# A STUDY OF SOME FUNGAL LEAFSPOT DISEASES OF *DACTYLIS GLOMERATA* IN THE MANAWATU

---

A Thesis Presented in Partial Fulfilment
of the Requirements for the Degree of
Master of Agricultural Science
in the University of New Zealand

---

by
G.C.M. Latch
Massey Agricultural College
August 1957

TABLE OF CONTENTS


| CHAPTER | | PAGE |
|---|---|---|
| I | INTRODUCTION ... ... ... | 1 |
| II | A SURVEY OF FUNGAL LEAF SPOT DISEASES OF COCKSFOOT IN THE MANAWATU | 8 |
| | A. Isolation Techniques ... ... ... | 8 |
| | 1. Surface Sterilisation of Leaf Tissues ... | 8 |
| | 2. Methods Involving the Use of Spore Suspensions ... | 9 |
| | B. Completion of Koch's Postulates ... ... | 10 |
| III | MATERIALS AND METHODS ... ... ... | 14 |
| | A. The Inducing of Sporulation ... ... ... | 14 |
| | 1. Sporulation on the Host ... ... | 14 |
| | 2. Sporulation on Culture Media ... ... | 15 |
| | B. Spore Germination Studies ... ... ... | 15 |
| | 1. On Water ... ... ... | 15 |
| | 2. On P.D.A. ... ... ... | 16 |
| | C. The Effect of Media on Colony Growth ... ... | 17 |
| | Preparation of Media ... ... | 18 |
| | (i) Laboratory Potato Dextrose Agar ... | 19 |
| | (ii) Difco P.D.A. ... ... | 19 |
| | (iii) Difco Prune Agar ... ... | 20 |
| | (iv) Oxoid Milk Agar ... ... | 20 |
| | (v) Malt Agar ... ... | 20 |
| | (vi) Soybean Agar ... ... | 20 |
| | (vii) Oatmeal Agar ... ... | 21 |
| | (viii) Cornmeal Agar ... ... | 21 |
| | (ix) Tomato Juice Agar ... ... | 21 |
| | (x) Water Agar ... ... | 21 |
| | (xi) Carrot Decoction Agar ... ... | 21 |
| | (xii) Cocksfoot Agar ... ... | 22 |
| | (xiii) Sterile Natural Media ... ... | 23 |

CHAPTER PAGE

III contd.

D. The Effect of Temperature on Colony Growth ... 24

E. The Effect of Hydrogen Ion Concentration on Colony Growth 25

F. The Construction of Temperature Cabinets ... ... 27

G. The Preservation of Diseased Leaves ... ... 28

IV A STUDY OF FIVE LEAF SPOT DISEASES AND THEIR CAUSAL FUNGI 29

A. Mastigosporium rubricosum (Dearn. & Barth.) Sprague ... 29

1. Taxonomy and Review of Literature ... ... 29

2. Symptoms ... ... ... 33

3. Spore Studies ... ... ... 33

(a) Sporulation and Spore Description ... ... 33
(b) Spore Germination ... ... ... 38

4. Physiological Studies ... ... ... 40

(a) The Effect of Media on Colony Growth ... 40
(b) The Effect of Temperature on Colony Growth ... 41
(c) The Effect of Hydrogen Ion Concentration on Colony Growth ... ... ... 42

B. Rhynchosporium orthosporum Caldwell ... ... 43

1. Taxonomy and Review of Literature ... ... 43

2. Symptoms ... ... ... 45

3. Spore Studies ... ... ... 46

(a) Sporulation and Spore Description ... ... 46
(b) Spore Germination ... ... ... 47

CHAPTER | PAGE

IV contd.


4. Physiological Studies ... ... ... 48

(a) The Effect of Media on Colony Growth ... 48
(b) The Effect of Temperature on Colony Growth ... 50
(c) The Effect of Hydrogen Ion Concentration on Colony Growth ... ... ... 50

C. Scolecotrichum graminis Fckl.

1. Taxonomy and Review of Literature ... ... 52

2. Symptoms ... ... ... 55

3. Spore Studies ... ... ... 56

(a) Sporulation and Spore Description ... ... 56
(b) Spore Germination ... ... ... 58

4. Physiological Studies ... ... ... 59

(a) The Effect of Media on Colony Growth ... 59
(b) The Effect of Temperature on Colony Growth ... 61
(c) The Effect of Hydrogen Ion Concentration on Colony Growth ... ... ... 62

D. Selenophoma donacis var. stomaticola (Bauml.) Sprague and A.G. Johnson.

1. Taxonomy and Review of Literature ... ... 63

2. Symptoms ... ... ... 67

3. The Possibility of Seed Infection ... ... 68

4. Spore Studies ... ... ... 69

(a) Sporulation and Spore Description ... ... 69
(b) Spore Germination ... ... ...
(i) On P.D.A. ... 74
(ii) On Distilled Water ... 76

5. Physiological Studies ... ... ... 76

(a) The Effect of Media on Colony Growth ... 76
(b) The Effect of Temperature on Colony Growth ... 79
(c) The Effect of Hydrogen Ion Concentration on Colony Growth ... ... ... 79

CHAPTER PAG

IV contd.

E. Stagonospora maculata (Grove) Sprague

1. Taxonomy and Review of Literature ... ... 81

2. Symptoms ... ... ... 82

3. Spore Studies ... ... ... 84

(a) Sporulation and Spore Description ... ... 84
(b) Spore Germination ... ... ... 86

4. Physiological Studies ... ... ... 87

(a) The Effect of Media on Colony Growth ... 87
(b) The Effect of Temperature on Colony Growth ... 89
(c) The Effect of Hydrogen Ion Concentration on Colony Growth ... ... ... 90

V THE SEASONAL SUCCESSION OF EACH DISEASE ... ... 91

A. Field Observations ... ... ... 91

1. General Survey of the Individual Diseases ... 92

(a) Leaf Fleck, caused by Mastigosporium rubricosum 92
(b) Scald, caused by Rhynchosporium orthosporum ... 92
(c) Leaf Streak, caused by Scolecotrichum graminis 92
(d) Eye Spot, caused by Selenophoma donacis var. stomaticola 93
(e) Purple Leaf Spot, caused by Stagonospora maculata 93

2. Observations on a Variety Trial ... ... 94

B. The Effect of Temperature on the Incubation Period and Severity of Attack ... ... 96

1. Results ... ... ... 97

(a) Leaf Fleck ... ... ... 97
(b) Scald ... ... ... 97
(c) Eye Spot ... ... ... 98
(d) Purple Leaf Spot ... ... ... 98

2. Discussion ... ... ... 98

REFERENCES 100

ACKNOWLEDGEMENTS

APPENDIX

LIST OF TABLES


| TABLE | | PAGE |
|---|---|---|
| I | Estimated Percentage Loss Caused by Leafspot Fungi in Washington in 1949 | 4 |
| II | Estimated Percentage Loss Caused by Leafspot Fungi in New York for the Years 1952, 53, 55 | 5 |
| III | Fungal Leafspot Diseases of Grass Species in New Zealand | 6 |
| IV | Comparison of M. rubricosum Spore Lengths on Various Media after Nine Days at 18±1°C. and of Spores from Natural Material | 36 |
| V | Effect of Temperature on "Spore Germination Time" of M. rubricosum in Distilled Water | 38 |
| VI | Effect of Temperature on Germ Tube Length of M. rubricosum in Distilled Water after 24 hours | 38 |
| VII | Effect of Media on Growth Rate of M. rubricosum after Nine Days at 18±1°C. | 40 |
| VIII | Effect of Temperature on Growth Rate of M. rubricosum on P.D.A. after Nine Days | 41 |
| IX | Effect of pH on Growth Rate of M. rubricosum on P.D.A. after Nine Days at 18±1°C. | 42 |
| X | Effect of Temperature on "Spore Germination Time" of R. orthosporum in Distilled Water | 47 |
| XI | Effect of Temperature on Germ Tube Length of R. orthosporum in Distilled Water after 24 hours | 48 |
| XII | Effect of Media on Growth Rate of R. orthosporum after Nine Days at 17±1°C. | 49 |
| XIII | Effect of Temperature on Growth Rate of R. orthosporum on P.D.A. after Nine Days | 50 |
| XIV | Effect of pH on the Growth Rate of R. orthosporum on P.D.A. after Nine Days at 17±1°C. | 51 |
| XV | Effect of Temperature on "Spore Germination Time" of S. graminis on P.D.A. and Distilled Water | 58 |
| XVI | Effect of Temperature on Germ Tube Length of S. graminis on P.D.A. and Distilled Water after Twelve hours | 59 |
| XVII | Effect of Media on Growth Rate of S. graminis after Nine Days at 24°±.5°C. | 60 |

| TABLE | | PAGE |
|---|---|---|
| XVIII | Effect of Temperature on Growth Rate of *S. graminis* on P.D.A. after Nine Days | 61 |
| XIX | Effect of pH on Growth Rate of *S. graminis* on P.D.A. after Nine Days at 24° ± .5° C. | 62 |
| XX | Seasonal Effect on Spore Dimensions of *S. donacis* var. *stomaticola* | 70 |
| XXI | Dimensions of Pycnidia and Pycnidiospores Recorded in Overseas Literature | 70 |
| XXII | Spore Lengths in Summer and Late Autumn of *S. donacis* var. *stomaticola* | 71 |
| XXIII | Analysis of Variation in Spore Lengths of *S. donacis* var. *stomaticola* | 72 |
| XXIV | Comparison of Spore Lengths on Various Media after Nine Days at 24° ± .5° C. | 73 |
| XXV | Effect of Temperature on "Spore Germination Time" of *S. donacis* var. *stomaticola* on P.D.A. | 75 |
| XXVI | Effect of Temperature on "Spore Germination Time" of *S. donacis* var. *stomaticola* on Distilled Water | 76 |
| XXVII | Effect of Media on Growth Rate of *S. donacis* var. *stomaticola* after Nine Days at 24° ± .5° C. | 77 |
| XXVIII | Effect of Temperature on Growth Rate of *S. donacis* var. *stomaticola* on P.D.A. after Nine Days | 79 |
| XXIX | Effect of pH on Growth Rate of *S. donacis* var. *stomaticola* on P.D.A. after Nine Days at 24° ± .5° C. | 79 |
| XXX | Effect of Temperature on "Spore Germination Time" of *S. maculata* on P.D.A. and Distilled Water | 86 |
| XXXI | Effect of Media on Growth Rate of *S. maculata* after Nine Days at 24° ± .5° C. | 88 |
| XXXII | Effect of Temperature on Growth Rate of *S. maculata* on P.D.A. after Nine Days | 89 |
| XXXIII | Effect of pH on Growth Rate of *S. maculata* on P.D.A. after Nine Days at 24° ± .5° C. | 90 |

# LIST OF FIGURES


| FIGURE | | AFTER PAGE |
|---|---|---|
| 1. | Germination of M. rubricosum in distilled water at 19° ± 1°C., time intervals (hours) are measured from the start of the experiment. | 39 |
| 2. | Effect of temperature on colony growth of the five fungi on P.D.A. after nine days. | 41 |
| 3. | Effect of temperature on colony growth of S. maculata on P.D.A. after nine days. | 41 |
| 4. | Effect of hydrogen ion concentration on colony growth of S. maculata on P.D.A. after nine days at 24° ± .5°C. | 42 |
| 5. | Effect of hydrogen ion concentration on colony growth of the fungi on P.D.A. after nine days. | 42 |
| 6. | Germination of R. orthosporum on P.D.A. at 19 ± 1°C., time intervals (hours) are measured from the start of the experiment. | 47 |
| 7. | Germination of S. graminis in distilled water at 24° ± .5°C., time intervals (hours) are measured from the start of the experiment. | 58 |
| 8. | Effect of temperature on germ tube growth of S. graminis on P.D.A. after twelve hours, comparison is made with colony growth on P.D.A. | 58 |
| 9. | Germination of S. donacia var. stomaticola on P.D.A. at 24° ± .5°C., time intervals (hours) are measured from the start of the experiment. | 75 |
| 10. | Germination of S. maculata on P.D.A. at 24° ± .5°C., time intervals (hours) are measured from the start of the experiment. | 86 |

# LIST OF PLATES


| PLATE | | FOLLOWING PAGE |
|---|---|---|
| 1. | Temperature cabinet. | 27 |
| 2. | Preservation of diseased leaves. | 27 |
| 3. | Lesions caused by *M. rubricosum* on leaves of Cocksfoot. | 32 |
| 4. | Conidia of *M. rubricosum* from P.D.A. | 32 |
| 5. | A twenty-one day old colony of *M. rubricosum* grown at 13°C. on P.D.A. | 40 |
| 6. | Lesions caused by *R. orthosporum* on leaves of Cocksfoot. | 45 |
| 7. | Conidia of *R. orthosporum* from Oatmeal agar. | 45 |
| 8. | A twenty-one day old colony of *R. orthosporum* grown at 13°C. on P.D.A. | 49 |
| 9. | Lesions caused by *S. graminis* on leaves of Cocksfoot. | 55 |
| 10. | Magnified view of lesions caused by *S. graminis* to show the characteristic fruiting bodies. | 55 |
| 11. | Fruiting bodies of *S. graminis* from Cocksfoot. | 56 |
| 12. | Conidia of *S. graminis* from Cocksfoot. | 56 |
| 13. | A twenty-one day old colony of *S. graminis* grown at 24°C. on P.D.A. | 60 |
| 14. | Lesions caused by *S. donacis* var. *stomaticola* on leaves of Cocksfoot. | 67 |
| 15. | Magnified view of one of the above lesions to show the pycnidia. | 67 |
| 16. | Lesions caused by *S. donacis* var. *stomaticola* on the flower stalks of Cocksfoot. | 68 |
| 17. | A pycnidium and pycnidiospores of *S. donacis* var. *stomaticola* from a lesion on Cocksfoot. | 69 |
| 18. | A twenty-One day old colony of *S. donacis* var. *stomaticola* grown at 24°C. on P.D.A. | 77 |
| 19. | Lesions caused by *S. maculata* on leaves of Cocksfoot. | 82 |
| 20. | Pycnidiospores of *S. maculata* from P.D.A. | 82 |
| 21. | An eleven day old colony of *S. maculata* grown at 24°C. on P.D.A. | 88 |
| 22. | An enlarged portion of a thirty-eight day old colony of *S. maculata* grown on P.D.A. | 88 |

# CHAPTER I

## INTRODUCTION

New Zealand is unique in that the entire basis of her national economy is based upon livestock-pastoral farming. Of the fortythree million acres in occupation for agricultural and pastoral purposes, seventeen and a half million are of sown pasture and about thirteen and a half million of natural grasslands. The seventeen and a half million acres of sown pasture are down in imported grasses of which approximately one half has been surface sown and the remainder sown on cultivated land with high producing English grasses. These have been selected for such qualities as leaf area and density of leaves, form of the plant, resistance to drought and many other desirable agronomic properties. Regarding the disease factor, there has been no attempt in New Zealand at breeding for resistance to disease with the exception of Blind seed disease of Ryegrass caused by Gloeotinia temulenta (Prill. et Delacr.) Wilson, Noble et Gray.

It has been observed that fungal leafspot diseases of grasses are widespread throughout the country. One can only speculate on the importance of these leafspot diseases with respect to pasture yield. It may well be that losses are not particularly great since only rarely do they attract the attention of the farmer or extension worker. However, according to Chester (1948) the farmer usually is not concerned with a plant disease until losses reach fifteen per cent of the crop, and rarely does he take action until

they amount to twentyfive percent. If this is so, it is reasonable to suggest that fungal leafspot diseases of pasture grasses are of some importance in this country.

Chester (1945) has made a special study of estimating disease losses and calculating their importance. Employing an artificial defoliation technique he was able to obtain evidence of the extent of losses sustained by crops that have lost foliage as a result of disease or insect attack. Wheat was found to have a yield reduction of twenty to twentyeight per cent if one quarter of its functional leaves were lost between rosette and boot stages. Yield reduction was greatest near the mid-season of the plant. This is understandable when one realises that in the early life of the plant the leaves that are removed or reduced are replaced by others, while as maturity approaches the leaves have largely outlived their usefulness. In mid-season the photosynthetic activity is most essential to the storage of food. His experimental work with cereals showed that losses of foliage in mid-season resulted in a very marked yield depression, ranging from thirtyseven to ninetynine per cent. It follows then, that so-called negligible or "trace" damage at this period is in fact quite serious.

As well as the reduction in yield Chester found there was a reduction in the quality of the grain produced. In addition, maturity was considerably delayed.

Jacques (1937) ascertained the effects of different intensities of defoliation on early root growth of ryegrass, cocksfoot and crested dogstail. He found that root deterioration increased with the severity of defoliation. Weinmann (1948) also demonstrated the close relationship between leaves and roots. Thus, foliage-yield

relationship is not entirely a direct one. Reduction in leaf area leads to reduction in root development and in turn to a reduction in water intake. This is reflected in further loss in quantity and quality of yield.

Chester experimented with cereals but his findings are equally applicable to grasses. The grass seed trade is of considerable importance to New Zealand. In 1955, over one and threequarter million bushels of Ryegrass and approximately thirteen million pounds of other pasture seeds were machine dressed in this country. Thus any factor which contributes towards a reduction of quality and quantity of harvested seed must be regarded as of some consequence.

The seed trade, however, is incidental to the primary purpose of growing grass in New Zealand. The maximum yield of dry matter per acre is aimed at in order that the maximum number of stock can be carried per acre. Whereas Chester was concerned mainly with the grain yield, the more important aspect to the pastoral farmer is the foliage yield. A reduction in photosynthetic tissue as caused by leaf spot diseases for example, will affect the formation of fresh leaves as is borne out by the old axiom "leaf makes leaf".

There are many reports indicating the importance of foliage diseases. Tabulated on the following page are two independent estimates of the percentage loss caused by some leaf spot fungi on common pasture grasses.

Table I

Estimated Percentage Loss Caused by Leafspot Fungi in Washington in 1949. (Sprague 1950)[3]

| Host | Pathogen | Percentage Disease Loss |
|---|---|---|
| Timothy | Selenophoma bromigena (Sacc.) Sprague and Johnson | 2.5 |
| | Rhynchosporium secalis (Oud.) Davis | 0.8 |
| | Scolecotrichum graminis (Fckl.) | 2.0 |
| Cocksfoot | S. bromigena | 2.5 |
| | R. orthosporum Caldwell | 1.5 |
| | S. graminis | 2.0 |
| | Mastigosporium rubricosum (Dearn. & Barth) Sprague | 3.0 |
| Brome | S. bromigena | 2.5 |
| | R. secalis | 0.8 |
| | S. graminis | 2.0 |

Table II

Estimated Percentage Loss Caused by Leafspot Fungi
in New York for the Years 1952,53,55.
(Roberts et al. 1952,54,56)

| Host | Pathogen | Percentage Disease Loss First Cut 1952 | 1953 | 1955 | Second Cut 1952 | 1953 | 1955 |
|---|---|---|---|---|---|---|---|
| Timothy | Heterosporium phlei Gregory | 2.6 | 1.0 | 1.4 | 1.7 | 1.0 | 0.5 |
| | Scolecotrichum graminis Fckl. | 0.5 | 0.4 | 0.2 | 0.5 | 0.2 | 0.3 |
| Cocksfoot | S. graminis | 2.0 | 0.4 | 1.5 | 0.6 | 0.6 | 2.0 |
| | Stagonospora maculata (G.) Sprague | 2.0 | 0.8 | 1.5 | 0.4 | 0.4 | 1.5 |
| | Rhynchosporium orthosporum Caldwell | 1.1 | 1.0 | 1.5 | 0.3 | 0.6 | 2.0 |
| Brome | Pyrenophora bromi (Died.) Drechsler | 1.7 | 1.5 | 1.2 | 2.5 | 1.0 | 1.0 |
| | Rhynchosporium secalis (Oud.) Davis | - | 1.0 | 0.5 | - | trace | 1.5 |

Leafspot diseases of grasses also have an effect on the palatability of the pasture. Thus MacVicar and Childers (1955) report that in midsummer the yield and palatability of Dactylis glomerata was seriously affected by Rhynchosporium secalis Caldwell and Scolecotrichum graminis Fckl. Further, Granti (1953) demonstrated that in a crop with fortyfour per cent infection of Scolecotrichum graminis there was an overall loss of nutritive value of approximately 25.22 per cent.

In view of our dependence on pastures, it is surprising that so little work has been carried out in this country on such an important subject. In Table III are presented the recorded fungal leaf spot diseases of grass species in New Zealand.

Table III

| Pathogen | Host | Authority |
|---|---|---|
| Mastigosporium rubricosum (Dearn. & Barth.) Sprague | Dactylis glomerata L. | Brien & Dingley 1951 |
| Phyllachora cunninghamii Syd. | Festuca elatior L. | Sydow 1924 |
| Rhynchosporium secalis (Oudem.) Davis | Agropyron repens Beauv. | Brien 1942 |
| Rhynchosporium secalis (Oudem.) Davis | Hordeum murinum L. | Brien 1942 |

These three organisms comprise the total number of grass leaf spot fungi identified in this country. Compared with the many hundreds of leaf spot fungi recorded overseas, this list is surprisingly small. Most fungi are not restricted to one host and there are cases where over one hundred different species are attacked by the one pathogen. In New Zealand we can cite only one fungus

(R. secalis), as a pathogen of two different grass species. American workers in particular, have devoted much study to this field and comple textbooks have been written on diseases of pasture grasses.

In the majority of cases the control of foliage diseases of pastures is by selection and breeding for resistance. A pre-requisite to any such programme is fundamental knowledge of the diseases involved. It has been observed that in the Manawatu cocksfoot (Dactylis glomerata) in particular expresses a variety of leaf spot symptoms throughout the year, suggesting that a number of pathogens are involved. The overall aim of this research project was to determine what fungi were in fact contributing to the symptom complex. The particular aims were:-

(i) To isolate and prove pathogenicity of fungi causing leaf spot diseases of cocksfoot in the Manawatu.

(ii) To study the symptoms as induced by each pathogen under field and glasshouse conditions.

(iii) To study each causal organism in pure culture.

(iv) To study the seasonal succession of each disease.

(v) To determine for each disease the influence of temperature on: (a) the incubation period (as defined by Walker 1950).
(b) the severity of attack.

# CHAPTER II

## A SURVEY OF FUNGAL LEAF SPOT DISEASES OF COCKSFOOT IN THE MANAWATU

### A. Isolation Techniques

During the year 1956, isolations were made from many leaf spot lesions of cocksfoot. The techniques used may be divided into two categories:-

(1) Surface sterilization of leaf tissues.

(2) Methods involving the use of spore suspensions.

(1) Surface Sterilization of Leaf Tissues.

Small pieces of tissue approximately twenty by ten millimetres were cut from the edges of young lesions and enclosed in a muslin bag, the top of which was secured with a rubber band. The bag was then placed in a 500 millilitre beaker with a coarse wire lid on top and a rubber hose from the tap passed just through the lid. The tap was turned on at a pressure sufficient to cause the bag to be vigorously agitated and so permit thorough surface washing of the tissue pieces. After eight to ten hours the bag was rinsed in sterile water and the diseased tissues removed and dried on sterile filter paper. The pieces were then set out on three plates of Potato Dextrose Agar, generally five pieces to a plate. In the early series of isolations the plates were incubated at 24° Centigrade. On further examination of the literature concerning leaf spot diseases of grasses it was found that several foliage pathogens of cocksfoot grew more rapidly at lower temperatures, in some instances 24° C. being recorded as their maximum growth temperatures. Thereafter plates were kept at room temperature which varied between 17° and 20° C.

Results from this method were poor. Contaminants tended to overgrow the slower growing pathogens and so this method was abandoned in favour of partial sterilization by means of chemicals. Two chemicals were tried, namely a solution of ten per cent sodium hypochlorite and a solution of .001 per cent mercuric chloride. After experimentation with varying times of sterilization the following technique was finally adopted.

The tissue pieces were placed in a muslin bag and pre-soaked in seventy per cent ethyl alcohol for twenty seconds and then transferred to a .001 per cent solution of mercuric chloride for one and a half minutes. The bag was thoroughly washed in tap water, followed by rinsing in sterile water. The tissue pieces were then dried and plated as outlined previously. Results were excellent, bacteria being the only contaminants.

When bacterial contamination did occur it was still possible to obtain the fungus in pure culture. Four van Tieghem cells were set in a Petri plate of P.D.A. while the medium was still liquid. A small agar square with the contaminated fungus was placed inside the van Tieghem cell and the plate incubated at room temperature. With the resumption of growth on this fresh medium the bacteria were restricted by the cell while the fungus grew down through the agar beneath the base of the van Tieghem cell to the surrounding agar.

(2) Methods Involving the Use of Spore Suspensions.

A lesioned area was examined under a binocular microscope and fruiting bodies, if present, were picked off with a sterile needle and transferred each to a separate drop of water. If the spores were contained in pycnidia they were set free by squashing the pycnidium with the tip of a sterile needle. The resulting spore suspension was then streaked across a plate of P.D.A. with a sterile loop. This method was much quicker than partial sterilization of tissues and

enabled individual colonies that developed from widely spaced spores to be transferred to test-tube slopes as stock cultures. As an aid to this procedure, lines were drawn on the bottom of the Petri dishes and the spore suspension streaked along the corresponding agar surfaces. Thus, by examining the plate under the binocular microscope it was possible to follow the spore line and readily locate the germinating spores. Individual spores were gouged out from the agar with a sterile needle and hence monosporous isolates were obtained.

During the initial isolations, both methods were employed in order to verify that the fruiting bodies did belong to the fungus causing the lesion, and not to a secondary invader. Once this had been established the spore suspension method was used wherever possible.

B. Completion of Koch's Postulates

In order to prove the relationship between a species of fungus or bacterium and a particular diseased condition, Plant Pathologists have adopted a set of rules which must be satisfied before any organism is accepted as the specific cause of a disease. These conditions were first stated by Robert Koch in 1882 and are known as Koch's Postulates.

Applied to fungi they are as follows:-

1. The specific fungus must be always associated with the disease.
2. It must be isolated from the diseased area and cultivated in pure culture.
3. The pure culture when inoculated back to a susceptible host must produce the same disease.
4. The specific fungus must be re-isolated in pure culture from the artificially diseased plants and proved identical with the original cultures.

Techniques for obtaining the pathogenic organisms in pure culture have already been described in Chapter I. The fulfilment of Koch's Postulates is described in the following section.

A strong spore suspension in water was made from those fungi which sporulated on culture media. For those fungi which could not be induced to sporulate on media, a suspension of mycelial fragments was prepared. The method consisted of picking off some of the mycelial thatch with a needle and breaking it up into fairly small fragments in a test-tube of sterile water. Ten to twenty small glass beads were introduced into the test-tube and the whole shaken vigorously for half a minute so that the mycelium was further broken up. Before inoculating the plants, a drop of the spore or mycelial suspension was always examined under the microscope so that an idea of the concentration could be gained.

The cocksfoot plants were grown in four inch pots filled with steam sterilized soil and kept in a glasshouse at temperatures ranging between 15° and 22° C. At the time of inoculation the plants varied in age from four weeks to five months so that any effect of age on disease incidence could be noted.

Plants about to be inoculated were first examined for symptoms of disease. This precaution was found necessary because several plants developed lesions of Scolecotrichum graminis (Fckl.) from drifting spores. The leaves of disease free plants were then rubbed between the finger and thumb in order to remove the "bloom". A better take of disease always resulted when this was done.

Two methods of inoculation were attempted. Plants were sprayed with a fine mist of the fungal suspension by means of a "Windex" spray attachment. The second method was to paint a fungal suspension on the leaves with a sterile brush. This latter procedure was slow and tedious and did not give any better results than spraying.

Usually six plants were inoculated with each fungal suspension and three control plants sprayed with pure water. Plants were then placed in a humidity cabinet for fortyeight hours. Shorter periods of high humidity gave a poorer take of disease, whereas longer periods were not advantageous.

The humidity cabinet measured 2 x 2 x 2½ feet and was panelled with glass on the sides and roof. A shallow tin tray covered the floor of the cabinet and was filled with one and a half inches of fresh water. The potted plants were placed in this tray and evapotranspiration quickly raised the atmospheric humidity of the cabinet to one hundred per cent. Cross contamination by water of guttation was avoided by spacing the plants well apart.

After fortyeight hours the pots were removed and placed in the glasshouse where the temperature varied between 15° and 22° C. Plants were examined each day for disease symptoms.

As a result of the above experimental work conducted between November 1955 and December 1956 the following fungi were shown to contribute to the leaf spot symptom complex of cocksfoot in the Manawatu.

Mastigosporium rubricosum (Dearn. and Barth.) Sprague
Rhynchosporium orthosporum Caldwell
Scolecotrichum graminis Fckl.
Selenophoma donacis var. stomaticola (Bauml.) Sprague and A.G. Johnson
Stagonospora maculata (G.) Sprague

Of these fungi, only Mastigosporium rubricosum has been previously recorded in New Zealand as a foliage pathogen of cocksfoot. (Brien and Dingley 1951).

A detailed study of each disease and its causal fungus is presented in subsequent chapters.

# CHAPTER III

## MATERIALS AND METHODS

### A. The Inducing of Sporulation.

Spore morphology and dimensions are essential for the positive identification of a fungus. Hence, the inducing of sporulation is a pre-requisite to the identification and formal study of an organism. It is preferable to obtain spores from the host for when the fungus is grown in pure culture, the composition of the media may have an effect on spore morphology. However, sporulation on host tissue has never been observed for some fungi; in such cases, sporulation on artificial media must suffice. In addition, large quantities of spores are sometimes required for experimental purposes. Thus, it is often very convenient to be able to obtain profuse sporulation on culture media.

(1) Sporulation on the Host.

Difficulty was at first experienced in obtaining spores from some types of lesions. By collecting lesions at all stages of development and subjecting them to various periods of high humidity, the most suitable conditions for maximum sporulation were generally found.

The technique consisted of covering the bottom of a Petri plate with a circle of filter paper. Sufficient sterile water was added to moisten the filter paper and then a sterile glass slide was positioned on it. Leaves with lesions at all stages of development were cut into one inch lengths and placed on the glass slide so that they were out of direct contact with the water. Plates were incubated at room temperature which varied between 16° and 21° C.

