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CENOCOCCUM GRANIFORME -- ITS DISTRIBUTION, ECOLOGY, MYCORRHIZA FORMATION, AND INHERENT VARIATION

by

JAMES MARTIN TRAPPE

A thesis submitted in partial fulfillment

of the requirements for the degree of

DOCTOR OF PHILOSOPHY

UNIVERSITY OF WASHINGTON

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Approved by _____ (Chairman of Supervisory Committee)

Department _____ (Department sponsoring candidate)

Date _____

UNIVERSITY OF WASHINGTON

Date: May 3, 1962

We have carefully read the thesis entitled "CENOCOCCUM GRANIFORME - ITS DISTRIBUTION, ECOLOGY, MICORRHIZA FORMATION AND INHERENT VARIATION" submitted by JAMES MARTIN TRAPPE in partial fulfillment of the requirements of the degree of Doctor of Philosophy and recommend its acceptance. In support of this recommendation we present the following joint statement of evaluation to be filed with the thesis.

The monographic treatment of Cenococcum graniforme in this thesis is unique in the literature and will be a major source of information for many years to come. The literature review is complete and comprehensive from a world standpoint. The thesis presents original data on many aspects of the life and biological function of the species studied.

In the course of the investigation Mr. Trappe perfected cultural techniques for isolating and growing Cenoccum in pure culture, techniques for growing the fungus in aseptic association with forest trees and following mycorrhizal development. These techniques will set patterns of future studies in the field of mycorrhizal studies.

The course of mycorrhizal infection of forest trees was carefully studied and good photographic detail is presented.

Many excellent photographs of Cenococcum growth stages are included. The evidence for the effect on host by Cenococcum is carefully reviewed and summarized with additions by the author. The complete listing of areas in which Cenococcum has been found, as well as all new areas included in this study will be of major reference value. The inclusion of a list of fungi species serving as ectotrophic mycorrhizal hosts is also of value.

The section on phenotypic variation and genotypic differences in Cenococcum is excellent. Results from the controlled environment studies account for the ability of Cenococcum to live in a wide range of habitats throughout the world and demonstrate the adaptability of the species through genotypic plasticity.

THESIS READING COMMITTEE:

We feel the author has made a major contribution to mycorrhizal fungi studies and that the thesis will be an important future reference.

In presenting this thesis in partial fulfillment of the requirements for an advanced degree at the University of Washington I agree that the Library shall make it freely available for inspection. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by my major professor, or, in his absence, by the Director of Libraries. It is understood that any copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Signature James M Skaggs

Date May 18, 1962

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ACKNOWLEDGEMENTS

No research project is conceived in a vacuum. This reassures me about my mental faculties; but, more important, it emphasizes the influence of ideas and approaches of others on the studies described in this thesis. Foremost among those who advised and consented were Professors S. P. Gessel, D. R. M. Scott, D. E. Stuntz, and R. B. Walker of the University of Washington. By using large amounts of their freely given time and thought, I was able to get the background necessary to proceed. Dr. R. F. Smith of Oregon State University provided facilities for microtechnique. And many others contributed substantially of their ideas and abilities.

Particular mention is deserved by those who collected and sent samples of soil and roots for my examination. They contributed time and effort even though they had before heard neither of me nor my studies: Dr. B. K. Bakshi, Forest Research Institute, Dehra Dun, India; Professor Dr. H. Kayacik, Forestry Faculty, Istanbul University, Turkey; Mr. D. G. M. Donald, Institute for Forestry and Wood Technology, University of Stellenbosch, South Africa; Dr. S. Y. Shin, Institute of Forest Genetics, Suwon, Korea; and a large number of employees, many anonymous, of the U. S. Forest Service throughout the country.

From the standpoint of financing, the University of Washington, College of Forestry, provided fellowships for my residence work. The Foundation for American Resource Management supplied funds for microscopy equipment, and the Pacific Northwest Forest and Range Experiment Station, U. S. Forest Service, not only furnished laboratory space and equipment, a growth chamber, means of travel, and the secretarial services of Miss McPhail and Mrs. Bailey, but even paid me a salary

to do the research!

Last but by no means least are the moral support, good meals, and typing given unstintingly by Esther Trappe, who by now is one of the great authorities on Cenococcum.

INTRODUCTION

Mycorrhizae are necessary for survival and good growth of ectotrophic host trees under most field conditions. Yet virtually nothing is known about the fate of mycorrhizae on nursery stock during and after outplanting. To insure best survival and rapid development of outplanted seedlings, their mycorrhizae theoretically should function immediately after planting. The mycorrhizal fungi should vigorously grow out into the soil so that a pervading absorption system is established before the onset of summer drought.

In the Pacific Northwest as in much of North America, tree seedlings are grown in a relatively few centralized nurseries for outplanting in a gamut of sites ranging from warm and humid to cold and wet to hot and dry. The seedlings, nurtured in fertilized and irrigated soils, are expected to survive when planted on till, pumice, serpentine, old fields, and any variety of other soils and situations. Seed sources suitable to outplanting sites are recognized as necessary for survival and subsequent development of trees. But little information has been developed on whether their mycorrhizal fungi are equipped to survive when transplanted from their native nursery environment to a radically different one.

The studies reported in this paper were undertaken as one step in clarifying the role of mycorrhizae in success or failure of tree outplanting. Their primary objective was to examine features of inherent variation of *in vitro* morphology, temperature response, and adaptability of selected provenances of a common mycorrhizal fungus,

Cenococcum graniforme (Sow.) Ferd. & Winge[1/]. To provide a satisfactory foundation on which to build and interpret experiments, the taxonomy, ecology, and distribution of the fungus were studied, with particular reference to the Pacific Northwest. Genetics per se of Cenococcum are not within the scope of this paper.

As is inevitably the case with studies of complex biological phenomena, more questions are raised than are answered. Results are intended to lay groundwork for additional research on the extent to which foresters must be concerned with proper provenance of fungi on nursery stock.

1/ Cenococcum being a monotypic form-genus, the species will be referred to simply as "Cenococcum" in the rest of this paper.

CENOCOCCUM AS A STUDY OBJECT

Cenococcum offers several advantages for a study of a mycorrhizal fungus. It is ubiquitous in natural forest areas of the Pacific Northwest and is easy to identify. Isolates can be obtained from sclerotia plucked from soil at any time of year. Considerable knowledge on its physiology and ecology has been developed, so that little effort was needed to devise culture methods, and its habits in the Northwest could be related to those in other areas.

Cenococcum does have disadvantages as an object of a study of this type. First, its taxonomic position is in question. Known only as a sterile mycelium, its relation to other mycorrhizal fungi is hard to assess. However, its behavior in mycorrhiza formation lies generally within the realm of variation shown by the mycorrhizal fungi as a group. Second, the mechanics of Cenococcum's inheritance are completely unknown. Because we are studying expressed genotypes rather than genetics, however, this offers no major problem.

TAXONOMY AND DISTRIBUTION OF CENOCOCCUM

NOMENCLATURE

The establishment of Cenococcum as a new genus by Fries (Ferdinandsen & Winge, 1925) seems more an accident of history than a logical separation in view of its close affinity to the genus Sclerotium (Ferdinandsen & Winge, 1925; Buchwald, 1939). But the taxonomy of Mycelia Sterilia, to which both Cenococcum and Sclerotium belong, presents an impossible situation in any event, so little can be gained by belaboring the issue.

Cenococcum was first isolated by Hatch (1934) who, not knowing the identity of the organism, provisionally termed it Mycelium radicis nigrostrigosum. This name still appears occasionally in current publications, even though Lihnell (1939) conclusively identified the fungus as the Cenococcum of mycological literature.

Pachlewski (1953 & 1954) suggests that Rhizoctonia silvestris Melin is synonymous with Cenococcum. While R. silvestris hyphae somewhat resemble Cenococcum in pure culture, the former grows much faster and does not produce the typical structure of Cenococcum mycorrhizae in laboratory syntheses (Hatch, 1934). Further, R. silvestris, as befits a Rhizoctonia by definition, tends to encrust host roots with a mass of fused sclerotia (as illustrated by Doak, 1955, fig. 18). While Cenococcum sclerotia may sometimes be fused to a mycorrhiza mantle as individuals or small groups (fig. 1), a massive encrustation has not been reported. Lihnell (1942) observed a similarity betweeen the unusual arrangement of cells in Cenococcum mycorrhiza mantles (Melin, 1927; Hatch, 1934; and

Figure 1: Cenococcum sclerotia attached to root fragments (2 lower groups) and as grown in soil unattached to a root. x5.

others) and that of perithecial walls of the Ascomycete genus Cephalotheca Fuckel. But the structures differ between the two genera in development and anatomical detail. The thread of relationship is tenuous at best. Dominik (1958c) believes that there may be more than one species or at least more than one form of Cenococcum because of the great variety of hyphae that the fungus presents in nature. Indeed, several forms were separated by early mycologists (Ferdinandsen & Winge, 1925). However, as revealed by Mikola (1948b) and later in this study, a pure culture of a single isolate may manifest all forms found in nature.

Despite more than a century and a half of study, no one has discovered a perfect stage of Cenococcum. Ferdinandsen and Winge (1925, p. 380) considered it "altogether improbable that Cenococcum graniforme should form any kind of spores or conidia." Thus there appears little hope that its phylogenetic position will be clarified.

Although not strictly related to taxonomic nomenclature, it seems useful at this point to mention special terminology used to designate mycorrhizae formed by Cenococcum. In his original broad, morphological classification of mycorrhizae, Melin (1927) termed

dark-brown to black individuals "Mycorrhiza D." Björkman (1937) subdivided Melin's type D, terming Cenococcum mycorrhizae "Dn" to separate them from other dark-colored mycorrhizae. Recently Dominik (1959) published a new system of morphological classification of mycorrhizae. This system, much more detailed than previous ones, designates mycorrhizae formed by Cenococcum alone as "Ga" and those in which Cenococcum forms a secondary mantle over another fungus as "Kb". In the literature, designations Dn, Ga, and Kb all denote participation by Cenococcum in mycorrhiza formation, whether or not the name of the fungus is specifically mentioned.

MORPHOLOGY

Although Cenococcum is not known to form a perfect stage and although there are myriad other species of dark fungi, identification of Cenococcum is rather straightforward. Referring to reports of fossil sclerotia of Cenococcum, Ferdinandsen and Winge (1925, P. 357) state, "The fossil Cenococcum is so characteristic that the records of its appearance in extant palaeontological literature may doubtless be taken as correct in all cases; it is hardly possible to make any mistake here." Hyphae, too, are readily identifiable when found in association with plant roots.

Although Cenococcum has been frequently described in mycological and mycorrhiza literature, the complete range of variation of hyphae and occurrence of chlamydospores has not been included in

any one description. Consequently, a redescription seems useful here[2/]:

Hyphae without clamps, septate, monokaryon, yellowish when young but soon turning brownish black to black. Under compound magnification they vary from nearly hyaline to nearly opaque purplish black, most a light burgundy to deep purple. Hyphae are straight to sinuate, often branching and occasionally fusing, often aggregating into short, loosely-organized strands but never forming true rhizomorphs. Hyphal diameters 1.5 to 8.2μ, mostly 3.5 to 6.5μ, the larger usually light colored new growth. Cells are cylindrical, 2.5 to 132μ long, mostly 30 to 70μ. Cell walls 0.2μ thick on young hyphae to 0.7μ on old. Cell surfaces bare to enclosed in a gelatinous to dry sheath which appears like a second wall up to 0.7μ thick and which often breaks up into bands, papillae, or granulations. Chlamydospores infrequent, usually intercalary or rarely terminal on setose hyphae, elliptical to globose to pyriform, 8 to 12.8 x 6 to 8μ. Walls 0.7μ thick at maturity.

Sclerotia spherical to irregularly lobed, the larger with a single navel-like hollow. Surface shiny-smooth and black to dull-roughened and brownish black. Outgrowing hyphae sparse to abundant, often absent on old or dead specimens. Diameters of sclerotia range from 0.05mm. to 7.0, most being 0.5mm. to 3.0. Interiors firm and solid at youth, black and moist from exuded oil when cut. Old specimens hollow, the cavity containing dark, granulated hyphae; hard and brittle, shattering when cut, the inner tissue dry and granular reddish-brown. Sclerotia composed of pseudoparenchyma, the inner cells polygonal, with diameters 3.2 to 16.3μ, mostly 5 to 12μ. Cell walls thin when young, soon darkening and thickening. Cells with pores, each pore surrounded by a detachable platelet that appears like a tiny spore when fallen off. Outer 2 to 6 layers of cells oblong rectangular, 4 to 8μ by 8 to 15μ, with hard, dark walls and filled with a homogeneous dark material. These elongate surface cells on young specimens are clustered in groups aligned alternately radially and tangentially.

2/ This redescription includes data both from published descriptions and from new observations of western North American material. These sources are discussed in detail following the redescription.

Hyphae

Usually the whole range of hyphal variation is found in a single collection, although predominance of particular forms may differ from one area to another. Hyphal colors have been variously reported as "dark brown" (Melin, 1927), "brownish black" (Lihnell, 1939), "violet-brown" (Aleskovsky, 1954), or as is more correctly the case, "light yellow to bronze to dark violet" (Pachlewski, 1953). Ferdinandsen & Winge (1925) consider darkening of color to be a matter of ageing. It is true that growing hyphal tips are hyaline to light yellow, while old cells are darker. But in my collections, the color on older hyphae sometimes switches abruptly from light bronze older cells to deep burgundy or purple younger cells (fig.2). Some collections have mostly light bronze to burgundy hyphae, with only a few darker. Others are mostly dark.

The extremes in size reported in the redescription above are from my examination of North American material. Both extremes occurred in hyaline to light bronze hyphae. Hyphal tips occasionally taper down to 1μ, but usually are blunter. Predominant hyphal cell dimensions differ from one collection to another--diameters have been reported 2 to 3μ (Melin, 1927), 3.5 to 5.2μ (Lihnell, 1939; Pachlewski & Pachlewska, 1960), 4 to 4.3μ (Aleskovsky, 1954), 4 to 5μ (Ferreira dos Santos, 1951), 4.5 to 6.0μ (Pachlewski & Pachlewska, 1960), 4.6 to 7.8μ (Masui, 1926).

The overall impression of a group of *Cenococcum* hyphae is one of stiffness and straightness. But close examination reveals many lengths of hyphae to be sinuate, sometimes strongly so. It is

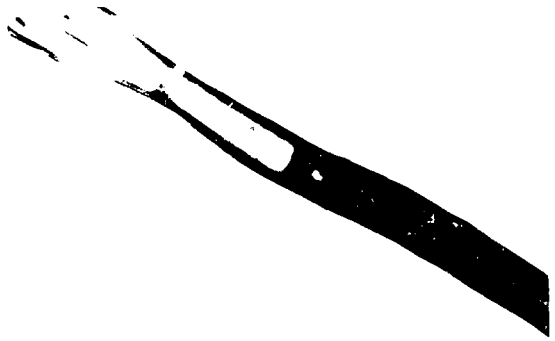


Figure 2: Abrupt color change of a Cenococcum hypha. Light colored cell is older than the dark one. x720.

tempting to suggest that growth around small soil grains accounts for most of the sinuosity. This is indeed true to a limited extent, but Cenococcum exhibits singular ability to force a straight hypha through a soil mass that to all appearances would prevent any straightness whatever. I have been unable to see any consistent difference in proportion of sinuate to straight hyphae between collections from coarse sandy soil and collections from silty clays.

The three forms of hyphal fusion described by Buller (1933)--excluding the fourth, clamp connections--occur between Cenococcum hyphae. Most common are peg-to-peg fusions, but hypha-to-peg and hypha-to-hypha may sometimes be found (fig. 3).

The gelatinous to dry sheathing of hyphae apparently originates as an exudation through cell walls. It may be found on hyphae of any size, color, or age. As with hyphal size and color, the predominant form assumed by hyphal sheaths varies between collections. When initially formed, the sheath appears as a hyaline gelatinous pellicle enveloping a hypha (fig. 4). If the hyphal cells are actively enlarging, the pellicle

Figure 3: Fusions between Cenococcum hyphae. Left, peg-to-peg (field collection); center, hypha-to-peg (in substrate of pure culture--note gelatinous exudate around hyphae); right, hypha-to-hypha. x1340.

is separated into regular to irregular papillae (fig. 5 & 6) which sometimes harden into surface granulations. Papillation is a result of cell growth rather than a micro-organismal decomposition of the pellicle, as attested by its common occurrence in pure cultures of Cenococcum. If hyphal cells do not enlarge at time of exudation, the pellicle hardens into a dark-colored, rigid encasement about the hypha, a feature that hypothetically could be useful for drought resistance. If hyphal cells elongate after the pellicle hardens, it ruptures circumferentially. Sometimes it then seems to slide along the elongating cell, leaving two separated halves. At other times it seems to adhere to the cell wall, breaking into a series of narrow concentric bands (fig. 7). Cells sometimes grow both diametrically and longitudinally, in which case the dried sheath breaks into neat, rectangular granulations.

Hyphae frequently aggregate into loose strands of a few to forty or fifty hyphae (fgs. 8 and 9), particularly near growth centers such as vigorous mycorrhizae. This seems a matter of chance grouping.

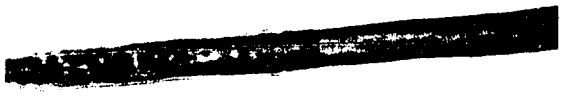


Figure 4: Cenococcum hypha with gelatinous pellicle overlying dark cell walls. x720.

Figure 5: Papillae on Cenococcum hyphae, showing plane view on horizontal hypha and cross-sectional view on vertical hypha. x720.

Figure 6: Cenococcum hypha with gelatinous pellicle grading into papillae. x1340.

Figure 7: Cenococcum hypha with pellicle broken into a series of concentric bands. x1340.

When these strands are dry, the adjoining hyphae cohere tightly. But if a dry strand is moistened, hyphae readily separate. The gelatinous sheaths evidently serve as adhesive (fig. 10). Only rarely do these structures exceed a centimeter in length before individual hyphae diverge. In the North American material examined no true rhizomorphs such as described by Siren and Bergman (1951) and Levisohn (1955) have been found. Their absence has been specifically noted by several European observers (Ferdinandsen & Winge, 1925; Pachlewski, 1953; Aleskovsky, 1954; Fritsch, 1956).

Chlamydospores

Chlamydospores, though common in pure cultures of Cenococcum, have been reported only once on a field collection (Dominik & Pachlewski, 1956). These were described as terminal chlamydospores, 2-celled, ovate-pyriform, arising on dark setose hyphae with swollen bases. Although Dominik and Pachlewski equated these specialized cells with those reported

Figure 8: Loose strand of Cenococcum hyphae near mycorrhiza tip. x150.

Figure 9: Strand of Cenococcum hyphae in pure culture. x150.

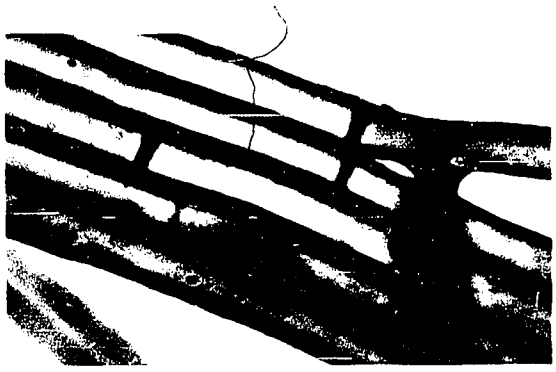


Figure 10: Strand of Cenococcum hyphae in pure culture. Gelatinous pellicles of adjacent hyphae fuse, gluing hyphae together when dried. x1340.

in pure cultures by Mikola (1948b), their description suggests conidia and conidiophores such as found in the Moniliales. Unfortunately they were unable to preserve the material for detailed study.

No such growth has been found in the North American material, but intercalary chlamydospores occur occasionally (fig. 11). These usually occur on dark hyphae, but sometimes on young, light-colored hyphae as well. Very often hyphae terminate in a trumpet shape suggestive of a chlamydospore base, but whether or not these are indeed points of chlamydospore detachment has not been established.

The power of broken hyphae to regenerate, characteristic of Cenococcum, is particularly noticeable on hyphae kept damp and examined several days after being broken. A new hyphal tip, usually hyaline, grows from the first septum behind the break, emerging with the same diameter as the inside of the broken cell (fig. 12) but soon (within a few microns as a rule) enlarging. Regrowth of this type is common on old, thick-walled hyphae.

Sclerotia

Sclerotium formation begins with one cell or a few adjacent cells on a hypha growing several lateral branches which rapidly become septate. Once a cell is walled off, it swells and produces additional branches (fig. 13), each forming more swelling cells. This continues until the sclerotium is fully formed and compact, the press of the mass of cells one on another resulting in the polygonal shape to which the walls finally harden (fig. 14). Surface cells of young specimens are elongate and irregularly arranged into clusters aligned alternately

Figure 11: Naturally occurring chlamydospore on Cenococcum hypha. x1340.

tangentially or radially. The result is a mosaic of radiate growths (fig. 15) similar to that of mycorrhizal mantles formed by Cenococcum (Melin, 1927; Hatch, 1934; Lihnell, 1939).

Several sclerotia may initiate close to each other on the same or on adjacent hyphae. As they enlarge to the point of contact, they fuse together; double or triple fusions are common where sclerotium formation is especially active (fig. 1). At the plane of contact several layers of dark elongate cells, similar to those on the outer surfaces, are laid down, separating the central polygonal cells of one from the other.

Live sclerotia have a very high content of ethanol-soluble oil, up to 35 percent of total dry weight. Sclerotia filled with this oil sink in water once all air bubbles have been removed from their outer cells. The dull-sheened to very shiny appearance of most young sclerotia is likely due to a high oil content of the outer cells. Live sclerotia are very firm but can be sliced readily with a sharp blade. When sliced in the air, their center has a moist, black appearance from oil exudation. If sliced under water, the cut surface appears

Figure 12: Regrowth from intine of broken Cenococcum hypha. x720.

milky blue from the emulsion formed by exuded oil with water.

If sclerotia rest for some time before germinating, the surface roughens and the sheen disappears, probably a result of the death of peripheral layers of cells. This may extend inward as much as a third of the sclerotial diameter, leaving only the center third alive. In this case the weathered portion peels off as a husk. A few specimens were found from which part of this "husk" had sloughed off; new hyphae were actively growing from the inner portion. Germination is accompanied by breakdown of the center tissue of a sclerotium, leaving it hollow.

Sclerotia that die without germinating remain solid but become very dry, hard, and brittle. As aptly described by Ferdinandsen & Winge (1925, p. 372), they "burst like bits of coal" when punched by a needle. Dead sclerotia can persist intact for several years in soil, hence their inclusion in the foregoing redescription of Cenococcum.

As yet, I have not found a North American sclerotium larger than 4 mm. in diameter (fig. 16), although specimens up to 7 mm. have been found in northern Europe (Ferdinandsen & Winge, 1925; Kreisel, 1957a). Not reported heretofore are the tiny (as small as 0.0[illegible] mm.

Figure 13: Initial cells of a Cenococcum sclerotium. Hyphal cells have rounded and are beginning to produce branches. x1340.

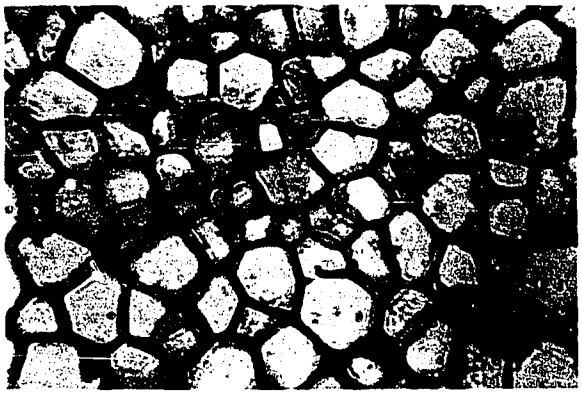


Figure 14: Interior cells of a mature Cenococcum sclerotium. The press of original rounded cells one on another result in polygonal cell shape. x600.

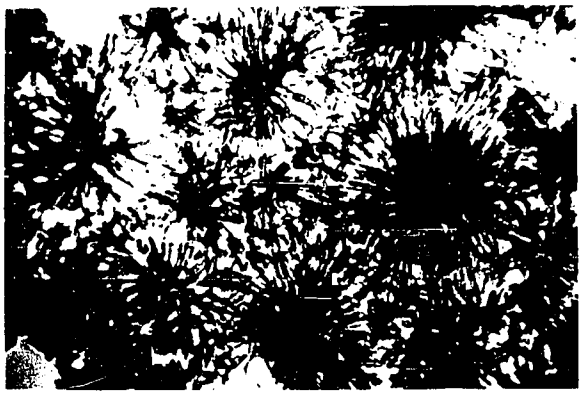


Figure 15: Arangement of cells on surface of young Cenococcum sclerotium (tangential section). x330.

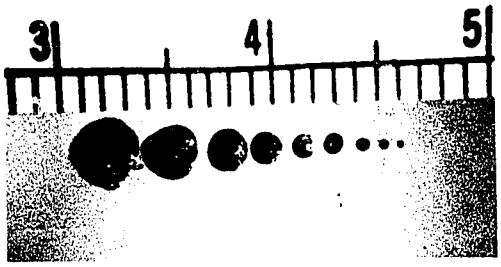


Figure 16: Mature Cenococcum sclerotia collected from soil in the Pacific Northwest (scale in millimeters). x2½.

