
Validation of the Biogenics Research Chamber for *Juniperus ashei* (mountain cedar) pollen

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Background: Pollen challenge chambers have been used to evaluate medication in allergic rhinoconjunctivitis under controlled conditions.

Objective: To validate a facility for the study of subjects' responses to inhalational challenges with *Juniperus ashei* (mountain cedar) pollen.

Methods: Two chambers, 307 m³ and 188 m³, seating 50 and 25 individuals, respectively, were constructed with clean room materials. The computer-controlled air handler used powered diffusers and exhausts to maintain a laminar flow. Pollen was delivered by a feeder into a vortex created by an eductor through a series of stainless steel tubes. Nonprimed mountain cedar sensitive and healthy control subjects were exposed to a masked sham air run and increasing increments of pollen during a 5-hour period outside the natural season. These individuals were then exposed for 2-hour periods for up to 4 sequential days at fixed pollen counts to demonstrate effects of priming.

Results: Airflow from diffusers and exhausts maintained 12 exchange cycles per hour. Pollen counts ranged from 1,300 to 12,000 grains/m³. None of the subjects responded during the masked sham run. Healthy controls did not respond. Nonprimed subjects had an inadequate response. Primed subjects responded symptomatically within a period adequate for entry into studies. There were no serious adverse responses.

Conclusions: The chambers functioned within the parameters for which they were designed. Subjects did not respond to a sham run. Priming runs were required to stimulate symptoms at levels in a timeframe sufficient for pharmacologic studies. Pollen counts 3 to 4 times the average seasonal counts were required to elicit significant symptoms.

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INTRODUCTION

Pollen challenge chambers have been used as a means to study medication in controlling signs and symptoms of allergic rhinoconjunctivitis.^{1–5} Traditional exposure assessment methods of using natural seasonal or perennial exposure to allergens for long periods in clinical drug trials are fraught with limitations.⁶ Natural exposures include large regional differences in pollen seasons and marked differences in pollen exposure due to subject lifestyles. Weather conditions may play a dominant role in pollen levels and exposure. Subject adherence to paper or electronic diaries may be questionable, and dropout rates may be high. Advantages⁶ of an allergen challenge chamber include the following: studies may be performed outside the time of natural pollination; the exposure is controlled and uniform for all participants; there is no effect of inclement weather; there is no effect of personal participant activity; there is ensured adherence, including medication administration and timeliness and completion of symptom assessments; and there are instantaneous and precisely timed symptom assessments. Because of these fac-

tors, the use of pollen challenge chambers may be a more accurate and effective method of studying the attributes of medication and the inflammatory responses.

Juniperus ashei (mountain cedar) pollen allergy is a robust model in the South Texas area that is commonly used in natural studies of pharmacologic efficacy.^{7–9} The Biogenics Research Chamber was designed to measure the pharmacologic effects and to aid in the study of the inflammatory and immunologic mechanisms of individuals' responses when challenged under controlled conditions. Certain design characteristics that distinguish these chambers include compact construction, configurable equipment and furnishings within each chamber, air-handling systems that are bifunctional, and an alternate electrical source. In addition, each chamber has a pollen feeding system that is highly controllable that does not rely on fans to adjust pollen levels within areas of the chamber. This is the first study, to our knowledge, of mountain cedar pollen in controlled conditions within a pollen challenge chamber.

METHODS

Phase I: Validation of the Mechanical Operation of the Chambers Without Subjects

Two chambers with a central control room and staging area were built using clean room technology. The large chamber is 307 m³ and designed to seat 50, and the small chamber is 188 m³ and designed to seat 25. Chairs and tables are movable to allow for ease in cleaning the chambers and for different

