CEPHALOSPORIUM SP.
AN ORGANISM ASSOCIATED WITH A CANKER OF WESTERN HEMLOCK[1]

by

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We accept this thesis as conforming to the standard required from candidates for the degree of MASTER OF ARTS

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Abstract

A canker on the main stem of oppressed western hemlock (*Tsuga heterophylla* (Rafn.) Sarg.), 1 to 3 inches in diameter at breast height, was found at Powell River and Turnour Island, British Columbia. The canker is irregularly elliptical, with a conspicuous resin exudation in the early stages. The canker appears to be annual. A species of *Cephalosporium* (Fungi Imperfecti, Moniliales) was consistently isolated from cankers collected at Powell River. The pathogenicity of the organism has not been proven to date. The growth and hyphal characters of the organism, and the temperature-growth relations of the organism in culture are described. The conidial apparatus of the organism was investigated. Attempts to produce the perfect stage in culture were unsuccessful.

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Introduction

Specimens of a canker on oppressed western hemlock (Tsuga heterophylla (Rafn.) Sarg.) were collected by Dr. D. C. Buckland, Associate Professor of Forest Pathology, University of British Columbia, at Powell River, B. C. in June, 1949. Cultures of a fungus were obtained from the cankers, but the organism was not identified.

An investigation of the canker and the organism associated with it was begun in April, 1950. To determine the distribution of the organism, specimens of cankers were collected from western hemlock at Campbell River, Port Alberni and Cameron Lake on Vancouver Island. These cankers were cultured, but none yielded the organism obtained from Powell River.

In June, 1950, a visit was made to the experimental forest at Powell River, where the canker was first reported. Here a fairly heavy infection of oppressed western hemlock was found in a small localized area. The overstory consisted of a Douglas-fir (Pseudotsuga taxifolia (Poir) Britt.) stand, 45 to 50 years old with some western hemlock, western red cedar (Thuja plicata D. Don) and grand fir (Abies grandis Lindley). The understory was chiefly western hemlock with some red cedar and grand fir. Cankers were collected in this locality. The organism isolated from these cankers was the

same as that originally obtained by Dr. Buckland. This collection formed the basis for the description of the canker and its associated organism.

Further collections of a canker on western hemlock were made by Mr. E. G. Marples, Forester, Powell River Company, on Turnour Island, B. C. in September, 1950. When received most of these specimens were too dry for successful culturing, but from two the original organism was obtained. The forest cover on Turnour Island was almost pure nemlock. The cankers came from oppressed material under scattered old-growth western hemlock and western red cedar.

Description of the Canker

Cankers occurred on the main stem of oppressed western hemlock, 1 to 3 inches in diameter at breast height. One to 4 or more cankers were found on each stem. The cankers were irregularly elliptical (Pl. I, Fig. 1 and 2), 2 to 6 inches in length. On some trees 2 or more cankers had fused to produce an elongate, irregular canker that extended for 1 to 2 feet along the stem.

The first indication of the presence of a canker was a conspicuous resin exudation from the margins of a slightly depressed area of bark. The bark otherwise appeared normal. The bark at the margin of these cankers was often unbroken, but sometimes the canker was outlined by cracking of the bark.

On some trees apparently abortive cankers were found. These appeared as a roughening of the bark over a small circular area up to 1 inch in diameter, with a small amount of resin exudation from the canker.

In later stages, the bark over the cankered area was noticeably depressed and firmly attached to the underlying wood except at the margins where, due to callus growth, the bark was cracked, raised, and in the process of being sloughed off. There was no resin exudation at this stage. The inner bark of the cankers was dark brown. The margin of

healthy and diseased bark was marked by a dark narrow line. The wood of 1 or 2 annual rings under the canker, in some cases was gray, but in most of the cankers was not appreciably different from normal wood.

All cankers that yielded the organism were at least 2 years old, and most were older, since there were from 2 to 9 rings of callus growth at the margin. It would appear that the organism is actively parasitic for 1 year only. There was no evidence of dieback of the callus, and all cankers examined had a smooth, curved margin of callus growth.

Cankers usually had a branch stub at the centre. This appeared to be the point of entrance of the canker organism.

Isolation of the Organism

Two of the 6 original cankers collected at Powell River, B. C. by Dr. D. C. Buckland were cultured. From 9 plantings on malt agar, 6 produced pure cultures of Cephalosporium sp. (Fungi Imperfecti, Moniliales). The remainder were sterile.

Plantings on malt agar were made from the inner bark near the margin of 12 cankers from Powell River. Three to 7 plantings were made from each canker. Of the 52 cultures

obtained: 26 were pure Cephalosporium, 12 were Cephalosporium mixed with some other organism, and the remainder comprised various molds and bacteria. Cephalosporium was isolated from 10 of the 12 cankers cultured. Cephalosporium was the only organism obtained consistently.

In spite of their dried condition, 2 cankers from Turnour Island were cultured. Four plantings on malt agar were made from each canker. This resulted in 1 pure culture of Cephalosporium from 1 canker, and 1 culture of Cephalosporium mixed with some other organism from the other. The rest of the plantings developed into a variety of molds.

Inoculation Experiments

Cephalosporium isolated from the cankers was used to inoculate western hemlock trees, 1 to 4 inches in diameter at breast height, at the University of British Columbia in May, 1950.

Inoculum was prepared as follows: Sterile inner bark chips of western hemlock were placed on malt agar in Petri dishes. The plates were inoculated with Cephalosporium and the cultures allowed to develop until the fungus had become well established on the chips. These bark chips were used as inoculum. Controls consisted of sterile bark chips and

malt agar only.

Six trees were inoculated on the main stem in the following manner: The area to be inoculated was swabbed with 70 percent alcohol. An inverted "U-shaped" incision (Pl. I, Fig. 3), about 1 inch square, penetrating to the cambium, was made with a sterile knife blade. The flap thus formed was raised slightly, and a piece of inoculum placed under the bark. A moistened sterile gauze pad was then placed over the inoculation, and fastened to the stem. Several layers of waxed paper were bound over the pad to retain moisture.

Inoculations were made over the stubs of small branches cut off flush with the bark, and under bark containing no branch stubs. Seventeen inoculations and 6 controls were made.

The inoculations were examined after 3 months. All inoculations showed some degree of fungus activity. Yellow or white mycelium was present in the cuts in the bark. On the smaller trees, there were small sunken areas in the bark around the inoculations (Pl. 1, Fig. 3). There was no evidence of callus growth in the inoculations, while callus growth was evident in the controls.

