THE LONGEVITY OF CONIDIA OF *SCLEROTINIA FRUCTICOLA* (WINT.) REHM UNDER FIELD CONDITIONS

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Summary

The longevity of conidia of *S. fructicola* was determined in an orchard environment. Less than 1% of conidia remained viable after exposure for 8 days in the tree canopy. In addition to reducing viability, exposure reduced both germ tube length and infection of mature fruit. Conidia prepared in a suspension in sterile water, to simulate those that are dispersed in rain, lost viability more rapidly on exposure than conidia that remained dry. When conidia were in contact with unsterilized soil for 24 hr they also lost viability.

A species of *Bacillus*, probably *B. cereus* Frankland & Frankland, was associated with exposed conidia. *B. cereus* was antagonistic to *S. fructicola* in plate cultures, and when introduced to the surface of mature fruits brown rot development was reduced. Cell-free filtrates prevented germination of conidia or caused germ tube aberrations.

The role of bacterial antagonism in the inactivation of conidia of *S. fructicola* and its significance on the build-up of inoculum on the fruit surface are discussed.

I. INTRODUCTION

The fungus *Sclerotinia fructicola* (Wint.) Rehm, which causes brown rot of stone fruits in Australia, is known to overwinter in mummified fruits, in infected fruit penuncles, and in wood cankers. Conidia are produced from these sites in the spring and give rise to infection of blossoms and green fruit. On these, in turn, sporulation occurs, thereby increasing the spore inoculum for subsequent infection of maturing fruits. Prolific numbers of conidia are produced at all sites of infection; these are dispersed aerially and in rain splash droplets (Jenkins 1965; Kable 1965).

Studies of longevity *in vitro* indicated that conidia of *S. fructicola* retain viability for several months when held under suitable conditions of temperature and humidity (Naqvi and Good 1957). The investigations reported in this paper were concerned with the longevity of conidia within the tree canopy and on the surface of the orchard floor. The apricot and peach fruits used in the experiments were from an area in north-western Victoria, where brown rot had not occurred, and thus were free of contamination with conidia and of latent infections.

Conidia harvested with a cyclone collector from 7-day-old potato dextrose agar plate cultures were used to simulate those which are aerially dispersed and deposited. A suspension of conidia in sterile water was used to simulate those which are dispersed in rain droplets.

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II. Experimental Procedure and Results

(a) *Effect of Exposure on Viability of Conidia*

Conidia of *S. fructicola* were introduced on 6 by 1 cm strips of glass fibre tape (Caldwell 1958) to various exposed and shaded sites within the tree canopy and samples of tape approximately 1 cm long were taken from each of four of these sites every 24 hr for 8 days. The conidia attached to the tapes were examined directly, and viability was determined using 0·1% orange juice made up in sterile distilled water as germinating medium, or by plating directly on to potato dextrose agar.

Percentages of viable conidia after periods of exposure of 0, 1, 2, 4, and 8 days within the tree canopy were 100, 100, 90, 80, and 1 respectively. After 2 days conidia were contaminated with bacteria, and the germ tubes were ensheathed with bacteria after germinating. After 4 days the contents had retracted from the walls of approximately 20% of conidia. The germ tubes of those which did germinate were ensheathed with bacteria, and lysis was observed. After 8 days conidia were difficult to find. Bacteria were present on those that could be distinguished and lysis was again observed.

(b) *Viability and Infectivity of Conidia Deposited within the Tree Canopy*

In three experiments carried out on different days in summer, simulated aerially dispersed conidia and conidia in suspension in sterile water were deposited on mature apricot fruits in exposed and shaded positions in an orchard. After periods ranging from 1 to 24 hr, the fruits were misted with water, and incubated at 25°C and 100% R.H. Brown rot development in the fruit was measured after 48 hr. The viability of conidia on glass fibre tapes placed adjacent to each group of fruit was determined after the same exposure periods as the fruits.

