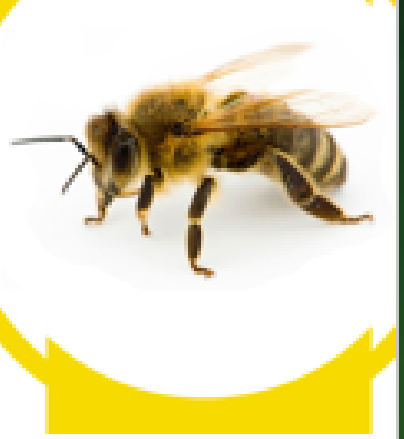
EFFECTS OF PESTICIDE USE ON THE DEVELOPMENT OF BEE DISEASES-ANALYTICAL AND ECOTOXICOLOGICAL THREATS AND CHALLENGES

National Institute for Agricultural and Food Research and Technology, INIA

7th- 8th Novembrer 2016



BEE FORUM PROGRAMME

Study of the effects of neonicotinoids on pollinator populations. Sunflower crop and seed treatment

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UNIVERSIDAD D CÓRDOBA

syngenta

BAYER Bayer CropScience

Context of the Project:

- **Field study – experiment "aimed at establishing cause-and-effect relationships"**

  field vs monitoring studies (MS): designed to satisfy different objectives.

  MS "to be aware of the state of a system, to observe a situation for any changes which may occur over time"

- **Higher tier, for a specific PPP and crop. Designed to study the effect of application of PPP on bee colonies under GAP, good agronomic practices**

  - **PPP:** Thiamethoxam, Chlotianidin. Restricted use since 2013 in EU
  - **Application:** coated seed
  - **Crop:** sunflower, one of the most important grass crop in Southern of Spain

- **Parameters of field studies: test requeriments defined by EPPO 170 (2010), revised by EFSA (2013)**

***EPPO**, European and Mediterranean Plant Protection Organisation; Guidance 170 (2010), "Efficacy evaluation of plant protection products. Side-effects on honeybees"*

***EFSA**; Guidance 2013 on the "Risk assessment of plant protection products, PPPs, on bees (Apis mellifera, Bombus spp. and solitary bees)*

