Cross-reactivity among edible nuts: double immunodiffusion, crossed immunoelectrophoresis, and human specific IgE serologic surveys

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Background: As many as one third of all food allergen anaphylactic events are related to tree nut ingestion. Although concurrent allergen sensitivity to tree nuts is common, cross-reactivity among nut antigens is less well defined.

Objective: To survey serologic cross-reactivities among 7 tree nuts (walnut, pecan, hazelnut, cashew, Brazil nut, pistachio, and almond) and peanut.

Methods: Human specific IgE enzyme-linked immunosorbent assay inhibition was used to identify nut allergen cross-reactivities. Single-nut rabbit antisera were used in double immunodiffusion, crossed-line immunoelectrophoresis, and crossed immunoelectrophoresis with intermediate gel studies of nut antigen cross-reactivity.

Results: Nut specific IgE enzyme-linked immunosorbent assay inhibition demonstrated no cross-reactivities between peanut and tree nuts. Among tree nuts, 2 groups with allergen cross-reactivity were defined: (1) walnut, pecan, and hazelnut and (2) hazelnut, cashew, Brazil nut, pistachio, and almond. Double immunodiffusion, crossed-line immunoelectrophoresis, and crossed immunoelectrophoresis with intermediate gel results supported the same groupings of cross-reactive tree nuts and identified several less prominent nut-nut antigen cross-reactivities between groups and with peanut.

Conclusion: With few exceptions (notably limited peanut cross-reactivity with pistachio and walnut), peanut antigens did not serologically cross-react with tree nuts. Walnut, pecan, and hazelnut form a group of strongly cross-reactive tree nuts. Hazelnut, cashew, Brazil nut, pistachio, and almond form a group of moderately cross-reactive tree nuts. Cross-reactivities between these groups are less pronounced (notably limited cross-reactivity of walnut and pecan with Brazil nut). The strongest cross-reactivities among tree nuts follow botanical family associations: (1) walnut and pecan in the family Juglandaceae and (2) cashew and pistachio in the family Anacardiaceae.

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INTRODUCTION

The prevalence of tree nut allergy in the US population is 0.5% to 1.0%.[1,2] Anaphylaxis to ingestion of tree nuts accounts for 10% to more than 30% of reported fatal and near-fatal food ingestions.[3] In a review[4] of 32 food anaphylaxis deaths in the United States between 1994 and 1999, 94% of the deaths were attributed to peanuts (63%) and tree nuts (31%).

Tree nut allergy is commonly reported to English walnut, pecan, hazelnut (filbert), cashew, Brazil nut, pistachio, and almond and less commonly to chestnut, black walnut, pine nut, macadamia nut, and coconut. Cross-reactivity among nuts may occur because of minor constituent panallergens (eg, profilins and lipid transfer proteins) or may involve major nut storage protein allergens (including albumins, legumins, and vicilins). Excellent reviews of tree nut allergy have been published recently.[3,5] Concurrent allergen sensitization to more than 1 tree nut is common in clinical reviews and nut ingestion challenge studies.[3,5,6] It is uncertain to what degree this concurrent nut allergen sensitization is a product of independent sensitization to more than 1 tree nut (co-sensitization), as opposed to cross-reactivity of proteins among different tree nuts. Different nut protein epitopes may have amino acid homology, but even a high degree of amino acid homology does not guarantee that 2 proteins will be cross-reactive.[3,5]

This qualitative survey of tree nut antigen cross-reactivity begins with human specific IgE enzyme-linked immunosorbent assay (ELISA) inhibition to identify allergen cross-reactivity among peanut and 7 tree nuts: English walnut, pecan, hazelnut, cashew, Brazil nut, pistachio, and almond. Human serologic investigation of nut cross-reactivities and an individual's primary nut sensitivities is complicated by the unpredictability of human exposure by time, quantity, and type of nut ingested. To evaluate nut cross-reactivity while controlling for primary nut sensitization, single-nut rabbit

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antisera techniques were used, including double immunodiffusion, crossed immunoelectrophoresis (CIE), crossed-line immunoelectrophoresis (CLIE), and CIE with intermediate gel (CIEWIG).

