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Functional relationships between airborne fungal spores and enviromental factors in Kitchener-Waterloo, Ontario, as detected by Canonical correspondence analysis

DE-WEI LI and BRYCE KENDRICK

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Outdoor air-sampling surveys were conducted in February, May, August and December 1992 with a Samplair-MK1 particle sampler at 50 randomly chosen sites in the Kitchener-Waterloo area of southern Ontario, Canada. Canonical Correspondence Analysis of the resulting data revealed that the influence of some environmental factors on the airborne fungal spora varied with the season of the year. Among the 16 environmental factors measured at each sampling time, the most important were found to be: relative humidity, rain, vegetation, cloud, temperature, and wind speed, in descending order. The composition of the airborne fungal spora also changed with the season. The dominant *Cladosporium, Alternaria* and *Aspergillus* + *Penicillium* were found at all seasons, but *Ganoderma, Leptosphaeria, Coprinus, and Polythrincium* occurred mainly in summer. Positive relations were revealed between, on the one hand, (1a) relative humidity, (1b) rain, (1c) cloud and (1d) temperature, and on the other hand, (2) high spore counts of *Leptosphaeria*, Xylariaceae, unidentified Ascomycetes, and *Ganoderma*. A similar relationship was detected between (1) vegetation and (2) *Alternaria* and *Oidium*. (1) Higher wind speeds were positively related with (2) hyphal fragments and relatively large spores, such as those of *Drechslera, Nigrospora, Periconia,* and *Oidium*. Canonical Correspondence Analysis provides both a new approach to the analysis of aeromycological data and informative graphical presentations of the results.

D.-W. Li and B. Kendrick, Department of Biology, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada.

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The air is seldom free of fungal spores (Lacey 1981). Most of the fungi observed in the air are those also found in the rhizosphere, the rhizoplane, and on both the dead and living leaves of plants (Kumar 1982). Airborne fungal spores originate from centaminated animals, birds, plants, soil, manure, decaying plants, and human activities (Al-Doory et al. 1980).

The composition and concentration of airborne fungal spora are, therefore, largely determined by geographical location, meteorological factors, vegetation, and human activities (Lacey 1981), as well as by a wide range of interrelated environmental and biological factors (Lyon et al. 1984). Concentrations of fungal spores in the atmosphere at any particular moment are influenced by the processes involved in their production, release, and deposition (Lyon et al. 1984). The eventual rate of spore deposition depends largely on meteorological factors and the size and shape of the spores (Lyon et al. 1984).

Many environmental factors are interrelated and it is often difficult to know which are the most significant (Skre 1981). A better understanding of the relative importance of these factors and their interrelationships would help determine the relationship of airborne spores to allergies caused by airborne fungal spores (Lyon et al. 1984).

Much of our knowledge of the behaviour of airborne spores comes from studying the epidemiology of plant diseases and of infectious diseases and allergies in man (Lacey 1981). More attention is now being paid to the fungal spores in the air and to the human health problems they cause.

Whenever the number of influential environmental factors is greater than two of three, is becomes difficult to obtain an overall graphical summary of multiple species-environment relationships using ordinary multiple regression (ter Braak 1987). To solve this problem, ter Braak (1986) introduced Canonical Correspondence Analysis (CCA) into community ecology. This form of analysis is an eigenvector ordination technique which also produces a multivariate direct gradient analysis (ter Braak 1987). Using CCA, axes are selected to be linear combinations of environmental factors, so that the species are related directly to a set of environmental factors (Dixit et al. 1989). Canonical correspondence analysis has been used both for detecting overall species-environment relationships and for tracing specific questions of individual species response to particular environmental factors (ter Braak 1987). The CCA ordination diagram is not in any way hampered by high correlations between species, or between environmental variables (ter Braak 1987). Canonical correspondence analysis permits the incorporation of existing knowledge about species-environment relationships into the

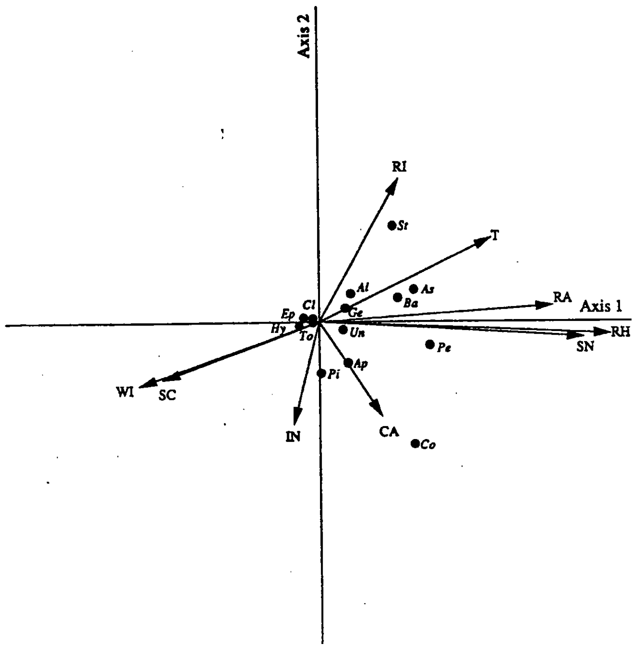

Fig. 5. Canonical correspondence analysis ordination biplot showing environmental factors (*arrows*) and airborne fungal genera (filled circles) from December. CA = car passing by, IN = industrial area, RA = rain, RH = relative humidity, RI = river/lake nearby, SC = snow cover, SN = snow, T = temperature and WI = wind speed; *Al = Alternaria, Ap = Aspergillus/Penicillium, As =* Ascomycetes, *Ba =* Basidiomycetes, *Cl = Cladosporium, Co = Coprinus, Ep = Epicoccum, Ge* = No. of fungal genera, *Hy* = hyphal fragments, *Pe = Periconia, Pi = Pithomyces, St = Stemphylium, To* = Total no. fungal spores and *Un* = unidentified spores.

mental factors (Fig. 5). *Periconia, Coprinus*, Ascomycetes, Basidiomycetes, and *Stemphylium* responded to higher RH. *Alternaria, Aspergillus/Penicillium, Pithomyces, Epicoccum* and hyphal fragments were most numerous at intermediate RH. The relationship between fungal taxa and the environmental factors snow, rain, and temperature were similar to those for RH. The response of fungal taxa to wind speed and snow cover was opposite to that for temperature.

