

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

A STUDY OF THE LEAFSPOT
DISEASE OF LETTUCE CAUSED
BY STEMPHYLIUM BOTRYOSUM WALLR.

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

by
Donald Arthur Slade
Massey Agricultural College
November 1961

CONTENTS.

	Page.
ACKNOWLEDGEMENTS	1
INTRODUCTION	2
MATERIALS AND METHODS	8
1. Used in the laboratory	8
2. Field survey: Records and methods	15
3. Production of lettuce plants	16
4. Glasshouse management	18
5. Preliminary experimental work	20
 <u>CHAPTER I, THE FUNGUS.</u>	
A. Artificial Media Studies	26
1. The influence of media on cultural features	27
2. The effect of medium pH on growth	35
3. The effect of temperature on growth	41
4. The effect of light on colonies	45
5. The effect of refrigeration on colonies	46
6. Pigmentation	47
7. Production of conidia	47
B. The taxonomy and nomenclature of <u>Stemphylium</u> <u>botryosum</u> Wallr.	
1. History of the genus <u>Stemphylium</u>	50
2. Studies on the taxonomy and nomenclature of New Zealand isolates from lettuce	55
 <u>CHAPTER II, FIELD OBSERVATIONS</u>	65
A. Discussion on observations made in each district	
1. Hawkes Bay	77
2. Manawatu	78
3. Horowhenua	80
B. Discussion on replies to questionnaire	85
C. Discussion on weather conditions observed which favoured leaf spot of lettuce.	86
Conclusions	87
 <u>CHAPTER III, THE DISEASED PLANT</u>	
Introduction	88
A. The Disease Cycle	
1. Spore germination	91
2. Penetration and infection	116
3. Incubation	137
4. Manifestation of symptoms of disease	149
5. Production of inoculum	159
6. Liberation and dispersal of inoculum	169
7. Survival during the summer months	177

contd.....

CHAPTER III contd.

B. Miscellaneous factors and their influence on the Disease Cycle	
1. Predisposition	207
2. Varietal susceptibility	213
3. Pathogenicity of isolates	215
4. Inoculum potential	216
C. Epidemiology	220
D. Conclusions	229

CHAPTER IV. THE THERAPEUTIC CONTROL OF LEAF SPOT.

1. Laboratory evaluation	231
	236
2. Glasshouse experiment	235
	238

BIBLIOGRAPHY

APPENDICES

LIST OF ILLUSTRATIONS.

Illustration.		After page.
1	Laboratory identification of lesions: High humidity treatment.	8
2	The glasshouse unit used during these studies.	8
3	Diagrammatic representation of the type of lettuce plant used for disease cycle studies.	22
4	The relative humidity cabinet	8
5	A 14 day old colony grown on P. D. A. at 24°C	35
6	A 14 day old colony grown on Natural Nutrient (lettuce) Medium.	35
7	Development of conidia.	48
8	Conidia of <u>Stemphylium botryosum</u> .	56
9	Conidiophores of <u>Stemphylium botryosum</u> .	57
10	Lesions caused by <u>Stemphylium botryosum</u> on lettuce growing at Otalci, March-April 1961	79
11	Lettuce remaining after harvest due to infection with <u>Stemphylium botryosum</u> .	81
12	Typical lesions caused by <u>Marcosonia</u> <u>panattoniana</u> . (Ring-spot.)	84
13	Typical lesions caused by <u>Stemphylium</u> <u>botryosum</u> , some of which may be confused with 'Ring-spot'.	84
14	Germination of conidia of <u>Stemphylium</u> <u>botryosum</u> at 24°C.	102
15	Growth of young hyphae from germinated conidia at 28°C.	102
16	Growth of young hyphae from germinated conidia at 32°C.	102
17	Method of maintaining constant relative humidities.	104

<u>Illustration.</u>		<u>After page.</u>
18	Host penetration.	120
19	Typical lesions caused by <u>Stemphylium botryosum</u> (Naturally infected leaf.)	150
20.	Apparatus used for spore dispersal studies.	170
21	Infections due to acrial dispersal of conidia.	201
22	Discharge of ascospores from Perithecium	201
23)	Infections of <u>Stemphylium botryosum</u> on plants	
24)	of various ages	2211

LIST OF FIGURES

Figure	After Page.
I. The effect of medium pH on colony growth	39
II. The effect of temperature on colony growth on P. D. A. after ten days.	42
III. Meteorological records for 1960: Levin	85
IV. Meteorological records for 1960: Napier	85
V. Meteorological records for 1960: Palmerston North	85
VI. Effect of P.D.A. pH on spore germination	96
VII. Rate of germination of various percentages of conidia at temperatures from 5°-40°C	101
VIII. The effect of temperature on the period required for penetration and infection	134
IX. Effect of incubation temperature on size of lesions	141
X. The relation of temperature to the length of the incubation period	146
XI. Standard centimeter squares for estimation of conidia densities	163
XII. Density of, and time required for production of conidia.	164
XIII. Optimum humidity and temperature requirements of the Disease Cycle	220

LIST OF TABLES.