The ability of aerially dispersed and splash-dispersed conidia to germinate and infect mature apricot fruits is shown in Table 1. More detailed analysis of the results of experiment 1 show that the viability of conidia decreased with increasing period of exposure, and that the viability also decreased if fruits were in a shaded location and if the conidia were initially moistened to simulate splash dispersal, as shown in the following tabulation (initial viability of conidia = 100%):

	% Viable		% Viable		% Viable
Exposed	74 (1·036)	Wet	43 (0·711)	Exposure period 2 hr	82 (1·137)
Shaded	45 (0·737)	Dry	76 (1·062)	Exposure period 4 hr	35 (0·636)

Values in parenthesis are transformed values [arc sin $V^{\frac{1}{2}}$, where V is the percentage viability]. For differences to be significant at the 1% level, these values must differ by at least 0·062.

In experiment 2, examination of the glass fibre tapes showed that there was lysis of germ tubes of wet conidia which had been exposed for 4 hr, but there was no lysis in the case of dry exposed, shaded wet, or shaded dry conidia after the same period.

In experiment 3, the germ tubes of wet conidia exposed for 18 hr were completely lysed. Dry conidia were contaminated with bacteria, but some long germ tubes developed. After 24 hr, no viable wet spores were detected in either exposed or shaded positions; less than 5% of dry spores were viable, and the lengths of their germ tubes were less than 100 μ after 24 hr in the germinating medium.

(c) *Suitability of Orchard Soil as Substrate for* S. fructicola

As a preliminary to determining the fate of conidia falling on to the orchard floor, it was established that conidia could germinate and establish colonies in sterilized orchard soil. Soil taken from the floor of a peach orchard in northern Victoria was sterilized in 250 c.c. Erlenmeyer flasks by autoclaving. The soil was a sandy clay loam, pH 5·3, with a moisture content of approximately 10% (w/w).

TABLE 1

INFECTIVITY OF CONIDIA ON MATURE APRICOT FRUITS LOCATED IN EXPOSED AND SHADED POSITIONS IN AN ORCHARD

Simulated splash-dispersed and air-dispersed conidial treatments are designated "wet" and "dry" respectively. C, control (fruits untreated)

Conidial Treatment of Fruit	Position in Orchard	No. of Fruit	No. of Fruit with Brown Rot after Exposure Period (hr) of:						
			0	1	2	4	6	18	24
			Experiment 1						
Wet	Exposed*	16	15		4	7	4		
Wet	Shaded†	16			16	15	16		
Dry	Exposed	16	15		12	16	16		
Dry	Shaded	16			16	16	16		
C	Both	16	0				0		
			Experiment 2						
Wet	Exposed	16	15		7	1	2		
Wet	Shaded	16			10	11	8		
Dry	Exposed	16	13		8	10	5		
Dry	Shaded	16			15	14	13		
C	Both	16	0				0		
			Experiment 3						
Wet	Exposed	16	16	5	1			0	0
Wet	Shaded	16		5	1			0	0
Dry	Exposed	16	16	8	4			2	1
Dry	Shaded	16		13	8			8	4
C	Both	16			0				0

* Received full radiation from the sun.

† Constantly shaded from direct radiation.

The sterilized soil was seeded with a disk of an actively growing culture of *S. fructicola* on potato dextrose agar, and incubated at 25°C. After 9 days, there was prolific growth of the fungus over the surface of the soil. To overcome the possible influence of the original disk of inoculum, crumbs of soil taken as far as possible from the site of the seeding were used to seed further flasks of sterilized soil. After 9 days, prolific growth was again observed over the surface of the soil. Positive identification as *S. fructicola* was established by culturing and examining the resulting isolates.

The same procedure was followed using soil from the same location, but without prior sterilization. After 9 days there was no obvious growth beyond the site of inoculation. Soil particles plated directly on potato dextrose agar did not yield isolates of *S. fructicola*, but there was considerable growth of other fungi and bacteria.

