# Determination of neonicotinoid insecticide residues in bees, pollen and nectar with LC-MS/MS

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# Seed coating with a neonicotinoid insecticide negatively affects wild bees

Nature (2015) 521: 77-80

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*[1]Lund University, [2]Swedish University of Agricultural Sciences, [3]Swedish Board of Agriculture*

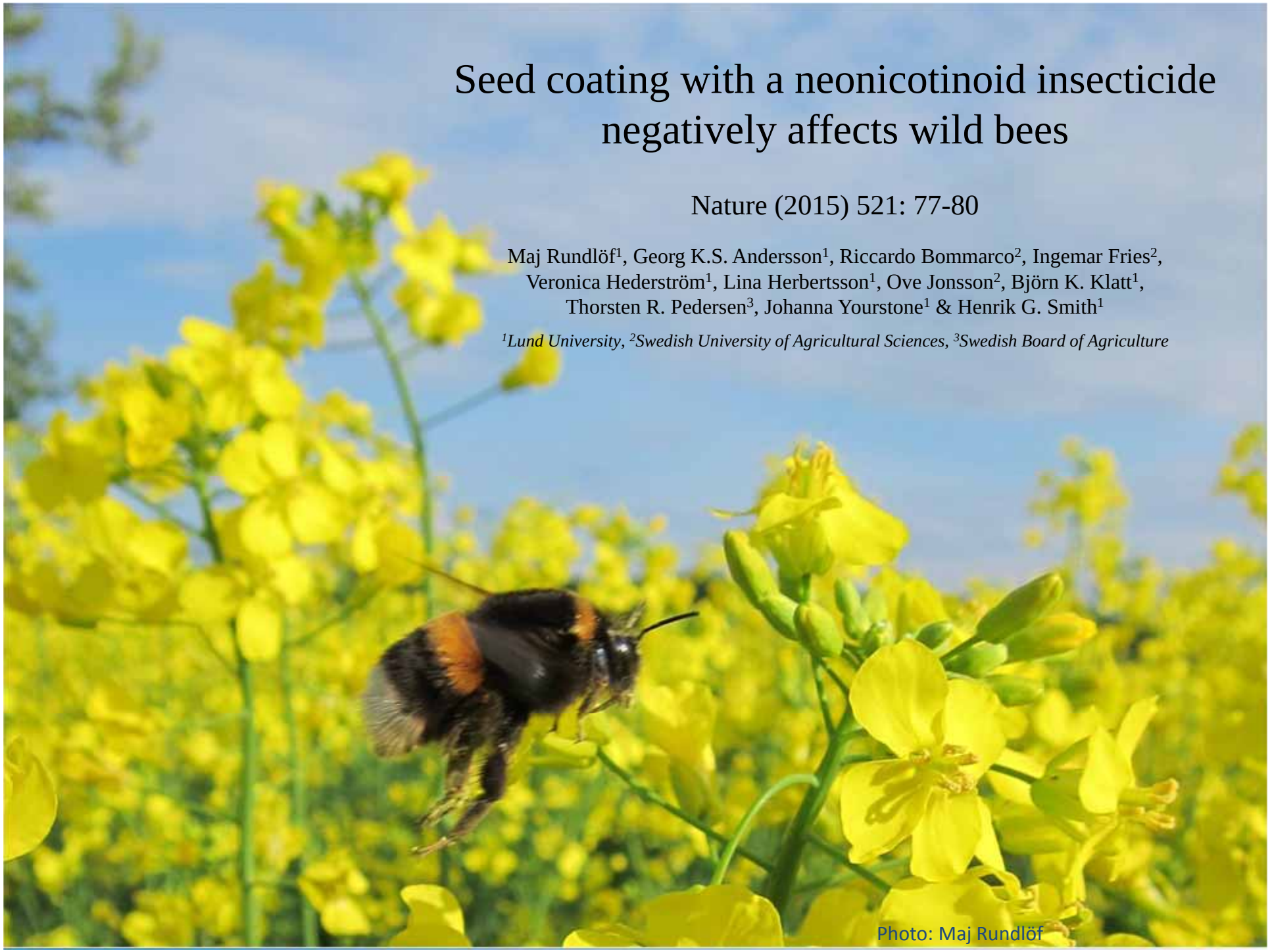


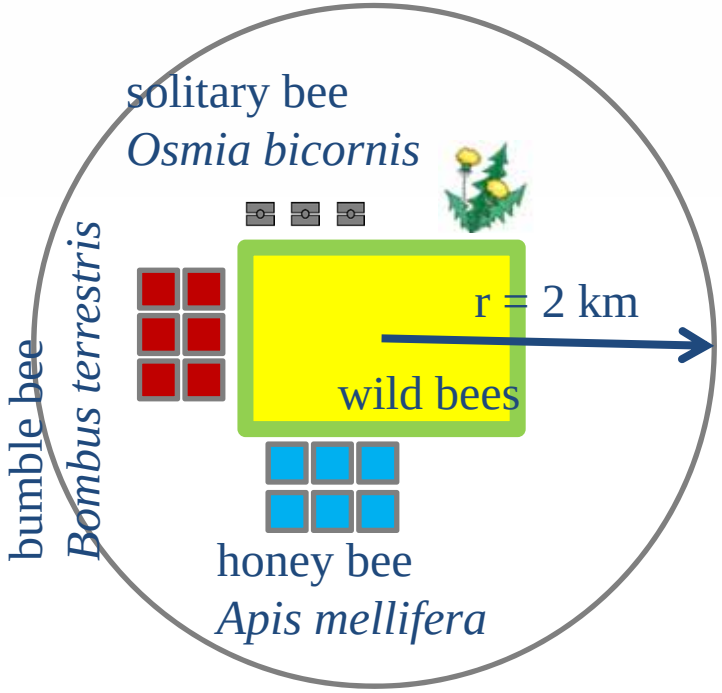
Photo: Maj Rundlöf

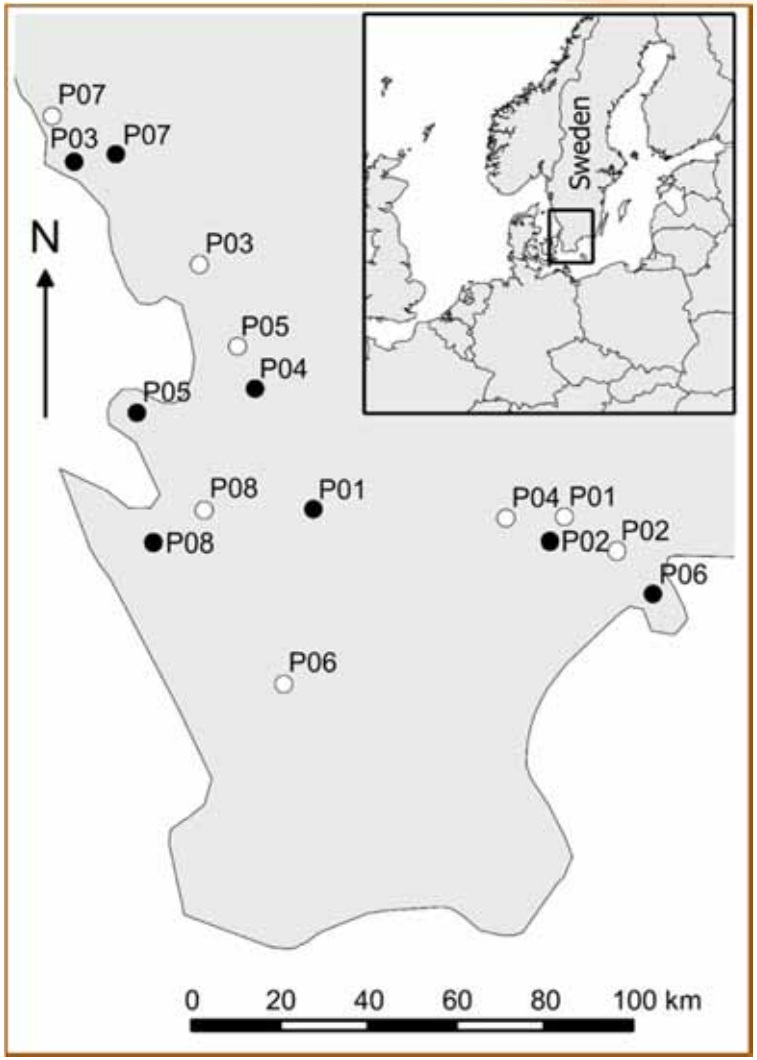
# Background

- Spring sown oilseed rape (just over 40 % in Sweden)
