THE DISEASE-SUPPORTING CAPABILITY OF SOILS OF DIFFERENT FUNGISTATIC CAPACITY

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ABSTRACT

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Disease incidence in three soils artificially infested with root-infecting fungi was compared with the degree of fungistasis in the soils. Incidence of tomato wilt, caused by Fusarium oxysporum f. sp. lycopersici, was in the order Capac loam > Boyer sandy loam > Colwood loam. This ranking was inversely correlated with that of fungistasis to the pathogen, as determined by nutrient titration. By contrast, incidence of radish wilt, caused by F. oxysporum f. sp. conglutinans was in the order Boyer sandy loam > Colwood loam > Capac loam. However, fungistasis was in the order Colwood loam > Boyer sandy loam > Capac loam. Total microbial biomass was twice as high in Colwood loam as in the other soils. The nutrient sinks of the soils, as measured by the rate of respiration of added glucose, was in the order Colwood loam > Capac loam > Boyer sandy loam. These two soil characteristics were partially correlated with fungistasis in the soils, and were inversely correlated with tomato wilt but not radish wilt development in the soils. These results suggest that factors in addition to fungistasis are involved in disease expression.

To my parents.

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TABLE OF CONTENTS

Discu

List

LIST OF TABLES

Figur

1

2

3

LIST OF FIGURES

FUNGISTASIS IN RELATION TO DISEASE-SUPPRESSIVENESS OF SOILS: A LITERATURE REVIEW

I. INTRODUCTION

The phenomenon of fungistasis in soils has been known for over three decades as a mechanism by which microbial propagules do not germinate and grow at an inopportune locations or times. Recent work, however, suggests that soil fungistasis imposed over a long term can also affect pathogen survival and virulence (Bristow and Lockwood, 1975; Filonow et al., 1983). Thus, soil fungistasis may play a greater role in the ecology of soil-borne plant pathogens than merely germination inhibition. The relationship between fungistasis and disease development in soils is the subject of this review.

As a prerequisite for examining the relationship of soil fungistasis and disease development, two concepts must be considered. First, although most soils are fungistatic, they differ in their ability to impose fungistasis, as shown by different germination rates when identical concentrations of nutrients are added to soils to annul fungistasis (Filonow and Lockwood, 1979). Second, soils differ in their ability to support disease development, as shown by differences in disease incidence or severity when identical quantities of inoculum of plant

pathogens are used to infest soils (Alabouvette et al., 1982). A narrow perspective is applied to soils differing in their ability to support disease when they are labelled either "disease-suppressive" or "disease-conducive". Much of the current research on "suppressive soils" implies that disease expression in soil is an "all or nothing" phenomenon and fails to acknowledge that disease differences are only relative. If all soils are considered, however, it should be seen that there is a continuum of response. In this review, fungistasis and disease development in soils will often be discussed using "disease-suppressive" and "disease-conducive" as convenient, comparative labels for soils which are far apart on the continuum of disease response.

The approach of this review will be to examine fungistasis as a component of disease-suppressiveness of soils. First, the occurrence and characteristics of disease-suppressive soils will be briefly described. This discussion will illustrate the diversity of the soils involved and serve as a basis for presenting generalizations. More inclusive reviews have been published (Cook and Baker, 1983; Hornby, 1983; Rovira and Wildermuth, 1981; Schneider, 1982; Schroth and Hancock, 1982). Next, fungistasis will be compared among soils with different degrees of disease suppression. Since fungistasis and disease-suppressiveness are primarily

induced by biotic factors the relation of the microflora involved in disease-suppression to the imposition of soil fungistasis will be discussed. The mechanisms by which the microflora exert their disease-suppressive effect will be compared to the mechanisms by which microflora impose fungistasis. Although it is recognized that fungistasis and disease-suppression are occasionally induced by abiotic factors, these factors will be discussed only when they influence the biotic factors in soil. It is hoped that this review will reveal generalizations about the level of fungistasis in soils relatively suppressive to disease and the deficiencies in knowledge that preclude generalizations.

II. DISEASE-SUPPRESSIVENESS IN SOIL:

Recognition that soil-borne diseases do not occur with the same incidence or severity in different soils arose originally from field observations made early in the history of plant pathology, as early investigators examined the many factors that affect disease. Atkinson (1892) is often credited with first recognizing "disease-suppressive" and "disease-conducive" soils. He noticed that cotton wilt, caused by Fusarium oxysporum f. sp. vasinfectum, was absent in certain soil types, but research on the nature of the differences was not done. Later workers can be credited with systematic examination

of conditions in soils leading to differences in disease.

These workers performed crop monoculture experiments in pathogen-infested soils to monitor disease development, rather than rely solely on field observations, as Atkinson did. Normally, monoculture should exacerbate disease because it provides conditions that favor inoculum increase. However, these early workers found that this did not occur in certain soils. After three seasons of monoculture, Jones (1923) observed that root rot of peas, caused by Fusarium solani f. sp. pisi, disappeared in a well-drained gravelly loam, while it remained severe in a nearby muck soil. Walker and Snyder (1934) also performed pea monoculture experiments with certain soils where pea wilt, caused by F. oxysporum f. sp. pisi, was absent, in spite of continuous pea cropping. In their greenhouse experiments, after the third monoculture, pea wilt incidence increased to 61% in a sandy loam, but only to 8% in a red clay. Thus, these workers established that certain soils suppress disease development, in spite of conditions such as monoculture that favor pathogen increase.

One consequence of suppressive conditions in soil is a less effective inoculum. The suppressive soils required a higher inoculum density to obtain a level of disease that would be present with lower inoculum densities in relatively conducive soils. In the case of muskmelon

wilt, caused by F. oxysporum f. sp. melonis, Wensley and McKeen (1963) found a direct relationship between population of the pathogen in soil and wilt incidence. However, they also observed more wilt in a Fox sandy loam than in a Colwood loam, even though the population of the pathogen was lower in the sandy loam.

Enumeration of pathogen populations in soils, in conjunction with disease evaluation from field surveys, can identify suppressive soils. However, such identification should be considered only tentative, since apparent population differences among soils, as revealed by differences in colony counts on agar media, could reflect differences in recovery of inoculum. Therefore, evaluation of disease after addition of a known quantity of inoculum to soils and monitoring survival of this inoculum is a better method for identifying suppressive soils. Differences in the amounts of inoculum required to cause disease in two different soils can be dramatic, if the soils are widely separated from each other on the continuum of response to plant pathogens. For example, chlamydospores of F. oxysporum f. sp. batatas gave 96% disease incidence when 5,000 propagules per gram (ppg) were incorporated into conducive Elkhorn sand, while 5,000 ppg gave a 67% disease incidence in suppressive Chualar sandy loam (Smith and Snyder, 1971).

However, there usually is a threshold at which

disease suppression in soil can be overcome by very high inoculum densities (Burke, 1965; Alabouvette et al., 1982). To prevent suppressiveness from being masked by excessive inoculum, soils should be compared using several levels of inoculum. With this approach, Alabouvette et al. (1982) found that curves of F. oxysporum f. spp. wilt incidence plotted against inoculum density were different for different soils. Disease incidence differed among soils over all inoculum densities, although differences were smaller at higher inoculum densities. Different concentrations of inoculum were also used to determine suppressiveness of soils to Rhizoctonia solani (Kinsbursky and Weinhold, 1983). This approach could be applied to evaluate suppressiveness of soils to other soil-borne plant pathogens. In most cases, plants growing in relatively suppressive soils can tolerate higher inoculum densities than plants in relatively conducive soils.

The decline, low incidence, or absence of disease in suppressive soils can often be attributed to a decline in inoculum. After 12 weeks, 58% of added chlamydospores of Phytophthora cinnamomi were recovered from Kioloa soil conducive to P. cinnamomi on many hosts, but only 14% of added chlamydospores were recovered from suppressive Tallaganda soil (Halsall, 1982a). Inoculum decline also occurs in some soils suppressive to Fusarium. As an example, two months after the addition of 3,000 ppg of F.

oxysporum f. sp. lini to two soils planted to flax, the population increased to 4,100 ppg in a soil with 97% wilt incidence, but declined to 300 ppg in a soil with 3% wilt incidence (Tu et al., 1978). This trend was observed in another suppressive and conducive "pair" of soils with three other form species of F. oxysporum (Komada, 1976).

Some types of propagules die more rapidly than other types in suppressive soils. After two months, numbers of viable macroconidia of F. solani f. sp phaseoli were 25% of that added to a soil conducive to bean root rot, compared to 0.1% in a soil suppressive to bean root rot. When chlamydospores were tested instead of macroconidia, 80% survived in the conducive soil, while 62% survived in the suppressive soil (Furuya, 1982). This great difference in survival between macroconidia and chlamydospores illustrates the importance of using survival propagules such as chlamydospores to compare population dynamics between soils that differ in disease suppressiveness, as they would better reflect the survival capability of the pathogen as it would occur in the field.

There are also many soils in which suppression occurs without pathogen decline; disease is absent in spite of the presence of a sufficient number of propagules to cause symptoms in other soils. To illustrate this point, populations of F. oxysporum f. sp. cepae in certain organic soils in which basal rot of onion did not occur,

were often the same as those in similar soils where basal rot was present (Abawi and Lorbeer, 1971). Additionally, a survey of 24 pea fields with a population range of 275-6,175 ppg of F. solani f. sp. pisi revealed no relationship between population and root rot severity in previous pea crops (Burke et al., 1970). These studies may not be sufficient to demonstrate suppression in spite of overwhelming inoculum, because differences among soils in propagule recovery for enumeration may distort the true population ranking of the soils. Addition of a known quantity of propagules and subsequent enumeration could be used to determine persistence of inoculum in suppressive soil, rather than relying solely on enumeration of indigenous pathogens. When this was done by adding 300 ppg of F. oxysporum f. sp. melonis to Chateaurenard suppressive soil planted to muskmelon, the population of the pathogen remained at about the same level at the end of the experiment without causing the level of disease that would occur in a conducive soil with the same amount of inoculum (Louvet et al., 1976).

On the other hand, pathogen populations generally increase in conducive soils, as in the case of F. oxysporum f. sp. batatas, which increased in the presence of sweet potato to twice the added 500 ppg (Smith and Snyder, 1971). The persistence of pathogens in some suppressive soils and their decline in other suppressive

soils suggests either different modes of suppression or different degrees of suppression.

In the majority of suppressive soils, the disease-suppressive factor is biological. Evidence for this is given by the elimination of suppression after biocidal treatments of soil (Burke, 1954; Menzies, 1959; Furuya, 1982). Sterilization, however, does not eliminate suppressiveness induced by abiotic factors, such as montmorillonite clays (Stotzky, 1972). It is important to use the least disruptive sterilization procedure to demonstrate that loss of suppression is associated with loss of the soil microflora and not with a change in the abiotic components of soil. Gamma irradiation is often thought to be the least disruptive, yet the changes in levels of extractable nutrients were greater in gamma-irradiated soils than in soils heated to 60°C for two hours (Mäntylahti and Yläranta, 1981). Thus, with sterilization techniques that alter soil characteristics, it becomes difficult to rule out abiotic contributions to suppressiveness.

Suppressiveness can be re-established in sterilized soils by introducing into them a small quantity of unsterilized suppressive soils (Menzies, 1959; Louvet et al., 1976; McCain et al., 1980), or only the microflora isolated from suppressive soil (Lin and Baker, 1980). However, a non-transferrable biologically-mediated

suppres-sion also has been described (Burke, 1954), suggesting that in some cases, suppression occurs through an interaction of the microflora and abiotic components unique to the soil.

In fact, certain abiotic soil components are closely associated with biotic components in suppressive soil. Montmorillonite clays, for example, are associated with soils suppressive to Panama wilt of banana, caused by F. oxysporum f. sp. cubense (Stover, 1962). Fine-textured soils are more suppressive to Pythium ultimum than coarse-textured soils (Hancock, 1979). In addition to texture, soil reaction can contribute to suppression. An acidic soil, which favored the growth of Trichoderma spp. was more suppressive to R. solani than an alkaline soil (Liu and Baker, 1980). Since abiotic soil components can affect pathogen behavior directly, these factors, as well as effects of the soil microflora, should be comparatively evaluated.

An under-appreciated aspect of suppressiveness is that it can be manipulated. A well-known example of this is "take-all decline," where soils with initially severe take-all disease become suppressive to Gaeummanomyces graminis var. tritici with continuous cereal monoculture. Take-all-suppressive soils arise from a change in soil microflora induced by cereal monoculture (Hornby, 1979), but unlike other cases of pathogen suppression which

appear to result from constitutive soil properties, take-all suppression can only arise in the presence of the pathogen (Wildermuth, 1980). Thus, take-all decline is probably a special case of an inducible suppression which involves a three-way interaction of host, pathogen, and microflora.

Suppressiveness to R. solani was induced in soils by weekly radish monoculture (Chet and Baker, 1980), but not by monoculture with other crops (Liu and Baker, 1980). The suppression was correlated with an enhanced population of Trichoderma harzianum. But while it is significant that a particular crop can influence the development of a suppressive microflora in, perhaps, any soil, the longevity of the suppressiveness in not known. Does the suppressiveness, once established, persist with crop rotation, or is it lost, like take-all suppressiveness is, once a non-cereal is planted?

There is also evidence that the microflora of soils can be manipulated to make soils suppressive to Fusarium wilt diseases by such procedures as cropping. Repeated planting in a conducive soil amended with 10% suppressive soil resulted in a decline of muskmelon wilt, caused by F. oxysporum f. sp. melonis (Alabouvette et al., 1980c). However, the presence of "suppressive" soil is not necessarily a prerequisite for inducing suppression, as suggested by a decline of carnation wilt, caused by F.

oxysporum f. sp. dianthi, in unamended conducive soil by repeated planting of carnation (Tramier et al., 1979). Nor does the induction of suppressiveness to a Fusarium wilt pathogen necessarily require previous cropping with its host. For example, a continuously-cropped Chualar sandy loam, which had never been planted to sweet potatoes, was more suppressive to wilt of sweet potatoes, caused by F. oxysporum f. sp. batatas, than the same soil that had never been cropped at all (Smith and Snyder, 1971). In addition to the plant pathogens discussed, the induction of disease-suppression by cropping or other agricultural practices which alter the soil microflora may also be applicable to other soil-borne plant pathogens.

While treatments that stimulate suppressiveness should be sought and incorporated into a cropping program, other treatments that decrease the suppressiveness of soil should be identified and avoided in a cropping program. For example, soil amendments of peat and manure increased incidence of carnation wilt (Tramier et al., 1979). Alternatively, treatments may affect the pathogen and the soil microflora, but have no effect on disease incidence. Monthly cropping of barley, followed by turning the crop under, increased populations of P. ultimum in suppressive soil, but the degree of suppressiveness did not change (Hancock, 1979). It can be concluded, based on the complexity of the plant host-pathogenic fungus-soil

system, that any attempts to manipulate suppressiveness of soil for biological control must be made on a somewhat individualized basis.

While knowledge of conditions that enhance suppressiveness is scant at best, the question of the range of species a given soil is suppressive to has, for the most part, remained unasked. Soils from five continents have been described as suppressive to a wide variety of plant pathogens, but few workers have evaluated suppressiveness of a particular soil to more than one plant pathogen. This tendency of specialization could lead to the incorrect assumption that a soil suppressive to one pathogen also is suppressive to others. This is known not to be true with the Chateaurenard, melon-wilt suppressive soil, which apparently suppresses only form species of F. oxysporum, but not the other Fusarium spp. or other genera of plant pathogens (Alabouvette et al., 1980a). Are other soils suppressive to F. oxysporum specifically suppressive to that fungus, or is there a wider range of pathogens inhibited? The identification of the range of pathogens affected by suppressive soils is an important step for developing approaches for advantageously manipulating suppressiveness.

