DEVELOPMENT OF *DIPLOCARPON ROSAE* ON DIFFERENT ROSE CULTIVARS

by

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A thesis presented in part fulfilment of the requirements for the Degree of Doctor of Philosophy in the Faculty of Science of the University of London.

DECEMBER 1975

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ABSTRACT

The development of Diplocarpon rosae Wolf was examined principally on five floribunda rose cultivars: Allgold, Frensham, Iceberg, Masquerade and Orange Sensation, both in the field and in the laboratory. A simple standard laboratory test using leaf discs was developed to evaluate the resistance of these cultivars to different isolates of D. rosae. The variability of isolates from different cultivars was demonstrated and distinct races or strains identified.

The development of the disease on the five cultivars was followed in a field trial over two successive seasons. Disease initiation and increase was associated with infection periods similar to those used for forecasting apple scab outbreaks, but no simple relationship could be demonstrated between the rate of disease increase and the weather preceding an assessment of disease. The onset of the epidemic was also related to the availability of strains of D. rosae able to grow on the cultivars. The results are discussed in relation to race-specific and race-non-specific resistance of these cultivars.

Experiments indicated that D. rosae could overwinter on fallen leaves either as conidia in existing acervuli or as a mycelium which gave rise to the perfect state. The collections of the acigerous stage of D. rosae made during the investigation were the first recorded in Britain.

The growth of one isolate of D. rosae was compared on the cultivars Frensham (susceptible) and Allgold (resistant). The resistance of Allgold to this isolate was associated with a reduction in the germination of conidia. This reduction appeared to be due to the production of an antifungal compound by the cultivar in response to substances from the conidia.

ACKNOWLEDGEMENTS

I wish to express my sincere thanks to my supervisor, Dr. B. E. J. Wheeler, for his constant advice, guidance and useful criticism throughout this work and in the preparation of this manuscript.

I wish to thank Professor R. K. S. Wood in whose department this work was undertaken.

I would also like to thank the staff at Imperial College Field Station, Silwood Park, in particular those of the Botany Department, Mr. E. Green, Mrs. C. Warren and Mrs. P. Tyler for technical assistance, and the gardeners.

I wish also to thank my parents for their continued support and encouragement.

Finally, I wish to acknowledge the financial assistance provided by the Royal National Rose Society.

CONTENTS

INTRODUCTION

Blackspot of roses, caused by the fungus Diplocarpon rosae Wolf is common wherever roses are grown, except in areas of high atmospheric pollution (Saunders, 1966 a). Few, if any, attempts have been made to use resistant cultivars; in general control measures have mainly involved the application of fungicides during the growing season and the use of sanitation methods in the winter. Considering the popularity of the rose and its importance to the horticultural industry there has been relatively little scientific work on the disease in this country.

For example, there is some uncertainty in this country how the fungus overwinters. Survival on fallen infected rose leaves appears to be the most common method, though few investigators have examined the various possibilities critically. Wolf (1912) demonstrated the involvement of the perfect state but it has not previously been found in Britain or Europe. The general lack of experimental data concerning the survival of D. rosae promoted an investigation into the overwintering on fallen leaves.

The present work had two basic aims:

- to examine disease development in the field and relate this to availability of inoculum, type of cultivar and the weather and
- to look more closely at blackspot resistance.

The latter aspect was developed mainly as a laboratory project involving the development of a standard test in which cultivar reactions could be compared. It was linked with the field work in the choice of cultivars tested and the isolates of D. rosae used.

I DEVELOPMENT OF *DIPLOCARPON ROSAE* ON ROSE CULTIVARS IN THE FIELD

A OVERWINTERING ON FALLEN ROSE LEAVES

INTRODUCTION

Blackspot is common wherever roses are grown, the disease being particularly prevalent in Europe and North America. According to Frick (1943) roses are indigenous to the northern hemisphere and garden roses are also cultivated in the southern hemisphere, particularly Australia and South America, and the disease occurs wherever roses are grown. It appears as circular, dark brownish-black spots which are most conspicuous on the upper surface of the leaves. These appear first as small dark leaf spots, from the edges of which radiating strands of the fungus grow out, which give the spots their characteristic fringe-like margin. Individual spots often reach a diameter of 1 cm., the spots may coalesce until large areas of the leaves are blackened. Infected leaves turn yellow and this causes premature defoliation, a characteristic symptom of the disease, which can cause buds, that normally remain dormant, to grow. The disease generally appears about the middle of July and infects all types of cultivated roses.

The causal fungus is an ascomycete, Diplocarpon rosae Wolf, but on the growing plant only the conidial state (previously described as Actinonema rosae by Wolf (1912)) is found within the lesions. The conidia which are formed in acervuli are the means by which the fungus enlarges its distribution on its host. The acervuli develop within the blackspot lesions, either scattered over the leaf surface or arranged, ring-like, at the margin.

Both the acigerous and conidial state may be involved in the perennation of the fungus, but it appears that the mode of survival

depends largely on locality and environment (Frick, 1943). Survival on fallen leaves appears to be the most common. The involvement of the acigerous state in this was demonstrated by Wolf (1912) at Ithaca in New York State, U.S.A. He placed leaves infected with the conidial state of D. rosae in wire cages, and left them to weather through the winter. In the following April he observed subepidermal apothecia on these diseased leaves from which asoospores were later discharged. This, indeed, was the first report of the perfect state of the fungus. There have subsequently been other reports of its occurence in New York by Aronescu (1934) and in Canada by Bisby (1938). However, even in North America it appears to be formed only rarely. Dodge (1931), for example, did not find it in observations on infected leaves overwintered in wire cages, in two consecutive seasons, in New York. Nor has it, until now, been reported in Britain or Europe though several investigators have searched for it. In Britain Alcock (1918), Shelly (1925) and Green (1931) examined, unsuccessfully, infected rose leaves that were overwintered under natural conditions, for the presence of the perfect state, but on no occasion were they able to detect it. Frick (1943) collected infected rose leaves in four successive years from various parts of Switzerland and overwintered them in Zurich, examination of these leaves in the spring for the presence of apothecia proved negative. She concluded that the formation of the perfect state seems to occur only rarely, and only under certain, still unknown, conditions.

In these situations, where blackspot occurs year after year it is obvious that the fungus must overwinter by other means. In terms of survival on infected, fallen rose leaves there are three other possibilities:

1. conidia overwinter in existing acervuli
2. existing acervuli survive and produce new conidia in the spring, and/or
3. the mycelium in the leaf survives and produces new acervuli in spring, in which conidia form.

Few investigators have examined these possibilities critically. Frick (1943), for example, suggested that the fungus hibernates in this situation as a saprophytic mycelium, and that new infections may be derived from conidia produced on fallen leaves. Leaves which were overwintered in the field, when placed in a humid chamber at room temperature produced fresh conidia in previously washed-out fruiting structures after 12 - 24h. These D. rosae conidia exhibit 94% germination, and when used to inoculate rose leaves produced blackspot lesions. On the other hand, conidia collected presumably from overwintering leaves in different seasons, failed to germinate in February and March. Though these conidia could survive low temperatures ($-3^{o}C$) for short periods other environmental factors, as well as temperatures around zero accounted for this loss of viability. She demonstrated that the life span of mature conidia appeared to be very short, even on leaves.

Survival of the fungus as conidial infections on young wood is a further possibility. Such infections were first discovered in England by Alcock (1918) and were further investigated by Green (1931). He found fully developed acervuli with viable conidia beneath the unbroken cuticle of young shoots in January and February, and suggested that these conidia might then be discharged later when vigorous growth of the rose plant is accompanied by a recommencement of fungus activity, with consequent rupture of the epidermis and release of the conidia. It was probable that the acervuli and

conidia were formed the previous autumn and remained dormant during the winter months. Some cultivars of rose appeared to be more susceptible than others to these shoot infections but the significance of this has not been fully investigated, but pruning and the resistance of certain cultivars may reduce the importance of these young wood infections as a source of inoculum.

Lastly, it was suggested both by Alcock (1918) and Bewley (1938) that the fungus might survive on an alternative host. This remains a matter of speculation.

The general lack of experimental data concerning the survival of D. rosae, through the winter in this country, prompted the following investigation.

EXPERIMENTAL

1. 1972 - 1973

a. Perennation of D. rosae on leaves under natural conditions

Leaves with lesions bearing acervuli of D. rosae were collected on 3rd November, 1972 from a planting of the cultivar Frensham at Silwood Park established in 1967 (Price, 1970). Those chosen were already showing the premature senescence associated with blackspot and could be removed almost at a touch from the shoots. The individual leaflets from these leaves were placed in three bags of terylene-net (30 x 27 cm.), 100 per bag, and the bags were then pinned to the soil between the rose bushes from which the leaflets were collected, by metal pegs at all four corners.

At intervals the leaflets were sampled and during the course of the experiment four things were tested:

1. the germination of spores already present,
2. their infectivity,
3. the ability of acervuli to produce fresh conidia,

and 4. their viability and infectivity.

Samples were taken at intervals, initially of two weeks and later of four weeks. The infectivity of conidia was assessed only towards the end of the experiment. Usually at each sampling five leaflets were removed from each bag. The total sample of fifteen leaflets were washed in 10 - 15 ml. sterile distilled water and large pieces of debris such as soil particles or bits of leaf tissue were first removed after allowing the suspension to settle briefly. The washings were then centrifuged at 650g for five minutes. The supernatent was discarded, and the sediment was resuspended in sterile distilled water and re-centrifuged under similar conditions, two to five times depending on the level of contamination. The cleaned washings were examined for conidia of D. rosae and the suspension was adjusted to about 200,000 conidia/ml., using a haemocytometer count as a guide. The washed leaves were then placed in a damp chamber for 24h. at 20°C, after which they were washed again using the same procedure as before, and examined for acervuli. The viability of D. rosae conidia obtained in these two operations were assessed in two ways:

1. Conidial suspensions were streaked over the surface of water agar in Petri dishes and germination of conidia determined after incubation at 20°C for 24h.
2. Drops of conidial suspensions were placed on discs cut from disease-free leaves of Frensham plants raised in the greenhouse as described on Page 64.

These discs were floated on water in small (5.5 x 3.5 x 2 cm.) polystyrene boxes and incubated at 20°C for fourteen days to allow development of lesions from viable conidia.

Some modifications of these techniques were necessary as the experiment progressed, due to the material weathering and microbial action. Greater care needed to be taken with the first washing to remove soil particles and leaf debris. Also in later samplings fewer conidia could be recovered so that the volumes of suspension containing a reasonable number of D. rosae conidia for tests became increasingly smaller and contained more spores of other fungi. Eventually, because of many contaminants, tests of the germination of D. rosae conidia were not possible and drops of the leaf washings were placed on leaf discs only. Some indication of the inoculum size of D. rosae was attempted by recording the number of discs infected and the size of lesion developing on them.

The results are summarised in Tables 1 and 2. Some conidia survived on overwintered leaves until mid-June. Although the numbers which did so were so small that their germination could not be assessed directly, nevertheless leaf discs of Frensham became infected when they were inoculated with drops of suspensions prepared from overwintered leaves. The lesions that develop were comparable with those produced by the same isolate in the standard leaf disc test on Frensham (Table 21). This clearly indicates that some viable inoculum remains in the field, potentially capable of infecting new growth on the rose bushes, even though by this time most of the overwintered leaves had rotted and disintegrated. No new acervuli appeared to develop on washed leaves

TABLE 1

GERMINATION OF D. ROSAE CONIDIA WASHED FROM OVERWINTERED LEAVES OF THE CULTIVAR FRENSHAM, 1972 - 1973

Sampling Date		Week Number	Mean % Germination	
			Sample A+	Sample B
1972	3 November	0	96.7	87.0
	17	2	93.3	83.7
	1 December	4	77.7	81.7
	15	6	42.8	41.5
	29	8	46.5	30.8
1973	26 January	12	4.4	2.0
	22 February	16	41.2	8.0
	23 March	20	21.4	- a
	20 April	24	- *	-
	18 May	28	- *	-
	15 June	33	- *	-

+ Sample A - Washings from leaves taken from the field.
Sample B - Further washings following incubation of washed leaves at 20°C for 24h.

* Infectivity test, see Table 2

a Conidia present, but too few for test

TABLE 2

INFECTIVITY OF D. ROSAE CONIDIA WASHED FROM OVERWINTERED LEAVES OF THE CULTIVAR FRENSHAM, 1972 - 1973

Sampling Date	Week Number	Sample+	Lesion diameter (mm.)* Box means						% leaf disc infection
			1	2	3	4	5	Mean	
1973 20 April	24	A	3.7	6.5	0	5.5	4.5	4.9	18
18 May	28	A	0	7.0	0	5.0	3.0	5.0	6
15 June	33	A	6.0	4.0	4.0	6.0	6.0	5.1	14

+ Sample A - Washings from leaves taken from the field.

* Ten leaf discs per box, means of those discs with lesions only.

kept for 24h. at 20°C, suggesting that generally this is not the means by which inoculum is derived in the spring. Also very few conidia were produced by existing acervuli after this treatment and few viable conidia could be obtained in this way after February. Where it occurred survival appeared to be largely as conidia already present in acervuli.

b. Numbers of spores derived from overwintered leaf tissue

Leaf discs, 1 cm^2, were cut using a standard leaf-punch from blackspot lesions on leaves of Frensham within the same planting at Silwood Park on 2nd November, 1972. Twenty to twenty-five of these discs were placed in each of five Terylene-net bags (10 x 10 cm.). These were pinned to strips of untreated wood, which were in turn pegged to the soil between the rose bushes of the planting from which the discs were derived. At intervals of first two weeks and later four weeks, one disc was removed from each bag and the five discs incubated in a damp chamber for 24h. at 20°C. They were then washed and washings centrifuged as described above, and the final suspension made up to 1 ml. The number of conidia of *D. rosae* in this suspension was assessed from haemocytometer counts and their ability to germinate determined by streaking drops on water agar as before. The amounts of conidia derived from the discs were expressed as numbers per cm^2 leaf tissue (Table 3).

The leaf discs did not survive well and by February 1973 most had disintegrated through weathering and microbial action. No conidia were obtained from these discs after six weeks and before this there was a rapid decline in the numbers of conidia obtained and in their viability.

TABLE 3

NUMBERS OF D. ROSAE CONIDIA WASHED FROM OVERWINTERED LEAF DISCS OF THE CULTIVAR FRENSHAM AND THEIR GERMINATION, 1972 - 1973

Sampling Date	Week Number	Number of conidia per cm^2.	Mean % Germination
1972 2 November	0	7,600	96.7
16	2	3,360	61.2
30	4	1,960	57.2
14 December	6	0	-
28	8	0	-
1973 25 January	12	0	-

2. 1973 - 1974

Leaves kept in Terylene-net bags during the winter of 1972/1973 were in close contact with one another, and following rain remained wet for long periods. These conditions probably accelerated their decomposition and did not correspond to those of leaves blown around the planting of rose bushes. To reproduce more closely these conditions leaves were overwintered in 1973/1974 in a large cage. This consisted of a frame (1 m^3) made from bamboo canes and covered with Netlon of $\frac{3}{4}$ in. (19 mm.) mesh. The netting was sunk into the ground on three sides and pinned to it on the fourth side to give easy access. Infected leaves selected as in 1972 from the Frensham bushes were placed in the cage in sufficient numbers to cover the bare soil at its base on 2nd November. These leaves were sampled as described for the previous winter.

The results (Tables 4 and 5) showed a similar pattern to that observed in the previous overwintering experiment, until the last sampling in May. However, the leaves did not decompose so rapidly and blackspot lesions could be found relatively easily throughout the experiment. In this respect keeping the leaves in the net cage had obvious advantages over placing them in the Terylene-net bags. Again acervuli appeared to produce few or no new conidia and the number that could be recovered from leaves declined progressively throughout the experiment and so did their ability to germinate (Table 4). By 22nd March, 1973 few conidia of *D. rosae* could be recovered and because of the large proportion of contaminants in the washings, germination of these could not be tested. However, in the last sampling on 9th May, 1973 this pattern changed. Many spores were recovered on washing the leaves from the field, more

TABLE 4

GERMINATION OF D. ROSAE SPORES WASHED FROM OVERWINTERED LEAVES OF THE CULTIVAR FRENSHAM, 1973 - 1974

Sampling Date		Week Number	Mean % Germination	
			Sample A[+]	Sample B
1973	30 November	4	83.2	78.3
	14 December	6	76.0	71.0
1974	25 January	12	45.6	27.0
	27 February	16	32.3	- [a]
	22 March	20	-	-
	26 April	24	-	-
	9 May	26	88.5*	90.3*

[+] Sample A - washings from leaves taken from the field

Sample B - further washings following incubation of washed leaves at 20°C for 24h.

* Infectivity test, see Table 5

[a] Conidia present, but too few for test

TABLE 5

INFECTIVITY OF D. ROSAE SPORES WASHED FROM OVERWINTERED LEAVES OF THE CULTIVAR FRENSHAM, 1973 - 1974

Sample Date	Week Number	Sample+	Lesion diameter (mm.)* Box means 1	2	3	4	5	Mean	% leaf disc infection
9 May 1974	26	A	7.4	7.0	7.0	8.0	6.8	7.5	92
			8.0	7.6	7.2	8.0	7.8		
		B	7.8	7.0	7.8	6.8	6.8	7.3	96

+ Sample A - Washings from leaves taken from the field

Sample B - Further washings following incubation of washed leaves at 20°C for 24h.

* Five leaf discs per box, means of those discs with lesions only.

were apparently produced on these leaves following incubation at 20°C, and many leaf discs of Frensham became infected following inoculations with these suspensions (Tables 4 and 5). The germination of these spores was also high (Table 4) and approached that observed for fresh collections of D. rosae from infected roses.

These results promoted a closer look at the leaf material from the field. Under a microscope balckspot lesions had what at first appeared to be many newly-burst acervuli with masses of conidia, but closer examination revealed these to be fruitifications with asci and discharged ascospores. Similar fruiting structures were found on other leaves then collected from the net cage. A comparison of this material with the description by Wolf (1912) suggested that it was the perfect state of D. rosae (Plates 1 and 2) and this was confirmed by the Commonwealth Mycological Institute, where a sample of the material is now deposited as IMI 185129. This is the first record of the perfect state for the British Isles. That the ascospores could initiate blackspot lesions was apparent from the pathogenicity tests on leaf discs on 9th May (Table 5), but further tests were carried out and these are detailed below.

OBSERVATIONS ON THE PERFECT STATE OF D. ROSAE

a. Development of apothecia

Leaves from the net cage were incubated in a damp chamber and kept in an illuminated cabinet at 20°C for two weeks. An examination of this material showed that a succession of apothecia developed within discernable blackspot lesions. Further observations on material collected at intervals from the field also indicated that

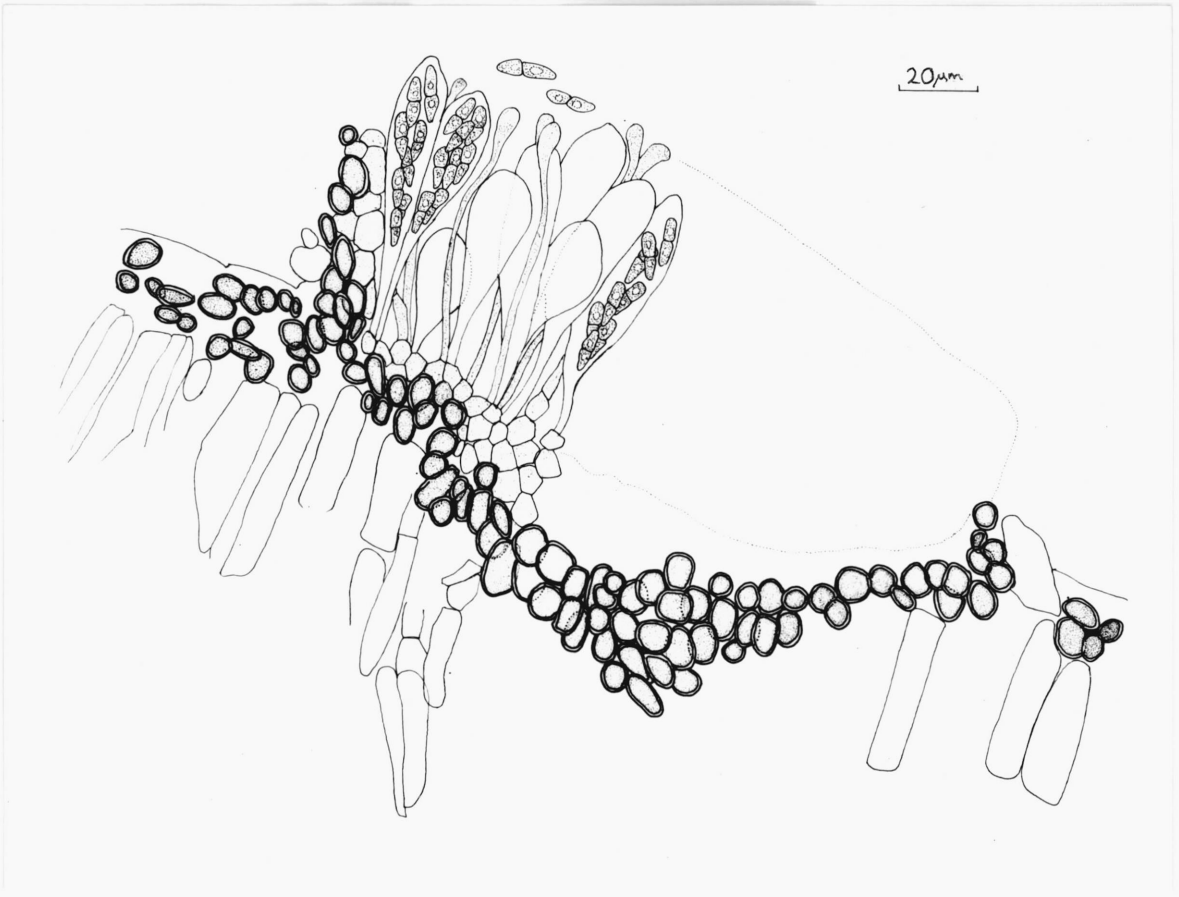
PLATE 1 PERFECT STATE OF DIPLOCARPON ROSAE.

SURFACE VIEW OF APOTHECIUM (a) ON AN OVERWINTERED LEAF

PLATE 2 PERFECT STATE OF D. ROSAE. T.S. APOTHECIUM

(Dr. B. E. J. Wheeler)


a
a


20μm

apothecia were produced on leaves there throughout May and June, a period of much new growth on the rose bushes.

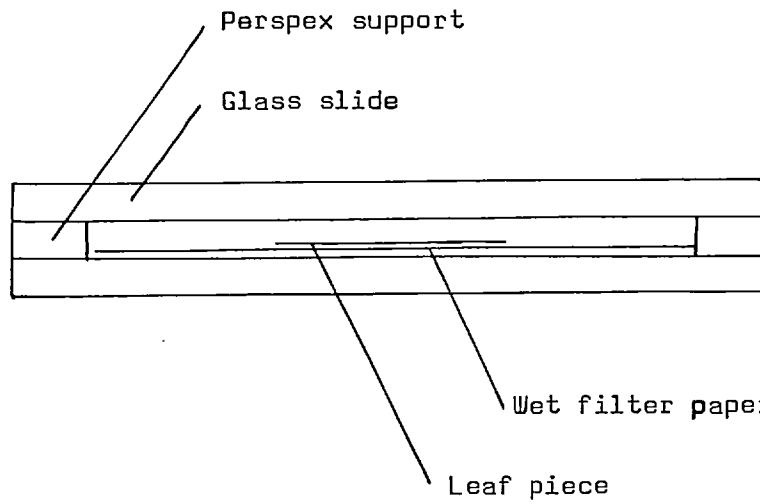
b. Ascospore discharge.

Wolf (1912) considered that ascospores were not violently discharged from the apothecium but piled up in a whitish mass, similar to the conidia in an acervulus. He assumed that ascospores were subsequently dispersed by rain splash. When apothecia from the overwintered material were examined either in squash preparations or in microtome sections, no extruded mass of spores were observed, so an experiment was designed to examine ascospore discharge.

The method was based on that of Cutler and Wheeler (1968), for looking at ascospore discharge from cleistocarps of powdery mildews. Pieces of leaves, collected from the field were first incubated in a damp chamber to encourage apothecia eruption. They were then placed on wet filter paper supported by a glass slide (7.5 x 2.5 cm.) which had at each end a piece of perspex, c. 5 mm. thick. These perspex pieces served to separate the slide from another placed over it and on which ascospores could be trapped (Figure 1). Pairs of slides were slotted into a standard glass staining trough turned on its side and this was supported on glass rods above tap water in a polystyrene sandwich box, and placed in an illuminated incubator at 20^{o}C. At 24h. intervals the slides above the leaf material were removed, replaced by clean slides, and were examined either directly or by adding a drop of cotton blue in lactophenol and placing a cover slip over the relevant area.

Examination of these slides showed ascospores to be present, mainly in groups of seven to eight or multiples thereof, above

FIGURE 1 ASCOSPORE DISCHARGE APPARATUS



apothecia present on the leaf piece below. Since asci of D. rosae contain eight spores this suggested that ascospores had been forcibly ejected. They appeared to adhere firmly to the trapslide.

c. Infectivity of ascospores

Wolf (1912) obtained blackspot lesions on rose leaves by inoculating them with spore masses from apothecia. In the present experiments further tests of ascospore infectivity were set up, in addition to that indicated by Table 5. In this apothecia were first picked off leaves with a needle, they were then either placed singly within a 0.005 ml. drop of sterile distilled water on a disc cut from healthy Frensham roses, or respectively two, five and ten such apothecia were crushed in sterile water in a cavity slide and each resulting suspension was used to inoculate five Frensham leaf discs. Out of the twenty leaf discs inoculated (Table 6), eleven became infected and showed typical blackspot lesions, some of which produced acervuli and conidia.

d. Germination of ascospores

In the experiments described by Wolf (1912), ascospores germinated on rose leaves but did not do so on artificial media or in water. He suggested that ascospores required some stimulus for germination from the living plant. The present observations do not support this, for example, ascospores germinated well on water agar (e.g. Table 4). They also germinated on the slides placed above apothecia in the experiment described in b. above.

TABLE 6

INFECTION OF FRENSHAM LEAF DISCS BY ASCOSPORES OF D. ROSAE

Ascospore inoculum Apothecia/drop+	% leaf discs infected	Number of leaf discs infected*
1	20	1
2	60	3
5	60	3
10	80	4

+ Apothecia either placed singly in the inoculum drop on the disc, or 2, 5 and 10 distributed amongst five inoculum drops.

* Five leaf discs inoculated per treatment.

e. Cultures from ascospores

Several attempts were made to produce cultures from ascospores using mass inocula and single spores. In the latter instance, spores were streaked on water agar, incubated for 24 h. at 20°C and single germinated spores then removed on a plug of agar using a dummy objective and transfered to different media, e.g. Yeast-malt agar, Potato Dextrose agar and filter paper cylinders soaked in Malt Extract (see page 53). All such attempts were unsuccessful.

3. 1974 - 1975

Two further experiments on the perennation of D. rosae on fallen leaves were set up in the autumn of 1974. These aimed to determine whether finding the perfect state on Frensham was owing to unusual conditions prevailing at that time, and to examine perennation of the fungus on five cultivars: Allgold, Frensham, Iceberg, Masquerade and Orange Sensation.

a. Overwintering on Frensham

Leaves with conidial D. rosae were picked from bushes and placed in a net cage within the planting of Frensham as in the 1973/1974 experiment. This cage was of similar design to that previously, but was slightly smaller (0.5 x 0.5 x 0.25 m.). The experiment was set up on 29th October and the leaves were sampled as before.

The results (Tables 7 and 8) were strikingly similar to those of the 1973/1974 experiment. Leaves again decomposed less rapidly than in the first (1972/1973) experiment, but increasingly through the experiment it became more difficult to recover conidia of D. rosae from leaves, not only because there were less present but

TABLE 7

GERMINATION OF D. ROSAE SPORES WASHED FROM OVERWINTERED LEAVES OF THE CULTIVAR FRENSHAM, 1974 - 1975

Sampling Date		Week Number	Mean % germination	
			Sample A+	Sample B
1974	26 November	4	76.9	76.1
	31 December	8	46.0*	28.9
1975	28 January	12	- [a]	-
	26 February	16	- *	-
	25 March	20	-	-
	23 April	24	85.3*	95.1*

+ Sample A - Washings from leaves taken from the field

Sample B - Further washings following incubation of washed leaves at 20°C for 24h.

* Infectivity test, see Table 8.

a Conidia present, but too few for test.

TABLE 8

INFECTIVITY OF D. ROSAE SPORES WASHED FROM OVERWINTERED LEAVES OF THE CULTIVAR FRENSHAM, 1974 - 1975

Sampling Date		Week Number	Sample+	Lesion Diameter (mm.)* Box means					% leaf disc infection
				1	2	3	4	Mean	
1974	31 December	8	A	4.5	3.0	-	-	3.8	30
1975	26 February	16	A	0	0	0	-	0	0
	23 April	24	A	7.3	7.5	5.3	4.8	6.2	100
			B	8.4	7.0	7.7	8.9	8.0	100

+ Sample A - Washings from leaves taken from the field.

Sample B - Further washings following incubation of washed leaves at 20°C for 24h.

* Five leaf discs per box, means of those discs with lesions only.

- No test.

also because the numbers of other micro-organisms, especially bacteria and fungi, increased. As a result of this, by 28th January, 1975 germination of D. rosae conidia could not be tested directly, but infectivity tests on leaf discs indicated that viable spores were present. By 26th February 1975, however, even these tests gave a negative result either because no viable conidia were present or the numbers were below the threshold for infection. On 23rd April 1975 there was a considerable increase in the number of D. rosae spores washed from leaves and in infectivity tests, substantially more leaf discs became infected than in previous assessments. Examination of this material revealed that many apothecia of D. rosae had erupted in the old blackspot lesions. When the washed leaf material was incubated at 20°C for 24h. even more apothecia burst through. Thus the number of ascospores obtained from washed leaf material from the field was c. 17,000/ml., after incubating the material the number of ascospores washed off was 350,000/ml.

The infectivity of this second collection of ascospores was tested on leaf discs of five cultivars: Allgold, Frensham, Iceberg, Masquerade and Orange Sensation. For comparison, discs of these cultivars were inoculated with conidia of D. rosae originally taken from the Frensham planting and stored as described on page 58. Slightly larger lesions developed on discs inoculated with ascospores compared with those inoculated with conidia, but otherwise the reactions of the cultivars to both inocula were similar (Table 9 and Appendix Table 1).

b. Overwintering on five cultivars

Leaves were collected from bushes of the cultivars: Allgold, Frensham, Iceberg, Masquerade and Orange Sensation, which were

TABLE 9

INFECTION OF LEAF DISCS OF FIVE ROSE CULTIVARS BY ASCOSPORES AND CONIDIA OF D. ROSAE

Cultivar	Ascospore inoculum+		Conidia inoculum*	
	Mean lesion diameter (mm.)	% leaf disc infection	Mean lesion diameter (mm.)	% leaf disc infection
Allgold	0	0	0	0
Frensham	8.0	100	6.9	90
Iceberg	7.6	100	7.2	100
Masquerade	0	0	0	0
Orange Sensation	8.3	100	7.0	100

+ Ascospore inoculum - Sample B from overwintered leaves, see Tables 7 and 8.

* Conidia inoculum - (F - 74 - Ash) isolate maintained in the deep freeze.

planted in the Walled Garden of Silwood Park at the beginning of 1973 and which, during the summer of 1974 had all become infected fairly severely with conidial D. rosae. These leaves were placed in five net cages, of similar construction to that indicated in a. above on 29th October 1974, one cage being used exclusively for the leaves of each cultivar. These leaves were then sampled at intervals and the viability of the conidia assessed by the methods previously described.

In contrast to the Frensham leaves in Experiment a., the leaves in this experiment soon rotted. This may have been owing to a different environment, the Walled Garden site being generally more low lying and wetter, the leaf material itself may have been more fragile. The leaves were small and had been infected early in their development by D. rosae. (When picked from the bushes they were still relatively young since all the older leaves had dropped off). Even after eight weeks (31st December 1974) relatively few leaflets remained. There were just sufficient of Allgold and Frensham to constitute a sample but not of the other cultivars. The infectivity of D. rosae conidia remaining on Allgold and Frensham leaflets was tested by inoculating healthy leaf discs of these two cultivars as in a. above. Some lesions developed on each from both types of inoculum (Table 10), showing that some viable conidia were present. However, contamination with other micro-organisms was considerable and inoculated discs browned and senesced rapidly, especially when the inoculum was derived from the overwintered leaflets of Allgold. Because of this rapid deteriation in the leaf material no further samples could be taken.

TABLE 10

INFECTIVITY OF D. ROSAE CONIDIA WASHED FROM OVERWINTERED LEAVES OF FRENSHAM AND ALLGOLD - WALLED GARDEN EXPERIMENT 1974 - 1975

Inoculum Source	Cultivar on which tested	Lesion Diameter (mm.)* Box mean 1	Box mean 2	Mean	% leaf discs infected
Frensham	Frensham	5.7	6.2	6.0	100
	Allgold	4.5	5.8	5.3	80
Allgold	Frensham	5.0	2.0	4.0	30
	Allgold	0	5.0	5.0	10

* Five leaf discs per box, means of those discs with lesions only

B DISEASE DEVELOPMENT

INTRODUCTION

Conidia of *D. rosae* are dispersed by water running over or splashing onto lesions on the leaves and stems (Wolf, 1912; Dodge, 1931; Frick, 1943; Saunders, 1966b). Apparently a very small amount of rain splash is required to release most of the conidia from a lesion (Saunders, 1966b). This author also suggested that man and animals may also distribute inoculum.