METHODS

Antigen Extracts

Twenty-five grams each of whole peanuts (Bolner's Fiesta Products, San Antonio, TX), almonds, pecans, walnuts, cashews, Brazil nuts, hazelnuts, and pistachios (Sunshine Nut Co, San Antonio) were ground before being added to 250 mL of 0.125M ammonium bicarbonate and stirred overnight at 4°C. Crude extracts were centrifuged, and middle aqueous layers were collected and filtered through 0.45-μm filters. Filtrates in 3,500-Da dialysis membranes (Spectrum Laboratories Inc, Rancho Dominguez, CA) were dialyzed against distilled water overnight at 4°C. Rye grass and short ragweed 1:10 aqueous extracts (Hollister-Stier, Spokane, WA) were dialyzed similarly. Extracts were lyophilized and stored at 4°C. Mountain cedar and *Bipolaris spicifera* extracts were prepared as previously described elsewhere.[7,8] Protein concentration was determined using the BCA Protein Assay (Pierce Chemical Co, Rockford, IL). The nut extracts ranged from 20% to 77% protein (pecan, 20%; walnut, 32%; peanut, 46%; almond, 60%; pistachio, 60%; hazel nut, 61%; cashew, 64%; and Brazil nut, 77%).

Human Serum Samples and IgE ELISA

Human serum samples were obtained with informed consent from 12 nut-allergic individuals and an atopic but nut-tolerant individual identified by retrospective clinical medical record review. Anonymous fetal cord serum was used as a negative control. Pooled human serum samples for IgE ELISA inhibition studies were prepared from 5 participants with the broadest or strongest representation of nut specific IgEs. An initial pool of 6 males and 6 females aged 16 to 49 years was identified retrospectively as being nut sensitive. Seven patients were clinically sensitive to peanut, 6 to walnut, 6 to pecan, 3 to hazelnut, 5 to cashew, 4 to Brazil nut, 3 to pistachio, and 7 to almond. When skin prick testing was accomplished for nut allergy, skin test responses most often were strongly confirmatory and concordant with history (data not shown).

Serum samples from the 12 nut-allergic individuals and the atopic but nut-tolerant individual and cord serum samples were assayed for IgE to the 8 nuts by specific IgE ELISA as described previously.[9] Cord serum and the atopic nut-tolerant control demonstrated no nut specific IgE. Two individuals showed moderate-to-high levels of specific IgE for each of the 8 nuts. One individual demonstrated significant IgE levels to all nuts except almond. These 3 individuals were included in the serum pool, along with 2 additional individuals among the remaining 9 participants demonstrating low-to-moderate specific IgE levels to at least 1 and as many as 4 nuts (Table 1).

Rabbit Antisera

Rabbit antisera to peanut, almond, pecan, walnut, cashew, Brazil nut, hazelnut, pistachio, and rye grass were raised in New Zealand white rabbits using 100 μg of extract protein in Hunter's TiterMax (CytRx Corp, Norcross, GA) and the TiterMax protocol. Antisera were pooled and used for Ouchterlony immunodiffusion, CIE, and CIEWIG assays. Rabbit antisera for mountain cedar pollen and *B spicifera* were prepared as described previously.[7,8] The care and use of animals adhered to the principles set forth in National Institutes of Health publication No. 86–23, *Guide for the Care*

Table 1. Individuals in the Serum Pool for Nut Specific IgE ELISA Inhibition Studies

	Patient No.				
	1	2	3	4	5
Age, y	33	28	35	27	35
Sex	F	M	F	M	M
Race	White	White	African American	African American	White
Atopic history	AS	AR, AS	Eczema	AR, AS	AR, AS
Sensitivity history	Pn, A, Br, W, Pc	Ps	Pn	Pn	Pn, C, W, Pc
Symptoms	H, AS, U	U, N, V	AE, U	AS, N, V	U, N, V
Peanut*	0.124	0.463	0.139	0.338	0.078
Almond*	0.499	0.715	0.015	0.000	0.117
Walnut*	0.679	0.277	0.204	0.000	0.007
Pecan*	0.656	0.385	0.193	0.067	0.010
Cashew*	0.667	0.055	0.375	0.000	0.030
Brazil nut*	0.588	0.079	0.225	0.016	0.012
Hazelnut*	0.810	0.458	0.320	0.044	0.029
Pistachio*	0.596	0.291	0.255	0.011	0.017

Abbreviations: A, almond; AE, angioedema (including laryngeal); AR, allergic rhinitis; AS, asthma; Br, Brazil nut; C, cashew; ELISA, enzyme-linked immunosorbent assay; H, hypotension; N, nausea; Pc, pecan; Pn, peanut; Ps, pistachio; U, urticaria; V, vomiting; W, walnut.
*Nut specific IgE ELISA optical density at 492 nm.

and Use of Laboratory Animals, and the Animal Welfare Act of 1966, as amended.

