HILGARDIA

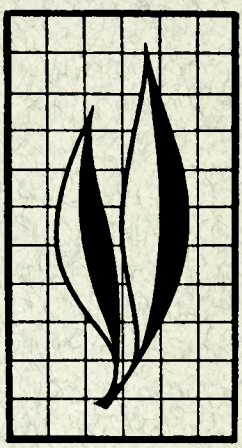
A JOURNAL OF AGRICULTURAL SCIENCE PUBLISHED BY THE CALIFORNIA AGRICULTURAL EXPERIMENT STATION

Volume 39, Number 4 · May, 1968

Phycomycetous Mycorrhizal Organisms Collected by Open-pot Culture Methods

A. E. Gilmore

UNIVERSITY OF CALIFORNIA DIVISION OF AGRICULTURAL SCIENCES



Phycomycetous mycorrhizal organisms, *Endogone* sp., were collected and maintained on strawberry roots in open-pot cultures by three methods: (1) transfer of a field-grown plant to a pot of steamed soil; (2) growing a strawberry runner for a time in potted, unsterilized field soil, followed by transplanting to steamed soil; and (3) inoculation of a strawberry runner in steamed soil with field-collected roots. After the mycorrhizae were well developed, extramatrical spores or vesicles were taken from the pots, disinfected, and used to establish permanent cultures on strawberry plants grown from runners in steamed soil. Eight morphologically different cultures were found among the 19 established. Six cultures are described.

THE AUTHOR:
A. E. Gilmore is Specialist in Pomology in the Experiment Station, Davis.

A. E. Gilmore

Phycomycetous Mycorrhizal Organisms Collected by Open-pot Culture Methods[1]

INTRODUCTION

ENDOTROPHIC MYCORRHIZAE, in which the fungal partner is aseptate (commonly known as phycomycetous or vesicular-arbuscular mycorrhizae), are formed in conjunction with fungi which exist in a remarkably intimate association with the cortex tissue of the plant root. The arbuscles, which are presumably fungal feeding structures, are located within the cells and surrounding the nuclei (Dangeard, 1900), which are apparently uninjured by the invasion. The nuclei remain and the cells appear normal when, after a time, the arbuscles decline and are eventually digested within the cells (Mosse, 1963). The fungi of vesicular-arbuscular mycorrhizae are believed by some to be the most common mycorrhizal organisms (Harley, 1959; Mosse, 1959*a*), and according to Butler (1939), they are "amongst the commonest of soil fungi."

The simple facts of the closeness and prevalence of the association indicate that the fungi involved should be extensively studied by both plant physiologists and mycologists. Yet this has not been the case. Phycomycetous mycorrhizae are not so well known nor understood as are the mycorrhizae with septate fungi, such as the ectotrophic forms on forest trees or the endotrophic kinds on orchids. The reason is, in part, that although phycomycetous mychorrhizae may sometimes be distinguished from nonmycorrhizal roots by their color (Barrett, 1961; Gerdemann, 1961, 1964), they usually do not show the gross morphological forms characteristic of many mycorrhizae with septate fungi, and are, therefore, usually unrecognized.

Furthermore, study of the aseptate mychorrhizal fungi has been hampered because they, unlike many of the septate forms, cannot readily be grown in pure culture on laboratory media. In a few cases they have been so grown, but the factors contributing to the success of those experiments are not understood.

This inability to culture the organisms by classical methods has, until recently, prevented their collection and study. However, work done over the years 1955 to 1963 by Mosse and others who followed her lead indicated the possibility of collecting these organisms as cultures on plant roots in open pots.

The experiment reported here was undertaken in 1964 to determine if open-pot methods were generally suitable for collection of diverse forms of endophytic mycorrhizal organisms.

Although Hawker *et al.* (1957) and Ham (1962) have isolated strains of *Pythium* sp. which form mycorrhizae with *Allium* sp. under certain conditions, *Pythium* is not the usual organism found in the phycomycetous-mycorrhizal association. Peyronel (1923–1924, 1937) was the first to show that *Endogone* sp. were fungal components of vesicular-arbuscular mycorrhizae. Butler (1939), Mosse (1953, 1956), Nicholson (1959), Dowding (1959), and Gerdemann (1961) have confirmed this. It is becoming increasingly ac-

[1] Submitted for publication March 1, 1967.

cepted that the usual fungal organisms associated with vesicular-arbuscular mycorrhizae are species of *Endogone*. However, some mycologists prefer to refer these endophytes to *Rhizophagus*, after Dangeard (1900).

Endogone, as now constituted, contains sporangiocarpic, zygosporic, and chlamydosporic species which are grouped together because their hyphae and spores are similar (Thaxter, 1922; Butler, 1939). On agar media, their asexual spores often germinate, but growth is usually limited (Butler, 1939; Gerdemann, 1955; Godfrey, 1957; Mosse, 1959*a*). Kanouse (1936) grew two species of *Endogone*, neither of which was demonstrated to be a mycorrhizal associate, through numerous transfers, in pure culture on laboratory media. One of these, *E. occidentalis*, which Kanouse described as a new species, was apparently obtained by her from nature only once. The other, *E. sphagnophila*, was available repeatedly, but in many additional trials was never cultured. In the culture of *E. sphagnophila*, sporangiospores, zygospores, and chlamydospores were produced, indicating that earlier mycologists were correct in grouping these diverse forms in a single genus.

Barrett (1947, 1961) isolated eleven cultures of fungi from mycorrhizal roots. The long and tedious procedure sometimes required as much as a year to obtain an isolate (personal communication). The organisms to which Barrett (1947) applied the name *Rhizophagus*, after Dangeard (1900), showed some similarities to *Endogone* in their colorless vegetative spores, and he thought it probable that they were members of that genus (Barrett, 1961). He achieved mycorrhizal synthesis with some of his cultures, and Mosse (1961) confirmed Barrett's synthesis experiments with two of them. According to Mosse (1963), Gerdemann isolated an organism, similar to those of Barrett, on a laboratory medium.

Specimens of *Endogone* fruiting bodies have been collected in various ways. They were first picked up as large sporocarps or spore masses (up to 2 cm in greatest dimension) either on or beneath the soil, or from mosses or organic litter (Thaxter, 1922). Dowding (1955) picked the large spores from the alimentary tracts of rodents at autopsy. Gerdemann (1955) recovered spores of *Endogone* by wet-sieving them from soil, and Ohms (1957) obtained them from soil by means of a density-gradient flotation system. Barrett's taxonomically doubtful *Rhizophagus* organisms were isolated by a hemp seed technique.

Mosse (1953) discovered a species of *Endogone* in association with a strawberry plant grown in a pot in the greenhouse, and began a series of experiments which demonstrated that the species could be maintained on plants grown in open pots. These experiments eventually led to the establishment of pure, two-membered mycorrhizal cultures of this *Endogone* with a number of plant genera (Mosse, 1961), thus proving conclusively the relationship of *Endogone* to vesicular-arbuscular mycorrhizae. During this period Mosse established three important points: (1) Mycorrhizae could easily be established on plants in open pots by inoculation with spores or sporocarps picked from another pot having a mycorrhizal plant. (2) The spores could be freed of contaminants, without injury, by treatment with suitable disinfectants (Mosse, 1953, 1956). These spores provided a pure inoculum which, although grown in mixed culture, would apparently be as useful as one produced in pure culture. (3) Development of the mycorrhizal *Endogone* was greatly stimulated by culture on plants grown in sterilized soil (Mosse, 1959*a*, *b*), thus providing a method for producing a vigorous growth from which spores or

sporocarps could be easily picked in quantity.

These findings of Mosse indicated that the open-pot method might prove generally useful for producing enriched cultures of endophytic mycorrhizal fungi from root or soil inocula, and that otherwise difficult-to-find fruiting bodies might be picked from such cultures with ease. Mosse apparently did not use the technique in this way—that is, to collect other forms or species of *Endogone*. Her findings were based on a single unnamed organism, which was referred to simply as an *"Endogone* sp." and which was an accidental associate of a greenhouse-grown strawberry plant. Mosse (1961) did introduce four additional organisms supplied by others, and obtained by various methods, into pot culture as mycorrhizal associates on various host plants. One of these, an organism similar to her *"Endogone* sp.," was supplied by Koch and had been collected by open-pot culture methods. Others were Gerdemann's "Urbana spores," and cultures RII and RXI of Barrett.

