Mold in Housing: Novel Detection Strategies from Improved Understanding of Fungal Function

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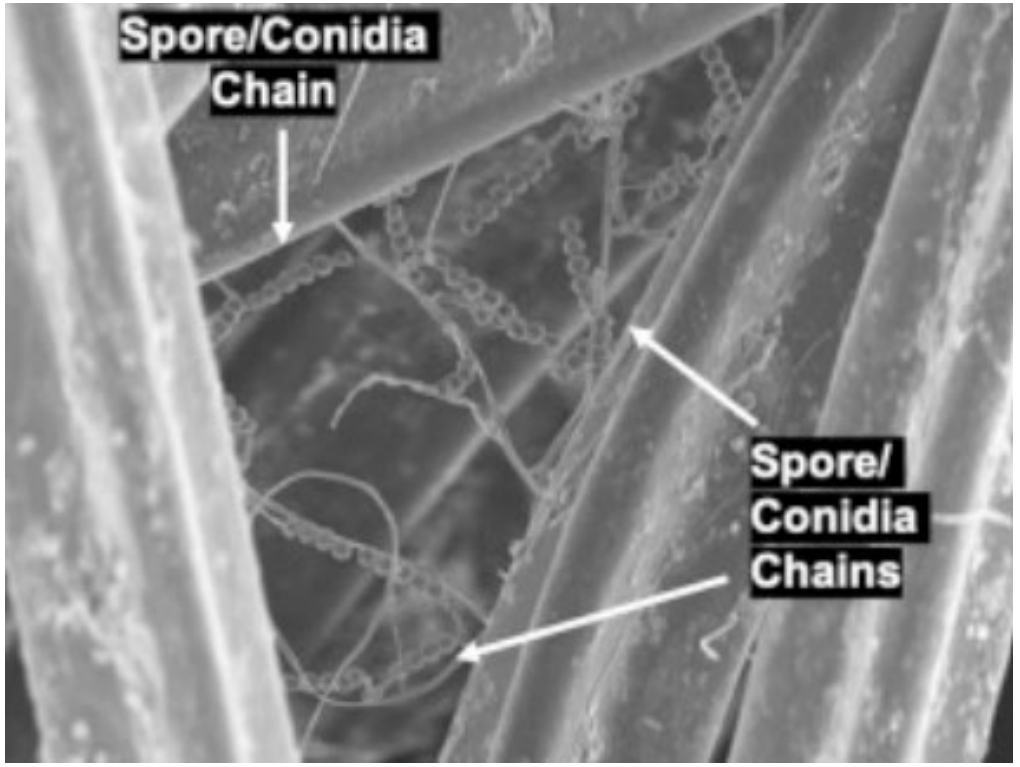
Associate Professor

@KarenCDannemill



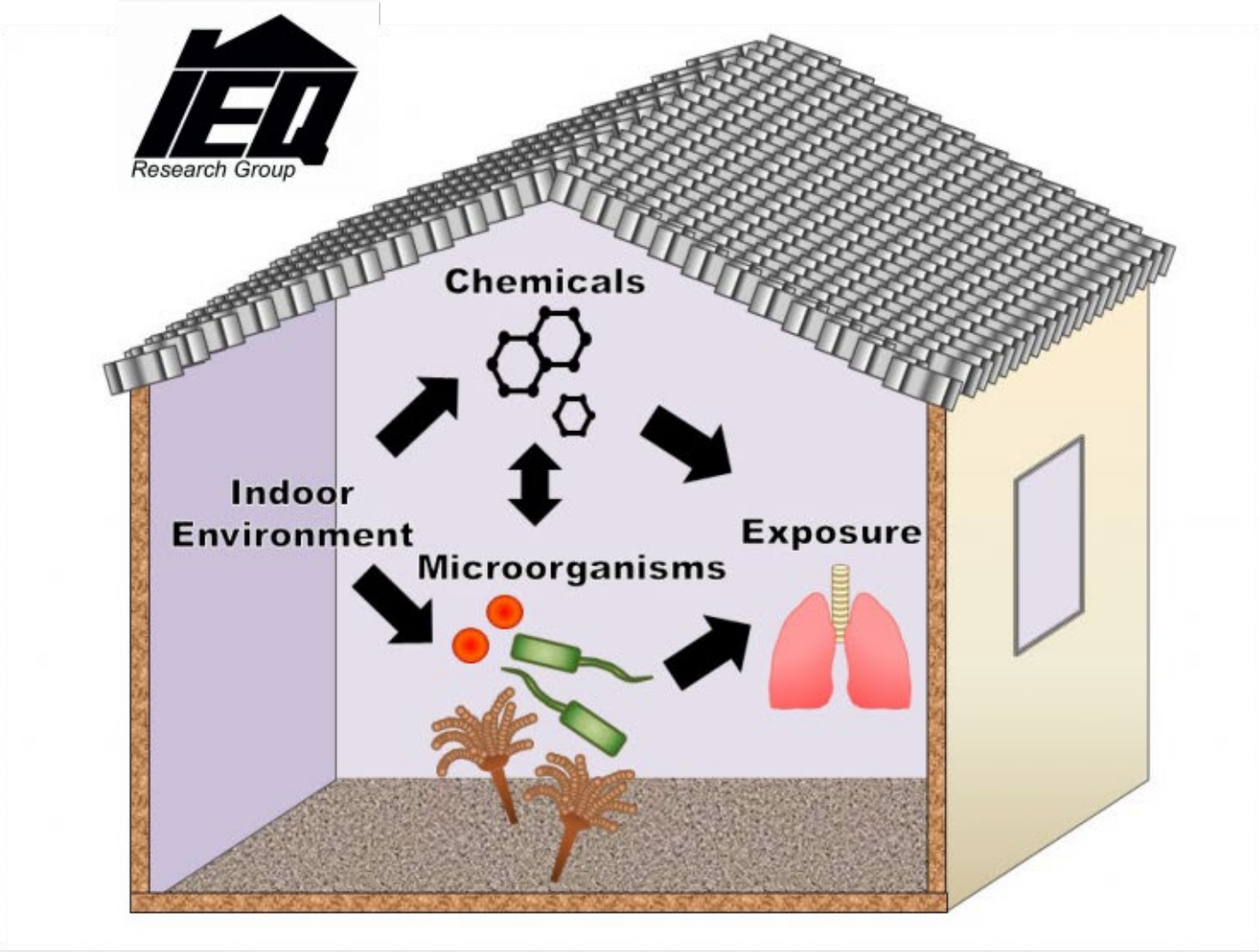
OHHN Conference

May 10, 2024

THE OHIO STATE UNIVERSITY

Image: CDC PHIL

Indoor Environmental Quality



What is a healthy indoor microbiome?

Hygiene hypothesis?

Detrimental taxa?

Good Neutral Bad

Today, exposure to mold in homes costs $22.4 billion per year

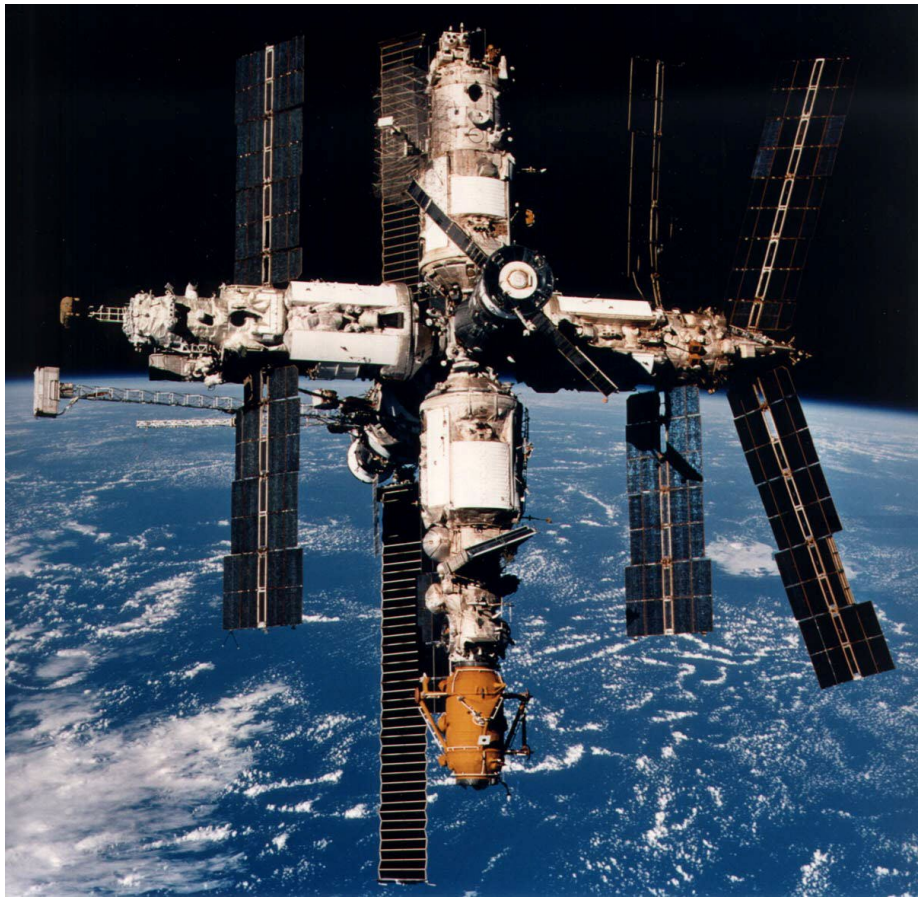
Artwork by Daniele Del Nero

16% of cost associated with:

- Allergic Rhinitis
- Acute Bronchitis
- Asthma

Mudarri, D., J Environ Public Health. 2016; 2016: 2386596.

Microbial growth is problematic on Earth and in space

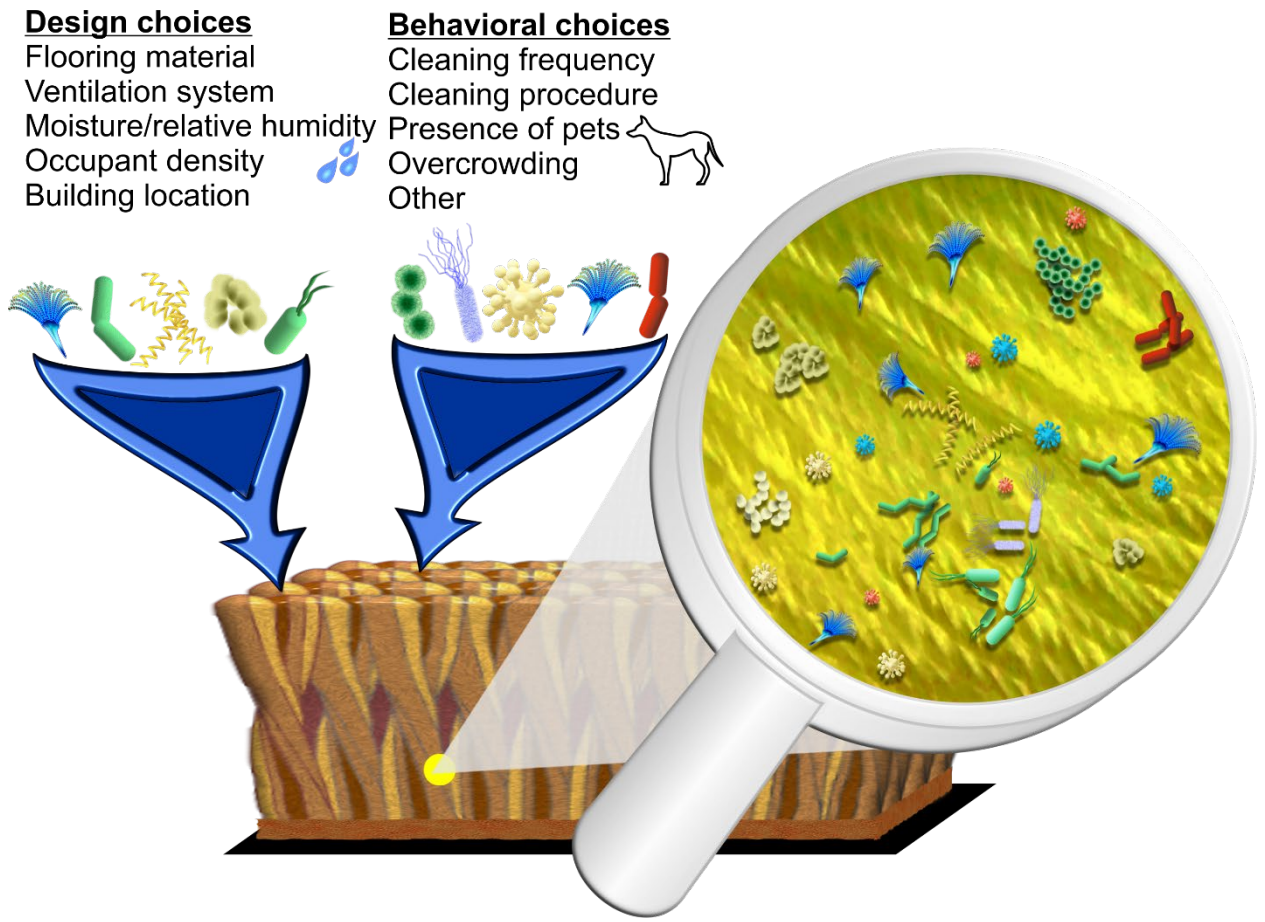


***Mir* suffered from microbial growth**

Mold growth on fabric panels on ISS from wet hanging towels

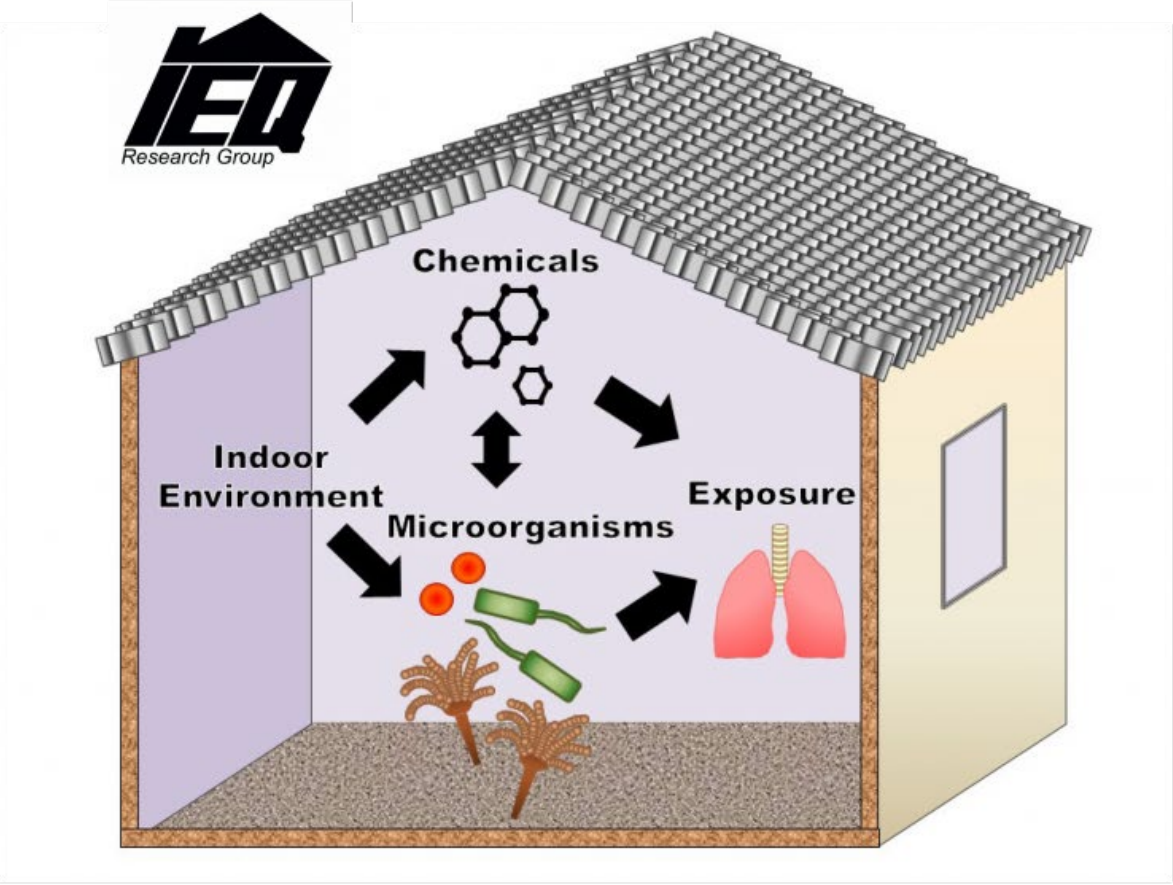
Microb. Ecol., vol. 47, no. 2, pp. 133–136, 2004
Res. Microbiol., vol. 159, no. 6, pp. 432–435, 2008
Photo credit: NASA.

We want to support a ***Healthy Indoor Microbiome***



Dannemiller, *mSystems*, 2019

Talk Outline
Healthy Indoor Spaces



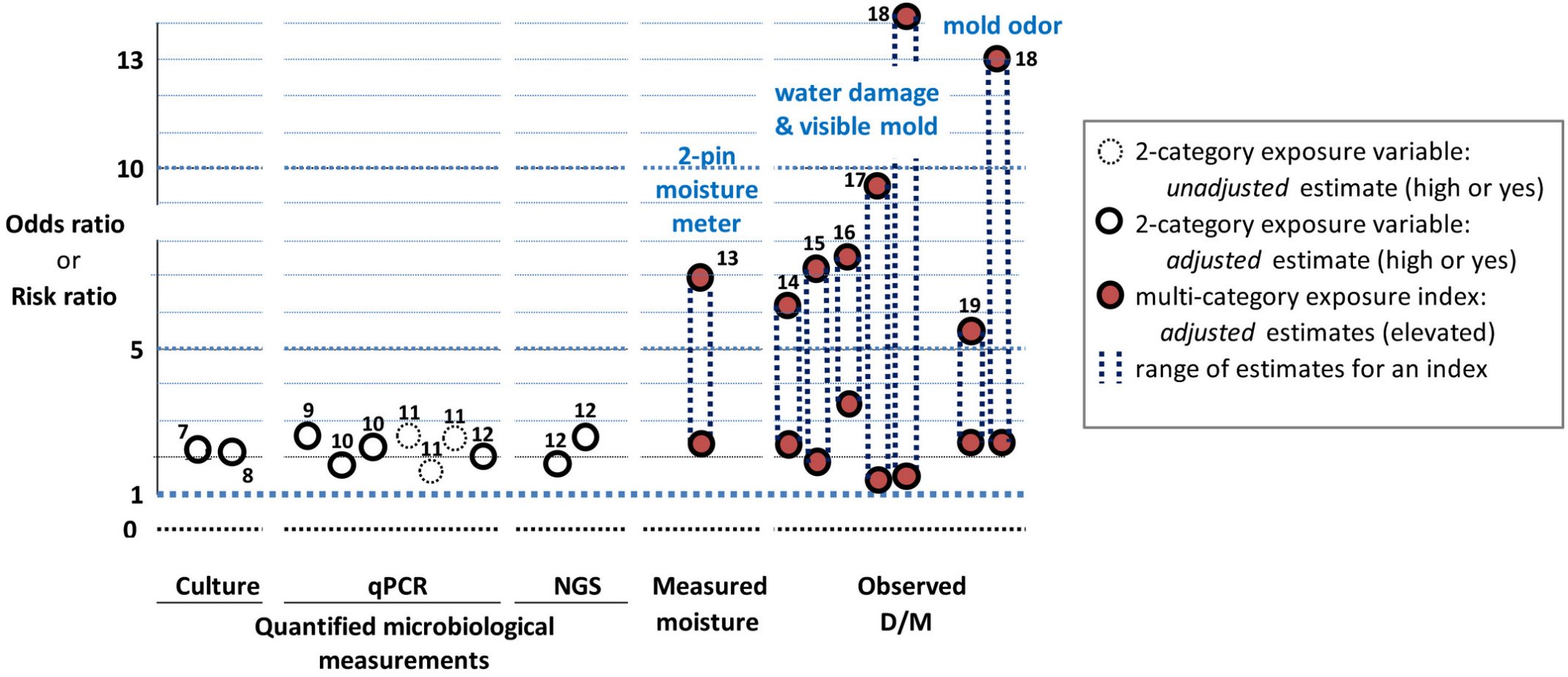
Part 1: New Mold Indicators

Part 2: Spacecraft

Part 3: Novel allergen sensors

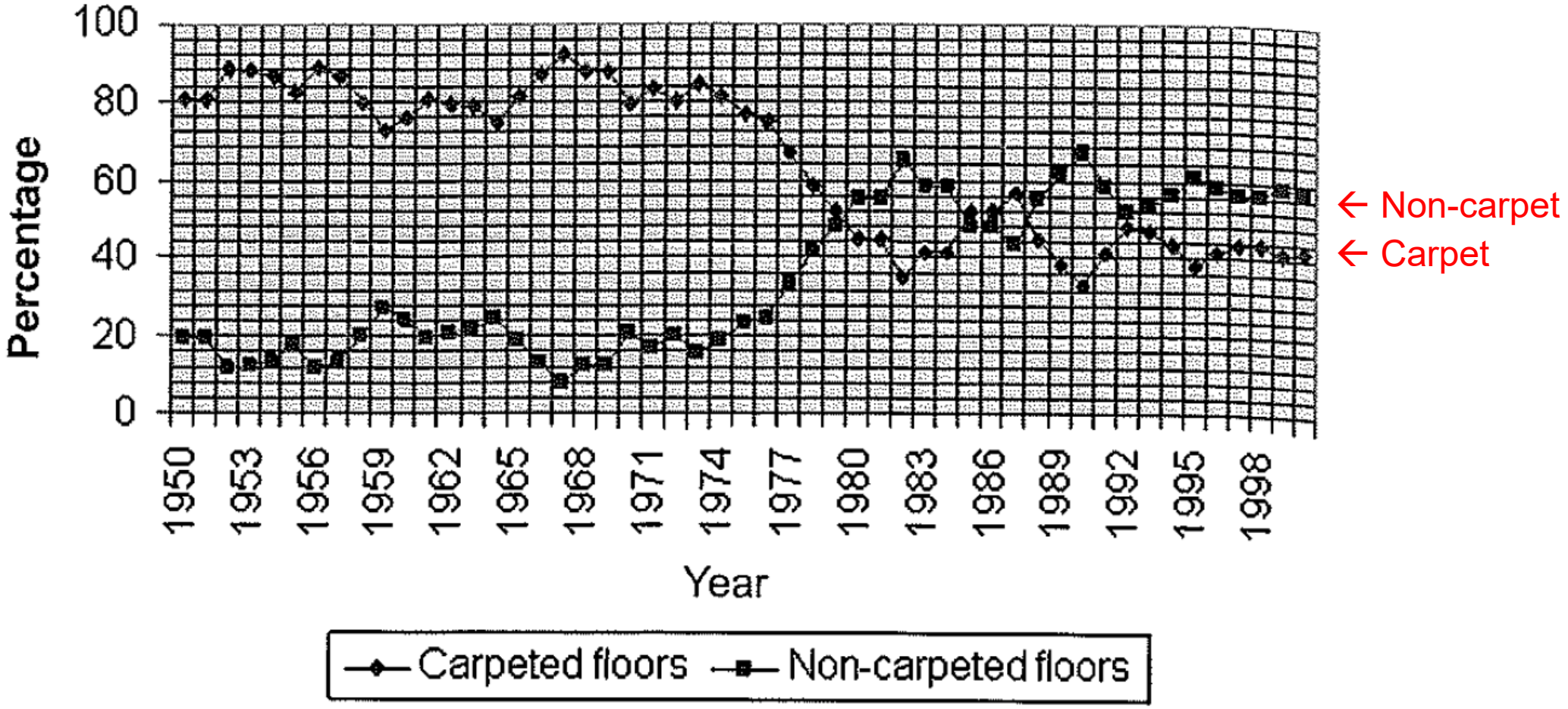
Part 1: Moving beyond a focus on only "Toxic Black Mold"

We need improved mold indicators to identify problems



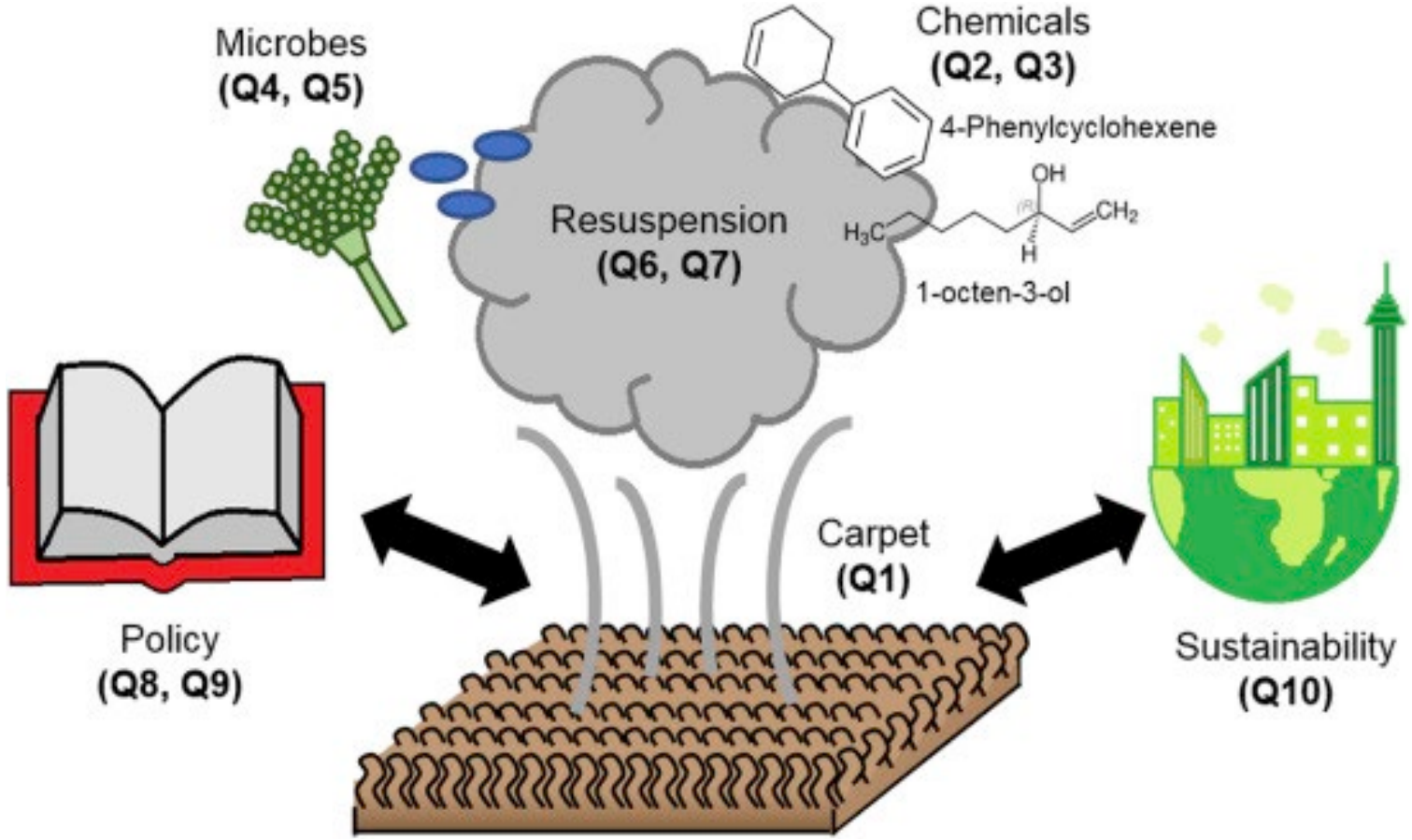
Mendell and Adams, Indoor Air, 2019

Carpet is prevalent in homes post-WW II



From: Ulrich and Lee, TJTI 2008, 99, 67-75

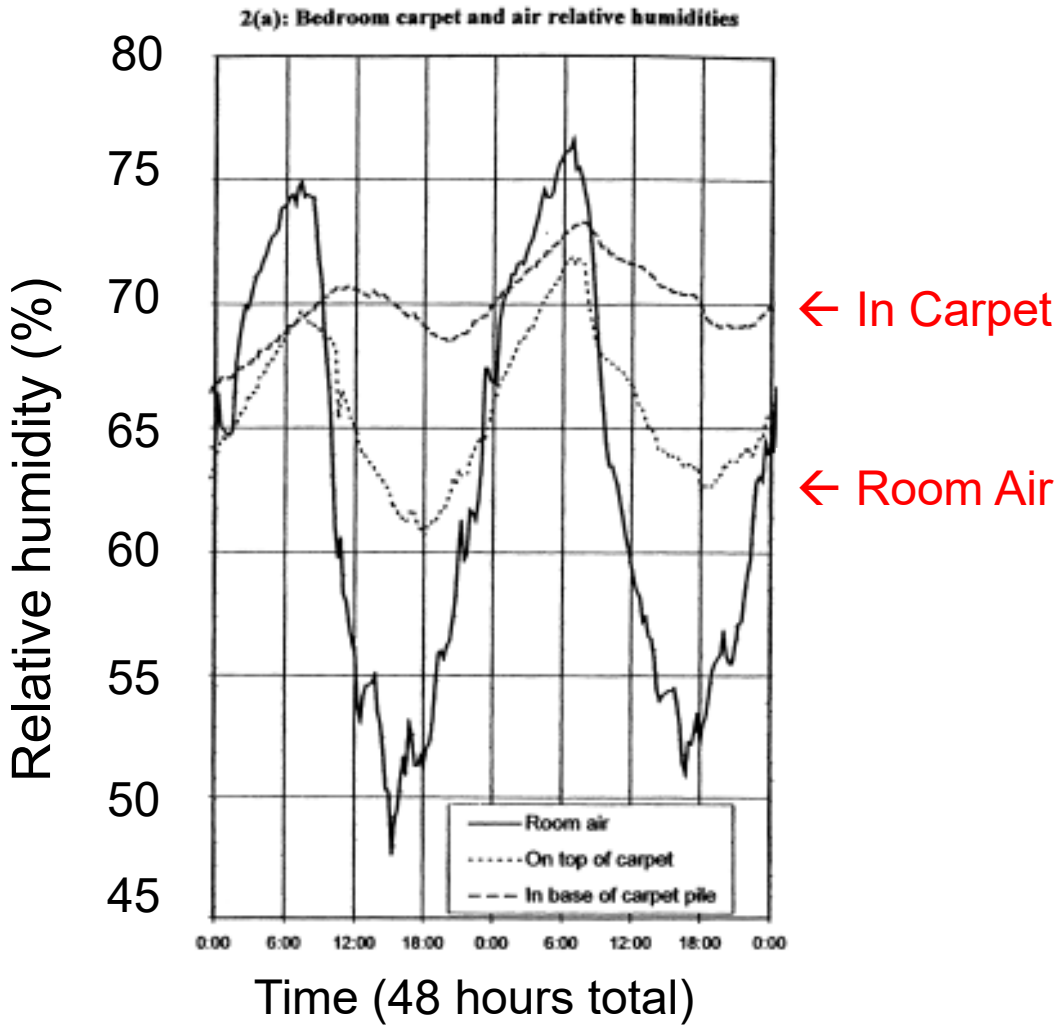
Carpet is an important reservoir for human exposure to dust



Haines et al, 2020, Building and Environment, https://doi.org/10.1016/j.buildenv.2019.106589

Relative humidity in carpet can be elevated above room air

- Dust mites
- Indoor chemistry
- Bacteria
- Fungi (← focus here)



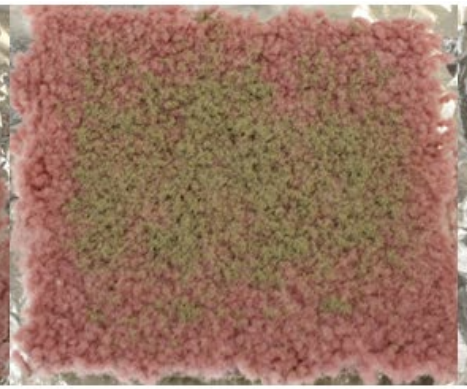
Cunningham, 1998 Clin Exp Allergy

Sample collection

- Collect carpet/dust from home
- Expose to relative humidity conditions
- Determine microbial growth
  - Rate
  - Function
  - Phthalate degradation

Dust is embedded in carpet

10 cm x 10 cm carpet coupon



Apply dust

Embed dust with modified ASTM F608−13 method

Dust is embedded

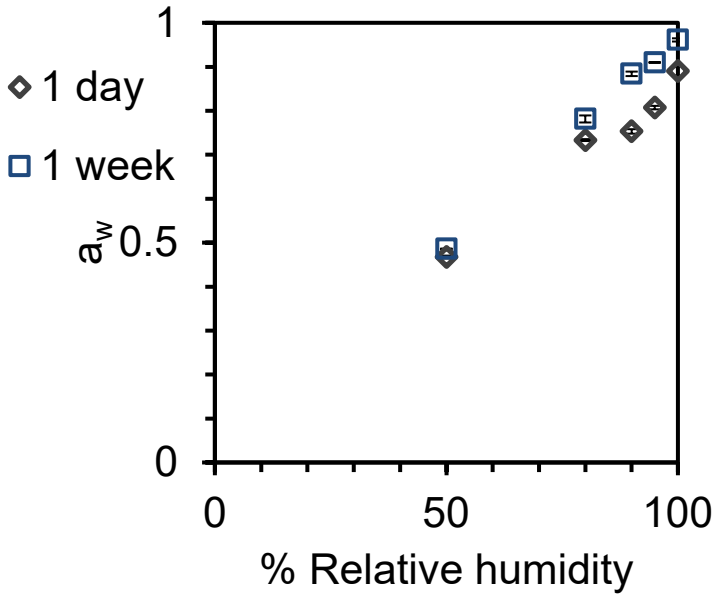
Place carpet coupon in temperature- & relative humidity-controlled chamber

