

Original Articles

INTRAMURAL DISSEMINATION OF SPORES OF HORMODENDRUM RESINAE*

CLYDE M. CHRISTENSEN,† ST. PAUL, MINN.

THE AIM of the work here reported was to determine the pattern of dispersal of fungus spores liberated within a building. By liberating, at a single point in the building, spores of a fungus definitely known not to be present in the air in detectable numbers from other sources, and by using a medium upon which these spores germinate and form visible colonies quickly, but upon which other fungi do not grow, it was possible to trace the travel of these spores throughout the building, to determine the approximate spore load in the air in various parts of the building at various times after the spores were liberated, and to determine how long appreciable numbers of spores remained in the air.

MATERIALS AND METHODS

Organism used.—The fungus used in these tests was *Hormodendrum resinae*. As described by Christensen, Kaufert, Schmitz, and Allison,¹ this fungus, in nature, is largely restricted to wood impregnated with coal tar creosote. Creosoted wood blocks, and agar containing creosote have been exposed from a few hours up to several days, indoors and out, at various times of the year, and on numerous occasions during the last ten years, and subsequently incubated for a week or more; no colonies of *H. resinae* have developed on these. This is fairly convincing proof that the fungus is not a normal inhabitant of the air. With the supporting evidence from the controls used in the tests described below, the possibility of contamination from unknown sources, in these experiments, would seem to be eliminated.

When grown on malt agar, *H. resinae* produces an abundance of powdery spores that can be readily brushed into the air, especially if the agar culture is allowed to dry for several hours before the spores are brushed off. The spores are a few microns in length, and in still air in an enclosed tube fall only a few centimeters per minute, and so are suited to transport by even slow-moving air currents. They retain their viability for days or weeks at the relative humidities ordinarily encountered within occupied buildings. So far as is known, the fungus is innocuous to man, animals, or plants, and therefore the liberation of considerable numbers of its spores into the air should result in no harm or inconvenience. In combination with the selective medium described below, the characteristics of this fungus make it very suitable for use as a "tracer" or "marker" organism in studies such as the present one.

*Paper No. 2556, Scientific Journal Series, Minnesota Agricultural Experiment Station, University of Minnesota, St. Paul, Minn.

†Professor of Plant Pathology, Division of Plant Pathology and Botany, University of Minnesota, St. Paul, Minn.

Selective medium.—To prepare the medium 20 Gm. of malt extract and 20 Gm. of agar were placed in 1 liter of distilled water, and the mixture boiled until the agar was dissolved. Five hundred ml. of this were poured into each of two 16 oz. medicine bottles, and autoclaved for fifteen minutes at 15 lbs. steam pressure. The liquid agar was cooled to approximately 60° C. in a water bath, and coal tar creosote equal to 0.5 to 0.75 per cent of the volume of the medium added. (Since coal tar creosotes differ greatly in composition, and are not standardized, one might, using different creosotes, need to use either slightly more or slightly less than the amounts here given.) The bottles were then shaken violently to emulsify the creosote in the agar, and the medium quickly poured into cool, sterile Petri dishes. After pouring a few dishes, the bottle of medium was shaken to maintain a fairly uniform emulsion of creosote.

This medium, hereafter called creosote agar, has the virtue of excluding almost all organisms but *H. resinae*. Other than an occasional colony of *Penicillium*, which usually can be easily distinguished from *H. resinae* without the use of a microscope, the only organisms found to develop on it have been a bacterium often isolated from creosoted wood along with *H. resinae*, and an unidentified mold isolated from asphalt street paving. Neither of the latter two have been isolated from the air. The writer has strewed soil, house dust, and decomposing plant debris on this agar, and not obtained a single colony of any organism. Since such materials abound in microorganisms of many kinds, this is evidence that the medium is sufficiently selective to serve the purpose for which it has been used in these tests. Colonies of *H. resinae* ordinarily develop rapidly enough on it, at temperatures of 20 to 25° C., to become macroscopically visible within three to four days.

Building in which tests were made.—Most of the tests were made in the Plant Pathology building on the St. Paul campus of the University of Minnesota. The building consists of four stories, with a hallway through the center of each floor, and rooms on either side of the hallways. At both ends of the hallways, interior stairways extend from the first to the fourth floors. The heat is from steam radiators placed against the walls near the floors, and there is no forced air circulating system.

