

Evidence of cross-reactivity between olive, ash, privet, and Russian olive tree pollen allergens

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In a clinical investigation, 103 Michigan residents with symptoms suggestive of allergic rhinitis or asthma were skin tested with olive (*Olea europaea*) pollen extract. Nineteen had positive reactions. Since the olive tree is not native to nor grown in Michigan, this study was undertaken to determine whether the skin test reactivity was the result of cross-reactivity among tree pollen allergens.

ELISAs were developed to measure olive, ash (*Fraxinus americana*), privet (*Ligustrum vulgare*), and Russian olive (*Elaeagnus angustifolia*) specific IgE antibodies. Inhibition studies were performed to determine whether pollen extracts from each of these tree species could inhibit IgE antibody binding to olive extracts.

Eleven of the 19 skin test-positive patients were olive-ELISA positive, eight either were ELISA-positive to ash, seven to privet and ten to Russian olive. There were significant correlations between the ELISA results to olive and each of the other three pollens. The inhibition studies demonstrated that all three of the tree pollens were capable of inhibiting the binding of IgE to olive extract in a dose-response fashion. IgE-immunoblot studies demonstrated several proteins common to olive, ash, and privet.

Twelve of the olive skin test-positive patients were contacted and 75% were exposed to one or more of the studied trees in their yards. Five patients had traveled to areas where olive trees are grown.

We conclude that there is a high degree of cross-reactivity among allergens from native Michigan trees and from olive trees. This cross-reactivity is the most likely reason for skin test reactivity to olive pollen extract in Michigan.

INTRODUCTION

European olive tree (*Olea europaea*) pollen is an important cause of allergic disease in Mediterranean countries.¹ In North America, this genus is found in the Southwest and typically flowers from April through June. The olive tree is not native to nor grown in Michigan.² It was therefore, somewhat unexpected that 18% of Michigan residents (19 of 103) with a history of allergic rhinitis or asthma were skin test positive to olive pollen extract in a clinical investigation comparing an in vitro test for allergen-specific IgE to skin test results. Previously investigators have found a high degree of cross-reactivity between four species of *Oleaceae* pollens—olive (*Olea europaea*), ash (*Fraxinus excelsior*), privet (*Ligustrum vulgare*), and a

bush (*Phillyrea angustifolia*)—commonly found in the Mediterranean area.^{3,4}

Ash and privet are also commonly found in Michigan.⁵ We suspected that the observed skin test reactivity to olive extract was the result of cross-reactivity among tree pollen allergens. Several types of studies were performed to investigate this possible cross-reactivity. Correlations between the ELISA results with specific ELISAs for olive, ash, privet, and Russian olive (*Elaeagnus angustifolia*) IgE antibodies were examined, inhibition experiments were performed, and immunoblots were compared. All of these studies suggest allergen cross-reactivity among the pollens examined.

PATIENTS

One hundred three Michigan residents were enrolled in a study comparing an in vitro test for allergen-specific IgE to skin tests results.⁶ All subjects had symptoms suggestive of allergic rhinitis or asthma and many

were known to have positive skin tests to common aeroallergens. A subject was considered to have a positive prick skin test to olive tree extract when erythema was greater than 20 mm and the subject had a positive histamine skin test. All subjects gave informed consent and the protocol was approved by the institutional Human Rights Committee. After skin testing, the subjects were asked to complete a questionnaire. This included questions regarding race, occupation, location and type of dwelling, whether a yard was present and the nature of its botanical constituents, allergic history, and travel history.

METHODS AND MATERIALS

Commercially available olive (*Olea europaea*), ash (*Fraxinus americana*), privet (*Ligustrum vulgare*), and Russian olive (*Elaeagnus angustifolia*) pollen extracts were used for ELISA (Hollister-Stier, Spokane, WA; 1:20 wt/vol, 50% glycerine). Olive (*Olea europaea*) pollen extract from Greer Laboratories, Len-

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oir, NC (1:20 wt/vol, 50% glycerine) was also used for both skin tests and ELISA.

Antigen-specific IgE for olive, ash privet, and Russian olive was determined using a biotin-avidin amplified enzyme-linked immunoabsorbent assay as described previously.⁷ Each extract was diluted to 1:100 in a 60 mmol/L carbonate/bicarbonate, pH 9.4 buffer and adsorbed to plastic microwells overnight at 4 °C. The coated wells were then blocked with 0.1% Tween/1.0% FCS in PBS. Sequentially, with interspersed washes, a study serum, biotin anti-human IgE, avidin labeled with alkaline phosphatase, diethanolamine buffer, and dinitrophenol phosphate were added. The resulting color change was read to maximal absorbance, approximately 60 minutes later, at wavelength 405 nm. Values were reported as the ratio of the mean of duplicate determinations for a study serum compared with the mean of triplicate determinations for a pool of negative control sera. ELISA values were expressed as ratios between the mean absorbance produced by a study serum and mean absorbance of a pool of negative control serum. Ratios greater than two were considered positive as previously reported.

