STUDIES ON FACTORS AFFECTING BOTRYTIS DISEASES ON CERTAIN CROP PLANTS

A thesis submitted to the University of Glasgow, for the Degree of Doctor of Philosophy in the Faculty of Science.

by

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SUMMARY

STUDIES ON FACTORS AFFECTING BOTRYTIS DISEASES ON CERTAIN CROP PLANTS

by

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The investigation forming the subject of this report was planned to study certain factors affecting Botrytis diseases on certain crop plants. Results are summarised in the following points:-

1. The optimum temperature for growth of Botrytis cinerea Pers., Botrytis cinerea f. lini, Botrytis fabae Sard., Botrytis galanthina Berk. & Br., Botrytis narcissicola Kleb., Botrytis paeoniae Oudem. and Botrytis squamosa Walker was 21°C., but there was no growth at 2°C. or at 31°C.

2. Botrytis cinerea Pers. as a disease on lettuce was more severe on 20°C. than 7°C. or 15°C.

3. Spores of Botrytis allii Munn, Botrytis cinerea Pers., Botrytis cinerea f. lini and Botrytis squamosa Walker germinated only at 100% relative humidity and there was no germination in any species at 95% relative humidity.

4. Botrytis cinerea Pers. was more severe on lettuce in very high relative humidities.

5. Length of day had no effect on the severity of Botrytis diseases.

6. Infection with Botrytis cinerea on lettuce and potato, Botrytis fabae on field beans and Botrytis allii on onions was more severe when plants were grown in more acid soils (below about pH 5.0). There was a significant correlation between acidity of the soil and severity of the diseases studied. One pot experiment with Chocolate Spot was made with soil ranging naturally from pH 4 to pH 7.2. It showed that growth of beans was greatest at about pH 5.7, when the amount of the disease was low. Above this value, the yield fell off with increasing soil alkalinity, but the amount of disease remained fairly constant at a low level. Below pH 5.7 the yield fell markedly and the disease increased considerably.

7. There was only a slight increase in the amount of disease resulting from artificial infection, as compared with natural infection on lettuce and beans, especially on the acid soils.

8. Infections of Botrytis cinerea on lettuce and tomato, Botrytis fabae on field beans and Botrytis allii on onions were all more severe on unmanured plots than on those treated with fertiliser. This effect was non-specific, and occurred with additions of phosphate, potash, organic nitrogen and even with normal dressings of ammonium sulphate. Differences in the amount of disease were highly significant in the experiment where statistical

treatment could be applied.

9. Normal applications of sodium nitrate increased the amount of Botrytis disease on several crops, and double applications of ammonium sulphate increased the amount of disease in one experiment with onions.

10. There was a significant inverse correlation between the severity of Chocolate Spot and available phosphorus (P_2O_5) in the soil.

11. There was no significant relation between yield of tomatoes in the untreated plots and those treated with varying amounts of hoof and horn. There was, however, a significant correlation between the yield and the percentage of the disease.

12. Ultra violet light checked the growth without killing the cultures of Botrytis allii Munn, Botrytis cinerea Pers., Botrytis fabae Sard., Botrytis paeoniae Ouden., Botrytis squamosa Walker and Botrytis tulipae (Lib.) Lind. It had no effect upon the time of sporulation except in cultures treated three days in succession. It then caused a slight delay of one day in sporulation.

13. No practical control of Grey Mould on lettuce by treatment with ultra violet light appeared to be possible. The irradiation, however, affected the seedlings' growth.

14. Painting tomato wounds with bitumen paint resulted in considerably less disease at the end of the growing season. The results showed that the painting of wounds was fully economic up to the beginning of July but not thereafter.

15. No control appeared from dusting established lettuce plants with pentachloronitrobenzene (Folosan).

CONTENTS

SECTION IV

INTRODUCTION

The fungus Botrytis cinerea Pers. is very widespread on living and dead plant material. It causes the most important disease of lettuce in Great Britain, and brings considerable losses on tomatoes. Other species of the genus have been found to cause damage to field beans (Vicia faba), onions and many other plants.

Temperature, humidity, light and nutritional conditions are some of the chief factors which may act on the parasite, or on the host, or on the two when brought into association. Temperature might be decisive in determining the presence and amount of disease, while humidity appears to act less frequently as a limiting factor in the distribution of parasitic fungi. Light is of much less significance in controlling the actual distribution of fungi than either temperature or relative humidity, but the length of day might occasionally be important in the incidence of disease.

Soil acidity and soil nutrition may affect the host plant in marked degree, whether by modification of structure or of physiological function, both of which may influence their resistance to disease. Natural soils differ considerably in their relative acidity (pH) which may affect plants directly by modifying the metabolism,

and indirectly, by varying the availability of inorganic material or the physical properties of soil. Variation in soil reaction may therefore also affect the susceptibility or resistance of the crop to a particular fungus disease.

Results of work on the influence of fertiliser and other nutrients applied to the soil show that the effect on crop disease varies for each host parasite relation. In general, however, high nitrogen increases susceptibility to many diseases affecting the green parts of plants, while potash usually increases resistance, and the influence of phosphate is variable.

The present investigation was planned to study the effect of these various factors on the severity of certain Botrytis diseases.

S E C T I O N I

The effect of external factors on *Botrytis* diseases

A. Temperature

(i) On the fungus in culture

(ii) On the severity of *Botrytis cinerea* Pers. on lettuce.

B. Relative humidity

(i) On the germination of *Botrytis* spores

(ii) On the severity of *Botrytis cinerea* Pers. on lettuce.

C. Length of day and *Botrytis* diseases

THE EFFECT OF EXTERNAL FACTORS ON BOTRYTIS DISEASES

This section considers the effect of temperature on the rate of growth of some species or forms in culture, and the relation between relative humidity and spore germination. It also considers the disease severity of Botrytis cinerea Pers. (Grey Mould) on lettuce in relation to these factors, and further estimates the effect of length of day on three Botrytis diseases.

PREVIOUS WORK

Temperature Studies

On the fungus in culture

Walker (1926) in his work on Botrytis allii Munn, Botrytis byssoidea Walker and Botrytis squamosa Walker, found that all the three species grew on potato dextrose agar over a range between 3°C. to 33°C., with greatest development between 20 and 25°C. Spore germination occured over a range between 3°C. and 27°C., but was most vigorous between 19° and 27°C. Newton and Hastings (1931), in their experiments on the production of conidia by the fungus Botrytis tulipae (Lib.) Lind., found that spores were rarely produced above 25°C. El Helaly (1938) stated that the fungus Botrytis fabae Sard. made its best growth at about 20°C.; the optimum temperature for germination of the conidia was about 21°C., but the thermal death point was 51°C. Timmermans (1946) mentioned that the minimum,

optimum and maximum temperatures for the growth of the fungus Botrytis gladiolorum Timmermans were below 3°, 20° to 22.5° and 30°C. respectively. Wade (1946) noticed that the optimum growth of the fungus Botrytis gladioli Kleb. was 21°C. and the maximum 30°C.

On the host

Lauritzen and Wright (1930) mentioned that the fungus Botrytis cinerea Pers. was able to attack chillies at temperatures from 0° to 13°C., but 4.5°C. was more favourable for infection than either 0°C. or 10°C. Guterman (1930) working on lily diseases, mentioned that the optimum temperature for growth of the fungus Botrytis elliptica (Berk.) Cooke on the host was 21°C. Inoculation experiments carried out by Gregory (1932) with narcissus bulbs of the variety "Emperor" showed that the optimum temperature of Botrytis narcissicola Kleb. was near 20°C. Abdel Salam (1934) mentioned that natural or artificial infection with Botrytis cinerea Pers. on lettuce was much less severe in warm greenhouses than in cold frames. Wilson (1937) noticed that the maximum temperature for infection with Chocolate Spot was close to 30°C., the optimum was clearly in the vicinity of 20°C. and the minimum was between +1 and -1°C. Moore (1944) found that air temperatures between 60°F. to 68°F. (15° to 20°C.) were

most favourable to the fungus Botrytis cinerea on field and broad beans. Ogilvie (1945) stated that rapid and extreme temperature changes were the most favourable factors to the incidence of Grey Mould on lettuce. McClellan, Baker and Gould (1949) found that infection of foliage of three varieties of gladiolus with Botrytis gladiolorum Timmermans was most severe at 13°C. and 18°C. They added that the optimum temperature for infection of freshly-harvested corms was at 2°C. Some infection also took place at 7°C., but none at 13°C. or higher.

Relative Humidity Studies

Most of the references found refer to fungi on the host. Sardina (1931) found that the minimum relative humidity for infection of beans by Botrytis fabae Sard. was 84-85 per cent. Abdel Salam (1934) suggested that high atmospheric humidity might probably be more conducive to the attack of Botrytis cinerea Pers. on lettuce than high soil humidity. Taylor (1935) showed that at 65°F. (18°C.) a relative atmospheric humidity of 97.7% was necessary to induce conidiophore production of Botrytis elliptica (Berk.) Cooke; at 80°F. (27°C.) an almost saturated atmosphere was required. Beaumont, Dillon Weston and Wallace (1936) found that a humidity between 90 and 100 per cent was necessary for spore formation of Botrytis tulipae Lind. (Lib.) Wilson (1937) mentioned that one of the optimum

conditions for aggressive infection was high humidity, and rain to maintain the water film on the foliage for some days. He added furthermore that Chocolate Spot of field and broad beans appeared only at a relative humidity between 100 per cent and 90 per cent but there was no infection at 85 per cent. Smieton and Brown (1940) found that under crowded enclosed conditions, giving high humidity in cold frames during winter, when ventilation was attended with risk from frost, lettuce was highly subject to Grey Mould. Moore (1944) stated that high humidity favoured Chocolate Spot. In greenhouses a high degree of humidity was one of the most favourable factors to the incidence of Grey Mould on lettuce (Ogilvie 1945). Hawker (1950) noted that when Botrytis cinerea Pers. was grown in a saturated atmosphere the conidiophores were long and indefinite and few spores were produced. In a dry atmosphere the conidiophores were very short and bore numerous spores. Intermediate humidities were most favourable to sporulation.

Length of Day and Botrytis Diseases

Wilson (1937) found that there was no difference in rate of development or of intensity of Chocolate Spot of broad beans (Vicia faba) in both plants kept in the dark and in diffuse daylight. Grainger (1949) mentioned that broad beans had similar amounts of Chocolate Spot, when grown in both short-day and long-day conditions.

GENERAL METHODS

Certain methods common to this section are dealt with here, but those applicable to particular investigations are mentioned later, in the appropriate place.

CULTURES

Malt agar was used throughout as a medium for culturing Botrytis fungi. For work on temperature, Petri dishes were inoculated with approximately equal portions of mycelium. Batches of four similar cultures were transferred to each temperature environment immediately after inoculation.

RELATIVE HUMIDITY AND SPORE GERMINATION

Spores of each fungus studied were scattered on cover glasses and then each was placed over an appropriate saturated solution within an enclosed tube kept at 20°C. The atmosphere over each saturated solution has a characteristic relative humidity as shown in table 1.

Table 1. Saturated solution and the relative humidity over each

Solution	% R.H.
(1) Water	100
(2) $NaHPO_4 12H_2O$	95
(3) $ZnSO_4 7H_2O$	90
(4) NH_4CL	79.5
(5) $NaCLO_3$	75

POT EXPERIMENTS

Eight-inch pots were filled with soil (medium loam) manured with a mixture of 1 ounce ammonium sulphate, 2 ounces of superphosphate and one ounce potassium sulphate per 1 cwt.

INOCULATION

Cultures of Botrytis cinerea Pers. (isolated from lettuce) and Botrytis fabae Sard. were used throughout the experimental studies. Several Petri dishes of malt agar were inoculated with the fungus and kept at 21°C. for two weeks. Spores were removed from the resulting growth by flooding the plates with sterile water and gently rubbing the surface of the medium with a sterilised finger. The water suspension was filtered to remove the mycelium, and was then sprayed upon the plants.

DISEASE ASSESSMENT

Field Beans (Vicia faba)

The amount of Chocolate Spot covering the leaf area was estimated as a percentage of the leaf area by a standard area diagram (Grainger, 1950). Three readings were taken, one among the lower leaves, another round the middle and the third at the top. The average of the three readings was taken as the percentage of infection for the

particular sample. Some of the infected leaves were kept under moist conditions. Botrytis fabae Sard. was identified by spore and sclerotia measurements, but Botrytis cinerea was also found.

Lettuce

The percentage of disease was calculated from counts of total and infected plants. Infected plants usually bore Botrytis fructifications, but if there was any doubt, plants were incubated a short time under moist conditions. If active Botrytis was present, spores developed within 24-48 hours, whereas chance contamination did not lead to sporulation before five days at least. No plant was counted as diseased unless it showed the presence of Botrytis fructifications.

Cultures of Botrytis cinerea from infected lettuce and of Botrytis fabae from beans were isolated and were sprayed on lettuce and beans. Sprayed plants showed the same symptoms.

RESULTS

TEMPERATURE

On Botrytis fungi in culture

Petri dish cultures were placed in incubators at the various temperatures shown in tables 2 to 7. The

diameter of growth was recorded daily and the area calculated from these measurements.

Table 2. Area of culture in sq. cm. at different temperatures. Botrytis cinerea Pers. from lettuce

Days after inoculation	2°C.	13°C.	18°C.	21°C.	26°C.	31°C.
1	0	0.12	0.12	1.2	0.12	0
2	0	0.78	2.01	4.52	3.14	0
3	0	3.14	10.17	16.61	16.61	0
4	0	8.04	22.89	26.40	22.89	0
5	0	19.62	38.46	45.38	40.69	0
6	0	24.69	45.38	59.06	52.78	0

Table 3. Area of culture in sq. cm. at different temperatures. B. cinerea f. lini.

Days after inoculation	2°C.	13°C.	18°C.	21°C.	26°C.	31°C.
1	0	0	0.12	0.12	0.12	0
2	0	0.12	0.79	0.79	0.79	0
3	0	0.50	2.01	9.07	5.31	0
4	0	1.13	4.52	16.61	13.85	0
5	0	4.52	12.56	32.15	26.46	0
6	0	8.04	21.22	50.24	42.78	0

Table 4. Area of culture in sq. cm. at different temperatures. *Botrytis fabae* Sard.

Days after inoculation	2°C.	13°C.	18°C.	21°C.	26°C.	31°C.
1	0	0.12	0.12	0.12	0.12	0
2	0	0.49	0.78	3.14	0.49	0
3	0	5.20	5.20	10.03	3.14	0
4	0	7.07	18.26	19.62	5.20	0
5	0	12.56	29.17	36.29	7.07	0
6	0	22.89	45.38	49.24	10.03	0

Table 5. Area of culture in sq. cm. at different temperatures. *Botrytis galanthina* Berk. & Br.

Days after inoculation	2°C.	13°C.	18°C.	21°C.	26°C.	31°C.
1	0	0	0.12	0.12	0.12	0
2	0	0	3.14	3.14	0.12	0
3	0	0.12	9.07	10.17	0.12	0
4	0	0.12	16.61	22.89	0.78	0
5	0	0.78	26.46	32.15	3.14	0
6	0	4.52	34.19	50.24	6.15	0

Table 6. Area of culture in sq. cm. at different temperatures. Botrytis narcissicola Kleb. (see also fig 3 p. 23A)

Days after inoculation	2°C.	13°C.	18°C.	21°C.	26°C.	31°C.
1	0	0	0	0.12	0	0
2	0	0.12	0.12	2.01	0.12	0
3	0	0.12	0.78	18.26	0.78	0
4	0	0.78	11.33	38.46	3.80	0
5	0	3.80	16.61	45.38	6.15	0
6	0	8.04	21.22	55.39	11.33	0

Table 7. Area of culture in sq. cm. at different temperatures. Botrytis paeoniae Oudem.

Days after inoculation	2°C.	13°C.	18°C.	21°C.	26°C.	31°C.
1	0	0	0.12	0.12	0.12	0
2	0	0.49	3.80	11.33	4.50	0
3	0	4.50	11.33	26.40	7.07	0
4	0	13.95	21.22	38.48	11.33	0
5	0	22.89	34.19	49.24	19.62	0
6	0	32.15	45.38	58.06	26.40	0

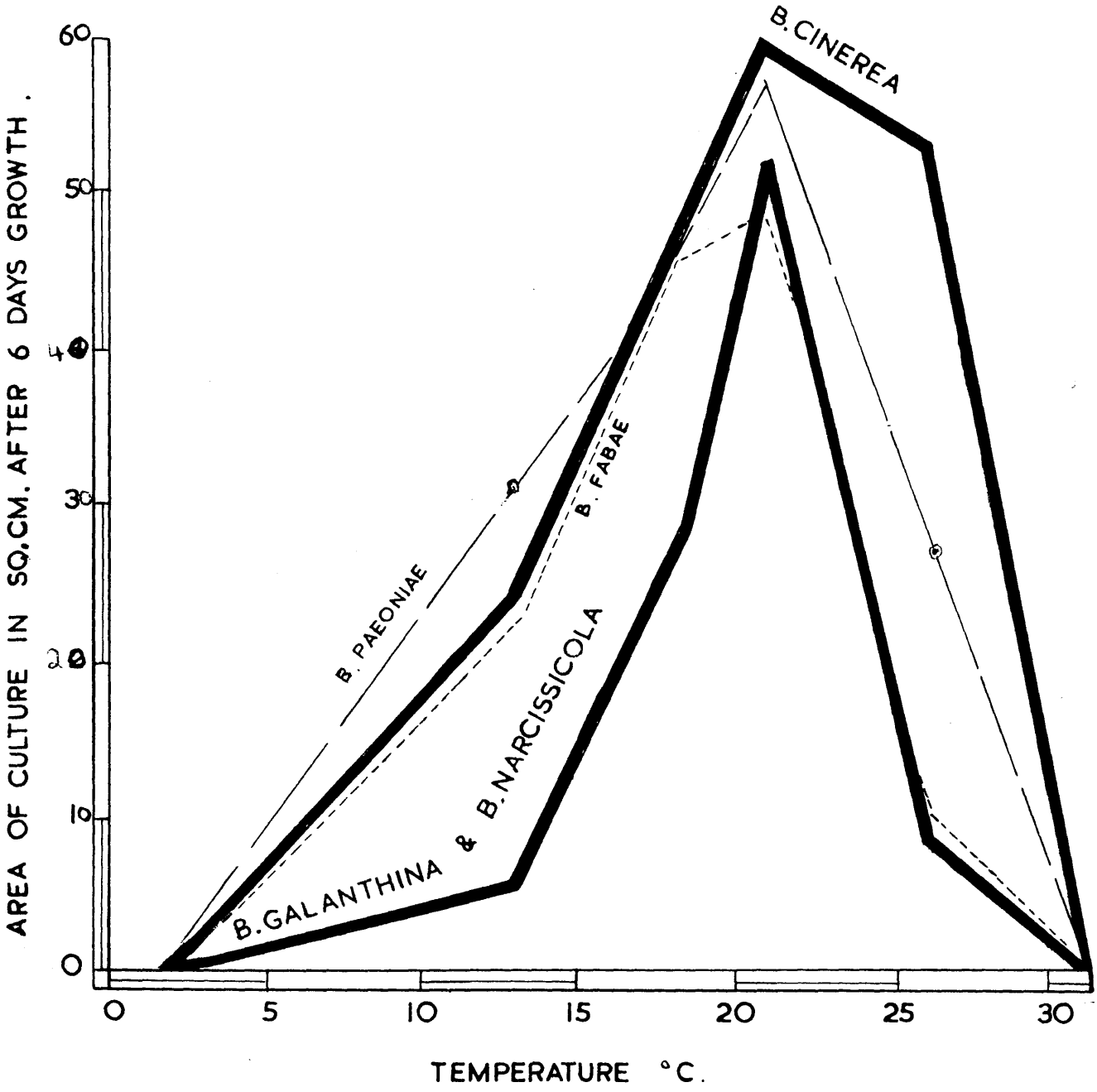


Fig. 1. The effect of temperature on mycelial growth.

It will be seen particularly from fig. 1 that all species investigated had a similar optimum temperature of growth round 21°C., and all agreed also in having no growth at 2°C. and 31°C. There were, however, certain qualitative differences between the species. Botrytis cinerea Pers. had a fairly "wide" temperature curve (fig. 1), i.e. it made relatively good growth at 13°C. and 26°C. Botrytis galanthina Berk. & Br. and Botrytis narcissicola Kleb. had a much "narrower" temperature curve and made relatively little growth at 13°C. and 26°C. Botrytis fabae Sard. and Botrytis paeoniae Oudem. were intermediate between these two types of reaction.

Temperature and the severity of Grey Mould on lettuce

Lettuce, variety New Market, was sown on October 19, 1952. Seedlings were transplanted on January 20, 1953, into 8-inch pots. 17 pots each containing three seedlings were placed in each of three greenhouses maintained at the average temperatures shown in table 8. They were inoculated with Botrytis cinerea. Results are given in table 8.

Table 8. Percentage infection of Grey Mould on lettuce at different temperatures

Average temperature in °C.	20	15	7
Percentage infection	27.4	13.7	9.8

RELATIVE HUMIDITY

The Effect on germination of fungus spores

Table 9 shows that spores of Botrytis species studied germinated only at 100% relative humidity, and there was no germination even at 95%.

