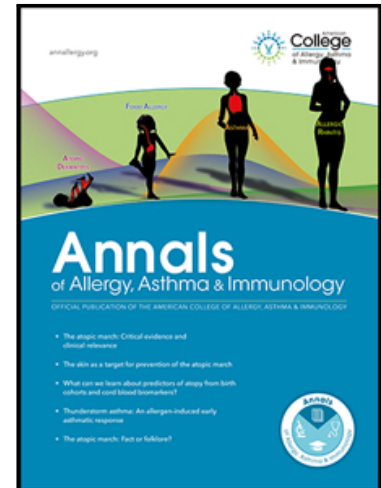


Environmental exposure unit simulates natural seasonal birch pollen exposures while maximizing change in allergic symptoms



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## Environmental exposure unit simulates natural seasonal birch pollen exposures while maximizing change in allergic symptoms

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### Conflicts of Interest

A.K. Ellis declares participation in advisory boards for ALK Abello, AstraZeneca, Aralez, Bausch Health, Circassia Ltd, GlaxoSmithKline, Johnson & Johnson, Merck, Mylan, Novartis, Pediapharm, and Pfizer; and has been a speaker for ALK Abello, Aralez, AstraZeneca, Boehringer-Ingelheim, Meda, Medexus, Mylan, Merck, Novartis, Pediapharm, Pfizer, and Takeda. Her institution has received research grants from ALK Abello, AstraZeneca, Bayer LLC, Circassia Ltd, Green Cross Pharmaceuticals, GlaxoSmithKline, Sun Pharma, Merck, Novartis, Pfizer, Regeneron Pharmaceuticals, Inc., and Sanofi. She has also served as an independent consultant to Allergy Therapeutics, Bayer LLC, Ora Inc., and Regeneron Pharmaceuticals, Inc. in the past.

L. Steacy, M. Suprun, D. Adams, J. Thiele, and T. Walker report no conflicts of interest.

M. DeVeaux, D. Ramesh, S. Langdon, C.Q. Wang, L.T. Perlee, and M.P. O'Brien are employees of and stakeholders in Regeneron Pharmaceuticals, Inc.

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**Key words (max 12):** allergens and epitopes; allergy treatment; biologics; challenge tests; immunologic tests; immunotherapy clinical; pollen

## Abbreviations/Acronyms

AUC, area under the curve; CSMS, combined medication and symptom score; DMS, daily medication score; EEU, environmental exposure unit; IgE, immunoglobulin E; OAS, oral allergy syndrome; PNIF, peak nasal inspiratory flow; RQLQ, Rhinoconjunctivitis Quality of Life Questionnaire; sIgE, serum levels of allergen-specific IgE; SD, standard deviation; SPT, skin prick test; TNSS, total nasal symptom score, TOSS, total ocular symptom score, TSS, total symptom score.

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## Author Contributions

AK Ellis, L Steacy, M Suprun, D Adams, J Thiele, and T Walker contributed to the study concept, data analysis and interpretation, and drafting and critical review of the manuscript, approved the final version for submission, and agree to be accountable for all aspects of the work related to its accuracy or integrity. M DeVeaux, D Ramesh, S Langdon, CQ Wang, LT Perlee, and Meagan P O'Brien were involved in the study concept, statistical analysis and interpretation of the data, provided critical review and revisions of all drafts of the manuscript and approved the final version for submission, and agree to be accountable for all aspects of the work related to its accuracy or integrity.

**Background:** Birch pollen is a prevalent aeroallergen during the springtime allergy season. In field studies, variable allergen exposure and environmental factors can impact data quality while environmental exposure units (EEUs) deliver controlled, standardized, reproducible allergen exposures.

**Objective:** To inform study design for EEU trials evaluating anti-allergic therapies. **Methods:** In this prospective study, 76 birch-allergic participants experienced 3 exposures to birch pollen: an out-of-season EEU challenge (two 3-hour sessions on consecutive days); a natural seasonal exposure; and an in-season EEU challenge (3hour exposure 2-weeks post birch pollen season initiation).

**Results:** Total nasal symptom score (TNSS), total ocular symptom score (TOSS), and total symptom score (TSS = TNSS + TOSS) were assessed every 30 minutes and daily during EEU and natural exposures, respectively. TSSs were highly associated between day 2 of the out-of-season and the in-season EEU challenges, with a good association between maximum TSS during the natural and in-season EEU challenges, and natural season and day 2 of the out-of-season EEU challenge ( $P < .001$  for all). Participants had higher maximum change from baseline TSS during day 2 of the out-of-season EEU challenge (12.4) vs the: first day (9.8); in-season EEU challenge (8.4); and natural seasonal exposure (7.6;  $P < .001$  for all).

Conclusion: Allergic symptoms are highly associated between participants exposed to birch pollen in the EEU (maximum change in symptom scores during day 2) and in the field. A hybrid trial design may be useful to demonstrate the clinical efficacy of novel anti-allergic therapies requiring fewer participants and shorter timelines, and expediting treatment availability.

