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Fungi of Flaxseed and of Flax-Sick Soil

by

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FOREWORD

STUDIES UPON FLAX WILT AND FLAX CANKER *(Anthracnose)*
NOT PREVIOUSLY PUBLISHED

By H. L. BOLLEY

IN early years of American experiment station investigation many findings were made, the relation of which to the practice of agriculture was not, at first, clearly apparent. Funds were limited and publication of details was often impossible.

The subject matter of this paper by Mr. T. F. Manns is but an illustration of much which lies locked in field and laboratory notebooks, photographic records and drawings.

In the autumn of 1890, I visited at the home of Dr. Otto Lugger at the Farm School and Experiment Station, St. Anthony Park, Minnesota. Dr. Lugger had a well equipped laboratory and gardens in which he conducted entomological and bacteriological studies. He explained that there was a destructive "blight" of flax which needed careful study and suggested that it was well that I was young because it might be a difficult problem. In the fields and gardens, I was shown what he had done and some of the outstanding field characteristics of the disease. Much impressed, I at once started structural and mycological and bacteriological studies based upon the flax plant, its seeds and soils upon which infection occurred. But slight progress was made during a number of years until the use of the physician's centrifuge was applied to the sedimentation of washings from flax-seed in the spring of 1900. Certain conidial spores were quite uniformly observed in the sediment from samples examined. These were none other than the spores of a fusarium often previously observed upon the dead roots and stems of wilted, dead, or dying flax plants and upon harvested flax lying unprotected in the fields.

A few days sufficed to produce distinctive cultures upon agar and later but a few weeks were necessary to procure pure cultures from the interior of the fibrovascular bundles of wilting but living flax plants, and to prove the pathogenic nature of this fusarium to flax seedlings by pure cultures applied to sterilized and virgin soil.[1] In June of 1900, the regular rotation plot 30 of the department of agronomy was assigned to the department of plant pathology and was used in some preliminary soil disinfection trials. During the spring of 1901 this old rotation plot was divided into 12 special beds for rotation and disinfection areas.

Mr. T. F. Manns worked as student aid during the years 1901 and 1902 and as assistant botanist during the summer of 1903 and until July, 1904. He presented a thesis, as here, in part, outlined, in June of 1903.

The laboratory studies covered a wide field and at that time the data was thought by station authorities to have but slight apparent agricultural merit. It was deemed best to await further investigations. Thus delayed, the thesis as a whole remains unpublished.

The present importance of the flax crop and the extensive work now being done upon flax disease control thru crop rotation and plant breeding seems to justify publication of those parts of Mr. Manns' thesis having direct bearing upon our studies upon flax-sick soil and particularly on the diseases known as flax wilt and flax canker, as done in this laboratory under my direction.

Dr. Manns has, accordingly, at our request, condensed the manuscript. I have reviewed the same and recommend this belated publication.

H. L. Bolley,
Botanist and Plant Pathologist.

Fargo, North Dakota
March, 1932.

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[1]Bulletin 50, North Dakota Agr. Exp. Sta. Dec. 1900.

Fungi of Flaxseed and of Flax-Sick Soil

By T. F. MANNS[1]

HISTORICAL SKETCH

SINCE the date of the early work of H. L. Bolley upon flax diseases and that of the data recorded in this paper, much effective work has been done, not only at the North Dakota Agricultural Experiment Station but in other states and flax producing countries. It is, therefore, thought important to summarize the studies since done upon flax wilt and flax canker. This has been well stated by Dr. Yoshihiko Tochinai in "Comparative Studies on the Physiology of Fusarium Lini and Colletotrichum Lini" in the Journal of the College of Agriculture, Hokkaido Imperial University, Vol. XIV, part 4, pages 173-176, Sapporo, Japan, 1926, as follows:

1. **Fusarium Lini** Bolley

Fusarium Lini causes the wilt-disease of flax. This disease must have existed in Europe and elsewhere for centuries before the discovery of its causal organism at the end of the nineteenth century in Japan.

In 1892, K. Miyabe first found that a species of *Fusarium* is concerned in the wilt-disease of flax, and under his direction N. Hiratsuka (48) investigated this disease. He confirmed the assumption that the casual organism of the wilt-disease of flax is a species of *Fusarium*, and explained the principle of the rotation of crops with long intervals in the cultivation of flax adopted by the cultivators in Europe. In America, H. L. Bolley (9) discovered, in 1901, quite independently of the researches by Miyabe and Hiratsuka, the causal fungus of the wilt-disease of flax and named it *Fusarium Lini*. Before them, O. Lugger (69) carried out an investigation on the wilt-disease of flax. He did not succeed in finding its causal organism.

Fusarium Lini attacks the flax plant at any stage of growth, but the greatest damage is done to the seedlings. The young flax plants are easily annihilated by the attack of this fungus, causing them to wither or fall down very rapidly. Grown plants, offering more resistance, never show such a rapid death.

Fusarium Lini is a facultative parasite. It can grow on organic matters in soil for many years, producing conidia and chlamydospores, and attacking the flax plants when grown on the same soil. In another way, the fungus disseminates itself adhering to the surface of flax seeds, or according to Hiura (50), the hyphae penetrate into the seedcoats and remain there in a dormant state, and attack the seedlings at the time of germination.

According to the authors experiments, the germ-tubes of the conidia or the hyphae attack the flax plants either by penetrating through their epidermis or passing through their stomatal slits. According to W. H. Tisdale (103), most cases of infection occur on the root hairs of flax.

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[1]An abridged statement of a thesis for the master's degree at the North Dakota Agricultural College, June, 1903, with additional investigations done as assistant botanist and plant pathologist, 1903 to July 1904, and including some later observations on the dissemination of flax diseases in a lighter soil region of North Dakota. Dr. T. F. Manns is now soil bacteriologist and plant pathologist at the Agricultural Experiment Station, University of Delaware. H. L. Bolley had not learned of Hiratsuka's work at the time of publishing "A Preliminary Note on the Cause of Flax Sick Soil", Proceedings 22nd Annual Meeting of the Society for Promotion of Agricultural Science, August, 1901, or when publishing Bulletin No. 50 of the North Dakota Agricultural Experiment Station, December, 1901.

2. Colletotrichum Lini (Westerdijk)

Colletotrichum Lini causes the anthracnose of flax. The anthracnose of flax began to attract the attention of phytopathologists at the beginning of this century. It had been overlooked by flax cultivators because the symptoms of this disease can not be distinguished from those of the wilt-disease without a careful examination.

This disease and its causal fungus were first noticed by T. F. Manns. He made an extensive research upon "Flax sick soil and flax seed" under Prof. Bolley of the North Dakota Agricultural College, from 1901 to 1903, and separated a species of *Colletotrichum* as a parasite of flax together with many other species of parasitic as well as saprophytic fungi. He named it *Colletotrichum Lini*. But for some reason this thesis unfortunately has never been published. By his courtesy, I was able to peruse this valuable paper, which was kindly sent to me at my request.

In 1903, Bolley (12) reported very briefly on a species of *Colletotrichum* parasitic on flax. Again in 1910, he (13) reported on this disease, and named the causal fungus *Colletotrichum Lini*, without giving any specific description, however, and again reporting two years later, in 1912, he described it as "Flax Canker" (14).

In 1915, Schoevers (98) reported on the occurrence of a species of Colletotrichum parasitic on flax in Holland. He found also a species of *Gloeosporium* on the seed-ball of flax, and thought of some possible relation between these two kinds of fungi.

In the same year, Westerdijk (116) described the causal fungus of this flax disease in Holland, and gave it the name *Gloeosporium Lini*. She reported on it again in 1918 (117).

In 1918, Pethybridge and Lafferty (86) published an important paper on the investigation of this disease in Ireland. They named the causal organism *Colletotrichum linocolum*, and gave a full description of it. They gave the reason for having treated this fungus as a new species by stating that, "It may be identical with the species already described (but not named) by Schoevers, and it is just possible that it may be Bolley's *Colletotrichum Lini*. Since, however, no description of this latter species has been published, the name cannot be regarded as valid." Pethybridge and Lafferty dwelt rather extensively upon the transmission of their fungus. According to them, the hyphae of the fungus penetrate into the epidermal cells of the seed-coat of flax, and remain there in a dormant state, attacking the seedlings after germination.

In 1922, Schilling (96) reported on the occurrence of anthracnose of flax in Germany. He adopted the name *Gloeosporium Lini* Westerdijk, and discussed the unreasonableness of separating the genus *Colletotrichum* from *Gloeosporium* by placing too much weight on the presence or absence of the setae. He treated *Colletotrichum linicolum*, Pethyb. et Laff. as a synonym of *Gloeosporium Lini* Westerdijk, regarding them to be identical.

In Japan, this disease was first noticed in 1918 by S. Ito and K. Katsufuji. T. Hemmi identified the causal fungus as *Colletotrichum Linicolum* Pethyb. et Laff., and he (45) spoke on the relation between this fungus and *Colletotrichum Lini* Bolley, having the opinion that, as Pethybridge and Lafferty had stated, this fungus is very probably the same as the causal organism of the so-called "Flax Canker," *Colletotrichum Lini* Bolley. But the identification of the two fungi is difficult, because we have no full description of the latter.

From T. F. Manns' manuscript, however, the author was able to establish the absolute identity of *Colletotrichum Lini* Bolley, *Gloeosporium Lini* Westerdijk, and *Colletotrichum linocadum* Pethyb. et Laff.

Manns' description of this fungus in his unpublished thesis presented to the Faculty of the North Dakota Agricultural College for the Master's degree in 1903 is as follows: "Vegetative hyphae abundantly septate; in deep tissue of host almost hyaline, near surface light to dark brown or sooty, 3 to 10 microns in diameter, average 3.5 microns. Acervules scattered, slightly erumpent; spores sessile on a compact matrix, surrounded by or interspersed with bristles (setae). Conidia biguttulate, hyaline, slightly curved, allantoid, 15 to 20 microns by 2 to 4.5 microns. Chlamydospores olive to brown, spherical to oval, 10 to 12 microns by 10 to 15 microns. This fungus is parasitic on flax, causing in seedlings a typical 'damping-off,' and in more mature stages weakening and browning of the plants, with a resulting shrivelling of seed at maturity. It is associated with *Fusarium Lini* in causing flax sickness in the Northwest flax districts of the United States, and is especially common in flax grown in European countries, as evidenced by specimens of sick flax; also the spores are readily carried upon flax seed. No perfect fruiting stage has been met or produced in cultures."

Bolley's *Colletotrichum Lini* is without any doubt the same as the one described and named by Manns. Bolley has never given any description of this fungus. But fortunately, the author was able to identify the fungus found in Japan, Ireland, Holland and Germany with *Colletotrichum Lini* of Bolley with the help of the full description of the fungus given by Manns in his unpublished thesis.

"Thus different names have been given to this fungus by several authors. Manns' *Colletotrichum Lini* has never been published, and Bolley's *Colletotrichum Lini* is a *nomen nudum*, Westerdijk named it *Gloeosporium Lini* in 1915, taking the genus in a broad sense. Pethybridge and Lafferty named this fungus *Colletotrichum linicolum* in 1918, apparently without knowing the work of Westerdijk.

As the author cannot agree with the proposal to abolish the genus *Colletotrichum* and to include it in *Gloeosporium*, he should like to call this fungus *Colletotrichum Lini* (Westerdijk). As a consequence, the names *Colletotrichum Lini* Bolley, *Gloeosporium Lini* Westerdijk and *Colletotrichum linicolum* Pethybridge et Lafferty should be treated as its synonyms."

The literature upon flax wilt and flax canker cited by Dr. Tochinai in his comparative studies is as follows:

9. Bolley, H. L.—A preliminary note on the cause of flax-sick soil. *Fusarium Lini* sp. nov. Proceedings Twenty-Second Annual Meeting of Society for Promotion of Agricultural Science, pp. 1-4. 1901.

10. Bolley, H. L.—Flax wilt and flax sick soil. North Dakota Agricultural College, Government Agricultural Experiment Station for North Dakota, Bulletin No. 50, pp. 27-57. 1901.

11. Bolley, H. L.—Preliminary efforts to develop a continuous process of seed disinfection by means of formaldehyde vapor. Proceedings Twenty-Third Annual Meeting of Society for Promotion of Agricultural Science. 1902.

12. Bolley, H. L.—Flax and flax seed selection. N. D. Agr. Coll. Governm. Agr. Exp. Stat. for North Dakota, Bull. No. 55, pp. 174-198. 1903.

13. Bolley, H. L.—Seed disinfection and crop production methods and types of machinery needed. N. D. Agr. Coll. Governm. Agr. Stat. for North Dakota, Bull. No. 87, pp. 131-166. 1910.

14. Bolley, H. L.—Flax canker. North Dakota Agr. Exp. Stat., Press Bull. No. 52, 1912.

45. Hemmi, T.—Kurze Mitteilung über drei Fälle von Anthraknose auf Pflanzen. Ann. Phytopath. Soc. of Japan. Vol. 1, pp. 13-21. 1920.

48. Hiratsuka, N.—Report of the Investigation on flax wilt-disease. Resources of Northern Japan, Vol. 48, 1896. (In Japanese.)

49. Hiratsuka, N.—On the cause of flax wilt-disease and its prevention. Bull. of the Agricultural Society of Hokkaido, Vol. 2, No. 25. 1903. (In Japanese.)

50. Hiura, M.—Studies on flax anthracnose. Jour. Soc. Agr. and Forest. Sapporo, Japan. Year 15, No. 64, pp. 1-23. 1923. (In Japanese.)

58. Jones, L. R. and Tisdale, W. B.—The influence of soil temperature upon the development of flax wilt. Phytopathology, Vol. 12, pp. 409-413. 1922.

69. Lugger, O.—Treatise on flax culture. Minnesota Agr. Exp. Stat. Bull. No. 13. 1890.

86. Pethybridge, G. H. and Lafferty, H. A.—A disease of flax seedlings caused by a species of *Colletotrichum*, and transmitted by infected seed. Scientific Proceedings of the Royal Dublin Society, Vol. 15, No. 30, pp. 359-384. 1918.

96. Schilling, E.—Beobachtung über eine durch *Gloeosporium Lini* verursachte Flachskrankheit in Deutschland. Faserforschung, Bd. 2, pp. 87-113. 1922.

98. Schövers, T. A. C.—Voorloopige medeeling over eine nog onbekende, wellicht niet ongeveerlyke van het vlas. Tijdsch. over Plantenziekten, Vol. 21, p. 100. 1915.

103. Tisdale, W. H.—Flax wilt: a study of the nature and inheritance of wilt resistance. Jour. Agr. Res., Vol. 11, pp. 573-607. 1917.

104. Tisdale, W. H.—Relation of temperature to the growth and infection power of *Fusarium Lini*. Phytopathology, Vol. 7, pp. 536-560. 1917.

105. Tochinai, Y.—On the causes of flax wilt-disease, seed disinfection and the effect of soil heating on the growth of the flax plant. Bull. Agr. Soc. Hokkaido, Vol. 22, No. 1, 1920. (In Japanese.)

106. Tochinai, Y.—Studies on the food relation of *Fusarium Lini*. Annals of the Phytopathological Society of Japan, Vol. 1, pp. 22-23. 1920.

107. Tochinai, Y.—On the fermentation of carbohydrates by *Fusarium Lini*. Journal of Plant Protection, Vol. 8, No. 2, pp. 71-78. 1921. (In Japanese.)

108. Tochinai, Y.—Studies on the physiology of *Fusarium Lini*. Transactions of the Sapporo Natural History Society, Vol. 8, pp. 19-42. 1921.

109. Tochinai, Y. and Enomoto, S.—On the dry heat sterilization of flaxseeds for the prevention of its anthracnose. Jour. Soc. Agr. and Forest., Sapporo, Japan. Year 16, No. 66, pp. 225-234. 1924. (In Japanese with English resume.)

116. Westerdijk, J.—Anthracnose van het vlas. Phytopath. Labor. Willie Commelin Scholten, Jaarverslag 1915, pp. 6-7.

117. Westerdijk, J.—Neueres über Flachskrankheiten. Jahrber. Vereinig. f. angew. Bot., Vol. 16, pp. 4-7. 1918.

PREVIOUS INVESTIGATIONS

There is in the flax producing districts of the world a condition in which the land fails to mature successive flax crops for even a few years. This is especially true in the older flax districts. Investigators have assigned different agents as the cause.

It seems fitting to cite some of the publications and, if possible, compare the conditions and manifestations of the flax sickness upon which they were working. The most important of these are as follows:

1. A TREATISE ON FLAX CULTURE by Otto Lugger, Bulletin 13, Minnesota Agricultural Experiment Station, Dec., 1890.
2. "Report on the Investigation of the Wilt Disease of Flax" by Naoji Hiratsuka, in *Resources of Northern Japan*. Vol. 48, 1896. (In Japanese.)
3. CHYTRIDINIE PARASITE OF FLAX by Emile Marchal. (Recherches Biologiques sur une Chytridinee du Lin.) L'Institut Agricole de l'Etat. Brussels. 1901. This parasite he recognized to be *Asterocystis radicis* de Wildeman.
4. FLAX WILT AND FLAX-SICK SOIL by H. L. Bolley. North Dakota Agricultural Experiment Station, Bulletin 50, Dec., 1901.