Table 9. Germination of Botrytis spores at different relative humidities

Species	Percentage relative humidity				
	100	95	90	79.5	75
Botrytis allii	+	-	-	-	-
Botrytis cinerea	+	-	-	-	-
Botrytis cinerea f. lini	+	-	-	-	-
Botrytis squamosa	+	-	-	-	-

\+ germination
\- no germination

Relative humidity and the severity of Grey Mould on lettuce

It has been shown that spores of several Botrytis species germinated only at 100% relative humidity. Fig. 2 shows some relation between the number of hours per week when the atmosphere was at 100% relative humidity and the amount of disease on lettuce and beans in the outdoor plots (1950). It would appear that weeks with only a small number of hours with saturated atmosphere did not have any great effect on the disease. It is noticeable that Choco-

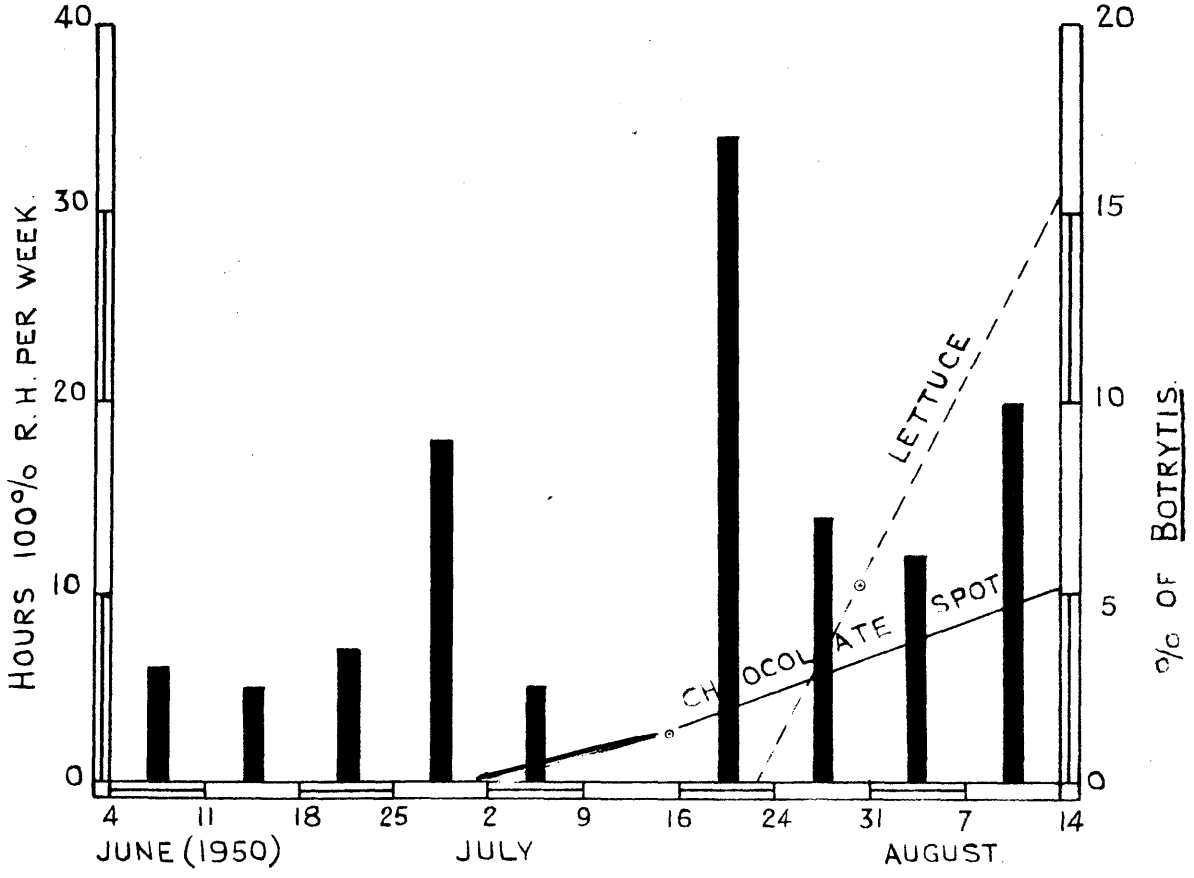


Fig. 2. Relation between Botrytis diseases and the number of hours 100% relative humidity (R.H.) per week. Vertical columns represent the numbers of hours per week with saturated atmosphere.

late Spot began its attack on field beans in the week following June 25 to July 2 when there were 18 hours during which the atmosphere was completely saturated. Botrytis attack on lettuce began after a week when there were 34 hours of complete saturation of the atmosphere.

In order to test these preliminary indications young lettuce plants were grown in pots which were placed within muslin cages. The muslin was supported on a framework of wire netting, and water was allowed to drip on the fabric. The relative humidity inside each cage was measured by inserting the thermometer sheath of an Assmann psychrometer through a hole left for the purpose. The hole was normally kept covered by the muslin. It was found that by varying the thickness of the muslin, altering the rate of water drip and changing the amount of ventilation at the lower end of each cage, the percentage relative humidity could be varied from about 86% to nearly 100%. Plants in three cages and also others at the relative humidity of the greenhouse (about 80%) were all inoculated with spores of Botrytis cinerea. Sixteen plants were included in each relative humidity with results which are given by table 10.

Table 10. Percentage infection at different relative humidities

Relative humidity, % (average of three readings a day)	98	94	90	80
Percentage infection	62.5	43.7	25.0	12.5

Length of day and Botrytis diseases

Ten pots were sown with field beans, variety Kilbride (four plants in each pot) on May 4, 1950, ten others with potato, variety Gladstone, (one plant in each pot) and ten others with Brussels Sprouts (3 plants in each pot). Five pots from each crop were left in the normal day length and the other five were exposed to a daily light period of only 8 hours, from 9 a.m. till 5 p.m. They were housed for the remaining part of each day in a well ventilated dark house.

Beans were sprayed on June 20, 1950, with a spore suspension of the fungus Botrytis fabae Sard.; potatoes and Brussels Sprouts were sprayed with the fungus Botrytis cinerea. The symptoms of Chocolate Spot appeared on both long-day and short-day plants during the first week of July. No disease appeared on either potato or Brussels Sprouts in both treatments. Table 11 gives the results for beans. Although there were notable differences between the fresh weights of all three kinds of plants, there was no effect of the length of day on the severity of Botrytis

diseases.

Table 11. The effect of length of day on Botrytis diseases

Crop	Pot No	LONG DAY % infection	LONG DAY Fresh wt. per plant in gms.	LONG DAY Time of flowering	SHORT DAY % infection	SHORT DAY Fresh wt. per plant in gms.	SHORT DAY Time of flowering
Beans	(1)	2.0	29	July 10, 1950	6.0	26	August 12, 1950
	(2)	6.0	31		6.0	26	
	(3)	5.0	30		7.0	25	
	(4)	7.0	30		7.0	22	
	(5)	6.0	34		6.0	24	
Average		5.2	30.8		6.4	24.6	

DISCUSSION AND CONCLUSIONS

All species of Botrytis studied in culture have, in general, similar relations to temperature and humidity. Tables 2 to 7 showed that optimum growth was made at 21°C., and there was no growth at 2°C. and 31°C. There were, however, certain qualitative differences between the species. Botrytis cinerea Pers. had a fairly "wide" temperature curve (fig. 1) i.e. it made relatively good growth at 13°C. and 26°C. Botrytis galanthina Berk. & Br. and Botrytis narcissicola Kleb., which attack monocotyledons, had a much "narrower" temperature curve and made relatively little growth at 13°C. and 26°C. Botrytis fabae Sard. and Botrytis paeoniae Oudem. were intermediate between these two types of reaction. It seems from the study of B. cinerea on the

~~severity of~~ lettuce that the disease was more severe at 20°C. than either 7°C. or 15°C., thus suggesting that temperature has a similar relation to the disease to that which it has on the fungus in culture. These results agree with Guterman (1930) on lily diseases, Gregory (1931) on narcissus, Wilson (1937) and Moore (1944) on field beans.

Spores of Botrytis cinerea, Botrytis cinerea f. lini, Botrytis allii and Botrytis squamosa germinated only at 100% relative humidity. The severity of Grey Mould on lettuce seems also to be severe in high average relative humidities. This agrees with all the workers mentioned in the paragraphs above on previous work.

A continuous record of relative humidity is rather difficult to obtain. Hair hygrographs are not very reliable, and duplex wet-and dry-bulb instruments do not record actual relative humidity. Readings with an Assmann psychrometer are reliable, but do not give an adequate idea of the relative humidity between readings. Average readings obtained by the latter method may therefore only be of use in the present problem insofar as they reflect the possible frequency of 100% relative humidity. This assumption would explain the apparent difference between the facts that Botrytis spores germinate only at 100%, but that substantial disease was found at an average relative humidity of 80% (table 10) p. 20.

Length of day had no effect on Botrytis disease. There was no difference in the amount of three Botrytis diseases on plants grown with eight hours illumination per day when compared with those exposed to the normal length of day. Scotland would thus appear to have no advantage or, indeed, disadvantage over regions to the south where the days are shorter in summer.

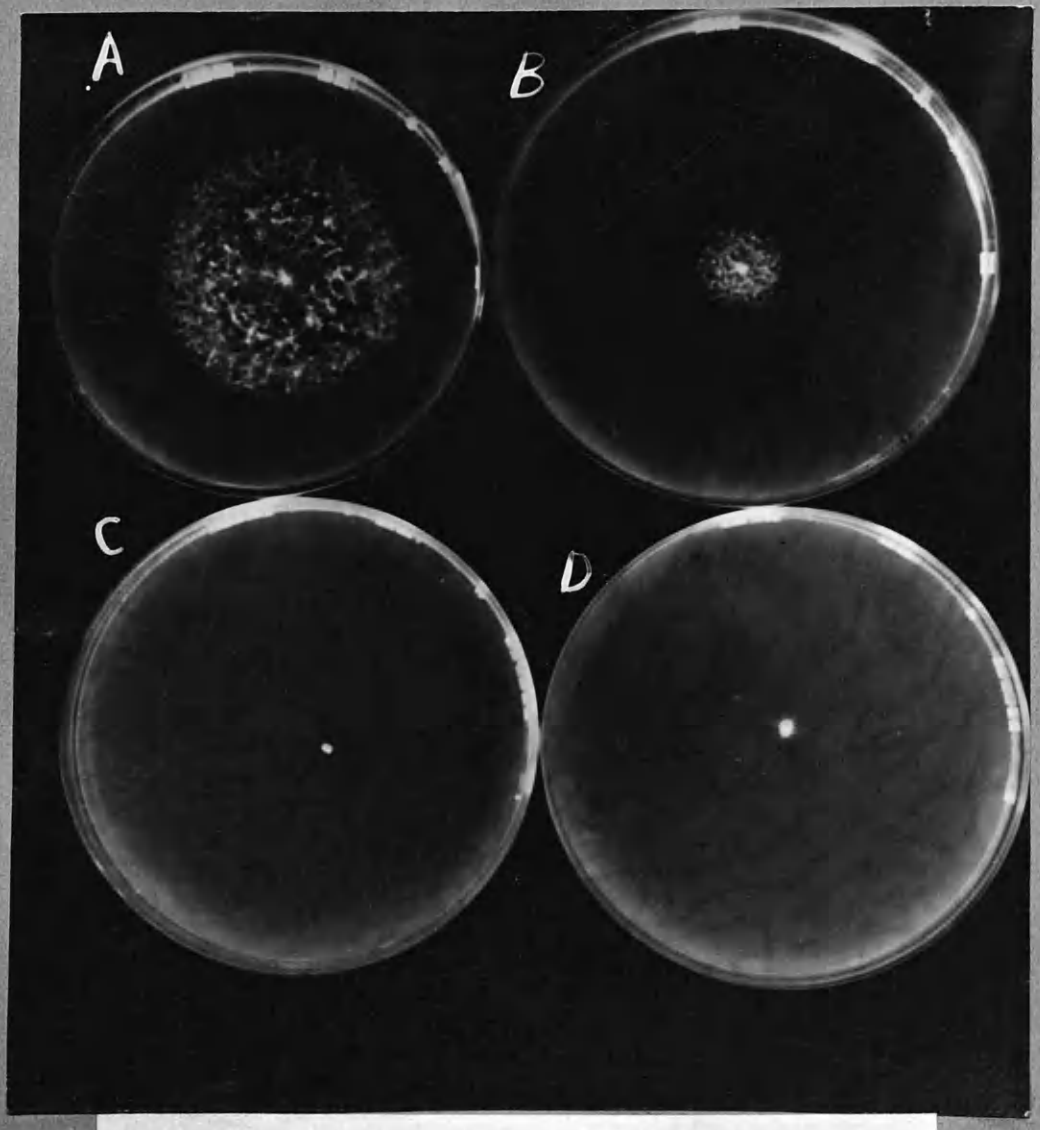


Fig. 3 The effect of temperature on the growth of Botrytis narcissicola: A 21°C, B 18°C, C 13°C and D 26°C. All cultures are four days old.

SECTION II

The effect of soil acidity on the severity of:-

A. *Botrytis fabae* Sard. on field beans (*Vicia faba*)

B. *Botrytis cinerea* Pers. on lettuce and potatoes

C. *Botrytis allii* Munn on onions

SOIL ACIDITY AND BOTRYTIS DISEASES

PREVIOUS WORK

Acidity of culture media

Sardina (1931) studied the fungus Botrytis fabae Sard. on a number of standard media and found that the optimum hydrogen ion concentrations for mycelial, conidial and sclerotial development were 5.3, 7.3 and 4.5 respectively in one strain of the fungus, but in another strain they were 7.3, 7.7 and 3.9. Laboratory studies of El Helaly (1938) showed that the fungus Botrytis fabae Sard. made its best growth near pH 4.5. Timmermans (1946) found that the optimum and maximum pH values for the fungus Botrytis gladiolorum Timmermans were 5 to 5.5 and 7.0 respectively.

Acidity of the soil on which the host plant is grown

Very few references to the relation between soil acidity and Botrytis diseases were found. Wilson (1937) mentioned that sour soils have been observed to increase the severity of Chocolate Spot on field beans, but no exact results were secured with regard to the influence of this factor. Glasscock, Ware and Pizer (1944) noticed that there was no significant relation between the severity of Chocolate Spot on beans and the acidity of soil between pH 4.8 and 7.5, but Warington (1950) noticed that susceptibility of Botrytis on lettuce was high on plants grown in acid solutions.

It was therefore decided to establish the relation between soil acidity and the severity of Botrytis fabae on field beans, Botrytis cinerea on lettuce and potatoes and Botrytis allii on onions.

GENERAL METHODS

POT EXPERIMENT

Six soils (medium loam) differing naturally in pH values between 4.0 and 7.2 were selected. They were filled into 8-inch pots and were used for growing field beans (Vicia faba), variety Kilbride (sown April 28, 1951). Five pots were used for each sample of soil. The relative acidities of the various soils with their subsequent disease behaviour are shown by tables 13 and 14 (p. 31 and 32). Four plants were grown in each pot.

FIELD EXPERIMENTS

Four plots each about 50 sq. yds. were brought to varying degrees of acidity by different rates of application of sulphur, and one untreated plot with pH 5.9 was left as a control. Sulphur has been widely used for making soil more acid (see later in discussion). The soil was a well-drained medium loam with the following mechanical analysis:-

	%		%
Coarse sand	22	Silt	12
Fine sand	34	Clay	16
Moisture and Loss on ignition	16		

The applications were made on April 24, 1950, and subsequent pH values at various times are recorded in table 12 and fig. 4.

Table 12. pH values before and after treatment with sulphur, April 24, 1950

Plots	A	B	C	D	E
pH before treatment	5.2	5.6	6.0	6.2	5.9
Treatment, oz. of sulphur added per sq. yd.	16	8	4	2	nil
pH value on June 14, 1950	3.7	4.1	4.3	5.2	5.9
pH value on Aug. 3, 1950	3.1	3.5	3.7	4.7	5.8
pH value on Feb. 13, 1951	3.9	4.0	4.2	4.5	5.9
pH value on May 6, 1951	3.5	3.9	4.0	4.5	5.9

The following crops were grown on the plots in 1950 and 1951:-

1950

FIELD BEANS, variety Kilbride, sown May 10
(One row 7 yds. long in each plot)

LETTUCE, variety New Market, sown May 10
(13 rows spaced at 14 inches, with plants at 12 inches. Approximately 220 plants per plot)

ONIONS, variety Ailsa Craig, sown May 10
(One row in each plot. Approximately 54 plants per plot)

POTATOES, varieties Kerr's Pink and Gladstone, planted May 12.
(3 rows spaced at 26 inches with 15 inches between plants. Approximately 54 plants per plot)

1951

FIELD BEANS, variety Kilbride, sown May 20
(Two rows 7 yds. long spaced at 18 inches in each plot)

LETTUCE, variety New Market, sown May 20
(The same as 1950)

ONIONS, variety Ailsa Craig, sown May 20
(The same as 1950)

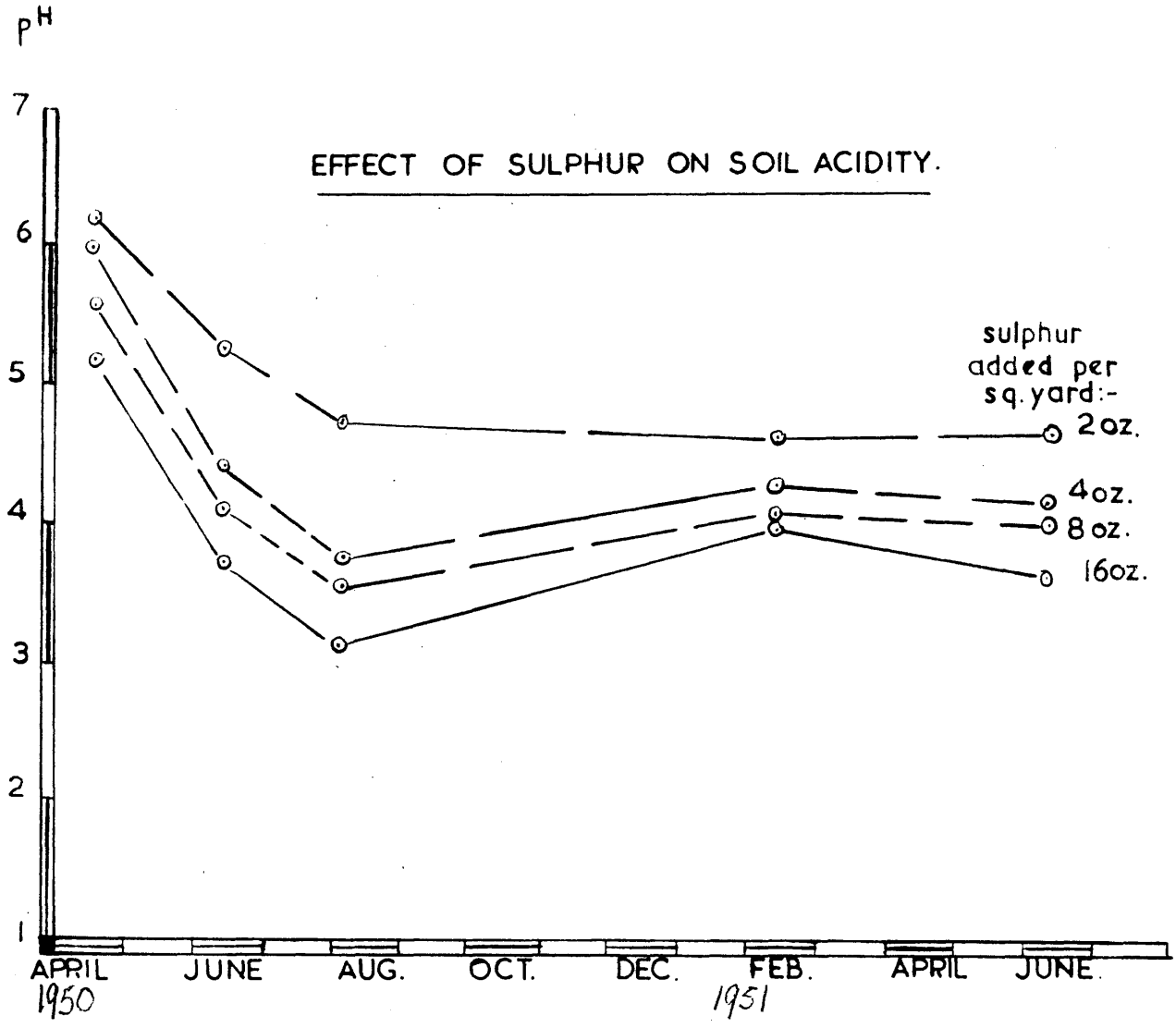


Fig. 4. Changes in soil acidity (pH) following application of sulphur at various rates on April 24, 1950.

With the exception of low numbers on some of the most acid plots mentioned later, the disease estimations are based on numbers of plants comparable to those given above.

Five additional plots (each about 40 sq. yds) - F, G, H, I and J - with a wide variation in soil acidity (resulting from varying applications of sulphur (2 to 16 oz. per sq. yd.) made in 1948) were also used in 1951 to estimate natural infection by the fungus Botrytis cinerea Pers. on lettuce. The relative acidities of the various soils with their subsequent disease behaviour are shown in table 20 (p.37).

INOCULATION AND DISEASE ASSESSMENT

Lettuce and field beans

The same methods for inoculation as those described (in section 1) were used for field beans and lettuce. In estimating the amount of Chocolate Spot in field plots, however, ten determinations were made per plot, and averaged to give the value for the plot.

Potatoes

Artificial infection was with Botrytis cinerea. Plants did not die as a result of infection but only some of the foliage showed lesions of disease. Those were kept for a short time under moist conditions, when fructifications of Botrytis cinerea covered all infected lesions.

