GROWTH AND DEVELOPEMENT OF *NIVATOGASTRIUM* A GASTROMYCETE RELATED TO *PHOLIOTA*

by

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INTRODUCTION

Nivatogastrium nubigenum (Harkness) Singer and Smith is an unusual gastromycete found fruiting on conifer logs near melting snow in the montane regions of Oregon, Washington, California and Idaho. It was originally placed in the genus Secotium by Harkness (1888), but Singer and Smith (1959) proposed it as the type species of the new genus Nivatogastrium. They derived the name from nivatus and gastrion. The first part of the name refers to the type locality in the Sierra Nevada and also to the fact that the mature sporocarp fades to white. This fungus is ovoid, somewhat flattened and has a very short stalk. When the fungus is cut in half it resembles an agaric (lamellate fungus) button which has not opened. In actuality the fungus is usually totally mature and the brown, contorted tramal plates vaguely resemble contorted lamellae. There is no forcible spore discharge when the gleba is suspended over agar (Miller, personal communication). This lack of forcible spore discharge along with the failure of the fruiting body to fully expand, exposing the gleba, are characteristics of gastromycetes. A fruiting body will occasionally undergo enough expansion of the pileus to partially reveal the gleba. In some of these caps nearly radially arranged tramal plates can be seen (Fig. 43).).

The fruiting habit of this fungus, at the snow line, and its similarity to agarics interested me. I found that Dr. Miller had pure cultures of this species and the dried fruiting bodies from which the cultures were made. Singer and Smith (1959) and Miller (1965) indicated that this fungus was probably a gastroid relative of Pholiota, a lamellate genus in the Cortinariaceae, which opens fully and forcibly discharges spores. The possibilty that a study of the morphology, and growth in culture of N. nubigenum might reveal additional evidence to support or refute the hypothesis lead me to undertake this study.

Four cultures of N. nubigenum were studied and compared with cultures of four species of Pholiota, P. decorata (Murr.) Smith and Hesler, P. scamba (Fr.) Moser., P. spumosa (Fr.) Singer, and P. subangularis Smith and Hesler. These Pholiotas were chosen on the basis of a number of features the fruiting bodies have in common with N. nubigenum. These include similar spores, cystidia, and a lignicolous habitat on conifers. These species are found in the montane regions of the Western United States, although most have a wider range than N. nubigenum.

Descriptions of the sporocarps of all species examined were available in the literature but further microscopic work was done on each collection to determine if any

variations in morphological features existed. The cultures have not been described in the literature before, so complete descriptions were prepared using the procedures of Davidson, Campbell, and Blaisdell (1942) and Nobles (1948, 1958 a&b).

LITERATURE REVIEW

Nivatogastrium nubigenum has gone through a number of name changes as the fungus has been better understood by taxonomists. First described by Harkness (1888) as Secotium nubigenum, Lloyd (1903) confused the name by reporting it in his Mycological Notes as S. rubigenum. He reported that nubigenum was a typographical error of other reporters (p. 139, 1903) however, sixty pages later he admits that the mistake was his misreading of Harkness' label on a specimen and that it was originally published as nubigenum. This error persisted in Coker and Couch's work (1928) on Gastromycetes of the Eastern United States and Canada. They list S. rubigenum (sic) as a synonum for Secotium agaricoides (Czern.) Hollos, along with Secotium warnei Peck and Secotium acuminatum Montagne. Zeller (1941) included all synonyms listed by Coker and Couch, except S. rubigenum (sic), under the name Endoptychum agaricoides Czern. A close study of its taxonomic characteristics has brought about the placement, by Singer and Smith (1959), of this fungus in the genus Nivatogastrium.

Nivatogastrium Singer and Smith (1959) is a monotypic genus with the type specimen nubigenum as described by Harkness (1888). It is distinct from the other members of Secotium since it is found fruiting on conifer logs, a fact which caused Lloyd (1903) to reject the possibility that it could be the same species as S. acuminatum, since the

latter is always found on the ground. Singer and Smith (1959) pointed out that it is the only secotiaceous fungus they know of that fruits on conifer wood. Separation of Thaxterogaster Singer (1951, 1958) and Endoptychum Singer and Smith (1958) from the genus Secotium was based in part on the description by Corda (1854) of original material from Kunze of the type species of the genus, Secotium gueninzii Kunze. He described the spores as smooth and slightly yellowish white. The spores of Nivatogastrium are smooth, however, they are honey brown. S. gueninzii also has a volva which is not found in Nivatogastrium. There are additional characteristics that separate Nivatogastrium from the other genera that have been labled Secotium by early authors, and these will be discussed as the genera are considered.

One genus easily distinguished from Nivatogastrium on the basis of spores is Thaxterogaster (Singer, 1951). This genus is based on the type species T. magellanicum Singer, which has ornamented spores of the type found in the Cortinariaceae. Coexistive distribution of Thaxterogaster and members of Pinaceae and Fagaceae suggests that the fungi form mycorrhizae (Singer & Smith, 1958).

Earlier in this paper it was mentioned that Coker and Couch (1928) listed S. rubigenum (sic) as a synonym for S. agaricoides. Czerniaiev (1845) originally published a description of this fungus under the name Endoptychum

agaricoides. Hollos (1903) changed the genus to Secotium only to have it changed back by Zeller (1941). Zeller (1949) even placed Endoptychum in a different family, Podaxaceae, from Secotium, in the Secotiaceae, on the basis of the condition of the gleba at maturity. Members of the Podaxaceae have powdery gleba at maturity and those in the Secotiaceae do not. Nivatogastrium not only lacks the powdery gleba at maturity but it differs from Endoptychum on several other points. Nivatogastrium has cystidia and spores with apical pores, whereas both are lacking in Endoptychum. The spores of Endoptychum are entire and deep sepia in color whereas those of Nivatogastrium are golden brown. Another important difference is that Endoptychum is not found on wood.

Weraroa Singer is the gasteromycete genus that should be most critically compared with Nivatogastrium according to Singer and Smith (1959). Weraroa has cystidia and smooth spores that are truncate at the apex with larger pores than those in Nivatogastrium. The cystidia of Weraroa are chrysocystidia, according to Singer and Smith (1959), whereas those of Nivatogastrium are leptocystidia. Weraroa has an overall physical appearance which differs greatly from Nivatogastrium. The fungi have slender stalks and conical caps in contrast to the short ovoid Nivatogastrium. Weraroa is found on the ground in grassy areas and not on conifer wood.

The gasteromycetes discussed so far in this paper and a number of others have been compared with various agaric genera, and it has been suggested that there are direct relationships between the two groups. Thaxterogaster has been compared with the genus Cortinarius (Singer, 1951, Singer and Smith, 1958a) on the basis of the warty brown spores, mycorrhizal associations and other characteristics the two genera hold in common. Singer (1951) stated, "There are some data which tend to favor the assumption that Cortinarius is derived from Thaxterogaster rather than vice versa."

Singer (1958) suggested that the genus Weraroa is the most likely ancestor of the Strophariaceae, implying that Stropharia and Psilocybe were probably derived from this genus. Smith (1965) described a specimen of Weraroa that was similar to a species that had been placed in the genus Galeropsis by Singer (1936). Thiers and Watling (1971) noted that the fungus described by Smith differed markedly from Galeropsis in gill and spore color, so they transferred it to Weraroa. Watling (1964) considered Galeropsis "to be an artificial assemblage of secotiaceous fungi." Galeropsis, with bright rusty-brown spores and a narrow germ pore, is similar to Nivatogastrium but, it differs in stature and has a thin gleba (Singer and Smith, 1959).

The similarities between Endoptychum and Agaricus have long been recognized. In 1915 Conard suggested putting

E. agaricoides into the Agaricaceae. Singer and Smith (1958b) stated, "The extremely short spores with their characteristic pigmentation and thick walls are in themselves indicative of Agaricus, and the general appearance of the carpophores corroborates the feeling that this is a gastroid representative of an evolutionary line of which both the genus Agaricus and the group to which Endoptychum depressum belongs partake." Some species of Endoptychum have light-colored spores, but some have spores that are green inside the walls, making them appear green in mass. This suggests Chlorophyllum molybdites, the only agaric with green, thick-walled spores (Singer and Smith, 1958b).

Nivatogastrium nubigenum is believed to be related closely to some species of Pholiota and to other closely related genera described as Pleuroflammula and Kuehneromyces (Singer and Smith, 1959). The species that Singer and Smith (1959) cite as being the most closely related to Nivatogastrium are Pleuroflammula fulvidula Singer, Kuehneromyces carbonicola Smith and "a third but as yet unpublished species with somewhat similar spores, in which the hymenopodium is gelatinous as in many species of Pholiota, and which has large cystidia of the Pholiota spumosa type, occurs in the Western United States." Miller (1965) stated that in morphological features, Nivatogastrium is closely related to the genus Pholiota.

Study of the hypogeous members of the genus Cortinarius by Thiers and Smith (1969) adds to the understanding of the relationship between dark spored agarics and the dark spored gastroid fungi mentioned in this paper. These unusual fungi, like Nivatogastrium, are found in the alpine and subalpine regions of California, Idaho and Oregon. In fact, both N. nubigenum and Cortinarius bigelowii Thiers and Smith (1959) have been collected in July near McCall, Idaho.

Thiers and Smith (1969) stated that these fungi represent species of Cortinarius in which the stipe elongates only slightly or not at all. They noted that in the alpine and subalpine habitat "well-known species have stipes tending to be shorter than those of basidiocarps of the same species collected at lower elevations." In these fungi the veils are membranous, instead of cobwebby, as in most species of Cortinarius, and the basidiocarps remain unexpanded. Thiers and Smith do not place these fungi in the gasteromycetes because they have found spore deposits on the inner surface of the veil. Obtaining spore deposits from some of the species has answered in part whether these spores were discharged or simply released as the basidia disintergrated. These species are described as Cortinarius, but C. wiebeae Thiers and Smith (1969) "shows progressions toward the level of the gastromycetes as represented by Thaxterogaster." These fungi are regarded as "somewhat reduced agaric

forms. Possibly they are agarics recently derived from Thaxterogaster, but we do not subscribe to this. To us who have seen the fungi in question they are Cortinarii in which the stipe elongation has been repressed and the veil considerably strengthened."

The possibility that N. nubigenum could be a reduced agaric, which has lost the ability to forcibly discharge spores is not an unusual theory, but it does bring up the controversy over the relationships between the Agaricales and the Gasteromycetes. Saville (1968) presented a table of fourteen connections between the Agaricales and the Gasteromycetes based on data from Heim (1948) and Singer and Smith (1960). This assemblage of fungi is found in the Secotiaceae, a family that, according to Saville, is not a natural family, but a "polyphyletic assemblage of organisms at nearly the same evolutionary level; i.e., an evolutionary grade, not a lineage." He presented arguments for both the origin of the Agaricales from the gasteromycetes and the opposite situation in which the gasteromycetes are derived from agarics through secotiaceous intermediates. He strongly favors the agaricoid origin stating that it was highly unlikely that the distinctive spore-discharge mechanism, connecting the hymenomycetes to most other basidiomycetes, was a chance developement that occured in fourteen different gasteromycete genera to give rise to agarics. Another point he makes is that some agarics

remain in the button stage, more or less hypogeous, while the spores mature. If mutations occurred that reinforced this tendency they would probably be adaptive for the agaric in the harsh environment, eventually leading to the formation of a gastroid population. Most gasteromycetes are found in dry or otherwise unfavorable conditions, for example Nivatogastrium in snowbanks, so this theory appears to be a likely explanation for the gastroid form. It has been suggested that some gastromycetes represent reduced agarics. Thiers and Watling (1971) indicated that they "believe that all Galeropsis-like fungi are xerophytic derivatives, many of which are, perhaps, quite restricted in distribution, of a whole series of familiar agaric groups." Evidence that these fungi are derived are the well-developed cuticular structure of the stipe and pileus, the well-developed stipe and cheilocystidia, and the lamellar nature of the gleba.

Singer (1951) along with Smith (Singer and Smith, 1960) favor a second theory which proposes derivation of Agaricales from the Gastromycetes. They cite as evidence their derivation of the Russulaceae from the astrogastraceous series of the Secotiaceae (1960). Their argument is based on the lack of connections at the agaric level to link the Russulaceae to any other agaric, so they say it must have been derived from gasteromycetes with similar spores. Heim (1948, 1971), however, believes that this group of fungi is also an example

of reduced agarics leading to the formation of gastero-mycetes.

Smith and Hesler (1968), in their monograph of Pholiota present Nivatogastrium as a gastroid extension of Flammuloides, stating, " It is difficult to visualize a lignicolous fungus fruiting under the cold conditions of the snow-line in the mountains having its direct ancestors hypogeous in the soil." The combination of anatomical characters connecting it to Flammuloides are not known in any hypogeous species, therefore, they regard the genus as a reduced agaric.

Heim (1971), in discussing the problems faced in attempting to find sufficient evidence for any theory of the origin of the gasteromycetes and agarics stated, "we believe that new arguments will spring out of pure cultures and of the experimental study in the laboratory of carpophores,of mutations and teratological cases," and he goes on to mention a need for a deeper knowledge of the chemistry, embryology, and spore walls and pores.

An example of the type of research Heim suggested is found in the study of Psilocybe merdaria (Fr.) Ricken, in culture by Watling (1971). He describes this dark spored agaric which develops normal fruiting bodies and a series of forms ranging from laterally stipitate to gasteromycetoid. The microscopic features of the sporocarps also varied. Giant basidiospores were found in low percentage among the

agaricoid forms and in great numbers in the gasteroid forms. The basidia ranged from normal shapes with two to five strigmata to aberrant forms with elongated sterigmata that had clavate heads. All the various forms of the fungus occurred under the same laboratory conditions and were not induced by reduced light or changing nutrient medium. McKnight (1955) induced Psilocybe mutans (unpublished species) to form gasteromycetoid fructi ications under suboptimal light conditions. He found complete intergradation between agaricoid and gasteroid carpophores within a single dikaryon under different light intensities.

Rosinski and Robinson (1968) reported successful crosses between Panus tigrinus (Bull. ex Fr.) Singer, a typical gilled fungus, and Lentodium squamulosum Morgan, a secotioid form. The question arose (Rosinski and Robinson, 1969) as to whether these two are sufficiently different for one to be identified as a gasteromycete or if this is within the range of genetic variability. They concluded "that the degree of divergence expressed by secotioid fungi from their hymenomycetous counterparts may be extremely subtle," and that "their disposition among the gasteromycetes cannot be made hastily." They suggested that secotioid fungi, including those that have lost their ability to discharge spores violently, should be examined ecologically, physiologically and genetically to gain a better understanding of divergence in secotioid and agaricoid fungi.

MATERIALS AND METHODS

Culture Study

Ten different cultures were studied and described. There were four cultures of Nivatogastrium nubigenum, three of Pholiota spumosa and one each of P. decorata, P. scamba and P. subangularis. These cultures were isolated by Dr. O. K. Miller, Jr. and maintained at the Center for Forest Mycology Research, Madison, Wisconsin. The cultures of P. spumosa (OKM-1547, OKM-1754 and OKM-2310), P. scamba (OKM-7770), P. subangularis (OKM-8341) and P. decorata (OKM-2597) were started from multispore isolates obtained from suspending a section of the pileus over agar with the gills oriented towards the agar. Forcible spore discharge from the gills resulted in a spore deposit on the agar. After the spores germinated, the mycelium was transferred to agar slants in glass, screw cap, test tubes and stored at 5 C as were the cultures of N. nubigenum. The cultures of N. nubigenum were obtained by planting portions of the gleba and trama from the peridium on the agar.

The cultures studied had been maintained by the Forest Mycology Research Labs on 1.5% malt agar. This agar was prepared according to Nobles' formula (1948) as follows:

Difco malt extract.....15 grams
Difco Bacto-agar.....20 grams
Distilled water.....1000 milliliters

It was prepared by adding the malt and agar to 1000 ml of water and autoclaving at 15 pounds pressure for 20 minutes.

This medium was used for determinations of all growth rates. Plastic petri dishes, 15 x 100 mm, were used for the growth studies. These were filled with 30 ml of agar, which was measured with a sterile syringe. Several plates, prepared as described above, were inoculated with mycelium of the fungi used in this study. By this method large amounts of actively growing hyphae, to be used as inoculum, could be grown and stored. These plates are referred to as starter plates.

The petri plates used in each experiment were inoculated with actively growing hyphae taken from the growing edge of the starter plates described above. All dishes were inoculated at the same time for any given study. The cultures used as the source of the inoculum were grown in the dark at 25 C for at least a week before use. A uniform inoculum plug was cut with a #4 cork borer. The plug was lifted from the plate and turned upside down on the fresh agar surface. This procedure sandwiched the hyphae between the two layers of agar. The plates were inoculated at the edge of the dish so that the advancing front could be measured across most of the agar surface. The four replicates of each culture were stacked and each stack of dishes was wrapped in aluminum foil to keep out light and avoid moisture loss.

Four replicates of each culture were grown at each of four constant temperatures, 10, 15, 20, and 25 C. Precision Scientific refrigerated incubators were used to maintain the constant temperatures. A line was drawn on the bottom of the plate from the inoculum plug, through the center, to the opposite side. Measurements were made by marking the bottom of the petri dish to correspond with the advancing margin and measuring from that point to a permanent mark at the edge of the inoculum plug. This distance is equivalent to the radius of the mat. The measurements, in millimeters, were taken every three days, and on days seven, fourteen and twenty-one. The first measureable growth appeared by the third day in most cultures. In addition to growth rates, lethal and/or inhibitory temperatures were found by placing four replicates of each culture at 30 and 35 C in incubators and 5 C in a refrigerator. They were observed for the presence of any growth on days seven, fourteen and twenty-one.

The descriptions of the fungal cultures consisted of growth rates at various temperatures along with various physical attributes of the cultures. These included color, texture, and topography of the fungal mat, odor and agar discoloration. This information is included in the results section of this paper along with the microscopic observations.

Davidson, Campbell and Blaisdell (1938) included in

their descriptions of wood rotting fungi their observations on the presence or abscence of an extracellular oxidase associated with white rots, a condition in which lignin is reduced by the fungus. It has been found that the presence of this enzyme or complex of enzymes can be determined by three methods. The first two methods are based on Bavendamm's (1928) observations that tannic and gallic acids form brown diffusion zones in the presence of this oxidase. Davidson, Campbell and Blaisdell (1938) developed the procedure used for these tests. The third method, described in Nobles (1958), used gum guaiac as the indicator.

The first two tests for the production of extracellular oxidase involve the formation of a brown discoloration zone in gallic or tannic acid agars. The amount of the extracellular oxidase varies in the different species of wood rots. The differences are recorded by rating the intensities of the brown discoloration. Brown rots show no discoloration and are rated as negative. White rots are rated on a scale of one to four. One is a light brown discoloration under the inoculum plug (Fig.1). A rating of four is given for a dark brown diffusion zone extending far beyond the inoculum plug (Fig. 6).

The medium used for these tests is 1.5% malt agar containing 0.5% gallic or tannic acid. Tannic and gallic acids are heat labile and must be added after the sterile agar cools to between 45 and 50 C. The tannic or gallic

acid was added to the agar in an aqueous solution made by cooling sterile distilled water to 45 C before adding the acid. This solution was added to the malt extract agar and mixed until a uniform color appeared. Petri plates were prepared with 30 ml of this agar. Each plate was inoculated in the center with actively growing hyphae cut into plugs with a #4 cork borer. Four replicates of each culture were made. The plates were wrapped in aluminum foil and incubated at 25 C. Observations were made on days seven, fourteen and twenty-one. The intensities of the reactions were recorded and the presence or absence of growth was noted. The extent of the growth and the location of the mycelium on the inoculum plug and plate were also noted. Distinct morphological variations between cultures occured on tannic acid agar. These were recorded photographically.

The third test for extracellular oxidase involved gum guaiac as the indicator. The procedures for this test were described by Nobles (1958). A solution of 0.5 grams of gum guaiac (obtained from Fisher Scientific Company) in 30 ml of 95% ethyl alcohol was used. This was filtered to remove the non-soluble residue. A drop or two of the solution was placed directly on the surface of actively growing cultures. The appearance of a blue color indicates the presence of laccase, which appears to be one of a complex of extracellular enzymes always produced by the fungi that cause white rot (Nobles, 1958). If the blue color did not appear

after an hour the tests were recorded as negative (Table III). The plates were observed closely for the first hour and then every few hours for twenty-four hours and changes were noted.

Microscopic observations were made of each culture and morphological features were recorded by drawings and photomicrographs. The drawings were made with the aid of a Leitz drawing tube on a Lietz SM-Lux microscope. A Leitz automatic camera mounted on an Ortho-Lux microscope was used to take the photomicrographs. Observations were made of cultures grown on 1.5% malt agar. The actively growing margin and older portions of the mat were examined. Drawings were made for each isolate at 25 C. The hyphae were observed in Melzer's solution, 3% potassium hydroxide, phloxine, and water. Color changes due to any of these reagents were noted.

A file pattern was compiled from growth rates, oxidase reactions, mat descriptions and microscopic features. A file pattern or file system is a card index system developed by the Center for Forest Mycology Research of the United States Department of Agriculture. The methods used in preparing the information for this system are discussed in studies of Davidson, Campbell, and Vaughn (1942, see Table I). Data collected from growth on media other than malt extract agar were not included as a part of the file pattern, but were appended to the culture description.

Fruiting Studies

Attempts were made to induce the formation of fruiting bodies in culture. Differences in the appearance of the mats and microscopic features were found during these studies and are reported in the discussion of the cultural characteristics. In the first attempts to induce fruiting the fungi were subjected to various temperatures and lighting conditions using two different media. The first was 1.5% malt extract agar with a layer of sawdust on top. The sawdust was saturated with water and sterilized before dropping 10 ml of it into a 250 ml flask with 40 ml of cool sterile agar. The second medium was prepared by adding the sawdust to 40 ml of agar before it was sterilized. Flasks of each culture were placed in indirect sunlight at room temperature and in the dark at 25, 20, 15 and 10 C. Other flasks were placed in a Sherer Model 2-112 growth chamber with 16 hours of light and 8 hours of dark. The day temperature was 25 and the night was 20 C.

In a second attempt to induce fruiting, wood of Picea engelmannii Parry was used in the media. This conifer was chosen since Nivatogastrium is found fruiting on it in nature. A number of small branches, with diameters of 2.5 cm and less, were broken into 5-8 cm lengths and placed in a liter of distilled water. Two liters were prepared in this way and then autoclaved for twenty minutes at fifteen pounds pressure. The flasks were allowed to stand for a day and

then autoclaved again. After they were autoclaved for a third time the resulting infusion was used in place of distilled water in preparing the agar mixtures. One liter of 1.5% malt extract agar was prepared with this infusion and 50 ml of the agar was put in each 250 ml flask. A piece of autoclaved wood was placed in each flask, with most of the wood sticking above the agar.

The second media was Engelmann spruce infusion combined with 12 grams of Difco Prune extract agar, 20 grams of Emerson's YpSs agar by Difco, and 5 grams of glucose. Extra distilled water was added to the infusion to bring it to a full liter before adding the other ingredients. Fungi were cultured in 250 ml flasks containing 50 ml of this agar. All flasks were inoculated and plugged with cotton and aluminum foil caps. These were placed in a Sherer CEL-25-7HL growth chamber with 12 hours of light and 12 of dark. The day temperature was 20 C and the night temperature was 15 C. The morphological features of the hyphal mats were recorded. Additional observations were made of cultures grown on media of Emerson's YpSs, Prune extract agar and glucose without the infusion. These cultures were grown in petri plates with 30 ml of agar. Plates were placed in the chamber and at 20 C for a month before recording the results.

Microscopic Observations of Sporocarps

The dried fruiting bodies from which the cultures were made were examined. Microscopic observations were made by

taking a section of the dried sporocarp, wetting it with 95% ethyl alcohol and soaking it in distilled water until pliable. The piece of the fruiting body could then be sectioned by hand. These sections were placed on glass slides and a drop of Melzer's solution, 3% KOH or distilled water was added and a cover slip placed on top. All sections were examined under oil emersion with a Leitz SM-Lux microscope. Cystidia, basidia, and spores were drawn with the aid of a camera lucida (Leitz drawing tube). A number of observations were made of the spores and cystidia to determine their size range. The texture of the context tissues and the reactions of the various cell types to each reagent were noted on cards along with the drawings. Comparisons of these observations with the descriptions of Smith and Hesler (1968) and Singer and Smith (1959) are included in descriptions of the perfect stage of each fungus studied. The descriptions appear in the results.

The number which accompanies each culture and sporocarp's name refers to the collection number of the fruiting body. All fungi are from the collection of Dr. O.K. Miller, Jr. and most are stored at the Center for Forest Mycology Research, Forest Products Laboratory, Madison, Wisconsin. The cultures have the same number as the sporocarp and are available from the Center for Forest Mycology Research (CFMR) and the Virginia Polytecnic Institute & State University Herbarium, (VPI).

SYSTEMATICS AND RESULTS

Taxonomic descriptions of the sporocarps are presented first, followed by the descriptions of the cultures. Drawings of microscopic features of both the fruiting body and cultures are placed after the descriptions of the cultures for each of the five species examined. Photographs of the reactions of cultures of N. nubigenum and P. spumosa on tannic acid agar are found after the description of N. nubigenum. Color terms used in describing the sporocarps and witin quotation marks are taken from Ridgway's Color Standards and Color Nomenclature (1912).

