Nosema treatment options

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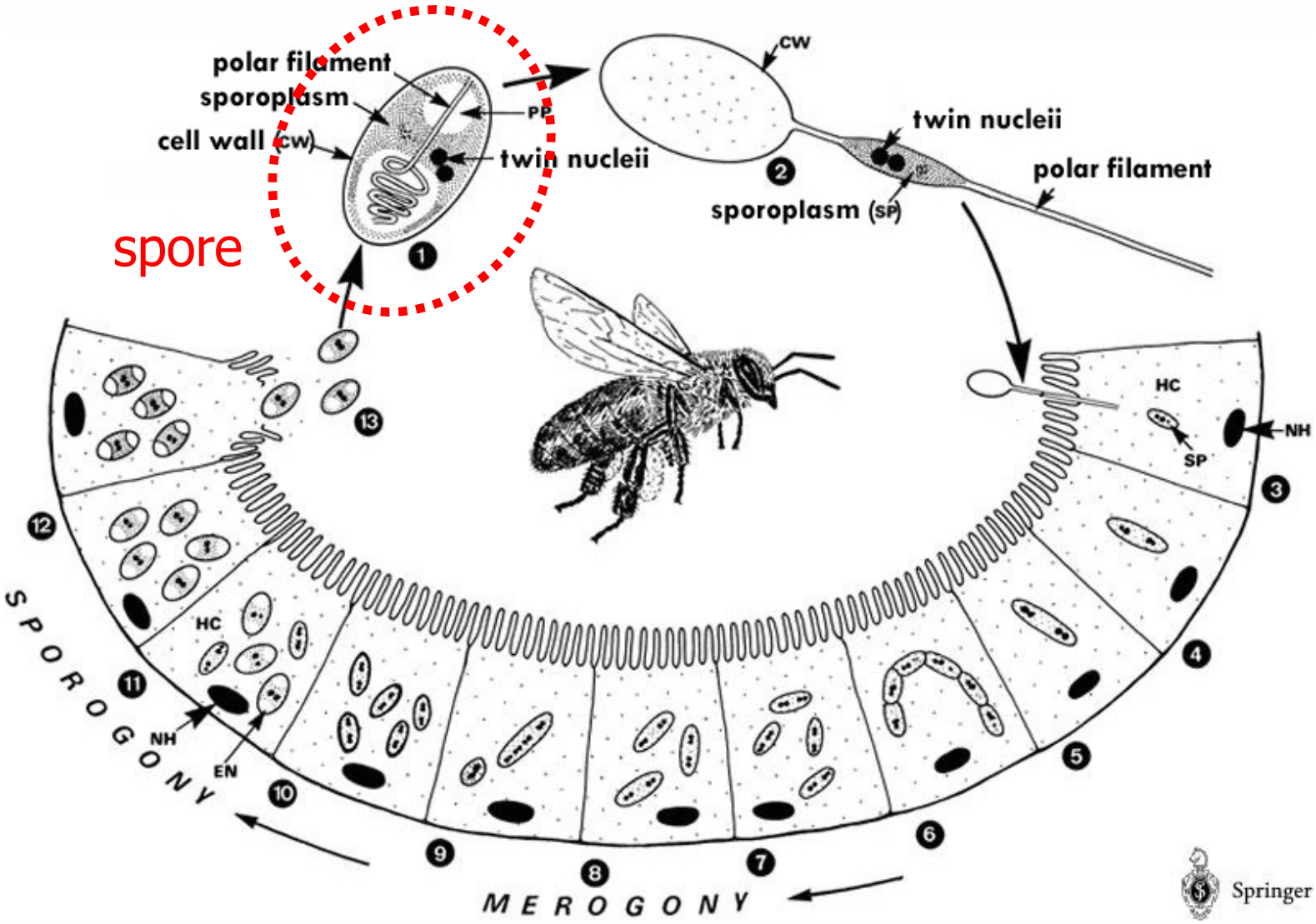
What is *Nosema*?

