MYCOSTASIS AND THE MICROBIAL NUTRIENT SINK OF SOIL
I. ASSESSMENT OF THE MICROBIAL NUTRIENT SINK OF FIVE SOILS
II. LOSS OF THE NUTRIENT-INDEPENDENT STATE OF FUNGAL PROPAGULES INCUBATED ON SOILS OR ON A MODEL SYSTEM SIMULATING SOIL-IMPOSED ENERGY STRESS

By

Alexander B. Filonow

A DISSERTATION

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

DOCTOR OF PHILOSOPHY

Department of Botany and Plant Pathology

1981

ABSTRACT

MYCOSTASIS AND THE MICROBIAL NUTRIENT SINK OF SOIL
I. ASSESSMENT OF THE MICROBIAL NUTRIENT SINK OF FIVE SOILS
II. LOSS OF THE NUTRIENT-INDEPENDENT STATE OF FUNGAL PROPAGULES INCUBATED ON SOILS OR ON A MODEL SYSTEM SIMULATING SOIL-IMPOSED ENERGY STRESS

By

Alexander B. Filonow

^{14}C-exudate lost over 4-24 h from sclerotia of Macrophomina phaseolina, chlamydospores of Thielaviopsis basicola, and conidia of Cochliobolus victoriae and Stemphylium sarcinaeforme to soils was studied in relation to mycostasis. Loss of ^{14}C-exudate from these nutrient-independent propagules to soils was sufficient to account for the inhibition of propagule germination. ^{14}C-exudate losses from the four fungi to two sandy loam soils and a loam were generally greater than losses to two clay loam soils. In contrast to the order of the fungal exudation, fine-textured soils required more nutrients to annul mycostasis than coarse-textured soils, and more ^{14}C-exudate was adsorbed by the clay loam soils than the loam and the sandy loam soils. Therefore, the adsorptive capacity of soil may be an important factor in controlling fungal utilization of soluble nutrients.

Conidia of C. victoriae and sclerotia of Sclerotium cepivorum became nutrient-dependent after 8 and 15 days, respectively, of extended diffusive stress aseptically imposed by a leached sand apparatus which simulated soil imposed diffusive stress. S. cepivorum sclerotia eventually lost their ability to respond to dilute concentrations of onion extract. Thirty-five days of stress made M. phaseolina sclerotia nutrient-dependent. Sclerotia of Verticillium dahliae lost little of

their nutrient-independence even after 45 days of diffusive stress. Conidia of C. victoriae gradually became nutrient-dependent when incubated for several weeks on each of five soils, and sclerotia of M. phaseolina also lost their nutrient-independence when incubated on four of the five soils. The germinability of nutrient-dependent conidia of C. victoriae in the absence of exogenous nutrients was significantly increased, when diffusive stress was interrupted by incubating the conidia in a non-germinated state at 4°C for 3.5 days. Nutrient-dependent conidia of C. victoriae incubated on soils labeled with ^{14}C-glucose, absorbed twice as much ^{14}C from three lighter-textured soils as from two clay loam soils. When incubated on four of the five ^{14}C-labeled soils, the germinability of the conidia in the absence of nutrients was significantly increased.

These results support the concept that persistent diffusive stress imposed by the microbial nutrient sink of a soil can convert nutrient-independent propagules to nutrient-dependent ones, and that an interruption in the stress may allow nutrient-dependent propagules to recoup nutrients from the soil solution or reorganize endogenous nutrients whereby the potential for germinability is increased.

To

Knawsie, who should be the scientist;

John, who is full of innocence and energy;

Ann, who likes to be free;

and

Me.

ACKNOWLEDGEMENTS

To Dr. John L. Lockwood I give my thanks for his patience, understanding, help, especially with this manuscript, and above all, friendship. I have learned a lot about many things just by talking with him.

To the members of my guidance committee: Drs. N. E. Good, R. P. Scheffer, and A. R. Wolcott, I express my thanks for their advice about matters scientific and not so scientific, some of which I fortunately retained. I appreciate their suggestions and their critical evaluation of my work and this manuscript.

I wish also to thank numerous people from this department and other departments for the use of equipment and more importantly, for their time.

Finally, I express my gladness to be part of a small and very funny little family, which has a lot of potential. Thanks for helping me grow.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LITERATURE REVIEW

Mycostasis is a descriptive term given to the phenomenon whereby the germination and growth of fungi in natural soils are much more restricted than would be expected under similar conditions of moisture, temperature, and pH in vitro. Dobbs and Hinson (1953) first described the phenomenon and since then a considerable volume of literature concerning mycostasis has accrued. The subject has been comprehensively reviewed by Lockwood (1964; 1977). An interpretive but less thorough review has been given by Watson and Ford (1972). Specific aspects of mycostasis have been reviewed more recently (Balis and Kouyeas, 1979; Griffin and Roth, 1979; Lockwood, 1979).

There are some generalizations concerning mycostasis which can be gleaned from the literature: (1) Mycostasis occurs in most soils throughout the world; (2) Propagules of most fungi exhibit mycostasis in natural soils, although some are not affected; (3) Mycostasis is almost always microbial in nature and partial or complete sterilization of soil will annul the effect; and (4) Mycostasis can be annulled by the addition of energy-yielding nutrients to soil. Mycostasis which persists in a few soils after heat sterilization and cannot be annulled by nutrients is termed 'residual mycostasis' to differentiated it from the more common 'microbial mycostasis'. In natural soil fungal propagules may germinate in the proximity of fresh organic matter, or in the rhizospheres or spermospheres of plants. Brief bursts of activity may occur in response

to inputs of plant and animal litter, such as decomposing roots, leaf fall, or following physical disturbances, including wetting and drying, or freezing and thawing that kill microorganisms or cause redistribution of nutrients.

Since mycostasis is mainly of microbial origin, it might be expected to occur in other habitats wherein microbial populations are high. In fact, mycostasis has also been demonstrated to occur on the surfaces of living plant leaves (Blakeman and Brodie, 1977) and in seawater (Kirk, 1980). Moreover, there is sufficient information to establish that the activity of bacteria (Brown, 1973; Davis, 1976; Ko and Chow, 1977), and actinomycetes (Lloyd, 1968; Mayfield et al., 1972) are also suppressed in soil. Thus, mycostasis most likely is a component of a more general microbiostasis operative in soil.

Sensitivity Of Fungi To Soil Mycostasis

Soil mycostasis is a form of fungal dormancy imposed via outside factors, and differs from a self-imposed constitutive dormancy found in several groups of fungi, e.g., carbonicolus fungi which require heat activation for ascospore germination (Wicklow and Zak, 1979).

A range of sensitivity to mycostasis exists among fungi (Lockwood, 1977). Sensitivity ranking depends on attenuating the mycostatic effect of soil by adding increasing amounts of nutrients to soil (Lockwood, 1964; 1977). Using germination data obtained from 29 fungal propagules incubated on varying mixtures of natural and sterilized soils, Steiner and Lockwood (1969) showed a strong correlation between propagule size and germination. Propagules most sensitive to mycostasis tended to be small and to require exogenous nutrients for germination

(nutrient-dependent), whereas the least sensitive propagules were larger and did not require exogenous nutrients for germination (nutrient-independent). Nutrient-dependent and nutrient-independent propagule types also have been termed, respectively, 'carbon-dependent and 'carbon-independent' (Griffin and Roth, 1979). For many nutrient-dependent propagules, glucose alone may satisfy the energy requirements for germination, while others may need some specific carbon source or form of nitrogen (Ko and Lockwood, 1967; Garrett, 1970; Griffin and Roth, 1979).

No nutrient-dependent fungal propagule which is insensitive to soil mycostasis has been identified. However, several large, nutrient-independent propagules are insensitive to soil mycostasis. These include urediospores of rusts and conidia of powdery mildews (Ko and Lockwood, 1967), activated ascospores of Neurospora and carbonicolus Ascomycetes (Ko and Lockwood, 1967; Wicklow and Zak, 1979), and conidia and ascospores of Calonectria crotolariae (Hwang and Ko, 1974). Different species within a genus (Griffiths, 1966; Steiner and Lockwood, 1969), different isolates of the same species (Chinn and Tinline, 1964), or different propagule types within a species (Papavizas and Adams, 1969; Steiner and Lockwood, 1969) may also differ in mycostasis sensitivity. Hyphae appear to be less sensitive than other propagules of the same species (Steiner and Lockwood, 1969). According to Steiner and Lockwood (1969) the difference in spore sensitivity to mycostasis is most likely due to the faster germination rates of large as compared to smaller spores. Early germ tube growth and vigor may be dependent on endogenous reserves in the spores prior to germination. Very early growth rates of germ tubes may differ from later growth rates (Trinci, 1971). However, later mycelial growth of a fungus is obviously independent of spore reserves, but it is dependent on the

availability of energy-yielding substrates in the immedite environment of the fungus.

Availability Of Energy-yielding Nutrients In Soil

Knowledge of the available supply of energy-yielding substrates in soil is necessary to evaluate mechanisms of soil mycostasis.

Although soil is inhabited by an abundant and diverse microflora (Gray and Williams, 1971; Alexander, 1977), the bulk of the microflora tends to be inactive as indicated by the minimal respiration rates measured for fallow, unamended soil (Clark, 1967; Clark and Paul, 1970). The production of CO_2 per day in stationary cultures of bacterial cells was nearly equivalent to the cell dry weight; however, a microbial biomass of 200 g/m^2 of soil was 35-70 times the daily weight of CO_2 produced in field soils (Clark and Paul, 1970). Moreover, 40% of the total soil respiration may be the result of fauna and roots (Kucera and Kirkham, 1971).

In addition, the number of microbial generations able to occur in soil is apparently low. Several studies show that microbial maintenance requirements would consume one-third or more of available substrate in soil. The substrate remaining for growth would be sufficient for only a few generations of cells (Babiuk and Paul, 1970; Clark and Paul, 1970; Shields et al., 1973; Flanagan and Bushnell, 1976; Flanagan and Van Cleve, 1977). Thus, insofar as carbon substrate is concerned, soil is an impoverished medium, in which a large proportion of the microbial population of the bulk soil is in a state of enforced quiescence (Gray and Williams, 1971; Lockwood, 1977; Lockwood and Filonow, in press).

Mechanisms Of Mycostasis

At the present there are two basic hypotheses to explain soil mycostasis: (1) the inhibitor hypothesis which postulates that inhibitory compounds from microbial metabolism in soil determine whether fungi germinate, and (2) the microbial nutrient sink hypothesis which postulates that energy-yielding nutrients are removed from the vicinity of fungal propagules by microorganisms intensely competing for these substrates.

Inhibitor hypothesis. Since Dobbs and Hinson (1953) first reported the presence of mycostasis in soil, considerable study has been directed towards substances inhibitory to fungal germination. Much of this literature must be viewed with caution, as the assay techniques employed do not rule out microbial competition for nutrients as an inhibitory mechanism (Lockwood, 1977). In addition, many of these reports describe the phenomenon of inhibition, but do not attempt to identify the inhibitory substances.

Nevertheless, despite these considerations, there are reports of chemically identified or characterized compounds native to soil which are inhibitory to fungi. These compounds may be classified into volatile and nonvolatile compounds for convenience.

Volatile inhibitors from soil which have been implicated in mycostasis are carbon dioxide, ammonia, and ethylene. A comprehensive review of the effects of O_2 and CO_2 on the growth and metabolism of fungi has been published (Tabak and Cooke, 1968). In addition, Griffin (1972) has discussed the many aspects of CO_2 chemistry in soil which affect fungal growth.

Ammonia has been shown to be toxic to fungal cells (Leach and Davy, 1935; Gilpatrick, 1969; Pavlica et al., 1978). It is a volatile waste

product of microbial metabolism and in the immediate vicinity of nitrogen-rich substrate, microbial degradation can release considerable quantities. The ammonia concentration in the soil solution is constantly regulated by adsorption and fixation reactions of ammonia and the ammonium ion with clay minerals and soil organic matter, immobilization of microbial nitrogen, volatilization of ammonia, and losses related to nitrification and subsequent removal of nitrate. Given these mechanisms of reduction and considering the dearth of energy-yielding nutrients in soil, ammonia most likely is not a primary antifungal substance operative in the majority of soils. Nevertheless, there are special situations where ammonia is present in soil at levels which are inhibitory to fungal development. Ammonia has been identified as an inhibitory compound in alkaline soils (Ko and Hora, 1972a; Ko et al., 1974; Pavlica et al., 1978). Anhydrous ammonia applied to soils has been shown to be inhibitory to fungi (Eno et al., 1955; Smiley et al., 1970), and ammonia released from nitrogenous amendments in soil (Lewis and Papavizas, 1974; Lewis, 1976; Tsao and Zentmeyer, 1979; Zakaria, Lockwood, and Filonow, 1980) also has been shown to be inhibitory.

Ethylene is an unsaturated hydrocarbon gas, which has been reported to cause soil mycostasis (Smith, 1973; 1976). Apart from Smith's work, which showed sensitivity of Scerlotium rolfsii to 1 ppm ethylene, most fungi to data do not appear to be sensitive to ethylene. Germination of Botrytis cinerea conidia was not reduced in a 10 ppm concentration of ethylene in the headspace of flasks which did or did not contain soil (Schippers et al., 1978). Likewise, Ioannou et al. (1977) found that ethylene concentrations of up to 35 ppm in soil did not effect any change in growth, sporulation, microsclerotia development, and microsclerotia

germination of Verticillium dahliae. Several other workers also have shown that very high levels of ethylene (>500 ppm) were needed to inhibit germination of some fungi (Conforth, 1975; Balis, 1976; Pavlica et al., 1978).

Evidence accumulated to date, therefore, suggests that ethylene is not a direct mycostatic agent in soil. However, ethylene in soil may induce the formation of other volatile toxic compounds (Balis, 1976). A 1% ethylene in air mixture in the headspace over soil produced another unsaturated hydrocarbon which was soluble in water condensates collected above the soil. The compounds was identified as allyl alcohol. It was not found in water condensates from soil incubated with air alone. In bioassays 4 ppm of allyl alcohol greatly inhibited germination of Arthrobotrys oligospora conidia. However, it is not known whether enough ethylene could accumulate in soil to produce allyl alcohol.

Nonvolatile inhibitors in soil derive from the inorganic component of the soil matrix, i.e., metals and salts of metals, and from organic soil components.

In acid soils, soluble aluminum may exhibit inhibitory properties (Ko and Hora, 1971; 1972b). Heat-activated ascospores of Neurospora tetrasperma, which are not sensitive to the common microbial soil mycostasis, were completely inhibited by an aqueous extract from a soil showing inhibitory properties (Ko and Hora, 1971). Of the several metals in the soil extract which were evaluated as possible inhibitory agents, aluminum was found to be active. Furthermore, a 0.65 ppm aqueous solution of aluminum salts gave complete inhibition of ascospores. Autoclaving this solution did not destroy its inhibitory nature, but inhibition was removed after the pH was raised to 7.0. Dormant

ascospores (not heat activated) were not affected by the fungitoxin.

Calcium carbonate in some limestone soils also inhibited fungi germination (Dobbs and Gash, 1965). In certain marine sands, exhibiting mycostasis, iron compounds were considered to be involved in the inhibition (Dobbs and Gash, 1965). The inhibitor could be removed from these sands with dilute HCL but not with hot water. Several cations were obtained in the acid leachate, but only iron compounds were inhibitory when added to acid-leached sand at concentrations found in sands prior to leaching.

Nitrous acid from nitrite in solution is an intermediate product of nitrification in soil, and has been shown to inhibit fungi. In urea-amended soils, both ammonia and nitrous acid were inhibitory to Phytophthora parasitica and Phytophthora cinnamomi (Tsao and Zentmyer, 1979). At alkaline pH, ammonia was the inhibitory agent, whereas at pH or less, nitrous acid was the inhibitor.

Nonvolatile fungal inhibitors have been demonstrated in molecular weight fractions of soil extracts (Vaartaja, 1974; 1977). Membrane filter-sterilized soil extracts were fractionated by use of molecular sieve columns, and fractions in agar were then bio-assayed for effects on fungal growth.

In one study, 66 soil extracts were analyzed (Vaartaja, 1974). Of the 54 inhibitory and 12 noninhibitory soil extracts, most contained one or more fractions inhibitory to growth of Pythium ultimum. A few of the extracts yielded only stimulatory fractions, whereas other extracts gave both stimulatory and inhibitory fractions. Of 36 inhibitory fractions, all except one were counteracted by nutrients.

In the discussion so far, examples of inhibitory substances produced

by one organism, but active against another, have been examined. These are examples of heteroinhibitors. Self-inhibitors in fungi also exist, and considerable literature concerning them has accumulated. Allen (1976) and Macko et al. (1976) offer admirable discussions of this subject.

Cook (1977) has proposed that soil mycostasis is caused by fungal self-inhibitors. According to this hypothesis, the biotic or abiotic environment of the fungus provides signals which regulate the response of a self-inhibitor in propagules. The signals may be activators of deactivators of germination.

The following lines of evidence militate against acceptance of this hypothesis: (1) It is not certain whether all fungi contain self-inhibitors. (2) Urediospores of rust fungi, some of which contain chemically identified and potent self-inhibitors, are not sensitive to soil mycostasis (Ko and Lockwood, 1967). (3) It is difficult to explain mycostasis occurring on a leaching apparatus (Ko and Lockwood, 1967; Hsu and Lockwood, 1973) by this mechanism since water flow would tend to remove or dilute self-inhibitors. (4) Finally, the high density of spores in soil required for expression of a self-inhibitory effect does not occur in normal populations (Griffin and Ford, 1974).

Workers investigating inhibitory substances have proposed that mycostasis in soil is a highly complex phenomenon involving a balance between inhibitory compounds and counteracting stimulatory substances (Dobbs and Gash; 1965; Linderman and Gilbert, 1973; Vaartaja, 1974; Watson and Ford, 1972; Smith and Cook, 1975). This view is based on reports of (1) several different kinds of inhibitors in the same or different soils, (2) the sometimes stimulatory nature of substances previously shown to be inhibitory, (3) the sporadic occurrence in time

and space of inhibitors and stimulators, and (4) the fact that in many cases the inhibition was very specific (Lockwood, 1977). According to Lockwood (1977), however, the complexity of such a hypothesis does not appear to be consistent with the broad spectrum effect manifested in nature nor is it consistent with the widely accepted concept of a dormant microbial population operating at or near starvation levels in soil.

Nutrient deprivation. Research on the status of energy-yielding nutrients in soils provides a solid basis for a concept that fungi and other microorganisms in soil exist in an environment deprived of available carbon needed for sustained growth. The proposition that nutrient deficiency in soil could account for mycostasis was advanced by Lockwood (1964) after earlier work had failed to show adequate evidence supporting the role of inhibitory substances in soil mycostasis. Lingappa and Lockwood (1964) demonstrated that nutrients leaking from fungal propagules activated microorganisms in the vicinity of the propagules, and suggested that microbial competition might deprive fungal propagules of nutrients needed for germination. More direct evidence that nutrient deprivation can restrict the germination of fungal propagules was later given by Ko and Lockwood (1967), who demonstrated that propagules that germinated poorly in distilled water also germinated poorly in soil. These propagules were nutrient-dependent and mycostasis-sensitive.

Several other propagules germinated in water and also germinated in soil. These propagules were nutrient-dependent, but mycostasis insensitive. A third group of propagules germinated in water and was mycostasis sensitive. Propagules from this group were strongly inhibited when incubated in a model system designed to create a steepened diffusion gradient away from the propagules, such as is thought to exist in soil

through microbial activity. In such a model system, propagules borne on membrane filters were exposed either to dripping water or salt solutions in a filter funnel, or were incubated on a bed of washed, sterile sand through which the solutions percolated. A nutrient solution used as a leaching medium supported germination, and activated ascospores of N. tetrasperma, which germinated freely on soil and in water were not inhibited in the leaching system (Ko and Lockwood, 1967). Thus, the leaching system, qualitatively, appeared to mimic the mycostatic effect of soil.

These findings have been confirmed and extended with other nutrient-independent but mycostasis-sensitive propagules. Germination of several propagules, including conidia (Ko and Lockwood, 1967; Sztejnberg and Blakeman, 1973; Hsu and Lockwood, 1973), chlamydospores (Adams et al., 1968; Filonow and Lockwood, 1981), and sclerotia (Emmatty and Green, 1969; Hsu and Lockwood, 1973; Ayanru and Green, 1974; Filonow and Lockwood, 1981) have been inhibited in various model systems imposing a diffusive stress on propagules or their substratum. Leaching with subsoil extract or salt solutions appeared to be as effective as distilled water in inhibiting germination (Ko and Lockwood, 1967; Sztejnberg and Blakeman, 1973; Bristow and Lockwood, 1975a).

The imposition of mycostasis by nutrient deprivation has been related to increased exudation losses from the propagules. Diffusive stress imposed on a leached sand apparatus induced greater losses of exudate from conidia of Cochliobolus victoriae, Curvularia lunata, and sclerotia of Sclerotium cepivorum than occurred on nonleached sand; intermittent diffusive stress induced greater losses than occurred during static periods (Bristow and Lockwood, 1975a; Sneh and Lockwood, 1975). Passage of exudates from the same species through the leached sand

apparatus stimulated partial germination nonspecifically, and the exudate was taken up by propagules made nutrient-dependent by prolonged diffusive stress (Britow and Lockwood, 1975a).