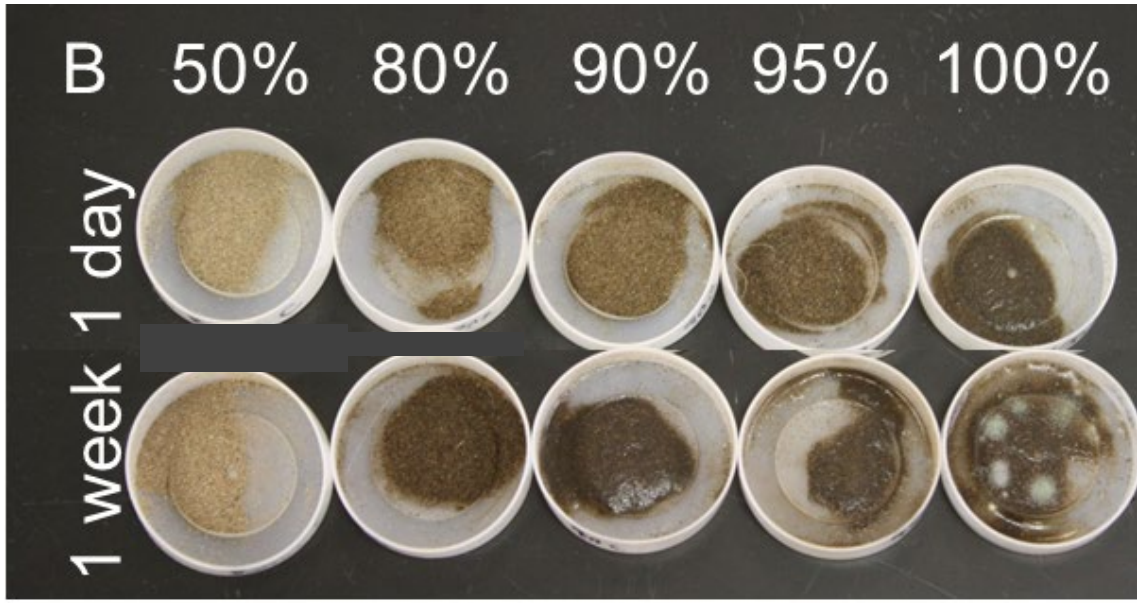
DNA/RNA-based measurement of microbial exposures in homes: Improved measurement

Dust → DNA → DNA Sequencing → Bioinformatics Tools

- ~Hundreds of thousands of DNA reads per sample
- Taxon identifications
- Quantification
- Diversity

Water activity (a_w) of dust equilibrates quickly with RH

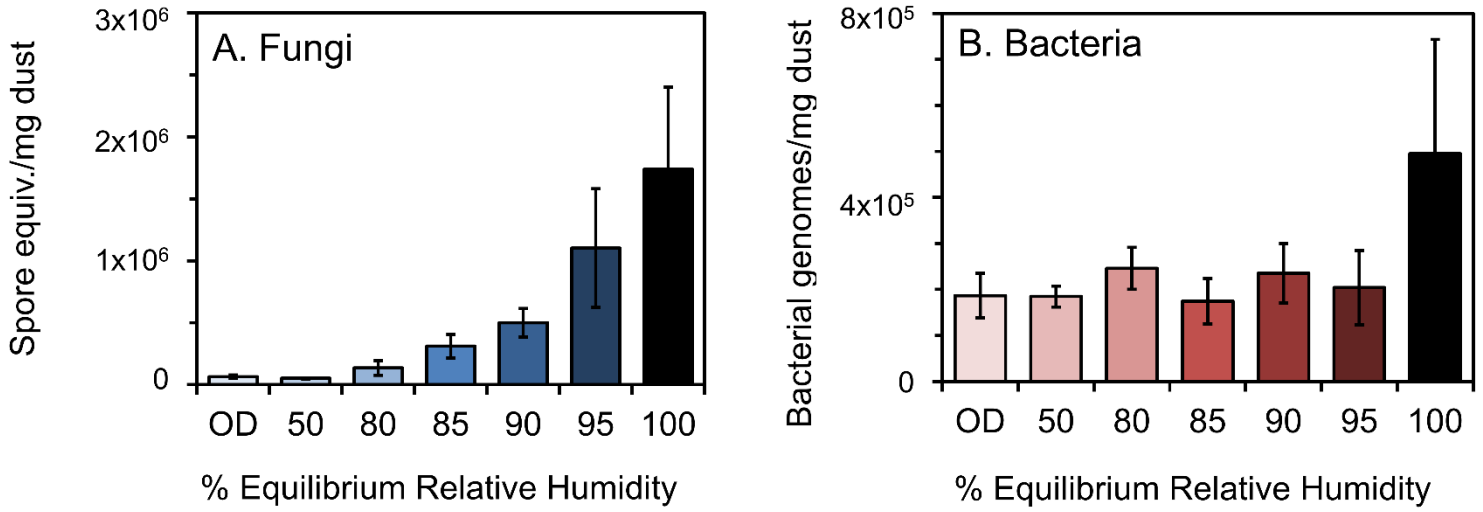


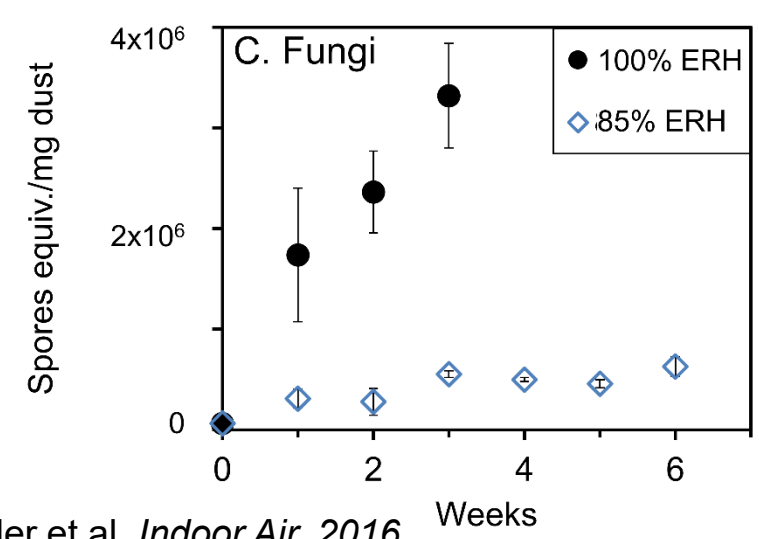


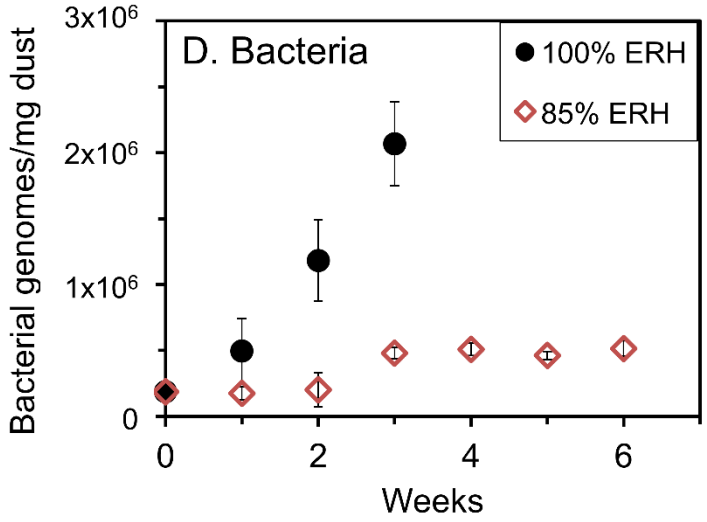
$$a_w = \frac{p_{dust}}{p_{water}}$$

$$Equilibrium\ RH = a_w \times 100\%$$

Microbial growth occurs above 80% relative humidity





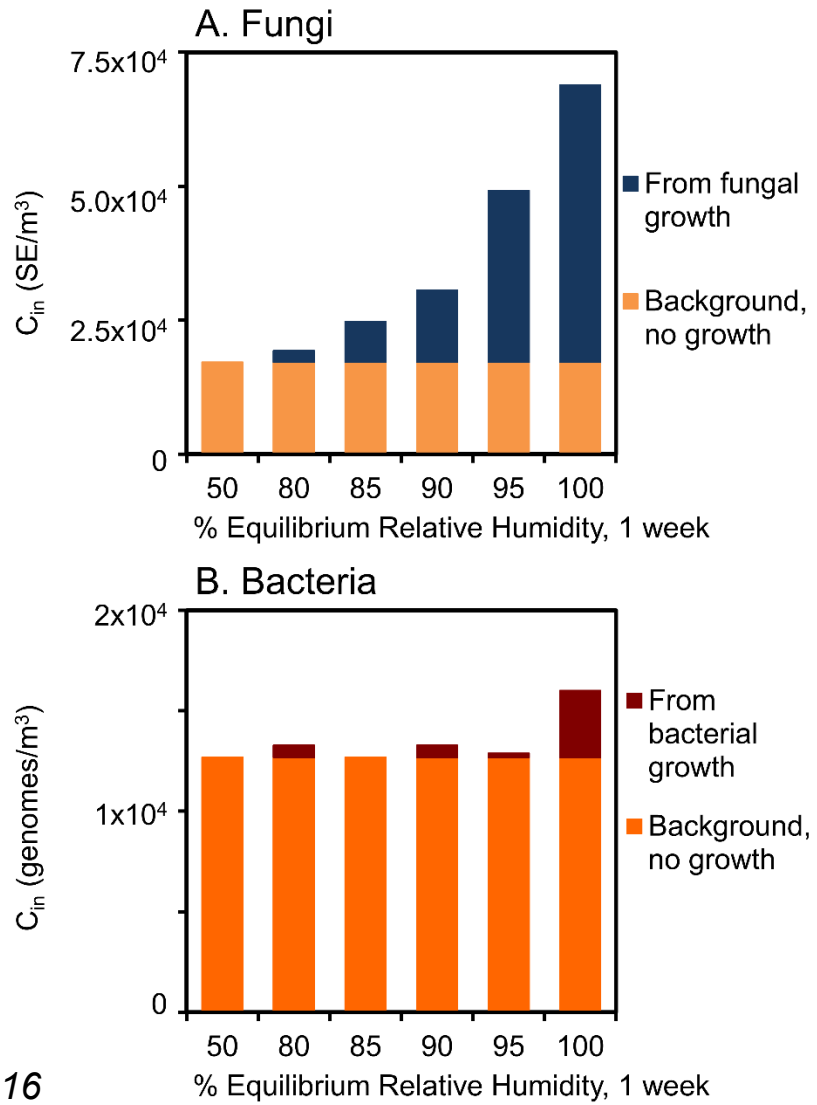


Dannemiller et al. *Indoor Air, 2016*

Moisture is the limiting factor for growth

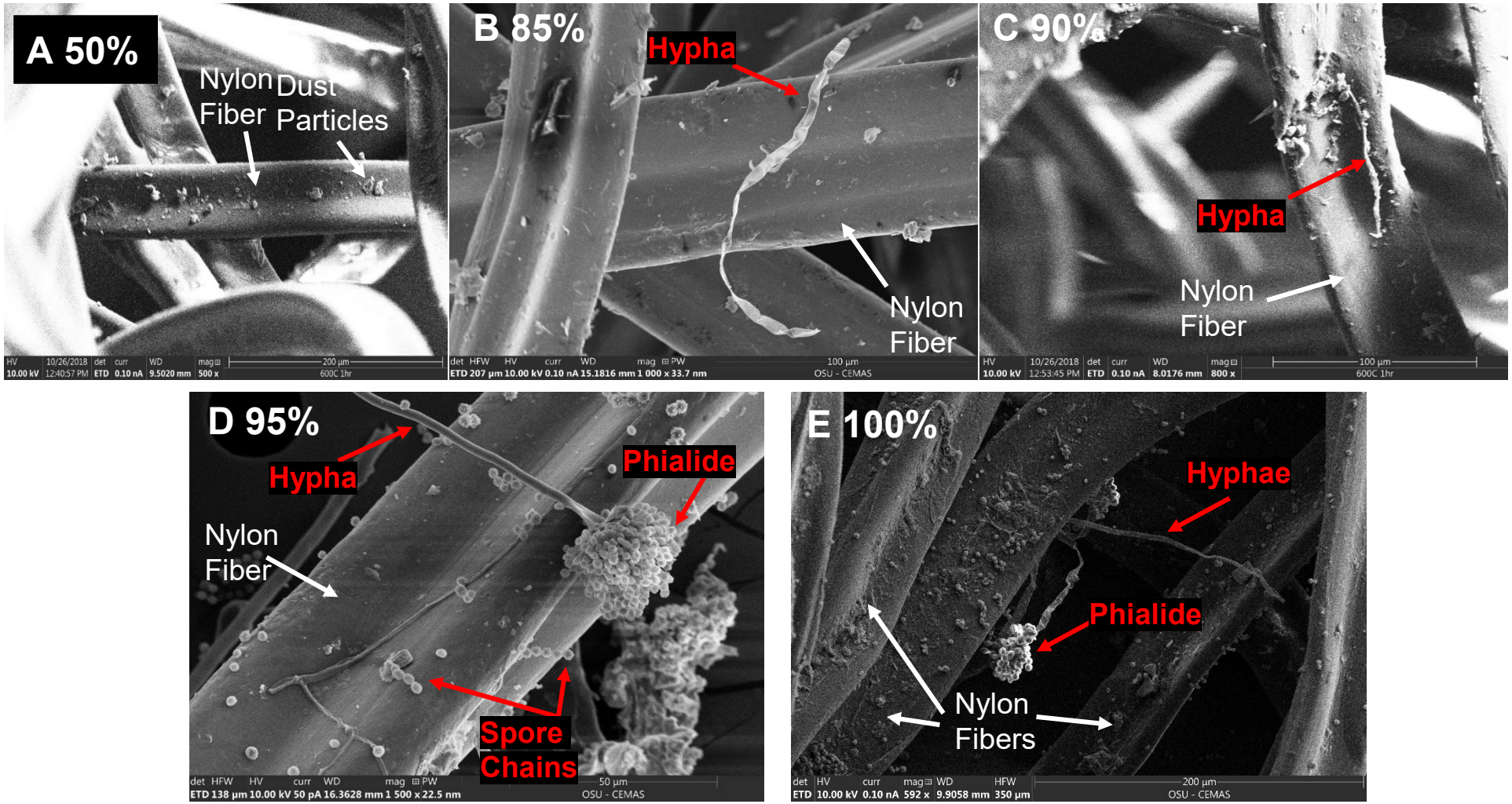
Nutrient/ Salt	Dissolvable amount in dust (mg/kg dust)	Estimated amount needed to support growth (mg/kg dust)
C	35000	7.2
N	5.7	1.3
P	7.9	0.22
S	9.1	0.058
Na	6300	-
K	2100	-
Ca	1600	-
Mg	220	-
NH_4	160	-
Cl	2400	-

Growth in dust contributes to human aerosol exposure



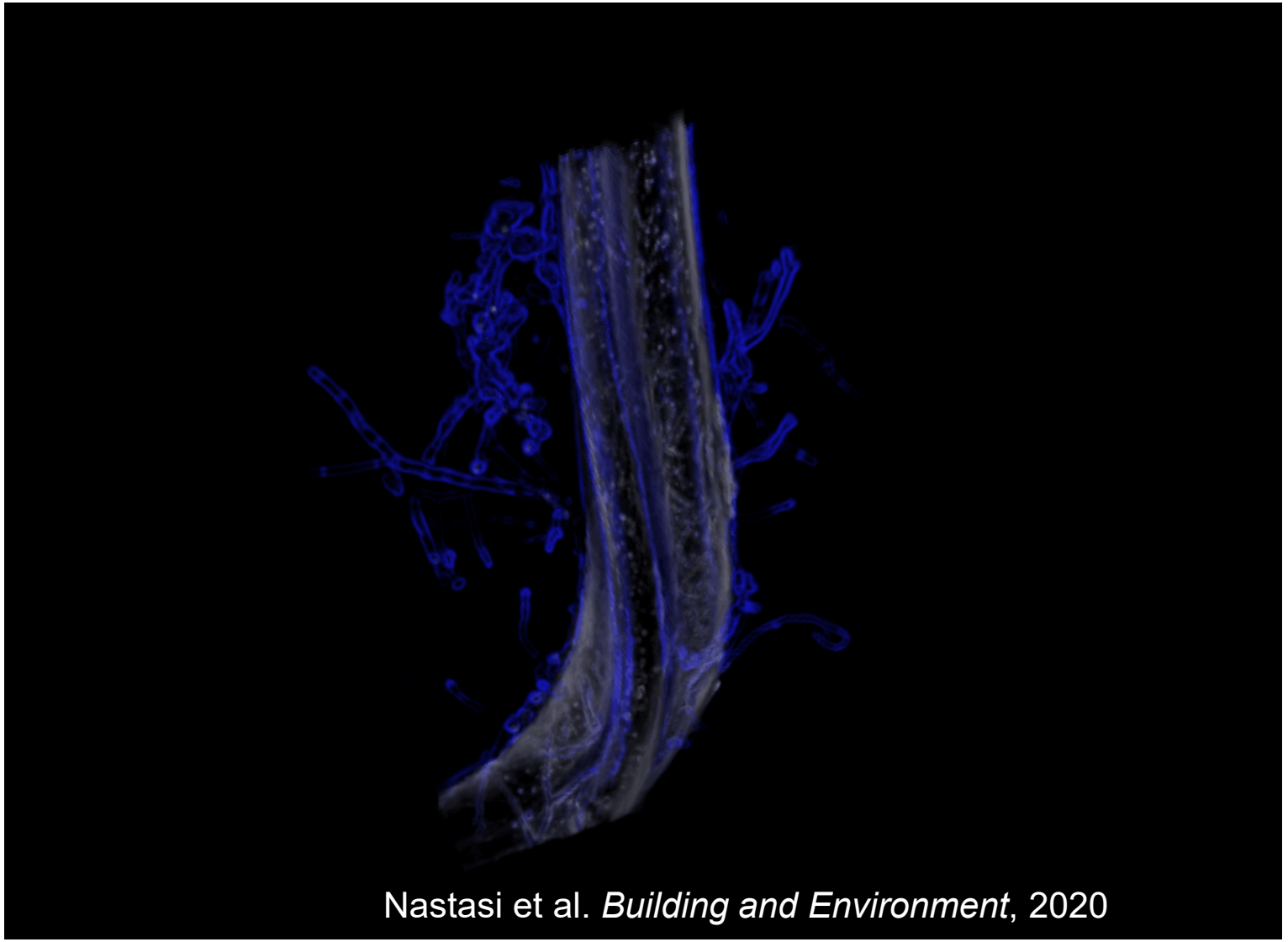
Dannemiller et al. *Indoor Air, 2016*

But what does it look like?
Low → High RH



Nastasi et al. *Building and Environment*, 2020

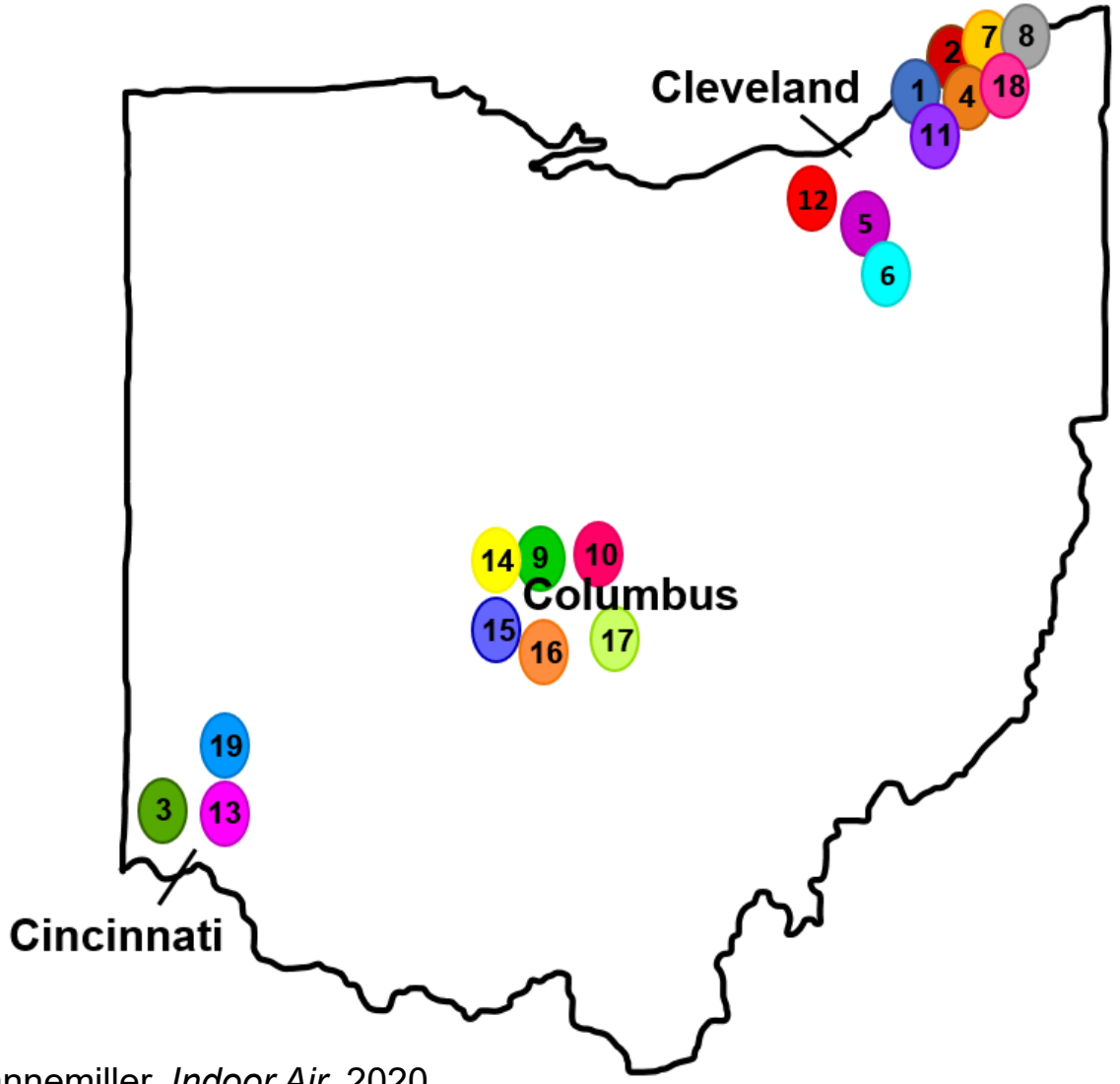
Fungal growth on carpet



Nastasi et al. *Building and Environment*, 2020

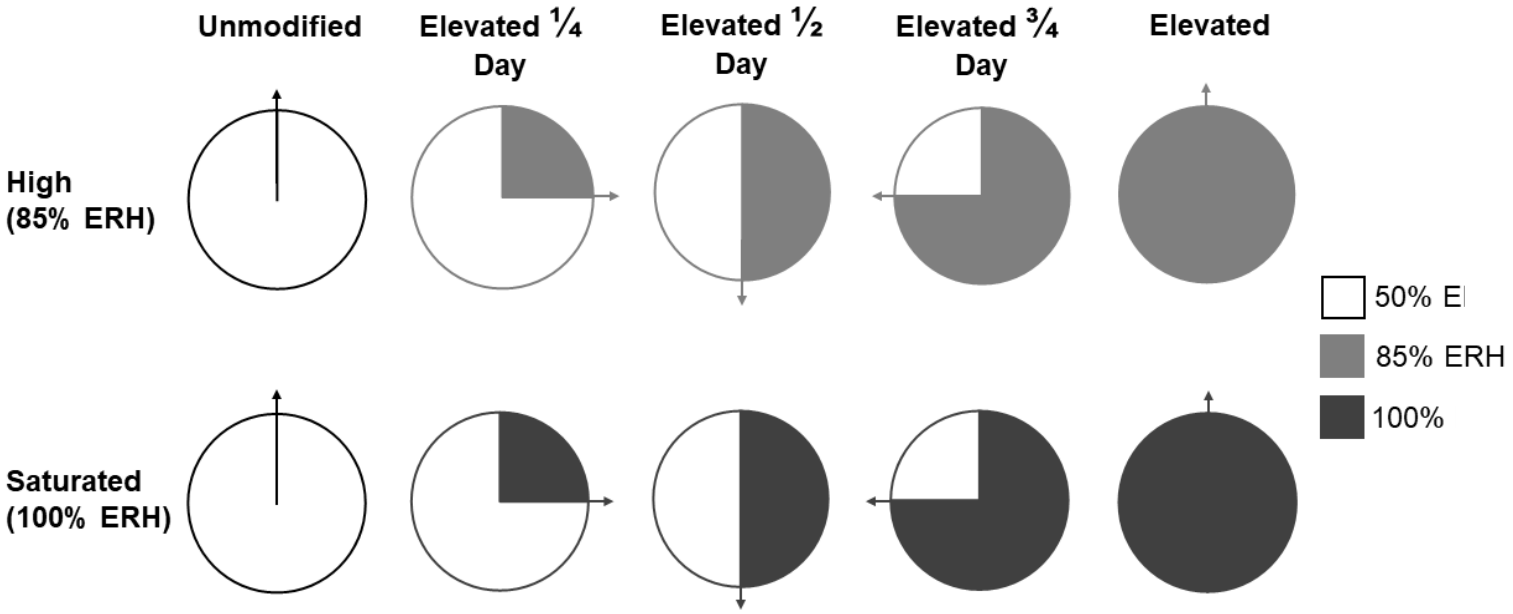
Next Goal: Determine how variations in RH affect fungal growth in carpet

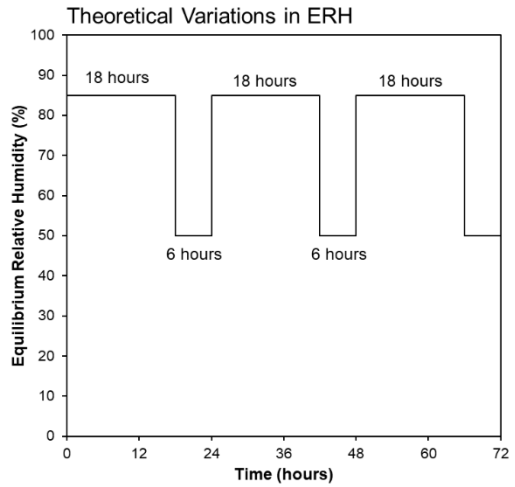
Sarah Haines

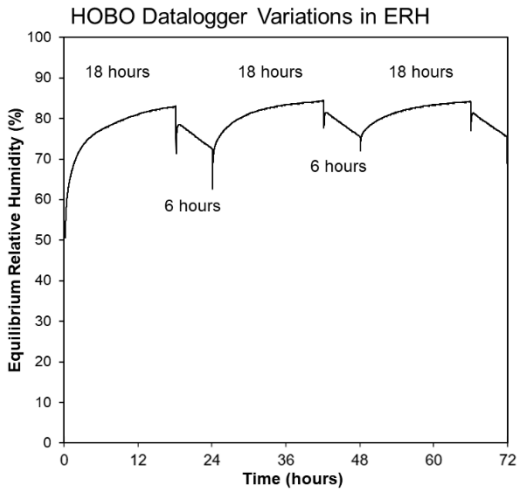


Haines, Siegel, Dannemiller. *Indoor Air.* 2020.

Goal: Determine how variations in RH affect fungal growth in carpet

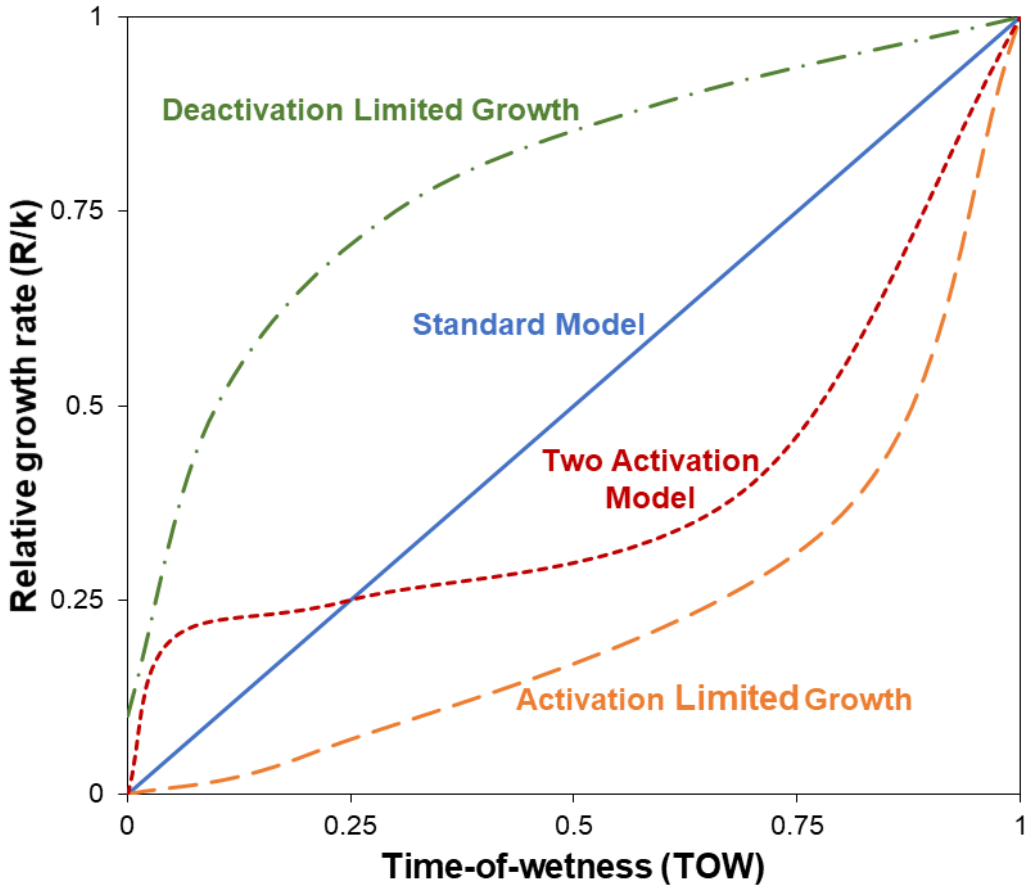






Haines, Siegel, Dannemiller. *Indoor Air.* 2020.

