Lack of correlation between regional pollen counts and percutaneous reactivity to tree pollen extracts in patients with seasonal allergic rhinitis

Jason F. White, MD*; Linda Levin, PhD†; Manuel Villareal, MD*; Karen Murphy, RRT‡; Raymond Biagini, PhD§; Lisa Wellinghoff, MS†; Harry G. St. Clair, BS¶; and David I. Bernstein, MD*

Background: Although seasonal patterns of tree pollination have been reported, it is unknown if aerobiologic data correlate with patterns of in vivo sensitization.

Objective: To evaluate the relationship between regional tree pollen exposure and patterns of in vivo percutaneous reactivity to specific tree pollen extracts in a local patient population with seasonal allergic rhinitis.

Methods: Patients with spring seasonal allergic rhinitis and percutaneous sensitivity to 1 or more regional tree pollens were studied. Tree pollen counts were collected at the same urban site from 1997 to 2002 and at a suburban site in 2002. Patients underwent skin prick testing with commercial extracts of 15 indigenous tree species. Serum specific IgE measurements were assayed in a subset of sensitized patients.

Results: Of 127 patients who reported symptoms consistent with seasonal allergic rhinitis during the spring pollen season, 93 qualified based on demonstration of at least 1 positive skin prick test result. Mean 5-year pollen counts (1997–2001) and 2002 urban counts were highly correlated (Spearman $r = 0.95$, $P < .001$), indicating that year-to-year pollen counts were consistent. No significant correlation was found between mean seasonal pollen counts (urban site, 1997–2001) and frequencies of skin prick test reactivity to specific tree pollen allergens (Spearman $r = -0.03$, $P = .93$). No significant relationship was found between 5-year mean tree pollen counts and positive serum specific IgE tests for specific tree pollens (Spearman $r = -0.42$, $P = .30$). Eight of 15 species elicited percutaneous reactions in more than 50% of patients (ie, satisfying definition of a major in vivo allergen). However, 6 of the 8 major tree allergens each represented 5% or less of 5-year mean total tree pollen counts.

Conclusion: No correlation was found between overall frequencies of in vivo sensitization to tree pollen allergens in a local population and regional pollen exposure data.

Ann Allergy Asthma Immunol. 2005;94:240–246.

INTRODUCTION

Wind-borne tree pollens are important causes of seasonal allergic rhinitis (SAR). Identification of relevant local pollen species is considered essential in selecting appropriate antigens both for diagnosis and treatment of seasonal allergic pollinosis and asthma. Traditionally, these decisions have been based largely on knowledge of regional patterns of pollination of allergenic tree pollen species.[1,2] The difficulty of this task is compounded by the dearth of data defining allergen cross-reactivity among tree pollen families, with the exception of the birch and beech families (ie, Betulaceae and Fagaceae).[1,3–5] Thus, multiple tree pollen extracts are often arbitrarily selected for use in diagnosis and treatment of patients with SAR. However, this approach is cumbersome and may lead to selection of some irrelevant or minor tree pollen antigens. There are no standard approaches that guide clinicians in discriminating major and minor tree pollen allergens and assist in selection of the most relevant tree pollens in a given geographic area. No studies have been performed to examine patterns of sensitization to tree pollen allergens among SAR patients combined with cumulative aerobiologic data of individual tree pollens. Therefore, we conducted such a study with the aim of developing a rational approach that would allow prioritization and selection of clinically important tree pollen allergens for clinical use.

METHODS

The study population was recruited from patients who presented to 2 regional allergy clinics with SAR symptoms that had lasted for at least 2 consecutive years during the tree pollen season (early March to mid May). To study a population representative of native atopic patients with active symptoms of SAR, we recruited patients 5 to 50 years of age who had lived for at least 50% of their lifespan and at least 50% of their first 16 years in the

* Division of Immunology-Allergy, Department of Medicine, University of Cincinnati, Cincinnati, Ohio.
† Department of Environmental Health, University of Cincinnati, Cincinnati, Ohio.
‡ Bernstein Clinical Research Center, Cincinnati, Ohio.
§ National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, Cincinnati, Ohio.
¶ Hamilton County Environmental Services, Cincinnati, Ohio.
This study was funded by grant R01 ES11170-01 from the National Institute of Environmental Health Sciences and Division of Immunology/Allergy, University of Cincinnati, Cincinnati, Ohio.
Received for publication May 16, 2004.
Accepted for publication in revised form October 3, 2004.

