

Hazelnut allergy across Europe dissected molecularly: A EuroPrevall outpatient clinic survey

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Background: Hazelnut allergy is birch pollen-driven in Northern/Western Europe and lipid transfer protein-driven in Spain and Italy. Little is known about other regions and other allergens.

Objective: Establishing a molecular map of hazelnut allergy across Europe.

Methods: In 12 European cities, subjects reporting reactions to hazelnut (n = 731) were evaluated and sensitization to 24 foods,

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This work was funded by the European Union through EuroPrevall (grant no. FP6-FOOD-CT-2005-514000). M.R.D., C.M., R.v.R., M.F.-R., and S.V. also received support from the EU 7th framework programme under grant agreement no. 312147.

Disclosure of potential conflict of interest: M. R. Datema has received research support from the European Commission (EC). L. Zuidmeer-Jongejan has received research support and travel support from the European Union (EU). L. Barreales has received research support and travel support from Fundación para la Investigación Biomédica del Hospital Clínico San Carlos. F. de Blay has received research support from Stallergènes and Chiesi; has received consultancy fees from and is a board member for Stallergènes, ALK, Mundipharma, and Novartis; and has received participation fees from Stallergènes and ALK. M. Clausen has received lecture fees from AstraZeneca and has received travel support from GlaxoSmithKline. M. L. Kowalski has received research support from the EC (6Framwork Programme Grant). A. C. Knulst has received research support and travel support from the EC. T.-M. Le has received research support from the EU. N. G. Papadopoulos reports grants from GSK, Nestlé, and Merck and personal fees from Abbvie, Sanofi, Novartis, Menarini, Meda, ALK-Abelló, Allergopharma, Uriach, GSK, Stallergènes, and MSD. G. Reese is employed by Allergopharma GmbH Co. KG. S. L. Seneviratne is employed by the Royal Free Hospital. S. Vieths has consultant arrangements with the Food Allergy Resource

and Research Program; has provided expert testimony for the Medical University of Vienna; has received research support from the Monsanto Company; has received payment for lectures from the American Academy of Allergy, Asthma & Immunology, Deutsche Dermatologische Gesellschaft, the Spanish Society of Allergy and Clinical Immunology, Westdeutsche Arbeitsgemeinschaft für pädiatrische Pneumologie und Allergologie e.V., Gesellschaft für pädiatrische Allergologie und Umweltmedizin, and Ärztesverband Deutscher Allergologen; has received royalties from Schattauer Allergologie Handbuch and Elsevier Nahrungsmittelallergien und Intoleranzen; and has received travel support from the German Research Foundation, the Federal Institute for Risk Assessment, the Austrian Society for Allergy and Immunology, the French Society of Allergy, the European Directorate for the Quality of Medicines and Health Care, the European Academy of Allergy and Clinical Immunology, the World Allergy Organization, Deutscher Allergie und Asthmabund, Association Monégasque pour le Perfectionnement des Connaissances des Médecins, the German Chemical Society, the Austrian Society for Dermatology and Venerology, and AKM Allergiekongress. C. Mills has received research support from the EU and Biological and Biotechnological Sciences Research Council; is a board member for Reacta Biotech Ltd; is employed by the University of Manchester and the Institute of Food Research; has received research support from the Biological and Biotechnological Sciences Research Council, the EU, the UK Food Standards Agency, the European Food Safety Authority, the UK Technology Strategy Board, DBV Technologies, and Novartis; has stock/stock options in Reacta Biotech Ltd; has received travel support from the International Life Sciences Institute, the European Academy of Allergy and Clinical Immunology (EAACI), Europa Bio, the Iceland Allergy Society, Fresenius, EuroFood Tox 2013, and the International Union of Nutritional Sciences Annual Meeting (Granada, Spain, September 2013); and is a lecturer in Allergy at Imperial College. J. Lidholm is employed by Thermo Fisher Scientific. K. Hoffmann-Sommergruber has received research support from the EC (Project EUROPREVALL and the Marie Curie Project); is employed by the Medical University of Vienna; and has received lecture fees from ThermoFisher, Milupa, and Meda. M. Fernández-Rivas has received research support and travel support from the EC, has received consultancy fees from DBV, and has received lecture fees from ALK and GSK. B. Ballmer-Weber has received research support and travel support from the EU and has received consultancy fees and lecture fees from Thermo Fisher Scientific. R. van Ree has received research support from the EC, is a member of the EAACI Board, and has received consultancy fees from HAL Allergy. The rest of the authors declare that they have no relevant conflicts of interest. Received for publication August 4, 2014; revised November 25, 2014; accepted for publication December 4, 2014.

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0091-6749/\$36.00

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<http://dx.doi.org/10.1016/j.jaci.2014.12.1949>

12 respiratory allergen sources, and latex was tested by using skin prick test and ImmunoCAP. A subset (124 of 731) underwent a double-blind placebo-controlled food challenge to hazelnut. Sera of 423 of 731 subjects were analyzed for IgE against 7 hazelnut allergens and cross-reactive carbohydrate determinants by ImmunoCAP.

Results: Hazelnut allergy was confirmed in 70% of those undergoing double-blind placebo-controlled food challenges. Birch pollen-driven hazelnut sensitization (Cor a 1) dominated in most cities, except in Reykjavik, Sofia, Athens, and Madrid, where reporting of hazelnut allergy was less frequent anyhow. In Athens, IgE against Cor a 8 dominated and strongly correlated with IgE against walnut, peach, and apple and against *Chenopodium*, plane tree, and mugwort pollen. Sensitization to seed storage proteins was observed in less than 10%, mainly in children, and correlated with IgE to nuts, seeds, and legumes. IgE to Cor a 12, observed in all cities (10% to 25%), correlated with IgE to nuts, seeds, and pollen. **Conclusions:** In adulthood, the importance of hazelnut sensitization to storage proteins, oleosin (Cor a 12), and Cor a 8 is diluted by the increased role of birch pollen cross-reactivity with Cor a 1. Cor a 8 sensitization in the Mediterranean is probably driven by diet in combination with pollen exposure. Hazelnut oleosin sensitization is prevalent across Europe; however, the clinical relevance remains to be established. (J Allergy Clin Immunol 2015;■■■:■■■-■■■.)

Key words: EuroPrevall, hazelnut allergy, component-resolved diagnosis, outpatient clinic

From 2005 to 2010, the multicenter and multidisciplinary EuroPrevall project was conducted, aiming to investigate the prevalence, cost, and basis of food allergy across Europe.¹ Several large-scale multicenter epidemiological surveys were performed, including birth cohort surveys,² cross-sectional community surveys in school-aged children and adults,³ and an outpatient clinic survey in 12 cities across Europe.⁴ The project studied 24 foods, but its most detailed investigations were directed toward 9 foods, that is, egg, milk, fish, shrimp, peanut, hazelnut, celery, peach, and apple.^{1,3} In the present article, we describe the main characteristics of hazelnut allergy across Europe.

Hazelnut allergy is one of the more common food allergies in Europe, but most studies so far have been limited to European countries such as Sweden, Germany, The Netherlands, Switzerland (North-western and Alpine), and Spain and Italy (Western Mediterranean).⁵⁻¹⁰ These studies have established that hazelnut allergy in the former countries is dominated by cross-reactivity between birch pollen Bet v 1 and hazelnut Cor a 1^{7,8} and in the latter by cross-reactivity between peach Pru p 3 and hazelnut Cor a 8.¹¹ Little is known about hazelnut allergy in Central and Eastern European countries and in North-Western (Iceland) and South-Eastern extremes of Europe (Greece). The EuroPrevall consortium set out to fill these gaps.

In addition, very little has been reported about the importance of hazelnut allergens other than Cor a 1 and Cor a 8 across Europe. Besides profilin (Cor a 2), first described around 2 decades ago,⁷ several allergens not related to pollen have now been identified and characterized. These include the seed storage proteins Cor a 9 (11S globulin),¹² Cor a 11 (7S vicilin-like),¹³ and Cor a 14 (2S albumin).¹⁴ More recently, oil-body-associated oleosins have also been identified as hazelnut allergens, that is, Cor a 12 and Cor a 13.^{15,16} Here, we investigate the full spectrum of

Abbreviations used

CRD: Component-resolved diagnosis
DBPCFC: Double-blind placebo-controlled food challenge
LTP: Lipid transfer protein
sIgE: Specific IgE
SPT: Skin prick test

hazelnut allergens as is known to date (Cor a 1, Cor a 2, Cor a 8, Cor a 9, Cor a 11, Cor a 12, and Cor a 14). The origin of sensitization to hazelnut Cor a 1 is generally accepted to be *Fagales* tree pollen, in particular birch pollen, but it is less well established for the other hazelnut allergens. Sensitization to profilin is thought to be closely linked to grass pollen sensitization, but a role for other pollens cannot be excluded.¹⁷ The concept of peach lipid transfer protein (LTP) Pru p 3 inducing sensitization to fruit, vegetable, nut, and seed LTPs is quite firmly established, but involvement of other foods or pollens as the primary sensitizer cannot be ruled out.¹⁸ In Northern China, mugwort pollen was recently shown to induce LTP-reactive IgE, resulting in cross-reactivity to peach.¹⁹

Some studies demonstrated that IgE responses to storage proteins are more common in children than in adults^{5,20} and pollen-related cross-sensitization first occurs at a later age. The age composition in the EuroPrevall population with around 17% children allowed us to verify this.

In 12 EuroPrevall outpatient clinic surveys, all enrolled subjects were tested by using skin prick test (SPT) and ImmunoCAP on 24 foods, 12 respiratory allergen sources, and latex. We aimed to identify differences in hazelnut sensitization patterns between European cities and possible associations between IgE against hazelnut components and IgE against pollen, latex, and/or other foods, providing insight into possible primary sensitizers. To our knowledge, this is the first detailed clinical and serological study of hazelnut allergy with such a broad geographic, socioeconomic, cultural, and lifestyle spectrum across Europe.

METHODS

Study design

This survey is part of the EuroPrevall project.¹ Subjects were prospectively recruited from 2006 toward the end of 2009 in outpatient clinics from 12 European cities: Madrid (Spain), Sofia, (Bulgaria), Reykjavik (Iceland), Athens (Greece), Prague (Czech Republic), Łódź (Poland), Utrecht (The Netherlands), Strasbourg (France), Manchester (United Kingdom), Milan (Italy), Zürich (Switzerland), and Vilnius (Lithuania). Participating subjects reported immediate adverse reactions occurring within 2 hours or less after the ingestion of any food. The population was further complemented with subjects enrolled in the EuroPrevall community surveys in adults and children.^{3,4,21} In the end, 2273 subjects were enrolled in the survey (see Fig 1; also see this article's [Methods](#) section in the Online Repository at [www.jacionline.org](#)). In the present study, we included 731 subjects reporting reactions to hazelnut. Local ethical committees approved all studies, and written informed consent was obtained from all subjects or their legal representatives.