The leached sand apparatus has been calibrated by comparing the amount of exudate lost from ^{14}C-labeled conidia of C. victoriae on soil and in the leaching system at various flow rates. The faster the rate of flow, the greater the amount of exudate lost, and the lower the germination (Sneh and Lockwood, 1975; Filonow and Lockwood, 1979). Conidia incubated on soil always lost larger amounts of exudate than they did during incubation on the leached sand apparatus at flow rates sufficient to impose mycostasis. This result was confirmed using the same fungus with eight different soils (Filonow and Lockwood, 1979). Exudation in the leaching system at flow rates sufficient to reduce germination to levels < 10%, was 20-70% of that from conidia incubated on seven of the eight soils, and was equal to that on the other soil. At least for C. victoriae, the leading system does not appear to impose an excessive stress on the propagules, as compared with that of soil. These results also show that each of the eight soils imposed a sufficient nutrient sink to account for mycostasis without the obligatory participation of other factors.

Exudate from fungal propagules contain nutrients readily utilized by microorganisms in soil (Lingappa and Lockwood, 1964; Dickinson and Coley-Smith, 1970; Gilbert and Linderman, 1971; Bristow and Lockwood, 1975a). The rapid metabolism of exudates from spores and sclerotia by microbes in soil has led to the concept that soil is a 'sink' for energy-yielding nutrients and that fungal propagules lose nutrients as exudate to a diffusive stress imposed by the 'sink' (Lingappa and Lockwood, 1964;

Ko and Lockwood, 1967; Steiner and Lockwood, 1970; Yoder and Lockwood, 1973; Bristow and Lockwood, 1975a; Filonow and Lockwood, 1979).

In order for mycostasis to operate via the microbial nutrient sink, microorganisms must quickly respond to and utilize nutrient inputs. Bristow and Lockwood (1975b) reported that $^{14}CO_2$ was detected within a few minutes of adding ^{14}C-labeled glucose or exudate from conidia of C. victoriae to soil. Other evidence for the existence in soil of an efficient microbial sink for nutrients is that loss of glucose from paper disks was more rapid on natural soil than on sterile soil (Lockwood, 1975). The mean glucose half-life on several natural soils was 80 minutes and for autoclaved soils, 240 minutes.

The role of the microbial nutrient sink in soil mycostasis is also indicated by experiments on the restoration of mycostasis by inoculating sterilized subsoil fortified with glucose, with various microorganisms (Steiner and Lockwood, 1970). Subsoil was used because it released no detectable carbohydrates on autoclaving, and thus allowed uncomplicated detection of glucose. Germination of conidia of C. sativus was correlated with the amount of glucose remaining in the soil at different times. By 55-70 hours after inoculation with microorganisms, glucose had disappeared from the subsoil. Nutrient-dependent spores did not germinate on the aqueous extracts, whereas nutrient-independent spores germinated freely.

The soil's microbial nutient sink can effect losses from fungal propagules not only of preformed materials, but also of substrate taken up during the course of germination. Propagules of several fungi were first exposed to sterile soil for an interval just short of that required for emergence of the germ tube, then were transferred to natural soil or

the leaching system (Yoder and Lockwood, 1973). After several hours or days they were transferred again to sterilized soil to measure any change in the time required for germination, as compared with control propagules incubated continually on sterlized soil. A few hours' exposure to soil or the leaching system caused no change in the germination status of the spores, but incubation for several days caused regression proportional to the time of incubation, i.e., the germination time was proportionately prolonged. When conidia of Penicillium frequentans were allowed to take up ^{14}C-labeled glucose, then were incubated on the leaching apparatus, regression as measured by germination time and by loss of radioactivity was closely parallel. The label lost was about equally divided between respiration and exudation.

The cellular mechanism by which nutrient deprivation supresses germination is not known. The enhanced exudation of energy-rich materials from propagules incubated in the presence of microorganisms in soil or on leaves, or in model leaching systems, may represent soluble energy reserves which are lost as a rate sufficient to prevent their being used to synthesize a germ tube (Lockwood, 1977). The large initial flushes of exudation (Bristow and Lockwood, 1975a; Brodie and Blakeman, 1975; Sneh and Lockwood, 1975) may be related to reorganization of the spore membrane on hydration (Blakeman and Brodie, 1977), but the extent to which these initial losses drain insoluble reserves is not known. The smaller losses incurred thereafter are very likely derived from insoluble reserves.

Propagules may differ in their ability to conserve materials under diffusive stress. Nutrient-independent conidia of C. sativus and C. victoriae (Bristow and Lockwood, 1975a), C. oryzae (Bhattacharya and

Samaddar, 1977), and B. cinerea (Sztejnberg and Blakeman, 1973) became nutrient-dependent after several days or weeks of incubation on soil or leached sand. Further incubation resulted in a progressive loss of viability (Bristow and Lockwood, 1975a; Bhattacharya and Samaddar, 1977). However, many types of propagules are known to survive for months or years in soil. Exhaustion of reserves in these fungi could be minimized by a feedback mechanism, whereby the propagules 'sense' the competitive status of the environment. If exudates are removed from the immediate vicinity of the propagule beyond a specific rate, enzymes hydrolyzing insoluble reserves could be repressed, conserving the reserves. However, under conditions of reduced competition the enzymes may be depressed and reserve materials, made available via hydrolysis could then be used to synthesize the germ tube. Such a mechanism would require a slow drain on the propagule's storage reserves to produce the minimum quantity of exudate necessary for 'sensing' the environment. Differences in longevity or in ability to maintain nutrient-independence could be functions of differences in amounts of exudation inherent in the species or of the efficiency of enzyme repression.

Nutrient-dependent propagules will remain in a quiescent state as long as energy-yielding substrate in their vicinity remains scarce. As shown by Bristow and Lockwood (1975a), nutrient-independent propagules under long-term diffusive stress may be converted to the nutrient-dependent state. Once converted, a diffusive stress may no longer be needed to maintain quiescence, as long as the propagule's immediate environment is clear of nutrients. Moreover, results of Bristow and Lockwood (1975a) suggest that nutrient-dependent propagules may reabsorb their own exudate or perhaps nutrients from their environment and thereby

replenish their supply of endogenous nutrients.

Ecological Sigificance Of Mycostasis And The Microbial Nutrient Sink Of Soil

Quiescence of fungal propagules in soil due to mycostasis is of survival value, preventing the wastage that would occur from germination in the absence of substrate (Garrett, 1970; Lockwood, 1977). This concept was experimentally confirmed by Tinline and Chinn (1964) who showed that isolates of C. sativus capable of germination spontaneously in soil disappeared rapidly, whereas isolates whose germination was restricted persisted. The apparent general ability of rust urediospores to germinate freely in soil (Ko and Lockwood, 1967) may contribute to their well-known inability to survive in soil from season to season.

It would be of interest to know the ecological significance in soil, if any, of the relationships demonstrated among mycostasis sensitivity, propagule size, and germination time (Steiner and Lockwood, 1969). Dix (1967) found a relationship between mycostasis sensitivity of 12 fungi colonizing bean roots and their colonization sites. The least sensitive were pioneer colonizers of the surfaces of rot tips; slightly more sensitive were secondary colonizers of the root tissues further back; still more sensitive were fungi invading moribund tissues. The most sensitive fungi were restricted to the rhizosphere, but were not found colonizing the root themselves. This colonizaton pattern was related to increasing amino acid exudation from the root tip back, with the greatest exudation occurring from invaded portions of the root.

It has been suggested that mycostasis might be manipulated to reduce the severity of root diseases (Papavizas and Lumsden, 1980). A reduction in the severity of V. dahliae wilt of strawberries by soil amendment with

0.2% chitin or laminarin was associated with increased soil mycostasis (Jordan et al., 1972). Moreover, certain root pathogens have been controlled in the laboratory by stimulating germination by organic amendments, thus rendering the pathogen germ tube vulnerable to lysis. Reduction in the population density of Thielaviopsis basicola by alfalfa meal and other dried, green plant material (Adams and Papavizas, 1969; Sneh et al., 1976), and of C. sativus by soybean meal (Chinn and Ledingham, 1961) have been reported. This approach is effective only for pathogens that do not readily form new persistent structures. Whether sufficient organic material can be practically incorporated to reduce pathogen populations in the field by this mechanism is not known.

Since soils differ in their ability to inhibit fungal germination, one might expect consequent ecological differences. Soils 'suppressive' or 'conducive' to certain root pathogens are known (Arjunarao, 1971; Smith and Snyder, 1972; Alabouvette et al., 1979; Hancock, 1979; Hornby, 1979; Papavizas and Lumsden, 1980), and may be related to the germinability of the pathogen in the soils (Arjunarao, 1971; Smith and Snyder, 1972; Alabouvette et al., 1979).

As soils differ in mycostatic capacity, so do they differ in responsiveness to nutrient additions to annul mycostasis. Filonow and Lockwood (1979) found that mycostasis of conidia of C. victoriae was more readily annulled by nutrient titration in coarse-textured soils than in fine-textured soils. By this method of assessing mycostasis, fine-textured soils were more mycostatic. However, more exudate was lost from these conidia in coarse-textured than in fine-textured soils. These findings raise the question of which aspect has the greater significance for the fungus - the ease with which it can germinate in response to nutrient

inputs, or its ability to retain endogenous reserves.

It is reasonable to assume that continued loss of endogenous substrate would reduce propagule longevity. Bristow and Lockwood (1975a) showed that loss of endogenous nutrients from C. victoriae conidia subjected to a diffusive stress imposed by a leached sand apparatus or by soil resulted in a change from nutrient independence to nutrient-dependence, and subsequent loss of viability. If such transformations are at the expense of endogenous carbon reserves, it is possible that other aspects of fungal biology, e.g., virulence may be affected. From the standpoint of long-term fungal survival and vigor, exposure to soils with less microbial nutrient sinks may offer the most favorable environment - a nutrient sink sufficient to impose mycostasis and insure against spontaneous germination, but maximum conservation of endogenous reserves.

LIST OF REFERENCES

LIST OF REFERENCES

ADAMS, P. B., LEWIS, J. A. and PAPAVIZAS, G. C. (1968) Survival of root-infected fungi in soil. IV. The nature of fungistasis in natural and cellulose-amended soil on chlamydospores of Fusarium solani f. sp. phaseoli. Phytopathology 58, 378-383.

ADAMS, P. B. and PAPAVIZAS, G. C. (1969) Survival of root-infecting fungi in soil. X. Sensitivity of propagules of Thielaviopsis basicola to soil fungistasis in natural and alfalfa-amended soil. Phytopathology 59, 135-138.

ALABOUVETTE, C., ROUXEL, F. and LOUVET, J. (1979) Characteristics of Fusarium wilt-suppressive soils and prospects for their utilization in biological control. In Soil-Borne Plant Pathogens (B. Schippers and W. Gams, eds.), pp. 165-182, Academic Press, London.

ALEXANDER, M. (1977) Introduction to Soil Microbiology. John Wiley and Sons, New York.

ALLEN, P. J. (1976) Spore germination and its regulation. In Physiological Plant Pathology Vol. IV (R. Heitefuss and P. H. Williams, eds.), pp. 51-85, Springer-Verlag, New York.

ARJUNARAO, V. (1971) Biological control of cotton wilt. I. Soil fungistasis and antibiosis in cotton field. Indian Academy of Sciences. Proceedings, Section B 73, 265-272.

AYANRU, D. K. G. and GREEN, R. J., Jr. (1974) Alterations of germination patterns of sclerotia of Macrophomina phaseolina on soil surfaces. Phytopathology 64, 595-601.

BABIUK, L. A. and PAUL, E. A. (1970) The use of fluorescein isothiocyanate in the determination of the bacterial biomass of grassland soil. Canadian Journal of Microbiology 16, 57-62.

BALIS, C. (1976) Ethylene-induced volatile inhibitors causing soil fungistasis. Nature 259, 112-114.

BALIS, C. and KOUYEAS, V. (1979) Contribution of chemical inhibitors to soil mycostasis. In Soil-Borne Plant Pathogens (B. Schippers and W. Gams, eds.), pp. 97-106, Academic Press, London.

BHATTACHARYA, M. and SAMADDAR, K. R. (1977) Fungistasis, lysis and survival of Pyricularia oryzae and Helminthosporium oryzae in soil. Phytopathologische Zeitschift 89, 60-67.

BLAKEMAN, J. P. and BRODIE, I. D. S. (1977) Competition for nutrients between epiphytic microorganisms and germination of spores of plant pathogens on beetroot leaves. Physiological Plant Pathology 10, 29-42.

BRODIE, I. D. S. and BLAKEMAN, J. P. (1975) Competition for carbon compounds by a leaf surface bacterium and conidia of Botrytis cinerea. Physiological Plant Pathology 6, 125-135.

BROWN, M. E. (1973) Soil bacteriostasis limitation in growth of soil and rhizosphere bacteria. Canadian Journal of Microbiology 19, 195-199.

BRISTOW, P. R. and LOCKWOOD, J. L. (1975a) Soil fungistasis: role of spore exudates in the inhibition of nutrient-independent propagules. Journal of General Microbiology 90, 140-146.

BRISTOW, P. R. and LOCKWOOD, J. L. (1975b) Soil fungistasis: role of the microbial nutrient sink of fungistatic substances in two soils. Journal of General Microbiology 90, 147-156.

CHINN, S. H. F. and LEDINGHAM, R. J. (1961) Mechanisms contributing to the eradicaton of spores of Helminthosporium sativum from amended soil. Canadian Journal of Botany 39, 739-748.

CHINN, S. H. F. and TINLINE, R. D. (1964) Inherent germinability and survival of spores of Cochliobolus sativus. Phytopathology 54, 349-352.

CLARK, F. E. (1967) Bacteria in soil. In Soil Biology (A. Burges and F. Raw, eds.), pp. 15-49, Academic Press, London.

CLARK, F. E. and PAUL, E. A. (1970) The microflora of grassland. Advances in Agronomy 22, 375-435.

CORNFORTH, I. S. (1975) The persistence of ethylene in aerobic soils. Plant and Soil 42, 85-96.

COOK, R. J. (1977) Management of the associated biota. In Plant Disease: An Advanced Treatise Vol I (J. G. Horsfall and E. B. Cowling, eds.), pp. 145-166, Academic Press, New York.

DAVIS, R. D. (1976) Soil bacteriostasis: relation to bacterial nutrition and active soil inhibition. Soil Biology and Biochemistry 8, 429-433.

DICKINSON, D. J. and COLEY-SMITH, J. R. (1970) Stimulation of soil bacterial by sclerotia of Sclerotium cepivorum Berk. in relation to fungistasis. Soil Biology and Biochemistry 2, 157-162.

DIX, N. J. (1967) Mycostasis and root exudation: factors influencing the colonization of bean rots by fungi. Transactions British Mycological society 50, 23-31.

DOBBS, C. G. and HINSON W. H. (1953) A widespread fungistasis in soils. Nature 172, 197-199.

DOBBS, C. G. and GASH, M. J. (1965) Microbial and residual mycostasis in soils. Nature 207, 1354-1356.

EMMATTY, D. A. and GREEN, R. J., Jr. (1969) Fungistasis and the behavior of microsclerotia of Verticillium albo-atrum in soil. Phytopathology 59, 1590-1595.

ENO, C. F., BLUE, W. G. and GOOD, J. M., Jr. (1955) The effect of anhydrous ammonia on nematodes, fungi, bacteria, and nitrification in some Florida soils. Proceedings of the Soil Science Society of America 19, 55-58.

FLANAGAN, P. W. and BUNNELL, F. L. (1976) Decomposition models based on climatic variables, substrate variables, microbial respiration and production. In The Role of Terrestrial and Aquatic Organisms in Decomposition Processes (J. M. Anderson and A. Macfadyen, eds.), pp. 437-457, Blackwell, Oxford.

FLANAGAN, P. W. and VAN CLEVE, K. (1977) Microbial biomass, respiration and nutrient cycling in a black spruce taiga ecosystem. In Soil Organisms as Components of Ecosystems (U. Lohm and T. Persson, eds.), pp. 261-273, Swedish Natural Science Research Council, Stockholm.

FILONOW, A. B. and LOCKWOOD, J. L. (1979) Conidial exudation by Cochliobolus victoriae on soils in relation to mycostasis. In Soil-Borne Plant Pathogens (B. Schippers and W. Gams, eds.), pp. 107-119, Academic Press, London.

FILONOW, A. B. and LOCKWOOD, J. L. (1981) ^{14}C-exudation by fungal propagules into soils of different textures in relation to fungistasis. Phytopathology 71, 216. (Abstract).

GARRETT, S. D. (1970) Pathogenic Root-Infecting Fungi. Cambridge University Press, London.

GILBERT, R. G. and LINDERMAN, R. G. (1971) Increased activity of soil microorganisms near sclerotia on Sclerotium rolfsii in soil. Canadian Journal of Microbiology 17, 557-562.

GILPATRICK, J. D. (1969) Role of ammonia in the control of avocado root rot with alfalfa meal soil amendment. Phytopathology 59, 973-978.

GRAY, T. R. G. and WILLIAMS, S. T. (1971) Microbial productivity in soil. In Microbes and Biological Productivity (D. E. Hughes and A. H. Rose, eds.), pp. 255-286, Cambridge University Press, London.

GRIFFIN, D. M. (1972) Ecology of Soil Fungi. Syracuse University Press, Syracuse.

GRIFFIN, G. J. and FORD, R. H. (1974) Soil fungistasis: fungus spore germination in soil at spore densities corresponding to natural population levels. Canadian Journal of Microbiology 20, 751-754.

GRIFFIN, G. J. and ROTH, D. A. (1979) Nutritional aspects of soil mycostasis. In Soil-Borne Plant Pathogens (B. Schippers and W. Gams, eds.), pp. 79-96, Academic Press, London.

GRIFFITHS, D. A. (1966) Sensitivity of Malayan isolates of Fusarium to soil fungistasis. Plant and Soil 24, 269-278.

HANCOCK, J. G. (1979) Occurrence of soils suppressive to Pythium ultimum. In Soil-Borne Plant Pathogens (B. Schippers and W. Gams, ed.), pp. 183-189, Academic Press, London.

HORNBY, D. (1979) Take-all decline: a theorist's paradise. In Soil-Borne Plant Pathogens (B. Schippers and W. Gams, eds.), pp. 132-156, Academic Press, London.

HSU, S. C. and LOCKWOOD, J. L. (1973) Soil fungistasis: behavior of nutrient-independent spores and sclerotia in a model system. Phytopathology 63, 334-337.

HWANG, S. C. and KO, W. H. (1974) Germination of Calonectria crotalariae conidia and ascospores on soil. Mycologia 66, 1053-1055.

IOANNOU, N., SCHNEIDER, R. W. and GROGAN, R. G. (1977) Effect of oxygen, carbon dioxide, and ethylene on growth, sporulation and production of microsclerotia by Verticillium dahliae. Phytopathology 67, 645-650.

JORDAN, V. W. L., SNEH, B. and EDDY, B. P. (1972) Influence of organic amendments on Verticillium dahliae and on the microbial composition of strawbery rhizosphere. Annals of Applied Biology 70, 139-148.

KIRK, P. W., Jr. (1980) The mycostatic effect of seawater on spores of terrestrial and marine higher fungi. Botana Marina 23, 233-238.

KO, W. H. and LOCKWOOD, J. L. (1967) Soil fungistasis: relation to fungal spore nutrition. Phytopathology 57, 894-901.

KO, W. H. and HORA, F. K. (1971) Fungitoxicity in certain Hawaiian soils. Soil Science 112, 276-279.

KO, W. H. and HORA, F. K. (1972a) The nature of a volatile inhibitor from alkaline soils. Phytopathology 62, 574-575.

KO, W. H. and HORA, F. K. (1972b) Identification of an Al ion as a soil fungitoxin. Soil Science 113, 42-45.

KO, W. H., HORA, F. K. and HERLICSKA, E. (1974) Isolation and identification of a volatile fungistatic substance from alkaline soil. Phytopathology 64, 1398-1400.

KO, W. H. and CHOW, F. K. (1977) Characteristics of bacteriostasis in natural soils. Journal of General Microbiology 102, 295-298.

KUCERA, C. L. and KIRKHAM, D. R. (1971) Soil respiration studies in tallgrass prairie in Missouri. Ecology 52, 912-915.

LEACH, L. D. and DAVEY, A. E. (1935) Toxicity of low concentrations of ammonia to mycelium and sclerotia of Sclerotium rolfsii. Phytopathology 25, 957-959.

LEWIS, J. A. and PAPAVIZAS, G. C. (1974) Effect of volatiles from decomposing plant tissues on pigmentation, growth, and survival of Rhizoctonia solani. Soil Science 118, 156-163.

LEWIS, J. A. (1976) Production of volatiles from decomposing plant tissue and effect of these volatiles on Rhizoctonia solani in culture. Canadian Journal of Microbiology 22, 1300-1306.

LINGAPPA, B. T. and LOCKWOOD, J. L. (1964) Activation of soil microflora by fungus spores in relation to soil fungistasis. Journal of General Microbiology 35, 215-227.

LINDERMAN, R. G. and GILBERT, R. G. (1973) Behavior of sclerotia of Sclerotium rolfsii produced in soil or in culture regarding germination stimulation by volatiles, fungistasis and sodium hypochlorite treatment. Phytopathology 63, 500-504.

LLOYD, A. B. (1968) Behavior of streptomycetes in soil. Journal of General Microbiology 56, 165-170.

LOCKWOOD, J. L. (1964) Soil fungistasis. Annual Review of Phytopathology 2, 341-362.

LOCKWOOD, J. L. (1975) Quantitative evaluation of a leaching model system for soil fungistasis. Phytopathology 65, 460-464.

LOCKWOOD, J. L. (1977) Fungistasis in soils. Biological Reviews 52, 1-43.

LOCKWOOD, J. L. (1979) Soil mycostasis: concluding remarks. In Soil-Borne Plant Pathogens (B. Schippers and W. Gams, eds.), pp. 121-129, Academic Press, London.

LOCKWOOD, J. L. and FILONOW, A. B. Responses of fungi to nutrient-limiting conditions and to inhibitory substances in natural habitats. Advances in Microbial Ecology, 5 (In press).