From the above discussion, disease-suppressiveness of soils can be defined as a property of soil whereby inoculum effectiveness is reduced; this is reflected in

less disease relative to other soils. This property is best identified by adding a known quantity of propagules of a pathogen to soils and comparing among them disease incidence or severity of a susceptible host under environmental conditions favorable to disease development. Since the primary determinant of suppressiveness is the soil microflora, it follows that all natural soils are more or less suppressive. Suppressiveness is eliminated by destroying the microflora and is restored by re-establishing the microflora. The range of suppressiveness of soils is related not only to the microfloral composition of the soil, but also to the physical and chemical components of soil and their interaction with the soil microflora. Evidence of different microflora among soils comes from the enhancement of suppressiveness by the transfer of microflora from a relatively suppressive soil to a relatively conducive soil (Alabouvette, et al., 1979). The suppressive microflora is dynamic and soil treatments such as cropping can alter it and lead to changes in disease expression. The requirement for successful manipulation of this microflora for biological control is an understanding of its active components and how these operate.

III. FUNGISTASIS IN DISEASE-SUPPRESSIVE SOILS:

The consequence of fungistasis in soils is dormancy of fungal propagules. Additionally, macroconidia of

Fusarium, which are not survival propagules, convert to chlamydospores under fungistatic conditions (Nash et al., 1961). Fungistasis occurs primarily because of a nutrient-deficient state of soil which is imposed by the soil microflora (Lockwood, 1977). The amounts of carbon and nitrogen required for germination of chlamydospores of Fusarium solani f. sp. phaseoli were reduced by the simultaneous addition of antibiotics to inhibit the microflora (Cook and Schroth, 1965). Although this decrease in nutrients required for germination suggests that the inhibition of the microflora reduced competition for nutrients, there is also the less likely possibility that microflora producing antifungal substances were inhibited.

Fungistasis is not imposed to the same degree in all parts of a soil. In the rhizosphere, it is reduced because of a greater supply of nutrients, as shown by germination of Fusarium macroconidia to produce tropic hyphal growth to the root, instead of forming chlamydospores (Jackson, 1957).

While all soils exhibit fungistasis, differences in fungistasis among soils can be observed by the addition of nutrients in concentrations that partially annul the effect (Filonow and Lockwood, 1979). Quantitative determination of nutrient concentrations that annul fungistasis is termed "nutrient titration". In practical

terms, propagules of plant pathogens in a highly fungistatic soil need to be placed closer to the root in order to germinate and grow to it; in effect, the inoculum potential is lowered. Quantitative differences in nutrient annulment of fungistasis are reflected not only by decreased germination of propagules, but also, by inhibition of hyphal growth, including germ tubes produced by propagules (Hsu and Lockwood, 1971).

Soils could be suppressive either because of an elevated level of fungistasis that prevents germination or, paradoxically, germination is promoted at a time or location that is inopportune for the pathogen. Germination promotion as a mechanism of suppression has not been widely reported. Burke (1965) described a Sagemoor sandy loam suppressive to F. solani f. sp. phaseoli which apparently was less fungistatic to macroconidia than a conducive Ritzville sandy loam. Macroconidia are less sensitive to fugnistasis than chlamydospores and will germinate more readily on soils in the absence of nutrients. growth from macroconidia was greater in the suppressive soil than in the conducive soil. However, this increased growth in the suppressive soil was ephemeral and was associated with relatively late formation of small chlamydospores. In contrast, germ tube growth was suppressed in the conducive soil. While there was no difference in the rapidity of lysis of mycelia and

germ tubes between the two soils, chlamydospores were larger and more numerous in the conducive soil. Thus, in the disease-suppressive soil, the pathogen germinated in the absence of hosts or nutrients that would sustain it. The end result was a depletion of nutrient reserves, resulting in a failure to form survival structures, leading to decreased survival. The contribution of the microflora, either as stimulants of germination or aggressive antagonists of germinated propagules, has not been determined.

A stimulation of germination was associated with the suppression of Fusarium in a coniferous forest soil. Biocidal treatments led to a decrease in macroconidial germination of Fusarium in the soil, suggesting the contribution of the microflora to this "stimulatory" suppression (Schisler and Linderman, 1984). However, abiotic soil components may play an important role in the stimulation of germination of Fusarium in coniferous forest soils. Evidence for this was stimulation of chlamydospore germination of F. oxysporum f. sp. lilii in nonsterile soil by the addition of five organic acids that were leachable from pine needles. Germination was followed by germ tube lysis with no new chlamydospore formation (Hammerschlag and Linderman, 1975). The role of soil microflora therefore is unclear. If they are not involved in germination stimulation, they could be

involved in preventing the reformation of chlamydospores. More research is needed to understand this mode of suppression in coniferous forest soils. The prevalence of germination stimulation as a mode of pathogen suppression in agricultural soils is not known, but it appears to be uncommon.

In contrast to germination stimulation, enhanced fungistasis apparently occurs in some disease-suppressive soils. This is shown by 42% germination of conidia of F. oxysporum f. sp. vasinfectum on water agar discs on "wilt-free" Kovilpatti soil, as compared with 84% and 87% germination on "wilt-sick" Palladam and Udamalpet soils, respectively (Arjunarao, 1971). Germ tubes produced in the suppressive soil were short and produced terminal chlamydospores, while germ tubes in the conducive soils were longer and produced intercalary chlamydospores. Ungerminated chlamydospores on the suppressive soil were not killed, as shown by their subsequent germination when agar discs bearing chlamydospores were transferred from soil to a glucose solution and root extract. Sterilization of the suppressive soil nullified germination inhibition, suggesting that the soil microflora are involved.

Enhanced fungistasis also was observed in a soil suppressive to bean root rot (Furuya, 1982). There was less germination of F. solani f. sp. phaseoli macroconidia

in suppressive Kitami soil, as compared with conducive Tokachi soil. Germ tubes in the suppressive soil were abnormal and lysed more quickly, and hyphal growth and formation of chlamydospores was less than in the conducive soil. Germination of chlamydospores on the laimosphere of detached bean hypocotyls was inhibited in the Kitami soil, suggesting that plant exudates could not overcome the inhibition. Suppression was biological in origin, as shown by nullification of inhibition by biocidal treatments, although a residual abiotic inhibition was suggested by a slower germination rate and abnormal germ tube morphology in these treated soils.

Fungistasis also appeared to be elevated in the Chateaurenard wilt-suppressive soil, as evidenced by greater inhibition of germination of chlamydospores of several form species of F. oxysporum and F. solani in this suppressive soil as compared with wilt-conducive soil (Alabouvette et al., 1979; 1980b) . The fungistasis could be annulled with biocidal treatments and nutrient amendments, suggesting involvement of the microflora. The requirement of more glucose to promote germination of F. oxysporum in wilt-suppressive than in conducive soils has been shown by other workers (Hwang et al., 1982; 1983). This differential germination also applies to the rhizosphere of the soils. Germination of the chlamydospores of F. oxysporum f. sp. tracheiphilum by

cotton root tips was 18% in conducive soil, but only 8% in suppressive soil (Smith, 1977). In suppressive soils, elevated fungistasis results in more intense competition for nutrients, which may restrict development of the pathogen.

There have been fewer studies on the relationship of fungistasis levels and disease suppression to pathogens other than Fusarium. In a suppressive soil, Pythium splendens sporangia coated with cucumber root extract germinated 15% compared with 90% germination in a soil conducive to P. splendens damping-off (Kao and Ko, 1982; 1983). However, this differential effect did not apply to R. solani, whose growth and the amount of mustard cabbage damping-off it caused, were identical in the P. splendens suppressive and conducive soils (Kao, 1982). Five out of 30 samples of soil were found to inhibit hyphal growth to less than 50% of that occurring in a conducive control soil, but disease suppressiveness of these soils to R. solani disease was not assessed (Ko and Ho, 1983). Chlamydospore germination of P. cinnamomi was 56% in an aqueous extract of P. cinnamomi-conducive Kioloa soil, but only 17% in an extract of suppressive Tallaganda soil (Halsall, 1982a). It appears, then, that soils suppressive to pathogens other than Fusarium are also more fungistatic to these pathogens than conducive soils, although the fungistatic effects of soils suppressive to

other pathogens are not yet known.

The long-term effects of elevated fungistasis in suppressive soils are not known. Recent work suggests that a greater loss of endogenous nutrient reserves in propagules maintained under fungistatic conditions was associated with a decrease in propagule germinability and virulence (Bristow and Lockwood, 1975; Filonow et al., 1983). This phenomenon could partially account for reduced disease in suppressive soils with a persistent, high population of pathogens. It could also account for disease suppression in soils in which the inoculum density declined, although this may reflect the operation of other types of antagonism, such as predation or hyperparasitism, in addition to competition.

Fungistasis elevation may occur with soil amendments used to induce suppression. One example is the reduction of tomato wilt, caused by F. oxysporum f. sp. lycopersici, with chicken manure. The pathogen population was not reduced, but its germination and growth were inhibited in the rhizosphere and the rhizoplane (Homma et al., 1979).

The above examples suggest that fungistasis in disease-supressive soils is either very strong or very weak. In soils with high fungistasis, the response of pathogen spores to nutrients is reduced. If these nutrients originate from a root, the consequence is a less effective inoculum which results in reduced disease. On

the other hand, a very low level of fungistasis allows precocious germination, with a consequent lower inoculum density because the pathogen becomes debilitated from unsustainable growth. If germination stimulants are involved, the identification of these chemicals and the microflora that produces them, suggest the potential for exploiting these for disease control. Soil fungistasis and disease suppression are both dependent on the soil microflora, yet the connection between disease suppression and fungistasis has not been explicitly evaluated.

With all the attention given to soil fungistasis, it must be emphasized that this phenomenon is only one aspect of a general microbiostasis operating in soil. So, while there is a relationship between the level of fungistasis imposed by a soil and disease expression in it, an intriguing question arises: can there not also be a relationship of inhibition of germination and growth in soil to suppression of diseases caused by prokaryotic as well as fungal soil-borne pathogens? An answer to this question is not possible at this time simply because there has been scant research in this area. While there are many reports of soils suppressive to fungal pathogens, there is only one report of a soil suppressive to an actinomycete, namely, Streptomyces scabies on potato (Menzies, 1959), and no reports of soils suppressive to soil-borne bacterial pathogens. As with fungi, inhibition

of germination and growth, primarily from a microbial-induced scarcity of nutrients in soil, has been shown with bacteria (Davis, 1976) and actinomycetes (Mayfield et al., 1972). It is conceivable that an enhanced microbiostasis in some soils may explain disease-suppression or inoculum decline of prokaryotic plant pathogens, but since these pathogens are of relatively minor importance as compared to fungal pathogens, this area of research has not so far garnered much attention. This is unfortunate. While such research may be of minor importance to the problems of plant pathology, it could make a significant contribution to the understanding of microbial interactions in soil.

IV. ROLE OF THE MICROFLORA IN SUPPRESSION:

It is clear that in the majority of cases of suppressiveness to a disease, as well as the elevated fungistasis associated with it, the soil microflora plays a primary role. Differences between suppressive and conducive soils usually consist of identifiable quantitative or qualitative soil microflora differences. There are exceptions, however. Burke (1965) found no specific microfloral differences between bean root rot-suppressive and -conducive soils. Rombouts (1953) found no difference between antagonism of banana rhizosphere microflora from conducive or suppressive soil. However, these negative results do not carry much weight because

the isolation techniques used by these workers may not accurately reflect active portions of the microflora that affect the pathogen.

Many workers found higher populations of a specific portion of the microflora - that which is antagonistic to the pathogen - in suppressive soils. Chet and Baker (1981) found that a soil in Colombia, suppressive to R. solani, contained 8 X 10^5 propagules (ppg) of Trichoderma hamatum. The same concentration of T. hamatum, when added to a conducive soil, rendered it suppressive. In cotton wilt-suppressive Kovilpatti soil, there was a greater number of antagonistic bacteria, fungi, and especially actinomycetes than in conducive soils (Arjunarao, 1971). Halsall (1982b) found more actinomycetes and antagonistic Streptomyces in a soil suppressive to P. cinnamomi than in a conducive soil. A soil suppressive to take-all disease supported higher populations of mycophagous amoebae in the rhizospheres and rhizoplanes of wheat plants growing in it than in conducive soil (Chakraborty, 1983). Indigenous bacteria, especially a species of Pseudomonas, were thought to be responsible for suppressiveness of a Metz sandy loam to F. oxysporum (Scher and Baker, 1980). This species of Pseudomonas, when used to infest a conducive Fort Collins clay loam, made it suppressive to flax wilt, caused by F. oxysporum f. sp. lini. Yellows of celery, caused by F. oxysporum f. sp. apii, was inhibited in the

presence of Corynebacterium sp. in soils of pH greater than 6.2 (Opgenorth and Endo, 1983). A sterilized soil, made suppressive to G. graminis by recontamination with non-sterilized soil, contained greater numbers of Trichoderma viride than its non-sterilized, take-all-conducive counterpart (Ludwig and Henry, 1943). In soil suppressive to P. cinnamomi, its host, Eucalyptus marginata, had fewer mycorrhizae associated with it than in a conducive soil (Malajczuk, 1979). Root microflora differences between suppressive and conducive soils were thought to be related to differences in the quantity of mycorrhizal roots between the two soils (Malajczuk and McComb, 1979).

In other cases, suppressiveness associated with greater numbers of microorganisms - a quantitative difference, rather than the presence of certain species, a qualitative difference - suggests enhanced nutrient competition from a larger biomass as the mode of action. Higher populations of bacteria and actinomycetes were found in Tamborine Mountain soil suppressive to P. cinnamomi root rot of avocado than in a conducive soil (Broadbent and Baker, 1974). Fungi, actinomycetes, and bacteria, when added to a sterilized soil, were all effective in reducing infection of wheat by Bipolaris sorokiniana and F. graminearum (Henry, 1931). Increased suppressiveness to Verticillium dahliae, induced by chitin

amendments, was associated with higher populations of bacteria and actinomycetes in the rhizosphere of strawberry (Jordan et al., 1972).

The opposite relationship, that disease suppression decreases with a decline in microflora, was shown with a suppressive soil stored for six months. A decline of the microbial population from 25 to 8 X 10^6 colony forming units per gram of soil was correlated with a 14% to 61% increase in germination rate of P. splendens sporangia coated with cucumber root extract and a concomitant decrease in disease suppression (Kao, 1982). A lower level of germination inhibition in subsoil as compared with topsoil was correlated with a lower total microbial population, but size of the population alone did not determine suppressiveness to P. splendens, since a re-infested conducive soil with an elevated population was still not rendered suppressive (Kao and Ko, 1983). Thus, critical analysis of the role of biomass size in disease suppression should be undertaken with only one soil, in which only the biomass is changed, while other soil characteristics are kept constant. However, this may be an unachievable ideal and so, disease and biomass comparisons between different soil types should not be condemned, as they may still illustrate valid relationships, especially with extreme differences in soils.