- Neonicotinoid (mostly clothianidin) seed dressing to protect seedlings
- Lack of well designed replicated field studies, particularly for non-honeybees

Flea beetle (*Phyllotreta* sp.)
Photo: Maj Rundlöf

# Landscape ecotoxicology experiment




- 8 replications (field pairs based on land-use)
- random assignment to treatment/control
- treatment blinded during field work

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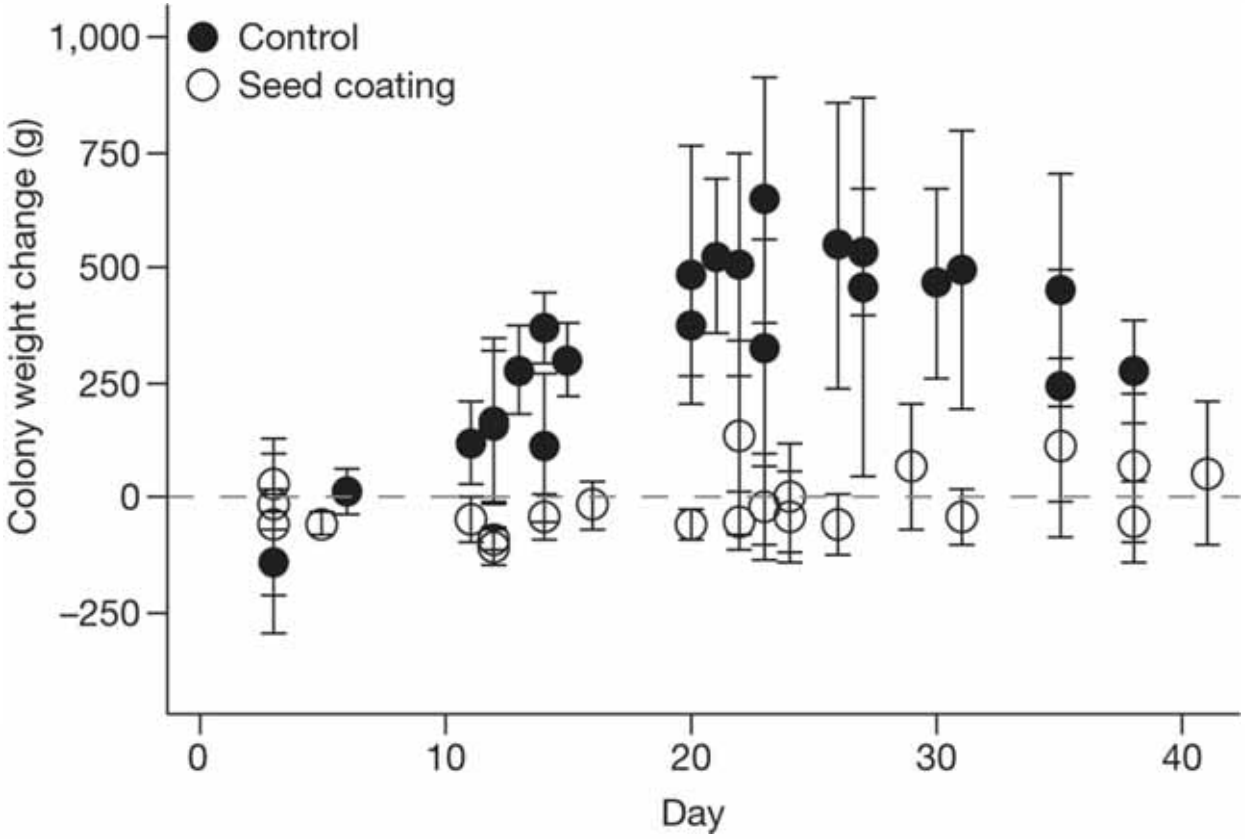
# Results

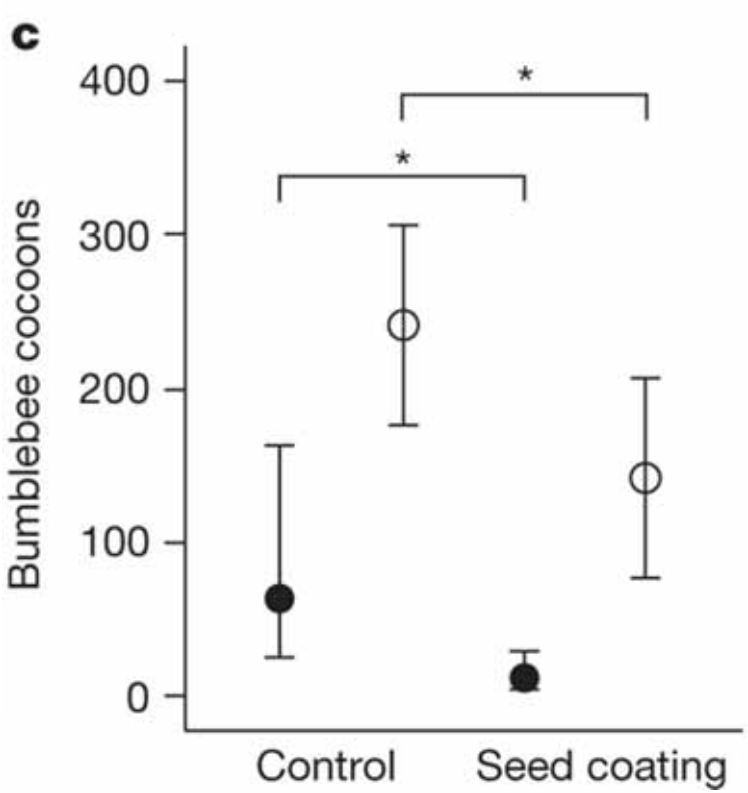
## The clothianidin treatment was negatively related to *Bombus terrestris* colony growth




Photos: Maj Rundlöf

Rundlöf et al. (2015) Nature 521: 77-80.

