# STUDY OF DISEASES OF NARCISSI

# A STUDY OF DISEASES OF NARCISSI.

THESIS.

Submitted by A. J. Hicks in partial fulfilment of the requirements for the degree of Master of Science, University of McGill.

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## INTRODUCTION.

In spite of the fact that for some time the commercial culture of ornamentals has been carried on extensively in the United States and many European countries, the diseases of ornamental plants have not received any great amount of attention.

Until recently this industry in Canada was of little economic importance, but the rapidly developing bulb industry in British Columbia, and such institutions as the Dale Estate, of Brampton, Ontario, impress one as being indicative of the fact that Canada's competition in the field is fast becoming a factor to be reckoned with.

It is well known that in the bulb industry, good quality and freedom from disease of the commodity produced, are absolutely essential to the creation and maintenance of home and foreign markets. For this reason, information regarding the symptoms and significance of certain of the more common and injurious diseases of bulbs, would be of great value to the Canadian grower.

In the summer and early fall of 1927, the writer became interested in certain bulb diseases which were brought to his attention. As time would not permit of the detailed investigation of all these diseases it was decided to concentrate attention on certain diseases of Narcissi. Work was initiated on a study of four diseases, the results of the investigation being incorporated in this thesis. Of these four, the first dealt with is a basal rot of the bulbs caused by a Fusarium species. The remaining three diseases are caused by sclerotium producing fungi and are designated as "Smoulder", "Type B", and "Type C" respectively. "Smoulder" is a serious disease of narcissus bulbs and is caused by Botrytis

narcissicola Kleb. The fungi found associated with the diseases designated as "Type B" and "Type C", have never been named as far as the writer is aware.

Since each of the four species of fungi concerned is a separate entity, it has been decided, for the sake of clarity, and in order to avoid confusion, to treat each separately. The summary at the end of this work is intended to be comprehensive enough to cover all the findings in the case of each of the four species of fungi studied.

# BASAL ROT.

## INTRODUCTION.

Basal rot, or "bol" rot as it is called by the Dutch, has been noticed by the writer to be extremely common on imported bulbs. At first this disease was confused with the eelworm disease of narcissi, but as will be shown, although the symptoms of disease in the bulbs appear to be similar there is in reality a marked difference in the primary and intermediate stages of decay. The condition of many bulbs in imported shipments of narcissi intercepted at the ports of entry because of the presence of basal rot left no doubt in the writer's mind that this disease is a quick developing and highly injurious rot. Cultures made from decayed portions of infected bulbs invariably yielded a species of Fusarium.

## HOSTS

### Plants Affected.

Bulbous plants of the genus Narcissus appear to be the only ones affected by basal rot.

## Varietal Susceptibility

As the writer has been unable to visit any of the large narcissus-growing centres of this continent the findings herein recorded must of necessity apply to examination of imported shipments and the results of artificial inoculation and storage experiments.

The Narcissus pseudonarcissus or Trumpet daffodil i.e. common daffodil group appear to be most susceptible to basal rot, the following varieties being especially so:- Bicolor Victoria, Bicolor Harbinger, Golden Spur, Spring Glory, Van Waverens Giant, Mme. de Graaf, Emperor, Empress, and Glory of Sassenheim.

The Incomparabilis and Poeticus groups are also occasionally susceptible, the variety Poeticus ornatus having been found to be the most susceptible variety in the latter group. The Jonquils and Paperwhites appear to be immune.

# THE DISEASE

## Names

In 1916 Westerdijk (27) described a disease of narcissus bulbs which she called "Bolziek", or bulb rot. The causal organism was given as Fusarium gemmiperda. Drayton (7) and Weiss (23) (24) described a disease of narcissus bulbs caused by a Fusarium species which they termed "Basal Rot". It is here considered advisable to use the term "Basal Rot" since that best describes the appearance and condition of bulbs infected with the Fusarium sp. studied.

## Review of Literature

Cooke and Massee (4) in 1887 described a species of Fusarium which they had found infecting narcissus bulbs in England, to which they gave the name Fusarium bulbigenum. At that time it was not recognized as a parasite.

Massee (14) later gave a further description of this organism. The same author (15) in 1913 observed a disease of narcissus bulbs which was then widespread in England and which he attributed to Fusarium bulbigenum.

Westerdijk, (27) in 1916 mentions the occurrence of Fusarium gemmiperda on narcissi in Holland and states that it causes a similar disease to that caused by F. bulbigenum. She writes, "De 2 soorten van Fusarium komen geheel overeen, wat vorm van conidien aangaat: alleen maakt gemmiperda blauw gekleurde ophoopingen van chlamydosporen, bulbigenum ongekleurde. Dit is in elk geval een constant verschils-kenmerk," thus differentiating the two species by the fact that in F. gemmiperda

the chlamydospores are blue in mass.

Drayton (7) in 1927 mentions the occurrence of basal rot on imported narcissus bulbs and states that it is caused by a Fusarium sp.

Weiss (23) (24) in 1929 gave a full account of the occurrence of basal rot in the eastern United States, and states that owing to the ravages of this disease the whole narcissus growing industry in those regions is threatened with extinction. This rot he attributes to an unidentified species of Fusarium. He refers to the occurrence of a similar narcissus rot in England in 1913, attributed to Fusarium bulbigenum (15) and states that later investigations showed that this species was merely a secondary organism following bulb injury caused by eelworm. The reference to the later investigations proving the secondary nature of Fusarium bulbigenum mentioned by Weiss (24), the writer has been unable to procure.

## Geographical Distribution

This disease has been found on shipments of narcissi from Holland, Germany, and England and is reported to be especially severe in the United States. Occasionally specimens have been received from Canadian growers but it is safe to presume that basal rot has not, as yet, become firmly established in this country. This is probably due to the fact that Canadian growers renew their stocks from year to year with imported bulbs, the development of basal rot in storage and transit being such that the infected ones can fairly readily be picked out on arrival.

## Economic Importance

Statistics are not available to show the loss to bulb growers

due to basal rot, but if one takes as a criterion the number of imported shipments either sorted or refused entry to Canada annually because of this disease one can readily see that the loss to foreign growers, especially the Dutch, must be severe. Weiss (23) states that in 1928 the loss due to this disease incurred by three growers in N.Y. State alone totalled $250,000.

## SYMPTOMOTOLOGY

### Morphological Symptoms

In the final stages of decay the bulbs are completely dried out, the inner tissue being reduced to a heterogenious powder-like mass. In the less advanced stages of decay the bulb is yielding to the touch, the inner fleshy scales are browned, soft and watery in appearance. (Plates 1 and 2 Fig.4). At this stage also a mass of whitish mycelium is often produced externally around the basal plate. The early stages of basal rot infection are more difficult to locate, but often when the outer suberized scales are stripped from a suspected bulb a purplish-coloured diseased area will be noticed, starting at the basal plate and spreading towards the nose of the bulb. A quick and efficacious way of determining as to whether or not basal rot is present to any extent in a bulb is to press the thumb into the basal plate. This portion of a healthy bulb is resilient to the touch, while in the case of a diseased bulb the thumb can generally be pressed into the tissue for some distance. The primary stages of decay are very difficult to locate, but even the smallest lesions have usually been detected when employing the two simple expedients mentioned above.

Cross sections of a diseased bulb, starting at the nose and working towards the basal plate show that the diseased area

increases as one cuts off the successive layers. Bulbs showing medium to light infection of basal rot have been sectioned in this way, the basal portion of the bulb being diseased, the upper portion of the bulb being perfectly healthy. Occasionally infection takes place between the side bulbs and the bulb proper (Plates 1 and 2, Fig.3). However, even so, the rot starts at the base and spreads towards the embryo flower parts and the nose. Many hundreds of infected bulbs have been examined by the writer during the past three or four years and invariably the above has been the case.

A weft of whitish fungus mycelium is characteristically found between the scales of infected bulbs. (Plate 3).

## Histological Symptoms

A cross section of a diseased fleshy scale reveals that the cellular structure is disintegrated and the individual cells collapsed. Another feature of the diseased cells is the enormous increase in number of starch grains over and above those found in healthy bulb cells.

# ETIOLOGY

## The Organism

### Isolation

Microscopical examination of portions of the mycelial weft, found between the scales of infected bulbs showed that large numbers of microconidia were present, as were also intercalary and terminal chlamydospores. Spore dilutions were made and poured on gelatine plates. The medium being clear, these spores could readily be located and, upon germination transferred to potato dextrose agar. On this medium a fairly dense white fluffy

growth of mycelium was produced, bluish coloured sclerotia appearing towards the bottom of the tube after two weeks growth (Plates 12 and 13). Although macrospores had not been observed it was believed that the fungus isolated was a species of Fusarium and efforts were made to produce these spores. Finally a few 3-septate Fusarium macrospores were found. The size and shape of the spores, the formation of blue sclerotia, and the production of both terminal and intercalary chlamydospores placed this species in the section Elegans according to Wollenweber's classification (28) and further investigation revealed that the fungus under observation was either Fusarium oxysporum Schlect. or some closely related species.

Cultural Studies

A study was made of the types of growth of the basal rot organism on different media, the results of which are given below:

POTATO DEXTROSE AGAR:- Good even growth of short, fluffy, white aerial mycelium. Bluish coloured sclerotia forming at the margin and on the upper surface of the agar near the bottom of the test tube. Sclerotial formation first became apparent on cultures ten to fourteen days old. The colour of the substratum ranged from no change to a very pale pink (Plates 12 and 13).

POTATO PLUG:- Good growth of mycelium. First white, turning to a faint salmon colour, later in parts turning blue. Bluish coloured sclerotia were formed early, being larger than those produced on potato dextrose agar.

PRUNE AGAR:- Good growth of short mycelium. Colour of mycelium was at first white to orange pink, in older colours ranging

from lilac to litho purple. Colour of medium greatly changed, in old cultures being madder violet. Dark violet coloured sclerotia were produced.

OATMEAL AGAR:- Fair growth of mycelium. Colour salmon-buff. Substratum becoming pale lavender violet in colour. Bluish coloured sclerotia formed early. A few sporodochia produced singly on old cultures.

MELILOTUS STEMS:- Fair growth of mycelium. At first white, later turning to La France pink. Bluish coloured sclerotia produced early. Sporodochia produced on old cultures, salmon-coloured.

PEACH STEMS:- Poor growth of mycelium. White to pinkish.

RICE:- Good growth of short, fluffy white mycelium. Later turning to flesh pink.

STERILIZED NARCISSUS LEAVES:- Fair growth of mycelium. White to pale achraceans-salmon. Numbers of small bluish coloured sclerotia produced.

NARCISSUS LEAVES:- (portions of living leaves) Poor growth.

Name, History and Classification of the Organism.

A comparison of the above cultural characteristics was made with Wollenweber's description of F. oxysporum Schl. (25). The similarity was striking, but examination of the few 3-septate macrospores produced at that time showed that the average size was a little larger than that given by Wollenweber for 3-septate macrospores of F. oxysporum Schl.

In March, 1930, the writer sent some pure cultures of this fungus to Dr. Wollenweber, the results of whose examination follow:

"Es stellte sich indes heraus, dass die Konidien etwa

> von der Grösse des F. oxysporum waren, während die des Vergleichspilzes länger und dünner sind. Ich halte den fraglichen Pilz für F. oxysporum Schlechtendahl forma 5 Wr. syn. F. oxysporum Schl. var. nicotiana Johnson. Die neue Bezeichnung als "forma 5" ist zum ersten Male in Fusaria autographice delineata Nr. 1012 gewählt, wobei gleichzeitig alle Fusarien der Sectio Elegans in 9 Arten zusammengefasst sind. F. oxysporum ist an Blumenzwiebeln schon mehrmals beobachtet worden, ohne dass die Pathogenität geklärt wurde."

Johnson (11) first gave this fungus the name F. oxysporum Schl. var. nicotianae and described it as causing a serious wilt of tobacco in the United States. Cultural characters of the organism studied by the writer tally very closely with those described by Johnson in the article afore mentioned, the only difference being very slight modifications in the size of the macrospores. However, according to Appel and Wollenweber (2) Thom (22) and Sherbakoff (20) spore size varies to some extent on different media and under different environmental conditions and this plus the probable error in measuring and averaging the spore size could easily account for the slight disparagement encountered.

The description of the organism is as follows:

Fusarium oxysporum Schl. form 5, Wr. syn.

Fusarium oxysporum Schl. var. nicotianae Johnson.

Isolated from narcissus bulbs infected with basal rot. Agrees with the description of Fusarium oxysporum Schl. var. nicotianae Johnson and identified by Wollenweber as being that organism.

Mycelium on most media pure white to a light pinkish tinge and of a powdery appearance due to the presence of numerous microconidia. Blue coloured sclerotia formed early on potato plugs and in two weeks on potato dextrose agar. No true pionnotes observed. Reduced pionnotes or "pseudopionnotes" obtained.

Sporodochia produced on Melilotus stems, oatmeal agar, and also on old cultures on potato dextrose agar and prune agar. Sporodochia salmon coloured and containing mostly 3-septate conidia. 4 and 5 septate conidia also occasionally found. These macroconidia are slightly larger than the corresponding macroconidia of Fusarium oxysporum Schl. Conidia in sporodochia and reduced pionnotes 3-septate up to 100%, 35.2µ x 4.1 (28.1 x 3.6µ to 43.4 x 4.6µ). Four septate up to 20%, 38.6 x 4.2µ (28.7 x 3.8µ to 48.5 x 4.6µ). Five septate up to 12%, 44.9 x 4.2 (37.4 x 3.9µ to 52.3 x 4.6µ). Six septate conidia have not been found by the writer. Non septate microspores from mycelium are very varied in size and shape averaging 8.7 x 3.3µ (3.5 x 2.8µ to 10.7 x 3.8µ). Chlamydospores terminal, intercalary and conidial, smooth, round, often thick walled, frequently in masses, 8.8µ (7.8 to 9.9µ). in diameter.

Pigment produced on some media.

HABITAT. This species is found in Europe and North America causing a basal rot of narcissus bulbs. All parts of the bulb are involved, the basal rot being the primary stage of decay. Diseased parts rot rapidly, affected tissue becoming disintegrated and the cells collapsed.

Also said to be parasitic in the fibro-vascular bundles of Nicotiana tabacum in Maryland and Ohio, U. S. A., where it causes a decided wilting of plants followed by death.

## Pathogenicity

## Inoculation Experiments

### Growing bulbs on artificially inoculated soil.

This experiment was conducted during the winter under greenhouse conditions. The soil was sterilized before the addition of the fungus and planting of the bulbs.

The following six varieties of narcissi were used, four bulbs of each variety being planted at the rate of two to a pot.

1. Golden Spur
2. Mme. de Graaf
3. Bicolor Victoria
4. Emperor
5. Spring Glory
6. Poeticus ornatus.

Three tube cultures of the basal rot Fusarium were added to each pot. All the bulbs, with the exception of one Emperor bulb and one bulb of the variety Mme. de Graaf produced healthy growth. The two bulbs mentioned were later lifted but were in such a badly decayed condition that a diagnosis of the cause of decay was impossible.

### Artificial inoculation of bulbs.

A bulb of the variety Emperor was taken and small pyramid-shaped portions removed from the sides with a sterile scalpel. A portion from a culture of the fungus was inserted in these holes, which were then plugged with the top layer of removed scale. The incision was sealed with hot paraffin wax. In a few days time the typical purplish colour began to appear around the outside of the wax, and in two weeks the rot had spread so rapidly that most of the bulb was affected. (Plate 7, A and B). The

typical white mycelial growth so commonly seen at the base of bulbs infected with basal rot also began to appear externally. Cultures were made from diseased portions of the bulb and in all cases yielded the fungus. This experiment was repeated at a later date with exactly similar results.

At this later date a healthy Emperor bulb was taken, incisions made as in the previous experiment, but in this case no fungus was added. The incision was sealed with hot wax. No rot developed, and removal of the wax later showed that the small pyramid-shaped portion of scale had dried up, as had also the inside cut surfaces.

