A RIPE FRUIT ROT OF THE STRAWBERRY CAUSED BY A SPECIES OF *GLOEOSPORIUM*.

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SUMMARY.

A species of Gloeosporium **causing a ripe fruit rot of the strawberry is described.**

During the spring and summer, seasonal temperatures beyond a mean daily temperature of 65 deg. F. are shown to be an important factor influencing economic levels of fruit wastage in diseased strawberry plantings.

I. INTRODUCTION.

Within horticultural districts surrounding Brisbane, commercial strawberry plantings have suffered a varying wastage of ripe fruit due to a species of *Gloeosporium* (Sturgess 1954). The disease was recorded in the first instance by R. B. Morwood within a strawberry clonal trial at Redlands Experiment Station, Ormiston, during the spring months of 1951. It was not until the following year that serious fruit losses were observed; these were in a commercial planting at Manly. Further losses occurred in many other strawberry-growing areas around Brisbane during the spring and early summer months of the next three years.

No similar ripe fruit rot of the strawberry appears to have been recorded. On reviewing the literature, it was noted that two species of the closely related form genera, *Gloeosporium* and *Colletotrichum*, have been described as strawberry pathogens. One, *Gloeosporium fragariae* (Lib) Mont., was briefly described by Saccardo (1884). It has caused a strawberry leaf spot disease in France, Great Britain, Belgium, Germany and North America. The other is *Colletotrichum fragariae* Brooks, which produced a leaf anthracnose and wilt condition of the strawberry in Florida (Brooks 1931, 1933). Descriptions of the two diseases and the morphology of their causal organisms do not conform to those of the ripe fruit rot disease.

II. DESCRIPTION OF THE DISEASE.

Under field conditions, the early formation of the rot, which is restricted to mature strawberry fruit, is characterized by the development of one or more tan or light-brown, water-soaked lesions. Shrivelling of affected tissues which is associated with the mycelial penetration of the fruit results in a compact depressed lesion, together with an intensification of the colour from tan to dark-brown. This is followed by the eruption of pink spore masses from acervuli immediately below the surface. With further deterioration the diseased fruit remains firm and the end-point is complete mummification. Occasionally a soft breakdown occurs, owing to the entry of secondary soft rot organisms.

Strawberry stolons, leaf stalks, peduncles and pedicels are also affected by the disease. The symptoms on these appear as black, slightly depressed, longitudinal lesions.

III. ECONOMIC SIGNIFICANCE OF THE DISEASE.

The strawberry cropping season may be broadly divided into three phases of production, depending on local seasonal conditions (Fig. 4).

During the first phase the plants set a bulk of large fruit during late winter, with a production peak in the early spring. The second phase consists of a short period of poor flowering and fruit set which is confined to the mid-spring section of the cropping period. During the third phase, which extends from late spring to midsummer, the plants flower profusely and set a large number of small strawberries which are classed as jam berries. The fruit production rises to a second peak during early summer. Following the development of stolons and runner plants from parent plants, fruit production tapers off during midsummer.

In diseased plantings the large winter strawberries, which are sent to local and interstate markets or to canning factories, are free from the ripe fruit rot. Only the smaller jam strawberries, which are set during the second and third phases of production, are subject to fruit wastage from this disease.

In spite of the fact that it is the late-season factory fruit which is mostly affected, the disease may reach a level of economic importance. A number of individual fruit counts and disease estimates illustrate the seriousness of the disease during the later stages of fruit production (Tables 1 and 8). For example, at Ormiston on Oct. 28, 1952, 33 per cent. of the harvested fruit, including ripe and part-coloured, was diseased. Seventeen days later, the incidence increased to 36 per cent. of the total sample, and on that occasion 44 per cent. of the ripe fruit, excluding part-coloured fruit, was affected.

Table 1.

STRAWBERRY FRUIT LOSSES FROM GLOEOSPORIUM SP.

Date.	Maturity.	No. Healthy.	No. Diseased.	Percentage Diseased.
28–10–52	Ripe and part-coloured	441	213	33
6–11–52	Ripe	615	491	44
	Part-coloured	264	0	0

Whole consignments of strawberry fruit have been condemned on arrival at the fresh fruit markets due to the appearance and spread within them of the ripe fruit rot. Invariably such losses have occurred when part-coloured fruit from diseased plantings have been consigned to interstate and intra-state markets.

