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Eidgenössisches Departement für
Wirtschaft, Bildung und Forschung WBF
Agroscope

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Methods in honey bee research

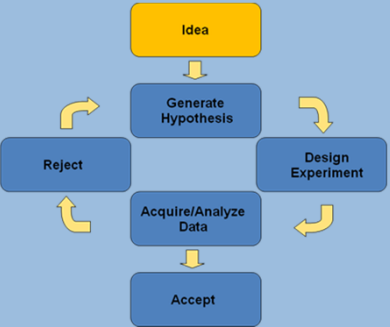


Gina Retschnig

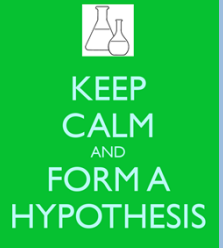
French German Summer School, 2nd of July 2015

Research Question

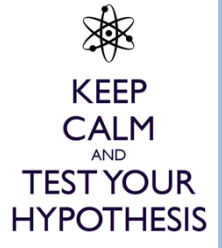
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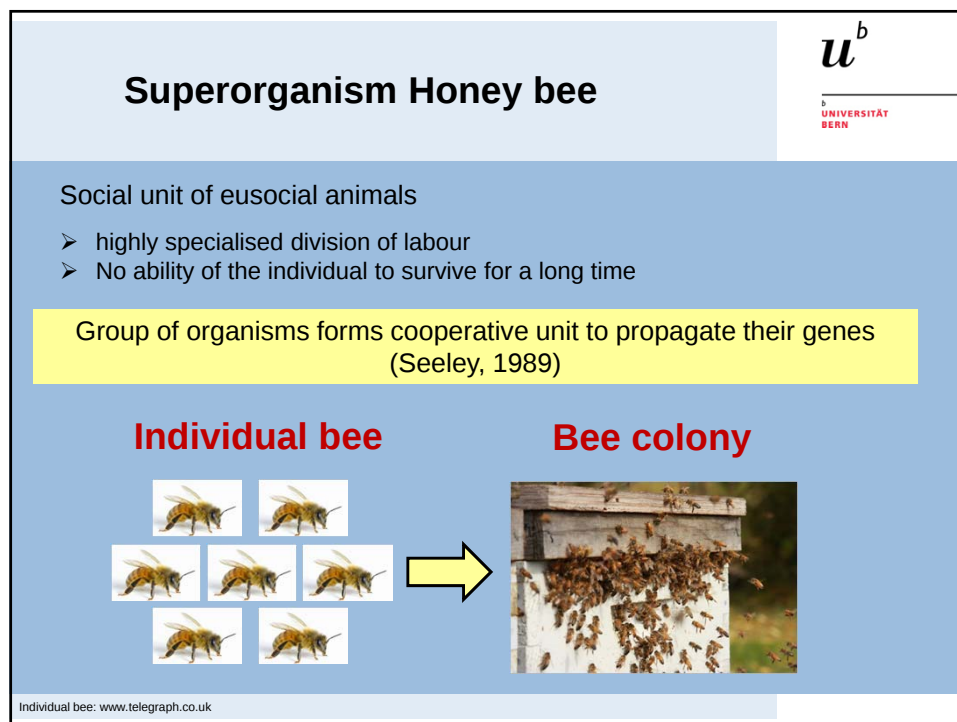
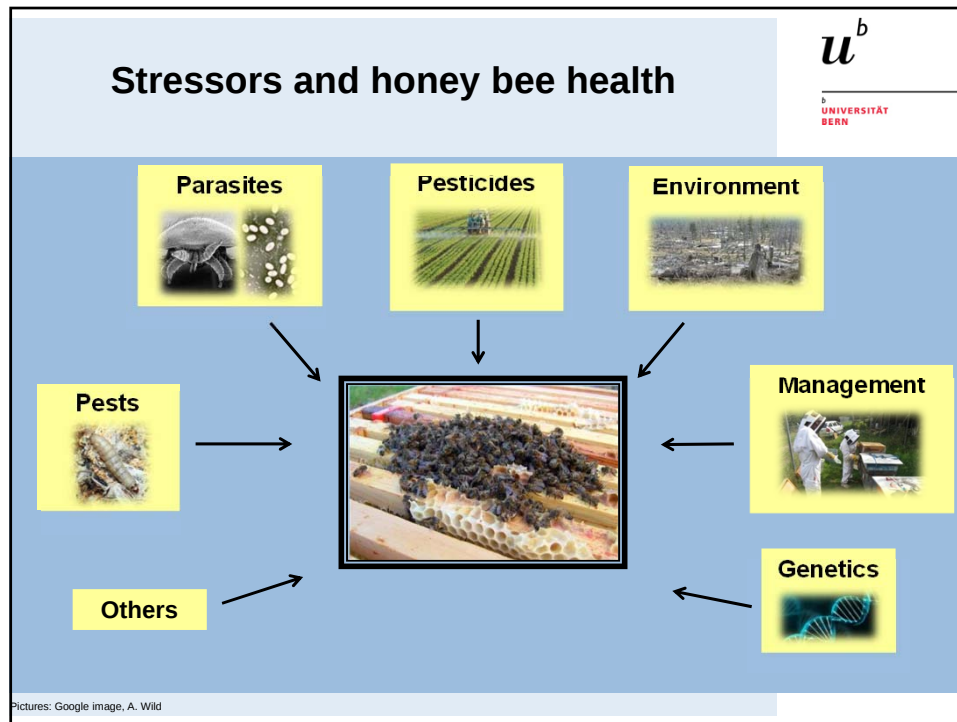
1st step:

