A COMMON LEAF SPOT
OF
IRIS IN QUEBEC

A Common Leaf Spot of Iris in Quebec.

by

C. Perrault

A thesis submitted to the Faculty of Graduate Studies and Research of McGill University in partial fulfilment of the requirements for the degree of Master of Science.

May 1927.

CONTENTS

ACKNOWLEDGEMENTS

To Professor Dr. B. T. Dickson, Head of the Department of Botany, Macdonald College, the writer is deeply indebted for many valuable suggestions and criticisms with regard to the work, and for placing at his disposal all laboratory equipment necessary as well as for the help in the arrangement of the manuscript.

To Mr. M. H.Howitt, lecturef in Horticulture, I am grateful for the material he provided me with to carry on my experiments.

To Mr. H. N. Racicot, I offer my thanks for the specimens he kindly sent me.

INTRODUCTION

Among the most important horticultural crops, the Iris is one of the most attractive and satisfactory for season-to-season effects in a garden. Its flowers of all shades of colors would satisfy any artist, and in landscape its foliage gives definite line and mass making gradual transition from the lawn to shrubbery or other architectural features.

Unfortunately a leaf spot disease attacks its foliage and causes its complete destruction wherever infestation is bad. At Macdonald College some two hundred and fifty varieties of Iris are grown. When walking through the plantation last Fall, the writer noticed that the foliage was more or less spotted, blotched and killed; this study was then undertaken in view of the general prevalence of the disease or diseases.

SCOPE OF INVESTIGATION.

The present studies were carried on in the Department of Plant Pathology at Macdonald College, during the year 1926/27 and were thus confined to the laboratory and greenhouse.

It is the writer's aim to show by this investigation;

1. The possible cause or causes of an Iris leaf spot in Quebec, its geographical distribution and economic importance.

2. The pathological effect of the fungus on the plant tissues.

3. The behaviour of the same pathogen in different environments.

4. The pathogenicity of the organism or organisms found associated in the disease.

5. The life-history of the pathogen in relation to pathogenesis.

6. Some of the possible factors of disease-resistance in Iris and especially in the Beardless Iris group.

Historical Review.

Leaf spot disease of Iris has been known for many years to mycologists but the attention of plant pathologists has been drawn to it only since 1893, when M. C. Cooke first recorded it in England on the living leaves of Iris germanica (3). Later Ritzema Bos reported investigations upon a disease of Narcissus which he attributed to the Iris organism.

In 1912, Gussow (10) reported the disease in Canada. He described the spotted lesions occurring on the leaves of the Bearded Iris and attributes the cause to Heterosporium gracile.

Ramsbottom (16) in 1915 undertook a study of the disease at the Wisley Laboratory (England). According to him an imperfect fungus Heterosporium gracile is the cause of the leaf blotch disease of Iris; no mention being made of the ascigerous stage. He conducted some infection experiments with Irises (The Bride, Albert Victor), Narcissi, Gladioli and Hemerocallis, but Irises only were found to be susceptible to the disease. He was thus raising the question of possible strains since Ritzema Bos and others before him had stated that the fungus could cause a serious disease of Narcissus (16).

In 1916 Tisdale (21) carried out his investigations at the University of Wisconsin where he connected the conidial stage Heterosporium gracile Sacc., with the ascigerous stage Didymellina iridis (Desm) v Hohn., thereby establishing the complete life cycle of the fungus. After a series of inoculations carried out with several species of Iris (I.germanica, I.variegata, I siberica, I.florentina), Hemerocallis sp. and Narcissus, var. Emperor, he concluded that groups of Iris

other than the broad-leaved group and also the species of Narcissus and Hemerocallis are evidently immune from the disease.

Hoare (15) in 1925 reported a Leaf spot or Leaf blotch in England occurring on the Bearded Iris, the description of which corresponds to those given by previous investigators. He attributed the cause to the same organism described by Tisdale (21) although he had not found the perfect stage of the fungus.

Hosts.

The range of hosts is very limited. According to Ramsbottom (16) and Tisdale (21) the organism is limited to the broad-leaved or German Irises. The present writer has verified this assertion in so far as broad-leaved Irises are concerned except that in the greenhouse, artificial infection of beardless D. K. Williamson, a narrow-leaved Iris, was obtained.

At Madison, Wisconsin, the species found affected by the organism are Iris germanica, I. florentina var. albicans, I. variegata var. honorabilis. Cooke reported the same fungus as occurring on Lychnis. Tisdale (21) mentions that Ritzema Bos attributed a serious disease of several varieties of Narcissus to the same fungus and that Ellis in 1889 collected at Newfield, New Jersey, a fungus which he identified as Heterosporium gracile Sacc., on Hemerocallis fulva. Lindau reported the same organism as occurring on Freesia, Antholyza, Gladiolus and Gemmingia (21).

The present writer obtained infection with the following horticultural varieties; Alcazar, Albert Victor, Caprici, Flavescens, Nokomis, Trojana, Walner, Nibelungen and under certain

conditions the Beardless Iris Dorothea K. Williamson.

The various results obtained by the different investigators on this fungus tend to prove the existence of physiologic species of the fungus.

Common Names.

The disease is most commonly known under the name of "Leaf spot" after its striking symptoms or as "Leaf blotch" which characterizes the more advanced stage of the malady when the spots coalesce and the leaves show large areas of necrotic tissues. It is also called "Leaf mould" denoting the hyphal growth and conidial production over the dead areas. This mouldy appearance seen over necrotic areas only, shows the saprophytic ability of the fungus. In some localities the disease is also termed "Leaf smut of Iris". In France the disease is known under the name of "Tache de la feuille de l'iris".

Symptomatology.

In the Province of Quebec the Iris leaf spot disease becomes particularly noticeable towards the end of the summer or in early autumn when the plants are in full bloom or nearing the end of the flowering season, but it is often present on the leaves in the spring.

The disease is evidently localized and limited to the foliage, the upper part of which seems to be most susceptible. It causes the blades to wither at the apex and in a very severe infestation, should optimum conditions for the propagation of the disease prevail, the upper half of the leaves subsequently turns brown and finally dry out.

Cross-sections have shown that the fungus is present in a comparatively small range in the dead areas. It does not spread rapidly around the point of infection and its growth is very limited. Nevertheless, its presence in the tissues may cause large deteriorations of the foliage.

In early stages of infection very small water-soaked spots can be easily detected. In two or three days these water-soaked areas, 1-2 mm. in size, soon lose their chlorophyllian colour. They are generally circular and gradually increase in size. When examined in a transmitted light they still appear slightly translucent or watery with a brownish spot about one millimeter in diameter in the center. (Pl.3, fig.3) The infected small areas soon become yellowish with a more or less brownish border forming definite spots 2-4 mm, in diameter.(Pl.6, fig.2,3) By this time the fungus has penetrated through the thickness of the leaf and caused the necrosis of the tissues on the opposite surface. From now on the diseased areas can be equally seen on both sides of the blade. Later, the centre of these spots turns to dark brown in colour partly due to necrosis and partly to the darkening of the mycelium and the beginning of hyphal formation underneath the epidermis.

As the growth of the organism progresses the spots become oval or elliptical in shape with an irregular margin. They spread more rapidly in the direction of the veins forming large necrotic areas of several square centimeters.(Pl.6,fig.1,4, Pl.5 D) The size and number of the spots vary with the various hosts and the mode of inoculation. (Pl.5 A.D. and Pl.3,fig.1,3). The continuous growth of the fungus into the healthy areas is

marked by an outer zone of freshly discoloured tissues. The older spots have a grayish brown center surrounded by a reddish brown border. Once the organism is well established in the tissues the necrotic areas, under suitable conditions, coalesce until the greater part of the leaf is affected.(Pl.3) The diseased areas are now very irregular, grayish-brown, blotched and bounded by darker brownish margins.

The spots viewed with a hand lens are seen to be covered by many small, black pin-point elevations which can even be seen by the naked eye. (Pl.4) The microscope reveals these to be tufts of dark coloured conidiophores with conidia, which have pushed forth through the stomata or raised the epidermis finally splitting it by the pressure exerted by their upward growth.

The symptoms are quite constant in their occurrence and any careful observer can easily distinguish them in the field.

Pathological Histology.

To understand the behaviour of the fungus towards the leaf tissues and to have a definite idea of the changes and damage caused to them by the pathogen, a knowledge of the anatomy and morphology of the Iris leaf is necessary. In his studies, the writer used free-hand sections which gave him very satisfactory results.

The sections were stained with Dalafield's Haematoxylin and Safranin. The fungus in the cellular tissues was stained with Cotton Blue, the formula of which follows:-

Solution A,
50 Gms. Phenol,
50 gms. Lactic acid,
50 gms. Glycerin,
100 cc. Distilled water,

Solution B,
Same as Solution A plus I gm. of Cotton Blue.

The material from water was placed in solution A for a few minutes, then transferred to solution B for 30 seconds to a minute and lastly, back to solution A for clearing. By this process the fungus stained deep blue and could easily be detected in the tissues whenever present.

The Iris leaf blade is built on a strong frame-work composed of wascular bundles which run almost parallel from the base toward the tip of the leaf. These bundles vary in the amount of their mechanical and conducting tissues according to their age and location in the spongy parenchyma. Since the leaf blade divides longitudinally into two halves as new meristematic tissue is formed there are therefore two definite rows of vascular bundles.

Immediately surrounding the vascular bundles are two or three layers of cells; the inner sheath is made of thick walled cells while the outer sheath is composed of thin walled parenchyma cells. The cells of the inner sheath lack chlorophyll completely while those of the inner layer contain some leucoplasts and chloroplasts, though in very small quantity.

Filling the space between the vascular bundles and the epidermis we find the mesophyll or photosynthetic tissue. This tissue is very dense beneath the epidermis but gradually looser towards the center. There is no strong palisade layer but

merely two or three layers of cells closely arranged, the cells of which are about equal in size to those of the epidermis. This tissue differs from the spongy mesophyll in that the latter contains many intercellular spaces as compared with the compactness of the former. The spongy parenchyma cells vary a great deal in size and shape while those of the outer tissues are nearly isodiametric.

The plastids are spherical to ovoid, the latter being most common. The parenchyma cells near the epidermis on both sides of the leaf are filled with these chloroplasts; their number gradually decreasing towards interior of the tissues. The cells of the center parenchyma are deprived of them or nearly so, except at the tip of the leaf where the entire parenchyma is filled with many dark chloroplasts. The cells of the inner parenchyma besides occasional chloroplasts may contain several leucoplasts which are mature, colorless plastids. In the meristematic tissue the plastids are roundish and smaller than in the mature cells of the inner tissues, where they are oval their size being 2.5-5 u, in the former case and 3.6-6 by 4 - 7.5 u in the latter. They are granular in texture and disintegrate easily when pressed under a cover glass.

The epidermal cells, rectangular in shape, are many times longer than wide, this character varying with the varieties of Iris. In a transverse section, the cells are nearly square; they do not vary very much in size except in the regions situated over the vascular bundles where they are smaller. This is more so in cases where the bundles are immediately

below the surface layers.

Chlorophyll is completely lacking in the epidermis. A thick coating of cutin covers the entire epidermal surface forming a strong protecting sheath for the cells underneath. This cuticle varies in thickness with the varieties, all environmental conditions being the same. Cutinization of the lateral and inner walls of these cells occurs in some varieties. This cutinization of the epidermal walls may play an important role in the varietal resistance of Iris to the disease.

Distributed all over the epidermis are the stomata. Their number and size vary somewhat with different varieties and even within the same variety according to their situation. (Table XXIX)

As soon as the germ tube has penetrated into the surface tissues it forms several mycelial branches which grow into the surrounding cells. The noxious effect of the pathogen can immediately be noticed. A toxic substance is excreted which penetrates the cell wall of the plant and acts upon the cellular contents.

The nature of this toxic substance was determined by an experiment in which the fungus was grown on various agar media containing a few drops of Phenolphthalein and Methyl Red. In every one of these cultures the fungus gave an acid reaction, showing the acidity of substance excreted by the organism.

The toxin spreads very rapidly and much ahead of the pathogen so that when the latter advances into the tissues

it finds a field already weakened. The chloroplasts which in the normal state of the tissues are thickly distributed throughout the cells and especially along the walls are plasmolysed by the action of the toxin. The entire cell contents agglomerate into a thick mass in the center of the cells. The Chloroplasts lose their dark chlorophyllian colour and become yellowish green.

The mycelium grows very slowly and is limited to a comparatively small area in the necrotic tissues. It is both inter- and intra-cellular, (Pl.8,B) wedging between the cells, separating them from each other after destroying the middle lamellae. These cells soon collapse and their contents, both starch and protoplasmic, gradually disintegrate into a loose, irregular granular mass.

