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Abundance of Airborne *Penicillium* CFU in Relation to Urbanization in Mexico City

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Air was sampled simultaneously at three localities in Mexico City differing in urbanization index and air pollution level on 22 days during a period covering both dry and rainy seasons. An Andersen two-stage microbial sampler was used for 15 min at 28 liters min^{-1} to isolate culturable fungi on malt extract agar. After exposure, plates were incubated at 25°C for 48 to 72 h before colonies were counted and identified to give concentrations of total fungal spores and of *Penicillium* spp., expressed as CFU per cubic meter of air. Total fungi numbered 91 to 602 CFU m^{-3} in Tlalpan Borough (southern area), 40 to 264 CFU m^{-3} in Cuauhtémoc Borough (downtown), and 26 to 495 CFU m^{-3} in Gustavo A. Madero Borough (northern area). Although *Penicillium* spp. were the second most frequently isolated fungal genus, concentrations were small, with a maximum of only 133 CFU m^{-3}. Twice as many colonies were isolated in the southern area, with an urbanization index of 0.25 (arithmetic mean, 41 CFU m^{-3}), as at other sampling stations with greater urbanization indices (arithmetic means, 19 and 20 CFU m^{-3}). In the downtown area, with an urbanization index of 1.0, *Penicillium* spp. were more numerous than any other genus and formed 25% of the total fungal count compared with 14 and 17% in the other areas. Concentrations of airborne *Penicillium* spp. did not differ significantly between rainy and dry seasons. However, their concentration was weakly negatively correlated with temperature ($r = 0.36$, $P < 0.01$), vapor pressure ($r = -0.47$, $P < 0.001$), and relative humidity ($r = -0.36$, $P < 0.001$). On average, 70% of *Penicillium* propagules were collected in the small-particle fraction (considered to be respirable on inhalation, <5-μm aerodynamic diameter). Of the eight *Penicillium* species identified, *P. aurantiogriseum*, *P. crustosum*, *P. chrysogenum*, and *P. spinulosum* were the most common. Their small numbers suggest that *Penicillium* spp. are not important outdoor aeroallergens in Mexico City, but total exposure cannot be assessed until indoor environments have been sampled.

Outdoors, airborne spores originate chiefly from vegetation and from soil (15). Consequently, in densely built-up and polluted urban areas, total numbers of fungal spores and the frequency of occurrence of some types are less than in rural areas (1, 13). By contrast, *Penicillium* spp. are often more numerous in city air (22, 23). *Penicillium* spp. are ubiquitous saprobes, able to colonize all types of organic materials common in urban environments (12). *Penicillium* spp. may form 2 to 15% of the culturable fungal spores in outdoor air in Britain, but they can account for 90% of the total in house dust (11). The degree of urbanization, as expressed by the percentage of constructed space, in different parts of Mexico City ranges from 100% in the city center (urbanization index [UI], 1.0) to only 25% in the south (UI, 0.25) (21). How different degrees of urbanization and air pollution might affect the occurrence of airborne fungal spores, especially *Penicillium* spp., is unknown, and we have therefore investigated their relationship in Mexico City.

MATERIALS AND METHODS

Sampling methods. A two-stage Andersen sampler (Andersen Sampler Inc., Atlanta, Ga.) was used to isolate culturable fungal propagules from the air. Each stage includes a plate with 200 holes of uniform diameter (1.5 mm in the first stage and 0.4 mm in the second stage) through which air is drawn at 28 liters min^{-1} to impact on petri dishes containing agar media immediately below. Airborne particles are separated into two fractions: a large-particle fraction which is retained in the upper airways on inhalation and is considered nonrespirable and a small-particle fraction which can penetrate to the gas exchange tissue of the lung and which is considered respirable. At each site, the sampler was mounted on a tower 2 m above ground level with the orifices facing into the wind. Duplicate 15-min samples were collected simultaneously at three sites at 10:00 a.m. at weekly intervals on 11 days during the dry season (February to May 1990) and on a further 11 days during the rainy season (June to October 1990). For each sample, the sampler was loaded with dishes containing malt-extract agar (MEA; Difco) and was operated for 15 min before plates were changed. After exposure, plates were incubated for 48 to 72 h at 25°C.

