ADVANCES IN MICROBIAL ECOLOGY

Edited by M. Alexander

Volume 5

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Preface

Advances in Microbial Ecology was established by the International Commission on Microbial Ecology to provide a vehicle for in-depth, critical, and, it is hoped, provocative reviews on aspects of both applied and basic microbial ecology. In the five years of its existence, *Advances* has achieved recognition as a major source of information and inspiration both for practicing and for prospective microbial ecologists. The majority of reviews published in *Advances* have been prepared by experts by invitation from the Editorial Board. Although the Board intends to continue its policy of soliciting reviews, some unsolicited review outlines have been approved and the authors invited to proceed with the preparation of manuscripts for publication in *Advances*. The Editorial Board continues to encourage microbial ecologists to submit unsolicited proposals on original topics, in outline form only, for consideration by the Board for subsequent publication.

Volume 5 of *Advances in Microbial Ecology* again covers a broad range of topics, with particular emphasis on the ecology of fungi and on the role that environmental extremes play in the overall behavior of microorganisms in natural habitats. J. G. Zeikus examines the biochemistry and ecological significance of lignins, with particular reference to the overall carbon cycle in nature. The responses of fungi to nutrient limitations and to inhibitory substances in natural habitats are considered in the review by J. L. Lockwood and A. B. Filonow. In a somewhat similar vein, J. S. Poindexter discusses the possible basis for oligotrophy, which describes the ability of certain bacteria to grow at very low nutrient concentrations. The contribution by D. M. Griffin looks at the stresses imposed upon microorganisms by water limitations in natural habitats. The mycorrhizal fungi and their beneficial contributions to plant growth are the subject of the review by B. Mosse, D. P. Stribley, and F. LeTacon.

The Editor and members of the Editorial Board of *Advances* are appointed by the International Commission on Microbial Ecology for fixed terms. With the publication of this volume, our colleague Hans Veldkamp

completes his term as a Board member. The Editor and members of the Editorial Board offer to Hans their sincerest thanks for his outstanding help and guidance since the inception of the series.

M. Alexander, Editor
T. Rosswall
K. C. Marshall
H. Veldkamp

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Responses of Fungi to Nutrient-Limiting Conditions and to Inhibitory Substances in Natural Habitats

JOHN L. LOCKWOOD AND ALEXANDER B. FILONOW

1. Introduction

Fungi, as heterotrophic microorganisms, coexist with numerous other microorganisms, with whom they must compete for a share of nutrients. Since such nutrients, particularly energy substrate, are often in short supply, adaptive traits have evolved that enhance survival (Lockwood, 1977). Moreover, numerous organic metabolic products are produced as a result of microbial degradation of various substrates. These, together with various mineral components of a habitat, also may affect microorganisms, including fungi. In this review we attempt to discuss the responses of fungi to nutrient competition with other microorganisms and to the presence of inhibitory substances that occur in natural environments.

Because of the great potential breadth of the subject matter encompassed by this topic, it has been necessary to restrict the scope of our review. Thus, we have confined the bulk of our discussion to the soil, since this environment has been by far the most studied with respect to the effects of nutrient limitation and toxic substances on fungal ecology. Where evidence exists for the expression in other environments of phenomena related to those occurring in soil, e.g., mycostasis on plant leaves, this also is discussed. We have restricted the cov-

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erage of nutrient effects to those imposed by nutrient deficiencies, rather than include responses to an abundance of nutrients, though it is granted that ecologically important responses to the presence of nutrients (in addition to vegetative growth), e.g., zoospore attraction to roots, are known. Since effects owing to inhibitory substances are manifestly negative ones, restriction of the subject matter concerning nutrients to responses to shortages allows a comparison of the effects of nutrient deprivation and toxic substances. We have attempted to evaluate the relative significance of each for a particular phenomenon. We also have dealt only with naturally occurring inhibitory substances, and have avoided consideration of pesticide residues, toxic pollutants from the atmosphere, or phytoalexins involved in suppressing fungal infection of plants. These are major subjects in themselves, and have been reviewed elsewhere (respectively, Goring and Hamaker, 1972; Heagle, 1973; Kuć, 1976).

For purposes of this review, our use of the term *inhibitory* is broad. It includes inhibitory and toxic substances and lytic enzymes of microbial origin that may degrade fungal cell walls.

Our coverage of fungal responses to nutrient limitation and inhibitory substances will deal primarily with mycostasis and mycolysis. Other fungal activities that will be discussed are regermination of propagules, appressorium formation, and persistent structure formation. Unfortunately, little work has been done regarding the effect of inhibitory substances on fungal activities other than mycostasis and mycolysis.