Interactions between a pathogen and a specific component of the microflora include not only hyperparasitism, predation, and antibiosis, but also, competition between closely-related species for the same ecological niche. This has been observed among Fusarium species. Fifty-four percent of isolations from roots of celery in areas suppressive to Fusarium yellows were infected with non-pathogenic strains of F. oxysporum while roots from areas conducive to disease had 6% incidence of saprophytes, suggesting exclusion of pathogenic Fusarium from infection sites by saprophytic Fusarium in suppressive soils (Schneider, 1983). Additional evidence for this is given in preliminary results, where the incorporation of some of these saprophytes into soil gave almost complete control of yellows of celery. Saprophytic F. oxysporum were also considered important in Chateaurenard soils suppressive to Fusarium wilts (Alabouvette et al., 1979). Melon root microflora in suppressive soil consisted primarily of Fusarium species, especially F. oxysporum (Alabouvette, 1983). Additional evidence for the role of non-pathogenic analogs of pathogens was the re-establishment of Fusarium wilt suppression in steamed soil with addition of saprophytic F. oxysporum and F. solani, but not with other fungi (Rouxel et al., 1979). One attribute of a successful competitor is a superior survival rate. In soil

suppressive to F. solani root rot of bean, macroconidia of F. solani f. sp. phaseoli declined to 0.1% of the original concentration in suppressive Kitami soil, but macroconidia of non-pathogenic F. solani declined to only 25% of the original in the same time period (Furuya, 1982). This suggests that in a suppressive soil, there will possibly be more saprophytic than pathogenic Fusaria to compete for the same infection sites. Competition between F. roseum and other fungi for nutrients in a specific niche was thought to be the mechanism of lentil disease suppression, as the introduction of actinomycetes and bacteria had no effect on disease (Lin and Cook, 1979). Apparently, saprophytic Fusarium spp. are better competitors for low concentrations of nutrients than pathogenic Fusarium spp.. For example, germination of chlamydospores of saprophytic isolates of F. oxysporum in unamended suppressive soil occurred more readily than germination of pathogenic F. oxysporum form species (Alabouvette et al., 1980b). In another wilt-suppressive soil, Fusarium saprophytes germinated better than pathogens in the presence of glucose and asparagine amendments (Smith and Snyder, 1972).

While there are many examples of a specific portion of the microflora, such as hyperparasites of the pathogen, responsible for disease suppression, competition for nutrients between the pathogen and either the general

microflora or a specific portion of it is probably a common mechanism of suppressiveness. Knowledge of the specific or general microflora that imposes suppressiveness, of the location of the pathogen-microflora interaction and, in the case of competition, the nature of substrates that generate the competition, are prerequisites for manipulation of suppression.

V. COMPETITION FOR NUTRIENTS IN SUPPRESSIVE SOILS:

The importance of nutrient competition on disease incidence was considered as long ago as 1931 by Henry who ascribed reduced disease incidence of B. sorokiniana on wheat in recontaminated sterilized soil to "exhaustion of food supplies". In natural soils, suppression of Fusarium wilt diseases has been attributed to unsuccessful competition of pathogenic F. oxysporum with soil microflora for carbon sources (Hwang et al., 1982; Smith and Snyder, 1972). Suppression of F. roseum on lentils in cellulose-amended soil is probably a result of competition for limiting nitrogen (Lin and Cook, 1979). Suppression of F. solani f. sp. phaseoli root rot of bean in cellulose-amended soil is also thought to occur through competition for limiting nitrogen (Baker and Nash, 1965).

Much attention has been given to iron as a limiting substrate. Successful competition for iron is thought to hinge on the ability to produce a siderophore that has a greater affinity for Fe^{3+} than the siderophore of

another competitor. In this respect, pathogenic Fusaria are poor competitors, while certain fluorescent Pseudomonads are better competitors. Thus, with the addition of a fluorescent Pseudomonas sp., or its siderophore, pseudobactin, a soil was made suppressive to F. oxysporum f. sp. lini and G. graminis (Kloepper et al., 1980). The pseudobactin bound available iron making it unavailable for pathogen utilization, although the pseudobactin-bound iron can be readily utilized by the Pseudomonas sp.

Metal chelators have been used instead of siderophores to manipulate suppressiveness. For example, the addition of Fe-EDDHA to soil conducive to radish wilt, caused by F. oxysporum f. sp. conglutinans, rendered it suppressive (Dupler et al., 1982). On the other hand, EDTA made a flax wilt-suppressive soil more conducive, presumably because it had less affinity for iron than the siderophore of F. oxysporum f. sp. lini (Scher and Baker, 1983).

The relationship of disease suppression manifested by iron competition and soil fungistasis is not clear. Germination of microconidia of F. oxysporum f. sp. lini was not inhibited by iron chelators, but germ tube elongation was inhibited. This growth inhibition was reversible with $FeCl_3$ amendments (Scher and Baker, 1982). NaFe-EDDHA had no effect on germination of Cochliobolus

victoriae and Botrytis cinerea in soil (Lockwood and Schippers, 1984). These results suggest that iron competition is not involved in propagule germination inhibition, but the role of iron in other phases of pathogen development should not be excluded. The contribution of competition for other elements to disease suppression has not been investigated.

The most important - and unanswered - question is whether the same nutrients are involved in competition leading to disease suppression as are involved in the competition resulting in fungistasis. If the same nutrients are involved, fungistasis assay, such as by nutrient titration, could be a short-cut for testing soils for relative disease suppressiveness (Ko and Ho, 1983). If different nutrients are involved, this would provide an explanation for a lack of correlation between amount of disease and levels of fungistasis.

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INTRODUCTION

In recent years, attempts have been made to identify the active components of "disease-suppressive" soils, with the ultimate objective of understanding how to manipulate "disease-conducive" soils so that disease is reduced in them (Cook and Baker, 1983; Schneider, 1982; Schroth and Hancock, 1982). Many workers studying suppressive soils have found that they impose a greater degree of fungistasis than disease-conducive soils, as evidenced by the higher quantity of nutrients required to annul germination inhibition in the disease-suppressive soils (Alabouvette, 1983; Arjunarao, 1971; Furuya, 1982; Hwang, 1982, 1983; Kao, 1982; Smith, 1977). With this possible relationship of elevated fungistasis and increased disease-suppressiveness in mind, Ko and Ho (1983) have proposed using a quantitative assay of soil fungistasis as a simple, rapid method for identifying suppressive soils.

The objective of this research was to determine the relationship between the degree of fungistasis imposed by three Michigan soils and the incidence or severity of soil-borne diseases occurring in them. Relative disease suppressiveness of these soils had not been previously determined, although they differed in their degree of fungistasis against Bipolaris victoriae and Mortierella

sp., as determined by adding different quantities of a glucose and peptone mixture to the soils (Lee and Lockwood, unpublished data). The hypothesis was that highly fungistatic soils should have lower disease incidence or severity than less fungistatic soils.

Relative disease suppressiveness was determined by adding propagules of four pathogens in increments to these soils and comparing disease incidence or severity of a susceptible host planted in them. Fungistasis imposed by the soils on these pathogens was quantified by measuring the degree of germination promotion by adding different quantities of a glucose and peptone mixture to the soils. Because fungistasis is microbial in origin, the qualitative microbial composition and the total microbial biomass were compared among the three soils. Also, the ability of the microflora of these soils to utilize added glucose was compared.

MATERIALS AND METHODS

Characterization of Soils

Three different agricultural soils from Michigan were used (Table 1). Soils were taken from the field, partially air-dried, passed through a 2 mm-mesh sieve and stored in large plastic bags at 4°C for the duration of all work. Elemental analyses, cation exchange capacity, and organic matter content were performed by the Soil Testing Laboratory, Michigan State University. Textural analyses were done by the hydrometer method (Day, 1965). The pH was measured by immersing a glass electrode into the supernatant of a 2:1 (v/w) mixture of 0.01 M $CaCl_2$:soil (Peech, 1965). Moisture characteristic curves for the soils were determined using a pressure-plate apparatus (Richards, 1965).

Soils were planted to radish (Raphanus sativus L. cv. 'Cherry Belle'), wheat (Triticum aestivum L. cv. 'Waldron'), tomato (Lycopersicum esculentum Mill. cv. 'Bonny Best'), and pea (Pisum sativum L. cv. 'Wando') to detect the presence of indigenous soil-borne pathogens. No indigenous pathogens were detected in any of the soils.

Microbial Characteristics of Soils

Microbial populations in soils were estimated by dilution plate counts, using serial dilutions made in 0.1% agar water blanks. The following media were used: trypticase soy broth agar (Martin, 1975), containing 50

Table 1. Characteristics of soils

Soil	%								PPM							
	Moisture*	Sand	Silt	Clay	Organic matter	Total nitrogen	pH	C.E.C.+ MEQ/100 G	P	K	Ca	Mg	Fe	Zn	Mn	Cu
Boyer Sandy Loam	3.7	77	6	15	2.26	0.04	6.3	6	151	143	2100	149	36	7	14	1
Capac Loam	11.6	43	32	24	5.26	0.12	5.4	17	247	304	3771	507	48	14	16	7
Colwood Loam	19.8	47	32	20	4.40	0.25	5.9	9	120	312	2200	364	44	7	16	3

*Stored soil

+C.E.C.=cation exchange capacity

ppm PCNB to inhibit actinomycetes (Farley and Lockwood, 1968), for aerobic bacteria; colloidal chitin-mineral salts agar (Hsu and Lockwood, 1975), for actinomycetes; and peptone-dextrose-rose bengal agar (Martin, 1950), for fungi. A bent glass rod was used to spread 0.2 ml of soil suspensions over the surface of the agar. Plates were incubated at 25° ±1°C for 5-7 days for bacteria and fungi and 10 days for actinomycetes. Anaerobic bacteria were estimated using the most-probable number method (Koch, 1981). One-ml aliquots from a two-fold dilution series were dispensed into 10, 15 cm X 1 cm screw cap test tubes containing 11 ml fluid thioglycollate medium (Difco, Detroit, MI 48226). Determinations were repeated at least once.

Populations of form species of F. oxysporum added to soil were determined by spreading 0.2 ml aliquots of soil dilutions onto Komada's medium (Komada, 1975). Plates were incubated under fluorescent lights ($63 \mu Em^{-2}S^{-1}$) at 25°C ±1°C for one week. Plates inoculated with pure cultures of F. oxysporum inocula were included for comparison of colony types to aid identification and dilutions from noninfested soils were included as controls.

Total soil biomass was determined using the chloroform fumigation method (Jenkinson and Powlson, 1976), with certain modifications. Soils were adjusted to

a matric potential of -30 kPa (-0.3 bars) before fumigation by spraying water onto soils with an atomizer. Soils were continuously agitated to ensure even wetting. Soils were incubated in closed 3.75 l (1 gallon) jars with 0.01 M NaOH to trap carbon dioxide. After incubation, 5 ml saturated $BaCl_2$ and 0.2 ml 1% phenolphthalein in 95% ethanol were added to the trap before titration with 0.01 M HCl. The weight of CO_2-C was calculated from the formula, mg CO_2-C = M HCl X (standard - sample) X 12, where "standard" represents the volume of acid in ml required to titrate base from incubation jars without soil, and "sample" represents the volume of acid required to titrate base from incubation jars containing fumigated or unfumigated soil. These values were used to calculate total carbon biomass according to the formula of Jenkinson and Powlson (1976), except that 0.411 was used for K, the proportion of biomass carbon mineralized (Anderson and Domsch, 1978). Soil biomass was determined twice.

Maintenance of Fungi

Cochliobolus victoriae Meehan and Murphy isolate CV_3 and C. sativus (Ho and Kurib.) Drechsl. ex Dastur isolate T_2T_1 were maintained on carrot agar containing per liter: 250 ml of carrot broth (30 g carrots in 250 ml water, autoclaved 10 min and filtered) and 20 g agar. Fusarium oxysporum Schlecht. f. sp. lycopersici (Sacc.) Snyd. and

Hansen isolate R5-6, F. oxysporum Schlecht. f. sp. conglutinans (Wr.) Snyd. and Hansen, and F. solani (Mart.) App. and Wr. f. sp. pisi (F.R. Jones) Snyd. and Hansen isolate F_{116} were stored in sterilized soil (Toussoun and Nelson, 1976). Single-spored isolates were grown on potato-dextrose broth containing per liter: 500 ml potato broth (200g potatoes in 500 ml water, autoclaved 30 min and strained through cheesecloth), 20g dextrose and 20g agar.

Production of Inoculum

Cochliobolus sativus: Inoculated carrot agar plates were incubated in the dark at 20°C for four weeks. Plates were flooded with distilled water and conidia were dislodged using a glass rod. The suspension was passed through a 153 mm sieve and concentrated to a volume of 30 ml by centrifugation. Viability was determined by counting germination of conidia incubated 6 hours on potato-dextrose agar.

Fusarium spp.: Chlamydospores were produced aseptically by growing mycelia in flasks containing 100 ml potato-dextrose broth. Mycelia were ground 30 sec using an Omni-Mixer (Ivan Sorvall, Norwalk, CT 06856) set at 9,600 rpm and washed by centrifugation three times. Mycelial fragments were placed in flasks containing 50 ml of salt solution (Quereshi and Page, 1970) and incubated at 25° ±1°C and ambient light on a rotary shaker for 4

weeks. Aggregates were then washed, ground with an Omni-Mixer for 3 minutes and chlamydospores in the suspension were enumerated with a haemocytometer.

Chlamydospores were also induced in the presence of soil. Mycelial fragments were prepared as previously described and incorporated into approximately 1 kg Boyer sandy loam (5% moisture content). The infested soil was placed in a shallow tray covered with aluminum foil and incubated 10 days. After this time, chlamydospores in mycelial fragments were seen in soil smears and the soil was allowed to air-dry. Population density was determined by dilution-plating the soil on Komada's agar. Inoculum produced in this manner was used only for disease assays in soils.

Assays of Disease

Fusarium oxysporum f. sp. *lycopersici*: Chlamydospores produced *in vitro* were added to 3 kg aliquots of sterilized and non-sterilized soil to give densities of 10,000, 25,000 and 50,000 propagules per gram (ppg). Soils were sterilized by autoclaving for 2 hours. Each aliquot of infested soil was distributed into 20, 6 cm diameter X 7 cm waxed paper cups, into which were transplanted 9-day-old 'Bonny Best' tomato seedlings. Seedlings were grown in a greenhouse at 18° to 24°C and were fertilized weekly with 2.5 gl^{-1} 20-20-20 Peters Soil

Test Fertilizer (Robert B. Peters Co., Allentown, PA 18105). Wilt incidence was recorded weekly. Plants were harvested after six weeks. Fresh shoot weight and shoot length were measured. Stems were cut longitudinally and the proportion of each stem length with vascular necrosis was rated as follows: 1=no necrosis; 2=necrosis confined to the taproot, below the soil line; 3=up to 50% necrosis of the stem length; 4=50-75% necrosis of the stem length; 5=more than 75% necrosis; 6=dead plant. Segments from symptomless plants, cut from the cotyledonary node to 1.5 cm above it, were surface-sterilized and plated on acidified potato-dextrose agar (10 ml 25% lactic acid added to one liter of medium after autoclaving). The experiment was repeated using 5.8×10^3, 2.9×10^4 and 1.45×10^5 ppg of chlamydospores induced in Boyer sandy loam, with 27 plants per treatment.

Fusarium solani f. sp. pisi: Chlamydospores produced in vitro were incorporated into 10 kg aliquots of each soil to obtain densities of 500 and 1000 ppg. Aliquots were distributed into five, 11 cm diameter X 15 cm waxed paper cups. Four, 5-day-old 'Wando' pea seedlings, grown in vermiculite, were transplanted into each container. Plants were grown in a growth chamber at 25°C with a 14 hour photoperiod and a quantum flux denisty (400-700 nm) of 240 $\mu Em^{-2}S^{-1}$. After four weeks, root, shoot and lesion lengths were measured and shoots were weighed.

Disease was rated, based on the sum of foliage, cotyledon and root ratings of each plant as follows: Foliage: 0=healthy, 1=wilt/some dead leaves, 2=dead; Cotyledon: 0=healthy, 1=trace necrosis, 2=extensive necrosis; Roots: 0=healthy, 1=less than 25% necrosis, 2=25-50% necrosis, 3=more than 50% necrosis. A similar experiment was performed with transplants grown in 2.5 cm diameter X 12.5 cm plastic containers containing soil infested with 500 or 5000 ppg. These containers were watered from below via holes in the bottom, by periodically setting them in a water bath.

