THE INCIDENCE OF AIRBORNE FUNGI IN THE TUCSON AREA

by

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ABSTRACT

Morning, afternoon and night air of the Tucson area was sampled for its viable fungus content during the summer months. A summer air-spora population profile was obtained for the Tucson Area. Periodic and cumulative colony counts were evaluated against individual weather factors reported by the United States Weather Bureau at the time of sample collection to identify any direct relationship which might exist. The data suggested that rain, humidity, and wind might exert the most directly observable influence on air-spora concentration. Population centers exhibited more concentrated air-spora than did remote areas; and night air was more heavily laden with air-spora than morning air which contained more than afternoon air. No evidence of differential effect of weather conditions on particular organisms was apparent.

INTRODUCTION

Air is a vehicle for the dispersal of microbes, including the fungi (23). These organisms produce an abundance of relatively resistant spores that are easily air-borne (56). Although it is known that mycelial fragments may also be present (44, 45, 47), the fungal elements in the atmosphere are sometimes referred to as "air-spora" (23). These elements are of great importance to all living things because they include organisms involved in the decay process without which life on earth cannot continue (13), as well as organisms that are responsible for disease of plants and animals. Some of the fungi function as allergens causing illness and distress to man (12, 14, 23, 53, 63). It is also recognized that air-spora could be extremely important as weapons of biological warfare (23).

It has been noted that different locations each have their own general air-spora population profile, and further, the profiles of most locations generally include, as major components, a small group of apparently ubiquitous genera (2, 5, 7, 8, 9, 11, 15, 25, 26, 27, 31, 35, 39, 41, 42, 49, 50, 53, 55, 57, 58). Only one survey appears to have been made of the numbers and types of air-borne fungi present in the Tucson area (11).

Many investigators have studied the effect of weather factors on the incidence of air-spora (1, 5, 15, 19, 22, 39, 42, 52, 53). Although there is general agreement on the influence of some weather conditions, conflicting conclusions have been reported pertaining to the role of humidity and wind in contributing to the concentration of air-spora (2, 15, 18, 42, 46, 52, 53).

The purpose of this study was to examine the Tucson area air-spora population profile and to investigate the relationship of weather conditions, particularly wind and relative humidity, in influencing that profile. In this study, "air-spora" refers to fungal elements (spores and vegetative cells), that produced colonies on the media employed under the conditions imposed, and, as such, it represents only a portion of the total number present.

HISTORICAL REVIEW OF THE LITERATURE

According to Gregory (22, 23), Porta is credited with having been the first to observe fungal spores around 1565, but it was not until after the development of the microscope that Micheli, in 1729, became the first to illustrate the spores of several fungi. Micheli further demonstrated that these spores served as "seeds" by inoculating fruit slices with spores of a parent mold and obtaining new growth resembling the parent. On noting that some of his control slices also became contaminated, he concluded that these spores were distributed throughout the air. Micheli's findings apparently received only passing interest. Little more research was done on air-spora until Miquel elaborated techniques that enabled him, throughout the last quarter of the 17th century, to initiate a systematic study of the microbiology of the atmosphere (22). Modern studies of aeromicrobiology began in the 1940's with the works of Meier who attempted to develop aerobiology as a separate branch of science.

Harsh and Allen (27) were among the first to quantitatively survey fungus contaminants in the air. Dunklin and Puck (10) reported that a relative humidity of about 50 percent exerted a lethal effect on certain air-borne bacteria. Following this, Lidwell and Lowberg (37), noted that daylight and ultraviolet light significantly enchanced the

death rate of bacteria at around 60 percent relative humidity. At higher and lower relative humidities, the action of these radiations was much slower.

In 1951, Ambler and Vernon (4) concluded that Penicillium was more prevalent in the air of large towns than in less built-up areas. That same year, Pady and Kapica (46) found that polar air contained fewer spores per unit volume than did tropical air. They further indicated that there was no diminution as distance from land increased, but that the numbers of spores obtained could be correlated with the movement of air masses which may carry the spores across the ocean.

Gregory (23) in 1952, recognized the need to develop new and improved techniques and equipment to gather more detailed and varied information. During the same period, Alvarez and Castro (2) observed that colony or spore deposition on open plates was increased by wind, that allergy symptoms were more common on windy days, and that the fungal concentration of the air is higher at night than during the day. They pointed out, however, that the spore-deposition-increase with wind-increase did not necessarily result from a greater concentration of spores per unit volume of air, but could be a result of their sampling technique which allowed a greater volume of air to pass over the surface of the plate under windy conditions. In a later study, Alvarez and Castro (3) related an increase in severity of asthma and sinusitis cases under observation to an increase in spore concentration in the air.

They noted that the predominant fungi were Cladosporium (Hormodendrum), Alternaria and yeasts, in that order. In 1953, Pady (44), using a volumetric sampler, observed that summer air-spora concentrations in Kansas were 10 to 35 times higher than winter concentrations and that many organisms show seasonal trends. He noted further that hyphal fragments were present throughout the year without seasonal peaks. Seasonal tendencies of various air-borne fungi were also reported on by numerous other investigators (1, 3, 7, 8, 9, 11, 30, 32, 34, 41, 44, 50, 53, 58). In a later study, Pady and Kramer (47) did observe a seasonal trend in the concentration of hyphal fragments with the peak occurring in the summer growing season and the smallest concentration occurring during the winter. Myers (42) stated in 1956 that the mold spore count of air in Honolulu, Hawaii, was much lower than that of mainland cities, with Cladosporium accounting for 33 percent of the total organisms collected. Although he used a volumetric air sampler to minimize the effect of the wind, he observed that the spore count tended to be higher on windy days, thus differing from the earlier conclusions by Alvarez and Castro (2). In 1957, Gregory and Hirst (25) reported that major changes in spore concentration of the air depend on weather and phenology of the local vegetation and its associated fungal flora. The following year Gregory and Sreeramulu (26) compared the air-spora of an estuary with that of agricultural areas and found that differences were primarily quantitative.