Onions

Artificial infection was with Botrytis allii. The percentage of disease was calculated from counts as with lettuce, but, as a different species of Botrytis was involved, steps were taken to see that Botrytis allii was actually the cause. In the field a few bulbs were infected with Neck Rot. These were kept under moist conditions for a short time. Microscopic examination then showed whether the species was Botrytis allii Munn or not. Spores of this species are elliptical 7-16 μ by 4-6 μ, and conidiophores are very short and unbranched, as is typical of this species. From a few bulbs, however, the fungus Botrytis cinerea was isolated, and those bulbs had similar symptoms to those which were infected by Botrytis allii. Sclerotia occurred on the older decayed tissue of some bulbs in storage. They were hard, black, rounded, kernel-like bodies, spherical, oblong or irregular and varying from 2-4 mm. Botrytis squamosa Walker, and Botrytis byssoidea Walker were not found during the experimental period.

In storage (wooden shed), the fungus caused the softening of the infected scale tissue which took a sunken, cooked appearance. Disease progressed most rapidly down the scales which had been originally infected in the neck. Diseased bulbs were periodically removed from storage, and kept under moist conditions. They also were examined to

determine the species which caused the infection. Measurements of spores and conidiophores suggested Botrytis allii Munn.

Cultures of Botrytis fabae from field beans, and potatoes Botrytis cinerea from lettuce and Botrytis allii from onions were isolated from plants in the pot and field experiments and were sprayed upon corresponding healthy plants. Sprayed plants showed the typical symptoms of the disease.

RESULTS

POT EXPERIMENTS

Field Beans (Vicia faba)

Artificial infection

Field beans were sprayed on May 27, 1951, with a spore suspension of the fungus Botrytis fabae Sard. until all surfaces were thoroughly wetted. Table 13 shows the percentage of Chocolate Spot and the yield of beans from series of varying acidity. There was but slight difference in the percentage of Chocolate Spot in the less acid series M, N, O and P (pH 5.1, 5.7, 7.1, 7.2 respectively). With further increase in acidity of the soil, however, there was a marked increase of the severity of Chocolate Spot (K and L; pH 4.0 and 4.5 respectively). The amount of growth, on the other hand, was at a maximum in series N (pH 5.7), and fell off above and below this value. Below about pH 5.7 the amount of disease rose and the growth fell,

but above this value, the growth fell, but the disease stayed uniform at a low value. It would therefore seem that pH affected growth and disease in two different ways (fig. 5).

Table 13. Percentage of infection and yield of beans growing in varying acidity - Artificial infection

Series		K	L	M	N	O	P
pH		4.0	4.5	5.1	5.7	7.1	7.2
	Pot No.						
Percentage infection	(1)	2.0	2.0	2.0	2.0	0.5	0.5
	(2)	2.0	3.0	2.0	2.0	2.0	0.5
July 21, 1951	(3)	5.0	2.0	2.0	2.0	2.0	0.2
	(4)	4.0	2.0	3.0	0.5	0.5	0.2
	(5)	5.0	4.0	0.5	1.0	1.0	0.4
Average infection on July 21		3.6	2.6	1.9	1.5	1.2	0.4
	(1)	40.0	17.0	6.0	5.0	8.0	6.0
	(2)	40.0	20.0	7.0	6.0	4.0	5.0
August 28, 1951	(3)	29.0	18.0	8.0	7.0	2.0	4.0
	(4)	25.0	30.0	10.0	4.0	3.0	7.0
	(5)	50.0	27.0	9.0	5.0	6.0	3.0
Average infection on August 28		36.8	22.4	8.0	5.4	4.6	5.0
Average fresh weight per plant, gms.		7.9	18.7	20.4	36.8	30.9	26.5

Natural infection

Natural infection gave results comparable to artificial infection (table 14), but percentages of disease were slightly lower. Thus series L (pH 4.5) had 22.4% of

disease (artificial infection) in August, but with natural infection it had 15.8% disease at the same time. Series N with pH 5.7 had 5.4% disease in artificial infection and 0.8% disease with natural infection.

Table 14. Percentage of Chocolate Spot and growth yield of beans growing in series of varying acidity

Natural infection

Series		K	L	M	N	O	P
pH		4.0	4.5	5.1	5.7	7.1	7.2
	Pot No.						
Percentage infection	(1)	20.0	14.0	1.0	1.0	1.0	1.5
	(2)	40.0	15.0	1.0	0.5	0.5	1.5
August 27, 1951	(3)	50.0	14.0	1.0	1.0	1.0	1.0
	(4)	35.0	20.0	1.0	0.8	1.0	1.0
	(5)	36.0	15.0	1.0	0.8	0.8	1.2
Average infection		36.2	15.6	1.0	0.8	0.8	1.2
Average weight per plant gms.		8.2	16.0	24.9	31.2	28.5	27.0

Table 15 shows that there was a significant correlation between percentage of disease at the end of the season and pH value for the average infection for the five pots of the six series K to P.

Table 15. Values of the correlation coefficient r, between percentage of disease and pH of soil

	Correlation coefficient	Range of P by t test	
Artificial infection	- 0.82	0.05 - 0.02	significant
Natural infection	- 0.75	0.1 - 0.05	significant

FIELD EXPERIMENTS

These plots were made acid by adding sulphur at different rates, as mentioned under "general methods".

Artificial infection

Field Beans

In 1950 beans failed in the most acid plots (pH 3.1), but plants in the other plots were sprayed with a spore suspension of Botrytis fabae Sard. on June 10, 1950. In 1951 plants appeared in the most acid soil (pH 3.5), and all plots were artificially infected with Botrytis fabae Sard. The symptoms of Chocolate Spot appeared during the fourth week of June in both years. Table 16 and fig. 6 show the results of this experiment. They were similar to those already recorded for soils naturally varying in pH (table 13) p 31.

YIELD & CHOCOLATE SPOT IN RELATION TO SOIL ACIDITY (Pot Experiments)

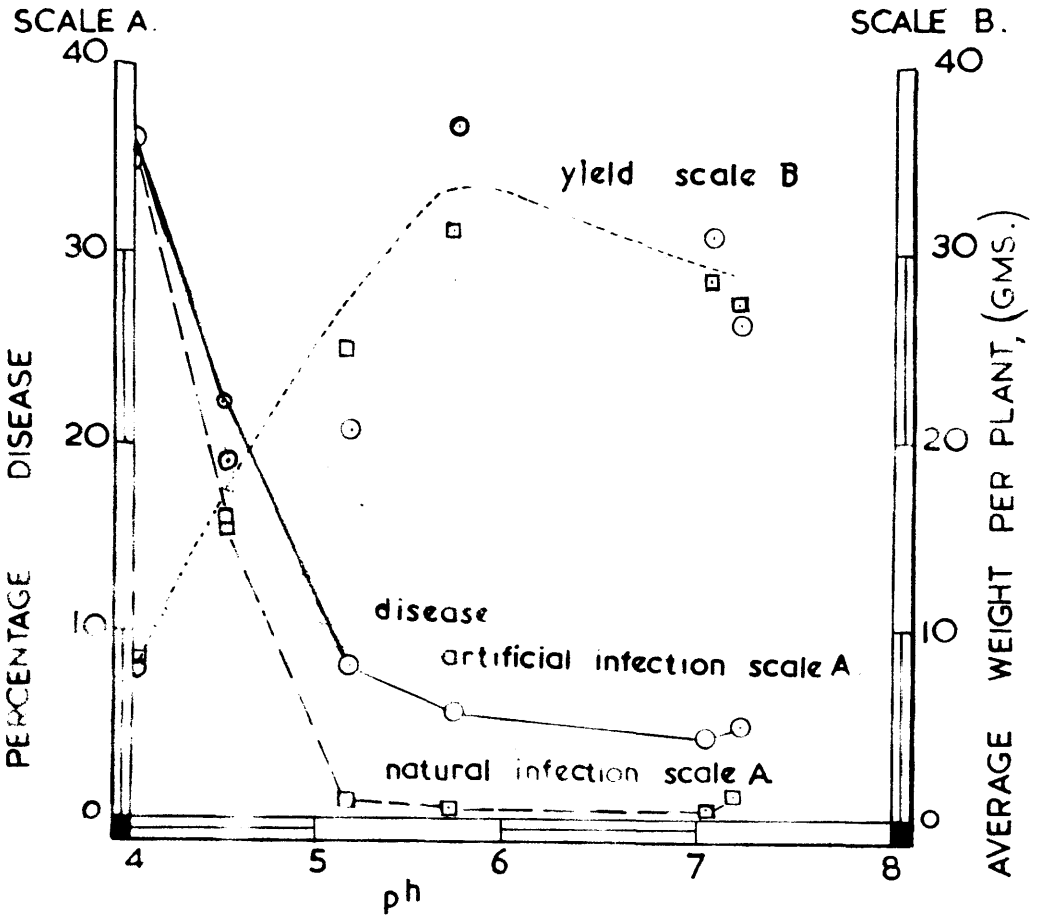


Fig. 5. Qualitative differences between the relation of yield and disease severity to pH of the soil in which beans are grown. Points on the graph within circles are results obtained from artificial infection, for both yield and the amount of disease.

Table 16. Percentage of infection of beans growing in soil of varying acidity

Plot	A	B	C	D	E
pH	3.1	3.5	3.7	4.7	5.9
1950					
Percentage infection					
July 15	-	5.0	5.0	3.5	2.0
August 28	-	32.0	30.0	20.0	8.0
1951					
pH	3.5	3.9	4.0	4.5	5.9
Percentage infection					
July 21	12.5	8.5	6.0	3.0	1.0
August 25	40.0	33.0	33.0	20.0	9.0

Table 17 shows that there was a significant correlation between the severity of Chocolate Spot on beans and the acidity (pH) for the four plots B to E in 1950, and for the five plots A to E in 1951.

Table 17. Values of the correlation coefficient r, between percentage of disease and pH of the soil

Year	Correlation coefficient	Range of P by t test	
1950	- 0.96	0.05 - 0.02	significant
1951	- 0.96	0.02 - 0.01	significant

SOIL ACIDITY & CHOCOLATE SPOT

FIELD EXPERIMENT

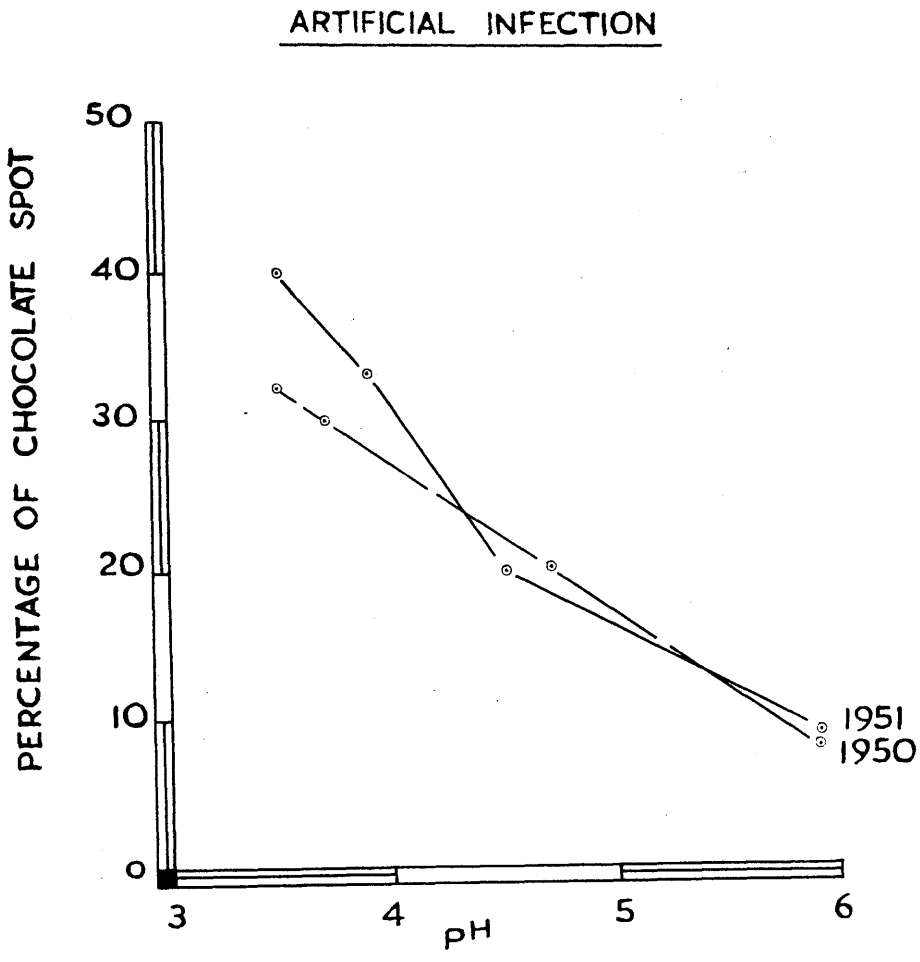


Fig. 6. The relation between pH of the soil and development of Chocolate Spot on beans grown thereon; artificial infection.

Lettuce

Artificial infection

Plants were sprayed with a spore suspension of the fungus Botrytis cinerea Pers. on June 22, 1950, and on June 25, 1951. Ten days after spraying the plants, the disease attacked young plants and infected many, particularly in the most acid soil. Most of the infected plants were subsequently covered with fructifications of the fungus, and were found by re-isolation and microscopic examination to be those of Botrytis cinerea Pers. Table 18 shows the relative incidence of disease on the plants during the two seasons.

Table 18. Relative incidence of Botrytis cinerea on lettuce growing in soils of varying acidity
Artificial infection, 1950 and 1951

plot	A	B	C	D	E
pH	3.1	3.5	3.7	4.7	5.9
1950					
Percentage infection					
July 15	50.0	8.2	3.2	1.4	1.1
July 30	62.5	24.5	19.1	15.4	9.4
August 15	75.0	52.0	38.6	33.7	26.4
Aver. fresh weight per plant, gms.	5.7	27.4	42.5	133.2	175.7
pH	3.5	3.9	4.0	4.5	5.9
1951					
Percentage infection					
July 30	36.0	10.7	7.3	5.0	2.5
August 15	62.0	46.4	37.7	32.4	20.0
Aver. fresh weight per plant, gms.	11.3	39.6	45.3	130.4	158.7

Table 19 shows that there was a significant correlation between increasing acidity (decreasing pH values) of the soil and increasing severity of Botrytis cinerea Pers. on lettuce in 1950 and 1951.

Table 19. Values of the correlation coefficient r, between percentage of disease and pH of the soil

Year	Correlation coefficient	Range of P by t test	
1950	- 0.83	0.1 - 0.05	Significant
1951	- 0.902	0.05 - 0.02	Significant

Natural infection

Natural infection gave comparable results with artificial infection. Table 20 shows the incidence of the disease. Botrytis cinerea was also more severe in very acid plots. A comparison of tables 18 (1951) and 20 shows, however, that the artificial infection was higher than with natural infection, particularly on the most acid soils (fig. 7). Thus plot F (table 20), with a pH of 3.4 and with natural infection, had 44.5% of disease in August; plot A (table 18) with artificial infection, with a pH of 3.5, had 62% at the same time. Plot J and E (both pH 5.9) had 13.4% and 20% of disease respectively. Plot J had natural infection, but plot E was artificially inoculated. Table 21 shows that there was a significant correlation between the acidity of soil and the severity of natural infection.

SOIL ACIDITY & BOTRYTIS DISEASE.

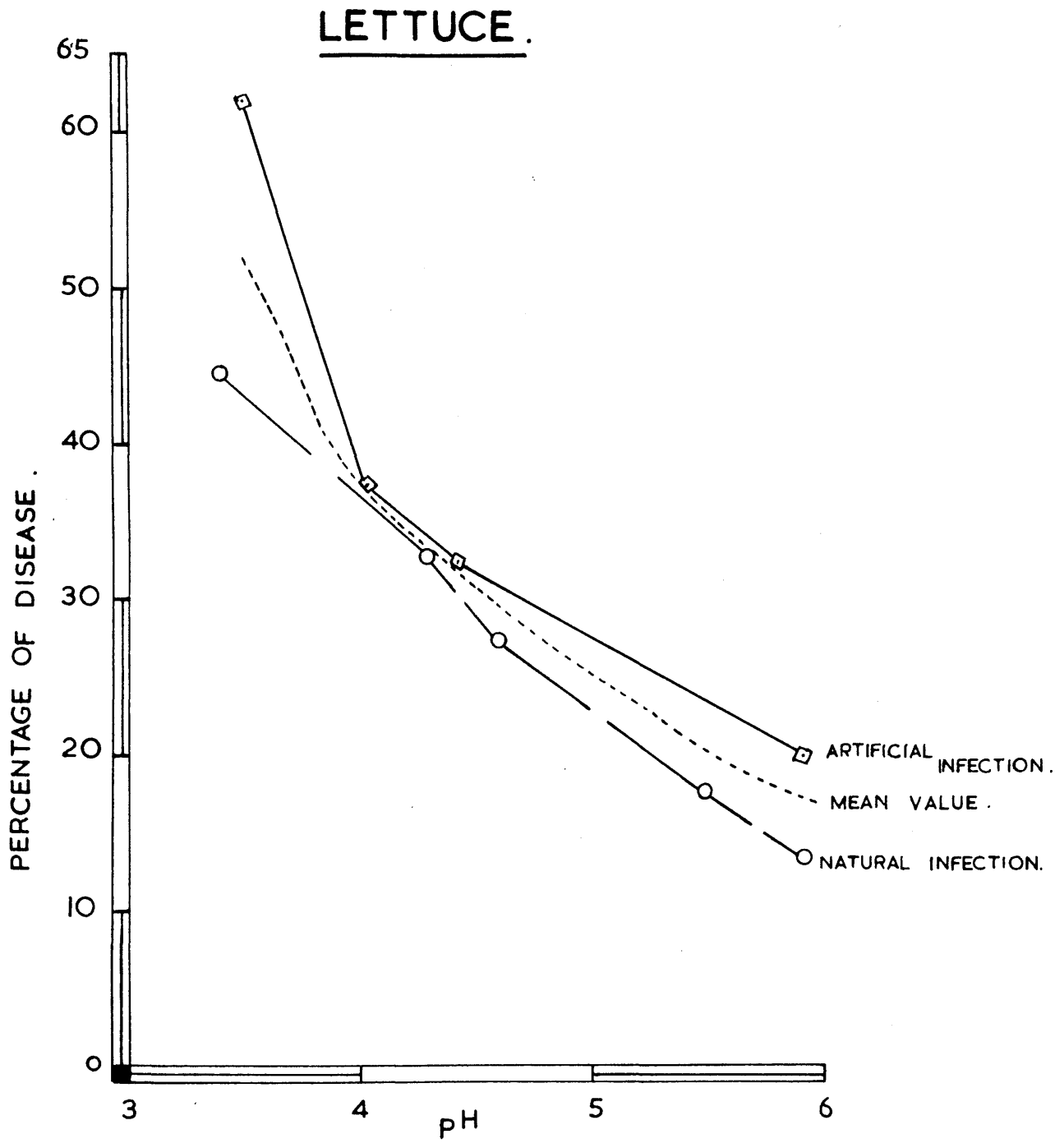


Fig. 7. The relation between pH of the soil and development of Botrytis cinerea on lettuce grown thereon; artificial and natural infection, 1951.

Table 20. Relative incidence of natural infection by Botrytis cinerea in soils of varying acidity

Plot	F	G	H	I	J
pH	3.4	4.3	4.7	5.5	5.9
Percentage infection					
July 15	12.4	8.2	6.1	2.2	2.1
July 30	20.4	11.4	10.5	4.3	3.7
August 15	44.5	32.4	28.8	17.6	13.4

Table 21. Values of the correlation coefficient r, between the percentage of disease and pH of soil

Correlation coefficient	Range of P by t test	
- 0.94	0.02-0.01	Significant

Potatoes

Artificial infection

A row from each of the varieties Kerr's Pink and Gladstone was sprayed on July 7, 1950, with sterilised water, another was sprayed with the fungus Botrytis cinerea and another was sprayed and scratched with the same fungus.

In the rows sprayed with water, very little disease appeared even in the very acid plots. In the other rows which were sprayed with a spore suspension of Botrytis cinerea scratched plants growing in the very

acid soil were severely infected with the fungus. Tubers in plot A (pH 3.1) were small and rough (fig. 9). Table 22 gives the incidence of the disease in the plots of different pH.

There were differences in yield from rows sprayed with water and those sprayed with the fungus Botrytis cinerea especially in the very acid plots. For example, the average yield of both varieties in plot A (pH 3.1) was 18.8 lb. in the rows sprayed with water but was 12.5 lb. in those sprayed with Botrytis spores. There was also a slight increase in the percentage of disease when plants were scratched before spraying with fungus spores.

Botrytis cinerea does not usually cause serious foliage destruction on potato but it seems from this study that it can be severe on very acid soils. Losses in yield of tubers following artificial infection at low pH values which may be encountered in practice (e.g. plot D, pH 4.7) were 11.7% (variety Gladstone) and 8.6% (variety Kerr's Pink). These approach the degree of damage which may be induced by Blight, Phytophthora infestans (Grainger 1950), and it is therefore fortunate that natural infection by Botrytis cinerea is naturally so low in the potato. The greatest difference between the amounts of disease resulting from natural and artificial infection in the present work is that recorded on this crop (table 22). Natural infections of

Botrytis cinerea are indeed found on field crops, but do not bring appreciable practical loss.