Specific IgE ELISA Inhibition and Double (Ouchterlony) Immunodiffusion

Specific IgE ELISA inhibition assays were accomplished as described previously[9] using the human pooled serum samples. Inhibiting extract proteins ranged from 0.1 to 100 μg protein/mL. Double immunodiffusion was accomplished as described previously.[7]

CIE, CLIE, and CIEWIG

CIE commenced by pipetting a 1% solution of agarose A in barbituric acid buffer onto an 8 $\times$ 8-cm leveled glass plate. Sample wells were punched and filled with 10 μL of nut antigen (1,000 μg of protein per milliliter of barbituric acid buffer). First-dimension immunoelectrophoresis (IEP) was accomplished on a cooled horizontal IEP unit (FBE-3000; Pharmacia, Piscataway, NJ), with voltage applied to maintain 10 V/cm across the gel for 30 minutes. Strips of the agarose gel were cut parallel to the first-dimension IEP. Two intermediate gels (1.1 mL of 1% agarose) were poured above the transferred strip. Above the intermediate gels, 1.8 mL of antiserum-agarose mixture (1:5–1:10) was poured. Second-dimension IEP was perpendicular to the first IEP, at 2 V/cm overnight. Gels were rinsed, pressed, and stained to identify protein precipitated rockets.

The CLIE was performed as for the CIE, except that the first intermediate gel contained 50 to 200 μg/mL of a second antigen (2 antigens and 1 antiserum). Cross-reactivities between the 2 antigens were identified by lines of identity between rockets of the primary (electrophoretically separated) antigen and the advancing antigen front of the second antigen in the intermediate gel.

The CIEWIG was performed as for the CIE, except that the second intermediate gel was a second antiserum-agarose mixture (1:5–1:20) (1 antigen and 2 antisera). Nut antigen cross-reactivities were inferred by precipitation of nut antigen in the secondary antiserum. Partial cross-reactivity resulted in a nut precipitin pattern "stretched" inferiorly into the intermediate gels or transposition of single precipitin lines inferiorly into the intermediate gels. A complete cross-reactivity transposed an otherwise unchanged nut precipitin pattern inferiorly into the intermediate gels.

RESULTS

IgE ELISA Inhibition

Representative of the IgE ELISA inhibition studies, the IgE ELISA inhibition of hazelnut is shown in Figure 1. Inhibition by 8 nuts and 4 control protein extracts is displayed for inhibiting protein per milliliter concentrations of 0.1 to 100 μg. The quality and quantity of cross-reactivity between 2 nuts depends on the shape (quality) and position (quantity) of the curves along the ordinate (inhibitor concentration). The more closely matched the slopes of 2 nut inhibition curves during the greatest rise in the percentage of inhibition (greatest slope), the more similar are the antigenic confirmations of

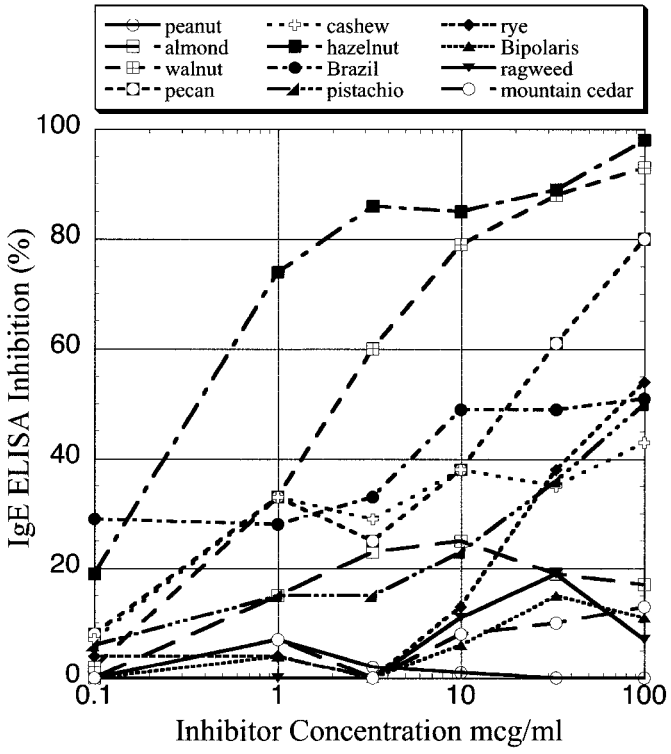

Figure 1. Hazelnut specific IgE enzyme-linked immunosorbent assay (ELISA) inhibited by 8 nuts and 4 control proteins.

