IDENTIFICATION OF CERTAIN SPECIES OF FUSARIUM ISOLATED FROM POTATO TUBERS IN MONTANA

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INTRODUCTION

The economic importance to this State of Fusarium wilt and various storage and field rots of potatoes has been recognized for several years. Between 1914 and 1918 isolations were made from affected tubers until nearly 100 cultures had been obtained. These isolations have been cultured on artificial media, being transferred about twice a year and kept in a refrigerator at about 10° C.

The purpose of this paper is to record the taxonomic work on these various Fusarium cultures. No especial endeavor was made to connect any of these species with an ascigeral stage, and in all our work no culture showed any form of growth suggesting such a stage.

SOURCE OF MATERIAL

The tubers from which the isolations were made came from many different localities in the State. In fact, practically all sections except the extreme northwestern are represented. The exact source of each culture will be found in Table I. Of the 97 original isolations only 70 were used.

There is little doubt that dryrot and Fusarium wilt are of large economic importance to all potato growers in the State. In 1917, this Station reported in the plant disease survey a loss of 4 per cent in the potato crop due to wilt and a loss of 3 per cent due to dryrot. The year 1917 is considered a normal one. These figures therefore represent the average yearly loss due to potato diseases caused by species of Fusarium. This loss is fairly evenly distributed over the State.

LITERATURE REVIEWED

A great deal of work has been done and a number of papers have been written on Fusarium troubles of potatoes, but the viewpoint in nearly all of this work is economic. However, in reporting on the economic phase many of the authors include some taxonomic data, and for this reason the following reviews are given. Occasional comment on individual papers has been made, but this has not been done consistently throughout. For convenience the various papers are taken up in chronological order.

Smith and Swingle (19) found that there was always present in the darkened vascular bundles of tubers affected with wilt, which had not at that time been separated from dryrot, a fungus which on culturing they found to be a species of Fusarium. In order to determine the characters of this fungus they grew it on about 40 different media and under various temperature conditions. The bulletin gives in detail the results of these

1 Accepted for publication July 24, 1922.
2 Reference is made by number (italic) to “Literature cited,” p. 363-364.

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studies. To this organism the authors applied the name *Fusarium oxysporum* Schlechtendal because they felt it “not at all certain” that the various names given to species of *Fusarium* growing on potatoes really stood for distinct forms. They therefore considered them as synonyms and used the earliest available name. This paper was the pioneer for *Fusarium* work in this country, and in addition to the careful work done, mycologists and pathologists are greatly indebted to its authors for the impetus given to the study of potato Fusaria, both in this country and abroad.

In 1910 Appel and Wollenweber (2) published a basis for a monograph of the genus *Fusarium* Link. This paper was the first to give a comparatively exhaustive treatment of the species of *Fusarium*. It is divided into two parts. In the first part the following subjects are discussed in detail: Methods, including media, inocula, nutrients, light, temperature, color standards, variation in forms appearing in the cultures, mycelium, etc., lack of distinction between microconidia, and macroconidia and characters which constitute a “normal” culture or “normal” spore. These authors give a description of the genus *Fusarium*, discuss its relationships, and list its synonymy.

The second part relates entirely to taxonomy. Thirteen known species are described with the greatest care and detail, each description being the record of a research problem in itself. This paper is, without doubt, the most fundamental in the literature on *Fusarium*. It is not here reviewed in proportion to its worth, because our experimental work was not directly influenced by it. The paper was published in Germany 10 years ago and did not include many of the common American species. Therefore, it was not well fitted for our identification work. The paper contains a very good bibliography of the *Fusarium* problem.

In 1912 Jamieson and Wollenweber (9) described the symptoms of a disease causing dryrot of potato tubers, first noted on tubers sent from Spokane, Wash., in February, 1910. To the causal organism of this disease Wollenweber gave the name *Fusarium trichotheciodes*, placing it in the Discolor group. Inoculation experiments proved the pathogenicity of this organism in producing the characteristic dryrot of tubers.

In 1912 Wilcox, Link, and Pool (20) published a research bulletin in which they described a rot which is practically identical with that described by Jamieson and Wollenweber (9), but due to the fact that the two papers were published so close together and that the investigational work was being carried on simultaneously and independently, these authors gave to the casual organism the name *Fusarium tuberivorum* Wile, and Link, which we know now to be a synonym of *F. trichothecioides* Wollenw. Wilcox, Link, and Pool’s paper is of particular value to our work because of its emphasis on taxonomy.

This paper gives the history and distribution of the dryrot of tubers and the economic importance and symptoms of the particular dryrot under discussion. The authors give a résumé of the genus *Fusarium* and allied genera from 1809 to date, concluding with Appel and Wollenweber’s description of it.

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various conditions which might influence the number of septations is discussed, and the results of actual investigations are recorded in detail.

In summarizing the taxonomic portion of the paper the authors endeavor to find a logical place for their species, and after several suggestions and comparisons they leave it unplaced, but give a detailed description of the organism. Some pathological studies are discussed with emphasis on the mode of infection, resistance, and susceptibility of varieties and methods of control.

Wollenweber (21), studying the genus Fusarium taxonomically, came to a few definite conclusions: The stroma as a taxonomic character in species determination is unreliable. A pure culture method which gives the normal stages is necessary, and the culture media recommended was steamed stems of trees, shrubs, or herbaceous plants for conidia and chlamydospore production and rice, potato tubers, and other starchy media for secondary characters, such as color, extra large sclerotia, and stromata. He is not sure that Fusarium is an obligate conidial stage of an ascomycete. He puts much emphasis on the importance of the proof of pathogenicity of the organisms, and in his discussions of each species he indicates the kind of parasite.

Wollenweber was the first to assemble into sections species of Fusarium having related characters. He considered a uniform shape of conidia the most important of the characters on which the division could be based. The sections which he described in this paper have been used in all taxonomic work with this genus since that time. Other sections, of course, have been added, but the original sections have for the most part never needed to be amended.

The 20 species of Fusarium described, of which only 3 are new, are grouped under the sections, practically all of which include wound parasites capable of destroying parenchymatous tissue, except the first, which includes vascular parasites only. Wollenweber's sections in their order are: Elegans, Martiella, Discolor, Gibbosum, Roseum, and Ventricosum.

Later Wollenweber (22), in morphological and pathological study of the divisions of fungi having cylindrical and crescent-shaped conidia, states definitely that fungi with cylindrical septate conidia fall outside the genus Fusarium, and belong, when the perfect form is known, to the genera Nectria, Hypomycos, and Mycosphaerella, and, when the perfect form is not known, to Cylindrocarpon when chlamydospores are absent and to Ramularia when they are present.

Lewis (11), working in Maine, carried on comparative studies with some 40 different cultures of Fusarium isolated from various hosts, including 7 isolated from potatoes. He noted how the growth of the cultures was effected by different media, different quantities of acid and alkali, and different temperatures. He tested for gas in fermentation tubes, but obtained negative results only. Tests of pathogenicity were carried on with all of the cultures, and considerable cross inoculation work was done. He made no attempt to identify the species, because the published descriptions were so incomplete as to make critical comparisons with them impossible. However, Wollenweber was in the United States at that time, and Lewis sent his cultures to Wollenweber, who identified most of them. His results were added as an appendix to the bulletin.

Four of these species—Fusarium poae, F. helianthi, F. conglutinans, and F. pirium—do not later appear in the literature as occurring on potato. Sherbakoff (18) says of the first two that they are closely
related to *F. sporotrichioides* n. sp. and belong to the section Sporotrichiella; the third species belongs to section Elegans and is closely related to *F. orthoceras*; and the fourth may belong to section Arthrosoriella. Sherbakoff does not recognize any of these species but disposes of them all in saying:

No technical description, except results of inoculations for potatoes, always negative, and certain characters of color and of colony growth, is given, and thus a proper identification is rendered impracticable.

Harter and Field (7) from the results of their work on the stemrot of the sweet potato concluded, as Appel and Wollenweber (2), that the type of inoculum—mycelium or spores—has a marked influence on the culture. They also proved the pathogenicity of *Fusarium hyperoxysporum* Wr. and *F. batatis* Wr. for the sweet potato and obtained negative results in their inoculation experiments with *F. oxysporum* Schlecht., *F. orthoceras* Ap. and Wr., *F. caudatum* Wr., and *F. radicicola* Wr. on the same host.

