THE DISTRIBUTION OF SOOTY-MOULD FUNGI AND ITS RELATION TO CERTAIN ASPECTS OF THEIR PHYSIOLOGY.

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(Plate iii; twelve Text-figures.)

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A sooty-mould colony usually consists of a number of different species growing together, as has been described in a previous paper (Fraser, 1933). The constituent fungi may be indiscriminately mixed, or may be more or less segregated. On a leaf or on adjoining leaves there may be colonies of a single species, and in other places several may be growing together.

The appearance of a sooty-mould colony is determined by the dominant fungus. *Capnodium salicinum*, for example, forms a thin black colony. *Limacinia concinna* and *Capnodium moniliformé* form thick felt-like moulds. *C. elegans* forms a thin cottony mould on account of the upright nature of the hyphae.

The appearance of the colony may vary with the habitat. On stems *Capnodium mucronatum* forms erect fascicles of hyphae up to 2 cm. high. Such a mass of mycelium could not be supported on a leaf, so that epiphyllous colonies of *C. mucronatum* are relatively thin and consist of loosely interwoven hyphae.

Sooty-moulds are found in all sorts of localities but not all the species are found throughout the whole range. Certain distinct associations are characteristic of sunny, shaded and densely shaded, and of dry and moist localities.

In this paper an attempt is made first to interpret this distribution in nature on the basis of the physiological properties of the individual species, and secondly to examine the reason for the limitation of sooty-mould-forming fungi to the excretions of scale insects.

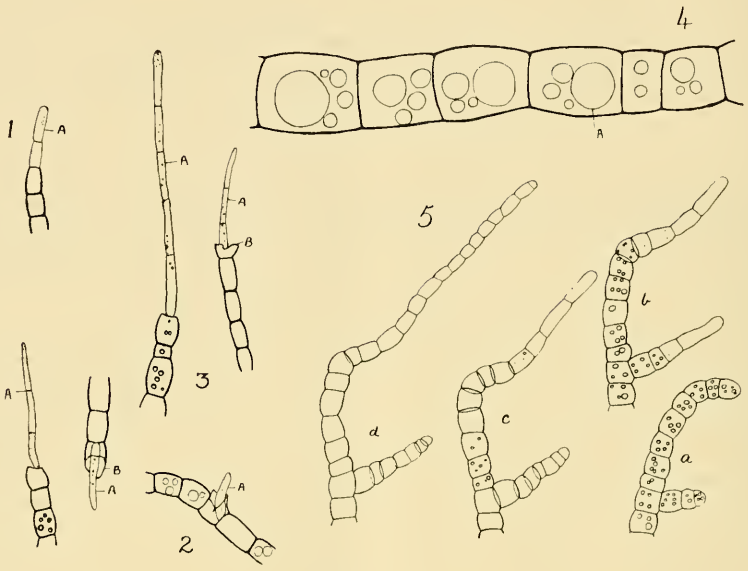
Methods of Growth of Naturally-Occurring Sooty-Mould Fungi.

A property shared by all sooty-mould-forming fungi is the ability to make use of intermittent moist conditions of the atmosphere for the purposes of growth. It is apparent that this must be a physiological factor of great importance.

If a fragment of sooty-mould is kept in a damp atmosphere or in water, growth takes place at all hyphal apices (Text-fig. 1), and from broken ends (Text-fig. 2). Text-figures 1 and 2 show the amount of new growth made in 12 hours by *Limacinia concinna*. The walls of the new cells are light coloured and therefore easily recognizable. Text-figure 3 shows the amount of growth made in 36 hours. Living sooty-mould cells contain large quantities of an oil-like substance (A in Text-fig. 4). The amount of this substance present in the cells behind the new growth is always found to be much decreased (Text-figs. 3, 5). Text-figure 5*a* shows a hypha as it appeared at the commencement of the growth test. The oil-like substance is present in all the cells. Text-figure 5*b* shows the amount of growth made in water after 12 hours, and Text-figures 5*c* and 5*d* show the amount of growth after 36 and 60 hours in water respectively. The food

reserve is then seen to be entirely depleted. Loss of food reserve takes place progressively from the cells nearest the new growth to those furthest from it.

If a sooty-mould mycelium growing under natural conditions is examined microscopically after a dewy night, evidence of fresh growth can be seen in the presence of thin-walled cells at the apices of the hyphae.



Text-figs. 1-5.

1.—A hypha of *Limacinia concinna* showing new growth (A) from the apex after 12 hours in water. × 285.

2.—Broken hyphae of *Limacinia concinna* showing new growth (A) after 12 hours in water, and the jagged appearance of the broken walls (B). × 285.

3.—Hyphae of *Limacinia concinna* showing the amount of new growth made after 36 hours in water (A), and the reduction in the amount of oil-like substance in the old cells adjoining the new growth. × 285.

4.—Cells of *Limacinia concinna* showing the presence of drops of an oil-like substance (A). × 1,000.

5.—A hypha of *Limacinia concinna* showing the disappearance of oil-like substance from the old cells with increase in number of new cells. 5*a*, original hypha. 5*b*, after 12 hours in water. 5*c*, after 36 hours in water. 5*d*, after 60 hours in water. × 285.

Growth of a sooty-mould colony seems therefore to take place as follows: the mould cell absorbs scale-insect excretion as it is available, and stores up food materials. Then when sufficient water is available, during rain or on a dewy night, growth is made and the reserve foods are drawn upon. It is obvious that little growth can be made during hot or dry weather.

The growth rate of sooty-moulds over a long period is therefore necessarily slow. This has been demonstrated in the case of *Brefeldiella brasiliensis*, for the growth rate of which exact data have been obtained. Twenty-two thalli of this species growing on leaves were measured at intervals. Measurements were taken always along the same two diameters at right angles. The average increase in diameter is given in Table 1.

TABLE 1.

Time in weeks from the commencement of the experiment	0	2	4	9	10	11
Average diameter of colony in mm.	0·87	0·96	1·0	1·2	1·24	1·27

Brefeldiella is specially suitable for such measurements as its flat thallus grows at the margin only and not in thickness, so that the total amount of growth made can be found by measuring the diameter from time to time. Exact measurements can not be made in the case of the members of the Capnodiaceae, which form mixed colonies growing in thickness as well as in diameter and from many points. The growth rate, in the field, of the members of the Capnodiaceae is faster than that of *Brefeldiella*. *Limacinia concinna*, for example, can form a thin mould over the surface of a leaf 7 × 2·5 cm. in size in two weeks during moist weather.

Natural Associations of Sooty-Mould Fungi.

The following situations are inhabited by characteristic associations of sooty-mould fungi.

(1). Sunny open habitats where sooty-moulds are exposed to maximum heat, light and desiccation.

Fungi: *Capnodium salicinum*, *C. salicinum* var. *uniseptatum*, *C. Walteri*, *C. anonae* (imperfect stage only), *C. fuliginodes* (imperfect stage only), *C. australe*, *Atichia glomerulosa*, *Dematium pullulans* and *Cladosporium herbarum*.

Hosts: *Bursaria spinosa* (attacked by *Ceroplastes destructor* and *Eriococcus eucalypti*), *Pittosporum undulatum* (*Ceroplastes destructor*), *Eugenia* sp. (*Ceroplastes rubens*), *Eucalyptus* spp. (*Ctenochiton eucalypti*), *Leptospermum flavescens*, *L. scoparium*, *L. lanigerum* (*Tachardia melaleucae*).

(2). Habitats which are moister than (1) and are exposed to light and heat for shorter periods.

Fungi: *Capnodium anonae* (perfect and imperfect stages), *C. anonae* var. *obscurum*, *C. fuliginodes* (perfect and imperfect stages), *C. fuliginodes* var. *grandisporum*, *Limacinia concinna*, *Aithaloderma ferruginea*, *Atichia Millardeti*, *Caldariomyces* sp. 1, *Brefeldiella brasiliensis*.

Hosts: *Ceratopetalum apetalum* (attacked by *Dactylopius* sp.), *Elaeodendron australe* (*Ceroplastes destructor*), *Eugenia* sp. (*Ceroplastes rubens*), *Synoum glandulosum* (*Ceroplastes destructor*), rarely *Bursaria spinosa* (*Ceroplastes destructor*).