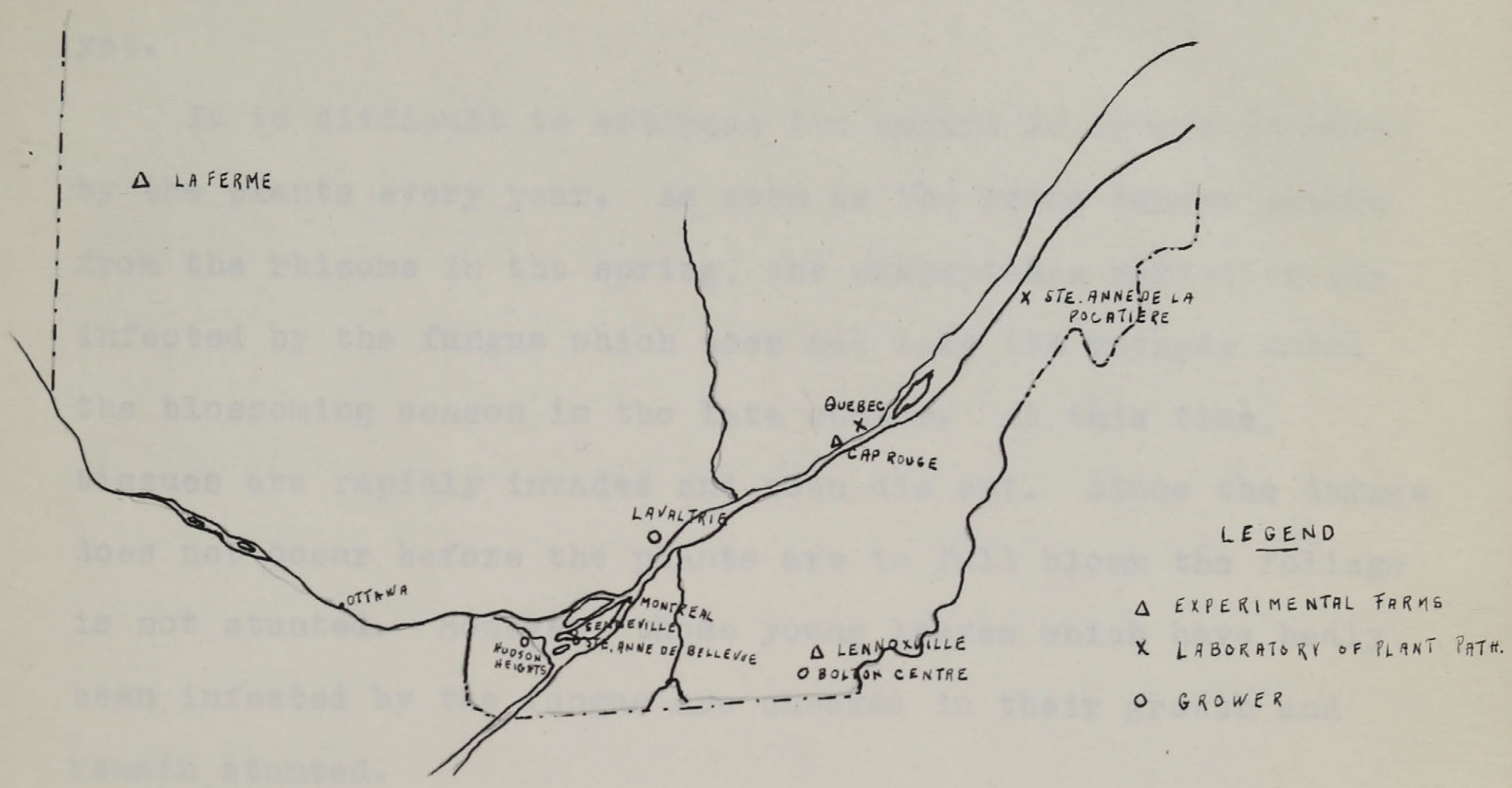
The fungus is not localized in any definite tissue but attacks every one of them and gradually makes its way through the spongy parenchyma, the mesophyll and the epidermis of the opposite surface, oxidizing and destroying the cell walls which become brown. The reddish brown colour of the tissues is the result of this oxidation. The reason for the fungus growing more rapidly in the longitudinal direction of the leaf is likely due to the fact that it finds open passage through the numerous vascular bundles. In the older regions of the leaf where a separation is formed between the upper and the lower halves, the organism has to grow through one or two layers of isodiametric cells somewhat resembling those of the epidermis. Once it has penetrated through these, it grows into the air space between the two halves invading the tissues

on the opposite side. After several days the mycelium develops into a thick mat of dark brown interwoven hyphae from which arise the conidiophores (Pl..8, A). These may come out through stomata or push the epidermis apart from the rest of the tissues breaking it, thus setting themselves free. The grayish appearance of the central part of the older leaf spots finds an explanation here in the air space formed between the epidermis and the parenchyma by the growing conidiophores.

Geographical distribution.

Last fall a survey was made by the writer throughout the Province of Quebec, by correspondence with the Heads of Experimental Farms, Pathological Laboratories and growers, both commercial and private. Answers to questionnaires revealed that the disease is prevalent especially in the South-Eastern part of the Province.

At la Ferme, Ste. Anne de la Pocatiere, Cap Rouge, and Lavaltrie, the plantations of Iris were apparently free from the disease. A small infestation was noticed in the region of Quebec City, Ayers Cliff, Lennoxville and Bolton Centre, while the plantations in Senneville, around Hudson Heights and at Macdonald College, Ste. Anne de Bellevue, were very badly infested with the Leaf Spot. (Pl.1,fig.1,2,3 4.) A more definite idea of the distribution of the disease will be obtained from the map below.



However, owing to the wide range of climatic conditions prevailing in the Province and the ease with which the fungus may spread over an Iris, plantation we may assume with certainty that the disease may develop wherever susceptible Iris plants are grown.

Outside the Province of Quebec, the disease is abundant in Manitoba and Ontario (7). It is also found in the United States (12,15) and practically all of the European countries (21). Ramsbottom (16) mentions the occurrence of the disease in Asia (Lydia), Africa (the Cape) and Australasia.

Economic importance.

The disease under discussion is no doubt the most serious fungous disease that attacks Irises not only in Quebec but wherever these are grown. Owing to the fact that Iris

culture is not very extensive in this province, Iris leaf spot has not attracted much attention from the pathologist as yet.

It is difficult to estimate the amount of damage incurred by the plants every year. As soon as the young leaves emerge from the rhizome in the spring, the susceptible varieties are infected by the fungus which does not make its ravages until the blossoming season in the late summer. At this time, tissues are rapidly invaded and soon die out. Since the damage does not occur before the plants are in full bloom the foliage is not stunted. However, those young leaves which have badly been infested by the fungus are checked in their growth and remain stunted.

According to several growers with whom the writer has been in touch, plants showing the disease for the first time will develop flowers normal in size, and their yield will not at all be affected. However, if the plants show the disease for several years in succession, their vigor will gradually be lessened and the flowers consequently reduced in size, due to a reduction in the starch contents of the rhizomes (5). Tisdale (21) states that "The premature death of the foliage due to the fungus reduces the amount of food stored up in the rhizomes and if the process continues from year to year, the death of the plants will ultimately follow. According to Cooke (3) the Leaf spot in England is one of the most troublesome and persistent diseases of Iris, the plants being killed in one or more years. Here at Macdonald College, the plantation of Bearded Iris as a whole was very badly infected. With the exception of a couple of varieties which showed great

resistance the foliage of all the plants was more or less destroyed by the disease. Plate 1, gives some idea of the infection of the College plantation in the Fall of 1926-27.

In concluding we may say that the sickly appearance of the leaf, its loss of elasticity and shriveling are definite evidence of the injury caused by the fungus. It has no immediate effect on the flowers and the rhizomes during the first year, but continuing over a period of years the divitalizing effect of the fungus may kill the plants by retarding photosynthesis and thus halting the storage of starch. The disease may be expected to become one of increasing importance in the Province since the Iris tends to be a more popular flower than what it has been in the past.

Insolation Studies.

Pure cultures were obtained from typical spots on the leaves of Iris gathered in early November in the College Iris plantation. Three methods of isolation were used:-

1. The plating out process.
2. The capillary tube method, (11).
3. Using lesions.

1. The plating out method:-

A thick spore suspension was made in distilled water by scraping off spores from spots on the leaves with a sterile needle. Five cc. of this solution were then pipetted into a melted potato dextrose agar slant and well stirred. Five cc. of this spore suspension in agar were again pipetted into another tube containing melted agar and stirred. The contents of these two tubes were then poured respectively into two sterile Petri dishes. Two days later little colonies of

whitish mycelium appeared in both plates; very abundant on the agar with the highest spore concentration while they were about fourty in number on the other medium. When eight days old a very marked difference was noticed among these colonies; various fungi were evidently present. With the aid of the microscope the writer was able to discover seven different fungi, namely, Heterosporium gracile, Macrosporium sp., Botrytis sp., Colletotrichum sp., Fusarium sp., and two other fungi which he has not identified but Heterosporium was predominant. Triplicate pure cultures of these various fungi were grown on potato dextrose agar slants for further investigations.

2. The capillary tube method:-

A piece of soft glass tubing was heated over a Bunsen flame and drawn out to an inside diameter of 30 microns which is 2 microns larger than the broadest Heterosporium spore observed. The prepared capillary tubes were broken up into lengths of about six to eight cms. and filled by means of capillary attraction with warm potato agar containing Heterosporium spores in suspension. These spores were embedded in the agar which solidified immediately in the capillary tubes. The tubes were then examined under the microscope and broken up according to the number and position of the spores observed. The broken pieces were immersed in 25% alcohol to sterilize the outside and placed in potato dextrose agar in Petri dishes.

Four plates were inoculated with these tube parts which were sowed in a triangle, therefore, three spores to each plate. But not more than two spores germinated in a plate,

the germ tubes coming out from both of the capillary tubes. In one of the plates no germination occurred and in another one, only one spore germinated. However, from those spores which germinated, colonies like those secured by the preceding method were obtained.

Spores from these various colonies were also compared with the original spores occurring on the leaves and were found to be absolutely identical.

3. Lesion method.

The writer received specimens of the disease from Lennoxville through the kindness of Mr. Racicot. The leaves had been collected in July 1926 and were evidently free from spores through manipulation. Parts of the necrotic lesions were put in Mercuric Chloride (I:1000) for three minutes, washed in sterile water and sowed in plates and slants of potato dextrose agar. No growth resulted from these inoculations. The necrotic lesions were dropped into water and after three days the hyphae from these tissues had germinated into long conidiophores. These necrotic parts were taken from the water and sowed once more on potato dextrose agar where they produced abundant growths. The spores developed in these cultures were Heterosporium and they were in every way identical with those found at Macdonald College.

Other isolations were made from gardens and beds of Iris in the neighborhood of Ste. Anne de Bellevue and with all of them the same organism was obtained.

Pathogenicity.

A preliminary experiment was conducted to determine the

pathogenicity of the various organisms isolated from leaf spots in the Fall.

Iris plants of the same variety were inoculated with the different pathogens or saprogens by wounding the leaves and inserting into them spores and bits of mycelia from the various organisms. The plants were placed under bell jars for three or four days after which they were uncovered and exposed to the normal conditions of the greenhouse.

From these inoculations positive results were obtained with Heterosporium gracile and macrosporium sp. The spots caused by the latter were a little larger than those caused by Heterosporium and spores were produced three or four days sooner also. The organisms were isolated from the diseased lesions on the leaves and grown on potato dextrose agar. The characteristics of these cultures corresponded to those of the original cultures.

Several experiments were made during the year to establish the pathogenicity of these two organisms but in view of the fact that Macrosporium was not constantly isolated in the first case and that time did not allow of a full study of both, it was decided that this investigation should be consentrated on Heterosporium leaving Macrosporium for further study. Nevertheless where comparative inoculation tests were made, the result with Macrosporium are noted and the results obtained indicate definitely the desirability of further work on that organism as it is possibly the cause of another leaf spot disease of Iris.

The writer has not been able to carry on field inocul-

ations since his work was conducted during the winter months. All his inoculation experiments were therefore, made in the College greenhouse. This had its advantages in so far as insuring against outside infection.

Rhizomes of several varieties were dug out in the Fall from the College plantation. The leaves of these plants were cut off and left in the field while the rhizomes were taken in and planted singly in pots of 7" in diameter by 8" deep. These plants grew vigorously and apparently normally, and signs of disease developed on the uninoculated plants during the whole period of experimentation.

After inoculation most of the plants were placed under bell jars for two or more days. These "cloches" were given a thorough cleaning with $HgCl_2$ after or before each inoculation. Inoculations by wounding were made with a sterile needle by cutting the epidermis transversely by the length of the leaf. The wounds varied from 1 to 3 millimeters in length. The greenhouse temperature during the course of these experiments ranged between 52 - 76° Fah.

Experiment 1

Two plants of the varieties Caprici, Albert Victor, Nibelungen were chosen for this experiment. Two or three leaves on each plant were wounded and then the entire plants sprayed with a spore suspension of Heterosporium made in tap water and in 5% Glucose. Other leaves on these plants were inoculated by inserting spores and bits of mycelium with a sterile needle. Three other plants were wounded and used as checks. The plants were placed under bell jars and uncovered after three days.

Table XXII - Results of Inoculation experiment No. I

Variety	Kind of Inoculation	No. of wounds	Positive results	Incub. period	Size of spots 2 weeks later	Plant covered
Caprici	Spray. with T.W.susp.	4	3	I2-I3 days	5-7 mm.	yes
	" " 5% glucose	4	4	9 "	5-8 "	"
	Inserting pathogen	4	4	I0-II "	5-9 "	no
Albert Victor	" "	6	2	I4-I6 "	4-7 "	"
	Spray. with T.W.susp.	4	I	I2 "	2-6 "	yes
	" " 5% glucose	5	2	I0 "	3-5 "	"
Nibelungen	" " " "	4	3	8 "	4-7 "	"
	" " T.W. susp.	6	4	8 "	3-6 "	"
	Inserting pathogen	4	4	9-I0 "	5-7 "	no

Experiment No. 2.

This experiment was intended to see whether the waxy coating on the leaves had any retarding influence on the penetration of the pathogen into the tissues.

Three plants of the variety Walner were inoculated; two with Heterosporium and one with Macrosporium spores. Two leaves on each plant were rubbed with a wet cheese-cloth and then two plants sprayed with spore suspension of each organism made in tap water. The other plant was sprayed with water and spores taken from a Heterosporium culture inoculated into the water drops on leaves. Water adhered much more easily to the foliage after the waxy layer had been rubbed off. The plants were covered with bell jars during three days after which they were removed. The following table shows the results of this experiment.

Table XXIII - Effect of rubbing on the penetration of the leaves by the fungus.

Inoculum.	Mode of inocul.	No.of leaves inoculated	No. of Inocul	Incubat. period	No.of spots at end of Ex
Heterosp.	Rubbing Before Spray.	2		6 days	7
"	Spraying only	9		-----	0
"	Rub.and Spores in water drops	2	12	6 days	5
"	Spores in drops only	6	21	9 "	3
Macrosp.	Rub.before spraying	2		4 "	11
"	Spraying alone	7		5 "	2

Rubbing off the greasy or waxy layer covering the epidermis

is apparently a helping factor in infection. The constant rubbing of the leaves in the field and a heavy rainfall may wash off this waxy coating on the Iris leaf and facilitate thereby the penetration of tissues by the fungus.

Experiment No. 3.

The following experiment was made with a mixture of Heterosporium and Macrosporium spores. Spore suspensions were made with tap water and a 5% Glucose solution. Three plants of the variety Trojana were used for this purpose; some of the leaves were wounded and sprayed with the inoculum. The results are in the following table.

Table XXIV - Results of inoculations with a mixture of Heterosporium and Macrosporium.

Inoculum	Spore Susp.	No.of wounds	No. of spots	Incubation period.
Het.& Macr.	Water	6	5	14 days
" "	Glucose	7	6	14 "
Heterosp.	"	5	3	16-17 "
"	Water	6	3	16-17 "
Mascrosporium	"	6	4	15 "
"	Glucose	5	4	14-15 "

The above table indicates that a mixture of both organisms may possibly infect the plants a little more readily than Macrosporium alone and two to three days earlier than Heterosporium but further work is necessary on this phase.

