
Comparison of outdoor allergenic particles and allergen levels

Charles Barnes, PhD, BS; Keith Schreiber, MS†; Freddy Pacheco, MS*; Julie Landuyt, BS*; Frank Hu, DVM*; and Jay Portnoy, MD*

Introduction: Spore and pollen counts have been used traditionally to determine aeroallergen exposure. Using a liquid based collector and enzyme immunoassays, we have developed methods for measuring airborne allergen concentrations. In this work we test the hypothesis that airborne allergen concentrations are directly related to spore and pollen counts.

Methods: Test samplers used included a high-volume cyclonic liquid impinger (SpinCon) and a standard spore trap (Burkard). Samples were collected on a weekly basis from May to October and were analyzed microscopically for spores and pollen grains. The liquid samples were analyzed by enzyme-linked immunoassay for the presence of allergens from *Alternaria*, *Cladosporium*, *Aspergillus*, oak, fescue, ragweed, and plantain. Specific *Alternaria* allergens Alt al and GP70 also were measured.

Results: Pollen counts for the SpinCon and Burkard collectors were similar, though spore counts were lower with the SpinCon. Detectable amounts of three of the seven allergenic species including fescue, ragweed, and *Alternaria* were present in air samples. Concentrations of pollens were seen in their respective seasons while fungal allergen levels varied throughout the period. Allergen levels generally agreed with particle counts, however peak allergen levels and peak particle counts for individual species did not correlate well.

Conclusions: At flow rates of 236 L/min, the SpinCon is comparable to the Burkard for counting airborne pollen and spores. Samples collected by the SpinCon permit quantitative determination of allergen levels in outdoor air. The poor correlation between measured airborne allergen and related particles indicates the potential for significant allergen exposure in the absence of identifiable particles in air.

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INTRODUCTION

Techniques for sampling airborne bio-particles have progressed from initial experiments involving inspection of gravity slides¹ and identification of proteins in the walls of pollen grains² to the complete bioaerosol survey undertaken by the AAAAI Aeroallergen Monitoring Network.³ These studies have mostly focused on particle identification by visual means and have

shown that allergy symptoms do correlate with some particle counts.⁴ Particularly, pollen counts have been associated with rhinitis symptoms while "basidiospore like" fungal spore counts as well as total airborne particulates have been associated with increased hospital asthma admission rates.⁵⁻⁷ Recently, day-to-day changes in asthma severity have been associated with total airborne fungal concentrations when weather conditions were controlled for.⁸ In many instances, however, peak asthma symptom scores differ temporally from peak particle levels.^{9,10} It is now understood that parts of an organism other than pollen grains or spores contain significant allergen concentrations that are readily disseminated via an airborne route.¹¹ In

other words, the particle itself does not necessarily cause the disease. Rather the particle acts only as a carrier for the protein agent that leads to the observed symptoms.¹²⁻¹⁷

A number of attempts have been made to measure the actual protein allergen content in environmental samples such as ragweed, dust mite, cat, and cockroach. Ragweed allergen has been detected in submicronic fractions collected during ragweed allergen season by both skin testing and enzyme immunoassay.¹⁸ Indoor studies of house dust have been generally more successful than outdoor efforts due to limitations on air sampling technology. Numerous authors have measured dust mite allergen levels in house dust and correlated high levels with allergic disease.¹⁹⁻²¹ Indoor levels of allergen have also been correlated with symptoms in cat and dog sensitive individuals.²²⁻²⁴ There have been fewer efforts to quantitate levels of outdoor allergenic proteins.^{25,26} A general correlation has been demonstrated for ragweed between periods of high allergen activity and elevated pollen counts and for *Alternaria* between periods of high allergen activity and elevated spore counts. These authors did note specifically that there were occasions when allergen levels were high in the absence of increased particle counts.²⁵ Likewise, it has often been observed that the necessity of obtaining viable or identifiable particles introduces a source of error in estimating exposure to fungal allergen.²⁷ Work by Solomon et al has demonstrated unequivocally that pollen allergens occur in fine particles much smaller than the grains themselves.²⁸ In addition, the major birch allergen, Bet vI, has been re-

* The Children's Mercy Hospital, Kansas City, Missouri.