Experimental procedure.—Cultures of *H. resinae* were grown on malt agar in Petri dishes or test tubes. When 2 to 4 weeks old, the surface of such cultures were covered with powdery spores. Before the spores were liberated, dishes of creosote agar were exposed for 1½ hour at various stations throughout the building, then covered and stored in the laboratory. These served as controls. In none of the tests did any colonies develop on the dishes exposed before spores were liberated. Another set of dishes containing creosote agar were then exposed, usually on all four floors, in the hallways, and in various offices and laboratories. As soon as these dishes were in place, a portion of the agar culture of *H. resinae* with a surface area of 1 to 4 sq. cm. was removed from a test tube or Petri dish, allowed to dry for a short time, and spores were brushed from the

surface by means of a bit of dry sterile cotton wound around a wire inoculating needle. In most tests, the spores were liberated within a room on the first floor, with the door to the hallway open.

At regular intervals after the spores had been brushed into the air, successive sets of plates were exposed at all stations, those previously exposed being covered and stacked in the laboratory. After liberating the spores and before handling any of the subsequent lots of plates, the operator changed his clothing, washed his hands thoroughly, and put on autoclaved gloves. This was to eliminate the possibility of contaminating the dishes from spores borne on the person. In some tests, one operator liberated the spores, while others, at the different stations, exposed the culture dishes at various times.

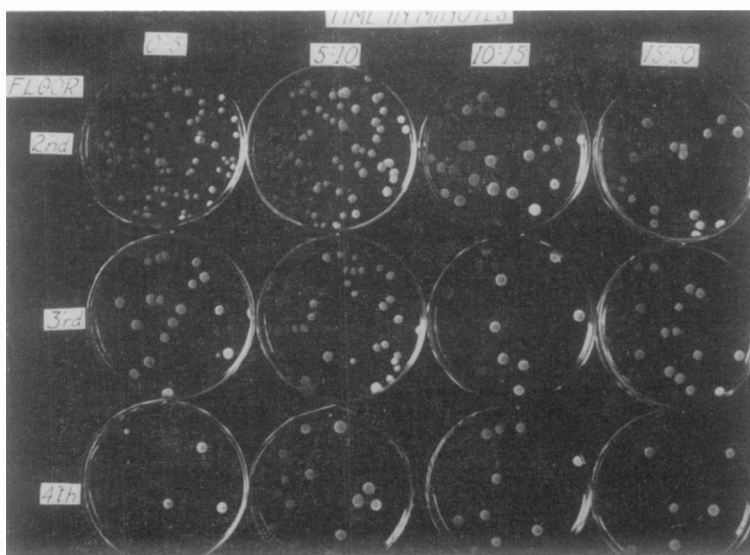


Fig. 1.—Colonies of *Hormodendrum resinae* that developed on plates of creosote agar exposed for successive five-minute periods on the second, third, and fourth floors of the Plant Pathology building after spores had been released in a room on the first floor.

RESULTS

Results of two typical exposures, out of several of those made over a period of several years, are given in Tables I and II, and the colonies which developed on plates exposed in one test are illustrated in Fig. 1.

TABLE I. NUMBER OF COLONIES OF *HORMODENDRUM RESINAE* ON PLATES OF CREOSOTE AGAR EXPOSED IN SUCCESSIVE FIVE-MINUTE PERIODS ON THE SECOND, THIRD, AND FOURTH FLOORS, AFTER SPORES WERE LIBERATED IN THE FIRST-FLOOR HALLWAY

FLOOR	NUMBER OF COLONIES PER DISH			
	FIRST 5 MINUTES	SECOND 5 MINUTES	THIRD 5 MINUTES	FOURTH 5 MINUTES
2	85	70	27	20
3	20	33	9	16
4	4	17	10	7

TABLE II. NUMBER OF COLONIES OF HORMODENDRUM RESINAE ON PLATES OF CREOSOTE AGAR EXPOSED DIFFERENT PERIODS OF TIME AFTER LIBERATING SPORES IN ROOM ON FIRST FLOOR

LOCATION OF EXPOSED PLATES	NUMBER OF COLONIES PER DISH							TOTAL
	0-5 MIN.*	5-10 MIN.	10-20 MIN.	20-30 MIN.	30-60 MIN.	60-120 MIN.	120-240 MIN.	
First floor hall	228	49	43	31	26	15	5	397
Second floor room, door closed	0	0	1	1	3	10	2	17
Third floor greenhouse connected to main bldg. by 30 ft. passage	0	0	0	0	1	0	1	2
Third floor laboratory, door open	8	0	5	19	55	28	8	123
Third floor hall	0	8	70	35	44	12	0	169
Fourth floor hall	68	78	50	34	51	7	3	291
Fourth floor room, door open	0	0	2	3	7	14	5	31
Totals	304	135	171	123	187	86	24	1030

*Number of minutes after spore release.