Fusarium oxysporum f. sp. conglutinans: Chlamydospores produced in Boyer sandy loam were used to infest 1 kg soil aliquots to obtain densities of 160, 800, 4000, 20,000, and 100,000 ppg. Each aliquot was distributed into four, 11.5 cm diameter X 5 cm waxed paper cups. Fifteen 'Cherry Belle' radish seeds, covered by 40 cc infested soil, were thinned to 12 seedlings after 5 days. Plants were grown in a greenhouse at 18° to 24°C. Plants dying from wilt were counted and removed from pots. Plants surviving after three weeks were harvested and upper stem segments were surface-sterilized and plated on acidified potato-dextrose agar. A similar experiment was performed using chlamydospores produced in vitro, then added to soils to give a density of 100,000 ppg.

The effect of a constant, -10 kPa (-0.1 bars) soil

matric potential on radish wilt incidence in the three soils was determined using tension plates (Duniway, 1976). Soil was infested with 20,000 and 100,000 ppg of F. oxysporum f. sp. conglutinans and 125 g (oven-dry weight) was placed in each 97 mm internal diameter (ID) Buchner funnel with a fritted glass plate of fine porosity, while 60 g was placed in each 67 mm (ID) funnel. Five replications were used with small funnels and three with large funnels. Soil was saturated with water and equilibrated overnight before planting radish seeds. Funnels were kept covered with plastic film. After four days, seedlings were thinned to 10 in small funnels and to 20 in large funnels. Plants were grown in a growth chamber at 28°C with a 14 hour photoperiod and a quantum flux density (400-700 nm) of 240 $\mu Em^{-2}s^{-1}$. The number of plants dying was recorded every second day for three weeks. The experiment was repeated several times.

Cochliobolus sativus: Conidia were applied to 250g aliquots of soil by spraying a conidial suspension into the soil in a plastic bag, using an atomizer. Sufficient volume was applied to obtain 750, 3750 and 18,750 conidia per g (oven-dry weight). Soils sprayed with water only served as controls. Spring wheat seeds (cv. 'Waldron') were surface-disinfested 5 min in 0.1% $AgNO_3$, rinsed twice in 0.5% NaCl, rinsed with distilled water and soaked in water three hours. Five cc sand was placed in a 1.5 cm

diameter X 10 cm plastic tube with a bottom drainage hole and wetted. One seed was placed on top of the sand and was covered to the top of the tube with soil. Tubes were placed in plastic bags for five days, which was the time required for coleoptile emergence. Tubes were watered from the top with a squeeze bottle to compact the soil at emergence, the sides were covered with aluminum foil to keep light away from the sub-crown internode, and each inoculum density for each soil type used was divided into four blocks of six seedlings each. Different blocks contained the same number of seedlings which were of similar size. Blocks were randomized in racks, which were kept in trays. Plants were grown at 25° ±1°C under fluorescent light with a 14 hour photoperiod and a quantum flux density (400-700 nm) of 63 $\mu Em^{-2}S^{-1}$, and were watered from the bottom as needed, by periodically setting trays in a water bath. After four weeks, the necrotic lesions on leaves were counted and the sub-crown internodes were rated as follows: 1=no symptoms; 2=slight disease (some necrotic flecks); 3=light disease (some necrotic portions); 4=moderate disease (necrosis with some healthy portions); 5=severe disease (extensive necrosis). The experiment was repeated once.

Quantitative Determination of Fungistasis

Propagules borne on 1 cm^2, 0.4 μm pore size polycarbonate membrane filters (Nuclepore Corp., Pleasanton, CA 94566) were placed on sterilized soil and natural soil amended with increasing amounts of glucose and peptone. Five-g samples of soils in 6-cm-diameter plastic dishes were wetted to -50 kPa (-0.5 bars) matric potential and were equilibrated for 16 to 24 hours before adding nutrients. Glucose concentrations ranged from 75 to 4800 g per gram of soil and peptone concentrations were 0.2 (w/w) that of glucose. The nutrients were added in a volume of water that brought all soils to -10 kPa (-0.1 bars). The soil was stirred and smoothed with a spatula and four membrane filters per species of fungus were placed on the surface. In order to detect fungistasis of abiotic orgin, filters bearing propagules were placed on wetted soils in glass dishes, which had been sterilized by autoclaving one hour. After 12 hours incubation for Fusarium spp. and 6 hours incubation for Cochliobolus spp., filters bearing these propagules were stained with phenolic rose bengal, destained in water and mounted on glass slides. Filters bearing Fusarium spp. were completely dried, cleared with a drop of clove oil, covered with a glass coverslip, and viewed with transmitted light at 150 X magnification. Conidia of Cochliobolus spp. were viewed on a white background with

incident illumination at 150 X magnification. Assays were repeated at least once.

Soil Solution Assay

The procedure of Filonow and Lockwood (1983) was used. Soils were wetted to -5 kPa (-0.05 bars) matric potential and equilibrated 24 hours before centrifugation at 5°C and 3000 X *g* for 40 min. Soil solutions were filter-sterilized (0.2 m pore diameter Nuclepore filters) and 0.1 ml aliquots were placed into double-welled microscope slides enclosed in Petri dishes. Nuclepore filters bearing fungal propagules were floated on the soil solutions. Incubation and examination of propagules were the same as previously described. Two experiments were performed.

Bioassay for Volatile Inhibitors

Twenty g (oven-dry weight) of soil was equilibrated 24 hours in a glass Petri dish at -5 kPa (-0.05 bars) matric potential. One-tenth ml aliquots of sterile, 1:100 solution (Bristow and Lockwood, 1975) were placed in depressions on a plastic plate suspended 2 mm above the soil surface. Nuclepore membrane filters bearing fungal propagules were floated on the Pfeffer's solution and the dishes were sealed with Parafilm (American Can Co., Greenwich, CT 06830). Incubation and examination of propagules were the same as previously described. To

ensure that this procedure was effective for detecting volatile spore germination inhibitors, filters bearing propagules were incubated over soil on which one drop of concentrated ammonium hydroxide (as a source of ammonia) was placed. The experiment was repeated once.

Soil Respiration of Glucose:

At -1 kPa (-0.01 bars) matric potential: The procedure of Filonow and Lockwood (1979) was used. Four-g aliquots of soil were pulsed with 0.05 μCi (1 μCi=37MBq) of uniformly labeled [^{14}C]-glucose (specific activity, 200 mCi/mM) and respired [^{14}C]-CO_2, collected in an ethanolamine scintillation cocktail at two-hour intervals for 8 hours, was measured using a liquid scintillation spectrometer. Counting was corrected using a [^{14}C]-toluene internal standard. Three replicates per soil were used and the experiment was repeated four times.

At -30 kPa (-0.3 bars) matric potential: One hundred g (oven-dry weight) soil samples in 400 ml beakers were equilibrated overnight at -30 kPa before adding 1% (w/w) glucose. Soils were incubated 48 hours in sealed 3.75 l (1 gallon) jars containing a beaker with 100 ml 0.25 M NaOH. Carbon dioxide production was determined by titration as previously described. The experiment was repeated using 100 ml 0.1 M NaOH titrated after 6, 12, 24 and 48 hours.

RESULTS

Disease Incidence in Three Soils in Relation to Their Level of Fungistasis

Fusarium oxysporum f. sp. *conglutinans*: In a preliminary experiment, radish mortality 25 days after planting was 78% in Boyer sandy loam, which was significantly greater (P=0.05) than that in Capac loam (44%) (Table 2). Colwood loam was intermediate (62%), but did not differ significantly (P=0.05) from the other two. The addition of the same quantity of inoculum to these soils which had previously been sterilized by autoclaving resulted in significantly (P=0.05) higher mortality than in the unsterilized soils, but mortality among the sterilized soils did not differ significantly (P=0.05) (Table 2). This preliminary experiment thus showed that the natural microflora of these soils suppress disease and the degree of this biologically-mediated suppression differed among the soils.

These results were confirmed in another experiment, in which development of radish wilt, first occurring 13 days after seeding, differed among the soils over the course of the experiment, 21 days (Figure 1). Mortality was significantly greater (P=0.05) in Boyer sandy loam than in Capac loam or Colwood loam after 16 days and remained so for the duration of the experiment.

Table 2. Incidence of Fusarium wilt of radish in three non-sterilized and sterilized soils, after 25 days[y].

Soil	Disease Incidence, %[z]	
	Non-sterilized soil	Sterilized soil
Boyer sandy loam	78 b	100 a
Capac loam	44 a	84 a
Colwood loam	62 ab	100 a

[y] Mean values, based on 4 replicates of 12 plants each per treatment.

[z] Based on mortality. Numbers within a column followed by the same letter are not significantly different ($P=0.05$) using Duncan's multiple range test.

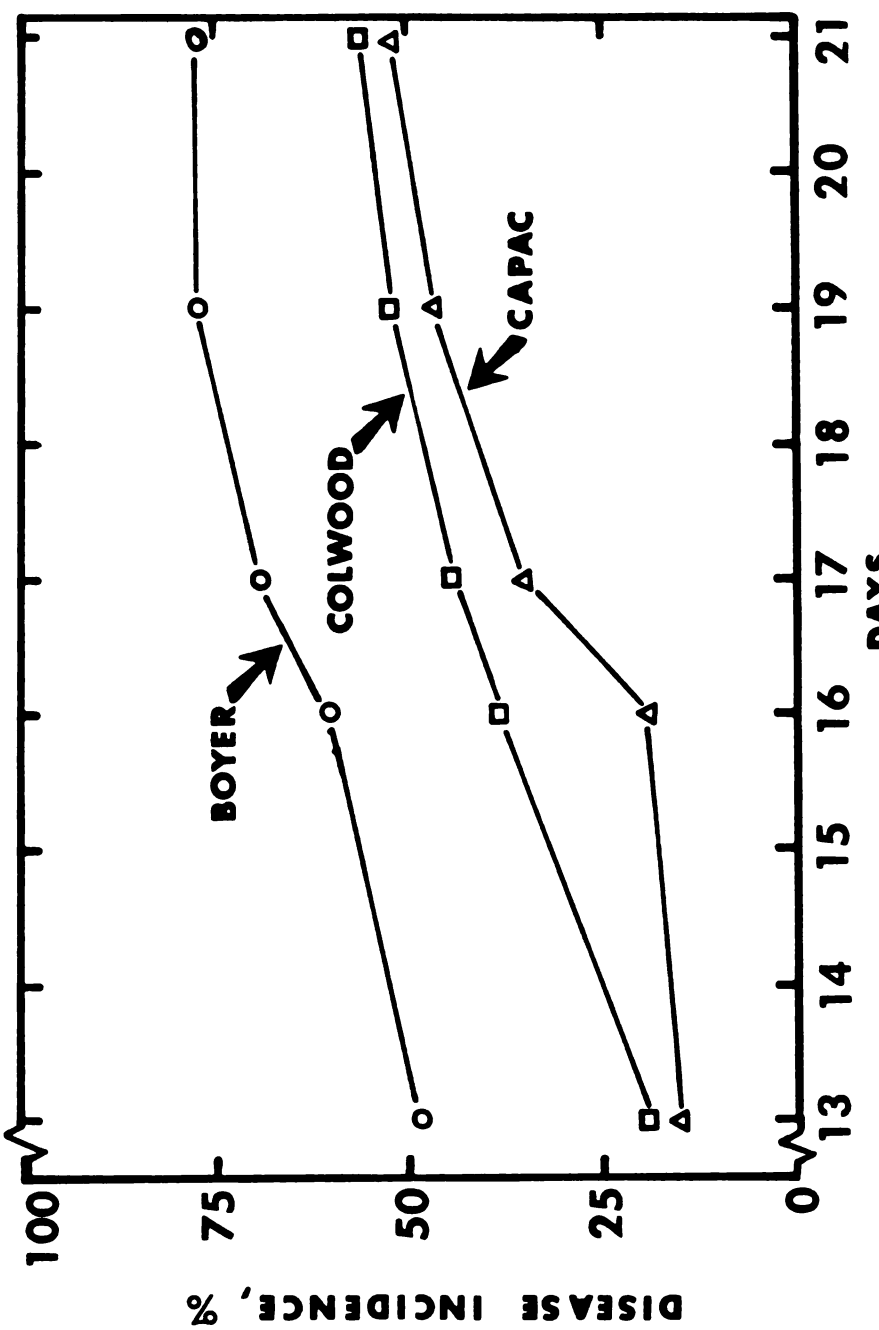

Figure 1. Effect of chlamydospores (2 X 10^5 per gram of soil) of Fusarium oxysporum f. sp. conglutinans on wilt incidence (mortality) of radish in three soils. Data represent mean values, based on 4 replicates of 12 plants each per treatment. Wilt incidence after 13 days was significantly greater (P=0.05) in Boyer sandy loam than in the other soils.

Mortality differed among soils when different inoculum densities were used. In one experiment, mortality was significantly greater (P=0.001) in Boyer sandy loam than in Capac loam at 100,000 propagules per gram (ppg) (Figure 2). Boyer sandy loam also had the greatest mortality among the soils at 20,000 ppg, 4,000 ppg, and 800 ppg, but the differences were not statistically significant (P=0.05). Populations of the pathogen, monitored over the course of the experiment in soils in which 100,000 ppg and 20,000 ppg had been added, either increased or remained constant (Table 3). Although initial recovered populations of the pathogen were similar in Capac loam and Colwood loam, the initial recovered population in Boyer sandy loam was approximately one-half that of the other soils. In all three soils, the populations of the pathogen enumerated by dilution-plating at the start of the experiment ranged from 10-58% of that estimated to have been originally added.

In greenhouse and growth chamber experiments, pots of radish seedlings were watered as needed. Since pots containing Boyer sandy loam tended to dry more quickly than other soils, experiments were performed in which all soils were maintained at -10 kPa (-0.1 bar) matric potential to determine if the greater likelihood of moisture stress in Boyer sandy loam could have accounted for the greater mortality in this soil than in the other two soils. In

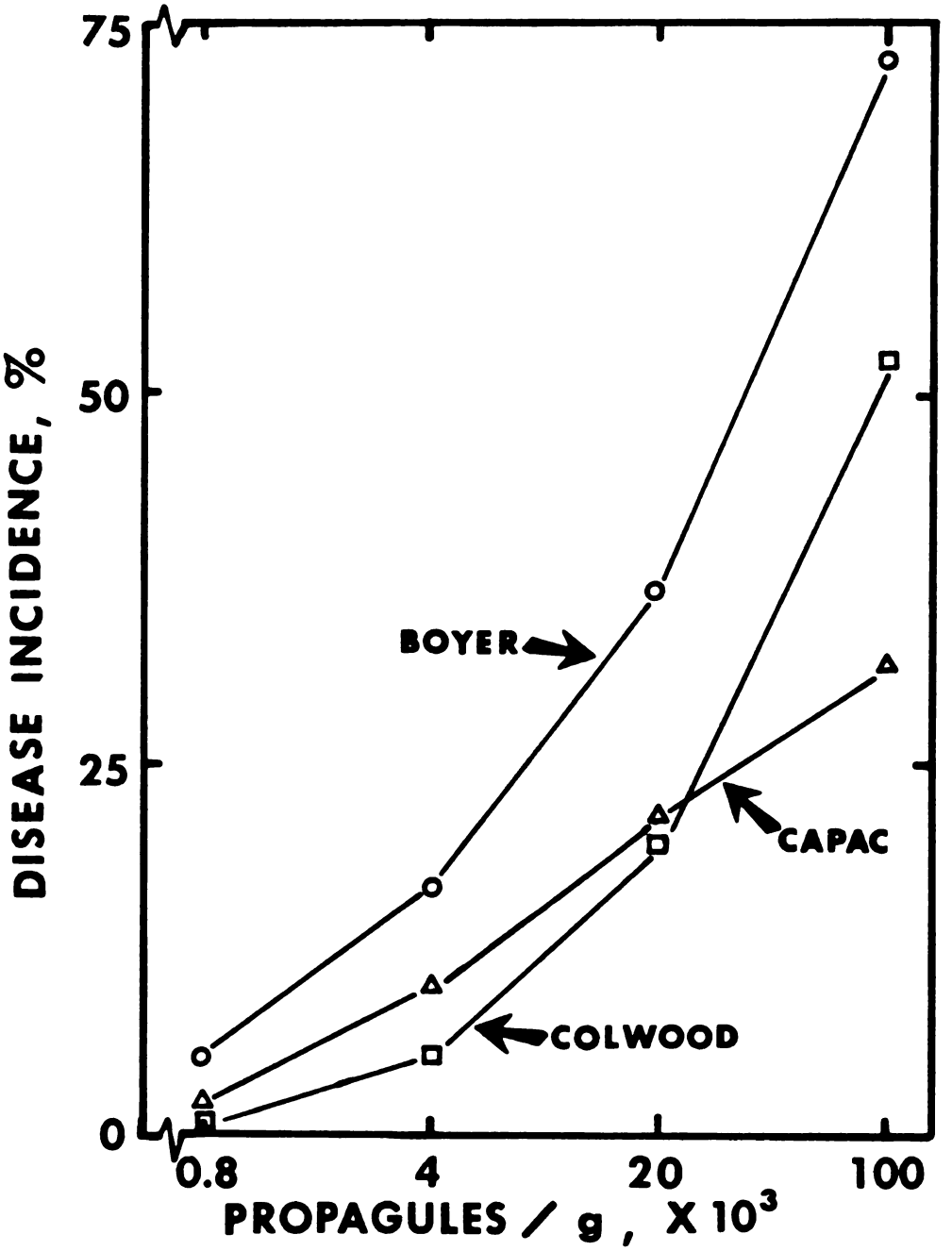

Figure 2. Effect of five inoculum densities of Fusarium oxysporum f. sp. conglutinans on wilt incidence (mortality) of radish in three soils, 24 days after planting. Data represent mean values, based on 4 replicates of 12 plants each per treatment. Wilt incidence at 100,000 propagules per gram of soil was significantly greater ($P=0.001$) in Boyer sandy loam than in the other soils.