After incubation, fungal colonies growing on each plate were counted and examined microscopically to determine their morphological characteristics. Counts of each morphological type were transformed, by tables provided by the sampler manufacturer, to account for multiple deposition of particles at individual impaction sites. Results were then expressed as CFU per cubic meter of air. *Penicillium* colonies were coded and subcultured for identification according to the methods, keys, and descriptions of Pitt (20) and Bridge (6).

Meteorological and pollution measurements. Wind speed and wet and dry bulb temperatures were measured at the time of sampling. The Mexican Air Quality Index (IMECA)

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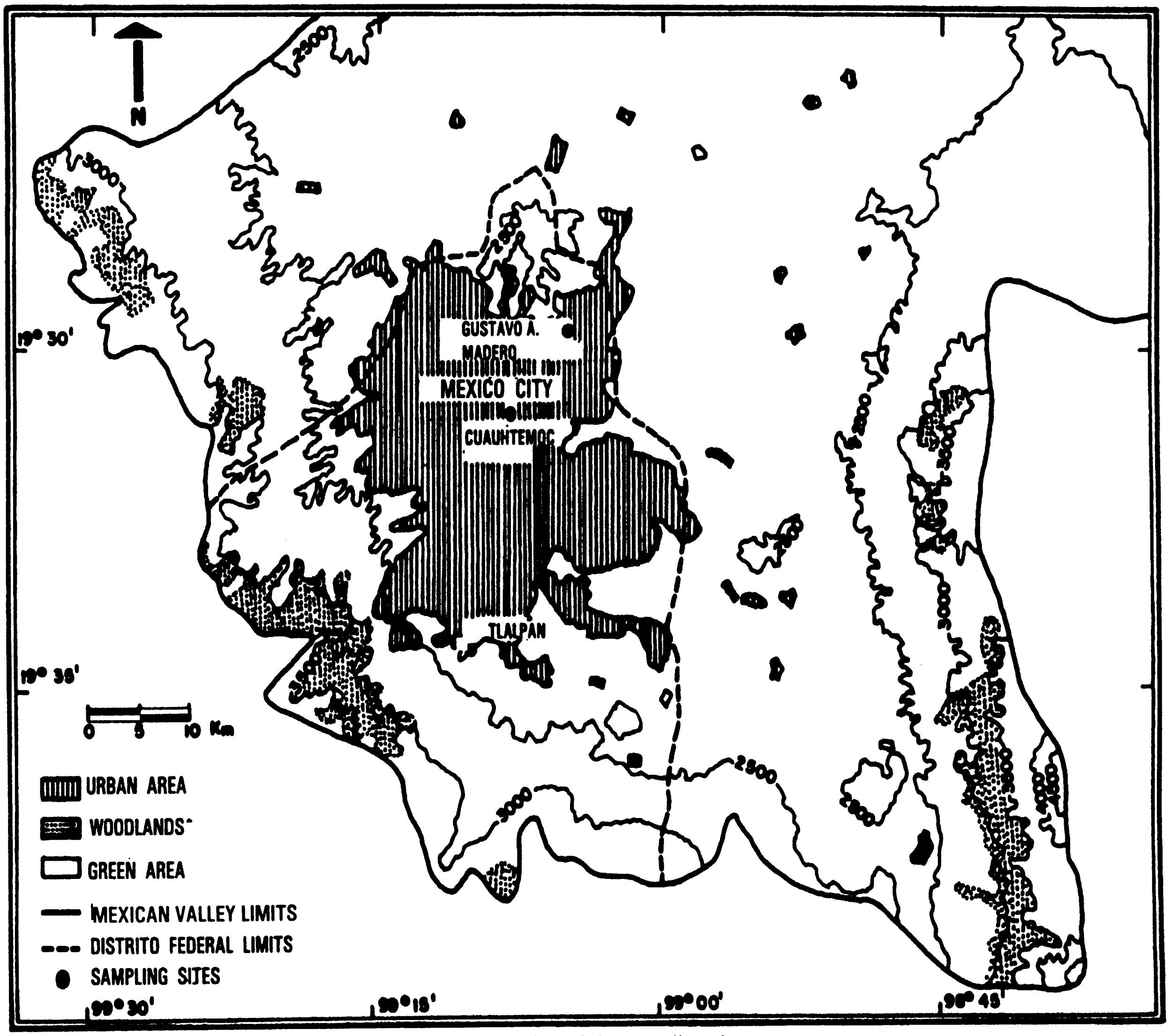

