



128
578
THS

POTATO WILT CAUSED
BY
FUSARIUM OXYSPORUM

THESIS FOR DEGREE OF M. S.

ROBERT W. GOSS

1915

128
513
TTC

LIBRARY
Michigan State
University

PLACE IN RETURN BOX to remove this checkout from your record.
TO AVOID FINES return on or before date due.
MAY BE RECALLED with earlier due date if requested.

DATE DUE	DATE DUE	DATE DUE

POTATO WILT CAUSED BY FUSARIUM OXYSPORUM.

Thesis for the Degree of Master of Science.

Michigan Agricultural College.

Robert W. Goss
Robert W. Goss

June

1915.

THESIS

TABLE OF CONTENTS.

History -----b	1
Geographic distribution.-----	13
Economic Importance-----	16
Field observations -- -----	19
Description of disease.-----	35
Etiology -----	43
Pathogenicity -----	45
Morphology -----	46
Cultural studies-----	54
Spore germination studies -----	77
Maximum and minimum growth.-----	81
Thermal death point. -----	84
Poisonous byproducts of the fungus -----.	85
Infection experiments. -----	88
Control - -----	103
Experiment with infected tubers. -----	105
Tests with chemicals -----	110
Effect of fungicides -----	111
Media formalae -----	116
Literature. -----	119.

POTATO WILT CAUSED BY FUSARIUM OXYSPORUM

HISTORY.

The Fusarium wilt of potatoes was probably present in this country for many years before the cause of the wilt was determined. The first note of the pathological condition caused by this disease in this country dates only as far back as 1895 when G.P. Clinton reported the disease in Illinois. Since that time it has been widely observed but until recent years it has probably been more or less confused with the other wilts of the potato. In most cases simply the presence of the condition caused by the organism was noted and very little attempt was made to determine the cause of this pathological condition. It was not until about 1904 that the disease came into prominence, when it first assumed economic importance in this country. At this time there appeared a bulletin by Smith and Swingle (1904) describing Fusarium oxysporum as the cause of the wilt of potatoes noted in Michigan and the District of Columbia.

No important epidemic periods or epochs in the history of the disease are found; this is probably owing to the slow and insidious nature of the infection of the potato crop, not causing a rapid rot of the plants but slowly

undermining the seed stock and gradually decreasing the yield.

Very little work was done on the disease in this country after 1904, until a widespread occurrence of the disease was reported in California in 1908, which was studied by Orton (1909), who also studied the diseases of potatoes in various parts of the country in 1909 and 1910 and abroad in 1911. He found *Fusarium wilt* to be very widespread in America but not recognized in Europe. Since that time very valuable work has been done by Dr. Wollenweber, especially on the morphology and classification of this species of *Fusarium*, and inoculation experiments have been conducted by Manns in Ohio.

The disease was first noted in Michigan in 1899, (Smith and Swingle 1904) and since that time very little has been done with the disease in this state. It was reported by G.H. Coons (1914) and was studied by the writer in various parts of the state during the past season and found to be of widespread occurrence, and while strictly speaking it has not caused an epidemic as yet in this state it is certainly assuming great economic importance.

PREVIOUS WORK.

The literature on the taxonomy of the genus *Fusarium* has been in a very chaotic state until recent years,

and necessarily that part of the literature in reference to the pathological conditions caused by these organisms has been in no better condition.

In regard to *Fusarium oxysporum* in particular, the literature has been very confusing, principally because no reliable cultural and morphological studies and inoculation experiments have been conducted with reference to the pathological conditions noted in the field.

Fusarium oxysporum had long been regarded as the cause of the dry rot of potatoes, even up to 1904 when Smith and Swingle l.c. wrote on the subject. In their bulletin the authors describe a species of *Fusarium* which they identify with the *Fusarium oxysporum* of Schlechtendal (1824) and which they report as causing a dry rot as well as the wilt of the potato plant. No inoculation experiments were made and it is very probable that they were working with more than one species, in the light of the recent studies of Wollenweber who has shown that *F. oxysporum* is a vascular parasite causing wilt and wintering over in the stem ends of the tubers but not producing a decay of the tuber.

Wilcox in 1912, (Wilcox, Link and Pool 1912,) working with a *Fusarium* causing the dry rot of the tuber compared his species with that which Smith sent him as *F. oxysporum* and found that the two were not identical. The *F. oxysporum* caused a wilt but not a decay, while his species

which he calls *F. tuberivorum* (sp. n. Wilcox and Link) was a saprophyte incapable of infecting any part of the potato plant except the tuber.

In fact previous to 1910 no reliable work had been done on the genus. In that year a publication by Wollenweber and Appel (1910) appeared which was the first authoritative publication giving a systematic treatment of the morphological characters and laying the foundation for the separation of the species.

Wollenweber in 1913* described *F. oxysporum* as a vascular parasite causing the wilt disease but not the tuber rot of *Solanum tuberosum* in the United States of America, possibly also in Southern Europe and the potato districts of South Africa, Australia, etc. Also found on various hosts, such as *Solanum lycopersicum*, *Vigna*, *Pisum* and *Ipomoea*. The disease associated with the fungus has been confused with "leaf roll" a disease called "Blattroll-krankheit" in Germany." (A fuller discussion of these two words is given under morphology.) The final result of Dr. Wollenweber's study of this genus will undoubtedly straighten out and simplify the chaotic condition of the present classification of this genus.

This description and classification is considered authoritative and is used as the basis for the following work.

* Wollenweber, H.W. Phytopath. 1913, p. 24.

It is not the purpose of this article to discuss the literature in regard to classification, nomenclature and morphological characteristics which are fully covered in Wollenweber's work as well as in Smith and Swingle's, where complete bibliographies can be found. The account which follows concerns itself with the publications upon the pathological aspect of the wilt in the field and the presence of the disease as noted in different sections of the country.

Probably the first note upon the pathological condition caused by this disease in this country was that by Clinton in 1895.* He describes a condition found in the tubers which he calls "bundle blackening". "This is a fungus trouble of stored potatoes which shows as small dots or lines a short distance from the surface----- These bundles originate at the stem end and send off a few bundles to each of the eyes. The fungus gains entrance, probably after the potatoes are gathered, through the dead stem, and proceeds from this through the bundles causing them to turn black as the result of its attack." He goes on to say that, "the fungus is quite similar to the one causing the following trouble." He then describes a condition of the tuber which he calls "dry end rot". "It affects all the tissue as it slowly advances forward, until, perhaps, the whole tuber is destroyed. As in the preceeding case the trouble begins at the stem end, the fungus gaining entrance after the rupture

* Clinton, C. P. p. 139, 1895.

of the tuber from the plant." He believed that the latter disease was due to *F. solani*. In none of his observations has he connected the pathological conditions in the field with the conditions he notes in storage, and he has probably confused the bundle blackening of the wilt with the stem end rot caused by another *Fusarium*.

Stewart in 1896 writing on "Another Stem Blight of the Potato" describes a wilt of the potato and in his paper says he was doubtful if any organism was responsible for the trouble. This has been considered in previous literature to have been one of the first reports of the *F. wilt* in this country and was thought by Smith and Swingle to be probably the same disease they were working on, but according to Orton # "The disease described by Stewart (1896) and thought by Smith and Swingle to be probably the same, was stated by Prof. Stewart at a recent meeting of the American Phytopathological Society to be not due to *Fusarium*."

Clinton in 1904* reports from Connecticut that "many vines wilted before the middle of July" probably caused by *F. oxysporum* described by Smith and Swingle l.c. He connects the wilt with the unusual amount of bundle blackening and rotting found in market potatoes. He apparently reports a condition similar to that described by Smith and

Orton, W.A. 1913, p.3.

* Clinton, G.P. 1904, p. 349.

Swingle in which he has confused the wilt with other organisms causing dry rot of the tuber.

Smith and Swingle l.c. in 1904 give the first accurate description of the symptoms of the disease, and it is to them that we owe much of the present knowledge of the *Fusarium* wilt in this country. In spite of the fact that Wollenweber has since proved that they were probably working with more than one organism, their conditions of the pathological conditions found in the field is still standard as well as much of their cultural work. It is well to note that the first tubers Smith observed and on which he worked were from Hubbardston, Michigan.

The next report of the disease comes from Maryland. Norton in 1906 * refers to a dry rot as caused by *F. oxysporum*. " The disease is indicated by the lighter colored and more or less rolled up condition of the leaves. When dug the potatoes may appear sound, but internally show black or brown streaks, and later are destroyed at least at one end by the dry rot." Here again we find the confusion of the wilted condition of the plants with the dry rot of the tubers.

The next report is by Orton in 1909 # from California and is probably the first report of the disease

* Norton, J.B.S. 1906, p. 67.

Orton, W.A. 1909, p. 4.

on the Pacific coast. He says, " The principal cause of the marked decrease in the yield of old potato land is the presence of a fungus disease, the wilt or dry rot- *Fusarium oxysporum* Schlecht. He gives a description of the wilt and dry rot similar to that by Smith and Swingle.

Again Orton in 1911 * describes the disease in regard to its geographical distribution, he says " the disease described by Smith and Swingle-----is now coming into prominence as one of the most wide spread and destructive maladies of this crop. It appears to occur throughout the United States, but is more injurious in the irrigated sections of the west and in the southern half of the potato belt." His description is the same as his previous one.

Morse in 1909 # reports the disease from Maine, saying, " *Fusarium* dry rot caused by the fungus *F. oxysporum* Schlecht. has been found for the first time in Maine during the past summer." He reports it as not being very widely distributed through Maine.

Loundsbury in 1909 ** describes a dry rot of the potato tuber found in Cape Colony as caused by *Nectria solani*. He says, " the infection is introduced into the soil

* Orton, W.A. 1911, p. 751.

Morse, W.J. 1909, p. 2.

** Loundsbury, C.P. 1909, p. 42.

with diseased tubers and it remains there from season to season, so that a crop from perfectly healthy seed may get infected if grown on land that previously bore an affected crop. The disease generally enters at the stem end. The fungus develops most rapidly in the vascular ring." From his reference to the wilt symptoms shown by affected vines it appears that he has confused the *Fusarium* wilt with another species causing a rot of the tuber.

Tidswell in 1910 describes a dry rot and wilt of the potato in New South Wales, which he says is caused by *F. solani*, an organism which he claims should be more properly called *F. oxysporum*. He also makes reference to the wilting of the tops, blackening of the vascular regions and root infection, showing that he probably confused one or more other diseases with the wilt of the plant.

Manns in 1911 published a bulletin on this disease in Ohio. He describes a condition in the field and storage similar to the one that Smith and Swingle reported. He goes a step further, however, by saying that the *Fusarium* found in the blackened vascular ring is identical with the *Fusarium* of dry rot. This he proved by isolating the organism from the two sources, and carrying them through artificial cultures and inoculation experiments. They were identical and both brought about wilting in the field. These inoculation experiments are probably the first reported in this country

and according to Orton (1914) cultures of the Ohio strain have been studied by Dr. H.W. Wollenweber in comparison with a large number of others and proved to be what we still call *F. oxysporum*.

However according to Wollenweber's work *Larns* must have been dealing with a dry rot organism in connection with the *F. oxysporum* which he had. The only point in his infection experiments which might be open to criticism is the fact that most of his inoculation experiments were conducted by growing healthy plants on badly infected soil, and he draws the conclusions that " The disease comes on much more definitely under the sick soil conditions than it did where artificial cultures had been used." There is a possibility that a great deal of the poor stand he speaks of in the sick soil may have been due to other organisms, because in carrying out an inoculation experiment, in order to draw definite and reliable conclusions, it would be necessary to use healthy seed grown in sterilized soil that had been infected with the disease artificially.

The next report of the wilt comes from Minnesota, Stakman in 1912 reports the wilt as one of the worst potato diseases in that state. He associates the wilting of the plants and the browning of the tubers with the storage rot , but says, " It is doubtful whether this rotting is caused by the same fungus which causes the wilting of the

vines. If it is not identical with the wilt fungus it is very closely related to it."

Jones in 1912* makes note of the disease in Wisconsin and describes it as a wilt and dry rot, which he reports as not occurring seriously anywhere in Wisconsin. "It seems most frequent in the southwest part of the state where the soil is warm and early potatoes are grown," He also mentions in the same paper a slight browning of the stem end of the tuber which is not caused by the wilt. "Apparently due to late autumnal growth or other cause, which interferes with the full normal maturing of the tubers."

Wollenweber in 1913 ** published another article on the genus *Fusarium*, which he divides into sections on the basis of form of conidia and other morphological characteristics. *F. oxysporum* he places in the section *Elegans* which contains all the vascular parasites causing wilt, and differentiates this section from the other *Fusaria*.

Wollenweber in 1913 l.c. also published in *Phytopathology*, the results of further work on *Fusaria*. In this he refers to the unreliability of the stroma as a taxonomic character owing to the variation of forms on the different substrata. He describes in detail the morphological characteristics of this species, which will be taken up later,

* Jones, L.R. 1912, p. 4.

** Wollenweber, H.W. Ber. d. deut. Bot. Ges. 1913.

and also states that the potato is the host of 30 different forms of Fusaria. This article refers to many inoculation experiments and also contains a complete bibliography.

The latest article in print on this subject is probably the one by Orton (1914) in which he takes up a general discussion of the symptoms, cause of the disease and control measures as well as the geographical distribution of the trouble, which he claims is found in all the potato growing districts of the United States and he also believes that there is no evidence that the American wilt disease occurs in Europe.

GEOGRAPHICAL DISTRIBUTION.

In the first extensive work done on this disease by Smith and Swingle l.c.*they report after examination of tubers from District of Columbia, Michigan, Virginia, Kansas, Nebraska, New York, Florida and Wisconsin that ,
" It seems safe to say that this disease extends north to Canada, east to the Atlantic, south to the Gulf of Mexico and west to Colorado. How much wider its distribution is in this country cannot be stated at the present time, but it is almost undoubtedly beyond the limits from which it has been so far reported."

This latter statement was soon proved by the widespread reports of the disease in subsequent years, especially from California where the disease had not been reported at that time.

Orton(1914)* after a study of the occurrence of the disease in the United States, concludes that " It is certain that the Fusarium wilt is a nation wide problem and one that will have a marked influence upon American agriculture." He also states that after a study trip through Germany, Austria and England in 1911 he believed that there was no evidence that the American wilt occurs in Europe. Reports of such occurrence have been confused with the leaf roll disease.

* Smith, E.F. and Swingle, D.B. 1904, p. 52

** Orton, W.A. 1914, p. 16.

Dr. Wollenweber in his morphological studies of *Fusaria* has been able to differentiate the *F. oxysporum* causing American potato wilt from the European form.

CLIMATIC RELATIONS.

Most of the *Fusaria* causing a wilt disease of other crops are apparently diseases of the warmer climates. The *Fusarium* wilt of potatoes is undoubtedly more serious and causes more loss in the southern states than in the north. In fact Orton (1914) states that *Fusarium* wilt is apparently a disease of warmer climates. The southern states with high summer temperatures seem to suffer more than the northern states. New England and New York are reported comparatively free from the disease, while Manns in Ohio reports a very wide distribution. Going west the fungus is found further north, many cases having been observed by the writer in the irrigated sections of Idaho and in eastern Oregon.

The results of a months study of the disease in Michigan showed the presence of the wilt even as far north as Houghton, and several sections of the Upper Peninsula were found to be heavily infected; the same amount of infection was found in the southern part of the Lower Peninsula, so that in the opinion of the writer the temperature conditions do not affect the distribution of the disease to any appreciable amount in the state of Michigan, and probably a closer inspection of potato crops in many of the other northern

potato growing states would reveal the presence of the wilt in many cases.

Neither do the moisture conditions present any definite distinction on the severity of infection, as many of the fields examined in the Upper Peninsula had been exposed to excessive rains in the later part of the summer, while fields similarly infected had been through a comparatively dry field in the Lower Peninsula. There is however, an affect , that the various moisture conditions would have on the severity of infection by means of weakening the plants and predisposing them to infection or by causing a breaking down of the tissues in the plant and making them more susceptible to invasion by the fungus.

ECONOMIC IMPORTANCE.

The economic importance of the disease in the United States varies greatly with the conditions found in the various localities, but it is undoubtedly much greater than was previously supposed, and the disease will continue to spread until more stringent methods are used for its control.

According to Orton (1914) the disease in the southern states while of possibly greater severity, is of minor importance owing to the fact that in most districts the Irish potato is a minor crop, while in the trucking sections of the south the main crop is the early one grown for northern markets and the infection of this crop is relatively slight compared with that of the fall crop. In the irrigated sections of the west the disease plays an important role because of the necessity for a longer rotation of crops less remunerative than the potato. In California the wilt is the principal factor in causing a reduction of the yield in the San Joaquin Valley, (Orton 1909). It is also serious in Oregon and a few other western states. In the middle west Ohio and Michigan are badly infected and recent reports would show that there is also a great deal of infection in Wisconsin.

