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Grana

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/sgra20>

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Published online: 01 Sep 2009.

To cite this article: E. Fernandez-Caldas, M. C. Swanson, J. Pravda, P. Welsh, J. W. Yunginger & C. E. Reed (1989): Immunochemical Demonstration of Red Oak Pollen Aeroallergens outside the Oak Pollination Season, Grana, 28:3, 205-209

To link to this article: <http://dx.doi.org/10.1080/00173138909427432>

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Immunochemical demonstration of red oak pollen aeroallergens outside the oak pollination season

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Fernandez-Caldas, E., Swanson, M. C., Pravda, J., Welsh, P., Yunginger, J. W. and Reed, C. E. 1989. Immunochemical demonstration of red oak pollen aeroallergens outside the oak pollination season. — *Grana* 28: 205–209, 1989. Uppsala 31 August 1989. ISSN 0017-3134.

Airborne red oak (*Quercus rubra*) allergens were collected with volumetric air samplers during 1983–1985 and quantitated immunochemically using red oak pollen proteins adsorbed to plastic microtiter plates and pooled IgE-containing sera from seven oak-sensitive persons. Peak levels of allergen occurred in late April to early May and coincided with peak oak pollen counts from Rotoslide collections. However, additional peaks of oak allergenic activity were measured during July through early October, at times when oak pollen was not airborne. Most of the allergen was in particles smaller than 5 microns even during the pollination period. These peaks coincided with changes in wind from the usual northwest direction to a southerly direction. Among 19 other tree, grass, weed, mold, or insect allergens tested, only birch, and, to a lesser extent, willow, cross-reacted with red oak. We postulate that bits of decaying leaves, male catkins during the summer and early fall and other plant particles become airborne during changes in wind direction and velocity. Because other allergens, such as ragweed, *Alternaria*, grass and insects, are also abundant in late summer and fall, it will be very difficult to determine the degree to which this oak allergen contributes to late summer hay fever and asthma.

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(Manuscript received 8 April 1988, revised version accepted 2 February 1989)

The length and intensity of the pollination season of allergen-producing plants is generally considered to be very important for diagnosing allergic rhinitis or allergic asthma. Pollen grains and mold spores are identified and counted microscopically and the results are expressed as pollen grains or spores per cubic meter of air (Solomon and Matthews 1983). We have developed an immunochemical method of quantifying aeroallergens that detects amorphous allergenic activity, as well as allergenic activity associated with morphologically distinct particles and using these methods ragweed allergens were detected at times outside the ragweed pollen season (Agarwal et al. 1981, 1983). The allergenic activity

was not always associated with pollen grains, but also occurred in smaller particles (Agarwal et al. 1984). These small amorphous particles, which carry allergenic activity, are easily respirable. The present study describes the immunochemical measurement of the oak allergen activity in the air in Rochester, Minnesota in 1983, 1984 and 1985. The presence of oak allergen activity outside the oak pollen season, and the demonstration of oak allergen activity in particles much smaller than intact pollen, suggests an oak allergen contribution from non pollen sources. Wind direction was considered a significant factor in determining relative aeroallergen concentrations. Red oak (*Quercus rubra*) pol-

len was selected for detailed study because this pollen contains the allergens of all oak species in this region (Pravda et al. in press).

MATERIALS AND METHODS

Air sampling

A high-volume air sampler (GMWS 2310 Accu-Vol; General Metal Work, Inc., Cleves, OH) operated at a constant air flow of 70.8 m³/h, was used to collect airborne particles on a filter sheet (20×25 cm). Sampling periods varied from 24 to 72 hours which represents a range from 1 699 m³/h to 5097.6 m³/h. During these studies, two types of filtration media were used: fiberglass sheets (Grade GMS-810, General Metal Works) and polytetrafluoroethylene membranes (PTFE) (W. L. Gore & Associates, Elkton, MD). The exposed filters were not examined microscopically, but were packed in plastic bags right after the exposure time to avoid any contamination, and stored at -20°C till they were eluted and assayed for oak aeroallergens.

Particle size was measured with an Accu-Vol air sampler equipped with an Andersen cascade impaction head that was operated near the standard Accu-Vol sampler. The allergenic activity was determined in five different stages with defined particle size ranges of >4.1 µm, between 4.1 µm and 2.3 µm, between 2.2 µm and 1.4 µm, between 1.3 µm and 0.8 µm and <0.8 µm. These samples were collected on fiberglass filters, 1 meter above ground level at an unobstructed site on the southern Minnesota prairie. Oak pollen counts were performed by light microscopy from samples obtained during the early spring and summer by a Rotoslide sampler located within 1 meter of the Accu-Vol samplers.