Dr. Lugger wrote,

> "The disease or whatever else it may be called, can be carried by means of water and by old straw or flax from infested land to soil not yet invaded. The young plants, when reaching the height of three inches, and very often much sooner, wilt, turn black and drop.

After experiments with different fungicides, Dr. Lugger concludes:

> "It is not a specific vegetable disease which affects the plants because one or other of the fungicides would have shown the effects of its application. . . . . Large numbers of dissections were made and thin sections stained in various ways, were studied, but a specific organism could not be found. . . . . We have not to deal with a disease, but the straw of the flax itself is the cause of the trouble."

Naoji Hiratsuka found the cause to be a *Fusarium*. During the summer of 1902, Dr. Kingo Miyabe, under whom Hiratsuka was a student, pronounced the fungus upon which Hiratsuka worked to be the same as H. L. Bolley found to be the cause of the disease and which he had named *Fusarium lini*, Bolley. Dr. Miyabe had also verified the descriptions and manifestations of the disease in the field as detailed by Bolley, and as herein outlined.

Dr. Marchal wrote:

> "It appeared in the fields most frequently in May, more rarely in the beginning of June, under the aspect frequently of circular spots, situated generally in those parts of the field most inclined. The plants, which constitute these spots, are checked in their development. They seldom exceed a height of 15 to 20 centimeters and present the following characteristics: The cotyledons are yellow, as also the lower leaves; the stems lose their rigidity, and the superior part falls wiltingly toward the ground. If one uncovers with precaution a clump of plantlets thus affected, it is seen that the furtherest terminations of the roots have a singular vitreous aspect, lose turgescence, and pull up with the greatest ease.
>
> "The author has observed that this disease is due to a Chytridinie, which exists in abundance in the roots or more exactly in the terminations of the more tenuous rootlets and which fungus is able to take the following description *Asterocystis radicis* de Wildeman, in An. Soc. Belge de Microscopie, t. 17, 1893."

Bolley wrote:

"The plants are attacked at all ages and die early or late in the stage of growth according to the time and intensity of the attack. If the soil is much infected, that is to say "flax-sick," most of the plants are killed before they get through the surface of the ground. Such areas appear in a field of flax as centers of disease, which enlarge throughout the summer as new plants sicken, wilt, and die down around the margins of the spots, finally giving the whole field a spotted appearance. Young plants, two to five inches in height, wilt suddenly, dry up and soon decay if the weather becomes moist. Older plants which are quite woody take on a sickly, weak yellowish appearance, wilt at the top, slowly die, turn brown and dry up. Nearly mature plants which are attacked, but not yet dead, are easily pulled up, the roots breaking off easily at about the furrow slice. Upon examination, most of the smaller branch roots are found to be dead, as well as the tap root below the point at which it breaks off. These dead roots and the parts of the tap root already have a very characteristic ashen gray color. Many nearly mature plants which are attacked late in life show this dead gray down one side of the tap root only. The leaves, side branches, and a strip of the main stem above this portion are dead, giving a peculiar one-sided blighting, similar to the appearance of a tree struck by lightning. . . . . By experiments in 1900 and 1901, I have been able to prove definitely that the disease is not due to soil impoverishment or to any chemical substance left in the soil by the decay of flax roots and stems. It is also proved that a definite species of fungus is the direct cause. The fungus which produces the disease belongs to a genus of minute plants, which botanists have called *Fusarium*. As it appears to be a species which is new to botanical descriptions, I shall call it *Fusarium lini*, after the plant which it attacks."

From these extracts from the different investigators, there are found given in each case similar symptoms and manifestations. Since some of the conclusions vary we may assume there are possibly several ailments which produce similar general field effects. Each investigator claims that the disease may remain in the soil for several years. Three claim the causative agent to be a fungus parasite. Two designate *Fusarium lini* Bolley as the fungus, while the third finds *Asterocystis radicis* de Wildeman as the cause. Dr. Lugger finds no specific organism, and assumes the cause to come from some property of the flax itself. This can hardly be accepted, as it is difficult to conceive a toxin or enzyme transmitted as a disease capable of aggressive spread. The conditions considered by Bolley were apparently not due to soil impoverishment, for soil which would raise no flax, when sterilized by steam, proved efficient for flax growth.

There is considerable evidence favoring a parasitic cause. From the preliminary studies there seems to be reason for further research on this flax sickness, with the aim of throwing more light on the subject. Three phases of work were undertaken:

(A) Separation and Classification of the Fungi in "Flax-sick" Soils or Rotation Plots 30 and 31, of the North Dakota Experiment Station, at Fargo.

(B) Separation and Classification of Fungi in Samples of Flax Seed[1] and on Diseased Plants[1] of Several Flax-sick Areas of Russia, Germany, Belgium, Austria and the United States.

[1]The materials for much of this work were secured by Prof. Bolley thru foreign correspondence or were selected and forwarded by him while on flax investigations in Europe in 1903.

(C) Pathological Effect of these Fungi on Flax thru Infection Work in the Laboratory Supplemented by field infection, using laboratory infected soil and pure cultures.

SEPARATING FUNGI FROM FLAX-SICK SOIL

The following methods were found suited for the investigations:

Taking Soil Samples: Dug with a spade, a narrow trench to the required depth; then with a sterile spatula at the desired depth, a composite of three samples of uniform size were taken and placed in sterile containers.

Media Used: The first efforts to obtain soil fungi were by means of gelatine media. On account of liquification, this proved

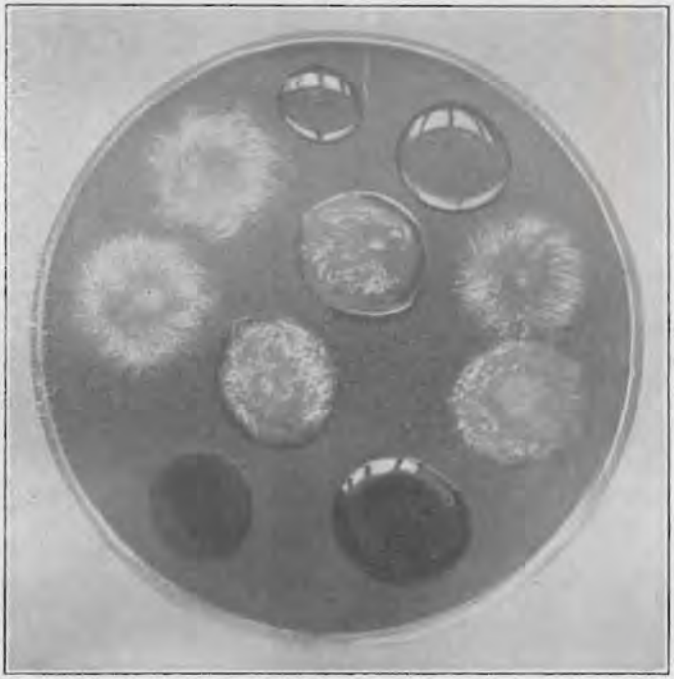

Figure 1. The nutrient glucose agar drop method of caring for fungus colonies from soil to await fruiting and identification.

The drops of agar are placed on the cover of the Petri dish as if they were hanging drops of water. The intervening glass insures reasonable separation of cultures.

unsatisfactory and was discarded for nutrient agar and nutrient glucose agar. The agar was used at the rate of 1.5 per cent, producing a firm medium that checked bacterial spreading and contamination.

TABLE 1: ORGANISMS FOUND BY CULTURE COUNT IN ONE GRAM OF FLAX-SICK SOIL, TAKEN AT SPECIAL DEPTHS FROM SPECIAL CROPPED BEDS, LAID OUT ON ROTATION PLOT 30 OF NORTH DAKOTA EXPERIMENT STATION. (CULTURES MADE IN 1903)

	Beds	1	2	3	4	5	6
Species—	Depth	1901—Fallow 1902—Corn	1901—Wheat 1902—Corn	1901—Potato 1902—Grass	1901—Beets 1902—Beets	1901—Fallow 1902—Peas	1901—Oats 1902—Fallow
Fusarium lini	2 inches	7,534	602	6,329	6,024	12,318	714
	5 inches					13,333	
Fusarium terrestris	2 inches	687	6,024		6,626	720	
	5 inches						
Alternaria sp.	2 inches	684		6,329			
	5 inches			25,970			
Hormodendrum sp.	2 inches	6,847	19,879	20,885	14,086	7,219	7,140
	5 inches	25,000				2,666	
Cephalosporium sp.	2 inches						
	5 inches						
Penicillium glaucum	2 inches		6,024	632	12,048	21,657	
	5 inches			12,985		13,333	
Mucor sp.	2 inches						
	5 inches						
Diplocladium sp.	2 inches	7,534	602	632			
	5 inches	25,000					
Fungus No. 16	2 inches				1,204		
	5 inches						
Fungus No. 53	2 inches						
	5 inches						
Fungus No. 50	2 inches						
	5 inches						
Aspergillus	2 inches						
	5 inches						
Fungus No. 54	2 inches						
	5 inches						
Zygodesmus	2 inches	6,487					
	5 inches		25,970				
Fungus No. 46	2 inches						
	5 inches						
Cephalothecium roseum	2 inches						
	5 inches						

TABLE 1: (Continued)

	Beds	7	8	9	10	11	12
Species—	Depth	1901—Oats 1902—Corn	1901—Flax 1902—Flax after 20 lbs. salt each year	1901—Flax 1902—Flax after 20 lbs. sulfur each year	1901—Flax 1902—Flax	1901—Corn 1902—Corn	1901—Idle 1902—Idle
Fusarium lini	2 inches	7,791	8,107	8,662	7,534	14,999	1,369
	5 inches	12,846			95,858	28,570	27,026
Fusarium	2 inches	16,231		8,662	14,381	17,856	
terretris	5 inches			14,285			
Alternaria sp.	2 inches		6,756		687	714	685
	5 inches		13,333	14,285	27,288	14,285	
Hormodendrum	2 inches	19,477	27,701	53,420	75,317		5,476
sp.	5 inches	38,538	26,666	14,285	27,288		13,513
Cephalosporium	2 inches						
sp.	5 inches					14,285	
Penicillium	2 inches			722	4,108	1,128	685
glaucum	5 inches				13,694	57,140	108,104
Mucor sp.	2 inches			7,219			685
	5 inches	12,846			13,694	14,285	
Diplocladium sp.	2 inches			722			2,054
	5 inches						
Fungus No. 16	2 inches						
	5 inches						
Fungus No. 53	2 inches		6,756				
	5 inches						
Fungus No. 50	2 inches			722			
	5 inches						
Aspergillus	2 inches						
	5 inches					14,285	
Fungus No. 54	2 inches	6,492	1,351	722			
	5 inches						
Zygodesmus	2 inches						
	5 inches						
Fungus No. 46	2 inches			7,219			
	5 inches						
Cephalothecium	2 inches			7,219			
roseum	5 inches						

Dilutions Used: From gram portions of the soil samples, the dilutions used for platings were 1/1000 and 1/10,000 gram of soil. The large numbers of fungus colonies necessitated rapid methods for isolation awaiting growth for identification. For this purpose, a so-called drop plate method was conceived and adopted. See Figure 1.

Over 100 samples of soil were cultured during the seasons of 1901 to 1904 from the flax-sick rotations 30 and 31 at depths varying from 2 inches to 20 inches, to learn at what depths and in what numbers *Fusarium lini* and other flax infecting fungi occupy the soil. Six samples were taken from bed 10, Rotation 30, at the surface, at 2, 4, 7, 12, and 20 inches in depth. In these preliminary trials it was found that the agar plate method was reliable and satisfactory to demonstrate the presence of *Fusarium lini*, other fungi and bacteria in the soil. Ten fungi other than *Fusarium lini* were isolated in this preliminary test. To get uniform results quantitatively, from three to five plates were made from each sample using both the 1/1000 and the 1/10,000 gram of soil.

A second series included the taking of 48 samples of soil from Rotation Plot 30.[1] This plot in 1900 had become thoroly "flax-sick" and was turned to the Botanical Department for investigations on the cause of the trouble. Accordingly, Professor Bolley divided the plot into 12 beds and started certain chemical treatments and rotations which, it was thought, might alleviate the trouble. Less than 2 years intervened previous to the taking of the samples to learn by a quantitative survey of *Fusarium lini* the value of the treatments. Two samples were taken from each of the 12 beds at the depths of 2 inches and 5 inches respectively. This was done in duplicate with good results. These are summarized in Table 1 and show that treatments with salt and sulfur and the crops had brought about very little if any change as far as *Fusarium lini* was concerned. The work as tabulated seems to show that the 2 years of change in crop had not given noticeable results; for *Fusarium lini*, the cause of flax wilt, was yet found in each of the beds, tho it was not so plentiful as in Bed 10 in which flax was being continued. Beds 6 and 2 showed the least *Fusarium lini* content. These beds were rotated in 1901 with oats and wheat respectively, and in 1902 with fallow and corn. Other fungi also were found quite constantly.

A third line of cultures were started in September 1902, as a quantitative survey of wilt on Rotation plots 30 and 31. On Rotation Plot 30, bed 10 was used for it had been continued in flax. On Plot 31, flax previously alternated with wheat. This plot was continued in flax when assigned to the Botanical Department, and was really thoroly wilt sick at the time of this comparison. The cultures

[1]The history of Rotation Plots 30 and 31 shows that the virgin prairie was probably first ploughed in 1882, and until the section was acquired by the Agricultural College and Experiment Station it was cropped continuously to wheat for approximately 10 years. In 1892 these plots were laid off with some 40 others to run a series of rotations. Plot 30 was to be flax continuously, and plot 31 adjacent was to be flax and wheat alternated. This plan was continued until 1900, when owing to the total failure of the flax by "flax-sick" soil, these two plots were assigned to the Botonical Department.

showed that practically the same fungi were in each plot and differ but little quantitatively. However, 2 years previous, in 1900, both plots were in flax At that time, plot 31 produced more flax than plot 30, showing that alternating with wheat retarded the complete infection of the land.

A fourth line of cultures was started February, 1903, to learn whether the flax wilt organism, *Fusarium lini* Bolley, survives the winter in frozen soil and, if so, in what numbers. The soil at time of taking these samples was frozen hard and was removed by means of a hatchet. Table 2 shows the *Fusarium lini* content of the plots to be about equal and that the wilt organism is most prevalent in the furrow slice.

TABLE 2: COMPARISON OF THE WILT CONTENT *Fusarium lini* IN ROTATION PLOT 30 IN 1903 AFTER CONTINUOUS FLAX, WITH THAT IN ROTATION PLOT 31, AFTER WHEAT ALTERNATED WITH FLAX

	At 2 inches	At 5 inches	At 10 inches
Rotation Plot 30	12,500[1]	1,666	None
Rotation Plot 31	6,666	16,666	None

[1]Colonies of *Fusarium lini* per gram of moist soil.

A further set of cultures carefully planned as to methods was begun May 5 and completed June 15, 1904. A quantitative and qualitative survey of the fungi and bacteria from the 12 beds of Rotation Plot 30 was made to learn if any of the different rotations practiced on the different beds or the different treatments applied, after continuing 4 years, were assisting in reducing the wilt content. If so, to what extent? Three plates were made from each soil sample, that is, two plates of 1/10,000 gram of damp soil, and, one plate of 1/1000 gram of damp soil from each bed. See Table 3.

TABLE 3: SHOWING THE NUMBER OF COLONIES OF *Fusarium lini* AND THAT OF VARIOUS BACTERIA AND OF OTHER FUNGI AFTER FOUR YEARS OF SPECIAL CROPPING ON THE BEDS LAID OUT ON ROTATION PLOT 30.[1] (CULTURES MADE SPRING AND SUMMER OF 1904)

	Beds	1	2	3	4	5	6
Species—	Depth	1901—Fallow 1902—Corn 1903—Wheat 1904—Rye	1901—Wheat 1902—Corn 1903—Grass 1904—Grass	1901—Potatoes 1902—Grass 1903—Grass 1904—Grass	1901—Beets 1902—Beets 1903—Flax 1904—Wheat	1901—Fallow 1902—Peas 1903—Flax 1904—Oats	1901—Oats 1902—Fallow 1903—Wheat 1904—Beans
Number of bacteria in 1 gram of dry soil	2 inches	3,075,757	1,368,421	10,416,666	1,282,895	8,153,226	3,658,730
	5 inches	1,794,521	25,000,000	1,257,143	2,365,789	2,316,177	3.880.597
	12 inches	198,718	1,298,701	250,000	437,500	140.000	6 [illegible].666
Fusarium lini	2 inches	45,454	6,579	6,944	*1,316	16,128	*1,596
	5 inches	*1,369	7,143		6,579	*4,412	*1,492
	12 inches		6,493				
Fusarium terrestris	2 inches			13,888		8.064	7.938
	5 inches			7,143	19,737	7.353	14.925
	12 inches	6,140					
Alternaria sp.	2 inches			6,944			
	5 inches			7,143			
	12 inches		6,493		18,750		
Hormodendrum sp.	2 inches						
	5 inches						
	12 inches						
Cephalosporium sp.	2 inches						
	5 inches						
	12 inches						
Penicillium glaucum	2 inches	37,875	13,158	20,832	32,900	8,064	
	5 inches	6,849		42,858	6.579	7,353	
	12 inches	12,280	12,987				6.666
Mucro sp.	2 inches						
	5 inches						
	12 inches	6,140					
Briarea sp.	2 inches				6,580		
	5 inches				13.158		
	12 inches						
Fungus No. 54	2 inches						
	5 inches			7,143			
	12 inches						

[1]From 1894 to 1900, Rotation Plot 30, from which the 12 beds were made had been continuously cropped with flax. The high bacterial content of each of these wilt-sick beds very clearly indicates that flax-wilt is probably not due to lost fertility.