Wollenweber (23) discussing the species of *Fusarium* occurring on sweet potatoes, points out the necessity of agreeing on the criteria by which a "normal" culture may be known, to avoid wide discrepancies in describing what is in reality the same species. He describes 11 species of *Fusarium*, two species of *Hypomyces* and one of *Gibberella* occurring on sweet potatoes. This included all the species of *Fusarium* then known to occur on sweet potatoes. Under each species is given a "diagnosis" or description of the type culture, habitat, and a general discussion of its history and relationships. In the case of new species, of which there are 6, the relationships are taken up with a great deal of care.

A descriptive key to all these species is included based upon the characteristics of pure cultures grown in daylight. Regarding the key the author makes the following comment:

This key might have been based entirely upon the morphological characters and curvature of the conidia but since the color reactions offer a simpler, though less trustworthy means of identification, they have been employed. This key, therefore, should be regarded only as an aid in identification, not as a guide to the morphology, which has been discussed in the diagnosis and illustrated in detail in the illustrations.

Sherbakoff (18) realizing the chaotic condition of the genus *Fusarium*, especially those species occurring on potato, conducted a research problem to discover on what basis the American species as well as those discussed by Wollenweber could best be separated.

In general, Sherbakoff verified the principles and the results of Appel and Wollenweber's (2) work in Germany. He believed, however, disagreeing with Appel and Wollenweber, that there should be a distinction drawn between microconidia and macroconidia, and that the presence or absence of the microconidia may be used in distinguishing species. He also disagrees with Appel and Wollenweber in believing that species can be distinguished—

when grown on almost any medium, including artificial media, provided that the medium is not extremely poor or rich in food materials, and also provided that the moisture supply in the medium is well regulated. Sherbakoff found no coremia nor typical pionnotal form of fructification as did Appel and Wollenweber.

The author outlined the scope of the work, discussed the source of material and methods of isolation, culture media, effect of light and temperature, variability in the species of *Fusarium*, relative taxonomic
importance of different characters, defined certain forms of fructification, reviewed the genus Fusarium, and explained the difficulties of identifying species of Fusarium with previously described species because of the scarcity of taxonomic detail in the descriptions. The greater part of the memoir is taken up by the description of sections, genera, species, and varieties. He listed 20 previously described species, including parasitic and nonparasitic forms—all that were known to occur on potatoes—and 41 new species and varieties. These 61 forms he distributed under Wollenweber's eight sections and three additional ones that he himself originated and defined. Three species of Ramularia, a genus closely related to Fusarium and also occurring on potatoes, are included. There are drawings of practically all species and varieties, showing conidia, conidiophores, and occasionally mycelium. Spore measurements are given for spores grown on various culture media.

Based on the ideas of Appel and Wollenweber, Sherbakoff (18) worked out a dichotomous key for the species of Fusarium described, which, while the best yet published, is far from perfect. Imperfections in keys are rather inevitable until methods used in the identification of the species are better standardized. Until standard methods are adopted, the boundaries of species can not be closely enough defined to prevent investigators from introducing numerous varieties, separating one from another on minor characters that are not stable under all conditions.

In the key, Sherbakoff uses septations and shape of conidia most often as differential characters. Presence or absence of microconidia, chlamydospores, sclerotia, sporodochia, type of fructification, and color and type of conidiophores are also used. Difficulty with the key arises most often in the case of varieties. Individual difficulties of this sort will be noted later. Confusion often arises from the misuse of the terms macroconidia and microconidia, but this may be due to typographical errors.

C. W. Carpenter (4), in a paper on tuber-rots caused by species of Fusarium, includes a section on taxonomy which gives the description of eight species. Among these one is new, _F. eumartii_, which falls into the section Marteilla.

Link (13) shows some very interesting results from physiological studies on _Fusarium oxysporum_ Schlect and _F. trichothecioides_ Wr. Comparisons of the two species are given to show temperature relations, growth, habit, and food requirements and pathogenicity to tubers and growing plants.

Pratt (15), in a paper on control of powdery dryrot caused by _Fusarium trichothecioides_ Wr., concludes that this species is of the highest economic importance of all of the Fusarium species in the irrigated portion of the West, and in another paper (14) notes that _F. radicicola_ Wr. is rather common in desert soils.

Hawkins (8), in studying the effect of certain species of Fusarium on the composition of the potato tuber, found that _Fusarium oxysporum_ and _F. radicicola_ secrete sucrass, maltase, xylase, and diastase. The last-mentioned enzym is apparently incapable of acting on the ungelatinized potato starch. The purpose of the study was to find out what constituents of the potato are most easily destroyed by the fungus and what compounds can not be utilized by it either in respiration or in building its own tissues. Their results are not conclusive as to whether kind or quantity of secretion is in the least specific, as only three organisms were used in their experiments, but this article introduces an interesting phase of the Fusarium problem.
Pratt (16), in studying the relation between soil fungi and diseases of the Irish potato in southern Idaho, isolated among many other fungi 14 species of Fusarium. Five of the strains isolated “apparently differed from all species heretofore described,” and Pratt, therefore, named them, giving in this publication the original descriptions, which include habitat, cultural characteristics, spore shape, septations, and size. Septations and size are given of spores grown on various media for different ages.

The new species described are: Section Gibbosum, *Fusarium lanceolatum*; Section Elegans, *F. elegantum* and *F. Idahoanum*; Section Discolor, *F. aridum* and *F. nigrum*.

The other nine species were identified, but in only one case, that where Pratt’s culture showed some differences from the “authentic culture,” which he used for comparison, are there any taxonomic notes.

Bisby (3) in his studies on Fusarium diseases in Minnesota notes that *Fusarium oxysporum* and *F. discolor* var. sulphureum are of large economic importance in Minnesota. His results with certain temperatures and media in studying these diseases are of interest to use, but otherwise the bulletin is strictly economic in its outlook.

Edson and Shapovalov (5) made a careful study of the relations of growth of certain species of Fusarium to temperature. The species they used in the studies were: *Fusarium discolor* var. sulphureum (Schlect) Ap. and Wollenw., *F. eumarii* Carp., *F. oxysporum* Schlect, *F. radicicola* Wollenw., *F. trichothecioides* Wollenw.

Two species of Verticillium were also used. For each of these species they made nine plate cultures and incubated them at nine different temperatures from 10°C up to 40°C at 5°C intervals, taking readings of the size of the colony at the end of each 24 hours. The results, aside from aiding in control determinations, proved to the authors that temperature tests in certain cases may serve as a useful supplementary method for the identification of fungi exhibiting contrasting thermal relations.

**THE GENUS FUSARIUM LINK.**

The genus Fusarium is classified according to Engler and Prantl *Naturliche Pflanzenfamilien* (6) as belonging to the section Mucediacae Pharagmosporae of the family Tuberculariaeae of the order Hyphomycetes of that heterogenous class known as the Fungi Imperfecti. It is, consequently, a form genus, and already the ascigerous stage of a number of its species has been found. A few of these are *Nectria solani* (Ren. and Bert.), which has been reported as the ascigerous stage of *Fusarium solani*; *Nectria graminicola* B and N., as the ascigerous stage of *F. nivale*; and *Gibberella saubinetii* (Durieu and Mont.) Sacc. to which species *F. tulmorum*, *F. avenaceum*, *F. hordei*, and *F. heterosporum* have all been referred. It seems very probable that more and more species of this genus will be connected with genera of the Ascomycetes, though as Wollenweber states (21)—

We are still far from having conclusive proof of the widely recognized theory that *Fusarium* is the obligate conidial stage of Ascomycetes.

The genus Fusarium was described in 1809 by Link (12), together with the allied genera Fusidium, Fusisporium, and Atractium. Later Link dropped one or the other or combined them in various ways. Schlectendahl, Corda, Fries, and Saccardo worked on this group of organisms and
classified them in various ways, but they all recognized the imperfections of the classification. In their monograph Appel and Wollenweber (2) have established the boundaries of the genus Fusarium, using Atractium Link, Fusidium Link, Fusisporium Link, Selenosporium Corda, Fusoma Corda, and Pionnotes Fries, either in toto or in part, as synonyms.

The synonymy of the genus Fusarium given by Appel and Wollenweber (2) is quoted below, and a translation is given of their description and notes.

