

Etienne Tardif

Independent Research

Honey bee colonies in the Yukon endure extreme winters, where prolonged cold and limited cleansing flights intensify disease pressures. This study examines the impact of *Nosema* and *Malpighamoeba mellificae* (Amoeba disease) infections on colony survival, emphasizing the role of late-season nutrition and hive management in reducing winter losses. Despite strong populations entering winter, subtle late-summer nutritional deficiencies often go unnoticed. In resource-scarce conditions, bees may forage on honeydew, fungal spores, and rust, compromising digestive health. These indigestible materials, combined with restricted winter flight opportunities, contribute to increased *Nosema* and Amoeba infections through fecal-oral transmission. Colonies that appear healthy in fall may collapse due to cumulative disease burdens in late winter and spring. Over multiple seasons, hive inspections and microscopic analyses tracked *Nosema* and Amoeba infection rates. Gut dissections and spore counts assessed disease progression. To analyze honey composition, samples were collected from Yukon's boreal regions and underwent Nuclear Magnetic Resonance (NMR) analysis to determine sugar composition and chemical profiles. Additionally, 10g of each honey sample was examined microscopically for pollen sources and honeydew elements. Data on hive management, forage availability, and honey production history were assessed for correlations with overwintering viability. Findings confirm that *Nosema* and Amoeba co-infections significantly increase winter mortality. Monthly monitoring of live bees and freshly dead bees at hive entrances revealed infection rates rising as winter progressed, with mortality notably higher in front of infected colonies. In spring, infected colonies failed to rear brood beyond early-stage larvae, collapsing within 2–4 weeks. However, when an infected colony's queen was introduced to a healthy queenless hive, she resumed laying, and new bees successfully emerged, demonstrating that colony failure stems from infected winter nurse bees' inability to raise brood rather than queen viability. Microscopy revealed substantial ingestion of non-pollen debris—especially rust and fungal spores—during late-summer/fall periods. Honey analysis indicated that certain honey types contribute to winter dysentery. Routine microscopic monitoring, evaluating honey stores, and supplementing late-summer and fall feeds can mitigate infection risks. Best practices—such as frame sterilization, strategic feeding, and optimized insulation—help subarctic beekeepers reduce winter stressors and improve colony resilience.

Microscopic Insights into Overwintering Disease Dynamics in Yukon's Subarctic by Etienne Tardif

Yukon winters impose long confinement and limited cleansing
flights

A focused “practical”, citizen-science approach to learning about
my bees



How often do your bees cleanse
in winter?



Background Context

- Long cold dark winters where bees are hive bound from mid-September to April (6-8 months)
- Little to no brood rearing this period (Detailed hive monitoring from 2018 to present)

Days from Bee Emergence

Typical Winter Bee	1st Patty	1st Natural Pollen	
New Bee	March 15, 2021	April 15, 2021	May 1, 2021
July 15, 2020	243	274	290
August 1, 2020	226	257	273
September 1, 2020	195	226	242
	Typical 1st Cleansing		

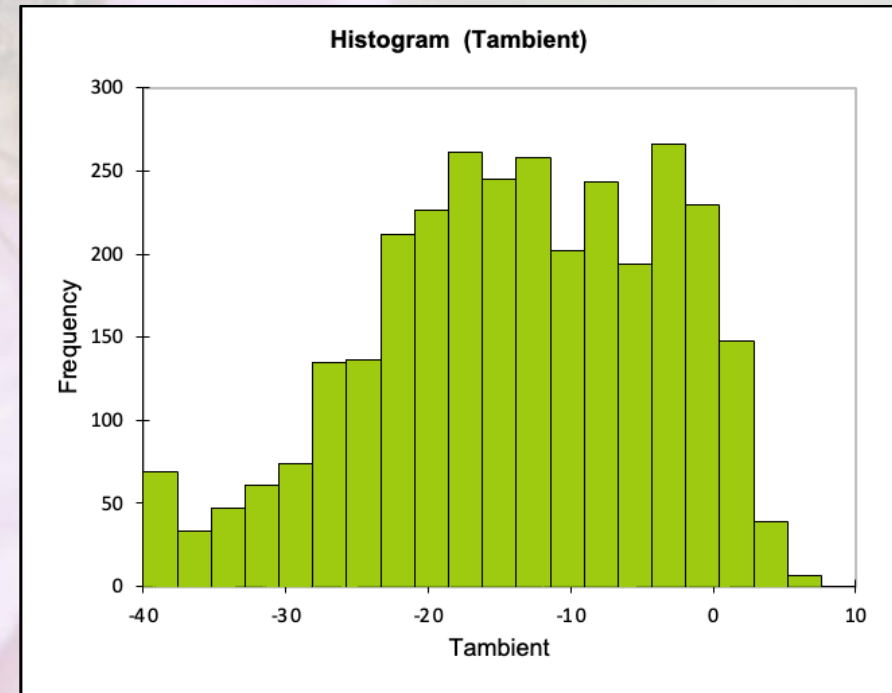
No late summer Pollen Patty equals:

early stop to queen laying

late start of brood rearing in Spring

lack of nutritional diversity

(Last significant pollen is early August)



My Questions

Why did I lose all my colonies in 2018?

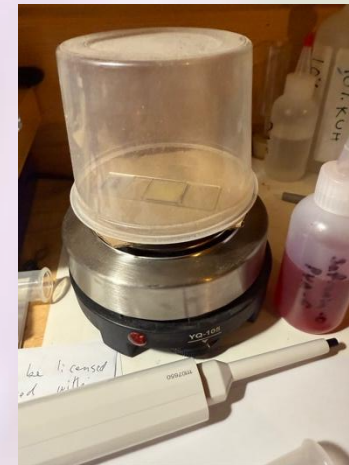
- Was it the food they had?
"It might be Honeydew Honey" – "What's that?"
- Was it Nosema?
"Nosema causes dysentery" (False)
- How do I clean things up after this type of event?
"Nosema spores will die at -45C!!" (Nope)

Subsequent Years:

- Why do I keep losing a single colony?

Methods Overview

- **Honey sampling:** Used NMR analysis for sugars, amino acids and some organic acids; pollen analysis to identify floral pollens and honeydew elements.
- **Bee sampling:** The 25-bee squeeze method (Infected Yes/No), 10-bee squeeze method (rate of infection) and individual dissections; differentiate midgut, ileum, Malpighian tubules and hindgut.
- **Feces sampling:** Collection from snow, frames, sensors and bottom boards; risk of equipment contamination.



Research Lineage: From Yukon Apiary to Peer-Reviewed Case Report

2017–2018
Field Observations

2018–2022
Microscopy Journey

2019–2023
Honey & Nutrition Studies

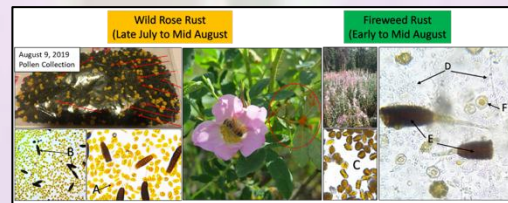
2022–2023
Research Synthesis

2023
Peer-Reviewed Validation

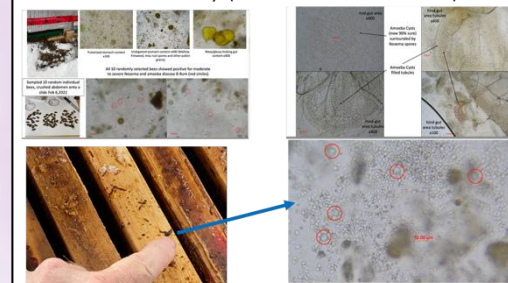
Unusual dysentery events
Spring colony losses
'Something missing'



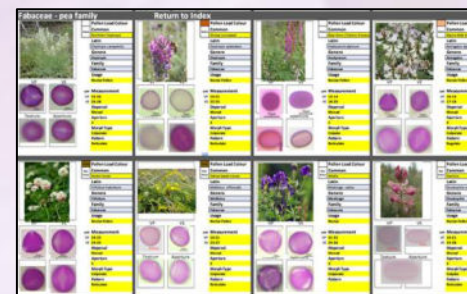
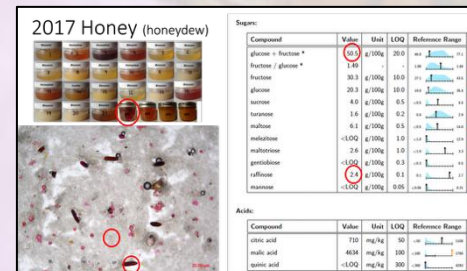
Nosema spores confirmed
Cysts in Malpighian tubules
Routine gut dissections



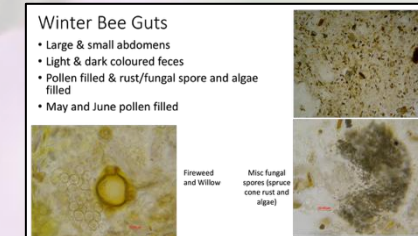
Amoeba Discovery (Nosema Co-Infection)



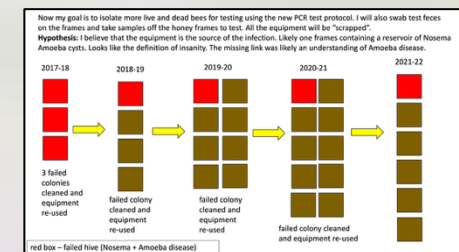
NMR honey analysis
Indigestibles & ash
Link to dysentery & spore loads



Microscopic Insights...
Colony failure via nurse bees



Veterinary Pathology case report
First modern NA amoeba confirmation with new PCR Protocol



Report of amoebic disease in a colony of Western honey bees (*Apis mellifera*)

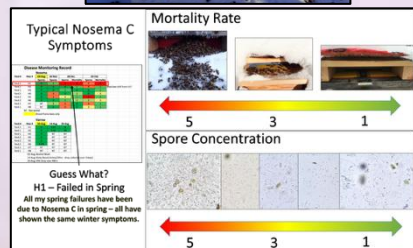
Marley E. Enders^{1,2}, Pedro H. O. Viadana^{2,3}, Kuttichanban Subramanian^{2,3}, Elziane Tardif⁴, Bryony C. Bonning¹, James D. Ellis¹

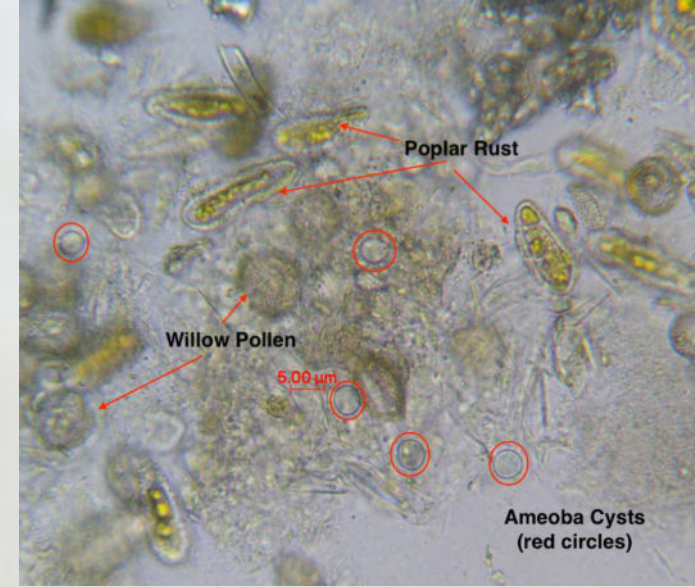
Affiliations: ¹ expand
PMID: 37313845 DOI: 10.1177/03009858231079956

Abstract

The amoeba *Malpighiella mellificae* is the etiologic agent of amoebic (amoeba) disease of Western honey bees (*Apis mellifera*). *M. mellificae* damages the Malpighian tubules, which is believed to weaken and kill the host bee. Here, the authors describe the detection of this organism in a honey bee colony in the Yukon Territory, Canada. The Malpighian tubules of 14% (7/50) of the adult worker bees were discolored dark brown. Fifteen bees were screened using conventional polymerase chain reaction for the 18S gene of *M. mellificae* were positive for the pathogen. Histologically, the lumens of Malpighian tubules were packed with amoebae, causing dilation of the tubules and attenuation and loss of the tubular epithelium. This phylogenetic analysis places *M. mellificae* in a new clade, a sister group to the Entamoebidae. This work provides a foundation for further investigation into the distribution, prevalence, and pathology associated with *M. mellificae* infection.