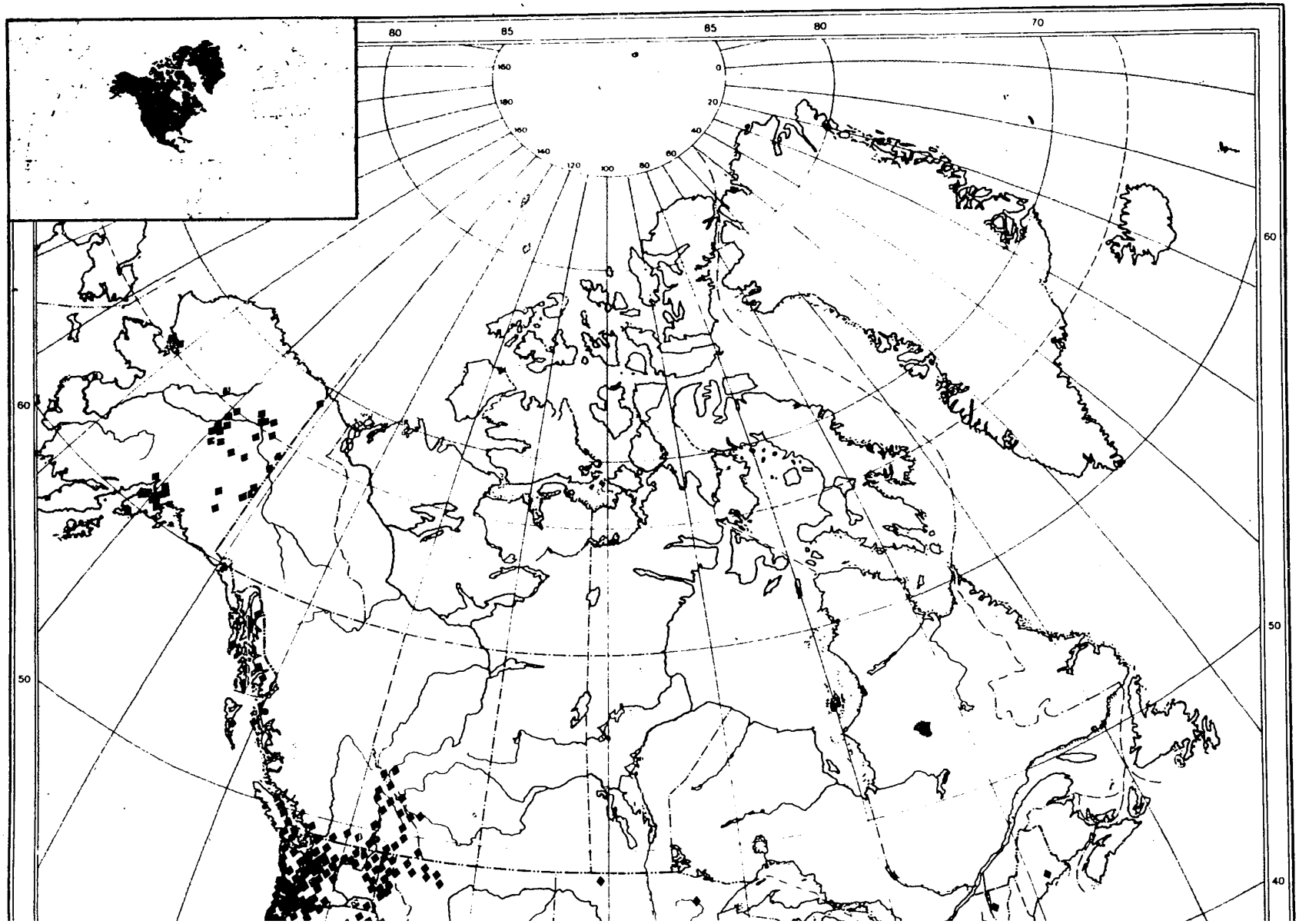
in diameter), mature sclerotia that are really rather common. They often may be found as tiny black specks floating on the surface of a slurry of Cenococcum-containing soil. Occasionally they are nested among hyphae growing from a mycorrhiza mantle.

DISTRIBUTION

Cenococcum is circumpolar in the northern hemisphere and occurs in the tropics and in South Africa as well (figs. 17 and 18). Field studies plus examinations of soil-and-root samples provided by cooperators from Korea, India, Turkey, South Africa, and many parts of North America have greatly extended its range beyond that known from the literature. Seventy-four representatives of several hundred new locations are listed in appendix I together with literature citations for previous reports.

As to its northern extension, Cenococcum was found in several samples from above the Arctic Circle in Alaska. The northernmost

Figure 17: New and previously reported locations of Cenococcum in North America.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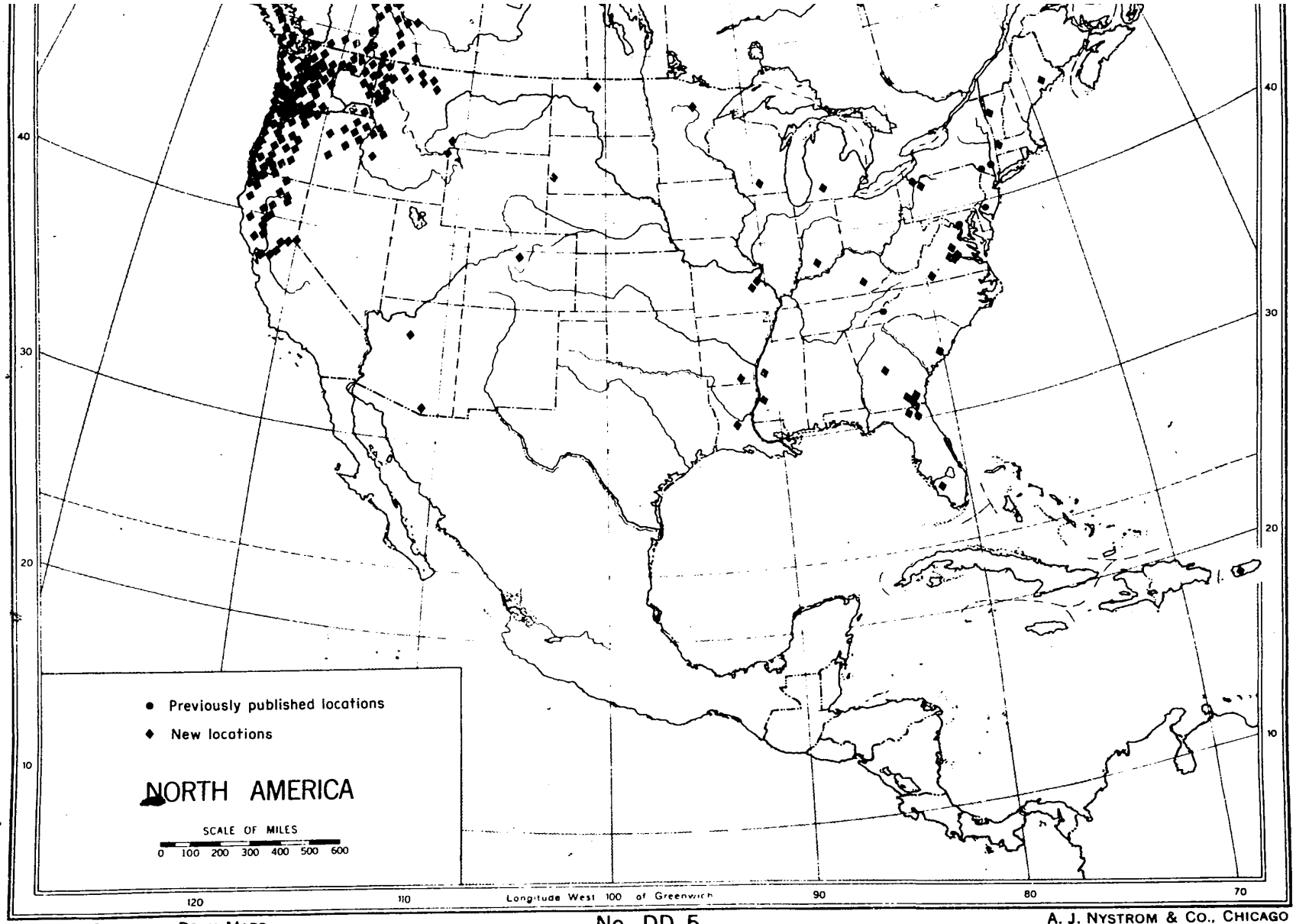

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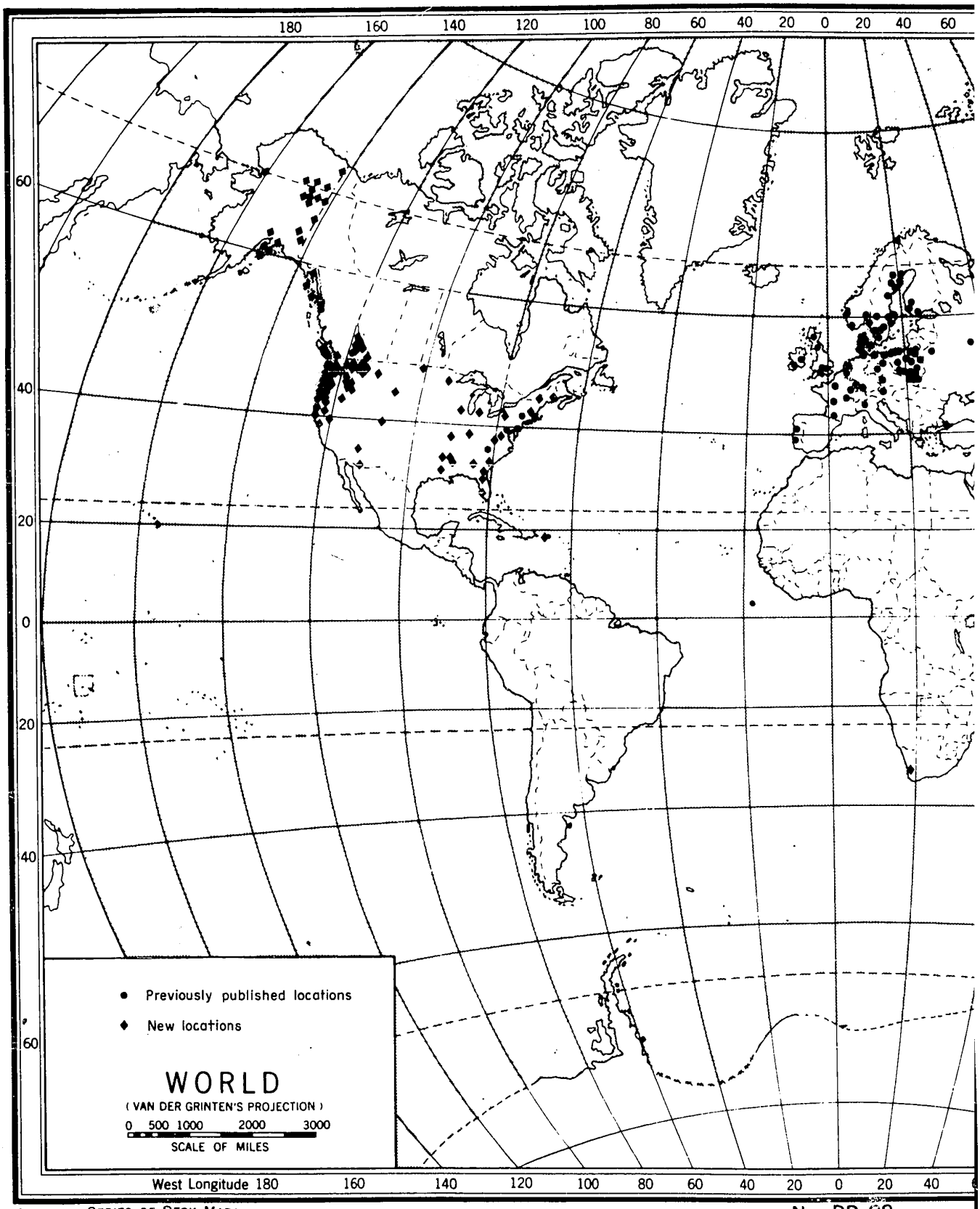
Figure 18: New and previously reported locations of Cenococcum in the world.



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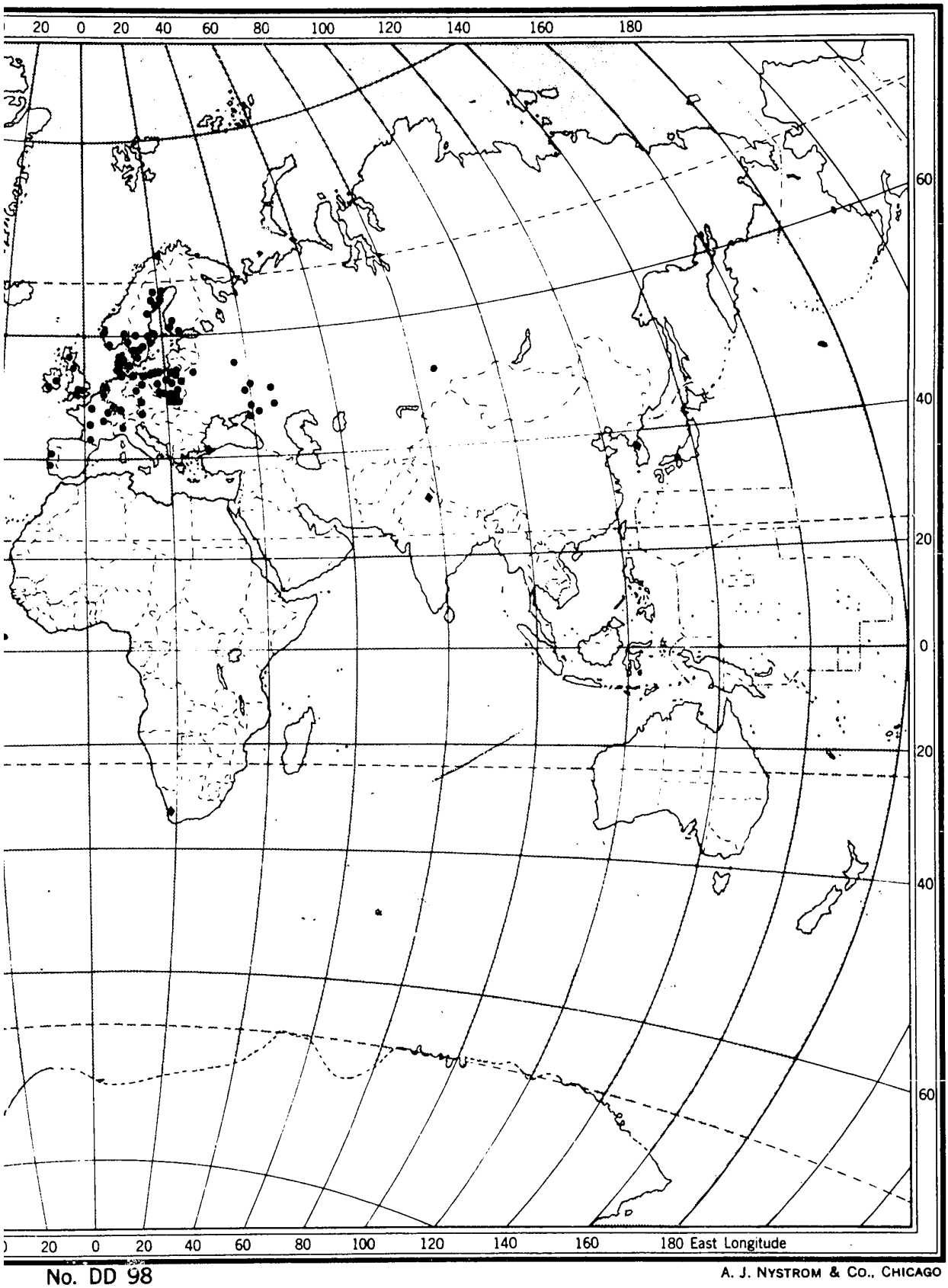

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sample came from the Firth River at 68°45' N. lat. Ferdinandsen & Winge (1925) found it at Tromsö, Norway at 69°39' N. lat. Its great abundance in my collections from the Firth River, from tundra at Kotzebue Sound, and from high elevations in the Cascade Mountains indicate its adaptability to the arctic-alpine environment. In all likelihood it is common throughout the Arctic where suitable host plants grow.

Heretofore there have been no reports of Cenococcum South of 35° N. lat. But samples from the southern United States revealed it to abound as far as 26° N. lat. in subtropical Florida. Moreover, it was found thriving in samples from 18° N. lat. in Puerto Rico, where it was probably introduced with humus imported from North Carolina in 1955. Probably it occurs within the tropics in the mountain ranges of Mexico as well.

The first discovery of Cenococcum in the southern hemisphere came from samples from Stellenbosch, South Africa, at 33°50' S. lat. These samples showed a great abundance of Cenococcum, whether native or exotic being unknown. It has not been encountered in Australia or New Zealand by researchers working with mycorrhizae[3/] but would probably thrive there if introduced with suitable hosts. Apparently no one has looked for it in South America as yet, so its presence there is problematical.

From Cenococcum's known distribution and from our knowledge of its habits (discussed in detail under "autecology"), it is safe to hypothesize that Cenococcum eventually will be found in most areas

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3/ Correspondence from G. B. Rawlings, G. D. Wade, B. C. Arnold, and M. R. Jacobs.

of the northern hemisphere that support its hosts. Information from the southern hemisphere is too scanty as yet to give any basis for generalization.

ISOLATION AND IN VITRO MORPHOLOGY OF CENOCOCCUM

TECHNIQUES

Cenococcum has been isolated from mycorrhizae, mycelial strands, and sclerotia. Attempts to isolate it from mycorrhizae, however, generally yield a low proportion of success in the experience of Hatch (1934), Mikola (1948a), and myself. Suitable mycelial strands as described by Levisohn (1955) are not easily found. But sclerotia are readily available and lend themselves well to surface sterilization.

To obtain sclerotia conveniently, small soil samples from under ectotrophic host trees are moistened to form a slurry, which is examined under a low-power stereomicroscope. With a little sloshing and breaking up of aggregates in the slurry, sclerotia can be easily detected, plucked out with tweezers, and transferred to a dish of water. They are then carefully cleaned of surface soil with a fine brush, the ones that float being discarded as probably non-viable.

After washing, they are ready for surface sterilization. The apparatus and technique devised by Slankis (1958) for surface sterilization of root tips serve admirably for this purpose (fig. 19). Tissue of healthy Cenococcum sclerotia is very compact and only slowly pervious. Consequently, a quite rigorous application of sterilant

Figure 19: The Slankis apparatus used for surface sterilization of Cenococcum sclerotia. A sclerotium is being inserted through an entry port onto a gauze-bottomed platform. The platform will then be turned upright and sterilant followed by a sterile water rinse will be dripped through. Photo by Wallace Guy.

can be applied. To obtain the Oregon isolates[4/], 0.1 percent mercuric chloride was dripped on sclerotia through the Slankis apparatus for

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4/ "Oregon isolates" refers to those I obtained, to differentiate them from literature reports woven into the discussions in this paper.

50 to 110 seconds, then washed off by dripping sterile water for 2 minutes. Application of mercuric chloride for 40 seconds or less often did not kill all surface contaminants, especially when outer cell layers of a sclerotium were weathered.

Once surface sterilized, sclerotia were transferred to test tube slant cultures. The medium used, based on studies by Melin and Mikola (1948), was:

Distilled H_2O		1 l.
d-glucose		20 gm.
KH_2PO_4		1 gm.
$MgSO_4 \cdot 7H_2O$		0.5 gm.
Ferric ammonium citrate	(1 percent solution)	0.5 ml.
$ZnSO_4$	"	0.5 ml.
$MnSO_4 \cdot 4H_2O$	"	0.5 ml.
$CaCl_2$	"	5.0 ml.
$(NH_4)_2C_4H_4O_6$		0.3 gm.
Casein hydrolysate (13 percent N)		2.0 gm.
Thiamin		50 mgm.
Biotin		1.0 mgm.

This formula served for all studies on the Oregon isolates, either alone, thickened with 2 gms. of agar, or gelled with 15 gms. of agar. All media were autoclaved at 115 pounds steam pressure for 15 minutes.

GERMINATION OF SCLEROTIA AND SUBSEQUENT GROWTH

At least 20 days elapsed before the first hyphal tips emerged from sclerotia. Most began detectable growth within 30 days, but three sat on the agar for 75 days before showing any signs of growth. On all isolates the first hyphae emerged at the sclerotium-agar interface. These hyphae had hyaline tips, but older cells soon darkened. A few days later white hyphae emerged from the aerial side of the sclerotium, at first appearing to be contaminants because of their complete lack of color. After growth of several days, however, these, too, darkened. Initial growth of all cultures was generally slow--on some, 2 months were required to produce a colony large enough to subculture. By 4 or 5 months after germination, growth rates of most isolates had increased substantially and had stabilized.

Certain isolates produced sclerotia in pure culture, either regularly or sporadically. These were identical in all respects to those found in nature. Sclerotium formation usually occurred on hyphae at or below the surface of agar slants. It was also frequent in cultures in vermiculite moistened with nutrient solutions. But no sclerotia were produced by any isolates when growing in a liquid culture.

Furthermore, sclerotia never formed on young cultures. Initiation usually began more than a month after inoculation. Attempts to induce earlier sclerotium formation by varying amounts and combinations of agar, glucose, and thiamin all failed. These experiments were on a small scale and cannot be considered conclusive, but left the impression that initiation and formation of sclerotia in pure culture is controlled by fungus-produced biochemicals that were not supplied by the nutrient medium.

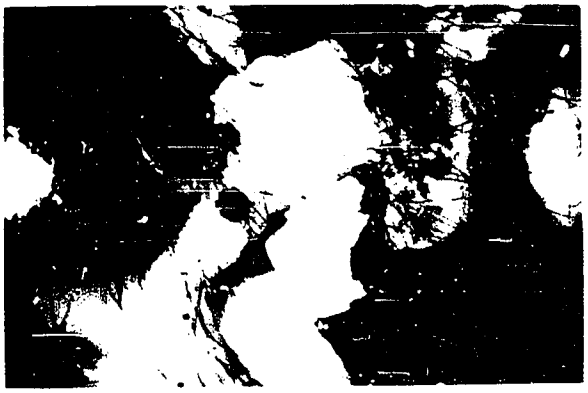


Figure 20: Hyphae and sclerotia of Cenococcum in vermiculite culture. These look identical to Cenococcum in situ. x10.

Only in vermiculite culture did hyphae look exactly like Cenococcum in nature, with very dark, stiff, thick-walled cells having sheaths, papillae, or granulations (fig. 20). Because the nutrient solution was the same in vermiculite as in agar, the physical structure of vermiculite (which more closely approaches that of soil than other substrates used) could possibly be a determining factor in "natural" hyphal development. Aeration and water molecules available to individual hyphae are undoubtedly important--even in liquid cultures the aerial hyphae more closely resemble natural Cenococcum than substrate hyphae do.

All of the Oregon isolates produced abundant chlamydospores, especially on substrate hyphae but occasionally on aerial hyphae as well. Chlamydospores varied in size and shape and were often produced at hyphal tips (fig. 21). But in general the terminal chlamydospores sprouted, and hyphal growth continued from them. Chlamydospores were also noted in pure cultures of Cenococcum by Hatch(1934), Lindquist (1939), and Mikola (1948b).

Substrate hyphae in liquid or agar cultures differed from natural hyphae primarily in color, the nature of the sheath, and production of chlamydospores. On the average, cell wall color is much less intense in

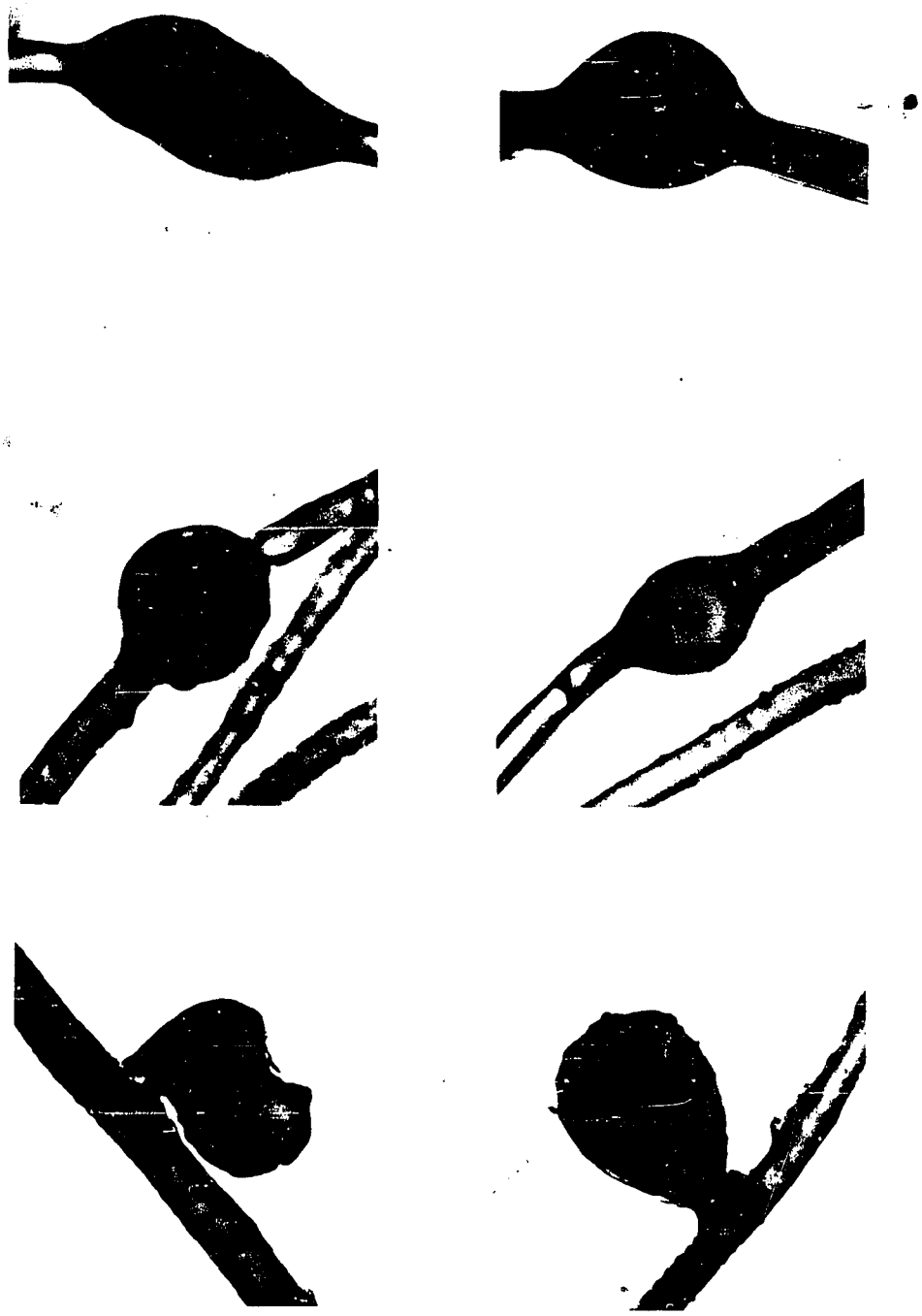


Figure 21: Various types of chlamydospores formed by Cenococcum in pure culture. x1340.

pure culture than in nature, especially on substrate hyphae. Aerial hyphae in pure culture form sheaths, papillae, and sometimes granulations. On substrate hyphae, in contrast, the exudate that normally forms these structures diffuses into the substrate. In agar this results in a dispersed film surrounding the hypha (fig. 3, center). In liquid culture it produces a cloud of the exudate, concentrated about the substrate mass of the colony.