In another test, spores were liberated in a laboratory, approximately 20 × 40 feet, on the first floor, in front of an electric fan, with the door closed except when the operator was entering or leaving to uncover or cover dishes. The results are given in Table III.

TABLE III. NUMBER OF COLONIES OF HORMODENDRUM RESINAE ON PLATES OF CREOSOTE AGAR EXPOSED AT SUCCESSIVE INTERVALS IN CLOSED ROOM IN WHICH SPORES WERE LIBERATED

TIME IN HOURS FROM SPORE LIBERATION TO BEGINNING OF EXPOSURE	DURATION OF EXPOSURE IN HOURS	NUMBER OF PLATES EXPOSED	AVERAGE NUMBER OF COLONIES PER PLATE
0	1.5	4	600
1.5	1.5	3	25
5	1	2	2
6	12	2	5

Spores were also liberated in the home of the writer, during the winter, when warm air was being circulated intermittently by a fan in the heating system. No windows were open during the course of the test. The results are given in Table IV.

TABLE IV. NUMBER OF COLONIES OF HORMODENDRUM RESINAE ON PLATES OF CREOSOTE AGAR EXPOSED AT VARIOUS INTERVALS AFTER LIBERATING SPORES IN LIVING ROOM OF WRITER'S HOME. ALL PLATES EXPOSED APPROXIMATELY 5 FEET ABOVE FLOOR

TIME IN HOURS FROM SPORE LIBERATION TO BEGINNING OF EXPOSURE	EXPOSURE IN DURATION OF HOURS	LOCATION OF PLATES	NUMBER OF COLONIES PER PLATE
0	2.5	First floor, living room	550
		Second floor, bedroom	160
3	1	Living room	2
3	4	Bedroom	0
24	12	Living room	8
36	12	Living room	5
48	1	Living room	2

DISCUSSION AND CONCLUSIONS

The most interesting fact gathered from these tests is that spores liberated in a room on the first floor of the Plant Pathology building were carried in con-

siderable numbers throughout the second, third, and fourth floors within a relatively few minutes. The experiments were made during the winter, when convection currents due to artificial heating were greater; even so, the speed and thoroughness with which the spores invaded all rooms with open doors was surprising. Very few colonies developed on dishes exposed in rooms with the doors kept closed during the time the dishes were uncovered.

In most of the tests described above, and in numerous others made since then, very few or no colonies have developed on dishes exposed twelve hours or more after spores were liberated. Those spores recovered in the writer's home two days after spores were liberated may well have come from dust dislodged from the air filter in the air circulating system, since dust taken from this filter more than a week after spores had been liberated, and scattered on creosote agar, gave rise to a very large number of colonies of *H. resinae*. The evidence available suggests that in a building such as that in which most of the tests were made, air-borne contaminants do not linger indefinitely, but largely disappear within a few hours at most. In one test, Petri dishes of creosote agar were exposed near an electric fan in a laboratory where spores had been liberated several days before. The fan presumably stirred up dust on walls, floors, tables, and other furniture. No colonies developed on the culture dishes. The fact that the spores could survive for some time in dust caught in the air filter in the writer's home, and the added fact that some of the spores remained viable for several years on dry material in the laboratory, suggest that the absence of colonies on these dishes was not due merely to death of spores by desiccation or other means. More probably, the circulation of air through the Plant Pathology building was sufficient to carry most of the spores out of doors in a short time. Those that remained probably adhered so firmly to the surfaces on which they landed that they were not easily dislodged.

If the spores of *H. resinae* are carried in large numbers throughout a four-story building within a few minutes after they are liberated in a room on the first floor, and enter in large numbers all of the rooms to which the air itself has free access, other air-borne fungi are probably spread just as effectively. Bakeries and other food processing plants where mold contamination is a problem might well institute studies to determine the major sources of air-borne contamination. Wherever air-borne dissemination of fungi within buildings is known or suspected to be of significance, whether in homes, hospitals, or factories, the above results might be considered.

REFERENCE

1. Christensen, C. M., Kaufert, F. H., Schmitz, H. S., and Allison, J. L.: *Horomodendrum resinae* (Lindau), an Inhabitant of Wood Impregnated With Creosote and Coal Tar, *Am. J. Botany* **29**: 552, 1942.