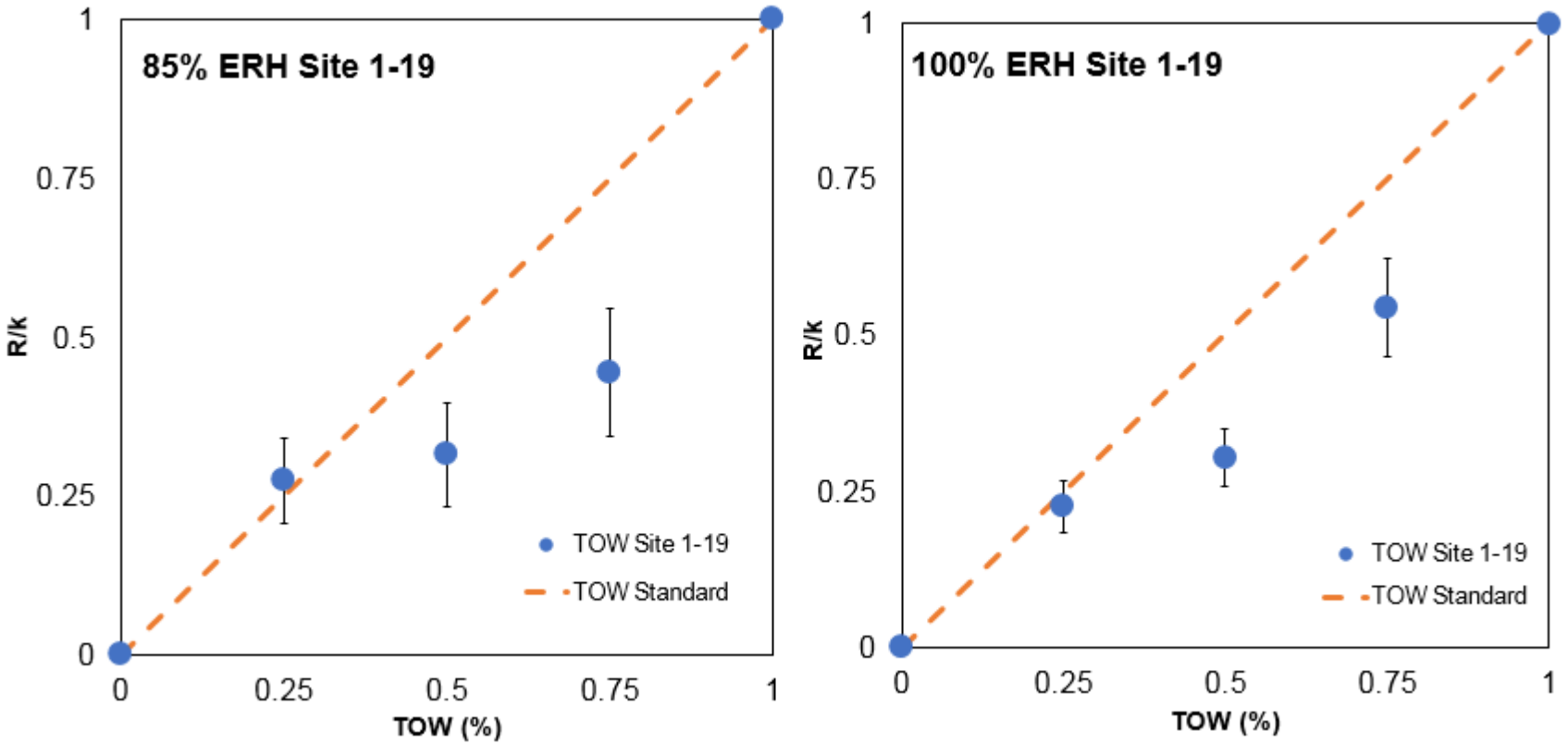
Apply Time of Wetness model



Adan, O., Huinink, H. "Fungal Growth & Humidity Fluctuations: A Toy Model. *Fundamentals of Mold Growth in Indoor Environments and Strategies for Healthy Living*. 2011

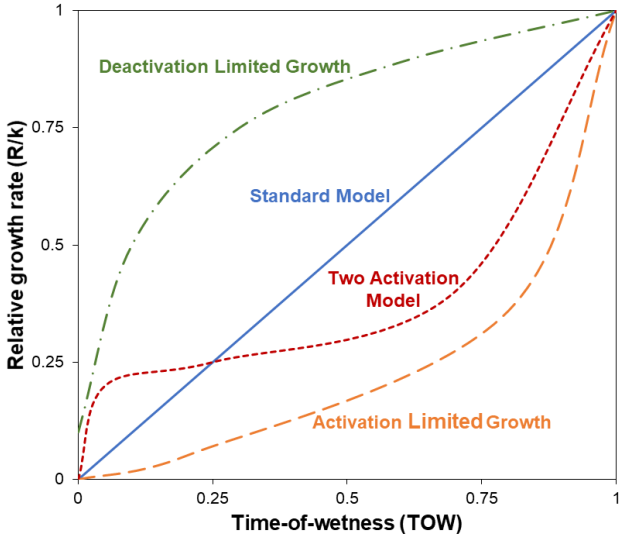
Fungal growth in carpet dust follows the activation limited growth model

Carpet TOW data



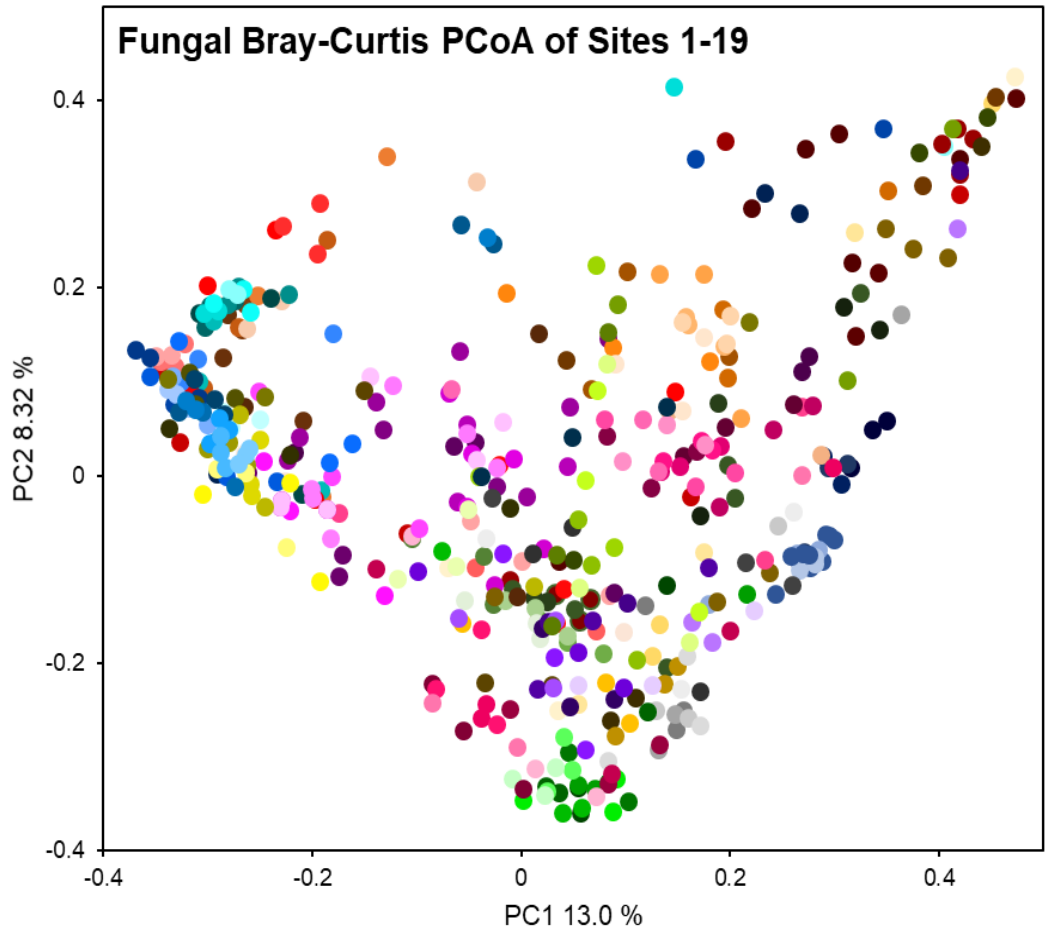
Haines, Siegel, Dannemiller. *Indoor Air.* 2020.

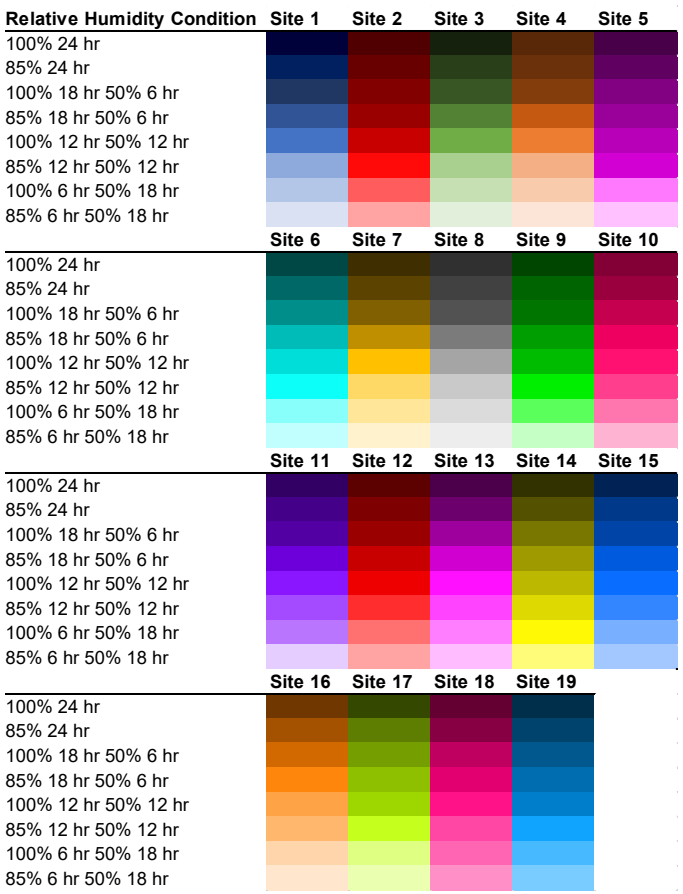
Standard TOW Model



Adan, Olaf C. G., and Robert A. Samson. (2011) "Fundamentals of Mold Growth in Indoor Environments and Strategies for Healthy Living", *Wageningen: Wageningen Academic*

Site-specific effects dominate moisture signature

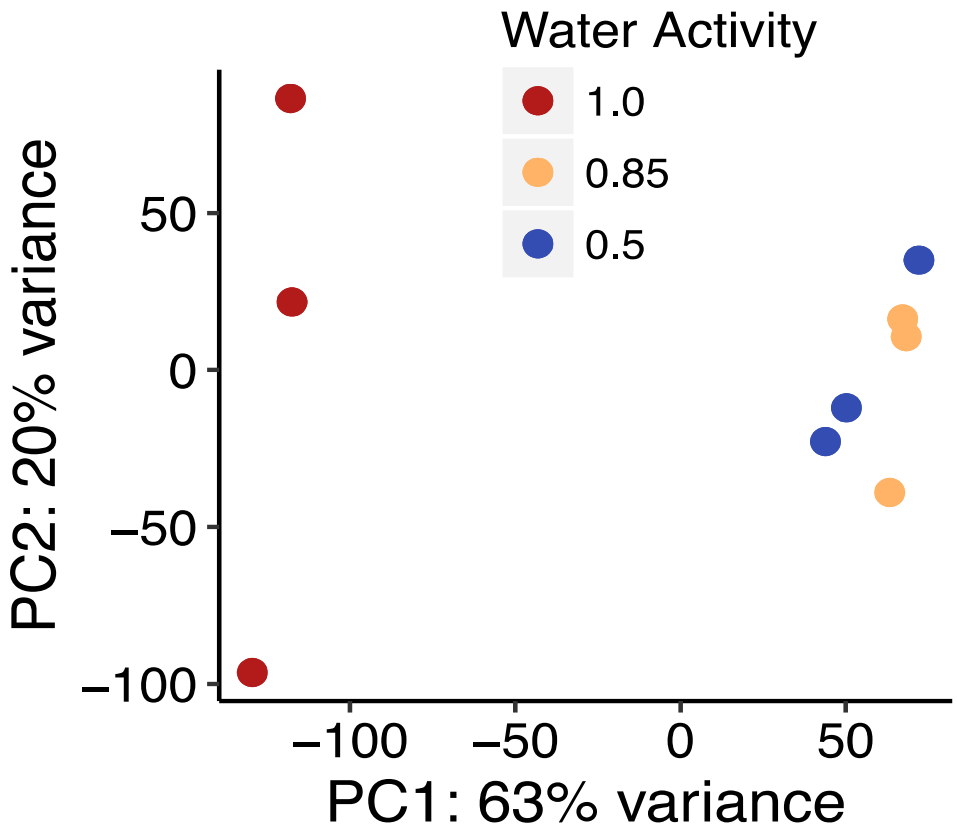




Haines, Siegel, Dannemiller. *Indoor Air.* 2020.

More moisture creates more “metabolic diversity”

Bridget Hegarty

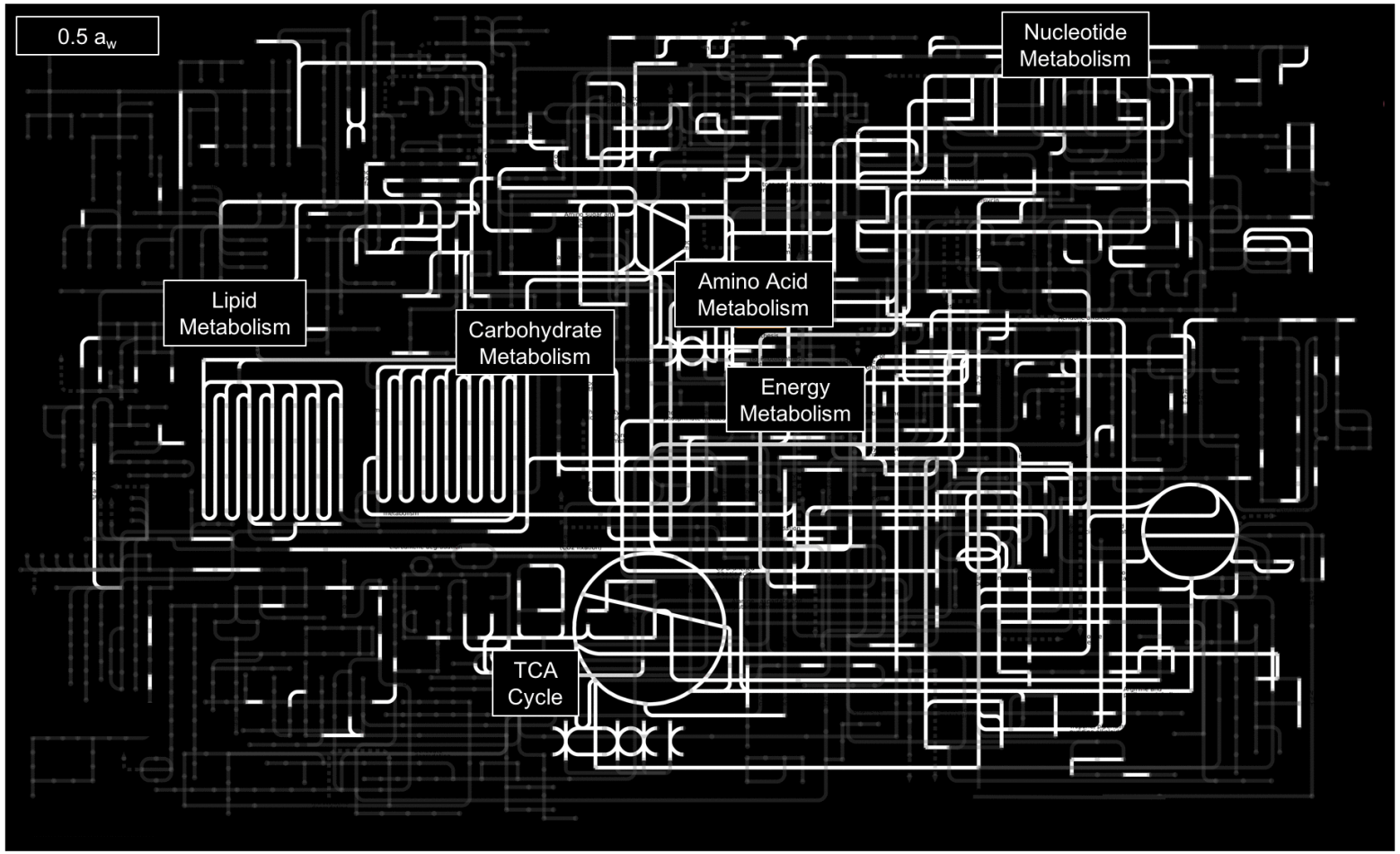


DNA → RNA → Protein

Gene expression

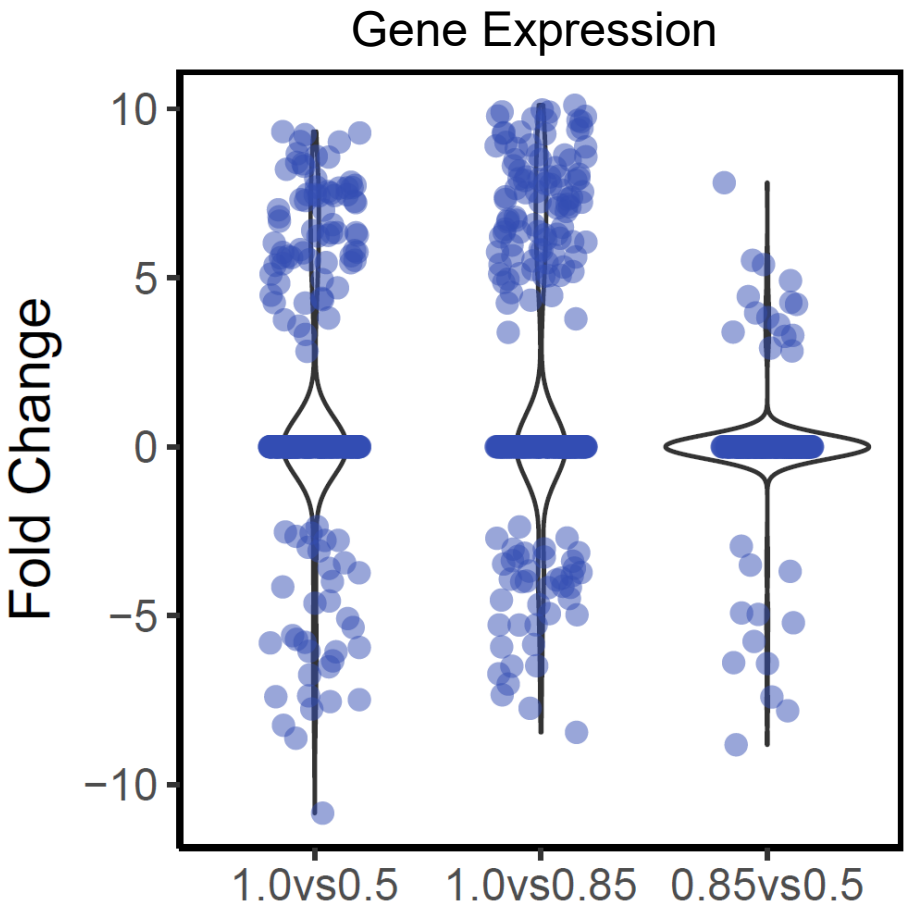
Hegarty, Dannemiller, & Peccia. *Indoor Air*

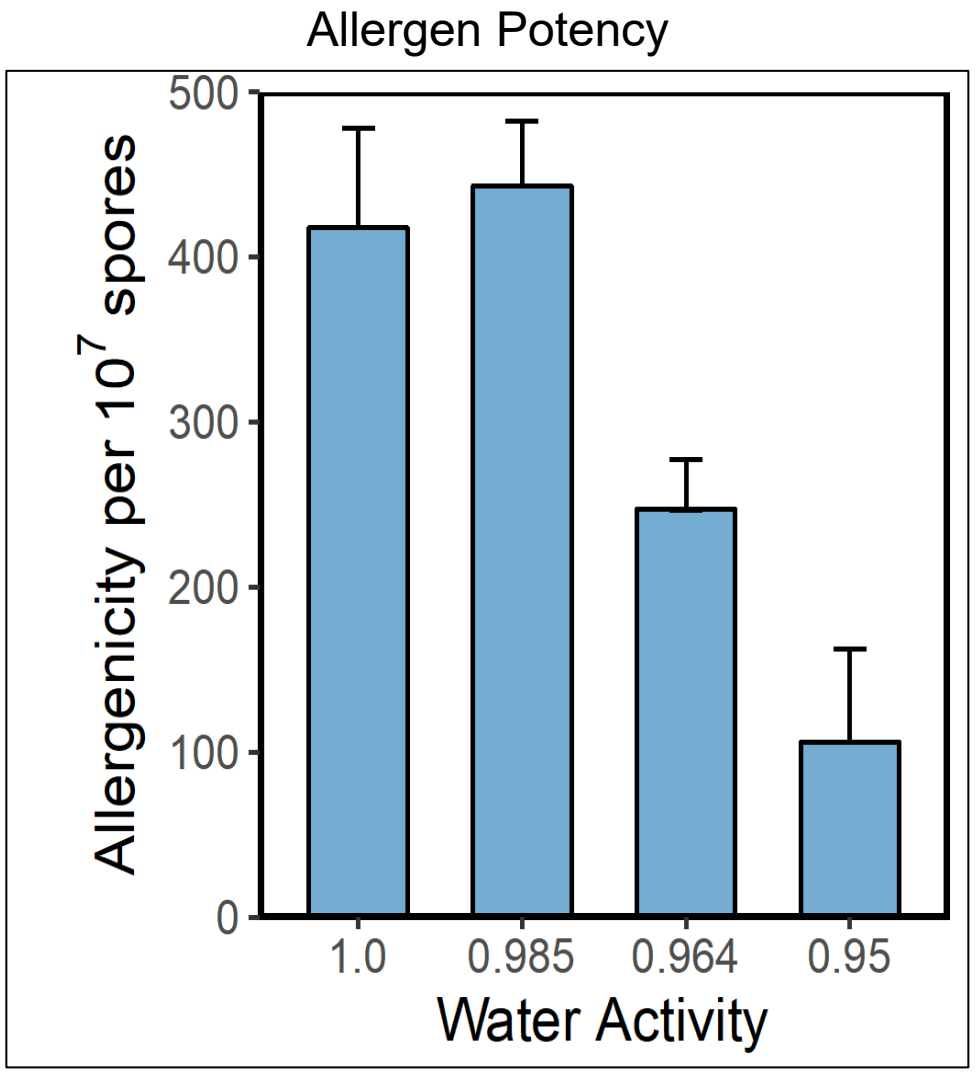
What happens in fungi as moisture availability increases?



Hegarty, Dannemiller, & Peccia. *Indoor Air*

Growth at increased water activity increases allergen potency



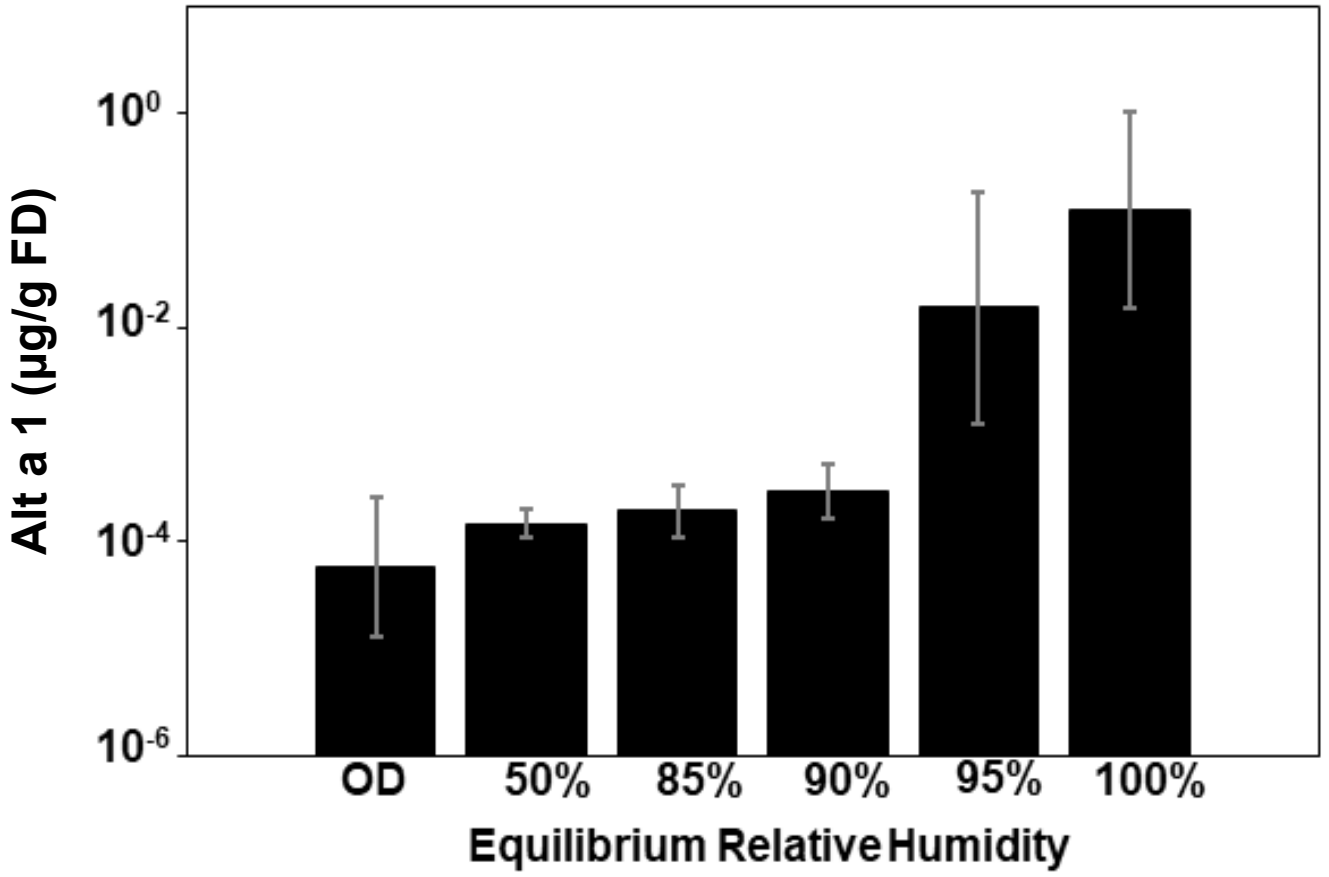


Hegarty, Dannemiller, & Peccia. *Indoor Air*

Relative humidity associated with increased allergen production

Nick Nastasi

Moisture > Dust > Fiber Type



Nastasi et al. *Building and Environment*, 2020

CAREER Project: Microbial indicators Of Latent Dampness (MOLD)

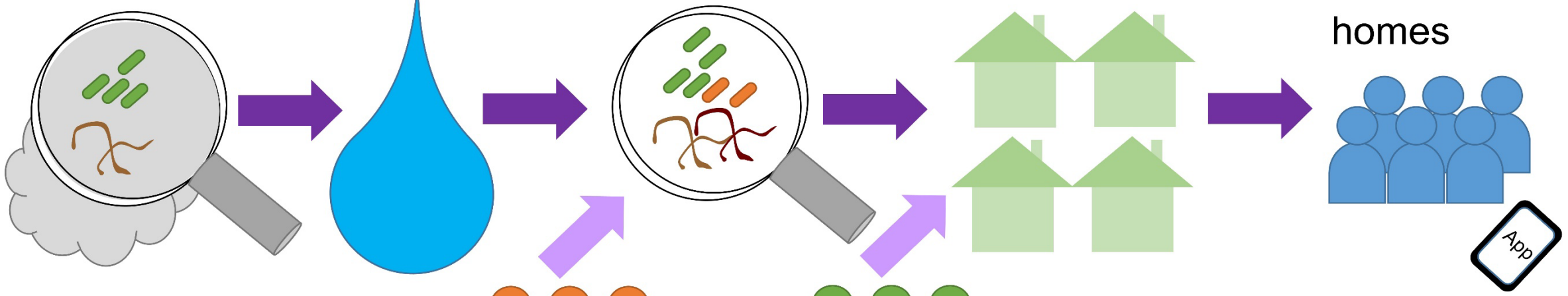
Prior Work: Microbial communities in dust

Now: Add Moisture

Objective 1: Identify common measures of growth

Objective 2: Validate measure in homes

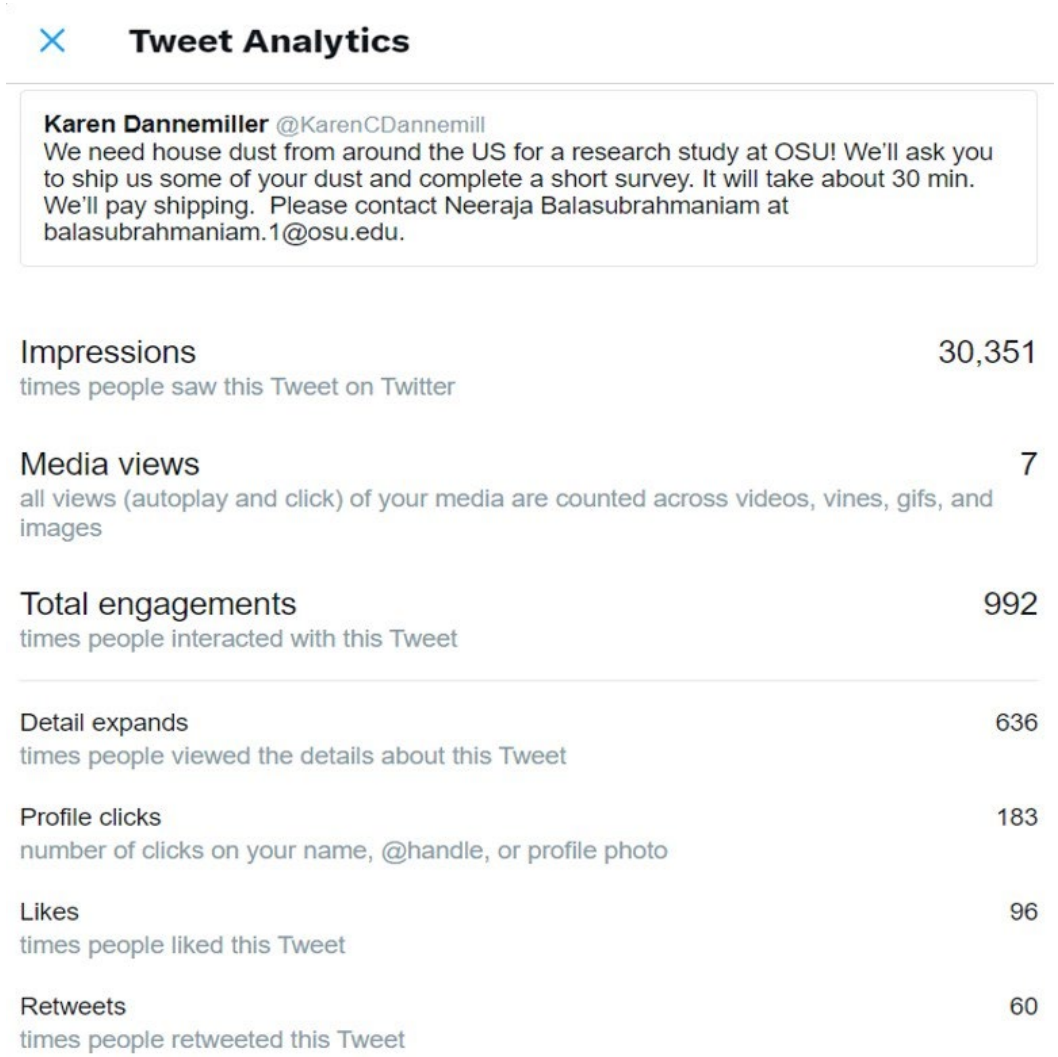
Future: Accessible, quantitative measurement method for mold in homes



Education: Students

Education: Practitioners

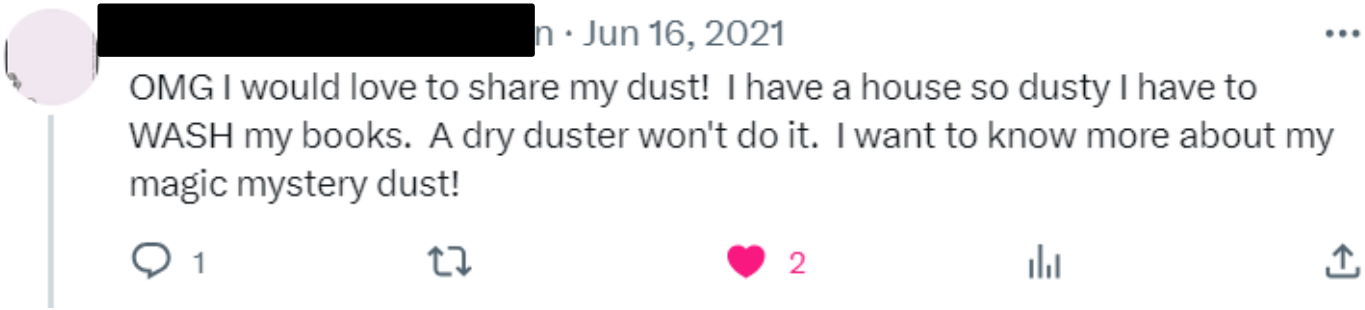
Twitter/X as a recruitment tool



Tweet Analytics

Karen Dannemiller @KarenCDannemill
We need house dust from around the US for a research study at OSU! We'll ask you to ship us some of your dust and complete a short survey. It will take about 30 min. We'll pay shipping. Please contact Neeraja Balasubrahmaniam at balasubrahmaniam.1@osu.edu.

Impressions times people saw this Tweet on Twitter	30,351
Media views all views (autoplay and click) of your media are counted across videos, vines, gifs, and images	7
Total engagements times people interacted with this Tweet	992
Detail expands times people viewed the details about this Tweet	636
Profile clicks number of clicks on your name, @handle, or profile photo	183
Likes times people liked this Tweet	96
Retweets times people retweeted this Tweet	60



[redacted] · Jun 16, 2021

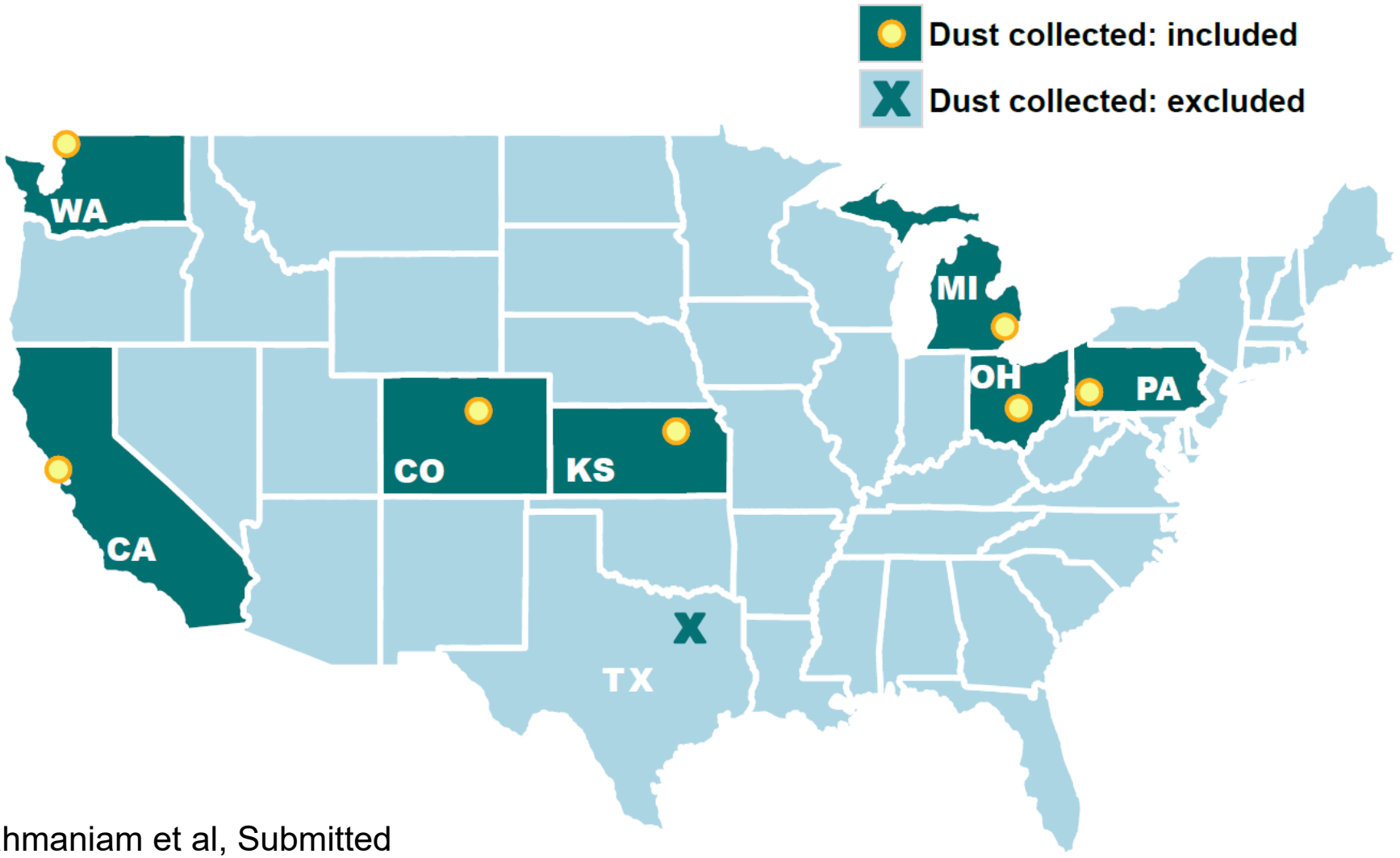
OMG I would love to share my dust! I have a house so dusty I have to WASH my books. A dry duster won't do it. I want to know more about my magic mystery dust!