2. The Soil Habitat

2.1. Energy Budgets

Fungi, and other heterotrophic microorganisms in soil, must subsist on (a) plant residues, such as root and leaf litter from trees, shrubs, herbs, and grasses; (b) animal bodies and excreta; (c) other microorganisms, in the case of mycoparasites; and (d) exudates from living roots. Humic materials are also present but probably do not serve as a major source of energy, since their turnover rate is very slow (Barber and Lynch, 1977).

There are few estimates of the amounts of these components in soils, but Lynch and Panting (1980) provide an estimate from an arable wheat field. Of the total annual substrate input of 3540 kg C/ha per year, 79% was straw residues, 11% decomposed roots, 7% root exudates, and 3% autotrophic microbes.

In soil, the supply of readily utilizable carbon is apparently severely limited (Clark, 1965; Lockwood, 1977) and hence is an object of intense competition. Recent studies relating microbial populations to substrate availability in soil verify this concept, and a consideration of these findings seems justified in

terms of its importance to fungal ecology. A number of investigators have related measurements of microbial biomass to the amount of substrate available. Much use has been made of the Monod growth equation (Marr *et al.*, 1963) to calculate the amount of substrate required for cell maintenance (functions other than growth):

$$dx/dt + ax = Y(ds/dt) \quad (1)$$

where a is the specific maintenance constant (per hour), x the concentration of cells in grams, Y the yield coefficient (efficiency of conversion of substrate to cells), and s the amount of substrate required for maintenance. Subtracting s from the total substrate input gives the amount available for growth. Values of a, or the maintenance coefficient m (g substrate/g dry weight per hr), have generally been obtained from laboratory studies. Values of a and m are interconvertible by $m = a/Y$. Estimates of a for aerobically grown bacteria in chemostats were 0.025–0.028/hr at 30°C and 0.005/hr at 15°C (Marr *et al.*, 1963), and 0.042/hr at 37°C (Pirt, 1965); Barber and Lynch (1977) advocate use of values of this order. However, when such values are used, maintenance energy requirements, in most cases, appear to consume far more energy than is available in the substrate (Barber and Lynch, 1977). Flanagan and Van Cleve (1977) obtained an m value of 0.00055 g/g substrate (equivalent to $a = 0.00022$). Most workers have used a values of the order of 0.001, and values of a obtained from experiments done in soil itself are of a similar magnitude (Shields *et al.*, 1973; Behera and Wagner, 1974).

The number of generations of microbial growth per year is given by the equation

$$Y(S + xR) = xR \quad (2)$$

(Gray and Williams, 1971), where Y and x are as in equation (1), S is the substrate available for growth, and R is the number of generations per year. This equation allows for the cells formed to serve as secondary substrates (Gray and Williams, 1971).

Values for Y for individual bacteria in chemostats are usually in the range 0.35–0.60 (Marr *et al.*, 1963; Payne, 1970; Flanagan and Bunnell, 1976; Flanagan and Van Cleve, 1977), and those in soil from 0.39–0.60 (Shields *et al.*, 1973; Behera and Wagner, 1974).

In nine studies tabulated (Table I), the annual number of generations of microbial growth in soil was determined to be very low. These estimates range from less than 1 to 36 generations/year, and strongly indicate that substrate limitations place severe restrictions on the ability of fungi (and other microorganisms) to grow in soil.

Measurements needed to estimate the number of generations occurring in

Table I. Predicted Numbers of Generations of Microbial Cells per Year and Maintenance Energy Requirements from Estimates of Substrate Inputs, Yield Coefficients, Biomass, and Specific Maintenance Constants in Several Soils