Clinical evaluation and double-blind placebo-controlled food challenge

Allergy specialists in the outpatient clinics applied standardized case-report forms to collect a detailed medical history.⁴ All subjects underwent SPT and serum IgE testing to detect sensitization to 24 foods, latex,

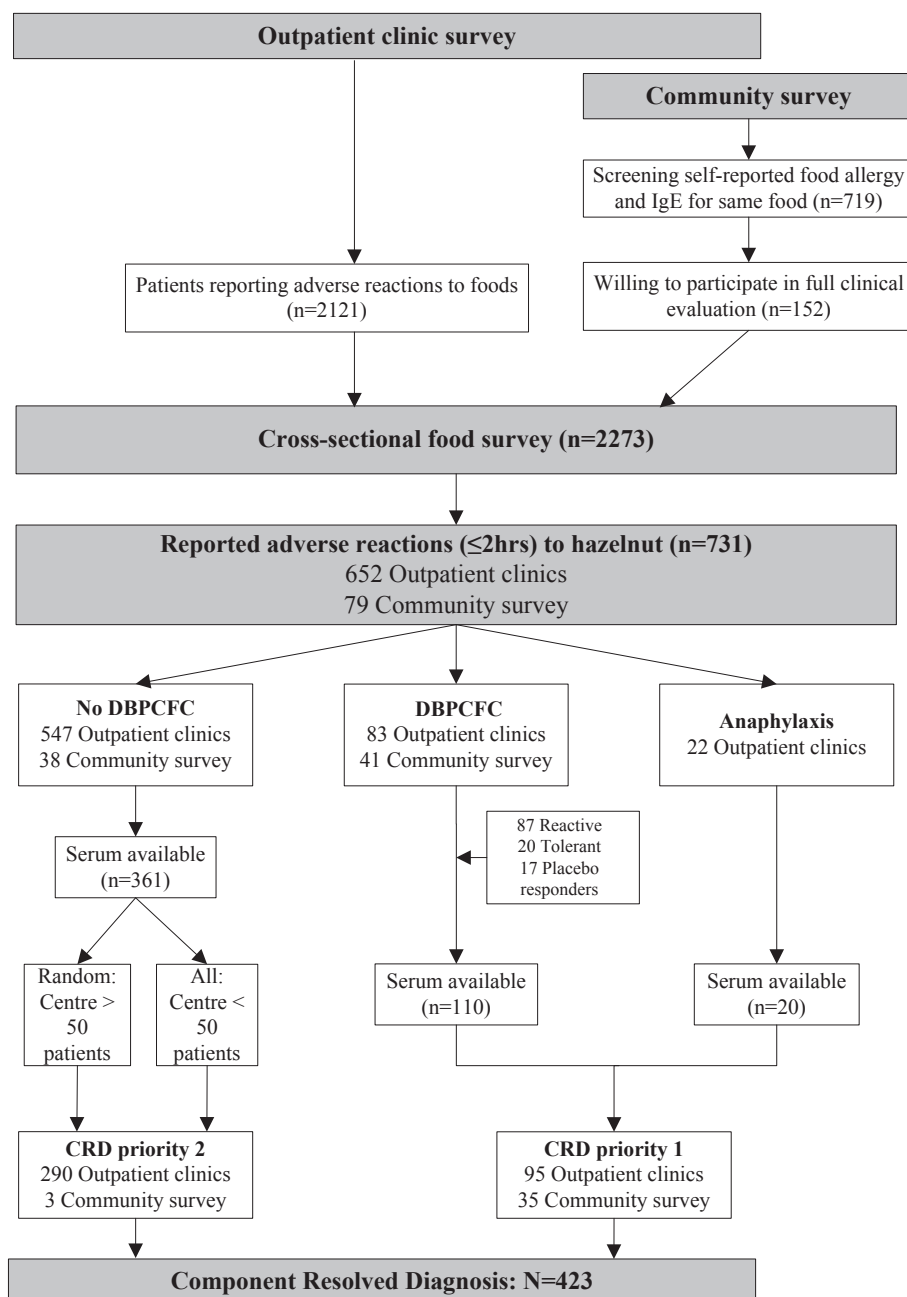


FIG 1. Flowchart showing the selection of subjects in the outpatient and community survey. The number of included subjects with reported adverse reactions (≥ 2 hours) to hazelnut. The full clinical evaluation included SPT and serum IgE testing for 24 foods, 12 inhalant sources, and latex. A subset also underwent a DBPCFC to the food to which they reported symptoms.

and 12 inhalant allergen sources (see [Table E1](#) in this article's Online Repository at www.jacionline.org). We asked all subjects to undergo a double-blind placebo-controlled food challenge (DBPCFC) to hazelnut and 124 consented (see also this article's [Methods](#) section in the Online Repository). Those with a history of severe anaphylaxis²² to hazelnut were excluded from the challenge ($n = 22$). Both a positive challenge and a history of severe anaphylaxis to hazelnut were considered as confirmed hazelnut allergy.

Skin prick testing

SPT reagents were kindly provided by ALK-Abelló (Madrid, Spain). Details of the procedure are described in this article's [Methods](#) section in the

Online Repository. SPT results were expressed as allergen/histamine wheal ratios, with a ratio of 0.5 or more designated as positive.

Specific IgE measurements

Specific IgE (sIgE) antibodies to foods and respiratory allergen sources and latex were tested by ImmunoCAP following the manufacturer's instructions (Thermo Fisher Scientific, Uppsala, Sweden). For component-resolved diagnosis (CRD), we tested the following hazelnut components: rCor a 1 (Bet v 1 homologue), rCor a 2 (profilin), rCor a 8 (LTP), nCor a 9 (11S globulin), nCor a 11 (7S globulin), nCor a 12 (oleosin), and rCor a 14 (2S albumin). In addition, IgE against bromelain was used as a measure for sIgE against cross-reactive carbohydrate determinants (CCDs).

TABLE I. Characteristics of the EuroPrevall hazelnut study population stratified by city

Characteristic	European city						
	All (N = 731)	Madrid (n = 15)	Sofia (n = 12)	Reykjavik (n = 13)	Athens (n = 23)	Prague (n = 29)	Łódź (n = 70)
Type of clinic/department		Allergy	Allergy	Pulmonology & Allergy	Allergy	Pediatrics	Immunology, Rheumatology, and Allergy
Age (y), mean $\pm$ SD	32.3 $\pm$ 14.8	24.7 $\pm$ 14.0	21.5 $\pm$ 15.0	32.7 $\pm$ 17.1	27.8 $\pm$ 12.7	17 $\pm$ 10.8	32.2 $\pm$ 18.1
<18 y, n/N (%)	120/731 (16.4)	5/15 (33.3)	6/12 (50.0)	3/13 (23.1)	5/23 (21.7)	16/29 (55.2)	18/70 (25.7)
Sex: female, n/N (%)	461/731 (63.1)	9/15 (60.0)	8/12 (66.7)	8/13 (61.5)	12/23 (52.2)	19/29 (65.5)	52/70 (74.3)
Hazelnut sensitization							
SPT, n/N (%)	556/718 (77.4)	11/15 (73.3)	3/12 (25.0)	10/13 (76.9)	20/23 (87)	20/29 (69.0)	37/70 (52.9)
sIgE, n/N (%)	585/669 (83.7)	10/15 (66.7)	3/11 (27.3)	10/12 (83.3)	14/21 (66.7)	23/28 (82.1)	41/69 (59.4)
DBPCFCs hazelnut							
Completed, n/N (%)	124/731 (16.9)	8/15 (53.3)	1/12 (8.3)	6/13 (46.2)	3/23 (13.0)	5/29 (17.2)	17/70 (25.0)
Reactive, n/N (%)	87/124 (70.2)	5/8 (62.5)	0/1 (0.0)	4/6 (66.7)	1/3 (33.3)	3/5 (60.0)	8/17 (47.1)
Anaphylaxis, n/N (%)	22/713 (3.0)	—	1/12 (8.3)	—	3/23 (13.0)	2/29 (6.9)	2/70 (2.9)

Characteristic	European city						P value*
	Utrecht (n = 80)	Strasbourg (n = 70)	Manchester (n = 51)	Milan (n = 50)	Zürich (n = 177)	Vilnius (n = 141)	
Type of clinic/department	Dermatology	Pulmonology	Allergy & Clinical Immunology	Allergy	Allergy/ Dermatology	Allergy	
Age (y), mean $\pm$ SD	32 $\pm$ 10.9	34.2 $\pm$ 13	31.2 $\pm$ 15.8	40.5 $\pm$ 14.2	36 $\pm$ 14.3	29.9 $\pm$ 13.7	<.001
<18 y, n/N (%)	5/80 (6.2)	7/70 (10.0)	11/51 (21.6)	1/50 (2.0)	14/177 (7.9)	30/141 (21.3)	<.001
Sex: female, n/N (%)	54/80 (67.5)	54/70 (77.1)	33/51 (64.7)	34/50 (68.0)	105/177 (59.3)	73/141 (51.8)	<.001
Hazelnut sensitization							
SPT, n/N (%)	62/73 (84.9)	60/69 (87.0)	38/51 (74.5)	48/50 (96.0)	129/173 (74.6)	118/140 (84.3)	<.001
sIgE, n/N (%)	75/80 (93.8)	63/68 (92.6)	40/48 (83.3)	47/50 (94.0)	149/176 (84.7)	110/121 (90.9)	<.001
DBPCFCs hazelnut							
Completed, n/N (%)	20/80 (25.0)	9/70 (12.9)	0/51 (0.0)	13/50 (26.0)	41/177 (23.2)	1/1441 (0.7)	<.001
Reactive, n/N (%)	15/20 (75.0)	8/9 (88.9)	—	8/13 (61.5)	34/41 (82.9)	1/1 (100)	
Anaphylaxis, n/N (%)	2/78 (2.6)	1/69 (1.4)	7/51 (13.7)	2/50 (4.0)	2/177 (1.1)	—	<.001

sIgE, Specific IgE $\geq$ 0.35kU_A/L.

*P values were calculated by using ANOVA and Pearson χ^2 test and show an overall difference in characteristics between the cities.

Details of serology measurements are presented in this article's [Methods](#) section in the Online Repository. IgE levels of 0.35kU_A/L or more were considered as positive.

Serum selection for CRD

Because of restricted availability of experimental custom-made ImmunoCAP tests, not all 731 sera were tested with CRD. Sera with sufficient volumes from subjects who had undergone a DBPCFC (110 of 124) and anaphylactic subjects (20 of 22) were tested with priority. Remaining ImmunoCAP tests were used for analysis of samples (n = 293) selected so as to achieve a more balanced representation of the 12 cities. In those with low numbers (<50) of subjects with sufficient serum volume (Sofia, Madrid, Reykjavik, Athens, Prague, Utrecht, Strasbourg, Manchester, and Milan), all sera were tested. A random sample from subjects of the remaining cities (Łódź, Zürich, and Vilnius) was drawn.

Allergens

Hazelnut allergens were produced and purified as described elsewhere.^{14,16,23,24}

Statistical analysis

Differences between cities in characteristics and proportions of positive and negative test results were tested using the Pearson chi-square test and ANOVA (age). We calculated medians and interquartile ranges and used the Kruskal-Wallis test to compare differences in IgE levels to hazelnut between

cities. Correlations between IgE levels to hazelnut and pollen of 9 species, 23 foods, and latex (see [Table E1](#) in this article's Online Repository) were analyzed using the Spearman correlation coefficient. To accommodate possible differences between cities affecting the overall results, we also assessed the correlations using random effects models. Because no significant differences between the 2 methods were observed, the Spearman rho (ρ) correlations are reported. P values of .05 or less were considered significant. For analyses including multiple comparisons, P values adjusted according to the Bonferroni method were calculated. We used R software (version 3.1.0 for all statistical analyses).

RESULTS

Population characteristics and hazelnut sensitization

Hazelnut was the most reported food allergy in the EuroPrevall outpatient clinic survey (32%). Differences in frequencies between European cities were however considerable, ranging from 68.4% in Vilnius to 5.7% in Madrid (see [Fig E1](#) in this article's Online Repository at www.jacionline.org). The population included more females (63.1%) than males (36.9%) ([Table I](#)). Most were adults (83.6%) and among the 120 children (<18 years), 22 were younger than 7 years (3-6 years).

