

Recognition of pollen and other particulate aeroantigens by immunoblot microscopy

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Most grass-pollen types appear identical by normal light microscopy. Restricted antigenic cross-reactivity of Cynodon dactylon (Bermuda grass) pollen allowed development of a new method to identify antigens associated with grass-pollen grains. Pollen applied to the surface of an adhesive tape was blotted onto nitrocellulose, and the blots were identified by anti-Bermuda-grass antibodies, second antibody, and fluorescence microscopy. Of the 44 species of grass pollen studied for specificity of the method, the only species to demonstrate uniformly bright staining were Cynodon dactylon, Elymus triticoides, Elymus cinereus, and Koeleria cristata. Thirty-one species were negative, and nine other species demonstrated occasional brightly fluorescent spots, suggesting contamination. The immunoblotting method was used to study Tucson air collected continuously by a Burkard pollen and spore trap throughout April 1986. Each 2-hour transect of the adhesive tape from the trap was examined by immunoblotting and by normal light microscopy to compare antigen particle counts with grass-pollen counts. The mean antigen-particle concentration, 52.8/m³ of air, was higher than the mean grass-pollen concentration, 21.9/m³ of air, suggesting presence of amorphous Bermuda-grass antigens in air samples. Antigen-particle concentration, not grass-pollen concentration, correlated significantly with wind velocity, temperature, and time of day. (J ALLERGY CLIN IMMUNOL 1988;82:608-16.)

Many, but not all, types of airborne pollen grains are distinguished by recognition of their unique morphologic features with light microscopy or electron microscopy. Some types can only be distinguished from one another by subtle morphologic differences identifiable by an experienced palynologist. For example, *Celtis* closely resembles *Cannabis*, and *Sal-sola* closely resembles *Amaranthus*. Pollen from many plants, for example, the grasses, cannot be identified at the genus level with light microscopy. Although immunochemical methods with improved species specificity have been applied to characterization of grass-pollen extracts,^{1, 2} these methods have not enabled enumeration of particles associated with species-specific pollen antigen. Thus, a new method for microscopic identification of pollen would be useful, particularly for genera that produce morphologically identical pollen simultaneously in the same geographic area.

Abbreviations used

FIB: Fluorescence immunoblotting
PBS: Phosphate-buffered saline
ha: Hectare

We have developed an immunochemical method to distinguish pollen grains of *Cynodon dactylon* (Bermuda grass) from morphologically identical grass-pollen types, based on the known immunologic uniqueness of Bermuda-grass allergens.^{3, 4} There were three intended applications of this method: (1) to study the proportion of total atmospheric grass pollen that was derived from Bermuda grass, (2) to study the relationships between atmospheric Bermuda grass-pollen concentration, time of day, and weather in southern Arizona, and (3) to relate the proportion of airborne grass-pollen grains that were positive for Bermuda antigen to the prevalence of Bermuda-grass plants in the region sampled. Contrary to expectations, we found that numbers of airborne particles associated with Bermuda-grass antigens were significantly greater than the total numbers of grass-pollen grains, suggesting the presence of morphologically unrecognizable grass-pollen antigens in the atmosphere of southern Arizona. It was found that the atmospheric

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TABLE I. Grasses exhibiting high cross-reactivity with anti-Bermuda-grass antiserum by FIB

	% Staining*	
	Hollister-Stier	International Biologicals
<i>Cynodon dactylon</i> (Bermuda grass)†	>98	ND
<i>Elymus triticoides</i> (alkali ryegrass, creeping wild rye)†	>98	77 (lot 8329) 95 (lot 9171)
<i>Elymus cinereus</i> (giant wild rye)‡	96	ND
<i>Koeleria cristata</i> (Kocler's grass, western June grass)†	>98	7 (lot 9861) 2 (lot 9996)

ND = not done.

*Total number of fluorescent blots counted as a percentage of total number of grass-pollen grains counted.

†Species found in the Tucson area.

‡This sample was collected and botanically authenticated in Spokane, Wash. The genus *Elymus* is found in the Tucson area.

concentration of Bermuda grass-antigen particles varied with time of day, temperature, and wind speed. This method for antigen identification, FIB, was also able to detect contamination of commercially available grass pollen with pollen from other grass types.

