67 Structural Characteristics and Validation of Biogenics Research Chamber to Juniperus asheii (Mt. Cedar) Pollen

Jacobs RL, Ramirez DA, Andrews CP.
Biogenics Research Chamber, San Antonio, Texas

Abstract

Rationale: To construct and validate a facility utilizing clean room characteristics for the study of subjects' responses to inhalational challenges of *Juniperus asheii* (Mt. Cedar) pollen.

Methods: Two compact chambers were constructed with clean room materials. The air handling system chilled outside air in a single pass. Powered, filtered diffusers delivered air and powered filtered exhausts removed equal amounts to maintain a laminar flow. Pollen was delivered by a feeder into a vortex created by an eductor thru a series of stainless-steel tubes. Mt. Cedar sensitive and non-allergic control subjects were exposed, outside the pollen season, to an air sham run and increasing increments of pollen over a 5 hour time period. Symptoms and pollen counts were recorded at 30 minute intervals.

Results: The chambers are 10846 and 6655 cubic feet and seats 50 subjects and 14 technicians and 25 subjects and 7 technicians respectively. Temperature and humidity were maintained at optimal levels When fully occupied. Air flow from diffusers measured 1.0 meter/second. Air flow out exhausts measured 2.65 meters/second. Pollen counts ranged from 1300-12500 grains/M3. No subjects responded to the air sham run. Mt. Cedar allergic subjects responded in increasing numbers and intensity as pollen levels were increased. Two non-sensitive subjects experienced low-level symptoms at high pollen counts.

Conclusions: The chambers functioned within the parameters for which they were designed. Subjects did not respond to an air sham run. Allergic subjects required much higher levels of pollen exposure than average natural exposure. Some non-sensitive subjects may experience symptoms at high pollen levels. Priming runs will be necessary.

Introduction

- Use of a pollen challenge chamber may be a more accurate and effective mechanism than natural exposure for studying the attributes if medication and the inflammatory responses of allergic rhinoconjunctivitis.
- Limitations of natural studies include differences in pollen exposure of subjects, uncontrollable weather conditions, and subject compliance. These factors are controlled in a pollen challenge chamber.
- *Juniperus asheii* (Mt. Cedar) pollen allergy is a robust model in South Texas commonly used in natural studies of pharmacologic efficacy. With a large, highly-sensitive population to Mt. Cedar pollen, a chamber enhances our ability to study the effects of medication and the inflammatory responses with greater precision than would be possible through natural exposures.

Objectives

- To determine air-flow characteristics of the air-handlers in two (2) independent pollen challenge chambers.
- To determine dispersal of pollen in order to achieve uniform distribution throughout the chambers.
- To determine the dynamics of the pollen feeder system in achieving and sustaining adequate levels of pollen within the chambers.
- To determine safe levels of pollen grains/M3 to stimulate nasal and ocular symptoms in Mt. Cedar sensitive subjects.
- To determine if there is a placebo effect during a blinded sham run.
- To determine if priming runs are required for Mt. Cedar sensitive subjects.

Methods: Mechanical

- Two chambers, with a central control room and staging area, were built with clean room technology.
- Air handlers are independently computer-controlled to adjust for full occupancy at maximal outside temperatures.
- Outside air is filtered and chilled or warmed in a single pass through the system and distributed from ducts by filtered, powered diffusers from the ceiling and exhausted by filtered, powered vents at the floor level. Air Exchange rates are 12 full cycles/hr.
- Pollen is delivered by a computer-controlled feeder into a vortex created by a blower augmented by an eductor.
- Pollen is dispersed through a series of stainless steel tubes with specially-bored ports and specially-machined joints locked by hand-cranked clamps that allow for directional adjustment of pollen levels.
- Pollen counts are performed utilizing two different manual Allergenco systems mounted on platforms at 46" from floor level.
- Chairs and tables within the chambers are movable to allow for ease of cleaning and for different configurations for different protocols.
- Audio and video systems are used for communication between the chambers and control room.
- An alternative energy source is a diesel-powered electrical generator with sufficient capacity to power both chambers under full operational mode for 24 continuous hours.

Methods: Operational without Subjects

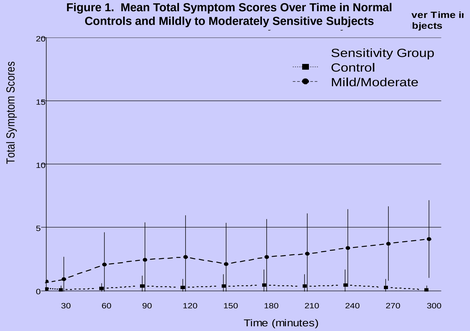
- Preliminary chamber runs were done without subjects with maximal equipment in place to determine air-flow characteristics and pollen dispersal

Methods: Operational with Subjects

- Mt. Cedar sensitive and normal control subjects, upon meeting entry criteria, were exposed to increasing amounts of pollen to determine levels sufficient to cause symptoms.
- Symptoms were self-scored on a scale of 0 (absent), 1 (mild), 2 (moderate), and 3 (severe).
- Symptoms that were scored included nasal congestion, sneezing, rhinorrhea, and itching. Ocular symptoms included redness, tearing, and itching.
- **Chamber Run #1**: Because of safety concerns, 26 mildly to moderately Mt. Cedar sensitive and 11 normal control subjects were selected for the initial non-primed chamber run.
- The pollen dispersal system was activated without pollen for an Air-only sham run to determine placebo effect.
- Pollen was delivered through the pollen dispersal system in a graduated fashion with counts in grains/M3 at 30 minute intervals
- Subjects recorded signs, symptoms, and adverse responses at baseline and 30 minute intervals on a manual chamber diary card.
- **Chamber Run #2**: Fifteen (15) highly-sensitive subjects to Mt. Cedar were selected for a non-primed chamber run utilizing higher pollen levels in graduated fashion.
- **Chamber Run #3:** The mildly to moderately sensitive, the highly sensitive, and normal control subjects were combined in a non-primed chamber run utilizing stable pollen counts.
- **Priming Chamber Runs:** Initiated upon recognition that priming would be necessary and high levels of pollen would be required.
- Priming runs were done on sequential days.
- Subjects were considered primed if meeting priming criteria (TNSS>6) on 2 consecutive days.