# …and *Bombus terrestris* reproduction



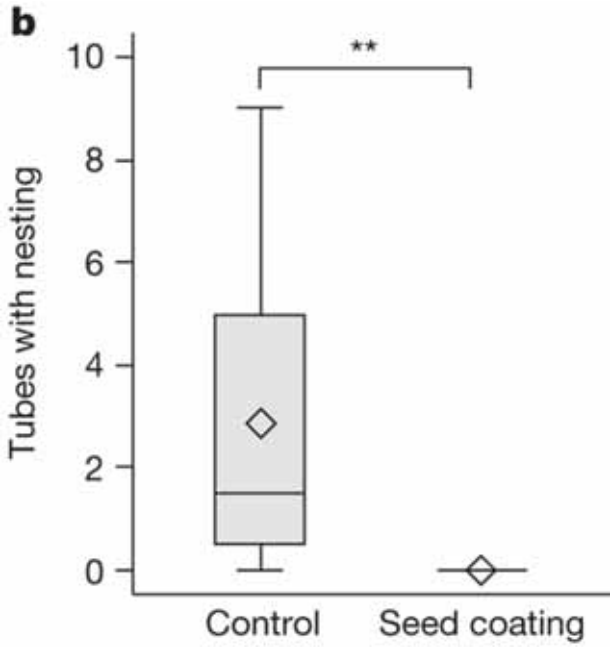

Queens
Workers/males

**Queens: -85%**

Photos: Maj Rundlöf

Rundlöf et al. (2015) Nature 521: 77-80.

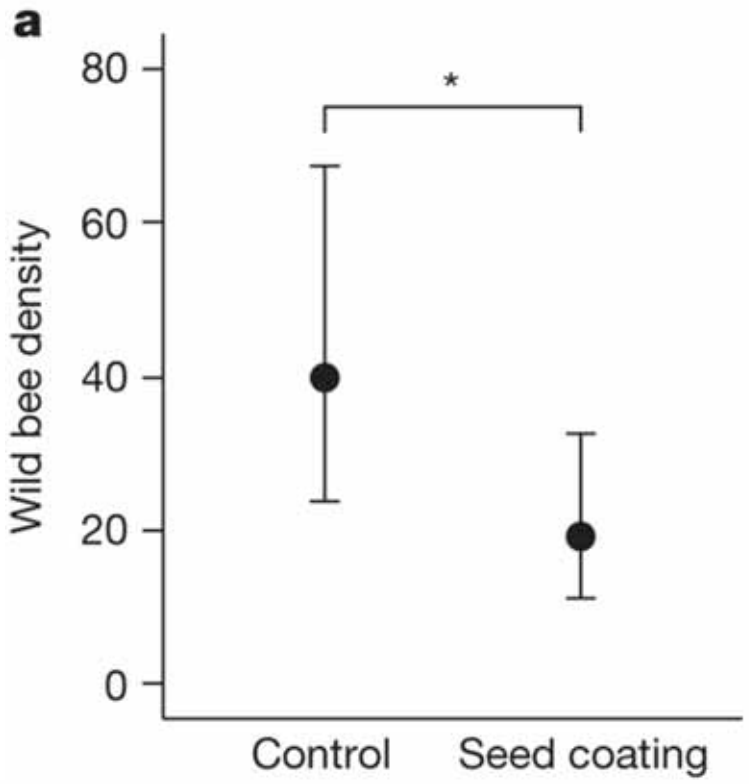
# Reduced nesting of solitary bee *Osmia bicornis* (red mason bee) in tubes placed close to the fields




Photos: Maj Rundlöf

Rundlöf et al. (2015) Nature 521: 77-80.

# Reduced wild bee density in treated fields




Wild bees are in Sweden bumble bees and solitary bees.


Photo: Maj Rundlöf


Photo: Morgan Boch


Photo: Maj Rundlöf

Rundlöf et al. (2015) Nature 521: 77-80.

# The clothianidin seed treatment had no significant influence on *Apis mellifera* colony strength

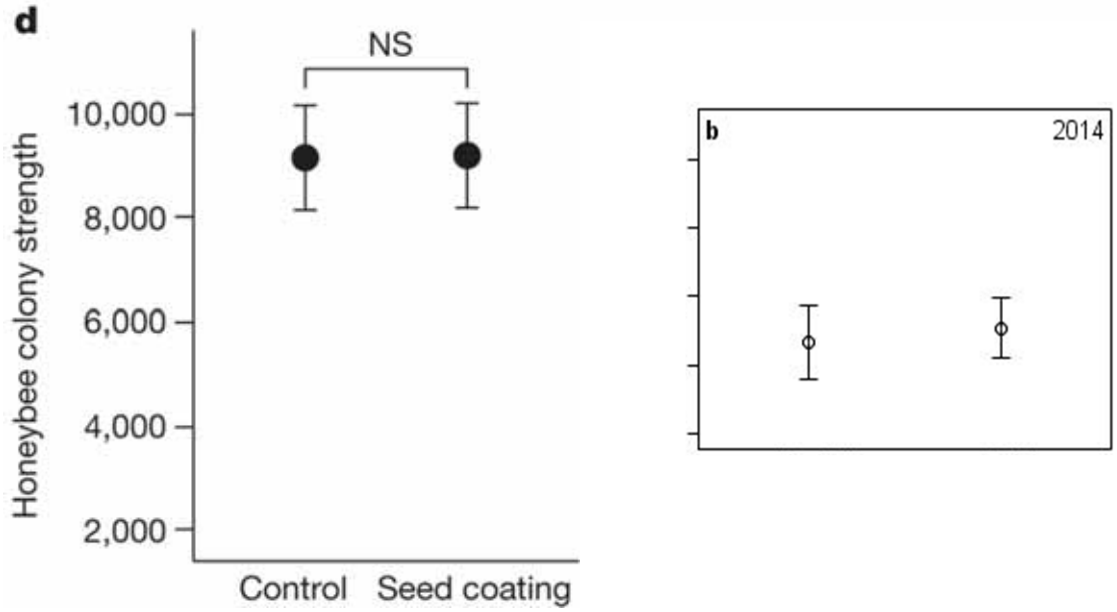




Photo: Maj Rundlöf

Not always possible to extrapolate ecotoxicology results for honey bees to wild bees or other insect species.