Synonomy:
Fusisporium Link in Spec. Plant 1, S. 30 (1824).
Selenosporium Corda Icon. I. S. 7 (1837).
Fusoma Corda Icon. I. S. 7 (1837).

Conidia more or less polar, mostly dorsiventral, seldom distinctly round (radiâr), more or less curved; when ripe usually septated; more or less colored when in masses; borne one after another in the same spot, but not connected in chains on the end of simple or branched septate conidiophores which appear spread out between the hyphae or joined as they are in coremia, or grouped together in sporodochia.

Conidia spread out in a powdery form between the hyphae or tubercular-like on a limited gelatinous sporodochia, a slimy layer or occasionally as pionnotes without definite boundaries. Chlamydosphores, oval or pear shaped, single or in bunches, in chains or bunched up, remaining joined for some time, terminal or intercalary, not more than one borne in the same place. The chlamydophore is not very different from the conidiophore, and it has no distinctive color. It never gathers in gelatinous layers.

Hyphae septate, variously branched epi- and endo-phytic, occurring sparingly or in great quantity, either isolated or together, curly or thick, partly like coremia, or especially like a stroma to plectychymatic form with definite shape or without definite shape, more often similar to an even growth all over, limited or spread out, often closed up together on the inside, occasionally building up brightly colored mycelium.

Note that it is undecided whether species that do not have septate conidia should be kept separate from the genus or be placed in a subgenus Fusamen according to Saccardo (17); but there is no question about those which have a tendency toward septation as F. orthoceras. It is also undecided in what order of importance the characters should be taken. The choice is between septations, dorsiventrality, polarity and the curve of the long axis of the conidia. It is very questionable whether Fusarium should be placed under Leptosporium as in Saccardo, and nothing but the study of the different forms can decide the boundaries of the genus. Concerning the color of the conidia masses it can be said that black does not appear normally, neither does black mycelium. Light orange and ochre colors predominate in the conidia. The mycelium also has yellow, red and blue. The term sclerotium as used in Fusarium is disputable. Researches have not shown that the term sclerotia used in Fusarium is dispositive. Researches have not shown that the term sclerotypia was justifiable for the plectychymatic structures found.

In 1913 Wollenweber (22) excluded from the genus all species having septocylindrical conidia. In bringing this genus to date, therefore, this fact should be incorporated. Sherbakoff (18) describes the genus concisely as follows:

Hyphomycetes, with from hyaline to bright, but never plain gray nor black, conidia and mycelium; conidia sickle-shaped, septeate (usually 3 or more septeate), apically pointed, mostly pedicillate, not appendiculate, noncatenulate; conidia scattered over substratum, in pseudopionnotes or in sporodochia, the latter without or with from flat to wart-like plectenchymatous substratum, and always without any differentiated enclosing or surrounding structures; conidiophores from simple to irregularly verticillate.
ISOLATION OF CULTURES

The original cultures were all obtained from infected tubers by the following method:

The infected tubers were thoroughly washed, dipped into a 1 to 1,000 solution of mercuric chloride, and cut open. In order not to contaminate the infected parts of the tuber, the healthy portion was cut almost to the edge of the discolored portion and the tuber then broken open, care being taken that nothing should touch the advancing margin of the fungus. A piece of discolored tissue was taken from the advancing margin and put into a tube of melted agar, which was then poured into a Petri dish and incubated at room temperature. Six to 10 plates were made from a single tuber. The plates were watched carefully to be sure that a pure culture was the result of the isolation. When this was assured a transfer was made to agar slants, and these were kept as stocks. It was surprising how very few contaminations appeared in the plates. Each culture was numbered, and all notes, including source, were kept under this number.

SINGLE-SPORE ISOLATIONS

In 1916 when detail work with these cultures was anticipated it seemed best to take every precaution to be assured of pure cultures, for a mixed culture of two or more species of Fusarium could easily pass unnoticed. Therefore, a single-spore isolation was made from each culture in the following manner:

A sterile platinum needle was used to transfer spores from the stock culture to tube 1, which contained 5 cc. of distilled sterile water. From this tube a series of five dilutions was made into tubes, each containing 5 cc. of sterile distilled water. Three 3-mm. loops of material were taken from tube 1 and put into tube 2; three 3-mm. loops of material were taken from tube 2 and put into tube 3; and three 3-mm. loops of material were taken from tube 3 and put into tube 4. Tubes of standard beef agar were melted and poured into Petri dishes and allowed to cool. From each dilution tube ½ cc. of material was made to flow over the surface of the plated agar. Any excess material was drained off. The plates were allowed to incubate at room temperature for about 16 hours, when they were searched with a microscope for germinating spores. All microscopic examination was made through the bottom of the inverted Petri dish.

Fusarium spores are for the most part hyaline and to locate them on a plate of clear agar is very difficult. The scheme was devised of sprinkling a few sterile spores of Tilletia foetans of wheat over the surface of the agar with a tiny sterile spatula. These spores were easy to locate and gave the plane of focus in which the Fusarium spores could be found. The use of the sterile smut spores proves to be a great timesaver.

Usually the search for spores began with the more heavily sown plates, down through the more dilute ones until a spore was found which was sufficiently isolated so that it alone could be removed. The position of this spore was marked by a ring of India ink on the glass and it was then cut out by means of a stiff platinum cylinder illustrated in Phytopathology (10) and placed on the upper end of an agar slant. It was carefully watched by means of the microscope to be sure that all growth came from the spore and not from a piece of mycelium from the edge of the piece of agar, as often happened when the germinating spores were not sufficiently isolated. When it was assured that all growth came
from the spore it was considered pure and the culture was kept as a stock. If there was any question as to the source of the growth the culture was discarded. Sometimes a number of attempts had to be made before the stock culture was obtained.

**COMPARATIVE CULTURAL STUDIES**

Since the purpose of our work was the identification of the species of Fusarium that we had found occurring on potatoes in Montana, we began by comparing the cultural characteristics of each isolation product, hoping to be able to group together those which belonged to one species so that we might eliminate unnecessary duplication with cultures of the same species. This hope was realized only in one group. After a very few trials with different media and at different ages, one group, containing about 20 per cent of all the cultures, separated itself out very constantly. Its growth was so characteristic that we then believed and have since proved that it was *Fusarium trichothecioides* Wr. There were other groups, some seven of them, each containing from 2 to 10 cultures; but the identity of the members of the groups was not sufficiently striking to warrant leaving any of them out of further studies. However, these groupings aided materially when actual identification work began. Seventeen series of cultures have been studied. A series consisted of all the transfers made at the same time and incubated under similar conditions for the purpose of comparative studies. At least four sets of individual notes were taken on each series, so as to bring in the influence of age on the characters.

The characters emphasized in these notes were color, amount, and nature of the growth of mycelium, absence or presence and color of pseudopionnotes or sporodochia. All color determinations were based on Ridgway's "Color Standards." 3

In practically all the series the cultures were inoculated in triplicate, so that when variations arose between cultures inoculated from the same stock culture a decision as to which was the more normal could be made. As a preliminary to note taking, those cultures which had sufficient similar cultural characters to suggest identity were put into groups. Each group was designated by a letter, and these were placed in a table in parallel vertical lines, a column for each note taking, so that gross group comparisons could easily be made. Notes on each group were made in detail at the end of the table. If a culture showed only a slight variance from a group it was put into the group, but with special additional notes, and was designated in the table by the group letter with a subnumber. In so far as possible the same letters were used throughout for the same group. After a few sets of notes were taken one or two cultures which seemed most typical of a group were chosen as type cultures, and these numbers were given the same letter each time and served as the nucleus for the group represented by that letter.

Some few cultures, the number varying with the medium on which they were grown, did not develop any distinctive microscopic characters; others developed distinctive characters on certain media; and occasionally the same culture when on one medium was placed in a certain group, while on another medium it would be placed in a different group, or, as

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often happened, would seem to fit none of the groups. These refractory cultures caused considerable trouble, and some of them have not yet been identified.

MICROSCOPIC STUDIES

USE OF PHOTOMICROGRAPHS

The microscopic studies that are most extensively used in classifying Fusaria are the size, shape, and septation of the spores. In view of the fact that different kinds of spores are produced and that in each kind there is much individual variation, the problem becomes in some cases rather complex.