Cultural tests were made on 12 of the inoculations from 5 of the 6 inoculated trees in October, 1950, approximately 5 months after inoculation. Several plantings were

made from each inoculation. Only four pure cultures of Cephalosporium, from 3 separate inoculations, were obtained.

As the above experiment was inconclusive, further work on inoculation is being carried out.

Description of the Organism

Method of Study

The terminology used by Pinkerton (11) in describing the fruiting structures of Cephalosporium is used here. The cephalosporium is the spore ball or conidial head, the cephalophore is the entire fructification, including spore ball and conidiophore, and the phialide is the end cell of a conidiophore.

Unless otherwise stated the organism was grown on malt agar, prepared according to the following formula:

Difco malt extract	12.5 gm.
Difco Bacto-agar	20.0 gm.
Distilled water	1000.0 c.c.

Ten cultures of Cephalosporium on malt agar in Petri dishes were used as the basis for the description of the gross features and growth rate of the organism in culture. The cultures were prepared by placing a piece of inoculum about 5 mm. square, taken from near the advancing zone of

an actively-growing culture, on the malt agar at the edge of the dish. The cultures were grown in the dark at room temperature (12° to 20° C.) for 6 weeks. They were brought out for examination at weekly intervals, at which times the radial growth, appearance and color of the cultures were noted. Photographs were taken at the end of 2, 4 and 6 weeks (Pl. II, Fig. 1 to 4). Nobles (10) method was followed for the description of the cultures. Munsell (9) color equivalents are given.

Hyphae and conidiophores were examined, and camera lucida drawings made by placing a cover slip directly over a portion of the culture on a Petri dish. Cephalosporia were difficult to examine since any movement of the conidiophore was sufficient to dislodge the conidia. Cephalophores were observed under low power of the microscope by direct examination of cultures, but this was not satisfactory as details of the structure were obscured by drops of condensed moisture, which usually formed around the conidial heads. For closer examination of cephalosporia, agar slide cultures were prepared by placing a drop of sterile, melted potato dextrose agar on a sterile slide, and immediately inoculating with conidia. When the agar had begun to set, a sterile cover slip was placed over the drop of agar. The cultures were incubated in a moist chamber. Cephalosporia were usually formed in 2 to 4 days, and could be examined under

the high dry objective. The cephalosporia formed in such cultures appeared typical except that fewer conidia were formed per head than on cultures in Petri dishes.

Measurements were taken with a filar micrometer from conidia mounted in 7 percent potassium hydroxide, with a small amount of phloxine added. Conidia were measured using the oil immersion objective.

Germination of conidia was observed by placing a drop of spore suspension on a small square of sterile cellophane on potato dextrose agar in a Petri dish. Germination took place within 24 hours. The slips of cellophane were then mounted on slides, and either examined directly or stained with gentian violet (12).

Repeated attempts to observe the formation of conidia in agar slide cultures under the microscope were unsuccessful.

Growth Characters

The growth of the organism is slow, varying from 2.5 to 4.1 cm. in 6 weeks (average, 3.6 cm.) (Pl. VI, Fig. 1; Pl. II, Fig. 3 and 4). The advancing zone is slightly bayed, hyaline and appressed, extending 1 to 5 mm. beyond the aerial mycelium. The mat is white and farinaceous, with hyaline droplets; becoming yellow (Munsell color equivalents: 10.0 YR 8.0/6.0 at 2 weeks; 10.0 YR 7.5/6.5, 10.0 YR 8.0/7.5 at 4 weeks; and

10.0 YR 7.0/7.0 at 6 weeks) and subfelty in older parts of the culture; occasionally with sectors of felty, yellowish to hyaline aerial mycelium (Pl. II, Fig. 4) from the inoculum to the advancing zone. There is no effect on the agar.

The white and farinaceous parts of the mat in the test cultures were faintly zonate (Pl. II, Fig. 2 to 4). The zoning is apparently due to exposure to light, since cultures grown in the dark, without exposure to light at intervals, do not have a zonate appearance.

Hyphal Characters

The hyphae have simple septa throughout. The advancing zone is composed of hyaline hyphae, 2 to 4 microns in diameter (Pl. III, Fig. 7). The aerial mycelium consists of: Narrow hyphae, 2 to 4 microns in diameter (Pl. III, Fig. 6), similar to the hyphae in the advancing zone; broad hyaline hyphae, 4 to 8 microns in diameter, cylindric (Pl. III, Fig. 2) to spheroid (Pl. III, Fig. 3), with the cells often enlarged at one end (Pl. III, Fig. 1), prostrate, or rising from the surface of the agar for a portion of their length near the tips; and smaller broad hyphae with amber-colored contents (Pl. III, Fig. 4). Occasionally in a broad hypha, some cells may be colored and others hyaline (Pl. III, Fig. 5). The amber-colored hyphae produce the color in the cultures.

The conidia are borne in roughly spherical heads

(cephalosporia) (Pl. IV, Fig. 1; Pl. V, Fig. 12), which are 7 to 27 microns in diameter (average 11 microns). The conidia are oval to obovate, occasionally cylindric, hyaline and one-celled, 2.4 to 4.5 by 4.1 to 6.7 microns (average of 50 spores from a culture on malt agar, 3.2 by 5.2 microns). Mucous is produced by the conidiophore, and is visible in the larger cephalosporia. Cultures grown on barley kernels on Sax's medium (8) produce a compact mat, with amber drops of mucous containing many spores. The phialides taper slightly, measuring 2.3 to 3.0 microns in diameter at the base by 14 to 28 microns in length (average 2.6 by 22.2 microns).

The conidiophores arise from prostrate or aerial hyphae. Near the advancing zone, simple or bifurcate (Pl. IV, Fig. 3) conidiophores arise from prostrate hyphae. In older parts of cultures, the conidiophores are usually compound (Pl. IV, Fig. 2 and 4), but may be simple, and arise from broad hyphae, which may be prostrate or aerial for part of their length. Simple conidiophores, arising from narrow prostrate hyphae, may also occur in older parts of cultures. Conidiophores and conidia are produced profusely over the surface of cultures, except in the hyaline, felty areas, referred to under the growth characters, where they are sparse.

Conidia germinate within 24 hours, when placed on a nutrient medium. The conidia swell to twice or three times their original size and send out 1 or 2 germ tubes.

