International Archives of Allergy and Immunology

Clinical Allergy

Int Arch Allergy Immunol
DOI: 10.1159/000551344

Received: November 28, 2025
Accepted: February 27, 2026
Published online: May 19, 2026

Downloaded from http://karger.com/iaa/article-pdf/doi/10.1159/000551344/4538628/000551344.pdf by Fachbibliothek Umwelt des Umweltbundesamtes user on 27 July 2026

Threshold Concentration of Ragweed Pollen for Inducing Allergic Symptoms: Initial Data Using an Allergen Challenge Chamber

Conny Höflich[a] Philipp Badorrek[b] Christina Gress[b, c] Meike Müller[b, c]
Wolfgang Straff[a] Jens M. Hohlfeld[b, c, d]

[a]German Environment Agency (UBA), Environmental Medicine and Health Effects Assessment, Berlin, Germany; [b]Fraunhofer Institute for Toxicology and Experimental Medicine ITEM, Hannover, Germany; [c]German Center for Lung Research (DZL), Biomedical Research in Endstage and Obstructive Lung Disease Hannover BREATH, Hannover, Germany; [d]Hannover Medical School, Department of Respiratory Medicine and Infectious Disease, Hannover, Germany

Keywords
Ragweed pollen · Allergic rhinitis · Allergen challenge chamber · Basophil activation test · Threshold concentration

Abstract
Introduction: Climate change may increase the spread of allergenic plants such as ragweed. Ragweed pollen is assumed to be more allergenic than birch pollen. Determining pollen threshold concentrations triggering symptoms is challenging. Technical modifications to the Fraunhofer allergen challenge chamber (ACC) now allow investigation of very low pollen concentrations. Based on Total Nasal Symptom Score (TNSS) data from 15 subjects with birch pollen allergic rhinitis (AR), a birch pollen threshold between 50 and 100 pollen grains/m^3 of air was previously derived. This study explored threshold concentrations of ragweed pollen. ***Methods:*** Two ragweed pollen AR patients were exposed to varying concentrations of ragweed pollen for 4 h. TNSS was recorded every 20 min, and nasal secretion and peak nasal inspiratory flow hourly. Area under the curve ($AUC_{0\text{-}240}$) values were calculated. Ex vivo basophil activation tests with varying concentrations of ragweed pollen, Amb a 1, birch pollen, and Bet v 1 were performed for both ragweed pollen AR patients and 6 of the 15 birch pollen AR patients. Mean effective concentrations (EC_{50}) for CD63 expression were calculated. ***Results:*** Ragweed pollen exposure resulted in the following TNSS $AUC_{0\text{-}240}$ values: 28/23 at 0, 18/17 at 50, 13/27 at 100, 40/40 at 200, 29/16 at 300, and 55/47 at 1,000 pollen grains/m^3. For CD63 expression, the mean EC_{50} was 277 pollen/mL for ragweed pollen, 884 ng/mL for Amb a 1, 11 pollen/mL for birch pollen, and 0.13 ng/mL for Bet v 1. On average, the proportion of Amb a 1 in ragweed pollen was 0.17%, and the proportion of Bet v 1 in birch pollen 1.96%. ***Conclusion:*** The modified Fraunhofer ACC can investigate pollen threshold concentrations under defined conditions. The assumed differences in threshold concentrations of ragweed and birch pollen for triggering symptoms were not confirmed. To derive reliable threshold concentrations for ragweed pollen, larger studies have to be followed.

© 2026 The Author(s).
Published by S. Karger AG, Basel

Edited by: H.-U. Simon, Bern.

karger@karger.com
www.karger.com/iaa

© 2026 The Author(s).
Published by S. Karger AG, Basel

This article is licensed under the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC) (https://karger.com/Services/OpenAccessLicense). Usage and distribution for commercial purposes requires written permission.

Correspondence to:
Conny Höflich, conny.hoeflich@uba.de

Table 1. Characteristics of participating subjects

Study population	Test received	Participant number	Gender	Age, years	Specific IgE to Amb a 1 (Ragweed pollen study population)/ Bet v 1 (Birch pollen study population), kU/L	FEV1, % pred	Mean TNSS 140–240 min at screening challenge
Ragweed pollen AR	ACC, BAT	1	Male	48	3.3	94.0	6
		2	Male	34	15.4	101.8	5.7
Ragweed pollen control group	BAT	4	Female	32	<0.1	–	–
		6	Female	29	0.68	–	–
Birch pollen AR	BAT	3	Female	58	11.1	105.9	1.5
		5	female	40	12.4	112.2	3.3
		7	Male	55	7.6	115.5	3.7
		9	Female	43	4.4	105.6	1.2
		11	Female	26	34.3	98.0	2.2
		12	Female	30	4.3	107.2	2.8
Birch pollen control group	BAT	20	Male	55	<0.1	–	–
		21	Male	49	0.91*	–	–
		22	Female	22	N/A	–	–
		23	Female	31	<0.1	–	–
		24	Female	29	0.31	–	–
		25	Female	22	N/A	–	–