All the fungi of (1) may also occur in this association, their fructifications being characteristically larger than in more open situations.

(3). Habitats which are moister than the preceding, obtaining as a rule in rain forests or in damp shady gullies where humidity is always high.

(*a*). Exposed to sunlight for at least part of the day.

Fungi: *Capnodium elegans*, *C. mucronatum*, *C. moniliforme*, *Henningsomyces affine*, *Scorias philippinensis*, *Microxyphium* sp. 1, *M.* sp. 2, *Caldariomyces* sp. 2, *Atichia Millardeti*. The fungi of (2) occur occasionally, those of (1) rarely.

Hosts: Rain forest trees attacked by the scale insects already mentioned, especially *Doryphora sassafras* attacked by *Aspidiotus rossi*.

(*b*). Not or rarely exposed to sunlight, often at some distance from the source of food.

Fungi: *Chaetothyrium* spp., *Atichia Millardeti*, *Trichopeltis reptans*, *Trichothallus hawaiiensis*, *Brefeldiella brasiliensis*, *Triposporium* sp., *Phycopsis vanillae*.

Hosts: Rain forest trees attacked by the scale insects mentioned above.

Certain species of fungi are not often associated with each other, though occurring in the same sort of situation. In some cases several species may occur on the same leaf but their mycelium does not become mixed and the colonies remain distinct though in contact at the edges. This has been observed in the case of some species of *Chaetothyrium*, especially when *C. fusisporum* is present (Plate iii, fig. 1).

In Table 2 a list is given of the species of sooty-mould fungi found growing with a selected number of types, to illustrate the associations recorded above.

TABLE 2.

Type.	Associated Fungi.	Number of Times Association has been Found.	Total Number of Times Type has been Collected.
Capnodium anonae	*Capnodium anonae* var. *obscurum* ..	5	61
	C. Walteri	21	
	C. salicinum	3	
	C. salicinum var. *uniseptatum* ..	12	
	C. moniliforme	6	
	C. fuliginodes	15	
	C. australe	1	
	C. elegans	2	
	C. mucronatum	2	
	Aithaloderma ferruginea	9	
	Caldariomyces sp. 1	1	
	Caldariomyces sp. 2	10	
	Atichia Millardeti	5	
	Microxyphium sp. 1	7	
	Microxyphium sp. 2	3	
	Chaetothyrium fusisporum	1	
	Henningsomyces affine	1	
	Limacinia concinna	6	
Capnodium Walteri	*Capnodium anonae*	21	33
	C. fuliginodes	9	
	C. salicinum var. *uniseptatum* ..	7	
	C. salicinum	3	
	C. australe	2	
	Limacinia concinna	2	
	Capnodium anonae var. *obscurum* ..	1	
	Aithaloderma ferruginea	1	
	Atichia Millardeti	1	
Aithaloderma ferruginea	*Capnodium anonae*	9	42
	Atichia Millardeti	9	
	Brefeldiella brasiliensis	6	
	Limacinia concinna	5	
	Caldariomyces sp. 2	3	
	Capnodium salicinum var. *uniseptatum*	3	
	Microxyphium sp. 1	3	
	Henningsomyces affine	2	
	Capnodium moniliforme	1	
	C. elegans	1	
	C. fuliginodes	1	

Capnodium elegans	*Capnodium mucronatum*	4	10
	C. moniliforme	4	
	C. anonae	2	
	Scorias philippinensis	2	
	Limacinia concinna	1	
	Aithaloderma ferruginea	1	
	Chaetothyrium roseosporum	1	
Capnodium moniliforme ..	*Capnodium mucronatum*	4	24
	C. elegans	4	
	C. anonae	6	
	Microxyphium sp. 1	4	
	Caldariomyces sp. 2	5	
	Atichia Millardeti	4	
	Trichopeltis reptans	2	
	Brefeldiella brasiliensis	1	
	Scorias philippinensis	1	
	Limacinia concinna	1	
Chaetothyrium roseosporum ..	*Atichia Millardeti*	2	11
	Chaetothyrium cinereum	2	
	Capnodium elegans	1	
	Trichopeltis reptans	1	
	Chaetothyrium fusisporum	1	
Atichia Millardeti	*Chaetothyrium fusisporum*	18	58
	Brefeldiella brasiliensis	9	
	Aithaloderma ferruginea	9	
	Trichopeltis reptans	6	
	Chaetothyrium griseolum	5	
	Capnodium anonae	5	
	C. moniliforme	4	
	Phycopsis vanillae	2	
	Caldariomyces sp. 2	2	
	Capnodium salicinum var. *uniseptatum*	1	
	C. mucronatum	1	
	C. Walteri	1	
	Microxyphium sp. 1	1	
	Limacinia concinna	1	
	Chaetothyrium depressum	1	
	C. fuscum	1	
	C. roseosporum	2	
	C. cinereum	1	

It has been found that *Capnodium anonae* is the commonest and most widespread sooty-mould species. It is found growing in many localities in all kinds of associations. This is shown in Table 2 by the number and variety of fungi associated with it. Other species are seen to be more limited in their associations. The species found growing with *Capnodium Walteri*, *C. elegans*, *C. moniliforme*, *Aithaloderma ferruginea*, *Chaetothyrium roseosporum*, and *Atichia Millardeti* are chiefly those of the same association class.

Heat Resistance of Naturally-Occurring Sooty-Mould Fungi.

As sooty-mould fungi show such a marked degree of natural grouping, an attempt was made to trace the cause. One probable reason seemed to be that some fungi might be more resistant to heat than others. Consequently as many sooty-mould fungi as were available were tested for their reactions to heat.

Methods.—After some experimenting the following method was adopted as being simple, quick and suitable for treating large numbers of fungi at the same

time. Fragments of the fungus to be tested were placed in four test-tubes, either dry or in water, according to whether dry or wet heat was to be used. The test-tubes were then placed in a water bath at the required temperature so that they were immersed to about half their height. The tubes were removed from the water bath after 5, 10, 20 and 40 minutes of heating. It was found that the temperature inside the tubes reached that of the water bath in approximately three and a half minutes, and this extra time was given in each case. Hanging-drop cultures were made of the treated fragments of mycelium and they were examined for signs of growth after one week.

In Tables 3 and 4 the results of these experiments are given.

TABLE 3.

Resistance of Sooty-Mould Fungi to Moist Heat.

Temperature in degrees Centigrade	30				35				40			
Time of treatment in minutes	5	10	20	40	5	10	20	40	5	10	20	40
Fungus.												
Limacinia concinna	3	3	3	3	3	3	3	3	2	1	—	—
Capnodium fuliginodes	3	3	3	3	3	3	3	3	2	2	—	—
Capnodium Walteri	3	3	3	3	3	3	3	3	1	—	—	—
Capnodium elegans	3	3	2	1	2	2	1	1	—	—	—	—
Capnodium moniliforme	2	2	1	1	—	—	—	—	—	—	—	—
Capnodium mucronatum	3	3	3	3	3	3	3	3	2	2	1	—
Capnodium salicinum	3	3	3	3	3	2	1	1	—	—	—	—
Capnodium anonae	3	3	3	3	3	3	3	2	2	2	1	—
Capnodium anonae var. *obscurum*	3	2	2	2	3	2	2	1	—	—	—	—
Chaetothyrium fusisporum	3	3	3	3	3	3	3	2	2	2	2	—
Aithaloderma ferruginea	3	3	3	3	3	3	3	3	3	2	1	—
Chaetothyrium cinereum	3	2	2	2	3	2	2	2	2	—	—	—
Microxyphium sp. 1	3	3	3	3	3	3	3	2	3	2	—	—
Trichopeltis reptans	3	3	3	3	3	3	3	2	—	—	—	—
Cladosporium herbarum	3	3	2	2	1	1	1	1	—	—	—	—
Penicillium expansum	3	2	2	1	1	—	—	—	—	—	—	—

The condition of the fungus after treatment is shown arbitrarily as follows:

— indicates that no growth has taken place in the hanging-drop culture and the fungus is considered to be dead.

3 indicates that growth equal to that of the untreated control has taken place.

2 indicates that a fair amount of growth has taken place.

1 indicates that very little growth has taken place, only an occasional hypha being alive.