† Midwest Research Institute, Kansas City, Missouri.

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corded in aerosol fractions even when no birch pollen grains were present.^{29,30}

We collected airborne allergens during one allergen season in the Midwest United States using a high-flow liquid-based cyclonic impinger.³¹ We counted the identifiable particles in these liquid samples microscopically. In these same samples, using polyclonal enzyme immunoassays, we measured the airborne level of immunogenic material from seven species known to produce aeroallergens. Also in these samples, using monoclonal enzyme immunoassays, we measured two specific aeroallergens associated with the fungus, *Alternaria alternata*.

MATERIALS AND METHODS

Materials

Air for visual identification of pollen grains and fungal spores was collected using a Hirst suction collector (Burkard Manufacturing Co Ltd, England). Air for antigen measurement and also for visual identification was collected using a spinning concentrator (SpinCon™, Midwest Research Institute, Kansas City, MO). Rabbit polyclonal antibodies specific to *Alternaria alternata*, *Aspergillus fumigatus* and *Cladosporium herbarum*, oak (*Quercus alba*), fescue (*Festuca elatior*), ragweed (*Ambrosia artemisiifolia*), and plantain (*Plantago lanceolata*) as well as standard allergens with known protein content were obtained from Greer Laboratories (Lenoir, NC). Polyclonal antibodies to short ragweed and short ragweed standard were obtained from the National Institutes of Health (NIAID Repository for Biological Reagents and Standards, Rockville, MD 20850). Conjugated antibodies, substrates, and other chemicals were obtained from Sigma Chemical Company (St. Louis, MO). Specific monoclonal antibodies to *Alternaria* allergens Alt al and GP70 were produced in our laboratories as described previously.^{32,33} The operation and performance of the cyclonic liquid impinger has also been described previously.^{31,34}

Bioaerosol Samplers

The cyclonic liquid impinger (SpinCon) is a high-volume collector developed for environmental monitoring situations.³⁴ For the current study, the SpinCon was adapted for bioaerosol sampling in the outdoor environment.³¹ Flow rates were calibrated to collect at 236 L/min that is approximately half the flow used in our previous study. This was done to test the hypothesis that high flow rate accounted for reduced collection efficiency of pollen possibly due to shearing forces destroying the grains. An initial volume of 10 mL of nanopure water was added to the SpinCon and kept constant by continually adding a controlled flow (1.5 mL/min) of nanopure water to the operating collector whenever necessary to retain the collecting liquid volume between 7 and 10 mL. At the end of the collection period, the collected volume was brought to 10 mL by the addition of additional nanopure water before the samples were processed.

The SpinCon units currently used for bioaerosol sampling in the outdoor environment as described in previous studies³¹ are made from clear acrylic or polycarbonate plastic. The SpinCon cylinder is machined with an air inlet slit cut the entire length of the main body and tangential to the wall. The top of the SpinCon unit is connected to a vacuum motor capable of drawing >2,000 L/min of air through the slit. This faster flow rate is unique to this type of sampling device. For these experiments the flow rate was adjusted to 236 L/min. When air enters, it spins inside the unit in a cyclonic fashion due to the tangential orientation of the slit adhering to the inside walls of the unit. The liquid sample is held in the collecting vessel by balancing centrifugal force created by tangential airflow with gravitational force. As long as flow rate is maintained below a critical limit the sample is retained. Since evaporation occurs rapidly at high flow rates, the initial volume of collecting liquid is constantly monitored and replenished as necessary. Since the loss is through evaporation, no non-volatile

material is lost from the collector. The device is not wind oriented, however since the input velocity approaches 42 mi/h even at 236 L/min flow rates, the unit is generally not sensitive to changes in wind direction and velocity.

