A PRELIMINARY STUDY ON CERTAIN SMUTS OF NATIVE GRASSES--ANDROPOGONEAE

by

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TABLE OF CONTENTS

Page

INTRODUCTION

During recent years there has been an increased interest in the development and improvement of grasses for various purposes. The Soil Conservation Service is interested in revegetation to prevent water and wind erosion; the Agricultural Adjustment Administration is interested primarily in the proper use of grasses in crop rotation to conserve the soil and improve yields; the Division of Forage Crops and Diseases is interested in selecting and breeding grasses adapted to humid regions, and other grasses suitable for the more arid localities; the Forest Service is interested in the revegetation of national forests. All of the above branches of the United States Department of Agriculture are cooperating with the state experiment stations and extension services of the Land Grant Colleges, in an effort to develop better balanced agriculture and for the improvement of the forage value of grasses. Naturally, anything that interferes with the normal development of grasses, such as diseases, should be of paramount importance.

Certain smuts of the native grasses of the tribe Andropogoneae may be of economic importance in reducing the yield of seed, the yield and palatability of the forage, winter hardiness, and the capacity of the plant to prevent erosion. As there seems to be a great deal of variation within each species of the Andropogoneae, all degrees of resistance and susceptibility to various smuts undoubtedly occur.

In breeding grasses, therefore, it would be valuable to make selections which would be resistant to smut and other diseases, besides having good agronomic qualities. In nature there is a tendency for susceptible strains to be eliminated or held in check. If no emphasis is placed on the phytopathological aspects, the plant breeder may propagate the susceptible as well as the resistant strains. Man may also destroy the biological balance by bringing in new smuts which may be very virulent on new grass strains.

Before resistant selections and hybrids can be obtained, it is necessary to know the prevalence, distribution, severity and biology of the organisms concerned. It has been the object of this investigation to identify the smuts which occur on certain native grasses of the Andropogoneae, to observe their distribution and severity, and to study certain phases of the biology of Sorosporium everhartii and Sphacelotheca holci.

SMUT COLLECTIONS

A number of smut collections was made during 1935 and 1936 on the tribe Andropogoneae by Donald Cornelius, C. L. Lefebvre, C. O. Johnston, and the writer. The collections are listed below according to their species, host, locality, collector, and date. Twenty of the specimens were collected in Kansas and one in Oklahoma. These were identified by the writer employing Zundel's monograph (1930), except for the smut on Sorghum halepense which was identified according to Jackson's monograph (1934). All of the identifications were checked by Dr. Lefebvre.

Smut Collections

Kansas

Sorosporium everhartii Ellis & Gall.

On Andropogon furcatus Muhl.

Shawnee Co.: 9 mi. n. Topeka (Cornelius, Sept. 25, 1935).

Wilson Co.: 3 mi. ne. Fredonia (Cornelius, Oct. 2, 1935).

Woodson Co.: 2 mi. n. Buffalo (Cornelius, Oct. 3, 1935).

Coffey Co.: 3 mi. sw. LeRoy (Cornelius, Oct. 4, 1935).

Riley Co.: 5 mi. sw. Manhattan (Lefebvre, Oct. 25, 1935).

Riley Co.: 5 mi. w. Manhattan (Lefebvre, Oct. 27, 1935).

Riley Co.: 2 mi. sw. Manhattan (Hansing, Nov. 3, 1935).

Riley Co.: Sunset Park, Manhattan (Hansing, Nov. 10, 1935).

On Andropogon scoparius Michx.

Coffey Co.: 3 mi. s. LeRoy (Cornelius, Oct. 5, 1935).

Sorosporium provinciale (Ellis & Gall.) Clinton.

On Andropogon furcatus Muhl.

Woodson Co.: 5 mi. ne. Vernon (Cornelius, July 12, 1935).

Woodson Co.: 2 mi. N. Vernon (Cornelius, July 25, 1936).

Allen Co.: 2 mi. nw. Iola (Cornelius, June 24, 1936).

Sphacelotheca andropogonis (Opiz) Bubak.

On Andropogon furcatus Muhl.

Woodson Co.: 2 mi. ne. Vernon (Cornelius, July 13, 1935).

Woodson Co.: 3 mi. s. LeRoy (Cornelius, Oct. 4, 1936).

On Andropogon scoparius Michx.

Elk Co.: 6 mi. n. Howard (Cornelius, July 20, 1935).

Sphacelotheca holci Jackson.

On Sorghum halepense (L.) Pers.

Elk Co.: 1 mi. ne. Howard (Cornelius, July 20, 1935).

Sphacelotheca occidentalis (Seym.) Clinton.

On Andropogon furcatus Muhl.

Wilson Co.: 4 mi. se. Fredonia (Cornelius, July 18, 1935).

Elk Co.: 12 mi. ne. Howard (Cornelius, July 20, 1935).

Woodson Co.: 5 mi. s. LeRoy (Cornelius, Aug. 2, 1935).

On Andropogon halii Hack.

Clark Co.: 5 mi. s. Ashland (Cornelius, July 12, 1936).

Oklahoma

Sphacelotheca holoi Jackson.

On Sorghum halepense (L.) Pers.

Love Co.: 10 mi. s. Ardmore (Johnston, May 25, 1936).

REVIEW OF LITERATURE

An attempt has been made to review all of the available literature pertaining to these smuts, namely: Sorosporium everhartii, Sorosporium provincials, Sphacelotheca andropogonis, S. holoi, and S. occidentalis. Practically all of the papers, however, have been of a taxonomic nature and it seems more feasible to refer to most of them under the descriptions of these smuts later in this paper.

Zundel (1930) made a monographic study of the Ustilaginales attacking Andropogon. Previous to his study, fifty-nine species of smuts were known to attack various grasses belonging to this genus. Zundel studied specimens from the Clinton herbarium together with his own personal specimens. He also obtained type specimens of certain species from the Royal Botanic Gardens, Kew; the Jardin Botanique de l'Etat, Bruxelles; the Museum d' Histoire Naturelle, Paris; the Botanischer Garden and Museum, Berlin-Dahlem; Department of Agriculture, Malta; the Bureau of Science, Manila, Philippine Islands, and from Dr. Rene Maire of Algiers. Zundel described eighteen new species in his monograph, most of which came from the Agricultural

Department of the Union of South Africa. He also transferred many of the species from one genus into another and excluded two species from the Ustilaginales. In his paper he reports seventy-six species of Ustilaginales as occurring on the genus Andropogon as follows:

Cintractia	1 species
Sorosporium	28 species
Sphacelotheca	39 species
Tolyposporella	3 species
Ustilago	5 species

Zundel uses the genus Andropogon as defined by Englor. Thus the smuts on Sorghum spp. of Hitchcock (1935) are also included in Zundel's monograph. It is very interesting to find that so many species of smut occur on Andropogon spp., and that all of them belong to the family Ustilaginaceae and none to the Tilletiaceae, the other family of the order Ustilaginales.

Brefelt (1883, p. 96) probably was the first to germinate the chlamydospores of Sphacelotheca andropogonis (Ustilago isohaemi). He obtained very good germination on nutrient solution but no germination on water. The chlamydospores produced promycelia upon which sporidia were produced. These sporidia budded or produced germ tubes and after a short time a large number of secondary sporidia were formed.

Boss (1927) studied the cytology of Sphacelotheca andropogonis (Ustilago isohaemi). He observed the entire developmental course of

the smut on culture media in the laboratory. The chlamydospores germinated and within a few weeks the multi-nuclear mycelium began to form spores. The mycelium swelled and the new chlamydospores were formed endogenously, each containing a single nucleus. Similarly, Boss found that the chlamydospores from the host also contain uniformly one nucleus. No sexual fusion was observed in his study. S. ischaemi, therefore, has an unusual development that is different from most of the other smuts that have been studied cytologically.

Outside of Brefelt's study on the germination of chlamydospores, Boss's investigation on the cytology of Sphacelotheca andropogonis, and certain papers of a taxonomic nature, the writer is unaware of any other publication relative to any experimental work on these five smuts.

DESCRIPTION OF SMUTS

The available literature pertaining to the descriptions of the various smuts that were collected was studied. Also new observations relative to the appearance of the sori, spore balls, and chlamydospores are included. The host range and the countries where each smut has been reported is listed. Hitchcock (1935) was followed in listing the various grass hosts which occur in the United States, but when the name was changed, the grass as listed in Zundel (1930) was given in parenthesis following Hitchcock's nomenclature. Whenever the

grass does not occur in the United States, the name is left as in Zundel's monograph.

Sorosporium everhartii Ellis & Gall.

Sorosporium everhartii was first described by Ellis and Galloway (1890, p. 32). It differs from S. ellisii Winter in its smaller spores, more compact spore balls and in attacking single florets instead of involving the entire inflorescence. Dietel in Engler and Prantl (1900, p. 14) placed the fungus in another genus and called it Tolyposporium everhartii, but Clinton (1906) did not accept Dietel's new classification and recognized the smut as Sorosporium everhartii.

Zundel (1930, p. 148) describes S. everhartii as follows: "Sori in the inflorescence, long linear, 1-2 cm. long, 0.5 cm. wide, at first concealed by the glumes, covered with an evident false membrane which dehisces from the apex revealing a granular dark brown spore mass; spore balls globose-ellipsoidal, opaque, dark reddish brown, rather permanent, consisting of many spores, 40-125 u diameter; spores globose-subglobose, somewhat irregular and angled, the spores on the outer part of the spore ball reddish brown, those on the inner part lighter (almost hyaline) in color, the free surface of the outer spores verruculose, otherwise smooth, under the oil immersion, 7-12 u diameter."

This smut is very well illustrated in Plates I and II and Plate III, figs. 1 and 2. The sori varied in length in the different

collections. The sori from the specimens collected five miles southwest of Manhattan, illustrated in Plate III, figs. 1 and 2, were distinctly longer than those from specimens of S. everhartii collected elsewhere. It is impossible to state whether the variation in length of these sori is due to the fungus, the host, environmental conditions or to a combination of these factors.

Not only the sessile and fertile but also the pedicellate and rudimentary ovaries were affected with this smut (see Plate I and II). Notes were taken on 148 diseased spikelets from the specimens collected five miles southwest of Manhattan in 1935 and on 219 affected spikelets from specimens collected at the grass nursery at the agronomy farm. Thirty-nine per cent of the sori occurred in the pedicellate spikelets in the former case and 32 per cent in the pedicellate spikelets in the latter case.

Separate infection of the staminate tissue occurred in both the sessile and pedicellate spikelets. These are very well illustrated in Plate I, figs. 5 and 8, and in Plate II. From one to four sori were observed in both types of spikelets. Usually the ovary and from one to three stamens were infected. Occasionally, however, sori from staminate tissue probably occurred and no sori from pistillate tissue were present. This is illustrated in Plate II, figs. 6, 7, and 16. Notes were taken on 91 sessile and 57 pedicellate affected spikelets from the specimens collected five miles southwest of Manhattan in the fall of 1935. Eighteen per cent of the former and 56 per cent

of the latter spikelets had independent sori from staminate tissue. Similarly notes were taken on 148 sessile and 71 pedicellate affected spikelets from the specimens collected at the grass nursery in 1936. Of these 4 per cent and 13 per cent respectively produced independent sori from staminate tissue. It appears that there is a greater tendency for sori to develop in the pedicellate than in the sessile spikelets. It is impossible to state whether the unusually high per cent of staminate sori in the specimens collected five miles southwest of Manhattan was due to the fungus, the host, the environment or a combination of these factors.

The sori of the specimens collected on Andropogon furcatus in Kansas during 1935 and 1936 were not entirely typical for Sorosporium everhartii, according to Zundel (1937). Zundel states that the sori may have been deformed by heat or other ecological factors. The spores in the center of the spore ball become colored because of heat, and in eastern United States there is usually not enough heat to color these chlamydospores. During the late summer and early fall of the last two years the temperatures in Kansas were extremely high so that the spores in the center of the spore balls were darker than usual.