AR, allergic rhinitis; ACC, allergen challenge chamber; BAT, basophil activation test; kU/L, kilounits/L; FEV1, forced expiratory volume in 1 s; % pred., percent of predicted normal; TNSS, Total Nasal Symptom Score; N/A, not available; –, no data collected. *Study participant 21 showed a slightly elevated specific IgE to Bet v 1 (CAP class 2). However, there was no allergy to birch pollen. The participant's in vitro results were comparable to those of the other healthy controls.

Introduction

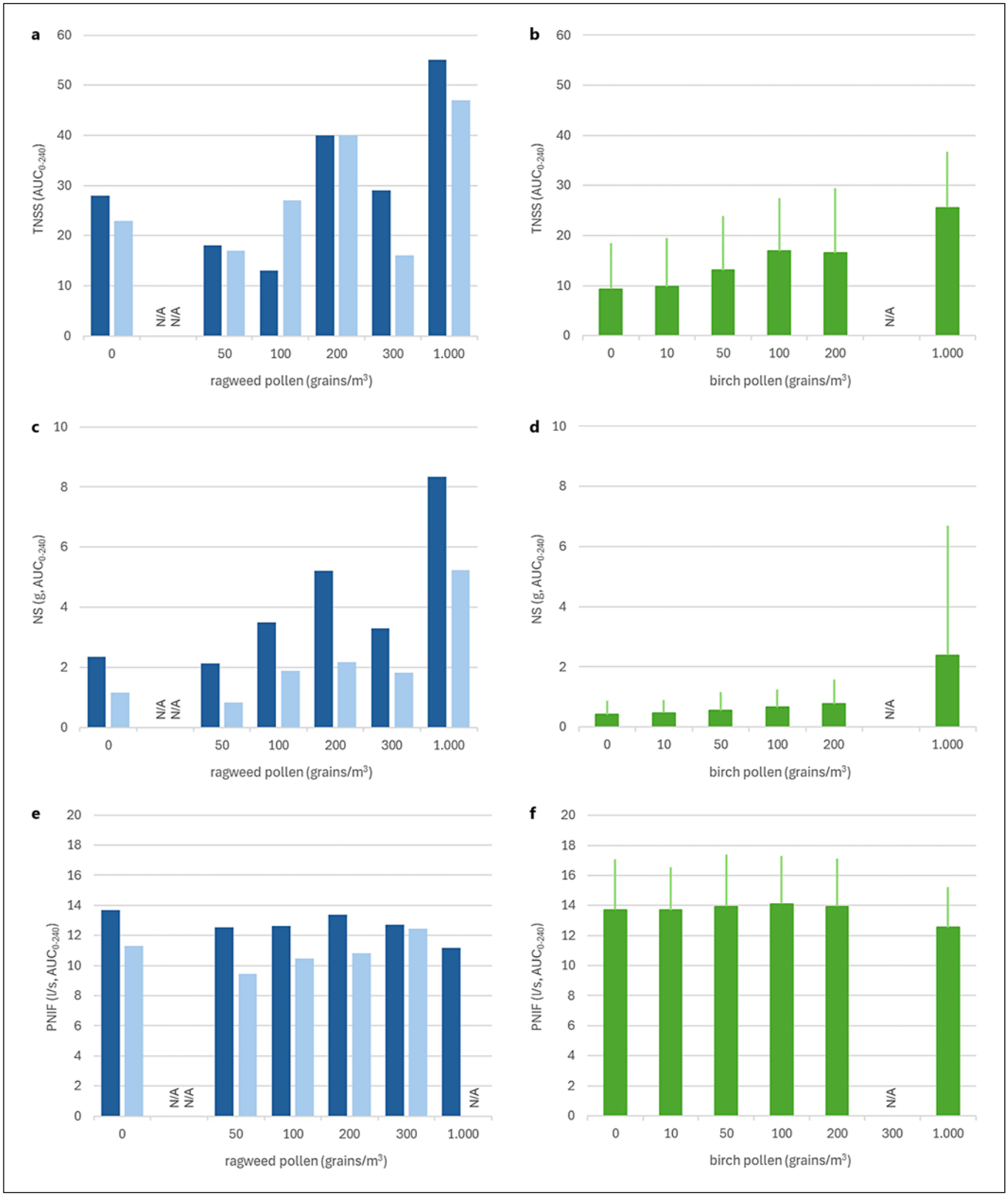
Ragweed, a plant native to North America, probably arrived in Europe via grain or clover seeds [1]. Today, it is mainly found in France (Rhone Valley), Italy (Po Valley), Hungary, and neighboring countries like Romania and Ukraine [1–3]. It was found growing wild in Germany as early as 1860 and was long considered unstable and rare, but it has been spreading for several years [1]. Nowadays, the region around Drebkau in south-eastern Brandenburg is considered a ragweed hotspot [4, 5].