A few other spots developed on the rest of the leaves but no attention was paid to them.

A considerable amount of moisture was given the plants

in this experiment which were kept continuously under bell jars.

Experiment No. 4.

The following experiment was carried out to get infection by spraying the plants with a spore suspension of Heterosporium. Nine varieties were tested; a plant from each variety growing single in potswas inoculated after having rubbed some of its leaves. The results of this experiment are tabulated below.

Table XXV -

Variety	No.of leaves rubbed	No. of other leaves	Incubation period rubbed leaves	Incubation period unrubbed leaves	Number of spots rub. leaves	Number of spots unrub. leaves
Nokomis	6	12	10	14	40	4
Dot.K.Williamson	8	15	-	-		
Alcazar	5	8		2		11
Flavescens	11	16	11	11	11	10
Walner	8	17	10	12	9	3
Caprici	8	12	-	-		
Albert Victor	7	14	-	-		
Trojana	5	11	-	-		
Nibelungen	9	13	11	12	24	11

The results show that five of the varieties inoculated proved to be susceptible at various degrees. The varieties Albert Victor, Caprici, Trojana would probably have shown infection had they been under bell jars like the others. Being exposed to the dry air of the greenhouse the spores dried out before they started to germinate. These three varieties are certainly susceptible since they have shown infection before.

Dot. K. Williamson which proved to be resistant in this experiment was again tested in a further experiment.

The above figures also show that rubbing the wax off the leaves helps infection to a very marked extent. Penetration of the tissues is sooner, and the number of germ-tubes penetrating them is greater.

Experiment No. 5.

Three plants of the variety Dorothy K. Williamson were inoculated with Heterosporium by inserting the fungus into the tissues with a sterile needle. The plants were well sprinkled with water and placed under bell jars for a week after which time these were removed. Of the twenty-four inoculations two white spots resulted from which the writer has not been able to isolate any fungus.

This variety belongs to the group of the Beardless Iris which are apparently immune to the disease, and while no positive infection occurred in this experiment in the next series five infections developed out of six inoculations as shown below in Table XXVI.

Experiment No. 6.

Another experiment was intended to compare the rate of development of the fungi Heterosporium and Macrosporium on various varieties. All inoculations were made by inserting the pathogens into the leaves of the plants. Some of the plants were covered with bell jars, others were left uncovered. The results are in the following table.

Table XXVI - Results of Inoculations of Several Varieties.

Variety	Inoculum	No. of leaves inoc.	No. of wounds	Incubation period	Positive results
Capriei	Macrosp.	3	8	3 days	8
	Heterosp.	2	5	3 "	5
Albert Victor	Macrosp.	1	4	3 "	4
	Heterosp.	1	4	5 "	2
Walner	Macrosp.	3	9	4 "	8
	Heterosp.	2	6	5 "	4
Dot.K.Williamson	Macrosp.	4	12	3 "	12
	Heterosp.	2	6	5 "	5
Alcazar	Macrosp.	2	5	3 "	5
	Heterosp.	1	3	3 "	3

The results of the above experiment show:

1. The Beardless variety Dorothea K. Williamson is susceptible when a large amount of inoculum is injected into the leaves.

2. The same variety seems to be more susceptible to Macrosporium than to Heterosporium. Positive results were obtained from all the inoculations with the former while only five out of six with Heterosporium. The lesions caused by Macrosporium appeared sooner and developed more rapidly than those caused by Heterosporium.

3. The varieties Albert Victor and Walner which were left uncovered showed symptoms of the disease on one or two days later.

Experiment No. 7.

This experiment was conducted in view of establishing the pathogenecity of the fungus Heterosporium gracile on Narcissus.

Twelve plants the foliage of which had been rubbed with a wet cheese-cloth to get rid of the waxy coating were wounded with a sterile needle and sprayed with a thick spore suspension. The plants were contained in four pots and kept under glass during a week after being inoculated. No positive results were obtained.

The same experiment was repeated under the same conditions with eight other plants. Inoculations were made by inserting the inoculum into the tissues with a sterile needle and covering the plants with bell jars for several days. But again no positive results were obtained.

A last trial was made on plants the foliage of which had burned yellow and which was nearly dead. The results were not more successful.

After this series of inoculations with Narcissus which were unsuccessful from the beginning to the end, the writer concludes that these plants are not susceptible to the pathogen causing leaf spot of Iris in Quebec.

Summary of results of inoculations.

1. The pathogenicity of Heterosporium gracile is an established fact, although its parasitic abilities are rather weak. In natural infection and in artificial inoculations by means of spraying, the pathogen spreads very slowly compared to artificial inoculations by means of inserting the fungus into the tissues. (Pl3, figs. 1,2,3).

2. The incubation period varies with the varieties, other conditions being equal. This is true also of the size of the spots (pl. 5, B.C.)

3. The factor of resistance in the Bearded Iris Dorothea K. Williamson is apparently one of physiological nature since plants of this variety showed infection only when they were injected with a large amount of the pathogen, overcoming the resisting action of the host; even here, infection was not 100% and the fungus growth was very slow, compared to varieties of the Bearded group.

4. The pathogenicity of the Macrosporium sp. found associated with Heterosporium gracile was definitely indicated on most of the varieties tested. It is noteworthy that Macrosporium is much more virulent on Dot. K. Williamson than is Heterosporium gracile, (Pl 5, A.B.) in the greenhouse with artificial inoculation.

Cultural Studies.

As stated above these studies are confined to Heterosporium for the reasons mentioned.

Media.

Two types of media were used for the cultural studies, solid and liquid media. The solid media were used in plates, tubes and cover glasses for germination studies. The liquid media were used in flasks and for germination tests in Van Tieghem cells.

The solid media used are the following:

Corn meal	agar
Czapek's	"
Daffodil	"
Iris	"
Nutrient	"
Oat meal	"
Potato Dextrose	"
Prune	"
Starch	"

The chemicals used in the making of these media were nearly all Merck's Standard Products.

The composition of the Iris and Daffodil agars used is as follows: Leaves from these two plants were chopped and dried in the oven at a constant temperature of about 70°C. This dry matter was separately soaked in distilled water in the proportion of 50 gms. to 1000 cc. and autoclaved at 15 lbs. pressure for 20 minutes. The decoctions were filtered and 2% agar added to them. The media were autoclaved once more at the same pressure and for the same length of time after which they were ready for use.

The following liquid media were used:

Currie's		solution
Knop's		"
Czapek's		"
Duggar's		"
Tubeuf's		"
Cohn's		"
Naegeli's		"
Dextrine	3%	"
Dextrose	"	"
Lactose	"	"
Saccharose	"	"
Maltose	"	"
Pfeffer's	"	"
Iris decoction		"
Daffodil "		"

Plates.

From the pure cultures obtained above the fungus was grown in plates on the nine different solid media mentioned. The general appearance of the cultures on these various media is quite constant for the same kind of medium and varies to a small extent with the different media. However, the rate of growth, the amount of, and time spores are produced varies somewhat with the medium which the fungus grows on.

In order to note the behaviour of the fungus toward the various media and to determine the number of times the cultures may be transferred without losing their vitality, the following experiment was made.

Eighteen sterilized Petri plates all equal in diameter and depth (90 mm. and 12 mm. respectively) were chosen for this purpose. 10 cc. of each of the solid media mentioned above were poured in a duplicate set of plates. The pathogen was grown six times in each of the culture media using pedigree cultures. The organism from F_6 culture on Pot. Dext. agar

was then inoculated on Iris and from there back on the medium for two more generations. Detailed observations were made every other day on changes which occurred. All cultures were grown at room temperature which was read three times a day and which ranged between 16.5°C and 24.5°C (61.7 - 76.1° Fah.) during the whole period this work was carried out.

On Czapek's agar the pathogen grows well and forms a circular, dense growth. The center is made of a thick, olive green to brown stroma on the surface of the medium. This black stroma is covered with a whitish aerial mycelium merging into a very thin white border around the entire growth. This mycelium when examined under the microscope shows many cell difformities (Pl 7, fig. E.). The general appearance of the culture is of a grayish colour with a thin whitish margin. No spores were formed on the 24th day after inoculation.

On potato dextrose agar the organism grows excellently and very rapidly, forming a very thick, dense and circular growth, greenish in colour with a creamy border. The stroma underneath is of a dark green colour merging into lighter shades of green and brown. On the 20th day after inoculation an abundance of spores were produced.

On corn meal agar a very poor and slow growth is produced; the mycelial mat formed is dusky and thin with irregular contours and a central tuft of aerial hyphae. The mycelium shows many difformities with no spore formation, (Pl. 7, Fig. H.).

On oat meal agar the organism grows very nicely forming a whitish uneven growth with a small dark green central stroma. The cultures examined 20 days after inoculation gave evidence of an abundant spore formation.

On prune agar a circular, danse, greenish inner growth is formed with a few concentric rings of darker green to brownish mycelium in most of the cultures. A large dark brown stroma extends nearly all over the surface of the medium covered by the growth. The cultures examined on the 20th day after inoculation showed spores to be present in considerable number.

On nutrient agar the organism forms a greenish, irregular, thin but dense growth, slightly thicker in the center from which arise white aerial tufts of hyphae. A dark green stroma about half the diameter of the entire growth is formed in the center. The cultures examined 21 days after inoculation showed very little spore formation. The mycelium was generally darker than in the other cultures.

On starch agar a thin circular, whitish growth is formed, turning greenish in places and merging into a white thin margin. In the center a large, olive green stroma is formed. Spores are scanty.

On Iris agar the organism forms a large, even, circular, dense grayish growth. A thick, very dark brown almost black stroma forms as the mycelium spreads over the medium. Numerous spores are produced.

On Daffodil, a greenish gray thick growth is produced spreading somewhat slower than on Iris agar. A dark brownish or dark green stroma is formed underneath. Numerous spores are produced.

Tubes.

Cultures with the same organism were carried on a parallel with those in plates.

On Czapek's agar a moderately thick, dense and even growth of light gray to greenish mycelium is produced, covering three-fourths of the surface. A thick dark brown to olive green stroma is formed underneath the aerial mycelium.

On starch agar a thin greenish gray growth, slightly thicker in the center from which arise a very few aerial hyphae. The central mycelium is whitish with one or two rings of darker mycelium appearing in some cultures. A large dark brown to olive green stroma is formed covering nearly the whole surface of medium over which the mycelial growth has spread.

On oat meal agar very rapid growth of iron gray mycelium covers the whole surface of the medium. Dark green stroma merging into light brown colour on the surface covered by the mycelium.

On potato agar a very abundant growth occurs. The whole surface is covered with a thick, dense, dark grayish green mycelium with creamy coloured areas dispersed anywhere on the surface. A large, thick, dark brown almost black stroma is formed.

On prune agar an evenly thick, dense, gray to greenish growth covering four fifths of the surface of medium is produced. Very dark green to dark brown stroma formed.

On corn meal an extremely small and thin growth of whitish mycelium is produced.

On nutrient agar the organism forms a moderately thick growth of whitish mycelium merging into a grayish green colour towards the center. A dark green stroma about half the size of the entire growth is formed in the center.

On Iris agar the growth resembles very much the one produced in the plates. The surface of the medium is covered by the mycelial growth which is gray in colour. The stroma has also covered the same area as the aerial mycelium.

On Daffodil agar a grayish growth with one or two darker spots occurring when the culture is getting old. The stroma is of a dark green to blackish brown colour.

Flasks.

The organism gave a very poor growth in the various solutions compared to the solid media. At the end of two weeks the following characteristics were observed.

Medium	Characteristics
Currie's sol.	Numerous small whitish growths.
Knop's "	Practically no growth; very small white colonies
Czapek's "	Abundant whitish growth.
Duggar's "	Small whitish growth.
Tubeuf's "	Few large white colonies.
Cohn's "	Extremely small white colonies.
Naegeli's "	Few small whitish colonies.
Pfeffer's "	Abundant whitish growth.
Dextrine 3%	Bottom covered with an abundant green growth.
Dextrose 3%	Several large blackish green colonies.
Lactose 3%	Very scarce white growth.
Maltose 3%	Numerous small colonies covering the bottom

with a greenish growth.

Saccharose 3%	Small and very thin whitish growth.
Iris decoction	Good growth of small, thick, dense colonies.
Daffodil "	Fair growth of fluffy mycelium.

The most abundant growth was obtained with Currie's Pfeffer's, Maltrose and Dextrine solutions.

Relation of Media

An experiment was carried to determine whether or not the fungous growth is much affected by the amount of medium on which it grows. The nine solid media above named were used for this purpose.

Sterilized Petri plates of equal diameter were filled with 5 cc. and 15 cc. quantities of each medium and inoculated with spores and bits of mycelium of F_1 generation. Each culture was duplicated and placed at laboratory temperature ranging between 19°C - 22°C (66.2 - 71.6° Fah.). Observations were made daily at first and then every second and third day. The results are tabulated as follows:-

Table I - Comparative rate of growth of mycelium in millimeters on two different amounts of media.

Medium	Amount of medium	3rd day	4th	5th	6th	7th	IOth	I3th	I5th	I7th	20 th
Czapek's	5 cc.	6.5	7.	8.	IO.5	I3	I6.	I6.	I7.	I8.	I9.5
	I5 "	5.	7.	8.	II.5	I4.5	I9.5	22.5	24.	26.	29.5
Potato	5 "	8.	II.	I4.	I6.	I9.	22.5	26.	27.	29.	32.
	I5 "	9.5	I2.5	I6.	I9.	22.5	29.	34.	35.5	37.	40.
Corn meal	5 "	4.5	8.	I3.	I3.5	I4.	I4.5	I6.5	I6.5	I6.5	I6.5
	I5 "	4.5	7.	II.	I2.5	I3.	I4.5	I6.5	I7.	I8.	I9.5
Oat meal	5 "	3.	7.	II.	I4.	I8.	22.5	29.	30.5	32.5	35.
	I5 "	9.	I3.	I6.	I9.	22.5	29.	34.	36.	37.	42.
Prune	5 "	6.5	9.	I3.	I4.5	I6.	I9.5	24.	24.	24.	24.
	I5 "	8.	II.	I4.5	I7.	I9.5	24.	30.5	33.	37.	42.
Nutrient A.	5 "	4.5	5.	6.5	7.5	9.5	I6.	I9.5	22.	24.	27.
	I5 "	4.5	5.5	6.5	9.	I3.	I9.5	22.5	24.	27.	29.