Koch (1961) successfully inoculated a number of plant species in open-pot culture, with soil and roots, and obtained two organisms, one of which was similar to Mosse's, the other, of a different type. He also grew, on plants in pot culture, an organism supplied by Mosse, which she had obtained from field-grown apple roots. Whether this fungus was the same as her *"Endogone* sp." is not clear, but it apparently was not, because Mosse (1959*b*) had pictured proliferating spores washed from field-grown mycorrhizal apple roots, and seemingly different from her *"Endogone* sp."

Barrett (1961) introduced his *Rhizophagus* cultures RII, RV, RVII, and RVIII into open-pot cultures.

After my work was begun in 1964, Stevenson (1964) obtained two forms of *Endogone* by transfer of field-grown plants to pots. One was similar to Mosse's *"Endogone* sp."

Gerdemann (1965) recovered *Endogone fasciculata* Thaxt. by growing corn (*Zea maize* L.) in pots of unsterilized soil.

Daft and Nicholson (1966) maintained three species of *Endogone,* from Scotland, on host plants in pots. These were obtained by wet-sieving of soil. One was similar to Mosse's *"Endogone* sp." and identical with one found by Gerdemann (1961) in Illinois. Thus, to date, if it is assumed that those cultures described as similar to Mosse's *"Endogone* sp." are a single species, but not identical with that of Mosse, and that the fungus recovered from apple roots by Mosse is also different, nine types of mycorrhizal-forming *Endogone* and five *Rhizophagus* organisms have been found that can be cultivated on plants in open-pot culture, and four types, at most, have been first obtained by the open-pot collection technique.

Although only two mycorrhizal endophytes had been obtained by deliberate use of open-pot methods at the time this work was undertaken, it seemed that this method might offer a simple and general means of collecting mycorrhizal fungi and thus be useful in determining their prevalence, morphological differences, host ranges, and influence on plant growth.

MATERIALS AND METHODS

The work was done in a general-purpose greenhouse where little effective sanitary discipline was practiced. Temperature control ranged from 24 to 27°C (with some variation above this in summer).

Clay pots, 6 inches in diameter, were supported by their rims in No. 10 cans

(15.5 cm diameter × 17.7 cm high) suitably punched in the bottoms and sides to allow drainage and ventilation. The cans kept the pot bases 5 to 6 cm above the bench, protecting them to some degree from bench-top contamination.

Fine alluvial sand was mixed 3:1 with Yolo loam. This was potted, covered with aluminum foil, and sterilized in the supporting cans at 15-pound gage steam pressure for 6 to 8 hours, after which the pots were stored on the bench until needed. This sterilization treatment insured kill of soil organisms but apparently did not make the medium unsuitable for growth of the strawberry runners, which could be planted as soon as the pots were cool.

The clone of strawberry plants was provided by Professor R. S. Bringhurst, who developed the plant (University of California, Davis, Pomology, Strawberry Accession No. 57. 91-2). It is a hybrid produced by a cross between a Bodega Bay, California, *Fragaria chiloensis* (L). Dene., an octoploid, and a European "alpine," *F. vesca* (L.) Dene., colchicine-induced tetraploid. The hybird is a semisterile, functional male with 42 chromosomes.

A nutrient solution was prepared, in tap water, from a commercial mixture (Ortho 16-16-8), plus calcium nitrate and an iron chelate, to approximate the following (meq./l): N, 10.2; P, 1.1; K, 0.8; Ca, 4.4; Mg, 4.0; plus Fe at 10 ppm. and B at 0.5 ppm. No other minor elements were added. This solution was applied at the rate of 125 cc/pot, at approximately two-week intervals, after the pots had been flushed with distilled water.

Sampling tubes for taking root samples from the pots were made from ¾-inch electrical metallic tubing as previously described (Gilmore, 1959).

Collection techniques

Mycorrhizal fungi were collected and maintained in open-pot cultures by three methods. In the first, strawberry plants were taken from the field or from storage at –3°C, their roots were washed to remove adhering soil, and they were potted in the sterilized sand-soil mix. After three to six months or more, when samplings showed sufficient development of mycorrhiza, extramatrical spores and/or vesicles were collected from the roots by picking them off with small tweezers. They were then transferred to the roots of other strawberry plants previously established from runners, in the sterilized medium. Three small holes were punched in the pot medium about half way between the center and edge of the pot, the inoculum was placed in the holes 2 to 3 cm below the soil surface, by means of a small spatula, and the holes were closed.

The spores or vesicles were transferred to the new host, either without disinfection or after being treated with chloramine T, as described by Mosse (1956). The latter procedure is preferable since plants in pots inoculated with spores not freed of contaminants, like plants brought from the field, may develop mild diseases which eventually reduce their vigor. This is not true of plants inoculated with decontaminated spores.

For the second method, soil collected in the field was placed in a sterilized pot, and a strawberry runner was established in it. Mycorrhizae were present at 37 days. The plant was removed, washed, and placed in a pot of sterilized soil. Handling was the same as for the field-grown plants.

In the third method, roots of plants from which the mycorrhizae were to be collected were washed and placed in a pot with an established strawberry plant. Handling was the same as for the field-grown plant.

Once they were well developed on the strawberry, mycorrhizae were in some cases examined to determine the purity of the endophyte. Extramatrical

spores of varying appearance may or may not be those of the same organism, and unless hyphal connection can be demonstrated such spores must be separated and inoculated into different plants to check identity. Likewise, vesicles in the roots may or may not be those of the fungus having the most prominent extramatrical spores. Thick-walled vesicles from old roots may sometimes be cleanly dissected out to check this. Usually 20 to 50 spores or vesicles were used as an inoculum, but on one occasion, when only a single spore of its type was available, it was used to establish a vigorous mycorrhiza.

RESULTS

The open-pot cultures were shown to be quite satisfactory for collecting a number of mycorrhizal organisms. Fourteen attempts were made, all successful, from which 19 cultures were recovered. Because a single plant, root, or soil sample may contain more than one mycorrhizal organism, the number of cultures recovered exceeded the number of samples cultured. Two cultures were from a single pot of garden soil, two were from two separate inoculations with peach roots, and the remaining 15 were from 11 field-grown strawberry plants. Of the 19 cultures, eight were morphologically different, and were retained in the collection; six are described below; two await further study.

Cultures were easily kept pure for the mycorrhizal fungus. In the present work, 39 inoculations were made with organisms picked from the collection pots. Sixteen check pots of strawberries were grown. None of the inoculated plants developed mycorrhizal organisms other than the inoculation type, and after a year, only one of the check pots developed a mycorrhiza. This pot had been accidentally splashed with soil from a neighboring culture by a too-fast hose stream. Only minimal greenhouse sanitation seems to be needed, the two most important requirements being first, to keep the pots off the bench by rim supports, and second, to prevent transfer of soil from pot to pot by careful watering and spraying techniques.

DISCUSSION

As indicated by the present study, and the works cited, the collection and maintenance of a number of endophytic phycomycetes as open-pot cultures on plant roots are apparently not difficult. Until shown to be otherwise, it may be assumed that, with a suitable choice of host plant most, if not all, such organisms can be collected and studied.

The open-pot technique for maintaining the cultures might be objected to because open pots soon become inoculated with a great variety of air-borne organisms. However, these apparently do not include pathogens or organisms that form mycorrhizae. Since pure inocula of many of the forms of mycorrhizal *Endogone* sp. can easily be obtained by treating septically grown spores with suitable disinfectants, it may even be advantageous to maintain these organisms under septic conditions. In inoculation experiments with aseptically grown plants, Mosse (1962) has shown that the presence of other organisms aids the entry of *Endogone* into the host root.

Although all attempts to collect the endophytes were successful, there is no assurance that all forms of the mycorrhizal organisms from sources other than strawberry grew on it, and that plant may not be a satisfactory medium for all endophytic mycorrhizal organ-

isms. My attempts to inoculate the plant with two of Barrett's *Rhizophagus* organisms (RV and RVII) failed; Mosse (1961) succeeded, in one instance, in growing Barrett's RII culture on strawberry, but was able to grow his RXI only on apple.

The strawberry plant is an excellent choice, however, for collection and maintenance of those mycorrhizal organisms that do grow on it. It is a suitable host for a number of forms of *Endogone*. It can be maintained for long periods of time without becoming large and unwieldy. If proper precautions are taken to harden the plant, it can be held in storage at –3°C, with its associated mycorrhiza, for up to a year without attention. The hybrid used in this study was found to have other advantages over some cultivars that were used at first. It is a small plant that requires less bench space and less watering than do the larger cultivars, and it retains its vigor from year to year without being subjected to winter chilling. It bears runners the year around, so that new plants can be established in quantity whenever needed.