## Determination of the rate of spread Of infection in Storage and Transit

This experiment was conducted in order to find to what extent healthy narcissus bulbs could become infected with basal rot by contact with diseased bulbs in storage and transit, and also to determine the length of time required for signs of basal rot infection to appear.

Five varieties of bulbs were employed, these being divided into three lots marked A, B, and C. Lot A were healthy untreated bulbs, lot B were healthy treated bulbs, and lot C constituted the check. The varieties of bulbs used in the experiment were as follows:-

1. Emperor
2. Bicolor Victoria
3. Mme de Graaf
4. Spring Glory
5. Golden Spur

Experimental methods and results.

Lot A. Three bulbs (healthy) of each of the above varieties were placed in separate paper bags and numbered, Emperor 1,

Bicolor Victoria 1, and so on. These bulbs were not skinned or injured in any way, and exemplified in their condition the usual appearance of healthy imported stock on its arrival at the Canadian ports of entry. To each of these lots of three was added a considerable amount of chopped up tissue from narcissus bulbs infected with basal rot. Each of the bags was then closed and an elastic band slipped around it.

Lot B. As in A, three bulbs of each of the aforementioned varieties were taken, but in this case the outer suberized scales were removed and small portions of the basal plate of each cut out with a sterile scalpel. The bulbs were then taken, still in lots of three, placed in separate bags, and numbered Emperor 2, etc. Repeating the procedure followed in A, portions of diseased bulbs were added to each bag which was then closed as above.

Lot C. Check. Two bulbs of each variety were taken as checks. One of the bulbs was injured as in B, the other uninjured as in A. These bulbs were then placed together, there thus being two bulbs of each variety, one injured, one uninjured, in each of five separate bags. The bags were closed and fastened as in A and B.

All of the bags were then taken and placed in a wooden box, being packed together as they would be in transit from Holland to this country. In order to further simulate conditions of storage and transit, a lid was fastened on the box, and the whole placed in a warm dark room. There it remained untouched for two weeks.

At the expiration of that period the bulbs were examined. There was every evidence of the start of decay at the bases of most bulbs in lot B. Some few bulbs in lot A also seemed to be

infected. The bulbs in the check were sound.

The bulbs were then left untouched for two months. The following results (Table 1) were obtained on examination of the bulbs at the termination of the two month period.

Table 1. Spread of Basal Rot in Storage.

| Variety | Treatment | Diseased | Healthy |
|---|---|---|---|
| Emperor | A | 1 | 2 |
| | B | 2 | 1 |
| | Check | - | 2 |
| Bicolor Victoria | A | 1 | 2 |
| | B | 3 | - |
| | Check | - | 2 |
| Mme de Graaf | A | 2 | 1 |
| | B | 3 | - |
| | Check | - | 2 |
| Spring Glory | A | - | 3 |
| | B | 2 | 1 |
| | Check | - | 2 |
| Golden Spur | A | 3 | - |
| | B | 3 | - |
| | Check | - | 2 |

Discussion

There is seen in the tables evidence of the fact that although wounding facilitates the chance of infection, sound bulbs are not immune from attack. The varieties Golden Spur and Mme de Graaf seem to be especially susceptible to basal rot infection. This has also been repeatedly observed by the writer in bulb inspection work. There is apparently a rapid spread of infection in storage, especially where healthy and diseased bulbs are packed tightly together and are subjected to warm humid conditions. In two weeks most of the bulbs which later were totally decayed were manifesting signs of basal rot infection.

## Physiological Reactions

### Effect of pH on the growth of the fungus.

In order to study the reactions of the fungus to various concentrations of H and O H ions the methods outlined by Karrer and Webb (12) were followed with slight modifications. Richards' modified solution, as described in the work above referred to, was used. A series of ten flasks was run in duplicate. As sugars react readily with acid or alkali when heated under pressure, the nutrient solutions and the acid and alkali were sterilized separately. Thirty C.C. of the nutrient solution together with the required amount of water, as indicated in the tables, were put into the flasks, plugged and sterilized. When cool the prescribed amounts of acid and alkali were added under sterile conditions by means of sterile pipettes. The solutions were then left standing for four days, and at the end of that time, being free from contamination each flask was inoculated with three drops of a heavy spore suspension of the fungus. Incubation was for ten days, in the light, at room temperature, 22°C. (Plate 31). At the completion of the incubation period the mycelial mats were filtered on dried weighed filter papers, oven-dried at 90°C and the weight determined. The pH values of the various solutions at the beginning of the experiment and after removal of the mycelial mat were determined electrometrically, using a Leeds and Northrup potentiometer, and colorometrically by means of a Hellige-Klett hydrogen-ion comparator.

As the experiment was run in duplicate the averages, for pH values and for weights of mycelium, are given in each case.

These with the dry weights of the mycelium are given in Table 2.

Table 2. Showing final dry weights of mycelium and pH values of solutions.

| Flask No. | N/5 HcL c.c. | N/5 K O H c.c. | Dist. $H_2O$ c.c. | Dry wt. of mycelium in grms. | pH Value | |
|---|---|---|---|---|---|---|
| | | | | | At beginning of exp. | On removal of mycelial mat. |
| 1 | 3.00 | | 17.00 | 0.00 | 2.0 | 1.96 |
| 2 | 1.00 | | 19.00 | 0.4715 | 2.4 | 2.62 |
| 3 | .50 | | 19.50 | 0.5535 | 2.8 | 3.00 |
| 4 | 0.00 | | 20.00 | 0.570 | 4.6 | 4.11 |
| 5 | | 1.00 | 19.00 | 0.5705 | 7.0 | 6.24 |
| 6 | | 1.50 | 18.50 | 0.478 | 7.4 | 6.58 |
| 7 | | 2.50 | 17.50 | 0.4365 | 7.8 | 6.93 |
| 8 | | 3.50 | 16.50 | 0.1475 | 8.2 | 7.56 |
| 9 | | 6.50 | 13.50 | 0.00 | 8.6 | 8.40 |
| 10 | | 15.00 | 5.00 | 0.00 | 9.0 | 8.60 |

The results show that the fungus makes good growth between pH 3.0 and pH 7.0, best growth being made between pH 4.6 and pH 7.0, the neutral point. There was a very quick drop in growth production between pH 7.0 and pH 8.2, no growth occurring at pH 8.0 and pH 9.0. It will thus be seen that the fungus grows best in an acid medium, the optimum occurring somewhere between pH 4.6 and pH 7.0. There was not any appreciable change in the reaction of the medium, even between pH 4.6 and pH 7.0, the trend being uniformly towards the acid side. The acidity of the cell sap of the fleshy scale tissue of mature bulbs was found to be approximately pH 5.8. The fungus produces good growth well beyond the limits of acidity encountered in host tissue. It does not appear to thrive on a very alkaline medium however, and the discovery of this fact led the writer to investigate the possibility of so treating the soil in which bulbs are grown that the soil conditions would inhibit the growth of the fungus and at the same time have no harmful effects on the bulbs. With this end in view a further experiment was conducted to find the effect of pH on the growth of

narcissus bulbs.

Effect of pH on the growth of narcissus bulbs.

A review of the literature showed that Hoagland (10), Duggar (8), McCall and Haag (16), Tarr and Noble (21) and others had grown seedlings on solutions of different pH values but so far as the writer is aware no record is available of attempts to grow bulbs under such conditions. Using the methods outlined by Tarr and Noble (21), an experiment was conducted, using narcissus bulbs of the varieties Emperor and Spring Glory. Twelve jars were used, Nos. 1-6, containing Emperor bulbs and Nos. 1a to 6a containing Spring Glory. The results of the experiment are found in Table 3.

Table 3. Growth of narcissus bulbs on solutions of different pH values.

| Variety | No. on jar | pH of solutions | Remarks re. growth |
|---|---|---|---|
| Emperor | 1 | 3 | Very poor growth. |
| | 2 | 4 | Poor growth. |
| | 3 | 5 | Good growth. |
| | 4 | 6 | Good growth. |
| | 5 | 7 | Fairly good growth. |
| | 6 | 8 | Very poor growth. |
| Spring Glory | 1 | 3 | Very poor growth. |
| | 2 | 4 | Poor growth. |
| | 3 | 5 | Good growth. |
| | 4 | 6 | Good growth. |
| | 5 | 7 | Fairly good growth. |
| | 6 | 8 | Very poor growth. |

It must be said that this method is not very satisfactory as far as bulbs are concerned. Preliminary trials showed that moulds developed very rapidly on the basal plates of the bulbs, even before roots had begun to form. Accordingly the bases of the bulbs used in this experiment were dipped in mercuric chloride 1-1000 for five minutes, and then washed in sterile water, before

being placed in the experimental bulb jars. Even these precautions did not prevent some growth of mould after two or three weeks, but by that time roots had formed and leaf tips began to show. The solutions were changed once a week during the course of the experiment. Best growth was made on solutions of pH 5 and pH 6.

Temperature Studies

Since temperature is an important factor in the control of fungus diseases it was decided to ascertain the optimum temperature for growth of the basal rot organism on artificial culture media. With this end in view the following experiments were conducted:

Growth of the fungus on hard potato dextrose agar plates.

This experiment was run in duplicate the plates being incubated at temperatures of 5°C, 10°C, 15°C, 20°C, 25°C, 30°C and 35°C. A small portion of mycelium from pure cultures was placed in the centre of each plate, the average diameter of the inoculum being 0.3 centimetres. The diameter of the growth of the fungus on the plate was measured every twenty-four hours. The experiment was run for five days at the end of which time the average growth at each temperature was recorded (Table 4).

Table 4. Growth of the fungus on hard potato dextrose agar plates.

| Temperature | Diam. of thallus in cm. | | Average diam. of thallus in cm. |
|---|---|---|---|
| | Original | Duplicate | |
| 5°C. | 0.6 | 0,8 | 6.70 |
| 10°C. | 1.0 | 0.9 | 0.95 |
| 15°C. | 2.5 | 2.6 | 2.55 |
| 20°C. | 5.5 | 5.7 | 5.60 |
| 25°C. | 6.2 | 6.2 | 6.20 |
| 30°C. | 6.0 | 6.0 | 6.00 |
| 35°C. | 2.1 | 2.3 | 2.20 |

From the aforementioned table it will be seen that the optimum temperature for growth is between 25°C. and 30°C, probably in the region of 28°C. An interesting feature of this experiment was the extraordinary type of growth of the fungus at 35°C. The mycelium either grew beneath the surface of the substratum or closely appressed to it. The appearance was slimy, in marked contrast to the white fluffy aerial growth of the fungus at the lower temperatures.

Growth of the fungus in Richards modified solution.

Modified Richards solution of a pH of 4.5 was used. 50 c.c. of the solution were placed in each of twelve Ehrlenmyer flasks, each flask being inoculated with three drops of a spore suspension of the fungus. The experiment was run in duplicate at six different temperatures. Duration of the experiment was for 10 days. (Plate 30B). At the end of that time the mycelial mats were removed, dried and weighed. The results of this experiment are tabulated below. (Table 5).

Table 5. Growth of the fungus in Richards modified solution.

| Temperature | Numbers on flasks | Dry weight of mycelial mat in grams. |
|---|---|---|
| 5°C. | 1 and 1a | 0.0000 |
| 10°C. | 2 and 2a | 0.1115 |
| 15°C. | 3 and 3a | 0.3580 |
| 20°C. | 4 and 4a | 0.3585 |
| 25°C. | 5 and 5a | 0.4035 |
| 30°C. | 6 and 6a | 0.5280 |

Unfortunately when this experiment was carried out the 35°C. incubator was not available. The results obtained however agreed favourably with those obtained in the previous experiment, best

growth being obtained at 30°C.

One of the flasks which had been held at 5°C. without growth appearing was kept at room temperature, 22°C. following the completion of the above experiment. The duplicate flask was left at 5°C. Profuse growth of the fungus resulted in the flask retained at room temperature. No growth appeared in the flask retained at 5°C., thus demonstrating that the fungus spores are not killed at a temperature of 5°C, and soon germinate when brought to a higher temperature. (Plate 30 A).

Effect of heat on spore germination.

A spore suspension of the fungus was made from a three weeks old culture, and kept at a temperature of 110°F. for 2½ hours. Plates of potato dextrose agar were poured, and a small quantity of the treated spore suspension added to each. Abundant growth of the fungus resulted.

Two flasks of Richards solution were later inoculated with a spore suspension of the fungus, and placed in the incubator at 37°C. - 98.5°F. for ten days. No growth resulted. The flasks were removed and kept at room temperature, without any sign of growth appearing. This would indicate that a temperature of 37°C. - 98.5°F. sustained for a period of ten days is sufficient to kill the spores of the fungus.

Germination of spores at different temperatures.

The spores used in this experiment were taken from two weeks old cultures. Counts of 100 spores were made in each case, this being done after 12 hours incubation. The spores were sown in hanging drops in van Tieghem cells, the individual cells were placed in moist chambers and incubated at the desired temperatures. Four different solutions were used, being designated as follows:

A. Tap water.

B. Distilled water.

C. Tap water + cell sap of the inner fleshy scales of narcissus bulbs.

D. Distilled water + cell sap of the inner fleshy scales of narcissus bulbs.

The results of this experiment are embodied in Table 6.

Table 6. Germination of spores at different temperatures on four separate culture media

| Temperature | Symbol | % of germinating spores. | % of ungerminating spores. |
|---|---|---|---|
| 10°C. | A | 5 | 95 |
| | B | 7 | 93 |
| | C | 11 | 89 |
| | D | 16 | 84 |
| 15°C. | A | 10 | 90 |
| | B | 14 | 86 |
| | C | 20 | 80 |
| | D | 31 | 69 |
| 20°C. | A | 38 | 62 |
| | B | 48 | 52 |
| | C | 84 | 16 |
| | D | 85 | 15 |
| 25°C. | A | 43 | 57 |
| | B | 70 | 30 |
| | C | 68 | 32 |
| | D | 62 | 38 |
| 30°C. | A | 49 | 51 |
| | B | 55 | 45 |
| | C | 66 | 34 |
| | D | 71 | 29 |

The spores of the fungus germinated best in solutions containing cell sap of the inner fleshy scales of narcissus bulbs. Their germination is favoured at temperatures ranging from 20°C. to 30°C. Best average germination occurs from 20°C. to 25°C. However, it was found that the germ tubes of the spores germinated at 30°C. were much longer than those produced at lower temperatures. It is deduced that a temperature of 30°C. is more favourable to mycelial growth than to spore germination.

## CONTROL

Although it is feared that little can be done to save bulbs when once they have become infected with basal rot, the writer offers a few practical suggestions regarding precautionary measures which may be adopted.

Sort over stocks of narcissus bulbs before storing and again before planting. Bulbs with soft or discoloured bases should be destroyed.

The hot water treatment for control of eelworm, two and one half hours at 110 F does not kill the basal rot fungus. Weiss (25) and McWhorter (17) found that the addition of a fungicide to the water prevented the spread of basal rot during the hot water treatment of bulbs. A 0.25% solution of Semesan seemed to give the best results.

Great care should be taken when digging the bulbs. Injury to the basal plate greatly increases the danger of infection. Bulbs should be dried immediately on lifting and, if possible, stored at a temperature of 10 C.

In view of the fact that the fungus causing a basal rot of narcissus bulbs also causes a wilt of tobacco, these two crops should not be grown in rotation.

Greenhouse men would be well advised either to discard or sterilize soil which has just grown a crop of bulbs.

Should the disease appear to any great extent in outdoor plantings, diseased plants and the soil surrounding them should be removed. If possible a three to five year rotation should be practiced.

# SMOULDER DISEASE OF NARCISSI.

## INTRODUCTION

Occasionally there were found, in imported shipments of narcissus bulbs, one or two which had fairly large black sclerotia attached to the outer scales. Sometimes the bulbs upon which these sclerotia were found would be completely decayed. Other bulbs with these sclerotia attached to the surface appeared to be perfectly healthy, only the outer dry scales apparently being infected. As it was not known whether the fungus concerned was a Botrytis or not a few studies were made of the habits of this organism, the results of which follow:

## HOSTS

### Plants Affected.

Bulbous plants of the genus Narcissus appear to be the only ones affected.