Estimates of the money losses caused by this disease are necessarily very hard to obtain, owing to the peculiar nature of the disease, and the small amount of information available. The loss is occasioned principally by

a reduction in the yield, and there are so many other factors which may cause a reduction of the yield that it is almost impossible to determine the exact amount of loss caused by the wilt.

Manns of the Ohio station reports in 1911 that the sick soil conditions may reduce the crop in many cases 50 % or more of the average yield. Their experiments on infected fields show a yield of 69 bu. per acre, while the average for the county was 186 bu.. The preceeding four years average for the station was 100 bu. and the county 101 bu. This shows the large amount of injury to the yield caused by the wilt, and figuring that only 5 % of the Ohio fields were infected, he would estimate that the loss would amount to over 870,000 bu. for the state.

In this state the loss could not be accurately ascertained from the field data. The fields examined showed an average of 30 % slight infection and 1.5 % serious; clearly indicating the large amount of loss due to the disease in this state. In many cases where the infection was bad the yield was reduced to under 100 bu. per acre, while there were very few other fungus troubles found in the same fields. The average given here is probably low for the entire state as this was taken from the best fields growing potatoes for seed purposes.

The disease is more important economically in this state in the seed growing section. The serious infection

found in the state during the last year was not large enough to be of great economic importance, but the fact that the high percentage of slight infection is present, is of vital interest to the seed grower as even the slight infection of the tubers without materially decreasing the yield for the present year would disqualify the potatoes for seed.

The widespread occurrence of the wilt in this state makes it undoubtedly one of the worst enemies of the potato grower, especially as it is hard for many growers to realize, that while the present years crop may not be badly damaged, by planting the infected tubers the following year the stand will probably be very poor and the yield materially reduced.

Thus it is seen that the disease is of the greatest economic importance in this state especially in those sections where the growing of potatoes for seed is being conducted, and it should be considered as such, because of the great difficulty in eradicating the trouble from the fields after it has once gained a foot hold.

FIELD OBSERVATIONS.

Early last fall it was decided to conduct a system of potato seed inspection and certification in this state by the cooperation of the Michigan State Potato Association and the Michigan Agricultural College. The writer attempted to conduct this work and during the month of September and October many of the most important seed potato producing centers were visited personally, in both the Upper and Lower Peninsulas.

A general survey of the potato diseases in the state were obtained in this way and the widespread presence of the Fusarium wilt was noted and the particular condition of the infected fields was observed by the writer and accordingly was made the subject of special study in the field and the continuence of the work on the problem during the winter.

The following data were obtained by the writer during September and October, one examination being made just before digging time. Only those fields were examined that were being grown for seed, so that the percentage of disease taken from these fields would probably not be as high as an average of the entire potato crop of the state. It was impossible to make examinations of all the potato growing sections in the state but those counties which were examined are probably a fair average for the entire state. Two counties were examined on the Upper Peninsula; the principal potato

growing sections in the northern part of the Lower Peninsula as well as St. Clair county in the southeast and several isolated fields of the larger growers in the central and western sections.

METHOD OF TABULATION.

The percentage of infection in a one-half acre field was obtained by a close examination of about 300 tops, a short examination of the entire field, and by digging up three or four groups of 10 consecutive hills in various parts of the field to obtain a good average. In all the hills dug up a cross section was made of the stem end of the tuber and also of the main stem of the plant a short distance above the tuber, and sections further up the stem were made to determine how far up the stem the fungus advanced. On larger fields a greater number of plants were examined, in proportion to the size of the field.

The percentage of slight infection does not relate to the depth of infection in the tuber but indicates that the plant was fully matured, all tubers formed, no wilting of the tops occurring and no material damage to the crop could be seen, although the browning of the vascular system in the stem and tuber was present.

The percentage of serious infection indicates that the plant had wilted and died before the tubers had matured thus greatly decreasing the yield.

Total infection indicates that all hills in the field showed infection, either slightly or serious as indicated in the tables. This is simply an approximation and would vary from 85 % - 100 %.

In all the examinations the entire field was taken as the unit and not the acreage.

ALPENA COUNTY.

No. Acres-Variety-[%]slight infect.-[%]serious other disease-frost date
 No. Acres-Variety-infect.-infect.-disease-killed-inspection.

1.-	^{Late} $\frac{1}{4}$ -Petosky--	^{1%} trace--	-----	-slight-	no--	Sept. 21.
2.-	^{Rural} $1\frac{1}{2}$ - N.Y. --	"	"	"	"	" "
3.-	$1\frac{1}{4}$ - "	2%	"	"	"	" "
4.-	^{Late} 8 -Petosky--	5 %	"	"	"	Oct. 25.
5.-	1- "	1 "	"	"	"	Sept. 12.
6.-	3- "	4 "	"	"	"	" "
7.-	2- "	-----	"	"	"	" "
8.-	1 - "	8 %	"	moderate	"	" "
9.-	$\frac{1}{2}$ - "	"	"	slight	"	" "
10-	^{Rural} 3 - N.Y.	4 %	"	serious	"	" "
11-	^{Late} $\frac{1}{2}$ -Petosky	"	"	moderate	"	" "
12-	$\frac{1}{2}$ - "	10 %	"	serious	"	" "

DELTA COUNTY.

1- 13- ^{Sir Walter}Raleigh- 15 %-- 25% moderate no Sept. 31.

ANTRIM COUNTY.

1-19	^{Late} -Petosky --	3%	----	moderate-	yes	Sept. 10.
2-3 $\frac{1}{2}$	- "	2 "	"	serious	no	" "
3-10	- "	1 "	"	"	"	" "

IRON COUNTY.

% %

slight serious other frost date

No. Acres-Variety-infect-infect.-disease-killed- inspection.

Sir Walter						
1 -	$\frac{3}{4}$	-Raleigh --	15 %	-- 2 %	slight	yes Sept. 21.
Early						
2.-	1	-Mackinaw--	20 "	----	"	" 23.
3 -	$\frac{1}{4}$	-S.W. Rel.--	6 "	2 %	"	" 18.
4 -	$\frac{3}{4}$	- "	3 "	----	moderate	no " 17.
5 -	$\frac{1}{4}$	- "	15 "	3 %	serious	" " 23.
6 -	$\frac{1}{4}$	- "	5 "	1 "	slight	yes " 18.
7 -	$\frac{1}{4}$	- "	6 "	4 "	moderate	" " 19.
8 -	$\frac{1}{2}$	- "	7 "	6 "	slight	" " 18.
9 -	1	- "	15 "	5 "	"	" " "
10-	$2\frac{1}{2}$	- "	100 "	----	moderate	" " 23.
11-	$1\frac{1}{2}$	- "	7 "	2 %	serious	no " "
12-	$1\frac{1}{8}$	- "	20 "	6 "	"	yes " 21.
13-	$1\frac{1}{2}$	- "	100 "	----	"	" " "
14-	$\frac{1}{4}$	- "	6 "	3 %	moderate	" " 18.
15-	1	- "	9 "	6 "	serious	" " "
16-	$\frac{3}{4}$	- "	8 "	----	moderate	" " 19.
17-	$\frac{1}{2}$	- "	5 "	----	serious	" " 17.
18-	$\frac{3}{4}$	- "	8 "	3 %	"	no " 18.
19-	$\frac{1}{2}$	- "	15 "	5 "	"	yes " 21.
20-	$\frac{1}{2}$	- "	35 "	4 "	"	" " "
21-	2	- "	6 "	3 "	slight	" " 19.
22-	1	- "	8 "	4 "	"	no " "

No-	Acres-	Variety-	% slight infect.	% serious infect.	other disease-	frost killed-	date inspection.
-----	--------	----------	------------------------	-------------------------	-------------------	------------------	---------------------

23-	½	S.W.Ral.-	35 %	----	slight	yes	Sept.23.
24-	¼	"	8 "	"	serious	"	" 17.
25-	2	"	6 "	"	moderate	"	" " .
26-	2	"	28 "	2	slight	"	" 21.
27-	1½	"	20 "	"	"	no	" 21.
28-	1½	"	3 "	"	moderate	yes	" 18.
29-	1½	"	100 "	"	"	"	" 22.
30-	1	"	12 "	"	slight	"	" 23.
31-	¼	"	8 "	2 %	serious	"	" 19.
32-	½	"	30 "	-----	moderate	"	" 21.
33-	¼	"	20 "	4 %	slight	"	" " .
34-	1	"	4 "	D "	"	no	" 19.
35-	2½	"	100 "	----	serious	yes	" 21.
36-	½	-Carmen# 3-	2 "	"	moderate	"	" 17.
37-	4-	Rural N.Y.-	18 "	"	"	"	" 23.
38-	23	"	5 "	2 %	"	"	" " .
39-	2	-S.W.Ral. -	10 "	----	"	"	" 17.
40-	1	"	100 "	"	serious	"	" " .
41-	½	"	2 "	"	slight	"	" 19.
42-	¼	"	6 "	2 %	"	"	" " .
43-	1	"	4 "	3 %	moderate	"	" " .
44-	½	"	30 "	4 "	serious	"	" 21.
45-	2	-Rural N.Y.	8 "	3 "	moderate	"	" " .

$\%$ $\%$
 slight serious other frost date
 No-Acres-Variety-infect.-infect.-diseases-killed.-inspection.

46-	^{Late} $\frac{1}{2}$ -Potosky--	8 %-	1 %-	moderate-	yes-	Sept. 19.
47-	$\frac{1}{2}$ -S.W.Ral.--	20 "	2 "	serious	"	" 21.
48-	$\frac{1}{4}$ - "	5 "	----	slight	"	" 18.
49-	2 - "	35 "	"	moderate	"	" 21.
50-	2 - "	20 "	3 %	"	no	" "
51-	1 - "	10 "	2 "	slight	"	" 23.
52-	$2\frac{1}{4}$ - "	4 "	----	serious	yes	" 17.
53-	1 - "	100 "	"	slight	"	" 22.
54-	$\frac{3}{4}$ - "	6 "	"	"	"	" 18.
55-	^{Green} 5 -Mountaine-	25 "	"	serious	no	Oct. 16.

HOUGHTON COUNTY.

$\%$ $\%$
 slight serious other frost date
 No-Acres-Variety-infect.-infect.-diseases-killed-inspection.

1	-	1½	-	Irish	-	15 %	----	moderate	no	Sept.25.
2	-	2	"	Cobbler	"	40 "	"	slight	"	" "
3	-	¼	-	S.W.Ral.	"	20 "	"	----	yes	" "
4	-	½	"	"	"	100 "	"	slight	"	" "
5	-	1/	"	"	"	30 "	"	"	no	" "
6	-	2	"	Rural N.Y.	"	----	25 %	"	"	" "
7	-	2	"	IRISH Cobbler	"	10 %	----	"	yes	" "
8	-	2	"	"	"	20 %	"	"	"	" 26.
9	-	1½	"	"	"	100 "	10 %	"	"	" "
10	-	¾	-	S.W.Ral.	"	100 "	----	"	"	" "
11	-	4	"	Early Ohio-Beauty of	"	100 "	3 %	"	"	" "
12	-	4½	"	Hebron	"	5 "	2 "	"	no	" "
13	-	3	"	S.W.Ral.	"	100 "	----	"	yes	" 27.
14	-	1	"	Irish Cobblers	"	4 "	----	"	"	" "
15	-	½	-	S.W.Ral.	"	30 "	"	moderate	no	" 28.
16-18	-		-	Rural N.Y.-	"	----	8 %	slight	"	" "
17	-	¾	-	S.W.Ral.	"	15 %	30 %	serious	yes	" "

ST. CLAIR COUNTY.

No-Acres	*Variety	% slight infect.-	% serious infect.-	other diseases	frost killed	date inspection.
----------	----------	-------------------------	--------------------------	-------------------	-----------------	---------------------

Green						
1 - 4½	Mountains-	11 %	----	moderate	yes	Oct. 3.
2 - 1	"	5 "	"	"	"	" 4.
Rural						
3- 10	Russets	100 "	"	slight	"	" "
4 - 2	Gr.Mount.	100 "	"	moderate	"	" "
5- 1	Irish Cobbler	"	"	"	"	" "
6 - 1	Peach Blow-	100 "	"	slight	"	" "
Early						
7 - ¾	Eureka	100 "	"	"	"	" 5.
8 - 1/8	Rural N.Y.	100 %	"	----	"	" "
9 - 10	Gr. Mount.	8 "	"	slight	"	" "
10- 10	Carmen #2-	100 "	"	serious	"	" "
11- ½	Early Mich.	100 "	"	slight	"	" "
12- 5-	Gr. Mount.	17 "	"	serious	"	" 6.
13- 4	"	6 "	"	slight	"	" "
14 - 4-	Rural N.Y.-	100 "	"	serious	"	" "
15- ¼-	Gr. Mount.	-----	"	slight	"	" "
16- 2	"	24 "	"	"	"	" 7.

BARAGA COUNTY.

1 - 1½-	S.W.Ral.	8 %	15 %	slight	yes	Sept.25.
2.- 6-	Carmen #3.-	8 "	1 "	"	no	" "

SUMMARY OF DATA.

Average
for
ALPENA-ST. CLAIR-HOUGHTON-IRON-State.

No. fields examined-----	12-	16-	17-	55-	100.
Total acreage-----	22.5-	56.	49.5	84.75-	212.
Aver. acreage per field-----	1.8	3.3	2.9	1.5	2.6
Aver % slight inf. per field.	4. %	60. %	40.5%	21.8%	30. %
" " " " " acre--	2.1"	17.3"	13.9"	14.2"	11. "
" " serious " " field-	----	----	4.5"	1.6"	1.5"
" " " " " acre--	----	----	1.5"	1. "	.6"
% fields with total inf.----	----	56. "	29. "	10. "	23. "
" " over 10% slight inf.	----	75. "	76. "	49. "	50. "
" " " " serious " .	----	----	3.5 "	----	.8"
" " slight & " " "	----	----	3.5 "	50. "	13.3"
" " total infect. sl.& ser.	----	----	40. "	----	10. "
" " serious infection.---	----	----	35. "	52. "	21.7"

DISCUSSION OF DATA.

It appears from the above data that there was a large amount of Fusarium wilt present in this state during the past season and that the greater part of it proved to be a slight infection which showed as a distinct browning of the stem and tuber in the vascular system. This infection showed

when the tops were still large, green and vigorous, but did not appear to materially injure the yield.

There is an average slight infection- taken with the field and not the acre as the unit- of 30 % while the average serious infection was only 1.5 %. Practically all the fields had some slight infection while only 21.7 % of the fields had any serious infection, and two of the four counties visited proved to be entirely free from any serious infection. The percentage of the fields having a total infection of a slight nature was 23 %.

This peculiar condition i.e. the large amount of slight infection has been made the subject of investigations conducted in the laboratory during the past winter.

Many of the plants infected with *Fusarium* early in the year appeared to have wilted down and then in the subsequent wet weather, a bacterial decay set in and caused a rotting of the stem and main root down to the tuber, so as to resemble black leg, yet distinguishable from it in most cases as the rotting was not as black as in the typical black leg and there was no rotting of the stem end of the tuber.

In all of the fields examined the soil conditions varied so greatly that no conclusive results could be obtained as to the effect of different soils and fertilizers on the amount of the disease present.

In some of the fields especially in Houghton County the disease appeared in the field in streaks and patches

giving an indication that there is a possibility of the disease being spread by means of cultivation.

FROSTING.

The question was brought up by many growers as to the effect of frost on the percentage and seriousness of the infection.

FIELD DATA.

Average
for
Alpena-St. Clair-Houghton-Iron- State.

Aver. slight inf. before frost-	4. %	----	17.5%	2.3%	5.9%
" serious " " "	----	----	4.3"	.2"	1.1"
" " " after "	----	----	4.7"	1.3"	1.5"
" slight " " "	----	60.%	61. "	19. "	35. "

The plants examined after frost appeared to have only the foliage killed, in most cases the main stem was still in a green condition.

Fields examined in August by Wm. Stuart and T.E. Johnson on their potato inspection trip in the Upper Peninsula and declared to be free from Fusarium wilt, were latter inspected by the county agent and all plants showing any symptoms of the disease on the tops were pulled out. When examined by the writer a few days after frost they showed

practically a total infection with Fusarium. The probability is that the above mentioned men had not dug up any of the plants and so had not noticed any of the browning of the stem and tuber, which may not have been accompanied by the wilting condition. In other fields examined by them and declared to be free, and examined by the writer before frost there was a large amount of slight infection not accompanied by the wilt.

In the above data we find that there is 35 % of slight and 1.5 % of serious infection found when the examination was made after the plants had been wilted by frost, against 5.9 % slight and 1.1 % serious infection found on plants examined before frost.

There are several conditions and possibilities that should be considered before definite conclusions can be drawn from these data.

From the first two counties we can draw no definite conclusions as in Alpena county the fields were all examined before frost, while in St. Clair county the entire examination was conducted after frost. In Houghton and in Iron counties the examination of the fields were made at practically the same time in the respective counties and the fields not frosted were due to their particular location and elevation. In these fields we find an average slight infection of 61 % in Houghton and 19 % in Iron county after frost, against 17.5 % and 2.3 % respectively before frost.