FIG. 1. Location of the sampling sites.

is determined daily by the Mexican Secretariat of Urban Development and Ecology Secretariat (SEDUE) Air Quality Network from the concentrations of different pollutants, especially carbon monoxide, sulfur dioxide, nitrogen oxides, ozone, total suspended particles, and particles smaller than 10 μm (4). IMECA values are reported daily, with the pollutant, usually ozone, present in the highest concentration indicated (10). IMECA values during the period of the study were obtained from SEDUE for each area and were compared with an acceptable IMECA value based on the Mexican air quality standards (7). The frequency (percent) by which the standard was exceeded was calculated for each sampling site.

Sampling areas. Three localities were selected for study (Fig. 1): (i) Tlalpan Borough, a residential area in the southern part of the city, 20 km from the city center, with a UI of 0.25 (21). The IMECA for this area was better than the standard IMECA for only 20% of the year (3). (ii) Cuauhtémoc Borough, a commercial-urban area situated in the city center, with a UI of 1.0 and an IMECA acceptable for 35% of the year. (iii) Gustavo A. Madero Borough, an industrial-urban area located in the northern part of the city, 40 km from the city center, with a UI of 0.64, and with an IMECA acceptable for 45% of the year.

Statistical methods. Because the raw data fitted neither a normal nor a log normal frequency distribution, but appeared closest to a Poisson distribution, a square root transformation ($\sqrt{n} + 0.5$) was applied to all counts before statistical analysis. Analysis of variance and Student's *t* test were applied to the transformed data to compare both total and *Penicillium* concentrations at the different sampling sites. Pearson's correlation coefficients were calculated to test for correlations between total or *Penicillium* counts and different environmental factors.

RESULTS

Geometric and arithmetic mean concentrations of total fungi and *Penicillium* spp., UI, and level of air pollution are given for each sampling site in Table 1, and changes in colony counts between sampling sites at dry and rainy seasons are given in Fig. 2. Table 2 shows the relative

TABLE 1. Concentrations of viable airborne fungi, UI, and air pollution level at sampling sites in Mexico City

Sampling site	No. of samples	Total fungi ($CFU\ m^{-3}$)			*Penicillium* spp. ($CFU\ m^{-3}$)			UI	% Days with acceptable IMECA
		Geometric mean	Arithmetic mean	Range	Geometric mean	Arithmetic mean	Range		
Tlalpan	22	254	293	91–602	24	41	0–133	0.25	20
Cuauhtémoc	22	85	96	40–264	16	19	2–39	1.0	35
G. A. Madero	22	93	108	26–495	14	20	2–62	0.64	45

abundance of the different fungal genera isolated at the three sites. Mycelia sterilia were isolated most frequently, usually followed in sequence by *Cladosporium*, *Penicillium*, *Alternaria*, *Aureobasidium*, and *Fusarium* spp. However, *Penicillium* spp. were the most common in the city center. Together, *Cladosporium* and *Penicillium* colonies accounted for about 45% of the total colonies isolated. The greatest mean concentrations of total fungi and *Penicillium* spp., 293 and 41 CFU m^{-3}, respectively, were both found in Tlalpan Borough, the site with the heaviest pollution and lowest UI. Fungal concentrations were greatest during the dry season, with peaks of 602, 264, and 495 CFU m^{-3} in southern, city center, and northern areas, respectively. In general, *Penicillium* spp. formed 14 to 25% of the total fungal count (Table 2). Thus, *Penicillium* spp. formed 17% of the 495 fungal CFU m^{-3} isolated in the city center. More than 70% of *Penicillium* colonies, especially in the city center, occurred in the