Relatively few workers have assessed blackspot in the field but two of note are Englehard (1969) and Saunders (1966b). Englehard's work was concerned mainly with the evaluation of fungicides for blackspot control. He estimated the disease as percentage leaflets infected with *D. rosae*. Saunder's work was concerned more with the spread of the disease in relation to environmental conditions. For this, he used a visual infection index based on grades of infection ranging from 0 to 10. The lowest (0 - 2) and highest grades (9 - 10) in this range designated specific phases of infection and the remainder the severity of the infection. He showed that the development of the disease followed a 'compound interest' or logarithmic pattern as defined by Van der Plank (1963). Foci of blackspot could be detected in rose plantings early in the growing season. Further spread occurred mainly in late summer. During his studies in South West England, August appeared to be the critical period for further disease development, rain and average daily temperature above 14^{o}C resulting in substantial blackspot development in September. He trapped many conidia of *D. rosae* on sticky glass slides during August and suggested, therefore, that chemical control during this period might achieve a great reduction

in inoculum.

This section described the disease development within a planting of five floribunda roses and one species of rose of differing susceptibility to blackspot. The work had two aims:

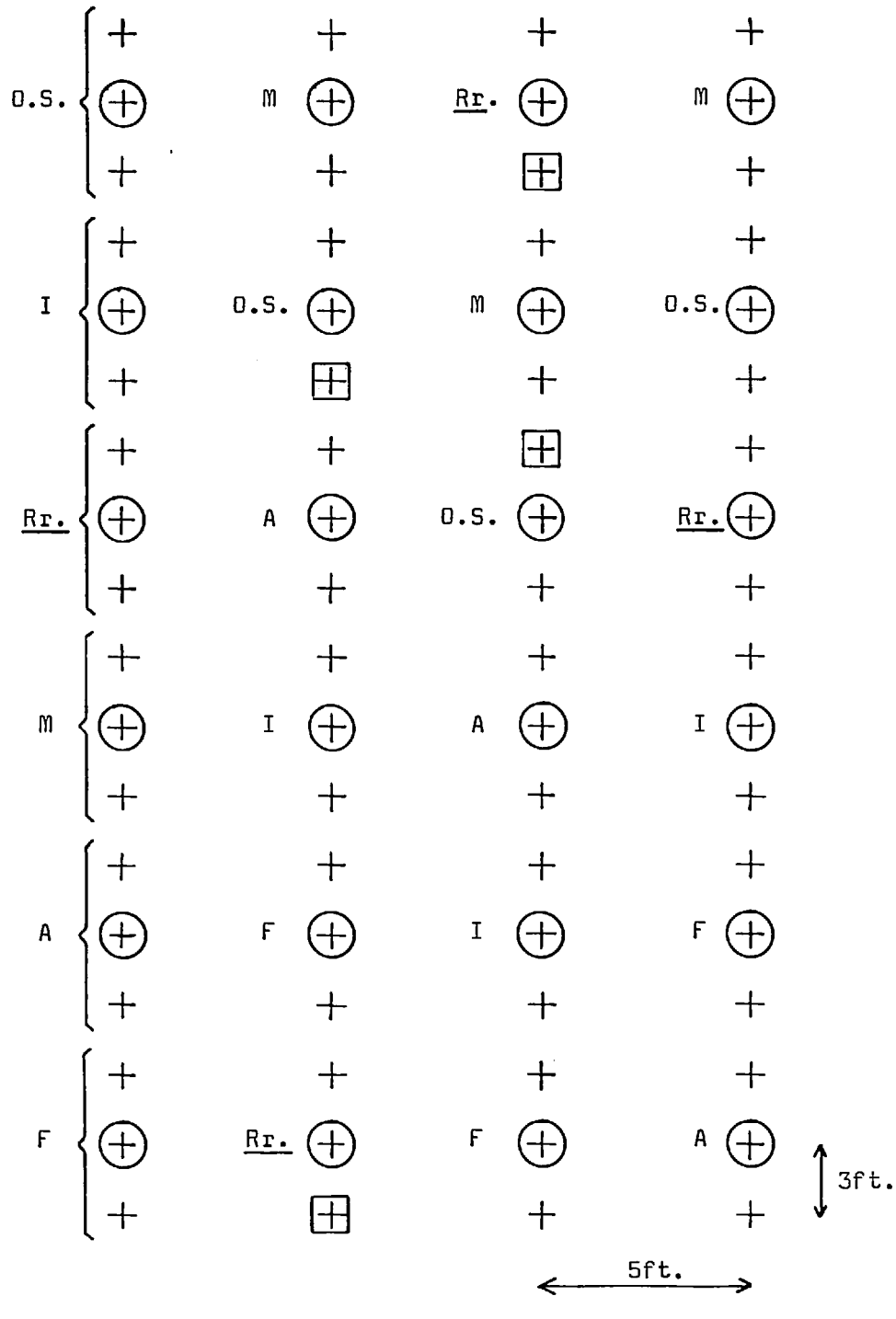
1. to relate disease development in the field to the laboratory assessments of resistance and susceptibility of these cultivars, and
2. to examine the weather conditions associated with disease development.

EXPERIMENTAL

1. General pattern of disease development

The disease development in the field and the reactions of different cultivars to *D. rosae* were examined over two seasons on a planting of roses in the Walled Garden at Silwood Park which was established during the Spring of 1973. This planting consisted of the cultivars Allgold, Frensham, Iceberg, Masquerade, Orange Sensation and the species *Rosa rugosa*. These were arranged in a randomised block design of four blocks or rows with three bushes of each type as a plot in each row as shown in Figure 2. The roses were pruned as required each spring and the planting weeded as necessary throughout the growing season. At pruning dead leaf material on the ground was also cleared as would be done in normal gardening practice. No positive attempt was made to ensure a large carry-over of inoculum from one season to the next.

FIGURE 2 FIELD TRIAL LAYOUT - Walled Garden, Silwood Park



No assessments of disease were carried out in 1973 because the rose bushes were then only newly-established and there was little disease present. Blackspot was noted only on two Frensham bushes in Rows 1 and 2. In 1974 and 1975 the disease was assessed, from 7 June and 20 June respectively, at weekly intervals where possible throughout the growing season on marked shoots of selected bushes of each plot. The total number of fully-expanded leaflets and those with visible blackspot lesions were recorded on each market shoot. These shoots were selected, at the beginning of each season, so as to range from the base to the top of the bush. Normally these were on the centre bush of each plot though, in a few instances, when this was not a suitable bush because of poor establishment another in the plot was chosen. The percentage leaflet infection per bush was calculated and a mean for the four replicates of each rose type derived.

In both seasons R. rugosa remained virtually disease-free. Some blackspot was noted on one shoot in 1974 near a bush of Orange Sensation in Row 4 but this did not develop further on this species. The cultivars could be divided into two groups as regards disease development in 1974. Figure 3 shows that the disease was present on Frensham, Iceberg and Orange Sensation from early June when assessments commenced. The disease levels remained fairly stable on these cultivars throughout June but then increased markedly throughout July. At the end of July the disease levels declined and this was associated with premature abscission and the fall of infected leaflets and leaves. Subsequently to the end of the season the levels of disease fluctuated. These fluctuations were associated in turn with the production and subsequent infection of new foliage and its abscission as a result of infection.

FIGURE 3 DISEASE DEVELOPMENT ON FRENSHAM, ICEBERG AND ORANGE SENSATION - SILWOOD PARK 1974

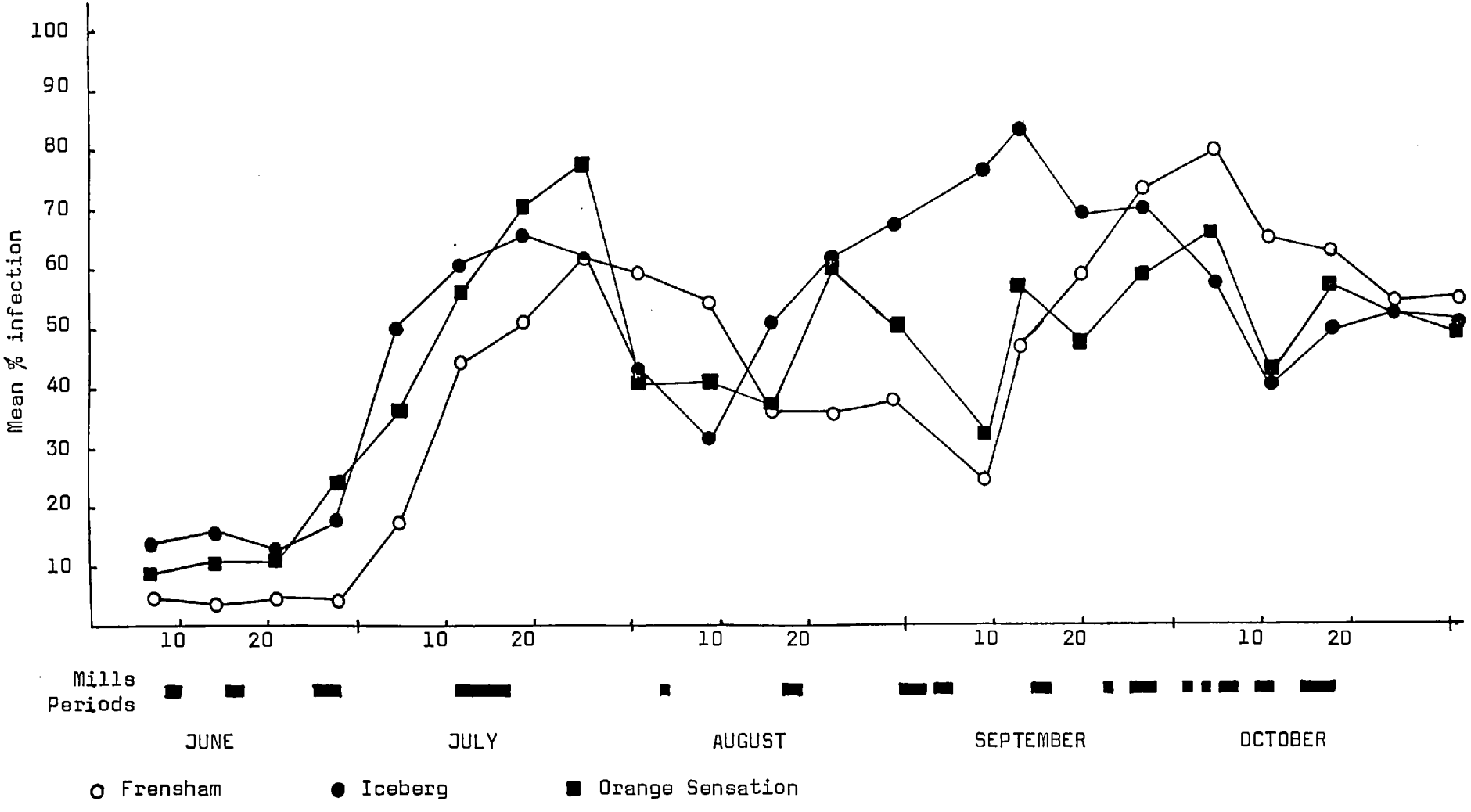


FIGURE 4 DISEASE DEVELOPMENT ON ALLGOLD AND MASQUERADE - SILWOOD PARK, 1974

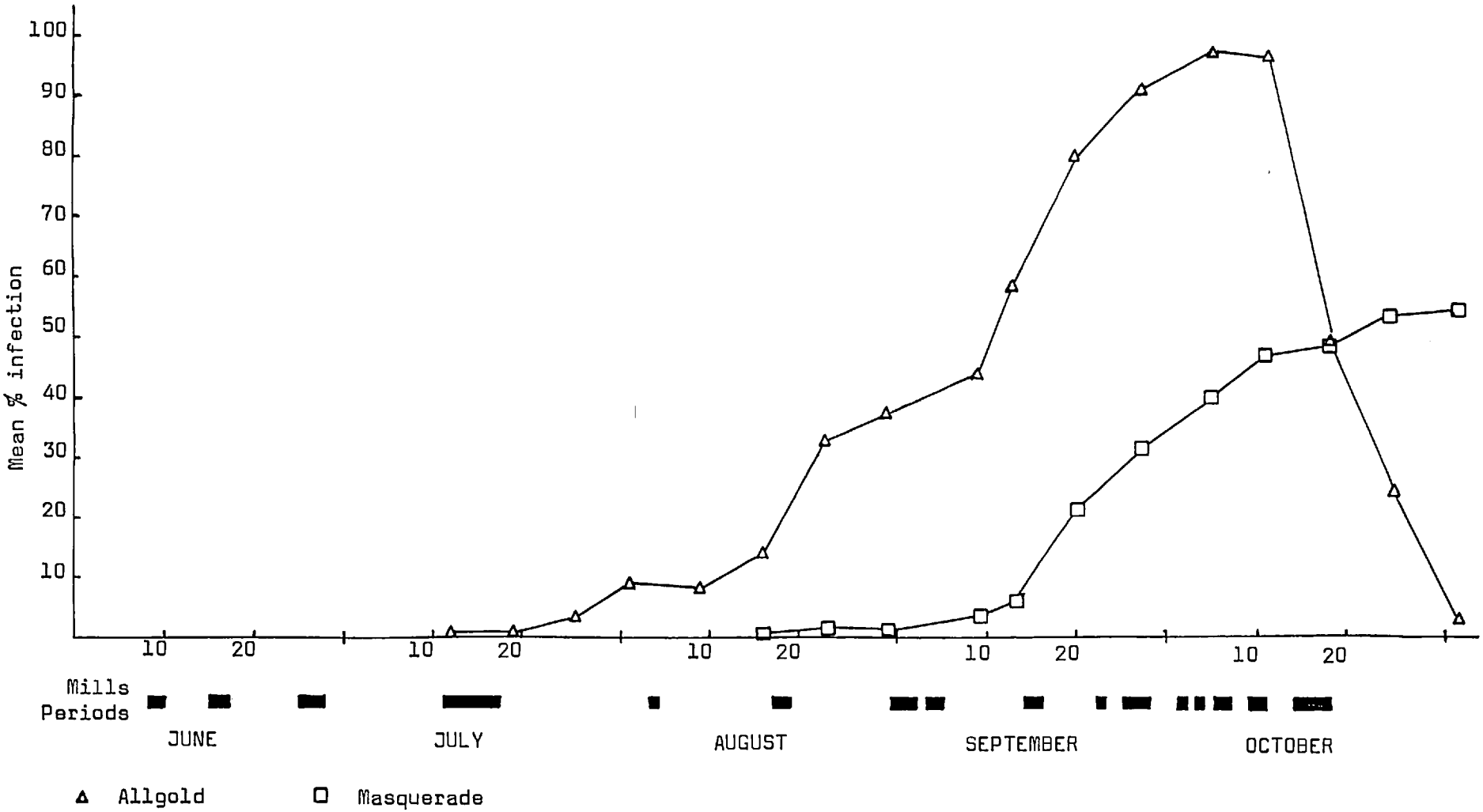


FIGURE 5 REGRESSION LINES OF INITIAL DISEASE DEVELOPMENT ON FIVE ROSE CULTIVARS - SILWOOD PARK 1974

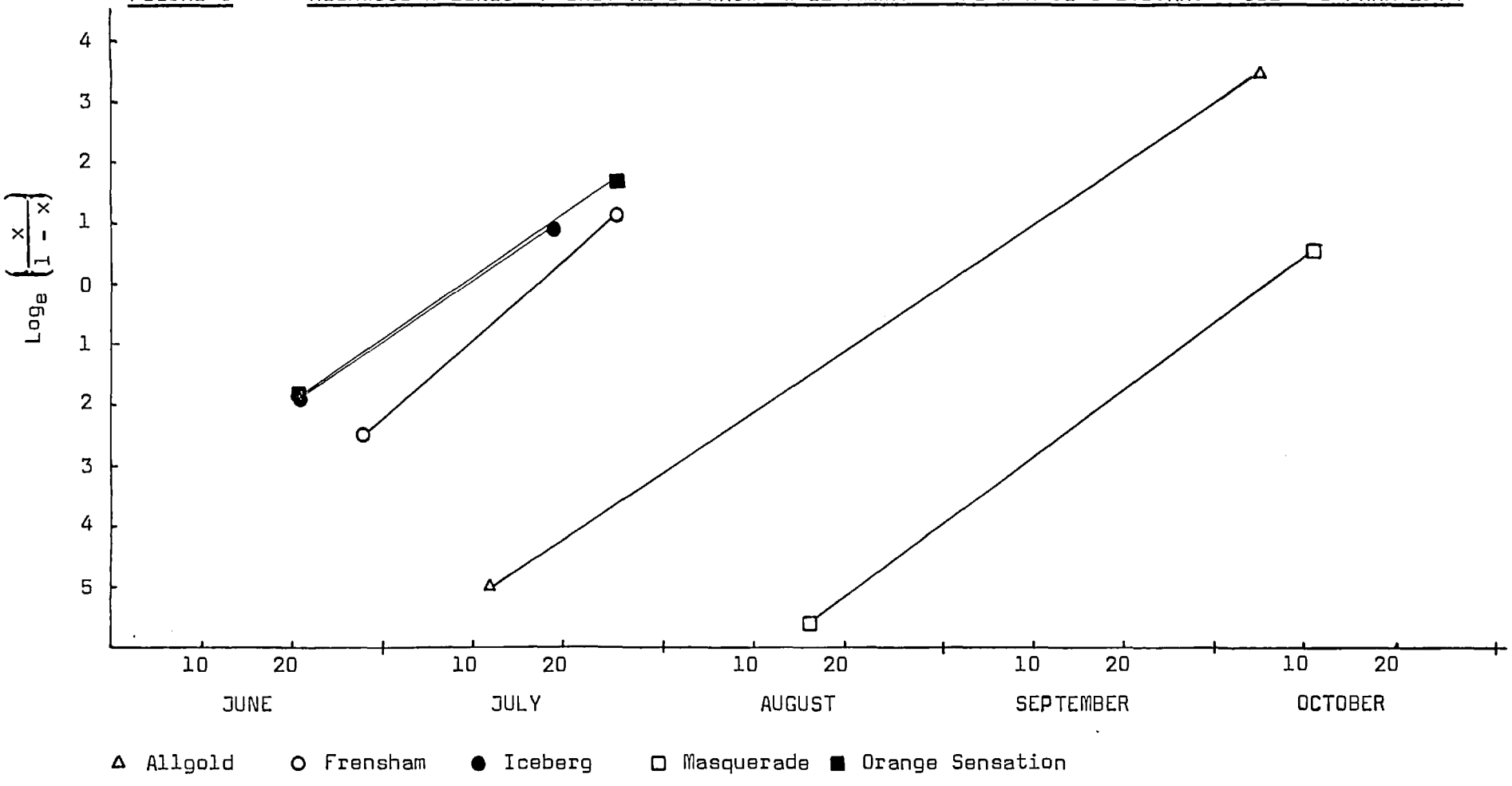


FIGURE 6 METEOROLOGICAL DATA AND MILLS INFECTION PERIODS - SILWOOD PARK 1974

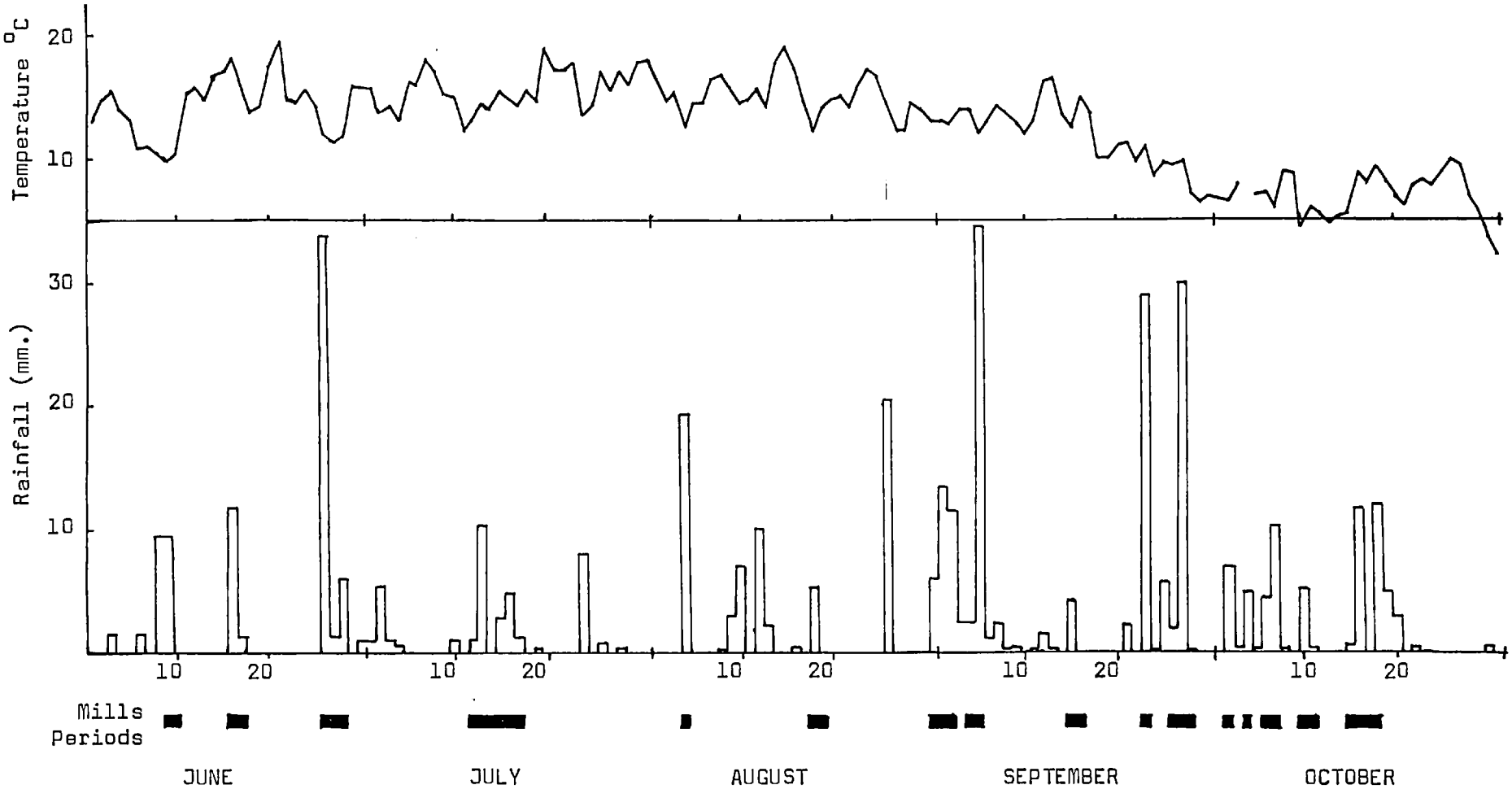


Figure 4 shows the development of blackspot on Allgold and Masquerade. In contrast to the other cultivars the disease did not appear until well into the growing season. It was first recorded on 12 July on Allgold and on 16 August on Masquerade. Despite the apparently favourable conditions for disease development on the other cultivars during July, the disease made little progress on Allgold during this period and obviously none on Masquerade. The disease eventually developed substantially on Allgold throughout August and September until, in October, there was considerable defoliation of the bushes giving an apparent marked decline in disease. By comparison the disease developed to a lesser extent on Masquerade with 50% of the leaflets infected by the end of October.

The difference between the two groups of cultivars was largely in the delay in onset of the epidemic. When the infection rates of D. rosae over the initial stages of disease development on the five cultivars were compared no substantial differences were found. This is illustrated in Figure 5. Here the figures for mean percentage leaflets infected have been converted to proportions of disease (x) and the transformation $\log_e (x/(1 - x))$ has been plotted against time, as suggested by Van der Plank (1963). The slopes of the fitted regression lines, which directly indicate infection rate (r) show no significant differences between the five cultivars. This was checked by an homogenicity test of the regression coefficients (Appendix Table 4). This implies that the differences in onset of disease results mainly from differences in initial inoculum. (Detailed data on disease development for all cultivars in 1974 are presented in Appendix Tables 2 and 3).