Southwest Ohio region or the 3-state region (Kentucky, Ohio, and Indiana) that surrounds Cincinnati. Patients with dermatologic conditions that prohibit interpretation of skin test results were excluded, as were patients currently receiving allergen immunotherapy. A screening questionnaire that listed the aforementioned inclusion criteria was used to determine eligibility. All patients signed a written informed consent statement approved by the University of Cincinnati Institutional Review Board.

Immunologic Studies

Qualified patients who were undergoing evaluation for SAR during spring and summer 2002 were recruited sequentially as they arrived at the clinics. Those who volunteered to participate underwent skin prick testing with 15 nonstandardized commercial tree pollen extracts, saline, and histamine phosphate (5 mg/mL; Allermed Laboratories, San Diego, CA) control reagents. Patients were instructed to withhold antihistaminic drugs for appropriate intervals before testing. Participants were tested with extracts of 15 pollens indigenous to the Midwest and Northern regions of the United States from identical manufacturers and antigen lots. The 15 tree antigens included the following: American sycamore (*Platanus occidentalis*), American elm (*Ulmus americana*), eastern cedar (*Juniperus virginiana*), box elder (*Acer negundo*), river birch (*Betula nigra*), white oak (*Quercus alba*), red oak (*Quercus rubra*), black willow (*Salix nigra)*, eastern cottonwood (*Populus deltoides*), red mulberry (*Moras rubrum*), black walnut (*Juglans nigra*), hackberry (*Celtis occidentalis*), and white poplar (*Populus alba*) (ALK Laboratories Inc, Wallingford, CT), and red maple (*Acer rubra*) and white ash (*Fraxinus americana*) (Greer Laboratories Inc, Lenoir, NC). A bifurcated disposable needle device (Duotip-Test, Lincoln Diagnostics Inc, Decatur, IL) was used. Skin prick test results were considered positive if the wheal diameter was 3 mm or higher than the saline control at 15 minutes and in the presence of a positive histamine control. Long and orthogonal diameters of wheal-and-flare responses were measured, although wheal and flare sizes were not analyzed as an outcome in this study.

Serum specific IgE for tree pollens was evaluated by the AlaSTAT microplate system (Diagnostics Products Corporation, Los Angeles, CA) with a panel of 14 available tree pollen antigens that included maple, birch, mountain cedar, oak, elm, olive, walnut, sycamore, willow, cottonwood, white ash, white pine, white mulberry, and oak mix using standards and reagents supplied by the manufacturer. Positive values were defined as greater or equal to a cutoff of 0.70 IU/mL, corresponding to a class II or greater response.

Aerobiologic Data

Tree pollen samples were collected with Rotorod Samplers (Multidata LLC, St Louis Park, MN) during March to May. During the 2002 tree pollen season, counts were collected at 2 sampling stations in an urban and a suburban location. All pollen measurements used for correlations and associations with skin tests and in vitro tests were collected via a Rotorod Sampler that was positioned at an urban collection site on a 40-ft unobstructed rooftop during the spring pollen seasons from 1997 to 2002. A second Rotorod Sampler was positioned 10 miles west of the urban site in a suburban, residential neighborhood. This sampler, which was used as a comparator to evaluate consistency with the first sampler positioned in the central urban location, was placed on a tripod at ground level within an apparently unobstructed sampling area. Experienced technicians at Hamilton County Environmental Health Services who performed urban counts were trained at McCrone Research Institute in Chicago, IL. Sixty percent of Rotorod slides were counted twice by 2 separate individuals, and data were reproducible within 15% to 20% for individual pollen species. These pollen measurements followed standards outlined by the Aeroallergen Monitoring Network of the American Academy of Allergy, Asthma and Immunology. Pollen counts of identifiable tree species were expressed as grains per cubic meter.