A file pattern, based on the work of Davidson, Campbell, and Vaughn (1942), is given at the beginning of each culture description. Table I on the next page lists the meanings of the numbers and letters used in the file pattern. The growth rates of the various cultures are illustrated in graphs with curves for the rates at 10, 15, 20, 25, and 30 C. A comparison of the growth rates at the different temperatures is given in Table II. Results of the oxidase tests are in Table III.

Observations follow each sporocarp and culture description and compare the different species studied. Differences and similarities of the species are discussed in the observations and again in the dicussion.

FILE PATTERN FORM

Table I. Based on Davidson, Campbell, and Vaughn (1942)

Petri dish data...days.

Color of mat

A. White.
B. White, then yellow or brown.
C. Yellow
D. Brown
E. Pink or orange.

Oxidase test

O. Negative gallic acid.
P. Positive gallic acid.
....Intensity 7 days.
....Mat Diam. 7 days.
....Intensity tannic acid.
....Growth tannic 7 days.

Growth rate

F. Rapid over 9 cm 7 days.
I. Moderately rapid over 9 cm 14 days.
M. Medium 5-9 cm 14 days.
S. Slow 2-5 cm 14 days.
V. Very slow less 2 cm.

Appearance of mat

App., raised, Intermediate.
....Aerial mycelium.
....No aerial mycelium
Texture
Margin

Constant temperatures

....20, ...25, ...30,
....35, ...40.
Optimum
Inhibitory

Microscopic features

1. Clamps
2. Chlamydospores
3. Conidia
4. Oidia
5. Basidia
6. Basidiospores
7. Setae
8. Vescicular cells

Microscopic features

9. Stag-horn branches
10. Both submerged and superficial hyphae staining with eosin.
11. Hyaline, fibrous non-staining hyphae present.
12. Sub merged
13. Superficial
14. Incrusted hyphae
15. Crystals
16. Special structures Amorphous refractive bodies.

Appressed.- Not raised above agar.
Raised.- Forming mound on agar.
Cottony.- Erect, rather long mycelium spreading in all directions.
Downy.- Short, fine hairs loosely scattered over surface.
Felty.- Matted with interwined hairs resembling felt.
Woolly.- A dense mass of mycelium of curly, twisted appearance.
Plumose.- Tufts of mycelium with central axis from which short hyphae radiate.
Silky.- Long parallel threads of mycelium like combed silk.
Nodulose.- Forming definite nodes or bunches.
Tufted.- Forming tufts.
Floccose.- Thin, cottony pubescence, collected in minute tufts.

Table I. cont.

Pulverulent.- Having a dusty or powdery appearance.

Constant temperature studies made on mats kept in dark. Inoculum taken from vigorously growing mycelium in Petri dishes. Plates kept at room temperature 24 hours before placing in incubator. Growth given as colony diameter.

The oxidase test is made by placing a square of inoculm on 1.5 percent malt agar in Petri dish to which has been added 0.5 percent gallic acid. White rot fungi form a brown discolored zone in agar; brown rot fungi form no zone.

Tannic acid medium made with 0.5 percent tannic acid.

Nivatogastrium nubigenum (Harkness) Figs. 18-32
Singer and Smith, Brittonia 11: 224. 1959.
Secotium nubigenum Harkness, Bull. Calif. Acad. Sci. 4: 251. 1888.

Sporophore 10-24 mm broad, 15-40 mm tall, subglobose at first to convex to plano-convex or depressed over the disc (Fig. 20), margin deeply incurved to meet the stipe-columella, area of attachment irregular to folded, the peridium rarely breaking away from the stipe-columella (Fig. 22). Peridium color evenly tan to ochraceous or with dark tawny to dark red brown streaks, fading to greyish or white in age, surface smooth, glabrous and slightly viscid. Contents fleshy, somewhat pliant, slighty watery at first, white when faded, odor fragrant, taste mild. Gleba loculate, vertically elongated chambers with mature tramal plates occasionally sublamellate, but not oriented vertically enough to allow spores to fall freely. Most tramal plates contorted, forming irregular chambers, not becoming pulverulent at maturity. Color rusty or dull cinnamon-brown. Stipe-columella, stipe portion equal to subbulbous, 5-25 mm long, 5-12 mm thick, context becoming rusty brown, this color not extending into the columella which is narrower than the stipe and nearly white in color (Fig. 21). Surface of stipe matted-fibrillose, dry, tan to ochraceous, fading to greyish white or buff in age, white rhizomorphs at the base. Veil white to buff, cortina like, occasionally breaking away to expose the irregular lamellate gleba. Columella attached to gleba

throughout or sometimes breaking away near the veil.

Spores 7-11 x 5-7 μ, smooth, elliptical, with a short eccentric sterigma and very narrow germ pore, thick-walled, rusty brown with a lighter endosporium, appearing golden ochraceous to honey color in 3% KOH (Figs. 24, 26, 28). Basidia 17-21 x 6-8.2 μ, hyaline, clavate, 4-spored or rarely 2-spored, sterigma apical to slightly curved inward, narrowly conic (Fig. 27). Cystidia abundant, protruding conspicuously above basidia(Fig. 18), fusoid-ventricose,narrowly to broadly clavate, some with extremely long necks and undulating walls, apex obtuse, walls mostly thin, some thick-walled cystidia in all collections except OKM-3090 (Figs. 22, 25, 29, 30). Peridium with an epicutis of narrow filamentous hyphae, 3-5 μ in diameter, gelatinous, forming a layer 15-35 μ thick, hyaline, yellowish in 3% KOH and Melzer's solution. Trama of the pileus of interwoven to subparallel, irregularly enlarged hyphae (Fig. 32), up to 16 μ in diameter, thin-walled, hyaline, appearing bright yellow to golden brown in 3% KOH. Trama of the lamellae made up of two layers (Fig. 19); the subhymenium of large inflated cells up to 20 μ in diameter, hyaline and highly refractive; interior to the subhymenium is a subparallel, floccose central strand of thin-walled, smooth hyphae, 5-10 μ in diameter, hyaline in H_2O, yellowing in 3% KOH.

All hyphae non-amyloid, clamp connections present.

Collections examined: U.S.A. Idaho OKM-3090, OKM-4003, OKM-4004, OKM-4006 (VPI)

Habit, hatitat and distribution: OKM-4003, 4004, and 4006 were found on conifer logs, either Picea engelmannii or Abies lasiocarpa (Hook.)Nutt.,near snow. Upper French Creek, Payette National Forest, Idaho, June 15, 1966. OKM-3090 was found on conifer limbs on the ground near melting snow. Brundage Mountain, McCall, Idaho, July 10, 1965. All collections made by Orson and Hope Miller.

Observation: The Nivatogastriums examined fit into the description of N. nubigenum of Singer and Smith (1959). A character not previously reported and observed in all but the OKM-3090 collection, was the presence of thick-walled cystidia. The majority are thin-walled cystidia, but the thick-walled ones are common enough to be seen in most slide mounts of sectioned material. They are hyaline and of the same size as the thin-walled cystidia. The wall thickenings are in the upper portion of the cystidia and do not extend into the apex. See figures 26 and 27 for comparisons of the two types of cystidia. Collections OKM-4003, 4004, and 4006 were collected on the same day and in the same general area, so it is possible that they are all from the same population. OKM-3090, which did not have any thick-walled cystidia, was collected the previous year in a different area.

Nivatogastrium nubigenum (Harkness) Singer & Smith (Figs. 1-4,9,10,14,15,33-68). File Pattern for cultures OKM-3090, OKM-4003, OKM-4004, and OKM-4006: APM 1,4,8,11,and 16.

Cultural Characteristics

Growth Characteristics- Growth rate at 25 C is medium, radius 3-4.2 cm in two weeks. OKM-3090 showed the lowest average growth rate for day 14 of 3 cm, followed by OKM-4006 with 3.3 cm growth. OKM-4003 and OKM-4004 both had an average rate of 4.2 cm on day 14 (Table II). Optimum temperature range 20-25 C (Figs. 33-36). Inhibitory temperature 35 C, showing no growth after two weeks. OKM-4003 and OKM-4004 grew after being removed from two weeks in a 35 C incubator to a 25 C box. OKM-3090 and OKM-4006 did not revive at 25 C.

Mat Appearance- Advancing zone even and very thin. Mat white, appressed, felty, and older portions becoming pulverulent. OKM-4004 becomes pulverent very near the edge of the mat, whereas, the others are only pulverulent in the area of the plug. Odor fruity at first becoming musty.

Oxidase Tests- Variable (Table III). All cultures showed a strong reaction on gallic acid agar. Only OKM-3090 showed any growth on the gallic acid media and this was only on the top of one inoculum plug. Tanic acid agar reaction varied. OKM-4003, OKM-4004 and OKM-4006 showed only slight discoloration under the inoculum (Figs. 1,2,8). Each of the replicates of OKM-3090 were different in their appearance.

About half of the tanic acid agar plates were weakly discolored, resembling the other three N. nubigenum cultures, but the rest showed strong discoloration with growth around the base of the inoculum plug (Figs. 3, 4, 14, 15). The gum guaiac tests also showed variation (Table III). OKM-3090 and OKM-4006 turned milky by the third minute. OKM-3090 was greenish-blue in 20 minutes and OKM-4006 took an hour to turn slightly greenish-blue. OKM-4003 and OKM-4004 became milky within 7 minutes and it took over 12 hours to turn a pale greenish-blue, and were recorded as negative reactions.

Morphological Characteristics- Hyphae of two types: 1. Cylindric, thin-walled hyphae 2-3 μ diameter, usually 20-30 μ in length, clamps rare (Figs. 37, 44, 51, 56, 63, 65, 66). 2. Large cylindric to inflated cells, enlarged where attached to the next cell in a line or where branched, thin-walled, 6-15 μ diamter, most are 50-75 μ long, without clamps (Figs. 39, 41 , 47-49, 58, 59). Both hyphal systems contain hyaline hyphae in 3% KOH. In Melzer's solution there are both hyaline and golden-brown hyphae. Phloxine stains some of the hyphae deep pink while others remain hyaline. Cystidial end cells present, variable in shape, usually inflated with tapering ends, thin-walled, hyaline in 3% KOH, golden-brown in Melzer's solution, and deep pink in phloxine (Figs. 40 , 46, 54, 55).

Oidia are present in all four cultures, variable in size, 5-8 x 1-2 μ, thin-walled, hyaline in 3% KOH and Melzer's

solution. Found in the advancing margin and in great abundance in the older portions of the mat. Figure 52 illustrates a section of hyphae breaking up to form oidia. This was readily visible when stained in Phloxine so the contents, stained deep pink, could be seen separating.

Amorphous refractive ovoid to round bodies are abundant ranging in size from 5-25 μ in diameter. They are both terminal on short stalks (Figs. 42 , 61) and intercalry (Figs. 43, 50, 60). A well defined wall never seems to develope and the contents appear to be irregular in density. Hyaline in 3% KOH, golden in Melzer's solution and deep pink in phloxine, some only staining centrally (Figs. 39, 50, 61).

Tissue types, as defined by Korf (1958) and adopted by Miller (1971) to apply to agaric culture tissues, are: 1. "Textura intricata"(Fig. 57) which is the dominate type in all four cultures, made up of irregularly shaped interwoven hyphae with space between the cells, hyaline in 3% KOH, golden brown in Melzer's solution and deep pink in Phloxine. These interwoven cells make the mat tough and when stained and viewed under high power, they give the appearance of strands of darker cells amongst the undifferentiated hyphae. 2. "Textura globosa"(Figs. 67, 68) made up of round to ovoid cells with thin-walls, intercellular spaces, together in a highly refractive substance, the entire mass hyaline in 3% KOH, golden brown in Melzer's solution, and deep pink in Phloxine. The tissue types are arranged very

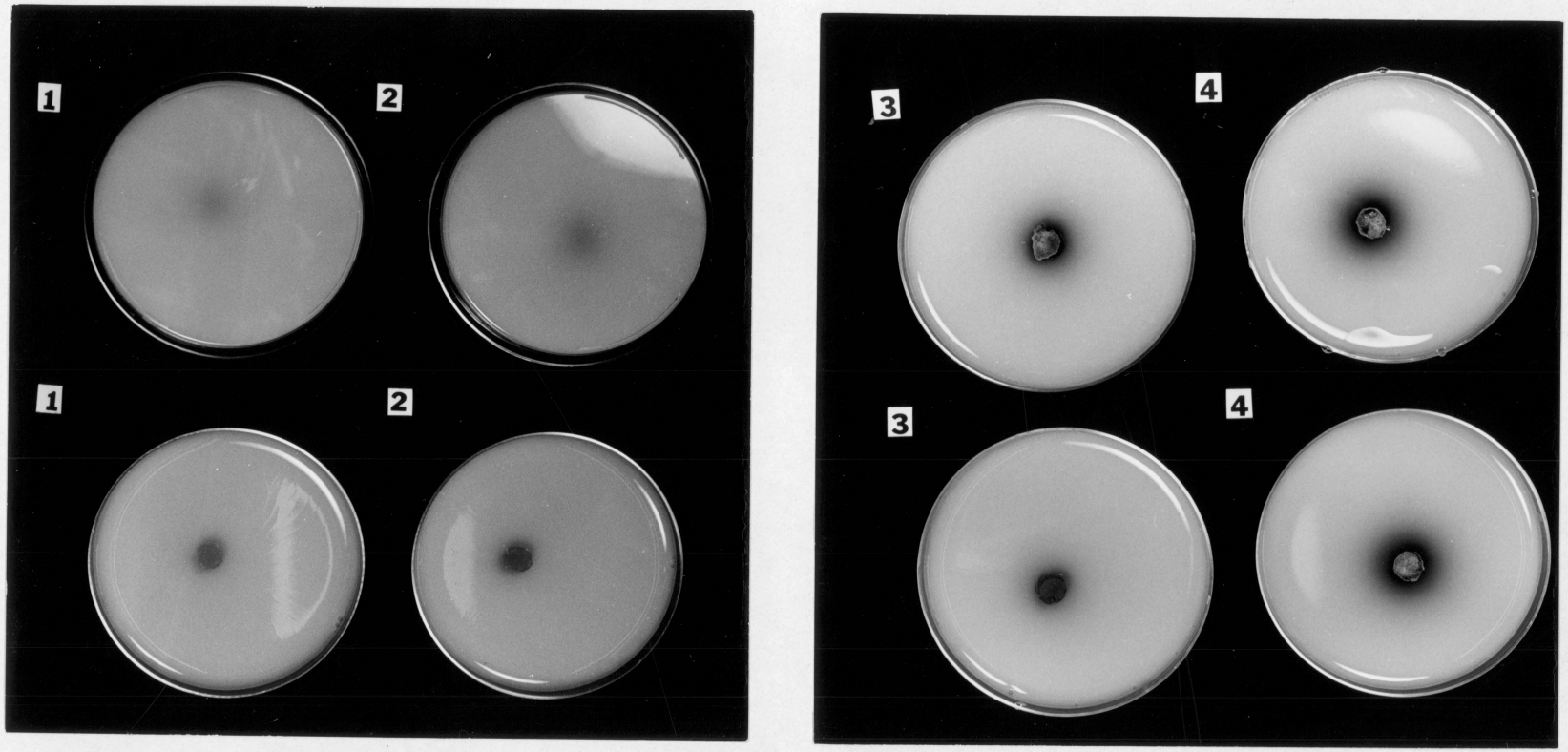
similarly to those illustrated for Pholiota spumosa culture OKM-1547 (Fig. 92).

Cultures grown on media other than the 1.5% malt agar, showed different growth habits. The first fruiting attempt was carried out on a mixture of sawdust and 1.5% malt agar. Flasks were placed at room temperature in indirect sunlight OKM-3090 was the only culture showing any singnficant difference in appearance from the plates grown for the growth study. White mounds on the mat began to appear by the sixth week. Some mounds were as large as 3 mm in diameter and 2 mm high, the majority were only 1-2 mm across. Microscopic examination of these mounds showed them to be made up of oidia and the smaller cylindric hyphae. Cultures of OKM-4004 and 4003 also showed these white mounds when grown in the chamber with 18-25 C temperatures and a cycle of 14 hours of light and 8 of dark. OKM-4006 remained appressed in both cases. OKM-3090 showed definite signs of clocking, rings of heavy white followed by thinner areas, in the chamber.

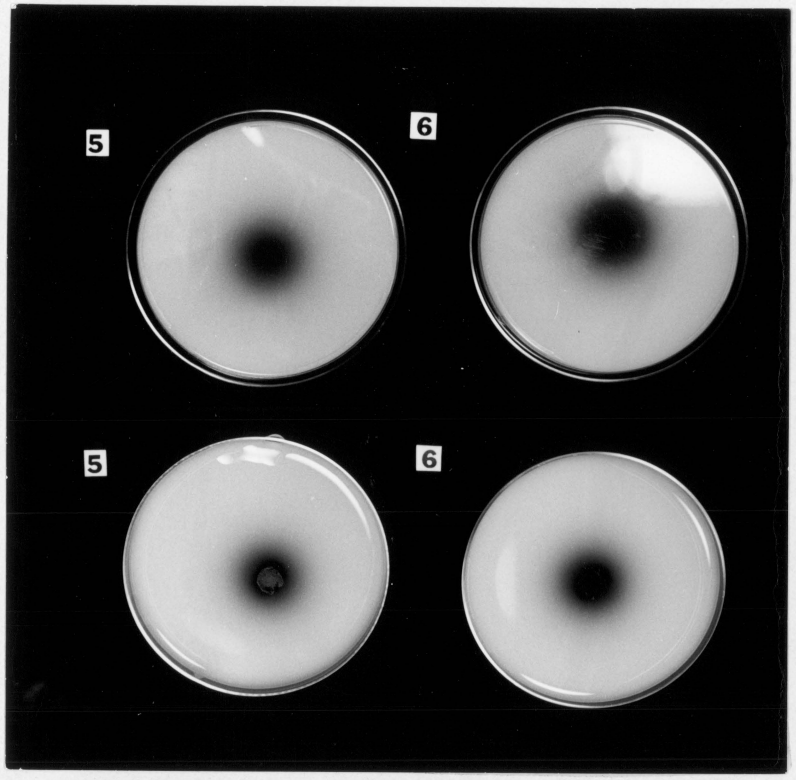
On both other media tested, 1.5% malt extract agar and Prune extract, Emerson's YpSs agar glucose medium, both with Engleman spruce infusion, OKM-3090 showed similar characteristics to its growth on malt extract and sawdust agar. On the agar with the infusion and Englemann spruce stick the mounds of oidia could be seen. On the other agar the clocking could be seen. Neither flask exhibited characteristics of the other even though they were grown in the same growth

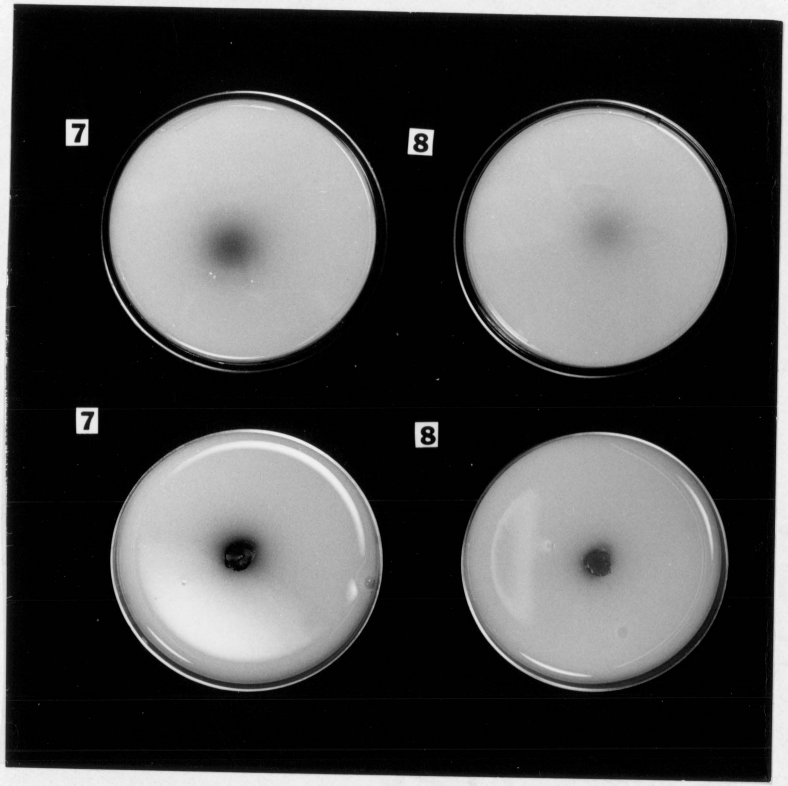
chamber at the same time. OKM-4006 did not changed any on either agar. OKM-4003 and 4004 developed large golden droplets on the surface of the mat in the malt extract agar with the Englemann spruce infusion and stick.

Observations: OKM-4003 and OKM-4004 appear to be nearly identical in all cultural characteristics examined. OKM-4006 was similar to the first two but had a slower growth rate at most temperatures and showed a slightly stronger reaction on tannic acid agar in a few cases where it was intensity two instead of only one. OKM-3090 had the slowest growth rate with the optimum temperature around 20 C instead of 25 C for the other three (See Table II). All four cultures were similar in microscopic characteristics and these in turn were similar to P. spumosa and P. decorata in the presence of oidia, inflated cells, amorphous refractive bodies and"textura intricata".

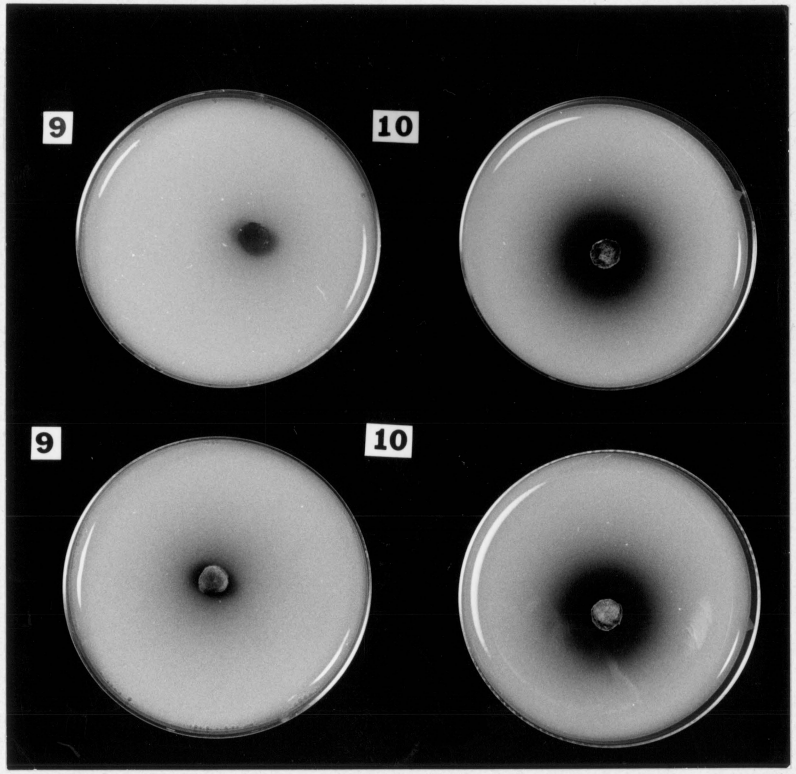


Figs. 1-4. N. nubigenum cultures on tannic acid agar after 7 days. Fig. 1. OKM-4003, zone intensity of one. Fig. 2. OKM-4004, zone intensity of one. Fig. 3. OKM-3090, growth on inoculum plug in top dish, zone intensity of three; no growth on inoculum plug in lower dish, zone intensity of one. Fig. 4. OKM-3090 subcultured from tannic acid agar, growth on both inoculum plugs, zone intensity of four.