In 1959, Gregory, Guthrie and Bunce (24) reported on a series of experiments on splash dispersal of fungus spores. They concluded that splash from raindrops at terminal velocity and from trees, leaves and other structures may act as a complete spore-dispersal mechanism in still air, or as a take-off mechanism leading to spore dispersal by wind. Their findings were later corroborated by Meredith (39), Hirst and Steadman (29), and Rich and Waggoner (52).

Walkington (63), using a slide spore trap method to study atmospheric allergens in the Phoenix, Arizona, area, reported in 1960 that Alternaria species contributed over 50 percent of his fungal isolates while Cladosporium species accounted for less than 10 percent. Frey and Durie (16) in Sydney, Australia and Shapiro, Eisenberg and Binder (54) in San Diego, California, compared the results of slide and culture sampling methods and both groups found that, although the slide trap method frequently indicated a predominance of Alternaria sp., volumetric sampling culture plate methods almost invariably resulted in Cladosporium being the predominant isolate. The latter group asserted that ". . . the only interpretation which can be placed on the gravity slide spore counts is that the spores were air-borne on a given day. . . ." The predominance of culturable Cladosporium colonies was further substantiated by numerous other investigators including Kramer, et al. (31), Derrick and McLennon (10), Pawsey (49),

Pawsey and Heath (50), Taylor and MacFadden (58) and Shapiro, Eisenberg and Binder (55).

Gregory (23), in 1961, made one of the most significant contributions in recent years by publishing, under one cover, a series of lectures in which he explains the dispersal and transport of air-spora, the effect of the atmosphere on transport and deposition of air-spora and numerous other aspects of the influence and properties of atmosphere and its associated microbial population.

In 1962, Barkai-Golen and Glazier (5) reported that the primary differences of air-borne fungi in arid Eilat, Israel, and more semi-tropical Tel Hashomer, Israel, tended to be quantitative more than qualitative. Low relative humidity was considered as a major factor contributing to the reduced fungal growth at Eilat.

Noble and Clayton (43), reporting in 1963 on a study of fungi in the air of hospital wards, observed that Aspergillus fumigatus was recovered on isolation plates at 37 C on each of the 78 days that samples were collected, and no other fungus was recovered with that regularity under the 37 C condition. They further reported that they could detect no dissemination of fungal particles by four patients with hypersensitivity type aspergillosis or by one patient with an aspergillus mycotama.

In 1964, Morrow, Meyer and Prince (41) published a summary of air-borne mold surveys conducted in 41 cities across the United States by the Asociation of Allergists for Mycological Investigation.

The results of these surveys showed that Alternaria predominated and Cladosporium (Hormodendrum) was the second most dominant fungus in 30 cities and Cladosporium (Hormodendrum) predominated at the other 11 cities while Alternaria was second in concentration in only four of these 11. It is notable, however, that the gravity plate collection method was used in all these surveys (40). Also in 1964, Adams (1) reported that most species displayed diurnal periodicity with those recorded most frequently in damp, humid weather peaking at night and those highest in concentration during dry, low-humidity periods peaking in the afternoons. Adams' findings were not in complete agreement with earlier reports by Kramer, Pady, and Wiley (34) and Pady, Kramer and Wiley (48), both of which indicated that a limited number of spore types demonstrated diurnal periodicity.

In 1966, Fulton and Mitchell (20), using new and greatly improved instrumentation (60), observed that the microbial population (micropopulation) of land air masses was significantly higher than that of marine influenced air masses. They also found that activities occurring within a city cause a significant increase in the micropopulation above the city, but this increase was small enough that it could not be detected when the city was under the influence of a land air mass. Fulton (18) further found that the land originating micropopulation of a land air mass traversing an ocean showed a consistent decrease at 152 meters altitude and an irregular patter at 1066 and 1981 meter altitudes,

as the distance from land increased. In addition, he noted that the fungal elements of the micropopulation appeared to be more stable and remain in the air mass longer than the other elements. Other contributions by Fulton include reports showing the relationship between altitude and micropopulation (17), and the effect of atmospheric frontal activity on the micropopulation at altitude (19).

Dworin (11) surveyed atmospheric fungi for one year employing the gravity plate technique. He observed that the predominant fungi were Alternaria, Pullularia, Hormodendrum, Aspergillus, Helminthosporium and Penicillium, in that order. He also saw a significant correlation between monthly spore count and relative humidity.

MATERIALS AND METHODS

Measured volumes of air were sampled for their viable fungal content at a specified location, (Site I--about 12 feet above ground on the Biological Sciences Building animal shelter roof), twice daily at 7:00 a.m. and 2:00 p.m. Monday through Saturday from 16 June 1964 to 15 September 1964, using a sieve sampler powered by a 110 vote AC electric vacuum pump calibrated to deliver one cubic foot of air per minute. Additional afternoon samples were collected on 10 and 24 July and 7 August at three remote locations, (Site II--a quiet, sparsely settled residential area in the Catalina foothills; Site III--Molina Basin at about the 4,500 foot level in the Catalina Mountains; and Site IV--Mt. Lemmon at about the 9,000 foot level in the Catalina Mountains), using the same type sieve sampler but employing a small portable 6 volt DC electric vacuum pump calibrated to deliver 0.5 cubic feet of air per minute.

Samples collected at the remote sites were taken with the sampling apparatus sitting essentially at ground level due to the absence of suitable structures which would permit elevated positioning of the sampler. At Site II the sampler was resting approximately one foot above the ground on a rock placed midway between to "bleeding" mesquite trees about 20 feet apart. These trees were considered as

possible suspects for harboring Cryptococcus neoformans in another study (64) being conducted during the period covered by this investigation. The Site III sample was collected with the sampler resting approximately three feet above the ground on a rock in hilly terrain and with small trees and shrubs present in the general area. The Site IV sample was taken with the sampler resting on the ground in a large level opening about 150 feet in diameter, but surrounded on all sides by tall pines and other mountain flora. Night samples were collected at Site I at 11:00 p.m. on days when remote samples were obtained. Isolation and culture media used were potato dextrose agar, (potatoes--200 gm, dextrose--20 gm, agar--20 gm, distilled water 1,000 ml), and American Sabaroud agar with chloramphenical, (neopeptone Difco-10 gm, dextrose--20 gm, agar--20 gm, distilled water 1,000 ml and chloramphenical--0.05 gm), hereafter referred to as PDA and SAB, respectively. One plate of each medium was exposed at each sampling, after which it was placed inverted at 28 Centigrade (C) for three to five days depending upon colony development. Counts of the number of colonies on each plate were made visually after which the plates were examined under a stereo-microscope to insure that slow-growing colonies were not overlooked. Mold colonies were identified to genus by microscopic examination on the isolation plates. Colonies not immediately identifiable were transferred to plates, slants and slide culture plates of sporulation and identification media including malt