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configurations for different protocols. There is oxygen, a crash cart, and a defibrillator for emergency use. Audio and video systems are used for communication between the chambers and the control room. There are viewing windows for monitoring from the control room into the chambers. The air-handling systems are independently computer controlled to adjust for the occupancy of the chambers. The design capacity of the air handler was calculated to control the chambers with an occupancy of 100 and 50 people in the large and small chambers, respectively, at maximal outside temperatures. In occupied mode, fresh outside air is drawn through highly filtered intakes, chilled in a single pass across the condenser, and distributed from a duct system by powered, filtered diffusers from the ceiling and exhausted by powered, filtered vents at the floor level to the outside environment air exchange rates are 12 full cycles per hour. This system delivers a slightly positive pressure inside the chamber to reduce the introduction of outside contaminants with opening of doors. When switched to an unoccupied mode, the system functions as standard air-conditioning recirculating the air. An alternate electrical source is a diesel-powered generator with sufficient capacity to power the chambers in full operational mode at maximum outside temperatures. The computer monitors the status of all filters, diffusers, and exhausts. Airflow through diffusers and exhausts is further monitored by manual measurements with a hot wire anemometer. The pollen is delivered by a computer-controlled feeder that delivers an adjustable weight of pollen in grams per hour into a vortex driven by an adjustable blower augmented by an eductor specially designed for this purpose. The pollen is dispersed through a series of grounded stainless steel tubes with specially bored ports and specially machined joints locked by hand-cranked clamps that allow for directional adjustment of pollen. Pollen counts are performed using 2 different manual Allergenco systems, MK-3 staged counters and Allergenco-D Posi-track disposable cassettes (Environmental Monitoring Systems Inc, Charleston, South Carolina), which are powered by suction pumps run at 15 L/min. Two different laser particle counters were used. The Allergenco systems are mounted on a platform at 46-in from floor level. The fixed Shinyei Laser Pollen Sensor (Shinyei Corp of America, New York, New York) is mounted on a platform at 46-in in the center of the subject seating area. The Kanomax Handheld Laser Particle Counter (Kanomax USA Inc, Andover, New Jersey) is used to measure particles at various locations within the chambers.

To initiate validation, the chambers were activated without subjects with maximal seating and equipment in place. Control settings on the air-handling system were determined to maintain acceptable temperature and humidity. Airflow measurements were taken from the diffusers and exhausts to ascertain balance and uniform laminar airflow through the chambers using a hot wire (Thermal) anemometer. The hot wire anemometer is used in many industrial and medical circumstances to measure very low air velocity, including flow from fume hoods¹⁰ and clean rooms. Airflow character-

istics of the chambers were determined using a MiniFog portable fog machine (HiFiSoundconnection, Nixa, Missouri). The pollen feeder was run at various feeding rates to determine pollen levels dispersed to the chambers. Pollen counts were determined for 1 minute at 2-minute intervals to determine the time for the chamber to reach levels suitable to challenge subjects. Pollen counts were determined for 10 minutes at 30-minute intervals to compare manual microscopic methods with laser methods.

Phase 2: Validation of the Air Handler and Pollen Feeder Systems With Mountain Cedar Allergic Subjects

This study was performed at Biogenics Research Chamber, San Antonio, Texas. All subjects provided written informed consent before enrollment in the study. The protocol, amendments, and informed consent documents were approved by IntegReview Institutional Review Board, Austin, Texas. The study was conducted according to Good Clinical Practice standards. This phase of the study was performed outside the mountain cedar season during the middle of the summer in which no pollen is present in the South Texas area. Sensitive subjects, males and females, with a 2-year or longer history of seasonal allergic rhinoconjunctivitis to mountain cedar and a 3-mm or larger positive response over a saline control by the skin prick test method and healthy control subjects, 18 to 65 years of age, were selected for entry into this phase of the validation studies. After meeting inclusion criteria, the subjects were issued a screening diary card with written and verbal instructions and the chamber visits were scheduled. Symptoms were self-scored with a Likert scale with the values of 0 (absent), 1 (mild), 2 (moderate), and 3 (severe). Symptoms that were scored included nasal congestion, sneezing, rhinorrhea, and itching. Ocular symptoms that were scored include redness, tearing, and itching. The total symptom score (TSS) was based on adding the scores of the 7 symptoms, yielding a minimum score of 0 and a maximum score of 21. Healthy control subjects were screened under the same inclusion criteria; however, they must have had no history suggestive of chronic or recurrent nasal or ocular signs and symptoms and negative skin test results. Control subjects, likewise, were issued a screening diary card and received verbal instructions and scheduled for the chamber visits.