One hundred per cent humidity was quickly attained within each Petri dish. The length of time under high humidity varied according to the age of the lesions, thirtysix hours being sufficient in most cases.

(2) Sporulation on Culture Media.

The majority of the fungi studied sporulated readily on most types of culture media. Two of the pathogens, however, proved very loathe to reproduce. Reports from overseas indicated that these two fungi had failed to sporulate on common laboratory media. Special media were described, however, on which limited success had been obtained. In addition, a treatment with ultra-violet light was described for Scolecotrichum graminis (Fckl.). Details of this technique and a modification used in this thesis will be given when dealing with this organism.

B. Spore Germination Studies.

Spore germination experiments were conducted over a range of temperatures in order to determine whether germ tube length and colony growth were correlated. It was also hoped that there would be a link with seasonal influence on disease prevalence.

Germination was determined in water and on P.D.A.

(1) On Water.

A drop of sterile water was placed on a sterile slide and between fifty and one hundred spores were added by means of a needle tip. Too many spores seemed to delay germination. Each slide was deposited in a separate Petri plate and kept at one hundred per cent humidity by virtue of a moistened filter paper on the bottom of the plate. To prevent the slide from becoming wet it was rested on a second slide.

(2) On P.D.A.

A thin film of P.D.A. was spread with a bent glass rod on to a sterile slide just prior to inoculation. This practice was necessary as the agar film dried up very quickly. Spores were placed on the agar either by stroking the surface with a spore laden needle or by applying them in the form of an aqueous suspension using a wire loop. The inoculated slides were kept under high humidity as described previously.

Duplicate plates of water and P.D.A. were set up for each temperature interval.

It has been common practice amongst Plant Pathologists to plot the percentage germination against time. Bonner (1948) pointed out that from the practical angle, it is much more important to know the germination time for the first few spores than to know the average time taken for all spores to germinate. His technique was to record the time taken for the first ten spores to germinate, calling this period the "germination time". Germination was defined as, "that stage of development where the sides of the germ tube are parallel".

In these studies a preliminary trial, consisting of single slides of P.D.A. and water at each temperature, preceded the main experiment. Slides were examined at four hourly intervals so that an estimate of Bonner's "germination time", could be obtained. In the main experiment, slides were examined during the estimated germination time ± one hour. Finally, after a suitable time had elapsed, the germ tube lengths at the various temperatures were measured.

All spore examinations were made with the low power objective of the microscope and spore measurements under high power. Observations were made as quickly as possible and the slides returned to their respective Petri plates to be returned to the appropriate incubators.

Germinating spores were drawn at suitable intervals of time in order that the mode of germination and germ tube branching could be clearly shown. The germination of particular spores could be followed by noting the readings on the two microscope stage scales when the spores were in the centre of the field. All drawings were made with the aid of a camera lucida.

C. The Effect of Media on Colony Growth.

Brancato and Golding (1953) have tested and evaluated the validity of using the diameter of the colony as a reliable indication of growth. They found that the thickness of the substrate did not affect the diameter but that the dry weight of the colony was materially altered. Hence, colony diameter does give a satisfactory measure of fungal growth.

The optimum temperature for colony growth on P.D.A. had been determined in a previous experiment. Ideally, to find the maximum effect of media on growth, all fungi should have been grown at their optimum temperature found on P.D.A.

Mastigosporium rubricosum unfortunately has an optimum temperature below summer room temperature. Thus, over the nine day experimental period, this fungus was kept at 17 ± 1°C. as refrigerated incubators were not available at the time.

It is realised that the optimum temperature on P.D.A. is not necessarily the optimum growth temperature on all media. It should be however, a good indication of the most favourable temperature.

The Petri plate inoculation technique first attempted consisted of cutting a cylinder, ~~forty~~ four millimetres in diameter from a stock culture on a plate of P.D.A. A cork borer proved ideal for the

operation. Results from this method were very unsatisfactory; growth was uneven and depended on whether the plug had been inverted or not. Thus correlation between colonies was poor.

A second technique was therefore attempted and proved eminently successful. M. rubricosum, R. orthosporum and S. donacis var. stomaticola are all prolific spore formers on P.D.A. so a sterilized needle tip was used to transfer a few spores from the stock culture to the experimental plates containing fifteen millilitres of media adjusted to a pH of 5.9. This latter adjustment was necessary so as to cancel out any effect of the hydrogen ion concentration. Sterile normal solutions of hydrochloric acid and sodium hydroxide were used to adjust the pH and all readings were made with the Beckman meter. The use of sterile acid and alkali obviated a second autoclaving with its accompanying effect of altering the pH. The agar surface was touched lightly with the spore laden needle tip, four colonies per plate, and duplicate plates set up at each temperature level. Nine days later, two diameters at right angles to one another were measured on each colony and the average taken.

A slight modification of the above method was necessary for S. graminis and S. maculata because of their poor sporulation on culture. A small area of mycelium, approximately eight to ten millimetres in diameter was picked off the stock culture with a needle. Four such pieces were placed on a Petri plate and two plates set up at each temperature as before. After nine days the colonies were measured and from this figure nine millimetres were subtracted, thus giving the mycelial growth over the experimental period. For want of a name this latter method of inoculation has been called the "Mycelial fragment technique" and will be referred to as such for the

remainder of the investigation.

The inoculum used in all the media experiments was one generation removed from natural material and had been cultured on P.D.A. for fourteen days.

Details of the various media used in this experiment are as follows:-

Preparation of Media

(i) Laboratory Potato Dextrose Agar. (P.D.A.)

| | |
|---|---|
| Peeled potatoes | 200 grams |
| Agar | 12 grams |
| Dextrose | 10 grams |
| Distilled water | 1000 millilitres |

Finely sliced potatoes were boiled in 500 mls. of distilled water for half an hour. After settling, the clear liquid was decanted through muslin and made up to 1000 mls. with distilled water. Agar and dextrose were added, the mixture boiled to dissolve the agar, and then poured into smaller flasks and autoclaved at fifteen pounds per square inch for twenty minutes. This was the standard autoclaving time and pressure for all media.

(ii) Difco P.D.A.

| | |
|---|---|
| Potato infusion | 200 grams |
| Bacto-Dextrose | 20 grams |
| Bacto-Agar | 15 grams |

Thirtynine grams of the mixture were suspended in 1000 mls. of distilled water and boiled to dissolve the medium. It was then autoclaved.

(iii) Difco Prune Agar.

| | |
|---|---|
| Prune infusion | 36 grams |
| Bacto-Agar | 15 grams |
| Distilled water | 1000 mls. |

Twentyfour grams of the mixture were suspended in 1000 mls. of distilled water and boiled to dissolve the medium. It was then autoclaved.

(iv) Oxoid Milk Agar.

| | |
|---|---|
| Bacteriological Yeast Extract | 3 grams |
| Bacteriological Peptone | 5 grams |
| Agar-Agar | 15 grams |

Milk equivalent to ten mls. of fresh milk. Adjusted from pH 7.2 to pH 5.9.

(v) Malt Agar.

| | |
|---|---|
| Malt extract | 20 grams |
| Agar | 25 grams |
| Distilled water | 1000 mls. |

(vi) Soybean Agar.

| | |
|---|---|
| Ground Soybeans | 100 grams |
| Agar | 17 grams |
| Distilled water | 1000 mls. |

The soybeans were soaked in 1000 mls. of tepid, distilled water for thirty minutes. They were steamed for thirty minutes and then filtered through muslin and the volume restored to 1000 mls. The agar was then added, the whole steamed and then autoclaved.

(vii) Oatmeal Agar.

| | |
|---|---|
| Oatmeal | 42 grams |
| Agar | 15 grams |
| Distilled water | 1000 mls. |

The oatmeal was cooked in fifty mls. of distilled water in a water bath at 60°C. for one hour, filtered through muslin so that a clear liquid was obtained. The liquid was boiled, the agar added and steamed till the agar melted. The whole was then autoclaved.

(viii) Cornmeal Agar.

| | |
|---|---|
| Cornmeal | 42 grams |
| Agar | 12 grams |
| Distilled water | 1000 mls. |

Prepared the same way as Oatmeal agar.

(ix) Tomato Juice Agar.

| | |
|---|---|
| Tinned tomato juice | 200 grams |
| Agar | 20 grams |
| Distilled water | 1000 mls. |

(x) Water Agar.

| | |
|---|---|
| Agar | 12 grams |
| Distilled water | 1000 mls. |

(xi) Carrot Decoction Agar. (Kilpatrick and Johnson 1956).

| | |
|---|---|
| Carrot leaves | 300 grams |
| Agar | 12 grams |
| Distilled water | 1000 mls. |

Three hundred grams of carrot leaves were finely ground and added to 500 mls. of distilled water. The material was steamed without pressure for one hour and then strained through two layers of

cheesecloth. The strained decoction was added to 500 mls. of distilled water containing twelve grams of dissolved agar, and the volume was adjusted to one litre with distilled water.

(xii) Cocksfoot Agar. Bollard's method (1950) modified.

| | |
|---|---|
| Cocksfoot leaves | 100 grams |
| Agar | 15 grams |
| Distilled water | 1000 mls. |

One hundred grams of green, disease-free cocksfoot leaves were chopped up and extracted in 1000 mls. of distilled water by steaming for three hours. The resulting extract was filtered and made up to a litre again. Fifteen grams of agar were added and after autoclaving the pH adjusted to 5.9.

This medium was more dilute than that of Bollard's because approximately 300 mls. of distilled water were added to the filtrate to make the quantity up to 1000 mls. again. Bollard added the agar only.

When this medium was first prepared it was thought that the volatile compounds driven off during the steaming may have been advantageous to growth. A double quantity was therefore prepared, one litre being steamed in an ordinary round flask and the other litre in a flask fitted with a condenser head so that the volatile compounds were returned.

No difference could be detected in growth of colonies between these two media. Hence, the condenser head was omitted in further cocksfoot preparations.

(xiii) Sterile Natural Material.

Cocksfoot leaves

Carrot Leaves

Pea Stems.

The only method of sterilization so far mentioned was by heat. The one serious disadvantage to sterilization by heat is that the chemical composition of the material may be profoundly altered.

Hansen and Snyder (1947) describe a method whereby natural material such as stems and leaves can be sterilized by fumigants instead of by heat. In this way the composition of the material remains unchanged. In addition, the advantages of using natural host tissue for culturing fungi are many. Snyder and Hansen (1947) discuss them with particular reference to the inducing of sporulation.

Hansen and Snyder's method of gaseous sterilization was modified and used in this investigation. The material to be sterilized was cut up into pieces no larger than thirty millimetres in length and put into small jars with tightly fitting lids. Into each jar a few square inches of moistened filter paper were dropped. This was to make sure that the material was not too dry although the precaution was probably unnecessary as the efficacy of the particular gas used in this experiment was not reduced by a low humidity. Hansen and Snyder used either ethylene or propylene oxide as the fumigant. Neither of these two gases was available so methyl bromide was used instead. Methyl bromide has a boiling point of 4.5°C.; hence a twenty cubic centimetre ampoule was placed in the freezing compartment of the refrigerator and cooled down to minus 10°C. It was then quickly removed, the tip broken with a pair of pliers

and the liquid poured into the jars. About one cc. of liquid was added for every 100cc. jar capacity. A second person immediately screwed down the jar lids so that the escape of gas was minimised. Methyl bromide is a very poisonous chemical and so the operation was carried out in a large, well-ventilated room. After twentyfour hours a little of the material was transferred, with the aid of sterile forceps, to sterile Petri dishes and then covered with 15-20 mls. of water agar. Plates were ready for use immediately the agar had hardened.

In general, sterilization was good. However, sometimes it was not one hundred per cent effective and the sterilization had to be repeated. The method outlined above was wasteful because a great deal of the fumigant vaporized on contact with the air. The use of some special type of closed circuit system would be an advantage if larger quantities of material required sterilizing.

Observations were made on the relative growth rates on various media, and also on appearance and intensity of sporulation, and spore dimensions.

D. The Effect of Temperature on Colony Growth

The medium used throughout these temperature experiments was P.D.A., prepared in the laboratory from potatoes and adjusted to pH 5.9 by the addition of normal hydrochloric acid and normal sodium hydroxide. The range of temperatures used were 5° - 36°C.

Large numbers of Petri plates were required for this experiment and so all the temperature recordings could not be run concurrently. Some difficulty was experienced in obtaining sub-room temperatures during the summer and hence some of the recordings had to be left

until the late autumn. This prolongation of the over all experiment was unfortunate because a fresh batch of P.D.A. had to be prepared. Growth on Difco P.D.A. varied from that on laboratory P.D.A. but would have given continuity between recordings over this lengthy period.

Plates containing fifteen millilitres of P.D.A. were inoculated in an identical manner to that for the media-growth experiment. Duplicate plates were incubated at each particular temperature for nine days before measuring the diameters. A time interval of nine days was chosen because it was a convenient compromise between the slower growing fungi becoming large enough for accurate measuring and the quickest growing fungus becoming too large for four colonies to fit on a plate without interference.

## E. The Effect of Hydrogen Ion Concentration on Colony Growth

Difficulty was encountered when altering the pH of the media in that the pH markedly changed after the final autoclaving, particularly in the more alkaline values. In addition, hydrolysis of the agar occurred in pH levels below 4.0 and hence they did not gel.

The method finally evolved is outlined below. Two litres of Difco P.D.A. were prepared and one hundred and fifty millilitre aliquots were poured into eleven 250 ml. conical flasks, autoclaved, and then placed in a waterbath at 50°C. Twenty ml. amounts of P.D.A. were poured into ten 30 m. beakers and placed in a waterbath held at 45°C. to prevent solidification of the agar. A measured amount of normal hydrochloric acid was run out from a

burette into one of the 20 ml. samples of P.D.A. and stirred vigorously. The temperature of the P.D.A. and acid was then allowed to fall to 28°C. and at this temperature the pH was read with a Beckman meter. Varying amounts of normal hydrochloric acid or tenth normal sodium hydroxide were run into the P.D.A. samples and a titration curve was built up. This rather cumbersome method of using separate P.D.A. samples each time was necessary because at a temperature of 28°C. the agar was beginning to solidify and a fresh addition of acid or alkali could not be mixed with it. If a higher temperature than 28°C. were used there would have been a danger of cracking the fine glass electrode. From this titration curve the amounts of acid or alkali required to give a range of values at approximately 0.5 unit intervals were estimated. Before adding the hydrochloric acid or sodium hydroxide both solutions were heated to approximately 90°C. and kept at this temperature for 15 minutes. This procedure was found necessary as a fungal contaminant occurred in the unheated alkali. These estimate amounts were added as quickly as possible to the aliquots of P.D.A. and then a ten millilitre sample was removed from each flask in order to determine the true hydrogen ion concentration. From each aliquot, eight plates, each containing 15 mls. of P.D.A. were poured for each level.

This method proved very satisfactory, estimated and final values being of approximately the same order. Contamination of plates was also very low.

## F. The Construction of Temperature Cabinets

These cabinets were a modified form of the temperature cabinets used in the Department of Plant Pathology, at the University of Wisconsin. The American cabinets measured 3 x 2 x 2½ feet, were covered with R-V-Lite, and consisted of four frames put together with screws to facilitate dismantling when not in use. The temperature was maintained by means of a 500 watt strip heater controlled by a thermo-switch.

A cabinet of these dimensions was constructed and covered with Windolite (an opaque, plastic re-inforced sheeting material). Heat was supplied by three 100 watt bulbs which rested on the floor of the cabinet. The plants were supported on a low fibrolite table.

Various thermostats were experimented with, the one giving the most sensitive temperature control being a TS2 Sunvic thermostat. This thermostat was then placed in various parts of the cabinet in order to find the region of maximum sensitivity. The minimum variation found was 2° Farenheit and this occurred when the instrument was placed just above the floor of the cabinet by the door.

The original cabinet had a folding blind of R-V-Lite instead of a door. The blind was found to be unsatisfactory as too much heat was lost around the edges and so it was replaced by a door, hinged at the top.

On experimentation with this modified cabinet it was found that "hot spots" occurred on the table just above the bulbs.

In addition, the cabinet was rather large and cumbersome.

Two smaller cabinets measuring 2'2 x 2'2 x 2' were constructed. To eliminate the hot spots, the table was covered above and below with fibrolite and the intervening space filled with vermiculite as

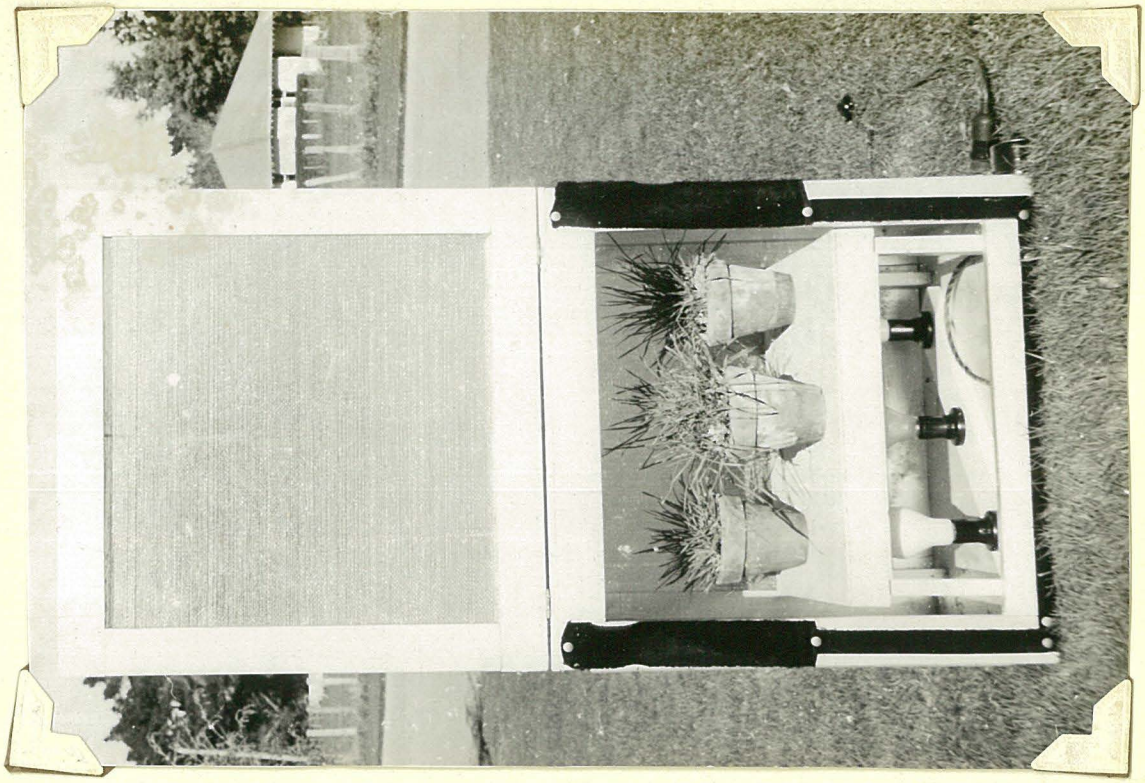


PLATE 1. Temperature cabinet with pots of Cocksfoot.

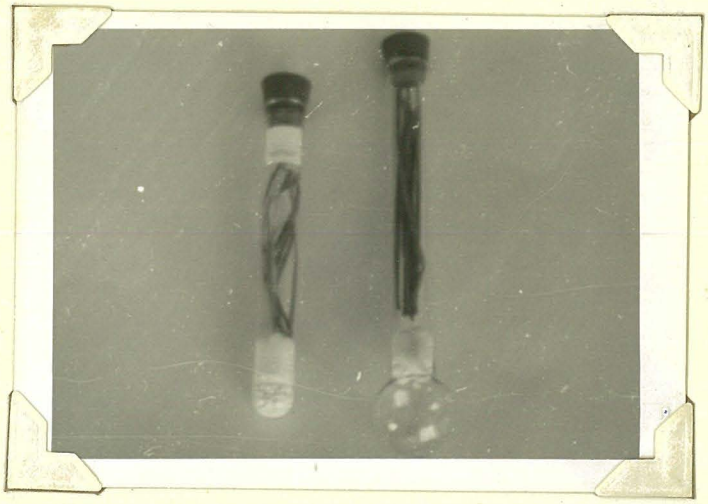


PLATE 2. The preservation of diseased leaves.

as an insulating medium. To make a better seal at the door, strips of black velvet were tacked on to the frame.

These latter two cabinets were very successful. A temperature of 28°C. could be maintained, even when the outside temperature dropped to 10°C.

G. The Preservation of Diseased Leaves

When first isolating out pathogenic fungi from lesions it was found necessary to keep some specimens of the lesions so that experience could be gained in identifying the causal organism in the field.

Diseased cocksfoot leaves were cut into six inch lengths and placed in special humidity tubes. These tubes were originally used for culturing anaerobic bacteria and measured nine inches in length and two inches in diameter and were widened into a bulb at the bottom. This bulb was one quarter filled with water and then a plug of cotton wool was pushed down the tube until positioned just above the bulb and held there by three glass projections. This plug served to keep the leaves out of contact with the water but allowed the humidity in the tube to be kept at a high level. When stored at 6-10°C. in the refrigerator, leaves kept fresh and natural for three to four weeks.

A second method was developed for preserving diseased leaves over a longer period. Large test-tubes were procured and one inch of powdered calcium chloride poured into them. A plug of cotton wool was forced down the tube to keep the chemical in position and then the leaves were placed in the tube and the rubber stopper fitted. Tubes were kept at 6°-10°C. in the refrigerator. With this method the leaves dried out fairly quickly and could be kept for an indefinite period.

# CHAPTER IV

## A STUDY OF FIVE LEAF SPOT DISEASES AND THEIR CAUSAL FUNGI

### A. Mastigosporium rubricosum (Dearn. and Barth.) Sprague.

#### (1) Taxonomy and Review of Literature.

The first record of a leaf spot disease caused by a species of the genus Mastigosporium was that by Fresenius (1852) when he described Mastigosporium album on several grasses. He noted that the conidia were 4- or 5-septate and bore one or more filiform appendages at their distil ends. Lindau (1907) has recorded this fungus on no fewer than nineteen species, but Sprague (1938) does not regard all these records as valid.

Sprague (1938) studied Mastigosporium leaf spots on Gramineae and compared the causal organisms with regard to their occurrence in nature, morphology, and pathogenic capability. As a result of this work he found that in addition to M. album with its appendaged conidia, there was a non-appendaged variety. By virtue of spore morphology and dimensions, colony characteristics and host range, Sprague recommended that these be differentiated as two distinct species. He noted that the earliest, though brief description of the non-appendaged organism was by Ellis and Davis in 1903, who named it M. album var. calvum Ellis and Davis. Sprague (1938) agreed with this description but considered the differences sufficient to warrant elevation of M. album var. calvum Ellis and Davis to specific rank and proposed the name M. calvum (Ellis and Davis) Sprague.

Dearness (1917), recorded Fusoma rubricosa Dearn. and Barth. as a new foliage pathogen of cocksfoot in North America, and Wakefield in 1918 recorded M. album var. muticum Sacc. as a new British fungus also on cocksfoot. However, from the descriptions of spore morphology, Sprague (1938) considered these two fungi as being identical with his species M. calvum. In this paper he listed the other synonyms so that the complete list is as follows;-

Mastigosporium album var. calvum Davis (1903)
M. album var. muticum Saccardo (1911)
Fusoma rubricosa Dearness (1917)
Amastigosporium graminicola Bondarzeva-Monteverde (1921)
Amastigis graminicola Bondarzeva-Monteverde (Clements) 1931.

In 1940 Sprague altered the name M. calvum to Mastigosporium rubricosum for, according to articles sixteen and sixty of the present International Rules of Botanical Nomenclature (1935), "varietal names raised to specific rank are not valid when a specific epithet is available". Hence the name now becomes Mastigosporium rubricosum (Dearn. and Barth.) Sprague.

Sprague also reported in this paper his finding of a third species of Mastigosporium on Bromus vulgaris (Hook). The spores differ from those of M. rubricosum in that they are not only much smaller but are regularly cylindrical instead of navicular or elliptical. He proposed the name Mastigosporium cylindricum Sprague, sp.nov. and gave a formal description of it.

Bollard (1950) made a general study of the genus Mastigosporium and confirmed Sprague's observation that the species Mastigosporium probably exists in different forms. Bollard found that the host range of his isolates of M. rubricosum was

not the same as those recorded by Sprague. However, he postulated that these differences may be due to differences in the genetic constitution of the host material and that it would be necessary to inoculate the same host material with both isolates to determine the true identity.

Sprague (1950) listed both Dactylis glomerata and Agrostis stolonifera as hosts of M. rubricosum. Bollard (1950) believed that isolates from these two grass species are distinct forms of the one species. He therefore proposed that since these differences are accompanied by slight changes in morphology, the name M. rubricosum should be retained for the fungus attacking species of Dactylis, and that the fungus attacking Agrostis stolonifera be designated as a new variety, M. rubricosum var. agrostidis.

No perfect stage has yet been found for any of the species. Fuckel (1869) suggested that M. album and Dilophospora alopecuri might be two stages of the one fungus. However, neither Sprague (1938), Sampson and Western (1939), nor Bollard (1950) could find any connection between these two fungi.

M. rubricosum is widespread over most of Europe and North America where it is reported to cause severe damage in some seasons, (Sprague 1950 and Creelman 1956). Stapledon and Jenkins (1922), Sampson (1924) and Bollard (1950) reported it causing considerable damage to Alopecurus pratensis in Britain, particularly in the late autumn and early spring. Sprague (1938) mentions that leaf damage varied from one to six per cent on Agrostis species in the U.S.A. M. rubricosum has also been reported by Sprague (1938, 1946, 1950), Stapledon and Jenkins (1922), Sampson and Western (1942) and Bollard (1950) as the cause of a serious leaf spot of Dactylis

glomerata. Observations in various parts of New Zealand by the writer tend to show that it is prevalent for only a very limited period of the year in this country.

Sprague (1950) lists twelve hosts of M. rubricosum of which seven are Agrostis species. Four new hosts have since been listed by Sprague (1955) all of which were found in Alaska. Details of these new hosts are given in Appendix I.

The perfect or sexual stage of reproduction has never been found for M. rubricosum. It is, therefore, classified in the Fungi Imperfecti by Clements and Shear (1931) as follows:

Fungi Imperfecti

Moniliales - conidia are neither borne in pycnidia nor acervuli but on conidiophores, either discreet or in groups.

Moniliaceae - hyphae hyaline or bright coloured, loose and cottony, rarely fasciculate; sterile and fertile hyphae or conidiophores both present as a rule, the latter differentiated by means of vescicles, whorls, basidia, sterigmata, etc. conidia concolourous, that is, hyaline or bright coloured.

Micronemeae - hyphae very short and little different from the conidia.

Mastigosporium - conidia not catenulate, ciliate at apex and upper septum, conidiophore not inflated, often obsolete.

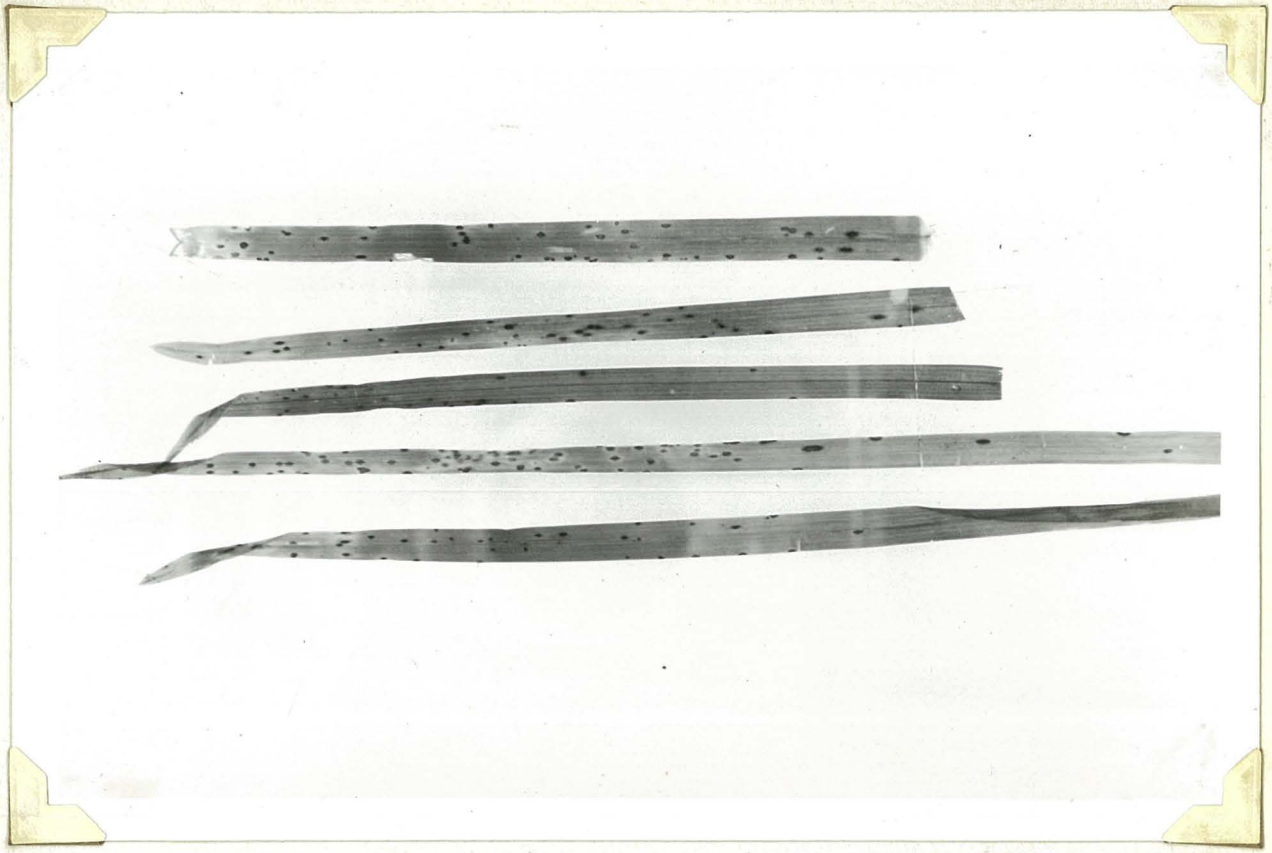


PLATE 3. Lesions caused by M. rubricosum on leaves of Cocksfoot.

