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Evaluation of the effectiveness of fumagillin and alternative therapies for the control of *Nosema ceranae*

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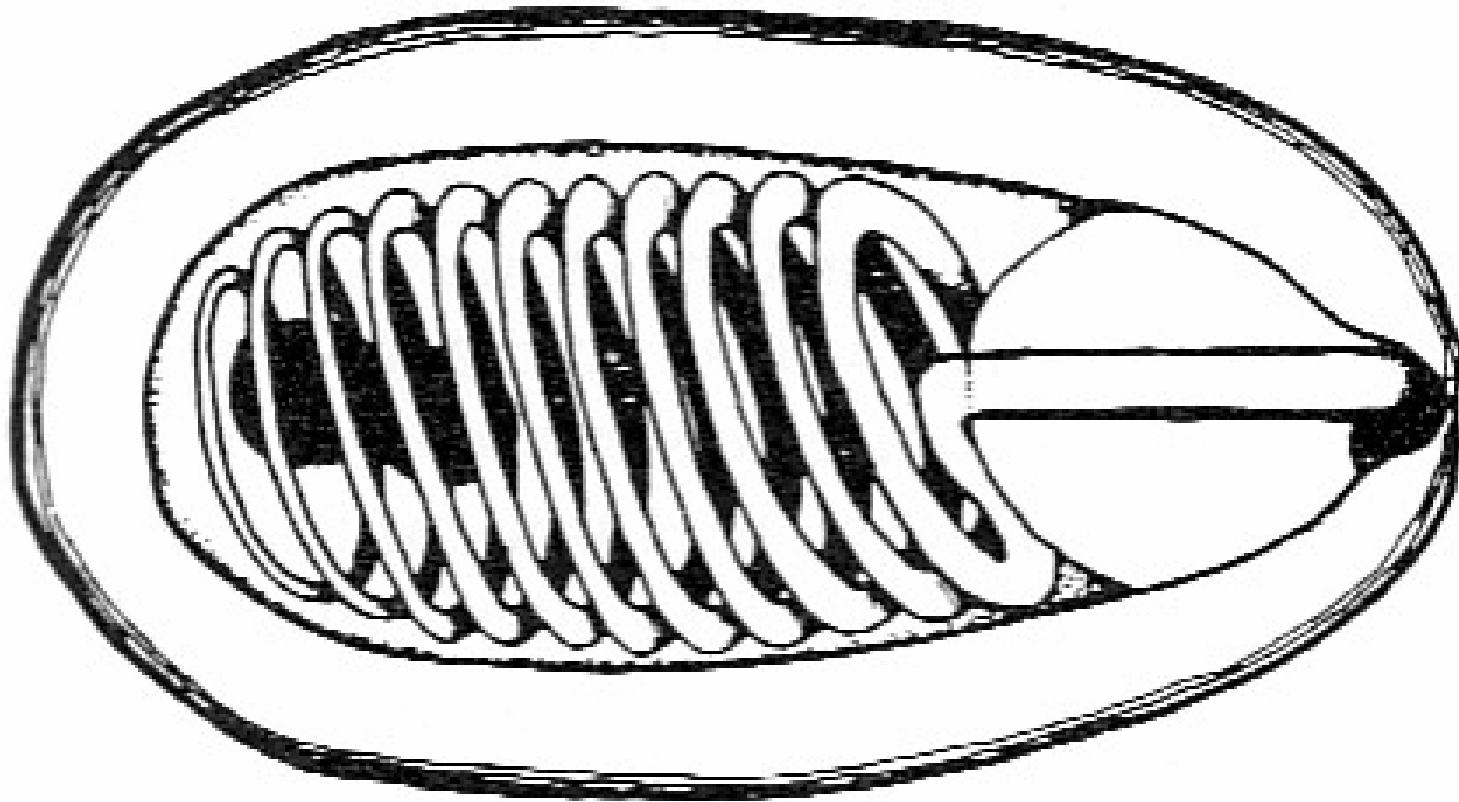
Canada

