wilhelm, 1955; Martinson and Horner, 1962),

this method also has disadvantages. Flaming of stubble helps prevent spread and increase of the disease but, again, is not a completely effective control. Deep plowing is temporarily effective in the muck soils of the Midwest but is not possible on the shallower mineral soils of Oregon.

The ideal way to control a disease of this kind is by development of resistant varieties. Because commercial peppermint is a male-sterile plant, conventional breeding procedures are difficult.

There exist fertile mint species resistant to Verticillium wilt which could be used in a breeding program, but neither the resistant parents nor their hybrid progeny produce mint oil of acceptable quality. One alternative remains open for development of resistant varieties. This is the creation of resistant mutants by irradiation.

Dr. M. J. Murray, of the A. M. Todd Company, Kalamazoo, Michigan, has been working for several years on this aspect of the problem. He has obtained strains of commercial peppermint which show field tolerance to Verticillium wilt by irradiating mint stolons, planting these under wilt conditions in the field, and selecting for resistance (Murray, 1967b).

The purposes of my study were:

(1) To test irradiated strains of mint for their reaction to Verticillium wilt under Oregon conditions.

(2) To compare these strains with Mitcham peppermint for

their reaction to root penetration and subsequent infection by *V. dahliae*.

(3) To determine sites and mechanisms of resistance that might be operating in the resistant strains.

LITERATURE REVIEW

Resistance

Resistance to a disease is defined by Wood (1967, p. 399) as the extent to which a plant does not become diseased when it is growing in association with a causal agent. Susceptibility is a measure of how much a plant does become diseased under conditions suitable for growth of a pathogen. High and low resistance correspond to low and high susceptibility.

Barnett (1959) defines resistance as the result of all the factors that tend to reduce the aggressive or pathogenic activities of the parasite after contact with a potential host has been made. He places resistance factors into two groups.

(1) Those which act to prevent penetration by the pathogen

(2) Those whose action is involved after penetration

However, as Kuc (1966) states, disease resistance is not limited to a single mechanism. Such factors as physical and chemical barriers to penetration, the presence of preformed inhibitors and the ability to establish a compatible metabolism between host and pathogen may all be important under some conditions. He continues to point out that all mechanisms for resistance or susceptibility are genetically controlled and that the presence of the genetic

code for resistance does not assure its expression or effectiveness in a particular host-parasite interaction. For example, a microorganism may be capable of producing a toxin which may overcome an otherwise effective resistance mechanism or an unsuitable environment may prevent expression of resistance by the host.

Kuc places mechanisms of disease resistance into three groups, and in doing so, recognizes that one or several of these factors may operate, depending on the situation. These factors are:

(1) Physical or chemical barriers including preformed inhibitors of microbial growth, detoxification of enzymes or toxins, or lack of necessary growth factors. These mechanisms have a minimum of specificity and provide general protection.

(2) Mechanisms based on physiological stress including production, liberation, or mobilization of compounds following infection or injury. These factors have a degree of specificity since different microorganisms as well as different modes of injury can induce different degrees of response.

(3) Unmasking of host DNA by a specific microorganism. This can give rise to specific RNA and hence alter metabolism of the diseased plant. Resistance to this type of attack is highly specific.

Wood (1967) has recently presented a summary of plant disease resistance. He places resistance factors into two main groups.

(1) Those present in the host before infection. These

include:

(a) Substances in plants that reduce or prevent growth of the parasite on or in the plant.

(b) Structures or types of growth which make it less likely that the plant will become infected after a potential parasite has made contact with it, or structures or types of growth which limit spread of the parasite after it has infected the plant.

(c) Absence from plants of substances needed by the parasite for growth, or absence of structures on which infection depends.

(d) Factors that inactivate substances which damage the host or nullify the effects of such substances.

(2) Resistance factors produced by the host in response to infection. Categories in this group are similar to (a) and (b) above except they become operable after infection rather than before.

The literature on resistance to plant diseases is voluminous, thus only the more significant findings on resistance to wilt diseases are presented here.

Sinha and Wood (1967) have suggested, on the basis of their work on the responses of resistant and susceptible tomato plants to Verticillium infection, that resistance is mainly of the type that develops after infection. When they inoculated susceptible plants through

the roots, the foliar symptoms and the amount of mycelium in the stem increased for some time. Then the mycelium began to disappear. This was accompanied by a check in the normal progress of symptoms and by tyloses formation. In resistant plants, a limited invasion of the roots and lower stems was accompanied by a rapid and extensive tyloses formation. The mycelium disappeared from the stem and the plants recovered from the initially mild symptoms. There was an inverse relationship between the amount of mycelium in the stem and the extent of tyloses in infected plants.

This work supports the hypothesis of Beckman (1966) which states that most vascular infections are successfully localized by a three step mechanism. This mechanism includes an initial screening out of mobile cells or spores from the transpiration stream followed by gelation and overgrowth of vasicentric parenchyma cells which eventually seal off the infected portion of the vessel. This type of localization occurs in a wide variety of host plants infected with a wide variety of microorganisms and is considered to be a general phenomenon.

In susceptible reactions, systemic distribution of pathogens occurs because they are able to disrupt this basic mechanism of resistance and disease results.

Beckman presents five hypothetical agents and the responses they might cause in the resistant host. These are:

(A) Parasite-produced substances that cause swelling of constituents of perforation plates and end-wall membranes. This is a non-specific agent.

(B) Parasite-produced substances which cause solation of gels. These are probably pectic enzymes. There may be some specificity of these substances.

(C) Parasite-produced substances that injure cells immediately adjacent to the infection site. These are non-specific substances.

(D) Substances which induce growth of host cells. These are hormone-like substances because they can move readily through living host cells.

(E) Parasite-produced substances that inhibit growth of host cells or initiate the release of inhibitors from host cells. These counteract the action of substance D in the vicinity of the infection, and are transported in the transpiration stream when gels are degraded rapidly as in the susceptible reaction.

Beckman summarizes by saying that in the susceptible-type reaction, compounds of the classes A-E are apparently produced since gel formation is initiated, gel degradation is very rapid, host cell browning in the vicinity of the reaction occurs, tylose formation begins, and growth in the vicinity of the infection is retarded. If the gels are rapidly degraded, they shear under the stress of transpiration and substances of class A, B, C, and E, as well as propagules

of the parasite, are transported within the transpiration stream at a rate that offsets the growth-stimulating effect of substance D.

In a later paper, Beckman (1968) pointed out that a number of resistance mechanisms might be operable that would determine the degree to which a plant is capable of resisting direct penetration by vascular parasites; however once a vascular infection has been established, resistance to a disease may depend upon:

(1) Poor growth of the parasite because of inadequate nutrition or direct inhibition by the host.

(2) Failure of an active parasite to produce specific toxic metabolites.

(3) An insensitivity of host tissues to the toxic metabolites or an inactivation of the toxic metabolites by the host.

(4) Physical localization of the parasite.

His experiments with leaves of broccoli, cotton, and tomato systemically inoculated with Fusarium oxysporum showed that the fungus grew equally well or better in resistant than in susceptible host tissues and that vascular occlusion and foliar symptom development were also as great or greater in the resistant type interactions as in the susceptible type interactions. He concluded that resistance depended primarily on a physical localization of the parasite. He suggested that systemic distribution of the pathogen in a susceptible type reaction was dependent upon fungal products which rapidly

degrade vascular gels and inhibit respiratory or growth metabolism of host cells.

It should be pointed out that tyloses have also been implicated in one phase of symptom expression, i.e., wilting, because the vessels may be blocked by these occlusions, thus cutting off the water supply to the host. Indeed, Beckman noted that resistant plants wilted even sooner than susceptible plants.

This apparent paradox can be resolved by assuming that vascular occlusion is a primary factor in both resistance and symptom induction. The time-space relationship is the determining factor. If a host responds quickly and violently to an infection, the infection can be effectively sealed off and the plant will not suffer, except perhaps for slight wilting from which it will recover. A resistant plant might respond in this way and thus be highly responsive to infection but resistant to the disease. This type of response could be analagous to a hypersensitive reaction.

If a susceptible host is infected in a normal way, through the roots, and it is not able to respond quickly to this infection, final occlusion does not occur until the parasite has become systemic. A large portion of the water transport capacity is cut off and wilting results. Thus, a susceptible plant responds more slowly to infection than a resistant one but suffers more serious damage.

Talboys (1958a, 1958b) found there was extensive growth of the

parasite in the vascular elements of Verticillium-infected hops with severe symptoms, but few tyloses were present. In plants showing mild symptoms, there was only limited growth of the pathogen but large numbers of tyloses were present. The pathogen secreted substances in culture which stimulated formation of tyloses in low but not in high concentration. Thus, the more the pathogen grows, the more metabolite is produced and fewer tyloses are formed. This facilitates movement of the pathogen through the vascular elements. If initial growth of the parasite is slow, the reverse takes place.

Blackhurst and Wood (1963) found that more tyloses were present in a resistant variety of tomato after infection by V. albo-atrum than in a susceptible variety, although healthy, susceptible plants developed more tyloses naturally than did healthy, resistant plants.

Tyloses may also be an important mechanism of resistance in Fusarium wilt of banana. The severity of the disease is related to the rate at which propagules of the fungus are carried in the transpiration stream. Occlusions which develop in the vascular system prevent or slow the passage of propagules. Resistance depends upon the rate at which occlusions are formed, resistance of gel plugs to degradation, or to penetration by the pathogen. The resistant variety, Lacatan, compared to the highly susceptible Gros Michel, formed gel plugs faster and more intensively. These plugs were also more resistant to degradation by the fungus (Beckman, Halmos, Mace,

1962).

The problem of where the mechanism of resistance operates has also received some attention.

Heinz and Andrus (1945) claimed that resistance was localized in the roots on the basis of their experiments with Fusarium wilt of tomato. They used varieties Bonny Best (susceptible) and Pan America (resistant) and inoculated grafted plants through the roots. Scions of both varieties on Bonny Best stocks were invaded by the pathogen and severe symptoms developed. On Pan America stocks, scions of both varieties did not develop symptoms and the fungus grew little or none at all.

Snyder et al. (1946) inoculated plants with Fusarium through cuts in the tap root and found that the pathogen grew much less in shoots of Pan America plants than Bonny Best.

Scheffer and Walker (1954) found that cuttings inoculated directly with Fusarium and then grown so adventitious roots developed, had severe symptoms on Bonny Best plants but not in Pan America plants. Thus, stems of the two varieties differ in resistance but adventitious roots might have an influence on resistance in the stem. To test this possibility, Scheffer (1957) used resistant and susceptible stems which had been approach grafted. By cutting the stems in appropriate places, the stem of one variety could be inoculated while being supported by the stock of the other variety. Under these

conditions, the stem retained the resistance of the whole plant.

Keyworth (1953) questioned the claim that stems of resistant and susceptible varieties are susceptible to wilt. He inoculated hop plants with V. albo-atrum, either through roots or directly into stems. In grafted plants, stems of a resistant and susceptible plant were both heavily colonized from infected roots of a susceptible variety, but both were colonized only a little from inoculated roots of a resistant variety. After direct inoculation of the stem of a susceptible variety, there was little growth of the fungus from a small inoculum but the stem was extensively colonized if a large inoculum was used.

Keyworth concluded from this that stems of both resistant and susceptible varieties are equally resistant and that the difference in resistance of the two varieties is caused by the amount of growth of the pathogen in the roots.

Berry and Thomas (1961) and Horner (1954) have presented evidence which indicates that resistance of mint to Verticillium wilt is present in stems as well as roots. Berry and Thomas showed that unrooted, inoculated cuttings had a range of resistance similar to rooted inoculated cuttings. Differential resistance was also expressed when wounded stems were inoculated. Isolates which were mildly virulent in root inoculations were more virulent when inoculated in stem wounds. They concluded that resistance was common

to stem and root but was greater in the stem.

Blackhurst and Wood (1963) found that resistant and susceptible tomato varieties were similarly colonized by Verticillium whether inoculated through roots or stems. Only slight disease symptoms appeared in the resistant plants and the fungus was not found in the upper petioles of resistant but was present in susceptible plants.

They also cross-grafted tomato plants and showed that stems as well as roots were resistant.

Keyworth (1963, 1964) also used grafted plants. He obtained somewhat unexpected results in that scions of resistant varieties on a susceptible rootstock showed symptoms more rapidly than scions of susceptible varieties on the same rootstock. There was also some difference in the symptoms. More vascular browning occurred in resistant scions, but the fungus grew more rapidly in susceptible than in resistant plants.

These results can be explained by assuming that the resistant stems are damaged more by metabolites of the pathogen but do not allow the fungus to invade. This suggests a form of hypersensitivity.

The preceding results emphasize the point made earlier that disease resistance is not a simple process but may consist of many interrelated processes. Neither the site nor the mechanism of resistance is likely the same for all host-pathogen relationships and this should be considered before making generalizations.

Talboys (1964) has discussed a hypothetical host-parasite relationship for Verticillium wilt diseases. He says that the two minimal requirements for the development of any vascular wilt disease are:

(1) that the pathogen shall enter the vascular system of the host and

(2) that it shall continue to colonize the vascular system to some minimum extent and with some minimum intensity.

Thus, any characteristic of the host that tends to exclude the pathogen from the vascular system or restrict its spread within it, is likely to contribute to the host's resistance. Any characteristic of the pathogen that facilitates its entry into the xylem, or its subsequent spread within it, contributes to its virulence.

The methods of exclusion by the host could include mechanical as well as physiological barriers. Talboys suggests that the extravascular host-parasite interactions constitute the "determinative phase" of the disease because the quantities of mycelium that become established in the xylem of the root determine the severity of the ultimate syndrome by determining the pattern of the subsequent host-parasite interaction. These interactions may culminate in the development of leaf symptoms which constitute the "expressive phase" of the disease.

Talboys further suggests there is a critical range of fungal metabolites necessary to cause tyloses or gum deposits which would

occur rapidly enough to occlude the pathogen and a corresponding critical range of quantity of vascular mycelium producing these substances. The quantity of mycelium necessary to exceed the critical range, thus preventing occlusion formation, is termed the "threshold value." Therefore, in the hypothetical disease, the quantity of mycelium exceeds the threshold level and suppresses the obstructive mechanisms. This allows unrestricted colonization of the whole vascular system, free movement of toxic metabolites, and development of acute symptoms.

In his discussion, Talboys did not postulate specific "wilt-resistance" factors in the host, but suggested that one or many of the several mechanical or biochemical factors already mentioned may be operable and still fit into his hypothetical scheme.

Other factors which could be mentioned in a discussion of resistance mechanisms include unsuitability of substrate, toxins, expressions of hypersensitivity, phytoalexins, wound responses, and pectic enzyme activities (Wood, pp. 479, 480, 487, 491, 462, 457). However, this thesis is not primarily concerned with a study of these factors and they will not be discussed here.

Two other factors of resistance, pertinent to this thesis, which can be grouped under the category of factors operating after penetration, are phenols and phenolases.

Phenols and substances derived from them may be important in

inhibiting fungus enzymes that degrade cell walls (Wood, p. 458). These compounds probably act by forming complexes with the enzymes in a non-specific manner. There is some evidence that enzyme inhibition by phenols is an important factor in disease resistance.

Byrde (1956, 1957) found that fruits of apple varieties with high resistance to Sclerotinia fructigena had a high tannin content. A positive relation was established between rate of discoloration and resistance. This indicates that resistance may depend, in part, on inactivation of enzymes by oxidized phenolic compounds.

When phenols are oxidized, quinones are formed, many of which are toxic in low concentrations to microorganisms. Quinones are highly reactive and react with other substances to form complexes which often are more toxic to microorganisms than the original phenols. As a result of phenol oxidation, cell walls become impregnated with substances that make them resistant to attack by most pathogens. This can also occur in wound reactions (Wood, p. 484).

Infected host tissue usually shows an increase in respiration, so it is not unusual that many workers have attempted to correlate resistance with increased oxidizing enzyme activity.

Potato tubers and leaves infected with Phytophthora infestans show an increase in polyphenoloxidase activity at and near the sites of infection (Rubin, Artsikhovskaya and Proskurnikova, 1947). These workers noted the resistant variety had high polyphenoloxidase

activity one to four days after inoculation. The susceptible variety showed no such increase in enzyme activity. This increase in polyphenoloxidase activity may be related to the wide variety of phenolic compounds which appear in resistant but not susceptible tubers as a result of infection.

Wilding (1960) found that extracts from pea plants resistant to Fusarium oxysporum f. pisi had about three times as much enzyme activity as extracts from susceptible plants. Menon and Schachinger (1957) noted similar results for tomato plants resistant to F. oxysporum f. lycopersici.

Highley (1968) showed that a wild species of mint resistant to Verticillium wilt had about twice as much polyphenoloxidase activity as a susceptible variety. This evidence seems to implicate phenol-oxidizing enzymes in disease resistance.

Cross-Protection

The literature on acquired immunity in plants is not extensive, especially in relation to cross-protection between fungal species.

Yarwood (1956) found cross-protection in rust diseases and concluded that a uredospore germination inhibitor was responsible for this phenomenon. Weber and Stahmann (1964) were able to cross-protect sweet potatoes against Ceratocystis rot by using a non-pathogenic strain of C. fimbriata. They found this acquired

resistance to be limited to a few cell layers adjacent to the inoculated layer.

Paxton and Chamberlain (1967) reported that soybean plants could be locally cross-protected against infection by Phytophthora megasperma var. sojae with P. cactorum. They implicated a phytoalexin in this cross-protection.

In 1964, Davis showed cross-protection in cabbage, carnation, tomato, watermelon, and flax seedlings grown on an agar substrate and inoculated with various combinations of Fusarium oxysporum f. vasinfectum, batatas, tracheiphilum, conglutinans race 1, dianthi, lycopersici, niveum, and lini. The non-pathogens penetrated the root a few millimeters from the point of inoculation indicating cross-infection was common. These experiments were done in test tubes. Schnathorst and Mathre (1966) thought either greenhouse or field studies were necessary to demonstrate cross-protection with a vascular fungus parasite.

They used a variety of cotton which showed differential symptoms to two strains of Verticillium. It was tolerant to infection by strain SS-4 but died when infected by strain T-1. The mild strain was used to inoculate plants in the greenhouse, followed one week later by a challenge inoculation with the severe strain. Up to 66% of the plants were protected from severe wilt. When plants were inoculated first with the severe strain and then challenged by the

mild strain one week later, only severe wilt developed.

When field soil naturally infested with the severe strain (5×10^3 viable propagules/gm) was supplemented with microsclerotial inoculum (10^5 propagules/gm) of the mild strain, 94% of the plants were protected. One-hundred percent severe wilt developed in non-supplemented field soil. If the two strains were mixed equally, severe wilt developed but when 10^5 viable propagules of the mild strain were mixed with 10^4 propagules of the severe strain, 50% of the plants were protected from severe wilt.

They concluded that demonstration of cross-protection depends upon amount of inoculum used and the timing of inoculation. Where mixtures of the two strains occur naturally, there may be a significant retarding effect on the buildup of the severe strain that is dependent on the proportion of each strain in the total inoculum in the soil.

Mutation Breeding

The use of irradiation to produce mutants that might have some agronomic value is relatively recent. Smith (1958) cites some of the early work in this area. Swedish workers began experiments in 1928 using irradiation to develop barley mutants. They successfully produced mutants in 1934-1935. Several Russian workers attempted to develop mutants of wheat in the 1930's and in the 1940's, German

breeders were attempting to obtain mutants of barley, wheat, flax, soybeans, and currants.

Work in the United States has been more recent. In 1954, Shebeski and Lawrence induced beneficial mutations which conferred stem rust resistance and stiffer straw to barley. Frey and Browning (1955) found mutations for stem rust resistance in x-irradiated oat seeds. Only one strain of 61 tested appeared to have a mutation that was different from known rust resistant genes.

Konzak (1956a, 1956b) initiated an irradiation program to study the genetics of Victoria blight susceptibility and crown rust resistance of oats. These characteristics are due to a complex genetic locus and Konzak hoped to determine if radiation might be useful for mutation of one of a number of closely linked genetic factors. He found that blight resistance was readily induced by irradiating seed with x-rays, fast neutrons, or thermal neutrons. Most of the blight resistant mutants were susceptible to crown rust. This confirmed that the characters were closely linked. He postulated that blight resistance could occur as a result of radiation-induced loss of the chromosomal segment carrying the loci under study.

Waggoner and Dimond (1956) used ionizing radiation to alter Fusarium wilt resistance of tomato. They found that if the whole plant was irradiated before inoculation, resistance increased. If the same treatment was given at the time of inoculation, resistance

decreased. Root irradiation before inoculation increased resistance significantly, but the same treatment at inoculation caused only a small increase in resistance. Shoot irradiation at any time decreased resistance but not significantly. These results suggested that irradiation may alter resistance by altering auxin levels. Other examples of induced mutations that have disease resistance or other characteristics of agronomic value are given by Frey (1955), Down and Anderson (1956), Larter and Elliot (1956), Gregory (1956), Hansel and Zakovsky (1956), Myers et al. (1956), Singleton et al. (1956), Brock (1957), and Osborne (1957).

MacKey (1956), Horner (1957), and Konzak (1959) reviewed and discussed the use of induced mutations in breeding programs. These things should be considered before using induced mutants.

(1) Artifically induced mutants should be different in their mutation spectrum from those of spontaneous origin.

(2) Induced mutations should be of positive value and should bring the evolution forward beyond what is already available.

(3) Constructive mutations should be frequent enough to pay for the effort.

Irradiation of plants to induce mutations seems to offer definite promise as a technique to improve characters that are controlled by one or a few genes, such as disease resistance. This is especially true for certain plants that cannot be manipulated with conventional

breeding techniques.

The decision to use induced mutations in a breeding program depends on the species of plant, the existing natural genetic variation, and available screening techniques (Horner, 1957). Peppermint should be a good plant to use for induced mutation studies. It is vegetatively propagated, thus natural variation is minimal. The commercial variety is male-sterile, so the usual crossing techniques cannot be used readily. Verticillium wilt resistance is easy to screen for by growing plants in a naturally infested soil.

Species of mint that show resistance to Verticillium wilt generally have oil of poor quality. Attempts to transfer wilt resistance to varieties with acceptable oil quality have not been successful (Murray, 1967b), indicating the genes controlling oil quality and wilt resistance may be closely linked. This is another reason why irradiation might be beneficial in a search for wilt-resistant mint. Perhaps wilt resistance could be induced without changing oil quality.

In 1955-1959, M. J. Murray subjected stolons of Mitcham peppermint to x-rays and neutrons. He planted a total of 103, 667 plants from irradiated material in a heavily wilt-infested field to select resistant plants. In the second year, more than six million plants were produced, but these were reduced by four years of natural selection to a 1% stand (Murray, 1964, 1965, 1966, 1967a, 1967b).

By 1966, he had selected 273 strains from the remaining 1%

that showed varying resistance to wilt. Of these, eight had high resistance and 59 were moderately resistant. The remainder had not been fully tested.

This thesis attempts to answer basic questions concerning the nature of resistance of seven of these strains. Nature of resistance is important to our understanding of the host-parasite relationship in Verticillium-wilt diseases; it will also help to determine which of these strains might ultimately replace Mitcham peppermint as a new commercial variety.

GENERAL METHODS AND MATERIALS

Plants

Commercial peppermint (Mentha piperita L. 'Mitcham') was used in this study as a susceptible control and is referred to as Mitcham in this thesis.

Mutant strains used are as follows:

92--High resistance, low vigor

58--High resistance, low vigor

1229--Moderate resistance, high vigor

2336--Moderate resistance, moderate vigor

1320--Moderate to low resistance, high vigor

3201--High resistance, moderate vigor

3202--High resistance, moderate vigor

Mutant strains were originally obtained from Dr. M. J. Murray, plant geneticist of the A. M. Todd Co. They are X-ray and neutron induced mutants of Mitcham peppermint stolons which were subjected to radiation at Brookhaven National Laboratory.

Dr. Murray planted these irradiated stolons under severe wilt conditions in Michigan to select for wilt resistance. The above seven strains have shown some tolerance to Verticillium wilt in the field.

Fungus

Verticillium dahliae Kleb. (peppermint strain) was isolated from diseased peppermint plants in a commercial field near Jefferson, Oregon, on June 1, 1966. This isolate is highly virulent to Mitcham peppermint and was used in all greenhouse and laboratory tests. It is designated as Mp-3.

An isolate of V. dahliae from cotton was used in some experiments. It was isolated in 1963 from cotton in a field near Lubbock, Texas. It is virulent to cotton. This isolate is designated as C-6.

Greenhouse Propagation

To insure that all plants were of uniform physiological age, tip cuttings were taken from all the strains of mint and rooted in sand. These rooted cuttings were then inoculated with the test fungus. Use of uniform plants eliminated differential susceptibility of the various strains of mint due to age differences.

The cuttings were watered weekly with a complete nutrient solution while in sand. After inoculation, they were transferred to greenhouse soil and fertilized with 16-20-0.

Greenhouse temperature was maintained near 25 C during all experiments. Supplemental lighting was used during spring and fall to maintain a 14 hour day.

Inoculation Methods

Rooted cuttings in the greenhouse were inoculated in one of three ways:

(1) Root dipping--Cuttings were removed from sand culture and the adhering sand was washed away. The roots were then dipped in a spore suspension of the fungus for up to four hrs. The plants were transferred to flats of greenhouse soil.