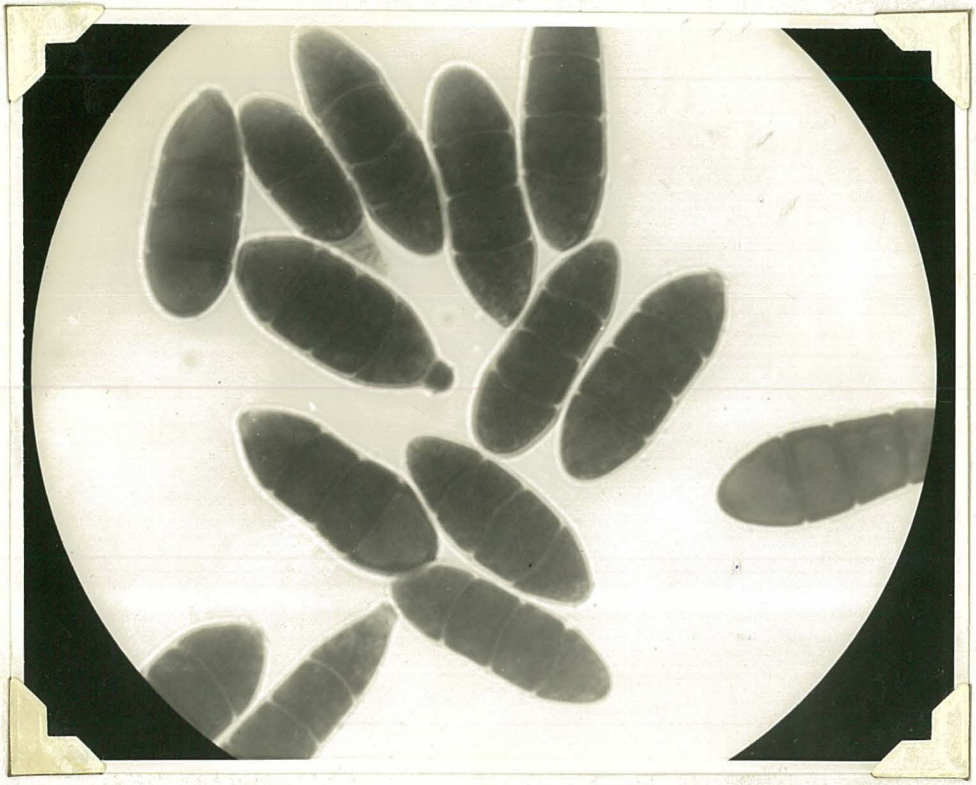


PLATE 4. Conidia of M. rubricosum from P.D.A., magnification, approximately 750.

## (2) SYMPTOMS

Diseased leaves are first covered with numerous dark brown to purple flecks with a water-soaked region surrounding them. As the lesions become older they increase in size up to 12 x 5 millimetres. (See Plate 3 .). Mature lesions are almost black in colour with a thin yellow-brown halo, while most have a small cream coloured area in the centre from which a single crop of spores is produced on conidiophores. Macroscopically they appear as a silvery mass which could at first be mistaken for the dead cuticle splayed back by the pathogen. These conidia are best seen on fresh lesions in the early morning when dew is present. On severely diseased leaves the individual flecks coalesce so that considerable reduction of photosynthetic tissue occurs.

Lesions caused by M. rubricosum are rather similar to those of Stagonospora maculata especially in the younger stages. However, as the lesions increase in size the symptoms may be distinguished since those of the former lighten in colour at the centre whereas the lesions caused by S. maculata remain dark throughout.

The diseased condition caused by M. rubricosum is commonly referred to as Leaf Fleck or Eye Spot.

## (3) Spore Studies

### (a) Sporulation and Spore Description.

It was found that the best time to collect Mastigosporium spores was early in the morning with the dew still on the young lesions. Alternatively, leaves with very young lesions were subjected to periods of high humidity in Petri plates.

Bollard (1950) records that M. rubricosum produces one crop

only of conidia. Observations on several occasions confirmed this statement.

The conidiophores are short, 11.6 - 16.5μ in length, single, and form the conidia by expansion of the distil portion and eventual abscission. The conidia are hyaline, elliptical, generally 3-septate with constrictions at the septa. A few spores are 2- or 4-septate. (Plate 4.).

Fifty spores, produced under conditions of high humidity in a Petri plate from naturally diseased material were measured. Their dimensions were as follows:-

| | | |
|---|---|---|
| Length | Mean | 45.3μ S.D. 2.4μ |
| | Mode | 44.6μ |
| | Range | 38.0 - 52.8μ |
| Width | Mean | 15.6μ |
| | Mode | 14.9μ |
| | Range | 11.6 - 16.5μ |

Dimensions of spores from natural material are recorded in overseas literature as follows:-

| | Sprague (1950)[1] | Bollard (1950) |
|---|---|---|
| Length | 29 - 60μ | 31 - 59μ S.D. 5.38μ |
| Width | 9 - 17μ | 10 - 14μ |

It is important to state the environmental conditions under which the spores are produced. Welles (1924) was one of the first to point out that the environment can greatly influence spore dimensions. He records that - "fruiting structures in the form-genus Cercospora were 50 - 150 per cent longer in the rainy season". Hence, it is quite possible that the production of spores under

conditions of high humidity may produce spores of significantly different dimensions from those in the field.

Likewise, when recording the dimensions of spores on artificial media, details of the composition of the substrate, age of the culture, the temperature at which it is grown, and perhaps the pH, should be given.

Measurements of spore lengths were taken in conjunction with an experiment determining the effect of media on colony growth. The inoculum was one generation removed from natural material and was grown for fourteen days on P.D.A. at 17°C. Each batch of media was adjusted to pH 5.9, inoculated, and then incubated at 17°C. for nine days. Fifty spores from each type of medium were measured under high power, being mounted in a drop of lactophenol acid fuchsin. The resulting data were analysed using Duncan's Multiple Range Test, (1955).

TABLE IV

Comparison of M. rubricosum of Spore Lengths on Various Media after Nine Days at 18°±1°C. and of Spores from Natural Material.

| Spore Length | Prune | Natural | Difco PDA. | Cornmeal | Oatmeal | Water |
|---|---|---|---|---|---|---|
| 20 | | | | | | 6 |
| 21 | | | | 1 | | 5 |
| 22 | 1 | | | 1 | | 9 |
| 23 | 2 | 1 | 1 | 1 | 3 | 13 |
| 24 | 1 | 2 | 3 | 4 | 5 | 6 |
| 25 | 4 | 5 | 8 | 6 | 4 | 7 |
| 26 | 6 | 5 | 6 | 3 | 11 | 2 |
| 27 | 8 | 13 | 11 | 10 | 12 | 2 |
| 28 | 7 | 9 | 6 | 11 | 5 | |
| 29 | 10 | 11 | 5 | 5 | 6 | |
| 30 | 9 | 1 | 7 | 5 | 3 | |
| 31 | 2 | 2 | 3 | 1 | 0 | |
| 32 | | 1 | | 2 | 1 | |
| n = | 50 | 50 | 50 | 50 | 50 | 50 |
| $\bar{X}$ = | 27.64 | 27.44 | 27.28 | 27.16 | 26.70 | 22.94 |

Analysis of Variance

| Source | df. | ss. | MS. | F |
|---|---|---|---|---|
| Total | 299 | 63150 | | |
| Between Media | 5 | 61896 | 12379 | 2902.2 xxx |
| Error | 294 | 1254 | 4.265 | |

$$S.E. = \sqrt{\frac{E.M.S.}{\text{number of items in the mean}}} = .2920$$

| | (2) | (3) | (4) | (5) | (6) | |
|---|---|---|---|---|---|---|
| 1% level | 3.71 | 3.86 | 3.98 | 4.06 | 4.11 | Significant studentized ranges. |
| | 1.083 | 1.186 | 1.162 | 1.200 | 1.162 | Shortest significa ranges (SSR x SE.) |

| Media | Prune | Natural | Difco PDA. | Cornmeal | Oatmeal | Water. |
|---|---|---|---|---|---|---|
| Mean | 27.64 | 27.44 | 27.28 | 27.16 | 26.70 | 22.94 |
| Mean in microns | 45.60 | 45.28 | 45.01 | 44.81 | 44.06 | 37.85 |

N.B. Any two means not underlined by the same line are significantly different.

Any two means underlined by the same line are not significantly different.

It can be seen from the above table that spores on all media were significantly greater at the one per cent level than those on water agar. Differences in spore length between the other media were not significant.

At the five per cent level spores on Prune were significantly greater in length than those on Oatmeal and Water agars. Differences between other media, except Water agar, were not significantly different.

(b) Spore Germination.

TABLE V.

Effect of Temperature on "Spore Germination Time" of M. rubricosum in Distilled Water

| Temperature in degrees centigrade | 5 | 11 | 19 | 24 | 28 |
|---|---|---|---|---|---|
| Germination time in hours | 36 | 24 | 4 | 18 | 24 |

It is clear from the above table that the optimum temperature for spore germination is in the region of 19°C. Prior to this experiment the effect of temperature on colony growth had been determined on P.D.A. The optimum temperature for growth of M. rubricosum colonies was found to be in the region of 11°C. Thus there is no positive correlation between germination time and rate of colony growth for this fungus.

TABLE VI.

Effect of Temperature on Germ Tube Length of M. rubricosum in Distilled Water after 24 hours.

| Temperature in degrees centigrade | 5 | 11 | 19 | 24 | 28 |
|---|---|---|---|---|---|
| Germ Tube length in microns | nil | trace | 61 | 12 | trace |

The length of the germ tubes after twentyfour hours again demonstrates that the optimum temperature for germination is in the vicinity of 19°C. and bears no relation to the optimum temperature for colony growth on P.D.A.

At a temperature of 19°C. the appearance of the germ tube was preceded by a swelling of the spore, generally three to four hours after preparing the spore suspension. One or two stout germ tubes were produced from the segments, not necessarily from the terminal ones. On some spores they soon branched profusely, while on others they remained as a straight tube for twelve to eighteen hours. Germ tubes which branch at an early stage in germination were irregular in shape and appeared to contain large vacuoles. (Fig. 1.).

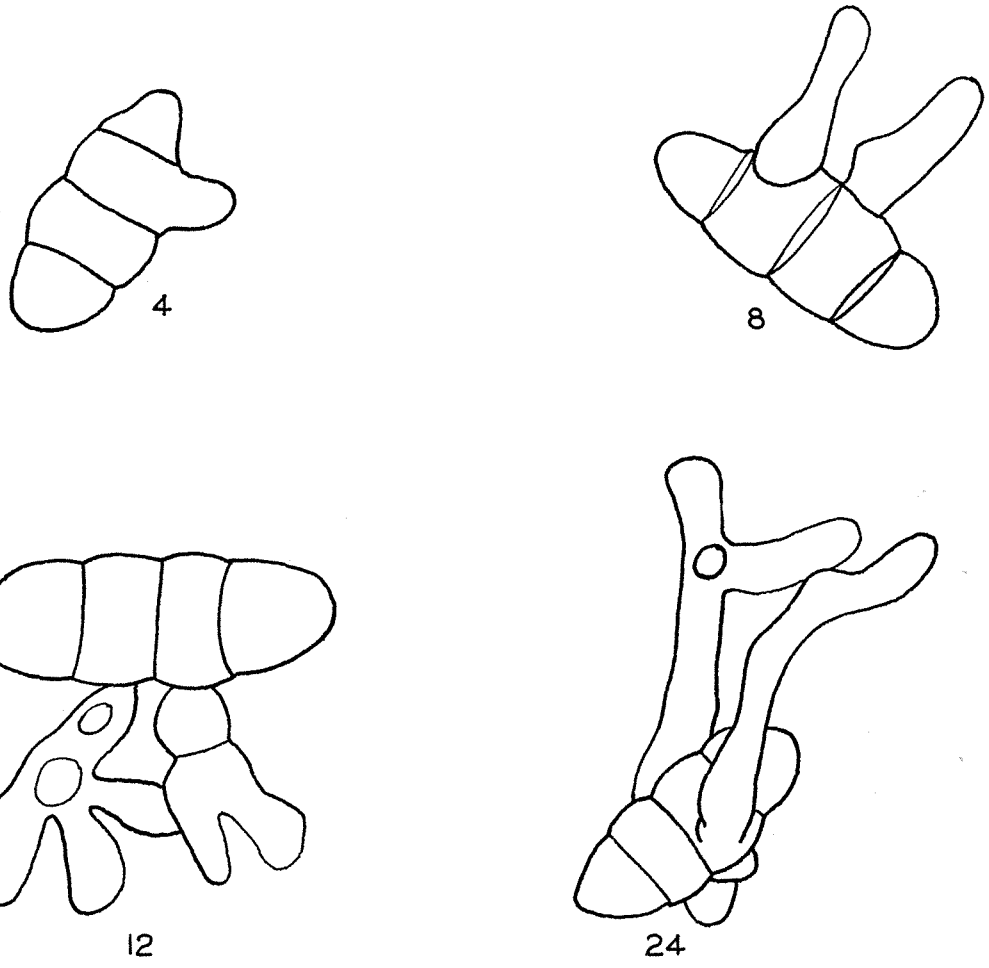



FIGURE 1

Germination of M. rubricosum in distilled water at 19° ± 1°C., time intervals (hours) are measured from the start of the experiment.

100μ

## (4) Physiological Studies

### (a) The Effect of Media on Colony Growth.

Duplicate plates were each touched four times with a spore laden needle tip so that four colonies per plate would result.

TABLE VII.

Effect of Media on Growth Rate of M. rubricosum after Nine Days at 18±1°C.

| Medium | Mean Colony Diameter in mms. |
|---|---|
| Oatmeal | 9·5 |
| Cornmeal | 8·5 |
| Tomato | 8·0 |
| Peastems | 7·1 |
| Carrot stems | 6·7 |
| Milk | 6·5 |
| Malt | 6·5 |
| Cocksfoot leaves | 5·8 |
| Difco P.D.A. | 5·7 |
| Lab. P.D.A. | 5·7 |
| Prune | 5·7 |
| Carrot | 4·4 |
| Soybean | 4·2 |
| Water | 3·8 |
| Cocksfoot | 3·7 |

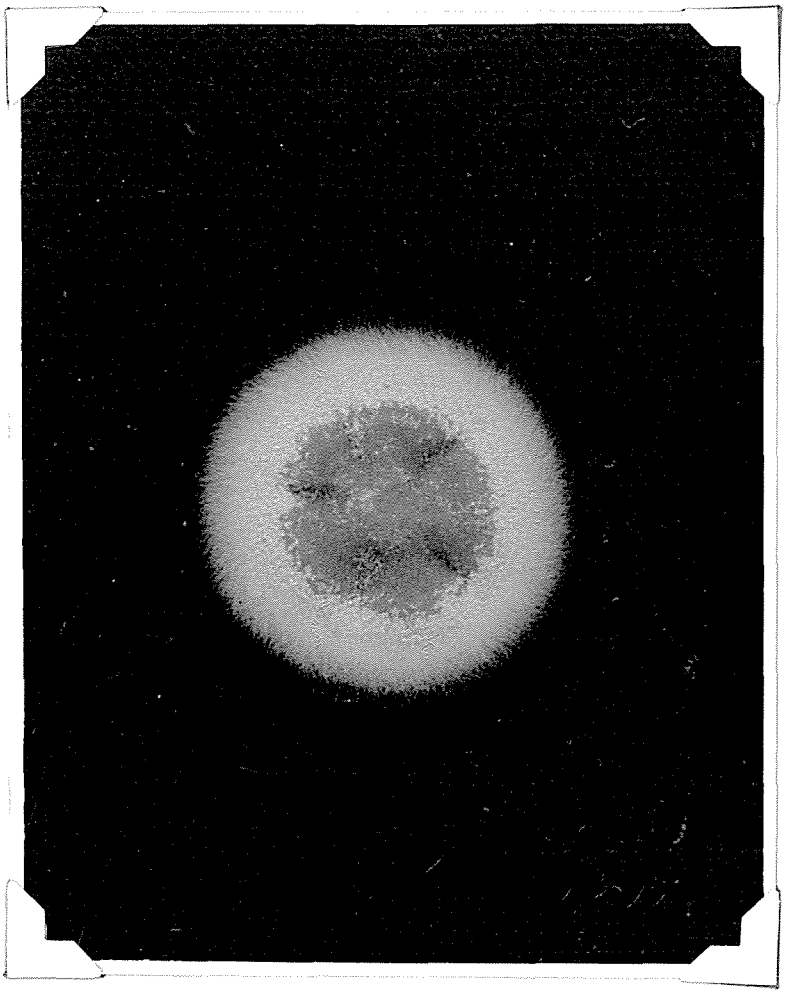


PLATE 5. A twenty-one day old colony of *M. rubricosum* grown at 13°C. on P.D.A., diameter = 17.5 mms.

Colonies of M. rubricosum on P.D.A. are slightly raised from the surface of the agar, the centre portion rising to a golden-brown, slimy knob of conidia. The mycelium grows into a creamy-yellow mat having a leathery texture and being free from aerial mycelium. After nine days growth slight convolutions were just discernible. Within a few days of further growth these ridges developed greatly. (Plate 5 .)

On the majority of media used the above description is applicable, apart from minor differences in colour and texture of the mycelium. Colonies on milk agar differed in that the mycelium was white and aerial with few spores present. On water agar the mycelium was sparse, as also was sporulation. In addition, spores were significantly smaller than those on other media.

Oatmeal was found to be the most satisfactory medium for culturing M. rubricosum because of its quick growth and prolific sporulation.

(b) The Effect of Temperature on Colony Growth.

TABLE VIII.

Effect of Temperature on Growth Rate of M. rubricosum on P.D.A. after Nine Days.

| Temperature in degrees centigrade | 5 | 8 | 11 | 14 | 17 | 20 | 24 | 28 | 32 |
|---|---|---|---|---|---|---|---|---|---|
| Average colony diameter in mms. | 28 | 43 | 67 | 59 | 57 | 45 | 23 | trace | nil |

Bollard (1950) records that on Cocksfoot agar 25°C. and 15°C. are respectively the maximum and optimum temperatures for growth.

FIGURE 2

Effect of temperature on colony growth of the five fungi on P.D.A. after nine days.

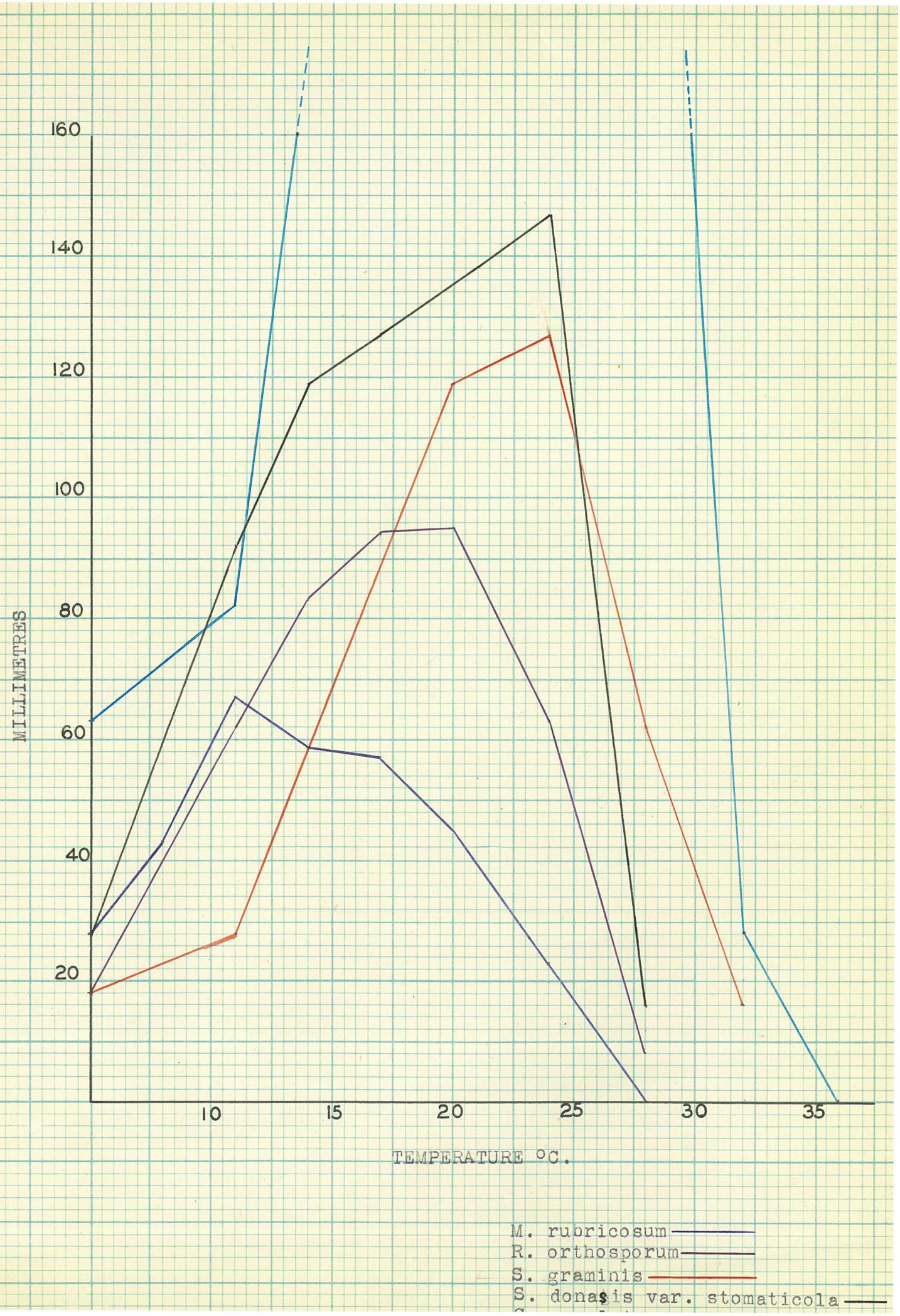
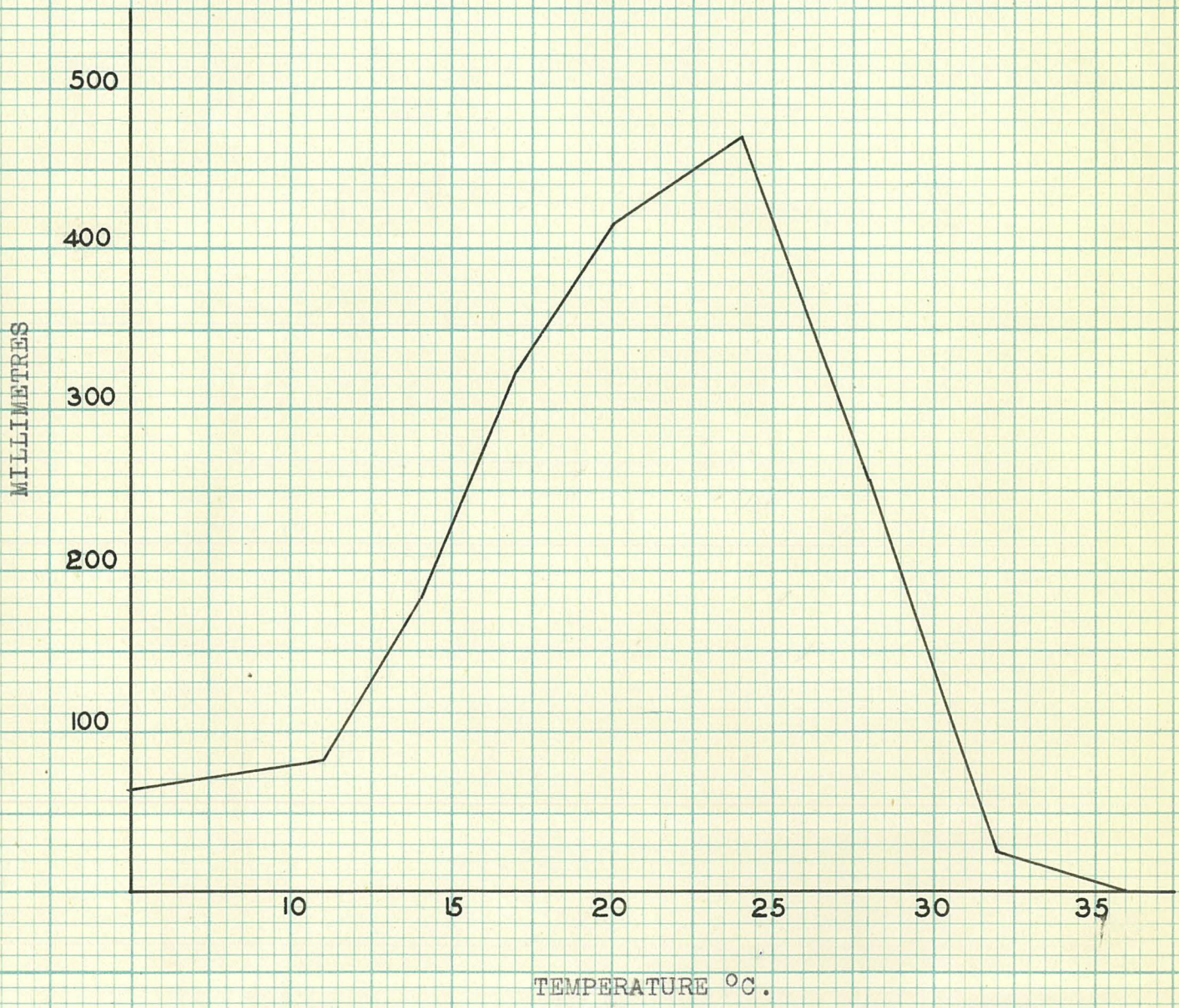

160
140
120
100
80
60
40
20
MILLIMETRES
10
15
20
25
30
35
TEMPERATURE °C.
M. rubricosum
R. orthosporum
S. graminis
S. donasis var. stomaticola

FIGURE 3

Effect of temperature on colony growth of *S. maculata* on P.D.A. after nine days.


500
400
300
200
100
MILLIMETRES
10
15
20
25
30
35
TEMPERATURE °C.

From Table VIII it can be seen that on P.D.A. 28°C. and approximately 11°C. are respectively the maximum and optimum temperatures for growth of M. rubricosum. With the aid of the binocular microscope a few small hyphae were seen at 28°C. Colonies grown at 24°C. were the typical yellow to off-white colour but were very much raised from the agar surface, strongly convoluted and with few spores. Sporulation was generally prolific, particularly at 5°C.

(c) The Effect of Hydrogen Ion Concentration on Colony Growth.

TABLE IX.

Effect of pH on Growth Rate of M. rubricosum on P.D.A. after Nine Days at 18°±1°C.

| pH | 3.0 | 3.4 | 3.9 | 4.4 | 5.0 | 5.9 | 6.7 | 7.6 | 8.3 | 8.6 | 9.0 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Mean colony diameter in mms. | 22 | 22 | 19 | 22 | 39 | 54 | 47 | 43 | 39 | 44 | 41 |

Over the range of pH 5.0 - 9.0, growth appears to be independent of pH except that growth at pH 5.9 is a little greater. Although the colonies at the higher hydrogen ion concentrations were smaller they contained a greater proportion of conidia than did the others. Apart from the differences in conidial concentration at the different pH levels, colony morphology remained very similar.

FIGURE 4.

Effect of hydrogen ion concentration on colony growth of *S. maculata* on P.D.A. after nine days at 24°±.5°C.