METHODS

Antiserum to Bermuda-grass pollen was prepared in rabbits, which were injected with a 1:10 extract of pollen emulsified with Freund's complete adjuvant and boosted with extract emulsified in Freund's incomplete adjuvant at 4-week intervals. Bleedings for antiserum were done 10 to 14 days after boosting. Pooled serum for immunoblotting produced at least 30 bands in crossed immunoelectrophoresis.

Particulate antigen in atmospheric samples was identified by FIB as follows: A Burkard seven-day volumetric pollen and spore trap (Burkard Manufacturing Co. Ltd., Rickmansworth, Hertfordshire, England) was calibrated for air sampling at 10 L/min and then loaded with a collecting assembly carrying a tape coated with a nonaqueous Dow Corning adhesive (Dow Corning Corp., Midland, Mich.) (280 series). After collection, the Burkard tapes were cut into 24-hour sections and marked at 2-hour intervals. Nitrocellulose membranes (pore size 0.45 μ m, Schleicher & Schuell Inc., Keene, N.H.), 2 by 6 cm, were marked at 2-hour transect points to correspond with identical marks on the adhesive tape and were saturated with 10 mmol/L of PBS, pH 7.2. The membranes were then applied to the surface of the particle-laden tape segments with the markings aligned, allowing 10 minutes for protein transfer. The membranes were removed and placed in pH 7 Tris-buffered saline containing 4% bovine serum albumin for 1 hour at 37° C. The membranes were then rinsed with PBS, overlaid with rabbit antiserum to Bermuda-grass pollen, incubated for 1 hour, and washed with large volumes of saline. Affinity-purified fluorescein isothiocyanate-labeled goat antirabbit IgG (Cooper Biomedical Inc., Malvern, Pa.,

1612-3151), diluted 1:400, was then applied to the membranes for 1 hour. After washing with PBS, the membranes were mounted on microscope slides, frozen at -20° C, and viewed within 48 hours with epifluorescence microscopy with a 25 X oil immersion objective. Substitution of anti-Bermuda-grass antiserum with normal rabbit serum or omission of fluorescent antiserum prevented fluorescent staining of Bermuda-grass pollen immunoblots on the membranes. Fluorescent spots were counted along alternate hourly transects of the membrane. The Burkard tape segments with their adherent pollen grains were mounted in basic fuchsin-glycerine jelly, and grass pollen was counted by light microscopy along equivalent transects. Each transect was equivalent to 15-minute collection by the trap. Pollen concentrations from Burkard collections were calculated as:

$$\text{Grains/m}^3 = \frac{P \times 1,000}{F \times M \times T}$$

where P = total number of pollen grains counted, F = flow rate in liters per minute (10), M = number of minutes per transect (15), T = number of transects counted (12), and 1000 = liters per cubic meter of air. Antigen particles per cubic meter of air sampled were calculated with the same formula, substituting the total number of fluorescent spots counted for the pollen-grain number.

The specificity of the Bermuda-pollen FIB system was tested by studying the reactivity of 44 species of nondefatted grass pollen applied to strips of Burkard trap sampling tapes coated with adhesive. Pollen samples from six of these species were obtained directly from plant specimens in the areas of sampling: *Aristida purpurea*, *Bromus rubens*, *Eragrostis lehmanniana*, *Hordeum leporinum*, *Poa annua*, and *Schismus spp.* (see Tables I to III for common names of grasses). The remaining 36 grass-pollen samples listed in Tables I to III were donated by Hollister-Stier Laboratories (Spokane, Wash.). Additional nondefatted pollen samples from the grasses *Agropyron repens*, *Dactylis glomerata*,

TABLE II. Grasses negative with anti-Bermuda grass FIB, except for low numbers of brightly fluorescent blots

	% Staining*	
	Hollister-Stier	International Biologicals
<i>Agropyron repens</i> (quack grass)†	<2	<2
<i>Dactylis glomerata</i> (orchard grass)	4.5	<2
<i>Festuca rubra</i> (red fescue)†	5.7	<2
<i>Poa compressa</i> (Canada bluegrass)†	9.8	<2
<i>Poa pratensis</i> (Kentucky bluegrass)†	4.5	<2
<i>Poa sandbergii</i> (Sandberg bluegrass)†	2.8	ND
<i>Sorghum vulgare</i> (common cultivated sorghum)†	<2	8.1
<i>Sorghum vulgare</i> var. <i>sudanense</i> (Sudan grass)†	7.2	3.6
<i>Zea mays</i> (cultivated corn)‡	<2	ND

ND = not done.