Whole year

Canonical axis 1 (48.3%) and axis 2 (25.2%) explained a significantly large quantity of the variance in the fungus-environment relationship verified by the Monte Carlo permutation Test (Table II). Axis 1 is principally defined by vegetation, and axis 2 by RH. The eigenvalues of axis 1 and axis 2 are 0.24 and 0.13. Among the 16 environmental factors measured at each sampling time, the most important, according to the arrow lengths, were found to be: RH, rain, vegetation, cloud, temperature and wind speed in descending order (Fig. 6).

Leptosphaeria responded to high RH. Xylariaceae, unidentified Ascomycetes, *Ganoderma, Bipolaris*, unidentified Basidiomycetes, *Alternaria, Coprinus, Torula, Stemphylium, Pithomyces, Arthrinium, Drechslera*, hyphal fragments, *Oidium, Nigrospora*, and *Periconia* occurred in that order along the RH ranking from medium to low medium (Fig. 6). Increasing wind speed and snow cover reversed this ranking. *Leptosphaeria* and *Drechslera* number increased during rain, but *Alternaria* spores peaked in mist or no rain. The other taxa reached their highest numbers in light rain. *Alternaria*

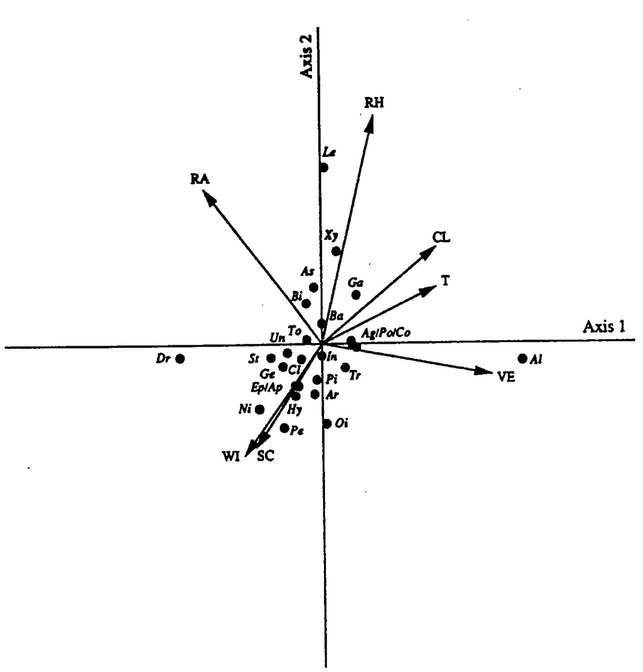

Fig. 6. Canonical correspondence analysis ordination biplot showing environmental factors (*arrows*) and airborne fungal genera (filled circles) form 1992. Cl = cloud, RA = rain, RH = relative humidity, SC = snow cover, T = temperature, VE = vegetation and WI = wind speed, *Ag = Agrocybe, Al = Alternaria, Ap = Aspergillus/Penicillium, Ar = Arthrinium, As =* Ascomycetes, *Ba =* Basidiomycetes, *Bi = Bipolaris, Cl = Cladosporium, Co = Coprinus, Dr = Drechslera, Ep = Epicoccum, Ga = Ganoderma, Ge* = No. of fungal genera, *Hy* = hyphal fragments, *In = Inocybe, Le = Leptosphaeria, Ni = Nigrospora, Oi = Oidium, Pe = Periconia, Pi = Pithomyces, Po = Polythrincium,. St = Stemphylium, To* = Total no. of fungal spores, *Tr = Torula, Un* = unidentified spores and *Xy* = Xylariaceae.

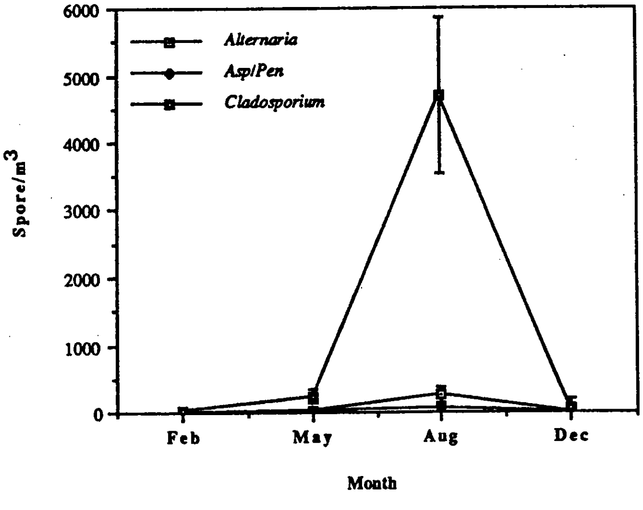

Fig. 7. Average spore concentrations of *Alternaria, Aspergillus/ Penicillum*, and *Cladosporium* in February, May, August and December of 1992.

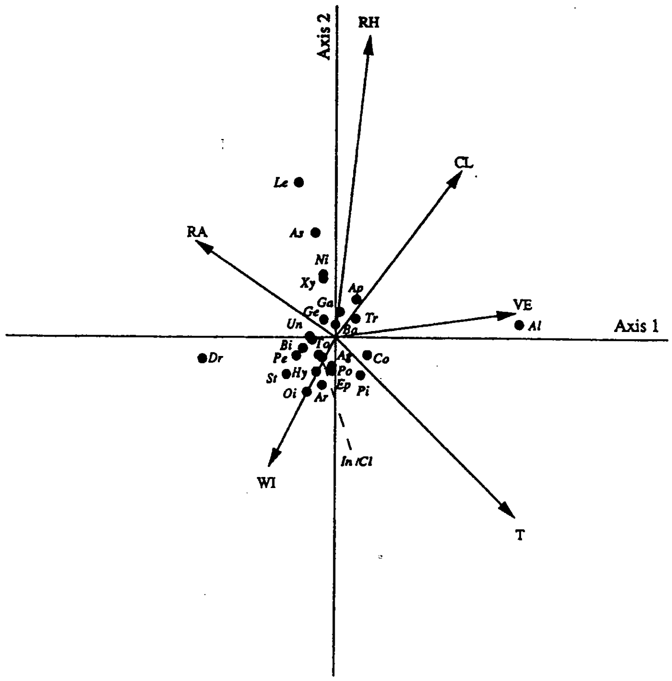

Fig. 4. Canonical correspondence analysis ordination biplot showing environmental factors (*arrows*) and airborne fungal genera (filled circles) from August. CL = cloud, RA = rain, RH = relative humidity, T = temperature, VE = vegetation, WI = wind speed; *Ag = Agrocybe, Al = Alternaria, Ap = Aspergillus/Penicillium, Ar = Arthrinium, As = Ascomycetes, Ba = Basidiomycetes, Bi = Bipolaris, Cl = Cladosporium, Co = Coprinus, Dr = Drechslera, Ep = Epicoccum, Ga = Ganoderma, Ge =* No. of genera, *Hy* = hyphal fragments, *In = Inocybe, Le = Leptosphaeria, Ni = Nigrospora, Oi = Oidium, Pe = Periconia, Pi = Pithomyces, Po = Polythrincium, St = Stemphylium, To* = Total no. of fungal spores, *Un* = unidentified spores and *Xy* = Xylariaceae.