Rundlöf et al. (2015) Nature 521: 77-80; Rundlöf et al. (2015) Project report, Lund university

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# Chemical analysis


5 neonicotinoid insecticides studied:

Acetamiprid
Imidacloprid
Clothianidin
Thiacloprid
Thiamethoxam

Internal standards:
Imidacloprid D4
Clothianidin D3
Isoproturone D6 (used for acetamipride, thiacloprid and thiamethoxam)

Agilent 6460 LC-MS/MS, on-line SPE (C18 and polymer)
C18 analytical column, methanol gradient in ammonium formate [1]

[1] C. Jansson and J. Kreuger 2010 J. AOAC Int., Volume 93, Number 6, 1732-1747

# Sample preparation bees (and pollen)

- 24 bees weighed and homogenized with drying agent, subsample corresponding to four bees
- internal standard to subsample homogenate
- extract twice under strong sonication with 7:3 mixture of acetone and ethyl acetate
- dispersive solid phase extraction, C18 and PSA
- evaporate to dryness at 40°C under nitrogen flow
- residue dissolved in 150 µl acetonitrile
- 10 µl injected on LC-MS/MS

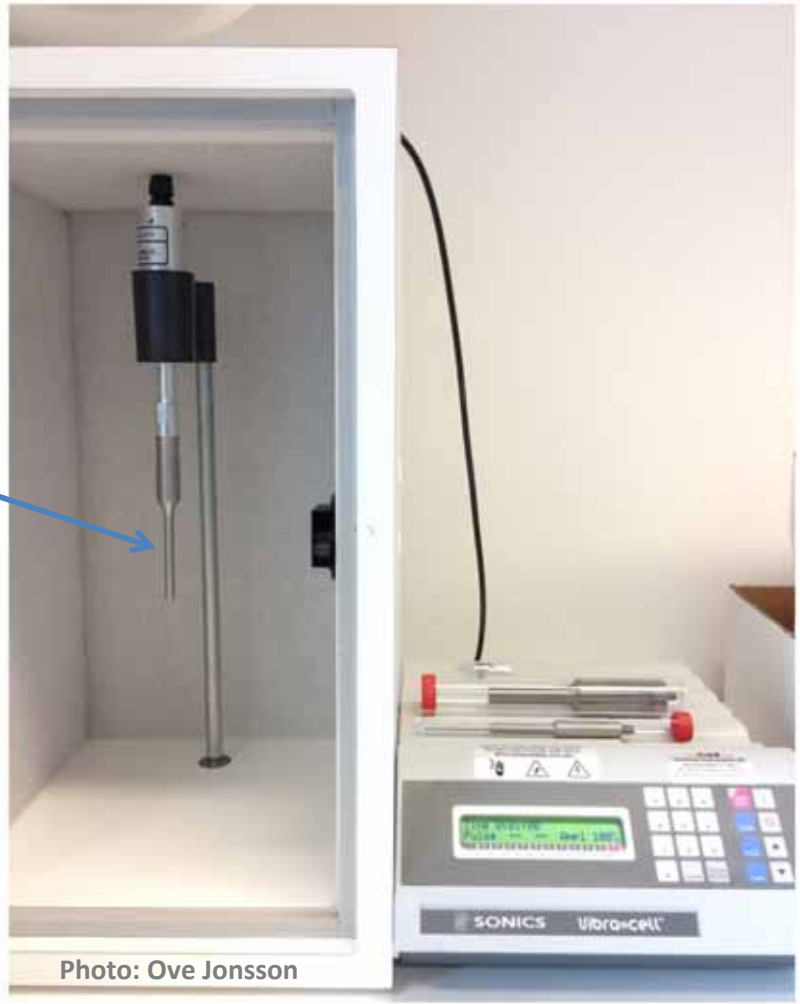
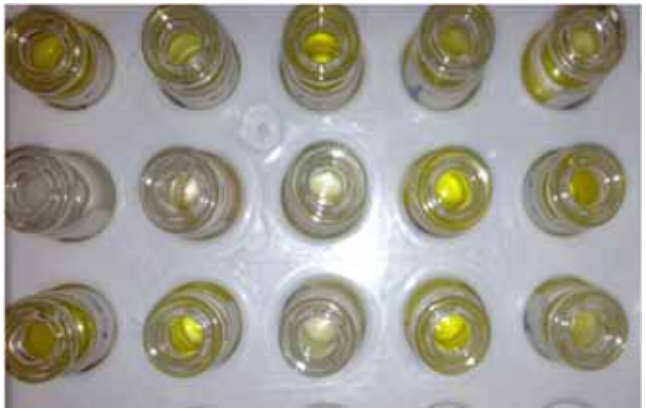



Photo: Ove Jonsson

Vibra-Cell VCX 130 (Sonics)

# Bees and pollen are varying matrices



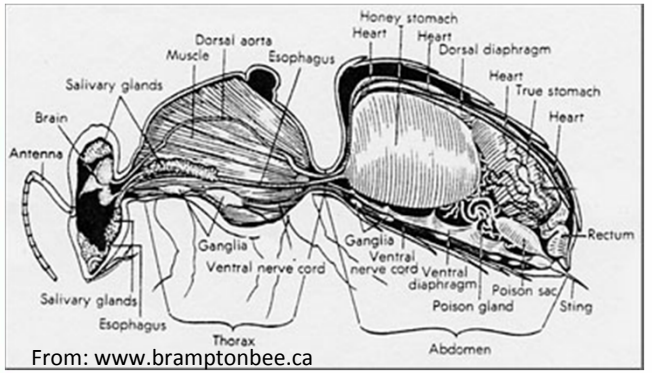
Photos: Ove Jonsson

Extracts from different bee samples.

Need for robust and selective methods!

# Collection and handling of nectar samples

Capillary with exact 8 µl nectar (deviation and %RSD <1%)


8 µl capillary in 1 ml tube
Capillary Microsampling (CMS) [1,2]


- IS washout solution
- acetonitrile protein crash
- LC-MS/MS


Intestine content

Nectar

Analysis of bee tissue and nectar from one single bee!

1. Capillary microsampling of 25 µl blood for the determination of toxicokinetic parameters in regulatory studies in animals. Jonsson O., Palma Villar R., Nilsson L.B., et al. Bioanalysis 2012, 4(6), page 661–674
2. Capillary microsampling. Jonsson O., in eBook: Microsampling in Pharmaceutical Bioanalysis, edited by Zane P. and Emmons G.T. Future medicine 2013

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# Clothianidin field exposure

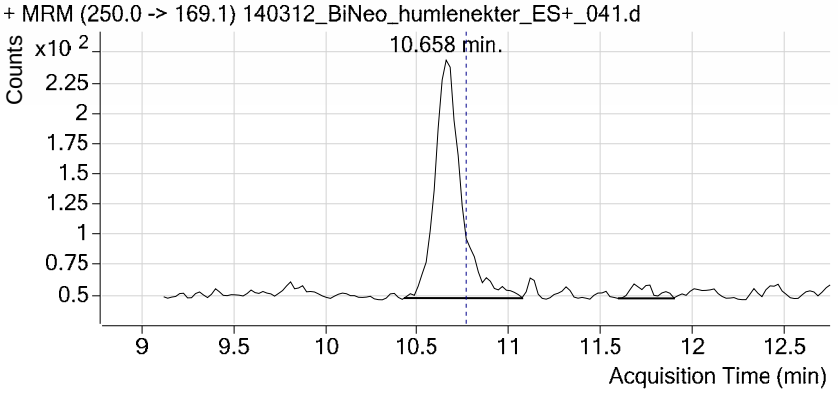
| (ng/g or ml) | Control (C) | | Seed coating (T) | | N (C/T) |
|---|---|---|---|---|---|
| **2013** | range | mean ± se | range | mean ± se | |
| **Honey bees** | <LOD-0,89 | 0,13 ± 0,11 | 0,35-4,9 | 2,4 ± 0,50 | 8/8 |
| **Pollen from honey bees** | <LOD | <LOD | 6,6-23 | 14 ± 1,8 | 6/8 |
| **Nectar from honey bees** | <LOD-0,61 | 0,11 ± 0,080 | 6,7-16 | 10 ± 1,3 | 8/8 |
| **2014** | | | | | |
| **Honey bees** | <LOD | <LOD | 0,15-1,5 | 1,1 ± 0,20 | 4/6 |
| **Pollen from honey bees** | <LOD | <LOD | 2,4-16 | 6,1 ± 2,0 | 4/6 |
| **Nectar from honey bees** | <LOD | <LOD | 2,6-9,8 | 4,9 ± 1,1 | 4/6 |