(2) Stem puncture--The rooted cuttings were transferred to flats of fertilized, limed soil. A drop of spore suspension (one million spores/ml) was placed just above the first node with a syringe. The stem was pricked with the syringe needle allowing the drop of spore suspension to be drawn into the plant. This method was used to bypass the root system to determine the role of stems in resistance.

(3) Infested soil--Two methods of infesting soil were used.

(a) A spore suspension, which had been grown in a shake culture of Czapek's-Dox broth plus yeast extract, was mixed with about one lb. of field soil which had been sifted through a 42 mesh soil screen. This soil was allowed to dry for several days and was again sifted through the 42 mesh screen. This was the stock inoculum. It was assayed by soil dilution

techniques on ethanol-streptomycin agar (ESA) (Nadakavukaren and Horner, 1959) to determine the number of viable propagules per gram of soil. Portions of this stock inoculum were then mixed with field soil in a cement mixer to obtain the desired number of viable propagules per gram of soil. Rooted cuttings were then planted in this infested soil.

(b) The second method of infesting soil used microsclerotia grown on steam-sterilized oat or mint straw to which was added two ml of Czapek-Dox broth (1%) per gram of straw. This served as a food base for the fungus. The fungus was grown axenically in 2000 ml flasks filled with 50 g straw, for about two months. The straw was then removed from the flasks and dried for about three days, after which it was ground in a Waring blendor (without water) and the straw was sifted through a series of soil screens. I found that an almost pure yield of microslerotia was obtained through a 200 mesh screen.

I determined the number of microsclerotia contained per gram in two ways:

(1) Dilution assays on ESA.

(2) Direct counting in a binocular microscope. This was done

by weighing out 25 mg of the microsclerotia and placing this in 50 ml water. One-tenth ml of this was placed on a filter paper. The water was absorbed leaving the microsclerotia which could be counted easily on the white background. I found there were approximately 1,500,000 microsclerotia per gram of debris that passed through a 200 mesh screen.

I preferred the preceding methods of inoculation by soil infestation when I desired a natural disease development in the greenhouse. Root-dip and stem puncture as well as inoculation of rootless cuttings were useful for certain other phases of these experiments to determine more about sites and mechanisms of resistance.

Propagule Assays

Assays were run at varying intervals after inoculation of the cuttings to determine the number of viable propagules per gram of tissue. This was used as an index of fungus development in the different strains of mint, especially in the early stages of the wilt syndrome before symptoms were manifested. After symptoms were visible, the numbers of propagules could be related to symptom severity.

The procedure for these assays was as follows:

Plants were collected and fresh weights were recorded. The leaves were then stripped from the stems and the stems weighed.

The stems were chopped finely with a razor blade then ground in an Omni-Mixer with sufficient distilled water to make 10 ml per gram tissue. Aliquots of this were removed and dilutions of 1:100 and 1:1000 were plated on ESA. The petri dishes were incubated at room temperature for about two weeks and the resulting Verticillium colonies were counted. Results were expressed as propagules per gram of tissue (fresh weight).

Roots were washed for 24 hrs. in running tap water and treated in the same manner as stems for assaying propagule numbers. Lacy's method (1965, p. 44) was used to detect penetration sites on roots. Results were expressed as colonies per gram of root (fresh weight).

Symptom Rating and Yield

Symptoms of diseased peppermint begin in the youngest leaves. Initial symptoms are a bronzing of leaf tips. The leaves then become asymetrical, turn yellow and, in cases of severe wilt, become necrotic and drop from the stem.

I assigned numerical values to these symptoms in order to relate disease severity to numbers of propagules in the stem and root and to measure disease progression after inoculation.

Ratings were:

1--slight symptoms. Leaf margins bronze, tip leaf beginning

to curl slightly.

2--moderate symptoms. Leaf margins yellow, all upper leaves asymetric.

3--severe symptoms. Some leaves becoming necrotic and falling from stem.

4--plants dead.

In some experiments, dry weights of stems and leaves were taken by drying the samples at 100 C for 24 hrs.

Polyphenoloxidase Assay

Because polyphenoloxidase has been implicated in disease resistance, I wished to determine if there was any difference in the level of this enzyme in healthy, field grown plants of the different strains of mint.

Plants were grown in the field and collected at weekly intervals from June 19, 1968, to August 28, 1968. The samples were placed in plastic bags and brought to the laboratory.

Five grams of stem tissue (leaves removed) were chopped and placed in an Omni-Mixer along with five grams of polyvinylpyrrolidone (PVP). Insoluble PVP was added to absorb the native phenols of the plant and thus obtain an active, soluble enzyme (Loomis and Battaile, 1966). Thirty ml of 0.1 M phosphate buffer (pH 7.0) were added to the above and the mixture was homogenized in the blendor

for two minutes at high speed.

The homogenate was filtered through cheesecloth. This was the crude enzyme extract. All the above steps were carried out at about 4 C.

Enzyme activity was determined colorimetrically (Matta and Dimond, 1963). One-tenth ml of the enzyme extract was added to 9 ml of a freshly prepared 0.5% catechol solution. Activity was measured at 400 mμ in terms of increase in optical density between 30 and 90 seconds after addition of the extract. Results were expressed as $^{\Delta}$OD per 0.1 ml plant extract.

Microsclerotial Assay in Dead Stems from Field Plots

Dead stems were collected in January and February, 1968 from field plots to determine the relative amounts of microsclerotia produced in the stems of resistant and susceptible strains of mint. Internodes were taken from these stems and the length measured in millimeters. Presence of microsclerotia was rated as follows:

1--no microsclerotia

2--< 100 microsclerotia per cm stem--slight invasion

3--100-1000 microsclerotia per cm stem--moderate invasion

4--> 1000 microsclerotia per cm stem--severe invasion

Field Studies

Four separate locations were established as field plots. Different types of data were taken from each. The locations were:

Hamlin farm: two miles SE Corvallis. Established in May, 1967. Plots consist of ca 0.25 acre of each variety. Plants for polyphenoloxidase assay were collected and data were taken on vigor and oil yield. Natural infestation of the soil with V. dahliae was very low in this field, thus wilt readings were not taken.

Chambers farm--plot number 1. Five miles NW Albany, Oregon. Established April, 1967. These plots consist of 6 ft long plantings of each of the previously described strains. In addition, about 65 other strains plus controls were planted for a wilt screening experiment. There are seven replications in this experiment. Data on incidence and severity of wilt were taken from this plot as there was a uniform, heavy natural soil infestation with Verticillium.

Chambers farm--plot number 2. Five miles NW Albany. Established April, 1968. These plots consist of 0.5 acre plantings of the previously mentioned strains except 1320 and 2236. Data on wilt incidence and severity as well as vigor and oil yield were taken.

Helms and Williams farm--three miles SE Jefferson, Oregon. Established May, 1966. Plots were 6 ft long and contained 75 strains of irradiated mint plus appropriate controls. This location yielded

data on incidence and severity of wilt and vigor of the plants for screening purposes. A severe infestation of symphyllans forced abandonment of this trial after one year.

RESULTS

Relative Resistance of Irradiated Strains in Field Tests

A series of field tests were made to establish the relative resistance of irradiated mint strains to Verticillium wilt. Strains were planted as rhizomes and data were taken on incidence of wilt (total percentage of foliage showing symptoms) and severity (degree of infection based on visible symptoms). Severity was rated on a numerical basis of 0-4 as previously described.

Data in this section show that the eight strains of mint previously described are less severely diseased than Mitcham when planted in a field naturally infested with V. dahliae.

1966 Experiments

In 1966, field experiments were on the Helms-Williams farm at Jefferson, Oregon. A number of these plots were severely infested with symphyllans, so the site was abandoned after one year. The data in Table 1 were taken from plots outside the symphyllan area.

These data show that all the irradiated strains were less severely infected with wilt than Mitcham. Based on this table, strain 58 is the most resistant followed in order by 3202, 3201, 92, 1229, 1320, 2236, and Mitcham.

Table 1. Summary of wilt severity and vigor of irradiated mint strains planted on Helms-Williams farm, 1966.

Strain	Symptom severity	Vigor index[a]
Mitcham	1.7	3.00
92	0.42	3.50
58	0.12	2.87
1229	0.50	5.00
3201	0.33	4.00
3202	0.16	3.20
2236	0.77	2.62
1320	0.66	4.00

[a]Vigor index rating:

1--poor growth
2--fair growth
3--normal growth (based on Mitcham)
4--good growth
5--very good growth

The vigor index shows strain 1229 to have very good growth. Strains 1320, 3201, and 92 had better growth than Mitcham. Strains 3202, 2236, and 58 were less vigorous in this experiment than Mitcham.

1967 Experiments

This experiment consisted of seven replications planted on the Chambers farm, Albany, Oregon. Within each replication, were three plots of Mitcham (six feet long) and two plots of the other strains. About 60 other mint strains were included in this experiment but data on their wilt reactions are not included here.

Table 2 shows the mean wilt incidence and severity of the eight mint strains taken at three dates during the growing season.

Mitcham was more severely diseased than any of the irradiated strains (Table 2). By September 19, 84% of the above ground parts of Mitcham had been completely killed. Strain 1320 was also severely infected but fewer plants were dead. Based on these data, strain 3202 is the most resistant. Strains 58 and 3201 are similar in their reactions to wilt and are among the least severely damaged. Strain 92 had good tolerance to wilt until after August 22, when it began to show severe symptoms.

The reading taken on September 19, is probably not as important as earlier readings. In practice, peppermint is harvested by

Table 2. Mean wilt incidence and severity of irradiated strains planted on Chambers farm, 1967.

Strain	July 18		Aug. 22		Sept. 19	
	Incidence[a]	Severity[b]	Incidence	Severity	Incidence	Severity
	%		%		%	
Mitcham	55	2.3	69	3.4	84	4.0
92	0	0	4	1.1	20	3.1
58	2	0.4	15	1.8	16	1.9
1229	9	0.7	29	1.7	34	3.1
3201	1	0.2	7	1.3	16	1.9
3202	1	0.1	7	0.7	16	1.6
2236	1	0.1	8	1.0	15	2.2
1320	29	1.7	39	2.8	62	3.9

[a]Percentage of foliage showing symptoms

[b]Severity ratings:

1--light symptoms
2--moderate symptoms
3--severe symptoms
4--plants dead

the end of August, thus readings taken after this will not be significant in assessing disease development in a field situation.

Based on the August 22 reading, Mitcham was the most severely affected by wilt. Strain 3202 had high tolerance followed closely by strains 2236 and 92. The other strains were somewhat less tolerant and the symptom severity in strain 1320 approached that of Mitcham.

Table 3 gives another index of vigor for these strains. These ratings are based on a reading of 100 for Mitcham.

Table 3. Vegetative vigor of irradiated mint strains planted on Chambers farm, 1967.

Strain	Vigor rating[a]
Mitcham	100
92	90
58	87
1229	143
3201	80
3202	92
2236	84

[a]Based on Mitcham = 100

All the strains appeared to be less vigorous than Mitcham except strain 1229. However, the final criterion of vigor should be the amount of oil yielded per unit area. No oil yield was taken from this experiment, thus these vigor ratings may not be significant in the overall selection program. They are useful to get an initial idea of the performance of these strains in the Willamette Valley.

1968 Experiments

The 1967 planting on the Chambers farm was allowed to remain. The data in Table 4 show incidence and severity of wilt during the second year. These data are means of six replications.

The relative ranking of the strains based on incidence and severity of wilt at each rating date is shown in Table 5.

Tables 4 and 5 show several things. (1) Mitcham was the most severely infected of all the strains tested based on both mean incidence and mean severity. A Duncan's Multiple Range test showed the wilt incidence of Mitcham was significantly higher at the 1% level than the wilt incidence of the irradiated strains. This was true each time data were taken. The irradiated strains did not differ significantly from each other in incidence or severity of wilt. (2) The strains showing the least wilt incidence vary from week to week. No one strain is always outstanding in its wilt tolerance when compared with the other strains. (3) Strains 58, 92, 3201, and 3202 are in a group with the least incidence of wilt compared to Mitcham. They can be referred to as high resistant strains. Strains 2236, 1320, and 1229 are more tolerant to wilt than Mitcham but fall below the other strains. They can be referred to as moderate resistant strains. (4) Readings for wilt incidence are probably more meaningful in selection of a new variety than are those for severity because

Table 4. Mean wilt incidence and severity of irradiated strains during second year on Chambers farm, 1968.

Strain	July 11		July 19		July 29		Aug. 8	
	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity
	%		%		%		%	
Mitcham	37.77	2.0	46.11	2.27	57.22	2.89	58.67	3.0
92	9.16	1.58	16.66	1.75	25.84	2.42	28.77	2.84
58	8.75	1.49	15.83	1.58	23.34	2.26	23.33	3.0
1229	9.58	1.24	14.16	2.16	37.51	2.59	30.01	2.84
3201	7.49	1.41	15.41	1.66	25.34	2.37	27.17	3.0
3202	7.08	1.24	17.50	1.50	24.17	2.59	27.92	3.0
2236	10.41	1.16	23.75	1.91	26.67	2.34	29.17	3.0
1320	12.08	1.75	22.08	1.66	26.17	2.25	29.24	2.99

Table 5. Mint strains showing least to most wilt based on incidence and severity.

July 11				July 19				July 29				Aug. 8			
Rank	Inc.	Rank	Sev.	Rank	Inc.	Rank	Sev.	Rank	Inc.	Rank	Sev.	Rank	Inc.	Rank	Sev.
1	3202	1	2236	1	1229	1	3202	1	58	1	1320	1	58	1	92
2	3201	2	3202[a]	2	3201	2	58	2	3202	2	58	2	3201	2	1229
3	58	3	1229	3	58	3	3201	3	3201	3	2236	3	3202	3	1320
4	92	4	3201	4	92	4	1320	4	92	4	3201	4	92	4	58
5	1229	5	58	5	3202	5	92	5	1320	5	92	5	2236	5	3201
6	2236	6	92	6	1320	6	3201	6	2236	6	3201	6	1320	6	3202
7	1320	7	1320	7	2236	7	2236	7	1229	7	1229	7	1229	7	2236
8	Mitcham	8	Mitcham	8	Mitcham	8	Mitcham	8	Mitcham	8	Mitcham	8	Mitcham	8	Mitcham

[a]Strains joined with a line had the same reading

differences in severity were not statistically significant. All the strains eventually developed moderate to severe symptoms but the percentage of plants showing these symptoms varied among the strains.

Wilt incidence in the large $\frac{1}{2}$ acre plots on the Chambers farm is shown in Table 6. The plots were not replicated so the results were not statistically analyzed. The data were taken on August 30, 1968.

Table 6. Incidence of wilt in $\frac{1}{2}$ acre plots on Chambers farm, 1968.

Strain	Number plants wilted	Number wilted/ 1000 sq. ft.	% of Mitcham
Mitcham	719	26.6	100.00
92	192	7.1	26.70
58	251	9.3	34.91
3201	210	7.8	29.21
3202	146	5.4	20.31
1229	118	4.4	16.41

Strain 1229 had the least incidence of wilt followed in increasing order by strains 3202, 92, 3201, 58, and Mitcham. All the irradiated strains had considerably less wilt than Mitcham, but the differences between the irradiated strains themselves are probably not significant. Incidence of wilt in Mitcham was low when compared to a severely diseased field. In all strains, symptoms of infected plants were moderate to severe. This supports other data which

indicate that selection for wilt incidence may be more significant than selection for wilt severity.

The hay from the $\frac{1}{2}$ acre plots was harvested and oil was distilled in a commercial distillery. Oil yields are shown in Table 7.

Table 7. Oil yields of irradiated strains from $\frac{1}{2}$ acre Chambers plots, 1968.

Strain	Oil yield/acre (lbs.)
Mitcham	49.4
92	49.2
58	64.4
1229	65.6
3201	61.6
3202	64.0

Oil yield is a more meaningful criterion of these strains than vegetative vigor ratings previously taken. Mitcham and 92 yielded the least amount of oil/acre. The other four strains yielded substantially more. A yield of 60 lbs. of oil/acre is considered very good for first-year mint.

These types of data are necessary to select a new variety to replace Mitcham. The yield is important but oil quality must also be considered because companies using this oil have rigid flavor standards. Flavor and quality tests are not reported in this thesis. Yield data are given to show that the irradiated strains are not depressed in yield when compared with Mitcham.

Presence of Microsclerotia of V. dahliae in Dead Stems of Mint Strains

Another measure of resistance is the amount of fungus in infected plants. In Mitcham, microsclerotia of V. dahliae form abundantly in dead stems. Dead stems from the small Chambers plots were collected and assayed for the presence of microsclerotia of V. dahliae as previously described. These results are in Figure 1 and Table 8. Almost 83% of the stems of Mitcham had microsclerotia (Figure 1). This was the highest of any of the strains tested. Of these stems, over 60% were in classes three and four (moderate to high numbers of microsclerotia (Table 8). Strain 2236 had the lowest percentage of stems with microsclerotia. Only 19.83% of the stems had microsclerotia (Figure 1) and of these, less than 10% were in classes three and four (Table 8). Strain 58 also had a low percentage of stems with microsclerotia (22.32%) (Figure 1). Strains 92 and 1229 had microsclerotia but only about half as many as Mitcham (Figure 1).

These data show that the irradiated strains are considerably more resistant to Verticillium infection than Mitcham. They are not, however, immune and fungus inoculum will still be maintained. One advantage of these strains seems to be that they allow fewer microsclerotia to be formed than does Mitcham. This means that a lower inoculum level will be maintained and eventually the fungus

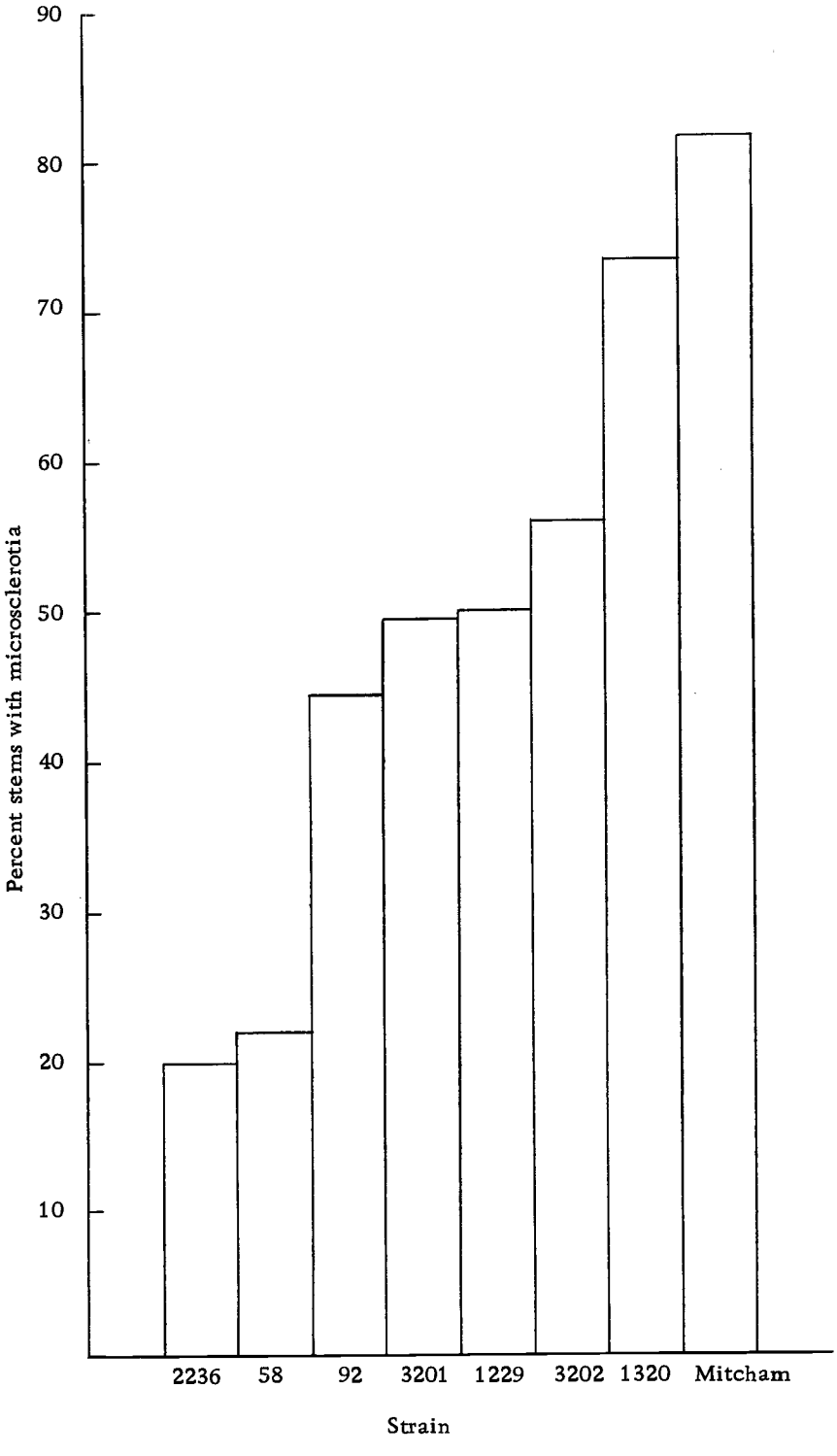

Figure 1. Percentage of dead stems of mint strains having microsclerotia.

could be reduced to a level which would no longer cause severe disease.

Table 8. Presence of microsclerotia of V. dahliae in dead stems of peppermint strains.

Strain	Class[a] 1	2	3	4
	%	%	%	%
Mitcham	17.38	19.64	31.42	31.56
92	55.94	13.66	15.76	14.64
58	77.68	9.89	5.22	7.21
1229	51.37	19.20	8.94	20.49
3201	51.63	27.61	12.22	8.54
3202	44.07	20.11	15.08	20.74
2236	80.17	10.98	4.84	4.01
1320	26.84	34.05	23.45	15.66

[a]Class rating:

1--no microsclerotia
2--less than 100 microsclerotia/cm stem
3--100-1000 microsclerotia/cm stem
4--more than 1000 microsclerotia/cm stem

Nature of Resistance

The preceding data have shown that certain of the irradiated strains have more tolerance in the field to Verticillium wilt than does Mitcham. This section deals with results of greenhouse and laboratory experiments to determine something of the nature of resistance, i.e., sites and mechanisms of resistance.

Growth of V. dahliae on Stem Pieces of Resistant and Susceptible Peppermint Strains

Experiments were set up to determine if there was a difference in growth of V. dahliae on stems of irradiated strains as compared with Mitcham. The hypothesis was that all strains would support the same amount of fungus growth. If this were true, it would suggest that resistance is not passive, i.e., nutrients such as carbon source produced by the susceptible strain do not differ from the resistant strains. On the other hand, positive differences would indicate that nutrient sources might play a role in resistance.

Experiment One

Stems of each of the irradiated strains and Mitcham were ground in an Omni-Mixer at a ratio of one gram stem to 10 ml water. This was added to cooled 2% water agar in suitable quantities to make a final dilution of 1:100 and 1:45. After the agar cooled, a 5 mm disc of a 3-week-old culture of V. dahliae was placed in the center of each of four replicate plates. The control was 2% water agar with distilled water added in place of plant extract.

Radial growth of the colonies, measured after five and 15 days, is shown in Table 9.

Table 9. Radial growth of V. dahliae on solid media with extract of irradiated mint strains.

Strain	Dilution	Ave growth in mm 5 days	15 days
Control	1:100	8.0	17.25
	1:45	8.5	19.23
Mitcham	1:100	0	0
	1:45	0.5	4.25
92	1:100	1.0	1.0
	1:45	0	0.5
58	1:100	6.0	6.0
	1:45	1.0	1.0
1229	1:100	1.0	1.0
	1:45	0	0
3201	1:100	0	0
	1:45	1.75	1.75
3202	1:100	1.0	4.75
	1:45	1.25	4.25
1320	1:100	2.75	2.75
	1:45	0	0.5
2236	1:100	1.0	1.0
	1:45	2.25	3.0

The extracts of all strains inhibited growth of the fungus to some degree. Mitcham extract supported no growth at 1:100 dilution, but at 1:45, growth was as much as any strain. However, this growth was only 22% of the control. Growth in extracts of the other strains was slight or none at 1:45. At 1:100, strain 58 supported the

most growth. Since other data show that 58 has high tolerance to Verticillium, resistance cannot be explained on a simple basis of nutrition. Extracts of all the strains suppressed growth, suggesting the presence of some inhibitory substances. The method of preparing the media could have released phenolic compounds or other substances which inhibited growth.

Experiment Two

An experiment was designed to test the growth of Verticillium on stems of resistant strains without release of inhibitors into the medium. Stem pieces were sterilized with propylene oxide and this allowed a more natural situation since the integrity of the cells could be maintained. Deese and Stahmann (1962) reported using propylene oxide sterilized tomato stem pieces to grow Fusarium for assay of enzyme production. They made no attempt to measure growth in their experiments.