Keywords: *Apis mellifera*, *Malpighiella mellificae*, amoebic disease, honey bee.

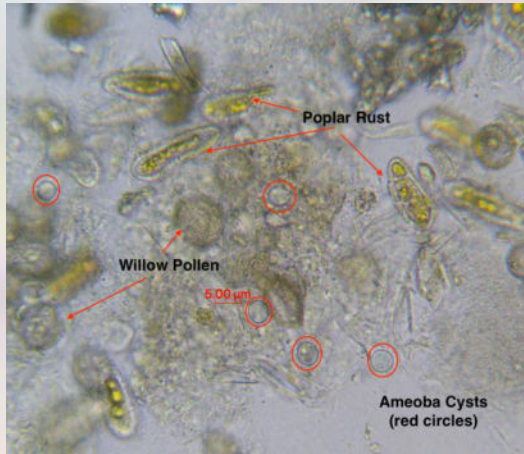




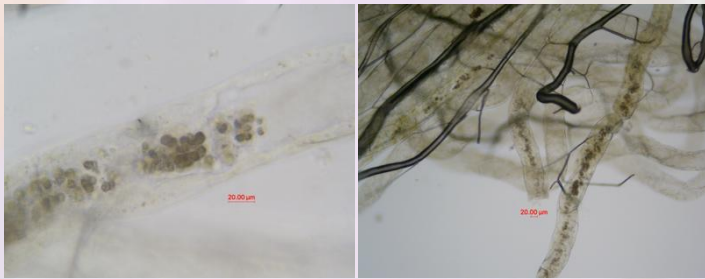
Interesting Findings

I bought my microscope in 2017 (My Christmas present)

Integrated Gut Overload Model in Yukon Honeybees



Jan 12, 2025
Amoeba cysts



Feb 4, 2025
Malpighian Tubule
Stones

Dec 30, 2024
Infected Malpighian
Tubule (Amoeba disease)



Winter Confinement
+ No Cleansing Flights

Intake of Pollen/Bee Bread
+ Indigestibles (e.g., Honeydew)

Gut Overload:
Full Midgut & Hindgut

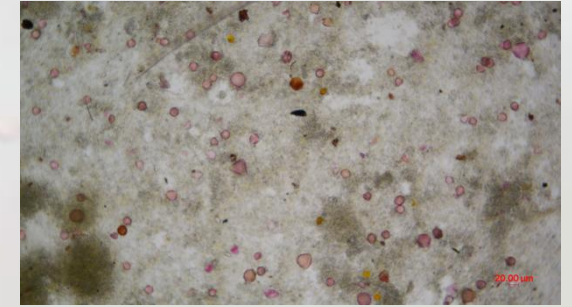
Malpighian Tubule Stones
→ Impaired Osmoregulation

Dysentery:
Premature Voiding Inside Hive

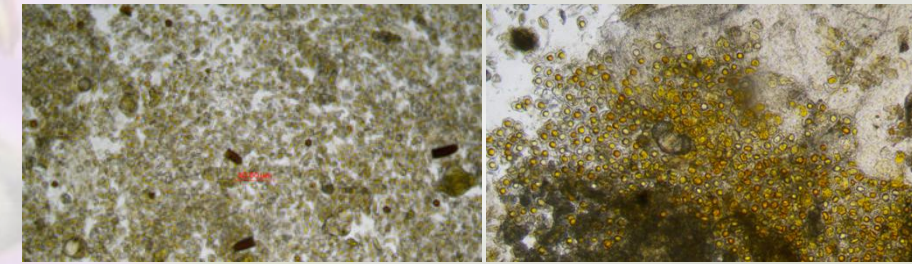
Fecal–Oral Transmission
of Nosema & Amoeba

Excessive Feeding in Infected Bees
+ Reinfection Loop

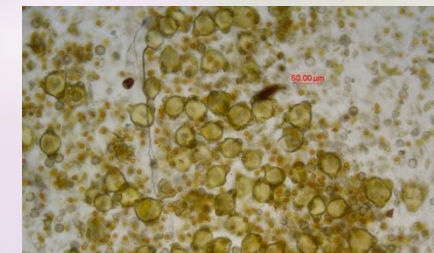
Escalating Gut Overload,
Tubule Damage & Colony Collapse



2024-25 Winter Honey (Honeydew)



Dec 05, 2024



Feb 4, 2025
Fireweed Pollen clumps
& Rust likely from Bee
Bread



Jan 12, 2025
feces in snow and x100 image

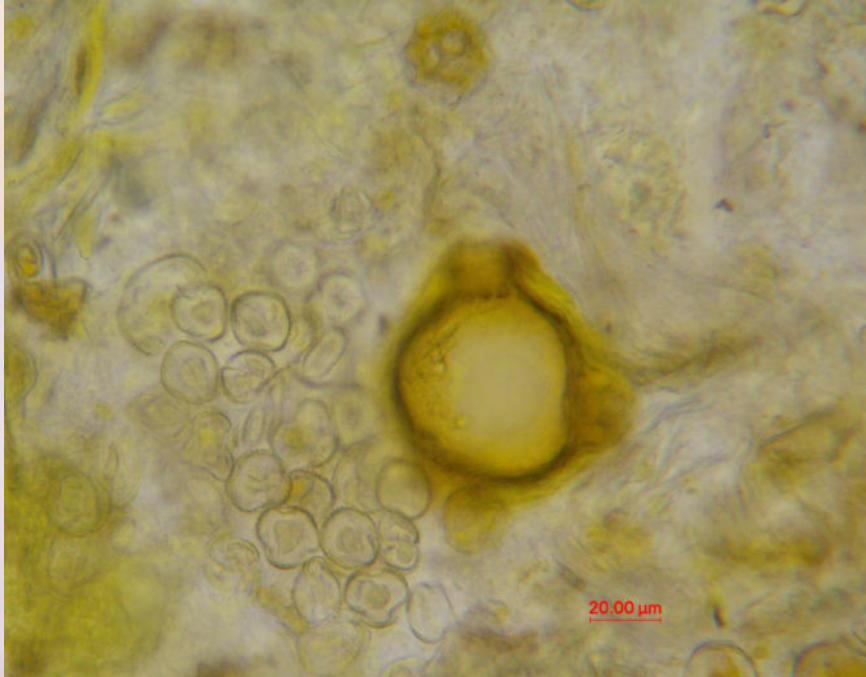
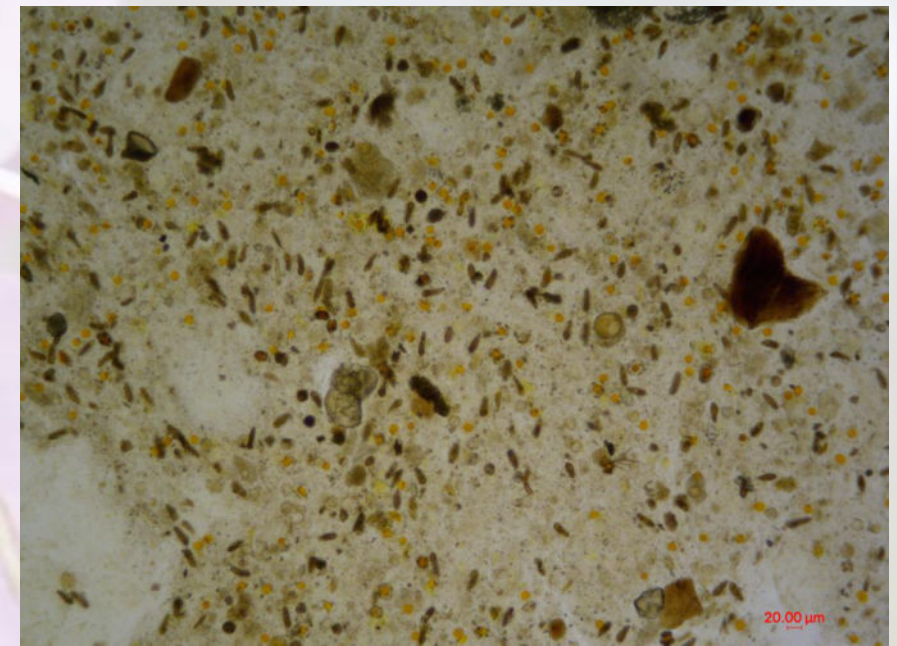
9 Findings

1. **Full Guts All Winter in Subarctic Bees (Even in good years)**
2. **Malpighian Tubule Stones (Uric Acid Concretions) as a Stress Marker**
3. **Honeydew Honey is Deadly in the Subarctic**
4. **Nosema Alone is Not the Killer**
5. **Amoeba Disease is Back — and Co-Infected with Nosema (Thrill of Discovery 😊)**
6. **Winter Consumption of Pollen/Stored Bee Bread is Widespread**
7. **Indigestibles and Ash Load Drive Gut Overload**
8. **Dysentery is Not Just a Symptom — It's a Disease Amplifier**
9. **Microscopy is an Accessible Citizen Science Tool**

1. Full Guts All Winter in Subarctic Bees

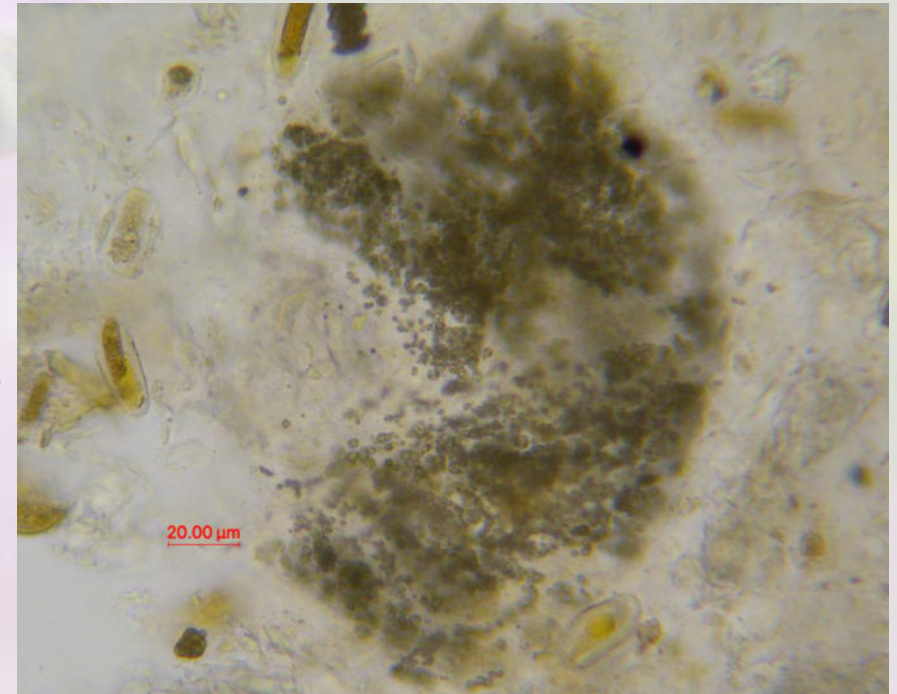
Unlike temperate colonies (which keep guts empty via cleansing flights), Yukon bees overwinter with **midgut and hindgut completely packed** with pollen, fungal spores, and indigestibles.

Challenge: Standard “empty gut winter bee” physiology models don’t apply north of 60°.