The plant flowers in late summer and early autumn. It poses a problem for both, agriculture and the health system, because it occurs as a "weed" and reduces crop yields, and because of its high sensitization and allergy potential, respectively [2, 3, 5].

According to a provocation study with ragweed pollen grains applied from gelatine capsules by nasal insufflation, only 10 ragweed pollen grains triggered allergic reactions [6]. A systematic literature analysis from the period July–August 2021 in the databases PubMed and Scopus identified 6 original studies describing threshold values for ragweed pollen and 6 describing threshold values for birch pollen [7]. In the majority of these studies, threshold values in the double-digit range were reported. However, due to differences in methodology, study population, and target organs, results can hardly be compared [7]. In 2022, a field study under natural conditions, conducted in Northern Italy, revealed ragweed pollen concentration thresholds of 1.33, 3.33, 14.5, and 50.67 pollen grains/m^3 for low, medium-low, medium-high, and high symptom severity levels, respectively [8]. Currently, in Germany a daily average of more than 10 ragweed pollen grains/m^3 is classified as high stress intensity, and the corresponding value for birch pollen, one of the main allergenic pollen in Germany, is 50 [9].

None of the above-cited studies used an allergen challenge chamber (ACC) approach for threshold investigation. ACC allows natural exposure under defined environmental conditions. Technically, they are aimed at ensuring pollen concentrations high enough to surely

Downloaded from http://karger.com/iaa/article-pdf/doi/10.1159/000551344/4538628/000551344.pdf by Fachbibliothek Umwelt des Umweltbundesamtes user on 27 July 2026



(For legend see next page.)

Downloaded from http://karger.com/iaa/article-pdf/doi/10.1159/000551344/4538628/000551344.pdf by Fachbibliothek Umwelt des Umweltbundesamtes user on 27 July 2026

induce allergic symptoms, and this might explain why ACC has not been used for threshold studies so far.

Technical modifications to the Fraunhofer ACC have made it possible to investigate the effects of very low pollen concentrations on humans. Based on Total Nasal Symptom Score (TNSS) data from 15 patients with birch pollen seasonal allergic rhinitis (AR), we recently derived a threshold concentration between 50 and 100 pollen grains/m^3 of air for birch pollen [10].

Next, we aimed at deriving an ACC-generated threshold value for ragweed pollen. Additionally, we wanted to compare allergenicity of ragweed and birch pollen using ex vivo basophil activation test (BAT).

Methods

Pollen

A single batch of ragweed pollen (105 g of batch number AMBE.0321) and a single batch of birch pollen (105 g of batch number BETP.1021) were purchased from Pharmallerga (Lisov, Czech Republic). Pollen was produced, tested, and released by the manufacturer according to ISO 9001 standards and stored at the study site under controlled conditions at 5 ± 3°C.

For use in ACC experiments, ragweed pollen aliquots were poured into the reservoir of the pollen dispenser, and the desired pollen concentration was generated as described by Struß et al. [10]. In the chamber, pollen concentrations were controlled using two Rotorod samplers (Rotorod, Multidata, Minneapolis, MN, USA).

For BAT experiments, pollen mass was converted into pollen count using a Neubauer counting chamber by counting pollen in 10 µL of a suspension of 2.5 mg pollen suspended in 1 mL phosphate-buffered saline (Fisher Scientific GmbH, Schwerte, Germany) supplemented with 0.5% bovine serum albumin (Sigma-Aldrich, Taufkirchen, Germany). The concentration of Amb a 1 and Bet v 1 in ragweed and birch pollen, respectively, was measured by ELISA (Indoor Biotechnologies, Charlottesville, USA).

ACC Experiments

Detailed information on the basic study design including targeted sample size, study population, ACC settings, exposure sequence, study endpoints, and statistics is given in Struß et al. [10]. Shortly, in analogy to birch pollen exposures a sample size of at least 15 adult patients with primary ragweed pollen AR was aimed at, the validated Fraunhofer ACC would be run according to site-specific standard operation procedures, allergen challenges would last for 4 h, exposure sequence would be partly customizable depending on data, primary ACC study endpoint would be TNSS data from 140 to 240 min, and secondary endpoints would be peak nasal inspiratory flow (PNIF) and nasal secretion (NS) [10].