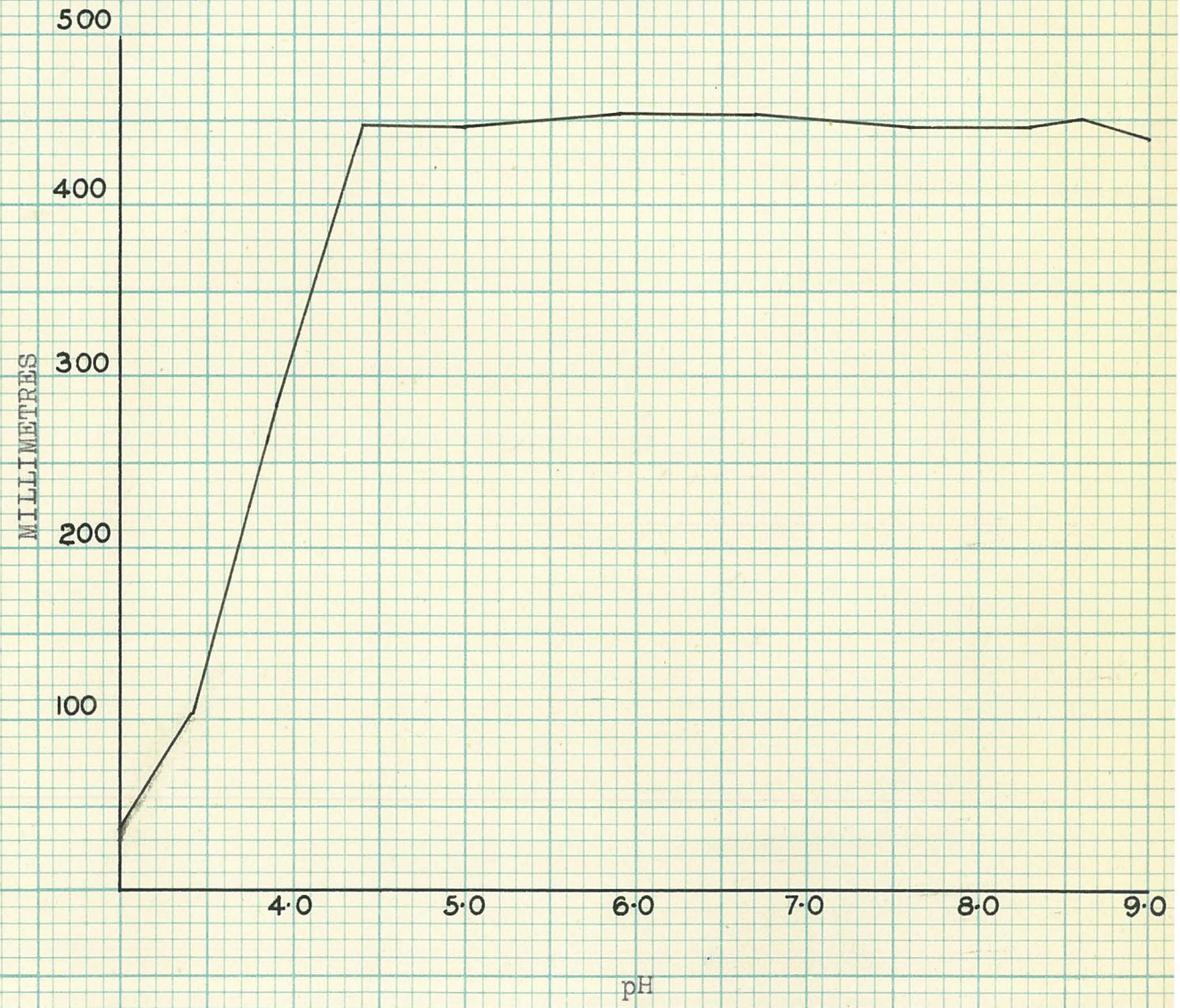
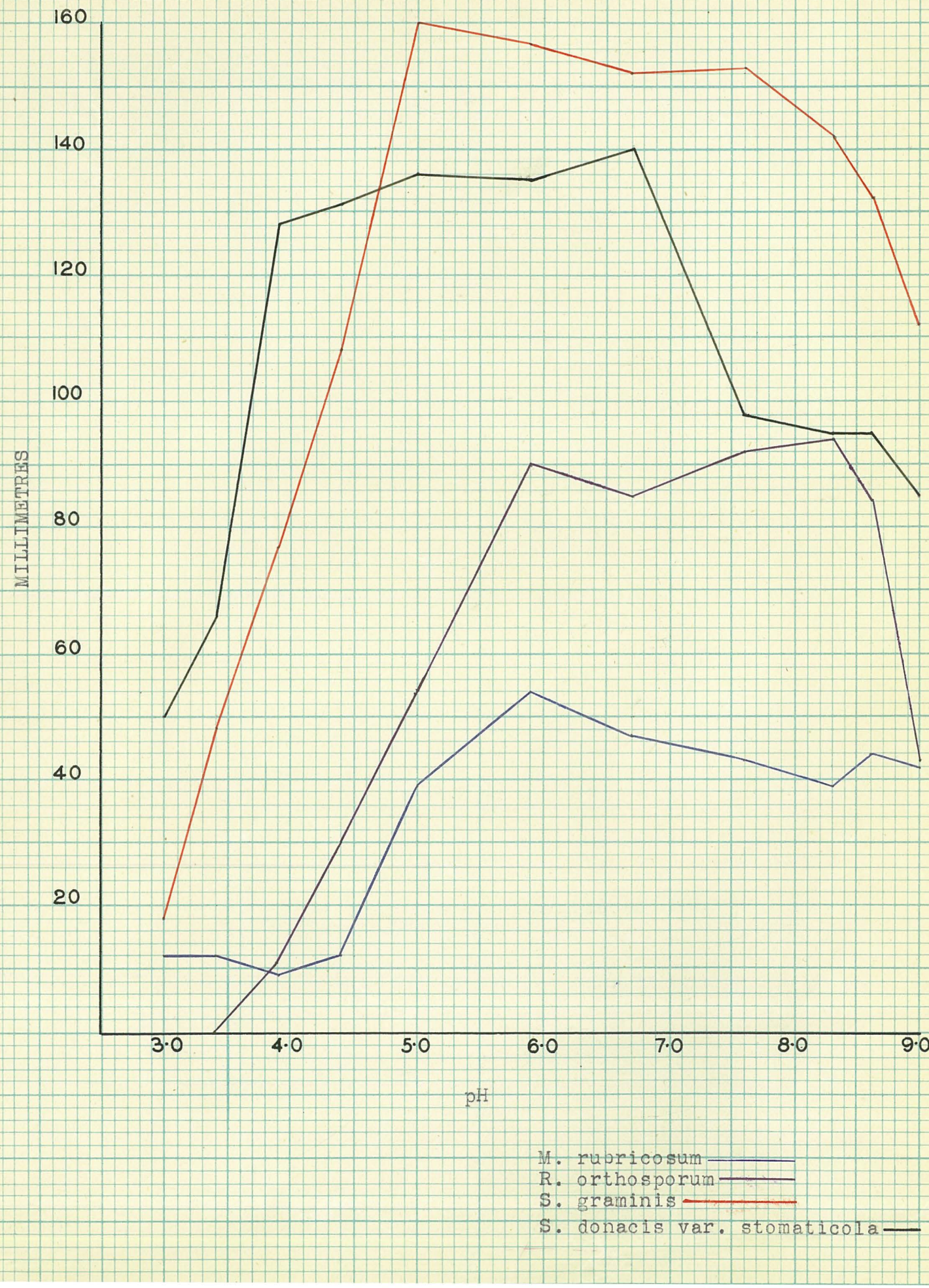

500
400
300
200
100
MILLIMETRES
4·0
5·0
6·0
7·0
8·0
9·0
pH

FIGURE 5

Effect of hydrogen ion concentration
on colony growth of the fungi on P.D.A.


160
140
120
100
80
60
40
20
MILLIMETRES
3·0
4·0
5·0
6·0
7·0
8·0
9·0
pH
M. rubricosum
R. orthosporum
S. graminis
S. donacis var. stomaticola

## B. Rhynchosporium orthosporum Caldwell.

### (1) Taxonomy and Review of Literature.

Oudemans (1897) recorded a leaf spot disease of Secale cereale and named the pathogen Marsonia secalis. The first mention of the generic name Rhynchosporium was made by Frank in 1897. He credited his associate, Heinsen, with the naming of the new genus. Frank observed that the disease caused by the above species on barley was of considerable economic importance, and that seedling plants were frequently killed in severe attacks. He failed to give a formal description of the genus or the species and this was left to Saccardo (1906).

The next record of the disease was in America in 1915, and since then it has been reported in Northern Europe, South America, Africa, Australia and New Zealand. In the U.S.A. yields of barley have been reduced by as much as twenty to thirty per cent (Caldwell 1937).

Davis (1919) believed that Oudemans had incorrectly classified the fungus in the Melanconiales and so he recognised Heinsen's genus Rhynchosporium in making the new combination Rhynchosporium secalis (Oud.) Davis.

Caldwell (1931) collected a new species of the genus Rhynchosporium attacking Dactylis glomerata. Symptoms were identical with those of R. secalis, the only morphological difference being in the shape of the conidia which were more uniformly cylindrical than in R. secalis. Caldwell named it Rhynchosporium orthosporum sp. nov.

Some doubt was expressed by Caldwell (1937) as to the validity of the genus in the family Moniliaceae. He stated that the "taxonomic position of the genus Rhynchosporium is somewhat debatable

since the genus appears to possess characteristics common to both orders, Moniliales and Melanconiales". However, on closer study Caldwell recognised Rhynchosporium as the true genus in the family Moniliaceae.

Since Caldwell (1937) described R. orthosporum on Dactylis glomerata other hosts have been found. Sprague (1950) names eight hosts and since making this list has found two more in Alaska, (Sprague (1955), and two more are listed by Meiners (1954).

It would appear, however, that the dividing line between R. secalis and R. orthosporum is not a sharp one. Caldwell based his division of the two species on parasitism and spore morphology. R. secalis is characterised by a beak whereas R orthosporum has straight cylindrical spores. Sprague (1950) described conidia of extremely variable size and shape on Agropyron subsecundum. A few had a slight tendency towards the formation of obliquely hooked apices as in R. secalis. He accredited this variability to changes in temperature. Sprague, however, examined Rhynchosporium collected on Phaleris arundinacea and stated that the spores were definitely intermediate between R. orthosporium and R. secalis, but assigned it to the latter because some of the spores tended to be obliquely hooked at the apex.

In view of the similarity of the two species and the difficulty in deciding to which species the Rhynchosporium on Phaleris arundinacea belongs, it may well be that Caldwell was somewhat premature in naming R. orthosporum as a new species. Snyder and Hansen (1954) in discussing the wide variability of fungi emphasise that it is not the differencesbetween individuals which provide the basis for speciation but rather the similarities which tie them together.

Hence, if more "border line" cases, similar to Rhynchosporium on Phaleris arundinacea are found, then reducing the species R. orthosporum to a variety of R. secalis should perhaps be considered.

The classification of R. orthosporum is given by Clements and Shear (1931) as follows:

Fungi Imperfecti

Moniliales - conidia are neither borne in pycnidia nor acervuli but on conidiophores, either discreet or in groups.

Moniliaceae - hyphae hyaline or bright coloured, loose and cottony, rarely fasciculate; sterile and fertile hyphae or conidiophores both present as a rule, the latter differentiated by means of vesicles, whorls, basidia, sterigmata, etc. conidia concolourous, that is, hyaline or bright coloured.

Hyalodidymae - conidia two celled, hyaline or bright coloured, globose to oblong or fusoid.

Rhynchosporium - conidia not catenate, obliquely beaked, cylindric.

(2) Symptoms

The first sign of the disease is the appearance of dark, water-soaked patches 1 - 3 centimetres in length or either surface of the leaves. These areas rapidly dry out and become light brown in colour with a darker brown margin, (Plate 6 .). The lesion enlarges radially so that a series of concentric rings characterise older

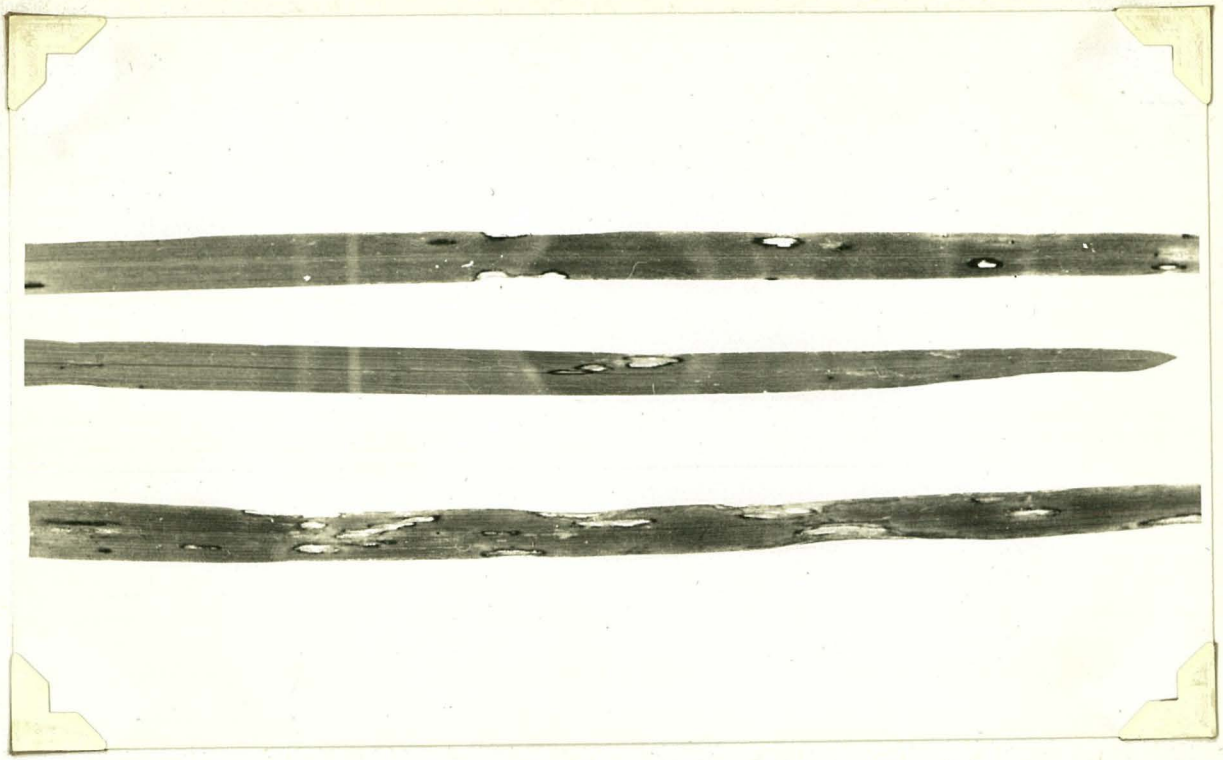


PLATE 6. Lesions caused by R. orthosporum on leaves of Cocksfoot.

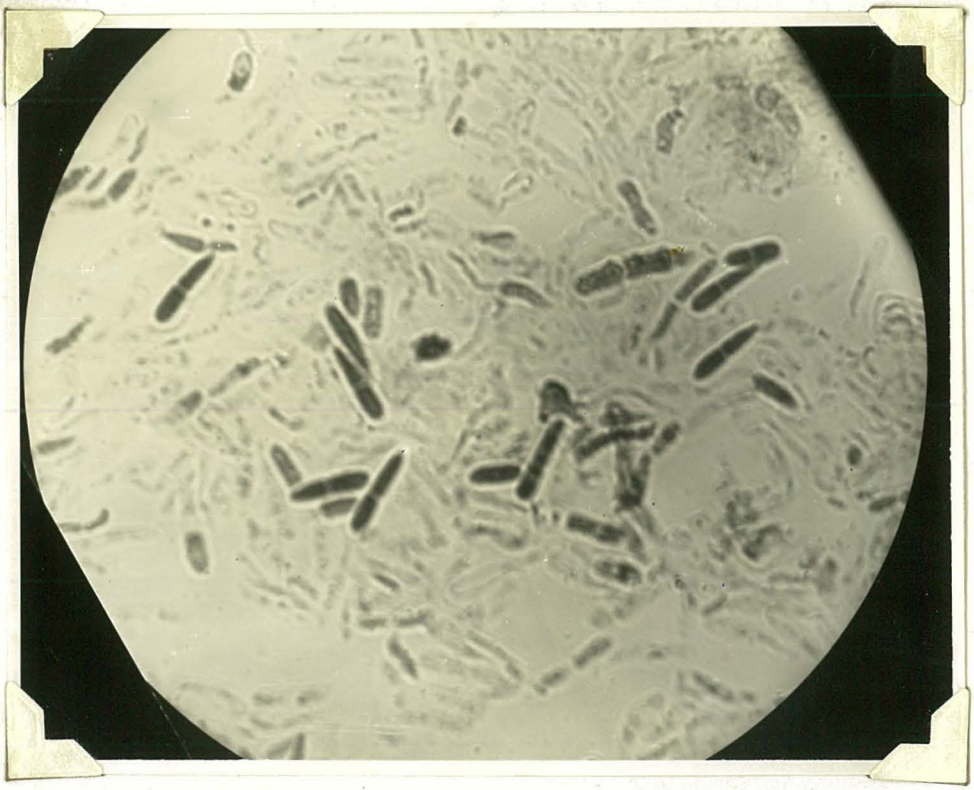


PLATE 7. Conidia of R. orthosporum from Oatmeal agar, magnification, approximately 800.

older lesions. Owing to the large dead, "scalded" patches which may be twenty centimetres in length, the leaves become wrinkled and twisted. The symptoms of this disease are very distinct and no confusion should arise in identifying it. The common name of Scald most adequately characterises the disease.

## (3) Spore Studies

### (a) Sporulation and Spore Description

Very few conidia were found on lesions in the field or in the glasshouse. Following the recommendation of Caldwell (1937), diseased leaves were subjected to high humidity in Petri plates held at 20°C. for several days. Conidia were produced in great numbers by this treatment. All told, 235 conidia were measured after being obtained by this method. For ease of measuring the spores were stained in lactophenol acid fuchsin.

| | | | |
|---|---|---|---|
| Length | Mean | 16.6μ | S.D. 1.6μ |
| | Mode | 16.4μ | |
| | Range | 13.1 - 21.3μ | |
| Width | Mean | 3.6μ | |
| | Range | 2.5 - 5.0μ | |

Caldwell (1937) records dimensions of the conidia as follows:-

| | |
|---|---|
| Length | 14.4 - 19.4μ |
| Width | 2.3 - 4.7μ |

The conidia are cylindrical and uni-septate, the septum dividing the spore into equal portions. There is no hilum apparent. Conidia are formed directly on the stroma in vast numbers and sporulation is most abundant in the central and most completely collapsed area of the leaf spot. Lesions may occur

on either surface but spores are formed only on the side where infection was initiated.

R. orthosporum sporulates freely on culture media. Caldwell noted that media with high levels of carbohydrate tended to produce conidia of diverse shape and size. Present investigations showed that of the various media used for culturing the fungus, only Cornmeal and Oatmeal agars produced conidia which closely resembled those from natural material. Conidia on other media were one to four septate, varied greatly in size, and many appeared to produce "buds" from the spores.

No comparison was made of spore dimensions on various media because of this great diversity of morphology.

(b) Spore Germination

TABLE X.

Effect of Temperature on "Spore Germination Time" of R. orthosporum in Distilled Water.

| Temperature in degrees centigrade | 5 | 10 | 19 | 24 | 28 |
|---|---|---|---|---|---|
| Germination time in hours | 21 | 17 | 13 | 19 | 24 |

The above table shows that the optimum temperature for germ tube growth on distilled water is in the vicinity of 19°C. Generally, a single, terminal germ tube is formed but occasionally two germ tubes may appear simultaneously from both ends of the spore. Unlike many of the other fungi, the tube curves and branches very shortly after its formation. This made an accurate measurement of its growth very difficult. (Fig.6.).

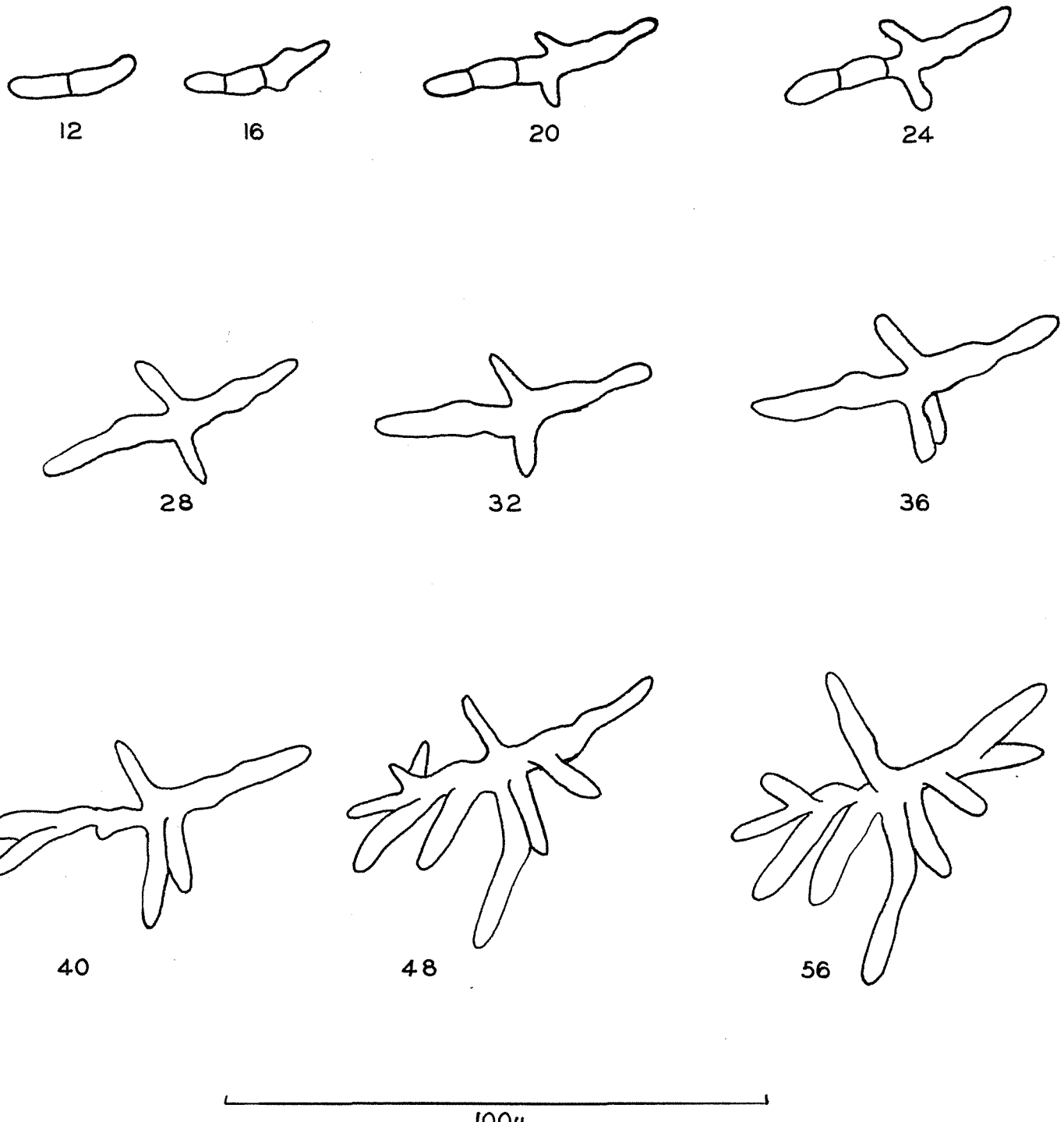



FIGURE 6

Germination of R. orthosporum on P.D.A. at 19° ± 1°C., time intervals (hours) are measured from the start of the experiment.

TABLE XI.

Effect of Temperature on Germ Tube Length of *R. orthosporum* in Distilled Water after 24 hours.

| Temperature in degrees centigrade | 5 | 10 | 19 | 24 | 28 |
|---|---|---|---|---|---|
| Germ tube length in microns | 3.6 | 6.0 | 16.2 | 6.6 | trace |

As expected, germ tube growth and "germination time" are positively correlated. Correlation between tube growth and growth on P.D.A. is poor. At the optimum temperature of 19°C., colony growth on P.D.A. is thirty per cent greater than at 24° and 10°C., whereas the respective germ tube growth on water is two hundred and fifty per cent greater.

## (4) Physiological Studies

### (a) The Effect of Media on Colony Growth

Petri plates were inoculated by the "spore needle-tip technique" and then held at 17° ±1°C. for nine days.

TABLE XII.

Effect of Media on Growth Rate of R. orthosporum after Nine Days at 17±1°C.

| Medium | Mean Colony Diameter in mms. |
|---|---|
| Oatmeal | 95 |
| LAB. P.D.A. | 94 |
| Cornmeal | 84 |
| Tomato | 76 |
| Prune | 69 |
| Cocksfoot | 62 |
| Milk | 55 |
| Soybean | 50 |
| Water | 47 |
| Carrot | 22 |

Nine day old colonies on P.D.A. are cream in colour with little aerial mycelium to raise them above the agar surface. They grow out from the inoculum in a uniformly radial manner and at the centre produce a slimy, cream mound of irregularly shaped conidia, (Plate 8.) These are roughly cylindrical in shape with one to three very pronounced septa and in many cases the spores appear to be composed of several semi-spherical balls. The central region of the colonies commences to darken several days later and in old colonies becomes a dark brown. After several weeks the entire colony develops a very dark pigment so that the mycelial thatch appears almost black.

Colonies on Milk, Prune and Cocksfoot agars are fairly similar to those on P.D.A. The conidia are all very irregular and on Milk

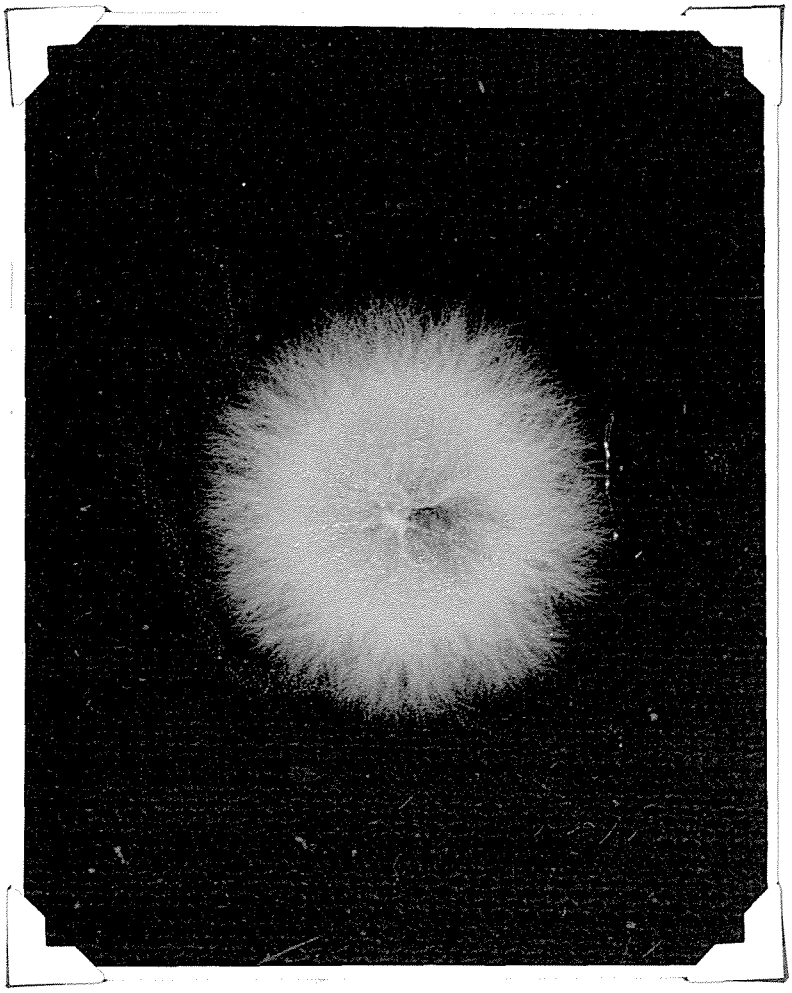


PLATE 8. A twenty-one day old colony of *R. orthosporum* grown at 13°C. on P.D.A., diameter = 185 mms.

agar they are branched, giving the appearance of producing buds.

On Tomato, Soybean, and Carrot agars colonies are produced in which the mycelium is aerial. Spores are scattered among this mycelium and do not occur as a mound at the centre.

On Cornmeal, Oatmeal and Water agars colonies are spread out over the agar surface. They all produce uni-septate cylindrical conidia similar to those occurring naturally. On the two former media the fungus sporulates profusely. On Water agar a very sparse mycelial network is formed which shows very clearly that the spores are attached directly to the hyphae in groups of up to six.

Caldwell (1937) records that on media with low sugar content R. orthosporum produces conidia which are similar to those from the host. This is borne out by the evidence above.

(b) The Effect of Temperature on Colony Growth.

TABLE XIII.

Effect of Temperature on Growth Rate of R. orthosporum on P.D.A. after Nine Days

| Temperature in degrees C. | 5 | 11 | 14 | 17 | 20 | 24 | 28 | 32 |
|---|---|---|---|---|---|---|---|---|
| Average colony diameter in mms. | 18 | 62 | 83 | 94 | 95 | 63 | 8 | nil |

As can be seen from the table, growth occurred between the temperature limits of 5° and 28°C. with the optimum between 17° and 20°C. Colony morphology did not seem to alter with the temperature and sporulation was abundant at all but the extreme temperatures.

(c) The Effect of Hydrogen Ion Concentration on Colony Growth.

TABLE XIV.

Effect of pH on Growth Rate of R. orthosporum on P.D.A. after Nine Days at 17° ±1°C.

| pH | 3.0 | 3.4 | 3.9 | 4.4 | 5.0 | 5.9 | 6.7 | 7.6 | 8.3 | 8.6 | 9.0 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Mean colony diameter in mms. | 0 | trace | 11 | 30 | 54 | 90 | 85 | 92 | 94 | 84 | 43 |

R. orthosporum had a more limited growth range than had any of the other four fungi. Growth was inhibited below pH 3.4 and restricted up to 5.9. From pH 5.9 to 8.7 it was unrestricted but at 9.0 the effects of alkalinity began to show.

The higher pH levels affected the morphological form of the colonies. The cone of spores that normally is found at the centre was enlarged and in many cases the mycelium did not grow out beyond this area. Colonies did not appear normal until the P.D.A. was adjusted to pH 5.0.

## C. *Scolecotrichum graminis* Fckl.

### (1) Taxonomy and Review of Literature.

Fuckel (1863) described a leaf spot fungus on *Dactylis glomerata* which he called *Scolecotrichum graminis*.

Lind (1913) examined the herbarium of Rostrup and commented that Rostrup believed *S. graminis* Fckl. represented the imperfect stage of *Mycosphaerella recutita*.

Von Hohnel (1923) pointed out that the genus *Scolecotrichum* is probably invalid. He argued that the genus *Scolecotrichum* Kze. is based on the type species *S. virescens* Kze., but stated that this fungus is of a very doubtful character and that *S. graminis* Fckl. is a *Passalora* with the perfect stage *Carlia recutita*. He therefore proposed the name *Passalora graminis* (Fckl.) Hohn, to replace *S. graminis* Fckl. Von Hohnel further stated that the following fungi are identical with *Passalora graminis* (Fckl.) Hohn.

*Scolecotrichum compressum* Allesch.

*Passalora hordei* Otth.

*Passalora punctiformis* Otth.

*Sphaeria recutita* Fr.

*Metasphaerella recutita* (Fr.) Sacc.

Subsequently, further confusion was caused by Horsfall (1930) when he suggested that the genus *Cercospora* seemed a more logical place for the fungus than *Passalora* as the latter has many-septate conidiophores, while those of *Passalora graminis* (Fckl.) Hohn. (=*Scolecotrichum graminis* Fckl.) are continuous and rarely septate. Horsfall therefore proposed the name *Cercospora graminis* (Fckl.) Horsfall.