Table	Page
1. Colony characteristics on various media	32
2. Quantities of acid and alkali added to buffered P. D. A. to obtain a range of media pH	36
3. Pathogenicity of lettuce isolates of <u>Stemphylium botryosum</u>	63
4. Pathogenicity of various isolates of <u>Stemphylium botryosum</u> to lettuce	65
5. Comparison of media for spore germination studies	95
6. Time required for germination of conidia of <u>Stemphylium botryosum</u> (100% R.H.)	101
7. Effect of high temperature on viability of conidia	102
8. Effect of relative humidity on spore germination	107
9. Average percentage of spores germinating after exposure to extremes of light and low temperature	109
10. The method of host penetration	120
11. Infection time on plants inoculated and exposed to various temperatures at 100% R. H.	124
12. The effect of light on penetration and infection	129
13. Description of lesions at completion of the incubation period at various temperatures	142
14. Effect of temperature during the incubation period on size of lesions	143
15. Effect of temperature and type of plant on manifestation	154
16. Infection resulting from infected soil applied to lettuce plants	190
17. Results of testing various soils using the soil dusting technique	191
18. Survival of <u>Stemphylium botryosum</u> as dormant mycelium	195

contd.....

Table	Page
19. Pathogenicity of <u>Stemphylium botryosum</u> isolated from hosts located during field surveys	196
20. Pathogenicity of <u>Stemphylium botryosum</u> isolated from headland weeds.	198
21. The effect of temperature on predisposition	209
22. The level of inoculum required for infection	217
23. Details of fungicides used during experimentation on control	232
24. Summary of results of experiments on age of deposit of fungicide and its efficacy and tenacity as measured by the glass slide technique	237
25. Summary of results: Glasshouse control experiment	239

ACKNOWLEDGEMENTS

Grateful acknowledgement is made to Mr. H. T. Wenham for his encouragement and guidance in the supervision of this study, and for helpful criticisms in the preparation of this thesis.

Thanks are also due to Mr. K. C. Hockey and the Horticultural Staff for loan of the coolstore for environmental studies and the use of equipment; the Horticulture Division Officers, Department of Agriculture, at Palmerston North, Hastings and Levin for assistance with field survey work; the Staff of the College Library for their assistance in obtaining numerous journals, and to Mrs. J. Little for typing this thesis.

For the duration of this study the author was assisted financially by a Public Service Study Award and the 1960 Shell Scholarship.

INTRODUCTION

Lettuce (Lactuca sativa L) is the most important salad crop and a principal vegetable of New Zealand where in many districts it can be grown out of doors all the year round. Winter lettuce growers commonly plant from $\frac{1}{2}$ - 5 acres of lettuce, and a good crop will cut 400 cases an acre. In late winter and early spring when other vegetables are scarce, returns to the grower from lettuce may be as high as 20/- to 60/- a case. Returns per acre from winter lettuce are therefore relatively high compared with other winter crops. The success of this crop is of major importance to the grower who commonly depends on it to cover the high winter and spring labour expenses which usually coincide with a period of low production.

From season to season, and even from week to week in the same season, the quality and quantity of lettuce available is a direct reflection of the prevailing weather conditions. Weather may act directly on the lettuce plant affecting its rate of maturation, appearance and plant size. Weather may also have a profound indirect effect by providing conditions conducive to disease development. It is well recognised that a disease may reach epiphytotic proportions only if weather conditions are favourable to the causal organism.

Several diseases of lettuce have been recorded in New Zealand (Appendix I). Of these the disease variously known as ring-spot, rust or anthracnose and caused by Marssonina panattoniana (Bert)

Magn., has long been considered the major foliage disorder affecting winter lettuce production in this country. This disease is world wide in occurrence and has been the subject of considerable research. (Chittenden 1912, Salmon and Wormald 1923, Stevenson 1939, Taylor and Li 1944).

Recently however Brooks (1958)* recorded Stemphylium botryosum Wallr. in its sexual phase on lettuce crops in the Auckland area, where large individual losses have occurred during the wet and cool winter months. This organism is known to be pathogenic to a wide range of unrelated hosts (Neergaard 1945) but comparatively little has been published on this fungus as the causal organism of a leafspot of lettuce.