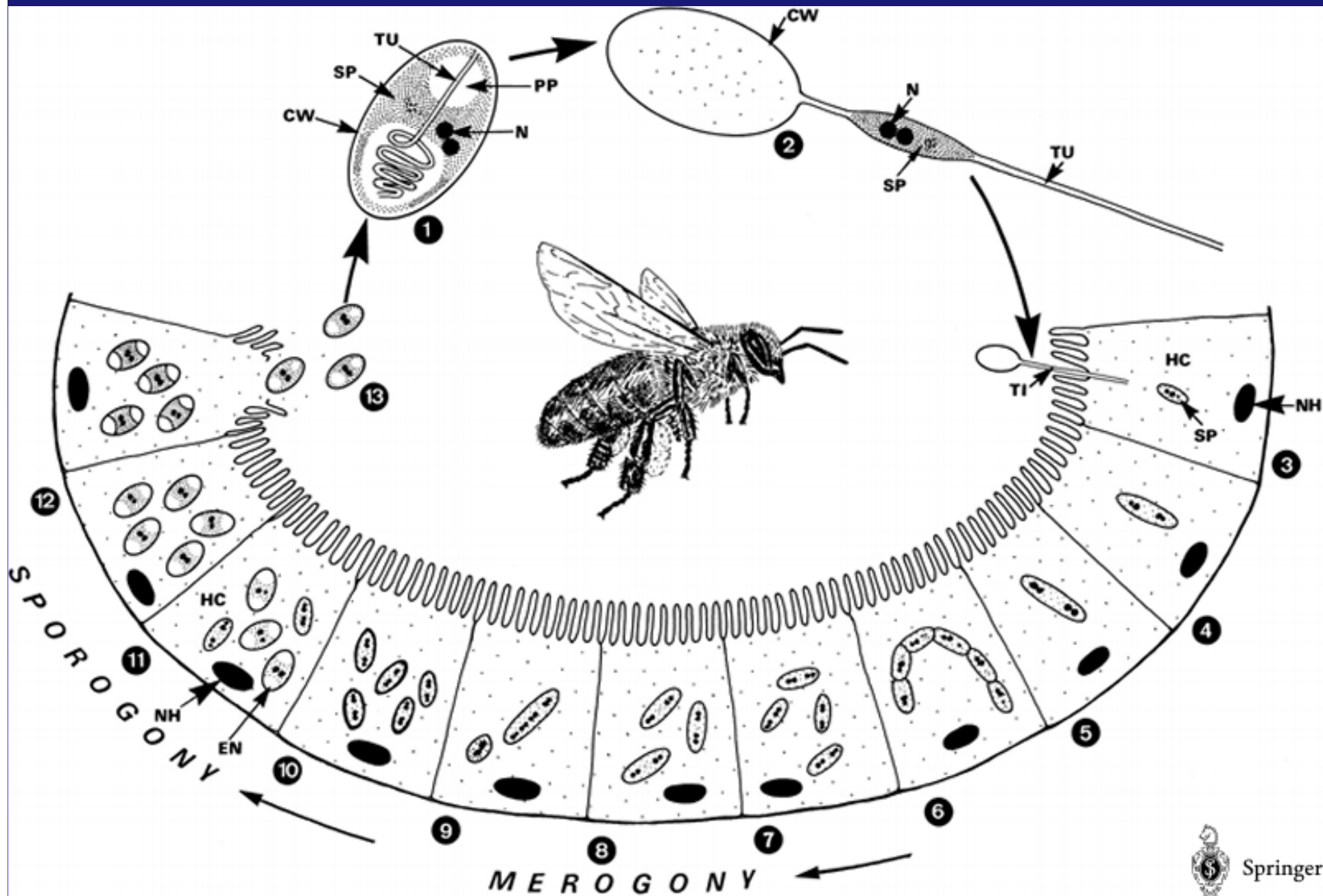
Overwintering Mortality in Canada 2009

Province	# Colonies Dead	Wintering Losses (% Provincial Total)
British Columbia	8,778	24
Alberta	98,700	44
Saskatchewan	24,130	25
Manitoba	24,000	30
Ontario	24,800	31
Quebec	10,800	32
Nova Scotia	5,694	29
New Brunswick	4,455	43
PEI	1,620	40
CANADA	202,977	34% (of National Total)

Nosema – microsporidia

Highly specialized parasitic fungi







Nosema Reduces:

Nursing

Lifespan

Pollen collection

Queen fertility

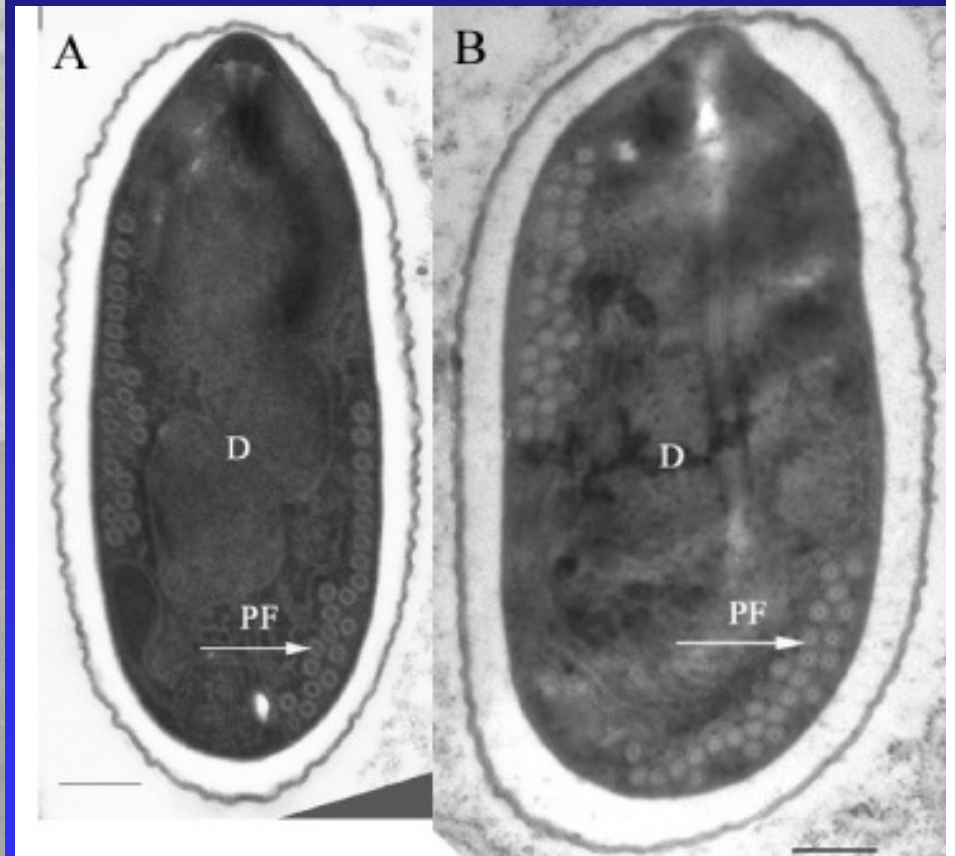
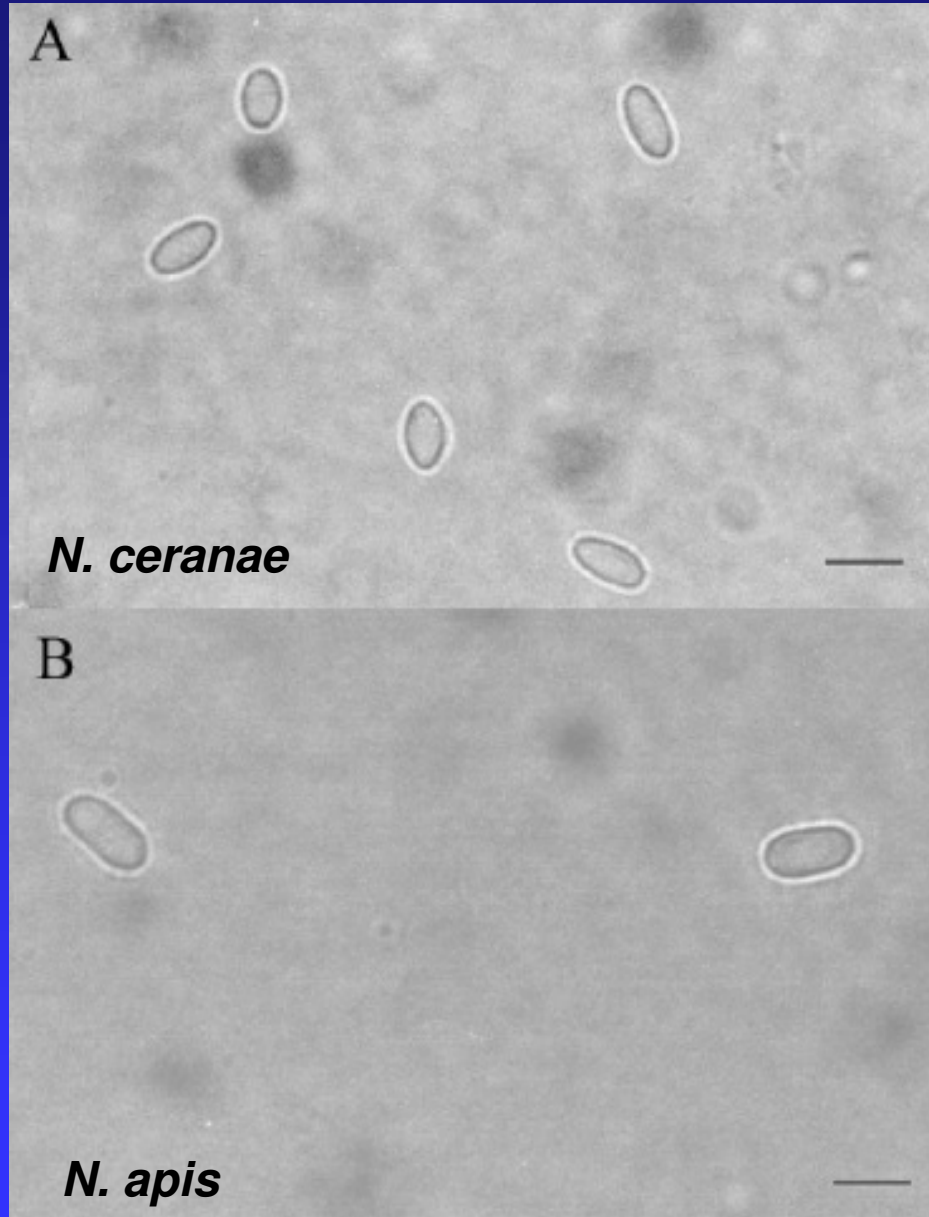
Queen acceptance

***Leads to wintering
mortality, poor spring
build-up and loss of
honey production.***

What is *Nosema ceranae*?

- Isolated from *Apis cerana* in China in 1996.
- Reported from *Apis mellifera* in Taiwan 2005 and later confirmed in Spain. Later confirmations from Germany and France.
- In Spain, *N. ceranae* characterized by gradual depopulation, higher fall/winter mortality, low honey production but NOT dysentery or crawling bees typically seen with *N. apis*.
- Above symptoms similar to “dry nosemosis” seen in France.
- Widespread in the U.S. and present in all Canadian Provinces.