Chamber Visits

Because of uncertainty concerning the level of reactivity in sensitive subjects, chamber runs were structured with safety as the foremost concern. Twenty-six mildly to moderately sensitive subjects, based on prior experience in natural seasons, and 11 healthy controls were selected for the initial chamber run. No priming runs were performed. Subjects reported to the chamber on the scheduled date with a completed screening diary card. The screening diary card was processed through the optical reader to confirm chamber randomization criteria. If qualified (TSS ≤ 5.0), the subject entered the chamber. The air conditioning system adjusted to maintain a 70°F to 72°F temperature with

a relative humidity between 40% and 50%. The pollen delivery system was activated without pollen for the first 30 minutes for a masked sham period. Then pollen was introduced in a graduated manner to supply increasing pollen counts. Pollen counts were measured at 30-minute intervals both electronically and manually.

Subjects recorded signs, symptoms, and adverse responses at 30-minute intervals on a manual chamber diary card throughout the 5-hour trial run. The diary cards were processed through an optical reader and results tabulated.

After the first chamber run, symptoms were lower than anticipated, with no adverse responses. Fifteen highly sensitive mountain cedar sensitive subjects were then selected and underwent a challenge 2 weeks later. These 15 subjects did not undergo a sham run, and pollen counts were increased by approximately 2-fold. Two weeks after the second chamber run, the mildly to moderately sensitive group, the highly sensitive group, and the healthy controls underwent a combined challenge with 2 levels of stable pollen counts.

On recognition that priming runs would be necessary and high levels of pollen would be required, priming runs were initiated. Runs were performed on 4 sequential days with a duration of 2 hours at pollen counts of 12,000 grains/m³ on 27 subjects.

Statistical Analysis

Simple descriptive statistics were used to summarize data. An independent *t* test was used to compare the 2 runs of diffuser and exhaust airflow. The TSSs were modeled using a general linear model repeated-measures analysis with an autoregressive covariance structure. Independent factors included pollen sensitivity group (controls, mild and moderate sensitivity, and severe sensitivity), time, and a group by time interaction term. In the absence of an interaction and with the interaction term removed from the model, the linear test for trend was based on the *P* value for the coefficient of time, with time entered into the model as a continuously distributed covariate. All statistical testing was 2-sided with a significance level of .05 and was performed using SAS statistical software (version 9.2, SAS Institute Inc, Cary, North Carolina). All figures were created using R Version 2.12.0 (Free Software Foundation, Boston, Massachusetts).

RESULTS

During the mechanical phase of operation, the chambers functioned according to the architectural and engineering design. Airflow from diffusers and outflow through exhausts are presented in Table 1. The computer-controlled air-handling system maintained temperature at 70°F to 72°F and relative humidity between 45% and 55%. The portable fog machine identified areas of airflow within the chamber that required adjustment of the pollen dispersal system. Pollen counts at 5 counting stations were uniform $\pm 20\%$. Counts were taken by an Allergenco MK-3 at 1-minute intervals and revealed a rapid stabilization within a 2-minute period after activation of the pollen dispersal system. Counts during unoccupied runs from the staged Allergenco MK-3 were con-

Table 1. Airflow From Powered Diffusers and Outflow Through Powered Exhausts^a

	Diffusers		Exhausts	
	Run 1 (1/19/10)	Run 2 (7/14/10)	Run 1 (1/19/10)	Run 2 (7/14/10)
1	1.0	1.0	2.7	2.8
2	1.0	1.0	2.6	2.7
3	1.0	1.0	2.4	2.6
4	1.0	1.0	2.0	2.4
5	1.0	1.0	2.4	2.9
6	0.9	1.0	2.1	2.5

^a Measurements were taken with a hot wire anemometer for low-flow air velocity in meters per second. The airflow measures were not significantly different between the 2 separate runs for the diffuser (*P* = .36, *t* test) or the powered exhaust (*P* = .06, *t* test).

sistently approximately 10% to 15% higher than counts from the Allergenco-D PosiTrack disposable cassette. Particle counts from the Shinyei Laser Pollen Sensor and Kanomax Handheld Laser Particle Counter were significantly lower and higher, respectively, than the counts from the Allergenco systems. Both laser particle counters had directional trends consistent with counts from the Allergenco systems.