- A Microsporidium (fungus-like organism)
- Reclassified in 1990s from a protozoan (animal) to a fungus
- A spore producing parasite of bees
- Can only be identified with a microscope

Nosema life cycle (~10-13 days)

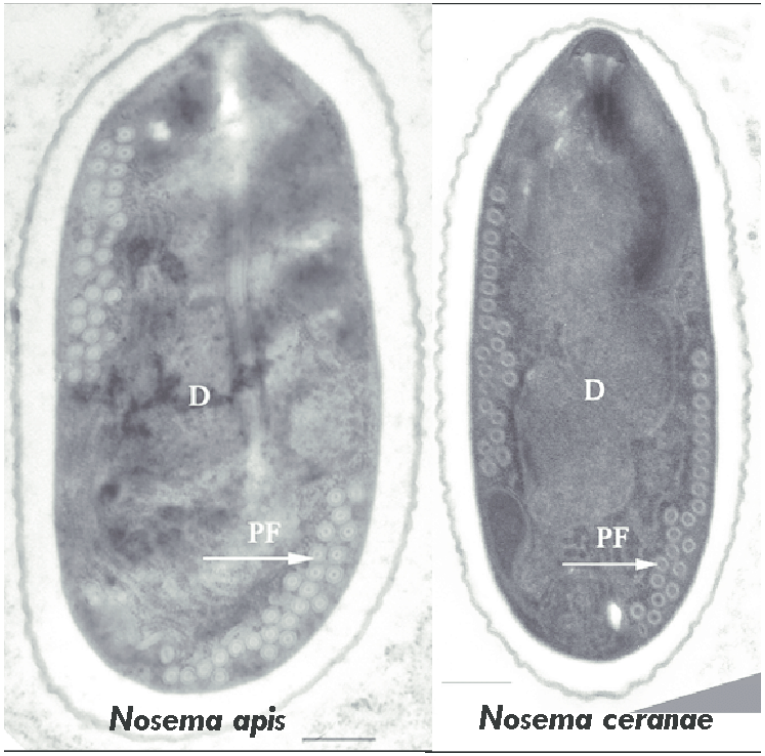


Mehlhorn H. (2015) Nosema apis. In: Mehlhorn H. (eds) Encyclopedia of Parasitology. Springer, Berlin, Heidelberg