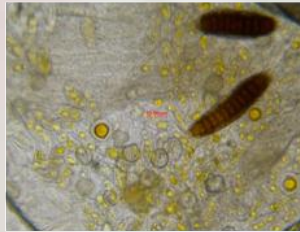
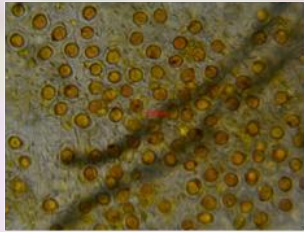
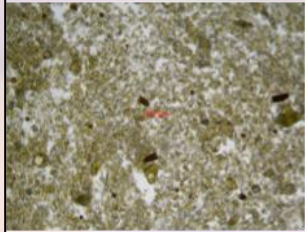
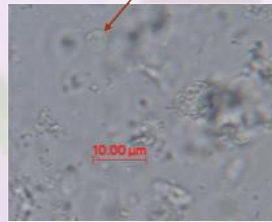
Fireweed
and Willow

Misc fungal
spores (spruce
cone rust and
algae)



Gut Sampling Approach – Monthly Collection

Bee Gut Sampling - Dec 5, 2024



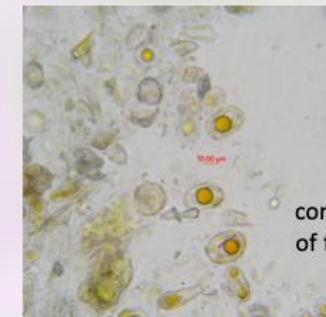
Bee Gut Honey Storytelling - Feb 25, 2024



Honeydew typically late
July/early August



High Nosema +
Low Amoeba



Lower
concentration
of fungal/rust
in feces

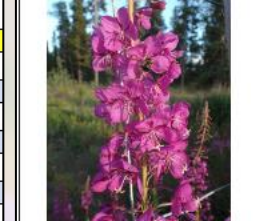


m. tubules stones
1st stones in Jan
samples

Bee Gut Honey Storytelling

October Guts

Mar-Apr Guts

	<table><tr><td>Grey</td><td>Pollen Load Colour</td></tr><tr><td>Non-Native</td><td>Common</td></tr><tr><td></td><td>Borago</td></tr><tr><td></td><td>Latin</td></tr><tr><td></td><td>Borago officinalis</td></tr><tr><td></td><td>Genera</td></tr><tr><td></td><td>Borago</td></tr><tr><td></td><td>Family</td></tr><tr><td></td><td>Boraginaceae</td></tr><tr><td></td><td>Usage</td></tr><tr><td></td><td>Nectar Pollen</td></tr><tr><td>um</td><td>Measurement</td></tr><tr><td>VP</td><td>30-32</td></tr><tr><td>VE</td><td>26-28</td></tr><tr><td></td><td>Dispersal</td></tr><tr><td></td><td>Monad</td></tr><tr><td></td><td>Aperture</td></tr><tr><td></td><td>9</td></tr><tr><td></td><td>Morph Type</td></tr><tr><td></td><td>Colpate</td></tr><tr><td></td><td>Pattern</td></tr><tr><td></td><td>Psilate</td></tr></table>	Grey	Pollen Load Colour	Non-Native	Common		Borago		Latin		Borago officinalis		Genera		Borago		Family		Boraginaceae		Usage		Nectar 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Comparative Gut Physiology in Wintering Honeybees

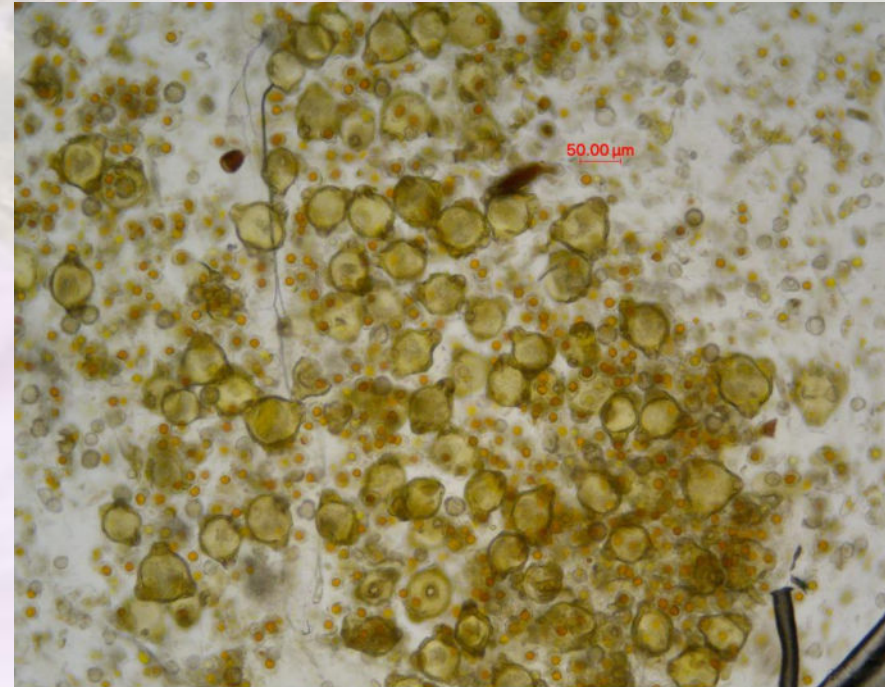
Temperate Bees (Kentucky)

- Honey-only diet (mainly carbs)
- Minimal pollen/protein intake
- Periodic cleansing flights → empty guts
- Disease pressure lower impact



Subarctic Bees (Yukon)

- Eat pollen all winter
- Guts fill with pollen, fungal spores, rust, indigestibles
- No cleansing flights for months
- Nosema & Amoeba worsen gut loading

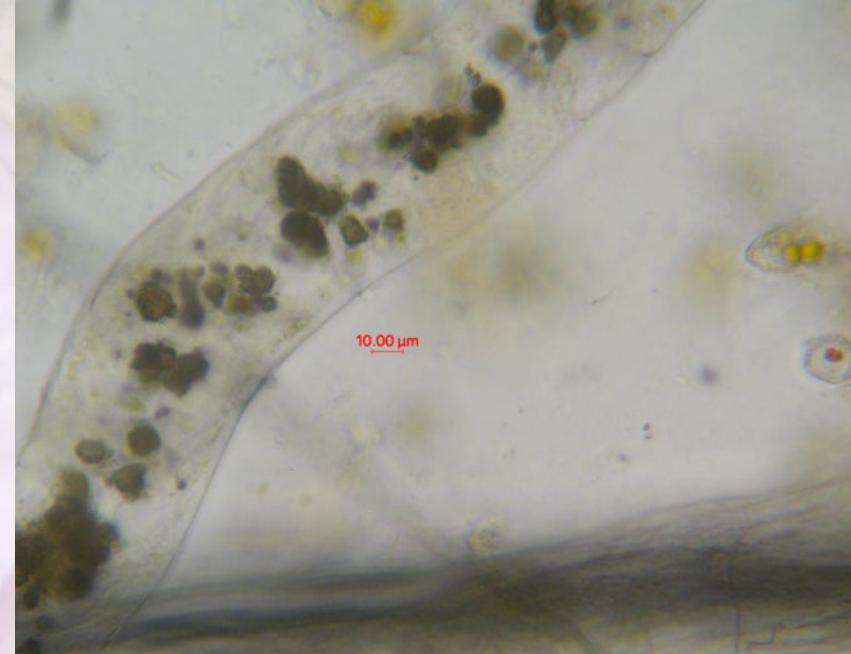


2. Malpighian Tubule Stones (Uric Acid Concretions) as a Stress Marker

Winter bees often develop “**kidney stones**” in Malpighian tubules.

These likely indicate renal overload and impaired osmoregulation — an important clue of wintering bee health when hive bound for 6-7 months. Amino Acids digestion generates the uric acid. **Lysine** (essential amino acid): $\text{C}_6\text{H}_{14}\text{N}_2\text{O}_2$

Challenge: Only way to observe is via gut dissection

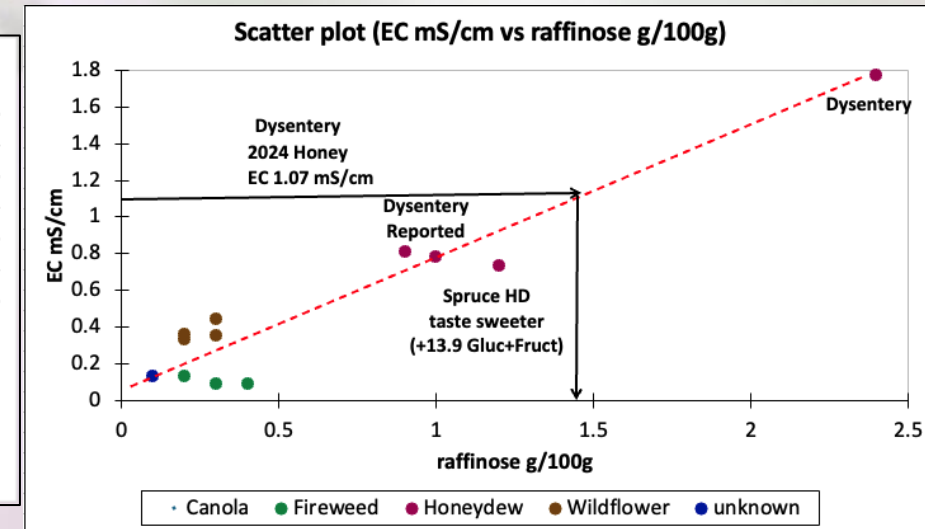
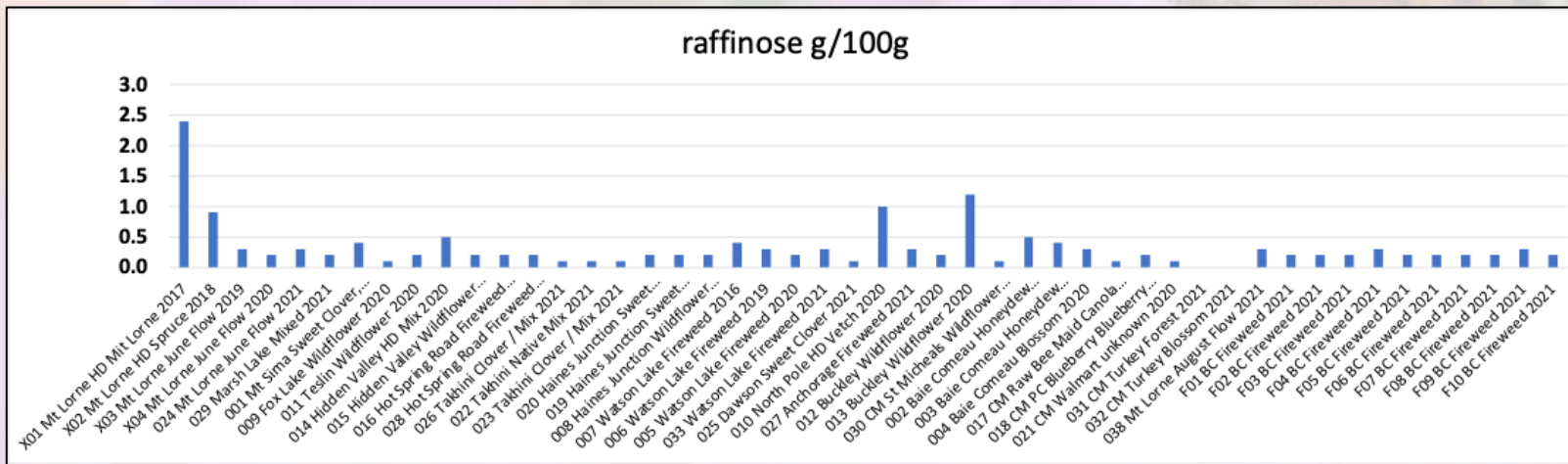