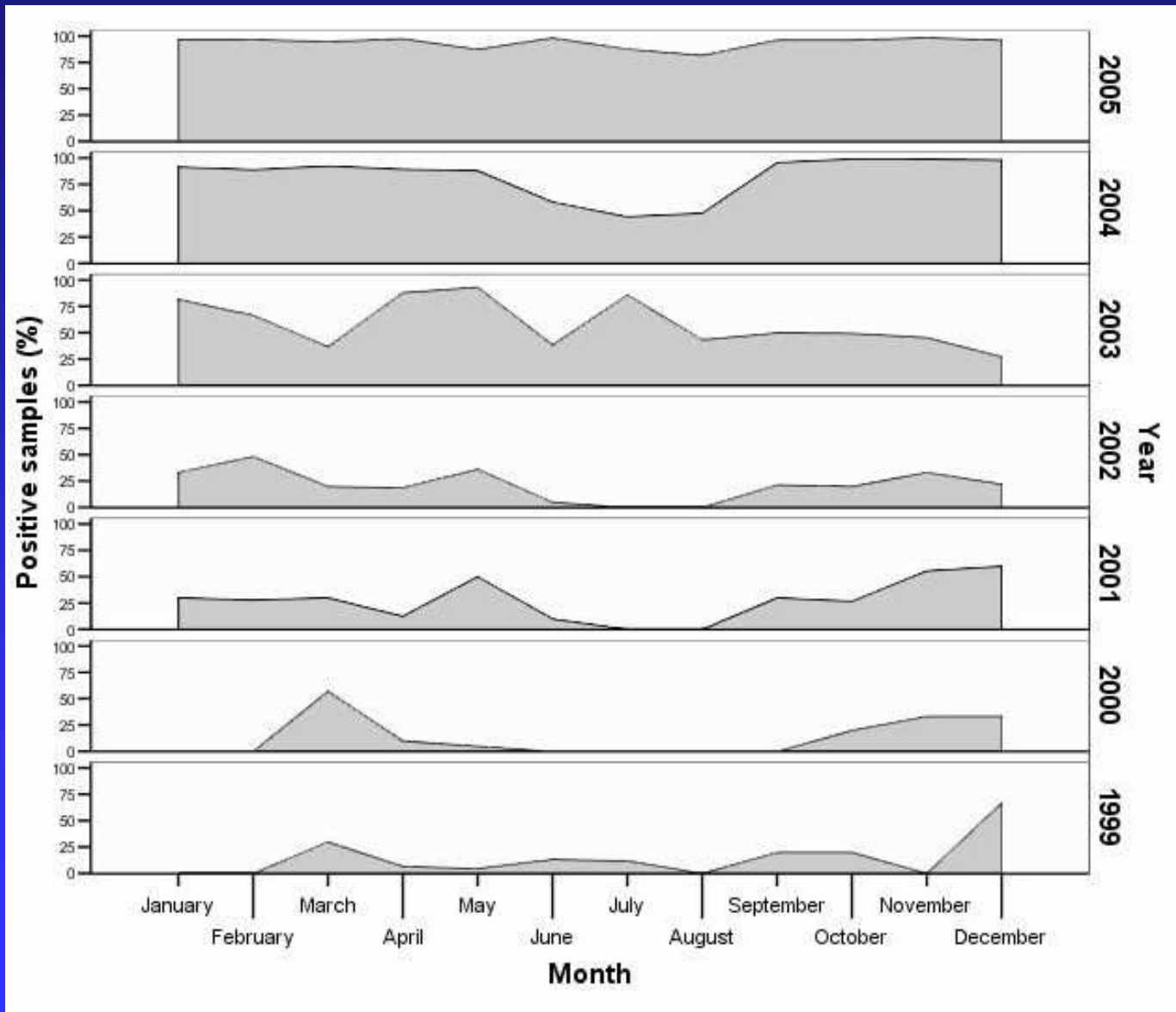




N. ceranae

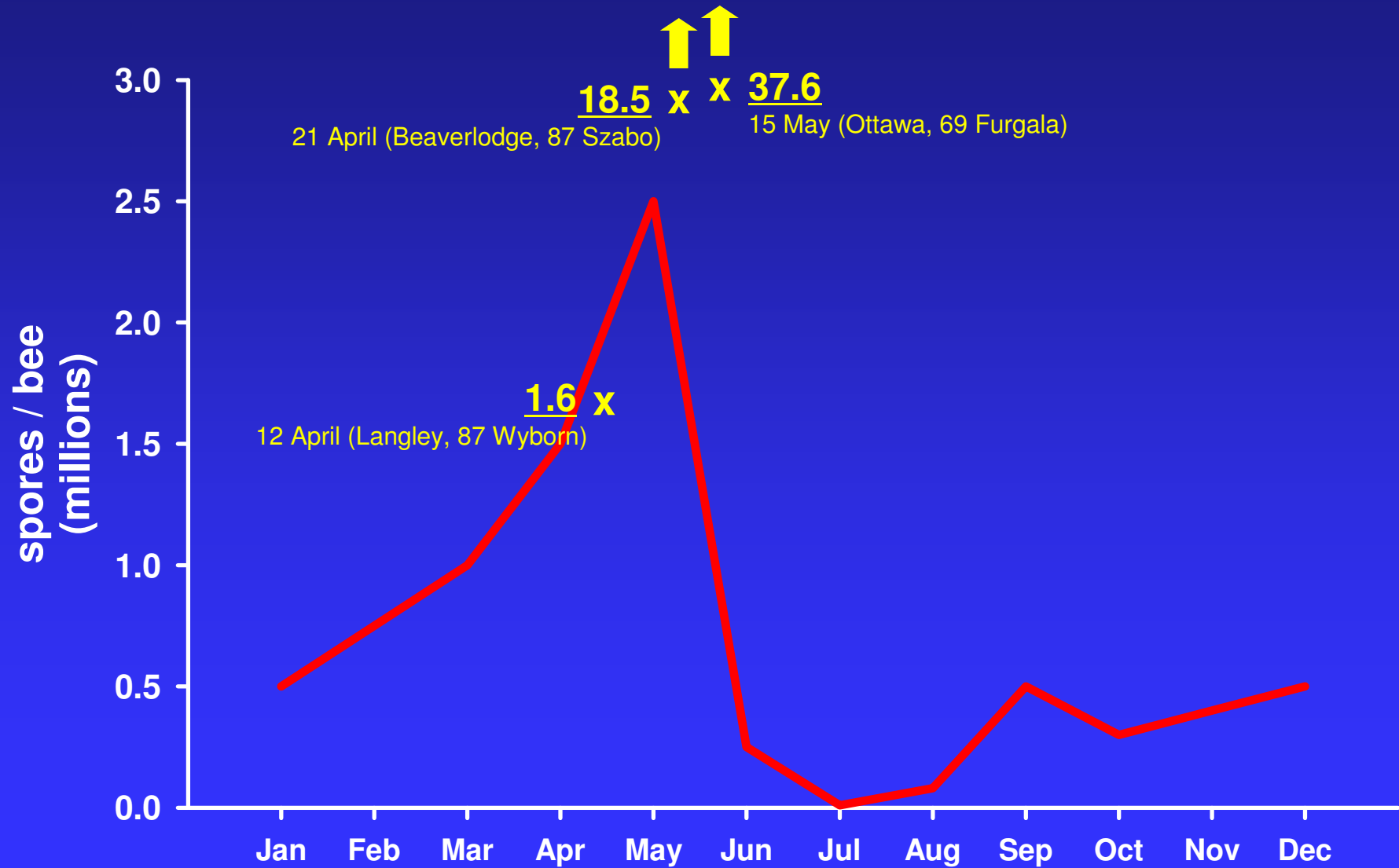
N. apis

Seasonal shift in *Nosema* incidence



Nosema apis

A problem in the winter and spring



Fries from Bailey and Ball

What is unknown about *N. ceranae*?

- Interactions / competition with *N. apis*.
- Transmission
- Survival / Infectivity of spores.
- Temporal phenology of symptoms.
- Most effective methods of management
- Use of current or new antibiotics.

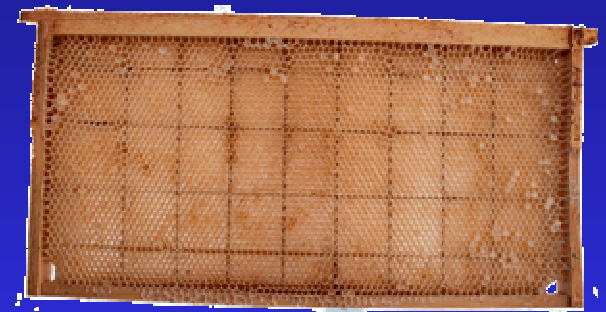


Objectives:

- To evaluate methods for disinfecting *N. ceranae* spores on comb.
- To evaluate different formulations of spring-applied fumagillin treatments for suppressing *N. ceranae* infections in overwintered colonies.

Comb Disinfection Experiment

- Treatments:
 - Inoculated, Irradiated
 - Inoculated, Acetic Acid Fumigated
 - Inoculated, Heat Treated
 - Inoculated, Untreated
 - Non-Inoculated, Untreated



12 Replicate colonies / treatment

Comb Disinfection Experiment

Inoculating Comb

4.51×10^8 *N. ceranae*
spores per colony



Comb Disinfection Experiment

Electron Beam Irradiation

FWT-602P Radiachromic film dosimeters



Comb Disinfection Experiment

Electron Beam Irradiation



10 kGy exposure, on tops and bottoms of boxes.

Comb Disinfection Experiment

Acetic acid Fumigation



Peter Jessing's Yard 22-29 April 2009

4.52 × 1.78 × 2.42 m high



Comb Disinfection Experiment

Acetic acid Fumigation



480 mL 80% acetic acid per four brood chambers

Joints between boxes sealed. Empty box placed on top of stack.