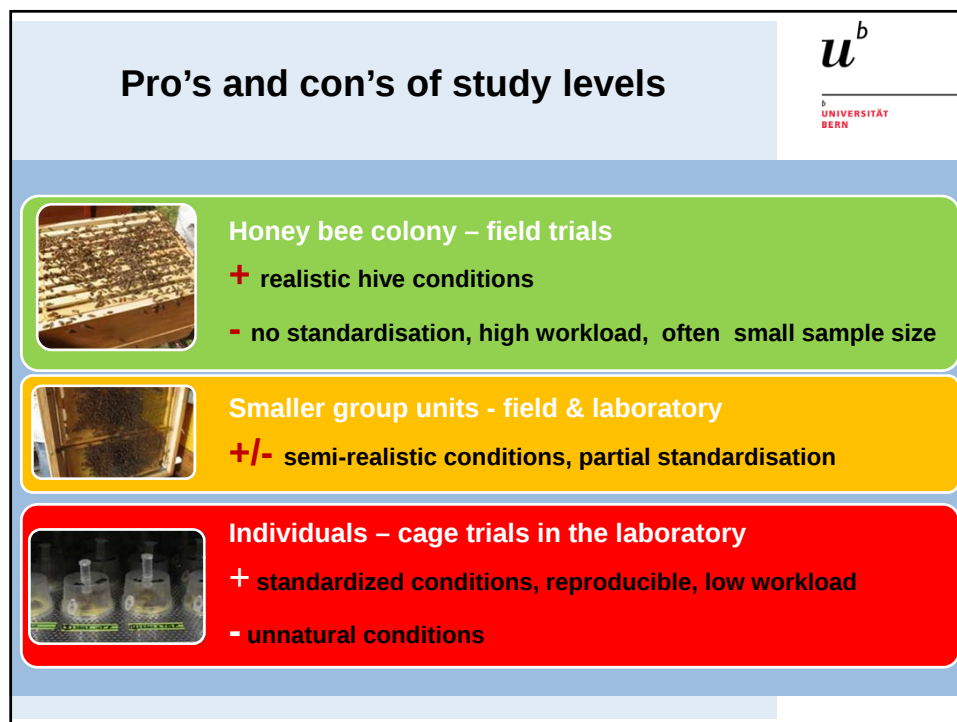
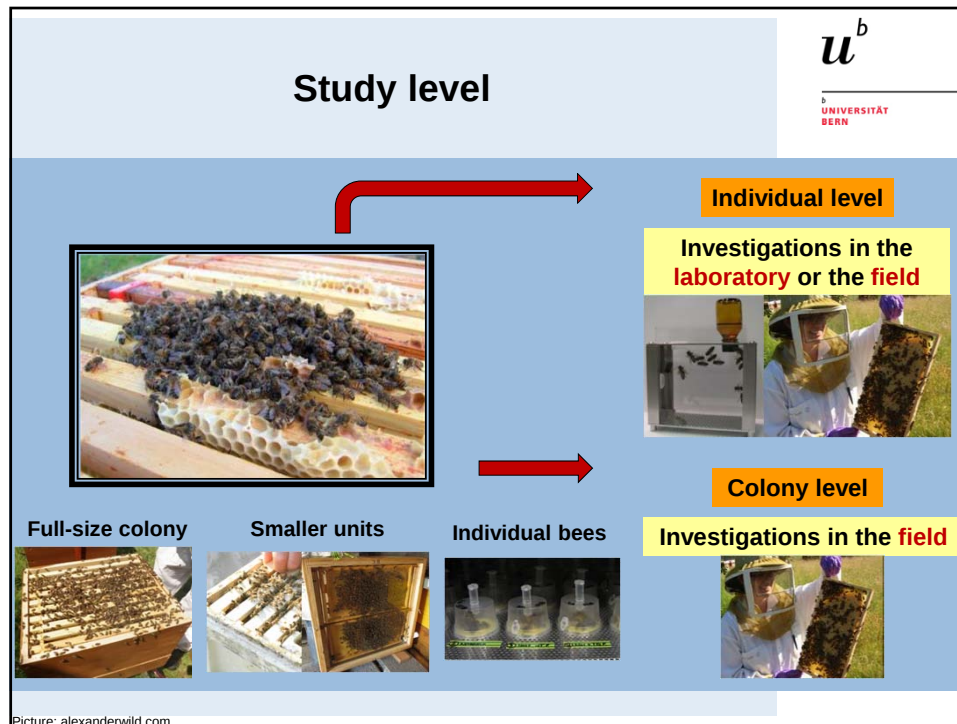