Weber and Foster (1928) in their bulletin on 'Disease of Lettuce, Romaine, Escarole and Endive' state that a leaf spot of Escarole, (Cichorium intybus, which is closely related to lettuce), is caused by an Alternaria spp. They state, "There is a possibility that with further study it will be found that this organism is the same as the one that causes a similar leaf spot on lettuce." The description of the leaf spot on Escarole

* See Dingley, Joan M. N.Z. Journ.Agric.Res. 3(3) 461-467 1960.

and the photograph of an infected leaf appear to be very similar to the symptoms of S. botryosum on lettuce. No further reports appear to have been published on subsequent work to determine the causal organism of this or the leafspot on lettuce.

Ogilvie and Mulligan (1931) were the first to describe a leaf spot disease of lettuce, from which they isolated Macrosporium sarcinula Berk. amend Holle. Identification was corroborated at that time by S. P. Wiltshire. The genus Macrosporium was later reduced to synonymy with Stemphylium by Wiltshire (1938), and M. sarcinula was thus synonymous with Stemphylium botryosum.

Ogilvie and Mulligan state that this previously undescribed leaf spot, from which they isolated and proved the pathogenicity to lettuce of the above fungus, was first noticed in April 1930. They later found the disease to be common on lettuce planted in greenhouses and out of doors in the West of England. The symptoms they describe appear to be similar to those caused by S. botryosum on lettuce in New Zealand.

While the disease was evidently not sufficiently serious to warrant control measures, they considered that under favourable environmental conditions it may become important.

Dippenaar (1939) reports that in 1935 he discovered lettuce grown on the Cape Flats, South Africa to be affected with a serious leaf spot disease previously unrecorded from South Africa. Further investigation showed that this was one

of the major diseases, if not the most important, affecting winter lettuce grown on the Cape Flats. Dippenaar considered the disease and the causal organism to be similar to that reported by Ogilvie and Mulligan (1931). He also successfully proved pathogenicity of the fungus isolated.

Neergaard (1945) states that spores of S. botryosum not infrequently occur on lettuce seed. In further infection experiments with seed borne fungi Neergaard found that this fungus attacked both sterile seedlings and wounded and unwounded six-week-old lettuce plants.

Gossens (1951) has reported a leaf spot disease of young lettuce caused by P. herbarum Rabenh (perfect stage of S. botryosum). Young lettuce plants in Dutch nurseries, especially about Rotterdam, were attacked during January-February 1950. Gossens states that infection was generally mild but in one case 70% of the crop was involved.

Pettinari (1951) obtained the P. herbarum stage of S. botryosum from leaves of lettuce growing at Apulia, Italy, but in the review of his article no mention is made of the extent of the infection, or how he obtained the perfect stage from infected leaves.

Padhi and Snyder (1954) state that a leaf spot of lettuce had been observed for at least twenty years prior to 1951 on lettuce crops in or near Colma, a district on the coast of

California, U.S.A. They considered the incitant to be a form of Stemphylium botryosum Wallr. an imperfect stage of P. herbarum.

Although the above mentioned report by Brooks (1958) is the only reference to Stemphylium botryosum causing a foliage disease of lettuce in New Zealand, it would appear that this disease has been present for many years in this country. Local instructors in the Department of Agriculture have been aware of the existence of two types of leaf spots on winter lettuce. One spot, characteristically small, caused by Marssonina panattoniana, was first recorded in New Zealand by Taylor and Li (1944). The other leaf spot disease, which under favourable conditions may develop larger lesions, is often present on winter lettuce and is commonly confused with ring spot. It would appear that this latter disease is in fact caused by Stemphylium botryosum.

The shothole effect, which occurs when the centre of a lesion collapses is characteristic of both diseases, but it is commonly associated with lesions caused by M. panattoniana. Many of the more observant growers in the Manawatu and Horowhenua district distinguish between the two diseases by calling one 'ring spot' and the other 'leaf spot'. One Chinese grower, who has observed the difference in the type of lesions, recalled that in the early years of World War II, probably 1942, he suffered substantial losses from the 'leaf spot' type disease, and in several seasons since has lost much of his late winter lettuce crops from this disorder. However, the majority of

growers consider that both diseases are similar.

Thus from discussions with the growers of winter lettuce in the Manawatu and Horowhenua district, local Instructors, and records of the Department of Agriculture on diseases of lettuce since 1948, it has not been possible to cite any definite period when the leaf spot of lettuce caused by S. botryosum was first observed. It seems probable, however, that the disease has been attacking winter lettuce for at least twenty years in this country.