Preliminary experiments showed that surface contaminants on mint stems could be killed with gaseous propylene oxide sterilization for eight hours. Stem pieces of the irradiated strains and Mitcham were collected from the field early in the season when they were actively growing. Stems were chopped into 5 mm pieces and divided into 2.5 g lots. These were sterilized with gaseous propylene oxide for eight hours, after which they were removed and added to a flask

containing 50 ml of a complete mineral solution with no carbon source. They were allowed to stand for two days to dissipate the residual propylene oxide before being inoculated.

Washed spores of a one-week-old shake culture of V. dahliae were used as inoculum. Spores were added at a rate of 1000/ml or 50,000/flask. The flasks were not shaken.

Growth was estimated by grinding the cultures in an Omni-Mixer, diluting, and plating on ESA. Numbers of viable propagules per ml of medium were recorded. Results of this experiment are in Figure 2.

Strains 1229, 3201 and 2236 all supported more growth than Mitcham. Strains 92 and 58 showed an increased growth (related to original inoculum) but this was considerably less than Mitcham. This could be interpreted to mean that inhibitory substances were produced by 92 and 58 which suppressed growth in relation to Mitcham. On the other hand, strain 1229 is considerably more resistant than Mitcham, yet supported more growth in this experiment.

The above results indicate that nutrition is not directly related to resistance to Verticillium. Growth takes place, to varying degrees, when the mint plants are used for a substrate. There is a possibility that the inhibition noted might be due to production of toxic substances, probably phenolic compounds, by intact cells or liberation of these substances by damaged cells.

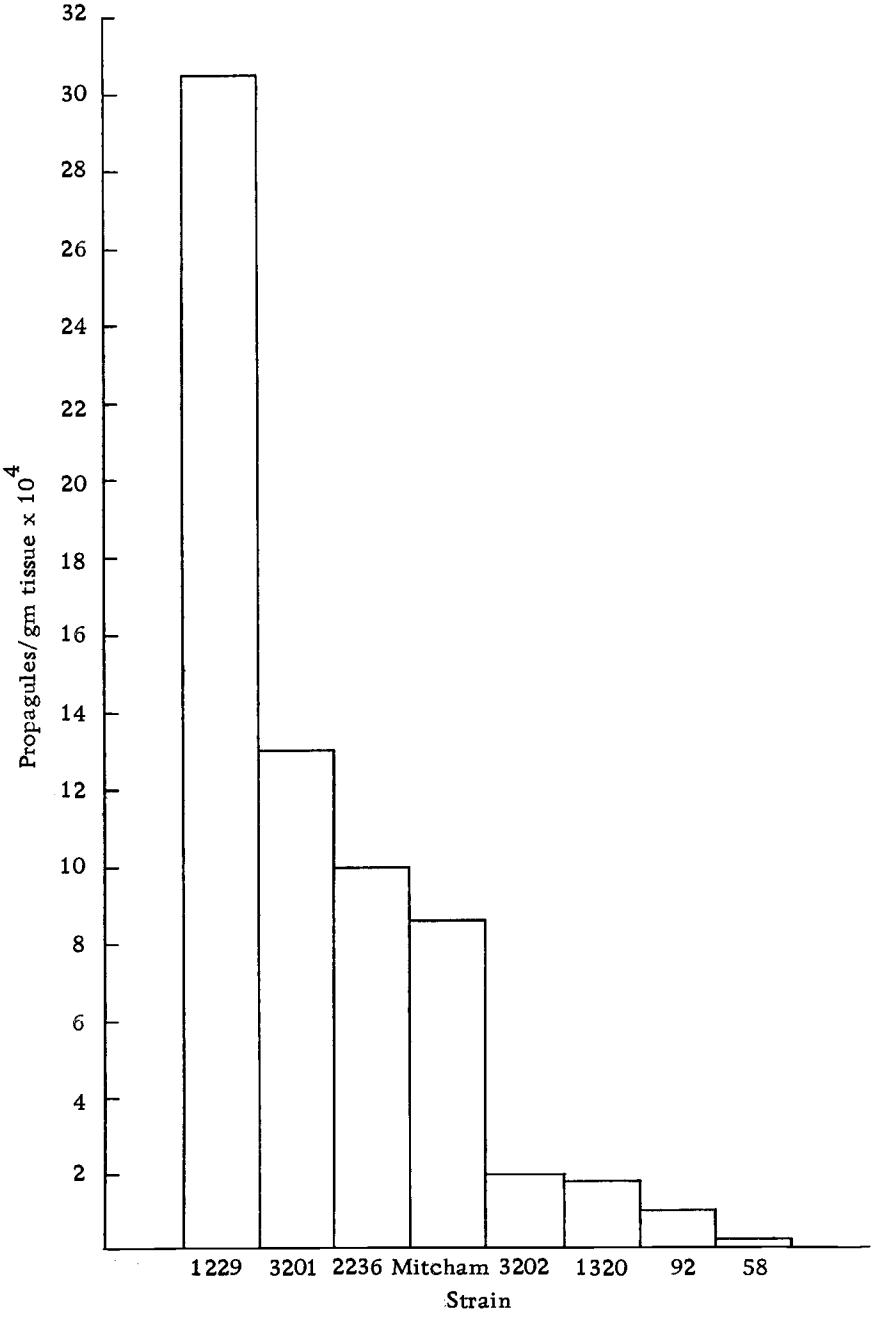

Figure 2. Growth of V. dahliae on stem pieces of peppermint strains. Assayed 3 weeks after inoculation.

Spore Movement in Inoculated Plants

This experiment was designed to determine if Verticillium moves throughout the mint plant as spores and if so, how fast these spores are distributed. I tested for differences in speed of spore movement in Mitcham, 92, and 1229. If there were differences in rapidity and extent of distribution, this could be an important mechanism of resistance.

I inoculated 45 uniform cuttings each of Mitcham, 92, and 1229 by root-dipping in a washed spore suspension of isolate Mp-3 (V. dahliae, mint strain). The inoculum was adjusted to one million spores/ml. The plants were assayed in groups of three by taking the whole plant, cutting it at each node, and plating each section on streptomycin potato dextrose agar (SPDA). Movement of the fungus was determined by noting where the colonies originated. This method allowed me to determine if the fungus was present or absent at each node of the plant. The plants were assayed at these times after inoculation: $\frac{1}{2}$ hour, 1 hour, $1\frac{1}{2}$, 2, $2\frac{1}{2}$, 3, 4, 5, 6, 7, 8, 10, 12, 24, and 48 hours. The distribution of the fungus after 30 minutes is shown in Table 10.

Table 10. Distribution of V. dahliae in mint plants 30 minutes after inoculation.

Strain	Plant	Nodes with fungus	Nodes without fungus
92	1	0	4
	2	0	4
	3	2 (top)	2 (bottom)
Mitcham	1	4	1 (bottom)
	2	0	4
	3	1 (mid)	3 (bottom and top)
1229	1	4	0
	2	3	0
	3	3 (mid)	2 (bottom and top)

These data show that it is possible for Verticillium to be systematically distributed in plants of all three strains within 30 minutes. Spores moved better through 1229 than through Mitcham or 92. Only 1/3 plants of strain 92 had the fungus while 2/3 plants of Mitcham and 3/3 of strain 1229 had the fungus.

The presence of the fungus was discontinuous at 30 minutes after inoculation. The top nodes had fungus and the bottom nodes did not in one plant of strain 92. One plant of Mitcham showed four of the top nodes with fungus while the bottom node was free of fungus. In another Mitcham plant, the middle node had fungus and the bottom and top nodes did not. In strain 1229, the middle nodes had the fungus and bottom and top nodes were free of fungus. The data

indicate that Verticillium can move throughout the host stem by conidia within 30 minutes. The discontinuity probably does not represent activation of a resistance mechanism because of the short time after inoculation.

Data in Figure 3 show the percentage of nodes infected at each assay time. After eight hours, the fungus disappeared in Mitcham and reappeared at 48 hours. This might indicate the point in time at which the fungus begins to multiply within the host. If there was a very small amount of Verticillium present between eight and 24 hours, it might have escaped detection. The highest number of nodes infected in strain 92 appeared ten hours after inoculation. After that time, the fungus gradually began to disappear. This may be due to a resistance mechanism operating after this time to reduce the amount of fungus in the plant. Other experiments, described later, showed that Verticillium will eventually disappear in strain 92.

Strain 1229 was more extensively invaded than either Mitcham or 92 at most of the assay times. The number of nodes with fungus also began to increase after 24 hours. Other experiments showed that the fungus persists in strain 1229 longer than in strain 92 and that it may even multiply. However, strain 1229 seems to be more tolerant of the same amount of fungus than Mitcham and the symptoms are not as severe as will be shown later.

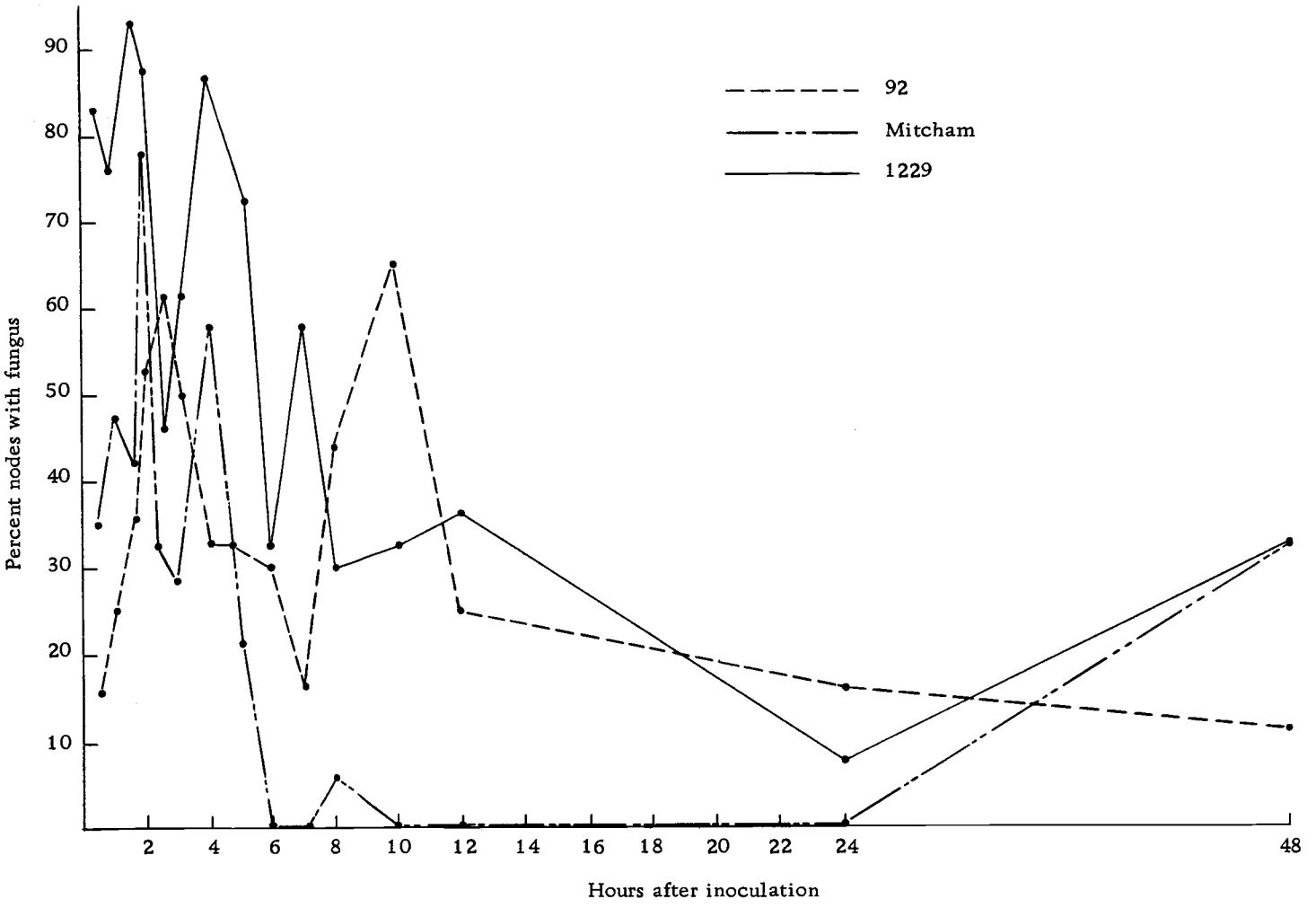

Figure 3. Percentage of nodes of mint strains showing Verticillium from 30 minutes to 48 hours after inoculation.

Polyphenoloxidase Assays

Highley (1968) found that healthy stems of resistant mint plants (Mentha crispa) had a higher polyphenoloxidase (PPO) level than susceptible M. piperita. Hybrid 148, intermediate in resistance to wilt, had an intermediate level of PPO. Highley used these three varieties to study PPO levels of infected plants and related this to fungus development. He found the level of PPO in uninoculated controls remained the same in a greenhouse experiment.

I wanted to determine what the level of PPO in the resistant strains was in relation to Mitcham and if the level of PPO in healthy plants of these strains fluctuated throughout the growing season. This would give an indication of the importance of PPO in resistance of these strains.

Healthy, field grown plants were collected at weekly intervals from June 19, 1968, to August 28, 1968. This corresponded to the time of active growth of the plants and also to the period when infection by Verticillium is most likely to take place.

The PPO levels, expressed as change in optical density of 9 ml of 0.5% catechol per minute per 0.1 ml plant extract, are given in Table 11 and expressed graphically in Figure 4.

Table 11. Polyphenoloxidase level of healthy, mint strains based on change in optical density at 400 mμ of 9 ml of 0.5% catechol between 30 and 90 seconds after adding 0.1 ml plant extract.

Strain	June 19	July 10	July 17	July 24	July 31	Aug. 7	Aug. 14	Aug. 21	Aug. 28
Mitcham	0.14	0.07	0.05	0.0425	0.037	0.087	0.115	0.052	0.107
92	--	0.125	0.082	0.0075	0.065	0.102	0.08	0.112	0.067
58	0.26	0.087	0.055	0.030	0.075	0.097	0.103	0.082	0.06
1229	0.07	0.065	0.052	0.01	0.031	0.092	0.095	0.045	0.075
3201	--	0.056	0.055	0.075	0.052	0.032	0.075	0.045	0.042
3202	0.19	0.0085	0.085	0.05	0.027	0.06	0.092	0.032	0.072
2236	0.23	0.082	0.054	0.075	0.055	0.142	0.062	0.045	0.074
1320	0.12	0.125	0.091	0.0481	0.020	0.01	0.04	0.015	0.045

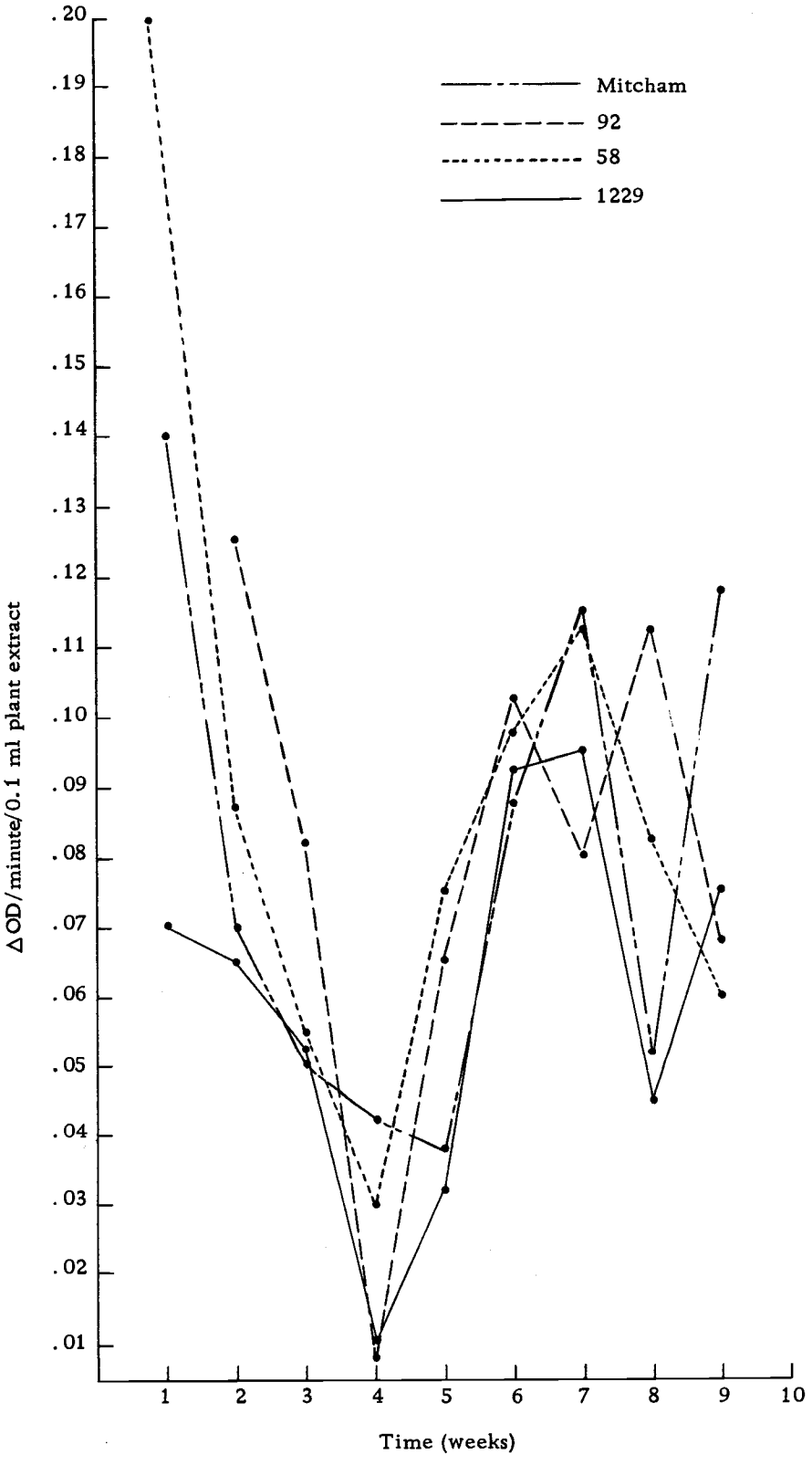

Figure 4. PPO level of healthy mint strains based on change in optical density of 0.5% catechol between 30 and 90 seconds after addition of 0.1 ml plant extract.

The level of PPO in Mitcham dropped to a low point at the fifth assay. This corresponded to the beginning of flowering in the field. Strains 92, 1229, and 58 reached the low point at the fourth assay. They also bloomed about a week earlier than Mitcham. The level of PPO then began to increase until the seventh and eighth assay, when it dropped again. At this time, flowers were well formed.

The level of PPO fluctuated markedly during this ten week period. There seemed to be no relation over the whole period of the assay between level of PPO and field resistance, although Mitcham was generally intermediate in position. PPO may be involved in resistance in combination with other factors.

The fact that PPO began to decrease at about the time of flowering indicated a possibility that plants in flower might be more susceptible to wilt than non-flowering plants or that resistance might break down if the plants were inoculated at the time of flowering. If so, this might give another reason to implicate PPO in resistance. If plants are more susceptible when in flower, it would be especially important to know this before selection of a resistant variety because a late flowering variety might be selected as having more resistance when in reality there was no difference (Berry and Thomas, 1961).

The following experiment was set up to determine if plants with buds or flowers were more susceptible to wilt than non-flowering plants and if resistance broke down among the strains upon flowering.

Ten rooted cuttings of strains 92, 1229, and Mitcham with buds or flowers were inoculated in the greenhouse by dipping the roots in a spore suspension of V. dahliae (mint strain) for 30 minutes. These plants were placed in greenhouse soil and symptom readings were made. Results are in Table 12.

Table 12. Symptom expression of cuttings with flowers inoculated with V. dahliae (mint strain).

Strain	Total no. with symptoms	Percent with symptoms	Average symptom reading
92	5/10	50	0.75
Mitcham	10/10	100	3.75
1229	9/9	100	2.87

Progression of symptoms over a five-week period is shown in Figure 5.

The earliest symptoms showed on strain 1229 six days after inoculation. Mitcham had symptoms ten days after inoculation but strain 92 showed symptoms only after 16 days. Only 50% of the plants of strain 92 showed symptoms while 100% of Mitcham and 1229 had symptoms (Table 12).

These data show that differential resistance is maintained after the cuttings have flowered. There seems to be no difference in overall susceptibility or symptom expression of these three strains when plants in flower are inoculated compared with non-flowering

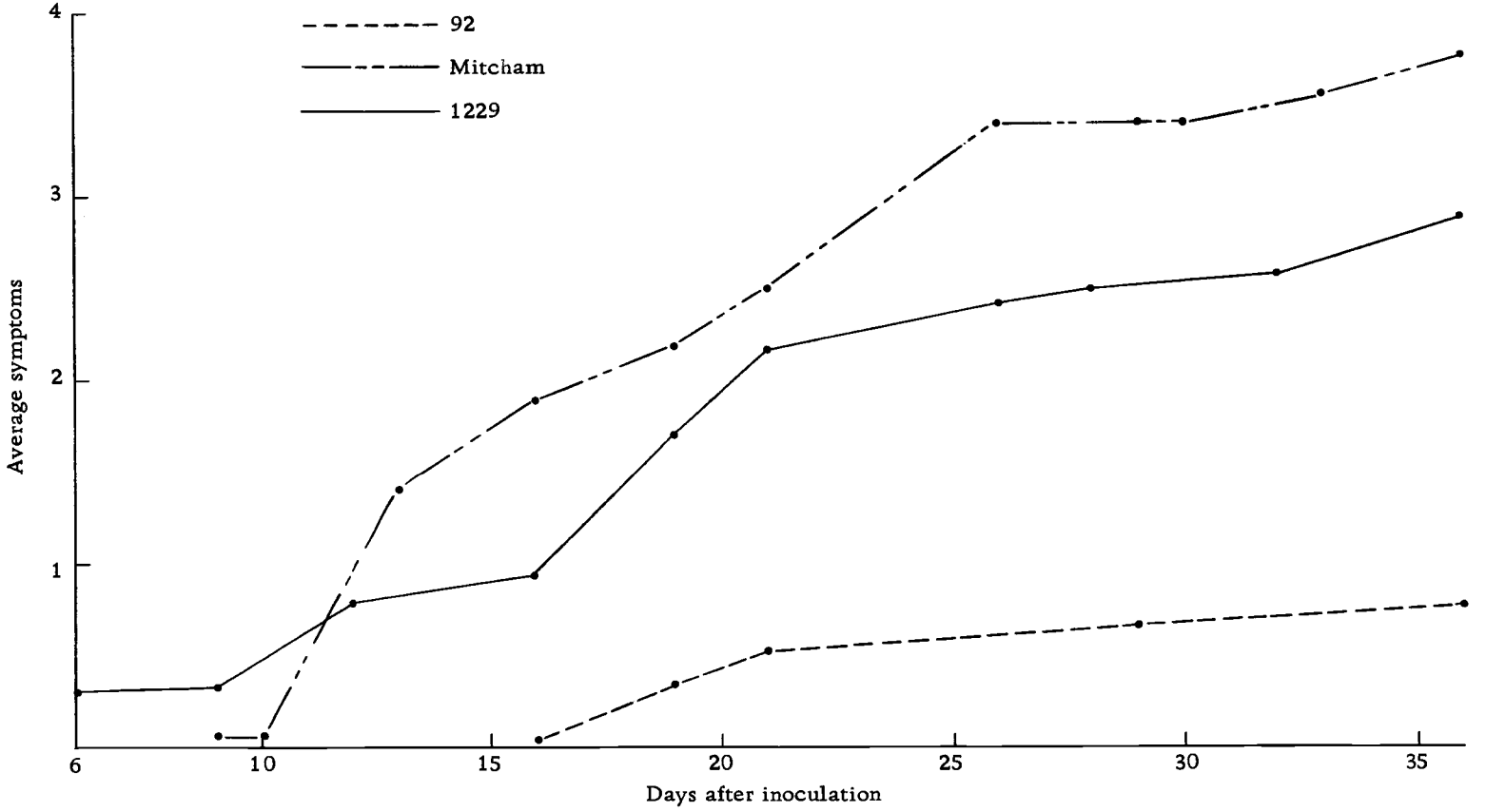

Figure 5. Symptom progression of mint cuttings with flowers inoculated with V. dahliae (mint strain).

plants. Therefore, flowering does not seem to influence susceptibility and selection of a resistant variety may be made without regard to flowering date.

Root Penetrations

Invasion of peppermint by Verticillium occurs naturally through the roots (Nelson, 1950, p. 65-69). The fungus then progresses to the xylem where it becomes established. Failure of mint plants to allow root penetration by the fungus could be an important resistance mechanism.

I wanted to determine if there were any differences in root penetrations in high, intermediate, and low resistant mint plants. Strains 92, 1229, and Mitcham were inoculated in three ways to check root penetration sites by V. dahliae. Plants were inoculated by root-dipping in a spore suspension, by planting in soil infested with conidia, or by planting in soil infested with straw on which Verticillium had been grown. Penetration sites were counted as previously described by plating root samples on ESA and counting colonies arising from these roots. Results are in Tables 13, 14, 15, and 16.