3. Honeydew Honey is Deadly in the Subarctic

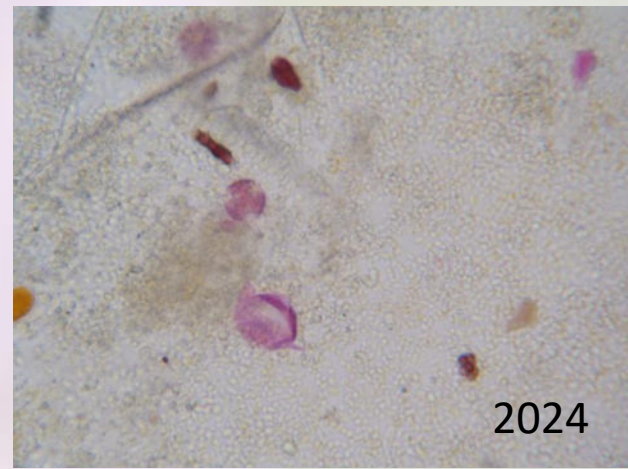
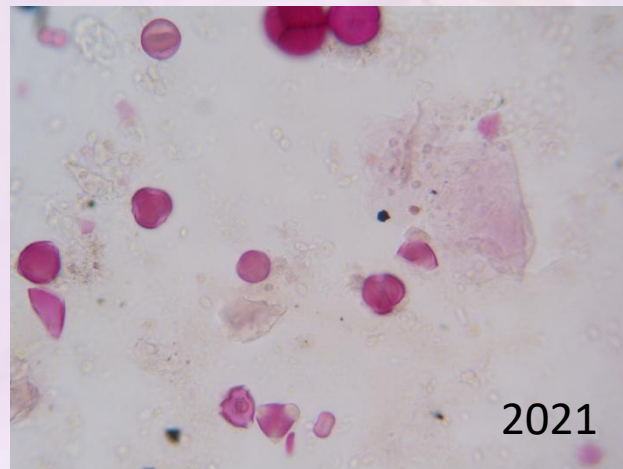
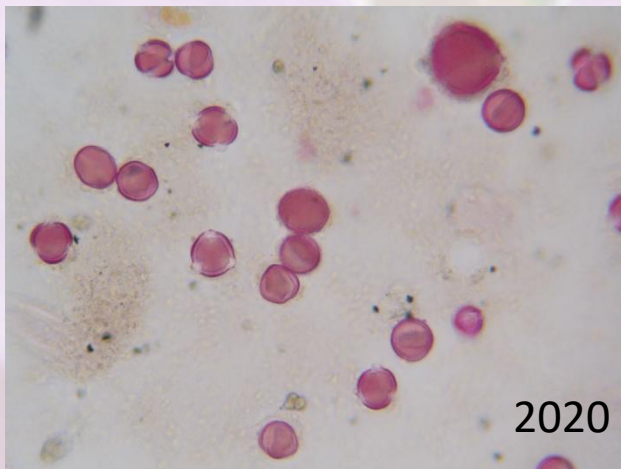
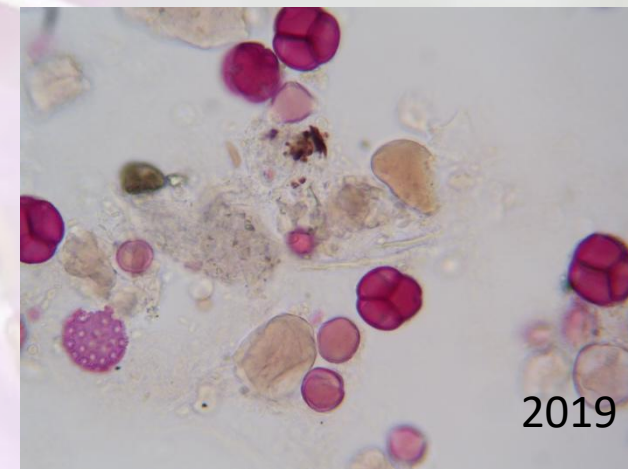
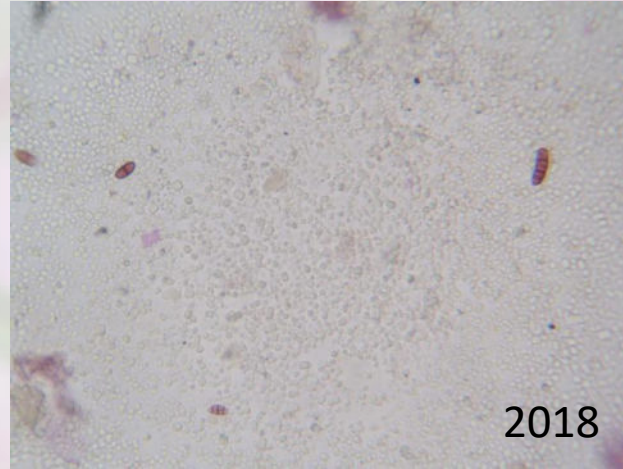
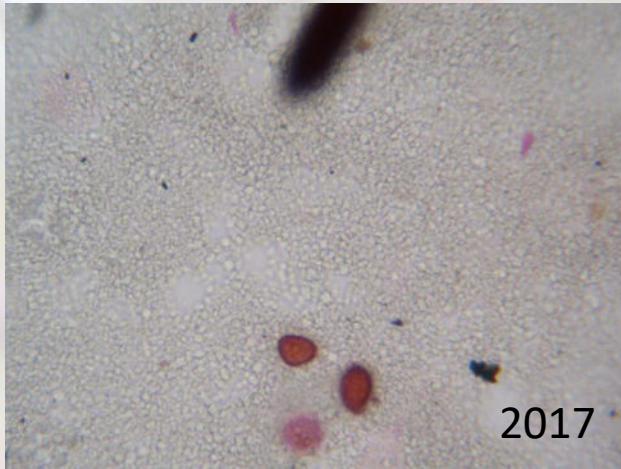
NMR data shows honeydew honeys with high ash and raffinose cause **severe dysentery** under long confinement.

In temperate climates, bees might survive; in Yukon, colonies collapse.

Challenge: The common wisdom that “honeydew is just another honey type” is context-dependent.



Honey Samples from same location (Mt Lorne YT)



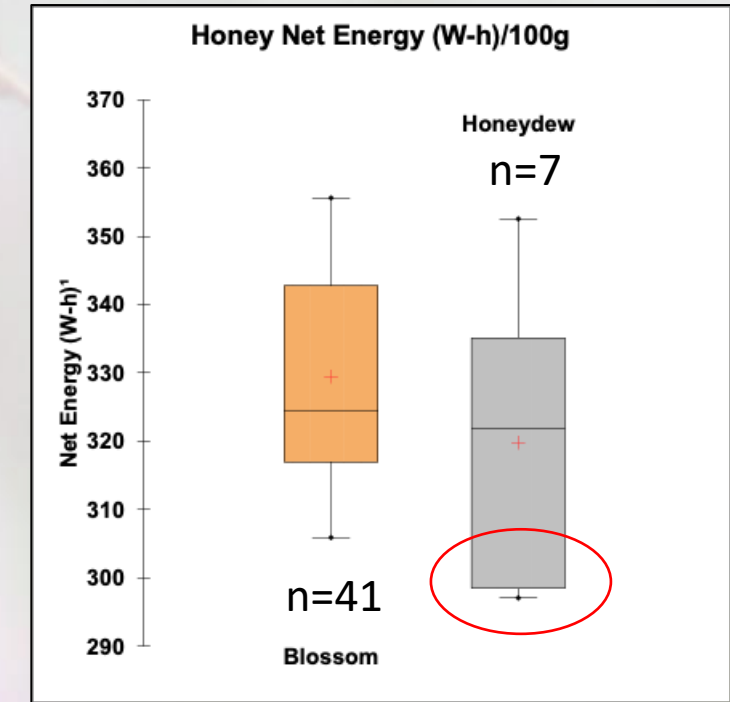
2017 to 2020 honey comparative

2017 Honeydew Honey (100g)

Component Description					
	Content g	Metaboliz Energy (W-h)	Metabolic Water (g)	Metabolic CO ₂ (g)	Nitrogenous Waste (Uric Acid) (g)
Monosaccharides					
Fructose	30.3	140.941	18.183	44.411	0.000
Glucose	20.3	94.461	12.182	29.754	0.000
Disaccharides					
Sucrose	4	16.741	2.316	6.172	0.000
Turanose	1.6	6.696	0.927	2.469	0.000
Maltose	6.1	25.530	3.533	9.412	0.000
Trisaccharides					
Maltotriose	2.6	10.882	1.486	4.083	0.000
Raffinose	2.4	0.000	0.000	0.000	0.000
Amino Acids					
Alanine	0.0009	0.004	0.001	0.001	0.0004
Proline	0.0406	0.189	0.028	0.078	0.0189
Organic Acids & Alcohols					
Citric Acid	0.071	0.206	0.027	0.098	0.000
Malic Acid	0.4634	1.291	0.158	0.609	0.000
Acetic Acid	0.005	0.020	0.003	0.007	0.000
Lactic Acid	0.0058	0.024	0.004	0.009	0.000
Formic Acid	0.014	0.019	0.006	0.013	0.000
Fumaric Acid	0.003	0.010	0.001	0.005	0.000
Pyruvic Acid	0.002	0.007	0.001	0.003	0.000
Succinic Acid	0.0614	0.215	0.031	0.092	0.000
Shikimic Acid	0.038	0.000	0.000	0.000	0.000
Ethanol	0.0011	0.009	0.001	0.002	0.000
Non-Energetic & Other					
Moisture Con	16	0.000	0.000	0.000	0.000
Totals	83.6462	297.546	38.785	97.033	0.019

2020 Blossom Honey (100g)

Component Description					
	Content g	Metaboliz Energy (W-h)	Metabolic Water (g)	Metabolic CO ₂ (g)	Nitrogenous Waste (Uric Acid) (g)
Monosaccharides					
Fructose	39.600	184.250	23.764	58.042	0.000
Glucose	31.000	144.221	18.603	45.437	0.000
Disaccharides					
Sucrose	0.500	2.093	0.290	0.772	0.000
Turanose	1.700	7.115	0.985	2.623	0.000
Maltose	2.600	10.882	1.506	4.012	0.000
Trisaccharides					
Raffinose	0.200	0.000	0.000	0.000	0.000
Amino Acids					
Alanine	0.001	0.004	0.001	0.001	0.0004
Proline	0.043	0.202	0.030	0.083	0.0201
Organic Acids & Alcohols					
Lactic Acid	0.001	0.006	0.001	0.002	0.000
Formic Acid	0.003	0.004	0.001	0.003	0.000
Pyruvic Acid	0.001	0.004	0.001	0.002	0.000
Succinic Acid	0.002	0.006	0.001	0.002	0.000
Ethanol	0.004	0.031	0.004	0.007	0.000
Non-Energetic & Other					
Moisture Con	16.500	0.000	0.000	0.000	0.000
Totals	92.155	348.826	45.185	110.984	0.021



**Lower energy per g
implies higher level of
indigestible
(Based on NMR results
and EC**)**

4. Nosema Alone is Not the Killer

Queens remain viable, but **infected nurse bees fail to rear brood** after winter.

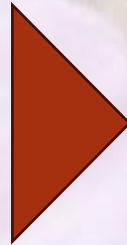
Collapse is due to *functional loss of winter nurses*, not queen failure.

Challenge: Shifts focus from “queen problems” to “winter nurse health.”

**Rapid
Spring
Decline**



April 1,
2019



May 9,
2019

5. Amoeba Disease is Back — and Co-Infects with Nosema

Malpighamoeba mellificae cysts are common in collapsing colonies; often co-present with Nosema.

Modern confirmation (Vet Pathology 2023) was based on my bees. 😊

Challenge: Amoeba disease was considered “rare or benign” — but in the north, it’s a major mortality driver.