(*d*) *Longevity of Conidia Deposited on the Orchard Floor*

Suspensions of conidia were added respectively to the surfaces of unsterilized and sterilized soil held in Erlenmeyer flasks. After 24 hr, re-isolation of *S. fructicola* was attempted using fruit traps. Plugs of tissue were taken from mature peach and apple fruits with a sterile cork borer (0·25 in. diam.); the resultant holes were filled with the soil and sealed with Vaseline. Some of the fruits were incubated at 25°C, which is close to the optimum for growth of *S. fructicola*, but at which temperature other fruit-rotting fungi, including *Rhizopus nigricans*, also grow well. Others were incubated at 3°C, at which temperature *S. fructicola* continues to grow, but *R. nigricans* and the other fruit-rotting fungi do not.

Brown rot developed in fruit traps which had been inoculated with sterilized soil and conidia, and incubated at either 25°C or 3°C; *S. fructicola* was confirmed by culturing and examining the resulting isolates. In the fruit traps inoculated with unsterilized soil and conidia and incubated at 25°C, fruit rots were caused by *Rhizopus nigricans* or *Penicillium* sp. In those incubated at 3°C, no rots developed.

Conidia on glass fibre tapes which had been introduced into sterilized soil were viable after 20 days. Those in unsterilized soil for 1 day failed to germinate, and lysis was observed. Bacteria were also present. After 5 days, conidia could not be detected.

The bacteria associated with the lysis of conidia and germ tubes were isolated by streaking previously exposed conidia on to nutrient agar. The resultant colonies were subcultured and maintained on nutrient agar slopes. The bacterium most frequently isolated from buried conidia and those exposed in the tree canopy was a *Bacillus* species, probably *B. cereus* Frankland & Frankland.

(*e*) *Effect of* Bacillus *sp. on* in vitro *Development of* S. fructicola

The following methods were used to determine the effects of *Bacillus* sp. on development of *S. fructicola*. Disks (approx. 1 cm diam.) of a culture of *S. fructicola* were placed centrally on six 9-cm Petri plates containing potato dextrose agar, and 0·3-cm disks of the *Bacillus* sp. were placed 2 cm away on either side. The plates were incubated at 25°C. The extent of growth of *S. fructicola* along the axis in common to the disks of *Bacillus* sp. and along the axis at right angles were measured at intervals, and the results expressed as percentage inhibition.

Twelve flasks, each containing 100 ml of a basic liquid medium [comprising dextrose 20 g; asparagine 2 g; K_2HPO_4 1 g; $MgSO_4$ 0·5 g; KCl 0·5 g; distilled water 100 ml (Vasudeva, Jain, and Nema 1952)] were uniformly seeded with *Bacillus* sp. and incubated at 30°C. After 5 days, the contents of six of the flasks were separately filtered through glass fibre and then through Millipore filters of pore diameter 0·45 μ. Each filtrate was halved, and the one-half autoclaved at 120°C and 15 lb/in^2. Flasks of unseeded basic medium were similarly treated. One millilitre of a suspension of conidia of *S. fructicola* in distilled water was added to 1 ml of each filtrate, and after incubation for 24 hr at 25°C viability and germ tube development were measured.

Colonies of *Bacillus* sp. inhibited the growth of *S. fructicola* on potato dextrose agar (Fig. 1). The percentage inhibition after 2, 4, and 6 days at 25°C was 10, 38, and 56 respectively.

Culture filtrates of *Bacillus* sp. inhibited germination of conidia, caused aberrant germ tube growth, and decreased germ tube length (Figs. 2 and 3). Average germination was 93% for autoclaved filtrate and 31% for filtrate that was not autoclaved. [Corresponding transformed values (arc sin $G^{\frac{1}{2}}$, where G is percentage

germination) are 1·303 and 0·593. Differences required for significance at the 5% and 1% levels are 0·123 and 0·174 respectively.] Germination of conidia in basic medium whether autoclaved or not was 100%. Similarly, average germ tube lengths were 50 and 30 μ respectively for autoclaved and non-autoclaved filtrate (differences