Hyphae of the Oregon isolates in pure culture generally resembled descriptions by earlier authors (Hatch, 1934; Lihnell, 1939; Lindquist, 1939; Mikola, 1948b). But the gross morphology of colonies varied considerably between isolates, both in color and form. These differences will be discussed later in connection with the studies on inherent variation.

PHYSIOLOGY OF CENOCOCCUM IN VITRO

Many facets of Cenococcum's physiology have been studied in vitro, but the results are difficult to equate with its life history. Inherent variation within the species is pronounced, as demonstrated by Mikola (1948b) and by experiments reported later in this paper. Consequently, generalization from studies of one or a few isolates can be hazardous. Many other features of study in vitro, e.g., composition of the nutrient solution (Kurtz, 1958), may induce response by an isolate to an imposed variable that is quite different from response in situ.

The available data on Cenococcum's physiology in vitro are briefly presented here with little attempt at interpretation. Where possible, application of this information to the biology of Cenococcum is elaborated in the chapter on autecology. All mention of growth of Cenococcum in pure culture refers to dry weight increment; colony dimensions bear no consistant relation to weight in this species.

TEMPERATURE

Two isolates of Cenococcum examined by Mikola (1948b) grew best at 25°C. In 25 days, neither grew at 5°C. and only one showed slight growth at 30°C.

AERATION

Many isolates of Cenococcum can grow well submerged in liquid culture, although a definite strain differentiation occurs between different Oregon isolates. Some grow nearly as well submerged as on the surface, while others grow hardly at all when submerged. Several have grown well for 3 months in tightly capped culture bottles. These same cultures, after 8 months in the bottles, commenced rapid regrowth when subcultured.

There is no evidence to link ability of isolates to grow under limiting oxygen supply to their provenance. For example, 8 isolates were obtained from sclerotia plucked from a single shovelful of soil at the ponderosa pine-desert ecotone of eastern Oregon. This soil, a pumice, is well aerated and receives less than 15 inches of precipitation a year. One of the 8 isolates, PPh, barely grew when submerged while the other 7 grew well. Surface colonies of PPh, in contrast, far outdistanced the other 7 in growth.

MOISTURE

Compared to other mycorrhizal fungi, Cenococcum has a remarkable ability to retrain life in old, dry cultures. As cultures age, drying proceeds inexorably unless the container is sealed, the total supply of nutrients available for new growth diminishes, and the concentration of metabolic wastes increases. The 22 isolates from Oregon withstood these environmental handicaps for unusually long periods. All subcultures were saved for a good many months and allowed to air-dry. After varying periods, pieces of each air-dried subculture were inoculated on fresh medium;

moisture content was determined for the remainder. All of those reinoculated in less than 6 months regrew on new medium. Aging beyond 6 months resulted in increasing proportion of failure to regrow. Beyond 11 months and 10 days no reinoculants resumed growth. Tabulated below for several isolates that resumed growing are age and moisture content (percent of oven dry weight) of subcultures at time of reinoculation:

Isolate	Age (days)	Moisture Content (%)
LPb	193	3.1
LPc	193	3.5
MHb	193	6.5
MHc	193	8.7
PPa	193	2.6
PPd	193	3.2
LPa	224	8.4
LPd	224	3.5
LPd	224	3.2
MHa	224	6.2
PPc	224	6.5
MHe	275	5.2
MHc	287	3.8
MHc	345	6.0

Old subcultures, dry and brittle, shattered into fragments when inocula were extracted. Regrowth on fresh medium emerged usually from fragments larger than a millimeter in diameter, but occasionally from smaller pieces or even from individual hyphal fragments. Small sclerotia included in some reinoculations sprouted readily.

Cenococcum's unusual retention of life after extreme drying and the other changes attendant to aging of cultures is illustrated by comparison with other mycorrhizal fungi similarly treated. Neither Amanita pantherina (D.C. ex Fr.)Secr., Lactarius sanguifluus(Paulet ex)Fr., Rhizopogon sp., Boletinus lakei(Murr.)Singer, Suillus gramulatus(L. ex Fr.)O. Kuntze, nor Xerocomus zelleri(Murr.)Snell regrew when cultured on fresh media after the age of the preceeding subculture exceeded 3 months.

CARBOHYDRATES

Experiments with single isolates of Cenococcum have revealed utilization of a variety of carbohydrates. Hexose monosaccharides, especially glucose and mannose, and certain disaccharides--maltose, cellobiose, sucrose, and trehalose--permit good growth. No polysaccharides studied proved particularly good as carbon sources for Cenococcum, but it was able to grow slowly on starch, glycogen, dextrin, and inulin (Mikola, 1948b; Keller, 1952).

Cenococcum has an unusually high economic coefficient, i.e., the ration of weight of substance produced to that consumed is relatively high. Its economic coefficient on glucose surpassed that of 18 other fungi, including both mycorrhizal and saprophytic species. Several isolates of Cenococcum were tested, their economic coefficients ranging from 0.40 to 0.49. Even the least efficient Cenococcum had a higher coefficient than the best of the other fungi (Mikola, 1956).

NITROGEN NUTRITION

Cenococcum is similar to many other soil fungi in its nitrogen nutrition. It does not use free nitrogen but grows well on NH_4^+, NO_3^-, and a variety of amino acids. Ammonium ions serve best as a single source and are preferentially absorbed by the fungus when other sources are included as well (Mikola, 1948b; Keller, 1952).

Although NH_4^+ serves well as a sole nitrogen source, better growth of one isolate resulted from a combination of NH_4-tartrate and casein hydrolysate (Melin & Mikola, 1948). In addition, certain amino acids found in casein hydrolysate stimulated growth of Cenococcum when used in combination with NH_4^+. Melin and Mikola hypothesized that Cenococcum cannot synthesize certain amino acids from NH_4^+ as rapidly as needed for growth.

Cenococcum has grown well over a considerable range of nitrogen contents in either substrate or mycelium. New cells have formed on media from which nitrogen was almost all used, so long as a good carbohydrate source was available. An apparent luxury consumption occurred in cultures containing abundant nitrogen, the fungus absorbing inorganic nitrogen and transforming it to organically bound nitrogen which then was secreted into the substrate (Keller, 1952).

VITAMINS AND OTHER GROWTH REGULATORS

Of a considerable number of vitamins tested on 6 isolates of Cenococcum, only thiamin consistently demonstrated a growth-promoting effect (Mikola, 1948b). Pyrimidin, one component of thiamin, stimulated growth of one isolate as much as complete thiamin did; but the other component, thiazol, produced no effect (Melin & Norkrans, 1942). Cenococcum responded mildly or not at all to other vitamins such as biotin, choline, nicotinic acid, and pantothenic acid, and response has varied between isolates (Melin & Nyman, 1940; Mikola, 1948b). Similarly, isolates have differed substantially in response to leaf extracts, some isolates being stimulated and others inhibited at given concentrations (Mikola, 1948b).

Thus Cenococcum in vitro appears autotrophic for a good many biochemicals and, indeed, produces some in excess. Bio-assays indicated that one isolate secretes pantothenic acid (Shemakhanova, 1957). And, although Shemakhanova's studies failed to detect heterozuxin secretion by Cenococcum and other mycorrhizal fungi, subsequent work reveals that Cenococcum, some of the other fungi studied by Shemakhanova, and many additional mycorrhizal fungi can produce detectable amounts of heteroauxin and other indole compounds (Moser, 1959a and personal communication; Ulrich, 1960). That Cenococcum can induce root forking on pine without actually forming mycorrhizae (Slankis, 1948; Levisohn, 1960) and can stimulate germination of certain fungus spores (Fries, 1943) further confirms its production of growth regulators.

Cenococcum has shown no antagonism to other organisms. Confrontations of several Oregon isolates with Amanita pantherina, Boletinus lakei, and Suillus gramulatus produced no reactions. All isolates of Cenococcum

used grew freely among hyphae of the other fungi. Alternaria tenuis, which antagonizes many mycorrhizal fungi, did not so affect Cenococcum (Levisohn and Parry, 1960). Bio-assays for antibiotic secretion by Cenococcum (Santoro & Casida, 1959a) gave negative results against several bacteria and yeasts. It also has been found more resistant to the antibiotic cyclohexamide than several other mycorrhizal fungi (Hacskaylo, 1961).

ACIDITY

Experiments indicate that one strain of Cenococcum is strongly acidophilic, with a well-defined optimum pH of 4. This isolate grew in a pH range of about 2.5 to 7.0, but growth was very slow outside of pH3.5 to 6.0 (Mikola, 1948b). The isolate studied by Keller (1952) grew relatively well at a pH even less than 3, however, so long as a good carbohydrate source was available.

CENOCOCCUM AS A MYCORRHIZAL ASSOCIATE

Müller (1879) first described the root inhabiting character of a unique but unidentified black fungus, later known to be Cenococcum. But until he read Frank's (1885) first paper on mycorrhizae, he did not recognize the true significance of the fungus-root association he had observed (Müller, 1886). Frank (1885, 1887) also discovered black mycorrhizae characterized by a dense outgrowth of black hyphae. Further reference to the phenomenon was scattered (Möller, 1903; Masui, 1926; Melin, 1927) until Hatch (1934) isolated the still unidentified causal fungus. Meanwhile Ferdinandsen and Winge (1925) had suggested that Cenococcum might form mycorrhizae. Lihnell (1939, 1942) resolved both questions by synthesis experiments between Cenococcum and several species of hosts: the same kind of black mycorrhiza as described by Müller and the others was formed.

HOST SPECIES

Cenococcum may well be the least specialized mycorrhizal fungus in respect to host species. It forms mycorrhizae with all known ectotrophic hosts within its range. Known hosts, listed in appendix II, comprise 56 species and varieties of gymnosperms, 97 of angiosperms, and 1 in the Filicineae, for a total of 154. Of these, 92 are reported for the first time here and 23 previously reported by others are confirmed. These facts strongly suggest that Cenococcum can form mycorrhizae with practically all ectotrophic hosts, other conditions being suitable.

In further emphasis of Cenococcum's remarkable adaptability, it occasionally forms ectotrophic mycorrhizae on hosts thought otherwise to be completely endotrophic: Acer pseudoplatanus in Aceraceae (Dominik and

Pachlewski, 1955 & 1956), Lactuca muralis in Compositeae (Boullard and Dominik, 1960), Vicia sylvatica in Leguminoseae (Dominik, 1957), Galium schultesii and G. rotundifolium in Rubiaceae (Dominik and Pachlewski, 1956). Moreover, the only report of an ectotrophic mycorrhiza in the entire order Pteridophyta involves Cenococcum on Dryopteris filixmas (Dominik and Pachlewski, 1956). Equally unusual is an endotrophic mycorrhiza formed by Cenococcum with Stellaria holostea (Dominik, 1957).

PURE CULTURE SYNTHESIS OF CENOCOCCUM MYCORRHIZAE

Apparatus

The usual pure culture synthesis method, first used by Fuchs (1911) and improved by Melin (1939) and Hacskaylo (1953), entails growing both fungus and host entirely enclosed within a cotton-stopped flask (fig. 22). Air circulation is minimized. As a result, the humidity and oxygen-carbon dioxide ratio around the seedling top are quite different than in nature, and a hothouse effect is hard to avoid. The various other techniques devised for mycorrhiza synthesis (Hatch, 1937; Shemakhanova, 1959; Lundeberg, 1960; and others) are either unduly complicated or are designed for special purposes.

The ideal system for synthesizing mycorrhizae in pure culture should approach the physical environment in nature as closely as possible. To do so, seedling tops should be in the open. Reasonably good substrate aeration is also desirable. Other important considerations include minimizing chance of contamination and providing an aseptic method of watering. To meet these requirements, I tried a number of different approaches in apparatus design and finally winnowed the choices down to s simple, inexpensive one, illustrated in figures 22 and 23.

Figure 22: New (left) and old (right) methods for pure culture synthesis of mycorrhizae.

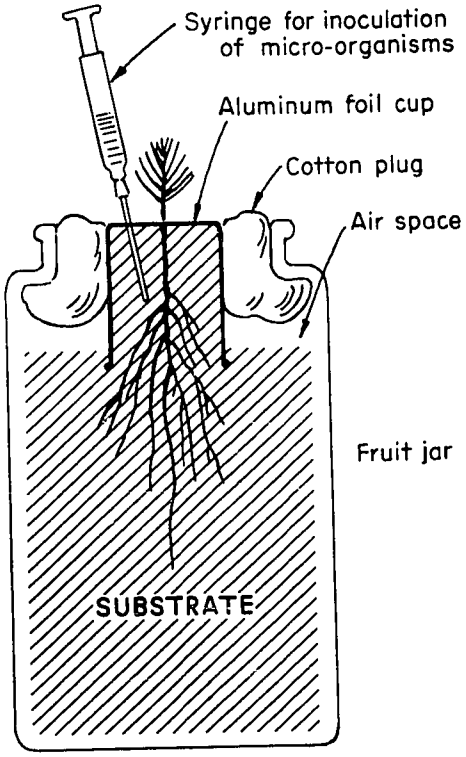


Figure 23: Diagram of new technique for pure culture synthesis of mycorrhizae. Seedling tops are in the open, roots in aseptic jar.

Any container of satisfactory size can be used--quart fruit jars serve ideally. Jars are filled to within 4 cm. of the rim with suitable substrate, vermiculite or expanded perlite being ideal (perlite is preferable if roots are to be sectioned on a microtome). A 3-cm.-deep cup of aluminum foil, formed by pressing the foil over a beaker about 2 cm. smaller in diameter than the jar opening, is also filled with the substrate. The open end of the foil cup is then covered with a single thickness of cheesecloth fastened by a rubber band. Nutrient solution is added to the jar until the bottom 2 to 5 cm. of its depth are saturated. Substrate in the foil cup is also moistened with nutrient solution to near saturation. The foil cup is then inverted onto the surface of the jar substrate and pressed down firmly to form good contact between jar and cup substrates. The solid end of the cup is now about 1 cm. below the jar rim. The space between cup and jar rim is stuffed with cotton, care being taken that the cotton does not touch the substrate. The jar lid is then put on, and the unit is autoclaved.

To prepare the fungus inoculum, techniques developed by Wikén et al (1951) or Santoro and Casida (1959b) serve well. The mycelium is grown in liquid culture with glass beads. Just before inoculation, the culture is shaken so that the mycelium is fragmented by the beads to provide a hyphal suspension.

To inoculate a jar, 5 or more cc. of hyphal suspension are drawn into a sterile, 15 cc. syringe, using a long needle with a 1 mm. bore. The lid of the jar is lifted and the needle inserted full length into the medium, punching it through the top of the foil cup (fig. 24). It is useful to insert the needle at an angle so that the first ejection of hyphae occurs against the jar wall. These hyphae can then be observed periodically to check how well they grow. As the remaining inoculum

is ejected, the syringe is slowly withdrawn. A long zone of inoculation results. The surface of the foil cup is then swabbed with ethanol and the lid replaced.

Seeds, surface sterilized with 30% H_2O_2 (Trappe, 1961), are then germinated on agar. Once seeds have germinated aseptically and their radicles have grown to about 1 cm. in length, they may be transferred to the culture jar. The jar lid is lifted and the radicle inserted into the hole in the foil cup. The lid is then replaced. In 2 or 3 days the radicle grows into the substrate and the hypocotyl becomes erect. At this point, latex cement (sterile by virtue of its solvent, usually benzene) is used to seal the hole around the seedling stem (fig. 25). The solvent soon evaporates, not harming the seedling and leaving a resilient seal around the stem. After this, the jar lid is left off permanently.

If jars are subsequently kept on sloping racks, resting on the side against which initial inoculum was ejected, the tree roots tend to grow down through the inoculation zone. Mycorrhizae will form against the glass, and their development can be followed nicely.

The seedling can be left more or less to its own devices for as much as 6 months, depending on size of jar and rapidity of transpiration. Amount of water in the jar at any time can be readily estimated by weighing (the increase of weight of the growing seedling is negligible compared to total weight of solution and jar). When water content in the jar becomes low, more can be added conveniently from a sterilized supply in a large stock jar fitted with a clamped siphon hose that has a hypodermic needle sealed into its outlet. The foil cup is swabbed with ethanol; the siphon needle is flamed and punched through the foil. Nutrient solution may be added in the same way. The hole is sealed with latex cement.

Figure 24: Inoculating hyphal suspension into substrate of new apparatus for pure culture synthesis of mycorrhizae. Photo by Wallace Guy.

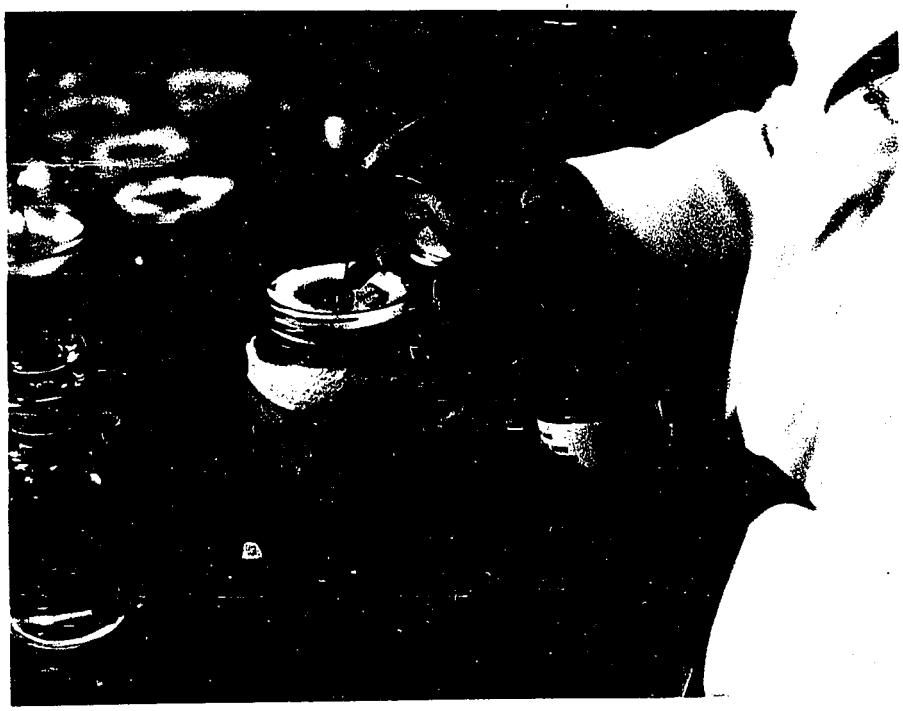


Figure 25: Applying seal of latex cement around stem of seedling growing from aseptic medium in jar for pure culture synthesis of mycorrhizae. Photo by Wallace Guy.

Superheating from radiation can be a problem in cultures kept under artificial light or in a greenhouse. Moreover, ambient air temperature is commonly higher than soil temperatures at which mycorrhizae normally form. To minimize radiation heating, the jars can be covered with aluminum foil, shiny side out. If air temperature is high, substrate temperature can be kept down a few degrees by covering jars with cheesecloth and keeping them in a shallow pan of water. Of course, controlled temperature baths provide the surest means of sustaining a desired substrate temperature.

One or more of the following features of this technique is advantageous over any other method of pure culture reported heretofore:

(1) Seedling tops are in the open. There is no spatial limitation to top growth, no hothouse effect, and neither air moisture nor other air constituents are held at artificial levels.

(2) Substrate aeration is adequate for normal growth of roots and fungi.

(3) Several seedlings can be grown in a single jar.

(4) The possibility of contamination is minimized. A 1 mm. hole is the only opening, and that is sealed for all but a few days.

(5) Seedlings can be grown for long periods. Large jars can be used for long-term studies. The elastic seal around stems permits expansion.

(6) A large area of substrate is inoculated, inducing faster contact between inoculum and roots.

(7) Water and nutrients can be added easily with little contamination risk.

(8) Roots can be grown along a glass wall for ready observation.

(9) Techniques are simple; no unusual or expensive equiment is needed.

(10) Nutrient change or continuous flow can be used by modifying the container to provide inlets and outlets below the cotton plug.

Nutrient Solution and Environment

From study of nutrient solutions ranging from 0.25 to 20.0 gms. of glucose per liter, Shemakhanova (1960) found the Suillus luteus formed the most mycorrhizae on pine in low light intensity when the nutrient solution contained 5.0 gms./l. of glucose, regardless of nitrogen and phosphorus levels. She further determined that levels of N and P best for mycorrhiza formation varied with light intensity. At a relatively low light intensity, Suillus luteus formed the most mycorrhizae on pine when N and P levels were maintained at about a 1:1 ratio. Comparable data are not available for Cenococcum, but it was decided to modify Melin's (1939) solution according to Shemakhanova's experience with Suillus luteus. Results were gratifying. The formula was:

Glucose	5.0	gms.
K_2HPO_4	0.25	gms.
$(NH_4)_2HPO_4$	0.125	gms.
$CaCl_2$	0.05	gms.
$MgSO_4 \cdot 7H_2O$	0.15	gms.
Fe-NH_4 citrate (1% solution)	1.2	ml.
Thiamin	50	mgm.
H_2O	1	l.

Solution was adjusted to pH4 as recommended by Hacskaylo (1953), the pH of vermiculite + nutrient solution rising to about 5 during autoclaving.

After the hole in the foil cup of a culture jar had been sealed around the seedling stem with latex cement, the jar was wrapped in aluminum foil and put into a controlled environment chamber on racks at a 45°

slope. The chamber was programmed on a cycle of 12 hours of light and 12 of darkness. Temperature was maintained at 65°F. Light striking seedling tops was about 700 foot-candles.

Microtechnique

Mycorrhizae were cleaned with a camel-hair brush and fixed in a mixture of chromic acid, glacial acetic acid, formalin, and ethanol, as described by Langlet (1946). Specimens were embedded in paraffin and sectioned to thicknesses of 4 to 8μ. Sections were stained either in Pianeze III-B (Vaughn, 1914) or 2% ferric citrate saturated with orseillin BB followed by 1% crystal violet in clove oil (Doak, 1955). The second procedure was particularly successful and was used for most slides.

Mycorrhiza Formation

Pinus radiata and Pseudotsuga menziesii var. glauca were used for synthesis experiments with three different isolates of Cenococcum: (1) MHc, from above timberline at 2,000m. elevation on Mt. Hood; (2) LPa, from a 1,200 m. elevation on Mt. Hood; and (3) PPb, from a 1,220 m. elevation in the pine-desert ecotone of eastern Oregon. The synthesis cultures were grown for 7 months without addition of water or nutrients. Seedlings grew very well for the first 6 months, after which symptoms of nutrient deficiency appeared. Mycorrhiza formation against sides of jars became evident in 4 to 5 months for Pinus and 6 to 7 for Pseudotsuga (fig. 26).

The course of mycorrhiza formation was observed at 120x magnification of roots adjacent to sides of culture jars, and by detailed examination of

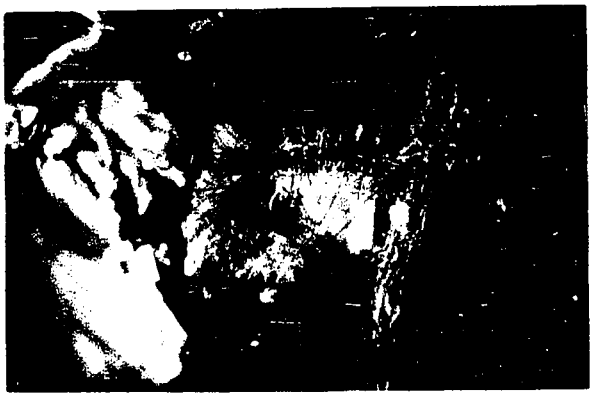


Figure 26: Mycorrhiza formed by Cenococcum with Pinus radiata in pure culture. x6.

40 sets of serial sections of mycorrhizae at all stages of development. The time sequence of formation began with contact between hyphal tips and noncutinized root epidermis. Hyphae then penetrated the epidermis. Concurrent development of Hartig net, intracellular infection, and mantle followed. All isolates performed the same on both host species.

Initial Infection

Cenococcum infected noncutinized distal portions of both long and short roots whether or not root hairs were present. When hyphae contacted noncutinized epidermis of roots, their growth habit changed abruptly from straight and black to nearly colorless and ramifying (figure 27). Having thus changed, hyphal tips soon penetrated the epidermis.

Development of Hartig Net

Development of the Hartig net proceeded promptly once the epidermis was pierced by hyphae. As the net of one entry court grew, it soon coalesced with nets from adjacent points of entry. Meanwhile, radial growth of the net continued to the endodermis in all mycorrhizae sectioned.

Hartig net hyphae were interwoven and much branched, usually hyaline, and very thin walled (figs. 28 & 29). They averaged about 2u in diameter, the individual cells reaching up to 10u in length. Occasionally a limited portion of some Hartig nets developed hyphae as wide as 5u with thick, dark walls. These structures seldom continued beyond the outermost layer of cortical cells and were usually associated with a massive growth of large, pseudoparenchymatic cells that filled a space vacated by demise of an outer cortical cell (fig. 30).

Hartig nets in several mycorrhizae grew a millimeter or more below the proximal end of the fungus mantle, sometimes reaching into the cortex of the parent long root. This extension was generally confined to the innermost two layers of cortical cells and was always accompanied by vigorous intracellular infection (fig. 31). But on one pine mycorrhiza it produced large masses of pseudoparenchyma that grew out to the epidermis. All cortical cells were filled and the epidermis was surrounded in some places, sloughed off in others. A typical Cenococcum mantle then formed on the surface. Judging from the degree of lignification of the stele of this root, it was relatively old. The significance of this type of formation is not clear.

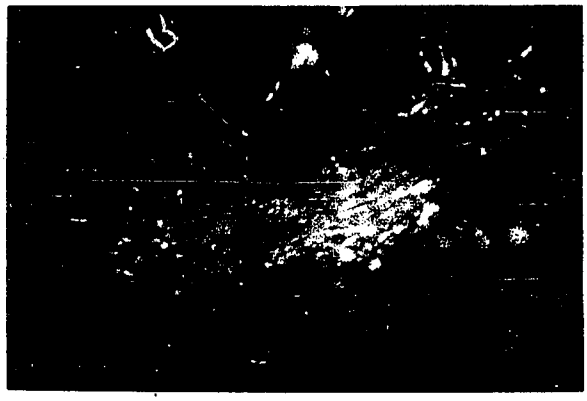


Figure 27: Initial infection by Cenococcum on root of Pinus radiata. Tips of the light-colored, ramifying hyphae have penetrated the noncutinized epidermis. x30.