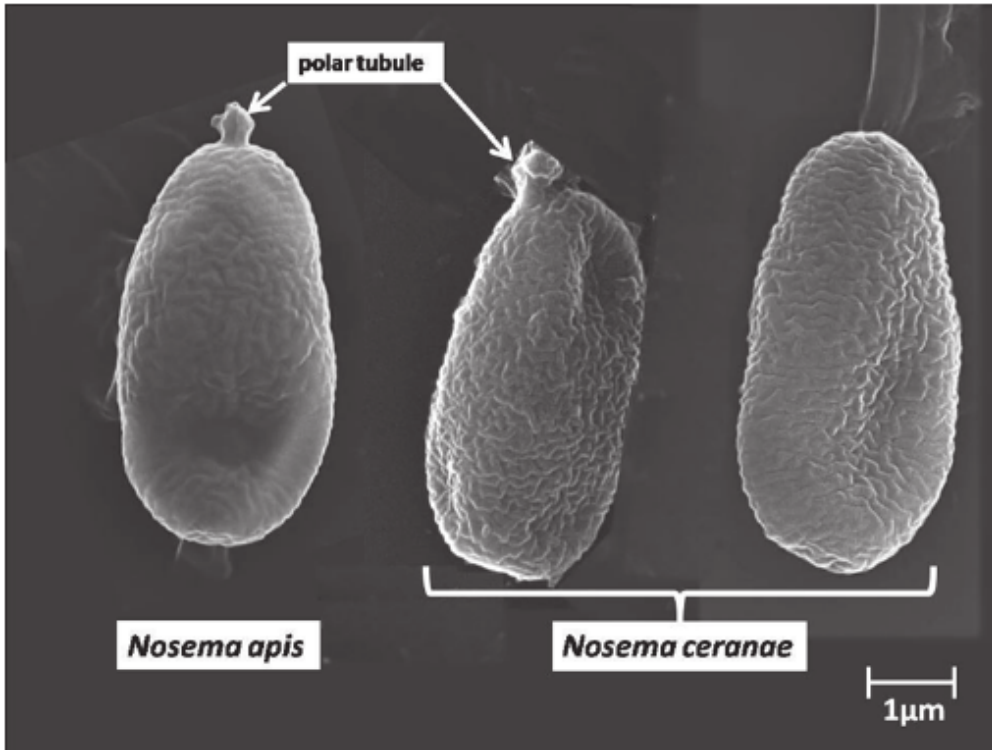
Species of *Nosema*

- ***Nosema apis*** – infects *Apis mellifera*, the European honeybee. Known to cause a potentially fatal disease condition, **nosemosis**
- ***Nosema ceranae*** – previously thought to only infect *Apis ceranae*, the Asian honeybee, was discovered infecting *Apis mellifera* in Taiwan, and in Spain (2006). Now widespread in North America
- ***Nosema bombus*** – infects *Apis bombus*, bumblebees and has been shown to be spread by wasps. Infects and kills cells throughout the bumblebee
- ***Nosema vespula*** – infects various members of the yellowjacket wasp family
- Numerous other species of *Nosema*