RESULTS

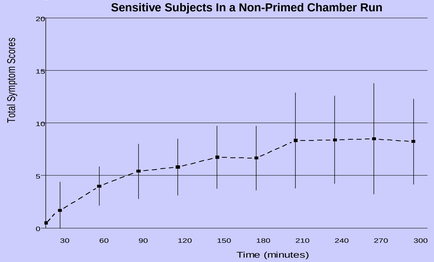
Figure 1. Mean Total Symptom Scores Over Time in Normal Controls and Mildly to Moderately Sensitive Subjects



0	1,600	2,230	3,050	3,725	4,334	4,450	5,734	6,330	7,100	Cassette Counts(m³)
0	1,950	2,150	3,080	4,150	4,250	4,650	6,660	8,000	8,000	Staged Counts (m³)
0	15	20	25	30	35	40	45	50	60	Feeder Rate(gr/hr)

There were no responses within the first thirty-minute sham interval when no pollen was being delivered. The mean Total Symptom Scores (TSS) are shown for the two sensitivity groups with symbols representing the mean and solid vertical lines representing the standard deviation (SD). A repeated-measures linear model of TSS in terms of group (normal controls, mildly to moderately sensitive subjects) and time found a significant group × time interaction (p=0.02) indicated by the non-parallel mean trajectories. Only two of eleven normal controls had minimal symptoms. Pollen grains/m3 were compared between 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 TSS gradually increased to modest levels with moderately increasing pollen counts (p<0.001, test for trend).

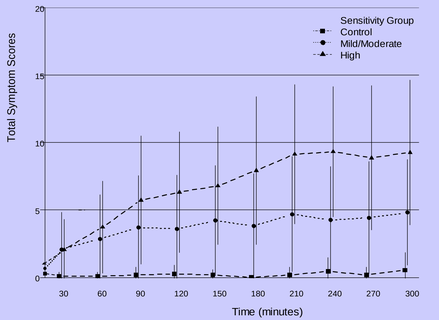
Figure 2. Mean Total Symptom Scores Over Time of Selected Mt. Cedar Highly Sensitive Subjects In a Non-Primed Chamber Run



1,981	3,431	4,325	5,424	6,550	7,711	9,233	8,621	10,177	11,167	Pollen Grain/m³

Highly sensitive subjects were selected based upon clinical, history, diary data from a previous natural trial, and size of a prick skin test. TSS increased over time in the chamber (p<0.001, test of trend). Their response to higher pollen counts over a shorter time period than those with mild to moderate sensitivity showed a two-fold increase in symptoms.

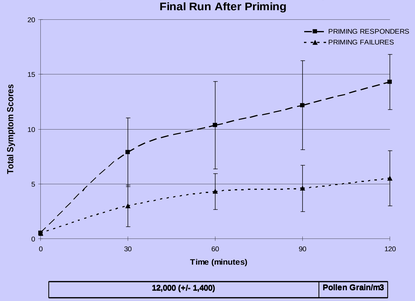
Figure 3. Mean Total Symptom Scores Over Time of Normal Controls, Mildly to Moderately and Highly Sensitive Subjects in a Non-Primed Chamber Run



4,425(+/-450)	6,600(+/-700)	Pollen Grain/m³

All subjects underwent a second chamber run utilizing intermediate pollen levels. There was an intermediate response with most patients reacting with symptoms similar to their first challenge. Only two of eleven normal controls had minimal symptoms. The mean TSS are shown for the three sensitivity groups (with SD). A repeated measures linear model of TSS in terms of group and time found a significant group × time interaction (p=0.008), indicated by the non-parallel mean trajectories. For the mildly to moderately sensitive subject group, the TSS gradually increased to modest levels with moderately increasing pollen counts (p<0.001, test for trend).

Figure 4. Mean Total Symptom Scores Over Time of Combined Mildly to Moderately and Highly Sensitive Subjects in a Final Run After Priming



12,000 (+/- 1,400)	Pollen Grain/m3

After priming runs, there were symptoms sufficient for pharmacologic and immunologic studies. TSS increased over time (0<0.001, test of trend).

Table #1: Air Flow from Diffusers and outflow through Exhausts.

Diffuser	Run #1 01/19/10	Run #2 07/14/10	Exhausts	Run #1 01/19/10	Run #2 07/14/10
#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

Measurements were taken with a hot wire anemometer for low flow/air velocity in meters/second. The air flow measures were not significantly different between the two separate runs for the diffuser (t-test, p=0.36) or the powered exhaust (t-test, p=0.06).

CONCLUSIONS

- Biogenics Research Chamber functioned according to engineering specifications.
- Air handlers maintained laminar flow and constant temperature and humidity control within target range.
- The pollen delivery system dispersed pollen in a uniform manner.
- No subjects responded during a blinded air-sham chamber run.
- Two of 11 non-allergic subjects responded with low-level, insignificant symptoms
- Non-priming runs were unsuccessful in triggering symptoms at adequate levels in a timely fashion in significant numbers of subjects.
- Priming chamber runs will be necessary to identify subjects suitable for study within the chamber.
- No serious adverse events occurred.
- Utilizing Mt. Cedar pollen in the chamber provides a new model for studying allergic responses.