### Varietal Susceptibility.

Most of the varieties of the Trumpet narcissus group have been found to be susceptible to this disease. The disease has not been found by the writer on any varieties in the Incomparabilis, Poetaz and Poeticus groups.

## THE DISEASE.

### Names.

This disease is known to the Dutch as "Smeul", and in England as "Smoulder". This is a descriptive name applied to the disease in the field, and it refers to the

burned and shrivelled appearance of the stems and leaves of diseased plants.

## Review of Literature.

Klebahn (13) in 1906 described a Botrytis which he isolated from diseased narcissus bulbs of the variety Narcissus pseudonarcissus. Artificial inoculations with potted bulbs proved the pathogenicity of the organism.

Westerdijk (27) states that infection of the leaves takes place at ground level. The leaves turn brown and wither and the fungus grows down into the bulb, in which black sclerotia, about 2 m.m. in diameter, are formed between both the inner and the outer scales.

Dowson (6) states that this disease is common in England on imported Narcissus bulbs.

Drayton (7) reports the occurrence of this disease on imported bulbs.

## Geographical Distribution.

Smoulder is reported as being severe in damp seasons in N. Germany and Holland. It is also found on imported bulbs in England and Canada.

## Economic Importance.

Serious losses are incurred in the bulb growing areas of Holland and N. Germany owing to the ravage of this disease. Details are not available as to the monetary value of the loss sustained.

## SYMPTOMOTOLOGY

### Morphological Symptoms.

Infected growing bulbs sent to the writer have dwarfed yellowed foliage, sclerotia being occasionally found on the bulb scales.

Black raised sclerotia of from 1 m.m. to 3 m.m. in diameter are found on and within the outer scales of infected imported bulbs. (Plate 4). Occasionally the sclerotia coalesce to form a sclerotial incrustation.

A bulb which became infected with the disease in one of the pot experiments conducted by the writer produced short leaves which became yellowed and dried at the tips. Sclerotia were formed at the neck of the bulb, being, as Klebahn found, smaller than those of the inoculum used.

### Histological Symptoms.

Cells of the outer bulb scales of infected bulbs were found to be collapsed. Cells of infected leaves were distorted and shrunken. Their chlorophyll content was greatly reduced as compared to that of cells of healthy narcissus leaves. Mycelium was found to be both intercellular and intracellular in the diseased leaf tissue.

## ETIOLOGY.

### The Organism.

#### Isolation.

Some of the sclerotia mentioned as being found on the scales of infected bulbs were removed, dipped in 95% alcohol, and immersed in 1-1000 mercuric chloride for three minutes. After being removed from the mercuric chloride they were washed in sterile water, each sclerotium cut in

half and the resulting pieces sown on acidified potato dextrose agar in Petrie plates. Mycelial growth soon developed from the portions of sclerotia, and, as soon as the various thalli were large enough, hyphal tips were cut off from the mycelial strands by means of a sterile scalpel, each tip being transferred to a separate tube of potato dextrose agar. Pure cultures of the fungus were thus obtained.

Cultural Studies.

For some considerable time it was found impossible to induce sporulation of this fungus. The organism was grown on a medium rich in dextrose, and transferred to one of a very low dextrose content and vice versa, but all to no avail. Finally typical conidia of the fungus Botrytis narcissicola Kleb. were found on a culture of the fungus on potato dextrose agar which had been placed outdoors and left there all winter.

The culture media used and type of growth on each are listed as follows:

POTATO DEXTROSE AGAR. Abundant growth of dense, white, compact mycelium was produced. Sclerotia begin to form as greenish coloured masses at the side of the test tube in one week. Older cultures produce many sclerotia, both on the surface of the agar and as sclerotial incrustations on the sides of the tubes. (Plates 16, 19, 20). Spores are occasionally produced on old or frozen cultures. Conidiophores generally arise directly from sclerotia.

MELILOTUS STEMS. Mycelial growth was sparse. Sclerotia were produced early. Some spore production was observed on old cultures.

PEACH STEMS. Mycelial growth was very light. Sclerotia were produced. No spores were observed.

STERILIZED NARCISSUS LEAVES. There was good growth of white, fairly dense mycelium on this medium. Sclerotia began to form in the leaf tissue in 4 - 5 days, (Plate 9).

OATMEAL AGAR. Mycelial growth was sparse. Large numbers of sclerotia were produced on this medium.

Name, History, and Classification of the Organism.

Botrytis narcissicola Kleb. causes a disease of narcissi known as "smoulder". Growth, on the media used, was white and generally compact and dense. Sclerotia are found on the scales of infected bulbs, and are produced freely in culture. They are black in colour, raised, and from 1 m.m. to 3 m.m. in diameter. Great variation was found in the width of the mycelial strands. Hyphal strands of young cultures were from 7u to 8u in diameter, and were hyaline in colour. Conidiophores often attain a length of about 1 m.m. and in the lower parts are as much as 17u to 18u in diameter. They are light brown in colour. The branches are approximately 10u in diameter, and are a little thicker than the young hyphal strands. Spores are borne at the tips of these branches on peculiar, short, knob-like attachments. The spores are oval in shape and are more or less acute at the point of attachment. (Plate 26).

The average spore size is 10u to 12u long, by 6u to 7u thick. The spores are smooth and very light brown in colour.

## Pathogenicity.

## Inoculation Experiments.

### Growing Bulbs on Artificially Inoculated Soil.

As in the corresponding basal rot experiment, six varieties of bulbs were used. The proceedure was identical. Four bulbs of each variety were planted, two being placed in each pot.

Varieties potted:

1. Golden Spur
2. Mme. de Graaf
3. Bicolor Victoria
4. Emperor
5. Spring Glory
6. Poeticus ornatus.

Sclerotia of the fungus were sown in the soil and on the surface. The bulbs all grew and flowered, and as far as could be observed no infection was produced.

At a later date four bulbs of the variety von Sion were procured and potted individually. Sclerotia were sown around the noses of the bulbs and they were then placed in a cool moist place. All the bulbs grew, three of them producing normal foliage, The fourth, however, produced sickly foliage which first turned yellow at the tips, and then became quite brown. Growth of the leaves did not exceed six inches. No flowers were produced. On taking out the infected bulb sclerotia were observed on the neck and

outer bulb scales. (Plate 32 A). The bulb was cut in two and examined carefully for eelworms and Fusarium spores, but none were found. Some of the sclerotia from the diseased bulb were treated, sown on potato dextrose agar, and yielded Botrytis narcissicola Kleb. in pure culture.

Artificial Inoculation of Leaves.

Attempts were made to secure infection on the leaves of healthy plants, but unfortunately spores of the fungus were not available at the time and so mycelium from tube cultures had to be used. Experiments conducted with wounded and unwounded leaves were not productive of any satisfactory results.

Late in the season leaves from the variety Emperor were secured, and surface sterilized with a solution of calcium hypochlorite. Pieces of these leaves were then placed in Petrie plates and inoculated by placing on them small portions of sterilized narcissus leaf on which the fungus had been cultured. (Plates 8B and 9). Growth of the fungus on the portions of healthy leaves soon ensued. Sclerotia began to form in less than a week, and spores began to be produced at the same time. Conidiophores were seen emerging from the stomatal openings, and also arising directly from the sclerotia produced (Plate 8B).

## Physiological Reactions.

### Temperature relations.

Growth studies were made with the fungus on poured plates of potato dextrose agar, and in flasks of Richard's solution. Both experiments were run in duplicate, the former for 5 days, the latter for 10 days. The results of

the experiments will be found in Tables 1 and 2.

TABLE 1. Linear growth of B. narcissicola on potato dextrose agar plates, at different temperatures.

| Fungus | Temperature | Ave. diam. of fungal thalli (final) |
|---|---|---|
| B. narcissicola | 5°C. | 3.3 cm. |
| | 10° | 6.0 |
| | 15° | 7.0 |
| | 20° | 8.0 |
| | 25° | 8.5 |
| | 30° | 6.5 |
| | 35° | 4.0 |
| | 40° | None |

TABLE 2. Growth of B. narcissicola in Richard's solution, at different temperatures.

| Fungus | Temperature | Ave. dry wt. of Mycelial mat. |
|---|---|---|
| B. narcissicola | 5°C. | 0.025 grams |
| | 10° | 0.086 |
| | 15° | 0.091 |
| | 20° | 0.095 |
| | 25° | 0.057 |
| | 30° | 0.053 |
| | 35° | 0.025 |
| | 40° | 0.013 |

The results of the two experiments indicate that the optimum temperature for growth is between 20°C. and 25°C. Very little growth takes place at 5°C. and it is practically inhibited at 40°C.

Experimental Methods.

This experiment was conducted in a long, specially constructed box, having at the one end a water tank equipped with a heating filament, and, at the opposite end an ice

box. A good range of steady temperature was obtained.

## CONTROL

Smoulder is unknown in Canada as a field disease, as far as the writer is aware. However, specimens of bulbs infected with smoulder have occasionally been received from greenhouse men. Botrytis narcissicola Kleb. is capable of over-wintering in this country, and there is no reason why it should not become established, if conditions are favourable.

Imported and home grown bulbs bearing the sclerotia on the scales should be destroyed. Bulbs producing sickly foliage are always suspect, and together with the soil around them, should be removed and burned.

## TYPE B. A CONDITION OF NARCISSI WITH WHICH IS ASSOCIATED A SCLEROTIUM PRODUCING FUNGUS.

### INTRODUCTION.

Small sclerotia have often been found on the outer scales of both healthy and diseased narcissus bulbs. Examination of the diseased bulbs generally revealed that they were infected with eelworms. The sclerotia were uniform in size and smaller than those of Botrytis narcissicola Kleb. The sclerotia were cultured and yielded similar sclerotia in culture, showing that they were not undeveloped sclerotia of B. narcissicola. Experiments were conducted to determine if possible, the role played by this fungus, and something of its behaviour at different tempeatures and on different culture media.

### HOSTS

#### Plants Affected and Varietal Susceptibility.

Sclerotia of this fungus have been found on the outer scales of narcissi, and as far as is known, do not occur on plants other than those found in the genus Narcissus. These sclerotia occur on bulbs of varieties of the Trumpet narcissus, Incomparabilis, Poetaz and Poeticus groups.

### THE DISEASE

#### Names

The condition with which this fungus is associated has never been described and no name has been given to it. Type B, the name given here is merely tentative.

## Review of Literature.

Only one reference, that of Dowson (5) was found, in which he described a fungus, Botrytis parasitica, as causing a disease of narcissi. The size of the sclerotia produced, however, 1/16 - 1/8 of an inch did not agree with the size of sclerotia of the fungus studied.

### Geographical Distribution and Economic Importance.

The fungus is often found on imported bulbs and also on home grown stock.

It does not appear to have any economical importance.

## SYMPTOMOTOLOGY

### Morphological Symptoms.

Small black partly-raised sclerotia were found partly embedded in the outer suberized scales of infected bulbs, (Plate 5). The writer has never found them in decaying tissue.

## ETIOLOGY

### The Organism.

Cultural Studies.

In the three years or so that the writer has studied this organism, no type of fructification has ever been observed to be produced. High and low temperatures, rich and poor media have been used without avail in an effort to induce sporulation. Clamp connections have never been observed. Some of the culture media used and the type of growth on each are listed below.

POTATO DEXTROSE AGAR. Profuse growth of white, aerial mycelium, with production of numerous small black sclerotia was observed on the surface of the agar,

and sides of the tube, after 8 days. (Plates 17, 19, 20).

MELILOTUS STEMS. Mycelial growth was quite profuse, aerial and white in colour. Large numbers of sclerotia were formed in the stem tissue.

PEACH STEMS. Mycelial growth was light. Sclerotia were produced, but not in abundance.

STERILIZED NARCISSUS LEAVES. Very good growth of dense, white aerial mycelium was produced on this medium. Sclerotia were formed in the leaf tissue in 3 - 4 days. (Plate 10).

OATMEAL AGAR. Very little mycelial growth and heavy production of sclerotia occurred.

Name, History and Classification.

The fungus associated with the condition of narcissus bulbs called Type B, has never been observed to produce spores in culture. The average diameter of the hyphae is 3.5u. The hyphal strands are hyaline. In the mass the mycelium is white coloured. The sclerotia are round, slightly raised, and black, averaging 1.5 m.m. in diameter. In cross section it is seen that the sclerotia are typical in that the plectenchyma appears tuberiform while the core is prosenchymatic in nature. (Plate 28).

## Pathogenicity.

### Inoculation Experiments.

Growing Bulbs on Artificially Inoculated Soil.

Exactly the same procedure was followed as in the case of the "basal rot" and "smoulder" experiments; four bulbs of each of six varieties being used. Resulting growth of the bulbs was healthy and none were found to be diseased.

Bulbs were wounded and pieces of inoculum appressed

to the injured parts without infection resulting.

Artificial Inoculation of Leaves.

Sections of living leaves treated as mentioned on page 34 were inoculated with small portions of sterilized narcissus leaf on which the fungus had been cultured. A fairly profuse mycelial growth, both on and in the living leaf tissue resulted. No spores were produced, however.

## Physiological Reactions.

Temperature relations.

Growth studies were made with the fungus on potato dextrose agar and in Richard's solution. The results of the experiments are tabulated below.

TABLE 1. Effect of temperature on growth of Type B. on potato dextrose agar plates. Experiment run in duplicate for 5 days.

| Fungus | Temperature | Ave. diam. of fungal thalli. |
|---|---|---|
| Type B. | 5°C | 4.0 cm. |
| | 10° | 5.0 |
| | 15° | 8.0 |
| | 20° | 8.0 |
| | 25° | 8.5 |
| | 30° | 8.8 |
| | 35° | 7.3 |
| | 40° | 6.2 |

TABLE 2. Effect of temperature on growth of Type B. in Richards modified solution. The experiment was conducted for 10 days.

| Fungus | Temperature | Ave. dry wt. of Mycelial mat. |
|---|---|---|
| Type B. | 5° C | 0.055 grams |
| | 10° | 0.070 |
| | 15° | 0.110 |
| | 20° | 0.115 |
| | 25° | 0.120 |
| | 30° | 0.120 |
| | 35° | 0.097 |
| | 40° | 0.085 |

The results of the two experiments indicate that the optimum temperature for growth is between 25° C. and 30° C. This experiment was conducted at the same time as that with B. narcissicola. It will be seen from the tables that the Type B. organism produced more mycelial growth than B. narcissicola at the higher and lower temperatures.

## DISCUSSION

It is believed that this fungus is a weak parasite which is almost invariably found attacking non-vigorous bulbs. The area of infection seems to be limited to the outer suberized scales. Although the writer believes that this fungus should take specific rank in the genus Sclerotium he has hesitated to take this step. Further efforts will be made to produce a spore stage, if such exists, before it is decided to give this fungus a name.

Bulbs with the fungus sclerotia on the outer scales should be viewed with suspicion. The probability is that they are not vigorous and will not produce good bloom.

## TYPE C, WITH WHICH IS ASSOCIATED A FUNGUS PRODUCING MICROSCLEROTIA ON THE OUTER SCALES OF NARCISSUS BULBS.

### HOSTS

This fungus was found to occur occasionally on outer scales of narcissus bulbs, often in association with Type B.

### THE DISEASE

#### Review of Literature.

Alcock (1) describes a fungus producing similar sclerotia on narcissus bulbs in England. He found the fungus to be a weak parasite. Drayton (7) found sclerotia of the fungus on scales of imported bulbs.

### ETIOLOGY

#### The Organism.

The fungus associated with Type C. has never been named. Cultural studies were undertaken with this organism on different media. White coloured aerial mycelium was produced in abundance. Large numbers of microsclerotia were produced in culture. These were from 88.5 u to 103.5 u in diameter, raised, round, and black.