Statistical Analysis

Statistical analysis was performed with SAS statistical software for Windows, version 8.2 (SAS Institute Inc, Cary, NC). Spearman correlations were calculated to evaluate the association between 2002 total pollen counts of Rotorod Samplers located at urban and suburban locations. Spearman correlations were also applied to evaluate correlations between pollen counts and numbers of patients with positive skin prick test and AlaSTAT results. The κ statistics were obtained to examine agreement between the number of positive SPT and AlaSTAT results for each tree antigen based on known patterns of cross-reactivity. A type 1 error level of $P = .05$ was considered statistically significant.[6]

RESULTS

Of 127 patients who reported symptoms consistent with SAR during the spring pollen season, 93 qualified based on demonstration of at least 1 positive skin prick test result. Frequencies of percutaneous sensitivity for specific tree species among the 93 patients with positive skin prick test results are shown in Figure 1. American sycamore, American elm, box elder, red maple, red oak, white ash, cottonwood, and black walnut were found to be major allergens, when major allergen is defined as percutaneous reactivity to a given tree pollen extract in more than 50% of all patients with positive skin prick test results.[7] Less than 50% of atopic patients reacted with eastern red cedar, river birch, red mulberry, black willow, white poplar, and hackberry.

Mean 5-year peak pollen counts, calculated with the urban Rotorod data from 1997 to 2001, were highest for oak (3,135 grains/mm^3), cedar (1,646 grains/mm^3), elm (1,254 grains/mm^3), and mulberry (1,054 grains/mm^3). The 2002 suburban Rotorod data showed the highest pollen counts for oak (7,221 grains/mm^3), cedar (1,636 grains/mm^3), mulberry (851

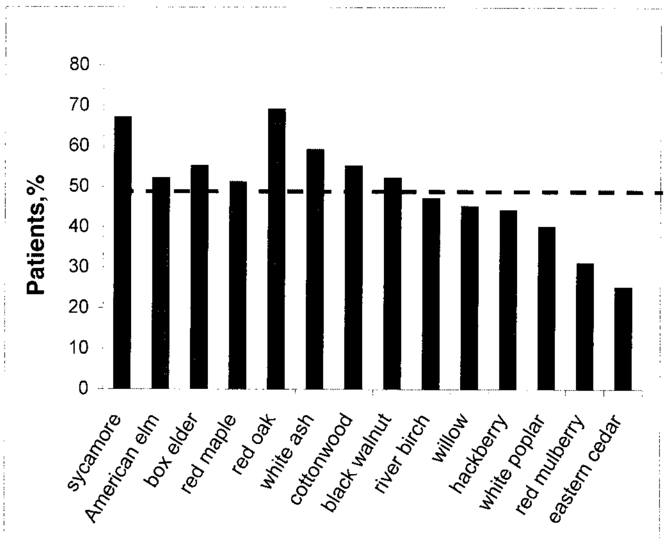

Figure 1. Percentage of patients with positive skin prick test results to 14 indigenous tree species (n = 93). Major allergenic trees that produced positive skin prick test results in more than 50% of patients (bars above the dashed line) include American sycamore, American elm, box elder, red maple, red oak, white ash, cottonwood, and black walnut. Forty-four percent of patients reacted to white oak (not shown).

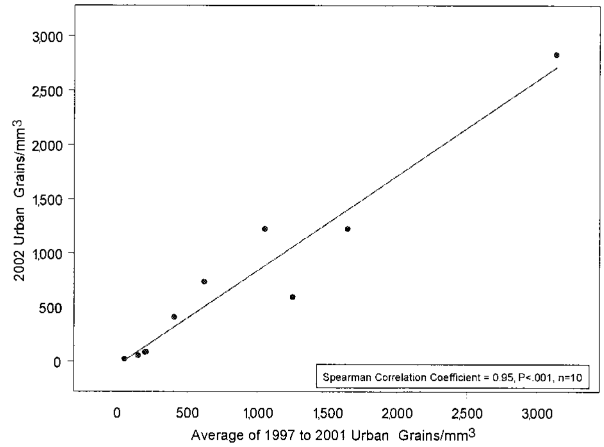

Figure 2. A Spearman correlation coefficient of 0.95 ($P < .001$) was found between the 5-year average pollen exposure at a centrally located, urban collection site and the 2002 pollen exposure at the urban collection site.