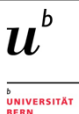
2nd step:



Research Question determines Research Methods







Parameters and measures

Effects

Lethal **Mortality**

Sublethal

Physiology:
e.g. immune system, body mass

Behaviour:
e.g. social behaviour, foraging

Reproduction:
e.g. fitness, sperm quality, etc.

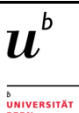
Other:
e.g. orientation, performance...




Strongest effect
Defined outcome

Different effect
levels

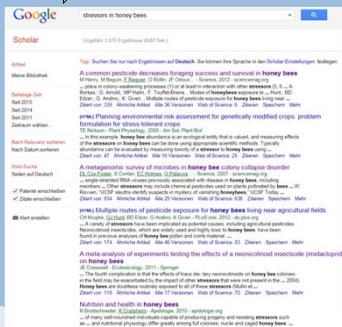
Picture: orist.org



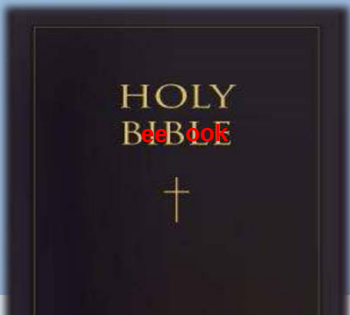
Search of the right method(s)

- Research question? ✓
- Study level (colony vs. Individual bee, queen, drone, worker)? ✓
- Parameters & measures (effects)? ✓
- Methods?

Scientific literature



Method collection

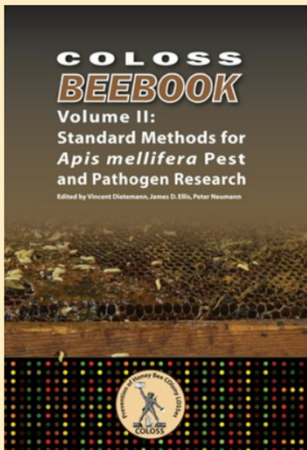


BEE BOOK

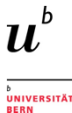
www.coloss.org/beebook



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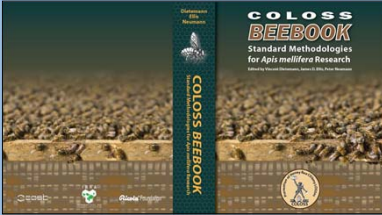
Standardization of bee research: COLOSS BEE BOOK



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Practical research
guidelines for direct use by
scientists, extension
specialists and
beekeepers