MACKO, V., STAPLES, R. C., YANIV, A. and GRANADOS, R. R. (1976) Self-inhibitors of fungal spore germination. In The Fungal Spore: Form and Function (D. J. Webber and W. M. Hess, eds.), pp. 73-98, John Wiley and Sons, New York.

MAYFIELD, C. I., WILLIAMS, S. T., RUDDICK, S. M. and HATFIELD, H. L. (1972) Studies on the ecology of actinomycetes in soil. IV. Observations on the form and growth of streptomycetes in soil. Soil Biology and Biochemistry 4, 79-91.

PAPAVIZAS, G. C. and ADAMS, P. B. (1969) Survival of root-infecting fungi in soil. XII. Germination and survival of endoconidia and chlamydospores of Thielaviopsis basicola in fallow soil and in soil adjacent to germinating bean seed. Phytopathology 59, 371-378.

PAPAVIZAS, G. C. and LUMSDEN, R. D. (1980) Biological control of soilborne fungal propagules. Annual Review of Phytopathology 18, 389-413.

PAVLICA, D. A., HORA, T. S., BRADSHAW, J. J., SKOGERBOE, R. K. and BAKER, R. (1978) Volatiles from soil influencing activities of soil fungi. Phytopathology 68, 758-765.

SCHIPPERS, B., BOERWINKEL, D. J. and KONINGS, H. (1978) Ethylene not responsible for inhibition of conidium germination by soil volatiles. Netherlands Journal of Plant Pathology 84, 101-107.

SHIELDS, J. A., PAUL, E. A. and LOWE, W. E. (1973) Turnover of microbial tissue in soil under field conditions. Soil Biology and Biochemistry 5, 753-764.

SMILEY, R. W., COOK, R. J. and PAPENDICK, R. I. (1970) Anhydrous ammonia as a soil fungicide against Fusarium and fungicidal activity in the ammonia retention zone. Phytopathology 60, 1227-1232.

SMITH, A. M. (1973) Ethylene as a cause of soil fungistasis. Nature 246, 311-313.

SMITH, A. M. (1976) Ethylene in soil biology. Annual Review of Phytopathology 14, 53-73.

SMITH, A. M. and COOK, R. J. (1974) Implications of ethylene production by bacteria for biological balance of soil. Nature 252, 703-705.

SMITH, S. N. and SYNDER, W. C. (1972) Germination of Fusarium oxysporum chlamydospores in soils favorable and unfavorable to wilt establishment. Phytopathology 62, 273-277.

SNEH, B. and LOCKWOOD, J. L. (1975) Quantitative evaluation of the microbial nutrient sink in soil in relation to a model system for soil fungistasis. Soil Biology and Biochemistry 8, 65-69.

SNEH, B., HOLDAWAY, B. F., HOOPER, G. R. and LOCKWOOD, J. L. (1976) Germination-lysis as a mechanism for biological control of Thielaviopsis basicola pathogenic on soybean. Canadian Journal of Botany 54, 1499-1508.

STEINER, G. W. and LOCKWOOD, J. L. (1969) Soil fungistasis: sensitivity of spores in relation to germination time and size. Phytopathology 59, 1084-1092.

STEINER, G. W. and LOCKWOOD, J. L. (1970) Soil fungistasis: mechanism in sterilized, reinoculated soil. Phytopathology 60, 89-91.

SZTEJNBERG, A. and BLAKEMAN, J. P. (1973) Studies on leaching of Botrytis cinerea conidia and dye absorption by bacteria in relation to competition for nutrients on leaves. Journal of General Microbiology 78, 15-22.

TABAK, H. H. and COOKE, W. B. (1968) The effects of gaseous environments on the growth and metabolism of fungi. Botanical Reviews 34, 126-252.

TRINCI, A. P. J. (1971) Exponential growth of germ tubes of fungal spores. Journal of General Microbiology 67, 345-348.

TSAO, P. H. and ZENTMYER, G. A. (1979) Suppression of Phytophthora cinnamoni and Ph. parasitica in urea-amended soils. In Soil-Borne Plant Pathogens (B. Schippers and W. Gams, eds.), pp. 191-199, Academic Press, London.

VAARTAJA, O. (1974) Inhibition of Pythium ultimum in molecular fractions from gel filtration of soil extracts. Canadian Journal of Microbiology 20, 1273-1280.

VAARTAJA, O. (1977) Responses of Pythium ultimum and other fungi to a soil extract containing an inhibitor with low molecular weight. Phytopathology 67, 67-71.

WATSON, A. G. and FORD, E. J. (1972) Soil fungistasis--a reappraisal. Annual Review of Phytopathology 10, 327-348.

WICKLOW, D. T. and ZAK, J. C. (1979) Ascospore germination of carbonicolous ascomycetes in fungistatic soils: an ecological interpretation. Mycologia 71, 238-242.

YODER, D. L. and LOCKWOOD, J. L. (1973) Fungal spore germination on natural and sterile soil. Journal of General Microbiology 74, 107-117.

ZAKARIA, M. A., LOCKWOOD, J. L. and FILONOW, A. B. (1980) Reduction in Fusarium population density in soil by volatile degradation products of oilseed meal amendments. Phytopathology 70, 495-499.

MYCOSTASIS AND THE MICROBIAL NUTRIENT SINK OF SOIL

I. ASSESSMENT OF THE MICROBIAL NUTRIENT SINK OF FIVE SOILS

MYCOSTASIS AND THE MICROBIAL NUTRIENT SINK OF SOIL

I. ASSESSMENT OF THE MICROBIAL NUTRIENT SINK OF FIVE SOILS

SUMMARY

^{14}C-exudate lost over 4-24 h from fungal propagules to 5 soils was studied in relation to mycostasis. Propagules were sclerotia of Macrophomina phaseolina, chlamydospores of Thielaviopsis basicola, and conidia of Cochliobolus victoriae, and Stemphylium sarcinaeforme. ^{14}C-exudate losses from the 4 fungi to two sandy loam soils and a loam were generally greater than losses to two clay loam soils. Respiration of soil microflora over 2-12 h, when soils were pulsed with ^{14}C-glucose or a mixture of ^{14}C-amino acids showed a similar trend. ^{14}C losses from M. phaseolina sclerotia and C. victoriae conidia incubated on soils were greater than diffusive losses imposed on these propagules during incubation aseptically, on a bed of sand through which water percolated at a flow rate sufficient to inhibit germination. In contrast to the order of fungal exudation, fine-textured soils required more nutrients to annul mycostasis than coarse-textured soils. Using γ-irradiated soils, more ^{14}C-exudate was adsorbed by the clay loam soils than the loam and sandy loam soils. The results suggest that the degree of adsorption in soil may be an important factor in controlling fungal utilization of soluble nutrients. These findings also raise the question as to which aspect has the greater influence on the life of a fungus--the ease with which it can germinate in response to nutrients, or the amount of endogenous substrate lost or retained.

INTRODUCTION

Mycostasis in soils has been attributed to the inherently low supply of energy-yielding substrate in soil and to the loss of endogenous nutrients essential for germination from fungal propagules to competing microorganisms in soil (Ko and Lockwood, 1967; Yoder and Lockwood, 1973; Lockwood, 1977). Nutrient-independent propagules which germinated only slight on natural soil also showed little germination when subjected to nutrient depletion stress on a sand bed udergoing continual leaching with water or dilute salt solutions (Ko and Lockwood, 1968; Hsu and Lockwood, 1973). Suppression of germination by nutrient depletion stress in the leached sand apparatus was related to loss of exudate from propagules (Bristow and Lockwood, 1975a,b; Filonow and Lockwood, 1979). The concept of an active soil microflora rapidly utilizing inputs of energy-yielding substrate, including endogenous nutrients from fungal propagules, has been termed the microbial nutrient sink of soil (Lockwood, 1977).

Recently, Filonow and Lockwood (1979) estimated the losses of ^{14}C-labeled exudate over 2-4 h from conidial of Cochliobolus victoriae to the microbial nutrient sinks of eight soils. By comparing such losses to those losses incurred in the leached sand apparatus, they found the microbial nutrient sink of soil was sufficient to account for soil mycostasis in all eight soils. Coarse-textured soils (soils with more sand and lesser amounts of clay) imposed a greater loss of ^{14}C-exudate

from C. victoriae conidia than did fine-textured soils.

The objectives of this work were (1) to evaluate over longer incubation periods the relative microbial nutrient sinks of five additional soils, using several test fungi, and (2) to assess the role of the microbial nutrient sinks in the five soils in imposing mycostasis.

MATERIALS AND METHODS

Characterization Of Soils

Five different agricultural soils from Michigan were used (Table 1). Soils were taken from the field, air-dried, passed through a 10 mm sieve and stored in large plastic bags at 4°C for the duration of all work. Subsamples were taken from the se soils and allowed to equilibrate at room temperature (24±1°C) for several days before use. Textural analyses were done by the hydrometer method (Day, 1965), total carbon was determined with a Leco Carbon Analyzer (Leco Corp., St. Joseph, MI), total nitrogen was determined by Kjeldhal analysis (Bremner, 1965), the cation exchange capacity was determined by ammonium saturation (Chapman, 1965), and pH of a soil-water paste (1:2, w/w) was measured using a glass electrode.

Maintenance Of Propagules

Cochliobolus victoriae Meehan and Murphy and *Stemphylium sarcinaeforme* Wiltshire (Cav.) were maintained on carrot broth containing per liter: 250 ml of carrot broth (30 g carrots in 250 ml water, autoclaved 10 min and filtered) and 20 g agar. *Penicillium frequentans* Westling, *Thielaviopsis basicola* (Berk. and Br.) Ferr. and *Macrophomina phaseolina* (Tassi) Goid. [*Macrophomina phaseoli* (Maubl.) Ashby] were maintained on potato-dextrose agar. Culture transfers were routinely derived from one parent culture of each fungus. Conidia of *C. victoriae* and

Table 1. Characteristics of soils*

Soil	Sand (%)	Silt (%)	Clay (%)	Total Carbon (%)	Total Nitrogen (%)	C.E.C.† meq/100 g	pH
Boyer sandy loam	81	4	15	1.03	0.07	3.96	6.3
Spinks sandy loam	77	8	15	1.18	0.07	3.56	7.3
Dryden loam	48	30	22	1.44	0.12	11.54	6.3
Parkhill clay loam	43	24	33	1.80	0.18	15.82	7.2
Brookston clay loam	36	26	38	5.74	0.55	33.01	6.2

*Results are means of duplicate determinations.

†C.E.C. = cation exchange capacity.

S. sarcinaeforme, chlamydospores of T. basicola, and sclerotia of M. phaseolina were nutrient-independent, whereas conidia of P. frequentans were nutrient-dependent.

^{14}C-labeled propagules were grown on ^{14}C-impregnated agar plates. One ml of sterile water containing 10 µCi of uniformly labeled ^{14}C-glucose or sucrose (sp. act. 210 mCi/mM or 360 mCi/mM, respectively; ICN, Irvine, CA) was spread on the agar surface and allowed to diffuse into the agar. After 3 days the plates were inoculated in the center, sealed with Parafilm and incubated at 24±1°C for at least one month prior to use.

Preparation Of Propagule Suspensions

The following procedure was routinely used to prepare propagule suspensions of all the fungi, except T. basicola. The surface of an agar culture was flooded with cold, sterile and dilute (10^{-2}) Pfeffer's salts solution (Bristow and Lockwood, 1975a). Propagules were gently dislodged with a bent glass rod, and the suspension was passed through a 250 µm stainless steel sieve into a cold (5°C) centrifuge tube. Propagules in the capped tube were washed 3 times by centrifugation (10^4 x g for 5 min at 5°C). The final suspension volume ranged from 10-20 ml and was held in ice during use. The density of conidia in suspensions was determined by microscopically counting sclerotia in several 0.1 ml drops of suspension.

Chlamydospores of T. basicola were separated from endoconidia and culture debris by a multiple sieving method. An agar culture of T. basicola was washed 3 times with dilute Pfeffer's solution to remove endoconidia. More salts solution was added and a glass rod was used to

dislodge the chlamydospores, which were transferred to a sterile glass tissue homogenizer with a loose fitting Teflon pestle. After trituration for 5 min, the material was poured through a 28 µm mesh nylon sieve atop a Buchner funnel with a 20 µm mesh nylon sieve on the perforated plate. Clusters of chlamydospore chains and hyphal debris were retained on the 28 µm mesh sieve, whereas single, intact chains of chlamydospores and individual chlamydospores were held on the 20 µm sieve. Endoconidia were washed through the 20 µm mesh sieve with gentle suction and several washes of sterile Pfeffer's solution. Chlamydospores on the 20 µm mesh sieve were dislodged by vortex-mixing in a centrifuge tube containing 10 ml of sterile Pfeffer's solution. Chlamydospore density was adjusted with a haemacytometer. Fungal material left on the 28 µm mesh sieve was triturated further to provide more single chains of chlamydospores, if the first yield was insufficient.

In all assays of propagule germination, propagules in suspension were vacuum deposited on 1.5 cm x 1.5 cm pieces of Nuclepore membrane filters (0.4 µm pore dia; Nuclepore Corp., Pleasanton, CA). Propagule densities on the membranes were as follows: ca. 10^3 for conidia of C. victoriae and S. sarcinaeforme and chlamydospores of T. basicola, 10^4 for conidia of P. frequentans, and ca. 10^2 for sclerotia of M. phaseolina.

Sensitivity Of Propagules To Soil Mycostasis

The germination of propagules was determined on sterilized soil and natural soil amended with increasing amounts of sucrose and peptone (Hsu and Lockwood, 1971), to determine their sensitivities to mycostasis. Ten g samples of the soils were wetted to -100 kPa (-0.1 bar) matric potential, and were equilibrated fro 16-24 h before use. Soils in 6 cm

dia glass dishes were sterilized by autoclaving for 1 h. Natural soils or amended soils were contained in 6 cm dia plastic dishes.

Sucrose concentrations ranged from 10^2 to 10^3 $\mu g \cdot g^{-1}$ soil and peptone concentrations were 1/5 (w/w) that of sucrose. The soil was mixed well with a spatula, smoothed, and allowed to equilibrate for 1 h. In each experiment, duplicate Nuclepore membranes bearing fungal propagules were placed on duplicate samples of untreated and treat soils. Conidia and chlamydospores were incubated on the soils for 12-18 hr prior to germination assay. Sclerotia were incubated for 24-36 h. Membranes bearing propagules were stained with phenolic rose bengal, destained in water, and mounted on glass slides with double sticky tape. Germination was counted microscopically with incident illumination. Three to four experiments were done.

Soil Solution Bioassay

Soils were wetted to about -50 kPa matric potential and equilibrated for 16-24 h prior to centrifugation at 5°C and 3000 x g for 40 min (Davies and Davies, 1963). Soil solutions were quickly filter-sterilized (0.2 µm pore dia. Nuclepore) and kept frozen until bioassay.

On hundred µl of sterile soil solution was placed into double-welled microscope slides enclosed in petri plates. Duplicate pieces of Nuclepore membrane bearing fungal propagules were floated on the soil solutions. Conidia of C. victoriae and S. sarcinaeforme were incubated on the soil solutions for 10-16 h, conidia of P. frequentans and chlamydospores of T. basicola for 24 h, and sclerotia of M. phaseolina for 48 h, prior to the counting of germination, as described above. There were 3 to 4 membranes on each soil solution, and the experiment was repeated once.

Bioassay For Volatile Inhibitors

Twenty grams of soil in a glass petri plate were wetted to about -50 kPa matric potential and equilibrated for 16-24 h. Double-welled microscope slides containing 100 µl of sterile Pfeffer's solution were placed on the sols. Small pieces of membrane filters bearing propagules were placed on the salt solution, the plates were covered, and wrapped in Parafilm. The germination bioassay was as described above. There were duplicate membranes per soil and duplicate soil plates. The experiment was repeated once.

Microbial Populations In Soils

Microbial populations in soils were estimated by dilution plate counts on the following media: chitin (Hsu and Lockwood, 1975) for actinomycetes; trypticase soy broth agar (Martin, 1975), containing 50 ppm of pentachloronitrobenzene to inhibit actinomycetes (Farley and Lockwood, 1968) for aerobic bacteria; and PDA supplemented with 250 mg of chloramphenicol and 0.5 ml of a detergent TMN (Union Carbide, New York, NY) for fungi. Plates were streaked with 0.2 ml of soil suspensions and incubated at 24°C for 5-7 days for bacteria and fungi and 10-12 days for actinomycetes. Anaerobic bacteria were determined using thioglycolate broth (Difco Co., Detroit, MI) and the most probable number technique (Alexander, 1965). Two to three determinations of microbial populations per soil were made.

ATP Content And Dehydrogenase Activity In Soils

ATP was extracted by the method of Paul and Johnson (1977), using $NaHCO_3$ as the extractant. Soils were wetted to about -50 kPa matric potential and equilibrated for 16-24 h prior to extraction. A

luciferase-luciferin preparation was made from 250 mg of Sigma FLE-250 (Sigma Chemical Co., St. Louis, MO) and 1 mg of luciferin in 25 ml of sterile distilled water. The preparation was incubated overnight in the dark, and centrifuged at 10 x g for 15 min. The enzyme preparation was kept at 5°C until use. Dilutions of ATP were prepared using a bicarbonate:Tris (1:5, v/v) diluent (Paul and Johnson, 1977). Two hundred µl of an ATP standard or sample were mixed with 400 µl of the luciferase-luciferin preparation in a glass scintillation vial, and ATP was measured in a Lab-Line ATP Photometer, Model 9140 (Lab-Line Instruments, Melrose Park, IL). Analyses were done in triplicate.

Dehydrogenase activity in soils was estimated by colorimetric measurement of 2,3,4-triphenyltetrazolium (TTF) formed by the reduction of 2,3,4-triphenyltetrazolium chloride (TTC) incubated in soils. A modified procedure combining aspects of the procedures of Casida (1977) and Domsch et al. (1979) was used.

To 10.0 g of soil in a 50 ml flask, enough 0.2 M Tris buffer (pH 7.6) was added so that the matric potential was about -100 kPa. The soils were then equilibrated for 16-20 h. Two ml of 1% TTC (Aldrich Chemical Co., Milwaukee, WI) and 2 ml of C. victoriae conidial exudate were added to the flasks and the contents were mixed with a sterile glass rod. Conidial exudate was obtained by soaking 10^7 conidia in 50 ml of sterile distilled water at 24°C for 4 h. The suspension was filtered through a 0.2 µm dia pore membrane filter. The flask was capped with a rubber serum cap and the air headspace replaced with nitrogen. The flasks were incubated anaerobically at 35°C for 24 h. Methanol extraction of the soils for TTF (Aldrich Chemical Co.) and quantitation was done according to the method of Casida (1977). Four determinations per soil were made.

Soil Respiration Using ^{14}C-substrates

Four g of soil in small incubation chambers were wetted to about -50 kPa matric potential and equilibrated for 16-24 h. Soils were then pulsed with uniformly labeled ^{14}C-glucose or a ^{14}C-amino acid mixture. Soils received 3 x 10^3 dpm of ^{14}C-glucose or 3.6 x 10^3 dpm of ^{14}C-amino acids. The respiration apparatus and $^{14}CO_2$ collection method were identical to those used in the ^{14}C-exudate method. There were two replicates per soil per experiment and two experiments were done.

^{14}C-exudate Lost From Propagules To Soils

Four g of soil were placed in a truncated polypropylene centrifuge tube (2.5 cm x 3.0 cm) which served as an incubation chamber. Soil was wetted to about -50 kPa matric potential and equilibrated for 16-24 h. A disk of nylon screen (80 μm mesh, 2.5 cm dia) was placed on the moist soil.

A membrane filter bearing ^{14}C-labeled propagules was centered on the nylon disc and the chamber was connected to a manifold which passed moist air at 50 ml·mm^{-1} over the soil. ^{14}C counts on the membranes were kept within a 10-20 x 10^3 cpm range. $^{14}CO_2$ was collected in 15 ml of ethanolamine scintillation cocktail via fine dispersion tubes (5 mm O.D x 10 mm x 135 mm; Ace Glass Co., Vineland, NJ). After incubation periods of 4-24 h, the dispersion tube was removed from the ethanolamine cocktail, and rinsed with 1-2 ml of methanol into the cocktail. The membrane was removed and placed on a small piece of paper tissue (2.5 cm x 2.5 cm) to absorb soil solution adhering to the soil side of the membrane. The membrane was dissolved in 0.3 ml of chloroform and the propagules were suspended in 10 ml of 4% Cab-O-Sil scintillation

cocktail. The tissue paper was placed inside the soil chamber, moistened, and the contents of the chamber were quickly frozen (3-5 min) in a dry ice-methanol bath. The chambers were kept frozen until soil oxidation.

^{14}C-exudate in soil was oxidized to $^{14}CO_2$ by acid dichromate (Filonow and Lockwood, 1979). Frozen soil was washed with distilled water into a boiling flask, and the flask was placed into a small heating mantle on a magnetic stirrer. Fifty ml of oxidant was slowly dripped into the flask (ca. 10 min for total delivery), with stirring. The reaction mixture was kept at 90°C and the soil was oxidized for 30 min. $^{14}CO_2$ was collected in ethanolamine.