Objects of the present Study.

In view of the lack of detailed research on leaf spot of lettuce caused by S. botryosum and its undoubted importance in many districts, the following study was undertaken, the principle objects of which were:-

1. To assess by way of field surveys the incidence and relative importance of the two foliage disorders of winter lettuce caused by Marssonina panattoniana (Bert) Magn., and Stemphylium botryosum Wallr.
2. To study in detail the morphology and physiology of S. botryosum.
3. To study the taxonomy and nomenclature of the New Zealand form of S. botryosum pathogenic to lettuce.
4. To study the influence of environmental factors on the disease cycle.
5. To investigate control of the disease by use of protective fungicides.

MATERIALS AND METHODS

The materials and methods which have been used for each experiment are described in detail in the relevant chapters of this study. Materials and methods which are applicable to several sections of this study are here outlined before this experimental work is described in detail.

1. MATERIALS AND METHODS USED IN THE LABORATORY

(a) Preparation of media

The details of the preparation of media are given in Appendix II. Stocks of potato dextrose agar (P.D.A.), prune agar, and water agar were prepared in 1 or 2 litre quantities. These were stored after sterilization in partly filled 250 ml. flasks stopped with cotton wool plugs. Other media were prepared as required.

(b) Pouring Plates

Petri plates, 90mm. in diameter, were used for culturing the fungus in the laboratory. Successful sporulation occurred when 15-20ml. of medium was used in each plate. Less media resulted in poor sporulation from 10-14 day colonies. Consequently, six petri plates were normally poured from 100ml. of media.

(c) Inoculation of Media

Difficulty was experienced in obtaining a form of inoculum which had no influence on the subsequent diameter of colonies. It was determined that as the size of the square of agar inoculum (containing mycelium) became smaller, the resulting



ILLUSTRATION 1.

Laboratory identification
of lesions: High humidity
treatment.

ILLUSTRATION 2.
The glasshouse unit used
during these studies.



ILLUSTRATION 4.

The relative humidity cabinet.

colony sizes became increasingly variable. If squares of agar containing mycelium measured 6 x 6 mm variation due to the size of inoculum did not occur. Therefore, during all experiments the inoculum consisted of a square of agar measuring 6 x 6 mm. taken from the fringe of an actively growing colony on P.D.A. This agar square was placed upside down on the media being inoculated. Growth rate was linear in respect to time. Therefore 6 mm. was subtracted from the diameter of colonies recorded. These are the diameters reported in this study.

(d) Design of Experiments

Where the diameter of colonies were to be measured, 3 - 5 colonies were used, according to the incubator space available and the type of experiment. Measurements were taken of two diameters at right angles and results were expressed as an average. Unless contaminants were present, the colony diameter for similar treatments were generally very uniform often agreeing to within 1 - 2 mm. If much variation in diameters for similar treatments was encountered the experiment was repeated until uniform results were obtained.

In experiments where a bias may have been introduced due to a previous knowledge of the treatments, the Petri plates were marked with a number to indicate the treatment. Records were kept of the code used which was selected from random tables available in the standard texts. After subjecting the colonies to required treatments the plates were removed from the incubator

and, prior to recording, arranged in the random order indicated by the numbers.

(e) Measurement of growth rate on Artificial Media

Brandt and Golding (1953) after testing and evaluating the effect of several environmental factors on colony diameter concluded that it may be used for measuring the rate of growth of fungus, comparing the influence of variously altered media on their growth, and investigating the effects of other environmental factors.

With the exception of the first twenty-four hours of growth, colony diameters increased at a constant and linear rate. Records of colony diameters were therefore made only once, normally ten days after inoculation.

(f) Incubators

Refrigerated incubators which operated at 5°, 10°, 12.5°, 15° and 20°C. were used for temperature studies of the fungus on culture media. Unfortunately the 5°C incubator failed to function correctly. Incubators were also available operating at intervals from 24°C - 50°C. The normal growth temperature of 24°C was selected because it was the lowest temperature at which the incubators would function without fluctuations occurring due to changes in room temperatures. All normal cultural work was undertaken in incubators operating at this temperature.

Artificial light was not provided in the incubators.

At times mite became a problem, frequently causing considerable

contamination. It was found that they were readily controlled if the shelves and walls of the incubator were occasionally wiped with a cloth soaked with kerosene.