Chupp (1954) rejected this latter argument on the basis that the conidia were minutely echinulate and could therefore not be classified as a Cercospora. Additional evidence was provided from the host range. Cercospora species are strongly limited in their host range, whereas this fungus has a particularly wide range of hosts.

Until von Hohnel's work is verified by a re-investigation of Scolecotrichum virescens Kze., neither Scolecotrichum graminis nor Passalora graminis can be definitely stated as being correct. It appears from the literature that the former name is favoured by American and Continental workers, and the latter by English workers.

Literature concerning the physiological status of S. graminis is almost non-existent. Sprague (1950) mentions that morphological varients occur (as on Poa ampla Merr.) but whether they are anything more than variations due to host and humidity is not known. This is surprising considering the widespread nature of the fungus.

The host range of S. graminis is unusually large. Sprague (1950) records 141 different hosts for this fungus in the U.S.A. and since this list was compiled, a further seven hosts have been recorded, six in Alaska (Sprague 1955) and one in the U.S.A. (Meiners 1954).

Brien and Dingley (1951) list Dactylis glomerata as the only host for this fungus in New Zealand. During the course of the present investigation a series of isolations were taken from lesions on many common pasture grass species. As a result of these isolations S. graminis was commonly found on Phleum pratense but contrary to expectations, this was the only other host of S. graminis which was detected.

There are many reports on the seriousness and wide host range

of S. graminis. Johnson and Hungerford (1917) state that in severe outbreaks of the disease almost all the leaves of affected plants are dried up during the flowering period. Sprague (1938) found forty per cent of the leaves of winter rye in Oregon to be diseased. Other reports on rye are by Guarch (1941), in Uruguay, Jankowska (1929) in Poland, Landaluze (1941) in Spain and Shitakova-Roussakova (1939) in Russia. This last worker reported that fifty per cent of leaf surface was affected and that in some districts it was no less serious than rusts.

A series of estimates of damage to Dactylis glomerata by S. graminis were published by Roberts et al. in 1952, 1954, 1956. They found that losses varied from one to two per cent and these figures are verified by Sprague (1950) who states that the fungus is one of the most important leaf spots on common pasture grasses on the East Coast of North America.

S. graminis has been reported overseas at all times of the year but appears to have its peak periods in the spring and autumn.

Several overseas research workers have claimed discovery of the sexual phase of S. graminis (Rostrup, cited by Lind 1913), Von Hohnel (1923). In no case has subsequent research confirmed the connection between the conidial stage, S. graminis and the postulated perfect stage, Carlia recutita. The practice is still to regard this fungus as a member of the Fungi Imperfecti, with classification as follows, (Clements and Shear) (1931).

Fungi Imperfecti

Moniliales - conidia are neither borne in pycnidia nor acervuli but on conidiophores, either discreet or in groups.

Dematiaceae - hyphae typically dark, olive to brown or black,

rarely hyaline but the conidia then dark, loosed and byssoid, more or less rigid, rarely fasciculate; sterile and fertile hyphae or conidiophores both present as a rule, the latter differentiated by means of vesicles, whorls, basidia, sterigmata,etc.; conidia typically dark, but sometimes hyaline.

Macronemeae - hyphae manifest and distinct from the conidia.

Scolecotrichum - conidia not ciliate, muriculate, capitate, catenate, not verrucose, but are acropleurogenous; conidiophores not flexuous or torulose, nor inflated but simple, short, fasciculate, mostly erect.

(2) Symptoms

Young lesions appear on the leaves as small chocolate-coloured dots surrounded by a pale yellow area. They very soon elongate and within a week or appearing have become up to 50mm. long and 6mm. wide. Some lesions stay at this size while others continue to elongate so that streaks 3 - 4 cms. in length are not uncommon; hence the common name of Leaf Streak for the disease. The degree of leaf infection can vary from a few lesions per leaf to many hundreds. In cases of severe infection the streaks coalesce so that a considerable portion of the leaf is killed. Older streaks are light brown and occupy the zones between one or two leaf veins. The veins appear as thin dark brown lines running along the lighter lesioned area. The original site of infection is darker and generally found at the centre of the long streak. Along the entire lesion are the fruiting bodies, (Plate 10). They are most noticeable as small black dots arranged in regular rows. Each fruiting body is a fascicle of unbranched, irregular conidiophores bearing bicelled

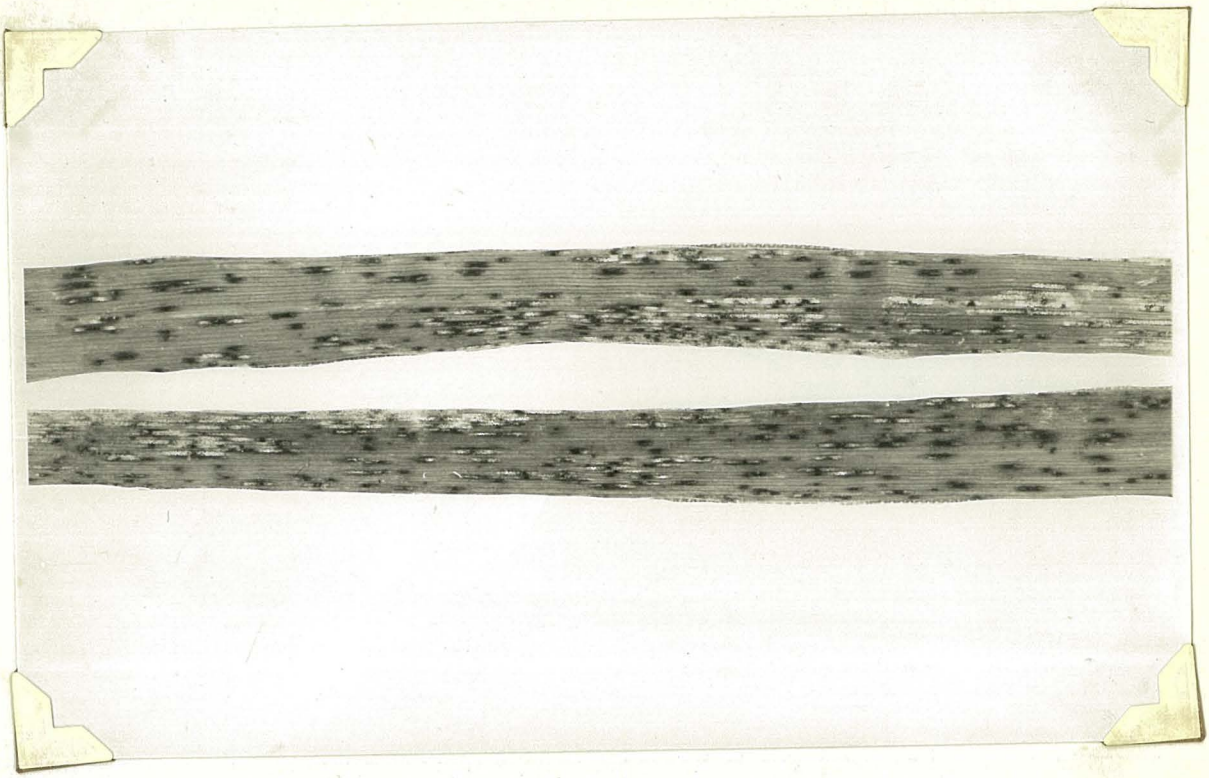


PLATE 9. Lesions caused by S. graminis on leaves of Cocksfoot.

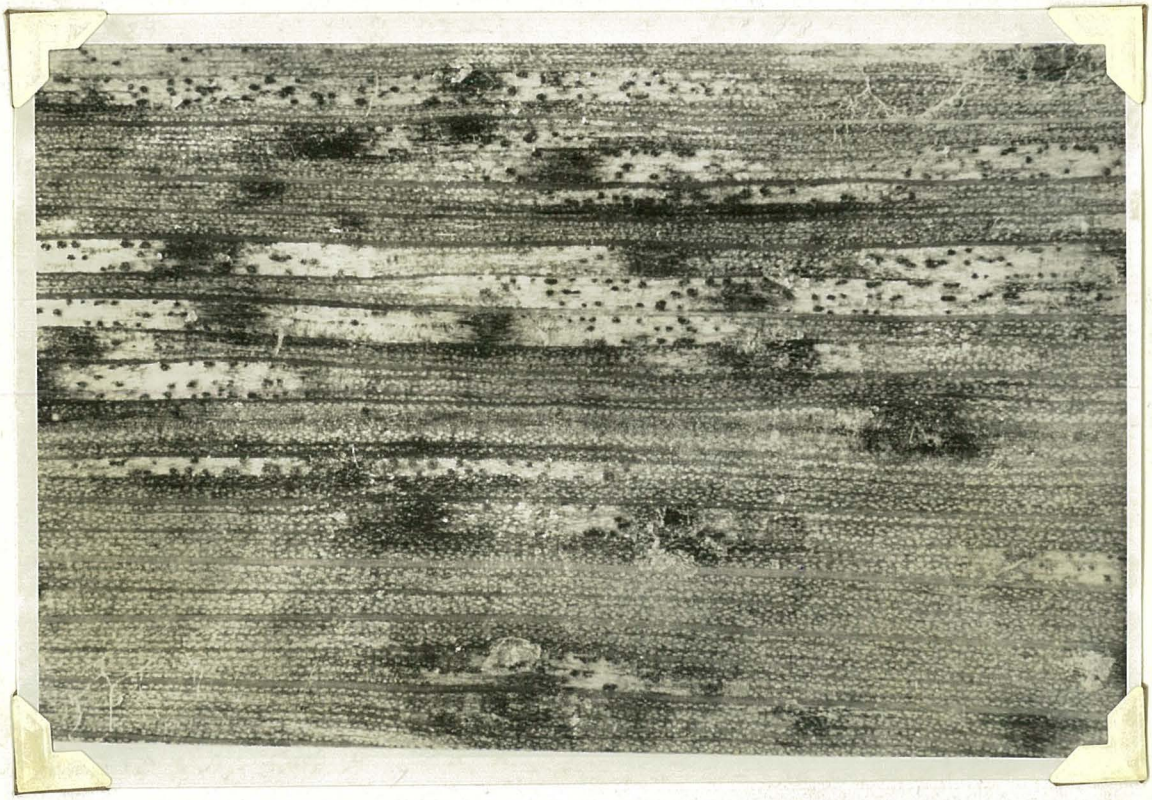


PLATE 10. Magnified view of lesions caused by S. graminis to show the characteristic fruiting bodies.

conidia. The fruiting bodies may be formed on both sides of the leaf, but generally occur only on the upper surface. Sampson and Western (1954) and Sprague (1950)' record that they found them only on the upper surface.

## (3) Spore Studies

### (a) Sporulation and Spore Description

Conidia occur in great numbers on young lesions. Mature lesions, when examined with the naked eye, appeared to have many spores present. A more detailed examination, however, showed that they were only fascicles of old conidiophores which had shed their conidia.

Natural material was gathered at various intervals throughout the year and 219 spores, in all, were measured with the following results:-

| | Range | Mode | Mean |
|---|---|---|---|
| Conidia | | | |
| Length | 28.1 - 46.2µ | 33.0µ | 35.9 S.D. 3.11µ |
| Width | 8.3 - 11.6µ | | 8.4µ |
| Conidiophores | 49.5 -107.3µ | | 90.8µ. |

Overseas Records.

| Authority | Sprague (1950)' | Guarch (1941) |
|---|---|---|
| Conidia | 35-45µ x 8-10µ | 32-34.5µ x 10-12.5µ |
| Conidiophores | 90-100µ x 6-8µ | - |

Fascicules are of dark, unbranched, geniculate, non-septate, conidiophores, which bear the conidia. The conidia are cylindrical, broadly rounded at the base and tapering slightly at the tip.

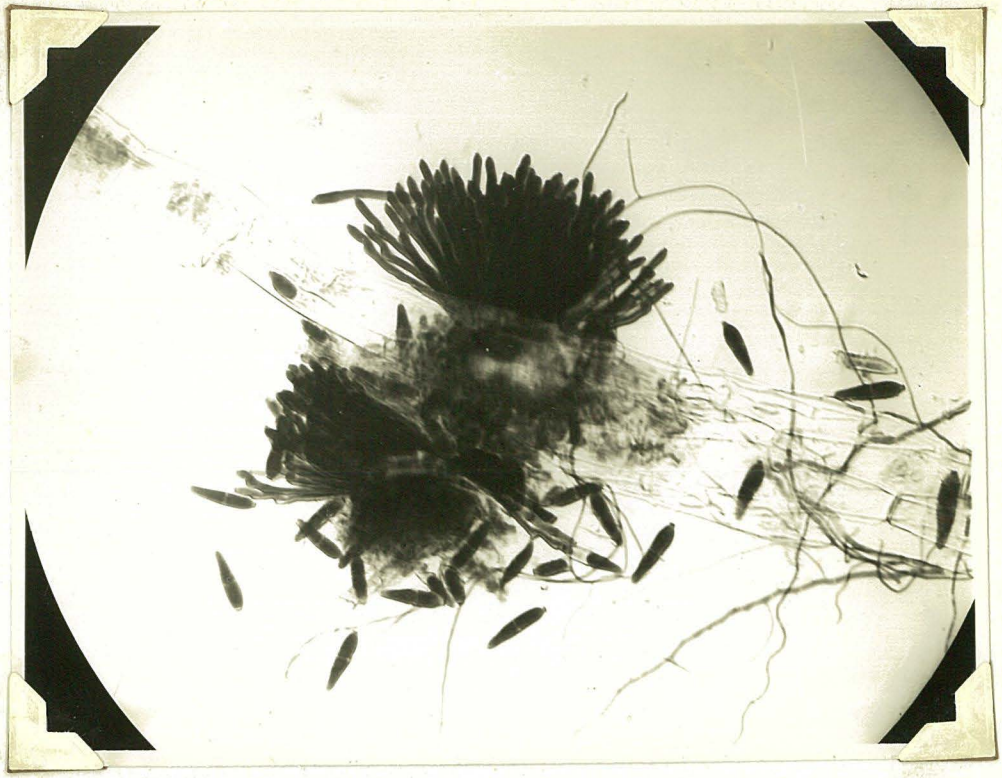


FIGURE 11. Fruiting bodies of S. graminis from Cocksfoot, conidia are detached from the conidiophores. x 250.

FIGURE 12. Conidia of S. graminis from Cocksfoot. x 750.

A well defined hilum marks the point of attachment to the conidiophore, (Plate 12 .). Under natural conditions the conidia are generally uni-septate, but when fresh lesions have been subjected to periods of high humidity in Petri plates, then a few conidia are two or even three septate.

Efforts to induce S. graminis to sporulate in culture by growing it on various media, by alternating light and dark conditions, by exposure to sunlight, and by varying the temperature, were unsuccessful. Braverman (1956) described a technique whereby he was successful in obtaining a few spores. He grew the fungus on a mixture of vegetable juices (commercially known as V-8 juice in the U.S.A.), for three weeks. The covered plates were then exposed to ultra-violet light for sixteen minutes at a distance of 36cms. from the light. Unfortunately V-8 juice was not available in New Zealand and tomato juice was used in its place. The technique was identical with Braverman's except that, whereas he used a copper Hewitt lamp of 900 watts, the only available lamp was a Westinghouse Sterilamp W.L. 782.

Sporulation could not be obtained on Tomato agar even when the time of exposure, distance away from the lamp, or concentration of the medium were altered. By using Cocksfoot agar and exposing the covered plates for five minutes at a distance of 10cms. from the ultra-violet lamp a few clumps of spores were observed after eight days. Only fifteen of these spores were measured so that their average length of 43.9 microns. is not a very reliable figure.

(b) Spore Germination

Germination of S. graminis was morphologically similar at all temperatures. A single germ tube emerged from the smaller, distil end of the spore and continued to grow for twelve to eighteen hours before branching. A second germ tube, from the other end of the spore, appeared six to twelve hours after the initial one. Higher temperatures tended to accelerate the rate of its appearance. Twelve hours after setting up the experiment nearly every spore at 24°C. had a second germ tube, whereas at 16°C. approximately fifty per cent had second germ tubes.

Septa formed across the hyphae at an early stage in their development.

TABLE XV.

Effect of Temperature on "Spore Germination Time" of S. graminis on P.D.A. and Distilled Water.

| Temperature in degrees centigrade | | 7 | 16 | 18 | 24 | 28 |
|---|---|---|---|---|---|---|
| Germination time in hours | P.D.A. | 12.0 | 3.5 | 3.0 | 2.5 | 3.5 |
| | Water | 28.0 | 12.0 | 6.0 | 4.0 | 36.0 |

The "germination time" was much longer on distilled water than on P.D.A., especially at the extremes of temperature. The time interval between the appearance of the first and second germ tubes was also correspondingly longer, and branching did not commence at such an early stage.

The optimum, maximum and minimum temperatures for spore

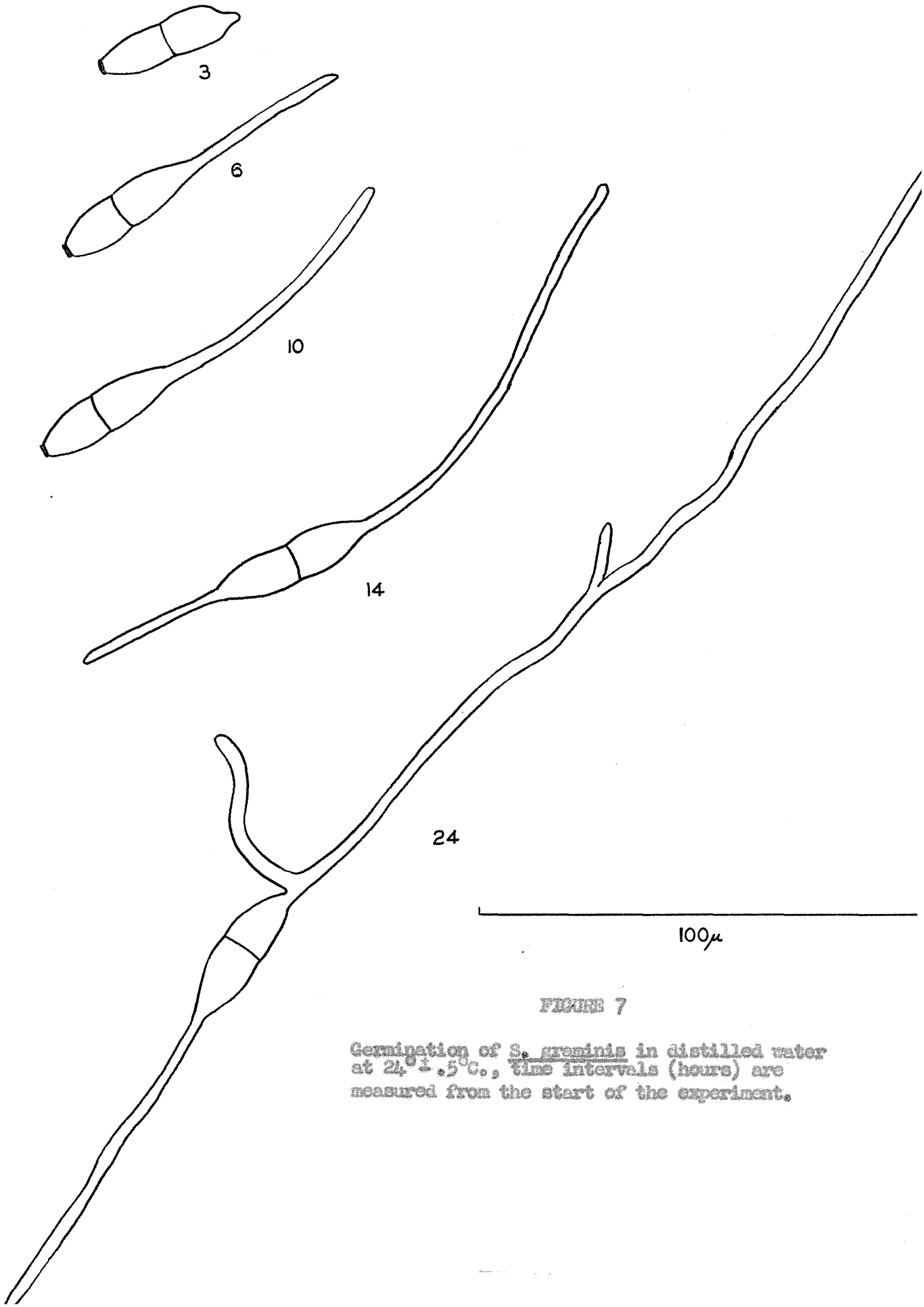
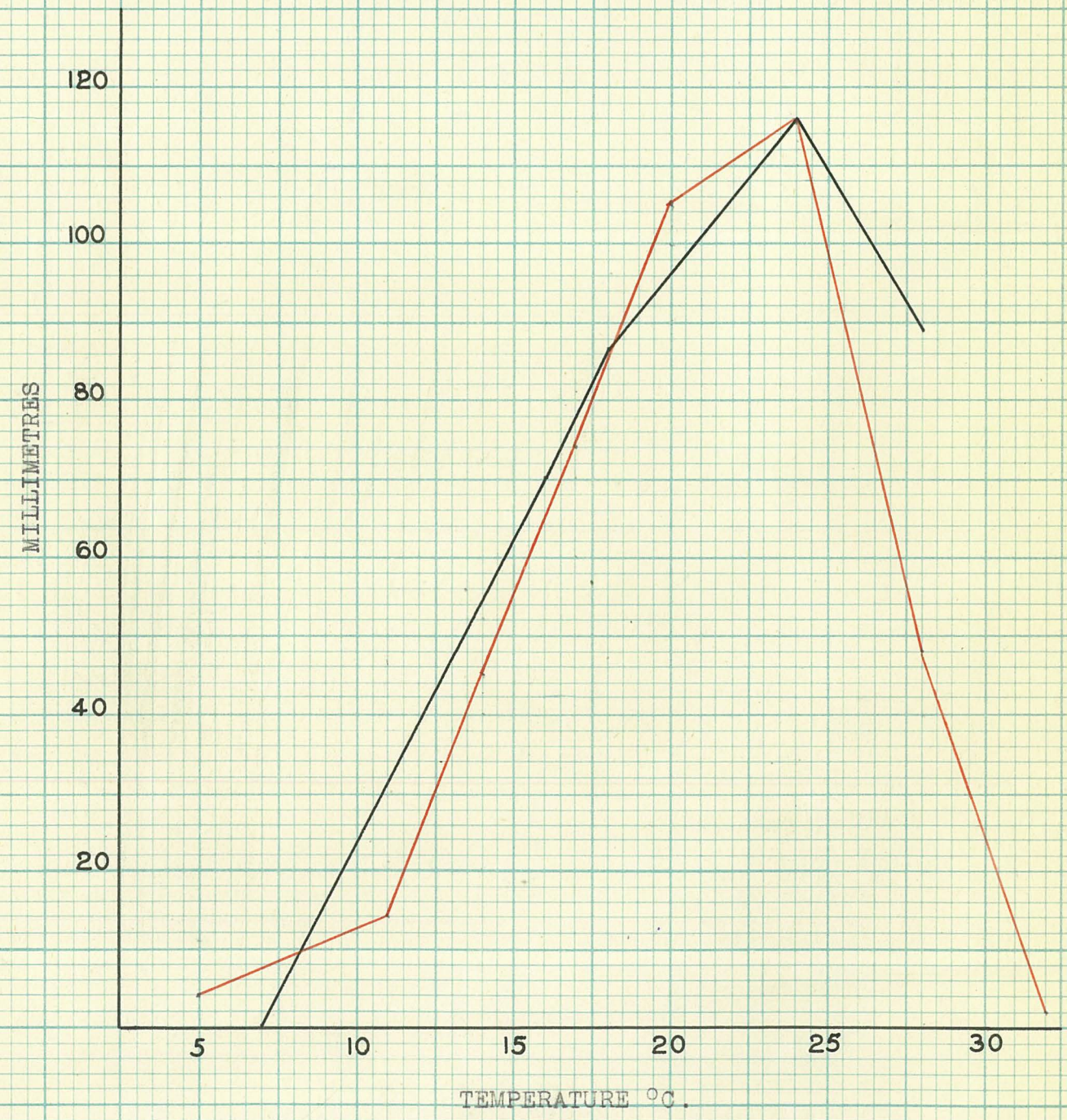



FIGURE 7

Germination of S. graminis in distilled water at 24.0 ± .5°C., time intervals (hours) are measured from the start of the experiment.

FIGURE 8

Effect of temperature on germ tube growth of *B. graminis* on P.D.A. after twelve hours, comparison is made with colony growth on P.D.A.


120
100
80
60
40
20
MILLIMETRES
5
10
15
20
25
30
TEMPERATURE °C.
Germ tube length
Relative colony growth

germination on distilled water closely followed the pattern observed on P.D.A.

TABLE XVI.

Effect of Temperature on Germ Tube Length of S. graminis on P.D.A. and Distilled Water after Twelve Hours.

| Temperature in degrees centigrade | | 7 | 16 | 18 | 24 | 28 |
|---|---|---|---|---|---|---|
| Germ tube length in microns | P.D.A. | nil | 70.2 | 86.8 | 112.9 | 79.2 |
| | Water | nil | trace | 47.1 | 55.0 | nil |

Both the "spore germination time" and the germ tube lengths show that 24°C. is the optimum temperature for this fungus on P.D.A. and distilled water. These results agree with the colony growth measurements on P.D.A. as presented on page 61.

(4) Physiological Studies

(a) The Effect of Media on Colony Growth

Petri plates were inoculated by the "mycelial fragment" technique. They were then held at 24° ± .5°c. for nine days.

TABLE XVII.

Effect of Media on Growth Rate of *S. graminis* after Nine Days at 24°±.5°C.

| Medium | Mean Colony diameter in mms. |
|---|---|
| Difco P.D.A. | 160 |
| Cocksfoot | 160 |
| Oatmeal | 152 |
| Cocksfoot leaves | 145 |
| Carrot leaves | 138 |
| Carrot | 132 |
| Prune | 130 |
| Lab. P.D.A. | 128 |
| Cornmeal | 112 |
| Milk | 101 |
| Water | 92 |
| Soybean | 86 |
| Tomato | 82 |
| Malt | 64 |
| Pea stems | 56 |

Growth on laboratory P.D.A. produces colonies which are composed of a dark green, slightly convoluted, mycelial mat of a tough, leathery nature. This mat is raised a little above the agar surface and covered by a carpet of short white aerial hyphae, (Plate 13.). No spores could be found on P.D.A. On commercially prepared Difco P.D.A. the aerial hyphae are much longer,

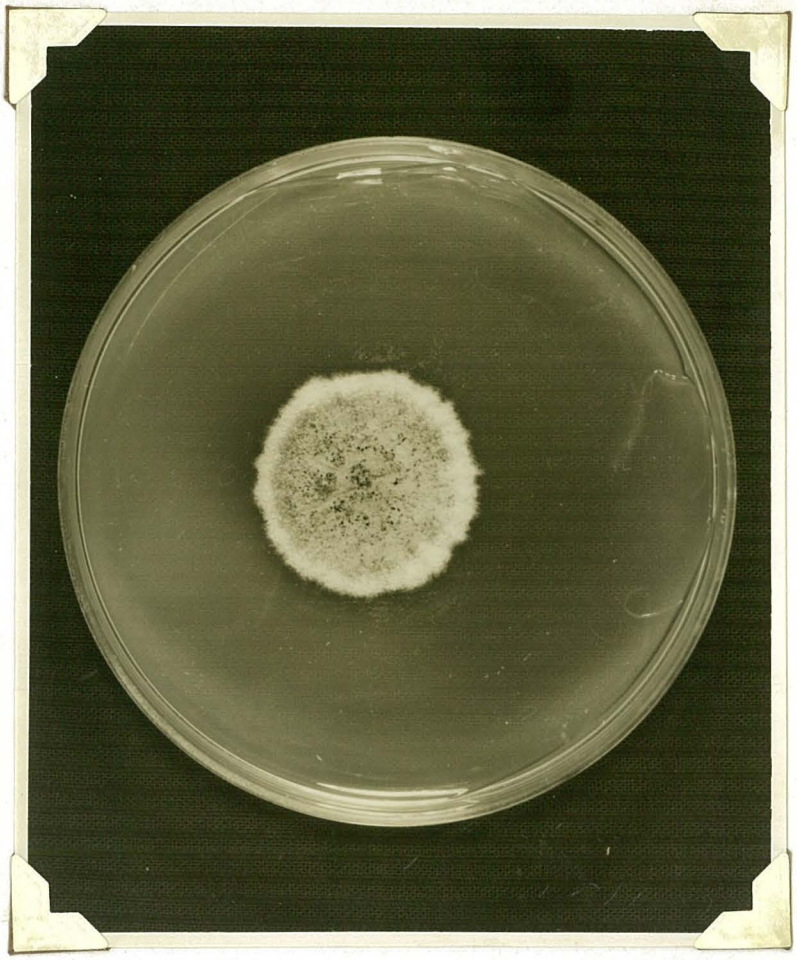


PLATE 13. A twenty-one day old colony of *S. graminis* grown at 24° C. on P.D.A., diameter = 320 mms.

especially around the periphery, thus giving the colonies a larger diameter.

On Oatmeal agar the fungus assumed a different growth form. The mycelium did not project above the surface, was not so thick, and the colonies were yellow at the centre and white around the edge.

On Cornmeal agar colonies were produced with white, aerial mycelium, quite unlike those on the other media.

(b) The Effect of Temperature on Colony Growth

TABLE XVIII.

Effect of Temperature on Growth Rate of *S. graminis* on P.D.A. after Nine Days.

| Temperature in degrees centigrade | 5 | 11 | 14 | 17 | 20 | 24 | 28 | 32 | 36 |
|---|---|---|---|---|---|---|---|---|---|
| Average colony diameter in mms. | 18 | 28 | 59 | 88 | 119 | 127 | 62 | 16 | nil |

At 32°C. growth was very slight and the colonies were extremely convoluted. At 28°C. they were still very convoluted and the under surface of the colonies badly cracked. At all other temperatures colonies were regular in outline and only slightly ridged. Sporulation did not occur at any of the temperatures.