There is considerable variation in the numbers of penetration sites, possibly because of variation in moisture level or temperature in the soil. It was not practical to maintain the water-holding

Table 13. Penetration sites on mint roots inoculated with infested soil.

Strain	Level of inoculum	Days after inoculation 27	43	64	Total
92	Low (58/gm)	1.54	0	0	1.54
	High (244/gm)	5.72	18.58	23.34	47.64
Mitcham	Low	29.2	0	10.86	40.06
	High	34.93	10.87	0	45.80
1229	Low	0	2.25	1.74	3.99
	High	84.4	0	5.70	90.1

Table 14. Penetration sites on mint roots inoculated by root-dipping in a spore suspension.

Strain	Level of inoculum	Days after inoculation 8	23	Total
92	Low (1 million/ml)	4.0	134.57	138.57
	High (10 million/ml)	5.5	47.61	53.11
Mitcham	Low	4.5	20.0	24.5
	High	0	7.65	7.65
1229	Low	2.5	85.71	88.21
	High	7.0	0	7.0

Table 15. Penetration sites on mint roots inoculated with infested soil.

Strain	Level of inoculum	Days after inoculation 14	21	28	42	Total
92	Low (1x)	0	0	0	0	0
	High (10x)	83.33	123.0	41.0	3.12	250.45
Mitcham	Low	0	0	0	0	0
	High	62.97	79.04	9.04	0	151.05
1229	Low	0	0	0	0	0
	High	130.36	17.5	29.64	0	167.50

Table 16. Penetration sites on mint roots inoculated with infested straw.

Strain	Level of inoculum	Days after inoculation 14	24	29	52	Total
92	Low (1x)	0	0	0	0	0
	High (10x)	65.02	4.0	0	4.0	73.02
Mitcham	Low	0	0	0	0	0
	High	13.65	0	5.08	26.0	44.73
1229	Low	0	0	1.36	0	1.36
	High	16.8	0	0	34.0	50.8

capacity or soil temperature at a constant level. For these data to be conclusive, environmental factors should be rigidly controlled.

It does seem, however, that there is no relation between number of penetrations and resistance. The data in Table 13, for example, show that strain 92 and Mitcham had about the same number of total penetrations at the high level of inoculum. Strain 1229 had almost twice as many. In some cases, strain 92 had more penetrations than Mitcham or 1229.

Propagule Assays

After the roots of mint plants are penetrated by the fungus, invasion of the xylem occurs. I wanted to determine the relative amount of fungus present in the roots and stems of inoculated mint plants. This would give information on development of the fungus within the plants. The amount of fungus could be related to symptom expression and this in turn could be related to overall resistance shown by the mint strains.

Experiment One

Plants of 92, Mitcham, and 1229 were inoculated by root-dipping in a spore suspension of *V. dahliae*, isolates Mp-3 and C-6. Isolate Mp-3 is a mint isolate and C-6 is one from cotton. Propagule numbers in inoculated plants were estimated by grinding stems and

roots of plants in an Omni-Mixer, making dilutions and plating on ESA, and counting colonies as previously described. Propagule numbers are shown in Table 17.

These data are graphed on a log scale in Figures 6 and 7. This shows the relation among the three mint strains when inoculated with two different Verticillium isolates. When inoculated with isolate C-6, strains 92 and 1229 had considerably fewer propagules than Mitcham after about two weeks. After inoculation with isolate Mp-3, Mitcham showed a large amount of fungus in the stems. Strains 92 and 1229 had fewer propagules.

Assays of root systems (Table 17) show that Mitcham was heavily invaded by isolate Mp-3 but only moderately invaded by isolate C-6. Strain 92 showed moderate numbers of root propagules of isolate C-6 and less than Mp-3. Strain 1229 had about the same amount of fungus in the roots at 35 days after inoculation when inoculated with either C-6 or Mp-3.

Symptom development for plants in this experiment is graphed in Figure 8. Strains 92 and 1229 showed no symptoms after inoculation with isolate C-6. Symptoms were light in 92 inoculated with Mp-3 and light to moderate in 1229. After 30 days, plants of strain 1229 began to recover from the symptoms. Mitcham showed moderate to severe symptoms when inoculated with either C-6 or Mp-3. Severe symptom development occurred about ten days earlier with

Table 17. Propagules of V. dahliae in stems and roots of inoculated mint plants.

Time after inoculation (days)	Plant part	Isolate, strain C-6, 92	C-6, M	C-6, 1229	Mp-3, 92	Mp-3, M	Mp-3, 1229
1	stems	9633	10,867	8850	1300	817	1017
3	stems	3917	6067	15,400	1300	4450	600
7	stems	2567	2417	4233	1167	2050	5383
21	stems	1000	3033	0	217	50,000	1333
	roots	513	767	1333	360	27,660	5800
35	stems	100	5225	0	5800	65,000	800
	roots	550	3700	367	0	2933	334

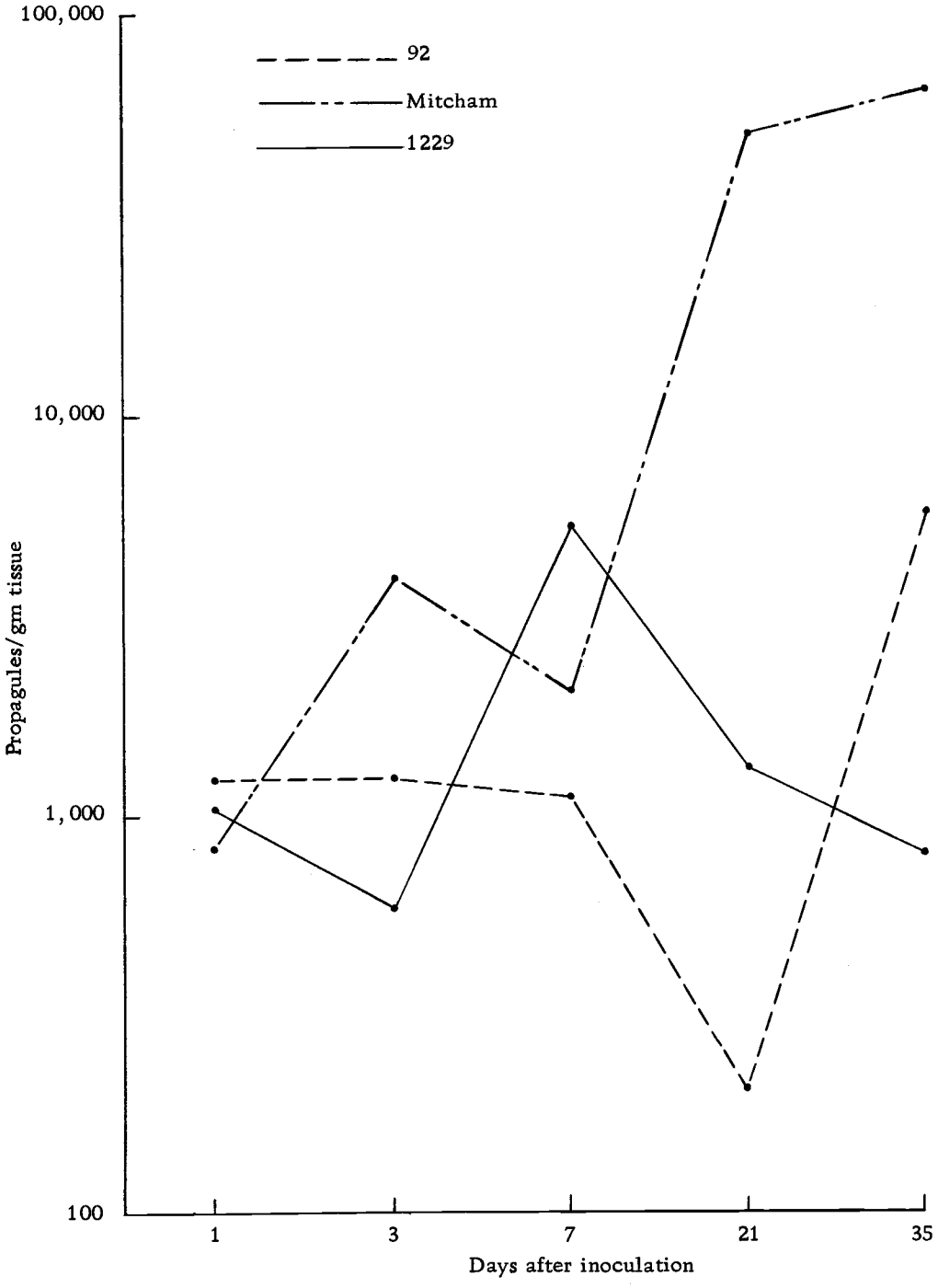

Figure 6. Propagules of V. dahliae (Mp-3) in stems of inoculated mint plants.

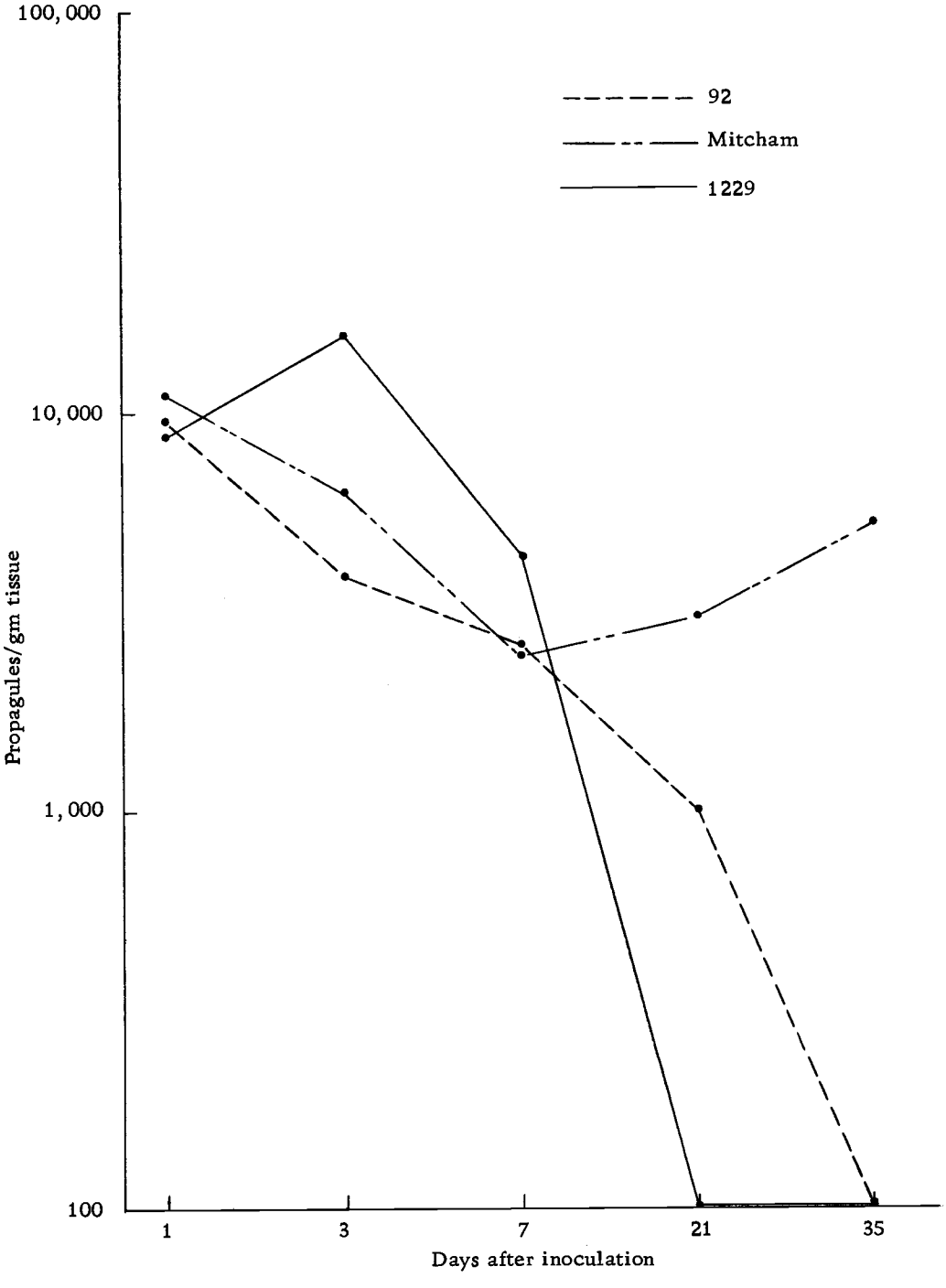

Figure 7. Propagules of V. dahliae (C-6) in stems of inoculated mint plants.

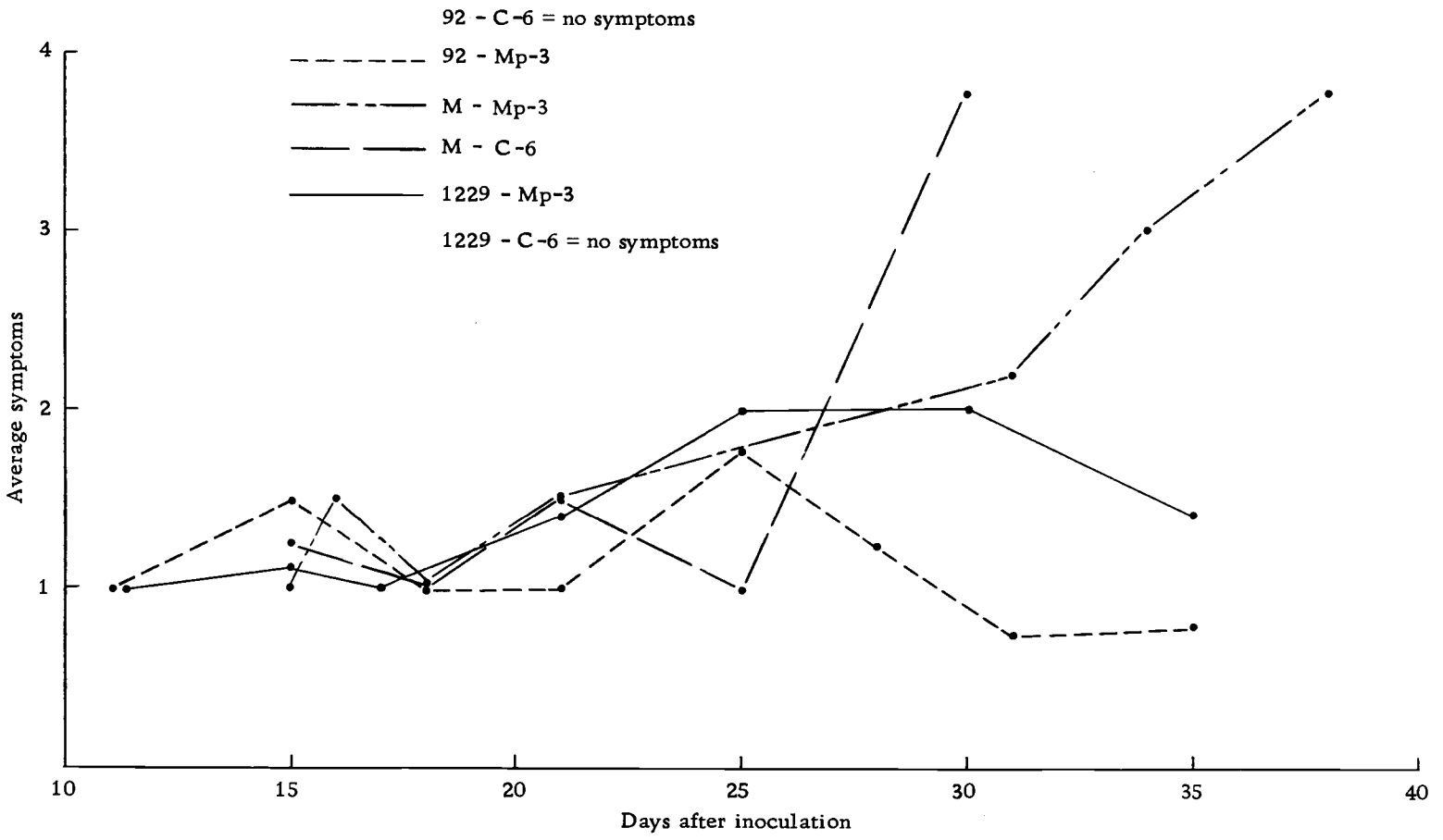

Figure 8. Symptoms of mint plants inoculated with mint (Mp-3) and cotton (C-6) isolate of V. dahliae.

isolate C-6.

Experiment Two

Plants in this experiment were inoculated by root-dipping in a spore suspension of isolate Mp-3. Propagules in stems and roots are shown in Table 18. The data are graphed in Figure 9.

Stem propagules of strains 1229 and 92 were highest at one day and dropped to a low point after 14 days. Propagules in strain 1229 began to increase before 42 days but never reached the high numbers shown by Mitcham. Numbers of propagules fluctuated in Mitcham but after three days, were always higher than propagules in 92 or 1229.

Root propagules of strain 92 and 1229 were higher at seven days than at 28 days after inoculation but root propagules of Mitcham were highest at 28 days.

Symptoms of inoculated plants in this experiment are graphed in Figure 10. Symptoms were light to moderate for all strains, but Mitcham showed the most severe symptom development. Symptoms of 1229 began to increase four weeks after inoculation. This corresponded to the time of increase of stem propagules.

Experiment Three

Plants of 92, 1229, and Mitcham were inoculated by root-dipping in a spore suspension of V. dahliae (Mp-3) adjusted to

Table 18. Propagules of V. dahliae (Mp-3) in stems and roots of mint plants inoculated with 100,000 spores/ml.

Strain	Time after inoculation	Propagules/gm stem	Propagules/gm root
92	1 day	14,433	--
	3 days	9983	--
	7 days	1633	5900
	14 days	100	5050
	28 days	0	550
	42 days	0	0
Mitcham	1 day	7283	--
	3 days	22,966	--
	7 days	3183	12,150
	14 days	3683	22,800
	28 days	29,334	24,667
	42 days	12,934	--
1229	1 day	9466	--
	3 days	3400	--
	7 days	267	12,616
	14 days	33	3633
	28 days	0	1300
	42 days	2200	--

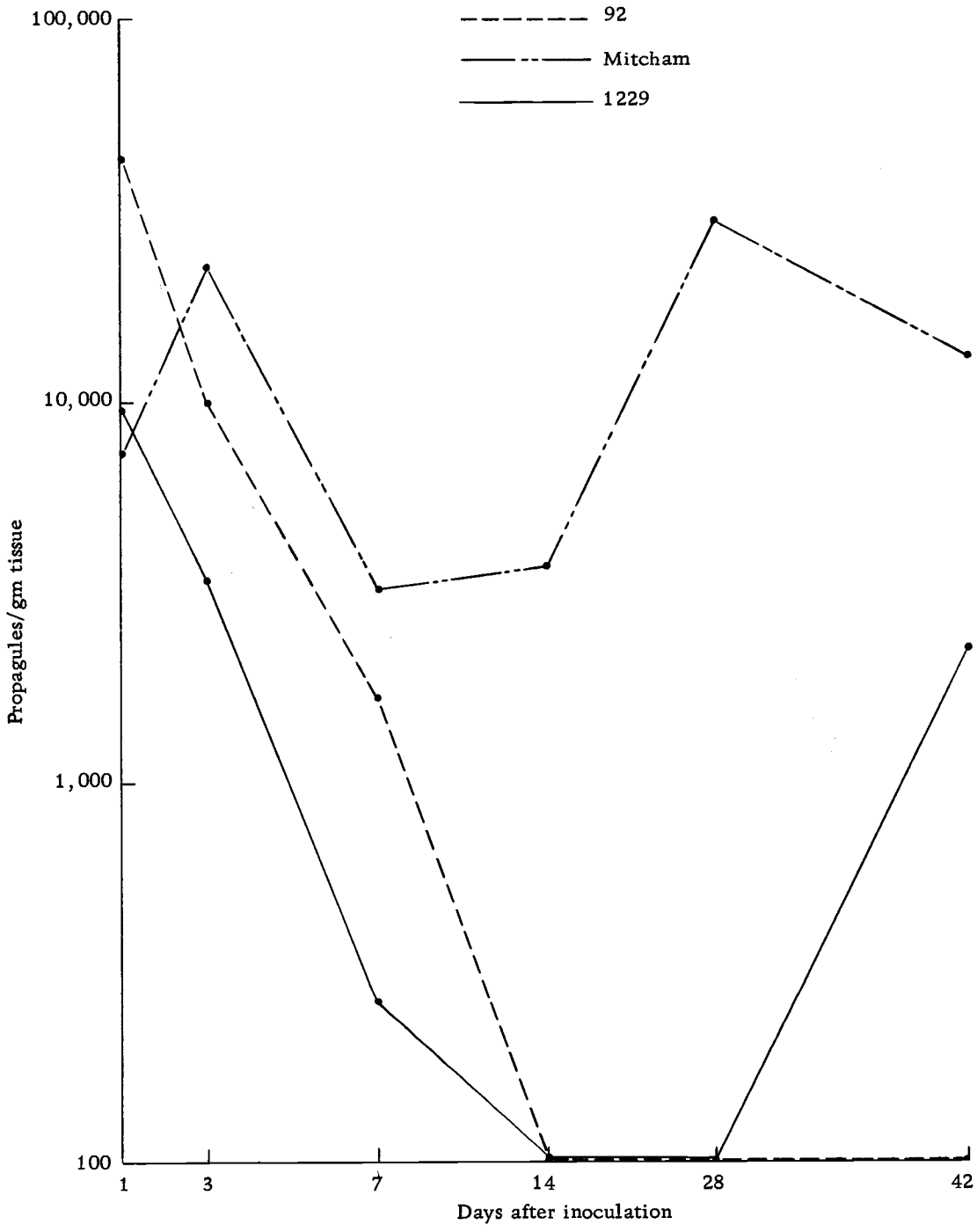

Figure 9. Propagules of V. dahliae (Mp-3) in stems of mint plants inoculated with 100,000 spores/ml.

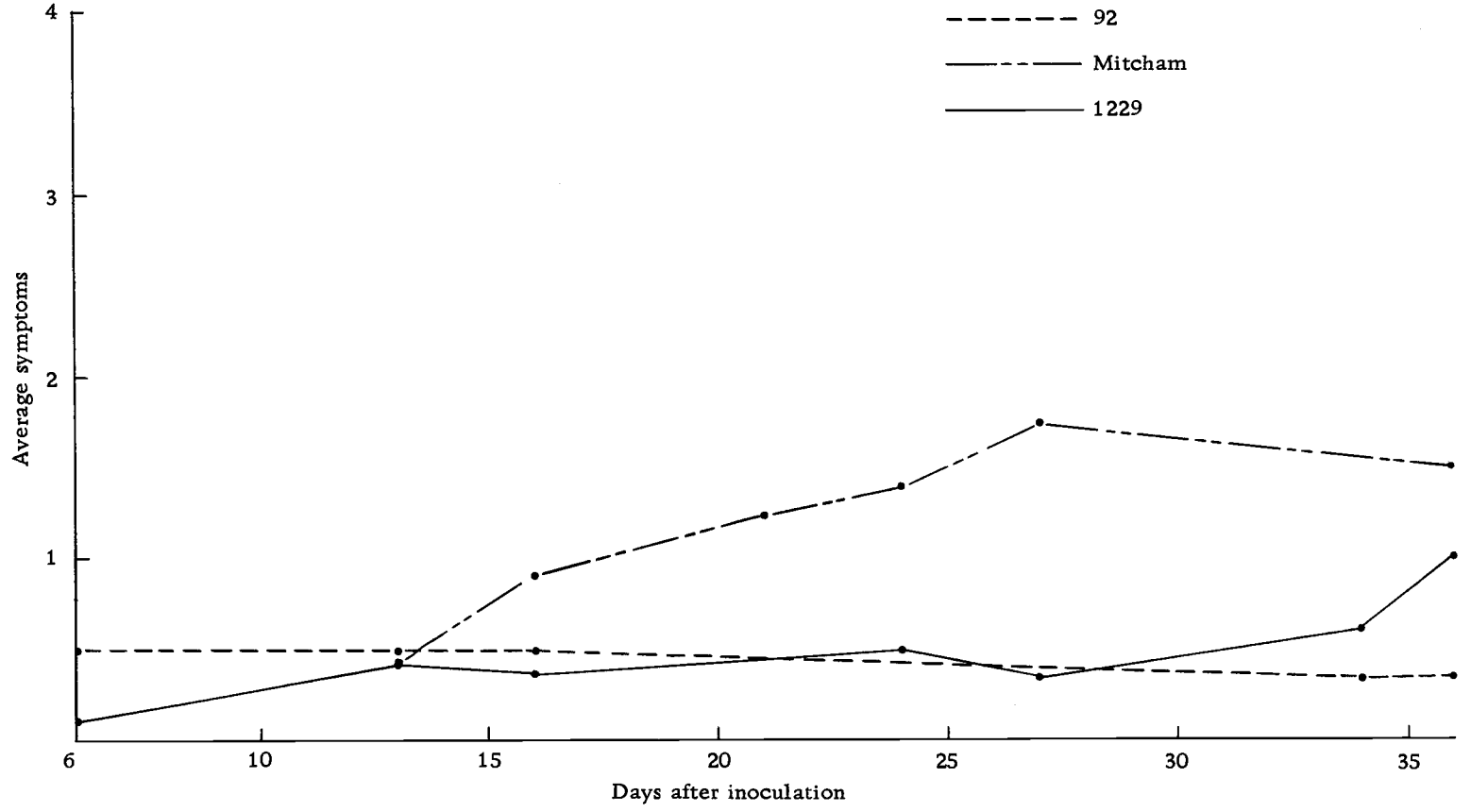

Figure 10. Symptoms of mint plants inoculated by root dipping in a spore suspension of V. dahliae (Mp-3).

three concentrations. The concentrations were: 475, 000 spores/ml (High); 47, 500 spores/ml (Medium); 4750 spores/ml (Low). This was to determine if initial inoculum concentration influenced symptom development and propagule numbers and if the differential inoculum potential would be maintained. Results of the stem propagule assay are shown in Table 19 and Figures 11, 12, and 13.