DESCRIPTION OF CULTURES

The findings of various authors over the last 10 to 15 years leave little doubt that there are numerous species of mycorrhizal *Endogone* and/or *Rhizophagus*. It is therefore important that workers in this field describe an organism as completely as is practical. Although such descriptions may not be adequate to establish new species, they can be of great aid to other workers.

Recent workers have not named new species of mycorrhizal *Endogone* or *Rhizophagus* that they have encountered, but have used numbers or letters to distinguish the various organisms. This has been due in part to inability to describe the organisms as cultures on laboratory media or perhaps, in the case of Barrett's pure *Rhizophagus* cultures, to his doubt as to their taxonomic position. The numbering convention is used in the following descriptions.

Culture E2

Source. The culture was from a strawberry plant having this organism only, taken from a field near Winters, California. The two types of spore and the sporocarps were shown by subculture to be developmental stages of a single fungus.

Fungus description. Spores were asexual, borne singly and naked in the soil at first or, when grown with other *Endogone* species, sometimes within their spore masses. In the latter, sporocarps were not seen. At first the spores were colorless, opaque, and pearlescent even after reaching full size. Later they became yellow transparent, and developed a partial, adhering hyphal tomentum from a hyphal branch or branches arising near the sporophore attachment. Characteristically the tomentum at first covered about half the spore and was positioned either lateral to the sporophore, or more or less centered on it. Other spores were then borne sequentially from hyphae arising within this tomentum or its later extensions or additions. The young, small, secondary spore was initially within or on the periphery of the tomentum of the first spore, but did not adhere to it. The young spore, as it matured, became covered or partially covered, and was incorporated into the developing sporocarp either through extension of the first tomentum or through development of a tomentum of its own intermingled with that of the first spore. In this way sporocarps were built, containing up to six spores—usually fewer. Some did not develop past the first spore stage, but

became completely covered. Spores measured 127 to 380μ (average, 240μ) with the shape generally spherical but occasionally departing from this somewhat (fig. 1).

The walls of spores removed from sporocarps consisted of two layers. The outer, colorless and transparent, about 2 μ thick, remained on the spore when it was removed from the sporocarp. This layer was easily scraped off in large flakes, leaving the yellow-amber spore with a wall 5 to 6 μ thick, which was bright when moist, dull and slightly frosted when dry. The spore's appearance was little different after removal of the outer wall. The contents could not be seen in the young, opaque, pearlescent stage. The mature, yellow, transparent spore was filled with innumerable oil drops of various sizes, distributed throughout the protoplasm (fig. 2). When E2 was grown with the E3 or E4 cultures (see below), an otherwise empty spore of E2 was sometimes seen filled with the smaller spores of the latter types, giving the superficial appearance of a sporangium.

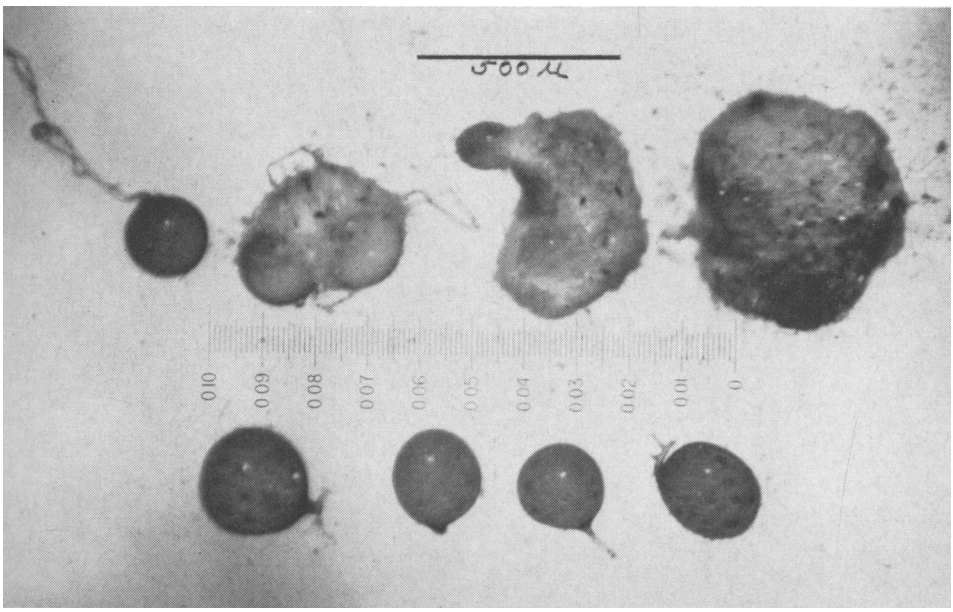


Fig. 1. Culture E2. Top: a single naked spore, and sporocarps in various stages of development. Bottom: spores removed from a sporocarp.

There was no evidence of an abscission mechanism at the spore's point of attachment to the sporophore. The latter was usually somewhat expanded, with thickening walls, as it approached the spore. Older spores often showed an internal thickening of the wall around the attachment, which tended to form a complete or interrupted ring around the opening. In the sporophore, and within the region of its thickened wall, a septum was present in older spores and sometimes in very young, colorless, obviously immature spores. In most young spores no septum was observable. In some maturing spores a plug of dense material occluded the sporophore. Above this plug and in contact with it the septum apparently formed at a later time (fig. 3, A).

Mosse (1956) interpreted the septum separating the spore contents from the sporophore in the organisms she obtained from strawberry as a continua-

tion of the endospore wall. Such appears to be the case with culture E2. Unlike the remainder of the endospore wall, however, which seems to be a static structure in the mature spore, the region defined by the septum and the spore wall near the attachment was the site of continuing activity. Evidence of this was the gradual development of the annular thickening within the spore surrounding the attachment. This structure did not compare with internal projections occurring elsewhere within the spores described by Mosse (1956), for there were no associated canals nor evidence of parasitism. Also,

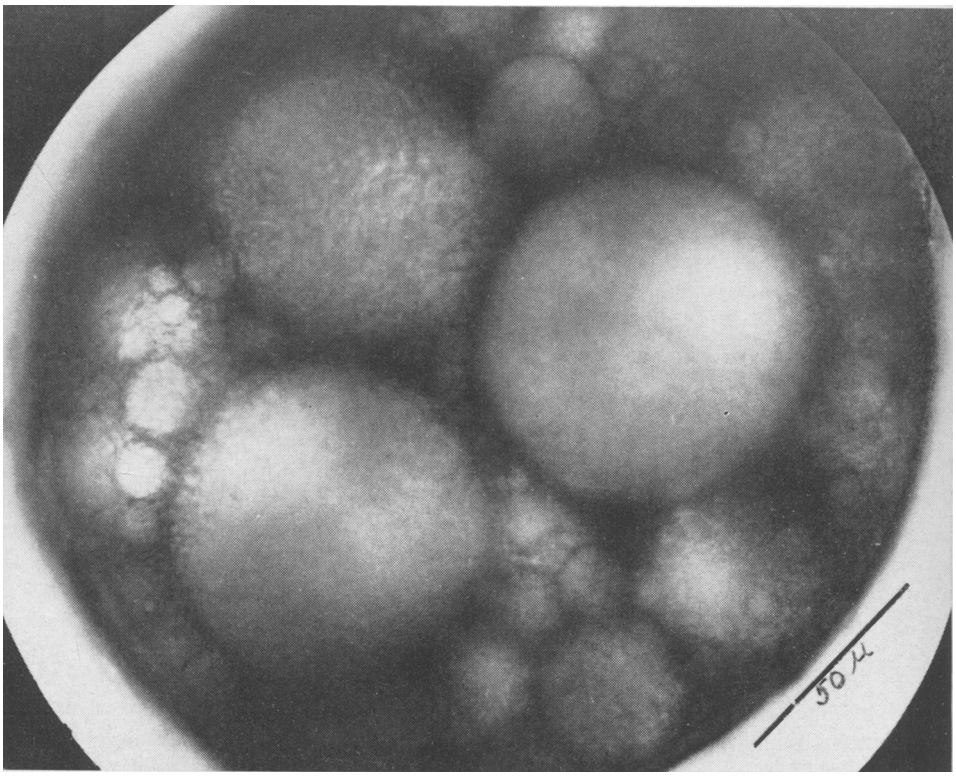


Fig. 2. Culture E2. Mature spore, containing large, round oil drops.

as previously mentioned, the time of appearance of the septum varied in relation to the apparent development of the spore. Furthermore, the septum was not a permanent structure, for a well-formed septum might open, in response to some demand from the organism, and later be replaced by a new one formed above the first (fig. 3, B, C, D).