Temperature Relations of the Organism in Culture

The growth of the organism in culture, in relation to temperature, was studied to find the minimum, maximum and optimum temperature for growth. The growth rates given are the average radial growth of 5 cultures on malt agar in Petri dishes (Pl. VI, Fig. 2). Plates were inoculated in the centre with a piece of inoculum about 5 mm. square, taken from near the advancing zone of an actively-growing culture on malt agar. The cultures were examined, and growth measurements recorded at weekly intervals for 3 weeks. Since there was no change in the trend of growth over the three-week period, the values given represent radial growth at the end of 3 weeks. Temperature variation of the incubators was within 1°C., with the exception of the 12°C. test, which tended to have a slightly greater variation. A limited number of constant temperature incubators was available, so that a complete temperature range could not be tested at one time.

Initially cultures were incubated at: 0°, 4°, 9°, 12°, 20°, 25° and 35°C. A second series was incubated at: 12°, 14°, 16° and 18°C., a third at: 12°, 14°, 16°, 18° and 20°C., and a fourth at: 20°, 22° and 25°C.

There was no growth at 0° or at 30°C., indicating that the minimum temperature for growth lies between 0° and

4°C., and the maximum between 25° and 30°C. There was no definite indication of an optimum temperature in this first series. However, since the rate of growth increased up to 20°C., and declined at 25°C.; it was thought that the optimum temperature lay between 12° and 20°C. The second series was set up to test this possibility. The growth rate at 14°C., in the second series, seemed to be much too high, and well above the trend of growth at adjacent temperatures (Pl. VI, Fig. 2). This was checked by the third series, in which the growth at 14° conformed with the general trend. There was still a possibility that the optimum temperature lay between 20° and 25°C. This was investigated in the fourth series, which was incubated for 11 days. In this series, growth was most rapid at 20°C, indicating that the optimum did not lie above 20°C. The optimum temperature, therefore, appears to lie between 18° and 20°C.

The mat of cultures incubated at the lower temperatures (below 12°C.) was thin and appressed. These cultures remained white, with no production of a yellow color over the three-week period. At temperatures above 20°, growth was slow, but abundant aerial mycelium was produced, giving a compact felty mat. There was no appressed advancing zone as at lower temperatures, and aerial mycelium was present to the limit of growth. The mat in such cultures was colored to the limit of growth.

Conidial Apparatus

Pinkerton (11) investigated the conidial apparatus of several human, parasitic species of Cephalosporium and some related Hyphomycetes. The conidial apparatus was found to be similar in the species investigated. The conidial apparatus of the organism under study was investigated in a similar manner to compare it with the species investigated by Pinkerton.

Agar slants of Difco Bacto potato dextrose agar in capsule vials were inoculated with Cephalosporium, and incubated for 10 days at 20°C. The cultures were killed and fixed with Hermann's fluid (5) applied directly to the slants. The vials were evacuated for 15 minutes in a vacuum dessicator, immediately following the application of the fixing fluid. The cultures were killed and fixed for 12 hours, then washed in slowly running water for 24 hours. The slants were then cut up into blocks, and dehydrated in an ethyl alcohol series. The blocks were then run through an alcohol-xylene series, and embedded in Fisher Tissuemat (m.p. 52° to 54°C.).

Sections were cut at 3, 5, and 15 microns. The sections were stained with Heidenhain's Iron-alum-haematoxylin (4) according to the following schedule: Hydration, 20 percent Merck Superoxol 1 hour, running water 30 minutes, 4 percent iron alum 2 hours, 2 changes of distilled water in 4 minutes,

1/2 percent quick ripened haematoxylin (6) 12 hours, 3 changes of tap water in 2 minutes, 2 percent iron alum 5 to 10 minutes, 2 changes of distilled water in 10 minutes, and running water 1 hour. The sections were then dehydrated in ethyl alcohol, cleared in xylene, and mounted in Canada balsam.

Sections cut at 5 microns were most satisfactory. The sections cut at 15 microns were too thick for either good staining or satisfactory observation under the oil immersion objective, which was necessary to see details of structure.

Conidial formation is first indicated by a narrowing of the cytoplasm, near the tip of the phialide into a slender thread, at the end of which is formed a small ball of cytoplasm (Pl. V, Fig. 1). This small ball enlarges to become a conidium (Pl. V, Fig. 1 to 4). Cytoplasm is apparently drawn through the thread into the developing spore, causing it to enlarge. The end wall of the phialide appears to be thicker than the lateral walls, and to contain a pore, penetrated by the thread of cytoplasm. After considerable enlargement, while still within the phialide, the spore is extruded through the end wall. The protoplasmic thread, connecting spore and phialide, is broken, and a wall is formed around the conidium (Pl. V, Fig. 5). Another spore begins to form, while the first-formed spore is still attached to the tip of the phialide (Pl. V, Fig. 6). The

extrusion of the next spore pushes the first-formed one aside. The process continues forming a cephalosporium.

The passage of a nucleus into the developing spore (Pl. V, Fig. 7), does not appear to take place when the spore is of a particular size, since in some cases, the spore may be small, and have a nucleus; while in others, may appear to be nearly full size and not yet possess one. The phialide is uninucleate. Nuclear division (Pl. V, Fig. 8) and migration of a daughter nucleus appears to take place as the spores are produced, since more than 2 nuclei were not observed in any phialide.

The cytoplasm of the phialide is at first dense, and without vacuoles. Vacuoles appear, forming first at the base of the phialide, as spores are produced and the cytoplasm used up, until the phialide contains several vacuoles (Pl. V, Fig. 9).

Attempts to Produce the Perfect Stage in Culture

On 2 cankers received from Turnour Island, B. C., small orange apothecia occurred. An attempt was made to obtain cultures from the bark of the cankers, and from the apothecia. None was obtained however, because of the dried condition of the specimens when received. No ascospores

were present in the apothecia. Since these were the only cankers on which fruiting occurred, it was not possible to tell whether the apothecia were the perfect stage of this species of Cephalosporium, or the perfect stage of a secondary organism.

In an attempt to induce the perfect stage in culture, the organism was grown on a variety of media, which included: oat, prune, potato-dextrose-peptone, and cornmeal agar; potato dextrose agar with biotin (2), Leonian's medium (7), and Badcock's medium (1). Badcock fructification flasks (1), using the inner bark of western hemlock, instead of sawdust was tested. Sax's nutrient agar (8) and barley kernels was also used as a medium. The perfect stage was not induced by any of the above media. The organism grew well on all the media except Leonian's.