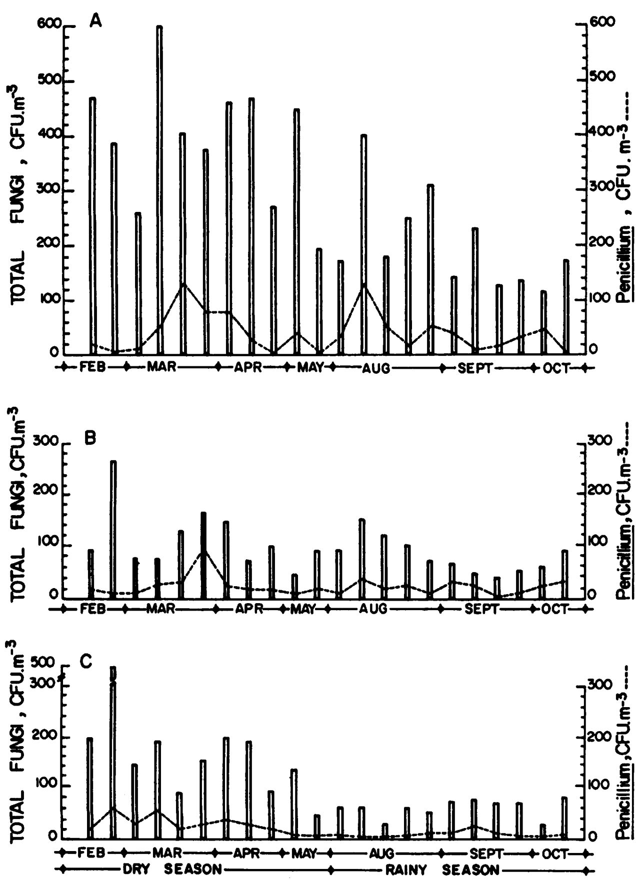

FIG. 2. Concentrations of total airborne fungi and *Penicillium* spp. at each sampling station. (A) Tlalpan Borough; (B) Cuauhtémoc Borough; (C) Gustavo A. Madero Borough.

small-particle fraction, isolated on the second stage of the Andersen sampler, and could be considered respirable (Fig. 3).

Unexpectedly, there were no significant differences in the concentrations of total fungi and *Penicillium* spp. between rainy and dry seasons. However, concentrations of both categories in the southern area (Tlalpan Borough) were significantly greater than in both northern (G. A. Madero Borough) and city center (Cuauhtémoc Borough) areas ($P < 0.001$). Concentrations of *Penicillium* CFU were weakly negatively correlated with temperature ($r = -0.36$, $P < 0.01$), vapor pressure ($r = 0.47$, $P < 0.001$), and relative humidity ($r = -0.36$, $P < 0.01$). The largest concentrations of *Penicillium* spp. usually occurred at low temperatures (15 to 20°C) (Fig. 4), especially in the southern area.

Eight *Penicillium* species were identified (Table 3). *P. aurantiogriseum*, *P. crustosum*, *P. chrysogenum*, and *P. spinulosum* were most often present in samples, especially in March and August. *P. aurantiogriseum* was the most frequently isolated species in Tlalpan and Cuauhtémoc Boroughs, and *P. chrysogenum* and *P. minioluteum* were the most frequently isolated species in Gustavo A. Madero Borough. However, although *P. chrysogenum* was isolated from few samples in the Tlalpan and Gustavo A. Madero Boroughs, it could occur in concentrations exceeding 50 CFU m^{-3}.

DISCUSSION

Airborne fungi can differ both quantitatively and qualitatively within urban areas, depending on population and building densities (2, 8). In the air of Mexico City, the *Penicillium* genus was the second most abundant fungal genus that could be cultured, with concentrations up to 133 CFU m^{-3} and forming 13.8 to 25% of the total fungi isolated. Their predominance is similar to that found previously in Mexico City (18). *Penicillium* spp. are ubiquitous in the air throughout the world, although mean concentrations of culturable propagules are usually small, within the range 3 to 56 CFU m^{-3} (2, 5, 16), although occasionally larger concentrations have been found, such as the 5,791 CFU m^{-3} found in California by Licorish et al. (17).