The figures in each case show the number of colonies per gram of soil estimated on an air dry basis. Samples of soil taken from 12 inches were of heavier type than those taken from near the surface. Cultures from the greater depths commonly showed a less number of organisms per gram. An asterik indicates that 1/1000 gram of soil was used in making the culture instead of 1/10,000 gram as used in the other cases.

TABLE 3: (Continued)

	Beds	7	8	9	10	11	12
Species—	Depth	1901—Oats 1902—Corn 1903—Corn 1904—Corn	1901 to 1904—Flax after 20 lbs. salt in 1901, 1902, 1903	1901 to 1904—Flax after 20 lbs. sulphur in 1901, 1902, 1903	1901—Flax 1902—Flax 1903—Flax 1904—Flax	1901—Corn 1902—Corn 1903—Flax 1904—Hemp	1901—Idle 1902—Idle 1903—Idle 1904—Idle
Number of bacteria in 1 gram of dry soil	2 inches	2,500,000	10,858,209	7,903,226	12,060,606	4,809,523	2,204,371
	5 inches	2,253,521	3,220,588	3,476,193	2,636,363	2,611,111	3,161,470
	12 inches	767,605	891,304	1,070,422	1,845,070	424,658	355,073
Fusarium lini	2 inches	*2,857	10,448	48,333	45,454	7,936	14,598
	5 inches	*1,408	*4,412	7,937	7,575	7,936	*2,941
	12 inches						
Fusarium terrestris	2 inches				30,303		
	5 inches	7,042	7,353				
	12 inches						
Alternaria sp.	2 inches						
	5 inches					7,936	
	12 inches						
Hormodendrum sp.	2 inches	7,143					
	5 inches						
	12 inches						
Cephalosporium sp.	2 inches				7,575		
	5 inches						*1,449
	12 inches			7,042	14,082		
Penicillium glaucum	2 inches		22,388				
	5 inches						
	12 inches						
Mucro sp.	2 inches						
	5 inches						
	12 inches						
Briarea sp.	2 inches						
	5 inches						
	12 inches						
Fungus No. 54	2 inches	7,143		16,111		15,872	
	5 inches			*3,174	15,151	7,936	7,352
	12 inches			7,042		20,548	

This table shows that *Fusarium lini* is still present in all the 12 beds of Rotation Plot 30, and that the content in the several beds differs but little. Thus far, there has been very little reduction thru the different rotations and treatments practiced. The data show, however, that beds 1, 5, 8, 9, 10, and 12 have somewhat the highest *Fusarium lini* content.

SEPARATING FUNGI FROM FLAX SEED BY MEANS OF THE PHYSICIAN'S CENTRIFUGE

Numerous attempts were made to learn what fungus spores or hyphae were being carried by flax-seed, and how carried, i. e., whether adhering to surface or if carried internally. The device used for quantitative work on spores adhering to the seed was the physician's centrifuge (Figure 2). This simple device was probably

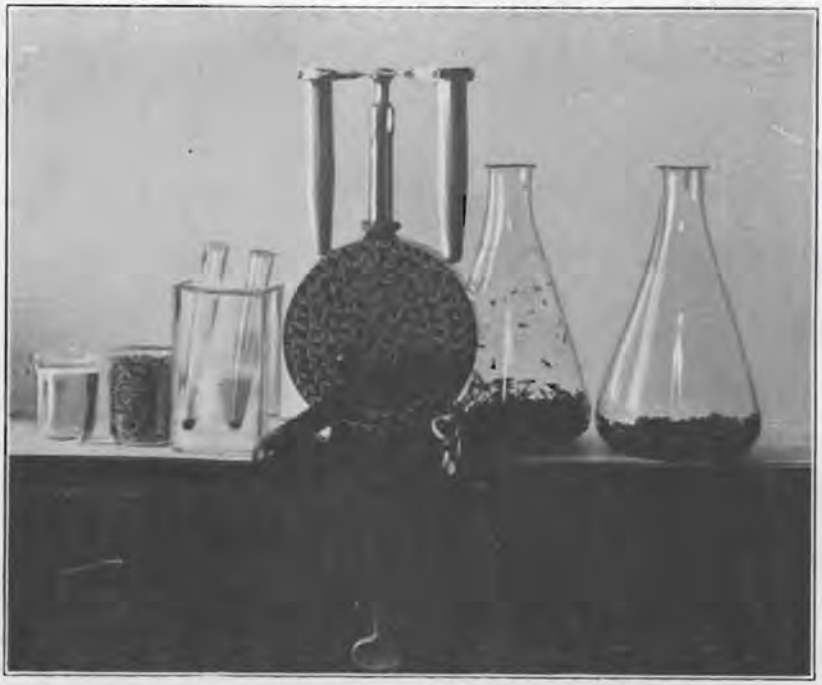

Figure 2. The physician's centrifuge and equipment used for precipitating spores carried in grain samples. The small beaker at the left contains 50 cc. of water, the amount necessary to use in washing the spores from 50 grams of oats, wheat, flax, and other seeds. The beaker filled with oats contains 50 grams. The two tubes show the amount of precipitate taken from 50 grams of badly smutted oats and wheat. The large flasks simplify washing the spores from the grain. See Table IV.

first used in detecting seed contaminated with fungus spores by Prof. H. L. Bolley while working on the smuts of oats and wheat (1896).[1] It is a means for insight into diseased condition of seed, too little known by station workers everywhere. All types of spores carried by the seed may be readily precipitated by this apparatus, and a micro-examination easily made. The following method was practiced by the writer with flax seed. About 50 grams of flax seed were placed in a 200 cc. flask. Into this was poured 40 to 50 cc. of sterile water; after thoroly shaking for 2 or 3 minutes, the liquid

[1]Bolley, H. L. "The Use of the Centrifuge in Diagnosing Plant Diseases." Proc. Soc. Prom. Agri. Science, 1902.

was poured into the centrifuge tubes. The same quantity of wheat and oats may be used in studies of smut and other spore types. Two samples were run at a time. The centrifuge was run to give about 2000 revolutions per minute for 4 to 5 minutes per test. These tests showed that the centrifuge precipitated the spores of *Fusarium lini, Alternaria, Colletotrichum* and other fungi.

Seventy American samples of flax were examined. The majority of these came from the Red River Valley district, some from Minnesota and several from other sources. With few exceptions, those from the Red River Valley were commonly much infected with the *Fusarium* of wilt. Other spores equal in size and appearance to the spores of *Colletotrichum lini*, n. sp. were often present. An *Alternaria* sp. was also quite constant. The flax from Minnesota also often showed *Fusarium lini* and the other fungi mentioned above.

An interesting history of one sample was obtained. This flax was from Riga, Russia, and had been imported to Northfield, Minnesota. We secured a sample of the original seed imported, also the seed produced from that grown at Northfield. A centrifuge examination of the original seed showed it to be infected with a *Fusarium* which had not been observed among Dakota samples. This *Fusarium* was later found plentifully in many Russian samples of flaxseed and is known in this work as "Russian" *Fusarium*. It is therein described under the proposed name *Fusarium russianum*, n. sp. The precipitate from the seed grown at Northfield was found to be as badly affected with this *Fusarium* as the parent seed from Riga, and in addition showed spores of *Fusarium lini*. Here is direct evidence of one means by which disease is carried from one country to another. We also learn that seed samples filled with spores of parasitic fungi can infect the following crop.

Examination of Russian seed samples forwarded by Bolley showed that *Fusarium lini* was also well distributed there. The centrifuge may be used to precipitate spores of fungi from seeds, from which precipitates plates may be made. It thus affords a simple method of quantitative and qualitative survey of disease content in seeds.

Use of Geneva Germinator to Detect Infected Seeds and Seedlings: Another device used in this laboratory with success in detecting disease-producing fungi in seedlings was the Geneva germinator. Figures 3a and 3b. This germinator consists of a copper box, tinned inside, 18 inches long, 12 inches wide and 4½ inches deep, with glass or metal cover. Two side strips and brass wires support cloth aprons having loops 2½ inches deep, into which seeds for germination are placed. By means of this apparatus, *Fusarium lini* was found to be infecting flax seedlings; also *Colletotrichum lini* was found to be a "damping off" fungus on young flax plants. An *Alternaria* was semi-parasitic, and occasionally caused wilting. The sick stems and roots were readily de-

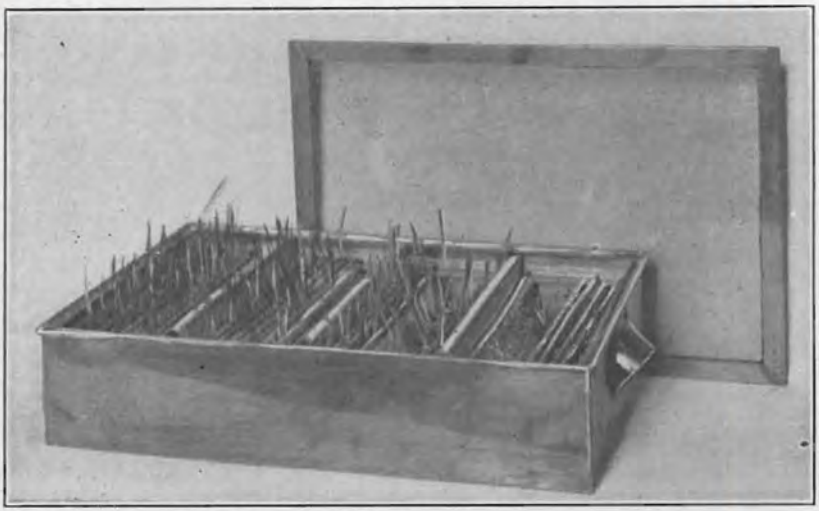

Figure 3a. Geneva germinator used in testing the germinating qualities of grains.

tected as they were free from soil, and could be removed without disturbing the relation of the fungus to the host.

Use of Sterilized Soil: Sterile soil in pots and boxes was used to study the root infecting power of the fungi. Different samples of seed were centrifuged, followed by Geneva germinator tests on infection. Artificial cultures were then made from the infected plants, after which plantings were made in steamed soils inoculated with cultures taken from the sick plants. See Table 4.

TABLE 4: FUNGI PARASITIC TO FLAX IN EUROPEAN AND AMERICAN FLAX SEED AS DETERMINED BY MEANS OF THE CENTRIFUGE, THE GENEVA GERMINATOR, AND BY GROWTH IN STERILIZED SOIL. SEE FIGURE 2. (AN * INDICATES THE FUNGUS FOUND.)

Source of Flax Sample	Centrifuge Studies				Geneva Germinator Tests				Growth in Sterilized Soil			
	Fusarium lini	Fusarium russianum	Colletotrichum lini	Alternaria sp.	Fusarium lini	Fusarium russianum	Colletotrichum lini	Alternaria sp.	Fusarium lini	Fusarium sp.	Colletotrichum lini	Alternaria sp.
1. North Dakota Exp. Sta. Farm Crop, 1901	*			*					(not run)			
2. No. 10, Dutilh & Co., Amsterdam, Crop 1902.			*						(not run)			
3. Magill Co., Fargo. No. 3, Crop 1901.			*						*			
4. Dr. Hecke, Vienna. 1902. No. 25.	*			*								
5. St. Petersburg, Crop 1902. No. 31.		*	*									
6. St. Petersburg, Crop 1903, No. 41.		*								*		
7. St. Petersburg, Crop 1902. No. 42.		*								*		
8. Riga, Russia, Crop 1900	*	*	*	*	*	*	*	*	*	*	*	*
9. Crop 1901, London, Ont. Crop 1902, Rochester, Minn. No. 8. (original seed)	*	*	*	*	*	*	*	*	*	*	*	*
10. Dutilh & Co., Amsterdam, 1902. No. 12.		*		*								
11. N. Dak. Exp. Sta. Farm Crop, 1902.	*		*		*		*	*	*		*	*
12. N. Dak. Exp. Sta. Rotation No. 31. Crop 1902.	*		*		*		*	*	(not run)			
13. F. J. Sprung, Ada, Minn. Crop 1901.	*			*	*			*	(not run)			
14. Brabant, Holland, Crop 1902. "Blue Blossom"	*		*				*		*		*	
15. Tver, Russia. Crop 1902. "Kasan Linseed"		*	*	*		*	*	*		*	*	*
16. Tver Gov't. Russia. St. Petersburg, Crop 1902. "Kasan Linseed."		*		*		*		*		*	*	*

Isolation of Parasitic Fungi from Sterilized Seeds: Nutrient agar and nutrient glucose agar were poured into sterile plates, upon which were placed surface sterilized seeds. This method aided us in the study of the internal seed troubles. No doubt certain para-

Figure 3b. Geneva germinator, view from above, used in taking seedling diseases from germinating flax seed. See Table 4.

sitic fungi are capable of penetrating the seed coats and possibly the cotyledons of flax seed and there await moisture and the growth of the plantlets, at which time the fungi may again grow. This was demonstrated thru plate cultures and our Geneva germinator tests. See Figures 4 and 5. Water cultures were used to some extent, but no reliable results were obtained by attempts at infection of these cultures by use of infected soil.

SEPARATION OF FUNGI FROM DISEASED PLANTS

The methods employed in study of the fungi upon and within sick plants were as follows:

(1) Direct macro- or micro-examination with low power lens showed external fruiting of fungi with characteristic lesions which could be determined. Thus the setae of the Colletotrichum or the compact masses of cream colored Macro-conidia of *Fusarium lini*, and the larger, more distributed groups of conidia of *Fusarium russianum*, each offered opportunity for detection of these troubles macroscopically or thru slight magnification.

(2.) Hand prepared sections, cut in pith, were extensively used.

(3) Teased portions of diseased stems and crushing stems of seedlings, affected roots and leaves proved useful in microscopical examinations.

(4) Staining with iodine aided in differentiating mycelia in host tissues.

Figure 4. Disinfected flax seed germinating on nutrient agar. Sterilized, bright, plump seed was used.

(5) Culture media and moist chambers were used on diseased plants which showed no sporulation. Surface sterilization was practiced before culturing by first dipping in alcohol, followed by mercuric chloride, 1 in 1000 or 2 in 1000, or by the direct use of 50 per cent alcoholic bichloride of mercury, 1-1000, 10 seconds to 1 minute. This treatment was immediately followed by three washings in sterile water. In most cases, cultures were made by crushing parts with sterile forceps into test tubes containing melted medium, which was then poured into the plates.

This work on diseased plants covered material from many different sources, most of which specimens were sent in by farmers who desired to learn the cause of sickness in fields of parts of North Dakota and Minnesota. Many were taken from the flax-sick soil on plots of the Experiment Station.

Seed from different sources planted in soil in the laboratory furnished opportunity for study of sick plants and the symptoms due to the different diseases.

Inoculation work on plants grown in sterile soil and under glass allowed observation on attack of the fungi, and the symptoms manifested by the infected plants.

Many diseased plants examined were gathered by Professor Bolley, botanist of the North Dakota Experiment Station, while studying in different European countries in 1903. Several diseased samples were sent in on request to U. S. Consuls in different parts of Europe, and by eminent investigators in plant breeding lines there, and one sample was received from Japan.

Many cultures were made from sick plants from North Dakota and Minnesota flax districts. Macroscopic and microscopic examinations, in nearly all cases, showed *Fusarium lini*. In several instances *Colletotrichum lini* was present. This fungus probably plays a minor part in the flax sickness. In only one case was *Fusarium russianum* obtained from Northwestern seed. This sample was grown in 1902 at Northfield, Minnesota, from seed which a year previous had been imported from Riga, Russia, and grown near London, Ontario, in 1901.

In germinating many European and American samples of flaxseed in the Geneva germinator, and in boxes containing soil, and in

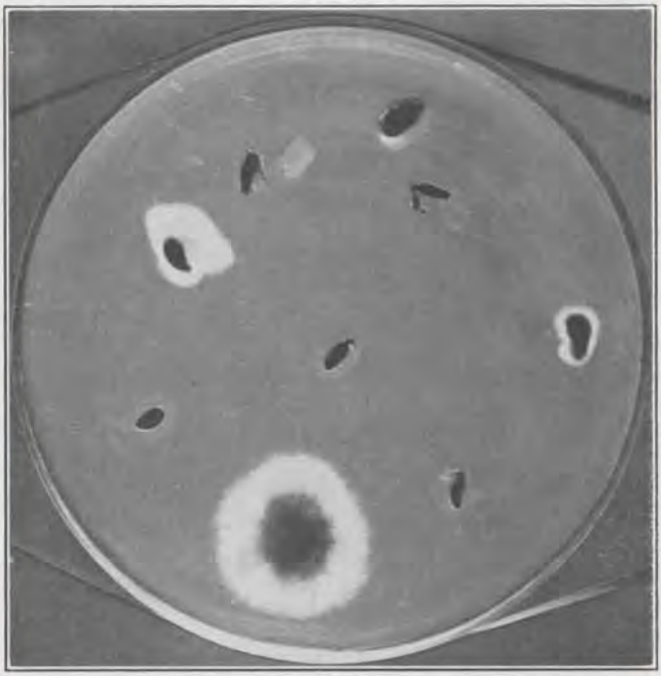

Figure 5. Shrunken and "streaked" kernels germinating on glucose agar in Petri dish. These were surface disinfected as in Figure 4. Fungi immerged from three of the seeds. The large colony is an *Alternaria* sp. The other two are of *Fusarium lini*.

planting over one hundred lots of European seed in the field, the diseased plants were studied as to cause of sickness. These studies afforded opportunities to observe the characteristic of each disease. The field inoculation work with pure cultures in virgin soil also gave further opportunity to study the disease and its symptoms.