extract agar (malt extract--20 gm. agar--20 gm, distilled water--1,000 ml); Czapek solution agar (sucrose--30 gm, sodium nitrate--2 gm, dipotassium phosphate--1 gm, magnesium sulfate--0.5 gm, potassium chloride- 0.5 gm, ferrous sulfate--0.01 gm, agar--15 gm, distilled water--1,000 ml); and potato-carrot agar (potatoes--100 gm, carrots--100 gm, agar--20 gm, distilled water--1,000 ml). These media will hereafter be referred to as ME, CZ, and PCA, respectively. The sporulation and identification cultures were incubated at 28 C for seven to 28 days, depending on colony and sporulation-apparatus development. Colonies not sporulating within 28 days were listed as "Not identified--no sporulation."

Yeasts suggestive of Cryptococcus neoformans were studied morphologically and physiologically according to the method of Wickerham (65) and identified according to the criteria of Lodder and Kreger-van Rij (38).

Aspergillus and Penicillium colonies were further studied on ME and CZ plates, according to the directions of Thom and Raper (59) and Raper and Thom (51), to determine species distribution within these genera at the sampling locations.

References and keys employed to identify the various groups of organisms include: Illustrated Genera of the Fungi Imperfecti--Barnett (6); The Yeasts, a Taxonomic Study--Lodder and Kreger-van Rij (38); Genera of the Mucorales with notes on their synonymy--Hesseltine (28);

A manual of soil fungi--Gilman (21); A manual of the Aspergilli--Thom and Raper (59); and A manual of the Penicillia-- Raper and Thom (51).

Following identification, the number and types of isolates were plotted against official daily weather factors which were recorded at the time of, or prior to, each sample collection (61, 62) in order to evaluate the effect or relationship of each factor with the incidence of specific types of fungi, as well as with total number encountered.

RESULTS

A total of 4,908 fungal colonies were isolated and studied during the 92-day period in which 320 culture plates were exposed to 552 cubic feet of air on 75 days. Of these 320 plates, two each were exposed on 75 mornings, 73 afternoons and three nights at Site I, and two each also at Sites II, III, and IV on three afternoons. Five of the plates, including two exposed to five cubic feet of air each on the first afternoon sampling, and three plates exposed to two cubic feet of air each during the first two night samplings, were discarded without study due to the collected colonies being so numerous and overcrowded that isolation and identification was impossible. An overall summary of the number of samples at each site and the average number of colonies per plate is given in Table 1.

Of the 4,908 isolates, 4,614 were identified to 88 genera. The remaining 294 colonies were composed of 94 unidentified molds and 200 yeasts. Site I collections accounted for all except 211 colonies, including 198 identified to 25 genera, nine unidentified molds, and four yeasts. The isolation distribution and frequency of occurence of all colonies is given in Table 2.

The percentage distribution of isolates showed that 12 genera made up 86 percent of the total collected. These included

TABLE 1. --Comparison of Numbers of Colonies Noted at Different Sampling Periods and Sites.

	Number of Samplings	Plates Exposed	Air Volume	No. of Colonies	Average Per Plate
Site I--AM	75	150	250 Cu/ft	2,535	16.9
Site I--PM	73	146*	254	2,026	8.0
Site I--Night	3	6**	12	136	45.3
Site II	3	6	12	61	10.2
Site III	3	6	12	66	11.0
Site IV	3	6	12	84	14.0
Totals	160	320	552	4,908	15.6

* Two plates exposed to ten cu/ft of air discarded due to overgrowth.

** Three plates exposed to six cu/ft of air discarded due to overgrowth.

TABLE 2. --Isolates and Their Distribution

Organism	Site I				% of	Site II	Site III	Site IV	Grand
	AM	PM	Night	Total	Total	PM	PM	PM	Total
Cladosporium	861	458	69	1,388	29.5	7	18	17	1,430
Alternaria	290	333	18	641	13.6	15	17	12	685
Helminthosporium	187	353	5	545	11.6	7	10		562
Penicillium	192	313	6	511	10.9		4		515
Aspergillus	187	63	7	257	5.7	7	1		265
Pullularia	87	55	8	150	3.2	2			152
Phoma	60	47	3	110	2.3	3	1	6	120
Fusarium	54	50		104	2.2				104
Curvularia	34	67		101	2.1				101
Cephalosporium	52	16	2	70	1.5	3	1	9	83
Oidiodendron	53	13		66	1.4	1		12	79
Verticillium	41	17	3	61	1.3		4	7	72
Aposphaeria	24	5		29	0.6			1	30
Paecilomyces	22	8		30	0.6				30
Stemphylium	10	18		28	0.6		1		29
Coniothyrium	12	11		23	0.5	2		3	28
Pleospora	18	7		25	0.5	1			26
Fusidium	7	12		19	0.4	1		2	22
Chaetomium	11	7		18	0.4	1			19
Rhizopus	10	3		13	0.3	1			14
Scopulariopsis	12	2		14	0.3				14
Botrytis	11	2		13	0.3				13
Cylindrocladium	4	5		9	0.2	2	2		13
Beauveria	12			12	0.3				12
Stachybotrys	6	5		11	0.2				11
Catinula	5	3	2	10	0.2				10
Diplodina	3	1	5	9	0.2				9