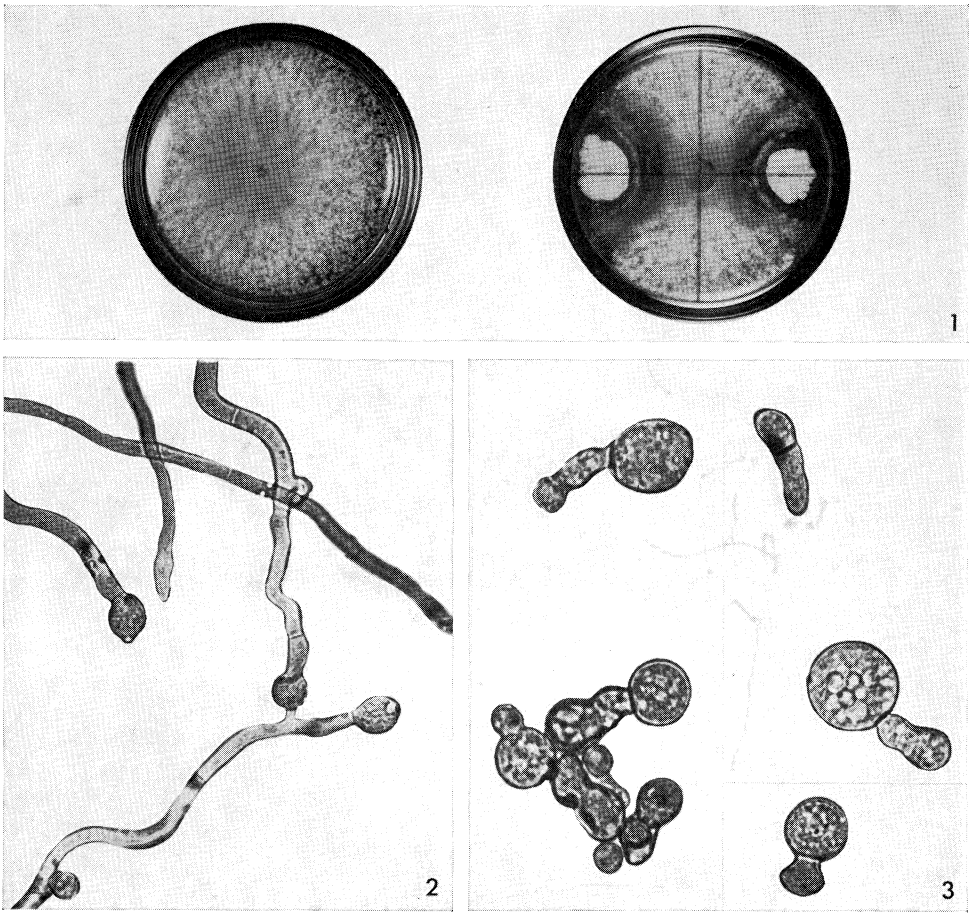

Fig. 1.—Left, normal growth of *S. fructicola*. Right, antagonism of *B. cereus* to *S. fructicola*.

Fig. 2.—Normal germination of conidia of *S. fructicola* in basic media (24 hr at 25°C).

Fig. 3.—Aberrant germ tubes of *S. fructicola* in filtrate of *B. cereus* culture in basic media (24 hr at 25°C).

required for significance at the 5% and 1% levels are 15 and 21 μ respectively). Average germ tube lengths of conidia germinating on basic medium were 800 μ, irrespective of whether the medium was autoclaved or not. A toxic component of the cell-free filtrates produced by *Bacillus* sp. was heat stable.

(*f*) *Effect of* Bacillus *sp. on Development of Brown Rot on Peach Fruits*

To determine the effect of *Bacillus* sp. on brown rot development, simulated air-dispersed (dry) and splash-dispersed (wet) conidia of *S. fructicola* were placed on marked areas on uninjured surfaces of mature peach fruits. Half the number of sites of inoculation had been contaminated with a suspension of *Bacillus* sp. in sterile water and allowed to dry prior to treatment with conidia.