value of axis 1 is 0.08 and of axis 2, 0.07. In light rain, the commonest spores were of unidentified fungi, *Leptosphaeria*, unidentified Ascomycetes, *Cladosporium*, *Ganoderma* unidentified Basidiomycetes, *Epicoccum*, hyphal fragments, *Coprinus*, *Nigrospora*, *Pithomyces*, and *Drechslera* (Fig. 2). In very light rain *Stemphylium* and *Aspergillus/Penicillium* were most numerous (Fig. 2). *Aspergillus/Penicillium*, *Stemphylium*, *Coprinus*, Basidiomycetes, Ascomycetes, hyphal fragments, and unidentified spores responded in descending order of occurrence to temperature from relatively higher to intermediate. The remainder of the taxa were recorded most often at lower temperatures. The relationship between wind speed and fungal taxa is inverse to that of temperature with fungal taxa. Rain and human population density resulted in similar rankings. The same congruence was also discernible with vegetation and river/lake proximity (Fig. 1).

May

In May, the dominant environmental factors, according to the arrow lengths, were vegetation, expressway nearby, industrial area and wind speed (Fig. 3). Axis 1 is defined by vegetation, human activities, and wind speed, axis 2 by expressway and industrial area. A significant proportion of the variance in the species-environment relations was interpreted on canonical axis 1 (29.8%) and axis 2 (15.1%) (Table II). The eigenvalues of axis 1 and axis 2 are 0.10 and 0.05 respectively. Conidia of *Torula* and *Nigrospora* occurred in well vegetated areas; *Periconia*, *Alternaria*, *Coprinus*, unidentified fungi, *Aspergillus/Penicillium*, hyphal fragments, Ascomycetes, and *Bipolaris* in intermediately vegetated areas. *Pithomyces*, *Oidium*, *Leptosphaeria*, and *Ganoderma* were highest in the least vegetated areas. The highest counts of *Pithomyces* and *Ganoderma* were found in industrial areas or near the expressway. *Ganoderma* and *Pithomyces* were most numerous under high wind speed conditions, while *Bipolaris*, *Leptosphaeria*, *Oidium*, Ascomycetes, *Aspergillus/Penicillium*, hyphal fragments, *Alternaria*, and *Periconia* responded to intermediate wind speeds, and *Nigrospora* and *Torula* to low wind speed (Fig. 3).

August

Canonical axis 1 (56.9%) and axis 2 (31.5%) explained a highly significant amount of the variance in the fungus-environment interactions verified by the Monte Carlo permutation Test (Table II). Axis 1 is defined by vegetation and temperature, axis 2 by RH. The eigenvalues of axis 1 and axis 2 are 0.33 and 0.19. On the basis of the environmental arrow lengths relative humidity, temperature, cloud, vegetation, rain, and wind speed, in descending order, were found to be the most important environmental factors (Fig. 4). Relative humidity, as the most significant factor, had a great influence on the number of airborne fungal spores. *Leptosphaeria* and Ascomycetes were commonest at higher RH; *Nigrospora*, Xylariaceae, *Aspergillus/Penicillium*, *Ganoderma* and *Alternaria*, *Coprinus*, *Drechslera*, *Stemphylium*, hyphal fragments, *Pithomyces*, *Arthrinium*, and *Oidium* at intermediate RH. Cloud cover had a similar relationship with those genera, but the rank of *Alternaria* changed from medium to high and *Drechslera* from medium to low. Wind speed had an opposite relation with these fungal taxa (Fig. 4). *Alternaria* responded to higher temperature than did *Pithomyces*, *Coprinus*, *Arthrinium*, *Oidium*, and hyphal fragments. With respect to rain, fungal taxa have an opposite relationship to that reported for temperature. *Alternaria* was commonest in well-vegetated areas while spores of the other taxa apparently drifted in the areas from intermediately to least vegetated (Fig. 4).

December

Canonical axis 1 (42.9%) and axis 2 (17.9%) captured a very significant proportion of the variance in species-environment relationships confirmed by the Monte Carlo Permutation Test (Table II). The first axis is explained by RH, snow, and rain, and the second axis by river/lake nearby. The eigenvalues of axis 1 and axis 2 are 0.08 and 0.03. According to the arrow lenghts, relative humidity, snow, rain, temperature, wind speed, snow cover, and nearby river/lake, in descending order were recognized as the most important environ-

Table I. *Variables used in Canonical correspondence analysis*

Variable	Measurement
Cloud	%
Commercial area	rank 0 – 4
Expressway nearby	m/100
Human activities	rank 0 – 5
Human population density	discrete data
Industrial area	rank 0 – 4
Lawn area	rank 0 – 20
Rain	rank 0 – 5
Relative humidity	%
River/Lake nearby	m/100
Snow fall	rank 0 – 5
Snow cover on the ground	%
Temperature	°C
Traffic density	discrete data
Vegetation	discrete data
Wind speed	m/sec

analysis and is thus potentially a poverful tool to advance analysis of this knowledge (ter Braak 1986). Fängström & Willén (1987), Stevenson et al. (1989) & Dixit et al. (1989), among others, showed that CCA is a useful ecological ordination technique in fresh-water ecology. Canonical Correspondence Analysis generates ordination bipolts displaying two types of entities together in one diagram from which one can easily visualize and quantify the environmental factors determining species distributions (Dixit et al. 1989, Smilauer 1991). It offers excellent potential for assessing the relative effects of multiple environmental factors on airborne fungal spores.

The objective of this study was to apply CCA to determine the relationship between airborne fungal spores and various environmental factors during different seasons of the year in Kitchener-Waterloo, southern Ontario.