Cultures isolated from several cankers were paired. The paired cultures intermingled freely, but the perfect stage was not formed.

Discussion

Christensen (3) studied a canker on balsam fir (Abies balsamea (L.) Mill.) in Minnesota, caused by a species of Cephalosporium. The description of the canker is very

similar to the one under consideration here. The author wrote to Dr. C. M. Christensen, Professor of Plant Pathology, University of Minnesota, for check cultures, but none were in existence. There are some differences between the two organisms in culture. In describing the organism found in Minnesota, Christensen reports: "On malt agar at temperatures from 20° to 30°C. it produces a faintly zonate culture of white, fluffy, aerial mycelium, ..." "Preliminary temperature studies indicate that it will not grow at 0° and 35°C., grows slowly at 15°C., and most rapidly at 27° to 30°C." In cultures of the organism found in British Columbia, a yellow color is a noticeable feature after 1 week, especially at temperatures from 20° to 30°C. There is no growth at 0°C., but the maximum temperature for growth lies between 25° and 30°C. Growth is quite rapid at 15°C., and the optimum lies between 18° and 20°C.

In describing the hyphal characters of the Minnesota organism, Christensen states: "The conidiophores taper to a slender tip, on which 1 to 3 conidia are borne simultaneously, either on short sterigmata or directly on the conidiophores. Each sterigma continues to produce conidia, and these remain clustered in a roughly spherical head about the tip of the conidiophore." In the organism found in British Columbia, the conidia are produced endogenously, and are extruded, one at a time through the tip of the

conidiophore. No sterigmata are present. Conidial measurements of the two organisms are: 2.8 to 5.7 microns in length (average 4.3 microns) for the Minnesota organism, compared with 4.1 to 6.7 microns in length (average 5.2 microns) for the British Columbia organism. In view of the above differences, the two organisms cannot be considered identical.

In comparing conidial formation in the species of Cephalosporium investigated by Pinkerton (11), and this organism, there are again some differences. However, Pinkerton made an extensive investigation of several species using both the celloidin and the paraffin method of embedding cultures, while only the paraffin method was employed in this study. In this connection, Pinkerton reports that accessory cytoplasmic inclusions were more noticeable in the celloidin material. This may account for the fact that certain structures in the phialide were not observed in the paraffin material studied. According to Pinkerton, a chain of small round granules collect longitudinally, and merge into several small rods at the tip of the phialide. These rods form a rigid collar, so that the end wall is weaker than the lateral walls at the tip of the phialide. As the spore is forced out, the middle of the tip is stretched and broken. These granules and rods were not observed in the paraffin material studied. Pinkerton observes, however: "Very thin

sections of paraffin material seem to indicate that the spore is abstricted as is the yeast bud. This appearance may be explained by assuming that one of the granules at the tip really stays in the center of the phialide and draws the cytoplasm as a narrow stream into the spore; and in some thin sections it is impossible to see any lateral granules." This appears to agree with the observations made on spore formation in this organism. Pinkerton found that the contents of the phialide cleave into segments, which eventually become spores. The cleavage may be only partial with the cleaved parts occurring only in small numbers towards the tip of the phialide. No suggestion of cleavage of the contents of the phialide was observed in this organism.

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Plate I

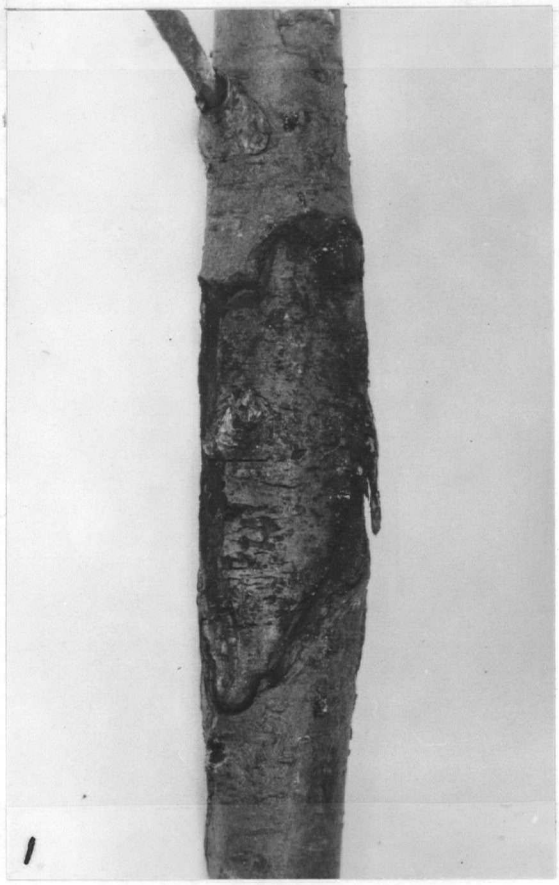
The Canker

Fig. 1. A natural canker on the main stem of western hemlock (Tsuga heterophylla (Rafn.) Sarg.). X 1/2.

Fig. 2. The same canker shown in Fig. 1 with the bark removed, showing the extent of the canker under the bark. X 1/2.

Fig. 3. A canker on the main stem of western hemlock, 5 months after inoculation. X 1/2.

PLATE I



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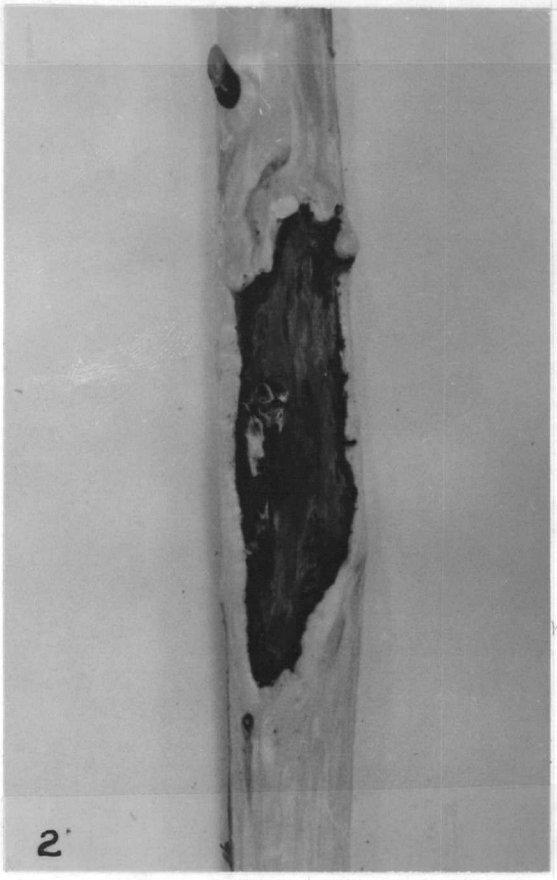