New branch hyphae arose near the attachment. Although branches commonly originated below the septum, they also grew from above it and from the base of the spore itself within the region defined by the thickening area surrounding the attachment of the sporophore. Spores with multiple hyphae attached to their bases were not thought to be zygospores because multiple hyphae were never observed on young or mature naked spores. They were not uncommon on older spores teased from sporocarps (fig. 3, E, F) and were interpreted as having resulted from branching. When spores germinated on agar, a hypha sometimes

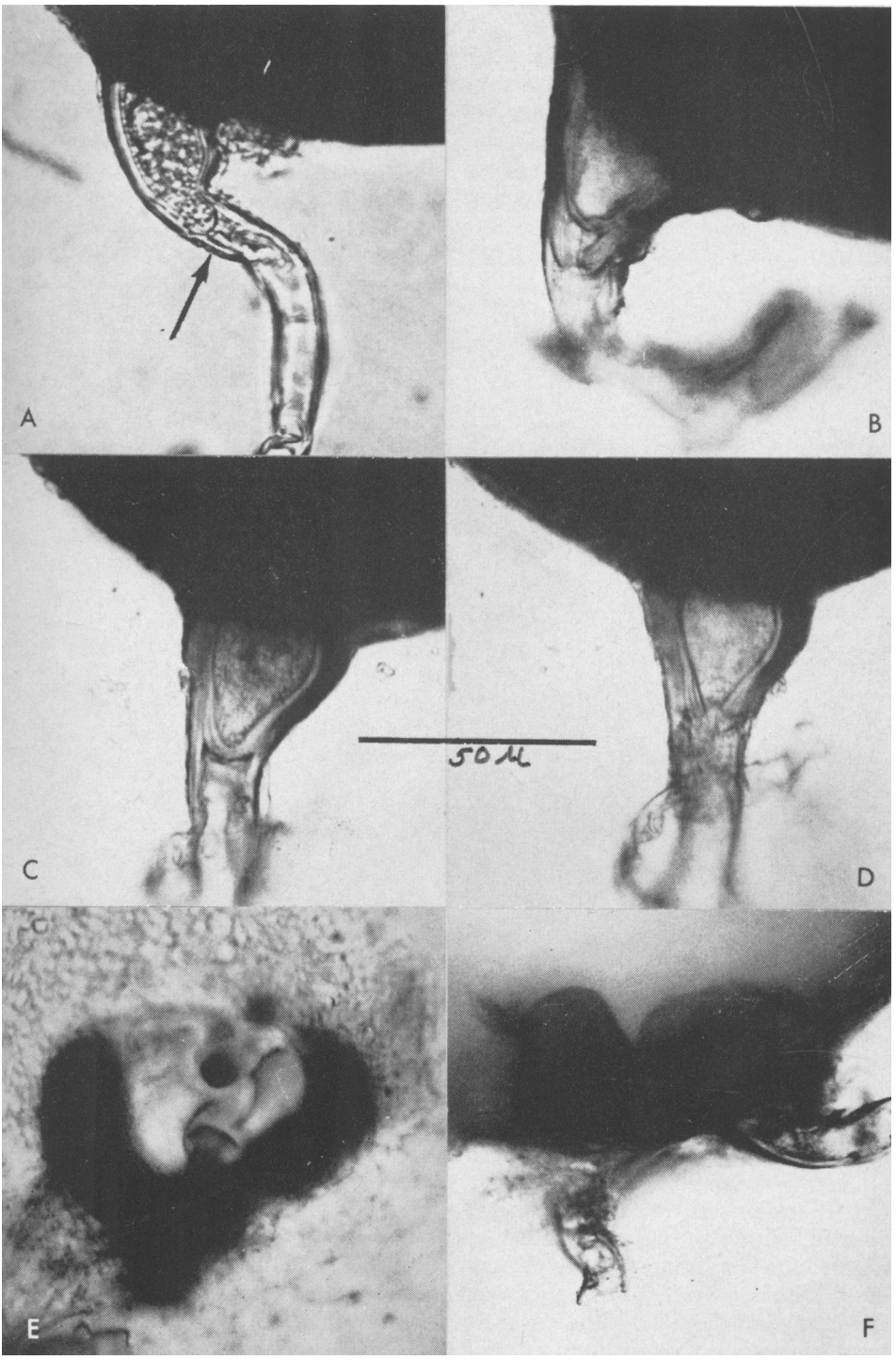


Fig. 3. Culture E2. A. Plug of dense material in sporophore; definite septum not yet formed. B. Sporophore with old septum that has opened and been replaced later by a new septum formed above it. C. Mature spore with a well-formed septum. D. Same spore as in C, after 19 days on water agar. The septum has opened and a new hypha (not shown) has emerged from broken end of sporophore. E. Inner spore wall, showing three hyphal openings and the partial ring around them. F. Lateral view of attachments shown in E. Two hyphae and the raised ring are visible.

emerged from the base of a spore after growth through the sporophore had begun.

The extramatrical hyphae were typical of *Endogone* (Mosse, 1959*b*); they were colorless, up to 18 μ in diameter, and thick-walled.

Large distributive hyphae and typical arbuscles were observed in the root cortex, but no vesicles were seen except for spore-like bodies found in dead and dying roots. These were considered to be spores formed from the fungus within the cortex in response to senescence and death of the latter.

Growth. On water agar the spores, especially the young naked ones, germinated readily even after treatment with chloramine T. Growth was limited, however, although many small spore primordia were formed within the medium. These reached their maximum size (less than 30 μ) within a day or two of initiation. Anastomoses occurred between hyphae, but were not frequent and seemed to depend on chance contact. The spores and their newly developed hyphae remained active for three and one-half months of observation, as evidenced by hyphal streaming to and from the spore.

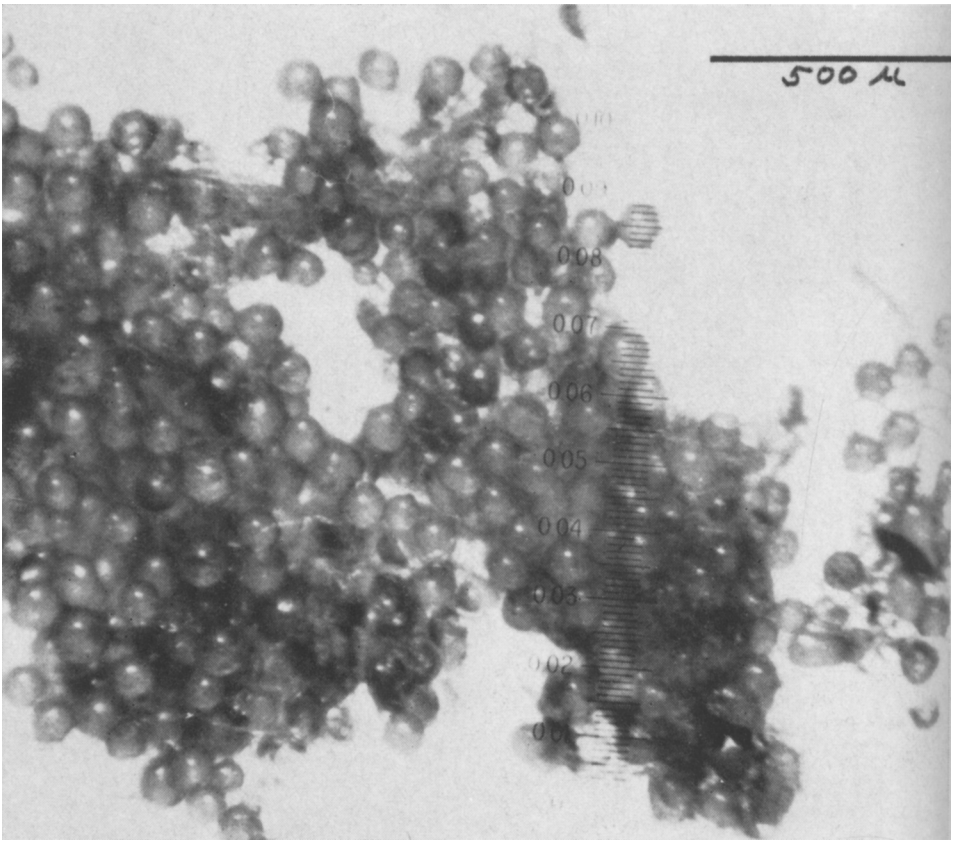


Fig. 4. Culture E3. Spore mass recovered from an otherwise empty seed of a Montia sp.