*Total number of fluorescent blots as a percentage of total number of grass-pollen grains.

†Genus found in Tucson area.

‡Species found in Tucson area.

Elymus triticoides, *Festuca rubra*, *Koeleria cristata*, *Poa compressa*, *Poa pratensis*, and *Sorghum vulgare*, and the trees *Olea europaea* (olive), *Morus alba* (white mulberry), *Fraxinus velutina* (Arizona ash), and the weed *Franseria deltoidea* (triangle leaf bursage, rabbit bush), were obtained from International Biologicals, Inc. (Piedmont, Okla.). *Amaranthus palmeri* (Palmer's amaranth, careless weed) pollen was from Greer Laboratories (Lenoir, N.C.). Pollen samples from *Prosopis juliflora* (mesquite), *Cercidium microphyllum* (palo verde), and *Juniperus deppeana* (alligator bark juniper) were collected directly from trees in the Tucson area. Pollen application was done by sprinkling the pollen from a toothpick 10 cm above the strips, inside a biologic safety hood. These tapes were then examined by FIB and light microscopy for enumeration of antigen positive spots and pollen grains, respectively. In many specimens, contiguous or clumped pollen grains were noted, artifactually decreasing the counts of FIB positive blots as a percentage of total grass-pollen grains by up to 0.7%. Pollen types that exhibited any degree of fluorescence in the FIB procedure were studied at least twice and usually three times.

RESULTS

Blotting of reference pollen types

The FIB method was tested by correlating grass-pollen and fluorescence counts for Bermuda-grass pol-

len sprinkled onto the adhesive-coated Burkard sampling tape in various concentrations. Fluorescent spots on the nitrocellulose membranes were perfectly round and were brightest centrally, fading exponentially to zero fluorescence toward the periphery (Fig. 1). The spots were generally twice the size of Bermuda grass-pollen grains. Although there was some variation in the fluorescence intensity of the spots, none of the spots was so faintly fluorescent that it required exclusion from the tally. The FIB counts correlated with grass pollen-grain counts up to a maximum count of 33 pollen grains per transect (Fig. 2). Above 33 grains per transect, the FIB counts tended to be lower than pollen-grain counts, and some spots were larger and more intensely fluorescent than others. This suggested the blotting of antigens from contiguous pollen grains as a single intense spot in the more heavily loaded adhesive tapes.

The method was also tested by mixing *Phleum pratense* pollen, negative by FIB, with Bermuda-grass pollen in various proportions by weight. Bermuda-pollen content of mixtures was estimated from FIB count times 100 divided by light microscopy count. In regression analysis of FIB count estimates of Bermuda-pollen content against the percent content

TABLE III. Grasses exhibiting no fluorescent blots with anti-Bermuda grass FIB

<i>Agropyron cristatum</i> (crested wheatgrass)*
<i>Agropyron smithii</i> (western wheatgrass)†
<i>Agropyron spicatum</i> (bluebunch wheatgrass)*
<i>Agrostis alba</i> (redtop)
<i>Agrostis tenuis</i> (colonial bent grass)
<i>Anthoxanthum odoratum</i> (sweet vernal grass)
<i>Aristida purpurea</i> (three-awn)†
<i>Arrhenatherum elatius</i> (tall oat grass)
<i>Avena fatua</i> (common wild oats)†
<i>Avena sativa</i> (common cultivated oats)*
<i>Bromus inermis</i> (smooth brome)*
<i>Bromus rubens</i> (foxtail brome)†
<i>Bromus secalinus</i> (cheat brome)*
<i>Bouteloua gracilis</i> (grama grass)†
<i>Distichlis stricta</i> (salt grass)†
<i>Eragrostis lehmanniana</i> (Lehmann's love grass)†
<i>Festuca elatior</i> (meadow fescue)
<i>Holcus lanatus</i> (velvet grass)
<i>Hordeum leporinum</i> (mouse barley grass)†
<i>Hordeum vulgare</i> (barley grass)*
<i>Lolium multiflorum</i> (Italian ryegrass)†
<i>Lolium perenne</i> (perennial ryegrass)†
<i>Paspalum notatum</i> (Bahia grass)*
<i>Phalaris arundinacea</i> (reed canary grass)*
<i>Phalaris canariensis</i> (canary grass)†
<i>Poa annua</i> (annual bluegrass)†
<i>Phleum pratense</i> (timothy grass)†
<i>Schismus spp.</i> †
<i>Secale cereale</i> (cultivated rye)
<i>Sorghum halepense</i> (Johnson grass)†
<i>Triticum aestivum</i> (cultivated wheat)

*Genus found in Tucson area.