Patients most commonly reported symptoms of the oral mucosa (84.4%), of which around half were without other symptoms. Upper airway (rhinitis, rhinoconjunctivitis), skin,

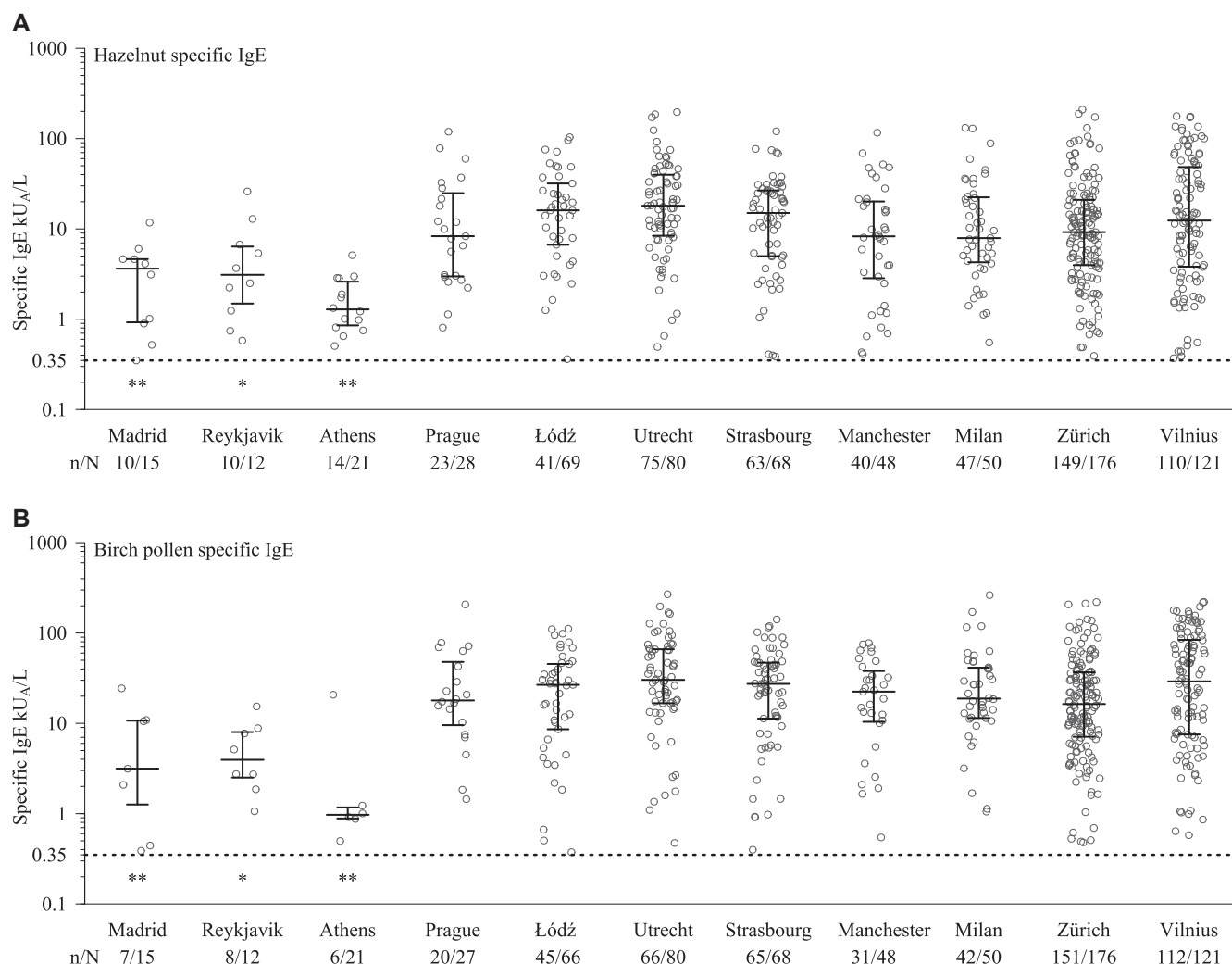


FIG 2. IgE levels of all subjects with sIgE to hazelnut (**A**) and birch (**B**) (≥ 0.35 kU_A/L). The black lines indicate the median IgE values and the interquartile range. For each city, the number of positive responders (n), total tested (N), and the proportion positives of the total (%) are shown. *Significantly different from Łódź, Utrecht, Strasbourg, Manchester, Milan, Zürich, and Vilnius. **Significantly different from Prague, Łódź, Utrecht, Strasbourg, Milan, Zürich, and Vilnius.

and digestive symptoms were reported by 20.7% to 35.4%. More severe symptoms were less often reported, with 13.5% reporting asthma and 3% reporting cardiovascular or neurological symptoms.

Symptoms to hazelnut were supported by sensitization for the vast majority (88.2%), as detected in 566 of 718 (77.4%) by SPT and 585 of 699 (83.7%) by ImmunoCAP (test results were positive in 496 subjects). In 11.8% of all tested subjects, we did not find evidence for hazelnut sensitization. On assessing sensitization by CRD in 423 patients (excluding CCD), 86.5% were positive. CRD detected IgE to individual hazelnut components in 15 of 68 (22%) with a negative hazelnut ImmunoCAP and 49 of 91 (54%) with a negative SPT result (see more details in this article's [Tables E2 and E3](#) in the Online Repository at www.jacionline.org). Because only 3 of 11 patients from Sofia had detectable IgE against hazelnut, they were excluded from further statistical analysis of serological data.

To confirm hazelnut allergy, 124 of 731 subjects underwent a DBPCFC (see [Table E4](#) in this article's Online Repository at

www.jacionline.org). Hazelnut DBPCFC was positive for 87 of 124 patients (70.2%). After including the 22 anaphylactic subjects, we confirmed hazelnut allergy in 109 patients, of which 95% had evidence for hazelnut sensitization by SPT (87.0%), ImmunoCAP (89.9%), or CRD (93.8%). Sensitivity of CRD was significantly higher than that of both other tests, but specificity was significantly lower (see details in [Tables E5 and E6](#) in this article's Online Repository at www.jacionline.org).

Patterns of recognition of individual hazelnut allergens in European cities

Hazelnut sIgE showed a clear variation across European cities ([Fig 2](#)). The pattern closely followed that of sensitization to birch pollen, and IgE levels significantly correlated ($\rho = 0.88$; $P < .001$). IgE levels to hazelnut were significantly lower in Athens and Madrid and, although not significantly, also in Reykjavik than in the other cities.

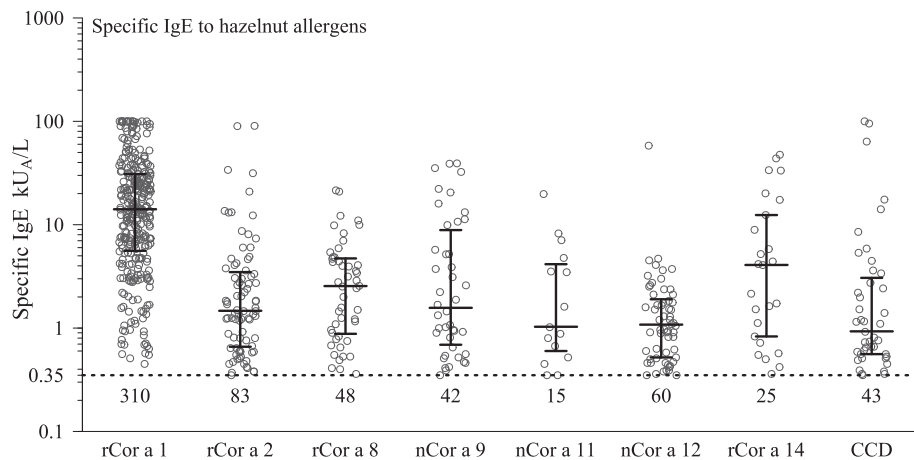


FIG 3. sIgE to hazelnut allergens in a subset of the population with hazelnut allergy ($n = 423$). Median sIgE values and interquartile range are indicated with black lines. The dotted lines indicate the cutoff IgE level at 0.35 kU/L. The number with positive IgE (≥ 0.35 kU/L) is indicated for each hazelnut allergen.

Fig 3 shows the frequency and level of sensitization to individual hazelnut allergens and CCD. Sensitization to Cor a 1 was most prevalent (74.3%), followed at distance by both other pollen-related allergens Cor a 2 (19.6%) and CCD (10.2%). IgE levels against Cor a 1 were 5 to 10 times higher than those against other hazelnut allergens. Cor a 1 was dominant ($\geq 60\%$) in all cities except Athens and Madrid ($<10\%$) (Fig 4). In contrast, sensitization to Cor a 8 dominated in Athens (15 of 18; 83%) and to a lesser extent Madrid (4 of 11; 36%), while this was rare in other cities ($<15\%$). Almost all patients sensitized to Cor a 14 were sensitized to Cor a 9 (20 of 22) and IgE levels closely correlated ($\rho = 0.74$; $P < .001$). Cor a 9 and/or Cor a 14 sensitization was more common in Prague, Reykjavik, Utrecht, Manchester, and Madrid (18.2% to 27.3%) than in other cities ($<7\%$). Sensitization to Cor a 11 reached a frequency of more than 10% only in Prague. Finally, sensitization to Cor a 12 was observed all over Europe in around 10% to 25% of the patients, except in Łódź and Strasbourg ($<8\%$).

Age differences in hazelnut sensitization

Details of age-related sensitization are given in Table E7 in this article's Online Repository at www.jacionline.org. Sensitization to Cor a 1 was less common in children (<18 years) than in adults (61.5% vs 76.2%; $P < .02$). Children (<18 years) were significantly more often sensitized to Cor a 9 and/or Cor a 14 than were adults (42.0% vs 5.8%; $P < .001$), with the exception of Utrecht, where 9 of 10 sensitized to Cor a 9/Cor a 14 were older than 18 years. In addition, children were more often sensitized to Cor a 12 than were adults (34% vs 11.4%; $P < .001$).

Correlations between IgE to pollens and hazelnut allergens

Sensitization to all pollen extracts was observed in all centers (see Table E8 in this article's Online Repository at www.jacionline.org). Birch pollen sensitization was the most frequent among the 9 pollen species tested (80.3%) followed by grass pollen sensitization, ranging from just under 50% to over 80%. To evaluate which of the 9 tested pollen species may be implicated in cross-reactivity to hazelnut allergens, IgE correlations were investigated. Fig 5, A, shows the strength of each correlation

between a hazelnut allergen and a pollen extract (for exact correlation coefficients, see Table E9 in this article's Online Repository at www.jacionline.org).

IgE against Cor a 1 correlated with IgE against birch pollen only ($\rho = 0.92$; $P < .001$), but such correlation was lacking in Athens and Madrid. Almost all birch-hazelnut cosensitized patients (301 of 318) were sensitized to Cor a 1 (median IgE, 14.60 kU/L; interquartile range, 6.08-31.20). However, 5 of 30 (16.1%) subjects sensitized to hazelnut but not birch pollen had IgE against Cor a 1 (median, 4.07 kU/L; interquartile range, 0.85-6.48).

IgE to Cor a 8 correlated weakly ($\rho < 0.55$) with that to *Chenopodium*, plane tree, mugwort, and *Parietaria* pollen. Sensitization to these pollen was frequent in Athens, Madrid, and Milan (see Table E8; also see Fig E2 in this article's Online Repository at www.jacionline.org), but only in Athens were these correlations stronger compared with the total population ($\rho = 0.78, 0.71, 0.71$, and 0.66 , respectively; $P = .001$).

Patients sensitized to profilin (Cor a 2) and to CCD were sensitized to virtually all pollen species (92%-100%). Surprisingly, correlations between IgE against Cor a 12 and pollens followed a very similar pattern. IgE to Cor a 9, Cor a 11, and Cor a 14 showed only very weak correlations to those against pollen.

Sensitization to other foods in a molecular perspective

IgE to other foods was observed in almost all subjects sensitized to hazelnut (92.9%), but the pattern of food sensitizations varied with the spectrum of hazelnut allergens recognized (Fig 5, B; see Table E10 in this article's Online Repository at www.jacionline.org). Peach and apple IgE correlated with Cor a 1 and Cor a 2, although not in Athens, Madrid, and Milan. In these cities, IgE against peach and apple correlated with that against Cor a 8 in Athens ($\rho = 0.95$ and 0.94 ; $P < .001$) and Milan ($\rho = 0.68$ and 0.66 ; $P < .001$). In Madrid, IgE only to peach correlated with Cor a 8 ($\rho = 0.63$; $P < .04$).