Table I - (continued)

Medium	Amount of medium	3rd day	4th	5th	6th	7th	I0th	I3th	I5th	I7th	20th
Starch	5 cc.	6.5	I2.	I8.	20.5	23.	26.	26.5	26.5	26.5	26.5
	I5 "	I0.	II.5	I3.	I6.	I8.	23.	29.	3I.	32.5	39.
Iris	5 "	5.	8.5	I3.	I5.	I9.	23.	25.	27.	29.	3I.
	I5 "	8.	I0.5	I6.	I9.	23.	29.	32.	34.	36.	37.
Daffodil	5 "	3.	4.5	6.5	8.	9.5	I5,	I9.	22.	25.	27.
	I5 "	4.5	6.	7.	I0.	I3.	I9.5	22.	23.5	26.	28.

The results show:

1. The organism on abundant medium generally takes advantage in its growth in the very beginning, over the same organism growing on a smaller amount of the same medium.

2. The difference between the two growths gradually increases as the cultures grow older. In other words, the growth on a large amount of medium is generally more rapid than on a smaller amount,other conditions being equal.

3. In two cases, with corn meal and starch agars, the growth on abundant media did not surpass the one on scanty media until the latter was checked in its growth by the drying out of the media underneath.

4. The best growth was secured on oat meal, potato dextrose, Prune agars.

See next page, Table II.

Table II - Growth characteristics on the different quantities of media

Medium	Amount of medium in cc.	Type of growth	Colour of growth	Peculiarit
Potato	5	fairly dense, thick, circular.	grayish merging into lighter green.	
	I5	very dense, thick, circular.	greenish with creamy border.	
Starch	5	very thin conchoidal growth.	greenish	dark gray regions irregularly distributed.
	I5	even, circular, dense,	greenish with white border.	
Nutrient A.	5	irregular, loose, thin.	dirty white.	tufts of aerial hyphae in center.
	I5	irregular, dense, moderately thick.	grayish with rings of greenish mycelium.	tufts of white hyphae in cent
Corn meal	5	irregular, very thin.	dirty white.	tufts of white hyphae in center.
	I5	irregular, thin, denser.	yellowish to brownish.	
Prune	5	thin, circular.	whitish with deep green central spot.	
	I5	thick, dense.	white outer ring, green inner; brownish ring, green center.	

Table II- (continued)

Medium	Amount of medium in cc.	Type of growth.	Colour of growth.	Peculiarit
Oat meal	5	thin, loose, circular.	whitish with greenish at places.	
	I5	dense, thick, oval.	whitish with slight greenish spots.	
Czapek's	5	thin and dense in center	green center merging into white border.	
	I5	thin, dense, circular.	olive green, to light brown, dirty white at edges.	
Iris	5	thin, fairly dense, circular.	grayish merging into white.	
	I5	evenly thick, dense, circular.	gray to olive green.	
Daffodil	5	thin, loose, irregular.	grayish merging into white.	
	I5	thick, fairly dense, even.	grayish to greenish with I to 2 rings of darker mycelium.	

A second experiment was conducted to compare the rate of growth of the organism on agar plates and on agar slants. The tubes and the plates used were equal in their respectful diameters. 10 cc. of the various media were poured in both plates and tubes, and the inoculations made on the same day, with spores and bits of mycelium of F_1 generation. The temperature range during the course of the experiment was 18°C - 24°C (64.4 - 75.2° Fah.) and the observations made at the same intervals as for the preceding experiment. The results of this experiment are tabulated on Table III and IV.

Table III - Rate of growth of the fungus in millimeters on various agar media. in plates

Medium	3rd day	4th.	5th	6th	7th	10th	13th	15th	17th	20th
Czapek's	3	5	7	8	10	16	22.5	25	29	36.5
Potato	8.5	9.5	12	17	21	30	32.5	35	38	42.
Corn meal	3	5	6.5	8	9	13	14.5	15	16	18
Starch	5	7	9	11.5	13	16	26	29	32	35
Prune	5	6.5	9	12	13	19.5	26	30	32.5	38.5
Nutrient	1.5	3	5	7	8	13	19.5	23	26	29
Oat meal	10	12.5	16	17	19.5	23	29	30	32.5	34
Iris	4	7	9.5	11	14	18.5	25	29	33	38
Daffodil	2	4	5.5	7	8.5	12	18	22	24.5	26

Table IV - Rate of growth of the fungus in millimeters on various agar media in tubes.

Medium	3rd day	4th	5th	6th	7th	10th	13th	15th	17th	20th
Czapek's	2	5	9	13	16	19	22.5	25	29.5	35
Potato	8	9	11	15	19.5	29	38.5	whole surface covered.		
Corn meal	2	4	6.5	7.5	9	10.5	13	13.5	14	15.5
Oat meal	4	8	13	16	19.5	26	whole surface covered.			
Starch	3	6.5	9	11	13	26	32	35	37	39.5
Prune	4.5	6	8	10.5	13	22.5	27	29	32	36
Nutrient	4.5	7	9	10	11	19.5	22.5	29	31	34
Iris	3	6	7.5	11	12.5	19.5	26	28.5	32	35
Daffodil	2	3	5.5	9	11.5	17	21	24	27	30

The figures in tables III and IV show:-

1. In the early stage of the organism growth was more rapid in the plates than in the tubes. Once the organism had reached the sides of the tubes and could not spread any more in that direction, growth became more rapid in lengthwise direction of the tubes than in the plates.

2. The best growth was obtained with potato dextrose, oat meal, and prune agars.

A duplicate experiment was conducted to determine the amount of dry matter produced by the fungus in solutions of definite pH concentrations and thereby extablished the range of pH most suitable for the growth of the fungus.

Pfeffer's nutrient solution was used for this purpose. 25 cc. of the solution were pipetted into sterilized Erlenmyer 125 cc. flasks. Fourteen solutions were made up to definite

pH ranging between 1.3 to 10.2 by means of the electrometric method described by Clarke (2). A spore suspension was made in sterile water and 2 cc. of this solution inoculated into the flasks with a sterile pipette, care being taken to avoid contamination when lifting the cotton plugs.

The cultures were numbered and left for two weeks in the laboratory at a temperature of 19°C - 26°C averaging about 22°C (71.6°Fah.). At the end of this time each culture was filtered on separate filter papers which had previously been numbered and weighed accurately. The cultures were therefore filtered through their corresponding filter paper. The filters were dried in the oven at a constant temperature of 80°C. and then weighed to find out the amount of dry matter produced in each solution. Having two samples for each definite pH the average production was calculated. The results of this experiment are tabulated below.

Table V - Amount of dry matter produced in solutions of definite pH.

Acidity of original solution	Weight of filters		Weight of filter plus dry matter.		Amount of dry matter		Average	No. of Sol.
	1st	2nd	1st	2nd	1st	2nd		
1.386	1.721	1.699	1.902	1.853	.181	.154	.1675	1
2.062	1.690	1.681	1.894	1.868	.204	.187	.1955	2
2.533	1.669	1.693	1.862	1.896	.193	.203	.1980	3
3.144	1.737	1.715	1.995	1.933	.258	.218	.2380	4
3.708	1.715	1.683	1.913	1.891	.198	.208	.2030	5
4.531	1.658	1.842	1.816	2.071	.158	.229	.1935	6
5.308	1.672	1.678	1.837	1.892	.165	.214	.1895	7

Acidity of original solution	weight of filters.		Weight of filter plus dry matter.		Amount of dry matter		Average	No. of Sol.
	1st	2nd	1st	2nd	1st	2nd		
5.478	1.695	1.682	1.962	1.935	.267	.253	.260	8
5.647	1.672	1.746	2.053	1.900	.381	.154	.2675	9
6.695	1.749	1.702	1.928	1.949	.179	.247	.213	10
7.557	1.718	1.729	1.961	1.932	.243	.203	.223	11
8.994	1.692	1.734	1.901	1.961	.209	.227	.218	12
9.755	1.738	1.740	1.874	1.872	.136	.132	.134	13
10.211	1.708	1.742	1.742	1.767	.034	.025	.0295	14

The results of this experiment are not conclusive because the cultures were not grown for a long enough time and as a consequence the difference in the various amounts of dry matter produced in the different solutions are not strongly pronounced. However, we may assume after the results obtained that the range in which the fungus seems to grow is between pH 3 and pH 9 with best growth about pH 5.5. In solutions No. 1 and 13 growth started three days later and in solutions 14 six days later than in the rest of them.

Another experiment similar to the previous one was carried out with the fifteen liquid solutions mentioned in this paper. The chief aim in mind was to determine the amount of acidity produced by the organism in the various media and establish the correlation between the pH value and the amount of dry matter produced.

Fifteen small Erlenmeyer flasks were filled in duplicate with 50 cc. of each of the solutions, the pH value of which had been determined by the electrometric method. A spore suspension was made in sterile water and 2 cc. of it were pipetted into the

various flasks. The cultures grew at room temperature for fifteen days after which they were filtered through Genuine Whatman filter papers which had previously been weighed. The filters were dried in the oven at a temperature of 75°C. Each filter was afterwards weighed again and the dry matter calculated.

The filtrate from each individual flask was tested electrometrically to determine how much the pH value of these solutions had been lowered.

Table VI - Amount of acid produced by the fungus in various solutions,

Medium	pH before growth	pH after		Amount of acid produced		Average
		1st	2nd	1st	2nd	
Currie	3.652	1.537	1.420	2.155	2.232	2.1735
Knop	3.956	3.463	3.618	.493	.338	.4155
Czapek	2.772	1.435	.895	1.337	1.877	1.607
Duggar+	2.785	2.840	3.397			
Tubeuf	2.029	1.876	1.724	.153	.305	.229
Cohn	3.719	3.024	2.840	.695	.879	.187
Naegeli	6.002	5.814	5.731	.188	.271	.2295
Dextrine	4.598	3.821	3.194	.777	1.404	1.0905
Dextrose	3.652	3.415	3.463	.237	.189	.213
Lactose	3.900	3.077	3.395	.823	.505	.664
Saccharose	2.975	2.449	1.995	.526	.980	.753
Maltose	3.685	2.753	3.160	.932	.525	.7285
Pfeffer	3.787	3.144	3.195	.643	.592	.6175
Iris decoc.+	3.787	5.697	5.697			
Daffodil	3.922	2.874	3.266	1.048	.656	.852

+ The pH value was raised in these two solutions contrary

to what occurred in the rest of the solutions. The writer wished to check these results but due to a lack of time he had to leave them as theywere.

Table VII - Amount of dry matter produced by the fungus on the various solutions.

Medium	Weight of filter paper		Weight of F.P. plus dry matter.		Dry matter produced		Average
	1st	2nd	1st	2nd	1st	2nd	
Currie	1.717	1.698	2.106	2.153	.389	.455	.422
Knop	1.684	1.737	1.694	1.744	.010	.007	.0085
Czapek	1.726	1.714	1.824	1.814	.098	.100	.099
Duggar	1.675	1.654	1.841	1.834	.166	.180	.173
Tubeuf	1.705	1.707	1.765	1.790	.060	.083	.0725
Cohn	1.716	1.692	1.756	1.736	.040	.044	.042
Naegeli	1.722	1.716	1.749	1.744	.027	.028	.0275
Dextrine	1.717	1.714	1.816	1.827	.099	.113	.106
Dextrose	1.651	1.677	1.750	1.768	.099	.091	.095
Lactose	1.675	1.644	1.770	1.737	.095	.093	.094
Saccharose	1.677	1.679	1.767	1.775	.090	.096	.093
Maltose	1.671	1.719	1.825	1.834	.154	.115	.1345
Pfeffer	1.678	1.723	1.928	1.964	.250	.241	.2455
Iris decoc.	1.692	1.697	1.798	1.755	.106	.058	.082
Daffodil"	1.708	1.693	1.783	1.758	.075	.065	.070

Table VIII - Correlation between the amount of dry matter and the quantity of acid produced by the fungus.

Medium	Average dry matter	Average pH	Amount of pH produced on the basis of 1.00 gm of dry matter
Currie	.422	2.1735	5.150
Knop	.0085	.4155	48.882
Czapek	.099	1.607	16.232
Duggar	.173		
Tubeuf	.0725	.229	3.16
Cohn	.042	.787	18.73
Naegeli	.0275	.2295	8.35
Dextrine	.106	1.0905	10.28
Dextrose	.095	.213	2.23
Lactose	.094	.664	7.06
Saccharose	.093	.753	8.09
Maltose	.1345	.7285	5.41
Pfeffer	.2455	.6175	2.51
Iris decoc.	.082		
Daffodil"	.070	.852	12.17

The figures on the preceding tables show that:-

1. The greatest pH variations were obtained with Currie's Czapek's and Dextrine solutions which have produced .422 gms., .099 gms., .106 gms., of dry matter respectively.

2. The largest amount of dry matter was produced with Currie's, Pfeffer's and Maltose solutions which gave a pH variation of 2.173, .617 and .134 respectively.

From these results we may conclude that if a luxuriant growth is produced on certain media it does not necessarily

follow that the pH value will vary proportionally. In other words the fungus giving a luxuriant growth on a medium will not necessarily give a large pH variation.

The size of spores and mycelial cells growing on different media was measured to determine whether or not variations occur with the media on which they are produced. The organism grown on six different media was examined between its 20th and 22nd day of growth. A count of 100 spores was made from every medium; in the case of mycelial cells careful observations were made of the mycelium seen under the microscope and measurements of the largest and the smallest cells recorded. The results are tabulated below.

Table IX - Size of spores in microns according to different media on which they are produced.

Medium	Length	Width	Average Length & Width		Ave.No.of septa per spore.
Nutrient Agar	81 -40.2	21 - 15	68.37	18.94	2.64
Czapek's "	-	-	-	-	-
Oat meal "	81.4 -39	21 - 14.4	67.36	18.16	3.0
Prune "	81.6 -43.2	21 - 14.1	67.23	18.3	3.68
Corn "	-	-	-	-	-
Starch "	84.3 -42	21 - 13.5	64.8	17.8	3.24

Table X - Size of mycelial cells in microns according to different media on which they are produced.

Medium	Length	Width
Nutrient Agar	108 - 18	9 - 3
Czapek's "	58.5 - 12	12 - 3.6
Oat meal "	105 - 21	10.5 - 6
Prune "	87 - 16.5	9 - 4.5
Corn "	92 - 12	10.2 - 3
Starch "	119 - 15	9.6 - 6

According to these figures the size of spores is about constant no matter the medium on which it grows. On the other hand the number of septa in the spores varies remarkably with the medium used. The size of mycelial cells varies also with different media. No spores were formed on Czapek's and corn agars when measurements were made.

An experiment was conducted to find out whether or not the size of spores and consequently, the vitality of the pathogen were much affected through several generations.