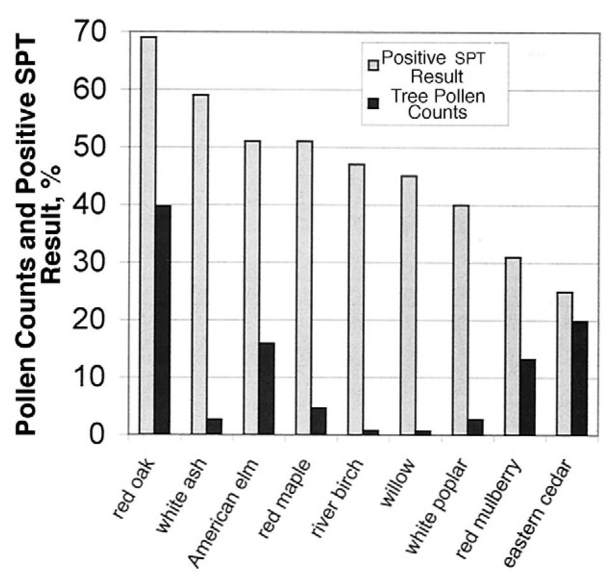

Figure 3. Mean 5-year counts of specific tree pollen expressed as percentage of mean total tree pollen counts for specific tree species and percentage of positive skin prick test (SPT) responses to individual tree pollen extracts among all tree pollen allergic patients (n = 93). No significant correlation was detected. Sycamore, walnut, and cottonwood species are not shown in this graph due to negligible 5-year pollen counts, and box elder was not distinguishable from maple species.

grains/mm^3), and willow (692 grains/mm^3). Neither the urban nor suburban collection sites recorded appreciable sycamore, cottonwood, or walnut pollens.

The Spearman correlation test statistic showed a moderate correlation ($r = 0.66$, $P = .04$) between the urban and suburban sites during the 2002 tree pollen season. Urban and suburban counts were not significantly different (paired *t* test, $P = .24$). The 5-year mean urban specific pollen counts (1997–2001) and the 2002 urban pollen count data were highly correlated ($r = 0.95$, $P < .001$) (Fig 2). The *P* value for testing the difference between urban counts from 1997 to 2002 was .06 based on a repeated-measures analysis of variance. There was a moderate correlation between the 5-year mean urban pollen counts (1997–2001) and the 2002 suburban counts (Spearman $r = 0.64$, $P = .05$). When urban pollen counts were evaluated for yearly consistency (1997–2002), the mean yearly pollen counts were not statistically significantly different ($P = .11$). The correlation between the yearly pollen counts was approximately 0.53.

No significant correlation was found between cumulative mean seasonal pollen counts (urban site, 1997–2001) and frequencies of skin prick test reactivity to specific tree pollen allergens (Spearman $r = -0.03$, $P = .93$). Figure 3 shows the obvious lack of any relationship between the frequency of positive skin prick test responses and pollen counts for individual pollen antigens. In fact, 8 of 15 species elicited percutaneous reactions in more than 50% of patients (ie, satisfying definition of a major in vivo allergen). However, each of 6 of these major allergens accounted for 5% or less of the total 5-year mean tree pollen count. No significant relationship was found between mean cumulative counts and positive serum specific IgE test (AlaSTAT) results for specific tree pollens (Spearman $r = -0.42$, $P = .30$).

The κ statistics were calculated to evaluate agreement between the skin prick test results (positive or negative) as given in Table 1. The scale of agreement κ scores were

Table 1. The κ Statistics Measuring Agreement Between Positive Skin Prick Test Results and Tree Allergens Among 93 of 127 Patients With at Least 1 Positive Skin Prick Test Result*