Respired ^{14}C plus residual ^{14}C recovered from soil by oxidation gave an estimate of total ^{14}C exuded by propagules to soils. $^{14}CO_2$ evolved from fungal respiration was not determined since the rate of respiration on all soils was assumed to be the same and thus would not significantly alter the relative amounts of $^{14}CO_2$ respired from the soils. Preliminary work with labeled conidia of C. victoriae showed that 0.9 to 1.4% of the ^{14}C was respired in 10 h by conidia incubated on a leached sand apparatus at 90 ml·min^{-1}. However, 3-10% of the conidia had germinated, whereas 0-2% germination was observed on the soils during the same incubation period. Moreover, respiratory output of a C. victoriae isolate had previously been estimated to be only 0.1% of the ^{14}C present in the conidia (Sneh and Lockwood, 1976). Total ^{14}C in the propagules prior to membrane contact with soil was estimated by the sum of ^{14}C exuded plus ^{14}C remaining in propagules on the membrane at the end of an experiment. Percent label lost in propagule exudate on soil was calculated from total ^{14}C exuded/total ^{14}C available x 100, and this value was

used to determine the efficiency of each soil in removing soluble nutrients from propagules. Using the ^{14}C-exudate method, recoveries of ^{14}C-glucose, ^{14}C-amino acids, and ^{14}C-exudate of C. victoriae applied to the soils ranged from 80% to 108%. Therefore, the method was considered to be essentially quantitative and results were not corrected for ^{14}C recoveries. For each fungal propagule type there were two replicates per soil per experiment. There were three to four experiments conducted for each incubation period.

^{14}C-exudate Adsorption To Soils

The ability of -irradiated soils to adsorb ^{14}C-exudate from C. victoriae was assessed. Soils were irradiated with 4.8 MRads of γ-rays from a ^{60}Co source. Sterility of soils was determined from crumbs of soil sprinkled onto nutrient agar. No colonies were detected after 1 week of incubation.

One g (O.D. weight) of soil was packed into disposable, sterile Pasteur pipets (5 mm i. d. x 14.5 cm) plugged at the bottom with glass wool. A sterile rubber serum cap was placed on top of the pipet. A 18-gauge needle connected to silicone medical tubing (1 mm i. d.) pierced the serum cap, and delivered sterile ^{14}C-exudate to the top of the soil columns. ^{14}C-exudate was obtained by soaking 10^7 conidia of C. victoriae in 50 ml of sterile water for 4 h. The conidia were removed by membrane filtration and the ^{14}C-exudate was diluted to 500 ml with sterile distilled water. Radioactivity in the diluted exudate was 194 cpm$\cdot$ml^{-1}. ^{14}C-exudate was pumped onto the soil columns at 0.1 ml$\cdot$min^{-1} using a peristaltic pump. Effluent (3.0 ml) from the columns was collected every 30 min into a scintillation vial, and 10 ml of aqueous scintillation

cocktail was added. Effluent was collected from duplicate columns per soil per experiment, and two experiments were done.

Loss Of ^{14}C-exudate From Propagules On Leached Sand Apparatus

The leached sand apparatus used was that described by Bristow and Lockwood (1975a). All components were autoclaved prior to each experiment. ^{14}C-labeled propagules borne on Nuclepore membrane filters (2.5 cm dia, 0.4 µm pore dia) were incubated on a sand bed through which a 10^{-2} dilution of Pfeffer's solution percolated. Flow rates ranged from 10-120 ml·min^{-1}. About 2 x 10^4 conidia of C. victoriae or ca. 300-500 microsclerotia of M. phaseolina per membrane were placed on the sand in the leaching dishes. Exudate form conidia of C. victoriae was collected for 6 h and exudate from microsclerotia was collected for 17 h. Exudate was collected in freeze-drying flasks set in an ice bath. Each flask contained 1-2 drops of 0.05% thimerosal to prevent microbial activity. Residual exudate in the leaching dishes was rinsed from the sand bed into the flask. Exudate in the flasks was frozen in a dry ice-methanol bath, and then concentrated via freeze-drying. The residue was taken up in 5-10 ml of water, transferred to a scintillation vial, and 10 ml of aqueous scintillation cocktail was added. Each experiment was repeated 2-3 times with similar trends in each experiment.

Scintillation Cocktails And ^{14}C Counting

A basic cocktail consisted of 4 g Omnifluor (New England Nuclear, Boston, MA) in one liter of toluene:methanol (3:1, v/v). A 15% ethanol-amine scintillation cocktail for collecting $^{14}CO_2$ consisted of 150 ml ethanolamine (Aldrich Chemical Co., Milwaukee, WI), 70 ml ethylene glycol (Piersolve, Pierce Chemical Co., Rockford, IL), and 780 ml of basic

scintillation cocktail. A 4% (w/v) Cab-O-Sil cocktail for suspending labeled propagules consisted of 4 g Cab-O-Sil (Research Products International, Elk Grove, IL) in 100 ml of basic scintillation cocktail. Cocktail for counting aqueous samples consisted of 4 g Omnifluor in one liter of toluene:Triton X-100 (2:1, v/v; Triton, Research Products International).

Radioactivity was measured in a Packard Tri-Carb liquid scintillation counter Model 578. For most experiments, counting was corrected using a ^{14}C-toluene internal standard.

Experimental results were subjected to analysis of variance and significant differences among means were determined using Duncan's Multiple Range Test.

RESULTS

Sensitivity Of Propagules To Mycostasis

On sterile Pfeffer's solution propagules of C. victoriae, S. sarcinaeforme, and M. phaseolina germinated 90%, whereas conidia of P. frequentans, a nutrient-dependent fungus, did not germinate. Intact chains of chlamydospores and clusters of chains of T. basicola routinely showed reduced germinabiity (30-59%) on Pfeffer's solution, whereas endoconidia from the same culture germinated greater than 80% of Pfeffer's solution.

Germination of all test propagules on natural soils was 0-2%, indicating that all the soils were mycostatic against the fungi. All the fungi germinated 90-100% on autoclaved soils and on PDA. The propagules differed in their germination response to nutrients added to the soils (Table 2). In general, more nutrients were required to promote germination on Parkhill clay loam and Brookston clay loam than the loam and the two sandy loam soils. However, S. sarcinaeforme conidia responded similarly to nutrients in all soils, except for Brookston clay loam, which required nearly twice the nutrient concentration to produce an equivalent germination response.

Germination Inhibitors And Stimulators In Soils

On sterile soil solution there was no inhibition of the nutrient-independent fungi (Table 3). Germination of P. frequentans was not

Table 2. Concentrations of sucrose and peptone required for 50% germination of fungal propagules on amended soils.

Soil	$\mu g \times 10^2$ sucrose g^{-1} soil for 50% germination*			
	Cochliobolus victoriae	*Stemphylium sarcinaeforme*	*Macrophomina phaseolina*	*Thielaviopsis basicola*
Parkhill clay loam	4.5	0.6	8.4	6.0
Brookston clay loam	2.2	1.1	8.0	5.9
Dryden loam	1.8	0.6	7.8	4.0
Spinks sandy loam	1.6	0.6	7.4	5.9
Boyer sandy loam	0.9	0.6	5.6	2.7

*Peptone was 1/5 the sucrose concentration.

Table 3. Germination of fungal propagules in soil solution extracted from soils by centrifugation and filter sterilized.

Soil	Germination (%)*				
	Cochliobolus victoriae	*Stemphylium sarcinaeforme*	*Thielaviopsis basicola*	*Macrophomina phaseolina*	*Penicillium frequentans*
Spinks sandy loam	100	98	34	100	0
Boyer sandy loam	99	99	30	92	0
Dryden loam	98	99	41	95	0
Parkhill clay loam	93	100	42	100	2
Brookston clay loam	96	100	49	100	3

*Germination of *T. basicola* chlamydospores on sterile dilute salts solution was 30-59%. All other nutrient-independent propagules germinated 90%, whereas conidia of *P. frequentans* did not germinate.

enhanced, suggesting that compounds stimulatory to germination were also absent. chlamydospores of *T. basicola* on soil solution germinated below 50%. This low germination appeared to be inherent in this fungus, as germination of Pfeffer's solution was also low (30-59%). There appeared to be no volatile inhibitors in the soils as germination of the fungi of Pfeffer's salts solution was not decreased when the fungi were incubated above moist soil in closed dishes.

Populations Of Microorganisms In Soils

Population densities of actinomycetes were not significantly different ($P=0.05$) in any of the soils (Table 4). Populations of fungi did not differ in the Spinks, Boyer, and Brookston soils; however, fungal numbers were significantly greater ($P=0.05$) in the Parkhill clay loam and the Dryden loam soils. Dryden loam also had the largest population of aerobic bacteria ($P=0.05$). Aerobic bacteria in the is soil were nearly twice the population in Boyer sandy loam, and 7-8 times that of the other soils. Numbers of aerobic bacteria in the Boyer sandy loam were significantly ($P=0.05$) greater than those in the Spinks sandy loam and the two clay loam soils.

Populations of anaerobic bacteria were significantly higher ($P=0.05$) in the Dryden loam and the clay loam soils than in the sandy loam soils.

ATP Content And Dehydrogenase Activity In Soils

ATP was higher in the two clay loam soils than in the other soils (Table 5), and the sandy loam soils had less ATP ($P=0.05$) than the Dryden loam soil. Dehydrogenase activity was 2-3 times greater in the Dryden and Spinks soils than in the other soils. The activity of Boyer sandy loam, however, was similar to that of Brookston and Parkhill clay loam soils.

Table 4. Populations of microorganisms in soils.

Soil	Colony forming units·g^{-1} soil			
	Actinomycetes x 10^6	Anaerobes x 10^5	Aerobes x 10^7	Fungi x 10^4
Spinks sandy loam	1.6 a	3.3 a	4.6 a	4.7 a
Boyer sandy loam	5.1 a	5.1 a	19.0 b	5.6 a
Dryden loam	3.2 a	34.0 b	37.0 c	18.0 b
Parkhill clay loam	1.2 a	36.0 b	4.8 a	14.0 b
Brookston clay loam	8.2 a	32.0 b	5.0 a	4.2 a

*Numbers within a column followed by the same letter are not significantly different (P=0.05) using Duncan's Multiple Range Test.

Table 5. ATP content and dehydrogenase activity of soils.

Soil	ATP $\mu g \cdot g^{-1}$ soil*	Dehydrogenase activity μg TTF $\cdot g^{-1}$ soil†
Spinks sandy loam	2.66 a	72 c
Boyer sandy loam	2.76 a	26 a
Dryden loam	3.60 b	44 b
Parkhill clay loam	5.48 c	28 ab
Brookston clay loam	5.35 c	22 a

*Mean of three determinations. Results were corrected for recoveries (84-105%) of ATP using an ATP internal standard.

†Mean of four determinations. TTF = 2,3,5-triphenyltetrazolium formazan.

Numbers within a column followed by the same letter are not significantly different ($P=0.05$) using Duncan's Multiple Range Test.

^{14}C-substrate Respiration By Soils

After 12 h the order of soil respiration was Dryden loam > Boyer sandy loam > Parkhill clay loam > Brookston clay loam and Spinks sandy loam (P=0.05). A similar pattern of respiration after 12 h was evident when the soils were pulsed with ^{14}C-amino acids (Figure 2), although respiration of the Boyer soil was not significantly greater (P=0.05) than the respiration responses of the Parkhill and Spinks soils. Brookston clay loam showed the least respiration of ^{14}C-amino acids.

^{14}C-exudate Lost From Propagules To Soils

^{14}C-exudate loss from *C. victoriae* was initially rapid until the 8th hour, when exudation began to slow. Dryden loam and Boyer sandy loam induced the greater losses of ^{14}C-exudate (P=0.05) than did the three other soils (Figure 3). Parkhill clay loam induced greater losses than Brookston clay loam and Spinks sandy loam soils. This trend was evident at 4 h and continued for all sampling intervals. Losses of ^{14}C-exudate from *C. victoriae* to Dryden loam during the 4-24 h incubation period ranged from 4.4% to 11.2%. Losses to Boyer sandy loam and Parkhill clay loam during this same time were 4.3% to 10.9% and 3.6% to 9.2%, respectively. Losses to Brookston clay loam and to Spinks sandy loam during the 4-24 h period were 3.3% to 8.0% and 2.8 to 8.3%, respectively. At 24 h overall losses of ^{14}C-exudate to the five soils ranged from 9.0% to 11.2%.

S. sarcinaeforme conidia lost exudate at a rapid rate during 4-8 h, followed by a decrease in the rate of exudation between 8-12 h (Figure 4). From 12-16 h the rate of loss increased again, and this was followed by another reduction in the exudation rate (16-24 h). Although

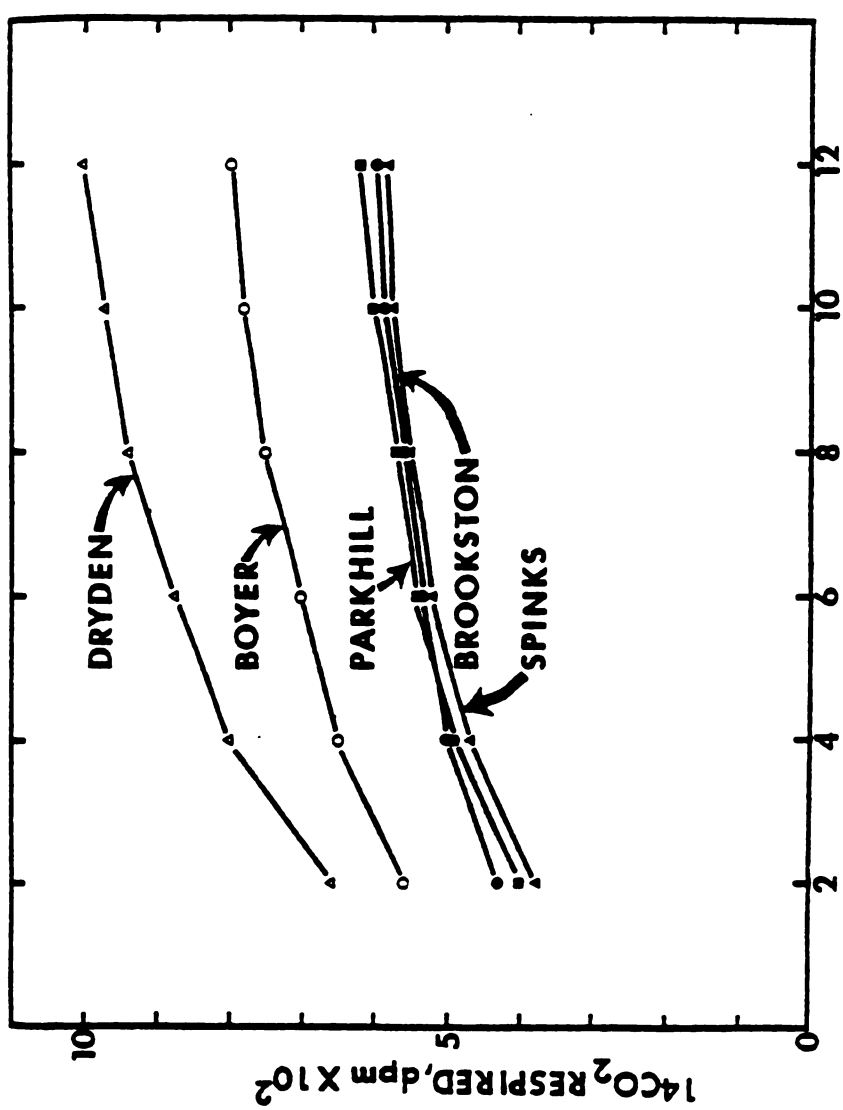

Figure 1. $^{14}CO_2$ respiration by soils pulsed with 3 x 10^3 dpm of ^{14}C-glucose. At 12 h Duncan's Multiple Range Test ($P=0.05$) showed the following significant differences among treatment means: Dryden[a], Boyer[b], Parkhill[c], Brookston[d] and Spinks[d]. Mean values of soils followed by the same letter did not differ significantly.

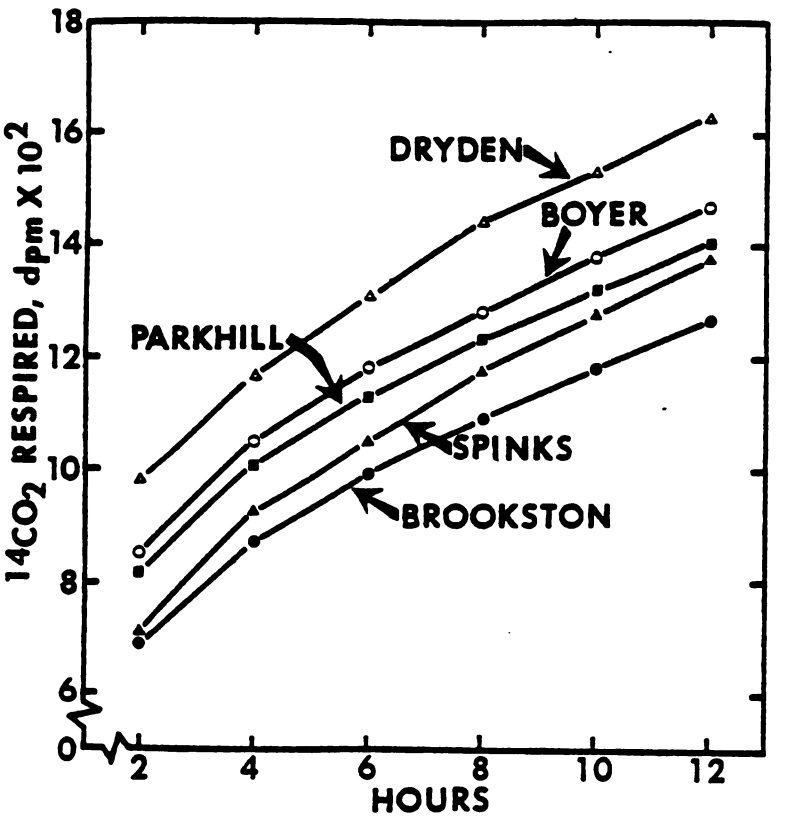

Figure 2. $^{14}CO_2$ respiration by soils pulsed with 3.6 x 10^3 dpm of a ^{14}C-amino acid mixture. At 12 h Duncan's Multiple Range Test ($P=0.05$) showed the following significant differences among treatment means: Dryden[a], Boyer[ab], Parkhill[b], Spinks[b], and Brookston[c]. Mean values of soils followed by the same letter did not differ significantly.

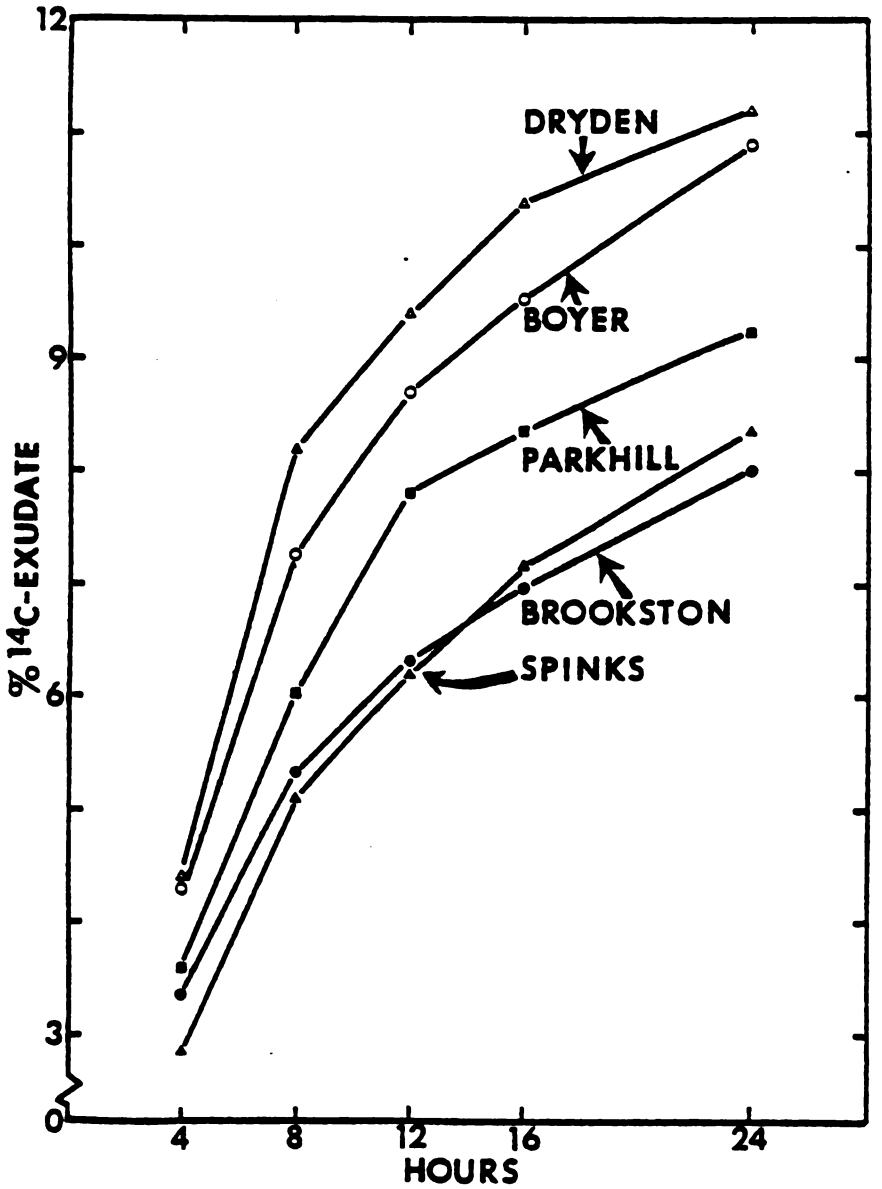

Figure 3. Percentage of ^{14}C exudate lost from Cochliobolus victoriae to soils. At 24 h Duncan's Multiple Range Test ($P=0.05$) showed the following significant differences among treatment means: Dryden[a], Boyer[ab], Parkhill[c], Spinks[d], and Brookston[d]. Mean values of soils followed by the same letter did not differ significantly.

