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Potential health risk of allergenic pollen with climate change associated spreading capacity: Ragweed and olive sensitization in two German federal states

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ABSTRACT

Background: Global climate changes may influence the geographical spread of allergenic plants thus causing new allergen challenges.
Objective: Allergy patients from two German federal states were compared for their status quo sensitization to ragweed, an establishing allergen, olive, a non-established allergen, and the native allergens birch, mugwort, and ash.
Methods: Between 2011 and 2013, 476 adult allergy patients per region were recruited. Patients completed a questionnaire, participated in a medical interview, and underwent skin prick testing and blood withdrawal for analysis of specific IgE to allergen components (ISAC technology). Data on regional pollen load from 2006 to 2011 were acquired from the German Pollen Information Service Foundation.
Results: Prick test reactivity to ragweed and ash, respectively, was lower in Bavaria than in NRW (ragweed: $p = 0.001$, aOR = 0.54; ash: $p = 0.001$, aOR = 0.59), whereas prick test reactivity to olive was higher ($p = 0.000$, aOR = 3.09). Prick test reactivity to birch and mugwort, respectively, did not significantly differ. 1% (1/127) of patients with prick test reactivity to ragweed showed sIgE to Amb a 1, and 65% (86/132) of olive-but-not-ash reactive patients showed sIgE to Ole e 1 (NRW: 67%, Bavaria: 65%; $p = 0.823$, OR = 0.91). Regional differences in sensitization pattern were neither explainable by cross-reactivity to pollen pan-allergens nor non-exposure variables nor by reported plant population or pollen data.
Conclusions: Spread of ragweed and particularly olive may result in prompt occurrence of allergic symptoms. Early identification of invasive allergens due to climate change does need time and spatial close meshed measurement of respective indicator allergens and sensitization pattern.

Abbreviations: CO_2, carbon dioxide; NRW, North Rhine-Westphalia; IgE, immunoglobulin E; sIgE, specific IgE; DEGS1, first wave of the German Health and Interview Survey for Adults; UBA, German Federal Environment Agency; GA^2LEN, Global Allergy and Asthma European Network; SBE, standardized biological units; IR, index of reactivity; G/V, weight/volume; ISAC, Immuno Solid-phase Allergen Chip; ISU, ISAC-standardized units; n.a., not analyzed; n/a, not applicable; PID, German Pollen Information Service Foundation; CASMIN, Comparative Analysis of Social Mobility in Industrial Nations; OR, odd's ratio; CI, confidence interval; min, minimum; max, maximum.

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1. Introduction

Pollen allergens are one of the main triggers of respiratory allergies, the latter being an increasing health problem worldwide (Beasley et al., 1998; Burney, 1996; Eder et al., 2006; Pearce et al., 2000). In Germany, currently 34% of adults are sensitized to inhalant allergens, and 15% and 9% suffer from hay fever and accordingly asthma at least once in their life (Langen et al., 2013). In children, 41% are sensitized to at least one of 20 tested allergens, and 11% and 5% suffer from hay fever and accordingly asthma at least once in their life (Schlaud et al., 2007). Allergic patients may have to face a progressive course of the disease and suffer from high psychological strain. Health care costs of allergic diseases are immense: in Germany, the total health care costs associated with asthma in the year 2008 were as high as 1.8 billion Euro (The Information System of the German Federal Health Monitoring, 2014).

Global climate changes, amongst other factors, have been discussed to play a role especially in the development of pollen-associated respiratory allergies (Beggs and Bambrick, 2005; Behrendt and Ring, 2012). Experimental and association studies have demonstrated a correlation between CO_2 and temperature, respectively, and pollen concentration or biomass production by allergenic plants (Kim et al., 2011; Negrini et al., 2011; Sicard et al., 2012; Song et al., 2012; Ziello et al., 2012; Ziska et al., 2003). An increase in pollen concentration may lead to an increase in allergic sensitization and/or severity of allergic respiratory symptoms (Breton et al., 2006; Innes Asher et al., 2010; Jäger, 2000; Kim et al., 2011). Furthermore, the geographical spread of allergenic plants might change, and this might cause new allergen challenges (Behrendt and Ring, 2012).

This study aimed at analyzing the status quo sensitization to potentially new allergens in two German federal states with different climatic conditions. Concretely, allergy patients from North Rhine-Westphalia (NRW) and Bavaria should be compared for their sensitization to ragweed, an establishing allergen, and olive, a non-established allergen. The native allergens birch, mugwort, and ash should serve as controls.

Ragweed, also referred to as common ragweed and scientifically named *Ambrosia artemisiifolia*, is an invasive plant from North America, which has spread across Europe especially in warmer climates (Starfinger, 2007). The spread of ragweed is caused by anthropogenic activities, but climate change has been discussed to promote this process (Karrer, 2014; Starfinger, 2007). In Germany, ragweed has been found to be growing wild for the last 150 years (Starfinger, 2007). Currently, in both NRW and Bavaria, ragweed is listed as an invasive plant (Botanical State Collection Munich and SNSB IT Center, 2015; North Rhine Westphalia State Environment Agency, 2014a). In 2012, Bavaria documented a higher number of ragweed crops than NRW (Bavarian State Ministry for the Environment and Health, 2013; North Rhine Westphalia State Environment Agency, 2014b). In Germany, currently 8% of adults have IgE to allergen extract from ragweed pollen, and 0.4% are positive for IgE to Amb a 1, the major allergen component of ragweed pollen (Haftenberger et al., 2013). Prick test data on adult allergy patients (study centres: Berlin and Munich) showed sensitization to allergen extract in 14% of patients (Heinzerling et al., 2009). Ragweed pollen is highly allergenic, thus ten pollen grains are efficient to induce nasal symptoms in adult patients with allergic rhinitis (Bergmann et al., 2008).