the 2 nut allergens by usual interpretation. The relative strength of the cross-reactivity is reflected in the maximal degree of inhibition achieved and the relative position of each curve at 50% of maximal inhibition. A qualitative strength of inhibition was assigned to each curve, taking into account maximal inhibition, relative slopes, and the positions of the curves. More simply, the qualitative strength of inhibition closely reflected the degree of inhibition in the range of 3 to 30 μg/mL inhibitor concentrations divided into quartiles: strong inhibition being the greatest quartile, moderate inhibition reflecting 50% to 75% inhibition, and weak and no inhibition representing the lowest 2 quartiles of inhibition.

Table 2 displays the qualitative nut cross-reactivities by IgE ELISA inhibition of each nut by the other nuts and by 4 unrelated protein extracts: *Bipolaris* mold, rye grass, ragweed, and mountain cedar pollens. For example, in Figure 1, hazelnut is inhibited moderately by walnut and weakly by pecan, cashew, and Brazil nut. Peanut is inhibited by rye grass but not by tree nuts. Tree nuts are not inhibited by peanut or by the 4 control proteins, except for the weak rye grass inhibition of Brazil nut and pistachio.

In Table 2, walnut and pecan display mutual strong cross-reactivity with each other, which is not unexpected owing to their close botanical relationship. Walnut and pecan also have moderate mutual cross-reactivity with hazelnut and together form a first group of strongly cross-reactive tree nuts. A second group of mutually cross-reactive tree nuts by IgE ELISA inhibition includes hazelnut, cashew, Brazil nut, pistachio, and almond. More isolated but notable nut-nut cross-reactivity (bidirectional ELISA inhibition) between these 2 major groupings included pecan–Brazil nut, walnut-almond, and walnut-cashew. Walnut and hazelnut inhibit all tree nuts while being the least inhibited by the other 5 tree nuts. This suggests that the serum pool used in the ELISA inhibitions may have included greater specific IgE representation for walnut and hazelnut than for the other 5 tree nuts.

Double (Ouchterlony) Immunodiffusion

Representative of the double immunodiffusion results, Figure 2 shows pistachio rabbit antisera (central well) double immunodiffusion precipitins formed with 5 nut extracts: pistachio, hazelnut, cashew, pecan, and walnut. Three precipitin bands are seen for pistachio extract/pistachio antisera. Cashew has a single strong precipitin identity with pistachio. Hazelnut forms 2 partial identities with the weaker precipitins of pistachio. Pecan and walnut demonstrate no identity with pistachio. Table 3 summarizes the double immunodiffusion results for 7 tree nuts, peanut, and rye grass. Walnut, pecan, and hazelnut have identities that describe mutual cross-reactivity. Hazelnut, cashew, Brazil nut, pistachio, and almond also show mutually cross-reactive identities among themselves. These groupings are similar to the human specific IgE ELISA results (Table 2). Notable nut-nut cross-reactivity between nuts of these 2 major groupings included walnut with Brazil nut. Pistachio has a partial identity with peanut.

CLIE and CIEWIG

The 2-dimensional nature of the CLIE and CIEWIG precipitins provides an opportunity for finer definitions of the nut extract/nut antisera precipitin patterns and cross-reactivities among nut proteins at the individual protein level. Cross-reactivity between nuts in each CLIE was assigned a qualitative strength based on the identities observed between proteins. Representative of these types of identities, Figure 3A presents the 9-protein CLIE pattern for pecan with a blank intermediate gel, and Figure 3B presents hazelnut in the first intermediate gel showing strong identity with more than 1 pecan protein. In addition to the strong cross-reactivity of pecan with hazelnut seen in Figure 3, pecan CLIE demonstrated strong (multiple identities) with walnut and a moderate (single identity) pattern with Brazil nut (data not shown). Pecan also demonstrated a weak (1 partial identity) pattern of cross-reactivity with pistachio and almond. Cashew, peanut,

Table 2. Qualitative Summary of Nut Cross-reactivities by Specific IgE ELISA Inhibition*

Inhibitor	Nut inhibited							
	Walnut	Pecan	Hazelnut	Cashew	Brazil nut	Pistachio	Almond	Peanut
Walnut		S	M	M	M	M	M	N
Pecan	S		W	W	W	W	W	N
Hazelnut	M	W		S	S	M	S	N
Cashew	W	N	W		M	M	M	N
Brazil nut	N	W	W	S		M	M	N
Pistachio	N	N	N	M	M		M	N
Almond	W	N	N	M	W	M		N
Peanut	N	N	N	N	N	N	N	
Rye grass	N	N	N	N	W	W	N	M
Ragweed	N	N	N	N	N	N	N	N
Bipolaris	N	N	N	N	N	N	N	N
Mountain cedar	N	N	N	N	N	N	N	N

Abbreviation: ELISA, enzyme-linked immunosorbent assay.