The plant selections of Andropogon furcatus in the grass nursery exhibited variability in susceptibility to Sorosporium everhartii. Although the smut infection was not very heavy, it was not uncommon to find a few diseased florets in about half of the panicles of the very susceptible plants. In some cases all of the plants in a family

seemed to be extremely resistant or immune while other families had from a few to many susceptible plants. Twenty per cent of the 200 plant selections started in the spring of 1935 were susceptible while 5 per cent of 1000 plants from seed of the first 45 plant selections, planted in the spring of 1936 were susceptible. If further observations are made in 1937 on the same 1000 plants it may be possible to find a higher per cent of the spikelets containing smut sori, because the plants of this perennial grass may have grown so fast in 1936 that the fungus was unable to follow and establish itself in the rapidly growing plant tissues. This situation is probably very similar to that found by Melchers (1935) on the belated development of kernel smut (Sphacelotheca sorghi) of sorghum.

General observations were made on the effect of infection by S. everhartii on the normal development of the plants of Andropogon furcatus. No difference in height or date of heading was noticed between the normal and effected plants, while collecting the smut two miles southwest of Manhattan during the fall of 1935. Similarly no differences were observed in these two respects on the grass nursery during the fall of 1936 at the Agronomy Farm, Kansas State College. During the last two years very few spikelets became infected, so it will be necessary to make further observations before stating definitely whether or not a heavier infection would affect the height and general vigor of the plant.

S. everhartii has been reported previously on Andropogon brachystachyus in United States (Florida); Andropogon diplandrus in Congo; Andropogon glomeratus (A. macrourus) in United States (Florida); Andropogon scoparius in United States (Alabama, Connecticut); Andropogon virginicus in United States (Alabama, Georgia, Mississippi, New Jersey); Cayana densiflora in Congo; and on Paspalum scrobiculatum in Congo. Outside of United States, the smut has been reported only in the Congo.

The collections in Kansas were the first reported in this state. Andropogon furcatus is a new host for this fungus.

Sorosporium provinciale (Ellis & Gall.) Clinton

Sorosporium provinciale was first described by Ellis and Galloway (1890, p. 31-32) as Sorosporium ellisii provinciale, a variety of Sorosporium ellisii Winter. It differs from the latter in its more regular-shaped spores, with a thicker spore wall and its larger spore balls. Clinton (1902, p. 145) gave this smut specific rank, naming it S. provinciale, because of its very strikingly uniform thick spore walls. It is the most distinctive of the related species of smut on Andropogon.

Zundel (1930, p. 152) describes S. provinciale as follows: "Sori in the inflorescence, long linear, 6-8 cm. long, entirely hidden by the sheath or sometimes the end partially protruding, covered by a false membrane which flakes away revealing a dark brown powdery spore mass; spore balls globose, somewhat irregular, containing more than

30-40 spores, reddish-dark reddish brown, sometimes opaque, fairly permanent, 45-105 u in diameter; spores reddish brown, globose-subglobose, evenly thickwalled, 3-4 u, verruculose under the oil immersion, 11-20 u diameter."

The sori of this smut are very well illustrated in Plate III, figs. 5 and 6. S. provinciale produces a striking disease in that the entire inflorescence and peduncle are replaced by the smut sorus.

S. provinciale has been reported previously on Andropogon furcatus (Andropogon provincialis) in United States (Missouri, Nebraska). The collections made in 1935 and 1936 in Kansas are the first for this state.

Sphacelotheca andropogonis (Opiz) Bubak

According to Savulescu (1936, p. 45) Sphacelotheca andropogonis was first described as Uredo (Ustilago) andropogi by Opiz in 1823-24. According to Clinton (1902, p. 140) Fuckel independently described another specimen of the fungus as Ustilago ischaemi in 1861. Peck (1882, p. 55) described another specimen of this smut as Ustilago cylindrica, because of the cylindrical appearance of the sori. According to Clinton (1902, p. 140) Sydow described another specimen as Cintractia ischaemi in 1901, thus placing it in a new genus. Clinton (1902, p. 140) placed the species in another genus naming it Sphacelotheca ischaemi since it possessed a definite false membrane. Clinton

undoubtedly, did not know of the description by Opiz. Finally, according to Savulescu (1936, p. 45) Bubak changed the binomial name to Sphacelotheca andropogonis in 1912. Thus, Bubak made the specific name fairly similar to the one given by Opiz.

Zundel (1930, p. 141) describes the smut as follows: "Sori usually involving entire inflorescence, long linear, hidden by sheath, 10-40 mm. long, 1-4 mm. wide, covered with a false membrane which flakes away disclosing a brown spore mass and a well developed columella: false membrane rather permanent, breaking up into large masses of tissue rather than individual cells; sterile tissue also scattered throughout the sori; sterile cells globose-subglobose, flattened when in contact with each other, 7-16 u diameter, usually hyaline or tinted brown en masse; spores medium reddish brown, globose-subglobose, smooth and minutely granular, 8-11 u diameter." This smut is illustrated in Plate IV, fig. 1.

Norton (1896, p. 231) observed that the affected plants were stunted. He quotes the late Dr. Bartholomew of Rooks County, Kansas as saying that the smut is undoubtedly perennial as it appears from year to year on a single plant on his farm.

Cornelius further observed, while making the collections during the last two years, that the affected plants were not only smaller than the normal ones, but that they apparently headed much earlier.

S. andropogonis has been previously reported on Andropogon distachyos in France and Spain; Andropogon foveolatus in Egypt; Andropogon

furcatus in United States (Kansas); Andropogon ischaemum in Austria, Bulgaria, Germany, Greece, Poland, Rumania, Spain, Switzerland, Yugoslavia, Congo, Persia; Andropogon ischaemum longiaristatum in Czechoslovakia, Hungary, Italy, Spain; Andropogon iwarancusa in Erythrea; Andropogon pubescens in Malta, Congo, Tunis, Syria, Palestine; Andropogon saccharoides (A. torreyanus) in United States (Arizona, Texas), Mexico; Andropogon scoparius in United States (Illinois); Andropogon sp. in United States (Arizona), Czechoslovakia, Central Africa, Congo, Tanganyika Territory, Tripoli, Union of South Africa; Cymbopogon excavatus in Union of South Africa; Ischaemum timorense in tropical Africa; Heteropogon contortus (Andropogon contortus) in United States (Arizona), Mexico, Erythrea, Philipine Islands; Hyparrhenia hirta (Andropogon hirtus, andropogon hirtus longiaristata, Andropogon hirtus pubescens) in Czechoslovakia, Spain, Anatolia, Congo; Pennisetum dichotomum in Egypt; Sorghum halepense (Andropogon halepensis) in Philippine Islands. S. andropogonis is thus widely distributed, having been reported in Kansas, Arizona, Illinois, Texas, and Mexico of North America and in South America, Europe, Africa, and Asia.

The specimen in Kansas on Andropogon scoparius was the first reported on this host for the state.

Sphaceloteca holci Jackson

Sphaceloteca holci was described by Jackson (1934, p. 259) as follows: "Sori in the ovaries, not exceeding the glumes, covered

at first with evident false membrane which soon ruptures and in old sori is not evident; spore mass dark brown soon becoming powdery, surrounding an evident, well developed columella; spores globose or subglobose, very finely but evidently verrucose-echinulate, 7.5-10 u in diameter (averaging 8-9 u); wall raddish brown, thin 1 u or less; sterile cells not evident."

This smut is illustrated in Plate IV, figs. 4-6; Plate V, figs. 1-14; and Plate VI, figs. 8-10. Not only are the sessile and fertile spikelets affected but also the pedicellate and staminate ones. These are clearly illustrated in Plate V. Notes were taken on a few hundred affected spikelets and about 60 per cent of the sori occurred in the pedicellate spikelets. In general all of the spikelets in the inflorescence were affected but occasionally at a single joint of the rachis the sessile and not the pedicellate spikelet had been infected while in other cases the latter and not the former had been infected.

Cornelius and Johnston, while making the collections of S. holci, observed that the affected plants were much smaller and headed earlier than the normal plants. This will be discussed later in the paper.

S. holci has been reported previously on Sorghum vulgare (Holcus sorghum) (Jackson, 1934, p. 259) "Aragua: Gardens at Las Delicias, near Maracay, 450 m., Chardon, Toro & Alamo 168, June 16, 1932." Zundel (1937) writes that he has just found this species among specimens from the Union of South Africa.

The specimens in Kansas and Oklahoma are the first collections of

this smut from North America. Also Sorghum halepense is a new host for this fungus.

An attempt was made to identify the specimens collected in Kansas and Oklahoma, but the smut did not conform with any of the 76 species described in Zundel's (1930) monograph on the Ustilaginales attacking Andropogon. The specimens were then sent to Dr. Zundel who identified the fungus as Sphacelotheca holci Jackson.

Sphacelotheca occidentalis (Seym.) Clinton

According to Zundel (1930, p. 134), Sphacelotheca occidentalis was first described by Seymour in Ellis and Everhart, North American Fungi 2265, 1889, as Sorosporium ellisii occidentalis, a variety of Sorosporium ellisii Winter. This fungus was independently described by Kellerman and Swingle (1889, p. 12-13) as Ustilago andropogonis. They observed that the pedicellate and staminate spikelets were affected as well as the sessile and fertile spikelets. They examined Seymour's specimens as well as young Kansas specimens but could not find the spore balls which characterize Sorosporium. They state that the species as far as can be determined without a knowledge of the germination of the spores, certainly seems to be a good Ustilago. Clinton (1902, p. 141) placed the fungus in the genus Sphacelotheca since it had a definite false membrane of sterile fungous cells, a central columella and no spore balls.

Zundel (1930, p. 134) describes the smut as follows: "Sori in the

ovary, linear, 0.5-1 cm. long, covered by an evident false membrane which dehisces from the apex disclosing a powdery mass of spores surrounding an evident, well developed columella; sterile cells hyaline, very variable in size and shape, 7-14 u diameter, globose-subglobose or rectangular; due to partial gelatinization of false membrane the cells are frequently rather indistinct; spores reddish brown, subglobose, often angled, variable in shape, sometimes mechanically hanging together in clusters but not forming spore balls, minutely verruculose under the oil immersion, 11-16 u diameter."

This smut is illustrated in Plate III, figs. 3 and 4. Notes were taken on a few hundred affected spikelets of Andropogon furcatus and A. hallii to determine how often the sori occurred in the pedicellate spikelets. In both cases 50 per cent of the sori occurred in the former and 50 per cent in the latter. In the majority of affected panicles all of the spikelets produced sori.

S. occidentalis has been reported previously on Andropogon furcatus (Andropogon provincialis) in United States (Kansas, Nebraska, North Dakota); Andropogon glomeratus (Andropogon macrourus) in United States (California); Andropogon hallii in United States (Kansas, Nebraska). This smut is not very widely distributed having been reported only in United States.

LABORATORY STUDIES ON SOROSPORIUM EVERHARTII

A study was made of the germination of chlamydospores and cultural aspects of monosporidial lines of Sorosporium everhartii. Previous to these investigations, the writer was unfamiliar with any study that had been made on Sorosporium everhartii except that pertaining to its taxonomy.

Materials and Methods

The primary sporidial lines used in these tests were isolated from chlamydospores of a single collection of Sorosporium everhartii made near Manhattan. The method used in isolating single sporidia was similar to that described by Dickinson (1926) and Hanna (1928) except that the needle was attached to a Bausch and Lomb micro-manipulator. Chlamydospores were taken from the center of the sorus and placed on a cover glass which was inverted over a partially broken van Tieghem cell on a glass slide, and placed under the microscope. The needle of the micromanipulator was adjusted to a point in focus with the low power of the microscope and slightly below the cover glass. By careful manipulation the point of the needle was raised until it touched a chlamydospore on the cover glass, a single spore was picked up and the needle lowered. A drop of potato-dextrose agar was then placed on another cover glass and inverted over another partially broken van Tieghem cell. An ink line was made on the top of the cover

glass from the center of the drop outward and the needle was carefully raised, placing the spore on the surface of the agar and in focus with the end of the line in the center of the drop. The ink line was used as an aid in finding the spore after it had germinated. The cover glass was then placed over a complete van-Tieghem cell in a petri dish containing sterilized distilled water and the next day periodic observations were made to see when the sporidia would be in the right stage to be removed from the promycelium. A few ink dots were made on the upper side of the cover glass at various points from the position of the chlamydospore; the cover glass was placed over the broken van Tieghem cell; and each primary sporidium was moved to a dot and recorded. The cover glass was placed back in the petri dish and after a visible monosporidial colony had developed it was transferred, by means of a sharply pointed needle, to a potato-dextrose agar slant in a test tube.