FIGURE 7 DISEASE DEVELOPMENT ON FRENSHAM, ICEBERG AND ORANGE SENSATION - SILWOOD PARK 1975

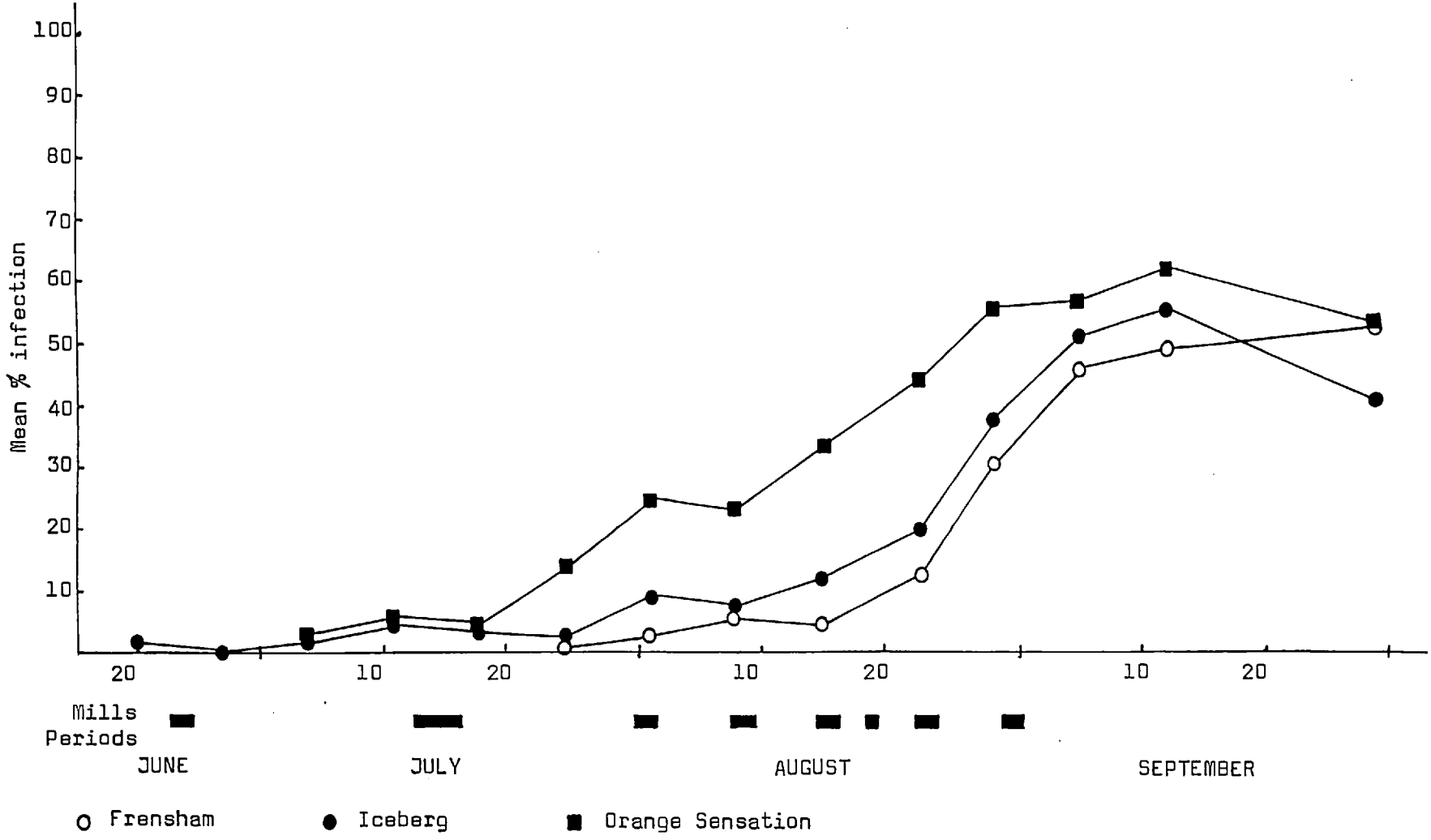


FIGURE 8 DISEASE DEVELOPMENT ON ALLGOLD AND MASQUERADE - SILWOOD PARK 1975

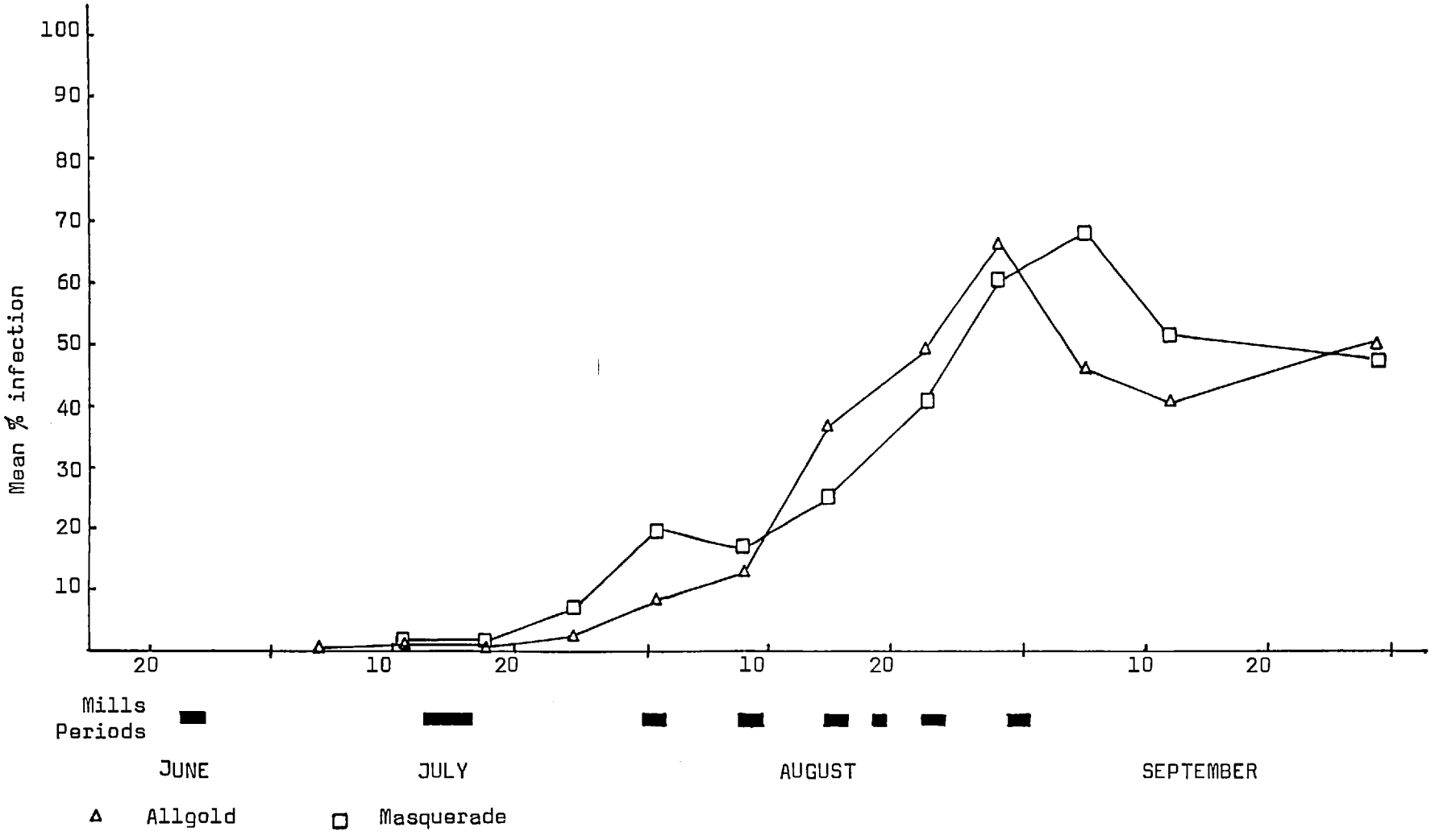


FIGURE 9 REGRESSION LINES OF INITIAL DISEASE DEVELOPMENT ON FIVE ROSE CULTIVARS - SILWOOD PARK 1975

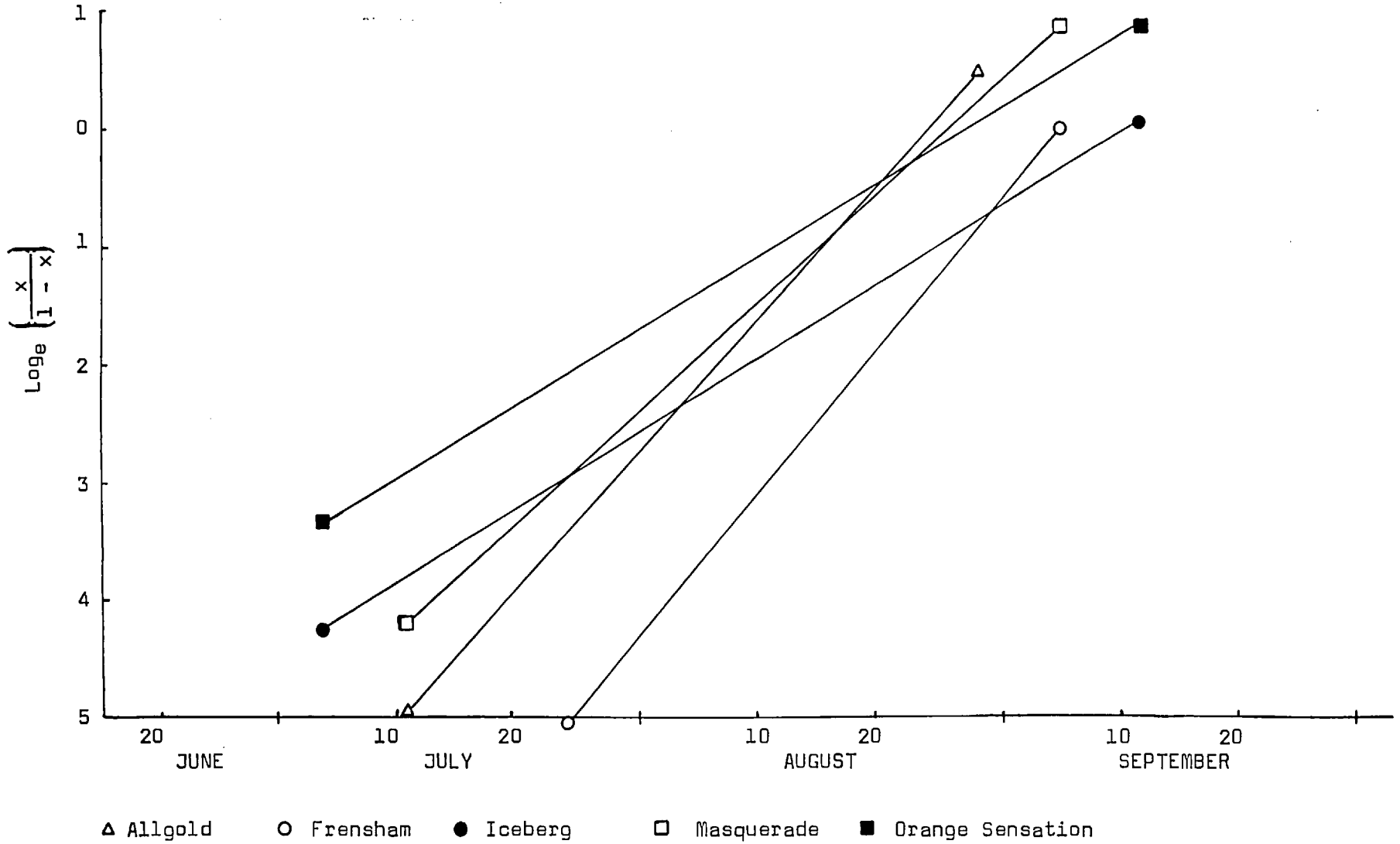
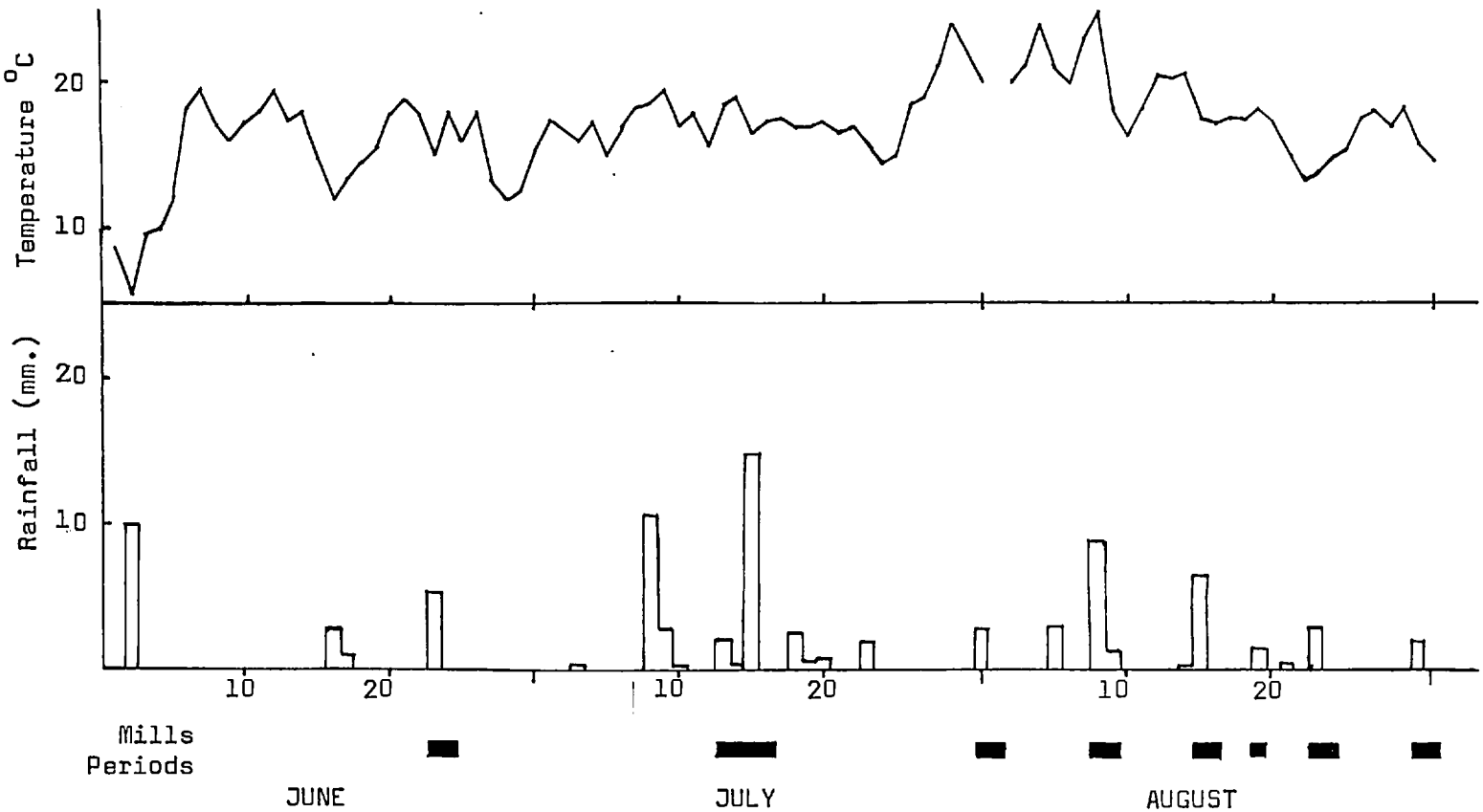


FIGURE 10 METEOROLOGICAL DATA AND MILLS INFECTION PERIODS - SILWOOD PARK 1975



In 1975 the disease situation was different, blackspot developed on all the cultivars at about the same time. The detailed results are present in Appendix Tables 5 and 6 and summarised in Figures 7, 8 and 9. Blackspot was noted on Iceberg when assessments began on 20 June. It was then first recorded on Allgold and Orange Sensation on 14 July, on Masquerade on 21 July and lastly on Frensham on 25 July. After an initial lag phase the disease increased fairly steadily on all the cultivars from the end of July to early September when the disease levels varied due to premature leaf fall and fresh leaf production.

An examination of infection rates of D. rosae on the five cultivars during the initial stages of the disease, using the same methods as with the 1974 results, now showed some significant differences between cultivars. In general the cultivars could be divided into three groups; one group (Allgold and Frensham) supporting a higher infection rate of D. rosae than the other (Iceberg and Orange Sensation) with Masquerade apparently intermediate between the two. This was determined by the use of t-tests comparing two regression coefficients (Appendix Table 7).

2. Disease development and the weather

Temperature, rainfall and relative humidity data, recorded at Silwood Park Main Meteorological Site (latitude 51° 28'N), c.200m. from the rose planting, were collected for the periods covering the assessment of blackspot in 1974 and 1975. Information on leaf surface wetness was derived from these data by adding the number of hours with rain to the hours following rain with 90%

relative humidity, allowing for short breaks in either of these (Preece and Smith, 1961; Preece, 1971). The combined data for 1974 and 1975 are presented in Figures 6 and 10 respectively and in detail in Appendix Tables 8 and 9 .

Generally 1974 was wetter (though cooler) than 1975 as indicated by the number of days with rain and a comparison of the amounts recorded. This might have accounted for the higher levels recorded in the early stages of disease development in 1974. It was apparent that increases in disease were associated with wet periods in both years so this was analysed further in two different ways.

The first approach was to determine, using the available meteorological data, the occurrence of Mills periods throughout each season, based on the following table:

Mean temperature over period (^{o}C)	5.6	7.2	10	11.7	15	15.6
Hours of leaf wetness	30	20	14	11	10	9.5

These periods are used as the basis for forecasting apple scab outbreaks and their use in this investigation was suggested by the similarity between the apple scab fungus, Venturia inaequalis, and Diplocarpon rosae both with regard to the need for water for dispersal and the way in which the respective host is first colonised. The occurrence of such infection periods was found to be closely associated with the initiation of blackspot on a particular cultivar in both years and also with disease increases. It was a striking feature that if such a period did not occur for some time then the level of blackspot declined as the lesions grew

from existing infections and caused the leaves to fall. New infections, resulting in a renewed increase in the disease only followed a further Mills period (Appendix Tables 8 and 9).

The second approach was to relate the rate of disease increase to the preceding weather. For this purpose, infection rates of individual cultivars were calculated as described by Van der Plank (1963) for the periods between successive assessments of disease particularly during the initial stages of disease development. These infection rates were then compared by simple regression analysis with total rainfall and the number of hours with rain over 7, 10, 14 and 7 - 14 day periods preceding the disease increases (Appendix Table 10). No simple, meaningful relationships could be detected between infection rates and these parameters and lack of time precluded further analysis by more sophisticated regression techniques.

II DEVELOPMENT OF *DIPLOCARPON ROSAE* ON DETACHED ROSE LEAVES

A THE PRODUCTION OF INOCULUM OF D. ROSAE

INTRODUCTION - REVIEW OF LITERATURE

Much of the phytopathological research on D. rosae requires viable conidia, and because these cannot always be obtained from natural infections in the field it is necessary to devise other ways of producing them. One method would be to inoculate rose bushes in the greenhouse and to use conidia from the lesions produced on these plants. This, however, is laborious and requires a lot of space to accommodate the plants, so it is not surprising that this method has been little used. The alternative, and the one preferred by most workers on this disease, is to grow the fungus on agar media and to obtain conidia from these. This method also has its limitations. The fungus grows slowly in culture, it has variously been reported to take six weeks (Shirakawa, 1955) and between one to two months (Frick, 1943) to achieve maximum growth. It appears also that while vegetative growth is good on several media e.g. Bean agar (Wolf, 1912), Malt agar (Frick, 1943), Yeast-Malt agar (Shirakawa, 1955), Pea agar (Jenkins, 1955; Palmer et al., 1966b) and Potato Dextrose agar (Saunders, 1967), sporulation is somewhat erratic. However, Shirakawa (1955) reported that spores could be obtained relatively quickly, in c. seven days, by spreading a dense conidial suspension over Yeast-Malt agar. Conidia produced in culture are less uniform than those produced on the plant, contain larger oil drops and appear to have thinner walls (Frick, 1943).

Loss of pathogenicity by isolates in culture is a more serious problem. Frick (1943) reported that her isolates lost their ability to infect roses within seven months and that conidia from old

cultures frequently showed abnormal germination. A similar decline in virulence of conidia was also reported by Lyle (1938) and also Jenkins (1954) who maintained single spore isolates in pure culture and used conidia derived from these to inoculate detached leaflets. This problem has been further discussed by Stewart and Semeniuk (1959). They point out that while cultures may still produce viable spores, these either do not germinate on rose leaves or the germ-tubes do not penetrate the leaves. They suggest that to maintain virulent inoculum, conidia from cultures should at intervals be used to inoculate roses and then re-isolate from the resulting infections. Suitable pathogenicity tests of the new isolates should be conducted on a standard range of rose cultivars.

Keeping cultures at low temperatures apparently has only limited use in maintaining pathogenicity. Palmer et al. (1966b) demonstrated that the virulence of conidia in pure culture, stored at 1 - 2°C, declined almost completely within a year, the use of such isolates thus being limited to the inoculation of a single generation of rose seedlings.

This section describes experiments, the aims of which were to find better methods of maintaining D. rosae and ensuring a ready supply of conidia.

1. Growth of D. rosae in axenic culture

Cultures of D. rosae were prepared as follows. Leaves bearing many acervuli were collected from field plots, these were washed and incubated in a damp chamber at 20°C for 24h. The new conidia

which developed during this incubation were removed by washing the leaves in sterile distilled water, and then drops of the resulting spore suspension were streaked over the surface of 2% water agar in a Petri dish. After 24h. at 20°C small plugs of agar each containing a single germinating conidium, were removed from the plate using a dummy objective and transferred to the various media under test. The main disadvantage of this method was that bacteria were invariably present following transfer. Attempts to suppress these involved streaking the conidia suspension out on acidified water agar or transferring conidia to nutrient medium plus streptomycin, but these were not successful because the conidia germinated and grew either very poorly or not at all. This problem of bacterial contamination was overcome by careful techniques, selecting the least apparently contaminated conidia for transfer and thus minimising the bacterial contamination so the fungus could outgrow it.

Using these techniques cultures were prepared and growth on the following media examined: Yeast-malt agar, Malt agar, V8 juice agar, Potato dextrose agar, Czapek-Dox medium and the latter plus the vitamins Thiamin hydrochloride and Mesonositol (Saunders, 1967). The most satisfactory media were Yeast-malt agar (YMA) and Potato dextrose agar (PDA). D. rosae grew well but slowly on both media, and YMA was particularly suitable for sporulation. Abundant conidia were obtained in 7 - 14 days on YMA slopes inoculated evenly with a dense conidia suspension.

D. rosae was also successfully cultured on cylinders of filter paper standing in 10% malt extract. This method was basically that described by Kirkham (1956) for culturing Venturia inaequalis.

The similarity of the growth habit of D. rosae in rose leaves to that of V. inaequalis on apple suggested that their cultural requirements might also be similar. Filter paper (Whatman 541) was cut into rectangles, 5 x 7 cm. which were rolled into cylinders. Each cylinder was held in place centrally by a glass ring (Microscope slide glass rings, 15mm. external diameter and 3mm. in depth), and placed singly into a screw-capped Universal container. This was sterilized at 120°C for 15 minutes, and subsequently 2 ml. of sterile 10% malt extract was added aseptically to each container and the filter paper cylinder was inoculated with a conidial suspension of D. rosae just sufficient to wet it (c. 0.5ml.). The tube was rotated to ensure an even distribution of medium and inoculum and then incubated in an upright position at 25°C. The fungus grew and sporulated on the filter paper cylinders in about 7 to 14 days. Conidial suspensions were obtained by removing the cylinders, placing them in sterile distilled water and shaking gently. This method produced many viable and infective conidia.

2. Maintenance of D. rosae on infected leaf material

Two experiments were designed to test the possibility of keeping D. rosae for long periods on infected rose leaf tissue.

In the first experiment, leaves of the cultivar Frensham with blackspot lesions were collected from the planting at Silwood Park on 24 October 1972. These leaves were washed and incubated in a damp chamber for 24h. at 20°C to induce sporulation and then discs (1 cm^2) were cut from these using a leaf punch. These

discs which bore many acervuli were put into polyethylene bags and these were placed in a deep freeze (-15°C). Samples, each of ten discs, were taken from the bags after 1, 2, 3 and 4 weeks and then once monthly. The discs were allowed to thaw out and the conidia washed from them with sterile distilled water. The resulting suspension was adjusted to 200,000 conidia/ml., drops of this suspension were streaked out on water agar and germination of the conidia assessed after 24h. at 20°C as described on Page 12. As Table 11 shows, conidia maintained on frozen leaf discs in this way retained their viability and germinated well for at least one year.

In the second experiment leaflets of Frensham with abundant D. rosae lesions were collected, and divided into two batches of 100 leaflets. One batch (A) of leaflets was incubated in a damp chamber for 24h. at 20°C, then allowed to dry at room temperature before placing them in a Terylene-net bag (30 x 27cm.). The other batch of leaflets (B) was dried at room temperature immediately after collecting from the field, and similarly was placed in a Terylene-net bag. Both bags of leaflets were stored at 5°C and samples, each of five leaflets per bag, were taken after 2 and 4 weeks and then once a month. Leaflets from sample A were placed in a damp chamber for a period just sufficient for them to absorb moisture. The conidia were then washed off and their germination on water agar assessed as before. Leaflets from sample B were first incubated in a damp chamber for 24h. at 20°C. They were then examined for acervuli and if they were present, conidia were washed off and their germination assessed. The results of these tests are summarized in Table 12. Conidia already present before the leaves were stored, remained viable and germinated well

TABLE 11

GERMINATION OF D. ROSAE CONIDIA TAKEN FROM INFECTED LEAF DISCS (CULTIVAR FRENSHAM) STORED AT -15°C

Weeks at -15°C	Mean % germination+
0	96.7
1	86.2
2	75.2
3	88.6
4	84.4
8	74.0
12	87.4
16	69.6
20	74.6
24	74.3
28	86.4
32	78.1
36	71.0
48	72.5

+ Mean of ten replicates.

TABLE 12

GERMINATION OF D. ROSAE CONIDIA FROM INFECTED LEAFLETS (CULTIVAR FRENSHAM) KEPT AT 5°C

Weeks at 5°C	Mean % germination*	
	Sample A+	Sample B
2	93.3	83.7
4	85.8	81.2
8	87.8	78.9
12	90.5	53.6
16	89.6	76.0
20	88.4	73.6
24	79.7	61.7
28	78.0	50.0
32	73.2	62.9
36	88.1	48.1

+ For details, see text.

A - leaves incubated in damp chamber before storage, allowed only to absorb moisture after storage.

B - leaves dried immediately after collection, incubated in a damp chamber after storage.

* Mean of ten replicates.

throughout the 36 weeks of storage during which tests were conducted. Conidia produced on leaves following storage were somewhat variable in their germination and relatively few conidia were produced by the acervuli in these instances, particularly after prolonged storage.

The ability of conidia obtained in both of the above experiments to infect rose leaves (cultivar Frensham) was also tested. Initially this was just recorded as presence or absence of lesions in inoculated leaf material, but from 20 weeks conidial suspensions on which germination was assessed were also used to inoculate leaf discs in a standard test as outlined on page 76. Each conidia suspension was tested on fifty leaf discs, floated on water in small polystyrene boxes (ten discs per box) and Lesion diameter was measured after fourteen days at 20°C. Table 13 shows the results of such tests conducted after storage of the material for 20 weeks. Conidia taken from leaf material stored at -15°C or 5°C were viable and capable of infecting Frensham. There were no further tests on the material stored at 5°C but a final test on 14 June 1973, using leaf discs stored in the deep freeze showed that the conidia on them were still both viable and infective, i.e. after c. 8 months in these conditions. In the course of further experiments, to be reported later (page 79), conidia derived from leaf discs kept for 15 months at -15°C were also found to be viable and able to initiate blackspot lesions.

The results of these experiments indicated that D. rosae could be maintained satisfactorily as conidia on infected leaf material, so this method was generally adopted to provide inoculum. The standard method was as follows: leaves with many acervuli were collected from the field, usually in October of each year. These

TABLE 13

INFECTIVITY OF D. ROSAE CONIDIA TAKEN FROM INFECTED FRENSHAM MATERIAL STORED FOR 20 WEEKS

Storage conditions and temperature		Mean* lesion diameter (mm.)	% leaf disc infected
Deep freeze: -15°C		5.9	77
Incubator: 5°C	A+	4.9	72
	B	5.4	78

+ For details, see text.

A - leaves incubated in a damp chamber before storage, allowed only to absorb moisture after storage.

B - leaves dried immediately after collection, incubated in a damp chamber after storage.

* means of 50 leaf discs (10 discs/box)

leaves were washed and then incubated in a damp chamber for 24h. at 20°C to allow a fresh crop of conidia to develop in the acervuli and thus provide inoculum of similar age. Either leaflets from these leaves or discs ($1cm^2$) cut from them, were placed in polyethylene bags which were stored in the deep freeze. Conidial suspensions were prepared from this stored material by washing either ten leaf discs or two leaflets in water and adjusting the number of conidia to the required concentration.

Infected leaves for storage were collected from three localities, Silwood Park-Ashurst (SP-A), Silwood Park-Walled Garden (SP-WG) and Betchworth (Bet), from different cultivars in the three years 1972 to 1974 as in Table 14. Cultures of D. rosae, derived from single conidia were also kept on slopes of Yeast-malt agar and on filter paper cylinders (page 52), but these were not used in inoculation experiments.

TABLE 14

ISOLATES OF D. ROSAE USED IN EXPERIMENTS

Year and date of collection	Date of storage at -15°C	Location+	Cultivar	Code
1972 24 October	25 October	S.P. - Ashurst	Frensham	F - 72 - Ash
1973 11 October	13 October	S.P. - Ashurst	Frensham	F - 73 - Ash
23 October	24 October			
6 October	9 October	Betchworth	Allgold	A - 73 - Bet
1974 31 October	4 November	S.P. - Ashurst	Frensham	F - 74 - Ash
1 October	2 October	S.P. - W.G.	Allgold	A - 74 - WG
2 October	3 October	S.P. - W.G.	Frensham	F - 74 - WG
2 October	3 October	S.P. - W.G.	Iceberg	I - 74 - WG
1 October	2 October	S.P. - W.G.	Masquerade	M - 74 - WG
2 October	3 October	S.P. - W.G.	Orange Sensation	O.S. - 74 - WG

+ S.P - Silwood Park

W.G. - Walled Garden

B. DEVELOPMENT OF A STANDARD LABORATORY TEST

INTRODUCTION

The overall aim of this part of the work was to develop a simple, standard laboratory test to evaluate the resistance of rose cultivars to *D. rosae*. Some indications of how this might be done were obtained from published papers, so a brief review of this literature is appropriate.

In the earliest-reported investigations, there were few attempts at standardisation. Leaves on plants were inoculated with conidial suspensions and the plants were then kept in a humid atmosphere. This was achieved usually by keeping them for two or three days in a closed chamber such as a bell-jar (Wolf, 1912), an 'iceless' refrigerator (Dodge, 1931, Aronescu, 1934) or a 'cubicle' (Frick, 1943). Jenkins (1955) was amongst the first to standardise the conditions of a test. He inoculated detached leaflets (from roses grown in the greenhouse) each with a drop of a suspension containing 200,000 - 250,000 conidia/ml. These leaflets were kept on thin cotton mats moistened with 2% sucrose solution in closed culture dishes for eight days at 24^{o}C. A similar technique was used by Palmer and Semeniuk (1960, 1961). They used leaflets taken from the first fully-expanded leaf on shoots of greenhouse grown plants. These leaflets were placed on sterile, absorbent cotton pads, soaked in 2% sucrose, in Petri dishes and they were inoculated with a drop of suspension containing 250,000 conidia/ml. using a 26 gauge needle. The conidia were derived from a single fungus-race/rose cultivar isolate. Contamination of the detached leaflet (with saprophytes) was reduced by removing inocula after 45h.

with sterile filter paper. Leaflets were then kept at 22.5°C (72°F) for 15 - 17 days. In a later paper (Palmer et al., 1966a) a few minor changes in the method were reported: leaflets were first washed and then blotted dry, a suspension containing 100,000 conidia/ml. was used and the inoculum drop was removed after 48h. Saunders (1970) used discs (11mm. diameter) punched from healthy leaflets of approximately the same size and age. These were washed for 1h. in running tap water and floated, adaxial-surface uppermost, on sterile deionised or distilled water in Petri dishes. They were then inoculated with a suspension containing 250,000 conidia/ml. and kept at 20°C in daylight for 12 days. Infection was assessed by measuring the diameter of lesions and/or calculating the percentage discs infected. The former gave a better separation of rose cultivars. Very little contamination or disorganisation of discs occurred and the test ensured maximum utilisation of available leaflets and space.

Certain other information from the literature is also relevant here since it concerns inoculum size and type of leaf material. There are five points of note:

1. Melching (1961) showed that the rapidity and degree of blackspot development were not noticeably altered by changes over the range 1,000 - 10,000 conidia per inoculum drop.
2. Infection is optimal, according to Aronescu (1934), in a saturated atmosphere at 24 - 26°C. Under these conditions spots appear on inoculated leaves after three days.
3. Leaves about 7 to 14 days old are most susceptible (Aronescu, 1934; Frick, 1943).

4. The disease produced ten days after inoculation on excised leaves is indistinguishable from that on attached leaves, provided that the leaves are inoculated 12h. or less after being detached (Melching, 1961).
5. The fungus infects both leaf surfaces equally well (Frick, 1943).

EXPERIMENTAL

a. Production of disease-free leaf material

Five floribunda roses of differing susceptibility to blackspot were selected for special study, this was based on data from Saunders (1970) and Palmer *et al.* (1966a). These were Allgold (extremely resistant), Frensham (very susceptible), Iceberg (resistant), Masquerade (intermediate) and Orange Sensation (susceptible). *Rosa rugosa* (very resistant) was also initially included in the tests. Initially these roses were grown, in pots, in the greenhouse but considerable difficulty was experienced with pests and diseases, particularly the mildew pathogen, *Sphaerotheca pannosa*, so the following technique was evolved. The roses were pruned hard and kept in a refrigerated cabinet at $5^{o}C$ to arrest shoot development. Some two weeks before leaf material was required the plants were removed from the cabinet, they were sprayed to run-off with Dodemorph (2.5g/l.; BASF Limited) to kill any mildew conidia on the shoots and they were fertilised with 'Baby Bio' (Pan Britannica Limited) at the prescribed rate. The pots were then transferred to a greenhouse

and placed on a sandbench with automatic watering within a filtered-air cabinet of the type described by Finney (1973). The greenhouse was illuminated for 16h. per day by thirty 80 watt daylight fluorescent tubes (Atlas) suspended above two filtered air cabinets. Plants so treated produced abundant shoot growth within two weeks and leaves from these shoots were used in the tests described that follow. Shoot growth ceased when flowers were produced and the plants were then removed from the cabinets and placed outside. After about six months or longer they were pruned and used again for leaf material as detailed above.

b. Factors influencing infection of D. Rosae

Leaves produced on plants in filtered-air cabinets were used in a series of experiments to find out which factors were most likely to affect a laboratory test of resistance. In most instances, discs (1 cm^2) punched from leaves were used.

i. Leaf age

A plant of the cultivar Frensham, was selected which had produced five new shoots in the filtered air cabinet, each of them with at least five leaves. Two discs, one either side of the mid-rib, were cut from the terminal leaflet of successive leaves on each of the five shoots so that, in all, five sets of ten discs were obtained representative of leaves of varying age down the shoot. The discs were washed for 0.5 - 1h. in running tap water and placed, adaxial surface uppermost on damp blotting paper. Each disc was inoculated centrally with 0.005 ml. of conidial suspension of D. rosae delivered by an Agla micrometer syringe. The conidial suspension was obtained from infected leaf material of Frensham collected in

1972 and stored in the deep freeze as described (F - 72 - Ash). The suspension was adjusted to 200,000 conidia/ml. so that each inoculum drop contained about 1,000 conidia. The two discs from each terminal leaflet were floated on sterile distilled water in a small, polystyrene box (5.5 x 3.5 x 2.0cm.). The boxes were placed on a tray at random and incubated in an illuminated cabinet (Gallenkamp 1H-280) at 20°C. Disease was assessed by measuring lesion diameter to the nearest 0.5mm., 14 days later.

Analysis of the results (Table 15 and Appendix Table 11) indicated that generally leaves 2, 3 and 4 (which included the penultimate fully-expanded leaf) were the most susceptible. Infection was least on the youngest leaf, at the tip of the shoot and also low on leaf 5, the oldest leaf tested. In the light of these results, the penultimate fully-expanded leaf was always selected for subsequent tests.

ii. Leaf/leaf and leaflet/leaflet variation

Five (penultimate fully-expanded) leaves were taken from shoots on three Frensham plants. The leaflets on each leaf were identified as in Figure 11, and one disc was then cut from each leaflet making a total of twenty-five discs (five discs from leaflet A, five from B, and so on). These discs were inoculated with D. rosae conidia (F - 72 - Ash), incubated and assessed for infection as described in Experiment i. above. An analysis of the results (Table 16) showed that there were no significant differences in lesion size either between leaves or between leaflets of the same leaf.

TABLE 15

EFFECT OF LEAF AGE (cv. FRENSHAM) ON SUSCEPTIBILITY

TO D. ROSAE

Leaf position on shoot	1 (Tip)	2	3	4	5
Mean lesion diameter (mm.)+	2.4	3.5	5.1	5.2	2.7

+ Means of ten discs per leaf position.

Means not underscored by the same line are significantly different ($p < 0.05$), the value for leaf 5 is not different from those for leaves 1 and 2 (based on Duncan's new multiple range test).

FIGURE 11 LEAF/LEAF AND LEAFLET/LEAFLET VARIATION

Leaflet identification diagram

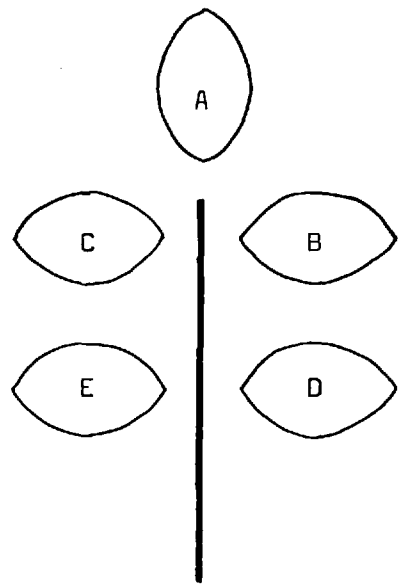


TABLE 16

EFFECT OF LEAF/LEAF AND LEAFLET/LEAFLET VARIABILITY

(CULTIVAR FRENSHAM) ON SUSCEPTIBILITY TO D. ROSAE

Leaflet position[+]	Lesion diameter (mm.) Leaf replicate number				
	1	2	3	4	5
A	6	4	6	5	5
B	5	8	6	8	8
C	6	9	8	7	4
D	5	6	8	7	7
E	6	7	7	6	8

Analysis of variance

Factor	D.F	S.S.	M.S.	F	
Leaves	4	5.84	1.46	0.84	N.S.
Leaflets	4	10.64	2.66	1.53	N.S.
Error	16		1.74		

S.E. = $\pm$ 0.59

[+] For details see text and Figure 11.

iii. Inoculum dose

Fifty discs were cut from (penultimate, fully-expanded) leaves of Frensham. Ten of these discs were inoculated with sterile distilled water and ten each with a suspension containing 10, 10^2, 10^3, or 10^4 conidia per inoculum drop. The ten discs of each treatment were distributed equally between two polystyrene boxes and these were incubated as described above. Lesion diameter was measured after 14 days.

The results (Table 17 and Appendix Table 12) showed that lesion diameter generally increased with inoculum dose. With ten conidia per inoculum drop, lesion diameter was very variable and on some discs no lesion formed. The mean sizes of lesions formed from inoculations with 10^3 and 10^4 conidia per ml. did not differ significantly. The density of hyphae and associated blackening of the host tissue increased with the inoculum dose, and on discs inoculated with 10^4 conidia the area beneath the inoculum drop became brown after 48h. The results indicated that maximum disease development could be achieved with 10^3 conidia, i.e. 200,000 conidia/ml.

iv. Inoculum drop and wettability of leaves

The five cultivars used varied in their leaf surface characteristics so that when discs were inoculated some retained the inoculum drop readily, others did not. For example, on Orange Sensation the inoculum drop remained almost spherical with little contact with the leaf surface. The possibility of using wetting agents to improve the contact between the inoculum and leaf and the effects of doing so on the lesion development, were therefore investigated in two experiments.

TABLE 17

EFFECT OF INOCULUM DOSE OF D. ROSAE CONIDIA

ON CULTIVAR FRENSHAM

Inoculum level	0	10	10^2	10^3	10^4
Mean lesion diameter (mm.)+	0	0.9	3.5	6.9	7.5

S.E. = $\pm$ 0.61

+ Means of ten discs per treatment

Means not underscored by the same line are significantly different ($P < 0.001$), based on Duncan's new multiple range test.

(a) The first experiment tested the susceptibility of four wetting agents, Tween 80 (Honeywill-Atlas Limited), Polyethylene glycol, Mannoxol (BDH Chemicals Limited) and Spreadite (Murphy Chemical Company). These were incorporated into conidial suspensions at two concentrations, 10^{-3} and 10^{-4}, as follows. A suspension of D. rosae conidia was prepared in the standard way from Frensham leaves collected at Ashurst in 1973 (F - 73 - Ash isolate). This was divided into ten portions of 0.5ml., each portion was centrifuged and the conidia then resuspended either in sterile distilled water only (control) or in sterile distilled water to which one of the wetting agents had been added to give a concentration of 10^{-3} or 10^{-4}/ml. The resulting ten suspensions were used each to inoculate five leaf discs of Orange Sensation, and each set of five discs was incubated in a polystyrene box. The degree of spreading of the inoculum drop was observed and after 14 days lesion diameter was measured.

Spread of the inoculum drop was greatest with Mannoxol (Table 18) and least with distilled water. In most instances lesion diameter was increased by the use of a wetter particularly at the lower concentrations.

(b) The second experiment investigated the effects of three concentrations of Mannoxol, 10^{-3}, 10^{-4}, and 10^{-5} on the spreading of the inoculum drop, the germination of conidia and lesion size. Mannoxol was incorporated into portions of conidial suspension (isolate F - 73 - Ash) at the appropriate concentration as described in (a). Ten discs cut from leaves of Orange Sensation were inoculated with a conidial suspension containing

TABLE 18

EFFECT OF SPREADERS ON LESION DEVELOPMENT ON ORANGE SENSATION

Wetting agent		Mean lesion diameter (mm.)	Spread of inoculum drop+
Distilled water (control)		5.8	1
Polyethylene glycol	10^{-3}	7.7	1
	10^{-4}	7.3	1
Tween 80	10^{-3}	6.0	3
	10^{-4}	7.3	2
Spreadite	10^{-3}	4.3	4
	10^{-4}	8.0	3
Mannoxol	10^{-3}	6.0	5
	10^{-4}	8.3	4

+ on arbitory scale, 1 (little) to 5 (considerable)

no Mannoxol, and further sets of ten discs were each inoculated with one of the conidial suspensions with Mannoxol. The discs were distributed, two per polystyrene box and were incubated as described previously. Drops of the five conidial suspensions were also streaked out on plates of 2% water agar. These were incubated at 20°C for 24h. and then germination of the conidia was assessed.