1 2

Dust collect from around the US for screening

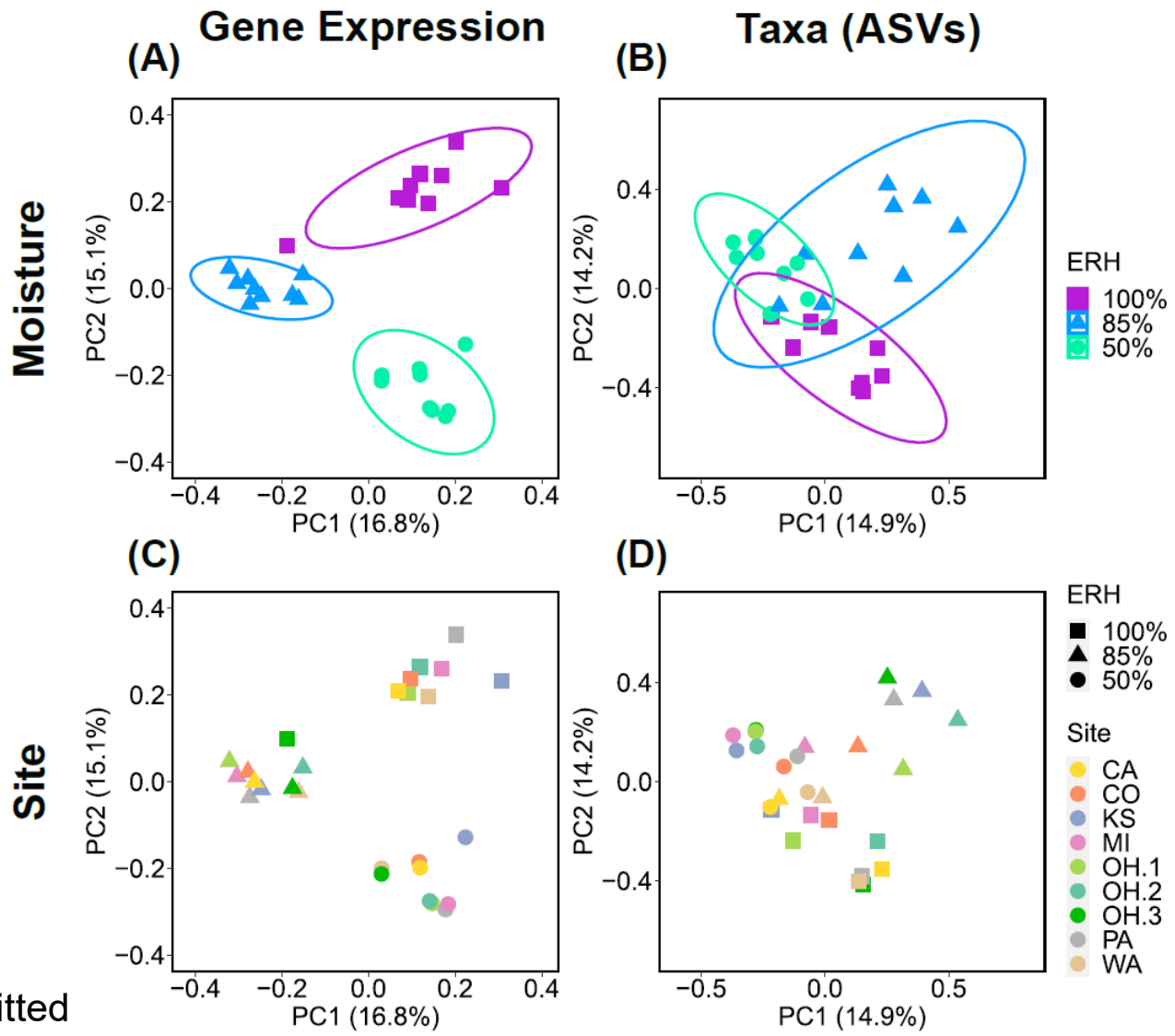
Neeraja Balasubrahmaniam

Jon King



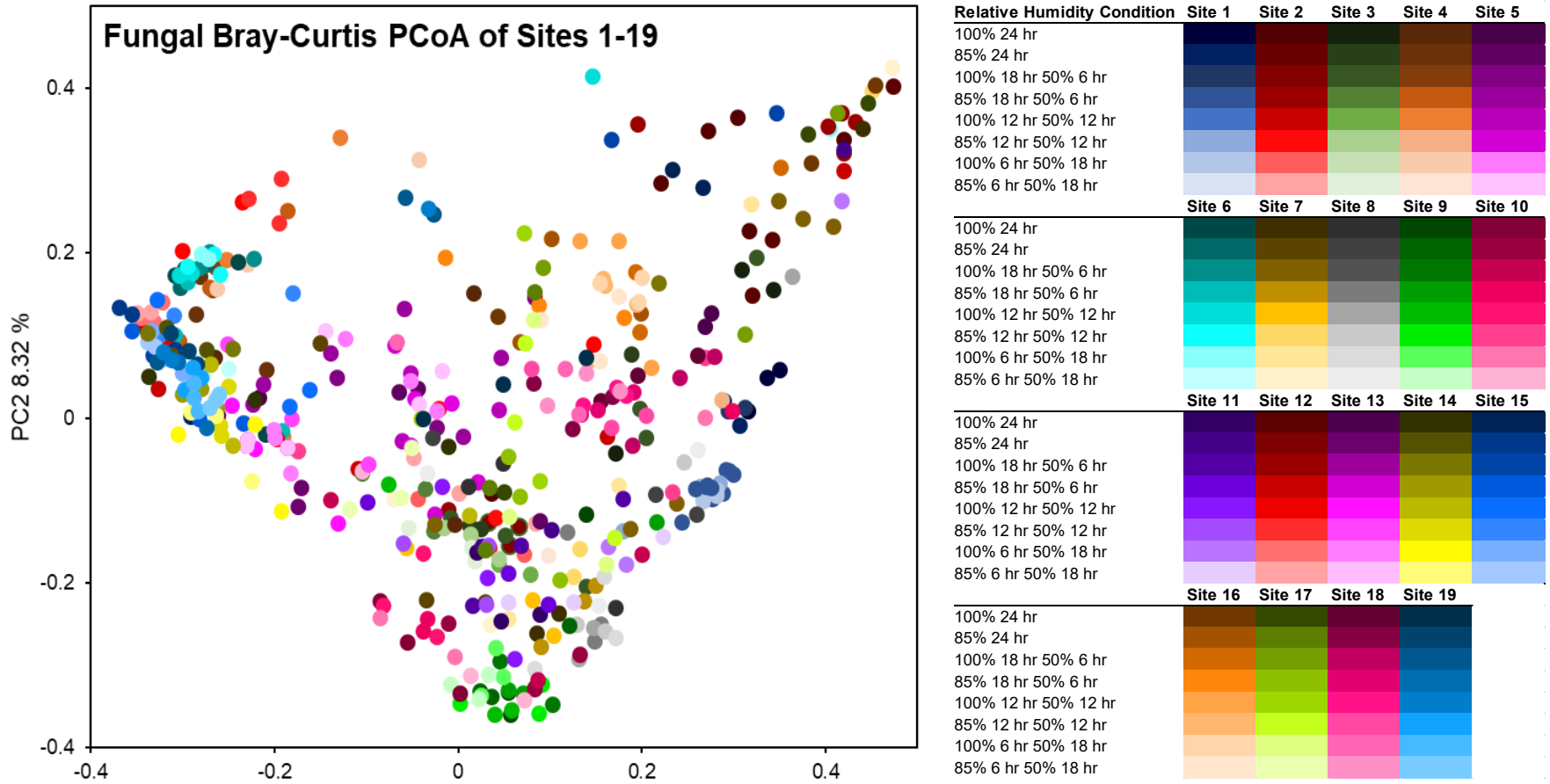
Balasubrahmaniam et al, Submitted

It’s so clear when we look at function



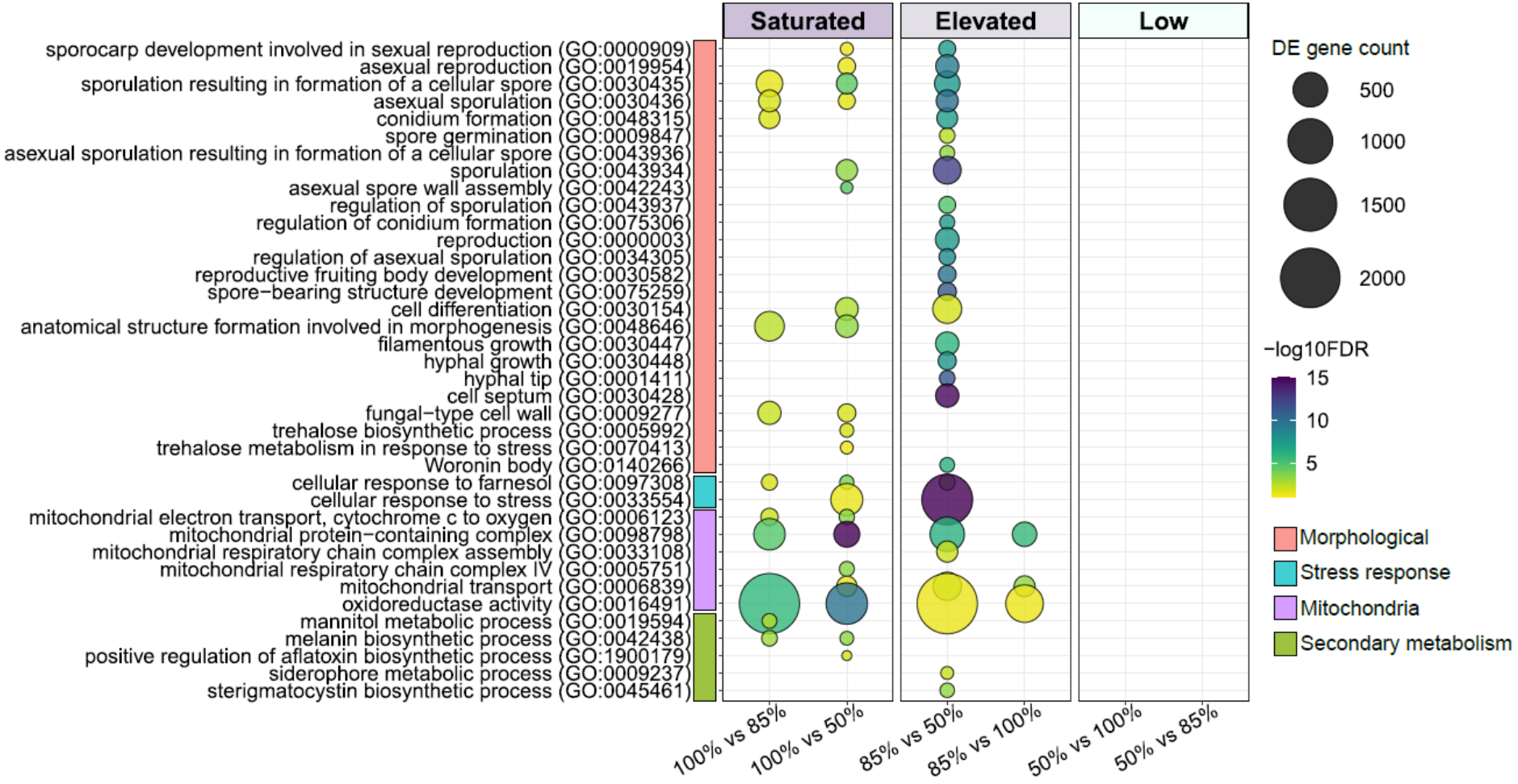
Balasubrahmaniam et al, Submitted

Recall: Site-specific effects dominate moisture signature for species



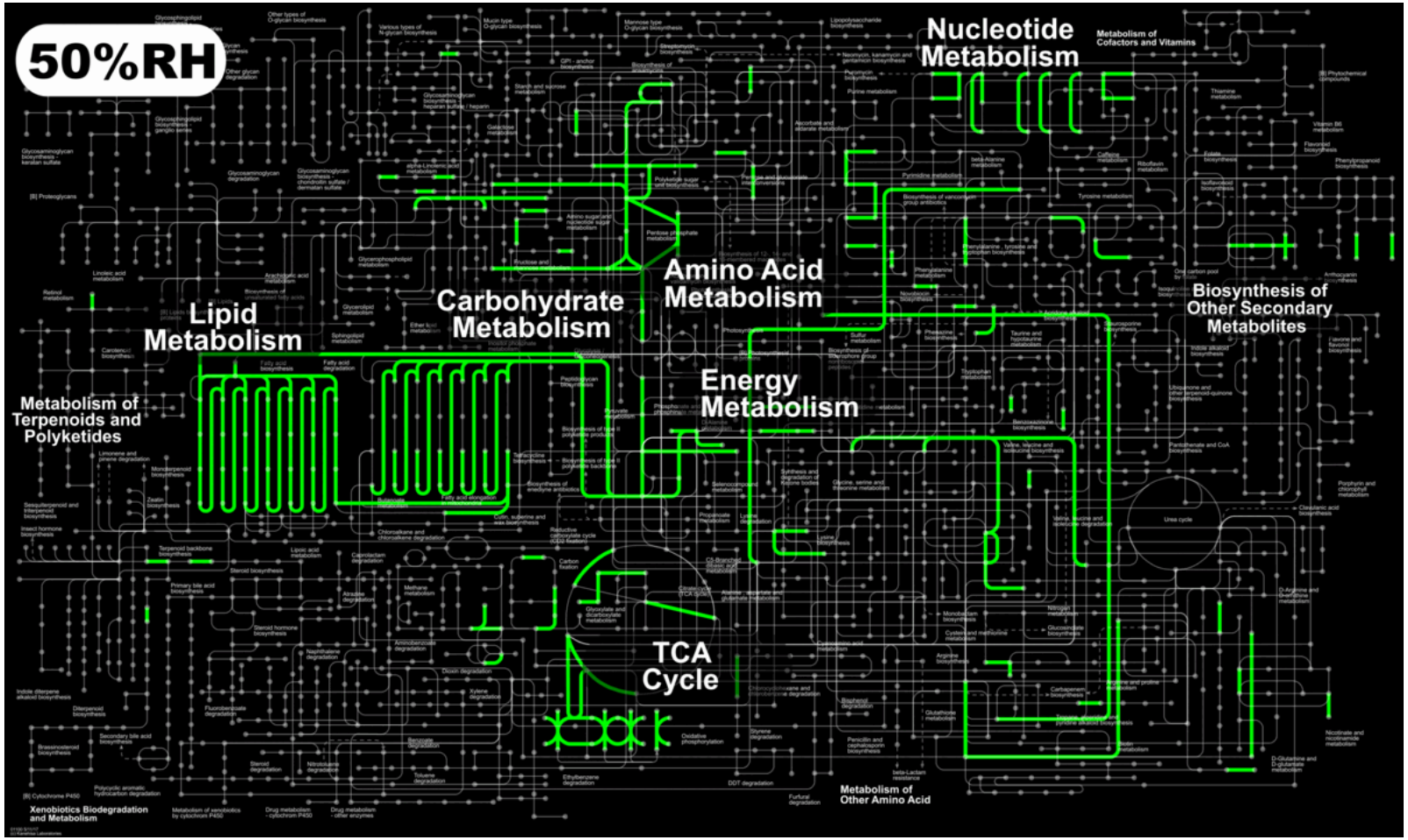
Haines, Siegel, Dannemiller. *Indoor Air.* 2020.

Growth-associated gene are upregulated at high RH



Balasubrahmaniam et al, Submitted

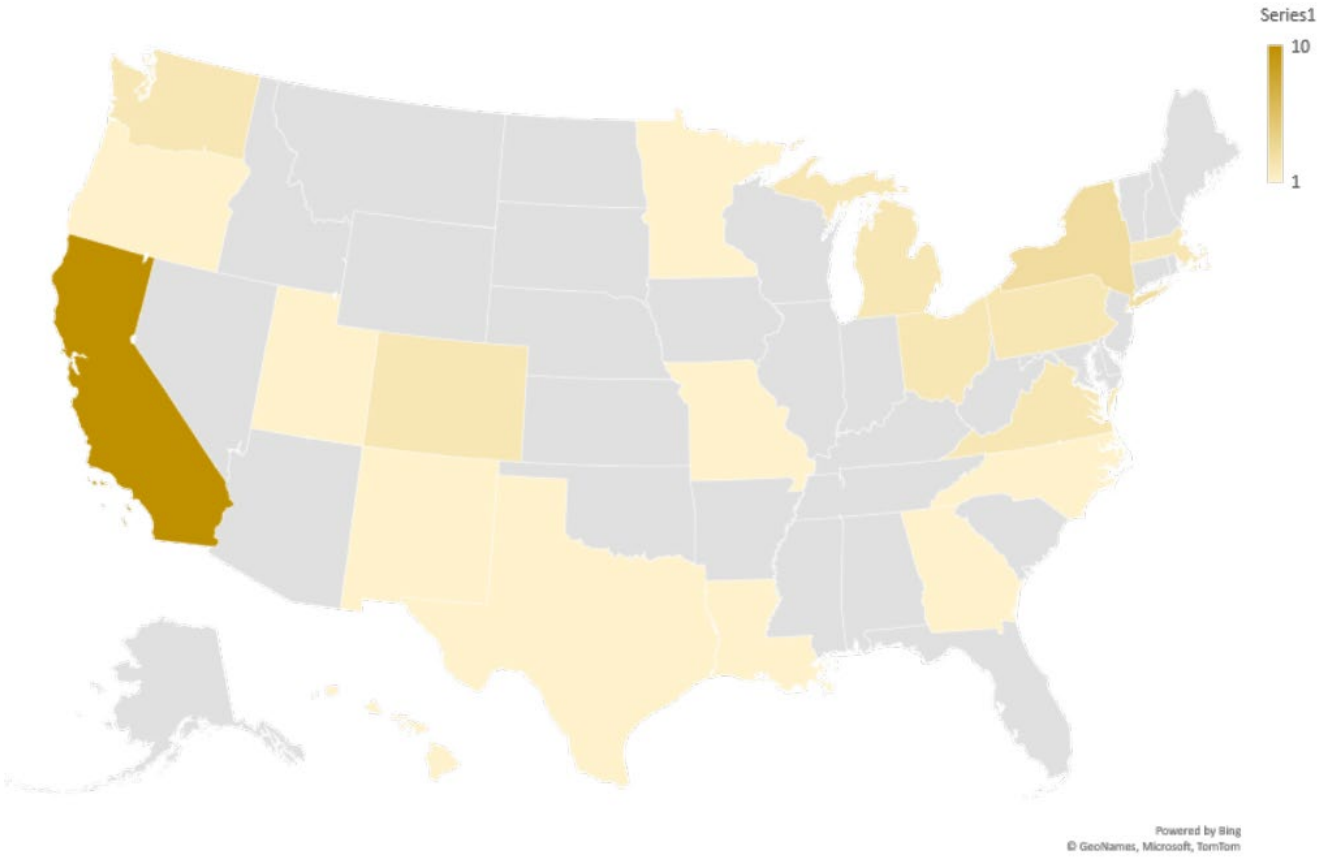
Secondary metabolic pathways upregulated at high RH



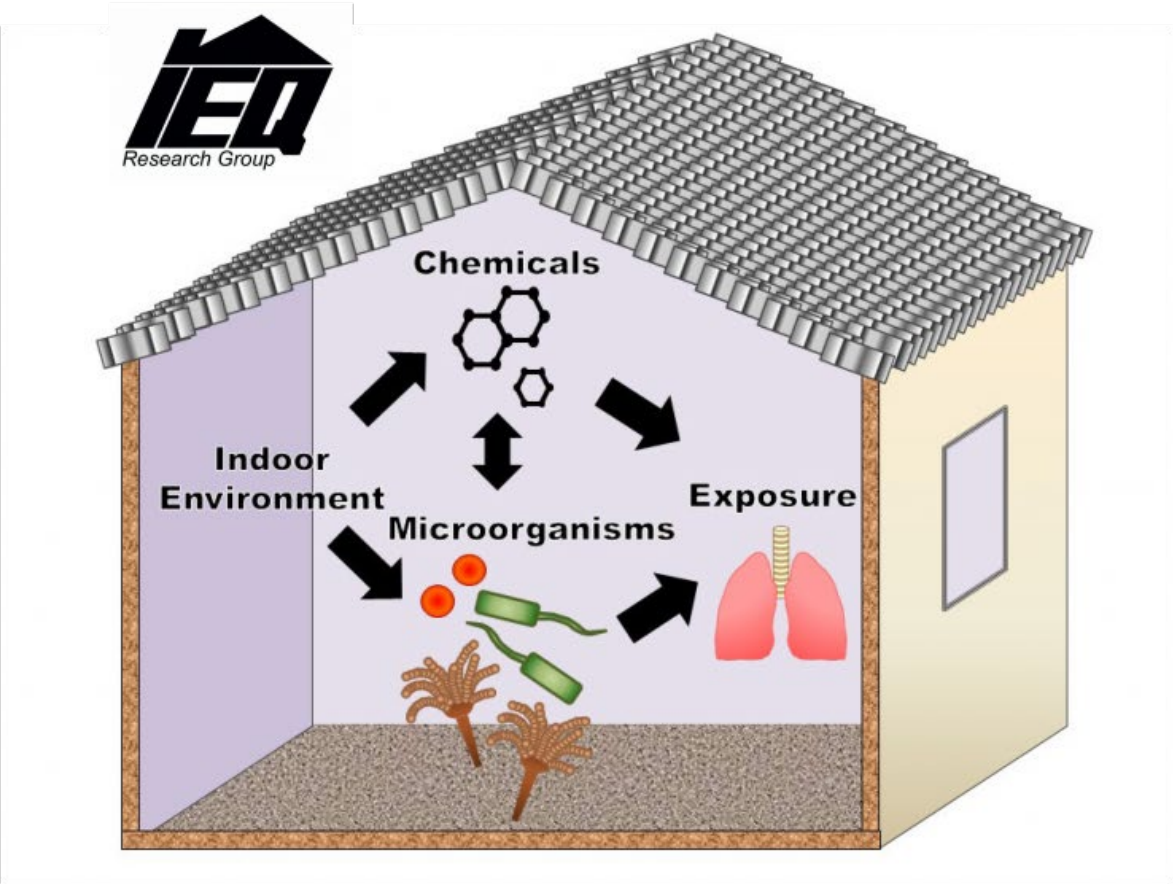
Balasubrahmaniam et al, Submitted

From these terms

- We can identify potential protein targets that will be consistently associated with mold growth in homes
  - Prioritized list of 29 proteins
- Those targets will be validated in 50 homes
- Currently applying for funding to develop an assay to test for top targets

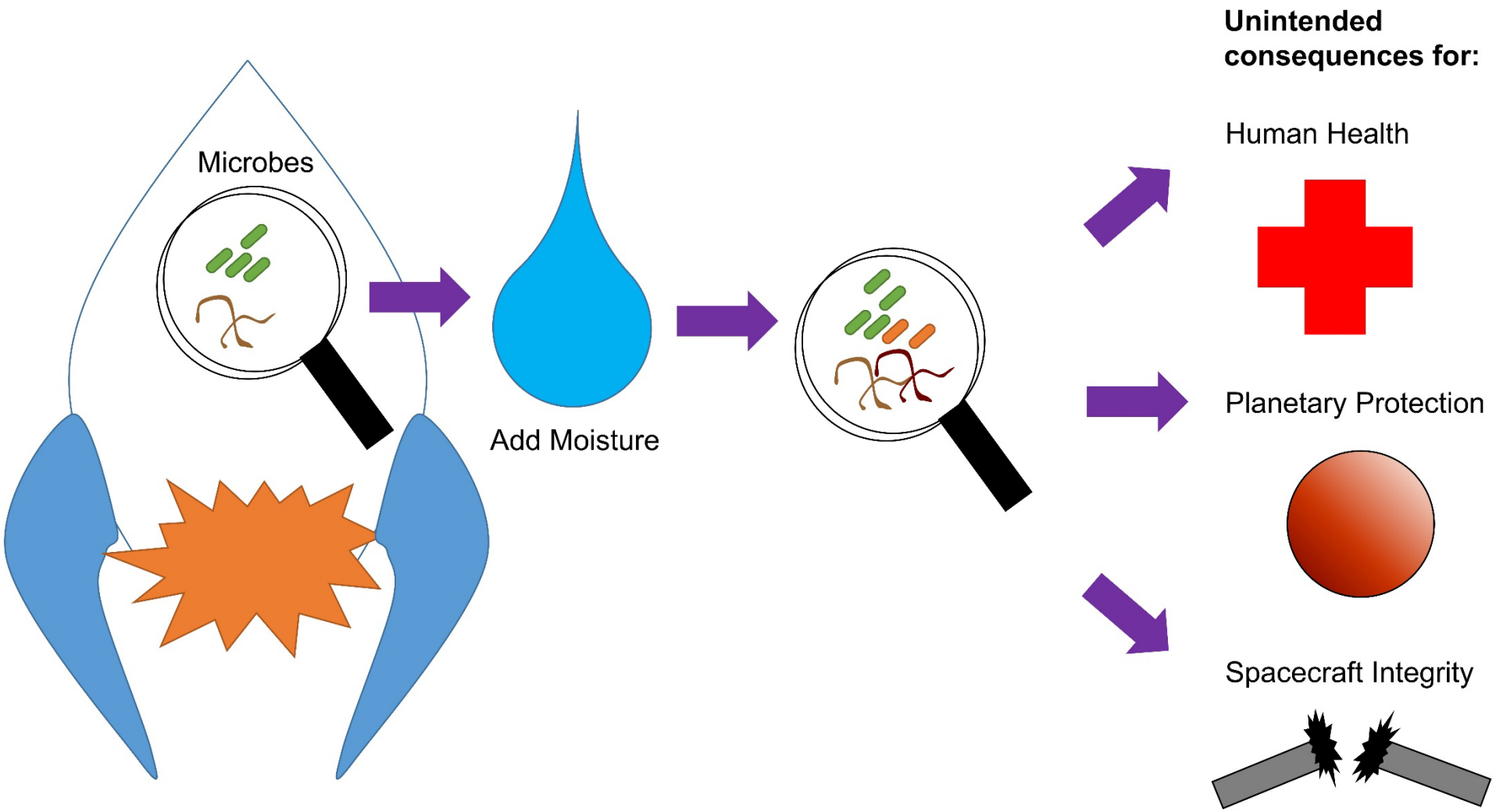


Part 1 Summary

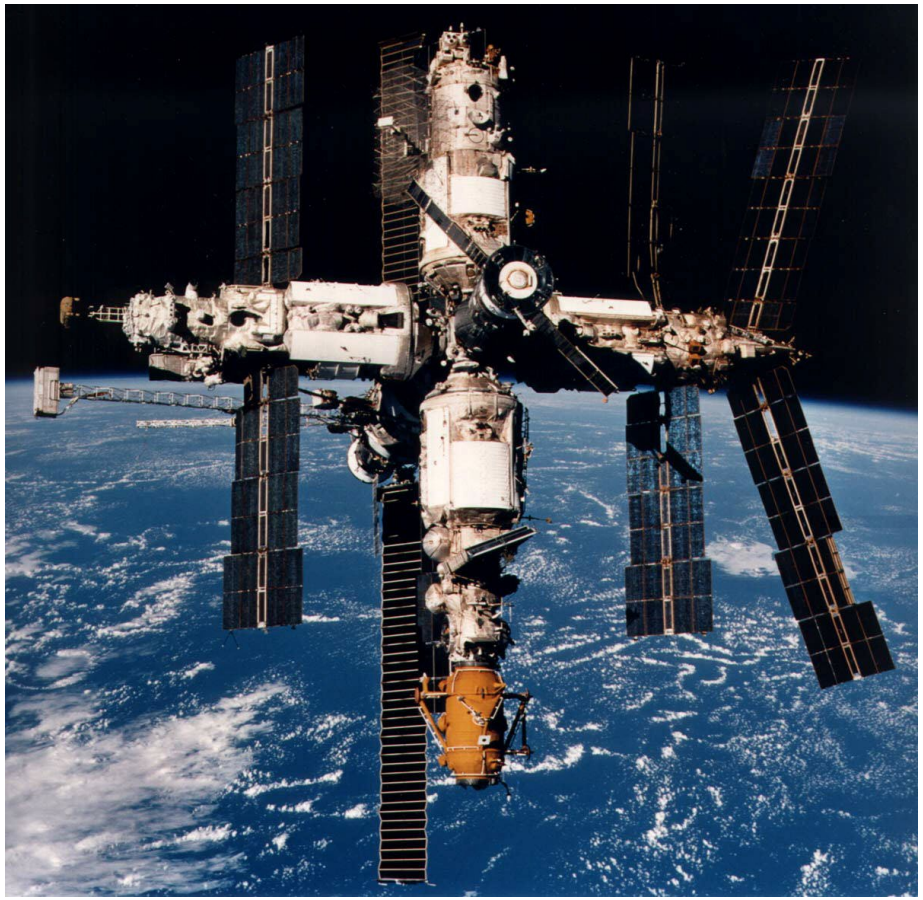


- Elevated moisture is sufficient to support microbial growth and function in dust
- We may be able to use this information to develop a better mold indicator

Part 2: Space Dust



Microbial growth is problematic on Earth and in space

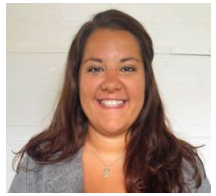


***Mir* suffered from microbial growth**

Mold growth on fabric panels on ISS from wet hanging towels

Microb. Ecol., vol. 47, no. 2, pp. 133–136, 2004
Res. Microbiol., vol. 159, no. 6, pp. 432–435, 2008
Photo credit: NASA.