MATERIALS AND METHODS

Fifty locations in the Kitchener-Waterloo area of southern Ontario were randomly chosen as outdoor air-sampling sites covering diverse areas from the city centre to the suburbs, up to 7 km east and west from Kitchener city hall and 10 km to north and south. At each location, on each survey data, one 10 min sample was taken with a Samplair-MK1 particle sampler (supplier: Allergenco/Blewstone Press, 403–7834 Broadway, San Antonio, TX, 78209, USA) on the top of a car. The sampler drew 15.5 L of air min^{-1} (factory calibration) and was run by a SUN-PAC MK1 rechargeable battery. The surveys were conducted in February, May, August, and December of 1992. Each survey took 3 to 5 days from 9:00 am to 9:00 pm to cover all 50 sampling sites. For each sample taken, 16 environmental factors were monitored concurrently: temperature, relative humidity (RH), rain, wind speed, cloud, vegetation, snow, snow cover on the ground, lawn area, human activities nearby, commercial area, industrial area, distance from the nearest river or lake, distance from the nearest expressway, human population density, and traffic density (Table I). Temperature and RH were measured with a hygrothermometer. Wind speed was measured with an airflow velometer. Rain and snowfall were recorded in 5 categories related to their amount. Cloud and snow cover were estimated according to the proportion of the sky or ground covered. Trees, shrubs, and hedges within 50 m were counted. Two trees less than 2 m high, two shrubs, or a 3 m-long hedge were each regarded as equivalent to one full-size tree. Lawn area and the distances from river/lake and expressway were estimated visually or by reference to a city map. Human activities included football training, over 20 school children playing, lawn mowing, and construction under way within 20 m. Commercial and industrial areas were located on a city map. Human population density data obtained from the Planning and Development Department of the Rigional Municipality of Waterloo. The environmental and species data need not be transformed.

The slides used sampling were coated with a thin layer of a mixture of 90% vaseline and 10% high melting point wax (by weight) and mounted with polyvinyl lactophenol. All fungal spores from the samples were counted and identified under the 40x objective of a Nikon light microscope equipped with phase contrast optics. The smaller fungal spores were counted and identified under the 100x objective. The following data were collected and analyzed, spores of: (1) twenty identified fungal genera: conidia of *Alternaria, Aspergillus/Penicillium, Arthrinium, Bipolaris, Cladosporium, Drechslera, Epicoccum, Nigrospora, Oidium, Periconia, Pithomyces, Polythrincium, Stemphylium,* and *Torula*; ascospores of *Leptosphaeria*, basidiospores of *Agrocybe, Coprinus, Ganoderma,* and *Inocybe*; (2) Xylariaceae, other Ascomycetes, and Basidiomycetes which could not be identified to genus; and (3) hyphal fragments, unidentified spores, total fungal spores and total number of genera. Since the spores of *Aspergillus* and *Penicillium* cannot be distinguished under a light microscope, these two genera were recorded as one pooled taxon.

Data from different months and for the whole year were subjected to Canonical correspondence analysis using the software CANOCO

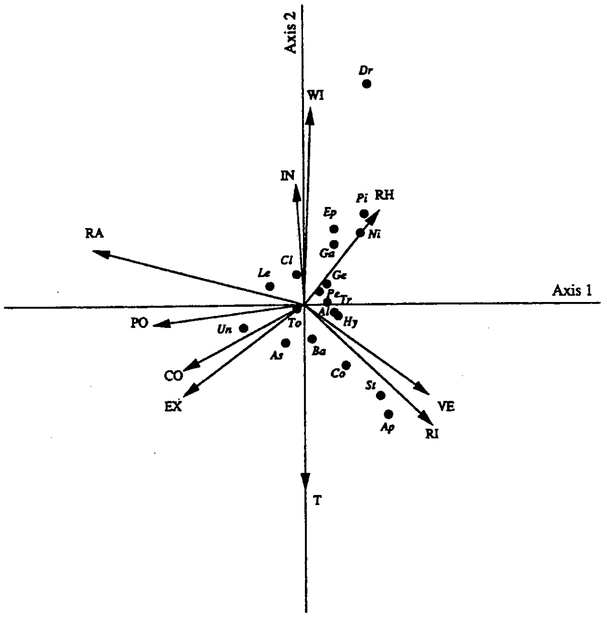

Fig. 1. Canonical correspondence analysis ordination biplot showing environmental factors (arrows) and airborne fungal genera (filled circles) from February. CO = commercial area, EX = expressway, IN = industrial area, PO = human population density, RA = rain, RH = relative humidity, RI = river/lake nearby, T = temperature, VE = vegetation and WI = wind speed; *Al* = *Alternaria Ap* = *Aspergillus/Penicillium, As* = *Ascomycetes, Ba* = *Basidiomycetes, Cl* = *Cladosporium, Co* = *Coprinus, Dr* = *Drechslera, Ep* = *Epicoccum, Ga* = *Ganoderma, Ge* = No. of genera, *Hy* = hyphal fragments, *Le* = *Leptosphaeria, Ni* = *Nigrospora, Pe* = *Periconia, Pi* = *Pithomyces, St* = *Stemphylium, To* = Total no. of fungal spores, *Tr* = *Torula,* and *Un* = unidentified spores.

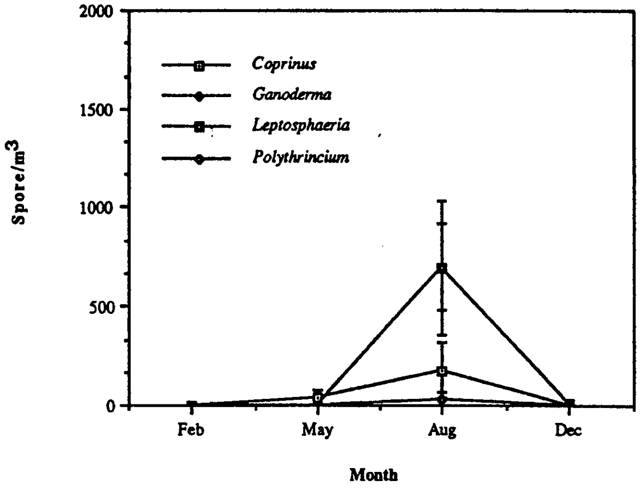

Fig. 8. Average spore concentrations of *Coprinus, Ganoderma, Leptosphaeria,* and *Polythrincium* in February, May, August and December of 1992.

was commonest in well-vegetated areas, while the remainder of the aeromycota were associated with less vegetated areas.