The next consideration is that the percentage

of infection be compared with that of the field from which the seed was obtained. This could only be done in Alpena and in Iron counties and the fields in Antrim and Delta counties from which the seed was obtained were examined in the same condition relative to frost as those in the former counties, and it was found that the percentage of infection was about the same.

The most important condition, that of the land on which the crop was grown was not a constant factor in any of the above mentioned fields.

In order to draw definite conclusions it would be necessary to examine crops from fields on which no potatoes had been grown for a long enough period to insure against the possibility of soil infection. The seed should be obtained from the same source and the fields should be examined at the same time, some being frosted and others still in the growing condition. As the conditions were not constant in any of the examined fields no definite conclusions could be drawn.

There is also the possibility of the frosted condition weakening the plant so as to make it easier for the entrance of the organism, but as all the fields were examined within a short time after frosting it does not seem possible for the organism to have made its appearance in such a short space of time.

The writer knows of no condition in which the frost, only killing the foliage of the plant, could cause a browning in the vascular system of the main stem for only a short distance above the surface of the ground and also in the root and stem end of the tuber, shown by the presence of *F. oxysporum* in tissue cultures taken from these tubers. So the possibility of frost being responsible for the large percent of slight infection found in this state has not been considered in the following investigations.

VARIETAL SUSCEPTIBILITY.

FIELD DATA.				Average for Alpena-St. Clair-Houghton-Iron- State.		
Rural New York, slight infect-	----	100. %	126.5 %	10. %	31. %	
" " " serious "	4.5%	----	4. "	1.6"	1.4"	
Late Petosky, slight "	2.3"	----	----	8. "	2.5"	
Green Mountain "	"	----, 21. "	----	25. "	11.5"	
Irish Cobbler "	"	---- 100. "	17.8"	----	29.4"	
Carmen "	"	---- 100. "	----	2. "	25.5"	
Sir Walter Ral. "	"	---- ----	58. "	23. "	20.2"	
" " " serious "	----	----	4.2"	1.7"	1.4"	

In regard to the susceptibility of the different varieties grown in the examined fields no reliable data could

be obtained from which definite conclusions could be drawn.

In order to obtain definite results on varietal susceptibility it would be necessary to have plots of the different varieties to be tested growing in the same infected field under the same soil and atmospheric conditions, all seed being free from the disease and the examination conducted at the same time on all the plots.

It is not possible to obtain this data so no conclusions are drawn as to varietal susceptibility, but this is a field in which very important results could be obtained.

SUMMARY OF FIELD OBSERVATIONS.

1. There is a large amount of Fusarium wilt in the state that appears late in the season causing no wilt of the plant and no observable decrease in the yield.

2. A much smaller percentage of serious infection.

3. Numerous fields showed a condition indicating a spreading of the disease through the soil by means of cultivation.

4. No definite conclusions could be drawn as to the effect of frosting, varietal susceptibility, or soil conditions upon the amount of infection in the field.

SYMPTOMOLOGIC ASPECTS.

DESCRIPTION OF DISEASED PLANTS.

The most characteristic symptoms of this disease are a change in the color of the foliage accompanied by a rolling and wilting of the leaves, causing a premature death of the foliage; and the occurrence of the fungus *F. oxysporum* in the vascular systems of the lower part of the stem and of the roots and tubers.

The first noticeable indications of the disease varies greatly with the seriousness of infection. In case very badly diseased seed stock has been used, the germination is poor and the stand is uneven. Although all the plants may appear above the ground they will vary in size and vigor. the disease progresses as in the following description but is more rapid owing to the early infection before the plants have obtained a good growth.

The more common occurrence of the disease is found however, in tubers not so seriously infected; the early growth may be retarded slightly, but the disease first becomes noticeable to the casual observer when the plants have attained the height of 8-14 inches. In many cases the plant does not show any marked symptoms till midsummer, and often wilting of the plants does not occur until a short time, two or three weeks, before the normal death of the plants.

In all of these serious types of infection the wilting of the top is the first symptom to be observed. This appears first as a change in the color of the foliage, usually to a lighter green than that of the healthy plants, particularly in the lower leaves. This lighter green appearance gradually changes to a yellow or brownish color, entirely covering the infected vines and giving the field a mottled appearance; or in cases where the infection is found in patches there will be a very conspicuous yellow patch of diseased hills contrasted against the normal green foliage of the healthy plants. In some varieties having a dark green foliage, the changes in color are not so prominent. This yellowing of the foliage is accompanied by a rolling of the leaves from the midrib upwards and inwards. The lower leaves of the plant are the first to die, they wilt over and remain attached to the main stem by a small strip of the stem. The upper leaves then roll up and wilt but do not drop; they appear very limp and die in a short time.

When the top of the plant has been entirely wilted the disease has usually progressed far enough to materially weaken the stem and the plant often droops over and lies on the ground, especially if the soil has not been hilled up. Usually when the plants reach this condition they can be pulled up very easily, the roots being very brittle and friable.

This premature death of the plant shortens the life of the crop from two to six weeks, thus materially decreasing the size of the tubers, which have not as yet matured, and appreciably decreasing the yield.

Another type of the disease which has appeared to a great extent in Michigan during the past year is characterized by a late or slight infection of the plants. In this case the early symptoms of yellowing, wilting and premature death of the plant are not observed. The disease does not appear until after the entire plant has matured, and there are no visible symptoms of the disease in the parts above ground. However when the plant is dug the characteristic browning of the vascular systems in the stem and tubers are found. This condition cannot be observed by a casual examination of the tops but can only be determined by digging the plants and cutting across the stem and the tuber. It is probably due to this fact that this stage of the disease has not been more widely noted, and the subtle and persistent nature of this manner of infection more seriously considered by plant pathologists.

This condition was briefly referred to by Smith and Swingle * who write, " In case the plants are not attacked until the stem and leaves have nearly reached maturity, the early symptoms of curling and dwarfing of the foliage do not appear. Even in such cases, however, if the roots are

* Smith, E.F. and Swingle, D.B. 1904, p.14.

entirely destroyed before the tops have died down there are the later symptoms of wilting, but often the fungus grows into the tuber without causing any visible symptoms in the parts above ground." Apparently the same condition was noted by Orton * who states, " It is not uncommon to find prematurely dead hills in infected fields which show comparatively slight vascular browning, while others remain living, yet when examined they prove to have both stems and tubers heavily infected with *F. oxysporum*. This apparent resistance may be explained by the fact that such hills were either accidental admixtures of other varieties, or bud sports, called 'run out hills'. In either case they are plants that remain in an active vegetative condition longer and thus resist the effects of the wilt. Still other hills are found which remain healthy till the normal time for maturity and are also free from fungus infection, thereby supporting the hope that resistant strains may be developed by selection." He goes on to state however, that in cases where selection had been made, they were all attacked by the wilt the following year.

The same condition can be noted in potato plants attacked with *Rhizoctonia* after reaching maturity, in which case the tops have a normal green color, and remain green even after the rest of the plants have died down, while the stem may be entirely rotted off a short distance below the surface of the ground and can be pulled up easily.

*Orton, W.A. 1914, p.6.

It would appear in the light of our present knowledge that there is a great possibility of this condition being due to a late infection attacking the plants after the tubers have matured. But it may also be possibly due to the presence of resistant strains or to a combination of both.

Whether this late infection is due to a slight infection through the seed stock or to an infection through the roots from the soil has not been determined as yet.

OCCURRENCE OF THE CAUSAL FUNGUS IN THE STEM.

The presence of the mycelium of the organism is shown in the vascular ring of the stem as a characteristic brown discoloration. This extends through the entire underground portion of the plant, and a short distance, several centimeters, in the above ground stem. The mycelium does not however extend up into the upper stem and leaves. Cultures made from the upper stem and foliage of infected plants showed no presence of *F. oxysporum* nor did a microscopic examination show the presence of any hyphae. However many cultures were made from the vascular ring in the stem, slightly below the surface of the ground, and at different points extending down to the tuber. These cultures yield almost always, a single species of fungus, *Fusarium oxysporum*, although other organisms are often present as may be expected.

By means of this distinct brown discoloration of the stem for a short distance above the surface of the

ground and extending into the roots, we are able to distinguish clearly between the Fusarium wilt herein described and the various other wilts and leaf rolls of the potato plant. In the case of Verticillium wilt the brown discoloration extends up into the top of the plant and is the chief means of distinguishing the two wilts which would otherwise be easily confused. The amount of hyphae present and the depth of discoloration varies greatly.

In many cases not all the shoots in the same hill show infection, and it is not unusual to find some shoots in a hill upright and healthy, while others have wilted and died. In the same manner we often find a number of potatoes in a hill showing infection while others are entirely free from the fungus. This fact would indicate that much of this infection comes through the soil and enters the plants through the roots and gradually invades the stem also working back to the seed piece but not rapidly enough to spread it to the other shoots in the hill.

OCCURENCE IN THE ROOT.

As stated above the fungus is evidently able to gain entrance to the plant through the smaller roots as well as through the seed piece. This entrance is usually made through wounds and is shown as a brown discoloration of the vascular system. Numerous cultures were made from these

discolored portions and in nearly all cases *F. oxysporum* was obtained. It is often accompanied by other species of fungi causing a white or pink growth of mycelium on the affected roots. The infected roots are usually much more brittle than normally and can be easily broken between the fingers, instead of being pliable as in the normal roots. The main root is also much more tender and brittle than normally. The fungus is also found extensively in the underground stem on which the tubers are borne, and extending into the tuber, but these portions of the plant do not appear as brittle as the roots.

The diseased condition of the root hairs and secondary roots is probably responsible for only a slight part of the checked development and death of the tops, although possibly causing the first stages of the disease.

Undoubtedly the greater damage is due rather to the invasion of the water conducting tissue of the plant by the mycelium of the fungus, or to a poisonous byproduct of the fungus or a combination of both.

OCCURRENCE IN THE TUBER.

The presence of the fungus in the tuber can always be observed by the distinct browning of the vascular ring shown by cutting across the stem end of the tuber.

The depth of the brown discoloration varies so greatly that in the cases where only a very slight discoloration is found it is hard to identify it as *F. oxysporum*

without the cultural studies or the accompanying symptoms on the tops. In nearly all cases tried where the brown ring extended for only 2-3 mm. into the tuber it was possible to obtain cultures of *F. oxysporum*. In the few cases which did not show the presence of the organism, bacteria or other fungi were always found, and had probably entered the tuber for a slight depth through the diseased stem.

The mycelium advances slightly into the tuber in storage. Smith and Swingle observed cases where the fungus advanced the entire length of the tuber, but during the past winter no cases were found here where the infection showed as a brown discoloration for more than half the length of the tuber, and the fungus did not appear to progress very rapidly through the tuber in storage.

Tubers of all sizes are attacked often showing the presence of the fungus before the tuber is mature.

The affected potatoes to all outward appearances seem perfectly sound, and the flesh inside and outside the vascular ring is white and perfectly normal. Such potatoes pass in the market and are often planted by the grower who is not familiar with these signs of the disease.

This tuber infection and the stem end rot condition were considered identical by Smith and Swingle but recently Wollenweber(1913) has proved that *F. oxysporum* is a strictly vascular parasite incapable of attacking the tuber through wounds or of producing a rot of the parenchyma.

ETIOLOGY

PRESENCE OF THE CAUSAL ORGANISM.

The fungus *Fusarium oxysporum* has been repeatedly isolated in the laboratory from plants showing infection by the characteristic wilting of the tops and vascular discoloration of the underground portions of the plant.

The isolations were made from the tubers as follows:- The suspected tubers were first washed with water thoroughly and then soaked in 1-1000 solution of HgCl_2 for about 10 minutes. A hot spatula was then used to sear the surface of the tuber where the cut was to be made, then by means of a hot knife the tuber was cut part way through, then broken across the rest of the way. With a sterile scalpel, small pieces about 2mm. in length were then removed from the infected vascular bundles on the broken surface. This piece of tissue was then transferred to a poured plate of agar. Usually potato agar was used and sometimes nutrient agar. The same methods were used in making isolations from the stem and roots. From these plates the fungus was then transferred to tubes of steamed potato plugs.

The fungus was isolated in this way from a large number of tubers obtained in various parts of the state and varying greatly in the depth of infection. In practically all cases the fungus appeared in a few days as a white mycelial growth, sparse at first, growing directly out of the

infected tissue and quickly spreading out into the media. All of these numerous isolations produced the same morphological characteristics in culture.

In a few cases bacteria appeared along with the fungus, when the tissue was taken from the extreme end of the tuber, the bacteria probably entering through the diseased stem in the same way as the fungus. Usually the fungus slowly overgrew the bacterial colonies and it was possible to transfer the culture to steamed potato plugs.

Many isolations were attempted from the tissue on either side of the discolored vascular bundle and also from the vascular system immediately in advance of the brown discoloration, but in no case was fungus growth obtained, while isolations of the discolored bundles even in cases of a very slight discoloration only a few millimeters deep produced a good growth in a few days.

Microscopical examination of sections of the vascular bundle showed the abundant presence of mycelium, while sections made from the tissue on either side, or in advance of the discoloration or even at the extreme end of the discoloration did not reveal the presence of any hyphae.

The same results were obtained by isolations made from the stem. These facts indicate that the fungus does not proceed in advance of the brown discoloration, but that the brown discoloration may proceed slightly in advance of the fungus. Also that the fungus does not spread out from the

bundles into the surrounding healthy parenchyma tissue.

PATHOGENICITY.

The pathogenicity of the organism worked with was proved by isolating the fungus from the tuber as described above, it was then grown in culture on steamed potato plugs or stems and later inoculated into healthy plants in various ways, (infection experiments p) The characteristic browning of the vascular system was produced and the fungus again isolated and grown in culture where the same morphological characters were observed as before.

The stock culture used in all the following experiments was obtained by tissue cultures made as described above, from potatoes showing only a slight infection obtained from farm near Paw Paw, Van Buren County, Michigan. The fungus was then transferred to potato agar slants and a single spore was transferred to a poured plate of agar. This was done on plates to make it possible to examine it under the microscope and determine that only one spore had been transferred. The single spore was obtained with a very fine platinum loop from the agar slant, observed under the microscope. This culture after a few days was then transferred to potato plugs, several sub cultures made and two of these sent to the Laboratory of Plant Pathology, Bureau of Plant Industry at Washington D.C. for identification. They were identified as *Fusarium oxysporum*. Cultures #3377 and # 3378.

MORPHOLOGY.

The fungus is found to grow readily on a wide variety of media but the form and density of the growth as well as the morphological characters of the spores vary greatly according to the substrata.

In the infected vascular bundle the mycelium shows as a slender colorless hyphae, frequently septate and only slightly branched. No spores were found in any of the sections examined.

Wollenweber * describes the fungus as follows;
" Sporodocia and a reduced pionnotes present, in masses salmon colored, the conidia of both being 3 septate up to 100%, 25-45 x 3.25- 4.5 microns; 4 septate up to 25 %, 5 septate up to 10 % where they are 40-50-x 3.5- 4.75 microns in size. Sclerotia blue on steamed potatoes. A slight lilac odor on steamed rice, milk, etc. "

These characteristics were observed in the cultural studies made, the most characteristic growth being obtained on steamed potato stems.

All the following observations made on morphological characters and spore germination as well as the drawings on Plates 1-6, were obtained in every case from a "Hoch-Kultur" which has been observed by Drs. Wollenweber and Appel (1910) to be the most characteristic form best suited for measurement and study. In their work they show

*Wollenweber, H.W. Phytopath. 1913,

that it is necessary to have a pure culture stage which shows the normal stages and if possible all the stages of the fungus. Thus in their classification of the genus *Fusarium* they differentiate the different cultures as follows. "An-Kultur" is the original culture obtained by isolation from the diseased plant, a transfer of this culture produces the "Norm-Kultur" which can be kept by repeated transfers until a change or degeneration sets in which they term the "Ab-Kultur". Again the "Norm-Kultur" is divided into the "Jung-Kulture" which has conidia of abnormal size and form, unsuitable for study, the "Hoch-Kultur" which forms after 8-14 days; in this culture the greatest uniformity is produced and will last for a number of weeks, from this form we have the poorly developed later forms known as the "Alt-Kultur".

In the following studies the "Hoch-Kultur" form was used and was grown on potato stems unless otherwise noted. In a study of the comparison of the different cultures described above, it was found that in the "An-Kultur" conidia were produced very sparingly although there was an abundant mycelial growth. The transfer from this showed abundant sporification.

In a study on poured plates of potato agar # 20, it was found that the spores germinated in about 6 hours. The mycelium was produced rapidly, the colonies being about 1 cm. in diameter at the end of 48 hours. The margin as a rule is circular or nearly so. The diameter gradually increases until when the colony is about 2 weeks old it will almost entirely

till the plate. Examining the colonies from the under side of the plates they appear to be formed by concentric rings of growth; the center of the colony appears as a rather dense tuft of mycelium about 2-3 mm. in height, surrounding the central tuft is a zone of mycelium with very short aerial hyphae which is itself surrounded by another zone of slightly taller hyphae than that in the center; outside of this is another one of short aerial hyphae made up of several concentric rings, each ring being marked by somewhat taller hyphae. Finally the entire colony is covered with this tall growth of mycelium.