In a goodly portion of the microscopic studies photomicrographs were used. The spores were mounted in water containing a small quantity of dilute Myer's flagella stain. The cover glass was blotted with a coarse filtered paper, pressed down, and sealed with paraffin to prevent evaporation and consequent movement of the spores. The pictures were taken soon after the slides were made, for the mounts gradually dried out. If they were put into a moist chamber they could sometimes be kept intact for 24 hours or more.

The Leitz horizontal photomicrographic apparatus IA No. 398, with Leitz microscope stand, having an apochromatic condenser, was used for this work. All pictures were taken magnified 500 diameters.

The photographic method proved very convenient, for by means of it actual reproductions of spore material were made when the spores were at their best and the data were studied when convenient. This permitted a massing of data by means of which close comparative studies of microscopic characters could be made, which without photographs could not have been done.

Drawings, of course, might have been used instead, but there is no doubt that the photographic method is far superior to that of drawing. In the first place, actual reproductions of selected fields of spores are made instead of a few spores selected in accord with the personal preference of the worker and idealized and perfected in the process of drawing. Drawings are often misleading in their fine definitions and detail. Secondly, if the worker is careful to photograph an average field, which in most cases is easy to do, a great deal of additional data are recorded on the picture, which the one who was drawing would have to add in notes that take too much time when the critical stage of a large group of cultures is demanding his attention. Such data, for instance, are the percentage of spores with a certain number of septa, the percentage of macroconidia and microconidia, limits of size of different types of spores, etc.

Two complete sets of pictures were taken, except for cultures that would not fruit. One was of the fungi from cultures about 47 days old, grown on oat agar, and the other of fungi grown on lima bean agar when the cultures were about 10 days old.

The spores of some cultures would not take the stain; others, particularly those having mostly microconidia, even though perfectly sealed exhibited Brownian movement, and still other cultures produced so few spores that a field suitable to photograph was impossible to find. Many attempts, some of which were successful, were made to grow these refractory cultures on a medium that would produce a greater abundance of spores, and whenever successful pictures were taken. Notable
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Advance was made with some of them when grown on sterilized tomato stems and leaves.

In order to see what effect age and medium had on spore characters, three species, each typical of a group, were chosen and about 10 photographs were taken of them, grown on different media and at different ages. Our conclusions are indicated on page 354.

MICROSCOPIC NOTES

The only key published which includes the greater number of American species of Fusarium is Sherbakoff's (18), and it was therefore used as a basis for this work. In determining the various sections this key was very useful, but owing to the fine distinctions made between species and especially between varieties much difficulty was experienced in identifying an unknown culture.

In order to secure the necessary data for the identification work microscopical study was made of all cultures grown on hard oat agar and on lima bean agar, in addition to the data secured from our microphotographs of the cultures grown upon the same media, but under somewhat different conditions. The cultures grown on hard oat agar were about 15 weeks old and had been kept in the refrigerator at 10°C. The cultures grown on lima bean agar were about 4 weeks old and had been kept in a dark incubator at about 21°C.

As with the series of cultures used for the microphotographs, we had difficulty here also with certain ones not fruiting; and additional notes were made for certain cultures at about 4 weeks of age when grown on potato glucose agar and at various ages, depending upon the organism, when grown on tomato stems, potato plug, and lima bean agar.

In these examinations particular care was taken to include some mycelium in order to determine whether or not chlamydospores were present, for in the photographic work it was natural to select a field filled with spores rather than one filled with mycelium, and the presence or absence of chlamydospores, which character Sherbakoff used considerably, was often overlooked in the earlier studies.

MEDIA USED

A limited number of media was used in our work, for the consensus of opinion of those investigators who have used a large variety, notably Smith and Swingle (10), Apple and Wollenweber (2), and Sherbakoff (18), seems to be that little is gained from so doing. Sherbakoff believed that all important characters were brought out on hard oat agar, certain vegetable stems, tuber plugs, and potato agar containing about 5 per cent glucose. We followed the suggestion of Sherbakoff but used a few additional media. The formulas of the media follow:

OAT AGAR.—One hundred gm. rolled oats were put in 1,000 cc. of water and cooked for an hour in an Arnold steamer which varied in temperature from 50° to 75°C.

The product was strained through cheesecloth and the volume of liquid was brought up to 1,000 cc. Thirty gm. of agar were added, and the mixture was put in the autoclave and the pressure allowed to rise gradually to 15 pounds, where it remained for 15 minutes. The material was then tubed, and the tubes were plugged and autoclaved.
LIMA BEAN AGAR.—This was made in the same way as the oat agar except that the decoction was made by heating 100 gm. of broken pieces of lima beans in 1,000 cc. of water.

POTATO GLUCOSE AGAR.—This medium was made in the same way as the oat agar except that the decoction was made from 100 gm. of sliced potato tubers, and just before the mixture was tubed 50 gm. of glucose (T. J. Baker's c. p.) was added.

RICE.—About 3 gm. of rice were put into each tube, 10 cc. of tap water were added, and the tubes were plugged and autoclaved.

POTATO PLUG.—Cylinders cut from potato tubers were slanted and placed in tubes with enough water added to about cover the cylinder. The tubes were plugged and autoclaved.

SWEET CLOVER STEMS.—Stems of sweet clover (Melilotus alba) were cut into convenient pieces. If the stems were large enough only one was placed in a tube. Most of them, however, were small and two to four pieces were used. The stems were dry, so water enough to cover them was added. The tubes were plugged and autoclaved.

TOMATO STEMS.—Stems and leaves of young tomato plants (Lycopersicum esculentum) were cut into convenient pieces, put into tubes with distilled water added to within about ½ inch of the top of the stems. The tubes were plugged and autoclaved.

Five series of cultures were grown on oat agar, five on potato glucose agar, three on lima bean agar, two on potato tuber plug and one each on the other media mentioned above.

TEMPERATURE AND LIGHT CONDITIONS USED

By far the greater number of the series of cultures were grown in the dark in an incubator regulated between 20° and 22° C. This temperature, according to data of various investigations, seemed to be nearest the optimum for the greater number of species.

In all cases where the "dark" incubator is mentioned it refers to one the temperature of which was regulated by burning a 20-watt carbon light automatically controlled by a thermostat in the lower portion of the incubator. The cultures were kept in cans on wire screen shelves, and the light given off from this bulb should possibly be considered as influencing the results, though it does not seem probable that it did as the temperature of the room was such that the bulb was lighted but a small portion of the time.

One series of cultures grown on potato glucose agar was kept in diffused light and incubated at room temperature which varies from about 18° to 25° C.

Another series grown on potato glucose agar was inoculated in quadruplicate, and two tubes of each culture were grown in the "dark" incubator at 20° C. and two similar tubes grown in an incubator with glass doors, designated as the "light" incubator, which was in the greenhouse so placed that the cultures were in strong diffused light during the day. The tubes were kept in glass beakers and only a few in each beaker. The temperature of the "light" incubator was kept at 21.5° C.

Still another comparison between cultures grown in light and darkness was made with lima bean agar. In this case two tubes of each culture were kept in the dark incubator at 20° C. and two each were kept in diffused light in a room at a temperature which varied between 18° and 25°.
GENERAL DISCUSSION OF METHODS

EFFECT OF MEDIA

No distinct effort was made to determine the comparative values of different media, for in order to determine such values a large number of trials should be made on each medium under observation. However, the results which we gained from a few trials on a few media may be of some value.

EFFECT ON CULTURAL CHARACTERS.—The growth made on sweet clover stems lacked color. In fact certain cultures (those ordinarily grouped under K, a Discolor group, and under D, an Elegans group) which constantly produced color on all other media did not exhibit any on sweet clover. The growth was scanty in proportion to that on other media. This may have been due to the fact that the stems used were old ones which had been very dry. However, Sherbakoff (16) notes that the presence of the epidermis on stems seems to lessen the development of aerial mycelium and to favor production of fewer but better developed sporodochia. We also found that sweet clover stems seemed to favor the production of sporodochia and pseudopionnotes. A few cultures which had not at any time formed sporodochia did so on this medium. The sweet clover stems were sometimes covered with a thick, tough layer of plectenchymatic-like tissue which seemed never to bear spores.

The little experience we had with tomato stems suggested that they, too, favored the production of spores. However, our work with this medium was very limited, as we used it only on refractory cultures.