Mitcham had more propagules at all levels of inoculum than did 92 or 1229. There did not seem to be any relation between initial level of inoculum and numbers of propagules in the stems of any of the strains. The fungus eventually disappeared from the stems of 92 and 1229 but did not from Mitcham regardless of the initial spore concentration.

Symptom development of inoculated plants in this experiment is shown in Figures 14, 15, and 16.

Strain 92 showed no symptoms with the low level of inoculum and symptoms were light at the other two levels. Symptoms began much later in 92 than in Mitcham or 1229, and the plants completely recovered after 30-37 days. After 42 days, strain 1229 showed no symptoms. Mitcham began to show symptoms ten days after inoculation with all levels of inoculum. Symptoms were light to moderate but generally were more severe than symptoms on 1229 or 92.

Table 19. Stem propagules in mint plants inoculated with three concentrations of V. dahliae (Mp-3).

Inoculum level	Strain	Time after inoculation days	Propagules/gm tissue	Inoculum level	Strain	Time after inoculation days	Propagules/gm tissue
High	92	1	2,600	High	1229	1	3,333
		3	7,050			3	7,083
		7	3,500			7	1,900
		28	0			28	167
		35	0			35	0
		42	0			42	0
Medium	92	1	10,350	Medium	1229	1	2,900
		3	3,450			3	9,313
		7	1,466			7	4,150
		28	0			28	0
		35	0			35	0
		42	0			42	0
Low	92	1	1,666	Low	1229	1	466
		3	2,633			3	2,500
		7	500			7	300
		28	0			28	0
		35	0			35	0
		42	0			42	0
High	Mitcham	1	11,700				
		3	27,600				
		7	14,213				
		28	666				
		35	2,500				
		42	1,133				
Medium	Mitcham	1	12,113				
		3	21,133				
		7	20,566				
		28	300				
		35	6,000				
		42	400				
Low	Mitcham	1	267				
		3	21,450				
		7	9,600				
		28	3,750				
		35	15,333				
		42	1,300				

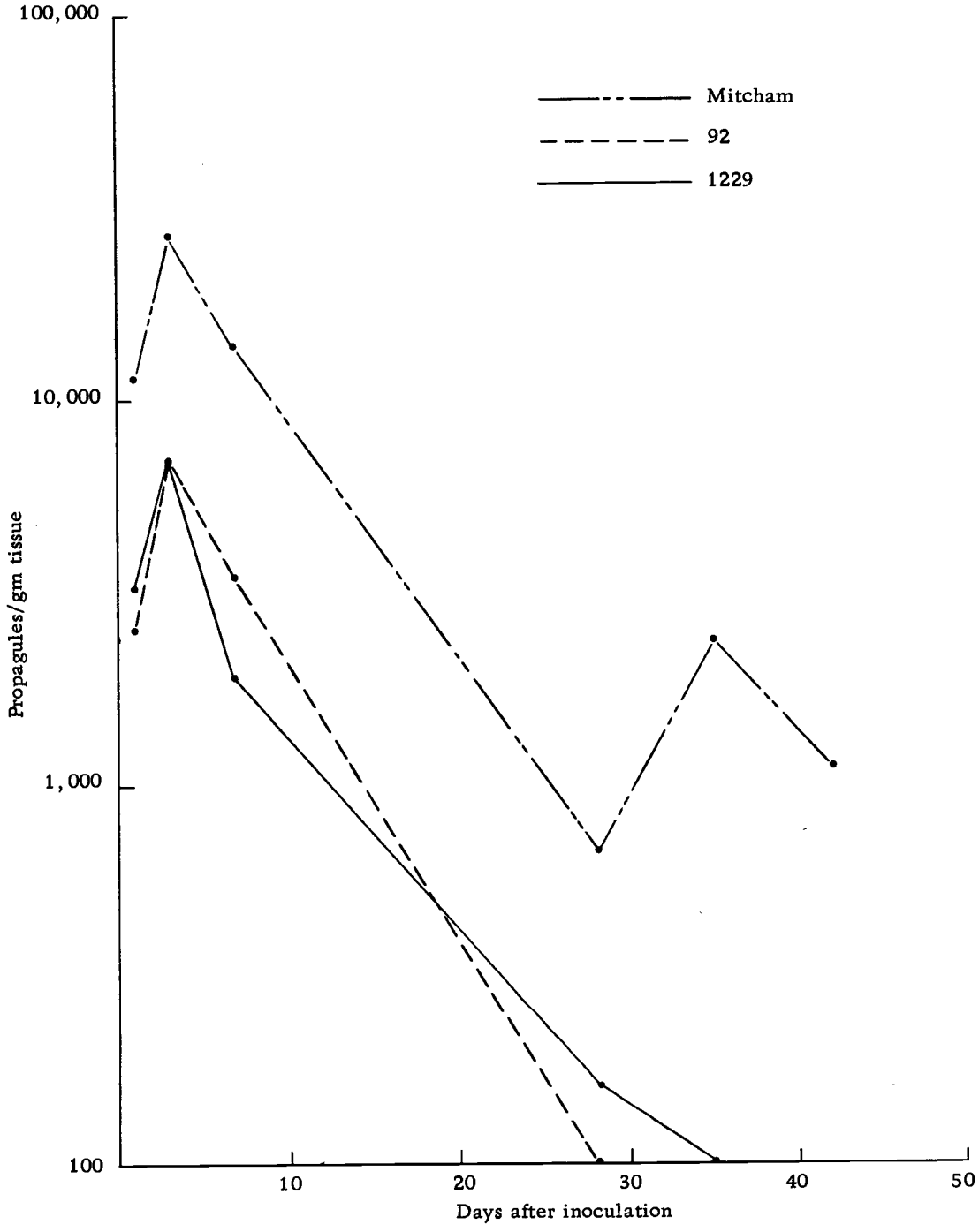

Figure 11. Propagules of V. dahliae (Mp-3) in stems of mint strains inoculated with 475,000 spores/ml.

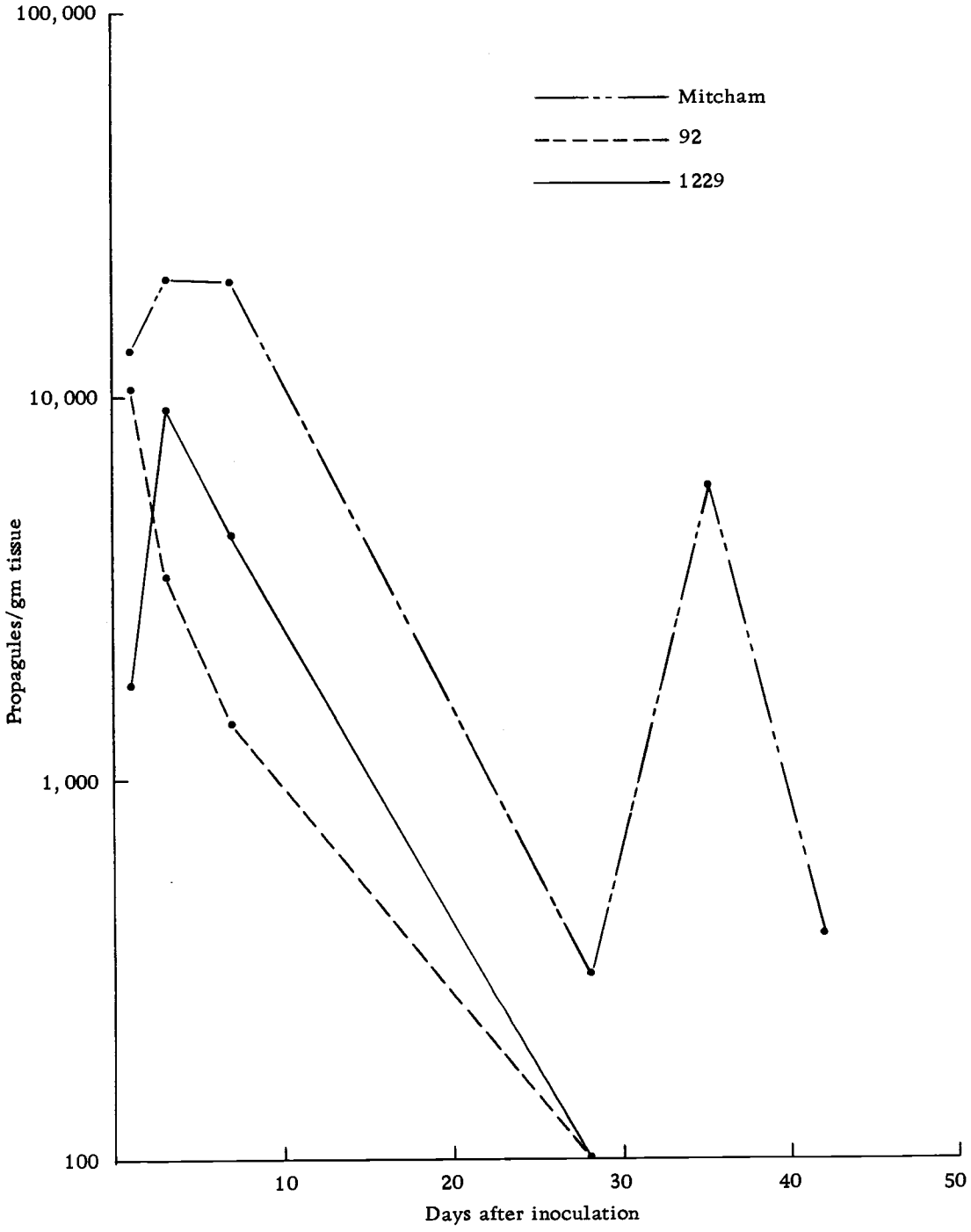

Figure 12. Propagules of V. dahliae (Mp-3) in stems of mint strains inoculated with 47,500 spores/ml.

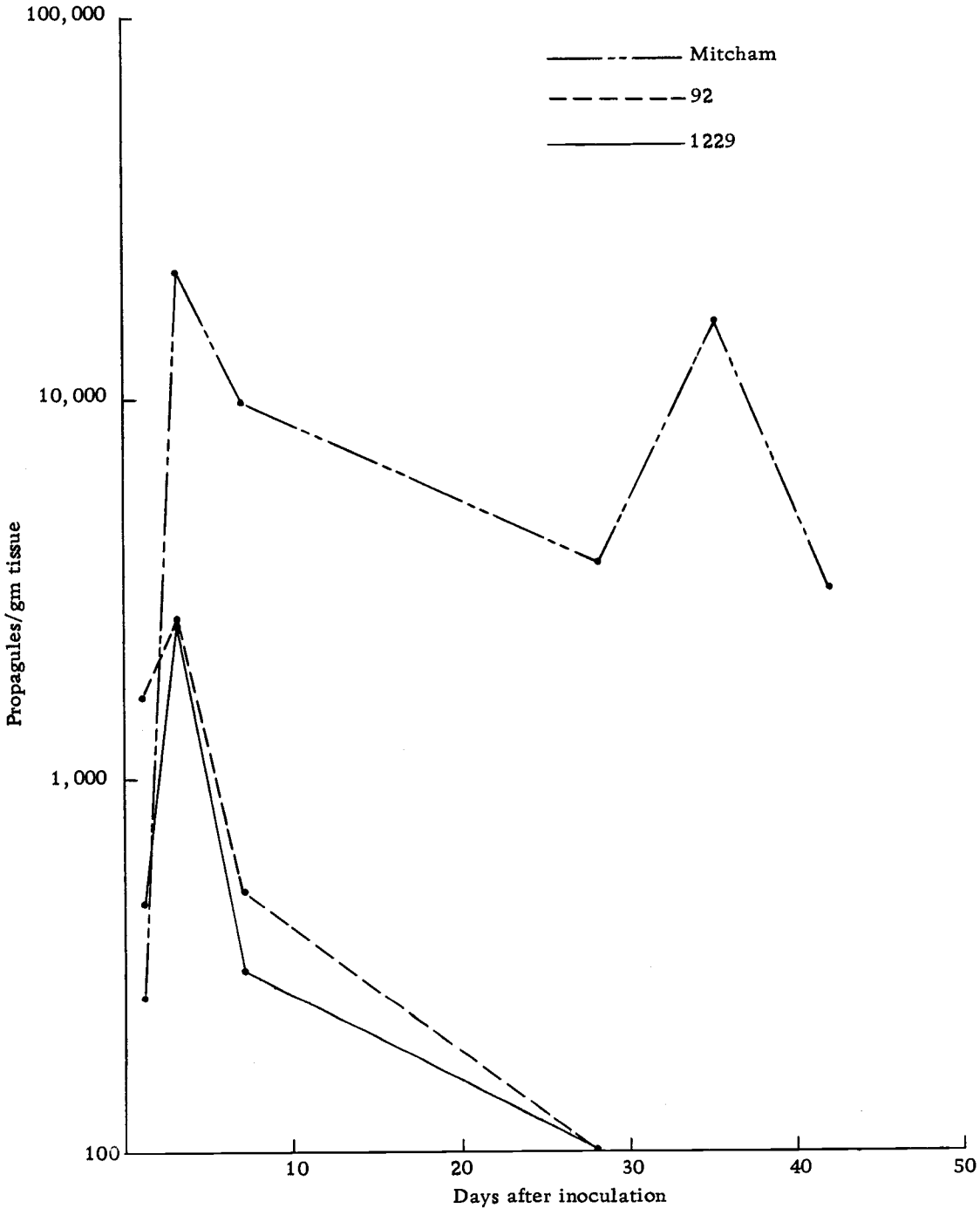

Figure 13. Propagules of V. dahliae (Mp-3) in stems of mint strains inoculated with 4,750 spores/ml.

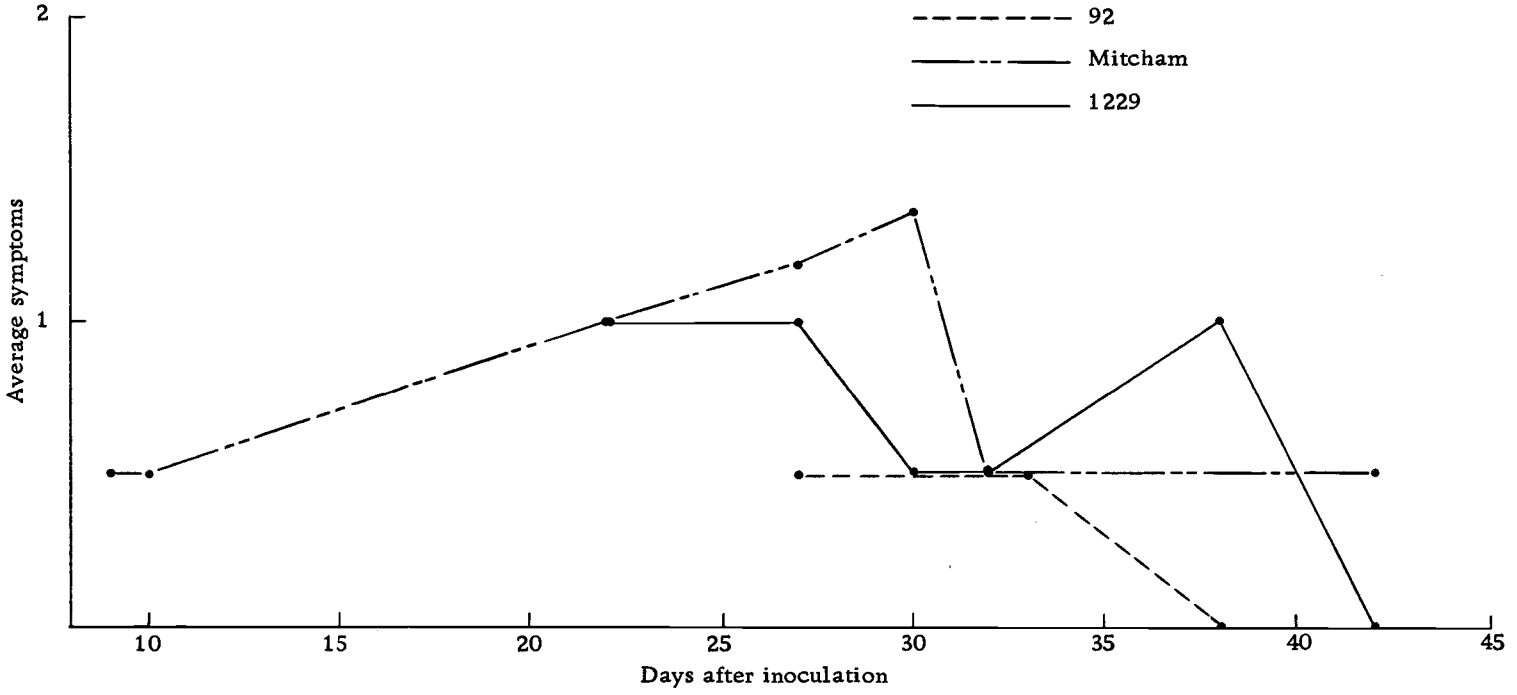

Figure 14. Symptom development of mint strains inoculated with 475,000 spores/ml of V. dahliae (Mp-3).

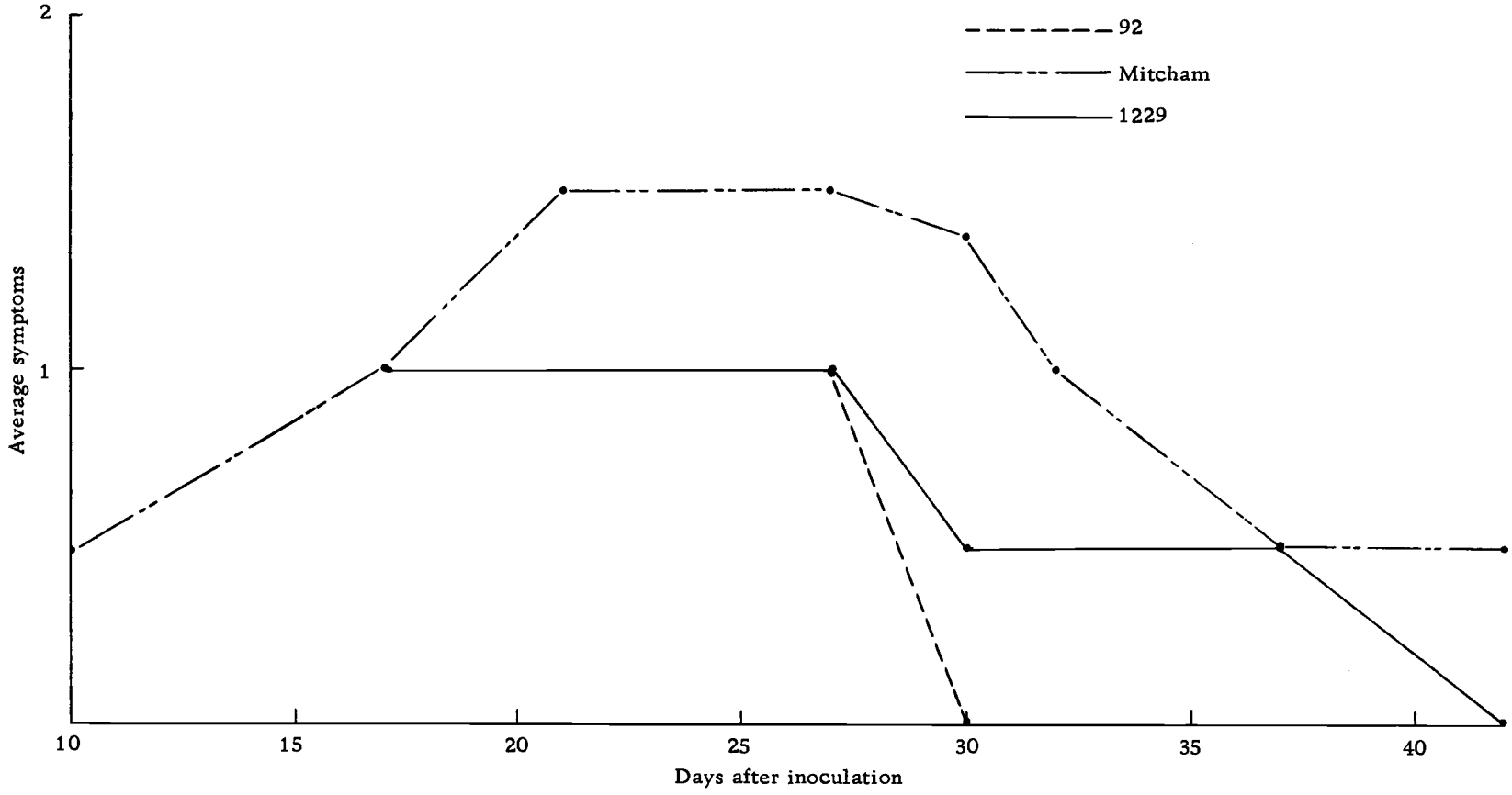

Figure 15. Symptom development of mint strains inoculated with 47,500 spores/ml of V. dahliae (Mp-3).

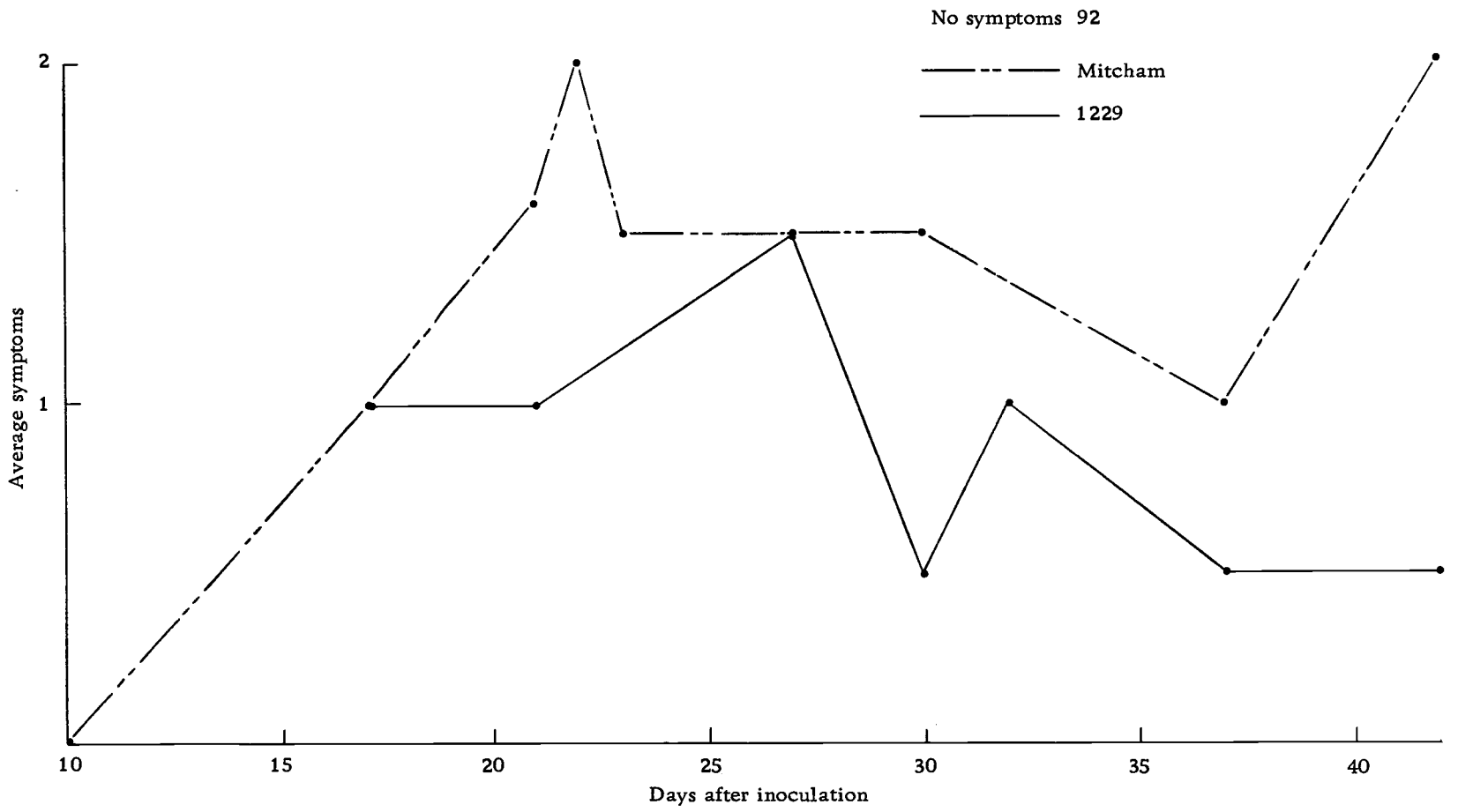

Figure 16. Symptom development of mint strains inoculated with 4,750 spores/ml. of V. dahliae (Mp-3).