In nature these sclerotia were only found on the outer papery bulb scales. Spores have never been produced by the writer.

#### Pathogenicity.

##### Inoculation Experiments.

Growing Bulbs on Artificially Inoculated Soil.

Exactly the same procedure was followed as in the case of the "basal rot", "smoulder" and "Type B" experiments; four bulbs of each of six varieties being used. Resulting growth of the bulbs was healthy and none were found to be diseased.

Artificial Inoculation of Bulbs and Leaves.

Bulbs were wounded and pieces of inoculum appressed to the injured parts without infection resulting.

Sections of living leaves treated as mentioned on page 34 were inoculated with small portions of sterilized narcissus leaf on which the fungus had been cultured. A profuse mycelial growth, both on and in the living leaf tissue resulted. No spores were produced, however.

## Physiological Reactions.

Temperature relations.

The fungus was grown on both hard potato dextrose agar plates and in Richards solution. Results of these experiments follow:-

TABLE 1. Growth of the Type C. fungus at different temperatures on hard potato dextrose agar plates. The experiment was in duplicate.

| Fungus | Temperature | Ave. diam. of fungal thalli after 5 days. |
|---|---|---|
| Type C | 5°C. | 2.2 cms. |
| | 10° | 3.0 |
| | 15° | 4.0 |
| | 20° | 7.5 |
| | 25° | 8.0 |
| | 30° | 7.5 |
| | 35° | 1.8 |
| | 40° | |

TABLE 2. Growth of Type C. in Richards modified solution. The experiment was in duplicate.

| Fungus | Temperature | Ave. diam. of fungal thalli after 10 days |
|---|---|---|
| Type C | 5°C. | 0.028 cms. |
| | 10° | 0.054 |
| | 15° | 0.158 |
| | 20° | 0.180 |
| | 25° | 0.210 |
| | 30° | 0.233 |
| | 35° | 0.150 |
| | 40° | 0.093 |

Optimum growth of the fungus is at 30°C. Experiment conducted at same time as that with B. narcissicola and the Type B. organism.

## DISCUSSION

The fungus is similar to that described by Alcock (1) and Drayton (7). The writer has been unable to cause disease in narcissus bulbs in inoculation experiments with the fungus, but secured good growth on inoculated portions of living leaves in petrie plates. This fungus does not appear to produce spores, and probably should, with the Type B. organism, be included in the genus Sclerotium. The fungus is a high temperature organism, and probably is a weak parasite occuring on heated bulbs and bulbs of low vigour.

## GENERAL DISCUSSION

Basal rot is a serious disease of narcissi in Holland, Germany, France, England, and the United States of America.

The prevalence of basal rot in imported shipments of narcissus bulbs induced the writer to investigate this disease. Fusarium oxysporum Schl. form 5 Wr. Syn. Fusarium oxysporum Schl. var. nicotianae Johnson was isolated from basal rot of narcissus bulbs. Inoculation and storage experiments proved that F. oxysporum Schl. form 5 Wr. caused a basal rot of the bulbs, and that, under favourable conditions, decay of infected bulbs was very rapid.

The fungus appears to grow best at high temperatures, the optimum temperature for growth being approximately 28°C. This probably is the reason why basal rot develops so rapidly in storage and transit, where low temperatures do not prevail.

Spores of the fungus have been found by the writer to be extremely resistant to drying and also to extremes of temperature. Tube cultures of this Fusarium were set outdoors for a whole winter, and, when examined in the spring were found to be still viable. This being the case, there would appear to be nothing to prevent the spread of narcissus basal rot in Canada if bulbs infected with this disease are planted. Most of the bulbs planted every year are imported, and this gives the growers an opportunity to pick out the diseased bulbs and to destroy them. Sorting of bulbs, rogueing of growing plants in the field, and careful storing of the bulbs are precautionary measures which should be adopted by all narcissus growers.

Smoulder, caused by Botrytis narcissicola Kleb., is a serious disease of narcissi in Europe. Although it is known to occur in

Canada, smoulder has not, as yet, developed into a serious disease. It is easier to detect the disease on the bulbs than it is to detect basal rot. The sclerotia of B. narcissicola are large and raised and are usually found on the neck and outer scales of infected bulbs.

Type B. and Type C. are not believed to be serious diseases.

The sclerotia of the fungi associated with these diseases are usually found on the outer scales of sickly bulbs. Type B. and Type C. do not appear to have any economic importance. The two species of fungi concerned are unnamed and have never produced spores in culture. The writer is of the opinion that much more must be known of the complete life history of each of the two fungi concerned before one can safely proceed to establish genuine species on a truly scientific basis. For this reason the writer has not created names for the Type B. and Type C. organisms.

## SUMMARY


I. Basal rot is a widespread and serious disease of narcissus bulbs. The Narcissus pseudonarcissus group is especially susceptible to this disease.

2. A species of Fusarium has repeatedly been isolated from bulbs infected with basal rot.

3. This fungus has been identified as Fusarium oxysporum Schlect. form 5 Wr. Syn. Fusarium oxysporum Schlect. var. nicotianae Johnson.

4. Good growth of characteristic white to pinkish mycelium is produced on most media employed. Bluish-green sclerotia are common. Reduced pionnotes or "Pseudopionnotes" have been observed in culture. Sporodochia containing 3 to 5 septate conidia are produced. Chlamydospores are terminal, intercalary and conidial. One-celled microspores are produced in abundance.

5. Optimum growth of the fungus was obtained between pH 4.6 and pH 7.

6. Best growth of bulbs was obtained at pH 5 to pH 6.

7. Mycelium was found to grow best at a temperature of approximately 28°C.

8. Spores were not killed at 5°C., but were killed when kept at a temperature of 37°C. for ten days.

9. Spores germinate best at 20°C. to 25°C., and are stimulated by additions of cell sap pressed from the inner fleshy scales of the bulb.

10. Storage and inoculation experiments show that although uninjured bulbs may become infected with basal rot, injured bulbs are much more susceptible.

11. Storage experiments demonstrate the need of care in shipping and storing bulbs. Diseased bulbs are a means of spreading infection to healthy bulbs. Injured bulbs were much more liable to infection from this source than uninjured bulbs. Basal rot was firmly established in two weeks in bulbs which were healthy at the start of the experiment.

12. Control measures are advocated.

13. Smoulder is a serious disease of narcissi in Holland and northern Germany. It has not, as yet, any economic significance in Canada.

14. Botrytis narcissicola Kleb., the causal organism, grows well on most culture media, producing typical oval shaped spores. The sclerotia are capable of overwintering in Canada in a living condition. Optimum growth of the fungus is between 20°C. and 25°C.

15. Inoculation experiments show that the fungus is capable of attacking bulbs, causing yellowing, decay and death of the leaves.

16. Control measures are advocated.

17. The Type B. organism is an unnamed, sclerotium-producing fungus occasionally found on the outer scales of narcissus bulbs.

18. Its economic importance is not known. It is found on both imported and home grown bulbs.

19. The temperature best suited to its growth is 25°C. to 30°C.

20. The mycelium grows profusely on most media, many small black sclerotia being produced. No spore stage has ever been observed.

21. Negative rsults were obtained in bulb inoculation experiments. Good growth was observed on petrie plate cultures of portions of living narcissus leaves.

22. The fungus is believed to play a weakly parasitic role.

23. The Type C. organism is an unnamed, sclerotium-producing fungus occasionally found on the outer scales of narcissus bulbs.

24. Its economic importance is not known. It is found on both imported and home grown bulbs.

25. The temperature best suited to its growth is 30°C.

26. Profuse growth of fine, white, aerial mycelium results on most media. Great numbers of microsclerotia are produced. No spore stage has ever been observed.

27. Negative results were obtained in bulb inoculation experiments. Good growth was observed on petrie plate cultures of portions of living narcissus leaves.

28. The fungus is believed to (pay) a weakly parasitic role as far as infection is concerned.

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25. _____ The use of fungicides in connection with the hot water treatment of narcissus bulbs. Florists Exchange 72:3. 1929.

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27. Westerdijk, J.: Phytopath, Laborat. W.C.S. Baarn, Jaarverslag. 1916.

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PLATE 1.

Narcissus bulbs infected with basal rot. Note diseased areas (dark coloured) at base.

PLATE I.

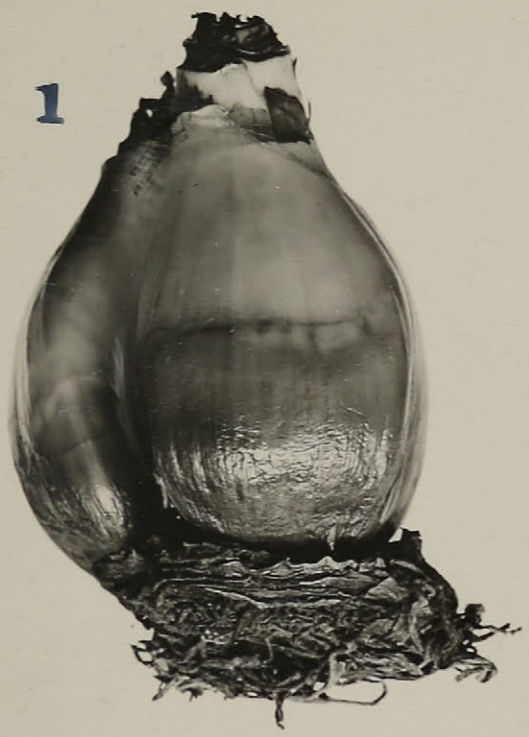
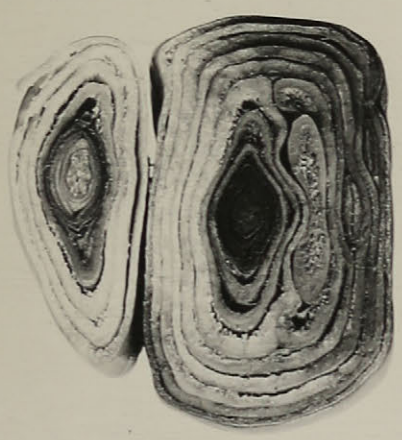





PLATE 11.

Cross section of bulbs shown in Plate No. 1.

PLATE III.



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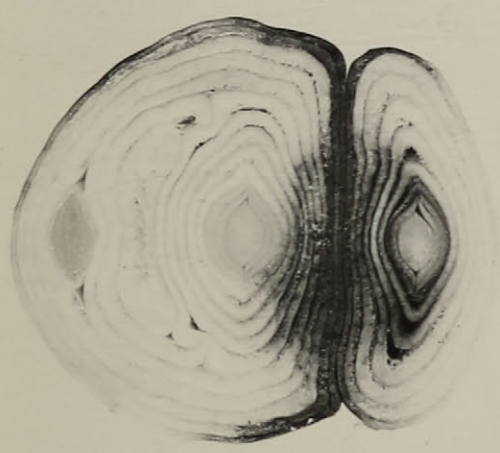

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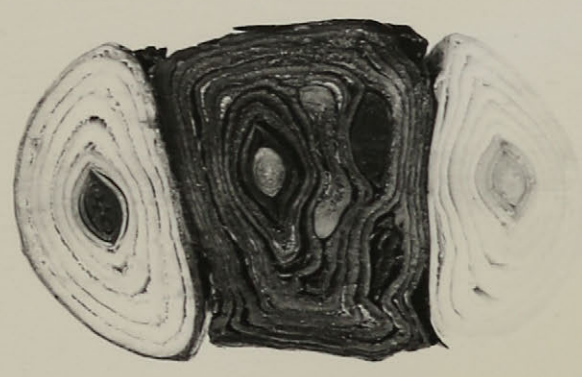


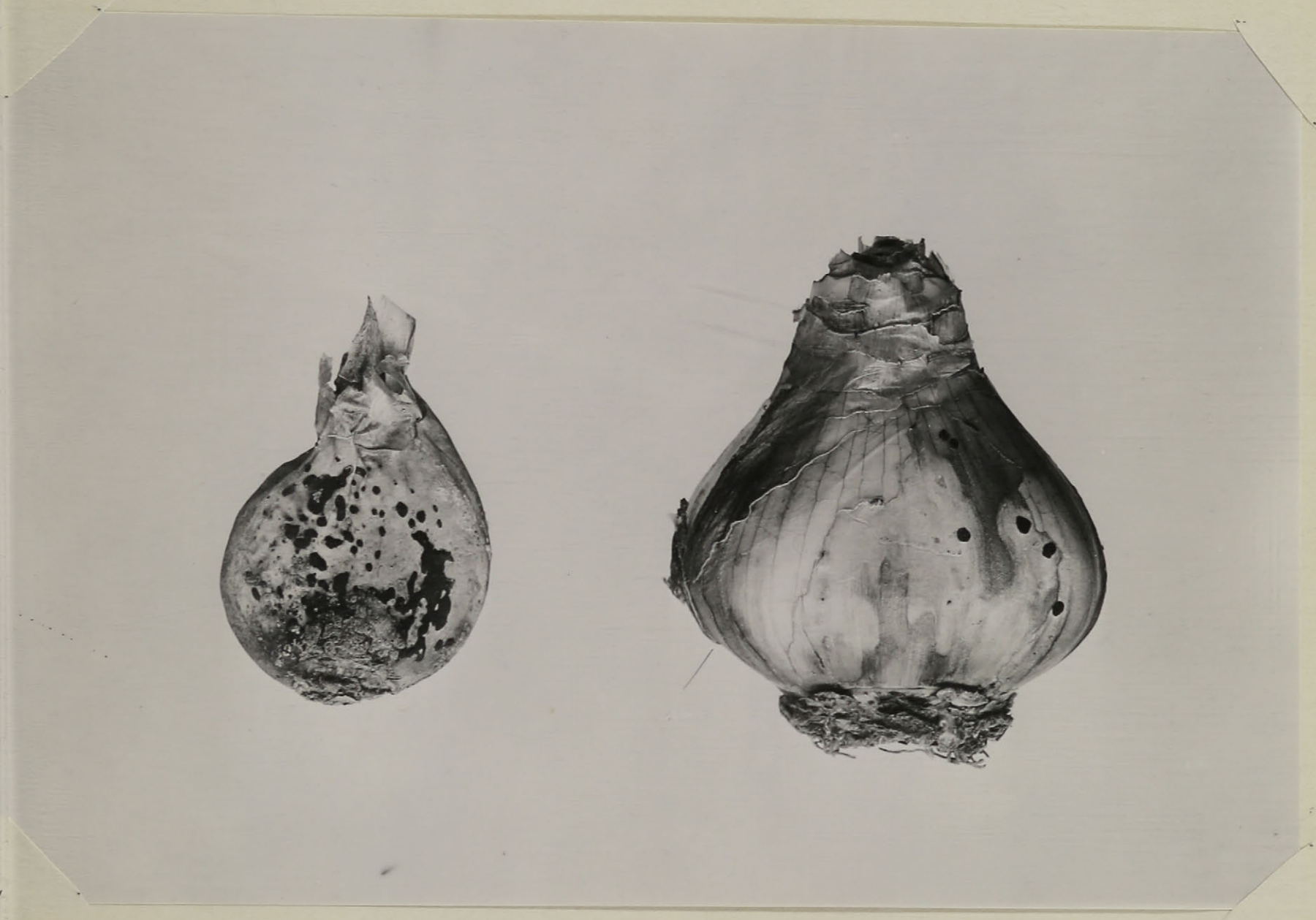
4

PLATE III.

## PLATE IV.

*Botrytis narcissicola* Kleb. Sclerotia shown on outer scales of Narcissus bulbs.

PLATE IV.



## PLATE V.

Type B. Sclerotia on outer scales of bulb.

PLATE V.

# PLATE V1.

Type C. Sclerotia on outer scales of bulb.

PLATE VI.

**PLATE V11.**

Photograph of bulb artificially inoculated with *Fusarium oxysporum* Schlect., *form 5* Wr. Note method of sealing incision with wax, also discoloured tissue around wax, and outgrowth of mycelium.

PLATE VII.

PLATE VIIIA.