(g) Production and Preparation of Inoculum

(i) Conidia

Isolates were selected for their ability to produce abundant conidia on culture media. This ability was usually related to the length of time the isolate had been maintained on culture media. Petri plates containing approximately twenty milli litres of P.D.A. were inoculated with squares of P.D.A. and mycelium obtained from other cultures, or mycelial fragments taken from stock cultures maintained on petri plates held in a refrigerator. The mycelial fragments proved most satisfactory. A sterile needle bent to form a small L was used to lift tufts of mycelium from the cultures. The needle was moved lightly across the surface of the P.D.A. to facilitate dislodging the mycelium fragments.

(Other methods of inoculation tested, but which failed to induce prolific production of conidia included streaking with suspensions of conidia or mycelial fragments, and incorporation of conidia or mycelial fragments into the medium while it was still liquid.)

After 10-14 days growth at 24°C. conidial suspensions were prepared by flooding the culture with sterile water and scraping off the superficial mycelium. This was placed in a flask with a dozen small glass beads and shaken vigorously for several

minutes. Usually six cultures were used to every 100 mls. of sterile water. The mycelial fragments and P.D.A. were removed by filtering the suspension through two layers of cheese cloth.

The concentration of conidia was estimated using a haemocytometer and was then adjusted, usually to contain 5000 conidia per ml.

(11) Ascospores:

Selected isolates were grown at 24°C. on P.D.A., prune agar or lettuce decoction agar for periods of up to 12 weeks. At this stage ascospore discharge commenced if the colonies were removed from the incubators. During the first ten days incubation colonies were frequently removed from the incubator and exposed to sunlight or ultra violet light to stimulate production of perithecia.

Perithecia were obtained by scraping off the aerial mycelium and perithecia on the surface of the P.D.A. Colonies were then washed with sterile water and the perithecia which remained partly imbedded in the media were scraped off. These were washed several times to remove as much agar as possible and finally shaken with glass beads until all mycelium attached to them was dislodged. After several further washes the perithecia were transferred with a minimum amount of water into a thick-walled test tube, where they were crushed with a close-fitting glass rod. The resulting fragments were suspended in water, filtered through cheese cloth and the

suspensions examined under the microscope. If conidia were present, the suspension was discarded and another prepared, taking further precautions to eliminate all conidia before perithecia were crushed.

(h) Identification of Organisms Causing Leaf Spot Symptoms

The method used to isolate fungi from leaf spots is shown in Illust. I. The petri dish, lined with moist filter paper and containing the lesions supported on glass slides, was incubated for 24 hours at 24°C. After this period of time B. betryosum produced numerous conidia. Identity was confirmed by microscopic examination of conidia and isolation of single spores on to P.D.A. with subsequent culturing and identification of the colonies which developed.

This method, used for rapid isolation and identification of specific organisms, was described by Stevenson (1939) for identifying Marssonina panattoniana on lettuce. The symptoms of these two diseases were sometimes similar, but once the lesions were subjected to this high humidity treatment, differentiation between the two types of spores produced was readily made and indicated with certainty which pathogen was involved.

2. FIELD SURVEY: RECORDS AND METHODS.

A card was prepared for each property visited during the survey and details of stage of growth of the crop, crop variety, and other relevant information was entered during each visit. An estimate of the infection and tentative identification of the foliar disorders present were also made. Provision was left for subsequent confirmation or amendment. If the crop was about to be harvested the percentage of heads rendered unsaleable due to leaf spot disease was estimated.

Specimens of leaf spots from lettuce or other suspected hosts were collected and placed individually in labelled plastic bags.

In the laboratory each leaf spot selected for identification was numbered consecutively and details entered in a register. This series of lesions were prefixed by "U" for "unknown" series (e.g. lesion U25). After successful isolation on to P.D.A. had occurred, selected colonies from the same lesion were designated where necessary with an appended letter (e.g. U23C). Identification was often possible at this stage. To ensure that future work on the isolate would not be complicated by contaminants, single spore isolates were prepared using a method similar to that described by Keitt (1915). These isolates were distinguished by a numeral preceding the U (e.g. 3U23C) to indicate which single spore isolate had been selected. This system of numbering and recording isolates

proved to be very satisfactory. (Two isolates were used in preliminary work before this system was established. These were the "A" and "P" isolates).

Stock cultures of most isolates of S. botryosum were prepared by inoculating P.D.A. slants in $\frac{5}{8}$ " test tubes with mycelial fragments from the single spores colonies. These slants were fitted with cotton wool plugs, labelled and incubated at 24°C until growth commenced. They were then removed from the incubator sealed with "Parafilm" (see p. 103) before storing at room temperature in the laboratory.

Kocks postulates were performed on several isolates from each district and in all cases the fungus S. botryosum isolated from lettuce was proved the incitant of the leaf spot disease.