Comb Disinfection Experiment

Acetic acid Fumigation



200 - 380 ppm; 7 day exposure at 30° C

Comb Disinfection Experiment *Heat Treatment*

$49 \pm 1^{\circ}\text{C}$ for 24 h

Melting Point of
Beeswax: $62\text{-}65^{\circ}\text{C}$





2 May 2009

Comb Disinfection Experiment

Sampling Bees for Spores



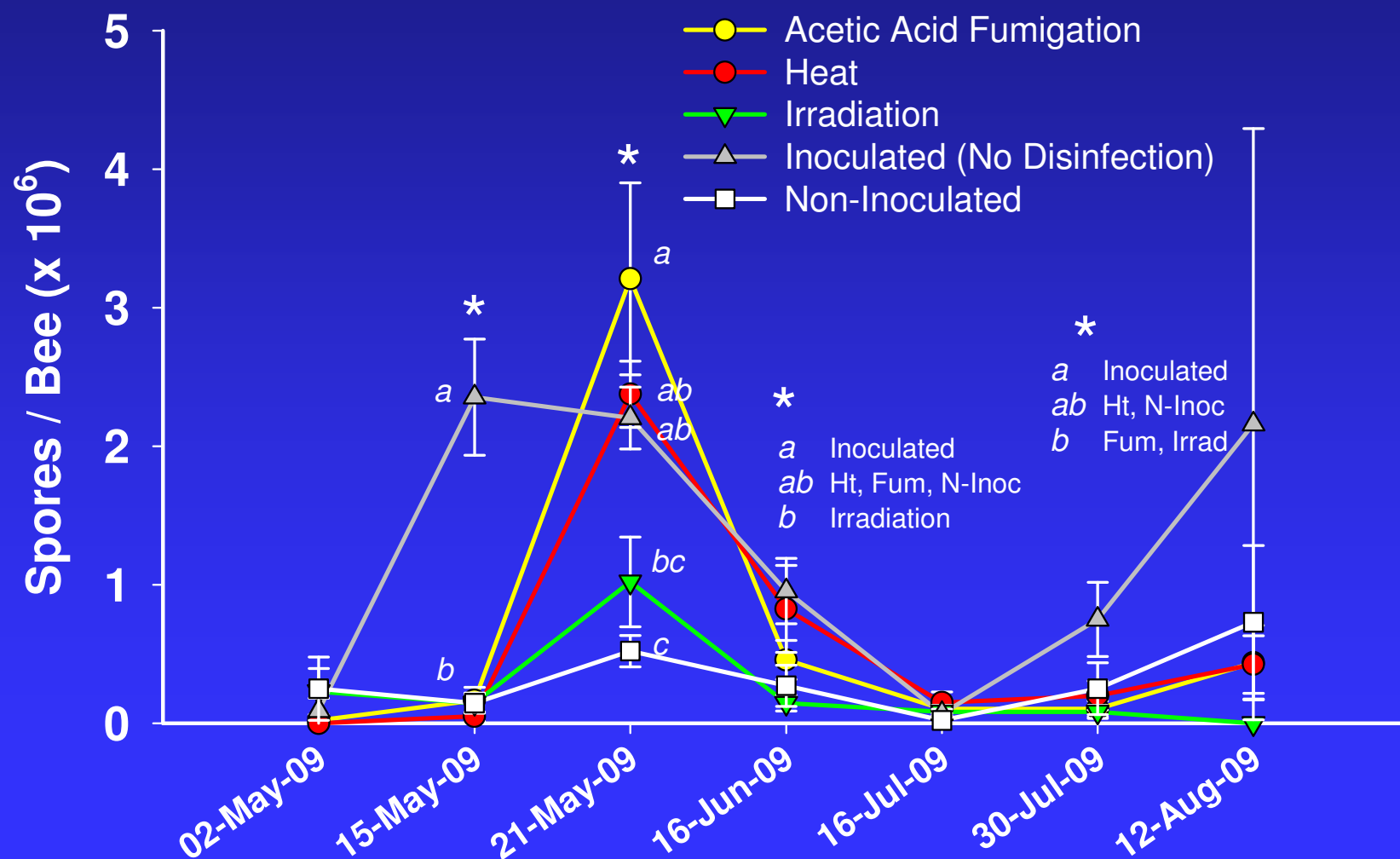
Weekly samples of 100 foragers (2 May - 4 June), then bi-weekly until end of October.

Spores enumerated microscopically at 400x.
Colony assessments 3 July, 31 Aug.



Comb Disinfection Experiment

Hived with New Zealand Packages: 2 May 2009



Fumagillin Efficacy Experiment

N. ceranae-infected overwintered singles: 22 April 2009



Pre-experimental infection level: $4.3 \pm 0.5 \times 10^6$ spores per bee

Fumagillin Efficacy Experiment

Treatments

(All containing 50 mg a.i. fumagillin and applied 2×):

- Sucrose Syrup (2 L)
- Sucrose Syrup (250 mL - Drench)
- Icing Sugar (20 g)
- Pollen Patties (100 g)
- Sucrose Syrup (2 L, unmedicated)
- 12 Replicate colonies / treatment



Fumagillin dicyclohexylammonium

Fumagillin Efficacy Experiment

Treatment Formulations – Spring 2009

DUSTING
Icing Sugar Dust



Colonies assessed
30 June, 26 Aug 09.