The sori were lettered A, B, C, D, etc., and the chlamydospores which were isolated from each sorus were numbered 1, 2, 3, 4, etc. The sporidia were lettered a, b, c, d, depending on the time they were isolated. Thus A20a would refer to the first sporidium isolated from the twentieth chlamydospore and from the first sorus A. Due to the irregular number and branching of the promycelia from each chlamydospore, it was thought that it would be better to disregard the position of the sporidia on the promycelia in this preliminary study.

A few of the monosporidial lines mutated occasionally. Four

mutants occurred in line A20a and these were given numbers as follows: A20a-1, A20a-2, A20a-3, and A20a-4. Some of these mutant cultures sectored and were given an additional number. Thus A20a-2-1 indicates the first mutation from A20a-2 which in turn is the second mutation from A20a.

The various isolates were grown in either 125 cc. or 250 cc. flasks to which 20 cc. or 40 cc. respectively of the culture medium had been added. The basic materials included in the various nutrient media were:

Potato-dextrose agar.

Distilled water	1000 cc.
Peeled and sliced potatoes	250 g.
Agar	20 g.
Dextrose	20 g.

Carrot-dextrose agar

Distilled water	1000 cc.
Peeled and sliced carrots	100 g.
Agar	20 g.
Dextrose	20 g.

Corn meal-dextrose agar.

Distilled water	1000 cc.
Corn meal	20 g.
Agar	20 g.
Dextrose	20 g.

Richard's agar.

Distilled water	1000.00 cc.
Potassium nitrate	10.00 g.
Potassium monobasic phosphate	5.00 g.
Magnesium sulphate	2.50 g.
Ferric chloride	0.02 g.
Sucrose	50.00 g.
Agar	20.00 g.

Difco standardized agar, and only high grade chemicals were used in the preparation of culture media. Erlenmeyer flasks of 250 cc. capacity were used for experiments involving a small number of flasks, otherwise 125 cc. flasks were used.

Young cultures were used for making transfers, the monoconidial cultures for any experiment being transferred to fresh potato-dextrose agar slants in tubes three to four days before they were used. Each colony was then transferred to a test tube containing about 5 cc. of sterilized distilled water. A small round loop was made on the end of a transferring needle and bent perpendicular to the platinum wire. One loop-full of sporidia from the suspension was placed on the agar in the center of the flask without disturbing the surface of the agar. The surplus water was absorbed by the culture medium and the sporidia would remain on the surface of the agar in a circular area of about 2 to 3 mm. It was thought that this method was superior to making uniform, initial transfers. These flasks were then left for 24 hours

at room temperature after which they were placed in constant temperature incubators where the temperatures were recorded three times a day.

Other data pertaining to the materials and methods will be included later in the text.

Germination of Chlamydospores

Chlamydospores of Sorosporium everhartii were placed on the surface of potato-dextrose agar, carrot-dextrose agar, and on sterile distilled water and left at room temperature. Very high germination occurred on the two agars and fairly high germination on the water. As the spores germinated on the nutrient agars, they produced from one to a few promycelia, each of which contained from one to four cells upon which primary sporidia were borne. These budded profusely and a large number of secondary sporidia was produced within 48 hours.

The germination on water was also very irregular in that usually more than one promycelium was produced from each spore, but instead of numerous sporidia being produced, each cell of the promycelium generally sent out a germ tube. Protoplasmic migration in the promycelium was observed under certain conditions on both of the agars and on the sterile water.

Effect of Temperature on the Germination of Chlamydospores of Sorosporium everhartii

A composite smut sample was obtained by washing a large number

of sori with a scalpel from part of the specimens that were collected near Manhattan during November, 1935. In February, 1936, sterile distilled water was added to sterilized syracuse dishes and another sterilized dish was placed over the top. These were placed at various temperatures ranging from 5°C. to 38°C. and two hours later, after the water was adjusted to the respective temperatures, a small quantity of chlamydospores was dusted on the surface of the water in each dish. The temperature of each incubator was recorded four times during this experiment.

Twenty-four hours after the spores had been added, the dishes were moved to the laboratory where a microscopic examination was made of each dish, after which they were quickly cooled to 1°C. One dish was removed at a time and 200 chlamydospores were counted, their germination recorded, and the per cent germination calculated. Rather high germination occurred between 24°C. and 29°C., the optimal temperature for germination being about 27°C. under the above conditions. The minimal temperature was below 12°C. and the maximal temperature was between 35°C. and 36°C. The percentage of germination gradually increased to the optimum and then rapidly decreased as the maximum temperature was approached. The results of this experiment are given in table 1 and graphically represented in figure 1.

Table 1.—Effect of temperature on the germination of chlamydospores of Sorosporium everhartii on the surface of sterile distilled water, after 24 hours, February, 1936.*

Temperature		Number spores counted	Number germinated	Per cent germination
Ave. °C.	Range** °C.			
3.4	2.5—4.0	200	0	0
6.0	5.5—6.5	200	0	0
8.9	8.5—9.5	200	0	0
12.2	12.0-12.5	200	2.0	1.0
14.9	14.0-16.0	200	9.0	4.5
18.2	17.5-19.0	200	27.0	13.5
20.0	19.5-21.0	200	24.0	12.0
23.6	23.0-24.5	200	59.0	29.5
27.5	27.0-28.0	200	82.0	41.0
29.1	28.5-30.0	200	53.0	26.5
33.2	32.5-34.0	200	4.0	2.0
35.8	34.5-37.0	200	0	0
38.5	37.0-40.0	200	0	0

*The water was allowed to adjust itself to the respective temperatures before the chlamydospores were added. A composite smut sample was used which was collected near Manhattan during November, 1935.

**The temperature of each incubator was recorded four times during the experiment.

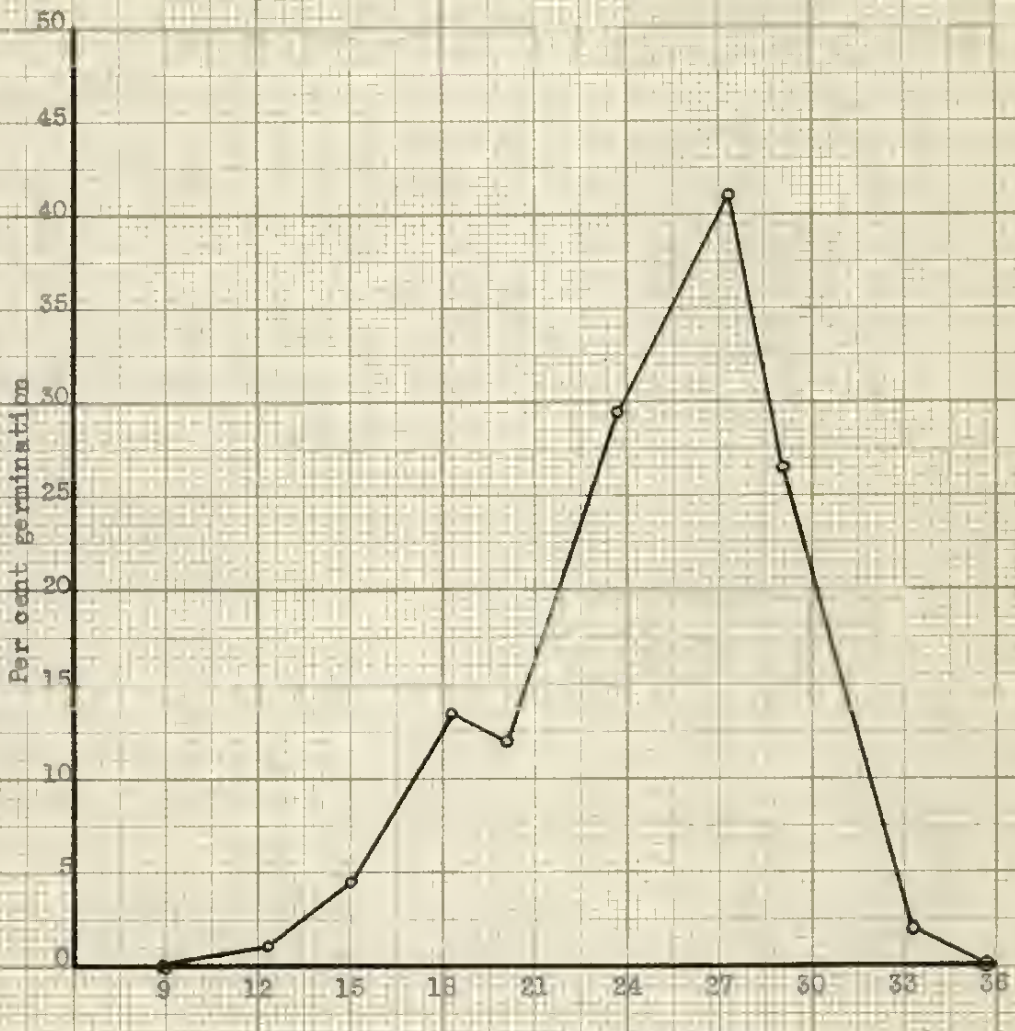

Fig. 1. Graph showing the effect of temperature on the germination of chlamydospores of Sorosporium everhartii on the surface of sterile distilled water, after 24 hours.

Effect of Temperature on the Rate of Growth of Monosporidial Lines of *Sorosporium everhartii*

A study was made to determine whether the monosporidial lines of *Sorosporium everhartii* could be distinguished by their rate of growth at various temperatures. Five monosporidial lines were grown each in a quintuplicate series of 125 cc. Erlenmeyer flasks on potato-dextrose agar at different temperatures ranging from 9°C. to 40°C.

The results of the experiment are summarized in table 2 and graphically represented in figure 2. Since there was very little difference in the diameters of the colonies in replicated cultures of the same line at the same temperature, only the averages are recorded in the table and graph. The temperature for distinguishing these lines by their radial growth varied according to the two lines compared. Line E13c could be easily differentiated from the other four lines at any temperature from 18°C. to 30°C., while lines A20a and D9c could be most easily differentiated from each other and from the other lines at 33°C.

Although the optimal radial rate of growth for the five lines varied, it was always between 30°C. and 33°C. The minimum temperature was considerably below 9°C. and the maximum between 36°C. and 40°C. There was a gradual increase in the rate of growth up to the optimum and then a very rapid decline to the maximal temperature. Although the optimal temperature for the radial rate of growth was very high,

the consistency of these cultures was more often yeastoid to bacteroid and the colonies were irregular in shape. General observations indicated that the optimal temperature for growing lines to be differentiated by their cultural characteristics was between 21°C. and 24°C.

Table 2.--Effect of temperature on the rate of growth of five monosporidial lines of Sorosporium everhartii grown in quintuplicate flasks on potato-dextrose agar, 12 days after inoculation, November 1936.*

Smut line :	Diameters of colonies in mm. at °C.										
	8.9	12.0	15.0	17.9	21.8	25.4	26.8	29.4	32.7	38.8	40.0
A20a	4.8	8.0	8.8	10.6	12.8	15.2	19.0**	20.8	21.4	15.2	0.0
B11a	8.4	9.6	10.2	12.2	13.8	15.2	19.8	21.4	31.2	20.8	0.0
D9c	9.8	11.4	13.2	14.8	15.6	17.2	20.4	28.8	37.6	20.8**	0.0
E9b	8.4	9.8	11.4	14.0	14.4**	15.4	20.8	28.8	32.0	17.8	0.0
E13c	9.8	12.4	14.2	19.2	21.0	24.6	22.6	37.4	32.6	16.2	0.0

*All flasks kept for 24 hours at room temperature after which they were kept for 11 days at the temperatures.