LOD: 0.080-0.50, LOQ: 0.25-1.5 ng/g or ng/ml

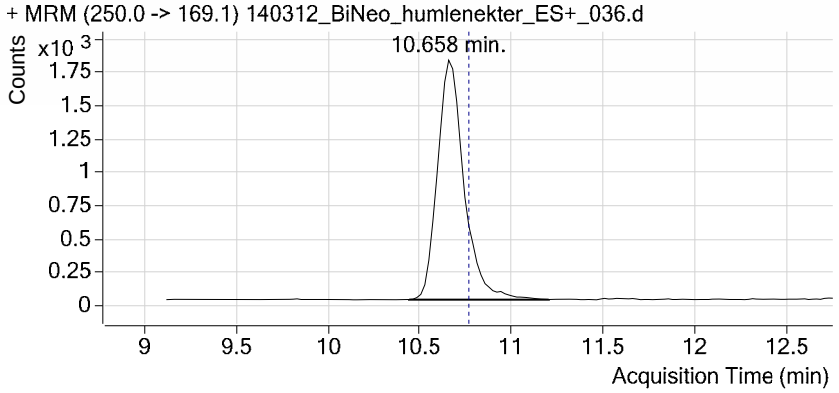
Rundlöf et al. (2015) Nature 521: 77-80;
Rundlöf et al. (2015) Project report, Lund university

# Clothianidin chromatogram from nectar samples

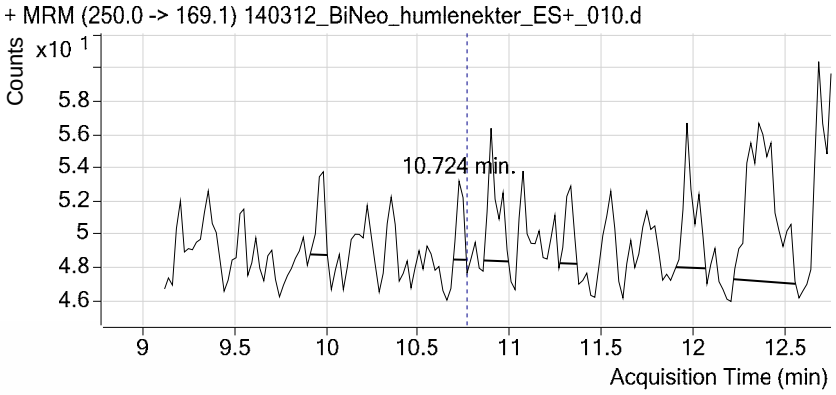
## Concentration 0.84 ng/ml



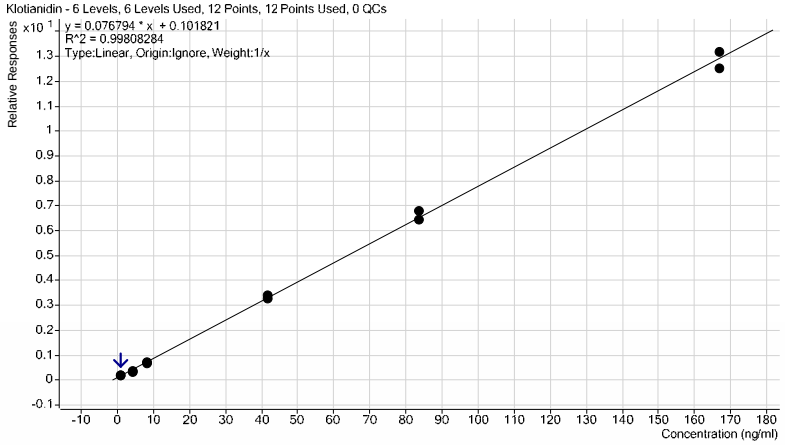

## Highest concentration in nectar sample 20 ng/ml




## Nectar sample from control field




## Calibration curve clothianidin in nectar

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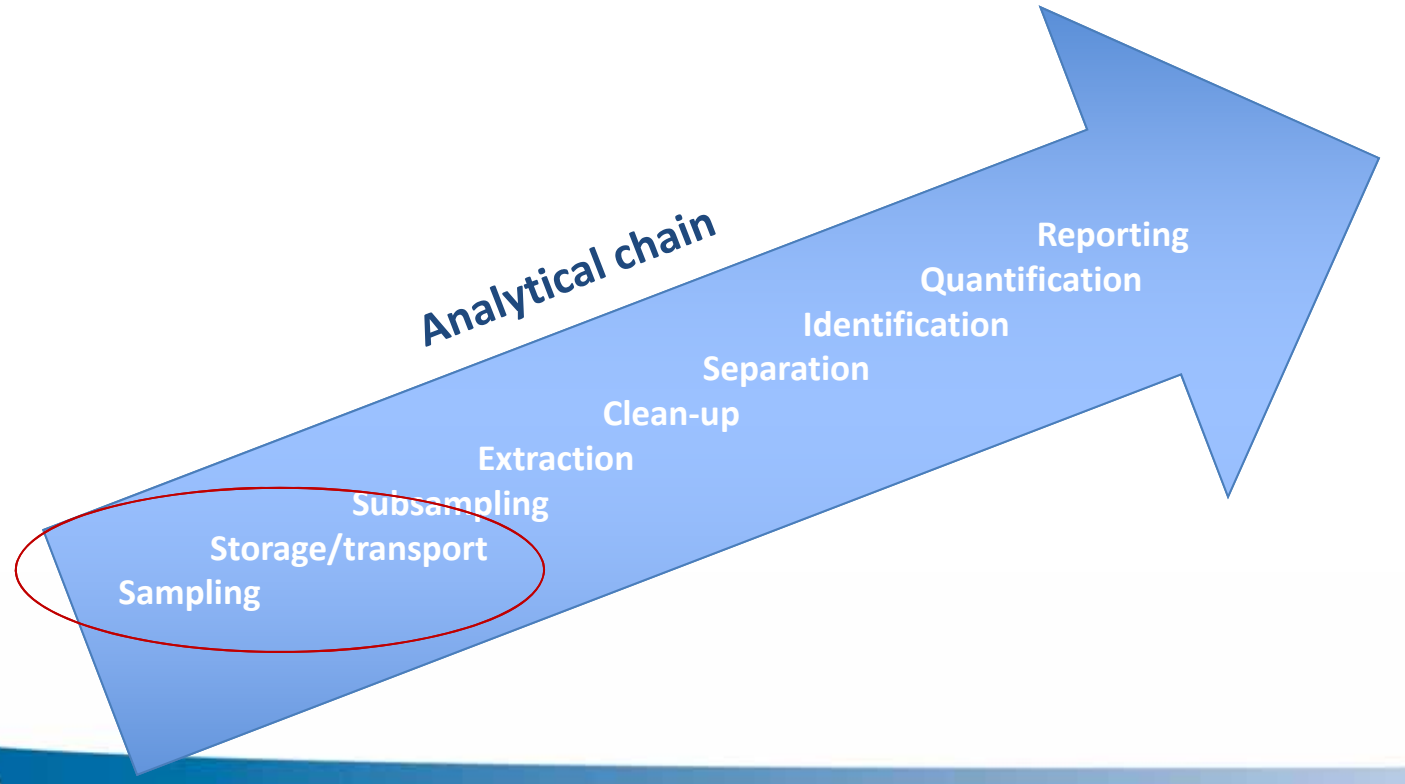
## 1. Individual exposure variation within a bee society