The results from the CCA clearly show that the influence of some environmental factors on the airborne fungal spora varied with the season of the year (Figs. 1, 3–6). The components of the aeromycospora also changed with the season. The dominant *Cladosporium, Alternaria,* and *Aspergillus/ Penicillum* were found in alll seasons, but *Genoderma, Leptosphaeria, Coprinus,* and *Polythrincium* were most numerous in summer (Figs. 7, 8). *Agrocybe, Arthrinium, Inocybe,* and *Oidium* were found only during the growing season (Figs. 1, 3–6).

DISCUSSION

Meteorological conditions clearly have profound influences on the production, dispersal, and desposition of fungal spores. Rain, wind speed, wind direction, humidity temperature, and flora and fauna in the testing area are among the major factors which affect the concentration of airborne fungal spores (Al-Doory et al. 1980). The influence of meteorological factors on the airborne fungal spore concentration appears to be additive, not independet (Munk 1981).

Our study indicates that PH, rain, vegetation, cloud, temperature, and wind speed, in that order of importance, effectively influence instantaneous values of airborne spora. Beaumont et al. (1985) concluded that the approximate order of importance of the meteorological factors is: rainfall, wind direction, daily maximum temperature, sunshine, windforce, and convection: these conclusions are not very different from ours, considering that wind direction and convection were not monitored in our study. The differences in research, areas, seasons, or years could all be responsible for the differences recorded. Our study revealed that the influence of some environmental factors on airborne fungal spores clearly varied with the season of the year.

We note that non-instantaneous factors such as rain, snow fall and very strong wind on previous days may have equally significant or even more significant effects on the fungal spore composition and level. These non-instantaneous effects need to be considered in future research.

Relative humidity and temperature

Relative humidity is, by definition, negatively correlated with temperature (Lyon et al. 1984). Our study indicated that higher spore counts of conidial fungi were associated with higher temperatures and lower RH, while those for Ascomycetes were greatest at higher RH and lower temperature (Fig. 4). Similar results have been obtained in other studies (Leach 1975, Beaumont et al. 1985, Davis & Main 1986). Some kinds of ascospores are released during the night, possibly in response to the increasing RH which accompanies falling temperatures (Burge 1986).

Controlled environment studies on *Drechslera turcica* and other conidial fungi have indicated that spore release is triggered off by decreasing RH and not by temperature changes (Leach 1975), but positive correlations were found between total aerospora, *Cladosporium* spores, and temperature (Beaumont et al 1985). Low temperatures were found to depress conidium release in *Cladosporium* and *Alternaria* (Halwagy 1989).

Relative humidity was correlated with Basidiomycetes spore numbers by Beaumont et al. (1985). High counts of basidiospores may have resulted from higher relative humidity and lower sunshine in 1978 in Galway, Ireland (McDonald & O'Driscoll 1980), which is quite consistent with the results from our study for the summer sample (Fig. 4). Lyon et al. (1984) suggested that adequate moisture is probably the most important factor in spore production.

No doubt temperature has a very important influence on the aeromycospora, but the rate of temperature change is even more important. Changes in temperature over short time intervals and in vertical space affect the degree of thermal turbulence, which can simultaneously dilute particle concentrations at ground level, and cause the release of more particles by mechanical disturbance (Stephen et al. 1990). The number of conidia of *Cerospora asparagi* trapped increased sharply when temperature increased and humidity fell below 90% (Cooperman et al. 1986). This suggests that the main mechanism bringing about spore release could be temperature change.

Temperature inversions play an important role in spore transport (Davis & Main 1986), and can from at heights of hundreds or even thousands of metres by large-scale atmospheric motion, or radiation from cloud or haze (Cox 1987). The turbulence resulting from temperature inversion is a significant force influencing fungal spore release and dispersal. In the first minutes after release, a spore cloud grows both vertically and horizonetally within the atmospheric boundary layer under the action of small-scale dispersive eddies (Davis & Main 1986), but its longer-term fate is much more difficult to forecast.

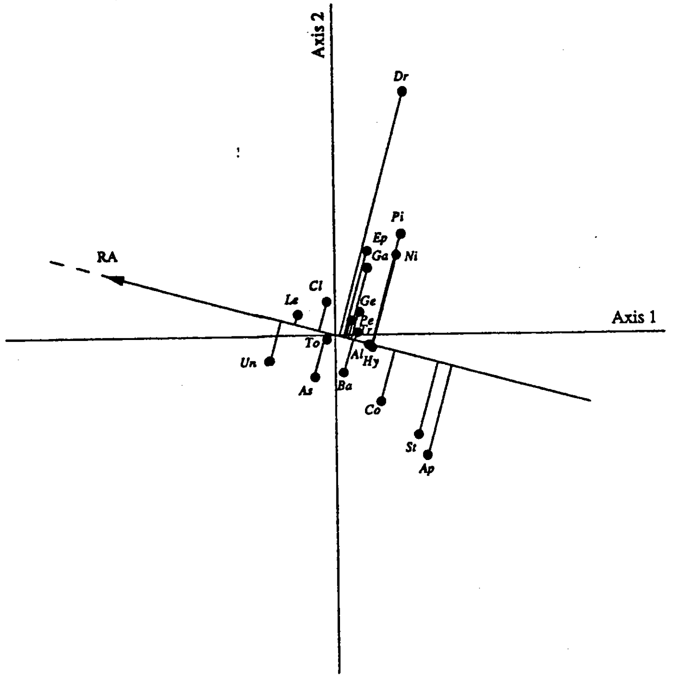

Fig. 2. The position of fungal genera along the rain arrow displays the approximate ranking of the weighted averages of taxa with respect to rain.