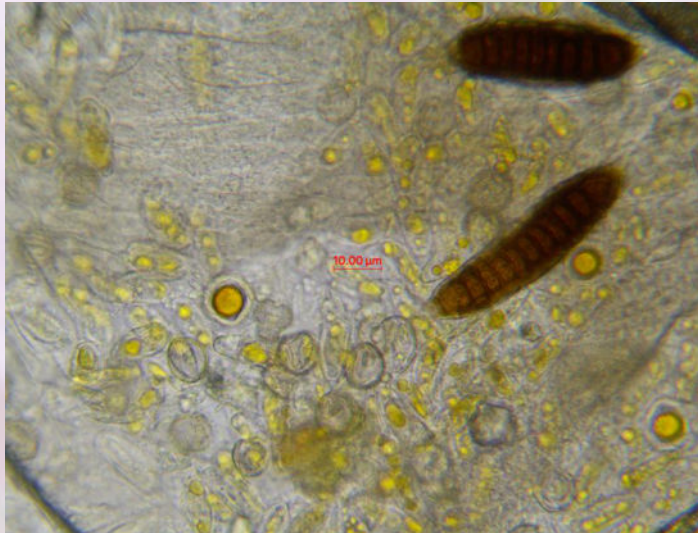
I have had a few more confirmation of the visual symptoms. “Puking bees”

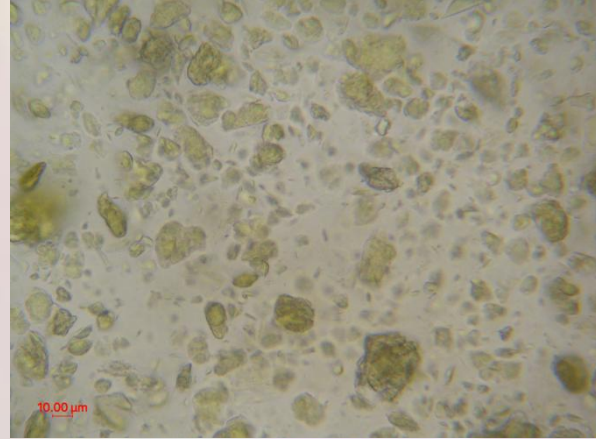
Visual Indication of Problem to Come



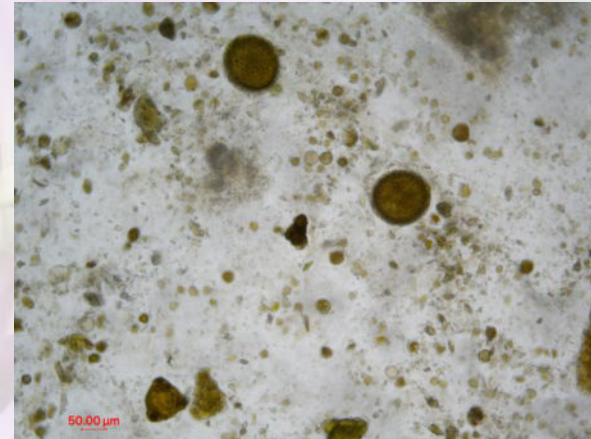
Learning to Dissect Honeybee Guts

Needed to confirm my diagnosis from cysts in feces





Pulverized stomach content
x100



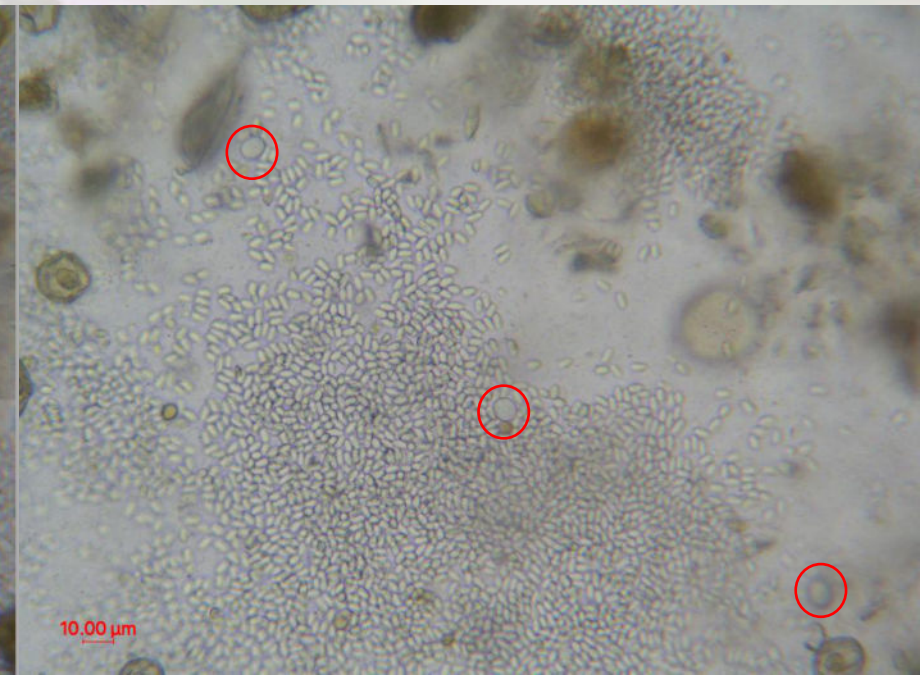
Undigested stomach content x400 (Mallow,
Fireweed, misc rust spores and other pollen
grains)

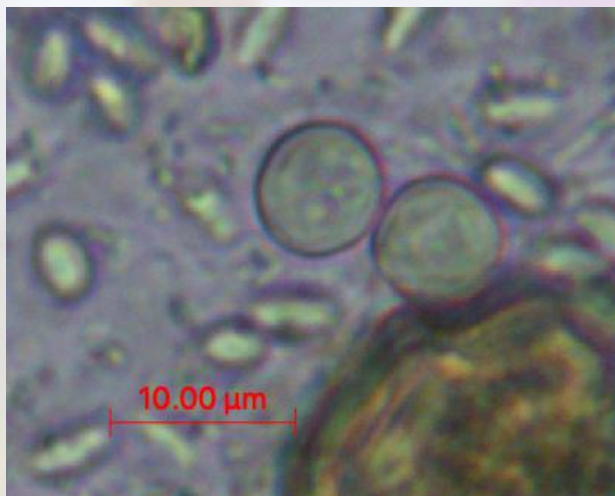
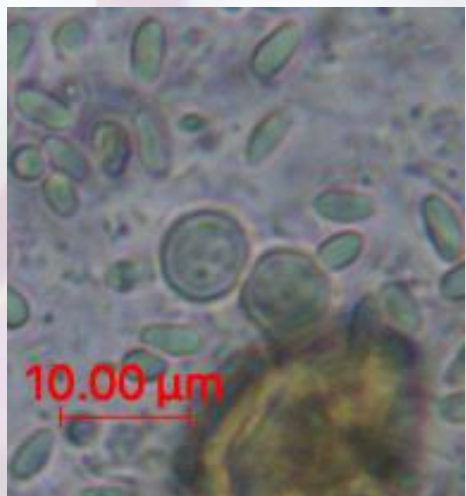


Waxy/glassy looking gut
content x400

All 10 randomly selected bees showed positive for moderate
to severe Nosema and amoeba disease 8-9um (red circles)

Sampled 10 random individual
bees, crushed abdomen onto a
slide Feb 6, 2022





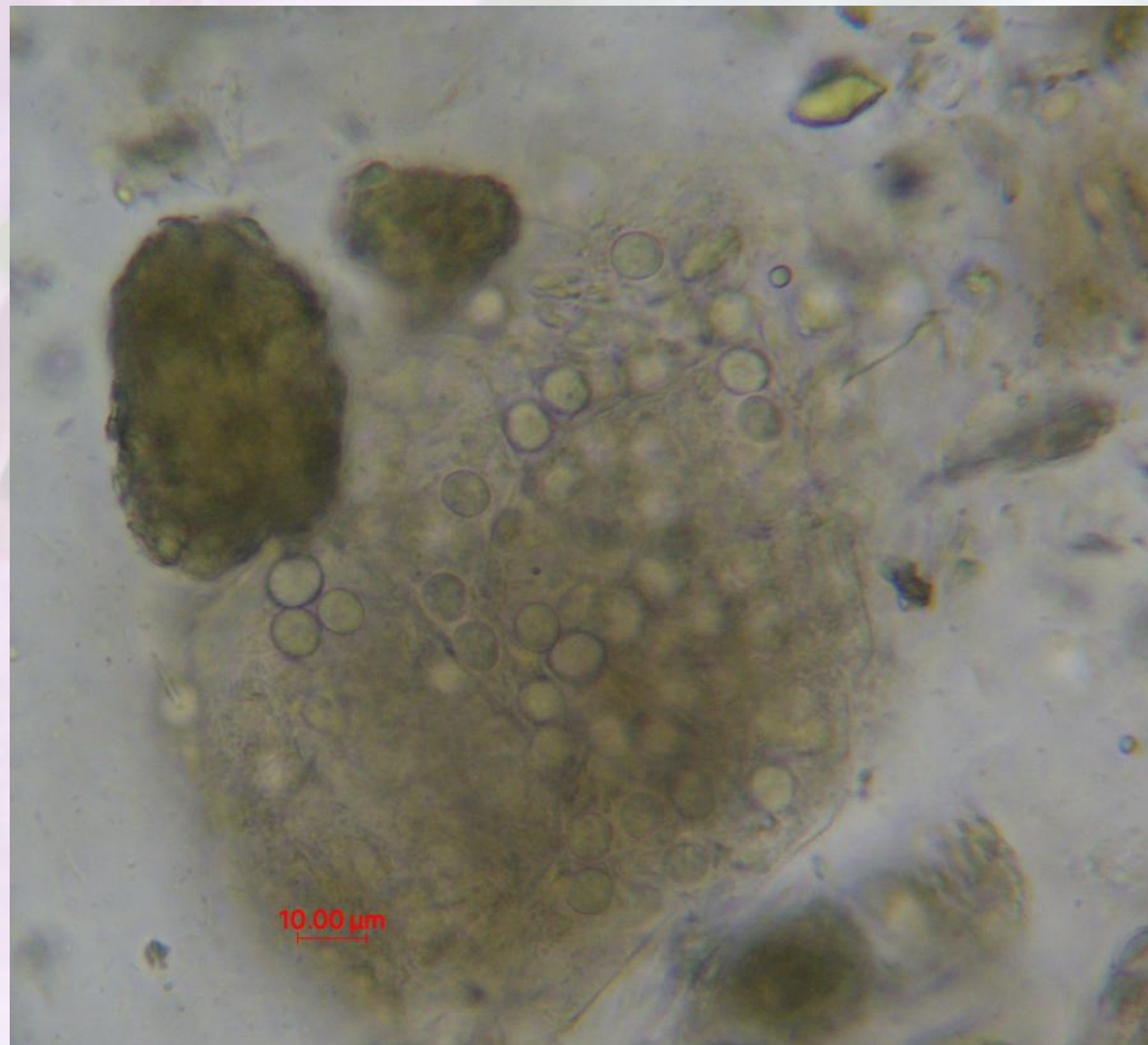
Are these amoeba cysts?