Experimental design

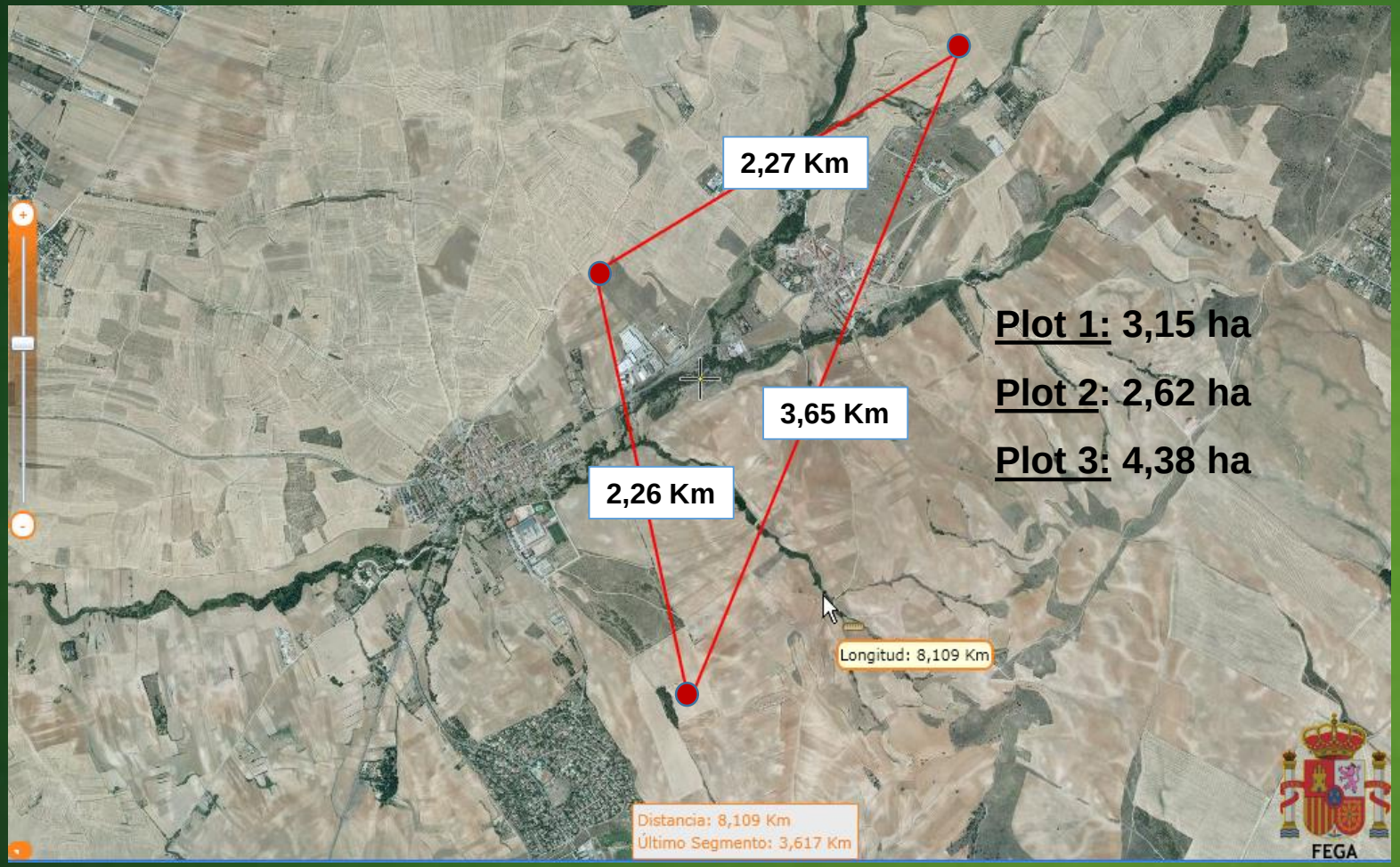
Under the current risk framework, defined by GD EPPO 170 and EFSA 2013, test requirements include the following parameters for full-field studies:

- **Proof of exposure:** fields should be chosen so that bees are exposed mainly to the treated field in which the hives are placed; 90th percentile of exposure);
  Trial conditions: surface of crop plots, distance between field plots; (test fields should be well separated to minimise bees foraging on alternative plants); treatments (study items and untreated control); replicates (number of hives per modality)

- **Exposure and effect assessment**:
  - Pollen analysis; food stored
  - Residue analysis
  - Several biological observations: study duration
    Primary assessment endpoints
    Secondary assessment endpoints

Proof of exposure:

Experimental unit 1: Valdeolmos, Madrid



Surface of plots: at least 2 ha

Spatial isolation: a minimum of 2 km
nearby fields:
-areas with non-attractive crops
-bloom periods not coincident (attractive crops)
-fewer opportunities to forage in the surroundings. in wild habitats