TABLE 2. --Continued

Organism	Site I AM	Site I PM	Site I Night	Site I Total	% of Total	Site II PM	Site III PM	Site IV PM	Grand Total
Trichoderma	4	1		5	0.1			4	9
Cylindrocephalum	8			8	0.2				8
Peyronellaea	4		3	7	0.1				7
Pyrenochaeta	3	4		7	0.1				7
Cylindrosporium							3	3	6
Diplodia	4	2		6	0.1				6
Sporotrichum	5	1		6	0.1				6
Acremonium	3	2		5	0.1				5
Acrotheca	1	4		5	0.1				5
Apiocarpella	1	4		5	0.1				5
Camerosporium	5			5	0.1				5
Gliocladium	3			3	0.06			2	5
Botryodiplodia	3	1		4	0.08				4
Cunninghamella		4		4	0.08				4
Sphaeronema	3	1		4	0.08				4
Ascochyta	2	1		3	0.06				3
Chaetomella	2	1		3	0.06				3
Chaetophoma	3			3	0.06				3
Circinella	3			3	0.06				3
Lomaantha	3			3	0.06				3
Myrothecium	2		1	3	0.06				3
Papularia	1	1		2	0.04			1	3
Pleiochaeta	2	1		3	0.06				3
Stagnospora	1	2		3	0.06				3
Trichothecium	1	2		3	0.06				3
Ambylosporium								2	2
Astromella	2			2	0.04				2

TABLE 2. --Continued

Organism	Site I AM	Site I PM	Site I Night	Site I Total	% of Total	Site II PM	Site III PM	Site IV PM	Grand Total
Auxarthrop	2			2	0.04				2
Botryophialophora	1		1	2	0.04				2
Botryotrichium	2			2	0.04				2
Chloridium	1	1		2	0.04				2
Dictyoarthrinopsis	2			2	0.04				2
Didymostible	2			2	0.04				2
Geotrichum	2			2	0.04				2
Mycotypha	2			2	0.04				2
Nigrospora	2			2	0.04				2
Oedocephalum	1	1		2	0.04				2
Piggotia	2			2	0.04				2
Piricularia		2		2	0.04				2
Shaeropsis	1	1		2	0.04				2
Aristotoma		1		1	0.02				1
Catenophora	1			1	0.02				1
Cylindrocarpos	1			1	0.02				1
Cytospora	1			1	0.02				1
Cytosporella	1			1	0.02				1
Diplorhinotrichum		1		1	0.02				1
Fusicoccum		1		1	0.02				1
Hendersonia		1		1	0.02				1
Heteropatella	1			1	0.02				1
Hormiscium	1			1	0.02				1
Idriella								1	1
Melanoma		1		1	0.02				1
Memnoniella	1			1	0.02				1

TABLE 2. --Continued

Organism	Site I AM	Site I PM	Site I Night	Site I Total	% of Total	Site II PM	Site III PM	Site IV PM	Grand Total
Perodiella	1			1	0.02				1
Periconia	1			1	0.02				1
Pseudogymnoascus	1			1	0.02				1
Rhynchophoma	1			1	0.02				1
Sphaerographum						1			1
Stichospora		1		1	0.02				1
Stysanus	1			1	0.02				1
Trichosphaeria	1			1	0.02				1
Yeasts	106	89	1	196	4.0	4			200
Not Ident--Molds	61	22	2	85	1.8	4	3	2	94
	2,535	2,026	136	4,697		61	66	84	4,908

Cladosporium--29.1%, Alternaria--13.9%, Helminthosporium--11.4%, Penicillium--10.5% (including the erratic samples), Aspergillus--5.4%, Pulluraria--3.1%, Phoma--2.4%, Fusarium--2.1%, Curvularia--2.1%, Cephalosporium--1.7%, Oidiodendron--1.6%, and Verticillium--1.5%. All other isolates each accounted for less than 1.5% of the total. Yeasts, as a group, were isolated on 200 occasions and accounted for 4.0% of the total, with Rhodotorula occurring most frequently. Cryptococcus neoformans and Candida albicans were not encountered.

Major component genera found more predominantly in the morning than in the afternoon included Cladosporium, Penicillium, Aspergillus, Pullularia, Cephalosporium, Oidiodendron, and Verticillium. Those occurring more frequently in the afternoon than in the morning included Alternaria, Helminthosporium, and Curvularia. Phoma and Fusarium showed no significant difference between the morning and afternoon samples. In the night collections, Cladosporium was the dominant isolate with Alternaria next. The yeasts were isolated at random and showed no discernable relation to time of sampling or to specific weather conditions.

Through the entire test period, there was a gradual monthly increase in the average number of colonies per cubic foot of air in both the morning and afternoon (Table 3). During the same inclusive period, the average monthly relative humidity rose from 19 percent

Table 3. --Monthly Average Number of Colonies Per Cubic Foot

	June*	July	August	September*	Total Averages
A. M.	6.6	9.3	13.3	13.9	10.8
P. M.	4.1	5.3	9.2	10.7	7.3
Daily	5.3	7.3	11.2	12.3	9.0

* Study includes only one-half of month.

in June to 43 percent in July, to 55 percent in August, to 59 percent in September.

The number of isolates obtained per plate ranged between two and 112 with medians of ten and seven per cubic foot and averages of 14 and eight per cubic foot in the mornings and afternoons, respectively. Extremely high counts were obtained at Site I on three consecutive afternoons in August. Although these erratic readings are important, the magnitude of their deviation from the mean and the average makes it infeasible to include them in general cumulative computations and relationships, and they will be dropped in further considerations of this nature. The night air at Site I exhibited high colony counts while air at the three remote sites contained relatively low fungal concentrations. No significant difference was noted in the numbers or types of isolates supported by the two media employed (Table 4). Night air appeared to have the highest concentration of viable fungal elements and afternoon air exhibited the lowest concentration (Table 5).

Table 4. --Colony Distribution by Medium, Site, and Time*

	Site I				Site II	Site III	Site IV		
	AM	PM	Night	Sub-Total				Sub-Total	Total
P D A	1,245	992	49	2,286	31	28	41	100	2,386
S A B	1,290	1,034	87	2,411	30	38	43	111	2,522
Total colonies	2,535	2,026	136	4,697	61	66	84	211	4,908
No. of plates studied	150	144	3	297	6	6	6	18	315
Vol. of air sampled (cu ft)	250	244	6	500	12	12	12	36	536

* Discarded plates not included in computations.

Table 5. --Periodic Cumulative Average Counts of Site I Air

Night air	22.7 colonies/cu. ft.
Morning air	10.8 colonies/cu. ft.
Afternoon air	7.3 colonies/cu. ft.