Section 1 – <i>A. mellifera</i> research methods	Section 2 – Pest & pathogen research methods
Behaviour	Epidemiology
Chemical ecology	Estimating colony losses
Molecular biology	<i>Acarapis woodi</i>
Physiology & biochemistry	<i>Aethina tumida</i>
Toxicology	<i>Ascophaera apis</i> & <i>Aspergillus</i> spp.
Cell culture	<i>Galleria mellonella</i>
Colony strength	<i>Melissococcus pulatunius</i> & associates
Endosymbionts	<i>Nosema apis</i> & <i>Nosema ceranae</i>
<i>In vitro</i> larval rearing	<i>Paenibacillus larvae</i>
Maintaining adults <i>in vitro</i>	<i>Tropilaelaps</i> spp.
Miscellaneous techniques	<i>Varroa</i> spp.
	Viruses

29 chapters by >170 authors

www.coloss.org/beebook



Method categories honey bee research



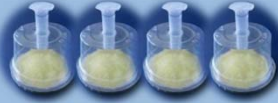
Field methods

Investigations of colonies, sub units of colonies or individual bees in a **colony setting**



Bee laboratory methods

Investigations of individual bees or groups of bees in a **laboratory setting**, laboratory measures



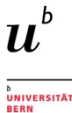
Molecular laboratory methods

Investigations of various **molecular measures**



dhruvlabs.com

Field methods: Exposure to stressors



Example: Pesticide exposure

➤ Depends on substance and application method

Sucrose solution: Feed treatment substance in sucrose solution

+ easy to prepare

- no control regarding dilution with nectar bees bring in

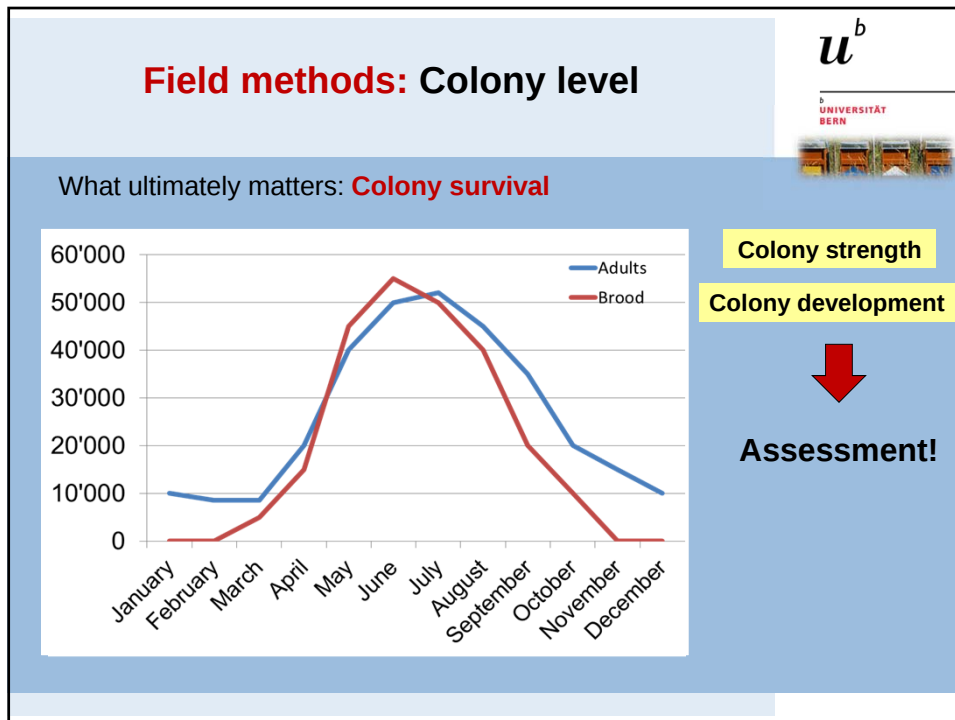
Pollen patties: Feed treatment substance in sucrose solution

+ no dilution (use of pollen traps), exposure of brood

- hard to standardize, expensive and availability




A. Troxler



Colony strength: Liebefelder estimation method

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Estimate colony strength by **COLONY ASSESSMENTS**

- Number of built up frames
- Number of bees
- Quantity of uncapped brood
- Quantity of capped (worker) brood
- Quantity of drone brood
- Quantity of honey stores
- Quantity of pollen stores

Full-size, nucs, apideas

cookevillebeekeepers.com cleveland.ces.ncsu.edu/pollen/ agroscope.admin.ch/imkereil/ scientificbeekeeping.com

Colony strength: Liebefelder estimation method





Field methods: Behaviour

Bees display a number of interesting behaviours:

In-hive:
Grooming, trophallaxis, undertaking, ...