Reference	Substrate input (S)	Yield coefficient (Y)	Biomass (x)	Specific maintenance constant (a)	Maintenance energy requirements (s)	Number of generations (R)
Clarholm and Rosswall (1980)	441 g/m^2	0.5[a]	35 g/m^2	0.001[a]	613 g/m^2	<1
Lynch and Panting (1980)	3540 kg C/ha	0.5[a]	200 kg C/ha	0.002[a]	3504 kg C/ha	<1
Gray and Williams (1971)	764 g/m^2	0.35	46 g/m^2	0.001	1156 g/m^2	<1
Babiuk and Paul (1970)	500 g/m^2	0.35	55 g/m^2[b]	0.001	1364 g/m^2	<1
Shields *et al.* (1973)	937 μg C/g	0.60	165 μg C/g	0.002	300 μg C/g	5.8
Flanagan and Van Cleve (1977)	398 g/m^2	0.40	5.7 g/m^2	0.00055	22.6 g/m^2	21
Flanagan and Bunnell (1976)	10 g/m^2[c]	0.54	0.45 g/m^2[d]	0.00032	1.2 g/m^2	23
Behera and Wagner (1974)	1040 kg/ha	0.39	8.4 kg/ha	0.0036	265 kg/ha	36
Gray *et al.* (1974)	706 g/m^2	0.35	3.7 g/m^2[b]	0.001	92 g/m^2	87
	706 g/m^2	0.35	11.1 g/m^2[e]	0.001	277 g/m^2	20

[a]Value supplied, or substituted for that in reference.
[b]Bacteria only.
[c]Amount available for microbial growth of annual input of 70 g/m^2.
[d]Fungi only.
[e]Assuming fungal biomass = 2 × bacterial biomass.

soil are subject to error. It is not reasonable, for example, that maintenance energy requirements should consume all of the substrate available (or more!) without allowing for any growth (Table I). This could result from estimates of Y or S being too low, or from those of x or a being too high. For example, in the study of Clarholm and Rosswall (1980), the estimated number of generations is given in Table I as <1. The authors gave no estimate of a, and our calculations used $a = 0.001$, which is an order of magnitude less than many estimates in chemostats. Actual specific maintenance might have been still lower. Direct counts of bacterial production revealed that the population approximately doubled after each of 12 seasonal rain events. Assuming that the fungi contributed equally to soil respiration and $Y = 0.50$, the biomass produced by the 12 generations (220 g C/m^2) would have consumed all of the substrate available (220 g C/m^2).

In the work of Lynch and Panting (1980), the annual number of generations was also calculated to be <1. Of the total 3540 kg C substrate input, 2800 kg C/ha was straw; this figure was used in the calculations, but straw is normally burned and thus was not available to support the microbial population. Omitting the substrate contributed by straw would result in an even lower estimate of the number of generations.

Soil microbial biomass could be overestimated when direct counts of bacterial cells are made and lengths of fungal hyphae are measured microscopically (Babiuk and Paul, 1970; Gray and Williams, 1971; Shields *et al.*, 1973; Flanagan and Bunnell, 1976; Flanagan and Van Cleve, 1977), because some proportion of dead cells is probably included. In some studies (Behera and Wagner, 1974; Gray *et al.*, 1974), only soil bacterial or fungal biomass was included. Including the other component would decrease the number of generations. For example, Gray *et al.* (1974) estimated 87 bacterial generations per year; assuming that the fungal biomass was twice the bacterial biomass—a conservative estimate—the number of generations would be reduced to 20 (Table I). If the ratio of fungal to bacterial biomass is taken to be 5:1, the number of generations would be 3.6 per year.

Clark and Paul (1970) looked at the problem in more general terms. Assuming a yield coefficient of 0.7 (higher than any reported), each gram of cell dry weight synthesized would require 1.4 g glucose. Thus, 20 g microbial biomass/m^2 would require 280 g glucose, or ca 1100 kcal energy, to produce a new generation of microbial cells. Since net annual production rarely exceeds 5000 kcal/m^2, reproduction could only occur a few times per year—not taking account of maintanance energy requirements.

Conclusions corroborating the above have been derived from a comparison of respiratory data from soil and laboratory (Clark, 1967; Clark and Paul, 1970). In stationary populations of bacterial cells, CO_2 production per day is nearly equivalent to cell dry weight (Clark and Paul, 1970). However, in field soils a microbial biomass of 200 g/m^2 is 35–70 times the weight of CO_2 pro-

duced daily; moreover, the weight of CO_2 produced also includes that respired by fauna and roots, which may amount to 40% of the total soil respiration (Kucera and Kirkham, 1971). Therefore, growth and activity must be severely limited in soil. Similar conclusions were drawn by Gray and Williams (1971) in comparing soil biomass, CO_2 evolution in the field, and hypothetical growth rates in soil. Clarholm and Rosswall (1980) reported that fungi respired 110 g C/m^2 per year in a forest soil. Assuming a yield coefficient of 0.5, the bacterial production of 105 g C/m^2 per year would require 105 g C/m^2 per year. Thus, combined bacterial and fungal respiration was equal to 215 g C/m^2 per year, accounting for all of the above- and belowground litter production, which amounted to about 220 g C/m^2 per year.