During this investigation, weather conditions showed no consistent relation to the fungal concentration. Although it was not a weather factor, but rather an indicator of past turbulent conditions, morning haziness routinely coincided with high colony counts (Fig. I). Of nine mornings observed to be hazy, seven gave significantly elevated counts with no immediate causative factor apparent. Only two of six hazy afternoons showed high counts. Rain fell in various degrees from light to heavy showers and light to heavy drizzles with the different types of rain exhibiting different effects on the associated air-spora. Wind fluctuated widely and appeared to be related to colony count in some instances but not always.

The number of genera isolated per sampling ranged from a low of four each on 2 and 30 July and 3 and 4 September, to a high of 18 on 20 July with an average and median of 11 in the morning, and 8 in the afternoon.

In the species identification studies, 26 Penicillium species and 18 Aspergillus species were identified. These species were distributed as indicated in Tables 6 and 7. Special mention must be made concerning Penicillium oxalicum, because, although it is the numerically predominant species, two erratic afternoon samples accounted for 215 of the 216 P. oxalicum isolates, with 101 colonies occurring on one plate alone, thereby distorting the overall results. Asperillus

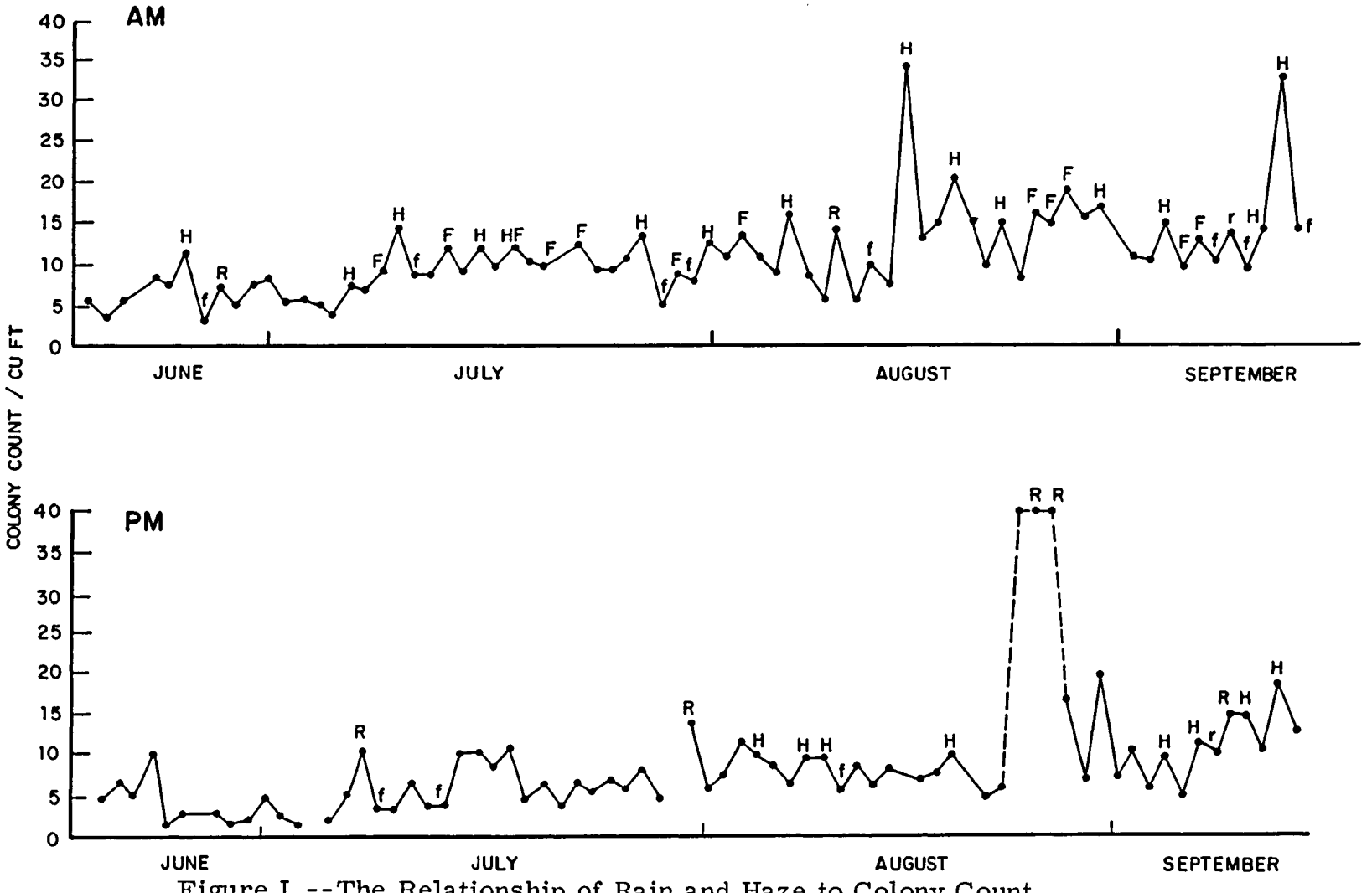

Figure I. --The Relationship of Rain and Haze to Colony Count

——Colony Count
---- Erratically High Count

H = Hazy
R = Rain Shower During Sampling
r = Drizzle During Sampling

F = Follows Shower
f = Follows Drizzle

TABLE 6. --Penicillium Species Distribution

Penicillium Sp.	AM	PM	Night	Remote Sites	Total
P. brevi-compactum	3	2			5
P. canescens	4				4
P. citrium	29	15		3	47
P. chrysogenum	18	8			26
P. commune		5			5
P. cyanofuluum		1			1
P. digitatum	13	4			17
P. diversum var.burenum	1		3		4
P. frequentans	28	28			56
P. godlewskii	1	1			2
P. janthinellum	1			1	2
P. jenseni	1	1			2
P. kapuscinskii	1				1
P. lanoso-coeruleum			3		3
P. lilacinum	2	2			4
P. myczynskii	19				19
P. nalgiovenskii	1				1
P. nigricans	2				2
P. notatum	2	2			4
P. oxalicum	3	216			219
P. raciborskii		1			1
P. rubrum	1				1
P. simplicissimum	1				1
P. stacki	1				1
P. stoloniferum	3				3
P. verruculosum	2				2
Penicillium sp*	55	27			82
Totals	192	313	6	4	515