For inhibition experiments aliquots of a 1:20 dilution of a pool of patient positive sera with detectable IgE antibodies to the four tree pollens were incubated with experimentally determined dilutions of individual pollen extracts or PBS control overnight at 4 °C. The serum-pollen extract mixtures were then assayed for specific IgE as above.

Multiple proteins in the olive, privet, Russian olive, and white ash extracts were separated by isoelectric focusing in 4.9% polyacrylamide gel in the presence of Biolyte (Bio-Rad, Richmond, CA), pH range 4 to 6 as described previously.⁸ Electrofocusing was performed at 4 °C with 4 W constant power (1000 V constant with 18 mamp initial current) for two hours.

After electrofocusing, the separated proteins were electrophoretically transferred to nitrocellulose membranes in a TransBlot cell (Bio-Rad).^{9,10} The electrophoretic conditions were 0.7% acetic acid buffer, pH 2.5, at 35 V for 16 hours. Under these composition conditions, we have found that most allergens transfer well.

After protein transfer to the nitrocellulose membranes, the membranes were blocked with 5% BSA in PBS and incubated with a 1:20 dilution of the pool of positive patient sera with detectable IgE antibodies to the four tree pollens. After washing, the IgE specifically binding to the separated proteins was visualized using a 1:1,000 dilution of rabbit anti-human IgE antibody followed by a 1:100 dilution of goat anti-rabbit IgG labeled with horseradish peroxidase (Bio-Rad) and then HRP color development reagent (Bio-Rad).

After enzymatic staining the membranes were scanned for density using an Ultrascan II Laser Densitometer (LKB Instruments, Inc., Houston, TX) interfaced with a micro computer. The resulting

scans were examined for peaks occurring at similar pI values suggesting shared allergens.

Allergen-specific IgE data were converted to natural logarithmic equivalents before calculation of correlation of coefficients by linear regression. The significance of correlation coefficients from zero was calculated by the *t* test; the significance of values in contingency tables was calculated by the chi-square statistic.

RESULTS

Of the 19 olive skin test-positive patients, 11 were ELISA positive to olive, eight were positive to ash, seven were positive to privet, and ten were positive to Russian olive. Figure 1 illustrates the correlations between IgE binding to olive extract and to ash, privet, and Russian olive extracts ($r = 0.93, 0.84, 0.60$, respectively). The correlations are all statistically significant ($P < .01$ for all correlations).

The olive, ash, privet, and Russian olive extracts readily inhibited the binding of the olive positive sera to olive-coated strips in a dose-dependent manner (Fig 2).

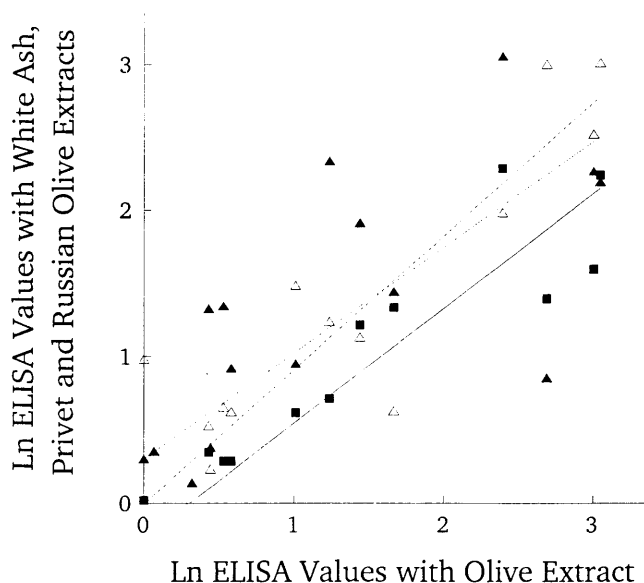


Figure 1. ELISA values with white ash, privet, and Russian olive extracts compared with ELISA values with olive extracts. (■ = privet extract, $r = .84$, solid line; △ = white ash extract, $r = .93$, long dashed line; ▲ = Russian olive extract, $r = .60$, dotted line).

Figures 3 and 4 illustrate the results observed with immunoblots using ash, olive, and privet extracts. There were five matching density peaks between ash and olive, three between privet and olive, and five between ash and privet.