Olive (*Olea europaea*) is found in all areas around the Mediterranean Sea and partly also around the Black Sea. In Europe, the northernmost tree population with more than 170 trees existed in Cologne, NRW, but due to the cold winters of 2009 and 2010, the plantation had to be given up (Olive E and Più Marzak KG, 2014). At present, field-grown olive is not established in Germany. However, in summer olive can be found as potted plant in cafeterias, restaurants, and balconies. Olive pollen represents a main cause for allergic respiratory problems in Mediterranean countries (Palomares et al., 2006; Villalba et al., 2014). In Germany, population based data on olive sensitization do not exist. Prick test data on adult allergy patients (study centres: Berlin and Munich) showed sensitization to allergen extract from olive pollen in 10% of patients (Heinzerling et al., 2009). These data, however, have been discussed to result from cross sensitization to ash (*Fraxinus excelsior*), a tree established in the temperate zones of Europe (Heinzerling et al., 2009; Palomares et al., 2006), as the major allergen component of olive, Ole e 1, has a homologous counterpart in Fra e 1, the major allergen component of ash (Palomares et al., 2006; Barderas et al., 2005).

NRW, geographically located in the west of Germany and the most populous federal state, is situated in the warm temperate climate zone of Europe with mainly maritime climate composed of relatively cool summers, mild winters and high atmospheric humidity. Bavaria, located in the southeast of Germany and the second most populous federal state, is situated in the transient region between the maritime climate of Western Europe and the continental climate of Eastern Europe. The latter is characterized by hot summers, cold winters and low atmospheric humidity (Bavarian Environment Agency, 2014). Specific data on climate details of both states are provided by the German Weather Service (German Weather Service, 2014).

The study should answer the following questions:

1. Do the two federal states differ with respect to sensitization to the mentioned inhalant allergens? If so, are these differences caused by pollen pan-allergens or non-exposure variables, or can these differences be related to the exposure variables plant population or pollen load?
2. What can be learned from these data with respect to necessary adaption measures to climate change?

2. Methods

2.1. Patients

476 patients from each state were included in the study. In NRW, the study was conducted at the Department of Dermatology and Allergology of the University Hospital of Aachen. In Bavaria, the Department of Otorhinolaryngology, Klinikum rechts der Isar, Technical University Munich, was responsible. The study was approved beforehand by the institutional review boards of both participating centres.

The patient number resulted from expected Amb a 1-sensitization rates of 1% in NRW and 4% in Bavaria which would have been significant with 476 patients in each state (Fisher's exact test, $p < 0.05$, power 0.8). Expected sensitization rates of 1 and 4%, respectively, were deviated from data on sensitization rates in school children from Baden-Württemberg (Baden-Württemberg Health Authority, 2008/9).

Recruitment occurred continually from spring 2011 to summer 2013. To become included, patients had to fulfil the following criteria: (i) age between 20 and 65 years, (ii) principal residence in NRW and accordingly Bavaria for the last 20 years, (iii) at least two of the symptoms of the upper respiratory tract put in parenthesis (sneeze, itchiness, running nose, itching eyes, obstructed nose, retronasal flow of mucus, cough, asthmatic attacks, affinity to infections, seasonal accumulation of symptoms).

Patients filled in a questionnaire, participated in a medical interview, and underwent skin prick testing as well as blood withdrawal for analysis of serum sIgE levels.

2.2. Questionnaire, medical interview, and patient data base

The questionnaire and the medical interview had been composed for this study and consisted amongst others of questions designed specifically for this study, e.g. travel habits,[1] and questions used in DEGS1, amongst others on origin, environmental living conditions, education, smoking habits, and allergic diseases (Gößwald et al., 2013; Kamtsiuris et al., 2013).

The completed questionnaires were scanned at the study centres and sent electronically to the UBA. At the UBA, data were entered manually into a patient database which had been designed using Access 2007 for Windows (Microsoft Corporation, Redmond, USA).

2.3. Sensitization

Sensitization was analyzed by means of skin prick testing with respective allergen extracts and by means of serum sIgE to the respective allergen components.

2.3.1. Skin prick testing

Skin prick testing was performed according to the GA^{2}LEN guidelines on harmonization of skin prick testing in Europe (Bousquet et al., 2012; Heinzerling et al., 2009). Besides other allergens, most of them included in the pan-European standard prick test panel advised by GA^{2}LEN (Heinzerling et al., 2009), the following inhalant allergens were tested: (i) *Ambrosia* (1:100 G/V; ALK-Abelló, Hamburg, Germany), (ii) olive (100 IR/ml; Stallergenes, Kamp-Lintfort, Germany), (iii) birch (50,000 SBE/ml; Allergopharma, Reinbek, Germany), (iv) *Artemisia* (100 IR/ml; Stallergenes), and (v) ash (1%; Leti-Novartis, Witten, Germany). Histamine dihydrochloride (10 mg/ml; ALK-Abelló) was used as positive control, diluent (ALK-Abelló) as negative control. Results were recorded after 15 min. Valid negative and positive controls provided (that is largest diameter of the negative control <2 mm and largest diameter of the positive control ≥3 mm), skin prick testing to an allergen extract was evaluated positive if the largest diameter of the wheal was ≥3 mm (Bousquet et al., 2012).