Species of *Nosema*

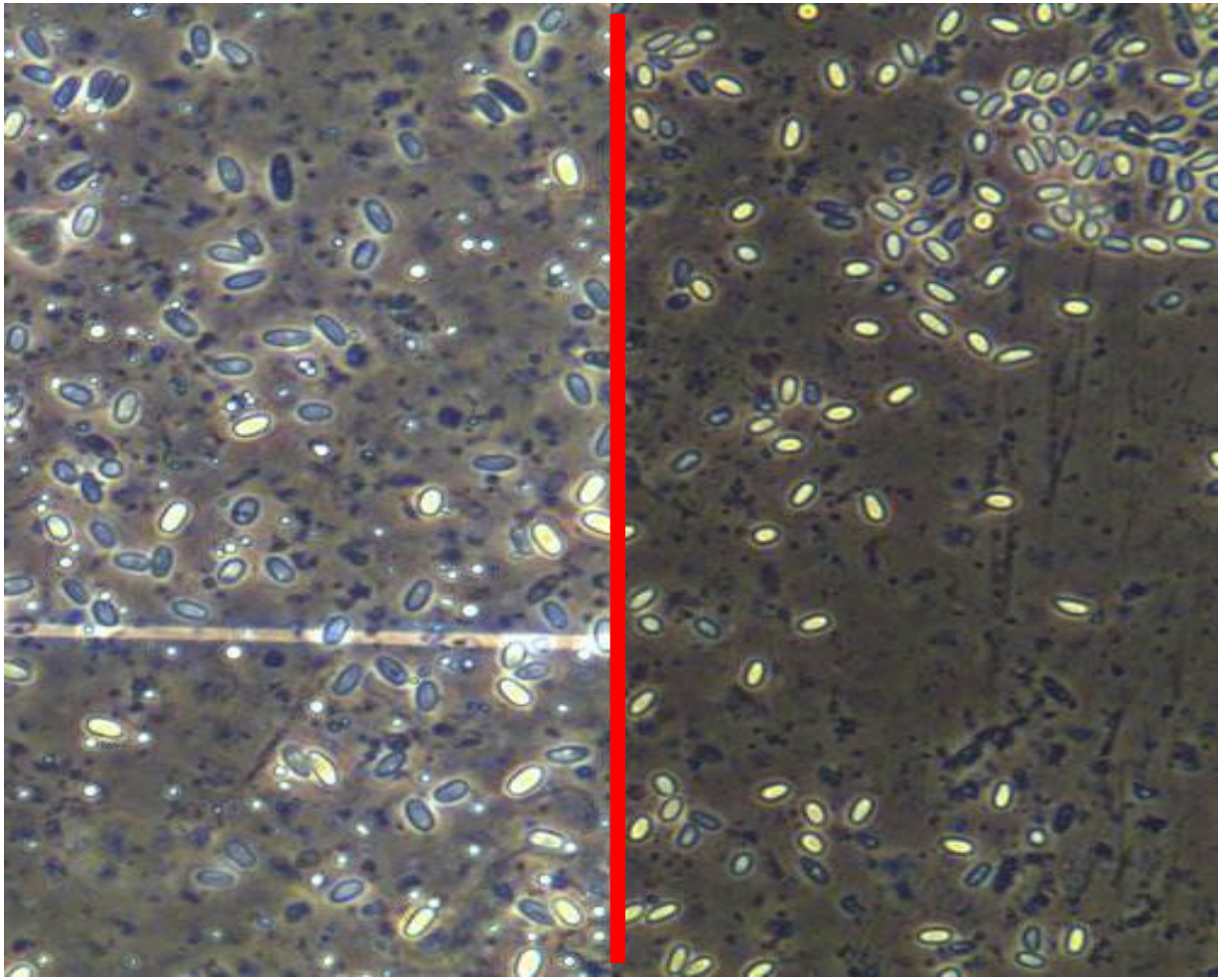


Transmission electron micrograph of *Nosema apis* and *Nosema ceranae* (Paxton, 2010)



Scanning electron micrograph of *Nosema apis* and *Nosema ceranae* (Ptaszyńska et al., 2012)

Species of *Nosema*



Spores of *Nosema apis* (left) and *Nosema ceranae* (right) (magnified 400x) using a compound microscope. *N. ceranae* is about 20% smaller and more almond shaped. (Zachary Huang)

Differences between *Nosema apis* and *Nosema ceranae*

	Nosema apis	*Nosema ceranae*
Spore size	6 x 3 µm	4.4 x 2.2 µm
Spore shape	round ends, symmetrical	sharp ends, less symmetrical
Polar filament coils	more than 30	18-21
Resistance to heat	low	high
Resistance to freezing	high	low
Seasonality	late winter/spring	none
Faeces in/on hive	heavy	slight to none
Colour of mid gut	milky	normal
Homing ability	probably reduced	reduced
Immuno-supression	no	pronounced

Huang

Nosema ceranae haplotypes

- *N. ceranae* has several haplotypes (strains) of varying virulence (Oliver)

- *N. ceranae* is a new pathogen and has more levels of virulence than *N. apis* (Xulio)

Relationships between *Nosema* species

- *N. ceranae* is more closely related to *N. vespula* than it is to *N. apis* (Chen 2009) and *N. bombus* (Shafer, 2009)

- Previously thought that *N. apis* and *N. ceranae* were similar enough to be treated with same methods.

  Study required to test this hypothesis

Nosemosis

- Caused by *Nosema apis*
- Symptom is extensive fecal streaking on hive exterior and interior frames.

Nosemosis

- Can cause early queen supercedure
- Made worse by cold and wet climate
- Usually appears in spring
- Spread by ingesting infected faeces
- Usually controlled by two doses of Fumagilin B (Fumadil B) in the fall

Nosema ceranae effect

- Caused by *Nosema ceranae*
- Symptom is slow build up of colony, poor production and eventual colony collapse
- Made worse by excessive heat
- Spread by normal food exchange
- Queen infected just prior to colony collapse
- **May show no obvious symptoms**

Why the concern?

- Both *Nosema apis* and *Nosema ceranae* have negative effects on our European honeybees
- There is reduced digestive activity in young bees infected with *Nosema apis*. (Molonea,1998) and *Nosema ceranae* (Higes, 2007) resulting in starvation even during a honey flow
- We are losing popular treatment (Fumagilin B)

Interaction with pesticides

- Production of glucose oxidase, a natural enzyme bees use to sterilize colony and brood food, is significantly decreased in the presence of pesticides ***and*** *Nosema*.
- Synergy between the two factors is suspected

What are treatment options?

Treatments

Fumadil B (Fumagilin B) – Antibiotic used with goal of decreasing *Nosema* spore counts. Banned in some countries and sole supplier, Medivet Pharm. Ltd, recently discontinued production.

Nozevit – "Natural herbal product" from Croatia made with bark extract, rich in plant polyphenols (likely tannins), essential oils, vitamins and citric acid. Claims by various sources that *Nosema* can be treated effectively.

Honey B Healthy – Feeding stimulant made with lemongrass and spearmint essential oils. No claims of efficacy against *Nosema*.