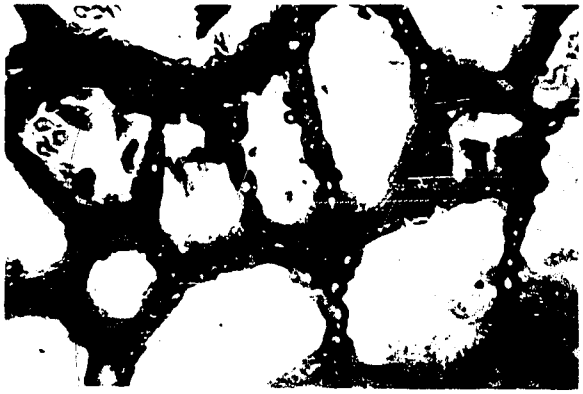


Figure 28: Cross section through Pseudotsuga mycorrhiza formed by Cenococcum. Hyphae of Hartig net form small cells separating large cells of root cortex. x600.

Figure 29: Plane view of a Cenococcum Hartig net separating walls of two cortical cells. x1340.

Intracellular Hyphae

Intracellular infection of cortical cells by Cenococcum occurred in all mycorrhizae sectioned. Root cells were penetrated at several points from the Hartig net, or, in a few cases, from adjacent infected cells without an intervening Hartig net. Intracellular hyphae, hyaline and up to 2.5μ in diameter, grew at first adjacent to the inner wall of the cortical cells. Density of these inner hyphae varied from sparse to completely lining the inside of the cell. Growing along the cell wall, they intermittantly sent plump, knobby, haustorium-like hyphae into the host cell cytoplasm and around the nucleus (fig. 32). The greater the number of "haustoria" within a cell, the smaller was their average diameter. In cells densely packed with these structures, hyphal diameter averaged only about 0.8μ in diameter. The smaller the diameter of "haustoria," the more deeply they stained with crystal violet.

In a few cortical cells these small, densely packed "haustoria" were in various stages of disintegration, leaving in their wake a deeply staining granular mass. The granular mass in turn was replaced by an evenly opaque material (fig. 33), orange to brown when unstained and deep violet when stained with crystal violet. This homogeneous material is similar to that which Rexhausen (1920) determined by histochemistry to be tannin(s). An identical granulation followed by formation of homogeneous opaque material also occurred in noninfected short and long roots, so it cannot be ascribed to action of hyphae. Moreover, the process is most advanced in older portions of roots, regardless of degree of infection. It would therefore seem to be a normal aging process that affects intracellular hyphae as well as the root cell itself.

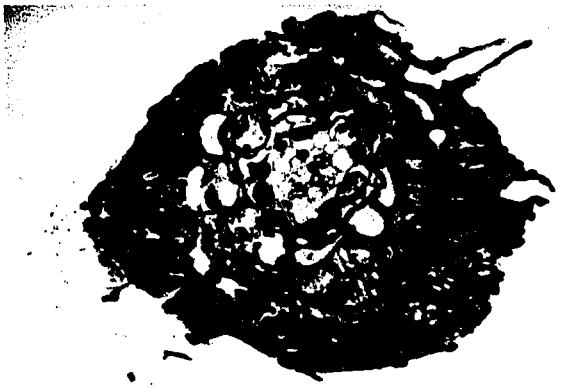


Figure 30: Cross section of Pinus radiata short root with unusual infection by Cenococcum. Outer cells are filled by large-celled hyphae. A typical fungus mantle is missing. x150.

Figure 31: Intracellular infection by Cenococcum in inner cortex of section below the mantle that covers the root tip. Epidermis at upper left, endodermis at right. x600.

Figure 32: "Haustoria" growing from hyphal lining of cortex cell. (The lining hypha pulled away from the cell wall during sectioning. x1340.

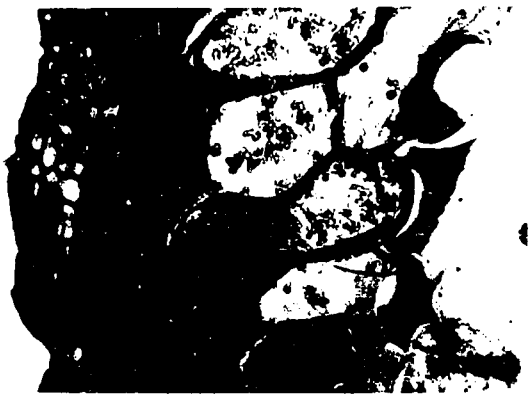


Figure 33: Cenococcum mycorrhiza. From left to right: fungus mantle, opaque outer cortex, granulated cortex cell, and cortex penetrated by hyphae. x330.

Figure 34: Tangential section of mycorrhiza mantle tissue formed by Cenococcum, showing radiate growth centers. Compare with tangential section of sclerotium, fig. 15. x330.

Figure 35: Young Cenococcum mycorrhiza (cross section). Hartig net and intracellular infection well advanced. Irregular periphery of mantle indicates it to be incomplete. x150.

Mantle

Mantle formation began with ramified growth of the first light-colored hyphae to penetrate the epidermis (fig. 27). These were in turn overlain by dark, thick-walled hyphae, resulting in an unorganized mantle two or three hyphae thick. Growth centers then appeared seemingly at random, the hyphae of one radiating out over the mantle surface to merge tip-to-tip with hyphae from another. The resultant mantle tissue forms a distinctive pattern that in tangential section appears as a mosaic of radiate growths (fig. 34). In cross section it presents irregularly alternating groups of hyphae, some sliced transversely, others longitudinally, and still others obliquely (fig. 33).

Terms used to describe this pattern have been singularly inappropriate. Melin (1927) described its tangential appearance as "nätformige stråligheten" (net-form radiations), conveying to the mind's eye a picture that is confusing at best. Hatch (1934) termed the cross-sectional appearance a "palisade" arrangement, which by classic definition it certainly is not. In a number of more recent papers it is called pseudoparenchyma, apparently referring to those hyphal groups that are transversely cut in sectioning. Again, this term is inaccurate. Careful study of sections clearly reveals the hyphal elements, so the mantle tissue is prosenchyma.

Both Hartig nets and intracellular infections had developed extensively before mantles were completed (fig. 35). But Mikola and Persidsky (1951) described the course of mycorrhiza formation by Cenococcum to be just the reverse, i.e., the mantle well formed before the Hartig net started. Their material was collected from seedlings grown in the nursery, so they could not actually observe the process as was done in my study. Possibly their

collections included mantled but uninfected roots as have been reported by Lindquist (1939) and Pachlewski and Gagalska (1953). Or perhaps Cenococcum mycorrhizae can be formed by more than one sequence of external and internal hyphal development.

Results Compared with Other Synthesis Experiments

The strong intracellular infection developed in this experiment proves that Cenococcum forms ectendotrophic mycorrhizae as well as ectotrophic. No synthesis experiments previously reported, either with Cenococcum (Hatch, 1934; Lihnell, 1942; Mikola, 1948b) or with other fungi (e.g., Melin, 1925; Hatch & Hatch, 1934; Modess, 1940) resulted in more than rudimentary intracellular infection. Because a new technique was used here, the reason(s) for this difference cannot be isolated without further experimentation. It is pleasing to note, however, that the synthesized mycorrhizae are identical with many Cenococcum mycorrhizae found in nature in the Northwest.

CENOCOCCUM MYCORRHIZAE IN NATURE

In the course of this study, thousands of Cenococcum mycorrhizae have been observed on the known hosts in Oregon and Washington. The many samples received from other parts of North America, Europe and Asia provided supplementary data.

Other fungi form black mycorrhizae, but the only ones similar enough to Cenococcum to cause confusion are Mycelium radicis atrovirens and similar

fungi. A little experience with both types, however, obviates any possibility of mistake by a perceptive observer. Cenococcum hyphae emerge from the mycorrhiza at right angles in orderly array, giving an impression of stiffness and straightness. In contrast, M. r. atrovirens emerges at all angles, winding about in tangled disarray. Furthermore, Cenococcum hyphae always average 4 or more microns in diameter and under high magnification (x100 or more) with transmitted light are warmly colored in tones of bronze, burgundy, and purple. M. r. atrovirens, on the other hand, never averages more than 3.5μ in diameter and under high magnification is colored in pallid grays or browns.

Gross Morphology

After a good many Cenococcum mycorrhizae from a wide variety of hosts had been examined, a broad morphological pattern was discernable. Hosts fell into three rather distinct groups based on configuration of their Cenococcum mycorrhizae (fig. 36):

(1) Mycorrhizae monopodial or occasionally branched. Often the mantle covers only the tip of the root. Hosts have both ectotrophic and endotrophic mycorrhizae, the latter often predominating. Short-root diameters average around 0.25 mm. Species in the Salicaceae, Betulaceae (except Corylus spp.), and ectotrophic genera of Rosaceae comprise this group.

(2) Mycorrhizae monopodial, dichotomous, or occasionally irregularly branched. Mantles usually cover all the short root. Hosts rarely form endotrophic mycorrhizae. Short-root diameters range from about 0.35 to 1.0 mm., averaging around 0.5 mm. All species of Pinus fall in this group.

(3) Mycorrhizae various--monopodial, racemose, irregularly branched, long or short. Mantles usually cover all or most of the short root. Some species form endotrophic mycorrhizae, but ectotrophic ones usually prevail. Depending on host species, short-root diameters range from 0.2 to 1.0 mm. and average from 0.3 to 0.6 mm. This group contains all species in the Fagaceae and Pinaceae (except Pinus spp.) plus the genus Corylus.

One can do little more than state that these groups of species differ in configuration of their Cenococcum mycorrhizae, because virtually no knowledge on comparative physiology of mycorrhiza formation has been developed. Environment and concomitant physiological effects on the host may also influence the form assumed by mycorrhizae. Pachlewski and Pachlewska (1961) found that Cenococcum mycorrhizae of pine were either simple or dichotomous in two biotypes but showed a great variety of forms in the third.

Anatomy

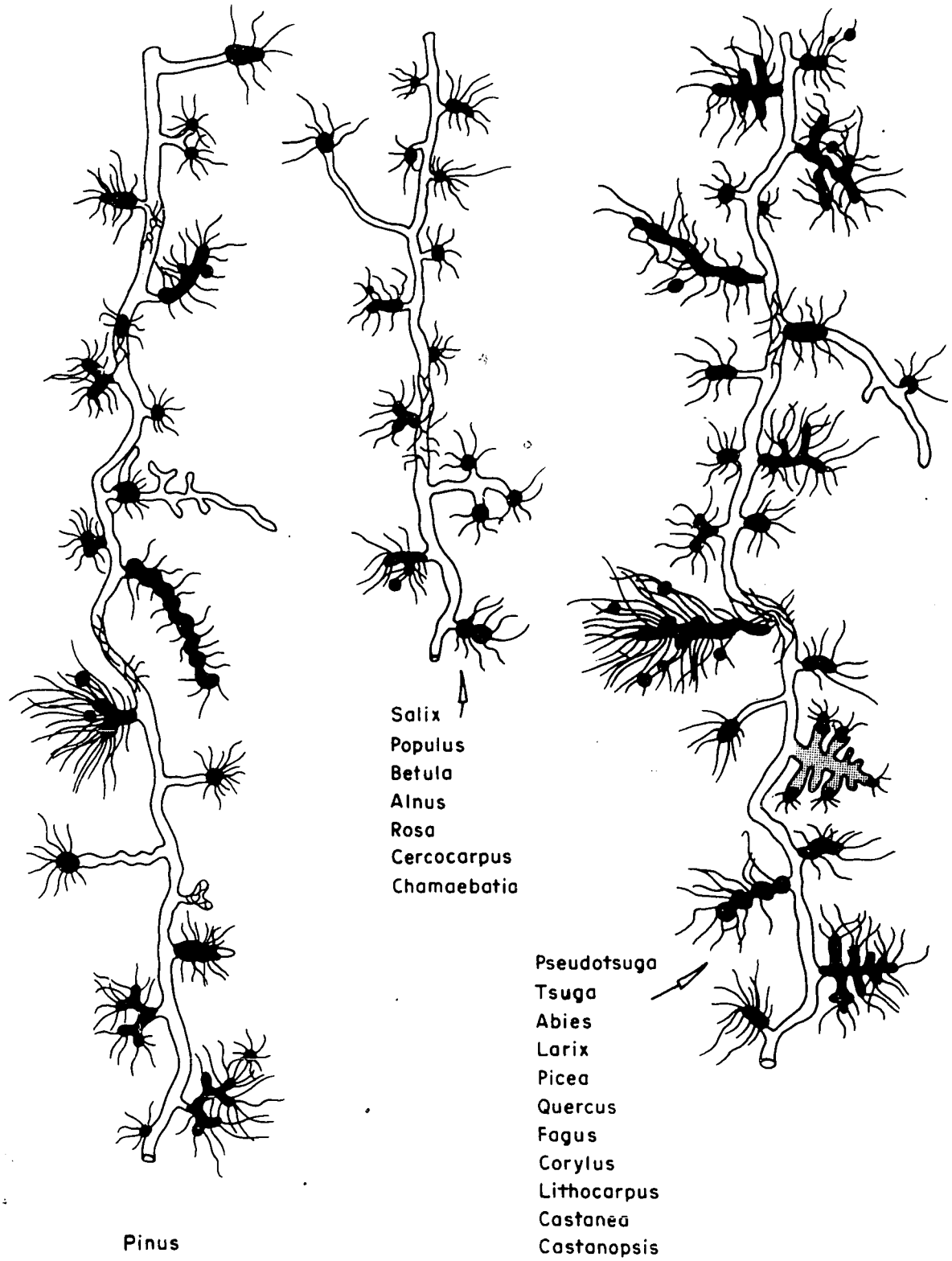
Series of sections of 95 Cenococcum mycorrhizae were studied in detail to determine the range of variability. Host species, according to the three morphological groups previously defined, were:

Group 1: Populus trichocarpa, Cercocarpus ledifolius.

Group 2: Pinus contorta, P. ponderosa, P. albicaulis, P. lambertiana, P. monticola.

Group 3: Abies amabilis, A. grandis, A. lasiocarpa, Picea sitchensis, Pseudotsuga menziesii vars. menziesii and glauca, Tsuga heterophylla, T. mertensiana, Castanopsis chrysophylla, Quercus garrayana.

Figure 36: Morphological forms of Cenococcum mycorrhizae.



MORPHOLOGICAL FORMS OF CENOCOCCUM MYCORRHIZAE

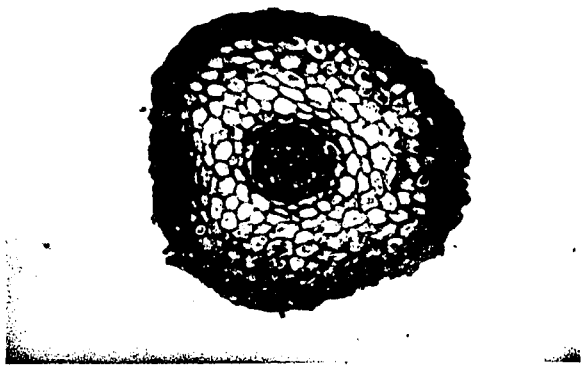


Figure 37: Cenococcum mycorrhiza of Cercocarpus ledifolius (cross section). Hartig net extends only to the third outer tier of cortical cells and intracellular penetration is nil. x150.

The Cenococcum mycorrhizae of group 1 species showed by far the least subepidermal infection (fig. 37). Their mantles averaged only 15 μ in thickness, much thinner than in groups 2 and 3. The Hartig net was never deeper than the third layer of cortical cells. Intracellular penetration was sparse and only in occasional cells. This agrees well with observations by others of Cenococcum mycorrhizae on hosts that have pronounced endotrophic tendency--Sorbus, Populus, and Ribes species (Dominik and Pachlewski, 1955; Dominik, 1957 and 1958b). Moreover, Cenococcum infection is weak on hosts that normally are completely endotrophic--Galium, Dryopteris, and Lactuca species (Dominik and Pachlewski, 1956; Boullard and Dominik, 1960). Apparently, the physiological factors that permit endotrophic infection of a host also erect or are accompanied by erection of barriers to strong penetration by Cenococcum.

Mycorrhiza anatomy of hosts in group 2 is similar to that of group 3 (figs. 38 and 39). Mantles range in thickness from 8 to 60μ, most averaging between 20 and 30. Mantle thickness at a single cross section varies substantially: a specimen of Pinus lambertiana ranged from 15 to 40μ.

These observations are comparable to previous experience (Hatch, 1934; Mikola, 1948a; Ferreira dos Santos, 1951; Pachlewski, 1953; and others). Regardless of the time of collection, most of my material from groups 2 and 3 showed a Hartig net extending to the innermost layer of cortical cells and strong intracellular infection throughout the cortex. Structure was identical to that formed in pure culture synthesis--ectendotrophic. Often all but the inner 2 or 3 layers of cortical cells had become filled with opaque material, and in a few cases all cortical cells were filled (fig. 40).

In 2 Cenococcum mycorrhizae of Pinus lambertiana, hyphae penetrated between endodermal cells and formed "haustoria" inside pholem cells. In both cases the xylem was strongly lignified, suggesting that the roots were relatively old. Intracellular infection was rife throughout the cortex, and outer cortical cells were crushed and dead. Pallid hyphae of the Mycelium radicis atrovirens type wound sparsely over the surface of the Cenococcum mantle in both cases. Melin (1927) noted a similar infection on long roots of Pinus sylvestris. Pachlewski (1953), too, noted steles of Cenococcum mycorrhizae on Larix sp. to be infected, though rarely.

Regrowth of Mycorrhiza Tips

Tips of Cenococcum mycorrhizae frequently break dormancy and resume growth (fig. 41). As meristematic cells divide and expand, the new tip bursts through the fungus mantle, which splits cleanly and is pushed aside in flaps. The young root tip is infected anew with a mycorrhizal fungus, most often Cenococcum. This infection comes from outside rather than up

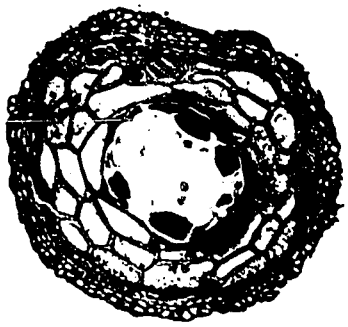


Figure 38: Cenococcum mycorrhiza of Pinus contorta (cross section). Hartig net extends to the endodermis and most cells have some intracellular infection in the cortex. x150.

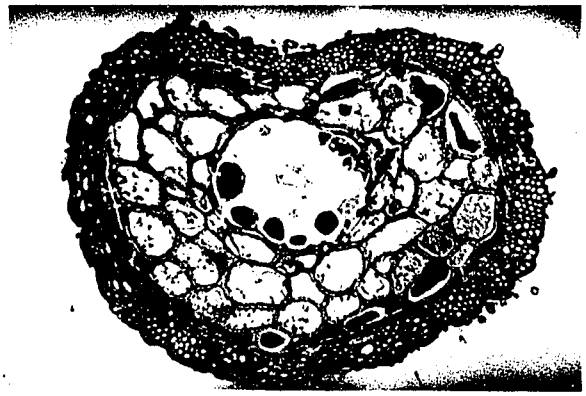


Figure 39: Cenococcum mycorrhiza of Pinus contorta (cross-section). Similar infection as shown in fig. 38 but on larger short root and with thicker mantle. x150.

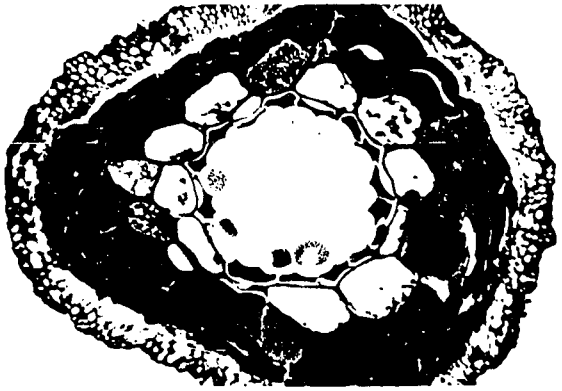


Figure 40: Cenococcum mycorrhiza of Pinus lambertiana (cross section). Outer cells of cortex crushed and opaque. Inner cells all with some hyphal penetration. x150.

Figure 41: Cenococcum mycorrhiza of Pinus contorta that has burst its mantle 3 times and been again covered after regrowth. Cortex of older part has been crushed. x25.

through the cortex of the older portion of the mycorrhiza, because the cortex is discontinuous. New cortex is separated from old by several layers of crushed, dead rootcap cells between which no hyphae may be seen. At the juncture of old tip and new, the mycorrhiza is constricted. This stricture is usually covered subsequently by fungal mantle. Regrowth may happen six or more times on a single mycorrhiza, giving it an elongate "necklace" appearance.

The Net Effect of Cenococcum on its Hosts

Parasitism by Cenococcum

Certain forms of Cenococcum infection have been interpreted as parasitic by Lindquist (1937, 1939), Mikola (1948a), and Dominik and Pachlewski (1955). In each case the conclusion was based on anatomy of infection. Cenococcum may indeed override the bounds of symbiosis on occasion, but these authors have not established that the anatomy observed indeed

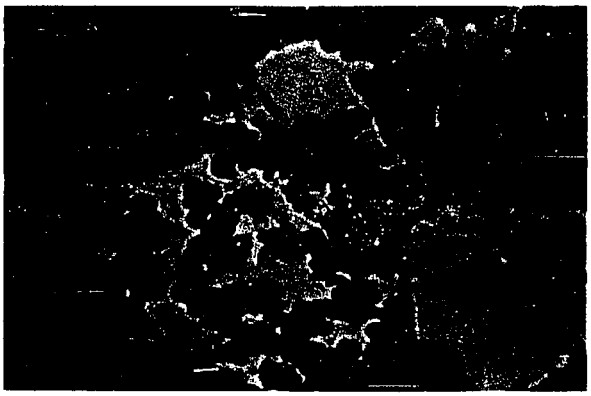


Figure 42: Intracellular infection by 2 fungi in a Cenococcum mycorrhiza. Cenococcum hyphae in lower cells, larger and darker hyphae of another fungus in upper cell. x600.

indicates parasitism. Nor is it sure whether the fungus caused the condition or just followed some other disturbance to the root.

Several things must be remembered when one encounters a Cenococcum mycorrhiza in which, judging from its anatomy, the fungus appears to be parasitic. First, Cenococcum is often accompanied by other fungi (fig. 42). Foremost among the associate fungi is Mycelium radicis atrovirens, known from pure culture synthesis to have parasitic ability (Melin, 1923). In my attempts to isolate Cenococcum from mycorrhizae, over half the cultures yielded M. r. atrovirens. Hatch (1934) and Manka and Truszkowska (1958) had similar results. Rayner and Neilson-Jones (1944) report an attack by M. r. atrovirens on Cenococcum mycorrhizae which were thereby transformed into pseudomycorrhizae. Some of the "parasitic" records of Cenococcum, therefore, may actually be attacks by other fungi.

Second, the Cenococcum mantle is exceedingly resistant to decay. I have found Cenococcum mycorrhizae on roots of Pseudotsuga stumps that had been cut 3 years before. Mantles and outgrown hyphae were intact, but a

light squeeze collapsed the structure. Root tissue inside was completely disintegrated. Thus, Cenococcum mycorrhizae which are dying from completely normal causes may contain necrotic tissue. Conceivably, the fungus could invade moribund tissue as a saprophyte.

Third, Cenococcum is capable of strong intracellular infection suggestive of parasitism. Indeed, the term "haustorium" is by definition an absorbing organ of a parasite (Snell and Dick, 1957). But there is no evidence whatever that intracellular infection in this case is detrimental to the hose. Björkman (1944, p. 10) candidly recognizes the lack of evidence by stating that a parasitic tendency "may well be imagined" for a mycorrhizal fungus that penetrates cortical cells. Strong intracellular infection in mycorrhizae could just as well be a structure that results in more efficient mutual exchange between host and fungus.

Late in the 19th century a debate raged on whether ectotrophism was a pathogenic or symbiotic phenomenon. At that time the arguments hinged on anatomy. Not until host response to ectotrophic infection was conclusively demonstrated to be beneficial was the question answered. We now face the same question about Cenococcum in respect to intracellular infections. Until the host response is unequivocally demonstrated, no solution can be offered. Meanwhile, the evidence at hand indicates that Cenococcum infection is on the whole very favorable to the host.

Figure 43: Sand dunes on Oregon coast supporting Pinus contorta and Picea sitchensis. Cenococcum forms nearly all their mycorrhizae.

Benefits to Hosts from Cenococcum

Perhaps the most impressive evidence that Cenococcum favorably affects growth and survival of its hosts is seen in the Pinus contorta--Picea sitchensis stands growing on sand dunes along the Oregon coast (fig. 43). These dunes are very low in fertility and water-holding capacity. Trees could hardly survive there if the Cenococcum that forms nearly all their mycorrhizae in the dunes were not beneficial. Dominik (1957, 1958b, 1961) records several analogous circumstances in which he believes that the host survives only by virtue of its Cenococcum infection.

Cenococcum can stimulate seedling growth comparably to other mycorrhizal fungi (Shemakhanove, 1961). Pure cultures of it, Suillus bovinus, and Suillus luteus were added to soils in which seedlings of Pinus sylvestris and Quercus robur were planted at five widely separated locations in the Soviet Union. Growth of seedlings treated with Cenococcum cultures

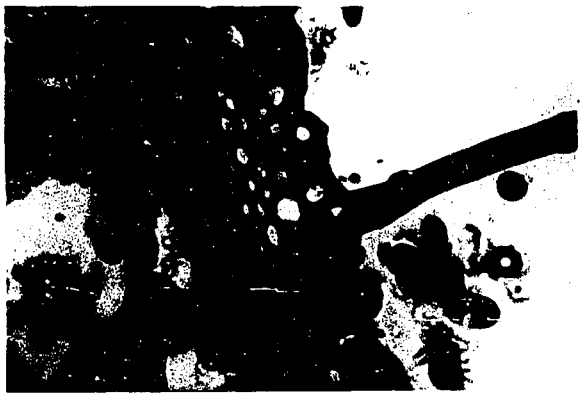


Figure 44: Cenococcum hypha, growing out from mycorrhiza mantle, connects soil with mycorrhiza's translocation system. x600.

was enhanced over controls as much or more as with the two Suillii, particularly on sandy soils.