Relation Between Symptoms at the Time of Assay and Propagule Numbers

Plants used in the preceding experiments were kept separate when assaying propagules, thus symptom development at the time of assay could be related to propagules/gm tissue. These results are in Table 20. These data were taken four to six weeks after inoculation to insure that symptoms were well developed.

There did not seem to be any relation between symptoms and propagule numbers. In Mitcham, symptoms ranged from 0.5-4.0. There were from 0 to over 50,000 propagules/gm tissue. Several plants of both Mitcham and 1229 showed light to moderate symptoms but no propagules could be detected at a 1:100 dilution. On the other hand, some plants of Mitcham with light to moderate symptoms had high numbers of propagules. This indicates that propagule numbers are more valuable in assessing fungus development in the host than is symptom development.

Stem Puncture Inoculation

Inoculation by stem puncture was used to bypass the root system to determine the role of the stem in resistance. A spore suspension (one million spores/ml) was drawn into a one ml hypodermic syringe. A drop was pushed out onto the tip of the needle and the

Table 20. Relation between symptoms at time of assay and propagule numbers in the stem of inoculated mint plants.

Strain	Symptoms	Propagules/gm	Strain	Symptoms	Propagules/gm
92	1.0	12,000	1229	1.0	100
	0.5	2,000		1.0	100
	0.5	3,400		1.0	0
	1.5	3,000		1.0	0
	1.0	0		3.0	2,200
Mitcham	1.5	62,000		1.0	500
	1.0	54,000		0.5	0
	1.5	50,000		0.5	0
	3.5	55,000		0.5	0
	3.0	84,000		1.0	0
	4.0	56,000		0.5	0
	1.0	5,500		1.0	0
	0.5	4,400		1.5	0
	0.5	1,600		1.5	0
	2.0	37,000		1.0	0
	2.0	24,000		0.5	0
	2.0	27,000		0.5	0
	0.5	0			
	4.0	38,000			
	0	800			
	1.0	200			
	0.5	0			
	1.5	1,800			
	0.5	0			
	2.0	7,500			
	2.0	0			
	1.0	3,400			
	2.0	500			
	0.5	400			
	1.5	0			
	2.0	16,000			
	0.5	0			
	1.0	200			
	0.5	1,000			
	1.0	0			
	0.5	200			
	2.0	850			
	2.0	10,400			
	1.5	0			
	2.0	13,000			
	2.0	31,000			
	2.0	0			
	1.0	210			
	1.0	3,700			

stem of the mint plant was pricked below the first node. Each stem was inoculated in two places. When the stem was pricked, the spore suspension was drawn into the plant by capillary action.

In one experiment, plants were inoculated with isolates C-6 and Mp-3. In the other experiment, only isolate Mp-3 was used. Results of stem propagule assays are in Tables 21 and 22 and Figures 17, 18, and 19.

Propagules in strain 92 were generally fewer than in Mitcham or 1229. Numbers of propagules were not as high, even in Mitcham, as when other methods of inoculation were used. This indicates that the stems of all strains have some resistance to both isolates of Verticillium. Symptoms in 92 were light in both experiments and moderate in Mitcham and 1229.

Roots of these plants were assayed at the same time as stems, but in no case, were propagules found. This shows that the fungus does not move down into the root system if it is originally introduced into the stem.

Shoot Inoculation

Shoots without roots were inoculated to provide another means of bypassing the root system. The cut ends of three-to-four-inch shoots were immersed in a spore suspension and placed in sand. Isolate Mp-3 was used to inoculate the plants. Table 23 shows the

Table 21. Propagules of V. dahliae (C-6 and Mp-3) in stems of mint plants inoculated by stem puncture.

Time after inoculation	Strain - isolate					
	92-C-6	92-Mp-3	M-C-6	M-Mp-3	1229-C-6	1229-Mp-3
7 days	1650	650	2100	4450	2550	950
21 days	50	200	50	325	50	50
35 days	100	0	0	650	50	250

Table 22. Propagules of V. dahliae (Mp-3) in stems of mint plants inoculated by stem puncture.

Time after inoculation	Strain		
	92	Mitcham	1229
1 day	916	7300	1567
3 days	733	1267	67
7 days	2300	317	3400
14 days	333	217	300
28 days	0	717	0
42 days	0	0	100

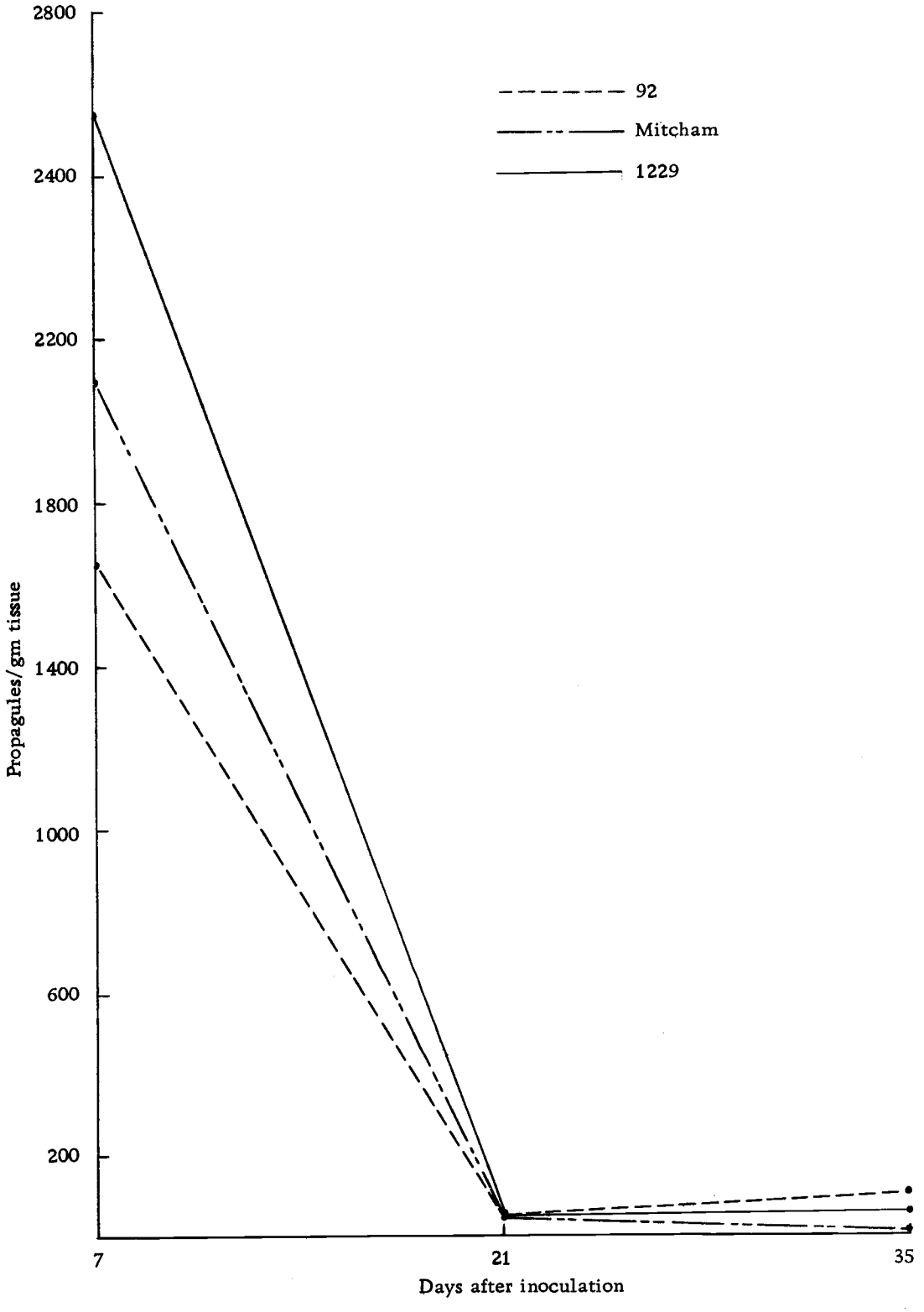

Figure 17. Propagules of V. dahliae (C-6) in stems of mint strains inoculated by stem puncture.

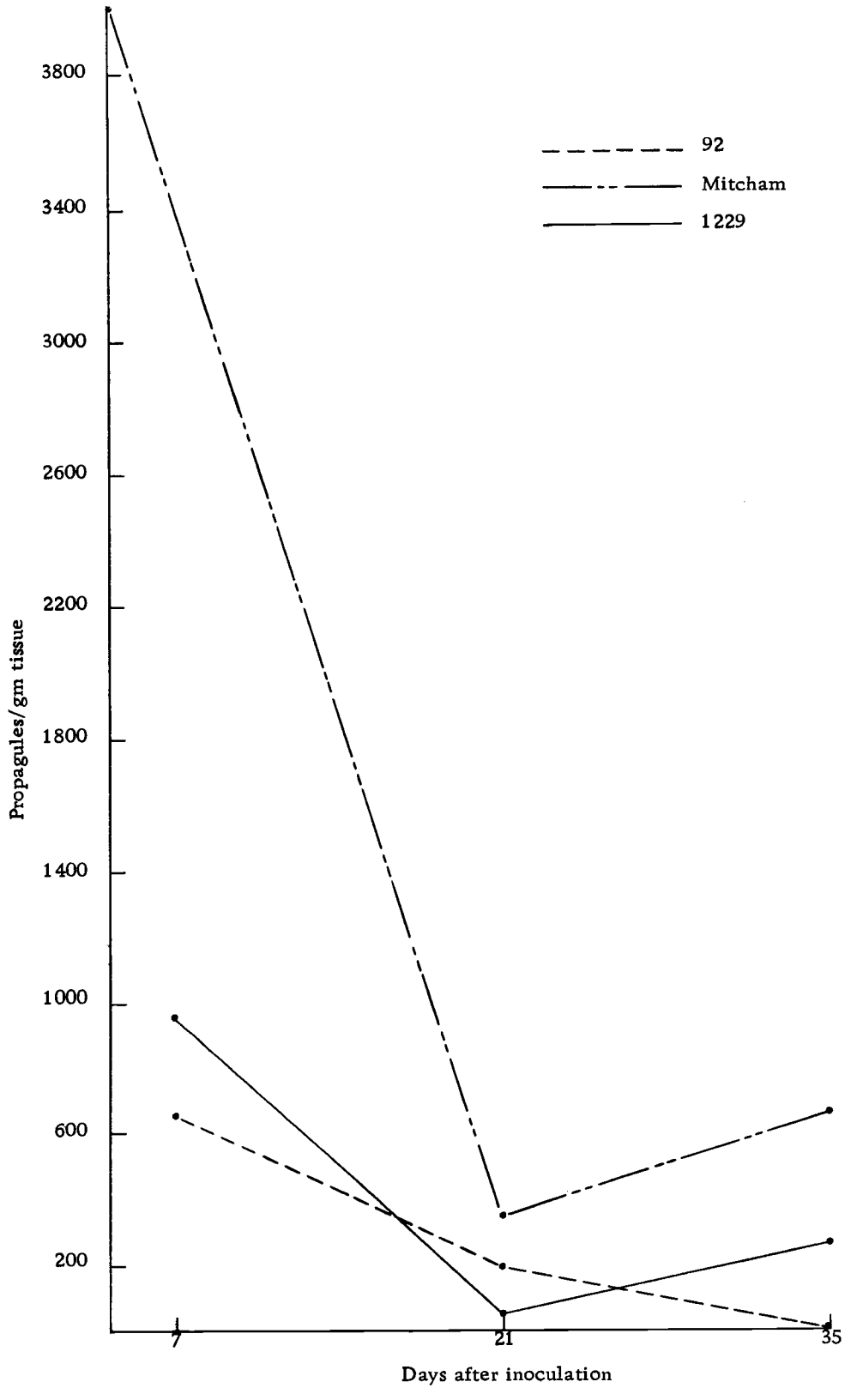

Figure 18. Propagules of V. dahliae (Mp-3) in stems of mint strains inoculated by stem puncture.

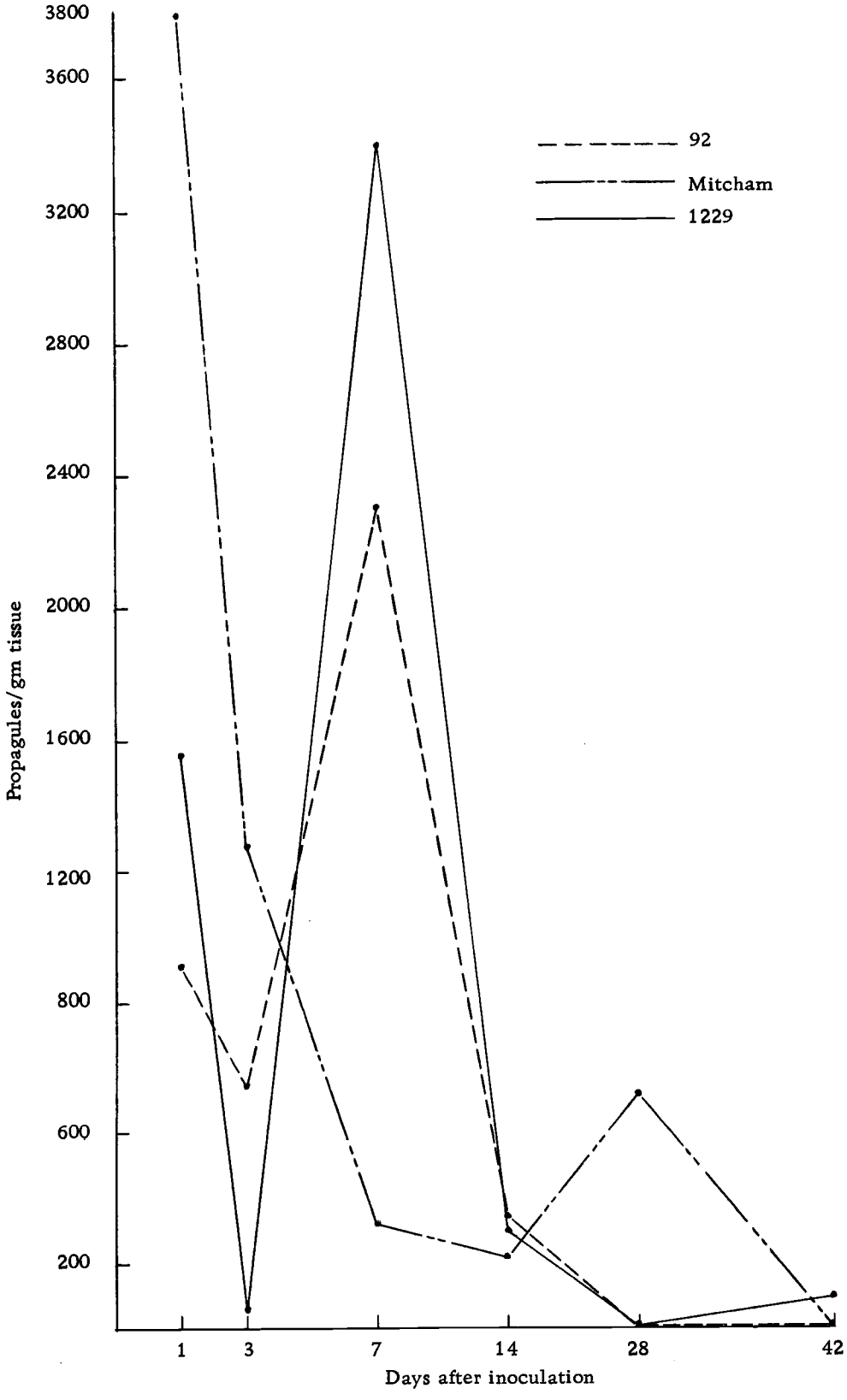

Figure 19. Propagules of V. dahliae (Mp-3) in stems of mint strains inoculated by stem puncture.

Table 23. Propagules of V. dahliae (Mp-3) in stems of mint strains inoculated through stems without roots.

Time after inoculation[a]	Strain 92	Mitcham	1229
1 day	43,300	47,666	16,150
5 days	17,533	35,333	14,450
7 days	21,833	43,666	8600
14 days	47,000	50,100	6900
28 days	26,000	56,666	23,013
42 days	56,666	35,000	34,000
Time after inoculation[b]			
1 day	4500	1913	866
3 days	7950	4413	3100
7 days	13,900	27,300	8950
28 days	46,000	21,133	3800
35 days	40,000	30,613	6013
42 days	43,000	1900	1166

[a]Inoculum = 1,000,000 spores/ml

[b]Inoculum = 4750 spores/ml

number of propagules in the stems of inoculated plants. The results are shown graphically in Figures 20 and 21.

Resistance to increase of the pathogen in stems was not evident with this method of inoculation. Strain 92 had as many or more propagules as Mitcham and 1229. The original level of inoculum seemed more important in determining propagules/gm of tissue than in the preceding experiments. There were generally more propagules in the plants inoculated with 1,000,000 spores/ml than with 4750 spores/ml.

Symptoms of plants inoculated through shoots are shown graphically in Figure 22.

Symptoms on all strains ranged from light to moderate. After nine days, symptoms were visible on all three strains. Strain 92 showed no symptoms after 37 days, even though propagules remained high. Symptoms of Mitcham were generally more severe than those of 1229 and 92.

Cross-Protection Experiment

I wanted to determine if cross-protection occurred in mint plants. If so, this would give more information on the nature of resistance to *Verticillium* wilt.

Earlier experiments showed that isolate C-6 of *V. dahliae* from cotton caused only mild symptoms in Mitcham peppermint

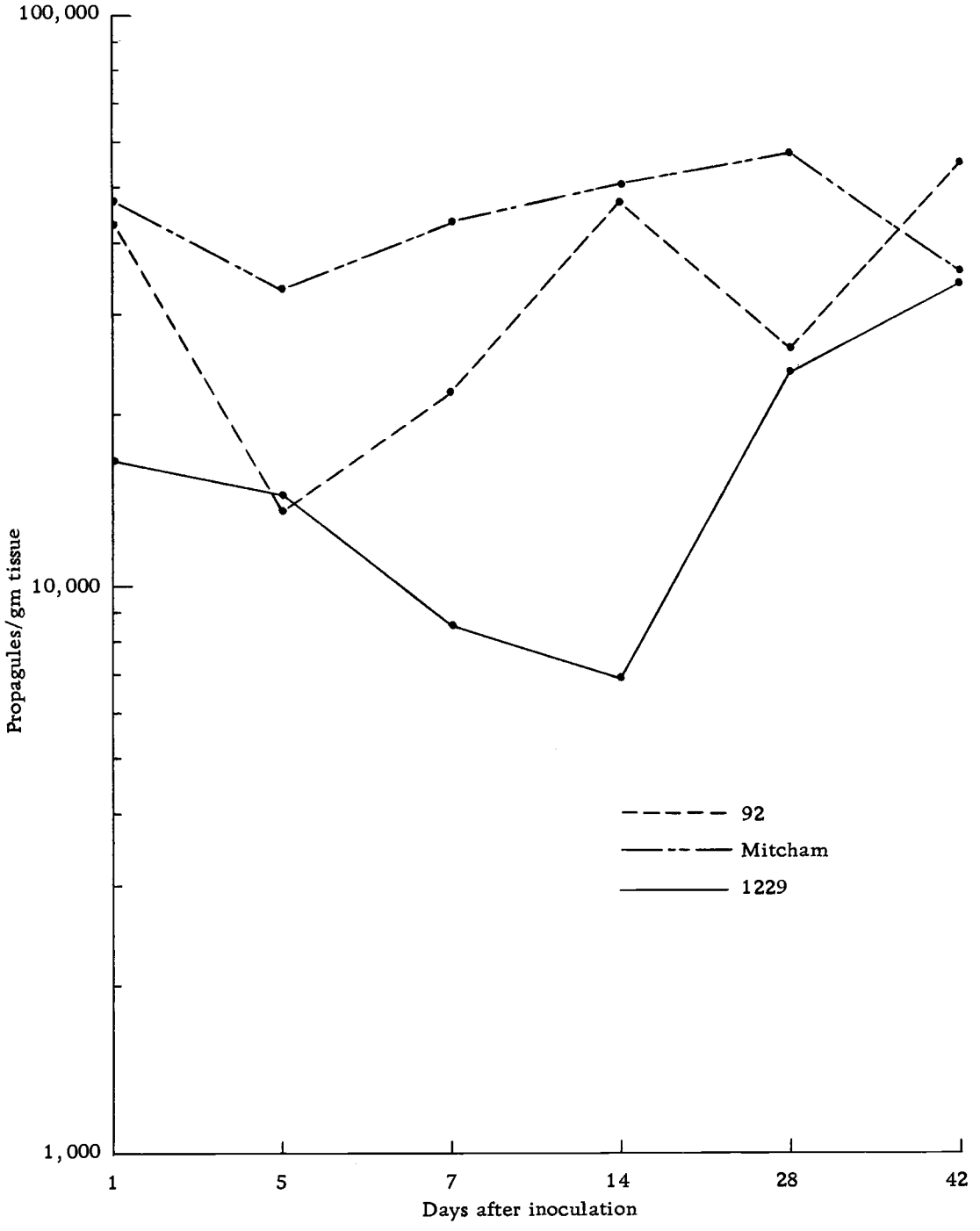

Figure 20. Propagules of V. dahliae (Mp-3) in stems of mint strains inoculated through stems without roots (inoculum = 1,000,000 spores/ml).

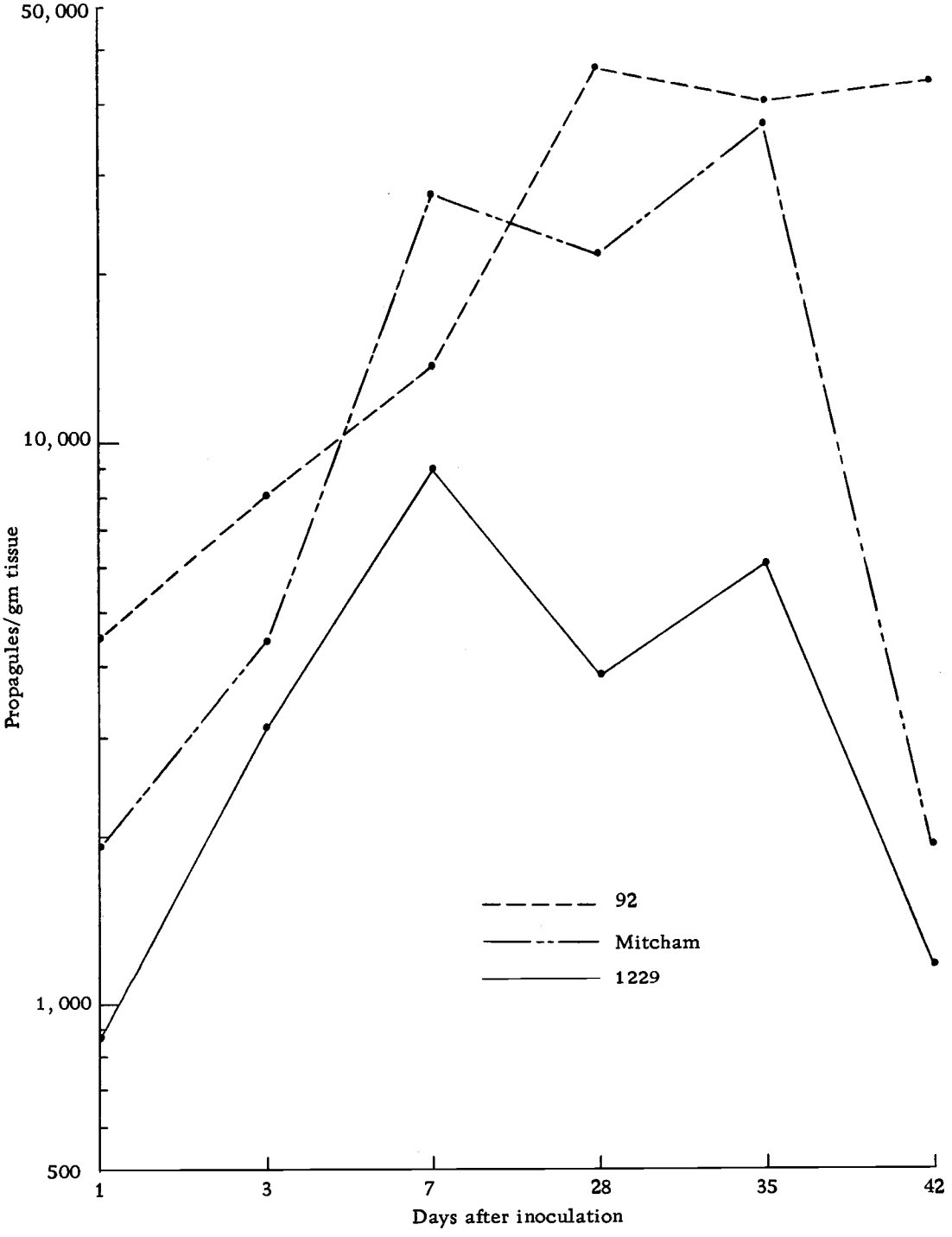

Figure 21. Propagules of V. dahliae (Mp-3) in stems of mint strains inoculated through stems without roots (inoculum = 4,750 spores/ml).