Our study of treatment options

Initial *Nosema* spore counts were done for colonies at three locations: Emma's Acres (Mission), Campbell's Gold apiary (Abbotsford), Kwantlen Polytechnic University (Langley)

Colonies were divided into control and treatment groups so that high (1M+), moderate (500k up to 1M) and low (under 500k) spore counts were included in each

Treatments were applied to bees by drench method, as per directions for product use

High-tech dosing device

Our study of treatment options

Doses:

Fumidil	20mL (4 tsp)/L (1:1 sugar syrup)
Nozevit	5mL (1 tsp)/L (1:1 sugar syrup)
HBH	5mL (1 tsp)/L (1:1 sugar syrup)

250mL (1 cup or 8oz.) applied per hive

Treatments were applied on the following dates:

treat 1 Sep 23
treat 2 Sep 26
treat 3 Oct 3
treat 4 Oct 7

Final *Nosema* spore counts were conducted Oct 14

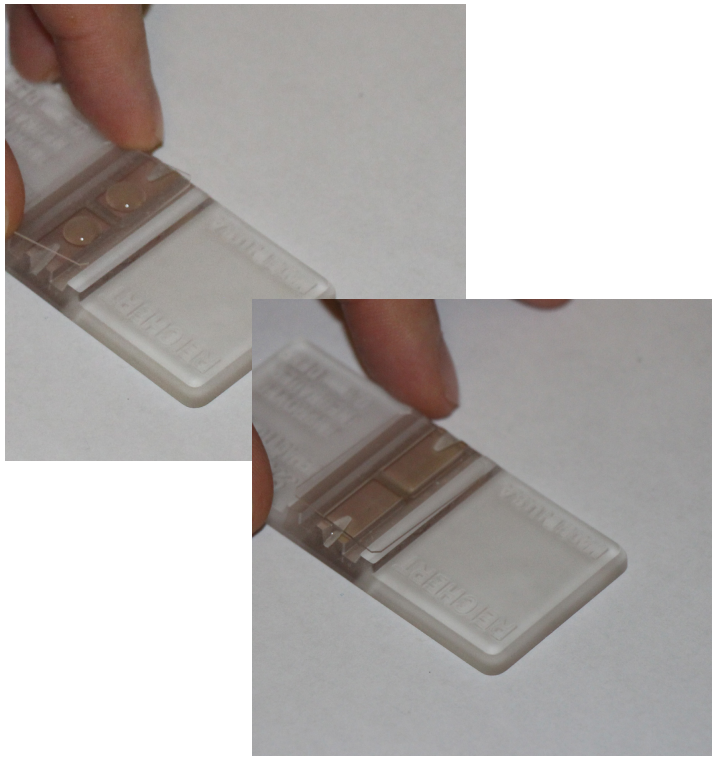
Sample collection

Foraging bees collected by vacuum directly into vial with ethanol

Sample processing

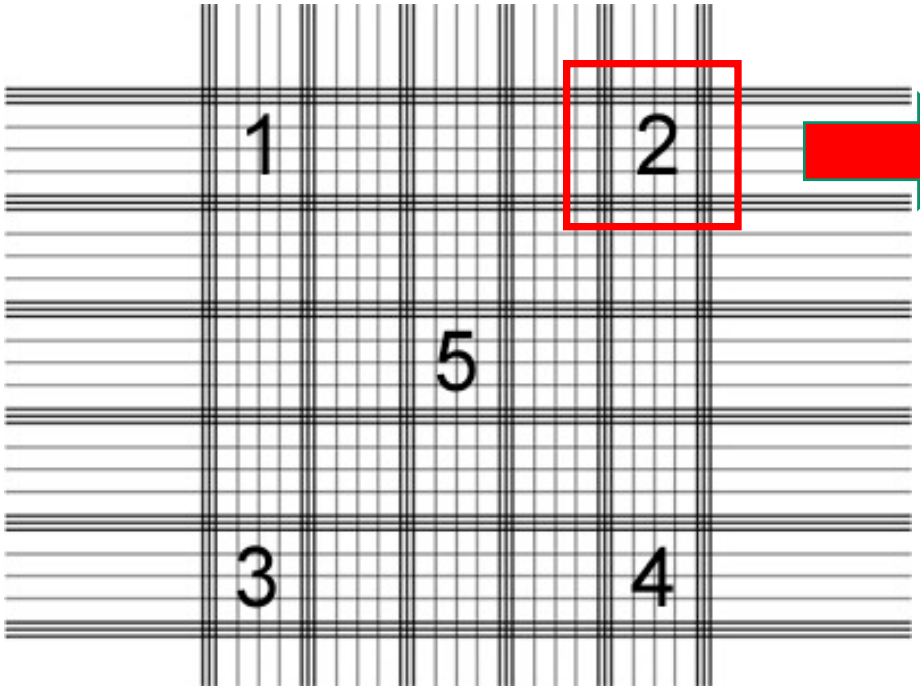
25 bee abdomens crushed and homogenized in 25 mL water