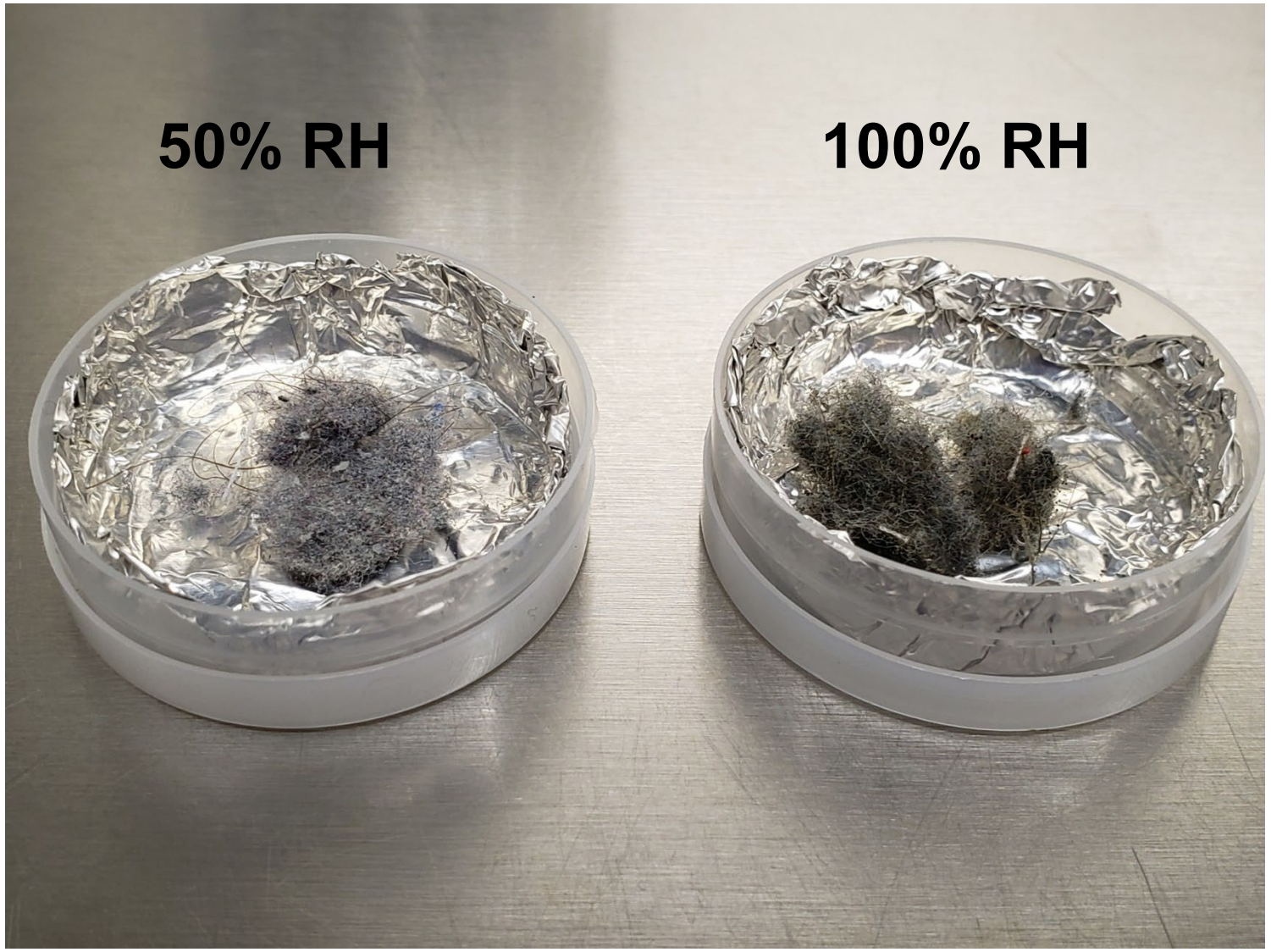
Relative humidity is very important to control on spacecraft


Ashleigh Bope

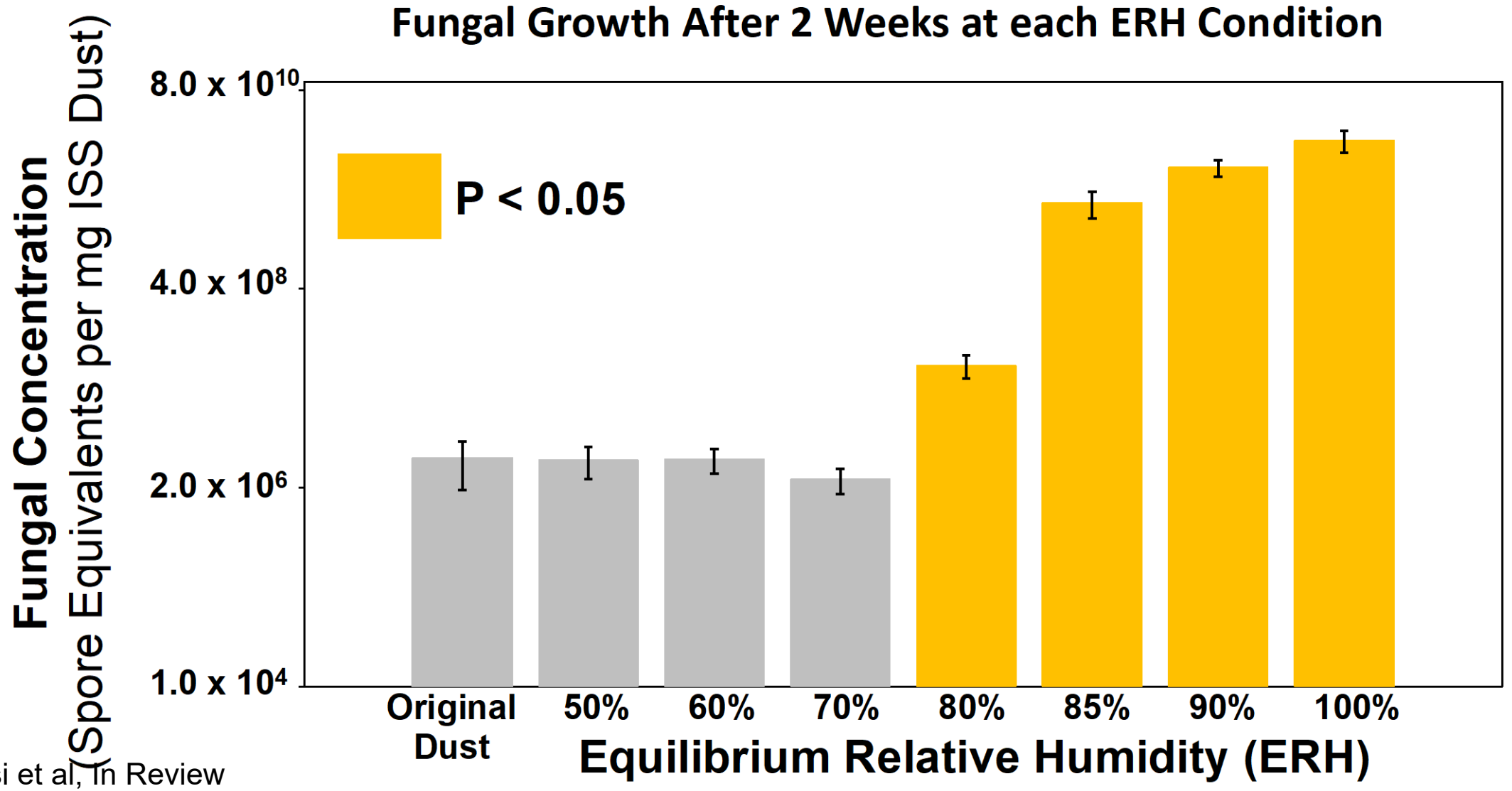

Nick Nastasi


Marit Meyer (NASA)


John Horack

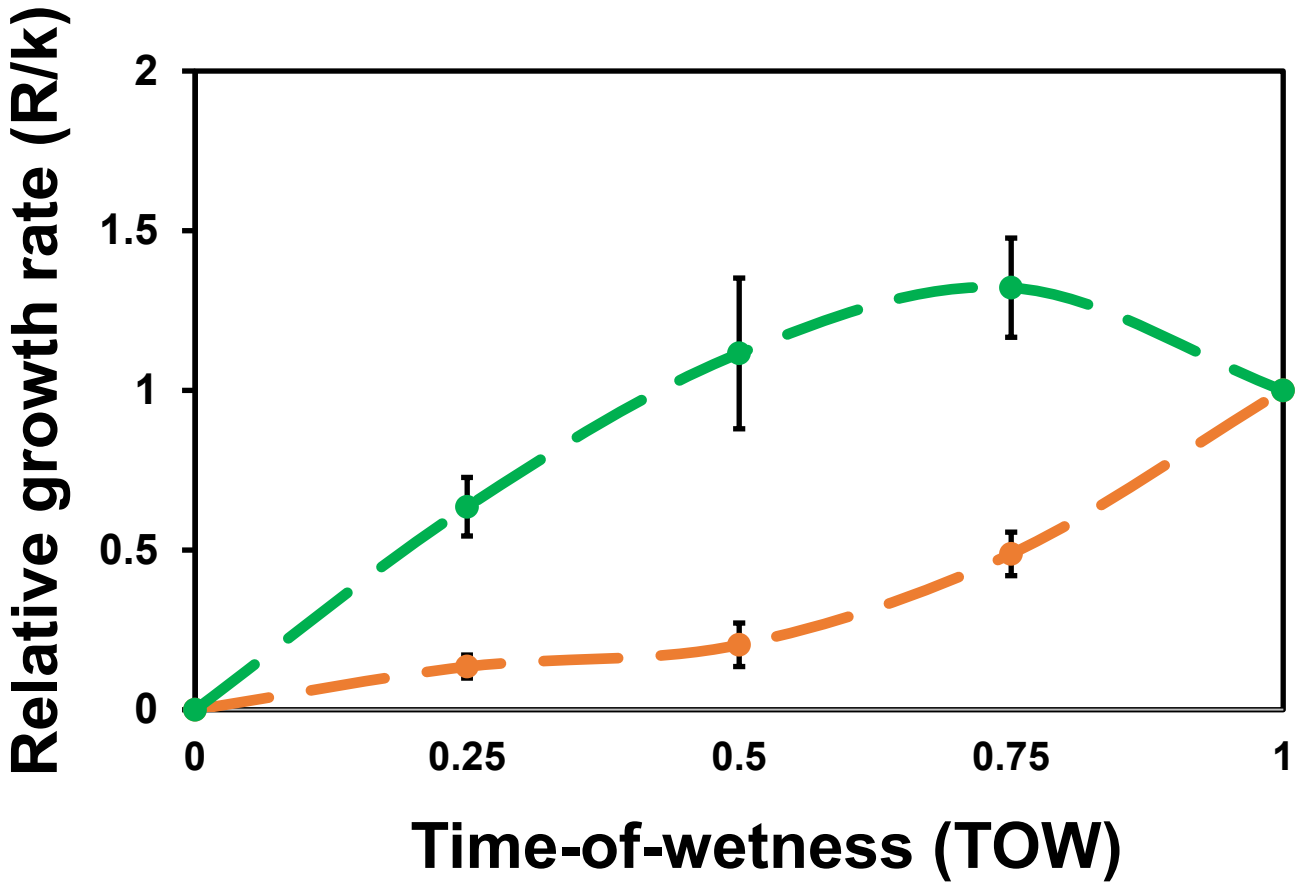


Fungal growth increases as RH increases



Nastasi et al, In Review

Growth can be modeled using the time-of-wetness framework, even if RH fluctuates



85% ERH

Activation Limited Growth

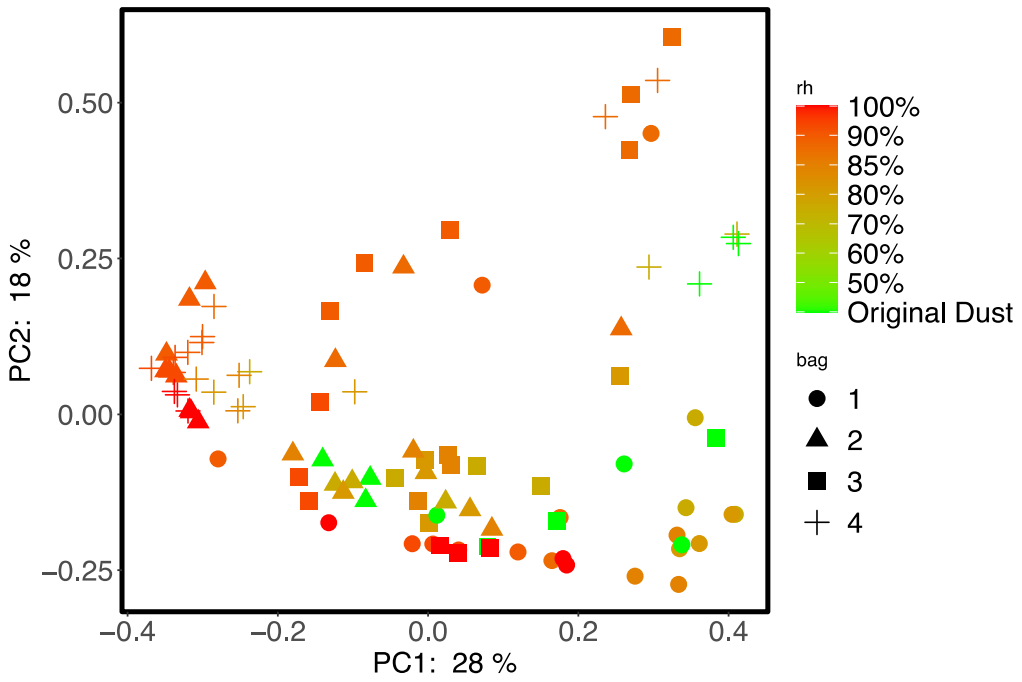
100% ERH

Deactivation Limited Growth

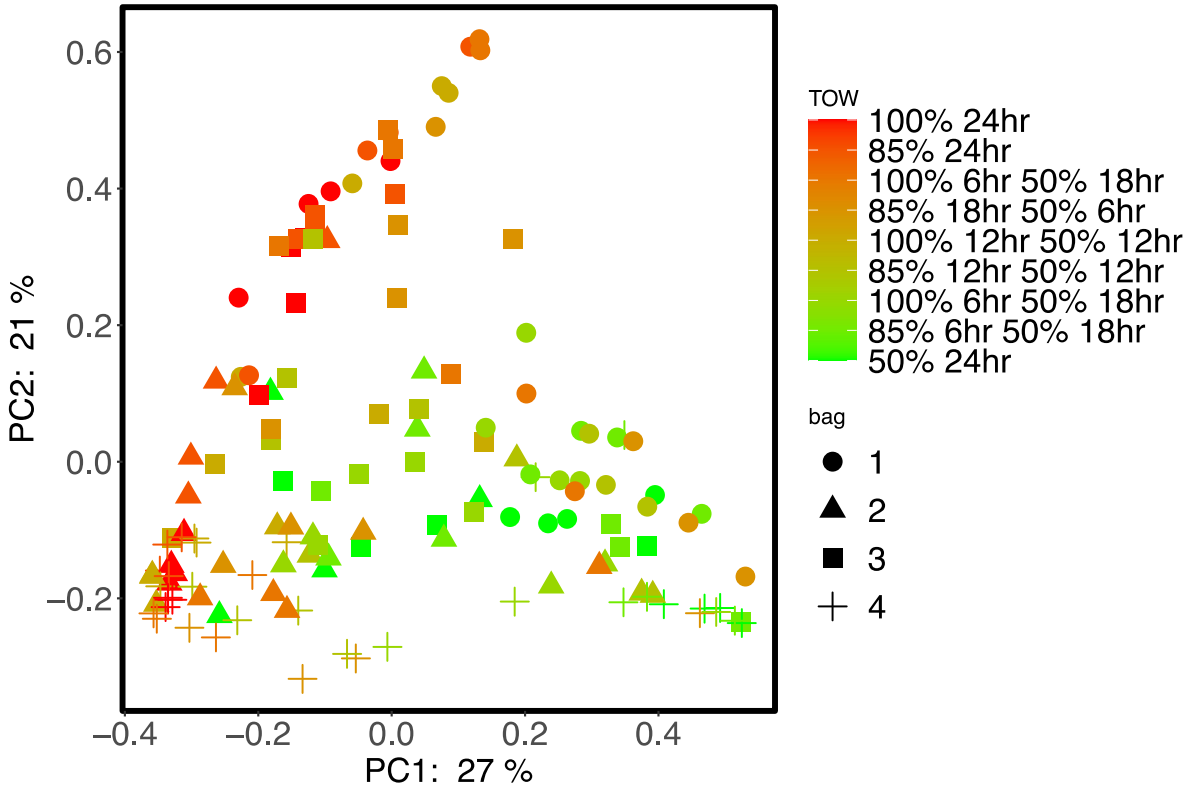
Nastasi et al, In Review

Species do change in a specific environment

(A) 2-Week Incubation Samples



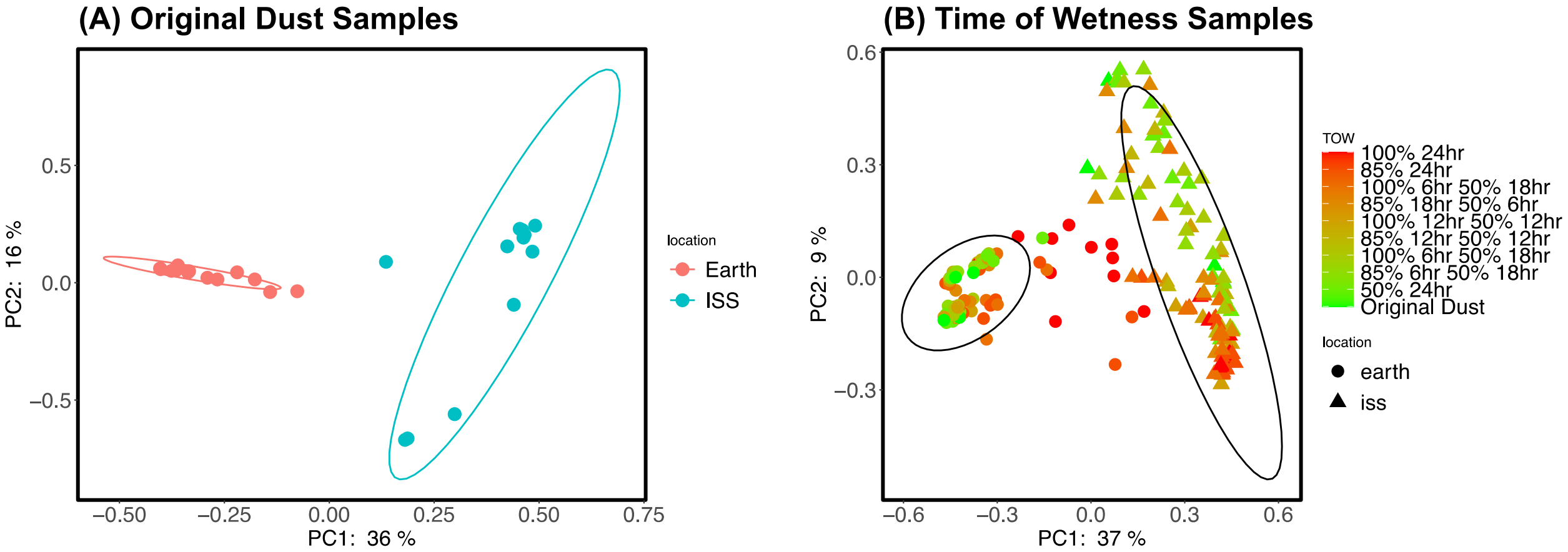
(B) Time-of-Wetness Samples



Nastasi et al, In Revision

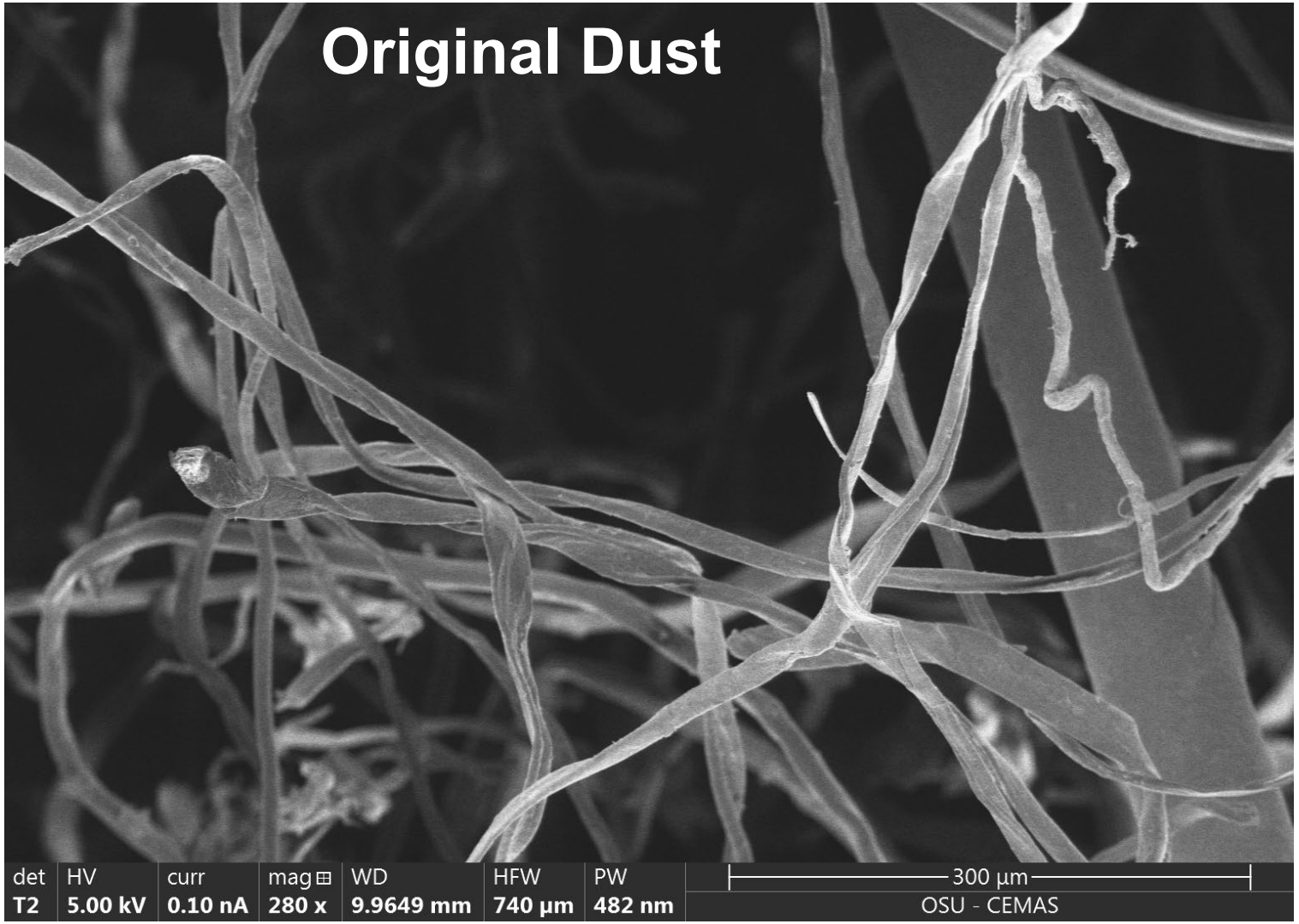
Comparison to Earth

Earth-ISS Fungal Community Comparisons

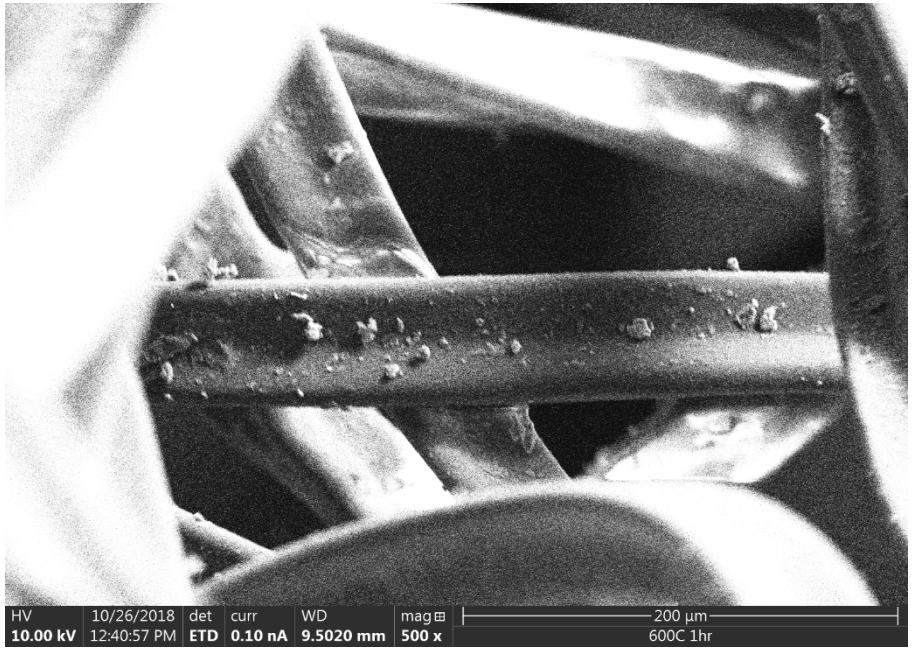


Nastasi et al, In Revision

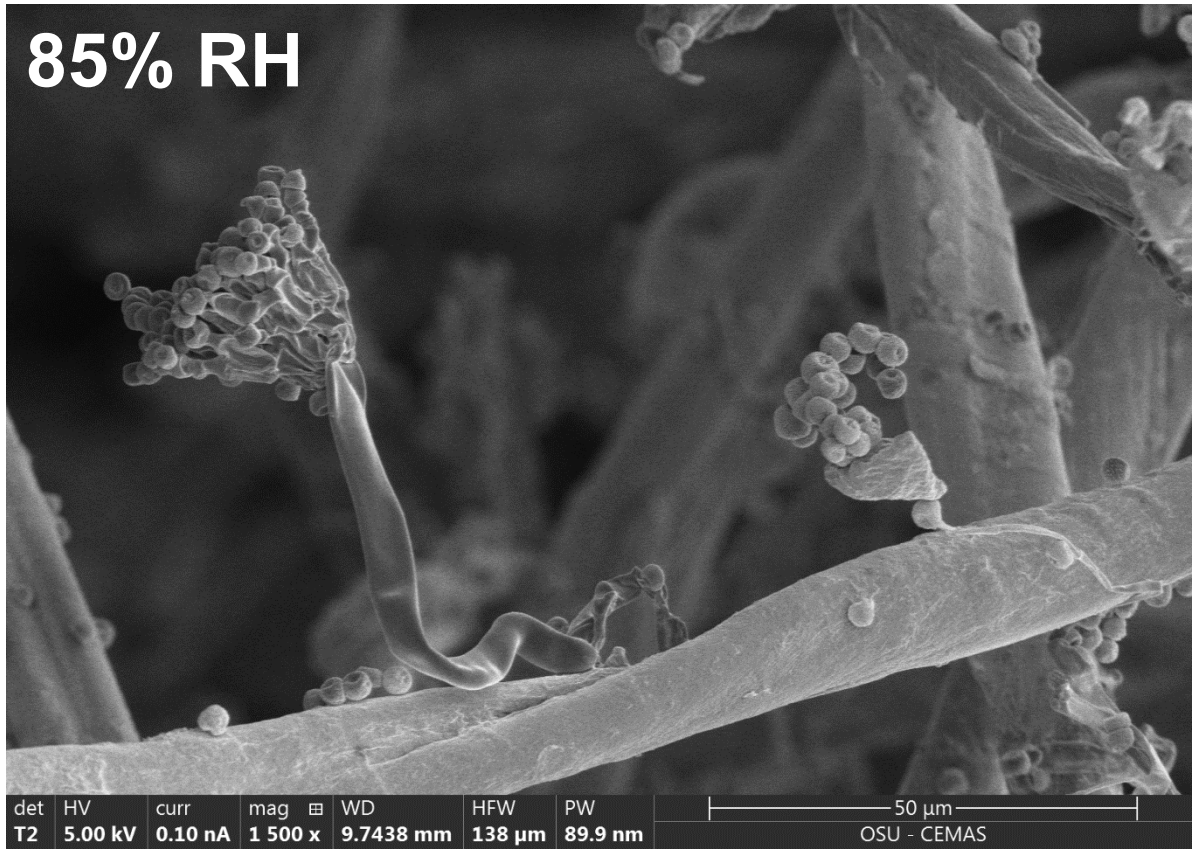
Original dust has few microbes; Looks similar to carpet

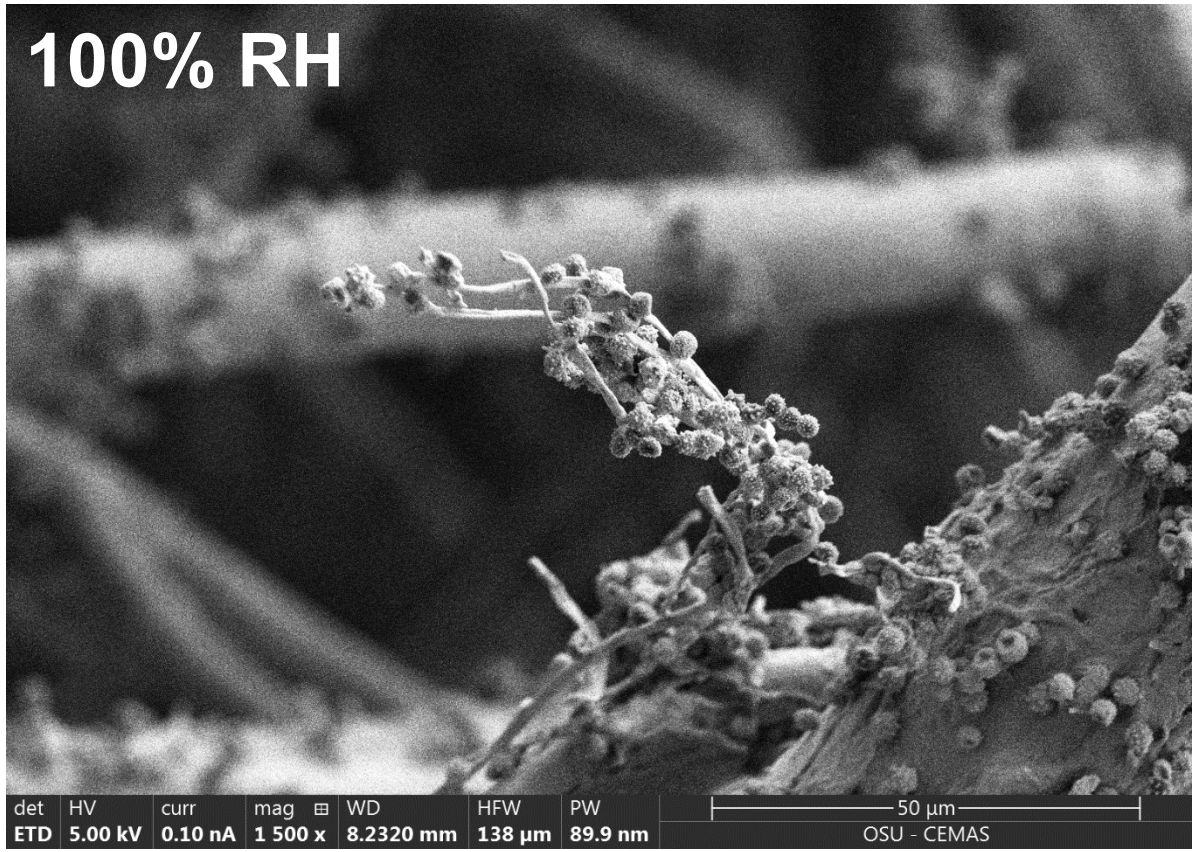


Carpet from Earth

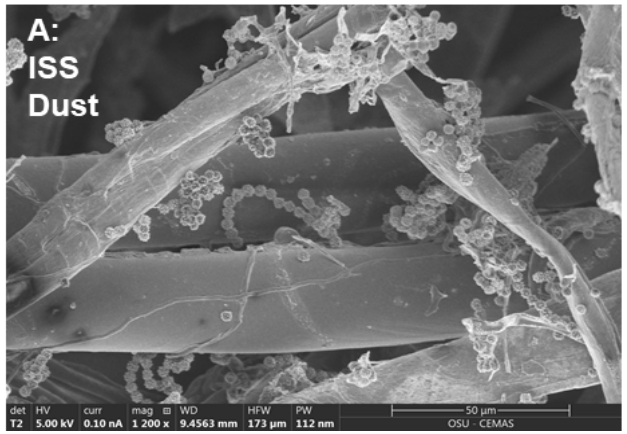


Dust incubated at higher RH has growth; Models a ventilation system failure scenario

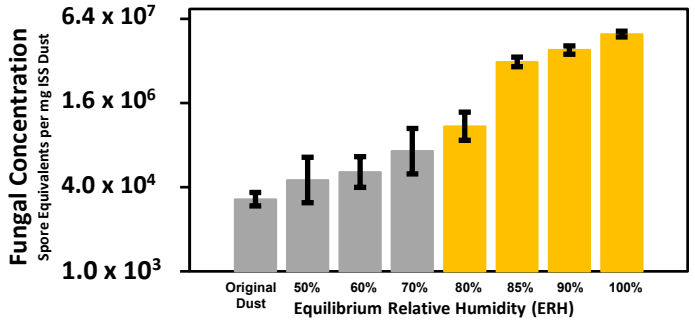


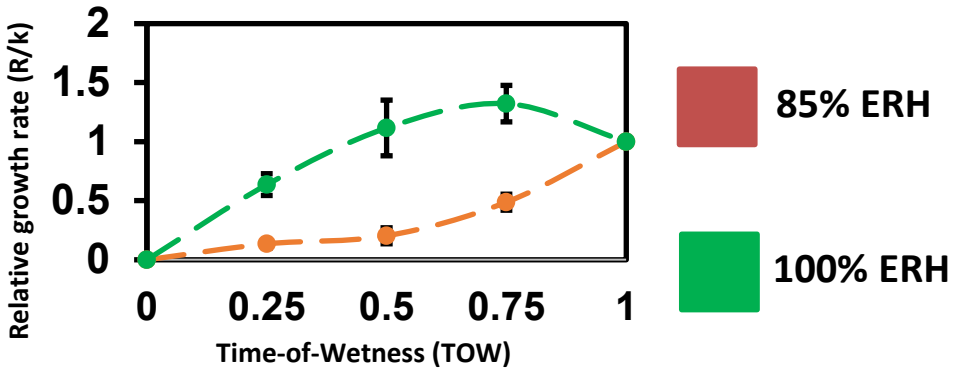


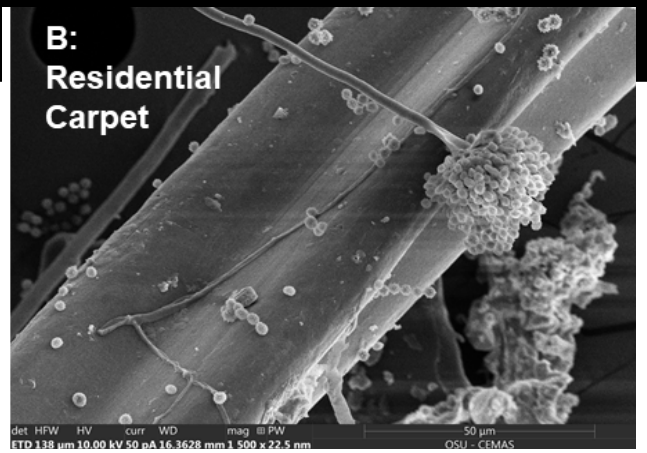
This relationship between dust, RH, and fungi is also seen in residential homes on Earth