Table 3. Recovered populations of *Fusarium oxysporum* f. sp. *conglutinans* from three soils infested with 20,000 and 100,000 propagules per gram (ppg), at the time of seeding and 24 days after seeding to radish[y].

	Colony forming units · g^{-1} soil, X 10^3			
	20,000 ppg		100,000 ppg	
Soil	0 days	24 days	0 days	24 days
Boyer sandy loam	2.6 b	5.1 a	27.2 b	59.9 a
Capac loam	5.7 a	9.2 a	57.6 a	48.4 a
Colwood loam	4.1 ab	8.2 a	55.0 a	65.3 a

[y] Mean values, based on 5 plates per treatment.

[z] Numbers within a column followed by the same letter are not significantly different ($P=0.05$) using Duncan's multiple range test.

these experiments, radish mortality was approximately twice as great (P=0.05) in Boyer sandy loam as in Capac loam at inoculum densities of 100,000 ppg and 20,000 ppg (Table 4). Populations of the pathogen, monitored in Boyer sandy loam and Capac loam to which 20,000 ppg had been added, had increased in both soils after 20 days, but there was no significant (P=0.05) difference between the final populations in these soils. Thus, these experiments demonstrated a significantly greater disease incidence in Boyer sandy loam than in Capac loam when matric potential was kept constant at -10 kPa (-0.1 bars).

In several experiments, the level of fungistasis was consistently highest in Colwood loam and lowest in Capac loam, as determined by the nutrient titration technique. For example, with the addition of 300 µg glucose and 60 µg peptone per gram of soil, germination of chlamydospores was significantly (P=0.05) higher in Capac loam than in Boyer sandy loam (Figure 3). Colwood loam had the lowest (P=0.05) germination of the soils. Thus, relative levels of fungistasis differed among the soils, but were not correlated with relative incidences of radish wilt.

Fusarium oxysporum f. sp. *lycopersici*: Disease incidence, based on wilting and vascular discoloration, was highest in Capac loam, followed by Boyer sandy loam, and

Table 4. Incidence of radish wilt in two soils infested with two concentrations of *Fusarium oxysporum* f. sp. *conglutinans* and maintained at -10 kPa (-0.1 bars) matric potential, and recovered populations of the pathogen, both three weeks after planting.

	Disease incidence, %[y]		Colony forming units of *Fusarium*,· g^{-1} soil, X 10^{3}[z]	
Soil	2 X 10^4 ppg	10^5 ppg	0 days	21 days
Boyer sandy loam	83	97	5.5	8.3
Capac loam	48	47	8.1	12.2

[y] Based on mortality. Mean values, based on 5 replicates of 6 plants each at 2 X 10^4 ppg and 3 replicates of 20 plants each at 10^5 ppg. Mean values within each column are significantly different (P=0.05) using Student's t-test.

[z] Mean values based on 5 replicates per treatment.

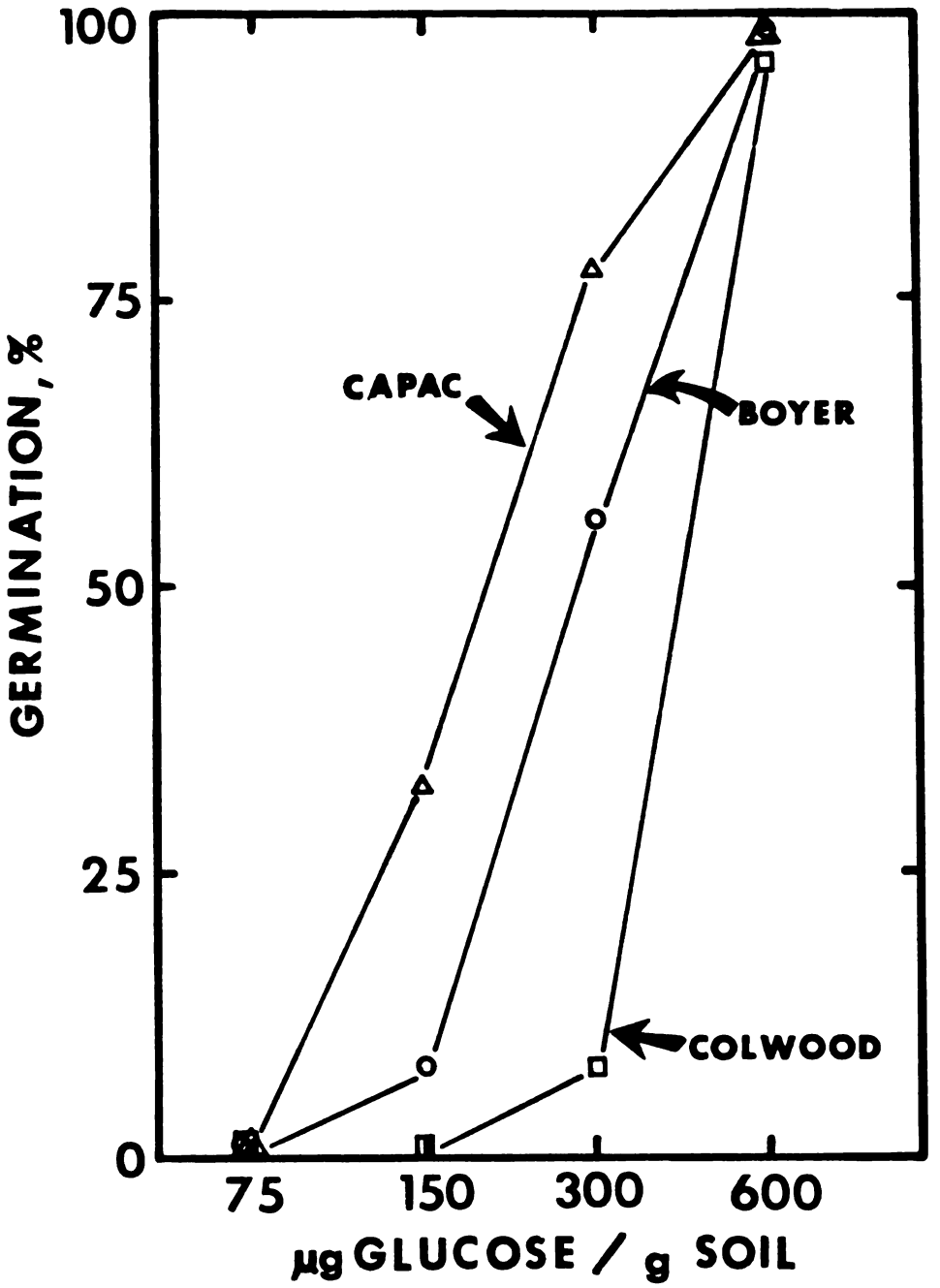

Figure 3. Germination of chlamydospores of Fusarium oxysporum f. sp. conglutinans on three soils amended with different concentrations of 5:1 (w/w) glucose:peptone, after 12 hours. Data represent mean values, based on 4 replicates per treatment; 100 spores were counted per replicate. Duncan's multiple range test showed the followng differences among means: 150 µg glucose/soil, Boyer^{b}, Capac^{b}, and Colwood^{a}; 300 µg glucose/soil, Boyer^{b}, Capac^{c}, and Colwood^{a}. Mean values of soils followed by the same letter did not differ significantly (P=0.05).

then Colwood loam (Figure 4). This trend was observed at all three inoculum densities used, but disease incidence was significantly (P=0.01) lower in Colwood loam than in the other soils only at an inoculum density of 25,000 chlamydospores per gram. Plants grown in Capac loam showed greater reduction of shoot height and weight in pathogen-infested soil, in relation to that in non-infested soils, than those in Colwood loam and Boyer sandy loam (Table 5). The proportions of vascular discoloration in stem lengths did not differ among the soils, indicating that this parameter was not useful for disease evaluation (Table 5). In a repeated experiment using 5,800, 29,000 and 145,000 ppg, no wilt symptoms were evident. The mean incidence of vascular discoloration was 1% in Colwood loam and Boyer sandy loam, as compared with 6% in Capac loam, but because of the low frequency of disease, these differences were not statistically significant. Thus, the ranking of disease incidence in these soils, with Capac loam the highest and Colwood loam the lowest, must be considered tentative. In other experiments, disease incidence in sterilized soils containing only 1,000 chlamydospores per gram was 100%. Thus, the presence of the natural microflora in these soils results in disease suppression and accounts for differences in disease incidence among the soils.

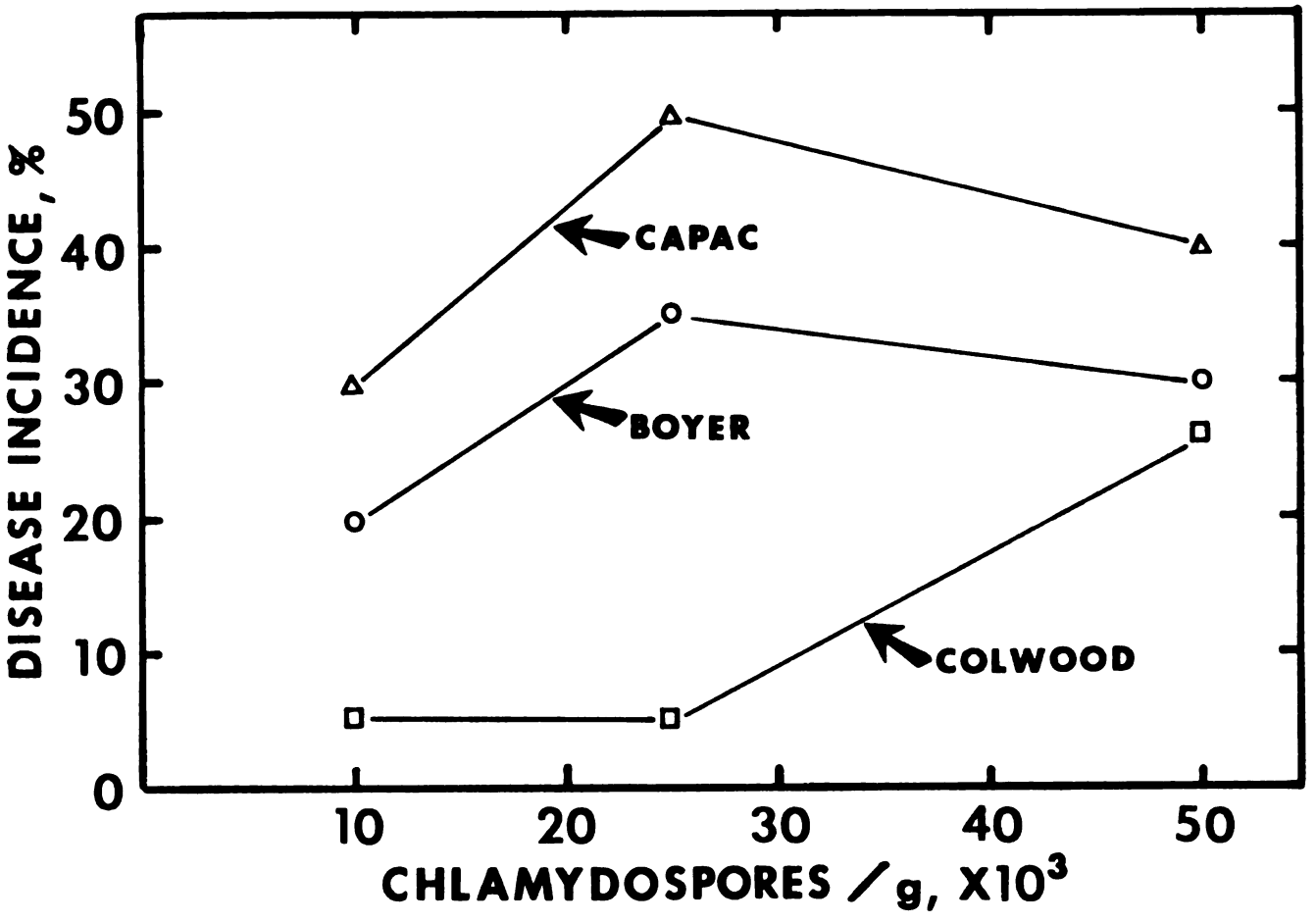

Figure 4. Effect of three inoculum densities of Fusarium oxysporum f. sp. lycopersici on wilt incidence of tomato in three soils, 6 weeks after transplanting. Data represent total incidence in 20 plants per treatment. Wilt incidence was significantly lower ($P=0.05$) in Colwood loam than in the other soils at 25,000 chlamydospores per g using chi-square analysis.

Table 5. Effect of Fusarium oxysporum f. sp. lycopersici at three inoculum densities (ppg) on tomato shoot length, fresh shoot weight, and vascular necrosis in three soils, 6 weeks after transplanting[y].

Soil	Shoot length, cm				Fresh shoot weight, g				Vascular necrosis[z]			
	0	10^4	2.5 X 10^4 (ppg)	5 X 10^4	0	10^4	2.5 X 10^4 (ppg)	5 X 10^4	0	10^4	2.5 X 10^4 (ppg)	5 X 10^4
Boyer sandy loam	9.1a	7.6a	7.6a	7.2a	2.9a	2.4a	2.7a	2.1a	1	1.7	2.1	2.0
Capac loam	11.2a	9.0 b	8.9 b	9.0 b	5.2 b	3.3ab	3.6ab	4.2 b	1	2.2	2.5	1.9
Colwood loam	10.2 b	9.5 b	9.9 b	9.0 b	4.7 b	4.2 b	4.1 b	4.1 b	1	1.1	1.1	1.8

[y] Mean values, based on 20 plants per treatment. Numbers within a column followed by the same letter are not significantly different ($P=0.05$) using Duncan's multiple range test. Analysis of variance revealed no significant differences ($P=0.05$) in mean ratings of vascular necrosis among soils.

[z] Severity of vascular necrosis: 1=no necrosis, 2=necrosis confined to taproot, 3=up to 50% stem necrosis, 4=50-75% necrosis, 5=75-100% necrosis, 6=dead.