Groups of fruit were treated in the following ways: inoculation with dry conidia; dry inoculation plus *Bacillus* sp.; wet inoculation; wet inoculation plus *Bacillus* sp.; *Bacillus* sp. only; no treatment.

Half of the fruits were injured by pricking with a sterile needle at the sites of inoculation immediately after treatment with conidia and incubated for 48 hr at 25°C and 100% R.H. The remaining fruits were allowed to stand exposed to direct radiation from the sun. After exposure for 24 hr, they were also injured, then incubated for 48 hr. After incubation, numbers of fruit that developed brown rot and the average diameters of rotted areas were measured.

The presence of *Bacillus* sp. on the surface of mature peach fruits which had been inoculated with conidia of *S. fructicola* resulted in a reduction of brown rot development. The results of two experiments are presented in Table 2.

TABLE 2

DEVELOPMENT OF BROWN ROT IN PEACHES INOCULATED WITH *BACILLUS* SP. AND DRY AND WET CONIDIA

Values in parenthesis are logarithms used for statistical analysis

Treatment of Fruit	No. of Fruit	No. of Fruit with Brown Rot		Av. Diam. (cm) of Rotted Area			
		Not Exposed	Exposed 24 hr	Not Exposed		Exposed 24 hr	
		Experiment 1					
Dry conidia	20	20	18	2·6	(0·415)	3·1	(0·486)
Dry conidia plus *Bacillus* sp.	20	20	12	1·3	(0·100)	0·5	(−0·326)
Diff. for significance, 1% level					(0·012)		(0·015)
Wet conidia	20	20	18	1·0	(0·015)	1·0	(0·015)
Wet conidia plus *Bacillus* sp.	20	13	13	0·6	(−0·263)	0·5	(−0·326)
Diff. for significance, 1% level					(0·008)		(0·016)
Bacillus sp. only	20	0	0		—		—
Controls	20	0	0		—		—
		Experiment 2					
Dry conidia	10	10	10	2·7	(0·432)	2·8	
Dry conidia plus *Bacillus* sp.	10	10	2	2·0	(0·310)	1·2	
Diff. for significance, 5% level					(0·009)		
Diff. for significance, 1% level					(0·012)		
Wet conidia	10	10	0	2·3	(n.s.)		—
Wet conidia plus *Bacillus* sp.	10	10	0	2·4			—
Bacillus sp. only	10	0	0		—		—
Controls	10	0	0		—		—

The effect of *Bacillus* sp. on brown rot development was greater for wet conidia than for dry conidia. There was an even more marked effect when *Bacillus* sp. and conidia were present on the fruit surface for 24 hr before conditions favourable for infection were imposed.

III. General Discussion and Conclusions

Conidia of *S. fructicola* are produced in abundance on mummified stone fruits and infected blossom tissue throughout the fruit-growing season, and are then dispersed in air and in splash droplets and deposited on the surfaces of developing fruits. Although conditions may occur which are favourable for germination, infections are seldom observed until the fruits approach picking maturity.

Haller (1952) posed the question of what becomes of spores that lodge on the surface of immature peaches, but fail to infect them, under conditions favourable for spore germination? Wade (1956) had demonstrated the occurrence of latent infections in green apricots, and Jenkins and Reinganum (1965) reported on the occurrence of quiescent infections in apricots and peaches. Jerome (1958) postulated that air-borne conidia, deposited on the fruit surface and concealed among the hairs of the surface, accumulate gradually and constitute a latent contamination of the immature fruit. She presented evidence that conidia are able to withstand extremes of temperature and humidity for quite long periods *in vitro* and remain viable.

Similarly Naqvi and Good (1957) demonstrated that conidia of *S. fructicola* show very considerable resistance to desiccation during storage in a wide range of temperatures; the drying withstood by the conidia was more drastic than would be encountered in nature. They concluded that it is probable that dehydration is not a significant factor in the inactivation of these spores, but that humidity is a more likely factor influencing senescence.