The specific reasons for enhancement of host growth and wellbeing by Cenococcum have received little study, but some good hypotheses can be made. Assuming that the hyphae absorb nutrients and water that are translocated to the host (as demonstrated for other fungi by Melin and Nilsson, 1958 and earlier papers), Cenococcum infection greatly increases absorbing surface of the host (fig. 44). I have traced hyphae from mycorrhizae in pure culture syntheses as far as 6 cm. through the substrate. Many of these fuse with other Cenococcum hyphae not directly connected with the mycorrhiza. Thus the entire hyphal system becomes interconnected. Moreover, sample counts show that from 200 to over 2,000 hyphae emerge from most Cenococcum mycorrhizae. Even if only a part of the length of these hyphae actively absorbs material from soil, the efficiency of the absorbing system is increased by astonishing amounts. A large soil mass can be tapped instead of just that part actually contacting the root.

Cenococcum-produced biochemicals undoubtedly affect growth of hosts. Extracts from Cenococcum cultures stimulated height growth, needle growth, long root length, number of short roots, and total root weight of Picea abies seedlings (Lindquist, 1939), and seed germination and seedling top growth of Pinus sylvestris (Shemakhanova, 1957). One of the active growth promoters produced by one isolate of Cenococcum was pantothenic acid (Shemakhanova, 1957), a vitamin believed to stimulate growth of host plants in legume-Rhizobium symbiosis (McBurney et al, 1935; Ratner and Dobrokhotova, 1958).

Summing up, two areas of evidence speak for a general beneficence to the host by Cenococcum infection. First, it commonly predominates on hosts that are healthy even though growing in adverse conditions. Second, the experimental work to date has always resulted in a positive host response to Cenococcum or its extracts. The possibility still exists that mycorrhizal fungi, including Cenococcum, can be deleterious to hosts in certain circumstances. In the case of Cenococcum this has yet to be demonstrated.

AUTECOLOGY OF CENOCOCCUM

PIONEERING ABILITY

Cenococcum is not only widespread but also is common in isolated areas, e.g., islands, mountains surrounded by deserts, and cinder pockets in large lava fields. Furthermore, it often is among the first mycorrhizal fungi to invade newly formed soils on which ectotrophic hosts become established. Many new soils--sand dunes; glacial moraines; volcanic ash, cinders and pumice--are initially devoid of organic matter and present an inhospitable habitat for mycorrhizal fungi. Indeed, most mycorrhizal fungi would seem unable to survive in these environments until a suitable host provides a root system to sustain their carbohydrate wants.

In cases like this, Cenococcum is often the first ectotrophic fungus to form mycorrhizae with the host and sometimes retains full possession of its mycorrhizal system for many years. Oregon and Washington provide many examples of pioneering by Cenococcum. Pinus contorta is often the first conifer to invade sand flats and dunes along the Oregon seacoast (fig. 43). Nearly all the mycorrhizae on these trees are formed initially by Cenococcum, which predominates thereafter for an indefinitely long period. A contrasting site, above timberline in the northern Cascade Mountains of Washington, presents an analogous situation. Receding glaciers have exposed many acres of soil newly ground from bedrock. Ectotrophic hosts invade these moraines within a decade or so after retreat of the ice overburden. In some cases, e.g., on moraine of a glacial remnant on the south slope of North Star Mountain in Chelan County, Cenococcum predominates on roots of the scattered, dwarfed Larix lyalli and Pinus albicaulis.

Pioneering by Cenococcum has been noted in other severe habitats. Dominik (1961) observed it to prevail initially on roots of Pinus sylvestris

and other species growing in crannies on sun-scorched cliffs. After a tree became well established and accumulated some humus around its base, other fungi followed to form mycorrhizae. Wright and Tarrant (1958) reported Cenococcum mycorrhizae to predominate on Pseudotsuga menziesii seedlings established on patches of severely burned soil of slash-burned clearcuts. An unusual example of pioneering by Cenococcum is recorded by Frydman (1957), who found it forming mycorrhizae along with other fungi on Betula verrucosa, B. alba, Populus tremula, and P. nigra which had invaded "soil" formed from brick and mortar rubble of World War II. Cenococcum can also invade field soils rapidly (Dominik, 1958b).

That Cenococcum invades not only by growth through soil is clear from its ready ability to jump barriers. The coastal dunes exemplify this, but even more strikingly do the ash-and-cinder pockets in lava flows of the Cascade Mountains and eastern Oregon (fig. 45). Many of these flows are less than 2 millennia old (e.g., at McKenzie Pass, Lava Butte, and Mount St. Helens). The cinder pockets may be separated by several hundred feet of bare basalt, yet the plants growing on them often have Cenococcum mycorrhizae.

MEANS OF DISPERSAL

The ability of Cenococcum hyphae to resist drought plus the common occurrance of viable fragments of similar hyphae in the air (Pady and Kramer, 1961) point to wind transport of hyphae as probably the most usual means of dispersal. Hyphae may be exposed to wind pickup by any soil disturbance--earth slide, erosion, animal burrowing, windthrowing of trees. But a more unique and dependable means of exposure is also utilized by

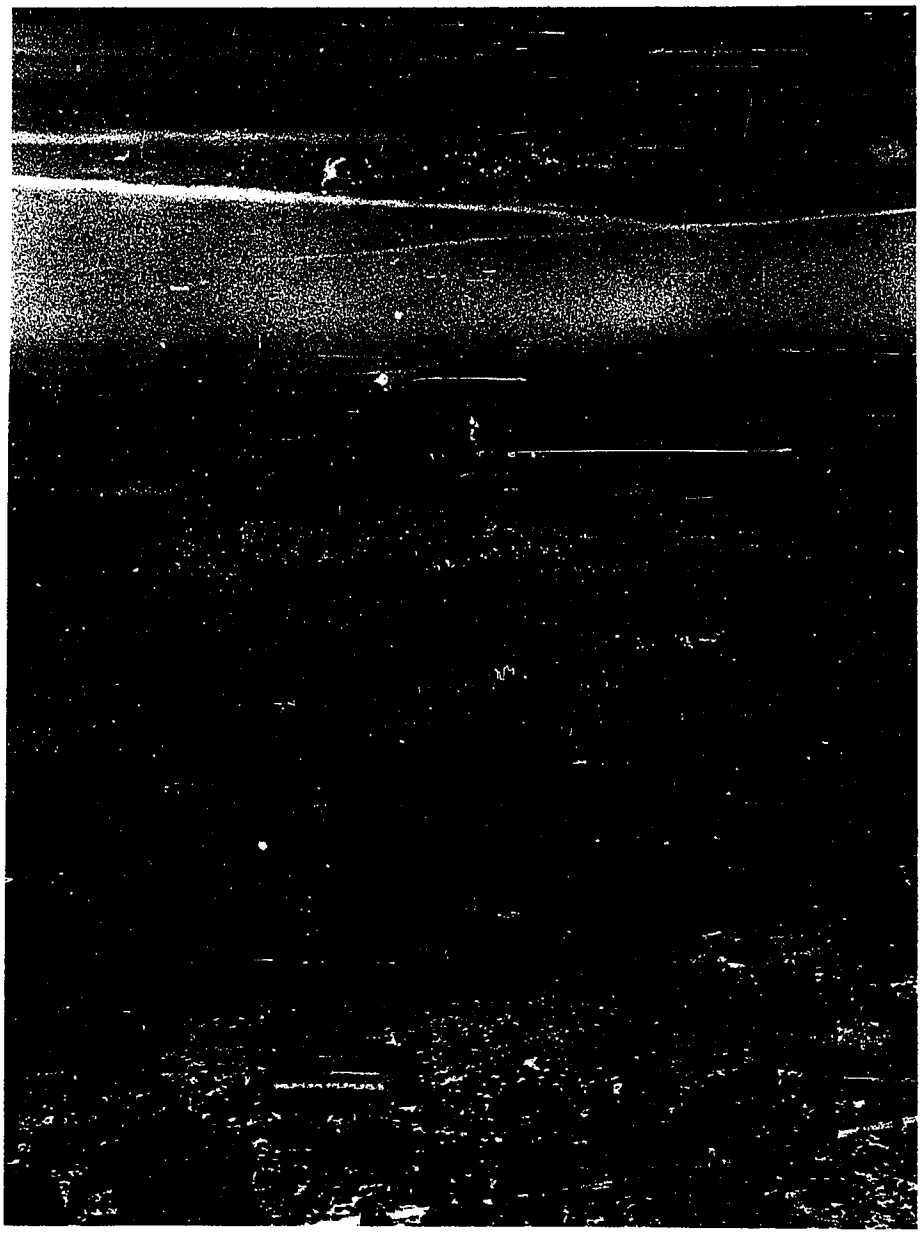


Figure 45: Cinder cone and lava beds of eastern Oregon. Cenococcum has jumped long stretches of bare basalt to become a mycorrhiza former in the tree islands. U. S. Forest Service photo.

Cenococcum: every spring and autumn masses of its hyphae and sclerotia are lifted by countless numbers on caps of mushrooms! It most often is lifted on mushrooms with rough or viscid caps, such as species of Suillus, Gomphidius, Amanita, and Cantharellus. But even smooth and dry-capped species such as Mycena expose Cenococcum to air transport by overturning clumps of humus.

Other agents, though less pervading than air movement, may also be effective. Water flow undoubtedly serves to distribute Cenococcum. Small mammals are known to eat various soil fungi (Whitaker, 1962); and Cenococcum, with a high fat content, could well be palatable to some. Miller (1879) found Cenococcum in guts and excrement of earthworms and larvae of insects, the hyphae seemingly unharmed by the digestive process.

SAPROPHYTIC ABILITY

The most obvious evidence of saprophytism by Cenococcum is its growth in pure culture. It does grow, though slowly, on some of the complex carbohydrates found in humus, and it utilizes nitrogen in a variety of forms. Further, it is relatively resistant to many antagonistic and antibiotic compounds (cf. pp. 35 to 37). But it has never been reported in nature in a situation where it was unambiguously saprophytic. Ferdinandsen and Winge (1925) found it growing amongst stems and rhizoids of mosses in a location that precluded ectotrophic symbiosis. But even here a "rhizoidosphere" effect and even some type of symbiosis with the moss was possible. Domański and Dzięciolowski (1955) believed they had isolated "Cenococcum sp." from advanced heart rot of roots and trunks of Pinus sylvestris. But this is highly questionable, because Cenococcum's slow growth in culture

precludes its detection in wood block, soil plate, or dilution methods; other fungi generally overrun a culture before Cenococcum starts growth. At present, its occurrence in nature as a pure saprophyte remains problematical.

SOIL MOISTURE

Cenococcum has gained particular renown for drought resistance. Hatch (1937) and Mikola (1955) found that thorough air-drying eliminated nearly all mycorrhizal fungi except Cenococcum from soil and humus. Moreover, Cenococcum can actively grow and form mycorrhizae better than many fungi under relatively dry conditions (Worley and Hacskaylo, 1959).

In the Pacific Northwest Cenococcum predominates on a variety of droughty sites (figs. 43 to 46). Coastal dunes in Oregon have only minute water-holding capacity. Mycorrhizae of the Pinus contorta and Picea sitchensis growing on them are nearly all formed by Cenococcum; dunes on the Baltic coast are similar in this respect (Dominik and Pachlewski, 1955). Shallow cinder pockets on lava beds in eastern Oregon--for example, at Lava Butte, where precipitation is less than 20 in. per year--support stunted Pinus contorta, Pinus ponderosa, and Populus tremuloides whose mycorrhizae are formed predominantly with Cenococcum.

Abrupt microsite changes provide striking illustrations of the relation between soil moisture and frequency of Cenococcum mycorrhizae. Mycorrhizae of three old-growth Pinus ponderosa were sampled in an open stand in eastern Oregon about 5 miles from the pine-desert ecotone, west of Fort Rock. Precipitation at this site averages less than 20 in. per year. Samples were taken at intervals of 1 meter along each of three

Figure 46: Pinus ponderosa at edge of eastern Oregon's desert. Over half of the mycorrhizae sampled from this tree were formed by Cenococcum.

transects, beginning at the stem of each tree and extending 3 meters beyond the crown drip line. Samples included A_0 and A_1 soil horizons and contained from 25 to 75 mycorrhizae each. Percentage of Cenococcum mycorrhizae was determined by examination with a low-power stereomicroscope in the field. Although sampling was done early in May, 1959, soil under the tree crowns was dry (it was moist in the open). Dryness under crowns was probably due both to interception of precipitation by crowns and to intensified absorption of soil moisture by the concentration of roots near the stems. The abrupt decrease in proportion of Cenococcum mycorrhizae from under the crown to out in the open, as shown in table 1, is undoubtedly due in large part to differences in moisture regime.

In contrast to droughty sites, Cenococcum generally forms a low proportion of the mycorrhizae in moist sites so long as other environmental

Table 1.--Percent of mycorrhizae formed by Cenococcum in samples taken at 1-meter intervals from stems of old-growth Pinus ponderosa. Vertical lines indicate location of crown drip line.

Tree No.	Transect No.	Distance from stem (meters) 0 m.	1 m.	2 m.	3 m.	4 m.	5 m.	6 m.	7 m.	8 m.	9 m.
1	1	75	90	100	95	100	100	60 |	30	20	25
	2	90	90	90	100	100	95	75 |	20	25	20
	3	85	100	100	100	100	95	80 |	45	20	20
2	1	80	85	80	100	90	95	80	50 |	15	30
	2	100	100	100	95	90	100	90	85 |	20	20
	3	100	90	95	95	90	90	85	85 |	25	0
3	1	85	85	80	75	80	100	65	80 |	15	15
	2	85	60	90	100	75	75	90	65 |	10	35
	3	100	90	95	85	75	80	85	50 |	25	15

factors are not extreme. High quality sites for Tsuga heterophylla and Picea sitchensis, whose soil rarely dries, are located within a mile of the sand dunes previously mentioned. Yet, less than one percent of the mycorrhizae examined there were formed by Cenococcum. Similarly, most of the better sites examined in the lower Willamette Valley and the Puget Sound region have a relatively low proportion of Cenococcum mycorrhizae.

Cenococcum mycorrhizae sometimes predominate in soils that are saturated much of the year. The Wickiup soil series of eastern Oregon, on which Pinus contorta is climax (Youngberg and Dyrness, 1959), exemplifies this. Its water table, permanent in many places at a depth of 60 cm. or less, often rises to within 30 cm. of the surface after the onset of autumn rains. Many places become ponded during spring snowmelt. Cenococcum mycorrhizae were common in all of many samples of this soil examined in the field. Sclerotia were often found in late summer at the low water table line.

Cenococcum was discovered to form typical mycorrhizae in several other wet soils. In each case, mycorrhizae were sectioned to determine if Cenococcum was indeed the fungus involved and if the mycorrhizae were normal. (1) At Bagle Springs near Pringle Falls Experimental Forest, several Cenococcum mycorrhizae were found on Populus tremuloides growing in a creek channel, the soil being saturated as a rule for at least 8 months of the year. (2) A sample of Tsuga heterophylla roots from a half-bog soil on Baranof Island, Alaska, contained mycorrhizae, about half of which were formed by Cenococcum. (3) A sample of Pinus elliottii roots from a Florida pond site, dry at time of collection but usually covered by about 6 inches of water, had 25 percent Cenococcum mycorrhizae. In addition, Cenococcum hyphae were noted on short roots of Taxodium ascendens included with the

sample. Mikola (1948) found a birch seedling in a swampy forest that had 30 percent of its short roots mycorrhizal by Cenococcum, the rest pseudomycorrhizal. It is pertinent to note that normal ectotrophic mycorrhizae can occur below the permanent water table. This was proven by McQuilken (1935) and reaffirmed by Wojciechowska (1960).

In summation, Cenococcum has been found over the gamut of moisture regimes that support suitable hosts. It becomes predominant where low soil moisture is limiting all or a good part of the year and sometimes where drainage is poor. Between these extremes it may be abundant or scarce.

SOIL AERATION

The ability of Cenococcum to grow and form mycorrhizae in both dry and wet soils suggests a wide tolerance to conditions of aeration. That some isolates grow well submerged or in tightly capped cultures confirms this. Additional evidence is provided by data on the depth of its occurrence.

Mycorrhizae are concentrated in the uppermost soil layers. Because of this, as well as the difficulty of studying roots deep in the soil, few data are available for depths greater than a few decimeters. To date, Cenococcum mycorrhizae have been reported from depths of 1.8 m. (Werlich and Lyr, 1957) and 1.25 m. (Sirén and Bergman, 1951). In the latter case, 10 out of 14 successful isolations of mycorrhizal fungi yielded Cenococcum. These, however, by no means indicate the greatest depth at which Cenococcum can grow. Study of roots in a borrow pit in coarse pumice soil in Crater Lake National Park revealed Pinus contorta mycorrhizae (albeit few) at a depth of 4 meters. Four of these were formed by Cenococcum. At a depth of 3 meters, Cenococcum mycorrhizae were common. This soil, being coarse in

texture, is no doubt exceptional. By way of contrast, Pseudotsuga roots were examined in excavations on a hillside above the Tualatin Valley near Portland, Oregon. This soil was a mottled clay with iron concretions, derived from basalt. The deepest long roots were found at 3 meters; the deepest mycorrhizae at about 1½ meters. One of about 20 mycorrhizae found at this depth was formed by Cenococcum. About a fourth of the mycorrhizae in the A_o horizon were Cenococcum-formed.

No doubt Cenococcum generally flourishes best in well-aerated soil. But it can occupy poorly aerated locations and successfully form normal mycorrhizae.

SOIL TEMPERATURE

As pointed out above, Cenococcum frequently predominates on hot, dry sites, e.g., the pine-desert ecotone and cinder pockets in lava beds of eastern Oregon. To succeed on such sites it must withstand unusually high soil temperatures as well as drought.

In contrast, it also prevails in relatively cold soils. Several samples supplied from above the Arctic Circle in central Alaska all contained an abundance of Cenococcum mycorrhizae and sclerotia. The sample from farthest North was collected from over permafrost at N. 68°45' latitude along the Firth River at an elevation of 600 m. It included many mycorrhizae of Picea glauca, about half of which were formed by Cenococcum. The soil of this sample contained an estimated 50 sclerotia per ml.! Lindquist (1937) found Cenococcum to be common in northern Sweden where soil remained frozen into midsummer and rarely reached 20°C.

Cenococcum frequently predominates on mycorrhizae of alpine hosts in the Cascade Mountains of Washington and Oregon (fig. 47). An extreme

example of this was found on the upper southwest slope of Mount Scott in Crater Lake National Park. A stand of "krummholz" Pinus albicaulis, Abies lasiocarpa, and Tsuga mertensiana occupy the coarse, pumice soil of this slope. Between the 2,500 m. level and the summit at 2,724 m. root samples from 40 Pinus, 10 Abies, and 10 Tsuga trees were examined. Ninety-five percent of the mycorrhizae were formed by Cenococcum on all three species. A hundred meters lower in more sheltered areas, the proportion of Cenococcum mycorrhizae was less than half. This slope, facing prevailing storm winds, probably is swept free of snow during much freezing weather. It would be subject both to unusual cooling and, at least in some summers, drought.

As was the case with soil moisture, Cenococcum is most common where soil temperatures reach the extremes. If other factors are not limiting, the proportion of Cenococcum mycorrhizae is lower, the more moderate the site.

SOIL ACIDITY

Because of its common occurrence in mor humus, especially that of Fagus and Picea, Cenococcum has gained a reputation as an acidophile (Ferdinandsen and Winge, 1925; Mikola, 1948b; Kreisel, 1957; and others). In truth, it frequents very acid soils, and isolates tested in pure culture grow best in acid media (Mikola, 1948b). But, as obtains for most environmental factors, it possesses wide amplitude of tolerance and can be found near both extremes. Here are some examples of pH's of soil samples containing an abundance of Cenococcum hyphae, sclerotia, and mycorrhizae (Numbers refer to locations listed in appendix I): 3.4 (#66), 4.4 (#64), 4.6 (#42), 5.2 (#45), 5.7 (#26), 6.0 (#65), 6.1 (#50), 7.0 (#23), 7.3 (#52),

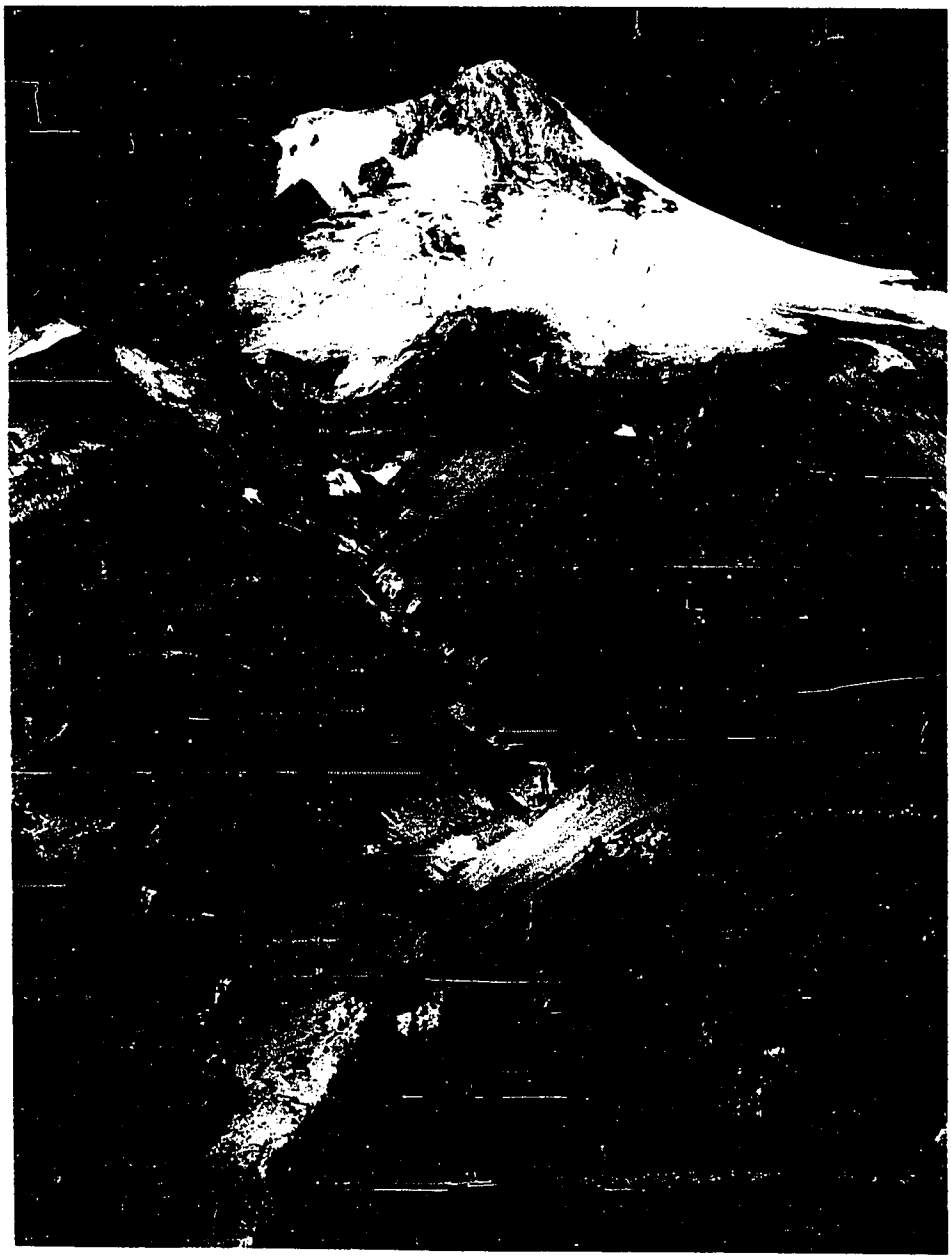


Figure 47: The highest shrubs of Pinus albicaulis, straggling up the ridges above timberline on Mt. Hood, have a majority of their mycorrhizae formed by Cenococcum. U. S. Forest Service photo.

and 7.5 (#11). Kreisel (1957a) failed to find Cenococcum on several calcareous soils in Germany, but its absence there must be attributed to other features than alkalinity per se--Dominik, Nespiak and Pachlewski (1954b) discovered Cenococcum mycorrhizae on several hosts species growing on limestone soils in Poland, and the brick and mortar rubble on which it was found by Frydman (1957) had a pH between 6 and 7.

HOST FACTORS

Cenococcum apparently can form mycorrhizae with all ectotrophic hosts, but it is more frequent on some species than others. Available data are completely inadequate to explain causes for this. It could relate to physiology of hosts, microsite differences associated with hosts, differences in numbers and kinds of competing rhizosphere organisms between hosts, etc. From extensive observations in the Pacific Northwest, a generalized impression of frequency of Cenococcum infection can be summed up for several genera of hosts, other things being equal: Abies = Picea = Pseudotsuga = Tsuga > Pinus = Larix = Quercus = Corylus = Lithocarpus = Castanopsis = Betula > Populus = Salix > Rosa > Alnus.

Experiments have shown that Cenococcum forms mycorrhizae more readily than various other fungi when the host is subjected to factors that reduce root carbohydrate reserves--low light intensity, adequate nitrogen and phosphorus (Björkman, 1942; Mikola, 1948b; Harley and Waid, 1955). Presumably, then, shading of the host would give Cenococcum an advantage in infecting the root system, other things being equal. But other things rarely seem to be equal in the Pacific Northwest, for no striking examples of heavily shaded trees having a markedly higher proportion of Cenococcum mycorrhizae

than their well-lighted neighbors have been seen. Literature reports of shade influencing proportion of Cenococcum mycorrhizae (e.g., Lindquist, 1937; Aleskovsky, 1954; Dominik and Pachlewski, 1955) are counterbalanced by instances where it does not (e.g., Pachlewski, 1953; Dominik, 1957 and 1958). Comprehensive studies in Poland provide strong evidence that many factors in the biocoenose combine to affect aggressiveness of infection by Cenococcum and that effects of light on the host are often overridden by other factors (see references by Dominik, Pachlewski, etc.).

No doubt physiological activity of the host other than carbohydrate production and translocation plays an important role in mycorrhiza formation by Cenococcum. The fungus does, for example, give positive response to thiamin in pure culture (Mikola, 1948b). Melin (1953) demonstrated that it would form mycorrhizae on excised roots even though abundant carbohydrate was available to it from the medium, suggesting that a root-produced growth substance induced infection. But at present the various host-originated stimuli to mycorrhiza infection are little understood.

DISCUSSION

So little is known about the soil microenvironment and the intricate biological battles waged therein, that fine details of Cenococcum's autecology are blurred. But two clear facts have emerged. First, although Cenococcum occurs on virtually all sites inhabited by its hosts in the Northwest, frequency of its mycorrhizae increases as one or more site factors become increasingly limiting. Only when such limitation becomes extreme does Cenococcum dominate mycorrhiza formation of a site. The conclusion is inescapable that because Cenococcum outreaches other mycorrhizal fungi in

tolerance to extremes of heat, cold, or drought it dominates in such environments by default. Second, Cenococcum does occur on extremely good sites with moderate physical environments and forms mycorrhizae there, albeit relatively infrequently compared to other fungi. Because thereis no reason to believe that Cenococcum cannot do relatively better in good environments than in poor, and because there is no evidence that it is inhibited by other organisms, its languid participation in mycorrhizal systems on the better sites apparently results from relatively low aggressiveness.