Overall, however, IgE to Cor a 8 most closely correlated with IgE to walnut, corn, and lentil. Walnut sensitization was very common in Athens (92.8%) and Madrid (100.0%) than in other cities (14.3% to 38%). IgE to walnut and Cor a 8 correlated highly in Athens ($\rho = 0.94$; $P < .001$), but no significant correlation was

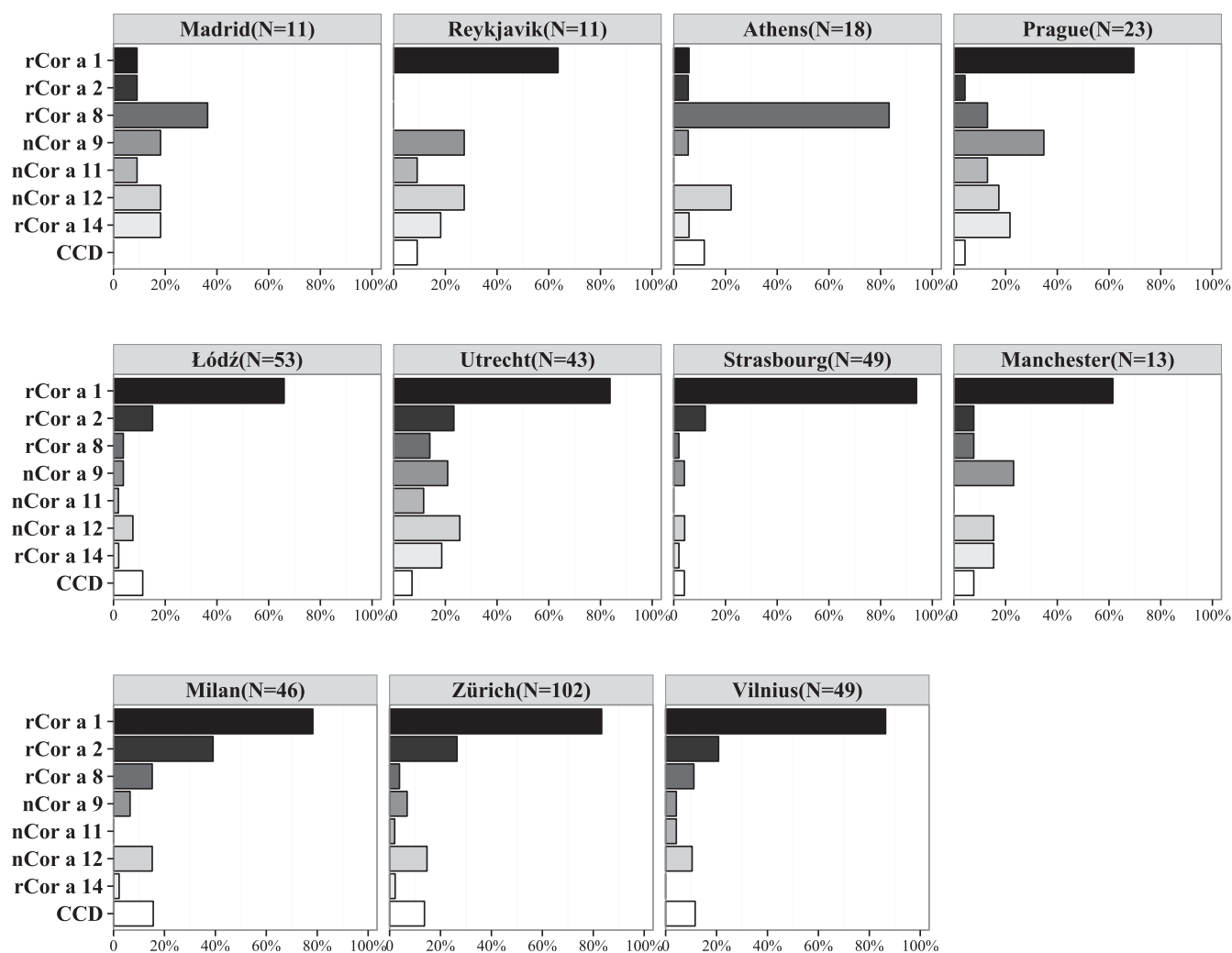


FIG 4. Sensitization to hazelnut allergens stratified by city. Sensitization to single hazelnut allergens was measured in a subpopulation (Sofia excluded) of the patients with allergy to hazelnut. The bars show the percentage of patients with positive test results for each allergen.

found in Madrid (see Fig E3 in this article's Online Repository at www.jacionline.org).

IgE responses to Cor a 9 and 14 showed weak correlations with those to tree nuts ($p \leq 0.57$), seeds, and legumes. IgE correlations between walnut and Cor a 9 were stronger in Utrecht and Prague ($p = 0.70$ and 0.78) than in the total population ($p = 0.57$). No such correlations were observed in Athens and Madrid.

IgE to Cor a 12 correlated moderately with IgE to oil-rich foods such as tree nuts, seeds, and legumes, and surprisingly also to melon and banana. No city-specific differences were observed for these correlations. Fig E4 in this article's Online Repository shows correlations between IgE to walnut and soybean and Cor a 9 and Cor a 12.

Finally, latex IgE correlated with all hazelnut allergens except Cor a 1 and was strongest for Cor a 12, Cor a 2, and CCD.

DISCUSSION

In the present study, the largest case series on hazelnut allergy ever performed was analyzed across Europe. Although the study was not a general population-based survey, the inclusion of

consecutive patients coming into the clinic over a longer period of time gives an indication of the magnitude of the problem of hazelnut allergy across Europe. This study indicates that hazelnut allergy is far less common in cities such as Athens, Madrid, Reykjavik, and Sofia than in other European cities, similar to what has been reported in population-based surveys.^{21,25} One probable explanation is the low exposure to birch pollen in these 4 cities²⁶ because IgE levels against hazelnut and birch are much lower than in other cities (median IgE, <4 vs $8-30$ kU_A/L).

Birch pollen exposure has a dominant role in the occurrence of hazelnut allergy.^{21,25} The high frequency and magnitude of IgE responses against Cor a 1 clearly support that, in most of our cities, birch pollen sensitization (ie, Bet v 1-Cor a 1 cross-reactivity) is the driving force. In a small group, Cor a 1 sensitization was observed in the absence of birch sensitization. This has also been reported in hazelnut-sensitized children from the Netherlands.²⁷ Although we cannot exclude direct food-driven sensitization to hazelnut Cor a 1, it is perhaps more likely that sensitization to pollen of other *Fagales* species, such as hazel, oak, alder, or beech, might induce IgE antibodies cross-reactive with Cor a 1.

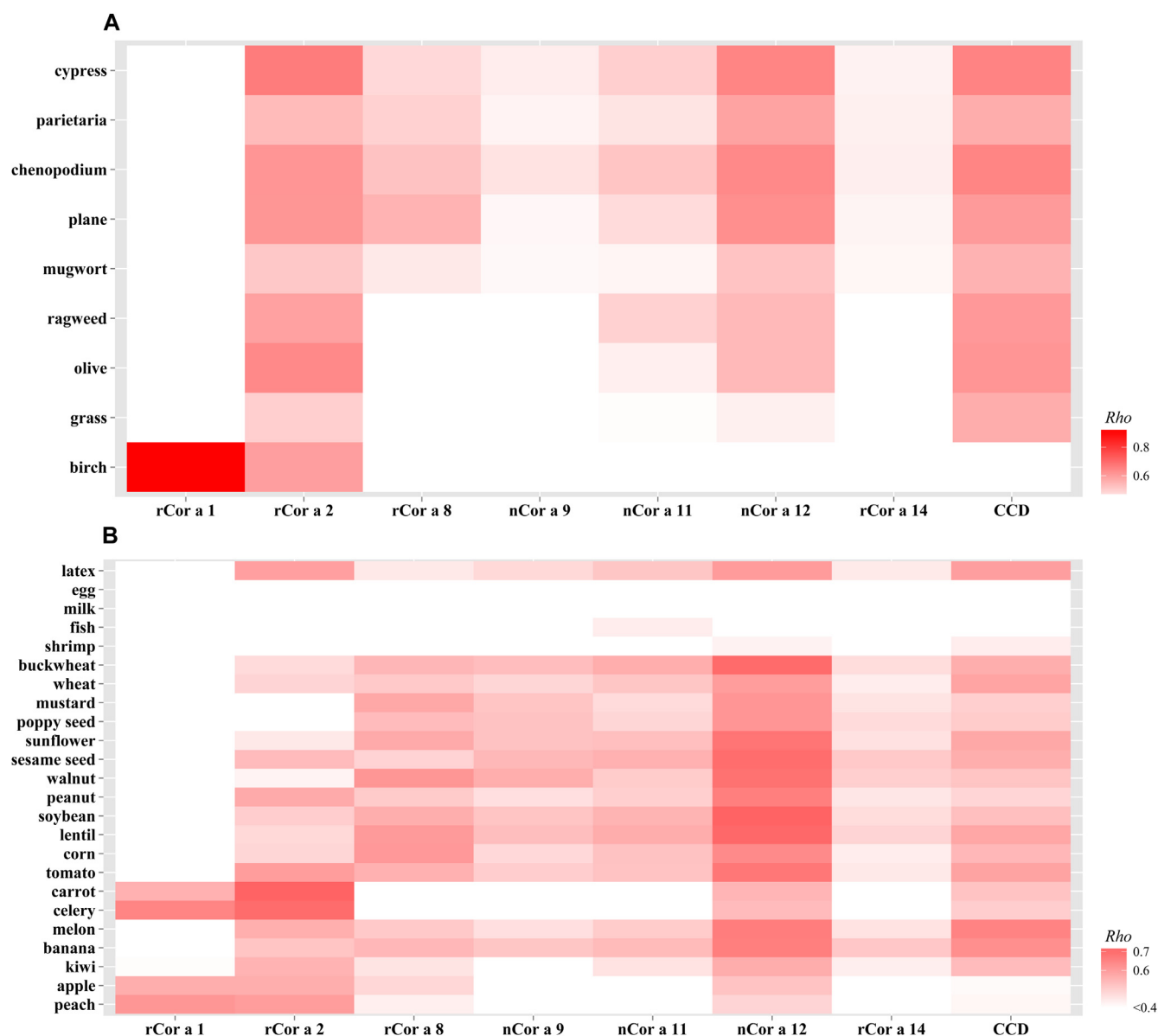


FIG 5. Heat plots. **A**, Correlation between IgE to individual hazelnut allergens and 9 pollens. **B**, Correlation between single hazelnut allergens and 23 foods and latex. Each color indicates the strength in correlation of IgE levels to hazelnut allergens with pollen, foods, and latex. White: Spearman $\rho < 0.4$; red: Spearman $\rho 0.4-0.92$.

Although the role of birch pollen as a dominant source of sensitization for Cor a 1 is not disputed, the situation is more complex for Cor a 8. A long-standing assumption is that sensitization to LTP is a Mediterranean phenomenon.⁶ The dominant Cor a 8 profile in Athens and Madrid confirms this. The currently prevailing opinion is that peach Pru p 3 induces sensitization to other food LTPs. In Athens and Madrid, IgE to Cor a 8 correlates strongly with that to peach ($\rho = 0.68$ and 0.95 , respectively). However, the high frequency of walnut sensitization (93%) and the strong correlation between Cor a 8 and walnut IgE ($\rho = 0.94$) in Athens suggest that a walnut-rich diet could also be relevant for hazelnut sensitization. However, several studies have demonstrated that sensitization to mugwort and plane tree pollen LTP also plays a role in the “LTP-syndrome.”^{19,28-30} We found correlations between IgE to

Cor a 8 and IgE to weed and tree pollen, although weaker than those to peach, apple, and walnut. Whether the associated foods or pollens act as the primary cause of sensitization to Cor a 8 cannot easily be inferred from available data. Mediterranean patients are perhaps geographically not likely to develop LTP-driven food allergies, but less prone to develop birch pollen-associated food allergies. Future studies, in particular IgE inhibition assays, are needed to unravel the possible primary source of sensitization to LTPs.