†Species found in Tucson area.

of Bermuda pollen by weight, the regression coefficient was 0.999 (Table IV).

In cross-reactivity studies, Bermuda-grass pollen and three other species of grass were strongly positive by FIB: *Koeleria cristata*, *Elymus triticoides*, and *E. cinereus*. *Elymus* from a different supplier elicited identical results, but *K. cristata* from International Biologicals Inc. demonstrated only a small percentage of pollen blots that fluoresced by FIB, indicating no cross-reactivity (Table I). Pollen from all three lots of *Koeleria* from the two suppliers were identical by size and color analysis. Eight other species of grass demonstrated occasional brightly fluorescent spots on the nitrocellulose membranes, ranging between <2% and 10% of the grass pollen-grain count, a finding that suggested contamination by pollen or antigen from Bermuda grass or cross-reacting grasses, such as *Koeleria* or *Elymus* (Table II). Other grasses, for

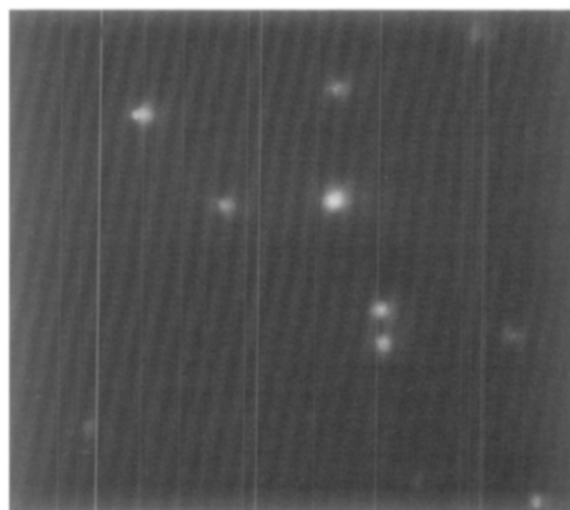


FIG. 1. Typical epifluorescence microscopy appearance of nitrocellulose membrane blotted with Bermuda-grass pollen adherent to Burkard trap tape. The membrane was treated with anti-Bermuda-grass antibodies and fluorescein isothiocyanate-labeled second antibody. Each spot is approximately twice the size of a grass-pollen grain. One spot near the center is brighter than others and may represent blotting of two contiguous pollen grains, but exact matching of fluorescence images to light images was not possible with this method.

example, *Eragrostis lehmanniana*, were entirely negative (Table III). The Bermuda-grass FIB method was also tested for cross-reactivity to tree and weed types that flower in April in Tucson: *Cercidium microphyllum*, *Franseria deltoidea*, *Fraxinus velutina*, *Juniperus deppeana*, *Morus alba*, *Olea europaea*, and *Prosopis juliflora*. None of these pollen types were positive.

Atmospheric sampling at different sites

A Burkard trap was placed on a rooftop, 4 m above ground level, at a site in central Tucson, elevation 730 m (University of Arizona Health Sciences Center). The site was adjacent to a playing field, area 0.58 ha (5800 m²), containing mainly Bermuda grass. A small minority of other grasses, including *Poa annua* and *Hordeum leporinum*, were flowering in the field. *Aristida purpurea*, *Bromus rubens*, and *Schismus* were flowering in the vicinity. The area is surrounded predominantly by single family dwellings on small lots, many of which possess Bermuda-grass lawns. The entire metropolitan area is intersected with unpaved alleys in which Bermuda grass grows as the ubiquitous weed.