**Only four colonies measured.

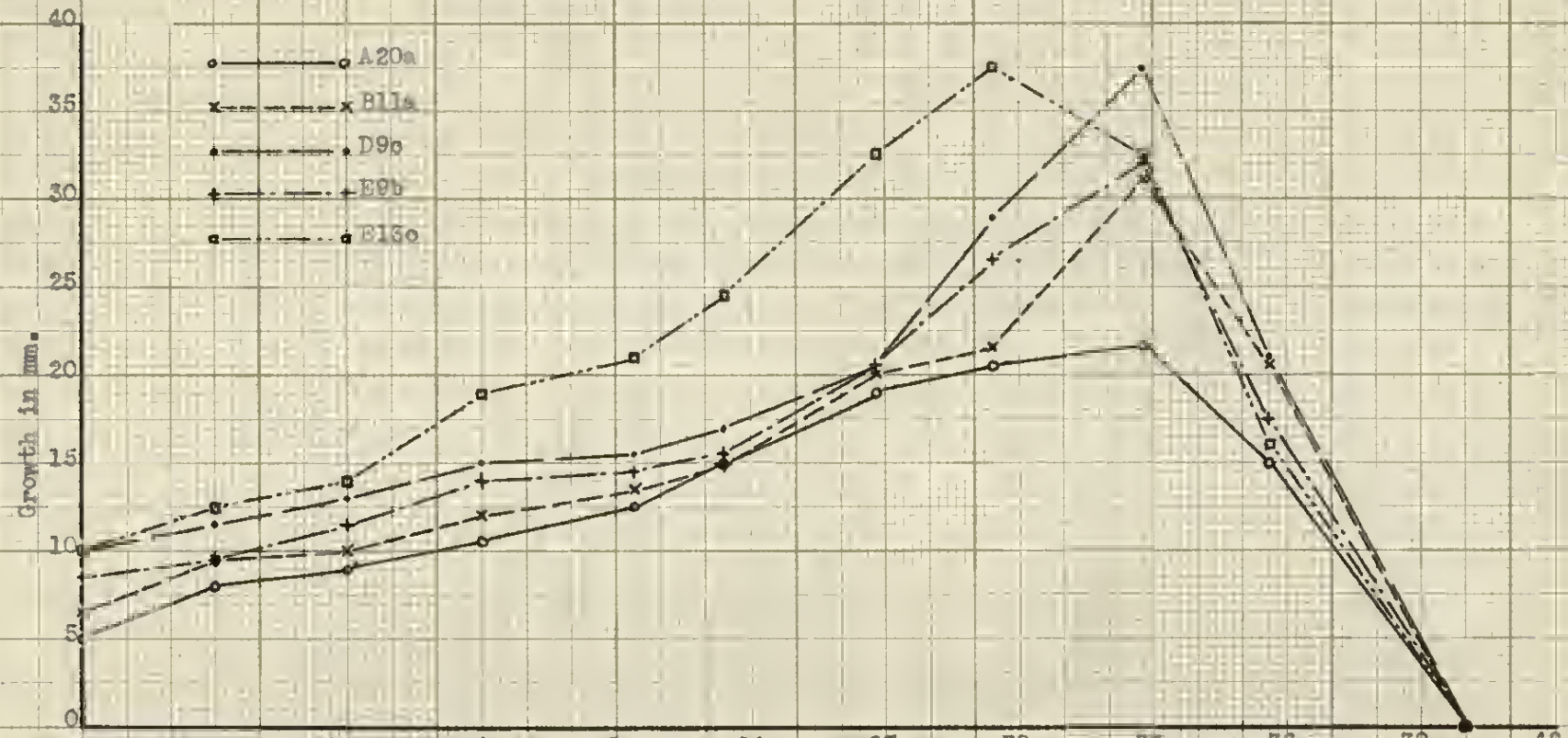

Fig. 2. Graph showing the effect of temperature on the average rate of growth of five monosporidial lines of Sorosporium everhartii, grown in quintuplicate flasks on potato-dextrose agar, 12 days after inoculation.

Effect of Temperature on the Daily Rate of Growth of a Monosporidial Line of *Scrosporium everhartii*

A study was made to determine the daily rate of growth of a monosporidial line of *Scrosporium everhartii*. Line A20a was grown in a quintuplicate series of 250 cc. Erlenmeyer flasks on potato-dextrose agar at temperatures ranging from 4°C. to 40°C.

The diameter of the colonies was measured every two days after they were 3 mm. or more in size. This was done by holding the flask up and measuring the diameter of the colony from the bottom of the flask with a glass scale. The results of this experiment are summarized in table 3 and graphically represented in figures 3 and 4. As there was very little difference in the diameters of the colonies in replicated cultures at the same temperature, only the averages are included in the table and graph. In general, the rate of growth was fairly constant throughout the 22 days. On the twenty first day after inoculation one flask from each series was photographed (see Plate VII). The colonies at the higher temperatures were yeastoid in consistency and very irregular in growth while those at the middle and lower temperatures were usually leathery and more regular in growth.

Table 6.—The effect of temperature on the average daily rate of growth of a monosporidial line A20a of Scrosporium everhartii grown in quintuplicate flasks on potato-dextrose agar at 12 different temperatures, during 22 days after inoculation,* 1936.

Temperature °C.		Diameters of colonies in mm. on November										
Average	Range	9	11	13	15	17	19	21	23	26	27	29
4.1	1.5-5.0	—	—	—	—	—	—	—	3.0	3.6	4.6	5.0
9.1	6.0-10.0	—	—	—	6.0	4.4	6.2	7.2	6.2	9.6	10.4	11.0
12.3	11.0-13.6	—	—	4.6	6.4	7.2	9.2	10.6	11.6	13.2	15.6	16.4
15.0	14.0-16.0	—	3.6	6.2	7.6	9.6	11.8	14.4	15.4	17.4	20.2	21.6
18.0	18.5-19.6	—	5.0	7.0	9.8	13.6	17.2	20.0	22.6	26.8	29.4	31.2
20.9	19.5-22.6	—	5.2	8.0	11.2	14.6	19.4	22.2	25.4	28.0	61.0	34.4
23.6	22.5-26.0	—	6.5	9.8	14.0	17.4	22.0	26.2	29.2	62.6	35.8	67.0
26.9	26.5-26.6	—	7.0	11.5	15.4	21.0	25.6	28.0	32.6	66.6	37.6	69.6
29.4	26.0-31.0	3.0	6.0	12.2	16.2	21.4	27.2	29.2	34.2	36.6	38.8	40.6
32.6	31.0-36.0	3.6	7.6	11.2	16.2	23.2	29.8	64.4	38.4	41.6	43.0	44.4
35.6	33.0-39.0	—	6.0	9.2	11.0	16.5	17.5	20.0	22.5	24.2	28.6	29.6
69.9	67.0-42.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

*The flasks were inoculated November 7 and kept for 24 hours at room temperature, after which they were kept for 22 days at various temperatures.

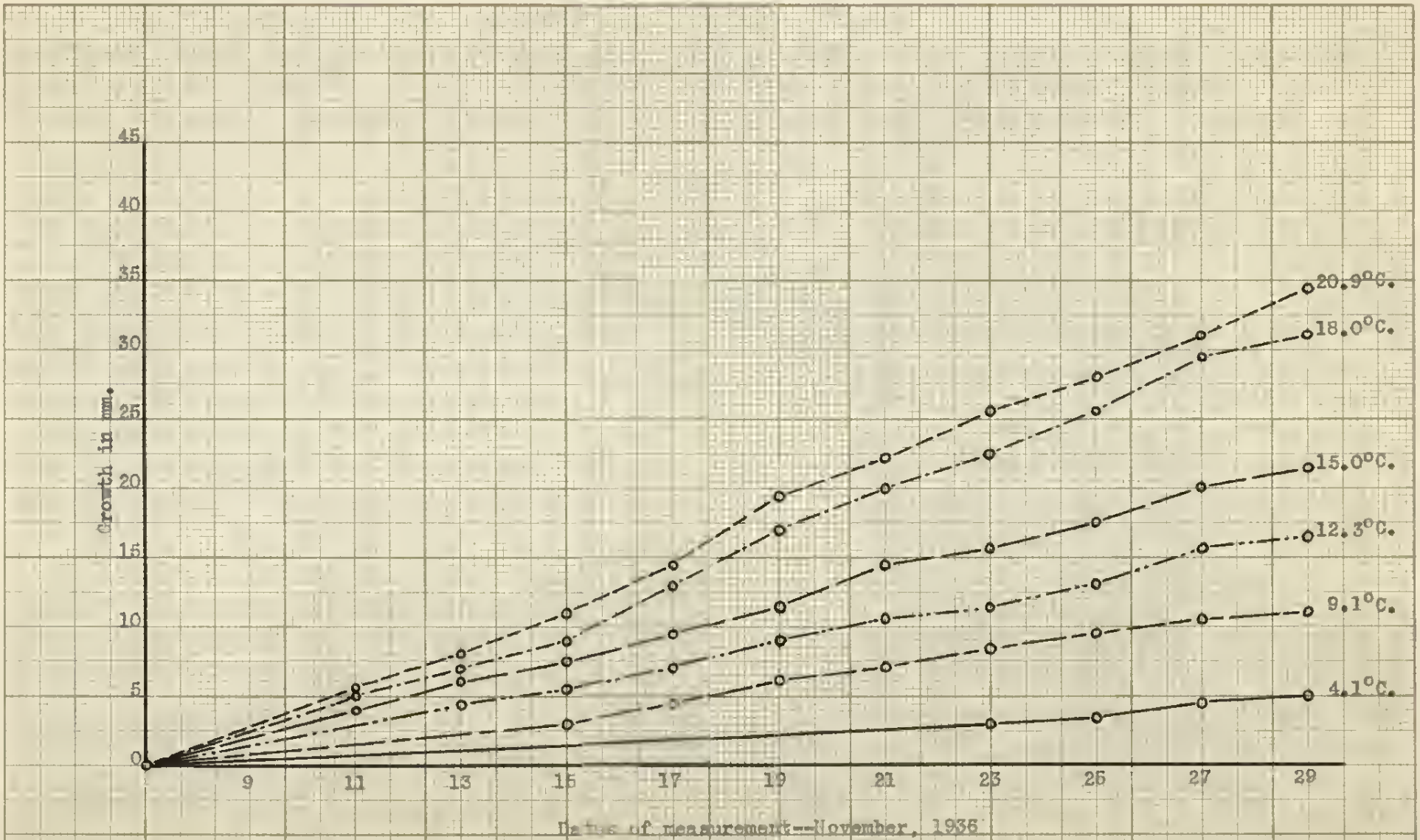

Fig. 5. Graph showing the effect of temperature on the average daily rate of growth of monosporidial line A20a grown in quintuplicate flasks on potato-dextrose agar at six different temperatures, during 22 days after inoculation.

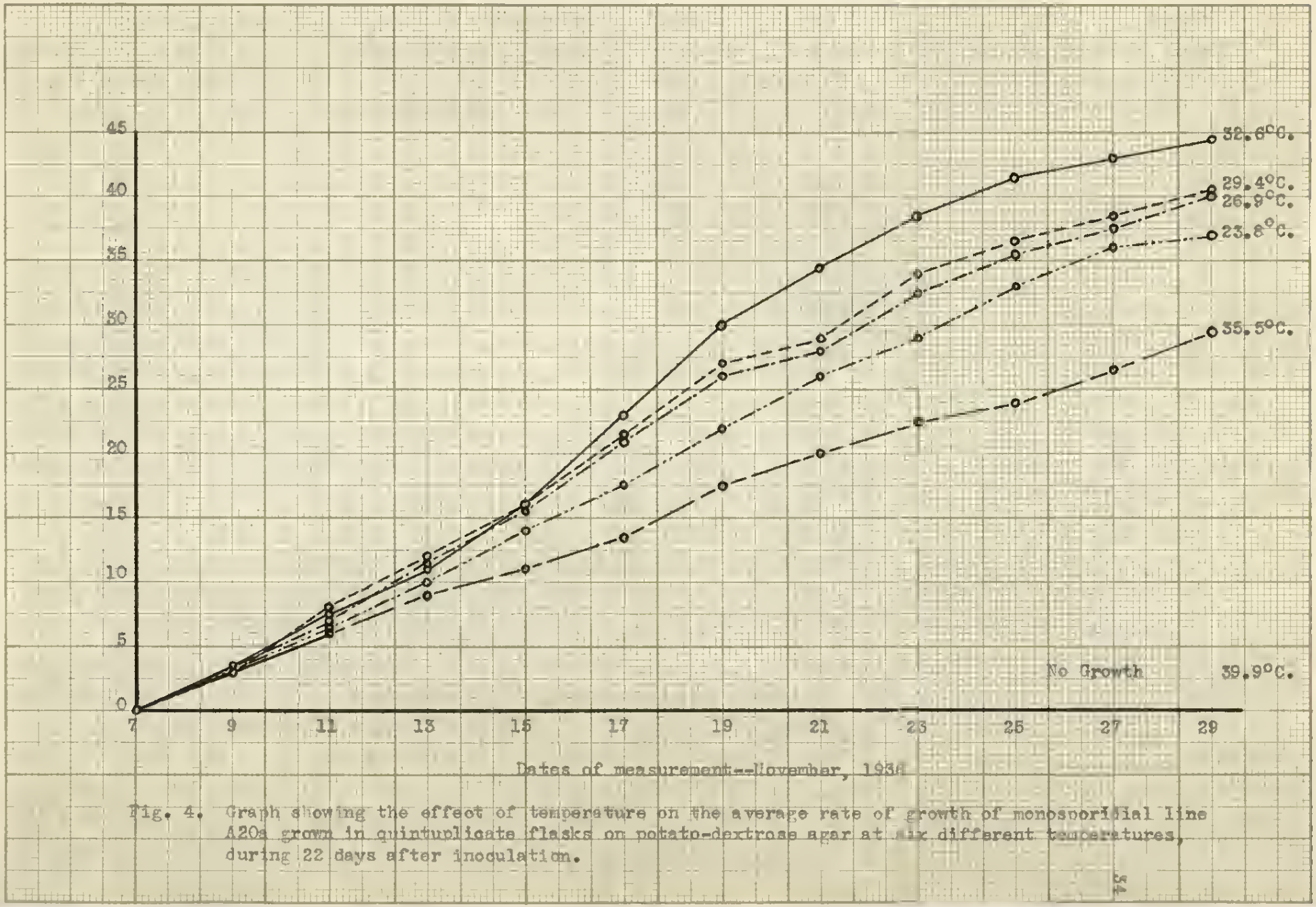

Fig. 4. Graph showing the effect of temperature on the average rate of growth of monosporidial line A20a grown in quintuplicate flasks on potato-dextrose agar at six different temperatures, during 22 days after inoculation.