ISS DUST

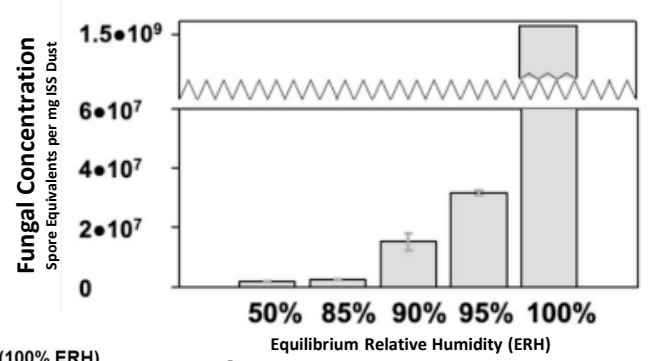




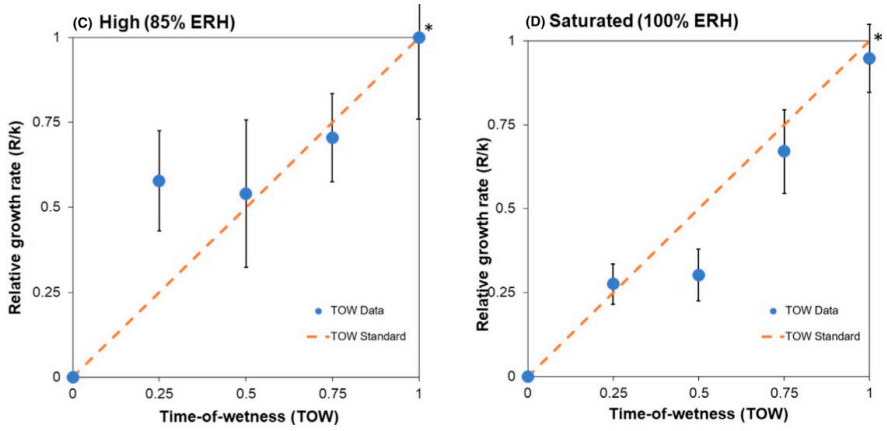


Earth-based Dust and Carpet

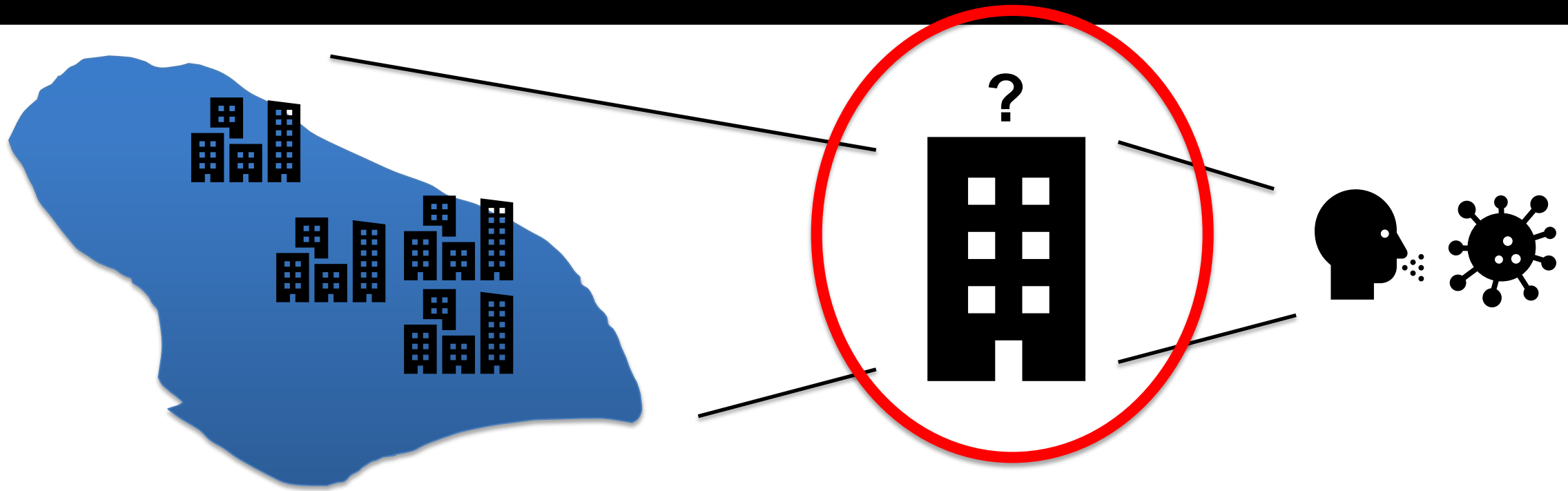
Nastasi et al., Building and Environment, 2020
https://doi.org/10.1016/j.buildenv.2020.106774



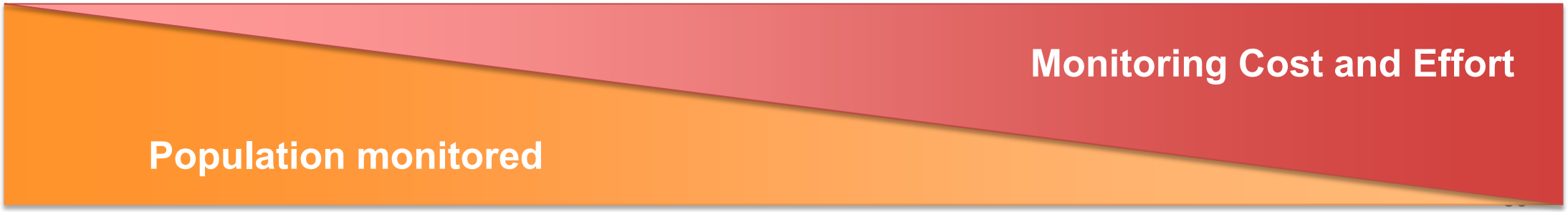
Haines et al., Indoor Air, 2020
https://doi.org/10.1111/ina.12686



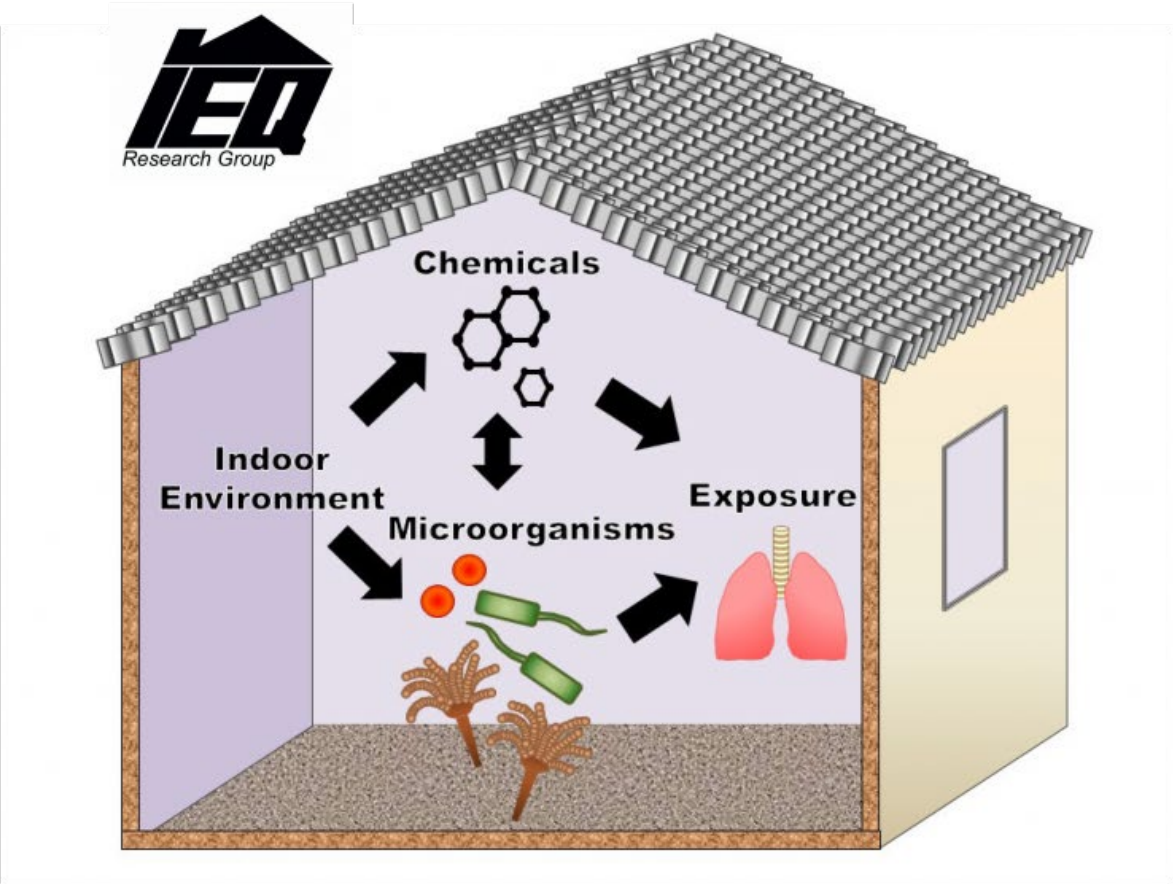
We need a new long-term surveillance solution



Solutions: Wastewater Monitoring | Building Monitoring???? | Individual Testing

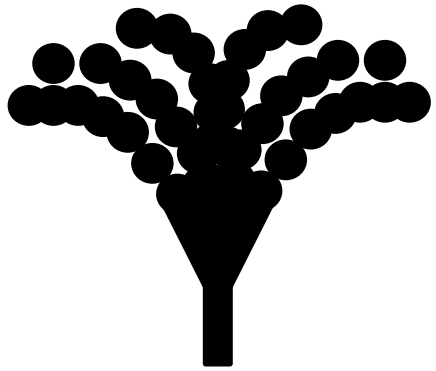


Part 2 Summary

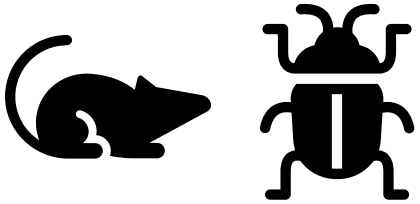


- Microbial growth on spacecraft is similar to microbial growth on Earth

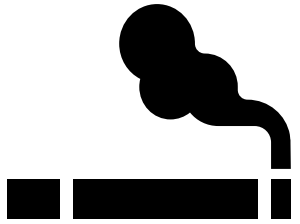
Part 3: Sensors - Multifactorial interventions are necessary to address housing quality



Mold/Dampness

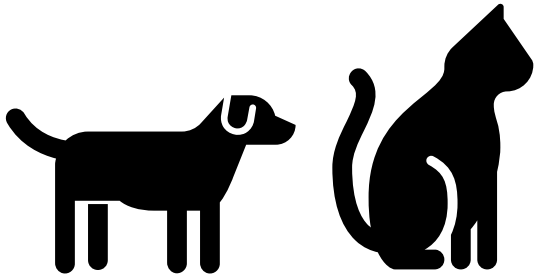


Pests

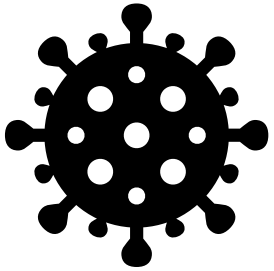


Environmental Tobacco Smoke

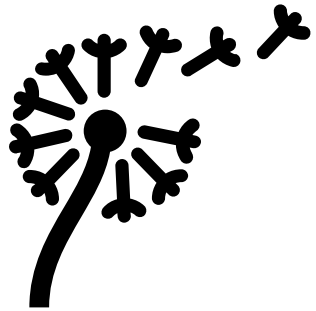
Pollution



Pets



Pathogens

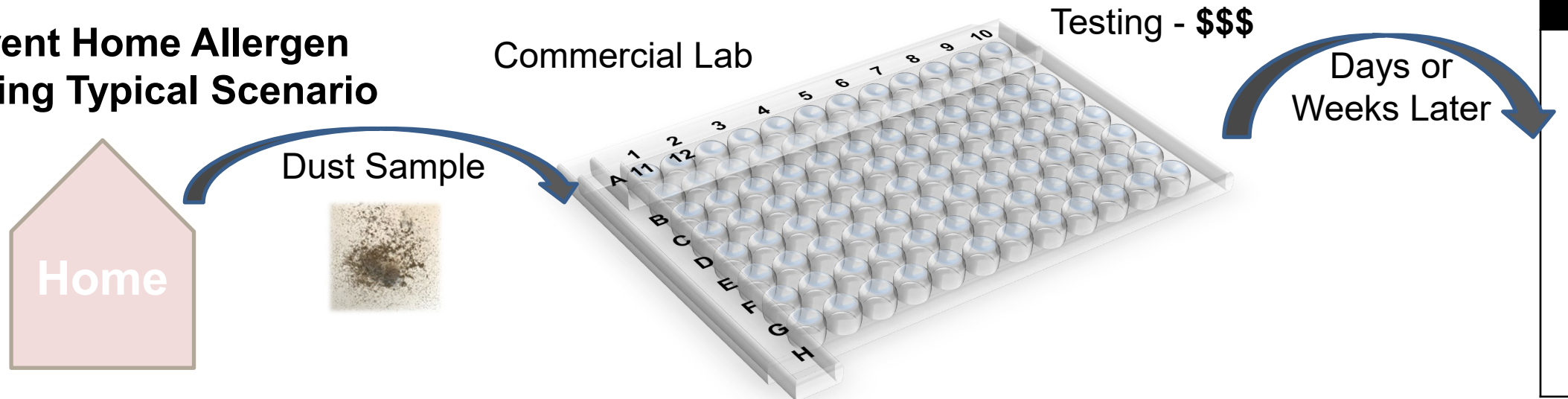


Pollen

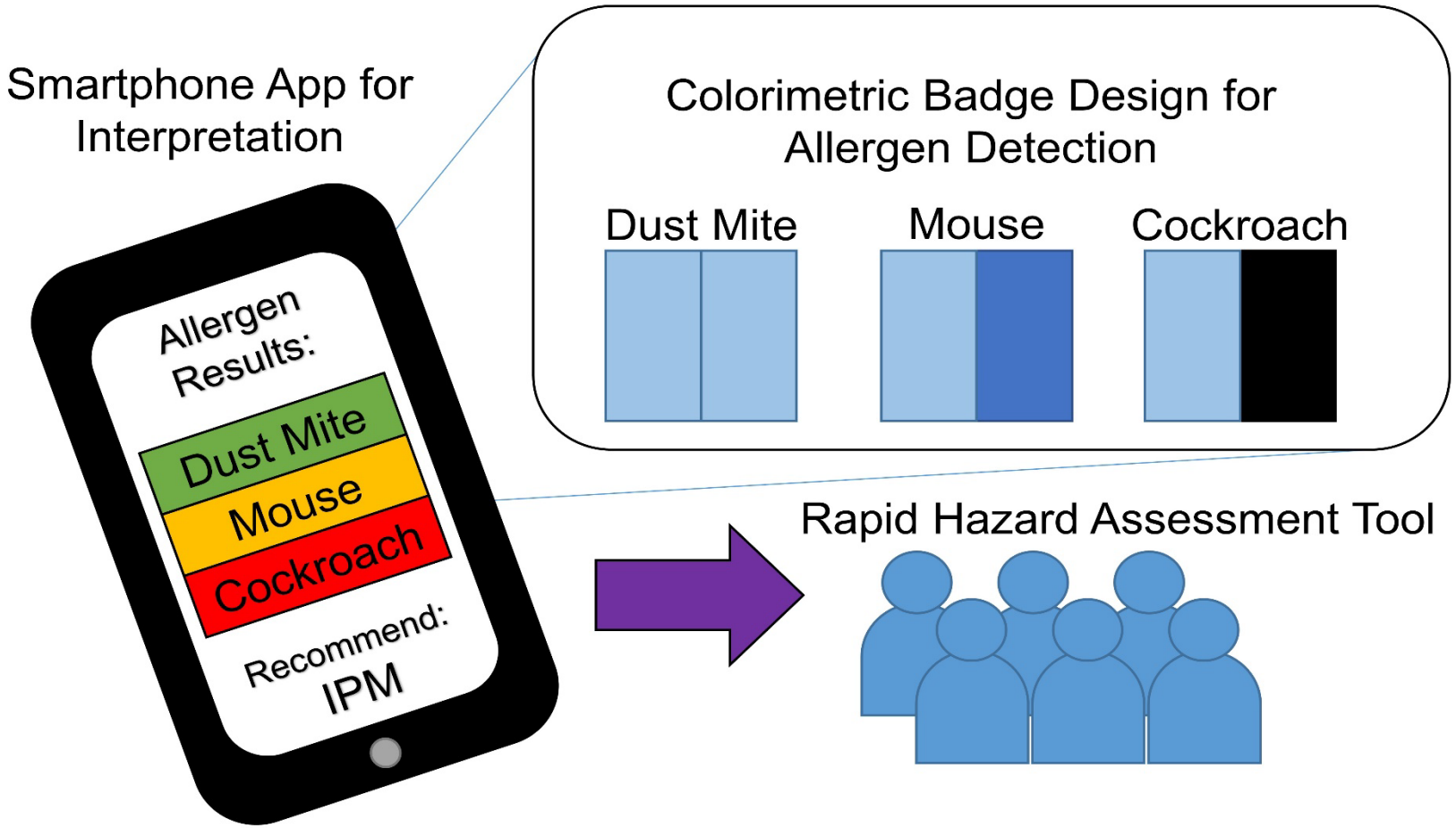
We need improved identification of hazards in each home for efficient resource allocation

- Children sensitized and exposed to allergens have worse outcomes
- Need to be able to measure allergens in homes
  - Current methods are difficult and results are delayed
  - Empower cost-effective asthma homecare programs

Current Home Allergen Testing Typical Scenario



Part 3: Develop smartphone-based system for point-of-care allergen detection



Collaboration with Asthma Express Program

Phase I (2021- 2023) Needs Assessment – 21 families

- Completed

Phase II (Started 2024) Field Testing – 100 families

- Home visits, sample collection, sensor unit and app testing in the field, allergen detection validation, patient education
- Exploring addition of spirometry for family participants

Home Allergens and Asthma:
Join Our Study

What's the purpose of the study?
Researchers at Nationwide Children's Hospital and The Ohio State University are studying allergens in the home and how they impact those with asthma.

Who can take part in this study?
Patients in the Asthma Express program between 5 and 13 years of age. Participants must be able to read and understand English. If your child has a disability but would like to participate, please contact us to learn more about accommodations and possible enrollment.

Participation is voluntary, and you can leave the study at any time. If you do not want to be in this study, your medical care will not be affected.

What will happen during the study?
Study participants will complete a survey and be interviewed by study staff. Participation will take about 90 minutes.

To participate, visit https://redcap.nchri.org/surveys/ and enter the code **33JL4LJFD,** or scan the QR code.

For more information, contact Dr. Chris Timan at (614) 722-4526

Principal Investigator: **Dr. Christopher Timan**

NATIONWIDE CHILDREN'S
When your child needs a hospital, everything matters.

BREATHE - Smart

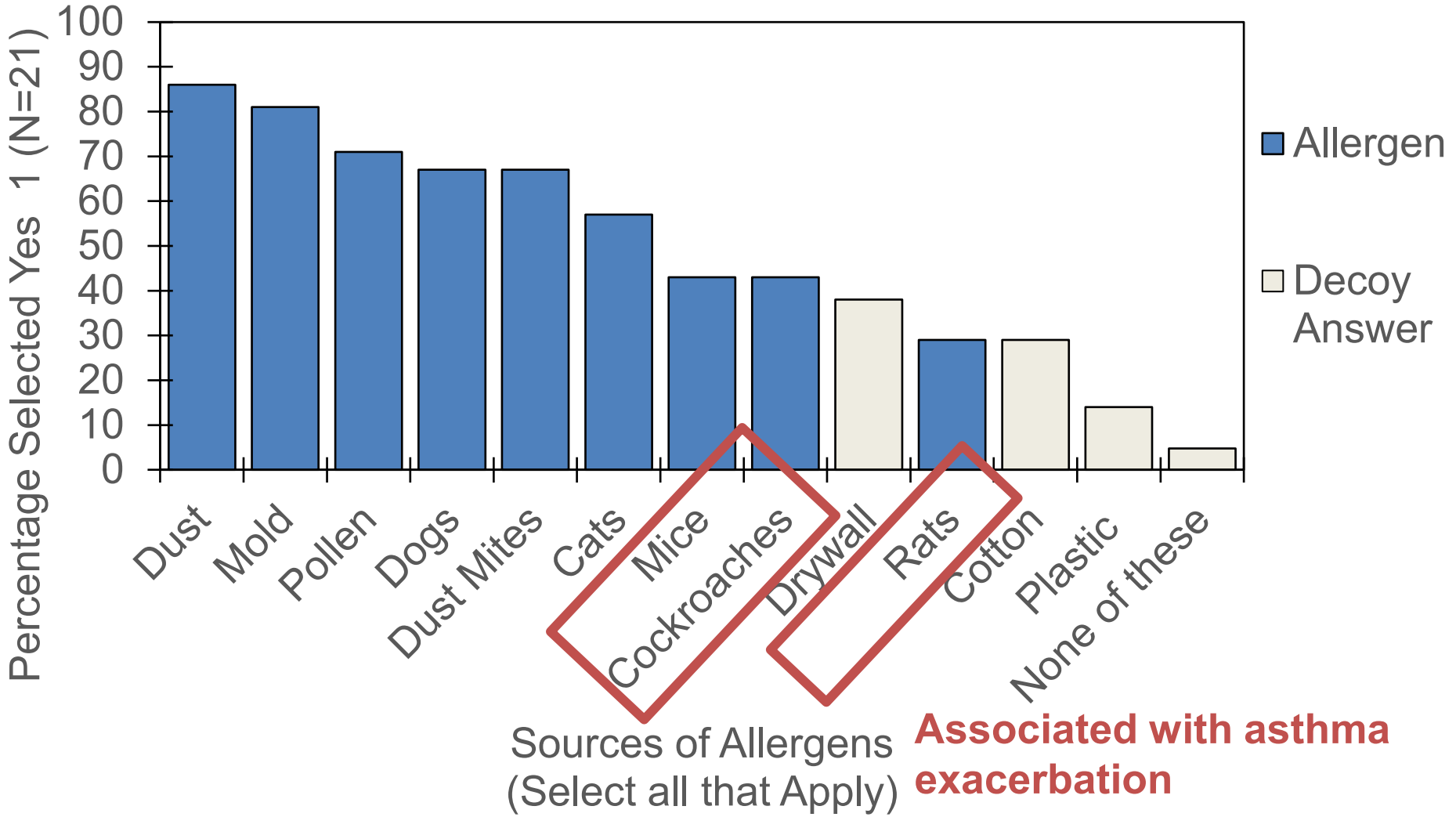
Needs Assessment: Complete

Child Characteristics	N	N=21[1]
Age	20	
5 years old or less		9 (45%)
6 to 10 years old		7 (35%)
11 years old or more		4 (20%)
Sees Asthma or Allergy Specialist	20	
Yes		17 (85%)
No		2 (10%)
Unsure		1 (5%)
Had Allergy Testing	20	
Yes		9 (45%)
No		10 (50%)
Unsure		1 (5%)
Attends Daycare/Preschool Most Days	21	
Yes		17(81%)
No		4 (19%)
Asthma Triggers (Select All That Apply)	21	
Illnesses		11 (52%)
Exercise		7 (33%)
Home Allergies		7 (33%)
Seasonal Allergies		11 (52%)
Smoke		3 (14%)
Weather Changes		12(57%)
Pets		5 (24%)
Strong Odors		2 (9.5%)
Other		3 (14%)

Parental Characteristics	N	N=21[1]
Relationship to Child	21	
Mother		18 (86%)
Father		3 (14%)
Caregiver Age	18	
25-29		6 (33%)
30-34		4 (22%)
35-39		4 (22%)
40-54		4 (22%)
Race/Ethnicity (Select all that Apply)	21	
White		13 (62%)
Black		7 (33%)
Hispanic/Latino		1 (4.8%)
Prefer Not to Answer		1 (4.8%)
Education Level	21	
Did Not Complete High School		3 (14%)
Graduated High School		3 (14%)
Technical/Vocational School or Some College		7 (33%)
Graduated College		6 (29%)
Graduate/Professional School		2 (9.5%)
[1]n (%)		

Young et al., *In Review*

Use of these system highlights need for education

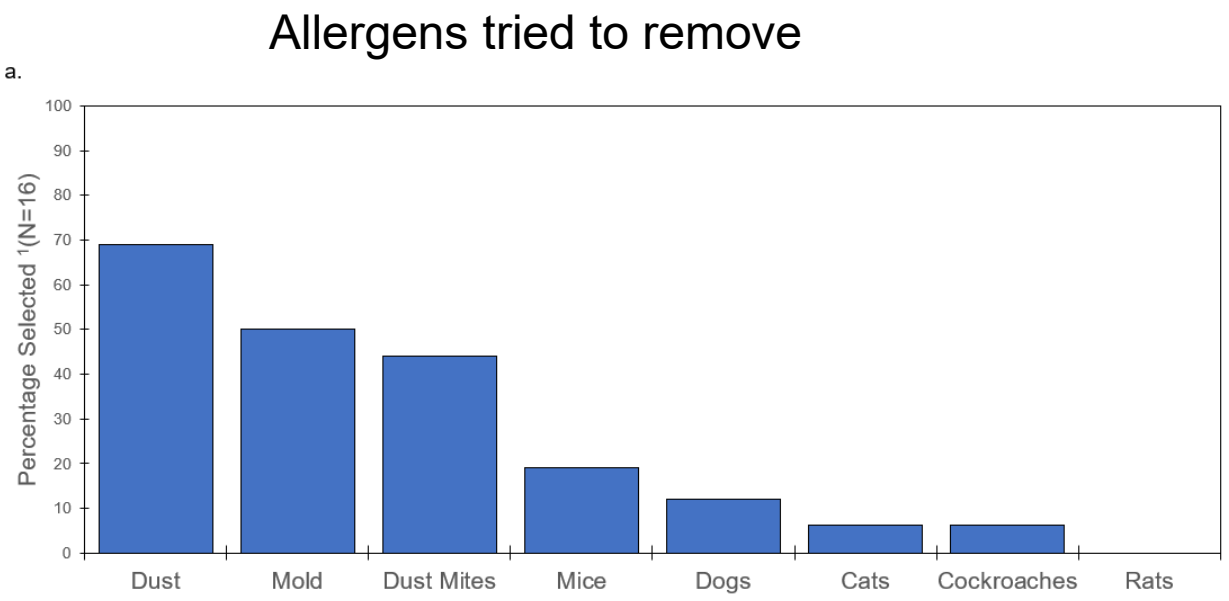


- 23% can define "allergen"
- 69% can list examples

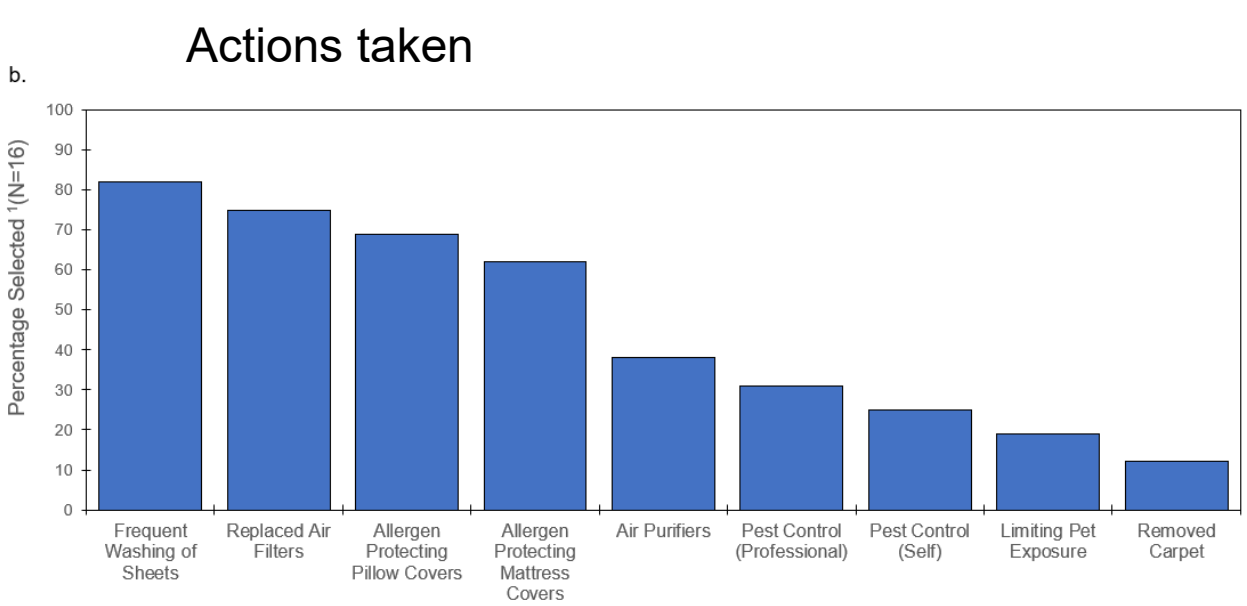
Young et al., *In Review*

The allergens families are focused on removing may not be the most associated with asthma

a.



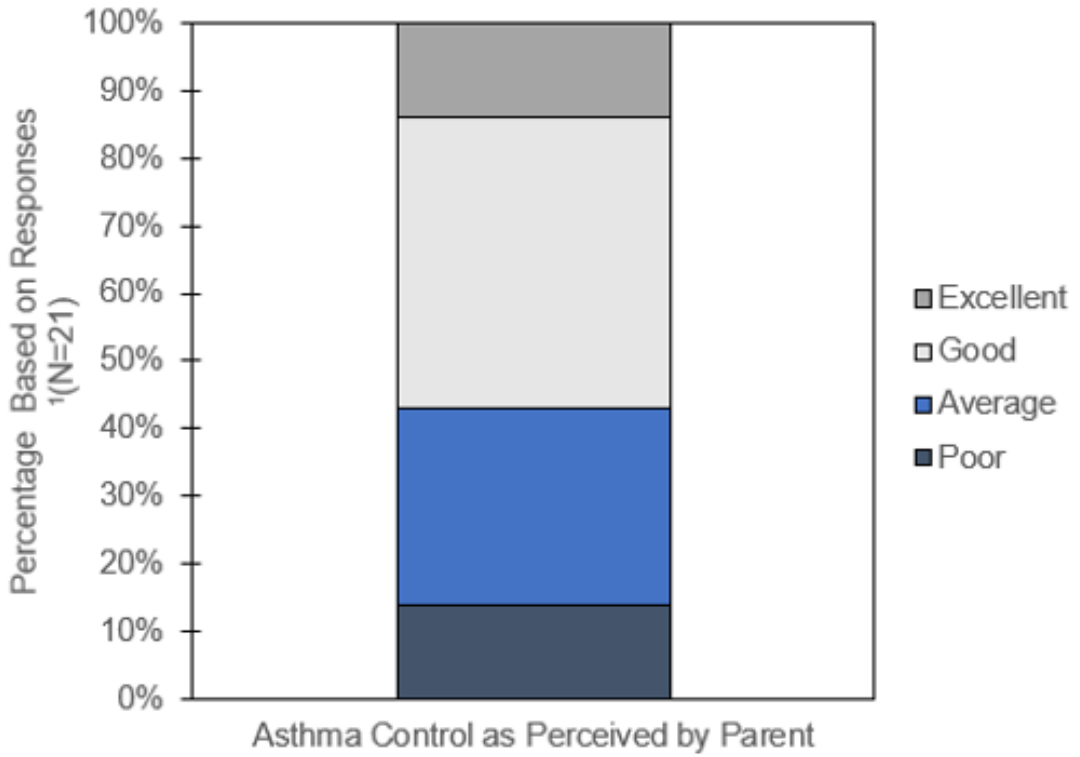
b.



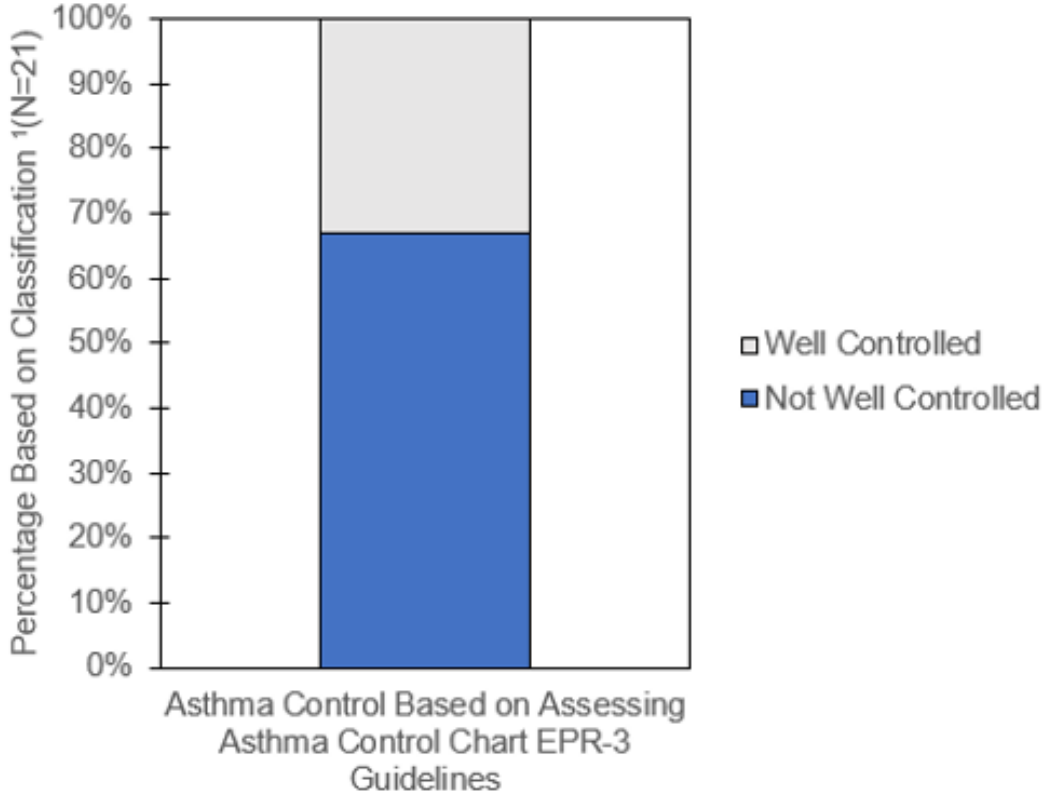
Young et al., *In Review*

Differences in asthma control vs. perception

c.



d.