TABLE 4.

Resistance of Sooty-Mould Fungi to Dry Heat.

Temperature in degrees Centigrade..	55				60				65				70				75			
Time of treatment in minutes ..	5	10	20	40	5	10	20	40	5	10	20	40	5	10	20	40	5	10	20	40
Fungus.																				
Limacinia concinna	2	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Capnodium fuliginodes	3	2	2	2	2	2	1	1	2	2	1	—	2	1	1	—	—	—	—	—
Capnodium Walteri	3	3	3	2	3	3	2	2	3	3	2	1	3	3	2	1	2	2	—	—
Capnodium elegans	3	3	2	2	3	2	2	2	2	2	1	1	—	—	—	—	—	—	—	—
Capnodium moniliforme	3	2	1	1	2	1	1	1	2	1	1	—	1	1	1	—	—	—	—	—
Capnodium mucronatum	3	3	3	3	3	3	3	3	3	3	2	2	3	3	2	1	—	—	—	—
Capnodium salicinum	3	3	3	3	3	3	3	3	3	3	3	3	3	2	2	2	2	1	1	1
Capnodium anonae	3	3	3	3	3	3	3	3	2	2	1	—	—	—	—	—	—	—	—	—
Capnodium anonae var. *obscurum*	3	3	3	2	3	3	2	1	2	2	—	—	2	1	—	—	—	—	—	—
Chaetothyrium fusisporum	3	3	3	3	3	3	3	3	2	2	2	1	2	1	1	1	2	1	—	—
Chaetothyrium cinereum	3	3	3	3	3	3	2	2	3	3	3	2	2	2	2	—	—	—	—	—
Chaetothyrium roseosporum ..	3	3	3	3	3	3	3	3	2	2	1	1	1	1	—	—	—	—	—	—
Aithaloderma ferruginea	3	3	2	2	3	2	2	2	2	2	2	1	2	2	2	—	—	—	—	—
Microxyphium sp. 1	3	3	2	2	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Trichopeltis reptans	3	3	3	3	3	3	3	1	2	2	2	1	—	—	—	—	—	—	—	—
Triposporium sp.	3	3	3	2	3	2	2	2	3	2	2	1	2	2	2	1	—	—	—	—
Cladosporium herbarum	3	3	3	3	3	2	2	2	2	2	1	1	1	1	—	—	—	—	—	—
Penicillium expansum	2	2	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

The condition of the fungus after treatment is shown arbitrarily as follows:

— indicates that no growth has taken place in the hanging-drop culture and the fungus is considered to be dead.

3 indicates that growth equal to that of the untreated control has taken place.

2 indicates that a fair amount of growth has taken place.

1 indicates that very little growth has taken place, only an occasional hypha being alive.

These tables show that there is a striking difference between the resistance shown by fungi to wet and dry heat. None of the fungi tested were able to withstand a temperature higher than 45° C. in water, even for so short a time as 5 minutes.

There does not appear to be any exact correlation between ability to resist wet heat and ability to resist dry heat. For example, *Limacinia concinna* can remain alive after 10 minutes' exposure to a temperature of 40° C., wet heat, but is killed by an exposure of 5 minutes to 60° C., dry heat, whereas *Capnodium*

elegans and *C. salicinum* are killed after 5 minutes at 40° C., wet heat, but are resistant to 65° C., dry heat, for 40 minutes.

The following classes of sooty-mould fungi can be distinguished on the basis of their resistance to dry heat:

(1).—Very resistant, comprising species which can withstand a temperature of 70° C. for 40 minutes.

Species: *Capnodium Walteri, C. mucronatum, C. salicinum, Chaetothyrium fusisporum, Triposporium* sp.

TABLE 5.

Resistance of Cultivated Sooty-Mould Fungi to Moist Heat.

Temperature in degrees Centigrade		30				35				40			
Time of treatment in minutes		5	10	20	40	5	10	20	40	5	10	20	40
Fungus.	Medium.												
Capnodium salicinum	S	3	2	2	2	2	2	2	2	1	1	—	—
	P	3	3	3	2	2	2	2	2	—	—	—	—
	G	2	2	—	—	1	—	—	—	—	—	—	—
Capnodium salicinum var. *uniseptatum*	S	3	3	3	3	3	3	2	2	3	3	2	1
	P	3	3	3	2	3	3	2	2	2	—	—	—
	G	2	2	—	—	1	—	—	—	1	—	—	—
Aithaloderma ferruginea	S	3	3	3	3	3	2	2	2	3	1	—	—
	P	3	3	2	2	2	1	—	—	—	—	—	—
	G	2	1	—	—	1	1	—	—	1	1	—	—
Capnodium fuliginodes	S	3	3	3	3	3	3	3	2	3	2	2	2
	P	3	3	3	3	3	3	3	2	2	2	2	1
	G	3	3	2	2	3	2	2	2	1	—	—	—
Capnodium Walteri	S	3	3	3	3	3	3	2	2	2	1	1	1
	P	3	3	3	3	3	2	2	2	1	1	1	—
	G	3	2	2	2	3	2	1	1	1	—	—	—
Chaetothyrium cinereum	S	3	3	3	3	3	3	3	2	3	3	1	1
	P	2	1	—	—	—	—	—	—	—	—	—	—
	G	1	1	—	—	—	—	—	—	—	—	—	—
Limacinia concinna	S	3	3	3	3	3	3	3	2	3	3	1	1
	P	2	1	—	—	—	—	—	—	—	—	—	—
	G	1	1	—	—	—	—	—	—	—	—	—	—
Triposporium sp.	S	3	3	3	3	2	2	2	—	2	—	—	—
	P	3	3	3	3	3	2	2	2	2	1	—	—
	G	3	1	—	—	1	1	—	—	1	1	—	—
Dematium pullulans	S	3	3	3	2	1	1	—	—	—	—	—	—
	P	3	3	3	2	3	2	2	2	1	1	1	—
	G	3	3	3	3	2	2	2	2	—	—	—	—

The condition of the fungus after treatment is shown arbitrarily as follows:

— indicates that no growth has taken place in the hanging-drop culture and the fungus is considered to be dead.

3 indicates that growth equal to that of the untreated control has taken place.

2 indicates that a fair amount of growth has taken place.

1 indicates that very little growth has taken place, only an occasional hypha being alive.

P, potato glucose solution.—S, unpurified adonite solution.—G, glucose salts solution.

(2).—Resistant, comprising species which can withstand a temperature of 65° C. for 20 minutes.

Species: *Capnodium fuliginodes, C. elegans, C. anonae, C. moniliforme, C. anonae* var. *obscurum, Aithaloderma ferruginea, Chaetothyrium roseosporum, C. cinereum, Trichopeltis reptans, Cladosporium herbarum.*

(3).—Not resistant, comprising species which can not withstand temperatures above 60° C. for more than 10 minutes.

TABLE 6.

Resistance of Cultivated Sooty-Mould Fungi to Dry Heat.

Temperature in degrees Centigrade		55				60				65				70			
Time of treatment in minutes		5	10	20	40	5	10	20	40	5	10	20	40	5	10	20	40
Fungus.	Medium.																
Capnodium salicinum	S	3	3	3	3	2	2	2	1	2	2	2	—	2	—	—	—
	P	3	3	3	3	2	2	2	1	3	2	2	—	2	2	2	—
	G	2	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—
Capnodium salicinum var. *uniseptatum*	S	3	3	3	3	3	3	2	2	3	3	2	2	3	3	2	2
	P	3	3	3	3	3	1	1	—	—	—	—	—	—	—	—	—
	G	3	3	1	—	2	2	—	—	2	2	—	—	—	—	—	—
Aithaloderma ferruginea	S	3	3	3	2	3	2	2	2	1	1	—	—	—	—	—	—
	P	1	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—
	G	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Capnodium fuliginodes	S	3	3	3	3	3	3	3	2	3	3	3	—	—	—	—	—
	P	3	3	3	3	3	3	2	—	3	3	2	—	—	—	—	—
	G	3	3	2	2	2	1	1	—	—	—	—	—	—	—	—	—
Capnodium Walteri	S	3	3	3	3	2	2	2	2	2	2	2	2	—	—	—	—
	P	3	3	2	2	2	1	1	1	1	1	1	—	—	—	—	—
	G	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Chaetothyrium cinereum	S	3	3	3	3	1	1	—	—	—	—	—	—	—	—	—	—
	P	3	3	3	2	1	1	—	—	—	—	—	—	—	—	—	—
	G	1	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—
Limacinia concinna	S	3	3	3	3	3	3	3	2	1	1	—	—	—	—	—	—
	P	1	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—
	G	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Triposporium sp.	S	3	3	3	2	2	2	2	1	1	1	1	—	—	—	—	—
	P	3	3	3	2	2	2	2	1	2	2	2	1	—	—	—	—
	G	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Dematium pullulans	S	3	2	1	—	1	—	—	—	—	—	—	—	—	—	—	—
	P	3	3	1	1	1	1	—	—	—	—	—	—	—	—	—	—
	G	3	3	3	3	3	3	3	3	3	3	3	3	—	—	—	—

The condition of the fungus after treatment is shown arbitrarily as follows:

— indicates that no growth has taken place in the hanging-drop culture and the fungus is considered to be dead.