INHERENT VARIATION IN CENOCOCCUM

Cenococcum's remarkable tolerance to site conditions and the diversity of its ectotrophic hosts raises a most interesting question: is the species separated into a large number of genotypes, each adapted to rather specific environment, or do its genotypes possess broad adaptability? This point bears on use of Cenococcum as inoculum in nurseries. If its genotypes are narrow in environmental tolerance or in ability to infect diverse hosts, it holds no advantage over many other ectotrophic fungi.

To gain insight into physiologic variation of Cenococcum, isolates were obtained from three widely differing habitats: (1) a "krummholz" island of Tsuga mertensiana above timberline on Mount Hood--the seven isolates are designated "MHa" through "MHg"; (2) a mixed stand of Pinus contorta, Abies amabilis, and Pseudotsuga menziesii at mid-elevation on Mount Hood--the seven isolates are designated "LPa" through "LPg"; (3) an open stand of Pinus ponderosa growing on the dry pumice plains of central Oregon--the eight isolates are designated "PPa" through "PPh." An additional source, the coastal fog belt, was originally planned for inclusion in the study, but isolation attempts yielded only one success out of 20 tries. This single isolate was included in all experiments, but careful study of its morphology in culture, meanwhile, lead to the conclusion that it was not Cenococcum. Consequently, it will not be discussed further. Soil samples collected for analysis and for obtaining sclerotia were from the A_o horizon at each habitat.

DESCRIPTION OF ISOLATE PROVENANCES

Alpine Habitat (MH)

The alpine habitat is situated at 45°21' N. lat., 121°42'W. long. on the south slope of Mount Hood at an elevation of 2,000 m., about 70 m. in elevation above timberline. Thirteen-year records from a U. S. Weather Bureau station at Timberline Lodge, about a half mile distant from the site of collection, show an average annual temperature of about 4°C. The high temperature of the year averages 28°, low -18°. There are no frost-free periods, but monthly average temperatures exceed freezing from April through November. Precipitation records are incomplete, but annual total is estimated at well over 250 cm. The larger portion of this is snow.

The soil from which sclerotia were collected was a 6-inch layer of volcanic ash overlain by raw humus. This ash was probably deposited about 1800 A. D. (Lawrence, 1948) and is now permeated with roots, alive and dead. Analysis showed it to be deficient in potassium but otherwise reasonably fertile (see table 2). Cenococcum formed about 75% of the mycorrhizae in the spots sampled.

Mid-elevation, Mount Hood (LP)

This habitat is located at an elevation of 1,220 m., about 3 miles southwest of the alpine habitat and 780m. lower in elevation, at N. 45° 18' lat., 121°45' W. long. The aspect is southwest. Seven-year U. S. Weather Bureau measurements taken a half mile away (Government Camp Station) show annual temperatures to average about 6°C., with an average high of the

year of 32° and a low of -18°. July and August are usually frost free. Precipitation averages about 225 cm. annually, with an average of 2.5 cm. in July, 5 in August, and more than 10 in all other months. Over half of this is probably in snow.

The stand of Pinus contorta, Pseudotsuga menziesii, and Abies amabilis from which sclerotia were collected is growing on a soil of volcanic origin, probably alluvium from a large mud flow thought to have plunged from near the summit of Mount Hood some 1,700 years ago (Lawrence & Lawrence, 1959). Soil from this habitat was not analyzed, because the isolates were not originally intended for inclusion in the experiments. The pH was 4.8. About 50% of the mycorrhizae collected here were formed by Cenococcum.

Dry Pumice Plains, Central Oregon (PP)

Situated at 43°20' N. lat., 121°10' W. long. and an elevation of 1,500 m., this habitat is subject to a dry, continental climate. U. S. Weather Bureau records (Fremont Station) from 4 miles to the east show an average temperature of about 6°C., based on 21 years' data, with an average high of the year of 36° and a low of -28°. There are no frost-free periods. Forty-six years of precipitation measurements average to an annual fall of 26 cm. Over half of this is usually snow between November and February. Seven months of the year average less than 2.5 cm. in precipitation.

Soil in this habitat is pumice, showered over the area several thousand years ago from the great Cascade volcanoes to the West. It has a relatively high content of exchangeable cations but is extremely low in nitrogen and organic matter (table 2). Soil moisture is limiting--this habitat is near the desert edge of the ponderosa pine-desert ecotone. Cenococcum formed about half of the mycorrhizae examined.

Table 2.--Soil analysis results of samples collected from the alpine and dry plains habitats[1/]

Habitats	pH	Readily available P	K	Ca	Mg	Na	Cation exchange capacity	N	Organic matter
		lbs./acre	me./100 gms.					Pct.	Pct.
Alpine (MH)	5.1	194	0.21	1.2	0.35	0.43	12.4	0.203	0.43
Dry plains (PP)	5.8	36	1.66	6.3	2.2	.15	10.8	.075	.15

1/ Soil analyses by Oregon State University Soil Testing Laboratory, using these methods: phosphorus by the sodium bicarbonate method; potassium, calcium, magnesium, and sodium by flame photometry; total nitrogen by Kjeldahl method; cation exchange capacity by the ammonium acetate method; and total organic matter by the Walkley-Black method.

HOST SPECIFICITY IN CENOCOCCUM

To determine whether host specificity existed in the isolates, one of each provenance was chosen for pure culture syntheses, as described earlier. Pseudotsuga menziesii var. glauca and Pinus radiata served as hosts, giving a total of six combinations:

(1) P. menziesii x Cenococcum MHc from alpine habitat about 700 m. higher than the highest native Pseudotsuga.

(2) P. menziesii x Cenococcum PPb from dry plains habitat, separated from the nearest Pseudotsuga by about 30 miles of low rainfall belt.

(3) P. menziesii x Cenococcum LPa from native Pseudotsuga habitat although collected from under Pinus contorta.

(4) P. radiata x Cenococcum MHc from alpine habitat containing Pinus albicaulis but collected from under Tsuga.

(5) P. radiata x Cenococcum PPo from high plains habitat supporting Pinus ponderosa.

(6) P. radiata x Cenococcum LPa from mid-elevation habitat supporting Pinus contorta.

Thus Pseudotsuga was paired with isolates of two provenances substantially removed from the nearest Pseudotsuga habitat by either elevation or distance and ecologically vastly different. The Pinus radiata was paired with isolates of three provenances containing pines of different species but ecologically very different from the native habitat of Pinus radiata. Results of the syntheses were positive in all cases, typical ectendotrophic mycorrhizae forming abundantly in each.

Certainly, no host specificity appeared, but conclusions cannot be induced beyond the isolates and hosts used without further evidence.

Such evidence is provided abundantly by arboreta in the Pacific Northwest: Portland City Park Arboretum, University of Washington Pack Forest Arboretum, U. S. Forest Service Wind River and Institute of Forest Genetics (Placerville) Arboreta. Each of these contains an abundance of exotic hosts that have formed mycorrhizae with the native Cenococcum. A striking example is the Tilia americana at Wind River. The nearest native member of the Tiliaceae grows half a continent away, yet the representatives raised from seed at Wind River have formed mycorrhizae with Cenococcum profusely. Thus all evidence points to a lack of inherent specificity for host in Cenococcum.

VARIATION IN MORPHOLOGY OF ISOLATES IN VITRO

Sclerotia that yielded the several Cenococcum isolates from each provenance were in each case plucked from a small volume of soil -- about 250 cc. All isolates within a habitat, therefore, originated within a few centimeters of each other. No phenotypic differences showed between Cenococcum of the three habitats as growing in situ. But in pure culture on agar, pronounced differences in colony morphology developed between provenances and between isolates within provenances. These differences have continued through three years of successive subculturing. It is, of course, impossible to relate most features of colonies grown in vitro to the life history of the fungus in situ. The following discussion rather serves to indicate the inherent variation of the population sampled.

Differences between isolate colonies on agar are most prominent in these characteristics (see table 3):

(1) Mat form developed in two distinct ways as a result of different growth habits. In one, hyphal tips formed at the interface between inoculum and substrate and grew down into the medium; cells at the perimeter of the interface elongated, raising older tissue off the substrate. Cells in this elevated tissue also continued growth, resulting in a hollow, strongly convoluted, roughly hemispherical mat. Growth of hyphal tips across the surface of the agar was very limited; radial spread of the colony resulted largely from a gradual rolling-out of periferal mat convolutions. In the second growth habit, hyphae grew out across the agar surface in the more usual way, forming an appressed mat.

(2) Tissue color of mats (underneath erect aerial hyphae) was either black or gray with scattered small inclusions of darker color. There seemed to be no gradient of color between light and dark types.

(3) Density of erect hyphae on mats varied from uniform pubescence to scattered tufts or individual erect hyphae.

(4) Color of erect hyphae was usually light at emergence from mat tissue, but then darkened with age. Some hyphae emerged with a nearly black color, however, and others emerged white and so remained. In most cases there was a mixture of light and dark, but one type strongly predominated.

(5) Length of erect hyphae arising from mat tissue varied substantially on most colonies, but either long or short (less than $\frac{1}{2}$ mm.) hyphae predominated. When longer hyphae predominated, they were always quite markedly longer than $\frac{1}{2}$ mm.

(6) Growing edge in the substrate was manifested in two distinct patterns. In one, the edge advanced uniformly with thickly growing hyphae that branched and intertwined to form a dense mass a millimeter or so behind the tips. In the other, the edge advanced in notably irregular fashion,

with individual hyphae growing here and there several millimeters beyond the main colony. These "runner" hyphae branched profusely, the branches extending laterally to merge with branch systems of adjacent "runner" hyphae. The main colony was thus extended raggedly by filling of gaps between "runner" hyphae.

(7) Substrate growth varied substantially between isolates, but in most it was relatively vigorous. A few isolates grew hardly at all in the substrate, penetrating below the surface only a millimeter or two at the most.

(8) Sclerotia were initiated by nearly all isolates but never matured in many. One isolate produced mature sclerotia only below the agar surface. Many formed them both in the agar and at the edge of the surface mat. A few formed them on the surface of the mat as well as at the other locations.

(9) Exudation refers neither to water nor to the gelatinous hyphal sheathing (both of which were exuded in quantity by all isolates), but to a dark, cloudy liquid. Exuded into the agar, this material formed a brownish cloud around the colony. In three cases it was exuded as large droplets on the surface of the mat tissue as well.

Comparison of isolates in respect to these characteristics (table 3) reveals some interesting patterns. First, most of the characteristics are shared by at least two provenances. For example, light colored mat tissue was produced by isolates LPg and PPh. All others were dark. Second, in some characteristics one habitat source differs substantially from the others. for example, all MH and LP isolates have a raised, convoluted mat, while six out of eight PP isolates have an appressed, spreading mat. All MH and LP isolates have an even, dense substrate growing edge while seven

Table 3.--Comparison of 1-month-old colonies of Cenococcum isolates from alpine (MH), mid-elevation (LP), and dry pumice plains (PP) habitats.

Characteristic		MH isolates							LP isolates							PP isolates							
		a	b	c	d	e	f	g	a	b	c	d	e	f	g	a	b	c	d	e	f	g	h
Mat tissue	(1) Raised, convoluted	x	x	x	x	x	x	x	x	x	x	x	x	x	x			x					x
	Appressed, spreading															x	x		x	x	x	x	
	(2) Uniformly dark	x	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x	
	Light with irregular dark spots														x								x
Erect hyphae	(3) Uniformly dense	x	x						x	x	x	x	x	x	x	x	x		x	x	x	x	
	Sparse, except in tufts			x	x	x	x	x										x					x
	(4) Dark from beginning			x			x							x									
	Initially light but turn dark	x			x	x		x	x	x	x	x	x		x	x	x		x	x	x	x	
	Remain light		x															x					x
	(5) Mostly longer than 0.5 mm.																x	x	x	x	x	x	
	Mostly shorter than 0 5 mm.	x	x	x	x	x	x	x	x	x	x	x	x	x	x								x
Growing edge	(6) Even and dense	x	x	x	x	x	x	x	x	x	x	x	x		x								x
	Irregular and sparse															x	x	x	x	x	x	x	
Substrate growth	(7) Vigorous	x	x	x	x	x	x	x	x	x	x	x	x			x	x	x	x	x	x	x	
	Slow													x	x								x
Sclerotia	(8) None initiated							x							x								
	Initiated but never matured								x		x			x		x	x	x	x	x	x	x	
	Mature, in substrate	x	x	x	x	x	x			x		x	x										x
	at mat edge	x	x	x	x	x	x			x													x
	on mat surface			x						x													x
Exudation	(9) Stains medium dark		x	x				x						x	x	x	x	x	x	x	x	x	x
	Dark droplets on surface							x						x									x
	None	x			x	x	x		x	x	x	x	x										

out of eight PP isolates have an irregular one. Third, MH isolates generally share more characteristics with LP isolates than either shares with the PP group. Fourth, the appressed mat and long, erect, aerial hyphae developed only on PP isolates.

Characteristics common to all isolates were mostly microscopic. The hyphae, chlamydospores, and sclerotial initials were much the same in both size range and structure. PP isolates tended to form more chlamydospores than LP or MH isolates in fresh cultures, but old cultures showed no such difference.

These morphological differences and similarities between isolates, having been retained through three years of culturing, are unquestionably inherent. They indicate a broad spectrum of variation in the species that is apparent only on agar or liquid culture. In general, there is greater similarity between MH and LP isolates than between either and PP.

CHANGES OF ISOLATES IN CULTURE

Mutations

During three years' culture of 22 isolates, 4 noticeable mutants sectored out-- one each on MHd, LPe, LPf, and PPb. Each of the four was obvious because of a marked color change from dark hyphae and black mat tissue to beige hyphae and light orange-brown mat tissue (figs. 48 to 50). Color change was due to loss or change of pigment in cell walls. In one case, LPf, color change was accompanied by a change in mat form from raised and convoluted to appressed and spreading. In LPe, the dense erect hyphae on the parent mat were completely, absent on the mutant. With these two exceptions, other observed features of the mutants remained the same as the parent isolate. Growth rates, though not measured, showed no obvious change.

Figure 48: Cenococcum isolate LPe--parent colony with erect, black hyphae and bare, light orange-brown mutant. x2.

Figure 49: Cenococcum isolate LPe. Parent colony with original mutation in center, mutant alone on left, parent alone on right. x3/4.

Figure 50: Brown sclerotia formed in pure culture by light-colored mutant of Cenococcum isolate LPe. Note dark parent hyphae intermixed with light mutant hyphae in substrate. x10.

The parent LPe was producing sclerotia at the time the mutant appeared. The LPe mutant produced sclerotia that were structurally identical but orange-brown rather than black (Fig. 50). As yet, the mutants have not been tested for mycorrhiza forming ability. None of them have shown any tendency to revert after 2 years in culture.

Frequency of mutation and mutant survival in nature is unknown. It is interesting to note that several times brown or orange-brown sclerotia have been found nestled amongst Cenococcum mycorrhizae.

Adaptation to Culture

Adaptation to the culture environment by Cenococcum was evident soon after isolation. Initial growth from sclerotia was extremely slow in all cases, particularly with the PP series and MHg. These required about 2 months to reach a colony diameter of 1 cm. During successive monthly subculturing, sample weighing of isolates showed that they gradually grew faster until about six months after isolation. By this time, growth seemed to stabilize for all but the PP series, which were faster growing than at the start but still slower than all other isolates except MHg. Growth rate of the PP series continued to increase until they stabilized at about 11 months after isolation. By this time, growth rate of the PP series averaged substantially more than the other two provenances.

RELATION OF ISOLATE TEMPERATURE RESPONSE TO HABITATE SOURCE

Temperature is one of the more important of many variables that affect growth of mycorrhizal fungi. Fungi in general are quite sensitive to temperature, and mycorrhizal fungi are no exception (Mikola, 1948b; Norkrans, 1950; Moser, 1958). Moreover, seedlings grown in nurseries have mycorrhizae formed by fungi adapted to temperature regimes often entirely different from those of outplanting areas. Determining Cenococcum's responses to different temperatures is a step in evaluating the importance of radical changes of soil temperatures brought about by transplanting on the functioning of Cenococcum mycorrhizae. The experiment described below was designed to provide information on the extent to which different provenances of Cenococcum vary in response to three temperatures.

Experimental Design and Analysis

A split plot experiment with whole plots completely randomized was designed to test effects of temperature on growth of selected isolates of Cenococcum. The experiment was conducted in a darkened National Appliance Company growth chamber, in which temperatures could be maintained within $\pm 1^{\circ}$ C. of those desired. Humidity was held at 80 $\pm$10 %.

The three whole "plots"--temperature treatments of 15°C., 22.5°, and 28°--were replicated twice. Only one growth chamber was available, so replications were run in this random order: 28°, 15°, 28°, 22.5°, 22.5°, and 15°. An additional temperature, 7° was originally planned as well. But during its first run no isolates grew during the allotted period, so it was not repeated.

Nine isolates of Cenococcum formed one set of "subplots." Three of these were the fastest growing, dark-colored isolates from each provenance. (MHc, LPc, and PPb) and three the slowest (MHg, LPa, and PPd), as determined by weighing six 30-day-old colonies of all 22 isolates just prior to the experiment. In addition, the two light-colored isolates (LPg and PPh) and the one isolate from the coastal provenance (later determined to be a different species) were included. Fastest and slowest growing isolates were used to give an idea of the range of variation within provenances without having to deal with the inordinately large number of cultures required if all 22 isolates were to be studied with adequate replication.

The second set of "subplots" was comprised of seven periods of four days each. Seven flasks of each isolate were put into the chamber at the beginning of each temperature run. Every fourth day a flask of each isolate was randomly selected and removed from the chamber. The colonies were immediately extracted, oven dried, and weighed. Thus weights were obtained for each isolate in each temperature run at 4, 8, 12, 16, 20, 24, and 28 days.

All isolate replicates were grown in 250 ml. Erlenmeyer flasks containing 50 mls. of the nutrient solution described on p. 27, thickened with 2 gms. of agar/l. to facilitate floating of inocula on the surface of the medium. Standardizing inocula was important to achieve reproducible results. Differences in age or amount of inoculum can markedly affect growth rates of fungi (Ward and Colotelo, 1960). Standard-sized plugs of colony growing edge are unsatisfactory for Cenococcum, because growing edges of this species vary in mycelial density from isolate to isolate. Moreover, subculturing from colony edges increases the possibility of selecting inherently faster growing hyphae. To avoid these problems,

one-cu.mm. pieces were cut from the surface of the center of each mat with a sharp inoculation spade. With practice, these pieces could be cut by visual estimation to consistently weigh 0.1±0.025 mg. oven dry. Three such pieces were inoculated in each replicate flask. Frequent checking of practice cuttings revealed that oven dry weight of such trio inocula always totaled between 0.30 and 0.35 mg.

Statistical Analysis -

Logarithmic transformation was necessary to equalize variances in the analysis, but even then the variance of the 28° whole plot differed from the other two. Consequently, variance was analyzed in two ways: (1) including only the 15° and 22.5°whole plots, and (2) including all three whole plots. The results of both analyses were identical, i.e., the inferences to be drawn from one paralleled those from the other. Moreover, growth of all isolates at 28° was clearly different from that at other temperatures (see fig. 51). Therefore, it was deemed justifiable to draw inferences from the analysis that included all three temperatures. Tables for both analyses of variance are given in appendix III.

Experimental Results

Results of the experiment clearly demonstrate that the population of Cenococcum sampled is comprised of a complex of different genotypes. These genotypes tend to resemble each other within more than between provenances. But even between isolates within provenances there exist substantial differences in growth patterns and reactions to given temperatures.

The complexity of variation between isolates in reaction to temperature is shown by the mean weights (antilogarithms) of all colonies of each isolate grown at each temperature (fig. 51). Four colonies averaged markedly heavier at 22.5°C. than at 15° or 28°. The other four averaged as heavy or heavier at 15° as at 22.5°. Alpine (MH) isolates did not grow at all at 28°, and the others did poorly.

The ranking of isolates by mean weight changed from one temperature to another (fig. 51), e.g., in many cases one isolate averaged heavier than another at 15° while the reverse was true at 22.5°. In other cases, two isolates that averaged close to the same weight at 15° and 28° differed appreciably at 22.5°. The test of the temperature-by-isolate interaction was significant at the 99% confidence level, so a few examples were tested by the Duncan multiple range test (appendix III). Thus isolate LPa weighed significantly more than PPb or PPd at 15°, but at 22.5° and 28° the reverse was true. MHc did not differ significantly from LPa at 15°, but at 22.5° it averaged heavier and at 28° lighter. Compared with PPb, MHc was significantly heavier at 15°, not different at 22.5°, and lighter at 28°. The evidence here for a great complexity of genotypic variation is strong.

Comparing patterns of growth of isolates at the three temperatures illustrates further this physiological diversity (figs. 52, 53, and 54 -- note that different ordinate scales were required to adequately spread curves in fig. 54 and to compress fig. 53 to page size). Degree of concavity of these growth curves is particularly important because it indicates the ability of an isolate to alternate enzyme pathways and other temperature dependent processes to cope with a new temperature. Of the isolates that grew at 28°, only PPb shows sign of marked steepening. Should

these curves continue at the same trend that shows in 28 days, PPb would before long be much the heaviest of the isolates. At 15°, in contrast, the curve of PPh is much more concave than any other, showing a substantial superiority of that isolate's ability to engage biochemical systems appropriate for that temperature. At 22.5° curves of all PP isolates steepen sharply, as to a lesser extent do those of MHc, LPa, and LPc. Neither MHg nor LPg produced concave curves at any temperature tested, suggesting that better growth occurs at other temperatures.

Isolates do sort out broadly by provenances. For example, neither alpine (MH) isolate was able to adapt sufficiently to a 28° temperature to grow in a 28 days' run. On the other hand, at 7° (not replicated, so not included in the statistical analysis or illustrated) only the alpine isolates grew in 40 days, though but slightly. The temperatures at which they can function thus range lower than those of either mid-elevation (LP) or dry plains (PP) isolates. Moreover if one averages growth of isolates, combining all temperatures (fig. 55), the alpine isolate MHg shows up poorest and dry plains isolate PPh best. Between these extremes, the others sorted into a series in which the mid-elevation (LP) isolates bridged a gap between fast-growing dry plains (PP) isolates and slow-growing alpine (MH) ones. In a multiple range test averaging out days and temperatures, they line up like this (isolates sharing any underline are not significantly different at the 95% confidence level--numerical data in appendix III):

Slowest ⟶ Fastest

MHg LPg MHc LPa LPc PPd PPb PPh

Figure 51: Average weights of Cenococcum isolates grown at 15.0°C., 22.5°, and 28.0°. Isolates MHc and g are from an alpine habitat; LPa, c, and g from a mid-elevation habitat; and PPb, d, and h from a dry pumice plains habitat.

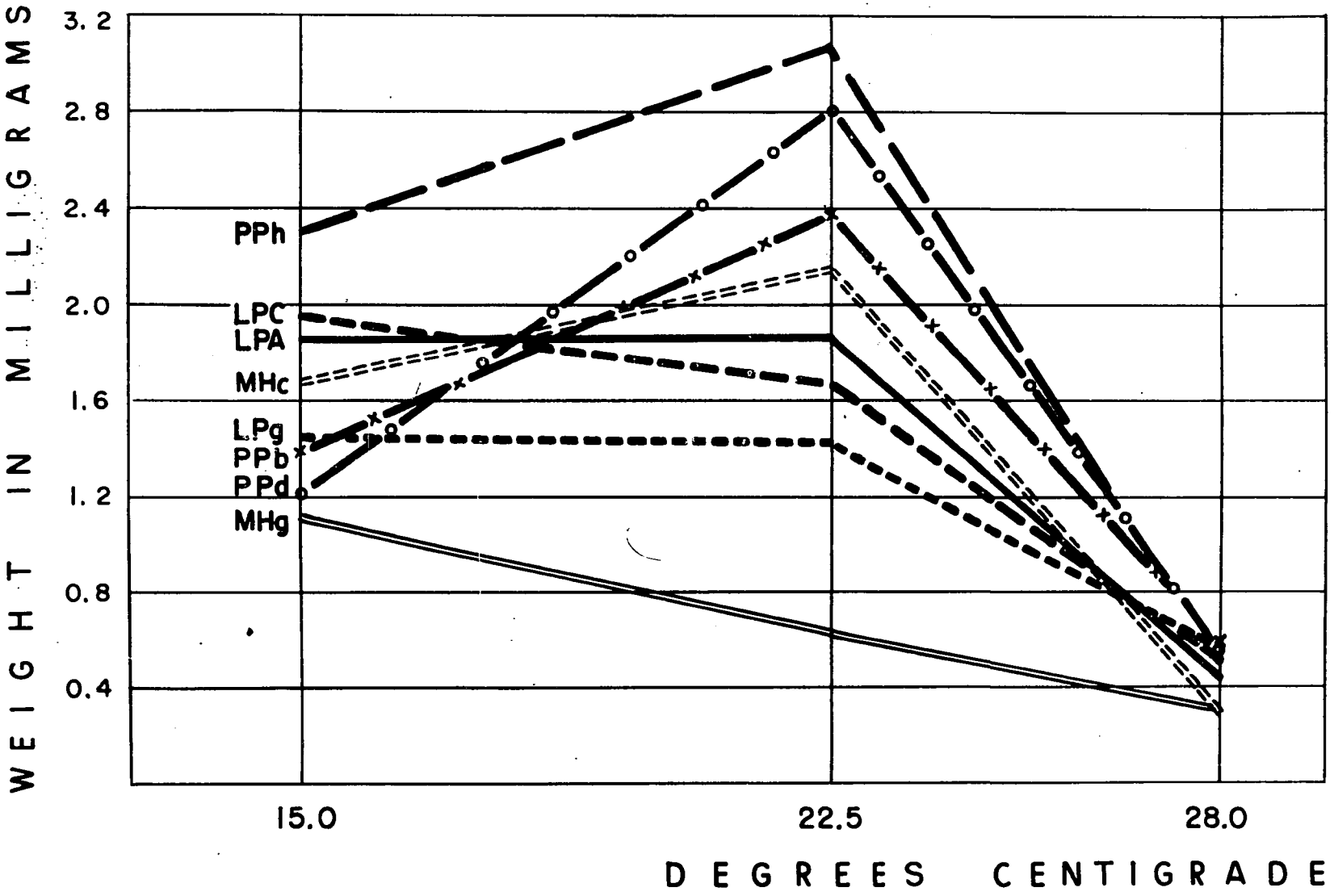

WEIGHT IN MILLIGRAMS
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
PPh
LPC
LPA
MHc
LPg
PPb
PPd
MHg
15.0
22.5
28.0
DEGREES CENTIGRADE

Figure 52: Average weights of Cenococcum isolates grown at 15° C. for 28 days. Isolates MHc and g are from an alpine habitat; LPa, c, and g from a mid-elevation habitat; and PPb, d, and h from a dry pumice plains habitat.