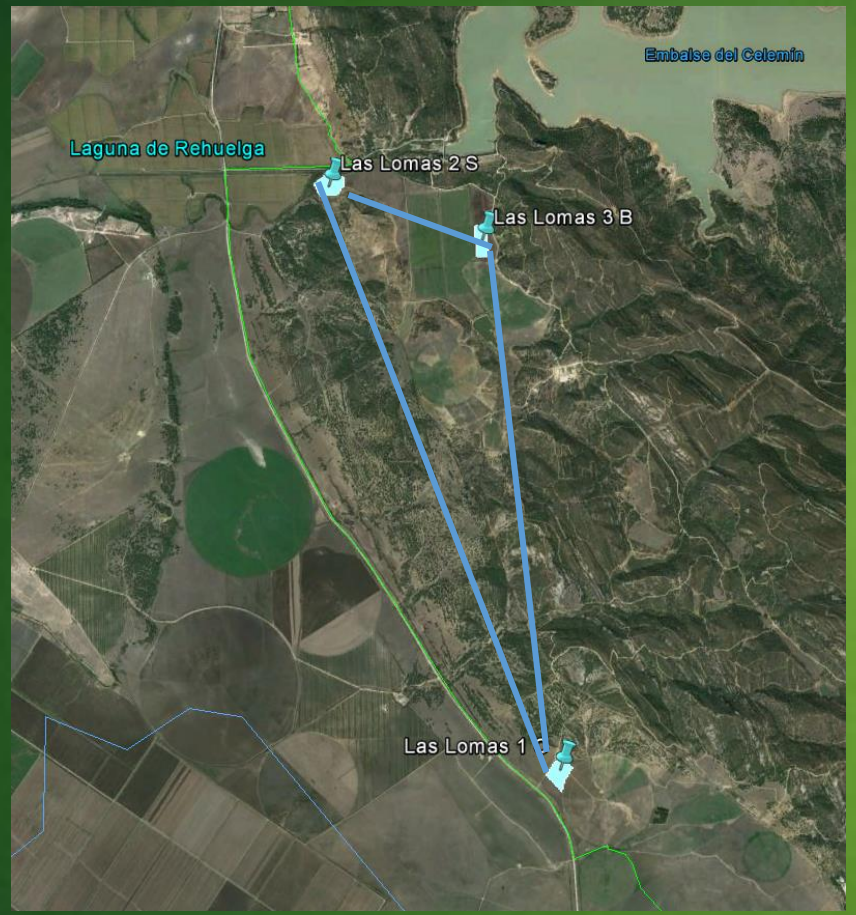
Cropping history:
-previous use of PPP: eg. herbicide residues, sulfonylureas in wheat

Topographic and climatic conditions:
Block of fields (study items and untreated control)