Twelve of 19 skin test positive subjects answered the questionnaire. Ten of 12 lived in a house with a yard. Nine were able to list the cross-reactive trees as being in their yards. Two experienced rhinitis after either trimming hedges or mowing the lawn. All were Caucasian, born in the USA and none was of Mediterranean descent. Three had traveled during the spring to the Mediterranean and two to southwestern USA (Arizona and Nevada).

DISCUSSION

We were initially surprised to find skin test reactivity to olive pollen extracts frequently among Michigan residents since olive trees do not grow in Michigan. Other trees of the *Oleaceae* family, ash and privet, are however, commonly found in this area. Despite having a similar common name, the Russian olive belongs to a different family (*Elaeagnaceae* versus *Oleaceae*) and order (*Elaeagnales* versus *Oleales*) than the olive tree. They both belong to the *Rosidae* subclass. We evaluated

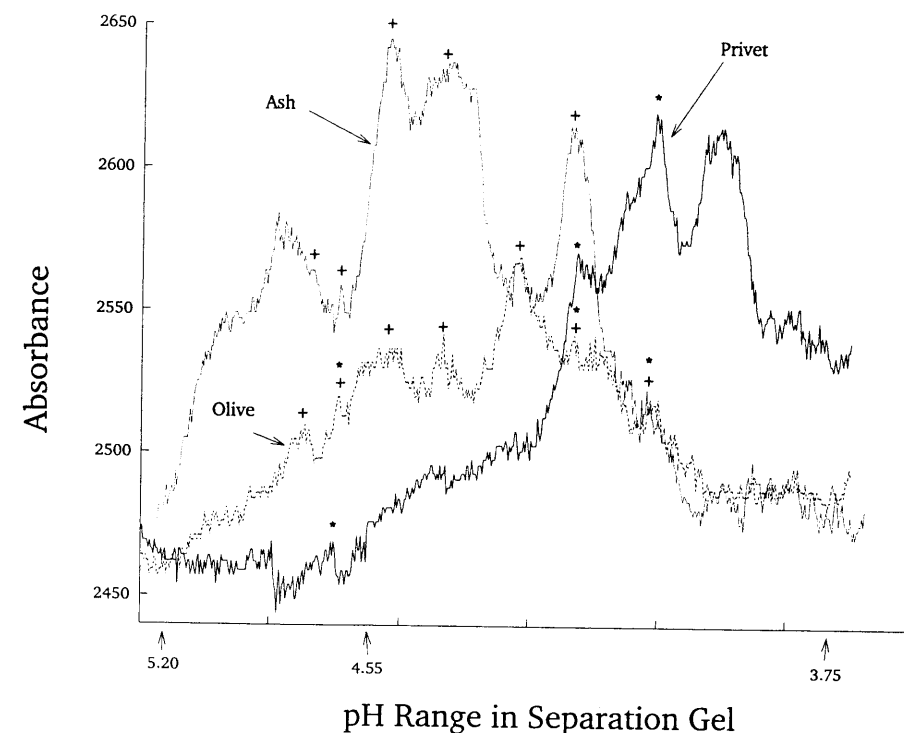


Figure 3. Absorbance versus pH range in nitrocellulose immunoblots for white ash, privet and olive proteins (+ = corresponding ash and olive peaks; * = corresponding privet and olive peaks).

Russian olive extract because several patients mentioned the presence of this commonly planted tree in their yards when told of their olive sensitivity. Our results suggest a high degree of cross-reactivity among olive, ash, privet, and Russian olive extracts. This agrees with

the report of cross-reactivity between olive, ash, privet and *Phillyrea angustifolia* by Bousquet et al.³ The major difference between the

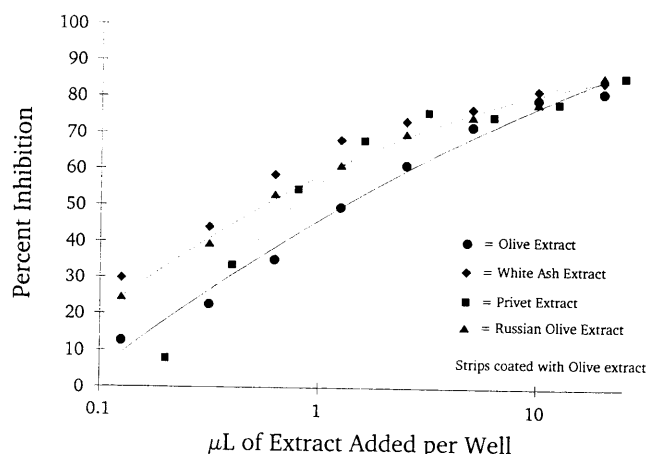


Figure 2. Percent ELISA inhibition of a pool of olive positive sera to olive-coated strips observed with olive (solid line), white ash (dotted line), privet (dashed line), and Russian olive (dot-dashed line) extracts.