(c) The Effect of Hydrogen Ion Concentration on Colony Growth.

TABLE XIX.

Effect of pH on Growth Rate of S. graminis on P.D.A. after Nine Days at 24°±.5°C.

| pH | 3.0 | 3.4 | 3.9 | 4.4 | 5.0 | 5.9 | 6.7 | 7.6 | 8.3 | 8.6 | 9.0 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Mean colony diameter in millimetres | 18 | 48 | 77 | 109 | 160 | 157 | 152 | 153 | 142 | 132 | 111 |

Below pH 4.4 the colonies of S. graminis were very similar to those grown at temperatures at 28°C. or higher, that is very convoluted and stunted with the dome shape of the central area more pronounced than at the other pH levels. Between pH 5.0 and pH 7.6 colonies were unrestricted by hydrogen ion concentration. From pH 7.6 to pH 9.0, although the macroscopic appearance of the colonies was normal, the effect of alkalinity became more apparent in its retarding effect on growth.

## D. *Selenophoma donacis* var. *stomaticola* (Bauml.) Sprague and A.G. Johnson.

### (1) Taxonomy and Review of Literature

Fungi classified under the genus *Selenophoma* were originally described as a species of *Septoria*. Passerini (1878) is cited by Sprague and Johnson (1950) as being the first to describe a sphaeropsidaceous species with non-septate, falcate spores, namely *Septoria donacis* Pass. on *Arundo donax*. *Septoria oxyspora* Penz. and Sacc. was recorded in 1884, also on *Arundo donax*. Sprague and Johnson (1950) compared the spore dimensions of the two and pronounced them as being only two collections of the same fungus.

Maire (1906) erected the genus *Selenophoma* in an endeavour to cover the pycnidial species with hyaline, falcate spores of *Septoria*. On the basis of Maire's description, Sprague and Johnson (1940) concluded that *Selenophoma* is the logical name for the genus. They considered that, -"certain fungi with non-septate, falcate spores borne in small globose pycnidia with coarse globose peridial cells, were more logically assigned to *Selenophoma* Maire than to *Septoria* Fries". In 1945 these two authors amended the genus also to include species with somewhat obtusely pointed spores, other chacteristics agreeing. In this paper they also proposed that *Phyllosticta stomaticola* Bauml. be included in *Selenophoma donacis* (Pass.) Sprague and A.G. Johnson and proposed the name *Selenophoma donacis* var. *stomaticola* (Bauml.) Sprague and A.G. Johnson.

Frandsen (1943) erected the genus *Lunospora* to include those species with spherical, compact pycnidia bearing falcate, hyaline spores. Frandsen's *Lunospora* was described during World War II when communication with other workers was greatly restricted. Petrak (1947) after a critical observation of the genus *Lunospora*

pronounced it as identical with Selenophoma Maire. The type species, Lunospora oxyspora (Penz. and Sacc.) Frandsen, based on Septoria oxyspora Penz. and Sacc. was also considered a synonym of Selenophoma donacis (Pass.) Sprague and A.G. Johnson.

Some confusion has arisen at various times by the finding of septate spores, for Selenophoma is fundamentally a genus with non-septate spores. Septa, when they occur, appear in spores which have been mature for a considerable period of time, usually after over-wintering on the dead host. Sprague and Johnson (1950) report the finding of septate spores from over-wintered material of S. donacis var. stomaticola on Elymus glaucus in Washington. However, septation of over-wintered spores is not particularly common. The type species Phyllosticta stomaticola Bauml. which is the basis for S. donacis var. stomaticola is itself based on winter conidia. In the original description, however, the spores were described as aseptate.

Sprague and Johnson (1947) list the following eight synonyms for Selenophoma donacis (Pass.) Sprague and A.G. Johnson:-

Septoria donacis Pass. 1878
Septoria oxyspora Penz. and Sacc. 1884
Septoria curva Karst. 1887
Rhabdospora curva Allesch. 1901
Selenophoma curva Petrak, 1940
Lunospora curva Frandsen, 1943
Lunospora oxyspora Frandsen, 1943
Lunospora baldingerae Frandsen, 1943

Within the species Selenophoma donacis are many fungi which cannot justifiably be separated into species because many of them grade from one group type to another. Petrak (1929) created the species Selenophoma drabae (Fckl.) Petrak to cover these groups. Sprague (1950)[1] believed that this species was "too inclusive to be practical". Sprague and Johnson (1947) grouped all the fungi in this complex under the heading Selenophoma donacis var. stomaticola (Bauml.) Sprague and A.G. Johnson. Synonyms for this variety are given by them as:-

Phyllosticta stomaticola Bauml., 1890
Septoria culmifida Lind, 1907
S. oxyspora var. culmorum Grove, 1916
S. oxyspora var. penniseti Trott., 1916
S. suboxyspora Lobik, 1928
S. lunata Grove, 1935
Lunospora culmifida Frandsen, 1943
L. culmorum Frandsen, 1943
L. suboxyspora Frandsen, 1943
L. lunata Frandsen, 1943
L. penniseti Frandsen, 1943
L. avenae Frandsen, 1943

In 1950 Sprague and Johnson divided S. donacis var. stomaticola into ten major groups on the basis of host range. This differentiation appears to be somewhat tentative. Cross inoculations between groups have been attempted on a few grasses with negative results but there are still a considerable number of known hosts which have

not been classified into groups.

Group two contains the races attacking Dactylis glomerata and Koeleria cristata. The isolates from the two hosts are morphologically identical and will cross inoculate.

Sprague (1950)[1] discussed the importance of S. donacis var. stomaticola and stated that the disease it causes on some clones was so severe as to reduce greatly seed set, with lesions occurring on all leaves and even on the heads. Apparently clones differ widely in their disease susceptibility. It has been noticed in the Manawatu that S. donacis var. stomaticola in extremely severe on the seed heads of some plants.

Allison (1945) and Sampson and Western (1954), reported that pycnidia and conidia are splashed about by rain, accounting for local spread of infection. Allison, however, reported the collecting of pycnidia half a mile away from infected plants. He theorizes that pycnidia may even be carried greater distances by the wind. This seems to be an extraordinarily great distance for whole pycnidia to be transported by air currents.

Seed infection of s. bromigena is reported by Allison (1945). In cases of severe infection the fungus fructifies in the glumes and occasional pycnidia are found enclosed between the lemma and palea in commercially dressed seed.

Selenophoma is classified in the Fungi Imperfecti by Clements and Shear (1931) as follows:-

Fungi Imperfecti

Phomales (Sphaeropsidales) - conidia borne in a pycnidium.

Phomaceae - pycnidia globoid, conic or lentiform, membranous,

carbonous or sub-coriaceous, innate, erumpent or superficial, ostiolate or astomous, separate or with a subicle or stroma, which is variously loculate, typically dark; conidia various, borne on simple or ramose basidia, or arising from the pycnidial wall.

Hyalosporae - conidia one celled, hyaline, globose, ovoid, ellipsoid or botuliform.

Selenophoma - pycnidia separate, sometimes cespitose, without subicle or stroma, not rostrate or cylindric, glabrous, ostiolate, not maculicole; conidia neither catenate not ciliate; basidia typically simple; spores lunate.

## (2) Symptoms

### Leaves

Lesions are of the eyespot type and may be 10 x 5mm. in size. Young spots are purple in colour and as they mature the borders darken and the centre becomes a dark cream. This area is composed of thin dead cells which can easily be lifted off. Sometimes small light brown pycnidia are embedded in this central area, but generally they occur on the under side of the leaf. Surrounding the complete lesion is a pale yellow area or halo. The lesions appear very similar on the lower leaf surface except that there are more pycnidia present. These are found between the leaf veins and often there are present only one or two large pycnidia; in other cases there may be up to six small pycnidia. They are very prominent and are only partially embedded in the dead tissue.

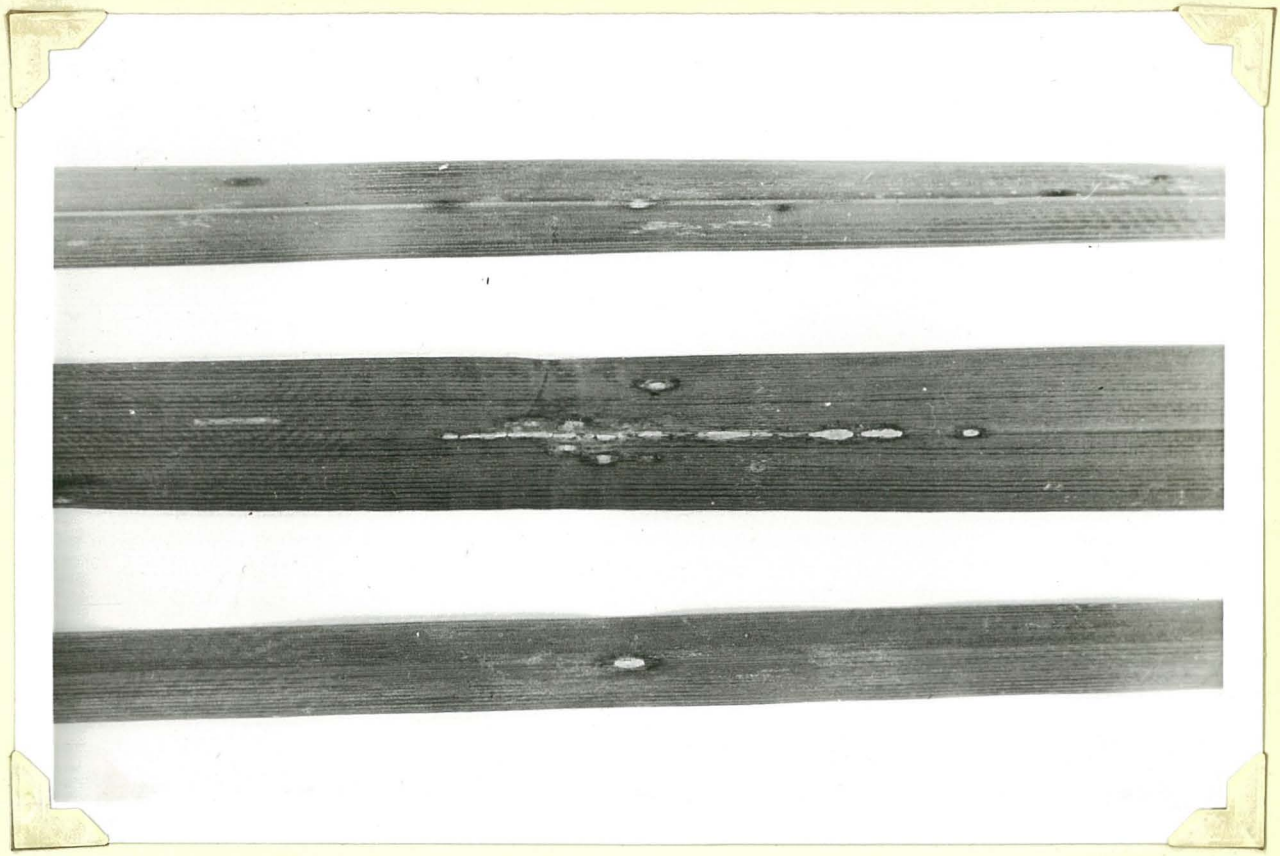


PLATE 14. Lesions caused by S. donacis var. stomaticola on leaves of Cocksfoot.

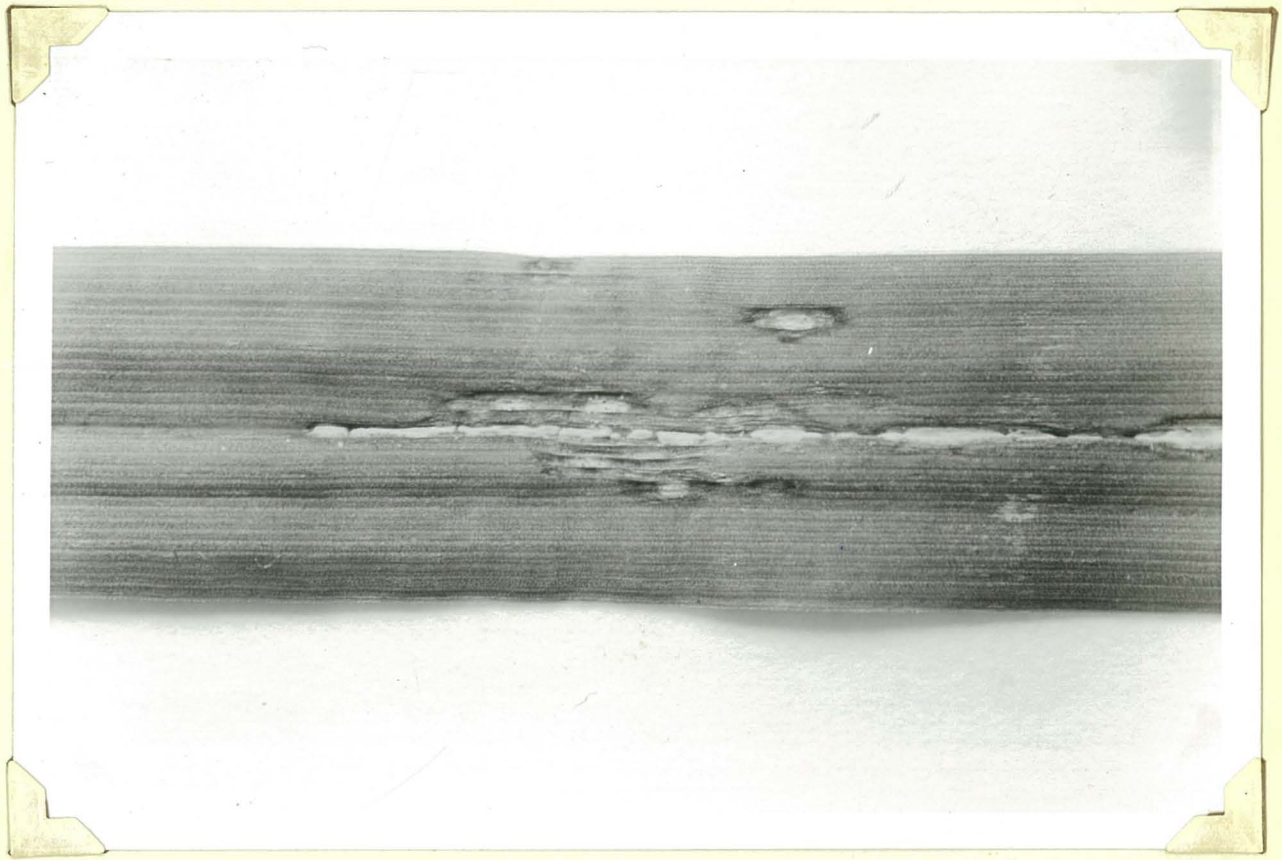


PLATE 15. Magnified view of one of the above lesions to show the pycnidia.

In some areas of the leaf the original lesions have spread so that large patches of tissue are killed and turn brown. In this straw-coloured area pycnidia are found in great numbers, even though there may not be any definite spots present. Sometimes the fruiting bodies of S. graminis may be found in these dead areas. It is very probable that Selenophoma was the original pathogen and S. graminis the secondary invader. This has been noted in artificially diseased plants. On severely diseased leaves the distil portion of the leaf withers but by the time this has happened most of the pycnidia have discharged their spores.

Culms

The pathogen is widely found on the seed stalks and lesions even occur amongst the florets. These lesions are restricted by the ribs on the stem and are creamy with light brown borders, being so numerous that quite often it is hard to find any tissue that is not diseased. The pycnidia are smaller than those on the leaves and are not restricted to the lesions.

Common names for this disease are Eye Spot or Halo Spot.

## (3) The Possibility of Seed Infection

Infection of cocksfoot by S. donacis var. stomaticola was severe on the seed heads of some plants, suggesting the possibility of seed infection. To test this hypothesis, seed (including the palea) from severely diseased seed heads were removed from the rachilla, and one half were surface sterilized. This was achieved by placing them in a muslin bag, pre-soaking for twenty seconds in seventy per cent ethyl alcohol, and then in .001 per cent mercuric chloride solution for one and a half minutes. They were then

PLATE 16. Lesions caused by S. donacis var. stomaticola on the flower stalks of Cocksfoot.

washed thoroughly in tap water and rinsed in sterile water. Both surface sterilized and non-treated seeds were plated out on P.D.A., approximately ten seeds per plate. They were incubated at 24°C. and observed each day.

Non-sterilized seed plated on to P.D.A. yielded a great variety of fungi and bacteria. Although over one hundred seeds were ex amined closely in no instance were colonies of S. donacis var. stomaticola observed.

Fungi of any type failed to grow out from surface sterilized seeds plated on P.D.A., suggesting either that no deep seated mycelium was present, or that this treatment was so severe as to kill the mycelium which may have been present in the seed. The former theory is favoured because this method of surface steriliz-ation was found to be the most satisfactory in tissue plating work, the surface contaminants being killed while the mycelium of the pathogen located within the host tissues was unaffected.

These limited results do not preclude the possibility of seed infection. It is pertinent to note that Allison (1945) working with a closely related species found that, "pycnidia may be seed borne in cases following severe infection when the fungus fruct-ifies on the glumes, as S. bromigena has been isolated from such seed". No record of seed infection of Dactylis glomerata by S. donacis var. stomaticola has been found in the literature.

## (4) Spore Studies

### (a) Sporulation and Spore Description

Pycnidia are readily found in the lesions on diseased material. They were gouged out, mounted in lactophenol fuchsin

PLATE 17. A pycnidium and pycnidiospores of S. donacis var. stomaticola from a lesion on Cocksfoot. Note the well defined ostiole. Magnification, approximately 600.

and their pycnidiospores measured. From observations on measurements made throughout the year, it appeared that the season influenced the dimensions of the spores. In view of this an analysis of variance was carried out, comparing spores measured in summer with those collected and measured in late autumn, with the following results:-

TABLE XX.

Seasonal Effect on Spore Dimensions of

S. donacis var. stomaticola

| Dimensions | Summer | Late Autumn |
|---|---|---|
| Mean length | 16.9µ SD. .34µ | 13.5µ SD. .30µ |
| Mode | 16.5µ | 13.2µ |
| Range | 14.9 - 21.5µ | 9.9 - 16.5µ |
| Mean width | 2.7µ | 2.5µ |
| Range | 1.7 - 3.3µ | 1.7 - 3.3µ |

Pycnidia On stalks 20 - 75µ x 25 - 95µ

On leaves 50 - 115µ x 65 - 140µ.

TABLE XXI.

Dimensions of Pycnidia and Pycnidiospores

Recorded in Overseas Literature

| Authority | Sampson and Western (1954) | Sprague (1950) | Shaw (1953) |
|---|---|---|---|
| Pycnidia | - | 40-150µ x 40-110µ | 60-100µ |
| Spores | 13-22µ x 3-4µ | 10-20µ x 1-3µ | 10-20µ x 2µ |

The pycnidia are dark brown and occur between the veins on either side of the lesion. They are round or slightly elongated with a prominent ostiole. The spores are falcate, non-septate and hyaline. On over-wintered material a septum is occasionally present.

Selenophoma donacis var. stomaticola sporulates readily in culture. When comparing spore dimensions from cultures grown on different media, differences were noticed between the means. The means were therefore statistically analysed and compared using Duncan's Range Test (1955), with the following results:-

TABLE XXII.

Spore Lengths in Summer and Late Autumn.

| Spore length | Summer | Late Autumn |
|---|---|---|
| X | f | f |
| 6 | | 2 |
| 7 | | 23 |
| 8 | | 99 |
| 9 | 26 | 53 |
| 10 | 75 | 6 |
| 11 | 39 | |
| 12 | 9 | |
| 13 | 1 | |

| | | | |
|---|---|---|---|
| $n$ | = | 150 | 183 |
| $X$ | = | 1534 | 1502 |
| $X^2$ | = | 15790 | 12428 |
| $\frac{X^2}{n}$ | = | 15688 | 12328 |

TABLE XXIII.

Analysis of Variation in Spore Lengths

| Source of Variation | d.f. | s.s. | m.s. | F |
|---|---|---|---|---|
| Between seasons | 1 | 336 | 336 | 210 p .001 xxx |
| Within seasons | 331 | 202 | 1.6 | |
| Total | 332 | 538 | | |

The result of this analysis shows that the differences in spore lengths gathered in the summer and those gathered in the late autumn were very highly significant. In view of these findings, the importance of stressing the period of the year when spore dimensions were taken cannot be overemphasized.

TABLE XXIV.

Comparison of Spore Lengths on Various Media

after Nine Days at 24° ± .5°C.

| Spore length | Tomato | P.D.A. | Oat | Corn | Milk |
|---|---|---|---|---|---|
| 6 | | | | | 2 |
| 7 | | | | | 3 |
| 8 | | 5 | 2 | 3 | 9 |
| 9 | 2 | 10 | 15 | 16 | 10 |
| 10 | 26 | 23 | 28 | 27 | 21 |
| 11 | 16 | 4 | 5 | 4 | 5 |
| 12 | 5 | 3 | | | |
| 13 | 1 | 3 | | | |
| 14 | | 2 | | | |
| n = | 50 | 50 | 50 | 50 | 50 |
| $\bar{x}$ = | 10.54 | 10.14 | 9.27 | 9.64 | 9.20 |

$$S.E. = \sqrt{\frac{E.M.S.}{\text{number of items in the mean}}} = .1465$$

Duncan's Range Test (1955)

| | (2) | (3) | (4) | (5) | |
|---|---|---|---|---|---|
| 1 % level | 3.71 | 3.86 | 3.98 | 4.06 | = Significant studentized ranges |
| | 0.54 | 0.57 | 0.58 | 0.59 | = Shortest significant ranges (S.S.R. x S.E.) |

| Media | Tomato | P.D.A. | Oat | Corn | Milk |
|---|---|---|---|---|---|
| Mean | 10.54 | 10.14 | 9.72 | 9.64 | 9.20 |
| Mean in microns | 17.39 | 16.73 | 16.04 | 15.90 | 15.18 |

N.B. Any two means not underlined by the same line are significantly different.

Any two means underlined by the same line are not significantly different.

Expressing these results in another form shows that:-

(i) The lengths of spores from colonies grown on Tomato agar are significantly greater than those grown on Oatmeal, Cornmeal and Milk agars.

(ii) Spores produced on P.D.A. are significantly greater in length than those from Milk agar.

(iii) Spores from colonies grown on P.D.A., Oatmeal and Cornmeal agars did not differ significantly.

(iv) Spores from colonies grown on Oatmeal, Cornmeal and Milk agars did not differ significantly.

(v) Spores from colonies grown on Tomato and P.D.A. did not differ significantly.

### (b) Spore Germination

#### (i) On P.D.A.

The same general description of germination on P.D.A. is applicable to all spores which germinate within the range of temperatures tested, that is $7^{o}$ - $28^{o}$C. The spore first swells and one or two

septa are formed. The swelling is usually confined to the ends but this is not invariable. Within the next few hours germ tubes develop from both ends of the spore and it very soon becomes impossible to distinguish the section which comprised the original spore. Lateral hyphae branch out from the germ tubes and septa form on the germ tubes and their branches.

Because of the inability to distinguish the spore soon after germination and also because of the profuse hyphal branching, it was impossible to measure the germ tube lengths after stated intervals of time had elapsed. Comparing the growth after eighteen hours, no difference could be detected between growth of spores at 20° and 24°C. although spores at the latter temperature germinated one and a half hours earlier. Spores incubated at 28°C. had the same germination time as those incubated at 20°C. but once they had sent out a germ tube of several microns in length they appeared almost to cease growing and remained at this stage during the following twentyfour hours.

At both 20° and 24°C., sporulation was observed to have occurred after thirtyeight hours. Between fifty and one hundred spores were produced in the central region of each thallus.

TABLE XXV.

Effect of Temperature on "Spore Germination Time" of

S. donacis var. stomaticola on P.D.A.

| Temperature in degrees centigrade | 7 | 14 | 20 | 24 | 28 | 32 |
|---|---|---|---|---|---|---|
| Time in hours | 16 | 12 | 8.5 | 7 | 8.5 | ∞ |

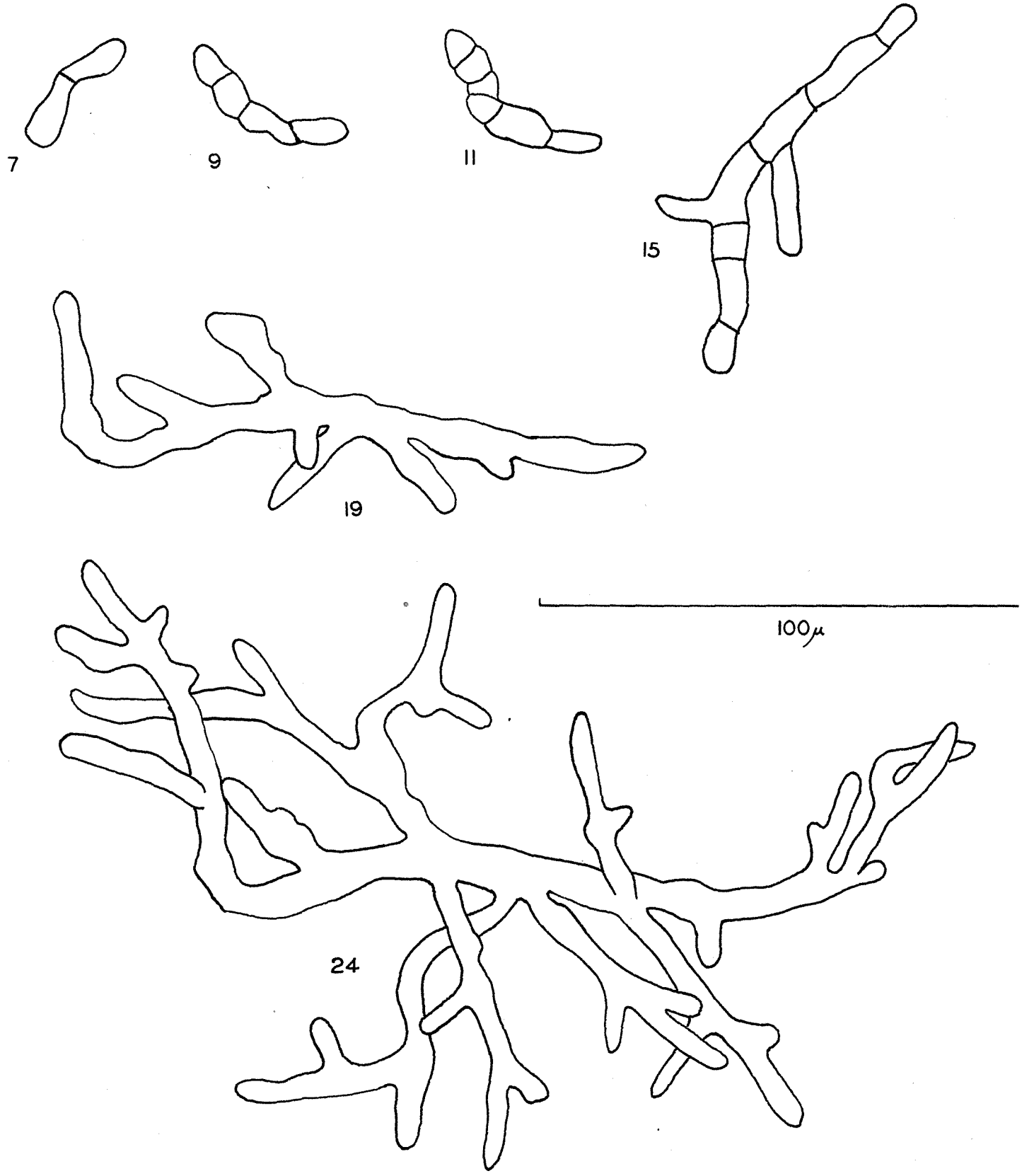



FIGURE 9

Germination of S. donacis var. stomaticola on P.D.A. at 24° ± .5°C., time intervals (hours) are measured from the start of the experiment.

(ii) On Distilled Water.

When spores of Selenophoma germinate in distilled water they usually send out a single, terminal germ tube which grows straight and unbranched for periods of up to twelve hours. The germination time at the various temperatures was as follows:-

TABLE XXVI.

Effect of Temperature on "Spore Germination Time" on S. donacis var. stomaticola in Distilled Water

| Temperature in degrees centigrade | 7 | 14 | 20 | 24 | 28 | 32 |
|---|---|---|---|---|---|---|
| Time in hours | 19 | 16 | 12 | 11 | 12 | $\infty$ |

Growth in distilled water was slow. After twentyfour hours the one or two terminal germ tubes remained unbranched and at 20°C. and 24°C. averaged about one hundred and fifty microns in length.

(5) Physiological Studies

(a) The Effect of Media on Colony Growth

Inoculation of plates was by the "spore needle-tip" technique. Duplicate plates were set up at 24° ±.5°C.

TABLE XXVII.

Effect of Media on Growth Rate of S. donacis var. stomaticola after Nine Days at 24°±.5°C.

| Medium | Mean Colony Diameter in mms. |
|---|---|
| Tomato | 160 |
| P.D.A. | 145 |
| Oatmeal | 127 |
| Carrot | 126 |
| Cocksfoot | 123 |
| Carrot leaves | 108 |
| Cornmeal | 101 |
| Malt | 98 |
| Soybean | 92 |
| Prune | 83 |
| Milk | 60 |
| Cocksfoot leaves | 53 |
| Water | 34 |

The inoculum for this experiment was one generation removed from the original spore suspension obtained from natural spores and had been cultured on P.D.A. for fourteen days at 24°C. This fungus cannot be cultured on artificial media for many generations without changing its form. On P.D.A. the first generation colonies are generally circular in outline, elevated a little above the agar surface, and slightly wrinkled. Masses of conidia are produced directly on the mycelium and the over all colour of the colonies is lavender with a pink area in the centre. Viewed

PLATE 18. A twenty-one day old colony of S. donacis var. stomaticola grown at 24°C. on P.D.A., diameter = 370 mms.

from the reverse side the colonies are pink with purple edges. The second generation (that is sub-cultures of the first generation) contains a few variant forms. By the time the fourth generation is reached there are few colonies similar to the original culture. The variants may be classified into three main types.