The completed prick test sheets were scanned at the study centres and sent electronically to the UBA. There, data were entered manually into the patient database mentioned above.

2.3.2. Measurement of specific IgE

Analysis of serum sIgE levels was done in the study centre in Aachen using ISAC technology (Fisher Scientific GmbH, Schwerte, Germany; 112 allergen components) (Hiller et al., 2002; Ott et al., 2006). Amongst others, sIgE levels to the following allergen components were analyzed: (i) Amb a 1, the major allergen component of ragweed, (ii) Ole e 1, the major allergen component of olive,[2] (iii) Bet v 1, the major allergen component of birch, (iv) Art v 1, the major allergen component of mugwort, and (v) Bet v 2, Hev b 8, Mer a 1, Phl p 12, Bet v 4, and Phl p 7, summarized as pollen pan-allergen components. Amb a 1, Ole e 1, and Art v 1 were purified native proteins, Bet v 1 and the pollen pan-allergen components were of recombinant origin. IgE data were displayed in ISU, and ISU-values ≥0.3 were evaluated positive. IgE data were recorded electronically (Excel 2007 for Windows, Microsoft Corporation) and electronically imported into the patient database mentioned above.

2.4. Data quality management

Except for the sIgE data, which were part of an internal quality management system, all data of the patient database were checked for (i) typing errors, (ii) plausibility, and (iii) completeness. Checking was done in 5 phases, phases 1–4 each with 100 and phase 5 with 76 patients from each study centre. In each phase, correctness of data entry was checked initially with 10% randomly chosen patients. If the error rate was above 0.3%, all patients of the respective phase were checked. Checking for plausibility and completeness was based on 124 and accordingly 100 queries and included all patients of the respective phase.

Data validation revealed typing error rates ≤0.3% in check phases 1, 3, 4, and 5 (error rates of 0.28, 0.12, 0.08, and 0.09%, respectively). In check phase 2, an error rate of 0.44% occurred and resulted in the double checking of all data entry in this phase. In all phases, rates of plausibility and completeness were found to be above 99%. The located typing errors and implausible data were corrected, and the resulting database dated July 15th, 2014 was used for data analysis.

2.5. Pollen data

From the PID, pollen data were acquired from the pollen reference counting stations in Mönchengladbach (NRW) and Munich (Bavaria). In Bavaria, the station in Munich was the only reference counting station (meaning that counting was done continually throughout the entire year), whereas in NRW, another reference counting station did exist in Bad Lippspringe, situated about 120 km (linear distance) northeast of the geographical centre of the Ruhr district. For reasons of comparability, data from only one NRW counting station were to be included. As half of the NRW patients were recruited from cities in or south the Ruhr district, the station in Mönchengladbach, situated about 60 km (linear distance) southwest of the geographical centre of the Ruhr district, was chosen.

Pollen was collected using Burkard traps. Data from 2006 to 2011 were acquired, as data on ragweed pollen have been available since 2006. Data consisted of the respective daily totals/m^3 air.

Pollen load was assessed by magnitude, calculated by the sum of daily totals/m^3 air per year, and duration, calculated by the sum of pollen-positive days per year. A day was defined pollen-positive, if the daily total/m^3 air was ≥1.

A draft of the geographical locations of NRW and Bavaria including the locations of the study centres and the pollen counting stations is given in Fig. 1.

2.6. Statistical analysis

Data analysis was performed using Excel (Excel 2007 for Windows, Microsoft Corporation) and SPSS (PASW Statistics 18, Hong Kong, China).

Bivariate testing for significant differences was performed with the Chi Square test (and Fisher's exact test, respectively) in case of patient data with categorical character, with the Mann–Whitney *U* test for unpaired samples in case of patient data with metric character, and with the Wilcoxon test for paired samples in case of pollen data.

To evaluate the effect of various independent variables on the dependent variable sensitization, multivariate analysis was performed using binary logistic regression (method: enter). The analysis included the following independent variables: *federal state* (categories: NRW/Bavaria), *age* (categories: ≤30 years/31–40

[1] Travel habits were enquired using the answer "yes" to the following statements: "I travel regularly to a specific region" or "I travel irregularly and to different regions of the world" or "I almost never/rarely travel". Patients, who affirmed the first or second statement where categorized as "travel yes", patients who affirmed the third statement were categorized as "travel no". See also Section 2.6.

[2] Ole e 1 has a homologous counterpart in Fra e 1, the major allergen component of ash, thus, Ole e 1 is a marker allergen for the diagnosis of olive and ash pollen allergy (Barderas et al., 2005; Palomares et al., 2006).