Table 22. Incidence of the fungus Botrytis cinerea on potatoes in acid soil (1950)

Plot	A		B		C		D	
pH	3.1		3.5		3.7		4.7	
	% Infection	Wt. of tubers in lb*	% Infection	Wt. of tubers in lb*	% Infection	Wt. of tubers in lb*	% Infec.	Wt. of tubers in lb*
VARIETY GLADSTONE								
Sprayed with water	11.1	22.0	5.5	25.0	0	28.0	0	30.0
Sprayed with Botrytis cinerea	72.2	12.5	66.6	17.0	38.8	23.0	27.7	26.5
Sprayed with Botrytis cinerea and scratched	83.3	12.0	66.6	16.0	44.4	23.0	33.3	26.0
VARIETY KERR'S PINK								
Sprayed with water	11.1	15.5	5.5	25.0	5.5	31.0	0	35.0
Sprayed with Botrytis cinerea	77.7	12.5	55.5	22.5	44.4	28.5	28.8	32.0
Sprayed with Botrytis cinerea and scratched	77.7	11.0	61.1	21.0	50.0	27.0	33.3	30.0

* Weight of the tubers from 18 plants in each case.

Onions

Artificial infection

Plants were sprayed on June 25, 1950, and on June 29, 1951, with a spore suspension of the fungus Botrytis allii Munn.

Neck Rot symptoms were found in the field; infection took place through the neck tissue and caused the death of some plants during the growing season and in storage, especially in the very acid plots.

Table 23 and fig. 8 show the incidence of the disease during the two seasons.

Table 23. Incidence of the fungus Botrytis allii on plots of varying acidity

Plot	A	B	C	D	E
pH	3.1	3.5	3.7	4.7	5.9
1950					
Percentage infection					
July 30	40.0	15.0	15.0	9.7	1.0
August 30	50.0	27.5	27.5	12.2	2.0
Sept. 30	75.0	50.0	42.2	19.5	8.0
March 15, 1951 (after storage)	100.0	77.5	72.5	48.7	37.0
pH	3.5	3.9	4.0	4.5	5.9
1951					
Percentage infection					
Sept. 30	40.0	30.0	30.0	20.0	5.0
March 15, 1952 (after storage)	80.0	60.0	55.0	45.0	25.0

There is a similarity between these results and

those recorded for beans (table 16, p. 34), lettuce (table 18, p. 35), and potatoes (table 22, p. 39). With increasing acidity of the soil in this very acid range there was an increase of the severity of Botrytis disease.

Table 24 shows that there was a significant correlation between the percentage of disease at the end of storage in March, and pH values for the five plots A to E.

Table 24. Values of the correlation coefficient r, between percentage of disease and acidity of soil

Year	Correlation coefficient	Range of P by t test	
1950	- 0.91	0.05 - 0.02	Significant
1951	- 0.95	0.02 - 0.01	Significant

SOIL ACIDITY & BOTRYTIS DISEASE.

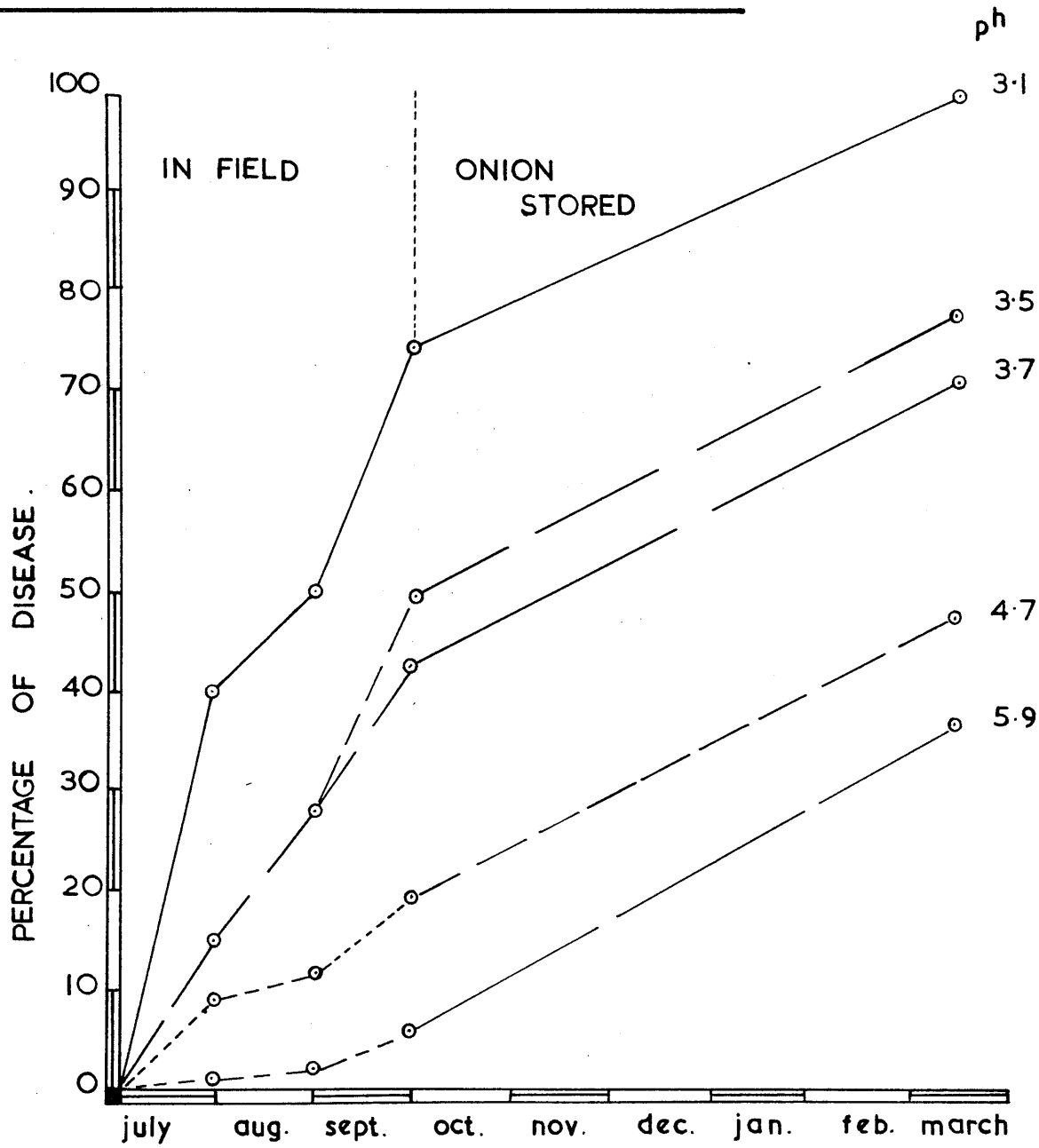


Fig. 8. The relation between pH of the soil and development of Botrytis allii on onions, grown thereon; artificial infection, 1950.

Table 25. Summary of relation between percentage of disease and amounts of P_2O_5 and K_2O in acid plots

Plot	A	B	C	D	E
pH	3.1	3.5	3.7	4.7	5.9
1950					
Percentage of Botrytis disease					
Beans - Botrytis fabae	-	32	30.0	20.0	8
Lettuce - Botrytis cinerea	75	52	38.6	33.7	26.4
Potatoes- Botrytis cinerea	77.7	62.4	44.4	30.8	-
Onions - Botrytis allii	100	77.5	72.5	48.7	37
Available P_2O_5 mg./100 g.	27.2	27.4	32.4	43.4	28
" K_2O mg./100 g.	5.0	8.2	11.0	10.6	8
pH	3.5	3.9	4.0	4.5	5.9
1951					
Percentage of Botrytis disease					
Beans - Botrytis fabae	40	33	33	20	9
Lettuce - Botrytis cinerea	62	46.4	37.7	32.4	20
Onions - Botrytis allii	80	60	55	45	25
Available P_2O_5 mg./100 g.	22	19	21	34	36
" K_2O mg./100 g.	9	12	11	15	6

DISCUSSION AND GENERAL CONCLUSIONS

SULPHUR AND SOIL ACIDITY

There is an extensive literature on the use of elemental sulphur to reduce pH of the soil, and also to investigate the indirect control of diseases which are severe on alkaline soils. Among the workers who have used sulphur for these purposes are:- Adams (1924); Stephenson and Powers (1924); Roach, Glynne, Brierley and Crowther (1925); Roach and Brierley (1926); Newton (1931); Younge (1931); Steinmann (1932); Larson, Albert and Walker (1938); Wallace (1941); Muncie, Moore, Tyson and Wheeler (1944); Owen (1944 and 1946); Bewley (1946) and Hooker and Kent (1950).

Dressings have been applied at rates of up to nearly 6 tons per acre (Stephenson and Powers 1924) and to glasshouse soils at rates up to 2 tons per acre (Owen 1946). The method is therefore well established, and in the present trials the limits given above have not even been approached; the heaviest dressings applied at Auchincruive did not exceed 2 tons per acre for field plots.

The fate of elemental sulphur in soil has been fully investigated by soil chemists (e.g. the useful summaries by Russell, 1950 and Waksman, 1931).

Under aerobic conditions sulphur is oxidised very rapidly to sulphuric acid, which reacts immediately with

carbonates and bicarbonates, producing sulphates of small biological consequence, and at the same time reducing the base level of the soil and making it more acid.

It would therefore seem that this is the important effect of adding sulphur to aerobic soils and the process is exactly similar to the acidifying action of sulphate-containing fertilisers which takes place under normal farming practice. No direct phytotoxicity of sulphur is therefore likely, though plant growth is poor on very acid soils.

Under anaerobic conditions the sulphur behaves quite differently; it is transfonned rapidly to sulphides and sulphuretted hydrogen. These compounds are extremely toxic to plants and result in immediate death. Anaerobic conditions are only encountered in ground permanently waterlogged, or under water, and this never happened on the experimental ground at Auchincruive.

It is therefore safe to assume that no sulphides were likely to be found and increasing acidity was the only important factor in these plots. There was, in fact, little difference in the amount of available phosphate or available potash in all the plots (table 25, p. 42). It is also important that the relation between pH and disease severity on field beans (Vicia faba) of naturally varying acidity was, moreover, the same as that on plots made acid with sulphur.

BOTRYTIS SEVERITY AND SOIL ACIDITY

It is not within the scope of the present invertigation to analyse the fundamental effect of pH on plant growth. This is almost certainly very complex, and it is a study of its own. The main point here was to study, if possible, the effect of disease severity on plants grown on soils of varying acidity.

The replicated trials of a considerable number of pot and field plants have shown statistically significant correlations between soil acidity and severity of damage by three species of Botrytis and four crops (tables 15 P.33, 17 P.34, 19 P.36, 21 P.37 and 24 P.41.) Disease on all crops was severe in soils below pH 5.

Glasscock, Ware and Pizer (1944) have examined the relation of Chocolate Spot severity and hydrogen ion concentration over a pH range of 4.8 to 7.5. They found no clear relationship. This corresponds generally with the results of the present work for such pH values, as there was indeed slight difference in severity of Chocolate Spot on beans grown in soils of pH 5.1 - 7.2 (tables 13, P.31 and 14, P.32). The pot experiment with Chocolate Spot on beans growing in soils varying naturally from pH 4.0 to pH 7.2 allowed more complete conclusions to be drawn. Growth of the host plants was greatest at pH 5.7 when the amount of disease was also low. Above this value the yield fell off with increasing

soil alkalinity, but the amount of disease remained fairly constant at a low level. Below pH 5.7, however, the yield fell markedly and the disease increased considerably. This complex relation is shown by fig. 5 which therefore suggested that pH 5.7 may be near the best value for growth of beans, likely to give not only the highest yield but also a minimum amount of disease. This seems to be an important practical result, but it is also fundamental to realise that pH appears to affect yield and disease in different ways. Present knowledge does not yet permit a full explanation of this finding.

In the field experiments there was a similarity in behaviour in four crops - lettuce, potatoes, beans and onions - towards infection by Botrytis with increasing soil acidity. This was in spite of the fact that there was little difference in the amounts of available P_2O_5 or K_2O (table 25, p. 42). In the potato it seems that Botrytis can be severe on very acid soils, though it usually causes little damage on field crops, and does not bring appreciable practical loss.

Low pH values of the soil had a limiting effect on growth of all crops in the present investigation. Nodule formation on beans was very poor at low pH levels and plants might suffer from nitrogen deficiency. Yield of all crops is diminished

with increasing soil acidity.

It is notoriously difficult to correlate fungal growth in culture with growth on a host plant. Botrytis fabae was reported by El Helaly (1938) to grow best at pH 4.5 but Sardina (1931) found optimum growth of mycelia and conidia at pH 5.3, 7.3 and 4.5 respectively in one strain but in another they were 7.3, 7.7 and 3.9.

It is very doubtful whether these figures have any relation to Botrytis growth on crops grown in soils of different pH levels. Plant cells are buffered to a narrow pH range. Small (1946), for example, mentioned that potato tuber juice was shifted from 6.23 to 5.93 with 20 per cent carbon dioxide and to pH 5.56 with 100 per cent carbon dioxide while distilled water shifted from pH 7.0 to 5.6 with 0.03 per cent carbon dioxide from the air, to pH 4.2 - 4.0 with 20 per cent, and to pH 3.8 - 3.6 with 100 per cent carbon dioxide. This would suggest that the fungus has the advantage of a nearly constant pH value in its host, whereas the plant may have to extract its mineral nutrients from a highly unfavourable medium. Infection might, therefore, depend on the vigour and health of the host. Soil acidity at the limiting levels found in these trials is a very significant factor controlling general vigour.

In the absence of any adequate explanation it would still appear to be a useful practical finding that

very acid soils are related to severe disease. One soil out of twenty in the west of Scotland has a pH below 5.0 (Dovaston, unpublished from a survey of 17,000 records from the Soils Laboratory, Auchincruive)

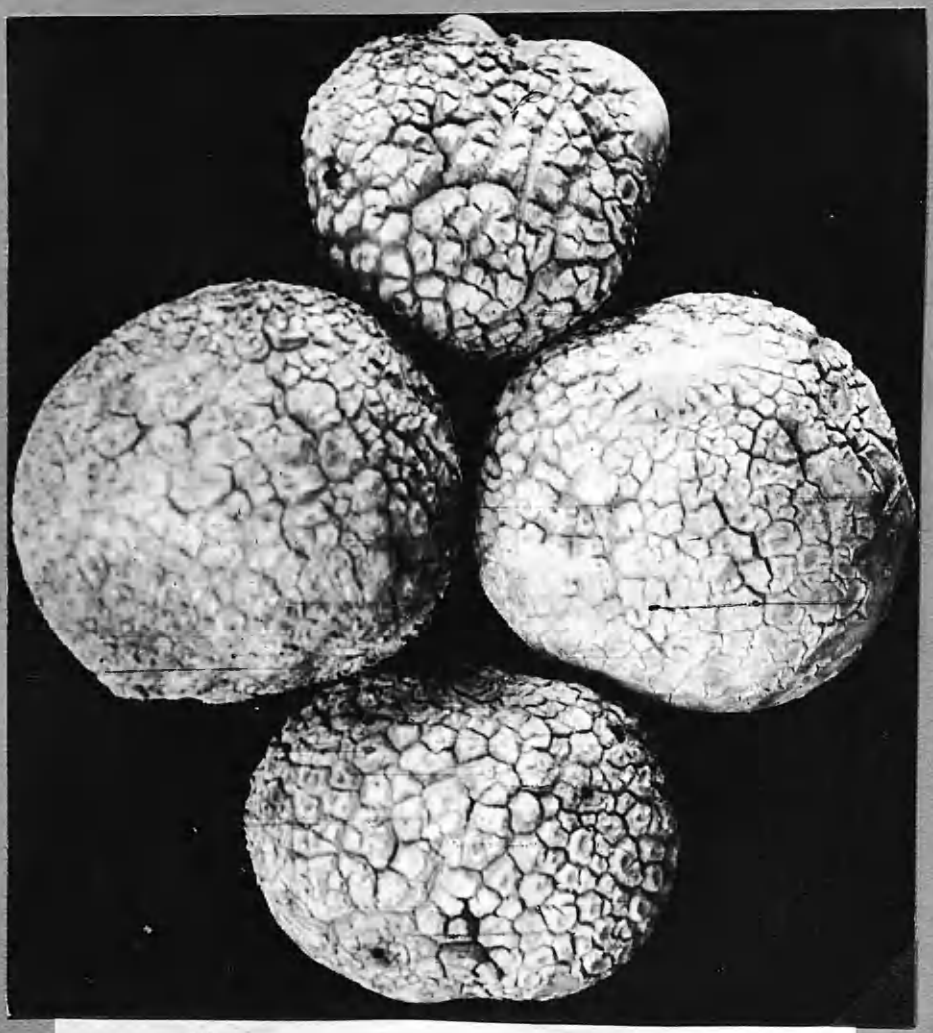


Fig. 9. Potato tubers grown in soil of pH 3.1. Tubers are small and rough.

S E C T I O N III

The effect of plant nutrition on:-

A. *Botrytis cinerea* Pers. on lettuce and tomato

B. *Botrytis fabae* Sard. on field beans (*Vicia faba*)

C. *Botrytis allii* Munn on onions

THE EFFECT OF PLANT NUTRITION ON BOTRYTIS DISEASES

PREVIOUS WORK

Several writers have mentioned that soil conditions influence the occurrence of Chocolate Spot on beans. Beaumont (1933) stated that soils where the disease occurred were deficient in potash or in the actual amount of available potash, or in the amount relative to the available phosphate. Cowie (1936), Scott Watson (1936), and Glynne (1936) produced evidence of a relation between the severity of Chocolate Spot and the amount of available potassium in the soil. Wilson (1937) concluded that any factor tending to weaken the beans, such as deficiency of potash or phosphate, rendered them more liable to aggressive infection. He also noticed that aggressive and non-aggressive infection was heavy on the plots treated with superphosphate or nitro-chalk, but aggressive infection was much reduced where muriate of potash had been applied. Glasscock, Ware and Pizer (1944), however, found that there was no significant relation between severity of attack by Chocolate Spot and available potassium. They also added that there was a highly significant relation between the severity of attack and the amount of available phosphorus in the soil. Damage by Chocolate Spot was generally slight on soils containing medium to high amounts of available phosphorus and was generally severe on soils

containing low amounts of available phosphorus.

Moore (1944) noticed that potash deficiency in the soil predisposes beans to attack, even when the deficiency was not serious enough to produce visible symptoms in the crop. He stated that the disease on beans tended to be worse on soils low in available phosphate. Ogilvie and Munro (1947) in their study on Chocolate Spot of field beans in the South west of England found that the disease appeared to be worse under conditions of unbalanced nutrition, especially where there was a deficiency of potash and phosphate. They also concluded that there was a relation between the severity of attack and the amount of available phosphorus in the soil.

Ogilvie (1945) recommended balanced fertilisers not too high in nitrogen to keep down Botrytis allii damage to onions. He stated furthermore that no manurial practice appeared to have any effect in mitigating Grey Mould on lettuce.

GENERAL METHODS

Soils in the pot and field experiments were medium loam (see p. 26).

POT EXPERIMENTS WITH FIELD BEANS (Vicia faba)

Eight-inch pots were used through the experiment. Eight treatments were given to the soil as follows:-

Treatment	Amount in ounces per cwt. of soil		
	Nitrogen	Phosphorus	Potash
1. Ammonium sulphate (N)	1	-	-
2. Potassium sulphate (K)	-	-	1
3. Superphosphate (P)	-	2	-
4. Ammonium sulphate and potassium sulphate (NK)	1	-	1
5. Ammonium sulphate and superphosphate (NP)	1	2	-
6. Potassium sulphate and superphosphate (KP)	-	2	1
7. Ammonium sulphate, superphosphate and potassium sulphate (NPK)	1	2	1
8. No treatment at all (0)	-	-	-

Samples from each soil were taken for analysis after adding the fertiliser. Table 26 gives the pH values, the amount of available P_2O_5 and available K_2O. Five pots were used for each sample of soil and four plants were grown in each pot.

Table 26. Analysis of soil samples for experimental pots

Treatment	pH	Available P_2O_5 mg. per 100 g.	Available K_2O mg. per 100 g.
Control	6.2	18	11
N	5.9	23	10
K	6.0	21	59
P	5.9	32	13
NK	5.9	19	69
NP	5.9	30	14
KP	5.7	30	38
NPK	5.6	24	42

FIELD EXPERIMENTS

Sixteen plots at Auchincruive were selected; the area of each was 30 sq. yds. = 1/161 acre (error = -10 sq. yd. per acre). Samples of soil from the plots were taken for chemical analysis and the results showed that there was no significant difference in the pH or available P_2O_5 or available K_2O before adding the fertiliser in 1950 and 1951 (table 27).

Table 27. Analysis of soil samples from experimental plots before treatment

Plot	pH values		Available P_2O_5 mg. per 100 g.		Available K_2O mg. per 100 gm.		pC*	
	1950	1951	1950	1951	1950	1951	1950	1951
1	5.7	5.7	30	36	11	11	3.75	3.96
2	5.7	5.7	29	45	14	14	4.02	4.08
3	5.7	5.7	28	36	8	6	4.08	4.02
4	5.7	5.7	30	36	14	14	4.09	4.01
5	5.7	5.7	27	33	9	9	4.18	4.08
6	5.6	5.4	26	36	8	8	4.02	4.17
7	5.7	5.7	27	27	14	16	4.07	4.13
8	5.6	5.6	26	26	8	8	4.02	4.23
9	5.5	5.4	30	39	11	11	4.13	4.03
10	5.7	5.7	33	33	15	15	4.06	4.08
11	5.8	5.8	34	36	14	22	4.07	4.23
12	6.0	6.0	30	30	8	6	4.06	4.13
13	6.1	6.1	30	30	10	19	4.07	4.08
14	6.2	6.2	32	39	10	10	4.02	4.08
15	5.9	6.3	35	42	11	11	4.18	4.01
16	5.9	6.3	34	42	14	15	4.13	4.02

* pC is the negative logarithm of the specific conductivity.