Narcissus bulb inoculated with *Botrytis narcissicola* Kleb. Notice stunted, yellowed leaves and production of sclerotia of the fungus on the neck and lower portions of the bulb.

PLATE VIII A.

PLATE VIIIB.

Inoculation of piece of living narcissus leaf in petrie plate with portion of pure culture of Botrytis narcissicola Kleb.

PLATE VIII B.

## PLATE 1X.

*Botrytis narcissicola* Kleb. Pure culture on autoclaved narcissus leaf in test tube.

PLATE IX.

## PLATE X.

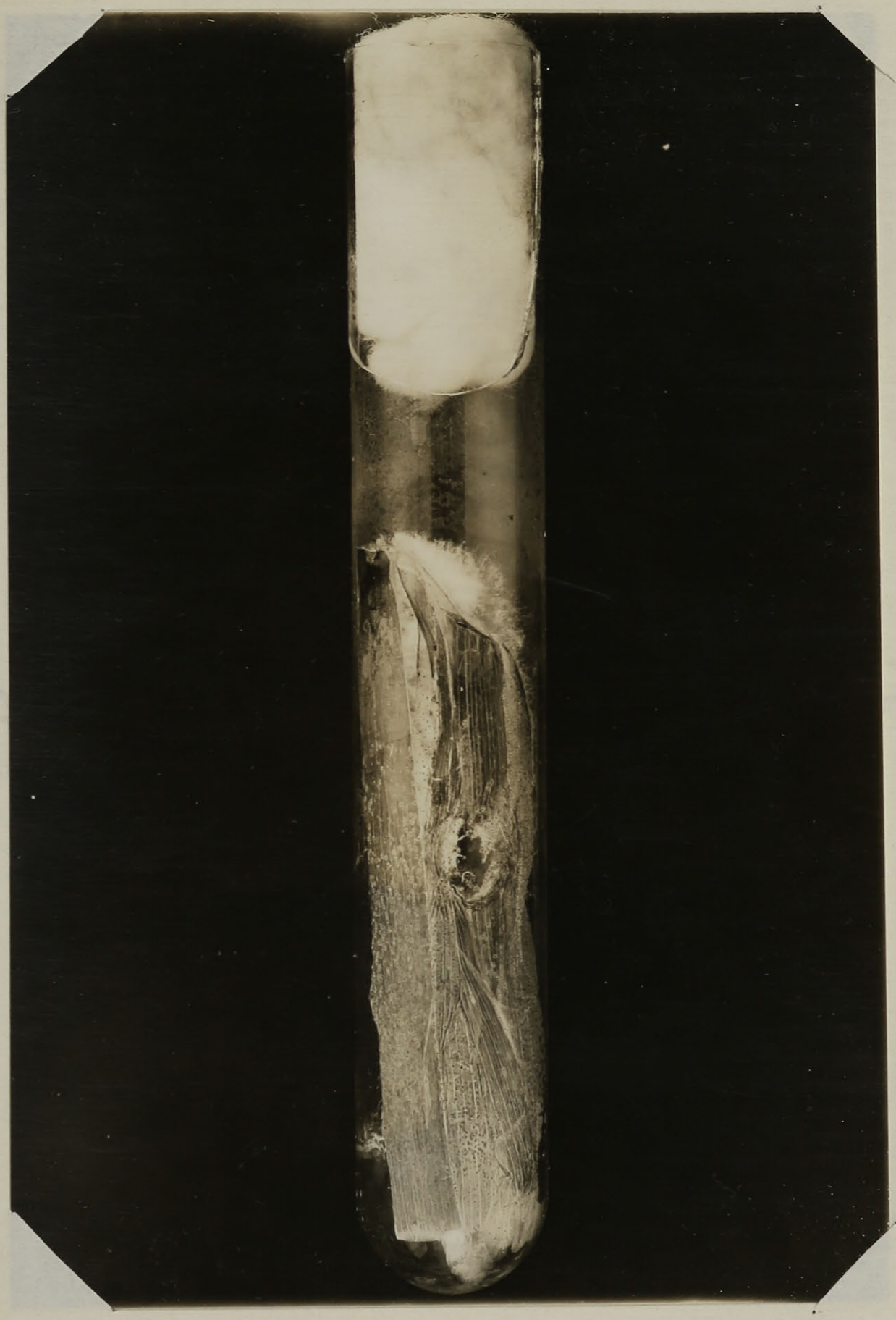
Type B. Pure culture on autoclaved narcissus leaf in test tube.

PLATE X.

# PLATE XI.

Type C. Pure culture on autoclaved narcissus leaf in test tube.

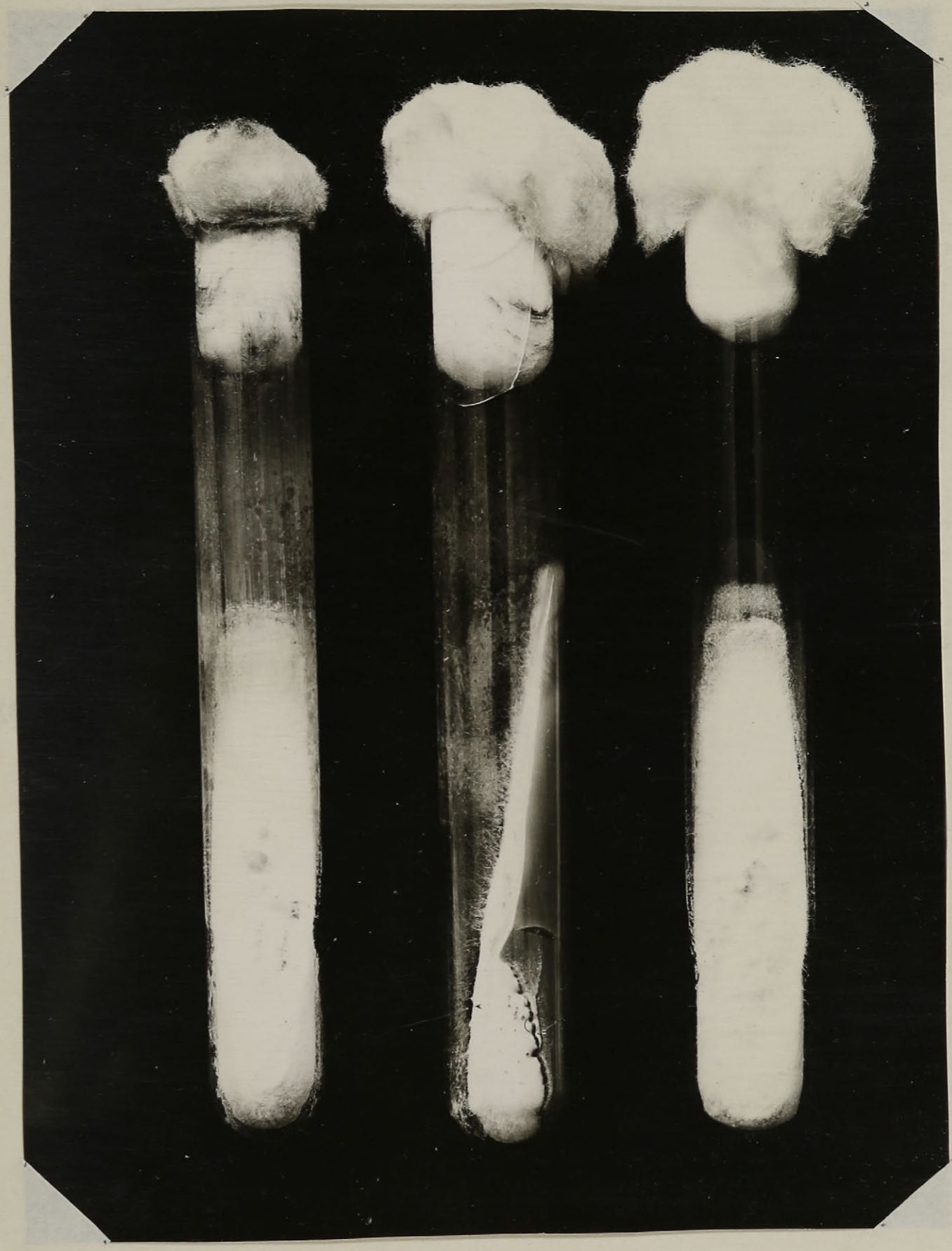
PLATE XI.



## PLATE XII.

*Fusarium*. Three weeks old cultures of basal rot organism on P. D. A.

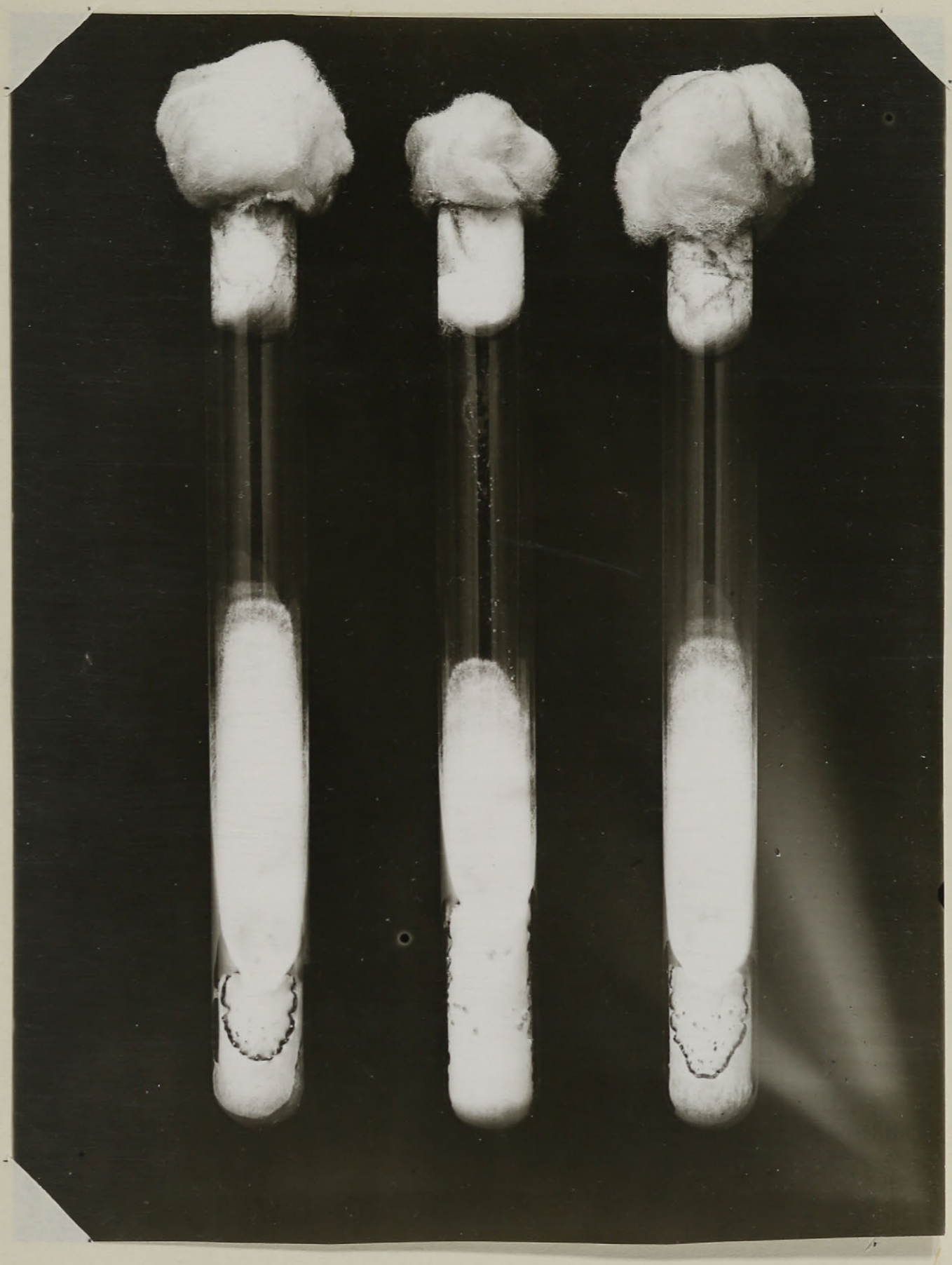
PLATE XII.



## PLATE X111.

*Fusarium*. Five weeks old cultures of basal rot organism on P. D. A.

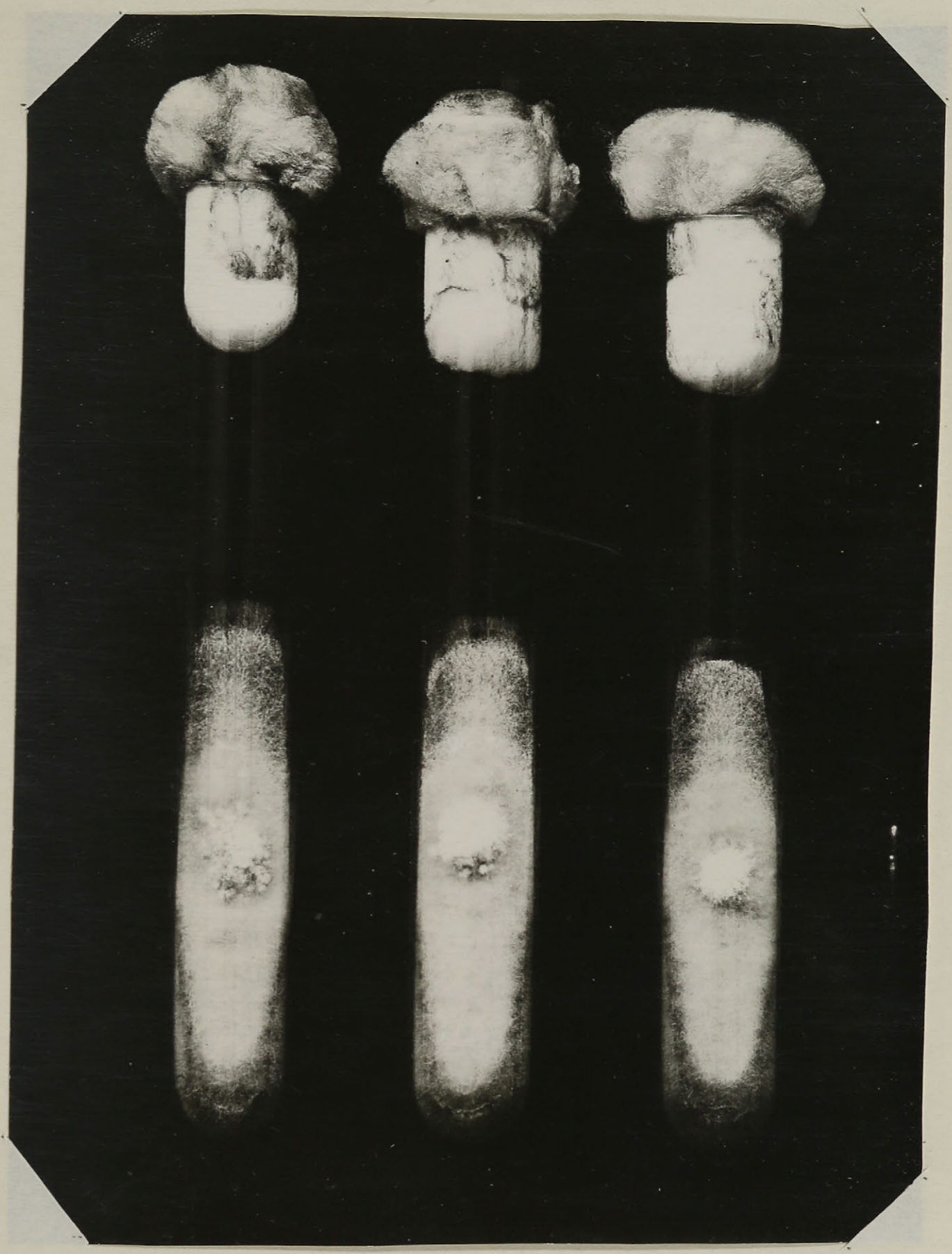
PLATE XIII.