During periods of wet weather, strawberry plantings which are established on poorly-drained soils are subject to the grey mould rot caused by a species of *Botrytis*. Fruit losses due to grey mould may occur throughout the harvesting period and such losses incurred during the late winter and early spring may be of greater economic significance than the later fruit wastage caused by the species of *Gloeosporium*.

IV. THE CAUSAL ORGANISM.

(1) Isolation.

Isolations were made from fruit lesions, stolons, leaf stalks and fruit stalks by tissue planting and conidial dilutions on to potato dextrose agar. Two isolates of the genus *Gloeosporium* which differed in morphology and cultural characters were obtained. One isolate, designated isolate *a*, was consistently obtained from all diseased tissues of the strawberry. The second isolate, isolate *b*, was obtained only on four occasions from diseased fruit and from leaf stalks. The relationship of this isolate to the disease is doubtful, and apart from a description of its morphological characteristics it receives only minor treatment in the discussion which follows.

(2) Morphology.

Strawberry isolate a.—Two types of mycelium are formed: (1) hyaline, thin-walled, septate, branched mycelium, 1–3 μ in diameter; and (2) dark-brown, thick-walled, branched mycelium with septations at short intervals, 6 μ in diameter. Conidia and chlamydospores are produced. Abundant conidia, which are unicellular, non-septate and cylindrical, develop directly from the mycelium. Oil drops are frequently formed within the conidia. Natural conidia vary in size, the range being 12.9–13 9 μ by 3.2–3.9 μ (range of conidial means). Non-setose acervuli form as a result of the compaction of mycelial strands. Masses of conidia, salmon-pink in mass, arise directly from the mycelium and appear as the acervulus erupts. Chlamydospores, which are irregular in shape and 4–6 μ in diameter, are produced as the dark-brown, thick-walled mycelium ages.

Strawberry isolate b.—The morphology of strawberry isolate *b* is similar to that of strawberry isolate *a*, except that conidia are formed in limited numbers and the size range is 7.0–8.8 μ by 2.3–2.8 μ (range of conidial means).

(3) Cultural Characters.

In strawberry isolate *a*, the aerial mycelium, in young colonies on potato dextrose agar, is white, compact and somewhat aggregated; in older colonies, a typical grey overgrowth develops. The substratum is pink-cream in young colonies, and scattered black areas with a dusky diffusion develop in older colonies. Pink spore masses influence the colour of the substratum.

(4) Pathogenicity Tests.

Tests using spore and mycelial inoculum demonstrated the pathogenicity of the two isolates. Pathogenicity ratings based on rate of lesion spread and mycelial penetration under identical conditions, using mycelial inoculum, showed that isolate *a* was more pathogenic than isolate *b*. The results of the strawberry inoculation are detailed in Table 2 and illustrated in Figs. 1 and 2.