The mycelium appears as somewhat slender colorless hyphae, many septate and much branched except at the extreme edges of the colony where the hyphae push out with little branching and no production of spores. The mycelium is densely filled with granular cytoplasm. Microconidia are produced both aerially and in the substrata, macrospores being produced in the older hyphae and only aerially, and the entomospores are formed a little later.

On steamed potato plugs # 3 and a few other vegetable media, blue sclerotia are formed in about one week.

The growth on all agar appeared white, while on potato plugs kept in bright daylight a faint salmon color was produced. On boiled rice the characteristic pink coloration was observed and a slight lilac odor, and when a little alkali was added the color of the growth turned a deep blue.

On boiled prunes, the reduced pionnotes formation was found, very little or no aerial hyphae being produced with an abundance of spores and chlamydo spores. On many plates of nutrient agar the pionnotes form was found to occur. (Plate # 7.)

MICROCONIDIA.

The formation of the microconidia was best observed in hanging blocks of agar or liquid media, used in Van Tieghem cells. The microconidia are produced in about 24-30 hours after the spores have germinated and the young mycelium formed.

The first sign of spore formation is a slight swelling along the hyphae at various points, (Plate #5) it was observed usually in 24-34 hours. These small swellings rapidly elongate until the normal size of the microconidia has been obtained when it is cut off and immediately the hyphae begins to push out past the newly formed spore and the same process is repeated. In many cases the microconidia seem to be cut off the end of small lateral branches by a constriction furrow, or the same process may take place on the terminal hyphae. The entire operation from the cutting off of one spore to the next usually does not take longer than one hour and in many cases it has been observed to take place in 45 minutes.

The microconidia formed are usually more or less oval in shape, thin walled, one celled and often slightly

curved. They vary in size from 5.5-15.5 microns in length, and from 1.7 - 5.5 microns in width. The most common size observed on potato stems was about 7.6 x 3.8 microns.

The time required for germination varies greatly in the different media and also for the different individuals in the same medium. This is shown on the tables following. Usually in 7-8 hours 50 % of the conidia had germinated but the hyphae are short and not branched. In 30 hours the branching was observed but not extensively.

In all the examinations conducted on the germination of conidia it has been observed that on all media there are always a few microconidia, up to 25 % that do not germinate even after 48 hours. It would thus seem that many of the microconidia cut off are incapable of sending out germ tubes and producing hyphae under the average cultural conditions cited below.

The germination of the microconidia is very simple, (Plate #3.) they first swell a little and in some cases become almost spherical, the germ tube is then pushed out usually from the end, although in some cases it was observed to take place from the side and frequently from both ends, although when they are sent out from both ends they are found to start at different times. Later the hyphae branch out and become septate.

MACROCONIDIA

The macroconidia are found to vary greatly on different media and also different individuals on the same medium. On potato stems they usually appear as 1-5 septate, mostly 3 septate. They are also found to vary in size on this medium from 9.5- 38 microns in length and 3.5 -7 microns in width, the average for the 3 septate conidia being about 27×3.8 microns, the 4 and 5 septate averaging larger and the two and one septate being smaller. The conidia vary in shape from nearly straight with slightly blunt ends, to a much curved form with one end blunt and the other with the characteristic foot, or pedicellate base. This type of conidia, slightly curved, 3 septate, more or less sickle shaped, and with the characteristic pedicellate base is the characteristic conidium used in the classification of this species. All the spores are thin walled.

The macroconidia were never observed being borne below the surface of the media and therefore the observation of the method of formation was very difficult. As far as could be observed the formation takes place at the end of short lateral branches, the ends of which are cut off by constriction furrows. Several hours after being cut off the septa begin to form.

The time of germination of the macroconidia varies greatly on different media and from different media. In sterile distilled water germination usually begins in

about 4 hours and in 7-8 hours practically all were germinating.

The germination of the macroconidia is more complicated than that of the microconidia. Usually the end cells germinate first but not always, sometime the germ tubes being sent out from the side of the spore, from the middle cells. Any of the cells are capable of sending out one or more than one germ tube and in many cases all of the cells in a 3 septate conidium have been observed to germinate, usually however only two germ tubes are sent out and sometimes three. The cell beginning germination slightly swells and pushes out a germ tube which rapidly elongates and in 7-10 hours is about 20 microns long, septation then takes place and in 32 hours conidia formation starts.

CHLAMYDOSPORES.

Chlamydospores are found as large, one or two celled spores, usually spherical, smooth with a heavy wall, and a brownish color. They are borne either terminally on the main hyphae or on its lateral branches, often intercalated within the mycelium and sometimes within the conidia. (Pl. # 6) Several of them may be formed along the same hyphae in chains. The contents appear as heavy rounded masses of cytoplasm.

The formation of the chlamydospore is first seen as an enlargement of tip of the hyphae or as a large swelling in the hyphae, usually at the point of septation. The swelling continues until the large characteristic form is

attained. It is cut off from the hyphae by cross walls before it reaches its mature form but does not always drop off. They vary in diameter from 6-11.4 microns, the usual size being 7.6 microns.

The number of chlamydospores produced varies greatly with the different media used, but on favorable media they are produced abundantly. The germination of the chlamydospore is similar in process to that of the microspore, a simple germ tube being sent out either from one end or from both, and it then proceeds the same as the hyphae from the other spores. In distilled water the germination begins in about 5 hours, at the end of 30 hours they were slightly branched and as in the case of microconidia many of them failed to germinate at all.

SCLEROTIA.

A nodule like mass of plectenchyma with a rough surface was found to grow on various of the vegetable media. On potato plugs they were found as blue green sclerotia and also on boiled white beans, sweet potato plugs and carrot plugs. In the latter they appeared more greenish. They develop at the surface of the medium and are partly covered over with the white mycelial growth. Examined under the microscope they appear only as tightly wound masses of mycelium, seeming to function as the resting stage.

CULTURAL STUDIES OF VARIOUS STRAINS.

This experiment was conducted with the following objects in view.

1. A study of the cultural characteristics of *F. oxysporum* on different media.
2. A comparative study of the different strains of *F. oxysporum* isolated from potatoes suspected of Fusarium wilt which were obtained from fields examined in different parts of the state, to show that the organism was identical in the numerous fields, and to substantiate the diagnosis made in the field from which the data on field observations were compiled.
3. A comparative study of cultures isolated from tubers showing a deep infection and from tubers showing a slight discoloration of the vascular ring or only a few millimeters.
4. A comparative study of cultures isolated from tubers having a white mycelial growth on the outside of tuber infected with *F. oxysporum* and cultures taken from the vascular ring.

In the following data all the media is numbered and formulae of them is given on p. All cultures were kept at room temperature in a northern light.

METHOD OF OBTAINING DATA.

The size of the spores was taken by observing several fields in each slide, several slides being made from each culture. The size of the spores varied so much that it

was impossible to obtain a fair average that would be a true indication of the size of the spores on the various media, therefore only the maximum and minimum length and thickness is given. In the same way the number of macrospores which are one, two or three septate is not given in percentage as it was not possible to make large enough counts to be able to give a definite percentage. However by observing three or four fields on each slide under low power it was found possible to make a fairly accurate estimation of the predominant method of septation, accordingly they have been comparatively estimated in the following data.

SOURCE OF THE DIFFERENT STRAINS USED.

1. Isolated from potatoes obtained from farm near Paw Paw Van Buren county. These showed a slight infection, the browning of the vascular ring being only about $\frac{1}{4}$ inch deep.
2. Isolated from slightly infected potatoes on College farm, browning only $\frac{1}{8}$ inch deep.
3. Isolated from potatoes from the same field as 1 but with a deep infection.
4. Isolated from potatoes from Stonington, Delta County. These were infected with late blight, a dry rot stage had set in, and white mycelial tufts were developed on the tubers when kept at room temperature.

5. Cultures made from the withered roots of infected potatoes that had been sterilized in HgCl_2 and sprouted in sterile sand in large moist chambers in the laboratory.

The ages of the various cultures used in this work varied greatly, they were as follows:-

#1.-14 days old	grown on media # 3- Potato plugs.
2.- 4 months, 9 days--	" " " "20- " agar
3.- 4 " 4 "	" " " " 3
4.- 3 "	" " " "20
5.- 2 " 24 "	" " " " 3.

NUTRIENT AGAR # I.

Height				
No--	Growth--	Density--	Color in light--	Remarks.
1--	2.5 mm.-	moderate--	white	growth slow, feathery.
2--	8. "	very dense-	"	" faster, "
3--	"	"	"	"
4--	"	moderate	"	"
5--	7 mm.-	"	"	"

No-- Microconidia-- Macroconidia-- Calamydospores.

1--	moderate 5.5-13.3 x 1.7-5.5	very few. I-3 septate mostly I " 11.4 x 3.8 to 22.8 x 5.2	present.
2--	many 7.6 x 3.8 to 14.4 x 5.5	few I-3 septate most I " 9.5 x 3.8 to 20.9 x 5.5	few- 7.6 diam.
3--	"	"	"
4--	"	few I-3 septate most I " 7.6x3.8 tp 38. x 7.6	"
5--	"	few I-4 septate most I " 11.4 x 3.8 to 18. x "	"

PEAR AGAR #10.

Height					
No.	Growth---	Density--	Color in light--	Remarks.	
1	2mm.	- Sparse	- White	characteristic foot. -macro & microconidia equal.	
2	"	"	"	"	
3	"	"	"	more macro than microconidia.	
4	"	"	"	macroconidia large, thicker.	
5-	"	very sparse	"	few large macrospores.	

No.	<u>Microconidia</u> ---	<u>Macroconidia</u> ---	<u>Chlamydospores</u>
1--	numerous 7.6 x 3.8microns-	numerous-26 x 5.5 many 3 septate few 2 "	numerous
2--	"	"	"
3--	"	"	"
4--	"	numerous 38 x 7.6 many 3 septate few 2 & 1 "	"
5--	"	few- many 1 & 2 septe 14.2 x 3.8 few 3 septate 22.4 x 3.8	"

PRUNE AGAR # II.

No.	Height Growth--	Density--	Color in light--	Remarks.
1--	I cm.	very dense	white	few spores.
2--	"	"	"	small macroconidia
3--	"	"	"	large "
4--	"	"	"	larger, thick, straight.
5--	"	"	"	few small macroconidia.

No--	Microconidia--	Macroconidia--	Chlamy dospores.
1--	very few 7.6 x 3.8	very few mostly 2 & 3 septate 12.5 3.8	numerous. 7 mic. diam.
2--	many 7.6 x 3.8	numerous-14.5 x 3.8 mostly 2 septate few 1 & 3 "	"
3--	"	numerous-28 x 3.8 mostly 3 septate few 2 & 1 " slender, sickle shape	"
4--	"	numerous-30 x 3.5 mostly 3 septate few 2 & 1 " very thick	"
5--	"	few-- 11.4x 3.8 mostly 1 septate few 2 & 3 "	none seen.

OAT AGAR # 12.

height		Density--	Color in light--	Remarks.
No--	growth--			
1--	1 cm.-	moderate-	White	mostly microconidia
3--	"	dense	"	macro. more numerous, larger.
4--	"	moderate	"	" thicker, blunt.
5--	2mm.-	very scarce	"	

No--	Microconidia--	macroconidia--	chlamydospores.
1--	numerous 7.6 x 3.8	very few-19 x 3.8 mostly 2 septate few 3 & 1 "	numerous 8.6 microns diam.
3--	"	numerous-28 x 3.8 mostly 3 septate few 4 & 5 " very " 2 & 1 "	"
4--	"	numerous-25.5 x 7 mostly 3 septate thick, blunt ends, no foot.	"
5--	"	numerous-31.5 x 3.8 mostly 3 septate	"

LETTUCE AGAR # 14.

No--	Height Growth--	Density--	Color in light--	Remarks.
1--	I cm.	very dense	white	
2--	"	"	"	
3--	"	"	"	
4--	"	"	"	Macroconidia thicker.

No--	Microconidia--	Macroconidia--	Chlamydoconidia--
1--	numerous 7.6 x 3.8	numerous many 3 septate 15 x 3.5	numerous, terminal 11.4 diam.
2--	"	" few 4 septate	"
3--	"	"	"
4--	"	numerous many 1 septate 19 x 6 few 2 & 3 23 x 7	very numerous

STEAMED POTATO STEMS # 2.

No--	Height growth--	Density--	color in light--	Remarks.
I--	4 mm.--	moderate--	white	equal no. of micro & macroconidia.
2--	"	"	"	more micro. than macro.
3--	"	"	"	macroconidia smaller.
4--	"	"	"	more macro. than micro. slender, more curved.
5--	"	"	"	"

No--	Microconidia--	Macroconidia --	Clamydospores.
I--	moderate 11.4 - 5.5 x 3.8	9.5-38 x 3.8 numerous I-3 septate most 3 "	numerous
2--	"	" most 3 septate few 4 & 5 "	very numerous.
3--	"	few 13.5 @ 26.5 x 3.8 I - 3 septate most 1 "	very few.
4--	"	numerous-15.2-30.6 x 3 mostly 3 septate slender, more curved.	"
5--	numerous	very few, mostly 1 septate small.	numerous.

STEAMED POTATO # 3

No.	Height Growth--	Density--	Color in light--	Remarks.
1--	I cm.	very dense--	white to light salmon-	blue sclerotia.
2--	"	"	"	"
4--	"	"	"	"
5--	"	sparse	"	no sclerotia.

No-- Microconidia-- Macroconidia-- Chlamydospores.

1--	numerous 9-7 x 3-3.8	numerous mostly 1 septate 11.4-22.8 x 3.8	numerous, terminal.
2--	" aver. 4.3 x 3	numerous	"
3--	"	mostly 2 septate few 3 " 11.4 x 3.8 to 22.5 x 7.4	few.
5--	"	"	"

BOILED CARROT. #13.

No--	Height Growth--	Density--	Color in light--	Remarks.
1--	1 cm.--	very dense--	white--	spore production profuse green-blue sclerotia. macroconidia small
2--	"	"	"	"
3--	"	dense	"	"
4--	"	"	"	"
5--	"	"	"	macro. larger, thick, straight. " long, narrow, curved sclerotia present.

No--	Microconidia--	Macroconidia--	Chlamydospores.
1--	very numerous-- 7.6 x 3.8	very few--14.4 x 3.8 mostly 2 septate	present.
2--	"	"	"
3--	"	"	"
4--	"	numerous--21 x 7.6 most 2 septate. thick blunt end.	"
5--	many	numerous 31 x 3 mostly 2 septate long, thin, with a distinct foot	"

BOILED CORN MEAL # 4.

No.	Height Growth	Density	Color in light	Remarks.
1--	1 cm.	very dense	light pink	mycelium very granular no typical macroconidia.
2--	"	"	"	"
3--	"	"	"	"
4--	"	"	"	more macroconidia, thicker.
5--	"	"	red	"

No.	Microconidia	Macroconidia	Chlamydospores.
1--	numerous 5.5-7.4 x 3.8	very few I- 3 septate most I " II.4-26.2 x 3.8	very few.
2--	"	most I septate	"
3--	"	"	"
4--	"	numerous I-3 septate most I " aver. 19.6 x 7.6 very thick, blunt ends.	"
5--	"	same as #3	

STEAMED BEAN STEMS # 5 ---

No--	Height Growth--	Density--	Color in light--	Remarks
1--	4mm.	dense--	white	
2--	"	"	"	
3--	"	"	"	
4--	2mm.	moderate	"	

No-- Microconidia-- Macroconidia-- Chlamydospores.

1--	numerous 7.5 x 3.8	numerous I-3 septate most I " II- 19 x 3.8 straight, blunt.	numerous
2--	"	"	"
3--	"	"	fewer.
4--	thicker	numerous most 3 septate 26 x 7.6 few 2 septate 18 x 7.2 few 1 septate 11 x 7.6	"

BOILED WHITE BEANS # 6

No--	Height Growth--	Density--	Color in light--	Remarks.
1--	1 cm.	very dense	white	blue green sclerotia.
2--	"	"	"	"
3--	"	"	"	"
4--	"	"	"	"
5--	5 mm.	scarce	"	"

No-- Microconidia-- Macroconidia-- Chlamydospores.

1--	numerous 7.6 x 3.8	few most 1 & 2 septate few 3 15 x 3.8	numerous
2--	"	"	"
3--	"	"	"
4--	"	numerous, thick many 2 septate few 3 & 1 " 2.1 x 6.5	few.
5--	"	same as # 3	

STEAMED LUPINE SPROUTS # 7 ---

Height				
No--	Growth--	Density--	Color in light--	Remarks.
1--	2mm.	moderate	white	
2--	"	dense	"	
3--	"	"	"	
4--	"	"	"	
5--	"	scarce	"	

No--	Microconidia--	Macroconidia--	C hlamydospores.
1--	numerous 7.6 x 3.8	numerous I- 3 septate most I " 15 x 3.8	very few.
2--	"	"	"
3--	"	"	"
4--	"	" most 2 septate 21 x 7.6	occasional
5--	few	very few	none seen.

