A study of airborne fungi in the Phoenix, Arizona, metropolitan area

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A survey of airborne fungi in the Phoenix metropolitan area, utilizing the culture-plate technique, has been conducted for a one-year period. The most prevalent fungi encountered in order of their frequency were: Alternaria, Pullularia, Hormodendrum, Aspergillus, Helminthosporium, and Penicillium. An additional genus found in relatively high concentration was Bispora. All of the predominant organisms showed seasonal variations at each location site. Homes with evaporative cooling consistently showed a higher fungus count than was found in refrigerated homes. The use of special media containing cycloheximide failed to result in the isolation from the atmosphere of spores of Coccidioides immitis, *a pathogenic fungus endemic to this area.*

While the spores of saprophytic fungi have been found repeatedly during the course of pollen surveys using slide collecting techniques, only those fungi whose spores can be identified microscopically have previously been reported in central Arizona.[1, 2] Environmental conditions such as temperature, substrate humidity, and prevailing winds have a predominant influence on mold growth, affecting the ecological and geographical distribution of fungi as well as their seasonal incidence. High humidity is one of the most significant influencing factors for prolific fungus growth. Thus, the desert area and its surroundings in central Arizona, with their relatively high temperatures and low atmospheric humidity, seem to be factors which might disfavor the development of fungi. Indeed, mold-sensitive patients are frequently sent by their physicians to the

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arid subtropical desert regions of Arizona. The greater Phoenix area contains more than half of the population of the entire state.

The objectives of this study are (1) to identify, by plate culture technique, the airborne fungal spores in the Phoenix metropolitan area, and (2) to compare the relative effectiveness of different cooling methods in reducing fungal concentration. Although Arizona is a highly endemic region for *Coccidioides immitis,* arthrospores have never been cultured from the air in this area. An attempt has been made during this investigation to isolate this organism from the atmosphere by the use of a selective culture medium.

This paper presents the results of a qualitative and quantitative study of airborne fungi in the Phoenix metropolitan area.

MATERIAL AND METHODS

Isolation procedures

The plate technique is widely accepted as the method of choice for atmospheric fungus surveys and has been utilized in this study. Trial exposures were made at 5, 10, 15, 20, 25, and 30 minute intervals to determine the optimal exposure time for obtaining adequate sampling with minimal spreading and overgrowth of colonies. The survey encompassed the one-year period from March 2, 1964, to Feb. 27, 1965. Sterile disposable Petri plates (100 by 15 mm.) containing Bacto Sabouraud dextrose agar (65 Gm. per liter) were exposed for 15 minute intervals, three times weekly on Monday, Wednesday, and Friday from 4:00 P.M. to 6:00 P.M. These plates were fortified with penicillin (20 units per milliliter) and streptomycin (40 units per milliliter) to prevent bacterial contamination. An additional plate containing Bacto Sabouraud dextrose agar (65 Gm. per liter), penicillin (20 units per milliliter), streptomycin (40 units per milliliter), and cycloheximide (1,000 mg. per milliliter) for isolation of *Coccidioides immitis* arthrospores was exposed at each atmospheric site for a one-hour period. Cycloheximide (Actidione) is an excellent medium for use in atmospheric studies in endemic areas because of its ability to selectively isolate *Coccidioides immitis* by inhibiting the growth of bacteria and saprophytic fungi.[3]

Exposure sites

Outdoor and indoor sites were selected in the greater Phoenix metropolitan area. Indoor sites included different types of cooling systems. Petri dishes were exposed indoors in the bedroom of each home at a point three feet above the floor, on a bureau or similar object. Outdoor exposures were made at three feet above the ground in an unobstructed area.

The plates were mailed to Arizona State University, Botany Department. The plates were then incubated at room temperature until sufficient growth occurred to enable study and identification of colonies. In most cases, there was suitable growth to permit identification within 72 to 96 hours. Plates were normally held 10 days to compensate for slow-growing fungi. Plates fortified with cycloheximide were held for a 30 day period. Identification and classifications were made to the level of genus only, with no attempt to identify species. An exception to this was made for differentiation of Monilia by use of chlamydospore medium, Nicherson's medium, and carbohydrate utilization.[4, 5]

The manuals entitled: *Illustrated Genera of Imperfect Fungi*[6], and *Morphology and Taxonomy of Fungi*[7] were used for identification of the isolated fungi. Permanent mounts prepared from the isolates were stained with lacto-phenol-cotton blue,[5] using coverslips ringed with fingernail polish.