The geographical distribution of sensitization to seed storage proteins (Cor a 9, Cor a 11, and Cor a 14) is less clear. Although relatively more subjects from Prague, Reykjavik, Utrecht, Manchester, and Madrid were sensitized to these allergens, the total numbers are low ($n = 2-9$). Other studies have shown sensitization for Cor a 9 and Cor a 14 to occur preferentially in

younger children,^{5,20,31} and we also observed significant differences between children and adults (42% vs 5.8%). These proportional differences can be explained by more Bet v-1-related sensitization later in life (adults showed a significantly higher proportion of Cor a 1 sensitization) causing a diluting effect of hazelnut storage protein sensitization in adults. Storage protein IgE correlates relatively weakly with other foods. This may indicate that sensitization to these proteins is driven by hazelnut consumption and sensitization to other foods are independent cosensitizations.

Of interest are the sensitization patterns observed for hazelnut oleosin (Cor a 12). To date, very little is known about allergic reactions to oleosin in foods and have so far been reported only for peanut,³² sesame seed,³³ and buckwheat.³⁴ Hazelnut oleosin was first described by Akkerdaas et al¹⁵ and has recently been associated with severe symptoms.¹⁶ Our data show that hazelnut oleosin sensitization is not uncommon in Europe. IgE against Cor a 12 correlated with sensitization to many foods, in particular oil-rich tree nuts, seeds, and legumes. Interestingly, the pattern of associations to pollen sensitization was very similar to that observed for Cor a 2 and CCD. Oleosins have been identified in pollen as well,³⁵ so it cannot be excluded that pollens play a role in sensitization to oleosins.

IgE against both Cor a 2 and CCD were associated with all pollen species as true panallergenic structures. Surprisingly, the closest correlation was with olive and cypress pollen and not with grass pollen. Foods that have previously been linked to profilin sensitization were closely associated in the present study as well: carrot, celery, peach, tomato, and melon.³⁶⁻⁴¹

Some limitations of the present study have to be considered. Although we performed the largest series of DBPCFCs for hazelnut, still 83% of those reporting hazelnut allergy were not challenged. Moreover, the number of challenges carried out was unbalanced between cities. However, this is the most comprehensive standardized study so far with respect to sensitization and allergy to hazelnut. What can we say about the place of the 3 tests for sensitization in an outpatient clinic setting? Sensitivity of CRD with 7 allergens together is higher than that of conventional hazelnut ImmunoCAP or SPT, but probably too costly for routine application. A more realistic approach, with a minimal loss of sensitivity (93.8% vs 91.2%), would be to test IgE against Cor a 1, Cor a 8, and Cor a 14. For simply assessing whether reported hazelnut allergy is supported by sensitization, SPT or hazelnut ImmunoCAP is most likely appropriate and more feasible. None of the 3 tests has a useful specificity (10% to 30%), maintaining that the DBPCFC still remains an important diagnostic procedure in cases in which clinical relevance needs to be established. Having said that, CRD has revealed associations between the outcome of a DBPCFC including severity and specific allergens.²⁰ This certainly is an added value of CRD, allowing better assessment of the risk of severe reactions. Currently, we are analyzing the patients' sensitization pattern reported here for associations of reported symptoms in real life and during DBPCFC with sIgE against individual allergens.

In conclusion, our study has mapped hazelnut allergy across Europe. Major differences in the number of cases were observed across Europe, which are largely explained by differences in exposure to birch pollen. This dominant cross-reactive phenomenon explains the lower sensitization rates to storage proteins, oleosins, and LTPs. A dominance of Cor a 8 (LTP) was confirmed

for the Mediterranean basin, in particular for Athens, but the source of primary sensitization is still not completely certain. Finally, oleosin sensitization is observed across the whole of Europe but whether pollen plays a role in sensitization to oleosins needs to be established.

We thank ALK-Abello for providing the SPT reagents for this study. We also thank Lothar Vogel for his involvement in the serum screening and selection and thank all the patients who volunteered to participate in the EuroPrevall studies.

Key messages

- Similar to what has been described for North-Western Europe, birch pollen exposure drives hazelnut allergy in Central and North-Eastern Europe.
- Sensitization to hazelnut storage proteins is observed across Europe, with its relative importance decreasing with age because of the increasing role of pollen cross-reactivity.
- As reported for Spain and Italy, hazelnut allergy in Greece is an LTP-driven phenomenon, closely associated not only with peach but also with walnut, and to a lesser extent with pollen sensitizations.

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Methods

Study design

The EuroPrevall outpatient clinic survey included 2273 subjects reporting adverse reactions to a food. Background and case-report forms were completed for 2261 subjects. This population consisted of 1615 adults (median age 32.9; IQR: 18.23) and 646 children below 18 years old (median age 8.3; IQR 10.70). Of those children, 148 were younger than 4 years.

Of the 2261, 740 food forms for hazelnut were completed. For this study, we included subjects when the reported time between the food intake and onset of symptoms was within 2 hours and 731 met these criteria.

Skin-prick testing

Commercially available extracts (ALK-Abelló, Madrid, Spain) were used to measure skin reactivity against 24 foods and 12 inhalants (Table E1). All centers were provided with the same batch of SPT reagents for the study. At each clinical center, a single investigator carried out all SPTs following the recommendations of the European Academy of Allergology and Clinical Immunology.¹ The same type of lancets were used in all centers (ALK-Abelló). Histamine hydrochloride (10 mg/mL) and saline were used as positive and negative controls, respectively.

Specific IgE measurements

Sera from patients were sent to the Paul Ehrlich institute (Germany). Using uniform batches of reagents, all sera were tested for the presence of IgE against 24 food extracts and 12 inhalant extracts (Table E1). All sera from the general population survey were first tested with five mixtures, containing 5 (in one case 6) different foods as described earlier (Table E1).² If a mix was positive ($> 0.35\text{kU}_\text{A}/\text{L}$), the individual foods present in that mix were tested separately. To save serum, the same procedure was performed for outpatient clinic subjects with limited serum volume ($< 2\text{ml}$).

For the component-resolved diagnosis, commercially available ImmunoCAPs were used to measure IgE levels to rCor a 1, rCor a 8 and MUXF3(CCD). Custom-made ImmunoCAPs were used to measure IgE against rCor a 2, nCor a 9, nCor a 11, nCor a 12 and rCor a 14. All CRD measurements were carried out at the Academic Medical Center (the Netherlands) on an ImmunoCAP 250 instrument.

DBPCFC

The active and placebo provocations with hazelnut were done on 2 different randomly assigned days. The first dose started at $3\mu\text{g}$ of hazelnut protein and the following 8 doses gradually increased until the top

dose of 3 g protein (corresponding to ~15 hazelnut kernels). The next dose was given after 20 minutes or if any previous reaction has disappeared. The challenge was stopped when an objective reaction occurred or convincing subjective reactions lasted for more than 45 minutes.

Results

Diagnostic performance of the SPT, ImmunoCAP and CRD

The differences in performance between the hazelnut SPT, ImmunoCAP and CRD (positive to any allergen) are shown in table E3. The majority of the allergic patients were positive for all test, with a sensitivity of 87.0% (95% CI: 79.2-92.7) by SPT, 89.9% (82.5-94.8) by ImmunoCAP and 93.8% by CRD using a cut-off of 0.35 kU/L. Among the tolerant cases, the percentage of those with positive IgE was high, resulting in a low specificity for SPT (30%), ImmunoCAP (25%) and CRD (16.7%). When using 0.1 kU/L as the cut-off IgE value, almost all tolerant patients (15/18) were classified as 'positive' resulting in a specificity of 10% (1.2-37.7%). Seven challenged patients that had IgE levels to hazelnut between 0.1 and 0.35 and 4/7 were reactive during the challenge. When combining the SPT and ImmunoCAP (cut-off 0.35), the NPV improved compared to the individual tests. The specificity for CRD (16.7%) was significantly lower compared to the SPT tests ($p = 0.001$) and ImmunoCAP($p = 0.04$).

1

Table E1. The 24 foods and 12 inhalant allergens (and latex) measured by Skin prick testing and ImmunoCAP

All foods	Composition of the food mixes		Non-food allergen sources and latex
Peach	Mix 1	Hazelnut	Birch [§]
Apple		Tomato	Plane tree ^{§*}
Kiwi		Carrot	Olive ^{§*}
Banana		Celery	Cypress ^{§*}
Melon		Walnut	Timothy grass [§]
Celery	Mix 2	Shrimp	Mugwort [§]
Carrot		Mustard	Parietaria [§]
Tomato		Poppy seed	Ragweed ^{§*}
Corn		Lentil	Chenopodium ^{§*}
Lentil		Sunflower	Dust mite
Soybean	Mix 3	Apple	Cat
Peanut		Kiwi	Dog [*]
Walnut		Melon	Latex ^{§*}
Hazelnut		Banana	
Sesame seed		Peach	
Sunflower	Mix 4	Egg white	
Poppy seed		Milk	
Mustard		Fish (cod)	
Wheat		Peanut	
Buckwheat		Soybean	
Shrimp	Mix 5	Wheat	
Fish		Maize, Corn	
Milk		Rice	
Egg		Sesame seed	
		Buckwheat	

Panel of foods, inhalants and latex that were tested for sensitization by by Skin Prick Testing and serum IgE. The mixes were only used for serological analysis in patients that were first selected in the general population survey or those with a limited serum volume (< 2ml)

[§]Analyzed for correlation between specific IgE and IgE against hazelnut allergens

^{*}Subjects from the general population survey were not tested for sensitization against these allergens

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TABLE E2. Sensitization to hazelnut measured by CRD, Skin prick testing and ImmunoCAP

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E2a. CRD vs Skin prick testing

	Skin Prick Test*	
	Positive	Negative
CRD Positive	316	49
CRD Negative	14	42
Total	330	91

* 2 subjects with missing data for SPT

8

E2b. CRD vs ImmunoCAP

	ImmunoCAP†	
	Positive	Negative
CRD Positive	349	15
CRD Negative	4	53
Total	353	68

†3 subjects with missing data for ImmunoCAP

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E2c. CRD vs ImmunoCAP

	SPT and/or ImmunoCAP*	
	Positive	Negative
CRD Positive	360	6
CRD Negative	16	41
Total	376	47

*Positive to SPT and/or ImmnoCAP, negative to both SPT and ImmunoCAP

All positive tests results were based on sIgE ≥ 0.35 kUA/L and SPT allergen/histamine ratio ≥ 0.5

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TABLE E3. Sensitization to hazelnut allergens in patients without detectable sIgE to hazelnut extract and negative SPT.

	Negative ImmunoCAP hazelnut		Negative SPT hazelnut		Negative ImmunoCap and SPT hazelnut	
	N	IgE levels		N	IgE levels	
		Median	(IQR)		Median	(IQR)
Total	68			91		
CRD positive	15			49		
rCor a 1	3	0.67	(0.63-4.00)	38	4.69	(1.34-13.68)
rCor a 2	7	2.22	(1.63-2.93)	13	1.79	(0.80-2.61)
rCor a 8	5	0.83	(0.68-1.10)	1	3.41	-
nCor a 9	1	1.07	-	9	0.96	(0.56-3.88)
rCor a 11	0	-	-	2	3.98	(2.43-5.53)
rCor a 12	0	-	-	12	1.40	(0.52-2.54)
rCor a 14	0	-	-	1	1.12	-
CCD	0	-	-	6	1.36	(0.80-2.92)

The number of subjects with IgE to a single hazelnut allergen but with negative tests results in the ImmunoCAP (sIgE < 0.35kUA/L) or SPT for hazelnut. The first rows shows the total number of negative ImmunoCAP and SPTs of the 423 CRD tested subjects. The second row shows the number of the negative tested subject that were positive in the CRD. Then for each allergens the number of positives to that allergen where shown. Some subjects responded to more than 1 hazelnut allergen.

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TABLE E4: Double blind placebo-controlled challenges to hazelnut and anaphylactic patients.