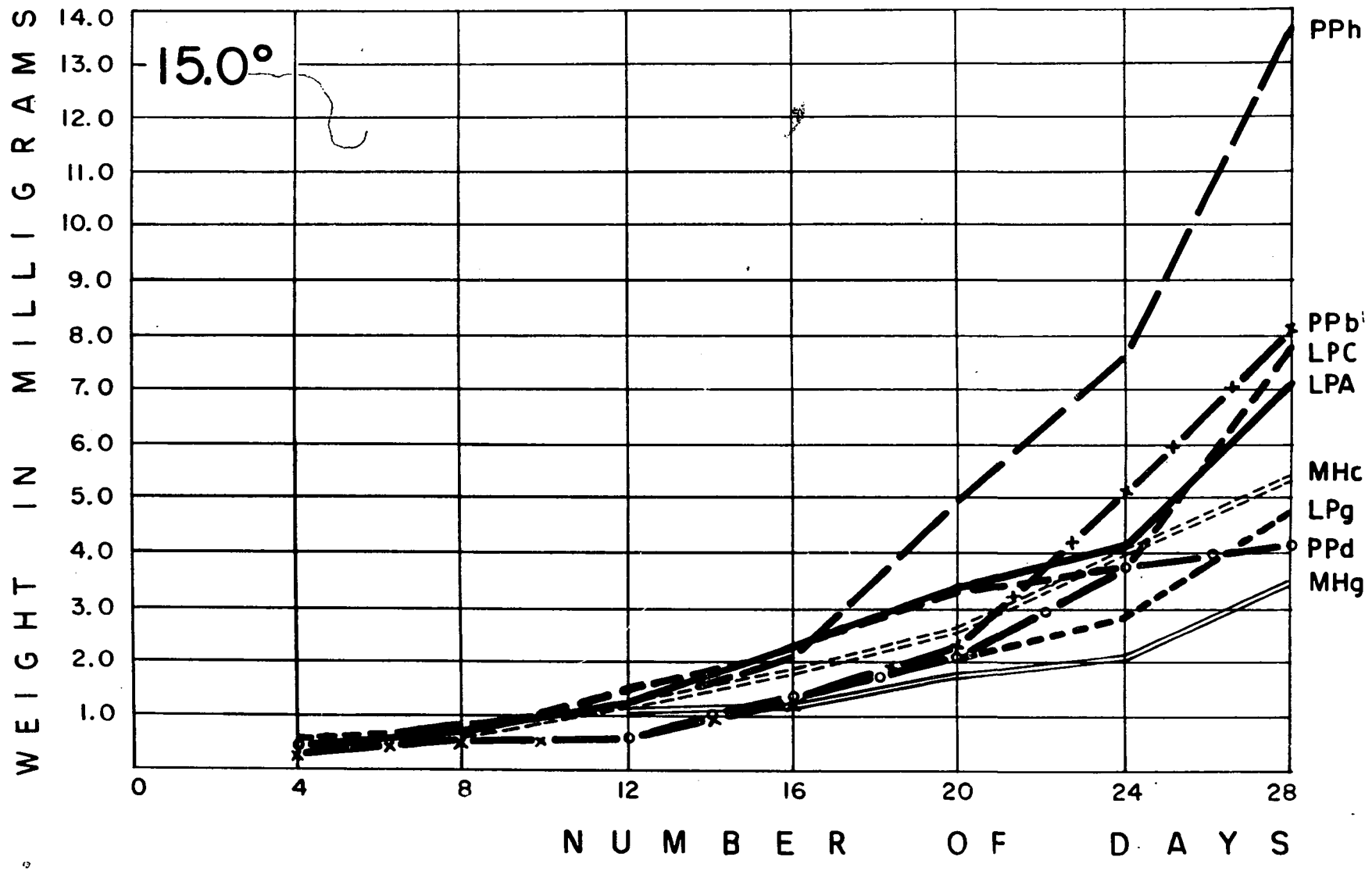

15.0°
WEIGHT IN MILLIGRAMS
14.0
13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
4
8
12
16
20
24
28
NUMBER OF DAYS
PPh
PPb
LPC
LPA
MHc
LPg
PPd
MHg

Figure 53: Average weights of Cenococcum isolates grown at 22.5°C. for 28 days. Isolates MHc and g are from an alpine habitat; LPa, c, and g from a mid-elevation habitat; and PPb, d, and h from a dry pumice plains habitat.

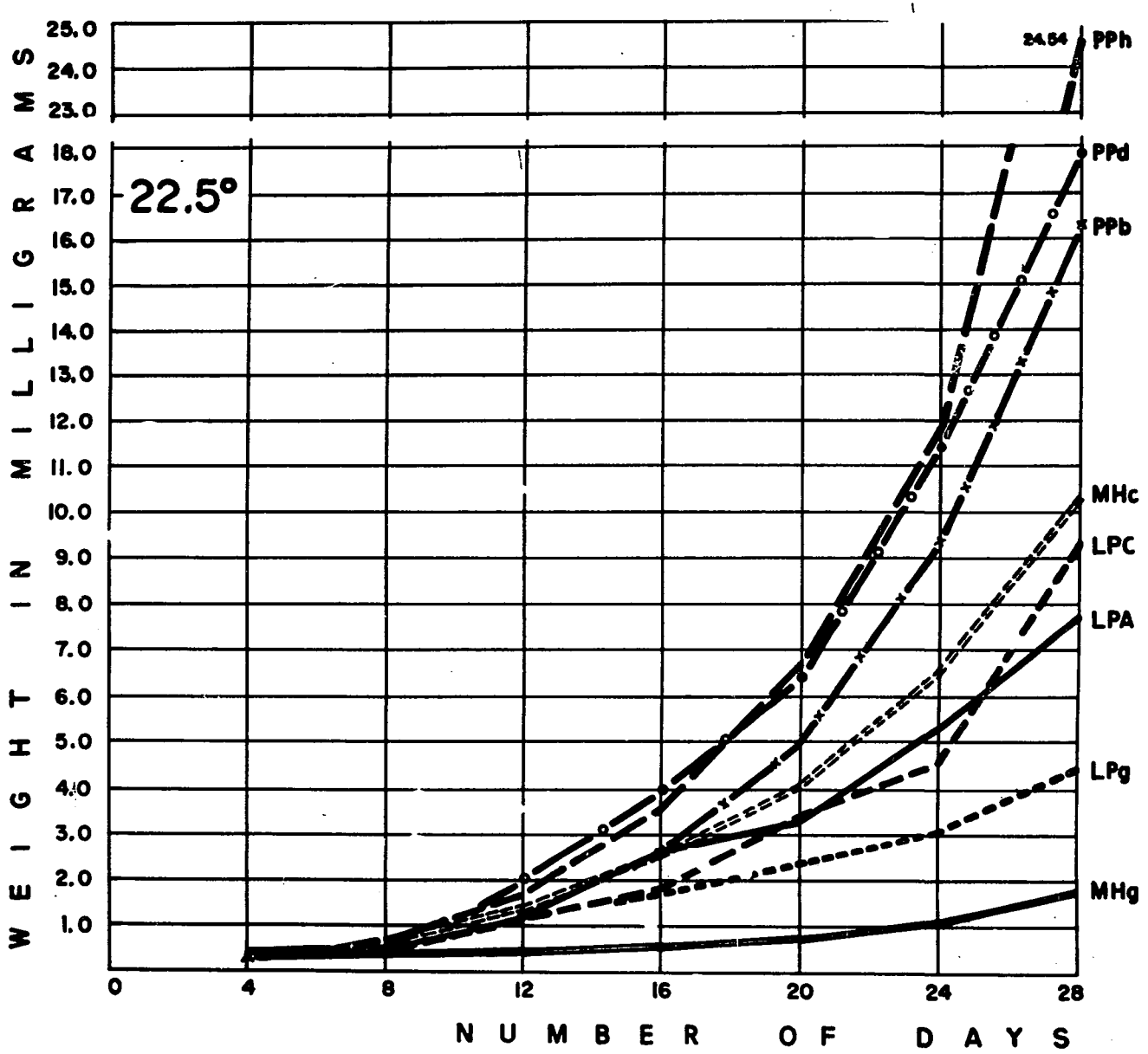

22.5°
24.64
PPh
PPd
PPb
MHc
LPC
LPA
LPg
MHg
WEIGHT IN MILLIGRAMS
25.0
24.0
23.0
18.0
17.0
16.0
15.0
14.0
13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
4
8
12
16
20
24
28
NUMBER OF DAYS

Figure 54: Average weights of Cenococcum isolates grown at 28°C. for 28 days. Isolates MHc and g are from an alpine habitat; LPa, c, and g from a mid-elevation habitat; and PPb, d, and h from a dry pumice plains habitat.

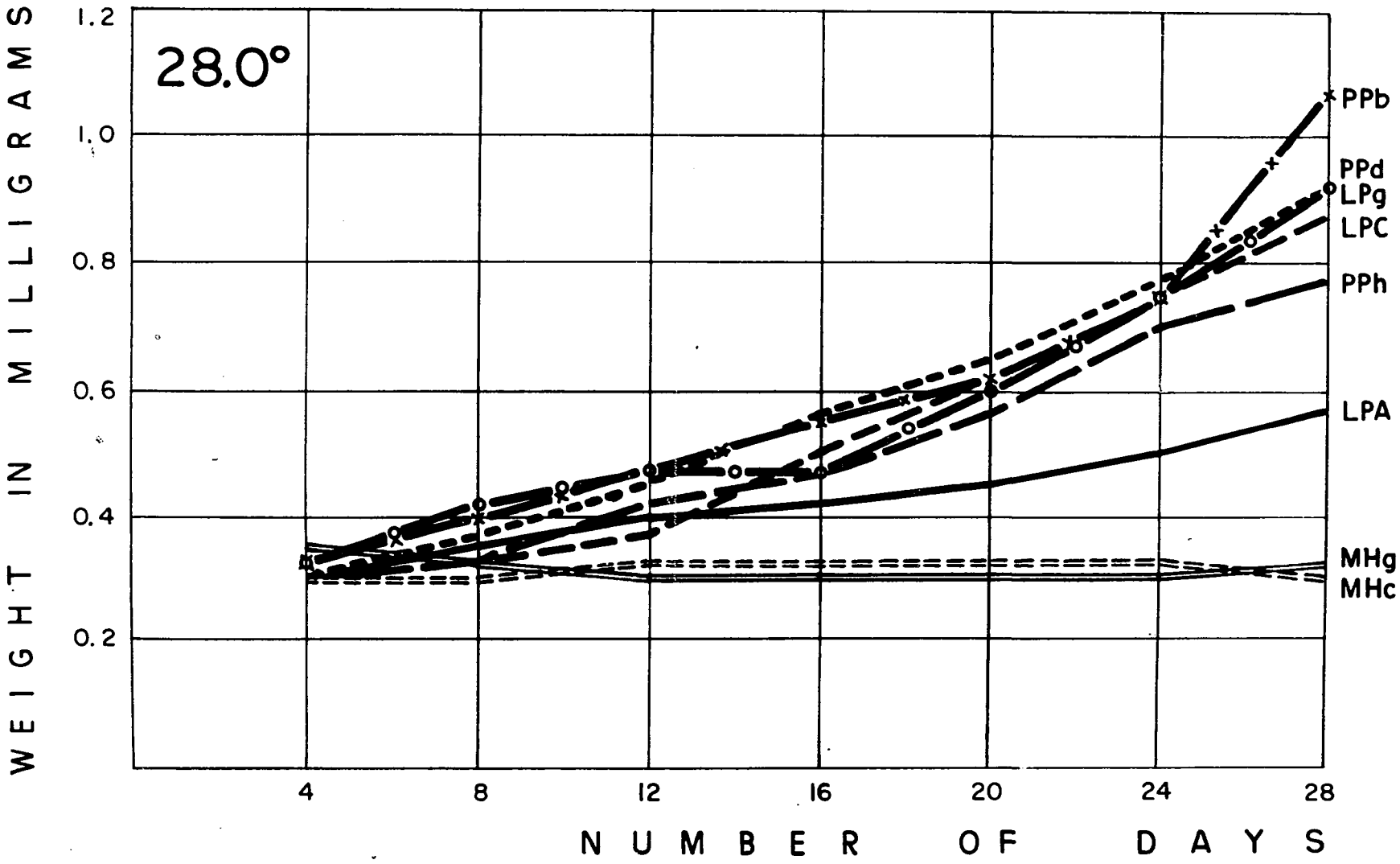
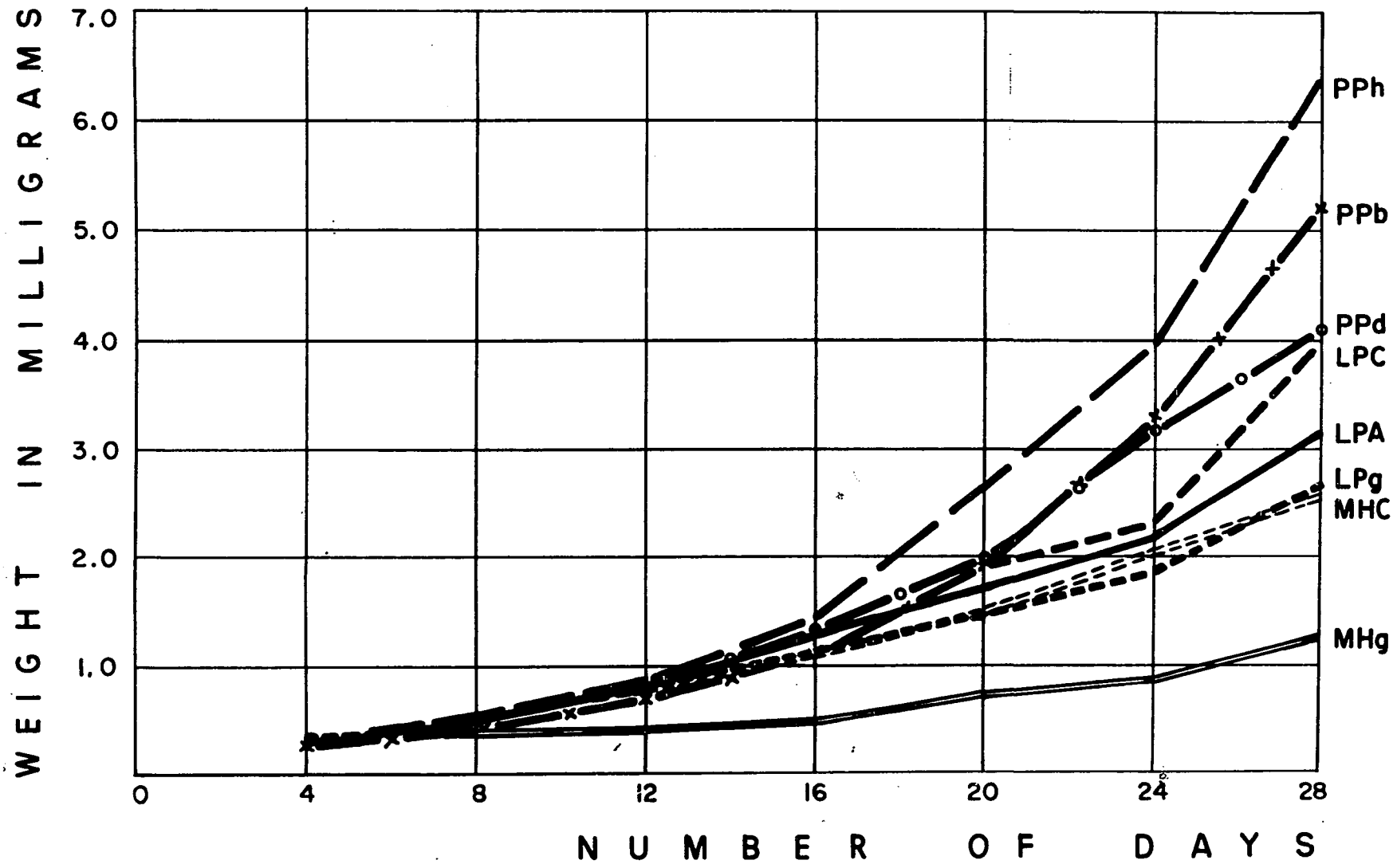

28.0°
WEIGHT IN MILLIGRAMS
1.2
1.0
0.8
0.6
0.4
0.2
4
8
12
16
20
24
28
NUMBER OF DAYS
PPb
PPd
LPg
LPC
PPh
LPA
MHg
MHc

Figure 55: Average weights of 8 Cenococcum isolates combined for three temperatures, grown for 28 days. Isolates MHc and g are from an alpine habitat; LPa, c, and g from a mid-elevation habitat; and PPb, d, and h from a dry pumice plains habitat.


WEIGHT IN MILLIGRAMS
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
4
8
12
16
20
24
28
NUMBER OF DAYS
PPh
PPb
PPd
LPC
LPA
LPg
MHC
MHg

DISCUSSION

EXPRESSION AND SIGNIFICANCE OF GENOTYPIC VARIATION IN CENOCOCCUM

Results of these studies reveal substantial physiological variation between genotypes of Cenococcum, even though differences are not readily evident in field collections. Temperature response is only part of the story--ability to adapt to drought, extremes of pH, limited aeration, and many other environmental factors may also vary between genotypes. Consequently it cannot be said that one isolate inherently grows better or is more able to adapt than another, except in reference to the particular environmental factors studied. Even then, we do not know at present if an isolate would respond in the same way to a given variable when other environmental factors are changed. For example, addition of a limiting metabolite to the substrate can change response of a plant to a given temperature.(Kurtz, 1958).

Having thus circumscribed the application of data from experiments in vitro, how can responses of the Oregon isolates of Cenococcum be interpreted in terms of Cenococcum's behavior in situ? First, its success in colonizing a remarkably broad range of habitats can be attributed in large part to its genetic diversity. Variation between isolates from each of the three habitats sampled was great enough that a large gene pool can be presumed. Moreover, the complexity of different characteristics and temperature responses within each of the habitats implies a means of gene recombination. Though Cenococcum is presently known only as an asexual, monokaryon organism, in all probability it can and does indulge in some type of sexual or parasexual process. One expression of gene recombination in organisms whose distribution is continuous over a wide range of habitats is clinal variation. Intergradation between the provenances of Cenococcum studied in

vitro shows clearly in terms of morphology and temperature response, the general trend being a gradation of character from dry plains isolates through mid-elevation to alpine. There are no indications of distinct ecotypes as would be expected for a completely asexual, monokaryon organism.

The meaning of this plus other available knowlege about *Cenococcum* in respect to nursery inoculation is clarified by recalling the prime objective in inoculating nursery soil with selected isolates of mycorrhizal fungi: to produce on the seedlings mycorrhizae that will function as absorbing organs immediately after outplanting and quickly extend the seedlings' absorbing system. To achieve this purpose efficiently, the ideal fungus for inoculation should be able to form an unequivocally beneficial symbiosis on all hosts raised in the nursery and should be able to adapt to all sites in which host seedlings will be planted. Moreover, as Moser (1958) points out, the fungus must grow rapidly so that nursery inoculum can be readily raised in quantity. No single isolate of any fungus will likely be able to meet all of these criteria. But *Cenococcum*, growing in a tremendous variety of environments and possessing a potentially large number of genotypes, offers good possibilities for finding isolates with unusually broad ability to adapt to environment. Its record as a pioneer fungus in new soils demonstrates that there are many genotypes able to adapt to severe habitats. Moreover, it meets other of the criteria well. It doubtless can form mycorrhizae with all ectotrophic hosts likely to be grown in nurseries. Techniques for growing it rapidly have been developed (personal communication from Dr. L. E. Casida, Jr.). And the non-speculative evidence to date indicates that it benefits the host trees as much or more than other mycorrhizal fungi with which it has been compared.

Much more research is needed, however, before particular isolates of *Cenococcum* can be considered unusually good for nursery inoculation.

Throughout this paper, gaps in knowledge about the phenomenon of mycorrhizae have been both stated and implied. Most of these gaps can be filled only by experimentation. Some of the more pressing questions are:

(1) Is intracellular infection in the cortex of a mycorrhiza a manifestation of strong parasitism by the fungus, or is it a structure promoting more efficient exchange between both symbionts?

(2) Do hyphae regrow vigorously from a mycorrhiza after they have been broken off when the host is transplanted?

(3) How does Cenococcum compare with other mycorrhizal fungi in benefitting the host?

(4) Does symbiotic association with a host change ability of a fungus to adapt to environmental changes?

These few questions offer real challenge to research. Until they are satisfactorily answered, the phenomenon cannot be put to work intelligently to improve survival and growth of planted seedlings.

SUMMARY

Cenococcum graniforme (Sow.)Ferd. & Winge is an important soil fungus because of its ability to form ectotrophic mycorrhizae on a wide number of hosts in a great variety of environments. Though known only as a sterile mycelium, its distinctive black hyphae on mycorrhizae and small, black sclerotia in soil are readily identifiable. Only one species is thought to exist in the genus.

Cenococcum has been reported to occur throughout most of Europe, but knowledge about its distribution from other parts of the world has been scanty or lacking. Field observations made in the Northwest during this study plus examination of soil and root samples supplied from many other parts of the world have resulted in an extension of its known range to Turkey, India, Korea, Puerto Rico, South Africa, and over most of the United States plus southwestern Canada. Its occurrence in the southern hemisphere is reported here for the first time.

In the Northwest, Cenococcum grows over a remarkable range of habitats. It is very common and often predominant on mycorrhizae of hosts growing above the Arctic Circle in Alaska or above timberline in the Cascade Range of Washington and Oregon. In contrast, it thrives as far south as subtropical Florida and even in tropical Puerto Rico. Ubiquitous on hosts in soils at the edge of the desert east of the Cascade Range, it also abounds along the Pacific coastal fog belt and in various soils that are ponded much of the year. It seems able to form mycorrhizae on nearly all ectotrophic hosts, and can occur wherever a host will grow. Ninety-two new hosts were discovered during the course of these studies.

Mycorrhiza formation by Cenococcum on Pinus radiata and Pseudotsuga menziesii var. glauca was observed in pure culture, using a simple,

inexpensive, new technique. This technique was designed to grow fungus and roots together in aseptic culture, while permitting seedling tops to grow in the open. Roots and fungus could be observed through the glass wall of the culture jar.

The course of mycorrhiza formation, as observed in the jar and by microscopic examination of sections of sample roots, began with penetration of hyphal tips through noncutinized root epidermis. This was followed by concurrent development of the fungus mantle around the root, the Hartig net, and strong intracellular infection of the cortex. The resulting ectendotrophic mycorrhiza was identical to many collected from a variety of sites in Oregon.

Although in the past there has been speculation that strong intracellular infection by a mycorrhizal fungus indicates parasitic tendencies, this has not been supported by any evidence. Cenococcum behaves this way on trees in many habitats so severe that the host could hardly survive if the net effect of infection were not beneficial. It can be hypothesized that intracellular infection is a structure promoting better exchange between host and fungus with equal or more justification than that the fungus is "more parasitic."

Because Cenococcum is found on such a variety of hosts and habitats, it has potential as a fungus for nursery inoculation. But since nursery inoculation entails use of one or a few isolates of a fungus, individual genotypes rather than the species as a whole must be considered. To gain insight into the patterns of variation within Cenococcum, isolates were obtained from three different habitats: above timberline on Mount Hood, mid-elevation on Mount Hood, and dry pumice plains of eastern Oregon. These isolates were compared in vitro for inherent differences in colony

morphology and growth at 15°C., 22.5° and 28°. Results showed that inherent variation in Cenococcum is very complex. Isolates varied substantially one from another within each provenance, although a general trend of clinal variation from one provenance to another was discernible. That is, types of colony morphology and temperature response tended to grade from the alpine through mid-elevation to dry pumice plains provenances. This overlapping complex of genotypes implies that Cenococcum some means of gene recombination, even though such has not yet been observed.

Because Cenococcum potentially is composed of a large number of genotypes and adapts to so diverse a range of environments, there is good chance to obtain isolates that will come relatively close to meeting the criteria for an ideal inoculum for nurseries. However, its effects on survival and growth of outplanted host seedlings must be compared with those of other mycorrhizal fungi before it can be recommended. Indeed, research in many aspects of the relation of nursery-formed mycorrhizae to seedling survival after outplanting must be thoroughly investigated before nursery inoculation can be intelligently applied in the Northwest.

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APPENDIX I

NEWLY DISCOVERED LOCATIONS AND HABITATS OF CENOCOCCUM

Mycorrhizae and soils from well over a thousand sites were examined for Cenococcum, resulting in substantial extension of its known range. Most of this was field study, particularly in western North America; but about 250 samples were supplied by various experiment stations of the U. S. Forest Service and by other agencies and individuals cited in the acknowledgements.

The habitats in which Cenococcum was found, as listed below, represent only a fraction of the total number. They were selected to indicate both the geographical range and ecological amplitude of Cenococcum. Tree species listed, unless otherwise indicated, had mycorrhizae with unmistakeable Cenococcum mantles.

Canada

(1) Alberta, N. 53° lat., W. 119° long. Jasper, elev. 1,070 m.; podzol on glacial till; on Picea engelmanni.

(2) British Columbia, N. 51° lat., W. 119° long. Mt. Revelstoke, elev. 1,980 m.; on Abies lasiocarpa.

(3) British Columbia, N. 50° lat., W. 118° long. Kaslo, elev. 550m.; on Pseudotsuga menziesii var. glauca.

(4) British Columbia, N. 50° lat., W. 126° long. Kelsey Bay, Vancouver Island, elev. 15 m.; on Picea sitchensis.

Korea

(5) N. 37°45' lat., E. 127°10' long. Kwang-noong Experimental Forest, Inst. of Forest Genetics, Suwan, Elev. 220m.; sandy loam on granite and gneiss; on Pinus koraiensis, Betula platyphylla var. japonica, and Fagus multinervis (samples collected by Dr. S. Y. Shin).

India

(6) N. 30°22' lat., E. 77°58' long. New Forest, Dehra Dun, elev. 700m.; in soil under Pinus roxburghii, but no Cenococcum mycorrhizae noted (samples collected by Dr. B. K. Bakshi).