Amounts of P_2O_5 were estimated by the colorimetric ammonium molybdate and stannous chloride method of William

and Stewart (1941); of K_2O by a colorimeter method with sodium cobaltinitrite (Whittles and Little, 1950). pH was measured by the quinhydrone method. All the soil analyses were kindly done by the Soil Department at Auchincruive.

In 1950 eight treatments were given (table 28) and each was replicated twice in randomised blocks.

Table 28. Treatment and amount of fertiliser given to the plots

Treatment	Amount of fertiliser in lb. per 30 sq. yds.		
	Nitrogen	Phosphorus	Potassium
1. Ammonium sulphate (N)	$7\frac{1}{4}$ (1)	-	-
2. Potassium sulphate (K)	-	-	$7\frac{1}{4}$
3. Superphosphate (P)	-	$14\frac{1}{2}$ (2)	-
4. Ammonium sulphate and potassium sulphate (NK)	$7\frac{1}{4}$	-	$7\frac{1}{4}$
5. Ammonium sulphate and superphosphate (NP)	$7\frac{1}{4}$	$14\frac{1}{2}$	-
6. Potassium sulphate and superphosphate (KP)	-	$14\frac{1}{2}$	$7\frac{1}{4}$
7. Ammonium sulphate, superphosphate and potassium sulphate (NPK)	$7\frac{1}{4}$	$14\frac{1}{2}$	$7\frac{1}{4}$
8. No treatment at all (0)	-	-	-

(1) approximately 10 cwt. per acre (actually 10 cwt. 41 lb. per acre)

(2) approximately 20 cwt. per acre (actually 20 cwt. 82 lb. per acre)

In 1951 an amount of 7¼ lb. of potassium sulphate and 14½ lb. of superphosphate were added to each plot. Four treatments with various amounts and forms of nitrogen were given, and each was replicated four times in randomised blocks:-

Treatment	Amount in lb. per 30 sq. yds.
1. Ammonium sulphate	7¼
2. Ammonium sulphate	14½
3. Sodium nitrate	7¼
4. No nitrogen at all	7¼

N.B. The 7¼ lb. of sodium nitrate was added to the soil at four intervals, while the other fertilisers were added before planting.

The same range of crops which were grown in 1950 and 1951 for experiments on soil acidity were grown in this section, except there were no potatoes.

GLASSHOUSE EXPERIMENT WITH TOMATOES

A house (200 x 15 ft.) at Mainholm Nursery near Auchincruive was chosen for an experiment on tomatoes. Sixteen plots (10 x 12 ft.) were set up and a distance of 2½ ft. was left between each plot to accommodate a buffer row. Samples of soil (medium loam) were taken from each plot and table 29 shows that there was no significant difference in

the pH values or available P_2O_5 or available K_2O and that the soil was rich in potash and phosphate. The whole house was given a dressing of 56 lb. potassium sulphate and the experiment was designed to investigate the effects of varying applications of slow-acting nitrogenous fertiliser. Four treatments as follows were given and each was replicated four times in randomised blocks:-

1. - 5 oz. hoof and horn meal per sq. yd.
2. - $2\frac{1}{2}$ oz. hoof and horn meal per sq. yd.
3. - $1\frac{1}{4}$ oz. hoof and horn meal per sq. yd.
4. - No nitrogen at all.

Hoof and horn meal was used to conform with normal practice; series 2 at $2\frac{1}{2}$ oz. per sq. yd. represents the standard rate of treatment at 52 lb. per house (200 x 15 ft.).

TOMATO, variety Ailsa Craig, was sown January 15, 1951. The seedlings were potted on January 26 and were planted in their permanent quarters on March 5. Plants were chosen for uniformity at planting time. All plots were given the same amount of water at all times through the period of growth. Each plot contained 40 plants, set 18 in. apart in rows 23 in. apart. Each manurial treatment was therefore given to a total of 160 plants in the four blocks of each treatment.

Table 29. Analyses of soil samples from experimental plots before treatment

Plot	pH value	Available P_2O_5 mg. per 100 g.	Available K_2O mg. per 100 g.
1	5.70	130	110
2	6.26	137	98
3	6.14	150	92
4	6.20	134	102
5	6.11	134	100
6	6.16	128	102
7	6.16	128	100
8	6.06	125	88
9	6.16	122	88
10	6.09	119	94
11	6.37	125	92
12	6.33	153	81
13	6.33	128	81
14	6.20	119	88
15	6.09	128	113
16	6.09	110	110

ARTIFICIAL INFECTION AND DISEASE ASSESSMENT

The same methods as those described (in sections I and II) were used for lettuce, beans and onions. Tomato plants were not sprayed but were left for natural infection; disease assessment was by

count, where the number of plants infected with Botrytis cinerea was expressed as a percentage of the total number. The fungus was very easily recognised on the stem. It was also isolated and sprayed upon tomato plants. Sprayed plants showed the same symptoms.

RESULTS

POT EXPERIMENT

Field Beans

Artificial infection

Beans were sprayed on May 16, 1950, with a spore suspension of the fungus Botrytis fabae Sard. until all surfaces were thoroughly wetted. A week after spraying the plants, the disease appeared. Infection was only of the non-aggressive type (as described by Wilson 1937) in all the pots. Table 30 and fig. 10 show the percentages of infection from the series of varying manurial treatments. The percentage of Chocolate Spot was higher in the untreated pots than the treated ones. Table 31 gives the average infection in plots with the varying treatments. There was little difference between the relative effects of nitrogen and potash, but there was a considerable difference between manuring and no manuring. A smaller difference between phosphate and no phosphate was also found.

Table 30. Percentage of infection from varying manurial treatments. Beans - pot experiment, 1950

Treatment		NPK	NK	NP	KP	N	K	P	O
	Pot No.								
Percentage infection June 19, 1950	(1)	1.0	0.5	1.0	1.0	1.0	1.0	0.5	1.0
	(2)	0.5	0.5	1.0	1.0	1.0	1.0	0.5	1.0
	(3)	0.5	0.5	1.0	1.0	1.0	1.0	0.5	1.0
	(4)	0.5	0.5	1.0	1.0	1.0	1.0	0.5	1.0
	(5)	0.5	0.5	1.0	1.0	1.0	1.0	0.5	1.0
Average infection on June 19, 1950		0.6	0.5	1.0	1.0	1.0	1.0	0.5	1.0
Percentage infection July 27, 1950	(1)	10.0	9.0	4.0	6.0	9.0	4.0	1.0	17.0
	(2)	6.0	12.0	6.0	7.0	6.0	6.0	4.0	10.0
	(3)	8.0	7.0	5.0	3.0	7.0	4.0	5.0	8.0
	(4)	6.0	6.0	4.0	5.0	5.0	5.0	3.0	18.0
	(5)	5.0	11.0	6.0	4.0	8.0	6.0	2.0	17.0
Average infection on July 27, 1950		7	9	5	5	7	5	3	14

Table 31. Average infection in the different treatments at July 27, 1950

Treatment	Average infection %	Treatment	Average infection %
Nitrogen	7.0	No nitrogen	6.75
Potash	6.5	No potash	7.25
Phosphate	5.0	No phosphate	8.75
Fertiliser	5.85	No fertiliser	14.00

By comparing the average amount of disease in the eight treatments (table 30) with the amounts of available

phosphate and potash in table 26 (p. 52) it was possible to find a significant correlation between phosphate and Chocolate Spot (table 32). The relation between available potassium and the amount of Chocolate Spot was not significant. A t-test of the average amounts of disease on pots with fertiliser and those on unmanured controls, however, was highly significant (P was between .01 and .001). This confirms the outstanding non-specific effect of fertiliser additions in reducing the amount of Chocolate Spot.

Table 32. Values of the correlation coefficient r, between the percentage of Chocolate Spot and the available P_2O_5.

Correlation coefficient r	Range of P by t test	
- 0.77	0.05 & 0.02	Significant

FIELD EXPERIMENT

Artificial infection

Beans were sprayed on June 10, 1950, and on June 12, 1951, with a spore suspension of the fungus Botrytis fabae Sard. Typical symptoms of Chocolate Spot appeared during the fourth week of June. Infection in all plots was only of the non-aggressive type. Tables 33 and 35 show the percentage of infection in the different treated plots.

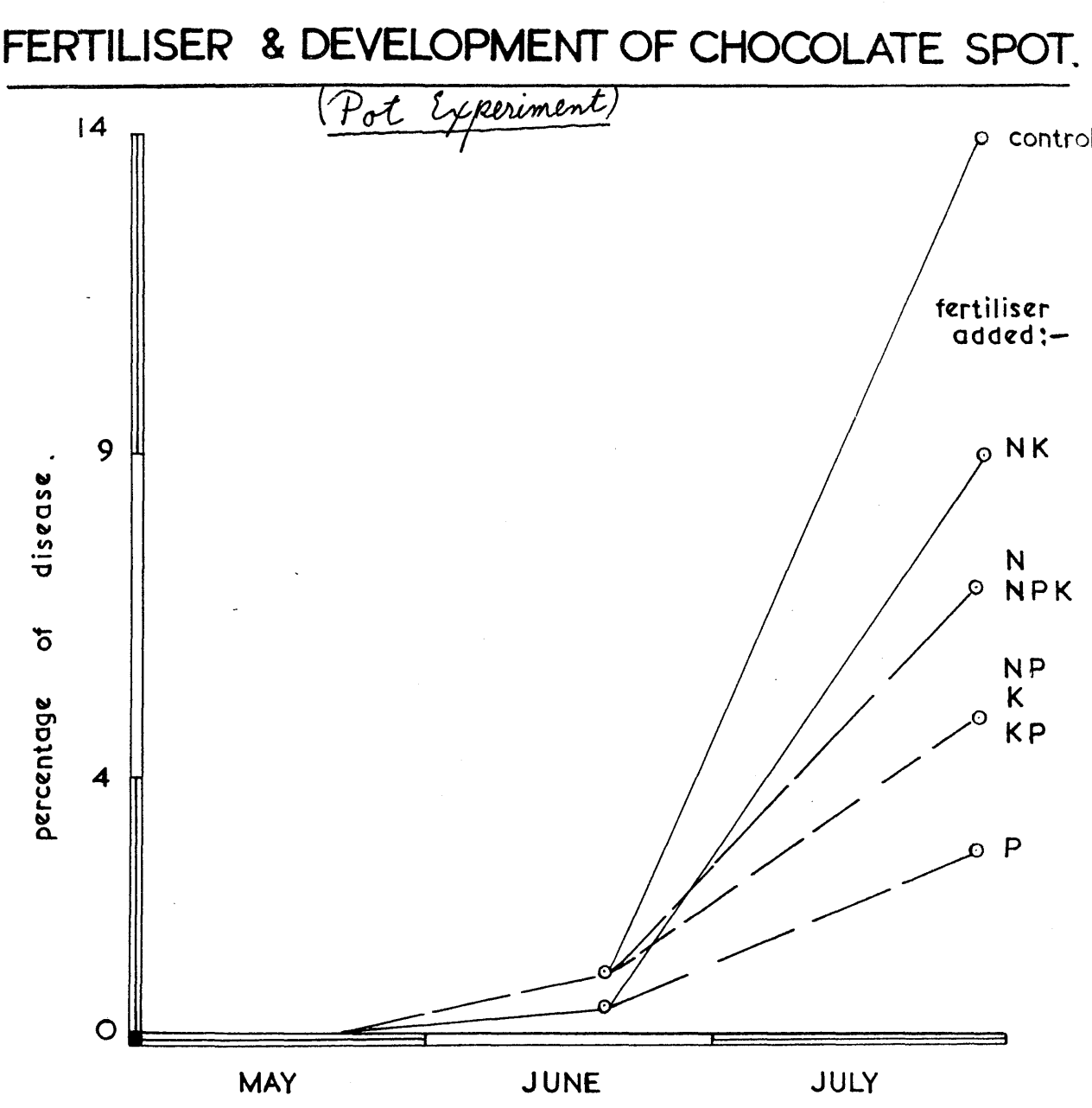


Fig. 10. The relation between additions of fertiliser and the amount of Chocolate Spot on field beans.

Table 33. Percentage of infection in the different treated plots in 1950

Treatment	NPK	NK	NP	KP	N	K	P	O
Percentage infection (average of two plots)								
July 15, 1950	2	1.5	1	1	2	1	1	2
August 27, 1950	5	4.5	4.5	4.5	5	4	4	9

Table 34 gives the average infections in plots with the varying treatments. There was again a considerable difference between manuring and no manuring, and but little difference with nitrogen, phosphate and potash.

Table 34. Average infection in the different treatments Beans - 1950

Treatment	Average infection %	Treatment	Average infection %
Nitrogen	4.75	No nitrogen	5.4
Potash	4.5	No potash	5.6
Phosphate	4.5	No phosphate	5.6
Fertiliser	4.5	No fertiliser	9

Table 35. Percentage of infection in the different treated plots in 1951

Treatment	Ammonium sulphate (1)	Ammonium sulphate (2)	Sodium nitrate (1)	Control
% of infection (average of four plots)	6	8	12	10

(1) amount added $7\frac{1}{4}$ lb. per plot
(2) amount added $14\frac{1}{2}$ " " "

These results show that there was more infection in the untreated plots than on those treated with single and double amounts of ammonium sulphate. There was, however, an increase of disease when sodium nitrate was added. The form of nitrogenous fertiliser would therefore seem to be important. Ammonium sulphate seemed to give the same effect in reduction of disease severity as did the application of any fertiliser in the 1950 experiments. Sodium nitrate on the other hand increased the amount of disease not only above the control plots but also above those with single and double amounts of ammonium sulphate (table 35).

Lettuce

Plants were sprayed with a spore suspension on June 22, 1950, and on June 27, 1951. Disease appeared in the third week of July. Tables 36 and 38 give the percentage of infection on the various plots.

Table 36. Amount of Botrytis disease on lettuce - 1950

Treatment	NPK	NK	NP	KP	N	K	P	0
Percentage infection (average of two plots)								
July 15	0	0	0	0	0	0	0	0
July 30	9.8	2.0	3.2	6.2	2.7	2.5	2.7	15.6
August 15	23.1	10.6	14.8	20.8	13.1	7.5	9.0	32.1

The fungus Botrytis cinerea Pers. was again more

severe in the plots with no manurial treatment than in the treated ones. Table 37 gives the average infections in plots with the varying treatments. There was again little difference between the relative effects of nitrogen, phosphate and potash, but there was a considerable difference between manuring and no manuring.

Table 37. Average infection in the different treatments lettuce - 1950

Treatment	Average infection %	Treatment	Average infection %
Nitrogen	15.4	No nitrogen	17.4
Potash	15.5	No potash	17.25
Phosphate	16.9	No phosphate	15.8
Fertiliser	14.1	No fertiliser	32.1

Table 38. Amount of Botrytis disease on lettuce - 1951

Treatment	Ammonium sulphate (1)	Ammonium sulphate (2)	Sodium nitrate (1)	Control
Percentage infection (average of four plots)	14.5	19.7	25.5	22.5
Yield of 10 lettuces in lb.	4.6	4.3	4.4	3.3
Yield per plant in lb.	0.46	0.43	0.44	0.33

(1) amount added $7\frac{1}{4}$ lb. per plot of 30 sq. yds.
(2) amount added $14\frac{1}{2}$ lb. per plot of 30 sq. yds.

The results with nitrogenous fertilizers (table 38) are also similar to those on bean (table 35, p.61); plots with single and double amounts of ammonium sulphate have again less infection than that in the untreated plots. There was, however, an increase of disease when sodium nitrate was added.

Onions

Plants were sprayed on June 15, 1950, and on June 22, 1951, with a spore suspension of the fungus Botrytis allii Munn.

Few bulbs were found in the field attacked by Neck Rot, but it developed during subsequent storage, as shown by tables 39 and 41.

Table 39. Percentage of infection with varying manurial treatments. Onions, 1950

Treatment	NPK	NK	NP	KP	N	K	P	O
Percentage infection (average two plots)								
September, 1950	5.5	4.0	5.5	8.0	3.0	5.0	6.0	8.0
March, 1951 (after storage)	24.0	18.2	17.0	26.2	9.5	16.0	24.0	37.0
Average weight per bulb in gms.	68.0	65.0	67.0	70.0	68.0	69.0	66.0	50.0

Table 40. Average infection in the different treatments in 1950

Treatment	Average infection % (March, 1951)	Treatment	Average infection % (March, 1951)
Nitrogen	17.2	No nitrogen	25.8
Potash	21.1	No potash	21.9
Phosphate	22.8	No phosphate	20.2
Fertiliser	19.2	No fertiliser	37.0

Table 41. Percentage of infection with varying manurial treatments. Onions, 1951

Treatment	Ammonium sulphate (1)	Ammonium sulphate (2)	Sodium nitrate (1)	Control
Percentage infection (average four plots)				
September, 1950	1.0	3.0	3.0	2.0
March, 1952	12.5	20.0	22.0	12.8
Average weight per bulb in gms.	70.9	68.0	68.0	39.7

(1) amount added $7\frac{1}{4}$ lb. per plot
(2) amount added $14\frac{1}{2}$ lb. per plot

These results were also similar to those recorded for beans (tables 33 p.61 and 35) and lettuce (tables 36 p.62 and 38 p.63). The non-specific reduction in the amount of disease by the addition of almost any fertilizer is again apparent, but sodium nitrate and double dressings of $14\frac{1}{2}$ lb. ammonium sulphate both increased the percentage of disease (table 41).

GLASSHOUSE EXPERIMENT

Tomato

Natural infection

The first appearance of Botrytis Grey Mould was recorded on May 28. Table 42 and fig. 11 show the development of the disease during the season in the different treatments. There was a small infection in June in all the plots. The infection became spectacular from the third week of July, and the highest incidence of Botrytis was again recorded from the control plots at the end of the season.

There is here no evidence that increasing amounts of added organic nitrogen (with the rate used in the present investigation) played a significant part in making Botrytis disease more severe. They rather tend to reduce it, as did applications of any fertiliser in other experiments here described. It is important, however, to determine whether the added nitrogen was sufficiently available as to be active in improving the crop. Table 43 shows that additions of hoof and horn meal did indeed increase the yield, but there was little difference between the three different rates of application.

Table 42. Incidence of Grey Mould on Tomato
Percentage infection

Date	Control No nitrogen	1¼ oz. hoof & horn per sq. yd.	2½ oz. hoof & horn per sq. yd.	5 oz. hoof & horn per sq. yd.
May 30	0.6	0	0	0.6
June 15	4.3	0.6	0.6	1.2
June 30	10.0	4.3	5.6	9.3
July 15	11.2	5.0	6.2	10.6
July 30	12.5	6.2	7.5	12.5
August 15	24.4	15.6	10.6	18.1
August 30	34.4	22.5	18.7	25.6
Sept. 15	43.1	31.2	28.7	32.5

Table 43. Crop yield in lb. per plant during the season in the different treatments

Treatment before planting	Crop yield in lb. per plant					Total yield in lb. per plant.	Total yield in lb. for four plots
	May	June	July	Aug.	Sept.		
Control (no treatment)	0.5	2.32	3.50	1.60	1.04	8.96	1433.6
1¼ oz. hoof & horn per sq. yd.	0.6	2.50	3.65	1.70	1.15	9.60	1536.0
2½ oz. hoof & horn per sq. yd.	0.6	2.50	3.80	1.84	1.25	9.99	1598.4
5 oz. hoof & horn per sq. yd.	0.7	2.50	3.60	1.70	0.99	9.49	1518.4

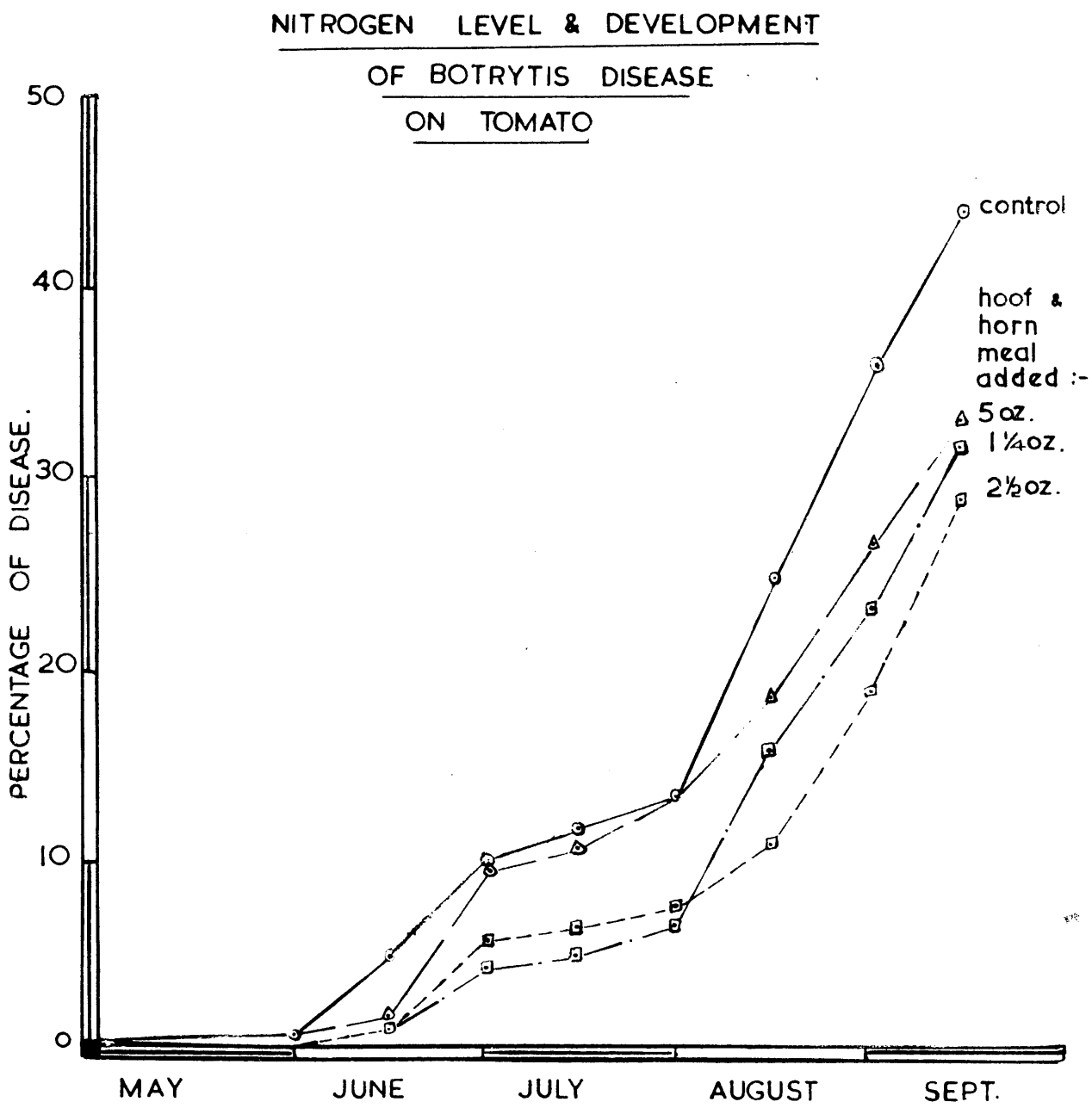


Fig. 11. The relation between additions of organic nitrogen fertiliser and the amount of Botrytis disease on tomato.