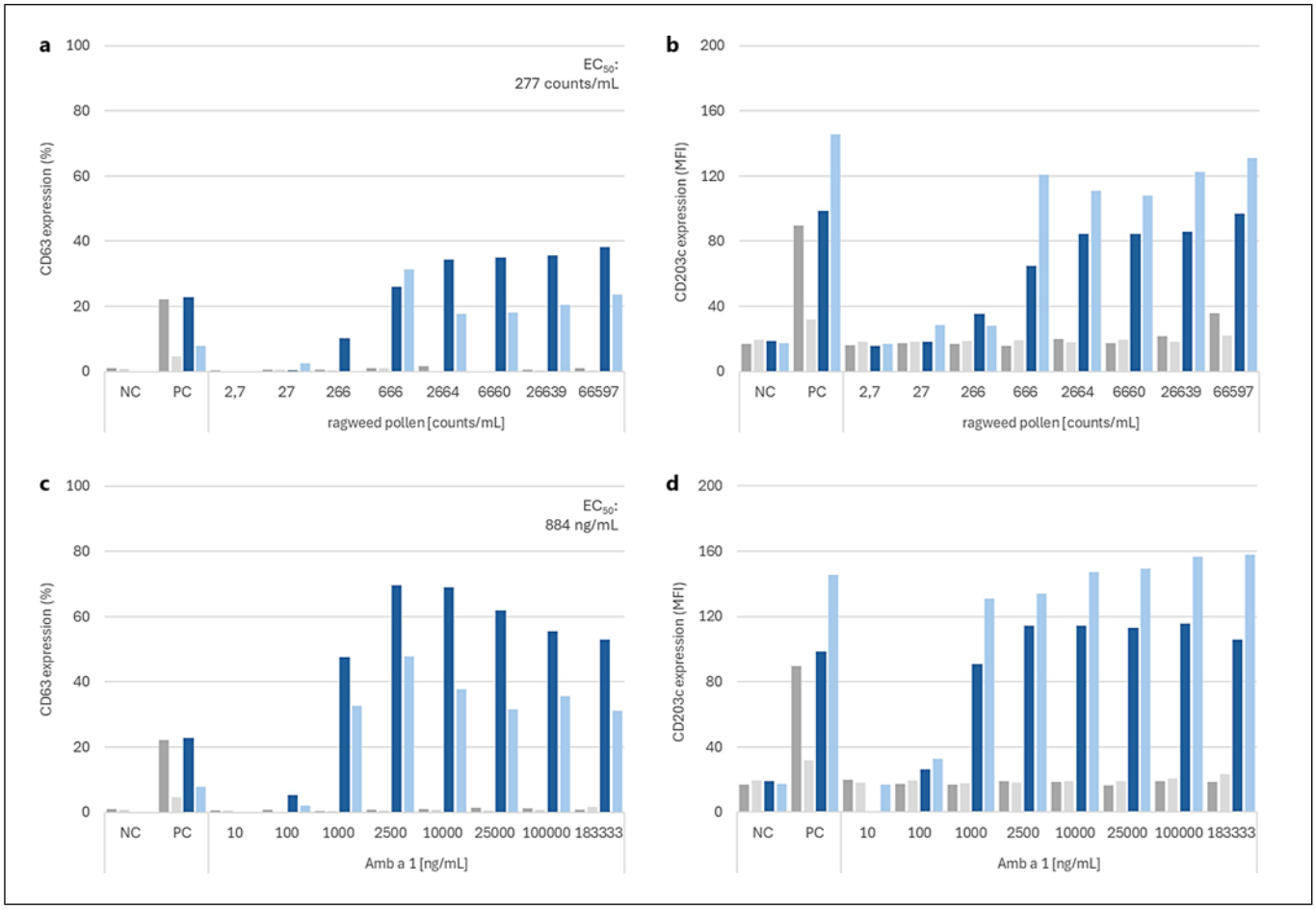
As in the greater Hanover area, only 6 patients with ragweed pollen AR could be identified over a period of 13 months despite intensive screening activities with social media campaigns and newspaper advertisements, and as only 2 of them could be allocated to screening exposure, adjustments had to be made to (i) sample size (n = 2), (ii) study population (negation of the upper limit of the inclusion criterion "mild to moderate rhinitis symptoms defined by a mean TNSS between 1 and 8 after the 4-hour screening challenge with 1,000 pollen/m^3"), (iii) exposure sequence (set at 0, 300, 50, 200, 100 pollen grains/m^3), and (iv) statistics (calculation of area under the curve [AUC] values from data from 0 to 240 min). Ragweed pollen exposures took place between October 2024 and December 2024. Nasal symptoms were evaluated as a patient-reported outcome prior to and every 20 min during allergen challenge using the TNSS. PNIF and NS were assessed hourly. Details for assessment of TNSS and NS are described by Struß et al. [10]. PNIF was measured using a pneumotach (Jaeger Medical GmbH, Höchberg, Germany) connected to a tight-sealing disposable face mask with a bacterial filter. Participants performed 2–3 tidal nasal breaths (mouth closed), a full exhalation, and a maximal nasal sniff. Three acceptable maneuvers were obtained, and the highest value was reported.