There was no clear relationship between the concentration of airborne *Penicillium* spp. and UI in our study, perhaps because the sampling sites were not widely separated. The prevailing northeast wind could possibly have blurred distinctions between areas, but this seems unlikely because the largest concentrations of both total fungi and *Penicillium* CFU were found in the southern area with the smallest UI and the largest green area. This appears to contradict earlier reports of larger numbers of airborne *Penicillium* spp. in urban than in rural areas (8). Concentrations of airborne *Penicillium* spp. have been reported to be independent of seasonal changes (1, 16), but we found a very weak but

TABLE 2. Abundance of fungi identified in air samples from Mexico City

Genus or stage	Tlalpan Borough		Cuauhtémoc Borough		G. A. Madero Borough	
	Arithmetic mean (CFU m^{-3})	Occurrence (%)	Arithmetic mean (CFU m^{-3})	Occurrence (%)	Arithmetic mean (CFU m^{-3})	Occurrence (%)
Alternaria	26.0	8.7	2.8	2.9	5.0	4.2
Aspergillus	5.0	1.7	6.0	6.3	4.0	3.3
Aureobasidium	8.0	2.7	3.8	4.0	2.6	2.2
Cladosporium	99.0	33.3	16.0	16.7	34.9	29.3
Epicoccum	0.7	0.3	0.3	0.3	0.2	0.2
Fusarium	8.0	2.7	1.5	1.6	2.6	2.2
Helminthosporium	3.0	1.0	1.0	1.0	0.7	0.6
Monilia	0.2	0.1	0.5	0.5	0.7	0.6
Paecilomyces	0.4	0.1	1.0	1.0	0.5	0.4
Penicillium	41.0	13.8	24.0	25.0	19.7	16.6
Phoma	0.7	0.2	0.3	0.3	0.5	0.4
Rhizopus	0.5	0.1	0.8	0.8	0.5	0.4
Trichoderma	2.0	0.7	0.0	0.0	0.1	0.1
Mycelia sterilia	102.5	34.6	38.0	39.6	47.0	39.5
Total fungi	297	100	96	100	119	100

significant negative correlation between concentration and temperature ($r = -0.36$, $P < 0.01$), as did Dransfield (9).

Penicillium propagules collected out-of-doors with the two-stage Andersen sampler were almost entirely (80%) in the small-particle fraction and should be considered respirable. Flannigan et al. (11) found a similar size distribution in indoor air. Fewer colonies occurred in the small-particle fraction in the southern area than in other areas, perhaps because the humidity at this sampling site was greater than that at the others, allowing some of the 3-μm-diameter spores to be dispersed in aggregates that would be impacted on the first stage of the sampler.

The most common *Penicillium* species were *P. aurantiogriseum*, *P. crustosum*, *P. chrysogenum*, and *P. spinulosum*. Of these, *P. chrysogenum* was also found to be most abundant outdoors in Scotland (11). Comparison of the incidences of *Penicillium* spp. in different studies is difficult because changes in the taxonomy of *Penicillium* spp. have produced many name changes and have reduced some previously accepted species to synonymy (20).

Earlier studies have shown that asthma can be provoked by the inhalation of both intact *Penicillium* spores and spore extracts (17, 24). Challenge with at least 6×10^4 *Penicillium* spores may be necessary to provoke asthma. To achieve this dose within 1 day would require uniform exposure to 6×10^3 CFU m^{-3}, assuming that an adult man inspires 10 m^3 of air in this period. This concentration is far greater than any concentration that we found. However, the total number of *Penicillium* spores, culturable and nonculturable but still allergenic, may be much greater, but assessment of their number is difficult because of their small size, the relatively inefficient collection by volumetric spore traps assessed microscopically, and because of the difficulty of separating