## INTRODUCTION

Birch trees produce a highly allergenic tree pollen during spring that causes allergic rhinitis in >100 million people worldwide. An estimated 20–30% of people with respiratory allergy in North America and Europe are sensitized to birch pollen.<sup>1–3</sup> Birch-related trees (including birch, alder, hazel, and oak) are classified under the order Fagales, and are all characterized by having Bet v 1 homologous allergens with high sequence identity and extensive cross-reactivity.<sup>4–6</sup> Thus, people who are sensitized to birch pollen are often also sensitized to alder, hazel, and oak trees, and may experience seasonal allergic symptoms including sneezing, nasal itching, nasal congestion, rhinorrhea, conjunctivitis, fatigue, malaise, irritability, and possibly neurocognitive deficits in response to pollen from the birch homologous group.<sup>7–10</sup> Up to 70% of people with pollen allergy also experience oral allergy syndrome (OAS), caused by oral cross-reactivity between proteins in fresh fruits, vegetables, and nuts, with pollens and associated foods.<sup>11, 12</sup> OAS symptoms typically manifest as itching, burning, tingling, and occasionally swelling of the lips, mouth, tongue, and throat, as well as angioedema.<sup>13, 14</sup>

There is a significant need to develop novel study designs to determine the efficacy of anti-allergen therapies. Regulatory agencies in the USA and Europe have required that clinical studies of allergen immunotherapies be conducted during the natural allergy season as field studies. However, variable allergen exposure, confounding environmental factors (rain, dust, other allergens, etc.), and allergen exposure avoidance by participants can influence the treatment effect in field studies.<sup>15–22</sup> Environmental exposure units (EEUs) have been developed to deliver controlled, standardized, and reproducible exposures of allergens.<sup>17, 23</sup> Pollen concentrations used in the EEU are typically up to 10 times higher than outdoor pollen counts measured during natural seasonal exposure. Outdoor pollen counts reflect the pollen burden per cubic meter in the outdoors as a mean over 24 hours and are therefore considerably lower. Although allergen concentrations used in the EEU are higher than those measured outdoors during natural seasonal exposure, a similar range of allergic symptoms is elicited.<sup>1, 16, 19, 23</sup> Compared with the first day, the second consecutive day of out-of-season EEU exposure typically elicits increased nasal and ocular symptoms. Consecutive 2-day EEU exposures mimic allergen priming that occurs during natural seasonal exposure, where there is a lowering of threshold allergen sensitivity due to activation of the allergic immune system.<sup>24</sup> There is limited data on the performance of EEU exposures during the allergen season,<sup>25, 26</sup> but an in-season EEU exposure may offer the advantage of natural priming where the participant has a naturally reduced threshold to allergic symptoms.

EEUs have been used routinely in phase 2 studies to evaluate the efficacy and dose ranges of pharmacotherapies and allergen immunotherapies in participants with allergic rhinitis and conjunctivitis;<sup>1, 27–31</sup> the European Academy of Allergy and Clinical Immunology supports the development of EEUs as an adjunct to natural exposure studies.<sup>32</sup> Previous publications have established a high correlation between

allergic symptoms elicited in the EEU and in the field,<sup>19, 33</sup> the ability of EEUs to be performed both in season and out-of-season,<sup>26</sup> and the use of 2 EEUs together in dual-center clinical trials.<sup>34</sup> EEU and field studies may thus be combined into a single hybrid trial design where the same anti-allergic therapy is investigated in the same population.

This study used a hybrid trial design to compare allergic symptoms to birch allergen elicited in an EEU with artificial priming vs an EEU after natural priming vs during natural seasonal exposure in birch-allergic participants in Ontario, Canada, to optimize the study design of future clinical trials testing novel anti-allergic therapies in a single hybrid study.

## METHODS

### Study participants

Participants were enrolled at a single site, the Allergy Research Unit at Kingston General Health Research Institute, Kingston, Ontario, Canada, and provided written informed consent, complied with study requirements, and met all inclusion criteria (eTable 1 in the eSupplement). Prior to screening and any EEU visits, participants were asked to observe washout periods for medications (eTable 2 in the eSupplement). The study was reviewed and ethical clearance granted by the Queen's University Health Sciences and Affiliated Teaching Hospitals Research Ethics Board.

### Birch pollen exposures

Participants were exposed to birch pollen during a series of challenge visits in an EEU (*Betula pendula*, Greer Laboratories, Lenoir, North Carolina) and during the natural birch pollen season of May 2019 in Kingston, Ontario. Pollen exposures in the EEU were conducted as previously described (maintained at a concentration of  $3500 \pm 500$  grains/m<sup>3</sup>).<sup>35</sup> Commercially available birch and grass pollen counts obtained using a Rotorod® sampler (IQUVIA™, Plymouth Meeting, Philadelphia) were obtained through Aerobiology Labs, taken from a remote air sampling site in Kingston, Ontario. The start and end of the birch and grass pollen season were defined according to Pfaar et al.<sup>36</sup> Birch pollen was also measured weekly at a second outdoor site in Kingston, Ontario, using an Andersen Cascade Impactor sampler (whole pollen grains and pollen fragments) and a Rotorod sampler (whole pollen grains); see also eMethods.

### Study design<sup>35</sup>

In this single-center, prospective, observational study, birch-allergic participants experienced 3 birch pollen exposure periods: (1) a 12-week screening period including an out-of-season 3-hour EEU exposure on 2 consecutive days; (2) a natural seasonal birch pollen exposure outpatient period; and (3) an in-season EEU 3-hour exposure 2 weeks after the start of the natural birch pollen season (Fig 1, eTable 3 in the eSupplement).