(i) Colonies that are more convoluted and raised from the agar than is the true form. They are a deep yellow colour and secrete a yellow substance into the agar so that the entire P.D.A. plate is tinged yellow.

(ii) Colonies that are dark brown with a very slight purple tint. The central region is convoluted into a black "rope" of conidia. The periphery of the colonies is lighter in colour and more aerial, giving the appearance of a light mycelial fringe. Both these variants have approximately the same dimensions as the true form.

(iii) Colonies that consist of a central area of vegetative mycelium and conidia from which a number of thick brown rhizomorph strands develop radially. This variant form is entirely dissimilar to any of the other forms.

From the above results, it is apparent that any presentation of results of investigations on the morphology and physiology of this pathogen are almost valueless unless accompanied by information indicating the number of generations removed from fungal isolations and the environmental conditions under which the organism was grown.

(b) The Effect of Temperature on Colony Growth

TABLE XXVIII.

Effect of Temperature on Growth Rate of

S. donacis var. stomaticola on P.D.A. after Nine Days.

| Temperature in degrees centigrade | 5 | 11 | 14 | 17 | 21 | 24 | 28 | 32 |
|---|---|---|---|---|---|---|---|---|
| Average colony diameter in mms. | 28 | 92 | 119 | 127 | 138 | 147 | 16 | nil |

The inoculum for this experiment was one generation removed from natural material and was grown on P.D.A. at 24°C. for fourteen days. Maximum growth was at 24°C. but dropped away very sharply at temperatures higher than this. Above 24°C. the colonies lost their circular outline and became extremely convoluted. Below 24°C. the rate of growth dropped away gradually. At 14°C. the hyphae became more aerial, the maximum amount being at 5°C. so that colonies had quite a different appearance from those grown at optimum temperature.

(c) The Effect of Hydrogen Ion Concentration on Colony Growth

TABLE XXIX.

Effect of pH on Growth Rate of S. donacis var. stomaticola

on P.D.A. after Nine Days at 24° ±.5°C.

| pH | 3.0 | 3.4 | 3.9 | 4.4 | 5.0 | 5.9 | 6.7 | 7.6 | 8.3 | 8.6 | 9.0 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Mean colony diameter in mms. | 50 | 66 | 128 | 131 | 136 | 135 | 140 | 98 | 95 | 95 | 85 |

As with Scolecotrichum graminis, high acidity levels caused the colonies to be stunted, irregular in shape, and convoluted. The optimum range for growth is from pH. 4.4 - 6.7 although, outside this optimum, growth does not fall off so rapidly as it does for the other four fungi.

E. Stagonospora maculata (Grove) Sprague

(1) Taxonomy and Review of Literature.

The genus Stagonospora was erected by Saccardo in 1884. Sprague(1950)states that the genus Stagonospora is separable from Septoria with some difficulty because dimensions of pycnidiospores are very similar. In such instances the species are left in the genus to which they have been customarily referred.

The species Stagonospora subseriata was first described by Desmazieres in 1846. He recorded finding it on Enodium coeruleum but named the pathogen Hendersonia subseriata Desm. When Saccardo created the genus Stagonospora he changed the name of this species to Stagonospora subseriata (Desm.) Sacc.

Grove (1935) recorded finding a distinct form of Stagonospora subseriata on Dactylis glomerata. The pycnidia were arranged in lines on sub-elongated blackish brown blotches, but they have spores similar to S. subseriata. He named this variant Stagonospora subseriata var. maculata Grove.

Sprague (1941) records finding this fungus on Dactylis glomerata in the U.S.A. The spores were fusiform, 3-4 septate, yellow with coarse contents, and 27-40 x 4.8-6.5μ, with a mean size of 33-5.5μ. They were borne in globose golden brown pycnidia with walls 5μ in thickness. Sprague observed that spores varied in the degree of constriction at the septa. Graham (1952) points out that the degree of constriction depends on the season. Conidia from over wintered tissue and from new spring leaves are usually constricted and contain large globules near the septa. Spores produced in culture and those gathered in the autumn have little or no constrictions.

Sprague (1948) compared S. subseriata and the variety maculata from the viewpoint of morphology, parasitic nature and type of symptoms produced on their respective hosts. He considered the differences to be of such a magnitude as to warrant elevation of the variety maculata to specific rank, and proposed the name Stagonospora maculata (Grove) Sprague.

Sprague (1948) has emphasised the importance of S. maculata in causing a leaf disease of cocksfoot in pastures in the middle Atlantic States of North America. Roberts et al. (1952, 1954, 1956) have estimated that losses in the region, of one to two per cent, can be accredited to this fungus. Graham (1952) stated that the fungus causes a major disease of cocksfoot in the North-west region of the U.S.A. He found leaf lesions on almost all cocksfoot plants throughout the growing season and often entire blades were killed.

The only formal work carried out on this organism is that by Graham (1952), and Graham and Sprague (1953). This is surprising in view of the recognition of its importance as a foliage pathogen of cocksfoot.

## (2) Symptoms

Lesions first appear as very small watery spots. These darken rapidly, almost to a black tint and at the same time enlarge up to five millimetres in length. (Plate 19.). Young watery lesions are very similar to those caused by Scolecotrichum graminis. As the lesions increase in size, however, they are easily distinguished because those caused by S. graminis elongate greatly, whereas those of S. maculata do not. Mature lesions of

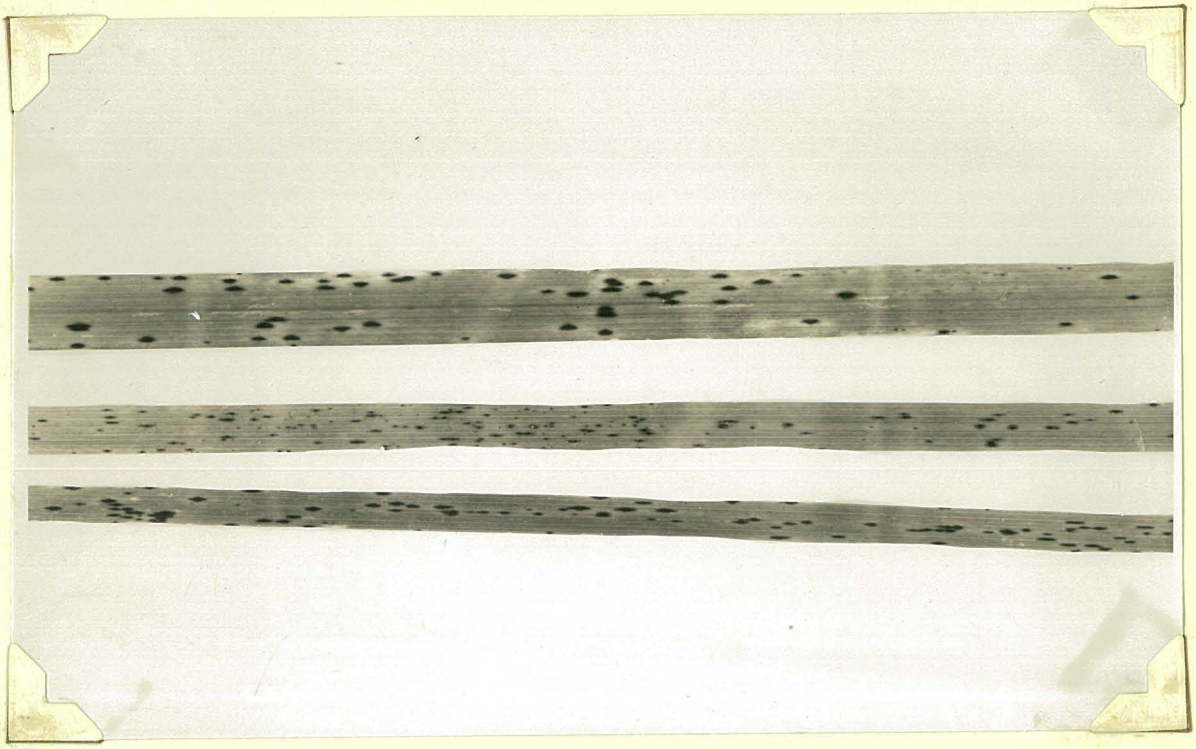


PLATE 19. Lesions caused by S. maculata on the leaves of Cocksfoot.

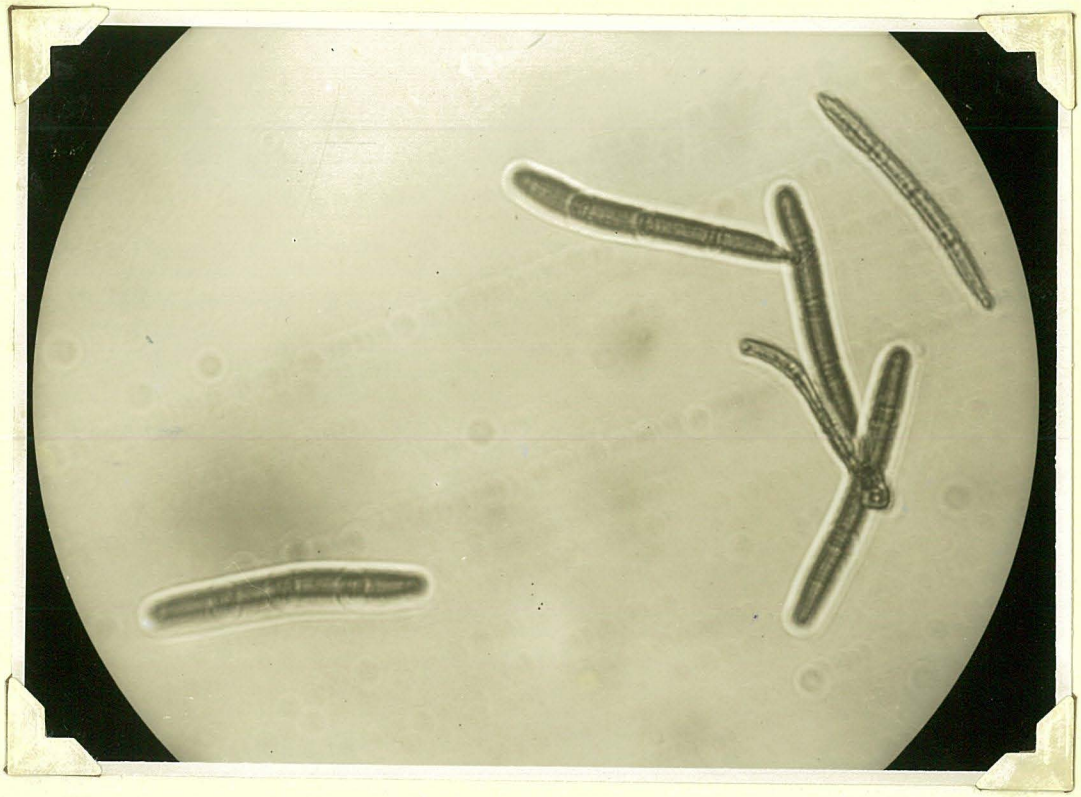


PLATE 20. Pycnidiospores of S. maculata from P.D.A. Magnification approximately 750.

S. maculata do not develop a lighter area in the centre but remain a purple-black colour; hence the derivation of the common name, Purple Leaf Spot.

Graham (1952) stated that "black pycnidia may be visible under the raised epidermis". Although the disease was under close observation for almost two years and scores of diseased leaves were collected and examined under the microscope, in no instance were pycnidia observed.

Stagonospora maculata is classified in the Fungi Imperfecti because the perfect stage of the fungus has never been detected. Clements and Shear (1931) classify it as follows:-

Fungi Imperfecti

Phomales (Sphaeropsidales.) - conidia are borne in a pycnidium

Phomaceae - pycnidia globoid, conic or lentiform, membranous, carbonous or sub-coriaceous, innate, erumpent or superficial, ostiolate or astomous, separate or with a subicle or stroma, which is variously loculate, typically dark; conidia various, borne on simple or ramose basidia, or arising from the pycnidial wall.

Hyalophragmiae - conidia x-celled, oblong to fusoid, typically with distinct septa.

Stagonospora - pycnidia separate, innate or erumpent, globose; conidia muticate and normal.

## (3) Spore Studies

### (a) Sporulation and Spore Description

Spores of S. maculata have not been detected on lesions in the field. Many lesions of varying stages of maturity and at all seasons of the year have been collected. Detailed microscopic examination was carried out, both before and after lesioned leaves had been subjected to varying periods of high humidity. Lesions were teased out with a needle into a drop of lactophenol acid fuchsin and the resulting tissue suspension examined under the microscope. On no occasion were pycnidia or conidia of this fungus observed.

Sporulation in culture was not profuse and took three to four weeks to develop. It was found that this period could be shortened by exposing five day old covered cultures to a W.L.782 Westinghouse ultra-violet lamp at a distance of fifteen centimentres for five minutes. Pycnidia appeared after eight to twelve days on P.D.A., Cornmeal, Oatmeal, Tomato, Malt and Carrot agars, also on sterile cocksfoot and pea stems.

The degree of sporulation depended upon the isolate. Many isolates studied were sterile. Graham (1952) records that S. maculata failed to sporulate on common laboratory media, and that one of the four isolates he studied was sterile on all media. He obtained occasional pycnidia on Cornmeal, Bean Pod, and Alfalfa Infusion agars, with good sporulation on Soyabean, Clover Infusion agars, and sterile birdsfoot trefoil stems. It is possible that the discrepancy between Graham's observations and those recorded here is due to differences in the sporulation ability of the various strains studied.

Pycnidia produced on P.D.A. were large and easily seen with the naked eye. They were found to vary in size from 215-320μ x 240-400μ.

Dimensions of pycnidiospores produced on P.D.A. are as follows:-

| | Range | Mode | Mean |
|---|---|---|---|
| Length | 26.4-44.6μ | 36.3μ | 36.6μ S.D. 3.0μ |
| Width | - | - | 4.3μ |

On P.D.A. the pycnidiospores are contained in reddish, globose pycnidia which are surrounded by a mycelial thatch. Pycnidiospores are long and narrow, two to seven septate, with the mode at three. They are not constricted at the septa. The spore contents are very granular, and the spores are pointed at the tip and blunt at the base.

Dimensions of conidia produced on naturally diseased material are recorded by overseas workers as follows:-

| Sprague (1941) | Petrak (1927) cited by Sprague (1941) | Grove (1935) |
|---|---|---|
| 27-40μ x 4.8-6.5μ | 23-44μ x 3-6μ | 25-40μ x 6.7μ |

(b) Spore Germination

TABLE XXX.

Effect of Temperature on Spore "Germination Time" of S. maculata on P.D.A. and Distilled Water

| Temperature in degrees centigrade | | 6 | 15 | 18 | 24 | 28 | 31 |
|---|---|---|---|---|---|---|---|
| Germination time in hours | P.D.A. | 2.0 | 1.5 | 1.5 | 1.0 | 1.5 | 1.5 |
| | Water | 2.5 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |

The above table shows that the "germination time " on P.D.A. was only half an hour less than the time taken for ten spores to germinate in distilled water.

A characteristic feature of the germination of S. maculata seems to be that a few spores germinate well ahead of the majority. This rendered a critical comparison of germ tube lengths at the various temperatures impracticable, more particularly as the "germination times" were equally short and similar.

Germ tube measurements were taken six hours after inoculation and a broad over all picture could be gained from them.

The comparable growth of S. maculata on P.D.A. and in distilled water in the first twelve hours is understandable when one realises that the large spore provided some nutrients in the early stages of growth and so the composition of the medium is of minor importance at this stage.

Growth was slightly greater on P.D.A. at all temperatures but the difference was only in the region of ten to fifteen per cent. The optimum temperature appeared to be 28°C. and this was closely followed by growth at 24° and 31°C. This is surprising because

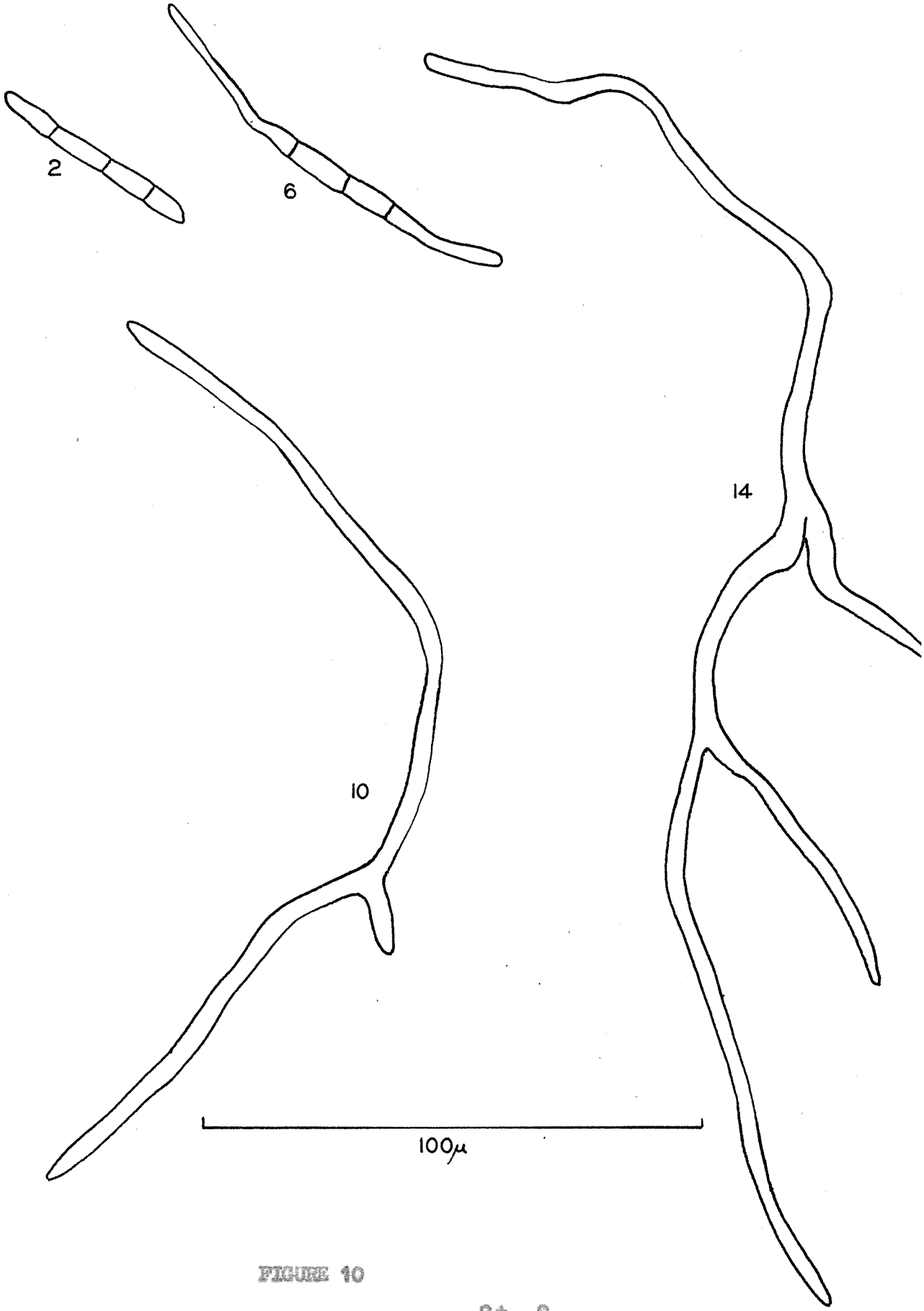



FIGURE 10

Germination of S. maculata on P.D.A. at 24° ± .5°C., time intervals (hours) are measured from the start of the experiment.

colony growth at 31°C. is almost negligible and at 28°C. is only a fraction of that at 24°C.

At 18°,15°, and 6°C. a very slight gradation in growth could be seen, although at each temperature there were individual spores developing germ tubes of much greater length than the average germ tube lengths at the other temperatures.

Germination on both P.D.A. and in distilled water took the form of one or two germ tubes emerging terminally from the spore. These tubes continued to grow unbranched for ten to twelve hours by which time the identity of the spore had been lost.

## (4) Physiological Studies

### (a) The Effect of Media on Colony Growth

Plates were inoculated by the "mycelial fragment" technique. All plates were incubated at 24°±.5°C. and the colony diameters measured after nine days.

TABLE XXXI.

Effect of Media on Growth Rate of S. maculata

after Nine Days at 24°±.5°C.

| Medium | Mean Colony Diameter in Millimetres |
|---|---|
| Cornmeal | 475 |
| Lab. P.D.A. | 469 |
| Difco P.D.A. | 447 |
| Carrot leaves | 440 |
| Cocksfoot | 433 |
| Oatmeal | 426 |
| Prune | 419 |
| Carrot | 407 |
| Pea stems | 342 |
| Cocksfoot leaves | 339 |
| Water | 316 |
| Soybean | 295 |
| Malt | 285 |
| Milk | 233 |

On P.D.A. the mycelium is white, aerial and dense but the colonies do not assume the form of a leathery mat as do the other four fungi on this medium. Most cultures develop a deep pink or red colouration at the centre and in many, these coloured areas may occur anywhere on the colony. Sporulation is very often associated with these areas.

Colonies on all media were very similar to those described on P.D.A. Minor differences were apparent, however, such as the

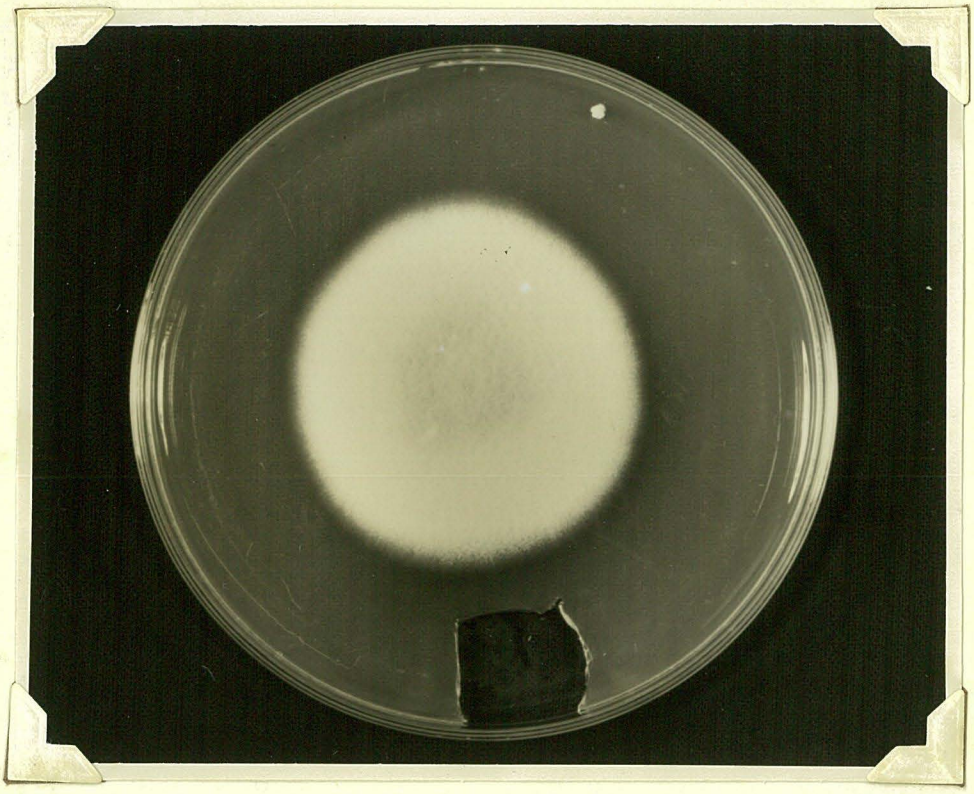


PLATE 21. An eleven day old colony of S. maculata grown at 24°C. on P.D.A. Diameter = 57.0 mms.

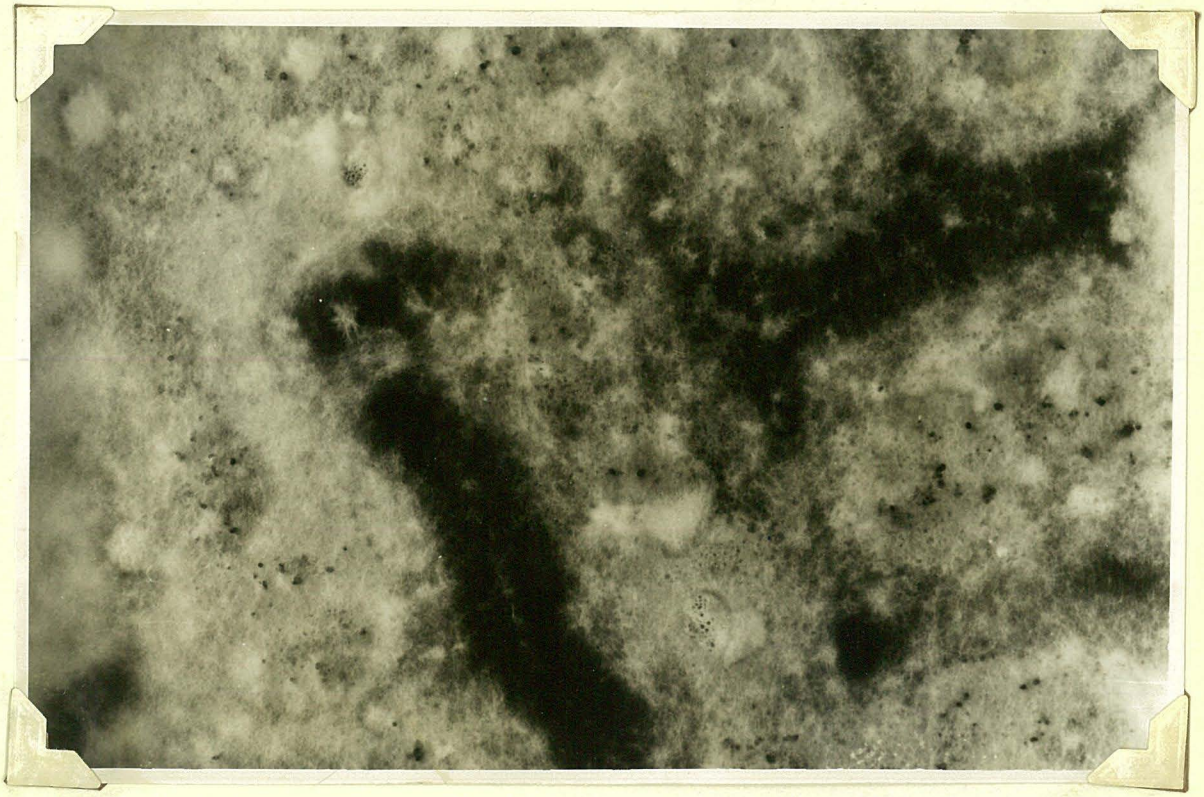


PLATE 22. An enlarged portion of a thirty-eight day old colony of S. maculata grown on P.D.A. Note the pycnidia.

lower density of mycelium on Cornmeal and Oatmeal agars, and the absence of colouration on many media.

(b) The Effect of Temperature on Colony Growth

TABLE XXXII.

Effect of Temperature on Growth Rate of *S. maculata* on P.D.A. after Nine Days.

| Temperature in degrees centigrade | 5 | 11 | 14 | 17 | 20 | 24 | 28 | 32 | 36 |
|---|---|---|---|---|---|---|---|---|---|
| Average colony diameter in mms. | 63 | 82 | 182 | 323 | 416 | 469 | 257 | 28 | trace |

Only the faintest suggestion of growth could be detected at 36°C.

At 32°C. the colonies were small and raised from the agar surface so that they appeared as balls of white mycelium resting on the agar. The under side of the colonies were deep red in colour.

The optimum temperature for growth was 24°C. and at lower temperatures growth fell away rapidly, although at 5°C. it was still quite appreciable.

Unfortunately, a sterile strain of *S. maculata* was used in this temperature experiment and recordings could not be taken of sporulation intensities at the various temperatures.

Comparison of the growth rate of the five fungi at their optimum temperatures for growth on P.D.A. reveals that *S. maculata* grows at three times the rate of *Selenophoma donacis* var. *stomaticola* and six times the rate of *Mastigosporium rubricosum*, the

fastest and the slowest growers, respectively. In addition, S. maculata has a much wider temperature-growth range than any of the others.

(c) The Effect of Hydrogen Ion Concentration on Colony Growth.

For each pH level one plate was inoculated at four, well separated, points and two plates were each inoculated in the centre. In this experiment, as with all the other pH experiments, Difco P.D.A. was used instead of the laboratory prepared P.D.A. Results are recorded in the following table:-

TABLE XXXIII.

Effect of pH on Growth Rate of S. maculata on P.D.A. after Nine Days at $24^{o} \pm .5^{o}$c.

| pH | 3.0 | 3.4 | 3.9 | 4.4 | 5.0 | 5.9 | 6.7 | 7.6 | 8.3 | 8.6 | 9.0 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Mean colony diameter in millimetres | 37 | 103 | 283 | 448 | 447 | 453 | 452 | 447 | 447 | 450 | 439 |

Antagonism occurred in those plates of pH range 4.4 - 9.0 which were inoculated at four points. This necessitated the taking of the average of only the two plates with the single inoculations. Hence a slight variation can be seen from pH 4.4 -8.7 where probably none should exist. This zone of unrestricted growth is greater for S. maculata than for any of the other four fungi.

The more acid media causes the fungus to form larger quantities of deep-red colouring than usual so that up to pH 3.4 the colonies have very conspicuous, large red centres.

# CHAPTER V

## THE SEASONAL SUCCESSION OF EACH DISEASE

### A. Field Observations

Observations on the seasonal occurrence of the various diseases were made at monthly intervals throughout the period November 1955 to July 1957. In the early stages, some difficulty was experienced in differentiating the five diseases on the basis of symptom expression in the field. However, by plating out the lesions and keeping dried specimens of similar lesions for comparison it was very soon possible to identify each individual disease.