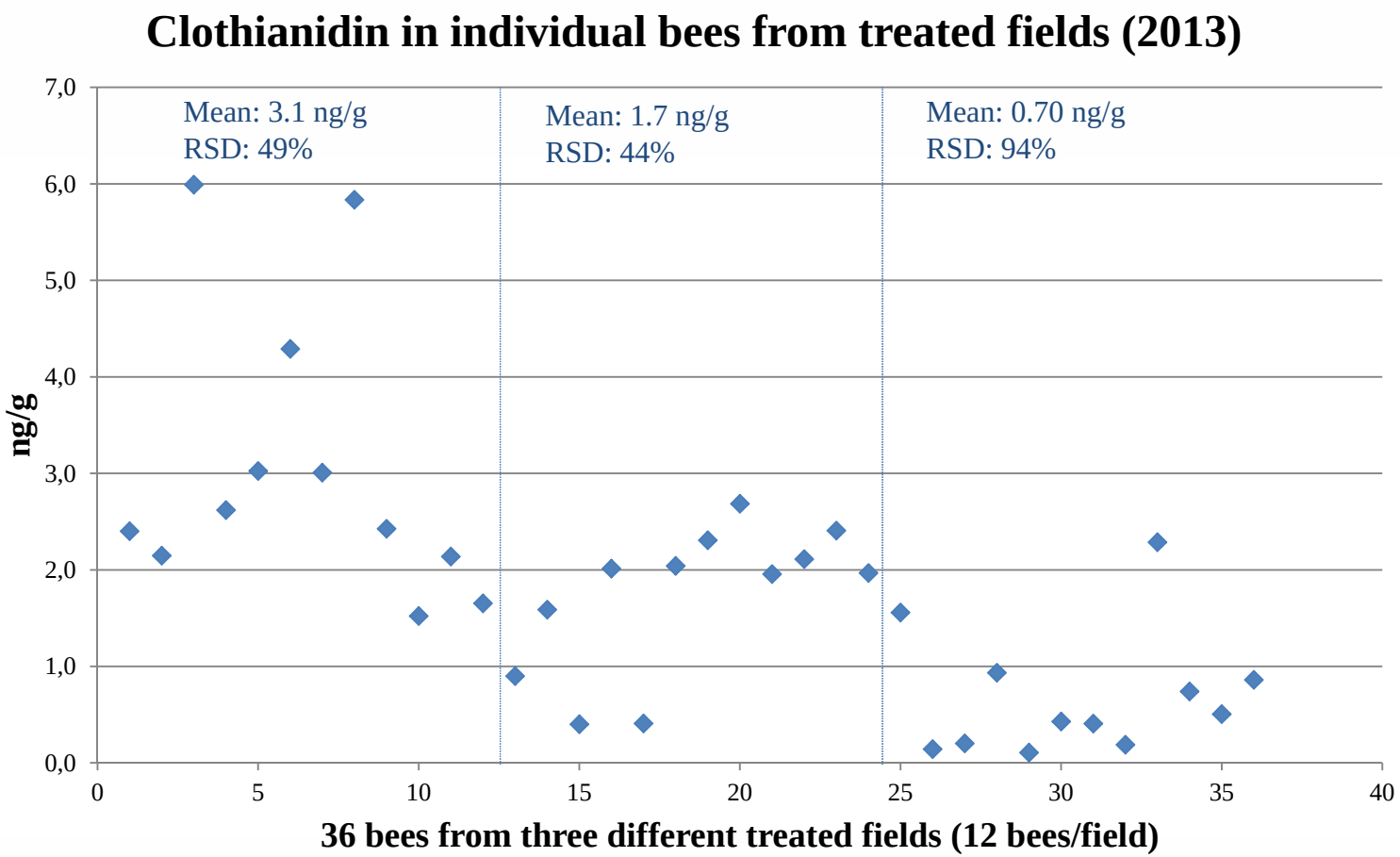
Representative sampling

## 2. Stability of pesticides in stored bees

Important step in quality assurance

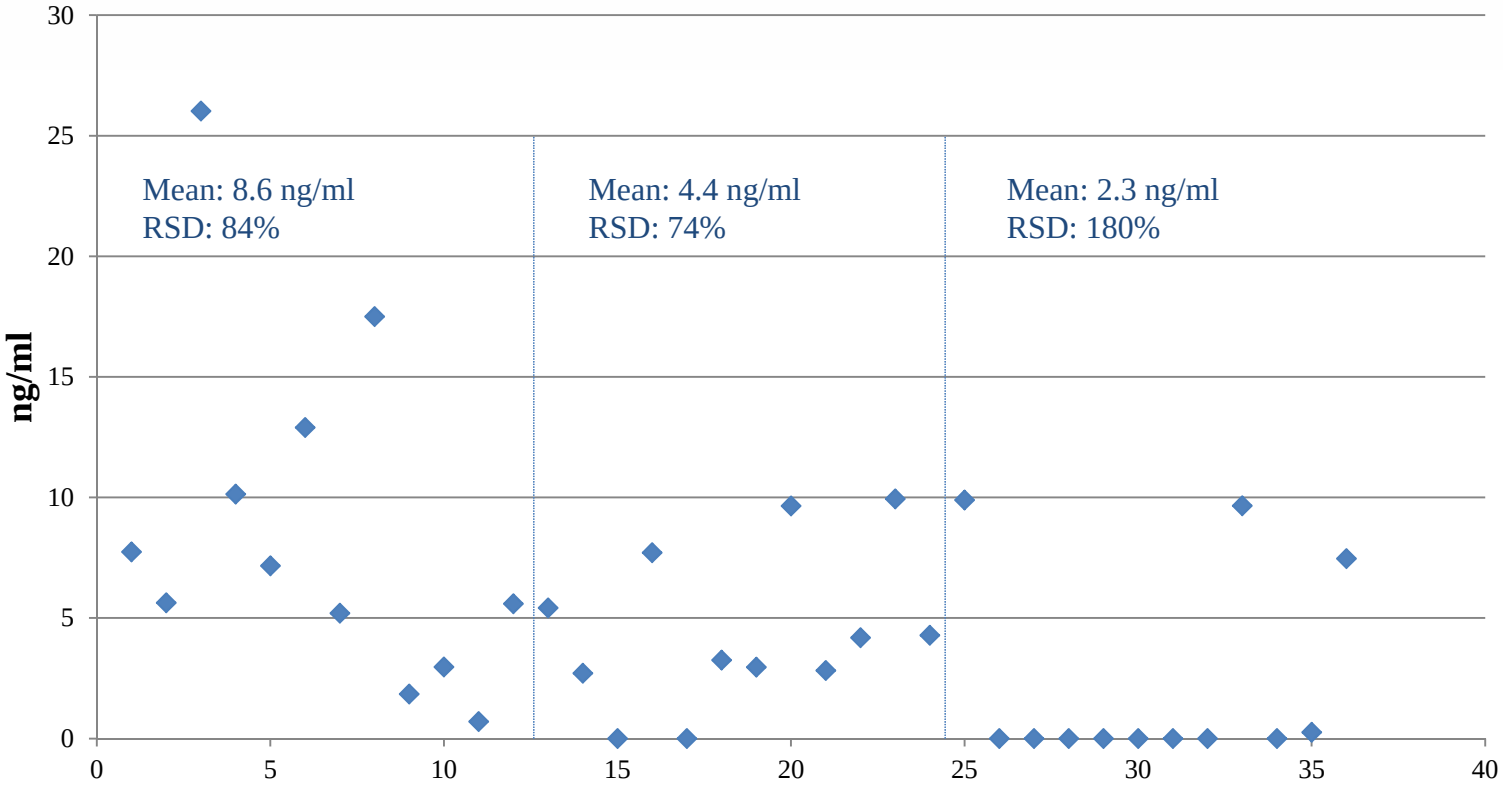


# Individual exposure




Overall mean: 1.8 ng/g
RSD: 77%

# Clothianidin in nectar from individual bees, collected in three