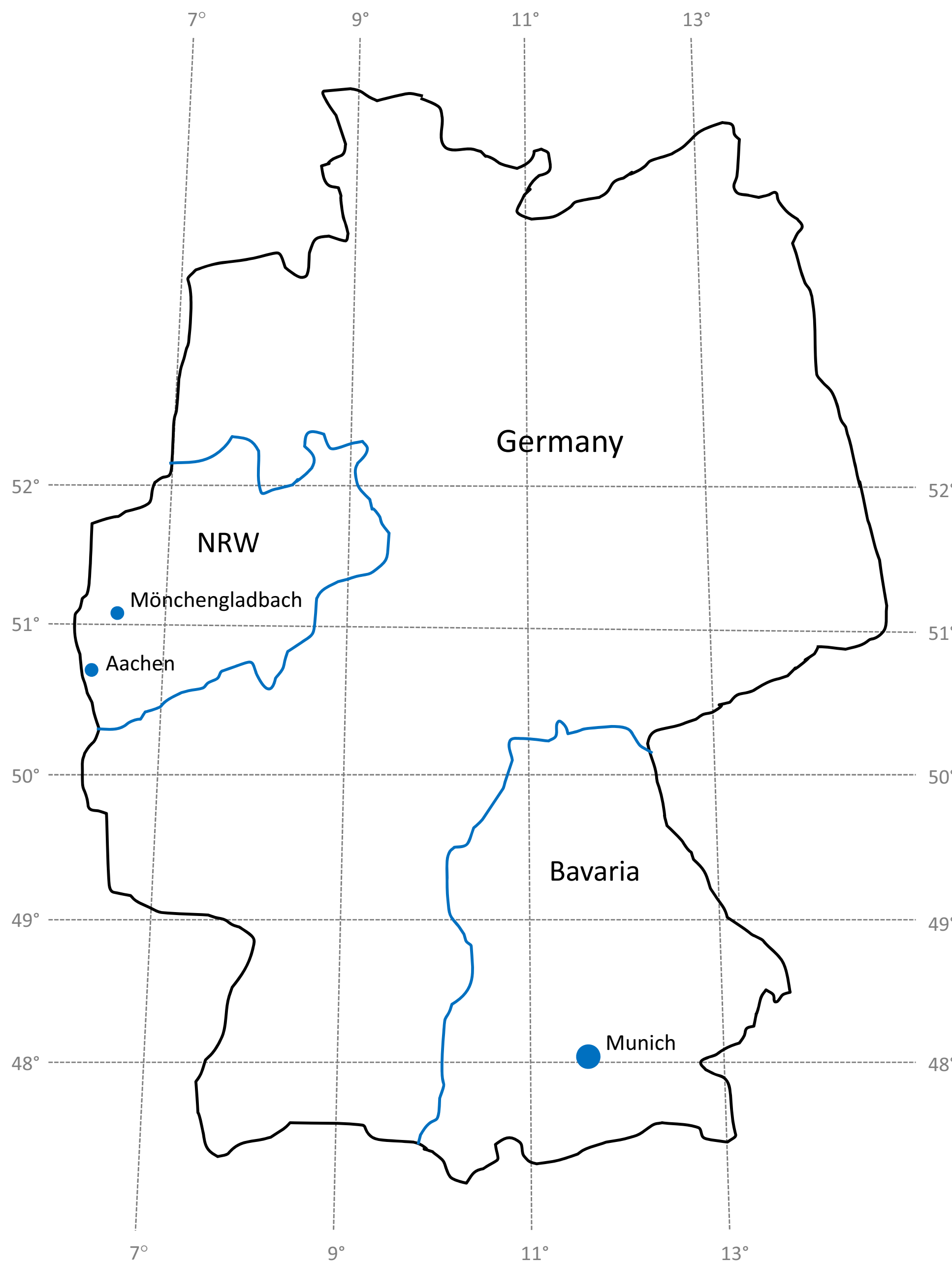

Fig. 1. Draft of the geographical locations of NRW and Bavaria. The draft was based on an administration map of Germany provided by the German Federal Agency for Cartography and Geodesy, dated January 1st 2014, scale 1:2,500,000. Vertical and horizontal degree values sketch the degrees of longitude and latitude, respectively. In NRW, the study centre was located in Aachen and the pollen counting station in Mönchengladbach. In Bavaria, the study centre and the pollen counting station were located in Munich.

years/41–50 years/>50 years), *sex* (categories: male/female), *German origin of the mother* (categories: no/yes), *living in a major city* (categories: no/yes), *travel* (categories: no/yes), *mould/dampness at home* (categories: no/yes), *pets* (categories: no/yes), *childhood on a farm* (categories: no/yes), *type of road of home address* (categories: very low traffic/moderate traffic/considerable traffic/high and accordingly very high traffic), *siblings* (categories: no/yes), *educational status* (categories: low/middle/high), *current smoking habits* (categories: not active and not passive/not active but passive/active), *allergies of the mother* (categories: no/yes/don't know), *allergies of the father* (categories: no/yes/don't know) (Haftenberger et al., 2013; Pénard-Morand et al., 2010; Robert Koch Institute and Federal Center for Health Education, 2008; Schlaud et al., 2007). Calculation of the variable *living in a major city* was based on data of cities with at least 100,000 residents on December 31st 2011, provided by the German Federal Statistical Office (German Federal Statistical Office, 2011). Calculation of the variable *educational status* was based on the CASMIN classification in which school leaving certificate and graduation are considered (Brauns and Steinmann, 1999).

Because of multiple testing (sensitization to 5 allergens and data on 5 pollen types, respectively), differences with p values <0.05 and $\geq$0.01 and accordingly OR with 95% CI <1 or >1 and =1 were described as significantly different *by trend*, and differences with p values <0.01 and accordingly OR with 95% CI <1 or >1 were described as significantly different (Bonferroni correction).