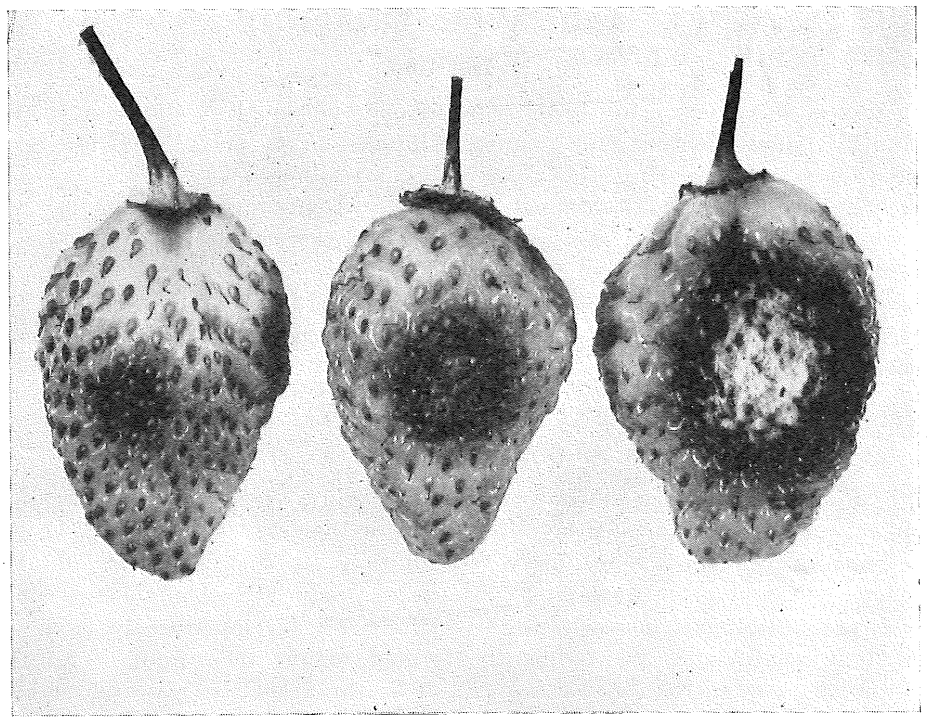

Fig. 1.

Strawberry Fruit Inoculated in the Laboratory with *Gloeosporium* Isolate *a*. Appearance of the disease at different stages of lesion development.

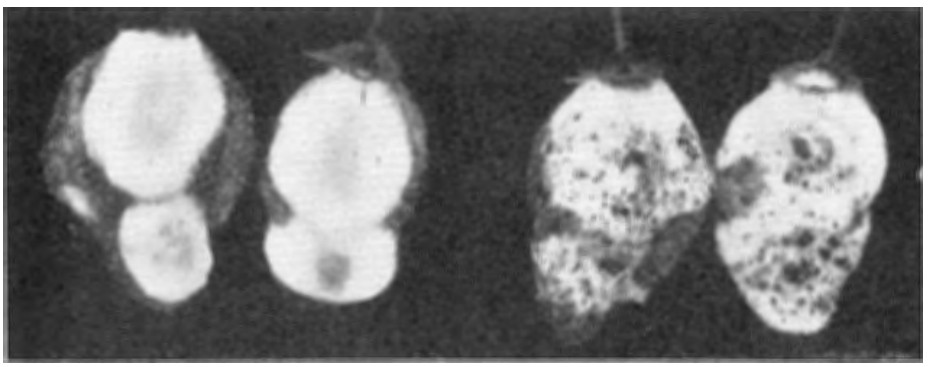

Fig. 2.

Laboratory Inoculation with *Gloeosporium* Isolate *a* (right) and Isolate *b* (left).

Table 2.

AVERAGE LESION DEVELOPMENT IN STRAWBERRIES INOCULATED WITH ISOLATES A AND B.

Maturity Group.	*Gloeosporium* Isolates.	Average Lesion Diameter (mm.) of 20 Fruit Inoculated.	
		4 days.	6 days.
Ripe fruit	*a*	6·2	15·0
	b	2·4	6·5
Green fruit (ripe in 3 days)	*a*	4·1	11·9
	b	1·7	3·6

(5) Cross-inoculation Studies.

For comparison with the strawberry organism two isolates of *Gloeosporium* from papaws naturally infected with ripe fruit rots were supplied by Mr. J. H. Simmonds, of the Science Branch. One of these, papaw isolate *a*, he considered to be morphologically similar to strawberry isolate *a*, while the other, papaw isolate *b*, was regarded as belonging to the *Glomerella cingulata* group.

(a) Strawberry Fruit Inoculations.

The strawberry and papaw isolates were tested for pathogenicity by inserting a P.D.A. culture into ripe fruit surface sterilized with 50 per cent. alcohol for 30–40 seconds. Papaw isolate *b* proved to be the most pathogenic isolate, while papaw isolate *a* and strawberry isolate *a* gave similar results in the cross-inoculation studies; these are summarised in Table 3.

Table 3.

RESULT OF INOCULATING GROUPS OF TWENTY STRAWBERRY FRUIT WITH FOUR GLOEOSPORIUM ISOLATES.

Gloeosporium Isolate.	Average Lesion Development.		Mycelial Growth Above Lesion.
	2 days.	5 days.	
Strawberry Isolate *a* ..	+	+++ ($\frac{1}{2}$)	Pinkish-white, 1–2 mm. elevated
Strawberry Isolate *b* ..	+	+++ ($\frac{1}{3}$)	Dense, white, 3–5 mm. elevated
Papaw Isolate *a* ..	+	+++ ($\frac{1}{2}$)	Pinkish-white, 1–2 mm. elevated
Papaw Isolate *b*	++	+++ (1)	Sparse, pinkish-grey, 0–$\frac{1}{2}$ mm. elevated

\+ White superficial mycelial *in situ* and advancing.
++ Lesion commencing to form.
+++ Typical sunken lesion with mycelial overgrowth.
(0–1) Approximate surface areas of fruit affected.