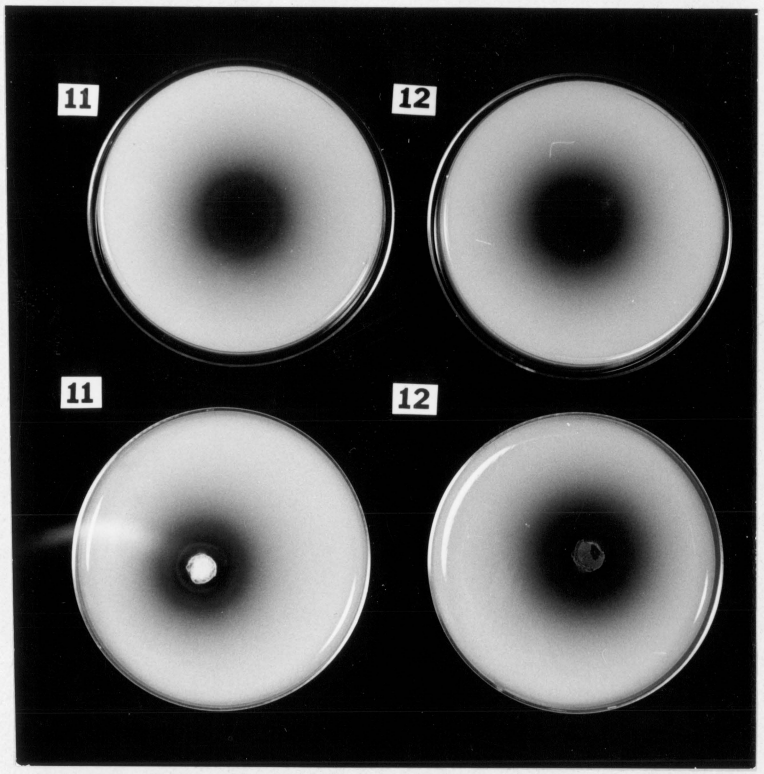


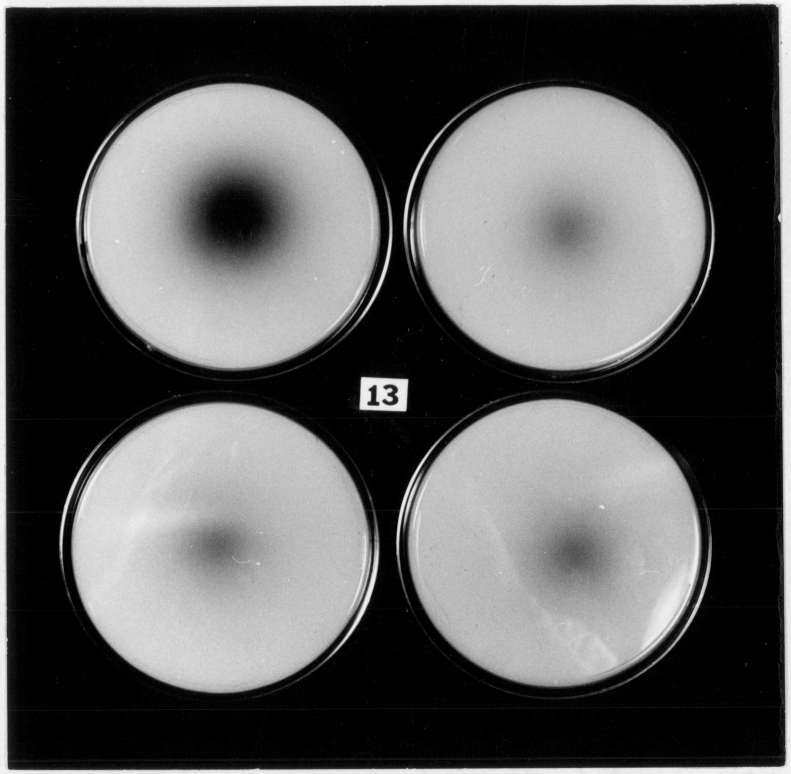


Figs. 5-8. Cultures on tannic acid agar after 7 days. Figs. 5-7. P. spumosa: Figs. 5. OKM-1754 zone intensity of four in both dishes; top dish shows under side of agar; lower dish shows fuzz on inoculum plug. Fig. 6. OKM-1547, zone intensity of four; growth too sparse to be seen. Fig. 7. OKM-2310, zone intensity of two; no growth. Fig. 8. N. nubigenum OKM-4006, zone intensity of one, no growth.

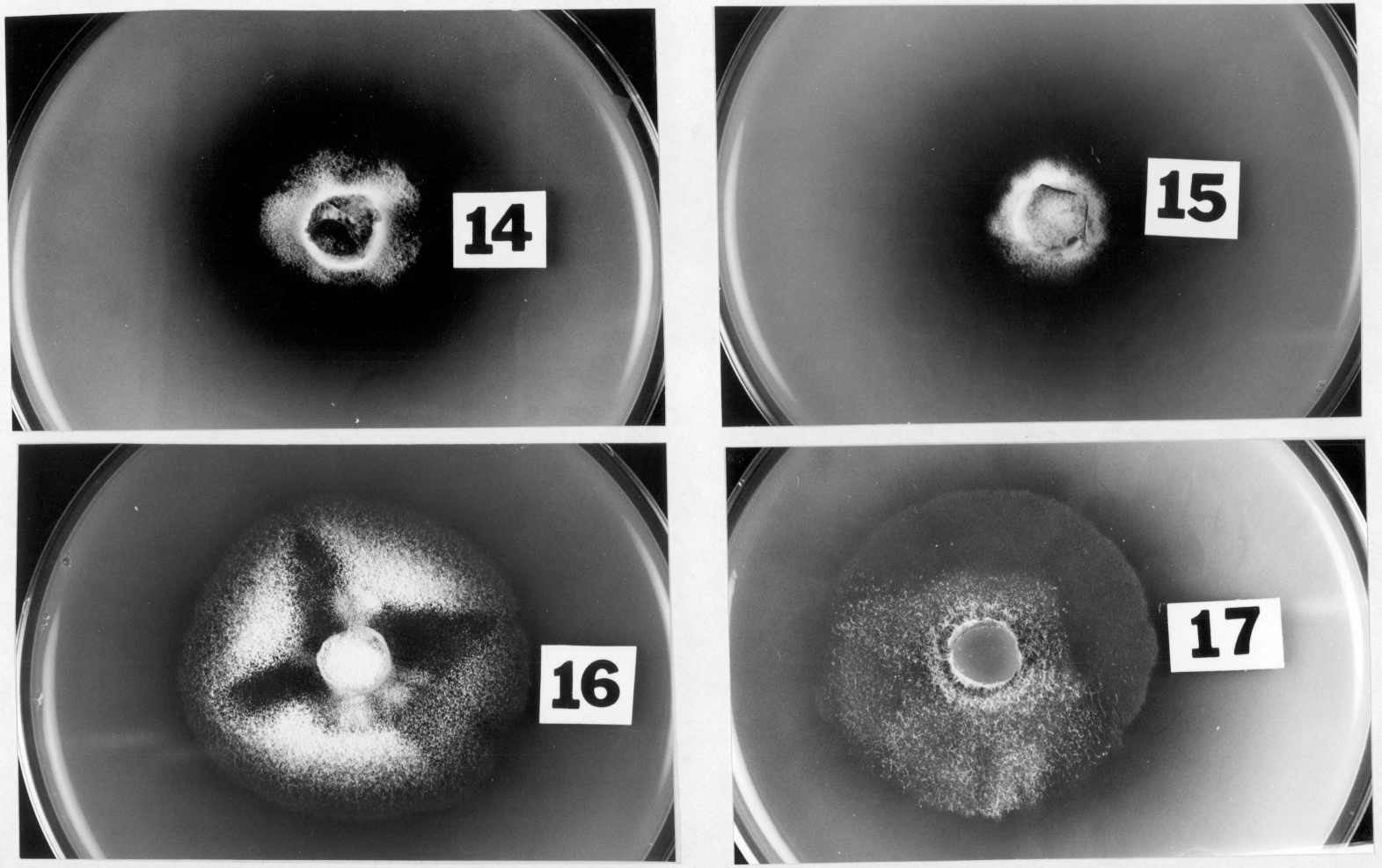


Figs. 9-10. N. nubigenum OKM-3090 cultures on tannic acid agar on day 14. Fig. 9. OKM-3090, top dish has no growth and a zone intensity of one; lower dish has fuzz on inoculum plug and a zone intensity of four. Fig. 10. OKM-3090 subculture from tannic acid agar, both dishes have zone intensity of four and fuzz on the inoculum plug.

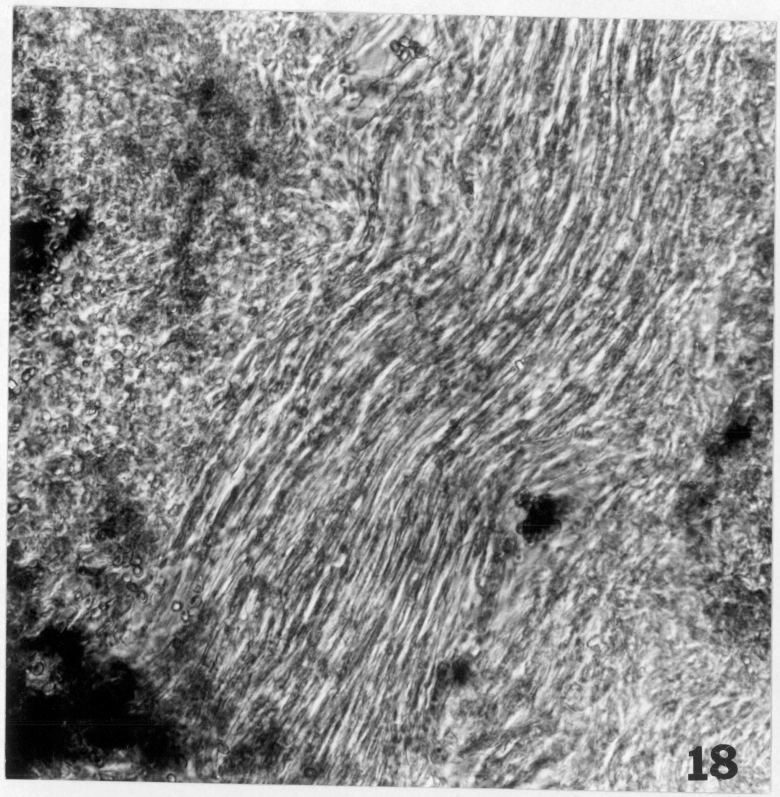


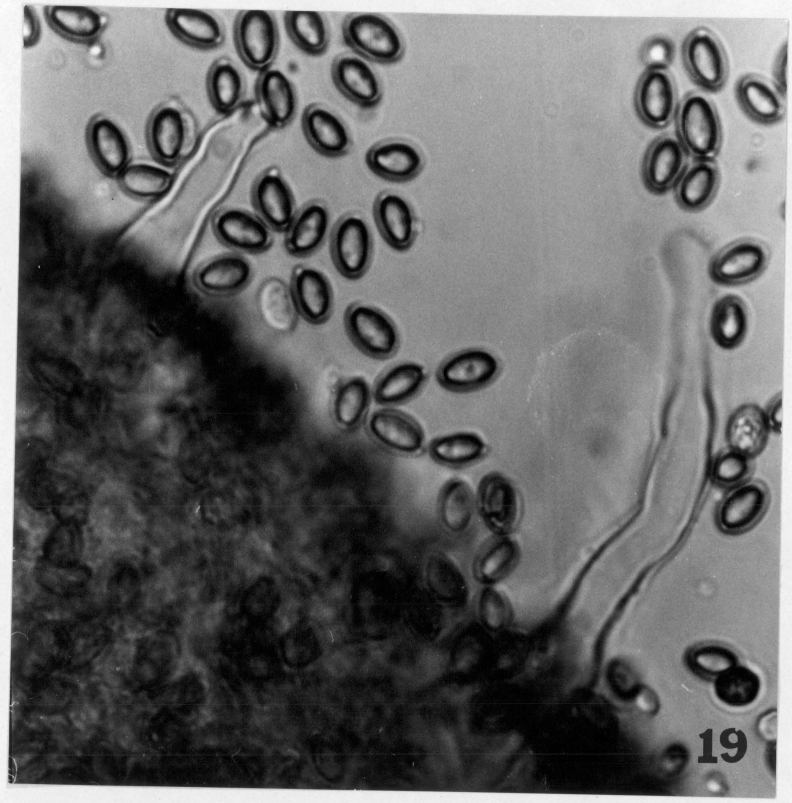


Figs. 11-13. P. spumosa culture on tannic acid agar on day 14. Fig. 11. OKM-1754, top dish shows under side of agar with zone intensity of four; lower dish has thick growth on inoculum plug. Fig. 12. OKM-1547, top dish shows under side of agar with zone intensity of four; lower dish inoculum plug with fuzz. Fig. 13. OKM-2310, under side of dishes showing variety in diffusion zones of intensity one, two and four.



Figs. 14-17. Cultures on tannic acid agar on day 42, showing differences in growth on inoculum plugs. Fig. 14. N. nubigenum OKM-3090 subcultured from tannic acid agar. Fig. 15. N. nubigenum OKM-3090. Fig. 16. P. spumosa OKM-1754. Fig. 17. P. spumosa OKM-1547.

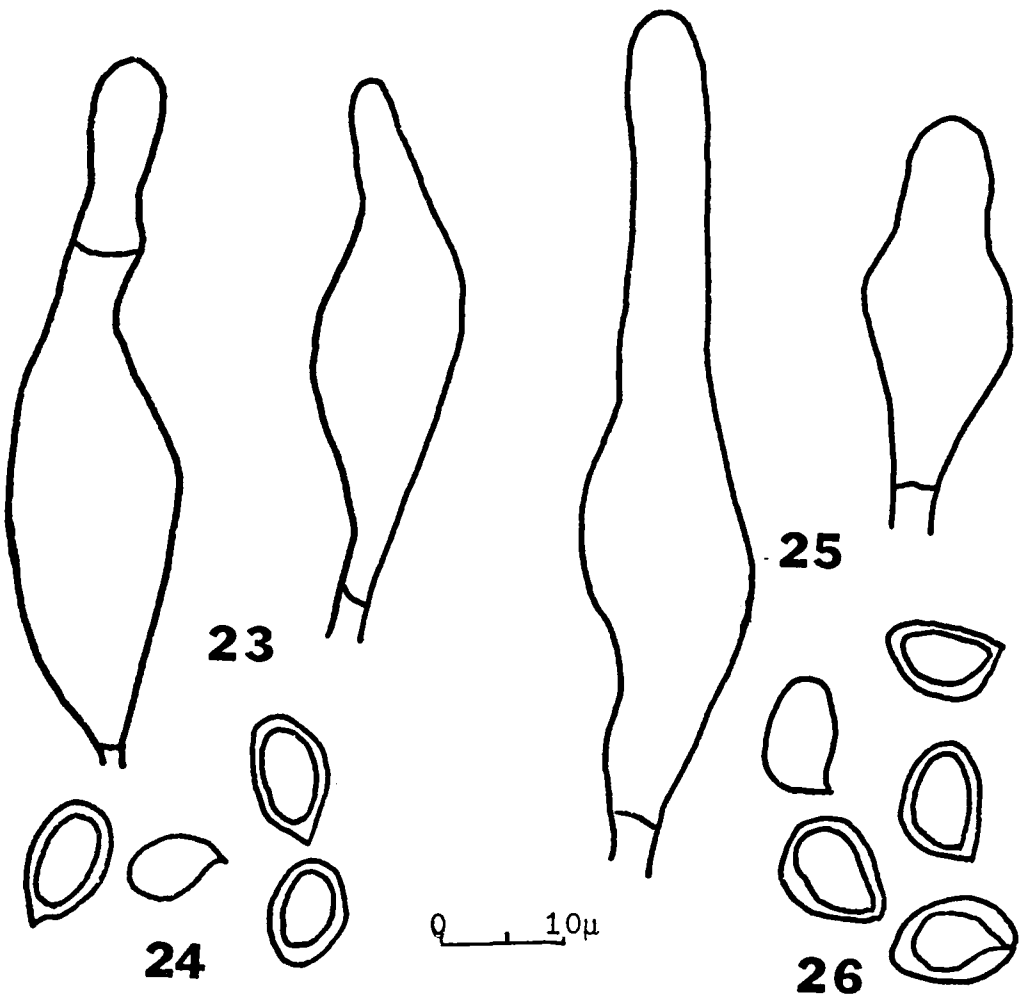




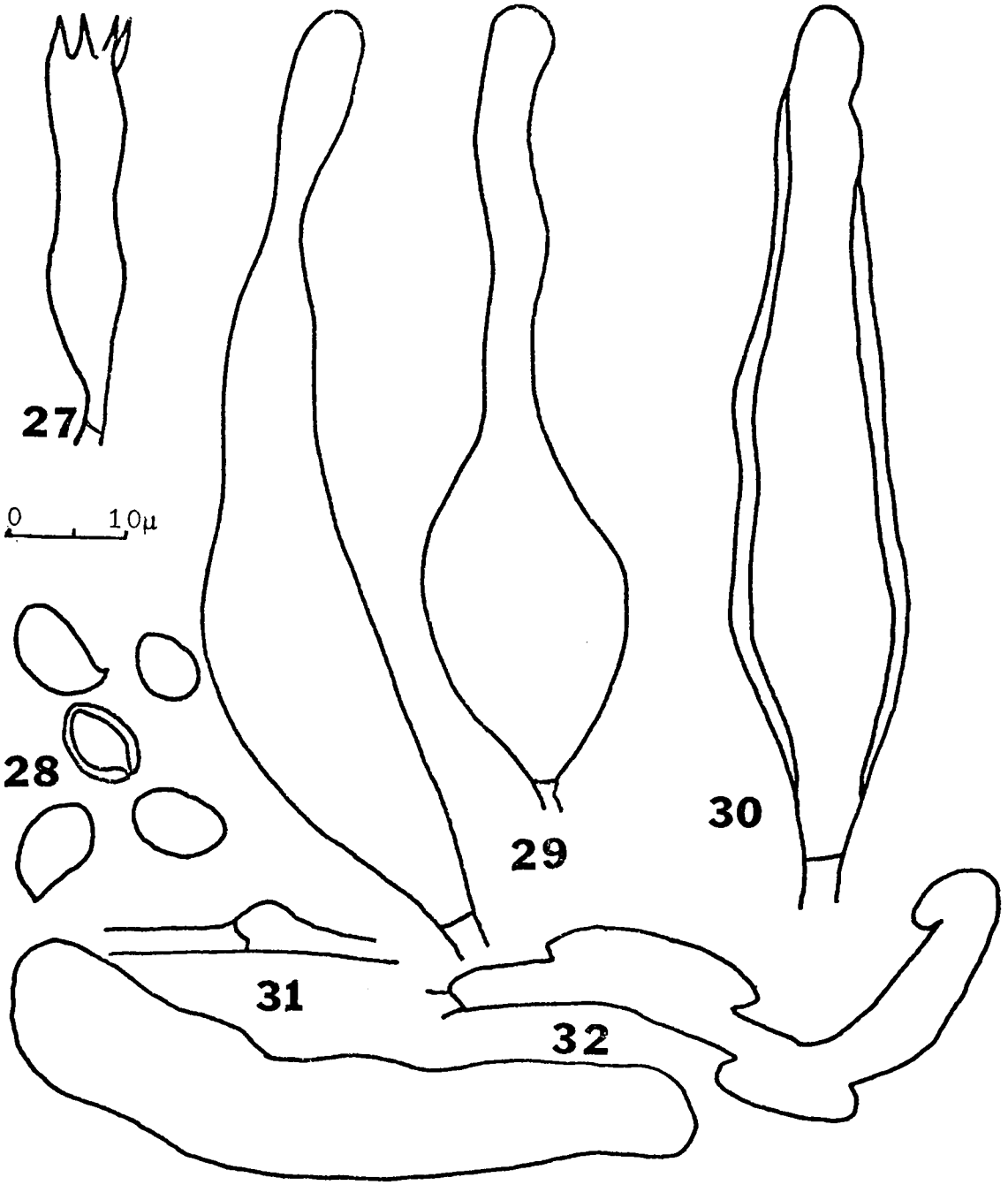
Figs. 18-19. Photomicrographs of sections of N. nubigenum OKM-4004 sporocarp. Fig. 18. Trama of the lamellae with two layers; the subhymenium of inflated cells in the upper left and lower right hand portions of the photograph; the interior region with subparallel, floccose hyphae. Fig. 19.Two thick-walled cystidia protruding above the hymenium; thick-walled spores.

Figs. 20-21. Sporocarp of N. nubigenum OKM-3090 on wood near melting snow. Fig. 20. Light colored peridium. Fig. 21. The gastrocarp slice open revealing the contorted tramal plates; deeply incurved margin; stipe-columella with dark base and white columella.

Fig. 22. Fruiting body of a N. nubigenum (OKM-3126) which expanded exposing radially arranged tramal plates.



Figs. 23-26. N. nubigenum: OKM-3090 Figs. 23-24. Fig.23 cystidia. Fig. 24. spores. OKM-4006 Figs.25-26. Fig. 25. cystidia. Fig. 26. spores.



Figs. 27-32. N. nubigenum: OKM-4003 Figs. 27-29 . Fig. 27 basidia. Fig. 28 . spores. Fig. 29 thin-walled cystidia. OKM-4004 Figs. 30-32 . Fig. 30 . thin-walled cystidia. Fig. 31 clamp connection. Fig. 32 inflated pileus context cells.

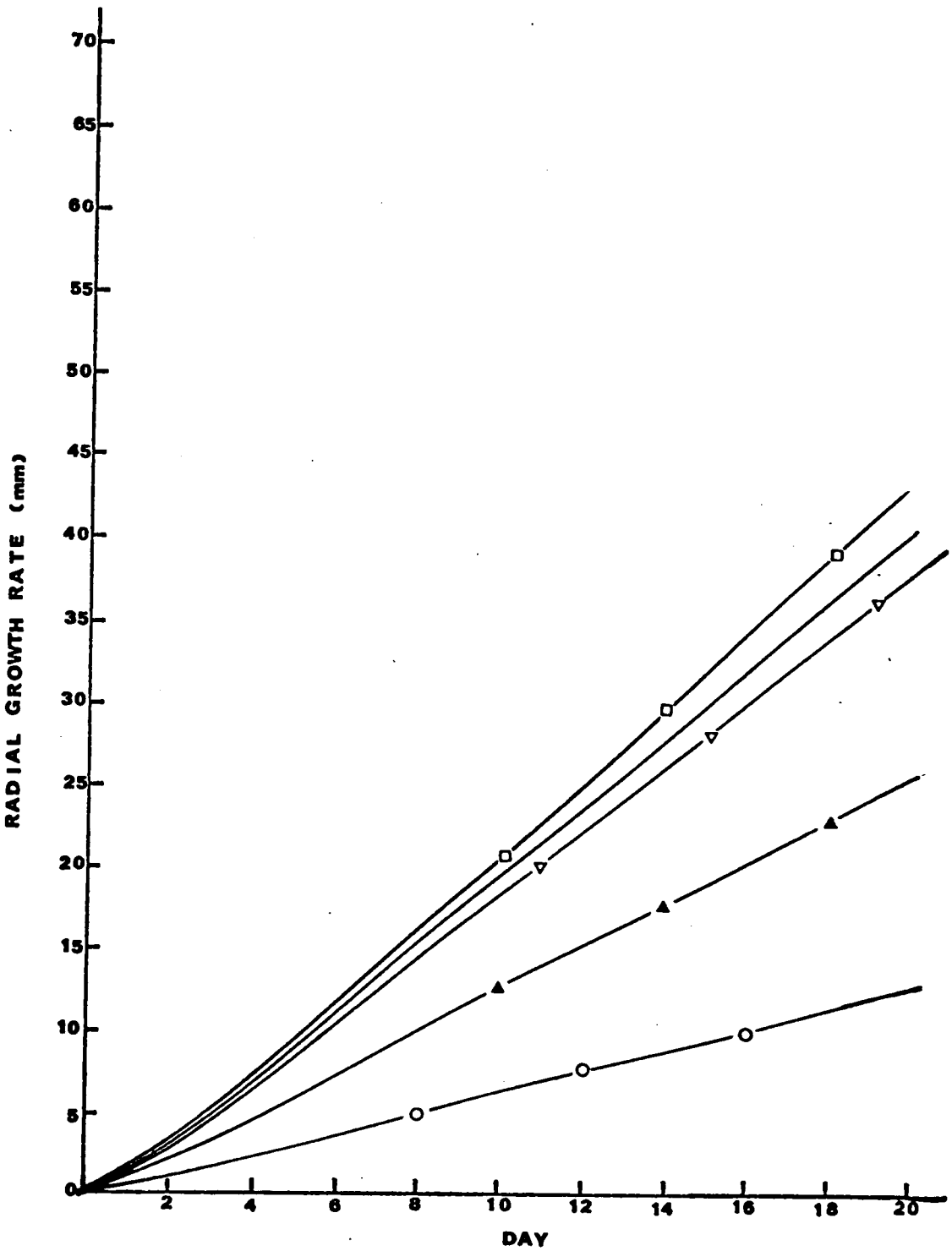


Figure 33. Linear growth of Nivatogastrium nubigenum OKM-3090 for five different temperatures in the dark on 1.5% malt extract agar. ——o—10; ——▲——15; ——□—20; ——— 25; —▽— 30 C.