During the combined occupied chamber run, the computer-controlled air-handling system adjusted for the 49 subjects and 9 technicians to maintain stability of temperature and humidity at the same consistency as with unoccupied and smaller occupied runs. Pollen counts taken with the Allergenco MK-3 at 3 stations and the Allergenco-D PosiTrack disposable cassette at 5 stations were consistent at various pollen dispersal levels (Fig 1). Microscopic examination of slides from the Allergenco systems during unoccupied runs had shown almost pure pollen grains with little other particulate matter; however, slides taken during the occupied chamber runs revealed heavy particulate material composed of epithelial cells, fibers, and dust particles associated with large numbers of subjects within the chamber. The laser counters were not useful during the occupied runs because of high particle counts from the additional debris.

Twenty-six mildly to moderately sensitive and 11 healthy control subjects recorded mean TSSs of 0.55 and 0.29, respectively, for the 4 previous days and the morning of the chamber run. During the sham run in which the pollen dispersal system was activated but no pollen was fed, the sensitive and control subjects recorded mean TSSs of 0.72 and 0.10, respectively. At 30 minutes into the chamber run, the pollen feeder was activated and symptoms increased in the most sensitive subjects. As shown in Figure 1, increasing levels of pollen led to mildly increasing symptoms in increasing numbers of subjects (*P* < .001, test of trend). No severe adverse events occurred. All sensitive subjects experienced symptoms, with 16 reacting mildly (TSSs of 1-6), 7 reacting at a moderate level (TSSs of 7-12), and 3 reacting severely (TSSs of 13-21).