TABLE 7. --Aspergillus Species Distribution

Aspergillus sp.	AM	PM	Night	Remote Sites	Total
A. amstellodami		1			1
A. caespitosus		1		1	2
A. candidus	6				6
A. flavipes		1			1
A. flavus	6			1	7
A. flavus-oryzae	1	1		1	3
A. fumigatus	14	3	5		22
A. granulosis	7				7
A. janus		1			1
A. nidulans	1				1
A. niger	110	43	2	5	160
A. ochraceous	8				8
A. sydowii	2				2
A. tamarii	1				1
A. unguis		2			2
A. ustus	2				2
A. variacolor	3	4			7
A. versicolor		2			2
Aspergillus sp.	26	4			30
Totals	187	63	7	8	265

niger, the predominant Aspergillus species, was encountered routinely throughout the entire study.

DISCUSSION

The primary sample site for this investigation (Site I) was selected due to its centralized location, easy accessibility and elevated position above ground level obstructions and activity. Sites II, III, and IV were chosen because they represented, respectively, an inhabitated area in the foothills with low population activity, a quiet unpopulated area at about the 4,500 foot level in the mountains and a quiet sparsely populated area at about the 9,000 foot level in the mountains. All four sites had distinctly different properties including population activity, local flora, terrain features, and climatic conditions. Data from Site I were intended to comprise the majority of this study with data from the remote sites being introduced for comparative purposes only.

The two routine sample collection times, 7:00 a.m. and 2:00 p.m., were selected because it was felt that they would project the air-spora population at Site I under a variety of daytime conditions. The morning sample results would indicate the profile when human, mechanical, and atmospheric activity, as well as temperature and ultraviolet radiations, were lowest and relative humidity, and actively dispersed spore concentrations were highest. The afternoon sample would indicate the fungal concentration at a time when the above conditions were reversed, and it was expected that the two samples would

show a significant variation both qualitatively and quantitatively. The night samples at Site I were intended to supplement the morning and afternoon results as well as furnish comparative data in evaluating the importance of time of sample collection during the 24-hour day. The composite of the three Site I samples might then be expected to give a reasonable indication of the overall air-spora population profile of the general area around Site I. Other surveys (7, 11, 15, 31, 41, 48, 49, 63) suggest that this study might be considered to give a reasonable indication of the air-borne fungi present during the period of the study. A somewhat similar study was carried out in the Tucson area by Dworin. While the sampling methods and the time periods differed from ours, the number of samplings were almost equal. The results bore a remarkable similarity in that eight genera were major components of both investigations. Predictable differences, based on the methods of sampling, were apparent within those major components.

Although three of the six plates exposed at Site I at night had to be discarded due to crowding and overgrowth, their crowded condition and the colony counts of the three analyzed plates are important because it suggests that night air is more heavily laden with viable spores than are the morning and mid-day air. As expected, a marked variation appeared between the morning and afternoon colony counts, but the differences were not as great as one might logically expect if afternoon turbulence were not so pronounced.

In light of Gregory's findings on the relationship of height above the ground and the spore concentration (23), the low colony counts obtained at the three remote sites (Table 1) indicates that the air-spora concentration in quiet, sparsely settled areas may be considerably less than that of cities. Little more of value can be derived from those remote samplings.

Following compilation of the air-spora population profile at Site I, various factors making up the official weather report were studied and compared with daily sample collection counts and major component genera incidence in an attempt to sort out any possible factors which would seem to exert noticeable influence on the concentration of these genera and total numbers. It was extremely difficult to see many meaningful general relationships, but some erratic counts appeared to be related to specific weather developments, and two weather-air-spora relationships did indicate some degree of association. These latter two were humidity and wind.

As reported by Rich and Waggoner (52), gusty, showery, rainy periods prior to or during sample collection were observed to give elevated counts while light misty and drizzly rain tended to decrease counts slightly (Fig. 1). Three extremely erratic afternoon samples were obtained on 25, 26, and 27 August, with Penicillium oxalicum completely dominating the 25 and 27 August samples, and Cladosporium contributing most heavily to the 26 August sample. No observable

condition was apparent to explain 101 P. oxalicum colonies on the 25th. It can be speculated that some minor local atmospheric disturbance greatly increased the concentration of those spores by agitating a sporulating colony in the immediate vicinity of the sampling apparatus. All other genera on that plate showed a more normal incidence rate. On the 26th, however, there was a more general increase, possibly due to the heavy rain showers that immediately preceded the sample collection. Extremely heavy P. oxalicum concentration was encountered again on 27th with a heavy rain shower and sample collection beginning concurrently. This sample, too gave more generally elevated counts but the great predominance of P. oxalicum isolated could indicate that an actively sporulating colony of P. oxalicum was present in the proximity of the sampling apparatus, and was the victim of a "direct hit" from one or more rain drops. No other colony counts of this magnitude were encountered, but the few other exceptionally high counts could also be associated with recent showery rain, with hazy conditions following windy periods, or, in the case of three plates, with night collection. The few instances where rain fell steadily over an extended period of time did not coincide with any dramatic reduction in colony count, but rather a token decrease.

The average monthly colony count per unit volume of air paralleled a monthly increased in the average relative humidity to a high

percentage level as indicated in Fig. II. This finding is in agreement with Dworin's observations.

Although there is no observable direct relationship between the daily isolation count and relative humidity computed in terms of humidity at sample collection time, average daily humidity and the cumulative average humidity two or three days prior to sample collection, this increase to a high average relative humidity and the associated increase in spore count could be interpreted as an indication that there is a direct overall relationship in which fungal colonies in arid areas utilize the moisture available in the atmosphere to grow and sporulate. Assuming that there is a critical low relative humidity below which fungal growth is greatly reduced or stopped and above which growth is active, it is possible for the air-spora to remain at a high level or continue to increase even when the relative humidity decreases so long as it does not drop below the favorable level. This proposed effect of relative humidity would only hold true for arid areas where water supplies are limited, and in that respect, might confirm the observations of Barkai-Golen and Glazer (5). A comparison between measured rainfall and increase in colony counts could not account for the gradual increase in air-spora concentrations.