STEAMED PARSNIPS # 15 ---

No--	Height Growth--	Density--	Color in light**	Remarks.
I--	4 mm.	moderate	white	mycelium with many vacuoles.
2--	1 cm.	very dense	"	"
3--	"	"	"	"
4--	"	"	"	"
5--	"	"	"	"

No-- Microconidia-- Macroconidia-- Chlamydospores.

I--	numerous 7.6 x 3	numerous mostly 2 & 1 septate 19 x 3.5	moderate
2--	"	"	numerous
3--	"	"	"
4--	"	"	"
		thicker 19 x 7	"
5--	"	3 septate 26 x 3 slender	"

BOILED RICE # 16.

Height				
No--	Growth--	Density--	Color in light --	Remarks.
1--	1.5 cm.	very dense	pink	lilac odor, pink turns blue when alkali is added.
2--	"	"	"	"
3--	"	"	"	"
4--	"	"	"	"
5--	"	"	"	"

No-- Microconidia-- Macroconidia-- Chlamydospores.			
1--	numerous 7.6 x 3	very few mostly 2 septate 19 x 3	numerous many terminal
2--	"	numerous	very numerous
3--	"	"	"
4--	"	many, thick, mostly 2 septate 25 x 6	"
5--	"	same as # 2.	

STEAMED SWEET POTATO # 17.

No.-	Height Browth--	Density--	Color in light--	Remarks.
1--	4 mm.	very dense	reddish gray	
2--	"	"	"	
3--	"	"	"	
4--	"	"	"	
5--	"	"	"	

No--	Microconidia--	Macroconidia--	Clamydospores.
1--	numerous 7.6 x 3	few, 19 x 3.8 mostly 2 septate few 3 & 1 "	numerous many terminal
2--	"	numerous "	"
3--	"	few "	"
4--	"	many 3 septate 19 x 6.	"
5--	"	same as # 1.	

BOILED LUPINES # 8.

No.-	Height Growth--	Density--	Color in light--	Remarks.
1--	2 mm.	scarce	white	
3--	"	"	"	

BOILED PRUNES # 9.

1--	2 mm.	dense	brownish white--	profuse pionnotes granulated cytoplasm.
2--	"	"	"	"
3--	"	"	"	"

LUPINES

No-- Microconidia-- Macroconidia-- Onlamydo spores.

1--	numerous 7.6 x 3.8	numerous most I & 2 septate few 3 15.x 3.8 cigar shape	none seen
3--	"	"	present, many terminal.

PRUNES.

1--	numerous 7.6 x 3	few, most I septate II x 3.5	numerous
2--	"	"	"
3--	"	"	"

DISCUSSION OF DATA.

From the above data it appears that all five cultures are identical. While they vary in some extent in the number and size of the microspores, macrospores and chlamydo-spores, it is safe to say after a careful study of the different cultures in which the mycelial growth, spore production, color of growth and especially the average size of the spores and their form, noting the presence of the characteristic foot present in the macrospores; that these various strains are all identical with culture # 1 which was the stock culture of *F. oxysporum*. What small differences are found can be accounted for to some extent by the great error possible in examining such a large number of spores comparatively, especially when only the maximum and minimum size is given. The color of all the cultures was the same on all the respective media.

According to Wollenweber l.c. *F. oxysporum* produces a characteristic pink color and lilac odor on boiled rice, this was found to be true in every case. When a strong alkali was added to this medium the pink color of this mycelial growth changed to a bright blue.

The blue sclerotia observed on potato plugs were found in every case. These were also observed on Sweet potato plugs as well as carrot plugs and boiled white beans.

The few differences that are observed between the several cultures are probably accounted for to some extent

by the great variation in the age of the cultures from which the transfers were made, the variety of the media the original cultures were grown on, as well as the possible differences in the moisture content and the composition of the substrata. There is also a possibility of differences in cultural characteristics being produced depending on whether the culture was started from mycelium, microspores or macrospores, in most cases probably all three were used.

The study of the results obtained by growing the same strain on different media bears out conclusively the statements made by Wollenweber l.c. and Smith and Swingle l.c. that the fungus grows readily on a wide variety of media. It varies greatly in density, color, height of growth, and the number, size and form of microspores, macrospores, and chlamydospores according to the substrata and environment. The growth on vegetable media seems to be the most favorable for a profuse growth and characteristic sporification. Potato plugs and especially potato stems are probably the best media for this fungus.

The cultures made in the above experiment were isolated from potatoes that varied greatly in the depth of infection from $1/8$ of an inch to a very deep discoloration. The possibility of the slight discoloration being *Fusarium* wilt has been questioned many times, but the above results point out that the organisms obtained from both the slight and the deep infection were the same. The same results have

also been obtained in a study of other cultures isolated from such slightly infected tubers, a large number of these have been made in the laboratory from tubers obtained from fields where the actual field conditions had been observed, and in practically all cases these cultures proved identical with the *F. oxysporum*. In the few cases where it was impossible to obtain cultures from the slightly infected tubers the chief cause was the presence of other organisms which had worked their way into the stem for a few millimeters.

When these slightly infected tubers were examined in the field work they were diagnosed as *F. oxysporum* if accompanied by a browning of the vascular ring of the stem, However numerous sets of two tubers each, from two infected fields on the college farm, one of which showed a slight infection and the other a deep browning of the vascular ring, brought back greatly varied answers when sent out for diagnosis to pathologists at other stations. This was done in an effort to determine how the different depths of browning would be diagnosed in the different states.

While there is undoubtedly a grave cause for doubt when only a slight browning of the tubers is found, it appears to the writer that when accompanied by the characteristic browning of the vascular ring in the stem for only a few inches above the surface of the ground and extending downwards to the tuber, these symptoms may be safely diagnosed

as Fusarium wilt caused by *F. oxysporum*. This statement is based on the large number of isolations made from such slightly infected tubers from various parts of the state, all of which appear identical with those given in the above data.

In examining Culture #4 we find that the same morphological characters are present as in the other cultures, while this culture was isolated from the white mycelial tufts on the outside of the tubers and the others were isolated from the vascular ring.

This would appear to bear out the statements made by Smith and Swingle l.c. that the fungus is capable of penetrating from the vascular ring of the potato tuber to the epidermis of the tuber.

According to Wollenweber l.c. *F. oxysporum* is strictly a vascular parasite, yet this work would indicate that there is a possibility of the fungus spreading through the tuber to the surface and then producing the white mycelial growth in a case where the tuber is attacked by another disease which produces a decay of the tissues between the vascular ring and the epidermis.

Similar results were obtained as the result of cultural and infection experiments by Manns l.c.

In any case it will take a good deal more careful investigating with particular study as to the progress of the mycelium in the tuber as well as careful cultural and inoculation work to prove that this fungus sends its mycelium through the tissue to the epidermis.

SPORE GERMINATION AT DIFFERENT TEMPERATURES.

This data was obtained by germinating the spores in distilled water in Van Tieghem cells. The various temperatures were obtained by using the same apparatus as that used for the determination of maximum and minimum growth. Spores were obtained from a stock culture on Potato stems.

No-	Temp.-	7 hours--	10 hours--	24 hours.
1--	8 C.	-no growth--	no growth	no growth
2--	10 "	"	"	few macroconidia
3--	14 "	-50% macro. 10 micro. tubes short	80% macroconidia 50 microconidia germ tubes 20 microns.	all macro most micro.
4--	18 "	"	"	longer growth
5--	24 "	" tubes 20 mic.	" tubes long	much branched.
6--	30 "	no growth	no growth	few macroconidia
7--	38 "	"	"	no growth.

It is seen from the above data that from 18 to 24 degrees C. is the most favorable temperature for the best spore germination, with a gradual decrease above and below.

GERMINATION OF SPORES ON VARIOUS MEDIA.

All spores obtained from a stock culture on potato stem and germinated in Van Tieghem cells at room temp.

Medium	Kind of spores-	Hours.			Condition at the end of 24 hours.
		10%-	50%-	All-	
Single distilled water	Macroconidia	4-	5-	6-	Many Microconidia not germinated, germ tubes short.
	Microconidia	4-	7----		
Double "	Macroconidia	4-	6-	9-	Germ tubes of macro. much branched, with spore formation.
	Microconidia	4-	9----		
Tap water	Macroconidia	4-	6-	9	"
	Microconidia	4-	6-	--	
Glycerinated Nutrient agar	Macroconidia	6-	7-	8-	" no spore formation. 75% germ.
	Microconidia	6-	9----		
Glycerinated Beer broth	Macroconidia	5-	6-	9	spore formation 75% germ.
	Microconidia	6-	9----		
Beef broth	Macroconidia	5	6	8	not branched spores formed. 75% germ.
	Microconidia	6	7----		
Nutrient agar	Macroconidia	5	6	8	much branched, spores formed. 75% germ.
	Microconidia	6	7----		
Synthetic agar #25	Macroconidia	6	7	8	" no spores formed.
	Microconidia	6	7----		
Synthetic medium #26	Macroconidia	4	6	7	" "
	Microconidia	5	7----		
Oat agar	Macroconidia	6	7	8	" spores formed.
	Microconidia	6	8---		
Hard Potato agar	Macroconidia	5	6	7	little no spores formed. branching
	Microconidia	6	7---		
Cucumber agar	Macroconidia	5	6	7	much branched-spores formed.
	Microconidia	6	7---		

SPORE GERMINATION FROM VARIOUS MEDIA.

The spores were obtained from cultures on various media 5 weeks old, germinated in distilled water in Van Tieghem cells, at room temperature.

Media	Kind of spores-	Hours.			Condition at end of 24 hours
		10%	50%	All-	
Nutrient agar-	Macroconidia	6-	7-	8	Much branched spores Many not germ. formed.
	Microconidia	6	11--		
Pear agar	Macroconidia	4	6	7	slight branching
	Microconidia	8	9	---	
Prune agar	Macroconidia	7	11	24	" "
	Microconidia	9	24	---	
Oat agar	Macroconidia	5	6	9	Much branched "
	Microconidia	6	24	---	
Cucumber agar	Macroconidia	6	7	8	" "
	Microconidia	7	8	---	
Boiled prunes	Macroconidia		8	9	" "
	Microconidia	4	7	----	
Boiled rice	Macroconidia	5	6	9	" "
	Microconidia	6	7		
Potato stems	Macroconidia	4	5	6	" "
	Microconidia	4	7	--	
Potato agar	Macroconidia	6	8	9	" "
	Microconidia	7	8	---	
Carrot plugs	Macroconidia	6	8	9	" "
	Microconidia	7	11	---	
Steamed potato	Macroconidia	6	8	9	" "
	Microconidia	4	7	---	

SUMMARY OF SPORE GERMINATION TESTS.

In the preceeding tables it is shown that the germination of spores from different media vary as well as spores on different media.

The spores taken from potato stems seem to have the greatest power for rapid germination, both for early and total germination. In most cases the spores taken from vegetable media seem to have greater vitality than those from agar.

In the data on germination on various media we find that for the most rapid germination once distilled water is the best while liquid syntnetic media # 26 starts the germination of macrospores at about the same time but the total germination is a little slower.

Since this experiment was conducted it has been found that Knudson's full nutrient liquid media # 27 will produce the same or perhaps slightly better results than distilled water, also causing a more rapid growth of the germ tubes and the production of conidia.

MAXIMUM AND MINIMUM TEMPERATURES FOR GROWTH.

The range of temperatures at which the organism grows best was tested by growing the fungus on potato plugs and nutrient agar as follows:-

Exp. 1. 8 test tubes were used containing nutrient agar. In each was placed a sterile thermometer to record the actual temperature, the bulb of the thermometer being placed near the point of inoculation on the agar. These tubes were placed in an apparatus consisting of a galvanized tin box about 2 inches deep filled with water, one end being in contact with the ice box the other with a similar box containing water kept at a constant temperature by means of an electric heater. The cultures were thus kept at a fairly constant temperature, within an error of 1 degree in 6 hours.

Temperature --	2 days	3 days	4 days.
1.- 24° C.	slight	medium	abundant
2.- 28 "	very "	slight	medium
3.- 30 "	" "	"	"
4.- 33 "	" "	"	"
5.- 34 "	no growth	very "	slight
6.- 36 "	"	no growth	very "
7.- 38 "	"	"	"

Other cultures were tried at higher temperatures but no growth was obtained in any case.

Exp.# 2. The same test as the above was also conducted using potato plugs as the medium. This was carried on in a smaller apparatus than the previous test but with a greater range of temperature. It consisted of an air chamber about 6 inches deep and 3 ft. long, thoroughly insulated, one end being in contact with an ice box the other with a similar box containing water kept at a constant high temperature. The air chamber was divided into 9 compartments each about 3 inches in width. The tubes stood upright, each with a thermometer next to it. All tubes were of the same size and thickness.

Temperature	3 days	5 days	1 week.
<hr/>	<hr/>	<hr/>	<hr/>
#1. 7° C.	no growth	no growth	no growth
2. 9 "	"	slight	slight
3.-12 "	"	"	medium
4.-18 "	slight	medium	abundant
5.-24 "	"	"	"
6.-29½ "	very slight	slight	slight
7.-37 "	"	"	"

None of the cultures placed at higher temperature produced any growth.

Exp. #3. The same test was again repeated by using potato stems, each tube being kept at a constant temperature in incubators and thermostats.

Temperature	2 days	4 days	1 week.
<hr/>	<hr/>	<hr/>	<hr/>
1.- 5 ° C.	no growth	no growth	no growth
2.-14 "	"	slight	medium
3.-23 "	medium	abundant	abundant
4.-29½ "	very slight	slight	medium
5.-34 "	"	"	slight
6.-37½ "	no growth	no growth	no growth.

RESULTS.

All three of these experiments show practically parallel results. We find that the fungus grows best in culture at a temperature of from 18-24 degrees. At 14 degrees the growth is slow, gradually decreasing with a decrease in the temperature until at 9 degrees there is very slight growth. The fungus was not observed to grow at all at 5 degrees. Above 24 degrees the growth became slower and slower as the temperature was raised, being slow at 30 degrees with only a very slight growth at 34 and 36. At 37 degrees the culture did not grow in any case.

The low temperature at which this fungus ceases growth may possibly be the cause of the inability of the fungus to penetrate into the tops of the plant.

THERMAL DEATH POINT.

The thermal death point of the organism was determined as follows.- Test tubes of equal size and thickness 18 x 150 millimeters, with thin walls were used containing nutrient agar # 1. A large water bath was used, the temperature being kept constant by regulating the flame of the heater with a thermo regulator. The water in the bath was kept at an even temperature by revolving fan kept in the bottom of the water bath and connected with a small motor. Two test tubes were always used together, one being used as a check on the temperature. A thermometer was placed in the agar in this tube and the temperature there observed was taken as the temperature of the inoculated tube placed adjacent to it. The check tube was of the same thickness as the tested tube and contained the same agar. The second tube was inoculated by transfer from a stock culture, after the agar in the tube had remained constant at the temperature to be tested for 10 minutes. The tube was then subjected to the temperature to be tested for 10 minutes and then poured into a petri dish. The first tubes to be tested were subjected to a temperature of 36 degrees, and the temperature was then raised one degree for each successive test until a temperature was reached that would kill the fungus.

RESULTS:- It was found that when the spores were subjected to a temperature of $61\frac{1}{2}$ degrees C. for 10 minutes, germination did not take place.

POISONOUS BY PRODUCTS OF THE FUNGUS.

Owing to the fact that many cases of the wilt have been observed where the foliage was entirely wilted, with only a slight trace of mycelium present in the stem and roots, the hypothesis was brought forward that the wilt may be due to a secretion of the fungus as well as to the invasion and blocking up of the water tubes by the mycelium. Accordingly tests were conducted as follows.

A suspension of the fungus was made by grinding up a stock culture of the fungus in water, this was then filtered through a Berkfeld filter. About 30 c.c. of the filtrate was then placed in large test tubes 1 x 8 inches, and the tops of healthy branches were cut and placed in these tubes of liquid to be tested. In the same way similar branches were inserted in tubes containing sterile water and another set in tubes containing a sterile water suspension of the fungus. All the tubes were kept in the greenhouse under the same conditions each being tightly plugged.

	3 days	1 week.
1. Filtrate	slightly wilted	badly wilted
2. " "	" "	" "
3. spore suspension	badly wilted---	completely wilted.
4. " " "	" "	" "
5. Sterile water	no wilt	no wilt
6. " "	" "	" "

It is seen that in three days the branches immersed in the spore suspension showed the first signs of

wilt closely followed by the tubes containing the filtrate, the checks remaining healthy and upright. At the end of one week the branches in the spore suspension were completely wilted and dead, while the ones in the filtrate were badly wilted and drooped over, the checks still remaining healthy. In ten days the checks had also wilted down owing to the water in the tubes having been completely taken up by the transpiration from the leaves. At this time the amount of liquid in the tubes containing spore suspension had been but very slightly reduced, while in the tubes containing the filtrate less than half of the liquid remained.