European city							
	All n=731	Madrid n=15	Sofia n=12	Reykjavik n=13	Athens n=23	Prague n=29	Łódź n=70
DBPCFCs hazelnut							
Completed n/N (%)	124/731 (16.9)	8/15 (53.3)	1/12 (8.3)	6/13 (46.2)	3/23 (13.0)	5/29 (17.2)	17/70 (25.0)
Reactive n/N (%)	87/124 (70.2)	5/8 (62.5)	0/1 (0.0)	4/6 (66.7)	1/3 (33.3)	3/5 (60.0)	8/17 (47.1)
Anaphylaxis n/N (%)	22/731 (3.0)	-	1/12 (8.3)	-	3/23(13.0)	2/29(6.9)	2/70(2.9)
European city							
	Utrecht n=80	Strasbourg n=70	Manchester n=51	Milan n=50	Zürich n=177	Vilnius n=141	p-value
DBPCFCs hazelnut							
Completed n/N (%)	20/80 (25.0)	9/70 (12.9)	0/51 (0)	13/50 (26.0)	41/177 (23.2)	1/144(0.7)	<0.001
Reactive n/N (%)	15/20(75.0)	8/9 (88.9)	-	8/13 (61.5)	34/41 (82.9)	1/1 (100.0)	
Anaphylaxis n/N (%)	2/78(2.6)	1/69(1.4)	7/51(13.7)	2/50(4.0)	2/177(1.1)	-	<0.001

*P-values were calculated by Pearson χ^2 test and show an overall difference between the cities.

20

TABLE E5 Sensitivity and Specificity of three different hazelnut tests to measure sensitization

		Allergic	Tolerant	Sensitivity (CI95%)	Specificity (CI95%)
SPT	Positive	94	14	87.0 (79.2-92.7)	30.0 (11.9-54.3)
	Negative	14	6		
ImmunoCAP (0.35)	Positive	97	15	89.8 (82.5-94.8)	25.0 (8.7-49.1)
	Negative	11	5		
ImmunoCAP (0.10)	Positive	102	18	94.4 (88.3-97.9)	10.0 (1.2-31.7)
	Negative	6	2		
SPT + ImmunoCAP (0.35)	Positive	106	16	97.2 (92.2-99.4)	20.0 (5.7-43.7)
	Negative	3	4		
CRD* (0.35)	Positive	91	6	93.8 (87.0-97.7)	16.7 (3.6-41.4)
	Negative	15	3		
CRD* (0.1)	Positive	94	17	96.9 (91.2-99.4)	5.6 (0.1-27.3)
	Negative	3	1		
Cora 1/Cor a 8/ Cor a 14 [§]	Positive	89	12	91.2 (84.3-96.4)	33.3 (13.3-59.0)
	Negative	8	16		

Sensitivity and specificity were calculated for ImmunoCAP and CRD using 0.35 and 0.1 kUA/L as a cut-off value.

*sensitization to any of the seven hazelnut allergens measured by component-resolved diagnosis (CRD)

[§] sensitization to Cor a 1, Cor a 8 or Cor a 14 using (0.35 ≥ kUA/L)

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TABLE E6: Sensitization against hazelnut allergens

	Reactors		Anaphylaxis		Tolerant	
	N	%	N	%	N	%
rCor a 1 (positive)	69/77	89.6	10/20*	50.0	11/18	61.1
rCor a 2 (positive)	14/77	18.2	3/20	15.0	4/18	22.2
rCor a 8 (positive)	7/77	9.1	5/20	25.0	1/18	5.6
nCor a 9 (positive)	5/77	6.5	5/20	25.0	3/18	16.7
rCor a 11 (positive)	5/77	6.5	2/20	10.0	1/18	5.6
nCor a 12 (positive)	9/77	11.7	6/20	30.0	2/18	11.1
rCor a 14 (positive)	5/72	6.9	5/17	29.4	0/17	0
CCD (positive)	4/77	5.2	5/20	25.0	2/18	11.1

* 7/10 subjects also had IgE against one of the non-pollen related allergens Cor a 8, Cor a 9, Cor a 11, Cor a 12 or Cor a 14, implying that 3 were selectively sensitized to Cor a 1 amongst the 7 allergens we tested.

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TABLE E7: Age related sensitization to single hazelnut allergens

	Adults		<18 years		< 7years	
	N	%	N	%	N	%
Extract (positive)	502/593	84.7	83/106	78.3	3/18	44.4
rCor a 1 (positive)	278/365	76.2	32/52	61.5	3/7	42.9
rCor a 2 (positive)	76/369	20.6	7/53	13.2	0/7	0
rCor a 8 (positive)	41/367	11.2	7/52	13.5	1/7	14.3
nCor a 9 (positive)	21/369	5.7	21/53	39.6	4/7	57.1
nCor a 11 (positive)	10/369	2.7	5/53	9.4	1/7	14.3
nCor a 12 (positive)	42/368	11.4	18/53	34.0	2/7	28.6
rCor a 14 (positive)	11/360	3.1	14/50	28.0	2/7	28.6
CCD (positive)	36/362	9.9	7/52	13.5	0/7	0

Sensitization to hazelnut Extract, individual allergens and CCD are shown for all adults, children younger than 18 years (including <7 years) and a group of subjects younger than 7 years.

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TABLE E8: Frequency of pollen sensitization stratified by city

European city													
	Total (%)	Athens (%)	Madrid (%)	Reykjavik (%)	Milan (%)	Manchester (%)	Prague (%)	Zürich (%)	Vilnius (%)	Strasbourg (%)	Łódź (%)	Utrecht (%)	p-value*
birch	80.3	28.6	46.7	66.7	84.0	64.6	74.1	85.8	92.6	95.6	68.2	82.5	<0.001
grass	65.0	61.9	80.0	50.0	68.0	81.2	66.7	71.0	62.8	58.8	47.0	68.8	<0.001
olive	58.0	61.9	66.7	33.3	70.0	64.6	55.6	71.2	52.9	55.9	29.7	62.8	<0.001
ragweed	47.0	57.1	66.7	41.7	76.0	31.2	51.9	50.4	55.4	23.5	35.9	37.2	<0.001
mugwort	46.3	66.7	60.0	25.0	66.0	31.2	59.3	44.9	61.2	26.5	42.4	35.0	0.001
plane tree	40.0	66.7	83.3	25.0	68.0	41.7	37.0	41.7	38.8	22.1	26.6	34.9	<0.001
chenopodium	35.1	57.1	58.3	25.0	50.0	29.2	40.7	41.0	35.5	17.6	21.9	34.9	<0.001
parietaria	34.4	66.7	60.0	16.7	56.0	29.2	29.6	37.5	33.1	25.0	24.2	27.5	<0.001
cypress	29.9	33.3	58.3	16.7	50.0	25.0	22.2	34.5	26.4	14.7	18.8	34.9	<0.001

*P-values were calculated by using Pearson χ^2 test and show the overall proportional differences in sensitization (IgE ≥ 0.35 kUA/L) between cities.

31 **TABLE E9:** Correlations between sIgE to hazelnut allergens and inhalants

	Hazelnut allergen							
	rCor a 1	rCor a 2	rCor a 8	nCor a 9	nCor a 11	nCor a 12	rCor a 14	CCD
birch	0.92	0.60	0.04 [§]	0.15	0.23	0.34	0.25	0.36
grass	0.20	0.50	0.31	0.33	0.40	0.43	0.35	0.57
olive	0.34	0.64	0.37	0.35	0.43	0.54	0.35	0.61
ragweed	0.23	0.59	0.38	0.40	0.49	0.54	0.37	0.61
mugwort	0.20	0.51	0.45	0.41	0.42	0.52	0.42	0.56
plane tree	0.21	0.61	0.55	0.42	0.47	0.63	0.42	0.61
chenopodium	0.17	0.61	0.52	0.46	0.52	0.64	0.43	0.65
parietaria	0.16	0.54	0.49	0.42	0.45	0.59	0.43	0.57
cypress	0.31	0.67	0.48	0.44	0.50	0.65	0.42	0.65

The values represent the Spearman's *Rho* correlation between IgE levels. All p-values <0.001. [§]no significant correlation

TABLE E10: Correlations between sIgE to hazelnut allergens and 23 foods and latex

	Hazelnut allergen							
	rCor a 1	rCor a 2	rCor a 8	nCor a 9	nCor a 11	nCor a 12	rCor a 14	CCD
peach	0.62	0.61	0.43	0.24	0.29	0.49	0.32	0.42
apple	0.57	0.57	0.48	0.27	0.30	0.53	0.32	0.41
kiwi	0.41	0.56	0.46	0.40	0.46	0.57	0.44	0.54
banana	0.21	0.52	0.55	0.52	0.54	0.67	0.52	0.63
melon	0.21	0.57	0.51	0.47	0.51	0.67	0.46	0.66
celery	0.66	0.71	0.25	0.26	0.37	0.54	0.33	0.50
carrot	0.56	0.73	0.23	0.30	0.40	0.55	0.35	0.53
tomato	0.22	0.61	0.56	0.50	0.53	0.68	0.45	0.59
corn	0.08 [§]	0.48	0.61	0.48	0.53	0.65	0.44	0.55
lentil	0.17	0.48	0.61	0.54	0.57	0.72	0.49	0.59
soybean	0.19	0.50	0.57	0.52	0.56	0.73	0.47	0.53
peanut	0.28	0.58	0.51	0.47	0.50	0.67	0.45	0.49
walnut	0.10 [*]	0.42	0.62	0.57	0.51	0.70	0.50	0.52
sesame seed	0.18 ^{**}	0.54	0.49	0.55	0.56	0.70	0.51	0.57
sunflower	0.11 [*]	0.45	0.58	0.53	0.53	0.69	0.46	0.58
poppy seed	0.04 [§]	0.36	0.54	0.53	0.49	0.62	0.47	0.51
mustard	0.07 [§]	0.31	0.58	0.52	0.47	0.62	0.46	0.50
wheat	0.12 [*]	0.49	0.51	0.48	0.52	0.61	0.44	0.59
buckwheat	0.12 [*]	0.48	0.55	0.54	0.57	0.71	0.47	0.57
shrimp	0.21	0.31	0.29	0.39	0.39	0.43	0.36	0.44
fish	0.19	0.30	0.26	0.28	0.44	0.40	0.33	0.36
milk	0.14 [*]	0.26	0.31	0.33	0.35	0.29	0.34	0.30
egg	0.14 [*]	0.24	0.34	0.39	0.37	0.34	0.37	0.29
latex	0.18	0.60	0.45	0.48	0.52	0.61	0.44	0.60

The values represent the Spearman's *Rho* correlation between IgE levels. All p-values <0.001; **p<0.01; *p<0.05; §no significant correlation;

Online figure legends

FIG E1: Frequency of subjects reporting hazelnut allergy. The proportion of patients reported hazelnut allergy for each clinical center is shown, with bars indicating the 95% confidence interval. The numbers below the cities show the number of subjects with convincing hazelnut allergy (n) and the total number of subjects included in the EuroPrevall survey (N).

Fig E2. Scatterplots showing the correlation between IgE against Cor a 8 and Chenopodium pollen and Mugwort pollen. The upper graphs present all subjects in the population, the lower graphs are specific for subjects from Athens. The x and y axis show the IgE levels on a logarithmic scale.