The SpinCon unit has been subjected to extensive quality control testing by Midwest Research Institute. Additional quality control testing included repetitive sampling at intervals varying from 15 minutes to 60 minutes. To test the absolute retentivity of the unit 10 mL of nanopure water was spiked with ragweed extract (Greer Labs, LeNoir, NC, USA) and introduced into the unit. The unit was operated for 60 minutes in the laboratory under conditions similar to those in the field. After a 60-minute run, the samples were assay by EIA for recovered ragweed allergen.

The Burkard sampler is a suction-type trap, which impacts particles on a sticky surface. For this study, the adhesive surface was on glass slides coated with lubriscal, which had been suspended in hexane and placed on a 24-hour collecting head. Air was drawn into the unit through a 2 × 14-mm slit at a rate of 10 L/min. Correct sampling orientation was accomplished by a wind vane attached to the drum housing which keeps the air inlet facing into the prevailing wind.

Sampling Protocol

The samplers were installed on the roof of the hospital, which is a 5-story building in the center of a metropolitan area. Air samples were collected in Kansas City, MO weekly between May and October 1995 which included the major pollen and mold season for the region. Samplers were operated in accordance with specifications given by the manufacturers for optimum performance. Flow rates of each unit were monitored throughout the sampling periods. SpinCon sampling took place for continuous periods lasting 60 minutes and the Burkard collector was operated continuously during that same period of time. Collectors were operated simultaneously on a given day and whenever counting comparisons were

made counts were generated to represent specific 60-minute intervals on the Burkard sampler collection record.

Pollen and Spore Counts

Samples from the Burkard collector and the SpinCon were examined microscopically and immunochemically. Liquid samples from the SpinCon were brought to a final volume of 10 mL with distilled water. After the liquid samples were removed from the unit and brought to a standard 10 mL volume the sample was mixed thoroughly and a 0.5-mL aliquot was taken from the diluted sample and dried onto a glass slide. All slides were stained with glycerin jelly containing 1.0% Calberla's solution, fitted with a coverslip, and examined microscopically. No factors reflecting a presumed collection efficiency of the sampler units were applied. Allergen and counting data were normalized to represent either particles or nanograms of allergen/m³ of air. Individuals doing the counting for this study have been certified by the Pollen and Mold Network of the American Academy of Allergy, Asthma, and Immunology.

Handling and Storage of Liquid Samples

After removal of an aliquot for spore and pollen counting, the remaining sample was divided into 2-mL aliquots and stored at -20°C until a later time when collected samples were assayed as a single batch. Caution was exercised to avoid exposing the samples to more than one freeze-thaw cycle. The collecting liquid contained no preservative; therefore, to avoid problems associated with germination of spores the total potential time of exposure to the liquid medium was limited to 2 hours.

Quality Control Methods

Two specific approaches were taken to assuring the quality of the data produced by the SpinCon device. First a sample of ragweed allergen with a known concentration was instilled into the liquid collection medium and the instrument was run for a 1-hour period. The liquid was then removed and the amount of allergen in the liquid was

assayed. This experiment was repeated in triplicate. This value was then compared with the known amount of allergen instilled and the triplicate runs were compared to one another. The second approach assumes that the airborne population of spores and pollens does not change rapidly during periods of stable weather. This approach, involved sampling with the same unit for sequential time periods from 10 to 30 minutes and comparing the values obtained. Quality control results for the immunoassays included precision, linearity, and linear range data.