**EEU:** The EEU provides reliable allergen exposure of up to 120 participants, irrespective of outdoor allergen and weather conditions. The dispersion of pollen is controlled from a single point of delivery by a custom-engineered device using computer and laser-aided system controls, with the pollen propelled over the seating area by directional fans located at the front of the room both at seated and ceiling height. Complete details have been described previously.<sup>35</sup>

*Screening:* Participants were screened during the winter months, January–March, prior to birch pollen season in the spring. At screening, informed consent, vitals, medical history, and blood samples were collected for serum birch and Bet v 1-specific immunoglobulin E (IgE). Skin prick test (SPT) for birch and other common allergens, SPT with serial birch allergen titration, and an OAS questionnaire were completed (refer to eTable 3 in the eSupplement for further details).<sup>37</sup> If OAS to birch allergen-related fruits or vegetables was reported, a prick-to-prick test for those available foods was completed. Participants were asked to refrain from taking specific prohibited medications (eTable 2 in the eSupplement) within the corresponding washout period preceding all screening and EEU visits.

*Out-of-season EEU exposure:* Participants were required to have a total nasal symptom score (TNSS)  $\leq 2$  to be eligible for the out-of-season EEU. An e-diary was used to grade nasal and ocular symptoms using TNSS and total ocular symptom score (TOSS) at baseline, at 30-minute intervals while in the EEU for 3 hours, then every 3 hours after leaving the EEU, for up to 24 hours (except during sleep hours). Peak nasal inspiratory flow (PNIF) was recorded at every EEU visit, at baseline, and every 30 minutes while in the EEU. Participants were required to reach a TNSS  $\geq 3$  during the 3-hour exposure on the second day (to demonstrate at least mild rhinitis symptoms provoked by birch pollen exposure in the EEU) to continue in the study.

*Natural season:* Following out-of-season EEU visits, data on allergic symptoms, anti-allergic medications for allergic rhinitis symptoms, and quality-of-life measures related to allergic symptoms were collected “in the field” prior to the start of and during the birch pollen season. Between the second out-of-season EEU and the in-season EEU study visit, participants recorded symptom scores daily in the evening and Rhinoconjunctivitis Quality of Life Questionnaires (RQLQ) weekly,<sup>38</sup> using an e-diary. Anti-allergic medications were provided: cetirizine (second-generation antihistamine), olopatadine (antihistamine eye drop), and mometasone furoate (intranasal steroid). Participants recorded their daily use by e-diary so that a daily medication score (DMS) and a combined medication and symptom score (CSMS) could be calculated.<sup>39–44</sup> In addition to SPT with serial birch allergen titration, a PNIF measurement, and prick-to-prick testing, participants with a history of OAS completed an in-season OAS questionnaire.

*In-season EEU exposure:* Approximately 2 weeks after the start of the birch pollen season, participants were exposed in the EEU for a single 3-hour session. Participants recorded symptom scores and PNIF in the same manner as the out-of-season EEU sessions. All participants refrained from taking prohibited anti-allergic medications.

### **Allergen exposure and safety assessments**

The primary study analyses were the differences between TSSs obtained during (1) the out-of-season EEU (during the second 3-hour exposure) and the natural seasonal exposure, (2) the out-of-season EEU (during the second 3-hour exposure) and the in-season EEU (during the single 3-hour exposure), and (3) the in-season EEU (during the single 3-hour exposure) and the natural seasonal exposure.

Secondary analyses included comparisons of TNSS and TOSS between all birch pollen exposure conditions, using maximum scores or area under the curve (AUC) as appropriate, and comparison of the maximum percent reduction in PNIF across EEU exposures. Change in serum levels of allergen-specific IgE (sIgE) levels to Bet v1, sIgE to other birch allergens, and sIgE to related tree antigens and related food allergens during natural exposure to birch pollen were also compared before the onset of natural birch pollen season and during birch pollen season using a non-parametric Wilcoxon signed-rank test.

This was in addition to the change in AUC of mean wheal diameters of the SPT with serial birch allergen titration calculated over the allergen concentrations.

Safety was assessed through physical examination findings and vital sign measurements (screening visit only), urine pregnancy tests as appropriate, and incidence and severity of adverse events.

## Statistical analyses

For a description of all statistical analyses performed for analysis of the primary and secondary assessments, see this article's eMethods. To explore the potential advantage of a hybrid interventional study design in terms of statistical power, sample size calculations were performed for 3 hypothetical clinical trial designs: (1) a traditional field study where the endpoint is average CSMS; (2) an EEU study where the endpoint is average TSS during an EEU challenge; and (3) a hybrid design where the CSMS from the natural birch season and the TSS from an EEU challenge are averaged together as a composite endpoint. Assumptions for study design (1) are based on a pooled estimate of average CSMS from 4 grass immunotherapy field studies,<sup>45</sup> and study design (2) is based on a completed EEU study for a similar grass immunotherapy treatment<sup>1</sup> (eTable 4 in the eSupplement). Statistical properties of a composite endpoint were estimated for (3) assuming a joint normal distribution and using the correlation coefficient observed between the average CSMS during the natural season and the average TSS during the in-season and out-of-season EEU exposures. Sample sizes necessary to achieve 90% power to detect differences in clinical endpoints, as described in eTable 4 in the eSupplement, were calculated assuming a 2-sided alpha of 0.05 and a 2-sample t-test.

## RESULTS

### Participant characteristics

Among 129 participants screened, 76 met the study inclusion criteria and were enrolled; 75 completed the out-of-season EEU visits and the field study portion of the study, and 72 completed the entire study. Descriptive characteristics of the enrolled participants are shown in Table 1 with detailed study criteria presented in eTable 1 in the eSupplement.