	American elm	American sycamore	Black walnut	Black willow	Box elder	Cotton-wood	Hack berry	Red cedar	Red maple	Red mulberry	Red oak	River birch	White ash	White oak	White poplar
American elm	1	0.55	0.59	0.50	0.55	**0.62**	0.45	0.28	0.33	0.54	0.34	0.50	0.37	0.38	0.47
American sycamore		1	**0.65**	**0.66**	0.57	0.50	0.45	0.24	0.23	**0.62**	0.53	0.58	0.58	**0.61**	0.44
Black walnut			1	0.49	**0.61**	**0.72**	0.57	0.24	0.27	**0.62**	0.50	**0.66**	0.55	0.48	0.55
Black willow				1	0.53	0.55	0.52	0.18	0.31	**0.61**	0.44	0.48	0.56	0.49	0.54
Box elder					1	0.59	0.57	0.07	0.36	**0.62**	0.46	0.57	0.42	0.44	0.47
Cottonwood						1	0.51	0.10	0.25	0.60	0.39	0.55	0.52	0.42	0.49
Hackberry							1	0.15	0.31	0.56	0.36	0.39	0.43	0.32	0.44
Red cedar								1	0.07	0.23	0.15	0.20	0.17	0.16	0.21
Red maple									1	0.31	0.18	0.44	0.13	0.10	0.41
Red mulberry										1	0.41	0.74	0.56	0.58	**0.62**
Red oak											1	0.40	0.50	0.44	0.32
River birch												1	0.48	**0.62**	0.53
White ash													1	0.41	0.38
White oak														1	0.35
White poplar															1

* Agreement may be considered slight if κ equals 0 to 0.20, fair if 0.21 to 0.40, moderate if 0.41 to 0.60, substantial if 0.61 to 0.80, and almost perfect if 0.81 to 1.00. The κ values that indicate a substantial agreement (0.61 to 0.80) are set in boldface type. Poor agreement exists between tree species with phylogenetic cross-reactivity. An exception is the similar skin prick test responses to red oak and river birch, species known to be highly cross-reactive.

defined as follows: slight, 0 to 0.20; fair, 0.21 to 0.40; moderate, 0.41 to 0.60; substantial, 0.61 to 0.80; and almost perfect, 0.81 to 1.00. Based on this scale, substantial agreement was found between river birch and white oak ($\kappa = 0.62$). Moderate agreement was found for the phylogenetically similar species white oak and red oak ($\kappa = 0.44$) and white poplar and cottonwood ($\kappa = 0.49$). Fair agreement was found for box elder and red maple ($\kappa = 0.36$). Unexpectedly substantial agreement existed between several tree species with no recognized cross-sensitivity, especially between mulberry and unrelated species (ie, sycamore, black walnut, willow, and box elder) and between sycamore and phylogenetically unrelated species, including black walnut, willow, and white oak.

Of the 93 patients with in vivo tree hypersensitivity, 64 consented to donate blood samples for serum specific IgE determinations by AlaSTAT. Serum samples from all patients were collected within 6 months after the onset of the 2002 tree pollen season. Positive values were defined as 0.70 IU/mL or higher, correlating to a class II or greater response. The AlaSTAT results were positive to at least 1 tree pollen allergen in 41 (64%) of the 64 patients. The percentage of 41 patients with a positive AlaSTAT response was greatest for olive (46%), followed by white ash (44%), birch (37%), walnut (37%), and maple (32%).

The κ statistics were calculated in 41 patients to evaluate agreement between the AlaSTAT results (positive or negative) as given in Table 2. Almost-perfect agreement was found between sycamore and mulberry ($\kappa = 0.93$), willow and mulberry ($\kappa = 0.85$), and cottonwood and mulberry ($\kappa = 0.85$). Perfect agreement was found between willow and cottonwood ($\kappa = 1.00$). Moderate agreement was found between birch and oak ($\kappa = 0.53$), and substantial agreement, as expected by known allergic cross-reactivity, was detected between olive pollen and white ash allergens ($\kappa = 0.66$).

DISCUSSION

Pollen allergen extracts are used for diagnostic evaluation and treatment of atopic patients with allergic respiratory disorders. Surprisingly, there are few published data that can be used to guide selection of clinically relevant pollen aeroallergens for specific geographic regions of North America. Traditionally, regional pollen counts have been relied on to determine major allergenic tree species. Historically, an exhaustive review of pollens and pollen allergy published by the botanist Oren Durham in 1946 became the standard for selection of clinically relevant aeroallergens in the northeastern and midwestern United States.[8] A recently published practice parameter for allergen immunotherapy recommended a list of the most clinically important pollen and indoor aeroallergens that could be considered for use in allergen vaccines in North America.[3] It is noteworthy, however, that selection of this list was based primarily on consensus opinion of experts rather than evidence derived from studies that evaluated relationships between pollen exposure and clinical sensitization.