For this purpose the organism was grown on potato dextrose agar from an isolation of leaf spots made in the Fall. Duplicate cultures were carried down to the sixth generation and from this generation the organism inoculated into Irises in the greenhouse. The fungus isolated from the plants was grown for two more generations on the same kind of medium. Measurements of 200 spores were taken and the results tabulated.

Table XI - Number of spores from Pedigree cultures tabulated according to their various lengths in microns.

Generation	30	35	40	45	50	55	60	65	70	75	80	81	82	83	84	85	86	87	88
Spores on original plant.	I	5	I8	3I	35	I6	22	25	27	I2	2	4		I		I			
F_I		6	5	28	37	4I	47	22	I2	6									
F_2	I	I	2	7	23	3I	65	35	23	I0	2								
F_3		4		4	I5	25	50	56	37	I3									
F_4		2		8	6	22	46	59	I3	I0			I		I				
F_5		4		5	I3	I2	22	I5	3	2									
F_6			No spores																
Spores on plant after artificial inoculation	I	3	II	22	25	36	30	37	22	7	3	I	I		I				
F_I				I6	22	50	4I	37	I8	I3	2								I
F_2	2			4	23	29	60	54	I3	I5									

Table XII - Number of spores from Pedigree cultures tabulated according to their various widths in microns.

Generation	I2	I3	I4	I5	I6	I7	I8	I9	20	2I	22	23	24	25	26	27	28	Numbe of spores
Spores on original plant.				6		I0	5	9	I7	69	33	I2	I5	II	9	2	2	200
F_I	7	I9	I4	78	2I	I5	34	4	3	3	2							200
F_2	6	5	I	5I	3I	I5	65	I4	9	3								200
F_3	2	5	3	37	55	I2	72	7	5	2								200
F_4	7	3		46	25	I7	49	9	7	5								I68
F_5		2		8	9	5	36	6	2	2								70
Spores on plant after artificial inoculation			I	5	6	9	8	7	23	55	35	I3	9	I8	I0	I		200
F_I	II	28	I0	67	30	II	39	4										200
F_2			2	24	3I	7	84	I5	7	I8	I0							200

Table XIII - Number of spores from Pedigree cultures tabulated according to their number of septa.

Generation	I	2	3	4	5	6	Average per spore.	Number of sp
Original spores	I5	8I	83	I4	5	2	2.59	200
F_I	2	I23	70	5			2.34	200
F_2	5	95	86	II	3		2.56	200
F_3	3	I0I	85	II			2.52	200
F_4	2	73	7I	22			2.67	I68
F_5	2	I9	24	22	3		3.07	70
F_6	No spores							
Spores from artificial inoculation	5	75	88	I9	I0	3	2.8I	200
F_I		96	75	2I	8		2.75	200
F_2	I9	II9	58	4			2.23	200

Table XIV - Average size of spores from Pedigree cultures.

Generation	Ave. length	Ave.Width	Ave.No.of septa	No. of spores
Original spores	67.36	22.45	2.59	200
F_1	60.34	16.50	2.34	200
F_2	65.22	17.80	2.56	200
F_3	67.17	17.83	2.52	200
F_4	66.32	17.74	2.67	168
F_5	60.52	18.31	3.07	70
F_6	No spores			
Spores from artificial inoc.	60.72	20.16	2.81	200
F_1	62.14	15.69	2.75	200
F_2	63.24	18.92	2.23	200

The preceding tables show:-

1. After the F_3 generation the production of spores is very much reduced. In the duplicate cultures F_4 and F_5 only 168 and 70 spores were found respectively. In F_6 no spores were formed.

2. By inoculating the apparently sterile organism from F_6 into Iris the fungus was regenerated; a large number of spores were produced on the plant and in the following two generations on media.

3. The width of the majority of spores ranges between 15 u and 19 u inclusively; spores formed on Iris are much broader than those formed on media.

4. The length of the majority of spores ranges between 50 u and 70 u inclusively.

5. The large majority of spores have 2 to 3 septa; spores with six septa were found from the plants only.

Temperature relations.

Twelve slants of potato dextrose agar were inoculated with apores and bits of mycelium and placed outdoors on a Monday at a temperature of several degrees below freezing point (22.2° - 37°Fah.). The following day, the slants were taken into the laboratory to see if they would grow or not. The cold weather seemed to have a retarding effect on the growth of the mycelium for the organism did not start to show signs of life before Friday of the same week.

Of the twelve cultures, nine only developed abundant mycelium and spores; the other three cultures did not grow at all. Seven days after growth had started or eleven days after inoculation, a thick mat of mycelium had covered one third of the surface of the slants. There were three distinct coloured areas; a purplish center 1/4 inch in diameter around which was seen a yellowish circle of about one eighth of an inch. The whole growth was surrounded by a grayish border. These three zones were more or less pronounced in some of the slants.

The cultures were examined under the microscope and the grayish mycelium only was found to have produced spores very few in number. The mycelium in the inner zones was completely sterile as yet. Three days later the cultures were examined again. The grayish mycelium which had spread a little more was still the only one to produce spores but this time in abundance.

The experiment was repeated in January with eight slants of the same medium. The temperature range outside was between 14° - 30°Fah. Four of the cultures were brought into the laboratory at a temperature range of 15°- 24°C. (59° - 75.2°Fah.)

after being outdoors one day.

The organism started to grow three days later. The cultures were of a yellowish colour with a grayish border, but the different zones in the mycelium were not as well marked as in the previous experiment; two of them had none at all. Spores were found among the grayish mycelium only.

The four slants which remained outside did not show any growth. Two of them were taken after a week but no growth resulted; the organism had apparently been killed.

Twelve cultures were used as checks for this experiment. The slants which had been inoculated on the same day were kept in the laboratory. The following day growth had started in seven of the tubes and on the second day growth was seen in every culture. The colour characteristics of the mycelium, nine days after inoculation were somewhat different. The general appearance of the cultures was grayish but in several of them concentric rings of darker mycelium could be seen. On nine of the cultures there was a yellowish center the diameter of which varied to some extent; in some of then this was as large as 10 mm. in diameter and in others it was barely visible. Around each growth a thin border of white mycelium was formed. Needless to say that spores were very abundant in these cultures but their complete absence from the yellow areas was still characteristic.

Six slants of potato dextrose agar inoculated with the same organism were placed in the incubator room where the temperature ranged between 29° - 32°C, but no growth resulted from any of these. Furthermore, the cultures were brought into

the laboratory but they had apparently been killed by the heat of the incubator.

Six other cultures were placed in the refrigerator at a temperature range of 4° - 10°C. Two days later, growth was noticeable in four of the slants and on the third day growth had started in the other two tubes. The cold temperature delayed growth but once the organism had started to develop, it spread just as rapidly as it did at room temperature.

The mycelial mat in these cultures was of a darker gray than in the cultures kept at room temperature. A very small circular yellowish area was formed in the center of only a few cultures. It would be very interesting to know that no spores were formed in any of these slants. Three tubes were left in the refrigerator and the other three were brought to the laboratory with the hope of having spores formed later. The cultures were examined six weeks after but none had produced spores as yet.

Another set of tubes were placed in the incubator at a temperature of 27° - 28°C., but no growth resulted.

Table Xv - Results of experiment with various temperatures.

No.of Cultures	Temp.range	Incubation period.	Spore formation
8	14°F. - 30°F.	no growth	
12	22°F. - 37°F.	" "	
6	29°C. - 32°C.	" "	
6	27°c. - 28°C.	" "	
6	15°C. - 24°C.	1-2 days	abundant
6	4°C. - 10°C.	2-3 "	none

The results of this experiment are very interesting.

They shew that under extreme temperature conditions the fungus is checked in its growth. A temperature range of 40°-10°C. which is favorable for the growth of the organism is unfavorable for its spore formation. The range of temperature at which the organism grows best is rather small being from 15° - 24°C.

Measurements of the spores from three of the cultures in the above experiment were taken to determine whether temperature had any effect on their size. From each culture 150 spores were measured. The results are shown in the following table.

Table XVI - Effect of temperature on the size of spores.

Temperature	Length of spores	Ave.	Width of spores	Ave	Average no. of septa.
1 day at 22° to 37° and taken at R.temp.	66.6-29	55.92	19.5-12	17.04	2.16
18°- 22°C.	83.7-45.3	70.47	21.3-13.8	18.89	2.92
7° - 15°C.	74.74-41.1	59.48	16.5-12.	13.87	2,

It is somewhat difficult to draw definite conclusions from the above table. Nevertheless one may say that the growth of spores is visibly affected by temperature conditions. A low temperature diminishes their length and width,while a moderate warmth favours their development in size. The number of septa in the spores seems to be considerably affected also. The figures show that where the cultures were placed outside at a temperature below freezing point and brought in the laboratory afterwards, the decrease in the length of spores is much greater relatively to the decrease in their width. The fact that the cultures were chilled seems to have affected their development in length more than in width.

Relation of light.

Twelve slants of potato dextrose agar were inoculated with spores and bits of mycelium of F_2 generation; six of them were wrapped up in black paper and left at laboratory temperature with the others. Three days after inoculation there was a very slight difference in the size of growth of the cultures left unwrapped. When examined on the fifteenth day the amount of growth was the same with small colour differentiation. The wrapped tubes had formed rings slightly darker than the rest of the growth while a yellow spot could be seen among the grayish growth of some of the cultures.

Summary of Cultural Studies.

1. The fungus thrives better on solid media and among these Pot. Dextrose, oat meal and Prune agars proved to be the most favora ble.

2. Growth is affected by the amount of medium available; large amounts favouring more growth.

3. The pathogen tolerates larger concentrations of acid than of alkali.

4. The size of spores varies but very little with the different media while the size of mycelial cells is very much affected.

5. The transfer of cultures for several generations tends to stop the formation of spores. A sterile culture is apparently regenerated by growing it back unto its host.

6. Temperature has a marked effect on the organism. The latter thrives best at medium temperature preferring low ones to high ones.

7. Spore production occurs in a rather small range of temp-

erature.

8. The presence or absence of light does not seem to affect growth to any large extent.

Germination Studies.

Spore germination has been carefully studied. The conidia for these studies were taken from pure cultures on potato dextrose agar. All cultures were made in hanging drops or films of several media in Van Tieghem cells kept at various temperatures.

The first definite indications of germination are slight protrusions in the cell walls which develop into definite germ tubes with more or less rapidity varying with the media in which the spore germinates. There is a slight increase in the size of the spore before germinating, this also varying on the kind of medium used.

Septa are formed in each tube at various distances from the germination cell depending also on the medium used. The distance at which septa form and the time for the first septum to form varies very little for the same kind of medium other conditions being equal. The number of germ tubes protruding from the spores vary with the medium in which the spores germinate. Germ tubes may arise from any one cell of the spore, the terminal cells being generally the first cells from which tubes protrude.

Relation of Media.

An experiment was made to compare the rate of germination of spores sowed in various liquid media and placed at room temperature ranging between 20° and 23°C. The results are tabulated below.

Table XVII - Comparative rate of germination of spores

Medium	No.of trials	Positive results	Time for germination in hours.
Tap water	13	9	3.05 - 4.45
Distilled water	12	6	3.15 - 4.35
Sterile water	8	2	3.25
Nutrient broth	5	5	2.45 - 3.05
Glucose 5%	3	2	5.45 - 7.15

The results from this first experiment show that Nutrient broth was the most suitable medium for the germination of spores and sterile water the poorest.

Germination of spores on solid media was also experimented. A very thin film of medium was made on one of the surfaces of cover slips. With the point of a sterile needle several spores were taken from a culture on potato dextrose agar and inoculated into the thin films of medium. The inoculations were made in the form of a triangle, the three points of which, have been cut off. These cover slips were then sealed with vaseline, film downward, over small glass rings also sealed to glass slides with paraffin. A drop of water was placed at the bottom of each cell to prevent the medium and spores from drying. Germination tests were tried several times with different media with negative results each time.

The same experiment was repeated with thicker films of media with the following results.

Table XVIII - Comparative rate of germination of spores in various solid media.

Medium	No. of trials	Positive results	Time for germination in hours.
Oat meal agar	4	3	3.05 - 3.15
Czapek's "	6	4	2.15 - 2.45
Prune "	6	4	2.20 - 2.30
Potato Dext"	6	2	4.30
Starch "	4	2	4.05
Nutrient "	6	3	2.30 - 2.50
Corn "	4	2	2.20
Iris "	4	3	3.35 - 3.50

Another experiment was conducted to compare the rate of germination in various solutions. The rate of growth was also determined in millimeters. The spores were sowed in Van Tieghem and placed in moist chambers at room temperature ranging between 17°C - 22°C. Observations were made every day during the three days following germination.

Table XIX - Rate of germination and growth in various solutions.

Medium	No.of trials	Positive results	Ave.rate of germination	% of germination.	Ave.rate of growth per Hr.
Tap water	2	2	3.20	89 & 100	14.29 microns
Pfeffer's	2	2	3.20	5 92	4.71
Czapek's	2	1	3.27	95	14.07
Duggar's	2	1	3.	88	4.53
Tap Water	2	1	3.20	95	3.09
Duggar's	2	2	4.23	90 & 30	.22
Knop's	2	2	2.35	100 & 72	14.87
Tubeuf's	2				
Naegeli's	2	2	3.50	100 & 30	13.29

Medium	No.of trials	Positive results	Ave.rate of germination	% of germination	Ave.rate of growth per Hr
Cohn's	2				
Sterile H_2O	2	1	3.10	94	8.01
Dextrine	2	2	3.35	71 & 91	15.5
Lactose	2	2	3.35	25 & 13	11.55
Maltose	2				
Dextrose	2	2	5.	61 & 53	1.52
Saccharose	2	2	4.20	67 & 62	2.82
Iris diluted	2	2	3.55	79 & 85	34.47
Iris conc.	2				
Daffodil conc.	2	1	22.33	28 & 35	1.68
Tap Water	2	2	4.29	89 & 92	12.62
Distilled H_2O	2	1	4.03	69	17.64

Mode of germination and tyoe if growth.

Medium	Mode of germination	Type of growth
Tap water	4-5 germ tubes at same time from any cell	Short hyphae with much branching,
Pfeffer's	1-3 germ tubes from end cells, none in center.	Growth almost stopped after germination
Czapek's	3-4 germ tubes;2 from each end cell generally.	Good growth
Duggar's	1-2 germ tubes at the ends	Very slow growth.
Knop's	1-2 tubes at either ends.	Fair and wavy growth; much branching.
Tubeuf's	No growth	
Naegeli's	2-3 tubes from any cell	Fair and wavy growth with little branching
Cohn's	No growth	
Sterile water	1-3 germ tubes from end cells	Very small growth and little branching.