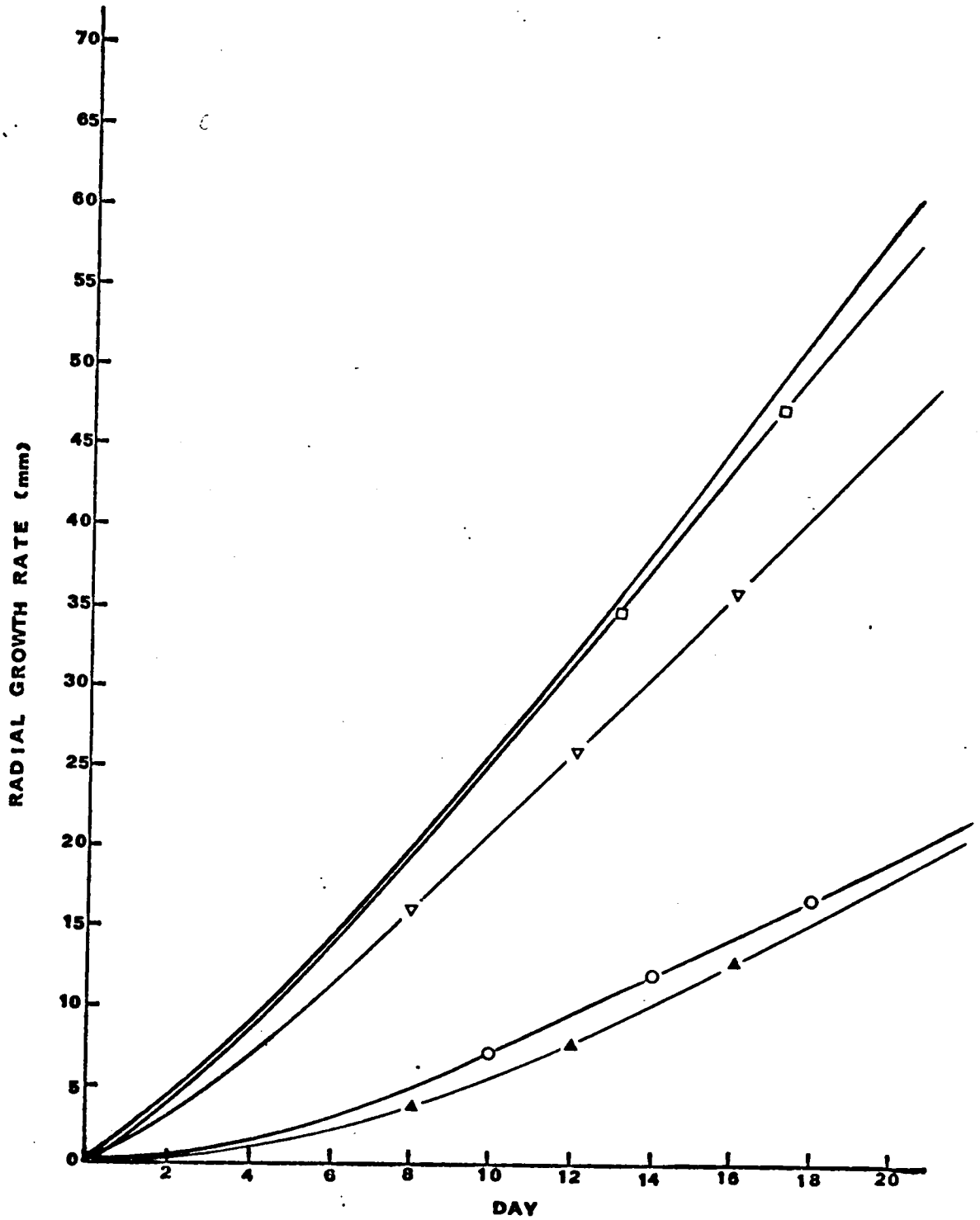


Figure 34. Linear growth of Nivatogastriun nubigenum OKM-4003 for five different temperatures in the dark on 1.5% malt extract agar. ——o—10; ——▲——15; ——□—20; ———— 25; —▽— 30 C.

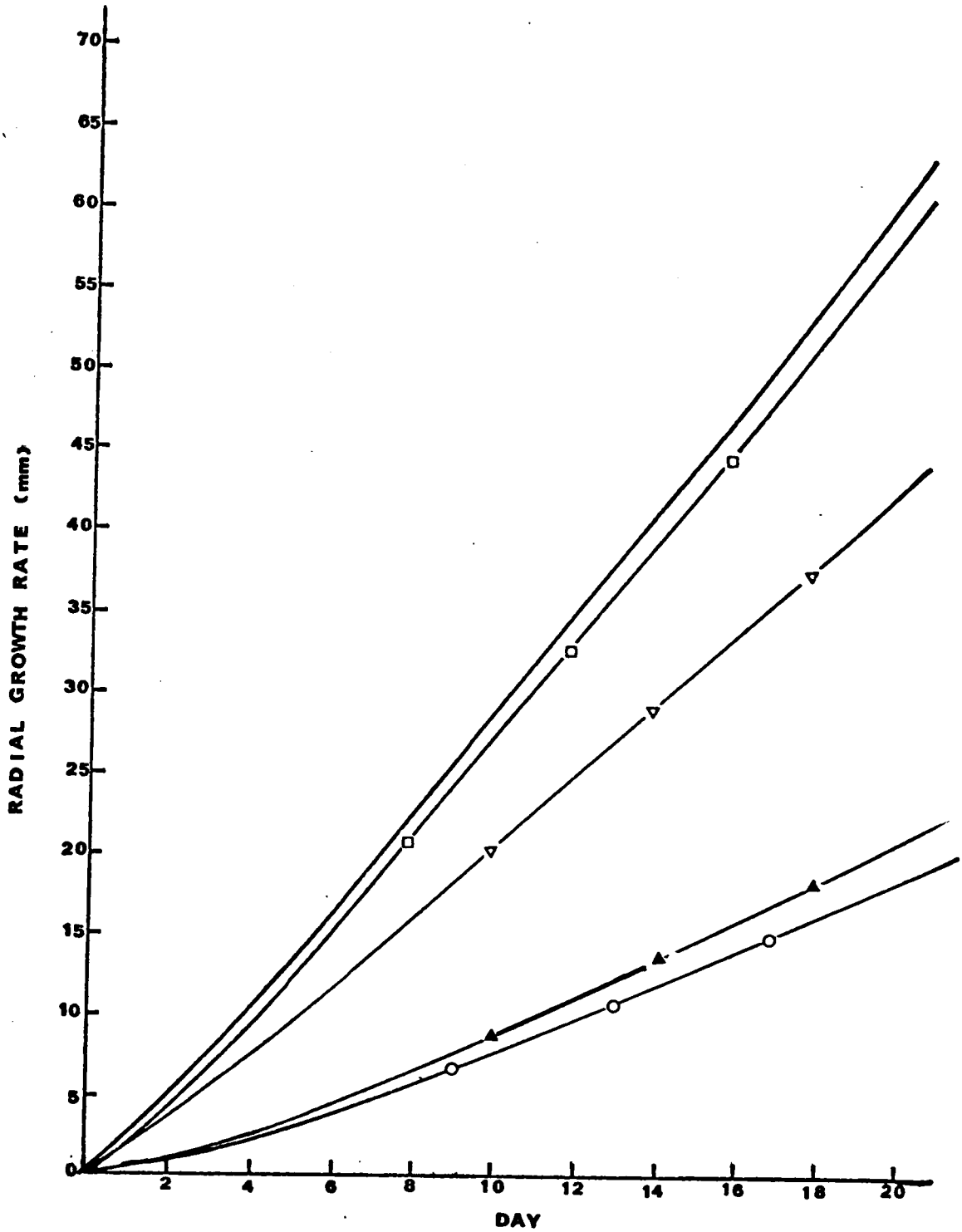


Figure 35. Linear growth of Nivatogastrium nubigenum OKM-4004 for five different temperatures in the dark on 1.5% malt extract agar. —o—10; —▲—15; —□—20; ——— 25; —▽— 30 C.

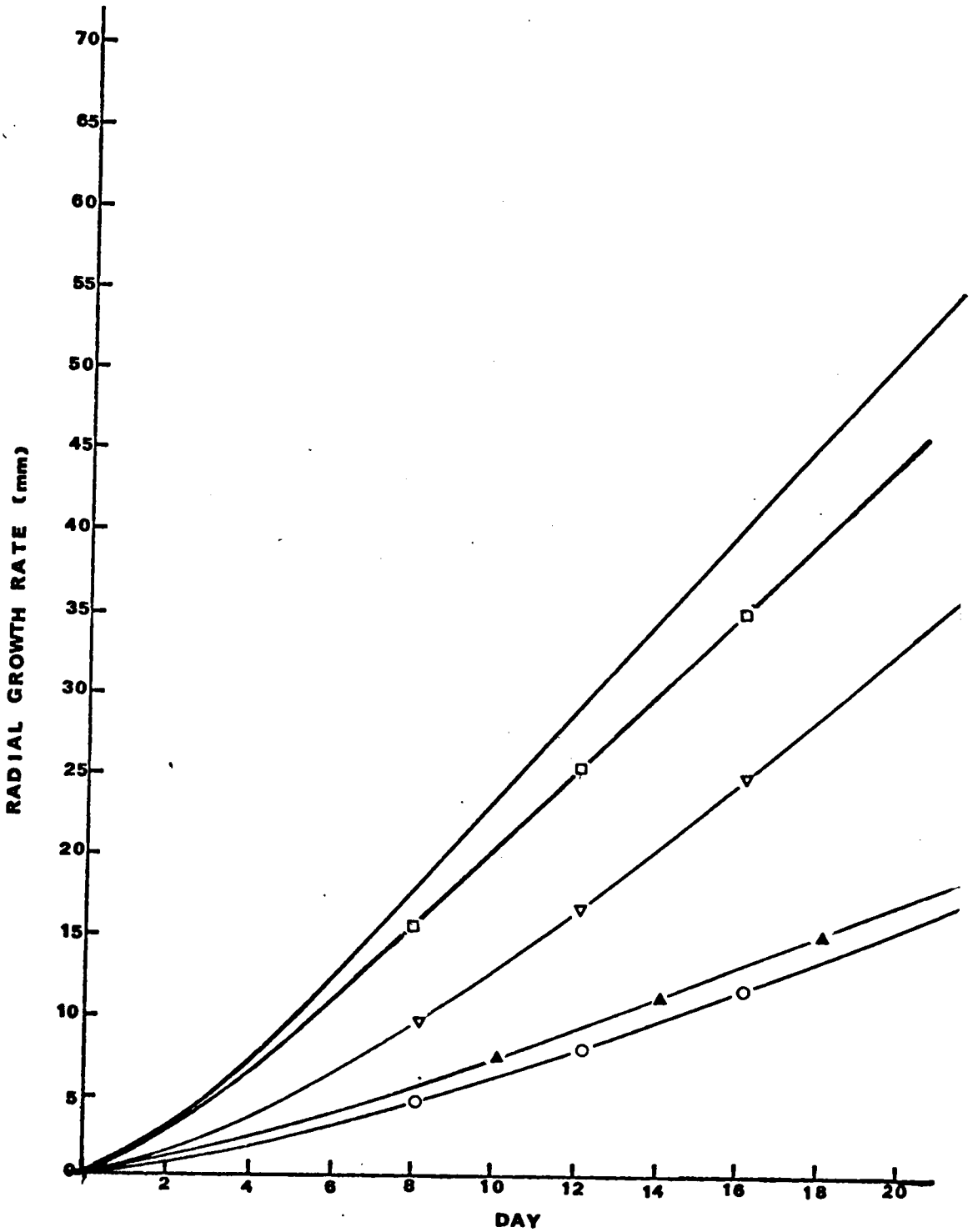
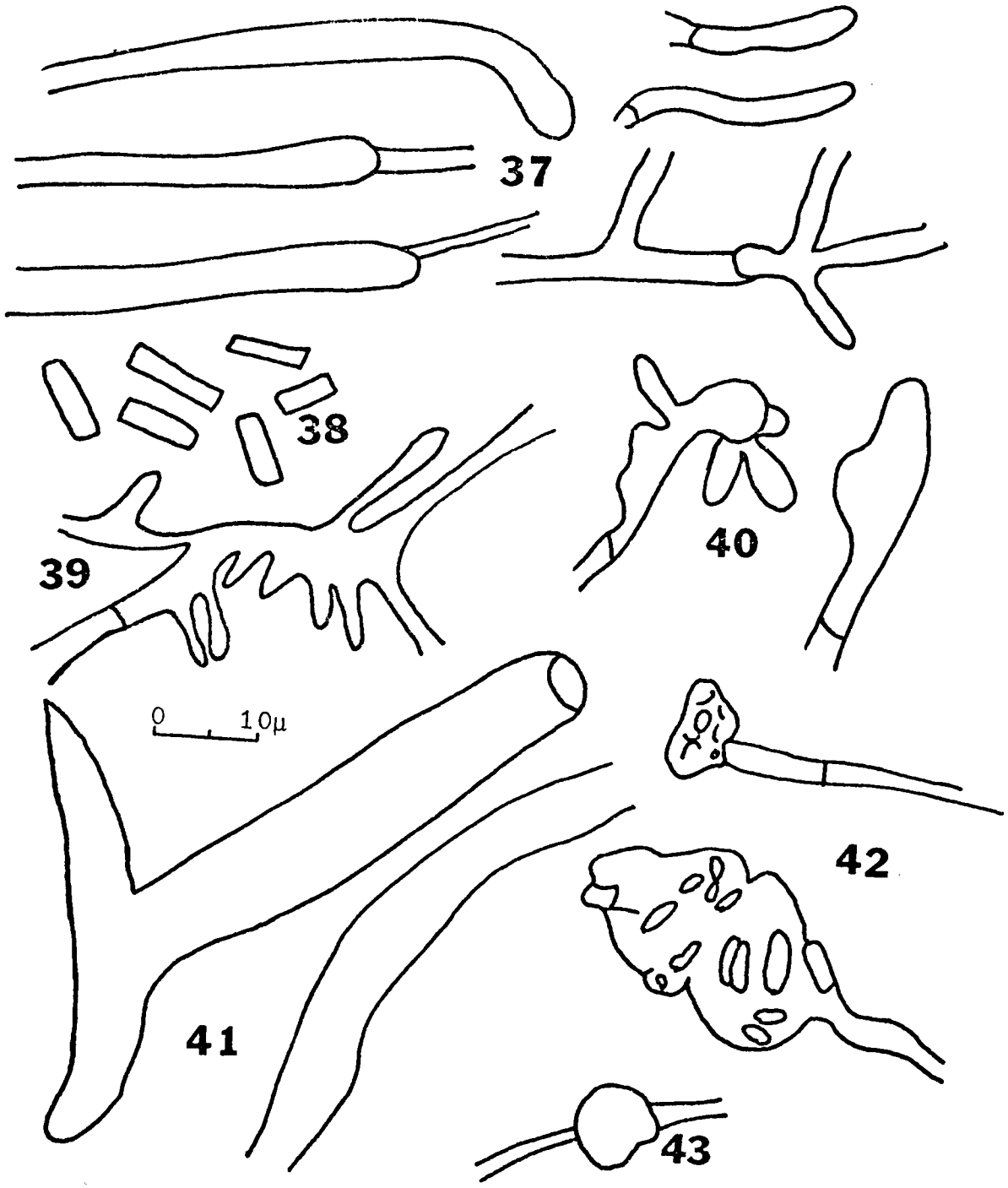
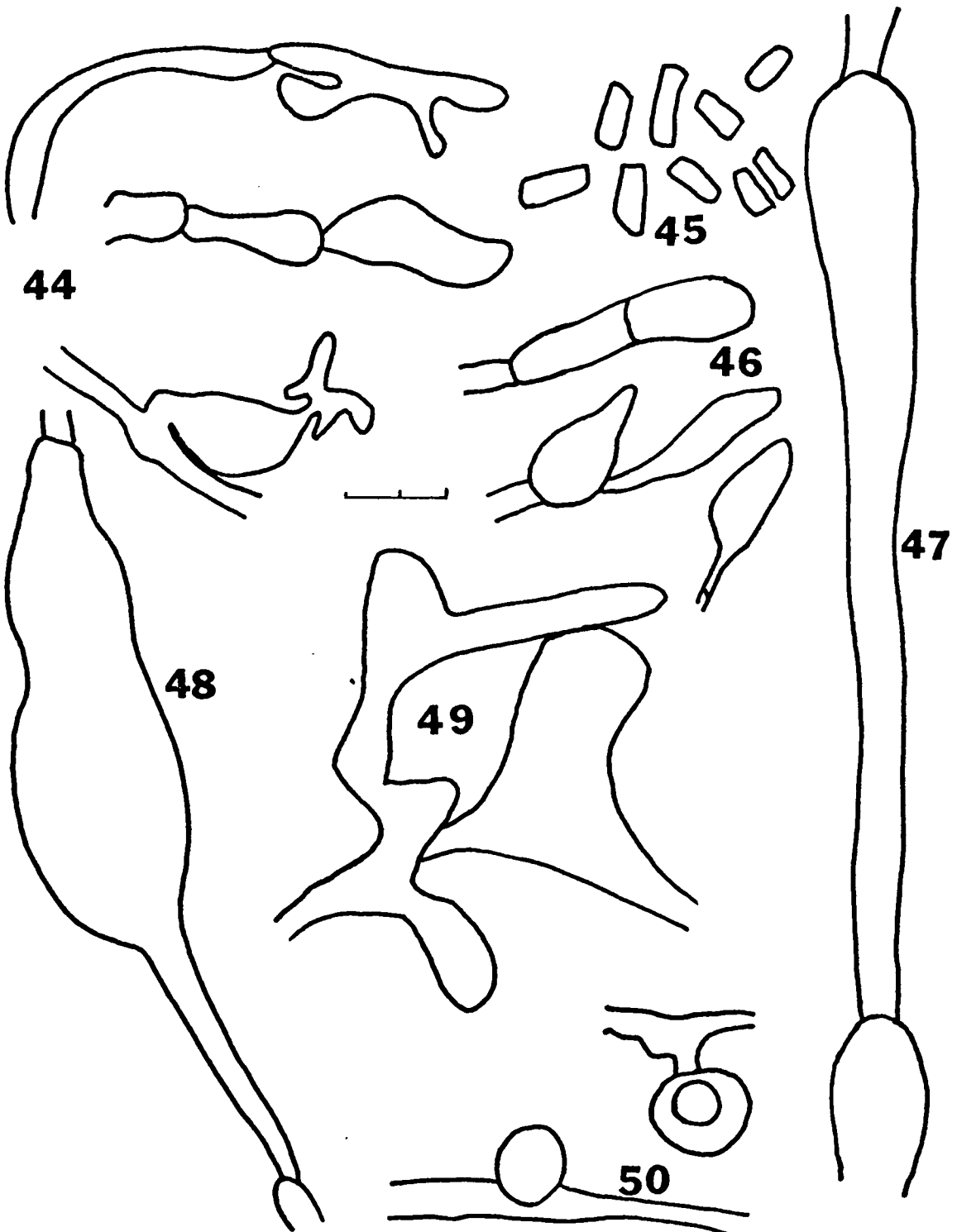


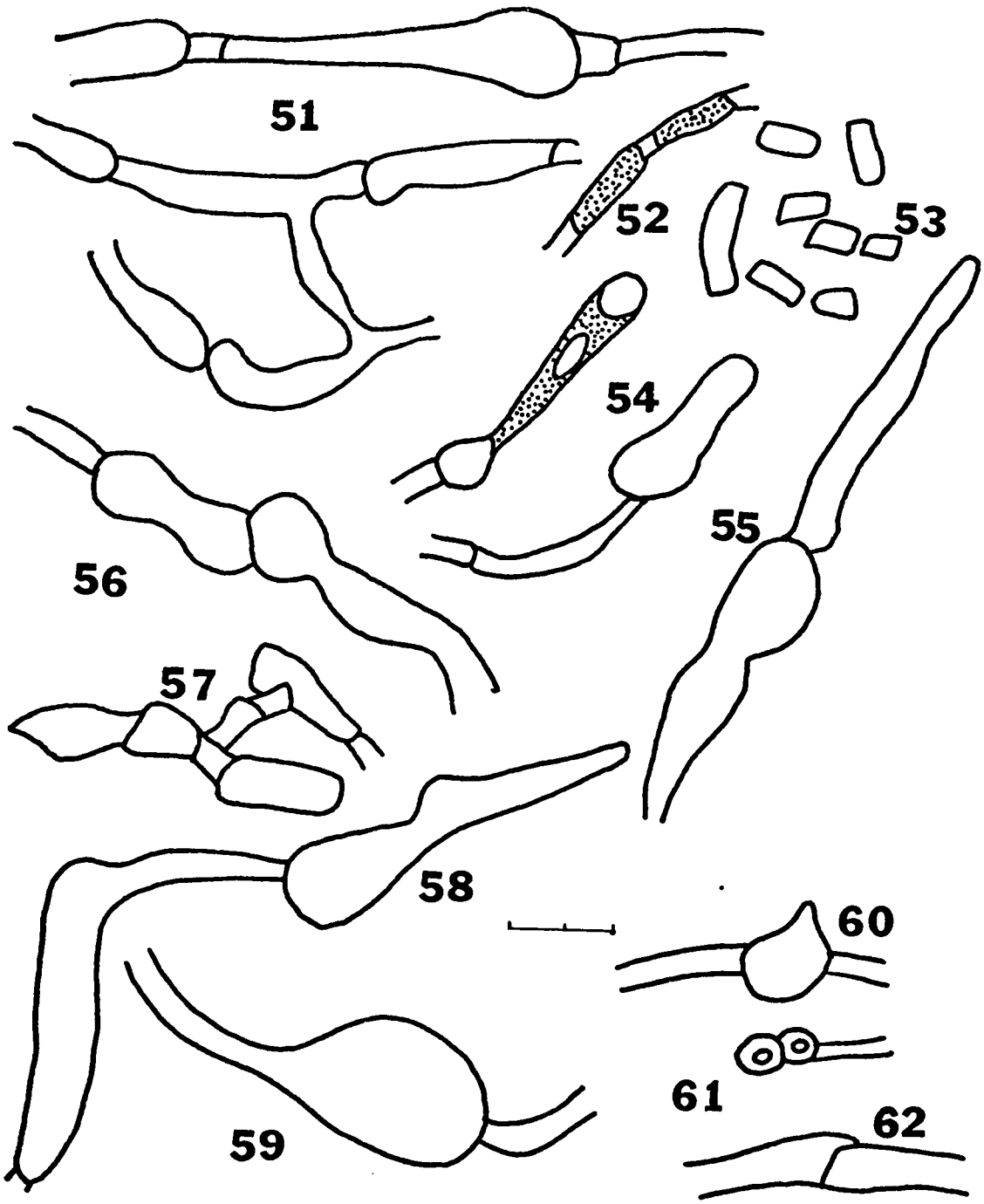
Figure 36. Linear growth of Nivatogastrium nubigenum OKM-4006 for five different temperatures in the dark on 1.5% malt extract agar. ——o——10; ——▲——15; ——□——20; ——— 25; ——▽—— 30 C.



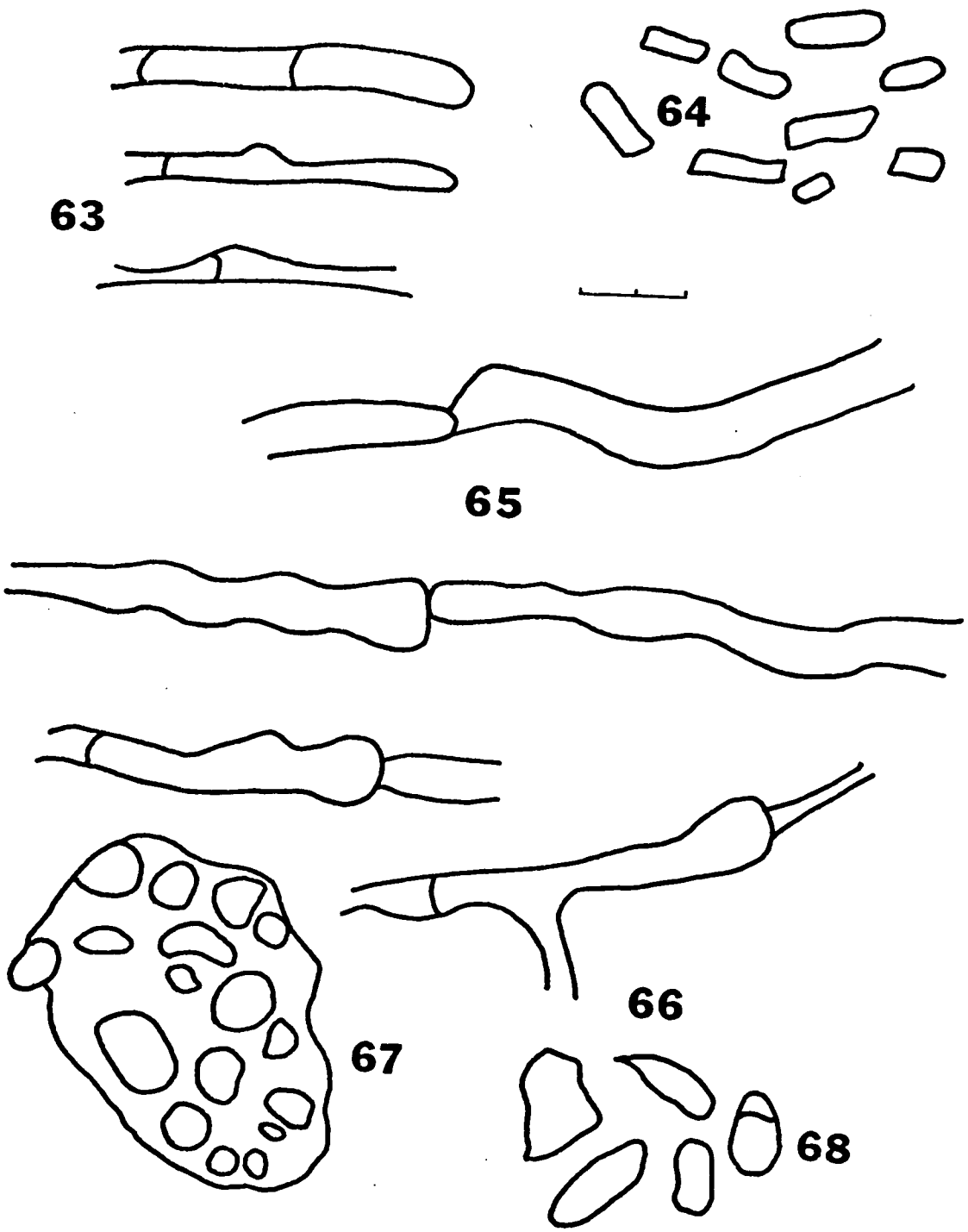
Figs. 37-43. N. nubigenum OKM-3090 cultural characteristics. Fig. 37. narrow mat hyphae. Fig. 38. oidia. Fig. 39. irregularly branching hyphae. Fig. 40-41. large hyphae in older portions. Figs. 42-43. amorphous refractive bodies.