Germination of conidia on the inoculated leaf discs was assessed after 48h. as follows. One disc was taken from each box, the inoculum drop was allowed to dry and a small strip of Sellotape was pressed onto the leaf surface. This was then removed and mounted in cotton blue in lactophenol. An assessment of germination was based on a minimum count of 100 spores in traverses corresponding to the width of the inoculum drop. Lesion diameter on the remaining leaf discs were measured 14 days after inoculation.

The results (Table 19 and Appendix Table 13) showed that a high concentration of Mannoxol (10^{-3}) was required to increase the spread of the inoculum drop but at this concentration significantly fewer conidia germinated on the leaf and smaller lesions formed. Conidia germination in 10^{-3} Mannoxol streaked onto water agar was only slightly lower than in the control, possibly due to a dilution of the wetting agent by surface water and the agar. The lower concentrations of Mannoxol (10^{-4} and 10^{-5}) had little effect on the spread of inoculum drops, on germination on water agar or on lesion development but did reduce germination of conidia on leaves. The results suggested that little benefit was to be derived from using a wetting agent.

TABLE 19

EFFECT OF MANNOXOL ON D. ROSAE ON LEAF DISCS

OF ORANGE SENSATION

Assessment		Treatment: Distilled water (control)	Mannoxol 10^{-5}	Mannoxol 10^{-4}	Mannoxol 10^{-3}	S.E.±
Diameter of inoculum drop (mm.)		2.0	2.1	2.9	5.4	0.24
% germination	water agar	75.3	74.8	73.5	69.5	1.46
	leaf discs	18.7	11.4	11.1	5.9	1.03
Lesion diameter (mm.)		7.0	7.4	7.0	2.0	0.73

v. Maintenance of leaf discs

Leaf discs of Frensham were inoculated with a conidial suspension (200,000 conidia/ml.) of the F - 72 - Ash isolate. The techniques were similar to those described in i. and ii. above but thirty discs were incubated on damp blotting paper in a Perspex seed germination tray (Stewart Plastics Limited) and thirty discs were floated on water in polystyrene boxes, ten discs per box. All discs were incubated at 20°C in an illuminated incubator. After 24h. fifteen discs were taken from each treatment (five from each box) and the germination of conidia on them was assessed using the Sellotape technique described in iv. above. The lesion diameter was measured on the remaining leaf discs after a further 13 days. The results (Table 20 and Appendix Table 14) indicated that more conidia germinated ($P < 0.01$) and larger lesions developed ($P < 0.05$) on discs floated on water.

vi. Standard test using leaf discs

As a consequence of the above experiments the following technique was adopted as standard for tests of resistance using leaf discs. Discs, $1cm.^2$, were cut using a leaf punch from either side of the mid-rib of leaflets comprising the penultimate leaf of each shoot. In comparisons involving different cultivars all discs were cut from single plants of the cultivars under test. The discs were washed for 0.5 - 1h. in running tap water, blotted dry and placed, adaxial surface uppermost on damp blotting paper. Each disc was inoculated centrally with 0.005ml. of a conidial suspension of *D. rosae* delivered by an Agla micrometer syringe, the glass syringe of which was previously sterilised by being soaked for at least 10 minutes in 70% ethanol and dried. The conidial suspensions were obtained from infected leaf material, stored in the deep freeze

TABLE 20

GERMINATION OF D. ROSAE CONIDIA AND LESION DEVELOPMENT ON LEAF DISCS (CULTIVAR FRENSHAM) INCUBATED ON DAMP BLOTTING PAPER OR ON WATER

	Blotting paper	Water	Difference between means
+ Mean % germination after 24h.	4.8	12.0	***
+ Mean lesion diameter (mm.) after 14 days	3.1	5.5	*

+ Means of fifteen leaf discs per treatment

Significant differences between means are indicated thus:

* $P < 0.05$, *** $P < 0.001$

as described, and they were adjusted to 200,000 conidia/ml. so that each inoculum drop contained about 1,000 conidia. The inoculated discs were floated on sterile distilled water in polystyrene boxes (5.5 x 3.5 x 2.0cm.) with lids, five or ten discs per box and they were incubated at 20^{o}C in an illuminated incubator. The disease was assessed after 14 days by measuring lesion diameter to the nearest 0.5mm.

C REACTIONS OF FIVE DIFFERENT CULTIVARS TO ISOLATES OF D. ROSAE DERIVED FROM A SINGLE PLANTING OF FRENSHAM

INTRODUCTION

The aim of this part of the work was to evaluate the laboratory test on five cultivars differing in their susceptibility to *D. rosae*. Because of the reports in the literature of the variability of *D. rosae*, a single source of the fungus was chosen and tests were carried out over three years. It was hoped by doing so that any effects of host variation would become evident and their significance in relation to the interpretation of the results could thus be determined. This was considered necessary because some reports suggested that cultivars reacted differently to a single isolate at different times of the year. For example, Palmer and Semeniuk (1961) compared the reactions of fifty species and hybrid roses in a test using excised leaves and reported that more cultivars were susceptible in tests carried out in late summer and early autumn. They suggested that this was due either to changes in the physiological state of the host or to an increase in virulence of the pathogen but their experiments did not enable them to determine which of these factors was responsible. Saunders (1970) also suggested that the physiological state of the host might affect the reactions of cultivars to *D. rosae* in a leaf disc test.

EXPERIMENTAL

The reactions of the five cultivars, Allgold, Frensham, Iceberg, Masquerade and Orange Sensation to *D. rosae* were examined in the standard laboratory test at intervals from May 1973 to May 1975.

Leaf discs of Rosa rugosa were also included in the first two tests. The three isolates of D. rosae collected from the planting at Ashurst, Silwood Park were used viz: F - 72 - Ash, F - 73 - Ash, F - 74 - Ash (Table 14). In all but two tests ten discs from each type of rose were divided equally between two polystyrene boxes. In the tests on 22 June and 20 November 1974 there were fifteen discs of each rose cultivar and these were divided equally amongst five boxes. In each test all boxes were completely randomised on a tray which was then placed in an illuminated incubator at 20°C.

The results of these tests on the five cultivars are summarised in Table 21 and are given in detail in Appendix Table 15 with the results of the two tests with R. rugosa. As Table 21 indicates the cultivars Allgold and Masquerade were generally more resistant to the isolates of D. rosae than were Frensham, Orange Sensation and Iceberg. Indeed in eight of the fourteen tests no lesions developed on Allgold and Masquerade. In these instances small brown flecks were generally evident within the area occupied by the inoculum drop. Only in three tests was the distinction between these two groups of cultivars blurred. These were: on 26 February 1974 where there was no significant differences ($p < 0.05$) between cultivars, on 5 December 1973 where the mean lesion development on Masquerade did not differ significantly from that on Orange Sensation and on 6 May 1974 when there were no significant differences in the mean lesions sizes on Allgold and Orange Sensation (Table 22). There was also a contrast between the variation within these two groups. The reaction of Allgold differed significantly from that of Masquerade only in the tests of

TABLE 21

LESION DEVELOPMENT (AS MEAN DIAMETER IN MM.) ON FIVE ROSE CULTIVARS INOCULATED WITH ISOLATES OF D. ROSAE FROM FRENSHAM

Isolate	Date of test	Cultivar					S.E. ±
		Allgold	Masquerade	Frensham	Orange Sensation	Iceberg	
F-72-Ash	23 May 1973	0	0	5.4	7.8	7.5	0.27
	14 June	0	0	3.6	5.8	4.0	0.16
F-73-Ash	5 December 1973	0	1.3	8.7	3.4	9.7	0.62
	26 February 1974	4.6	5.3	6.4	7.7	7.9	1.05
	6 May	4.9	6.6	7.5	4.4	9.1	0.4
	22 June	1.3	1.1	9.6	7.2	7.6	0.56
	23 July	2.9	5.3	7.4	7.7	9.3	0.42
	28 August	1.4	1.5	7.7	6.7	9.5	0.37
	8 January 1975	0	0	7.6	3.7	9.0	0.15
F-74-Ash	20 November 1974	0	0	7.0	5.4	4.7	0.75
	8 January 1975	0	0	6.9	2.3	5.7	0.71
	5 February	0	0	5.2	6.7	8.2	0.3
	22 April	0	0	6.9	7.0	7.2	0.25
	24 May	0	0	4.1	8.5	6.8	0.34
All tests		1.1	1.5	6.7	6.0	7.6	
(Mean % discs infected)		(20.0)	(20.2)	(92.4)	(99.3)	(94.5)	

TABLE 22

ANALYSIS* OF CULTIVAR REACTIONS IN THE TESTS DETAILED IN TABLE 21

Isolate	Date of test	Cultivars (A = Allgold, M = Masquerade, F = Frensham, O.S. = Orange Sensation, I = Iceberg)
F -72-Ash	23 May 1973	A M F I O.S.
	14 June	A M F I O.S.
F -73-Ash	5 December 1973	A M O.S. F I
	26 February 1974	A M F O.S. I
	6 May	O.S. A M F I
	22 June	M A O.S. I F
	23 July	A M F O.S. I
	28 August	A M O.S. F I
	8 January 1975	A M O.S. F I
F -74-Ash	20 November 1974	A M I O.S. F
	8 January 1975	A M O.S. I F
	5 February	A M F O.S. I
	22 April	A M F O.S. I
	24 May	A M F I O.S.

* Analysis based on Duncan's new multiple range test. Cultivars not underscored by the same line differed significantly ($P < 0.05$) in respect of the growth of *D. rosae* which they supported (as measured by lesion size).

6 May and 23 July 1974. There was, however, considerable variation in the relative ratings of Frensham, Orange Sensation and Iceberg. Thus in four tests (6 May, 1974 and 8 January, 5 February, 24 May 1975) each of these three cultivars differed signficantly in their reaction to D. rosae but in two tests (26 February 1974 and 22 April 1975) there were no significant differences at all. In most of the remaining tests the reactions of one cultivar were different to those given by the other two cultivars but again not consistently so. For example, the reactions of Frensham were significantly different in two tests (23 May 1973 and 22 June 1974), those of Orange Sensation in three tests (14 June and 5 December,1973, 8 January 1975) and those of Iceberg in two tests (23 July and 28 August, 1974). In one test (20 November, 1974) Frensham and Iceberg differed from each other but not from Orange Sensation.

However, the most striking feature indicated in Table 21 is the change in the reactions of Allgold and Masquerade (and to a lesser extent in the reaction of the other cultivars) in the tests with the 1973 Frensham isolate. The development of blackspot lesions on these cultivars in the tests 26 February to 28 August 1974 was in marked contrast to the results of all other tests except that of 5 December 1973 when some lesions developed on Masquerade. The changes in susceptibility of these cultivars noted in the tests with the 1973 isolate which was stored at -15^{o}C suggests that the conditions under which the host material was grown were responsible and that the differences in susceptibility were related to differences in the physiological state of the host. Table 23 gives details of the tests with the 1973 Frensham isolate. This shows that the

TABLE 23

INFECTION OF LEAF DISCS OF ALLGOLD AND MASQUERADE WITH

ISOLATE F - 73 - Ash

Date of test	Number of discs infected (mean lesion diameter in mm. of infected discs)	
	Allgold	Masquerade
5 December 1973	0 (0)	2 (6.5)
26 February 1974	9 (5.1)	7 (7.3)
6 May	9 (5.4)	9 (8.5)
* 22 June	3 (6.7)	2 (7.5)
23 July	5 (5.8)	7 (7.3)
28 August	3 (4.5)	2 (7.3)
8 January 1975	0 (0)	0 (0)

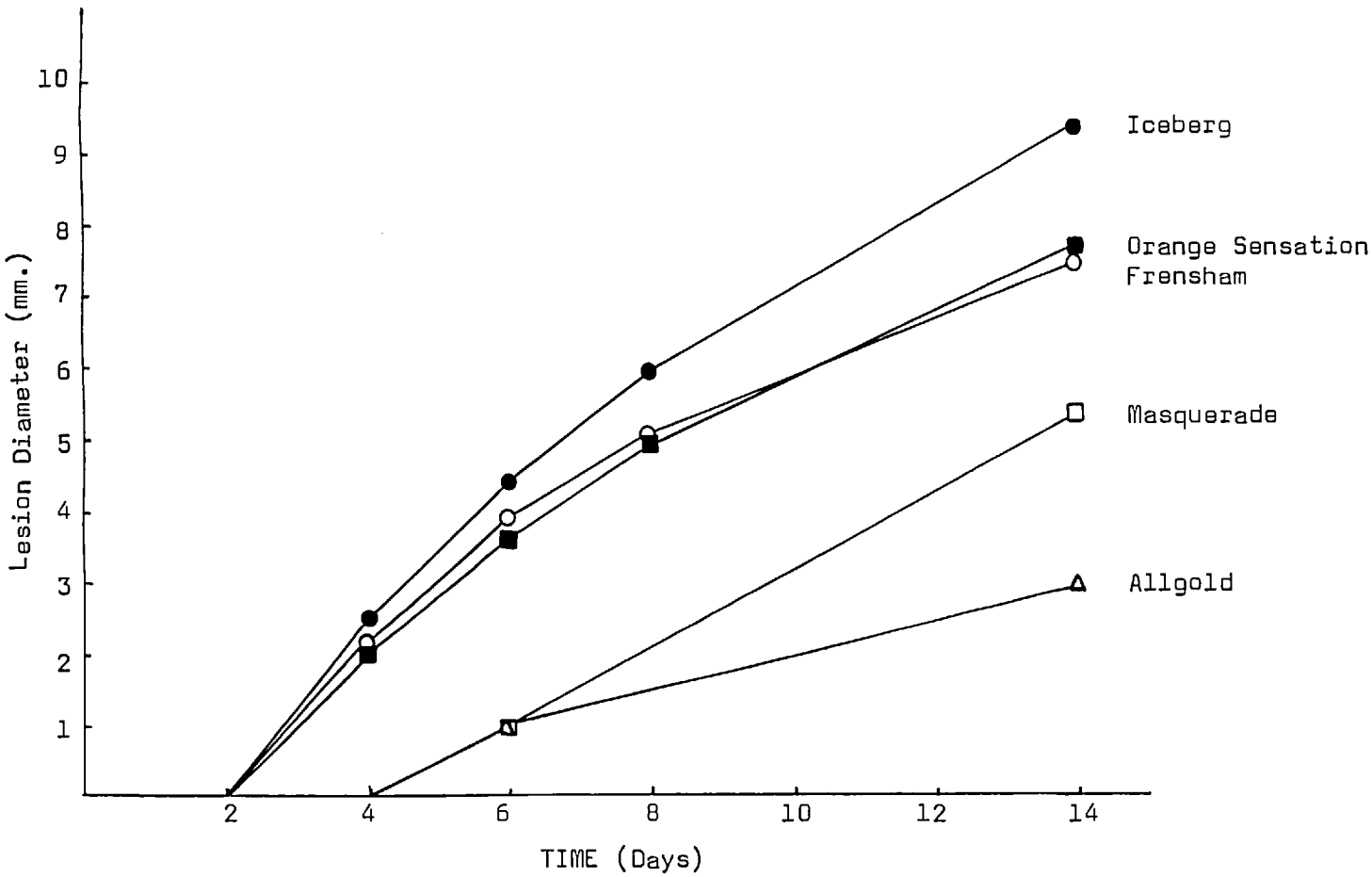
* total of fifteen discs used in this test, whereas ten discs were used in the rest.

ability of the isolate to grow in a leaf disc as measured by lesion size, increased from 5 December 1973 to 22 June 1974 and thereafter declined to zero on 8 January 1975. Initially the increased susceptibility of the two cultivars as indicated in Table 21 could also be partly attributed to the formation of a lesion on most discs that were inoculated but by the 28 August 1974 lesions developed only on a few discs. In most instances, however, if a disc was infected at all lesion development was considerable. For example, only two discs of Masquerade were infected in the test of 28 August yet the mean lesion size was 7.3 mm. In the same test only three discs of Allgold were infected butthe mean lesion size was 4.5 mm. and this was virtually the lowest recorded in any test where lesions developed.

Lesion development on the five cultivars was examined in detail in the test of 23 July 1974, that is during the period in which there appeared to be an increase in the susceptibility of Allgold and Masquerade. This indicated (Figure 12) that not only did lesions develop more slowly on Allgold and Masquerade but their development on these two cultivars was delayed and this contributed to the smaller lesion size recorded at day 14.

The two tests with R. rugosa (Appendix Table 15) indicated that this was somewhat intermediate in susceptibility. But because it was difficult to cut out discs of a reasonable size and flatness from such a narrow leaf or to inoculate them satisfactorily because they were so hairy, no further tests were carried out with this species.

FIGURE 12 LESION DEVELOPMENT ON FIVE ROSE CULTIVARS OF F - 73 - ASH ISOLATE (24 JULY 1974)



D THE VARIABILITY OF D. ROSAE ISOLATES

INTRODUCTION

The results of the previous section indicated that while there was some variation in lesion development, in general the five cultivars reacted similarly in successive tests with isolates of D. rosae derived from a single planting of Frensham. In this sense a 'Frensham isolate' of D. rosae was characterised by its behaviour on the five cultivars. However, in the literature there are several reports which indicate that isolates of D. rosae from different localities differ in their pathogenicity. Jenkins (1955), for example, tested twenty-two isolates from various localities on twenty different types of rose. He found significant differences between all the isolates in respect of their behaviour on the range of host material tested. Similar results were obtained by Palmer et al. (1966a) They showed that collections of conidia (described by the authors as 'polysporus inoculum') from seven geographic locations within the U.S.A., each produced lesions on a different and characteristic range of roses amongst the fifty types tested. In neither of these papers are the cultivars named from which isolates were derived, so there is no information on the cultivar - cultivar variation of isolates, though Jenkins (1955) did report that some isolates from the same locality differed in their pathogenicity which suggests some variation due to the cultivar of origin. Palmer et al. (1966a) in their studies also had some evidence that conidial inoculum from two different cultivars growing in the same field were of unequal virulence.

EXPERIMENTAL

a Isolate from Allgold at Betchworth

The behaviour of the *D. rosae* conidia collected at Betchworth in 1973 was examined on the standard five cultivars in four tests. The obvious feature of these conidia is that they were produced within lesions on Allgold, a cultivar resistant to the Frensham isolate. The results of the tests are given in Table 24 and Appendix Table 16. These confirmed that the isolate could infect Allgold but its ability to form a lesion on this and the other cultivars was most variable. Part of this variation could be attributed to the host material, the test of 25 June showing a similar increase in lesion size on the five cultivars as was noted for this period with the Frensham isolate (Table 21). The remainder of the tests and indeed the overall results of all tests suggest that Frensham has considerable resistance to this isolate and so does Masquerade. In general the isolate grew rather better on Orange Sensation and less well on Iceberg than on Allgold. However, differences between cultivars were not well marked with this isolate (Table 25).

b Isolates from the five cultivars in the Walled Garden at Silwood Park

The collections of conidia from Allgold, Frensham, Iceberg, Masquerade and Orange Sensation within the trial at Silwood Park (page 35) were each tested in the standard way on leaf discs of these cultivars in the autumn of 1974. Some discs within each test were used for the assessment of conidia germination, that is fifteen discs per cultivar were inoculated, five discs per box, two discs removed for germination assessment after 48h., the remainder used

TABLE 24

LESION DEVELOPMENT (AS MEAN DIAMETER IN MM.) ON FIVE ROSE CULTIVARS INOCULATED WITH AN ISOLATE OF D. ROSAE FROM ALLGOLD (A - 73 - 8et)

Date of Test	Cultivar					S.E. ±
	Allgold	Masquerade	Frensham	Orange Sensation	Iceberg	
5 December 1974	0.7	1.1	0	2.3	1.4	0.6
23 January 1974	3.9	0	0	3.5	1.4	0.8
29 January	2.8	0	0.5	0.7	0.2	0.4
25 June	2.1	4.8	1.9	6.1	5.3	0.7
All tests	2.4	1.5	0.6	3.2	2.1	
(Mean % discs infected)	(43.3)	(20.0)	(8.7)	(69.8)	(12.0)	

TABLE 25

ANALYSIS* OF CULTIVAR REACTIONS IN THE TESTS DETAILED IN TABLE 24

Date of test	Cultivars+				
5 December 1973	F	A	M	I	O.S.
23 January 1974	F	M	I	O.S.	A
29 January	M	I	F	O.S.	A
25 January	F	A	M	I	O.S.

* Analysis based on Duncan's new multiple range test. Cultivars not underscored by the same line differed significantly ($P < 0.05$) in respect of the growth of *D. rosae* (as measured by lesion size).

\+ Names of cultivars abbreviated as in Table 22.

for lesion assessment (Appendix Table 19). The conidia germination results are considered in Section E, those relating to lesion development are shown in Table 26. There are several points of interest. The isolate derived from Orange Sensation clearly differed from the others in its inability to produce lesions either on Allgold or Masquerade. In this respect it was similar to the isolates obtained from Frensham at Ashurst whose reactions are detailed in Table 21. These contrast markedly with the isolate from Frensham in the Walled Garden which appeared capable of attacking all five cultivars with similar severity. The isolate from Allgold, also, infected all cultivars and to a much greater extent than the isolate from this cultivar at Betchworth (Table 24). On Frensham it was somewhat less vigorous and only in this respect did it resemble the Betchworth isolate. Similarly, the isolates from Masquerade and Iceberg were capable of forming lesions on the five cultivars tests but it is noteworthy that they formed the largest lesions on the cultivar from which they were derived. However, on the results of these tests it is difficult to distinguish between the isolates from Frensham, Allgold, Masquerade and Iceberg and this is emphasised by the table of ranked means (Table 27).

c Effects on the virulence of isolates of passing them through their cultivar of origin or through other cultivars

The variability of isolates was examined further in three experiments. In each of these, conidia of each isolate were first transferred either to a leaf disc of the cultivar from which the isolate was derived or to a leaf disc from a different cultivar. Lesions were allowed to develop on the inoculated leaf discs and then conidia produced in these lesions were tested on the standard

TABLE 26

LESION DEVELOPMENT (AS MEAN DIAMETER IN MM.) ON FIVE ROSE CULTIVARS INOCULATED WITH ISOLATES OF D. ROSAE DERIVED FROM THEM

Isolate derived from	Date of test (1974)	Cultivars tested on					S.E. ±
		Allgold	Masquerade	Frensham	Orange Sensation	Iceberg	
Frensham	23 October	8.5	8.9	8.6	7.9	9.4	0.26
Allgold	9 November	8.1	9.2	5.2	6.2	8.9	0.25
Masquerade	16 November	6.4	9.9	6.2	7.0	9.4	0.26
Iceberg	18 November	4.5	5.0	5.6	7.7	9.1	0.43
Orange Sensation	19 November	0	0	4.2	6.7	6.6	0.12

TABLE 27

ANALYSIS* OF CULTIVARS REACTIONS IN THE TESTS DETAILED IN TABLE 26

Isolate derived from	Ranking of cultivars tested+				
Frensham	O.S.	A	F	M	I
Allgold	F	O.S.	A	I	M
Masquerade	F	A	O.S.	I	M
Iceberg	A	M	F	O.S.	I
Orange Sensation	A	M	F	I	O.S.

* Analysis based on Duncan's new multiple range test. Cultivars not underscored by the same line differed significantly ($P < 0.05$) in respect of the growth of *D. rosae* (as measured by lesion size).

\+ Names of cultivars abbreviated as in Table 22.

five cultivars.

In the first experiment, three isolates from Frensham (F - 73 - Ash, F - 74 - Ash and F - 74 - WG) were first transferred to leaf discs of Frensham and the conidia produced on these then transferred to discs of all five cultivars. The two isolates from Ashurst developed on the cultivars as they did in previous tests (Table 28), producing lesions on Frensham, Iceberg and Orange Sensation but not on Allgold. The 1973 isolate produced some lesions on Masquerade but the 1974 isolate did not. The Walled Garden isolate in this test gave similar reactions to the 1974 Ashurst isolate. This contrasted with the previous test with this isolate as recorded in Table 26. In this instance, passing the isolate through Frensham markedly affected its ability to infect Allgold and Masquerade.

In the second experiment, the five isolates from the cultivars in the Walled Garden was first transferred to leaf discs of their respective cultivars before being tested in the usual manner. The results are shown in Table 29 and Appendix Table 17. The isolate from Orange Sensation again failed to induce lesions on Allgold and Masquerade but produced lesions on the other cultivars. It thus behaved similarly to the previous test given in Table 26 as did the isolates from Allgold, Frensham and Masquerade in so far as they produced lesions on all cultivars. The isolate from Iceberg behaves quite differently in this test. It failed to induce lesions on Allgold and Masquerade, thus resembling the isolate from Orange Sensation. The results of the test with Frensham differed from that recorded above where one passage of the Walled Garden isolate through Frensham resulted in a marked change in its ability to infect

TABLE 28

DEVELOPMENT* OF FRENSHAM D. ROSAE ISOLATES ON FIVE ROSE CULTIVARS AFTER ONE TRANSFER THROUGH FRENSHAM

Isolate	Cultivar					S.E. ±
	Allgold	Masquerade	Frensham	Orange Sensation	Iceberg	
F - 73 - Ash	0	2.9	3.9	4.1	6.8	0.83
F - 74 - Ash	0	0	4.2	6.7	9.6	0.88
F - 74 - WG	0	0	4.1	6.5	9.4	0.71

* Lesion development (as mean diameter in mm.)

TABLE 29

DEVELOPMENT OF D. ROSAE ISOLATES ON FIVE ROSE CULTIVARS

AFTER ONE TRANSFER THROUGH THEIR RESPECTIVE HOSTS

Origin of isolate (Walled Garden 1974)	Grown on	Tested on					S.E. ±
		Allgold	Masquerade	Frensham	Orange Sensation	Iceberg	
(a)							
Allgold	Allgold	3.8	3.0	1.4	4.5	5.0	0.9
Frensham	Frensham	2.4	3.1	4.2	6.7	3.6	1.1
Iceberg	Iceberg	0	0	1.9	6.6	4.7	0.29
Masquerade	Masquerade	4.6	2.5	3.0	4.0	3.4	0.55
Orange Sensation	Orange Sensation	0	0	2.7	5.5	6.6	0.48
(b)							
Allgold	Allgold	50	40	20	100	70	
Frensham	Frensham	40	40	70	100	80	
Iceberg	Iceberg	0	0	50	100	90	
Masquerade	Masquerade	60	30	70	90	90	
Orange Sensation	Orange Sensation	0	0	70	100	90	

(a) Lesion development (as mean diameter in mm.)

(b) % leaf discs infected

Allgold and Masquerade. However, in this test it was less vigorous on these two cultivars, infecting few leaf discs and producing smaller lesions on those discs which it did infect.

In the third experiment the isolate from Frensham in the Walled Garden (F - 74 - WG) was first grown on leaf discs of Allgold and then the conidia produced were used to inoculate discs of the five cultivars. Similarly the isolate from Allgold in the Walled Garden (A - 74 - WG) was grown on Frensham before being evaluated in the standard test.

The Allgold isolate again produced lesions on all the cultivars (Table 30 and Appendix Table 18) but its passage through Frensham markedly reduced its virulence. Passage of the Frensham isolate through Allgold reduced its virulence so much that it produced a lesion (7mm. diameter) only on one leaf disc. It is also worth noting that in this experiment the production of conidia by each isolate was poor when grown initially on the cultivar different to its origin. This effect was removed by the normal standardisation of inocula.

TABLE 30

DEVELOPMENT* OF D. ROSAE ISOLATES ON FIVE ROSE CULTIVARS

AFTER ONE TRANSFER THROUGH ANOTHER CULTIVAR

Origin of isolate (Walled Garden 1974)	Grown on	Tested on				
		Allgold	Masquerade	Frensham	Orange Sensation	Iceberg
Allgold	Frensham	2.8	1.6	1.7	1.6	2.4
Frensham	Allgold	0	0	0.7	0	0

* Lesion development as mean diameter in mm.

E THE RESISTANCE OF ROSE CULTIVARS TO D. ROSAE

INTRODUCTION

The experiments of Jenkins (1955), Palmer and Semeniuk (1961), Palmer et al. (1966a) and Saunders (1970) conducted with detached leaves, leaflets and leaf discs, as well as the tests detailed above, indicate that rose cultivars differ in their susceptibility to D. rosae. On some cultivars e.g. Goldilocks (Palmer and Semeniuk, 1961) and Allgold (Saunders, 1970) few or no lesions develop following inoculation, though as the present work indicates this lack of reactions may be restricted to certain isolates of the fungus. The nature of the resistance in these instances has not been much studied. Green (1931) suggested that in general the resistance of some cultivars was associated with thick and tough leaves. Frick (1943) also considered that resistance was linked with the physical nature of the leaf surface. She suggested that the wax covering was important in this respect possibly because the waxy surfaces repelled water drops charged with conidia. These observations were not, however, supported by any experimental data. The only serious attempt to determine the mechanism of the resistance to blackspot has been that of Saunders (1967 and 1970). He showed that there was no apparent link between resistance and the amino acid content of cultivars nor with the amounts of phenolic compounds in the leaf either before or following infection. Further experiments involving the cultivar Allgold (resistant) and Orangeade (susceptible) showed that germination on Allgold was reduced in a manner that suggested this cultivar might produce some antifungal substance in response to the fungus, that is a phytoalexin (Cruickshank, 1963 and

Deveral, 1972). These experiments were, however, only exploratory. The behaviour of selected isolates on the five cultivars used in the tests described was examined in more detail, in an attempt to elucidate some of the factors involved in blackspot resistance.

EXPERIMENTAL

1 Conidial Germination

Conidial germination was examined first in a series of five experiments using the cultivars Allgold, Frensham, Iceberg and Orange Sensation and two isolates of *D. rosae* that from Frensham at Ashurst, collected in 1973, and that from Allgold at Betchworth. The behaviour of these isolates on the cultivars as indicated in Tables 21 and 24 may be summarised as follows:

Isolate	Cultivar			
	Allgold	Frensham	Iceberg	Orange Sensation
F - 73 - Ash	R	S	S	S
A - 73 - Bet	S	(R)	S	S

where R = virtually complete resistance (no lesion); (R) = resistance (few lesions) and S = susceptible (many, large lesions).

In each test there were twenty leaf discs of each cultivar which were divided equally amongst four polystyrene boxes. Each disc was inoculated with 1,000 conidia in a 0.005 ml. drop and the other conditions were also as those described in the standard laboratory test (Section B). Germination was assessed after 24h and 48h. On each occasion small pieces of polypropylene tape (E 1201 - Acrylate) were pressed onto the surface of ten discs.

The outline of each disc was marked on the covering tape (to facilitate scanning of the area later), this was then removed and each piece of tape was mounted in cotton-blue-lactophenol on a separate microscope slide. At least 100 conidia were counted in complete traverses of the area occupied by the inoculum drop on the leaf disc.

In each experiment the germination of one isolate was examined on two cultivars; in all but one experiment these cultivars were respectively susceptible and resistant to the isolate. The results are summarised in Table 32. There are three points of interest. The first is that, overall, germination on the leaf discs was relatively low with a maximum in one test of only 23% after 48h. Further experiments (to be reported later) indicate that this is a feature of germination on leaf discs and that similar inocula were potentially capable of some 70 - 90% germination on water agar. The second feature of note is that after 24h. germination was similar on both cultivars under test, the only exception being experiment 3 where significantly more spores germinated on Frensham than on Allgold inoculated with the Frensham isolate. The third, and most important feature, is that after 48h. the levels of germination did to a large extent reflect the differences (where present) in the resistance of the cultivars. Thus, in experiments 1, 2 and 3 significantly more conidia germinated on the susceptible cultivar (Orange Sensation, Iceberg and Frensham respectively) than on the resistant Allgold. In these instances the differences between the germination on the two cultivars were a result of further germination on the susceptible cultivar and a complete cessation of germination on the resistant cultivar with the second 24h. of the experiment. By contrast in experiment 4 where both cultivars were susceptible to the isolate

TABLE 32

CONIDIAL GERMINATION OF TWO ISOLATES OF D. ROSAE ON RESISTANT AND SUSCEPTIBLE CULTIVARS

Experiment Number	Isolate and date of test	Cultivars tested	Mean % germination 24h.		Mean % germination 48h.	
1	F - 73 - Ash	Allgold	9.8	n.s.	10.2	***
	16 Oct. 1973	Orange Sensation	13.8		23.3	
2	F - 73 - Ash	Allgold	8.2	n.s.	8.2	**
	14 Feb. 1974	Iceberg	9.6		13.6	
3	F - 73 - Ash	Allgold	9.3	**	8.8	***
	20 Feb. 1974	Frensham	12.2		16.4	
4	A - 73 - Bet	Allgold	14.1	n.s.	16.1	n.s.
	21 Jan. 1974	Orange Sensation	13.3		17.0	
5	A - 73 - Bet	Allgold	9.7	n.s.	13.2	n.s.
	26 Feb. 1974	Frensham	10.5		13.2	

Probability that means differ is indicated thus:

** $P < 0.01$ and *** $P < 0.001$

n.s. = means do not differ significantly

used there was no difference in germination. Nor was there in experiment 5, suggesting that any resistance of Frensham to the Allgold isolate does not depend on inhibition of germination. This might be expected since some lesions formed on this cultivar when it was inoculated with this isolate (Table 24). It is also noteworthy that in experiments 4 and 5 the germination of the Allgold isolate was similar on the three cultivars tested: Allgold, Frensham and Orange Sensation.