Lima bean agar proved to be another medium that did not stimulate color production. For instance on Group F (a Martiella group) notes taken 12 days after inoculation read, "a slight appearance of greenish blue growth," while cultures of the same group grown on oat at 11 days showed "various combinations of blues, greens, and purples." The D group (an Elegans group) which was mulberry purple on oat showed white or gray on lima bean.

That series of cultures (p. 350) growing on lima bean which was divided and part grown in the diffused light and part in the dark incubator showed practically no difference in color production. Only two cultures produced color in the dark that did not produce any in the light. The amount of mycelium on the lima bean cultures varied somewhat with the groups.

The mycelial growth on potato plug was abundant, and color appeared in varying degrees. That is, more color was produced on potato plugs than on lima bean or on the stems of sweet clover, but less than on the oat or potato glucose agars. Thick, tough layers of plectenchymatic tissue formed over the tuber plugs, as it did over the stems of sweet clover, but sporodochial growth was rare.

The greatest variety of colors was produced on rice, and finer group distinctions were brought out on this medium than on any other used in the cultural studies, but the colors were very mixed and they seemed not to stay true to group. Very little reliance was put on these results at the time the notes were taken. It is interesting to look back and see how nearly true to group the color determinations were. However, the mixture of colors produced was so difficult to describe that except for grouping purposes it is doubtful if rice as a cultural medium would have any specific value.
Again and again in the literature is noted the fact that for color production agar with glucose is the medium to use. Our results showed that the cultures grown on potato glucose agar did develop color, but not much more so than did those on oat agar. The color on the glucose agar in some cases was deeper than on the oat. For instance, notes taken at practically the same age on both media give for the color of group A (a Discolor group) on potato glucose "vinaceous cinnamon to orange cinnamon" and on oat it is "salmon buff to salmon color." For group C (a Discolor group) it reads "Bordeaux" on the glucose and "spinel pink" on the oat. However, the deepness of color is not constant throughout for special notes on culture No. 16 give "seashell pink" as the color on glucose and "cinnamon" as the color on oat. Our results would indicate that the two media are about equal in color production value and the amount of growth is practically the same, being abundant on both.

**Effect on Microscopic Characters.**—No striking difference microscopically was noted between spores grown on lima bean, potato glucose, or oat agars (Pl. 1, A and B; 2, C). We found that the series of oat cultures kept in the refrigerator was in the best condition for spore study of all with which we worked, but it seems probable that this was due to the temperature rather than the culture medium, as the other oat series was about equal to the lima bean.

The few refractory cultures that were grown on tomato stems led us to believe that that medium might prove to be very good. At least it would be worth while to try it out further.

Appel and Wollenweber (2) concluded from their work that agar media were by no means so sure of producing normal conidia as the tubers, and the stems were found to be the most satisfactory of all. Wilcox, Link, and Pool (20) state that cultures grown on gelatin and agar media are not normal and can not be used in the determination of characters. Our results do not support these conclusions, but they confirm a statement made by Sherbakoff (18):

An agar, especially such a one as oat hard agar, often gives all the forms of fructification for these fungi, with "normal" spores and more or less typical and brilliant color production.

It might be well to add here a footnote given by Sherbakoff (18) in explanation of the variance between his results and those of Appel and Wollenweber (2):

This observation is apparently in some contradiction to the observations of Appel and Wollenweber (1910: 12–13), but indeed it is not so; because, judging by the "artificial" media actually used by them, their observations of unfitness of such media for study of "normal" growth of the Fusaria was based on "soft" agars too rich in sugar. The writer also found that such agars produced abnormal growth.

We found that oat agar more than any other medium used—combined the qualities necessary to produce good cultural characters such as growth and color and normal spores.

**Effect of Light**

So little comparative work was done upon the effect of light on growth and color that our results are of rather limited value. The effect of light on Fusarium has never been thoroughly studied. Our conclusions and those of a few other investigators are included below.

Using lima bean agar for a medium, we found practically no difference in the amount of growth or the amount of color produced in cultures...
Species of *Fusarium* Isolated from Potato Tubers

grown in the dark incubator and those grown in diffused light, but the *lima bean* is not a good color producer under any condition.

With potato glucose agar there was some difference, though it was not striking. These comparisons were drawn, however, from cultures grown in the light incubator and others grown in the dark incubator (p. 350). Our notes taken when the cultures were a week old show that in the dark group A produced rufous pseudopinnotes; in the light they were ferruginous. A more intense purple color showed in group D when grown in the light than when grown in the dark. The most striking comparison, in fact practically the only striking one, was in the case of culture No. 69 which produced carmine mycelium in the light and white mycelium when grown in the dark.

Notes taken on a complete series of week-old cultures grown on potato glucose in diffused light showed practically the same results as those taken on the cultures grown in the dark incubator.

Smith and Swingle (19) found that often cultures which produced—a beautiful, rich salmon colored mycelium when grown in sunlight produced white mycelium when grown in a dark closet.

A difference in color was not noted on all media tried. Except for a difference in color these men concluded that light had no material effect on the growth.

Appel and Wollenweber (2) noted that conidia masses were much richer in color when grown in the light than in the dark. They also noted that when cultures were grown in the dark, poorly developed conidia with uneven septations and form appeared. Although direct sunlight was not exactly injurious the diffused daylight was most favorable in every way for the product of morphological characters.

**EFFECT OF TEMPERATURE**

We did no work with the effect of temperature on the fungi, but a summary of the conclusions of other workers may be of value.

In working with *Fusarium oxysporum*, Smith and Swingle (19) found that the fungus grows well on boiled potatoes at a temperature of from 15° to a little above 30° C. Below 15° the growth became slower and slower until 5° was reached, when practically no growth took place. Above 371/2° no growth took place.

Link (15) did some detailed work on temperature relations of *Fusarium oxysporum* and *F. trichothecioides*. The optimum temperatures for the two are different. However, at temperatures between 15° and 20° C. a good growth was made by both.

Lewis (11) in his work with 24 cultures of Fusaria found that 20° to 25° C. seemed to be the best range of temperature for most of the cultures.

Appel and Wollenweber (2) in their summary of conditions which will guarantee a “normal growth” say that room temperature should be used, that is, “between 12° and 25° C. neither higher nor lower.”

Edson and Shapovalov (5), working on temperature relations of six of the more common species of Fusarium, found that growth took place in varying amounts between 2° and 38° C. The minimum for growth was shown by *F. discolor* var. *sulphureum*, the maximum by *F. radioiocola*. The maximum growth for all cultures took place between 25° and 30°, though growth was abundant between 15° and 30°.
A temperature of between 19° and 22° C. was used for practically all the work reported in this paper. Incidentally it was found that cultures kept in the refrigerator at 10° showed an unusually good spore condition. It was very likely due to the fact that so low a temperature inhibited the growth and was about the optimum temperature for preservation of spores after their formation. Cultures kept in the refrigerator were always kept at room temperature for about a week after they were inoculated.

During the later spring months the light incubator in the greenhouse warmed up in the middle of the day to 30° or 35° C. We found that cultures kept in this incubator during that time deteriorated quickly.

The conclusion drawn from our own experience and that of others was that cultures can be grown as well at room temperature as at a fixed temperature, provided the temperature does not go lower than 12° or higher than 25° C. In case it is desirable to keep spores in a normal condition for a longer period of time than is possible at room temperature this can be done by keeping them in a refrigerator at 10° or less.

EFFECT OF AGE

Careful study of the series of cultures grown for the purpose of noting the effect of age on spore formation was in some measure disappointing. Isolated examples could be found that would illustrate practically any theory one might wish to propound. Too many factors enter in, such as moisture, nutrients, temperature, etc., for one to be able to make definite conclusions as to the effect of age. It seems that if all conditions are right to produce a "hoch" stage of a normal culture, age does not enter in more than that a very young culture or a very old one can not produce a "Hochkulture." Only relative age then, would seem to be of importance.

CONCLUSIONS AND SUGGESTIONS ON METHODS

The effects of media, temperature, light, and age, though not always very great on cultural and spore characters, are sufficient to make it advisable in describing characters to describe the culture media and conditions so freely as to make the repetition of the culture upon the same medium and under approximately the same conditions easily possible, to note under what conditions these results were observed, and to keep them within certain limits.

Our experience would suggest that cultures grown on a hard oat agar, in diffuse light, and at room temperature will give the best satisfaction.