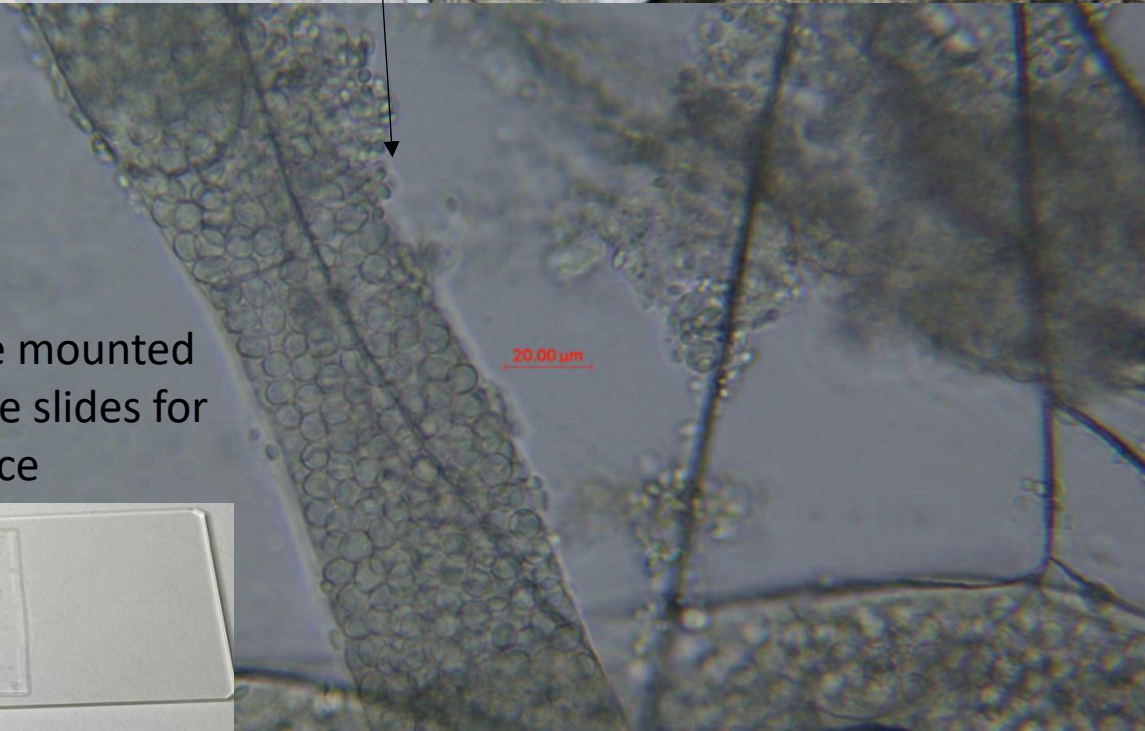
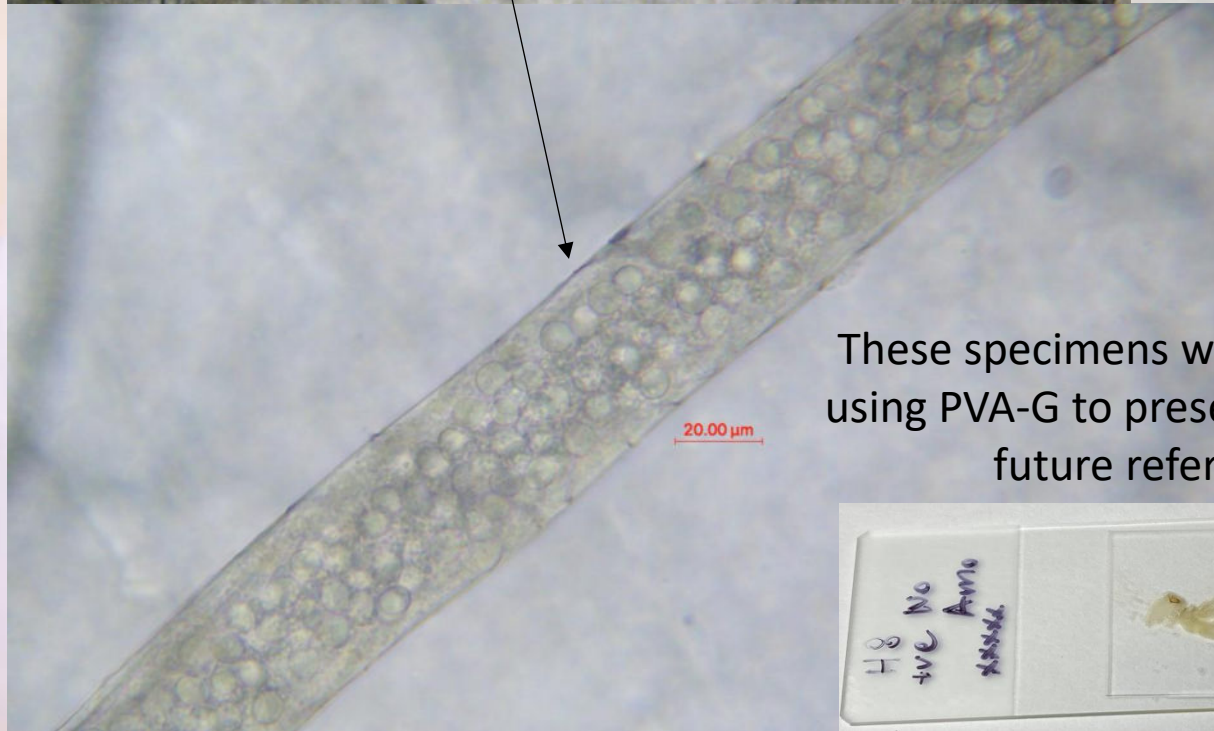
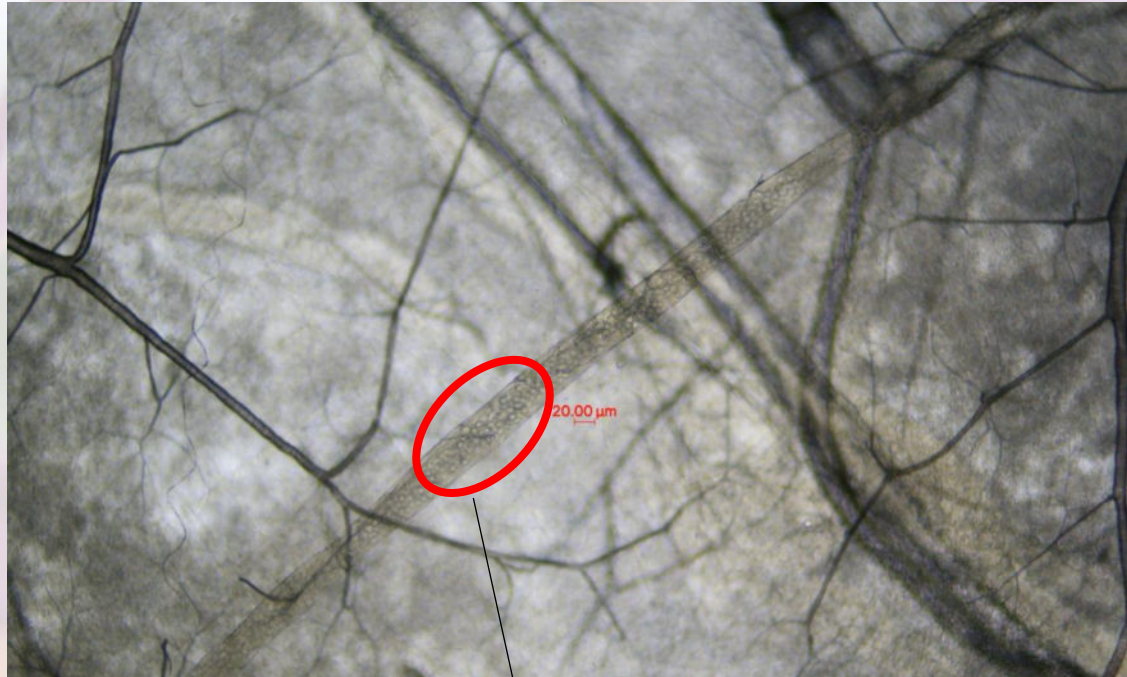
Sampled 10 random bees from colony with high individual bee mortality.

All had moderate to severe Nosema. All had these round shaped cysts 7-9µm in size.



Nosema spore
"grey zone
clumps" within
the fecal matter





These specimens were mounted
using PVA-G to preserve slides for
future reference

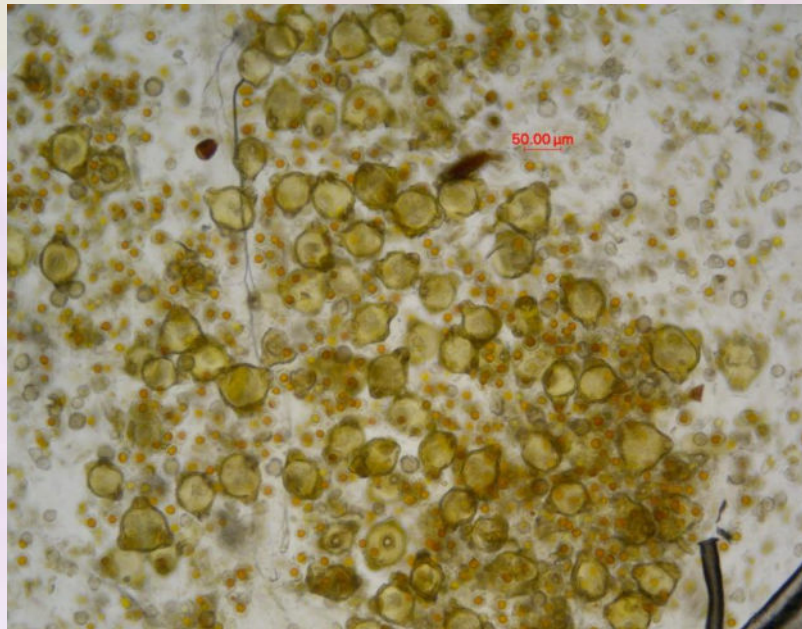


6. Winter Consumption of Pollen/Stored Bee Bread is Widespread

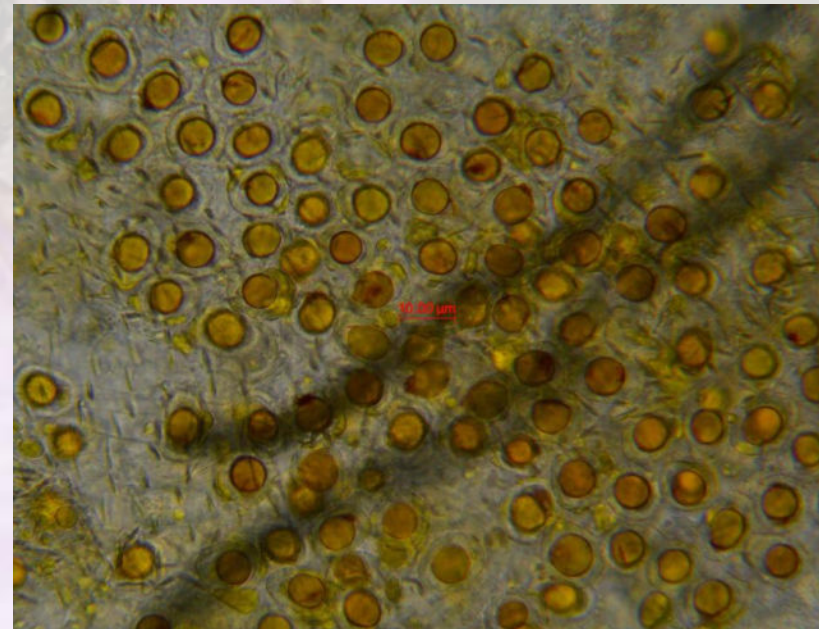
Microscopy shows **dense clumps of single-source pollens** (e.g., fireweed + rust), not just scattered grains in honey.

Indicates bees actively consume bee bread in mid-winter.

Challenge: Textbooks assume bees avoid protein digestion in winter.



Likely from Bee bread due to Fireweed pollen being very underrepresented in honey – “Wet” bee bread



Likely from rust collect “like” pollen during fall

7. Indigestibles and Ash Load Drive Gut Overload

Energy-to-indigestible ratios (from NMR work) show floral honeys provide >2× more usable energy than honeydew honeys per gram of waste processed.

Challenge: Not all calories are equal — winter survival is about “digestive efficiency,” not just energy density.

Ash content in honey is measured after burning off all organic matter. What remains is **inorganic mineral residue** (K, Ca, Mg, Na, Fe, etc.).

Algae, fungal spores, and plant debris found in honeydew honeys are organic → they are incinerated and do *not* directly contribute to the ash weight. Therefore, using Ash content to determine honey indigestibles likely under values.

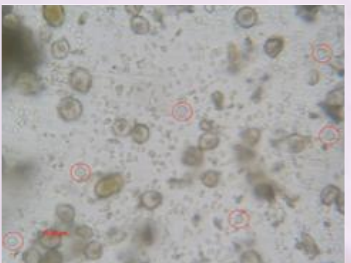
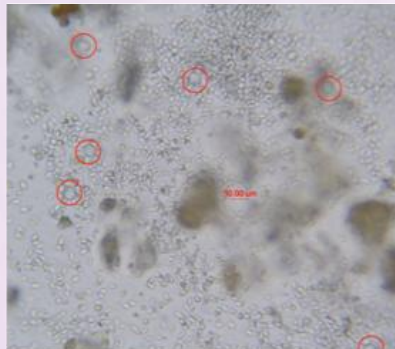
Their *mineral skeleton* contributes to ash content — but the organic bulk does not.

8. Dysentery is Not Just a Symptom — It's a Disease Amplifier

Once bees void in-hive, fecal–oral transmission explodes *Nosema* and *Amoeba* cycles.

Dysentery = **pathogen multiplier**, not just a messy by-product.