Table 44. Significance of comparison between yield in the control plots and yield with different treatments

Comparison A	with B	Range of P by t test	
Yield of control	Yield with $1\frac{1}{4}$ oz. hoof & horn per sq. yd.	0.5 & 0.4	Not significant
Yield of control	Yield with $2\frac{1}{2}$ oz. hoof & horn per sq. yd.	0.5 & 0.4	Not significant
Yield of control	Yield with 5 oz. hoof & horn per sq. yd.	0.5	Not significant

Table 45. Correlation between the percentage of the disease and the yield in the different treatments

Comparison A	with B	Correlation coefficient	Range of P by t test	
Yield of control	% of disease	- 0.96	0.05 & 0.02	Significant
Yield with $1\frac{1}{4}$ oz. hoof and horn per sq. yd.	% of disease	- 0.98	0.05 & 0.02	Significant
Yield with $2\frac{1}{2}$ oz. hoof and horn per sq. yd.	% of disease	- 0.99	0.02 & 0.01	Significant
Yield with 5 oz. hoof and horn per sq. yd.	% of disease	- 0.76	0.3 & 0.2	Not significant

The relation between yield in control plots and yield in the different treated plots were not mathematically significant (table 44) but there was a significant correlation between percentage of disease and yield in the

treated plots, except for those with 5 oz. hoof and horn (table 45).

Table 46. Summary of relations between the severity of Botrytis and manurial treatments (1950)

Manurial treatment	NPK	NK	NP	KP	N	K	P	O
Percentage infection:-								
Beans (pot experiment)	7.0	9.0	5.0	5.0	7.0	5.0	3.0	14.0
Beans (field experiment)	5.0	4.5	4.5	4.5	5.0	4.0	4.0	9.0
Lettuce	23.1	10.6	14.6	20.8	13.1	7.5	9.0	32.1
Onions	24.0	18.2	17.0	26.2	9.5	16.0	24.0	37.0

Table 47. Summary of relations between the severity of Botrytis and nitrogenous manurial treatments (1951)

Manurial treatment	Ammonium sulphate (1)	Ammonium sulphate (2)	Sodium nitrate (1)	Control
Percentage infection:-				
Beans	6.0	8.0	12.0	10.0
Lettuce	14.5	19.7	25.5	22.5
Onions	12.5	20.0	22.0	12.8

(1) amount added $7\frac{1}{4}$ lb. per plot
(2) amount added $14\frac{1}{2}$ lb. per plot

Table 48. Organic nitrogen level and severity of Botrytis disease on tomato (1951)

Manurial treatment	1¼ oz. hoof & horn per sq. yd.	2½ oz. hoof & horn per sq. yd.	5 oz. hoof & horn per sq. yd.	Control
Percentage infection	31.2	28.7	32.5	43.1

DISCUSSION AND GENERAL CONCLUSIONS

The most consistent result of this section is that there was less disease in the plots receiving fertiliser than in unmanured controls. This non-specific action of added fertilisers is new as an exact statement, though it is in line with earlier conclusions that disease is generally smaller in amount when conditions are best for the growth of the crop. These consistent results on four crops would seem to suggest that the fact of adding fertiliser was of more importance in determining the amount of disease than the relative amounts of nitrogen, phosphate and potash, with the exception of sodium nitrate, and of high amounts of ammonium sulphate on onion. This crop, indeed, seems to be rather more susceptible to the effects of ammonium sulphate than are the others.

The only treatment which consistently raised the amount of disease in the manured plots above that in the controls was with sodium nitrate, but even here the overall effect was relatively slight. Ammonium sulphate tended to decrease the amount of disease on lettuce and beans, but with onions a double application did indeed make the disease more severe in 1951. Organic nitrogenous

manures such as the hoof and horn meal used commonly on tomato also decreased the amount of disease and improved the crops. These results suggest that the form of nitrogenous fertiliser is important.

With the exception of sodium nitrate and of the onion crop, where a double application of ammonium sulphate increased the amount of Botrytis disease in 1951, all the seven experiments on fertiliser treatment brought large differences in the amount of disease. These differences were found to be highly significant in the pot experiment on beans (p. 60). Field experiments were not always amenable to statistical analysis, but if the percentage differences of disease between manured and unmanured treatments in all seven experiments are compared, they also are significantly different (P is less than .001 by t-test). Of treatment with fertiliser elements, only the amount of available phosphate (P_2O_5) gave a significant correlation with the amount of disease in a pot experiment on beans (table 32, p. 60). The amount of available potash (K_2O) was not significantly correlated with the amount of disease.

In the previous work several writers mentioned that Chocolate Spot was more severe in soils with a deficiency of potassium or phosphorus. The present results agree with regard to phosphorus, but further

suggest that a deficiency of any or all of the major elements may have a similar but greater effect. Glasscock, Ware and Pizer (1944), moreover, found no significant correlation between the amount of available potash and the severity of Chocolate Spot on beans.

Other workers have suggested that variations in inorganic manuring of the host have an effect upon the amounts of disease. Grainger (1947), for example, reported that nitrogen and potash tended to make the mildew fungus Erysiphe graminis more severe on oats while the correction of phosphate deficiency reduced the amount of disease. There was, moreover, a definite effect of increased level of manuring in raising the amount of disease. In the long-established and classical experiments at Rothamsted, the higher nitrogen plots were the most susceptible to rusts, and the same was true in the plots on the lighter soil of Woburn, which have been established for many years (Butler and Jones, 1949).

It is becoming increasingly clear that variation of inorganic manuring of the host affects particular host-parasite relations in different ways. Each such relation must be studied, but a more extensive investigation of this matter is likely to give important practical results.

Fig. 12 Tomato Grey Mould caused by the fungus Botrytis cinerea.

Fig.13 A severe Grey Mould of tomato at ground level caused by the fungus Botrytis cinerea.

Fig. 14 Chocolate Spot of broad beans.

SECTION IV

Studies on methods of direct control

1. The effect of ultra violet light on *Botrytis* species in culture and on host plants.
2. The control of tomato Grey Mould by painting wounds with bitumen paint.
3. The economics of Grey Mould control on tomato.
4. The effect of dusting lettuce plants with pentachloronitrobenzene.

STUDIES ON METHODS OF DIRECT CONTROL

Laboratory, greenhouse and field experiments were established to investigate the effect of ultra violet light on Botrytis species in culture and on host plants, of controlling Botrytis Grey Mould of the tomato by painting wounds with bitumen paint, and of dusting lettuce plants with pentachloronitrobenzene.

PREVIOUS WORK

Ultra violet irradiation

Ultra violet light has been used for the control of undesirable fungi in various commercial and industrial processes, e.g. in cheese manufacture and meat storage (Ewell, 1942), and in the baking industry (Read, 1934). On the host plant Hey and Carter (1931) found young hyphae of Erysiphe graminis were more susceptible to ultra violet light than older mycelia. English and Gerhardt (1945), using a wave length of 2537 Angstrom, found that spores of the more common fungi causing decay in sweet cherries could be destroyed if the requirements for proper wave lengths, intensity and period of exposure were fulfilled.

Painting tomato wounds

Moore (1948) stated that complete control of Grey Mould was obtained on one nursery in the east Midlands by rubbing the cut surfaces of the leaf stalks immediately after pruning with copper sulphate or liver of sulphur.

Dusting lettuce foliage with pentachloronitrobenzene (Folosan)

Brown (1935) reported that "Folosan" dust reduced Botrytis attack but checked plant growth. Smieton and Brown (1940) suggested that Folosan provided a useful control; it could be worked into the soil to a depth of 2 inches at the rate of 1 oz. per square yard, or it could be sprinkled on the surface after sowing. If the mould appeared when the seedlings were very young they should be dusted very lightly, but older ones can stand dusting at intervals of 3 to 4 weeks preferably when they are slightly moist, a final dusting being given before the plants were lifted.

GENERAL METHODS

ULTRA VIOLET LIGHT

Action on culture

An Ergon quartz mercury vapour lamp with an output of ultra violet rays between 2150 and 3000 Angstrom units, the maximum lying around 2500, was used as a source of irradiation. Malt agar was used as a medium for culturing the fungi in Petri dishes. The top covers of the dishes were removed so that the glass did not screen the ultra violet light during treatment. All treated plates were placed at a distance of 8 inches from the source of the lamp and were irradiated in an inverted position to prevent contamination.

Four dishes were irradiated for the periods and intervals shown in tables 49, 55, 57 and fig. 15. The effects of irradiation on the time of sporulation were also studied.

Action on lettuce

Lettuce, variety New Market, was sown on October 19, 1952, and was transplanted on January 20, 1953, in 8-inch pots. Two pots each containing four healthy plants were exposed and placed at a distance of 24 inches from the same lamp for the periods and intervals shown in table 60. Plants infected with the fungus Botrytis cinerea Pers. were also given similar treatment. Two pots containing healthy plants and others containing some infected plants were kept as controls.

PAINTING TOMATO WOUNDS

Two houses (2oo ft. x 15 ft.) were chosen for the experiment. Soil and plants were as those mentioned before in the plant nutrition section (p. 55).

Where Grey Mould was noticed all diseased tissues were cut away with a sharp knife and the cut surface was painted with bitumen paint. This was done through the growing season in one of the houses and the other was left without treatment.

THE EFFECT OF DUSTING LETTUCE PLANTS WITH PENTACHLORONITRO-BENZENE (FOLOSAN)

Four plots (soil, medium loam) each about 6 sq. yd. were used for the experiment. Lettuce, variety New Market, was sown on March 15, 1950; plants were thinned to a distance of 12 inches, with the rows 14 inches apart. Table 63 (p. 88) gives the number of applications of dust.

RESULTS

ULTRA VIOLET LIGHT

Action on Culture

Treatment one day after inoculation

One day after treatment the ultra violet irradiation checked all the treated cultures. Two days after treatment the treated cultures grew rapidly but still the controls were larger than the treated. Four days after treatment there were but slight differences between the controls and the treated plates. Tables 49 to 54 and fig. 15 show the area of culture in sq. cm. in the control and treated cultures.

Table 49. Area of culture in sq. cm. in the control and treated cultures

Botrytis allii Munn

	Control	15 min.	30 min.	60 min.
Before treatment	0.12	0.12	0.12	0.12
Days after treatment:-				
1	1.22	0.12	0.12	0.49
2	3.14	2.44	1.52	1.22
3	7.07	4.52	3.79	3.14
4	13.19	12.66	11.26	10.17
5	22.89	21.22	19.62	18.66

Table 50. Area of culture in sq. cm. in the control and treated cultures

Botrytis cinerea Pers.

	Control	15 min.	30 min.	60 min.
Before treatment	0.12	0.12	0.12	0.12
Days after treatment:-				
1	4.52	0.28	0.28	0.28
2	16.61	9.27	7.07	6.15
3	26.40	21.23	16.61	15.20
4	45.34	42.99	40.69	38.46
5	59.06	52.78	50.24	45.38

Table 51. Area of culture in sq. cm. in the control and treated cultures

Botrytis fabae Sard.

	Control	15 min.	30 min.	60 min.
Before treatment	0.12	0.12	0.12	0.12
Days after treatment:-				
1	3.14	0.12	0.12	0.12
2	10.03	4.52	3.80	3.14
3	19.62	10.05	9.07	8.03
4	36.29	29.17	26.40	24.62
5	49.24	36.27	32.25	30.17

Table 52. Area of culture in sq. cm. in the control and treated cultures

Botrytis paeoniae Oudem. (see also fig. 16) p. 84 A

	Control	15 min.	30 min.	60 min.
Before treatment	0.12	0.12	0.12	0.12
Days after treatment:-				
1	11.33	7.07	4.50	3.80
2	26.40	21.22	19.62	16.60
3	38.48	34.19	32.28	28.26
4	49.24	47.76	45.34	40.69
5	58.06	50.24	50.24	47.76

Table 53. Area of culture in sq. cm. in the control and treated cultures p. 84B

Botrytis squamosa Walker (see also fig. 17)

	Control	15 min.	30 min.	60 min.
Before treatment	0.12	0.12	0.12	0.12
Days after treatment:-				
1	3.80	0.12	0.12	0.12
2	13.19	9.07	8.04	6.15
3	21.22	18.08	16.61	11.33
4	42.78	38.48	36.29	32.25
5	50.24	47.76	45.34	38.46

Table 54. Area of culture in sq. cm. in the control and treated cultures p. 84C

Botrytis tulipae (Lib.) Lind (see also fig. 18)

	Control	15 min.	30 min.	60 min.
Before treatment	0.03	0.03	0.03	0.03
Days after treatment:-				
1	5.20	0.28	0.28	0.28
2	13.19	12.66	11.26	9.07
3	21.12	19.62	18.08	16.61
4	32.25	28.26	26.40	24.62
5	42.99	38.46	36.29	34.19

Treatment immediately after inoculation

Tables 55 to 58 show that the ultra violet irradiation did not kill the cultures even with the longest exposure (2 hours three days in succession).

Table 55. Area of culture in sq. cm. in the control and in Petri dishes treated immediately after inoculation

Botrytis allii Munn

Days after treatment	Control	Single treatments 30 min.	60 min.	120 min.
1	0.12	0	0	0
2	1.22	0.03	0.03	0
3	3.14	1.22	0.79	0.28
4	7.07	4.52	3.14	1.12
5	13.19	9.07	7.07	6.15
6	22.89	18.08	16.61	13.19

Table 56. Area of culture in sq. cm. in controls and in Petri dishes treated immediately after inoculation

Botrytis cinerea Pers.

Days after treatment	Control	Single treatments 30 min.	60 min.	120 min.
1	0.12	0	0	0
2	4.52	0.12	0.12	0.12
3	16.61	4.52	0.79	0.28
4	26.40	19.62	4.52	0.79
5	45.34	26.40	16.61	7.07
6	59.06	45.38	36.29	26.40

Table 57. Area of culture in sq. cm. in controls and in Petri dishes treated three days in succession after inoculation

Botrytis allii Munn

Days after treatment	Control	Length of daily treatment 30 min.	60 min.	120 min.
1	0.12	0	0	0
2	1.22	0	0	0
3	3.14	0.12	0.12	0.12
4	7.07	0.78	0.28	0.12
5	13.19	4.52	3.14	2.01
6	22.89	10.03	8.04	4.52

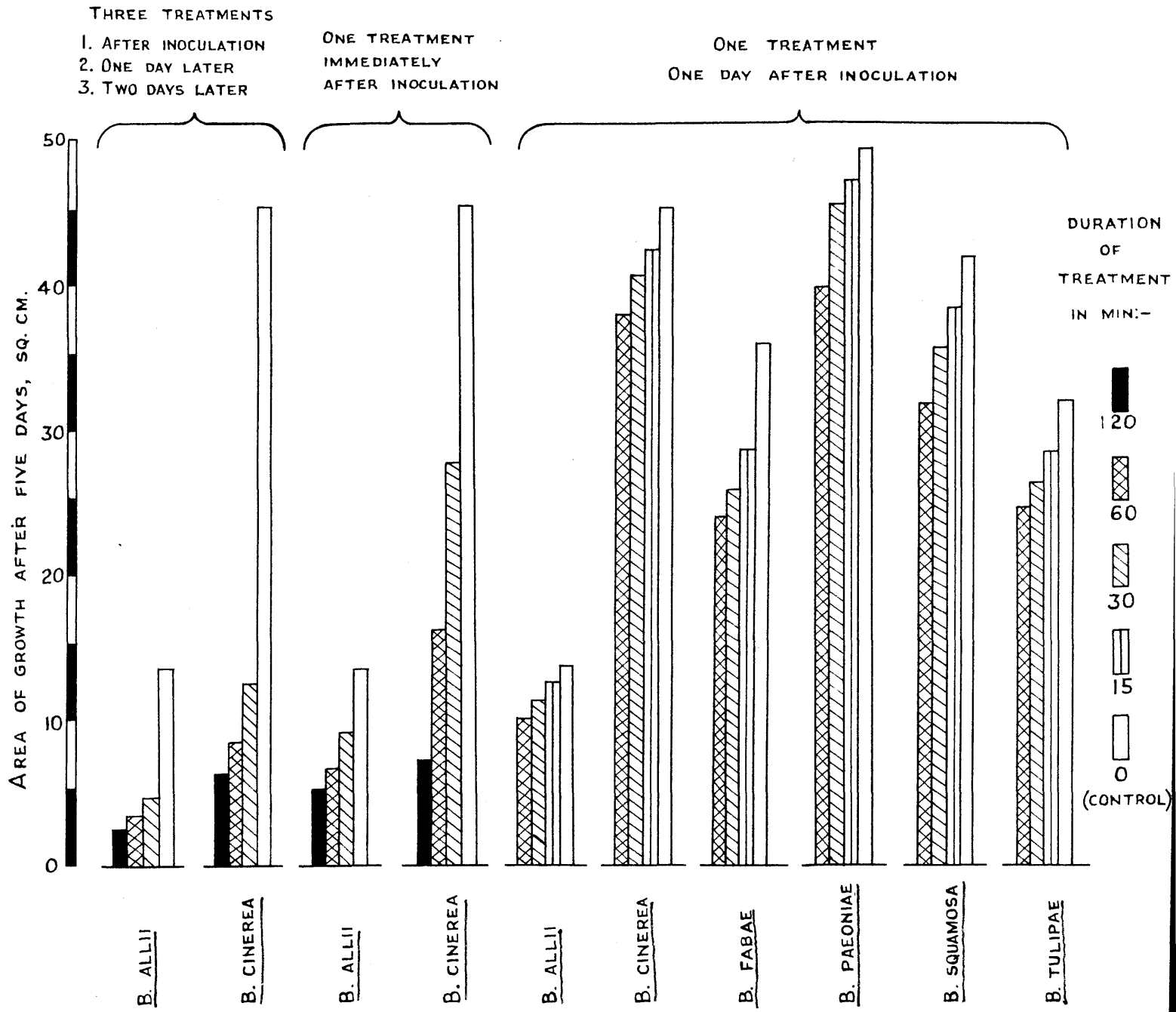
Table 58. Area of culture in sq. cm. in controls and in plates treated three days in succession after inoculation

Botrytis cinerea Pers.

Days after treatment	Control	Length of daily treatment 30 min.	60 min.	120 min.
1	0.12	0	0	0
2	4.52	0	0	0
3	16.61	1.2	0	0
4	26.40	4.52	2.01	0.78
5	45.34	12.66	8.04	6.15
6	59.06	29.17	16.61	10.17

Tables 55, 56, 57 and 58 and fig. 15 show that ultra violet irradiation immediately after inoculation checked the growth of the fungus more severely than if

Fig. 15. The effect of ultra violet irradiation on the growth of Botrytis cultures.



treatment was given one day after inoculation. Treatment for three days in succession checked the growth of mycelium more markedly than did a single treatment. Growth was, moreover, in inverse proportion to the time of exposure.