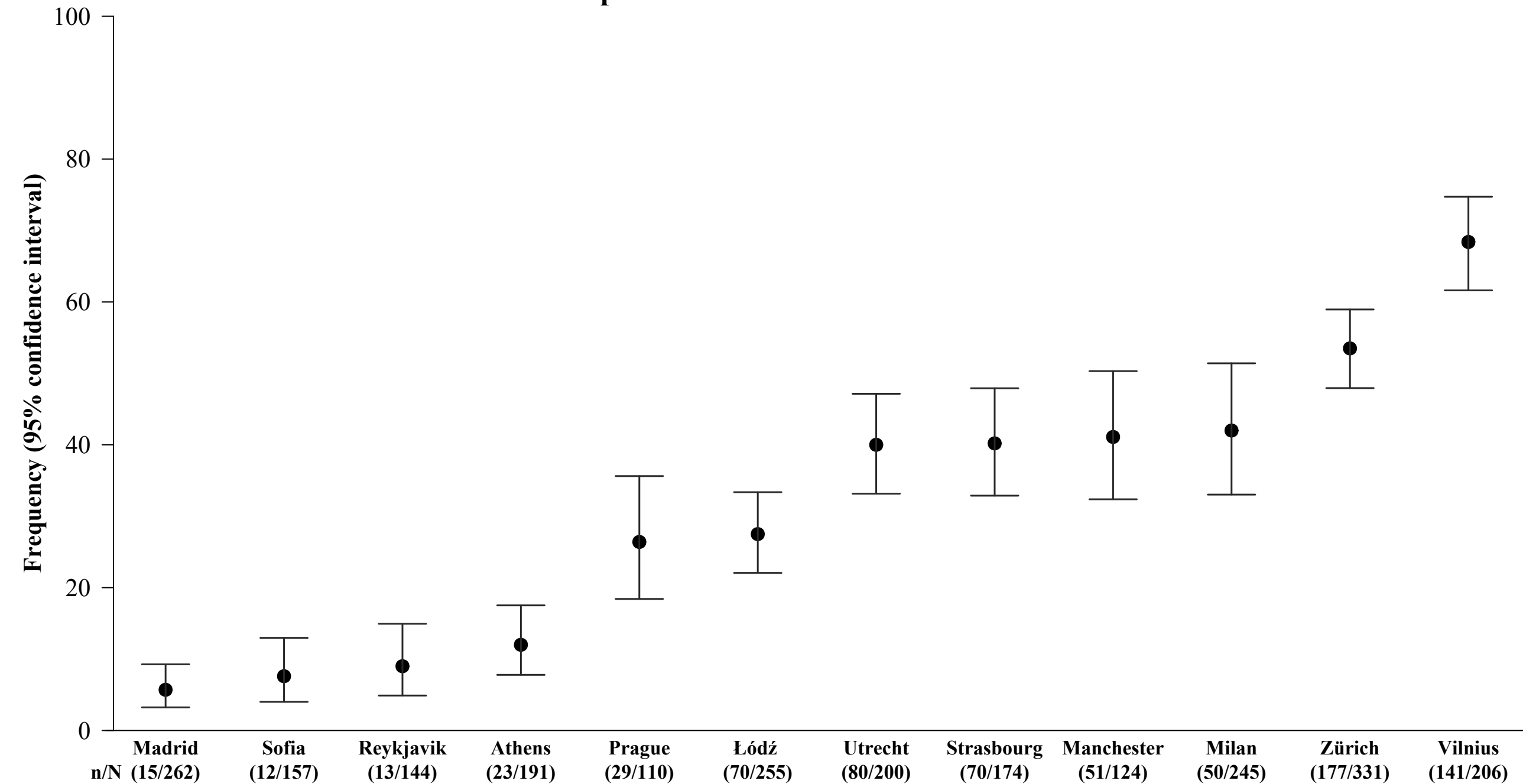
Fig E3. Scatterplots showing the correlation between IgE against Cor a 8 and walnut in the total population and in Athens. The x and y axis show the IgE levels on a logarithmic scale

Fig E4. Scatterplots showing the correlation between IgE against hazelnut allergens Cor a 9 and Cor a 12 with IgE against walnut and soybean. The x and y axis show the IgE levels on a logarithmic scale.

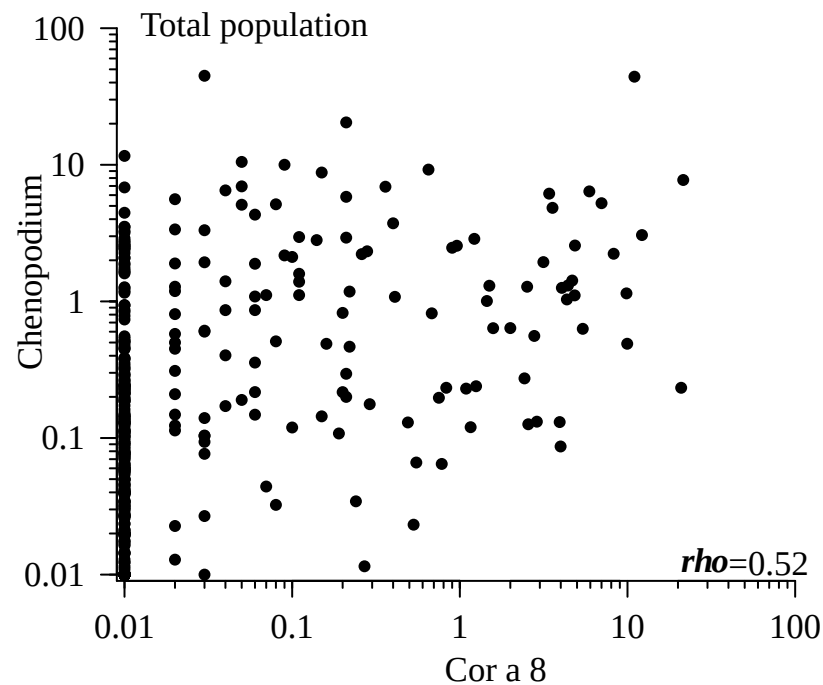
51 **Supplemental References**

52

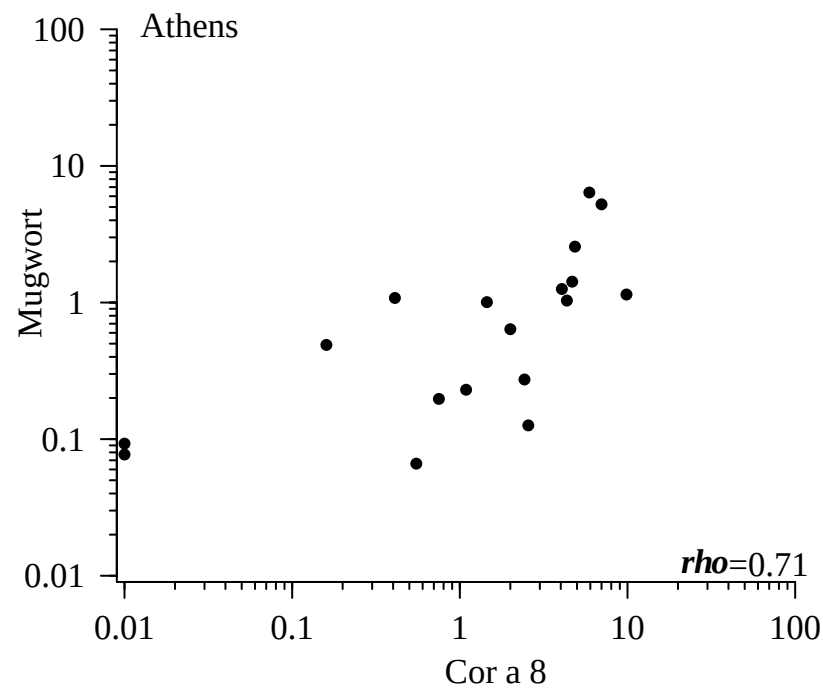
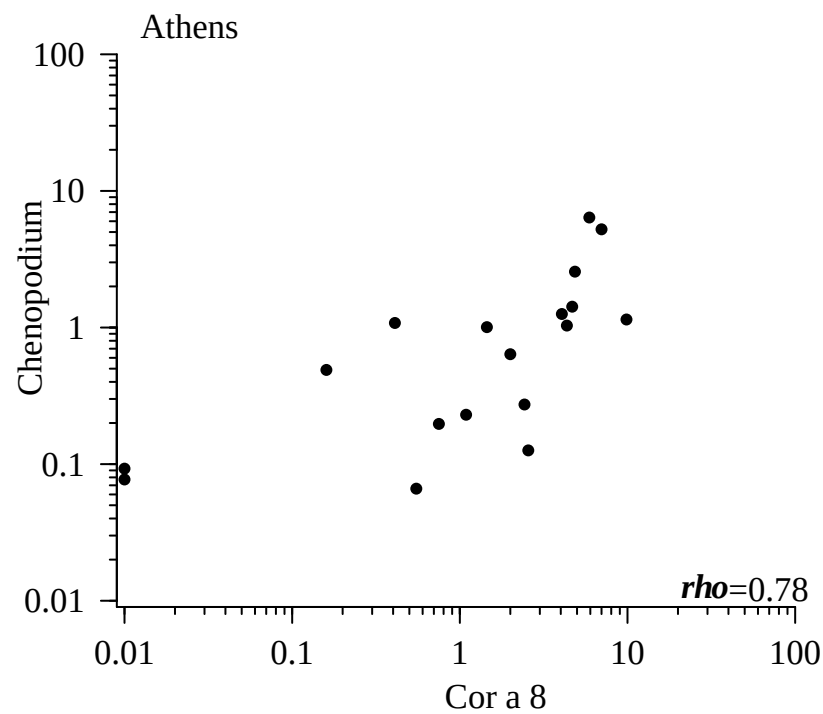
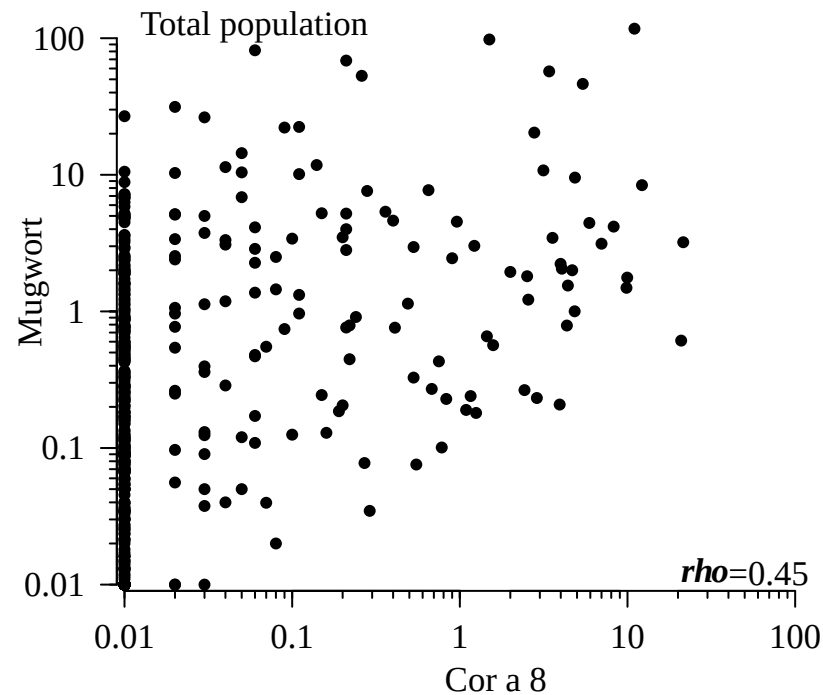
Reported adverse reactions to hazelnut