### Out-of-season EEU exposure(s)

All 75 participants (100%) who underwent the 3-hour exposures in the EEU on 2 consecutive days attained at least a TNSS  $\geq 3$  during the 3-hour exposure on the second day, meeting inclusion criteria; 66 (88%) attained a TNSS  $\geq 6$ . Although more than 80 participants were exposed to birch pollen in 1 large EEU simultaneously, each participant exhibited a unique pattern of allergic symptoms, comparing the two 3-hour exposures on consecutive days (eFig 1 in the eSupplement). Symptom scores were highly associated between the two 3-hour exposures for the AUC from 0 to 3 hours ( $AUC_{0-3hr}$ ) for TNSS ( $r=0.73$ ,  $P < .001$ ), TOSS ( $r=0.77$ ,  $P < .001$ ), and TSS (Fig 2A) and maximum TNSS ( $r=0.64$ ,  $P < .001$ ), TOSS ( $r=0.71$ ,  $P < .001$ ), and TSS (Fig 2A); however, mean and median symptoms scores were higher during the second 3-hour exposure for the  $AUC_{0-3hr}$  of TSS, average TSS, and maximum TSS, but with similar variability (Table 2). A summary of participants who returned to their baseline symptom scores is described in eTable 5 in the eSupplement; median time to return to baseline TSS was 12 hours for both out-of-season EEU exposures.

Significant decreases in PNIF (an objective measure of nasal congestion), which was measured every 30 minutes while in the EEU, were observed during the first and second out-of-season EEU exposure, with a mean (standard deviation [SD]) maximum percent reduction in PNIF of 26.6% (17.4%),  $P < .001$  and 30.5% (14.9%),  $P < .001$ , respectively. The association between the maximum nasal congestion score of the TNSS and the maximum percent reduction in PNIF was moderate for both the first and second out-of-season EEU exposures (Spearman's  $\rho = -0.48$ ,  $P < .001$ ; Spearman's  $\rho = -0.41$ ,  $P < .001$ , respectively), demonstrating a moderate association between nasal congestion symptoms and PNIF.

### **Natural season exposure**

Approximately 4.5 weeks of pre-season data and 2 weeks of in-season data was recorded. The start date of the birch pollen season was ~2–3 weeks later than anticipated, based on historical local birch pollen counts, therefore more pre-season and less in-season data were collected than planned. Compared with pre-season, mean TNSS, TOSS, TSS, and RQLQ scores increased significantly within-participants during birch pollen season (Table 3).

### **In-season EEU exposure**

A summary and timeframe of participants returning to baseline TSS and TNSS after EEU challenges is described in eTable 5 in the eSupplement. The median time to return to baseline TSS was 12 hours following the in-season EEU exposure. A significant decrease in PNIF was observed, with a mean (SD) maximum percent reduction in PNIF of 26.8% (16.1%),  $P < .001$ . The association between the maximum nasal congestion score component of the TNSS and PNIF was moderate (Spearman's  $\rho = -0.48$ ,  $P < .001$ ).

### **Medication use**

Out-of-season and in-season DMS were calculated from participants' daily diaries and summarized per participant as a mean and maximum score. Fifty-three percent of participants (40/75) used medications out of season: average mean DMS, 0.9 (SD=1.1) and maximum DMS, 7.3 (SD=2.8). Fifteen percent (11/75) used medications in season: average mean DMS, 1.4 (SD=1.1) and average maximum DMS, 6.8 (SD=2.5). On average, subjects used medications on 7.6% of out-of-season days and this decreased to 3.4% of in-season days ( $P < .001$ ).

### **EEU exposure comparisons**

#### **Allergic symptoms elicited in the out-of-season EEU compared with in-season EEU**

TSSs were highly associated between the out-of-season day 2 EEU and the in-season EEU exposures (Fig 2B). A similar association was seen between individual symptom scores for TNSS (max:  $r = 0.72$ ,  $P < .001$ ;  $AUC_{0-3hr}$ :  $r = 0.76$ ,  $P < .001$ ) and TOSS (max:  $r = 0.73$ ,  $P < .001$ ;  $AUC_{0-3hr}$ :  $r = 0.72$ ;  $P < .001$ ).

#### **Allergic symptoms elicited in EEU(s) compared with in season**

There was a good association between the maximum TSS during the natural season and the in-season EEU exposure, and during natural season and the out-of-season day 2 EEU exposure (Fig 2C), with most of the participants clustering with low, medium, or high symptoms. Similar correlations were observed between the average CSMS during the natural season and the average TSS during the out-of-season EEU ( $r = 0.52$ ,  $P < .001$ ) and the in-season EEU exposure ( $r = 0.72$ ,  $P < .001$ ). Similar associations were seen

for TNSS during natural season and the in-season EEU exposure ( $r=0.72$ ,  $P < .001$ ), during natural season and the out-of-season day 2 EEU exposure ( $r=0.53$ ,  $P < .001$ ), for TOSS during natural season and the in-season EEU exposure ( $r=0.77$ ,  $P < .001$ ), during natural season and the out-of-season day 2 EEU exposure ( $r=0.47$ ,  $P < .001$ ).