Effect of Low Temperature on the Rate of Growth of Monosporidial Lines of *Sorosporium everhartii*

A study was made to determine the minimal temperature for the growth of monosporidial lines of *Sorosporium everhartii* and to determine whether monosporidial lines could be distinguished by their radial rate of growth at this temperature. Eight lines were grown each in a quintuplicate series of 125 cc. Erlenmeyer flasks on potato-dextrose agar at an average temperature of 3.8°C. with a range of 0.0°C. to 4.5°C.

At the end of 80 days the diameters of the colonies were measured. The results of the experiment are recorded in table 4 and graphically represented in figure 5. The diameters of each replicated series were very uniform. As every monosporidial line grew at these temperatures (0.0°C. to 4.5°C.), it is evident that the minimal temperature for growth lies below 4.5°C. Some of the lines can be easily distinguished at low temperatures while others cannot be. Line E8d grew about three times as fast as line E11a. In general the cultures were rugose to rugulose, mycelioid, convex to raised, and had an irregular margin.

Table 4.--The effect of low temperature* on the rate of growth of eight monosporidial lines of Sorosporium everhartii grown in quintuplicate flasks on potato-dextrose agar, 60 days after inoculation.**

Line	Diameters of colonies in mm.					
	1	2	3	4	5	Average
A20a	9	11	10	10	10	10.0
B11a	4	6	8	7	7	6.4
D9a	9	10	9	11	9	9.6
D9c	7	7	8	7	8	7.4
D9d	10	10	12	11	11	10.8
E8d	21	19	20	19	20	19.8
E9b	9	11	10	11	12	10.6
E13c	11	12	12	12	13	12.0

*Average temperature 3.8°C. Range 0.0°C. - 4.5°C.

**The flasks were placed in the low temperature room immediately after inoculation.

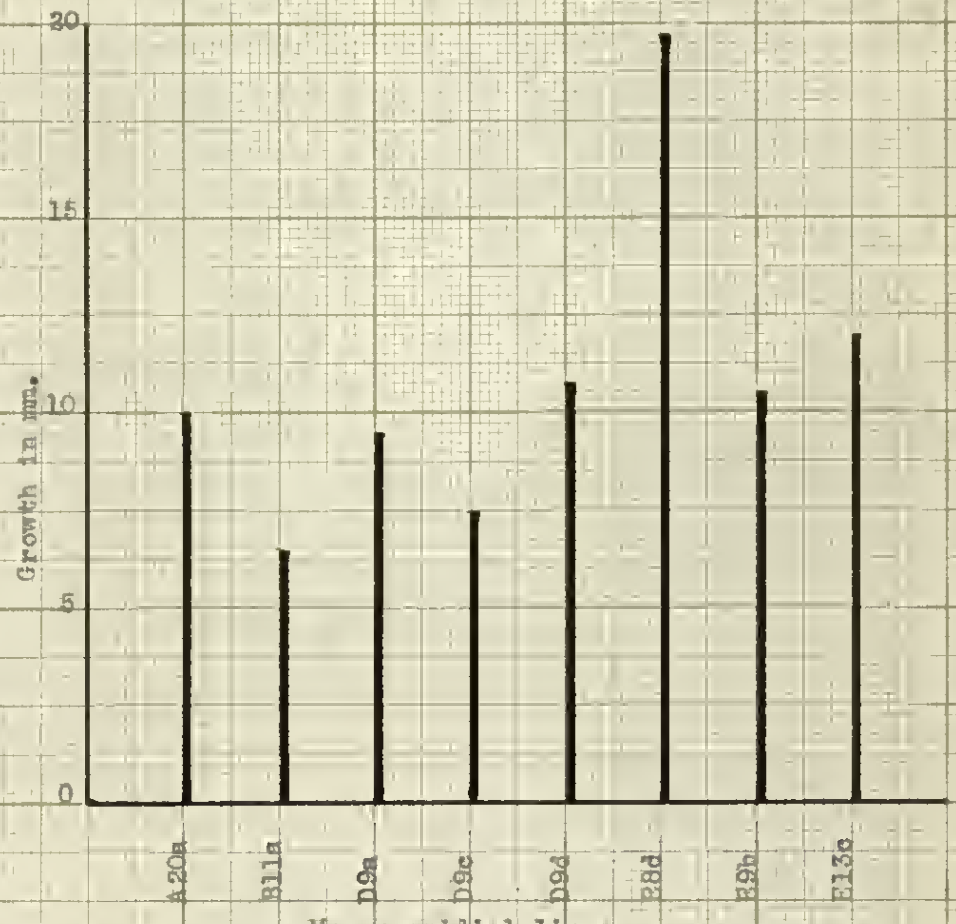

Fig. 5. Graph showing the effect of low temperature (0.0°C. to 4.5°C.) on the average rate of growth of eight monosporidial lines of Sorosporium everhartii grown in quintuplicate flasks on potato-dextrose agar, 60 days after inoculation.

Cultural Characters of Monosporidial Lines on Potato-Dextrose Agar

Thirty-five primary sporidia were isolated from germinating chlamydospores of Sorosporium everhartii during the winter of 1935-36. Each of these was grown in triplicate 250 cc. Erlenmeyer flasks on potato-dextrose agar in order that the cultural characters of the different lines might be studied. Twenty-one days after inoculation such data as the diameter, color, elevation, luster, surface and topography, consistency, and margin of the colonies were recorded. The data concerning six of the lines are tabulated in tables 5 to 7 and illustrated in Plate VIII and Plate IX, figs. 1 and 4. Two colonies of D9d (Plate VIII, figs. 3 and 4) and two of D9a (Plate VIII, figs. 5 and 6) were photographed to show the consistency of cultural characteristics.

There were considerable differences between the rates of growth of different lines. Some grew very rapidly while others grew more slowly; D9a grew to a diameter of 51 mm. while B8d grew to a diameter of 34 mm. Some lines grew somewhat more rapidly than D9a and others grew somewhat more slowly than B8d but the growth rates of most of them varied between these two points.

Ridgway's (1912) color chart was used to determine the color of each culture. Light buff predominated, although other colors were observed as warm buff, lilac grey, smoke grey, pearl grey, and pallid

neutral grey. Some cultures were uniformly colored while others were zonate, one area being of one color and another zone being of some other color.

Most of the lines were convex while others were umbonate, pulvinate, or raised. Some of the lines were cretaceous in luster while others were dull, waxy, or glossy. The surface and topography varied considerably between different lines, all degrees being found from smooth to very rough. Many were zonate, the central area being of one type, while the marginal area being of still another type. The consistency of the cultures was either leathery, yeastoid, bacteroid, mycelioid, or combinations of these. The margin of the colonies was either entire, fimbriate, lobate, undulate, erose or otherwise. The cultural characters of the triplicate series of each line were very uniform.

There are an indefinite number of monosporidial lines of S. everhartii and in isolating primary sporidia from a mass of chlamydospores new cultural lines are more often obtained than ones similar to those previously isolated. This smut is undoubtedly moderately heterozygous in nature based on the cultural characters of sporidial lines from the same chlamydospores and of the same sorus. The differences of the distinct monosporidial lines of D9d and D9a are illustrated in Plate VIII and D9c in Plate IX. There is as much difference between these cultural lines as between other lines from different chlamydospores and from different sori.

This phenomenon is common in certain of the other smuts.

Rodenhiser (1932) isolated 12 primary sporidia from three chlamydospores of the same sorus, and of the same physiologic race of the Sphacelotheca sorghi and found that 10 of the 12 were distinctly different in type of growth on artificial media.

Table 5.--Cultural characters of four monosporidial lines of Sorosporium everhartii growing in triplicate flasks of potato-dextrose agar, 21 days after inoculation.

Line	Diam. in mm.	Color	Elevation	Luster	Surface and topography	Consistency	Margin
E8d	34	Central area light buff, masked by mycelium; margin of 2 mm. white	Slightly convex	Dull to cretaceous	Central area verrucose; surrounded by irregular radiating folds which terminate 2 mm. from the margin; marginal zone narrow, flat	Mycelioid and leathery	Lobate and mycelioid
B11a	48	Central area light buff, masked by mycelium; margin of 7 mm. white	Broadly umbonate	Dull to cretaceous	Central area verrucose grading into radial folds which terminate in a circular ridge; marginal zone broad, flat	Mycelioid and leathery	Undulate and mycelioid
D9d	47	Light buff; margin of 7 mm. partially masked by white pulverulent growth	Slightly convex	Waxy; marginal band pulverulent	Smooth	Leathery	Entire
D9a	51	Central area light buff; surrounded by pale smoke grey; margin of 8 mm. white	Broadly umbonate	Dull to cretaceous	Central area verrucose to verruculose, surrounded by a circular furrow and many short faint radiating ridges and a few longer radiating folds which terminate in a discontinuous faint circular ridge; marginal zone broad, flat	Mycelioid and leathery	Entire and mycelioid

Table 6.--Cultural characters of a monosporidial line of *Sorosporium everhartii* and two of its mutants growing in triplicate flasks of potato-dextrose agar, 21 days after inoculation.

Line	Diam. in mm.	Color	Elevation	Luster	Surface and topography	Consistency	Margin
D9c	50	Central area light buff masked by mycelium; margin of 7 mm. white	Broadly umbonate	Dull to cretaceous	Central area verruculose to verrucose grading into radial folds which terminate in a circular ridge; marginal zone broad, flat	Mycelioid and leathery	Entire and mycelioid
D9c-1	44	Central area warm buff; margin of 3 mm. white	Slightly convex	Waxy	Central area rugulose; marginal zone irregular, flat	Yeast-like and granular	Irregularly lobate and mycelioid
D9c-2	46	Light buff partially masked by mycelium	Slightly convex	Waxy to cretaceous	Smooth	Partially mycelioid and leathery	Entire

Table 7.--Cultural characters of a monosporidial line of Sorosporium everhartii and two of its mutants growing in triplicate flasks of potato-dextrose agar, 21 days after inoculation.

Line	Diam. in mm.	Color	Elevation	Luster	Surface and topography	Consistency	Margin
E9b	43	Light buff partially masked by mycelium	Slightly convex	Waxy to cretaceous	Smooth	Partially mycelioid and leathery	Entire and mycelioid
E9b-1	44	Light buff masked by mycelium; margin of 5 mm. white	Broadly umbonate	Dull to cretaceous	Central area verrucose to rugose grading into radial folds which terminate in a circular ridge; marginal zone irregular, flat	Mycelioid and leathery	Slightly lobate and mycelioid
E9b-1-1	45	Pinkish buff	Slightly convex	Waxy to glossy	Central area rugulose grading into radial folds which usually terminate at the margin	Yeast-like and granular	Erose and bacteroid

Mutations

Bauch (1926) in his studies on the physiology of Ustilago bromivora was the first to record a mutation in the smut fungi. Since then, mutations have been observed in most of the smuts that have been studied extensively.