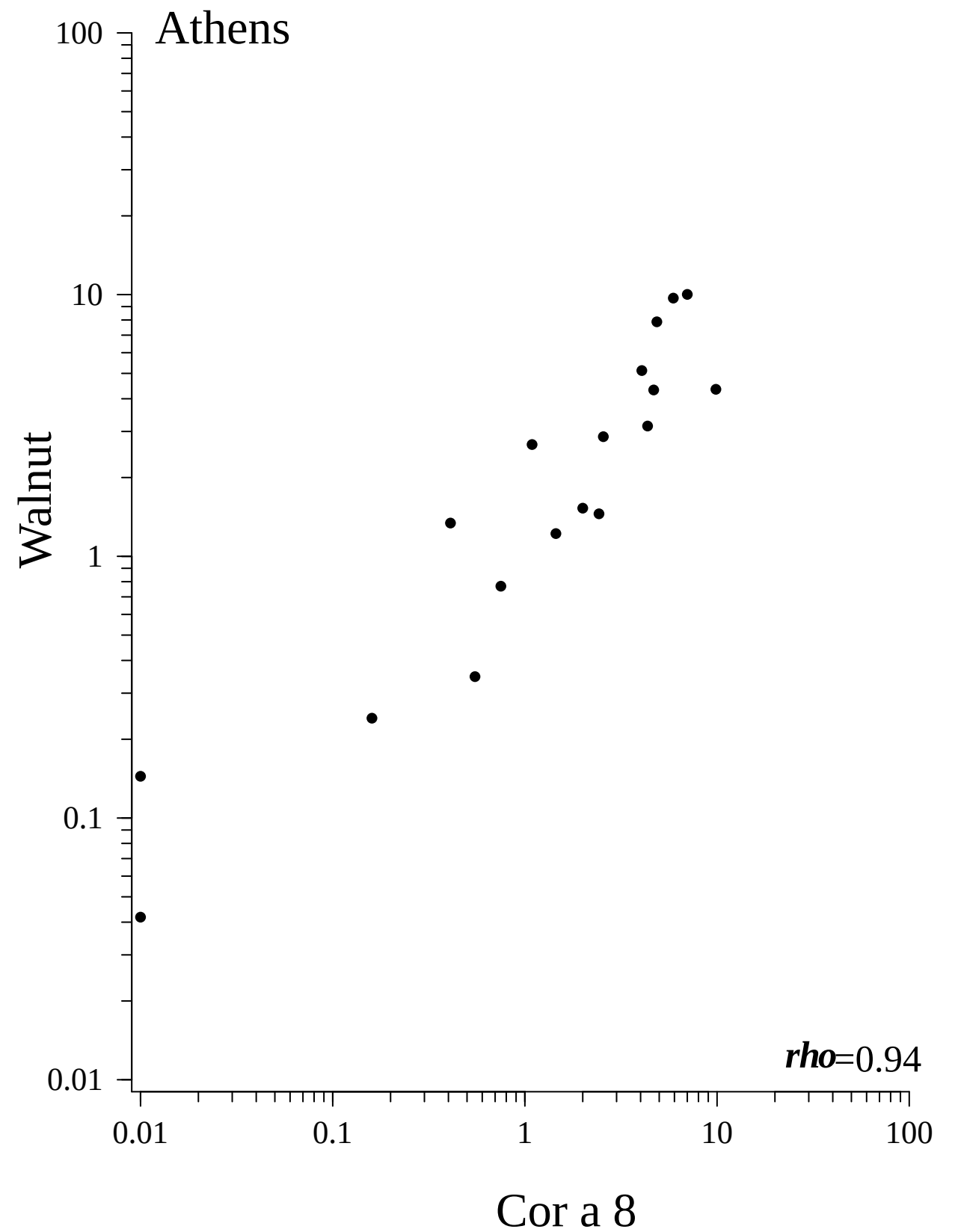
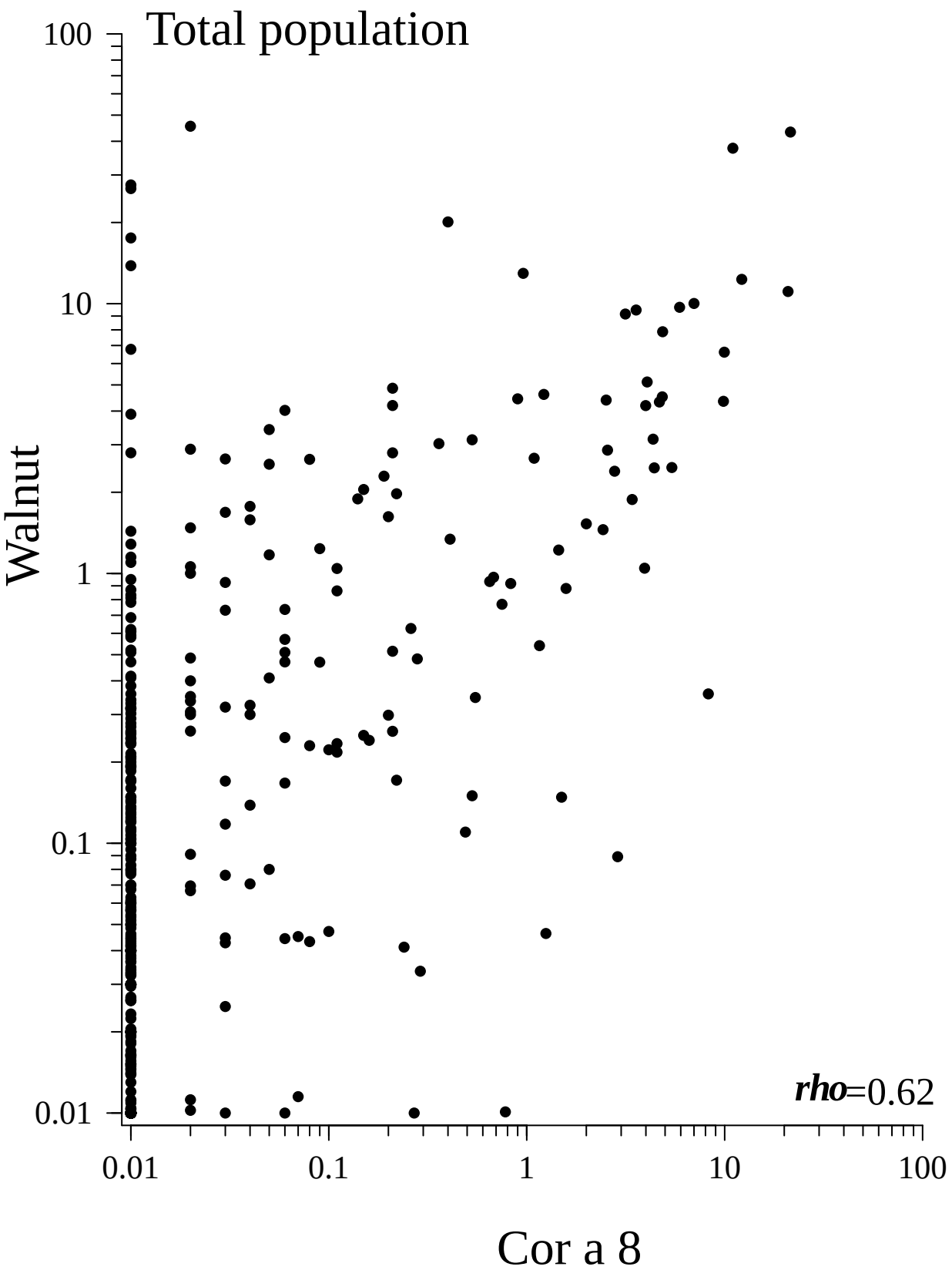
Correlation Cor a 8 – Chenopodium



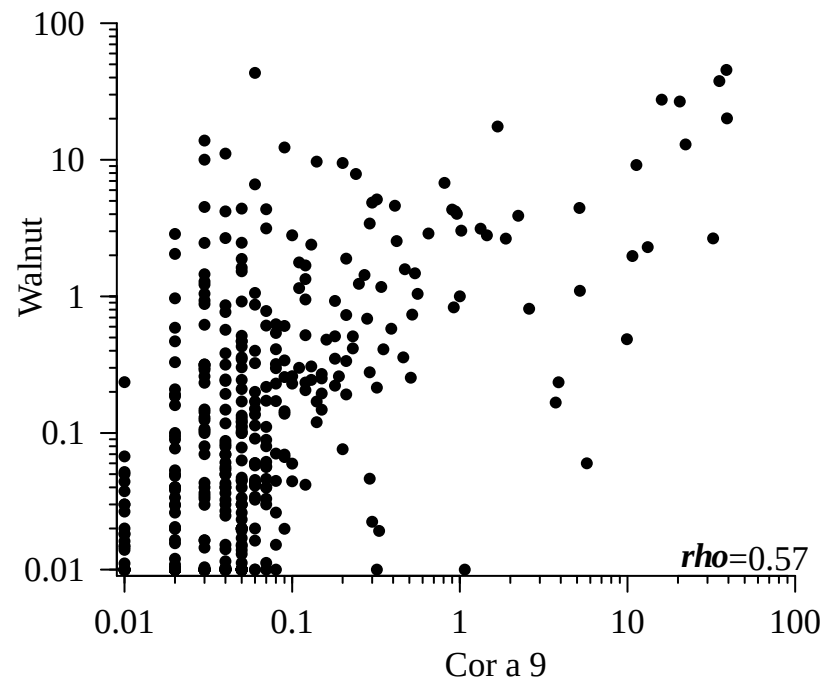
Correlation Cor a 8 – Mugwort



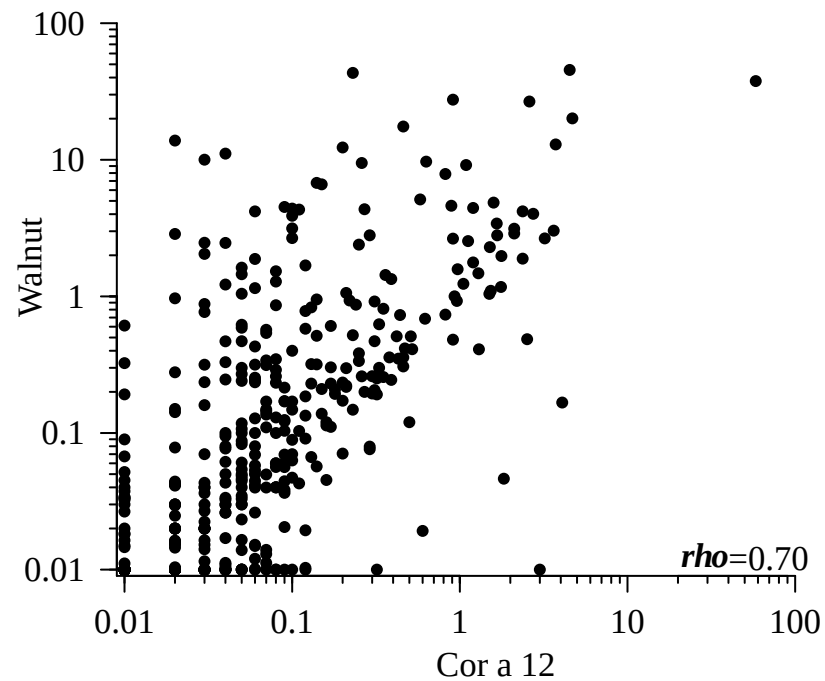
Correlation Cor a 8 – Walnut



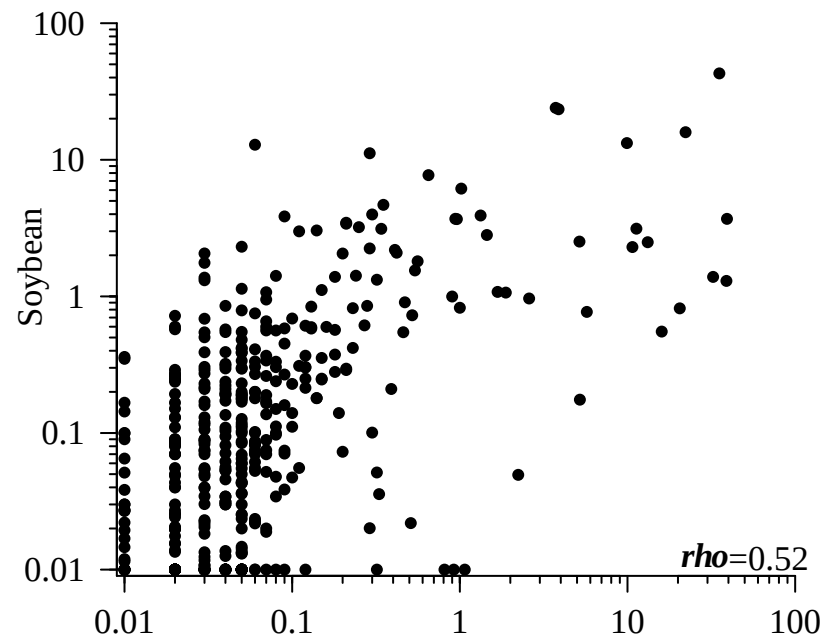
Cor a 9 – Walnut



Cor a 12 – Walnut



Cor a 9 – Soybean



Cor a 12 – Soybean