BAT Experiments

BAT was performed as described by Carstensen et al. [11]. Peripheral blood was used from (i) patients with ragweed pollen AR, (ii) patients with birch pollen AR, and (iii) respective healthy controls.

Fig. 1. $AUC_{0\text{-}240}$ values for TNSS (**a**, **b**), NS (**c**, **d**), and PNIF (**e**, **f**). Dark and light blue bars show values of participant 1 and participant 2 with ragweed pollen AR, respectively. Green bars and vertical lines show mean values and standard deviation, respectively, of 15 participants with birch pollen AR, described in detail [10]. Due to a technical error, the PNIF value for 1,000 pollen/m^3 was not available for participant 2 with ragweed pollen AR. $AUC_{0\text{-}240}$, area under the curve 0–240 min; TNSS, Total Nasal Symptom Score; NS, nasal secretion; g, gram; PNIF, peak nasal inspiratory flow; L/s, liter per second; N/A, not available.

Downloaded from http://karger.com/iaa/article-pdf/doi/10.1159/000551344/4538628/000551344.pdf by Fachbibliothek Umwelt des Umweltbundesamtes user on 27 July 2026



(Figure continued on next page.)

Ragweed pollen was tested at final concentrations of 250,000, 100,000, 25,000, 10,000, 1,000, 100, and 10 ng/mL corresponding to 66,597, 26,639, 6,660, 2,664, 666, 266, 27, and 2.7 pollen/mL. Amb a 1 (BIOZOL Diagnostica Vertrieb GmbH, Hamburg, Germany) was tested at final concentrations of 183,333, 100,000, 25,000, 10,000, 2,500, 1,000, 100, and 10 ng/mL. Birch pollen was tested at final concentrations of 2,500, 1,000, 500, 250, 100, 25, 10, and 1 ng/mL, corresponding to 509.7, 203.9, 101.9, 51.0, 20.4, 5.1, 2.0, and 0.2 pollen/mL. Bet v 1 (Hölzel Diagnostika Handels GmbH, Cologne, Germany) was tested at final concentrations of 10, 2.5, 1.0, 0.75, 0.5, 0.25, and 0.1 ng/mL. Anti-IgE (HP6061, final concentration 0.45 µg/mL; SouthernBiotech, Birmingham, UK) was used as positive control, and assay buffer as negative control. Basophilic expression of CD63 (% positive cells) and CD203c (mean fluorescence intensity) was used as readout parameters. BATs with birch pollen and Bet v 1 were done between January 2023 and March 2023, BATs with ragweed pollen and Amb a 1 in February 2025.

Statistics

For ACC data, AUC values from 0 to 240 min were calculated by summing the corresponding TNSS, PNIF, and NS data, respectively. For BAT data, GraphPad Prism 10.4.1 was used to calculate EC_{50} values by implementing a four-parameter, nonlinear agonist regression model.

Results

Participants and Demographics

Characteristics of participating subjects are given in Table 1. For BAT, the 6 participants with birch pollen AR were recruited from the study population of 15 patients participating in the birch pollen ACC experiments [10].