Table 5 gives the source of some of the foreign samples and the fungi found or taken from the diseased plants by culture methods. Practically all diseased plants sent in from North Dakota and Minnesota showed *Fusarium lini* as cause of wilt. Occasionally *Colletotrichum lini* was found to be an associated trouble.

TABLE 5: PARASITIC FUNGI FOUND ON SICK PLANTS FROM EUROPEAN FLAX DISTRICTS

Source—	By macro and micro Examination				By cultures			
	Fusarium lini	Fusarium russianum	Colletotrichum lini	Alternaria sp.	Fusarium lini	Fusarium russianum	Colletotrichum lini	Alternaria sp.
1. From H. L. Bolley at Usquert, Holland, 1903.		*		*				
2. From H. L. Bolley from Groningen, Holland. Flax 4-6 wks. old—1903					*			
3. From H. L. Bolley, at Olai near Riga, Russia; plants 8 weeks old—1903					*		*	
4. From H. L. Bolley, Smolenk's Govt.-Steppen, 15 kilm. N. of Gshatsk, Russia plants 8 weeks old—1903	*				*			
5. From H. L. Bolley, Vologda Region, Russia, Sept. 1, 1903.			*	*			*	
6. From U.S. Consul-General, St. Petersburg from Tver Govt. Russia. 1902.	*	*	*		*	*	*	
7. From U.S. Consul-General, St. Petersburg. "Rjiff Linseed." Crop 1902. Tver Govt.-Russia.		*				*	*	
8. Dr. Hecke, Vienna, Austria. 1902 "from a very sick field"		*	*		*	*	*	
9. By Kingo-Miyabe, Japan in 1902. Collected in 1897.	*					No growth		

From this summarization, it is evident that *Fusarium lini* is not confined to the United States but is well distributed in Europe and is also found in Japan. *Fusarium russianum* n. sp. and *Colletotrichum lini*, n. sp. are also generally distributed in Europe and in the United States.

With this data obtained above from diseased plants, reinforced by laboratory and field infections, it appears that flax sickness is not distinctly confined to a specific organism but there are several organisms which may cause somewhat similar types of damage to the crop. There is little doubt that it is possible for *Fusarium lini*, *Fusarium russianum* and *Colletotrichum lini* all to be present in "flax-sick" soils. This was verified by a sample of flax seed sent to us by Dr. Hecke from "a very sick field near Vienna, Austria", thru several other samples of European flax seed, and by one sample of American grown seed.

SEPARATING FUNGI FROM INTERIOR OF FLAX SEEDS

Tests have been made which indicate that certain samples of flaxseed, worked upon, either carried spores on the exterior of the

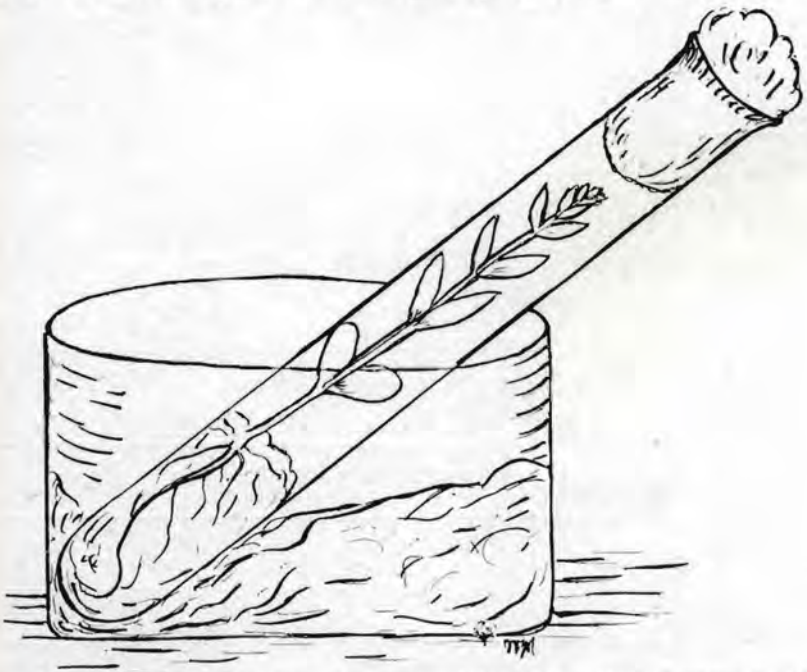

Figure 6. This tube has the bottom filled with cotton, moistened with tap water. When sterilized it can be used for growing sterile flax plants for seedling inoculation. The seedling will make normal growth for about 2 weeks. It is useful also for taking internal fungi from seed.

seed coats which withstood thoro formaldehyde treatment at a strength of 1 pound of formaldehyde to 40 gallons of water, or the fungi were located internally. Evidence of the internal location was obtained in 1902 during attempts at disinfection of flaxseed known to be carrying conidia of *Fusarium lini*. Several strengths of formaldehyde and corrosive sublimate were tried on different samples. The treated seed was planted on virgin soil lands which had

never produced flax. Scattered plants showed evidence of *Fusarium lini* and *Colletotrichum lini* n. sp. It could hardly be possible that these were in the soil. All the treatments showed some dying. The two strongest treatments used were mercuric bichloride, 2½ grams in 1000 cc. of water and formaldehyde 5 cc. in 1000 cc. of water.

A Geneva germinator was sterilized by boiling water in it for 2 hours. The seeds were treated in large test tubes. About 8 grams of seed were used. Full strength commercial ethyl alcohol was poured in and shaken until the seeds were fully moistened; then the disinfectant, formaldehyde, 5 parts in 1000, was added. The tube was shaken for 15 minutes, after which the solution was poured off. The seeds were then rinsed three times in sterile water and placed into the folds of the germinator.

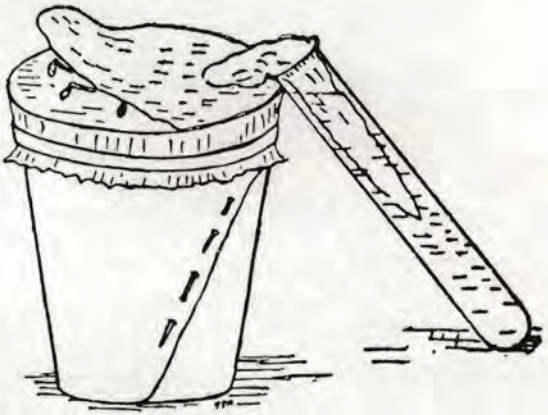

Figure 7. Showing tumbler filled with Sach's nutrient solution, covered below with black paper; a cloth is fastened over the top by a rubber band. To cause germination, the flax seed is covered by a sterile cloth which is raised to show germination.

Nine different samples were treated, each of which by the soil, germination, and centrifuge tests had shown the presence of detrimental organisms in or on the seed.

Table 6 gives the source of seed, fungus spores previously noted, and also the fungi taken from germinator following the treatments.

It is quite evident that some of the fungi were not destroyed by the treatment or were internal to such depth that the disinfectant failed to reach them. These treatments were duplicated with five samples planted in sterilized soil, steamed twice, on separate days, 4 hours. Formaldehyde, 2 in 1000, and corrosive sublimate,

TABLE 6: PARASITIC FUNGI TAKEN FROM SEED WHICH HAD BEEN STRONGLY DISINFECTED—USING THE GENEVA GERMINATOR

Source of Seed—	Fungi present before treatment				Fungi which killed flax in Geneva germinator after disinfection[1]			
	Fusarium lini	Fusarium russianum	Colletotrichum lini	Alternaria sp.	Fusarium lini	Fusarium russianum	Colletotrichum lini	Alternaria sp.
1. From N. Dak. Exp. Sta. Crop 1901.	*		*	*		*	*	*
2. Magill & Co., Fargo, N. D. Crop 1901.			*		*		*	
3. U. S. D. A. 2142, Crop 1902.	*							
4. N. Dak. Exp. Sta. 31. Rot. 31. 1902.	*		*	*				*
5. Dutilh and Co. Rotterdam[1] Holland					(See note below[1])			
6. Dutilh and Co. Rotterdam[1] Holland					(See note below[1])			
7. Sample 18 from Riga, Russia, 1902	*	*	*	*	*			*
8. Dr. Hecke, Vienna. 1902.	*	*						
9. U. S. Consul-General, Belgium, Crop 1902.			*				*	

[1]A Rhinotrichum sp. caused a wet rotting in the Geneva germinator

2 in 1000, were used as seed disinfectants. Fifty seeds of each lot were planted in 7-inch pots.

TABLE 7: VALUE OF FORMALDEHYDE AND CORROSIVE SUBLIMATE SOLUTIONS AS DISINFECTANTS SHOWING AVERAGE RESULTS FROM FIVE SAMPLES OF SEED

Seed Treatment	Average number of plantlets from 50 seeds in Geneva germinator	Number of plants which came up in 5 pots of sterile soil of 50 seeds each	Number of plants in sterile soil which died after coming up	Number of plants in sterile soil alive at end of one month
Untreated seed	20	99	19	80
Corrosive sublimate	21	105	16	89
Formaldehyde	34	171	18	153

Conclusions:—

1. After disinfection with either the corrosive sublimate or the formaldehyde, all flax samples contained considerable infection.

2. Formaldehyde at 2 in 1000 was more effective than corrosive sublimate at 2 in 1000.

3. The formaldehyde treatment favors germination of diseased flax seeds.

4. It is probable the corrosive sublimate injured the germination in some of the weak seeds.

5. Poor germination in the untreated seed was due to disease.

6. The cotyledons showed a small percentage of injury before emerging. These injuries were due to an *Alternaria* sp. and *Colletotrichum lini.*

GROWING DISINFECTED FLAX ON CULTURE MEDIA IN PLATES

Agar plates were run to learn whether disinfected seed would, if dissected or whole, develop fungi when placed on a sterile medium. Seeds were selected from Rotation plot 31, crop 1902, as follows: 17 plump seeds, 17 dark seeds, and 17 scabby seeds. These seeds were washed in distilled water 15 minutes, then treated in a 5 to 1000 solution of formaldehyde. When dry these were placed on agar, some being dissected. Plump seeds gave no fungus growth; dark and scabby seed brought *Alternaria* and *Fusarium lini.* See Figure 5.

It is evident that some of the parasitic fungi are carried internally in diseased flaxseed.

Infection Work with Pure Cultures of Fungi Separated From Flax-Sick Soil, Flax Seed and from Sick Flax Plants

Methods: Infection is a difficult phase of mycology to control, owing to spore distribution and the difficulties of sterilization of seeds and soils. Soil thoroly sterilized by live steam may, perhaps, be so changed in physical and chemical nature that both the parasite and the host have different properties with which to contend. Yet, however faulty steam sterilized soil may be as a medium for growing plants, it is superior to the use of sterile water cultures.

The most satisfactory medium was found to be virgin prairie soil, the upper inch of sod being skinned off, the lower part finely pulverized and used without sterilization. Flax thruout Northwestern districts makes excellent growths in virgin soil. In the use of such soil slight difficulty was met. The checks usually showed normal growth, unless some specific organism was introduced by the seed. Infection by the seed was revealed by micro-examination and culture work.

The Geneva germinator sterilized by live steam is an effective apparatus in which, with separate sterile germination cloths, to conduct infection experiments. Any one of these specific flax diseases readily manifested its presence when introduced among the germinating plants in the germinator.

Whenever one of the fungi showed specific affinity for the flax

plant, the laboratory trials were supplemented by field infections on virgin soil.

Infection tests were made in boxes 9 inches square and 6 inches deep. Virgin sandy loam soil was placed in these boxes. They were then wrapped in heavy paper, and heated for about 2 hours on each of two consecutive days in live steam without pressure. The temperature reached 98°C. (See Figure 8). After the seeds were planted and infected, the boxes were placed in a sterile cage. See Figure 9. This cage was used to eliminate infection from spores in the air. A number of tests could be run in close proximity. A gravity watering attachment supplied sterile tap water. See A in Figure 9. Two rows of seed were placed in each box, 25 seeds per row.

Figure 8. Apparatus fitted with thermometer, used for steaming soil in pots. Its dimensions are such as to furnish space for 20 six-inch flower pots. Steaming four hours on each of two consecutive days gave good results.

Disinfection of Seeds: The following method of seed disinfection proved successful. The seeds were moistened in full strength commercial ethyl alcohol for 5 minutes, then water was poured off, and a solution of 2½ grams of mercuric bichloride in 1000 cc. of equal parts of commercial alcohol and water was poured on. After 10 minutes' treatment, the seeds were washed in sterile water. These treatments were carried out in sterile tubes. A similar treatment was tried using 3 cc. of water in place of the bichloride of mercury. The alcoholic corrosive sublimate method proved most efficient.

After a number of preliminary treatments and after confirming the usefulness of this method, a number of tests were made using *Fusarium lini* and *Colletotrichum lini* as infecting agents. Plantings not infected were used as checks. It was thus proved conclusively that both these fungi kill flax, the *Fusarium lini* causing a definite wilting, the plants being attacked at all stages, but the greatest destruction takes place when the plants reach an age between 10 and 25 days. The *Colletotrichum* caused a definite "damping off" in seedlings, with browning and early maturing in late infections. The disinfectant occasionally failed to kill all the *Alternaria* present, but proved useful in this line.

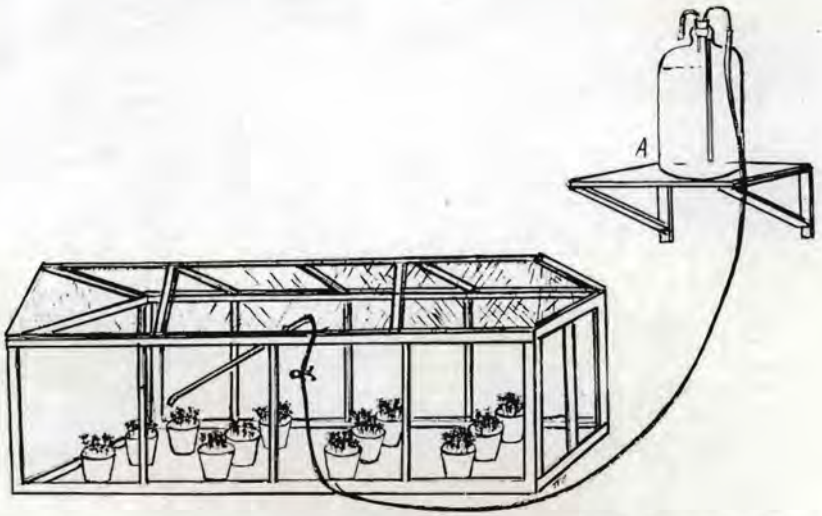

Figure 9. Showing the sterile culture cage (glass) in which inoculation work with sterile soil was carried out. The space between the raised door and the cage is covered with a close weave cloth, permitting ventilation and preventing entrance of fungus spores. The apparatus (A) was used to furnish sterile tap water.

LABORATORY INFECTION WITH VARIOUS FUNGI SEPARATED FROM SOIL, SEED, AND SICK PLANTS

A series of infection tests in pots of virgin soil, sterilized, were done under cover, and growth was continued in the sterile cage. Fifty seeds each were planted in 7-inch pots. The seed for the first 21 pots, except untreated checks, was treated with 3 cc. of 40 per cent formaldehyde in 1000 cc. of equal parts of water and commercial alcohol. The seed lots used in the remaining experiments were treated in a solution of two grams of bichloride of mercury in 1000 cc. of equal parts of water and commercial alcohol. Only select, healthy looking seeds were used.

(1) *Fusarium lini* was used in triplicate as an infecting agent, using in one instance *Fusarium lini* of European source. In that case, the results were as destructive as when the same fungus of American sources was used. All plants excepting one were dead in 20 days. Pots planted were as follows: 2, check; 3, *Fusarium lini* from North Dakota; 4, *Fusarium russianum* from Russia; 5, *Colletotrichum lini* from Europe; 26, *Fusarium lini* from Russia; 27, *Alternaria* sp. and 28, also an *Alternaria* sp.

The following notes show the progress and nature of the infections:

Pot 1. 6-22-03. Check. Not infected. (See Table 8.)

Pot 2. 6-22-03. Seed treated—not infected—check on untreated, and on infected. 6/25. Many plants coming up. No fungous growth is seen. 6/28. There are several spots of fungous growth. No plants are

sick. 6/29. Plants are healthy. 7/17. No plants sick—Several spots of fungus growth seen. 7/9. All plants up—no plants dead or dying. Several spots of fungous growth noticeable. 7/10. Two plants dying. Fungous growth on surface of one. No fungous on the other. 7/11. Two dead plants show fungous growth on stems. 7/27. Micro-examination show dead plants were killed by *Alternaria*.