treated fields (2013)




**Nectar from 36 bees from three treated fields, (12 from each field)**

Overall mean: 5.1 ng/ml
RSD: 111%

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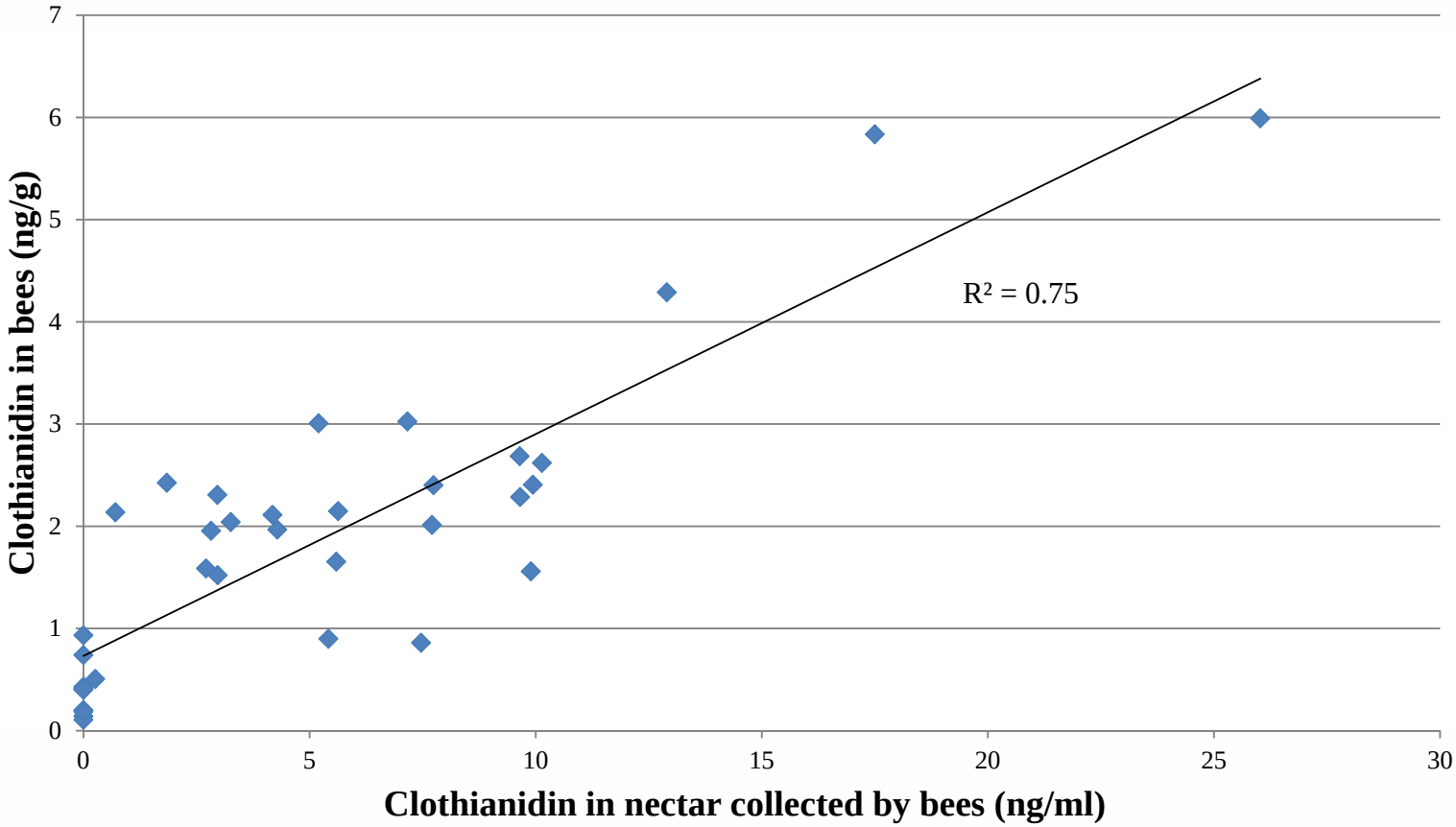
# Clothianidin concentrations in 36 bees and the nectar they collected from treated fields