CLIMATOLOGICAL DATA

Phoenix is located in about the center of the Salt River Valley, a broad, oval-shaped, nearly flat plain, at an elevation of about 1,100 feet. The Salt River runs from east to west through the valley but, owing to impounding dams upstream, it is usually dry. The climate is of a desert type, with low annual rainfall and low relative humidity.

The central floor of the Salt River Valley is irrigated by water from dams built on the Salt River system. There is considerable agricultural land irrigated by pump water. There is no evidence that the irrigation has in any way affected the relative humidity in the valley.[8] The average daytime relative humidity is 30 per cent.

There are two separate rainfall seasons. The first occurs during the winter months from November to March. The second rainfall period occurs during July and August, when Arizona is subjected to widespread thunderstorm activity whose moisture supply originates in the Gulf of Mexico. The normal total precipitation is 7.40 inches per year.

Phoenix lies in the zone receiving more sunshine than any other section of the United States, an average of 86 per cent of the possible amount for the year—an unusually high normal. During the winter there are, on the average, only 6 or 7 cloudy days per month, but the remainder of the time skies are sunny and daytime temperatures mild. In spring, sunshine is at its best, averaging between 80 and 90 per cent of the possible amount during the 3 months of March, April, and May. Beginning in June, daytime weather is hot. During July and August, there is often considerable afternoon cloudiness associated with cumulus clouds building up over the nearby mountains.

The autumn season, beginning during the latter part of September, is characterized by sudden changes in temperature. The change from the heat of summer to mild winter temperatures usually occurs during October. The normal temperature change from the beginning to the end of this month is the greatest of the 12 months in central Arizona. By November, the mild winter season is definitely established in the Salt River Valley region.

The average daily maximum temperature is 84.7° F. and minimum 53.3° F. The average daily extremes of relative humidity vary from 51 per cent at 5:00 A.M. to 22 per cent at 5:00 P.M.

RESULTS

A total of 23 genera were isolated and identified during the one year encompassed by this study. These can be seen in Table I. The following genera: Alternaria, Helminthosporium, Aspergillus, Bispora, Hormodendrum, and Pullularia were isolated from each exposure site.

The fungi most often isolated were: Alternaria, Pullularia, Hormodendrum,

Table I. *Total counts of fungi*

Alternaria	2,893	Botryodiplodia	17
Pullularia	1,251	*Monilia sitophilia*	8
Hormodendrum	1,157	Epicoccum	3
Aspergillus	800	Nigrospora	1
Helminthosporium	698	Phoma	1
Penicillium	595	Catinula	1
Curvularia	170	Camarosporium	1
Fusarium	162	Diplodia	1
Bispora	119	Cephalsoporium	1
Rhizopus	76	Syncephalastrum	1
Candida	51	Chaetophoma	1
Stemphylium	25	Unidentified	187
Total		8,220	

Aspergillus, Helminthosporium, and Penicillium. Other genera are listed in order of decreasing counts. The peak months in January, February, October, and December were greatly influenced by an increase in the counts of Pullularia which showed a low count in the months of June and July, compared to counts in January, February, and December.

It can be seen in Figs. 1 and 2 that Alternaria is present in this area throughout the year, with peaks in January and February. Helminthosporium reaches a peak in June and July; Hormodendrum in October and November; Penicillium in July, August, and September. In the column marked "Other" sharp increases during November, December, January, and February are due to an increase in Pullularia with peak months in January and February. Bispora, not generally listed as a common air contaminant, reached a peak concentration during the months of April, May, and June. Other genera also showed seasonal variations and peak months.

For the most part, climatic conditions were apparently insignificant in influencing the number or types of fungi isolated which represented the most prevalent genera. Unfortunately, Arizona, with its limited rainfall, offered very few days in which plates exposed during periods of rain could be studied, and the results which were obtained showed little change from normal days, except for an increase in the number of unidentifiable organisms. A similar condition existed for plates exposed during dust storms.