In spite of the errors inherent in estimations of the data required for calculations such as those cited in Table I, it seems clear that the soil must be viewed as an impoverished medium insofar as carbon substrate is concerned. Inevitably then, a large proportion of the microbial population of the bulk soil must for the most part be confined to a state of enforced quiescence. Brief bursts of activity may occur in response to inputs of plant and animal litter (such as decomposing roots or leaf fall) or following physical disturbances (including wetting and drying, freezing and thawing, and addition of water) that kill microorganisms or cause redistribution of nutrients isolated from microbial contact with microsites. It may be argued that humic materials provide sources of energy for microbial growth (Griffin and Roth, 1979), but this seems unlikely in view of the average residence time of humus of over 1000 years (Barber and Lynch, 1977) and the fact that humus itself is ultimately derived from plant litter.

2.1.1. Root Exudates

Of the loci capable of supporting microbial growth in soil, probably that which offers the best opportunity for sustained growth of microorganisms is the rhizosphere, since it is here that energy sources are available more or less continuously for the life of the root. It is well established that roots release into soil a wide range of sugars, amino acids, proteins, organic acids, and other compounds which may be used by microorganisms. The rhizosphere is of particular significance to root-infecting fungi, since root exudates provide the nutrients which stimulate the fungal propagules to germinate and to grow toward the root. The exudates also provide the energy required for infection and may attract zoospores of Oomycetous fungi to the root (Schroth and Hildebrand, 1964). The rhizosphere has been the object of much study, and readers are referred to reviews for general information (Rovira and Davey, 1974; Bowen and Rovira, 1976; Hale *et al.*, 1978; Bowen, 1979). However, recent

information on sites of exudation and on the quantities of photosynthate released from roots seem pertinent to this review.

Sites of exudation have been identified using plants grown with $^{14}CO_2$. Methods of detection include localizing the radioactivity of the roots by autoradiography, by collection of exudate on filter papers and scanning for radioactivity, and by hot ethanol extraction of roots cut into segments followed by counting the radioactivity by scintillation. Such studies have revealed, for wheat seedling roots, a very early (first 2 min) intense locus of exudation from the apices of emerging lateral roots (McDougall and Rovira, 1970). This represented about half of the exudate released during a 2-hr period. The discreteness of the spots in autoradiograms suggested that the labeled material is nondiffusible, and that it may consist of root cap cells and mucilaginous material. Later, a more diffuse zone of radioactivity appeared along the axis of the primary root and to a lesser extent along the axes of the lateral roots. These were thought to represent lower-molecular-weight diffusible substances. The area of major release was in the zone of elongation distal to the root tip.

Sloughed cells and tissues apparently constitute a major portion of the organic carbon and nitrogen in the rhizosphere. Of the carbon released from wheat roots into the soil, insoluble mucilaginous material including sloughed root cap cells accounted for about four times as much as water-soluble exudate (Bowen and Rovira, 1973; Martin, 1977a). Griffin *et al.* (1976) have attempted to determine directly the amount of sloughed cells and tissues released from the roots of solution-grown peanut seedlings. After 2–4 weeks, root caps, cortical tissue fragments and sheets, and individual cells were seen microscopically. Sloughed material ranged from 0.26 to 0.73 mg/plant per week and ca 1.5 mg/g root dry weight per week. Thus, about 0.15% of the root tissue was sloughed per week.

Total materials lost from roots, including exudates, lysates, mucigel, and cellular material, may constitute a surprisingly large amount of the carbon fixed. Martin (1975) grew wheat, clover, and ryegrass plants in nonsterile soil in an atmosphere continuously supplied with $^{14}CO_2$. The amount of ^{14}C lost from the roots after 8 weeks was 3.1–5.8% of the total ^{14}C fixed and 10.4–38.4% of that translocated to the roots. Roots of wheat and barley plants grown under nonsterile conditions released about twice as much material as did roots grown axenically for 3 weeks (Barber and Martin, 1976). Roots of wheat and barley grown in nonsterile soil released 18–21% and 25%, respectively, of the total dry matter production. Corresponding values as proportions of root dry matter production were 48–63% for wheat and 74% for barley. Most of the lost material was in the insoluble fraction. To account for the greater ^{14}C loss from roots grown in nonsterile soil, Martin (1977a,b) hypothesized that soil microorganisms induced autolysis of cortical root cells, and that this accounted for release of sugars and amino acids from the roots. The roots apparently were