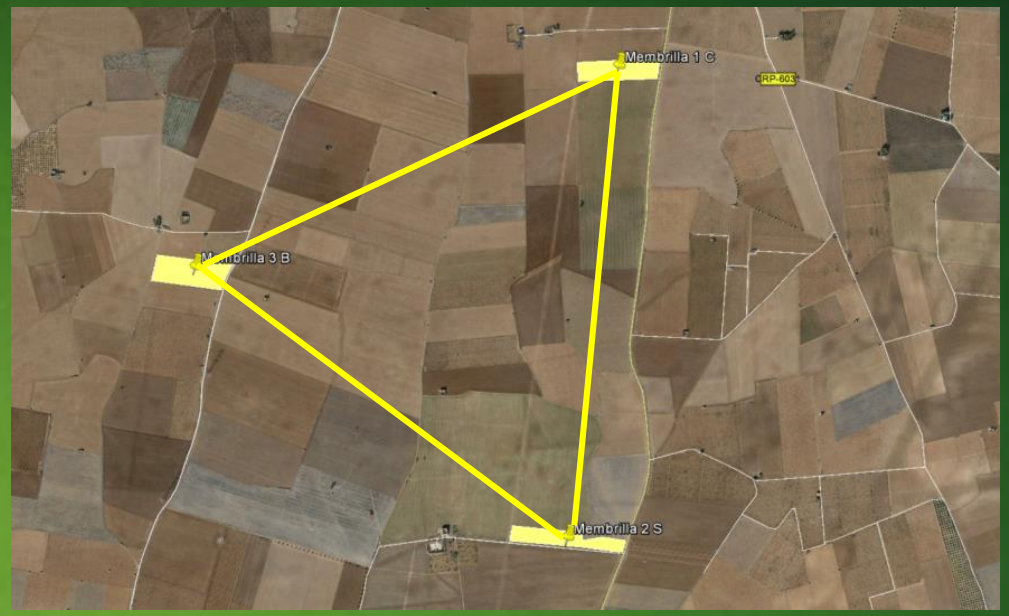
Variety of sunflower seed

Approval by the Competent Administration
Authority to conduct a research study

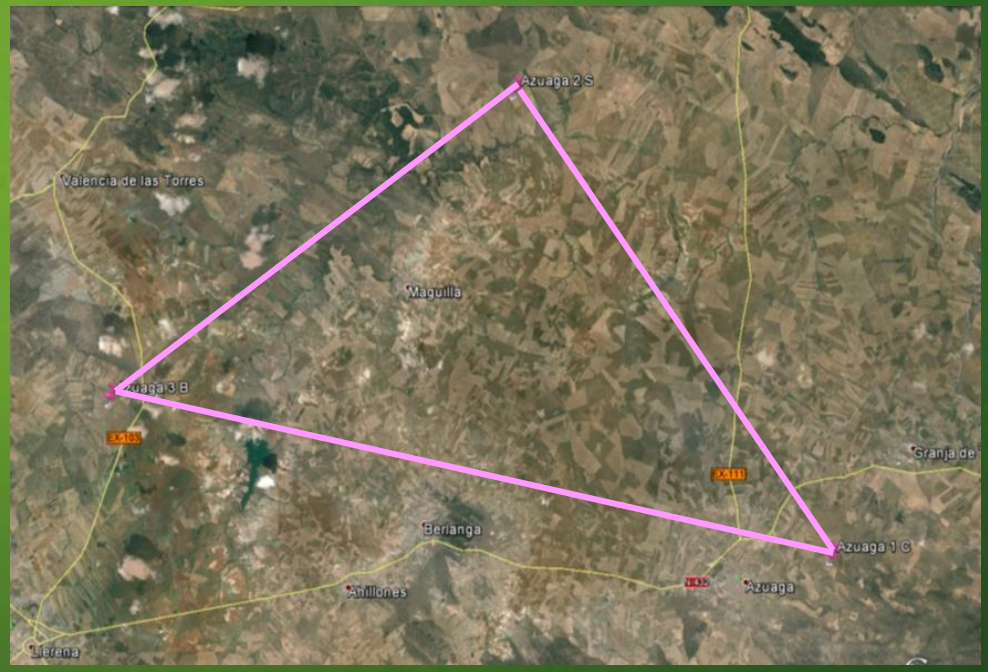
Experimental unit 2: Las Lomas, Cádiz



Experimental unit 3: Membrilla, Ciudad Real



Experimental unit 4: Azuaga, Badajoz



Replicates, blocks and number of hives per modality

Protection goal for field studies:

(i) the magnitude of effects on colonies should not exceed 7%.
(ii) foragers mortality should not be increased compared with controls by a factor 1.5 for 6 days, a factor of 2 for 3 days, or a factor of 3 for 2 days.
(iii) over-wintering success

	Number of replicate blocks (n)											n for	n for	Variance	
	3	4	5	6	7	8	9	10	12	15	20	7 % ES	20 % ES	τ^2	σ^2
Honeybees															
Peak colony strength (T1)	>20	>20	>20	19.9	18.6	17.5	16.5	15.8	**14.5**	13.1	11.5	**55**	**6**	0.013	0.053
Rate of colony strength increase (T1)	>20	>20	>20	>20	>20	>20	>20	>20	**>20**	>20	>20	**765**	**80**	0.178	0.742
Overwintering colony weight (T1)	15.9	13.9	12.5	11.5	10.7	10	9.5	9	**8.3**	7.5	6.5	**17**	**2 ***	0.004	0.016
Overwintering colony strength (T1)	>20	>20	>20	>20	>20	20	18.9	18	**16.6**	15.0	13.2	**75**	**20**	0.017	0.075
Mean colony strength	>20	>20	20	18.5	17.4	16.3	15.5	14.7	**13.5**	12.2	10.7	**51**	**5**	0.012	0.049
Dead bees	>20	>20	>20	>20	>20	>20	>20	>20	**>20**	>20	>20	**242**	**26**	0.076	0.137
Counts on OSR	>20	>20	>20	>20	>20	>20	>20	>20	**>20**	>20	>20	**264**	**28**	0.096	0.083
Colony weight average	17.2	15.2	13.7	12.6	11.7	11	10.4	9.9	**9.1**	8.2	7.1	**20**	**3**	0.004	0.023
Overwintering % nectar cell area	>20	>20	>20	>20	>20	>20	>20	>20	**19.8**	17.9	15.7	**112**	**12**	0.040	0.038
Overwintering % pollen cell area	>20	>20	>20	>20	>20	>20	>20	>20	**>20**	>20	>20	**348**	**37**	0.089	0.298
Overwintering % empty cell area	18.5	16.3	14.7	13.5	12.5	11.8	11.2	10.6	**9.7**	7.6	7.6	**24.8**	**3**	0.009	0.008