Participants had a higher maximum change from baseline TSS during the out-of-season day 2 exposure vs the out-of-season day 1 exposure vs during in-season EEU exposure vs during a natural seasonal exposure, respectively (TSS max change mean [SD]: 12.4 [5.4] vs 9.8 [4.5] vs 8.4 [4.4] vs 7.6 [4.5], all  $P < .001$  as compared to out-of-season day 2 exposure).

### **Comparison of the individual components of allergic symptom scores**

For all EEU exposures and the natural birch season, the TNSS contributed 63–67% on average to the maximum observed values for TSS (Fig 3A).

### **Participants with discordant symptoms between the EEU and the natural season**

As can be visualized in Fig 2C, a few participants were outliers, either with moderate symptoms in the EEU and high symptoms in the natural season, or with high symptoms in the EEU and low symptoms in the natural season. Participants belonging to these clusters were further investigated to understand the discordance (eTable 6 in the eSupplement). Participants who had high symptoms in the EEU and low symptoms in the natural season ( $n = 2$ , blue cluster) were more likely than the overall population to report low time spent outdoors during the natural season (median of 2.6 maximum hours/day). These participants had low specific IgE to ragweed, compared to the rest of the participants. Participants who had moderate symptoms in the EEU and high symptoms in the natural season ( $n = 4$ , purple cluster) were more likely than the overall population to report more time spent outdoors (median of 12 maximum hours/day), and had higher specific IgE levels to oak, silver birch, rye, and timothy grass (eTable 6 in the eSupplement). As a sensitivity analysis, we clustered CSMS in natural season with TSS in EEU (eFig 2 in the eSupplement), the results were consistent with TSS in natural season analysis, which is likely driven by low medication use during the natural season.

### **Skin prick testing and oral allergy syndrome**

Participants had poly-allergy as demonstrated by positive skin wheal responses after SPTs to common allergens (eTable 7 in the eSupplement). Birch SPT with serial birch allergen titration was performed to compare seasonal differences in birch allergen skin prick reactivity. There was a statistically significant increase in the AUC of the mean wheal diameters from out of season ( $9.1 \pm 3.5$ ) to in season ( $10.1 \pm 3.9$ ;  $P < 0.05$ ), and a significant intra-participant increase in serum allergen-specific concentrations for birch, Bet v 1, and hornbeam during the birch season, and a significant decrease in Timothy grass IgE (eTable 8 in the eSupplement). Similar to other reports, the size of the screening birch SPT was not associated with symptom scores in EEU exposures or during the natural season (all  $P > .05$ ). A weak association was observed between the AUC of the mean wheal diameters from in season to the in-season EEU exposure TSS AUC<sub>0-3hr</sub> ( $r=0.23$ ,  $P = .048$ ).

Overall, 40.8% (31/76) of participants reported having OAS to birch pollen-related foods. These participants did not differ in symptom scores (TSS) compared with participants without OAS across all exposure conditions ( $P > .25$  for comparisons of TSS in those with and without OAS in all EEU exposures and in the natural season). Most (83.5%) reported OAS symptoms only to raw OAS-related food(s). The most common symptom experienced was “itching in the mouth” (100%), with the majority

reporting symptom onset within 5 minutes. Of the participants with self-reported OAS, all underwent prick-to-prick testing using the food to which they reported symptoms twice during the study, once each prior to and during the birch pollen season. Across most foods tested, there was a trend for an increase in skin prick mean wheal diameter during birch pollen season that was not statistically significant, with the largest median intra-participant differences in prick-to-prick mean wheal diameters observed for apples (1.5 mm,  $P = .06$ ), plums (2.8 mm,  $P = .06$ ), and strawberries (2.5 mm,  $P = .25$ ; Fig 3B).

### Pollen counting comparisons

Natural season pollen counts varied between 10 and 642 particles/m<sup>3</sup>, measured daily using aeroallergen rotation impaction samplers at a single outdoor site in Kingston Ontario by Aerobiology Research Laboratories, Ottawa, Ontario (Fig 1). Similar peaks were observed in daily birch pollen counts obtained from an outdoor Rotorod sampler that was used in the study as compared to the Kingston official pollen counts. The fragments of Bet v 1 protein measured using the Andersen Cascade Impactor sampler were consistent between the outdoor and EEU samples (4.7 to >7.0  $\mu$ m), suggesting that both natural and EEU birch pollen exposure is predominantly represented by whole pollen grains, not pollen fragments, and likely affects the upper rather than the lower respiratory tract.

## DISCUSSION

The purpose of this study was to understand the intra-participant variability in allergic symptoms (TSS) between different birch pollen exposure conditions, in order to inform the design of a potential hybrid study to determine the efficacy of a novel anti-allergy treatment. TSS was highly associated between the 2 out-of-season 3-hour EEU exposures, the second day of the out-of-season 3-hour EEU exposure, and the single in-season 3-hour EEU exposure, suggesting high intra-participant concordance between these EEU conditions, as previously described.<sup>17</sup> TSS was less well associated between the out-of-season EEU exposures and the natural exposure, supporting the observation that allergic symptoms in field studies are more variable than in EEU studies.