Figs. 44-50. N. nubigenum OKM-4003 cultural characteristics. Fig. 44 narrow mat hyphae. Fig. 45 oidia. Fig. 46. cystidial end cells. Figs. 47-49. enlarged hyphae. Fig. 50. amorphous refractive bodies.



Figs. 51-62. N. nubigenum OKM-4004. Fig. 51. small hyphae with enlarged areas. Fig. 52. hyphal strand breaking up to form oidia. Fig. 53. oidia. Figs. 54-55. hyphal end cells. Fig. 56. hyphae enlarged at point of attachment. Fig. 57."textura intracata" Figs. 58-59. enlarged hyphae. Fig. 60-61. amorphous bodies. Fig. 62. clamp.



Figs. 63-68. N. nubigenum OKM-4006. Fig. 63. narrow mat hyphae. Fig. 64. oidia. Figs. 65-66. slightly enlarged hyphae. Figs. 67-68. "textura globosa"; Fig. 67 in a gelatinous mass, Fig. 68. cells separate.

Pholiota spumosa (Fr.) Singer Figs.69-83.
Singer Lilloa 22:517. 1951.
Agaricus spumosa Fries, Syst. Myc. 1:252. 1821
Flammula spumosa (Fr.) Kummer, Der Fuhrer in die Pilzkunde, p.81. 1871.
Dryophila spumosa (Fr.) Quilet, Enchir. Fung. p.70. 1886.

Pileus 3-6 cm broad, slightly umbonate, yellow-brown to a light reddish-brown, often with a faint greenish hue. Disc darker and with more of a brownish tint, margin more yellowish, viscid when fresh, or glabrous, slight prescence of fibrils gives a streaked look. Context yellow to yellow-green, soft. Odor mild. Lamellae adnate to adnexed, close, medium broad, mustard yellow to buff when young, becoming rusty or cinnamon-brown in age, but often retaining a slight greenish hue. Stipe 3-5 cm long, 4-5 mm wide, equal becoming hollow, cream to buff above with spots of yellow, the base reddish-brown, often tinted green. Remnants of the veil on the surface often leave a thin layer of yellow fibrils.

Spores 5.5-8 x 3.5-5.5 μ, smooth elliptical, with a distinct germ pore, thick-walled, rusty-brown, dull tawny in 3% KOH, paler and more cinnamon in Melzer's reagent. (Figs. 73, 80, 83) Basidia 26-32 x 6-7 μ, 4-spored, narrowly clavate, hyaline to yellowish in 3% KOH, yellowish in Melzer's reagent (Fig. 82). Pleurocystidia 40-60 x 7-14 μ, fusoid-ventricose with an obtuse apex, sometimes with irregular swellings on the side, or broad, blunt apices, thin-walled, contents hyaline or with irregularly

distributed material appearing greenish-yellow in 3% KOH. (Figs. 69-71, 77-79, 81). Cheilocystidia 35-55 x 9-13 μ, fusoid-ventricose to broadly subfusoid, thin-walled, hyaline to yellowish in 3% KOH (Fig. 72). Cuticle of the pileus a thick gelatinous pellicle of smooth, narrow, thin-walled hyphae, 2-3 μ in diameter, hyaline to yellowish in 3% KOH. A layer of thin-walled, incrusted hyphae which appear brown in 3% KOH, is found just below the cuticle. Trama of the pileus of interwoven, inflated, up to 12 μ in diameter, thin-walled, smooth hyphae, hyaline to yellowish in 3% KOH (Figs.74,75). Trama of the lamellae with a central area of floccose, subparallel hyphae, loosely arranged, smooth, thin-walled, some inflated up to 10 μ diameter, yellowish in 3% KOH (Fig.76). All hyphae non-amyloid. Clamp connections present.

Collections examined: U. S. A.: Idaho. OKM-1547, OKM-1754, and OKM-2310. (VPI)

Habit, habitat and distribution- OKM-1547, found on dead wood under Abies lasiocarpa, Pseudotsuga menziesii (Mirb.) Franco, and Pinus ponderosa Laws. Brundage Mountain Road, Payette National Forest, Idaho. June 21, 1962. OKM-1754, on wood. Seven Devils Mountain, Nez Pierce National Forest, Idaho. July 13, 1962. OKM-2310, on ground amongst dead wood. Priest River Forest, Idaho. June 23, 1964.

Observations: This species is in the subgenus Flammuloides, and the stirps Spumosa according to Smith and Hesler (1968). The entire "spumosa complex" is described as being extremely variable, with P. spumosa the most variable of all the species. The three collections examined in this study showed the most variation in the shape of the pleurocystidia and spore size. OKM-2310 is the closest to the three to the major description presented by Smith and Hesler. The spores are in the larger range of 7-9 x 4-4.5 μ, and the pleurocystidia are regular in shape as described (Figs. 81-83). OKM-1754 has some pleurocystidia with irregular swelling near the apex (Fig. 71) and OKM-1547 has some very broad pleurocystidia. The spores in both of these collections are slightly smaller than in the main description by Smith and Hesler.

Smith and Hesler (1968) explain in their discussion that their microscopic data for the species description was based on a collection made in Sweden by Nannfeldt, in which the spore size is 7-9 x 4-5.5 μ. "In most collections under the name Flammula spumosa in American herbaria (we have not studied all of them) the spores measure 5.8-8 x 4-4.5 μ." (Smith and Hesler, 1968). The spores of collection OKM-1547 and OKM-1754 fit into the latter category.

Pholiota spumosa (Fr.) Singer Figs. 84-111

File Pattern for cultures OKM-1547 and OKM-1754: APM, 4, 8, 10, 11, 16. Culture OKM-2310: BPM, 4, 8, 10, 11, 16.

Cultural Characteristics

Growth Characteristics- Growth rate at 25 C is medium, radius 4.2-4.5 cm in two weeks. Average mat radius for different constant temperatures are in figures 84-86. Optimum temperature range from 25-30 C. Inhibitory temperature 35 C, lethal in 50% of the cultures tested by returning them to 25 C to see if any growth occurs.

Mat Appearance- Advancing zone even and thin. Mat white except in culture OKM-2310 which is white turning yellow in age. Appressed margins with patches of intermediate to raised mycelium in the older portions. Texture subfelty to downy with wooly patches. Odor fruity in young cultures, musty in older ones.

Oxidase Tests (Table III)- All cultures showed a strong reaction, intensity of 4, on gallic acid agar. No growth on gallic acid agar except in one petri plate of OKM-1754 which had sparse growth on day 21. Tannic acid agar reactions were strong, intensity 4, for OKM-1754 and OKM-1547, with growth onto the agar by day 14 (Figs. 11, 12). OKM-1754 had the thickest growth and by week six had developed sectors (Fig. 16). OKM-1547 developed a thin layer of mycelium on the agar with a pulverulent section (Fig. 17). OKM-2310 varied in reactions on tannic acid agar from a weak

diffusion zone with no growth to a strong diffusion zone with dense growth on the inoculum plug (Fig. 13). Gum guaiac reactions were variable. A white, milky reaction occured by the second minute, but the occurance of blue varied from five minutes in the rapid reactions on some of the mats of OKM-1754 to an hour in OKM-2310.

Morphological Characteristics- Hyphae of two types, without clamps; 1. Cylindric to inflated, thin-walled hyphae, 2-4 μ diameter, 15-40 μ long (Figs. 88, 94, 102). 2. Large cylindric to inflated cells, irregular enlargments, often inflated where attached to the next cell, thin-walled, 6-12 μ in diameter, 25-100 μ long (Figs. 90, 97, 104, 108). Both hyphal systems contain hyaline hyphae in 3% KOH, with amyloid inclusions in some cells (Figs. 98, 109). Hyaline and golden-brown hyphae in Melzer's solution. Phloxine stains some of the hyphae deep pink while others remain hyaline. Cystidial end cells short, variable in shape, club-shaped, tapering or irregularly inflated, thin-walled (Figs. 96, 105).

Oidia are present in all three cultures, 6-12 x 1-2.5 μ, thin-walled, hyaline in 3% KOH and Melzer's solution, stained deep pink in Phloxine (Figs. 89, 95, 103). Most abundant in older portions, but found in advancing margin.

Amorphous refractive bodies, ovoid to round, abundant, 5-20 μ in diameter, terminal and intercalary (Figs. 91, 99, 100, 107). A well defined wall never developes and the

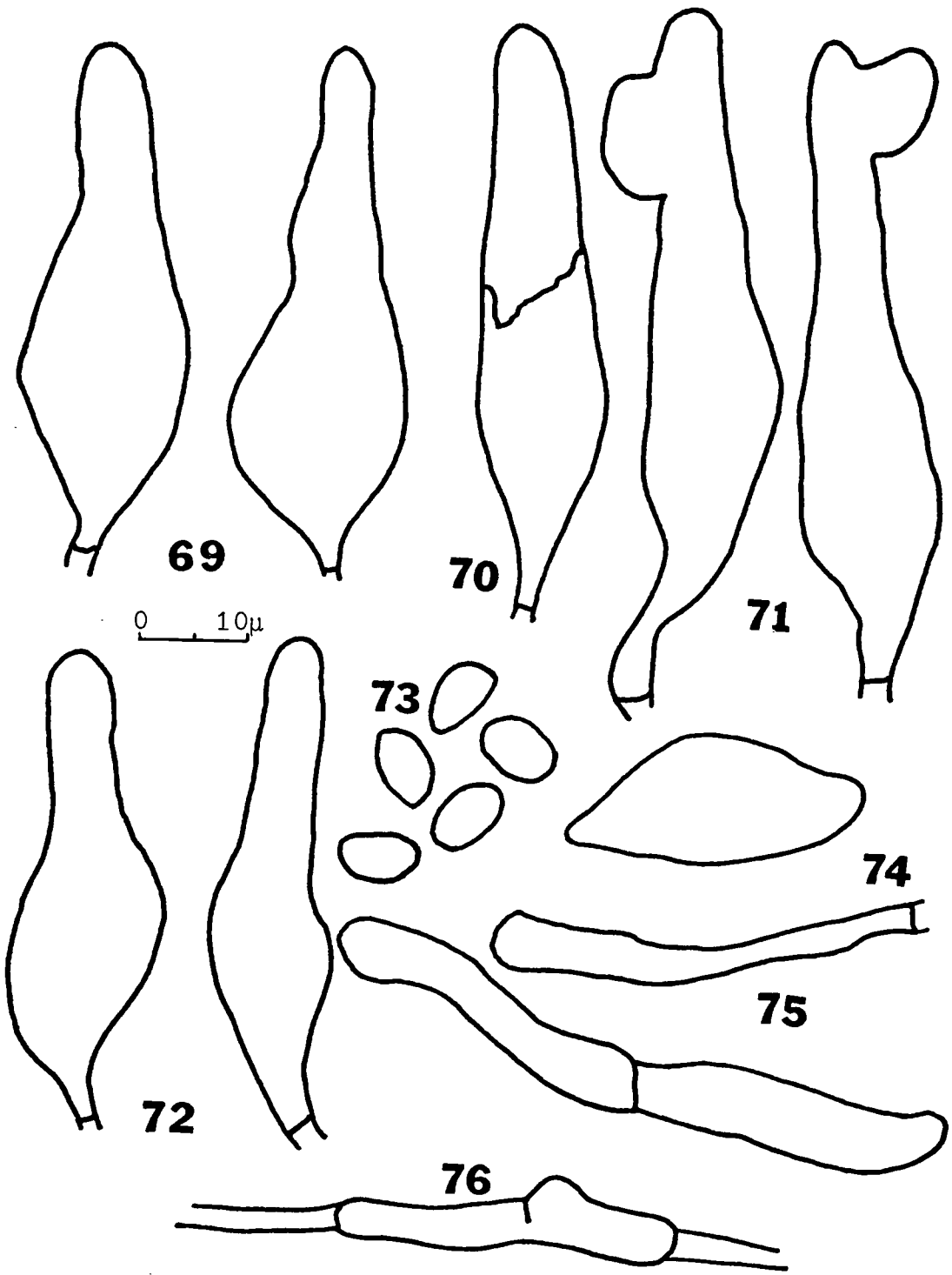
contents appear to be irregular in density. Hyaline in 3% KOH, golden in Melzer's solution and deep pink in phloxine. Thin spheres terminal and intercalry, are found in all three cultures. These do not stain and may be oil droplets (Figs. 93, 101, 106).

Tissue types (Korf 1958, Miller 1971) found in all three cultures are of the Textura intricata type with some Textura globosa. Figure 92 illustrates the tissue arrangement. These cells are deep pink in Phloxine. Found in older portions of the cultures.

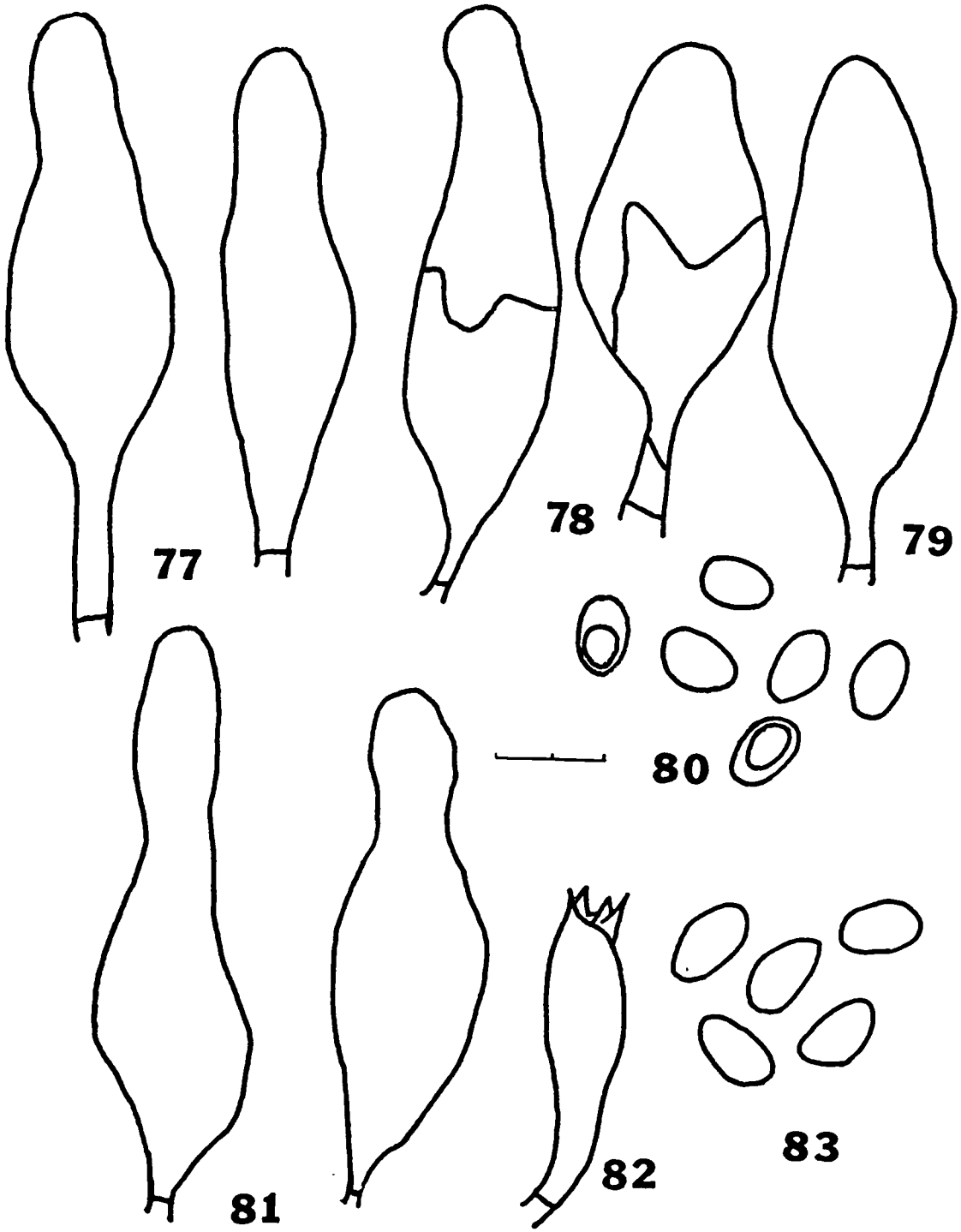
Cultures grown on media other than 3% malt extract agar showed different morphological features in the over all appearance of the mat and in some microscopic features. OKM-2310, grown on Prune extract, Emerson's YpSs agar and glucose mixture showed deep furrows with dark brown dots on the mat.

When these were examined microscopically, hyphae with dark brown wall thickenings were found. Some hyphae had solid wall thickenings and others were only brown in patches (Figs. 110, 111). The other two cultures of P. spumosa did not have these brown areas in the mats and no thick-walled cells were found. OKM-1754 did, however, have the deep furrows around the inoculum plug as seen in OKM-2310. OKM-1547 and OKM-1754 both had furrows around the plug when grown in a petri dish with the Prune extract, Emerson's YpSs, glucose agar, however, when OKM-1547 was grown in the flask with Englemann spruce infusion, the hyphae were submerged.

Observations: The growth rates of all three cultures were very close, showing less standard deviation at day 14 for all temperatures compared (Table II). These cultures had the greatest mat diameter by the second week at 30 C. The only other culture that had such a rapid growth rate was P. decorata, but its optimum temperature was a 25 C. Within the cultures of P. spumosa the greatest difference was seen in the appearance of the mat of OKM-2310 which was white turning to yellow. The other cultures remained white. The three cultures were similar in microscopic features on malt extract agar and these similarities were seen in P. decorata and N. nubigenum, i.e., the oidia, inflated cells, and amorphous refractive bodies. The developement of the tissue type"textura intricata" was seen in all three species. The developement of thick-walled hyphae in OKM-2310 on Prune extract, Emerson's YpSs agar, glucose medium was an important observable difference. The amyloid inclusions in OKM-2310 and OKM-1754 were not seen in any other cultures examined.



Figs. 69-76. P. spumosa OKM-1754. Figs. 69-71. pleurocystidia. Fig. 72. cheilocystidia. Fig. 73. spores. Figs. 74-75. inflated cells from contexts of pileus. Fig. 76. hyphae from trama of lamellae.



Figs. 77-83. P. spumosa: OKM-1547 Figs.77-80. Figs. 77-79 cystidia. Fig. 80 spores. OKM-2310 Figs. 81-83. Fig. 81. pleurocystidia. Fig. 82 basidia. Fig. 83. spores.

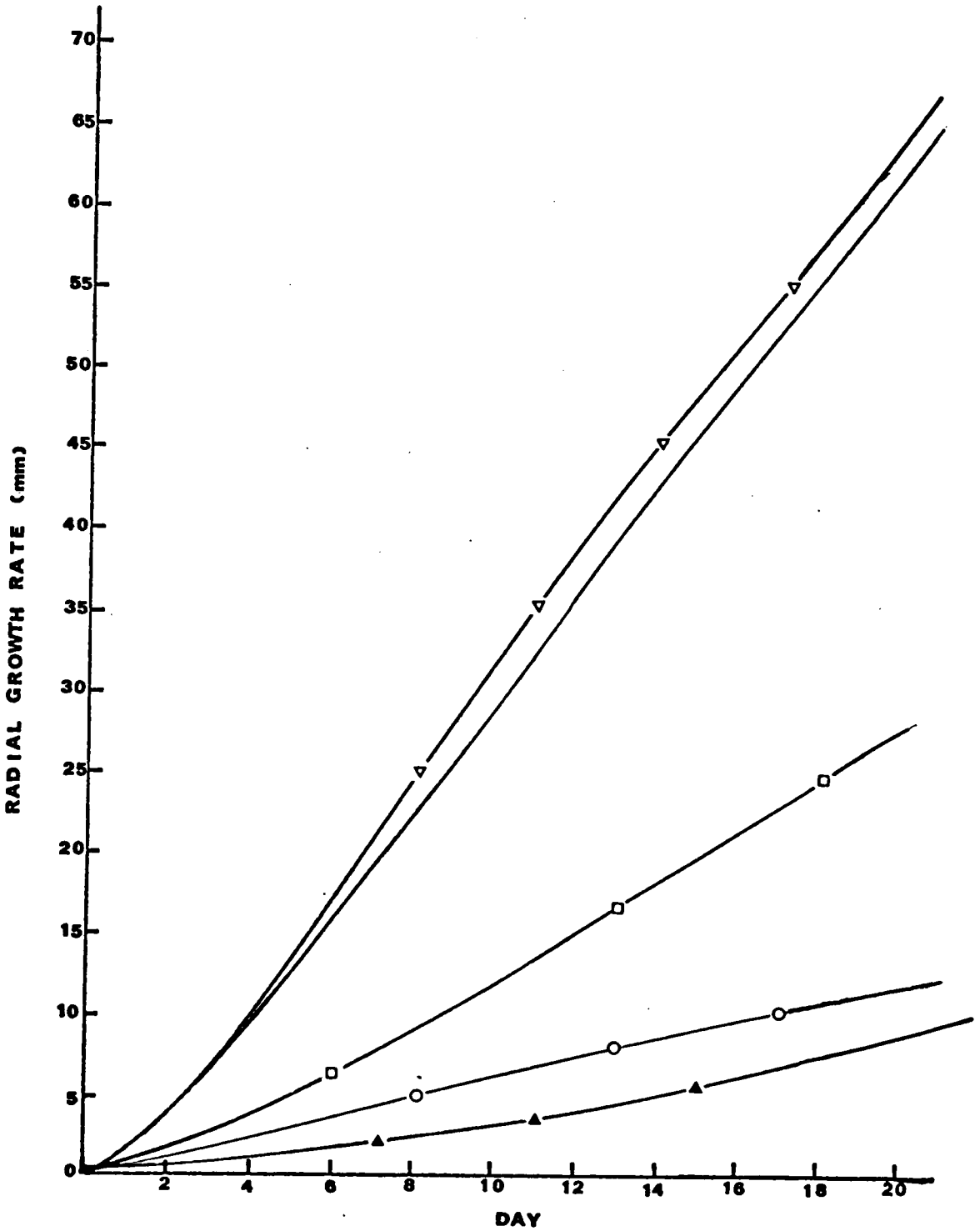


Figure 84. Linear growth of Pholiota spumosa OKM-1547 for five different temperatures in the dark on 1.5% malt extract agar. —o—10; —▲—15; —□—20; ——— 25; —▽— 30 C.

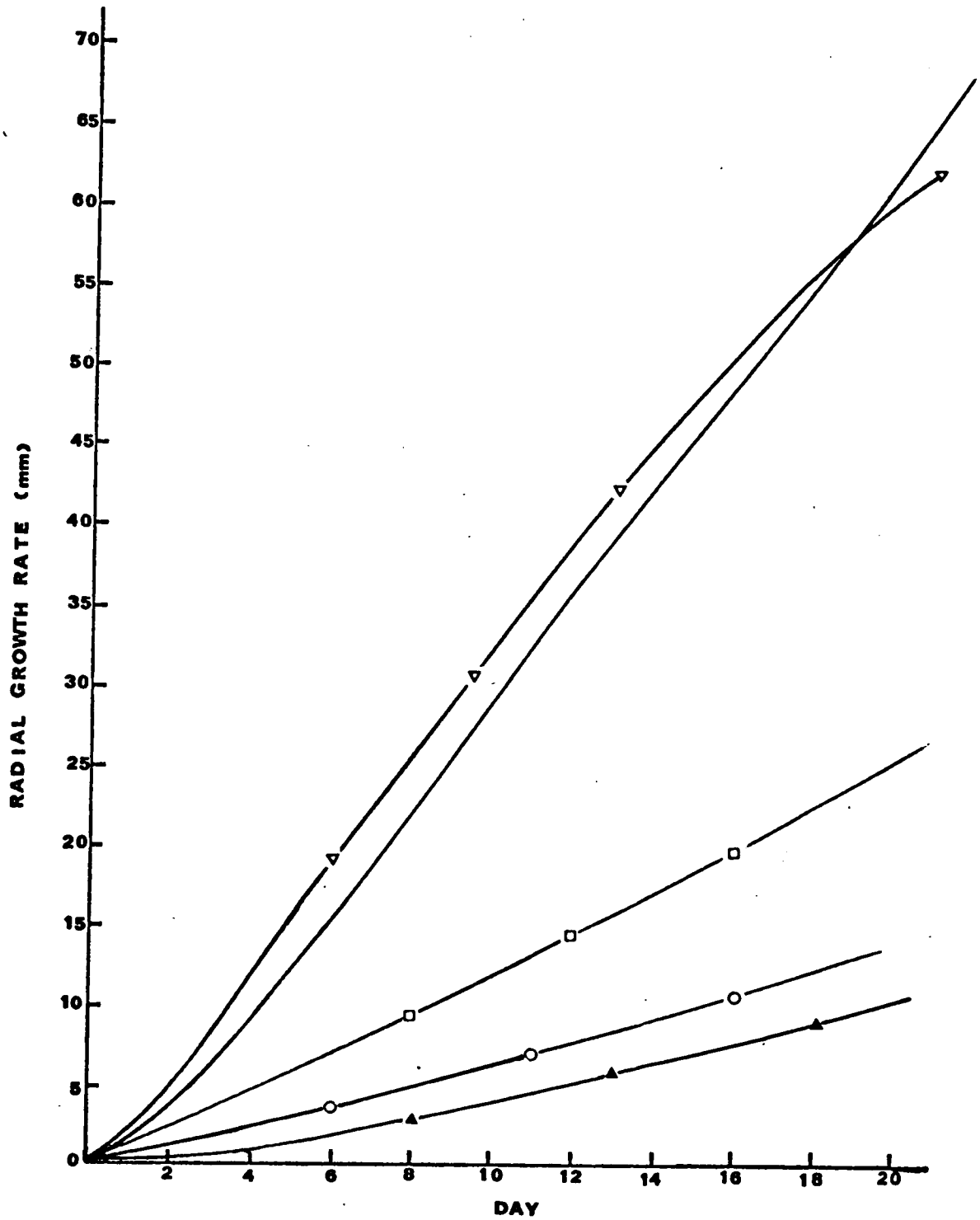


Figure 85. Linear growth of Pholiota spumosa OKM-1754 for five different temperatures in the dark on 1.5% malt extract agar. —o—10; —▲—15; —□—20; ——— 25; —▽— 30 C.