Entrance/outside of hive:
flight activity, guarding, drifting, ...









What is necessary for observations of specific bees?

Scheiner et al. 2013, Standard methods for behavioural studies of *Apis mellifera*, BEEBOOK

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Labelling bees for **field** and **lab**

Another example: **Longevity of stressor-exposed honey bee workers in colonies (summer and winter bees)**

Color code (group-specific)

Platelet no. (individual)

Daily or weekly collection and counts of marked bees

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Field methods: Orientation & Navigation

Harmonic radar: Tool to track bees over the range of several hundred meters (up to 3 km)

A Radar

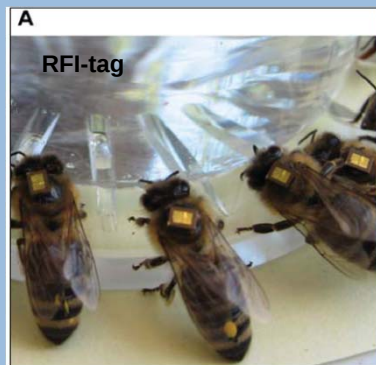
B transponder

Scheiner et al. 2013, Standard methods for behavioural studies of *Apis mellifera*, **BEEBOOK**

Field methods: Flight activity



Radio Frequency Identification:
registration of tagged bees,
e.g. at hive entrance: life monitoring



Scheiner et al. 2013, Standard methods for behavioural studies of *Apis mellifera*, BEEBOOK

Lab methods: Exposure to stressors



Example: Artificial inoculation of experimental bees with parasites

Individual inoculation:



+ standardized dosage

- time-consuming – sample size

Group feeding:



+ high sample size possible

- Variable intake

Other example: injection of viral particles using a microinjector

Lab methods: Individual level dissections

Workers, queens and drones

Examples: size, appearance etc. of specific organs (e.g. midgut) or collection of tissue for histological investigations



Carreck et al. 2013, Standard methods for *Apis mellifera* anatomy and dissection, **BEEBOOK**

Lab method:

Olfactory conditioning of workers

Examples: impact of stressors on learning ability and memory

A Specific odour



**Naïve bee –
no proboscis extension**

B Specific odour



**Conditioned proboscis
extension**

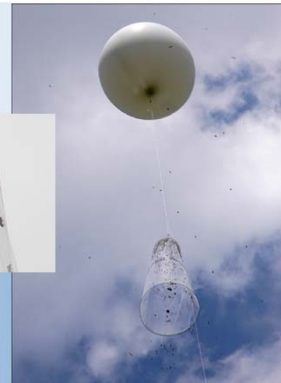
Conditioning by pairing the odour with a sucrose droplet

Scheiner et al. 2013, Standard methods for behavioural studies of *Apis mellifera*, **BEEBOOK**

Field and lab methods: Drones

Example field: Collection of drones

- in colonies
- On drone congregation areas (DCA's)



Example lab: measures of sperm quality

- Sperm collection
- Sperm counts (microscopic)
- Sperm viability



L. Straub

Pictures DCA: Scheiner et al. 2013

Field and lab methods: Queens

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Example field: Mating behaviour and performance

- Mating flights, mating success
- Queen survival, production of offspring...



Example lab: Queen rearing and physiology

- Grafting for queen rearing
- Physiology of reproductive organs, spermathecae, ...