Sampling at this site was carried out continuously throughout the month of April and early May 1986 for subsequent analysis by FIB and by light micros-

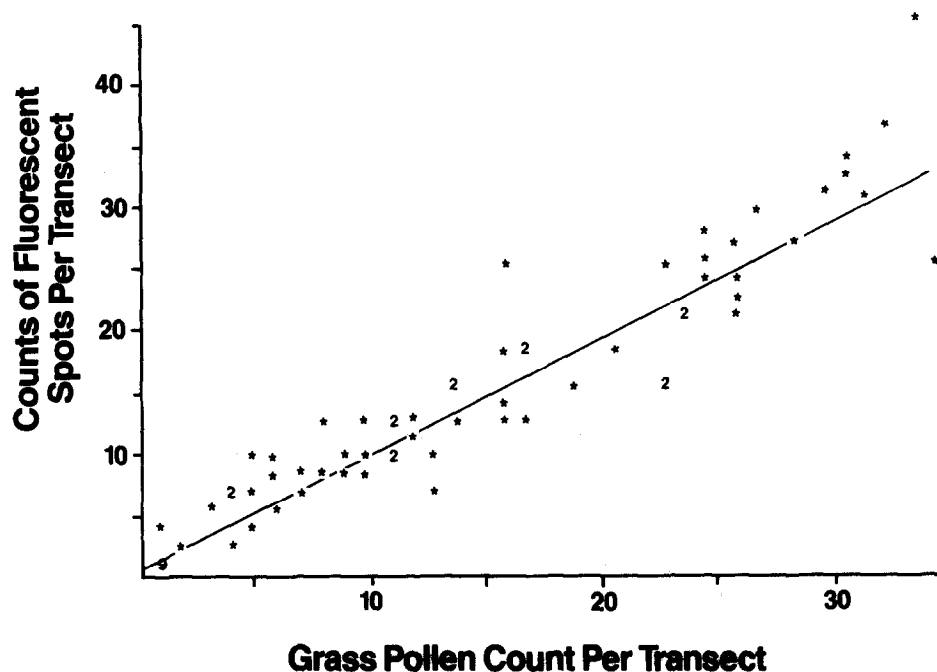


FIG. 2. Correlation between grass-pollen counts per transect and counts of fluorescent spots per transect transferred by FIB, with Bermuda-grass pollen applied directly to adhesive tape. Linear regression: $r = 0.95$, slope = 0.93, y intercept = 0.8, and $p = 0.00001$.

TABLE IV. Accuracy of prediction of Bermuda-grass content of mixed grass pollen with FIB

% Of pollen by weight	0	10	30	50	90	100
Estimate of % Bermuda-pollen content by FIB*	<2	12	41	47	92	>98

*Calculated from mean of FIB counts across 12 transects of nitrocellulose times 100 divided by mean of pollen-grain counts by light microscopy across 12 transects of adhesive tape. Regression analysis: coefficient = 0.999 and $r = 0.994$.

copy. Appearances of fluorescent spots on nitrocellulose membranes blotted with the Burkard trap tapes were qualitatively indistinguishable from membranes that had been applied to tapes sprinkled with authentic Bermuda-grass pollen. The mean antigen concentration, 53.2 particles per cubic meter of air, was significantly higher than the grass-pollen concentration, 22.1 grains per cubic meter of air ($p < 0.0001$), suggesting the presence of large numbers of particles that contained Bermuda-grass antigen but were not recognizable as pollen by light microscopy. One grass-pollen count was high at 35 grains per transect. The next highest grass-pollen count per transect, 22, was well within the range in which counting of pollen blots was found reliable. High FIB counts were often associated with unusually bright or large spots, suggesting blotting of contiguous particles. Since the error in matching Burkard tapes to their corresponding blotted nitrocellulose tapes was ± 0.5 mm, it was not possible to match individual fluorescent spots with

individual pollen grains or other particles from which they were blotted.

In an experiment designed to sample grass pollen relatively free of Bermuda-grass antigen, the trap was moved to a site on the Santa Rita Experimental Range (U.S. Forest Service) in Arizona, elevation 1260 m, 52 km south of Tucson and 16 km from the nearest small town, Green Valley, Ariz. (population 8500). The range encompasses 22,300 ha (223 km²) and is dominated by *Eragrostis lehmanniana*. The grasses *E. superba*, *Bouteloua hirsuta* (hairy grama grass), and *Andropogon barbinodis* (cane beard grass) were also flowering at the site in the same period. *K. cristata* was not found near the trap but grows in the region. There appeared to be no Bermuda grass in the area aside from a small lawn, area 100 m², 1.3 km southeast of the site. The trap was located at ground level and was surrounded by pollinating grass approximately 0.8 m tall. The trap operated continuously on Sept. 7 and 8, 1986, when *Eragrostis* was polli-

TABLE V. Mean antigen-particulate concentrations by FIB and mean daily grass-pollen concentrations (per cubic meter of air) at three locations in Arizona

	Tucson	Santa Rita Range	Gadsden
Month	April	September	September
Fluorescence counts	52.8	333	891
Grass pollen counts	21.9	2574	26.9

nating at its peak, and there was no rainfall. FIB and light microscopy of the tape from this collection demonstrated that the FIB counts were only 13% of the total grass-pollen counts (Table V).