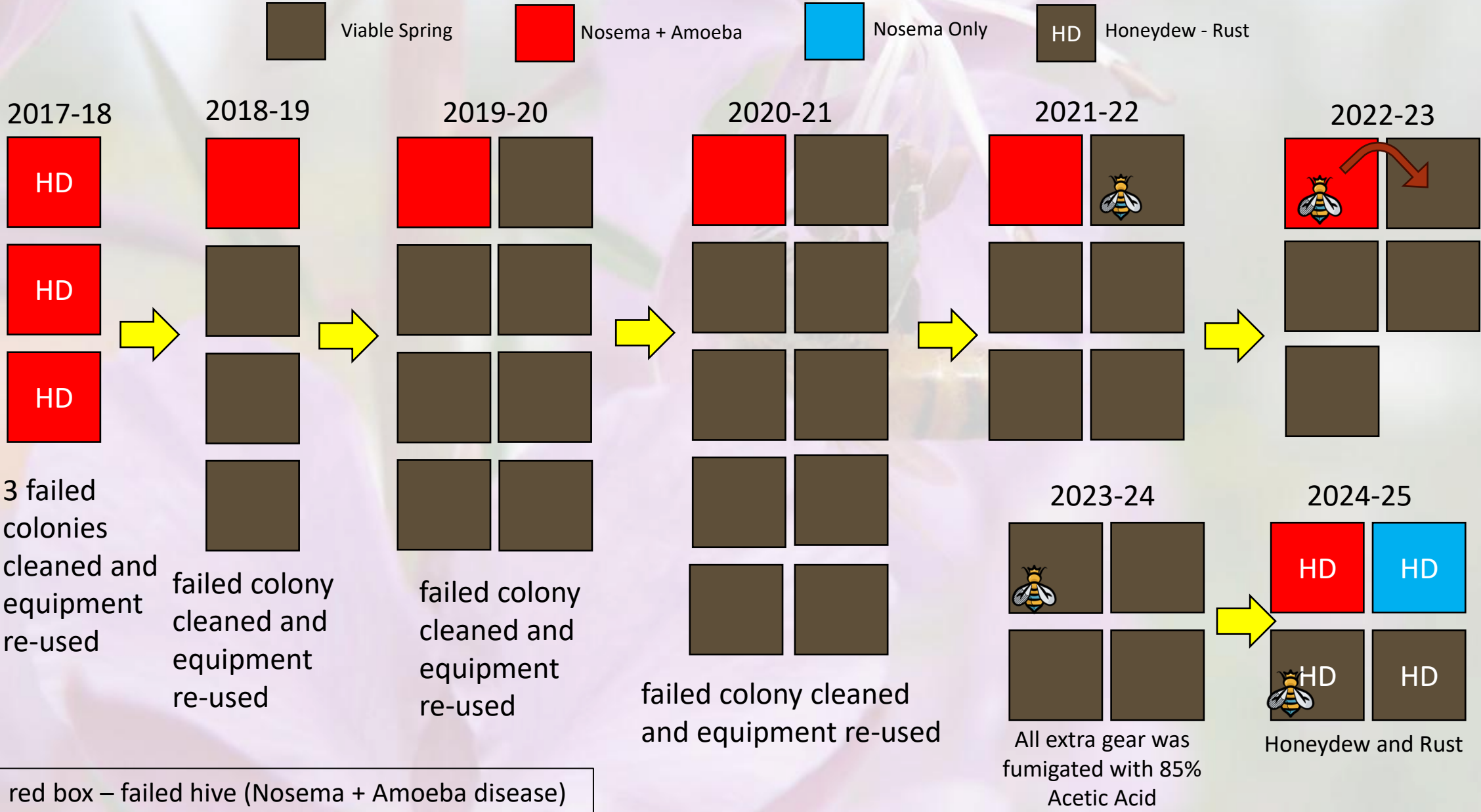
Challenge: Shifts dysentery from cosmetic to central pathology. Low honey consumption, clean winter feed and clean equipment with no residual disease load are needed.



Amoeba disease

Malpighamoeba mellificae Prell was studied at Rothamsted first by Hassanein (1952b), who confirmed that an annual epidemic, similar in character to that of *Nosema apis*, occurs in adult bees of endemically infected colonies. This annual epidemic appears to have the same explanation as that of *Nosema apis*; transference of infected colonies to non-contaminated combs in early summer eliminated infection, and transference of combs from infected to uninfected colonies in autumn introduced infection which became epidemic the following spring (Bailey, 1957d). Cysts of *M. mellificae* on combs were killed as readily as spores of *N. apis* by vapour of acetic acid, but fumagillin did not affect the development of *M. mellificae* in infected bees (Bailey, 1955d).

Hypothesis: I believe that the equipment is the source of the infection. Likely one frames containing a reservoir of Nosema Amoeba cysts. The missing link was likely an understanding of Amoeba disease. Initial infection was in 2017-18



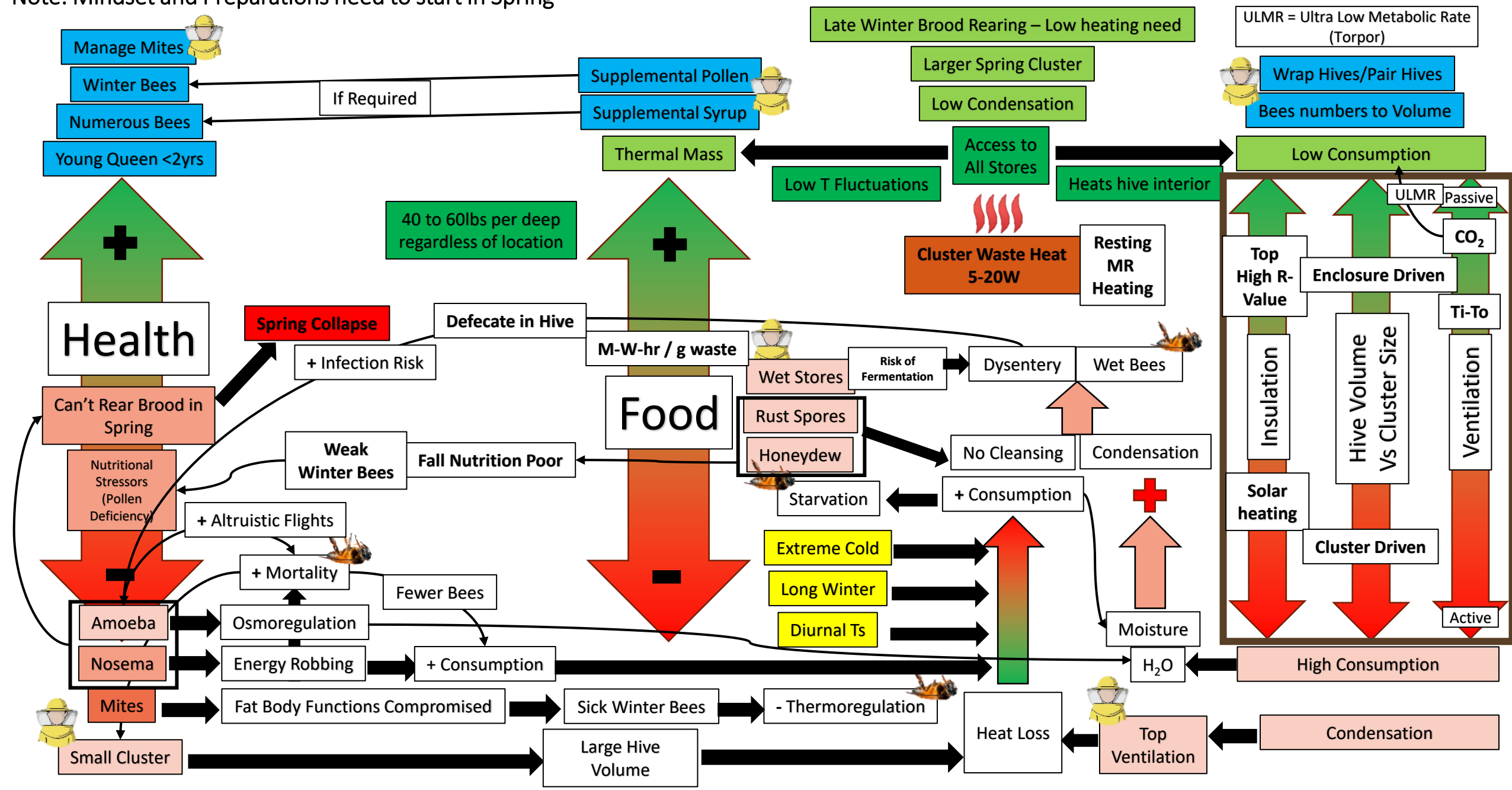
Best Practices & Recommendations

- Avoid wintering bees on honeydew honey; test stores via colour/taste/smell, pollen analysis and/or EC.
- Provide high-quality floral honey or sugar syrup and ensure stores are low in ash.
- Maintain insulated; using caution when re-using soiled equipment, sterilize frames and boxes to reduce transmission (WARNING – PPE – CAUTION).
- Use the 25-bee squeeze method or dissections to monitor Nosema and amoeba.
- Use individual x 10 bees to understand rate of infection

If your bees have **regular winter cleansing flights** the risk is low. However, **extreme honeydew and rust** can **overwhelm** the colonies capability to manage waste effectively.

Winter Survival Levers

Note: Mindset and Preparations need to start in Spring





By Etienne Tardif
yukonhoneybees@gmail.com
www.northof60beekeeping.com
<https://www.facebook.com/groups/yukonbees>



A close-up photograph of a bee on a pink flower. The bee is positioned in the center-right of the frame, facing left. It has a yellow and black striped abdomen and a fuzzy thorax. The flower is a light pink color with several petals visible. The background is a soft, out-of-focus green and yellow, suggesting a natural outdoor setting. The word "Extras" is written in a large, black, sans-serif font on the left side of the image, partially overlapping the flower petals.

Extras

Why bees consume pollen during long winters?

- Simplest is they **don't cleanse** so it is easy to observe.

Other possible reasons:

- **Maintain vitellogenin (Vg) & longevity:** Vg is a storage/antioxidant protein that buffers oxidative stress and underpins winter-bee lifespan
- **Keep hypopharyngeal glands “serviceable”:** so nurses can quickly produce brood food late winter.
- **Immune support under pathogen load:** pollen improves survival and immune enzyme activity; diversity helps under infection.
- **Tissue repair & enzyme turnover:** digestion/absorption of protein occurs in the midgut and requires dietary amino acids.
- **Essential sterols (can't synthesize):** Bees must obtain sterols from pollen for membranes and hormone precursors; some intake is needed year-round.