Allergens

Twelve nondefatted oak pollens were purchased from Greer Laboratories, Inc., Lenoir, NC. One gram of each oak pollen species was defatted with anhydrous ether in a Soxhlet apparatus for 4 hours, and then extracted (1:10 w/v) in 200 mM ammonium bicarbonate overnight. After centrifugation the supernatants were dialyzed in 3500 MW cutoff castings (Spectrapor-3; Spectrum Medical Laboratories, Inc., Los Angeles, CA) against distilled water. After the second centrifugation, 1 cc aliquots of each extract were diluted with an equal volume of glycerine and stored at -20°C. The oak species included white oak (*Q. alba*), scrub oak (*Q. dumosa*), gambel oak (*Q. gambelli*), West white oak (*Q. garryana*), California black oak (*Q. kelloggii*), California live oak (*Q. lobata*), burr oak (*Q. macrocarpa*), water oak (*Q. nigra*), red oak (*Q. rubra*), post oak (*Q. stellata*), black oak (*Q. velutina*), and Virginia live oak (*Q. virginiana*). Protein content was measured on all these extracts by the method of Lowry et al. (1951).

Extracts of oak tree parts

Red oak bark, leaves, branches and birch leaves were collected in the late summer, washed thoroughly, ground with a mortar and pestle, then extracted in a fashion identical to the pollens. The allergenic reactivities of each

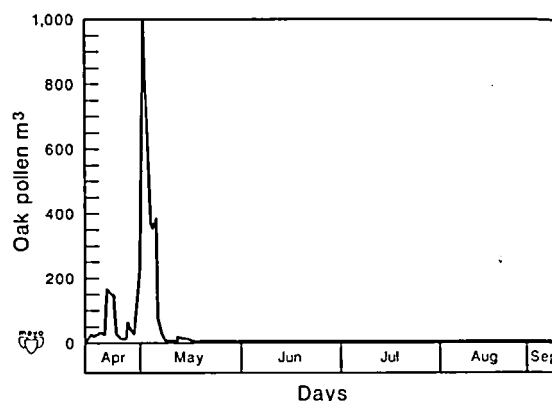


Fig. 1. Oak pollen counts for April–September 1985 using a Rotoslide pollen collector. Results expressed as pollen grains per cubic meter of air.

oak extract was evaluated by the radioallergosorbent test (RAST) inhibition technique.

Extraction of air filter samples

Allergens were eluted from the fiberglass sheets with 100 mM ammonium bicarbonate by descending chromatography in a large polyethylene tank (Agarwal et al. 1981). The 20×25 cm fiberglass filters were cut into four symmetrical triangles and one of the four was eluted. The elute was recovered in a collection reservoir, lyophilized and reconstituted in RAST diluent to a total volume of 1.5 cc for allergen assay. The filters from the Anderson sampler were processed similarly with minor modifications (Agarwal et al. 1984). PTFE membranes were cut in half and delaminated. The exposed membrane was then extracted in RAST diluent (0.1 M phosphate buffer, 0.2% bovine serum albumin, 0.1% Tween 20) for 10 hours by soaking and gentle mixing. Air filter extracts were then placed in Brinkmann tubes (Brinkmann Instruments Co., Westbury, NY) with a Pasteur transfer pipette, centrifuged and the pellet discarded. The PTFE membrane facilitated rapid sample processing, provided more efficient recovery of allergens from the filtration media, and eliminated the need for lyophilization due to the recovery of small volumes of already concentrated extracts.

Serum source

A human serum pool containing elevated specific IgE for oak pollen allergens was used. All the patients were from the Rochester, Minnesota area.

Solid phase

The oak pollen allergens were adsorbed to the surface of Immunolon-2 microtiter Removawell strips (Dynatech Laboratories Inc., Alexandria, VA). Maximal allergen adsorption to the surface of the microtiter plates was achieved by using 2.6 micrograms of protein in 100 microliters of 100 mM/l of sodium bicarbonate pH 9.2. The plates were incubated overnight at room temperature and washed three times with RAST wash (0.1 mol/l of phosphate buffer, 1% Tween 20).

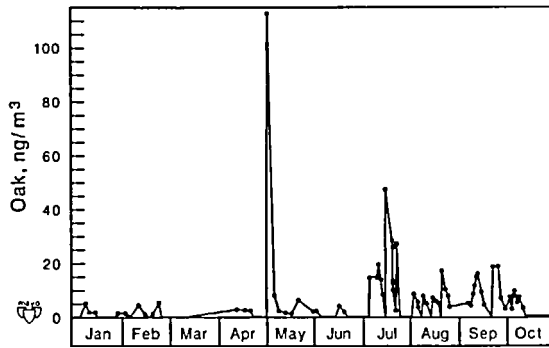


Fig. 2. Oak aeroallergenic activity for January–October 1985 using human sera containing IgE antibodies to oak allergens. Results expressed as oak protein per cubic meter of air.