Another approach in evaluating the effect of relative humitity on air-spora was stimulated by Dunklin and Puck's (10) observation that some bacteria suffer a lethal effect from relative humidities in the intermediate

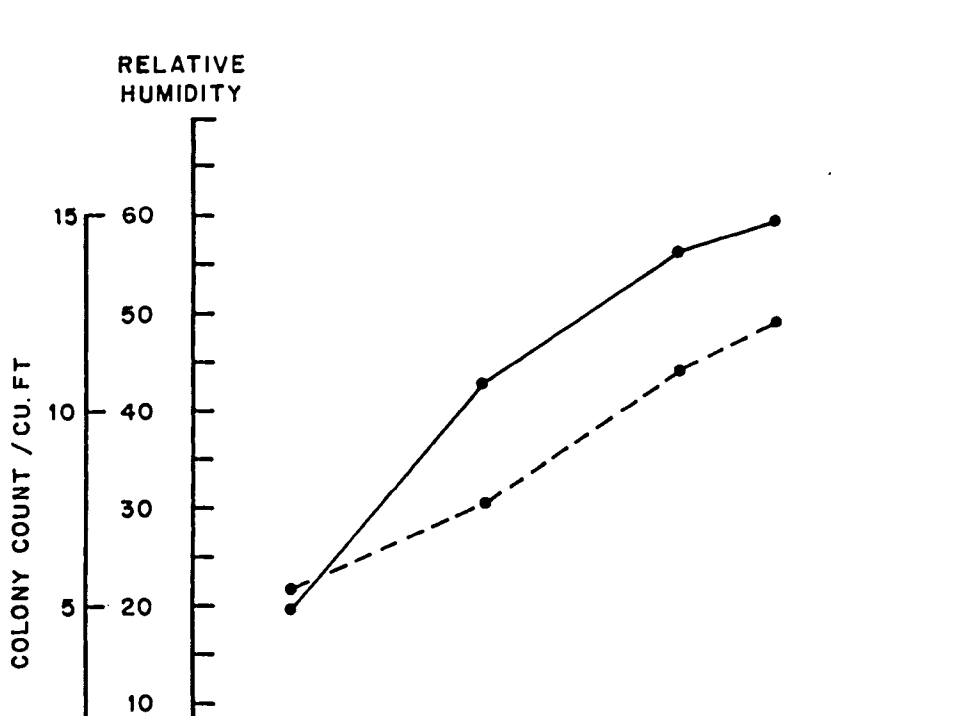

Figure II. --Average Monthly Count Versus Relative Humidity

——Relative Humidity
----Colony Count

(50%) range. An evaluation of relative humidity versus colony count gave no indication that there was any critical relative humidity for fungal spores.

Wind is another factor which has reportedly been a contributing force in increasing air-spora concentration. Although plots of air-spora concentration versus wind speed, wind speed change and wind direction gave a few correlated peaks, the relationship appeared to be negative almost as frequently as it was positive. Since the effects of wind are cumulative to a certain extent, a system was devised whereby wind speeds were segregated into the three categories of low = 0--6 miles per hour (mph), medium = 7--14 mph, and high = 15 mph and up (abbreviated L, M, and H, respectively). Hourly readings at two hours before sampling, one hour before sampling, and the reading at sample time, were assigned increasingly larger scaled values (Table 8). In

TABLE 8. --Wind Value Computation System

	2 hrs before sample	1 hr before sample	At sample time
Low--0 to 6 mph	0	0	0
Medium--7 to 14 mph	1	2	3
High--15 mph and up	3	5	7

this system, L was scored 0 points for the three consecutive readings, M was scored 1, 2, and 3 points respectively, and H was scored 3, 5, and 7 points, respectively. The three speed-categories each with their

three values, made possible 27 combinations with scaled values ranging from LLL = 0 to HHH = 15. Using this method, each sample collection period was scored and plotted, with the morning collections and afternoon collections plotted separately (Fig. III). When the morning colony count plot was overlayed on the morning wind plot, the results were contradictory as was previously noted in the direct comparison above. When the afternoon colony count plot was overlayed on its related wind plot, however, 52 of the 72 possible points showed parallel increase-decrease relationships in amplitude, but not necessarily in magnitude. The fact that a possible relationship is apparent in 72 percent of the total points indicates that this approach might warrant further investigation. It is not enough of a relationship, however, to make any definite conclusions. Therefore, it can be said that wind might be shown to have a direct effect on fluctuations in the daily concentration of airspora, but the indication is that its effect is more noticeable in the afternoon than it is in the morning. Due to the centralized location of Site I, no qualitative variation in colonies was expected to result from changes in wind direction, and none was observed.

Sky cover was studied in relation to daily fluctuations in colony counts to determine if the ultraviolet radiation of clear days would show any discernible reduction in total numbers with a comparative increase becoming apparent on cloudy days. There seemed to be no correlation, however, and daily colony counts fluctuated independent of