## PLATE XIV.

Fusarium. Three weeks old cultures of basal rot organism on prune agar.

PLATE XIV.



## PLATE XV.

*Fusarium*. Culture of basal rot organism on potato plug.

PLATE XV.

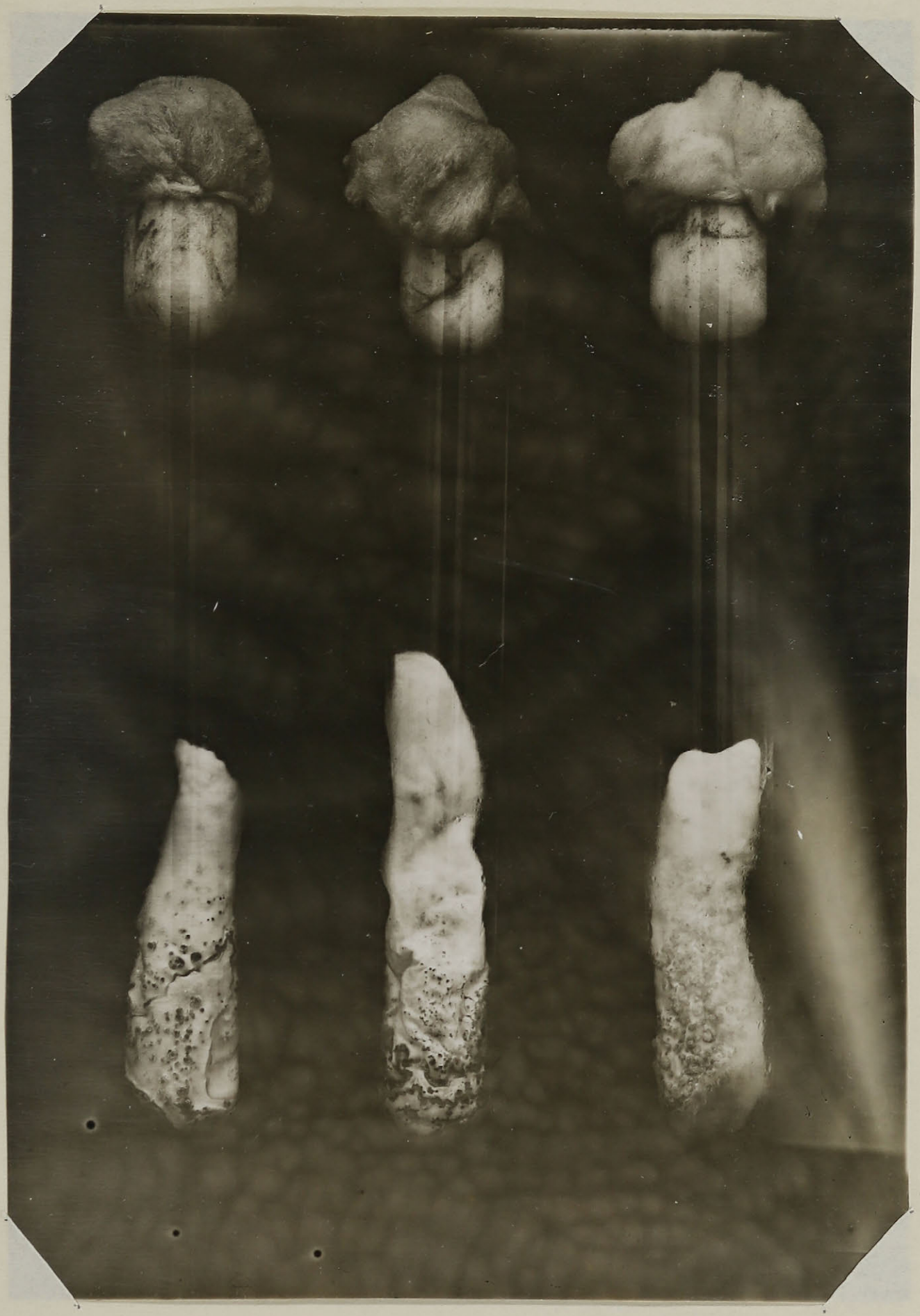


PLATE XV1.

*Botrytis narcissicola* Kleb. Culture on P. D. A.

PLATE XVI.

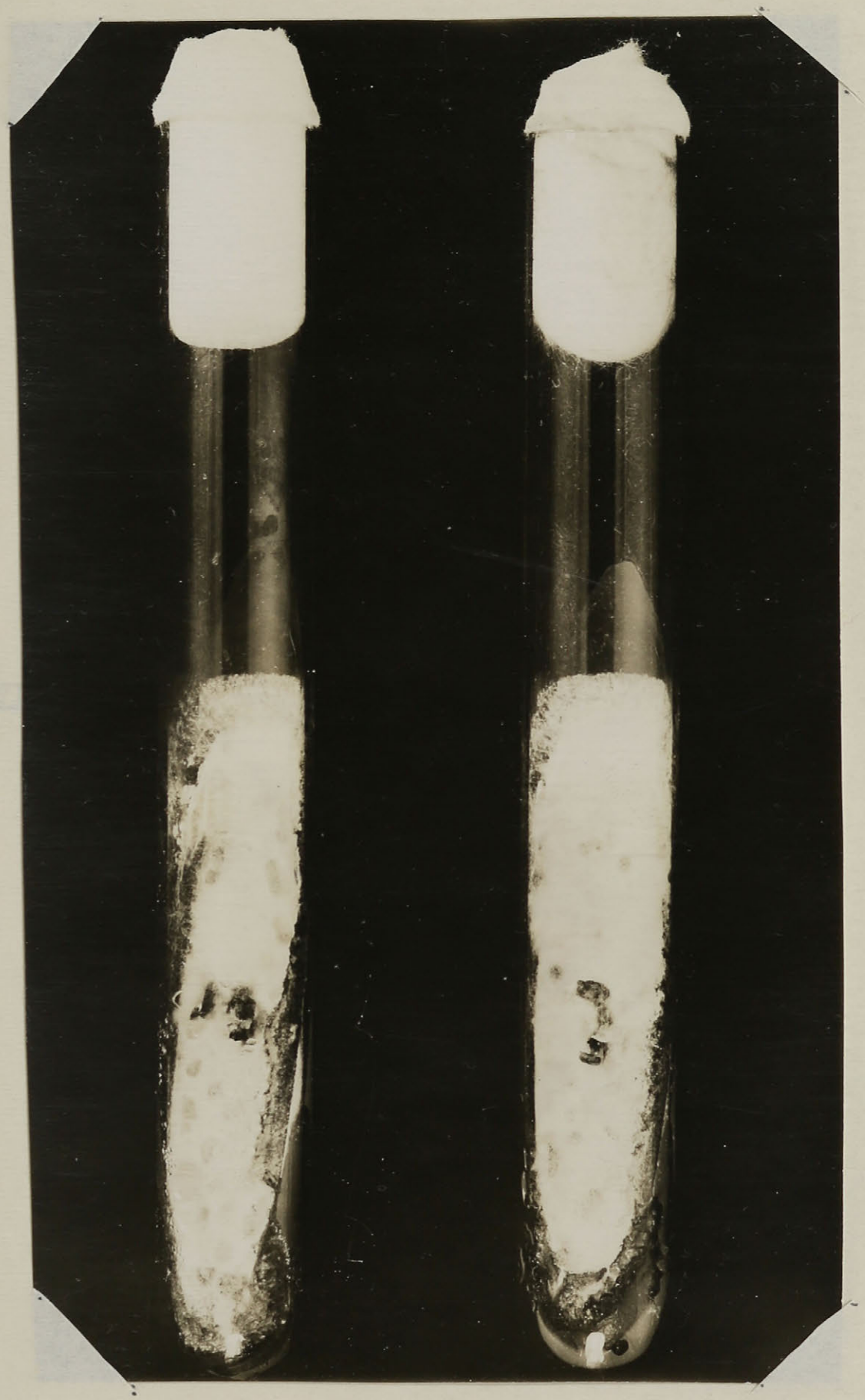


PLATE XVII.

Type B. Culture on P. D. A.

PLATE XVII.

PLATE XVIII.

Type C. Culture on P. D. A.

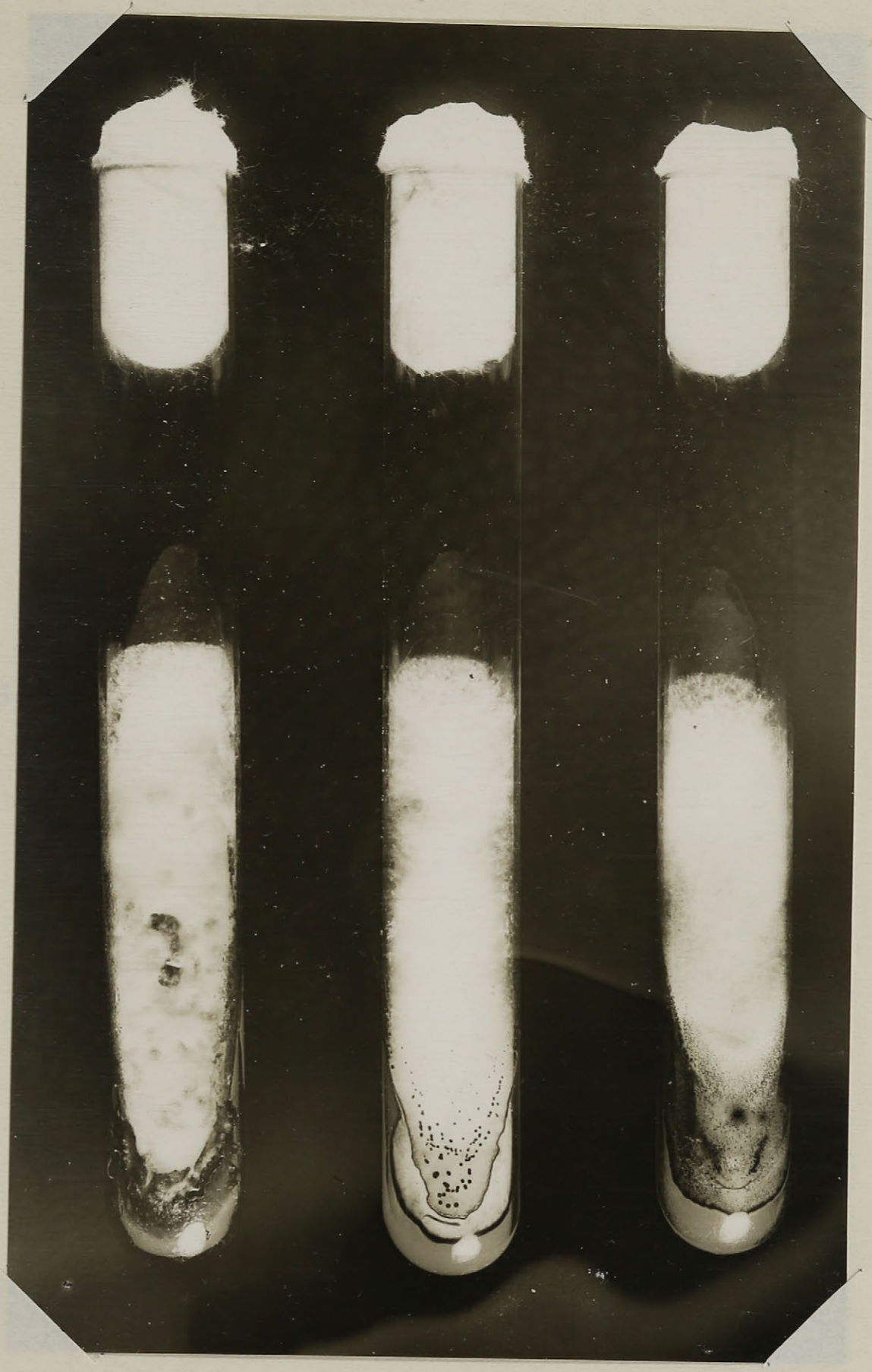
PLATE XVIII.

## PLATE XIX.

*Botrytis narcissicola* Kleb., *Type B* and *Type C*, on P. D. A. Front view.

| | | |
|---|---|---|
| *Botrytis narcissicola* | - | No. 1. |
| Type B. | - | No. 2. |
| Type C. | - | No. 3. |

PLATE XIX.



1 2 3

## PLATE XX.

Same as Plate XIX. Side view.

PLATE XX.

1 2 3

## PLATE 21.

All the photomicrographs were made with a Spencer microscope, using a 10X eyepiece and a 4 m.m. dry objective. The camera was a 5 x 7 Eastman studio camera, equipped with a Bausch and Lamb - Ziess and Tessor f. 6.3 lens.

Photomicrograph showing 3 - septate macrospore of *Fusarium oxysporum* Schlect. *form 5* Wr. syn. *Fusarium oxysporum* Schlect. var. *nicotianae* Johnson.

PLATE XXI.

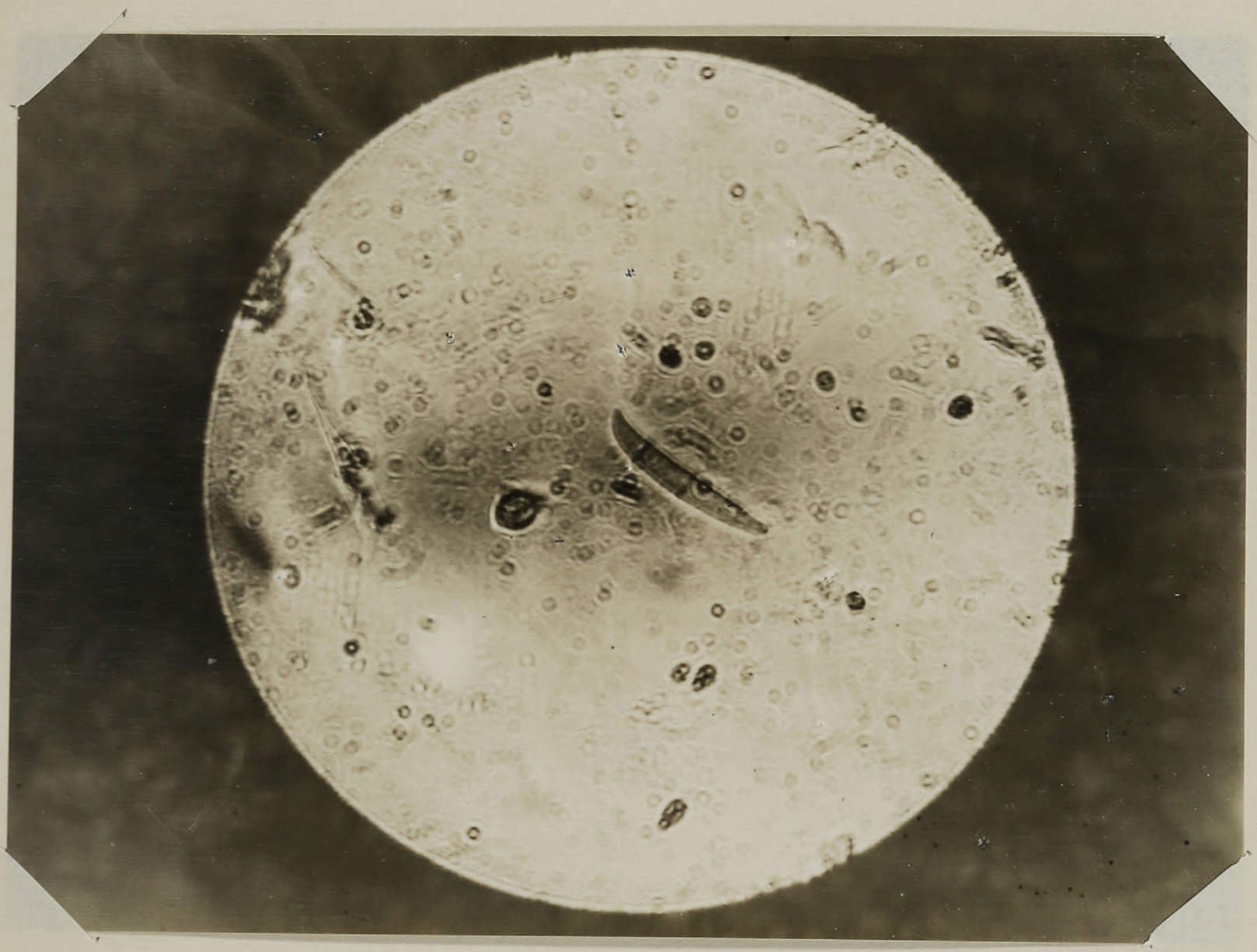


PLATE 22.