Mutations were observed in monosporidial cultures of Sorosporium everhartii either as sectors or as patch mutants, the former predominating. A sector mutant is illustrated in Plate IX, fig. 1 and in Plate X. Mutations occur as a wedge or a fan shaped zone in the culture and start at the edge of the colony while patch mutants occur later and start between the margin and center of the colony. Sector mutants are more easily recognized and isolated than the patch mutants. Twenty-two such variations have been isolated and compared with the parental colonies under similar conditions. Duplicate cultures were very consistent in cultural characters and varied from their parental cultures in radial growth, color, elevation, luster, surface and topography, consistency, and margin. Most of the mutants were so distinct in their characters from the parental cultures that it was impossible to tell from which line they were isolated. This is shown by the appearance of D9c-1 and D9c-2, the first and second mutants of D9c (see table 6 and Plate IX). D9c-3 is just developing as a sector in the colony D9c illustrated in the photograph Plate IX, fig. 1.

A mutant may sector giving rise to another mutant which is

distinctly different from either the parent mutant or the original parental line. Thus E9b-1 is a mutant from E9b while E9b-1-1 is a mutant from the mutant. These are described in table 7 and illustrated in Plate IX, figs. 4-6.

Some of the lines mutated several times during the 15 months that they were in culture while others did not mutate at all. Line A20a did not mutate for one year, then suddenly mutated four times within three weeks. No mutable lines were observed similar to Stakman's et al. (1929) cultures of Ustilago zeae in which as many as 220 mutants occurred from a single line within a year. Two mutants per flask were the most observed in this study of Sorosporium everhartii. It is possible that if more monosporidial isolations had been made and studied in greater detail, that a more mutable line might have been secured.

Cultural Characters of Monosporidial Lines and their Derivatives on Four Different Agars

Eighteen monosporidial lines and 12 of their derivatives were grown each in duplicate 125 cc. Erlenmeyer flasks on potato-dextrose, carrot dextrose, corn meal-dextrose, and Richard's agar to study their cultural characters on various agars.

Fourteen days after inoculation, notes were taken on such cultural characters as radial growth, color, elevation, luster, surface and topography, consistency, and marginal characters. The cultural char-

sctors of E8a, E13b, and E13a are given in tables 8, 9, and 10 respectively, and illustrated in Plate XI, while those of the four derivatives of A20a are given in tables 11 to 14 and illustrated in Plate XII.

In general, the radial rate of growth was greatest for the cultures on the corn meal-dextrose agar, slightly less on the carrot-dextrose agar, still less on the potato-dextrose agar, and least for those on Richard's agar. It must be remembered, however, that the radial rate of growth is not a true index of the total growth. Although the cultures on the corn meal-dextrose agar were the greatest in diameter, on the average, the growth was in the nature of a thin mycelioid layer over the surface of the agar, and their total growth was undoubtedly the least.

The color of the different lines varied most on the potato agar, less on the carrot, and hardly at all on the corn meal and Richard's agar. The colonies on the potato and Richard's agar were generally convex, on the carrot agar umbonate, and on the corn meal agar flat. The luster of the lines varied most on the potato and carrot agars and least on the corn meal and Richard's agar.

The surface and topography of the lines varied most on the potato agar. Some cultures were smooth while others were very rough. The cultures on the carrot agar were usually rough in the center but had a broad flat marginal area. Some of the cultures had radiating ridges or furrows while others were clockwise in this respect. The short radiating slightly clockwise furrows of E13a is well illustrated in

Plate XI, fig. 10, and those of A20a-3 in Plate XII, fig. 8. Although this phenomenon was observed in this monosporidial line and mutant on the carrot agar, it did not manifest itself when grown on the other three agars. Stakman et al. (1929, pp. 18-19) while working with monosporidial lines of Ustilago zeae (Beckm.) Ung. and their mutants found that when the cultures exhibited strictly radial growth, the hyphae grew straight; but when the mycelium had a tendency to grow in a clockwise or counter-clockwise direction the hyphae curled one way or the other depending on the direction of growth.

In consistency, the cultures on the potato, carrot, and Richard's agar were usually leathery and mycelioid, while those on the corn meal agar had a narrow yeastoid center and the remainder of the culture was mycelioid. The margin of the colonies on the various agars did not vary to any great extent. The greatest total variation occurred between the different lines on the potato-dextrose agar.

A number of the flasks was mixed and an attempt was made to group them in their respective order according to the duplicate cultures of the same line on the same agar, the same line on the four different agars, and the different lines on the same agar. It was fairly easy to group most of the duplicate cultures of those lines and mutants grown on the potato and carrot agars, but it was impossible to classify the cultures grown on corn meal and Richard's agar. In general the cultural characteristics of the same sporidial line or mutant were very different on the four agars. Consequently, it was impossible to place

the duplicate flasks of one line on one agar with those of the same line on another agar. It was possible, however, to group all of the cultures on corn meal agar and all of those on Richard's agar, and in the majority of cases, it was possible to tell the cultures on potato-dextrose agar from those on carrot agar. Potato-dextrose agar and carrot-dextrose agar are very satisfactory for differentiating monosporidial lines and their mutants while corn meal-dextrose agar and Richards agar are unsuitable for this purpose.

Table 8.--Cultural characters of monosporidial line E8a of Sorosporium everhartii growing in triplicate flasks on four different agars, 14 days after inoculation.

Agar*	Diam. in mm.	Color	Elevation	Luster	Surface and topography	Consistency	Margin
PDA	25	Light buff masked by mycelium	Slightly convex	Waxy to cretaceous	Rugose	Slightly mycelioid and leathery	Entire
CDA	31	Central area of 10 mm. light buff; marginal area white with circular powdery areas	Umbonate	Waxy to cretaceous	Central area of 11 mm. rugulose; marginal area flat with many concentric pulverulent areas	Slightly mycelioid and leathery	Entire and mycelioid
CMDA	30	Central area of 7 mm. light buff; marginal zone white	Flat	Dull	Flat with many faint concentric rings	Central area yeastlike; marginal zone mycelioid	Entire and mycelioid
RA	20	Cream-buff	Convex	Waxy	Coarsely pulverulent	Leathery	Entire

*PDA - Potato-dextrose agar
CDA - Carrot- " "
CMDA - Corn meal-dextrose agar
RA - Richard's agar

Table 9.--Cultural characters of monosporidial line E13b of Sorosporium overhartii growing in triplicate flasks on four different agars, 14 days after inoculation.

Agar*	Diam. in mm.	Color	Elevation	Luster	Surface and topography	Consistency	Margin
PDA	27	Central area light buff masked by mycelium; marginal zone of 2 mm. white	Convex	Waxy to cretaceous	Central area verruculose grading into irregular radiating ridges which terminate 2 mm. from the margin; marginal zone flat	Mycelioid and leathery	Slightly lobate, fimbriate and mycelioid
CDA	39	Central area of 17 mm. light buff masked by mycelium; marginal zone white	Umbonate	Cretaceous	Central area with radiating ridges which terminate 10 mm. from the margin; marginal zone flat with many faint concentric rings	Mycelioid and leathery	Entire and mycelioid
CMDA	39	Central area of 4 mm. light buff; marginal zone white	Flat	Dull	Flat with many faint concentric rings	Central area yeast-like; marginal zone mycelioid	Entire to slightly lobate and mycelioid
RA	23	Cream-buff	Convex	Waxy	Coarsely pulverulent to smooth	Leathery	Entire

*PDA - Potato-dextrose agar
CDA - Carrot- " "
CMDA - Corn meal-dextrose agar
RA - Richard's agar

Table 10.--Cultural characters of a monosporidial line E15a of Sorosporium everhartii growing in triplicate flasks on four different agars, 14 days after inoculation.

Agar*	Diam. in mms	Color	Elevation	Luster	Surface and topography	Consistency	Margin
PDA	31	Light buff masked by mycelium	Convex	Cretaceous	Central area smooth grading into a few broad radiating ridges which terminate 2 mm. from the margin, marginal zone flat	Mycelioid and leathery	Entire, fimbriate and mycelioid
CDA	39	Central area of 17 mm. light buff masked by mycelium, marginal area white	Umbonate	Cretaceous	Central area crater-like; surrounded by short, clockwise radiating furrows which terminate 11 mm. from the margin; marginal zone flat with many concentric rings	Mycelioid and leathery	Entire and mycelioid
CMDA	40	Central area of 4 mm. light buff masked by mycelium; marginal area white	Flat	Dull	Flat with many faint concentric rings	Central area yeast-like; marginal zone mycelioid	Entire and mycelioid
RA	21	Cream-buff	Convex	Waxy	Coarsely pulverulent to smooth	Leathery	Entire

*PDA - Potato-dextrose agar
CDA - Carrot- " "
CMDA - Corn meal-dextrose agar
RA - Richard's agar

Table 11.--Cultural characters of the first mutant from a monosporidial line A20a of *Sorosporium everhartii* growing in triplicate flasks on four different agars, 14 days after inoculation.

Agar*	Diam. in mm.	Color	Elevation	Luster	Surface and topography	Consistency	Margin
PDA	27	Light buff partially covered with irregular scattered patches of white powder	Slightly convex	Waxy	Smooth; partially covered with irregular powdery areas	Leathery	Entire
CDA	30	Light buff central area masked by mycelium	Slightly convex	Waxy to cretaceous	Smooth to finely regulose	Mycelioid and leathery	Entire
CMDA	33	White	Flat	Dull	Flat with one or two pits in the center and a few faint concentric rings	Mycelioid	Entire and mycelioid
RA	21	Light buff masked by fine white powder	Convex	Dull to cretaceous	Finely pulverulent	Leathery	Entire

*PDA - Potato-dextrose agar
CDA - Carrot- " "
CMDA - Corn meal-dextrose agar
RA - Richard's agar

Table 12.--Cultural characters of the second mutant from a monosporidial line A20a of Scrosporium everhartii growing in triplicate flasks on four different agars, 14 days after inoculation.

Agar*	Diam. in mm.	Color	Elevation	Luster	Surface and topography	Consistency	Margin
PDA	33	Lilac gray partially masked by mycelium	Slightly convex	Waxy to cretaceous	Central area verruculose; surrounded by rugose zone which grades into irregular radiating ridges that terminate 2 mm. from the margin; marginal zone flat	Mycelioid and leathery	Slightly lobate and mycelioid
CDA	37	Central area light buff masked by mycelium; margin of 10 mm. white	Umbonate	Cretaceous	Central area rugulose; surrounded by radiating ridges which terminate 11 mm. from the margin; marginal zone broad, flat with a few faint concentric rings.	Mycelioid and leathery	Entire and mycelioid
CMDA	42	Central area of 7 mm. light buff; marginal zone white	Flat	Dull	Flat with a few faint concentric rings	Central area yeast-like; marginal zone mycelioid	Entire and mycelioid
RA	24	Light buff masked by fine white powder	Convex	Dull to cretaceous	Coarsely pulverulent	Leathery	Entire

*PDA - Potato-dextrose agar
CDA - Carrot- " "
CMDA - Corn meal-dextrose agar
RA - Richard's agar

Table 13.--Cultural characters of the third mutant from monosporidial line A20a of *Sorosporium everhartii* growing in triplicate flasks on four different agars, 12 days after inoculation.

Agar*	Diam. in mm.	Color	Elevation	Luster	Surface and topography	Consistency	Margin
PDA	36	Central area light buff; surrounded by smoke grey; partially masked by mycelium	Slightly convex	Waxy to cretaceous	Central area rugose to rugulose grading into radiating ridges which terminate 3 mm. from the margin; marginal zone flat	Mycelioid and leathery	Slightly lobate, fimbriate and mycelioid
CDA	37	Central area pallid mouse grey partially masked by mycelium; margin of 9 mm. white	Umbonate	Cretaceous	Central area of 7 mm. rugulose; surrounded by clockwise radiating furrows which terminate 11 mm. from the margin, marginal zone broad, flat, with many concentric rings	Mycelioid and leathery	Entire and mycelioid
CMDA	38	Central area of 8 mm. light buff; marginal zone white with radiating drab-grey bands 2 mm. wide	Flat	Dull	Flat with many concentric rings	Central area yeast-like; marginal zone mycelioid	Entire and mycelioid
RA	30	Light buff masked by mycelium	Convex	Dull	Verruculose to powdery	Leathery	Entire

*PDA - Potato-dextrose agar
CDA - Carrot- " "
CMDA - Corn meal-dextrose agar
RA - Richard's agar

Table 14.--Cultural characters of the fourth mutant from monosporidial line A20a of Sorosporium everhartii growing in triplicate flasks on four different agars, 14 days after inoculation.