Downloaded from http://karger.com/iaa/article-pdf/doi/10.1159/000551344/4538628/000551344.pdf by Fachbibliothek Umwelt des Umweltbundesamtes user on 27 July 2026

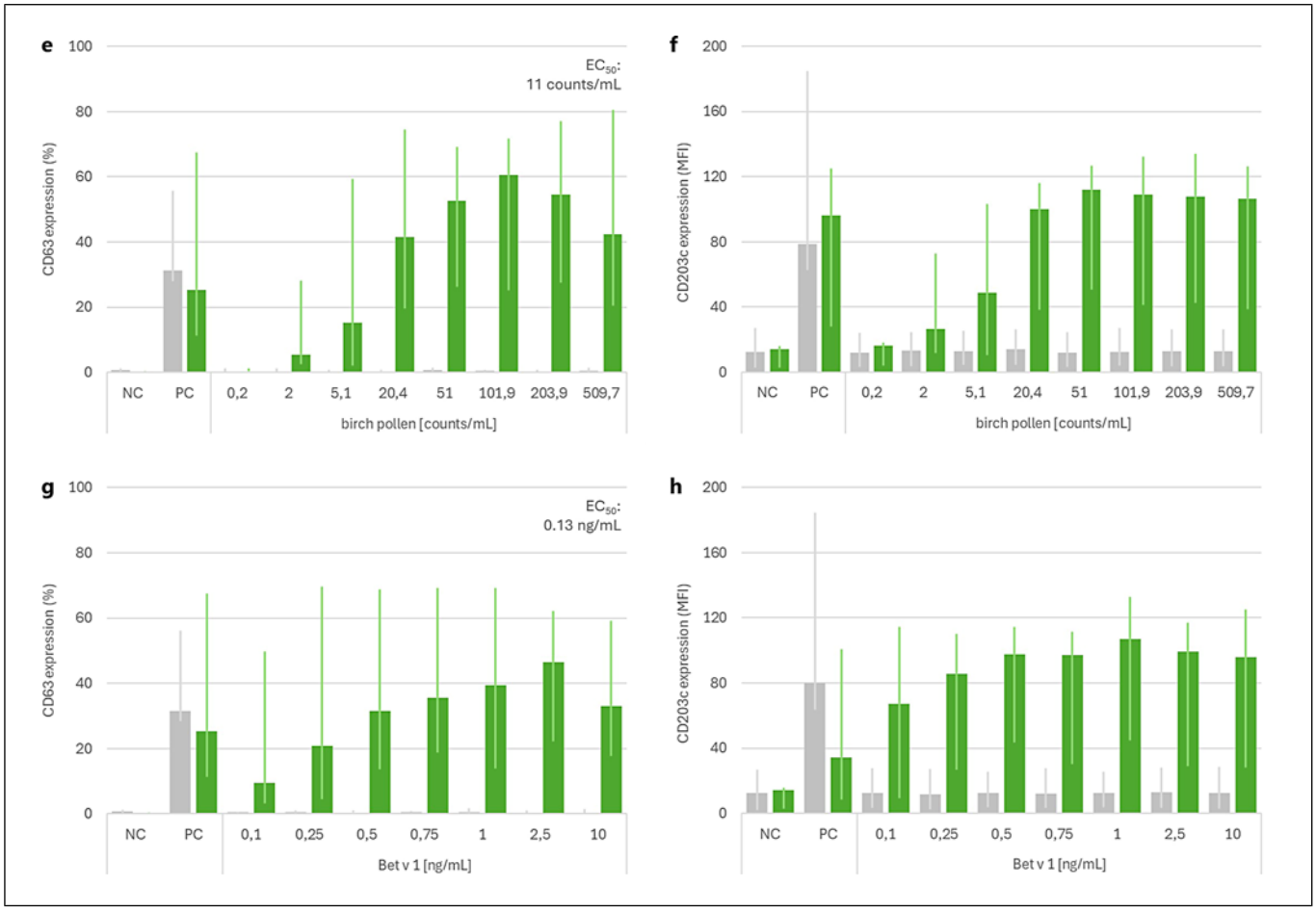


Fig. 2. Basophilic CD63 and CD203c expression in response to ragweed pollen (**a**, **b**), Amb a 1 (**c**, **d**), birch pollen (**e**, **f**), and Bet v 1 (**g**, **h**). Dark and light blue bars show values of participant 1 and participant 2 with ragweed pollen AR, respectively. Green bars show values of participants with birch pollen AR. Gray bars show values of respective healthy controls. In **e–h**, bars indicate median values and vertical lines respective minimum and maximum values of $n = 6$ each. EC_{50}, mean effective concentration for respective patient data; NC, negative control; PC, positive control; MFI, mean fluorescence intensity.

Participants of the respective healthy control groups were recruited from the Fraunhofer ITEM study participant database.

ACC Data

The evaluation of the two Rotorod samplers yielded the following pollen chamber concentrations (mean pollen grains/m^3 ± % deviation), and respective target concentrations (pollen grains/m^3) are given in square brackets: 0 ± 0 [0], 47.0 ± 6.0 [50], 96.5 ± 3.5 [100], 212.0 ± 6.0 [200], 311.5 ± 3.8 [300], and 1,016 ± 1.6 [1,000]. For the corresponding pollen concentrations, outcomes for TNSS, NS, and PNIF are shown as $AUC_{0\text{-}240}$ values in Figure 1. In addition to data from the 2 patients with ragweed pollen AR, respective mean $AUC_{0\text{-}240}$ values from the birch pollen AR cohort, described in [10], are shown. As expected, highest TNSS and NS values and lowest PNIF values were found at the highest pollen concentration, 1,000 pollen grains/m^3.