Similar to other reports, the highest magnitude of change of TSS was observed during the second 3-hour exposure.<sup>26, 46-48</sup> For the majority of participants, the TSS in the EEU exposure and the natural season were highly concordant with the majority of participants having low, moderate, or high symptoms in both the EEU and the natural season; however, we identified 2 small clusters of participants with symptoms higher in either EEU exposure or the natural season. Upon further exploration, participants with high natural season TSS could be characterized by having a number of other aeroallergies, ie, ragweed, oak, and grass, as well as spending more time outdoors. By contrast, participants with high EEU but low natural season symptoms were less likely to spend time outdoors. While these results address the discordant symptom scores, they are mostly descriptive, due to the small number of participants in those two groups. Collectively, these data help address the challenges associated with performing EEU and field studies separately.

Approximately 40.8% of birch-allergic participants experienced OAS, with apple, nectarines, and tree nuts identified as the most common triggers. These figures are lower than in previously reported studies;<sup>46</sup> however, they underscore immunologic cross-reactivity between birch pollen and structurally related food allergens. IgE antibodies specific for the birch pollen allergen Bet v 1 have been shown to cross-react with homologous proteins identified in different stone fruits, such as apple (Mal d 1)<sup>49</sup> and

other stone fruits, as well as tree nuts. There was a trend for an increase in skin prick mean wheal diameter that was not statistically significant during birch pollen season for apples, plums, and strawberries, using prick-to-prick testing, suggesting that cross-reactive food allergy in birch-allergic participants may worsen in pollen season.

Given that allergic symptom scores during the natural season correlate well with scores in the EEU both in season and out of season (following artificial priming), hybrid studies can effectively compare effects of allergen therapies both in the EEU (either out of season or in season) and in the field. Collection of allergic symptoms for up to 24 hours after leaving the EEU in our study demonstrated that allergic symptoms return to baseline in most participants, suggesting that symptoms elicited in an in-season EEU exposure should minimally impact the daily symptom scores assessment of the field study.

Based on the correlations observed between symptoms elicited in the EEU and in the natural season in our study, as well as in published studies of grass immunotherapy trials,<sup>45</sup> statistical properties of a composite endpoint for a hypothetical hybrid interventional study design were modeled. While a traditional field study would require ~225 participants/arm to detect a treatment effect of ~20% between drug and placebo, which is the recommended treatment effect thought to confer clinical benefit in allergic rhinitis,<sup>45</sup> a composite endpoint in a hybrid study design has more power, as evidenced by a requirement of only 72 participants per arm in season, or 80 per arm out of season, to achieve 90% power.

Limitations of this analysis include the relatively small number of participants and the fact that this is a single center study.

A hybrid approach combining an EEU challenge and a field study provides a robust model to determine efficacy of an anti-allergy intervention. The composite endpoint of this hybrid study model takes advantage of the larger magnitude of symptoms experienced during an EEU challenge combined with lower variability, leading to a more powerful endpoint. It may also fulfil the regulatory requirement of a field study while providing protection against the unpredictability of a natural allergy season.

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**Table 1.** Baseline demographic characteristics of study participants

| Characteristics at screening                                 | Total (N = 76) |
|--------------------------------------------------------------|----------------|
| Age, years, mean ( $\pm$ SD)                                 | 40.9 (12.5)    |
| Male, <sup>a</sup> n (%)                                     | 26 (34.2)      |
| Ethnicity, <sup>b</sup> n (%)                                |                |
| Non-Hispanic white, n (%)                                    | 74 (97.4)      |
| Screening birch test diameter (mm), mean ( $\pm$ SD)         | 8.7 (2.9)      |
| Subjects poly-sensitized to >2 allergens, <sup>c</sup> n (%) | 75 (98.7)      |
| Ragweed, n (%)                                               | 67 (88.2)      |
| Birch silver sIgE (kU/L), mean ( $\pm$ SD)                   | 5.6 (10.9)     |
| rBet v 1 Birch sIgE (kU/L), mean ( $\pm$ SD)                 | 3.3 (6)        |
| Asthma history, <sup>d</sup> n (%)                           | 12 (15.8)      |
| Oral allergy syndrome, <sup>e</sup> n (%)                    | 31 (40.8)      |

<sup>a</sup>Participants were categorized into male sex versus the rest.

<sup>b</sup>Participants were categorized into non-Hispanic white versus the rest.

<sup>c</sup>Participants were categorized into those with poly-sensitization to other allergens (in addition to birch) based on skin prick test at screening.

<sup>d</sup>Participants were categorized into those with or without a history of asthma at screening.

<sup>e</sup>Participants were categorized into those who had experienced OAS versus those who had not at screening.

OAS, oral allergy syndrome; SD, standard deviation; sIgE, allergen-specific immunoglobulin E.

**Table 2.** Descriptive statistics of symptom scores during the out-of-season EEU exposures, and during the in-season EEU and during natural exposure in season