The aim of this study was to evaluate relationships between regional tree pollen counts and specific patterns of sensitization to tree pollen extracts and determine how these data could be used to identify the most relevant tree pollen allergens for use in clinical practice. Patients were recruited with considerable lifetime exposure to indigenous trees (ie, resided for at least 50% of their lifespan and 50%

Table 2. The κ Statistics Measuring Agreement Between Positive AlaSTAT Results and Tree Allergens Among 41 of 64 Patients With at Least 1 Positive AlaSTAT Outcome*

	Maple	**Birch**	**Cedar**	**Oak**	**Elm**	**Olive**	**Walnut**	**Sycamore**	**Willow**	**Cotton-wood**	**White ash**	**White pine**	**White mulberry**	**Oak mix**
Maple	1	0.35	0.10	0.23	0.24	0.60	**0.68**	**0.70**	0.49	0.49	0.54	0.46	**0.63**	0.37
Birch		1	0.19	0.53	0.32	0.20	0.47	0.60	0.53	0.53	0.34	0.26	0.54	**0.73**
Cedar			1	0.27	0.45	0.09	0.19	0.34	0.27	0.27	0.13	0.17	0.20	0.12
Oak				1	**0.69**	0.28	0.41	0.48	**0.66**	**0.66**	0.42	0.48	0.54	**0.66**
Elm					1	0.12	0.32	0.50	**0.69**	**0.69**	0.24	0.50	0.56	0.41
Olive						1	0.40	0.34	0.28	0.28	**0.66**	0.24	0.39	0.45
Walnut							1	**0.72**	0.53	0.53	0.45	0.38	**0.66**	0.51
Sycamore								1	**0.78**	**0.78**	0.48	0.60	**0.93**	**0.63**
Willow									1	**1.00**	0.42	**0.63**	**0.85**	**0.66**
Cottonwood										1	0.42	**0.63**	**0.85**	**0.66**
White ash											1	0.38	0.42	0.38
White pine												1	**0.66**	0.38
White mulberry													1	**0.68**
Oak mix														1

* Agreement is considered to be slight if κ equals 0 to 0.20, fair if 0.21 to 0.40, moderate if 0.41 to 0.60, substantial if 0.61 to 0.80, and almost perfect if 0.81 to 1.00. The κ values that indicate a substantial agreement or better ($\kappa \geq 0.61$) are set in boldface type.

of their childhood in the local area). Skin prick testing data were used as the primary end point for defining prevalences of sensitization to specific tree pollen allergens. Serum specific IgE data, obtained in a limited number of patients, was considered less robust for correlating with pollen exposure. Estimation of cumulative ambient exposure was based on pollen counts collected at a single, centrally located urban site from 1997 to 2001. These counts were considered reflective of exposure in the entire region in that 2002 data were highly correlated with mean tree pollen counts at the same site during the preceding 5 years and correlated well with the 2002 pollen counts collected at a distant site. In contrast to these results, significant variability among counting stations have been described in Philadelphia, New Jersey, Central California, and Washington, DC.[9–11] Although explanations for discrepant findings with these studies are unclear, we have confirmed in a different study that tree pollen counts are highly correlated when collected at 3 different sites in the Cincinnati region when samples were collected with a continuous aerosol "button" sampling device.[12] Thus, our aerobiologic data collected at a single site could be extrapolated as a reasonable index of regional exposure to specific tree pollens.

We postulated that wind-borne pollen counts could be associated with frequencies of in vivo sensitization to seasonal tree aeroallergens among patients who reside in a given geographic region for most of their lives. However, specific pollen counts failed to correlate with prevalences of skin test reactivity to those same allergens in an atopic population with symptoms of SAR that resided in Greater Cincinnati. This was also illustrated by our results (Fig 3), which demonstrate that pollinating tree species with negligible mean pollen counts during the preceding 5 years (ie, American sycamore red maple, box elder, eastern cottonwood, white ash, and black walnut) were major in vivo allergens. On the other hand, eastern red cedar (*J virginiana*), which represented 20% of total tree pollen counts, was a minor in vivo allergen.