Pot 3. 6-22-03. Seed treated. Infected with *Fusarium lini* from pure cultures from conidia on straw, plants taken from sick area on Rotation plot 31, N. Dak. Exp. Sta. Used for infection, washings of conidia from six cultures about 4 months old. 6/25. Few plants coming up. No fungous growth visible. 6/26. Many plants coming up. Some fungous showing. 6/27. Nearly all plants up. Much fungous growth noticeable. 6/29. Six plants attacked. 6/30. About twenty plants attacked. 7/1. Only five plants remain alive. 7/2. Five plants left. 7/3. Five plants left. 7/5. Two plants left. 7/7. Only one plant left standing. The rest have wilted. 7/9. One plant survives. A whitish fungous growth shows all over dead plants. 7/10. One plant still surviving. 7/11. One plant surviving. Evidently a case of resistance to this disease. Micro-examination shows abundance of *Fusarium lini* spores on dead plants, and mycelium penetrates the stems. No other fungus seen.

Field Infections: 7/11/03. In Science Hall garden. Seed disinfected as for laboratory experiment. One six-foot row infected with the upper three inches of soil from pot 3 above. Also sowed a check row, seed treated, but not infected. 7/20. All plants up nicely. In the infected row fully one-half of the plants are already dead, many more dying. In check row all plants are healthy, none have died. 7/22. Four-fifths of plants in infected row are dead. Plants in check row are healthy. 7/24. Micro-examination of dead plants shows all killed by *Fusarium lini*. All dead plants have an abundance of wilt spores on the wilted roots.

Pot 4. 6-22-03. Seed treated. Infected with *Fusarium russianum* Nov. sp. cultured from Russian seed. Used washings from six cultures containing many spores. 6/25. Many plants coming up. 6/26. Some fungal growth seen. 6/27. More fungal growth seen. 7/1. Four plants attacked. 7/2. Six plants attacked, apparently like *Fusarium lini* wilt. 7/3. Ten plants wilting. 7/5. Twelve plants wilting. 7/6. Eighteen plants wilting. 7/7. Twenty-two plants wilting. 7/9. Nineteen plants still vigorous. The rest are dead. This fungus is a true "wilter." 7/10. Nineteen plants still living. A grayish fungus is visible on dead plants. 7/11. Three more plants have wilted. Only sixteen plants remaining. 7/12. Only twelve plants left. 7/24. Micro-examination of a number of the dead plants showed *Fusarium russianum* in all. Two also showed an *Alternaria* sp. and one showed *Penicillium glaucum*.

Field Infection. 7-24. (Science Hall Garden). Seed treated the same as in laboratory experiments. Infected one six-foot row with soil of pot 4; also sowed a check row. 8/5. Three plants dead in the infected row. None dead in the check row. Micro-examination of these dead plants shows much mycelium, also bacteria, and nematode worms. 8/10. One more plant wilted. Agar culture showed *Fusarium lini*, probably from seed. 8/14. Three other plants have wilted. Gluscose agar cultures show plants dead from *Fusarium lini*. 8/26. Two more plants have died. Plate cultures made from these show *Fusarium lini* as cause of dying.

It is evident that *Fusarium russianum* was not as active in the field infections of this sample of flax as in the laboratory.

Pot 5. 6-22-03. Seed treated. Infected with *Colletotrichum lini*, n. sp., taken from European sample 6, washings of which contained many spores. Six tubes used.

6/25. Many plants coming up. No fungous growth visible. 6/27. Some fungous growth seen. 6/28. Three plants "damping-off." 6/29. Twelve plants "damping off." 6/30. About twenty-four plants attacked. Some show only seed injury to cotyledons. 7/1. Only seven plants left. 7/2. Six plants left. One has only one seed leaf attacked. 7/3. Five plants remain. 7/5. Only four plants left. 7/7. Only three plants healthy. 7/9. Three plants still survive. Soon after plants "damp off" they dry up and turn brown. 7/10. Two plants survive. Some of the dead plants show growths of fungi on them and others do not. *Colletotrichum lini* does not show microscopically on the plant until the setae come out. The conspicuous fungal growth must be a contamination. 7/11. Two plants are still living, though one is attacked. Micro-examination shows that *Cephalothecium roseum* and *Penicillium glaucum* are present as contaminations.

Field Infection. 7-11-03. In Science Hall garden. Seed treated same as for laboratory experiments. Infected one six-foot row with soil and sick plants from pot 5. Planted also a non-infected row for check.

7/20. Plants are all up two-thirds of an inch high. Of the infected row, one-third are dead from "damping off" and many others are dying. The check is perfectly healthy. 7/24. Micro-examination of many dead plants show all are killed by *Colletotrichum lini.*

Pot 26. 10-12-03. Seed treated. Infected with *Fusarium lini* from Russia, received from Professor Bolley. 10/16. 45 up, none wilting. 10/22. Seven wilting. 10/26. Twenty-three dead or wilting. 10/28. Twenty-six dead or wilting. This rapidity of dying is characteristic of Bolley's *Fusarium lini.* 11/5. All dead. Micro-examination showed all dead from *Fusarium lini.*

Pot 27. 10-12-03. Seed treated, infected with an *Alternaria* sp. 10/21. Forty-eight plants up, all strong. 10/28. None dying. 11/5. Two dead. 11/20. Fifteen dead; all the rest healthy.

Micro-examination show no contamination in this experiment. This shows that this *Alternaria* sp. may infect seedlings.

Pot 28. 10-28-08. Seed treated. An *Alternaria* sp. taken from seedlings with cotyledon injury upon germination. The cultures were taken directly from the injured surface of sterile cotyledon and indicated that the fungus was an internal infection.

10/21. Thirty-four plants up, none dying. 10/26. Four dying. 10/28. Eight dead or wilting. 11/5. All dead but seven. 11/20. All dead but one.

TABLE 8: PARASITISM OF THE FUNGI ON FLAX IN ABOVE SOIL INFECTIONS
The checks are also given

Exp. No.	How Infected	Number dead in days stated									Contaminations
		Days of Growth									
		5	7	8	10	15	20	25	30	35	
1.	Seed treated—check	0	0	0	0	4	9	9	9	12	Alternaria sp. Cephalothecium roseum
2.	Seed treated—check	0	0	0	0	0	2	2	2	2	Alternaria sp.
3.	Fusarium lini, source North Dakota	0	6	20	all dead but 5	all dead but 1	1 remains	1 left	1 left	1 left	No contamination
4.	Fusarium russianum	0	0	0	6	22	10 left	10 left	10 left	10 left	Alternaria on two Penic. glacum
5.	Colletotrichum lini	0	12	24	6 left	3 left	2 left	2 left	2 left	2 left	Ceph. roseum and Penic. glaucum
6.	Fusarium lini	0	7	–	26	all dead	–	–	–	–	No contamination
14.	Seed treated—check	0	0	0	0	9	32	46	–	–	Fusarium lini
19.	Seed treated—check	0	0	0	0	12	24	all dying	–	–	Fusarium lini
25.	Seed treated—check	0	0	0	1	3	–	6 left	–	19 left	Alternaria sp.
26.	Fusarium lini European source	0	0	0	7	23	26	all dead	–	–	No contamination
27.	Alternaria sp.	0	0	0	0	0	–	2	–	15	No contamination
28.	Alternaria sp.	4	8	–	–	27	–	–	33	1 left	Cephalothecium roseum

Summary of Infection Work in Soil: Fusarium lini Bolley in experiments 3, 6, and 26 is an active parasite on flax. Pure cultures of different ages on nutrient agar or nutrient glucose agar, of which washings from 6 tubes were used in pot 3; 3 tubes in pot 6; and 2 tubes in pot 26, each proved sufficient to kill practically all the plants in less than 25 days. Field infections with *Fusarium lini* resulted similarly. Many of the plants died early and others continued to wilt.

Wherever *Fusarium lini* became a contamination due probably to resistance of chlamydospores or to growth from internally infected seed, it readily spread and soon took the majority of the plants. The characteristics of the wilt fungus, *Fusarium lini* Bolley, is well described by Professor Bolley in Bulletin 50, North Dakota Experiment Station 1901, and need not be repeated here.

Colletotrichum: The causal fungus here given the name *Colletotrichum lini* is a destructive "damping-off" parasite of flax. In 15 days, it had "damped-off" 47 plants out of 50.

In the field infection, the percentage was not so great, but its attack was severe and characteristic. This fungus "damps-off" plants at the seedling stage, and later is responsible for much shrivelling of seed by causing early partial maturity. The stems of the partially matured plants are of unnatural brown color and are covered with acervulae containing abundant spores with scattered setae.

The *Fusarium russianum* n. sp. is parasitic on flax, but slower in its action than either *Fusarium lini* or *Colletotrichum lini*. In seedling stages it causes wilting, and it causes premature ripening which results in shrivelled, immature seeds. This was observed in samples received from Professor Bolley while in Russia and from infected plants forwarded from other countries.

The sporodochia and macro-conidia form a broken creamy mass over the lower parts of the stem, similar to that produced by *Fusarium lini*, but less continuous. Flax stems attacked by this Fusarium have an unnatural brown color at maturity.

The *Alternaria* sp. of experiments 1, 2, 7, 8, 25, and 29 caused slow wilting of seedlings. In older plants this symptom seemed to disappear. Other fungi found upon flax and illustrated in Figures 60, 61, Plate 8, may be parasitic but they have not been tested in infection.

OTHER FUNGI SEPARATED

With one exception, Figure 64, pl. 9, the perfect or perithecial stage of fruiting was not obtained tho various cultures were run to bring this stage. Most of the organisms were taken from flax-sick soil, not previously intensively studied as to the fungal flora. It thus has been difficult to find literature applicable to our studies. Classification of imperfect fungi is based but slightly upon characteristics

of the growth on artificial media; therefore, some difficulty was met from a cultural standpoint. The growths obtained have been studied and, if fruiting appeared, an attempt at classification was made. In case parasitic on flax, the specific name was sought, or one has been proposed if not previously described. No attempt was made to learn the specific name of the *Alternaria* found parasitic to seedlings. Two new specific names have been given, viz., *Colletotrichum lini* and *Fusarium russianum*.

In case of *Fusarium lini* Bolley, see Bulletin 50, North Dakota Experimental Station, Fargo, 1901, and Figures 1 to 11, Plates I and II in this paper. This fungus was determined by Professor H. L. Bolley to be the cause of flax wilt and flax-sick soil. He separated the organism and classified it as a *Fusarium* and gave it the specific name, *Fusarium lini*. His description is again verified by the writer.

Fusarium russianum Manns n. sp. Pl. II and III, Figs. 12 to 18. Description: Vegetative hyphae septate, hyaline, 1.5 to 7μ in diameter, averaging about 2μ, branching irregularly. Sporodochia erumpent, cream colored, scattered or grouped, produced upon the stems of flax. Sporophores short, branched, with clavate cells producing numerous conidia.

Conidia 35μ to 50μ by 4 to 5μ, fusiform to slender, crescent shaped, in compact, sessile masses of sporodochia on stems of host. In artificial media the spores are borne upon sporophores more elevated and open. Conidia normally 5 septate, though 3, 4, 6 and 7 septa are found. Micro-conidia seldom produced.

On nutrient 2% glucose agar, the fungus produces a Port wine red color. The fungus exists upon humus, and is parasitic on flax, causing a wilt of seedlings and shrivelled seeds in maturing plants. Readily carried upon the seed, it produces flax sickness in soil and is often associated with *Fusarium lini* Bolley, in "flax sick" areas.

This fungus can readily be obtained from plate cultures. **On Standard Nutrient Agar** one week old, aerial growth is profuse. White to grayish, cottony, growths almost fills culture tubes above the medium. Substratum not colored, macro-conidia few. **On Nutrient 2% Glucose Agar**, one week old, aerial growth profuse, at first white, in 5 days pink near surface of medium, and the upper substratum soon becomes Port wine red. Conidia plentiful, and more thickened than those upon the host.

On Potato Plug, the aerial growth is profuse, at first, grayish to white, and downy, later pink near medium, the surface of potato later becoming Port wine red. Some conidia much thickened.

Soil when artificially infected from cultures causes wilt of seedling flax plants. The spores of this *Fusarium* have been found in abundance in European flax seed. See Table IV. No perfect fruiting was produced in the cultures.

Fusarium terrestris Manns, n. sp. Plate IV and V, Figures 31 to 39. Description: Vegetative hyphae septate, hyaline, 2 to 5μ in diameter, with an average of 2½μ, irregularly branching, living saptrophytically upon humus in the soil. Sporophores in artificial medium scattered, much branched; spores borne irregularly on slender sporophores, 5 to 10μ in length, borne irregularly along the hyphae. Conidia normally 5-septate, tho 3, 4, 6, and even 7 septa are common. Conidia hyaline, fusiform or crescent shaped, 30 to 48μ by 3.5 to 5μ with an average of 40 by 4μ. Chlamydospores (Figs. 37-38, Pl. 5) sperical 10 to 15μ in diameter, heavy walled, quite plentiful in artificial medium hyphae torulose. A non-fertile pseudo-oogonial-like structure, spherical, 25 to 30μ in diameter, is produced at the ends of the hyphae (Fig. 34, 35c, 36c, Pl. 5). This fungus produces no color on glucose media. It has not been proved to be parasitic on flax, tho taken plentifully from flax soil (see Table 1); readily

taken pure from Petri dish cultures of soil samples from flax-sick land. No perfect stage was found, though this fungus was given a thoro trial on different media.

On nutrient agar, one to two weeks old, copious, cottony, white to gray and in older cultures grayish brown; conidia plentiful; on nutrient glucose agar, as on nutrient agar, substratum somewhat more brown. Conidia plentiful, chlamydospores abundant, hyphae torulose; pseudo-oogonial structures frequently formed at ends of the hyphae. On potato plug, the aerial growth is profuse, in beginning colonies white to gray, but later turning grayish yellow to grayish brown—conidia quite plentiful.

This Fusarium in our inoculation tests on flax in sterile soil gave only negative results.

Colletotrichum lini n. sp. Manns and Bolley. (See Plates III and IV, Figs. 19 to 31.) Description: Vegetative hyphae abundantly septate, in deep tissue of host almost hyaline, near surface light to dark or sooty, 3 to 10μ in diameter, average 3.5μ. Acervulae scattered on stems of host, slightly erumpent; spores sessile on a compact matrix, surrounded by or interspersed with setae. Setae 2 to 3 septate, dark brown, 70 to 130μ in length. Acervulae scattered, spores produced in abundance. Conidia biguttulate, hyaline, slightly curved, allantoid, 15 to 20μ by 2 to 4.5μ abundant on wilting seedling, particularly on stems of more mature flax. Chlamydospores olive to brown, spherical to oval, 10 to 12μ by 10 to 15μ.

Parasitic on flax, causing in seedlings typical "damping-off" in mature stages weakening the plants, and shrivelling of seed. It is associated with *Fusarium lini* Bolley in causing flax sickness in flax districts of the United States and in European countries. The spores are carried in and on flax seed. No perfect fruiting was found or produced in cultures.

Growth on Artificial Media: This *Colletotrichum* is difficult to obtain in pure culture owing to the damp growth, which favors bacterial association.

On Nutrient Agar—Colonies spread slowly, moist, mycelium gray to sooty, conidia abundant over moist areas, setae few and scattered. Conidia clear or biguttulate. **On Nutrient 2% Glucose Agar.** Growth rapid over surface, moist, slight aerial mycelium, abundant conidia give the surface a rusty color, setae scarce. **On Potato Plug**—Slow growing, characters similar to those on Glucose Agar, spores abundant, rusty, tending towards yellow. On the surface, the growth later assumes a wrinkled, wavy appearance as in cultures of Avian tuberculosis.

Inoculation tests in laboratory and field caused typical "damping-off" in seedlings. Germinating plantlets show setae projecting from stems, associated with abundant conidia. Growing plants show blighting effects and shrivelling of seed. The acervulae are distributed along the lower stem. Setae and spores are plentiful.

Other fungi separated from "flax sick" soil, flax seed and sick plants are illustrated in Fugures 31 to 63, but are not here described.

Melampsora lini, Figures 65 and 66, the rust of flax was commonly found in the field studies but this disease has not, as yet, been very destructive in Northwest flax districts.[1]

[1]This rust of flax is a seasonal disease which varies in intensity according to weather conditions, the varieties in common use, and with the intensity of consecutive culture of non-resistant sorts. There have been years since 1903, when this thesis was written, when the rust of flax has been very destructive in parts of the Northwestern states. Since this disease does not attack certain strains and varieties, it is possible that it can in the future be almost wholly controlled thru proper breeding, selection and use of rust resistant varieties. H. L. B.

MISCELLANEOUS EXPERIMENTS

IMMUNITY TESTS IN LABORATORY

It has previously been demonstrated by H. L. Bolley that a rather definite type of resistance or immunity to flax wilt may be shown by certain individual flax plants. During the summer of 1903 plants showing considerable resistance were selected and planted. In the crop, from some 30 numbered plants, No. 10 was about as resistant as any. The object of the tests was to see if frozen flax sick soil would produce wilt and to learn, in laboratory practice, if it be possible to distinguish resistant qualities in flax plants. The seed used for the check crop was known as Magill No. 3 and was grown in 1902 near the station. Daily records were taken on the growths and infection results. The experiments and results are as follows:

Experiment 1, December 11, 1903. Frozen soil from Rotation Plot 31, thawed, placed in box and was planted with 40 seeds from "Immune" Plant No. 10, crop 1903. 12/18. Thirty-eight plants up, all healthy. 1/3/04. Thirty-eight plants, all remain healthy.