Medium	Mode of germination	Type of Growth.
Dextrine	1-3 tubes; 1 or 2 at each end	Fair,wavy growth with much branching.
Lactos	1-3 tubes;generally at the ends,seldom in center.	Moderate growth with much branching.
Maltrose	No growth	
Dextrose	1-2 tubes; one at each end.	No growth
Saccharose	1-2 tubes; one at each end and aften 2 at one end.	2-8 germ tubes formed later very small growth
Iris diluted	1-5 tubes from any cell	Very best growth;wavy but not much branching
Iris Conc.	No growth	
Daffodil conc.	1 tube very seldom 2	Very slow growth;tubes big but very short.
Distilled H_2O	1-4 tubes from any cell	Long wavy growth but little branching.

Relation of temperature

The following experiment was conducted to determine the effect of temperature on the germination of spores. Spores were sowed in hanging-drops of water and Nutrient broth and placed at various temperatures. Table XX shows the comparative rate of germination of these spores under such conditions.

Table XX - Rate of germination of spores under different temperatures..

Medium.	No. of trials	Temperature	Positive results	Germination in hours from time of inoculation.
Tap water	4	21°C	4	4.30 - 4.40
Nutrient	4	"	4	3.25 - 3.45
T. water	4	30°C	4	3.50 - 4.55
N. broth	4	"	4	4. - 4.25
Tap water	4	35°C	-	
N. Broth	4	"		

Medium	No. of trials	Temperature	Positive results	Germination in hours from time of inoculation.
T. water	4	10°C	3	4.15 - 5.10
N. broth	4	"	2	4.40 - 4.50
Glucose 5%	2	"	1	4.35
T. water	2	7°C	2	6.45 - 6.55
N. broth	2	"	1	6.05

The above experiment shows that spores germinate over a wide range of temperature.

Relation of light.

An experiment was made to find out whether or not spore germination was at all affected by light. Spores were sowed in drops of water at room temperature ranging between 17°C - 21°C. The cultures were kept under moist chambers, some in complete obscurity, others in day-light. The results of this experiment are tabulated below.

Table XXI - Effect of light on spore germination.

Medium	No. of trials	Germination
Light	4	3.50 - 425 hours
"	4	4. - 4.55 "
Dark	4	4. - 4.45 "
"	4	3.40 - 4.10 "

Although the above figures are not very conclusive they tend to show that germination is not much affected by the absence or presence of ordinary day-light.

Summary of germination studies.

1. Spore germination varies with the different media. Solid media gave an earlier germination in general.

2. The number of germ tubes protruding from a spore is apparent-

ly different on various media.

3. Temperatures away from the optimum retard spore germination; extremes stop it.

4. The presence or absence of day-light apparently has no effect on germination.

Taxonomy of the causal organism.

The fungus causing the Leaf spot of Iris under consideration in this study, is known as Heterosporium gracile Sacc. The first mention of this fungus in literature is made by Saccardo who gave it the name of Heterosporium echinulatum in 1881 (16 & 21), but owing to the fact that the specific name had been used for a fungus called Helminthosporium echinulatum (B & Br.) attacking carnations, and that the same fungus had been included by M. C. Cooke in the genus Heterosporium in 1877 (16), Saccardo changed the name to Heterosporium gracile (17) the description of which follows.

"Hyphis in areolis foliorum arescentibus, fusco-cinctis enascentibus, crassis, septulatis, 70 by 10 - 11, sursum nodulosis subolivaceis; conidiis tereti-oblongis, 40 - 60 by 18 - 20, saepius 2-3 septatis atque constrictis, distincte muriculatis, pallide olivaceis.

"Hab. in foliis Iridis germanicae in Italica bor. et in Arduennis."

Tisdale (21) in 1920 found an ascomycete on the Iris leaf and related it to Heterosporium gracile Sacc., to which corresponds with the description of the conidial stage of his organism.

The fungus under consideration does not correspond in

every respect to the above description. The spores are much larger, 30-88 by 12-28 microns and are 2-6 septate.

Morphology and Physiology

The mycelium is slender, hyaline, inter-and extracellular, branching considerably with the different media on which it grows. It is generally uniform in its width although its diameter may vary from 3 u to 14 u. The mycelial wall is colorless and thin especially so in its young stage. The cells may vary excessively in length which may range between 7 u to 160 u and more. As the mycelium grows older, the protoplasmic contents become more granular and vacuolate. The cell walls grows thicker and turns brown to olive green or almost blackish brown, depending on the substratum. This type of mycelium forms a thick and dense mat of interwoven hyphae from which develop the conidiophores.

The conidiophores vary in size with the substratum on which they grow and the amount of nutrients available. Tisdale states that the conidiophores borne in the spots during the summer average about 11 u by 70 u, while those developing in the spring on the apices of sterile perithecial average about 12 u by 150 u. They are concolorous with the hyphae beneath and more or less zigzag in shape at the upper end, due to the production of the spores.

When the conidiophore has attained about 40 u to 60 u the first conidium starts to develop by the progressive bulging of the apex, (Pl.9 E.F.) which constricts itself gradually to form the conidium. The development of conidia is relatively slow and sometimes before the first conidium has

attained its full size a second one starts to grow. A swelling on the side of the conidiophore near the tip, about almost under the conidium is due to the conidiophore continuing its growth. As it grows larger it shoves aside the conidium and elongates in a somewhat oblique direction due to the pressure of the conidium. (Pl 9 I.J.) After it has reached 30 u to 60 u in length another conidium develops at its apex in the same manner as the first (Pl 9 K.). When the spore has reached about half its normal size it ~~is~~ gradually constricts at its base until it has reached its maturity. By this time it is larger than the conidiophore to which it is attached by a very short pedicel about 1 u to 2 u in length. (Pl 7, D .E.). The spore formation goes on until two or three or probably more conidia are formed on the same conidiophore, (Pl.9, L.)

The spores are easily broken off but unlike those of Heterosporium echinulatum and H. betae (6) they are not shed as soon as they are formed. So far as the writer is aware there is only one kind of conidium; the chain-like spore formation as it occurs in H. echinulatum and H. betae has not been observed as yet. The formation of conidia causes on the conidiphore peculiar shapes called geniculations, giving it the zigzag appearance mentioned above (Pl.9 L)

The conidia are echinulate and septate, (Pl 7, D.E.F.) These two characters appear on the spores when the latter have almost or quite reached their full size. There may be from one to six septa in the same spore. The writer on two occasions has noticed a seven septate spore. This is one indication that the organism under investigation may be a different strain from the one described by Saccardo (17), Stevens (20) and Tisdale (21),

their spores having 2 to 3 septa only. Another reason for believing in the possibility of a different strain is the fact that the size of the spores under consideration (30 - 88 u by 12 - 28 u) varies greatly from the size of the spores given by these authors which is 40 - 60 u by 13.7 by 18.7 u. The range in size of the spores by Ramsbottom (16) approaches very much that of the spores described in this paper.

These spores are concolorous with the conidiophores, cylindrical with rounded ends, and slightly constricted at the cross-walls.

2 Ascigerous stage:

The writer on account of being away early in the spring has not been able to ascertain whether or not there is a perfect stage in the life cycle of the pathogen under investigation.

Seasonal Development of the host

The Iris unlike most other plants developsrather late in the season. Young leaves will start to shoot up in May or early June according to the climate conditions and the different varieties grown. In the province of Quebec, plants have generally produced their first leaves by the end of May and by the month of July, the foliage has practically all developed, offering a good field for the fungous growth.

Seasonal development of the disease

As soon as the first leaves develop in the early summer the plants are susceptible to the attacks of the pathogen. However, due to climatic conditions the disease very seldom appears to any serious extent before the plants are in bloom or after the blossoming season. The temperature which is not so high at that time of the year is most favourable for the growth of the pathogen. The fungus in natural infection develops very slowly; being a weak parasite naturally, its noxious effects are very much retarded by weather conditions. It is during the months of September, October and early November that the damage caused by the disease is most noticeable.

Production of Spores.

The conidia are apparently the disseminators of the disease. Heterosporium spores may be produced during the whole season of growth, favourable conditions prevailing. These spores develop on conidiophores arising from the hyphal mats which have caused the necrotic lesions on the leaves. They are generally produced in the center of the spots but in bad infestations they may be seen covering nearly the entire surface of the spots.

They are very easily broken off from their conidiophores and for this reason are dispersed by the wind with great facility. The writer has not been able to observe on artificially inoculated plants or any medium more than three conidia produced on the same conidiophore.

Tisdale has recorded the production of ascospores during one of the summers he investigated the disease in Wisconsin but the formation of these is so unstable that no large importance is attached to that special stage of the fungus. In Canada, as well as in other countries, the production of these spores is not necessary to assure the propagation of the pathogen.

Viability and longevity of the fungus.

There is but very little data on the subject mentioned. The writer has performed several experiments on the relation of the fungus to various conditions and the results show that the pathogen can resist adverse conditions to a large extent.

Due to their thick wall, spores can resist very cold temperatures. Ramsbottom (16) mentions that spores which had been submitted for several months to winter temperatures germinated with a good percentage when sowed in hanging drops. The writer has collected leaves from the field immediately after the snow had gone and examined the spots for spores; to his surprise some of these were still present on the leaves. Several germination tests were made in hanging drops of tap water and of 5% Glucose solution in Van Tieghem cells at a room temperature of about 24°C. The percentage of germination was found to vary between 52 and 86.

Two cultures of the F_3 generation on potato dextrose agar

were kept outside all winter on the surface of the snow and exposed to the inclemencies of the weather and lowest temperatures At the beginning of April spore germination tests in hanging drops of tap water and of 5% glucose solution were made from these two cultures. In every case a fair percentage of germination was obtained, ranging between 23 and 78%. As checks for these two experiments, germination tests were made in the same kind of media with spores obtained on spots of dried leaves and cultures of potato dextrose agar which were kept in the laboratory all winter at a temperature range of 17°C to 24°C. The percentage of germination ranging between 48 to 93 was somewhat higher than in the two previous cases.

Low temperatures do not kill the mycelium altogether. Young cultures on potato dextrose agar which had begun their growth in the laboratory were placed outdoors for several weeks at a temperature of several degrees below freezing point. When they were taken inside they delayed their growth for two or three days but developed later just as good growth as the cultures kept inside and produced spores as well.

In one of the preceding experiments on the relation of cold temperature to the growth of the fungus the latter has been killed when placed in the cold immediately after being inoculated on the medium. The reason for this is that the fungus had not been given time to start its growth which would have enabled it to withstand low temperatures.

High temperatures unlike low ones kill spores and mycelia more readily. An experiment was made to see whether or not spores could withstand much desiccation. Spores were sowed in

drops of water in the center of cover slips and air dried in the laboratory at a temperature of 21°C. After definite lengths of time drops of water and 5% Glucose solution were placed with a platinum loop over the dried spores. The hanging drops were then turned over Van Tieghem cells and the germination observed. The results of the experiment are as follows:

Table XXVII - Effect of desiccation on the viability of Heterosporium spores.

Medium	Length of desiccation	No. of trials	Positive results	Incubation period
Glucose 5%	15 min.	2	2	3.55 hrs.
T. Water	" "	2	2	3.40 "
" "	20 "	2	2	4.05 "
Glucose 5%	" "	2	1	4.25 "
" "	25 "	2	1	4.45 "
T. Water	" "	2	1	4.50 "
" "	30 "	4	-	
Glucose 5%	" "	4	-	
" "	no desiccation	2	2	3.05 "
T. Water	" "	2	2	3.15 "

From the above figures we may assume that spores are very sensitive to drying temperatures. Wetted spores do not germinate after being desiccated for thirty minutes. On the other hand spores which had been kept for months on dried up media and leaves exposed to the same temperature did germinate.

Two reasons may account for this difference in the percentage of germination. (I) The spores while attached to their conidiophores may be able to withstand a long period of heat

as long as the living plasm of the hyphae reaches them.
(2) The drying of spores immersed in water may cause a thickening and hardening of their wall with the plasmolysis of their protoplasm.

If these reasons are correct the fact has a very practical importance in natural infection. It means that spores falling in drops of water on the leaves have very little chance to germinate once the hot rays of the sun have evaporated the water and heated the spores.

Dissemination of spores.

The spores are very easily disseminated; a rainfall or wind will free them from the leaves and at the same time spread them by the splashing of the rain drops. Faulwetter (91) shows the great possibility of spores in a film of water to be splashed several feet away especially when a wind is blowing. A drop of 0.02 cc. in volume falling 16 feet upon a thin film of water splashed a relative abundance of water to a distance of 8 feet, moderate quantities as far as 12 feet and slight amounts to 16 feet. One can readily understand the possibilities of spores contained in a film of water to be splashed to several feet away and from there farther still. If the height of the fall and the velocity of the wind are taken into consideration the chances for dissemination are very much increased.

The investigations on the dissemination of rust spores by the wind clear away all doubts on a means such as the wind is a powerful carrier of spores. Strong winds bring healthy leaves in contact with diseased ones and thus they become

inoculated, especially when wet with rain or dew.

Another means of dissemination is the grower himself by walking through his infested plantation with carelessness. Insects may possibly carry the disease from one plant to another. With these means the fungus is rapidly disseminated throughout a plantation and from field to field.

Period of incubation.

Several factors influence the length of the incubation period.

1. Different varieties of Iris are more or less susceptible to the organism, the incubation period being therefore longer in some varieties than in others.

2. The mode of inoculation influences the length of the incubation period. The disease takes more time to show on plants which have been naturally infected or simply sprayed with the organism in suspension than on plants which have been inoculated through wounds.

3. Environmental conditions have a marked effect on the period of incubation. Plants kept under bell jars after inoculation showed infection sooner than those which always remained uncovered. In the case of inoculations by means of spraying with spores in suspension without wounding, the writer was never successful in obtaining infection with those plants which were not placed under bell jars for a few days.

Natural infection

Natural infection may take place any time during the growing season. Infection develops very slowly indeed on plants which have not been injured previously. In the Province of Quebec a primary infection may occur in the late spring or

early summer.

There are three possible sources of inoculum for the various infections.

1. Overwintered conidia on either leaves or soil is the first possible source of infection. But on account of the leaves developing so late in the spring this source of inoculum is rather inoffensive. The spores may be destroyed by the adverse climatic conditions which may prevail while they are waiting for a suitable medium to grow on.