Re-isolation from the lesions confirmed the presence of the inoculated isolates.

(b) Papaw Fruit Inoculations.

Strawberry isolate *a*, papaw isolate *a* and papaw isolate *b* produced similar ripe fruit rot lesions when inoculated on a random basis into surface cuts on ripe papaw fruits. Strawberry isolate *b* was unable to form a typical lesion when inoculated into the same papaw fruits. The mycelium either remained dormant *in situ* or formed a small, dry, superficial lesion with little or no penetration. The results of the cross-inoculation into papaw fruits are summarised in Table 4. The inoculated organism was re-isolated.

Table 4.

RESULTS OF INOCULATING PAPAW FRUITS WITH FOUR GLOESOSPORIUM ISOLATES.

Gloeosporium Isolate.	No. of Inoculation Sites.	Dormant Mycelium *in situ*.	Slight Penetration of Tissues.	Ripe Fruit Rot Lesion.
Strawberry Isolate *a*	46	1	0	45
Strawberry Isolate *b*	99	51	40	0
Papaw Isolate *a*	41	0	0	41
Papaw Isolate *b*	19	0	0	19

(c) Comparison of Spore Sizes.

Conidia of strawberry isolate *a*, papaw isolate *a* and papaw isolate *b*, which developed on three different sporulation substrates (P.D.A. and inoculated strawberry and papaw fruit), were used. Five groups of 30 spores were measured in each case; the range of means and overall mean values are summarised in Table 5.

Table 5.

SUMMARY OF SPORE MEASUREMENTS (μ).

Gloeosporium Isolate.	Length.			Width.		
	P. D. A.	Strawberry.	Papaw.	P. D. A.	Strawberry.	Papaw.
Strawberry Isolate *a* . .	(10·2–11·8) 10·8	(10·9–11·5) 11·2	(10·5–13·2) 11·9	(2·8–3·3) 3·1	(3·4–3·7) 3·5	(2·8–3·4) 3·2
Papaw Isolate *a*	(9·9–10·9) 10·5	(10·2–12·4) 11·1	(9·8–12·1) 10·9	(2·8–3·2) 3·0	(3·5–3·7) 3·6	(3·0–3·4) 3·2
Papaw Isolate *b*	(13·1–14·8) 13·9	(12·7–15·7) 14·1	(14·2–15·5) 14·7	(3·3–3·4) 3·3	(3·8–4·5) 4·2	(4·0–4·3) 4·1

The mean values were analysed statistically to determine the significance of differences between the three isolates. There were no significant differences in length or width of strawberry isolate *a* and papaw isolate *a* on the same medium and overall media. Papaw isolate *b* significantly exceeded the other two isolates in length and width at the 1 per cent. level.

As a result of the cross-inoculation studies with four isolates of *Gloeosporium* and the comparison of spore measurements, it is concluded that strawberry isolate *a* and papaw isolate *a* are identical organisms and cause ripe fruit rots of the strawberry and papaw.

(6) Taxonomy.

Frequent examinations of naturally infected strawberry tissues did not reveal the perfect stage of the causal organism. It was not formed in laboratory culture nor was it induced by applying the technique described by Chona and Srivastava (1952).

In view of the results of the genetic studies of Chilton and Wheeler (1949), it is conceivable that the perfect stage of the strawberry isolates does not exist in nature. They found that conidial strains of *Glomerella cingulata* which do not possess genes or gene factors necessary for the development of the perithecial stage arise in culture as a result of gene mutations. A similar condition may arise in nature with the species of *Gloeosporium* causing the ripe fruit rot of the strawberry.

In the absence of the perfect stage it is not considered desirable to assign a binomial to the conidial stage without further investigating possible relationships with other species belonging to the form genus *Gloeosporium*.

(7) Latent Infection.

Circumstantial evidence noted throughout the strawberry ripe fruit rot investigations strongly suggested a mechanism for latent infection by spores of the strawberry organism. A latent infection in various fruit rot organisms has been postulated by a number of workers, while Simmonds (1940) illustrated the mechanism involved in the case of *Gloeosporium* spp. on banana, mango and papaw.