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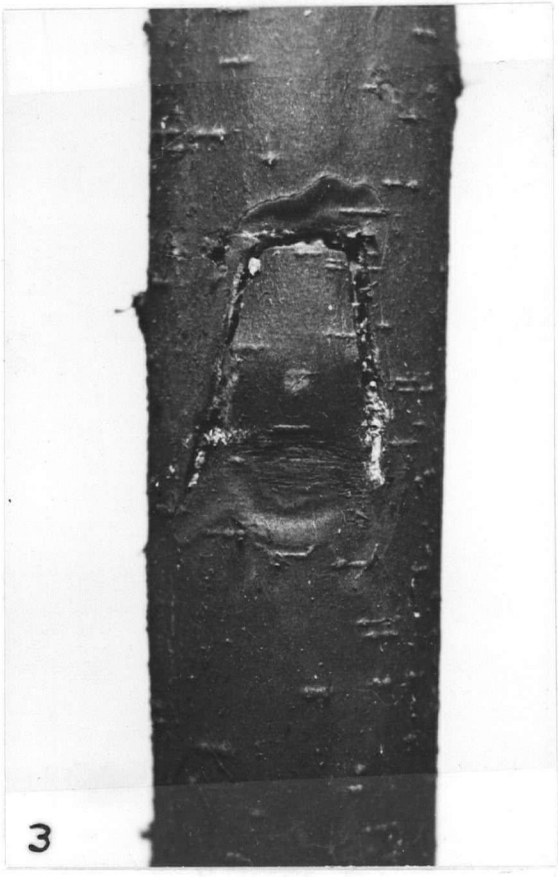


3

Plate II

Cephalosporium sp. on Malt Agar, Grown in the Dark in 90 mm. Petri Dishes

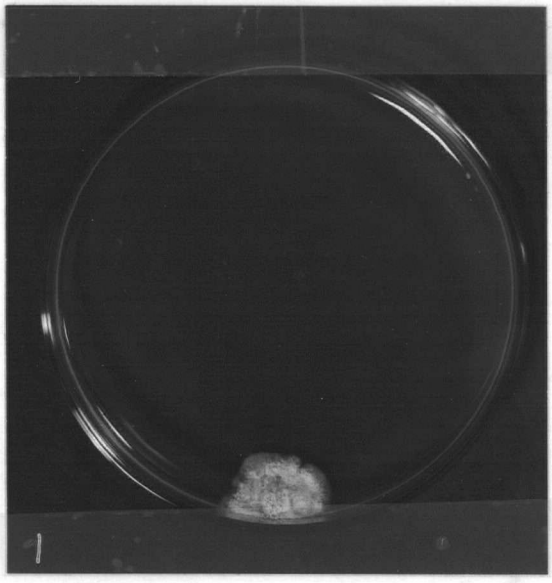
Fig. 1. Growth after 2 weeks.

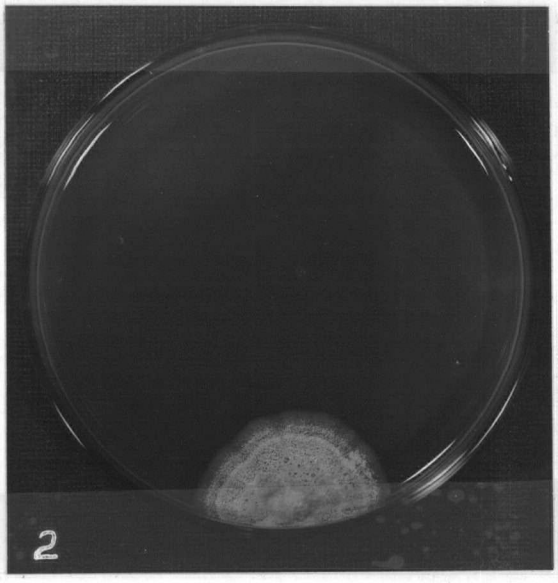
Fig. 2. Growth after 4 weeks.

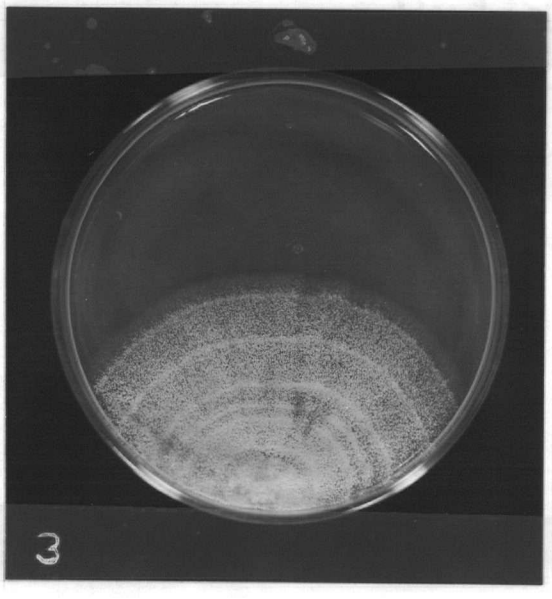
Fig. 3. Growth after 6 weeks.

Fig. 4. Growth after 6 weeks, with a hyaline sector on the left.

PLATE II







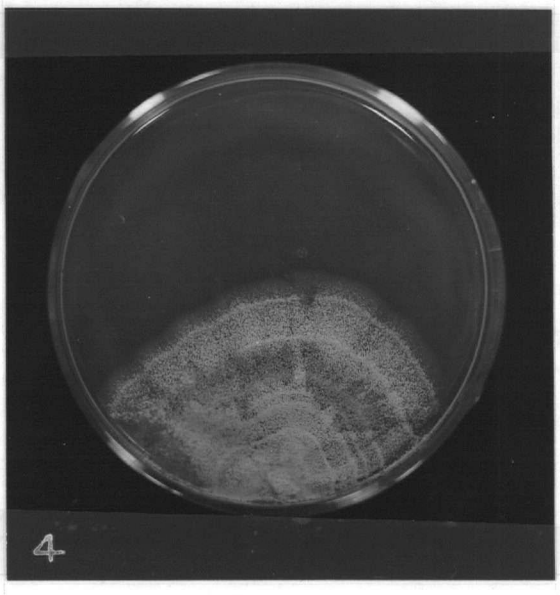


Plate III

Camera Lucida Drawings of the Organism from Cultures on Malt Agar

Fig. 1. Broad hyaline hyphae, cells with swollen ends.