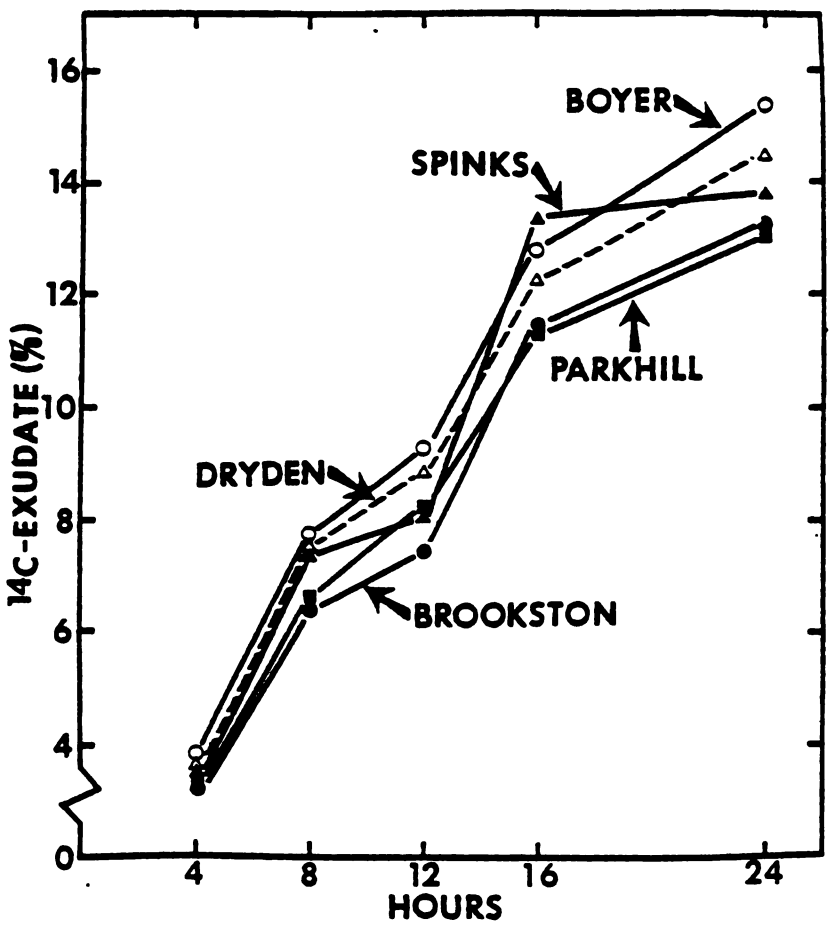

Figure 4. Percentage of ^{14}C exudate lost from Stemphylium sarcinaeforme conidia to soils. No significant differences ($P=0.05$) among soils were observed during 4-24 h.

^{14}C-exudate losses to Boyer sandy loam and Dryden loam were higher than losses to the other soils, there were no significant differences (P=0.05) in the abilities of the soils to remove ^{14}C-exudate. Losses of ^{14}C from S. sarcinaeforme conidia to the soils over 4-24 h were as follows: Boyer, 3.8-15.3%; Dryden, 3.6-14.4%; Spinks, 3.6-13.6%; Brookston, 3.6-13.3%; and Parkhill, 3.7-13.2%. At 24 h ^{14}C-exudate losses to the five soils ranged from 13.2% to 15.3%.

Chlamydospores of T. basicola lost approximately the same amount of exudate to the five soils during the first 16 h of incubation (Figure 5). Only at 24 h did soils begin to separate in this regard; Dryden loam and Spinks sandy loam differed significantly (P=0.05) from the Parkhill clay loam and Brookston clay loam soils. Losses of ^{14}C from T. basicola chlamydospores to the soils over 4-24 h were as follows: Dryden, 1.9-11.2%; Spinks, 1.9-11.2%; Boyer, 1.8-10.9%; Parkhill, 1.8-10.0%; and Brookston, 1.9-9.5%. At 24 h ^{14}C-exudate losses to the five soils ranged from 9.5% to 11.2%.

Except for the Spinks soil, the rate of ^{14}C-exudation from M. phaseolina sclerotia to the soils was the same up to 12 h, then the exudation rates began to quickly decline on the Brookston and Parkhill soils (Figure 6). The decrease in exudation after 12 h was not as abrupt on the Boyer and Dryden soils. Spinks sandy loam induced an initial rapid loss of ^{14}C-exudate from sclerotia, and from the 8th hour on, a slightly lower rate of exudation persisted.

Spinks and Boyer sandy loams induced the greatest loss (P=0.05) of ^{14}C-exudate from M. phaseolina sclerotia (Figure 6). Dryden loam, which was intermediate, induced greater loss (P=0.05) than the Brookston and

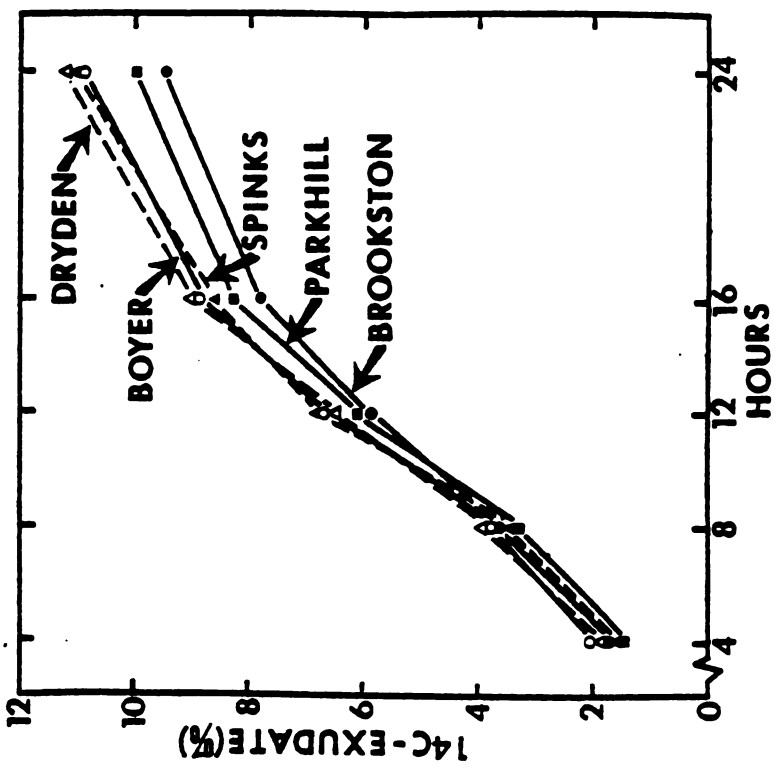

Figure 5. Percentage of ^{14}C exudate lost from Thielaviopsis basicola chlamydospores to soils. At 24 h Duncan's Multiple Range Test ($P=0.05$) showed the following significant differences among treatment means: Dryden[a], Spinksab, Boyerbc, Parkhill[cd], and Brookston[d]. Mean values of soils followed by the same letter did not differ significantly.

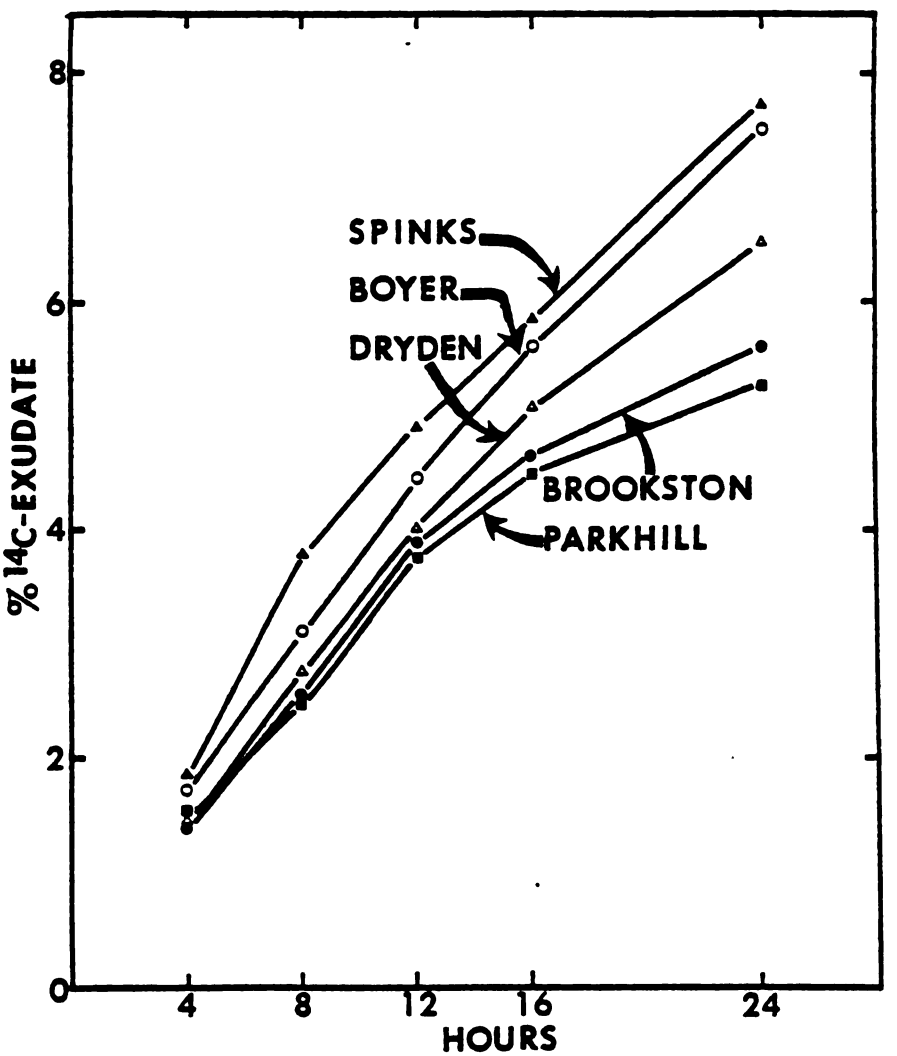

Figure 6. Percentage of ^{14}C exudate lost from Macrophomina phaseolina sclerotia to soils. At 24 h Duncan's Multiple Range Test ($P=0.05$) showed the following significant differences among treatment means: Spinks[a], Boyer[ab], Dryden[c], Brookston[d], and Parkhill[d]. Mean values of soils followed by the same letter did not differ significantly.

Parkhill clay loams. This trend was evident at 8 h and continued up to 24 h.

During the 4-24 h exudation period, losses of ^{14}C-exudate from sclerotia to the soils were as follows: Spinks, 1.9-7.7%; Boyer, 1.7-7.5%; Dryden, 1.6-6.5%; Brookston, 1.4-5.6%; and Parkhill, 1.6-5.2%. At 24 h the overall losses of ^{14}C-exudate from sclerotia to the five soils were 5.2% to 7.7%. These losses were considerably less than losses from the other test propagules to these soils.

^{14}C-exudate Adsorbed By Soils

After 60 min the soils showed distinct differences in their ability to adsorb ^{14}C exudate obtained from C. victoriae conidia (Figure 7). Brookston and Parkhill clay loams adsorbed 25% and 24%, respectively. Dryden loam adsorbed 18%, and the sandy loams both adsorbed 10% of the ^{14}C which percolated through the soil columns. Adsorption reached a peak at 60 min and thereafter declined. Saturation of the soils, therefore, was rapid, and was followed by a slow and steady desorption of ^{14}C-exudate. After 3 h the order of greatest retention of ^{14}C-exudate was still the clay loams, followed by the loam, and the sandy loam soils. The adsorption of ^{14}C-exudate by these soils appeared to be directly related to the cation exchange capacity of the soils (Table 1).

^{14}C-exudation From Propagules On Leached Sand

Percentage of ^{14}C-exudate removed from propagules by the diffussive stress imposed by the leached sand apparatus was calculated according to the following: % ^{14}C-exudate = Exuded ^{14}C/Total ^{14}C x 100. Total ^{14}C available prior to the imposition of stress was estimated by summing ^{14}C in exudate plus ^{14}C remaining in the propagules on the membrane at the end of incubation.

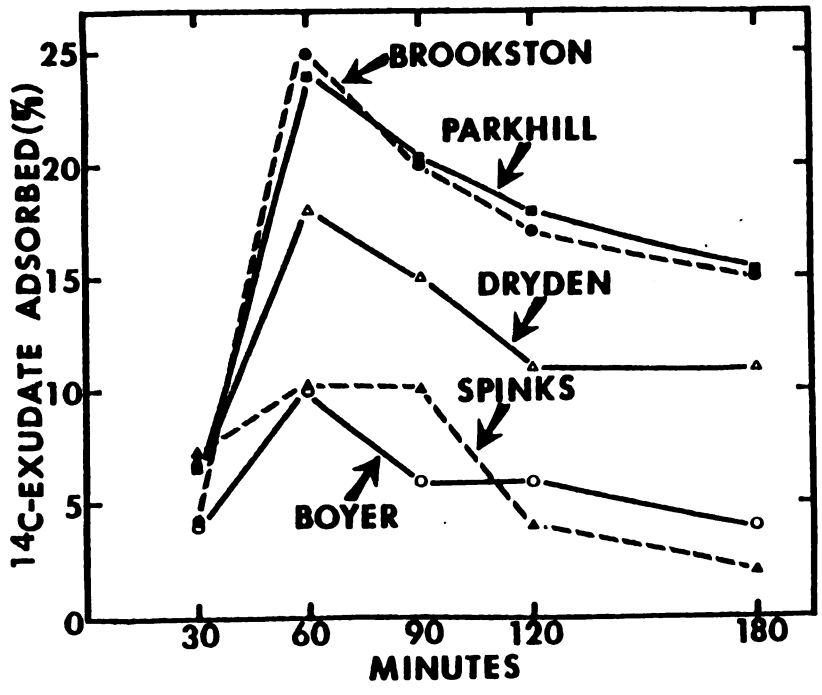

Figure 7. Adsorption by γ-irradiated soils of sterile ^{14}C-exudate obtained from labeled conidia of Cochliobolus victoriae. At 180 min Duncan's Multiple Range Test (P=0.05) showed the following significant differences among treatment means: Parkhill[a], Brookston[a], Dryden[b], Boyer[c], and Spinks[c]. Mean values of soils followed by the same letter did not differ significantly.

Exudation from C. victoriae conidia and M. phaseolina sclerotia increased as the diffusive stress, i.e., the flow rate, increased beneath the propagules (Figures 8 and 9). As exudation increased, propagule germination decreased in accord with results shown previously by Sneh and Lockwood (1976) and Filonow and Lockwood (1979). Germination of C. victoriae conidia incubated on leached sand for 12 h at 12, 25, 80, and 120 $ml \cdot h^{-1}$ was 97%, 32%, 16%, and 9%, respectively. No germination of conidia was observed on the natural, unamended soils after a similar incubation time (12-16 h). After 6 h of ^{14}C-exudate collection, conidia of C. victoriae had lost 1.3%, 2.6%, 3.7%, and 3.1% of their ^{14}C when stressed at 12, 45, 80, and 120 $ml \cdot h^{-1}$, respectively. Germination of conidia incubated for 12 h on saturated (non-leached) sterile sand was >80%.

Sclerotia of M. phaseolina under 48 h of diffusive stress germinated 84%, 50%, 33%, and 24% at respective flow rates of 15, 45, 70, and 105 $ml \cdot h^{-1}$. ^{14}C-exudate losses incurred at these flow rates during the first 17 hours of incubation were 1.2%, 3.4%, 4.2%, and 4.8%. Sclerotia germinated 82% on saturated sterile sand after 48 h incubation. Only 0-2% germination of sclerotia occurred on the five soils after 48 h.

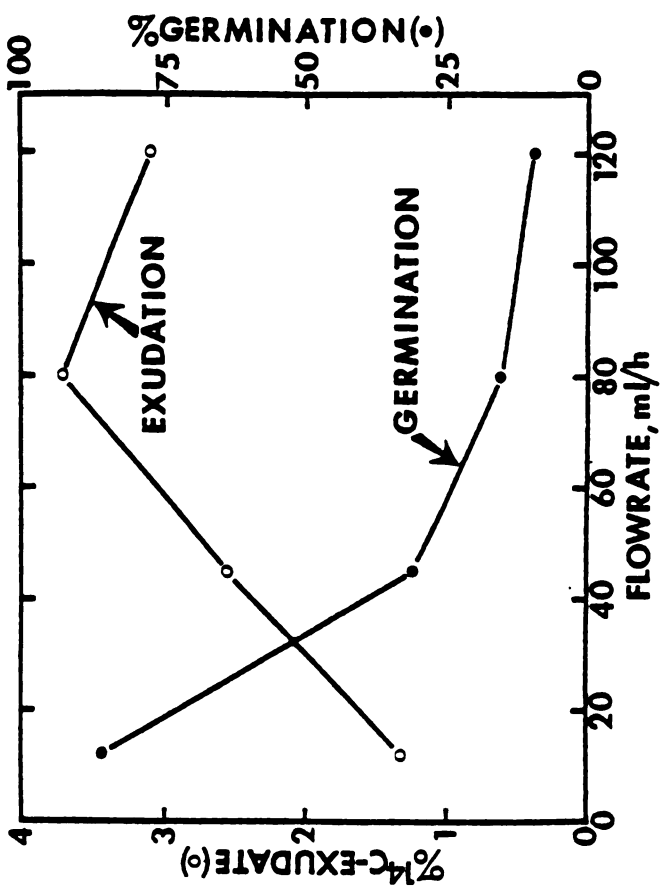

Figure 8. Germination and loss of ^{14}C by labeled conidia of Cochliobolus victoriae on sterile sand and through which a dilute salts solution percolated. ^{14}C-exudate was collected for 6 h, conidial germination was determined after 12 h.

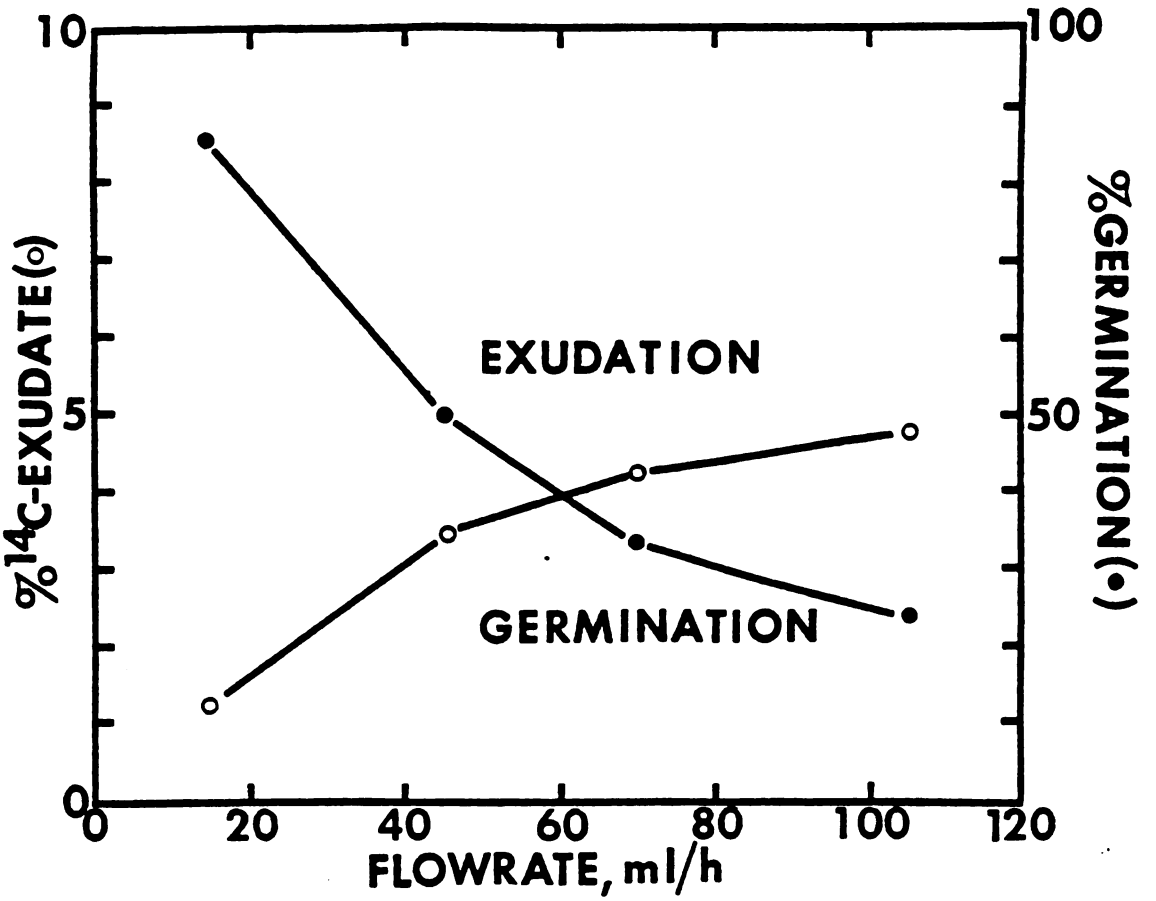

Figure 9. Germination and loss of ^{14}C by labeled sclerotia of Macrophomina phaseolina on sterile sand through which a dilute salts solution percolated. ^{14}C-exudate was collected for 17 h, and sclerotial germination was determined after 48 h.