Following the technique developed by Simmonds, three groups of 30 green, fully developed fruit were surface-sterilized and treated as follows:— (1) incubated at 25·5 deg. C.; (2) immersed in a conidial suspension of strawberry isolate *a*, then one hour later surface-sterilized with 1/1,000 mercuric chloride; and (3) immersed in a conidial suspension, then incubated for 27

Table 6.

LATENT INFECTION STUDIES WITH STRAWBERRY FRUIT.

Group.	Healthy Fruit.	Ripe Fruit Rot (*Gloeosporium* sp.)
Uninoculated; surface sterilized	29 (1 *Botrytis* sp.)	0
Conidial suspension; surface sterilization	29	1
Conidial suspension; 27 hours' incubation; surface sterilization	10	20

Fig. 3.

Six Days' Growth of Strawberry Isolate *a* on P.D.A. Slopes at a Series of Temperatures (°C.).

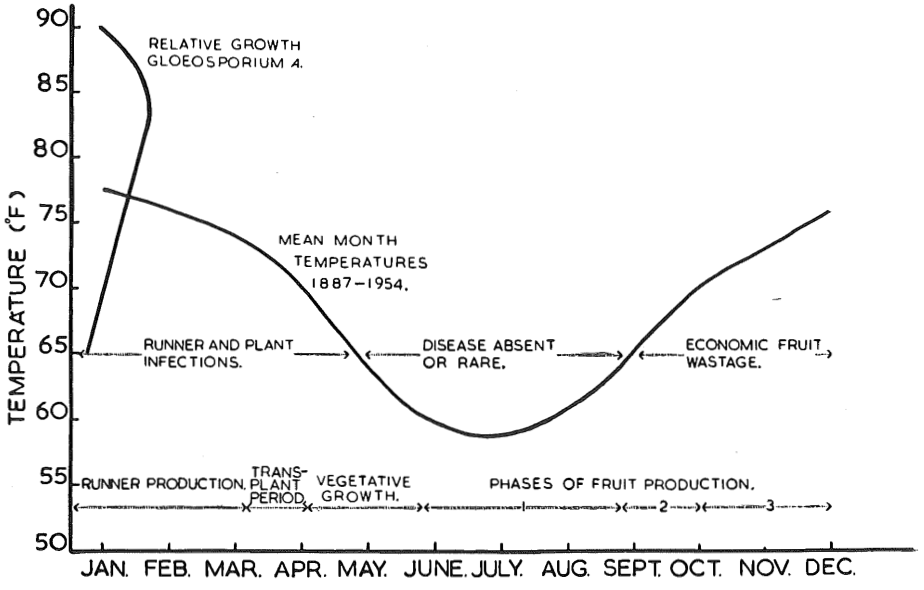

Fig. 4.

Correlation of Strawberry Ripe Fruit Rot Incidence with Seasonal Fruit Production and Temperature.

hours at 25·5 deg. C., then sterilized by mercuric chloride and further incubated at 25·5 deg. C. The fruit were examined when ripe five days later. The results, which are summarised in Table 6, suggest that spores are able to penetrate the epidermis of the green fruit within 27 hours after inoculation and form typical lesions when the fruit are ripening.

(8) Temperature Studies.

Single-spore colonies of the two strawberry isolates were cultured in small, flat-sided bottles containing 15 ml. P.D.A. The bottles were incubated in a multiple temperature incubator at 13 different temperatures from 14 deg. to 33·5 deg. C., and growth measurements were recorded daily for seven days. Over this temperature range the growth-temperature curves for each isolate were similar, although the growth rate of strawberry isolate *a* exceeded that of strawberry isolate *b*. Mycelial growth steadily increased with increasing temperature from 14·0 deg. C. (57·2 deg. F.) to the optimum temperature of 28·0 deg. C. (82·4 deg. F.), and beyond that temperature mycelial growth rapidly decreased with increasing temperature. The growth-temperature relationships of strawberry isolate *a* are illustrated in Figs. 3 and 4.

P.D.A. cultures of strawberry isolate *a* were able to withstand temperatures below freezing point. Spores and mycelium remained viable when exposed to —35 deg. C. for three days. When heated for 10 minutes in a constant temperature water-bath, strawberry isolate *a* was shown to have an upper thermal death point between 50 deg. and 60 deg. C.