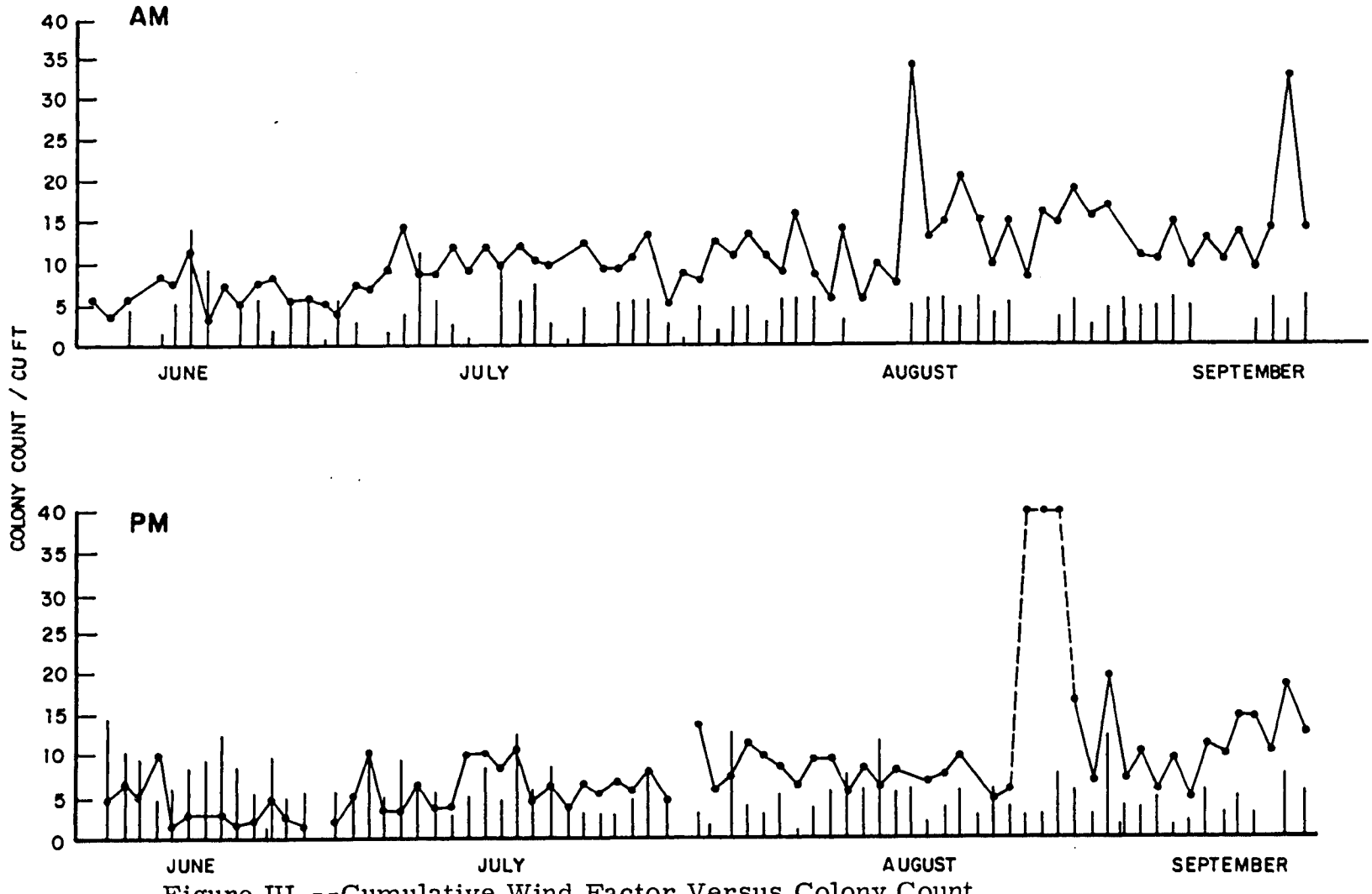

Figure III. --Cumulative Wind Factor Versus Colony Count

——Colony Count
| Wind Factor

sky cover, regardless of the length and intensity of the cloudy and clear periods. The routinely higher morning counts and the absence of daylight with the associated high colony count obtained in the night samples raise the speculation that air-spora could be adversely affected by daylight, as were bacteria in the study by Lidwell and Lowberg (37). The relationship of relative humidity to this proposed susceptibility could not be evaluated due to the limited data obtained. This area might warrant further investigation in a more controlled study in order to provide a better understanding of the factors that grossly affect air-spora viability.

Other weather factors investigated in relation to their effect on daily viable air-spora concentrations included station pressure, dry bulb temperature, wet bulb temperature and dew point. Although these factors are undoubtedly interrelated or influenced among themselves and the previously discussed factors, no clear or indicated correlations of effect on individual or cumulative counts could be identifed. For any meaningful information to be obtained on the singular effect of each of the above factors, controlled laboratory studies in which the majority of unpredictable variables can be eliminated will probably give us more reliable and meaningful results.

Due to the known presence of the potential pathogens Coccidiodes immitis (11) and Cryptococcus neoformans (36, 64) in the Tucson area, it was anticipated that these organisms might be encountered in this

survey, but neither was observed. Failure to isolate either one, however, has no significance other than the fact that viable spores or cells did not appear to be present in the air passing through the sampling apparatus. Of greater but still limited significance is the fact that Aspergillus fumigatus, the most frequently incriminated causative agent in aspergillosis (12), was isolated on 22 occasions from samples collected at Site I during morning, afternoon and night samplings.

SUMMARY

A survey of air-borne fungi in the Tucson area was obtained for the summer months with Cladosporium, Alternaria, Helminthosporium, Penicillium and Aspergillus, in that order, being the most frequently isolated organisms.

Attempts to determine the effects of individual weather factors on the total fungal concentration as well as on major component genera revealed no direct correlation. An overall increase in relative humidity coincided with an overall increase in air-spora, but attempts to relate fluctuations in these two variables did not give strongly indiccative results. Cumulative data suggest that they may be closely related, especially in an arid desert location such as the Tucson area. A correlation was suggested between cumulative wind conditions and the fungal content of air in the afternoon, but no positive relationship could be detected for the morning samplings. Rain showers were observed to occur coincidentally with significantly elevated colony counts, while steady, drizzling rains were noted to coincide with slightly reduced counts. Morning haziness, although not an active factor, appeared to give a fairly reliable indication that elevated viable spore count could be expected. Variations in barometric pressure, sky cover, dry and

wet bulb temperatures, dew point, wind speed, and wind direction could not be correlated with variations in daily air-spora concentrations.

Night air appeared to have considerably more viable elements than early morning air, which, in turn, contained more than midday air. Also, the air of sparsely populated areas, with reduced human and mechanical activity, appeared to contain less viable air-spora than the air of more heavily populated areas.

Aspergillus fumigatus, encountered on 22 occasions, was the only recognized potential pathogen. Coccidioides immitis, Cryptococcus neoformans and Candida albicans were not observed.

The results of this investigation indicate that controlled laboratory studies might be more productive in determining the direct effect of individual weather factors on the incidence of air-spora. This approach might thereby provide a greater understanding of the overall relationship between weather and air-borne fungi.

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