2. Another source for primary infection and very likely the most important one is the first crop of conidia produced as soon as the weather is favourable. These conidia are produced on the spots of the old leaves which have overwintered on the ground.

Dead leaves were brought in the laboratory in the spring and the necrotic lesions examined. The microscope revealed the presence of a thick mat of interwoven hyphae formed of large, brown, thick walled cells. Few conidia were found but none were attached to their conidiophores. Some of the leaves were kept in the laboratory under moist chambers at a temperature varying between 20 and 25°C. Three days later numerous conidia had developed in the old spots.

This tends to prove that several crops of conidia may be produced during the growing season under favourable conditions. Conidia produced on the primary lesions serve as inoculum for secondary infection.

The writer has made several observations on the dead leaves in the field during the month of April which was fairly

warm toward the end and has not been able to see any spore formation as yet. He is therefore not in a position to say how early conidia are produced in the spring. However, it is very plausible to say that by the time Irises have developed their first leaves there is a good crop of conidia to cause their infection.

3. A third means of infection is the ascospores. It is regretful that no definite data can be given concerning this kind of infection. However, mention may be made of Tisdale's views on the subject. Due to the fact that ascospores are rarely formed he concludes that under such erratic occurrence the spores play a very minor role in the early infection of new leaves. According to him, sterile perithecia develop tufts of four to twelve conidiophores on the apex or ostiole which later produced conidia. These conidia presumably are similar to the others and may also be a source of infection.

Mode of infection

Ramsbottom states that infection takes place through the epidermis and also through the stomata. Tisdale on the other hand was unable to observe penetration of the tissues otherwise than through the stomata. An experiment was conducted in the laboratory to determine whether or not both means of infection are possible.

Leaves of five different varieties were cut into small parts of two inches long and washed with $HgCl_2$ (1:1000) for three minutes, then dipped into sterile water. These leaf parts were placed on glass slides in sterile Petri plates at the bottom of which sterile water was poured to keep the

tissues moist.

The leaf parts were inoculated with spores (F_3) in suspension in water by smearing the leaf surface with the suspension. The leaves examined six hours later through transmitted light showed that a good majority of spores had germinated but none of the germ tubes had penetrated the tissues apparently. On the following day the leaves were again examined by means of a strong beam of light reflected on the mirror of the microscope through the leaf on the stage. The writer observed then, that some of the germ-tubes had penetrated stomata on the leaf surface (Pl.7 C), of three varieties only namely, Caprici, Walner and Alcazar while no infection could be seen on the varieties Dorothea K. Williamson and Albert Victor.

The experiment was repeated with the last two varieties under the same conditions but negative results were again obtained. Another trial was made by wounding the leaves without any success.

On no occasion was the pathogen seen to penetrate through the epidermis.

Overwintering of the organism

The pathogen may overwinter in a resting state either in the form of thick walled mycelium in the leaves or as spores on the ground which will start to grow or germinate in the spring under suitable conditions. Experiments have shown that both mycelium and spores can stand the coldest winter temperatures of the Province without suffering much injury. It is therefore of the utmost importance that a thorough

cleaning of the plantation should be made in the Fall wherever the disease has been noticed.

Environment in relation to the disease.

The conditions under which the plants are grown together with the cultural practices are factors which influence to a large extent their susceptibility to the disease.

The general opinion has been for a long time that the Iris required a lot of moisture and consequently grew best in wet lands. Fortunately, due to careful investigations and observations of Iris growers, this idea has given place to the contrary, which is true.

The disease is most prevalent in wet and fairly cool seasons during which the disease spreads very rapidly. It would be therefore, of great preference to have the Iris plantation on an elevated land or on a slope toward the South East rather than on a low and wet soil in a shady place. Sunshine should have an easy access to the plants especially if the soil is naturally wet and lacking drainage. Since the fungus is favored in its growth by a wet and rather cool environment while these same conditions are unfavorable to the Iris, one can easily see the great advantages for the fungus to have a rapid growth.

Iris grows practically on all soils, provided that there is a certain amount of lime present in them. Ramsbottom's statement that the disease is prevalent on lime-loving varieties growing in soils deficient of this substance shows the necessity of having this element present in the soil. The most preferable soils for the cultures of Irises are the chalky loams and clay

loams, provided that the latter are given good dressings of lime and chalk.

The atmospheric conditions such as moisture, coolness and winds play a great part in the spreading of the disease. However these conditions can easily be reduced to minimum importance provided that the soil conditions and cultural practices are favourable to the growth of the Iris.

Disease Resistance.

The Bearded Iris seems to be the only group of Iris susceptible to the disease; the Beardless Iris is apparently immune to this disease under natural conditions. From the observations made last Fall in the College plantation the following varieties show different degrees of disease resistance.

Name of Variety	Resistance
Albert Victor	Very resistant
Albicans	Susceptible
Alcazar	Very susceptible
Ambassador	Extremely susceptible
Anne Farr	Very susceptible
Benanciencis	Extremely susceptible
Benlow	Fairly resistant
Bridesmaid	Susceptible
Carthusian	Very susceptible
Cherubin	Extremely susceptible
Chester J. Hunt	" "
Cliny	Fairly resistant
Coquette	Very susceptible
Cretonne	Extremely "
Crusader	" "
Dalmatica	" "
Dawn	" "
Drake	Fairly resistant
Dwarf Purple	Extremely susceptible
Florentina	" "
Flavescens	Very "
Fritzol	Susceptible
Greenish White Falls	Fairly susceptible
Innocenza	Very "
Isoline	Fairly resistant
Kharput	Susceptible
Khedne	"
Mme Chereau	"
Mary Garden	Extremely susceptible

Name of varieties	Resistance
Marengo	Very susceptible
Mandraliscae	Susceptible
Lord of June	Extremely susceptible
Mme Paquet	" "
Morwill	Very susceptible
Neptune	Extremely "
Mrs. Darwin	Susceptible
Lord Seymour	Very susceptible
Nibelungen	Extremely "
Nokomis	" "
Opera	" "
Pallida Dalmatica Spea	Very resistant
Parc de Neuilly	Extremely susceptible
Paxtouny	" "
Pal Glory of Reading	Susceptible
Pumila compacta	Very resistant
Purple King	Susceptible
Oriental	Resistant
Princess Victoria Louise	Extremely susceptible
Rembrandt	Extremely "
Phein Nixe	" "
Shrewsbury	Very susceptible
Swatara	Extremely "
Sweet Lavender	Very "
Rubella	" "
Tamalane	" "
The Bride	" resistant
Tinia honorabilis	Susceptible
Tranelieb	Very "
Trojana	Susceptible
Var Agathe	"
Virshner (Mme Chereau)	Resistant
Violaceae grandifolia	Fairly resistant
White Knight	" "
Wyomissing (Grachus)	Susceptible

There are several possible factors which may combine to make the Beardless Iris immune. Among those factors the writer thought of investigating the following: (I) thickness of the epidermal layer, (2) thickness of the cuticle, (3) Compactness of the cells forming the tissues, and (4) size and number of the plant tissues. The results are tabulated below.

Table XXVIII - Thickness of epidermis and cuticle of several varieties, (microns)

Variety	Thickness of epidermis				Thickness of cuticle			
	Base of leaf	Ave.	Tip of leaf	Ave.	Base of leaf	Ave.	Tip of leaf	Ave.
Dot. K. Williamson	18. -27.3	21.3	17.4-24.3	20	3.1-6.3	4.5	2.8-5.8	4.
Caprici	27.- 46.	31-35	24 -39	29-32	5. -9.	6.5	4.5-7.5	6.
Walner	27. -45.	34-35	27 -45	31-33	5. -10.	6.5	4.5-9.5	6.5
Flavescens	21.1-30.4	24.2	16.8-27.6	21.5	6. -9.3	7.8	5.4-9.3	6.5
Albert Victor	27.4-43.5	35-38	23.8-39.2	32.4	7.2-9.6	8.3	7.2-9.	7.8

Table XXIX - Size and number of stomata in various varieties

Variety	Size of Stomata		Number of Stomata	
	Tip of leaf	Base of leaf	Base of leaf	Tip of leaf
Dot. K. Williamson	34.5-40.5x11.4-13.2	36.-43.5 x 12.-14.5	172	218
Caprici	33 - 45	35 - 45	325	375
Walner	37.5 - 48.3	42.-51.x 36.-39.	176	453
Flavescans	42.1 - 50.4	43.4 - 51.4	134	237
Albert Victor	36.-43.5 x 37.5-45.1	35.8-40.5 x 39.2-45.7	261	427

The figures in these tables show that the high degree of resistance in Dorothea K. Williamson is not due to the thickness of its epidermis or its cuticle, since these are very much thinner on this plant than they are on the susceptible varieties of the group Bearded Iris studied. Resistance is not likely due to the size of its stomata, since these are about equivalent to those on the other varieties. The number of stomata may be a small factor in resistance. The opening of the stomata could have been investigated and as a matter of fact the writer started some work on this but due to lack of time he discontinued. Generally speaking there is no difference in the compactness and size of the cells. However, there are very large air spaces in the leaf of Dot. K. Williamson which the writer has very seldom noticed in the Bearded varieties.

According to the above experiments immunity in Dot. K. Williamson is apparently not due to any of the mechanical factors mentioned except to the number of stomata on the leaf surface which may assist the unknown factors of resistance.

Control Measures

The leaf spot of Iris is a disease relatively difficult to control satisfactorily on account of the heavy bloom covering the Iris leaves and which makes it difficult for a spray mixture to adhere uniformily. Furthermore during the flowering stage of some varieties it is practically impossible to reach all parts of the leaves with a spray solution.

Very little work has been done as to the means of controlling the disease and very little satisfaction has apparent-

ly been obtained. Cooke (3) and Gussow (10) recommended the removal of the dead infected leaves. Ramsbottom (16) advocated the addition of lime to soils deficient in this substance stating that only the lime-loving species were attacked by the disease and these were susceptible only when grown in soils lacking calcium. Tisdale (21) conducted experiments with two methods of control in view, namely:

1. The removal of dead leaves prior to the development of young leaves in the spring.
2. The protection of plants with a coating of some copper fungicide during their development.

The results of his experiments indicate that primary infection was prevented but that the plants were not continuously protected by the spray mixture. However, the best control was obtained by removing all dead debris in the spring before the fungus and the new foliage started to grow.

No work on control has been done in this investigation.

SUMMARY

1. Two fungi may cause Leaf spot of Iris in Quebec, namely, Heterosporium gracile and Macrosporium sp. The former only is concerned with in this manuscript.
2. The range of hosts attacked by Heterosporium gracile is apparently limited to the Bearded Iris group in the field.
3. The disease is common in the South-Western part of the province while practically unknown in the East.
4. An acid toxin is secreted by the fungus; it discolours the tissues and causes as much damage as the mycelium itself.
5. Several organisms were isolated from leaf spots, but Heterosporium gracile was decidedly predominant.
6. Heterosporium gracile and Macrosporium sp. are the only two organisms which, in this investigation, are shown to be definitely pathogenic on Iris in the experimental greenhouse.
7. Heterosporium gracile is a weak parasite; in natural infection and artificial inoculations under certain conditions the pathogen spreads very slowly.
8. The Beardless Iris, Dorothea K. Williamson, was infected by Heterosporium gracile and more so with Macrosporium sp. when infected with a large amount of inoculum in the greenhouse.
9. The factor for resistance in the Beardless group was not definitely determined.
10. For cultural purposes solid media gave best results. Large amounts of media were more favorable to growth than small

amounts.

11. The pathogen grows better on a medium of a hydrogen-ion concentration of about 5.5, tolerating larger concentrations of acid than of alkali.

12. Spores vary but very little in size according to the media on which they grow, while mycelial cells are much affected.

13. The fungus is apparently made sterile when grown on artificial media through several generations, but is regenerated when grown back on Iris.

14. A low temperature is more favourable to the growth of the organism than a high one, but a range of 15°-24°C seems to be preferred. Spore production occurred only in the temperature range above mentioned.

15. Spores and mycelium are more readily killed by high temperatures than by low ones.

16. Growth is just as abundant in the presence or absence of light.

17. Spore germination is affected by the various media being better on solid media.

18. Spore germination is sensitive to temperature but apparently not to the presence or absence of day-light.

19. Spores resist dryness to a large extent as long as they are attached to their conidiophores.

20. The period of incubation is affected by temperature, moisture, amount of inoculum, method of inoculation and the variety of Iris

21. Infection takes place through stomata.

22. The pathogen overwinters in the form of conidia which may germinate in the spring.

BIBLIOGRAPHY

1. Bailey, L.H., (ed): Standard Cyclopedia of Horticulture. 1923.
2. Clarke, W.M., The Determination of Hydrogen - ions. 1920.
3. Cooke, M.C., Iris leaf blotch. Jour.Roy. Hort. Soc.(London) 26: 450- 551. 1901.
4. Copeland, E.B., The Mechanism of Stomata. Ann. of Bot. 16: 327-364. 1902.
5. Correspondence.
6. Dowson, W.J., On two species of Heterosporium, particularly Heterosporium echinulatum. Mycol.Centr.Bd. II, Heft I. Jan. 1913.
7. Drayton, F.L., A Summary of the Prevalence of Plant Diseases in the Dominion of Canada, 1920-24 Dominion Expt.Farms, Bull. 71 N.S. 1926.
8. Eames and McDaniels. Introduction to Plant Anatomy. 1925.
9. Faulwetter, R.C., Jour.Agri. Res. 10: 639-648, 1917.
10. Gussow, H.F., Leaf Spot of Iris. Can.Expt.Farms Rpts.(1911) 1912: 208.
11. Hansen, H.N., A simple Method of Obtaining Single Spore Cultures. Science N.S. 64, Oct. 15, 1926.
12. Heald, F.D., Manual of Plant Disease. 1926.
13. Hoare, A.H., Iris Diseases. Jour. Ministry of Agri. 32: 454-457. Aug. 1925.
14. Hohmel von, Mycologische Fragmente. Ann.Mycol.16, 1918.
15. Parasitic Fungi of New Jersey. N.J. Agri.Exp.Sta. Bull 313, 1917.

16. Ramsbottom, J.K., Iris Leaf Blotch Disease. Jour.Roy. Hort. Soc. 40, April 1915.

17. Saccardo, P.A., Sylloge fungorum 4: 480,

18. Sand, A.W.W., Bearded Iris. A perennial Suited to all Gardens. Cornell Ext. Bull. 112, June 1925.

19. Stevens, F.L., The Fungi Which Cause Plant Diseases. 1913.

20. Stevens, F.L., Plant Disease Fungi, 1925.

21. Tisdale, W.B., Iris Leaf Spot Caused by Didymellina Iridis. Phytopath 10: 148. 1920.

22. Wister, J.C., The Iris. 1927.

ILLUSTRATIONS

Plate I.