3. PRODUCTION OF LETTUCE PLANTS

The glasshouse and workroom used for plant production is shown in Illustration 2. This same glasshouse unit was used for handling plants during the disease cycle studies.

(a) Soil Composts

Loam was obtained when possible from turf which had been stacked for eighteen months. At other times a clay loam from the College soil heap was used. This clay loam was heat sterilized (by electrode method) before use. The loam obtained from the turf was treated with methyl bromide prior to the preparation of seedling composts. It was not treated in this way when it was to be used in potting composts.

Seedling composts, based on John Innes mix, contained 2 parts of loam, 1 part of peat and $1\frac{1}{2}$ parts of sand. To every bushel of this mixture was added $1\frac{1}{2}$ ozs superphosphate and $\frac{1}{2}$ ozs of ground limestone.

Pricking out and growing on composts finally adopted consisted of three parts of turf-loam passed through a $\frac{1}{2}$ inch sieve, and one part of sand. To every bushel of this mixture ^{was added} $1\frac{1}{2}$ ounces of ground limestone and four ounces of a mixture consisting of $\frac{1}{4}$ part sulphate of ammonia, $1\frac{1}{2}$ parts superphosphate and $\frac{1}{4}$ part of sulphate of potash.

(b) Seed:

The variety of lettuce used during all studies of the disease cycle was Black Seeded Triumph (ex Coopers, Lot 745/1960). This was sown in small seed trays at weekly intervals to ensure a continuing supply of plants.

Seed of other varieties of lettuce and that used for host range studies, if not available from lines of seed held in the Micro-biology Department, were purchased from chain stores.

(c) Pricking Out and Potting.

When two true leaves had formed and expanded the young plants were pricked out into 3" clay pots or seedling trays. Approximately 100 plants were potted each week, the actual numbers depending on the requirements of the experiments. A 10% surplus of plants was provided above the requirements of the experiment to allow for plant losses and discarding atypical plants.

(d) Supplementary Nutrients:

During periods when water loss from the pots was high, constant watering leached soluble nitrogen from the potted and boxed plants. Plant growth became slow and hard. The base fertilizers were supplemented during these periods by applying a liquid fertilizer to the plants. This was prepared by dissolving one rounded tablespoon of sulphate of ammonia or a similar quantity of "Zest" plant food in one gallon of water. This was applied to the foliage of the plants at weekly intervals.

4. GLASSHOUSE MANAGEMENT

(a) Temperature

Minimum temperatures were maintained at or above 50°F. by electrical radiators fixed under the glasshouse benches. During winter the glasshouse minimum and maximum were usually between 50°F. and 70°F. In summer the maximum temperature at times rose to above 90°F. By heavy shading and maximum ventilation consistent with the weather conditions prevailing, it was usually possible to hold the temperatures down to 80°F.

(b) Relative Humidity and Watering

The benches in the glasshouse were originally fitted with 2½" slats. When the heaters operated the pots became overheated and the relative humidity about the plants dropped, causing excessive moisture loss and plant damage. The benches were then fitted with asbestos sheeting and the amount of water required by the potted plants was considerably reduced. Thus during winter it was usually necessary to water the potted

plants at weekly intervals. In summer the plants were watered every morning and if temperatures were high and a wind blowing it was found advisable to damp the house down during the afternoon.

(c) Control of Miscellaneous Pests and Diseases

(i) Insects.

Aphis were the only insects which attacked the lettuce plants. The glasshouse was fumigated with Lindane at weekly intervals by burning one "Nexa" paper (commercial size) in each glasshouse.

This was done in the evenings so that the house could be shut down without undue temperature rises occurring.

(ii) Fungi.

Considerable plant losses were experienced due to Botrytis cinerea Pers. ex Fr. attacking the young potted plants. Losses were reduced or eliminated by replacing the dried blood in the base fertilizer with sulphate of ammonia; by improving glasshouse hygiene and removing of all dead plant parts in an effort to eliminate the source of primary inoculum; and by eliminating root damage which occurred when the heaters were operating for any length of time by covering the slit benches with sheets of asbestos.

(iii) Bacteria

During early investigations bacterial infection of fungal lesions caused a masking of symptoms of disease incited by S. botryosum, particularly at the higher temperatures used in the growth cabinets.

It was believed that the bacteria were multiplying in

the hoses used for watering the glasshouse. Samples of water taken from the hose had a very high count of bacteria. Once the water had been running for a short time samples taken had either very low counts or were free from bacteria.