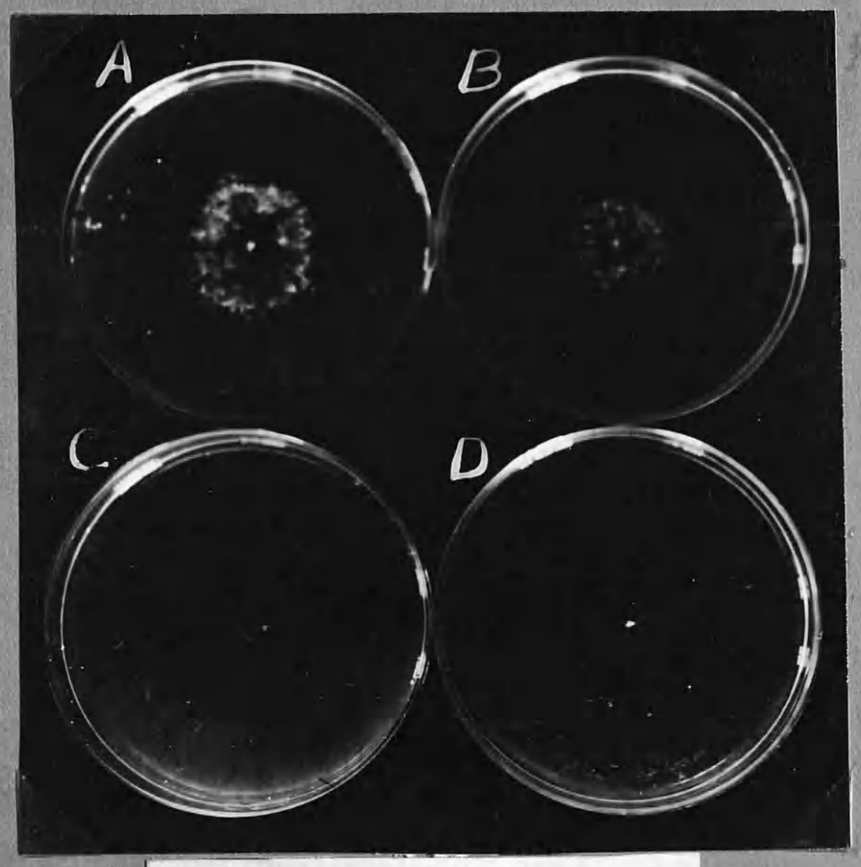
Action on sporulation

Table 59 shows the time of sporulation in the control and treated cultures. The ultra violet light had no marked effect on the time of sporulation except in cultures treated three days in succession after inoculation. It then caused a slight delay of one day in sporulation in these cultures.

Table 59. Age of culture in days before sporulation occurred in controls and in irradiated cultures

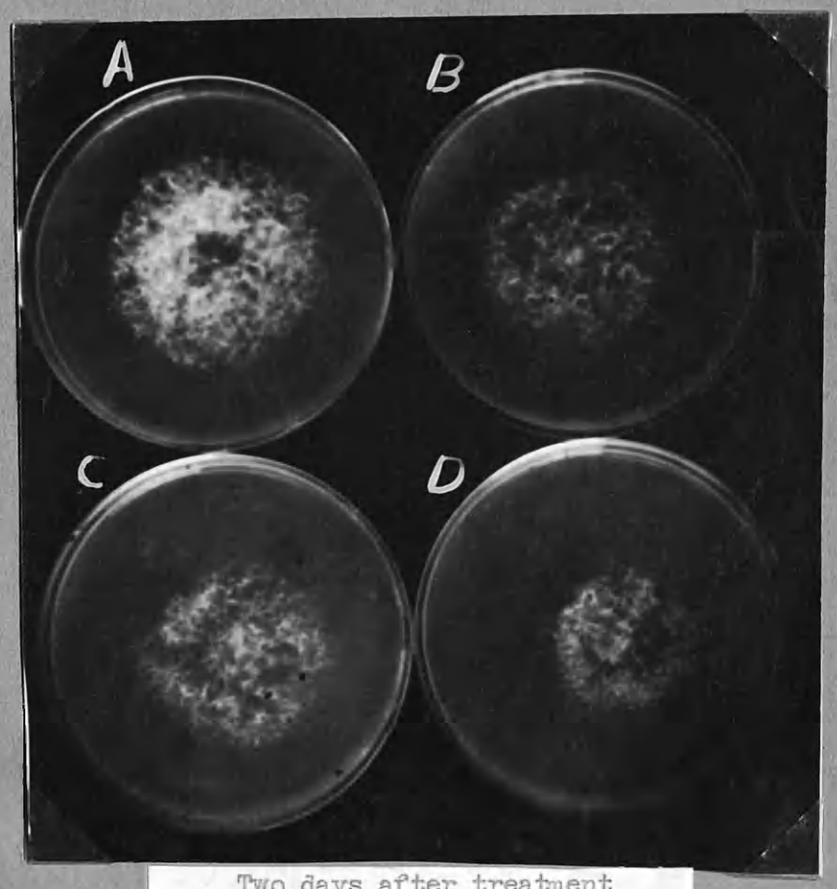
	Period of irradiation									
	No treatments	15 min. one day after inoculation	30 min. one day after inoculation	60 min. one day after inoculation	30 min. after inoculation	60 min. after inoculation	120 min. after inoculation	30 min. three days in succession after inoculation	60 min. three days in succession after inoculation	120 min. three days in succession after inoculation
Botrytis allii	4	4	4	4	4	4	4	5	5	5
Botrytis cinerea	5	5	5	5	5	5	5	6	6	6

Fig. 16 The effect of ultra violet irradiation on cultures of Botrytis paeoniae treated one day after inoculation.



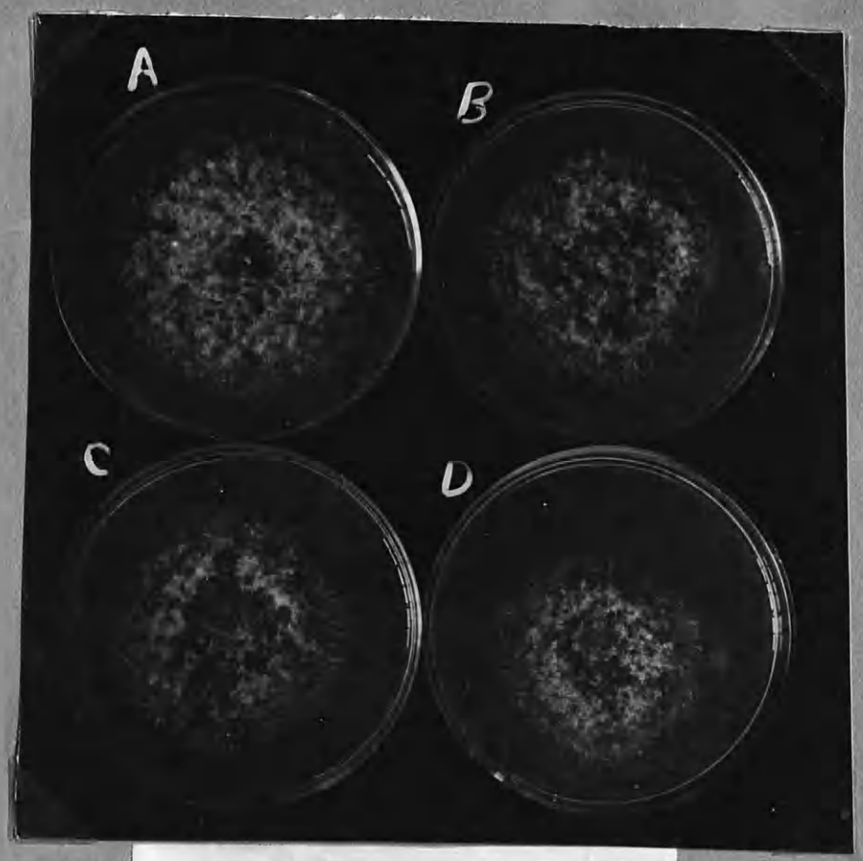
One day after treatment

A. Control B. 15 minutes
C. 30 minutes D. 60 minutes



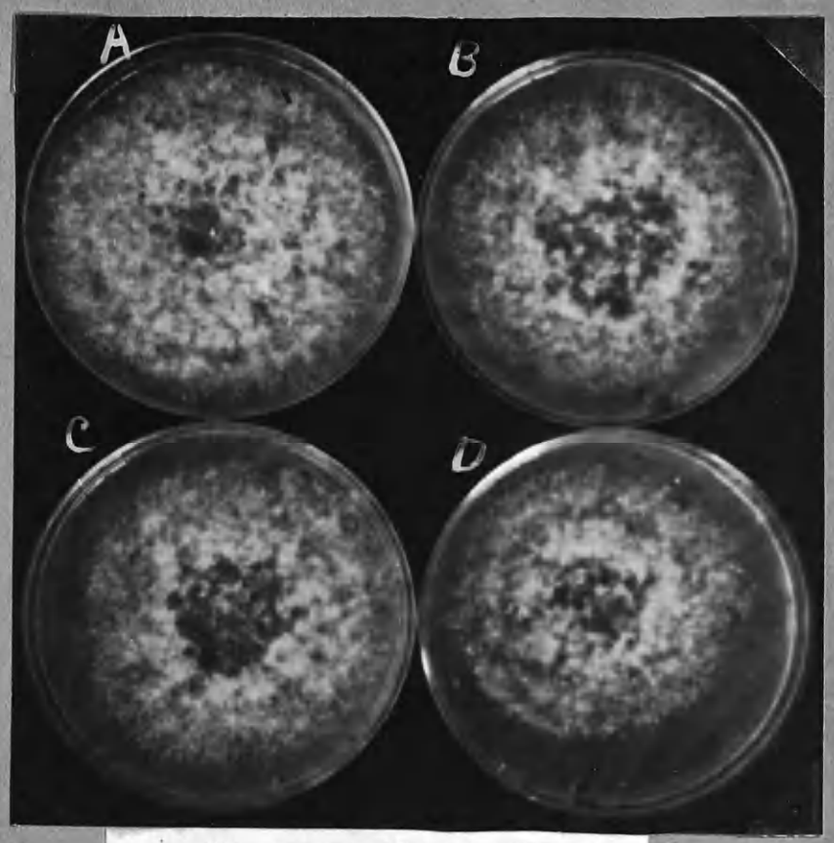
Two days after treatment

A. Control B. 15 minutes
C. 30 minutes D. 60 minutes



Three days after treatment

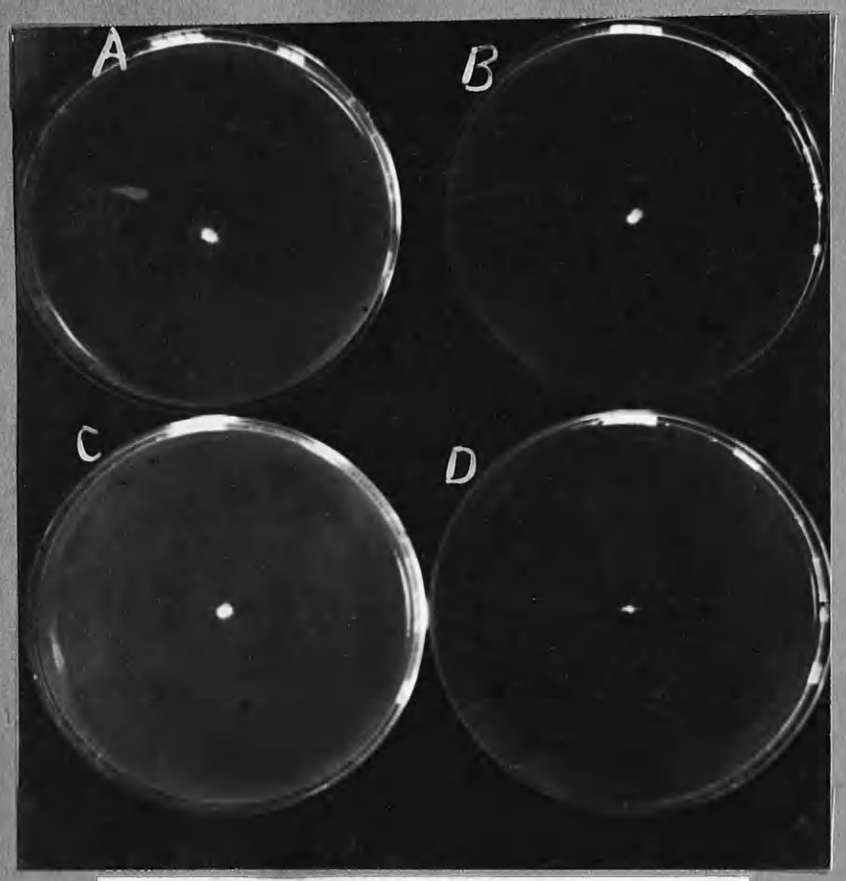
A. Control B. 15 minutes
C. 30 minutes D. 60 minutes



Four days after treatment

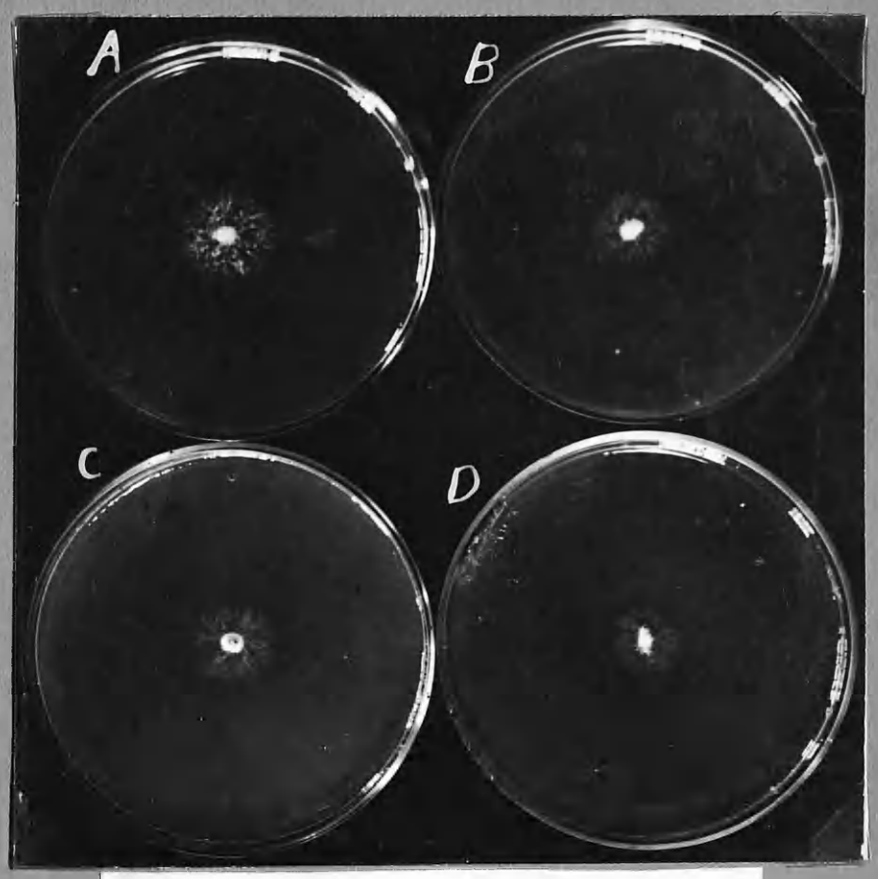
A. Control B. 15 minutes
C. 30 minutes D. 60 minutes

Fig. 17 The effect of ultra violet irradiation on cultures of Botrytis squamosa treated one day after inoculation.



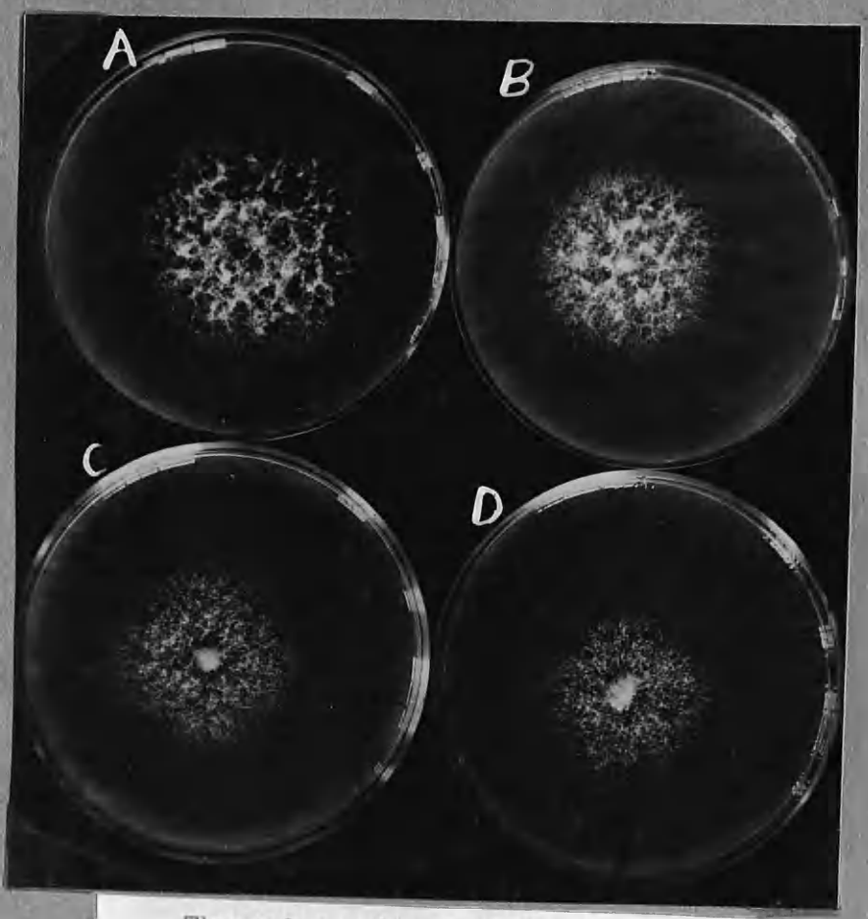
One day after treatment

A. Control B. 15 minutes
C. 30 minutes D. 60 minutes



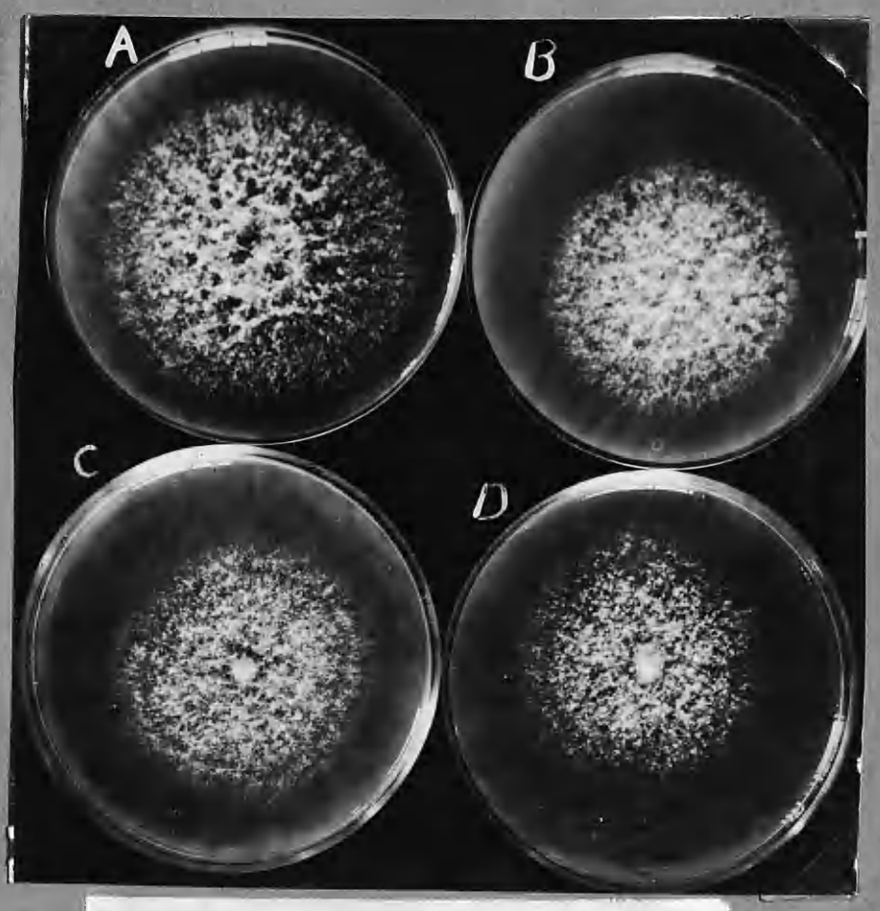
Two days after treatment

A. Control B. 15 minutes
C. 30 minutes D. 60 minutes



Three days after treatment

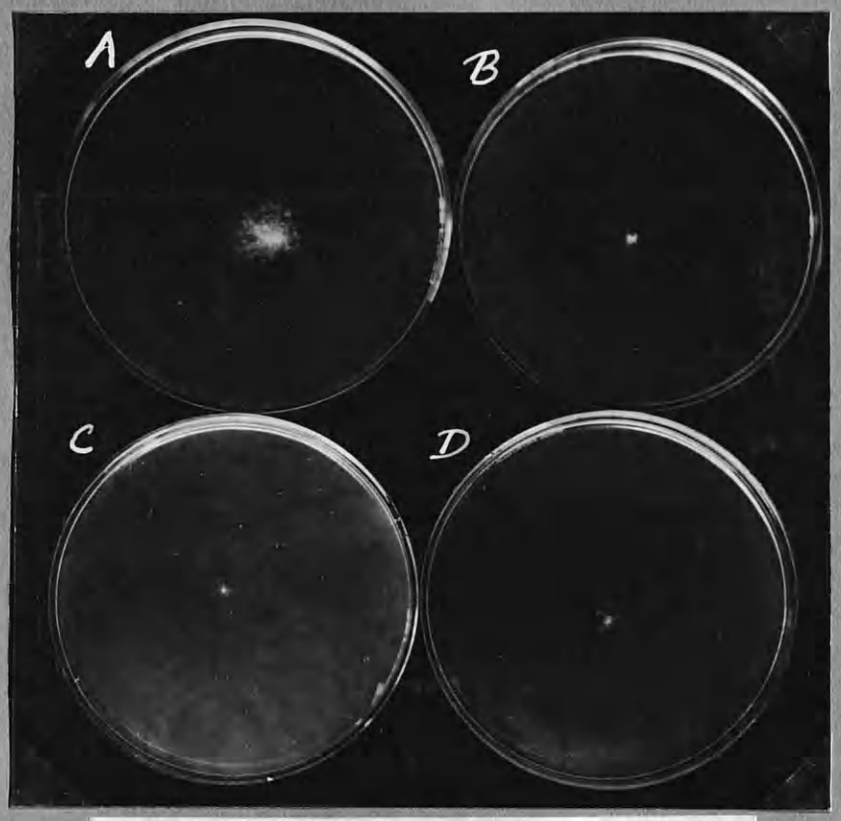
A. Control B. 15 minutes
C. 30 minutes D. 60 minutes



Four days after treatment

A. Control B. 15 minutes
C. 30 minutes D. 60 minutes

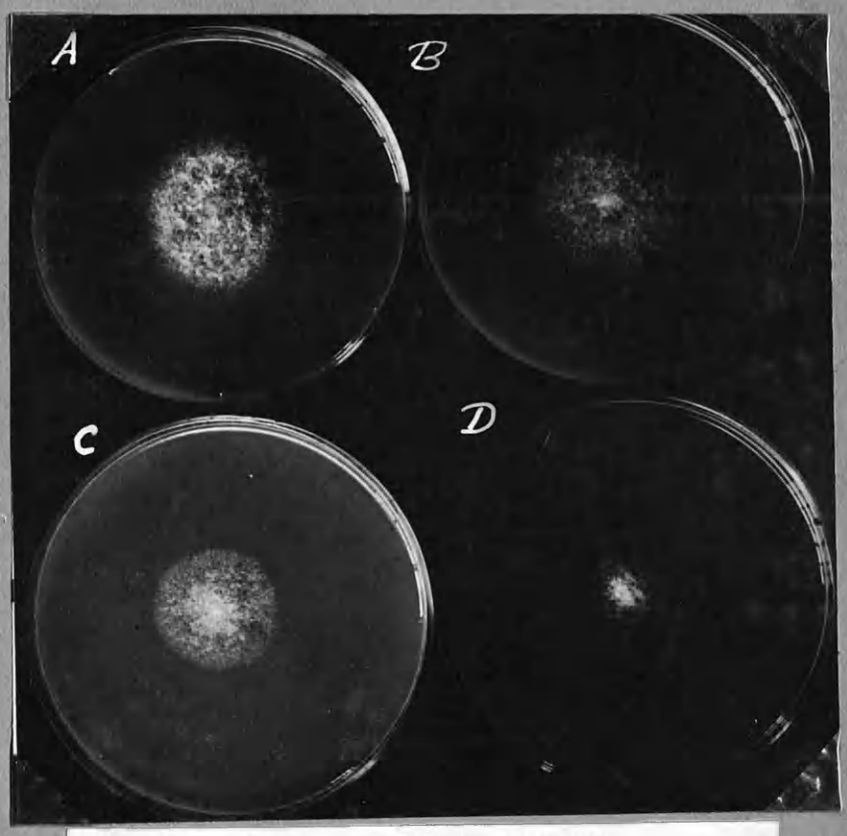
Fig. 18. The effect of ultra violet irradiation on cultures of Botrytis tulipae treated one day after inoculation.