Rust Spores in the August Pollen

Recommendation: Start feeding pollen supplements to ensure continued brood rearing and healthy bees

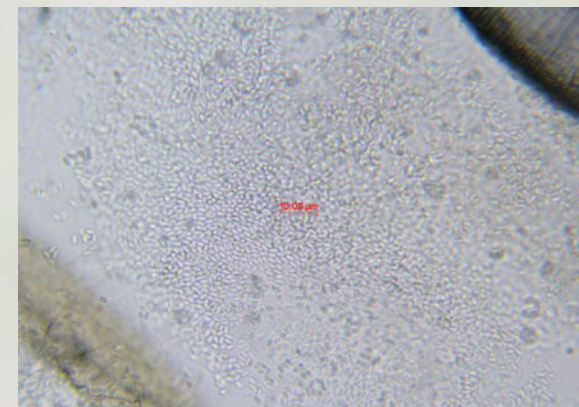
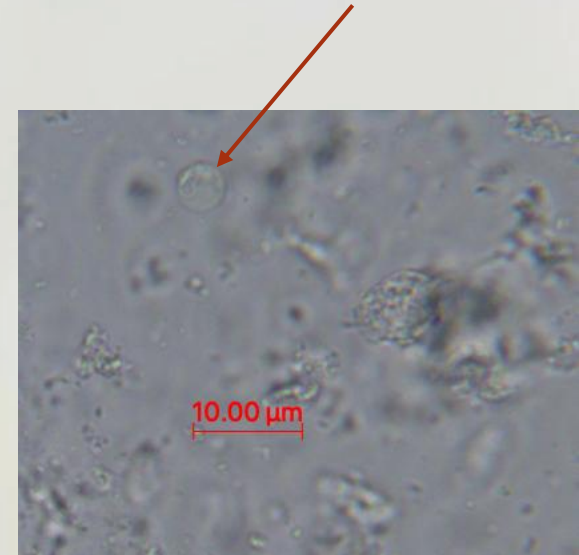
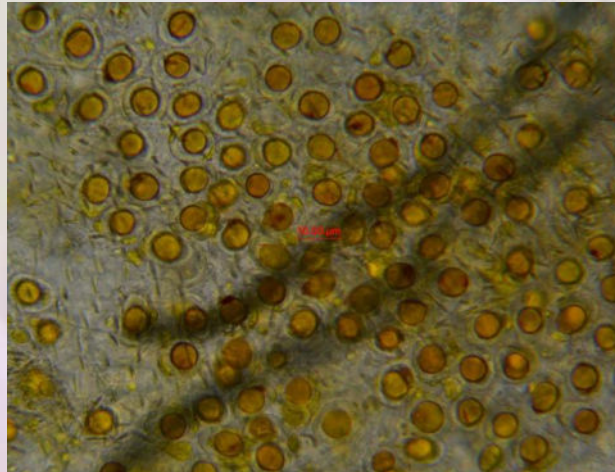
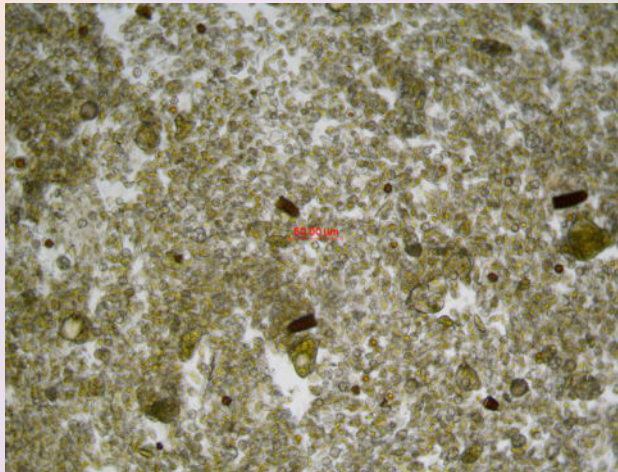
Wild Rose Rust
(Late July to Mid August)

Fireweed Rust
(Early to Mid August)



Figure: Photos on left - smaller, yellowish ones are A - urediniospores (summer spores), and the larger, longer, septate dark ones are teliospores (winter spores) of a rust that grows on members of the rose family called B - phragmidium - Center Photos C - *Pucciniastrum epilobii* (Fireweed Rust); Photo on right - Microscopic photo x400 mag of dead bee gut content showing severe D - *Nosema C* infection, E - phragmidium spores, smaller F - uredidiospores and a few pollen grains. Hive had severe dysentery and *Nosema C* infection

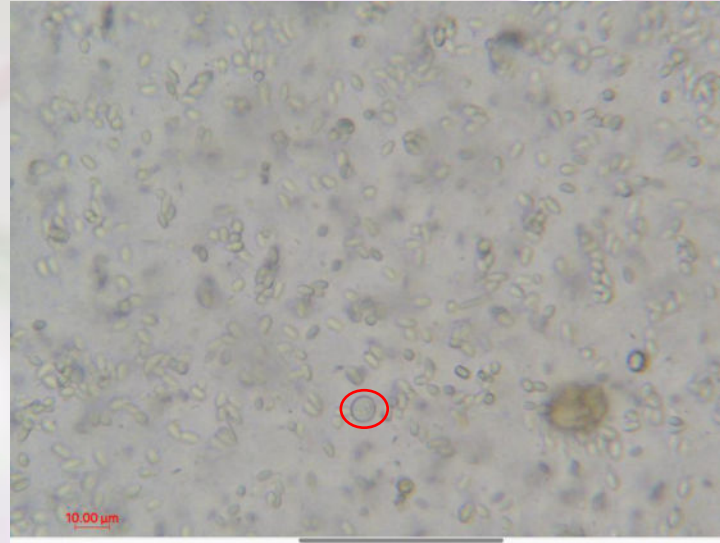
Bee Gut Sampling - Dec 5, 2024



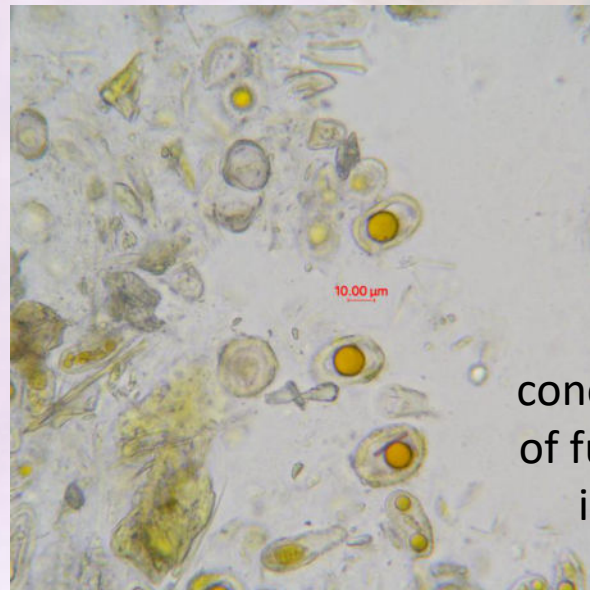
Bee Gut Honey Storytelling - Feb 25, 2024



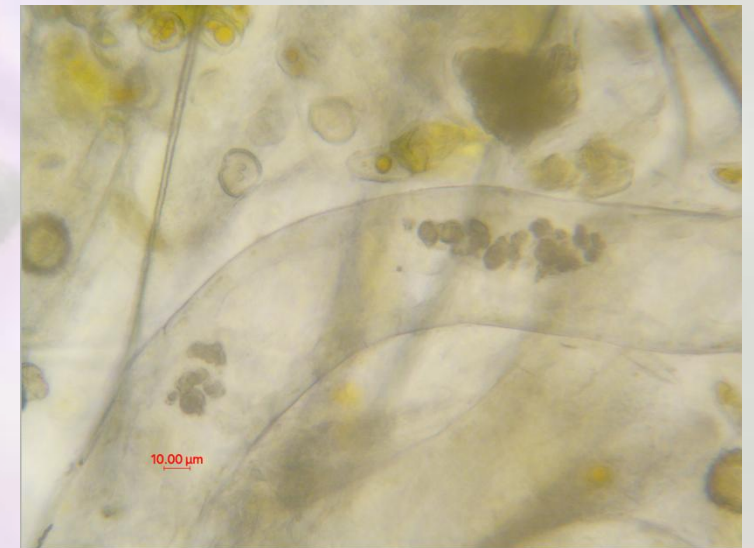
**Honeydew typically late
July/early August**



**High Nosema +
Low Amoeba**



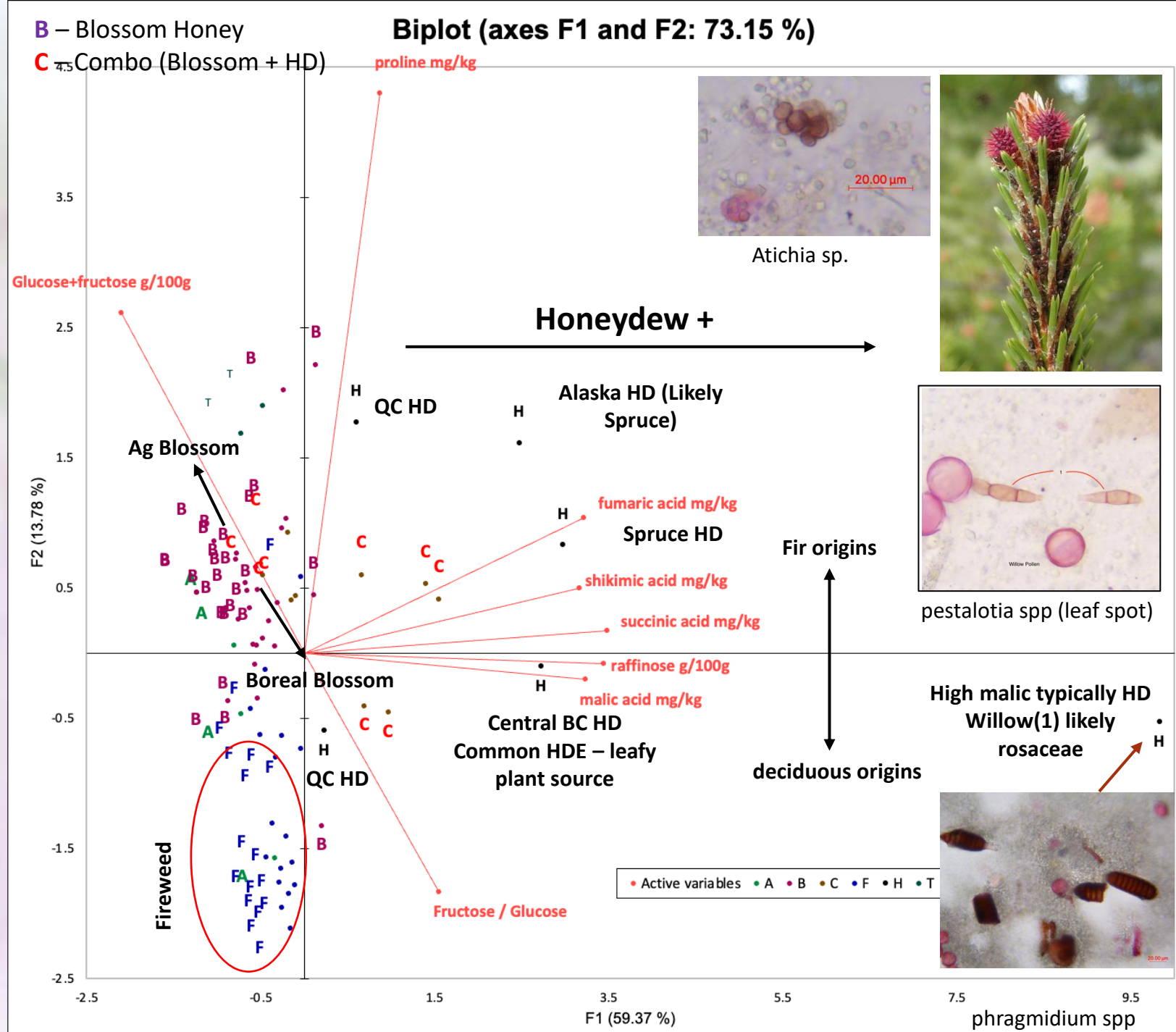
**Lower
concentration
of fungal/rust
in feces**



**m. tubules stones
1st stones in Jan
samples**

NMR Honey Stats

- **H** - Honeydew Indicators
 - High malic (Willow)
 - Higher raffinose
 - Shikimic and succinic acids
 - High HDE/Pollen (P.A.) + Fungal spore type
 - Colour, smell and flavour (Sensory)
- **F** - Fireweed
 - **Low Proline (under ripe)**
 - Variable Leucine
 - Trigger NMR on low Proline
- Boreal Vs Ag Honey
 - Higher G/F
 - Lower G+F
 - Honeydew component
 - HDE – Algae and misc fungal spores
- Dark Honey
 - higher phenolic content
 - HMF / Processing / Heat
- **A** - Sucrose Syrup Adulterated Honey
 - 10% to 25% Sucrose Syrup falls in middle of fireweed honey cluster (only hard NMR fail)
 - 1% to 5% falls in blossom honeys



Can Bees Digest Algae in Honeydew?

- Bees have a **limited enzymatic toolkit**: invertase, amylase, glucose oxidase — all focused on breaking down sugars.
- They cannot digest **complex algal cell walls** (cellulose, chitin, silica, etc.).
- In honeydew honeys, algae appear as **intact microscopic elements** in sediment (e.g., Fumago, Cladosporium, green algae).
- These function much like **pollen husks**: indigestible, pass into the hindgut.
- Instead, algae (and fungal spores) increase the **indigestible load** → promoting gut filling and dysentery risk during confinement.