Immunoassays

The liquid samples provided by the SpinCon were analyzed by inhibition enzyme immunoassay for *Alternaria alternata*, *Aspergillus fumigatus* and *Cladosporium herbarum*, oak (*Quercus alba*), fescue (*Festuca elatior*), and plantain (*Plantago lanceolata*). Ragweed allergen (*Ambrosia artemisiifolia*) was measured by a double polyclonal sandwich immunoassay and the specific *Alternaria* allergens Alt al and GP70 were measured using a double monoclonal antibody-based sandwich assay as described previously.^{32,33}

For the inhibition assays, polyclonal rabbit antibodies raised against whole commercially available allergen extracts were used. These assays were standardized using the total protein content of the allergen extract supplied by the manufacturer or calculated from an amino acid analysis of the extract. Polyclonal rabbit anti ragweed antibody obtained from the NIH repository was biotinylated and used in a sandwich type EIA. The first layer for the ragweed assay was rabbit anti-ragweed and the top layer was biotinylated-rabbit anti-ragweed. All assays were developed using alkaline phosphatase conjugated goat anti-rabbit antibody or alkaline phosphatase conjugated streptavidin and alkaline phosphatase substrate. Optical densities were measured on a MR7000 (Dynatech, Chantilly, VA) microtiter plate reader and standards were fitted to either a linear or sigmoidal curve using DIAS software. Allergen values were calculated

directly from standard curves by the software. Quality control procedures for the immunoassays included determinations of precision, linearity and sensitivity.

RESULTS

Quality control data for three consecutive runs of the SpinCon unit on 3 separate days produced an average coefficient of variance of 30% for pollens and 19% for spores. On days with higher pollen counts (>200 particles per cubic meter) the CV was 17% and on days with lower pollen counts (>50 particles/m³) the CV was 47%. Quality control results for retention of allergen content showed a CV of 2% between the three individual runs and samples diluted but not run through the unit with an average retention of 97% of the instilled allergen. In the inhibition enzyme immunoassay for *Cladosporium*, *Aspergillus*, oak, fescue, and plantain sensitivity ranged from 2.0 to 0.03 µg/m³ with a linear range of 2.7 µg/mL to 0.1 µg/mL. Precision values expressed in CV for these assays varied from 9 to 28. The double polyclonal sandwich immunoassay used for ragweed allergen was sensitive to 0.33 µg/m³ and had a linear range from 1.5 µg/mL to 0.5 µg/mL. The average CV for six sequential determinations was 18. *Alternaria* allergens Alt al and GP70 measured using a double monoclonal antibody-based sandwich assay were sensitive to 0.33 µg/m³ with a linear range (correlation = 0.93) of 10 µg/mL to 0.5 µg/mL and an average CV of 17 for 6 sequential determinations.

Pollen and spore counts for both the SpinCon and Burkard collectors during the allergen season of 1995 are displayed in Figure 1. Counts obtained with the SpinCon correlated well with counts taken from the Burkard collector both for pollen grains ($R^2 = 0.76$, $P < .05$) and fungal spores ($R^2 = 0.64$, $P < .05$). The relative performance of the SpinCon and Burkard collectors related to total numbers of pollen particles is very good. Except for two data points, the pollen counts were nearly identical for the two devices. Spore