Upon examination the checks showed no presence of fungus growth, the wilt being entirely due to the drying out of the water in the tubes. The branches in spore suspension when cut open showed the presence of the mycelium in the water conducting tissue for an inch above the level of the liquid, the loss of the liquid probably being due to the transpiration of the plant before the mycelium in the water tubes had become dense enough to entirely plug them up; but as the wilt began earlier and was more rapid than that of the filtrate it would seem that it was caused by a combination of both the mycelium invasion of the water conducting tissue and a poisonous byproduct secreted by the fungus.

In the branch kept in the filtrate no fungus presence was noted and as there was still a large amount of liquid in the tube after the top had completely wilted, it

would seem that the wilt was probably due to some poisonous secretion by the organism.

More complete work with this problem is to be carried on as it appears to open up a new field of experiment with the *Fusarium* wilt.

W.H. Whetzel has reported verbally to Dr. E. A. Bessey that in working a similar experiment using the fungus that causes the *Verticillium* wilt of that particular plant he was working with, the Ginseng, that he obtained results similar to the above mentioned experiment.

INFECTION EXPERIMENTS.

While a great deal of study has been given in the last few years to *Fusarium oxysporum* very few artificial infection experiments have been reported.

Smith and Swingle l.c. report the injuries to the plant caused by a *Fusaria* in the vascular ring of the seed piece, but they do not report inoculation experiments, although their work has often been so interpreted and has even been referred to in literature as such, for example, Duggar* says, " Smith and Swingle have by careful cultural and inoculation experiments demonstrated the causal connection of a *Fusarium* with these types of the disease."

Wollenweber **established the pathogenicity of the fungus stating, in reference to *F. oxysporum*, that, " The pathogenicity of this fungus established by Smith and Swingle has been confirmed by this author for the strain upon which the diagnosis given above is based."

* *
Manns * reports successful inoculations by wounding the roots, but he also states that " The organism is however, productive of infection in the absence of any root disturbance or stem injury, as was shown by a number of experiments." But he states that inoculations by means of

* Duggar, B.M. 1909, p. 317.

** Wollenweber, H.W. Phytopath. 1913, p. 42.

*** Manns, T.F. 1911, p. 317.

injuries brought about the most rapid infection. His infection experiments however were conducted by growing the plant in " sick soil " which he reports produces a greater infection than the use of pure cultures. He states " The disease came on much more definitely under sick soil infections than it did where pure artificial cultures were used without incisions or root injury. The great difference between sick soil infection and that from pure cultures or even internal seed infection, is that in the use of sick soil the roots are attacked at practically every point, while with pure cultures or seed internally infected, the fungus attacks only in close proximity to the main root while most of the secondary and root hairs remain healthy."

The chief objection to these experiments is the fact that the " sick soil " will probably contain a great number of organisms other than that of the fungus in question. In the following infection experiments in the greenhouse, these possibly undesirable conditions have been eliminated by using thoroughly sterilized soil and inoculating it with *F. oxysporum*, leaving the sick soil experiments to be conducted in the field.

The experiments were conducted using various methods of inoculations, as follows.

1. Inoculations of seedlings on healthy and injured roots.
2. Inoculation in soil of pure culture with roots sound.
3. " " " " " " " injured.

4. Inoculation in soil of pure culture with roots sound and also with the addition of Rhizoctonia.
5. Inoculated by wounding the stem and inserting mycelium and spores of a pure culture.
6. Inoculate by placing the stem end of infected tubers in the pots with sound roots.
7. " " injured roots.

INFECTION OF SEEDLINGS.

This experiment was conducted to determine the ability of the fungus to invade sound roots of seedlings.

In using seedlings it was realized that the natural conditions of the field were not obtained, but there were several distinct advantages in this method, such as the exclusion of all fungi from the seed by more efficient sterilization than could be obtained in using mature tubers, as well as the perfect sterile conditions of the soil that could be obtained by using large test tubes 1 x 8 inches, and also the prevention of injuries to the roots.

For these experiments potato seed were obtained from Wm. Stuart, United States Department of Agriculture, from Houlton, Maine. Various methods of sterilization were used, as it was found to be very difficult to obtain nearly perfect sterilization without injury to the seed.

Treatment with 95 % alcohol for 10 minutes, concentrated sulphuric acid for 15 minutes and 1-200 mercuric chloride for 1½ - 3 minutes resulted either in the death of

the seed or very poor germination.

The seeds used in this experiment were treated by soaking the seed overnight in running water, washing in distilled and sterile water and then dipping in 1-200 mercuric chloride for a few seconds. These were then placed on nutrient agar and allowed to germinate. Practically all germinated and the few seed that showed contamination were removed. The remaining were placed in various tubes and grown in the greenhouse.

Seed were placed in large test tubes 1 x 8 inches, of soil extract agar # 28 and after a growth of several inches had been obtained they were inoculated by transferring a small loopful of mycelium and spores from a stock culture to the surface of the agar.

1.-	inoculated 4-8-15. -----	Examined 4-19-15- the seedlings were all
2.	" "	dead, with a heavy mycelial
3--	" "	growth covering the stem
4.-	" "	and great spore formation.
5.-	" "	
6.-	check -----	good healthy grown, 3
7.-	"	inches in height.
8.-	"	
9.-	"	

DISCUSSION OF RESULTS OF SEEDLING INFECTION.

In Experiment # 1 all the seedlings inoculated

with the fungus died inside of 10 days while the checks still remained healthy. The roots were not sectioned and stained so that the presence of the fungus in the vascular system of the seedlings was not determined. It was attempted to cut sections with the freezing microtome but owing to the profuse mycelial growth and the luxuriant sporification which entirely covered the seedlings it was impossible to get good results.

The death of the seedlings may have been due to the invasion of the water conducting tissue, or possibly to a poisonous byproduct of the fungus or simply to the profuse growth which covered the young and tender sprout. This condition is parallel to that found in the field conditions where the fungus attacks the plant when the young and tender shoots are first produced, causing the early death of the plant, or to an attack of the root hairs at a later period probably resulting in the first wilting of the tops, by means of a general weakening of the vitality of the plant caused by the invasion of the root system.

INFECTION EXPERIMENTS WITH GROWING PLANTS.

Artificial infection experiments were begun in January under greenhouse conditions.

Paraffined baskets were used holding about one cubic foot of soil. These baskets were all washed in mercuric chloride after paraffining and were then filled

with soil that had been sterilized at 22 lbs. pressure for 2 hours.

Two sets of checks were used and these were placed in various parts of the greenhouse with the infected plants.

In baskets 1-50 inclusive the tubers used were procured from Frank Lowell, Gardner, Maine and are known as the Lowell Green Mountain variety, they were obtained through the kindness of Dr. W.J. Morse of the Maine Experiment Station. All these tubers were examined for Fusarium and found to be free. They were then treated in various ways with formaldehyde and mercuric chloride. This was done for use in another experiment on the effect of disinfection on the sprouting of the tubers, and then were then used for this infection experiment, if anything they aid in the work as only those tubers which produced the most healthy and vigorous shoots were used for this experiment.

All tubers were planted January 25 th. they were inoculated March 1 st, and dug May 10th.

The formaldehyde treatment was as follows; the sound tubers were washed in water thoroughly and then soaked in a solution of formaldehyde, 1 pint to 30 gallons of water, using the 40% formaldehyde, for $1\frac{1}{2}$ - 2 hours, these were then allowed to dry before planting.

Mercuric chloride treatment was for the same length of time in a 1-1000 solution of mercuric chloride.

INOCULATIONS.

The plants at the time of inoculation were all about 1-2 ft. in height, growing vigorously.

The spore suspension was made by grinding up a stock culture of *F. oxysporum* in sterile water, this suspension was then put on the soil. The cut roots were injured by inserting a trowel in the soil at different points thus injuring and breaking the roots, but not severly enough to materially injure the vigor of the plants.

The *Rhizoctonia* suspension was made up in the same way.

Where diseased pieces of infected potatoes were used, the stem end which was deeply infected was cut off and placed in the basket from which the soil had been removed around the roots and the diseased pieces were then placed in close proximity to the main roots and the soil put back so as not to injure the roots.

Where mycelium was introduced into the stem below the surface, the soil was gently removed so as not to injure the roots, the stem was then washed in mercuric chloride and the mycelium introduced by means of a sterile platinum inoculation spade.

It was found impossible to make a diagnosis of the foliage for wilt symptoms as owing to the crowded condition in the greenhouse the plants were set too close together and resulted in a spindling growth, which soon wilted

down owing to the excessive heat in the greenhouse in the spring and the changes in moisture conditions, which in some cases even caused a rotting of the seed piece and stem. The tubers produced were very small and scarce and it was impossible in many cases to diagnose the disease in this way. The following data were obtained by observing the condition of the stem and following it down to the roots and tuber if possible.

Any cases which were slight enough to make a diagnosis very doubtful were taken into the laboratory and tissue cultures were made from these and the results shown in the tables.

INFECTED PLANTS.

METHOD OF INOCULATION	SEED TREATMENT	SIZE OF SEED.
1. spore suspension	formaldehyde	single eye
2. sound roots	"	"
3. "	"	"
4. "	"	"
5. "	"	"
6. "	mercuric chloride	"
7. "	"	quartered
8. "	"	"
9. "	"	"
10. spore suspension injured roots	formaldehyde	single eye
11. "	"	quartered
12. "	"	"
13. "	mercuric chloride	halved
14. "	"	"
15. "	"	"
16. "	"	single eye
17. "	"	"
18. mycelium in contact with injured roots.	formaldehyde	quartered.
19. F. & Rhiz. " "	"	"
20. " " sound roots	"	"
21. "	"	"
22. "	"	"
23. "	"	"
24. "	"	halved.

METHOD OF INOCULATION		SEED TREATMENT	SINGLE EYE
25.	Check	formaldehyde	halved
26.	"	"	"
27.	"	"	"
28.	"	"	"
29.	"	"	"
30.	"	"	"
31.	"	"	"
32.	"	mercuric chloride	single eye
33.	"	"	"
34.	"	"	"
35.	"	"	"
36.	"	"	"
37.	placed diseased pieces of--	formaldehyde	halved
38.	int. plants in pot,	"	single eye
39.	sound roots.	"	"
40.	"	mercuric chloride	quartered
41.	"	"	"
42.	same as above with	"	"
43.	injured roots	"	"
44.	"	"	single eye
45.	"	"	"
46.	"	"	"
47.	insert mycelium in stem		quartered.
48.	below ground	"	halved
49.	"	"	"
50.	"	"	"

RESULTS.

DIAGNOSIS

TISSUE CULTURES.

1. No browning of either stem, roots or tuber.-- no growth
2. " "
3. " "
4. " "
5. " "
6. Stem showed wet rot due to moisture-conditions- no growth
7. No infection of stem, roots or tuber. "
8. " "
9. " "
10. Slight infection of stem and tuber-- good growth.
11. Stem and roots wet rotted.
12. " "
13. No infection
14. Slight infection of the stem.
15. " "
16. Deep infection of stem, roots and tuber.
17. " "
18. Slight infection of stem---- good growth.
19. Deep infection of stem, no Rhizoctonia. "
20. No infection, Rhizoctonia on stem and roots-slight.
21. " " no Rhizoc. lesions.
22. Wet rot of stem and roots.
23. " "
24. No infection, few slight Rhizoc. lesions on stems and roots.

RESULTS

DIAGNOSIS

CULTURE.

- | | | |
|---------------------------------------|-------|--------------|
| 25. Wet rot | | |
| 26. No infection. | | |
| 27. " | | |
| 28 Wet rot. | | |
| 29. No infection. | | |
| 30. " | | |
| 31. " | | |
| 32. Slight infection----- | ----- | good growth. |
| 33. No infection. | | |
| 34. " | | |
| 35. " | | |
| 36. "" | | |
| 37. " | | |
| 38. Wet rot. | | |
| 39. Very slight browning of stem----- | | suspected. |
| 40. No infection. | | |
| 41. " | | |
| 42. Deep infection. | | |
| 43 " | | |
| 44. Wet rot ----- | ----- | no growth |
| 45. Very slight infection. ----- | | suspected |
| 46. " | | |
| 47. Slight infection. | | |
| 48. Deep infection | | |
| 49. " | | |
| 50. " ----- | ----- | good growth. |

SUMMARY OF RESULTS OF INFECTION EXPERIMENTS.

In considering the results of these inoculation experiments, the different methods of cutting the seed pieces and sterilization have been disregarded, only the various methods of inoculations have been considered.

The plants practically all died down before digging, but it was a premature death caused by the poor cultural conditions in the greenhouse and the tubers were by no means mature when the examination was made. Thus if the plants had been grown under better conditions and the growing period maintained for a longer time, infection might have taken place later in cases where the inoculations did not succeed, or it may have been present very slightly in the roots at the time the examination was made but owing to the poor condition of the plants for diagnosis it was impossible to observe them.

In all cases where the inoculation was carried on by means of using a water suspension of the spores on the sound roots, no infection occurred and no cultures made in the laboratory showed the presence of the fungus. This shows the inability of the fungus to penetrate the sound roots of the plant quickly after a vigorous growth had been obtained. It is very possible that if 'sick soil' had been used or even pure culture inoculations made in the soil before the plants had made a good growth, that the fungus would be able to penetrate the smaller roots and bring about the slight infection which Manns reports l.c.

In the case of the spore suspension used with injured roots, infection takes place rapidly and 5 out of 8 of the plants show the characteristic browning of the stem, from which cultures were obtained in the laboratory. Two died through other causes making it impossible to determine the presence of the fungus, and only one showed no infection. This is probably the cause of the rapid spreading of the disease in the field either through infected tubers or by means of badly infected soil. The cultivation of the crop undoubtedly injures the roots to some extent and the entrance of the fungus is made much easier.

The object of the experiment where inoculations were made first with *Rhizoctonia* and later with *Fusarium* was to determine the effect the *Rhizoctonia* lesions on the roots have to the entrance of *Fusarium*. However only two out of six plants showed the presence of *Rhizoctonia* lesions on the roots and these but slightly, thus practically the same conditions were found as in the inoculation experiments on the sound roots and no infection was found in any case.

Where diseased pieces of infected potatoes were used with sound roots, only one showed a very slight browning, suspected to be an infection while 4 showed no infection, these results are practically the same as in the other experiments on sound roots.

Where the same method as above was used with injured roots there were four infections out of five, one dying of the wet rot, two of these four cases showed a very deep infection. This experiment shows the degree of infection to be about the same as in the case where the spore suspension was used on injured roots.

All of the plants inoculated by introducing the mycelium into the stem below the surface of the ground showed infection, all but one being a very deep infection from which the organism was isolated in the laboratory.

SUMMARY.

1. The parasitism of the fungus on the seedlings.
2. The inability of the fungus to penetrate the sound roots of the plant quickly under the conditions of the experiment.
3. The rapidity with which the fungus gains entrance through root injuries.
4. Inoculation with spore suspension caused the disease as quickly as the diseased pieces of infected tubers.
5. Pathogenicity of the organism, shown by the infection produced by the inoculation of the mycelium into the stem.

CONTROL.

Taking up the study of the remedial measures necessary in the control of this disease the chief factor to be observed is the method of infection. From the preceeding experiments it can be seen that the fungus enters the plant through the underground system at any time during the season, and it is shown in the following experiments that it is capable of living over in the vascular system of the host and infecting the young sprouts in the spring. These two methods of infection make the disease one of the hardest potato troubles to control.

It can be readily seen that a spray such as Bordeaux mixture applied to the tops of the plants will have no affect on the progress of the fungus in the underground system of the plant. In the same way it is seen that the ordinary formaldehyde or mercuric chloride dips applied to the outside of the potato tuber before planting will have absolutely no effect on the control of the fungus. Thus we find that a control of this disease must be accomplished either by the eradication, exclusion, or protection from the causal factor. The following experiments were conducted with this object in view.

CONTROL EXPERIMENTS.

Work on the control of this disease by means of various methods of treatment with the infected tubers

was done by Manns * l.c. who conducted his work under field conditions. He reports that " The increased yield brought about by cutting away the infection and treating the seed varied just about in proportion to the amount and serverity of the Fusarium infection.----- Treatment alone without cutting away the infection as a rule gave no better results than the untrested, showing that the chief factor in reducing the yield was the Fusarium infection, which was internal in the seed and the treatment did not reach it."

In the following experiments conducted in the greenhouse the condition the condition of injured roots caused by cultivation and the possibility of spreading from hill to hill is done away with as each plant was kept in sepearte baskets prepared in the same way as in the infection experiments, using sterile soil in all cases , thus making a better controlled experiment than could be conducted in the field.