Figure 4. Immunoblots using privet (lane 1), olive (lane 2) and ash (lane 3) extracts.

reports is that the patients studied by Bousquet were probably sensitized primarily to olive pollen while our patients were most likely sensitized by ash or privet pollen. Differences in the cross-reactivity patterns detected could be due to which pollen primarily sensitized each group of patients. For example, a patient primarily sensitized to olive tree pollen may not cross-react with Russian olive. We could not find a report of cross-reactivity between allergens from *Oleaceae* and *Elaeagnaceae*. The frequency of exposure to these trees in the patient's yards suggests that local exposure may be related to the risk of sensitization.

Our data do not provide direct information concerning the clinical significance of the observed cross-reactivity, but the presence of a high degree of cross-reactivity does suggest that some patients may have symptoms during tree pollen seasons in widely different geographic areas. The frequency of reactivity to privet may also explain why some individuals experience increased symptoms while around privet hedges, especially when trimming

such a hedge.

In summary, our results confirm Bousquet's observation of broad cross-reactivity between *Oleaceae* pollens and extend the cross-reactivity to at least one member of the *Elaeagnaceae*.

REFERENCES

1. D'Amato G, Lobefalo G. Allergenic pollens in the southern Mediterranean area. *J Allergy Clin Immunol* 1989;83:116-22.
2. Solomon WR, Mathews KP. Aerobiology and inhalant allergens. In: *Allergy principles and practice*. 3rd edition. Middleton, E Jr, Reed CE, Ellis EF, Adkinson NF, Jr, Yunginger JW, eds. St Louis MO: CV Mosby Co, 1988:312-72.
3. Bousquet J, Guerin B, Hewitt B, et al. Allergy in the Mediterranean area III: cross reactivity among *Oleaceae* pollens. *Clin Allergy* 1985; 15:439-48.
4. Weber RW. Cross reactivity among pollens. *Ann Allergy* 1981;46: 208-15.
5. Lewis WH, Vinay P, Zenger VE. In: *Airborne and allergenic pollens of North America*. Baltimore MD: Johns Hopkins Univ Press, 1983; 73-9.
6. Frank PM, Margaret D, Adkinson NF, et al. Clinical evaluation of Abbott Matrix Aero [Abstract]. *J Allergy Clin Immunol* 1991;87:331.
7. Enberg RN, Leickly FE, McCullough J, et al. Watermelon and ragweed share allergens. *J Allergy Clin Immunol* 1987;79:867-75.
8. Winter A, Perelmutter H, Davies H. Preparative flatbed electrofocusing in a granulated gel with the LKB 2117 M-14 phos (product application note). Bromma, Sweden: LKB, 1975:198.
9. Towbin H, Stachelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA* 1979;76:4350-4.
10. Sutton R, Wrigley CW, Baldo BA. Detection of IgE and IgG binding proteins after electrophoretic transfer from polyacrylamide gels. *J Immunol Meth* 1985;52:183-94.

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ASSOCIATION OF ANTIBIOTIC ALLERGY WITH SYSTEMIC LUPUS ERYTHEMATOSUS

In order to investigate the frequency of antibiotic allergy in patients with systemic lupus erythematosus (SLE), interviewers administered questionnaires to 221 patients and to two sets of control subjects selected by the patients: 178 relatives of the same sex and 186 friends of the same sex and race and similar age. Allergy to antibiotics was common in the 214 patients with systemic lupus erythematosus who reported having received them in the past: 27% for penicillin or cephalosporin, 31% for sulfonamide, 7% tetracycline, and 13% erythromycin. Allergy to antibiotics was significantly more frequent among patients with SLE who had received them than among controls who had received them (odds ratio 2.3, 95% CI 1.5-3.6 for penicillin or cephalosporin; odds ratio 2.4, 95% CI 1.2-4.7 for sulfonamides; odds ratio 4.8, 95% CI 1.5-14.9 for erythromycin).

Worsening of SLE was confirmed by the patients' physicians or chart review to have been associated with treatment with sulfonamides in 21% of the reported allergic reactions to sulfonamide.

These data confirm the previously suspected association of antibiotic allergy with SLE and provocation of exacerbations of SLE by sulfonamide.

—RMS

Petri M., Allbritton J. Antibiotic allergy in systemic lupus erythematosus: a case-control study. *J Rheumatol* 1992;19:265-9.