Table 1
Rates of prick test reactive patients in NRW and Bavaria. Prick test reactivity to allergen extracts was tested in adult patients with a suspected respiratory allergy (n = 935, NRW: n = 472, Bavaria: n = 463). Bivariate analysis (Chi squared test; OR, non-adjusted) was used to test for significant group differences. OR refer to test-positive patients from Bavaria in relation to test-positive patients from NRW.

Prick test positive to	NRW % (n)	Bavaria % (n)	p	OR (95% CI)
Ragweed	**18** (87)	**11** (50)	0.001	0.54 (0.37–0.78)
Ragweed-but-not-mugwort	**7** (32)	**4** (17)	0.036	0.52 (0.29–0.96)
Olive	**17** (80)	**36** (166)	0.000	2.74 (2.02–3.72)
Olive-but-not-ash	**8** (36)	**21** (96)	0.000	3.17 (2.12–4.76)
Birch	62 (294)	59 (271)	0.240	0.85 (0.66–1.11)
Mugwort	21 (99)	18 (84)	0.275	0.84 (0.60–1.16)
Ash	**28** (130)	**19** (86)	0.001	0.60 (0.44–0.82)

Bold: $p < 0.05$, OR with 95% CI ≤1 or ≥1.

3. Results

3.1. Patient characteristics

Of all 952 patients included, the average age was 41 years (min/max: 20/65 years) (NRW: 40, Bavaria: 42, p = 0.039, Mann–Whitney U test). In NRW, patients were recruited from 75 different residences, with 41.2% (196/476) of the patients living in Aachen. In Bavaria, patients were recruited from 97 different residences, with 63.7% (302/474; two patients did not answer the question on their current residence) living in Munich. 99.6% (948/952) of the patients were born in Germany (NRW: 99.4% [473/476], Bavaria: 99.8% [475/476], p = 0.250, Fisher's exact test), and 64.4% (613/952) were female (NRW: 64.7% [308/476], Bavaria: 64.1% [305/476], p = 0.925, Chi squared test). 77.1% (734/952) of the patients reported to have suffered or to suffer from asthma and/or allergic rhinitis, diagnosed by a physician (NRW: 78.2% [372/476], Bavaria: 76.1% [362/476], p = 0.782, Chi squared test).

Due to invalid prick test data (reasons: invalid test control [n = 12], Urticaria factitia [n = 3], suspected irritative reaction [n = 1], antihistamines intake within the last 2 days [n = 1]), the number of valid patients was reduced down from 952 to 935. From these 935 patients, 84.8% (793/935) reacted positive to at least one of the allergen extracts tested (NRW: 84.3% (398/472), Bavaria: 85.3% (395/463), p = 0.904, Chi squared test).

In multivariate analysis only those patients were to be included who could be precisely assigned to all independent variables listed in Section 2. Therefore, in multivariate analysis the number of valid cases was reduced down further from 935 to 855. With respect to the need for precise assignment (e.g. "no" or "yes") one exception was made: the variables *allergies of the mother* and *allergies of the father* consisted of the categories "no", "yes" and additionally "don't know". Otherwise, too many patients would have been lost.

3.2. Sensitization data

3.2.1. Prick test reactivity in patients from NRW and Bavaria

Prick test reactivity to ragweed was seen in 15% (137/935) of the patients. In Bavaria, the percentage of ragweed reactive patients was about 40% lower than in NRW (p = 0.001, Chi squared test) (Table 1). The difference was also seen if possible cross sensitization to mugwort was excluded (p = 0.036, Chi squared test) (due to the lowered case numbers only significant by trend).

Prick test reactivity to olive was seen in 26% (246/935) of the patients. In Bavaria, the percentage of olive reactive patients was about twice as high compared to NRW (p = 0.000, Chi squared test) (Table 1). The difference increased to almost three times if possible cross sensitization to ash was excluded (p = 0.000, Chi squared test).

Prick test reactivity to birch and mugwort was seen in 60% (565/935) and 20% (183/935) of the patients, respectively, and the percentages did not significantly differ between both federal states (Table 1).

Prick test reactivity to ash was seen in 23% (216/935) of the patients (Table 1). In Bavaria, the percentage of ash reactive patients was about one third lower than in NRW (p = 0.001, Chi squared test).

3.2.2. Sensitization to the respective major allergen component in prick test reactive patients from NRW and Bavaria

The rates of sensitization to the respective major allergen component in prick test reactive patients from NRW and Bavaria are given in Table 2. Note, that sensitization to Ole e 1 was analyzed in patients with prick test reactivity to olive-but-not-ash and ash-but-not-olive, as Ole e 1 is a marker for both olive as well as ash sensitization.

With respect to ragweed, olive, birch, and mugwort, no significant differences were found between both federal states. With respect to ash, the frequency of ash-but-not-olive reactive patients with sIgE to Ole e 1 was significantly lower compared to

Table 2
Sensitization to the respective major allergen component in prick test reactive patients from NRW and Bavaria. Prick test reactivity to allergen extracts was tested in adult patients with a suspected respiratory allergy (n = 935, NRW: n = 472, Bavaria: n = 463). Serum IgE to the respective major allergen components (ragweed: Amb a 1; olive/ash: Ole e 1; birch: Bet v 1; mugwort: Art v 1) was measured using ImmunoCAP ISAC technology. As Ole e 1 is a marker for both sensitization to olive as well as to ash, Ole e 1-positivity was analyzed in patients with prick test reactivity to olive-but-not-ash and ash-but-not-olive, respectively. Bivariate analysis (Chi squared test and Fisher's exact test, respectively; OR, non-adjusted) was used to test for significant group differences. OR refer to IgE-positivity in prick test positive-patients from Bavaria in relation to IgE-positivity in prick test-positive patients from NRW.