4 Appel and Wollenweber (2) in their attempt to find distinctive terms by which to designate the degree of development and the age of the cultures created six terms which are briefly defined as follows:

ANKULTURE: A little mycelium from the original substance of some Fusaria is inoculated on tubers or stems. A pure, rich mycelial culture results in which there are either no conidia or only a very few, and these are apt to be irregular in shape and septation and are not suitable for morphological research.

NORMKULTURE: A culture which produces conidia readily and in which the spores are regular in form and septation.

ABKULTURE: A culture in which deterioration has set in, and the spores which have not disintegrated are small and usually have fewer septations than do those in the Normculture. The Normkulture is subdivided into three stages:

The Jungkulture, usually less than 8 days old, is one in which the spores have not reached a constant form of development, and spores of varying sizes and septations are found.

In the Hochkulture the spores are truly normal, that is, comparatively even in size, shape, and septations.

In the Altkulture the spores, due to lack of moisture or food, shrink a little in size; or if new spores are formed they are undersized, yet not deteriorated enough in form to belong to the Abkulture.

Plate 1. C and D illustrate the "alt" and "hoch" stage of the Normkulture as seen in our work.
The cultures may be kept in a normal condition for 12 weeks or more by keeping them at a temperature of 10° C. or less.

Were the standardization work suggested on page 356 to be attempted, we would suggest as the media to be tested out a hard oat agar, potato stems, potato plugs, and possibly potato glucose agar. It would be advisable to try several regulated temperatures, together with a room temperature, the limits of which should be given, probably 12° to 25° C. Tests should be made to determine that time nearest which all the species reach the normal stage of their growth, and if possible some method should be devised to standardize moisture and humidity conditions.

PITFALLS IN IDENTIFICATION WORK WITH SPECIES OF FUSARIUM

The greatest obstacle in the way of accurate determination of species of Fusarium is the lack of a good monograph of the genus, and this lack is due in part to nonstandardization of the methods used in identification work, especially as regards kinds of media, environmental conditions, and the relative value ascribed to various characteristics of the fungus when grown in pure culture under laboratory conditions. The species and varieties intergrade and the differential characters used in the keys are not sufficiently distinct to permit any but an experienced investigator to use the key. To become an authority one must work long enough and with large enough numbers of species so that he can create within himself a conception of the species. In other words, he judges to what species the fungus in question belongs rather than actually identifying it.

Pathologists in various parts of the world often in connection with some pathological studies isolate a species of Fusarium. In their eagerness to name the organism which is causing economic loss they describe it so incomprehensively that future workers are not able to identify their cultures with it and therefore more new names appear.

Appel and Wollenweber (2) made a good beginning toward a monograph, but it was merely a beginning. Sherbakoff (18) has helped the situation somewhat with his "Fusaria of Potatoes," but his key is deserving of the criticism given above. He has split species up into so many varieties that to identify a specimen beyond its section becomes a tedious task of scientific guessing.

In the near future the botanical world, especially the mycological world, must determine and actively promote some policy with regard to trinomial nomenclature. If all the flowering plants were split into varieties on as many minor characters as are the fungi, binomial nomenclature would before long be a thing of the past. The American Code of Botanical Nomenclature (7) states in regard to categories of classification that the terms "subspecies" and "subgenus," etc., may be used when additional categories are necessary for the convenience in presentation of relationships, but "the term variety is relegated to horticultural usage."

The mycologist's difficulty arises largely from lack of perspective. The person who has worked on a single group for some time sees very real, fine distinctions which would not be at all significant to other mycologists. These distinctions may not be of enough importance to justify his making a new species, but they are too real to him to be overlooked, and he therefore originates a variety. Would it not be better to keep in mind that a species must have more or less flexible boundaries due to evolution which has taken and is now taking place,
and in describing new species or in identifying new cultures allow for a certain amount of variance? The code quoted just above defines species as “connected or coherent groups of individuals.”

There is little doubt that in some cases physiological strains of species must be recognized by some system to be agreed upon, possibly by making new species, but until formally adopted by a representative body varietal names, especially those based on morphological characters, should be avoided.

SUGGESTION FOR A STANDARD METHOD FOR FUSARIUM STUDY

An important step in taxonomic work on Fusarium would be to standardize the methods for growing Fusarium species in somewhat the same way that certain bacteriological methods are standardized.

In order to do this it would first be necessary to carry on a comprehensive preliminary study. Interested workers in different localities would grow a large number of species of Fusarium, preferably subcultures from common stocks. The conditions of media composition, light, temperature, and humidity should be as nearly uniform as possible, selecting those suggested by the results of previous workers.

The method of note taking in the work should be sufficiently uniform to facilitate comparative studies of the results. From these comparative studies it could be concluded what conditions proved most satisfactory in growing species of Fusarium.

Selecting the most promising method thus obtained as a provisional standard, cultural work should again be carried on by a very large number of workers and with a very large number of species, and notes taken in a uniform manner. A comparison of the various notes on single species would determine whether or not the method used could be adopted as “a permanent standard.”

By careful study of the various notes taken on all species of Fusarium used, the most stable characters could be determined and a really workable key made.

Such a procedure would involve a large expenditure of time and money and much care on the part of the workers. To find enough interested workers with the time to devote to such a study might in itself be a difficult task. However, until a key based on comprehensive data of this kind is made we see little hope for accuracy in identification. (See also page 354.)

RESULTS OF IDENTIFICATION WORK

The cultural and microscopic data, acquired as described above, were carefully studied and with the aid of Sherbakoff’s (18) key many of the cultures under investigation were identified. Their descriptions and identifications follow according to groups. Practically all the cultures were found to be included under Wollenweber’s three sections: Elegans, Discolor, and Martiella. Notable exceptions to this are No. 20, 69, and 75. For various reasons some few of the cultures are only provisionally identified, and two are not identified at all. The summary given in Table I will show which these are and give the reason for indecision. (See page 362.)

Since Sherbakoff’s key was used, the descriptions of species given by him were taken as the standard in most cases. If questions arose about
individual characters, comparisons were made with descriptions by other investigators whenever such descriptions were available.

**SECTION ELEGANS**

One group (Group D) fell within this section. It included No. 21, 24, 25, 27, 28, 29, 31, 45, 46, 58, and 59. This was a difficult group to identify because of the scarcity of macrospores and the variations in color. Microscopically the group (with the exception of No. 59) falls into the species *Fusarium oxysporum*, but no tube culture produced sclerotia, which according to Smith and Swingle (19) were green in color and always found in cultures grown on potato plug, and according to Sherbakoff (18) were:

Bluish black in color, constantly present on potato tuber plug and sometimes on different agars.

In plate cultures grown on potato agar with 5 per cent of glucose, a few of the numbers (21, 28, and 46) produced small, dark purple spots, which on examination proved to be masses of nonsporing mycelium; but after four weeks of growth these small masses of mycelium seemed too loose to be called sclerotia. The fact that they did not form consistently throughout the group also suggests that they are not the sclerotia noted by the authors mentioned above.

The color of our cultures, also, does not quite agree with former descriptions of the species. Sherbakoff gives “macroconidia in mass usually of pinkish buff color” but neglects to state on what medium this is true.

On potato-glucose agar plates kept in the light most of our cultures (No. 24, 25, 27, 29, 45, 58, and 59) showed salmon coloring varying from light buff to ochraceous salmon, but shades of purple are typically found. Combinations with pinks and sometimes with greens occur but a greater or less amount of purple was characteristic of the group under all conditions. The only media used in common with Smith and Swingle were boiled rice and potato tuber plugs. Smith and Swingle found the color on the former when grown in the dark “mixed pink and lilac shading into white.” Our notes show a production of purple (true) to resolane purple. On potato tuber plug these authors noted the growth when made in the darkness was “pure white changing to creamy white.” We noted a slight development of a pinkish and purple pigment when grown in the dark.

However, these discrepancies in color do not seem sufficient to throw these cultures out of *Fusarium oxysporum*, but the lack of sclerotia seems important. We would, therefore, identify these cultures as *F. oxysporum* var. *ascerotium*, a variety described by Sherbakoff which differs from *F. oxysporum*—by the absence of sclerotia, and definite plectenchymic sporodochia, in color of the mycelium and somewhat longer and narrower macroconidia.