3 indicates that growth equal to that of the untreated control has taken place.

2 indicates that a fair amount of growth has taken place.

1 indicates that very little growth has taken place, only an occasional hypha being alive.

P, potato glucose solution.—S, unpurified adonite solution.—G, glucose salts solution.

Species: *Limacinia concinna*, *Microxyphium* sp. 1, *Penicillium expansum* (control).

It can be seen from Table 3 that the distribution of sooty-mould fungi can not be explained by their powers of heat resistance alone, for, although the species of the non-resistant class occur in the less exposed situations in nature, others which occur in similar situations are strikingly resistant to heat, e.g., *Chaetothyrium* spp.

Resistance of Cultivated Sooty-Mould Fungi to High Temperatures.

In this series of experiments three media were used for the cultivation of the fungi, as it was thought that the composition of the medium might influence the resistance of the fungus to some extent. The media were as follows:

(1) Unpurified adonite 2 gm., water 100 c.c. (S in Table 5).

(2) Standard potato glucose solution (P in Table 5).

(3) Glucose 2 gm., sodium nitrate 2 gm., potassium dihydrogen phosphate 0·5 gm., magnesium sulphate 0·25 gm., water 100 c.c. (G in Table 5).

The fungi were grown for three weeks on glass-wool soaked with the culture medium in Petri dishes, and were then allowed to become air-dry at laboratory temperature and humidity under aseptic conditions before testing for heat resistance. The treatment adopted was the same as for the naturally-occurring sooty-moulds. The results are given in Tables 5 and 6.

TABLE 7.

Resistance of Sooty-Mould Fungi Grown on Media of Different Concentrations to Dry Heat.

Temperature in degrees Centigrade		45				50				55				60			
Time of treatment in minutes		5	10	20	40	5	10	20	40	5	10	20	40	5	10	20	40
Fungus.	Concentration of Glucose in Medium.																
	%																
Caldariomyces sp. 1	0	3	3	3	2	2	1	1	1	1	—	—	—	—	—	—	—
	0·5	3	3	3	2	1	1	1	1	2	2	—	—	—	—	—	—
	2·0	3	3	2	2	3	2	1	1	2	2	1	—	2	—	—	—
	10·0	3	2	2	1	2	1	1	—	2	2	1	—	2	2	—	—
	25·0	2	1	—	—	1	1	—	—	1	1	—	—	—	—	—	—
Capnodium fuliginodes	0	3	3	3	3	2	3	3	3	2	1	—	—	—	—	—	—
	0·5	3	3	3	2	3	2	1	1	3	2	1	1	1	—	—	—
	2·0	3	3	3	2	3	3	3	2	3	2	1	—	1	—	—	—
	10·0	3	2	2	—	1	—	—	—	—	—	—	—	—	—	—	—
	25·0	3	2	2	—	—	—	—	—	—	—	—	—	—	—	—	—
Dematium pullulans	0	2	1	—	—	2	1	—	—	2	—	—	—	—	—	—	—
	0·5	2	2	—	—	1	—	—	—	1	—	—	—	—	—	—	—
	2·0	3	3	1	1	3	2	1	1	2	1	1	1	1	—	—	—
	10·0	3	2	2	2	3	2	1	1	2	2	1	1	2	2	—	—
	25·0	3	3	2	1	3	2	1	—	2	2	—	—	—	—	—	—

The condition of the fungus after treatment is shown arbitrarily as follows:

— indicates that no growth has taken place in the hanging-drop culture and the fungus is considered to be dead.

3 indicates that growth equal to that of the untreated control has taken place.

2 indicates that a fair amount of growth has taken place.

1 indicates that very little growth has taken place, only an occasional hypha being alive.

The results obtained for resistance to wet heat are similar to those obtained for naturally-occurring sooty-mould species. All species except *Dematium pullulans* showed greater resistance on unpurified adonite media than on potato solution or glucose salts solution. From an examination of Table 7 it can be seen that this was also the case when dry heat was tested.

On the whole, fungi in culture are less resistant to heating than are the same species when growing in their natural habitat. An exception to this is *Limacinia concinna*, which is more resistant in culture.

The Effect of Altering the Concentration of the Culture Medium on the Heat Resistance of Sooty-Mould Fungi.

It is well known that certain higher plants, e.g., *Rhus*, *Peganum*, etc. (see Maximov, 1929, p. 271 et seq.), which can endure long periods of desiccation unharmed, are characterized by cell sap of high osmotic pressure. The osmotic pressure of the cell sap of naturally-occurring sooty-moulds has been found to vary from 70 to 95 atmospheres. If the high osmotic pressure has any direct bearing on the heat-resisting powers of the cell, it should be possible, by raising or lowering the osmotic pressure, to increase or decrease the degree of resistance. This is most readily done by raising or lowering the concentration of the culture medium. The powers of heat-resistance of mycelium grown in solutions of various concentrations of glucose were therefore tested. Potato extract solutions were used with 0%, 0·5%, 2%, 10%, and 25% sugar. The fungi used in these experiments were *Capnodium fuliginodes*, *Caldariomyces* sp. 1, and *Dematium pullulans*. One set of cultures three weeks old was used for tests with wet heat. Another set of the same age was allowed to dry slowly at laboratory temperature and humidity. These were then used for tests with dry heat.

In Table 7 the result is shown of experiments using dry heat. It can be seen that resistance was slightly less in media of high and low sugar concentration than in media of medium concentration in the case of *Capnodium fuliginodes* and *Caldariomyces* sp. 1. For *Capnodium* the optimum concentration is 0·5–2·0%, and for *Caldariomyces* 2–10%. In the case of *Dematium pullulans* low concentrations reduced the powers of heat-resistance to a greater extent than in the other species, but high concentrations reduced it to a lesser extent. The optimum concentration was 10%.

Similar results were obtained using wet heat, but, as before, the temperature necessary to cause death was lower.

It appears, therefore, that in the case of these fungi there is no direct relationship between osmotic pressure and heat resistance. For each species there is an optimum concentration of medium, above and below which heat-resistance falls off.

A series of experiments in which different concentrations of nitrogen were used was made. The results showed that high and low concentrations reduced the heat-resistance of all species to about the same extent.

Resistance of Sooty-Mould Fungi to Low Temperatures.

The species of naturally-occurring and cultivated sooty-moulds which had been tested for heat-resistance were subjected to low temperatures to ascertain their powers of resistance to cold. The procedure adopted was similar to that used in the heat-resistance experiments. Pieces of mycelium were placed in test-tubes, dry, or with a little water, according to whether dry or wet temperatures were to be tested, and were partly immersed in a water bath. The temperature

of the water bath was controlled by the addition of ice and salt. The following temperatures were used: −15° C., 0° C., 2° C., 5° C.

All the species were able to withstand these temperatures without injury, both in the wet and in the dry condition.

Resistance of Sooty-Mould Fungi to Desiccation.

Material of the species of fungi which had been collected for heat-resistance tests was kept at laboratory temperature and tested weekly for viability. Material of the cultivated species used in the heat tests was also treated in this way.