One day after treatment

A. Control B. 15 minutes

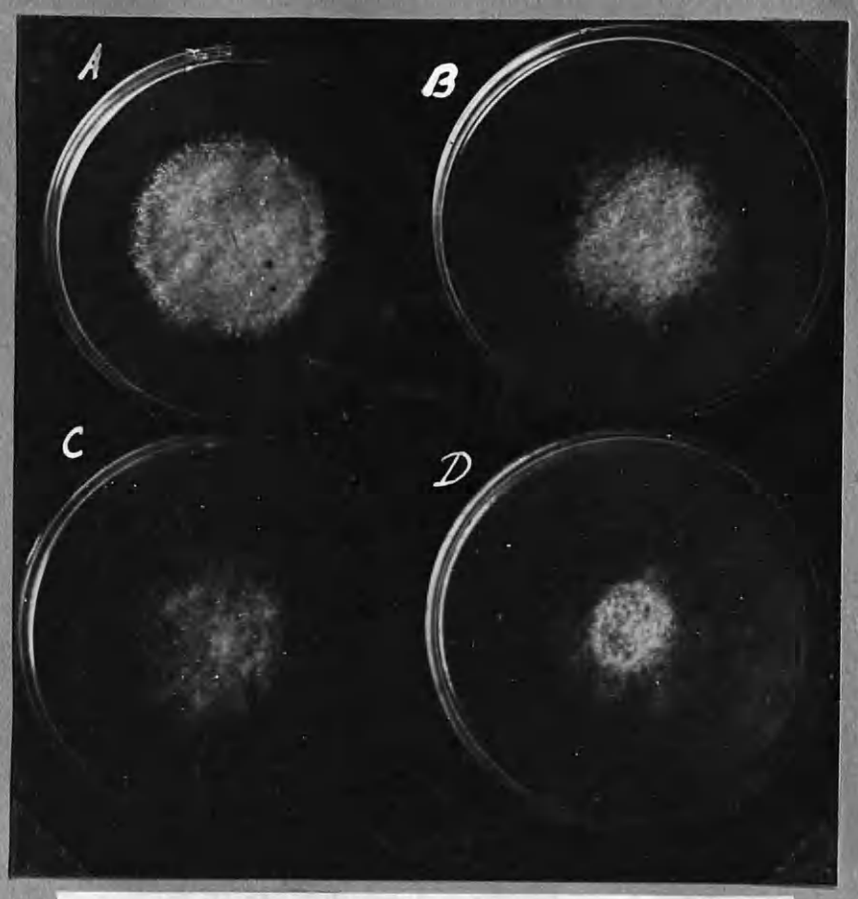
C. 30 minutes D. 60 minutes



Two days after treatment

A. Control B. 15 minutes

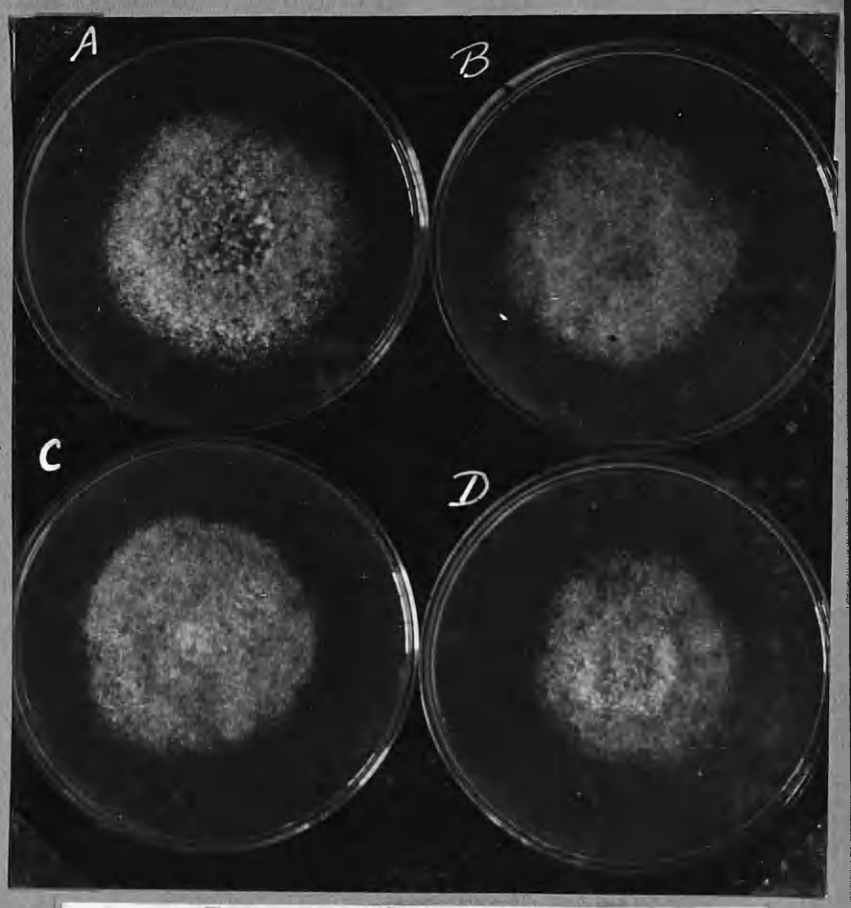
C. 30 minutes D. 60 minutes



Three days after treatment

A. Control B. 15 minutes

C. 30 minutes D. 60 minutes



Four days after treatment

A. Control B. 15 minutes

C. 30 minutes D. 60 minutes

Action on lettuce

Table 60 shows that the ultra violet light had nearly no effect on the fungus Botrytis cinerea on lettuce but it checked the plant growth.

Table 60. Effect of ultra violet light on lettuce seedlings

One treatment in minutes	Effect of irradiation:- on the fungus	on plants
1	-	-
3	-	-
5	-	-
15	-	-
30	-	++
60	-	+++
Three treatments three days in succession in minutes		
1	-	-
3	-	-
5	-	-
15	-	++
30	-	+++
60	+	+++

- No effect ++ Moderate (seedlings were not killed)
+ Slight +++ Severe (some seedlings killed)

PAINTING TOMATO WOUNDS

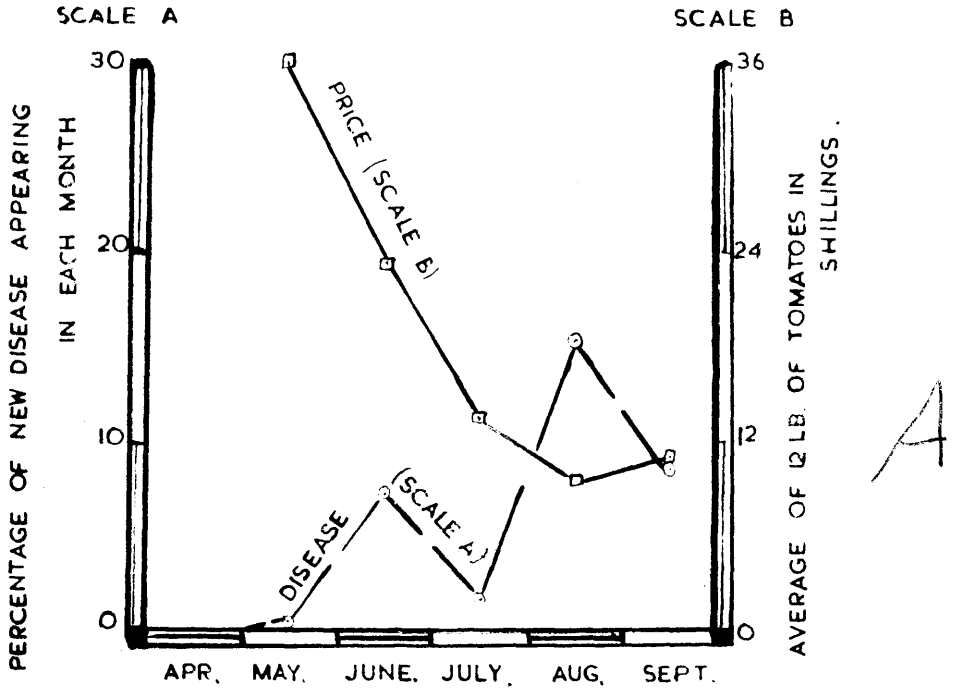
Painting wounds resulted in considerably less disease at the end of the growing season. This was in spite of the fact that the "painted" house had a higher initial incidence of disease in June than the control (table 61).

Table 61. Wound painting and Botrytis disease on tomato

	May	June	July	Aug.	Sept.
Painted					
Percentage of infection	0	3.5	6.9	30.3	40.3
Not painted					
Percentage of infection	0 (824 plants in each house)	1.4	4.8	40.6	63.7

Economics of Grey Mould control on tomato

It is interesting that the grower in whose glasshouses the work on tomato was done regards the painting of wounds as fully economic up to the beginning of July, but not thereafter. The "painted" and "unpainted" glasshouses had no Grey Mould in May, and but little in June, when, moreover, the "painted" house had a higher initial infection than had the "unpainted". Replicated plots in the house devoted to manurial experiments (section III, p. 65), however, had sufficient disease to allow the making of correlation diagrams between yield and the amount of infection in four months covering the main fruit production period (fig. 19B). When the amounts of new infection appearing in each month are plotted (fig. 19A) there were definite peaks in June and August. Prices are high in May and June, but fall through July and August (fig. 19A). The relative effects of these factors are brought together in table 62 which shows that when there is an appreciable amount of



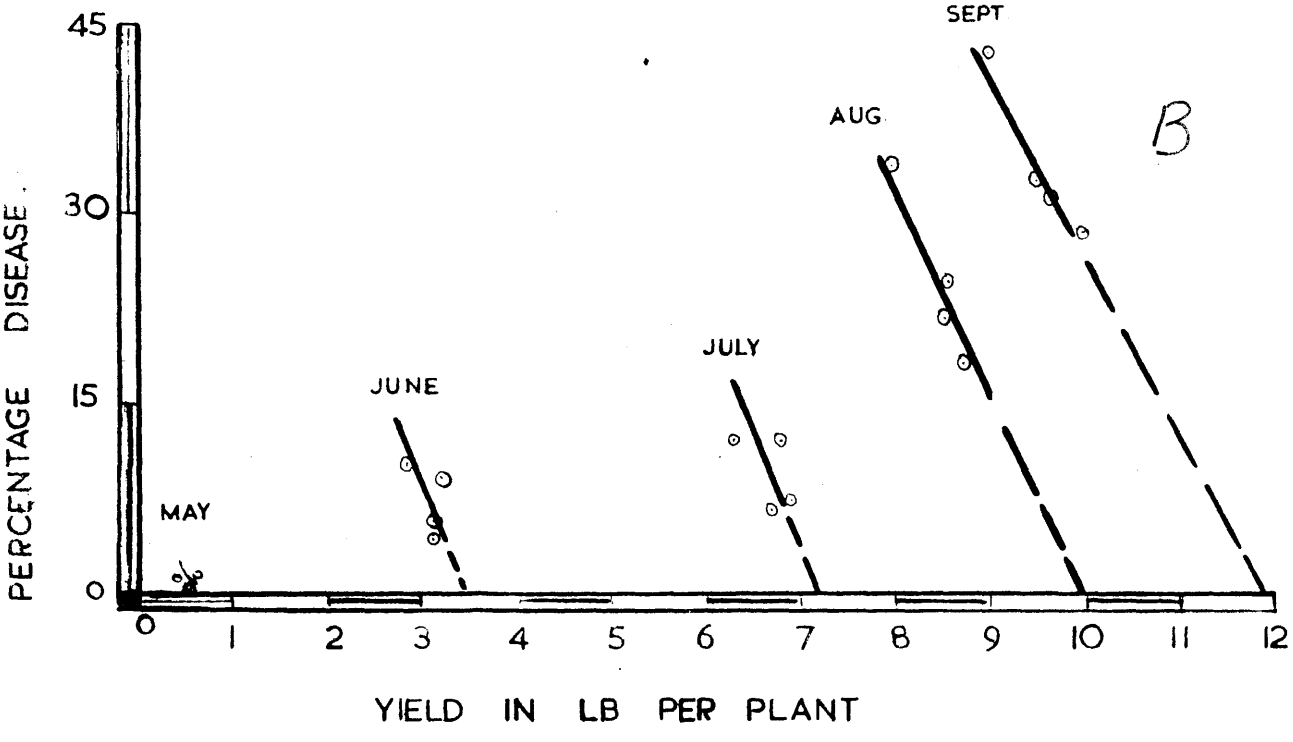


Fig. 19. A (above) The amount of new disease appearing in each month compared with the average price of tomato at Glasgow market, 1951. B (below) Relations between the amount of disease and the yield, for different months.

Botrytis disease in June, it does indeed bring a heavier financial loss than does a larger amount of the disease in later months.

Table 62. Average values for percentage of Botrytis disease, yield, loss and price level - Tomatoes 1951

	Yield, no. of 12 lb. chips per house*	Aver. % Botrytis	% loss of yield calculated from fig.B(12 lb. chips per house)	Calculated yield if healthy; (12 lb. chips per house)	Estimated loss due to Botrytis(12lb. chips per house)	Price of fruit; shillings per 12 lb. chip	Values of fruit lost by Botrytis; shillings per house.
May	41.2	0.3	0	41.2	0	36	0
June	164.6	7.4	8.8	178.6	14.0	23.4	327.6
July	247.2	9.8	8.3	267.7	20.5	13.3	272.6
Aug.	116.6	25.1	16.8	136.1	19.5	9.5	185.2
Sept.	76.2	33.9	20.8	91.4	15.2	11.0	167.2

* This house is 200 ft. by 15 ft.

Dusting Folosan on lettuce

Plants were dusted on May 15 and May 30 on two plots, another was dusted on May 15, 30 and June 15 and the fourth was left without treatment. Table 63 shows that there was no significant difference between treated and control plots. Some plants already infected with Grey Mould were also dusted and no control was obtained.

Table 63. Percentage infection in treated and control plots

	Rate of each application in ounces per sq. yd.			
Treatment	½	1	1	0
Number of applications	2	2	3	-
Percentage infection	16	14	12	14

(50 plants in each plot)

DISCUSSION AND CONCLUSIONS

Ultra violet irradiation checked the growth of Botrytis species but did not kill the cultures. The degree of check was, moreover, in direct proportion to the time of exposure. Ultra violet light had no effect upon the time of sporulation except in cultures treated three days in succession. It then caused a slight delay of one day in sporulation. The irradiation seems also to have no effect on the fungus Botrytis cinerea on lettuce, except in long exposures, but it had a severe effect on seedlings, except with short exposures. It would thus appear that ultra violet irradiation cannot be used in practice to control Botrytis cinerea on lettuce in a manner similar to that suggested for cherry diseases by English and Gerhardt (1945).

It seems from painting tomato wounds with bitumen paint that there was considerably less disease at the end of the season in the "painted" house at the end of the growing season. This was in spite of the fact that the

"painted" house had a higher initial incidence of disease in June than had the control. Painting wounds was found to be fully economic up to the beginning of July, but not thereafter (table 62).

It is already known that dusting Folosan on lettuce seedlings, applied to the soil or sprinkled on the surface after sowing, gave a good control (Smieton and Brown 1940). The main point in the present investigation was to see the effect of dusting older lettuce plants with the substance. Results showed that there was no significant difference between dusted and control plots. Some other plants infected with the fungus Botrytis cinerea were also dusted but there was no effect on controlling the disease.

SUMMARY

1. The optimum temperature for growth of Botrytis cinerea Pers., Botrytis cinerea f. lini, Botrytis fabae Sard., Botrytis galanthina Berk. & Br., Botrytis narcissicola Kleb., Botrytis paeoniae Oudem. and Botrytis squamosa Walker was 21°C., but there was no growth at 2°C. or at 31°C.

2. Botrytis cinerea Pers. as a disease on lettuce was more severe on 20°C. than 7°C. or 15°C.

3. Spores of Botrytis allii Munn, Botrytis cinerea Pers., Botrytis cinerea f. lini and Botrytis squamosa Walker germinated only at 100% relative humidity and there was no germination in any species at 95% relative humidity.

4. Botrytis cinerea Pers. was more severe on lettuce in very high relative humidities.

5. Length of day had no effect on the severity of Botrytis diseases.

6. Infection with Botrytis cinerea on lettuce and potato, Botrytis fabae on field beans and Botrytis allii on onions was more severe when plants were grown in more acid soils (below about pH 5.0). There was a significant correlation between acidity of the soil and severity of the diseases studied. One pot experiment with Chocolate Spot was made with soil ranging naturally from pH 4 to pH 7.2. It

showed that growth of beans was greatest at about pH 5.7, when the amount of the disease was low. Above this value, the yield fell off with increasing soil alkalinity, but the amount of disease ~~remained~~ remained fairly constant at a low level. Below pH 5.7 the yield fell markedly and the disease increased considerably.

7. There was only a slight increase in the amount of disease resulting from artificial infection, as compared with natural infection on lettuce and beans, especially on the acid soils.

8. Infections of Botrytis cinerea on lettuce and tomato, Botrytis fabae on field beans and Botrytis allii on onions were all more severe on unmanured plots than on those treated with fertiliser. This effect was non-specific, and occurred with additions of phosphate, potash, organic nitrogen and even with normal dressings of ammonium sulphate. Differences in the amount of disease were highly significant in the experiment where statistical treatment could be applied.

9. Normal applications of sodium nitrate increased the amount of Botrytis disease on several crops, and double applications of ammonium sulphate increased the amount of disease in one experiment with onions.

10. There was a significant inverse correlation between the severity of Chocolate Spot and available phosphorus (P_2O_5) in the soil.

11. There was no significant relation between yield of tomatoes in the untreated plots and those treated with varying amounts of hoof and horn meal. There was, however, a significant correlation between the yield and the percentage of the disease.

12. Ultra violet light checked the growth without killing the cultures of Botrytis allii Munn, Botrytis cinerea Pers., Botrytis fabae Sard., Botrytis paeoniae Oudem., Botrytis squamosa Walker and Botrytis tulipae (Lib.) Lind. It had no effect upon the time of sporulation except in cultures treated three days in succession. It then caused a slight delay of one day in sporulation.

13. No practical control of Grey Mould on lettuce by treatment with ultra violet light appeared to be possible. The irradiation, however, affected the seedlings' growth.

14. Painting tomato wounds with bitumen paint resulted in considerably less disease at the end of the growing season. The results showed that the painting of wounds was fully economic up to the beginning of July but not thereafter.

15. No control appeared from dusting established lettuce plants with pentachloronitrobenzene (Folosan).

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