Fig. 2. Broad hyaline hypha with regular cells.

Fig. 3. Broad hyaline hypha with irregular cells.

Fig. 4. Narrow hypha with amber-colored contents.

Fig. 5. Hypha with a narrow, amber-colored cell, and a broad hyaline cell with bases of conidiophores.

Fig. 6. Narrow hyaline hypha.

Fig. 7. Hyphae from the advancing zone.

PLATE III

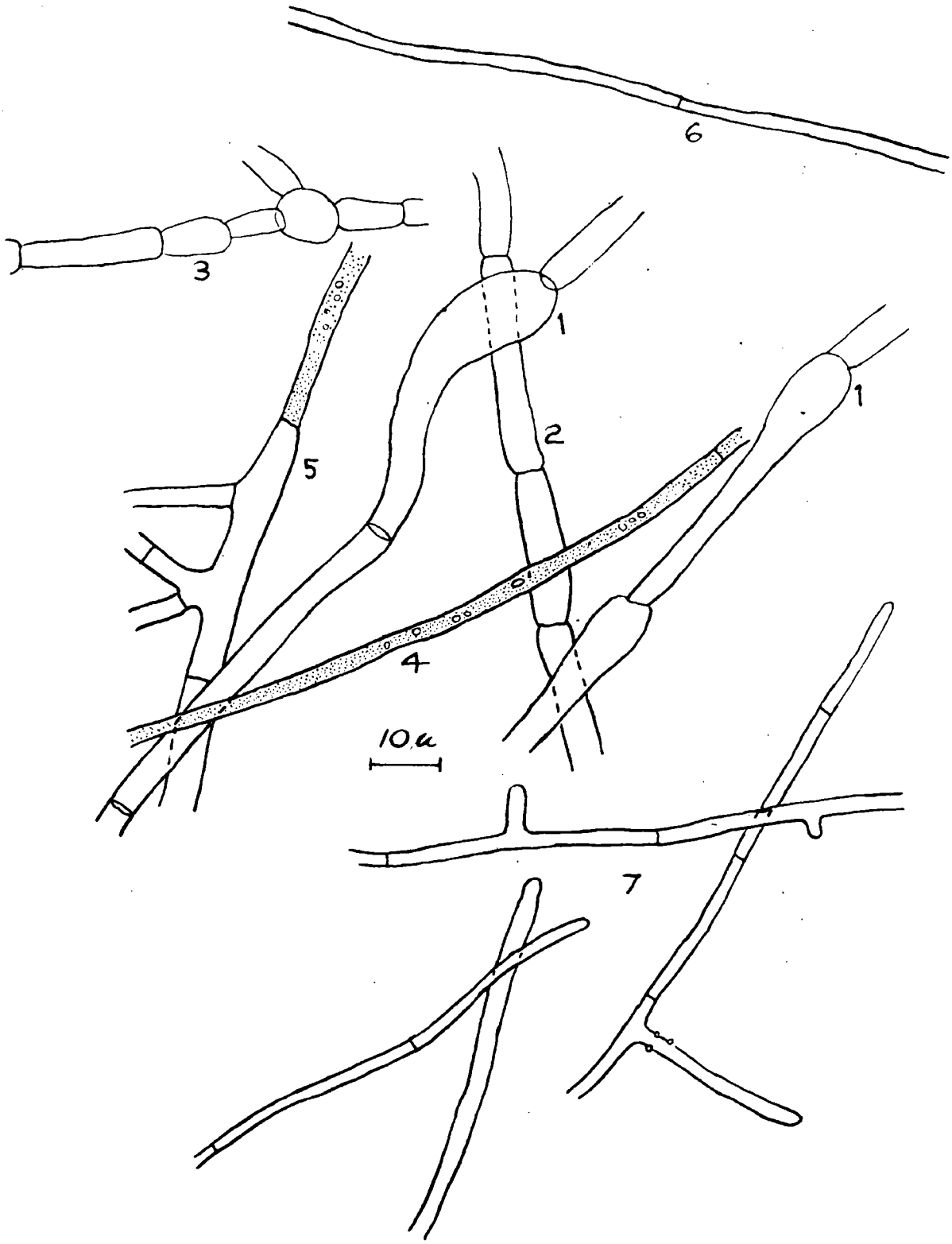


Plate IV

Camera Lucida Drawings of the Organism

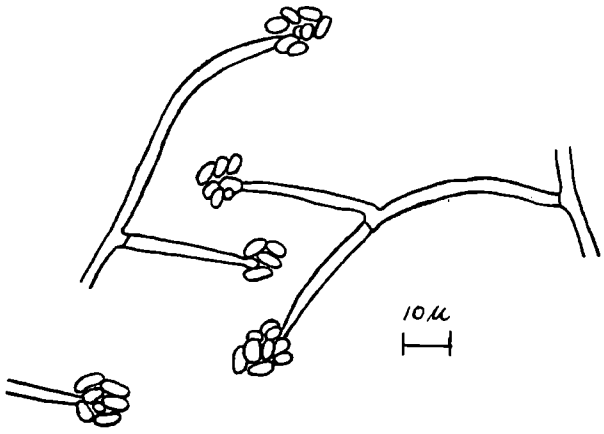
Fig. 1. Cephalophores from an agar slide culture on potato dextrose agar.

Fig. 2. Compound conidiophore, with conidial heads removed, from a culture on malt agar.

Fig. 3. Conidiophores, with conidial heads removed, from near the advancing zone of a culture on malt agar.

Fig. 4. Compound conidiophore, with conidial heads removed, from a culture on malt agar.