Colwood loam was significantly (P=0.05) more fungistatic to chlamydospores of *F. oxysporum* f. sp. *lycopersici* than Boyer sandy loam, which was significantly (P=0.01) more fungistatic than Capac loam, as determined in soils amended with 150 µg glucose and 30 µg peptone per gram of soil (Figure 5). Thus, the levels of fungistasis to *F. oxysporum* f. sp. *lycopersici* were inversely correlated with observed incidences of disease. Chlamydospores of *F. oxysporum* f. sp. *lycopersici* germinated more readily in soil in the presence of nutrients than those of *F. oxysporum* f. sp. *lycopersici*.

Cochliobolus sativus: Incidence of sub-crown internode lesions was compared among the three soils in two experiments. Leaf lesions also were enumerated in one experiment, but did not occur in the other experiment. Results of the two experiments were contradictory. In the first experiment, sub-crown internode lesions were significantly (P=0.05) more severe in Boyer sandy loam than in Capac loam at inoculum densities of 3,750 and 750 conidia per gram (Table 6). At both inoculum densities, the ranking of Colwood loam was intermediate, but did not differ significantly from either of the other soils. There was no significant difference in sub-crown internode lesion severity among soils at an inoculum density of 18,750 conidia per gram. There was no significant difference in

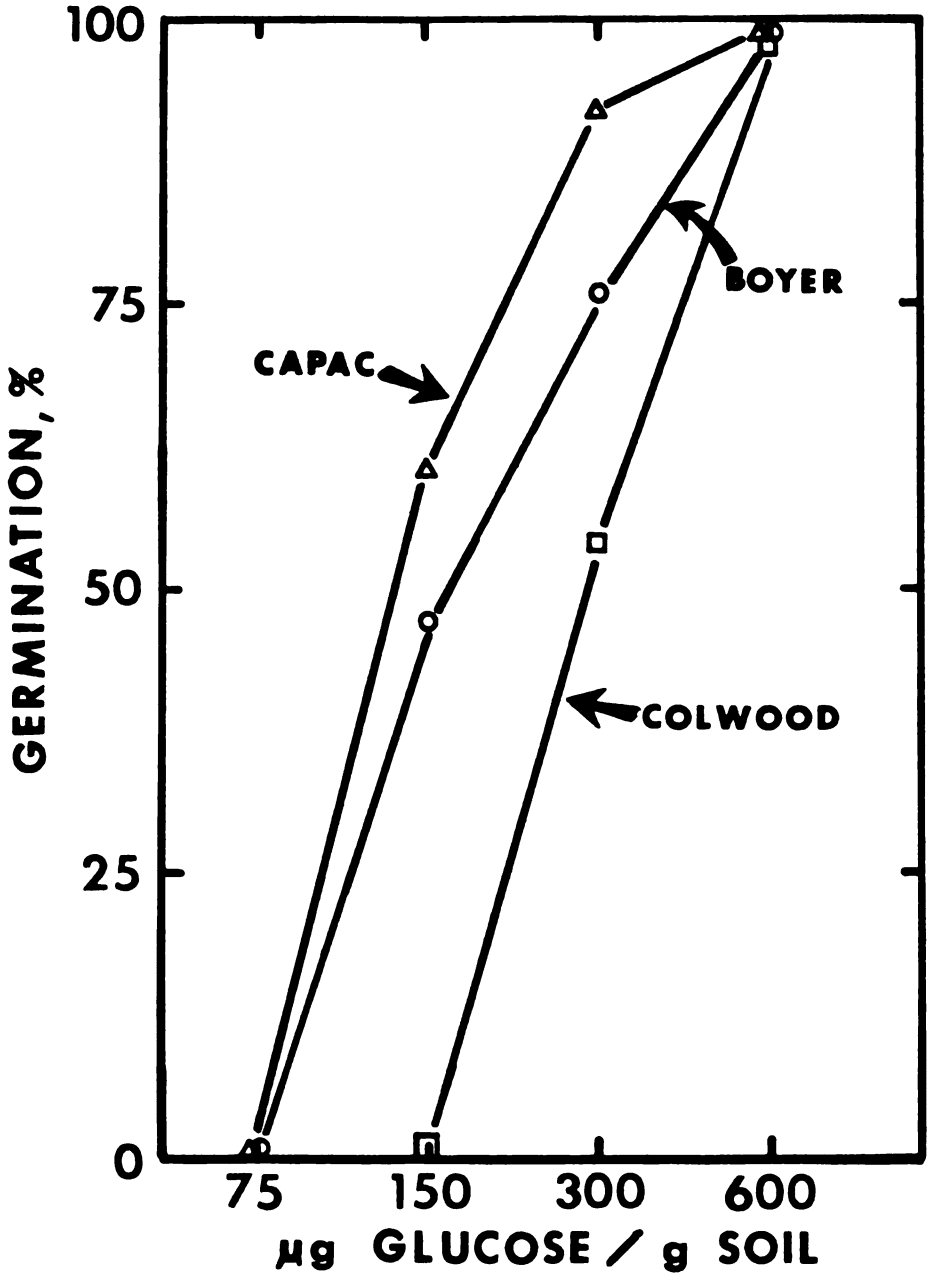

Figure 5. Germination of chlamydospores of Fusarium oxysporum f. sp. lycopersici on three soils amended with different concentrations of 5:1 (w/w) glucose:peptone, after 12 hours. Data represent mean values, based on 4 replicates per treatment; 100 spores were counted per replicate. Duncan's multiple range test showed the following differences among means: 150 µg glucose/soil, Boyer^{b}, Capac^{c}, and Colwood^{a}; 300 µg glucose/soi, Boyer ^{a}, Capac^{b}, Colwood^{c}. Mean values of soils followed by the same letter did not differ significantly (P=0.05).

Table 6. Effect of Cochliobolus sativus at three inoculum densities on severity of sub-crown internode disease and numbers of leaf lesions on wheat in three soils, four weeks after seeding[x].

Soil	Disease index[y]			Leaf lesions, no.[z]		
	750 ppg	3750 ppg	18,750 ppg	750 ppg	3750 ppg	18,750 ppg
Boyer sandy loam	3.1 a	3.7 a	3.4 a	0.4	0.7	3.1
Capac loam	2.4 b	2.7 b	3.1 a	0.1	0.6	2.6
Colwood loam	2.9 ab	3.3 ab	3.3 a	0.2	0.3	2.5

[x] Mean values, based on 4 replicates of 6 plants each per treatment.

[y] Severity of necrosis of sub-crown internode: 1=no necrosis, 2=slight (some flecks), 3=light (some necrotic portions), 4=moderate (necrosis with some healthy portions), 5=severe (extensive necrosis). Numbers within a column followed by the same letter are not significantly different (P=0.05) using Duncan's multiple range test.

[z] Analysis of variance showed no significant differences (P=0.05) among treatments.

leaf lesion numbers among soils at all three inoculum densities.

In the second experiment, sub-crown internode lesions were significantly (P=0.01) more severe in Capac loam than in Colwood loam at an inoculum density of 8,000 conidia per gram (Table 7). Boyer sandy loam was intermediate, but did not differ significantly from either of the other soils. There was no significant difference in lesion severity among soils at an inoculum density of 80 or 800 conidia per gram of soil. The ranking of severity of *C. sativus* root rot in these soils based on these experiments is therefore inconclusive.

As determined by the nutrient titration technique, the level of fungistasis against *C. sativus* was significantly (P=0.05) lower in Capac loam than in Colwood loam or in Boyer sandy loam (Figure 6). Nutrient titration of these soils using conidia of *C. victoriae* gave similar results.

Fusarium solani f. sp. *pisi*: Results of disease incidence determinations for two experiments were contradictory. In the first experiment, root lengths, lesion lengths, and disease ratings were not significantly (P=0.05) different among the soils at 500 and 1000 chlamydospores per gram (Table 8). However, shoot length and fresh weight were significantly (P=0.01) lower in Boyer

Table 7. Effect of Cochliobolus sativus at three inoculum densities on severity of sub-crown internode disease on wheat in three soils, four weeks after seeding[x].

Soil	Disease index[y]		
	80 ppg	800 ppg	8000 ppg
Boyer sandy loam	2.1 a	2.5 a	3.3 ab
Capac loam	1.7 a	3.1 a	3.7 a
Colwood loam	1.7 a	2.4 a	2.7 b

[x] Mean values, based on 4 replicates of 6 plants each per treatment.

[y] Severity of necrosis of sub-crown internode: 1=no necrosis, 2=slight (some flecks), 3=light (some necrotic portions), 4=moderate (necrosis with some healthy portions), 5=severe (extensive necrosis). Numbers within a column followed by the same letter are not significantly different (P=0.05) using Duncan's multiple range test.

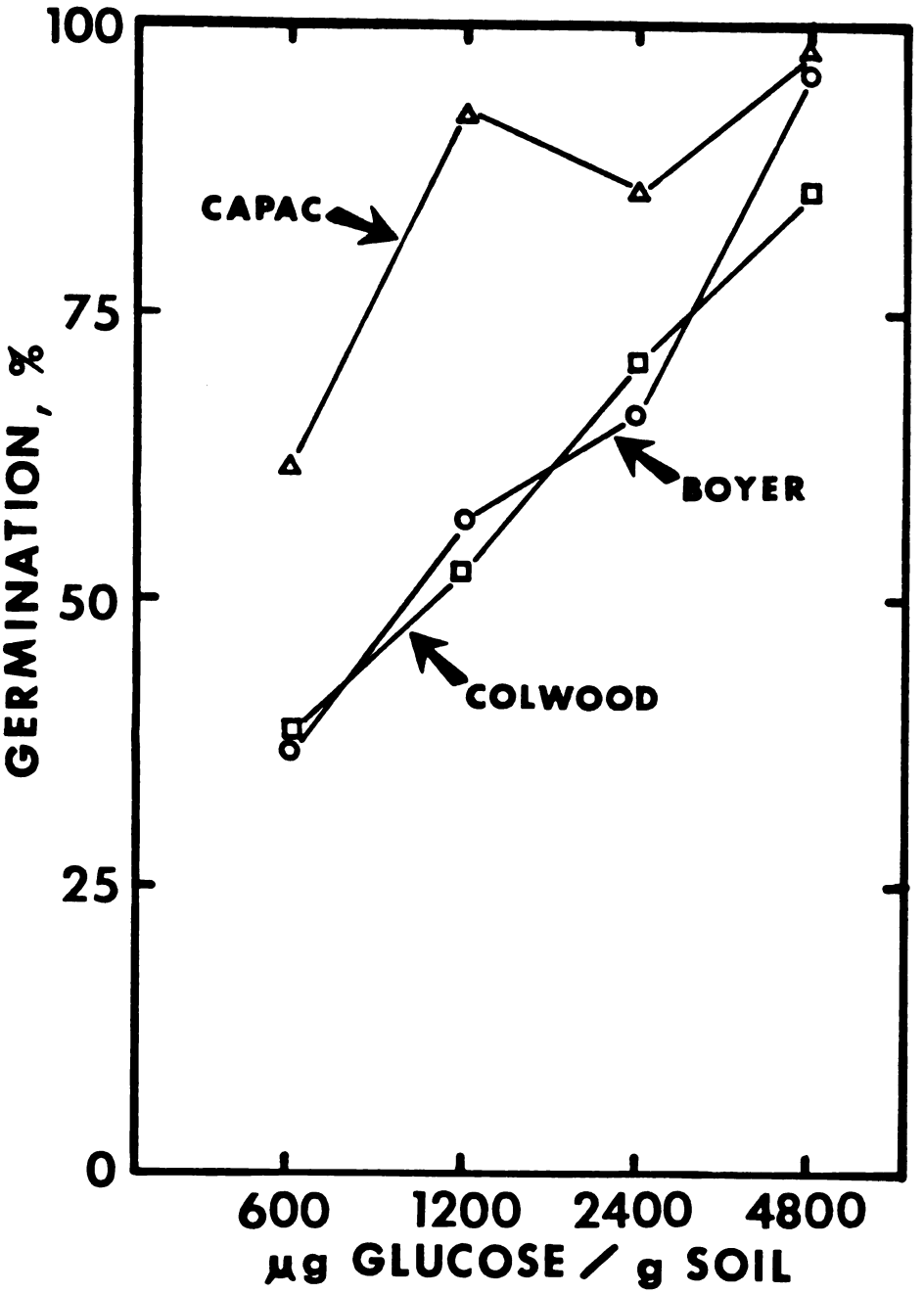

Figure 6. Germination of conidia of Cochliobolus sativus on three soils amended with different concentrations of 5:1 (w/w) glucose:peptone, after 6 hours. Data represent mean values, based on 4 replicates per treatment; 100 spores were counted per replicate. Duncan's multiple range test showed the following differences among means: 600, 1200, and 2400 µg glucose/soil, Boyer[a], Capac[b], and Colwood[a]. Mean values of soils followed by the same letter did not differ significantly (P=0.05).

Table 8. Effect of *Fusarium solani* f. sp. *pisi* at two inoculum densities on root, shoot, and lesion lengths, fresh shoot weights and disease severity on pea seedling in three soils, 4 weeks after transplanting[y].

	Root length, cm		Shoot length, cm		Lesion length, mm		Fresh shoot wt., g		Disease index[z]	
Soil	500 ppg	1000 ppg	500 ppg	1000 ppg	500 ppg	1000 ppg	500 ppg	1000 ppg	500 ppg	1000 ppg
Boyer sandy loam	13.6	14.3	13.0	13.9	43	45	1.3	1.3	4.1	4.7
Capac loam	12.9	13.0	21.2	19.3	41	57	3.1	3.0	4.3	4.7
Colwood loam	11.7	13.1	23.9	24.8	32	48	3.2	3.3	3.9	4.6

[y] Mean values, based on 5 replicates of 4 plants each per treatment. Analysis of variance revealed no significant differences ($P=0.05$) among mean root and lesion lengths and disease severities among soils, but shoot weights and lengths of plants in Boyer sandy loam were significantly less by Duncan's multiple range test ($P=0.05$) then those in Capac and Colwood lesions.

[z] Mean values, based on the sum of foliage, cotyledon and root ratings of each plant as follows: Foliage: 0=healthy, 1=wilt/some dead leaves, 2=dead; Cotyledon: 0=healthy, 1=trace necrosis, 2=extensive necrosis; Roots: 0=healthy, 1=less than 25% necrosis, 2=25=50% necrosis, 3=more than 50% necrosis.

sandy loam than in Capac loam or Colwood loam. In the second experiment, lesion length was significantly (P=0.05) less in Capac loam than in Boyer sandy loam or Colwood loam at an inoculum density of 500 chlamydospores per gram (Table 9). However, differences were not significant at an inoculum density of 5000 chlamydospores per gram. The ranking of severity of *F. solani* f. sp. *pisi* root rot in these soils based on these experiments is therefore inconclusive. Disease incidence in sterilized soils did not differ at either 500 or 5000 chlamydospores per gram of soil.

The level of fungistasis against *F. solani* f. sp. *pisi* in Capac loam was significantly (P=0.05) lower than that in Colwood loam and Boyer sandy loam, which did not differ significantly (P=0.05) (Figure 7).

Germination of Propagules on Natural and Sterilized Soils

Propagules of all the fungi used in this work failed to germinate on all three, unamended soils. However, when these same soils were autoclaved for 1 1/2 hours, propagules germinated 99-100%.

Assay of Soil Solutions for Germination Inhibitors

Germination of chlamydospores of *F. oxysporum* f. sp. *congultinans* was 99-100% in sterile aqueous extracts of the three soils, as compared with 99% germination in 1:100

Table 9. Effect of *Fusarium solani* f. sp. *pisi* at two inoculum densities on shoot and lesion lengths and fresh shoot weights on pea seedlings in three soils, four weeks after transplanting[z].