The depth of the infection in the tubers used in this experiment varied very little, usually about $\frac{1}{4}$ -1 inch in depth. In all cases where the infection was cut away the cut was made as far as the brown discoloration appeared. The treatment with formaldehyde and mercuric chloride was the same as in the infection experiments. When the stem ends were removed the treatments with the disinfectants was made after

cutting. If the tuber was cut in half for butt and stem end experiments the treatment was made before cutting and in all cases the tubers were allowed to dry before planting.

When the tubers were cut the knife was sterilized before each cut, preventing the possibility of carrying the infection from some of the tubers to the others.

The tubers used were of the Sir Walter Raleigh variety grown on a badly infected field that showed practically a total infection with a premature death of the plant several weeks early.

All tubers were planted in the baskets February 9th and examined May 8th. In most cases the foliage was still green and the tubers had not matured.

TREATMENT OF TUBERS

1. Infection not cut away, tubers not treated.
2. " "
3. " "
4. " "
5. " "
6. " " ----- stem end
7. " " ----- butt end
8. Infected stem end removed, treated HgCl_2
9. " "
10. " "
11. " "
12. " "

TREATMENT OF TUBERS.

13.	Infected stem end removed, treated formaldehyde.		
14.	"	"	
15.	"	"	
16.	"	"	
17.	Infection not cut away----- treated HgCl ₂		
18.	"	"	
19.	" ----- treated formaldehyde.		
20.	"	"	
21.	"	"	
22.	" -----stem end		
23.	" butt "		
24.	" ----- HgCl ₂ treatment stem "		
25.	" butt "		
26.	" ----- not treated. stem "		
27.	" butt "		
28.	" stem "		
29.	" butt "		
30.	" stem "		
31.	" butt "		
32.	" stem "		
33.	" butt "		
34.	" stem "		
35.	" butt "		



CONDITION OF PLANTS MAY 8. ---

Lab. culture.

1. Slight infection, few roots, no tubers.
2. "
3. No infection.
4. Deep infection, no tubers. ----- good growth.
5. Sprout only 1 inch in height, stunted.
6. Deep infection, no tubers.
7. No infection, growth of plant very slow.
8. Top wilted, suspected infection, no tubers.-- no growth.
9. Top dead, very slight infection. ----- suspected.
10. " deep infection. "
11. " " "
12. No infection.
13. Suspected infection, no tubers ----- no growth.
14. No infection.
15. Stunted sprout, only 2 inches high.
16. No infection.
17. No " no tubers.
18. Deep infection of stem and roots, no tubers.
19. Slight infection in the tubers.
20. No infection in stem or tubers, stunted growth.
21. "
22. Deep infection in stem, slight in tuber.--- slight growth.
23. Very slight browning in stem, suspected.--- no growth.

CONDITION OF PLANTS.

Lab. culture.

24. Top dead, no tubers, deep infection in stem.

25. Stunted, sprout only 2 inches, no roots.

26. Entire plant dead and rotted, no diagnosis.

27. "

28. No infection.

29. "

30. Deep infection.

31. No "

32. Deep "

33. No "

34. Deep "

35. Wey rot.

DISCUSSION OF RESULTS.

Tubers not treated or cut show infection in all cases, one of these being so badly infected that the sprouting was retarded so much that only a growth a few inches long was made, (Plate 8) the seed piece remaining sound but no roots being formed. The same tubers show that the stem end of such infected tubers produces a deep infection while the plant from the butt end is healthy.

When the stem end was removed and the tubers treated with $HgCl_2$ we find that the infection was slight in 3 out of 5 cases, and when treated in the same way with formaldehyde only one infection was found, showing the better

fungicidal effect of formaldehyde, while the cutting of the stem ends controls the disease greatly.

These experiments while not conducted on a large enough scale to give conclusive data on control under field conditions, are indicative of certain results, and parallel experiments will be run in the field this summer.

Where HgCl_2 and formaldehyde were used without cutting off the stem end we find that practically all the plants, 7 out of 9 show infection, which clearly shows as may be expected that fungicides applied to the outside of the tuber cannot materially injure the fungus growth on the inside.

To recapitulate:-

It is seen that the cutting off of the infected ends of the tubers is the greatest means of controlling the disease, especially if a formaldehyde dip is used.

The stem end of the infected tuber is the chief factor in producing the disease unless the infection is very deep.

In a few cases sprouting was retarded to such an extent that no tops resulted. This condition in the field would greatly injure the crop causing an uneven stand and a poor yield, a condition which has often been noted by the writer in Michigan.

TESTS WITH CHEMICALS.

It was thought that by adding chemicals to the soil as fertilizers the disease might be controlled in the soil, so attempts were made to test this out in the laboratory.

This was done by growing the fungus on potato stems, sterilized, to which the chemicals to be tested were added.

Potato stems weighing 2.5 gr. were put in test tubes with 3.5 cc. of distilled water, to two tubes each were added .1 gr. of the chemical to be tested thus making the chemical added 1.6 % of the total substance in each case.

	<u>3 days</u>	<u>1 week.</u>
1. Potassium chloride,--	abundant	abundant
2. " sulfate --	vary slight	"
3. Flowers of sulfur --	abundant	"
4. Check --	"	"

No appreciable differences in the different growths were observed, showing that the fungus is capable of growing abundantly in the presence of these chemicals in much larger amounts than would ordinarily be found under field conditions, and therefore it may be concluded that the use of these chemicals are useless in controlling this disease in the soil.

EFFECT OF FUNGICIDES.

The experiments conducted in the greenhouse indicated that the disinfectants used in treating the seed are capable of controlling the disease to a slight extent if brought in direct contact with the organism, i.e. by treating the cut ends of infected tubers. This work was carried on in the laboratory to determine just what effect the disinfectants used have on the organism.

An abundant culture of both mycelium and spores was obtained by growing in a flask of Knudson's Full Nutrient media #27. Several of the large colonies formed were then transferred to tubes of HgCl_2 1-1000 sol. and to tubes of formaldehyde, 1pt. to 30 gal. water. They were allowed to remain in these tubes for $1\frac{1}{2}$ hours and then the colonies were transferred to tubes of nutrient agar and plates poured.

RESULTS.

1. HgCl_2 -- 2 tubes ----- no growth at end of week.
- 2; Formaldehyde--- 2 tubes-- "
3. Check, no treatment----- good growth.

While it is impossible for the disinfectants to be of any use in controlling the disease when a simple dip is used, owing to the fact that the fungus is in the vascular system of the tuber, there is no doubt that a dip used after the infected ends of the tuber have been cut away, will

necessarily penetrate a small distance into the vascular system and kill the fungus present, which may have been overlooked by the grower who is not familiar with the disease and might possibly fail to cut deep enough.

There is also the possibility that if the disinfectant penetrated the vascular system enough to kill the fungus it would also penetrate deep enough to prevent germination, but the usual time allowed for dipping has been used in many localities after cutting the seed stock without great danger, although it is rather a risky practice without further experimental data.

SUMMARY OF CONTROL MEASURES.

After a study of the disease in the field and the preceeding experiments conducted during the winter, the following recommendations are made for the control of the disease in this state.

The first most fundamental and important control method to be recommended is the exclusion of the causal factor. To accomplish this the grower must become familiar with the symptoms of the disease in the field. The plants showing wilt in the field should be staked out and harvested separate from the rest of the crop. Again at digging time numerous tubers should be examined to determine the possibility of the organism being present in the tubers that had not showed any signs of the wilt on the tops in the field. In this way the percentage of infection in the crop can be obtained and the course to be followed in the next years planting can be laid out.

If possible enough healthy plants should be selected from the infected field to start a small seed plot the next year on land that is free from infection, preferably land that has never been planted to potatoes. The infected seed should not be sold or used for seed purposes, but the infection unless very serious does not materially injure the tubers for market purposes and they can be disposed of in this way. From the seed plot the next year there should be

a large enough yield of healthy tubers to plant the next years crop. In this way potatoes not only free from the Fusarium wilt but also from the other potato diseases may be obtained as well as a method of hill selection which should be used to keep up the standard of quality and to increase the yield. By using this method the grower is enabled to produce his own seed and raise the standard of his quality. This is greatly to be recommended over the practice of selling the entire infected crop and buying new seed from some unknown source. The amount of Fusarium wilt found in this state, and in the entire country in fact is so great that in most cases it is much safer to select your own seed than to risk buying it from others.

The organism has been proved to live over in the soil for a number of years and therefore a longer rotation than three years should be used. Badly infected fields should not be planted again to potatoes for 6 years and a longer period is better. Cultivated crops will naturally help in spreading the fungus through the soil and should not be used on infected fields.

From the few small experiments conducted with chemical fertilizers in the laboratory and from the large amount of work done along this line by Smith and Swingle i.c. it appears that the use of adding chemical fertilizers to the soil to help control the spread of the fungus is useless

and no evidence as yet has been presented to show that it controls the disease in any degree.

TREATMENT OF INFECTED SEED.

If it is necessary to plant seed that shows a slight infection the following methods are to be recommended. All tubers should be examined by cutting across the stem end and ascertaining the depth of the infection. This infected piece should be cut away as far as the brown discoloration appears and the tuber then treated with a formaldehyde dip, 1 pint of 40% formaldehyde to 30 gallons of water, for 1½ hours allowed to dry and then planted.

There is a slight danger of injuring the seed germination by treating with disinfectants after cutting, and although it is used in many localities without any visible injury to the stand it is a method that is not safe to advise without further experimental data.

If the infection is slight and the cut made deep enough to remove all the brown discoloration, it is better to use the dip before cutting, then the seed may be dried out and the depth of infection easily observed at the time of cutting the seed. The chief objection to this method is the great loss of treating a large number of potatoes and then having them thrown out.

The safest plan in the control of this disease is the use of sound seed on clean soil.

MEDIA FORMULAE#1. Nutrient agar.

Dissolve 3 gm. extract of beef in 500 cc. water, add 10 gm. peptone, add to 500 cc. water in which has been dissolved 15 gm. agar. Steam 30 min. cool to 60°, add egg albumen, boil 10 min. over free flame, filter, tube, autoclave.

#2. Steamed potato stems.

Potato stems washed thoroughly and placed in test tubes with small amount of distilled water, heated 3 successive days for 10 min. at 100° C.

#3. Steamed potato plugs.

Large smooth tubers thoroughly washed and pared thick, cut into plugs 2 inches long with knife, trim to long slant, wash over night in running water, wash in distilled water, and place in tubes with small amount of distilled water. Heated 3 successive days at 100°C. for 10 min.

#4. Boiled corn meal

$\frac{1}{4}$ inch corn meal in tube, add 10 cc. water, soak over night, autoclave 15 min. at 15 lb.

#5. Steamed bean stems.

Same as #2.

#6. Boiled white beans.

Fill tube $\frac{3}{4}$ inch, coarsely cracked white navy beans, add 10 cc. water, soak well, autoclave 15# for 15 mi

#7. Steamed lupine sprouts.

Lupine sprouts washed thoroughly, placed in tube with distilled water, steam 3 days at 100° C.

#8.
Boiled lupines

Same as # 6.

9. Boiled prunes.

Cut prunes in half, add water, autoclave
for 15 min. at 15 lb.

10. Pear agar.

500 cc. pear twig decoction, 500 cc. water
in which was dissolved 10 gm. agar and 10 gm. pentone, steam
for 1 hour, filter, tube, autoclave, after adding egg abumen.

#11. Prune agar.

120 gm. prune juice, 1000 cc. distilled
water. 12 gm. agar flour, 10 gm. egg albumen. Steam, filter,
tube and autoclave.

12. Oat agar.

50 gm. ground oats, 350 cc. distilled water.
steam in cooker, strain through chees cloth, 10 gm. agar
heated with 150 cc. water. Mix thoroughly and boil in steamer,
filter, tube and autoclave, 15 min.

13. Carrot plugs.

Same as # 3.

#14. Lettuce agar.

15 gm. agar flour, 1000 cc. water, 10 gm
pentone, 2 lb. chopped lettuce, 10 gm. egg albumen.

15. Steamed parsnips.
Same as # 3.

16. Boiled rice.
2½ gm clean rice, add 10 cc. water,
autoclave, 15 min. at 15 lb.

17. Sweet potato plugs.
Same as # 3.

20. Potato agar.
Potatoes washed, pared thick, cut in thin
slices, 300 gm. potato, 500 cc. water. Cook in steamer for 1½ hr.
Strain, dissolve 15 g agar in 500 cc. water, mix with infusion
add 20 gm. glucose, autoclave 45 min. filter, tube, autoclave.

28. Soil extract. agar.
Soil extract 1000 cc. 15 gm. agar, K_2HPO_4
tube, autoclave.

#26 Synthetic medium.
1000 cc. water, $MgSO_4$.4932 gm.-- KH_2PO_4 1.362 gm.
 Na_2CO_3 1.061-- Maltase .7204-- Asparagin .2644.

27 Knudson's Full Nutrient.
1000 cc. water , 1 gr. HNO_3 --.5 gr. KH_2PO_4
.25 gr. $MgSO_4$ -- .002 gr. Fe_2Cl_3 --5 gr. cane sugar.

34. Cucumber agar.
Grind cucumbers in chopper, resout juice,
filter through cheesecloth, to this add 10 gr. agar dissolved
in 600 cc. water. Add egg albumen, steam for hour, filter,
tube and autoclave.

*
LITERATURE.

- 1895---Clinton, G.P. Dry End Rot. Ill. Agr. Exp.
Bull. # 40, p 139-140
- 1896---Stewart, F.C. Another Stem Blight of the Potato.
N.Y. Gen. Exp. Sta. Bul. 101, p 83 and 84.
- 1897--- " " The Communicability of Potato Stem Blight
N.Y. Gen. Exp. Sta. Bul. 138, p 632 and 634.
- 1904--- Clinton, G.P. Dry rot of the *Fusarium oxysporum*.
Rpt. from the Conn. Exp. Sta. 1903; fig b on pl. 22
- 1904--- Smith, E.F. and Swingle D.B. The Dry Rot of Potatoes
due to *Fusarium oxysporum*. Bur. Pl. Ind. U.S.
Dept. Agr. Bul. 55, pp i-62. 8 pl. 2 text fig.
- 1906 -- Norton, J.B.S.
Irish Potato Diseases. Maryland Agr.
Exp. Sta. Bul. 108. p. 67.
- 1909--- Tidswell, E
Dry Rot. Rpt. Gov. New South Wales.
Bur. Microbiology, 60-61. Illus.
- 1909--- Morse, W.J.
Potato diseases in 1908.
Maine Agr. Exp. Sta. Bul. 164-p.2.
- 1909--- Loundsbury, C.P.
Dry Rot of the Potato.
Agr. Jour. Cape of Good Hope 35: 42-48. 3 fig.
- 1909 ---Duggar, B.M.
Fungus Diseases of Plants.p.317 & 318

- 1909--- Orton, W.A.
Potato Diseases in San Jouquin County,
Cal. U.S.Dept. Agr. Bur. Plant Ind. Cir. 23
p 4-8.
- 1910--- Appel, O and Wollenweber, H.W.
Grundlagen einer Monographie der Gattung
Fusarium. Arb.k. biol. Anst. Land -u- Forstew.
Band 18- 8 that is not right 1-207.
Pls. 1-3. fig.-1-2.
- 1910--- Orton, W.A.
Science Vol. 31. p. 751.
- 1911--- Manns, T.F.
The Fusarium Blight and Dry rot of the Potato
Ohio, Agr. Exp. Sta. 229, p.p.299-336. Pl.1-15.
- 1912--- Jones, L.R.
Potato diseases in Wisconsin and their
Control. Wis. Agr. Exp. Sta. Cir. 36. 9, 4.
- 1912--- Stakeman, E.C.
Potato Diseases.
Minn. Agr. Exp. Sta. Bul. 35
- 1912--- Wilcox, E.M.- Link, G.K.K. and Coll V.W.
Dry Rot of the Irish Potato Tuber.
Neb. Agr. Exp. Sta. Research Bul,1, pp 1-88
- 1913--- Wollenweber H.W.
Pilzparasitäre Welkenkrankheiten
der Kulturpflanzen. Ber. d. Deut. Bot. Ges.
Hert 1. p 17-33.

1913--- Wollenweber H.W.

Studies on the Fusarium Problem

Phytopath. vol 13. no 1, p 24-51, pl and 1 text fig.

1914--- Orton, W.A.

Potato Leaf Roll and Related Diseases.

U.S. Dept. Agr. Bur. PL. Ind. Bul. 64 professional

pl. figure 1 and 2.

1914--- Wollenweber, H.W.

Identification of Species of

Fusarium occurring on the Sweet Potato, Ipomea Batat

Jour. Agr. Research. vol 2. No, 4 , 251-286.

1914--- Coons, G.H.

Potato Diseases of Michigan.

Mich. Agr. Exp. Sta. Bot. Dept. Sp, Bul # 66.

* No attempt has been made to give a complete bibliography. This work includes all of the recent works on the subject and reports of the disease from the different localities.

EXPLANATIONS OF PLATES.