In an effort to obtain air samples from an area where pollinating Bermuda grass is the overwhelmingly predominant grass species, the trap was placed at ground level at Gadsden, Ariz., near Yuma, and 380 km west of Tucson. The trap was located in a field of pollinating Bermuda grass, area 15 ha, between Sept. 22 and 26, 1986. The field is traversed by a canal with occasional clumps of the grass *Sorghum halepense* on its banks. Heavy rainfall occurred during the first 3 days of collection. Tapes from this collection demonstrated that the antigen-particle concentrations by FIB were an average of 33 times the grass-pollen concentration by light microscopy, and at times exceeded the limits of the method for accurate counting (Table V).

Diurnal variation of FIB results and correlation with weather

In the April to May collection in Tucson, day-to-day variation in the FIB count did not correlate with day-to-day variation in the grass-pollen count (Fig. 3). However, antigen-particle concentrations demonstrated a clear diurnal variation, with peak counts occurring at midday and low counts occurring between 9 PM and 3 AM (Fig. 4). By contrast, a diurnal pattern of grass-pollen concentrations was not evident. Furthermore, there was no significant correlation between grass-pollen counts and FIB counts.

Relationships between antigen-particle concentrations, grass-pollen concentrations, and meteorologic data were studied by correlation analysis. A significant correlation was found between wind velocity and antigen-particle concentrations ($r = 0.49$; Fig. 5), but the relationship between wind and grass-pollen concentrations was not significant ($r = 0.06$). Although grass-pollen concentrations could not be correlated with temperature, a significant negative correlation

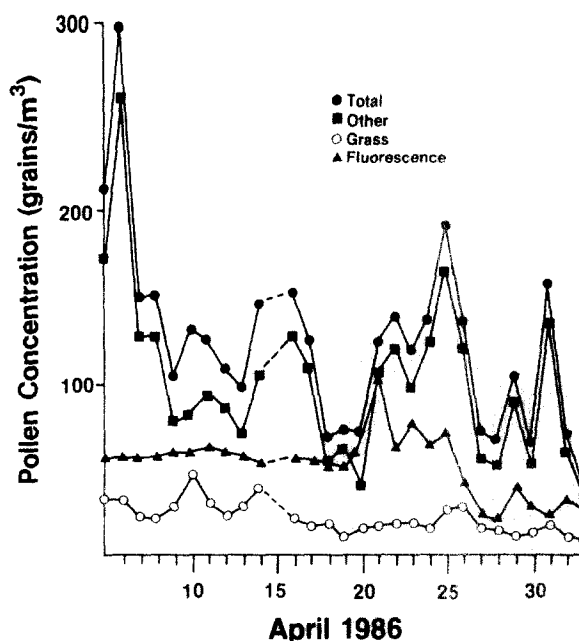


FIG. 3. Variation in total pollen concentrations, grass-pollen concentrations, and Bermuda-grass antigen-particle concentrations by FIB throughout April 1986.

was found between antigen-particle concentrations and temperature 10 hours previously with a lag-analysis method (Table VI). No correlation could be found with grass-pollen concentrations or antigen-particle concentrations and dew point. Aside from a trace of precipitation on 1 day, there was no rainfall throughout the monitoring period in Tucson.

DISCUSSION

The FIB method distinguishes between presence and absence of Bermuda-grass antigen in pollen samples. As only three of 43 nonBermuda species of grass tested were uniformly positive for Bermuda-grass antigen, the test has use in identifying contamination of most nonBermuda-grass species with grass-pollen grains that contain Bermuda-grass antigen. It is unlikely that cold-climate grasses, for example, *Poa*, would be contaminated with Bermuda-grass pollen, but grasses, including *Elymus*, which cross-react in our system, could have caused the contamination. There was a wide discrepancy between two pollen suppliers in regard to reactivity of Koeler's grass (*K. cristata*) by FIB. Although the discrepancy is unexplained by available data, it demonstrates the potential of the method for identifying major antigenic differences between apparently identical pollen types. Cross-reactivity between Bermuda grass and *Distichlis* (salt grass), previously demonstrated by RAST inhibition,⁵ was not found with our rabbit IgG antibody system, possibly because human IgE antibodies and