|                   |                        | TNSS                |        | TOSS                |        | TSS                 |        | PNIF                |
|-------------------|------------------------|---------------------|--------|---------------------|--------|---------------------|--------|---------------------|
|                   |                        | Mean<br>( $\pm$ SD) | Median | Mean<br>( $\pm$ SD) | Median | Mean<br>( $\pm$ SD) | Median | Mean<br>( $\pm$ SD) |
| Out-of-season EEU |                        |                     |        |                     |        |                     |        |                     |
| First day         | Average<br>(0–3 hours) | 4.6<br>(2.1)        | 4.2    | 2.3<br>(1.8)        | 2.3    | 7.0<br>(3.5)        | 6.5    | 110.6<br>(36.4)     |
|                   | Maximum<br>(0–3 hours) | 6.9<br>(2.6)        | 7.0    | 4.1<br>(2.8)        | 4.0    | 10.7<br>(4.8)       | 10.0   |                     |
|                   | AUC<br>(0–3 hours)     | 4.9<br>(2.2)        | 4.5    | 2.4<br>(1.8)        | 2.4    | 7.3<br>(3.6)        | 6.7    |                     |
| Second day        | Average<br>(0–3 hours) | 6.3<br>(2.1)        | 6.3    | 3.8<br>(2.3)        | 3.9    | 10.2<br>(3.9)       | 10.4   | 102.4<br>(30.8)     |
|                   | Maximum<br>(0–3 hours) | 8.8<br>(2.6)        | 9.0    | 5.7<br>(3.0)        | 6.0    | 14.3<br>(5.0)       | 15.0   |                     |
|                   | AUC<br>(0–3 hours)     | 6.6<br>(2.2)        | 6.5    | 4.0<br>(2.4)        | 4.2    | 10.6<br>(4.1)       | 10.7   |                     |
| In-season EEU     | Average<br>(0–3 hours) | 6.5<br>(2.6)        | 6.3    | 4.1<br>(2.6)        | 3.4    | 10.6<br>(4.8)       | 10.1   | 108.8<br>(35.3)     |
|                   | Maximum<br>(0–3 hours) | 8.4<br>(2.7)        | 9.0    | 5.6<br>(3.1)        | 5.0    | 13.8<br>(5.4)       | 14.0   |                     |
|                   | AUC<br>(0–3 hours)     | 6.6<br>(2.6)        | 6.4    | 4.2<br>(2.6)        | 3.6    | 10.8<br>(5.0)       | 10.4   |                     |

AUC, area under the curve; EEU, environmental exposure unit; PNIF, peak nasal inspiratory flow; SD, standard deviation TNSS, total nasal symptom score TOSS, total ocular symptom score; TSS, total symptom score.

**Table 3.** Summary of the average and maximum symptom and medication scores, and quality of life assessments, measured during the pre-season and natural exposure in season, with intra-subject differences

| Natural season measures |         | 4.5 weeks pre-season<br>(mean $\pm$ SD) | 2 weeks in season<br>(mean $\pm$ SD) | Within-subject<br>change from<br>preseason<br>(mean $\pm$ SD) | Significance vs<br>pre-season score<br>(of within-subject<br>differences,<br>paired t-test) |
|-------------------------|---------|-----------------------------------------|--------------------------------------|---------------------------------------------------------------|---------------------------------------------------------------------------------------------|
| TNSS                    | Average | 3.2 (2.1)                               | 5.1 (2.6)                            | +1.8 (1.6)                                                    | $P < .001$                                                                                  |
|                         | Maximum | 7.3 (2.9)                               | 7.5 (3.0)                            | +0.2 (2.3)                                                    | $P = .204$                                                                                  |
| TOSS                    | Average | 1.4 (1.5)                               | 2.9 (2.6)                            | +1.5 (1.8)                                                    | $P < .001$                                                                                  |
|                         | Maximum | 4.6 (3.0)                               | 5.1 (3.5)                            | +0.4 (3.0)                                                    | $P = .450$                                                                                  |
| TSS                     | Average | 4.6 (3.3)                               | 7.9 (4.9)                            | +3.3 (3.1)                                                    | $P < .001$                                                                                  |
|                         | Maximum | 11.2 (5.3)                              | 12.2 (6.0)                           | +1.0 (4.6)                                                    | $P = .077$                                                                                  |
| DMS                     | Average | 0.5 (0.9)                               | 0.2 (0.6)                            | -0.3 (0.6)                                                    | $P < .001$                                                                                  |
|                         | Maximum | 3.9 (4.2)                               | 1 (2.6)                              | -2.9 (4.0)                                                    | $P < .001$                                                                                  |
| CSMS                    | Average | 5.2 (3.7)                               | 8.3 (5.3)                            | +3.1 (3.2)                                                    | $P < .001$                                                                                  |
|                         | Maximum | 13.7 (7.2)                              | 12.8 (6.8)                           | -0.9 (6.0)                                                    | $P = .209$                                                                                  |
| RQLQ                    | Average | 0.8 (0.7)                               | 1.4 (1.0)                            | +0.6 (0.7)                                                    | $P < .001$                                                                                  |

DMS, daily medication score; CSMS, combined medication and symptom score; RQLQ, Rhinoconjunctivitis Quality of Life Questionnaire; SD, standard deviation; TNSS, total nasal symptom score; TOSS, total ocular symptom score; TSS, total nasal symptom score.