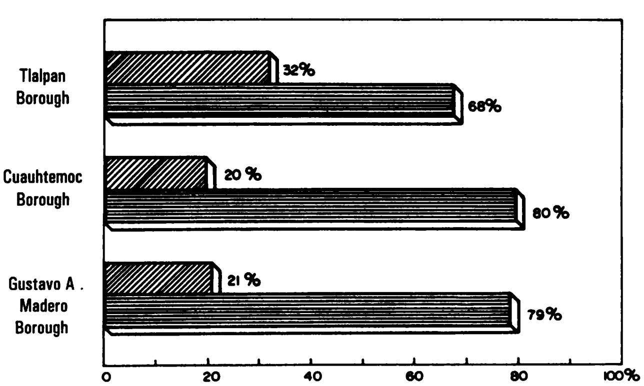

FIG. 3. Distribution of *Penicillium* propagules at each sampling site between the two stages of the Andersen sampler. ▨, first stage, large-particle fraction; ▤, second stage, small-particle fraction.

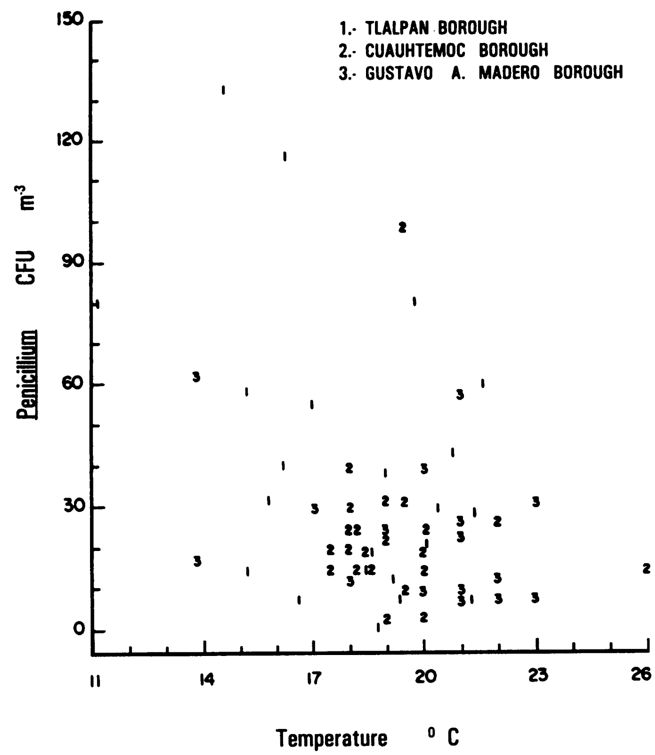

FIG. 4. Concentrations of airborne *Penicillium* propagules in relation to temperature at the time of sampling.

TABLE 3. Frequency of isolation of different *Penicillium* spp. at different sites in Mexico City

Species	Frequency of isolation at sampling site[a]:		
	Tlalpan	Cuauhtémoc	Gustavo A. Madero
P. aurantiogriseum	f	f	o
P. chrysogenum	o	f	f
P. citreonigrum	–	–	o
P. claviforme	o	o	o
P. crustosum	o	f	o
P. miniolutеum	–	o	f
P. purpurogenum	–	o	o
P. spinulosum	f	o	o

[a] Key: –, absent; o, isolated occasionally; f, isolated frequently.

spores from the many inorganic particles present in the air of Mexico City. Nevertheless, our results suggest that *Penicillium* spp. are unlikely to be important allergens in Mexico City unless subjects are exposed to locally intense sources at home or at work.

Penicillium spp. often dominate the indoor air spora in concentrations often greatly exceeding those outdoors. They grow on damp surfaces, on food particles, house dust, or other organic matter and in stored products (14, 17, 19). Assessment of the total exposure of the general population thus requires simultaneous measurement of concentrations both indoors and outdoors.