Fusarium. Photomicrograph showing macrospores.

PLATE XXII.

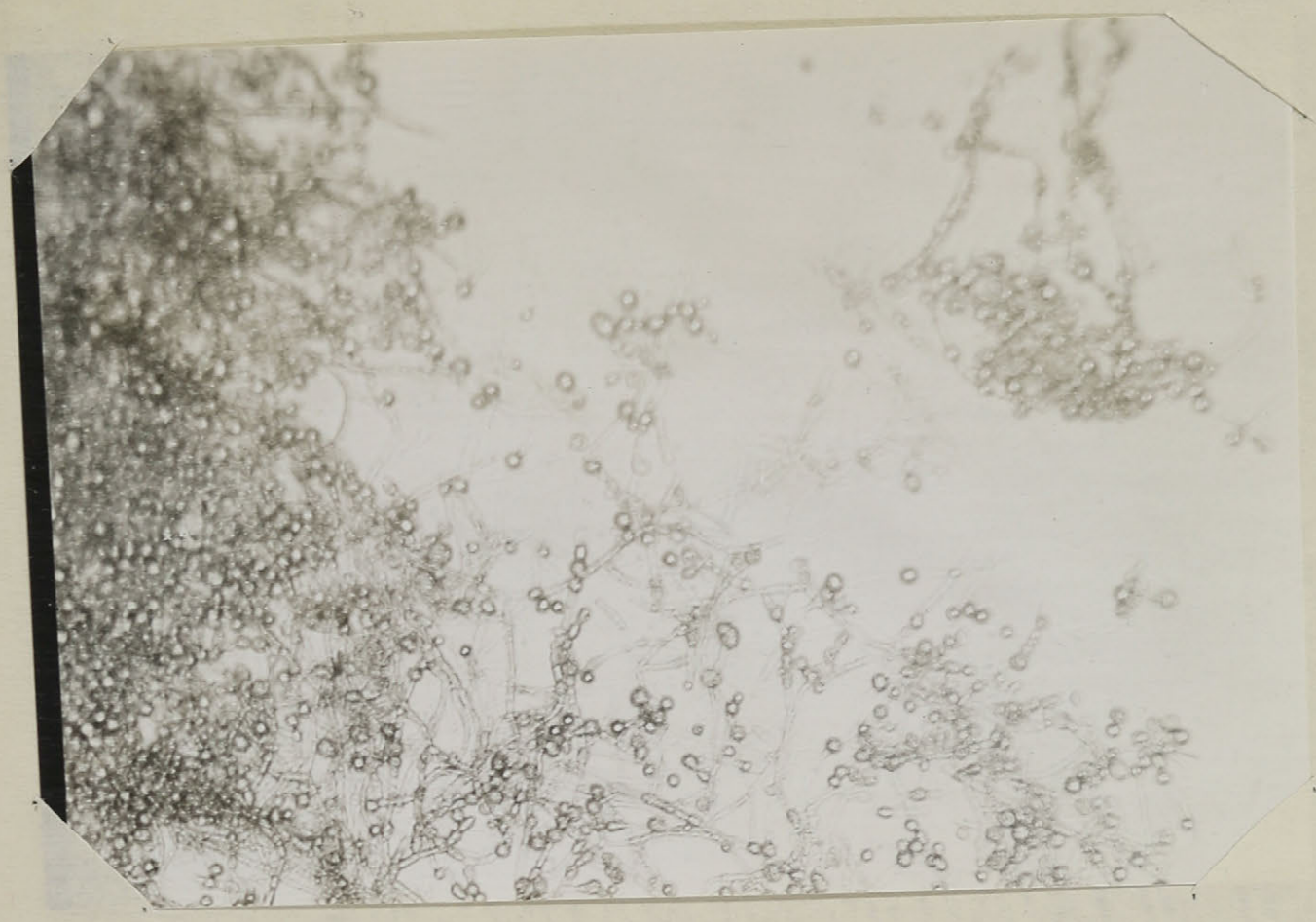
PLATE 23.

**Fusarium.** Photomicrograph showing

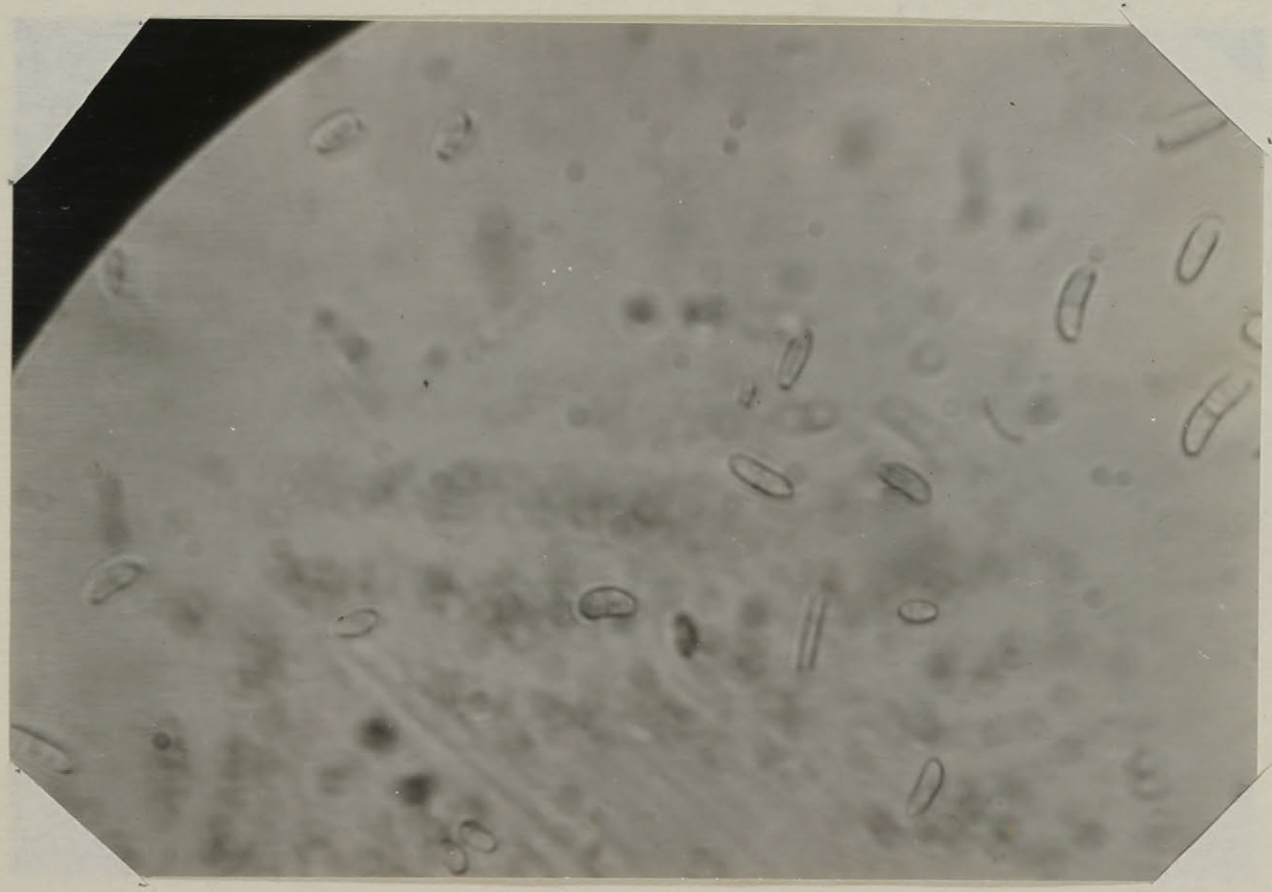
A. Chlamydospores and mycelium.

B. Microspores.

# PLATE XXIII.



A



B

PLATE 24.

*Fusarium*. Mycelium and Chlamydospores.

PLATE XXIV.

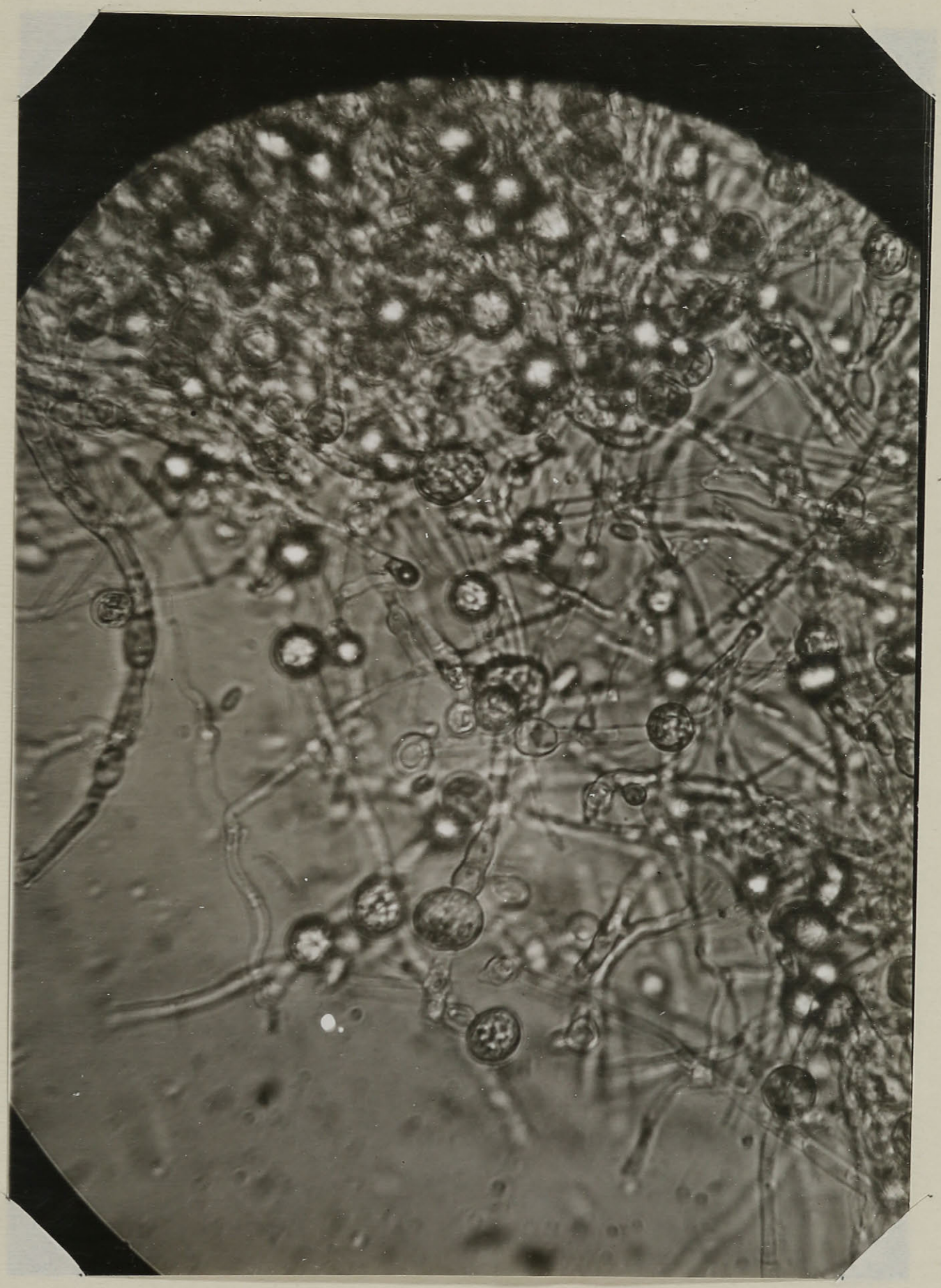


PLATE 25.

*Fusarium*. Mycelium and Chlamydospores.

PLATE XXV.

PLATE 26.

*Botrytis narcissicola* Kleb.

Photomicrograph. Spores and conidiophore.

PLATE XXVI.

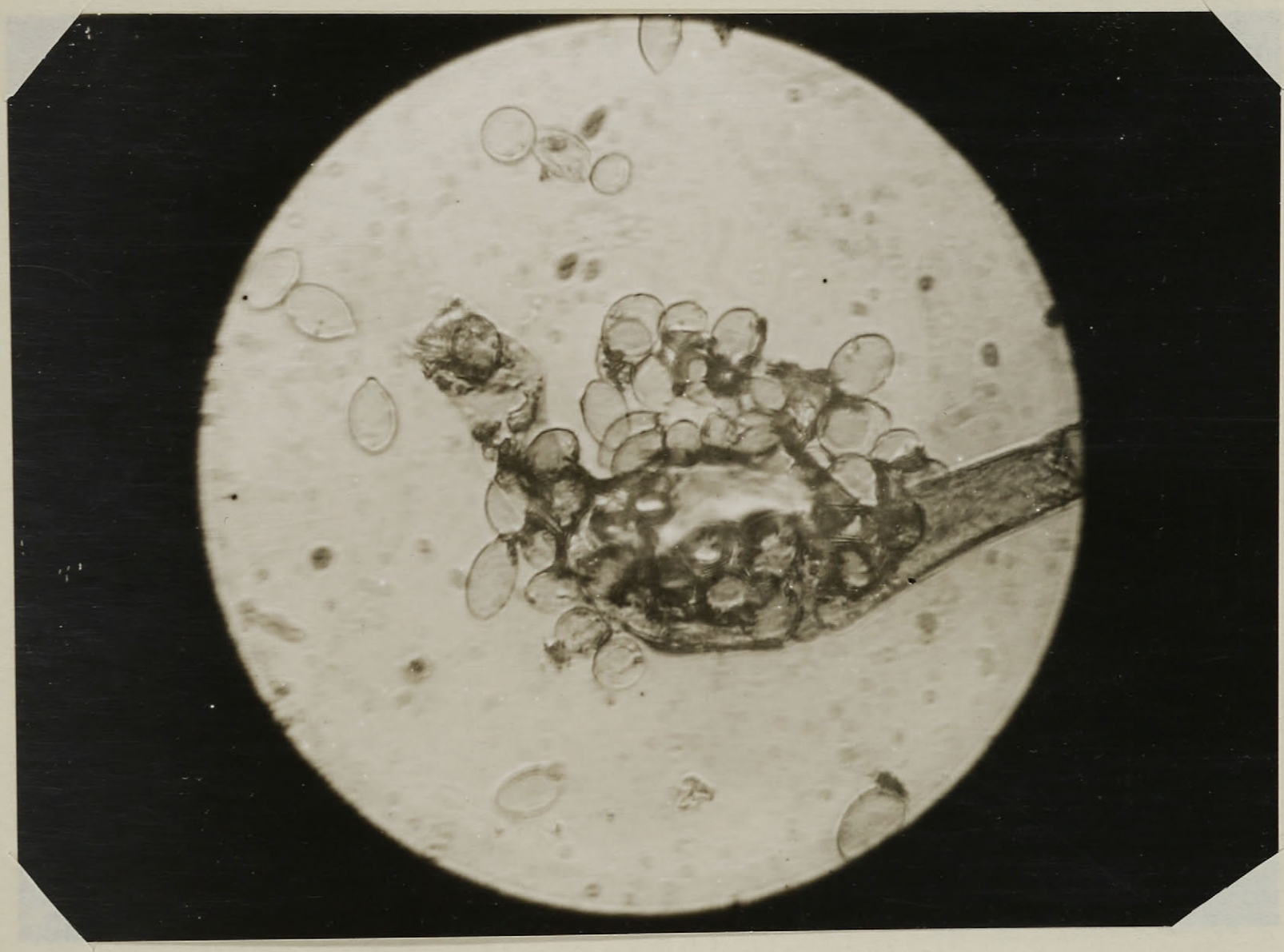


PLATE 27.