Pictures: G.Williams

Bees during development

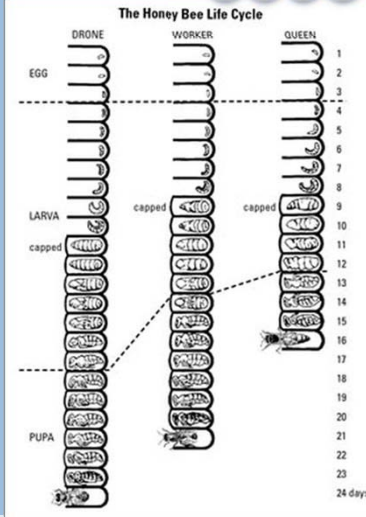


- Effects of stressors during development
- Investigations of larval diseases (e.g. EFB)

Artificial larval rearing

- ✓ Move eggs into artificial cells
- ✓ Development in incubator (temp. % humidity!)
- ✓ Enables observation, manipulation and collection of bee larvae and pupae





Molecular lab methods



Some fields of applications:

Species determination
Pathogen quantification
Genomics, proteomics, ...

A very wide range of methods...

Sample collection

Sample transport

Sample storage

Sample homogenization

RNA extraction

RT-qPCR

DNA extraction

Classical PCR

RNAi

Microsatellites

Sequencing

Hybridization methods

Phylogenetic analyses

Etc....

Etc....



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Field, lab and molecular methods: Parasite quantification

Ectoparasite *Varroa destructor*

Mite counts on bottom board of the hive

Mite washes of collected bees

Ratio of infested drone brood cells

mites in colony

mites on bees

mites in cells – all life stages

Pictures: Sandra Lanz

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Field, lab and molecular methods: Parasite quantification

Endoparasite *Nosema* spp.

Microscopic spore counts using hemocytometer (Cantwell (1970), Fries *et al.* (2013))

quantitative PCR

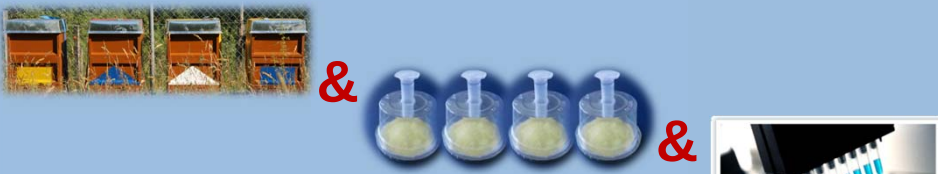
spores - viable stage

genetic material

A = sting apparatus; B = rectum; C = small intestine; and D = ventriculus (midgut) (Dade, 2009)

Picture pestle: scientificbeekeeping.com

Combination of field and lab methods



Investigations:

Exposure in the field – outcome measures in the lab
 Exposure in the lab - outcome measures in the field

Bee health assessment:

Sample collection in the field
 Health measures in the field & lab



Example 1: Interactions of pesticides and *Nosema ceranae* in honey bees?

Control
(4 colonies)



Control:

- ✓ sucrose solution

Treatment

Bern
Switzerland

Pesticides
(4 colonies)



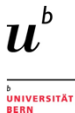
Pesticide 1:

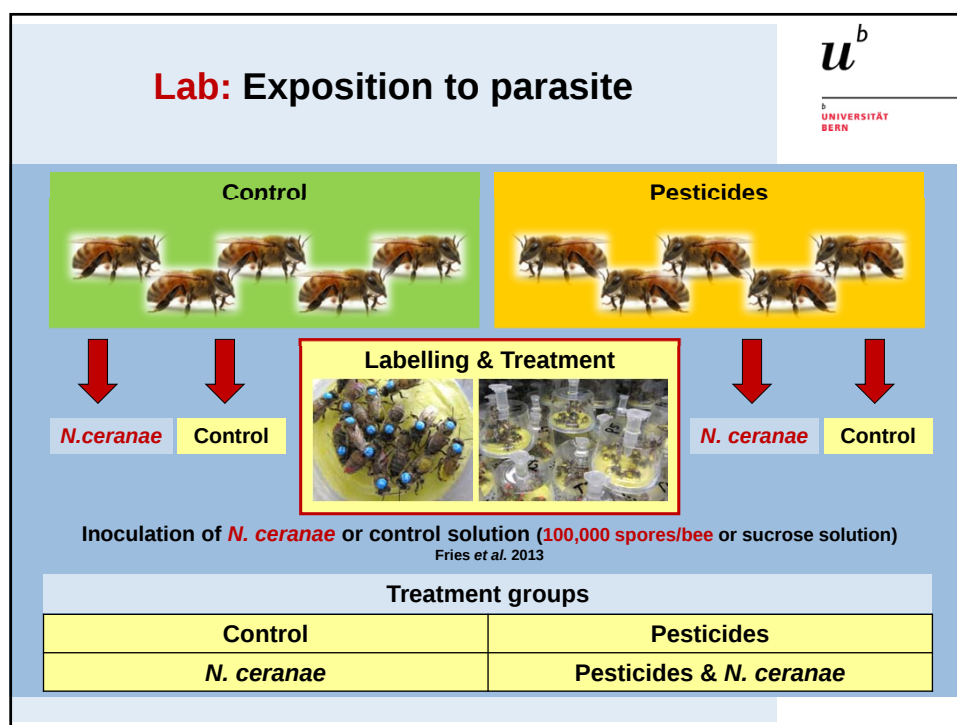
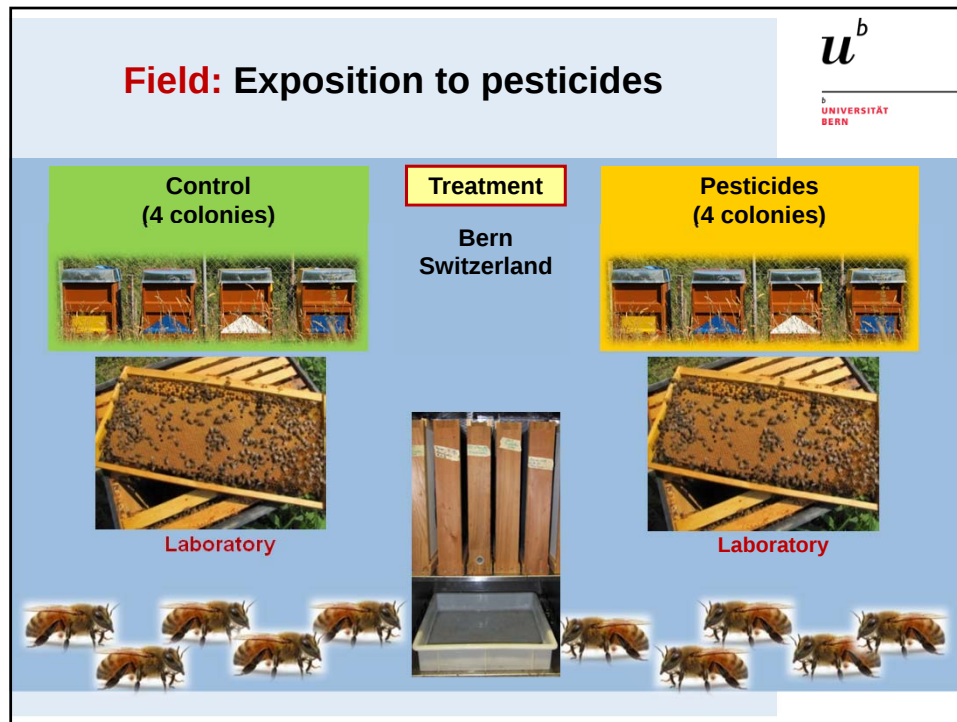
- ✓ 1000 ppb
- ✓ 1 kg/week
- ✓ in-hive feeder

Pesticide 2:

- ✓ 2 Apistan® strips (1.6 g)/box
- ✓ According to instructions







Field: Observations and data collection

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198 workers/ treatment group/ hive

14 days

1. Worker mortality 2. In-hive behaviour
Daily records Daily observations

Scheiner et al. 2013

Example 2: Bee health surveys

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Epidemiological monitoring of honey bee colony health on a national or international level

Example: German Bee Monitoring

German bee monitoring : Field screening and sampling

~ 1200 colonies
Ongoing since 2004

Stand: 28.01.2013

www.bienenmonitoring.org/

Take home message

-  Methods depend on research question
-  Field methods, bee lab methods & molecular lab methods
-  Huge range of methods – **standardization!**
-  Specific methods for queens and drones
-  Each method has pro's and con's – trade-off
-  Most studies combine field, lab and molecular methods

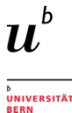


- Study level
- Parameters & measures









... for your attention!