Agar*	Diam. in mm.	Color	Elevation	Luster	Surface and topography	Consistency	Margin
PDA	34	Light buff partially masked by mycelium	Slightly convex	Waxy to cretaceous	Central area verruculose; surrounded by rugose zone; marginal zone of 3 mm. flat	Mycelioid and leathery	Slightly lobate and mycelioid
CDA	39	Central area light buff partially masked by mycelium; margin of 13 mm. white	Umbonate	Waxy to cretaceous	Central area rugulose; surrounded by short radiating ridges which terminate 12 mm. from the margin; marginal zone broad, flat with many concentric rings	Mycelioid and leathery	Entire and mycelioid
CMDA	38	Central area of 7 mm. light buff; marginal zone white	Flat	Dull	Flat with many concentric rings	Central area yeast-like; marginal zone mycelioid	Entire and mycelioid
RA	28	Light buff masked by white powder	Convex	Dull	Pulverulent	Leathery	Entire

*PDA - Potato-dextrose agar
CDA - Carrot- " "
CMDA - Corn meal-dextrose agar
RA - Richard's agar

STUDIES ON SPHACELOTHECA HOLCI

Germination of Chlamydospores of Sphacelotheca holci

The chlamydospores of S. holci germinate readily within 24 hours, on potato-dextrose agar. On this medium the germinating chlamydospore generally produces a promycelium containing three septa dividing it into four cells. Each cell gives rise to a primary sporidium which may bud profusely, producing a large number of secondary sporidia.

Susceptibility and Morphology of Sorghum halepense Inoculated with Sphacelotheca holci

A small quantity of seed of Sorghum halepense was dusted with chlamydospores of Sphacelotheca holci and placed on moist blotting paper in petri dishes to germinate in an incubator at about 24°C. Similarly, non-inoculated seed was germinated as a check. This seed was obtained through the courtesy of I. M. Atkins, Texas Substation No. 6, Denton, Texas. The seed germinated rather irregularly but when the plumules were from two to three centimeters long the germinated seed was transplanted to twelve-inch pots in the greenhouse. Fifty-five plants from the inoculated seed grew to maturity and 49 plants from the non-inoculated seed. Forty-seven per cent of the former group of plants was infected with smut while all of the checks were normal. The smutted plants averaged about 40 per cent of the height of the healthy plants. This is illustrated in Plate VI. The

affected plants also have narrower culms, smaller leaves, not as well developed root systems and headed earlier than the non-smutted plants.

Microscopic Comparison of Chlamydospores, Promycelia, and Sporidia of *Sphacelotheca holci* and *S. cruenta*

A comparison was made in the laboratory of the chlamydospores, promycelia, and sporidia of *Sphacelotheca holci* and *S. cruenta* (Kuhn) Potter to study the similarity of these two smuts. Both of these fungi cause loose kernel smuts of *Sorghum vulgare* and *S. halepense*; infection takes place in the seedling stage; and the infected plants head earlier and are much smaller than the normal plants. Dimensions of the chlamydospores of *S. holci* and *S. cruenta* were determined by microscopic measurements of 200 spores of each. The spores were suspended in a drop of water on a glass slide and measured with the aid of an eye piece micrometer. These measurements are given in tables 15 and 16 and graphically represented in figure 6.

Chlamydospores of these fungi were germinated on the surface of hanging drops of potato-dextrose agar in van Tieghem cells. After twenty-four hours measurements were made of the length and width of mature promycelia (those containing four cells) and primary sporidia that were apparently fully developed (see table 15). The chlamydospores of *S. holci* are slightly larger and germinate producing larger promycelia and sporidia than those of *S. cruenta*. The spores of the former are also verruculose-echinulate while those of the latter are

smooth. There are definite morphological differences between these smuts but the two are undoubtedly closely related.

Table 15.—Comparison of diameter of chlamydospores in water of Sphacelotheca holci and S. cruenta, and length and width of their promycelia and primary sporidia when germinated on the surface of a drop of potato-dextrose agar underneath a cover glass, in a van Tieghem cell, February, 1935.

Measurement	Number measured	S. holci		S. cruenta	
		Range	Ave.	Range	Ave.
		μ	μ	μ	μ
Diameter of chlamydospores	200	6.4-10.2	8.2	5.1- 8.3	6.3
Length of promycelia	25	20.5-34.8	28.7	24.3-29.4	26.0
Length of sporidia	25	9.0-12.8	10.2	7.7-10.2	9.0
Width* of promycelia	25	3.6- 6.4	4.1	2.6- 3.9	3.1
Width* of sporidia	25	2.6- 3.8	3.3	2.6- 3.2	2.8

*Approximate measurements.

Table 16.--The frequency in classes of the diameters of chlamydospores of Sphaceloth̄ea holci and S. cruenta.

Class in microns	Frequency	
	S. holci	S. cruenta
4- 4.99	0	0
5- 5.99	0	59
6- 6.99	9	104
7- 7.99	66	35
8- 8.99	115	2
9- 9.99	8	0
10-10.99	2	0
11-11.99	0	0

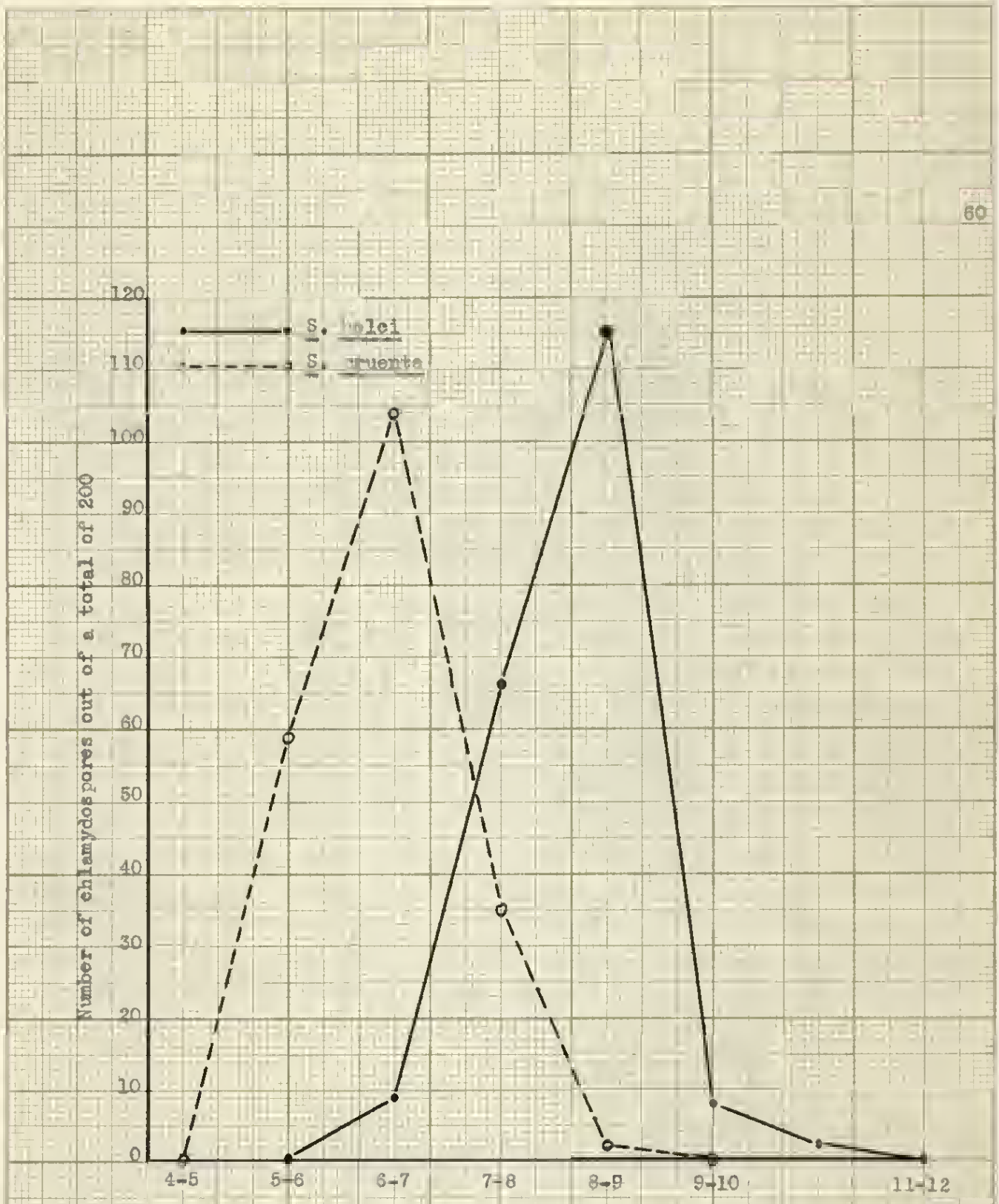

Fig. 4. Graph showing comparison of the diameters of chlamydospores of Sphacelotheca holci and S. cruenta.

Reaction of Sorghum vulgare to Sphacelotheca holci

In the spring of 1936, the seed of six varieties and hybrids of Sorghum vulgare Pers. was dusted with the spores of Sphacelotheca holci and planted in the field to study the susceptibility of the sorghums to this smut. These varieties apparently were not susceptible to the collections of this smut from Sorghum halepense as no infection occurred (see table 17).

A few smutted secondary panicles arose by branching of the stalks on Red Amber x feterita and Pink kafir, but the general appearance of the sori and microscopic examination of the chlamydospores from these panicles proved that the causal organism was S. sorghi (Link) Clinton. These smutted panicles did not appear until late in the season after heads infected by S. sorghi were numerous in adjacent experimental plots. It seems, therefore, that such infections were the result of local infections of lateral buds by wind-carried spores of S. sorghi as was found to be the case for S. cruenta by Reed and Faris (1924, p. 534). The conditions for infection were ideal as about 80 per cent of the heads of Pink kafir were infected with S. sorghi in another experiment. The chlamydospores of S. holci were known to be viable from germination tests and because high infection was secured in inoculated plants of Johnson grass in the greenhouse. It appears therefore, that S. holci does not readily attack these sorghums. The writer plans to test varieties of sorghums which are more closely re

related to Sorghum halepense and those which are more susceptible to Sphacelotheca cruenta.

Table 17.--Reaction of sorghum varieties and hybrids to Sphacelotheca holci, 1936.

Variety or hybrid	Accession number	Date planted	Number plants	Per cent infection
Red Amber x feterita	K.B. 2570	May 5	77	0*
Dwarf Yellow milo	C.I. 332	May 5	62	0
Pierce kaferita	K.B. 2547	May 5	57	0
White Yolo	K.B. 2525	May 5	43	0
Kafir x feterita	K.B. 2686	May 5	52	0
Pink kafir	K.B. 3706	May 5	65	0*

*A few secondary panicles were infected by Sphacelotheca sorghi, probably due to local infection of lateral buds.

SUMMARY AND CONCLUSIONS

Identification of Smuts on the Andropogoneae

Twenty-one different smut collections on the tribe Andropogoneae were made in Kansas and Oklahoma during 1935 and 1936 and identified. The following facts regarding these collections and the causal organisms are of particular interest:

Sorosporium everhartii was collected in Kansas for the first time. Andropogon furcatus was discovered to be a new host for this fungus. The sori occurred in the pedicellate and staminate spikelets almost as often as in the sessile and fertile spikelets. Separate infection of the staminate tissue frequently occurred in both the sessile and pedicellate spikelets.

Sorosporium provinciale was also collected in Kansas for the first time. It produced a striking disease in that the inflorescence and the entire peduncle was replaced by the smut sorus.

Sphacelotheca andropogonis was collected on Andropogon scoparius in Kansas for the first time.