An ancillary study was conducted concerned with the relative efficacy of different air-conditioning units, and what role each played in contributing to the total number of spores found in various homes. The data indicate that the type of refrigeration unit is a critical influencing factor. The results of such a study, and a similar, more comprehensive indoor study now in progress, will be reported in a future communication.

DISCUSSION

Considerable interest has developed in the past forty years concerning the relationship of fungi to the etiology of asthma, hay fever, and other allergic responses. The occurrence of fungus diseases and their etiologic agents are a function of the incidence, geographic distribution, and areas of endemicity of

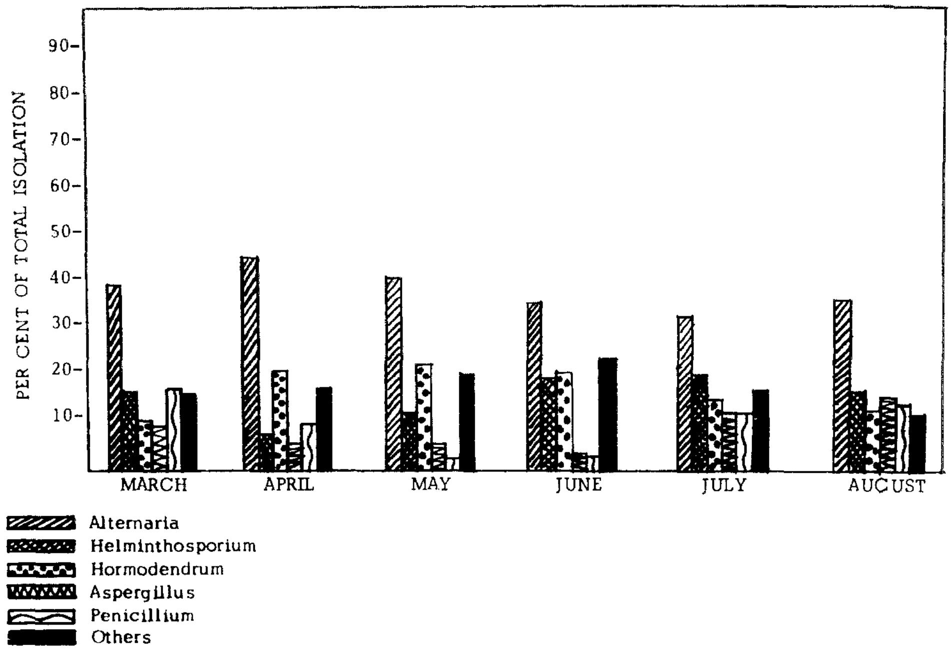

Fig. 1

Fungus isolates from atmospheric and indoor sites in southern Arizona (March to August).

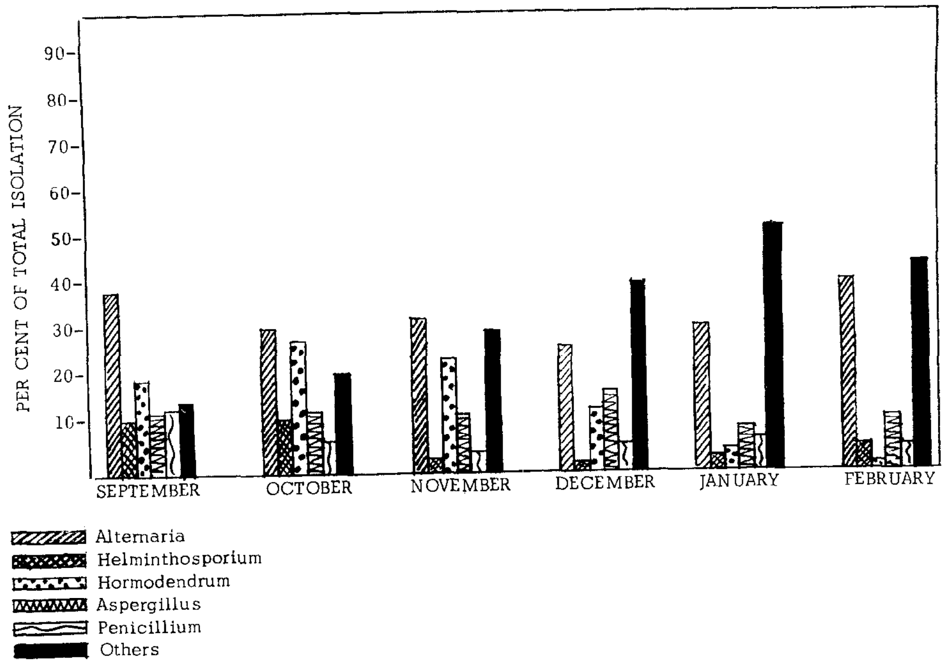

Fig. 2

Fungus isolates from atmospheric and indoor sites in southern Arizona (September to February).