Prick test positive to	sIgE positive		p	OR (95% CI)
	NRW % (n/n)	Bavaria % (n/n)		
Ragweed	0 (0/87)	2 (1/50)	0.310	5.30 (0.21–132.67)
Olive-but-not-ash	67 (24/36)	65 (62/96)	0.823	0.91 (0.41–2.05)
Birch	90 (264/294)	92 (249/271)	0.392	1.29 (0.72–2.29)
Mugwort	37 (37/99)	35 (29/84)	0.689	0.88 (0.48–1.62)
Ash-but-not-olive	**34** (29/86)	**6** (1/16)	0.035	0.13 (0.02–1.04)

Bold: $p < 0.05$, OR with 95% CI ≤1 or ≥1.

Table 3
Rates of prick test reactive patients in NRW and Bavaria after exclusion of patients with sIgE to pollen pan-allergen components. Prick test reactivity to allergen extracts was analyzed in adult patients with a suspected respiratory allergy and negative sIgE to the pollen pan-allergen components Bet v 2, Hev b 8, Mer a 1, Phl p 12, Bet v 4, and Phl p 7 (n = 803, NRW: n = 411, Bavaria: n = 392). Bivariate analysis (Chi squared test; OR, non-adjusted) was used to test for significant group differences. OR refer to test-positive patients from Bavaria in relation to test-positive patients from NRW.

Prick test positive to	NRW % (n)	Bavaria % (n)	p	OR (95% CI)
Ragweed	**10** (43)	**5** (21)	0.001	0.48 (0.28–0.83)
Ragweed-but-not-mugwort	**7** (27)	**3** (11)	0.015	0.41 (0.20–0.84)
Olive	**12** (50)	**31** (121)	0.000	3.22 (2.24–4.65)
Olive-but-not-ash	**8** (32)	**21** (82)	0.000	3.13 (2.03–4.84)
Birch	57 (233)	55 (215)	0.599	0.93 (0.70–1.23)
Mugwort	12 (51)	13 (49)	1.000	1.01 (0.66–1.53)
Ash	**19** (80)	**13** (49)	0.007	0.59 (0.40–0.87)

Bold: $p < 0.05$, OR with 95% CI ≤1 or ≥1.

Table 4
Sensitization to the respective major allergen component in prick test reactive patients from NRW and Bavaria after exclusion of patients with sIgE to pollen pan-allergen components. Sensitization to the respective major allergen component was analyzed in prick test reactive patients with negative sIgE to the pollen pan-allergen components Bet v 2, Hev b 8, Mer a 1, Phl p 12, Bet v 4, and Phl p 7 (n = 803, NRW: n = 411, Bavaria: n = 392). Serum IgE to the respective major allergen components (ragweed: Amb a 1; olive/ash: Ole e 1; birch: Bet v 1; mugwort: Art v 1) was measured using ImmunoCAP ISAC technology. As Ole e 1 is a marker for both sensitization to olive as well as to ash, Ole e 1-positivity was analyzed in patients with prick test reactivity to olive-but-not-ash and ash-but-not-olive, respectively. Bivariate analysis (Chi squared test and Fisher's exact test, respectively; OR, non-adjusted) was used to test for significant group differences. OR refer to IgE-positivity in prick test positive-patients from Bavaria in relation to IgE-positivity in prick test-positive patients from NRW.

Prick test positive to	sIgE positive		p	OR (95% CI)
	NRW % (n/n)	Bavaria % (n/n)		
Ragweed	0 (0/43)	0 (0/21)	0.727	2.02 (0.00–105.49)
Olive-but-not-ash	66 (21/32)	62 (51/82)	0.733	0.86 (0.37–2.03)
Birch	92 (215/233)	93 (199/215)	0.910	1.04 (0.52–2.10)
Mugwort	47 (24/51)	37 (18/49)	0.296	0.65 (0.29–1.45)
Ash-but-not-olive	34 (21/62)	10 (1/10)	0.161	0.22 (0.03–1.83)

olive-but-not-ash reactive patients (29% and 65%, respectively; $p < 0.001$, Chi squared test). Furthermore, only 6% (1/16) of the Bavarian patients with ash-but-not-olive reactivity showed sensitization to Ole e 1 in contrast to 34% (29/86) of the NRW patients ($p = 0.035$, Fisher's exact test).

Data on the association of specific IgE to the major allergen component and respective prick reactivity are given in supplemental data Table 1.

3.2.3. Effect of sIgE to pollen pan-allergen components on the sensitization pattern

14% (132/935) of the patients with valid prick test data (NRW: 7% [61/935], Bavaria: 8% [71/935]; $p = 0.290$, Chi squared test) were sIgE positive to at least one of the pollen pan-allergen components Bet v 2, Hev b 8, Mer a 1, Phl p 12, Bet v 4, or Phl p 7. Exclusion of these patients from analysis did not significantly change the regional differences in sensitization to ragweed, olive, birch, mugwort, and ash (Tables 3 and 4). Due to the lowered case numbers, regional differences in sensitization to Ole e 1 in ash-but-not-olive reactive patients did not reach statistical significance.