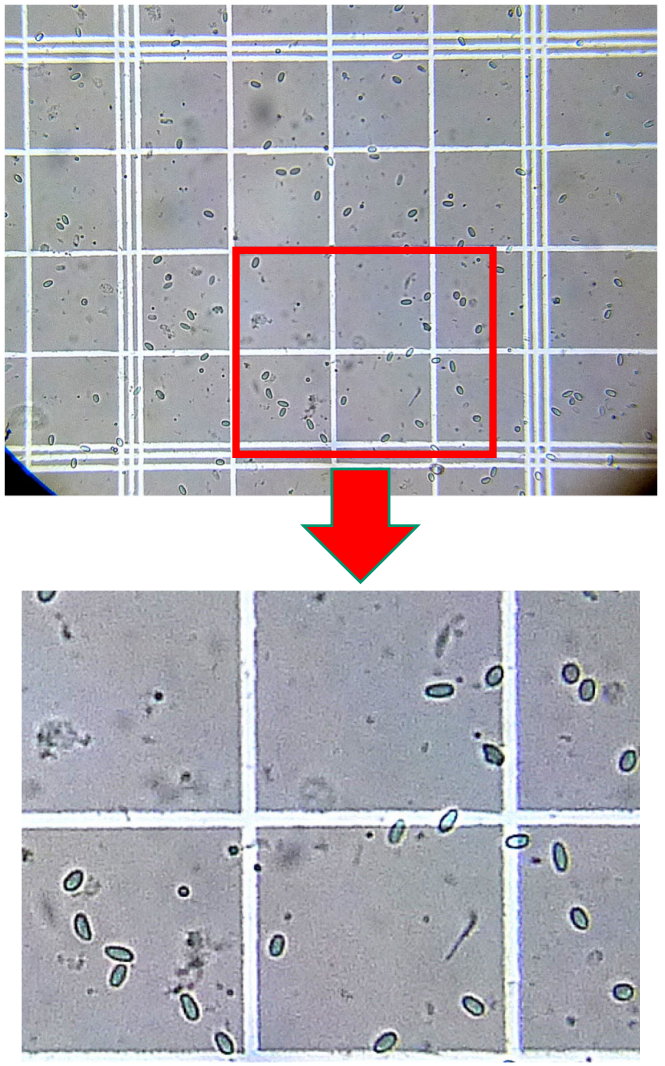
Spore counts



Bee abdomen homogenate added to two hemacytometer (cell counting slide) chambers and cover slip placed

Compound microscope with 400x magnification





Spores in five squares counted for each chamber. Average number of spores for the two chambers multiplied by 50,000 to find the spore load per bee in the sample

Results:

- Wide variety of spore counts at beginning of trial
- Range
  - from 50,000 spores
  - To 5,500,000 spores

Control

	%incr	ave % incr	total d/o	init spore	final spore counts <2 M	2-3 M	3-5 M	5 M +	% survival
control		**198**	**8**						**56**
1	-	-	~~yj~~	4.6 M					
2	-		-	2.1 M	-				
3	-		-	1.1 M	-				
4	191.2			850k	x (1.6M)				
5	110.3			725k	x (800k)				
6	344.4			675k		x			
7	154.2			600k	x (925k)	-			
8	561.1			450k		x			
9	-		~~yj~~	425k	-				
10	-6.667			375k	x (350k)	-			
11	-		~~yj~~	350k	-	-			
12	321.4			350k	x (1.1M)				
13	-		-	225k	-	-			
14	-87.5			200k	x (25k)				
15	-33.33			150k	x (100k)	-			
16	-		~~yj~~	125k	-				
17	433.3			75k	x (325k)				
18	-		-	25k	-	-			

survival over 1M initial **0%**

survival over 500k initial **57%**

survival under 500k initial **55%**

Fumagilin B (Fumadil B)