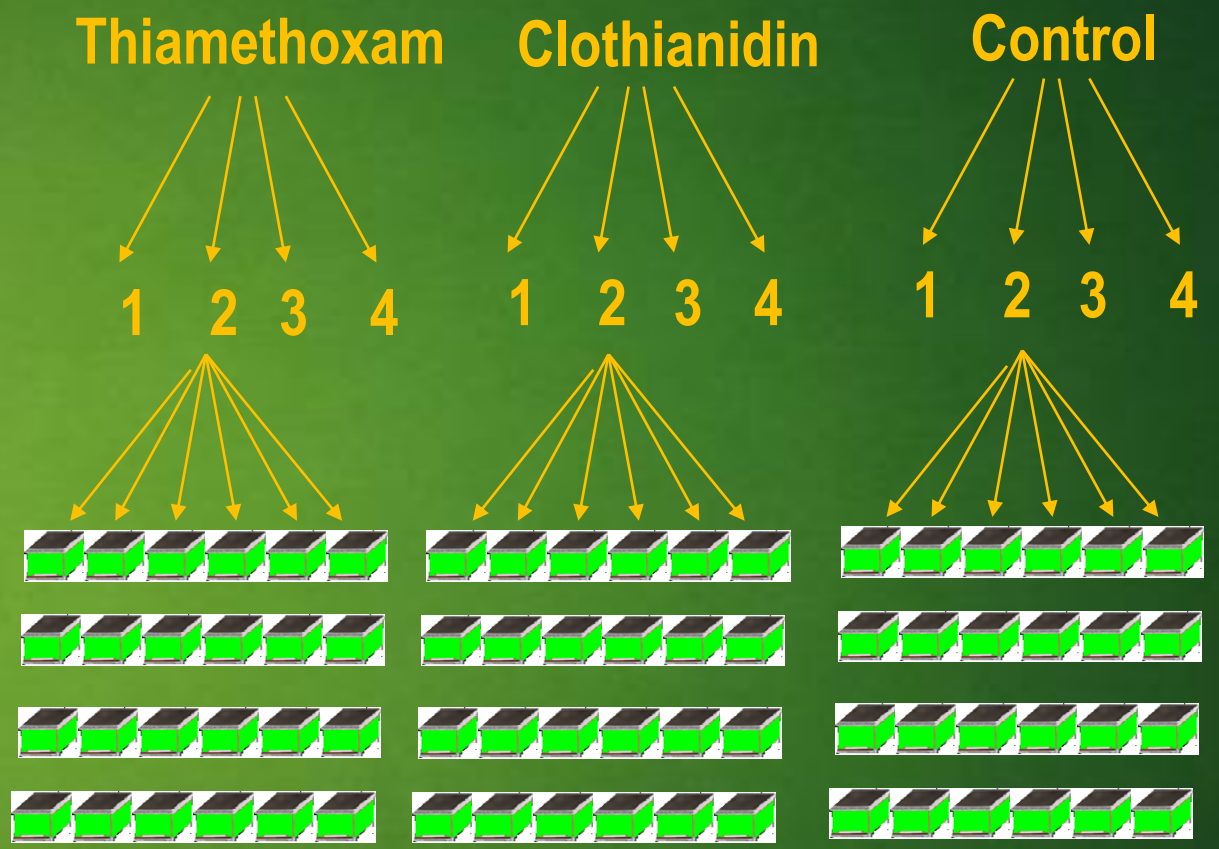
* Note a min. of three replicate required; †Assuming 5 × honeybee; n = 20% is the number of blocks to find 20% effect size.