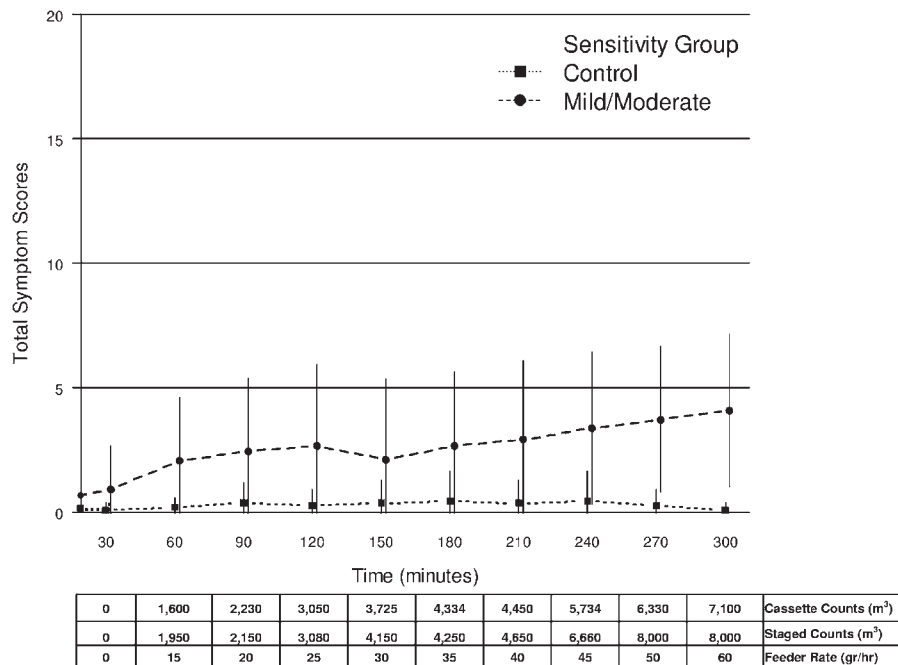


Figure 1. Mean Total Symptom Scores (TSSs) over time in healthy controls and mildly to moderately sensitive subjects. There were no increased responses within the first 30-minute sham interval when no pollen was being delivered. The mean TSSs are shown for the 2 sensitivity groups, with symbols representing the mean and solid vertical lines representing the SD. A repeated-measures linear model of TSSs in terms of group (healthy controls, mildly to moderately sensitive subjects) and time found a significant group by time interaction ($P = .02$) indicated by the nonparallel mean trajectories. Only 2 of 11 healthy controls had minimal symptoms. Pollen counts were compared among the 3 Allergenco MK-3 movable stage systems and 5 Allergenco PosiTrack disposable cassette systems at fixed locations. The Allergenco MK-3 system tended to closely track the cassette system at increasing feeder rates with variations of approximately 10% at high rates. For the mildly to moderately sensitive subjects, the TSSs gradually increased to modest levels, with moderately increasing pollen counts ($P < .001$, test for trend).

The mean TSS was 4.19. In contrast, only 2 of 11 nonsensitive subjects experienced minimal symptoms, demonstrating a statistically significant difference in trajectory of TSS over time for the nonsensitive vs the mildly and moderately sensitive subjects ($P = .02$).

Fifteen highly sensitive subjects underwent a chamber run without priming with higher levels of pollen exposure. No sham run was performed. Mean TSSs of 0.50 were recorded for the 4 previous days and the morning of the chamber run. All subjects experienced symptoms at a moderately higher level than in the mild to moderate group and symptoms increased over time ($P < .001$, test of trend). Nine subjects reacted at a moderate level (TSSs of 7-12), and 6 subjects reacted severely (TSSs of 13-21). The mean TSS for the group was 9.26 (Fig 2).

Both sensitive groups and the healthy controls were exposed in a combined run using intermediate levels of pollen and compared with their individual response to the initial exposures. There was 1 screen failure with excessive symptoms and 2 subjects were lost to follow-up. Mean TSSs of 0.96, 0.89, and 0.64 were recorded for the 4 previous days and the morning of the chamber run for the highly sensitive, mildly to moderately sensitive, and healthy control subjects, respectively. The same 2 of 11 healthy control subjects again experienced minimal symptoms. All sensitive subjects experienced symptoms, with 16 reacting mildly (TSSs of 1-6), 12

subjects reacted moderately (TSSs of 7-12), and 10 subjects reacting severely (TSSs of 13-21) (Fig 3). Of the 38 sensitive subjects, 30 reacted with similar TSSs, 3 reacted with less intensity, and 5 reacted with higher intensity on the combined chamber run. The 3 groups had significant trajectories of increasing symptoms over time ($P = .008$). There was a significant test of trend for increasing symptoms over time for the mild to moderately sensitive subjects ($P < .001$).

Subsequently, 27 subjects underwent up to 4 priming runs, with 17 (63%) meeting criteria (TSS ≥ 11 within a 2-hour period) to qualify for entry into chamber studies. Mean TSSs of 0.41 were recorded for the 4 previous days and the morning of the first priming chamber run. Seven subjects qualified during the first priming run, 7 qualified during the second priming run, 2 subjects qualified during the third priming run, and 1 subject qualified during the fourth priming run, with 10 failing to meet priming criteria. The TSSs increased over time ($P < .001$, test of trend), and symptoms experienced during the 2-hour priming runs were 2-fold increased over the nonprimed runs in less than half the time (Fig 4).