each of the various strains. The migration of thousands of persons from various areas of the United States to Arizona for reasons of health, tourism, military service, and the movement of migrant farm laborers, has made it important to establish and document the airborne fungi of Arizona.

Previous investigations in this geographical area have been concerned only with the identification of those spores which are characteristically distinguishable upon microscopic examination of exposed slides coated with glycerin jelly.[1, 2] The culture-plate technique utilized in this investigation has permitted positive identification of a much more comprehensive sampling of the airborne fungi found within this area. However, those fungi which are obligate parasites will not propagate on synthetic media and thus are not measured using this technique. Furthermore, the addition to the medium of antibiotics to inhibit bacterial and mold contamination tends to reduce the total number of fungi isolated, including yeasts. Rooks and Shapiro,[9] were of the opinion that plate counts using a sampling device do not reflect with any degree of accuracy the actual incidence of organisms for a given day, and a sudden daily increase or decrease in the number of organisms isolated by this method is of no significance. However, when the monthly totals are computed and expressed in terms of a daily average, these readings suggest that the culture-plate method is of value in that it does indicate a seasonal trend in the incidence of the organisms isolated. Thus, monthly totals expressed in terms of a daily average are of some value for comparing the incidence of organisms in one area with those in another comparable area. Further studies in which exposure plates may be used without the addition of antibiotics to the medium may reveal other genera not isolated during the course of this study.

Morrow, Meyer, and Prince,[10] have predicted the existence of basic groups of universally dominant genera of airborne fungi. The results of the present study tend to confirm this in that the most prevalent genera found in the Phoenix area were included in this scheme. Representative genera of the most prevalent fungi were isolated from each exposure site, and each demonstrated a characteristic seasonal variation. Pullularia, as reported by Prince and Morrow,[11] produced large showers of fungi during the months of December, January, and February which were influential in producing the highest incidence of fungus concentration during these months. The occurrence of showers of Pullularia may have continued into later months during certain years and this may influence monthly totals. A survey encompassing a period of 5 to 7 years would thereby serve to establish a more accurate seasonal variation. Significant changes which may occur would be reflected as a true seasonal phenomenon or point up sporadic occurrences of certain organisms. Bispora was isolated at each exposure site, and atmospheric samplings revealed its presence in 11 of the 12 months covered by the survey. It may, therefore, be considered to be a dominant genus peculiar to central Arizona. Although species from other genera were frequently isolated, they were not found in sufficient quantities to determine seasonal variations during this one-year period.

While the geographical and ecological location of each exposure site played a significant role in determining the total numbers of spores, little variation in the type of the most predominant genera was noted during the one-year survey.

Meteorological factors were generally inconclusive in effectively influencing the number of spores isolated. In the presence of active wind currents, with accompanying dust or rain, the most predominant organisms isolated were unidentifiable.

Arthrospores of *Coccidioides immitis* were not isolated in this study. A review of the literature indicates that other investigations have been similarly unsuccessful in isolating the arthrospores from the atmosphere. Since *Coccidioides immitis* cultures can be readily propagated on synthetic media in the laboratory, it would seem logical that isolations could be made from the atmosphere in an endemic area such as central Arizona. Several conditions may serve to explain this apparent enigma. Persons may become infected with the disease by inhalation because of the comparatively large volume of air inhaled. The total spore content inhaled would be large, although the spore content per unit volume of air would be small, and thus spores might not be seen on an exposed plate during a 15 minute exposure period. The use of a high-volume air sampler may be necessary for subsequent air surveys of the incidence of *Coccidioides immitis* arthrospores. Such a sampling may provide a much more reliable index of both the seasonal variations and total volume of arthrospores in this highly endemic area.

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