There are several possible explanations for this apparent disconnection between allergen exposure and sensitization. Intrinsic differences between allergenicity of different pollens may be more significant determinants of sensitization potential rather than actual quantitative exposure to ambient particles. An example of this phenomenon is the relatively low in vivo allergenicity of *Pinus* species despite high dispersion in regions throughout the world.[13,14] Although Davies[15] reported that 50 grains/m^3 of grass pollen is needed to elicit symptoms of SAR, Florido et al[16] estimated an exposure threshold of 400 grains/m^3 of olive pollen (*Olea*) to obtain mild symptoms. Although as yet unproven, specific immune response genes could be associated with increased expression of specific IgE responses for specific tree pollen allergens, as has been demonstrated for ragweed Amb a V.[17–20]

Cross-reactive allergens shared among tree families could account for poor correlations observed between specific tree pollen counts and patterns of percutaneous reactivity. For example, the high frequency of birch pollen reactivity (>40%), despite low regional birch pollen counts, could be explained by known cross-reactivity between purified allergens of Betulaceae and Fagaceae (oak species).[1,5,21,22] The high frequencies of cutaneous reactivity to low pollinating species (eg, white ash, American sycamore, and cottonwood) could be due to unknown and heretofore undefined cross-reactive allergens. Although cross-reactivity between tree allergen of different species was not investigated or proven in our study, we found substantial concordance between those patients with positive skin test results to white oak and those with positive results to river birch. In addition, unexpected concordance was detected between red mulberry and several phylogenetically unrelated trees, including sycamore, black walnut, black willow, and box elder (Table 1). Similarly, strong agreement was found between in vitro serum specific IgE responses to unrelated tree pollens, especially between mulberry and sycamore (Table 2). Thus, it is intriguing to speculate from these observations that pan-allergens common to unrelated tree species could explain why species with negligible pollen counts elicit such high frequencies of percutaneous reactivity. In fact, recent studies have identified a 10.8-kDa pan-allergen derived from olive tree pollen (Ole e 10), which exhibits extensive cross-reactivity between unrelated Betulaceae and Cupressaceae tree families, as well as with allergenic weed pollens.[23] Other investigators have shown allergenic cross-reactivity between ash tree pollen (*Fraxinus*) and timothy grass (*Phleum pratense*) allergens.[24] Examination of concordance (Tables 1 and 2) between results of percutaneous tests and serum specific IgE for specific tree pollen species, although interesting, do not demonstrate allergenic cross-reactivity; this would require purification of allergens and characterization for most native North American tree species. Finally, high frequencies of percutaneous reactivity to low pollinating tree species could also be explained by greater exposure to tree pollens experienced by patients residing or traveling to other regions of the country.

In conclusion, these results showed that no association exists between regional pollen data and frequency of skin test reactivity to specific tree pollen allergens. These findings may be uniquely relevant to the Southwestern Ohio region and cannot be universally applied to other geographic areas. However, this experience would suggest that regional ambient pollen counts cannot be used exclusively to guide selection of specific tree pollen for skin testing and allergen immunotherapy. Such decisions should be based on patterns of in vivo hypersensitivity established in a representative group of indigenous patients with confirmed diagnoses of allergic rhinitis. Prioritization of antigens identified as major tree pollen allergens (percutaneous sensitization in >50% of patients) should be strongly considered for clinical use and even for those tree species that are historically associated with low seasonal ambient pollen counts. As has been the case for northern grasses, future studies may allow characterization of cross-reactive tree pollen allergens shared by North Americans species, which might reduce the number of antigens required for testing and immunotherapy.

ACKNOWLEDGMENTS

We thank Jan Shulman for assistance with the manuscript and Carol Abbot, RN, for recruitment of the patients.