DISCUSSION

The Biogenics Research Chamber has been constructed to study the symptomatic and inflammatory effects of sensitive subjects to inhaled pollen in a controlled manner. *J ashei*

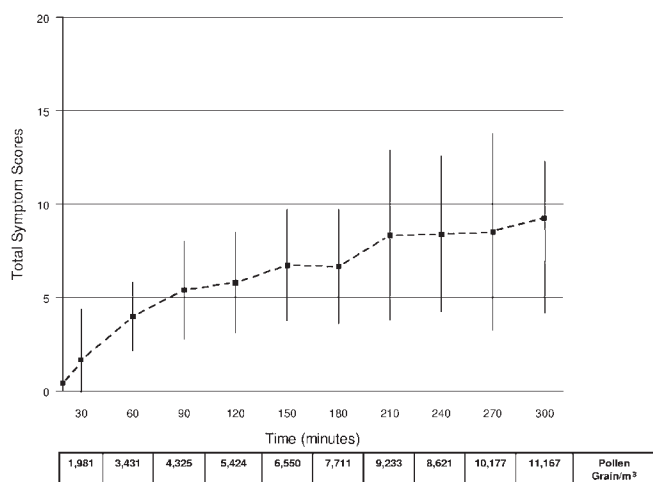


Figure 2. Mean Total Symptom Scores (TSSs) over time of selected subjects highly sensitive to mountain cedar in a nonprimed chamber run. Highly sensitive subjects were selected based on clinical history, diary data from a previous natural trial, and size of the skin prick test result. The TSSs increased over time in the chamber ($P < .001$, test of trend). Their response to higher pollen counts during a shorter period than those with mild to moderate sensitivity showed a 2-fold increase in symptoms.

(mountain cedar) pollen was chosen as the initial pollen for standardization in the South Texas area because of the intensity of pollen exposure, the severity and intensity of symptoms, and the large numbers of sensitive subjects available for study.

These chambers were engineered to produce desired testing conditions. The volume in cubic feet of these chambers is intermediate, allowing for seating that is relatively large but logistically manageable. Airflow characteristics and pollen dispersal in such chambers may be easier to control, obviating the need to use fan systems and thereby lessening ocular and nasal symptoms that may be induced by air movement alone. These chambers were constructed with no fixed furnishings. All equipment, tables, and chairs are removed from the chambers after each run, which allows for more efficient and rapid cleaning. A central filtered vacuum cleaning system is used to exhaust pollen and other debris to the outside. The computer-controlled air-handler is a single-pass system in which filtered outside air moves across the condensers through a duct system with filtered, powered diffusers and exits through filtered, powered exhausts in a laminar flow.

Pollen counts were performed with the Allergenco MK-3 movable stage counter and Allergenco-D disposable cassettes. The Allergenco MK-3 staged counters allow for collecting the entire run on a single slide to be used as a permanent record. The Allergenco-D disposable cassette is brought into the control room for immediate reading. The staged Allergenco MK-3 has been compared favorably with the Burkard staged system.¹¹

Two laser counters were used during unoccupied chamber runs to help understand pollen dispersal. The stationary

Shinyei model was helpful in revealing rapid increases in particle counts as the pollen dispersal was initiated. The counts, however, were low when compared with counts from the Allergenco systems. The Kanomax hand-held laser particle counter was also used in the unoccupied chamber; however, counts from this device were high compared with the Allergenco systems. As a person approached this device within approximately 2 ft, counts increased significantly, indicating that the cloud of debris surrounding a person can be easily detected and confounds the use of this device in the occupied chamber.

In the first occupied run, subjects were brought into the chamber and seated, and the blower and eductor were activated without the pollen feeder for a masked sham period. The noise created by the blower and eductor are the same with or without the feeder being active. No subjects recorded any increased symptoms during the masked sham run. We believe that the masked sham run was important to demonstrate a lack of a placebo response.

Because of safety concerns, low levels of pollen were delivered in the initial phase. With no adverse effects occurring and symptoms remaining relatively low, counts were increased to levels higher than had been anticipated. The high pollen counts are similar to counts reported to be tolerated by subjects in the study by Hashiguchi et al,⁴ which used a