DISCUSSION

Losses of ^{14}C-exudate from C. victoriae conidia to the five soils (Figure 3) after 6 h ranged from 3.9-6.2% compared to a maximum of 2.7% lost from conidia after 6h in the leached sand apparatus (Figure 8). During this time conidial germination in the leached sand apparatus was reduced to 9%, whereas no germination occurred on the soils. Similarly, losses of ^{14}C-exudate from M. phaseolina sclerotia to the soils (Figure 6) after 17 h ranged from 4.7-6.1% compared to a maximum of 4.8% lost from sclerotia after 17 h on the leached sand apparatus (Figure 9). During this time sclerotial germination in the leached sand apparatus was reduced to 24%, whereas germination on soils was 0-2%. Therefore, losses of ^{14}C-exudate from M. phaseolina sclerotia and C. victoriae conidia incubated on soils were greater in almost all cases than ^{14}C-exudate losses from these propagules undergoing a simulated diffusive stress sufficient to reduce germination to levels approximating (but not equal to) germination levels on soils. Moreover, nutrients released from propagules during germination most likely contributed to the ^{14}C found in exudate obtained from propagules incubated on the leached sand apparatus. Although exudate losses from T. basicola chlamydospores and S. sarcinaeforme conidia to the soils were not compared against losses to simulated diffusive stress, the percentage of exudate removed from these propagules by the soils was as high or higher than that removed from C. victoriae. Based on these results and the apparent absence of inhibitory

substances in these soils, the microbial nutrient sinks of these soils were sufficient to account for soil mycostasis.

Sclerotia of M. phaseolina incubated on soils lost less exudate as a proportion of total label, during the same incubation time, than did the propagules of the other fungi. For example, at 24 h the sclerotia had lost 7.7% of their ^{14}C, whereas propagules of T. basicola, C. victoriae and S. sarcinaeforme lost 11.2%, 11.2%, and 15.3%, respectively. In addition, the exudation rate of C. victoriae conidia at high simulated diffusive stress (3.7% ^{14}C lost in 6 h) was greater than that from sclerotia under a similar stress (4.8% ^{14}C lost in 17 h), which agreed with results from the soil studies. The lower exudation rate of the sclerotia may be a factor in their relatively longer persistence in soil than conidia. Sclerotia of M. phaseolina in soil have been reported to survive for one year or more (Watanabe, 1973; Papavizas, 1977) whereas conidia of Cochliobolus survived only weeks to a few months (Chinn and Tinline, 1964). Microsclerotia of Verticillium dahliae have been reported to survive up to 82 weeks in soil, whereas conidia and hyphae disappear within 2-3 weeks (Schreiber and Green, 1962). Therefore, it is reasonable to assume that a low rate of exudation by a fungal propagule may increase its chances of survival in soil; conversely, propagules with higher exudation rates may not be able to persist as long.

More soluble exudate was removed from propagules of one or more of the four test fungi when they were incubated on the loam and sandy loam soils than when the fungal propagules were incubated on the clay loam soils. These results suggest that the microbial nutrient sinks of the coarse-textured soils were more active in withdrawing exudate from the propagules than those of the fine-textured soils. Similar findings

regarding the intensity of the microbial nutrient sink in relation to soil texture had been demonstrated by Filonow and Lockwood (1979), using eight soils different from those in the present work, and C. victoriae conidia as the test propagule.

The responses of soils to soluble nutrients from fungal propagules were also similar to their respiratory responses when soils were pulsed with ^{14}C-glucose and ^{14}C-amino acids. Glucose and various amino acids are major constituents of exudate from C. victoriae conidia and M. phaseolina sclerotia (Bristow and Lockwood, 1975a; Filonow, unpublished). Dryden loam and Boyer sandy loam showed considerably more respiration than other soils, most notably, the clay loams. Similar results using other soils were noticed by Filonow and Lockwood (1979) and Bristow and Lockwood (1975b).

In contrast to the order of nutrient sink activity as shown by fungal exudation, fine-textured soils generally required more nutrients to annul mycostasis than did coarse-textured soils. A similar finding was also reported by Filonow and Lockwood (1979) in a study of eight other soils. An examination of ^{14}C-exudate adsorption by the five soils shows that the two clay loams adsorbed more ^{14}C-exudate than the loam or sandy loam soils, which explains the greater amounts of nutrients required to annul mycostasis in clay loam soils.

Adsorption by soil cannot solely account for the removal of soluble nutrients from fungal propagules, since the adsorptive capacities of these γ-irradiated soils were, in general, the inverse of their ability to remove ^{14}C-exudate from propagules. Thus, a living component of the soil is required for the removal of nutrients from fungal propagules. The existence in soil of an efficient microbial sink for nutrients was

shown by Lockwood (1975). Glucose was more rapidly removed from paper discs incubated on natural soil than on sterile soil. The mean glucose half-life on several natural soils was 80 minutes vs. 240 minutes for autoclaved soils.

In this regard, populations of aerobic bacteria were significantly greater in the Dryden and Boyer soils than in the clay loam soils, and this fact may explain the more efficient microbial nutrient sinks in the former soils compared to the latter soils. Although numbers of anaerobic bacteria in the clay loams were significantly greater than in the other soils, the clay loam soils were consistently low in their ability to remove exudate from fungal propagules. Therefore, anaerobic bacteria in the clay loam soils did not appear to be important in removing exudate from the propagules tested.

ATP results were the inverse of those obtained for ^{14}C-exudate, but they generally agreed with results from nutrient titration. ATP results did not agree with microbial populations, however. Dehydrogenase activity showed some relationship to the microbial nutrient sink, when fungal exudate was used as a substrate in the assay. Dryden loam, which consistently had a greater microbial nutrient sink than the other soils, and Spinks sandy loam which sometimes had a greater sink (depending on the test propagule) had 2-3 times more dehydrogenase activity than the other soils.

It is quite possible that the plate counts under-estimated the microbial populations in the clay loam soils, and that the greater ATP content in these soils reflects a much greater microbial biomass. If so, the low response of the microflora in these soils to soluble nutrients such as glucose, amino acids or fungal exudate may, in part, be due to a

diminished metabolic surface area in these soils than in the loam and sandy loam soils. Occlusive coatings such as argillans and organans (Brewer, 1964) may partially or totally cover microorganisms in the clay loam soils and thereby restrict the microbial surface area available for interaction with soluble substrate in the soil solution (Marshall, 1976; Filonow and Lockwood, 1979).

The above results indicate that the degree of adsorption in soils is an important factor controlling fungal utilization of soluble nutrients. Moreover, the findings raise the question as to which aspect has the greatest influence on the life of a fungus in soil--the ease with which it can germinate in response to nutrients, or the amount of endogenous substrate lost or retained. It is reasonable to assume that continued loss of endogenous substrate would reduce propagule longevity. Loss of endogenous nutrients from C. victoriae conidia to a diffusive stress imposed by a leached sand apparatus or by soil resulted in a change from nutrient-independence to nutrient-dependence, and a subsequent loss of viability (Bristow and Lockwood, 1975a). If such transformations are at the expense of endogenous carbon reserves, it is possible that other aspects of fungal biology, e.g., virulence, may be affected. From the standpoint of long-term fungal survival and vigor, exposure to fine-textured soils with lesser microbial nutrient sinks may offer the most favorable environment - one in which nutrient sinks are sufficiently strong to impose mycostasis and deter spontaneous germination, but weak enough to result in the maximum conservation of endogenous nutrients.

LIST OF REFERENCES

LIST OF REFERENCES

ALLISON, L. E., BOLLEN, W. B. and MOODIE, C. D. (1965) Total carbon. In Methods of Soil Analysis (C. A. Black, Ed.), Vol. 2, pp. 1346-1366. American Society of Agronomy, Madison.

BREMNER, J. M. (1965) Total nitrogen. In Methods of Soil Analysis (C. A. Black, Ed.), Vol 2, pp. 1149-1178. American Society of Agronomy, Madison.

BREWER, R. (1964) Fabric and Mineral Analysis of Soils. 470 pp. John Wiley and Sons, New York.

BRISTOW, P. R. and LOCKWOOD, J. L. (1975a) Soil fungistasis: Role of the microbial nutrient sink and of fungistatic substances in two soils. Journal of General Microbiology 90, 147-156.

CASIDA, L. E., Jr. (1977) Microbial metabolic activity in soil as measured by dehydrogenase determinations. Applied and Environmental Microbiology 34, 630-636.

CHAPMAN, H. D. (1965) Cation-exchange capacity. In Methods of Soil Analysis (C. A. Black, Ed.), Vol 2, pp. 891-901. American Society of Agronomy, Madison.

CHINN, S. H. F. and TINLINE, R. D. (1964) Inherent germinability and survival of spores of Cochliobolus sativus. Phytopathology 54, 349-352.

DAY, P. R. (1965) Particle fractionation and particle-size analysis. In Methods of Soil Analysis (C. A. Black, Ed.), Vol 1, pp. 545-567. American Society of Agronomy, Madison.

DOMSCH, K. H., BECK, T. H., ANDERSON, J. P. E., SODERSTROM, B., PARKINSON, D. and TROLDENIER, G. (1979) A comparison of methods for soil microbial population and biomass studies. Zeitschrift fuer Pflanzenernaebrung Bodenkunde 142, 520-533.

FILONOW, A. B. and LOCKWOOD, J. L. (1979) Conidial exudation by Cochliobolus victoriae on soils in relation to soil mycostasis. In Soil Borne Plant Pathogens (B. Schippers and W. Gams, Eds.). pp. 107-119. Academic Press, New York.

HSU, S. C. and LOCKWOOD, J. L. (1973) Soil fungistasis: behavior of nutrient-independent spores and sclerotia in a model system. Phytopathology 63, 334-337.

HSU, S. C. and LOCKWOOD, J. L. (1975) Powdered chitin agar as a selective medium for enumeration of actinomycetes in water soil. Applied microbiology 29, 422-426.

KO, W. H. and LOCKWOOD, J. L. (1967) Soil fungistasis: relation to fungal spore nutrition. Phytopathology 57, 894-901.

LOCKWOOD, J. L. (1977) Fungistasis in soils. Biological Reviews 52, 1-43.

MARSHALL, K. C. (1976) Interfaces In Microbial Ecology. Harvard University Press, Cambridge, Massachusetts.

MARTIN, J. W. (1975) Comparison of agar media for counts of viable soil bacteria. Soil Biology and Biochemistry 7, 401-402.

PAPAVIZAS, G. C. (1977) Some factors affecting survival of sclerotia of Macrophomina phaseolina in soil. Soil Biology and Biochemistry 9, 337-341.

PAUL, E. A. and JOHNSON, R. L. (1977) Microscopic counting and adenosine 5'-triphosphate measurement in determining microbial growth in soils. Applied and Environmental Microbiology 34, 263-269.

SNEH, B. and LOCKWOOD, J. L. (1976) Quantitative evaluation of the microbial nutrient sink in soil in relation to a model system for soil fungistasis. Soil Biology and Biochemistry 8, 65-69.

YODER, D. L. and LOCKWOOD, J. L. (1973) Fungal spore germination on natural and sterile soils. Journal of General Microbiology 74, 107-117.

MYCOSTASIS AND THE MICROBIAL NUTRIENT SINK OF SOIL

II. LOSS OF THE NUTRIENT-INDEPENDENT STATE OF FUNGAL PROPAGULES INCUBATED ON SOILS OR ON A MODEL SYSTEM SIMULATING SOIL-IMPOSED ENERGY STRESS

MYCOSTASIS AND THE MICROBIAL NUTRIENT SINK OF SOIL

II. LOSS OF THE NUTRIENT-INDEPENDENT STATE OF FUNGAL PROPAGULES INCUBATED ON SOILS OR ON A MODEL SYSTEM SIMULATING SOIL-IMPOSED ENERGY STRESS

SUMMARY

Conidia of Cochliobolus victoriae and sclerotia of Sclerotium cepivorum, Macrophomina phaseolina, and Verticillium dahliae were subjected to extended diffusive stress aseptically imposed by a leached sand apparatus through which a dilute salts solution percolated at a flow rate sufficient to inhibit germination. C. victoriae conidia and S. cepivorum sclerotia both germinated only 6% in the absence of exogenous nutrients after 8 and 15 days, respectively, of diffusive stress. The ability of S. cepivorum sclerotia to germinate in response to dilute concentrations of onion extract was progressively reduced with diffusive stress, commencing at 25 days. Thirty-five days of diffusive stress were required to attenuate the nutrient-independent status of M. phaseolina sclerotia to a level comparable with C. victoriae. Sclerotia of V. dahliae lost little of their nutrient-independent status even after 45 days of diffusive sress. Viability of the four propagules was not seriously affected by 45 days of extended diffusive stress imposed by the leached sand apparatus. Conidia of C. victoriae gradually became nutrient-dependent when incubated for several weeks on five soils. Dryden loam, Boyer sandy loam, and Spinks sandy loam soils were more effective in decreasing the nutrient-independent status of propagules. Sclerotia of M. phaseolina also lost their nutrient-independent state when incubated on four of the five soils. Dryden loam and Spinks sandy

loam soils were more effective than the other soils, but Boyer sandy loam did not appreciably reduce the nutrient-independent state of the sclerotia even after 70 days of incubation.

Interruption of artifically imposed diffusive stress, followed by a return to stress resulted in a pronounced increase in ^{14}C-exudate from conidia of C. victoriae and sclerotia of M. phaseolina. The germinability on salts solution of C. victoriae conidia made nutrient-dependent was significantly increased in the absence of exogenous nutrients, when diffusive stress was interrupted and the conidia were kept in a non-germinated state at 4°C for 3 1/2 days prior to the germinability assay.

Conidia of C. victoriae made nutrient-dependent after 9 days of artificially imposed diffusive stress, then incubated on soils labeled with ^{14}C-glucose, absorbed twice as much ^{14}C from a loam and two sandy loam soils as from two clay loam soils. When incubated on four of the five ^{14}C-labeled soils, the germinability of the conidia in the absence of nutrients was significantly increased over the germinability of nutrient-dependent conidia not incubated on these soils.

These results support the concept that continued diffusive stress imposed by the microbial nutrient sink of a soil can reduce the potential germinability of nutrient-independent fungal propagules. The results also suggest that a continued minimal nutrient stress may be needed to maintain the nutrient-dependent status of fungal propagules in soil. The interruption of nutrient stress appears to allow these nutrient-dependent propagules to recoup nutrients from the soil solution or reorganize endogenous nutrients whereby the potential for germinability is increased.

INTRODUCTION

The concept of an activated soil microflora rapidly utilizing inputs of energy-yielding substrate, including endogenous nutrients from fungal propagules has been termed the microbial sink of soil (Lockwood, 1977). A microbial nutrient sink sufficient to account for mycostasis has been demonstrated to exist in several soils (Bristow and Lockwood, 1975a; Sneh and Lockwood, 1976; Filonow and Lockwood, 1979; submitted). The amount of ^{14}C-exudate from nutrient-independent propagules was generally higher in loamy sand, sandy loam, and loam soils than in clay loam soils (Filonow and Lockwood, 1979; submitted), suggesting that coarse-textured soils possessed a greater capacity to withdraw nutrients from the test propagules. Moreover, sclerotia of Macrophomina phaseolina had a lower ^{14}C-exudation response to soils than conidia of Cochliobolus victoriae.

It is generally accepted that quiescence of fungal propagules due to mycostasis is of survival value, preventing the wastage that would occur from germination in the absence of substrte (Garrett, 1970; Lockwood, 1977). Chinn and Tinline (1964) experimentally confirmed this concept. Isolates of C. sativus which germinated spontaneously in soil disappeared rapidly, whereas isolates sensitive to soil mycostasis persisted. However, an advantage in the short run may be lost or overcome in the long-run. For C. victoriae conidia prolonged exposure to the competitive stress imposed by a loam soil or by the diffusive stress artificially imposed by a leached sand model system, resulted in the loss of the

ability to germinate without exogenous nutrients (Bristow and Lockwood, 1975a). Viability of the conidia was also reduced after 3 weeks' incubation on the loam soil. Thus, long-term microbial competitive stress in soil may reduce the ability of fungal propagules to germinate under conditions of decreased competitive stress, or in response to nutrient inputs, e.g., from root or seed exudate. Lack of or inhibition of fungal pathogen germination has been shown to be a factor involved in disease suppressive soils (Alabouvette et al., 1979).

The purpose of this research was to evaluate the role of the microbial nutrient sink in attenuating the nutrient-independent state of fungal propagules incubated on five soils of known mycostatic capacities or on a leached sand apparatus, imposing artificial diffusive stress.

MATERIALS AND METHODS

Soils

The following soils were used: Brookston clay loam, Parkhill clay loam, Dryden loam, Boyer sandy loam, and Spinks sandy loam. Soils were passed through a 2 mm sieve prior to use. Characteristics of these soils have been described elsewhere (Filonow and Lockwood, submitted).

Maintenance Of Fungi

C. victoriae Meehan and Murphy was maintained on carrot broth agar containing per liter: 250 ml of carrot broth (30 g of carrots in 250 ml water, autoclaved 10 min and filtered) and 20 g agar. M. phaseolina (Tassi) Goid. [Macrophomina phaseoli (Maubl. Ashby], Verticillium dahliae Kleb., and Sclerotium cepivorum Berk. were maintained on potato-dextrose agar.

Propagule Suspensions

Suspensions were prepared by flooding agar cultures with cold (5°C), sterile and dilute (1/100) Pfeffer's salts solution (Bristow and Lockwood, 1975a). Propagules were gently dislodged with a bent glass rod, and the suspension was passed through a 250 μm stainless steel sieve into a cold polycarbonate centrifuge tube. Propagules in the capped tube were washed 3 times by repeated centrifugation (10^4 x g for 5 min at 5°C). The final suspension volume ranged from 10-20 ml and was held in ice during its use. The density of conidia in suspensions was determined

with a haemacytometer, whereas the density of sclerotia was determined microscopically by counting sclerotia in several 0.1 ml drops of suspension.

Incubation Of Propagules On Soils

Fungal propagules were vacuum-deposited on 1.5 x 1.5 cm pieces of 0.4 µm pore dia Nuclepore membrane filters (Nuclepore Crop., Pleasanton, CA). Propagule densities were ca. 10^3 for conidia of C. victoriae and 10^2 for sclerotia of M. phaseolina per membrane. Five membranes bearing propagules were aseptically placed inside a sterilized incubation chamber (Figure 1), constructed from a 250 ml polypropylene bottle (60 mm dia x 125 mm) with the botton removed. A 65 x 65 mm piece of Nuclepore membrane (0.4 µm dia pore) was sealed to the bottom with silicone rubber cement. The middle of the chamber was nearly cut through, so that the top half could be easily opened. The chamber was sealed in the middle with tape. The chamber was placed on 20.0 g (O.D. weight) soil wetted to about -50 kPa (0.05 bar) matric potential contained in a 90 x 15 mm plastic petri plate. There were two plates for each of the five soils. Soil plates plus chambers were placed inside a large polypropylene tray (52 x 42 x 15 cm), a little water was poured into the tray to maintain high humidity, and the tray was covered with a large plastic bag containing several small pinholes. Every 4 to 5 days 0.5 to 1.0 ml of water was added to each soil plate to maintain contact between the membrane filter inside the chamber and the soil solution.

At various times several membranes were removed from the chambers to determine germination. Two or three of these were floated in 1.0 ml of dilute (10^{-2}) Pfeffer's solution in stainless sttel planchets (2.4 cm dia

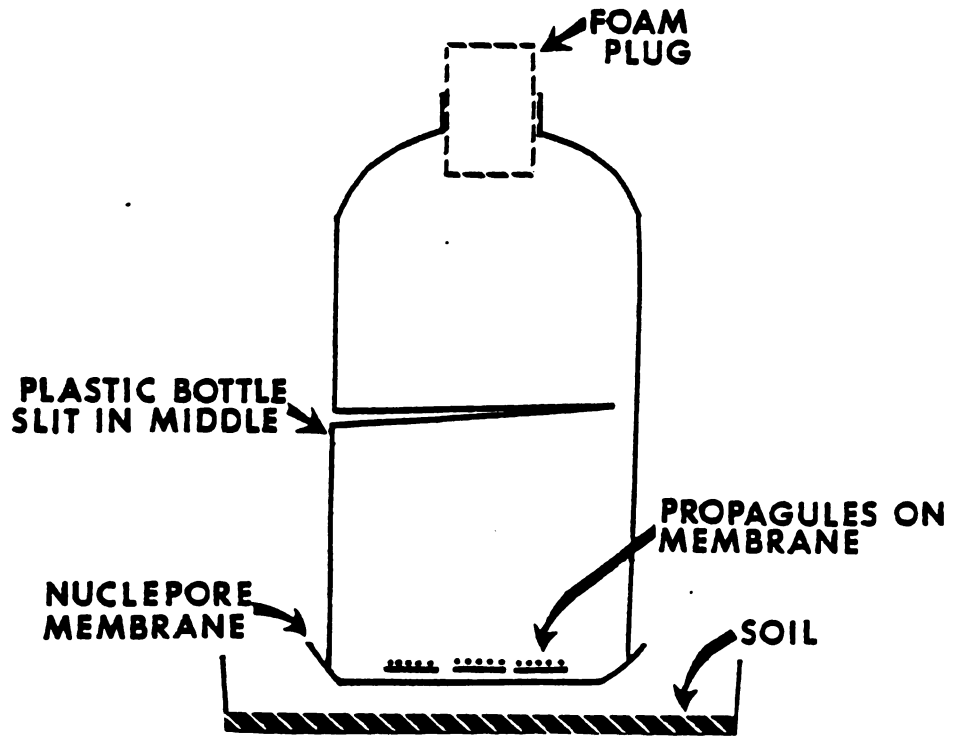

Figure 1. Polypropylene plastic incubation chamber. The bottom of the bottle was replaced with a Nuclepore membrane (0.4 µm dia pore) and rested on moist soil in a petri dish. Propagules on Nuclepore membranes inside the chamber were in contact with the soil solution.

x 0.6 cm). Conidia of C. victoriae were incubated on Pfeffer's solution for 8-10 h, whereas sclerotia of M. phaseolina were incubated for 16-24 h. Propagules were stained in phenolic rose bengal, destained on a moist paper towel, and germination determined. In addition, one of the membranes was stained with rose bengal to determine the germination which had occurred on soil.