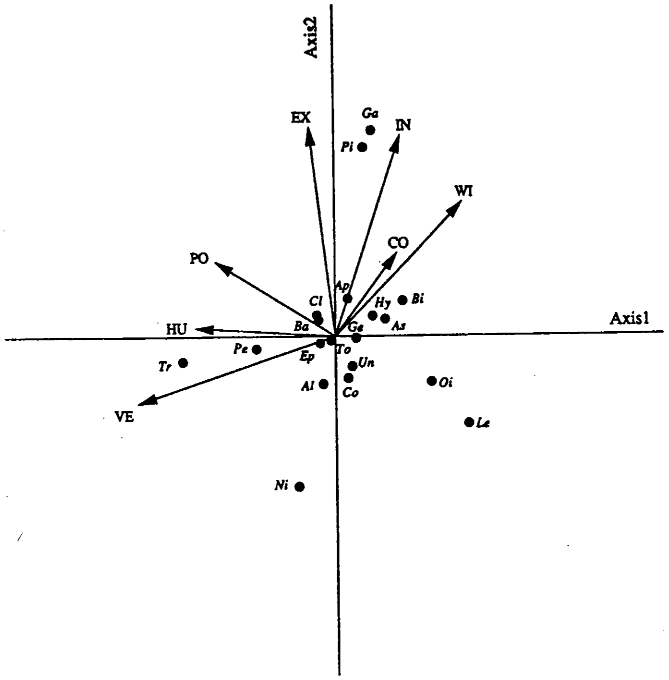

Fig. 3. Canonical correspondence analysis ordination biplot showing environmental factors (*arrows*) and airborne fungal genera (filled circles) from May. CO = commercial area, EX = expressway nearby, HU = human activities, IN = industrial area, PO = human population density, VE = vegetation, WI = wind speed, *Al* = *Alternaria, Ap* = *Aspergillus/Penicillium, As* = *Ascomycetes, Ba* = *Basidiomycetes, Bi* = *Bipolaris, Cl* = *Cladosporium, Co* = *Coprinus, Ep* = *Epicoccum, Ga* = *Ganoderma, Ge* = No. of genera, *Hy* = hyphal fragments, *Le* = *Leptosphaeria, Ni* = *Nigrospora, Oi* = *Oidium, Pe* = *Periconia, Pi* = *Pithomyces, To* = Total no. of fungal spores, *Tr* = *Torula* and *Un* = unidentified spores.

(version 3.12) (ter Braak 1992) and CANODRAW (version 2.20) respectively (Smilauer 1991), based on the fact that the variation of the fungal spore population through a year is over a wide range, i.e. gradient length > 2 Standard Deviations, which indicated that CCA is appropriate (ter Braak & Prentice 1988).

Canonical correspondence analysis results are displayed by an ordination diagram in which environmental variables are bepicted by arrows and species by points (Fig. 1) (Dixit et al. 1989). The CCA biplot can be interpreted as follows: each arrow representing an environmental factor determines a direction or axis in the diagram (Fig. 1); the species points can be perpendicularly projected onto this axis (Fig. 2) (ter Braak 1987). The order of the projection points corresponds approximately to the ranking of the weighted averages of the species with respect to that environmental factor. The weighted average indicates the position of a species' distribution along an environmental factor (ter Braak 1987). Arrow length indicates the importance of each factor in data interpretation. Important environmental factors therefore tend to be represented by longer arrows. The position on the environmental arrow depends on the eigenvalues of the axes and the intraset correlations of that environmental arrow (ter Braak 1986).

RESULTS

February

In February the most important environmental factors on the basis of the arrows, were found to be rain, wind speed, temperature, river or lake nearby, vegetation, human population density, and expressway nearby, in that order (Fig. 1). The first axis is defined by rain, the second axis by temperature and wind speed. Canonical axis 1 and axis 2 explained the variance of 24.1% and 20.4% in the species-environment relationship respectively (Table II). The eigen-

Table II. *Summary of Canonical correspondence analysis of airborne fungal spores and environmental factors in Kitchener-Waterloo, Southern Ontario*

Sampling time	February		May		August		December		Year of 92	
Axes	1	2	1	2	1	2	1	2	1	2
Eigenvalues	0.08	0.07	0.10	0.05	0.33	0.19	0.08	0.03	0.24	0.13
Species-environment correlations	0.75	0.74	0.77	0.69	0.77	0.93	0.87	0.70	0.69	0.77
Percentage variance of species data	9.0	7.6	8.9	4.6	33.3	18.4	19.1	8.0	16.1	8.4
of species-environment relationship	24.1	20.4	29.8	15.1	56.9	31.5	42.9	17.9	48.3	25.2

During fungal sporulation, RH and temperature are the most important factors influencing fungal spore release, but since meteorological factors are always correlated with each other to some degree, none of them should be evaluated alone.

Rain

The effect of rain on airborne spore concentrations can be simultaneously positive and negative, and is related more droplet size and droplet frequency than to amount (Stephen et al. 1990). Although rain has a "wash-out" effect on many kinds of fungal spores, particularly those of conidial fungi, ascospore release is often triggered off by rain (Burge 1986). This is why our study recorded high numbers of *Leptosphaeria* and unidentified Ascomycetes during light rain, while the numbers of conidia were depressed. Burge (1986) noted that when rain begins to fall on warm afternoons in the growing season, the characteristic dry spora is washed slowly from the air, and within minutes, levels of ascospores, such as *Leptosphaeria* begin to increase and remain high (up to 100 000 m^{-3} of air) as long as the rain persists.

The dispersal of basidiospores is dependent on a specialized active discharge process that requires the presence of free water, and their numbers are known to increase during periods of rainfall and dampness (Salvaggio & Aukrust 1981, Lyon et al. 1984, Salvaggio 1986). Precipitation was reported to be more highly correlated with spore release and dispersal in the Basidiomycetes than humidity (Lyon et al. 1984, Palmas & Cosentino 1990). Our results indicated that basidiomycete spores were most numerous during medium RH and rain (Figs. 4, 6). Hyphomycetes (conidial fungi) responded negatively to rain (Figs. 4, 6). Precipitation was also significantly negatively correlated with spore counts of *Cladosporium* and *Alternaria* in Sanluri, Italy (Palmas & Cosentino 1990).

Because the Samplair MK1 particle sampler is not waterproof, surveys were interrupted by moderate to heavy rain and snow. Thus, the effects of these kinds of precipitation on the airborne fungal spora could not be determined. It would be worth defining the relationship between heavy rain and the aeromycota, but the sampler requires modification before such data could be collected.

Rain always has an important effect on the airborne fungal spora. May was the only season in which there was no rain during the survey, though rain is generally expected to be an important environmental factor at that time.

Vegetation

Vegetation did not appear as an important influence in December for several reasons: (1). The deciduous plants had lost their leaves, (2) any leaves which did exist were covered by snow, (3) temperatures reduced fungal growth and sporulation to negligible levels, (4) when the survey was resumed after heavy snow, the ground and the crowns of the woody plants were still covered by snow. In February, the crowns of the woody plants were not covered by snow during the sampling period, and although growth and sporulation were still very low, wind was able to resuspend spores previously developed or deposited on plants. In May vegetation became the most important influence on the numbers of airborne fungal spores, especially those of conidial fungi such as *Torula, Periconia, Nigrospora*, and *Alternaria*. Those fungi are saprobes or plant pathogens. Although May is not the season for sporulation of most fungi, some Hyphomycetes can release conidia from spring through to fall whenever environmental factors are favourable. Once fungi have grown on or in plants, fungal spore production and release are mainly determined by RH and temperature, which were highest in August. *Alternaria* species are pathogenic to many plants and also grow on dead plant meterial, which explains why this genus was recovered mainly in well vegetated areas in summer. Vegetation is a major substrate for fungi, so it understandably has an important relationship to the airborne fungal spora.