South Africa

(7) S. 33°50' lat., E. 19°7' long. 150-yr.-old plantation, elev. 405m.; on Quercus robur (samples collected by Mr. D. G. M. Donald).

(8) S. 33°50' lat., E. 19°7' long. 5-yr.-old windbreak, elev. 395m.; on Salix babylonica (sample collected by Mr. D. G. M. Donald).

(9) S. 33°56' lat., E. 18°50' long. Municipal forest, at least 100 yrs. old, elev. 180m.; in soil under Pinus pinea, but no Cenococcum mycorrhizae noted (sample collected by Mr. D. G. M. Donald).

Turkey

(10) N. 41°11' lat., E. 28°59' long. Belgrad Forest, Elev. 100m.; on Fagus orientalis, Quercus sessiliflora, Castanea sativa, Carpinus betulus, Pinus sylvestris, Pinus pinea, and Pinus pinaster (samples collected by Prof. Dr. H. Kayacik).

United States

(11) Alaska, N. 68°45' lat., W. 104°0' long. Firth River, elev. 610m.; young azonal soil derived from limestone, pH 7.2-7.5; near northern limit of tree growth; on Picea glauca and Salix sp.

(12) Alaska, N. 67° lat., W. 163° long. Kotzebue Sound, elev. 8 m.; arctic tundra; on Salix sp.

(13) Alaska, N. 67° lat., W. 147° long. Chandler River, elev. 270 m.; on Populus trichocarpa.

(14) Alaska, N. 65° lat., W. 142° long. Washington River, elev. 490 m.; on Betula papyrifera.

(15) Alaska, N. 63° lat., W. 142° long. Ladue River, elev. 910m.; on Salix sp.

(16) Alaska, N. 66° lat., W. 148° long. Purgatory, elev. 300m.; on Populus tremuloides.

(17) Alaska, N. 63° lat., W. 142° long. Northway, elev. 530m.; on Salix sp.

(18) Alaska, N. 61° lat., W. 151° long. Kenai, elev. 45m.; on Betula papyrifera and Populus tremuloides.

(19) Alaska, N. 58° lat., W. 135° long. Admiralty Island, elev. 230 m.; lithosol on sedimentary parent material; on Picea sitchensis.

(20) Alaska, N. 57° lat., W. 135° long. Baranof Island, elev. 40 m.; half bog on glacial till; on Tsuga heterophylla.

(21) Alaska, N. 56° lat., W. 133° long. Hollis, Prince of Wales Island, elev. 320 m.; lithosol on argillite parent material; on Tsuga heterophylla.

(22) Alaska, N. 56° lat., W. 133° long. Salmon Lake, Prince of Wales Island, elev. 80m.; podzol on glacial till; on Tsuga heterophylla.

(23) Arizona, N. 35° lat., W. 112° long. Fort Valley, elev. 2,290 m.; volcanic cinder soil, pH 7.0; on Populus tremuloides.

(24) Arizona, N. 32° lat., W. 111° long. Madern Canyon, Santa Rita Mountains, elev. 1,830 m.; on Quercus oblongifolia.

(25) Arkansas, N. 33° lat., W. 92° long. Crossett, elev. 55 m.; Grenada silt loam, pH 4.7; on Quercus falcata.

(26) California, N. 42° lat., W. 121° long. Lassen National Park, elev. 1,615 m.; soil pH 5.7; on Abies concolor.

(27) California, N. 41° lat., W. 122° long. Mineral, elev. 1,620 m.; soil pH 5.6; on Pinus lambertiana.

(28) California, N. 38° lat., W. 121° long. South Fork, American River, elev. 975 m.; on Chamaebatia foliolosa.

(29) Colorado, N. 40° lat., W. 106° long. Front Range, elev. 2,740 m.; on Picea pungens.

(30) Florida, N. 30° lat., W. 92° long. Osceola National Forest, elev. 45 m.; flood plain of small stream; on Pinus glabra, Carpinus caroliniana, and Quercus prinus, also in rhizosphere of Liquidambar styraciflua and Ilex opaca.

(31) Florida, N. 30° lat., W. 92° long. Osceola National Forest, elev. 45m.; Plummer fine sand; on Pinus serotina.

(32) Florida, N. 30° lat., W. 92° long. Osceola National Forest, elev. 45m.; pond site, dry at time of collection but usually covered with 6 in. of water; on Pinus elliottii and in rhizosphere of Taxodium ascendens.

(33) Florida, N. 26° lat., W. 82° long. Corkscrew Experimental Forest, elev. 8 m.; sand over organic pan; on Pinus elliottii var. densa.

(34) Georgia, N. 31° lat., W. 82° long. Wayne County, elev. 30 m.; Leon fine sand, soft organic pan phase; on Pinus palustris.

(35) Georgia, N. 31° lat., W. 82° long. Pierce County, elev. 15 m.; flood plain of Santilla River; on Betula nigra.

(36) Georgia, N. 31° lat., W. 82° long. Charlton County, elev. 30 m.; on Quercus laevis.

(37) Idaho, N. 49° lat., W. 117° long. Bonners Ferry, elev. 550 m.; on Larix occidentalis, Pinus contorta, and Pinus monticola.

(38) Idaho, N. 44° lat., W. 116° long. Idaho City, elev. 1,220 m.; on Pinus ponderosa.

(39) Indiana, N. 38° lat., W. 87° long. Paoli, elev. 240 m.; in soil under Pinus echinata but no Cenococcum mycorrhizae noted.

(40) Kentucky, N. 37° lat., W. 84° long. Baldrock Experimental Forest, elev. 400 m.; soil pH 4.9; on Quercus alba.

(41) Louisiana, N. 31° lat., W. 92° long. Near Alexandria, elev. 35 m.; on Pinus palustris and P. elliottii.

(42) Maine, N. 45° lat., W. 69° long. Bradley, elev. 40 m.; on Thorndike stoney silt loam, pH 4.6; on Pinus strobus, Quercus rubra, and Abies balsamea, also in rhizosphere of Acer rubrum.

(43) Massachusetts, N. 42° lat., W. 73° long. Hopkins Experimental Forest, elev. 305 m.; poor, stoney till soil on steep slope, pH 3.5; on Betula lutea, B. lenta, and B. papyrifera.

(44) Michigan, N. 43° lat., W. 90° long. Calhoun County, elev. 250 m.; on Bellefontaine Series, gray-brown podzol; on Pinus banksiana, P. strobus, and P. resinosa.

(45) Minnesota, N. 47° lat., W. 94° long. Itasca County, elev. 400 m.; glacial outwash, pH 5.2; on Abies balsamea, Quercus rubra, Corylus cornuta, and Salix humulis.

(46) Mississippi, N. 33° lat., W. 91° long. Benoit, elev. 30 m.; Dundee silty clay loam, river alluvium; on Quercus sp.

(47) Mississippi, N. 32° lat., W. 91° long. Vicksburg, elev. 90 m.; Memphis silt loam, loess; on Fagus grandifolia and Quercus sp.

(48) Missouri, N. 38° lat., W. 91° long. Dent County, elev. 90 m.; soil pH 4.8; on Pinus echinata.

(49) Montana, N. 48° lat., W. 113° long. Kiowa, elev. 1,370 m.; aspen groves at prairie ecotone; on Populus tremuloides.

(50) Montana, N. 45° lat., W. 111° long. Hegben Lake, elev. 2,040 m.; soil pH 6.1; on Pinus contorta.

(51) Nevada, N. 39° lat., W. 120° long. Dog Valley, elev. 1,770 m.; on Pinus jeffreyi.

(52) Oregon, N. 45° lat., W. 124° long. Sand Lake, elev. 5 m.; stabilized beach dunes, Cenococcum the sole mycorrhizal fungus; on stunted Picea sitchensis and Pinus contorta.

(53) North Dakota, N. 49° lat., W. 101° long. Turtle Mountain, elev. 670 m.; soil pH 7.3; on Quercus macrocarpa.

(54) Oregon, N.45° lat., W. 124° long. Aloha, elev. 120 m.; 55-year-old Thuja-Pseudotsuga stand; on Pseudotsuga menziesii var. menziesii and Corylus cornuta var. californica.

(55) Oregon, N. 45° lat., W. 123° long. Timberline, Mount Hood, elev. 1,980m.; azonal pumice; on Pinus albicaulis, Abies lasiocarpa, and Tsuga mertensiana.

(56) Oregon, N. 45° lat., W. 118° long. Starkey, Blue Mountains, elev. 1,680m.; Tolo Series; on Pinus contorta, Larix occidentalis, and Pseudotsuga menziesii var. glauca.

(57) Oregon, N. 45° lat., W. 117° long. Wallowa Mountains, elev. 2,290m.; on Abies Lasiocarpa.

(58) Oregon, N. 44° lat., W. 123° long. Crater Lake National Park, Mount Scott, elev. 2,680m.; Cenococcum the sole mycorrhizal fungus; on Pinus albicaulis and Tsuga mertensiana.

(59) Oregon, N. 44° lat., W. 121° long. Fort Rock, elev. 1,520m.; on Cercocarpus ledifolius and Pinus ponderosa.

(60) Oregon, N. 43° lat., W. 124° long. Bandon, elev. 15m.; on Pinus [illegible], Tsuga [illegible], and Picea [illegible].

(61) Oregon, N. 43° lat., W. 123° long. Rogue River, Siskiyou Mountains, elev. 910m.; seep mine soil; on Pinus jeffreyi.

(62) Pennsylvania, N. 42° lat., W. 79° long. Irving, elev. 365 m.; on Pinus strobus, Quercus alba, and Quercus borealis.

(63) Puerto Rico, N. 18° lat., W. 67° long. Maricao Insular Forest; on Pinus sp.

(64) South Carolina, N. 33° lat., W. 80° long. Francis Marion National Forest, elev. 30 m.; plastic clay, pH 4.4; on Fagus grandifolia.

(65) South Dakota, N. 45° lat., W. 103° long. Black Hills, elev. 1,710 m.; soil derived from quartzite and shist, pH 6.0; on Betula papyrifera.

(66) Vermont, N. 44° lat., W. 73° long. Univ. of Vermont Research Forest, Jerico, elev. 180 m.; dry, shallow-soiled ridge, soil pH 3.4; on Fagus grandifolia.

(67) Virginia, N. 38° lat., W. 78° long. Near Charlottesville, elev. 170 m.; on Pinus virginiana and P. echinata.

(68) Virginia, N. 37° lat., W. 80° long. Chatham, elev. 210 m.; on Pinus virginiana and P. echinata.

(69) Washington, N. 48° lat., W. 123° long. Whidbey Island, elev. 45 m.; On Pseudotsuga menziesii var. menziesii.

(70) Washington, N. 48° lat., W. 121° long. North Star Mtn., elev. 2,410 m.; azonal colluvium, 450 m. above timberline; on Larix lyalli.

(71) Washington, N. 47° lat., W. 118° long. Sprague, elev. 610 m.; basalt scabland at ecotone of ponderosa pine savannah and Artemesia semidesert; On Pinus ponderosa and Populus tremuloides (the latter in a moist swale.)

(72) Washington, N. 47° lat., W. 122° long. Mt. Rainier, Yakima Park, elev. 2,440 m.; azonal pumice at 450 m. above timberline; on low, shrubby Abies lasiocarpa, 40 meters from any other vegetation.

(73) Washington, N. 47° lat., W. 124° long. Hoquiam, elev. 60 m.; on Pinus monticola, Pinus contorta, Picea sitchensis, Pseudotsuga menziesii var. menziesii, Tsuga heterophylla, Abies grandis.

(74) Washington, N. 46° lat., W. 122° long. Wind River Experimental Forest, elev. 760 m.; on all of a great many sites and soils examined; on all native and exotic hosts examined.

LOCATIONS OF CENOCOCCUM REPORTED IN THE LITERATURE

The following literature citations account probably for all locations of Cenococcum published heretofore, as shown in figures 17 and 18. Reports in which the identity of Cenococcum is clear from descriptions or illustrations, but the fungus is not specifically named, are asterisked.

Austria: Ferdinandsen and Winge (1925)

Belgium: Ferdinandsen and Winge (1925), Levisohn (1957)

Canada: Rayner and Neilson-Jones (1944), McMinn (1954 and 1955)*

Czechoslovakia: Ferdinandsen and Winge (1925), Sen (1961)

Denmark: Müller (1878 and 1886)*; Ferdinandsen and Winge (1925), Lihnell (1942)

Finland: Ferdinandsen and Winge (1925), Mikola (1948a and 1948b), Siren and Bergman (1951), Heikurainen (1957)*

France: Ferdinandsen and Winge (1925), Boullard and Dominik (1958), Boullard (1959a and 1959b)

Germany: Koert and Weber (1899), Möller (1903)*, Ferdinandsen and Winge (1925), Fritsch (1956)*, Werlich and Lyr (1957), Kreisel (1957a and 1957b)

Great Britain and Ireland: Ferdinandsen and Winge (1925), Rayner and Levisohn (1941), Rayner and Neilson-Jones (1944), Harley and Waid (1955)

Italy: Ferdinandsen and Winge (1925)

Japan: Masui (1926)*

Netherlands: Oudemans (1904)

Norway: Ferdinandsen and Winge (1925)

Poland: Dominik (1957 to 1961b), Dominik and Nespiak (1953), Dominik, Nespiak and Pachlewski (1954a and 1954b), Dominik and Pachlewski (1955 and 1956), Dominik and Ferchau (1956), Pachlewski (1953 to 1955), Pachlewski and Gagalska (1953), Pachlewski and Pachlewska (1960), Truszkowska (1953), Manka and Truszkowska (1958), Frydman (1957), Boullard and Dominik (1958 and 1960), Wojciechowska (1960)

Portugal: Ferreira dos Santos (1951 and 1957), Ferreira dos Santos and Clotilde dos Santos (1951)

Soviet Union: Ferdinandsen and Winge (1925), Aleskovsky (1954)*, Chastukhin (1954)*, Gorbunova (1955), Shcherbakova (1955), Vlasov (1955)*, Trubetskova and Mikhalevskaya (1955)*, Shemakhanova (1957 and 1961), Lobanov (1957)

Sweden: Sernander (1918), Ferdinandsen and Winge (1925), Melin (1927)*, Hatch and Hatch (1933), Hatch (1934), Lindquist (1937), Lihnell (1939 and 1942), Arnborg (1940), Björkman (1942), Mikola (1948b)

United States:

Maryland: Palmer (1954), Hacskaylo and Snow (1959), Worley and Hacskaylo (1959)

New Jersey: Ferdinandsen and Winge (1925), Hatch (1934)

New York: Hatch (1934 and 1937), Kelley (1960)

North Carolina: Dominik and Ferchau (1958)

Oregon: Wright and Tarrant (1958), Lavender and Wright (1960)

Washington: Wright and Tarrant (1958)

Wisconsin: Mikola and Persidsky (1951), Mikola (1955)

APPENDIX II

ECOTROPHIC MYCORRHIZAL HOSTS OF *CENOCOCCUM*

The ectotrophic hosts of *Cenococcum* published previously plus those I observed (asterisked) are listed below. Species not accompanied by author citations are reported for the first time here. Most such species not native to the western states were examined from root samples identified and sent by cooperators previously acknowledged, although some were observed in the Wind river Arboretum.

Gymnospermae

Pinaceae

Abies alba (Pachlewski, 1955; Fritsch, 1956; and others)

- *A. amabilis*
- *A. balsamea*
- *A. concolor*
- *A. grandis*
- *A. lasiocarpa*
- *A. magnifica* var. *shastensis*
- *A. sachalinensis*

Larix decidua (Rayner and Levisohn, 1941)

- *L. decidua* var. *polonica* (Pachlewski, 1953)
- *L. decidua* var. *sudetica* (Pachlewski, 1953)
- *L. laricina*
- *L. lyalli*
- *L. occidentalis*

*Picea abies (Lihnell, 1942; Wojciechowska, 1960; Dominik, 1961a; and others)

*P. breweriana

*P. engelmanni

*P. glauca

*P. mariana

*P. orientalis

*P. pungens

*P. sitchensis (Rayner and Levisohn, 1941)

*Pinus albicaulis

*P. banksiana (Mikola and Persidsky, 1951; Mikola, 1955; Dominik, 1958b)

*P. contorta

*P. coulteri

*P. echinata

*P. elliotii var. elliotii (Zak, 1962)

*P. elliotii var. densa

*P. glabra

*P. jeffreyi

*P. koraiensis

*P. lambertiana

*P. monophylla

*P. monticola (McMinn, 1955)

*P. mugo (Dominik, Nespiak and Pachlewski, 1954a; Dominik and Pachlewski, 1956)

*P. nigra var. poiretiana (Rayner and Neilson-Jones, 1944)

*P. palustris

*P. pinaster

*P. pinea

*P. ponderosa

*P. radiata

*P. resinosa (Hatch, 1934)

P. rigida (Dominik and Ferchau, 1958)

*P. serotina

*P. strobus (Hatch, 1934; Boullard and Dominik, 1958; Dominik and Ferchau, 1958; Boullard, 1959; and others)

*P. sylvestris (Lihnell, 1942; Björkman, 1942; Dominik, 1958; Pachlewski and Pachlewska, 1961; and others)

*P. taeda (Hacskaylo and Snow, 1959)

*P. virginiana (Hacskaylo and Snow, 1959, Worley and Hacskaylo, 1959)

*Pseudotsuga menziesii var. menziesii (Ferreira dos Santos and Clotilde dos Santos, 1951; Wright and Tarrant, 1958; Lavender and Wright, 1960; and others)

*P. menziesii var. glauca

*P. macrocarpa

*Tsuga canadensis (Dominik and Ferchau, 1958)

*T. heterophylla

*T. mertensiana

Cupressaceae

Juniperus communis (Lihnell, 1939)

Angiospermae

Aceraceae

Acer pseudoplatanus (Dominik and Pachlewski, 1955 and 1956)

Betulaceae

Alnus firma (Masui, 1926)

A. glutinosa (Lihnell, 1942; Truszkowska, 1953)

*A. rubra

*A. sinuata

*A. tenuifolia

*Betula fontinalis

*B. glandulosa

*B. lenta

*B. lutea

*B. nigra

*B. papyrifera

B. pendula (Mikola, 1948a; Gorbunova, 1955; Dominik, 1961; and others)

*B. platyphylla var. japonica

B. pubescens (Mikola, 1948a and 1948b)

*B. pumila

*Carpinus betulus (Truszkowska, 1953; Dominik, 1961a)

*C. caroliniana

Corylus avellana (Gorbunova, 1955; Truszkowska, 1953; Dominik, 1957; Wojciechowska, 1960)

*C. cornuta var. cornuta (Hatch, 1934)

*C. cornuta var. californica

Compositae

Lactuca muralis (Boullard and Dominik, 1960)

Fagaceae

*Castanea dentata

*C. sativa

*Castanopsis chrysophylla

*C. sempervirens

*Fagus grandifolia (Kelley, 1960)

*F. multinervis

*F. orientalis

F. sylvatica (Müller, 1886; Dominik, 1957; Boullard and Dominik, 1960; and others)

*Lithocarpus densiflora

*Quercus agrifolia

*Q. alba

*Q. borealis

*Q. breweri

*Q. chrysolepis

Q. faginea (Ferreira dos Santos, 1957)

*Q. falcata

*Q. garryana

*Q. kelloggii

*Q. laevis

*Q. macrocarpa

*Q. moreha

*Q. oblongifolia

*Q. petraea (Dominik, 1957; Wojciechowska, 1960)

*Q. prinus

*Q. robur (Pachlewski and Gagalska, 1953; Aleskovsky, 1954; Shcherbakova, 1955; and others)

*Q. sadleriana

Q. suber (Ferreira dos Santos, 1951 and 1957)

*Q. vaccinifolia

*Q. virginiana

Juglandaceae

Carya spp. (Hatch, 1934)

Leguminosae

Vicia sylvatica (Dominik, 1957)

Rhamnaceae

Rhamnus frangula (Truszkowska, 1953; Dominik and Pachlewski, 1955)

Rosaceae

*Cercocarpus ledifolius

*Chamaebatia foliolosa

Crataegus monogyna (Dominik, 1957)

Dryas octopetala (Dominik, Nespiak and Pachlewski, 1954a)

Rosa pendulina (Dominik, Nespiak and Pachlewski, 1954b; Dominik, 1961b)

*R. gymnocarpa

Sorbus aria (Dominik, 1961b)

*S. sitchensis

S. aucuparia (Truszkowska, 1953; Dominik, Nespiak and Pachlewski, 1954a; Dominik, 1957; and others)

Rubiaceae

Galium schultesii (Dominik and Pachlewski, 1956)

G. rotundifolium (Dominik and Pachlewski, 1956)

Salicaceae

Populus alba (Dominik, 1958c)

*P. angustifolia

*P. balsamifera (Dominik, 1958c)

P. canadensis (Dominik, 1958c)

P. canescens (Dominik, 1958c)

*P. deltoides

P. nigra (Frydman, 1957; Dominik, 1958c)

P. robusta (Dominik, 1958c)

P. tremula (Lihnell, 1942; Siren and Bergman, 1951; Dominik, 1958c; and others)

*P. tremuloides

*P. trichocarpa

*Salix bebbiana

*S. bella

S. caprea (Dominik, Nespiak and Pachlewski, 1954a and 1954b; Dominik, 1961b)

*S. coulteri

*S. geyeriana

*S. hookeriana

*S. humilis

S. kitaibeliana (Dominik and Nespiak, 1953)

*S. lasiandra

*S. lutea

S. myrsinites (Dominik, Nespiak and Pachlewski, 1954a)

*S. petrophila

*S. piperi

S. repens (Dominik and Pachlewski, 1955)

S. reticulata (Dominik, Nespiak and Pachlewski, 1954a)

*S. scouleriana

S. silesiaca (Dominik and Nespiak, 1953; Dominik, Nespiak and Pachlewski, 1954a; Dominik and Pachlewski, 1956)

Saxifragaceae

Ribes alpinum (Dominik, 1957)

Tiliaceae

*Tilia americana

*T. cordata (Lihnell, 1942; Gorbunova, 1955; Wojciechowska, 1960; Dominik, 1961b)

T. europaea (Sen, 1961)

Filicineae

Polypodiaceae

*Dryopteris filix-mas (Dominik and Pachlewski, 1956)

APPENDIX III

ANALYSIS OF VARIANCE TABLES

Split Plot with 15° and 22.5°C. Whole Plots

Source	DF	SS	MS	F
Total	251	60.2303		
Whole Plot	(3)	(.4169)		
Temperatures (T)	1	.4064	.4064	77.1 *
Error a	2	.1054	.0053	
Subplot	(248)	(59.8134)		
Isolates (I)	8	5.4562	.6820	127.9**
Days (D)	(6)	(47.8117)	(7.9686)	1,494 **
Linear	1	47.6794	47.6794	8,940 **
Quadratic	1	.0298	.0298	5.6*
Cubic	1	.0390	.0390	7.3**
Quartic	1	.0634	.0634	11.9**
Residual	2	.000058	.000029	n.s.
I x D	48	2.6130	.0544	10.2**
T x I	8	1.8727	.2341	43.9**
T x D	6	.7887	.1314	24.6**
T x I x D	48	.6097	.0127	2.4**
Error b	124	.6614	.0053	

Split Plot with 15°, 22.5°, and 28°C. Whole Plots

Source	DF	SS	MS	F
Total	377	88.8116		
Whole plot	(5)	(25.9325)		
Temperature (T)	2	25.9212	12.9606	3,452 **
Error a	3	.01126	.0038	
Subplot	(372)	(62.8791)		
Isolates (I)	8	4.8015	.6002	157.3 **
Days (D)	(6)	(40.0828)	6.6805	1,751 **
Linear	1	40.0025	40.0025	10,485 **
Quadratic	1	.0176	.0176	4.62*
Cubic	1	.0207	.0207	5.43*
Quartic	1	.0418	.0418	10.96**
Residual	2	.00017	.00008	n.s
I x D	48	2.4375	.0508	13.3 **
T x I	16	3.5634	.2227	58.3 **
T x D	12	9.9340	.8278	217.0 **
T x D x I	96	1.3498	.01406	3.68**
Error b[1/]	186	.7095	.0038	

1/ Error b is not homogeneous.

MULTIPLE RANGE TESTS

Isolates, Temperatures, and Days Combined

	MHg	SS1/	LPg	MHc	LPa	LPc	PPd	PPb	PPh
Antilogs:	.604	.761	1.037	1.044	1.132	1.177	1.223	1.226	1.501

Comparisons of Individual Isolates

LPa vs. MHc

28.0°: LPa (0.42 mg.) > MHc (0.31 mg.)

22.5°: LPa (1.87 mg.) < MHc (2.16 mg.)

15.0°: LPa (1.85 mg.) = MHc (1.67 mg.)

LPa vs. PPb

28.0°: LPa (0.42 mg.) < PPb (0.56 mg.)

22.5°: LPa (1.87 mg.) < PPb (2.38 mg.)

15.0°: LPa (1.85 mg.) > PPb (1.38 mg.)

MHc vs. PPb

28.0°: MHc (0.31 mg.) < PPb (0.56 mg.)

22.5°: MHc (2.16 mg.) = PPb (2.38 mg.)

15.0°: MHc (1.67 mg.) > PPb (1.38 mg.)

LPc vs. MHg

28.0°: LPc (0.50 mg.) > MHg (0.31 mg.)

22.5°: LPc (1.67 mg.) > MHg (0.63 mg.)

15.0°: LPc (1.96 mg.) > MHg (1.12 mg.)

1/ Not *Cenococcum*.

LPc vs. PPd

28.0°: LPc (0.50 mg.) = PPd (0.54 mg.)

22.5°: LPc (1.67 mg.) < PPd (2.81 mg.)

15.0°: LPc (1.96 mg.) > PPd (1.38 mg.)

MHg vs. PPd

28.0°: MHg (0.31 mg.) < PPd (0.54 mg.)

22.5°: MHg (0.63 mg.) < PPd (2.81 mg.)

15.0°: MHg (1.12 mg.) = PPd (1.38 mg.)

VITA

James Martin Trappe was born in Spokane, Washington, on August 16, 1931, of Esther Louise Koss (Trappe) and Martin C. Trappe. He attended grade school and high school in Spokane, graduating from Lewis and Clark High School in June, 1949. He entered the University of Washington in September, 1949. Work for a Bachelor of Science in Forestry was completed in June, 1953. In June, 1955, he received a degree of Master of Forestry from New York State University, College of Forestry, at Syracuse.

Since graduation in 1953 he has been employed by the U. S. Forest Service.