The second series of experiments on conidial germination were linked with the tests with the Walled Garden isolates of D. rosae and the five cultivars shown in Table 26 (Appendix Table 19). In each experiment ten discs of each cultivar were taken after 48h. and conidia germination was assessed on these using the stripping technique detailed above. For comparison there were three experiments in which conidia germination of the 1973 and 1974 isolates of D. rosae from Frensham at Ashurst (F - 73 - Ash and F - 74 - Ash) and the Allgold isolate from Betchworth (A - 73 - Bet) were examined on the five cultivars. The results are summarised and grouped by isolate in Table 33. In terms of germination the isolates can be divided into two groups. One group (1), comprising all the isolates from Frensham and those from Orange Sensation and Iceberg is characterised by significantly lower germination on Allgold and Masquerade than on the other cultivars, the only slight exception being that germination of the isolate from Iceberg (I - 74 - WG) was not significantly different either from that on Allgold and Masquerade or, correspondingly, from that on Orange Sensation and Frensham. The other group (2) comprises isolates which germinated equally well on all five cultivars, differing only in the level of germination attained. This group contained both isolates from Allgold and that from Masquerade.

TABLE 33

CONIDIAL GERMINATION (AS MEAN % GERMINATION AFTER 48h.)

OF EIGHT ISOLATES OF D. ROSAE ON FIVE ROSE CULTIVARS

Experiment Number	Date of test (1974)	Isolate	Cultivars					S.E. ±
			Allgold	Masquerade	Frensham	Orange Sensation	Iceberg	
1	22 June	F -73-Ash	16.4	11.7	23.2	35.6	26.9	0.76
2	20 November	F -74-Ash	13.2	14.2	21.2	31.2	23.7	0.16
3	23 October	F -74-WG	16.1	11.8	26.2	22.6	21.5	0.89
4	25 June	A -73-Bet	33.7	32.0	30.0	32.9	33.8	0.56
5	9 November	A -74-WG	10.5	9.6	10.7	10.9	9.5	0.83
6	16 November	M -74-WG	20.7	18.8	22.4	20.4	19.6	1.53
7	19 November	OS-74-WG	12.2	11.0	18.0	20.5	16.6	1.06
8	18 November	I -74-WG	13.5	13.0	18.7	21.8	16.6	1.34

TABLE 34

ANALYSIS* OF CULTIVAR REACTIONS IN THE TESTS DETAILED IN TABLE 33

Experiment Number	Isolate	Cultivars+				
1	F - 73 - Ash	M	A	F	I	O.S.
2	F - 74 - Ash	A	M	F	I	O.S.
3	F - 74 - WG	M	A	I	O.S.	F
4	A - 73 - Bet	F	M	O.S.	A	I
5	A - 74 - WG	I	M	A	F	O.S.
6	M - 74 - WG	M	I	O.S.	A	F
7	O.S. - 74 - WG	M	A	I	F	O.S.
8	I - 74 - WG	M	A	I	F	O.S.

* Analysis based on Duncan's new multiple range test (see Table 22)

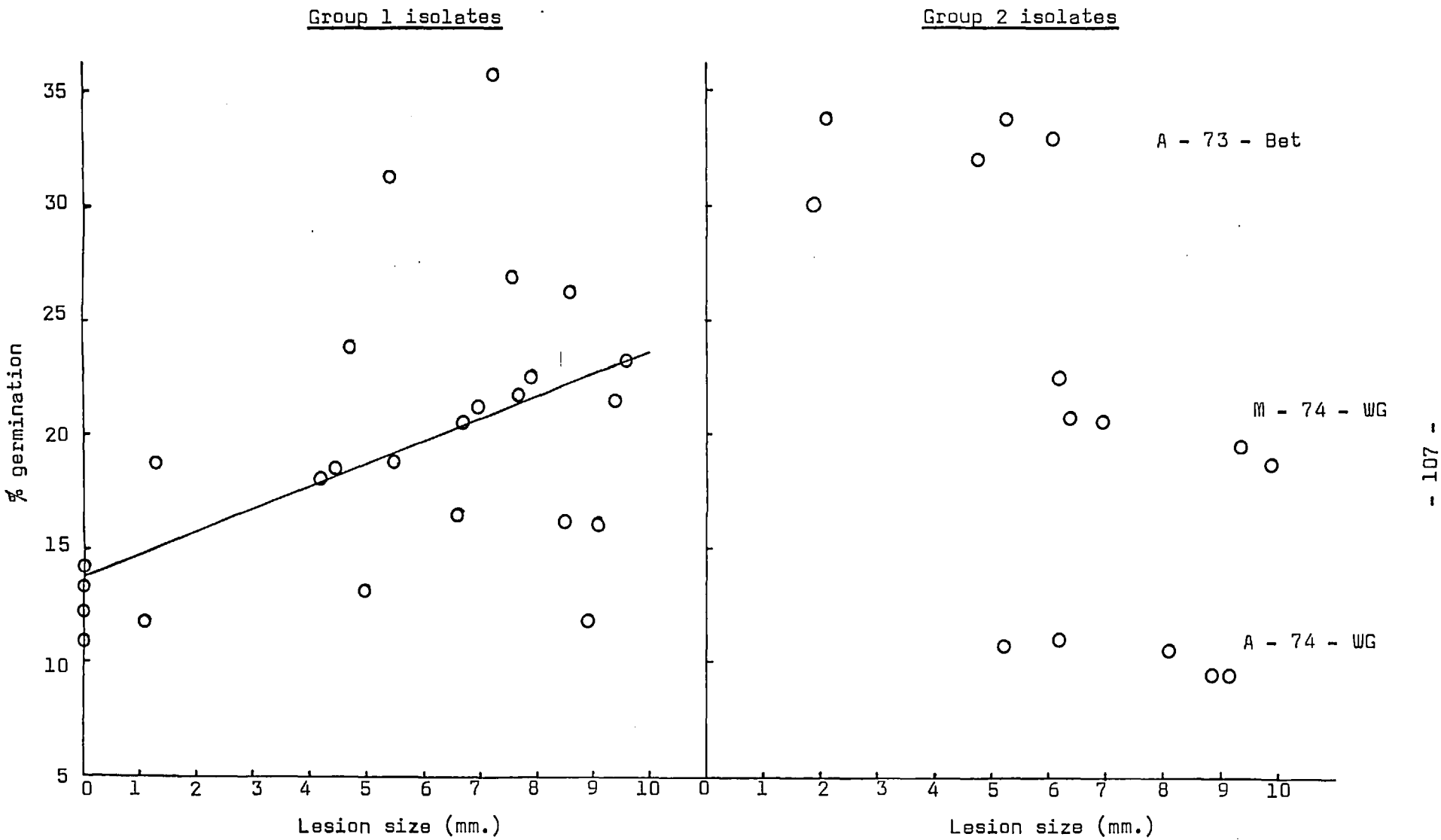
\+ Names of cultivars abbreviated as in Table 22.

Only the germination behaviour of the group 1 isolates appeared to be linked in a general way to the resistance of the cultivars as judged by lesion size. In previous tests (Tables 21 and 26) four of the isolates in this group (F - 73 - Ash, F - 74 - Ash, I - 74 - WG and O.S. - 74 - WG) either produced no lesions on Allgold and Masquerade or they produced lesions which were smaller than on the other cultivars. Only the 1974 isolate from Frensham in the Walled Garden (F - 74 - WG) differed in that it produced lesions equally well on all cultivars. The contrast between the two groups of isolates is best shown by relating, for each group, the percentage germination with the size of lesion obtained on other discs in the same experiment as detailed in Appendix Table 19 (Figure 13). With the group 1 isolates there is clearly a general relationship between the amount of germination and the size of lesion produced. The regression of percentage germination on lesion size is highly significant ($t = 2.84$, $P < 0.01$, Regression Coefficients $bo = 13.7$ and $b1 = 0.99$ where $y = bo + b1x$). With the group 2 isolates the situation is different. The level of germination is clearly a function of the isolate. What is interesting here is that the isolate which germinated best on the five cultivars (A - 73 - Bet), in fact produced the smallest lesions and the similar range of lesion size produced by the other two isolates was similarly associated with quite different germination levels. Clearly, therefore, within this group lesion size is not determined by the amount of germination but by other factors.

2 Nature of the effects on conidial germination

The results of section I above showed that with one group of *D. rosae* isolates, there was a general link between the amount of

FIGURE 13 RELATIONSHIP BETWEEN % CONIDIA GERMINATION AND RESULTING LESION SIZE OF EIGHT ISOLATES OF D. ROSAE ON FIVE ROSE CULTIVARS



conidial germination on the leaf and the subsequent size of lesion formed. The experiments reported here aimed to investigate this link in detail using a combination of isolates and cultivars which gave extreme reactions in terms of lesion development. The particular isolates chosen for this purpose were those collected on Frensham at Ashurst in 1973 and 1974. They formed no lesion (F - 74 - Ash) or few lesions (F - 73 - Ash) on Allgold and Masquerade, and also germinated less well on these cultivars. In later experiments the isolate collected in 1974 from Frensham in the Walled Garden was also used because its germination was relatively low on Allgold and Masquerade although it formed substantial lesions on these cultivars. The isolates and cultivars used and their main features are summarised in Table 35. There were, in all, eight experiments or groups of experiments.

Experiment 1

The first experiment was designed to test the hypothesis that substances passing from the leaf into the inoculum drop were responsible for the differences obtained in the germination of D. rosae conidia. Water drops were allowed to stand on leaves of Frensham and Allgold for 24h. and conidial germination of the Frensham isolate (F - 73 - Ash) was assessed in this water on glass slides.

Standard leaflets were floated on sterile distilled water in glass petri dishes. Measured drops (0.005 ml.) of sterile distilled water were then placed on these leaflets, so that as much of the leaflet surface as possible was covered. The petri dish lids were then sealed down. These were incubated for 24h. at 20°C in an illuminated incubator, after which the drops were collected using

TABLE 35

SUMMARY OF THE BEHAVIOUR OF CERTAIN ISOLATE/CULTIVAR COMBINATIONS

Isolate	Cultivar		
	Frensham	Allgold	Masquerade
F - 73 - Ash	S - 7.4 - 23.2	R - 2.2 - 16.4	R - 3.0 - 11.9
F - 74 - Ash	S - 6.0 - 21.2	R - 0 - 13.2	R - 0 - 14.2
F - 74 - WG	S - 8.6 - 26.2	S - 8.5 - 16.1	S - 8.9 - 11.8

Key: S = susceptible R = resistant

The first figure relates to mean size of lesion. Those for isolates F - 73 - Ash and F - 74 - Ash are the means for the experiments with these isolates which are recorded in Table 21; those for F - 74 - WG are the results given in Table 26.

The second figure relates to mean % germination of conidia; they are taken (for all isolates) from Table 33.

sterile pasteur pipetts and bulked for each cultivar.

0.5 ml. aliquots of a standard conidial suspension (F - 73 - Ash) were centrifuged (650 g. for 5 minutes) and the supernatant carefully poured off. An equal volume of the bulked leaf collection water, from each cultivar, was used to resuspend the conidia, to give a standard suspension in each cultivar/leaf collection. Similarly, as a control, 0.5 ml. of the standard conidial suspension was centrifuged as above and an equal volume of sterile distilled water used to resuspend the conidia.

Thirty, new glass coverslips (22mm. diameter) were secured to glass slides (two per slide) by a spot of vaseline at one edge. Drops of the test conidial suspensions (0.01 ml.) were placed in the centre of each coverslip. There were ten replicates per treatment in a completely randomized design, on the slides which were equally spread in a sealed, humid chamber. This was incubated at 20°C for 24h.

Conidial germination was assessed by removing the slides and allowing the drops to dry. The coverslips were removed and inverted on clean slides with a drop of cotton blue in lactophenol. A minimum of 100 conidia were counted in traverses through the area occupied by the drop, to allow for any variation in germination between the edge and the centre of the drop.

Conidial germination in each treatment was also assessed on 2% water agar. Drops of the suspensions were streaked over the agar surface and the plates were incubated at 20°C for 24h. A minimum of 100 conidia were counted through complete traverses of the streak.

TABLE 36

GERMINATION OF D. ROSAE CONIDIA (F - 73 - Ash) IN WATER

ALLOWED TO STAND ON LEAFLETS OF FRENSHAM AND ALLGOLD FOR 24 HOURS

	Mean % germination			S.E. ±
	Control[+]	Allgold	Frensham	
Glass coverslips	18.7	40.5	42.3	1.11
Water agar	60.6	65.6	65.5	

[+] Treatments: Control-standard conidial suspension; Allgold and Frensham - water collected from the respective cultivars.

The results are summarised in Table 36. Germination on water agar was higher than on coverslips with all three conidial suspensions but the pattern was similar in both instances, germination in the water from leaves being better than in the corresponding control. The difference was most marked with suspensions placed on coverslips. Clearly, water allowed to stand on the leaves for 24h. stimulated germination, indicating that the diffusion of substances already present in leaves into inoculum drops were unlikely to account for the differences in conidial germination on these two cultivars shown for this isolate in Table 35.

Experiment 2

This second experiment was designed to determine whether the presence of conidia in the inoculum drop could itself stimulate the production by the leaflets of substances which would reduce germination.

Leaflets of Frensham and Allgold were floated on sterile distilled water in glass petri dishes and were inoculated with drops of either sterile water or a standard conidial suspension. Two drop sizes were used: 0.005 and 0.01 ml. The smaller drops were collected and bulked to give a separate sample from each cultivar after 24h.; the larger drops were collected and bulked similarly after 48h. Samples containing conidia were centrifuged (650 g. for 5 minutes) the supernatant was carefully decanted and the conidia were discarded. As in the previous experiment, 0.5 ml. portions of each sample, plus 0.5 ml. sterile water as a control, were used to resuspend fresh conidia of the isolate (F - 73 - Ash) so that both after 24h. and 48h. five conidial suspensions were obtained in water derived as

follows:

1. Sterile distilled water (Control)
2. Water only on Frensham leaflets
3. Conidial suspension on Frensham leaflets
4. Water only on Allgold leaflets
5. Conidial suspension on Allgold leaflets.

Germination of conidia in drops of these suspensions placed on coverslips (as in experiment 1) was assessed after 24h. The results are shown in Table 37.

As in experiment 1, germination in water allowed to stand on leaflets for 24h. was significantly better than in the sterile water control. So too, was germination in water derived from the conidial suspensions on leaflets. However, it is the comparisons for each cultivar between germination in water derived from conidial suspensions on leaflets and that from water only on leaflets which is most interesting. With Frensham, there was no significant difference in the germination of conidia in these two treatments. With Allgold, germination was significantly reduced in water derived from conidial suspensions on leaves.

The reduction in germination was even more marked in the second series using water which had been allowed to stand for 48h. on leaves. Germination in water derived from conidial suspensions on Allgold was only about one-third of the corresponding 'water only' treatment. Even with Frensham there was a significant difference between these two treatments. Only germination in the 'water only' treatments of Frensham and Allgold were now better than the sterile water control. Thus from this 48h. series, it appeared that there was a stimulation of germination in water derived from leaflets but an inhibition where conidia were also present on leaves, this

TABLE 37

MEAN % GERMINATION OF D. ROSAE CONIDIA (F - 73 - Ash)

IN WATER ALLOWED TO STAND ON LEAFLETS OF ALLGOLD AND

FRENSHAM FOR 24 HOURS OR 48 HOURS WITH OR WITHOUT CONIDIA

Period on leaf	Treatment+				Sterile water control
	F	F + C	A	A + C	
24h	36.5	34.0	40.8	29.1	14.9
48h	29.0	17.3	33.6	10.3	22.6

Differences between treatments*

(data as angular transformation of % germination)

						S.E. ±
24h	C	A + C	F + C	F	A	
	23.76	32.61	35.63	37.17	39.63	0.81
48h	A + C	F + C	C	F	A	
	18.63	24.4	28.28	32.52	35.39	0.98

+ Key: F = water only on Frensham
F + C = water and conidia on Frensham
A = water only on Allgold
A + C = water and conidia on Allgold
C = sterile water control

* Analysis based on Duncan's new multiple range test. Treatments not underscored by the same line differed signficantly ($P < 0.05$)

inhibition being most marked with Allgold.

Experiment 3

This experiment had similar aims to those of Experiment 2 and was of similar design but 1974 isolate from Frensham at Ashurst (F - 74 - Ash) was used and the two cultivars Frensham and Masquerade, the latter cultivar also being resistant to this isolate. Drops of water, with or without conidia, were allowed to stand on leaflets of the two cultivars for 48h. and then the germination of fresh conidia was examined in water derived as follows:

1. Sterile distilled water (Control)
2. Water only on Frensham leaflets
3. Conidial suspensions on Frensham leaflets
4. Water only on Masquerade leaflets
5. Conidial suspension on Masquerade leaflets.

Germination of conidia in 0.02 ml. drops of these suspensions placed on coverslips was assessed after 48h. as in Experiment 1 and the following experiments.

In this experiment germination in all treatments was less than in the water control (Table 38) and there was not, therefore, the apparent stimulation by water allowed to stand on leaflets as in the previous two experiments. The presence of conidia on leaflets of Masquerade made the water inhibitory to subsequent conidial germination.

Experiment 4

In this experiment drops of water and drops of conidial suspension of the Frensham isolate from the Walled Garden (F - 74 - WG) were placed on leaflets of Frensham and Allgold. After 48h. these drops

TABLE 38

MEAN % GERMINATION OF D. ROSAE CONIDIA (F - 74 - Ash)

IN WATER ALLOWED TO STAND ON LEAFLETS OF MASQUERADE

AND FRENSHAM FOR 48 HOURS WITH OR WITHOUT CONIDIA

Treatment+				Sterile water control	S.E. ±
F	F + C	M	M + C		
49.0	45.2	45.8	34.6	53.1	1.23

Differences between treatments*

M + C	F + C	M	F	C
	———	———	———	

+ Key: F = water only on Frensham
F + C = water and conidia on Frensham
M = water only on Masquerade
M + C = water and conidia on Masquerade
C = sterile water control

* Analysis based on Duncan's new multiple range test. Treatments not underscored by the same line differed significantly ($P < 0.05$).

were recovered, the conidia were removed where necessary and the germination of fresh conidia was examined in the water samples so obtained.

The results are shown in Table 39. Germination in water allowed to stand on Allgold and Frensham was not significantly different to that in the sterile distilled water control. Water derived from conidial suspensions on the leaflets inhibited germination, that from Allgold more so than that from Frensham.

Experiment 5

This was essentially an extension of the previous experiment. Leaflets of Allgold were inoculated with conidial suspensions of isolate F - 74 - WG and after 48h. the drops were collected and the conidia were removed. The germination of two isolates (F - 74 - WG and F - 74 - Ash) was compared in this water. Conidial suspensions of these two isolates in sterile distilled water were used as controls. The results (Table 40) show that the germination of both isolates was reduced in the water derived from the inoculation of leaflets.

Experiment 6

The experiments so far described in the section suggest that when drops of conidial suspensions are placed on Allgold or Masquerade, substances which can inhibit germination diffuse from the leaflets into the drops. Such substances appear to form only in response to conidia, because little or no inhibition could be detected in water only allowed to remain on leaves for a comparable period. This experiment was designed to determine whether live

TABLE 39

MEAN % GERMINATION OF D. ROSAE CONIDIA (F - 74 - WG) IN WATER ALLOWED TO STAND ON LEAFLETS OF ALLGOLD AND FRENSHAM FOR 48 HOURS WITH OR WITHOUT CONIDIA

Treatment+				Sterile water control
F	F + C	A	A + C	
22.1	13.5	24.7	10.3	24.2

Differences between treatments*

(data as angular transformation of % germination)

					S.E. ±
A + C	F + C	F	C	A	
18.42	21.36	28.01	29.35	29.71	0.97

+ Key: F = water only on Frensham
F + C = water and conidia on Frensham
A = water only on Allgold
A + C = water and conidia on Allgold
C = sterile water control

* Analysis based on Duncan's new multiple range test. Treatments not underscored by the same line differed significantly ($P < 0.05$)

TABLE 40

MEAN % GERMINATION OF D. ROSAE CONIDIA (F - 74 - Ash AND F - 74 - WG) IN WATER ALLOWED TO STAND ON LEAFLETS OF ALLGOLD FOR 48 HOURS WITH CONIDIA (F - 74 - WG)

Treatment+			
A + C	C	A + C	C
F - 74 - Ash	F - 74 - Ash	F - 74 - WG	F - 74 - WG

Differences between treatments*

(data as angular transformation of % germination)

				S.E. ±
A + C	A + C	C	C	
F - 74 - WG	F - 74 - Ash	F - 74 - WG	F - 74 - Ash	
26.27	29.37	31.35	34.27	0.75

+ Key: C = sterile water control
A + C = water and conidia

* Analysis based on Duncan's new multiple range test.

Treatments not underscored by the same line differed significantly ($P < 0.05$)

conidia were required to induce these substances.

Leaflets of Allgold and Frensham were inoculated with water, with conidial suspensions of isolate F - 74 - Ash or with conidial suspensions of this isolate which had been autoclaved at 120°C for 20 minutes. Samples of the autoclaved conidial suspensions were streaked on water agar to ensure that all conidia were dead. The various kinds of drops were allowed to remain on the leaflets for 48h., then they were collected and conidia removed where necessary. The germination of fresh conidia of isolate F - 74 - Ash was examined in the six samples of water so obtained and compared with germination in sterile distilled water, these were as follows:

1. Sterile distilled water (control)
2. Water only on Frensham leaflets
3. Conidial suspension on Frensham leaflets
4. Killed conidial suspension on Frensham leaflets
5. Water only on Allgold leaflets
6. Conidial suspension on Allgold leaflets
7. Killed conidial suspension on Allgold leaflets.

The results (Table 41) showed clearly that on Allgold killed conidia induced the formation of substances inhibiting germination just as well as live conidia. On Frensham neither live nor dead conidia induced any response over and above that of water alone, though germination in all three samples of water was below that of the sterile water control.

Experiment 7

The previous experiment indicated that both live and dead conidia could induce germination inhibitors on Allgold. This experiment

TABLE 41

MEAN % GERMINATION OF D. ROSAE CONIDIA (F - 74 - Ash) IN WATER ALLOWED TO STAND ON LEAFLETS OF ALLGOLD AND FRENSHAM FOR 48 HOURS WITH OR WITHOUT LIVE AND KILLED CONIDIA

Treatment[+]						Sterile water control	S.E. ±
F	F + C	F + C (k)	A	A + C	A + C (k)		
46.8	45.4	46.8	51.5	33.4	30.6	51.6	1.5

Differences between treatments*

A + C (k)	A + C	F + C	F + C (k)	F	A	C
———	———	———	———	———	———	———

[+] Key:
- F = water only on Frensham
- F + C = water and conidia on Frensham
- F + C (k) = water and killed conidia on Frensham
- A = water only on Allgold
- A + C = water and conidia on Allgold
- A + C (k) = water and killed conidia on Allgold
- C = sterile water control

* Analysis based on Duncan's new multiple range test.

Treatments not underscored by the same line differed significantly ($P < 0.05$)

was designed to test the hypothesis that substances idffusing from conidia could also act as 'inducers'. The experiment was in two parts. In the first part a conidial suspension of isolate F - 74 - Ash was incubated at 20°C for 48h. This suspension was shaken gently at intervals throughout the incubation. After 48h. they were centrifuged at 650g for 5 minutes and the supernatant used to prepare a suspension of fresh conidia. Other conidia from the same batch were suspended in sterile distilled water. Drops (0.02ml) of both suspensions were incubated on clean glass coverslips at 20°C. Ten replicates of each were completely randomised. Germination of conidia was assessed after 24h. and was found to be similar in each suspension (Table 42).

In the second part of the experiment leaflets of Frensham were inoculated with drops of (i) sterile water (ii) a conidial suspension of isolate F - 74 - Ash (iii) water in which conidia of this isolate had previously been suspended as in the first part of the experiment. After 48h. the drops were collected and the conidia were removed where necessary. The presence of inhibition in the six samples of water so obtained was examined by suspending fresh conidia in them and determining germination after 24h. These were as follows:

1. Sterile distilled water (control)
2. Water only on Frensham leaflets
3. Conidial suspension on Frensham leaflets
4. 'Conidia water' on Frensham leaflets
5. Water only on Allgold leaflets
6. Conidial suspension on Allgold leaflets
7. 'Conidia water' on Allgold leaflets.

TABLE 42

GERMINATION OF D. ROSAE CONIDIA (F - 74 - Ash) IN WATER AND WATER IN WHICH CONIDIA WERE INCUBATED FOR 48 HOURS

Replicates	% germination		
	Water		'Conidia Water'
1	13.0		16.9
2	17.6		17.8
3	26.9		21.0
4	18.5		21.0
5	14.7		21.7
6	19.7		20.8
7	17.5		22.3
8	17.1		13.4
9	23.0		17.1
10	27.7		18.5
Mean	19.6	n.s.+	19.0

+ Analysis (as angular transformation of % germination) based on a t - test

n.s. = not significantly different

TABLE 43

MEAN % GERMINATION OF D. ROSAE CONIDIA (F - 74 - Ash) IN WATER ALLOWED TO STAND ON LEAFLETS OF ALLGOLD AND FRENSHAM FOR 48 HOURS WITH OR WITHOUT CONIDIA AND 'CONIDIA WATER'

Treatment+						Sterile water control	S.E. ±
F	F + C	F + C (w)	A	A + C	A + C (w)		
36.1	34.4	32.7	40.3	26.7	24.1	34.0	0.89

Differences between treatments*

A + C (w)	A + C	F + C (w)	C	F + C	F	A

+ Key:
- F = water only on Frensham
- F + C = water and conidia on Frensham
- F + C (w) = 'conidia water' on Frensham
- A = water only on Allgold
- A + C = water and conidia on Allgold
- A + C (w) = 'conidia water' on Allgold
- C = sterile water control

* Analysis based on Duncan's new multiple range test

Treatments not underscored by the same line differed significantly ($p < 0.05$)

Table 43 summarised the results. On Allgold conidial suspensions and water in which conidia had germinated apparently induced a similar level of inhibitors. On Frensham these treatments were also similar but in a completely different sense in that they did not appear to induce any inhibitors; germination in water from these sources was not significantly different from that in the sterile water control. The germination of conidia in water which had remained on Allgold for 48h. significantly stimulated germination.

Experiment 8

It could be inferred from the previous experiments that antifungal substances are produced by Allgold and Masquerade in response to certain isolates of D. rosae. This section describes four preliminary investigations into the nature of the 'inhibitors' and 'inducers'.

(a) Some observations during the previous experiments indicated that the inhibitory effect of water derived from conidial suspensions on leaflets of Allgold declined if it was stored. The following experiment was designed to examine this. Water collected from conidial suspensions of isolate F - 74 - Ash on Allgold in Experiment 6 was stored (after the removal of the conidia) for two months at $5^{o}C$. Fresh conidia of the same isolate were then prepared in this water and germination compared with that of conidia in sterile distilled water. The results (Table 44) indicated that the stored material no longer inhibited germination; indeed it now stimulated germination slightly.

The amount of bacterial contamination in the stored material was checked by streaking drops out on nutrient agar plates and incubating

TABLE 44

GERMINATION OF D. ROSAE CONIDIA (F - 74 - Ash) IN WATER ALLOWED TO STAND ON LEAFLETS OF ALLGOLD FOR 48 HOURS AFTER STORAGE AT 5°C FOR TWO MONTHS

Replicates	% germination	
	Sterile water control	Allgold conidia and water
1	29.8	37.4
2	34.0	31.2
3	29.6	34.8
4	32.5	35.8
5	29.5	37.0
6	34.0	35.4
7	36.0	33.6
8	35.6	41.0
9	26.8	33.0
10	29.7	34.7
Mean	31.7 *+	35.4

+ Analysis based on a t - test

* = $\underline{P} < 0.05$

these for 24h. at 25°C. Also drops were dried and fixed on slides and the smears stained by Gram's method. There appeared to be no more bacteria present than in water freshly derived from suspensions on leaves.

(b) Many antifungal compounds which are formed by plants in response to fungi (phytoalexins) have characteristic u.v. absorption spectra. So the absorbance of some preparations from leaves was determined on a Beckman D.B. Spectrophotometer. The material was derived from Experiment 7 and consisted of:

i. Water derived from a conidial suspension of isolate F - 74 - Ash placed on Allgold

ii. Water derived from a conidial suspension of the same isolate placed on Frensham

iii. Water in which conidia of this isolate had germinated.

Each sample was concentrated under reduced pressure in a rotary-film evaporator at 40°C and the absorbance of the original strength material and that of a x10 concentration were determined.

The results (Appendix Table 27) indicated that there was no real difference, at either concentration, between water derived from suspensions on Allgold or from suspensions on Frensham. However, these differed in magnitude to the absorbance pattern of water in which conidia had germinated suggesting that substances other than those in the 'conidia water' were contributing to the spectra.

(c) An attempt was also made to determine particular types of substances in the materials tested above, using thin-layer chromatography. In particular water in which conidia had germinated was tested, at original strength and at a x10 concentration for

amino acids and sugars and the water samples derived from conidial suspensions were tested for phenolic compounds (Saunders, 1967; Stahl, 1969; Edwards, 1970) The methods, using Silica-gell G plates, were as follows:

i. Amino acids, plates run in n-butand: glacial acetic acid: water (80:20:20, by vol.) and developed by spraying with ninhydrin in acetone.

ii. Sugars, plates run in n-propanol: water (85:15, by vol.) and developed in a silver nitrate reagent or anisaldehyde-sulphuric acid reagent.

iii. Phenolics, plates run in chloroform: Methanol (97:3, by vol.) or isobutanol: methanol: water (80:5:15, by vol.) and examined under u.v. light with and without ammonia fumes.

No positive identifications could be made in any liquid.

(d) Blakeman and Faser (1971) showed that the poor conidial germination of Botrytis cinerea on chrysanthemum leaves was due to the development of bacteria in the inoculum drop, rather than the production of an antifungal compound by the leaves in response to the fungus. The bacterial contamination of the materials from previous experiments was checked by streaking drops on nutrient agar plates and by staining fixed smears with Gram's method. There appeared to be no marked difference in the level of bacteria in water derived from conidial suspensions on the two cultivars, Frensham and Allgold or indeed in water only which had been placed on these two cultivars.

3 Penetration

The process of conidial germination and subsequent penetration by *D. rosae* was described by Aronescu (1934). In summary, after germination of the conidium either a narrow infection peg from an appressorium or the end of the germ tube penetrates the cuticle and immediately afterwards enlarges into a subcuticular hypha. An attempt was made in these investigations to examine penetration quantitatively on Frensham and Allgold using isolates which germinated to different degrees on these two cultivars. For this purpose leaf discs were inoculated with either isolate F - 73 - Ash or isolate F - 74 - Ash and these were incubated at 20°C for 24h. or 48h. Two techniques were used to examine penetration on these leaf discs.

(a) Light microscopy

Various staining methods were used before examining discs by the light microscope. None of the stains gave really satisfactory results. The best of those tried was boiling the discs in 95% ethanol followed by staining in cotton-blue-lactophenol. Aronescu's method (1934) of boiling the material in 95% ethanol for 15 minutes then leaving it in 70% ethanol to regain its softness before staining in cotton-blue-lactophenol gave good results. Other methods tried were the Periodic Acid-Schiff's staining technique (Dring, 1955), the whole leaf clearing and staining technique of Shipton and Brown (1962) and the differential staining technique of Andren and Rowell (1962).

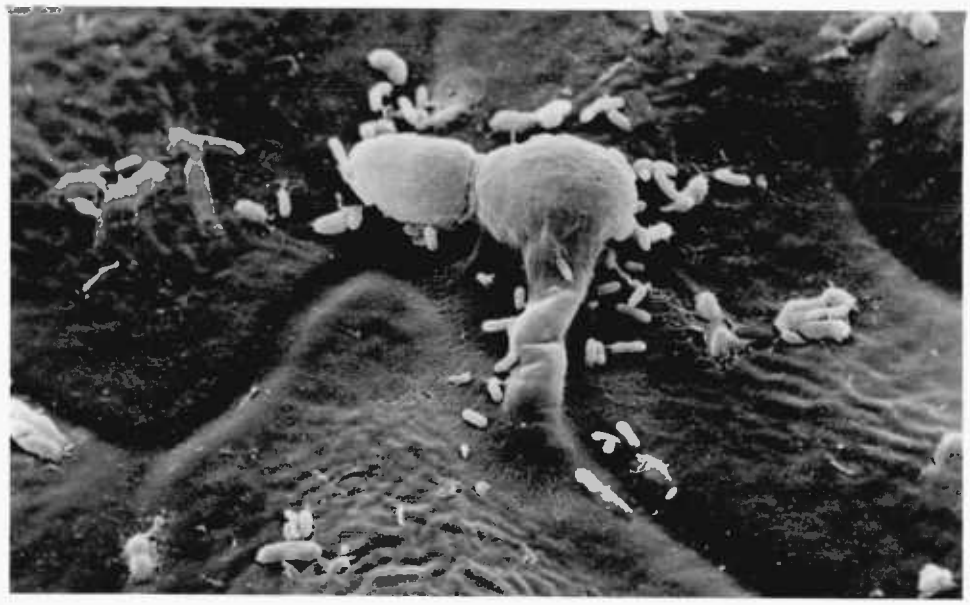
(b) Scanning electron microscopy

Material for examination was fixed in 2.5% glutaraldehyde for periods up to 48h. It was then dehydrated by passing through

ethanol of increasing concentration, 30%, 60%, 90% and 100%, two changes of 100%, allowing 15 - 20 minutes at each stage. The leaf discs were stuck on to stubs, critically-point dried (Polaron E 3,000) and coated with gold (Polaron E 5,000 splutter coater). The leaf discs were then examined in a Cambridge Stereoscan Mk. 2 scanning electron microscope.

This technique proved most satisfactory for observing the fungus on the surface - as conidia, hyphal strands and acervuli (Plate 3). A quantitative evaluation of penetration within an inoculum drop, however, proved to be difficult, mainly owing to contamination by bacteria and debris of the drop. Conidia taken directly from material stored at -15^{o}C were generally too contaminated for these E.M. studies, particularly those from leaf discs. Some improvement in inoculum was obtained by first obtaining a fresh collection of conidia from inoculated leaf discs and using these conidia as inoculum. It does seem likely that the scanning electron microscope could be a useful tool in examining penetration but more work is required on the technique and there was not sufficient time for this.