Wind

Wind is the most unpredictable agent in the dispersal of fungal spores. Many fungi are adapted for aerial transport (Tilak 1984). Increasing wind speed expands the turbulent boundary layer of the atmosphere (Gregory 1973) which efficiently lifts and redisperses fungal spores previously deposited on the surface of the ground, buildings, plants, etc. This is especially true for larger spores such those of *Drechslera, Pithomyces, Epicoccum*, and *Nigrospora* (Fig. 1). Wind speed was a significant factor in all of our surveys. A spore cloud of variable density, up to millions of spores per m^3, may form over a crop. A nearly logarithmic vertical spore density is established, and those spores which are transported horizontally may travel 100 km (Tilak 1984).

Cladosporium and *Alternaria* are released by wind, and increase in concentration in with diminishing RH and increasing airflow (Salvaggio & Aukrust 1981). In the conidial fungi, spore release is often influenced by wind speed (Lyon et al. 1984). Our study showed that basidiospores were most numerous at intermediate to low wind speed (Figs. 1–6). *Alternaria, Periconia, Nigrospora*, and *Torula* also responded to low wind speed, but were found mainly in well planted areas in May (Fig. 3), suggesting that spores of these taxa were mainly being released from local sources. However, we found that the resuspending action of high winds was signigicant for many other kinds of spores.

Although wind direction had a profound effect on the airborne fungal spore count in Galway, Ireland (McDonald & O'Driscoll 1980), it was not recorded as a parameter in our study. Wind direction is clearly an important factor if large, concentrated spore sources, such as infected crops, or crop residues, are involved.

Wind speed is always one of the top five most improtant factors at all seasons (Figs. 1, 3–6) playing a role in spore

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release and dispersal. In winter and early spring, wind mainly resuspends or redisperses fungal spores in Southern Ontario, since these seasons are not suitable for fungal sporulation, and there is no new supply of spores. From late spring to late fall, wind affects both spore release and dispersal.

Although the Samplair MK1 is an efficient spore trap, it cannot be orientated with respect to wind direction, and it appears likely that the efficiency of sampling may be affected by air movement. Wind may in fact resuspend spores, but the rapid movement of air over the collection slot may reduce the efficiency of the sampler as compared with its performance in still air. Unfortunately there appears to be no useful data on this effect for this sampler, so it must be noted as an imponderable, and presented as a problem to be tackled by future research.

The sampling efficiency of automatic volumetic spore samplers changes with wind speed (Lacey, 1981). Under certain conditions the bioaerosol concentration may be significantly over- or under-estimated, when sampled with serveral samplers including both wind-oriented and non-wind-oriented samplers (Chang et al. 1992). Since no sampler is perfect, the decision in choosing a sampler may have to be made, in addition to sampling efficiency, on cost, convenience of operation and reliability.

Cloud and solar radiation

In August, cloud moved up to become the third most important factor. The effect of cloud was actually the inverse of the effect of solar radiation. Figure 4 clearly shows that most of the conidial fungi prefer sunshine and dry conditions, while Ascomycetes favoured higher RH and low solar radiation. It has been observed that light triggers spore release in several fungi (Leach 1975). Not Ascomycetes were negatively correlated with solar radiation. In many of the bitunicate fungi, light is required to initiate ascospore discharge (Lyon et al. 1984). Spore release in *Erysiphe graminis* was also found to be positively correlated with total solar radiation (Munk 1981). The results of our study were consistent with the earlier observation that conidia of *Cladosporium* and *Alternaria* are often abundant during midday periods with maximal sunlight (Salvaggio & Aukrust 1981).

Radiation from cloud may establish temperature inversions that could influence the dynamics of airborne fungal spores, but since cloud was an important factor only in August, the effect of cloud (inverse effect of solar radiation) is probably mainly on spore release.

Snow and snow cover

The ground in Kitchener-Waterloo is generally covered by snow from December to March. Although most of the airborne spores were correlated, to different degrees, with high RH, light rain, and light snow, snow cover efficiently kept the count of fungal spores low. This is why most taxa appear on the right side of Axis 2 (Fig. 5). The effect of snow has been largely ignored in the aeromycological literature. The fact that spore counts after snow are very low suggests that snowflakes could bring down the airborne fungal spores in a "wash-out" effect, but more research is necessary to verify this. Snow cover effectively prevented fungal spores produced on soil and plant debris from being introduced into the atmosphere.

Seasonal variation

Although airborne fungal spores have been considered to lack clearly defined seasonal patterns (Burge 1986, Salvaggio 1986), more and more research has, in fact, found such patterns for certain fungi. Our results provide additional evidence for this, as one might expect in a country like Canada, which has such distinct seasons.

Winter in temperate and cold regions is a dormant season for most fungi. Meteorological factors, such as rain, wind, and temperature were the most important factors in this season, playing crucial roles in affecting the aerodynamics of fungal spores in Kitchener-Waterloo. The fungal spores in the air in winter are mainly those remaining from the growing season or from long distance dispersal. There is no major local spore source for recruitment. The generic diversity of the airborne spora and spore counts were both much lower than in other seasons.

In August, RH became the most important factor in our study. In summer fungal diversity and spore population densities became much greater than at any other time of the year. *Ganoderma, Leptosphaeria, Coprinus, Polythrincium, Agrocybe, Arthrinium, Inocybe*, and *Oidium* were found only during the growing season (Figs. 1, 3–6). At the end of the growing season, the basidiomata of agarics such as *Coprinus, Agrocybe*, and *Inocybe* vanished. Since *Leptosphaeria, Polythrincium, Arthrinium*, and *Oidium* are highly correlated with the growth of their plant hosts, it is understandable that they also displayed seasonal patterns.

Summer is the most important season in the aerospora calendar. The pattern for the whole year and its relationships with environmental factors are mostly determined in summer (Figs. 4, 6). When the biplots of the whole year and August are compared, one obvious general trend can be noticed. Ascomycetes and Basidiomycetes responded to medium to high RH, on the upper side of Axis 1, while hyphomycetes responded to medium to low RH, on the lower side of Axis 1.