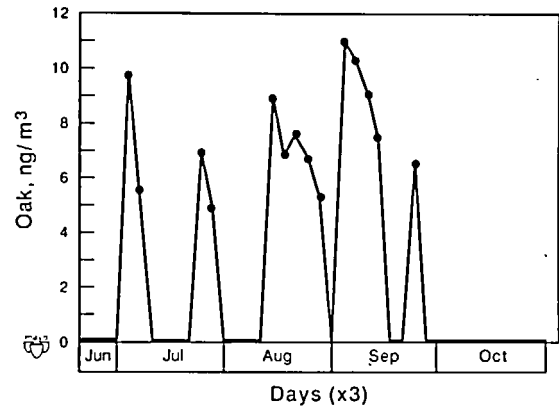


Fig. 4. Oak allergenic activity for June–October, 1983. Results expressed as oak protein per cubic meter of air.

Measurement of allergenic activity

The quantitation of the airborne red oak allergens was done immunochemically using plate-RIA inhibition assays. Fifty microliters of the red oak pollen reference standard at various concentrations (33 ng to 866 ng) were delivered into the wells of the allergen-coated microtiter plates along with 50 microliters of specific human antisera diluted 1:2. Fifty microliter aliquots of the air filter extracts were placed in other wells along with the specific antisera. The plates were incubated overnight in a humid chamber, washed three times, and 100 microliters (20 ng) of ^{125} I-antihuman IgE was added to each well. The plate was incubated again at room temperature overnight in a humid chamber, washed three times with buffer, and the Removawell strips were broken apart, placed into 16×125 mm glass test tubes and counted in a gamma scintillation counter. Plate-RIA inhibition results were analyzed by regression analysis using a Hewlett-Packard 9845B computer (Hewlett-Packard Co., Palo Alto, CA). Oak allergen

concentrations are expressed as nanograms of oak protein per cubic meter of air.

RESULTS

In April and May, 1985, the immunochemically measured oak allergen activity coincided with the observed oak pollen counts in late April and early May. Pollen counts reached 1000 pollen grains per cubic meter of air (Fig. 1). The maximum oak allergen activity measured was 112 ng/m³ (Fig. 1). The maximum oak allergen activity measured was 112 ng/m³ (Fig. 2). Additional peaks of oak allergenic activity were measured when there was no airborne oak pollen, during July through early October not only in 1985 but also in 1984 and 1983 (Figs. 3, 4).

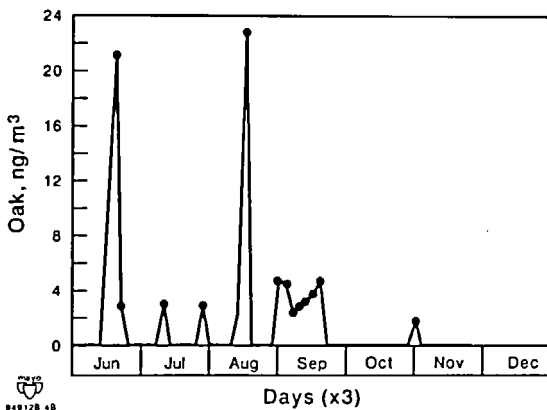


Fig. 3. Oak allergenic activity for June–November, 1984. Results expressed as oak protein per cubic meter of air.

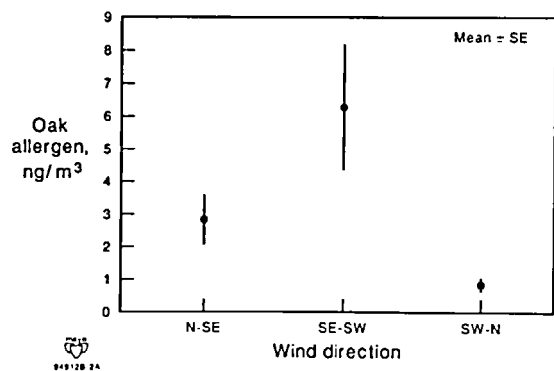


Fig. 5. Directional effects of wind on airborne oak allergen concentrations in 1985.