Variance parameters are determined from data within Pilling et al. (2013: PLoS ONE **8**:e77193) and focus on oilseed rape associated bee colonies only, where τ^2 = between site variance in response parameter, equivalent to the CV^2 and σ^2 = within site between colony variance, equivalent to $ln(1+CV^2)$. Power analysis is based on a **modification** of that proposed by EFSA (2013: EFSA Journal **11:3295**:266). Where effect sizes exceed the minimum 20 % detection rate (indicated by >20) the required number of replicate blocks to detect both a 7 (column 'n for 7 % ES') and 20 % effect size (column 'n for 20 % ES') are given. Note that all measures of colony strength for honeybees are recorded using the Liebefeld method and the rate of increase in colony strength is based on a Pearsons correlation coefficient of the increase in Liebefeld colony strength over the first 5 weeks of monitoring in a given year. Variables with the suffix T1 are considered to be core Tier 1 parameters of key importance in assessing honeybee responses to Neonicotinoids.

4 Experimental units: blocks
3 years

Locations of blocks: 1st year

Replicates:
blocks of plots and number of beehives



24 hives

24 hives

24 hives

Exposure and effect assessment:

Pollen composition:

At least 23% of pollen in honey samples from almost all experimental colonies originated from *Helianthus annuus*

Large areas of monoculture could have detrimental effects on the nutrition and health of bees.

Poor nutrition could have major implication in causality.
Poor nutrition makes them more vulnerable, weaken their immune system.

As side-effect on health of bees, poor nutrition therefore is liable to interfere with the establishment of a direct cause and effect relationships, as proposed in this study

Honey bees require a balanced diet of sugar, protein, vitamins and minerals.

Exposure and effect assessment:

Residue analysis:

The assessment of PPP exposure resulting from neonicotinoid seed treatment, **requires of a high sensitive analytical methodology**. To support statistical analysis, for establishing cause-effect relationship, analytical study of PPP residues comprises a large number of samples

- **Bee wax:** foundation wax used for brood frames
- **seed treated:** determination of experimental doses
- **stored hive products (pollen, nectar)**
- **larvae and bee**

nectar

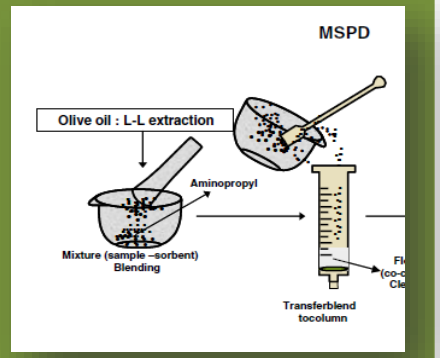
pollen

larvae

forager bee

4 samples x 4 (t exp) x 72 hives= 1152 samples per year

SAMPLE TREATMENT



Extraction recovery of pesticide residues from bee matrices

Acceptable: > 70%

e_1

UNCERTAINTY

$$e_c = \sqrt{(e_1)^2 + (e_2)^2}$$

Questionable > 50%

e_2

MASS SPECTROMETRY ANALYSIS

Reproducibility of measurements (LOQs)

Acceptable ≤20%

Thiamethoxam
Rec = 79%, RSD= 12%

Chlotianidin
Rec = 85%, RSD= 16%

Thiamethoxam
MQL, LC-QqQ-MS=0.1 ppb (μg.Kg^{-1})
LC-Orbitrap-MS, semi-quant < 0.1 ppb