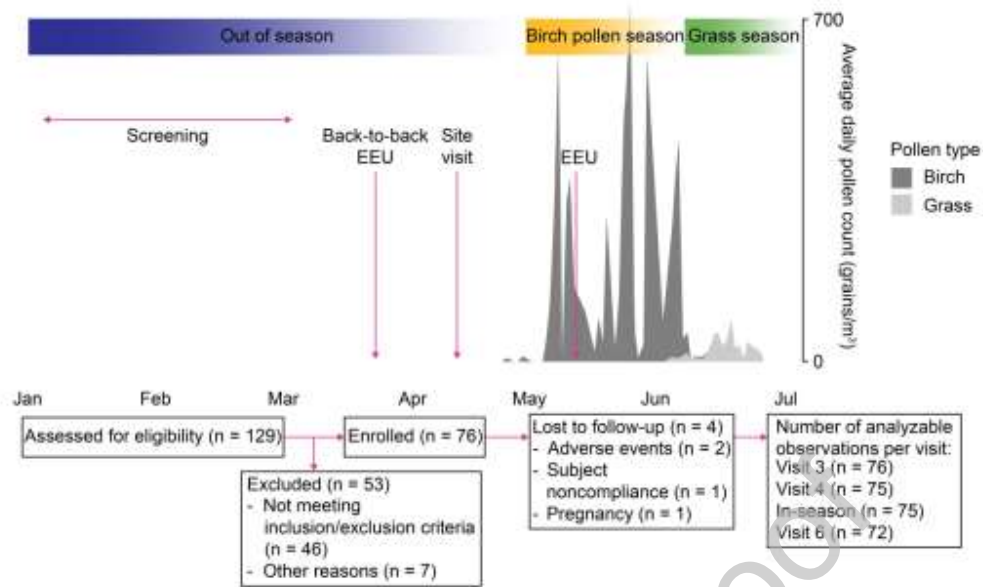


Figure 1. Study design

EEU, environmental exposure unit.

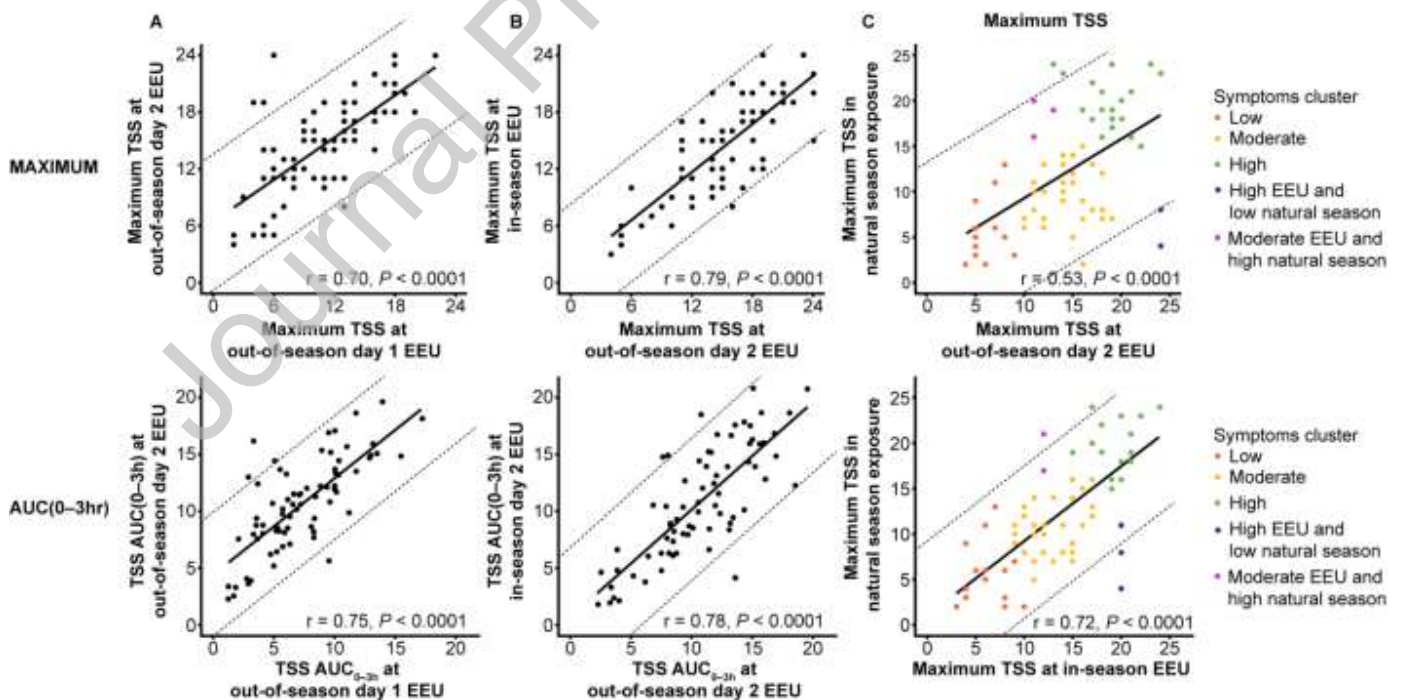


**Figure 2.** Association between TSS in EEU out of season exposures, during the natural season, and during in-season EEU exposure. Scatterplots showing association of maximum or  $AUC_{0-3hr}$  of A) in EEU during out-of-season days 1 vs day 2; and B) in in-season EEU vs out-of-season EEU at day 2. C) Participants were clustered based on their maximum TSS during natural season and out-of-season EEU exposure at day 2 (top panel) or in-season EEU exposure (bottom panel). Five clusters are highlighted; each plot shows a fitted regression line ( $\pm 2SDs$ ) and Pearson correlation.

AUC, area under the curve; EEU, environmental exposure unit; SD, standard deviation; TSS, total symptom score.

**Figure 3.** A. The distribution of maximum TNSS, TOSS, and TSS experienced by participants in the EEU and in the natural season; the TNSS contributes more to the TSS than the TOSS. B. Mean wheal diameters from prick-to-prick testing of selected fruits in and out of the birch season for participants who reported OAS.

EEU, environmental exposure unit; OAS, oral allergy syndrome; TNSS, total nasal symptom score; TOSS, total ocular symptom score; TSS, total symptom score.