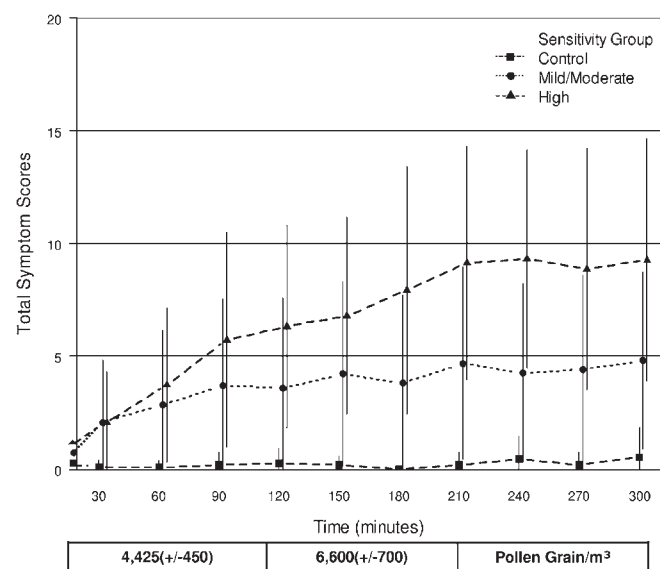


Figure 3. Mean Total Symptom Scores (TSSs) over time for healthy controls, mildly to moderately sensitive subjects, and highly sensitive subjects in a nonprimed chamber run. All subjects underwent a second chamber run using intermediate pollen levels. There was an intermediate response, with most patients reacting with symptoms similar to their first challenge. Only 2 of 11 healthy controls had minimal symptoms. The mean TSSs are shown for the 3 sensitivity groups (with SDs). A repeated-measures linear model of TSSs in terms of group and time found a significant group by time interaction ($P = .008$), indicated by the nonparallel mean trajectories. For the mildly to moderately sensitive subject group, the TSSs gradually increased to modest levels with moderately increasing pollen counts ($P < .001$, test for trend).

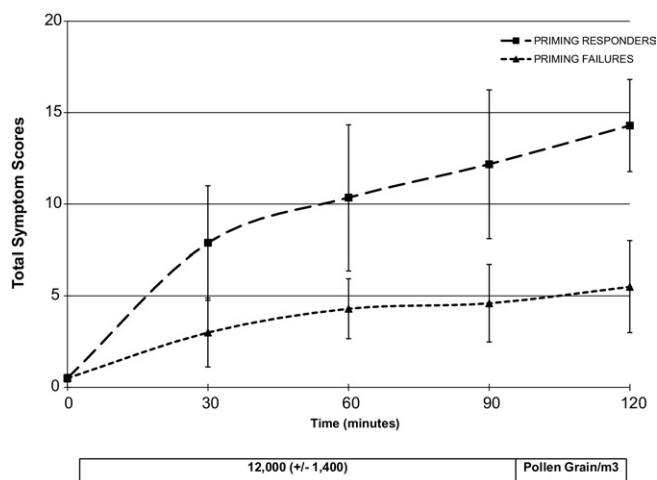


Figure 4. Mean Total Symptom Scores (TSSs) over time of combined mildly to moderately and highly sensitive subjects in a final run after priming. After priming runs, there were sufficient symptoms in priming responders for pharmacologic and immunologic studies. The TSSs increased over time ($P < .001$, test of trend).

similar pollen, *Cryptomeria japonica* (Japanese cedar). Hashiguchi et al reported on subjects chosen for the chamber studies by symptomatic history, positive radioallergosorbent assay results, and nasal challenge using a paper disk impregnated with Japanese cedar extract. Their population responded equally at pollen counts of 4,000, 8,000, and 12,000 grains/m³. No priming runs were performed; however, their use of a nasal challenge may have preselected more sensitive subjects than in the general allergic population.

We selected an intermediate level of pollen for the repeat chamber run that was delivered during the first 3 hours to determine effect to a continuous stable count. When the subjects' responses were determined to be inadequate, the pollen levels were increased by 50% for the final 2 hours of the run (Fig 3). These symptoms at both pollen levels were deemed inadequate and too slow in onset to be used in clinical trials.