	Shoot length, cm		Lesion length, mm		Fresh shoot wt., g	
Soil	500 ppg	5000 ppg	500 ppg	5000 ppg	500 ppg	5000 ppg
Boyer sandy loam	9.0 a	7.9 b	26 a	32 a	0.8 a	0.7 b
Capac loam	11.1 a	10.7 a	20 b	33 a	1.2 a	1.1 a
Colwood loam	10.7 a	10.9 a	30 a	34 a	1.1 a	1.2 a

[z] Mean values, based on 4 replicates of 5 plants each per treatment. Numbers within a column followed by the same letter are not significantly different ($P=0.05$) using Duncan's multiple range test.

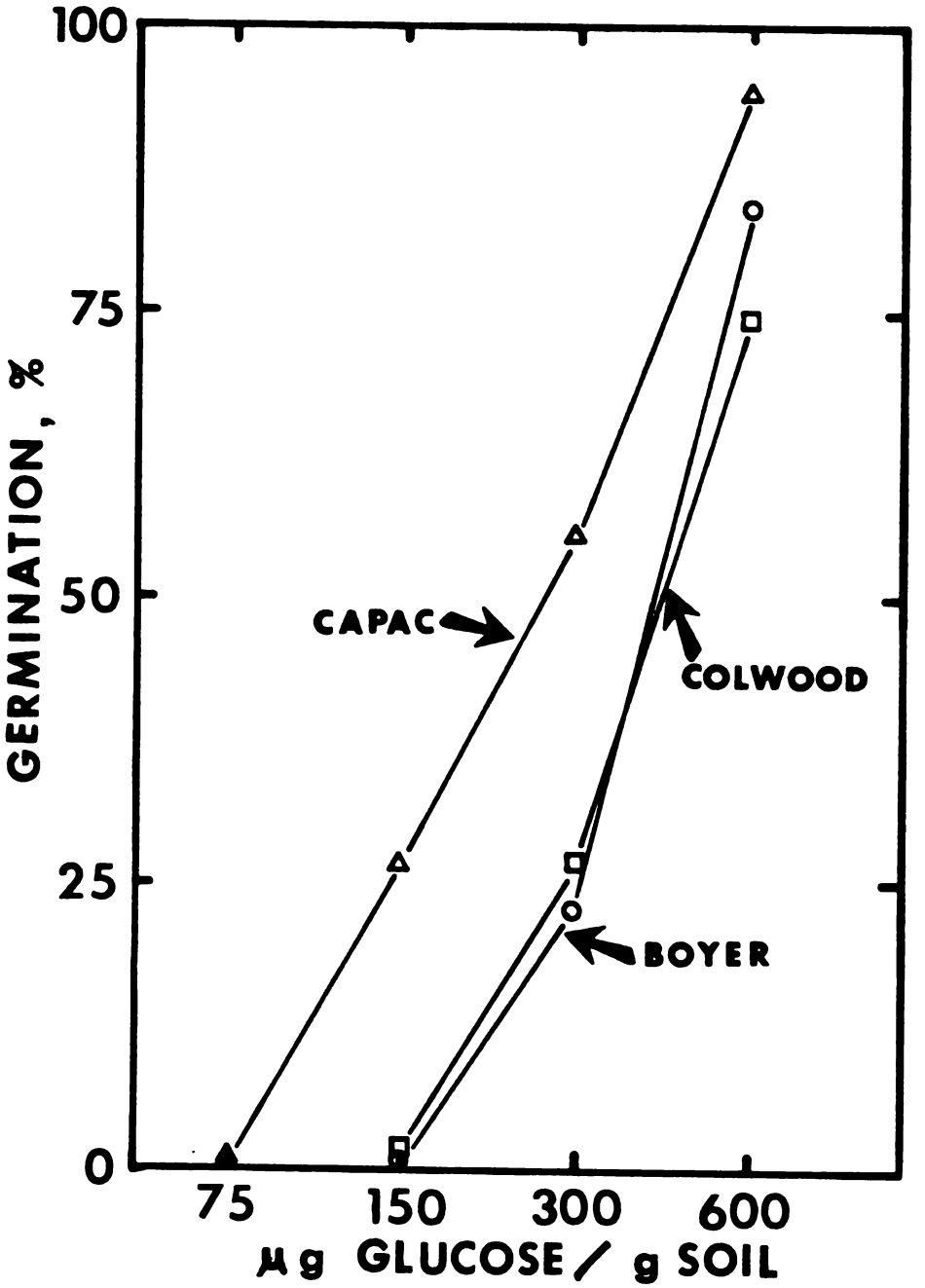

Figure 7. Germination of chlamydospores of Fusarium solani f. sp. pisi on three soils amended with different concentrations of 5:1 (w/w) glucose:peptone, after 12 hours. Data represent mean values, based on 4 replicates per treatment; 100 spores were counted per replicate. Duncan's multiple range test showed the following differences among means: 150 and 300 µg glucose/soil, Boyer^{a}, Capac^{b}, and Colwood^{a}; 600 µg glucose/soil, Boyer^{a,b}, Capac^{b}, and Colwood ^{a}. Mean values of soils followed by the same letter did not differ significantly (P=0.05).

Pfeffer's solution. Conidia of C. sativus germinated 98% in sterile extracts of the three soils, as compared with 66% germination in 1:100 Pfeffer's solution.

Assay of Soils for Volatile Inhibitors

Chlamydospores of F. oxysporum f. sp. conglutinans and conidia of C. sativus germinated 99% and 60%, respectively, in Pfeffer's solution incubated over soil. In both cases, spore germination over soil did not differ from spore germination over Pfeffer's solution. The technique used was effective at trapping inhibitory volatile compounds, as shown by nearly 100% inhibition of spore germination of these fungi incubated over soil to which 1500 ppm ammonia was added.

Microbial Biomass and Microbial Composition of the Soils

The biomass of Colwood loam, as determined by the chloroform fumigation method, was 107.8 mg C/100g soil - twice that of Capac loam and Boyer sandy loam (Table 10). Colwood loam had significantly (P=0.05) more aerobic bacteria than Capac loam, while Boyer sandy loam ranked intermediate (Table 10). Populations of actinomycetes were significantly (P=0.05) greater in Colwood loam and Boyer sandy loam than in Capac loam (Table 10). Populations of anaerobic bacteria were significantly (P=0.05) greater in

Table 10. Biomass and microbial populations in three soils.

Soil	Total biomass[x], mg C/100 g soil	Colony forming units · g^{-1} soil[w]			
		Actinomycetes[y], X 10^5	Aerobic[y] bacteria, X 10^6	Anaerobic[z] bacteria, X 10^5	Fungi[y], X 10^4
Boyer sandy loam	53.2 a	71.9 b	16.5 ab	10.0 a	15.6 a
Capac loam	53.5 a	50.3 a	12.2 a	19.2 b	25.9 b
Colwood loam	107.8 b	74.7 b	21.2 b	24.7 b	44.9 c

[w] Numbers within a column followed by the same letter are not significantly different ($P=0.05$) using Duncan's multiple range test.

[x] Determined by chloroform fumigation. Mean values, based on 4 replicates per soil.

[y] Determined by dilution-plating on selective media. Mean values, based on 5 replicates per soil.

[z] Determined by most probable number technique, using three serial, two-fold dilutions of 10 replicates each.

Capac loam and Colwood loam than in Boyer sandy loam (Table 10). Populations of fungi were significantly (P=0.05) greater in Colwood loam than in Capac loam, while populations in Boyer sandy loam were least (P=0.05) (Table 10).

Nutrient Sinks of Three Soils

Using an incubation chamber continually flushed with air, there was no significant (P=0.05) difference in utilization of ^{14}C-labelled glucose among soils adjusted to -1 kPa (-0.01 bars) (Figure 8). Most of the respiration occurred within four hours after the addition of label. In contrast, using a closed, large-volume system, significant differences (P=0.05) in utilization of glucose among soils adjusted to -1 kPa (-0.01 bars) were shown after 24 hours of incubation, but not after only 12 hours (Figure 9). Between 12 and 24 hours, significantly (P=0.05) more carbon dioxide was respired by Colwood loam and Capac loam than by Boyer sandy loam. When the closed, large-volume system was used to measure respiration of soils adjusted to a matric potential of -30 kPa (-0.3 bars), there was 2- to 4-fold greater respiration in all soils after 12 hours, than in soils adjusted to -1 kPa (-0.01 bars). Respiration in Colwood loam after 24 hours was 5 times greater (P=0.05) than that in Boyer sandy loam and 3 times greater than that

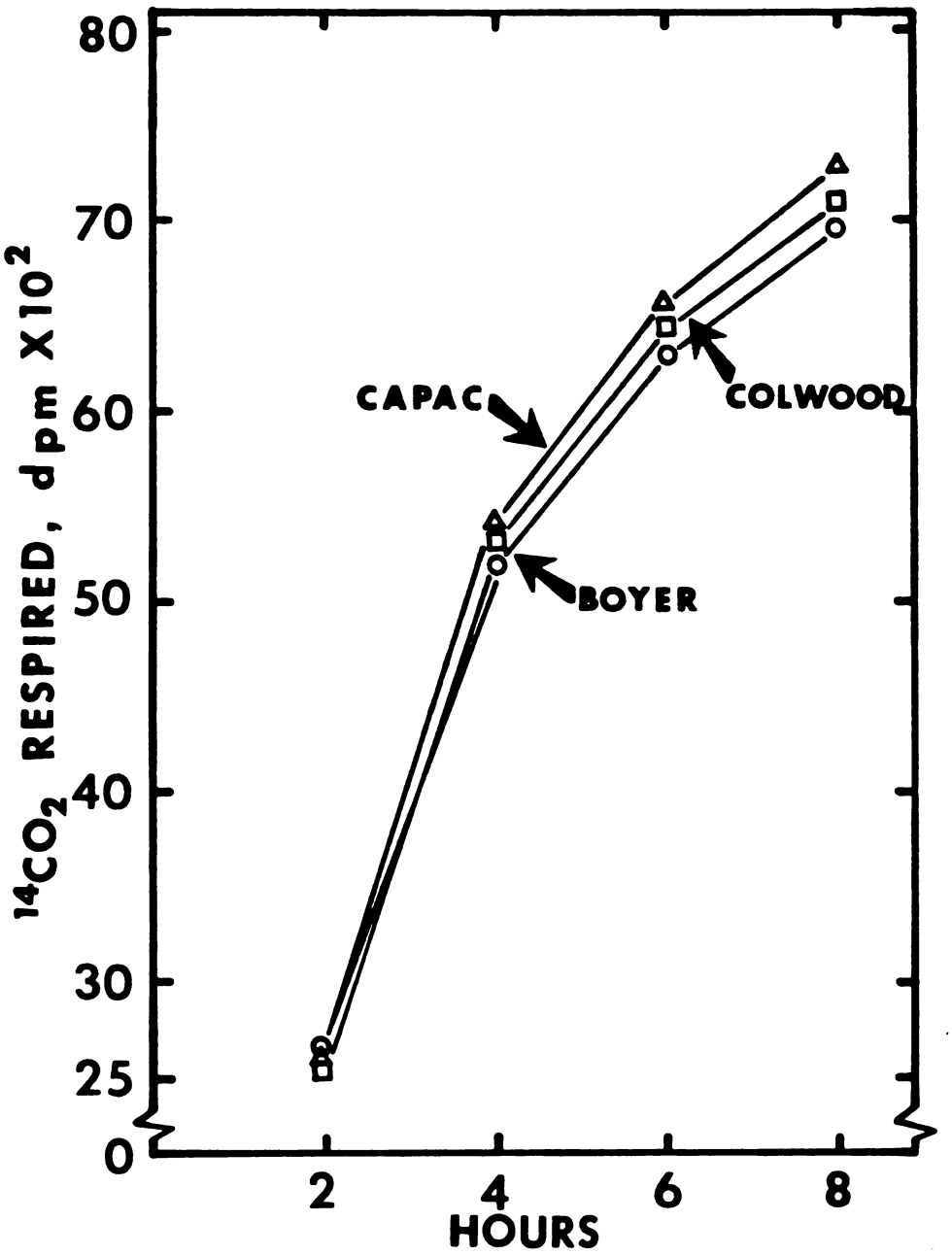

Figure 8. Cumulative $^{14}CO_2$ respiration by three soils at -1 kPa (-0.01 bars) matric potential, pulsed with 0.05 µCi (1.85 MBq) of ^{14}C-glucose. Data represent mean values, based on 3 replicates per soil. No significant differences ($\underline{P}$=0.05) were observed among the soils during 2-8 hours.

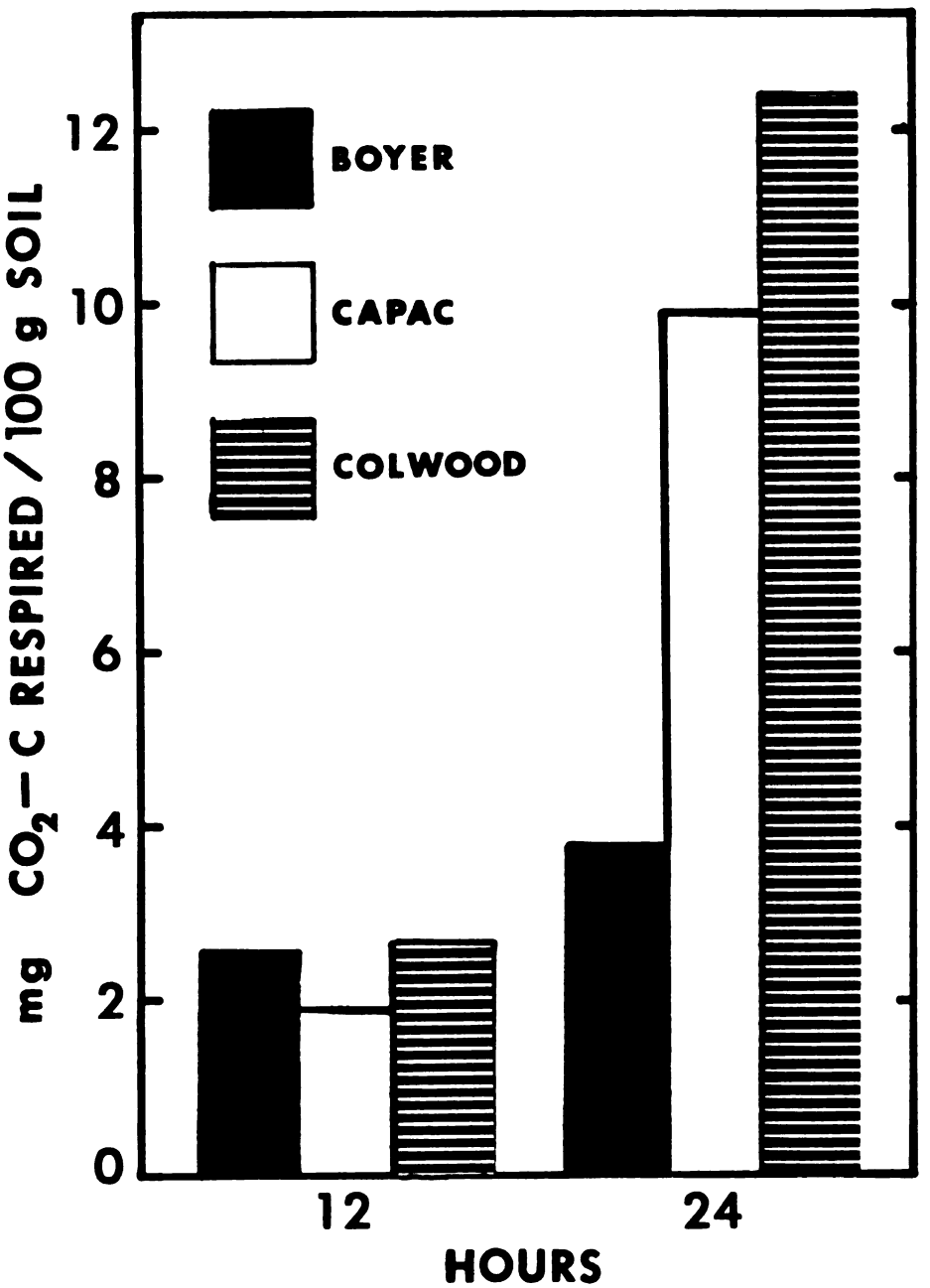

Figure 9. CO_2 respiration by three soils at -1 kPa (-0.01 bars) matric potential measured 12 and 24 hours after the addition of 1% (w/w) glucose. Data represent mean values, based on 4 replicates per soil. Duncan's multiple range test showed the following differences among means after 24 hours: Boyer^b, Capac^a, Colwood^a. Mean values of soils followed by the same letter did not differ significantly ($\underline{P}$=0.05).

in Capac loam (P=0.05) (Figure 10).