Long-term Incubation Of Propagules On The Leached Sand Apparatus

The microbial nutrient sink of soil was simulated by aseptically incubating propagules on a bed of sand through which a dilute salt solution percolated at a flow rate sufficient to inhibit germination. The apparatus has been described elsewhere (Bristow and Lockwood, 1975a). A long-term diffusive stress was placed on the propagules by percolating a 10^{-2} dilution of Pfeffer's salt solution through the sand bed at a flow rate of 60 $ml \cdot h^{-1}$ for several weeks.

Propagules were borne on 1 x 1 cm Nuclepore membrane filters (0.4 µm pore dia), and 10-12 membranes per fungus were placed in a row across the middle of a 14 cm dia leaching dish. There were two leaching dishes per fungus. After various incubation times up to 45 days, two membranes from each dish were removed and the propagules on three membranes assayed for germinability on dilute Pfeffer's solution. One membrane was placed on PDA to assess viability.

In addition to germination tests on Pfeffer's solution and PDA, leached sclerotia of S. cepivorum were also tested for their ability to respond to dilute onion extract, which contains organic sulfides which are germination activators (King and Coley-Smith, 1968; Coley-Smith and King, 1969). Dilute onion extract was prepared by steaming for 1 h, 15 g

of peeled and chopped onion (Allium cepa L. 'Golden Globe Danvers') in 300 ml of distilled water. The extract was filtered through cheese cloth and membrane filter sterilized (0.4 μm dia pore, Nuclepore). Dilutions were made with sterile water to 1/10, 1/20, 1/40, 1/80, and 1/100 (v/v). Germination of unleached and leached S. cepivorum sclerotia was determined by floating small membranes bearing sclerotia on 1.0 ml of onion extract at the various dilutions. Propagules were incubated for varying times up to several days.

Unleached sclerotia of S. cepivorum incubated for 36 h on undiluted onion extract germinated completely. Germinated sclerotia usually showed profuse hyphal development and often produced a dense mycelial plug (Coley-Smith, 1960). Incubation of unleached sclerotia on the 1/10 dilution gave complete germination (>95%) sometimes by 36 h, but most often by 48 h. Unleached sclerotia incubated on dilutions ranging from 1/20 to 1/100 also showed high germination (>90%), but the incubation period for maximum germination was 48-72 h.

Interruption Of Artificially Imposed Diffusive Stress

Bristow and Lockwood (1975a) had shown that conidia of three fungi exuded more ^{14}C-label when they were incubated on leached sand than when they were incubated on buffer-saturated sand. The conidia were alternately incubated on leached sand followed by incubation on saturated sand without leaching for a similar time. This line of work was extended by studying the effects of interrupting the diffusive stress on the loss of exudate from propagules.

^{14}C-labeled conidia of C. victoriae and sclerotia of M. phaesolina were prepared and collected for suspension according to the method of

Filonow and Lockwood (submitted). ^{14}C-labeled propagules on membrane filters (2.5 cm dia; 0.4 μm dia pore) were incubated on leached sand through which dilute Pfeffer's salt solution percolated at 60 ml·h^{-1}. One membrane was incubated in each dish, and there were to dishes per fungus. Ten ml fractions of eluant from the dishes were collected continuously in scintillation vials (22 ml capacity) for several hours. At various times the flow of leaching fluid was stopped, and the propagules were aseptically removed from the dishes and transferred to small (60 x 15 mm) plastic petri dishes and stored for 12 h at 4°C to retard germination. After storage the propagules were returned to the leaching dishes, and the diffusive stress (60 ml·h^{-1}) resumed. Ten ml fractions from the dishes were then collected for an additional 30-60 min. Sclerotia of M. phaseolina were subjected to four cycles of diffusive stress interruption, whereas diffusive stress on C. victoriae conidia was interrupted only once.

Ten ml of aqueous scintillation cocktail (Filonow and Lockwood, submitted) were added to each eluant fraction. Membranes bearing ^{14}C-labeled propagules were placed in scintillation vials, dissolved in 0.3 ml chloroform, and the propagules suspended in 10 ml of 4% Cab-O-Sil (Research Products International, Elk Grove, IL) in a toluene/methanol scintillation cocktail (Filonow and Lockwood, submitted). Radioactivity was measured in a Packard Tri-Carb liquid scintillation counter Model 578. ^{14}C-toluene was used as an internal standard to correct for counting efficiency.

Uptake Of ^{14}C From Soil By Nutrient-dependent Conidia Of C. victoriae

Conidia of C. victoriae borne on 2.5 cm dia Nuclepore membranes (0.4 µm dia pore) were converted to nutrient-dependence after 8 days of continuous diffusive stress on the leached sand apparatus. There were 15 x 10^3 conidia on each membrane and germination of these conidia on dilute Pfeffer's solution was 20±3% (mean ± s.d.) after 24 h at 24°C.

^{14}C-labeled soils were prepared by adding 1.0 ml of sterile water containing 6 µCi of ^{14}C-glucose (210 mCi/mM) to 10.0 g of air-dried soil in a 90 x 15 mm plastic petri dish. Mycostasis was not annulled in soils by the addition of this quantity of glucose. Distilled water was added to adjust soil moisture to -50 kPa (0.05 bar) matric potential. The ^{14}C-labeled soils were mixed well with a spatula and allowed to equilibrate for 36 h at 24°C. After equilibration a sterilized incubation chamber (Figure 1) containing two 2.5 cm dia membranes was placed atop each soil. One membrane held nutrient-dependent conidia and the other, a control with no conidia was used to determine ^{14}C adsorbed to the membranes. There were four replicate plates per soil. After 36 h of incubation on soils, the chambers were removed, and adhering soil solution on the bottom of each chamber was washed back into the respective soil plate with 2-3 ml of distilled water. Membranes within the chambers were removed and each was aseptically washed twice, using gentle suction, with 1.5 ml of cold (5°C), dilute, sterile Pfeffer's solution. Using a template, a one-quarter sector of each membrane was excixed with a sterile razor blad, and place don 1.0 ml of dilute Pfeffer's solution in sterile stainless steel planchets. Germination of conidia on these membranes was determined after 24 h at 24°C. The remaining portion of a membrane was placed in a scintillation vial, and dissolved in 0.3 ml of chloroform. Ten ml of 4% Cab-O-Sil cocktail was added and ^{14}C in each vial was determined.

At the end of the experiment, the soil solution from a 30.0 g (O.D. weight) composite of each soil was extracted using centrifugation (Davies and Davies, 1963). The volumes of soil solution collected from each soil were adjusted to 10.0 ml and mixed well. To 1.0 ml of soil solution in a scintillation vial, 10.0 ml of aqueous cocktail was added, and ^{14}C determined.

In each experiment all treatments were replicated 2-4 times and each experiment was repeated at least once. Experimental results were subjected to analysis of variance and significant differences among means were determined using Duncan's Multiple Range Test.

RESULTS

Germinability Of Propagules After Incubation On Soils

Incubation of C. victoriae conidia on soils gradually decreased their ability to germinate on sterile Pfeffer's solution (Figure 2). After 7 days of incubation on the five soils, germination ranged from 51% for conidia incubated on Dryden loam to 74% for conidia incubated on Brookston clay loam. Germination after 30 days ranged from 11% for conidia taken from Dryden loam to 33% for those from Brookston clay loam. However, conidia were viable after 30 days incubation on the five soils, germinating greater than 90% on PDA.

The soils differed in their ability to reduce the nutrient-independent state of conidia incubated on them. Dryden loam and Spinks sandy loam attenuated the ability of conidia to germinate on Pfeffer's solution significantly more than the two clay loam soils ($P=0.05$). Boyer sandy loam was intermediate in this regard. Conidia incubated on this soil consistently showed less germination ($P=0.05$) than those from Brookston and Parkhill clay loams; however, differences between the Brookston and Parkhill soil were sometimes not significant ($P=0.05$). Incubation of conidia on the coarse-textured soils, then, produced the greatest decreases in the nutrient-independent status of the conidia.

After ten days of incubation on soils, sclerotia of M. phaseolina still retained their nutrient-independent state as shown by greater than 90% germination of Pfeffer's solution (Figure 3). At 20 days incubation,

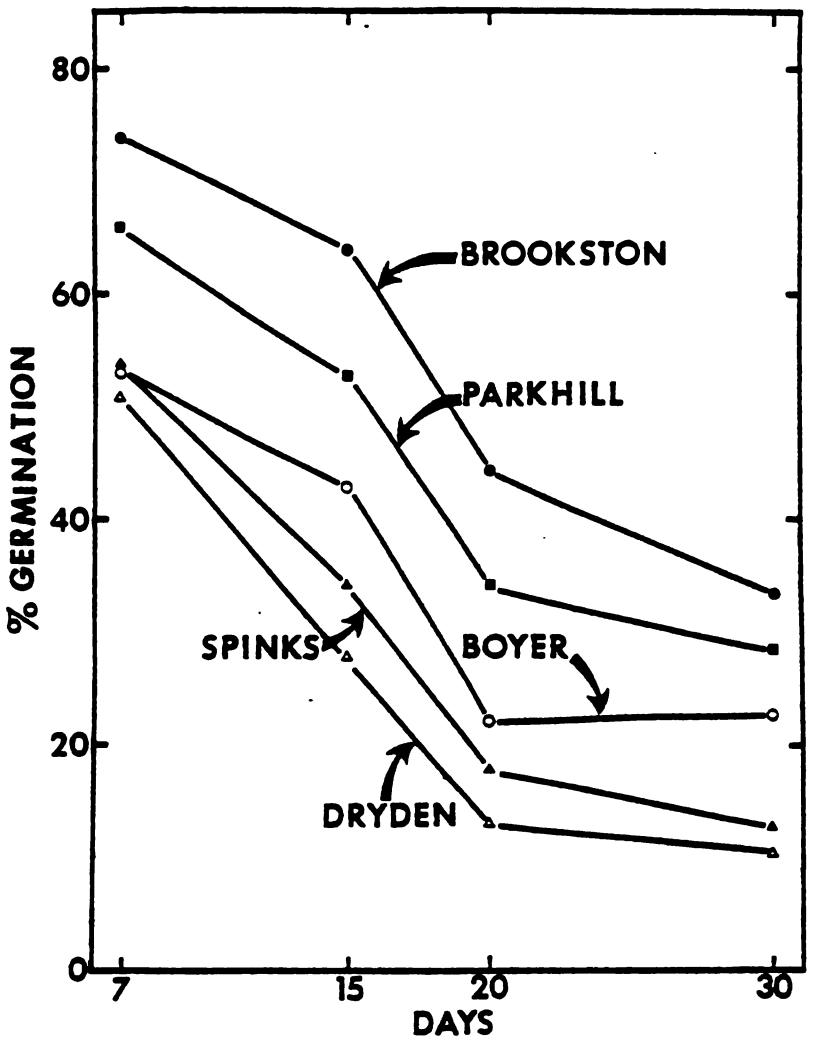

Figure 2. Germination of Cochliobolus victoriae conidia on sterile, dilute salt solution after prior incubation on soils for up to 30 days. At 30 day Duncan's Multiple Range Test ($P=0.05$) showed the following significant differences among means: Dryden[a], Spinks[a], Boyer[b], Parkhill[bc], and Brookston[c]. Mean values of soils followed by the same letter did not differ significantly.

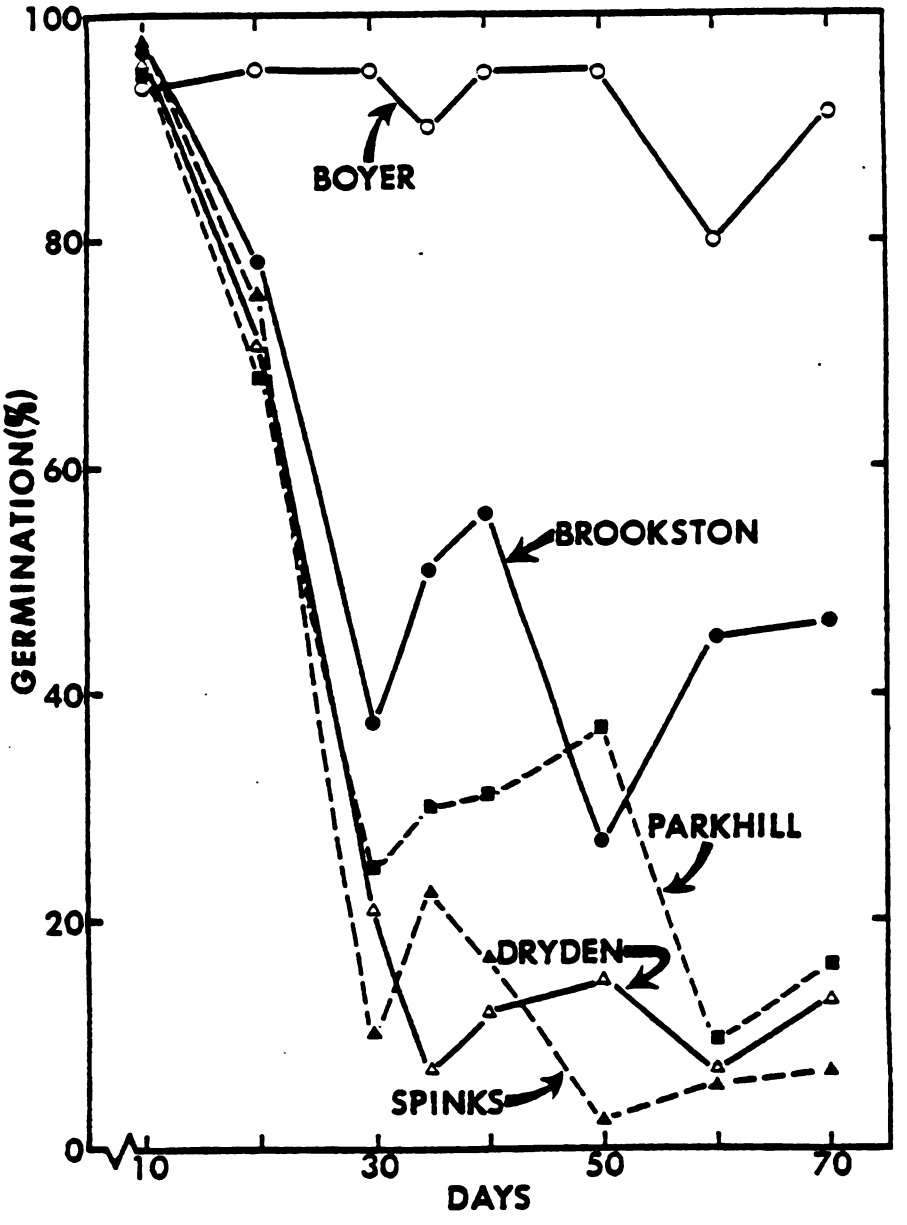

Figure 3. Germination of Macrophomina phaseolina sclerotia on sterile, dilute salt solution after prior incubation on soils for up to 70 days. At 70 day Duncan's Multiple Range Test ($P=0.05$) showed the following significant differences among means: Spinks[a], Dryden[a], Parkhill[a], Brookston[b], and Boyer[c]. Mean values of soils followed by the same letter did not differ significantly.

all of the soils, except Boyer sandy loam, slightly decreased germinability of the sclerotia. However, by 30 days, a precipitous decline in sclerotial germinability was induced by all soils, except Boyer sandy loam. The Boyer soil did not appreciably reduce the germinability of the sclerotia even after 70 days of incubation. Following the 30 day sampling, germination on Pfeffer's solution of sclerotia from soils other than Boyer sandy loam showed great fluctuations, with the greatest fluctuations occurring amongst the clay loam soils. Nevertheless, two coarse-textured soils, Dryden loam and Spinks sandy loam consistently ($P=0.05$) reduced the germinability of sclerotia when compared to germinability of sclerotia incubated on at least one of the clay loam soils.

Effects Of Long-term Incubation Of Propagules In A Leached Sand Apparatus

Bristow and Lockwood (1975a) had reported that conidia of C. victoriae incubated in a leached sand apparatus became nutrient-dependent after 7 days. These results were confirmed in the present study using a different isolate of C. victoriae, which germinated only 6% on Pfeffer's solution after 9 days of diffusive stress. Germination on PDA after 14 h was 55%, whereas the germination of unleached conidia on PDA was greater than 90% in the same period of time. However, by 24 h the germination of leached conidia had increased to 90%, indicating that viability was not greatly affected.

In another experiment, ^{14}C-labeled conidia of C. victoriae were made nutrient-dependent after 8 days of incubation on the leaching apparatus. Radioactivity at 2-10 cpm above the scintillation cocktail background (25 cpm) was still detected in the leachate after 8 days, suggesting that

the conidia continued to exude at a low level for an extended period. Germination of these conidia on dilute Pfeffer's solution was 20% after 18 h and germination on PDA was 64% after 24 h.

Several membranes bearing these nutrient-dependent conidia were aseptically placed on 1.0 ml dilute Pfeffer's solution in stainless steel planchets enclosed in a 90 x 15 mm plastic petri dish and incubated for 3.5 days at 4°C to retard germination. After 3.5 days of incubation at 4°C, only 2% of the conidia had germinated; however, conidia incubated in the cold and then allowed to incubate at 24°C, germinated 32%. Thus, a 3.5 day storage of 4°C allowed C. victoriae conidia to regain a portion of the nutrient-independent state they had previously lost ($P=0.05$).

Sclerotia of S. cepivorum lost the ability to germinate on Pfeffer's solution after 15 days of diffusive stress (Table 1). Thereafter, no germination was observed even after four days' incubation on Pfeffer's solution. Viability of sclerotia (90% germination on PDA) was not significantly decreased ($P=0.05$) until 35 days of stress, and even after 45 days of diffusive stress the viability remained high (81% germination on PDA).

There were no differences in the responses of stressed versus unstressed S. cepivorum sclerotia to onion extract until 25 days of leaching. From then on further diffusive stress diminished the ability of the sclerotia to respond to increasing dilutions of onion extract. After 25 days of leaching a 1/10 and 1/20 dilution gave complete (>95%) germination of stressed and unstressed sclerotia after 72 h incubation. However, on 1/40 dilution of onion extract stressed sclerotia germinated 30% after 45 h incubation on the extract, whereas unstressed sclerotia germinated completely. After 35 days of diffusive stress, the leached

Table 1. Germination of sclerotia of *Sclerotium cepivorum*, *Macrophomina phaseolina*, and *Verticillium dahliae* on sterile, dilute salts solution or on PDA after prior incubation on a leached sand apparatus imposing diffusive stress for different time periods.

	Germination (%)					
	S. cepivorum		*M. phaseolina*		*V. dahliae*	
Days	Salts Solution	PDA	Salts Solution	PDA	Salts Solution	PDA
0	81 a	100 a	88 a	100 a	96 a	100 a
15	6 b	100 a	62 b	100 a	95 a	100 a
25	0 c	100 a	51 b	100 a	96 a	100 a
35	0 c	90 b	30 c	100 a	97 a	100 a
45	0 c	81 b	28 c	100 a	82 b	100 a

Numbers within a column followed by the same letter are not significantly different ($P=0.05$) using Duncan's Multiple Range Test.

sclerotia germinated only 24% on a 1/80 dilution even after 5 days of incubation. On the same dilution, unstressed sclerotia germinated 94% after only 72 h of incubation. By 45 days of diffusive stress, sclerotia did not germinate on a 1/100 dilution, whereas unstressed sclerotia germinated 90% after 96 h of incubation.

Prolonged stress on M. phaseolina sclerotia gradually reduced their ability to germinate on Pfeffer's solution (Table 1). After 15 days of diffusive stress the germinability (62%) of M. phaseolina sclerotia was significantly less ($P=0.05$) than at the start of the experiment and after 45 days of diffusive stress, the sclerotia germinated only 28% of Pfeffer's solution. However, the viability of these sclerotia was not affected.

In contrast to S. cepivorum and M. phaseolina sclerotia, V. dahliae microsclerotia did not begin to lose nutrient-independence until 45 days of stress, and germinability was reduced to only 82% ($P=0.05$). Viability, however, was not affected.

Interruption Of Artificially Imposed Diffusive Stress

^{14}C-labeled conidia of C. victoriae were removed from the leached sand apparatus after 4 h, and incubated for 12 h at 4°C to retard germination. Upon return of the conidia to the leached sand apparatus, there was a burst of ^{14}C-exudate, followed by a quick return to the exudate rate which existed prior to the interruption of the diffusive stress (Figure 4).