Experiment 2, December 11, 1903. Some soil planted with 40 seeds from check samples No. 3, Magills', crop 1902 which was previously grown on virgin soil lands. 12/18. Thirty-nine plants up, all healthy. 12/27. Two plants wilting. 12/28. Three plants wilting. 12/29. Eleven plants wilting. 12/31. All plants wilting. 1/3/04. **All plants dead.**

Conclusions: 1. Plant 10, crop 1903, from the original immune selects was fully immune to the flax wilt disease; 2, the check seed was not from an immune strain; 3, test of immunity can be successfully carried out in the laboratory; 4, the frozen soil contained the wilt fungus and it became active at proper temperature.

EFFECT OF COMPOSTING FLAX STRAW FROM WILT INFECTED CROP IN BARNYARD MANURES

Professor Bolley in 1902 had gathered considerable diseased flax straw. This was tied in small bundles and in January placed at different depths in fresh stable manures in order to learn the results of composting on the wilt fungi.

August 29, 1903. Removed first bundle from east end of compost heap at a depth of about 1 foot.

Pieces of straw from different parts of the bundle were placed in 20 sterile tubes, adding sterile water. In two large tubes larger amounts of material and sterile water were used. The straw in some parts was well rotted while in other places it was partly dry rotted and brittle.

9/5. A white mold resembling *Fusarium lini* came out of many straws. Micro-examination showed that it was not *Fusarium lini*. 9/20. Examination of all the tubes on this date showed no *Fusarium lini*. 10/7. Careful examination of all tubes showed no *Fusarium lini*.

Conclusion: In well composted flax straw, danger of disease dissemination is lessened.

Spread of Wilt and Other Diseases in the Soil: From the experiments as outlined, it is apparent that there are two or more

seed and soil infecting fungi which account for the spotted appearance of the flax crop upon lands newly planted. These, in large part, account for many of the failures experienced in consecutive or close flax cropping.

SUMMARY

1. There are several fungi associated with flax wilt and soil sickness in flax. The most important of these are *Fusarium lini* Bolley, *Fusarium russianum*, Manns n. sp., *Colletotrichum lini*, Manns and Bolley, n. sp., *Alternaria* sp. and *Melampsora lini* DC Tul, the latter causing the rust of flax.

2. These root destroying fungi are also present in all of the chief flax districts studied. In new areas of flax growing, the chief infections are rapidly spreading.

3. All of these fungi overwinter in the soil or carry from crop to crop, either as saprophytes, in or on the seed, and in or on the roots, stems, and other parts of the flax crop.

4. Soils become "flax-sick" due to the presence of **specific** disease-producing fungi.

5. The presence of the most important of these organisms may be demonstrated in "flax-sick" soils by plating out on artificial media in Petri dishes.

6. By use of the physician's centrifuge, the spores of several of these diseases may be precipitated from flax seed and directly examined under the microscope.

7. Each of these diseases, with exception of *Melampsora lini*, may be obtained in pure culture from plates made of precipitated spores.

8. Each of the parasitic types, excepting *Melampsora lini*, may be taken in pure cultures from infected seedlings grown in the laboratory either in the Geneva germinator or in soil in pots. They may be taken also from any part of sick plants from the field, after surface sterilization, then planted on a sterile medium in tubes or plates or by crushing and maceration in liquified agar and plated in culture dishes.

9. The pathogenic nature of each of the parasitic types was demonstrated by infecting graded and treated flax seed, planted and grown in sterile soil.

10. The organisms gain entrance to the interior of the flax seed. This may be seen in shrunken spots or streaks on the seed coats and in seed leaf injury. Lesions are evident when the cotyledons break from the testa. These internal organisms may be taken in pure culture from such infected seeds by sterilizing the seed coats and removal of the embryos or cotyledons from the testas to sterile media.

11. Soil which has become "flax-sick" thru infection by these organisms will probably require several years of rotation and cultivation with flax omitted.[1]

12. The fact that "flax-sickness" is not fertility depletion is proved by successful production of other crops and by the high bacterial content of such soils.

13. Sulphur and salt as applied in Department tests to "flax-sick" soil, after heavy applications each year for 4 years appear to have no effect in reducing the sickness.[2]

14. *Fusarium lini* Bolley penetrates the soil to a depth of at least 12 inches, but is taken most abundantly at depths not exceeding 5 inches. Under continuous cropping of flax in sick soil, as many as 45,000 colonies of the wilt fungus *(Fusarium lini)* may be taken from a single gram of soil.

15. It is demonstrated that many European flax samples contain several and sometimes all of the parasitic fungi mentioned and it has been possible to observe the symptoms manifested by flax attacked by each.

16. Sick plants from European seed samples showed one or several of these specific diseases upon them.

17. Growth of flax in water cultures and in soil failed to show that *Asterocystis* sp. is instrumental in causing any type of flax sickness.

18. Flax plants grown from seed from many different sources indicate that *Fusarium lini* Bolley is the most destructive and widely distributed parasite of flax. This fungus is aided in producing "flax-sickness" by *Fusarium russianum* Manns n. sp. and by *Colletotrichum lini* Manns and Bolley n. sp. and also by an *Alternaria* sp.

19. Formaldehyde treatment for disinfection of flax seed appears to be the most effective and satisfactory agent. Careful grading and treatment of flax seed retard flax-sickness.

20. Sterilized sandy loam soil is the best medium in which to grow sterile flax plants for infection work.

21. Plants may be selected which show resistance to these specific diseases. The resistance may be demonstrated in the laboratory by the pot methods.

22. These different fungi readily overwinter in the soil.

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[1]Since 1903 there have been extended series of crop rotation tests applied to flax sick or partially infected soil. No evidence has accumulated at the Experiment Station or in the general farming operations in the State which indicate that the flax root diseases can with any certainty be controlled by any type of crop rotation now known. H. L. B.

[2]Since 1903 sulfur and salt applications at the rate of 20 pounds per square rod were continued on these separate plots for approximately 20 years. There has been no apparent control of the root diseases of flax or wheat. Several other plots have received heavy applications of various fertilizers, natural and artificial. None of these have materially aided in the control of flax wilt nor gave noticeable benefit thru increase of crop, until resistant varieties of flax were used on such fertilized plots. H. L. B.

23. Carefully composted barnyard manures known to contain flax straw may lessen the chances for soil infection.

24. These specific flax diseases require but 2 to 3 years to cause "flax-sick" soil. Sandy loams are extremely susceptible to flax sickness.

25. About 80 percent of Northwestern flax seed samples carry the wilt disease; hence the importance of seed treatment is evident. Resistant strains of seed offer possible opportunity for control in flax sick areas.

RAPID SPREAD OF FLAX-SICK SOIL CONDITION

Since 1903, when the thesis herein abstracted was prepared, the writer has had opportunity of seeing the aggressive action of *Fusarium lini* and associated fungi in producing "flax-sick" conditions in virgin soils. The spread is surprisingly rapid. A field 1½ miles southeast of Ypsilanti, Stutsman County, North Dakota, broken from virgin sod in the spring of 1906 and seeded to flax suffered considerable loss in the first cropping. The writer visited the field in 1907; it was again in flax. On July 6 fully one-third of the seedlings had wilted and the disease was general. The soil in which such rapid spread had taken place was light sandy loam.

Another field visited is located 6 miles east of Montpelier, Stutsman county. Virgin sod was broken in 1902. There had been three flax crops, the third being planted in 1907. In August the crop was so spotted by wilt that it was not worth harvesting. It had virtually turned into a weed patch. This disease does not seem to check the weeds.

On a farm managed by my brothers, care had been exercised to prevent the incoming of flax wilt. Seed was thoroly cleaned and graded each season, discarding in these processes fully one-fourth in light and small seed. As soon as treatment by aqueous formaldehyde solution was worked out, 1902, seed disinfection was practiced. A number of these fields, in all amounting to 400 or 500 acres, were carefully examined and I found none free from wilt. In the older areas, those cropped with flax three or four times, spots containing 2 to 10 square rods were common .

It is evident that these light soils, sandy loams, known to be well adapted to flax raising, are also prolific in the production of flax root diseases; much more so than the heavier soils of the Red River Valley region. Nothing short of long seried rotations, extra care in seed cleaning and grading, together with seed treatment of flax can prevent the rapid sickening of the soils of the central part of the State. Immune or highly resistant strains may, of course, prove a great boon to every flax district.

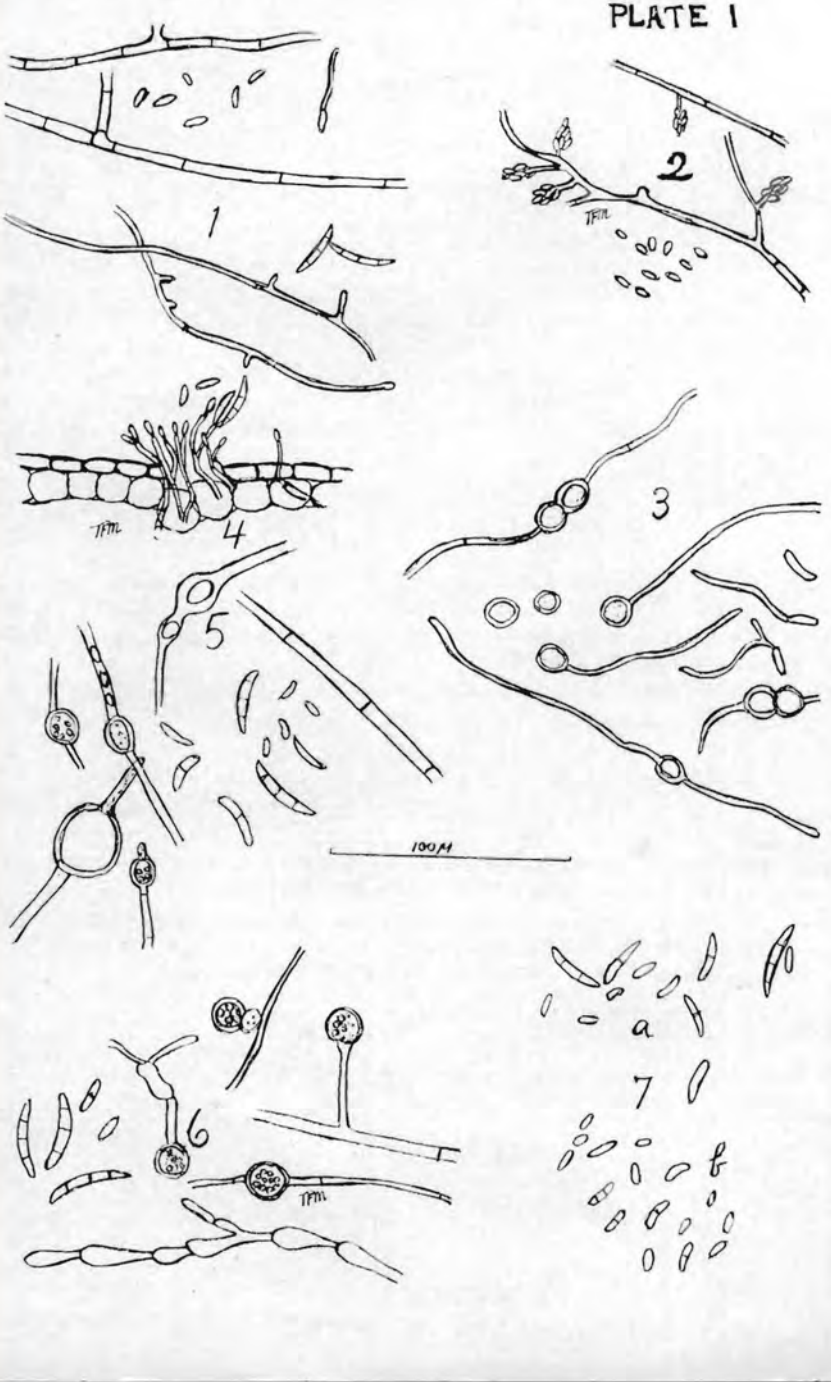
PLATE I
1
2
3
4
5
6
7
a
b
100 μ

EXPLANATION OF PLATES

All figures in the following plates, 1 to 9, are from original drawings and, except as otherwise stated or indicated on the plates, are engraved to a scale approximately 330 diameters.

Plates 6, 7, 8, and 9 include figures and descriptions of various types of fungi other than the fusaria of wilt and the colletotrichum of canker. Many of these fungi were found in the seed associated with *Fusarium lini* while others were taken by pure cultures from flax-sick soil of Rotation Plot 30 of the North Dakota Agricultural Experiment Station. Numerous inoculation tests were made with some of these associated fungi but none were, at the time, proved to be parasitic on flax or directly instrumental in causing flax-sick soil.

For botanical description of *Fusarium lini* Bolley, see Bulletin 50, North Dakota Agricultural Experiment Station, December, 1901.

PLATE 1

Figure 1. Macro-conidia, from nutrient agar; plated from "flax-sick" soil of Rotation Plot 30, N. Dak. Exp. Sta.

Figure 2. Mycelium, sporophores, and micro-conidia, from nutrient agar and plated from sick soil. An early formation of micro-conidia as shown is very characteristic.

Figure 3. Chlamydospores and micro-conidia germinating; both types of spores germinated well after the cultures were air dried four months. Originally plated from "flax-sick" soil.

Figure 4. Beginning of sporodochium on a young plant, grown from seed direct from Tver, Russia. The seed was sown in sterile soil.

Figure 5. Hyphae, chlamydospores, micro and macro-conidia from sick plant grown in Geneva germinator infected by fungus transferred to nutrient agar from seed grown on sick soil of Rotation Plot 31, N. Dak. Exp. Sta.

Figure 6. Hyphae, chlamydospores, micro and macro-conidia, grown on nutrient agar and as taken from sick plant grown in Geneva germinator. Original seed from F. J. Sprung, Ada, Minn.

Figure 7. Micro and macro-conidia; (a), on nutrient agar; (b), on nutrient glucose agar; culture from plant grown in Geneva germinator, seed from Northfield, Minn. 1902.

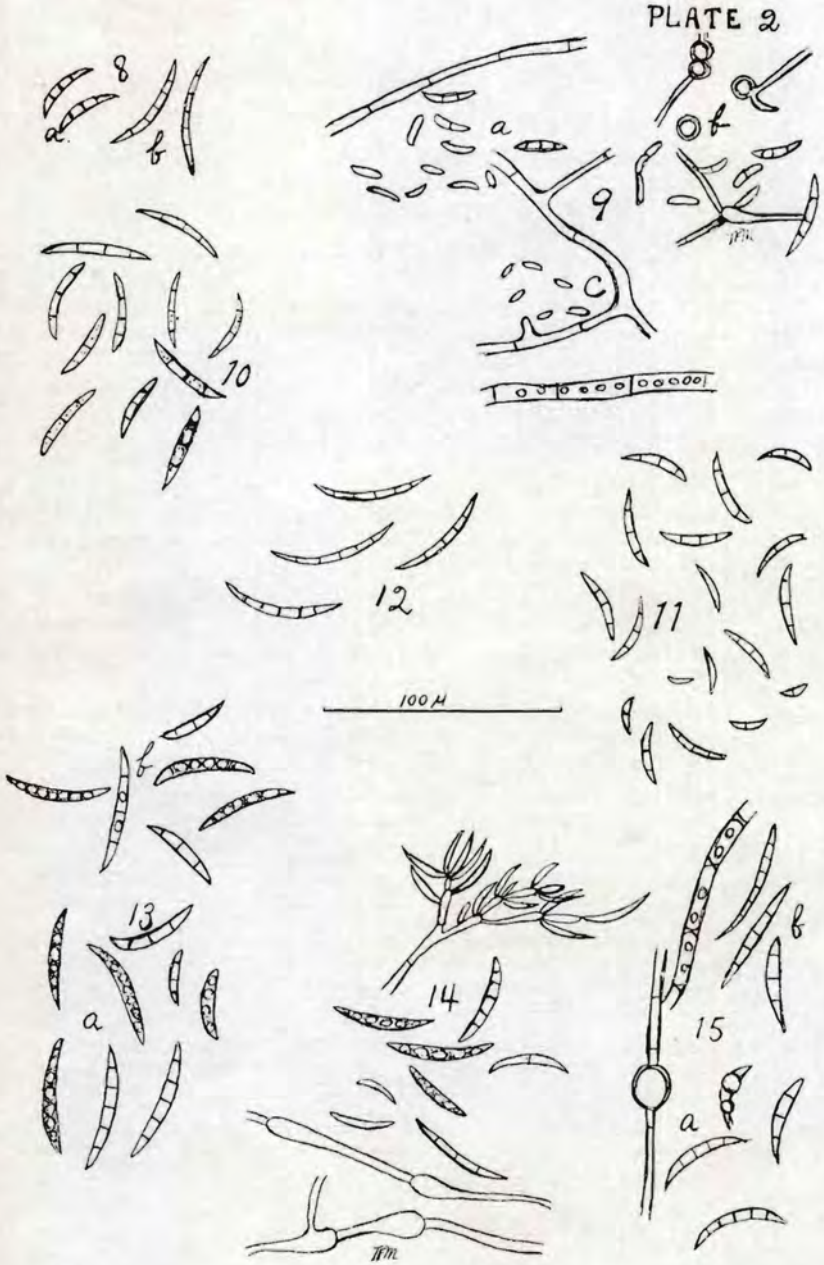
PLATE 2
8
a
b
9
c
10
12
11
100 μ
13
14
15

PLATE 2

Figures 8 to 15 inclusive show the chief comparative features of *Fusarium lini* Bolley and *Fusarium russianum* Manns.