PLATE 1. Fig. 1. Showing the different methods of septation in macrospores from 1 to 5 septate, and the characteristic forms found in 2 weeks old culture on potato stem.

Fig. 2. Average forms found of microconidia.

PLATE 2. Showing different methods of germination of the macroconidia from the same culture.

Fig. 1 and 5 , 8 hour old showing method of starting germ tubes.

Fig, 2,3 and 4 , all 24 hours old.

PLATE 3. Fig. 1. Method of microconidia germination.

Fig. 2. Abnormal forms of macroconidia obtained from an old culture.

PLATE 4. Shows the growth of the same conidia through the various stages of growth to the spore formation, shown on PLATE 5.

PLATE 6. Fig. 1 Terminal chlamydospore.

Fig. 2 terminal and intercalated in same hyphae.

Fig. 3. Intercalated in conidia.

Fig. 4. Chlamydospores germinating.

Plate 2.

Fig. 1

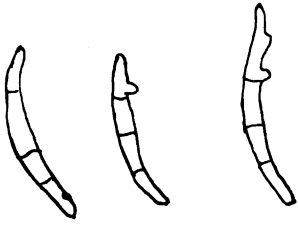


Fig. 5.

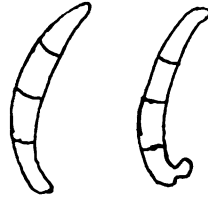


Fig. 2

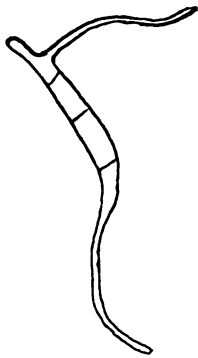


Fig. 3.

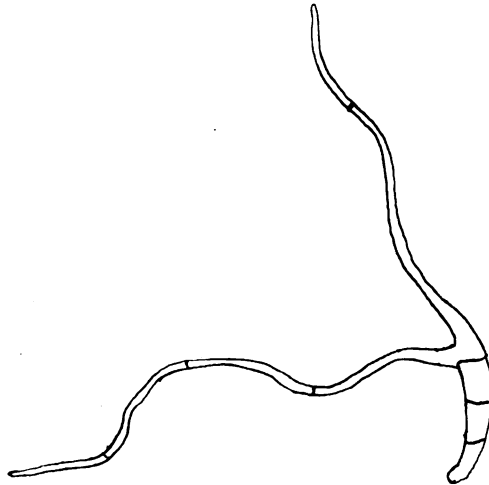


Fig. 4.

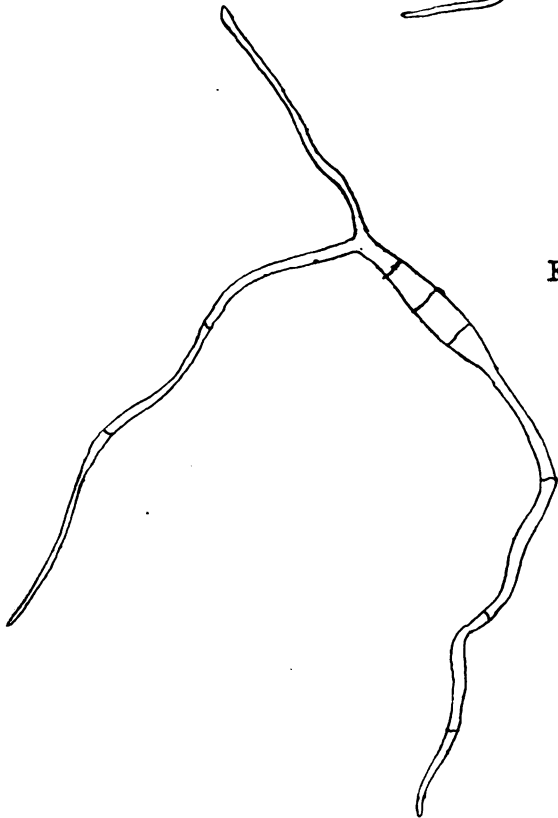


Plate 3.

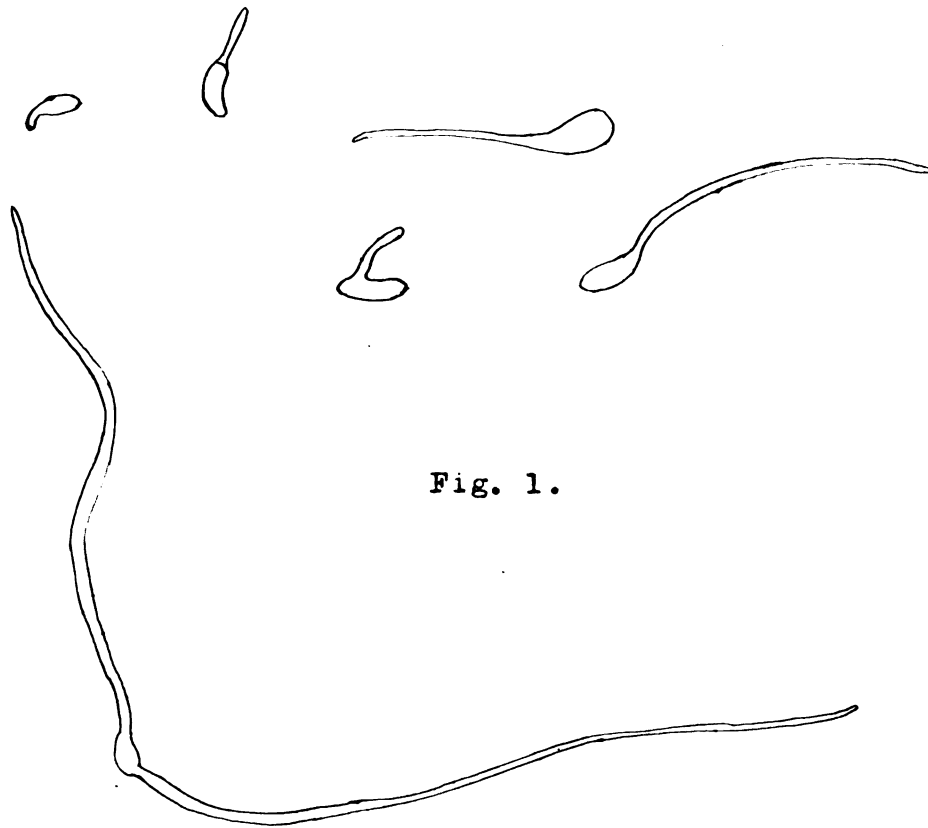


Fig. 1.

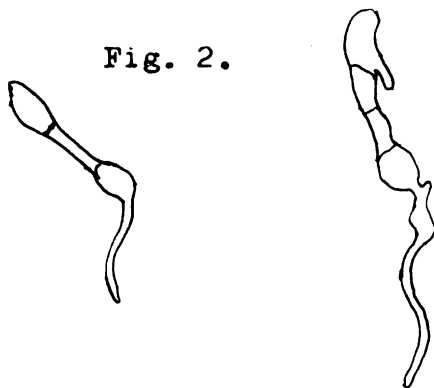
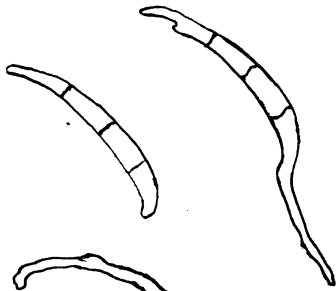


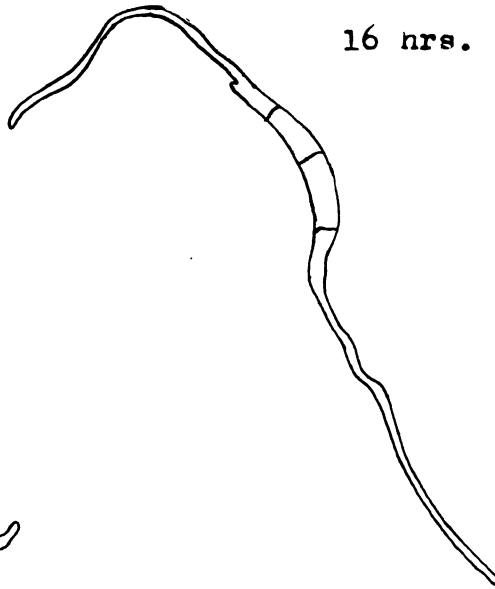
Fig. 2.

Plate 4.

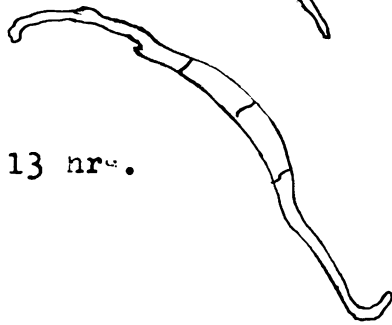
10 hrs.



16 hrs.



13 hrs.



30 hrs.

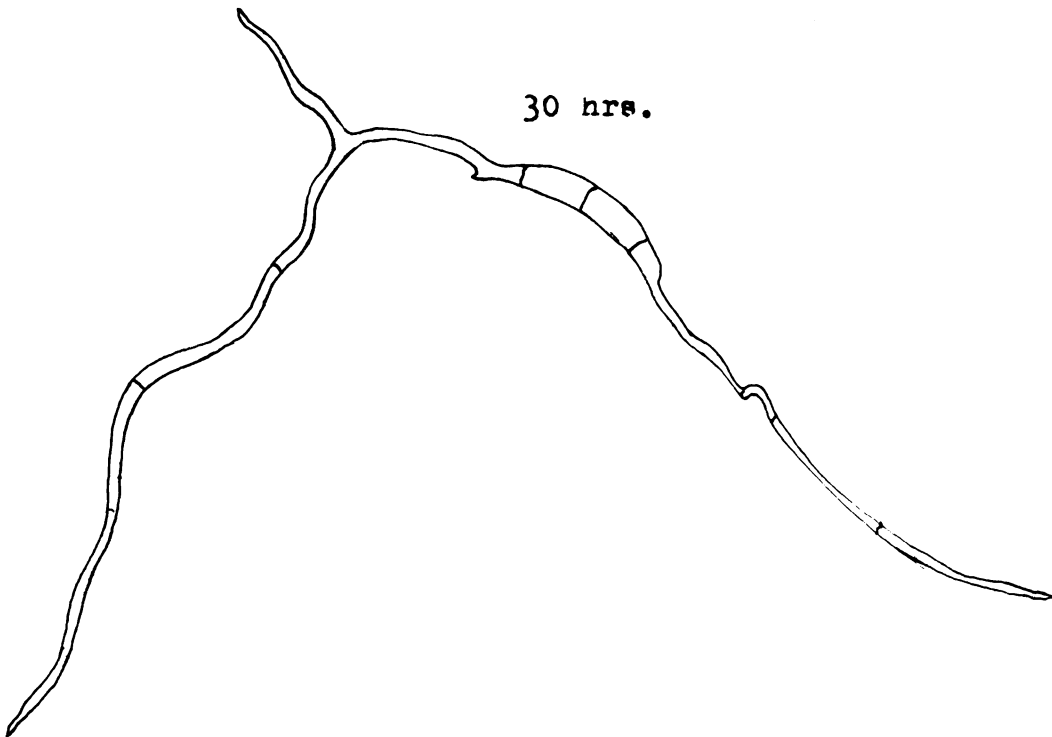
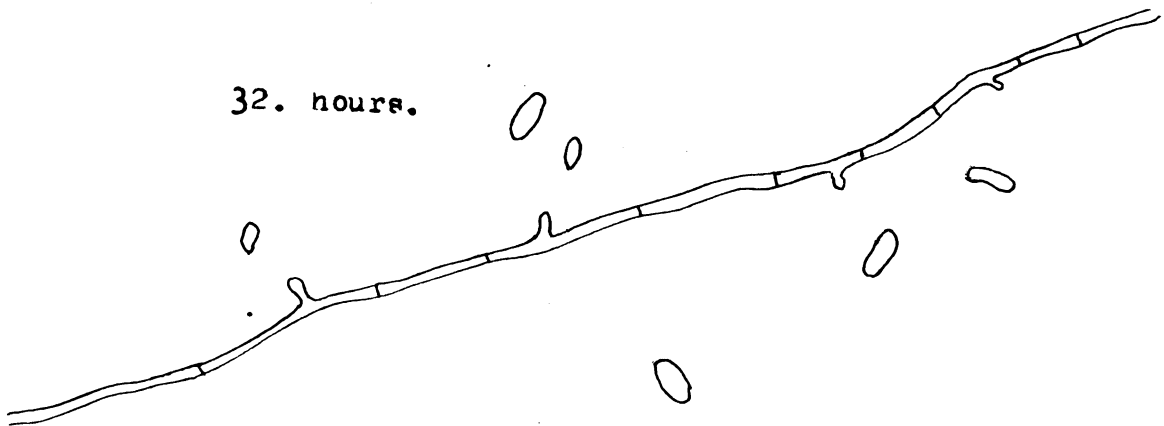


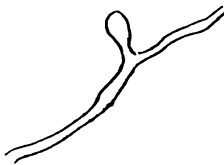
Plate 5.



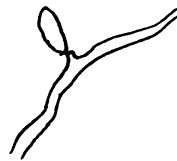
7.15



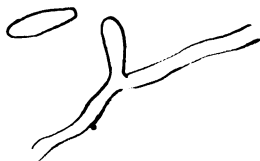
7.45



8.15



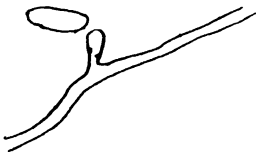
9.10



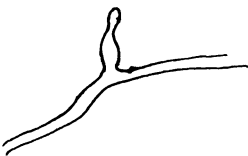
9.30



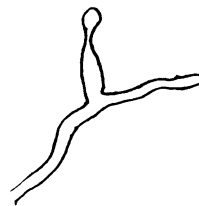
8.45



9.50



10.15



10.45

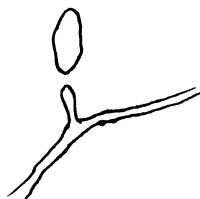


Plate 6.

Fig. 1

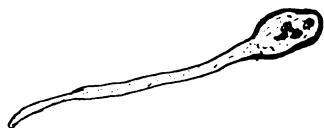


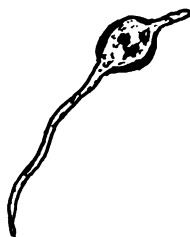
Fig. 2



Fig. 3.



Fig. 4.



EXPLANATION OF FILMS 7-10

(substituted for following sheet.)

Plate 7.

- Fig. 1. Tissue culture isolation on agar from infected tubers.
- Fig. 2. Plate poured in Nutrient agar after 10 minutes immersion at 50° c.

Plate 8.

- Fig. 1. Fusarium wilted potato plant.
- Fig. 2. Infected seed planted in rows in the foreground, checks in background.

Plate 9.

- Fig. 1. Row of infected seed planted between check rows.
- Fig. 2. Three rows of infected seed (left) center row planted from bud ends and both wilted rows planted from stem ends.

EXPLANATION OF PLATES 7-10

PLATE 7. Fig. 1. Culture 1 week old, showing pionnotes form on nutrient agar.

Fig. 2. Plate poured in nutrient agar at 56 ° C when testing for thermal death point.

PLATE 8. Fig. 1. Badly infected tubers, 3 months after planting, in the greenhouse.

Fig. 2. Tubers showing the brown discoloration of the vascular system in the stem end.

PLATE 9. Showing cultures in test tubes.

Fig.1. On nutrient agar.

Fig 2. " boiled rice.

Fig. 3. " potato stem.

PLATE. 9. Tissue culture made from tubers of plants inoculated in the greenhouse with mycelium introduced in the stem ,

2 F. D. H.
Tissue
20/1/40
4 days old

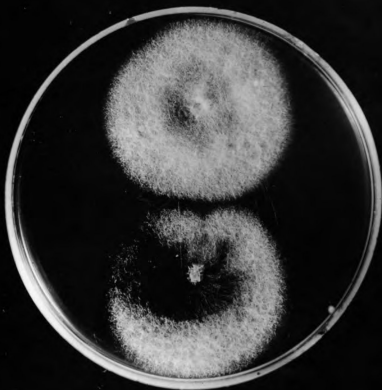


Fig. 2

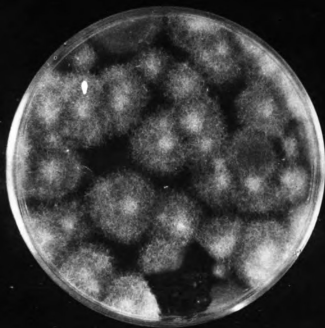




Fig. 2.

PLATE 9

PLATE 10.

ROOM USE ONLY



MICHIGAN STATE UNIVERSITY LIBRARIES



3 1293 02670 9703



MICHIGAN STATE UNIVERSITY LIBRARIES



3 1293 02670 9703



MICHIGAN STATE UNIVERSITY LIBRARIES



3 1293 02670 9703