The results are given in Tables 8 and 9. From these it can be seen that the naturally-occurring sooty-mould fungi can be grouped into the following classes on the basis of their ability to resist periods of desiccation:

(1).—Very Resistant, comprising species viable after 10 weeks without water.
Species: *Capnodium salicinum, C. Walteri, C. mucronatum.*

(2).—Resistant, comprising species viable after 5 weeks without water.
Species: *Capnodium elegans, C. anonae, C. moniliforme, Microxyphium* sp. 1.

(3).—Not Resistant, comprising species which are dead after 4 weeks without water.
Species: *Limacinia concinna, Chaetothyrium roseosporum, C. fusisporum, C. cinereum, Trichopeltis reptans, Aithaloderma ferruginea, Triposporium* sp.

It can be seen that with a few exceptions, the distribution of those species whose associations could not be explained on the basis of their powers of heat-resistance can be explained on the basis of their resistance or susceptibility to desiccation.

TABLE 8.

The Resistance to Desiccation of Naturally-Occurring Sooty-Mould Fungi.

Period of desiccation in weeks ..	1	2	3	4	5	6	7	8	9	10	11
Fungus.											
Capnodium salicinum	3	3	3	3	3	3	3	3	3	2	—
Capnodium elegans	3	3	3	2	2	1	1	1	1	—	—
Capnodium moniliforme	3	3	2	2	1	1	1	—	—	—	—
Capnodium anonae	3	3	2	2	2	2	2	1	—	—	—
Capnodium Walteri	3	3	3	3	3	3	3	2	2	1	—
Capnodium mucronatum	3	3	3	3	2	2	1	1	1	1	1
Limacinia concinna	3	3	2	1	—	—	—	—	—	—	—
Chaetothyrium fusisporum	3	—	—	—	—	—	—	—	—	—	—
Chaetothyrium roseosporum	3	2	—	—	—	—	—	—	—	—	—
Chaetothyrium cinereum	2	1	—	—	—	—	—	—	—	—	—
Triposporium sp.	3	3	1	—	—	—	—	—	—	—	—
Aithaloderma ferruginea	3	3	3	—	—	—	—	—	—	—	—
Trichopeltis reptans	3	—	—	—	—	—	—	—	—	—	—

The condition of the fungus after treatment is shown arbitrarily as follows:

— indicates that no growth has taken place in the hanging-drop culture and the fungus is considered to be dead.

3 indicates that growth equal to that of the untreated control has taken place.

2 indicates that a fair amount of growth has taken place.

1 indicates that very little growth has taken place, only an occasional hypha being alive.

TABLE 9.

The Resistance to Desiccation of Cultivated Sooty-Mould Fungi.

Period of desiccation in weeks ..		1	2	3	4	5	6	7	8	9	10	11
Fungus.	Medium.											
Capnodium salicinum ..	S	3	3	3	3	3	3	3	3	3	2	—
	P	3	3	3	3	2	2	2	1	1	—	—
	G	3	—	—	—	—	—	—	—	—	—	—
Capnodium salicinum var. *uniseptatum*	S	3	3	3	3	3	2	2	2	1	—	—
	P	3	3	3	2	2	2	1	—	—	—	—
	G	3	2	1	1	1	—	—	—	—	—	—
Capnodium fuliginodes ..	S	3	2	2	2	2	2	2	2	2	2	2
	P	3	3	3	3	2	2	2	2	1	1	1
	G	3	3	1	—	—	—	—	—	—	—	—
Capnodium Walteri ..	S	3	3	3	3	3	2	2	2	2	2	2
	P	2	1	1	1	1	1	—	—	—	—	—
	G	2	2	1	1	1	—	—	—	—	—	—
Aithaloderma ferruginea	S	3	2	2	2	2	—	—	—	—	—	—
	P	3	2	1	—	—	—	—	—	—	—	—
	G	3	2	1	—	—	—	—	—	—	—	—
Limacinia concinna ..	S	3	3	3	3	2	2	2	2	2	2	2
	P	2	2	1	1	1	1	—	—	—	—	—
	G	2	2	2	1	1	—	—	—	—	—	—
Chaetothyrium cinereum ..	S	3	3	3	3	3	2	2	2	2	2	2
	P	2	1	1	1	—	—	—	—	—	—	—
	G	2	1	1	—	—	—	—	—	—	—	—
Triposporium sp. ..	S	3	3	3	3	3	2	2	2	2	2	—
	P	3	3	2	2	2	2	1	1	1	1	—
	G	3	2	—	—	—	—	—	—	—	—	—
Dematium pullulans ..	S	3	3	3	2	2	1	—	—	—	—	—
	P	3	3	2	2	2	2	2	2	—	—	—
	G	3	3	3	3	3	2	2	2	2	2	2

The condition of the fungus after treatment is shown arbitrarily as follows:

— indicates that no growth has taken place in the hanging-drop culture and the fungus is considered to be dead.

3 indicates that growth equal to that of the untreated control has taken place.

2 indicates that a fair amount of growth has taken place.

1 indicates that very little growth has taken place, only an occasional hypha being alive.

P, potato glucose solution.—S, unpurified adonite solution.—G, glucose salts solution.

Reaction of Individual Species of Sooty-Mould Fungi to Special Conditions of Nutrition.

It appeared significant that only a limited number of species of fungi should occur in sooty-mould colonies, and that most omnivorous moulds such as *Penicillium* spp. should be relatively unimportant. There seemed to be several possible reasons for the paucity of these common saprophytes. Either they might not be able to utilize "honey dew", on which sooty-moulds grow in nature, or they might not be able to withstand the conditions of desiccation, high temperature

and strong sunlight to which they would be subjected in a sooty-mould colony, or their growth might be prevented by the production of staling substances by the sooty-mould fungi. It was thought also that there might be two reasons why the Capnodiaceae, Atichiaceae and Trichopeltaceae are found only in sooty-mould colonies. Either they might be restricted to "honey dew" as a source of food, or they might be too slow-growing to compete with mould fungi in any other habitat.

Experiments have been recorded in an earlier paper (Fraser, 1934) which showed that the limitation of most sooty-mould-forming species in nature to the excretions of scale insects does not appear to be due to their inability to make use of different types of food materials.

a. Reaction to Adonite.

The exact nature of the food materials available to the sooty-mould fungi was apparently not known to previous workers. Arnaud (1911) alone referred to the composition of "honey dew". He considered it to be a watery solution of dextrin, gums, etc. It has been shown by Dr. V. Trikojus* that the "honey dew" produced by the scale insect *Ceroplastes destructor* is a nearly-pure aqueous solution of adonite.

A small quantity of purified adonite was made available to the writer, and preliminary experiments were made to ascertain its effect on the growth of sooty-mould fungi. The results obtained indicated that adonite was probably a specially suitable medium for the growth of sooty-mould fungi, but it did not appear to be very suitable for the growth of *Penicillium*.

More extensive experiments were accordingly planned. Pure B.D.H. adonite of plant origin was obtained. It was thought that it might also be necessary to test adonite of scale-insect origin, so a large quantity of *Ceroplastes destructor* growing on a host tree, *Melia Azedarach* var. *australasica*, was collected. The insects were scraped off the host and heated until the wax melted and the adonite solution present in its meshes was liberated. This was strained off, filtered and evaporated to dryness. The residue consisted almost entirely of adonite, and it was not considered necessary to purify it.

The following agars were used:

(1). Unpurified adonite agar.—Unpurified adonite extracted from *Ceroplastes destructor* 2 gm., agar 2 gm., water 100 c.c.

(2). Unpurified adonite agar with the addition of salts.—Unpurified adonite 2 gm., sodium nitrate 2 gm., magnesium sulphate 0·25 gm., potassium dihydrogen phosphate 0·5 gm., agar 2 gm., water 100 c.c.

(3). Purified adonite agar.—B.D.H. adonite 2 gm., sodium nitrate 2 gm., magnesium sulphate 0·25 gm., potassium dihydrogen phosphate 0·5 gm., agar 2 gm., water 100 c.c.