Infected Iris plantation at Macdonald College.

A - Row of Iris very badly infected by the disease.

B - Variety of Iris completely killed by the fungus.

C - Very susceptible variety of Iris.

D - Variety showing great resistance to the disease.

E - Row of Iris almost destroyed.

Plate I

fig. 1

fig. 2

fig.3

fig.4

Plate II.

Iris leaves showing coalescence of large spots and the consequent blotching and complete drying of the tissues.

Plate II

Plate III.

Plants inoculated on the same day with Heterosporium gracile and Macrosporium sp.

Fig.1 A - Inoculations through wounds with Heterosporium gracile; the region between the spots is blotched, showing the great susceptibility of the plant to the pathogen compared to B.

B - Inoculations through wounds with Macrosporium sp. Notice the small size of the spots compared to those in A.

C - Check showing no infection.

Fig.2 D,E- Plant equally susceptible to both Heterosporium gracile and Macrosporium sp.

Fig.3 F - Leaf showing the slow spreading of Heterosporium in the tissues when inoculated by spraying.

Plate III

fig. 1

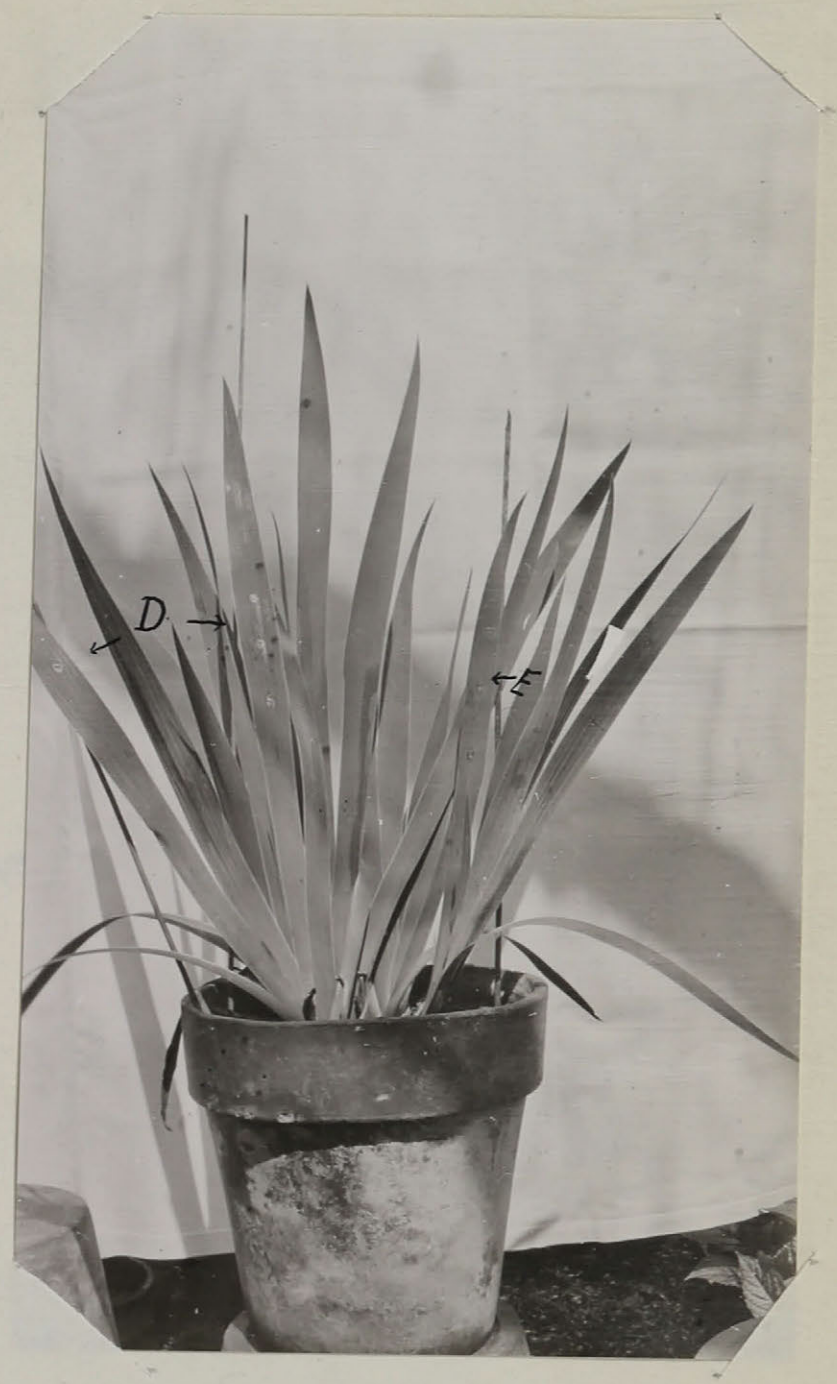


fig. 2

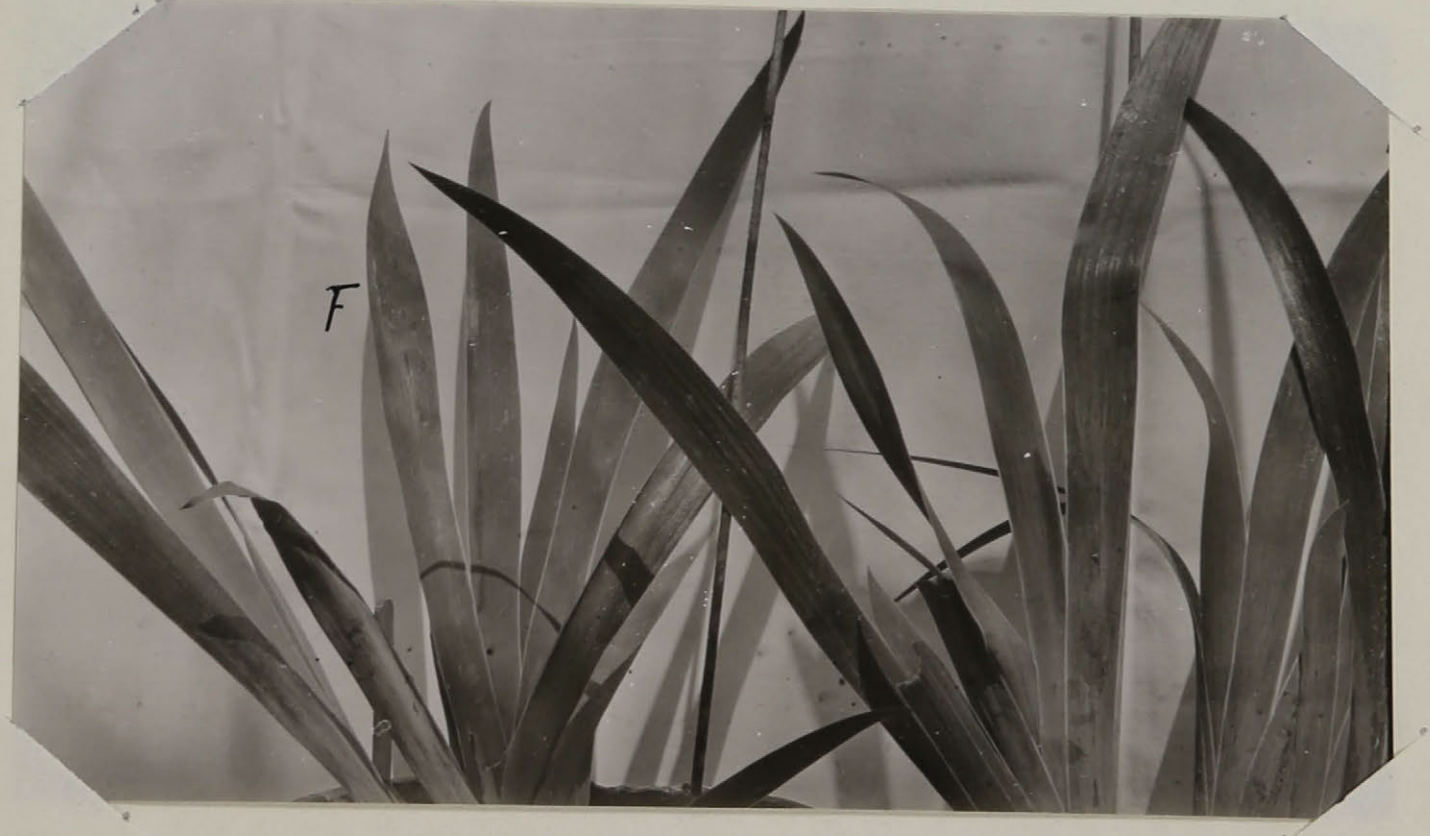


fig. 3

Plate IV.

Magnified spots showing the pimples caused by tufts of conidiophores.

Plate IV

Plate V.

A - Leaf of Dorothea K. Williamson infected with Macrosporium sp.

B - Leaf of Dorothea K. Williamson infected with Heterosporium gracile. Notice the difference in the size of the spots.

C - Leaf of Walner infected with Macrosporium sp. Notice the wounds between the spots showing the checks which remained healthy.

D - Leaf of Walner showing infection with Heterosporium gracile.

Plate V

Plate VI.

Fig. 1, A.B.C. Infection with Heterosporium gracile. Notice the drying and bending of top part of the leaves in the advanced stage of the disease in C.

Fig. 2, Lesions caused by Heterosporium and Macrosporium on Dot. K. Williamson showing the difference in the size of spots.

B. Infection of Dot. K. Williamson with Heterosporium gracile.

E & F. Infection of Dot. K. Williamson with Macrosporium sp.

Fig. 3, G. Infection by spraying with a spore suspension of Heterosporium gracile.

Plate VI

fig. 1

fig. 2

fig.3

Plate VII.—

A - Diagramatic view of a stoma (superior aspect)

B - " " of the same stoma (sub-epidermal aspect)

C - Diagram showing the entrance of the pathogen through stomata.

D - Echinulated spores.

E - Spore diagrams showing the various sizes and number of septa.

F - Deformed spores.

G - Deformities of mycelial cells on Czapek's agar.

H - " " " " " corn meal agar.

Plate VII

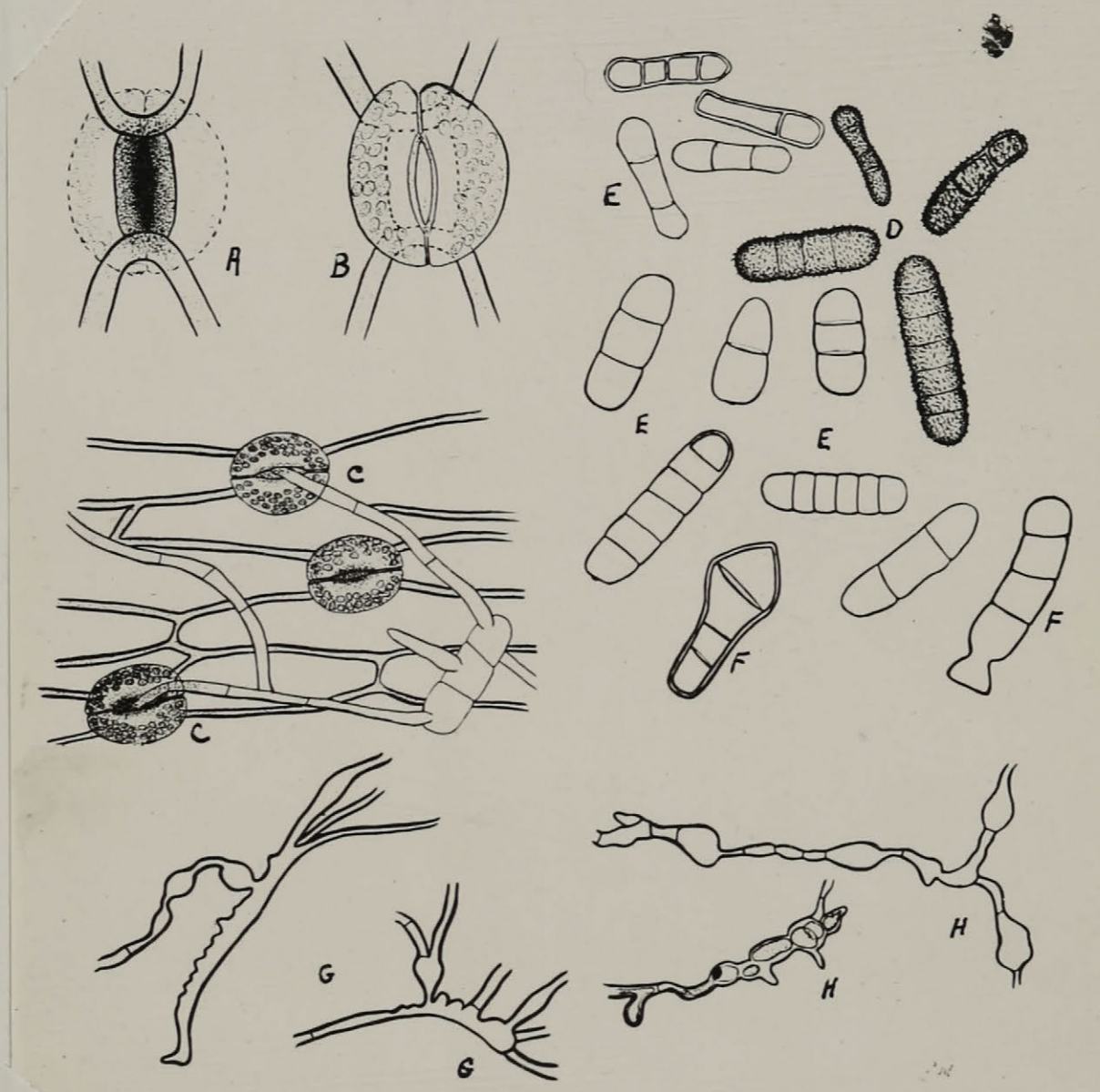


Plate VIII.

A - Section through a spot showing a mass of conidiophores arising from the interwoven hyphae beneath.

B - Section of leaf parenchyma showing growth of mycelium through the cells.

Plate VIII

fig. 1

fig. 2

Plate IX.

A to D - Progressive growth of a conidium on a hypha.

E to L - Progressive growth of conidia on a conidiophore.

M to Q - Germination of a spore in water.

R to V - Germination of a spore in nutrient broth.

Plate IX