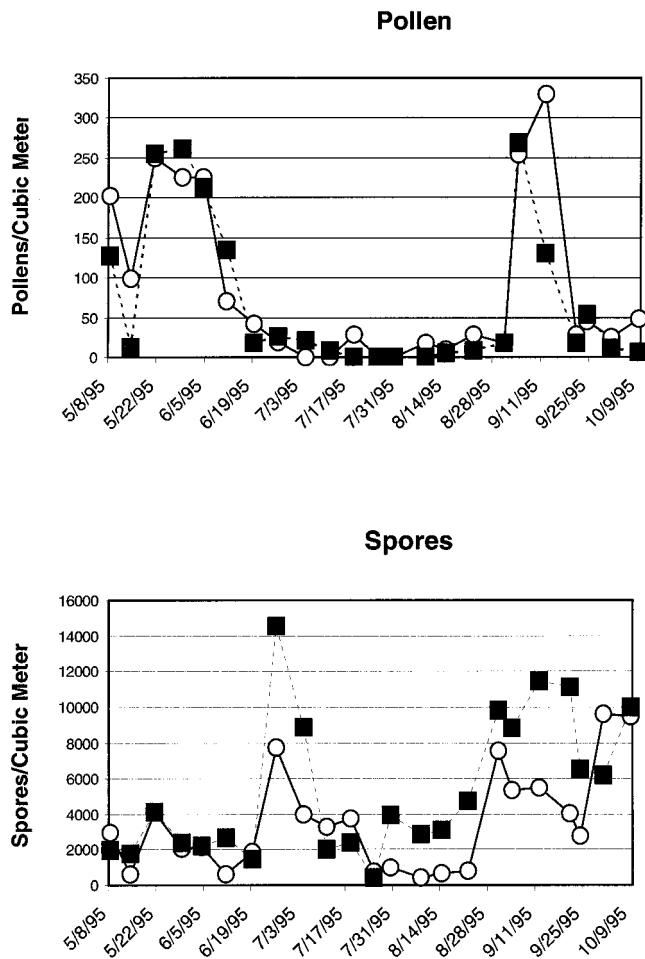


Figure 1. Weekly pollen and spore counts for SpinCon and Burkard samplers. Values are expressed as particles/m³ of air. —○— SpinCon and ---■--- Burkard.

counts generated by the two collectors although different at several points showed similar trends. At this lower flow rate the SpinCon collector often produced spore counts lower than the Burkard. Additionally, we saw no evidence that germination of spores occurred during the SpinCon collection.

Both collectors demonstrated a pollen peak in the spring representing tree and grass season and a second pollen peak in the fall representing the weed and especially ragweed season. Spore counts showed a less clear pattern with a sharp peak in June and a broad elevation in September occurring for both collectors. We were unable to determine the specific reasons for these increases in spore count. Detectable air-

borne concentrations of allergens were present for fescue, ragweed, and Alternaria.

Spring samples contained substantial amounts of fescue as the predominant allergenic material (Fig 2). Particle and antigen concentrations increased simultaneously on May 15 and reached their peaks on May 22. Following that, the pollen numbers remained elevated for several more weeks while the antigen concentration dropped quickly. Airborne fescue antigen concentration was more sustained, however, remaining detectable until July 5 while pollen grains were virtually absent by June 20. Ragweed allergen was identified in the fall starting on August 23 which was a full week before substantial

numbers of pollen grains were seen. In addition, ragweed antigen content remained elevated and even exceeded its initial early September peak on September 25 when the pollen counts were already on the decline.

Fungal spores were found in the air throughout the year. Detectable levels of Alternaria allergens were measured with peaks occurring in the spring and fall respectively (Fig 3). When the specific Alternaria allergens Alt al and GP70 were measured, peak concentrations were found in the fall in late August for GP70 and mid September for Alt al.

The two measurements of Alternaria (Alt al and GP70) did not correlate with each other. The comparison of Alternaria spore counts with the two measured Alternaria allergen levels (Fig 3) shows that peak spore counts on July 26 and September 7 are related to increased levels of Alt al on July 19 and September 6. These same peaks in spore numbers are both preceded by elevations of GP70 on July 10 and August 30.

DISCUSSION

Overall, these data relate specific airborne particle counts and airborne protein levels of three different aeroallergen producing species throughout the spring, summer and fall of one allergen season. For 4 of the species tested airborne allergen levels were below detection limits. The allergen levels generally agree with the particle counts in that allergen is not detected in the absence of the related particles. During the time when particles are expected to be in the air, however, specific allergen levels and particle counts did not generally correlate. There were notable instances when allergen levels increased one sample period prior to particle counts as for fescue and where allergen levels remain high even though specific particle counts were greatly reduced as for Alternaria.