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REFERENCES

1. **Adams, K. I.** 1964. Year to year variation in the fungus spore content of the atmosphere. Acta Allergol. **19:**11–50.
2. **Al-Doory, Y., F. J. Domson, and J. Best.** 1982. Further studies of the airborne fungi and pollens of the Washington, D.C. Metropolitan area. Ann. Allergy **49:**265–269.
3. **Anonymous.** 1990. Programa integral contra la contaminación atmosférica, documento criterio. Secretariat of Urban Development and Ecology Secretariat, Mexico City.
4. **Anonymous.** 1992. Boletín informativo de la calidad del aire (1986–1992). Secretaría de Desarrollo Social, Instituto Nacional de Ecología, Mexico City.
5. **Beaumont, F., H. F. Kauffman, T. H. van der Mark, H. J. Sluiter, and K. de Vries.** 1985. Volumetric aerobiological survey of conidial fungi in the north-east Netherlands. Allergy **40:**173–180.
6. **Bridge, P. D.** 1985. An evaluation of some physiological and biochemical methods as an aid to the characterization of species of *Penicillium* Subsection Fasciculata. J. Gen. Microbiol. **131:** 1887–1895.
7. **Cicero-Fernández, P.** 1991. Prioritizing air pollutant controls for urban centers in developing countries: Mexico City as a case study. Air Waste Management Association Paper 91-115.10.
8. **Davies, R. R., M. Denny, and L. Newton.** 1963. A comparison between the summer and autumn airsporas at London and Liverpool. Acta Allergol. **18:**131–147.
9. **Dransfield, M.** 1966. The fungal air-spora at Samaru, northern Nigeria. Trans. Br. Mycol. Soc. **49:**121–132.
10. **Escurra, E.** 1991. Qué mide el IMECA? Ciencias **22:**41–43.
11. **Flannigan, B., E. M. McCabe, and F. McGarry.** 1991. Allergenic and toxigenic micro-organisms in houses. J. Appl. Bacteriol. **70**(Suppl.):61S–73S.
12. **Gravesen, S.** 1978. Identification and prevalence of culturable mesophilic microfungi in house dust from 100 Danish homes. Allergy **33:**268–272.
13. **Hamilton, E. D.** 1959. Studies on the air spora. Acta Allergol. **13:**143–175.
14. **Hyde, H. A.** 1973. Atmospheric pollen grains and spores in relation to allergy. II. Clin. Allergy **3:**109–126.
15. **Lacey, J.** 1987. Airborne fungus spores in outdoor air, p. 125–128. *In* D. W. Griffiths (ed.), Aerosols, their generation, behaviour and application. The Aerosol Society, Loughborough, United Kingdom.
16. **Larsen, L. S.** 1981. A three-year-survey of microfungi in the air of Copenhagen 1977–79. Allergy **39:**15–22.
17. **Licorish, K., H. S. Novey, P. Kozak, R. D. Fairshter, and A. F. Wilson.** 1985. Role of *Alternaria* and *Penicillium* spores in the pathogenesis of asthma. J. Allergy Clin. Immunol. **76:**819–825.
18. **López Martínez, R., D. Ruiz Sánchez, J. G. Huerta, A. Esquenaze, and M. Álvarez.** 1986. Variación estacional de hongos productores de alergia en el sur de la Ciudad de México. Allergol. Immunopathol. **14:**43–48.
19. **Lumpkins, E. D., S. L. Corbit, and G. M. Tiedeman.** 1973. Airborne fungi survey. Culture plate method of the home environment. Ann. Allergy **31:**361–370.
20. **Pitt, J. I.** 1979. The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*. Academic Press, London.
21. **Rapoport, H. E., E. M. Díaz-Betancourt, and R. I. López-Moreno.** 1983. Aspectos de la ecología urbana de la Ciudad de México. Editorial Limusa, Mexico City.
22. **Richards, M.** 1954. Seasonal periodicity in atmospheric mould spore concentrations. Trans. Br. Mycol. Soc. **37:**357–366.
23. **Richards, M.** 1956. A census of mould spores in the air over Britain in 1952. Trans. Br. Mycol. Soc. **30:**431–441.
24. **Salvaggio, J., and I. Aukrust.** 1981. Mold induced asthma. J. Allergy Clin. Immunol. **68:**327–346.