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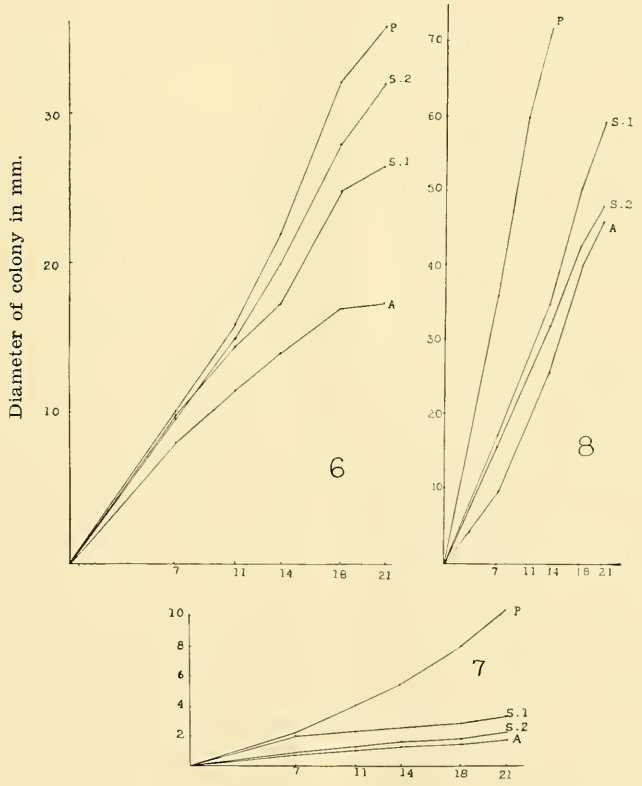
* Dr. Trikojus kindly made available to the writer the unpublished results of his investigations on the excretions of *Ceroplastes destructor*. This insect is commonly associated with sooty-moulds. It attaches itself at an early age to the leaf or twig of a host plant and remains there throughout its life, absorbing food materials by means of thin suckers called "stylets". It excretes a waxy covering of spongy texture, which becomes several millimetres thick. The insect also produces a watery solution, the "honey dew", which contains certain by-products of its metabolism. The "honey dew" fills the meshes of the waxy covering and runs out on to the leaf or twig. Adonite (or adonitol) is a pentahydric alcohol of the constitution $C_5H_{12}O_5$. In fresh "honey dew" it occurs in a concentration of 6%.

(4). Potato extract agar.—Sodium nitrate 2 gm., magnesium sulphate 0·25 gm., potassium dihydrogen phosphate 0·5 gm., agar 2 gm., potato extract (200 gm. potato in 1 litre of water, boiled and filtered) 100 c.c. This was used as a control.

Petri dishes 9 cm. in diameter were poured with 10 c.c. of the required medium and inoculated with the species to be tested. The cultures were incubated at 25° C. in darkness for 21 days. All experiments were made in triplicate and the growth rate was obtained by measuring the diameters of the colonies in two directions at right angles three times weekly.

The following fungi were chosen for experiment, as they represented the two most important groups of sooty-mould fungi, the Capnodiaceae and the Fungi Imperfecti: *Capnodium fuliginodes*, *C. salicinum*, *Caldariomyces* sp. 1, *Aithaloderma ferruginea*, *Chaetothyrium griseolum* (Capnodiaceae), *Dematium pullulans*, *Penicillium expansum* (Fungi Imperfecti). *Penicillium* was included as a control.

All the fungi used were able to make a certain amount of growth on the agars on which they were tested.



Text-figs. 6-12.

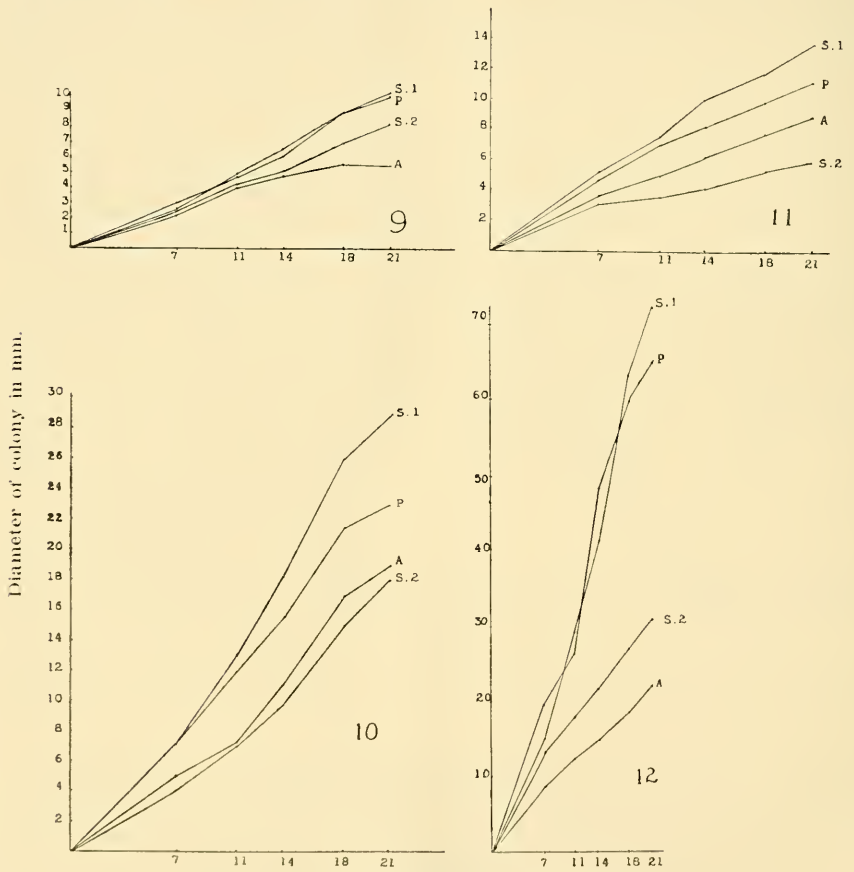
6-12.—Graphs to show growth rates on adonite (A), unpurified adonite (S. 1), unpurified adonite with the addition of salts (S. 2) and potato extract (P) agars: 6, *Caldariomyces* sp. 1; 7, *Chaetothyrium cinereum*; 8, *Penicillium expansum*; 9, *Aithaloderma ferruginea*; 10, *Capnodium fuliginodes*; 11, *Capnodium salicinum;* 12, *Dematium pullulans.*

Unpurified adonite proved a very satisfactory source of food for all the sooty-mould fungi except *Chaetothyrium*. It was found more satisfactory than the control (potato extract) for *Dematium* (S.1 in Text-fig. 12), *Capnodium fuliginodes* (S.1 in Text-fig. 10) and *C. salicinum* (S.1 in Text-fig. 11). The growth of *Penicillium* was poorer than on the control agar (Text-fig. 8).

The addition of salts (S.2 in Text-figs. 6–12) to unpurified adonite made it less suitable for all the fungi except *Caldariomyces* (Text-fig. 7).

Purified adonite was found to be less suitable for growth than unpurified adonite or potato extract (A in Text-figs. 6–12). In the case of *Caldariomyces*, *Aithaloderma* and *Chaetothyrium*, staling became more pronounced after 14 days, as shown by the flattening of the growth curve (Text-figs. 6, 7, 9).

Potato-extract agar was well utilized by all the fungi. *Penicillium*, *Caldariomyces*, and especially *Chaetothyrium* (Text-figs. 6, 7, 8) made better growth on this medium than on unpurified adonite.



From these experiments it appears that adonite excreted by *Ceroplastes destructor* is not a very suitable medium for the growth of the mould *Penicillium*, which is not a common constituent of naturally-occurring sooty moulds. On the

other hand, it was very satisfactory for the growth of all sooty-mould species tested except *Chaetothyrium*. It is also apparent that purified adonite was not so satisfactory as unpurified adonite.

So far the nature of the "honey dew" on one scale insect only has been determined, *Ceroplastes destructor* on *Bursaria spinosa*. It is quite possible that other species of scale-insect may secrete slightly different substances and that some species of sooty-mould fungi may grow particularly well on one special type of secretion.

b. Staling Phenomena shown by Sooty-Mould Fungi.

In the case of soil fungi the influence of the species on each other's growth is well known. Garrett (1934) has recently summarized and extended the knowledge on this subject. Comparatively little attention, however, has been paid to the influence of other saprophytic fungi on each other in nature.