Figure 8. Contrasting macro-conidia of *Fusarium lini* Bolley with macro-conidia of *Fusarium russianum* Manns Nov. Sp., both taken from the same sample of seed from Tver, Russia. Both fungi parasitic on flax.

Figure 9. *Fusarium lini*, (a), from culture on potato plug; (b) from nutrient agar; (c) from nutrient glucose agar. Original cultures from sick soil of Rotation Plot 31, N. Dak. Exp, Sta.

Figure 10. *Fusarium lini* showing macro-conidia from nearly mature sick plant from sick soil of Rotation Plot 31, N. Dak. Exp. Sta.

Figure 11. *Fusarium lini* macro-conidia from sick plant from Dr. Kingo Miyabe, Agric. Coll., Sapporo, Japan.

Figure 12. *Fusarium russianum*. Macro-conidia. These are usually 5-septate while those of *Fusarium lini* are 3-septate. From Russian seed samples by centrifuge.

Figure 13. *Fusarium russianum*. Macro-conidia; (a) from nutrient agar; (b) from glucose agar. From seed from Northfield, Minn.

Figure 14. *Fusarium russianum*. Conidia, hyphae, and sporophore from cultures. Seed from Tver, Russia.

Figure 15. *Fusarium russianum*. Hyphae and conidia; (a) from nutrient agar; (b) from glucose agar. From seed from Russian samples.

PLATE 3

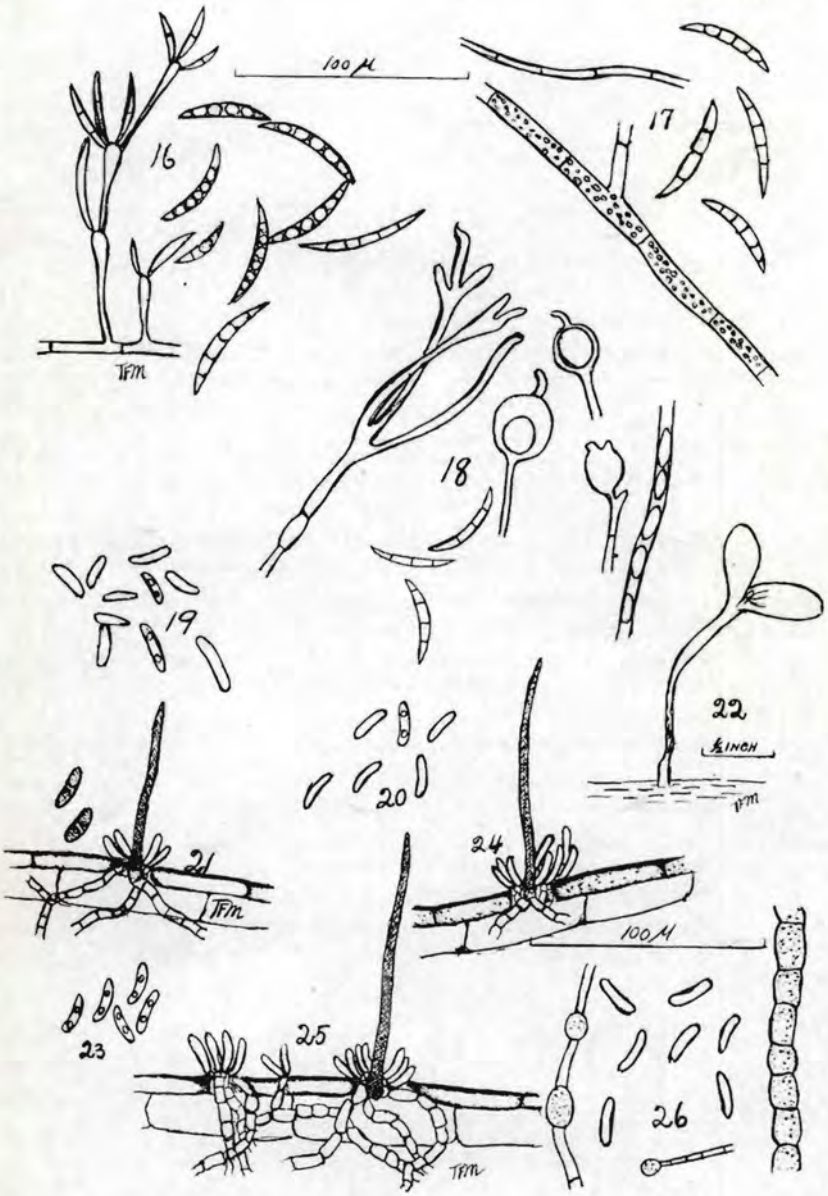

PLATE 3

Showing characteristic form of *Fusarium russianum* Manns in Figures 16 to 18 and of *Colletotrichum lini* Manns and Bolley in Figures 19 to 26.

Figure 16. Sporophore, hyphae, and conidia from nutrient agar, original cultures from flax seed grown at Northfield, Minn. Crop 1902.

Figure 17. Macrospores and hyphae same source as Fig. 16.

Figure 18. *Fusarium russianum.* Same source as Fig. 16, grown on potato plug.

Figure 19. *Colletotrichum lini.* Showing conidia from diseased plant grown in sterile soil; seed from Riga, Russia.

Figure 20. *Colletotrichum lini.* Conidia from a diseased plant from Dr. Hecke, Vienna, Austria. Crop in South Hungary.

Figure 21. *Colletotrichum lini.* Setum, conidia and tissue of seedling flax "damped-off" by this fungus.

Figure 22. Seedling flax plant "damping-off" from effects of *Colletotrichum lini.* Seed from Rotation Plot 30, N. Dak. Exp. Sta.

Figure 23. *Colletotrichum lini* showing guttulate conidia from precipitate in centrifuge from a Russian seed sample.

Figure 24. *Colletotrichum lini.* Setum and conidia from sick plant from Geneva germinator. Seed from Northfield, Minn. Crop 1902.

Figure 25. *Colletotrichum lini.* Hyphae, conidia and setum from sick plant; seed from Brabant, Holland. Sent by Consul-General L. Listoe, Rotterdam.

Figure 26. *Colletotrichum lini.* Chlamydospores grown on nutrient agar; seed from Brabant, Holland.

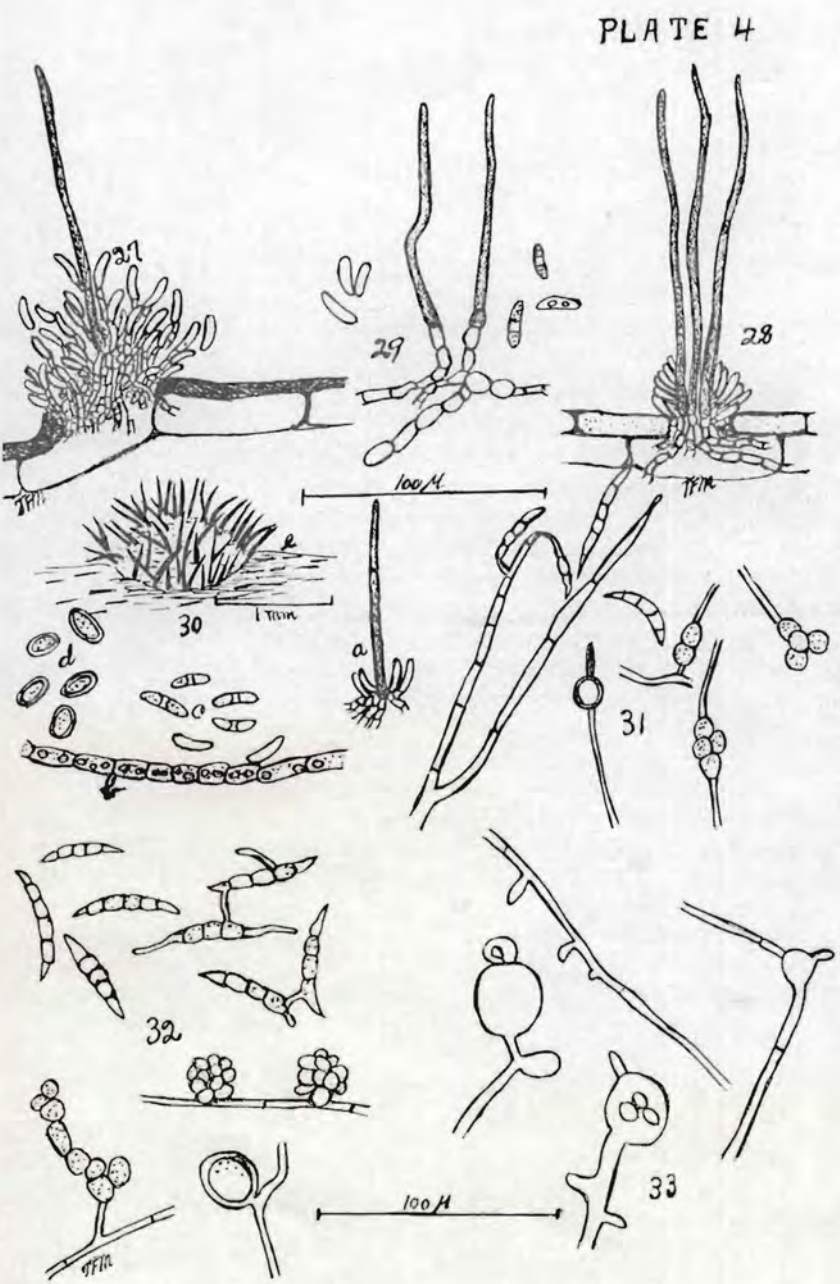
PLATE 4
27
29
28
100 μ
30
1 mm
a
d
c
31
32
33
100 μ

PLATE 4

Characteristic growth of *Colletotrichum lini* Manns and Bolley, Figures 27 to 30, and *Fusarium terrestris* Manns, n. sp., Figures 31 to 33.

Figure 27. *Colletotrichum lini.* Showing sporodochium with setum; from sick plant sent by Dr. Hecke, Vienna, Austria.

Figure 28. *Colletotrichum lini.* Setae and conidia from young plant from Geneva germinator; seed from South Hungary.

Figure 29. *Colletotrichum lini.* Setae, hyphae, and conidia grown on nutrient agar. Cultures one month old.

Figure 30. *Colletotrichum lini.* (a) Single setum with matrix on which conidia are borne (sessile); (b) hyphae; (c) conidia; (d) a type of spore not fully understood, possibly chlamydospores; (e) group of setae in acervulus. From nutrient glucose agar, in tube culture.

Figure 31. *Fusarium terrestris* Manns, n. sp. This fungus was found plentifully in flax sick soil, but is not proved to be parasitic on flax. Hyphae, conidia, and chlamydospores taken from "flax sick" soil.

Figure 32. *Fusarium terrestris* Hyphae, conidia and chlamydospores, from nutrient agar. Originally cultures were from "flax sick" soil of Rotation Plot 30, N. Dak. Exp. Sta.

Figure 33. *Fusarium terrestris* Pseudo-oogonial-like structures formed at terminals of hyphae. Grown on nutrient agar. Possibly distorted chlamydospores vegeative before mature.

PLATE 5

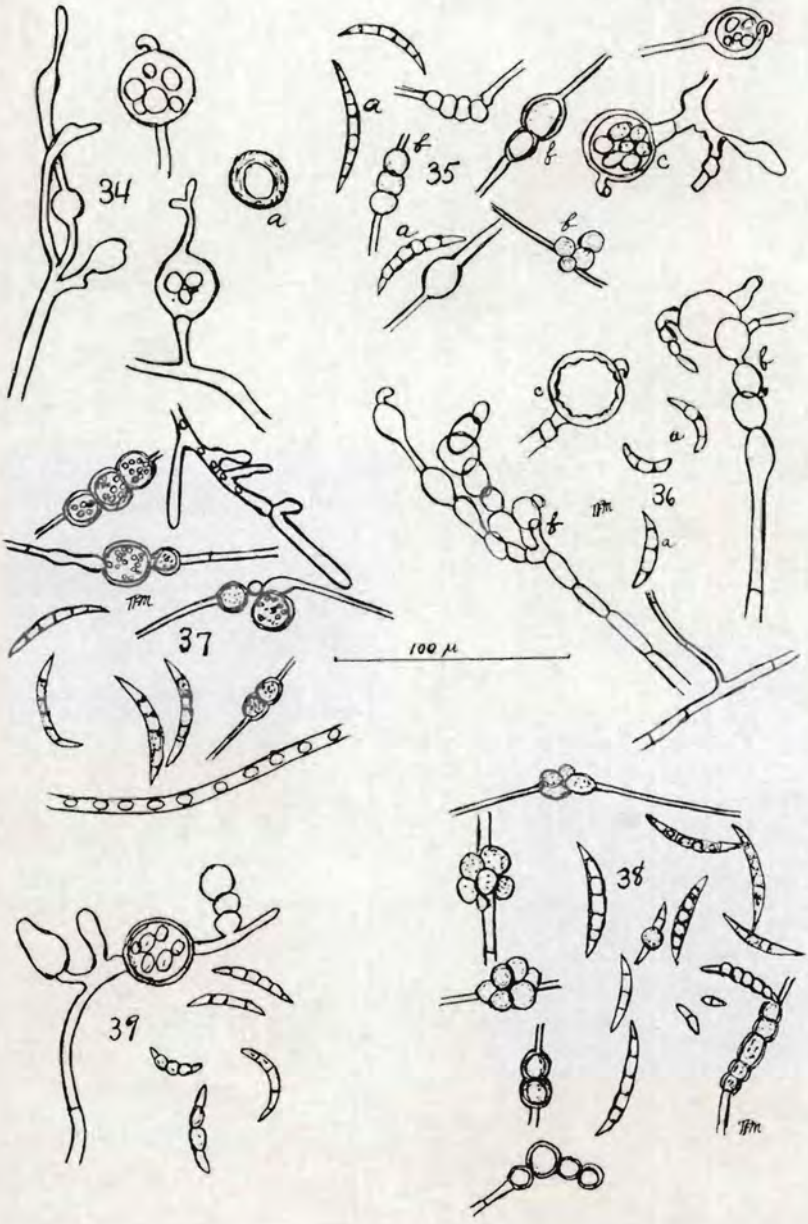

PLATE 5

Figures 34 to 39 illustrate various characteristics of *Fusarium terrestris* Manns n. sp.

Figure 34. Pseudo-oogonial-like structures. (a) One of these had turned brown with thickened wall, possibly forming a chlamydospore or resting spore of some type. From "flax-sick" soil.

Figure 35. (a) Conidia, (b) chlamydospores, (c) oogonial-like structures grown on potato plug. Cultures originally from "flax-sick" soil, Rotation Plot 30, N. Dak. Exp. Sta.

Figure 36. (a) Conidia, (b) torulose hyphae, and (c) oogonial-like structures, grown on nutrient agar. From "flax-sick" soil.

Figure 37. Spores and hyphae from same source as Fig. 36, grown on nutrient glucose agar.

Figure 38. Spores and hyphae parts from same source as Fig. 36, grown on potato plug.

Figure 39. Conidia and oogonial-like structure, taken from flax sample, grown on "flax-sick" soil on Rotation Plot 30, N. Dak. Exp. Sta.