PLATE 3 GERMINATION AND PENETRATION OF D. ROSAE CONIDIUM (FRENSHAM ISOLATE) ON FRENSHAM (x 2200)



DISCUSSION

OVERWINTERING ON FALLEN ROSE LEAVES

The experiments investigating the overwintering of the fungus on fallen rose leaves were promoted by the general lack of experimental data concerning the survival of D. rosae through the winter in this country. The results of these experiments clearly indicated that some viable and potentially infective conidia could be recovered throughout the winter and, in 1973, some could still be recovered in mid-June when rose bushes were growing vigorously. However, the numbers of conidia which survived were low relative to those initially on the fallen leaves. Several factors account for this. The conidia which survived appeared to be those already present in the acervuli, many of these were undoubtedly lost through the washing action of rain and also much of the leaf material bearing the acervuli was decomposed by micro-organisms. This was particularly so during the winter of 1972 - 1973 when the leaves were kept in Terylene-net bags and as a result they remained wet and packed together for long periods. Even when leaves were kept in large cages during the winter of 1973 - 1974 and 1974 - 1975 and the conditions for microbial decomposition were not so extreme, there was little indication that either fresh acervuli were produced on the leaves in the spring or that new conidia were formed in existing acervuli.

These results were supported by those from laboratory experiments in which leaf material was stored at $5^{o}C$. Abundant conidia were only recovered from leaves on which the production of conidia had been stimulated by keeping the leaf material in a damp chamber at

20°C for 24h. prior to storage. There was again no evidence that new acervuli were produced or few if any new conidia were produced in existing acervuli, after storage even when the leaves were transferred to suitable conditions.

These results contrast with those of Frick (1943). She considered that the fungus hibernated on fallen leaves as a saprophytic mycelium and produced new conidia in acervuli already present and from which the existing conidia had been washed by rain. She did not make clear, however, the conditions under which she kept these leaves nor their state of decomposition. Possibly where decomposition of the leaflets is minimal the fungus can survive in this way. It could be that in Switzerland where Frick conducted her experiments, that the local conditions were less favourable to leaf decomposition than were those which pervailed during the present experiments. Frick also maintained that the conidia were short-lived and indeed were adversely affected by environmental factors such as low temperatures. The present studies suggest that this is not so. Indeed, conidia on leaves could be stored quite successfully at very low temperatures and at 5°C on dry leaves, indicating that they may be considerably resilient to the varying conditions encountered in the field. The survival of conidia on fallen leaves in the field therefore appeared to depend much more on the extent of leaf decomposition than on any direct effect of temperature or drying though these factors would affect the activity of micro-organisms and thus, indirectly, the survival of D. rosae.

While even a few conidia may be sufficient to initiate blackspot lesions on the new growth of the rose, clearly the survival of conidia on fallen leaves can be, for the fungus, a hazardous mode of

perennation particularly in winters like those commonly encountered in Britain where frequent rain is likely to deplete existing acervuli of conidia.

The discovery of the perfect state of D. rosae during the overwintering experiments of 1973 - 1974 and 1974 - 1975 is especially significant in this and other respects. The material collected in May 1974 was the first record of the perfect state in this country. That similar material was collected in the following year suggests that it may be far more common than previously supposed, though it must be emphasised that at present it has only been found at one locality and leaves from a few other areas were examined for the ascigerous state without success. It would be of considerable interest to widen the search for such material, especially as ascospore inoculum has two distinct advantages over conidia which survive the winter. These are that many ascospores are freshly-produced within the apothecia which erupt on the fallen leaves and they form in abundance during April, May and June when conditions are generally much more favourable for the infection of new rose leaves. To these may be added another advantage related to the way in which ascospores are dispersed. The present work showed that, in common with many Ascomycetes of this type, the ascospores are violently discharged. This is in direct contrast to that found by Wolf (1912) who described the discharge as a passive process as with conidia in acervuli, though he did not detail his experiments which may not have been able to detect this in practice. The ascospores are, therefore, apparently discharged actively from the ascus to above the surface of the apothecium where normal methods of dispersal will then operate. This indicates a mechanism which might be more effective in getting spores onto lower leaves than

splashing raindrops on the relatively few conidia present on the leaves. In addition many ascospores are produced in the apothecium which may result in a large local inoculum, whereas the few conidia remaining in the acervuli may only provide a small inoculum in terms of spores. In some situations the production of the perfect state may account for the variability of the fungus, due to sexual recombination and the rapidity in which the fungus may apparently form new strains or races.

DISEASE DEVELOPMENT

In both seasons the initial development of the disease followed a logarithmic pattern, conforming to the 'compound interest' type as described by Van der Plank (1963). This agrees with the work of Saunders (1966b) on blackspot development on different rose types in the field. The disease development on individual cultivars appeared to consist of an initial lag phase, a rapid disease development phase and a decline in disease levels. These phases are traditionally associated with the limitations in inoculum for the lag phase, the expression of cultivar-strain-environment interaction for the log phase (assuming there is neither limitation due to inoculum or susceptible host tissue) and lastly the lack of susceptible host tissue. The pattern of disease development is somewhat confused with blackspot by its defoliation effect, which induces new leaves to form and these then, in turn, become infected. The initial S-shaped curve thus changes to a fluctuating pattern which reflects the fall of infected leaves and leaflets due to premature abscission, the production of new leaf tissue, its infection and subsequent abscission as a result of further infections.

While the general pattern of disease development was similar in both seasons there were differences in the onset of the epidemics on the cultivars and the rates at which these subsequently developed. In 1974 it was the onset of the disease on the five cultivars which was strikingly different. The two groups of cultivars could be distinguished: there were those (Allgold and Masquerade - Group 1) on which the onset of disease was considerably delayed and those (Frensham, Iceberg and Orange Sensation - Group 2) on which disease developed early in the season. The implication of this is that while there was inoculum capable of infection Group 2 cultivars early in the season either there was no inoculum present capable of infecting Group 1 cultivars or it was below the threshold level for infection, other conditions such as the weather appeared to be equal. In this connection, it is of interest that in 1973 the only cultivar to become infected was Frensham and laboratory studies showed that the isolate derived from this cultivar can generally only infect Group 2 cultivars. It seems therefore that either the inoculum or a proportion of it infecting the Group 2 cultivars changed in some way or inoculum was introduced from outside. No Allgold was grown on the field station other than within the experimental site but it is possible that there are bushes of this cultivar nearby in private gardens. On the other hand the nearest planting of Masquerade is about 150 yards away, which could have provided suitable inoculum since again laboratory tests showed that there are isolates of D. rosae which particularly infect this cultivar and Allgold.

In 1975 there were no such differences in the onset of the epidemic suggesting that sufficient inocula compatible with each cultivar successfully overwintered. There were however striking differences between the infection rates of D. rosae on the five

cultivars. On this basis three groups could be distinguished which are not related to the two distinct groups evident in 1974. These were:

i. Iceberg and Orange Sensation

ii. Frensham and Allgold, and

iii. Masquerade.

It is interesting to speculate why this should be so when the infection rates were similar in 1974. Firstly it is possible that the pattern of the strains of D. rosae may differ in the two years. There may have been throughout 1974 a selection of strains particularly adapted to each cultivar. There is, in fact, some indication with the isolates from the Walled Garden that these can show preferences for a particular cultivar. The similarity in the infection rates in 1974 might therefore be somewhat misleading in that they do not reflect fully the cultivar-strain reaction. But by 1975, with a particular strain selected out there is a truer picture of this effect so that their different infection rates become apparent on the cultivars. Secondly the overwintering process itself may have selected more vigorous strains on some cultivars. In addition it must also be borne in mind that the weather conditions differed in the two years and in 1975 these might have provided conditions which alter slightly the relative susceptibility of the cultivars and might favour the development of some strains over others.

The combined results for 1974 and 1975 indicated that there were two components of resistance operating. Vertical or race specific resistance, which as a general rule delays the start of an epidemic and after the initial infection the rate of disease

increase is not reduced by the resistance. The results in 1974 imply such vertical resistance, the epidemic was delayed on Allgold and Masquerade but the rate of development was similar on all the cultivars. This suggests that Allgold and Masquerade were not infected early in the season because of the lack of suitable inoculum. It is interesting that by reducing the initial inoculum this will delay the onset of the epidemic, particularly so on resistant cultivars. This would be of significance for the application of fungicides and use of sanitation methods to reduce the initial inoculum. Vertical resistance may also involve active responses of the host that are physiological and biochemical defence mechanisms, for example, hypersensitivity and the production of phytoalexins. In this respect the resistance of Allgold and Masquerade to various isolates, notably those of Frensham, appears to involve processes which are characteristic of vertically resistant hosts. The second component may involve horizontal or race non-specific resistance where cultivars react similarly to all races but these develop at different rates on cultivars, that is the rate of disease increase is slowed. There is some evidence for this in the 1975 results in that the disease developed on all the cultivars at about the same time but the rate of disease development was not the same on all the cultivars.

By examining the disease development and the weather it was apparent that disease increases were associated with wet periods in both 1974 and 1975. This would not be unexpected as it has previously been shown that the conidia of D. rosae require water for successful dispersal and germination (Saunders, 1966b and Frick, 1943).

It was found that the occurrence of infection periods, similar to those used for forecasting apple scab outbreaks (Mills periods), were closely associated with both the initiation of blackspot on a particular cultivar and with disease increases. Apart from its initiation, the continued development of the disease also appeared to be dependant on the occurrence of infection periods. If such a period did not occur for a while the disease levels declined and picked up again only after the occurrence of a further infection period. There is the possibility of using Mills periods and linking their occurrence with spraying, as they are already monitored. This information could easily be extended to rose growers. No simple relationship between the rate of disease development and the preceding weather could be detected but a more detailed analysis might well reveal some useful relationship which would be of value in predicting epidemics and of benefit for control practices.

DEVELOPMENT OF D. ROSAE ON DETACHED ROSE LEAVES

Standard Laboratory Test

The ability of D. rosae conidia to survive on leaf material kept in the deep freeze provided a ready source of spores for laboratory tests. These were apparently normal, viable and infective and thus able to initiate blackspot lesions, even after long periods of storage. This method has the further advantage of overcoming the variability of isolates in culture particularly with regard to pathogenicity.

Some problems, however, with the variability of host tissue remain, since there was an indication that the tests in 1974 with

one isolate varied with the time of year. It seems most likely that this was a host effect due to changes in its physiological state and therefore it may be that for completely standard results the plants should be grown in controlled environment cabinets. The use of leaf discs ensures a maximum utilisation of available leaflets and space, provides almost optimum humidity and temperature for infection and eliminates most differences in host material due to age and conditions of growth. There is very little contamination or disorganisation of the discs.

The leaf disc test appears to be a reasonable way of assessing the ability of the fungus to grow on the cultivar in the field. This agrees with Saunders (1970) findings. The laboratory test does not, however, indicate some factors which may be important in the field. It does not, for example, indicate the rate of defoliation. Some cultivars may absciss leaves with only small lesions. Indeed, Palmer and Semeniuk (1961) indicated that two cultivars may show comparable infections but differ considerably in their defoliation characteristics. It also does not indicate very well those features of the whole plant which affect disease development in the field, for example, the ability of leaves to retain inoculum drops or otherwise, the growth habit which may or may not favour the trapping of water-borne spores or influence the disease development through its associated microclimate.

The variability in isolates of D. rosae which the present and other work indicates imposes some limitations on the leaf disc test. These are that it would be necessary to keep a standard collection of both cultivars and isolates in order to provide

standards for which the reactions had been characterised, with which either new cultivars or isolates of D. rosae from other areas could be compared. Because of this it seems unlikely that a laboratory test could ever replace completely the field assessments of new cultivars but it could provide valuable information during the early stages in the development of a cultivar of its tissue susceptibility to D. rosae. Indeed, Saunders (1970) suggested that such laboratory tests should be backed up by field tests and assessments.

VARIABILITY OF ISOLATES

In the past tests of the susceptibility of rose cultivars to D. rosae have proved difficult and to a large extent unsatisfactory because of the variability of the fungus. Collections have been made from different localities and different cultivars and these have been insufficiently characterised. The present work indicates some of the problems involved and possible ways in which some of these may be overcome.

The isolates from Frensham at Ashurst appeared to be relatively stable and uniform in successive tests on the five cultivars. It seems that in a long established planting of a single cultivar that a fairly stable isolate of D. rosae may be maintained. The stability of this isolate is in contrast with those obtained from the cultivars in the Walled Garden where the different roses were grown in close proximity in a mixed planting. Here there are indications that the so-called isolates are of very mixed abilities with regard to infection of the five cultivars concerned.

In these situations, passing isolates through the cultivars from which they were derived might help to produce isolates with relatively stable infection patterns on a range of cultivars. The limited work on this aspect was sufficiently encouraging to warrant further experiments and they did suggest that it may be possible to stabilise isolates and so characterise them.

Keeping D. rosae on leaf material at - 15°C does at least ensure that the pathogenicity of the isolate is maintained and with the techniques used here it was possible to demonstrate the existence of quite distinct strains or races of the fungus. Thus two groups of isolates could be defined and the general cultivar reactions in this situation compared to those for which they were originally chosen (Table 45).

The rose is a long and much cultivated garden plant and it seems that its long development has been matched by the development of D. rosae. So just as the modern rose is a complex of genetic material derived from several wild species so now D. rosae is equally complex in its matching of any factors for resistance. It is therefore not unreasonable to suppose that within a population of D. rosae there is some potential for infecting most modern cultivars, and it remains only a matter of selection to develop a particular 'isolate' able to infect a particular cultivar. In addition to this, is the feature common to most fungi and other organisms of change by chance mutation.

This does not necessarily mean that distinct isolates cannot be characterised. The present work shows that they can, as other work also suggests for example Jenkins (1955) and Palmer et al. (1966a)

TABLE 45

SUMMARY OF CULTIVAR AND ISOLATE CLASSIFICATION

	Cultivar classification[+]		Isolate classification*	
	Saunders (1970) and Palmer *et al.* (1966a)	Frensham isolate	Conidial germination	Group
Allgold	Extremely Resistant	Resistant	Equal	1
Masquerade	Intermediate	Resistant	Equal	
Frensham	Very susceptible	Susceptible	Unequal	2
Iceberg	Resistant	Susceptible	Unequal	
Orange Sensation	Susceptible	Susceptible	Unequal	

[+] Grading of resistance/susceptibility based on lesion development

* Based on conidia germination of the isolate on the five cultivars

that some isolates from the same locality may differ in their pathogenicity which suggests some variation due to the cultivar of origin. This is borne out by this work but that isolates from different localities may differ in their pathogenicity cannot be adequately commented on here. What needs to be done is to make many more collections of the fungus and to extend the testing to a wider range of cultivars. The parentage of these cultivars needs to be known and any common links investigated in terms of resistance to D. rosae, so that the main sources of blackspot resistance in modern roses could be evaluated.

DISEASE RESISTANCE

Because of the obvious variability of D. rosae studies of resistance inevitably involves a drastic selection of both the fungal isolate and cultivar to be examined, because only in a well defined disease situation can resistance be evaluated. Thus the Frensham isolate from Ashurst was chosen and the cultivars Frensham and Allgold because the reaction of this isolate on these cultivars was clearly distinct (i.e. Group 2 isolate on a cultivar from Group 1 and 2).

This difference in resistance was linked with differences in germination of conidia in an inoculum drop on the leaf surface. It was also associated with the presence of small, brown flecks in the area occupied by the inoculum drop on Allgold, indicating a hypersensitive type of reaction on this cultivar. Resistance may thus result from a combination of factors, the inhibition of conidia being only the first of these. Though conidia germination

may have been reduced, some penetration may have taken place and the fungus was then killed or stopped in the host tissue.

This resistant reaction is linked with two factors. Firstly, the reaction is induced by substances from the conidia (though not necessarily live conidia) and secondly, the resistant cultivar is induced to produce a germination inhibitor. This latter reaction appears to be relatively slow, since some conidia germinate in the first 24h. on the resistant cultivar but none apparently do so in the 24 - 48h. period. Presumably sufficient quantities of the inhibitors have to accumulate before germination is impaired. It might be supposed that because some conidia germinate and yet no lesions develop that there are also other mechanisms of resistance operating. This is not necessarily so as it has not been established that the germinated conidia are still viable and it is possible that they may be killed after penetration be accumulation of the same anti-fungal material. There was no indication of the nature of the inducers other than they are probably not enzymic as the reaction is still induced after heat killing of the conidia, or of the inhibitors. These may be phytoalexin type substances as suggested by Saunders (1970).

There are two other points of interest. First, it is possible that resistance may vary with the amount of phytoalexin produced. This could account for the variation between cultivars or even on the same cultivar which was experienced in tests. Second, Group 1 isolates which generally infect all cultivars and exhibit equal germination on all cultivars may not produce the necessary inducer or they are immune to any inhibitor produced in response to an

inducer. Any degree of resistance to these isolates may then be determined by other factors.

REFERENCES

ANDREN, A. S. and ROWELL, J. B. (1962). Duration of protective activity in wheat seedlings of various compounds against stem rust. Phytopathology 52, 909 - 913.

ALCOCK, N. L. (1918). Studies on the life history of the rose blotch fungus. Kew Bulletin 6, 193 - 197.

ARONESCU, A. (1934). Diplocarpon rosae: from spore germination to haustoria formation. Bulletin of the Torrey Botanical Club, 61, 291 - 293.

BEWLEY, W. F. (1938). Recent work on rose diseases. Scientific Horticulture 6, 97 - 101

BISBY, G. R. (1938). The fungi of Manitoba and Saskatchewa (Canada).

BLAKEMAN, J. P. and FRASER, A. K. (1971). Inhibition of Botrytis cinerea spores by bacteria on the surface of chrysantemum leaves. Physiological Plant Pathology 1, 45 - 54.

CRUICKSHANK, I. A. M. (1963). Phytoalexins. Annual Review of Phytopathology 1, 351 - 374.

CUTTER, E. C. and WHEELER, B. E. J. (1968). Effect of temperature on ascospore discharge from cleistocarps of Erysiphe cichoracearum on Arctium lappa. Transactions of the British Mycological Society, 51, 791 - 812.

DEVERALL, B. J. (1972) Phytalexins and disease resistance. Proceedings of the Royal Society of London B, 181, 233 - 246

DODGE, B. O. (1931). A further study of the morphology and life history of the rose blackspot fungus. Mycologia 23, 446 - 462.

DRING, D. M. (1955). A periodic acid - Schiff technique for staining fungi in higher plants. New Phytologist 54, 277 - 279.

EDWARDS, D. I. (1970). Chromatography: principles and techniques London: Butterworths.

ENGELHARD, A. W. (1969). Preventive and residual fungicidal activity of three Benzimidazole compounds and Zinc ion and Maneb against Diplocarpon rosae on two rose cultivars. Plant Disease Reporter 53, 537 - 540

FINNEY, M. E. (1973). Growth analysis of Barley (c.v. Zephyr) infected with Erysiphe graminis DC. Ph.D. Thesis, University of London.

FRICK, L. (1943). Untersuchungen Über biologie und pathogentät von Diplocarpon rosae (Lib.) Wolf. Phytopathologische Zeitschrift 14, 525 - 591

GREEN, D. E. (1931). Experiments and observations on the incidence and control of the blackspot disease of roses. Journal of the Royal Horticultural Society 56, 18 - 30.

JENKINS, W. R. (1954). Variation in pathogenicity and physiology of Diplocarpon rosae (Lib.) Wolf, the rose blackspot pathogen. Ph.D. Thesis, University of Maryland.

JENKINS, W. R. (1955). Variation of pathogenicity and physiology of Diplocarpon rosae Wolf, the rose blackspot fungus. American Rose Annual 40, 92 - 97.

KIRKHAM, D. S. (1956). A culture technique for Venturia spp. and a turbidimetric method for the estimation of comparative sporulation East Malling Research Station Annual Report 1956, 125 - 127.

LYLE, E. W. (1938). The blackspot disease of roses and its control under greenhouse conditions. Cornell Agricultural Experimental Station Bulletin, 690 - 721.

MELCHING, J. S. (1961). The effects of environmental factors and of selected chemicals upon the development of blackspot of roses. Dissertation Abstracts 22, 3803 -.

PALMER, J. G. and SEMENIUK, P. (1960). Relative susceptibilities to blackspot of some rose varieties in excised leaflet assay during 1959. Phytopathology 50, 572.

PALMER, J. G. and SEMENIUK, P. (1961). Comparable susceptibilties of fifty species and hybrid roses inoculated with blackspot fungus from plants, field grown in Maryland in 1959. American Rose Annual 46, 125 - 133.

PALMER, J. G. and SEMENIUK, P. and STEWART, R. N. (1966a). Roses and blackspot. I. Pathogenicity to excised leaflets to Diplocarpon rosae from seven geographic locations. Phytopathology 56, 1277 - 1282.

PALMER, J. G. and SEMENIUK, P. and STEWART, R. N. (1966b). Roses and blackspot. II. Seasonal variation in host susceptibility and decline of virulence in culture of conidia from Diplocarpon rosae. Phytopathology 56, 1283 - 1286.

PREECE, T. F. (1971). Some environmental and microscopical procedures useful in leaf surface studies. Ecology of leaf surface micro-organisms, Ed. Preece, T. F. and Dickenson, C. H. Academic Press.

PREECE, T. F. and SMITH, L. P. (1961). Apple scab infection weather in England and Wales, 1956 - 1960. Plant Pathology 10, 43 - 51.

PRICE, T. V. (1970). Epidemiology and control of powdery mildew (Sphaerotheca pannosa) on roses. Annals of Applied Biology 65, 231 - 248.

SAUNDERS, P. J. W. (1966a). The toxicity of sulphur dioxide to Diplocarpon rosae Wolf causing blackspot of roses. Annals of Applied Biology 58, 103 - 114.

SAUNDERS, P. J. W. (1966b). Epidemiological aspects of blackspot disease of roses caused by Diplocarpon rosae Wolf. Annals of Applied Biology 58, 115 - 122.

SAUNDERS, P. J. W. (1967). Host/parasite interaction in blackspot disease of roses caused by Diplocarpon rosae Wolf. Annals of Applied Biology 60, 129 - 136.

SAUNDERS, P. J. W. (1970). The resistance of some cultivars and species of Rosa to Diplocarpon rosae Wolf causing blackspot disease. The Rose Annual 1970, 118 - 128.

SHELLEY, A. D. G. (1925). Blackspot. The Rose Annual 1925, 133 - 138.

SHIPTON, W. A. and BROWN, J. F. (1962). A whole-leaf clearing and staining technique to demonstrate host-pathogen relationships of wheat stemrust. Phytopathology 52, 1313.

SHIRAKAWA, H. S. (1955). The nutrition of Diplocarpon rosae. American Journal of Botany 42, 379 - 384.

STAHL, E. (1967). Ed. Thin-layer chromatography. George Allen and Unwin.

STEEL, R. G. D. and TORRIE, J. H. (1960). Principles and procedures of statistics. McGraw-Hill.

STEWART, R. N. and SEMENIUK, P. (1959). The search for better roses. American Rose Annual 44, 15 - 18.

VAN DER PLANK, J. E. (1963). Plant disaeses: epidemics and control. London: Academic Press.

WOLF, F. A. (1912). The perfect stage of Actinonema rosae. The Botanical Gazette 54, 218 - 234.

APPENDIX

Key to abbreviations:

(1) D.F. - Degrees of freedom

S.S. - Sums of squares

M.S. - Mean square

F. - F test

(2) Significance levels

N.S. - not significant

* - $\underline{P} < 0.05$

** - $\underline{P} < 0.01$

*** - $\underline{P} < 0.001$

SPORE DISPERSAL

INTRODUCTION

Conidia of D. rosae are dispersed mainly by rain splash, this was confirmed by Saunders (1966b), he also attempted to examine this in the field by trapping conidia on sticky slides, assessing numbers on alternate months and relating this to disease development. Although there are no satisfactory methods for collecting spores distributed by water splash attempts were made in this investigation to collect them in water traps and assess numbers on a daily basis. An experiment was designed to relate the release and dispersal of conidia to the amount and duration of rain, and the inoculum rain splash between rose bushes and those released into rain water draining from an infected rose bush.

EXPERIMENTAL

Two adjacent rose bushes of the cultivar Frensham from the middle of the planting at Ashurst were selected. Under each was placed a right angle shaped piece of plastic guttering (11cm. x 65cm. outer arm length), at the join was a hole under which was attached a funnel that drained into a glass flask. The guttering was angled slightly so that rain water dripping from the bush above drained into the collecting container. In between these two bushes was placed a device to capture the lateral rain splash, this consisted of an upright section of plastic piping (11cm. diameter x 30cm.) suspended 50cm. above the ground over a rain gauge funnel (13cm. diameter) which drained into a large boiling tube where rain splash impinging on the piping was collected. The whole apparatus was shielded above to exclude direct rainfall.

Disease development was assessed on a marked shoot above the guttering for each bush at weekly intervals as previously described and the rain water collected daily, the experiment was set up in the field on 5 June 1974 and disease assessments commenced on 5 August. The total volume of rain water was recorded and reduced by centrifugation to 1 ml., which could then be stored at 5°C. The number of conidia per ml. in each sample was assessed by haemocytometer counts but this proved rather unsatisfactory and impractical as only very small numbers of conidia were involved from sometimes large volumes of water and considerable contamination was invariably present. Assessments were therefore discontinued, the disease development is shown in Appendix Table I. It is noteworthy that this followed a similar pattern to that described previously, though in this case the development on Frensham was later than that in the Walled Garden.

TABLE I

DISEASE DEVELOPMENT ON FRENSHAM - ASHURST 1974

Date	% Leaflets infected	
	Bush A[+]	Bush B
5 August	0.27	4.02
15	0.32	4.67
20	0.27	5.96
28	0.27	12.87
11 September	3.88	14.18
18	3.44	22.96
25	10.95	22.54
9 October	29.31	47.03
16	45.24	60.0
23	56.72	62.1
30	63.05	68.79
6 November	65.07	69.67
13	64.1	65.63
28	73.79	63.64

[+] see text for details

TABLE 1

INFECTION OF LEAF DISCS OF FIVE ROSE CULTIVARS BY ASCOSPORES AND CONIDIA OF D. ROSAE

Cultivar	Ascospore inoculum - Lesion Diameter (mm.) Box means					% leaf disc infection	Conidia inoculum - Lesion Diameter (mm.) Box means			% leaf disc infection
	1	2	3	4	Mean		1	2	Mean	
Allgold	0	0	0	0	0	0	0	0	0	0
Frensham	8.4	7.7	7.0	8.9	8.0	100	6.9	6.9	6.9	90
Iceberg	7.3	7.6	7.9	7.5	7.6	100	7.7	6.6	7.2	100
Masquerade	0	0	0	0	0	0	0	0	0	0
Orange Sensation	8.9	7.9	8.1	8.5	8.3	100	7.1	8.1	7.0	100

TABLE 2

DISEASE DEVELOPMENT ON FIVE ROSE CULTIVARS

- SILWOOD PARK 1974

	Date	Days	Allgold		Frensham		Iceberg	
			Mean % leaflets infected	$\text{Log}_e \left\{\frac{x}{1-x}\right\}$	Mean % leaflets infected	$\text{Log}_e \left\{\frac{x}{1-x}\right\}$	Mean % leaflets infected	$\text{Log}_e \left\{\frac{x}{1-x}\right\}$
7	June	0	0	-	4.86	-2.974	14.23	-1.796
14		7	0	-	3.75	-3.245	15.94	-1.66
21		14	0	-	4.99	-2.946	12.89	-1.91
28		21	0	-	4.37	-3.085	18.2	-1.5
5	July	28	0	-	17.38	-1.558	50.27	0.01
12		35	0.42	-5.468	44.3	-0.228	60.99	0.446
19		42	0.57	-5.161	51.2	0.048	65.98	0.662
26		49	3.19	-3.412	62.19	0.497	62.74	0.521
1	August	56	8.99	-2.314	59.57	0.387	43.11	-0.277
9		63	7.85	-2.462	54.45	0.178	31.27	-0.788
16		70	13.85	-1.827	36.3	-0.562	51.17	0.047
23		77	32.46	-0.732	35.73	-0.587	62.22	0.449
30		84	37.21	-0.523	37.99	-0.489	67.36	0.725
9	September	94	43.39	-0.265	24.29	-1.136	76.61	1.186
13		98	58.53	0.344	46.81	-0.127	83.5	1.621
20		105	79.55	1.358	59.19	0.371	69.02	0.801
27		112	91.0	2.314	73.34	1.012	70.52	0.872
5	October	120	96.98	3.469	80.17	1.397	57.62	0.307
11		127	95.94	3.163	64.87	0.613	41.13	-0.359
18		134	48.47	-0.061	62.91	0.528	49.65	-0.014
25		141	24.0	-1.153	54.34	0.174	52.02	0.081
1	November	149	3.13	-3.432	55.19	0.208	50.38	0.015

TABLE 2 (CONTD.)

Date	Days	Masquerade		Orange Sensation	
		Mean % leaflets infected	$\log_e \left\{\frac{x}{1-x}\right\}$	Mean % leaflets infected	$\log_e \left\{\frac{x}{1-x}\right\}$
7 June	0	0	-	9.15	-2.295
14	7	0	-	10.99	-2.092
21	14	0	-	10.93	-2.098
28	21	0	-	24.56	-1.122
5 July	28	0	-	36.74	-0.543
12	35	0	-	56.09	0.243
19	42	0	-	70.55	0.874
26	49	0	-	77.44	1.233
1 August	56	0	-	40.34	-0.391
9	63	0	-	41.04	-0.362
16	70	0.17	-6.375	36.91	-0.536
23	77	1.32	-4.314	59.98	0.405
30	84	1.14	-4.462	50.64	0.026
9 September	94	3.39	-3.349	32.66	-0.724
13	98	6.07	-2.739	56.75	0.019
20	105	20.83	-1.335	47.66	-0.093
27	112	31.21	-0.79	59.17	0.371
5 October	120	39.74	-0.416	66.03	0.665
11	129	46.25	-0.15	43.13	-0.277
18	134	48.24	-0.07	57.11	0.286
25	141	52.94	0.118	51.78	0.071
1 November	149	54.2	0.168	49.15	-0.034

TABLE 3

MEAN[+] TOTAL LEAFLETS RECORDED 1974

		Allgold	Frensham	Iceberg	Masquerade	Orange Sensation
7	June	116.25	218.0	160.5	144.5	116.25
14		159.5	196.0	192.75	144.5	123.25
21		194.5	195.0	180.75	157.25	133.0
28		200.75	202.75	188.5	161.5	144.0
5	July	210.5	209.5	179.75	177.5	124.5
12		205.75	219.0	175.75	179.0	104.75
19		203.5	172.5	155.0	182.75	83.0
26		198.25	131.25	127.0	187.25	54.75
1	August	227.5	97.0	162.25	202.25	63.5
9		280.75	82.5	214.5	207.25	64.5
16		306.0	68.5	202.25	226.5	76.5
23		321.0	74.5	204.75	210.5	74.25
30		260.5	78.5	153.75	224.75	45.5
9	September	195.5	95.25	109.5	237.5	39.75
13		194.75	72.5	107.25	225.25	39.5
20		109.25	72.5	66.75	211.75	41.75
27		59.75	46.5	34.25	169.5	21.5
5	October	41.5	45.0	43.5	142.75	25.0
11		28.75	40.5	40.5	123.5	17.5
18		16.25	41.75	51.25	107.75	24.5
25		8.25	38.75	48.75	95.5	20.75
1	November	2.0	30.5	39.25	91.0	16.5

[+] Mean of four replicates

TABLE 4

REGRESSION ANALYSIS OF $\log_E \left\{\frac{x}{1-x}\right\}$ AGAINST TIME FOR

INITIAL DISEASE DEVELOPMENT - SILWOOD PARK 1974

Days	Cultivar				
	Allgold	Frensham	Iceberg	Masquerade	Orange Sensation
14			-1.91		-2.098
21		-3.085	-1.5		-1.122
28		-1.558	0.01		-0.543
35	-5.468	-0.228	0.446		0.245
42	-5.161	0.048	0.662		0.874
49	-3.412	0.497			1.233
56	-2.314				
63	-2.462				
70	-1.827			-6.375	
77	-0.732			-4.314	
84	-0.523			-4.462	
94	-0.265			-3.349	
98	0.344			-2.739	
103	1.358			-1.335	
112	2.314			-0.79	
120	3.469			-0.416	
127				-0.15	

Cultivar	Regression coefficients bo	bl	Correlation	F value
Allgold	-8.55	0.1	0.98	***
Frensham	-5.25	0.13	0.95	*
Iceberg	-3.29	0.1	0.96	*
Masquerade	-3.25	0.1	0.99	***
Orange Sensation	-13.3	0.11	0.98	***

$y = bo + blx$

TABLE 4 (CONTD.)

HOMOGENEITY OF REGRESSION COEFFICIENTS+

	df.	x^2	xy	y^2	df.	Reduced S.S.
Allgold	12	9094.77	873.43	86.59	11	2.7
Frensham	4	490	61.39	8.5	3	0.81
Iceberg	4	490	49.63	5.48	3	0.46
Masquerade	8	3004.22	324.35	36.85	7	1.84
Orange Sensation	5	857.5	82.01	7.97	4	0.13
Residuals from individual regressions					28	5.93
Totals for single regressions	33	13936.49	1390.81	145.39	34	6.6
Difference for homogeneity of regressions					4	0.66

To test homogeneity of regression

F = 0.78 with 4 and 28 d.f. N.S.