Many workers, notably Brown (1923) and Pratt (1924*a*, 1924*b*) have discussed the problem of staling caused by the growth of fungi in agar media. As the fungus grows it produces decomposition products which diffuse out into the surrounding agar. These may accumulate in such quantities as to retard or finally stop the growth of the fungus itself, and to retard or stop the growth of another fungus growing near it.

When a fungus is grown on nutrient agar, growth takes place as a rule at the margins only, so that a flat circular colony is produced. It has been shown by Pratt (1924*a*) that the agar in the centre is not depleted of food materials but contains staling substances which render it unfit for further growth.

As indicated here and in an earlier paper (Fraser, 1934), sooty-mould fungi do not produce staling substances which retard their own growth to any great extent, except when the nitrogen content of the culture medium is high, or when unfavourable nitrogen compounds are present in the agar.

Many sooty-mould fungi do not form flat even colonies on agar media. They may be ridged, domed or very much raised in the centre. Moderate examples of this are shown in Plate iii, figures 2 and 4, where the colonies are domed and furrowed respectively. In extreme cases the colony may become as thick as it is wide. This is due to continued growth and branching of the hyphae in the older parts, which seem to continue until all available food material is exhausted. The formation of a thick colony is especially marked on agar containing a high concentration of sugar. This method of growth furnishes additional proof that the species of sooty-moulds do not form substances which stale their own growth to any extent.

There is less likelihood of the accumulation of staling substances on a leaf surface, where they could be washed off by rain, than in an agar medium. It is evident, however, that if no rain were to fall over a period of a week or more, and if sufficient dew for growth to be made were available each night, a considerable amount of staling substances could accumulate.

To obtain further light on the problem of staling reactions, sooty-mould species were grown together in pairs on thin agar media, as staling is more readily detected in thin agar than in thick.

A series of experiments was made using potato glucose agar. The results so obtained were checked by an experiment in which unpurified adonite agar was used.

Six possible types of reaction may result when fungi are grown together in pairs on nutrient agar:

(1). A stops growth of B, but is not itself affected by B.
(2). A decreases the growth of B, but is not itself affected by B.
(3). A stops or nearly stops the growth of B, and is itself slowed down by B.
(4). A and B slow down and stop each other's growth.
(5). A and B slow down each other's growth, but do not stop, continuing to grow over each other: 5*a*. Mutual effect slight; 5*b*. Mutual effect fairly strong.
(6). A and B have no mutual effect, but grow over each other with undiminished vigour.

On potato dextrose agar the reactions of the pairs of fungi fall into the following classes:

Class 2. A	B
Capnodium anonae	and *Penicillium expansum*

Class 3. A	B
Microxyphium sp. 1	and *Penicillium expansum*
,, ,, ,,	,, *Dematium pullulans*
Caldariomyces sp. 1	,, *Penicillium expansum*
Dematium pullulans	,, ,, ,,
Aithaloderma ferruginea	,, ,, ,,
Triposporium sp.	,, ,, ,,
Capnodium salicinum var. *uniseptatum*	,, ,, ,,
Capnodium fuliginodes	,, ,, ,,
Limacinia concinna	,, ,, ,,
Triposporium sp.	,, *Limacinia concinna*

Class 4. A	B
Chaetothyrium cinereum	and *Caldariomyces* sp. 1
Aithaloderma ferruginea	,, *Dematium pullulans*
Microxyphium sp. 1	,, ,, ,,
Caldariomyces sp. 1	,, *Capnodium salicinum* var. *uniseptatum*

Class 5a. A	B
Caldariomyces sp. 1	and *Limacinia concinna*
Dematium pullulans	,, *Capnodium anonae*
Cladosporium herbarum	,, *Aithaloderma ferruginea*
,, ,,	,, *Capnodium Walteri*
Chaetothyrium cinereum	,, *Penicillium expansum*
Capnodium fuliginodes	,, *Capnodium Walteri*
,, ,,	,, *Cladosporium herbarum*
,, ,,	,, *Capnodium fuliginodes*
Limacinia concinna	,, *Cladosporium herbarum*
,, ,,	,, *Capnodium fuliginodes*
Aithaloderma ferruginea	,, ,, ,,
,, ,,	,, *Cladosporium herbarum*
Penicillium expansum	,, ,, ,,
,, ,,	,, *Penicillium expansum*
Capnodium anonae	,, *Cladosporium herbarum*
,, ,,	,, *Capnodium Walteri*
,, ,,	,, *Capnodium fuliginodes*
,, ,,	,, *Dematium pullulans*
,, ,,	,, *Caldariomyces* sp. 1
,, ,,	,, *Capnodium salicinum* var. *uniseptatum*
Chaetothyrium cinereum	,, *Dematium pullulans*
Microxyphium sp. 1	,, *Capnodium fuliginodes*
,, ,, ,,	,, *Capnodium salicinum* var. *uniseptatum*
,, ,, ,,	,, *Microxyphium* sp. 1
,, ,, ,,	,, *Capnodium Walteri*
Cladosporium herbarum	,, *Dematium pullulans*
,, ,,	,, *Capnodium salicinum* var. *uniseptatum*
,, ,,	,, *Triposporium* sp.

Dematium pullulans	,, *Capnodium Walteri*
,, ,,	,, *Dematium pullulans*

Class 5b. A	B
Caldariomyces sp. 1	and *Dematium pullulans*
,, ,, ,,	,, *Limacinia concinna*
,, ,, ,,	,, *Capnodium Walteri*
,, ,, ,,	,, *Aithaloderma ferruginea*
,, ,, ,,	,, *Triposporium* sp.
,, ,, ,,	,, *Capnodium salicinum* var. *uniseptatum*
,, ,, ,,	,, *Caldariomyces* sp. 1
,, ,, ,,	,, *Microxyphium* sp. 1
,, ,, ,,	,, *Capnodium fuliginodes*
,, ,, ,,	,, *Cladosporium herbarum*
Dematium pullulans	,, *Triposporium* sp.
,, ,,	,, *Capnodium fuliginodes*
,, ,,	,, *Capnodium salicinum* var. *uniseptatum*
Triposporium sp.	,, *Aithaloderma ferruginea*
,, ,,	,, *Capnodium fuliginodes*
Limacinia concinna	,, *Capnodium salicinum* var. *uniseptatum*
Capnodium fuliginodes	,, *Chaetothyrium cinereum*
,, ,,	,, *Capnodium salicinum* var. *uniseptatum*

Class 6. A	B
Limacinia concinna	and *Cladosporium herbarum*
Capnodium Walteri	,, *Dematium pullulans*
Chaetothyrium cinereum	,, *Cladosporium herbarum*
Capnodium anonae	,, ,, ,,

From this it can be seen that the majority of sooty-mould fungi cause only slight staling effects on each other. *Caldariomyces* sp. 1 and *Microxyphium* sp. 1 cause more staling than any other species.

Plate iii, figure 2, shows a colony of *Cladosporium* growing over a colony of *Capnodium anonae* (Class 6). There appears to have been little or no slowing down of the growth rate of either fungus.

Plate iii, figure 3, shows an example of slight staling. The growth rate of both fungi, *Caldariomyces* sp. 1 and *Limacinia concinna*, has been slowed slightly in the adjacent parts of the colonies (Class 5*a*). A slightly greater degree of staling is shown in Plate iii, figures 4 and 5. In Plate iii, figure 4, *Capnodium fuliginodes* and *Caldariomyces* sp. 1 are shown causing fairly strong mutual slowing in adjacent parts of the colonies. In Plate iii, figure 5, *Capnodium Walteri* and *Caldariomyces* show a similar effect (Class 5*b*).

An example of stronger staling is shown in Plate iii, figure 6, representative of Class 4. Growth has almost entirely ceased in adjacent parts of the colonies.

In Plate iii, figure 7*a*, is shown an example of Class 3. *Limacinia concinna* has caused the growth of *Penicillium expansum* to cease abruptly. Plate iii, figure 7*b*, shows the same colonies two weeks later. It can be seen that the *Penicillium* colony has not grown round the *Limacinia* colony to any extent. The *Limacinia* colony, on the other hand, has continued to enlarge and is growing over the edge of the *Penicillium* colony, but at a slightly slower rate than at the edge farthest from it.