Because there had been no adverse effects to intermediate levels of pollen during a 5-hour chamber run and symptoms were believed to be inadequate, we chose to do priming runs and to significantly increase pollen levels to those reported by Hashiguchi et al⁴ using Japanese cedar. The Japanese cedar is a similar pollen to the mountain cedar pollen, with extensive cross-reactivity of proteins and, presumably, similar allergenic cross-reactivity.¹²

Mountain cedar pollen counts average approximately 3,200 grains/m³ during a natural season; however, counts may reach very high levels of 50,000 grains/m³ during the peak of the season on windy days. These levels are tolerated by highly sensitive subjects for short periods. Therefore, levels of 12,000 grains/m³ (3–4 times the natural seasonal average) for short periods in the chamber do not lead to

overwhelming signs or symptoms and will trigger symptoms sufficient to study allergic mechanisms.

The Biogenics Research Chamber functioned according to engineering specifications. The air-handling systems controlled temperature and humidity within target ranges. The pollen delivery system was adjusted to deliver measured amounts of pollen in a uniform manner at levels to trigger symptoms sufficient for use in the study of the effects of medication and/or changes in inflammatory markers. Symptoms recorded during 2-hour priming runs were 2-fold increased over subjects challenged during 5-hour nonprimed runs. No subjects responded to a sham run. Healthy controls did not experience significant symptoms. Priming runs were necessary to trigger significant symptoms in a timely fashion for practical use in controlled studies. No adverse effects were recorded. Using mountain cedar pollen in the chamber provides a new model for studying allergic responses.

REFERENCES

1. Renborg SM, Mosbech H, Johnsen CR, Poulsen LK. Exposure chamber for allergen challenge: the development and validation of a new concept. *Allergy*. 1996;51:82–88.
2. Day JH, Briscoe MP. Environmental exposure unit: a system to test anti-allergic treatment. *Ann Allergy Asthma Immunol*. 1999;83:83–93.
3. Hashiguchi K, Tang H, Fujita T, et al. Pilot study of Japanese cedar pollen exposure using a novel artificial exposure chamber (OHIO Chamber). *Clin Exp Allergy Rev*. 2008;8:30–36.
4. Hashiguchi K, Tang H, Fujita T, et al. Validation study of the OHIO Chamber in patients with Japanese Cedar pollenosis. *Int Arch Allergy Immunol*. 2009;149:141–149.
5. Berkowitz RB, Woodworth GG, Lutz C, et al. Onset of action, efficacy, and safety of fexofenadine 60 mg/pseudoephedrine 120 mg versus placebo in the Atlanta allergen exposure unit. *Ann Allergy Asthma Immunol*. 2002;89:38–45.
6. Day JH, Horak F, Briscoe MP, et al. The role of allergen challenge chambers in the evaluation of anti-allergic medication: an international consensus paper. *Clin Exp Allergy Rev*. 2006;6:31–58.
7. Ratner PH, Hampel FC, Amar NJ, et al. Safety and efficacy of olopatadine hydrochloride nasal spray in the treatment of seasonal allergic rhinitis to mountain cedar. *Ann Allergy Asthma Immunol*. 2005;95:474–479.
8. Andrews CP, Martin BG, Jacobs RL, et al. Fluticasone furoate nasal spray is more effective than fexofenadine for nighttime symptoms of seasonal allergy. *Allergy Asthma Proc*. 2009;30:128–138.
9. Jacobs R, Martin B, Hampel F, et al. Effectiveness of fluticasone furoate 110 microg once daily in the treatment of nasal and ocular symptoms of seasonal allergic rhinitis in adults and adolescents sensitized to mountain cedar pollen. *Curr Med Res Opin*. 2009;25:1393–401.
10. Tseng LC, Fung R, Chen CC. Significance of face velocity fluctuation in relation to laboratory fume hood performance. *Ind Health*. 2010;48:43–51.
11. Portnoy J, Landuyt J, Pacheco F, et al. Comparison of the Burkard and Allergenco MK-3 volumetric collectors. *Ann Allergy Asthma Immunol*. 2000;84:19–24.
12. Schwieth LA, Goetz DW, Whisman B, et al. Cross-reactivity among conifer pollens. *Ann Allergy Asthma Immunol*. 2000;84:87–93.

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