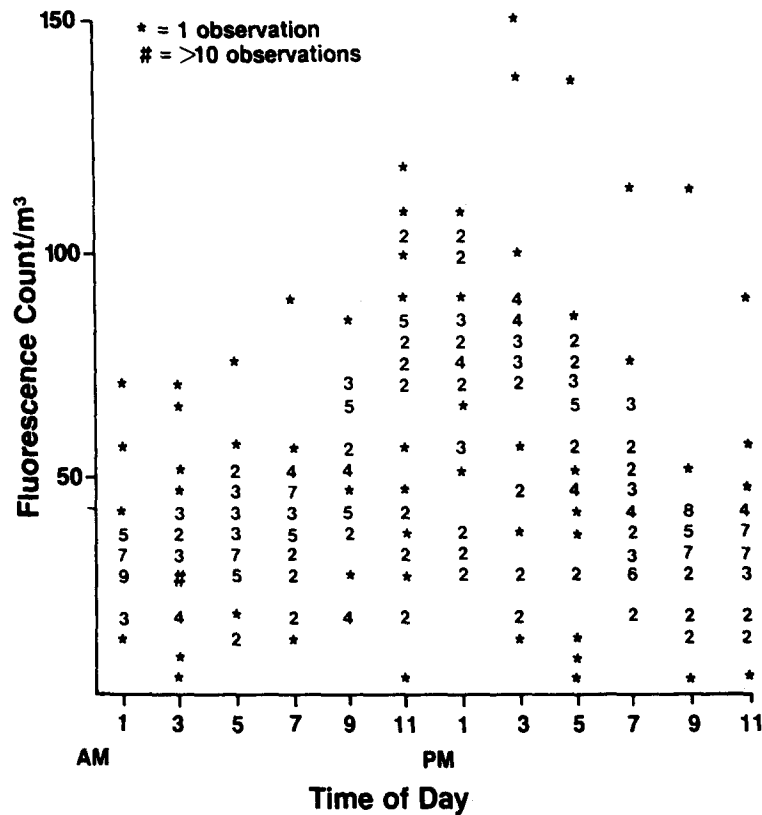


FIG. 4. Variation of Bermuda-grass antigen-particle concentrations by FIB with time of day.

rabbit IgG antibodies may be predominantly specific for different components of Bermuda-grass pollen extracts. The reliability of the method for quantitative transfer of antigen from adhesive tapes was tested and found to elicit accurate results for FIB counts <33 per transect, well above the grass-pollen counts obtained during the air-sampling period.

Antigen-particle concentrations were consistently higher than total grass-pollen concentrations at two different geographic locations. There was a discrepancy between antigen-particle concentrations and grass-pollen concentrations that averaged 2.4-fold during the month of April 1986 in Tucson, and a tenfold discrepancy between antigen-particle and grass-pollen concentrations in Gadsden, Ariz., indicating the presence of airborne Bermuda antigen-containing particles that could not be recognized as pollen. Even on the days in Tucson when the FIB count was only a little higher than the grass-pollen count, it is likely that Bermuda grass comprised only a proportion of the total grass-pollen concentration, because several other species of grass were pollinating in the vicinity of the trap. Thus, amorphous airborne grass antigen was present at all times during April in Tucson. It is

possible that many of the amorphous particles collected by the Burkard trap are paucimicronic in size. Some investigators suggest that this trap may preferentially capture airborne particles in this size range.⁶ A thorough investigation of the size distribution of amorphous particles that contain Bermuda antigens was beyond the scope of our study.

It was not possible to fulfill the original aim of the study, that is, to differentiate morphologically identical grass-pollen types in air samples, because the FIB count represents the sum of blots from an antibody-reactive pollen type and the blots from amorphous particulate antigen derived from the same grass. Since the ratio of grass-pollen numbers to antigen-particle concentrations is usually unknown, it is impossible to estimate proportions of two or more grass pollens in the same sample.