The quality control values for the SpinCon device are quite good. The low coefficient of variation in the spiked samples and the 97% recovery for these samples indicates that allergen is not lost

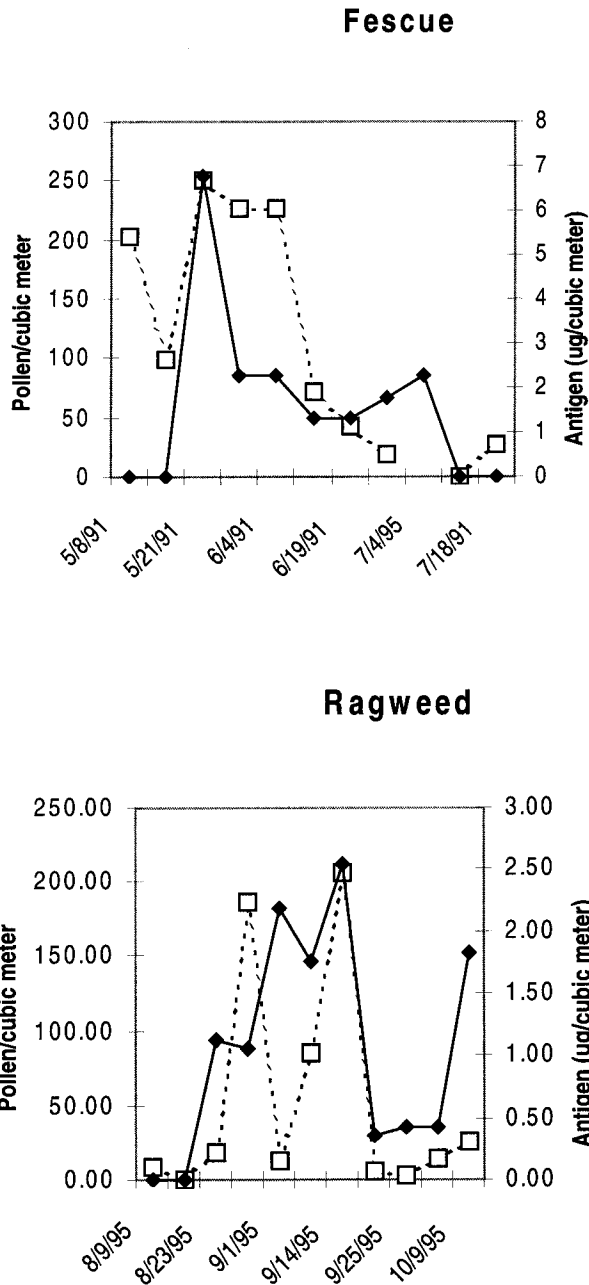


Figure 2. Pollen counts and antigen concentrations collected by the SpinCon for fescue and ragweed. --□-- pollen and —◆— antigen.

by the device either through evaporation or aspiration. The variation in field trials although considerably higher is quite similar to that of other samplers under actual conditions.³¹ The improvement in variation coefficient with increasing numbers is predicted by sampling the-

ory. It should be noted that the 60--minute sampling time introduces some potential variables. Namely, because of the hour-to-hour variation in pollen shedding, and the variation in the dispersion of airborne particles that arises from changes in wind direction and velocity,

rain and other weather conditions, a 1-hour sampling is not necessarily sufficient to determine average exposures. Additionally, for low levels of allergen a sampling time of 1 hour at the rate used with the SpinCon may not have adequate sensitivity. Even though the quality control data is quite good, these factors must be taken into consideration when evaluating the results.

The particle collection data indicate that when the flow rate on the SpinCon was reduced from that used in our previous study, pollen collection efficiency was comparable to the Burkard. At this same flow rate, however, the SpinCon became less efficient at collecting spores,³¹ particularly under conditions of low spore counts. The seasonal variation of the pollen and mold counts is very much as we expected.