During the first 4 h, 3.6-4.0% of the total ^{14}C in the conidia was lost as exudate prior to the interruption of diffusive stress. The burst of ^{14}C lost following return of conidia to diffusive stress was an additional 0.2-0.4%. When the diffusive stress imposed on M. phaseolina

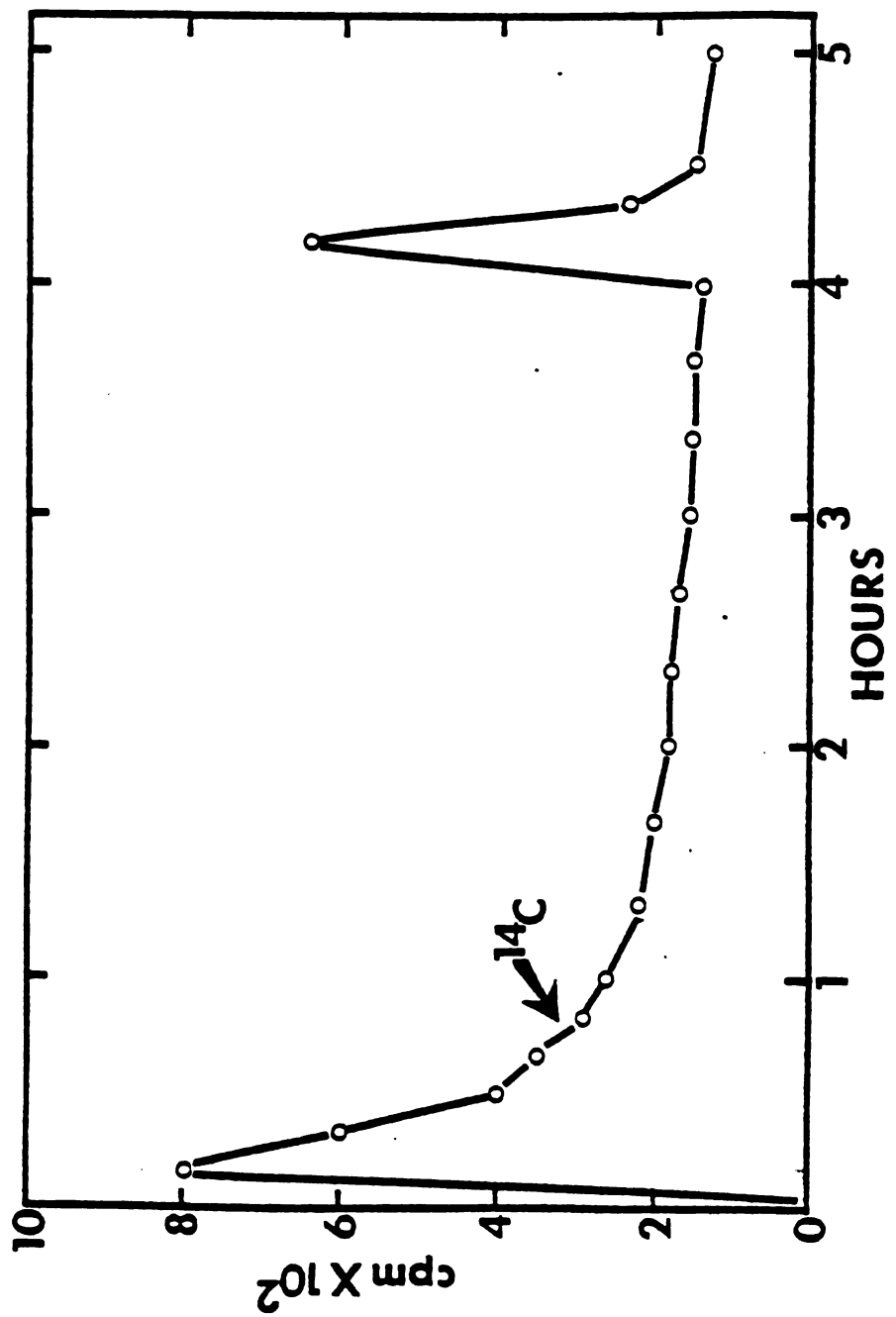

Figure 4. Exudation of ^{14}C from labeled conidia of Cochliobolus victoriae incubated on a leached sand apparatus prior to and following an interruption during which conidia were incubated in non-leaching conditions at 4°C for 12 h. The burst of ^{14}C after 4 h was from conidia returned to the leached sand apparatus.

sclerotia was successively interrupted several times (Figure 5), there was a corresponding burst in ^{14}C-exudate each time the sclerotia were returned to diffusive stress. Total ^{14}C lost in the combined bursts increased ^{14}C-exudate from 0.9-1.2% prior to the first burst to 1.3-1.5% after a fourth burst.

It was of interest to determine how much of the ^{14}C in a burst of exudate from C. victoriae conidia was released from the conidia during incubation at 4°C. It was assumed that this amount of ^{14}C would be easily washed off the conidia and would not represent endogenous nutrients lost to diffusive stress. Membranes were removed from cold storage after 12 h, and prior to placing them on the leached sand apparatus, some were repeatedly washed via vacuum suction with 3 ml volumes of cold (5°C) dilute Pfeffer's solution. ^{14}C in each 3 ml washing was determined. After 3-4 washings, radioactivity removed was considered neglibible, and the membranes were returned to diffusive stress and collection of ^{14}C-exudate resumed. Other membranes went directly from cold storage to the leached sand apparatus, and exudate collection was resumed. Approximately 40% of the total ^{14}C lost from conidia during a burst in exudation was removed by washing the conidia prior to returning them to the leached sand apparatus. About 60% of the ^{14}C lost, therefore, came from within the conidia after they were returned to diffusive stress. This suggests that an interruption in diffusive stress may allow conidia of C. victoriae an opportunity to reorganize or replenish internal nutrient pools, which had become depleted via diffusive stress.

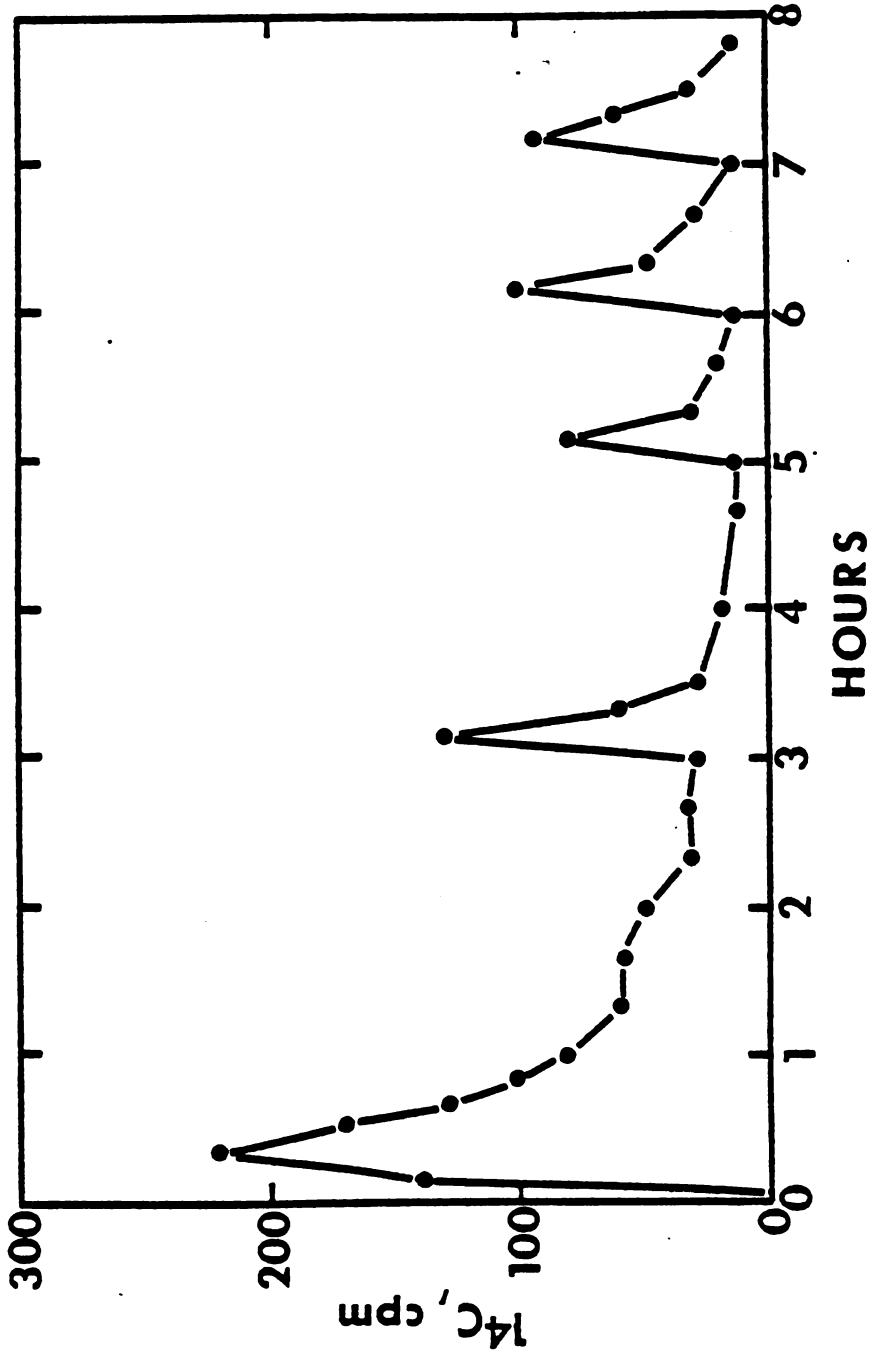

Figure 5. Exudation of ^{14}C from labeled sclerotia of Macrophomina phaseolina incubated on a leached sand apparatus prior to and following several interruptions during which sclerotia were incubated in non-leaching conditions at 4°C for 12 h. The bursts of ^{14}C after 3, 4, 5, and 7 h were from sclerotia returned to the leached sand apparatus.

^{14}C Uptake By C. victoriae Conidia Incubated On Soils

Nutrient-dependent conidia absorbed about twice as much ^{14}C during 36 h of incubation on Dryden loam, Boyer sandy loam, and Spinks sandy loam soils as on the two clay loam soils (Table 2). In addition two to three times more ^{14}C was found in the soil solutions of the coarse-textured soils than of the clay loam soils. The germination of nutrient-dependent conidia incubated for 36 h on four of the five soils significantly increased ($P=0.05$) from 20% prior to incubation to 35-44% after incubation on soils. The germination of conidia incubated on Brookston clay loam did not increase.

Conidia incubated on the four other soils had significantly greater ($P=0.05$) germination than conidia incubated on Brookston clay loam.

Table 2. Amount of ^{14}C extractable from soils treated with ^{14}C glucose and uptake of ^{14}C and conidial germination of Cochliobolus victoriae on the soils.

Soil	Conidia* dpm	Soil Solution x 10^3 dpm†	Germination (%)‡
Brookston clay loam	58 a	6.1 a	22 a
Parkhill clay loam	60 a	6.9 a	35 b
Spinks loamy sand	105 b	15.4 b	44 b
Boyer loamy sand	120 b	21.8 c	38 b
Dryden loam	128 b	20.0 c	38 b

*Conidia were made nutrient-dependent after eight days of incubation on the leached sand apparatus. Germination of conidia after 24 h on sterile, dilute salt solution was 20%. ^{14}C in conidia was determined after 36 h incubation on ^{14}C labeled soils.

†Soils were centrifuged (Davies and Davies, 1963) and the volumes of the extracted soil solutions adjusted to 10.0 ml. ^{14}C in a 1.0 ml sample of soil solution was determined.

‡Germination was determined after 24 h on sterile, dilute salts solution.

Numbers within a column by the same letter are not significantly different ($P=0.05$) using Duncan's Multiple Range Test.

DISCUSSION

The conversion of fungal propagules from a nutrient-independent state to one of dependence suggests that endogenous nutrients needed for propagule germination were lost to a diffusive stress imposed by the soils or by the leached sand apparatus. A diffusive stress intense enough to account for mycostasis has been demonstrated in several soils using several different types of fungal propagules (Bristow and Lockwood, 1975a,b; Sneh and Lockwood, 1976; Filonow and Lockwood, 1979; submitted).

Fungal propagules differ in their sensitivities to diffusive stress imposed artificially or by soils (Bristow and Lockwood, 1975a; Filonow and Lockwood, submitted). In the present study, conidia of C. victoriae lost their nutrient-independence within 9 days on the leached sand apparatus, whereas sclerotia of M. phaseolina required nearly 4 times longer for a comparable reduction in nutrient-independence. Sclerotia of V. dahliae lost only a portion of their nutrient-independent state in 45 days of continued stress, whereas sclerotia of S. cepivorum rapidly became nutrient-dependent and the response of sclerotia to germination stimulators in onion extract was progressively reduced.

Factors involved in the varied responses of fungal propagules to diffusive stress in soil have yet to be studied. Prolonged exposure to the diffusive stress imposed by soil may deplete endogenous reserves in fungal propagules to below a threshold level needed for germination. Qualitative changes in the endogenous reserves among fungi also may be

involved, although the nature and extent of such changes are not yet determined. Moreover, the extent of endogenous respiration by fungal propagules in soil requires study, as prolonged respiratory loss of carbon by propagules may also contribute to the loss of nutrient-independence. It is reasonable to assume, however, that exudation rates would be a factor in the survival of propagules in soil, since propagules with low exudation rates would lose less endogenous nutrients over the same time than those with higher exudation rates. M. phaseolina sclerotia lost less ^{14}C-exudate than conidia of C. victoriae when these propagules were subjected to a short-term diffusive stress imposed by soils or by a leached sand apparatus (Filonow and Lockwood, submitted). In the present study, however, C. victoriae conidia and M. phaseolina sclerotia attained similar levels of nutrient-dependence after 30 days on incubation on soils. These results suggest that exudation rates of fungal propagules are only one of many factors controlling the survival of fungal propagules in soils.

It is reasonable to assume that a nutrient-independent propagule once made nutrient-dependent no longer needs to remain under diffusive stress to maintain a quiescent condition, as long as the propagule's immediate environment is devoid of nutrients which could be utilized for germination. Most likely this situation exists in many microsites in soil, espectially in light of the inherent scarcity of readily available nutrients in soil for fungal germination (Clark and Paul, 1970; Shields, et al., 1973; Lockwood, 1977). However, since soil is a collection of microsites, all of which, to some degree are constantly changing, there most likely exist microsites where fungal propagules with varying nutritional requirements for germination contact nutrients in the soil

solution (Stevenson, 1956; Birch, 1953; Allison, 1973; Sørensen, 1974; Adu and Oades, 1978; Lund and Goksøyr, 1980). In the present study propagules were incubated on soils in which soil moisture was not kept constant. Every 4 or 5 days, about 0.5 ml to 1.0 ml of water was added to the soils to insure wetting of the membranes and continuity with the soil solution. Soil moisture was allowed to fluctuate between saturation and about -300 kPa (0.3 bar), the lower limit of soil moisture at which discontinuities in the membrane-soil solution interface was observed. Consequently, during these fluctuations in soil moisture, small quantities of soluble nutrients may have been released into the soil solution of the different soils. Results from Bristow and Lockwood (1975a) and results reported herein, showing ^{14}C uptake from soil by nutrient-dependent conidia of C. victoriae suggest that nutrient-dependent propagules may reabsorb their own exudate or take up nutrients released into the soil solution and thereby replenish their supply of endogenous nutrients. A nearly constant soil moisture would minimize the release of nutrients into the soil solution and therefore the quantity absorbed by fungal propagules. Work by Papavizas (1977) suggests this: survival of M. phaseolina sclerotia in a sandy loam soil kept constantly moist (50% m.h.c.) for 20 weeks was reduced by 43%, whereas alternate drying and wetting of the soil did not appreciably reduce survival of the sclerotia.

Extreme fluctuations in drying and wetting of soil may result in sharp decrease in fungal propagule survival, however. Air-dried and rewetted sclerotia (Smith, 1972; Coley-Smith, 1979; Gladders and Coley-Smith, 1980) and conidia (Tsuneda and Skoropad, 1978) have been shown to leak considerably more nutrients than undried ones. The increased nutrient leakage elevated microbial activity around these

propagules, which may have been causally related to decreased longevity.

Fluctuations in the soil environment such as drying and wetting are disruptive of soil structure and increase the decomposition of organic materials in soil aggregates (Allison, 1973; Adu and Oades, 1978), and interrupt the diffusive stress imposed by soil microorganisms. Results from the present study suggest that interruptions in diffusive stress may allow fungal propagules to reorganize their endogenous nutrients and thereby enhance their potential germinability. Conidia of C. victoriae and sclerotia of M. phaseolina showed increased ^{14}C-exudation following a 12 h respite from diffusive stress. Moreover, nutrient-dependent conidia of C. victoriae, which were removed from diffusive stress and kept in a non-germinated state (4°C) for 3.5 days showed reduced dependence on exogenous nutrients for germination. Interruptions in diffusive stress may also allow nutrient-dependent propagules greater opportunity to become scavengers of small quantities of nutrients released into the soil solution. In this regard, the high adsorptive capacity of clay loam soils may restrict availability of nutrients in the soil solution, making it more difficult for fungal propagules to recoup lost nutrients in these soils, whereas uptake of nutrients may be facilitated in coarse-textured soils with their lower adsorptive capacities (Filonow and Lockwood, submitted).

Results of this study support a concept of a 'give and take' operating between many nutrient-independent propagules and components of their microhabitats in soil. Fungal propagules in soil 'give up' endogenous nutrients to a diffusive stress imposed by microorganisms intensely competing for carbon substrate. A persistent stress reduces the potential germinability of many propagules, perhaps to such an extent

that they fail to germinate without exogenous nutrients, or perhaps a continued stress may even lead to death. Cyclic fluctuations in the soil environment, e.g., drying and wetting, disrupt microhabitats and interrupt the diffusive stress imposed on many fungal propagules. An interruption of diffusive stress may allow many propagules an opportunity to 'take up' small amounts of nutrients released in the soil solution and to reorganize internal nutrient pools, whereby the potential for germinability and, ultimately, survival is increased.

LIST OF REFERENCES

LIST OF REFERENCES

ADU, J. K. and OADES, J. M. (1978) Physical factors influencing decomposition of organic materials in soil aggregates. Soil Biology and Biochemistry 10, 109-115.

ALABOUVETTE, C., ROUXEL, F. and LOUVET, J. (1979) Characteristics of Fusarium wilt-suppressive soils and prospects for their utilization in biological control. In Soil Borne Plant Pathogens (B. Schippers and W. Gams, Eds.). pp. 166-182. Academic Press, New York.

ALLISON, F. E. (1973) Soil Organic Matter and Its Role In Crop Production. Elsivier Scientific Publishing Co., New York.

BIRCH, H. F. (1958) The effect of soil drying on humus decomposition and nitrogen availability. Plant and Soil 10, 9-31.

BRISTOW, P. R. and LOCKWOOD, J. L. (1975a) Soil fungistasis: Role of spore exudates in the inhibition of nutient-independent propagules. Journal of General Microbiology 90, 140-146.

BRISTOW, P. R. and LOCKWOOD, J. L. (1975b) Soil fungistasis: Role of the microbial nutrient sink and of fungistatic substances in two soils. Journal of General Microbiology 90, 147-156.

CLARK, F. E. and PAUL, E. A. (1970) The microflora of grassland. Advances in Agronomy 22, 375-435.

CHINN, S. H. F. and TINLINE, R. D. (1964) Inherent germinability and survival of spores of Cochliobolus sativus. Phytopathology 54, 349-352.

COLEY-SMITH, J. R. (1960) Studies of the biology of Scerotium cepivorum Berk. IV. Germination of sclerotia. Annals of Applied Biology 48, 8-18.

COLEY-SMITH, J. R. and KING, J. E. (1969) The production by species of Allium of alkyl sulphides and their effect on germination of sclerotia of Sclerotium cepivorum Berk. Annals of Applied Biology 64, 289-301.

COLEY-SMITH, J. R. (1979) Survival of plant pathogenic fungi in soil and in the absence of host plants. In Soil Borne Plant Pathogens (B. Schippers and W. Gams, Eds.). pp. 40-57. Academic Press, New York.

DAVIES, B. E. and DAVIES, R. I. (1963) A simple centrifugation method for obtaining small samples of soil solutions. Nature 198, 216-217.

FILONOW, A. B. and LOCKWOOD, J. L. (1979) Conidial exudation by Cochliobolus victoriae on soils in relation to soil mycostasis. In Soil Borne Plant Pathogens (B. Schippers and W. Gams, Eds.). pp. 107-119. Academic Press, New York.

FILONOW, A. B. and LOCKWOOD, J. L. Mycostasis and the microbial sink of soil. I. Assessment of the microbial sink of five soils. Soil Biology and Biochemistry. (submitted).

GARRET, S. D. (1970) Pathogenic Root-Infecting Fungi. pp. 1-294. Cambridge University Press, London.

GLADDERS, P. and COLEY-SMITH, J. R. (1980) Interactions between Rhizoctonia tuliparum sclerotia and soil microorganisms. Transactions of the British Mycological Society 74, 579-586.

KING, J. E. and COLEY-SMITH, J. R. (1968) Effects of volatile products of Allium species and their extracts on germination of sclerotia of Sclerotium cepivorum Berk. Annals of Applied Biology 61, 407-414.

LOCKWOOD, J. L. (1977) Fungistasis in soils. Biological Reviews 52, 1-43.

LUND, V. and GOKSØYR, J. (1980) Effects of water fluctuations on microbial mass and activity in soil. Microbial Ecology 6, 115-123.

PAPAVIZAS, G. (1977) Survival of sclerotia of Macrophomina phaseolina and Sclerotium cepivorum ater drying and wetting treatments. Soil Biology and Biochemistry 9, 343-348.

SHIELDS, J. A., PAUL, E. A., LOWE, W. E. and PARKINSON, D. (1973) Turnover of microbial tissue in soil under field conditions. Soil Biology and Biochemisry 5, 753-764.

SMITH, A. M. (1972) Drying and wetting sclerotia promotes biological control of Sclerotium rolfsii Sacc. Soil Biology and Biochemistry 4, 119-123.

SNEH, B. and LOCKWOOD, J. L. (1976) Quantitative evaluation of the microbial nutrient sink in soil in relation to a model system for soil fungistasis. Soil Biology and Biochemistry 8, 65-69.

SØRENSEN, L. H. (1974) Rate of decomposition of organic matter in soil as influenced by repeated air drying-rewetting and repeated additions of organic material. Soil Biology and Biochemistry 6, 287-292.

STEVENSON, I. L. (1956) Some observations on the microbial activity in remoistened air-dried soils. Plant and Soil 8, 170-182.

TSUNEDA, A. and SKOROPAD, W. P. (1978) Nutrient leakage from dried and rewetted conidia of Alternaria brassicae and its effects on the mycoparasite Nectria inventa. Canadian Journal of Botany 56, 1341-1345.