Discussion. This organism was readily distinguished from a similar one, described by Mosse (1956), by its larger spore size and sequential production of spores to form a sporocarp. It showed some resemblance to a fungus pictured by Koch (1961, fig. 7*a*) but insuffi-

ciently described to establish identity. Gerdemann (1961) has also noted an organism of like appearance.

Culture E3

Source. The culture was recovered from a strawberry plant taken from a field near Winters, California. The plant contained the one type of mycorrhizal organism only, as established through subcultures of lemon-shaped vesicles dissected from the root cortex, and extramatrical spores.

Fungus description. Asexual spores borne free in the soil were difficult to find in young cultures up to about one year, but became more numerous after that time. A distinctive characteristic was the bearing of extramatrical spores in protected situations. They could first be found in old seed coats, which the spore masses often filled, then in soft, coherent organic matter, such as rotted plant fragments, and finally in noncoherent groups of a few to hundreds borne free in the soil (fig. 4). Size range was 51 to 100 μ (average, 73 μ). Spores were essentially spherical, but many had somewhat distorted shapes.

The spore wall consisted of a single colorless to light-yellow, transparent layer, usually bright and shining in appearance. However, spores were seen with an adhering coat of bacteria and soil particles, giving the superficial appearance of an exospore. Wall thickness ranged from 1 to 3 μ.

The transparent spore was filled with numerous oil drops of varying sizes. In many spores (approaching 50 per cent in some samples), a very large oil drop was present, that nearly equaled or exceeded half the spore diameter, and was usually near or touching the spore wall. Other spores varied from this extreme type to ones filled with drops more or less uniform in size (fig. 5, A, B, C). Both types of spores were observed to have hyphal connection.

At the attachment there was no evidence of an abscission mechanism nor was there a septum, although in some instances the sporophore lumen was more or less restricted by internal wall thickening. In some cases the sporophore wall met the spore wall abruptly and without enlargement; in others, it had enlarged, and the sporophore met the spore wall in a well-rounded curve.

Hyphae were typical of *Endogone* and agreed closely with the illustration of Peyronel (1923, p. 469). They were abundant, colorless, up to 12 μ in diameter, and thick-walled.

In the root cortex, arbuscles and vesicles were abundant. Vesicles were up to 65×80 μ in size, formed on many intercellular hyphae. They were usually terminal, but were sometimes within the hyphae, and frequently occurred in such numbers as to tear the cortex apart. There was no apparent difference between these and the extramatrical spores except that those formed deep in the cortex were generally lemon-shaped. Linear arrays of spores frequently seen in mature cultures, with some old, decayed root tissues threading through them, were obviously masses of vesicles from which the root tissue had largely disappeared. The lemon-shaped spores or vesicles, which were less noticeable in these arrays apparently rounded after release.

Growth. When spores were placed on water agar without disinfection, some new hyphae emerged from the broken ends of sporophores or attached, old hyphae. Growth of new hyphae was limited; anastomoses were common and obviously not dependent on chance, for frequently a hypha was seen to have turned abruptly or branched and extended directly to contact another through several hundred μ of intervening agar. New spores were formed occasionally on these new hyphae, and were observed to grow slowly, apparently by transfer of materials from the spores originally plated. At three and

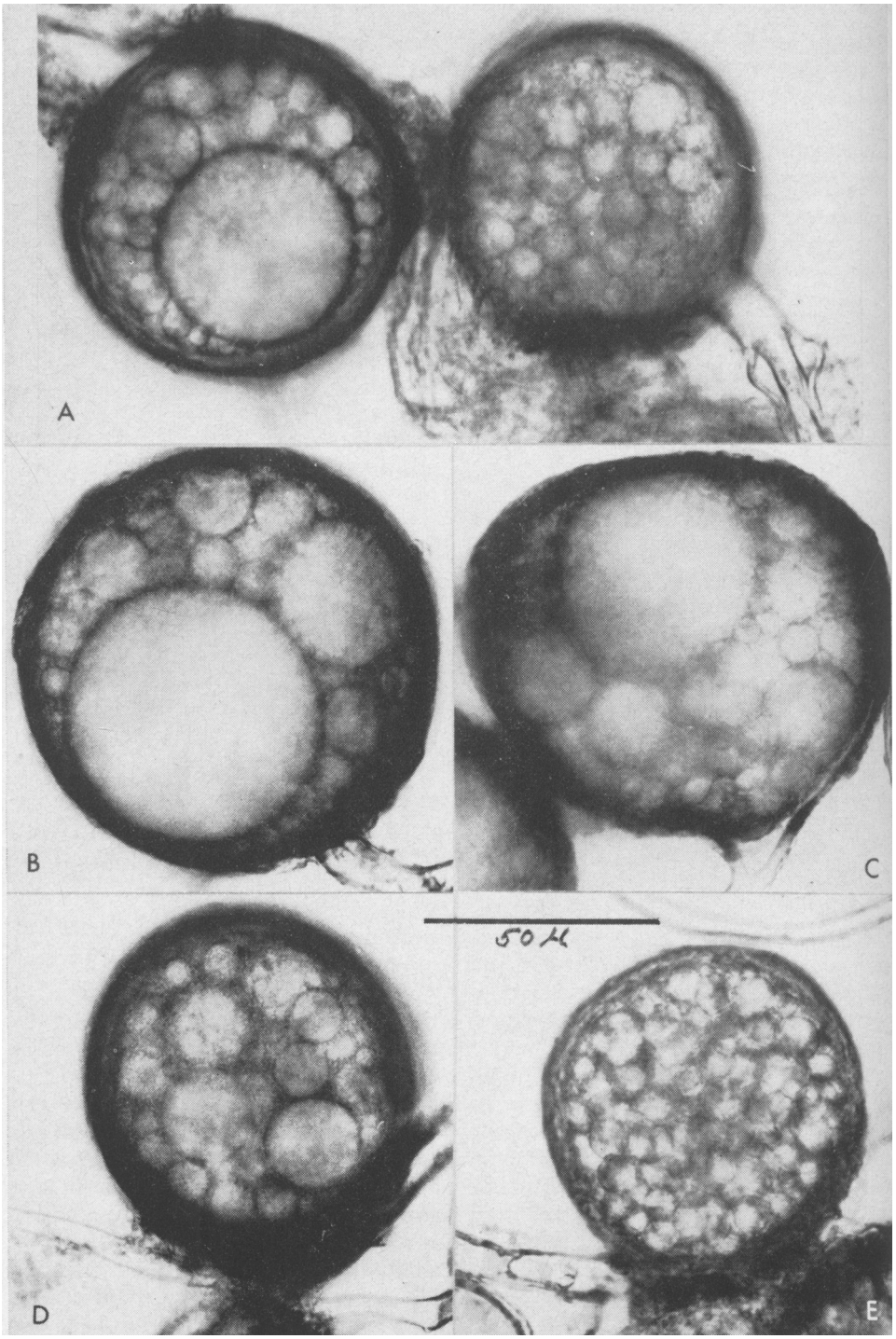


Fig. 5. A, B, C, Culture E3. Various spore types and sporophore attachments. A, right. Collapsed sporophore wall with apparent regeneration. D, E, Culture E4, showing predominant spore types.

one-half months some had reached diameters of 50 to 65 μ and were approaching maturity, as indicated by their accumulation of oil drops.

Culture E4

Source. The culture was from a strawberry containing this mycorrhizal organism only, and grown in a field near Davis, California. Culture E4 can best be described by pointing out a number of small differences between it and Culture E3, which it resembled. Although the differences may eventually be shown to be trivial, they are at present believed not to be so.

Fungus description. As compared with E3, spores were slightly smaller (30 to 93 μ; average, 63 μ), with wall thickness the same. Color was a deeper yellow. The contents covered the range described for E3, but fewer spores contained the very large oil drops. It was on this basis alone that the cultures were first judged to be distinct (fig. 5, D, E). The tendency to bear extramatrical spores in protected situations was present but not so marked as in E3, and masses of noncoherent, free-borne spores were larger, spreading to 4×7 mm on a flat surface, and appeared much earlier (four to six months) after inoculation.