These data do represent a year with a late spring and a relatively dry summer and fall. The actual rainfall in this part of the state in 1995 approached drought conditions during the late fall and winter with little recorded precipitation from September through the end of the year. Fungal spore count peaks exceeding 20,000 are not uncommon during a normal fall whereas the highest recorded level for this period in 1995 did not exceed 15,000. The dry conditions were not severe enough to affect ragweed that thrived on adequate rainfall earlier in the spring and produced pollen levels around 250 grains/m³ which approximates levels seen in other years.³

There were no totally unexpected findings in the measured allergen levels. The pollen related allergens we were able to measure appeared, as expected, when the respective pollen grains were visible in our collectors. Fescue, which is the predominant grass in this region and is cultivated for both lawns and cattle feed, appeared around the middle of May and persisted until the second week of July. Although we are not able to distinguish fescue pollen from other grasses, it is safe to assume that the majority of the airborne pollen from mid May to mid June is fescue. Measured allergen lev-

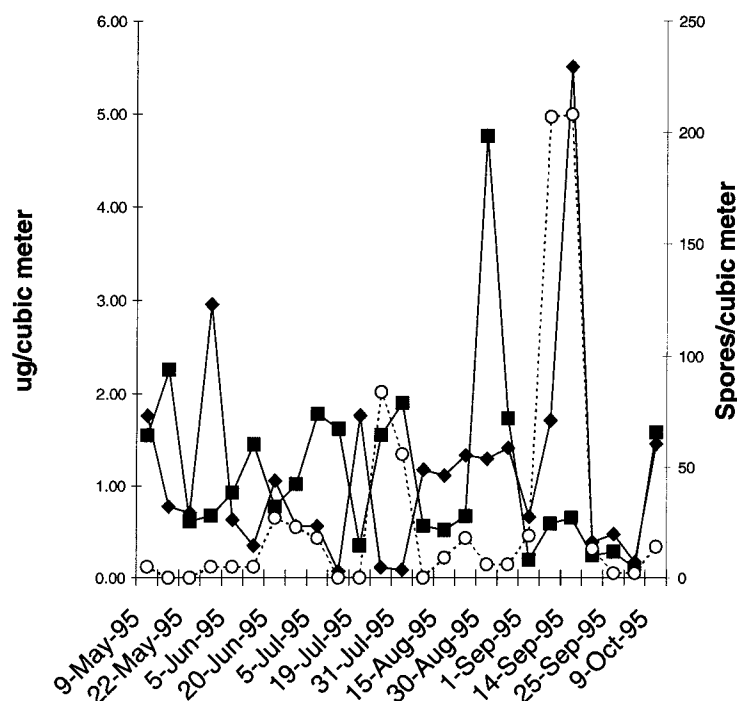


Figure 3. *Alternaria* spore counts in spores per cubic meter of air versus concentrations of the specific *Alternaria* allergens Alt a1 and GP70 in $\mu\text{g}/\text{m}^3$ of air. —■— GP70, —◆— Alt a 1, and --○-- Alt Spores/ m^3 .

els for fescue exceeded $1 \mu\text{g}$ of allergen/ m^3 of air for all periods sampled from the second week of May until the second week of July. Since these levels were measured at a downtown location we can assume that levels in outlying areas may have been substantially higher.

Measured concentrations of Ragweed antigen first appeared during the second week of August and persisted until the onset of cold weather. Concentrations were found to be in the 1 to $2 \mu\text{g}/\text{m}^3$ of air range. This level is clearly adequate to cause symptoms in sensitive individuals since ragweed allergy is one of the most common allergies in this area. The comparison of measured ragweed allergen to ragweed pollen counts provides an interesting study. Ragweed allergen levels are seen to rise both on August 23 and September 5. These increases both preceded increases in pollen levels seen in the subsequent measurement period. The two peaks in the pollen counts

may represent the two types of ragweed found in our area (*A. artemisiifolia* and *A. trifida*), but the measured airborne allergen concentrations do not exhibit such clear separation. The reason why measured allergen concentrations might precede particle counts is not clear. It has been speculated that early pollen might contain more allergen than late pollen or that the weathering of pollen as it matures on the plant itself might reduce the allergen either in amount or potency. The current data do not support one hypothesis over the other.