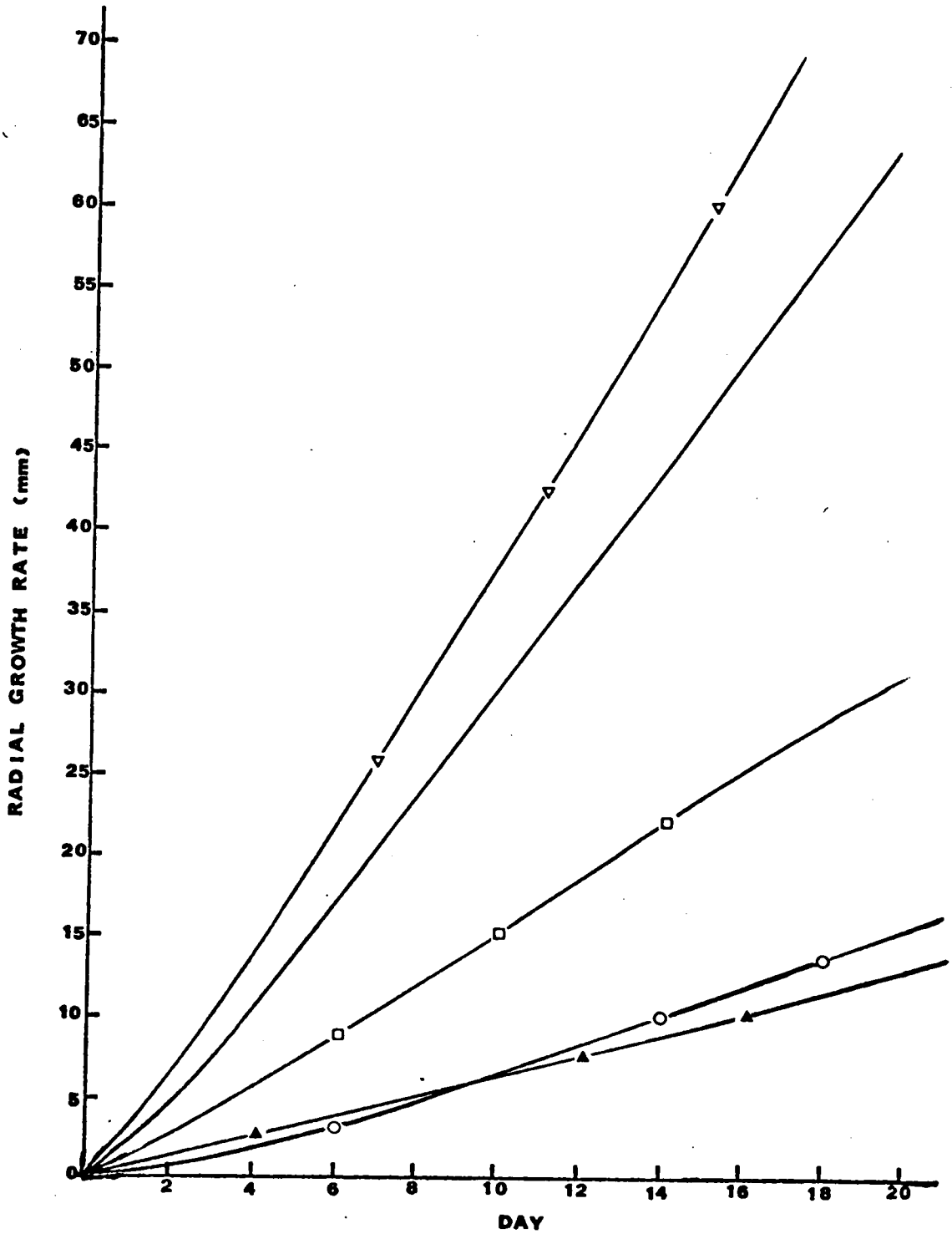
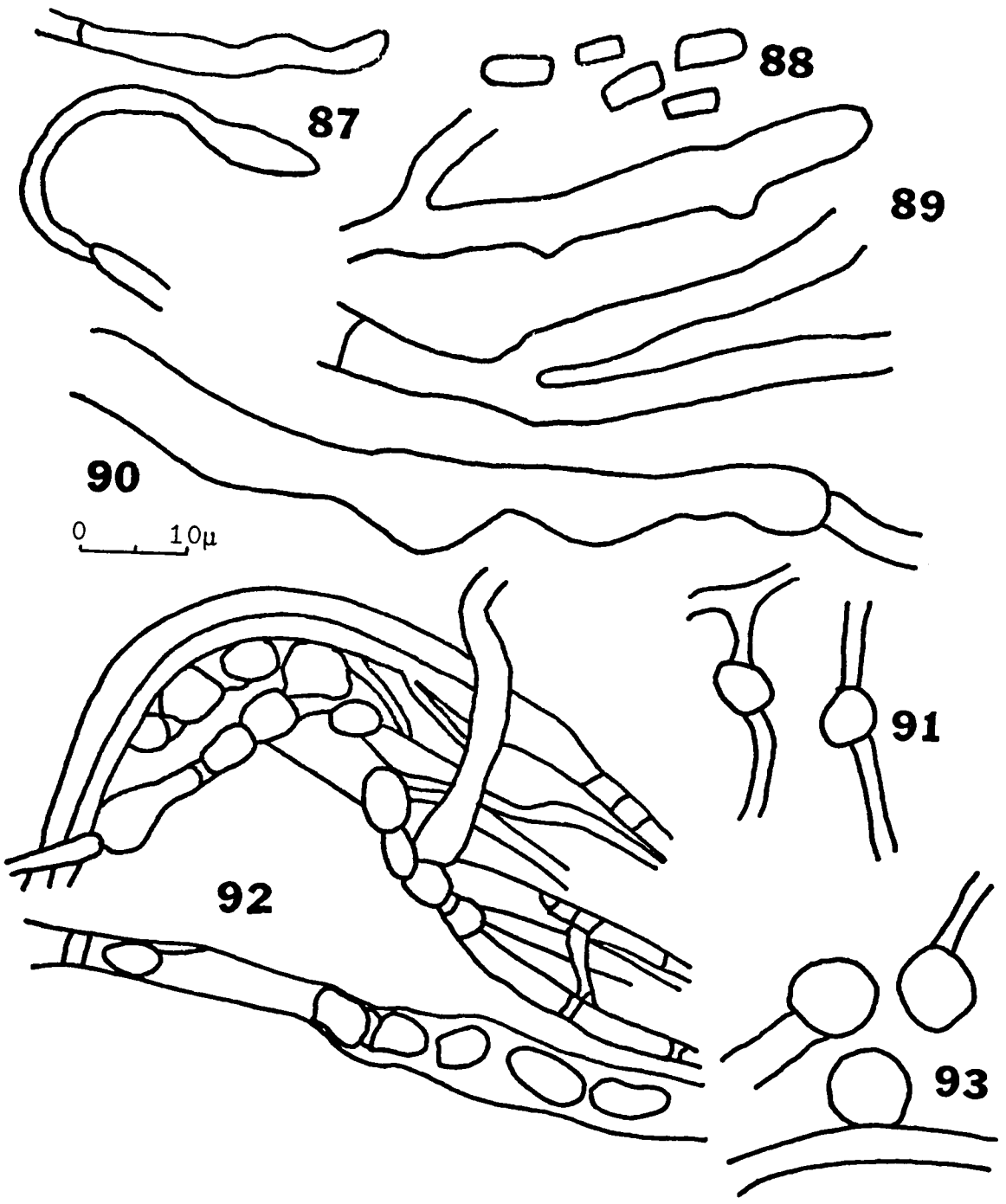
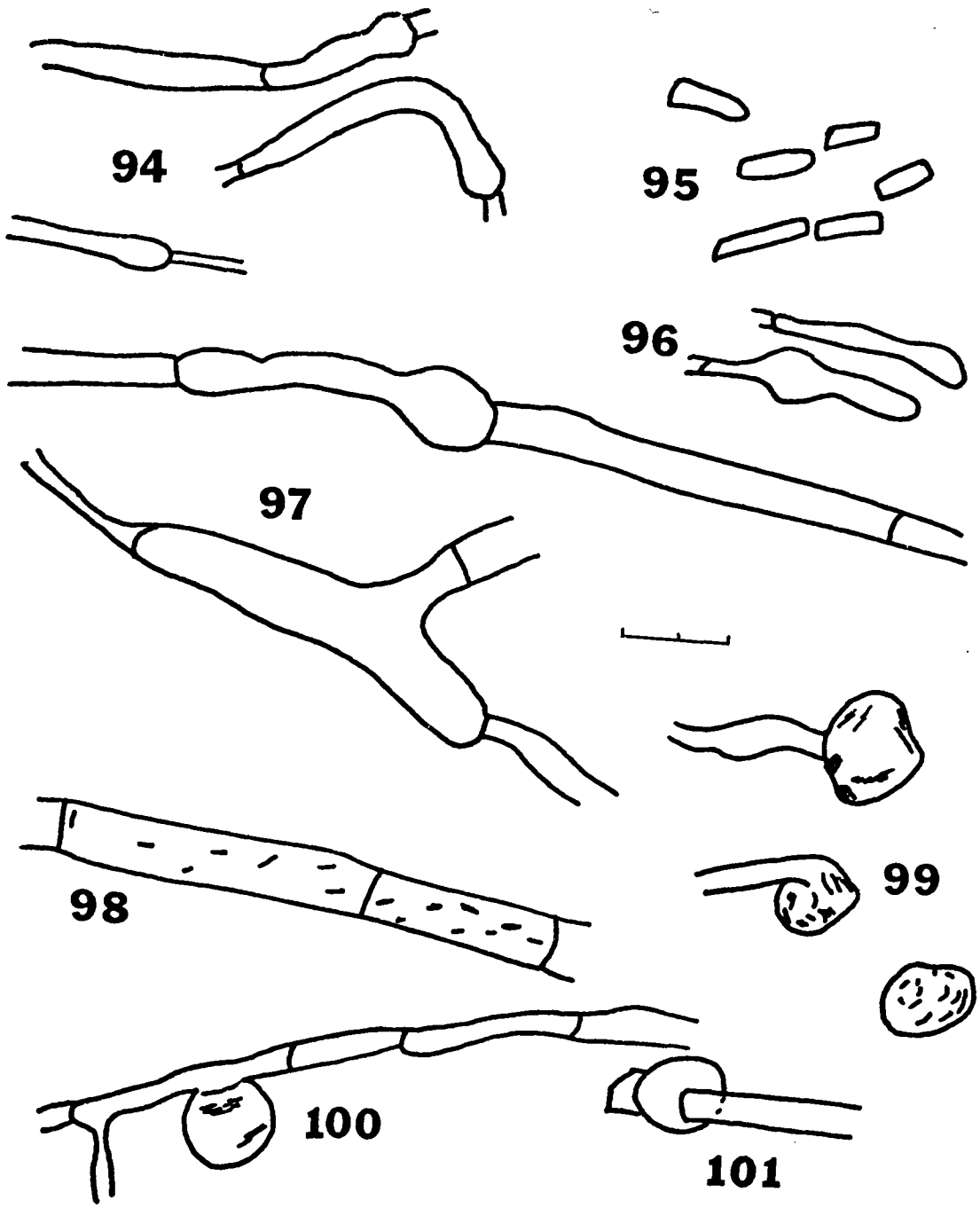


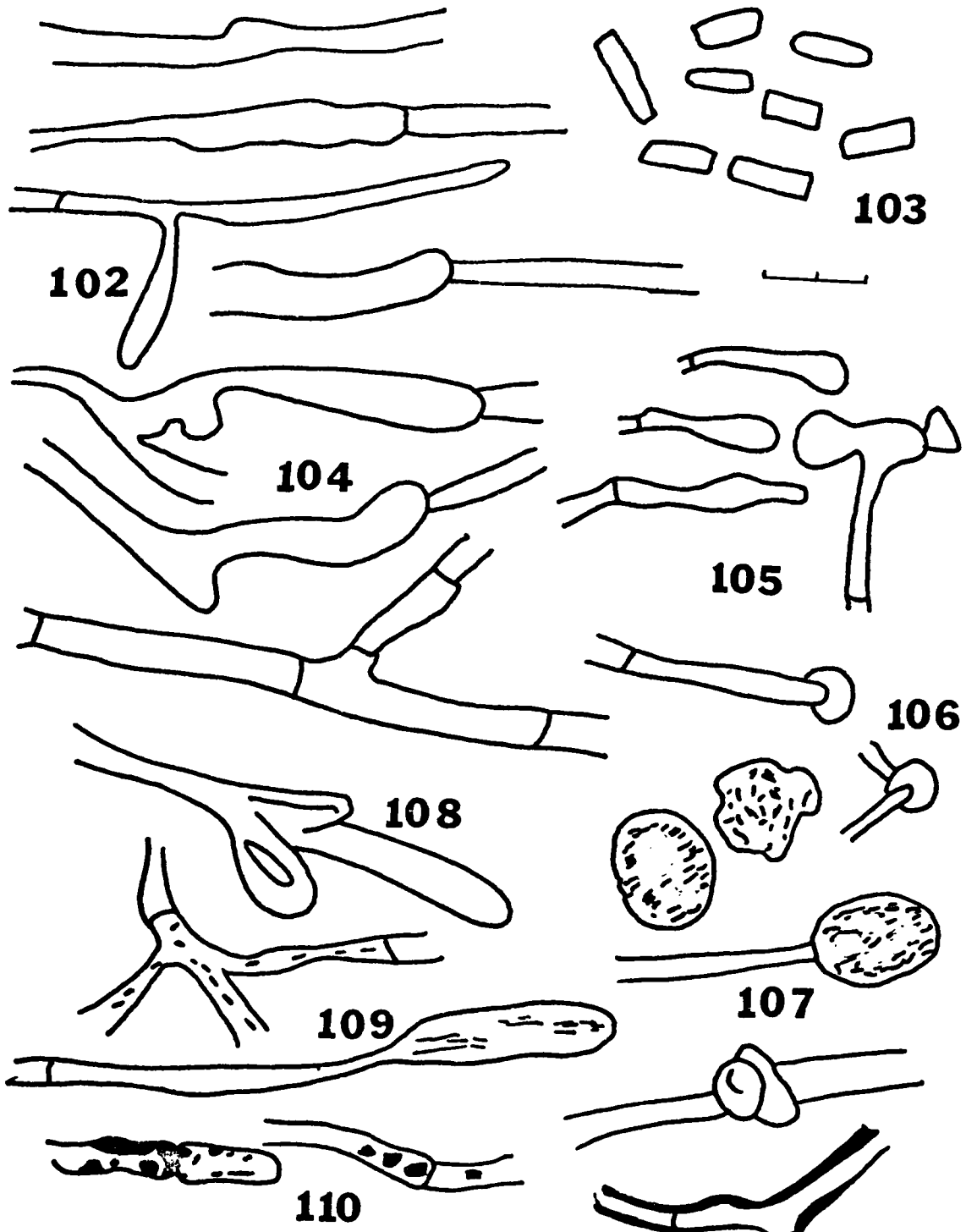
Figure 86. Linear growth of Pholiota spumosa OKM-2310 for five different temperatures in the dark on 1.5% malt extract agar. ——o——10; ——▲——15; ——□——20; ———— 25; ——▽—— 30 C.



Figs. 87-93. P. spumosa OKM-1547 cultural characteristics. Fig. 87. narrow mat hyphae. Fig. 88. oidia. Fig. 89 branched hyphae. Fig. 90. enlarged hyphae. Fig. 91. intercalary amorphous refractive body. Fig. 92. "textura intricata". Fig. 93. amorphous refractive bodies.



Figs. 94-101. P. spumosa OKM-1754 cultural characteristics. Fig. 94. narrow mat hyphae. Fig. 95. oidia. Fig. 96. cystidial end cells. Fig. 97. inflated hyphae. Fig. 98. hyphae with amyloid inclusions. Figs. 99- 100. amorphous refractive bodies. Fig. 101. sphere around hyphae of unknown origin.



Figs 102-111. P. spumosa OKM-2310 cultural characteristics. Fig.102. narrow mat hyphae. Fig. 103. oidia. Fig. 104. branching hyphae. Fig.106 spheres on hyphae. Fig. 107. amorphous bodies. Fig. 108.branching. Fig. 109. amyloid inclusions. Fig. 110-111. thick-walled brown hyphae.

Pholiota decorata (Murr.) Smith & Hesler Figs. 112-114.
The N. Am. Species of Pholiota: 254. 1968.
Gymnopilus decoratus Murril, Mycologia 4: 251. 1912.
Flammula decotata (Murr.) Murrill, Mycologia 4:262.1912.

Pileus 3.4 cm broad, faintly umbonate at disc, mostly buff on margin, radiating fibrils towards disc "fawn color" to "natal brown" or dull red-brown just over the disc, viscid from a gelatinous pellicle except at the disc where a few cream-colored fibrils protrude through the glutinous surface. In age the scales frequently disappear leaving a glabrous or fibrillose-streaked appearance beneath the glutin. Context thin, fleshy, near white to pale buff in some areas. Odor not distinctive. Lamellae adnate, close, moderately broad, "pale vinaceous-fawn" to vineceous-buff, darking in age to "avellaneous" and finally "wood brown". Stipe 3.5-5.5 cm long, 3-8 mm thick, buff, with reddish brown fibrils below the veil which becomes whitish in age; subviscid to dry. Contexts as in the cap but darkening to light-brown near the base.

Spores 5.5-7 x 3-4.5 μ, elliptical, smooth, thin-walled, with a minute apical pore, rusty-brown to yellow-brown in 3% KOH, paler ochraceous in Melzer's reagent. Basidia 23-27 x 5-7 μ, 4-spored, clavate, hyaline, some becoming yellowish in 3% KOH and Melzer's reagent. Pleurocystidia 45-90 x 6-18 μ, fusoid-ventricose, obtuse apex, thin-walled or with wall thickenings up to 2 μ, hyaline or with yellowish contents in 3% KOH and Melzer's. Cheilocystidia 30-50 x 8-12 μ, subfusoid to nearly clavate, thin-walled or rarely

thick-walled, hyaline in 3% KOH and Melzer's reagent. Cuticle of the pileus a gelatinous layer of narrow hyphae, around 2.5 μ in diameter, smooth, thin-walled, hyaline, scattered within the gelatinous layer. Trama of the pileus thin-walled, smooth, inflated hyphae with a diameter up to 12 μ, slightly yellow or hyaline in 3% KOH. Trama of the lamellae with a central strand of floccose, subparallel, thin-walled, hyphae, hyaline to ochraceous in 3% KOH.

All hyphae non-amyloid. Clamp connections present.

Collection examined: U.S.A.: Idaho. OKM-2597 (VPI).

Habit, habitat and distribution: On conifer logs, Benton Creek, Priest River Experimental Forest, Idaho. September 17, 1964.

Observations: This species of Pholiota is placed in the stirps Decorata in the section Flammuloides by Smith and Hesler (1968), on the basis of the dark brown coloration on the disc and the spore size of less than 7-9 x 3.5-4.5 μ. The section Flammuloides itself is separated from the other sections of the subgenus Flammuloides on the basis of the prescence of pleurocystidia with thickened walls. The other Pholiotas, P. spumosa and P. scamba, in the subgenus Flammuloides are lighter in color over the disc and have larger spores and thin-walled cystidia. Although N. nubigenum has larger spores and a lighter color than P. decorata, it has similar thick-walled cystidia with

an overlapping size range. N. nubigenum has cystidia ranging from 60-100 x 15-25 μ, while those of P. decorata range from 45-90 x 6-18 μ and are the largest cystidia of all the Pholiotas examined in this paper.

Pholiota decorata (Murr.) Figs. 115-124

File Pattern for culture OKM-2597: API,1,4,8,11, and 16.

Cultural Characteristics

Growth Characteristics- Growth rate at 25 C is moderately rapid, radius 9.9 cm in two weeks. Optimum temperature is 25-30 C. Inhibitory temperature 35 C, lethal since the cultures did not revive when returned to 25 C. Average mat radius in 14 days at constant temperatures are found in Table II. See figure 115 for growth rates.

Mat Appearance- Advancing zone even. Mat white, appresse to intermediate. Texture felty to slightly downy. Odor sharp fruity.

Oxidase Tests- Gallic acid agar showed a strong reaction of intensity four, growth confined to a few small patches of fuzz on the agar, less than 5 mm growth in three weeks. Tannic acid agar reaction intensity of four, growth dense on the inoculum plug by the second week and onto the agar by the third week. Gum guaiac reaction time, 8 minutes to become milky and 60 minutes for an even blue color.

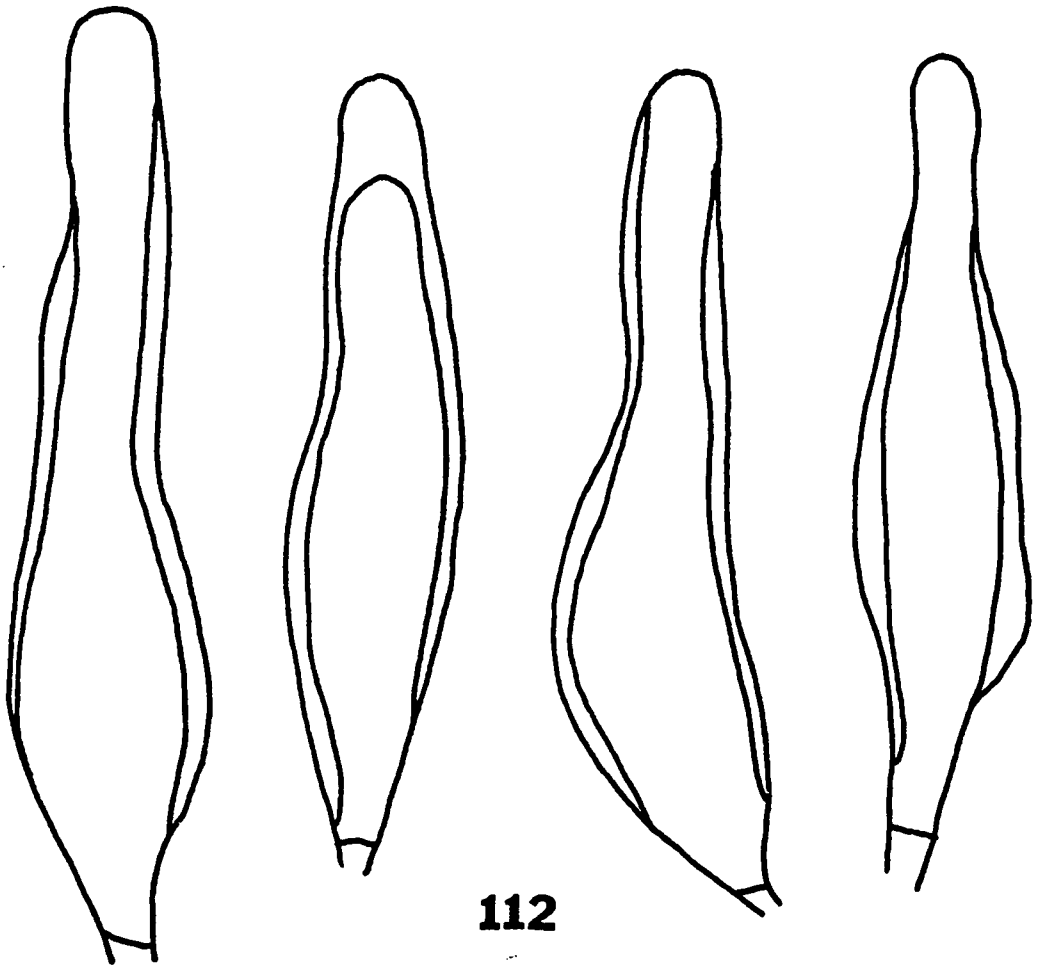
Morphological Characteristics- Hyphae of two types: 1.Cylindric to inflated or irregular, thin walled hyphae 2-3 μ diameter, 15-30 μ long, clamps abundant (Figs. 117-118). Submerged hyphae are of the same size but often appear spiraled (Fig.119). 2. Large cylindric to inflated cells, enlarged where branched or attached to another cell,

thin-walled, 6-15 μ diameter, 20-30 μ long, without clamps (Fig. 121). Both hyphal systems contain hyaline hyphae in 3% KOH. In Melzer's solution there are both hyaline and golden brown hyphae. Cystidial end cells present, variable in shape, usually inflated, club shaped, some with oily contents, thin-walled, hyaline in 3% KOH and golden brown in Melzer's reagent, deep pink in Phloxine (Fig. 120).