*Qualitative strength of inhibition and cross-reactivity, corresponding to percentage of inhibition in the range of 3 to 30 μg/mL inhibitor concentration: S (strong) indicates the greatest quartile; M (moderate), 50% to 75% inhibition; W (weak), 25% to 50% inhibition; and N (none), the lowest quartile.

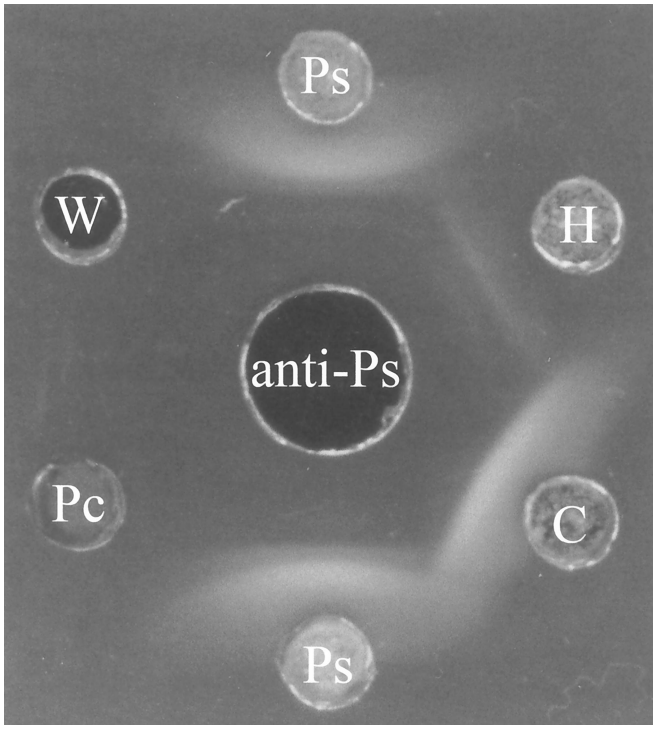

Figure 2. Double immunodiffusion with pistachio antisera (anti-Ps) in the central well. Nut extracts are positioned at 12 and 6 o'clock, pistachio (Ps); 2 o'clock, hazelnut (H); 4 o'clock, cashew (C); 8 o'clock, pecan (Pc); and 10 o'clock, walnut (W).

and the 4 controls demonstrated no cross-reactivity in the pecan CLIE studies. Table 4 summarizes the results of all the nut CLIE studies.

In CIEWIG, the qualitative strength of cross-reactivity reflected early precipitation of a nut protein in the intermediate gel containing the second nut antisera. In Figure 4, hazelnut CIEWIG shows 4 precipitin curves. Hazelnut CIEWIG with the intermediate gel containing pecan antisera demonstrates early precipitation of the wings of the most prominent hazelnut protein and complete displacement of a prominent wide precipitin into the intermediate gel (moderate cross-reactivity). Figure 5 shows another moderate pattern of cross-reactivity in the peanut CIEWIG. With blank intermediate gel, peanut CIEWIG shows 5 precipitin arcs. With pistachio antisera intermediate gel, moderate cross-reactivity is demonstrated by a single peanut protein precipitated early, between the intermediate gels. Table 4 summarizes the results of all the nut CIEWIG studies. Strong cross-reactivity (more than 1 protein precipitated early in the intermediate gels) was seen only for cashew-pistachio (data not shown). Weak cross-reactivity was defined as 1 or more protein precipitin wings moved into the intermediate gel.

The CLIE and CIEWIG results for all combinations of nuts and nut antisera are displayed together in Table 4 and can be compared with the double immunodiffusion results in Table 3 and the IgE ELISA inhibition results in Table 2. The pattern of 2 groups of nut cross-reactivities seen in Tables 2 and 3 is continued in Table 4, with less clearly delineated boundaries for the second group of nuts. Cashew-pistachio cross-reactivity is of special note for its strength. Notable lesser cross-reactivities between and outside these groups are seen with CLIE or CIEWIG for walnut and pecan with the nuts in the second group (most prominently with Brazil nut), for walnut with peanut, and for almond with rye grass.