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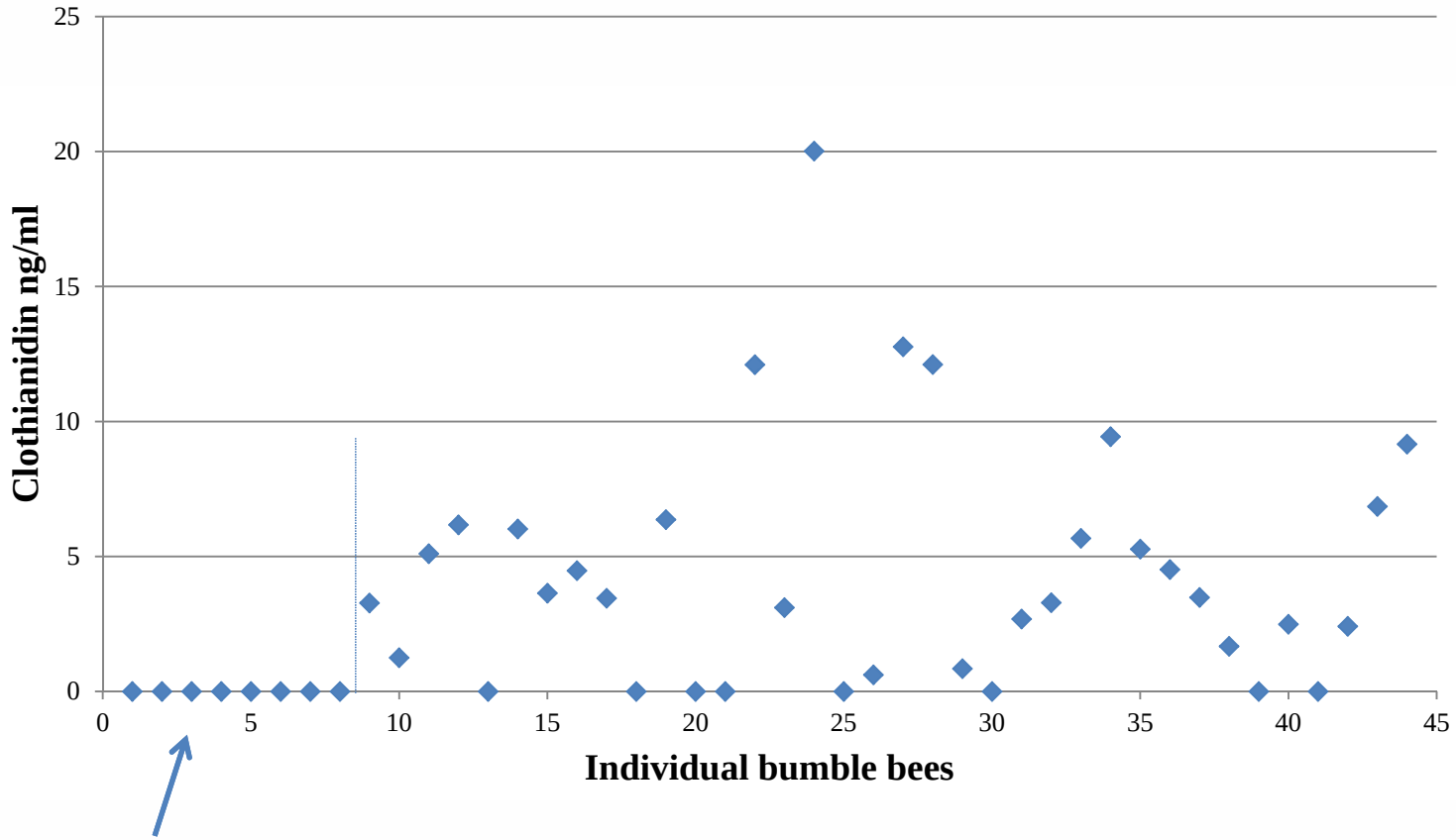
## Clothianidin in nectar collected by bumble bees




Pooled samples from
8 control fields

Overall mean treated fields: 4.4 ng/ml
RSD: 103%

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# Pesticide stability in bees after individual feeding

Individual feeding with 10 µl sugar solution containing pesticide mix – Relevant spiking!

Evaluate stability *in vivo*, in room temperature (dead bees) and after long time freeze storage at -20°C


Professor Ingemar Fries, SLU, feeds a bee.

Freezer and room temp:
All five neonicotinoids were stable in freezer for 21 months and at room temperature for 22 hours.

*In vivo:*
Clothianidin was stable for 1 h while acetamiprid, imidacloprid, thiacloprid and thiamethoxam showed degradation with 44, 44, 43 and 22%, respectively.

Each estimate was based on triplicate samples and four bees per sample (in total 12 bees)

# Acknowledgement

**Swedish EPA and the Centre for Chemical Pesticides for financial support to develop analytical methods**

**Maj Rundlöf for support with field study slides**

**Prof. Ingemar Fries, SLU, for feeding bees**

**Jenny Kreuger, CKB and SLU, for support and scientific discussions**

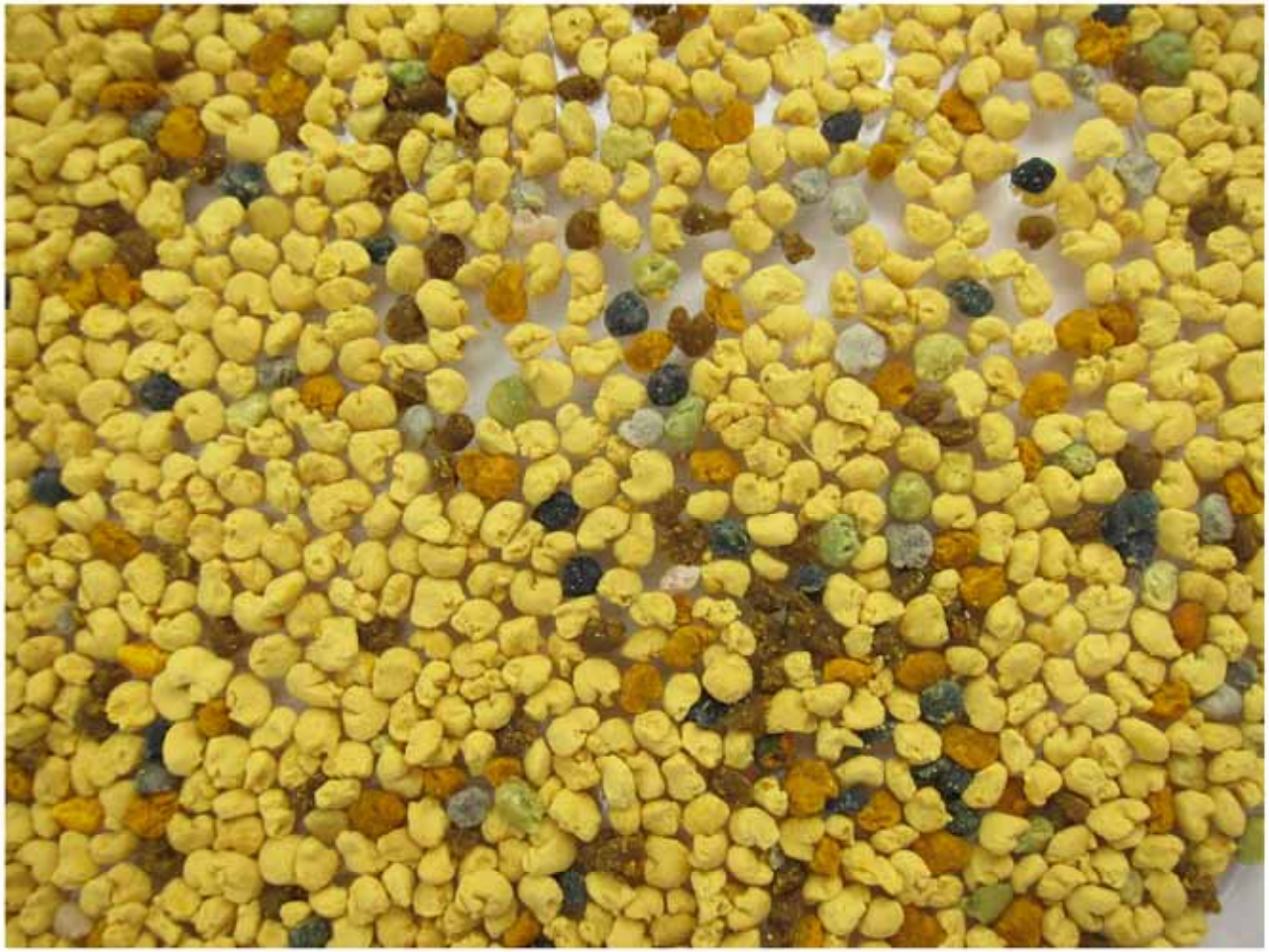

**Photo: Maj Rundlöf**

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Thank you
for your time!