The fungus in the root appeared much the same, but there was not the massive cortex vesiculation characteristic of E3. Proliferation of hyphae into empty spores was seen occasionally.

Growth. On water agar there was limited hyphal growth, and new spores seemed to be produced somewhat more freely than in E3. The largest seen measured 91 μ (equaling the largest of the parent spores) at three and one-half months after plating and one and one-half months after germination of the parent spores was noted.

Culture E5

Source. The culture was taken from peach roots collected near Winters, California. Only this endophyte developed. Observations were made on the pot originally inoculated, because two attempts to transfer the organism failed.

Fungus description. Four months after inoculation, there were extramatrical, immature asexual spores (38 to 51 μ), and at six to eight months many mature spores were evident. These measured 50 to 90 μ (average, 71 μ), and were spherical to slightly longer than broad, in loose, noncoherent clusters. At 20 months there were many large, coherent masses measuring up to $8 \times 5 \times 5$ mm, in jelly-like to rigid matrices incorporating sand and soil and without an external tomentum, so that spore surfaces were seen clean and bright, one against the other. When the spore masses were teased apart, the interstitial spaces were found to be filled with fine hyphae and fine soil particles, that were not adhering to the spores. Most sporophores broke a short distance from the spores when they were teased from the more rigid masses.

The spore wall consisted of a single yellow to deep brownish-yellow layer, up to 6 μ thick. In some older spores the wall was slightly rough.

Mature spores were filled with oil drops of various sizes (fig. 6, A, B), but had none of the large drops that were prominent in E3. Characteristic of this culture was the considerable number of empty spores. In the young culture, at the stage when tendency to cluster was first observable, some of the larger but still thin-walled spores had collapsed as though their contents had been sucked out, pulling one hemisphere into the other and leaving a hemispherical cup

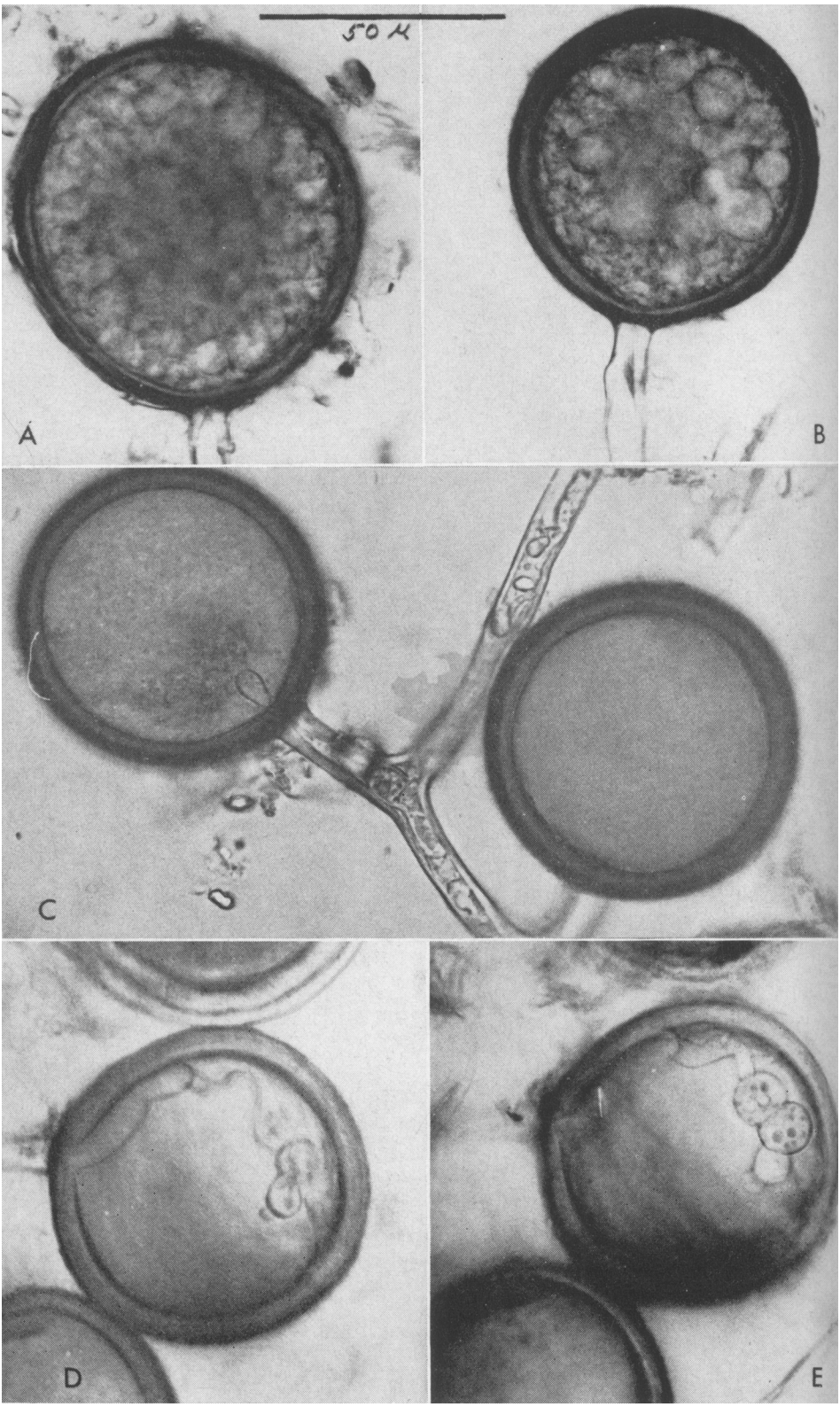


Fig. 6. Culture E5. A, B. Contents of spores. C. Empty spores on protoplasm-filled hyphae. A hyphal tip is entering spore on left. D, E. An empty spore, at two foci, showing entrance of a new hypha from the sporophore, and formation of small, spore-like bodies within the old spore.

attached to the sporophore. In parts of the large spore masses, appearing later, many spores—sometimes more than half—were empty. These older, thick-walled spores were not collapsed and were frequently attached to protoplasm-filled hyphae which at times proliferated into the empty spores (fig. 6, C, D, E).

The appearance of collapsed and empty spores inevitably led to speculation that in addition to their role as propagules, the spores in this culture must also act as simple storage organs,