3.2.4. Effect of "non-exposure" variables on the sensitization pattern

Detailed data on the distribution of non-exposure variables in NRW and Bavaria are given in supplemental data Table 2. Patients in NRW and Bavaria significantly differed in *age* ($p = 0.001$, Chi squared test), *living in a major city* ($p = 0.002$, Chi squared test), *pets* ($p = 0.001$, Chi squared test), *type of road of home address* ($p = 0.026$, Chi squared test), *educational status* ($p = 0.005$, Chi squared test), *allergies of the mother* ($p = 0.001$, Chi squared test), and *allergies of the father* ($p = 0.001$, Chi squared test). However, inclusion of non-exposure variables into data analysis did not essentially affect the regional sensitization pattern of ragweed, olive, birch, mugwort, and ash, as seen by comparison of the respective OR and adjusted OR (Table 5).

3.3. Pollen data

Ragweed, olive, birch, mugwort, and ash pollen data from 2006 to 2011, collected at the PID pollen counting stations in Mönchengladbach (NRW) and Munich (Bavaria), are summarized in Table 6. Ragweed pollen load was comparably low in both counting stations. Due to its rare appearance, data on olive pollen had not been collected systematically and were assumed to approach zero in both counting stations. Birch pollen load was comparably high in both counting stations. With respect to mugwort pollen load, the magnitude was about two times higher and the duration was about two times longer at the pollen counting station in NRW ($p = 0.028$ and $p = 0.027$, respectively, Wilcoxon test). With respect to ash pollen load, the magnitude was about four times higher at the pollen counting station in Bavaria ($p = 0.043$, Wilcoxon test).

4. Discussion

The study analyzed the status quo sensitization to ragweed, olive, birch, mugwort, and ash in adult allergy patients from two German federal states with different climatic conditions. The study revealed unexpected regional differences in the sensitization pattern, which were neither explainable by cross-reactivity to pollen pan-allergen components or non-exposure variables nor by reported plant population or pollen data: sensitization to ragweed measured by prick test reactivity was higher in NRW compared to Bavaria, and sensitization to Amb a 1 in ragweed reactive patients was de facto negative in both federal states, although (i) the reported ragweed population was higher in Bavaria than in NRW and (ii) pollen data showed a comparably low ragweed pollen load in both federal states. Sensitization to olive measured by prick test reactivity was higher in Bavaria than in NRW, and sensitization to Ole e 1 in olive-but-not-ash reactive patients was comparably high in both federal states, although (i) field grown olive is not established in Germany and (ii) pollen load was assumed to approach zero in both federal states. Sensitization to ash measured by prick

Table 5
Effect of “non-exposure” variables on the sensitization pattern of ragweed, olive, birch, mugwort, and ash. Prick test reactivity to allergen extracts was analyzed in adult patients with a suspected respiratory allergy and precise assignment to all non-exposure variables ($n = 855$, NRW: $n = 428$, Bavaria: $n = 427$). Bivariate analysis (Chi squared test; OR, non-adjusted) and multivariate analysis (binary logistic regression; aOR, adjusted) were used to test for significant group differences. Non-exposure variables included age, sex, German origin of the mother, living in a major city, travel, mould/dampness at home, pets, childhood on a farm, type of road of home address, siblings, educational status, current smoking habits, allergies of the mother, and allergies of the father. OR and aOR, respectively, refer to test-positive patients from Bavaria in relation to test-positive patients from NRW.

Prick test positive to	NRW % (n)	Bavaria % (n)	OR (95% CI)	aOR (95% CI)
Ragweed	**19** (80)	**11** (49)	0.56 (0.38–0.83)	0.54 (0.36–0.82)
Ragweed-but-not-mugwort	**7** (29)	**4** (16)	0.54 (0.29–1.00)	0.51 (0.26–1.00)
Olive	**17** (74)	**37** (157)	2.78 (2.02–3.83)	3.09 (2.20–4.35)
Olive-but-not-ash	**7** (32)	**21** (90)	3.31 (2.15–5.07)	3.97 (2.51–6.28)
Birch	63 (271)	59 (254)	0.97 (0.74–1.27)	0.87 (0.64–1.16)
Mugwort	21 (90)	18 (78)	0.84 (0.60–1.18)	0.85 (0.59–1.21)
Ash	**29** (123)	**19** (81)	0.58 (0.42–0.80)	0.59 (0.42–0.82)

Bold: OR and aOR, respectively, with 95% CI ≤1 or ≥1.

Table 6
Pollen data from ragweed, olive, birch, mugwort, and ash from 2006 to 2011, collected at the PID pollen counting stations in Mönchengladbach (NRW) and Munich (Bavaria). (A) Magnitude: sum of daily pollen totals/m^3 air per year, (B) duration: sum of pollen-positive days per year.