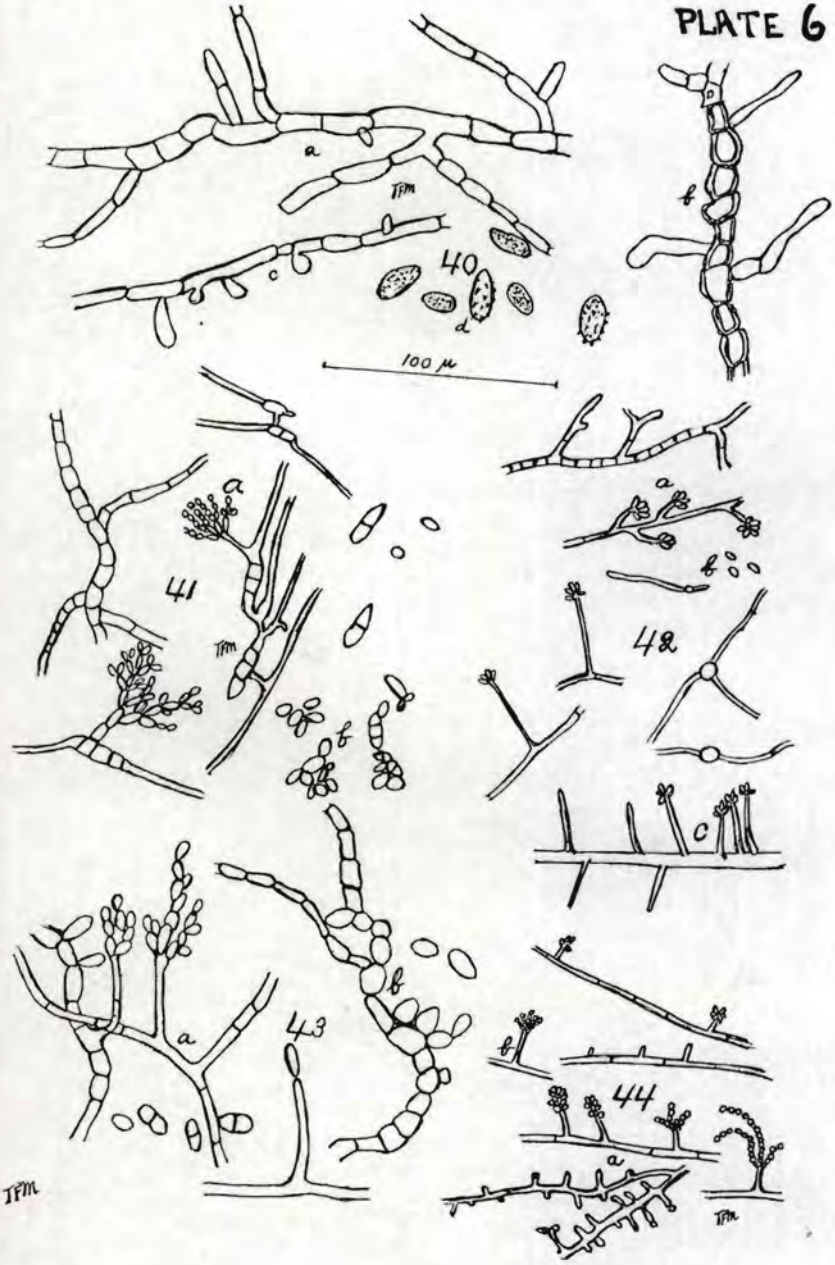
PLATE 6
a
IFM
c
40
d
100 μ
b
41
a
IFM
b
42
a
b
c
43
a
b
IFM
44
b
a
IFM

PLATE 6

Various fungi, taken from flax-sick soil in pure cultures but not proved to be parasitic on flax.

Figure 40. *Zygodesmus* sp. (a) Hyphae, (b) older hyphae, (c) conidial formation, (d) spiny conidia. Grown on nutrient agar.

Figure 41. *Hormodendrum* sp. (a) Fruiting hyphae from nutrient agar culture, (b) conidia from potato plug.

Figure 42. *Cephalosporium* sp.. (a) sporophore and (b) conidia, from nutrient agar, (c) sporofore from potato plug.

Figure 43. *Hormodendrum* sp. (a) Hyphae, sporophore, and conidia, from nutrient agar culture, (b) hyphae from potato culture.

Figure 44. *Briarea* sp. (a) Hyphae, sporofore, and conidia from nutrient agar, (b) Hyphae, sporofore and conidia from potato.

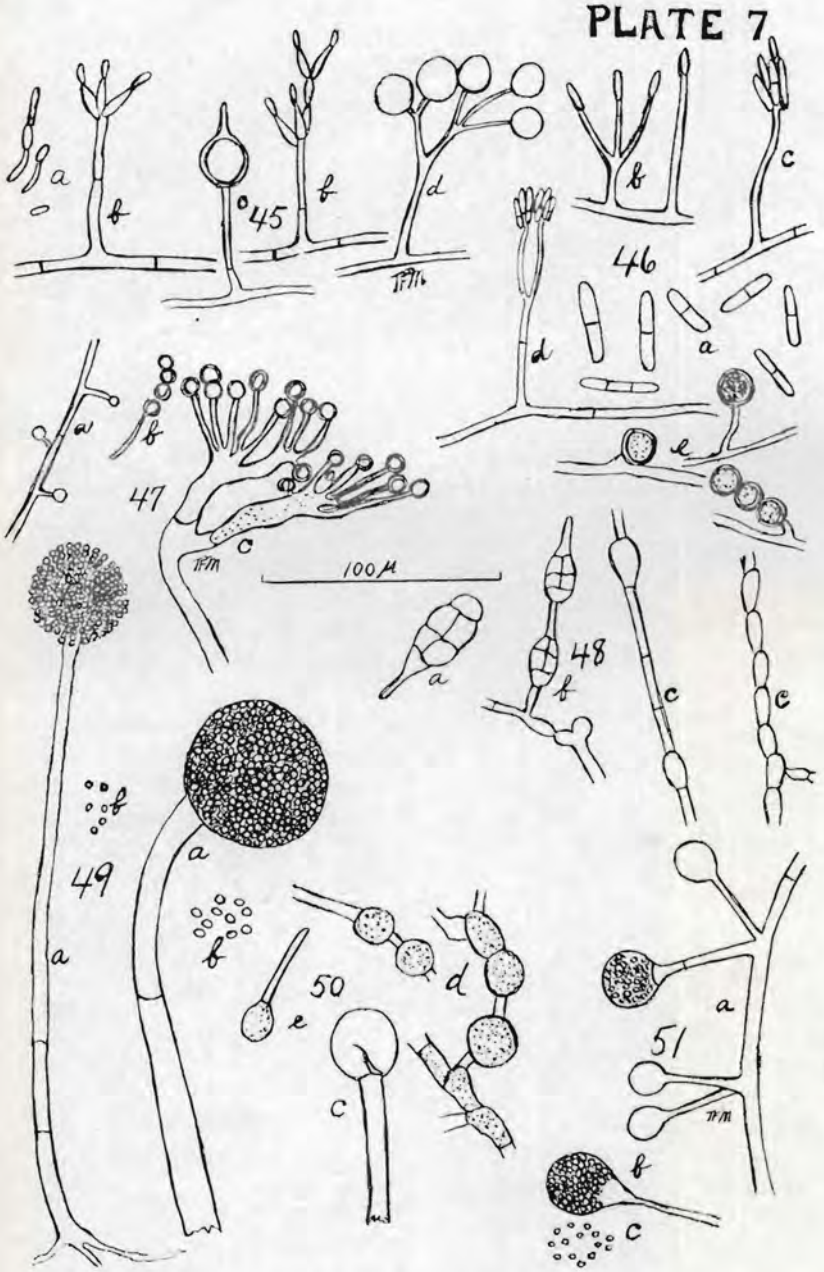
PLATE 7
45
46
47
48
49
50
51
100 μ

PLATE 7

Various fungi taken from flax-sick soil, the relation of which to flax cropping was not fully determined, except in case of No. 48 in which the tests were positive.

Figure 45. *Monosporium* sp. (a) Conidia germinating, (b) sporofore, (c) and (d) sterile growths.

Figure 46. *Diplocladium* sp. (a) Conidia, (b) early growth of sporophores, (c) mature sporofore, showing conidia, (d) branched sporofore, (e) chlamydospores.

Figure 47. This fungus is known in our cultures as No. 16. (a) Hyphae and sporofores, (b) conidia germinating on nutrient agar, (c) sporofore on potato plug.

Figure 48. *Alternaria* sp., parasitic on flax; (a) mature conidia, (b) sporophore, (c) hyphae.

Figure 49. *Aspergillus* sp. (a) Sporophore, (b) conidia.

Figure 50. *Mucor* sp. (a) sporophore and sporangium, (b) conidia, (c) sporophore shorn of fruit, (d) chlamydospores, (e) chlamydospore germinating.

Figure 51. *Mucor* sp. (a) Carpophores, (b) mature fruit, (c) conidia.

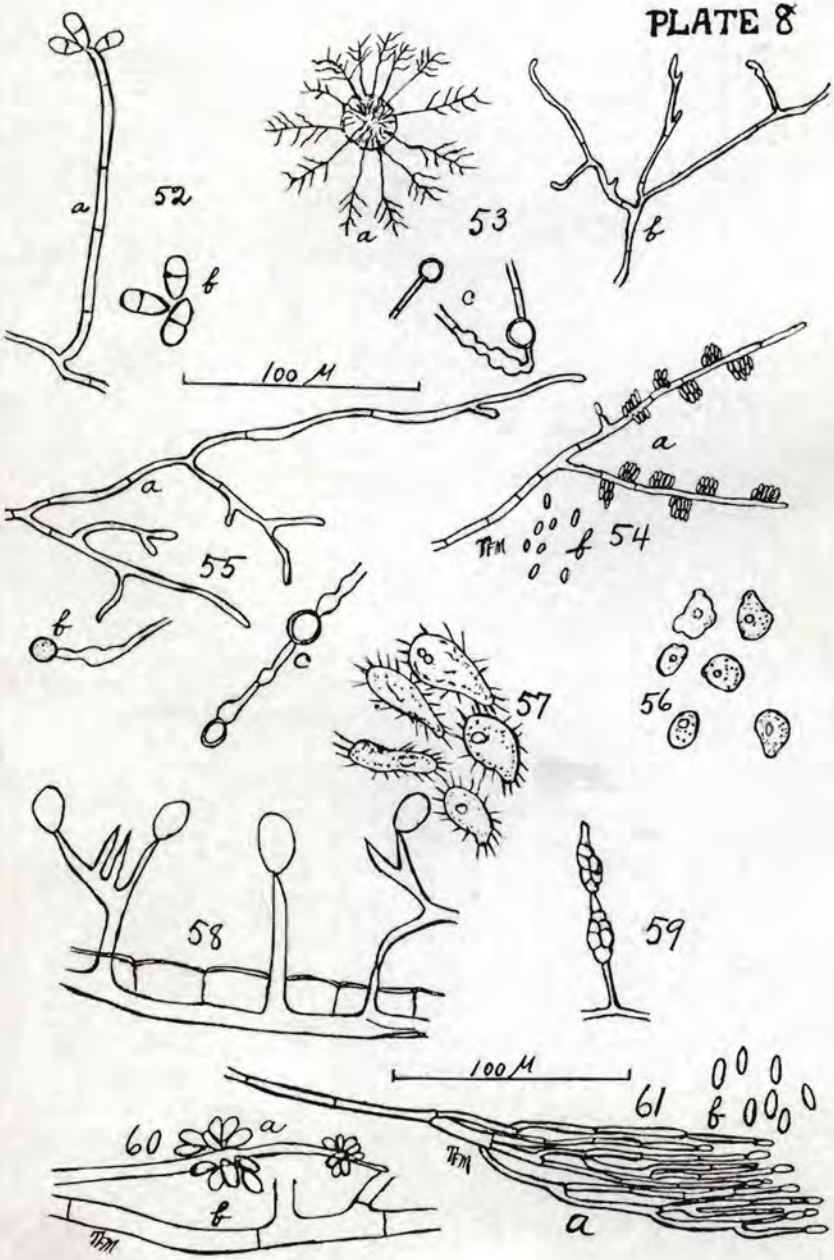
PLATE 8
a
52
b
a
53
b
c
100 μ
a
a
55
b
c
a
TFM
b
54
56
57
58
59
100 μ
61
b
60
a
b
TFM
TFM
a

PLATE 8

Various fungi taken in pure cultures during these investigations, but of unknown relation to flax cropping, excepting as stated under No. 59.

Figure 52. *Cephalothecium roseum.* (a) Sporofore with conidia, (b) conidia. This, as indicated, was taken from flax-sick soil on Rotation Plot 30, N. Dak. Exp. Sta. and the spores of the same were also found abundantly in certain flax seed samples and seemed to cause a type of moldy grain.

Figure 53. Known in our cultures as No. 50. A sterile type from the family *Tuberculariaceae.* (a) Type of colony magnified about two diameters, (b) hyphae, (c) chlamydosphores.

Figure 54. Our fungus No. 53. Probably a member of the family *Tuberculariaceae.* The conidia appear to be somewhat similar to those of *Fusarium lini* when in early growth, but soon differ. The conidia are sessile. (a) hyphae and conidia, (b) conidia.

Figure 55. Fungus No. 54. Much branching, sterile, not certainly determined. Apparently belongs to the family *Mucedinaceae.* (a) hyphae, (b) and (c) types of chlamydospores.

Figure 56. *Amoeba* sp. These were found associated in the "damping off" of flax by *Colletotrichum lini.* The seed in which this was found came in the Russian harvest of 1901, Austrian grown in 1902. This sample of flax seed produced these amoebas in abundance whenever the seed was germinated.

Figure 57. *Paramoecium* sp. These were found associated with the foregoing types of amoeba upon certain samples which were "damped off" by *Colletotrichum lini.* The sample of flaxseed was grown in Russia in 1903.

Figure 58. *Peronospora* sp. This fungus was associated with *Colletotrichum lini* and with *Fusarium russianum* in cultures made from a certain Russian seed sample. It was not tested as to its pathologic nature.

Figure 59. *Alternaria* sp. This fungus was associated with *Fusarium russianum* on certain diseased flax plants. It seems to be semiparasitic on flax and caused a slight loss to seedlings. This was taken from a Russian sample of flaxseed. A similar fungus appears plentifully on American grown flax and causes a dying in seedlings. Bolley attributes a special seed boll blight to this *Alternaria.* (See Bulletin 55, 1903, N. Dak. Exp. Sta.)

Figure 60. *Rhinotrichum* sp. (a) Sporophore and conidia, (b) older hyphae. This fungus was associated with *Colletotrichum lini* in "damping off" of the seedlings from a Russian seed sample.

Figure 61. *Spicaria* sp. (a) Sporophore, (b) conidia. This was commonly found associated with *Fusarium lini* on seed from sick plants grown on Plot 30. This was also taken out of European grown seed samples and was apparently active in killing flax seedlings in the germinators.

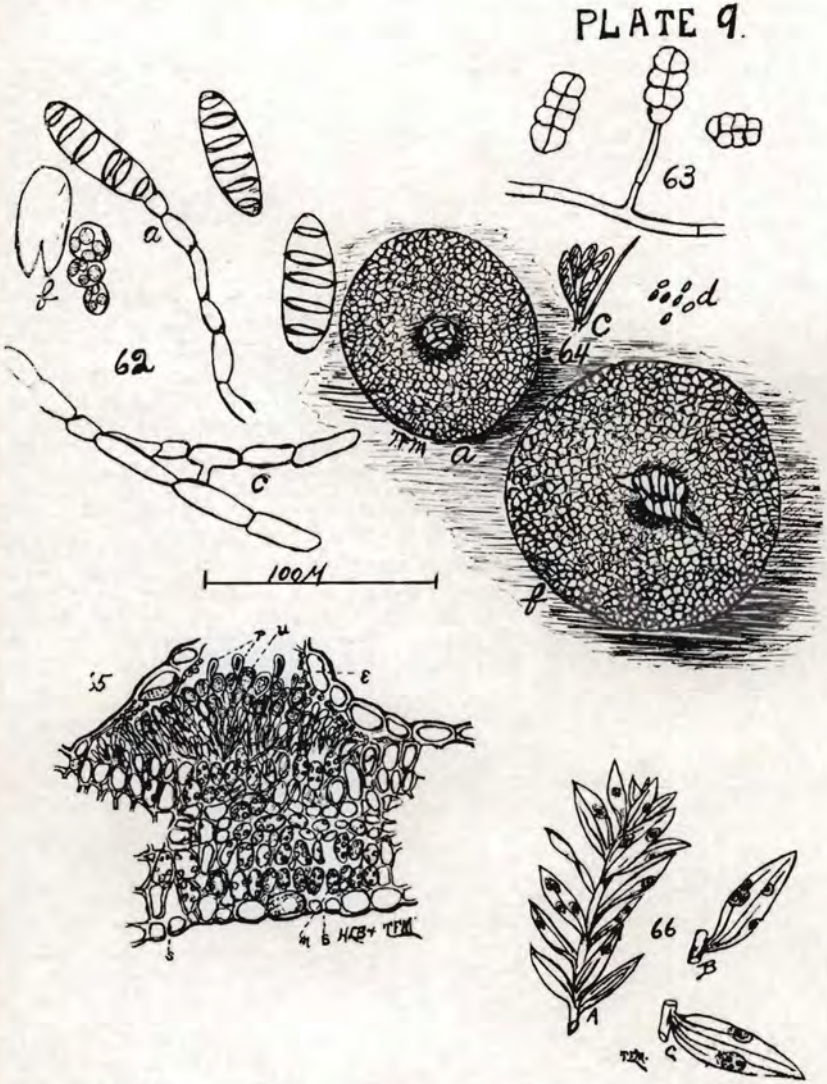
PLATE 9.
63
a
b
62
c
64
c
d
a
b
100μ
65
e
66
A
B
C

PLATE 9

Various fungi from flax and flax seed of unknown or undetermined relation to flax sickness—Figures 62 to 66.

Figure 62. *Brachyosporium* sp. Found in flax seed grown at the N. Dak. Exp. Sta., crop 1901. (a) Conidiophore, (b) conidia, showing contents, (c) hyphae. Not tested as to parasitism.

Figure 63. *Macrosporium* sp. Spores and sporophore from flax grown in Belgium. Not tested as to parasitism.

Figure 64. *Coelosphaeria* sp. (a) and (b) perithecia; (c) asci, (d) ascospores. The fruiting of this fungus was abundant on flax from a field near Vologda, Russia. Professor Bolley wrote, August, 1903, "¾ of the field is dead from what appears to be a 'Boll and stem' disease." Failed to get pure culture of this organism.

Figure 65. *Melampsora lini* (D. C.) The cross section of young pustule in which the uredospores are nearly full size, about 150 diameters.

Figure 66. *Melampsora lini* (D. C.) The severe attack on a two weeks old plant, natural size. (b) Upper surface of leaf, (c) under surface of leaf. Approximately natural size.