Sherbakoff neglects to state in what way the color differs.

Macrospores were very scarce in all the cultures of this group, and in No. 24 none at all were found. In the other numbers they varied.

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6 It might seem inconsistent after the discussion on binomial nomenclature on page 355 to make use of varietal names in our classification. We recognize the disadvantages of trinomial nomenclature but feel justified in following it to avoid still further confusion of the names used in this genus. We feel, however, that some official recommendations and action should be taken upon the important question by societies qualified to represent mycology.
considerably in size. This variation in size seemed to bear no consistent relationship to numbers, age, media, or temperature. We are therefore including in the limits given the various sizes of the spores measured on the different cultures.

*Fusarium oxysporum* Schlect. var. *asclerotium* Sherb. (Description taken from No 21, 24, 25, 27, 28, 29, 31, 45, 46, and 58.

Macroconidia typically dorsiventral, dovesiveness sometimes slight, usually distinct, and if so, ventrally curved. More or less uniform in diameter, with more or less abruptly attenuated apex; base pedicillate.

Microconidia very abundant. Oftentimes no macroconidia present, especially in cultures that have been kept for some time in stock. Chlamydospores common, mycelial intercalary and terminal, conidial intercalary.

Mycelium abundant, fine and long, from white to slight purple tint to haematoxylin violet to mulberry purple on hard oat agar grown in the dark; white to slight development of pinkish pigment and sometimes purple color on potato plug grown in dark; white to slight purple tint on potato-glucose agar grown in darkness and white to cameo pink to petunia violet when grown in the light. Substratum colorless to purple to dull purplish black on potato-glucose agar.

The conidial measurements are as follows:
1-septate, few, 22.5 by 4.5 microns.
2-septate, rare, none measured.
3-septate, 50 to 100 per cent, 34 by 4 (22 to 52 by 3 to 5 microns).
4-septate, 0 to 40 per cent, 48 by 4.5 (35 to 60 by 4 to 5 microns).
5-septate, 0 to 20 per cent, 48 by 4.5 (35 to 60 by 4 to 5 microns).

*Fusarium sclerotioiides* Sherb. var. *brevius* Sherb. (Description from No. 59.) See Plate 2, A.

Macroconidia typically dorsiventral; dorsiventrality distinct, ventrally curved, dorsally elliptic, typically broader toward apex, indistinctly pedicillate; gradually attenuated, pointed apex.

No sclerotia, no plectenchymic sporodochia.

Microconidia abundant, oval, o- and 1-septate.

Intercalary mycelial chlamydospores common. Mycelium well developed, white to mulberry purple on hard oat agar; white to cameo pink and petunia violet when grown in the light on potato glucose agar; slight development of pinkish pigment on potato plug. Substratum on potato glucose agar livid pink to dark maroon purple.

The conidia measurements are as follows:
1-septate rare, 22 by 4 microns.
2-septate rare, no measurements made.
3-septate 50 to 80 per cent, 36 by 5 (25 to 40 by 4.5 to 5.5) microns.

**SECTION DISCOLOR**

Three distinct groups, namely A, B, K, and one group C, which varied considerably within itself, contained species included in Wollenweber's section Discolor. The description of these cultures with their identification follows.

*Fusarium trichothecioides* Wr.; (Description taken from No. 1, 3, 5, 6, 7, 8, 9, 10, 11, 12, 14, 33, 38, 61, 63, 64, 76, 79, 85, 86, 91, and 97.)

Conidia not differentiated into macroconidia and microconidia, but there are two types of spores, the comma and the discolor types. The discolor type, that is conidia shaped like those of *Fusarium discolor*, is very rare. Comma type of spore slightly dorsiventral to straight, diameter more or less uniform, apex and base not differentiated rounded. (Pl. 2, B.)

Spores in powdery masses, at first on aerial mycelium, which soon covers the surface completely.

Terminal and intercalary chlamydospores occasionally noted. Mycelium abundant at first and white, soon becoming covered with powdery spore masses, which vary from pale flesh, salmon buff, chamois to buckthorn brown on potato-glucose agar; pale flesh to pale pink to pale pinkish buff on potato plug; white to pale flesh to safrano pink to light ochre to salmon buff on oat agar. Substratum colorless to somewhat darkened on potato-glucose agar. This species is easily identified by its very characteristic powdery growth.
The conidial measurements of the comma type of spore are as follows:

- **0-septate**: 11 by 4 (6 to 19 by 3.5 to 4.5) microns.
- **1-septate**: 15 by 4 (12 to 24 by 3.5 to 5) microns.
- **2-septate**: 20 by 4.5 (18 to 26 by 4 to 5) microns.
- **3-septate**: 26 by 5 (19 to 34 by 4.5 to 6) microns.
- **4-septate**: 32 by 5 microns.

The percentages of 0-, 1-, 2-, and 3-septate conidia vary in different cultures. In most cases 1-septate conidia predominate.

**Fusarium subpallidum** var. **roseum** Sherb. (Description taken from No. 15, 17, 22, and 40.)

Macroconidia typically dorsiventral, dorsiventrality distinct, ventrally curved, more or less uniform diameter, apex not long, rounded; base pedicillate.

Spores in small salmon-colored sporodochia, occasionally merging into pseudopionnotes. Microconidia absent. Chalmydospores sometimes found, both conidial and mycelial intercalary. Mycelium somewhat varied in color, white above to salmon orange below with some shades of purple on potato plug; flesh pink to light coral pink and rose pink with occasional slight tint of purple or yellow on potato agar with 5 per cent glucose.

Substratum, colorless to rose pink on potato agar with glucose.

The conidial measurements are as follows:

- **1-septate**: 0 to 20 per cent, 18 by 4 microns.
- **2-septate**: rare (no measurements made).
- **3-septate**: 50 to 90 per cent, 25 by 4.5 (16 to 30 by 3.5 to 6) microns.
- **4-septate**: 0 to 15 per cent, 34 by 5 microns.
- **5-septate**: 0 to 12 per cent, 34 by 5 microns.

It is doubtful whether there is enough difference between the species **Fusarium subpallidum**, **F. subpallidum** var. **roseum**, **F. clavatum**, and **F. discolor** to warrant more than one species.

**Fusarium clavatum** Sherb. (Description taken from No. 23, 41, 42, and 43.)

Macroconidia typically dorsiventral, dorsiventrality distinct, ventrally curved, slightly broader toward the apex, apex rather abruptly attenuated, base distinctly pedicillate.

Spores in small sporodochia, later merging into pseudopionnotes from pale flesh to salmon colored on oat and lima bean; light coral red to coral red on potato agar with 5 per cent glucose.

Microconidia absent.

Intercalary conidial chlamydospores sometimes present, mycelial intercalary chlamydospores occasionally found or scattered.

These cultures showed unusually close identity culturally and microscopically throughout.

The conidial measurements are as follows:

- **1-septate**: rare.
- **2-septate**: 2 to 6 per cent.
- **3-septate**: 75 to 90 per cent, 27 by 4.5 (16 to 40 by 4 to 6) microns. Average limits 20 to 30 by 4 to 5 microns.
- **4-septate**: 15 to 30 per cent, 30 by 5 microns.
- **5-septate**: 5 to 15 per cent, 30 by 5 microns.

Practically speaking, the spore measurements and the percentages of the variously septated spores varied no more between the different media (potato glucose, oat, and lima bean agars) than between different cultures grown on the same medium.

No. 16 seems to vary between **F. clavatum** and **F. subpallidum** var. **roseum** and **F. discolor** in color characters, but the spores agree with **F. discolor** in shape and size, and sometimes in average number septations. We, therefore, are identifying it as that species.

---

4 Practically no normal spores were found in any of the cultures of No. 17 on any of the media used. The culture seems attenuated. However, from the few spores and from earlier cultural notes we identified it with this species.
Fusarium discolor Ap. and Wr. var. sulphureum (Schlect) Ap. and Wr. (Description taken from No. 77 and 85.)

Macroconidia typically dorsiventral, dorsiventrality distinct, ventrally curved, more or less uniform in diameter. Apex not long, typically slightly broader toward the apex, more or less abruptly attenuated, base distinctly pedicillate.

Spores in pseudopionnotes, flesh ochre to salmon color on all media used.

Microconidia absent.