It is apparent that *Penicillium* is fairly strongly affected by the growth of most sooty-mould fungi. *Cladosporium* is scarcely affected by the growth of the members of the Capnodiaceae, *Dematium* is affected by some, but not at all by others.

Since staling is a function of the medium, it is not possible to assume from their behaviour on potato glucose agar that the fungi will behave similarly on "honey dew".

Consequently a representative group of fungi comprising some found to be mutually staling, slightly staling and not staling on potato glucose agar, were grown on agar of the composition 2% unpurified adonite, 2% agar.

The results showed that mutual retardation of growth by sooty-mould species on unpurified adonite is less marked than on potato glucose agar. Members of the Capnodiaceae show little or no sign of mutual effect (Class 6, Plate iii, fig. 8, *Capnodium fuliginodes* and *Triposporium* sp.; Plate iii, fig. 9, *Capnodium fuliginodes* and *Chaetothyrium cinereum*). Only those species which show the strongest effects (Class 5*b*) on potato glucose agar show slight retarding effects (Class 5*a*) on adonite agar. The growth of *Penicillium* is retarded more or less strongly by sooty-mould fungi on adonite agar.

Conclusions.

The distribution of each species of sooty-mould fungus appears to be dependent on one or more factors. All the fungi occurring together in similar positions are not limited to them for the same reasons. *Capnodium salicinum*, *C. Walteri* and, to a less extent, *C. anonae* are resistant both to heat and desiccation, and in nature occupy the most exposed habitats. *Limacinia*, *Aithaloderma* and *Microxyphium* sp. 1 are limited to favourable habitats by susceptibility both to heat and desiccation. The members of the Chaetothyrieae, *Triposporium* and *Trichopeltis*, though strongly resistant to heat, are restricted to moist localities by their susceptibility to desiccation.

Capnodium elegans, *C. mucronatum* and *C. moniliforme* form a group by themselves, since they are resistant both to heat and desiccation, yet in nature occur in rain-forest areas only. Either they may be restricted to the excretions of certain specific scale insects of limited distribution, or they may require a very moist atmosphere for growth. These species could not be obtained in culture and, therefore, experiments could not be made to test the hypotheses.

Heat and desiccation appear to be the most important factors influencing the distribution of sooty-mould species in nature, cold evidently having no effect.

The results of the tests on the heat-resisting and desiccation-resisting powers of sooty-mould species in culture largely confirm those obtained for naturally-occurring material. Several species are, however, more resistant both to heat and to desiccation in culture than in nature. It appears probable that the factors for resistance are specific to each fungus species. It appears also from the experiments that the composition of the media in which the fungi are grown may considerably modify their powers of resistance both to heat and to desiccation.

True sooty-mould fungi are able to withstand very considerable temperatures in the dry condition but are killed quickly by exposure to moist heat. This has also been found to be the case with certain wood-destroying fungi by Snell (1923), and is known to be the case with lichens (see Smith, 1921).

It seems reasonable to assume that when growing on excretions of *Ceroplastes destructor* most true sooty-mould fungi do not form staling substances in sufficient quantities to retard each other's growth noticeably. Since the sooty-mould fungi do produce staling substances which retard the growth of *Penicillium* strongly, it seems probable that a colony of sooty-moulds, once established, could prevent to some extent the growth of *Penicillium* in it.

It also appears likely that some of the Capnodiaceae could not invade a sooty-mould colony in which *Dematium* is well established, but many species, such as *Capnodium anonae*, could do so without difficulty.

Several species of sooty-moulds have been found to be mutually antagonistic in culture, notably *Caldariomyces* sp. 1 and *Microxyphium* sp. 1, and these have not been found associated in nature. Some species, therefore, which belong to the same ecological class, may not occur together because of their mutually antagonistic effect.

The relative paucity of the common saprophytic moulds, of which *Penicillium expansum* has been taken as the type, appears to be due to a number of causes. The chief of these is probably their inability to withstand high temperatures and prolonged desiccation. Another cause may be that the composition of the food material available is not specially suitable for their growth. Finally it appears that the staling substances produced by the true sooty-mould fungi have a retarding effect on their growth. This effect may be lessened during periods of wet weather, since the staling substances would be likely to be washed out of the mould. Actually it has been found that *Penicillium* spp., *Alternaria* spp., *Fusarium* spp., etc., are most abundant in sooty-mould colonies in wet weather, and while this is probably largely due to the absence of strong evaporation, it may in part be due to the absence of staling substances.

The limitation of most sooty-mould-forming species in nature to the excretions of scale insects appears to be due to their extremely slow growth rate. Sooty-moulds appear to be specially adapted to an epiphytic life on account of their ability to withstand heat and dryness, and to grow slowly, making use of any slight amount of water available for this purpose.

It has been found by Zeller and Schmitz (1919), Asthana and Hawker (1936), Mix (1933), and others, that the growth substances produced by a fungus in culture may have the effect of increasing the sporulation of other species as well as retarding their growth. This effect has been observed in mixed cultures of sooty-mould fungi, and may be one of the reasons why, in nature, sooty-mould fungi are mostly found in a fruiting condition. Another factor which is probably of importance in this connection is the ultra-violet radiation of sunlight. Ramsey and Bailey (1930), Stevens (1928), and others, have found that ultra-violet radiation increases sporulation in fungi.

Summary.

In nature sooty-mould fungi grow very slowly, as they can grow only during periods of damp weather. They store up an oil-like substance, which is drawn upon when growth is made.

Associations of sooty-mould fungi characteristic of certain habitats are described.

The powers of resistance to heat, cold and desiccation shown by a number of species are recorded, and their bearing on the distribution of the fungi in nature is discussed.

The influence of different types and different concentrations of culture media on the powers of resistance to heat and desiccation of sooty-mould fungi grown in culture is described.

Adonite, the chief constituent of the "honey dew" of *Ceroplastes destructor*, is very suitable for the growth of most sooty-mould fungi. It is not specially suitable for the growth of *Penicillium*. Unpurified adonite of scale-insect origin is more suitable for the growth of sooty-mould fungi than purified adonite of plant origin.

Most true sooty-mould fungi do not stale potato glucose agar to any great extent for their own growth. *Caldariomyces* sp. 1 and *Microxyphium* sp. 1 cause

the greatest amount of staling, *Capnodium anonae* the least. On unpurified adonite agar, staling is even less marked than on potato glucose agar.

Substances are produced by sooty-mould fungi in both media which retard the growth of *Penicillium* fairly strongly. Some species retard the growth of *Dematium* also.

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DESCRIPTION OF PLATE III.

Fig. 1.—Leaves of *Cryptocarya glaucescens* showing colonies of *Chaetothyrium fusisporum* (A) and *C. roseosporum* (B). × 0·56.

Fig. 2.—Colonies of *Capnodium anonae* (A) and *Cladosporium* (B) showing no mutual antagonism. × 0·8.

Fig. 3.—Colonies of *Caldariomyces* sp. 1 (A) and *Limacinia concinna* (B) showing slight mutual antagonism. × 0·8.

Fig. 4.—Colonies of *Capnodium fuliginodes* (A) and *Caldariomyces* sp. 1 (B) showing fairly strong mutual antagonism. × 0·8.

Fig. 5.—Colonies of *Caldariomyces* sp. 1 (A) and *Capnodium Walteri* (B) showing fairly strong mutual antagonism. × 0·8.

Fig. 6.—Colonies of *Caldariomyces* sp. 1 (A) and *Capnodium salicinum* var. *uniseptatum* (B) showing strong mutual antagonism. × 0·8.

Fig. 7*a*.—A colony of *Penicillium expansum* (B) whose growth has been checked by the growth of a colony of *Limacinia concinna* (A). × 0·8.

Fig. 7*b*.—The same colonies two weeks later showing that the colony of *Limacinia concinna* has continued to grow and that the colony of *Penicillium expansum* has remained almost stationary. × 0·8.

Fig. 8.—Colonies of *Triposporium* sp. (A) and *Capnodium fuliginodes* (B) showing no mutual antagonism on unpurified adonite agar. × 0·8.

Fig. 9.—Colonies of *Chaetothyrium cinereum* (A) and *Capnodium fuliginodes* (B) showing no mutual antagonism on unpurified adonite agar. × 0·8.