Groups of 10 ripe strawberry fruit surface-sterilized in 50 per cent. alcohol were immersed in a conidial suspension of strawberry isolate *a* and incubated at different temperatures. At a temperature of 27·8 deg. C., which approximates the optimum temperature for mycelial growth, strawberry isolate *a* initiated a ripe fruit rot lesion in two days and complete mycelial penetration followed in a further 2–3 days. Table 7 details the results for lesion initiation and mycelial penetration of ripe fruit when incubated at temperatures ranging from 15·5 deg. to 27·8 deg. C.

Table 7.

PERIOD REQUIRED FOR LESION INITIATION AND COMPLETE MYCELIAL PENETRATION OF STRAWBERRY FRUIT AT VARIOUS TEMPERATURES.

Temperature. °C.	(°F.)	Lesion Initiation (days).	Complete Mycelial Penetration (days).
15·5	(59·9)	7	11–14*
17·0	(62·6)	6	9–11
18·6	(65·5)	5	9–10
21·2	(70·1)	4	6–7
24·8	(76·6)	3	4–6
27·8	(82·0)	2	4–5

* Range indicates time taken from first to last fruit in the groups of 10.

V. FIELD CONDITIONS INFLUENCING THE SEASONAL OCCURRENCE OF THE DISEASE.

The absence of the strawberry ripe fruit rot each year during the winter period of fruit harvest and the outbreaks of the disease which followed the appearance of localised traces of infection during the early spring directed attention towards the prevailing climatic conditions. The fluctuating order of disease incidence from the spring until the end of summer suggested that the increasing seasonal temperatures were an important factor conditioning the development of the disease.

Twelve estimates of fruit losses which were made in field plantings during the years 1952 to 1954, together with associated meteorological data, are summarised in Table 8. The mean daily temperatures are averaged for the week preceding each estimate of the disease, and the amount of rain and number of wet days for the fortnight preceding each estimate are given. The records of the disease were taken from diseased strawberry plantings located within a radius of 20 miles of the Brisbane Meteorological Station.

From Table 8, a mean daily temperature of 65 deg. F. (18·3 deg. C.) was selected as the critical temperature above which economic levels of fruit wastage (greater than 10 per cent. loss) may be expected to occur. In Table 9, the mean monthly temperature for 1951–1954 shows that the months of June, July and August experience temperatures below the arbitrary level of 65 deg. F., and apart from the last week or two in August, the disease has not been observed during that period of early fruit production. Figure 4 summarises the relationship of ripe fruit losses to temperature.

Table 8.

STRAWBERRY RIPE FRUIT ROT ESTIMATES AND METEOROLOGICAL DATA.

Date.	Fortnightly Total.		Weekly Average Mean Daily Temp. (°F.).	Fruit Rot. (%)	District.
	Rainfall (in.).	Wet days.			
24–8–54	4·10	5	63	Trace	Sunnybank Wellington Point
1–9–53	3·50	5	62	3	Mitchelton
10–9–53	3·70	5	64	Trace	Ormiston
4–10–54	1·30	7	65	60	Manly
22–10–52	1·10	5	68	77	Ormiston
28–10–52	1·10	6	68	33	Ormiston
6–11–52	·80	5	79	44	Ormiston
11–11–54	3·00	8	71	>50	Brisbane
1–12–53	2·20	4	76	>50	Brisbane
7–12–53	2·20	4	75	20	Mount Gravatt
21–12–54	1·20	5	74	>50	Sunnybank
22–12–52	·90	4	74	Trace	Ormiston

Table 9.

MEAN MONTHLY TEMPERATURES (°F.)—BRISBANE (1951–1954.)

Month.	1951.	1952.	1953.	1954.	1887–1954.
January	73	79	74	74	77
February	75	76	75	76	76
March	74	74	73	77	74
April	68	71	72	71	70
May	62	64	65	65	64
June	61	61	60	60	60
July	58	60	59	62	59
August	59	62	60	61	61
September	64	65	65	64	65
October	69	69	70	68	70
November	74	75	74	73	73
December	76	76	78	76	76

Another field condition affecting the strawberry microclimate during the susceptible period of the year is plant humidity, which is influenced by the amount and duration of rainfall, dews and the prevailing winds. Although with few exceptions overhead irrigation is applied to strawberry plantings in the districts surrounding Brisbane, serious outbreaks of the disease tend to follow rainfall which extends over several days. Given suitable temperature conditions, rainfall appears to be associated with the outbreaks of strawberry ripe fruit rot, but rainfall alone is clearly insufficient.

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