Sphacelotheca holci was collected in North America for the first time. Sorghum halepense is a new host for this fungus. The pedicellate and staminate spikelets were affected as well as the sessile and fertile ones.

The pedicellate and staminate spikelets of both Andropogon furcatus and A. hallii were affected by Sphacelotheca occidentalis to

almost as great an extent as those of the sessile and fertile spikelets.

Field observations indicated that the plants which were affected with Sorosporium provinciale, Sphacelotheca andropogonis, Sphacelotheca holci, and Sphacelotheca occidentalis were smaller and headed earlier than the apparently normal plants.

Studies on Sorosporium everhartii Ellis & Gall.

Laboratory studies were made on Sorosporium everhartii in relation to such phases as the germination of chlamydospores, and cultural aspects of monosporidial lines. It was found that mature chlamydospores germinate readily on potato-dextrose agar, carrot-dextrose agar, and on the surface of water. The spores germinate very irregularly, producing from one to several promycelia each of which has from one to three septa. Sporidia are usually produced on nutrient agar, whereas on water each cell of the promycelium generally sends out a germ tube. As germination progresses and under certain conditions, protoplasmic migration in the promycelium occurs. The optimal temperature for the germination of chlamydospores on water at the end of 24 hours was about 27°C., the minimum below 12°C., and the maximum between 33°C. and 36°C.

Sorosporium everhartii comprises an indefinite number of monosporidial or haploid lines as demonstrated by their cultural characteristics. These lines may differ from other lines in rate of growth, color, elevation, luster, surface and topography, consistency, and marginal characters of the colonies. From this study it appears that

chlamydospores are undoubtedly heterozygous in nature as demonstrated by the cultural characteristics of different haploid lines from the same spore. The optimal temperature for the radial rate of growth of the monosporidial lines on two per cent potato-dextrose agar lies between 30°C. and 33°C., the minimum is below 4°C. and the maximum between 35°C. and 40°C.

Mutants occur as sectors in most of the lines. Patch mutants may also occur but less frequently than sectors. These mutants may differ from their parents or other mutants in any of the cultural characteristics in which monosporidial lines differ.

Potato-dextrose agar and carrot-dextrose agar are satisfactory for studying cultural characteristics while Richard's agar and corn meal-dextrose agar are unsuitable for differentiating lines. On different agars, however, the cultural characteristics of the same monosporidial line or mutant are entirely different.

Studies on *Sphacelotheca holci* Jackson

The germination of chlamydospores was observed and a preliminary study was made on the life history of *Sphacelotheca holci* on *Sorghum halepense*. The glumes were removed from the seed to facilitate higher infections. A small quantity of seed was dusted with chlamydospores and germinated on moist blotting paper in petri dishes in an incubator at about 24°C. Similarly, non-inoculated seed was germinated as checks. The seed was then transplanted to twelve-inch pots in the greenhouse.

The chlamydospores germinate readily on potato-dextrose agar. On this medium the germinating chlamydospore generally produces a promycelium containing three septa. Each cell gives rise to a primary sporidium that may bud profusely, producing a large number of secondary sporidia.

Infection of the host takes place in the seedling stage. The infected plants of Sorghum halepense head earlier than normal plants. They are also shorter in stature, the stems are smaller in diameter, and the leaves are narrower than those of normal plants. A comparison was made in the laboratory of the chlamydospores, promycelia, and sporidia of Sphacelotheca holci and S. cruenta. From these studies and greenhouse observations it appears that the two smuts are very closely related. There are, however, definite morphological and physiological differences that make the two distinct species.

In the spring of 1936, the seed of six varieties and hybrids of Sorghum vulgare Pers. was dusted with the spores of Sphacelotheca holci and planted in the field to study the susceptibility of the sorghums to this smut. These varieties apparently were not susceptible to the collections of the smut from Sorghum halepense as no infection occurred.

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Plate I. Sorosporium everhartii Ellis and Gall.
on Andropogon furcatus (x2).

1. Normal inflorescence.

2. Normal seed.

3, 5, 7, and 9. Infected inflorescences.
The sori from the staminate tissue are
especially apparent in the central
part of the right raceme in 5.

4. Immature sori from pistillate tissue.

6. Mature sori from pistillate tissue.

8. Sori from staminate tissue.


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Plate II. *Sorosporium everhartii* on *Andropogon furcatus* (x3).

1. Sessile spikelet with one sorus.
2. " " " two sori.
3. " " " three sori.
4. " " " four sori.
5. Pedicellate " " one sorus.
6. " " " two sori.
7. " " " three sori.
8. " " " four sori.
9. Normal seed of *Andropogon furcatus*.
10. One sorus from a sessile spikelet.
11. Two sori " " " "
12. Three sori " " " "
13. Four " " " " "
14. One sorus " " pedicellate "
15. Two sori " " " "
16. Three sori " " " "
17. Four " " " " "

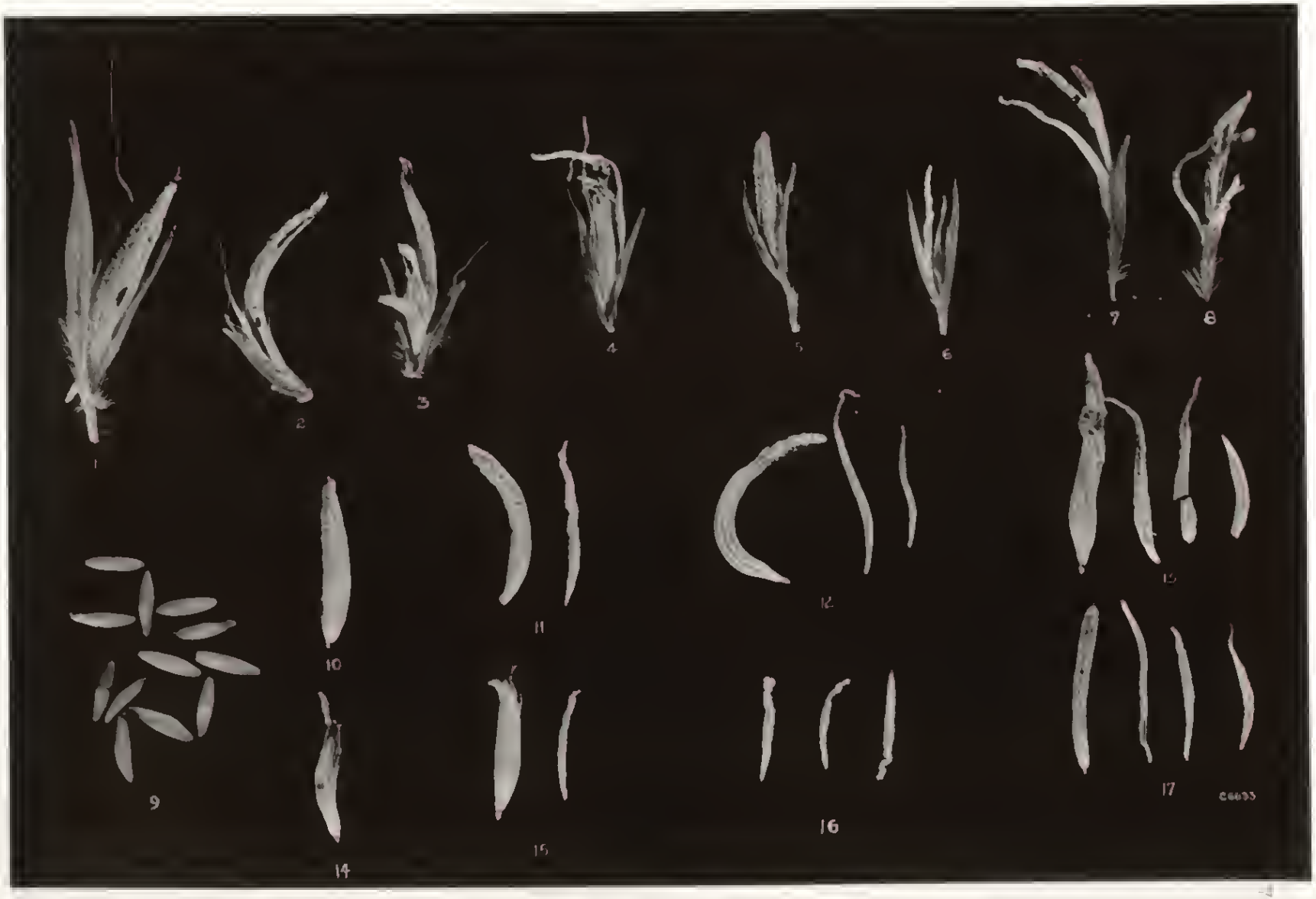
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Plate III. Three different smuts on Andropogon furcatus (x2).

1 and 2. Sorosporium everhartii Ellis & Gall.

3 and 4. Sphacelotheca occidentalis (Seym.) Clinton.

5 and 6. Sorosporium provinciale (Ellis & Gall.) Clinton.

Plate IV. Two different Sphacelotheca smuts on *Andropogon furcatus* and *Sorghum halepense* (x 1.5).

1. *Sphacelotheca andropogonis* (Opiz) Bubak on *Andropogon furcatus*.
2. Normal seed of *Sorghum halepense*.
3. Normal inflorescence of *Sorghum halepense*.
4, 5, and 6. *Sphacelotheca holci* Jackson on *Sorghum halepense*. Different stages of maturity. Note the well developed central columellae in 4 and 5. The sori in 6 are just appearing above the glumes.

Plate V. Sphacelotheca holci on Sorghum halepense (x5).

1 to 4. Immature infected sessile and pedicellate spikelets.

5 to 8. Mature infected sessile spikelets and immature infected pedicellate spikelets. Although the sori of the former are just appearing above the glumes, the peridia have started to flake away.

9 to 11. Branches of the paniole with infected sessile and pedicellate spikelets.

12 to 14. Infected sessile and pedicellate spikelets in a later stage, showing the prominent columellae. Most of the peridia have fallen away.

15. Normal seed of Sorghum halepense.


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10

Plate VI. Effect of Sphacelotheca holci on the normal development of Sorghum halepense (x0.15).

1 to 5. Normal non-inoculated plants of S. halepense.

6 to 10. Smutted plants of S. halepense. Some of the infected plants are stunted considerably while others are only partially reduced in height.

Plate VII. The effect of temperature on the rate of growth of a monosporidial line A20a of Scrosporium everhartii grown on potato-dextrose agar at 12 different temperatures, 21 days after inoculation (x0.45).

Temperature

1. 4.1°± C.
2. 9.1°± C.
3. 12.3°± C.
4. 15.0°± C.
5. 18.0°± C.
6. 20.9°± C.
7. 23.8°± C.
8. 26.9°± C.
9. 29.4°± C.
10. 32.6°± C.
11. 35.5°± C.
12. 39.9°± C.


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Plate VIII. Cultural differences between four monosporidial lines of Scrosporium overhartii, grown on potato-dextrose agar, 21 days after inoculation. Two are shown in duplicate to show the consistency of cultural characteristics (x1).

1. E8d
2. E11a
3. D9d
4. D9d
5. D9a
6. D9a


1
2
3
4
5
6
C6646.

Plate IX. Cultural differences between two monosporidial lines D9c and E9b of Sorosporium everhartii and four of their mutants grown on potato-dextrose agar, 21 days after inoculation (x1).

1. D9c
2. D9c-1
3. D9c-2
4. E9b
5. E9b-1
6. E9b-1-1

A mutant is just developing as a sector in the colony D9c.


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C6647.

Plate X. A mutant A20a-1 developing as a sector in a monosporidial culture A20a of *Scrosporium everhartii* (x1).

Plate XI. Cultural differences between three monosporidial lines of Scrospora overhartii grown on four different agars, 14 days after inoculation (x1).

	E8a	E13b	E13a
Potato-dextrose agar	1	5	9
Carrot-dextrose agar	2	6	10
Corn meal-dextrose agar	3	7	11
Richard's agar	4	8	12


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Plate XII. Cultural differences between four mutants from a monosporidial line A20a of Sorosporium everhartii grown on three different agars, 14 days after inoculation (xl).

	Potato-dextrose agar	Carrot-dextrose agar	Corn meal-dextrose agar
A20a-1	1	2	3
A20a-2	4	5	6
A20a-3	7	8	9
A20a-4	10	11	12


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