Extra precautions were taken to ensure that no practice was facilitating the introduction of bacteria on to the lesions. Before watering or damping down the house, the water in the hose was run off and the hose flushed for several minutes before use. The high humidity cabinet was washed out at weekly intervals using a 1:120 dilution of "Savlon"*. The plastic sheets used were for covering the plants/treated in a similar manner prior to use. The seed trays and flower pots were also sprayed with "Savlon" 1:120. After these precautions had been taken no further trouble was experienced with bacterial contamination.

5. PRELIMINARY EXPERIMENTAL WORK

Before any detailed studies of the effect of disease incited by S. botryosum on lettuce was undertaken, preliminary experimental work was necessary to determine a method suitable for recording and measuring the effect of disease on the lettuce plants.

It was essential that symptoms of disease should be immediately recognised. The preliminary work provided an

* I.C.I. Trade Name for Disinfectant.

opportunity for observation of the lesions from first appearance until death of the leaf occurred. The importance of post recording identification of the incitant of the disease was demonstrated when bacterial infection of the leaf occurred following penetration by S. botryosum. Consequently it was considered necessary to take samples of the leaf spots from each experiment and subject them to 100% relative humidity. If conidia of S. botryosum developed from these lesions, it was considered that similar infections had resulted on the remainder of the plants. Where bacterial infection occurred, sporulation was prevented. It was necessary to repeat these experiments where bacterial infection occurred.

Inoculum prepared as described previously was most satisfactorily applied to the lettuce plants by evenly spraying all plants to the point of run-off with the spore suspension, using a small hand atomiser.

The type and age of plant introduced considerable variation in expression of the symptoms of disease. It became apparent early in the studies that the desirable type of plant was one which had made rapid growth after being potted or pricked out and had expanded a total of five true leaves, in addition to the two lower leaves and the cotyledons. The type of plant used is shown diagrammatically in Illustration 3.

Two criteria were used to record the effect of the disease on the plants. Where a measure of the number of lesions

produced was required, actual counts were made of lesions occurring on the lower five leaves (No. 1 - 5, Illustration 3) excluding the cotyledons, and first two true leaves. During normal growth conditions in the glasshouse these recordings were made between the seventh and tenth days from inoculation.

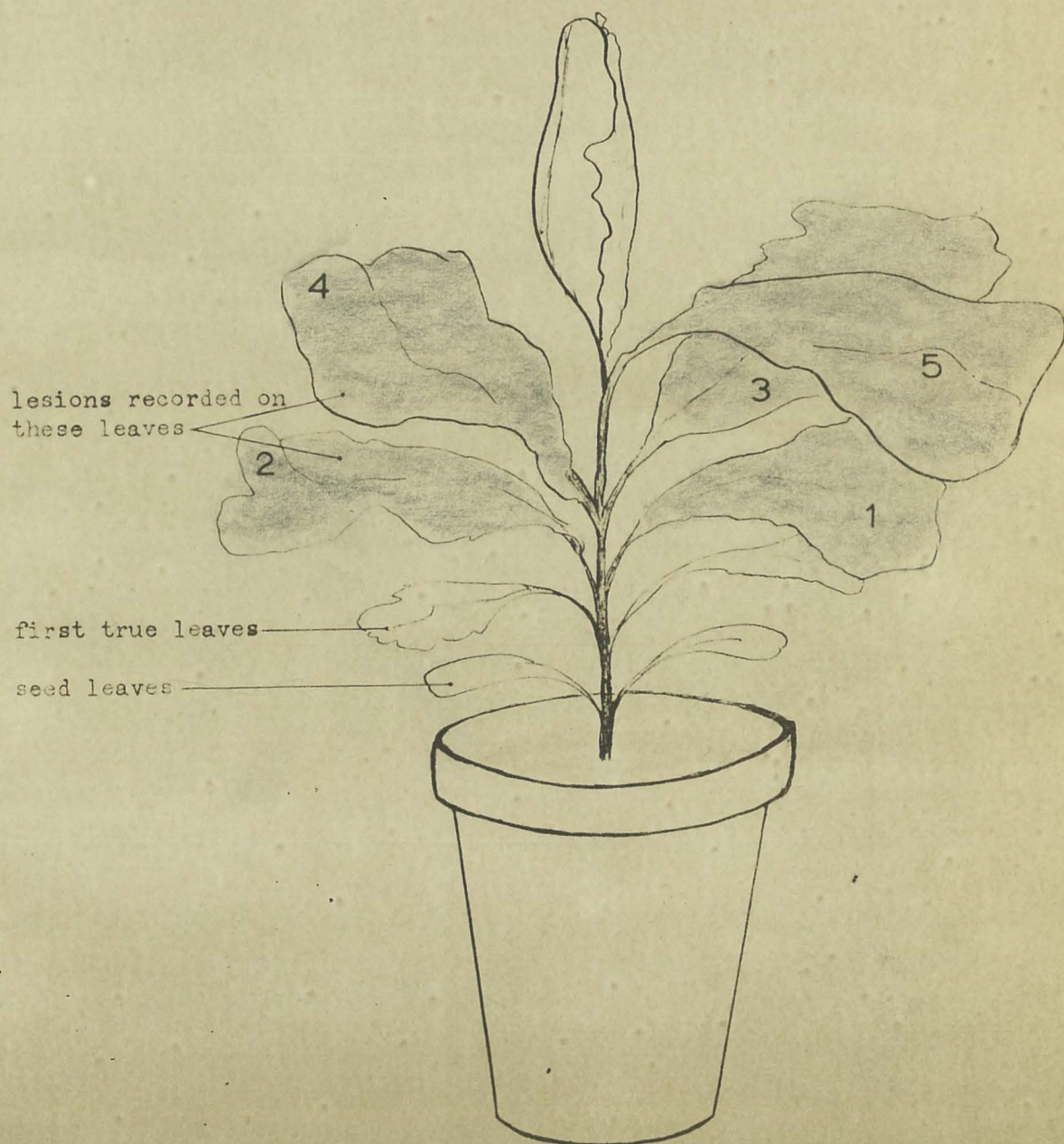
When a measure of the effect of environment on the expression of symptoms was required the individual leaves were removed from the plants and placed into one of ten classes indicated by photographs of diseased leaves which were ranked into ten classes. Leaves free from lesions were rated 0, and those leaves which were dead were rated 10. Therefore a score for a healthy plant was 0 and the maximum score for a dead plant was 50, there being five leaves recorded on each plant.