Oidia are present in most areas, varible in size, 4-9 x 1-3 μ, thin-walled, hyaline in 3% KOH and Melzer's reagent. Deep pink in Phloxine. (Fig. 116).

Tissue type appears to be "textura intricata" as shown in Figure 123. This type of cell arrangement is found in older portions of the mat in areas where the hyphae seem to be intertwining or radiating out from tight sections (Fig.112).

The mat appearance of P. decorata on media other than malt extract agar showed some variations. The appearance on Prune extract agar, Emmerson's YpSs agar, glucose mixture was not much different than on malt extract agar other than it was more cotteny in appearance, with more raised hyphae. When Englemann spruce extract was added to this mixture and used in growing the culture in a flask in the growth chamber, the only difference was the appearance of golden brown droplets on the mat. The greatest variation occured in the flask containing 1.5% malt extract agar with Englemann spruce infusion and stick. The hyphal mat was still fluffy white but golden brown threads of hyphae radiated across the surface.



Figs. 112-114. P. decorata OKM-2597. Fig. 112. thick-walled cystidia. Fig. 113. basidia. Fig. 114. spores.

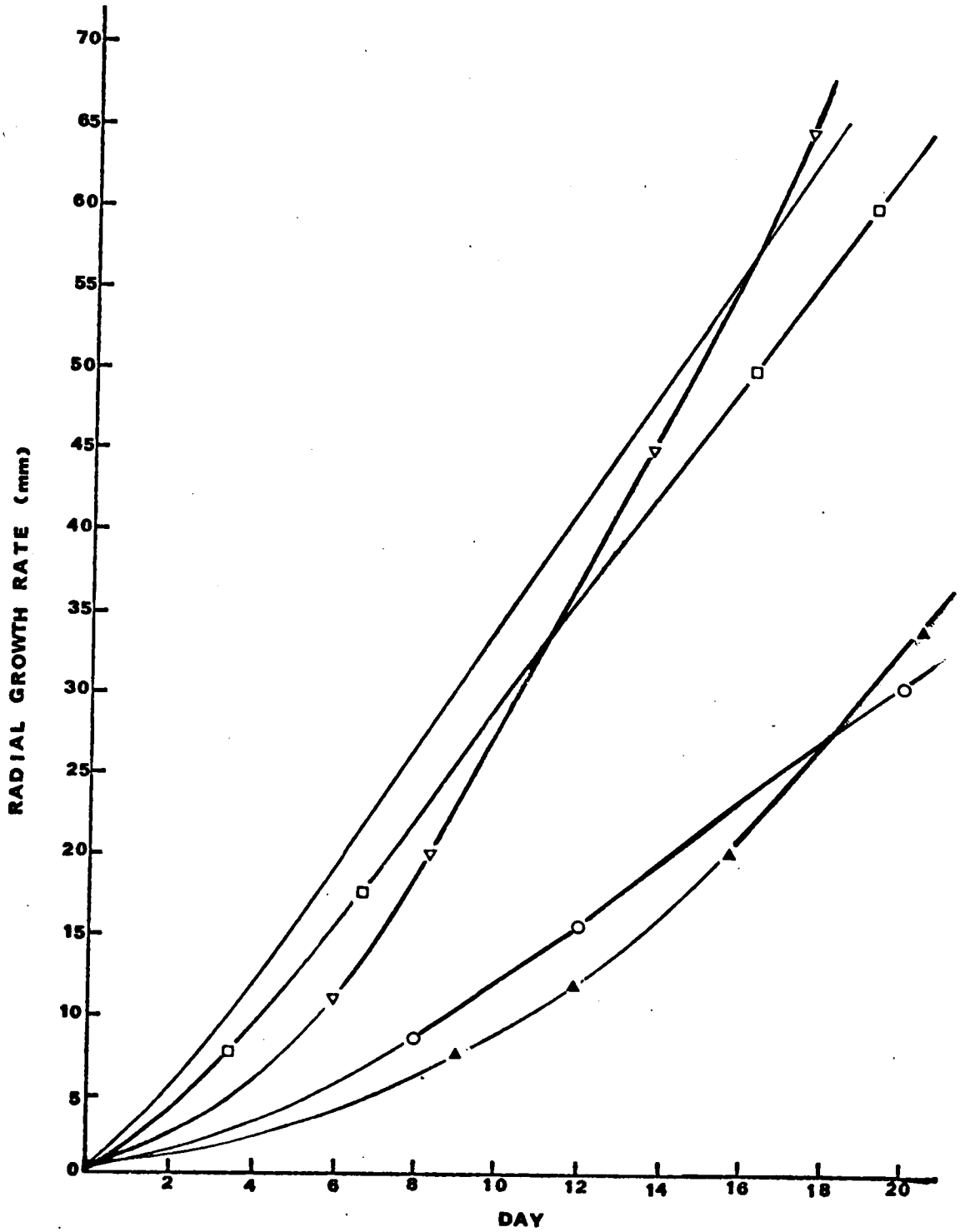
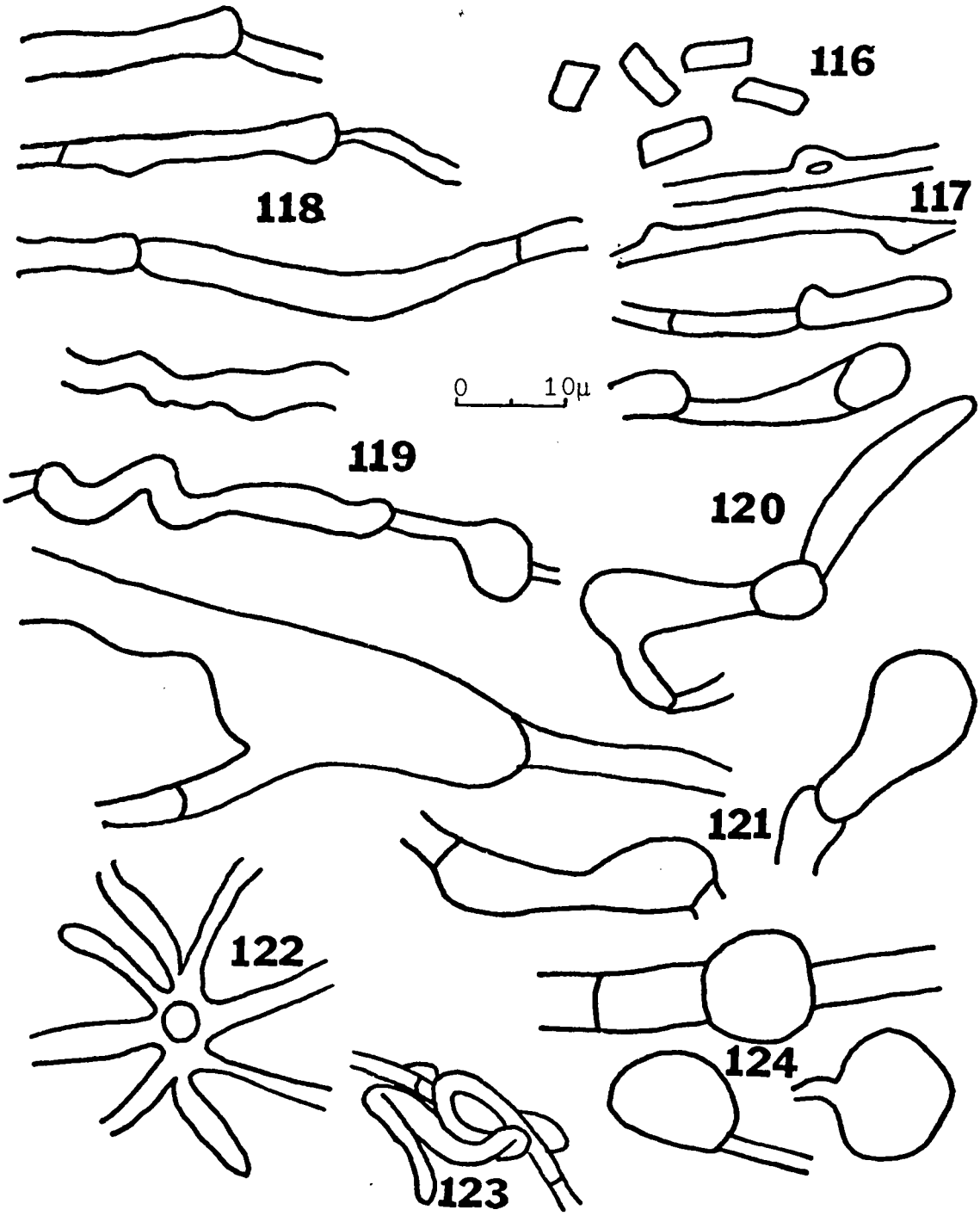


Figure 115. Linear growth of Pholiota decorata OKM-2597 for five different temperatures in the dark on 1.5% malt extract agar. ——o—10; ——▲——15; ——▫—20; ——— 25; —▿— 30 C.



Figs. 116-124. P. decorata OKM-2597 cultural characteristics. Fig. 116 oidia. Figs. 117-118. narrow mat hyphae. Fig. 120. cystidial end cells. Fig. 121. inflated hyphae. Figs.122-123. arrangements of hyphae in older sections. Fig. 124. amorphous refractive bodies.

Pholiota scamba (Fr.) Moser. Figs. 125-127
in Helmut Gams Kleine Kryptogamenflora II.p.228. 1955.
Agaricus scambus Fries, Epicr. Myc., p. 184. 1836-38.

Pileus 8-12 mm broad, convex, pallid, viscid, with coarse appressed fibrils (as seen with the hand lense), fibrils appear silky when dry; pinkish buff, with pinkish cinnamon fibrils. Context yellowish, very soft, watery. Odor faintly fragrant. Lamellae adnate, subdistant, medium broad, alternating with lamellule, buff becoming brown in age. Stipe 16-20 mm long, 1.5-2.5 mm thick, equal, buff, dry, lighter at apex,base with clay brown, wooly fibrils.

Spores 7-9 x 4.5-5.5 μ, thin-walled (0.25 μ thick), smooth, elliptical, with a small apical pore, sometimes appearing truncate, dull cinnamon in 3% KOH, a pale reddish cinnamon in Melzer's (Fig.127). Basidia 18-24 x 7-9 μ, 2- to 4-spored, clavate, hyaline, some appear yellowish in 3% KOH and Melzer's (Fig.126). Pleurocystidia 28-40 x 8-14 μ, thin-walled fusoid-ventricose with obtuse apex, most have short necks, hyaline of with yellowish contents in 3% KOH and Melzer's (Fig.125). Cheilocystidia 24-33 x 7-10 μ, subovate or fusoid-ventricose with a short neck, thin-walled, yellowish contents in 3% KOH and Melzer's reagent.

Cuticle of the pileus a layer of subgelatinous, thin-walled hyphae, 3-5 μ in diameter, some with yellow-brown encrusting material. Trama of the pileus with compactly arranged, smooth, yellow to yellow-brown hyphae 3-8 μ in diameter, thin-walled, floccose hyphae near the cuticle and

thin-walled, inflated hyphae in the context, hyaline to yellow in 3% KOH. Trama of the lamellae with a central area of floccose, loosely interwoven hyphae, thin-walled, some inflated to 3-8 μ, hyaline in 3% KOH and evenly yellowish in Melzer's reagent. All hyphae non-amyloid. Clamp connections present.

Collection examined: U.S.A.:Alaska. OKM-7770 (VPI).

Habit, Habitat and Distribution- On dead hemlock snag. Douglas Island, Juneau, Alaska. July 28, 1969.
Smith and Hesler (1968) reported it on conifer logs, from June to October, Michigan, Idaho, Washington and Canada.

Observations: This species is smaller than the others studied and has smaller pleurocystidia and cheilocystidia than the others, however the spores have the same size range as P. spumosa. The gelatinous subhymenium is characteristic of the subgenus Flammuloides to which it belongs. Smith and Hesler (1968) described the pleurocystidia as being between chrysocystidia and leptocystidia.

Pholiota scamba (Fr.) Moser. Figs. 128-132

File Pattern for culture OKM-7770: CPV, 1, 8, 10, 11.

Cultural Characteristics- Growth rate at 25 C is very slow, radius 1.9 cm in two weeks. Optimum temperature 20 C. Inhibitory temperature 30 C, lethal 35 C. (Fig. 127, Table II).

Mat Appearance- Advancing zone even and thin. Mat yellow, appressed, older portions may be slightly raised and a dark yellow, almost brown. Texture felty to silky. Odor fruity.

Oxidase tests- Strong diffusion zones, intensity of four, on gallic and tannic acid agars. No growth on gallic acid agar. Growth appeared by day 7 on the tannic acid agar, confined to the inoculum plug throughout the three week study. Gum guaiac reaction very strong, 30 seconds to become greenish, one minute for dark blue to appear.

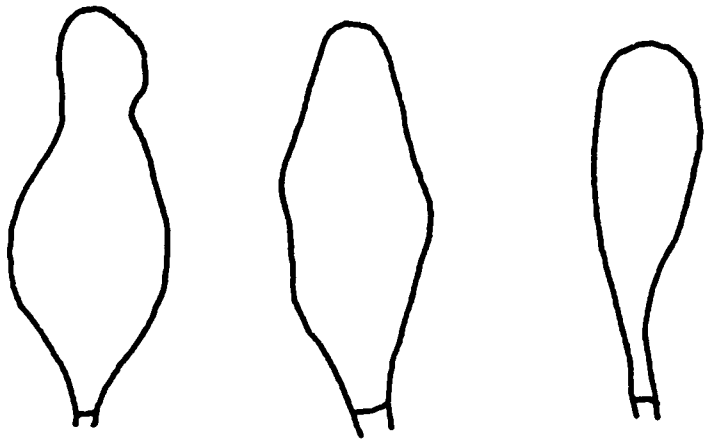
Morphological characteristics- Hyphae of two types, both with abundant clamp connections. 1. Cylindric, thin walled hyphae, 2-5 μ in diameter, even or only slight variations in diameter (Figs.129-130). 2.Vesicular hyphae, thin walled, 5-10 μ in diameter, enlarged at areas of branching and with enlarged areas irregularly spaced through the cells, giving unusual shapes (Fig. 131) Both hyphal systems are hyaline in 3% KOH and Melzer's solution, with some cells turning golden brown in Melzer's. Red inclusions are seen occasionally in Melzer's solution.

These inclusions are usually together in a small group of cells. These cells do not appear to be differentiated in any way other than having the dextrinoid reaction.(Fig.130)

No asexual spores nor amorphous refractive bodies were seen in any cultures examined.

"Textura oblita", parallel, thick walled, dark brown cells were seen in the culture grown on the Prune extract agar with Emerson's YpSs agar, glucose and Picea engelmanii infusion (Fig.132). The areas where these cells developed could be seen as dark brown dots on the hyphal mat. These cells were not found on any other medium. The area around the inoculum plug was deeply furrowed, and it was in this area that the brown cells were found. There was little differentiation on the 1.5% malt extract agar with the Englemann spruce infusion. There was dense growth around the stick.

Observations: This was the slowest growing culture. It was the only one with a yellow mat at all times although P. spumosa OKM-2310 did turn yellow as it aged. The deep furrows around the inoculum plug in the Prune extract, Emerson's YpSs agar, glucose medium was very similar to the furrows seen in P. spumosa, as were the appearance of brown dots with thick-walled hyphae on the mats in OKM-2310. The dense growth around the Englemann spruce stick was similar to that of P. subangularis. The dextrinoid inclusions in the hyphae were the most outstanding cultural character.



125

0 10μ

126

127

Figs. 125-127. P. scamba OKM-7770. Fig. 125. cystidia Fig. 126. basidia. Fig. 127. spores.

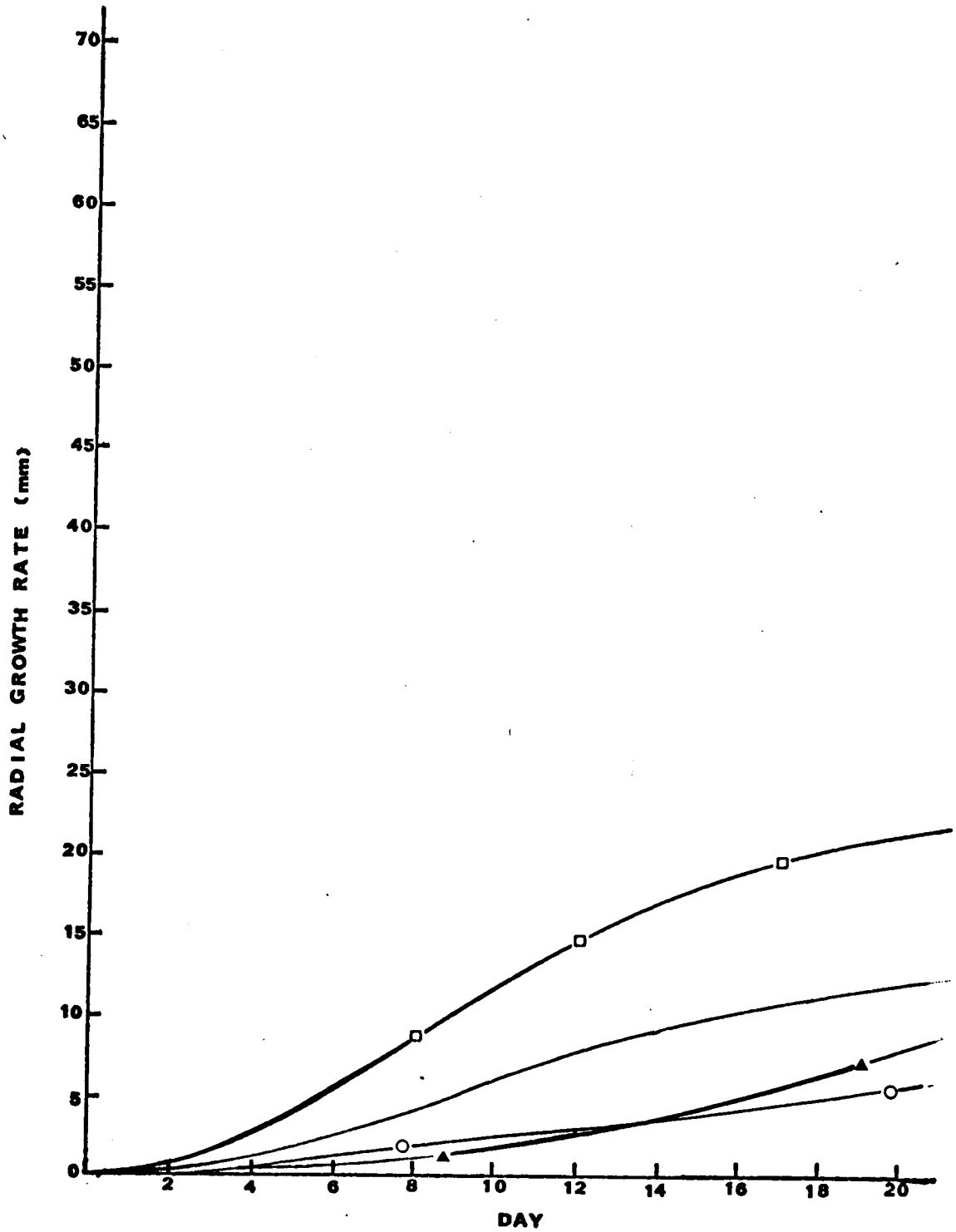
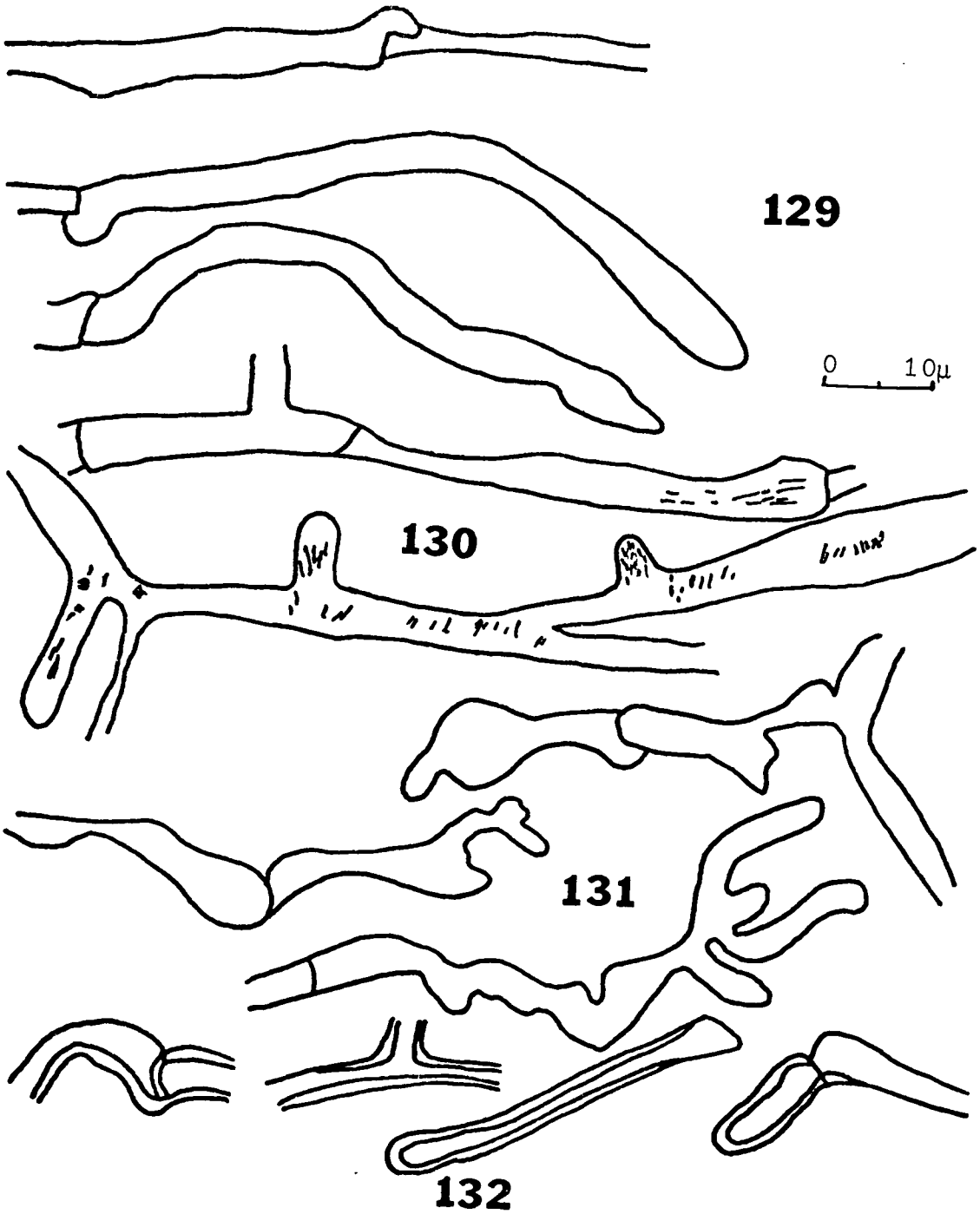


Figure 128. Linear growth of Pholiota scamba OKM-7770 for five different temperatures in the dark on 1.5% malt extract agar. ——o—10; ——▲——15; ——□—20; ——— 25; ——▽— 30 C.



Figs. 129-132. P. scamba OKM-7770 cultural characteristics. Fig. 129. narrow hyphae with clamp connections. Fig. 130. hyphae with dextrinoid inclusions. Fig. 131. contorted hyphae in older portions. Fig. 132. thick-walled hyphae.

Pholiota subangularis Smith & Hesler Figs.133-135
The N. Am. Species of Pholiota:p.44. 1968.
Kuehneromyces carbonicola Smith, Sydowia Suppl.1:53.1957.

Pileus 2-5 cm broad, convex-umbonate, nearly plane in age, some with upturned margins, evenly colored deep mahogany brown, glabrous to subviscid, a marginal zone of light brown fibrils from the veil, which is thin, white and fibrillose in the button stage. Context thin, watery brown, fading to buff in age. Odor none. Lamellae adnexed to to adnate or emarginate, close to subdistant, thin, two tiers of lamellule, cinnamon brown, maturing to hazel or "kaiser brown", edges fimbriate Stipe 3-7 cm long, 2.5-3.5 cm wide, lower three-fourths fibrillose giving a silky appearance, due to the remnants of the veil, a fibrillar superior annular zone appears with a brown coloration from the spores, the rest of the stipe is a ground color. Veil thin, white, fibrillose.