+ After Steel and Torrie (1960)

TABLE 5

DISEASE DEVELOPMENT ON FIVE ROSE CULTIVARS - SILWOOD PARK 1975

Date	Days	Allgold		Frensham		Iceberg	
		Mean % leaflets infected	$\log_e \left\{\frac{x}{1-x}\right\}$	Mean % leaflets infected	$\log_e \left\{\frac{x}{1-x}\right\}$	Mean % leaflets infected	$\log_e \left\{\frac{x}{1-x}\right\}$
20 June	0	0	-	0	-	1.51	-4.178
27	7	0	-	0	-	0.2	-8.517
4 July	14	0.45	-5.399	0	-	1.61	-4.113
11	21	1.39	-4.262	0	-	4.38	-3.083
18	28	0.73	-4.913	0	-	3.55	-3.302
25	35	2.35	-3.727	0.33	-5.711	2.39	-3.71
1 August	42	8.65	-2.357	2.58	-3.631	9.19	-2.291
8	49	12.44	-1.951	5.48	-2.848	7.54	-2.507
15	56	36.47	-0.555	4.02	-3.173	12.18	-1.976
23	63	49.3	-0.028	12.7	-1.928	20.11	-1.379
29	70	66.14	0.67	30.58	-0.82	37.95	-0.492
5 September	77	45.65	-0.174	46.06	-0.158	51.44	0.058
12	84	39.78	-0.415	48.97	-0.415	55.73	0.23
26	98	49.33	-0.027	52.51	0.1	41.05	-0.362

TABLE 5 (CONTD.)

Date	Days	Masquerade		Orange Sensation	
		Mean % leaflets infected	$\log_e \left\{\frac{x}{1-x}\right\}$	Mean % leaflets infected	$\log_e \left\{\frac{x}{1-x}\right\}$
20 June	0	0	-	0	-
27	7	0	-	0	-
4 July	14	0	-	2.86	-3.525
11	21	1.64	-4.094	5.33	-2.877
18	28	1.35	-4.291	4.32	-3.098
25	35	6.44	-2.676	13.31	-1.874
1 August	42	19.41	-1.424	24.78	-1.11
8	49	16.57	-1.616	22.87	-1.216
15	56	24.76	-1.111	33.34	-0.693
23	63	40.47	-0.386	43.94	-0.244
29	70	60.14	0.411	55.29	0.212
5 September	77	68.08	0.757	56.51	0.262
12	84	50.7	0.028	62.14	0.495
26	98	47.3	-0.108	53.2	0.128

TABLE 6

MEAN[+] TOTAL LEAFLETS RECORDED 1975

	Allgold	Frensham	Iceberg	Masquerade	Orange Sensation
20 June	78.25	73.0	70.75	121.75	69.5
27	84.5	79.25	84.5	126.5	124.5
4 July	96.0	94.75	100.25	124.5	191.25
11	114.75	121.5	127.25	126.5	219.5
18	121.75	126.25	152.0	127.5	232.75
25	123.0	128.5	158.75	118.75	216.0
1 August	126.25	129.25	161.0	112.5	218.5
8	120.75	133.0	161.0	110.5	238.25
15	108.25	126.75	166.5	104.0	226.75
23	97.0	117.0	164.25	81.5	203.0
29	86.0	132.25	162.25	74.75	181.25
5 September	76.0	134.75	164.5	58.0	155.0
12	59.75	136.0	169.0	46.0	135.0
26	52.0	117.0	150.75	27.75	103.25

[+] Mean of four replicates

TABLE 7

REGRESSION ANALYSIS OF $LOG_E \left\{\frac{x}{1-x}\right\}$ AGAINST TIME FOR

INITIAL DISEASE DEVELOPMENT - SILWOOD PARK 1975

Days	Cultivar				
	Allgold	Frensham	Iceberg	Masquerade	Orange Sensation
14	-5.399		-4.113		-3.525
21	-4.262		-3.083	-4.094	-2.877
28	-4.913		-3.302	-4.291	-3.098
35	-3.727	-5.711	-3.71	-2.676	-1.874
42	-2.357	-3.631	-2.291	-1.424	-1.11
49	-1.951	-2.848	-2.507	-1.616	-1.216
56	-0.555	-3.173	-1.976	-1.111	-0.693
63	-0.28	-1.928	-1.379	-0.386	-0.244
70	0.67	-0.82	-0.492	0.411	0.212
77		-0.158	0.058	0.757	0.262
84			0.23		0.495

Cultivar	Regression coefficients bo	bl	Correlation	F value (significance)
Allgold	-7.25	0.11	0.98	***
Frensham	-9.24	0.12	0.96	***
Iceberg	-5.09	0.06	0.96	***
Masquerade	-6.08	0.09	0.97	***
Orange Sensation	-4.19	0.06	0.98	***

$$y = bo + blx$$

TABLE 7 (CONTD.)

(A) HOMOGENEITY OF REGRESSION COEFFICIENTS+

	df.	x^2	xy	y^2	df.	Reduced S.S.
Allgold	8	2940	326.99	37.92	7	1.55
Frensham	6	1372	162.41	20.71	5	1.49
Iceberg	10	5390	333.8	22.44	9	1.77
Masquerade	8	2940	268.82	25.96	7	1.38
Orange Sensation	10	5390	323.84	20.44	9	0.98
Residuals					37	7.17
Totals	42	18032	1415.87	127.47	43	16.3
Difference					4	9.13

To test homogeneity of regression

F = 11.77 with 4 and 37 d.f. ***

(B) COMPARISONS OF TWO REGRESSION COEFFICIENTS+

i.	Frensham and Allgold	t = 1.44 12 df.	N.S.
ii.	Allgold and Masquerade	t = 9.21 14 df.	***
iii.	Iceberg and Orange Sensation	t = 1.49 18 df.	N.S.
iv.	Allgold and Iceberg	t = 26.29 16 df.	***
v.	Masquerade and Iceberg	t = 15.43 16 df.	***

+ After Steel and Torrie (1960)

TABLE 8

METEOROLOGICAL DATA - SILWOOD PARK 1974

Date	Mean daily temp. °C	Rain (mm.)	Leaf surface wetness+		Mills periods+	
			Rain	90% r.h.	Leaf wetness (hours)	Mean temp. over period (°C)
1 June	13.0	0				
2	14.7	0				
3	15.8	1.4	00.30 - 03.30	00.30 - 07.30		
4	13.8	0				
5	13.0	0				
6	10.7	1.5				
7	11.0	0				
8	10.4	8.9	02.45 - 09.30	03.30 - 09.30		
9	9.7	8.9	09.30 - 17.30	20.00 - 24.00*		
10	10.3	0		00.00 - 06.00*	18.0	9.75
11	15.3	0				
12	15.7	0				
13	14.7	0				
14	16.8	0				
15	17.1	0				
16	18.0	11.8	19.15 - 22.45	20.15 - 24.00*		
17	15.8	1.2	03.00 - 06.15	00.00 - 10.00*	14.5	16.25
18	13.8	0				
19	14.3	0				
20	17.4	0				
21	19.7	0				
22	14.7	0				
23	14.4	0				
24	15.4	0				
25	14.3	0				

TABLE 8 (CONTD.)

Date	Mean daily temp. °C	Rain (mm.)	Leaf surface wetness+		Mills periods+	
			Rain	90% r.h.	Leaf wetness (hours)	Mean temp. over period (°C)
26 June	12.0	33.8	04.30 - 14.30	03.00 - 24.00*		
27	11.3	1.2	11.00 - 24.00	00.00 - 24.00*		
28	12.7	6.0	00.00 - 04.00	00.00 - 10.00*	53.0	11.5
			12.30 - 18.45	21.00 - 24.00*		
29	16.0	0		00.00 - 08.00*	19.5	13.75
30	15.7	0.9	04.45 - 08.00	04.45 - 09.00		
1 July	15.7	1.0	04.00 - 06.15	04.00 - 05.30		
2	13.7	5.3	18.15 - 19.45	18.30 - 23.45		
3	14.2	0.9				
4	12.9	0.5				
5	16.2	0				
6	15.9	0				
7	16.4	0				
8	18.3	0				
9	17.1	0				
10	15.3	1.0				
11	15.1	0				
12	12.2	1.1	17.15 - 24.00	20.30 - 24.00*		
13	13.1	10.3	00.00 - 13.00	00.00 - 13.30*	17.0	13.1
			20.00 - 20.30	21.30 - 24.00*		
14	14.4	0		00.00 - 07.00*	11.0	11.75
15	13.9	2.8	02.45 - 10.00	04.00 - 04.00*		
				07.30 - 15.00*	11.0	14.0
			20.00 - 21.00	20.30 - 21.30		
16	14.9	4.8	13.45 - 14.30			
			21.30 - 22.00	22.00 - 24.00*		
17	14.2	1.2	00.30 - 01.30	00.00 - 06.45*	17.0	14.5
			21.45 - 22.15	22.30 - 24.00		
18	15.6	0		00.00 - 05.45		
19	14.8	0.3				
20	18.9	0				

TABLE 8 (CONTD.)

Date	Mean daily temp. °C	Rain (mm.)	Leaf surface wetness+		Mills periods+	
			Rain	90% r.h.	Leaf wetness (hours)	Mean temp. over period (°C)
21 July	17.2	0				
22	17.3	0				
23	17.8	0				
24	13.5	8.1	09.15 - 12.30	09.30 - 12.45		
25	14.5	0				
26	16.9	0.7				
27	15.5	0				
28	16.9	0.3				
29	16.1	0				
30	17.8	0				
31	18.0	0				
1 August	16.4	0				
2	14.8	0				
3	15.3	0				
4	12.3	18.2	03.15 - 15.30	09.00 - 13.00*	12.0	13.0
			21.15 - 22.30			
5	14.5	0				
6	14.6	0				
7	16.4	0.1				
8	16.7	0.3				
9	15.8	2.9				
10	14.4	7.0				
11	14.7	0				
12	15.8	10.1	00.15 - 09.15			
13	14.2	2.2	01.15 - 06.00			
14	17.8	0				
15	19.0	0				
16	17.3	0.4				
17	14.5	0				
18	12.4	5.3	03.30 - 09.30	03.30 - 11.30		
			18.30 - 21.45	22.30 - 24.00*		

TABLE 8 (CONTD.)

Date	Mean daily temp. °C	Rain (mm.)	Leaf surface wetness+		Mills periods+	
			Rain	90% r.h.	Leaf wetness (hours)	Mean temp. over period (°C)
19 August	14.0	0		00.00 - 08.30*	14.0	13.0
20	14.8	0				
21	14.9	0				
22	14.2	0				
23	15.8	0				
24	17.3	0				
25	16.8	0				
26	14.5	20.5	01.00 - 10.00	01.45 - 09.30		
27	12.3	0				
28	12.2	0				
29	14.4	0				
30	14.1	0				
31	13.1	5.9	02.45 - 07.00	02.45 - 09.00		
			19.00 - 24.00	*		
1 Sept.	12.9	13.5	24.00 - 01.30	00.30 - 10.00*	14.0	12.5
			08.00 - 09.00			
			11.45 - 15.45			
2	12.6	11.4	04.30 - 05.00	05.00 - 10.00*		
			09.45 - 23.45	*	19.5	13.25
3	14.1	2.6	13.00 - 16.00			
4	14.1	2.7	09.30 - 11.45	10.30 - 12.30		
			22.00 - 24.00	*		
5	12.1	34.7	00.00 - 17.00	00.30 - 15.30*	19.0	12.25
6	12.9	1.2				
7	14.2	2.2	03.00 - 06.45	04.45 - 07.00		
8	13.8	0.2				
9	13.0	0.4				
10	12.1	0				
11	13.3	0.2				
12	16.3	1.8	04.30 - 06.30	05.00 - 09.45		
			12.30 - 15.45	20.30 - 22.30		

TABLE 8 (CONTD.)

Date	Mean daily temp. °C	Rain (mm.)	Leaf surface wetness+		Mills periods+	
			Rain	90% r.h.	Leaf wetness (hours)	Mean temp. over period (°C)
13 Sept.	16.4	0.3	03.30 - 04.00	03.30 - 07.30		
14	13.7	0				
15	12.6	4.3	13.00 - 18.30	18.30 - 24.00*		
16	14.9	0		00.00 - 10.30*	21.5	14.0
17	13.8	0				
18	9.9	0				
19	9.9	0				
20	11.0	0				
21	11.3	2.3	11.00 - 13.30			
22	9.8	0				
23	11.0	29.0	00.45 - 20.30	17.30 - 24.00*		
24	8.4	0.5		00.00 - 01.30*	25.0	12.0
25	9.8	5.7	00.00 - 04.45	02.00 - 07.15		
26	9.5	2.1	22.00 - 24.00	23.00 - 24.00*		
27	9.7	30.0	00.00 - 17.00	00.00 - 24.00*		
28	7.2	0.5		00.00 - 04.30*	19.0	11.25
29	6.5	0				
30	7.0	0				
1 Oct.	6.8	0				
2	6.5	7.1	05.00 - 24.00	05.00 - 13.30*		
				23.00 - 24.00*		
3	8.0	0.6	00.00 - 05.00	00.00 - 06.00*	25.0	6.75
4		5.0	01.45 - 20.30	07.30 - 24.00*		
5	5.0	0.2		00.00 - 08.30*	18.0	7.5
6	7.3	4.6	13.00 - 18.15	17.30 - 24.00*		
7	5.9	10.3	07.45 - 11.00	00.00 - 09.15*		
			14.45 - 18.15	*	29.0	6.5
8	8.9	0.3				
9	8.8	0				

TABLE 8 (CONTD.)

Date	Mean daily temp. °C	Rain (mm.)	Leaf surface wetness+		Mills periods+	
			Rain	90% r.h.	Leaf wetness (hours)	Mean temp. over period (°C)
10 Oct.	5.0	5.2	08.00 - 22.00	22.00 - 24.00*		
11	6.1	0.6		00.00 - 08.30*	24.5	6.25
12	5.4	0				
13	4.8	0				
14	5.0	0				
15	5.5	0.7	20.00 - 24.00	*		
16	8.8	11.8	00.00 - 11.15	00.00 - 24.00*		
			15.00 - 20.00	*		
17	8.0	0.1		00.00 - 12.30*	40.0	8.25
18	9.2	11.5	07.00 - 17.30			
19	8.1	4.9	17.00 - 21.30	22.00 - 24.00		
20	6.9	3.0	15.15 - 18.45	00.00 - 04.30		
21	6.2	0				
22	7.8	0.6				
23	8.3	0				
24	7.8	0				
25	9.0	0				
26	10.0	0				
27	9.4	0				
28	7.0	0				
29	5.8	0				
30	3.4	0.6	16.00 - 18.30	18.30 - 24.00		
31	2.0	0		00.00 - 11.30		

+ See text for details

Leaf surface wetness data, summary of periods of rain and 90% r.h. following rain with minor periods omitted.

Mills periods data, direct from daily record charts.

TABLE 9

METEOROLOGICAL DATA - SILWOOD PARK 1975

Date	Mean daily temp. °C	Rain (mm.)	Leaf surface wetness+		Mills periods+	
			Rain	90% r.h.	Leaf wetness (hours)	Mean temp. over period (°C)
15 June	14.75	0				
16	12.0	2.8	11.15 - 11.30			
17	13.3	1.1	04.30 - 05.30	04.45 - 07.15		
18	14.6	0				
19	15.6	0				
20	17.8	0				
21	18.8	0				
22	17.8	0				
23	15.0	5.3	16.00 - 19.15	17.00 - 24.00*		
24	18.1	0		00.00 - 04.45*	11.5	14.0
25	16.0	0				
26	18.1	L				
27	13.2	0				
28	12.0	0				
29	12.6	0				
30	15.2	0				
1 July	17.5	0				
2	16.7	0				
3	16.1	0.3				
4	17.3	0				
5	15.1	0				
6	17.0	0				
7	18.2	0				
8	18.5	10.5	04.00 - 06.00	04.15 - 10.15		
9	19.5	2.8				
10	17.0	0.2				
11	18.0	0				
12	15.6	0				

TABLE 9 (CONTD.)

Date	Mean daily temp. °C	Rain (mm.)	Leaf surface wetness+		Mills periods+	
			Rain	90% r.h.	Leaf wetness (hours)	Mean temp. over period (°C)
13 July	18.5	1.9	14.45 - 24.00	17.45 - 24.00*		
14	18.9	0.2	00.00 - 02.30	00.00 - 09.00*	18.25	18.5
15	16.5	14.7	15.00 - 19.45	22.30 - 24.00*		
16	17.3	0		00.00 - 09.30*	15.5	16.0
17	17.5	0				
18	17.1	2.6				
19	16.9	0.5				
20	17.3	0.7				
21	16.5	0				
22	17.0	0				
23	15.7	1.4	11.15 - 12.30	10.30 -13.00		
24	14.5	0				
25	15.0	0				
26	18.5	0				
27	19.1	0				
28	21.0	0				
29	23.9	0				
30	22.0	0				
31	20.0	2.7	18.15 - 21.30	19.30 - 24.00*		
1 Aug.	-	0		00.00 - 07.30*	13.5	16.75
2	20.0	0				
3	21.0	0				
4	24.0	0				
5	21.0	3.0	05.00 - 08.45	05.00 - 08.30		
6	20.0	0				
7	23.0	0				
8	25.0	8.8	22.15 - 24.00	22.30 - 24.00*		
9	18.0	1.2	00.00 - 01.00	00.00 - 11.00*	12.0	12.75
10	16.2	0.1				

TABLE 9 (CONTD.)

Date	Mean daily temp. °C	Rain (mm.)	Leaf surface wetness+		Mills periods+	
			Rain	90% r.h.	Leaf wetness (hours)	Mean temp. over period (°C)
11 Aug.	18.2	0				
12	20.5	0				
13	20.2	0				
14	20.4	0.3	23.45 - 24.00			
15	17.5	6.6	00.00 - 05.00	00.00 - 09.00		
			16.30 - 21.15	18.00 - 24.00*		
16	17.2	0		00.00 - 06.45*	14.0	16.75
17	17.4	0.1				
18	17.5	0				
19	18.2	1.6	01.30 - 06.30	01.30 - 12.15*	10.5	15.75
20	17.3	0.1				
21	15.3	0.6				
22	13.6	0				
23	13.8	3.1	19.45 - 23.45	19.45 - 24.00*		
24	14.7	0.1		00.00 - 09.30*	13.25	14.5
25	15.4	0				
26	17.6	0				
27	18.1	0				
28	17.1	0				
29	18.5	0				
30	15.8	0				
31	14.8	2.2	05.00 - 05.45	05.00 - 17.00*	12.0	15.0

+ see text for details

Leaf surface wetness data, summary of periods of rain and 90% r.h. following rain, with minor periods omitted.

Mills periods data, direct from daily record charts.

TABLE 10

COMPARISON OF THE RATE OF DISEASE INCREASE ON SAMPLE CULTIVARS

TO THE PRECEDING WEATHER

	Infection rate		Period preceding infection rate							
			7 Days Rainfall		10 Days Rainfall		14 Days Rainfall		7 - 14 Days Rainfall	
	Orange Sensation	Iceberg	Total (mm.)	hrs.	Total (mm.)	hrs.	Total (mm.)	hrs.	Total (mm.)	hrs.
1+	-0.029	0.019	2.9	3.8	2.9	2.6	3.3	4.2	0.4	0.4
2	-0.001	-0.035	17.8	6.5	19.3	8.0	20.7	10.3	2.9	3.8
3	0.139	0.058	21.0	2.6	21.0	13.2	30.8	9.1	17.8	6.5
4	0.083	0.215	35.0	13.2	35.0	22.1	48.0	15.8	13.0	2.6
5	0.112	0.062	14.6	8.9	49.6	5.5	49.6	22.7	35.0	13.2
6	0.089	0.03	1.0	1.5	7.7	17.2	15.6	10.4	14.6	8.9
7	0.51		20.2	5.7	21.2	17.2	21.2	17.2	1.0	1.5

+ Disease development, 27 June - 26 July 1974

TABLE 11

EFFECT OF LEAF AGE (cv. FRENSHAM) ON SUSCEPTIBILITY TO D. ROSAE

Leaf position on shoot		1 (Tip)	2	3	4	5
Shoot mean lesion diameter (mm.)	1	2	4.5	8	5.5	6.5
	2	2	4	0	0	0
	3	5	5	8.5	6.5	3.5
	4	2	2.5	3	7.5	3.5
	5	1	1.5	6	6.5	0

Analysis of Variance

Factor	D.F.	S.S.	M.S.	F	
Leaves	4	34.54	8.64	1.28	N.S.
Error	20		6.75		
Total		169.54			

S.E. = $\pm$ 0.58

TABLE 12

EFFECT OF INOCULUM DOSE OF D. ROSAE CONIDIA ON cv. FRENSHAM

Inoculum Level	Mean lesion diameter (mm.) Box means 1	2	Mean
0	0	0	0
10	1.2	0.6	0.9
10^2	4.8	2.2	3.5
10^3	6.6	7.2	6.9
10^4	7.4	7.6	7.5

Analysis of variance

Factor	D.F.	S.S.	M.S.	F.	
Treatment	4	92.46	23.12	30.74	***
Error	5		0.75		
Total		96.22			

S.E. = $\pm$ 0.61

TABLE 13

EFFECT OF MANNOXOL ON D. ROSAE ON LEAF DISCS OF ORANGE SENSATION

(A) Diameter of inoculum drop

Treatment		Inoculum drop diameter (mm.) Box mean 1	2	3	4	5	Mean
Distilled water (control)		2	2	2	2	2	2.0
Mannoxol	10^{-5}	2	2	2	2.5	2	2.1
	10^{-4}	2.8	2.8	3	3	3	2.9
	10^{-3}	5.5	6.8	6	4.5	4.3	5.4

Analysis of variance

Factor	D.F.	S.S.	M.S.	F.	
Treatments	3	37.7	12.57	43.71	***
Error	16		0.29		
Total		42.3			

S.E. = $\pm$ 0.24

Differences between treatments[+] (5% level)

Control	Mannoxol 10^{-5}	10^{-4}	10^{-3}

[+] based on Duncan's new multiple range test, treatments not underscored by the same line differed significantly.

TABLE 13 (CONTD.)

(B) Conidia germination on water agar after 24 hours

Replicate	% germination			
	Distilled water (control)	Mannoxol 10^{-5}	Mannoxol 10^{-4}	Mannoxol 10^{-3}
1	74	77	74	71.2
2	74	79	79	65.5
3	72	76	73	71.7
4	77	71.3	76	61.5
5	76	75.6	60.9	68
6	75.8	72	70.1	68.2
7	73	73	77	75.3
8	77	67	76	62.1
9	75	84	74	80.4
10	79	73	74.5	71.1
Mean	75.3	74.8	73.5	69.5

Analysis of variance

Factor	D.F.	S.S.	M.S.	F.	
Treatment	3	205.95	68.65	3.23	*
Error	36		21.25		
Total		970.9			

Differences between treatments[+] (5% level)

Mannoxol	10^{-3}	10^{-4}	10^{-5}	Control

TABLE 13 (CONTD.)

(C) Conidia germination on leaf discs after 48 hours

Treatment	% germination Angular transformation						Mean
	Box						
	1	2	3	4	5	Mean	
Control	23.03	29.0	25.4	24.35	26.13	25.58	18.7
Mannoxol 10^{-5}	19.46	20.96	16.43	21.05	20.36	19.65	11.4
10^{-4}	19.19	20.96	20.69	18.72	18.24	19.44	11.1
10^{-5}	12.92	9.63	18.63	15.0	12.38	13.71	5.9

Analysis of Variance

Factor	D.F.	S.S.	M.S.	F.	
Treatment	3	352.41	117.47	22.3	***
Error	16		5.27		
Total		436.7			

S.E. = $\pm$ 1.03

Differences between treatments $^{+}$ (5% level)

Mannoxol	10^{-3}	10^{-4}	10^{-5}	Control

TABLE 13 (CONTD.)

(D) Lesion diameter after 14 days

Treatment	Lesion diameter (mm.)					
	Box					Mean
	1	2	3	4	5	
Control	6	8	7	7	7	7.0
Mannoxol 10^{-5}	9	8	6	7	7	7.4
10^{-4}	8	6	7	6	8	7.0
10^{-3}	4	6	0	0	0	2.0

Analysis of variance

Factor	D.F.	S.S.	M.S.	F.	
Treatment	3	99.35	33.12	12.27	***
Error	16		2.7		
Total		142.55			

S.E. = $\pm$ 0.73

Differences between treatments $^{+}$ (5% level)

Mannoxol	10^{-3}	10^{-4}	Control	10^{-5}

TABLE 14

GERMINATION OF D. ROSAE CONIDIA AND LESION DEVELOPMENT ON LEAF DISCS (CULTIVAR FRENSHAM) INCUBATED ON DAMP BLOTTING PAPER OR ON WATER

Replicate	% germination		Angular transformation+		Lesion diameter (mm.)	
	Blotting Paper	Water	Blotting Paper	Water	Blotting Paper	Water
1	5	9	12.92	17.46	o	8
2	6	18	14.18	25.1	0	3
3	4	15	11.54	22.79	9	4
4	4	12	11.54	20.27	5	5
5	1	11	5.74	19.37	2	5
6	5	14	12.92	21.97	3	0
7	5	5	19.92	12.92	0	8
8	4		11.54		1	5
9	4		11.54		5	6
10	3		9.97		0	8
11	9		17.46		6	7
12	3		9.97		6	5
13	6		14.18		1	6
14	2		8.13		7	7
15	9		17.46		2	6
Mean	4.8	12.0	12.13	19.98	3.1	5.5

t =	15.786	t = 2.54
df =	20	df = 28
P =	< 0.001	P = < 0.05

+ Angular transformation of % germination data

TABLE 15

LESION DEVELOPMENT ON FIVE ROSE CULTIVARS

INOCULATED WITH ISOLATES OF D. ROSAE FROM FRENSHAM

(1) F - 72 - Ash isolate (23 May 1973)

Cultivar	Lesion diameter (mm.) Box means				Mean	% leaf discs Infected
	1	2	3	4		
Allgold	0	0	0	0	0	0
Frensham	5.8	5.4	5.8	4.6	5.4	100
Iceberg	7.2	7.0	7.8	8.0	7.5	100
Masquerade	0	0	0	0	0	0
Orange Sensation	7.4	7.6	8.6	7.6	7.8	100

Analysis of variance

Factor	D.F.	S.S.	M.S.	F.	
Cultivars	5	243.66	48.73	168.95	***
Boxes	3	0.27	0.09		
Error	15		0.29	0.32	N.S.
Total		248.26			

S.E. = $\pm$ 0.27

TABLE 15 (CONTD.)

(2) F - 72 - Ash isolate (14 June 1973)

Cultivar	Lesion diameter (mm.) Box means 1	2	Mean	% leaf disc infection
Allgold	0	0	0	0
Frensham	3.8	3.4	3.6	90
Iceberg	3.8	4.2	4.0	90
Masquerade	0	0	0	0
Orange Sensation	5.6	6.0	5.8	100

Analysis of variance

Factor	D.F.	S.S.	M.S.	F.	
Cultivars	5	62.82	12.56	244.74	***
Boxes	1	0.003	0.003	0.07	N.S.
Error	5		0.5		
Total		63.08			

S.E. = $\pm$ 0.16

(3) F - 73 - Ash isolate (5 December 1973)

Cultivar	Lesion diameter (mm.) Box means 1	2	Mean	% leaf disc infection
Allgold	0	0	0	0
Frensham	9.0	8.4	8.7	100
Iceberg	10.0	9.4	9.7	100
Masquerade	2.6	0	1.3	20
Orange Sensation	3.6	3.2	3.4	100

Analysis of variance

Factor	D.F.	S.S.	M.S.	F.	
Cultivars	4	152.62	38.15	49.94	***
Error	5		0.76		
Total		156.44			

S.E. = $\pm$ 0.62

TABLE 15 (CONTD.)

(4) F - 73 - Ash isolate (26 February 1974)

Cultivar	Lesion diameter (mm.) Box means 1	2	Mean	% leaf disc infection
Allgold	4.0	5.2	4.6	90
Frensham	8.4	4.4	6.4	80
Iceberg	8.2	7.6	7.9	100
Masquerade	6.2	4.4	5.3	70
Orange Sensation	8.2	7.2	7.7	100

Analysis of variance

Factor	D.F.	S.S.	M.S.	F	
Cultivars	4	16.78	4.19	1.9	N.S.
Error	5		2.2		
Total		27.79			

S.E. = $\pm$ 1.05

(5) F - 73 - Ash isolate (6 May 1974)

Cultivar	Lesion diameter (mm.) Box means 1	2	Mean	% leaf disc infection
Allgold	4.2	5.6	4.9	90
Frensham	7.8	7.2	7.5	100
Iceberg	9.2	9.0	9.1	100
Masquerade	7.0	6.2	6.6	90
Orange Sensation	4.2	4.6	4.4	100

Analysis of variance

Factor	D.F.	S.S.	M.S.	F.	
Cultivars	4	29.48	7.37	23.32	**
Error	5		0.32		
Total		31.06			

S.E. = $\pm$ 0.4

TABLE 15 (CONTD.)

(6) F - 73 - Ash isolate (22 June 1974) - see Appendix Table 19

(7) F - 73 - Ash isolate (23 July 1974)

Cultivar	Lesion diameter (mm.) Box means 1	2	Mean	% leaf disc infection
Allgold	2.4	3.4	2.9	50
Frensham	7.6	7.2	7.4	100
Iceberg	9.4	9.2	9.3	100
Masquerade	4.6	6.0	5.3	70
Orange Sensation	8.0	7.4	7.7	100

Analysis of variance

Factor	D.F.	S.S.	M.S.	F.	
Cultivars	4	48.98	12.24	34.78	***
Error	5		0.35		
Total		50.74			

S.E. = $\pm$ 0.42

(8) F - 73 - Ash isolate (28 August 1974)

Cultivar	Lesion diameter (mm.) Box means 1	2	Mean	% leaf disc infection
Allgold	1.8	0.9	1.4	30
Frensham	7.0	8.3	7.7	100
Iceberg	9.6	9.4	9.5	100
Masquerade	1.4	1.5	1.5	20
Orange Sensation	6.5	6.9	6.7	100

Analysis of variance

Factor	D.F.	S.S.	M.S.	F.	
Cultivars	4	111.09	27.77	102.48	***
Error	5		0.27		
Total		112.44			

S.E. = $\pm$ 0.37

TABLE 15 (CONTD.)

(9) F - 73 - Ash isolate (8 January 1975)

Cultivar	Lesion diameter (mm.) Box means 1	2	Mean	% leaf disc infection
Allgold	0	0	0	0
Frensham	7.5	7.6	7.6	100
Iceberg	8.8	9.1	9.0	100
Masquerade	0	0	0	0
Orange Sensation	3.4	4.0	3.7	100

Analysis of variance

Factor	D.F.	S.S.	M.S.	F.	
Cultivars	4	138.37	34.59	752.03	***
Error	5		0.05		
Total		138.6			

S.E. = $\pm$ 0.15

(10) F - 74 - Ash isolate (20 November 1974) - see Appendix Table 19

(11) F - 74 - Ash isolate (8 January 1975)

Cultivar	Lesion diameter (mm.) Box means 1	2	Mean	% leaf disc infection
Allgold	0	0	0	0
Frensham	5.9	7.8	6.9	100
Iceberg	6.9	4.4	5.7	100
Masquerade	0	0	0	0
Orange Sensation	2.5	2.0	2.3	100

Analysis of variance

Factor	D.F.	S.S.	M.S.	F.	
Cultivars	4	80.79	20.2	19.97	**
Error	5		1.01		
Total		85.85			

S.E. = $\pm$ 0.71

TABLE 15 (CONTD.)

(12) F - 74 - Ash isolate (5 February 1974)

Cultivar	Lesion diameter (mm.) Box means 1	2	Mean	% leaf disc infection
Allgold	0	0	0	0
Frensham	4.9	5.5	5.2	90
Iceberg	7.6	8.8	8.2	100
Masquerade	0	0	0	0
Orange Sensation	6.6	6.7	6.7	100

Analysis of variance

Factor	D.F.	S.S.	M.S.	F.	
Cultivars	4	116.2	29.05	160.5	***
Error	5		0.18		
Total		117.11			

S.E. = $\pm$ 0.3

(13) F - 74 - Ash isolate (22 April 1975)

Cultivar	Lesion diameter (mm.) Box means 1	2	Mean	% leaf disc infection
Allgold	0	0	0	0
Frensham	6.9	6.9	6.9	90
Iceberg	7.7	6.6	7.2	100
Masquerade	0	0	0	0
Orange Sensation	7.1	6.8	7.0	100

Analysis of variance

Factor	D.F.	S.S.	M.S.	F.	
Cultivars	4	117.67	29.42	226.29	***
Error	5		0.13		
Total		118.32			

S.E. = $\pm$ 0.25

TABLE 15 (CONTD.)