As was anticipated, the pattern of fungal allergen levels throughout the season is quite complex. Most puzzling is the observation that the two measurements of *Alternaria* antigen (Alt a1 and GP70) did not correlate well with each other. These two monoclonal antibody-based assays are both highly sensitive and specific. One possible explanation for why the antigens correlate poorly with spore counts re-

lates to reports that At al-like immunologic activity is present in fungi that are taxonomically related to *Alternaria* such as *Stemphyllium*.³⁵ GP70 has also been measured in other fungal species such as *Ulocladium*.³² We may therefore be detecting antigens from several species that may not all be contributing the same amount of activity to the integrated sample we have measured. A possible explanation for why concentrations of GP70 and Alt a1 are poorly correlated to each other may relate to the function and location of each protein in the fungus. GP70, for example, is usually associated with rapidly growing hyphae and appears to be secreted into the environment³² whereas Alt a1 is frequently associated with spores.³⁶

As a result, when GP70 was high on August 30, the fungi may have been going through a rapid expansion phase in response to favorable rainfall and temperature conditions that were observed at that time. Alt a1, which was high on September 14, may have been produced as the fungi came under stress due to low moisture conditions. At this point the fungi would have been more likely to produce spores to survive until the next period of weather favorable to growth occurred. Our comparison of *Alternaria* spore counts with measured allergen levels is consistent with this possibility, though it does not prove it. Peak *Alternaria* spore counts on July 19 and September 1 correlated well with increased levels of Alt a1 which were preceded by elevations of GP70 allergen from rapidly expanding mycelia.

There are several overall comparisons that can be made between particle counting and EIA allergen measurement when used as a tool for estimating human allergen exposure. Counting methods can be quite precise, and specific pollen grains or fungal spores can be absolutely identified in most cases. EIA analysis methods, on the other hand, do not distinguish between similar epitopes on mugwort, timothy grass and ragweed.³⁷ The argument can be made, however, that the EIA result is more closely related to the reaction

mounted by the individual in response to an allergen. Unfortunately, enzyme immunoassay methods are not available for most outdoor aeroallergens. The question of whether pollen and spore counts, or direct immunoassay produces the closest correlation with human symptoms will only be answered after much additional investigation.

CONCLUSIONS

The advantage of the SpinCon over the Burkard collector is the ability to measure allergen concentrations in air samples. Since the collection efficiencies are comparable for pollen at a flow rate of 236 L/min and for spores at a flow rate of about 500 L/min,³¹ different rates may be needed for pollen and spore collection. This high flow rate ensures that enough air is sampled in a short time to collect detectable amounts of allergen. The addition of an automated fraction collection capability to the SpinCon makes it an ideal device for collecting air when antigens are to be measured over time.

This work demonstrates the feasibility of direct immunoassay measurement of allergenic material in the environment collected into a liquid sample. We were able to detect allergen in two pollens that were expected to be in abundance in this area and in the one of the most abundant fungi, *Alternaria*. Measured concentrations were generally in the range expected to cause symptoms in allergic individuals and correlated with pollen counts for the respective species.

The relationship between airborne bioparticles and their associated antigen concentrations appears to be complex. Our data indicate that there is a good relationship between pollen grains and pollen allergens, but not as much agreement between spore counts and fungal allergens. Fungal counts do give an accurate measure of exposure to fungal spores, but the patterns of fungal allergen exposure are not yet clear. Perhaps visual observation of mold spores does not give as accurate an indication of exposure as visual observation of pollen grains does. Since

it is the allergens that likely cause allergic symptoms, further study of the airborne concentration of allergens is indicated.

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Request for reprints should be addressed to:
Jay Portnoy, MD
Chief, Section of Allergy/Immunology
The Children's Mercy Hospital
Kansas City, MO 64108