Other workers have found that *Aspergillus fumigatus* spore concentrations in Cardiff, Wales, and St. Louis, Missouri showed seasonal variations, with highest concentrations during the winter (Mullins et al. 1984). *Cladosporium, Alternaria*, and *Aspergillus/Penicillium* were found in all seasons in our study. Those genera can utilize the widest range of substrates for their growth and are ubiquitous. *Aspergillus* is not a well identified taxon in our study, therefore, it is hard to compare it with other results from different areas and years.

was not affected by this factor. However, many airborne fungal spores remain to be characterized (Salvaggio & Aukrust 1981, Salvaggio 1986, Hawksworth 1991), so the precision of our data still leaves something to be desired. Isolated basidiospores are often almost impossible to identify with any degree of certainty (Kendrick 1990), and clearly require intensive study.

This study has demonstrated that CCA provides a novel approach to aeromycological research and generates ordination biplots from which the environmental factors determining or influencing species occurrence and numbers can be easily visualized, quantified, and compared. The relative importance of multiple environmental factors on airborne fungal spores can be analyzed with confidence by reference to the graphical summaries.

In order to monitor the aeromycota and its dynamics, two aspects need to be studied, (1) local and distant fungal spore sources, and (2) factors related to growth, release and dispersal of fungal spores. For long-distance dispersal of fungal spores, it is necessary to apply Atmospheric Trajectory Analysis to trace the original spore source, and always to record wind direction.

More and more regions and cities will soon provide information on airborne fungal spore and pollen concentrations along with the weather forecast, but in most parts of Canada and around the world geographical maps and calendars of airborne fungal spores are not yet available. We may expect to be asked to provide them.

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Luley & McNabb (1991) reported that ascospore production showed a distinct seasonal periodicity in Iowa during the growing seasons of 1984 and 1985. Their results are quite consistent with ours. In our study, *Leptosphaeria* and identified ascospores were numerous in August with higher RH or after rain.

Salvaggio & Aukrust (1981) reported that basidiospores are present throughout the year, with peaks during the moist late summer and early fall months. Our results verified their finding. *Ganoderma, Coprinus, Agrocybe*, and *Inocybe* were mainly retrieved during the summer, in our study. Occasionally, *Ganoderma* and *Coprinus* were found in other seasons. Unidentified basidiospores were found in all seasons in our study, but were most numerous in August.

Although the periodicities of certain species have been delineated in temperate regions, overall seasonal patterns have not well defined. One reason is that the concentration and composition of the outdoor airborne fungal spora are determined by the interaction of many variables: meteorological factors, biological factors, environmental factors, geographical variation, human activities, and the biological characteristics of fungi (Salvaggio 1986). The interactions of these factors still need to be intensively researched. Most research has dealt with fungi at the generic level because it is extremely difficult to identify many spores to species. Different but overlapping life cycles of congeneric species (e.g. in *Penicillium* and *Aspergillus*) present extended and unclear periodicities.

Geographical variation

Geographical variation among fungi is common phenomenon. In the United States the composition of the air spora was different in different states or within different areas of the same state (Meyer et al. 1983). Al-Doory et al. (1980) indicated that in almost all air surveys reported in North America, conidia of *Alternaria, Cladosporium, Stemphylium, Phoma, Aspergillus*, and *Penicillium* have been reported as the most numerous, whether indoors or outdoors and even at high altitudes. In Kitchener-Waterloo the dominant taxa were as follows: *Cladosporium, Alternaria*, and *Aspergillus/Penicillium*. In Toronto *Cladosporium* and *Alternaria* were the most common moulds (Collins-Williams et al. 1973). In Hamilton, 70 km from Toronto and 60 km from Kitchener-Waterloo, *Ganoderma* was the most common, followed by *Cladosporium* and *Coprinus*, from 1972 to 1974 (Tarlo 1979). basidiospore counts were higher in woodlands than in cities (Koivikko & Savolainen 1988). Lacey (1981) found 2.6 times as many spores in a valley, close to a stream, as on a nearby exposed hill. Our results from February and December also showed that river/lake was an important factor in that period and some mycota seem to occur mainly near water. River and lakes may have different effects on the airborne spora, and these two factors should be recorded separately in future research.

When the results from different studies and different areas are compared, yearly variation should be kept in mind. An outbreak of a plant disease, even if the causal fungus was usually of only secondary importance in the air spora, could boost its ranking for that particular year.

Other factors

May is a prime season for assimilative fungal growth (i.e. hyphal growth), but most of the fungi did not sporulate at that time in our study. In this period, meteorological factors no longer dominate the airborne fungal spora, and the importance of human factors such as expressway, industrial area, human population density, and human activities, increased (Fig. 3). The reason that *Pithomyces* and *Ganoderma* spores were numerous near the expressway and in nearby industrial areas could be that the traffic on the expressway formed a very strong horizontal airflow resuspending and dispersing these spores.

Vehicles passing by the sampling site became a significant factor in December. When snow did not cover the ground completely, airflow accelerated by cars resuspended fungal spores from the road and the ground.

Problems and perspectives

The eigenvalues for February and December are the lowest in the present study (Table II), indicating that the importance of the first two axes are not as great at those times as in May, August, and over the whole year. February and December are not seasons for fungal growth. Some overriding factors may not have been included in the present study.

Unidentified fungal spores, *Cladosporium*, and total fungal spores always positioned around the centre of the ordination diagram (Figs. 1, 3–6). *Cladosporium* is ubiquitous (Infante-Garcia-Pantaleton & Dominguez-Vilches 1988). It is also a large genus, in which over 500 species have been described (Infante-Garcia-Pantaleton & Dominguez-Vilches 1988, Morgan-Jones & Mckemy 1990). Many species of *Cladosporium* are obviously pooled together, and this accretion phenomenon may be responsible for its ranking at the centres of the biplots in our study. The same reasoning applies to total fungal spores and unidentified fungal spores. Whenever taxa appear around the centre of the biplot, caution should be exercised in interpreting the data, because the most important practical shortcoming of CCA is that species unrelated to the ordination axes tend to be placed in the centre of the ordination diagram, and are not distinguished from species that have true optima there. This problem can be circumvented by looking at individual species and sites arranged in order of their scores along one of the ordination axes (ter Braak 1987).

The great majority of basidiomycetes, like many ascomycetes, do not grow on common laboratory media (Salvaggio & Aukrust 1981). That has obviously affected those studies recording colony-forming units (CFU). The present study was based on direct observations of trapped spores, and so