(14) F - 74 - Ash isolate (24 May 1975)

Cultivar	Lesion diameter (mm.) Box means			% leaf disc infection
	1	2	Mean	
Allgold	0	0	0	0
Frensham	4.8	3.4	4.1	60
Iceberg	7.0	6.5	6.8	80
Masquerade	0	0	0	0
Orange Sensation	8.6	8.3	8.5	100

Analysis of variance

Factor	D.F.	S.S.	M.S.	F.	
Cultivar	4	118.55	29.64	128.86	***
Error	5		0.23		
Total		119.7			

S.E. = $\pm$ 0.34

TABLE 16

LESION DEVELOPMENT ON FIVE ROSE CULTIVARS INOCULATED WITH AN ISOLATE OF D. ROSAE FROM ALLGOLD (A - 73 - Bet)

(1) 5 December 1973

Cultivar	Lesion diameter (mm.) Box means			% leaf disc infection
	1	2	Mean	
Allgold	0	1.4	0.7	10
Frensham	0	0	0	0
Iceberg	1.2	1.6	1.4	30
Masquerade	0	2.2	1.1	20
Orange Sensation	2.2	2.4	2.3	100

Analysis of Variance

Factor	D.F.	S.S.	M.S.	F.	
Cultivars	4	5.8	1.45	2.07	N.S.
Error	5		0.7		
Total		9.3			

S.E. = $\pm$ 0.59

(2) 23 January 1974

Cultivar	Lesion diameter (mm.) Box means			% leaf disc infection
	1	2	Mean	
Allgold	2.4	5.4	3.9	90
Frensham	0	0	0	0
Iceberg	2.4	0.4	1.4	40
Masquerade	0	0	0	0
Orange Sensation	3.4	3.6	3.5	70

Analysis of variance

Factor	D.F.	S.S.	M.S.	F.	
Cultivars	4	27.86	6.97	5.34	*
Error	5		1.3		
Total		34.38			

S.E. = $\pm$ 0.81

TABLE 16 (CONTD.)

(3) 29 January 1974

Cultivar	Lesion diameter (mm.) Box means 1	2	3	4	Mean	% leaf disc infection
Allgold	2.4	1.8	5.0	1.8	2.8	40
Frensham	1.2	0	0	0.6	0.5	8
Iceberg	0.4	0.4	0	0	0.2	12
Masquerade	0	0	0	0	0	0
Orange Sensation	1.2	0	1.2	0.4	0.7	16

Analysis of variance

Factor	D.F.	S.S.	M.S.	F.	
Cultivars	4	19.73	4.93	8.03	***
Error	15		0.61		
Total		28.95			

S.E. = $\pm$ 0.39

(4) 25 June 1974 (see Appendix Table 19)

TABLE 17

DEVELOPMENT OF D. ROSAE ISOLATES ON FIVE ROSE

CULTIVARS AFTER ONE TRANSFER THROUGH THEIR RESPECTIVE HOSTS

(1) F - 73 - Ash isolate grown on Frensham (9 January 1975)

Cultivar	Lesion diameter (mm.) Box means			% leaf disc infection
	1	2	Mean	
Allgold	0	0	0	0
Frensham	4.3	3.5	3.9	90
Iceberg	6.7	6.8	6.8	100
Masquerade	4.6	1.2	2.9	40
Orange Sensation	3.4	4.7	4.1	100

Analysis of variance

Factor	D.F.	S.S.	M.S.	F.	
Cultivars	4	47.27	11.82	8.5	*
Error	5		1.39		
Total		54.22			

S.E. = $\pm$ 0.83

Differences between cultivars $^{+}$ (5% level)

A	M	F	O.S.	I

TABLE 17 (CONTD.)

(2) F - 74 - Ash isolate grown on Frensham (14 January 1975)

Cultivar	Lesion diameter (mm.) Box means			% leaf disc infection
	1	2	Mean	
Allgold	0	0	0	0
Frensham	2.2	6.1	4.2	70
Iceberg	9.8	9.4	9.6	100
Masquerade	0	0	0	0
Orange Sensation	6.5	6.8	6.7	100

Analysis of variance

Factor	D.F.	S.S.	M.S.	F.	
Cultivars	4	140.75	35.19	22.76	**
Error	5		1.55		
Total		148.48			

S.E. = $\pm$ 0.88

Differences between cultivars[+] (5% level)

A	M	F	O.S.	I

(3) F - 74 - WG isolate grown on Frensham (14 January 1975)

Cultivar	Lesion diameter (mm.) Box means			% leaf disc infection
	1	2	Mean	
Allgold	0	0	0	0
Frensham	4.3	3.9	4.1	70
Iceberg	9.3	9.5	9.4	100
Masquerade	0	0	0	0
Orange Sensation	6.0	6.9	6.5	100

Analysis of variance

Factor	D.F.	S.S.	M.S.	F.	
Cultivars	4	134.34	233.59	332.53	***
Error	5		1.01		
Total		134.85			

S.E. = $\pm$ 0.71

Differences between cultivars[+] (5% level)

A	M	F	O.S.	I

TABLE 17 (CONTD.)

(4) A - 74 - WG isolate grown on Allgold (21 February 1975)

Cultivar	Lesion diameter (mm.) Box means 1	2	Mean	% leaf disc infection
Allgold	2.7	4.9	3.8	50
Frensham	2.8	0	1.4	20
Iceberg	4.2	5.8	5.0	70
Masquerade	3.0	2.9	3.0	40
Orange Sensation	4.9	4.0	4.5	100

Analysis of variance

Factor	D.F.	S.S.	M.S.	F.	
Cultivar	4	15.91	3.98	2.48	N.S.
Error	5		1.61		
Total		23.94			

S.E. = $\pm$ 0.9

Differences between cultivars^{+} (5% level)

F M A O.S. I

(5) F - 74 - WG isolate grown on Frensham (19 February 1975)

Cultivar	Lesion diameter (mm.) Box means 1	2	Mean	% leaf disc infection
Allgold	1.2	3.6	2.4	40
Frensham	5.4	3.0	4.2	70
Iceberg	3.6	3.6	3.6	80
Masquerade	4.9	1.3	3.1	40
Orange Sensation	6.4	6.7	6.6	100

Analysis of variance

Factor	D.F.	S.S.	M.S.	F.	
Cultivars	4	20.14	5.03	2.05	N.S.
Error	5		2.46		
Total		32.42			

S.E. = $\pm$ 1.1

Differences between cultivars^{+} (5% level)

A M I F O.S.

TABLE 17 (CONTD.)

(6) I - 74 - WG isolate grown on Iceberg (24 February 1975)

Cultivar	Lesion diameter (mm.) Box means 1	2	Mean	% leaf disc infection
Allgold	0	0	0	0
Frensham	2.5	1.2	1.9	50
Iceberg	4.7	4.7	4.7	90
Masquerade	0	0	0	0
Orange Sensation	6.7	6.5	6.6	100

Analysis of variance

Factor	D.F.	S.S.	M.S.	F.	
Cultivars	4	68.98	17.24	99.68	***
Error	5		0.71		
Total		69.84			

S.E. = ± 0.29

Differences between cultivars[+] (5% level)

A M F I O.S.

(7) M - 74 - WG isolate grown on Masquerade (19 February 1975)

Cultivar	Lesion diameter (mm.) Box means 1	2	Mean	% leaf disc infection
Allgold	4.6	4.5	4.6	60
Frensham	3.8	2.1	3.0	70
Iceberg	3.1	3.6	3.4	90
Masquerade	1.6	3.3	2.5	30
Orange Sensation	3.9	4.1	4.0	90

Analysis of variance

Factor	D.F.	S.S.	M.S.	F.	
Cultivar	4	5.54	1.39	2.28	N.S.
Error	5		0.61		
Total		8.58			

S.E. = ± 0.55

Differences between cultivars[+] (5% level)

M F I O.S. A

TABLE 17 (CONTD.)

(B) O.S. - 74 - WG isolate grown on Orange Sensation (21 February 1975)

Cultivar	Lesion diameter (mm.) Box mean 1	2	Mean	% leaf disc infection
Allgold	0	0	0	0
Frensham	3.4	1.9	2.7	70
Iceberg	6.1	7.1	6.6	90
Masquerade	0	0	0	0
Orange Sensation	6.1	4.9	5.5	100

Analysis of variance

Factor	D.F.	S.S.	M.S.	F.	
Cultivar	4	74.64	18.66	39.79	***
Error	5		0.47		
Total		79.99			

S.E. = $\pm$ 0.48

Differences between cultivars[+] (5% level)

A	M	F	O.S.	I

[+] based on Duncan's new multiple range test, cultivars not underscored by the same line differed significantly.

TABLE 18

DEVELOPMENT OF D. ROSAE ISOLATES ON FIVE ROSE CULTIVARS AFTER ONE TRANSFER THROUGH ANOTHER CULTIVAR

(1) A - 74 - Ash isolate grown on Frensham, tested on (21 April 1975)

Cultivars	Lesion development (mm.) Box means*			% leaf disc infection
	1	2	Mean	
Allgold	4.3	1.2	2.3	40
Frensham	1.6	1.7	1.7	20
Iceberg	2.7	2.1	2.4	40
Masquerade	3.1	0	1.6	30
Orange Sensation	0	3.2	1.6	30

(2) F - 74 - Ash isolate grown on Allgold, tested on (21 April 1975)

Cultivars	Lesion development (mm.) Box means*			% leaf disc infection
	1	2	Mean	
Allgold	0	0	0	0
Frensham	0	1.4	0.7	10
Iceberg	0	0	0	0
Masquerade	0	0	0	0
Orange Sensation	0	0	0	0

* means of five discs/box

TABLE 19

CONIDIAL GERMINATION AND LESION DEVELOPMENT OF EIGHT

D. ROSAE ISOLATES ON FIVE ROSE CULTIVARS

(1) F - 73 - Ash (22 June 1974) - a. Lesion development

Cultivar	Lesion diameter (mm.) Box means (3 discs/box)						% leaf disc infection
	1	2	3	4	5	Mean	
Allgold	0	0	4.3	0	2.3	1.3	20
Frensham	8.7	9.7	10.7	9.3	9.7	9.6	100
Iceberg	7.3	7.0	8.3	7.3	8.0	7.6	100
Masquerade	2.7	0	0	3.0	0	1.1	13.3
Orange Sensation	7.6	8.0	5.6	7.0	7.6	7.2	100

Analysis of variance

Factors	D.F.	S.S.	M.S.	F.	
Cultivar	4	302.03	75.51	47.59	***
Error	20		1.59		
Total		333.77			

S.E. = $\pm$ 0.56

(1) F - 73 - Ash (22 June 1974) - b. Conidial germination

Cultivar	% germination Box means (2 discs/box)					
	1	2	3	4	5	Mean
Allgold	14.7	16.2	16.7	15.8	18.5	16.4
Frensham	26.2	23.6	25.8	19.9	20.6	23.2
Iceberg	27.5	28.2	25.7	28.6	24.3	26.9
Masquerade	13.2	12.8	11.2	11.0	10.1	11.7
Orange Sensation	29.3	39.7	34.5	35.0	39.6	35.6

Analysis of variance

Factor	D.F.	S.S.	M.S.	F.	
Cultivar	4	837.21	209.3	71.89	***
Error	20		2.91		
Total		895.44			

S.E. = $\pm$ 0.76

Analysis based on angular transformation of % germination data

TABLE 19 (CONTD.)

(2) A - 73 - Bet (25 June 1974) - a. Lesion development

Cultivar	Lesion diameter (mm.) Box means (2 discs/box) 1	2	3	4	5	Mean	% leaf disc infection
Allgold	2.3	2.3	2.3	1.3	2.3	2.1	33.3
Frensham	0	2.7	4.7	2.3	0	1.9	26.7
Iceberg	7.3	5.0	6.7	4.7	3.0	5.3	86.7
Masquerade	7.7	3.0	2.3	5.7	5.3	4.8	60
Orange Sensation	5.0	5.7	6.7	7.0	6.0	6.1	93.3

Analysis of variance

Factor	D.F.	S.S.	M.S.	F.	
Cultivar	4	72.16	18.04	7.33	***
Error	20		2.46		
Total		121.38			

S.E. = ± 0.7

(2) A - 73 - Bet (25 June 1974) - b. Conidial germination

Cultivar	% germination Box means (2 discs/box) 1	2	3	4	5	Mean
Allgold	33.9	31.2	33.5	34.8	35.1	33.7
Frensham	32.6	31.4	26.6	30.9	28.4	30.0
Iceberg	31.8	32.4	31.7	35.3	37.6	33.8
Masquerade	32.3	30.4	32.4	33.6	31.4	22.0
Orange Sensation	32.6	35.3	33.2	33.9	29.8	32.9

Analysis of variance (angular transformation of % germination data)

Factor	D.F.	S.S.	M.S.	F.	
Cultivar	4	18.68	4.67	3.01	*
Error	20		1.55		
Total		49.7			

S.E. = ± 0.56

Analysis based on angular transformation of % germination data

TABLE 19 (CONTD.)

(3) F - 74 - Ash (20 November 1974) - a. Lesion development

Cultivar	Lesion diameter (mm.) Box means (3 discs/box) 1	2	3	4	5	Mean	% leaf disc infection
Allgold	0	0	0	0	0	0	0
Frensham	8.2	3.8	7.5	8.5	7.0	7.0	93.3
Iceberg	2.3	4.5	1.5	5.2	9.8	4.7	73.3
Masquerade	0	0	0	0	0	0	0
Orange Sensation	5.2	5.5	5.2	5.2	5.8	5.4	100

Analysis of variance

Factors	D.F	S.S.	M.S.	F.	
Cultivar	4	207.56	51.89	18.31	***
Error	20		2.83		
Total		264.24			

S.E. = $\pm$ 0.75

(3) F - 74 - Ash (20 November 1974) - b. Conidial germination

Cultivar	% germination Box means (2 discs/box) 1	2	3	4	5	Mean
Allgold	14.0	9.6	14.1	15.2	13.2	13.2
Frensham	17.1	28.9	19.9	21.5	19.0	21.2
Iceberg	23.0	23.9	26.8	23.8	21.1	23.7
Masquerade	16.2	15.4	14.6	12.7	12.3	14.2
Orange Sensation	35.7	39.3	30.1	28.7	22.4	31.2

Analysis of variance (angular transformation of % germination data)

Factor	D.F.	S.S.	M.S.	F.	
Cultivar	4	540.42	135.11	20.03	***
Error	20		6.75		
Total		675.34			

S.E. = $\pm$ 1.16

Analysis based on angular transformations of % germination data.

TABLE 19 (CONTD.)

(4) A - 74 - WG (9 November 1974) - a. Lesion development

Cultivar	Lesion diameter (mm.) Box means (3 discs/box)						% leaf disc infection
	1	2	3	4	5	Mean	
Allgold	8.5	8.3	8.2	8.0	7.8	8.1	100
Frensham	5.5	6.0	5.2	4.5	4.7	5.2	100
Iceberg	9.0	8.8	9.5	8.8	8.3	8.9	100
Masquerade	9.5	9.3	9.2	8.8	9.0	9.2	100
Orange Sensation	7.8	6.0	5.7	5.8	5.5	6.2	100

Analysis of variance

Factor	D.F.	S.S.	M.S.	F.	
Cultivar	4	62.03	15.51	48.62	***
Error	20		0.32		
Total		68.41			

S.E. = ± 0.25

(4) A - 74 - WG (9 November 1974) - b. Conidial germination

Cultivar	% germination Box means (2 discs/box)					
	1	2	3	4	5	Mean
Allgold	10.0	12.0	9.5	8.4	12.9	10.5
Frensham	11.8	13.9	10.9	9.0	7.8	10.7
Iceberg	10.7	8.6	7.0	12.4	8.7	9.5
Masquerade	12.0	7.3	10.2	9.9	8.8	9.6
Orange Sensation	12.8	9.9	10.5	12.4	9.0	10.9

Analysis of variance (angular transformation of % germination data)

Factor	D.F.	S.S.	M.S.	F.	
Cultivar	4	7.8	1.95	0.57	N.S.
Error	20		3.44		
Total		76.52			

S.E. = ± 0.83

Analysis based on angular transformations of % germination data

TABLE 19 (CONTD.)

(5) F - 74 - WG (23 October 1974) - a. Lesion development

Cultivar	Lesion diameter (mm.) Box means (3 discs/box)						% leaf disc infection
	1	2	3	4	5	Mean	
Allgold	8.7	9.0	8.0	7.5	9.2	8.5	100
Frensham	8.7	9.2	7.8	8.7	8.8	8.6	100
Iceberg	8.8	9.7	9.3	10.3	8.3	9.4	100
Masquerade	7.7	8.8	9.5	9.3	9.3	8.9	100
Orange Sensation	8.3	7.8	8.0	7.8	7.7	7.9	100

Analysis of variance

Factor	D.F.	S.S.	M.S.	F.	
Cultivar	4	5.93	1.48	4.22	*
Error	20		0.35		
Total		12.96			

S.E. = ± 0.26

(5) F - 74 - WG (23 October 1974) - b. Conidial germination

Cultivar	% germination Box means (2 discs/box)					
	1	2	3	4	5	Mean
Allgold	17.3	17.9	18.4	13.9	13.1	16.1
Frensham	31.9	27.9	23.7	24.6	22.7	26.2
Iceberg	22.4	19.1	20.7	23.5	21.8	21.5
Masquerade	13.5	13.4	12.9	10.5	10.9	11.8
Orange Sensation	25.2	25.8	20.5	24.8	16.7	22.6

Analysis of variance (angular transformation of % germination data)

Factor	D.F.	S.S.	M.S.	F.	
Cultivar	4	352.72	88.18	22.24	***
Error	20		3.97		
Total		432.03			

S.E. = ± 0.89

Analysis based on angular transformations of % germination data

TABLE 19 (CONTD.)

(6) I - 74 - WG (8 November 1974) - a. Lesion development

Cultivar	Lesion diameter (mm.) Box means (3 discs/box) 1	2	3	4	5	Mean	% leaf disc infection
Allgold	3.7	5.0	5.2	3.0	5.8	4.5	93.3
Frensham	5.8	5.0	5.3	5.8	6.2	5.6	100
Iceberg	7.8	9.8	9.5	9.3	9.0	9.1	100
Masquerade	4.8	6.5	2.7	4.7	6.2	5.0	60
Orange Sensation	8.2	7.3	7.7	7.7	7.7	7.7	100

Analysis of variance

Factor	D.F.	S.S.	M.S.	F.	
Cultivar	4	75.55	18.89	20.77	***
Error	20	-	0.91		
Total		93.73			

S.E. = ± 0.43

(6) I - 74 - WG (8 November 1974) - b. Conidial germination

Cultivar	% germination Box means (2 discs/box) 1	2	3	4	5	Mean
Allgold	18.9	9.4	13.9	14.4	10.8	13.5
Frensham	14.1	18.4	14.6	23.4	22.9	18.7
Iceberg	14.4	14.6	19.1	17.8	17.3	16.6
Masquerade	15.5	9.4	14.6	14.0	11.3	13.0
Orange Sensation	14.5	24.7	29.7	22.6	17.6	21.8

Analysis of varinace

Factor	D.F.	S.S.	M.S.	F.	
Cultivar	4	156.78	39.19	4.36	*
Error	20		8.98		
Total		336.4			

S.E. = ± 1.34

Analysis based on angular transformations of % germination data

TABLE 19 (CONTD.)

(7) M - 74 - WG (16 November 1974) - a. Lesion development

Cultivar	Lesion diameter (mm.) Box means (3 discs/box) 1	2	3	4	5	Mean	% leaf disc infection
Allgold	5.2	6.8	7.2	6.5	6.5	6.4	100
Frensham	7.0	7.7	6.0	7.0	5.3	6.2	100
Iceberg	9.2	9.5	9.8	9.0	9.7	9.4	100
Masquerade	10.0	10.5	9.8	9.3	9.8	9.9	100
Orange Sensation	7.2	6.8	7.0	7.0	6.8	7.0	100

Analysis of variance

Factor	D.F.	S.S.	M.S.	F.	
Cultivars	4	55.52	13.88	40.06	***
Error	20		0.35		
Total		62.45			

S.E. = ± 0.26

(7) M - 74 - WG (16 November 1974) - b. Conidial germination after 48h.

Cultivar	% germination Box means (2 discs/box) 1	2	3	4	5	Mean
Allgold	12.6	16.2	20.3	25.9	28.9	20.7
Frensham	26.2	21.1	22.0	21.7	21.1	22.4
Iceberg	21.4	18.2	14.7	17.2	26.6	19.6
Masquerade	23.2	14.5	13.1	16.6	26.5	18.8
Orange Sensation	25.3	17.5	17.3	21.5	20.3	20.4

Analysis of variance

Factor	D.F.	S.S.	M.S.	F.	
Cultivars	4	20.77	5.19	0.44	N.S.
Error	20		11.74		
Total		25.47			

S.E. = ± 1.53

Analysis based on angular transformations of % germination data

TABLE 19 (CONTD.)

(8) O.S. - 74 - WG (19 November 1974) - a. Lesion development

Cultivar	Lesion diameter (mm.) Box means (2 discs/box)						% leaf disc infection
	1	2	3	4	5	Mean	
Allgold	0	0	0	0	0	0	0
Frensham	4.5	4.3	4.3	4.2	3.7	4.2	100
Iceberg	6.3	6.5	7.2	6.3	6.5	6.6	100
Masquerade	0	0	0	0	0	0	0
Orange Sensation	6.5	6.7	6.8	7.3	6.3	6.7	100

Analysis of variance

Factor	D.F.	S.S.	M.S.	F.	
Cultivars	4	224.03	56.01	758.06	***
Error	20		0.74		
Total		225.51			

S.E. = $\pm$ 0.12

(8) O.S. - 74 - WG (19 November 1974) - b. Conidial germination

Cultivar	% germination Box means (2 discs/box)					
	1	2	3	4	5	Mean
Allgold	10.1	11.1	12.7	14.6	12.4	12.2
Frensham	14.7	18.8	16.5	20.2	20.1	18.0
Iceberg	17.8	15.0	19.9	17.2	13.4	16.6
Masquerade	11.0	8.7	9.2	14.0	11.9	11.0
Orange Sensation	19.0	12.3	23.5	22.1	25.8	20.5

Analysis of variance

Factor	D.F.	S.S.	M.S.	F.	
Cultivars	4	202.88	50.72	8.98	***
Error	20		5.65		
Total		315.88			

S.E. = $\pm$ 1.1

TABLE 20

GERMINATION OF D. ROSAE CONIDIA (F - 73 - Ash) IN WATER

ALLOWED TO STAND ON LEAFLETS OF FRENSHAM AND ALLGOLD FOR 24 HOURS

	% germination					
	Glass coverslips			Water agar		
	Control+	Frensham	Allgold	Control	Frensham	Allgold
1	16.6	46.7	43.3	65.4	66.2	71.6
2	19.1	38.0	47.7	63.9	58.8	65.7
3	20.4	41.9	38.8	57.3	64.6	70.1
4	15.3	40.0	38.3	55.7	66.1	66.7
5	21.0	38.3	40.2	61.4	64.1	70.3
6	20.7	50.0	40.2	63.3	70.6	68.6
7	17.0	47.8	35.9	63.0	70.8	61.3
8	16.0	37.8	42.7	61.7	67.3	62.5
9	22.9	39.8	39.8	55.8	64.3	63.0
10	18.2	43.0	37.5	58.8	62.1	56.0
Mean	18.7	42.0	40.5	60.6	65.5	65.6

Glass coverslips - Analysis of variance

Factor	D.F.	S.S.	M.S.	F.	
Treatments	2	3442.54	1721.27	138.63	***
Error	37		12.42		
Total		3777.78			

S.E. = $\pm$ 1.11

Difference between treatments (Duncan's new multiple range test - 1% level)

C A F

+ Key: for details see Table 36.

TABLE 21

GERMINATION OF D. ROSAE CONIDIA (F - 73 - Ash) IN WATER

ALLOWED TO STAND ON LEAFLETS OF ALLGOLD AND FRENSHAM

FOR 24 HOURS and 48 HOURS WITH OR WITHOUT CONIDIA

(1) 24 hours

Replicate	% germination				
	C^+	F	F + C	A	A + C
1	17.9	34.3	34.3	41.3	28.8
2	13.8	38.1	38.1	44.4	23.3
3	14.0	34.5	34.5	39.1	26.0
4	17.0	34.6	34.6	44.0	26.4
5	18.0	42.3	42.3	40.6	26.9
6	14.8	41.0	41.0	25.4	30.1
7	14.3	35.7	35.7	45.8	34.4
8	17.9	32.5	32.5	40.8	30.7
9	21.1	33.1	33.1	42.6	26.2
10	14.2	39.3	39.3	43.7	38.5
Mean	14.9	36.5	36.5	40.8	29.1

Analysis (based on angular transformation of% germination values)

Analysis of variance

Factor	D.F.	S.S.	M.S.	F.	
Treatments	4	1508.11	377.03	57.26	***
Error	45		6.59		
Total		1804.43			

S.E. = $\pm$ 0.81

Differences between cultivars (Duncan's new multiple range test)

	C	A + C	F + C	F	A
1% level					
5% level					

TABLE 21 (CONTD.)

(2) 48 hours

Replicate	% germination				
	C+	F	F + C	A	A + C
1	13.6	22.3	18.1	24.3	9.2
2	25.9	24.5	15.5	36.0	14.0
3	21.7	28.1	23.5	28.7	8.1
4	25.2	30.8	15.0	38.0	10.2
5	20.6	25.6	20.4	32.8	9.8
6	22.3	35.1	14.3	31.9	6.5
7	22.1	36.1	25.0	38.8	9.2
8	28.2	22.4	17.7	39.6	9.3
9	19.3	34.0	15.2	31.7	13.2
10	27.0	31.3	8.6	34.4	13.7
Mean	22.6	29.0	17.3	33.6	10.3

Analysis (based on angular transformation of % germination values)

Analysis of variance

Factors	D.F.	S.S.	M.S.	F.	
Treatments	4	1757.3	439.33	45.64	***
Error	45		9.63		
Total		2190.46			

S.E. = ± 0.98

Differences between treatments (Duncan's new multiple range test)

	A + C	F + C	C	F	A
1% level				———	
5% level					

+ Key: for details see Table 37.

TABLE 22

GERMINATION OF D. ROSAE CONIDIA (F - 74 - Ash) IN WATER

ALLOWED TO STAND ON LEAFLETS OF FRENSHAM AND MASQUERADE

FOR 48 HOURS WITH OR WITHOUT CONIDIA

Replicate	% germination				
	C+	F	F + C	M	M + C
1	47.6	50.8	47.0	48.4	34.0
2	52.6	41.6	46.2	43.1	36.3
3	53.6	46.9	43.4	48.9	30.4
4	50.3	52.7	47.3	45.3	41.2
5	49.0	52.9	39.7	44.8	28.4
6	47.2	47.6	45.0	45.9	37.8
7	48.8	46.8	45.2	44.0	35.7
8	61.3	45.7	46.4	43.2	30.6
9	64.0	52.1	46.5	48.7	36.1
10	56.6	53.3	45.2	50.0	35.3
Mean	53.1	49.0	45.2	45.8	

Analysis

Analysis of variance

Factor	D.F.	S.S.	M.S.	F.	
Treatments	4	1891.14	472.79	31.51	***
Error	45		15.0		
Total		2566.36			

S.E. = $\pm$ 1.23

Difference between treatments (Duncan's new multiple range test)

	M + C	F + C	M	F	C
1% level					
5% level					

+ Key: for details see Table 38.

TABLE 23

GERMINATION OF D. ROSAE CONIDIA (F - 74 - WG) IN WATER ALLOWED TO STAND ON LEAFLETS OF FRENSHAM AND ALLGOLD FOR 48 HOURS WITH OR WITHOUT CONIDIA

Replicate	% germination				
	C+	F	F + C	A	A + C
1	32.7	21.3	19.6	28.8	11.3
2	22.1	21.6	10.5	31.6	7.0
3	22.3	24.3	14.6	28.8	16.2
4	31.7	16.6	13.1	21.4	12.3
5	19.5	19.7	18.6	26.6	17.4
6	21.2	23.1	16.9	25.0	6.4
7	21.1	26.6	11.1	25.8	7.0
8	24.2	22.2	8.6	21.9	9.9
9	20.0	21.8	13.2	16.7	7.8
10	26.7	23.8	8.6	20.3	7.4
Mean	24.2	22.1	13.5	24.7	10.3

Analysis (based on angular transformation of % germination values)

Analysis of variance

Factor	D.F.	S.S.	M.S.	F.	
Treatments	4	1059.87	264.97	28.35	***
Error	45		9.35		
Total		1480.45			

S.E. = $\pm$ 0.97

Differences between treatments (Duncan's new multiple range test)

	A + C	F + C	F	C	A
1% level					
5% level					

+ Key: for details see Table 39

TABLE 24

GERMINATION OF D. ROSAE CONIDIA (F - 74 - Ash and F - 74 - WG)

IN WATER ALLOWED TO STAND ON LEAFLETS OF ALLGOLD

FOR 48 HOURS WITH CONIDIA (F - 74 - WG)

Replicate	% germination			
	F - 74 - Ash C+	F - 74 - Ash A + C	F - 74 - WG C	F - 74 - WG A + C
1	29.8	26.0	27.5	19.6
2	34.1	24.0	32.5	13.0
3	29.6	22.4	25.4	16.7
4	32.5	25.0	20.2	24.0
5	29.5	23.9	26.7	19.5
6	34.0	25.9	28.5	22.8
7	36.0	32.5	25.6	20.5
8	35.6	18.7	27.1	22.9
9	26.8	19.0	32.5	15.3
10	29.7	24.1	25.4	22.3
Mean	31.7	24.1	27.1	19.7

Analysis (based on angular transformation of % germination values)

Analysis of variance

Factor	D.F.	S.S.	M.S.	F.	
Treatments	3	340.51	113.5	20.02	***
Error	36		5.67		
Total		544.57			

S.E. = $\pm$ 0.75

Difference between treatments (Duncan's new multiple range test)

	F - 74 - WG A + C	F - 74 - Ash A + C	F - 74 - WG C	F - 74 - Ash C
1% level				
5% level				

+ Key: for details see Table 40.

TABLE 25

GERMINATION OF D. ROSAE CONIDIA (F - 74 - Ash) IN WATER

ALLOWED TO STAND ON LEAFLETS OF ALLGOLD AND FRENSHAM

FOR 48 HOURS WITH OR WITHOUT LIVE AND KILLED CONIDIA

Replicate	% germination						
	C+	F	F + C	F + C(k)	A	A + C	A + C(k)
1	52.9	44.9	51.0	55.0	56.7	24.2	22.5
2	55.2	45.8	52.0	42.5	56.3	37.8	30.4
3	49.6	45.0	49.0	54.8	49.5	34.8	27.3
4	48.5	45.1	47.9	45.0	50.0	37.2	35.1
5	56.1	46.4	44.3	52.8	54.6	36.2	38.1
6	48.1	49.6	51.0	46.7	53.5	30.8	29.9
7	51.4	46.5	43.3	55.1	50.7	34.9	32.0
8	50.3	47.1	42.9	40.0	50.4	35.0	27.9
9	49.6	45.7	37.6	37.1	49.6	34.5	34.1
10	53.8	52.5	35.0	38.0	42.57	28.2	28.3
Mean	51.6	46.8	45.4	46.7	51.4	33.4	30.6

Analysis

Analysis of variance

Factor	D.F.	S.S	M.S.	F.	
Treatments	6	4222.02	703.67	31.44	***
Error	63		22.38		
Total		5632.13			

S.E. = ± 1.5

Differences between treatments (Duncan's new multiple range test)

	A + C (k)	A + C	F + C	F + C (k)	F	A	C
1% level							
5% level							

+ Key: for details see Table 41.

TABLE 26

GERMINATION OF D. ROSAE CONIDIA (F - 74 - Ash) IN WATER

ALLOWED TO STAND ON LEAFLETS OF ALLGOLD AND FRENSHAM

FOR 48 HOURS WITH OR WITHOUT CONIDIA AND 'CONIDIA WATER'

Replicates	% germination						
	C+	F	F + C	F + C(w)	A	A + C	A + C(w)
1	34.2	40.5	36.1	31.7	39.1	23.2	23.8
2	30.8	35.4	34.5	33.3	37.3	25.0	22.9
3	36.9	34.6	32.2	31.4	38.8	24.3	22.0
4	35.2	31.4	38.1	31.9	43.8	32.5	28.3
5	38.3	39.1	30.9	33.8	44.5	30.0	21.5
6	34.9	34.9	39.0	34.6	38.1	29.6	22.1
7	30.1	34.6	30.7	30.5	44.0	24.6	27.8
8	33.3	38.7	33.3	33.3	35.9	25.5	22.0
9	37.6	36.6	33.6	30.0	41.4	23.6	24.8
10	29.0	35.6	35.8	36.3	39.6	28.8	25.6
Mean	34.0	36.1	34.4	32.7	40.3	26.7	24.1

Analysis

Analysis of variance

Factor	D.F.	S.S.	M.S.	F.	
Treatments	6	1836.99	306.16	39.04	***
Error	63		7.84		
Total		2331.0			

S.E. = ± 0.89

Differences between treatments (Duncan's new multiple range test)

	A + C (w)	A + C	F + C (w)	C	F + C	F	A
1% level			———				
5% level			———	———			

+ Key: for details see Table 43.

TABLE 27

U/V ABSORPTION SPECTRA OF SOME PREPARATIONS FROM LEAVES

(a) Original concentration

Wavelength (mm)	Absorbance		
	'Conidia water'	Frensham	Allgold+
320	0.1225	0.105	0.105
310	0.1375	0.115	0.115
300	0.145	0.125	0.12
290	0.154	0.138	0.135
280	0.1675	0.1515	0.15
270	0.18	0.155	0.165
260	0.19	0.19	0.1875
250	0.209	0.24	0.24
240	0.235	0.325	0.33
230	0.285	0.495	0.51
220	0.335	0.68	0.71
210	0.3825	0.72	0.75
200	0.355	0.51	0.51

(b) x10 concentration

Wavelength (mm)	Absorbance		
	'Conidia water'	Frensham	Allgold+
320	0.675	0.77	0.74
310	0.735	0.84	0.82
300	0.82	0.92	0.89
290	0.91	1.025	0.99
280	1.025	1.15	1.1
270	1.1	1.225	1.19
260	1.15	1.325	1.3
250	1.25	1.55	1.5
240	1.35	1.8	1.75
230	1.5	2.0	1.9
220	1.6	1.75	1.7
210	1.4	1.4	1.4
200	1.05	1.025	1.025

+ Key: 'Conidia water' - water in which conidia had germinated
Frensham and Allgold - water derived from a conidial suspension placed in Frensham and Allgold respectively.