Unlike grass-pollen concentrations, antigen-particle concentrations correlated significantly with time of day, wind velocity, and temperature. Failure to find a similar correlation with total grass-pollen concentrations may reflect flowering of multiple grass species that vary in optimal environmental conditions for pollen release. Another explanation for the difference

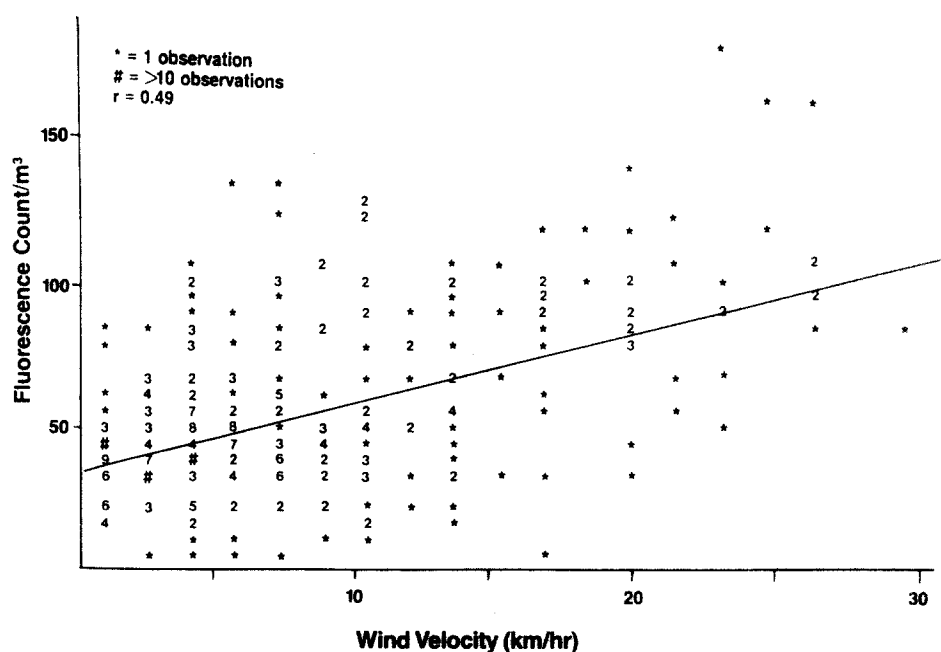


FIG. 5. Variation of Bermuda-grass antigen-particle concentrations by FIB with wind velocity. Linear correlation: $r = 0.498$, slope = 2.36, y intercept = 36, and $p = 0.00001$.

TABLE VI. Correlation coefficients in multiple regression analysis of effects of wind velocity and temperature on fluorescence counts and grass-pollen counts*

	Wind	Temp	Temperature lagged (hr)								
			2	4	6	8	10	12	14	16	18
Fluorescence counts	0.49	0.27	0.16	-0.05	-0.28	-0.47	-0.54	-0.48	-0.32	-0.13	0.07
Grass pollen counts	0.06	0.02	-----NS-----								

NS = not significant (coefficients ranged between -0.01 and -0.13).

*Analysis based on 12 transects per 24 hours for 30 days.

could be that amorphous pollen antigen may be, on the average, smaller than pollen grains and therefore more easily dispersed in the atmosphere than pollen. Additionally, the Burkard trap may have captured smaller particles more efficiently than pollen grains.

Since antigen-bearing particles may vary considerably in size, there may not be a good correlation between antigen-bearing particles and total allergenic activity per unit volume of air estimated by RAST or ELISA. These immunoassay methods have been used by several investigators⁶⁻¹⁰ but have not yet become sensitive enough to quantify allergen in air samples at 2-hour intervals. Nevertheless, studies correlating quantitative allergen assays with particle counts are

necessary to assess the significance of various particle concentration for allergic individuals.

The greatest discrepancy between antigen-particle concentration and grass-pollen concentration was encountered in a field of pollinating Bermuda grass when rain was falling during the sampling period. These results confirm Australian observations of increased concentrations of paucimicronic grass-pollen allergen during days after rain.¹¹ Therefore, rain may not only have the effect of reducing atmospheric pollen counts but also may actually increase antigen load in the atmosphere through liberating allergens from pollen grains or plant surfaces for subsequent dispersal by wind.

In conclusion, these studies demonstrate that antigen in morphologically unrecognizable particles may occur in the atmosphere in numbers that are several fold greater than the numbers of recognizable pollen grains derived from the same species. These observations, together with published data on the presence of paucimicronic ragweed antigen⁶⁻¹⁰ and ryegrass-pollen antigen,¹¹ indicate the potential for these particles to cause allergic airway disease, particularly in the lower respiratory tract, the site of deposition of particles <10 μm .

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