BAT Data

Data on basophil activation measured by CD63 and CD203c expression are shown in Figure 2. In blood from AR patients, the mean EC_{50} for CD63 expression was 277 pollen/mL for ragweed pollen, 884 ng/mL for Amb a 1, 11 pollen/mL for birch pollen, and 0.13 ng/mL for Bet v 1. In blood from control participants, no basophil activation was observed. On average, 1,000 ng ragweed pollen contained 1.7 ng Amb a 1 and 1,000 ng birch pollen contained 19.6 ng Bet v 1 meaning an Amb a 1 proportion of 0.17% and a Bet v 1 proportion of 1.96%, respectively.

Downloaded from http://karger.com/iaa/article-pdf/doi/10.1159/000551344/4538628/000551344.pdf by Fachbibliothek Umwelt des Umweltbundesamtes user on 27 July 2026

Discussion

Within the study period, in the greater Hanover area only 2 patients with ragweed pollen AR could be allocated to screening exposure. Like most parts of Germany, the greater Hanover area is not yet a ragweed hotspot. It is possible that a larger number of patients with ragweed pollen AR would have been found in the ragweed hotspot region around Drebkau in south-eastern Brandenburg [4, 5]. However, searching for patients in this region and testing patients from this region in the Fraunhofer ACC would have exceeded the time, and financial and logistical scope of this study.

Due to the limited number of patients with ragweed pollen AR, respective ACC and BAT experiments could only provide initial indicative data on ragweed pollen thresholds. Interestingly, these preliminary data do not support the assumed difference in the allergenic potential of ragweed and birch pollen: (1) while the threshold concentration of birch pollen for inducing symptoms in patients with birch pollen AR was between 50 and 100 pollen/m^3 [10], both patients with ragweed pollen AR showed relevant clinical symptoms at concentrations of 200 ragweed pollen/m^3. (2) The concentrations of ragweed pollen and Amb a 1 required to activate basophils in the BAT were many times higher than those of birch pollen and Bet v 1, respectively.

To obtain reliable data on ragweed pollen thresholds, studies with larger groups of test subjects have to be followed. Furthermore, our experiments are valid for the groups of participating subjects and pollen batches only and need to be confirmed by other study centers.

Besides intrinsic factors, extrinsic factors like air quality and climate change affect the allergenicity of pollen and the allergic predisposition of people [12]. Both ACC and BAT cannot reflect the complexity of the real world, as field studies can, but can provide data under controlled environmental conditions and, in the case of ACC, under natural exposure conditions. Thus, ACC and BAT are powerful tools for comparing the allergenic potential of pollen, for instance, in the context of climate change-associated changes in pollen allergy.

Statement of Ethics

The study was reviewed and approved by the Local Ethics Committee of Hannover Medical School, Approval No. 10024_BO_S_2021, and was conducted in compliance with the Declaration of Helsinki (7th revision, Fortaleza, Brazil), International Council for Harmonisation Guideline for Good Clinical Practice (E6, Step 4, November 2016), and all national laws and regulations. Written informed consent was obtained from the participants for participation in the study and publication of their medical details.

Conflict of Interest Statement

Conny Höflich, Wolfgang Straff, Meike Müller, and Christina Gress have no conflict of interest to disclose. Philipp Badorrek reports personal fees from LETI Pharma GmbH, which are outside the submitted work. Jens M. Hohlfeld reports grants to his institution from Altamira Pharma GmbH, Astellas Pharma GmbH, AstraZeneca AB, Bayer AG, Beiersdorf AG, Boehringer Ingelheim Pharma GmbH & Co. KG, CSL Behring GmbH, Desitin Arzneimittel GmbH, F. Hoffmann-La Roche AG, Genentech, Inc., GlaxoSmithKline GmbH & Co. KG, Janssen Pharmaceutica NV, M&P Pharma AG, Novartis AG, Sanofi-Aventis Deutschland GmbH, and UCB Pharma GmbH, and personal fees from Boehringer Ingelheim Pharma GmbH & Co. KG, CSL Behring GmbH, Merck & Co, Inc., Nocion Therapeutics, Inc., HAL Allergy Group, Novartis AG, and Roche, all of which are outside the submitted work.