Grindle and Good (1961) demonstrated that germinated conidia *in vitro* are capable of withstanding at least one prolonged period of drying and can still retain their ability to grow, when suitable conditions return.

In the field, however, there are factors operating other than temperature, humidity, and moisture. Thanos (1951) showed that ultraviolet radiation significantly reduced viability of conidia, and Shepherd (unpublished data) demonstrated the inactivating effects of ultraviolet radiation of intensities likely to be experienced by exposed conidia on peach fruits in orchards in the Murrumbidgee Irrigation Area of New South Wales in midsummer.

From the results of the experiments reported herein it appears that biological factors also can influence longevity and infectivity.

The failure of *S. fructicola* to establish or persist in natural soil could be expected; Lockwood (1960) included *S. fructicola* in tests using mycelia of soil-inhabiting, and non-soil-inhabiting, plant-pathogenic fungi, and demonstrated complete lysis of living mycelium and almost complete lysis of dead mycelium by soil extracts.

The effects of specific bacteria as antagonists to *S. fructicola* were reviewed by Wormald (1954). He referred to the work of Michener and Snell (1949) and of Keil (1950). The former found that *Bacillus subtilis* cultures secrete two substances antibiotic to a number of fungi including *S. fructicola*; the latter reported that two bacteria, *Sporobacterium fungostaticum* and *Bacillus antimycoides*, isolated from air, strongly inhibited growth of *Monilia cinerea* on molasses agar cultures.

Bacillus cereus, which is widely distributed in soil, dust, and on plant surfaces [see "Bergey's Manual of Determinative Bacteriology," 7th Ed., p. 617 (1957)], was found to be a common contaminant of buds and fruits in orchards in Victoria.

This species of *Bacillus* is antagonistic to members of Dematiaciae (Kingsland 1965), and causes fungistatis, germ tube aberrations, and inhibition of vegetative development of *Helminthosporium specificerum*.

Mitchell and Alexander (1963) found that the enzymes chitinase and laminarinase were associated with the mycolysis of mycelium of *Fusarium oxysporum* by *B. cereus*.

The difficulties in identification of *B. subtilis*, arising from variation in cultural characteristics exhibited by various strains, the distribution of mislabelled cultures, and confusion with *B. cereus* are known (see "Bergey's Manual", p. 620). It is possible that some of the inhibitory effects reported for *B. subtilis* should be attributed to *B. cereus*.

Last (1955, 1961) proposed the term "phyllosphere" for populations of saprophytes on the surfaces of plants, and he referred to the presence of leaf-inhabiting microorganisms which are antagonistic to other microorganisms.

The results of work on longevity of conidia in orchards in Victoria reported herein demonstrate the presence of at least one microorganism common to the soil and the phyllosphere which is antagonistic to *S. fructicola*.

There was a marked effect of exposure on viability of conidia of *S. fructicola*, and, in addition to reducing viability, exposure reduced germ tube length and infection of mature fruit. The loss of infectivity of conidia had been observed in inoculation experiments with green apricot fruits. Trevatt apricots, inoculated with a suspension of conidia in talc approximately 4 weeks after petal fall, developed quiescent infections when rain followed 2 days after inoculation (Jenkins and Reinganum 1965). No infection occurred at sites inoculated 14 or 21 days previously although 100% viable spores had been used for the inoculation.

Thus, notwithstanding the apparent resistance of conidia of *S. fructicola* to desiccation and temperature extremes as indicated by experiments conducted *in vitro*, in orchards in Victoria the conidia are relatively short lived.

In view of the results of these experiments, it is unlikely that conidia produced during the summer and autumn on infected mature fruit are of any consequence in initiating blossom infection in the spring. Similarly, it is unlikely that there is a long-term build-up of inoculum, without infection, on the fruit surface during the period of fruit growth.

Latent or quiescent infections of green fruits may result following germination of newly produced and deposited conidia. Ungerminated conidia, or germinated conidia which do not infect, are subject to environmental factors resulting in desiccation and inactivation through ultraviolet radiation, and to the effects of bacterial antagonists.

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