Young et al., *In Review*

Participants interested in allergen detection

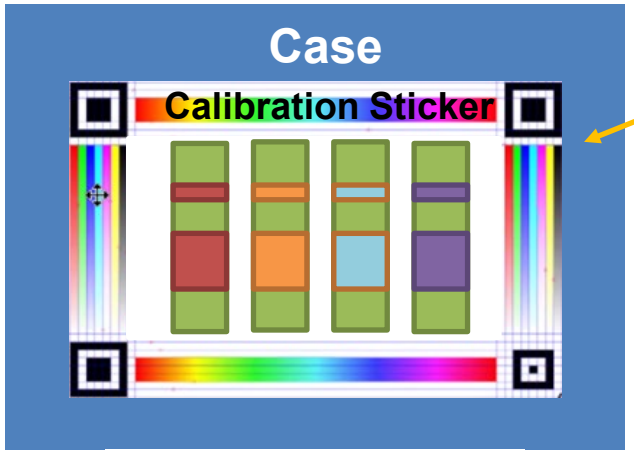
- 71% - “extremely important” to be able to detect allergens
- 52% - allergen education is “extremely important”
- 71% - allergen mitigation is “extremely important”

- Participants were especially interested in help resolving landlord/tenant issues

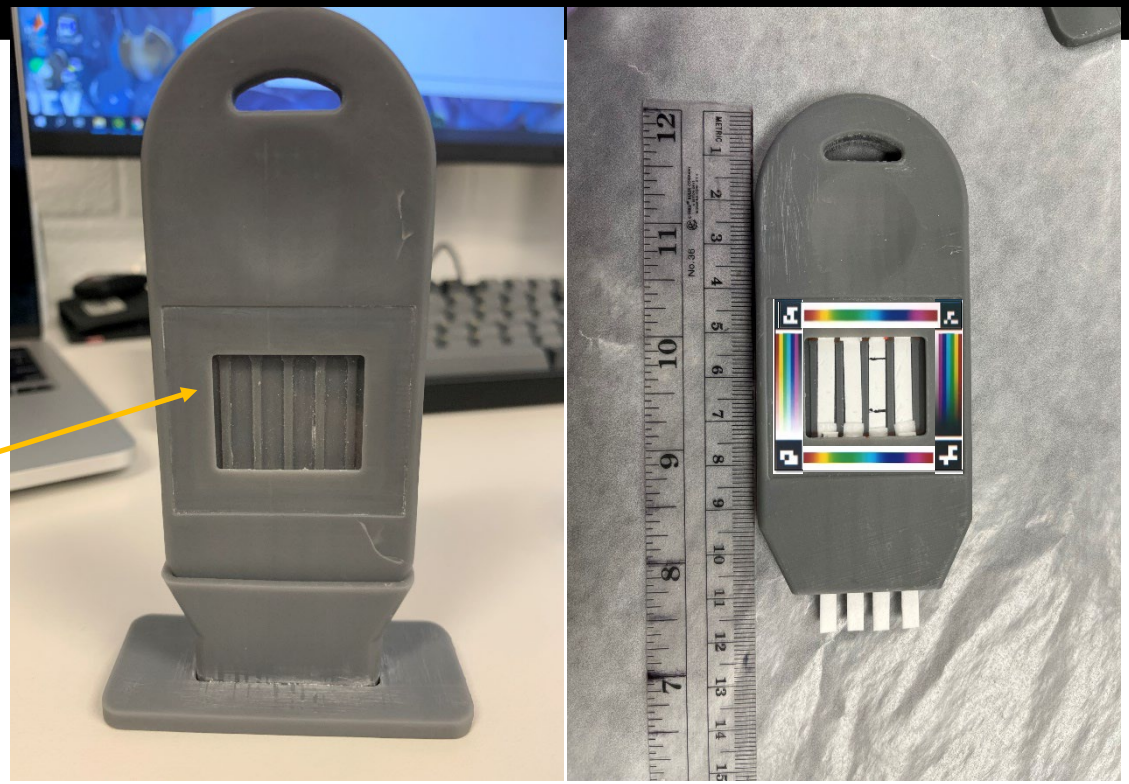
Young et al., *In Review*

BREATHE-Smart Test Unit under development

- Case prototype printed
  - Produced by the Center for Design and Manufacturing Excellence (CDME) at OSU
  - Will house the sensor

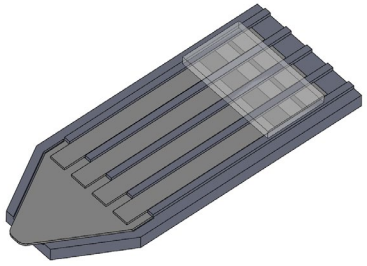


Results window



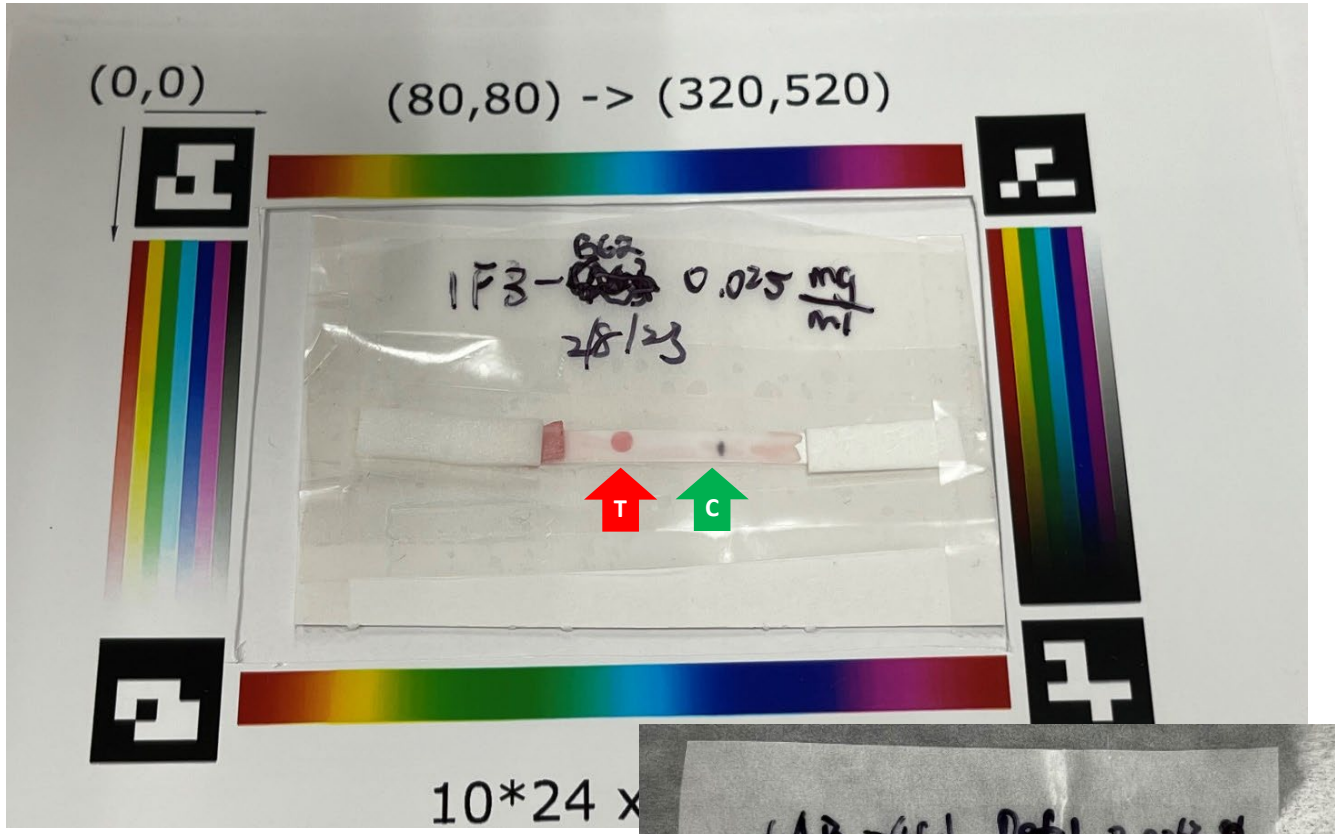
Complete sensor unit with case. Left: case only (3D-printed from synthetic resin), Right: the complete sensor inside the case

interior of the test unit (drawing)

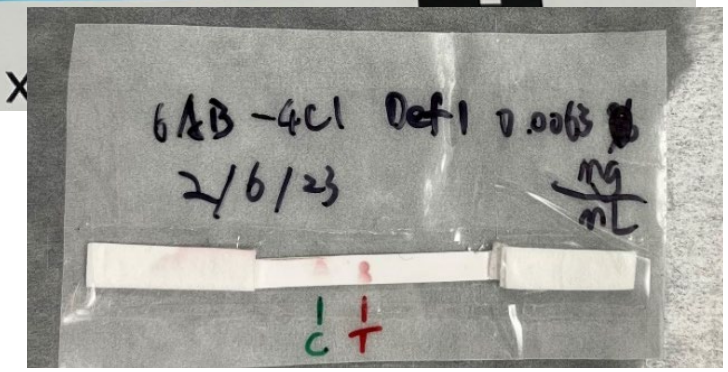


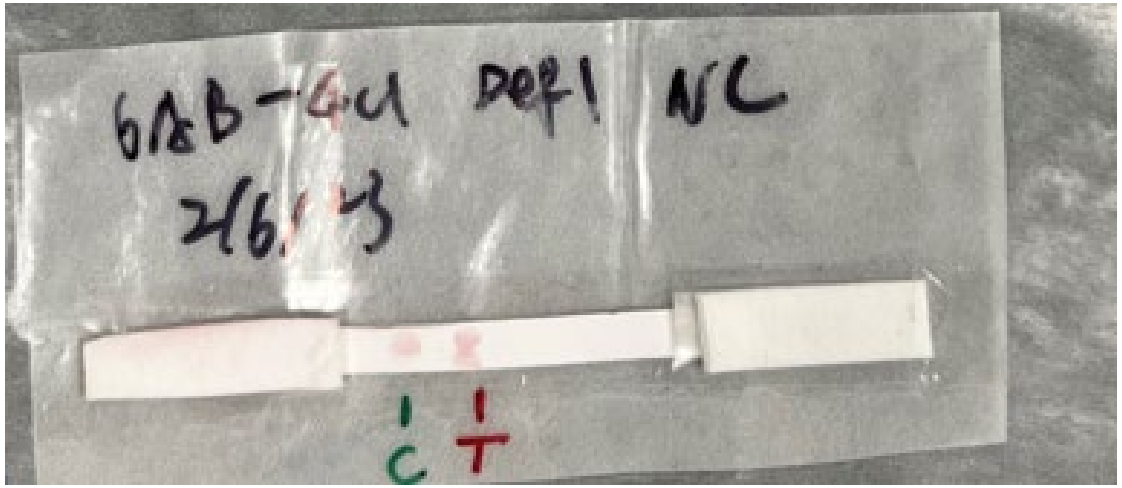
Walt VanCleave, CDME, 2021
Dannemiller and Panescu, 2021

Novel Electrospun Sensor



Cockroach sensor

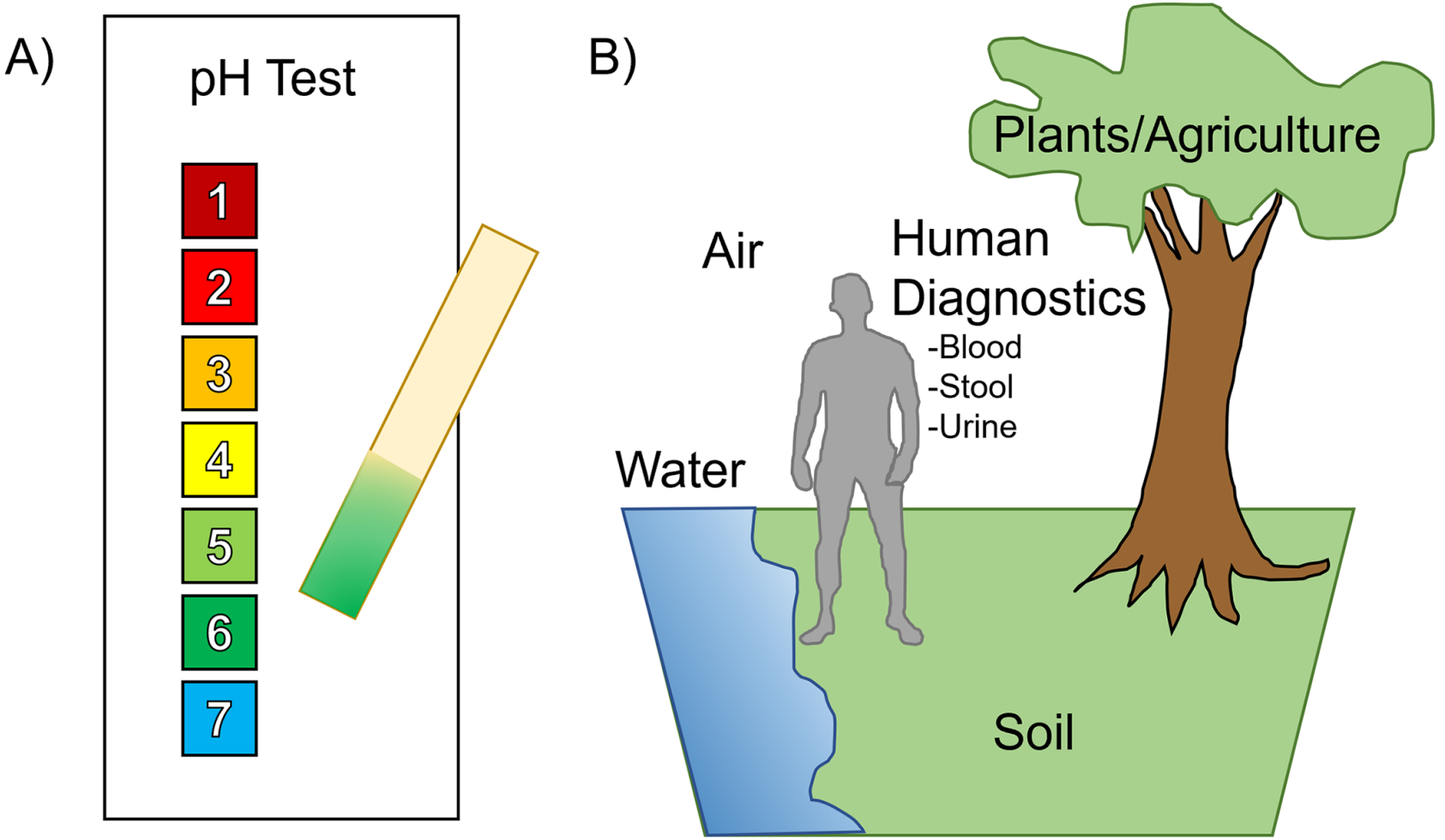




Left: **Cockroach sensor** complementary monoclonal antibody pair

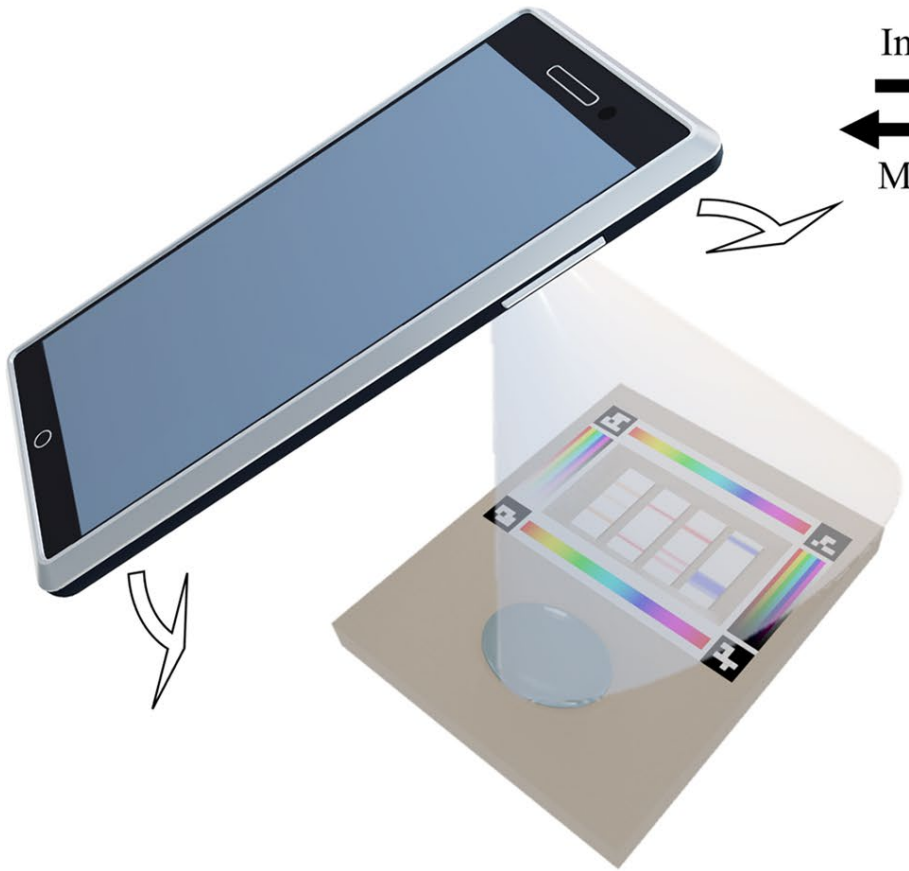
Top: **Dust mite Der f 1** complementary monoclonal antibody pair – false positive result

System dependent on color detection like many other tests

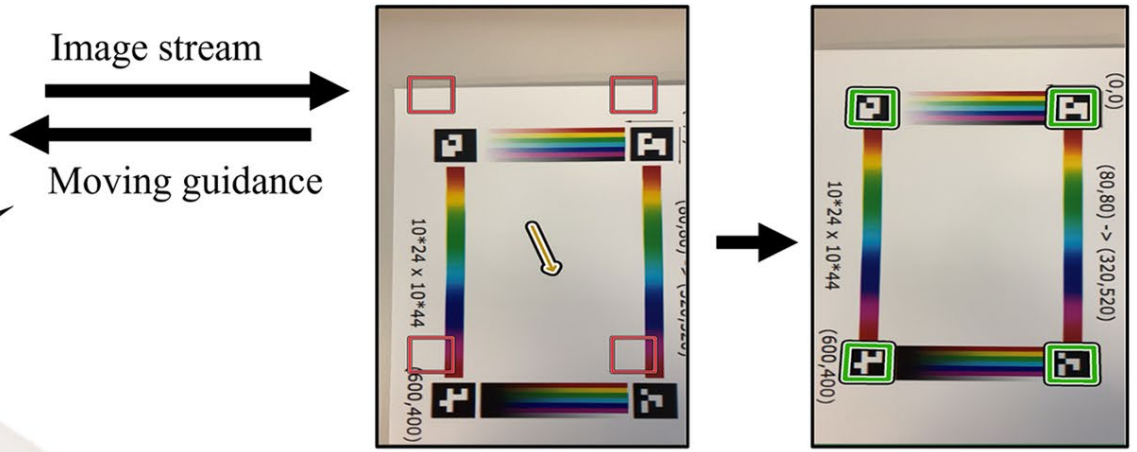


Zhang et al., *PLOS ONE*, 2023

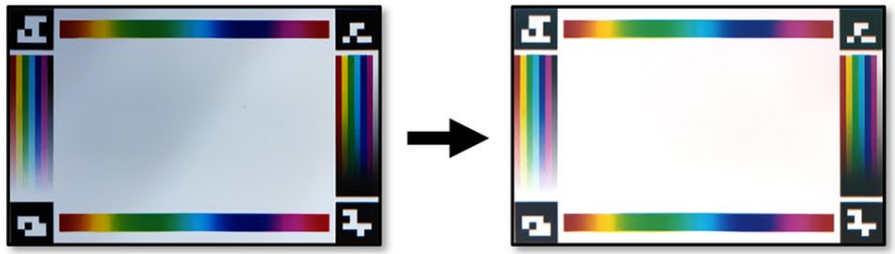
Color detection and quantification enhanced



(A) **Color reference board:** A general-purpose board design can be integrated to test kits.



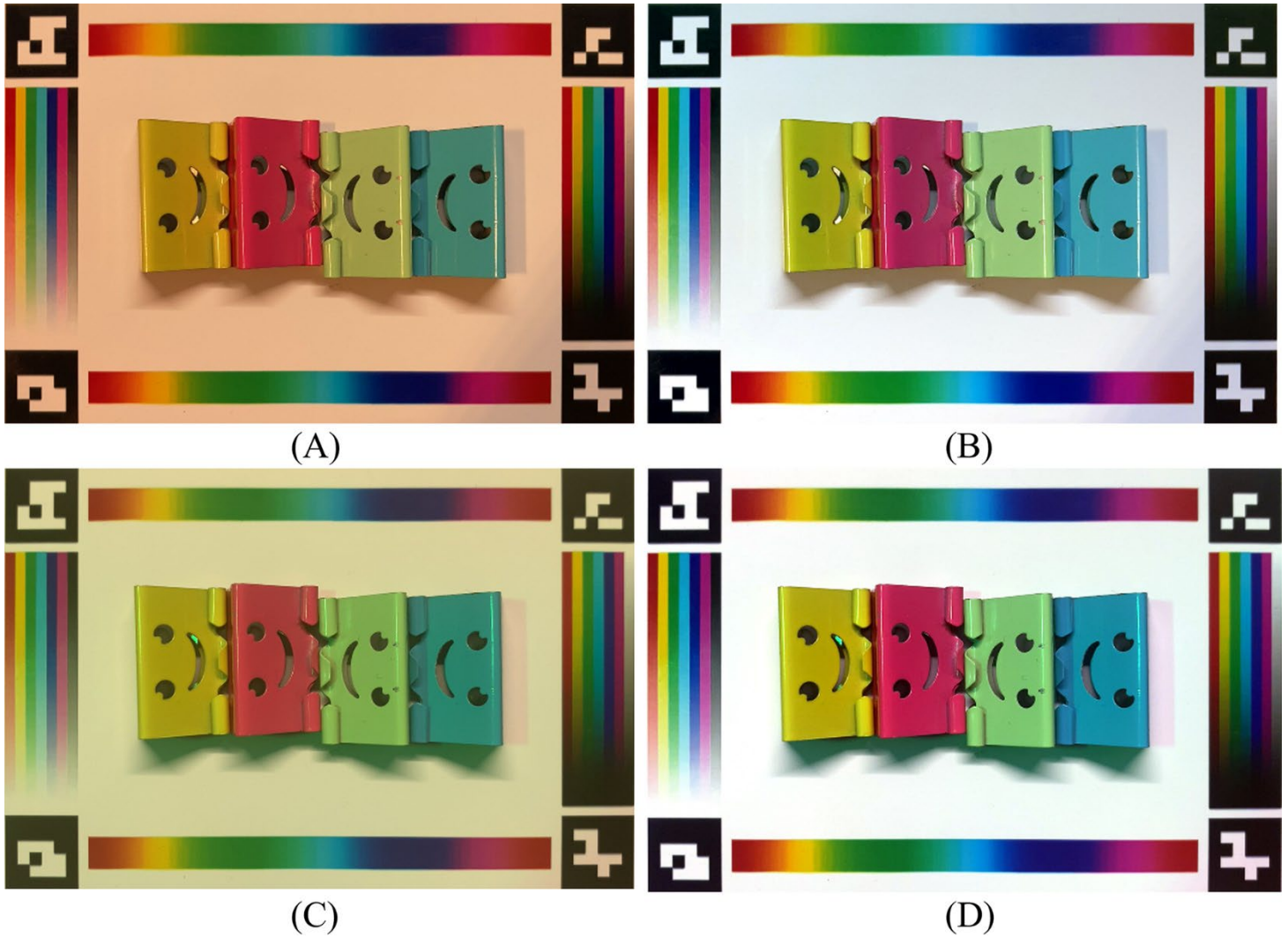
(B) AR-based image capture module: Guiding user device movement until optimal position reached.



(C) Color Correction Algorithm: Align captured images with the standard color reference board

Zhang et al., *PLOS ONE*, 2023

Correction of poor lighting conditions



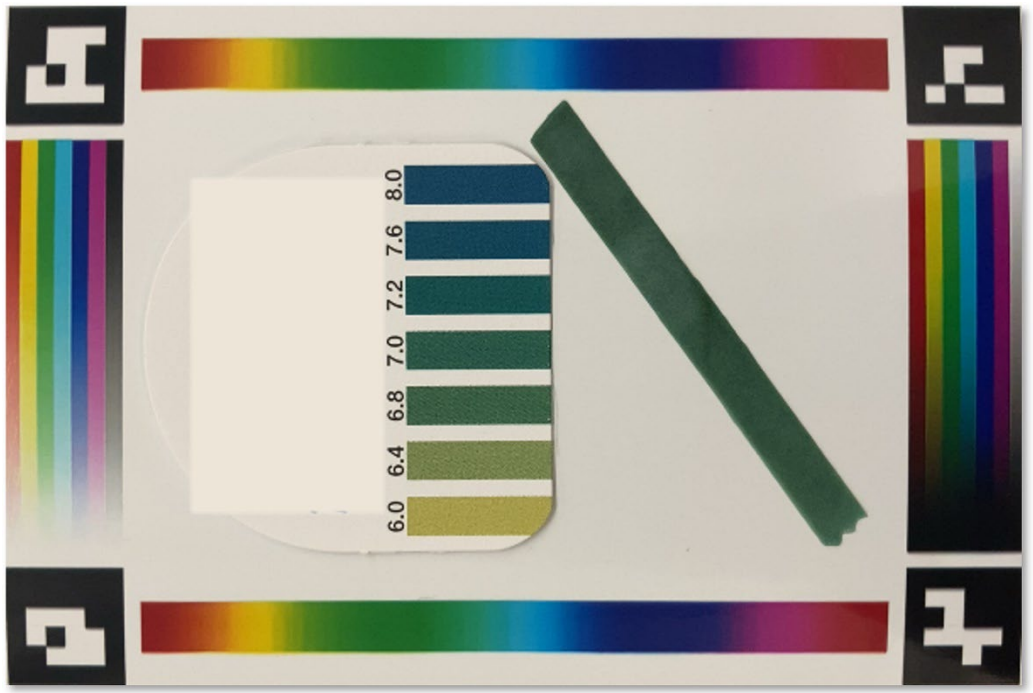
Zhang et al., *PLOS ONE*, 2023

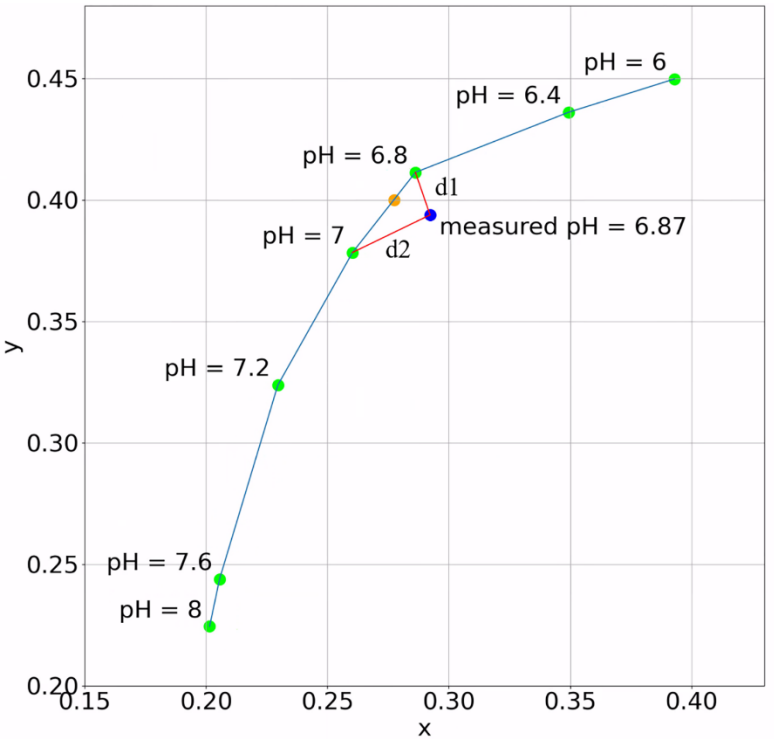
Integration of warnings and shadow correction

(A) (B) (C)

Zhang et al., *PLOS ONE*, 2023

Improved color determination: pH strip demo



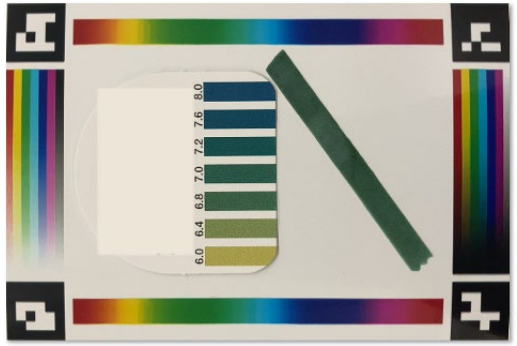


Zhang et al., *PLOS ONE*, 2023

Smartphone measurements were 3x better than human eye

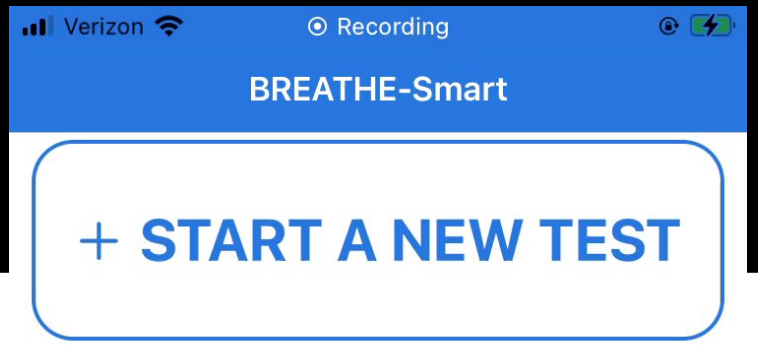
	Reference–pH test paper and color chart co-located (color chart and pH test paper under the same illumination)			Color chart free pH reading (color chart and pH test paper under different illumination)	
Ref. pH value	w/o correction	w/ correction	avg. human readings	w/o correction	w/ correction
3.00	+0.16 (3.16)	+0.15 (3.15)	**-0.07** (2.93)	+0.16 (3.16)	**+0.13** (3.13)
6.86	-0.17 (6.69)	-0.20 (6.66)	**-0.06** (6.80)	-0.24 (6.62)	**-0.20** (6.66)
7.00	-0.10 (6.90)	-0.13 (6.87)	**-0.08** (6.92)	-0.10 (6.90)	**-0.09** (6.91)
7.80	**+0.05** (7.85)	+0.07 (7.87)	+0.17 (7.97)	+0.09 (7.89)	**+0.06** (7.86)
9.00	**+0.13** (9.13)	-0.15 (8.85)	+0.45 (9.45)	-0.22 (8.78)	**-0.20** (8.80)
9.18	+0.12 (9.30)	**0.00** (9.18)	+0.67 (9.85)	-0.06 (9.12)	**-0.04** (9.14)
MAE	**0.12**	**0.12**	0.37	0.15	**0.12**

https://doi.org/10.1371/journal.pone.0287099.t002



Zhang et al., *PLOS ONE*, 2023

User-friendly App Development



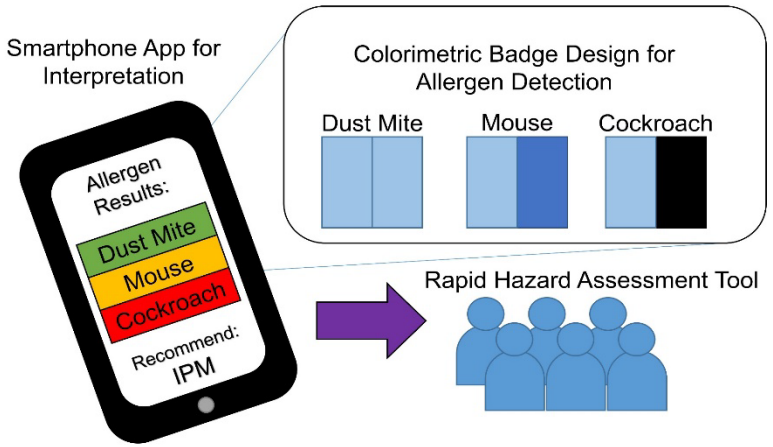
The app for the iPhone platform is completed

- Refinements
- Accessibility
- Will be tested with the sensor once it is completed

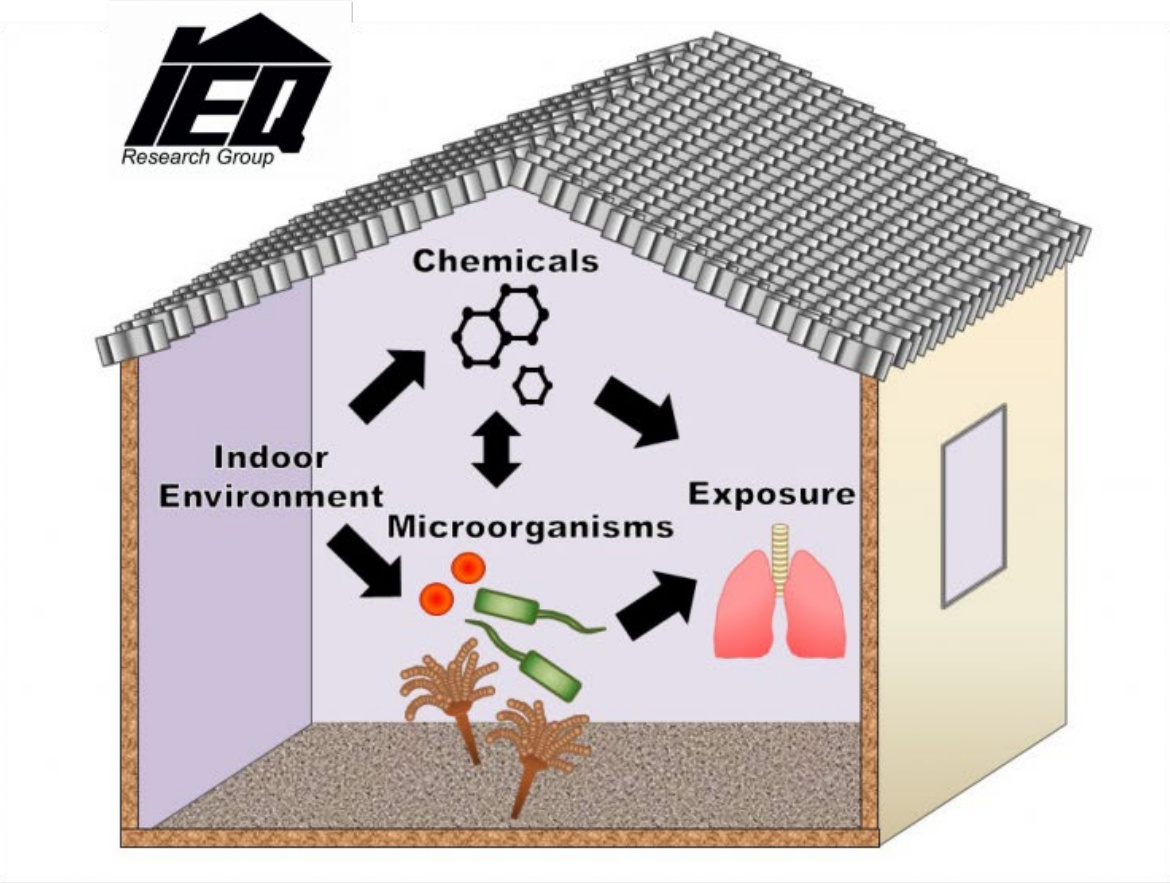
Song, Zhang and Qin, unpublished research, 2023

Part 3 summary: A new tool for asthma homecare programs

- Patient education: Many common allergens associated with asthma exacerbations are underrecognized
- Lateral flow assays offer opportunity for point-of-care allergen testing
- Smartphone technology can improve measurement accuracy



Talk Summary



Part 1: New Mold Indicators from Function

Part 2: Spacecraft are similar to built environments on Earth

Part 3: Novel allergen sensors provide an opportunity for point-of-care testing

Indoor Air 2024!