Funding Sources

This study was funded by the German Federal Ministry for the Environment, Climate Action, Nature Conservation and Nuclear Safety (departmental research plan/Ressortforschungsplan, research Grant No. 3720622030).

Author Contributions

Conny Höflich: conceptualization, supervision, methodology, project administration, resources, writing original draft, review, and editing, and visualization. Philipp Badorrek: conceptualization, methodology, investigation, resources, supervision, project administration, funding acquisition, and writing – review and editing. Christina Gress: methodology, investigation, formal analysis, and writing – review and editing. Meike Müller: conceptualization, methodology, supervision, and writing – review and editing. Wolfgang Straff: conceptualization, methodology, project administration, and resources. Jens M. Hohlfeld: conceptualization, methodology, formal analysis, supervision, project administration, funding acquisition, and writing – review and editing. All authors read, revised, and approved the submitted manuscript.

Data Availability Statement

All data analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author (C.H.).

Downloaded from http://karger.com/iaa/article-pdf/doi/10.1159/000551344/4538628/000551344.pdf by Fachbibliothek Umwelt des Umweltbundesamtes user on 27 July 2026

References

1 Starfinger U. The German Action Program Ambrosia - can the spread of ragweed still be halted? Umwelt + Mensch Informationsdienst (UMID). 2007;3:27–30.

2 Buters J, Alberternst B, Nawrath S, Wimmer M, Traidl-Hoffmann C, Starfinger U, et al. Ambrosia artemisiifolia (ragweed) in Germany. Current presence, allergologic relevance and containment procedure. Allergo J Int. 2015;24:108–20. https://doi.org/10.1007/s40629-015-0060-6

3 Chen KW, Marusciac L, Tamas PT, Valenta R, Panaitescu C. Ragweed pollen allergy: burden, characteristics, and management of an imported allergen source in Europe. Int Arch Allergy Immunol. 2018;176(3–4):163–80. https://doi.org/10.1159/000487997

4 Gereke U. Study to record the frequency of sensitisation and the occurrence of allergic symptoms caused by ragweed in the Drebkau area. Berlin: Freie Universität; 2025. https://refubium.fu-berlin.de/handle/fub188/45486

5 Höflich C. Pollen associated allergic diseases in times of climate change–new data on the development in Germany. Umwelt + Mensch Informationsdienst (UMID). 2018;1:5–14. https://www.umweltbundesamt.de/system/files/medien/360/publikationen/allergische_erkrankungen_klimawandel.pdf

6 Bergmann KC, Werchan D, Maurer M, Zuberbier T. The threshold value for number of ambrosia pollen inducing acute nasal reactions is very low. Allergo J. 2008;17:375–6.

7 Badorrek P, Carstensen S, Dieter S, Gress C, Hohlfeld JM, Müller M. Struß N. Investigation of the pathogenic mechanisms of action of newly emerging pollen allergens using Ambrosia artemisiifolia as an example. Dessau-Roßlau: Umweltbundesamt; submitted.

8 Bonini M, Monti GS, Pelagatti MM, Ceriotti V, Re EE, Bramè B, et al. Ragweed pollen concentration predicts seasonal rhino-conjunctivitis and asthma severity in patients allergic to ragweed. Sci Rep. 2022;12(1):15921. https://doi.org/10.1038/s41598-022-20069-y

9 Deutscher Wetterdienst [Internet]. Freiburg: classification of stress intensities. *[cited 2025 Sep 9]. Available from:* https://www.dwd.de/DE/leistungen/gefahrenindizespollen/erklaerungen.html?nn=16102&lsbId=463856

10 Struß N, Badorrek P, Schwarz K, Windt H, Straff W, Höflich C, et al. Determining the threshold concentration of birch pollen for inducing allergic symptoms using an allergen challenge chamber. Int Arch Allergy Immunol. 2025;6:1–10. https://doi.org/10.1159/000545509

11 Carstensen S, Benediktus E, Litzenburger T, Hohlfeld JM, Müller M. Basophil activation test: assay precision and BI 1002494 SYK inhibition in healthy and mild asthmatics. Cytometry A. 2022;101(1):86–94. https://doi.org/10.1002/cyto.a.24338

12 Bergmann KC, Brehler R, Endler C, Höflich C, Kespohl S, Plaza M, et al. Impact of climate change on allergic diseases in Germany. J Health Monit. 2023;8(Suppl 4):76–102. https://doi.org/10.25646/11654

Downloaded from http://karger.com/iaa/article-pdf/doi/10.1159/000551344/4538628/000551344.pdf by Fachbibliothek Umwelt des Umweltbundesamtes user on 27 July 2026