It must be emphasised that the survey covered all situations under which cocksfoot normally grows. Observations were made in open paddocks where the shelter from sun and wind was at a minimum, in cocksfoot seed paddocks where the vegetation was much more rank and luxuriant, and in sheltered areas such as under trees and next to buildings. Plants growing in these last mentioned situations were generally attacked by leaf spot fungi to a greater extent, presumably because the microclimate was more favourable to the pathogens. Plants were often sheltered from the direct rays of the sun and the atmosphere was more humid.

1. General Survey of the Individual Diseases.

(a) Leaf Fleck, caused by Mastigosporium rubricosum.

This fungus was found to have a very limited season. It first appeared in mid-August, causing very severe lesioning, and was very abundant until early November. Lesions have not been found at any other period of the year. As demonstrated by Bollard (1950) and verified in this work, spores of M. rubricosum will not survive desiccation and so the hot, dry summer months tend to prevent the spread and growth of spores. Overseas reports (Bollard 1950, Sampson and Western 1952), indicate that the fungus is prevalent in the autumn as well as in early spring. Presumably the autumn is too dry and hot in the Manawatu district for infection by this fungus.

(b) Scald, caused by Rhynchosporium orthosporum

Lesions caused by R. orthosporum are never very common during any month of the year. A few diseased plants have been found at all times but one cannot state any particular season as being the peak period. Caldwell (1937) records that little disease was evident in Wisconsin during the summer or winter. The extremes of climate are not so pronounced in the Manawatu as in Wisconsin and this would, perhaps, explain the discrepancy between the two sets of observations.

(c) Leaf Streak, caused by Scolecotrichum graminis

The peak period for Leaf Streak disease is from mid-autumn until late spring. It diminishes in intensity during November and in the following two months occurs mainly on plants which are

sheltered from the sun. A few lesions appear in February and the disease gradually builds up in intensity, once more becoming common in April. Sporulation is not profuse on lesions produced during the summer. The cooler, moist autumn weather appears to favour sporulation.

S. graminis caused by far the most important leaf spot disease of cocksfoot in this district. Brief observations in Whangarei, Auckland, Wellington, Christchurch and Dunedin at various times of the year have shown that the foregoing statement is probably true for most of New Zealand.

(d) Eye Spot, caused by Selenophoma donacis var. stomaticola.

A few leaf spot caused by this fungus can be found at any month of the year although they are never very common until the spring. During October, November and December, lesions are fairly plentiful and in some cases may coalesce to kill the entire leaf. Cocksfoot plants flower in December and January and although lesions are hard to find on the leaves during these months, they may cover the flower stalks of many plants. The spread of pycnidiospores is mainly by rain splash and therefore a localised "flare-up" of the disease may occur during the summer months after wet periods.

(d) Purple Leaf Spot, caused by Stagonospora maculata.

Lesions were not observed during the winter and early spring. The earliest this disease has been detected was towards the end of November. The disease becomes fairly common in December but almost disappears during the next two months. It increases in

intensity during March and quickly reaches a peak, gradually tailing off until it disappears at the end of May. Like M. rubricosum, S. maculata causes a considerable loss of photosynthetic tissue at its peak, but this peak period does not last for many weeks.

## 2. Observations on a Variety Trial

The disease susceptibility of nineteen varieties of Cocksfoot was noted over a period of five months, from early November 1956 until mid-March 1957. The following varieties were involved:

| | |
|---|---|
| English Imported | C.B. Netherlands |
| Minerva II Sweden. | S. 143 Wales |
| Oron Canada | S. 37 Wales |
| Taradus II Sweden | S. 26 Wales |
| V. Kamekes Germany | Hercules Canada |
| Roskilde Denmark | Akaroa U.S.A. |
| Brage Sweden | Akaroa New Zealand |
| Daeno II Imported | C. 23 Lincoln N.Z. |
| S.V. Frode Sweden | Certified New Zealand |
| Danish Commercial | |

The plants were grown as separate clones, thirty for each variety, in a fairly open paddock and grazed down by sheep when the majority of plants had reached a height of approximately eighteen inches.

The disease incidence on these plants corresponded with observations made for the district as a whole, except that lesioning was never as severe as in the more favourable microclimates.

During the observation period the weather was generally hot

and dry and considered to be unfavourable for leaf spot development.

In late January and February the expression of leaf spot symptoms was almost negligible but leaf rust, caused by Uromyces dactylidis Otth. was very bad. In cases of severe infection entire plants were killed by this disease.

Examination of the experimental records showed that varieties did not differ very greatly in their disease susceptibility. The effect of the great divergence of growth form and time of maturity between varieties was partly nullified by the experimental conditions. If the grazing had been more lenient and infrequent, the time of flowering would have differed more markedly between varieties and possibly would have affected the disease incidence.

In spite of these circumstances, all New Zealand lines were affected by S. graminis to a greater extent than were imported lines. In particular, New Zealand Akaroa was severely attacked by this fungus with some plants estimated as having over fifty per cent of the leaf area lesioned. The greater incidence of infection on New Zealand varieties is an interesting feature of the experiment. It may well be that S. graminis in New Zealand has become adapted to growth on local varieties and therefore lost its general property of infecting all varieties equally.

Individual plants in some lines stood out from the rest as being badly diseased. It seems, therefore, as though resistance is a genetical factor within varieties. Hence breeding for resistance could be a possible way of controlling the disease.

## B. The Effect of Temperature on the Incubation Period and Severity of Attack

Walker (1950) defines the incubation period as the interval between infection (establishment of the pathogen within the host following penetration) and the appearance of the disease.

In order to determine the effect of temperature on the incubation period of the fungi the following experimental work was conducted.

Nine pots of cocksfoot plants, varying in age from eight weeks to six months, were inoculated with a spore suspension of M. rubricosum. Similar plants were inoculated with R. orthosporum, S. donacis var. stomaticola and S. maculata, making thirtysix pots in all. In addition, six control plants were sprayed with distilled water. S. graminis was not used in this experiment. On culture media it fails to sporulate, and when mycelial fragments are used as inoculum there is no establishment of infection.

Previous work with germination of spores in distilled water had shown that the "germination time" at room temperature (16° - 20°C.) was no longer than eighteen hours for any of the fungi. Other work had shown that, when plants were subjected to high humidity for twentyfour hours after inoculation, a very low incidence of disease resulted, whereas, after fortyeight hours of high humidity disease incidence was high. It was postulated therefore, that infection should have been established within fortyeight hours from the time of inoculation.

All fortytwo pots of cocksfoot were kept under high humidity for fortyeight hours and then removed to temperature cabinets.

These cabinets were held at temperatures of 17°, 24° and 28°C. Into each cabinet were placed fourteen pots, these being four plants inoculated with each organism and two control plants. Both young and old plants were held at each temperature so that age effect, if any, could be recorded.

1. Results

(a) Leaf Fleck (Mastigosporium rubricosum)

Three days after inoculation (that is, one day after placing the plants in the temperature cabinets) minute, water-soaked areas appeared on all inoculated plants held at temperatures of 17° or 24°C. Within twentyfour hours these areas turned brown in the centre and developed into the typical lesions of the disease. Disease incidence was severe at both these temperatures, perhaps slightly more so at 17°C., and within ten days of manifestation, resulted in the death of those leaves which were badly infected. The age of the plant did not appear to affect its susceptibility to the fungus.

None of the plants held at 28°C. developed lesions over the next twentyone days. One pot was removed on the fourth day and placed again in the humidity cabinet at room temperature. Five days later (that is, nine days after inoculation) a few lesions were seen. Apparently a temperature of 28°C. inactivates the pathogen but does not kill it immediately.

(b) Scald (Rhynchosporium orthosporum)

The earliest evidence of infection appeared fifteen days after inoculation. Like M. rubricosum, this fungus caused the appearance of symptoms simultaneously at temperatures of 17° and 24°C. and was equally intense on both old and young plants. On

no plant, however, was infection severe, seven or eight lesions per plant being the maximum. No disease was observed at 28°C., even after twentyone days.

(c) Eye Spot (Selenophoma donacis var. stomaticola)

Symptoms of the disease were discernable seven days after inoculation at both 17° and 24°C. The disease took the form of small chlorotic areas which developed into the characteristic eye spot within three to four days of first appearing. At neither temperature were there many leaf spots. This seems to be a characteristic of S. donacis var. stomaticola as infection in the field never approximates the importance of the other diseases. This disease became apparent at 28°C. ten to eleven days after inoculation. Infection was slight, there being only five lesions noted on the three plants inoculated.

(d) Purple Leaf Spot (Stagonosphora maculata)

Six days after inoculation, disease symptoms were noticed simultaneously at both 17° and 24°C. Two days later the disease was perceptible in the 28°C. series. Infection was so severe at the two lower temperatures that many of the leaf blades were killed. At 28°C. plants were only moderately diseased.

## 2. Discussion

The principal comment that can be made on the above results is that temperatures of 17° and 24°C. equally affect both the incubation period and severity of attack for each organism. M. rubricosum was the only exception as it was slightly more pathogenic at 17° than at 24°C. This is understandable when one realises that the maximum growth temperature for this fungus in pure culture is not much above 24°C.

M. rubricosum was the slowest growing fungus on culture media and yet was the first to cause manifestation of the disease. Obviously the retarding effect of the host plant is negligible. R. orthosporum, on the other hand, is almost as slow growing as M. rubricosum but at 17° and 24°C. it takes fifteen days to complete the incubation period. The reaction between host and pathogen must be considerable to cause this delay of expression. S. donacis var. stomaticola and S. maculata have fairly similar incubation periods at the two lower temperatures, but the effect of the host must be a great deal more inhibitory for the latter pathogen because it has a quicker "Spore germination time", and grows very much faster on culture media.

As would be expected, those fungi which did not grow above 28°C. on culture media did not develop in the host. S. donacis var. stomaticola which has a maximum growth temperature slightly above this figure was able to mildly infect cocksfoot. S. maculata grows quite vigorously at 28°C. on culture media and was able to cause moderate infection at the higher temperatures.

Although only one aspect of the environment, namely temperature, has been studied, it is interesting to note that S. maculata and S. graminis are the dominant fungi over the summer, provided the conditions are not too dry. S. donacis var. stomaticola is common up until late December and then falls in disease intensity over the summer. M. rubricosum and R. orthosporum are not warm weather fungi and so the former, in particular, is restricted to the moist, relatively cool spring.

---

# R E F E R E N C E S

Ahlgren G.H. (1949)"Forage Crops". McGraw Hill Book Co.

Ainsworth G.C. & Bisby G.R. (1954) "A Dictionary of the Fungi". Commonwealth Mycol. Inst. Kew Surrey.

Alexopoulos C.J. (1952) "Introductory Mycology". John Wiley and Sons. New York.

Allison J.L. (1939) "Studies on Septoria bromigena Leaf Spot on Bromis inermis". Phytopathology 29:1.

------ (1939) "Studies on Monosporous Cultures of Septoria bromigena". Phytopathology 29:554-556.

------ (1945) "Selenophoma bromigena Leaf Spot on Bromis inermis". Phytopathology 35:233-240.

------ & Chamberlain D.W. (1946) "Distinguishing Characteristics of some Forage-grass Diseases Prevalent in the North Central States". Circular 747 U.S.D.A. Wisconsin.

Armolik K. & Dickson J.G. (1956) "Minimum Humidity for Germination of Conidia of Fungi Associated with Storage of Grains". Phytopathology 46:462-465.

Barnett H.L. (1955) "Illustrated Genera of Imperfect Fungi". Burgess Publ. Co. Minneapolis.

Bisby G.R. (1945) "An Introduction to the Taxonomy and Nomenclature of Fungi". Imp. Mycol. Inst. Kew Surrey.

Bollard E.G. (1950) "Studies on the Genus Mastigosporium". Trans. Brit. Mycol. Soc. 33:250-275.

Bonner J.T. (1948) "A Study of the Temperature and Humidity Requirements of Aspergillus niger". Mycologia 40:728-738.

Brancato F.P. & Golding N.S. (1953) "The Diameter of the Mold Colony as a Reliable Measure of Growth". Mycologia 45:848-864

Braverman S.W. (1956) "Host Relationship, Morphology and Sporulation in Culture of Scolecotrichum graminis". Personal Communication.

Brien R.M. (1942) "First Supplement to a List of Plant Diseases Recorded in New Zealand". N.Z. Journ. Sc. Tech. A24:62-64

------ & Dingley J.M. (1951) "A Revised List of Plant Diseases Recorded in New Zealand". N.Z. Dept. Sc. Indus. Res. Bull.101.

Caldwell R.M. (1931) "Host Specialization and Parasitism of the Genus Rhynchosporium". (Abstract). Phytopathology 21:109-110.

Caldwell R.M. (1937) "Rhynchosporium Scald of Barley, Rye and Other Grasses". Journ. Agr. Res. 55:175-198.

Charlton K.M. (1953) "The Sporulation of Alternaria solani in Culture". Trans. Brit. Mycol. Soc. 36:349-355.

Chester K.S. (1945) "Defoliation and Crop Loss". U.S.D.A. Pl. Dis. Reptr. 29:162.

Chupp C. (1954) "A Monograph of the Fungus Genus Cercospora". Ithaca New York.

Clements F.E. & Shear C.L. (1931) "The Genera of Fungi". H.W. Wilson and Co. New York.

Conners I.L. & Savile D.B.O. (1951) Thirty-first Annual Report of the Canadian Plant Disease Survey 1951 XIX 122pp. 1952.

Creelman D.W. (1956) "The Unusual Occurrence of Three Leaf-Spotting Fungi on Grasses in Nova Scotia". U.S.D.A. Pl. Dis.Reptr. 40:510-512.

Darley E.F. (1941) "Spore Germination of Selenophoma bromigena". Phytopathology 31:953-954.

Davis J.J. (1903) "Third Supplementary List of Parasitic Fungi of Wisconsin". Wis. Acad. Sci., Arts, First Suppl. by Davis 1893, Second 1897. Vols. VI, IX, XI.

----- (1919) "Notes on Parasitic Fungi in Wisconsin". - VI". Wis. Acad. Sci., Arts, and Letters, Trans. 19:705-727.

Dearness J. (1917) "New or Noteworthy North American Fungi". Mycologia 9:345-364.

Dennis R.W.G. & Foister C.E. (1942) "List of Diseases of Economic Plants Recorded in Scotland". Brit. Mycol. Soc. Trans. 25:266-306.

Dickson J.G. (1947) "Diseases of Field Crops". McGraw Hill Book Co.

Diener U.L. (1955) "Sporulation in Pure Culture by Stemphylium solani". Phytopathology 45:141-145.

Elliott E.S. (1954) "Notes on Forage Plant Diseases Observed in North West Virginia During 1953". U.S.D.A. Pl. Dis.Reptr. 38:279-281.

Fischer G.W., Sprague R., Johnson H.W. & Hardison J.R. (1942) "Host and Pathogen Indices to the Diseases Observed on Grasses in Certain Western States During 1941". U.S.D.A. Pl. Dis. Reptr. Suppl. 137 pp.87-144.

Frandsen N.O. (1943) "Septoria-arten des Getreides und anderer Graser in Danemark". Kgl. Vet. und Landw. Hochsch. Kopenhagen Mitt. N. 26, 92pp.
(cited Sprague R. and Johnson A.G. 1947).

Frank A.B. (1896) "Mastigosporium Riess". Krankheiten der Pflanzen, Aufl. 2, v. 2, pp. 356-357.
(cited by Sprague R. 1938).

Fresenius G. (1852) Beitrage zur Mykologie. Frankfurt.
(cited by Bollard E.G. 1950).

Fuckel L. (1863) "Scolecotrichum graminis". Fungi Rhenani Exsiccati. Hedwigia 2:134. (cited by Horsfall J.G. 1930).

----- (1869) "Symbolae mycologicae. Beitrage zur Kenntris der Rheinische Pilze, pp.130. (cited by Bollard E.G. 1950).

Garren K.H. (1955) "Disease Development and Seasonal Succession of Pathogens of White Clover. Part II".
U.S.D.A. Pl. Dis. Reptr. 39:339-341.

Graham J.H. (1952) "Purple Leafspot of Orchard Grass".
Phytopathology 42:653-656.

Graham J.H. & Sprague V.G. (1953) "The Effects of Controlled Periods of High and Low Humidities on the Development of Purple Leafspot of Orchard Grass".
Phytopathology 43:642-643.

Granti A. (1953) "Observations on the Changes and Injuries Caused by Scolecotrichum graminis in Dactylis glomerata".
Nuovo G. Bot. Ital., N.S., 60, 3 pp.563-578.
(R.A.M. 33:606-607 1954).

Grove W.B. (1935) "British Stem-and Leaf-Fungi". Vol. I
Cambridge Uni. Press. 488pp.

Guarch A.M. (1941) "Phytopathological Notes". Rev. Fac. Agron. Univ., Montevideo. (R.A.M. 21:129-130 1942).

Hadfield J.W. (1952) "Arable Farm Crops of New Zealand".
N.Z. Dept. Sc. and Indus. Res. Bull. 5.

Hansen H.N. & Snyder W.C. (1947) "Gaseous Sterilization of Biological Materials for the Use as Culture Media".
Phytopathology 37:369-371.

Hawker L.E. (1950) "Physiology of Fungi". Uni. of London Press.

Hohnel von F. (1923) (By Weese.) "Studien uber Hyphomyzeten".
Centralbl. fur Bakt. Abt. 2, 60:1-26.
(R.A.M. 3:102-103 1924).

Horsfall J.G. (1930) "A Study of Meadow Crop Diseases in New York". Cornell Uni. Agr. Expt. Sta.Mem. 130. 139pp.

Howard F.L., Rowell J.B. & Keil H.L. (1951) "Fungus Diseases of Turf Grasses". Agric. Exptl. Sta. Uni. Rhode Island. Kingston Bull. 308 Contrib. 764.

Jacques W.A. (1937) "The Effect of Intensity of Defoliation on Root Development and Production in Some Pasture-grass Species". Proc. 6th. Ann. Conf. N.Z. Grassland Assoc.

Johnson A.G. and Hungerford C.W. (1917) "Scolecotrichum graminis on Timothy, Orchard Grass and Other Grasses". Phytopathology 7:69.

Kilpatrick R.A. & Johnson H.W. (1956) "Sporulation of Cercospora Species on Carrot Leaf Decoction Agar". Phytopathology 46:180-181.

Kreitlow K.W., Graham J.H. & Garber R.J. (1953) "Diseases of Forage Grasses and Legumes in the Northeastern States". Penn. Sta. Uni. Bull. 573 pp.42.

Lind J. (1913) "Danish Fungi as Represented in the Herbarium of E. Rostrup". Copenhagan 1913.

Lindau G. (1907) "Fungi Imperfecti : Hyphomycetes". Rabenhorst L., Kryptogamen-flora von Deutschland, Oesterreich und der Schweiz. Aufl. 2, Abt. 8 Leipzig.
(cited by Sprague R. 1938).

McKay R. (1946) "A Study of Septoria oxyspora Penz. and Sacc. Isolated from Diseased Barley".
Sci. Proc. Roy. Dublin Soc. N.S. 2499-110.

MacVicar R.M. & Childers W.R. (1955) "Progress Report of the Forage Crops Division, Central Exptl. Farm, Ottawa, Ontario 1949-53-55". 61pp.

Maire R. (1906) "Contribution a l'etude de la flore mycologique de L'Afrique du Nord". Soc. Bot. France Bull. 53:180-215.
(cited by Sprague R. and Johnson A.G. 1947).

Meiners J.P. (1954) "Grass Diseases on New Hosts in the Pullman Nursery Unit of the Soil Conservation Nurseries".
U.S.D.A. Pl. Dis. Reptr. 38:277.

Muller W.H. (1956) "The Influence of Temperature on Growth and Sporulation of Certain Fungi".
Bot. Gazette 117:(4) 336-343.

New Plant Diseases in N.S.W. Agricultural Gazette of N.S.W. 65:102-103. 1954.

Oudemans C.A.J.E. (1897) "Observations Mycologiques". K. Akad. Wetensch. Amsterdam, Verslag. Wis em Natuurk. Afd. 6:86-92.

Owen H. (1952) "Leaf Blotch of Cocksfoot". Plant Path. 1:122.

Pammel L.H., Weems J.B. & Lamson-Scribner F. (1901) "The Grasses of Iowa". Iowa Geological Surv. Bull. No. 1 Des Moines.

Park J.Y. and Sprague R. (1953) "Studies on Some Selenophoma Species on Gramineae". Mycologia 45:260-275.

Petrak F. (1929) Mykologische Notizen X Ann. Mycol.27:324 (cited by Sprague R. 1950).

----- (1047) "Critical Observations on Some Ascomycetes and Fungi Imperfecti Recently Described as New". Sydowia, i, 1-3,pp.61-79. (R.A.M. 27:99 1948).

----- (1951) "Results of a Revision of the Basic Types of Various Ascomycetes and Fungi Imperfecti". Sydowia 5:3-6,pp.328-356. (R.A.M. 31:353 1952).

Preston D.A. (1944) "Diseases of Grasses in Oklahoma". U.S.D.A. Pl. Dis. Reptr. 28:562.

Report on the Farm Production Statistics in New Zealand 1954-55. Dept. of Statistics Wellington New Zealand. Owen 1956.

Riker A.J. & Riker R.S. (1936) "Introduction to Research on Plant Diseases". John Swift Co. Wisconsin.

Roberts D.A., Fezer K.D. & Sherwood R.T. (1952) "Diseases of Forage Crops in New York 1952". U.S.D.A. Pl. Dis. Reptr. 36:416-418.

----- (1954) "Diseases of Forage Crops in New York 1953". U.S.D.A. Pl. Dis. Reptr. 38:30-31.

----- Fezer K.D. & Ramanurthi C.S. (1956) "Diseases of Forage Crops in New York". U.S.D.A. PL. Dis. Reptr. 40:219-221.

Saccardo P.A. (1889) Sylloge fungorum omnium hucusque cognitorum. Vol.14:Pavia.

----- (1906) Sylloge fungorum Vol.18, pp.540. (cited by Caldwell 1937).

Sampson K. (1924) "Seasonal Notes on the Fungus Diseases of Grasses in the Aberystwyth District". Agric. Progr. 1:106.

Sampson K. & Western J.H. (1954) "Diseases of British Grasses and Herbage Legumes". Cambridge Uni. Press. 118pp.

Shaw D.E. (1953)[1] "Cytology of Septoria and Selenophoma Spores". Proc. Linn. Soc. N.S.W. 78:122-130.

----- (1953)[2] "The Genus Selenophoma on Gramineae in Australia". Proc. Linn. Soc. N.S.W. 78:151-159.

Shitakova-Roussakova, A.A. (1939) "The Susceptibility of Rye Varieties to the Spot Disease (Scolecotrichum graminis) Under Conditions at the Otrada-Kuban Selection Farm". Pl. Prot., Leningrad. 18:71-76. (R.A.M. 18:587 1939).

Snedecor. G.W. (1956) "Statistical Methods". Iowa State Coll. Press Ames Iowa.

Snyder W.C. & Hansen H.N. (1947) "Advantages of Natural Media and Environments in the Culture of Fungi". Phytopathology 37:420-421.

----- (1954) "Variation and Speciation in the Genus Fusarium". Annals New York Acad. Sc. 60:16-23.

Sprague R. (1938) "Two Mastigosporium Leaf Spots on Gramineae". Journ. Agr. Res. 57:287-299.

----- (1938) "Notes on Diseases of Cereals and Grasses in Oregon and Adjacent Counties in Washington During the Spring of 1938". U.S.D.A. Pl. Dis. Reptr. 22:174-175.

----- (1940) "A Third Species of Mastigosporium on Gramineae". Mycologia 32:43-45.

----- (1941) "Stagonospora arenaria on Grasses". Mycologia 33:371-379.

----- (1941) "Some Leaf Spot Fungi on Western Gramineae". Mycologia 33:371-379.

----- (1942) "Cercosporella Eyespot of Kentucky Bluegrass". Phytopathology 32:737-738.

----- (1946) "Rootrots and Leafspots of Grains and Grasses in the Northern Great Plains and Western States". U.S.D.A. Pl. Dis. Reptr. Supp. 163 167pp.

----- (1948) "Some Leaf Spot Fungi on Western Gramineae II". Mycologia 40:177-193.

----- (1948) "Some Leaf Spot Fungi on Western Gramineae III". Mycologia 40:295-313.

Sprague R. (1949) "Some Leaf Spot Fungi on Western Gramineae IV". Mycologia 41:493-504.

Sprague R. (1949) "Selenophoma Spot, a New Wheat Disease for North America". Phytopathology. 39:23 Abs.

----- (1950)[1] "Diseases of Cereals and Grasses in North America". Ronald Press Co. New York. 538pp.

----- (1950[2] "Some Leafspot Fungi on Western Gramineae V". Mycologia 42:758-771.

----- (1950)[3] "Forage Crop Disease in Washington 1949". U.S.D.A. Pl. Dis. Reptr. 34: 142-143.

----- (1954) "Some Leafspot Fungi on Western Gramineae VII". Mycologia 46:76-88.

----- (1955) "Check List of the Diseases of Grasses and Cereals in Alaska 1955". U.S.D.A. Pl. Dis. Reptr. Suppl. 232.

----- & Johnson A.G. (1940) "Selenophoma on Grasses I". Mycologia 32:415.

----- (1945) "Selenophoma on Grasses II". Mycologia 37:638-639.

----- (1947) "Selenophoma on Grasses III". Mycologia 39:737-742.

----- (1950) "Species of Selenophoma on North American Grasses". Oreg. State Coll. Monog. Bot.

Sprague R. & Meiners J.P. (1949) "Some New Disease Records of Gramineae in the Western United States III". U.S.D.A. Pl. Dis. Reptr. 33:259-270.

Stapledon R.G. & Jenkin T.J. (1922) "Preliminary Investigations With Herbage Plants". Bull. Welsh Pl. Breed. Sta.1919-21. Ser. H, no.1, p.47.

Sydow H. (1924) (Ann. Mycol. 22:293-317. (cited by Brien & Dingley 1951).

Thomas S. (1930) "Bacteriology". McGraw-Hill Bk. Co.

Tiffany L.H. (1955) "Fungus Leaf Spots of Brome Grass in Iowa". Iowa Sta. Coll. Journ. Sc. 30:21-32.

Tracy S.M. & Earle F.S. (1895) "New Species of Parasitic Fungi". Bull. Torrey Bot. Club.22:179.

Valder P.G. & Shaw D.E. (1953) "Mastigosporium rubricosum on Grasses in N.S.W." Aust. Pl. Dis. Recorder 5(3)22.

Wakefield E.M. (1918) "New and Rare British Fungi". Roy. Bot. Gard. Kew Bull. Misc. Infor. 6:229-233.

Walker J.C. (1950) "Plant Pathology". McGraw-Hill Bk. Co.

Weinmann H. (1948) "Underground Development and Reserves of Grasses". Journ. Brit. Grassl. Soc. 3:115-140.

Welles C.G. (1924) "Observations on Taxonomic Factors Used in the Genus Cercospora". Science LIX.

Wilson M., Noble M., & Gray E. (1954) "Gloeotinia - A New Genus of the Sclerotiniaceae". Trans. Brit. Mycol. Soc. 37:29-32.

# A P P E N D I X

# APPENDIX

## Host Range.

The following is the list of new hosts reported since Sprague 1950.

*Mastigosporium rubricosum*. (Dearn. and Barth.) Sprague

Agrostis aequivalvis Alaska. Sprague.
A. borealis Alaska. Sprague.
Calmagrostis nutkaensis Alaska. Sprague.
Phleum alpinum Alaska. Sprague.

*Rhynchosporium orthosporum*. Caldwell.

Agrostis exarata Alaska. Sprague.
Alopecurus arundinaceus U.S.A. Meiners.
Calamagrostis canadensis var. scabra Alaska. Sprague.
Lolium remotum U.S.A. Meiners.

*Scolecotrichum graminis* Fckl.

Agropyron latiglume Alaska. Sprague.
A. sericeum Alaska. Sprague.
A. trichophorum. Meiners.
A. exarata var. ampla Alaska. Sprague and Meiners.
Arctagrostis latifolia Alaska. Sprague.
Hordeum brachyantherum Alaska. Sprague.
Puccinellia paupercula var. alaskana Alaska. Sprague.

*Selenophoma donacis var. stomaticola*. (Bauml.) Sprague and A.G.Johnson.

Agropyron scabrum Aust.*
A. trachycaulum Alaska. Sprague.
A. wheat hybrid Aust.
Amphibromus neesii Aust.
Anisopogon avenaceum Aust.
Aristida vagans Aust.
Avena fatua Cañada. Conners and Savile.
Danthonia caespitosa Aust.
D. pallida Aust.
D. penisillata Aust.
D. ramosa Aust.
Deschampsia canthonioides U.S.A. Sprague and Meiners.
Deyeuxia monticola var. valida Aust.
Dichelachne rara Aust. Shaw.

* New hosts of *S. donacis* var. *stomaticola* noted in Australia but with no authority quoted were recorded on page 102 of the Agricultural Gazette of New South Wales, Feb. 1954.

Microlaena stipoides Aust. Shaw.
Neurachne Muelleri Aust. Shaw.
Poa caespitosa Aust. Shaw.
P. stenantha Alaska. Sprague.
Puccinella paupercula var. alaskana Alaska. Sprague.
Sporobolus capensis Aust. Shaw.
S. elongatus Aust. Shaw.
Stipa aristiglumis Aust. Shaw.
S. variabilis Aust. Shaw.
Tristum spicatum Alaska. Sprague.
Triticum vulgare Aust. Shaw.
Vulpia Myuros Aust. Shaw.

Stagonospora maculata. (Grove) Sprague.

Nil.

ACKNOWLEDGEMENTS

Grateful acknowledgement is made to Mr. H.T. Wenham of the Microbiology Department, Massey Agricultural College, for his interest, assistance and guidance in this project.

The author is indebted to Mr. H. Drake and to Mr. L. Thompson for their assistance in connection with much of the photographic work.

Also, thanks are due to the Staff of the College Library for their efforts in obtaining numerous journals on loan from other institutions.