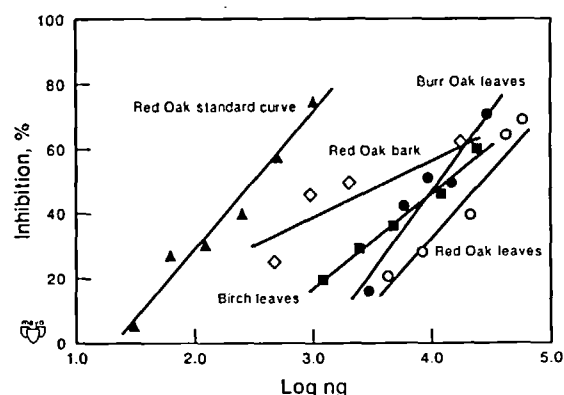


Fig. 6. Red oak RAST inhibition with red oak pollen: standard extract (▲), red oak bark (◇), red oak leaves (○), burr oak leaves (●), and birch leaves (■). The concentrations are expressed in total protein.

Wind direction seemed to play a role in the overall incidence of observed oak allergen activity especially in late summer of 1985 (Fig. 5). No cross-allergenicity could be demonstrated between red oak pollen and 19 other trees, grasses, weeds, molds or insect allergens except birch pollen and willow (Table I). Oak allergens were found in different parts of the oak tree.

Cross-reactivities between oak pollen and oak tree parts were found in some cases to produce parallel inhibition curves with the oak pollen standard (Fig. 6). Birch leaves contained cross-reacting allergen.

Oak allergens associated with particles of defined sizes were evident throughout all size ranges. As would be expected early in the season when the

Table I. Red oak allergen specificity by RAST inhibition, red oak allergens in the solid phase, and pooled sera from oak sensitive individuals

Other tree pollens (cottonwood, maple, poplar, black walnut, elm and ash) showed no significant inhibition at 2000 PNU

Inhibitor	Amount ^a (ng)	% Inhibition
Red Oak	33	9
	43	21
	65	26
	130	47
	260	64
	520	68
White Birch	4 000	57
Willow	3 000	45

^a Protein in nanograms by Lowry assay.

oaks pollinate more allergen activity was observed to be associated with larger particles (Table II).

DISCUSSION

The immunochemical quantitation of aeroallergens disclosed that oak allergens are present not only during the pollen season, but also, unexpectedly, in the summer and fall of 1983, 1984 and 1985.

The oak aeroallergens detected during the summer and fall are not likely to be cross-reacting allergens from weed or grass pollen, molds, mite or insect sources, because these allergens do not inhibit the binding of IgE from the serum pool to the oak solid phase we employed. Birch, and to a lesser extent willow, do cross-react, but these pollens are

Table II. Distribution of airborne allergen activity in particles of defined sizes-oak ng/m³

LLD = lower limit of detection

Date	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
5/5-5/8	3.0	3.2	3.3	1.4	LLD
5/8-5/11	2.0	1.7	1.3	2.3	LLD
5/26-5/30	1.9	3.0	2.7	2.1	LLD
5/30-6/3	2.8	2.1	2.3	2.2	LLD
6/30-7/3	LLD	0.7	0.2	0.42	LLD
7/7-7/10	LLD	0.52	LLD	1.1	0.36
7/10-7/13	LLD	0.5	LLD	0.41	0.43
7/13-7/15	0.45	0.5	LLD	0.36	LLD
7/15-7/18	1.4	0.7	LLD	0.38	LLD

not in the air in the summer and fall. In contrast we did find cross-reacting allergens in oak leaves, bark and wood.

These other oak plant parts, particularly decomposed leaves and male catkins (shed during the main growing season and found in substantial quantities in leeward shelters), are probable sources of the airborne oak allergen in the summer and fall. These particles would appear as amorphous plant fragments on Rotorod and Burkard samples and the allergenic activity would not be detected. The highest levels of airborne allergen concentration appeared on the days when the wind changed direction from the prevailing northwest and gusts from the south. This correlation is consistent with the idea of resuspension of particles that have settled out in the leeward of shelters from the prevailing wind.

Some authors have suggested that patients may suffer allergic symptoms not only from tree pollen but also from the leaves, leaf hairs, or sap, as well (Fonde 1935, Rudolph and Cohen 1932, Ostrov 1984).

Our findings are consistent with this suggestion. However, the simultaneous occurrence of ragweed, grass pollen, *Alternaria* spores and insect debris, and the coincidental development of sensitivity to several of these allergens in the same patient, will make it more difficult to assign a causal relationship to a particular allergen in the presence of exposure to the others.

ACKNOWLEDGEMENTS

We wish to thank Samuel C. Bukantz, M.D. for his critical review of the manuscript and Pat Walsh for her secretarial assistance. This paper was supported by grants from the National Institute of Allergy and Infectious Diseases, AI 21255, and by the Mayo Foundation.

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