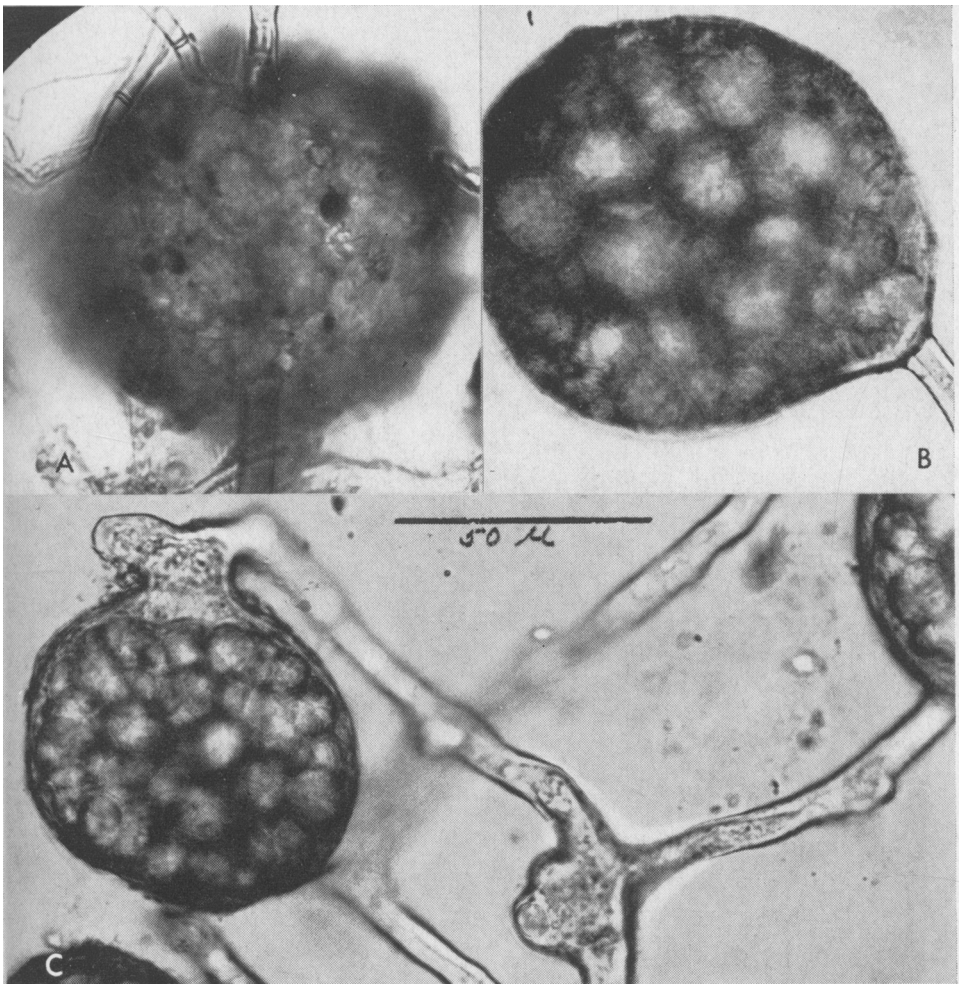


Fig. 7. Culture E6. A. Spore, as found in soil, with its adhering coating. B, C. Spores and hyphae after being cleaned by abrasion. The attachment (C, left) is unusual.

giving up their substance to more recently formed spores or perhaps to some other need of the organism.

The sporophore at the point of attachment to the spore showed no evidence of an abscission mechanism nor did it enlarge as it approached the spore, and its meeting with the spore was abrupt. Internal sporophore walls were thickened near the point of attachment, but were not completely closed. An occasional spore was seen with two

hyphae attached, but there was no other evidence that these were zygospores.

Hyphae were typical of *Endogone*, up to 13 μ in diameter, colorless, and thick-walled.

Arbuscles and vesicles were present in the plant.

Growth. On water agar, unsterilized spores put out hyphae which produced only limited growth and, like cultures E3 and E4, tended to seek other hyphae and anastomose with them. Sometimes a few new spores were produced in the medium. These grew slowly for five to six weeks and finally accumulated some oil drops, but none was seen over 40 μ in diameter.

Discussion. This culture resembles *Endogone fasciculata* in many respects, but the upper limit of spore size was less than that of an organism attributed by Dowding (1959) to *E. fasciculata*. The spore walls were not so thick as those of Dowding's or Thaxter's (1922) organisms. Culture E5 was slower to develop than was that of Gerdemann (1965), and no zygospores were present. Gerdemann (1965), however, recently examined the type material of *E. fasciculata* and concluded that its zygospores were those of another fungus. He therefore chose a spore mass of the same collection, but without zygospores, as the lectotype of the species.

Culture E6

Source. Inoculum, provided by Professor C. J. Hansen, was from a large greenhouse soil tank in which peach seedlings were being tested for resistance to the nematode *Meloidogyne incognita*. In 1953 the tank had been filled with soil treated first, in the field, with "D-D" (dichloropropane-dichloropropene mixture) and then, in cans, with chloropicrin. Grapes infested with the nematode were then grown in the tank as a source of inoculum, after which (from 1955 to the present) it has been cropped yearly, first with tomatoes and then with peach seedlings to be tested. Peach roots taken in November, 1964, were used to inoculate a strawberry plant growing in steamed soil. Only this endophyte developed on the strawberry.

Fungus description. The asexual spores were borne singly and in small, very loose clusters of a few to 20 to 30. They were distinguished by a layer of fine, adherent soil particles which usually, but not always, was so heavy as to obscure the contents (fig. 7, A). The adhering soil could be removed by rather vigorous abrading without injuring the very strong spores, which were spherical to subglobose in form, colorless to medium yellow, and transparent. Spore size was 40 to 91 μ (average, 57 μ).

If an exospore wall were present, it was not recognizable, and came away with the soil layer. The cleaned spore walls were pearlescent, vellum-like, and 2 μ or less in thickness. The readily seen spore contents (fig. 7, B) consisted of protoplasm filled with oil drops of various sizes.

The sporophore wall usually was not expanded as it approached the spore, but it sometimes had odd-shaped swellings at the attachment, or the spore departed from its generally spherical form to meet the sporophore (fig. 7, C). There was no septum nor abscission mechanism.

Hyphae were typical of *Endogone*, up to 13 μ in diameter, and had irregular walls with thickenings intruding into the lumen. They were light yellow in color, thick-walled, and abundant.

Arbuscles and many vesicles were present in the root cortex.

Growth. Weak, limited growth took place on water agar. No new spore primordia were produced.

Culture E7

Source. This culture was from a field-grown strawberry collected at Davis,

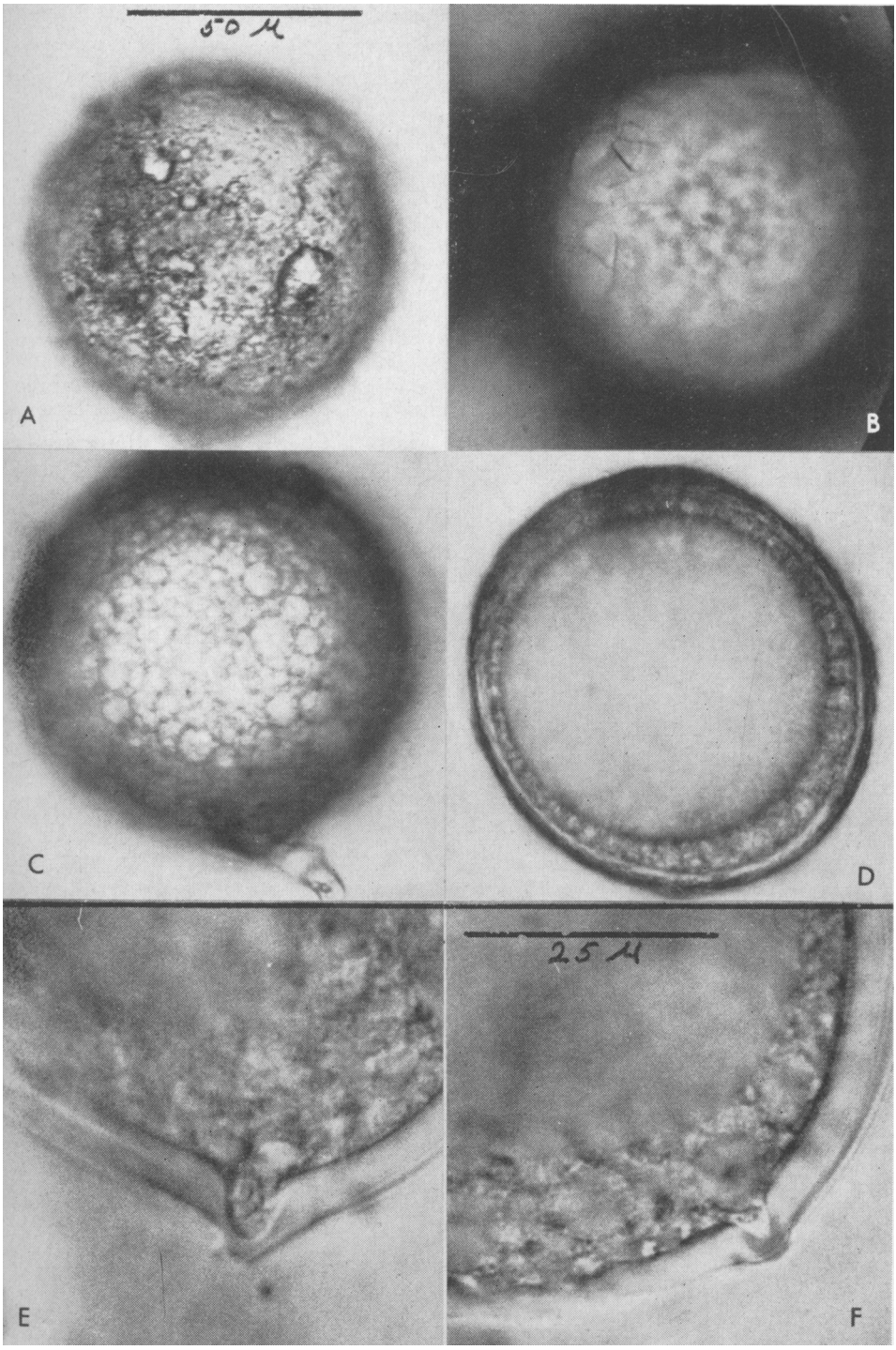


Fig. 8. Culture E7. A. Encrusted spore. B. Spore with parchment-like covering; note break and cracks at left. C. Small oil drops in protoplasm next to wall, high focus. D. Large central oil drop in spore, central focus. E, F. Well-cleaned spores, showing point of sporophore attachment. Note spore wall has formed a septum across the opening.