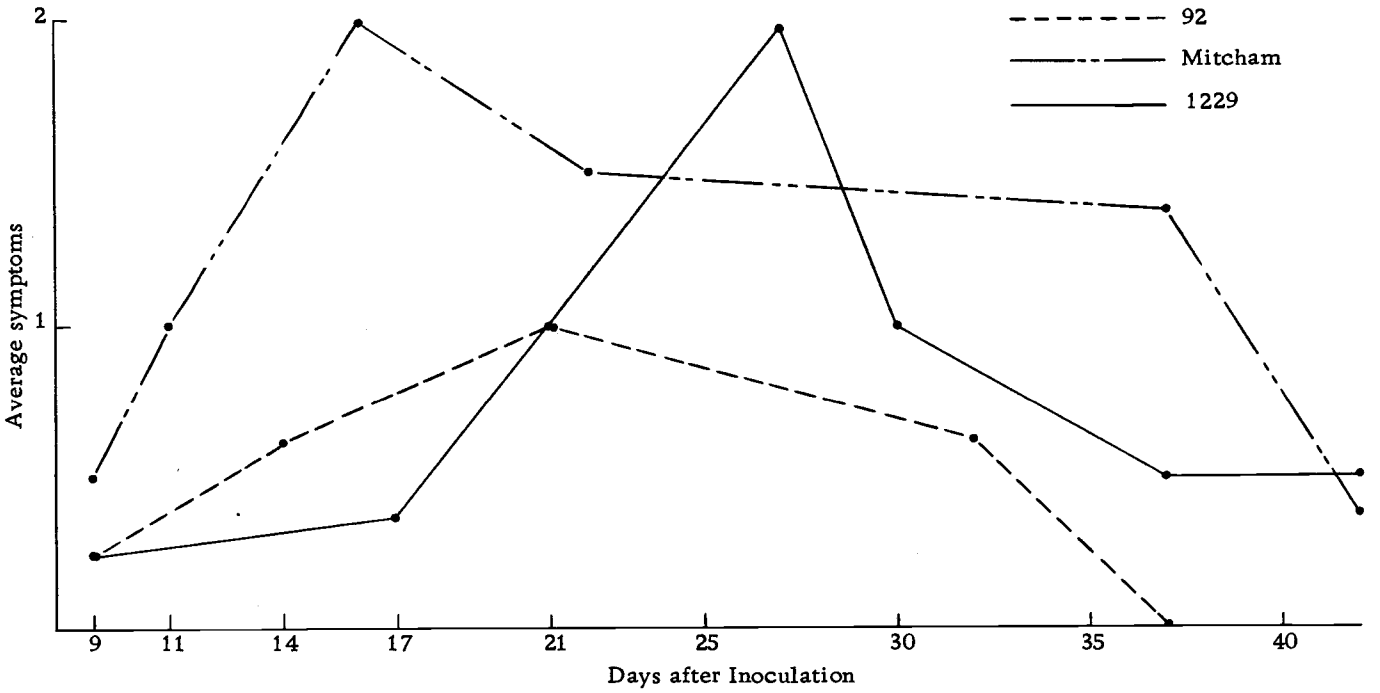

Figure 22. Symptoms of mint plants inoculated through shoots without roots.

plants. The plants eventually recovered. Isolate Mp-3 of V. dahliae from mint caused severe symptoms in the Mitcham strain, usually resulting in death of the plants. These two fungus isolates were used to inoculate Mitcham and strains 92 and 1229.

The plants were inoculated by root-dipping in a conidial suspension of 100,000 spores/ml. Each of the mint strains was inoculated with the following treatments.

1. Mp-3 alone
2. C-6 alone
3. Mp-3 followed one week later by C-6
4. C-6 followed one week later by Mp-3
5. Distilled water as control

Schnathorst and Mathre (1966) believe that inoculum potential and time of challenge inoculation are important in demonstrating cross-protection. In this experiment, the inoculum was adjusted to the same number of spores/ml in all inoculations. The challenge inoculation was applied seven days after the original inoculation. After this time, the fungus was presumably established in the plant but no symptoms were evident.

Table 24 shows the number of plants with symptoms at 41 days.

Table 24. Plants of cross-protection experiment with symptoms at 41 days after inoculation.

Treatment	Strain	Total with symptoms	Percent
C-6 alone	92	0/8	0
	M	8/9	88
	1229	5/9	55
Mp-3 alone	92	8/8	100
	M	9/9	100
	1229	9/9	100
Mp-3 + C-6	92	7/9	77
	M	9/9	100
	1229	10/10	100
C-6 + Mp-3	92	1/10	10
	M	10/10	100
	1229	5/9	55

All of the plants of each strain inoculated with Mp-3 alone had symptoms. Isolate C-6 alone caused symptoms on 88% of Mitcham plants, 55% of strain 1229, but none on strain 92. Symptoms were severe on Mitcham, inoculated with Mp-3 alone (Figure 23), but less so on strains 1229 and 92. Figure 24 shows that Mitcham had only moderate symptoms after inoculation with C-6; strain 1229 had light symptoms and strain 92 had no symptoms. This indicates that isolate C-6 is only mildly virulent on Mitcham or 1229 and is avirulent (with regard to symptoms) on 92. However, other data, previously presented, showed that strain 92 may be infected by C-6 and manifest no symptoms. Inoculation of the strains with Mp-3 alone

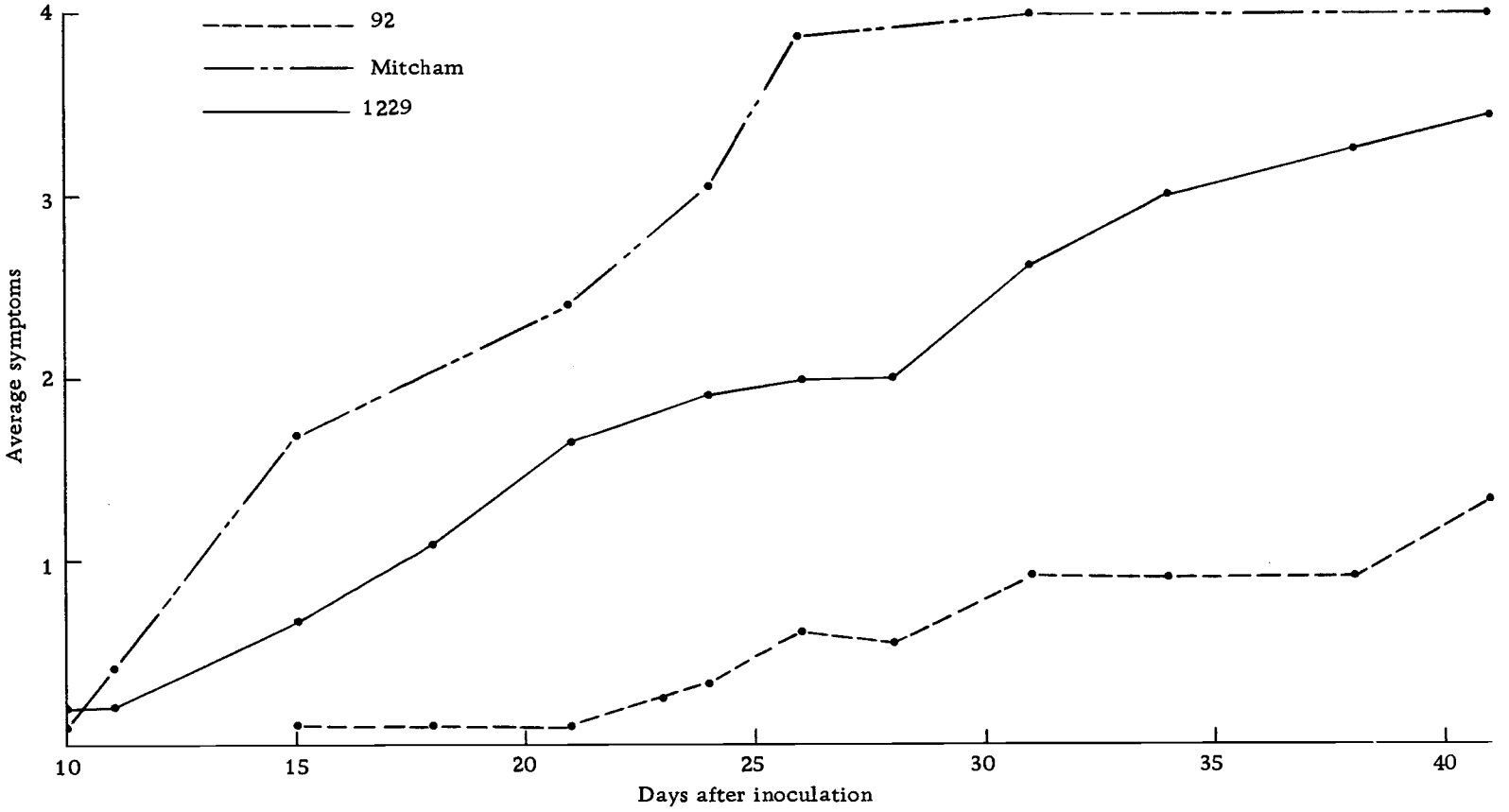

Figure 23. Symptoms of mint strains inoculated with Mp-3 alone.

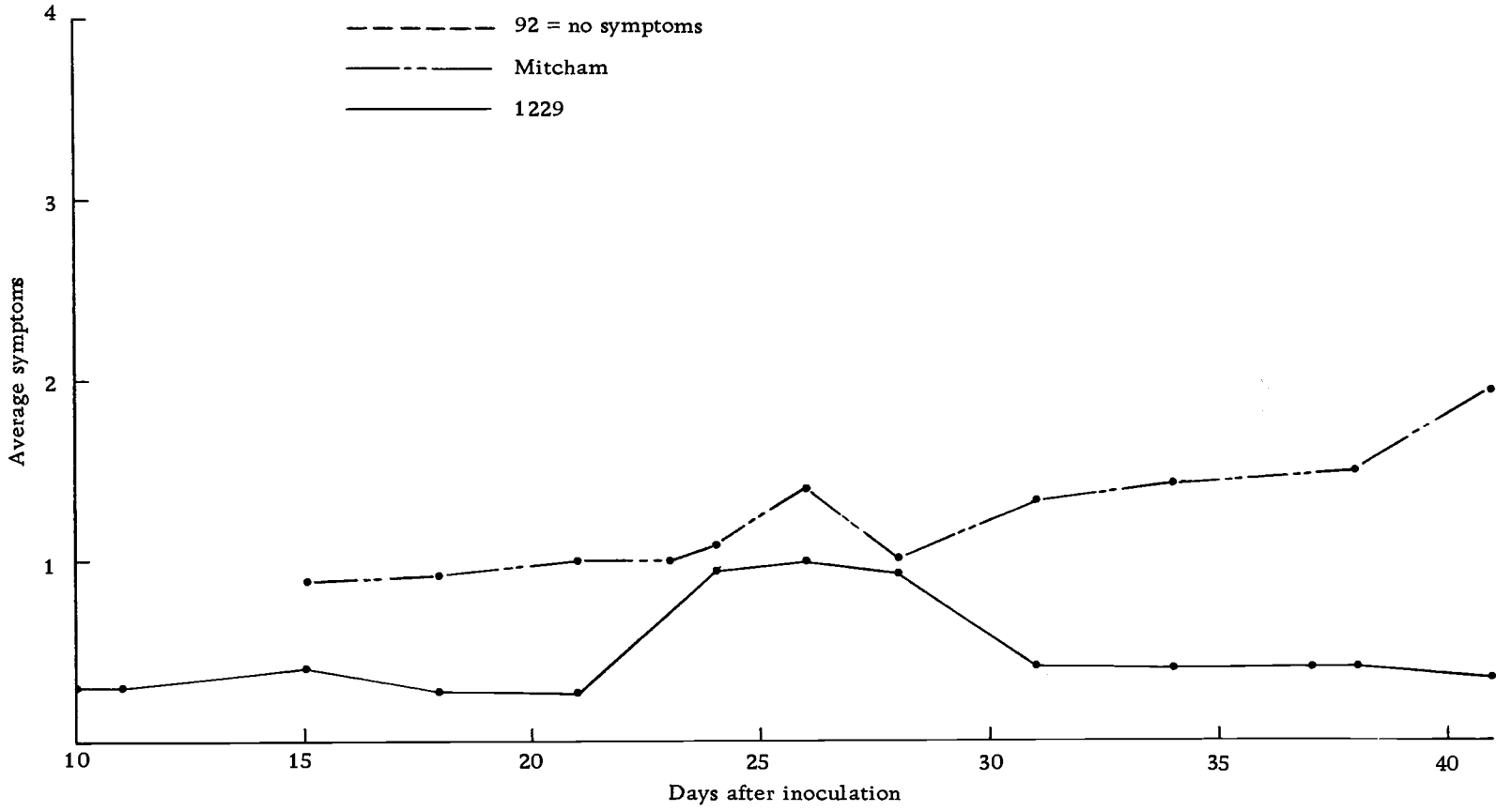

Figure 24. Symptoms of mint strains inoculated with C-6 alone.

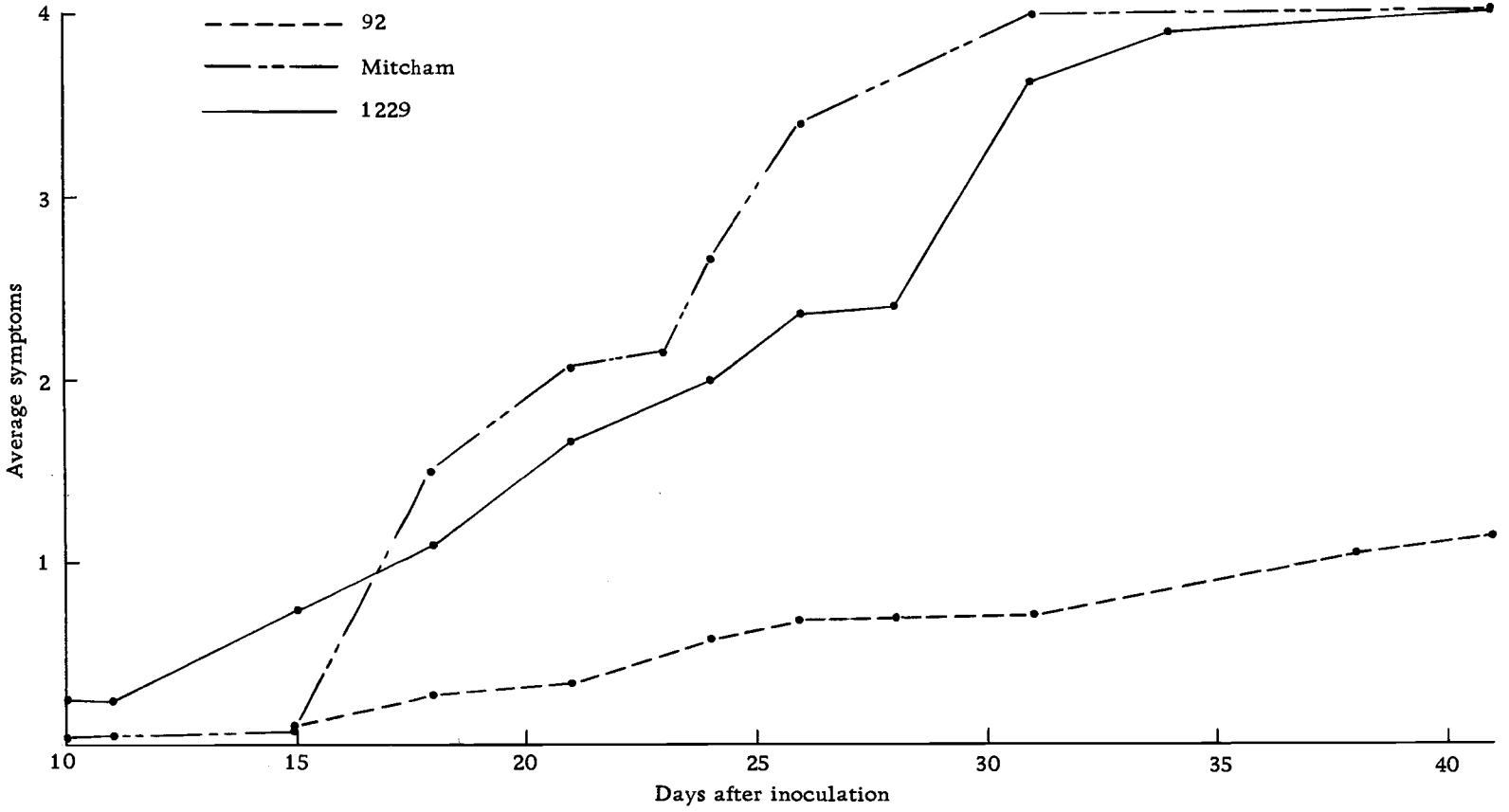

Figure 25. Symptoms of mint strains inoculated with Mp-3 followed by C-6.

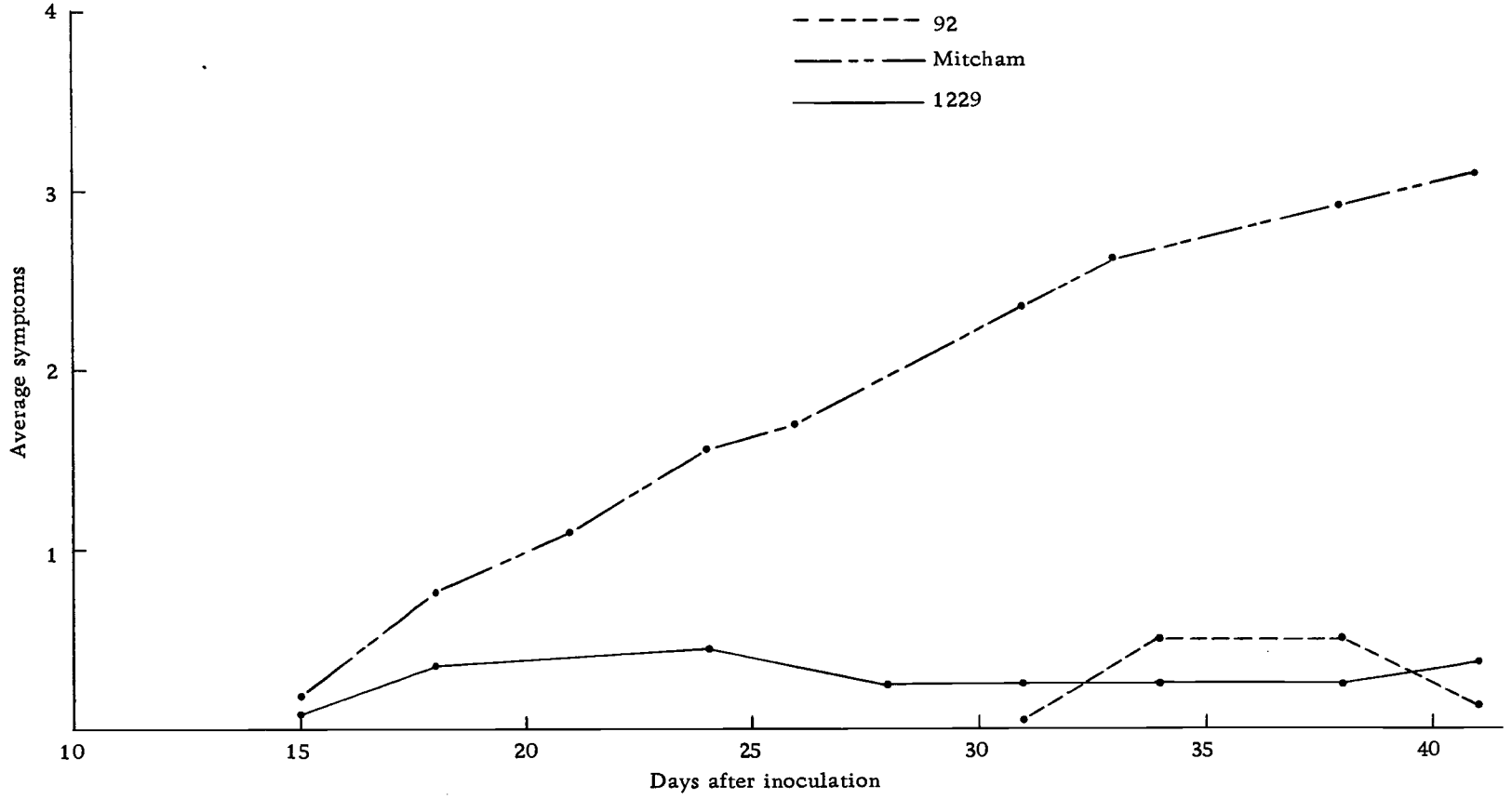

Figure 26. Symptoms of mint strains inoculated with C-6 followed by Mp-3.

and C-6 alone gives a basis to compare the other two treatments.

When inoculation with Mp-3 was followed by C-6, 77% of the plants of strain 92 showed symptoms (Table 24) but the symptoms were more severe than when inoculated with Mp-3 alone (Figure 25). One hundred percent of the plants of Mitcham and strain 1229 were killed by Mp-3 followed by C-6 (Table 24 and Figure 25). Strain 1229 was not so severely affected by Mp-3 alone (Figure 23). This indicates that there may be a synergistic effect causing more severe symptoms when the two strains are together.

When inoculation by C-6 was followed by Mp-3, there was a reduction in symptom severity (Figure 26). All the mint strains showed some symptoms, but severity was less than with Mp-3 alone (Figure 23) or Mp-3 + C-6 (Figure 25). This is noticeable on Mitcham and 92 but is especially striking on strain 1229. The number of plants of 1229 showing symptoms was reduced by one-half (Table 24). Only 10% of the plants of 92 showed symptoms. One hundred percent of the Mitcham plants showed symptoms but the severity was not as great as with Mp-3 alone or Mp-3 followed by C-6.

Strain 1229 showed symptoms earlier than Mitcham or 92 and initially these symptoms were slightly more severe when inoculated with C-6 alone (Figure 24), Mp-3 alone (Figure 23), or Mp-3 + C-6 (Figure 25). This is especially true of plants inoculated with C-6 alone. Results suggest that strain 1229 responds more quickly and

violently to infection and this may be a significant resistance mechanism.

Three plants from each treatment were assayed at 26 days after inoculation for propagules/gm tissue. Data are in Figure 27.

Strain 92 showed no propagules and no symptoms (Figure 24) when inoculated with C-6 alone. Strain 92 was immune to C-6 in this experiment. Mitcham had more than 10,000 propagules/gm and moderate symptoms (Figure 24) when inoculated with C-6 alone. Strain 1229 had less severe symptoms (Figure 24) than Mitcham and had considerably fewer propagules/gm tissue (Figure 27).

When inoculated with Mp-3 alone, strain 92 had almost 1000 propagules/gm (Figure 27) but only light symptoms (Figure 23). Symptoms were severe (Figure 23) and propagule numbers high (Figure 27) in Mitcham inoculated with Mp-3 alone. Strain 1229 was intermediate in response to Mp-3 alone.

The numbers of propagules found in 92 after inoculation with Mp-3 followed by C-6 was the highest of any treatment (Figure 27) but the symptoms remained light (Figure 25). Propagules in Mitcham are somewhat lower in this treatment than with Mp-3 alone but the symptoms are comparable (Figure 25). Strain 1229 had a very high amount of fungus when inoculated with Mp-3 followed by C-6 but symptoms were still only moderate (Figure 25).