	%incr	ave % incr	total d/o	init spore	final spore counts: <2 M	2-3 M	3-5 M	5 M +	% survival
Treatment 1		**533**	**4**						**73**
1	-93.27			5.6M	x (375k)	-			
2	318.1			3.2M				x	
3	-		-	2.9M	-	-			
4	-53.06			1.2M	x (575k)				
5	208.7			1.2M		x			
6	129.4			850k	x (1.1M)				
7	327.6			725k		x			
8	-		~~yj~~	675k	-				
9	495.7			575k		x			
10	600			475k		x			
11	311.8			425k	x (1.3M)				
12	-		-	225k	-				
13	-		-	175k	-				
14	-75			100k	x (25k)				
15	3533			75k		x			
16	700			25k	x (175k)				

survival over 1M initial **80%**

survival over 500k initial **78%**

survival under 500k initial **71%**

Nozevit

	%incr	ave % incr	total d/o	init spore	final spore counts: <2 M	2-3 M	3-5 M	5 M +	% survival
Treatment 2		**2693**	**2**						**88**
1	309.5			5.6M				x	
2	116.4			3.4M			x		
3	-100			1.6M	x (o)				
4	663.8			1.2M				x	
5	-65.91			1.1M	x (375k)				
6	-75.76			825k	x (200k)				
7	1783			750k				x	
8	1456			625k				x	
9	795.5			550k			x		
10	2222			450k		x			
11	1844			400k				x	
12	-		-	275k	-				
13	4950			250k				x	
14	5456			225k				x	
15	1956			225k			x		
16	12450			100k				x	
17	9333			75k				x	
18	-		-	25k	-				