The sum of leaf totals were termed plant totals. The most successful method of recording investigated was where four plants were used for each plot, and plot totals subjected to statistical analyses.

Most experiments involved four or five treatments. Four or five replications of these treatments applied to plots of four plants enabled differences likely to be important in nature to be measured by the normal statistical procedures (Snedecor, 1956) Cochran and Cox (1957) and Duncan (1953)) and permitted maximum use of glasshouse facilities. Missing plants or plots were estimated using the methods described by Cochran and Cox (1957).

ILLUSTRATION 3

DIAGRAMATIC REPRESENTATION
OF TYPE OF LETTUCE PLANT
USED FOR DISEASE CYCLE
STUDIES



During preliminary experimentation, it was determined that the optimum temperature for growth was above 40°F and below 60°F, the minimum temperature was below 40°F and the maximum approximately 80°F. Therefore the temperature range investigated during the studies of the disease cycle was between 40° - 70°F.

Temperatures were controlled by growing the plants in temperature controlled cabinets. The construction of the cabinets used in this study has been described by Latch (1957). They are a modified form of those used in the Department of Plant Pathology at the University of Wisconsin. One larger cabinet measured 3 x 2 x 2½ feet. Due to development of local "hot spots" in this cabinet it was operated at temperatures closest to the surrounding air temperature. The other cabinets measured 2½" x 2'2" x 2' and were more efficient. A fan was installed in the cabinet which operated at temperatures 20°F. or more above the surrounding air temperatures.

By placing the cabinets in a cool store operating at 40°F, temperatures ranging from 40° - 80°F. were obtained.

The temperatures in the cabinets were controlled by TS2 Sunvic thermostats connected in series with three 100-watt bulbs. Providing the contacts in the thermostats were kept clean and adjusted correctly, temperatures were controlled to within 2°F. of the required temperature.

Cabinets were operated for twelve hours before the plants were introduced with the thermographs in place to check on the operation of the thermostats. Thermographs were also placed in the cabinets when space was available. As soon as an increase in the temperature differential was noticed, the contact points were cleaned or replaced, and re-adjusted.

If the plants were to remain in the cabinets for several days light was provided for twelve hours a day. One 150 Watt reflector type floodlight was suspended 12" above each cabinet. To minimise the heating effect of the lamp, a sheet of glass was placed 2" above the cabinet. The lights were operated during the night by a time switch.

After inoculation, prior to placing the plants in the incubator they were held for 24 hours in 100% relative humidity.

A glass cabinet measuring approximately 2'3" x 3' x 2'6" was fitted with a rack to support two shallow metal trays. Following inoculation the plants were placed on the metal trays inside the cabinet and the trays filled with $1\frac{1}{2}$ " of water. The door was tight fitting and when closed it enabled a relative humidity of 100% to be maintained.

When larger numbers of plants were involved, they were placed in trays on a bench and covered with polythene sheeting which was kept in contact with the wet bench by weighted timber.