DISCUSSION

Oral food challenges, skin testing, and human serologic studies have been used in the investigation of food antigen cross-reactivities. Because each involves varying aspects of the human immunologic response to food antigens, they have provided complementary but sometimes differing results. Food challenges are the most clinically relevant, usually producing the fewest "true" cross-reactivities. Human serologic studies often define a greater number of potential food cross-reactivities, many of which seem to be unexpressed clinically except in more unusual individual clinical cases. Beyond human studies, zoonotic serologic studies and molecular studies of food allergens have extended the comparisons of allergens. Homology among food allergens is frequently identified at the molecular level, but these results are often most distantly related to clinical cross-reactivity in human patients. Teuber et al[3] and Roux et al[5] provided excellent extensive reviews of current nut cross-reactivity studies.

The present study broadly surveys the nut antigen cross-reactivities of 7 tree nuts and peanut using different but complementary techniques, including human nut specific IgE ELISA inhibition and rabbit nut specific antisera methods (double immunodiffusion, CLIE, and CIEWIG). Comparing and contrasting results, we draw 3 general cross-reactivity conclusions supported by all the serologic methods (Tables 2, 3, and 4). In addition, each method also highlighted a few individual nut-nut cross-reactivities for which the experimental support is more limited.

The first general conclusion is support of the generally held belief[5] that, with few exceptions, peanut antigens do not serologically cross-react with true nuts. The strongest evidence for isolated exceptions include the pistachio antisera early precipitation of a single peanut protein in the CIEWIG (Fig 5 and Table 4) and a single peanut protein identity with walnut in the walnut CLIE (Table 4). The pistachio-peanut cross-reactivity is also supported by the double immunodiffusion result (Table 3). At least 1 weak CIE interaction was also seen for the other tree nuts with peanut, but these were not supported by double immunodiffusion or human specific IgE ELISA. Human specific IgE ELISA demonstrated no cross-reactivity among the 7 nuts with peanut (Table 2).

The second general conclusion is that walnut, pecan, and hazelnut form a group of strongly cross-reactive tree nuts. This conclusion is supported by human specific IgE and

Table 3. Qualitative Summary of Nut Cross-reactivities by Double Immunodiffusion*

Antigen	Nut antisera								
	Walnut	Pecan	Hazelnut	Cashew	Brazil nut	Pistachio	Almond	Peanut	Rye grass
Walnut		M	N	N	M	N	N	N	N
Pecan	M		N	N	M	N	N	N	N
Hazelnut	M	M		W	W	M	M	N	N
Cashew	W	N	M		W	S	M	N	N
Brazil nut	W	N	M	W		W	W	N	N
Pistachio	W	W	M	W	W		W	N	N
Almond	N	M	M	W	S	W		N	N
Peanut	N	N	N	N	N	W	N		N
Rye grass	N	N	N	N	N	N	N	N	

*Qualitative strength of identities: S (strong) indicates more than 1 identity; M (moderate), 1 identity or more than 1 partial identity; W (weak), 1 partial identity; and N (none), no identity.

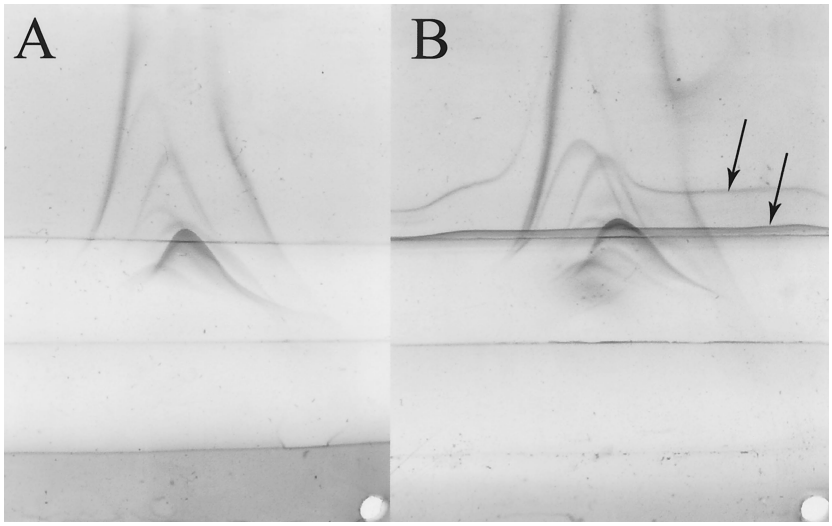

Figure 3. Pecan crossed-line immunoelectrophoresis with blank intermediate gel (A) and intermediate gel containing hazelnut (B). Arrows indicate lines of identity.