PLATE IV



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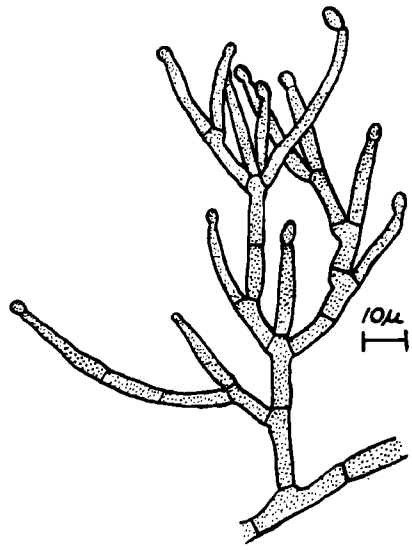

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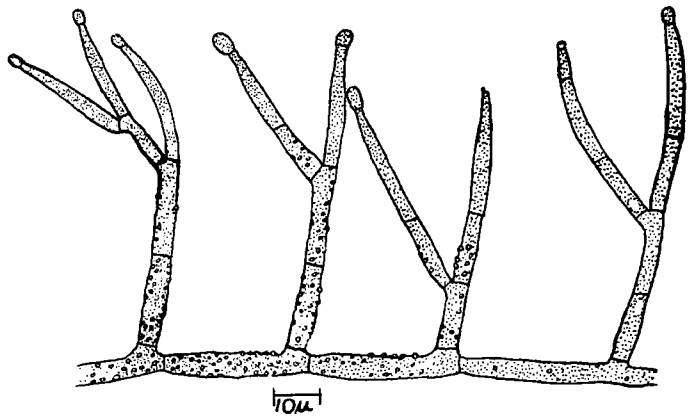

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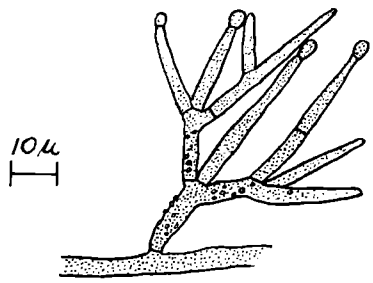


4

Plate V

Camera Lucida Drawings of Conidial Formation in *Cephalosporium* sp.

Fig. 1-3. Development of a conidium within the tip of the phialide.

Fig. 4. Extrusion of the spore through the tip of the phialide.

Fig. 5. Phialide, showing the connection between conidium and phialide broken.

Fig. 6. Mature conidium, with another spore beginning to form in the tip of the phialide.

Fig. 7. Passage of a nucleus into a conidium.

Fig. 8. Nuclear division in a phialide.

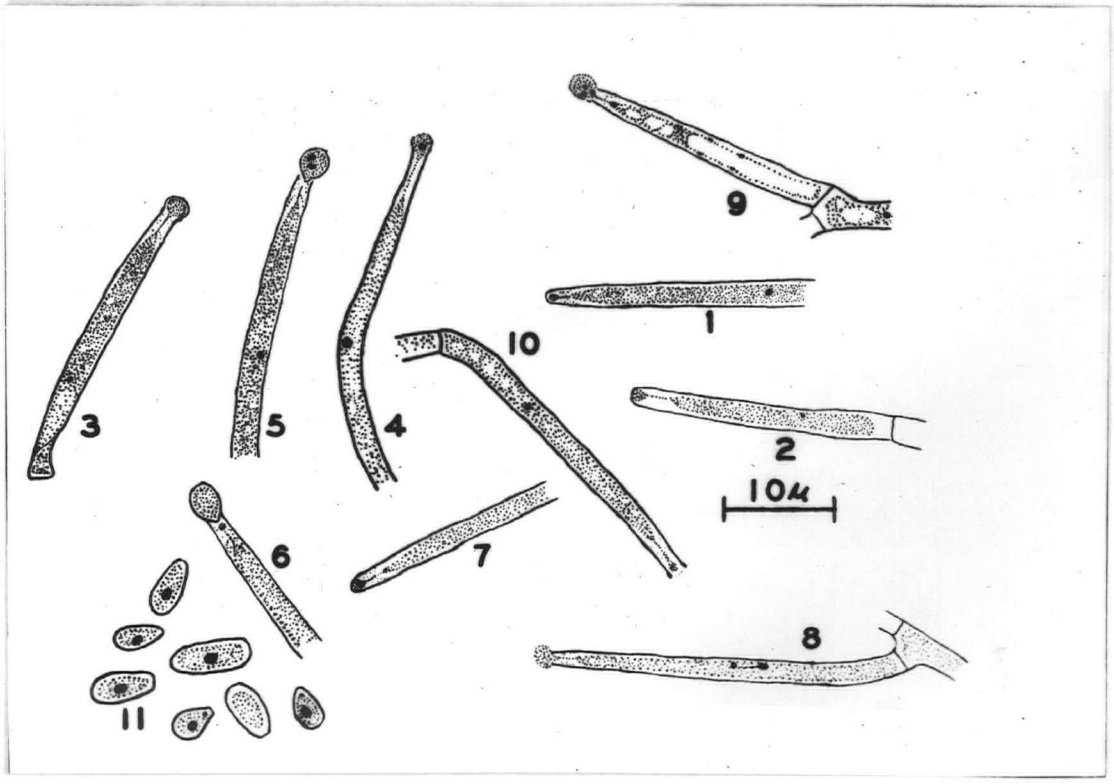
Fig. 9. Phialide, showing vacuoles.

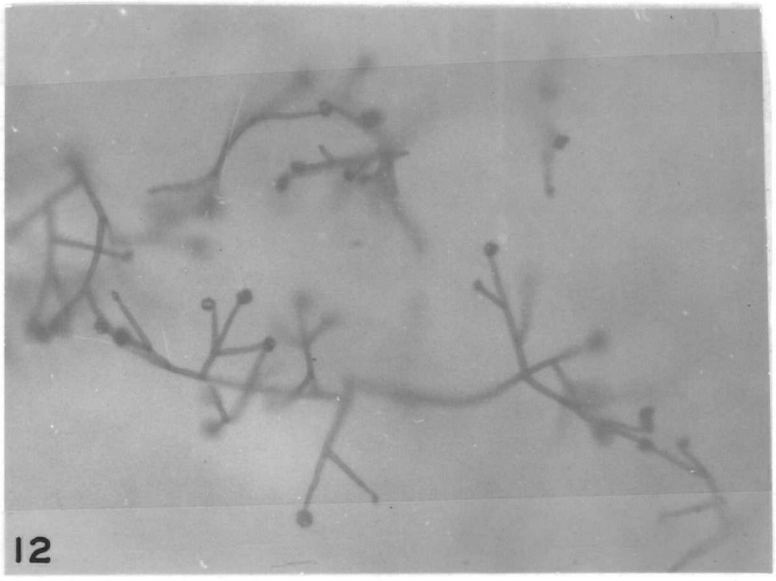
Fig. 10. Phialide with spore broken off, and a new spore beginning to form.