PATTY
Pollen, Soy Flour, Syrup



SYRUP
1:1 Sucrose
Drench vs. Full
Volume

Fumagillin Efficacy Experiment

Sampling Residues

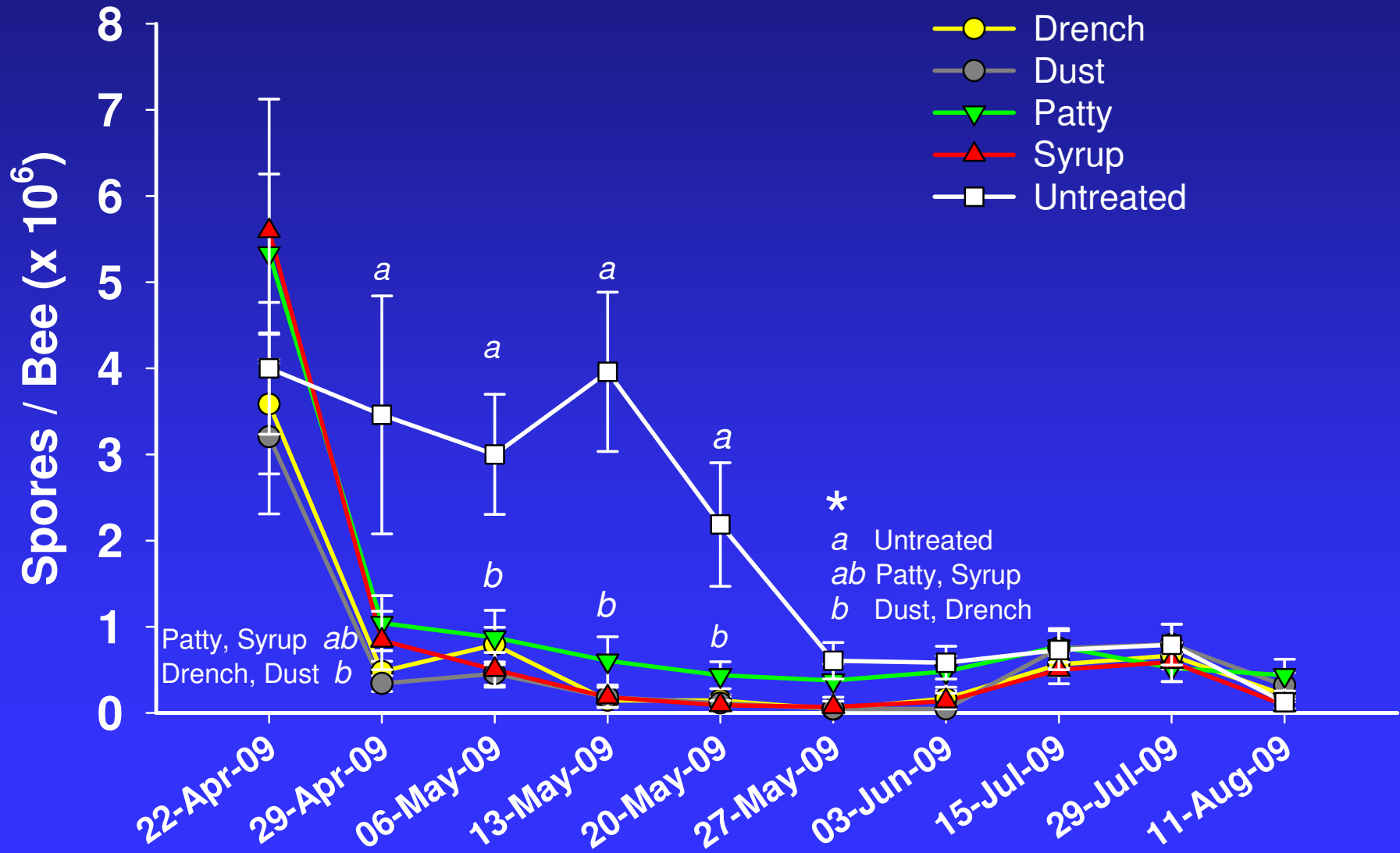


15 g honey samples

Development of LC-MS/MS technique to determine residue levels of Fumagillin and degradation products.