rabbit antisera serologic studies. Walnut, pecan, and hazelnut are in the same botanical subclass, Hamamelididae. Walnut and pecan show the greatest cross-reactivity, which is not surprising because they are in the same botanical family, Juglandaceae, and are the most closely related among the nuts. Hazelnut, also known as filbert, is in a separate family, Betulaceae (birch family), within the same subclass, Hamamelididae, and is less strongly cross-reactive with walnut and pecan. Using ELISA and immunoblot inhibition techniques, Asero et al[10] similarly demonstrated cross-reactivity between several walnut and hazelnut proteins.

The third general conclusion is that hazelnut, cashew, Brazil nut, pistachio, and almond form a group of moderately cross-reactive tree nuts. Using CIE studies, cashew and pistachio are more strongly cross-reactive together than either with the other 3 nuts (Table 4), which may reflect their membership together in the family Anacardiaceae. Using the other serologic methods, the cross-reactivities are more evenly distributed among the nut pairs (Tables 2 and 3). Brazil nut is in the family Lecythidaceae, and almond is in the family Rosaceae. Brazil nut, almond, cashew, and pistachio are in the same botanical subclass, Rosidae (as is peanut).

Cross-reactivity of walnut and pecan with the second group of tree nuts is limited and varies among the different methods (except for hazelnut, which is included in the first and second groups). Of these minor cross-reactivities, walnut and pecan with Brazil nut is most notable. Cross-reactivity of walnut with Brazil nut has previously been reported.[10]

Of the 4 control proteins (rye grass, ragweed, *Bipolaris*, and mountain cedar), only rye grass showed occasional weak cross-reactivity with tree nuts by specific IgE ELISA (Brazil nut and pistachio) (Table 2), CLIE (Brazil nut, pistachio, and almond), or CIEWIG (walnut, pecan, and almond) (Table 4). Limited cross-reactivity of peanut and grass was also demonstrated by ELISA (Table 2) and CLIE (Table 4) and has previously been reported.[11]

Table 4. Qualitative Summary of Nut Cross-reactivities by CLIE and CIEWIG*

	Walnut	Pecan	Hazelnut	Cashew	Brazil nut	Pistachio	Almond	Peanut	Rye grass
Walnut									
Pecan	SS/MM								
Hazelnut	MM/WM	MS/MM							
Cashew	NN/WM	WN/NM	MN/NW						
Brazil nut	WN/NM	NM/NM	NW/WW	NW/WW					
Pistachio	NN/NM	NW/NM	NN/NW	MM/MS	WW/WM				
Almond	NN/WW	NW/WW	NN/WN	NN/WN	WN/WN	NW/WN			
Peanut	WM/WW	NN/NW	NW/NN	NW/NN	NW/NN	NW/MN	WW/NW		
Rye grass	NN/NW	NN/NW	NN/NN	NN/NN	MN/NN	WN/NN	WW/NM	NW/NN	

Abbreviations: CIEWIG, crossed immunoelectrophoresis with intermediate gel; CLIE, crossed-line immunoelectrophoresis.
*Results are given in the following order: 2 CLIE cross-reactivities/2 CIEWIG cross-reactivities for each nut pair. Qualitative strength of cross-reactivity: S (strong) indicates more than 1 CLIE identity or CIEWIG full intermediate gel precipitin; M (moderate), 1 CLIE identity or more than 1 CLIE partial identity or CIEWIG partial intermediate gel precipitin; W (weak), 1 CLIE partial identity or CIEWIG only precipitin wings in the intermediate gels; and N (none), no cross-reactivity.