REFERENCES

1. Weber RW. Cross-reactivity of plant and animal allergens. *Clin Rev Allergy Immunol*. 2001;21:153–202.
2. White JF, Bernstein DI. Key pollen allergens in North America. *Ann Allergy Asthma Immunol*. 2003;91:425–435; quiz 35–36, 92.
3. Joint Task Force on Practice Parameters. Allergen immunotherapy: a practice parameter. *Ann Allergy Asthma Immunol*. 2003;90:1–40.
4. Wensing M, Akkerdaas JH, van Leeuwen WA, et al. IgE to Bet v 1 and profilin: cross-reactivity patterns and clinical relevance. *J Allergy Clin Immunol*. 2002;110:435–442.
5. Eriksson NE, Wihl JA, Arrendal H, Strandhede SO. Tree pollen allergy, III: cross reactions based on results from skin prick tests and the RAST in hay fever patients: a multi-centre study. *Allergy*. 1987;42:205–214.
6. Dawson BTR. Clinical decision making. In: Dawson BTR, ed. *Basic and Clinical Statistics.* 3rd ed. New York, NY: McGraw-Hill; 2001:274–276.
7. King TP, Hoffman D, Lowenstein H, Marsh DG, Platts-Mills TA, Thomas W. Allergen nomenclature. *Allergy*. 1995;50: 765–774.
8. Durham OC. Pollens and pollen allergy. In: Feinberg SM, ed. *Allergy: In Practice*. 2nd ed. Chicago, IL: Year Book Publishers; 1946:111–215.
9. Kosisky SE, Carpenter GB. Predominant tree aeroallergens of the Washington, DC area: a six year survey (1989–1994). *Ann Allergy Asthma Immunol*. 1997;78:381–392.
10. McLean AC, Parker L, von Reis J. Airborne pollen and fungal spore sampling on the central California coast: the San Luis Obispo pollen project. *Ann Allergy*. 1991;67:441–447.
11. Dvorin DJ, Lee JJ, Belecanech GA, Goldstein MF, Dunsky EH. A comparative, volumetric survey of airborne pollen in Philadelphia, Pennsylvania (1991–1997) and Cherry Hill, New Jersey (1995–1997). *Ann Allergy Asthma Immunol*. 2001;87: 394–404.
12. Grinshpun SA. Assessment of exposure to diesel exhaust particles and biological aerosols in the metropolitan area with intense interstate highway traffic. *Am J Respir Crit Care Med*. 2003;167:A106.
13. Subiza J, Jerez M, Jimenez JA, et al. Allergenic pollen pollinosis in Madrid. *J Allergy Clin Immunol*. 1995;96:15–23.
14. Freeman GL. Pine pollen allergy in northern Arizona. *Ann Allergy*. 1993;70:491–494.
15. Davies HJ. Exposure of hay fever subjects to an indoor environmental grass pollen challenge system. *Clin Allergy*. 1985; 15:419–427.
16. Florido JF, Delgado PG, de San Pedro BS, et al. High levels of *Olea europaea* pollen and relation with clinical findings. *Int Arch Allergy Immunol*. 1999;119:133–137.
17. Huang SK, Zwollo P, Marsh DG. Class II major histocompatibility complex restriction of human T cell responses to short ragweed allergen, Amb a V. *Eur J Immunol*. 1991;21: 1469–1473.
18. Huang SK, Marsh DG. Human T-cell responses to ragweed allergens: Amb V homologues. *Immunology*. 1991;73: 363–365.
19. Marsh DG, Zwollo P, Huang SK. Molecular and cellular studies of human immune responsiveness to the short ragweed allergen, Amb a V. *Eur Respir J*. 1991;13(suppl):60s–67s.
20. Zwollo P, Ehrlich-Kautzky E, Scharf SJ, Ansari AA, Erlich HA, Marsh DG. Sequencing of HLA-D in responders and nonresponders to short ragweed allergen, Amb a V. *Immunogenetics*. 1991;33:141–151.
21. Weber RW, Nelson HS. Pollen allergens and their interrelationships. *Clin Rev Allergy*. 1985;3:291–318.
22. Bernstein IL, Perera M, Gallagher J, Michael JG, Johansson SG. In vitro cross-allergenicity of major aeroallergenic pollens by

the radioallergosorbent technique. *J Allergy Clin Immunol*. 1976;57:141–152.
23. Barral P, Batanero E, Palomares O, Quiralte J, Villalba M, Rodriguez R. A major allergen from pollen defines a novel family of plant proteins and shows intra- and interspecies cross-reactivity. *J Immunol*. 2004;172:3644–3651.
24. Niederberger V, Purohit A, Oster JP, Spitzauer S, Valenta R, Pauli G. The allergen profile of ash (*Fraxinus excelsior*) pollen: cross-reactivity with allergens from various plant species. *Clin Exp Allergy*. 2002;32:933–941.

Requests for reprints should be addressed to:
David I. Bernstein, MD
Division of Immunology/Allergy
University of Cincinnati College of Medicine
231 Albert Sabin Way
Cincinnati, OH 45267-0563
E-mail: bernstdd@ucmail.edu