survival over 1M initial **100%**

survival over 500k initial **100%**

survival under 500k initial **78%**

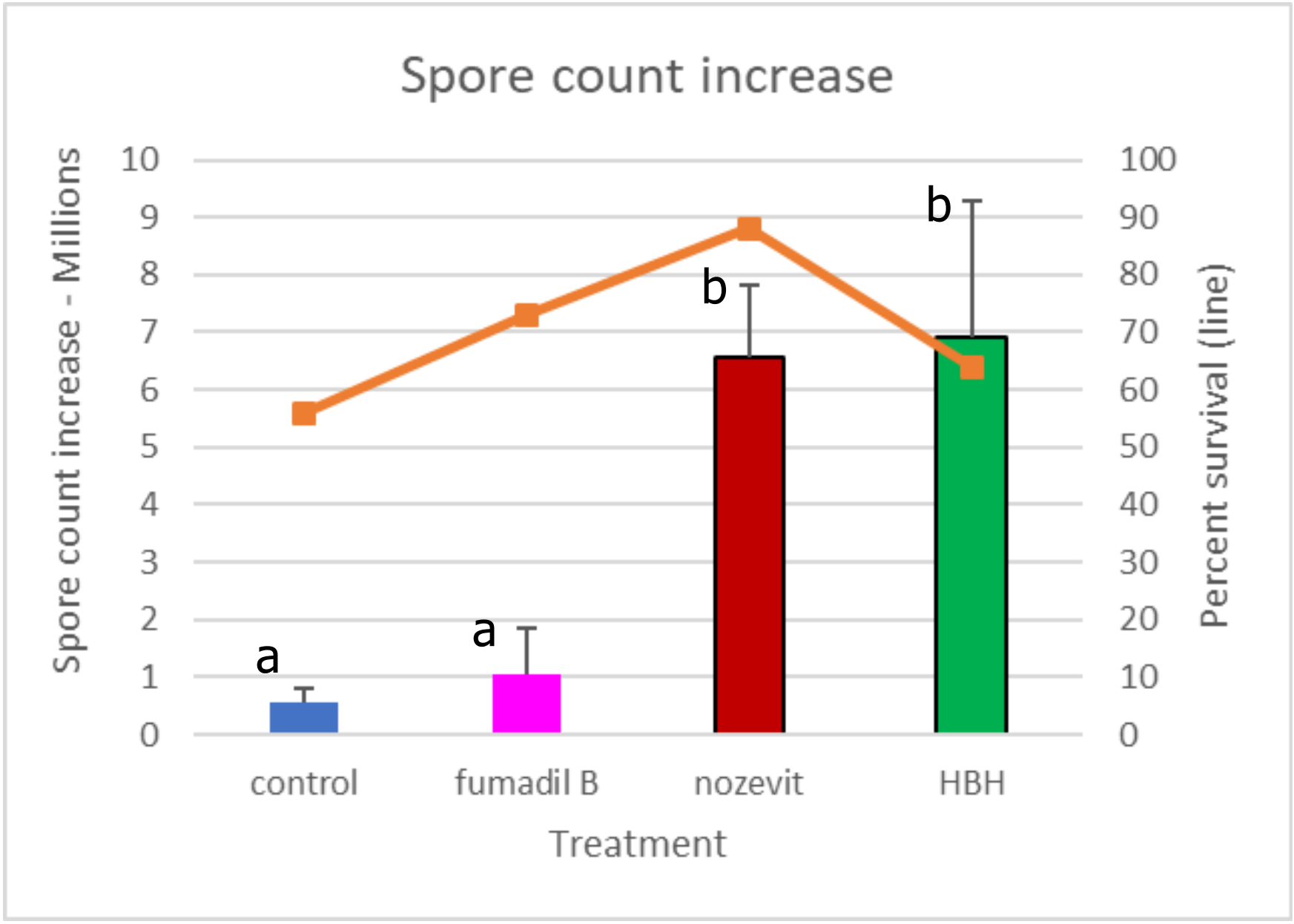
HBH

	%incr	ave % incr	total d/o	init spore	final spore counts <2 M	2-3 M	3-5 M	5 M +	% survival
Treatment 3		**2863**	**5**						**64**
1	102.1			3.6M			x		
2	-		~~yj~~	3.5M	-				
3	-		-	1.1M	-				
4	1992			950k		-		x	
5	390			750k		x			
6	-		~~yj~~	700k	-				
7	-34.62			650k	x (425k)				
8	-91.3			575k	x (50k)				
9	2116			475k		-		x	
10	1456			**450k**			x		
11	-		-	375k	-				
12	1171			350k			x		
13	8230			250k				x	
14	-		~~yj~~	150k	-				
15	13300			75k				x	

survival over
1M initial **33%**

survival over
500k initial **62%**

survival under
500k initial **71%**



Mean + SEM, bars with same letter not significantly different

Deadouts by treatment:

- Controls 44%
- Fumidil 25%
- Nozevit 12%
- Honey Bee Healthy 33%

Discussion/Conclusions

- From our data, there does not seem to be a strong correlation between increased spore counts and colony mortality going into winter.
- Fumadil B appeared to be the only treatment to significantly control *Nosema* spore production in surviving colonies.
- Nosemosis and the *Nosema Ceranae* effect should be considered as different maladies
- We cannot predict how results will extend into next season. However, we will continue to track spore counts and mortality of study colonies heading into spring buildup.

Further Study Required: Some Issues

- Relationship between varroa infestation and nosema
- Relationships with pesticides
- Determination whether there is *Nosema Ceranae* larval infestation and if there is, if it is important
- Speed at which *Nosema Ceranae* develops
- Easier and earlier detection methods
- Determination of relationship between spore counts and level of hive damage
- Variations of *Nosema Ceranae* haplotypes

Do yellowjacket wasps vector *Nosema*?

- We found no evidence of *Nosema sp*. in the guts of yellowjacket wasps sampled (n=25) at any of the three study sites.

- We found no significant difference in spore counts between hygenic and non-hygenic bee stock.

Disclaimer

The Government of British Columbia is committed to working with industry partners. Opinions expressed in this document are those of [the authors] and not necessarily those of the Government of British Columbia or the Investment Agriculture Foundation of BC.

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Thanks for your attention!

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