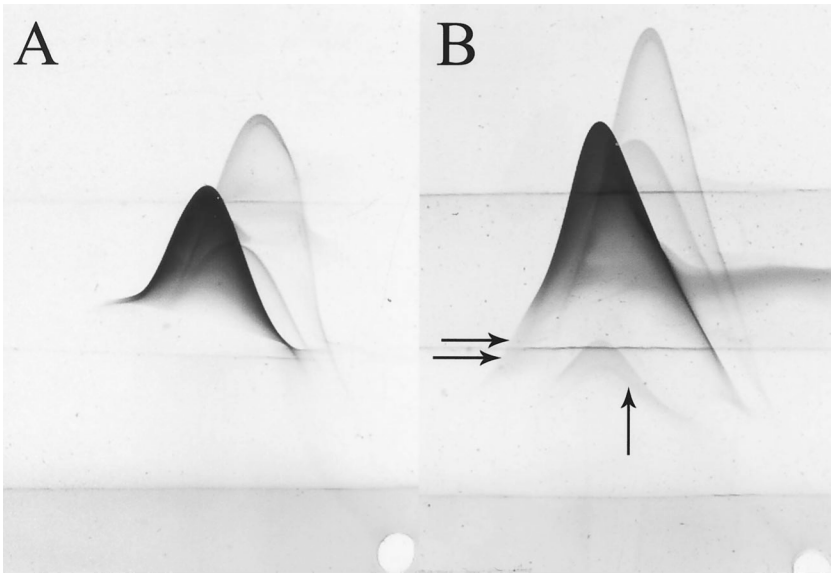

Figure 4. Hazelnut crossed immunoelectrophoresis with intermediate gel with blank intermediate gel (A) and intermediate gel containing pecan antisera (B). Arrow indicates hazelnut antigen precipitated early by pecan antisera; double arrow, early precipitation of the wings of the most prominent hazelnut protein by pecan antisera.

The small size of the human serum pool, on which the ELISA results depend, is a major limitation of this technique. The more limited antibody diversity of a small pool of human sera may account for the poorer reciprocal cross-reactivity seen among nuts with weaker cross-reactivities in Table 2 compared with Tables 3 and 4. The added diversity of parallel rabbit antisera studies provided complementary evidence of antigen cross-reactivities among the nuts. Some cross-reactivities (such as walnut and pecan) were strong and were observed by every serologic technique. Other nut cross-reactivities were observed only by certain experimental permutations (eg, positive peanut CIEWIG with anti-pistachio sera intermediate gel but negative pistachio CIEWIG with anti-peanut sera intermediate gel and negative human ELISA inhibition study results for peanut-pistachio). Despite the potential differences in human and rabbit serologic responses to nuts, it is interesting to note the similarities in results that allowed us to draw general conclusions from this assembly of different methods. In their review of almond allergens, Roux et al[5] note similar immunologic recognition across species.

In summary, this serologic survey of tree nut cross-reactivities confirmed the generally held belief that, with few exceptions (eg, pistachio-peanut and walnut-peanut single antigens), peanut is not cross-reactive with tree nuts. Tree nuts can be grouped into 2 general groups of cross-reactive nuts: (1) walnut, pecan, and hazelnut and (2) hazelnut, cashew, Brazil nut, pistachio, and almond. Cross-reactivities within these groups are generally stronger than between the 2

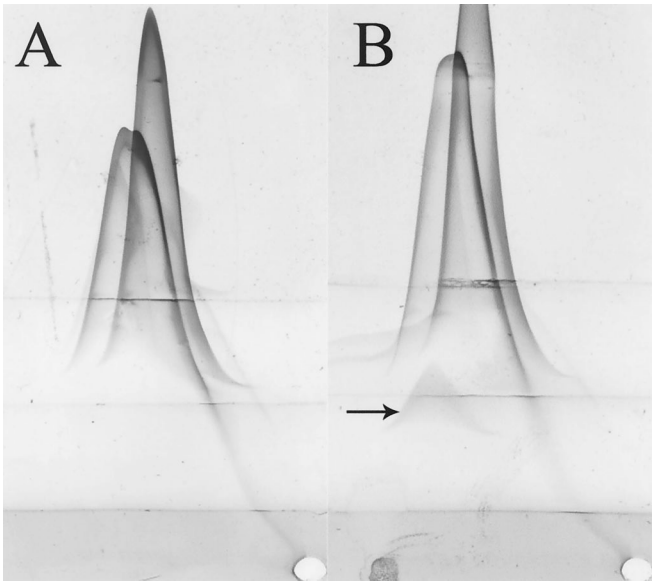

Figure 5. Peanut crossed immunoelectrophoresis with intermediate gel with blank intermediate gel (A) and intermediate gel containing pistachio antisera (B). Arrow indicates peanut antigen precipitated early by pistachio antisera.

groups (eg, lesser cross-reactivity of walnut and pecan with Brazil nut). The strongest cross-reactivities among tree nuts follow botanical family associations: (1) walnut and pecan in the family Juglandaceae and (2) cashew and pistachio in the family Anacardiaceae.

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