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INDOOR AIR 2024

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Questions?

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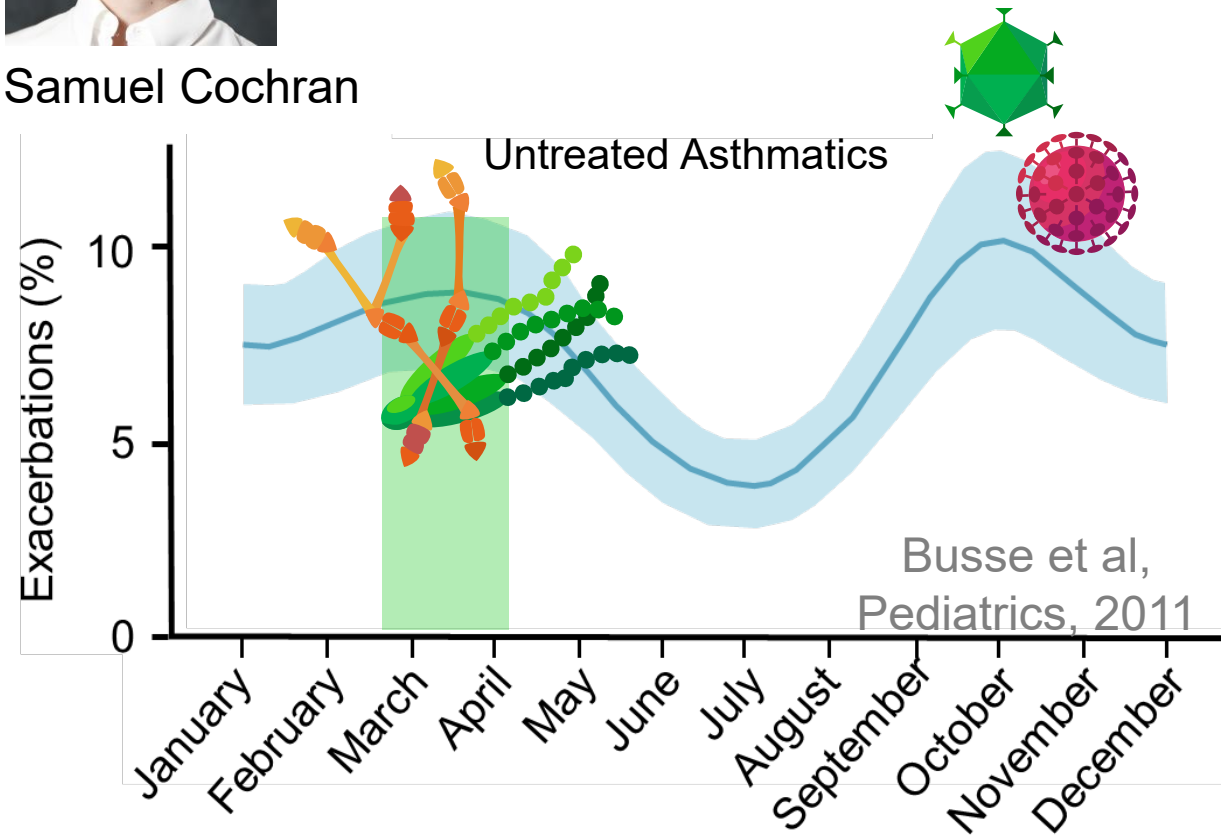
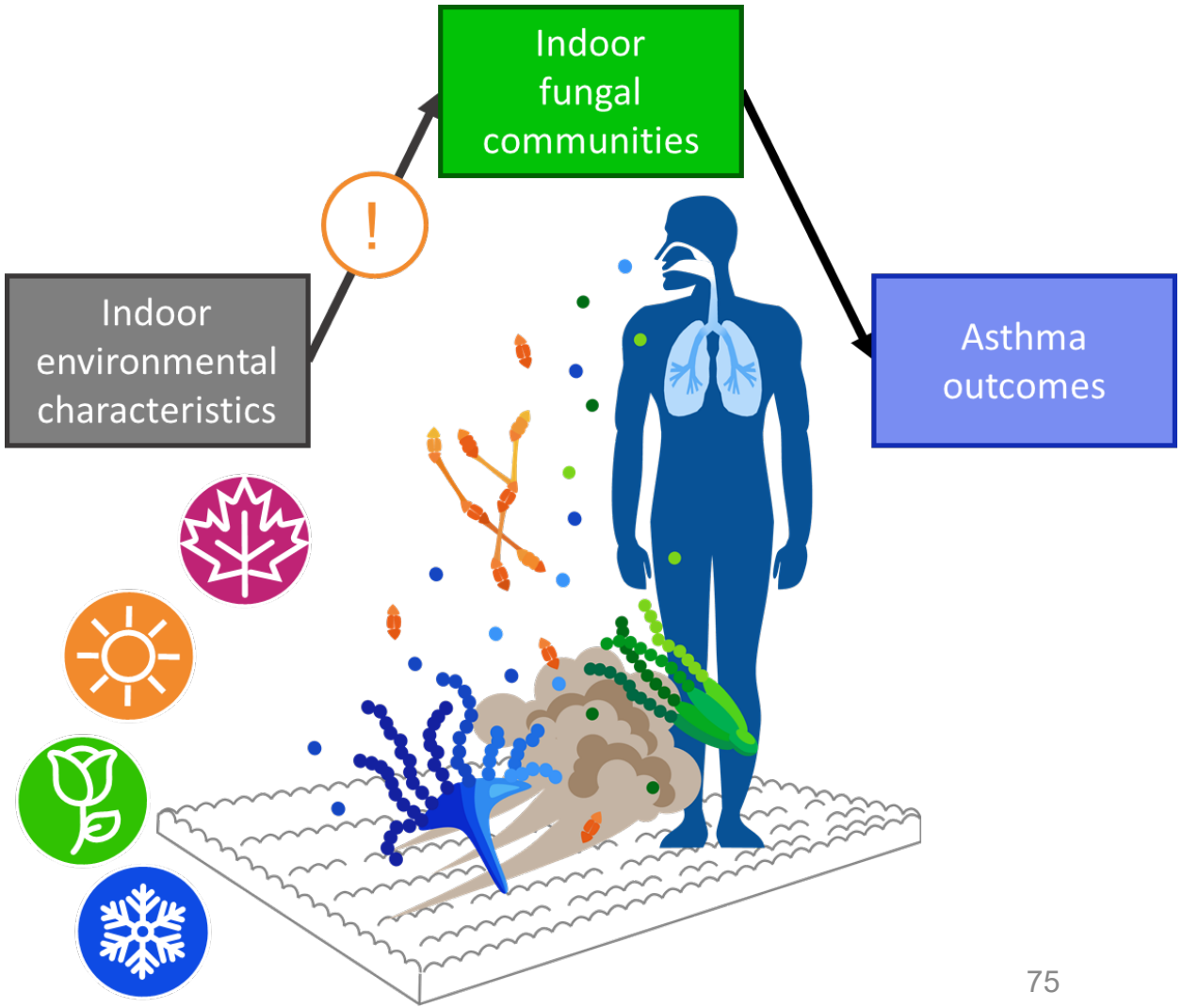
Part 4: Seasonal variation in indoor fungi might contribute to patterns in adverse respiratory outcomes, but is understudied

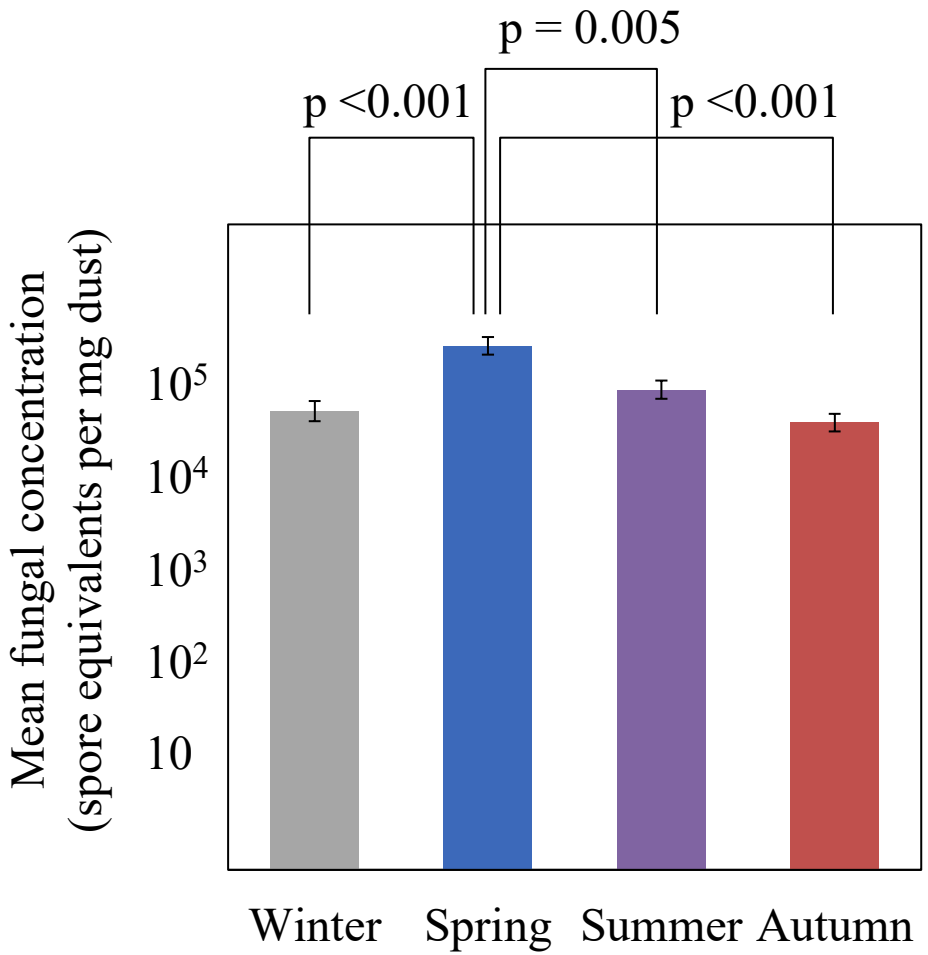
Samuel Cochran



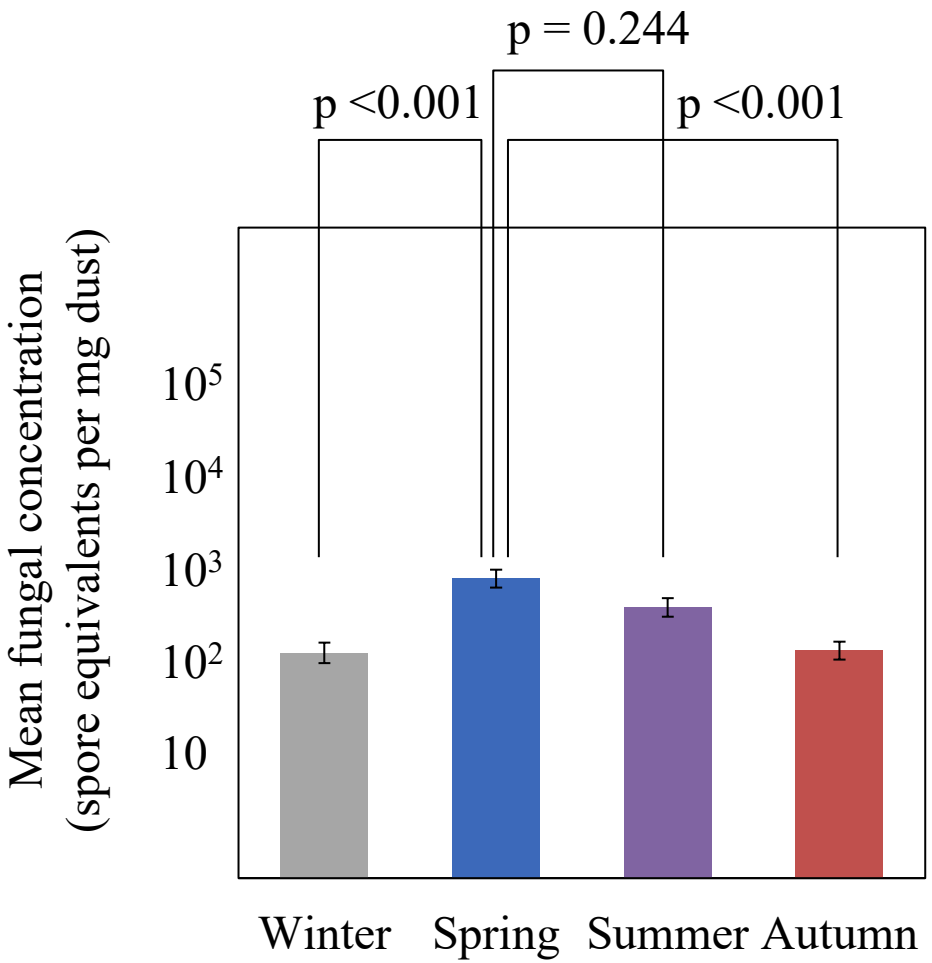


Concentrations of summed fungal species significantly higher in spring (similar trend for summed allergenic species)

a) Seasonal variation in total fungal concentrations

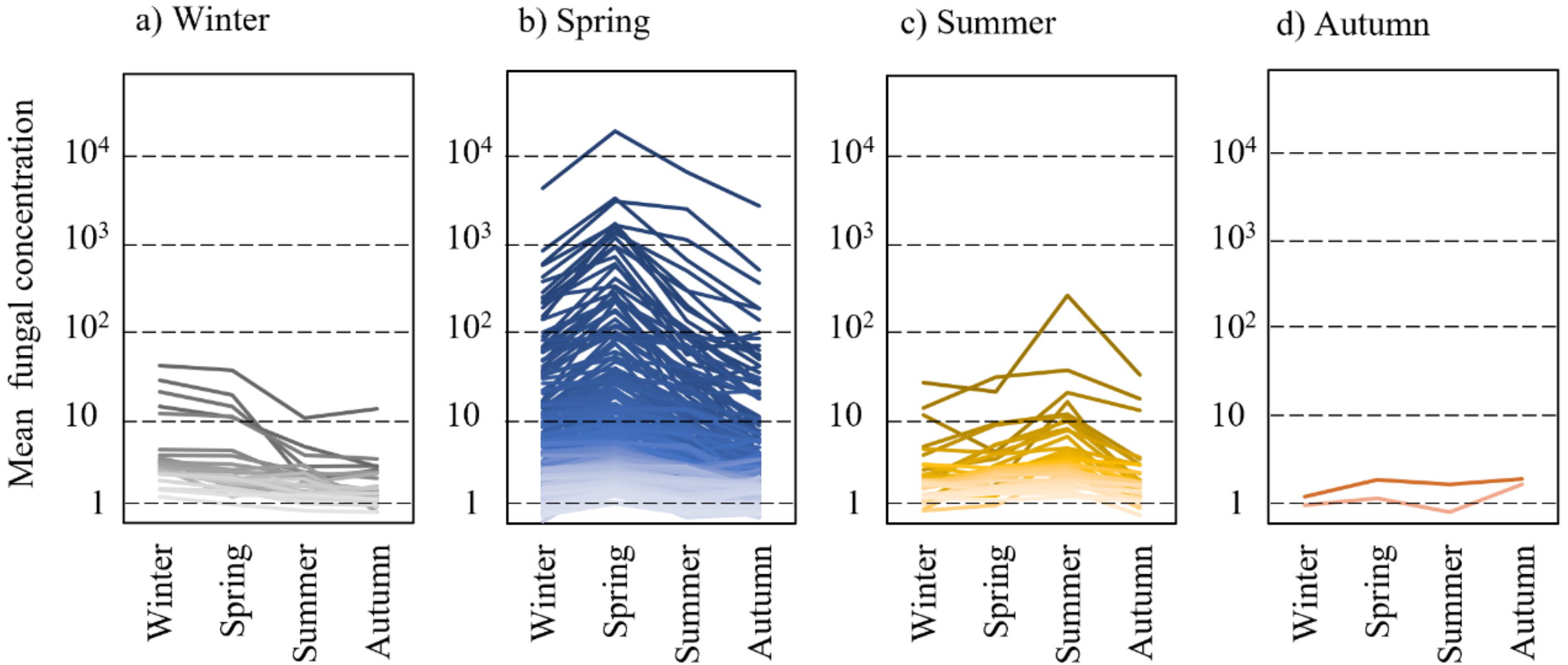


b) Seasonal variation in summed allergenic fungal concentrations



Cochran et al, Building and Environment, 2022

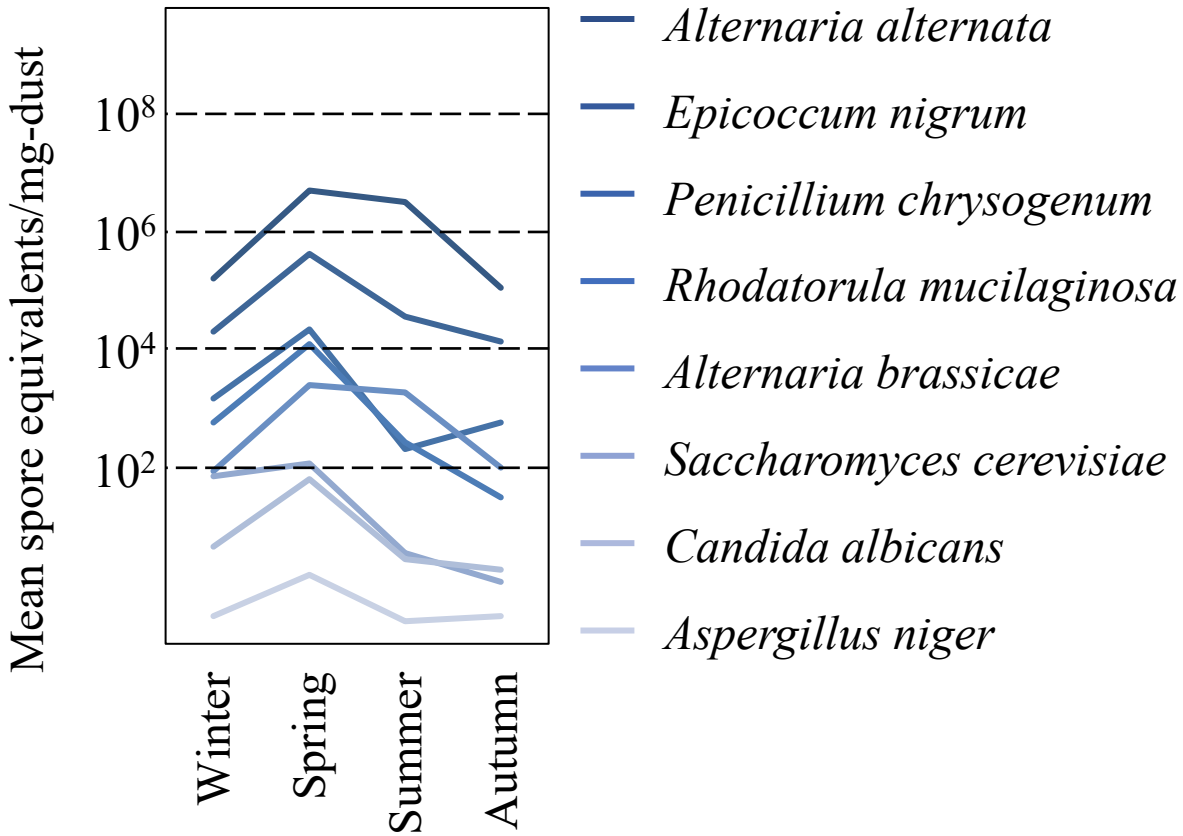
Trends in concentration for 227 fungal species showed spring peaks (significant for 77 species)



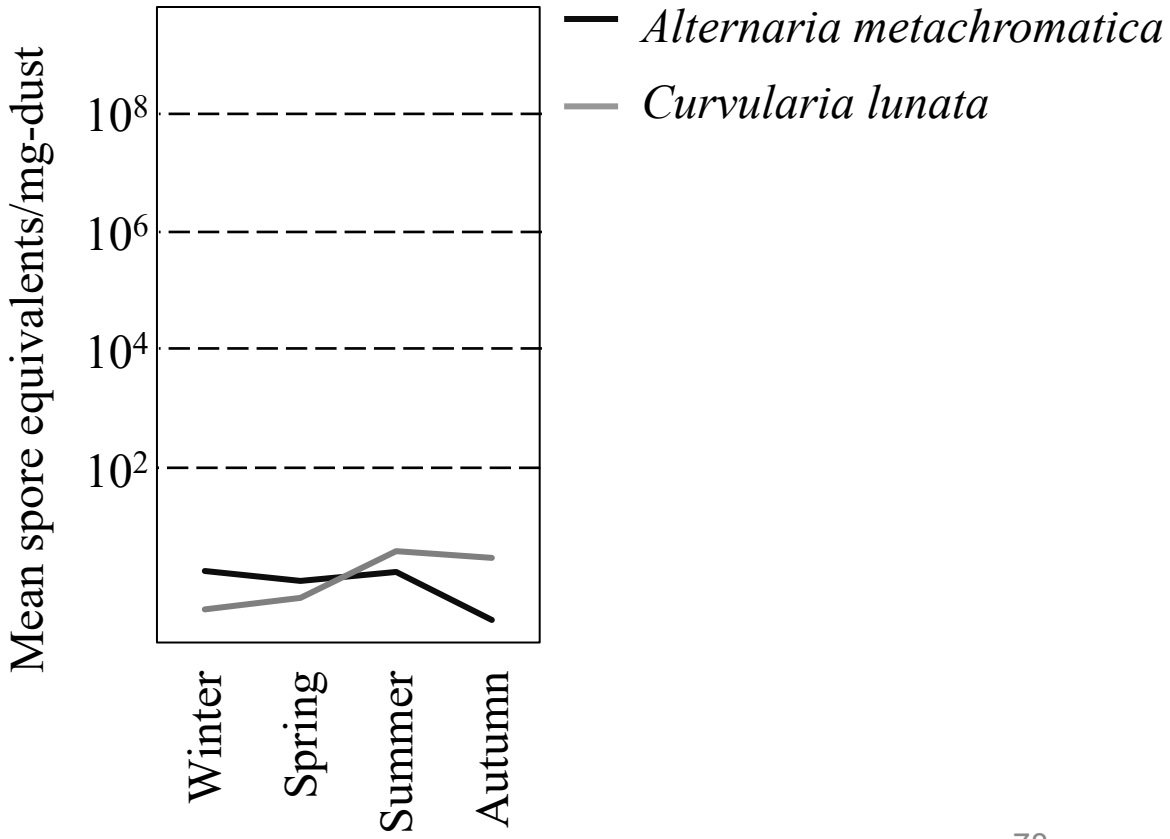
Cochran et al, Building and Environment, 2022

Seasonal variations offer insight to patterns in fungal allergens in NYC (with implications for asthma control)

a) Allergenic species, springtime > 2 or 3 seasons



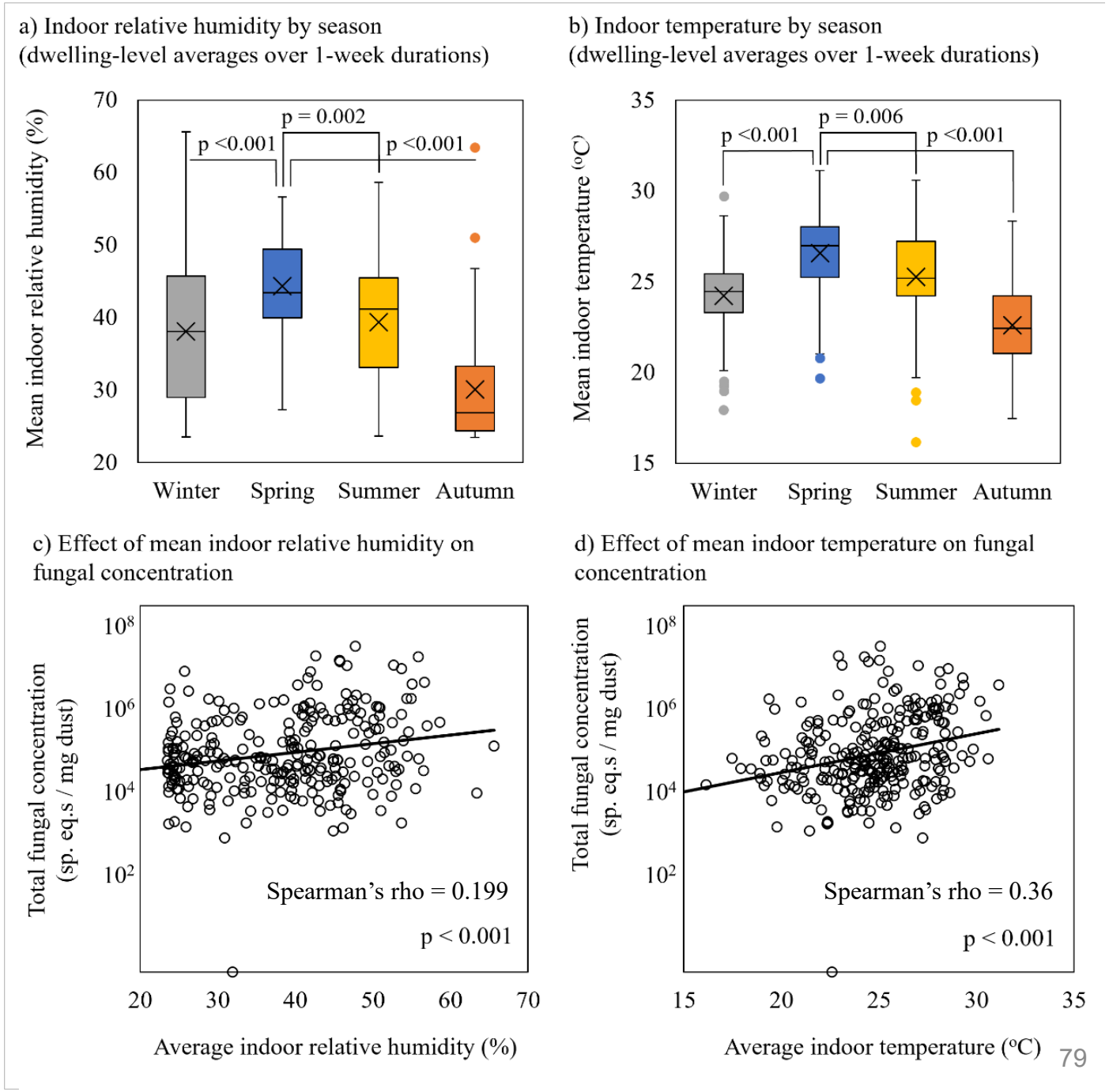
b) Allergenic species, Other seasonal associations



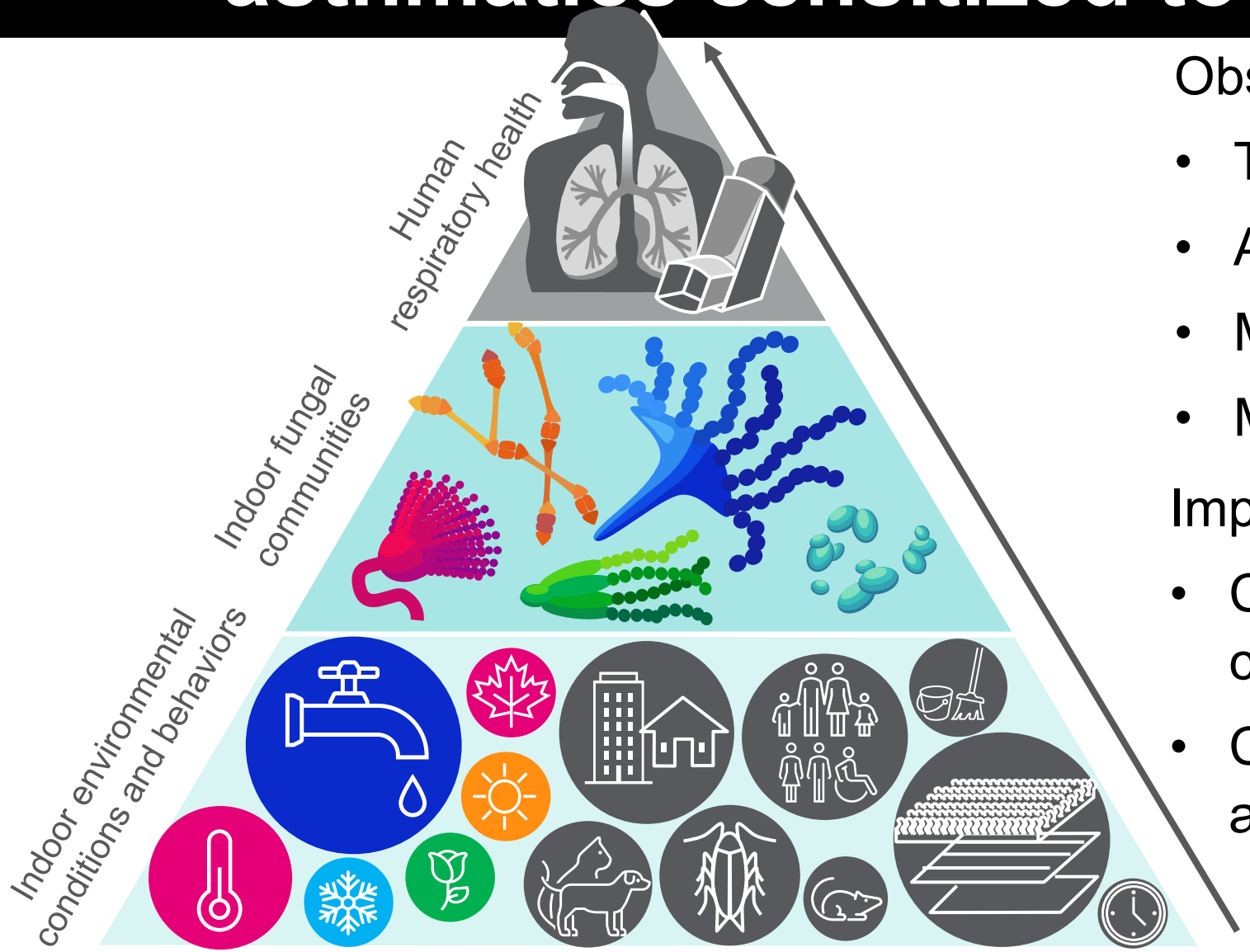
Cochran et al, Building and Environment, 2022

Mean indoor relative humidity and temperature may be a driving factors in the association between season and fungal concentration

Cochran et al, B&E, 2022



Spring may represent an important season for asthmatics sensitized to fungi in NYC



Observed springtime increases in:

- Total fungal concentration
- Allergenic species concentration
- Mean indoor relative humidity
- Mean indoor temperature

Implications

- Controlling indoor conditions to control fungi
- Characterizing trends in seasonal allergens for asthma control