Fig. 11. Conidia.

Fig. 12. Photomicrograph of cephalophores from a culture on malt agar. X 200.

PLATE V





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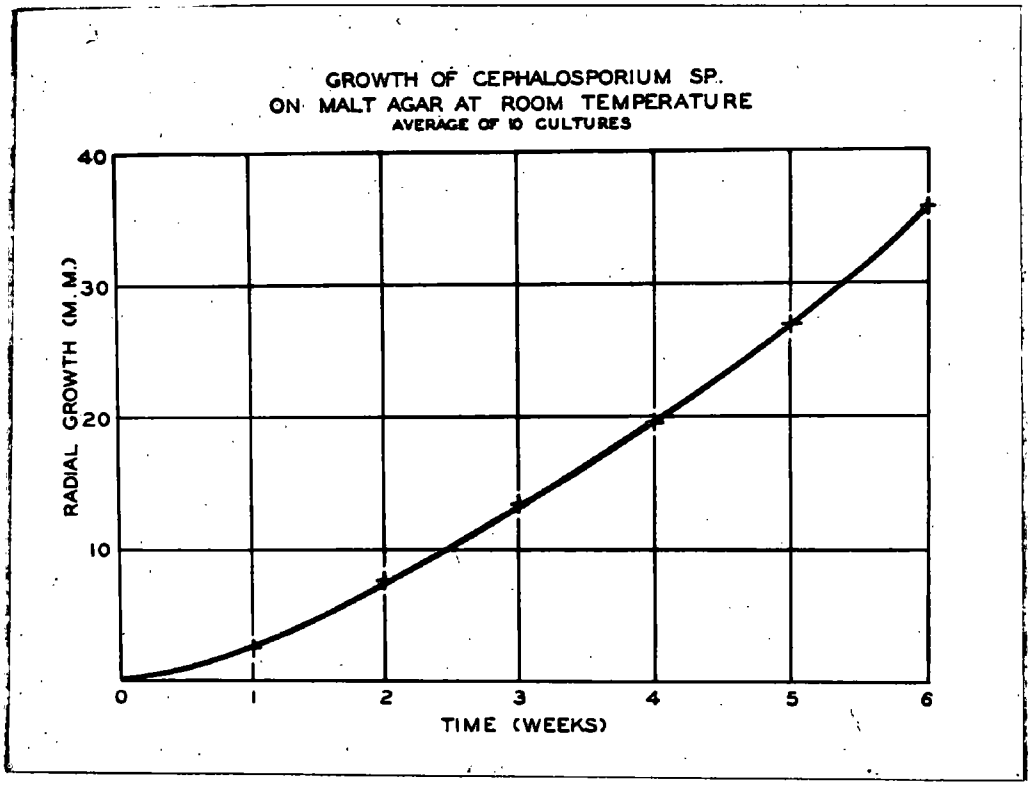
GROWTH OF CEPHALOSPORIUM SP.
ON MALT AGAR AT ROOM TEMPERATURE
AVERAGE OF 10 CULTURES
RADIAL GROWTH (M.M.)
40
30
20
10
0
1
2
3
4
5
6
TIME (WEEKS)

FIG. 1

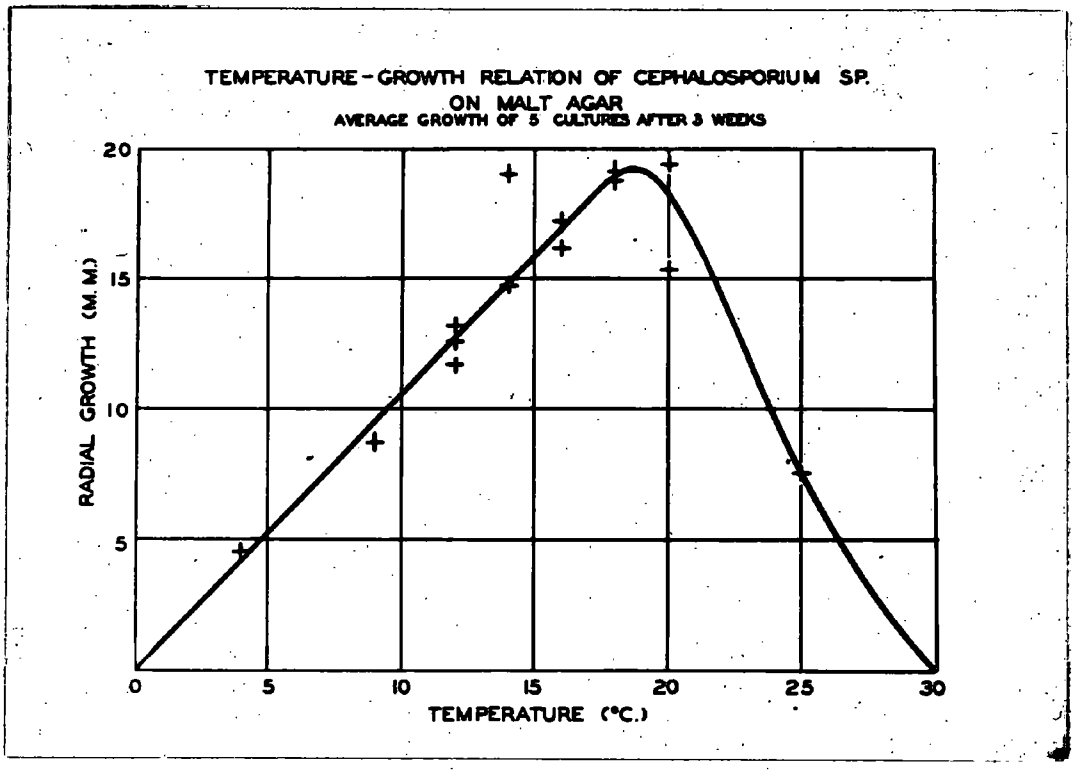
TEMPERATURE-GROWTH RELATION OF CEPHALOSPORIUM SP.
ON MALT AGAR
AVERAGE GROWTH OF 5 CULTURES AFTER 3 WEEKS
RADIAL GROWTH (M.M.)
20
15
10
5
0
5
10
15
20
25
30
TEMPERATURE (°C.)

FIG. 2