Pollen type/year	Ragweed		Olive		Birch		Mugwort		Ash	
	NRW	Bavaria	NRW	Bavaria	NRW	Bavaria	NRW	Bavaria	NRW	Bavaria
A: Magnitude										
2006	20	5	Ø	Ø	10,936	9,073	81	69	n.a.	11,386
2007	4	2	Ø	Ø	7,513	10,186	162	42	133	2,173
2008	0	16	Ø	Ø	8248	9,727	102	30	1,033	3,806
2009	1	0	Ø	Ø	6195	5,751	70	48	446	4,026
2010	5	6	Ø	Ø	6600	5,752	86	10	1,495	4,066
2011	8	1	Ø	Ø	7963	3,379	113	47	1,852	2,596
Median	5	4	Ø	Ø	7,738	7,413	**98**	**45**	**1,033**	**3,916**
(p)	(0.528)		(n/a)		(0.600)		(0.028)		(0.043)	
B: Duration										
2006	11	3	Ø	Ø	44	43	28	21	n.a.	37
2007	4	2	Ø	Ø	49	54	36	14	17	43
2008	0	6	Ø	Ø	69	49	27	5	71	48
2009	1	0	Ø	Ø	46	40	25	10	23	14
2010	5	5	Ø	Ø	62	42	23	3	43	32
2011	6	1	Ø	Ø	69	28	37	13	51	28
Median	5	3	Ø	Ø	56	43	**28**	**12**	43	35
(p)	(0.345)		(n/a)		(0.074)		(0.027)		(0.498)	

Ø: due to the rare appearance of olive pollen, data were not systematically collected and were assumed to approach zero; n.a.: not analyzed, n/a: not applicable. *Bold*: $p < 0.05$, Wilcoxon test for paired samples.

test reactivity was higher in NRW than in Bavaria, and so was sensitization to Ole e 1 in ash-but-not-olive reactive patients, although pollen data showed a higher pollen load in Bavaria (population data on ash were not available). Furthermore, sensitization to mugwort measured by prick test reactivity was comparable in NRW and Bavaria, and so was sensitization to Art v 1 in mugwort reactive patients, although pollen data showed a higher pollen load in NRW (population data on mugwort were not available).

When discussing plant population or pollen data in relation to sensitization data, however, the following aspects need to be considered:

- Data on plant population may be based on voluntary communication of locality and not on a systematic field mapping. At present, in Germany this is the case for data on ragweed population.
- It might take 10–15 years from the first detection of new pollen types and the incidence of relevant sensitization rates (and another 5 years for the development of symptoms) (Jäger, 2000; Tosi et al., 2011).
- Pollen data collected at pollen counting stations show the *potential* of individual pollen exposure. Statements on *genuine* individual pollen exposure can only be gained by personalized measures.
- Pollen measurements taken at pollen counting stations are punctual measurements. More pollen counting stations would ensure a more accurate statement on the potential of individual exposition. In this study, pollen data from only one station from each federal state were analyzed. Patients, however, were recruited from the entire region of the respective federal state.
- Preliminary data suggest that quantitative pollen data alone might not be the optimal marker for allergen exposure (Buters et al., 2010; Galan et al., 2013).

Regarding sensitization to Amb a 1, the major allergen component of ragweed, one has to keep in mind the reduced sensitivity of the ISAC technology compared to the more frequently used ImmunoCAP technology (Melioli et al., 2011; Wöhrl et al., 2006). If the reduced sensitivity of the ISAC test did not account for the very low frequency of Amb a 1-positive patients, it would mean that neither the regional ragweed population nor the regional ragweed pollen load nor travel activities had (so far) led to an exposure effective enough to induce genuine ragweed sensitization.

With respect to olive, we had to choose a pragmatic approach to discriminate between olive and ash sensitization. This approach cannot completely compensate the lack of marker allergen component(s) to distinguish primary olive from primary ash sensitization. However, our prick test data indicate that in Germany olive sensitization independently from ash sensitization does exist. One might speculate that prick test solutions from olive and ash used in this study might display different allergenic potential (a problem which, due to the lack of overall comparability between standardized test solution products, applies effectively to all skin prick test data).

But then, olive-but-not-ash OR ash-but-not-olive reactive patients would not have been detectable, if olive reactivity would be a mere result of overlapping ash reactivity.

The high rate of olive-but-not-ash reactive patients with sensitization to Ole e 1 might suggest previous contact with olive pollen. In Germany, field-grown olive does not exist and significant olive pollen load has not been found so far, meaning that on-site sensitization could hardly have happened. Thus, sensitization may have been induced by travelling to respective pollution areas. As Ole e 1 is a marker for all trees of the family *Oleaceae*, which besides olive and ash, both wind-pollinated, also includes the insect-pollinated genera forsythia, lilac, privet, and jasmine, another explanation may be sensitization to one of these plants (Asero, 2011; Palomares et al., 2006).

5. Conclusions

Regarding the allergic potential of plants not (yet) native in Germany, the following conclusions may be drawn from the study results:

- Establishing allergic plants like ragweed can have an allergic risk potential due to cross reactivity, even if its population/pollen load is not (yet) large enough to induce genuine sensitization.
- Not established allergic plants like olive can have an allergic risk potential due to genuine sensitization. If these plants would start to establish this could result in prompt occurrence of allergic symptoms.

Climate change associated changes in the allergenic pollen and sensitization spectrum should be identified as early as possible to adapt (i) prevention measures (e.g. avoidance of respective allergenic plants in the public area, specific removal), (ii) diagnostic measures (e.g. provision with respective test allergens), and (iii) therapy measures (e.g. development of respective allergen components for specific immunotherapy). According to the data presented, an early identification would need the following systematic time and spatial close meshed monitoring measures (with the degree of *close meshed* defined by scientists in cooperation with policy makers):

- field mapping of indicator plants,
- monitoring of exposure to indicator allergens,
- monitoring of sensitization to indicator allergens, both with allergen extract as well as with major allergen components.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijheh.2016.01.007.

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