*B. narcissicola* Kleb.

Photomicrograph. Cross section of sclerotium.

PLATE XXVII.

PLATE 28.

Type B. Cross section of sclerotium.

PLATE XXVIII.

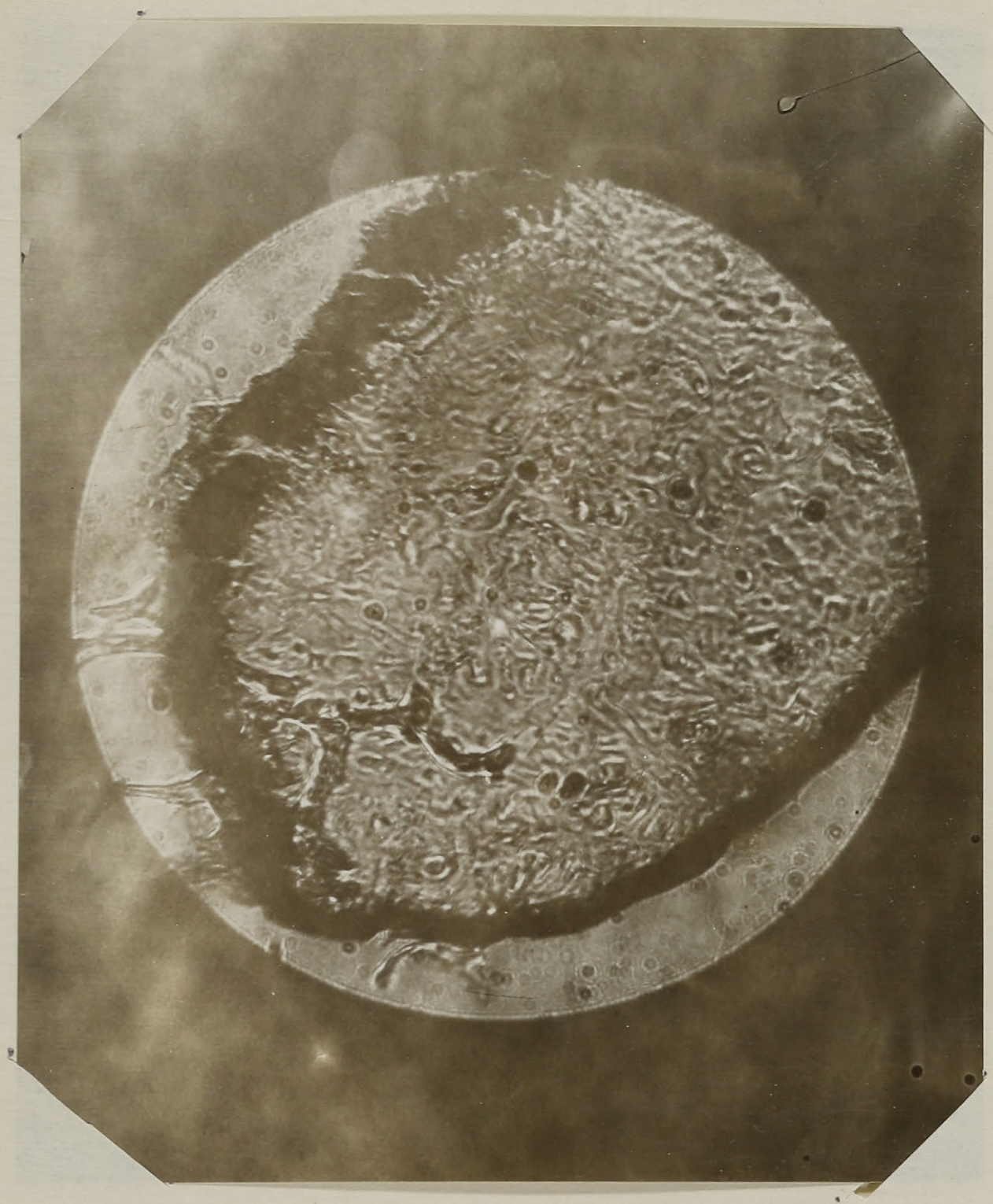
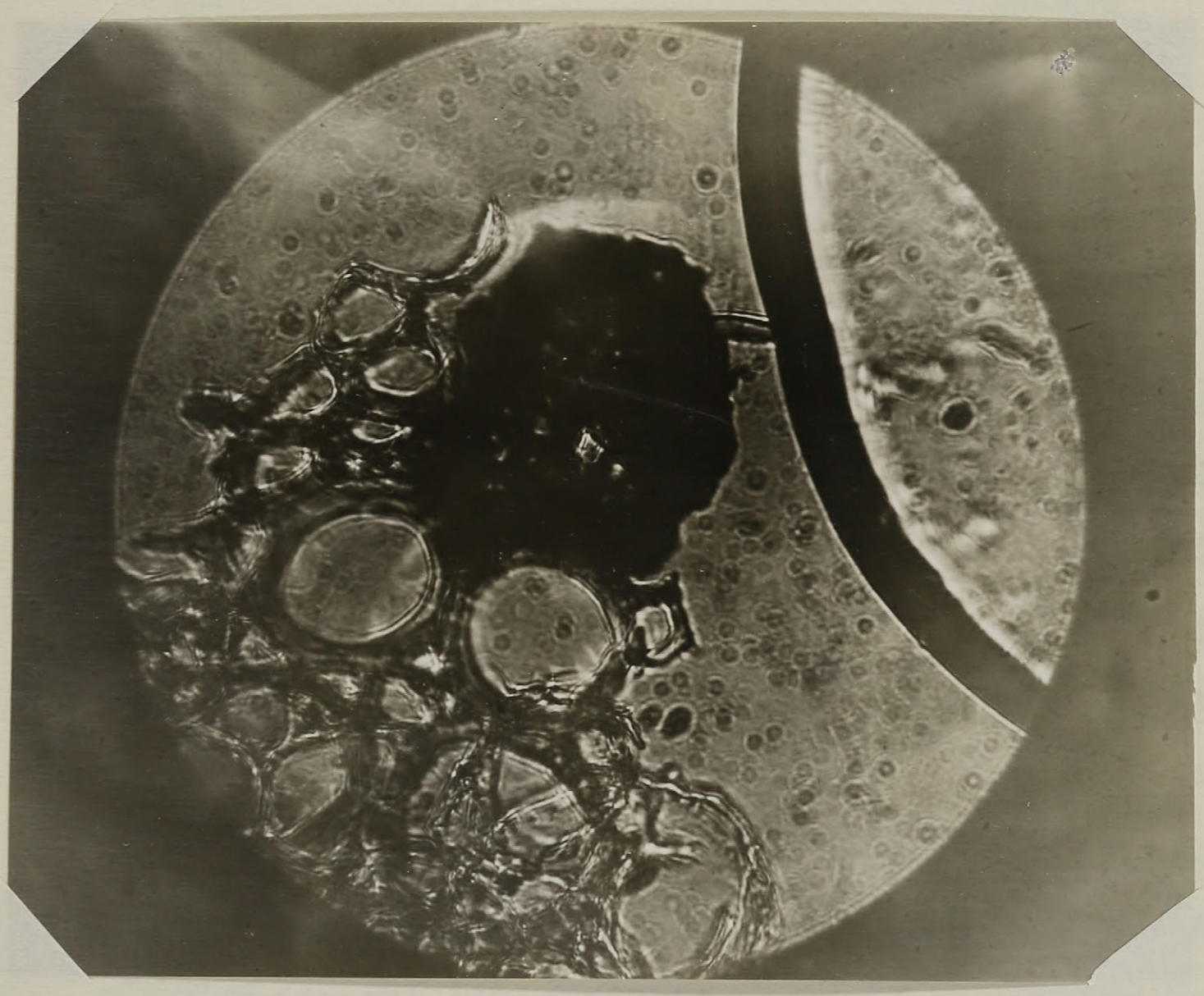


PLATE 29.

Type C. Cross section of sclerotium.

PLATE XXIX.



## PLATE 30.

Fusarium. Growth temperature studies

A. 1. Growth of fungus on Richards solution in flask inoculated with spore suspension. This culture was incubated first at 25 degrees centigrade, where no growth occurred, and later at room temperature, 22 degrees centigrade.

2. Flask containing Richards solution, inoculated at same time as above, but kept at 5 degrees centigrade.

B. Growth of fungus on Richards solution at different temperatures.

PLATE XXX.

1 2

A

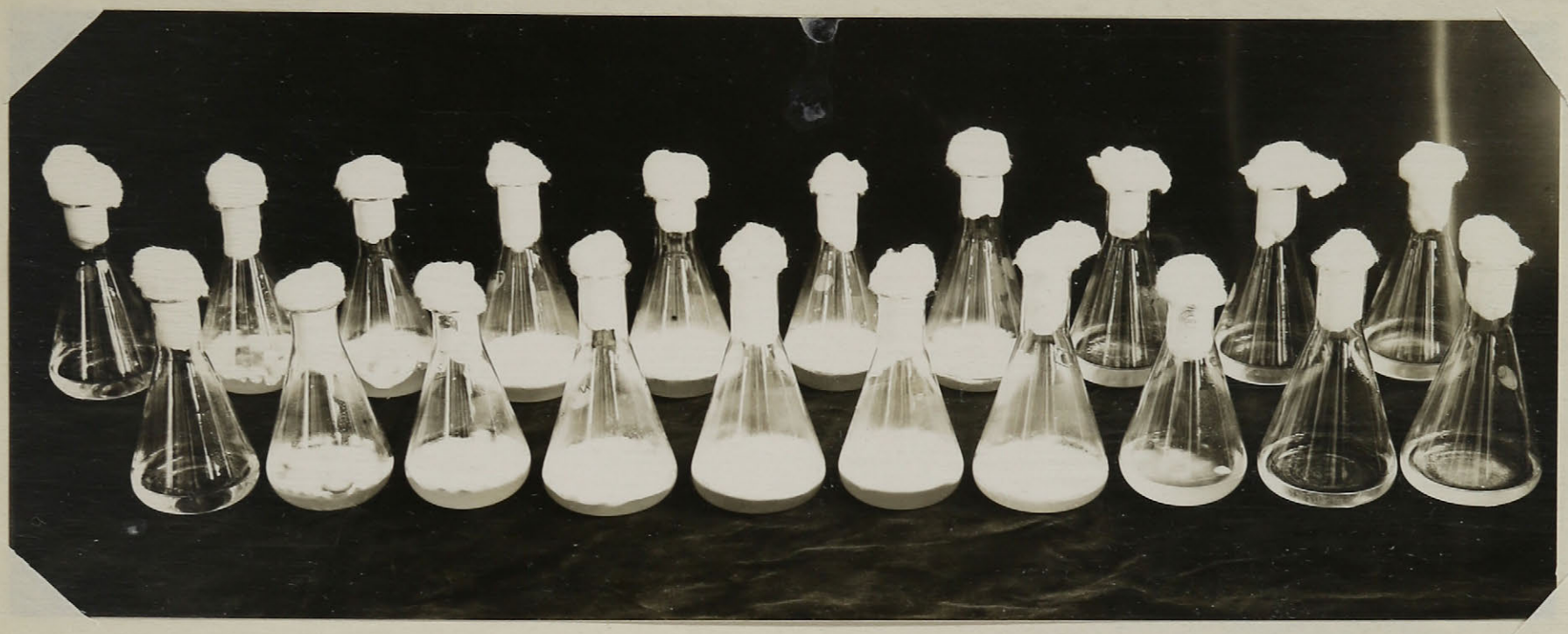
B

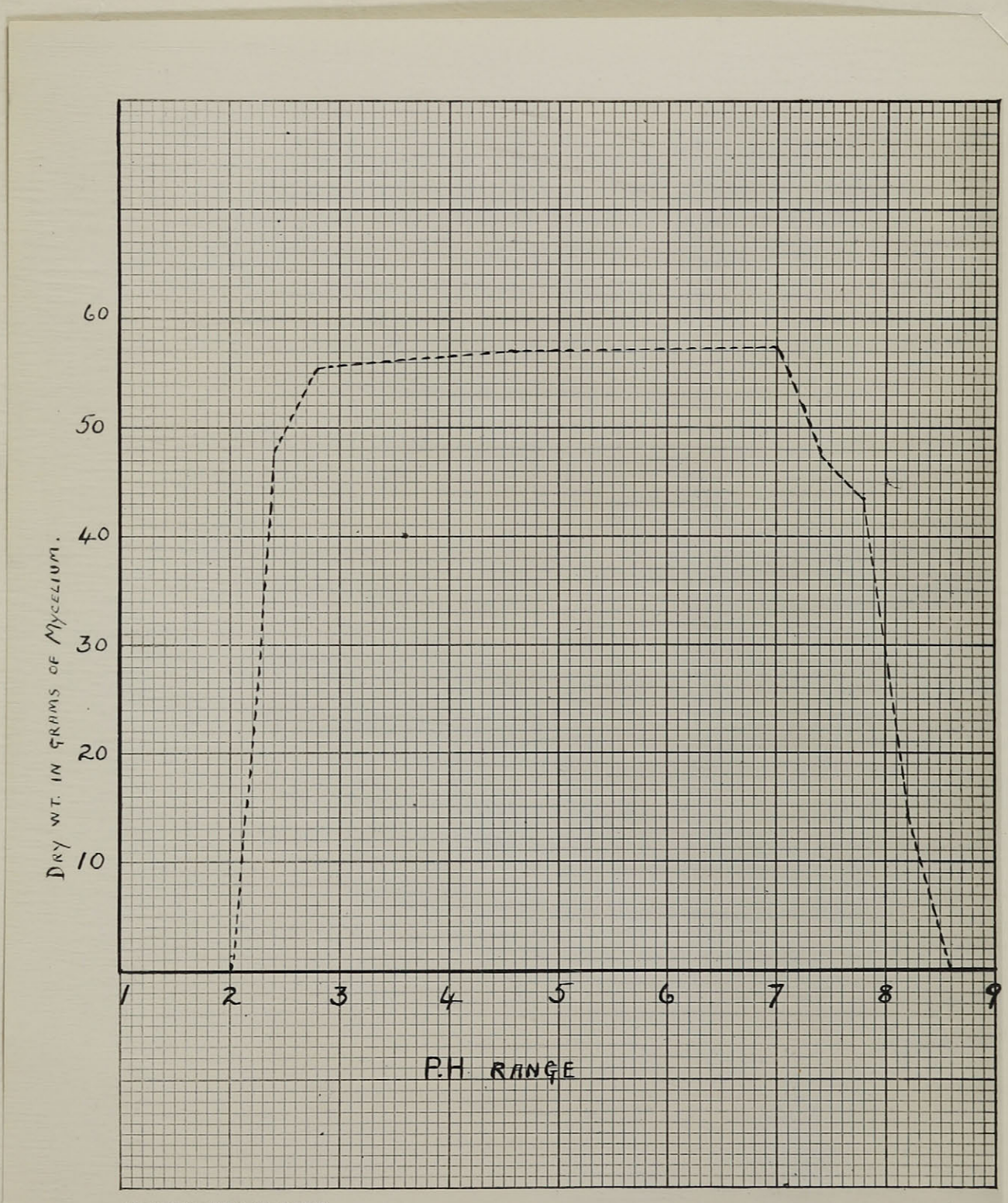
PLATE 31.

Fusarium. A. Growth of the fungus on Richards solution adjusted to different values of P.H.

B. Graph illustrating weight of mycelial mat, in grams, of the cultures photographed above.

PLATE XXXI.

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DRY WT. IN GRAMS OF MYCELIUM.
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P.H RANGE
THE HUGHES OWENS CO., LIMITED
NO. 315 B 10 LINES 1 INCH