Spores 8-11 x 6.5-9 μ, elliptical to unequally ovoid, smooth, thick-walled, (1-1.5 μ), distinct apical pore, slightly truncate, deep cinnamon-red in 3% KOH and Melzer's solution (Fig. 135). Basidia 23-26 x 8-10 μ, 4-spored, clavate, hyaline in 3% KOH, yellow in Melzer's reagent (Fig. 134). Pleurocystidia none. Cheilocystidia 25-39 x 8-13 μ, abundant, fusiform with short necks, smooth, thin-walled, hyaline (Fig. 133). Cuticle of the pileus composed of a layer of errect compact hyphal end cells, 4.4-13.5 μ in diameter, cylindric, fusiform to ineqular, smooth walls

giving an ochraceous color in water, a deep red-brown in 3% KOH, yellow in Melzer's reagent. Trama of the lamellae parallel, thin-walled hyphae, some incrusted with hyaline particles, the cells pale cinnamon in 3% KOH. All hyphae non-amyloid. Clamp connections present.

Collection examined: U.S.A.: Idaho. OKM-8341.

Habit, habitat and distribution: Gregarious on the soil in areas where wood has been burned. One mile below goose Lake, Payette National Forest, Idaho. June 22, 1970.

Observations: The lack of pleurocystidia, the extremely thick walls of the spores, the reddish cinnamon wall coloration of the spores in 3% KOH and Melzer's, and the dark brown pileus distinguish this fungus from the others in this study. The spores are much larger than the other Pholiotas studied, however, they are within the range on N. nubigenum.

Pholiota subangularis (Singer) Smith & Hesler Figs. 136-140

File Pattern for culture OKM-8341: APV, 1, 8, 10, 16.

Cultural Characteristics

Growth Characteristics- Growth rate at 25 C is very slow, radius 1.9 cm in two weeks. Optimum temperature 20 C. Inhibitory temperature 30 C, lethal 35 C. (Fig. 136).

Mat Appearance- Advancing zone even. Mat white, slightly raised, cottony to downy. Odor fruity.

Oxidase Tests- Gallic and tannic acid agar diffusion zones weak, intensity of one. No growth on either agar. Gum guaiac substrate turned milky in 8 minutes and it took an hour to turn blue.

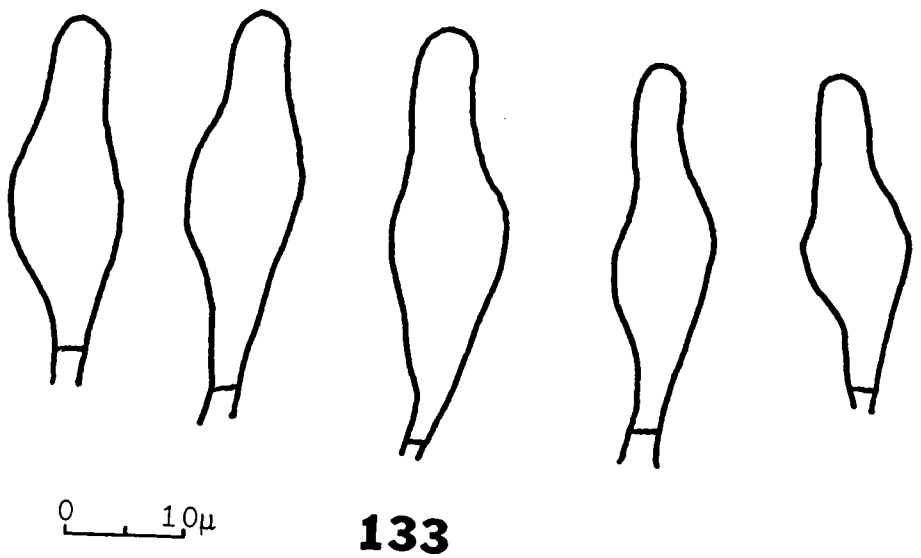
Morphological Characteristics- Hyphae cylindric to inflated of irregular, thin walled 2-3 μ in diameter, except where inflated up to 10 μ. Enlargements usually occur where two cells come together or where branching. The inflated cell type is dominant over regular cylindric cells. Hyphae hyaline in 3% KOH. Hyaline or golden brown in Melzer's solution.Most hyphae stain deep pink in Phloxine, some remain hyaline. Clamps are abundant.

No asexual spores nor amorphous refractive bodies seen in any of the cultures examined.

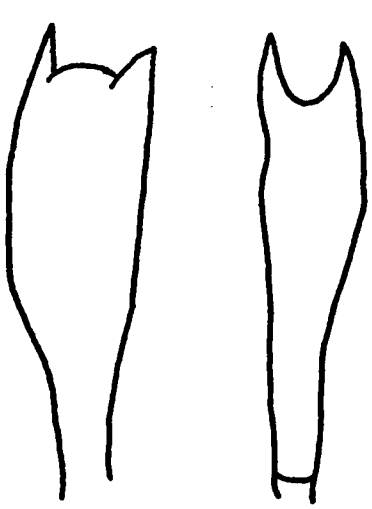
Hyphae tend to grow closely in a tight bundle, in the older portions of the mat, giving the appearance of threads growing through the hyphal mat as seen under the microscope. These bundles stain pink in Phloxine and brown in Melzer's.

When grown on different media P. subangularis did not differentiat much. On Prune extract, Emerson's YpSs agar, glucose mixture in a flask the mat remained white in color but it developed deep furrows radiating out from the inoculum plug further than seen in any other culture. The response to the malt extract agar with the infusion and stick from Englemann spruce was slow fluffy growth, with the heaviest growth on the stick.

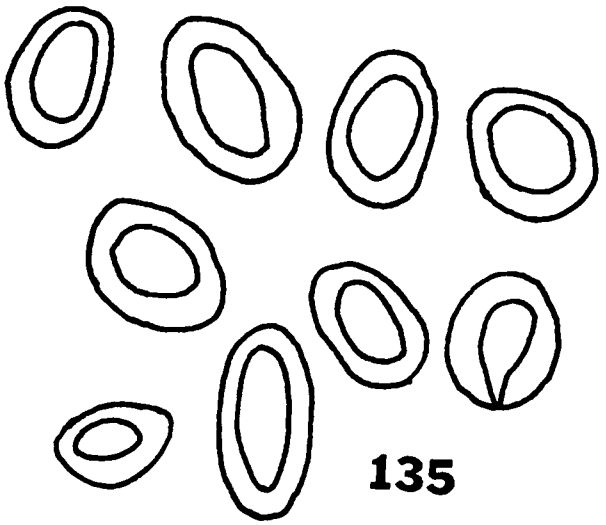
Observations: This culture along with P. scamba had the slowest growth rate. The oxidase tests were weak as were those of P. spumosa and some cultures of N. nubigenum. This culture showed the least differation in microscopic features and mat appearance, on all agar types, of all the cultures.



133



134



135

Figs. 133-135. P. subangularis OKM-8341. Fig. 133. cheilocystidia. Fig. 134. basidia. Fig. 135. spores.

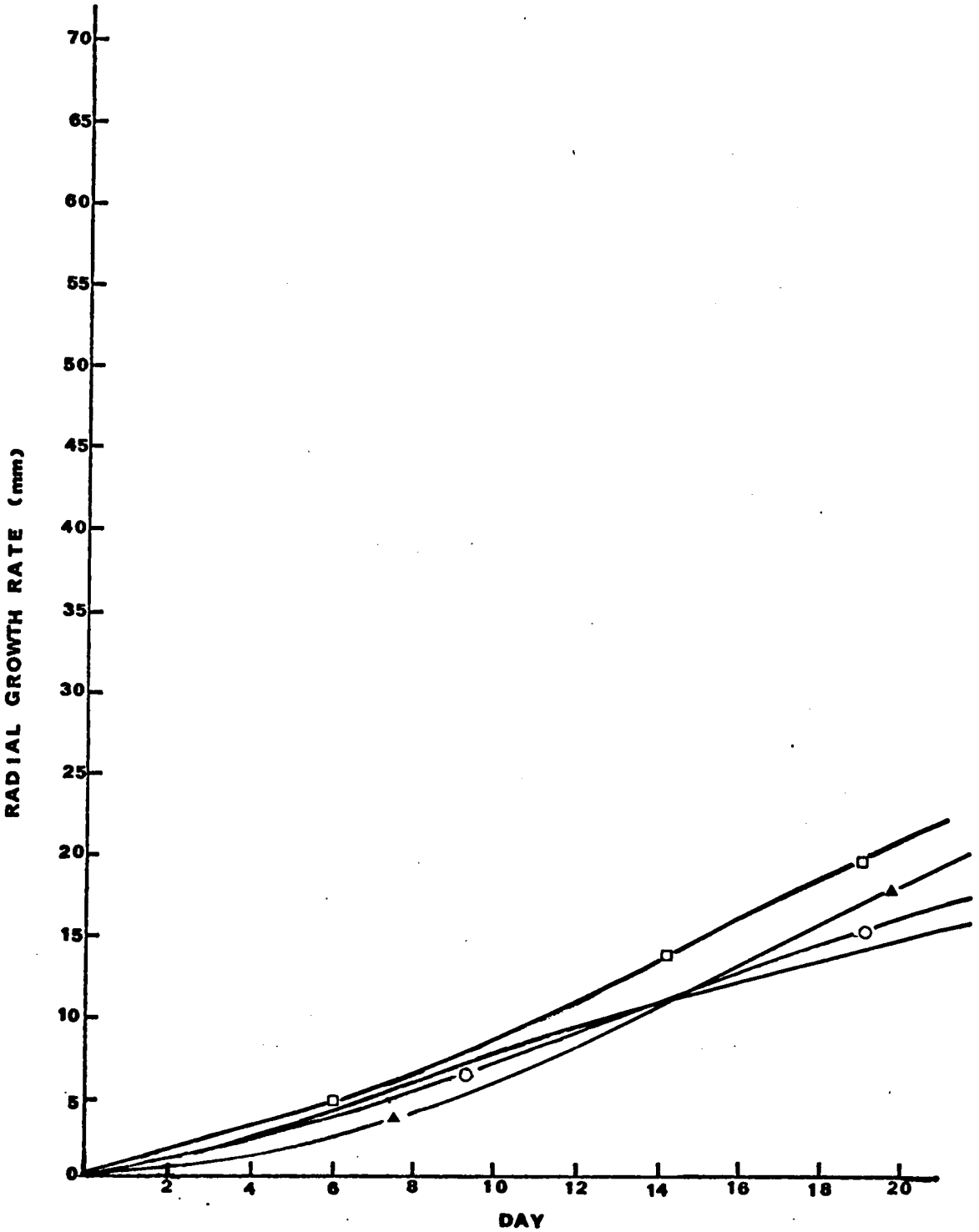
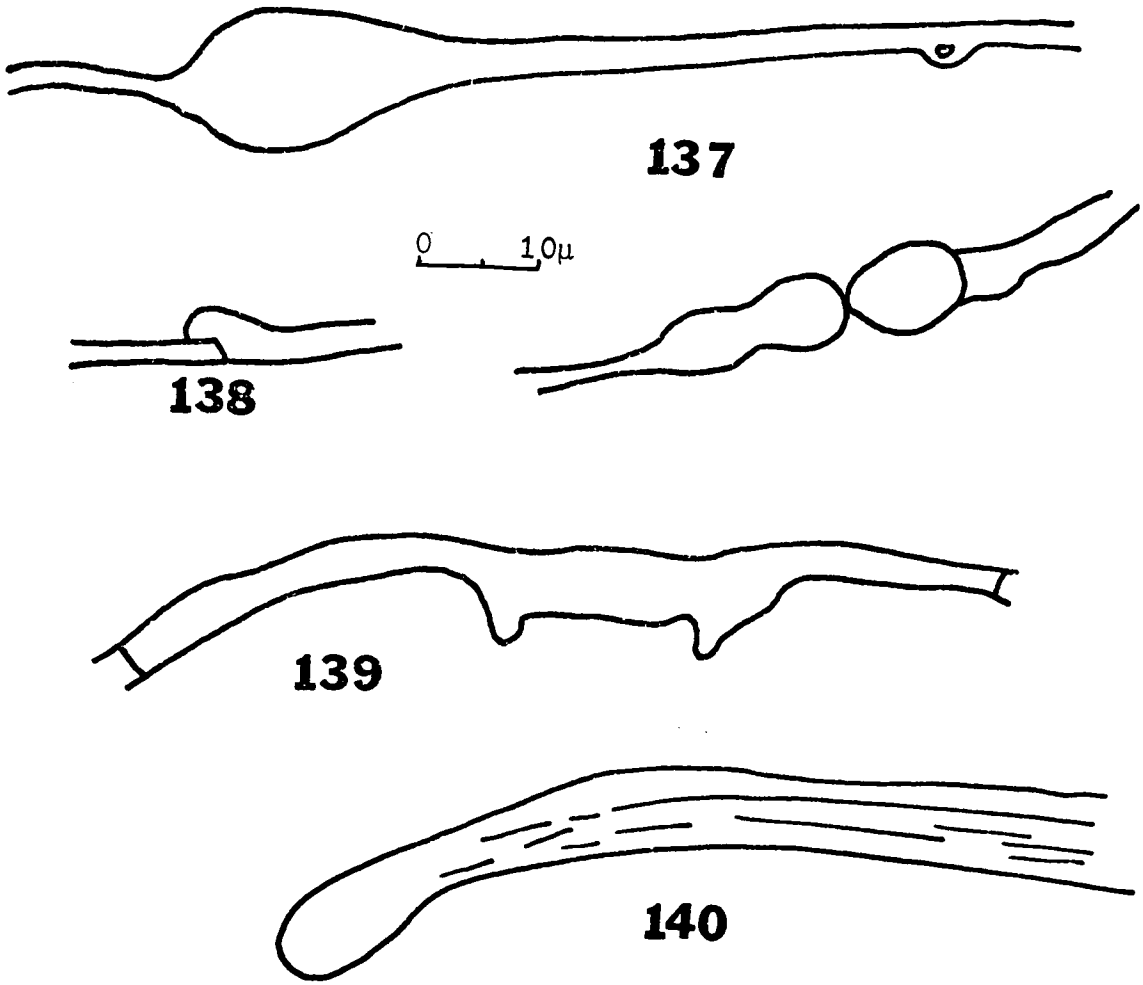


Figure 136. Linear growth of Pholiota subangularis OKM-8341 for five different temperatures in the dark on 1.5% malt extract agar. ——o—10; ——▲——15; ——□—20; ——— 25; —▽— 30 C.



Figs. 137-140. P. subangularis OKM-8341 cultural characteristics. Fig. 137. inflated hyphae and major cell type. Fig. 138. clamp. Fig. 139. branching. Fig. 140. bundle of hyphae.

Table II. Average radius of mycelial mat in mm after 14 days at five different temperatures, on malt extract agar.

Isolate	Temperature (C) 10	15	20	25	30
N. nubigenum					
OKM-3090	7.4	18.8	31.5	30.0	27.6
OKM-4003	12.5	11.0	38.0	39.5	31.0
OKM-4004	11.5	12.8	38.0	41.0	29.3
OKM-4006	10.0	12.2	28.0	39.0	21.0
Mean	10.4	13.7	33.9	37.5	27.2
S.D.*	2.22	3.48	4.97	4.99	4.38
S.E.*	1.11	1.74	2.49	2.49	2.19
P.spumosa					
OKM-1547	8.5	5.0	18.0	42.5	45.5
OKM-1754	9.0	7.3	16.8	42.0	45.2
OKM-2310	10.0	8.0	22.2	44.0	54.0
Mean	9.2	6.8	19.0	42.8	48.2
S.D.	0.76	1.57	2.83	1.04	5.0
S.E.	0.44	0.90	1.64	0.60	2.88
P. decorata					
OKM-2597	20.2	17.0	42.5	49.0	47.5
P. scamba					
OKM-7770	4.0	4.0	17.0	8.0	0
P. subangularis					
OKM-8341	11.0	10.3	18.0	10.3	0

* S.D.= Standard deviation
S.E.= Standard error

Table III. OXIDASE TESTS

Isolate	Gallic Acid Reaction	Growth on Gallic Acid	Tannic Acid Reaction	Growth on Tannic Acid	Gum Guaiac Reaction Time
N. nubigenum OKM-3090	4+	Fuzz on 1 plug day 14	1-4+	Fuzz on top of plug day 7. Fuzz on agar day 21	Positive weak-medium 3 min. milky, 6 min. green tint 20 min. greenish blue
OKM-4003	4+	None	1+	None	Neg. 7 min. milky, 24 hrs. to turn blue
OKM-4004	4+	None	1+	None	Neg. 7 min. milky, 24 hrs. to turn blue
OKM-4006	4+	None	1-2+	None	Positive-weak 3 min. milky 1 hr. greenish blue
P. spumosa OKM-1547	4+	None	4+	Fuzz on plug, day 7. several mm growth on agar day 21	Positive medium 2 min. milky, lt. blue 2-15 min. Intense blue
OKM-1754	4+	Fuzz on 1 plug day 21	4+	Fuzz on top of plug day 7. 5 mm on agar day 21	Positive, weak-to medium 2 min. milky 5-60 min. blue

1+ = slight, discolored area under inoculum
2+ = medium, discolored area confined to portion under mat
3+ = discolored zone extending beyond margin of mat
4+ = strong, discolored zone dark brown, opaque, extending much beyond mat

Table III. cont.

Isolate	Gallic Acid Reaction	Growth on Gallic Acid	Tannic Acid Reaction	Growth on Tannic Acid	Gum Guaiac Reaction Time
P. spumosa OKM-1547	4+	None	1-4+	Fuzz on 1 plug, day 14. 1 plug covered day 21 no growth on others	Positive
P. decorata OKM-2597	4+	Fuzz on agar day 14	4+	Fuzz on plug by day 7 Fuzz on agar day 21	Positive-weak 8 min. milky 1 hr. blue
P. scamba	4+	None	4+	Fuzz at base of plug day 7	Positive, strong 30 sec. greenish 1 min. dark blue
P. subangularis OKM-8341	1+	None	1+	None	Positive-weak 8 min. milky 1 hr. blue

DISCUSSION

The search for the possible relatives of the gasteromycete Nivatogastrium nubigenum, lead to a careful consideration of the members of the Secotiaceae. It was found that there are several dark spored gastroid fungi with contorted tramal plates. The characteristics of Endoptychum, Weraroa, Thaxterogaster and Galeropsis were compared with those of Nivatogastrium. Weraroa was found to have the most in common with Nivatogastrium but differed markedly in spore coloration and morphology, sporocarp appearance and Weraroa was not found on conifer wood. Since all of the gasteromycetes considered were compared to agaric genera, the suggestion of Singer and Smith (1959) that Nivatogastrium was related to the agaricales, seemed to be a reasonable line to pursue. They suggested the genera Pholiota, Pleuroflammula and Kuehneromyces. These genera are now listed under Pholiota (Smith and Hesler, 1968).

Comparing the various subgenera of the genus Pholiota as described by Smith and Hesler (1968), it is found that the subgenus Flammuloides has the largest number of characteristics in common with Nivatogastrium. The works of Nobles (1948, 1958), Miller (1971) and Watling (1971) have shown that the study of cultural characteristics can lead to a better understanding of relationships between genera and within them. Members of the subgenus Flammuloides were available in culture as were collections of Nivatogastrium

and Pholiota subangularis (Kuehneromyces carbonicola Smith, a suggested relative of N. nubigenum, Singer and Smith, 1959). Pholiota scamba, P. decorata and P. spumosa are all members of the subgenus Flammuloides (Smith and Hesler,1968).

The study of the cultures showed a number of characteristics that can be used to support the hypothesis that Nivatogastrium is related to Pholiota, the subgenus Flammuloides in particular. The suggestion by Singer and Smith (1959) that Kuehneromyces carbonicola (P. subangularis), was a possible relative is not supported by the data from the culture study. This species is in the subgenus Hygrotrama (Smith and Hesler, 1968). The sporocarp is much darker than that of N. nubigenum and members of the subgenus Flammuloides. P. subangularis lacks pleurocystidia. The cheilocystidia are much smaller (20-26 x 8-13 μ) than the cystidia of N. nubigenum (60-110 x 15-25 μ), which are found on the sides of the tramal plates and would be equivalent to pleurocystidia in an agaric. Optimum temperature for growth of P. subangularis in culture is 20 C with 18 mm of radial growth in 14 days, for N. nubigenum it is 25 C and 37.5 mm growth. P. scamba has a low growth rate, 17 mm in 14 days at 20 C. In 14 days P. decorata grew 49 mm at 25 C and P. spumosa grew 48 mm at 30 C.

The growth rates of P. spumosa and P. decorata are greater than that of N. nubigenum, however, there are other characteristics of the culture that support the idea that

they are related. The presence of oidia, inflated cells, amorphous refractive bodies, "textura intricata", positive oxidase tests and the growth of hyphae on the tannic acid agar are all characteristics that P. spumosa, P. decorata, and N. nubigenum have in common. There are characteristics that vary within the same species, such as the reaction to tannic acid by the cultures of N. nubigenum and P. spumosa (Table III and Figs. 1-17). The white mat turning yellow in culture OKM-2310 of P. spumosa and the occurrence of amyloid inclusions in this culture and culture OKM-1754 are other examples of variations within species. The occurrence of thick-walled, brown hyphae in P. scamba and P. spumosa (OKM-2310) along with the yellow of the mat may link these two species in the subgenus Flammuloides. The similar cultural characteristics of P. decorata and N. nubigenum led me to reexamine the sporocarps for other possible similarities. It was at that time that I first became aware of the thick-walled cystidia in N. nubigenum which very closely resembled P. decorata.

The file pattern of the cultural characteristics could be used to separate all the species in this study, however, if more cultures were available and additional species within the Flammuloides were studied, there may be an overlapping of features in the file pattern. The only difference between N. nubigenum and P. spumosa in the file pattern is the presence of clamp connections (microscopic feature 1,Table I).

The P. spumosa cultures could have reverted to a monocaryon and fresh cultures may well have an abundance of clamps. The morphological characteristics are necessary in making a a distinction between the cultures. Several different media should be used to induce the formation of as full a range of differentiation in the hyphae as possible. The oxidase tests were all positive in the file pattern and little difference could be seen in the gallic acid agar tests. The tannic acid agar showed the greatest diversity in intensity of the diffusion zone and growth on the agar. Gum guaiac reactions were slower than those reported by Nobles (1958), in most cases. The reactions were not constant in reaction time or intensity of color. The appearance of a blue-green color after several hours suggests that the enzymes were induced to form by the gum guaiac substrate. This test does not appear to be constant enough to use for this group of fungi.

Additional members of the subgenus Flammuloides need to be studied in culture to see how variable the group is as a whole in cultural characteristics. Information on other members of the genus Pholiota is needed before one can be sure that N. nubigenum is related to the subgenus Flammuloides as suggested by Smith and Hesler (1968), however, the data from this experiment supports the hypothesis.

Attempts to induce fruiting, although unsuccessful, did provide additional data for comparisons. Deep furrows on the

surface of the agar were seen in cultures of N. nubigenum, P. spumosa and P. scamba. The golden brown threads that occurred on the mat of P. decorata could perhaps be used as an identifying characteristic. Use of additional media may eventually lead to fruiting, however, even if it does not, it may lead to finding a larger set of identifying characteristics. At the time attempts were made to induce fruiting in these cultures, the growing conditions were probalbly not optimum. A number of species of Pholiota have been induced to fruit in culture and the cultures studied may yet fruit if the proper humidity, temperature and light conditions are found. A mixture of cornmeal, cornstarch and malt extract (Etter, 1929) might produce fruiting since other Pholiotas are fruiting on it (Farr,1973). It would be desirable to get the developement of fruiting bodies so single spore isolates can be tested for the presence of compatible mating strains between different cultures of the same species and different species. This would also aid in studying the difference between the monocaryotic and dicaryotic stages in culture.

CONCLUSION

The gastromycete Nivatogastrium nubigenum appears to be closely related to the subgenus Flammuloides, in Pholiota, a genus of the Cortinariaceae. This relationship is seen in the similar characteristics of the sporocarp and in the cultural characteristics examined. More cultural studies need to be made on other members of Pholiota to better determine the lines of relationship, but the closest relative will probably be similar to P. spumosa or P. decorata and will most likely be found in the Western United States as are P. decorata, P. spumosa and N. nubigenum.

The use of cultural studies appears to be a useful tool in determining relationships between members of the subgenus Flammuloides. It is suggested that more studies be carried out before concluding just how useful the various cultural characteristics are in determining relationships.

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GROWTH AND DEVELOPMENT OF *NIVATOGASTRIUM* A GASTROMYCETE RELATED TO *PHOLIOTA*

by

Ilene Baxter Ray

(ABSTRACT)

The morphological features of *Nivatogastrium nubigenum* are compared with closely related gastromycetes and lamellate agarics. Only one genus, *Pholiota* in the Cortinaricaeae, was found to have many features similar to *N. nubigenum*. The subgenus *Flammuloides* has many features in common with this gasteromycete.

The fruiting bodies and cultural characteristics of *P. decorata*, *P. scamba*, *P. spumosa*, and *P. subangularis* are compared with *N. nubigenum*. All of the fungi studied are found in the western United States on conifer wood. Smooth, brown, thick-walled spores, large cystidia, and a somewhat visicid pileus are found in these species. Thick-walled cystidia, similar to those found in *P. decorata*, are reported for the first time in *N. nubigenum*.

Growth rates, oxidase reactions, and morphological features were found to be similar for *N. nubigenum*, *P. decorata*. and *P. spumosa*. Positive oxidase tests, oidia, amorphous refractive bodies, "textura intricata" and inflated hyphal cells were found in these three similar species. Different media were used and additional morphological features were found that can be used to distinguish the species.