Conidal chlamydospores often found; mycelial chlamydospores never noted, due, perhaps, to scarcity of mycelium.

Mycelium white at first, but soon becoming entirely covered by pseudopionnotes. Substratum colorless to slight salmon coloring.7

(For photograph of No. 77, see Pl. 1, C and D.)

The conidial measurements are as follows:

- 2-septate, rare.
- 3-septate, 15 per cent, 24 by 4 (22 to 32 by 4 to 4.5) microns.
- 4-septate, 15 per cent, 35 by 5 microns.
- 5-septate, 70 per cent, 40 by 5 (35 to 45 by 4 to 5) microns.
- 6-septate, rare, 42 by 6 microns.

The percentages of the different septate spores vary in different cultures. For instance, 5-septate spores were 97 per cent on 7-day culture on lima bean and only 40 per cent on oat about 15 weeks old. The size also varies. The cause of difference in size would seem to depend on temperature and moisture conditions quite as much as on the medium used.

No. 84 was much the same as No. 77 and 85 but showed the following variations in spore measurements:

- 3-septate, rare.
- 2-septate, 4 per cent.
- 3-septate, 40 per cent, 26 by 4.5 microns.
- 4-septate, 30 per cent, 32 by 4.5 microns.
- 5-septate, 25 per cent, 36 by 5 microns.
- 6-septate, rare, 38 by 5 microns.

Color of growth lighter on potato glucose, orange tinge with bacterial-like growth below.

No. 52 was much the same as No. 77 and 85 but showed the following variations in spore measurements:

- 3-septate, 12 per cent, 25 to 40 by 4.5 microns.
- 4-septate, 8 per cent, 38 by 4.6 microns.
- 5-septate, 80 per cent, 42 by 5 (35 to 52 by 5) microns.
- 6-septate, rare.

Spores seem to be slightly less curved than in No. 77, though they grade into each other.

Color of growth slightly different, apricot orange rather than flesh ochre on all media used. On lima bean mycelium was medium in growth, contrasted with its scarcity in No. 77.

The darkening of the medium mentioned in note on Fusarium discolor var. sulphureum was never noticed in cultures of this number.

F. culmorum (W. Smith) Sacc. (Description taken from No. 84, which was the only isolation made of this species.) See Plate 2, D.

Macroconidia dorsiventral, ventrally straight or very slightly curved, slight constriction at the apical end and the pedicillate base, quite uniform diameter throughout, typically 5-septate 36 by 6 microns. 3- and 4-septate conidia are not uncommon. Conidia have thick membranes and very pronounced septa. Orange-colored sporodochia found.

Conidial chlamydospores abundant on lima bean agar, age 175 days.

On potato glucose agar mycelium abundant, bright pink above, carmine to ox-blood red below.

Substratum ox-blood red.

SECTION MARTILLA

The members of the one group (F) that fell within this section were not identical in their cultural characters but were sufficiently similar to suggest a group. Microscopically it is quite easy to recognize the group

1 On potato glucose agar, both in the light and in the dark, the medium sometimes darkened to a brown black and the growth became more or less powdery, from Sanford’s brown to a nigger brown in color, the pseudopionnotes disappearing. Spores mounted from such cultures appeared more or less disintegrated.
Species of Fusarium Isolated from Potato Tubers

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Martiella, for the blunt spores are very characteristic. The size of the spores and number of septations vary considerably, and to identify the species and varieties offers many difficulties.

Sherbakoff (18), in this group also, has made varieties that could have been avoided had he made his species a little more comprehensive, and from our experience the characters of some of his species are not sufficiently stable. Such characters are comparative width and length of spores, "somewhat narrower macroconidia," "color of conidia and substratum usually paler," "frequent occurrence of bluish plectenchyma," etc.

After considerable comparative study we have identified No. 30, 35, 36, 47, 49, 51, 53, 71, 74, 80, 89, 90, 94, and 95 as Fusarium solani. Slight differences occur in these cultures, which, judging from a single photograph or a single set of notes, might suggest a variety of F. solani or of F. Martii or even a new species, but study of all of the data shows that these cultures do not have sufficiently stable characters to identify them as varieties or species. The cultures vary from one another and from the descriptions of F. solani only in minor details. Some of these variations are shown in the two photographs of F. solani. (Pl. 3, A and B.)

Fusarium solani (Mart. p. par.) Ap. et. Wr. (Description taken from No. 95 as a type.)

Macroconidia typically somewhat broader in upper half, rounded to slightly constricted apex, slightly if at all pedicillate. Normally 3-septate 28.75 by 4.5 microns (limits 27 to 38.5 by 4 to 5 microns), sometimes 2- and 4-septate, rarely 5-septate.
Pseudopionnotes and sporodochia occur commonly on most media.

Microconidia may or may not be present. When present usually abundant, round or oval in shape. Chlamydospores in mycelium terminal and intercalary, common in old cultures.

Aerial mycelium weak to well developed, typically white, neutral gray, sometimes with a purple tint.

Substratum on potato glucose agar usually from deep purplish vinaceous to dull violet black. Color on oat agar a mixture of blue, green, and purple.

Fusarium coeruleum (Lib.) Sacc. (Description taken from No. 55, which was the only isolation made of this species.)

Macroconidia dorsiventral, slightly ventrally curved. Basal end distinctly pedicillate. Apex rounded, more or less abruptly attenuated. Uniform diameter throughout. Three-septate spores dominant, quite variable, 31 to 42 microns by 5 to 6 microns.

Plectenchymatic tissue and substratum on potato glucose agar violet to indigo blue and bluish black.

Chlamydospores very abundant in old cultures, terminal and intercalary and in long chains and masses.

OTHER SECTIONS AND UNIDENTIFIED ISOLATIONS

The few cultures that fell outside of the three sections just discussed were identified by means of Sherbakoff's key and descriptions (18), but since we did not have known cultures for comparison, no descriptions of them are included here. The identification of each as we determined it is as follows:

Section Gibbosum: No. 20, Fusarium gibbosum.

Section Roseum: No. 13, F. subulatum var. brevius.

Section Arthrosporiella: No. 69, F. arthrosporioides; No. 72, F. anguioides? (Chlamydospores were sometimes found.)

Section Ferruginosum: No. 75, F. bullatum (may be variety roseum).
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<th>Date of isolation</th>
<th>Suspected disease</th>
<th>Determination</th>
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See notes on Elegans group p. 357.

* There is some confusion in the record of the source of this culture. It is recorded in one place as Big Arm and in another as Lewistown. Circumstantial evidence points to the latter as correct.


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<th>Isolation No.</th>
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**LITERATURE CITED**

(11) Lewis, Charles E.  

(12) Link, Heinrich Friedrich.  

(13) Link, George K. K.  

(14) Pratt, O. A.  

(15)  

(16)  

(17) Saccardo, P. A.  

(18) Sherbakoff, C. D.  

(19) Smith, Erwin F., and Swingle, Deane B.  

(20) Wilcox, E. Mead, Link, George K. K., and Pool, Venus W.  

(21) Wollenweber, H. W.  

(22)  

(23)  

(24)  
PLATE 1

A.—*Fusarium clavatum*, No. 41, grown on potato glucose agar. Age 43 days.
B.—*Fusarium clavatum*, No. 41, grown on oat agar. Age 47 days.
C.—*Fusarium discolor* var. *sulphureum*, No. 77, grown on lima bean agar. Age 7 days. (Hochkulture.)
D.—*Fusarium discolor* var. *sulphureum*, No. 77, grown on lima bean agar. Age 91 days. (Altkulture.)
Species of *Fusarium* isolated from Potato Tubers

PLATE 1

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Washington, D. C.
PLATE 2

A.—*Fusarium sclerotoides* var. *brevius*, No. 59, grown on lima bean agar. Age 9 days.
B.—*Fusarium trichothecioides*, No. 38, grown on oat agar. Age 45 days.
C.—*Fusarium clavatum*, No. 41, grown on lima bean agar. Age 12 days.
D.—*Fusarium culmorum*, No. 48, grown on lima bean agar. Age 10 days.
PLATE 3

A.—*Fusarium solani*, No. 95, grown on tomato leaves and stems. Age 37 days.
B.—*Fusarium solani*, No. 47, grown on tomato leaves and stems. Age 34 days.
Species of Fusarium Isolated from Potato Tubers

PLATE 3

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Washington, D. C.