In the last treatment, C-6 followed by Mp-3, strain 92 again

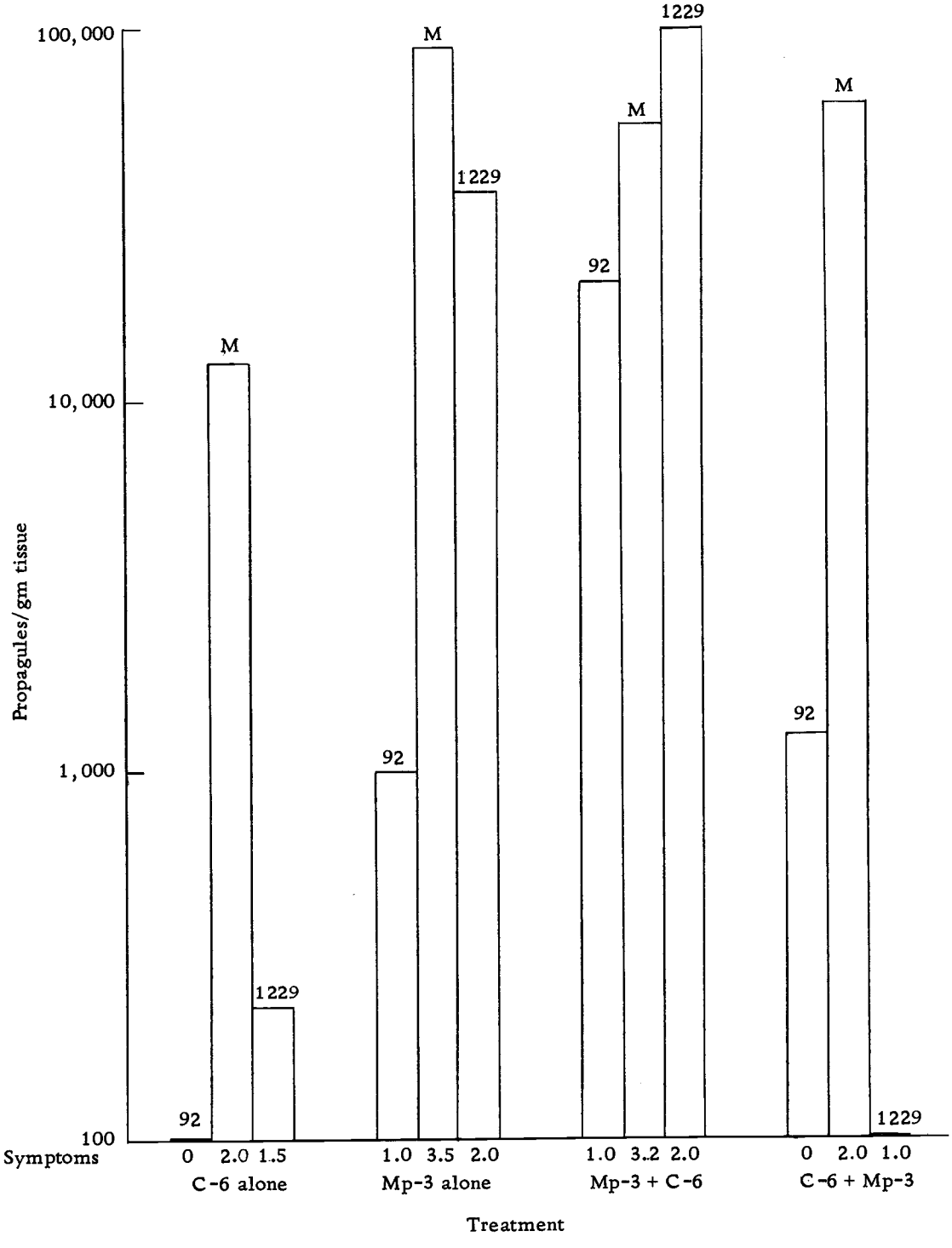

Figure 27. Propagules/gm stem tissue and symptoms of mint plants at 26 days after inoculation.

showed no symptoms (Figure 26) but in this case, there were over 1000 propagules/gm tissue (Figure 27). This indicates that the fungus can be present within the host with no symptoms evident and supports data previously presented. The propagules in Mitcham are comparable to Mp-3 + C-6 but the symptoms are less severe (Figure 26), indicating cross-protection on the basis of symptoms but not on propagules. Cross-protection is most evident in strain 1229 when inoculated with C-6 followed by Mp-3. The symptoms are light (Figure 26) but the amount of fungus is reduced to almost zero (Figure 27).

DISCUSSION AND CONCLUSIONS

Field experiments showed that the peppermint strains derived from irradiated stolons had more tolerance to Verticillium wilt than did the non-irradiated commercial strain. This was especially noticeable when incidence of the disease was compared in plots of the irradiated strains against plots of Mitcham. Disease severity in individual plants was sometimes as great in irradiated strains as in Mitcham. This indicates that, for purposes of field selection of a tolerant variety, disease incidence is a more important criterion than disease severity.

None of the irradiated strains was immune to Verticillium wilt but all tolerated infection better than Mitcham. There were two groups of these resistant strains. One group, consisting of strains 92, 58, 3201, and 3202, showed a high tolerance to wilt. Strains 1229, 2236, and 1320 showed a moderate tolerance.

Vigor and oil yield data showed that some strains were more vigorous and yielded more oil per unit of area than did Mitcham. These yields may not be significantly greater, but the data showed that none of the strains was depressed in vigor or yield. This, coupled with the wilt resistance shown, should make one of these strains satisfactory as a replacement for Mitcham, if the oil quality is of acceptable standards.

The mint strains that showed wilt tolerance in the field also had fewer microsclerotia present in dead stems than did Mitcham. This is a measure of resistance and shows that these strains were tolerant but not immune to Verticillium infection. They maintained a lower level of the fungus in their tissues than Mitcham. This could conceivably result in a level of inoculum low enough so severe disease would not develop.

Growth of V. dahliae on stem pieces and sap-extract media of the irradiated strains indicated that resistance was not due simply to differences in nutrition. Differences in nutritional effect on growth would not be expected since Verticillium is a facultative saprophyte able to grow on a wide variety of substrates. Growth of the fungus occurred when all the strains were used as substrate but was always inhibited when compared to the control. This suggests that some inhibitory substances, possibly phenolic compounds, are present. These may be native to the plants or may be synthesized by them in response to infection. It would be interesting to study the phenolic composition of these plants, before and after infection. This might give more insight into the mechanism of resistance.

Phenolic compounds must be present in these plants because all show phenol oxidase activity to some degree. The level of the enzyme fluctuated throughout the growing season and dropped to a

low point at about the time of flowering. The level of PPO *per se* did not seem to be related directly to resistance, because at certain times Mitcham had a higher level than some of the more resistant strains. However, the role of this enzyme might be related to the types of phenolic compounds in the plants. Resistance could be due to oxidized phenols present in one strain but absent or at a lower level in another strain.

The low level of PPO at the time of flowering suggested that plants with flowers might be more susceptible to wilt than non-flowering ones. McLean (1955) reported that one type of physiological resistance in potato was related to the time of tuber set. Flowers were also set about this time. Potatoes which set tubers late in the season were more resistant to wilt than those which set tubers early. Tolmsoff and Young (1957) found that root infection did not occur until after tuber initiation. Busch and Edgington (1967) showed that potatoes are more susceptible to *Verticillium* wilt at the time of tuber initiation. They were able to control wilt symptoms by altering the photoperiod. Plants grown in a long-night photoperiod formed tubers and developed moderate to severe wilt symptoms when inoculated. Plants grown in a long-day photoperiod or in a long-night period broken by a short light period, showed no disease symptoms when inoculated with *Verticillium*. They postulated that a change in sugar-starch ratio was related to resistance. When tubers are

initiated, the sugar is converted to starch. This allows the synthesis of cellulase, which had been inhibited by the high sugar level. This enzyme could then lyse cell walls of the xylem parenchyma, releasing toxic substances which would produce wilt symptoms. They concluded that a photoperiod which induces fruit or storage organ development would increase susceptibility and they wondered if other plants which are hosts of Verticillium and sensitive to photoperiod reacted in this way.

Flower formation in peppermint is related to photoperiod and food is stored in rhizomes. However, my data show that differential resistance is maintained in the mint plants after they have flowered. High resistant strain 92 showed only slight symptoms while low resistant Mitcham was almost killed by wilt when cuttings with flowers were inoculated with Verticillium. Intermediate resistant strain 1229 remained intermediate in response. Thus, flower initiation has no influence on wilt susceptibility in peppermint and selection of a wilt resistant strain can be made without regard to flowering date.

This further suggests that generalizations should not be made concerning mechanisms of resistance, even in wilt diseases caused by Verticillium. Each host-parasite interaction must be studied individually to see where similarities and differences exist between it and other situations.

Results of the spore movement experiment were similar to those of other workers. Sewell and Wilson (1964) found that Verticillium may be distributed through the hop plant as conidia. Hop plants have very large vessels so it is not surprising that spores would move freely in the xylem sap. These workers detected conidia in the vessels three weeks after inoculation. This delay was because they inoculated plants with infested stem fragments placed in soil.

Presley et al. (1966) also found that conidia of Verticillium move freely in the cotton plant and they concluded that dispersal of the fungus within the plant is primarily by conidia. Schnathorst, Presley and Carns (1967) had similar conclusions. In addition, they noted that there were fewer viable conidia present in resistant cotton varieties than in susceptible. Their first sample was taken six hours after inoculation and they found conidia at that time.

My data show that all three strains of mint were invaded by Verticillium conidia within 30 minutes after inoculation. Strain 92 began to react after about ten hours to reduce the amount of fungus present in the plants, while the number of nodes invaded increased in Mitcham and 1229. If this experiment had been continued for a longer time, the fungus would probably have disappeared from strain 1229 but may have continued to build up in Mitcham.

Other conclusions about the nature of resistance in Verticillium wilt of mint can be related to wilt diseases in other plants.

Schnathorst, Presley and Carns (1967) found that 24 hours after inoculation of cotton plants with Verticillium, the numbers of conidia increased logarithmically but the total numbers were 20-fold less in the tolerant variety. Miller and Cannon (1967) found that distribution of Verticillium in resistant tomato plants was not as extensive as in susceptible plants. My results show this is also true for resistant mint plants developed by irradiation mutation. Propagules of the fungus declined and eventually disappeared in high resistant strain 92 and also in moderate resistant strain 1229. In susceptible Mitcham, the numbers of propagules in the stem continued to increase as symptoms progressed.

Some differences also exist in physiologic strains of the pathogen. Griffiths and Isaac (1966) established a close relationship between virulence of six isolates of Verticillium and their ability to penetrate and colonize sterile tomato seedlings grown in culture. The highly pathogenic species rapidly colonized host tissue in culture while the mildly pathogenic strains penetrated the roots more slowly. The variations in virulence in the field between two isolates of V. dahliae suggested they were different physiological strains but they induced no difference during the first stage of invasion reaction.

The cotton isolate of V. dahliae that I tested is morphologically similar to the mint isolate. However, differences in virulence were shown toward all three mint strains inoculated. Strain 92 was highly

resistant to the cotton isolate based on both symptoms and propagules in the stem. Strain 1229 developed light symptoms and Mitcham had moderate symptoms when inoculated with the cotton isolate. The conclusion, based on symptoms and propagule numbers, is that the cotton isolate of V. dahliae is physiologically different from the mint isolate though they are morphologically similar.

Lacy and Horner (1967) found that root infections were greater on susceptible mint plants than on resistant ones; however, the resistant roots were still heavily infected. They concluded that the resistance of M. crispa (resistant) and M. sylvestris (moderate resistant) was not correlated with resistance to root infection.

My data show that roots of resistant, moderately resistant, and susceptible plants can be penetrated by V. dahliae. There was considerable variation in these experiments, but the evidence suggests that resistance is not wholly dependent on resistance to root penetration. The fungus generally reached higher levels in the roots of susceptible varieties indicating factors in resistant strains which operate to reduce the fungus population after establishment in the roots.

Resistance to Verticillium is present in stems of mint plants. When the root system was bypassed by direct stem inoculation, with a drop of spore suspension through a hypodermic syringe, strains 92, 1229, and Mitcham had resistance to the fungus. The total

numbers of propagules in the stems were not as high as when inoculation was by root-dipping. This may mean that less fungus was originally present in the stem.

When shoots without roots were inoculated by allowing the cut shoots to stand in a spore suspension, propagule numbers were high in all strains. However, strain 92 eventually recovered from the initially light symptoms. This is another indication that an active resistance mechanism is present in the stem to enable the host to overcome effects of the pathogen.

Cross-protection was demonstrated when mint strains were inoculated with a cotton isolate of Verticillium and challenged one week later with a mint isolate. This further suggests that resistance mechanisms in peppermint are active and can be initiated by an avirulent strain of the fungus. Two possible mechanisms of resistance might be:

(1) Tylose formation. This has been demonstrated in other plants infected by vascular wilt fungi. I have no data to support or eliminate this as a possibility in mint wilt. More work should be done with the mint-Verticillium interaction to determine if tylose formation is of significance in resistance to this disease.

(2) Phytoalexins. These are inhibitory substances formed in response to attack by a pathogen. My data suggest that inhibitory substances are present in the mint strains. The resistant strains

tended to have a lower amount of fungus present after a period of time and they also recovered from wilt symptoms. This means that the resistance mechanisms are retarding growth of the fungus and eventually eliminating it from the plant. Such action strongly suggests the presence of inhibitory substances rather than tyloses as a means of resistance. However, as previously discussed, resistance to a fungus of such ubiquitous habits as Verticillium is probably due to more than one factor.

To fully understand the mint-Verticillium host-parasite interaction, more research in detail is needed on such things as tyloses, phenolic compounds and phenol oxidases in relation to infection, and physiologic specialization of strains of the pathogens.

BIBLIOGRAPHY

Barnett, H. L. 1959. Plant disease resistance. Annual Review of Microbiology 13:191-210.

Beckman, C. H. 1966. Cell irritability and localization of vascular infections in plants. Phytopathology 56:821-824.

Beckman, C. H. 1968. An evaluation of possible resistance mechanisms in broccoli, cotton, and tomato to vascular infection by Fusarium oxysporum. Phytopathology 58:429-433.

Beckman, C. H., S. Halmos and M. E. Mace. 1962. The interaction of host, pathogen, and soil temperature in relation to susceptibility to Fusarium wilt of bananas. Phytopathology 52: 134-140.

Berry, S. Z. and C. A. Thomas. 1961. Influence of soil temperature, isolates, and method of inoculation on resistance of mint to Verticillium wilt. Phytopathology 51:169-174.

Blackhurst, F. M. and R. K. S. Wood. 1963. Resistance of tomato plants to Verticillium albo-atrum. Transactions of the British Mycological Society 46:385-392.

Brock, R. D. 1957. Mutation plant breeding. Journal of the Australian Institute of Agricultural Science 23:39-50.

Busch, L. V. and L. V. Edgington. 1967. Correlation of photoperiod with tuberization and susceptibility of potato to Verticillium albo-atrum. Canadian Journal of Botany 45: 691-693.

Byrde, R. J. W. 1956. The varietal resistance of fruits to brown rot. I. Infection experiment with Sclerotinia fructigena Aderh. and Ruhl. on certain dessert, culinary, and cider varieties of apple. Journal of Horticultural Science 31:188-195.

Byrde, R. J. W. 1957. The varietal resistance of fruits to brown rot. II. The nature of resistance in some varieties of cider apple. Journal of Horticultural Science 32:227-238.

Davis, D. 1964. Cross-protection of Fusarium wilt suscepts with eight formae specialis of Fusarium oxysporum. (Abstract) Phytopathology 54:891.

Deese, D. C. and M. A. Stahmann. 1962. Pectic enzyme formation by Fusarium oxysporum f. lycopersici on susceptible and resistant tomato stems. Journal of Agricultural and Food Chemistry 10:145-150.

Down, E. E. and A. L. Anderson. 1956. Agronomic use of an x-ray induced mutant. Science 124:223-224.

Frey, K. J. 1955. Agronomic mutations in oats induced by x-ray treatment. Agronomy Journal 47:207-210.

Frey, K. J. and J. A. Browning. 1955. Mutations for stem rust resistance induced in oats by x-ray treatment. Phytopathology 45:490-492.

Green, R. J. 1958. Deep-plowing for controlling Verticillium wilt of mint in muck soils. Phytopathology 48:575-577.

Gregory, W. C. 1956. Induction of useful mutations in peanuts. In: Genetics in Plant Breeding. Brookhaven Symposium in Biology, no. 9, p. 177-190.

Griffiths, D. A. and Ivor Isaac. 1966. Host-parasite relationships between tomato and pathogenic isolates of Verticillium. Annals of Applied Biology 58:259-272.

Hansel, H. and J. Zakovsky. 1956. Mildew-resistant barley mutants induced by x-rays. Euphytica 5:347-352.

Heinze, P. H. and C. F. Andrus. 1945. Apparent localization of Fusarium wilt resistance in the Pan America tomato. American Journal of Botany 32:62-66.

Highley, T. L. 1968. Comparison of pectic enzymes in resistant and susceptible mints infected by Verticillium dahliae. Ph.D. thesis. Corvallis, Oregon State University. 93 numb. leaves.

Horner, C. E. 1954. Pathogenicity of Verticillium isolates to peppermint. Phytopathology 44:239-242.

Horner, C. E. 1962. Oregon State University mint wilt research project. Proceedings of the 13th Annual Meeting of the Oregon Essential Oil Growers League, 1962, p. 6-16.

Horner, C. E. and H. L. Dooley. 1965. Propane flaming kills Verticillium dahliae in peppermint stubble. Plant Disease Reporter 49:581-582.

Horner, E. S. 1957. The utilization of artificially induced mutations in plant breeding programs. Proceedings of the Soil and Crop Science Society of Florida 17:130-136.

Keyworth, W. G. 1953. Verticillium wilt of the hop. VI. The relative roles of root and stem in the determination of wilt severity. Annals of Applied Biology 40:344-361.

Keyworth, W. G. 1963. The reaction of monogenic resistant and susceptible varieties of tomato to inoculation with Fusarium oxysporum f. lycopersici into stems or through Bonny Best rootstock. Annals of Applied Biology 52:257-270.

Keyworth, W. G. 1964. Hypersensitivity of monogenic resistant tomato scions to toxins produced in Bonny Best rootstocks invaded by Fusarium oxysporum f. lycopersici. Annals of Applied Biology 54:99-105.

Konzak, C. F. 1956a. Induction of mutations for disease resistance in cereals. In: Genetics in Plant Breeding. Brookhaven Symposium in Biology, no. 9, p. 157-176.

Konzak, C. F. 1956b. A note on the use of radiation for the production of mutations for Victoria blight resistance in oats. Phytopathology 46:177-178.

Konzak, C. F. 1959. Induced mutations in host plants for the study of host-parasite interactions. In: Plant pathology: problems and progress, 1908-1958, ed. by C. S. Holton et al. Madison, University of Wisconsin Press. p. 202-214.

Kuc, J. 1966. Resistance of plants to infectious agents. Annual Review of Microbiology 20:337-365.

Lacy, M. L. 1965. Influence of host and non-host plants upon populations of Verticillium dahliae in soil. Ph.D. thesis. Corvallis, Oregon State University. 95 numb. leaves.

Lacy, M. L. and C. E. Horner. 1967. Reproduction of Verticillium in the rhizosphere and subsequent root infection of resistant and susceptible mint species. (Abstract) Phytopathology 52: 739.

Larter, E. N. and F. C. Elliot. 1956. An evaluation of different ionizing radiations for possible use in the genetic transfer of bunt resistance from Agropyron to wheat. Canadian Journal of Botany 34:817-823.

Loomis, W. D. and J. Battaile. 1966. Plant phenolic compounds and the isolation of plant enzymes. Phytochemistry 5:423-438.

Mackey, J. 1956. Mutation breeding in Europe. In: Genetics in Plant Breeding. Brookhaven Symposium in Biology, no. 9, p. 177-190.

McLean, J. G. 1955. Selecting and breeding potatoes for field resistance to Verticillium wilt in Idaho. Moscow. p. 36-51. (Idaho. Agricultural Experiment Station. Bulletin 30)

Martinson, C. A. and C. E. Horner. 1962. Importance of non-hosts in maintaining the inoculum potential of Verticillium. (Abstract) Phytopathology 52:742.

Matta, A. and A. E. Dimond. 1963. Symptoms of Fusarium wilt in relation to quantity of fungus and fungus activity in tomato stems. Phytopathology 53:574-578.

Menon, R. and L. Schachinger. 1957. Die Rolle des Phenols bei der Widerstandsfähigkeit von Tomatenpflanzen gegen Infectionen. Berichte Deutschen Botanischen Gesellschaft 40:11-20.

Miller, T. D. and O. S. Cannon, 1967. Penetration and distribution of Verticillium dahliae in tomato. (Abstract) Phytopathology 57:1007.

Murray, M. J. 1964. Irradiation breeding in peppermint. A progress report illustrating present results. Paper presented at Purdue University, Lafayette, Indiana, December 2, 1964. 6 numb. leaves.

Murray, M. J. 1965. Verticillium wilt resistant peppermint varieties obtained by radiation breeding. Proceedings of the 16th Annual Meeting of the Oregon Essential Oil Growers League, 1965, p. 2-3.

Murray, M. J. 1966. Irradiation breeding in peppermint. A brief progress report illustrating present results. Paper presented at the Biennial Mint Meeting, Chicago, Illinois, December 7, 1966. 3 numb. leaves.

Murray, M. J. 1967a. The 1966 status of our program to obtain Verticillium wilt resistant strains of peppermint by irradiation breeding. Proceedings of the 18th Annual Meeting of the Oregon Essential Oil Growers League, 1967, p. 9-13.

Murray, M. J. 1967b. Successful use of irradiation breeding to obtain Verticillium resistant strains of peppermint. Paper presented at the 59th Annual Meeting of the American Society of Agronomy, Washington, D.C., November 5-10, 1967. (Abstracted in Agronomy Abstracts, 1967, p. 15)

Myers, W. M., E. R. Ansemus, F. K. S. Koo and K. J. Hsu. 1956. Resistance to rust induced by ionizing radiations in wheat roots. Proceedings of the International Conference on the Peaceful Uses of Atomic Energy 12:60-62.

Nadakavukaren, M. J. and C. E. Horner. 1959. An alcohol agar medium selective for determining Verticillium microsclerotia in soil. Phytopathology 49:527-528.

Nelson, R. 1950. Verticillium wilt of peppermint. East Lansing, 259 p. (Michigan. Agricultural Experiment Station. Technical Bulletin 221)

Osborne, T. S. 1957. Mutation production by ionizing radiation. Proceedings of the Soil and Crop Science Society of Florida 17:91-107.

Paxton, J. D. and D. W. Chamberlain. 1967. Acquired local resistance of soybean plants to Phytophthora spp. Phytopathology 57: 352-353.

Presley, J. T., H. R. Carns, E. E. Taylor and W. C. Schnathorst, 1966. Movement of conidia of Verticillium albo-atrum in cotton plants. Phytopathology 56:375.

Rubin, B. A., E. V. Artsikovskaya and T. A. Proskurnikova. 1947. Oxidative conversions of phenols and their role in the phenomena of potato resistance to Phytophthora infestans. Biokhimia 12: 141-152.

Schnathorst, W. C. and D. E. Mathre. 1966. Cross-protection in cotton with strains of Verticillium albo-atrum. Phytopathology 56:1204-1209.

Schnathorst, W. C., J. T. Presley and H. R. Carns. 1967. Determination of the internal inoculum potential of Verticillium albo-atrum in cotton plants. (Abstract) Phytopathology 57: 101-102.

Sewell, G. W. F. and J. F. Wilson. 1964. Occurrence and dispersal of Verticillium conidia in xylem sap of the hop (Humulus lupulus L.). Nature 204:901.

Shebeski, L. H. and T. Lawrence. 1954. The production of beneficial mutations in barley by irradiation. Canadian Journal of Agricultural Science 34:1-9.

Scheffer, R. P. 1957. Analysis of Fusarium resistance in tomato by grafting experiments. Phytopathology 47:328-331.

Scheffer, R. P. and J. C. Walker. 1954. Distribution and nature of Fusarium resistance in the tomato plant. Phytopathology 44:94-101.

Singleton, W. R., C. F. Konzak, S. Shapiro and A. H. Sparrow. The contribution of radiation genetics to crop improvement. Proceedings of the International Conference on the Peaceful Uses of Atomic Energy 12:25-30.

Sinha, A. K. and R. K. S. Wood. 1967. An analysis of responses of resistant and of susceptible tomato plants to Verticillium infection. Annals of Applied Biology 59:143-154.

Smith, H. H. 1958. Radiation in the production of useful mutations. Botanical Review 24:1-24.

Snyder, W. C., K. F. Baker and H. N. Hansen. 1946. Interpretation of resistance to Fusarium wilt in tomato. Science 103:707-708.

Talboys, P. W. 1958a. Association of tylosis and hyperplasia of the xylem with vascular invasion of the hop by Verticillium albo-atrum. Transactions of the British Mycological Society 41: 248-260.

Talboys, P. W. 1958b. Some mechanisms contributing to Verticillium resistance in the hop root. Transactions of the British Mycological Society 41:227-241.

Talboys, P. W. 1964. A concept of the host-parasite relationship in Verticillium wilt diseases. Nature 202:361-364.

Tolmsoff, W. J. and R. A. Young. 1957. Relation of inoculum potential of Verticillium albo-atrum to development and severity of wilt in potatoes. (Abstract) Phytopathology 47:536.

Waggoner, P. E. and A. E. Dimond. 1956. Altering disease resistance with ionizing radiation. Phytopathology 46:125-127.

Weber, D. J. and M. A. Stahmann. 1964. Ceratocystis infection in sweet potato: Its effect on proteins, isozymes, and acquired immunity. Science 146:929-931.

Wilding, M. D. 1960. A study of the free amino acids in alfalfa and red clover roots as related to winter-hardiness and the occurrence of hydroxylysine and hydroxyaspartic acid in alfalfa roots. Biochemical studies on resistance in peas to Fusarium wilt. Ph. D. thesis. Madison, University of Wisconsin. 123 numb. leaves. (Abstracted in Dissertation Abstracts 21:45-46. 1960)

Wilhelm, S. 1955. Longevity of the Verticillium wilt fungus in the laboratory and field. Phytopathology 45:180-181.

Wood, R. K. S. 1967. Physiological plant pathology. Oxford, Blackwell Scientific Publications. 570 p.

Yarwood, C. E. 1956. Cross-protection with two rust fungi. Phytopathology 46:540-544.