California, and having only this endophyte.

Fungus description. The asexual spores were borne singly in the soil, were not clustered or numerous, and generally were encrusted with adhering material so that the contents could not be seen (fig. 8, A). Sometimes they were fairly clean. Abrasion removed much of the adhering material, which at times was associated with a thin, parchment-like layer which came away during cleaning. Whether this layer was an exospore or material of other origin that had formed on the spore wall was not determined (fig. 8, B), and its presence could not always be demonstrated. The spore was 60 to 100 μ (average, 71 μ) in size, generally spherical, with various minor deviations.

The cleaned wall was fairly smooth and transparent on some spores; on others it was coarse, frosted, or pebbled and quite impossible to see through. It was colorless, delicate, and 1.2 to 5 μ thick.

The contents were a thin protoplasm filled with small oil drops of various sizes surrounding a larger oil drop sometimes exceeding four-fifths of the spore diameter (fig. 8, C, D).

Attachment was less firm than in the other organisms, suggesting the possibility of an abscission mechanism. When spores were pulled from the hyphal plexis, a short piece of the sporophore was sometimes attached. The spore, if well cleaned, showed only a small, thin protrusion of its wall, which evidently indicated the point of attachment and served as a septum to cut off the sporophore (fig. 8, E, F).

The hyphae were not seen to exceed 6 μ in diameter; they were thin-walled, delicate, and abundant. Many of them were less than 3 μ in diameter, had much adhering soil, and also tended to stick to each other so that after a sample was picked from the roots and placed on a slide, it was difficult to spread for examination. The larger hyphae looked much like *Endogone* in that they showed more or less typical angular projections and irregular cystic enlargements.

No vesicles were seen in the plant, but there were numerous arbuscles and large, inflated hyphae equaling or exceeding the size of the largest extramatrical hyphae.

Growth. No germination took place on agar.

LITERATURE CITED

BARRETT, J. T.
1947. Observations on the root endophyte *Rhizophagus* in culture. Phytopathology 37: 359–60.
1961. Isolation, culture, and host relation of the phycomycetoid vesicular-arbuscular mycorrhizal endophyte *Rhizophagus*. Recent Adv. in Bot. 2:1725–27, University of Toronto Press.

BUTLER, E. J.
1939. The occurrences and systematic position of the vesicular-arbuscular type of mycorrhizal fungi. Trans. Brit. Mycol. Soc. 22:274–301.

DAFT, M. J., and T. H. NICHOLSON
1966. Effect of *Endogone* mycorrhiza on plant growth. New Phytologist 65:343–50.

DANGEARD, P. A.
1900. Le "*Rhizophagus populinis*" Dangeard. Botaniste Ser. VII:285–91.

DOWDING, E. S.
1955. *Endogone* in Canadian rodents. Mycologia 47(1):51–57.
1959. Ecology of *Endogone*. Trans. Brit. Mycol. Soc. 42(4): 449–57.

GERDEMANN, J. W.
1955. Relation of a large soil-borne spore to phycomycetous mycorrhizal infections. Mycologia 47(5): 619–32.

1961. A species of *Endogone* from corn causing vesicular-arbuscular mycorrhiza. Mycologia **53**(3):254–61.
1964. The effect of mycorrhiza on the growth of maize. Mycologia **56**(3):342–49.
1965. Vesicular-arbuscular mycorrhizae formed on maize and tulip tree by *Endogone fasciculata*. Mycologia **57**(4):562–75.

GILMORE, A. E.
1959. A soil sampling tube for microbiology. Soil Sci. **87**(2):95–99.

GODFREY, R. M.
1957. Studies on British species of *Endogone*. III. Germination of spores. Trans. Brit. Mycol. Soc. **40**(2):203–10.

HAM, ANGELA M.
1962. Studies on vesicular-arbuscular endophytes. IV. Inoculation of species of *Allium* and of *Lactuca sativa* with *Pythium* isolates. Trans. Brit. Mycol. Soc. **45**(2):178–89.

HARLEY, J. L.
1959. The biology of mycorrhiza. London: Leonard Hill [Books] Limited, p. 184.

HAWKER, LILLIAN E., R. W. HARRISON, VALERIE O. NICHOLS, and ANGELA M. HAM
1957. Studies on vesicular-arbuscular endophytes. I. A strain of *Pythium ultimum* Trow. in roots of *Allium ursum* L. and other plants. Trans. Brit. Mycol. Soc. **40**(3):375–90.

KANOUSE, B. B.
1936. Studies of two species of *Endogone* in culture. Mycologia **28**:47–62.

KOCH, H.
1961. Untersuchungen über die Mykorrhiza der Kulturpflanzen unter besonderer Berücksichtigung von *Althaea officinalis* L., *Atropa belladonna* L., *Helianthus annus* L., und *Solanum lycopersicum* L., Gartenbauwissenschaft **26**(8):5–32.

MOSSE, B.
1953. Fructifications associated with mycorrhizal strawberry roots. Nature (London) 171: 974.
1956. Fructifications of an *Endogone* species causing endotrophic mycorrhiza in fruit plants. Ann. Bot. Lond., N. S. **20**:349–62.
1959*a*. The regular germination of resting spores and some observations on the growth requirements on an *Endogone* sp. causing vesicular-arbuscular mycorrhiza. Trans. Brit. Mycol. Soc. **42**(3):273–86.
1959*b*. Observations on the extra-matrical mycelium of a vesicular-arbuscular endophyte. Trans. Brit. Mycol. Soc. **42**(4):439–48.
1961. Experimental techniques for obtaining a pure inoculum of an *Endogone* sp., and some observations on the vesicular-arbuscular infections caused by it and other fungi. Recent Adv. in Bot. **2**:1728–32, University of Toronto Press.
1962. The establishment of vesicular-arbuscular mycorrhiza under aseptic conditions. Jour. Gen. Microbiol. **27**:509–20.
1963. Vesicular-arbuscular mycorrhiza: an extreme form of fungal adaptation. Symposia of The Society for General Microbiology. No. **XIII**, Symbiotic Associations. London: Cambridge University Press.

NICHOLSON, T. H.
1959. Mycorrhiza in the Gramineae. I. Vesicular-arbuscular endophytes, with special reference to the external phase. Trans. Brit. Mycol. Soc. **42**(4):421–38.

OHMS, R. E.
1957. A flotation method for collecting spores of a phycomycetous mycorrhizal parasite from soil. Phytopathology **47**(12):751–52.

PEYRONEL, B.
1923–24. Prime ricerche sulle micorrize endotrofiche e sulla microflora radicicola normale delle Fanerogame. Riv. Biol. **5**(4):463–85, and **6**(6):17–53.
1937. Le *"Endogone"* quali produttrici di micorrize endotrofiche nelle Fanerogame alpestri. Nuovo G. Bot. Ital. N. S. **44**(3):584–86.

STEVENSON, GRETA
1964. Growth of seedlings of some pioneer plants and micro-organisms associated with their roots. Trans. Brit. Mycol. Soc. **47**(3):331–39.

THAXTER, R.
1922. A revision of the Endogoneae. Proc. Amer. Acad. Arts. Sci. **57**(12):291–346.

4*m*-5,'68(H9214)L.L.

The journal HILGARDIA is published at irregular intervals, in volumes of about 650 to 700 pages. The number of issues per volume varies.

Single copies of any issue may be obtained free, as long as the supply lasts; please request by volume and issue number from:

Agricultural Publications
University Hall
University of California
Berkeley, California 94720

The limit to nonresidents of California is 10 separate titles. The limit to California residents is 20 separate titles.

The journal will be sent regularly to libraries, schools, or institutions in one of the following ways:

1. In exchange for similar published material on research.
2. As a gift to qualified repository libraries only.
3. On a subscription basis—$7.50 a year paid in advance. All subscriptions will be started with the first number issued during a calendar year. Subscribers starting during any given year will be sent back numbers to the first of that year and will be billed for the ensuing year the following January. Make checks or money orders payable to The Regents of The University of California; send payment with order to Agricultural Publications at above address.