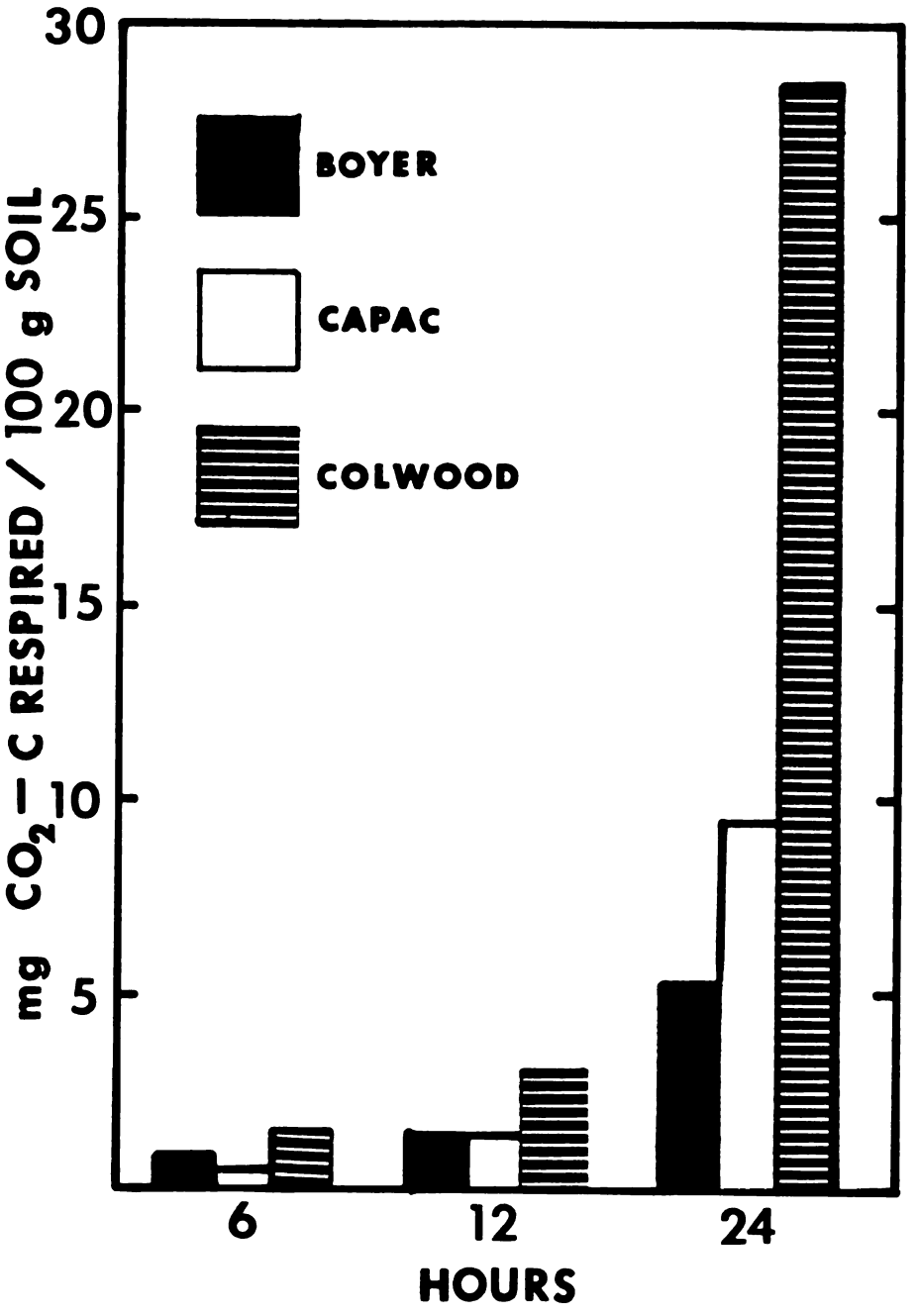

Figure 10. CO_2 respiration by three soils at -30 kPa (-0.3 bars) matric potential measured 6, 12, and 24 hours after the addition of 1% (w/w) glucose. Data represent mean values, based on 4 replicates per soil. Duncan's multiple range test showed the following difference among means: 12 hours, Boyer^{ab}, Capac^{b}, Colwood^{a}; 24 hours, Boyer ^{a}, Capac^{b}, and Colwood^{c}. Means values of soils followed by the same letter did not differ significantly.

DISCUSSION

The objective of this work was to determine if a relationship exists between levels of fungistasis in soils and disease incidence or severity occurring in them. This question had not been explicitly addressed previously. Results with Fusarium oxysporum f. sp. lycopersici supported this hypothesis in that highly fungistatic soils had lower disease incidence than less fungistatic soils. Other workers have observed that disease-suppressive soils are apparently more fungistatic than disease-conducive soils (Alabouvette, 1983; Arjunarao, 1971; Furuya, 1982; Hwang et al., 1983; Kao and Ko, 1983; Smith, 1977). However, results with Fusarium wilt of radish did not show this relationship: the lowest incidence of wilt, caused by F. oxysporum f. sp. conglutinans, was in Capac loam, which had the lowest level of fungistasis to the pathogen, suggesting that factors other than fungistasis are involved in disease expression in the case of this pathogen (Table 11). Possibly, differences in fungistasis in the rhizosphere may be correlated with differences in disease in soils, or other mechanisms of antagonism may be involved. However, the low incidence of radish wilt in Capac loam cannot be explained by hyperparasitism, or other lethal antagonisms, as the population of the pathogen did not decrease during the

Table 11. Ranking of soils with respect to their microbial biomasses, nutrient sinks, levels of fungistasis, and incidences of radish and tomato wilt[u].

Soil	Radish wilt incidence[v]	Tomato wilt incidence[w]	Total biomass	Nutrient sink	Level of fungistasis[z]
Boyer sandy loam	1	2	2	3	2
Capac loam	3	1	2	2	3
Colwood loam	2	3	1	1	1

[u] 1 = highest, 2 = intermediate, 3 = lowest

[v] Based on several experiments

[w] Tentative, based on one unconfirmed experiment

[x] Determined by chloroform fumigation

[y] Based on measurement of CO_2 production from unlabelled glucose in large, closed containers.

[z] Determined by the nutrient titration.

course of the experiments. Results obtained with Cochliobolus sativus causing root rot of wheat and with F. solani f. sp. pisi causing root rot of pea where inconsistent and so conclusions cannot be drawn from these experiments.

Where competition for nutrients is the mechanism of disease suppression in a soil, nutrient titration, or an analogous technique for determining differences in fungistasis, should be effective in identifying suppressive soils. Such an approach was used by Ko and Ho (1983), who found that soils highly fungistatic to R. solani and P. splendens, suppressed disease caused by these fungi. However, in my work, radish wilt development was least in Capac loam, which was not as fungistatic to F. oxysporum f. sp. conglutinans as the other soils. Therefore, the method of Ko and Ho may not be applicable in all cases, since other mechanisms may be involved in suppression.

All pathogens used in this work caused greater incidences of disease in soils which had been sterilized, than in non-sterile soils, showing that the natural microflora played an important role in suppressing disease development. The similar levels of disease observed in the sterilized soils suggest that abiotic factors were not responsible for disease differences in non-sterile soils. However, abiotic factors, such as fertility and soil texture

as it affects water availability, may indirectly affect the ability to detect differences in disease among soils, because they can affect host susceptibility. Lack of consistent results when comparing pea and wheat root rot severity among soils could be ascribed to variability in water potential, since this factor was not controlled. However, the order of severity of radish wilt among the three soils remained consistent, whether plants were grown at constant or fluctuating matric potentials or temperatures. Thus, some plant/pathogen systems, like Fusarium wilt of radish, are more suitable for detecting differences in disease among soils than others. Alabouvette et al. (1982) also found that differences in suppressiveness among soils could be more readily shown using Fusarium wilt of flax, than Fusarium wilt of muskmelon because of less variation in results with flax wilt. Additional advantages to the flax wilt system, which were also seen with the radish wilt system, included rapid development of wilt and a small plant size, which made increased replication possible. Other workers have used damping-off fungi such as Rhizoctonia solani on radish (Kinsbursky and Weinhold, 1983), and Pythium splendens on cucumber (Kao and Ko, 1983), as rapid, repeatable systems for identifying differences in disease development in soils.

Soil fertility is another important factor that can affect disease comparisons among different soil types. As shown in Table 1, the concentrations of mineral nutrients among the three soils were different. Since differences in fertility may affect disease development, supplementary fertilization was used. However, supplementary fertilization could not fully compensate for differences among soils, as shown by tomato shoot lengths and weights that were significantly lower (P=0.05) in non-pathogen-infested Boyer sandy loam than plants grown in the other two non-infested soils (Table 5). Thus, comparisons of these growth parameters could not be directly made among pathogen-infested soils, but rather, had to be made from proportional growth differences in relation to the non-infested controls. In contrast, disease incidence of tomato was a parameter less influenced by soil fertility than by biotic factors, as shown by differences among non-sterilized, but not among sterilized soils.

The importance of using several inoculum densities to measure disease incidence in soils was demonstrated in this work. My results with radish and tomato wilts, and the results of Alabouvette et al. (1982) with flax and muskmelon wilt, demonstrated that statistically significant differences in disease can be demonstrated only at certain inoculum concentrations.

Total microbial biomass was greatest in Colwood loam, which had the lowest incidence of Fusarium wilt of tomato and the highest degree of fungistasis of the three soils (Table 11). Biomass and fungistasis were less in Boyer sandy loam and Capac loam, which had greater incidences of tomato wilt. These results suggested a possible relationship between biomass and the suppression of the pathogen. However, the order of the incidence of radish wilt in these soils differed from that of tomato, suggesting that other factors have a greater effect on development of this disease than microbial biomass. Counts of specific microbial components of soils were imperfectly correlated with total biomass. The ranking of soils with respect to counts of fungi was similar to the ranking of total biomass values, which reflects the fact that fungi account for 75% of the total microbial biomass (Anderson and Domsch, 1978). Populations of actinomycetes and bacteria also were greatest in Colwood loam. However, Capac loam had a higher population of anaerobic bacteria and a lower population of actinomycetes, than Boyer sandy loam, demonstrating a lack of correlation of these microbial components with total biomass.

One objective of dilution plate enumeration of the microflora was to detect the presence of specific components

of the microflora which may be important determinants of disease suppression. In other work (Chet and Baker, 1981), dilution plating revealed the presence of high numbers of Trichoderma viride, which, although representing only 0.8% of the total number of fungi, accounted for disease suppression. However, there were no obvious differences in the composition of the microflora of the three soils examined.

The nutrient sinks of the soils, as determined by measuring the respiration of added glucose, showed some correlation with total biomass (Table 11). The nutrient sinks and biomasses were greatest in Colwood loam, but the nutrient sink of Capac loam was greater than that of Boyer sandy loam, although their mass values were identical. From the work of Filonow and Lockwood (1983), Boyer sandy loam had a greater nutrient sink than several other soils whose composition was similar to that of Capac loam. By contrast, Boyer sandy loam had a relatively low level of fungistasis as compared with these soils. The reasons for the discrepancies between their results and the present results are not known, although there may be differences in the microflora between samples of soils used in the two studies.

Results of nutrient sink determinations using a closed, large volume system and a continuous collection system

differed. The continuous collection system, using ^{14}C-glucose, permits a high degree of sensitivity, so that responses to added glucose could be measured in the same time frame required to evaluate spore germination. However, differences between the nutrient sinks of the soils were not shown by this method. Respiration reached a maximum in all soils within four hours after the addition of label, and decreased thereafter at similar rates. In contrast, differences in respiration among soils were shown only after 12 hours using the closed, large volume system. Further evaluation of these techniques is necessary to account for the lack of correlation of results between them.

It is noteworthy that the order of fungistasis among the soils was similar for all the fungi tested. Colwood loam was the most fungistatic of the soils, while Capac loam was the least fungistatic (Table 11). Germination of all fungi was inhibited on natural, unamended soils, whereas they germinated unimpeded on sterilized soils. No volatile compounds or inhibitors from sterile soils solutions were detected in any of the soils. These results suggest that nutrient deprivation is the primary mechanism of fungistasis in these soils (Lockwood, 1977).

Conidia of C. victoriae and C. sativus required an amendment of 4800 µg glucose and 960 µg peptone per gram of

soil for approximately 98% germination, while chlamydospores of F. oxysporum f. sp. lycopersici, F. oxysporum f. sp. conglutinans and F. solani f. sp. pisi required only 600 µg glucose and 120 µg peptone per gram of soil to germinate to the same extent. The greater sensitivity of Cochliobolus spp. conidia is an exception to the general relationship of decreasing sensitivity to fungistasis with increasing spore volume (Steiner and Lockwood, 1969). Also, the conidia of Cochliobolus spp. required less than half the time required for germination of chlamydospores of Fusarium spp., whereas propagules more sensitive to fungistasis generally required a longer germination time (Steiner and Lockwood, 1969). It is not known why Cochliobolus spp. was more sensitive to fungistasis than Fusarium spp. The sensitivity of Fusarium spp. in this work was similar to that reported by others (Alabouvette, 1983; Smith, 1977). However, previous workers, using the same isolate of C. victoriae and the same soil types, found that an amendment of only 1000 µg glucose and 200 µg peptone per gram of soil resulted in 60-90% germination (Lee and Lockwood, unpublished). Differences in the production of conidia or mutation of the culture may account for the high sensitivity to fungistasis of the Cochliobolus spp. used in this work.

Chlamydospores of F. oxysporum f. sp. lycopersici and F. oxysporum f. sp. conglutinans were nutrient-independent,

as shown by their high germination (80% and 99%, respectively) on 1:100 Pfeffer's solution, which contrasts with previous reports of 0-1% germination of chlamydospores of F. oxysporum f. spp. in the absence of nutrients (Smith and Snyder, 1972). However, chlamydospores of F. solani f. sp. pisi germinated only 1% on 1:100 Pfeffer's solution, which is similar to the results of Griffin (1970). Conidia of C. victoriae and C. sativus were partially nutrient independent, germinating 30% and 50%, respectively. This is in contrast to other workers reporting 97% germination of these fungi in the absence of nutrients (Hsu and Lockwood, 1973).

In conclusion, this work did not show a consistent relationship between the level of fungistasis in soils and disease incidence or severity occurring in them. However, because of the inherent difficulties in ruling out factors other than the soil microflora as an explanation for disease differences, the hypothesis that highly fungistatic soils have lower incidence or severity of diseases cannot yet be rejected. There may be differences in fungistasis occurring within the rhizospheres of these soils that could account for differences between radish and tomato wilt incidences. Instead of comparing disease in different soils with different levels of fungistasis, another approach might have

been to alter the fungistasis of one soil and observe changes in disease. The problem with this approach is finding a treatment that would affect fungistasis, but not other properties of the soil. Prolonged storage could be such a treatment.

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