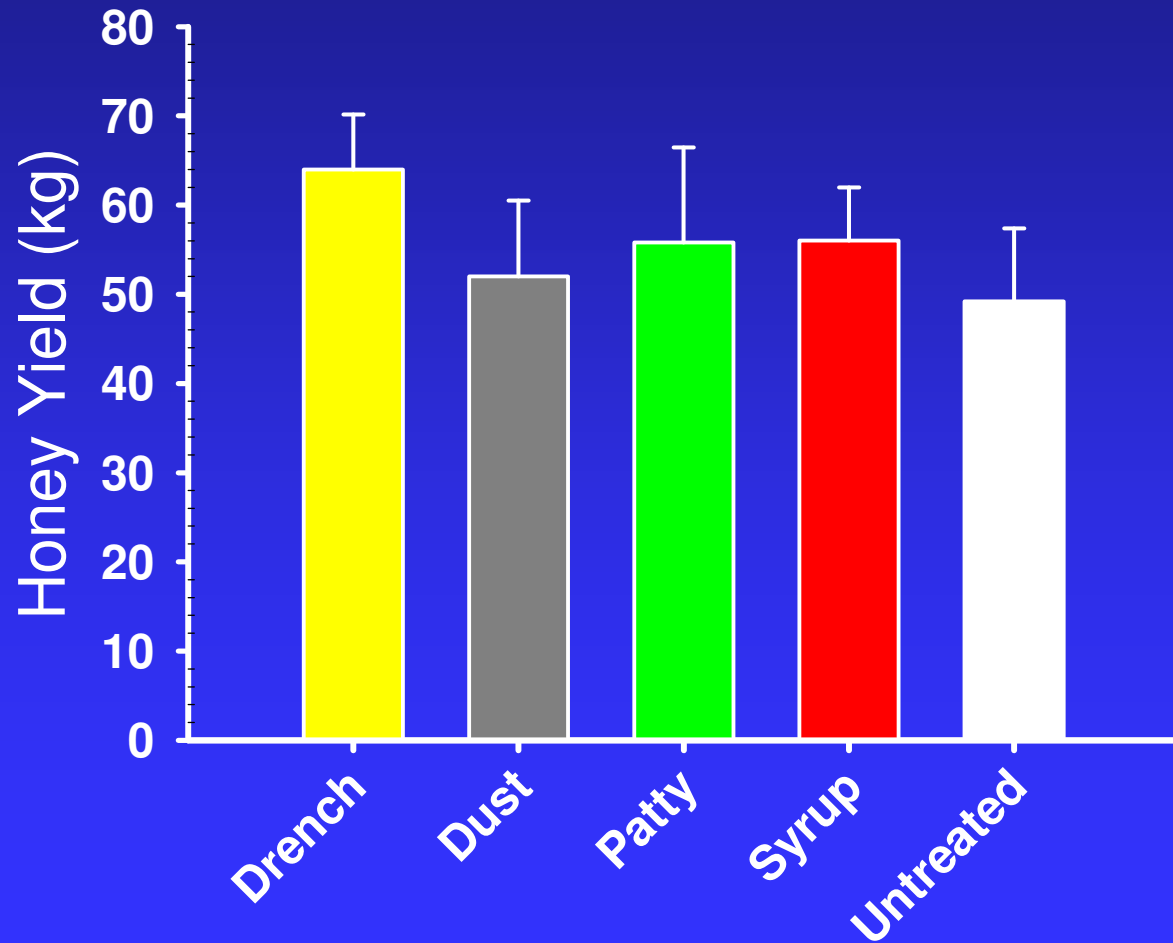
Fumagillin Efficacy Experiment

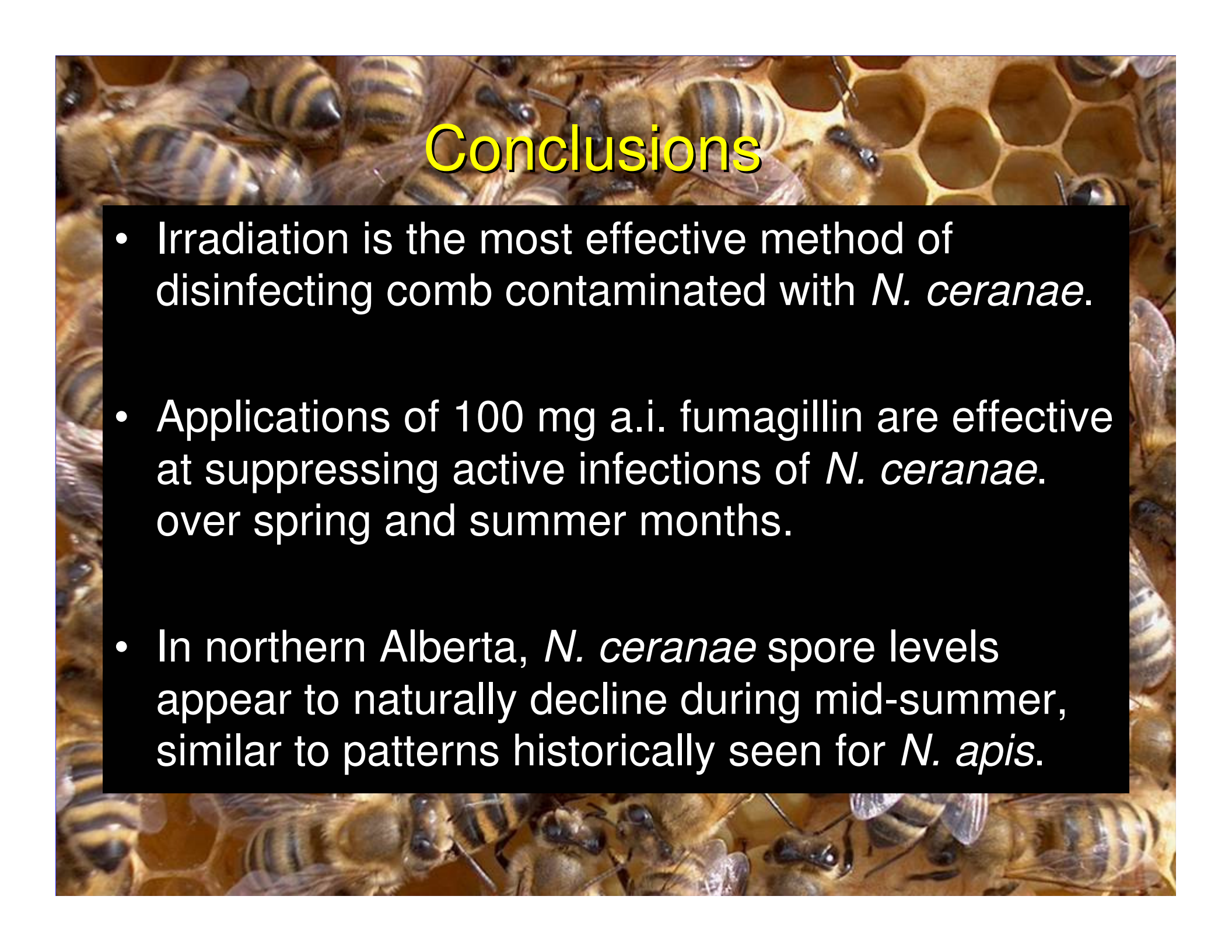
Treatments applied 22 April, 6 May 2009; Total a.i. = 100 mg



Fumagillin Efficacy Experiment

Honey Production



A close-up photograph of several bees working on a honeycomb. The bees are yellow and black striped, and the honeycomb is a golden-brown color with hexagonal cells. The bees are clustered together, some facing the camera and others with their backs to it.

Conclusions

- Irradiation is the most effective method of disinfecting comb contaminated with *N. ceranae*.
- Applications of 100 mg a.i. fumagillin are effective at suppressing active infections of *N. ceranae* over spring and summer months.
- In northern Alberta, *N. ceranae* spore levels appear to naturally decline during mid-summer, similar to patterns historically seen for *N. apis*.

Acknowledgements

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