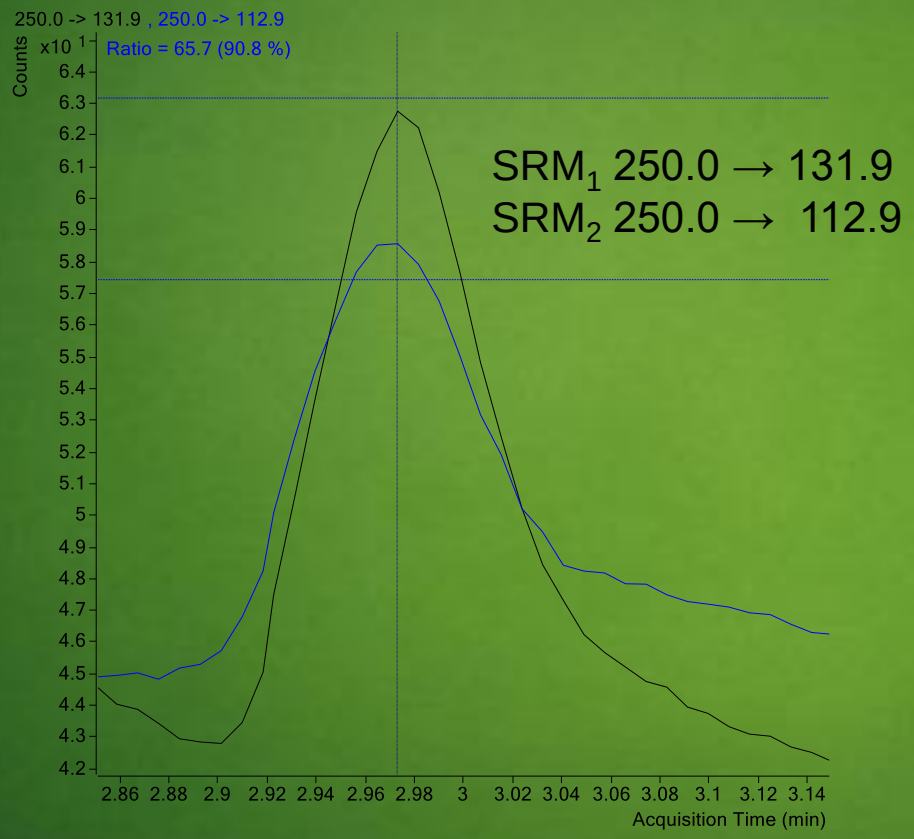
Chlotianidin
MQL, LC-QqQ-MS=0.5 ppb (μg.Kg^{-1})
LC-Orbitrap-MS, semi-quant < 0.5 ppb

LC-QqQ-MS

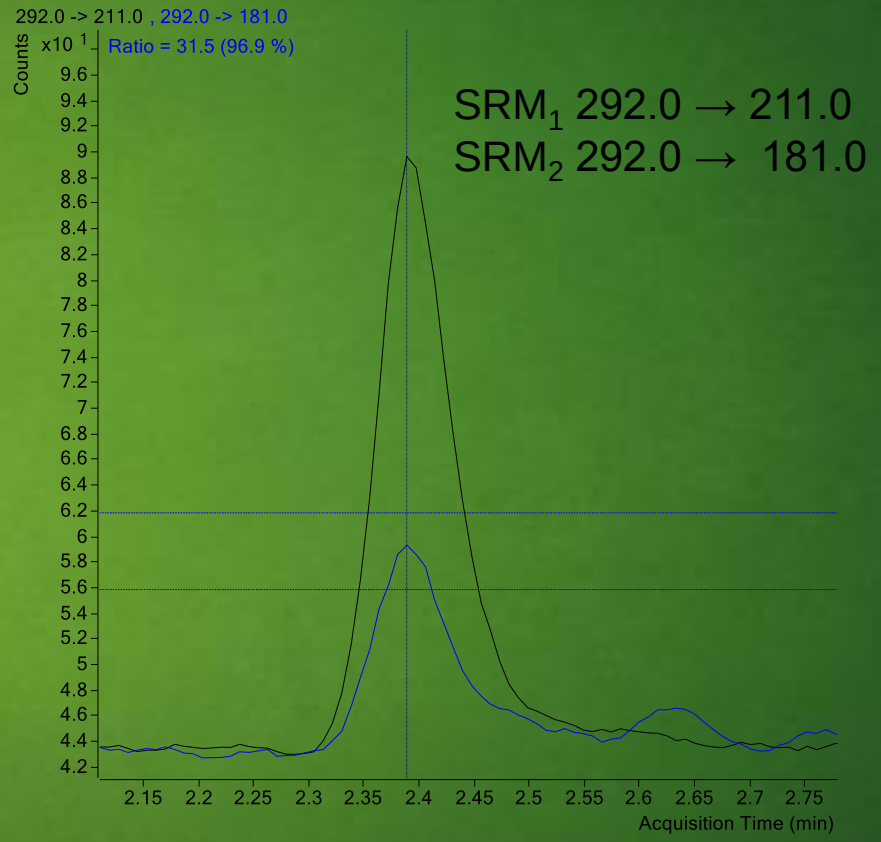
Mass spectrum example

Bees (sample dilution 12.5 times before injection)

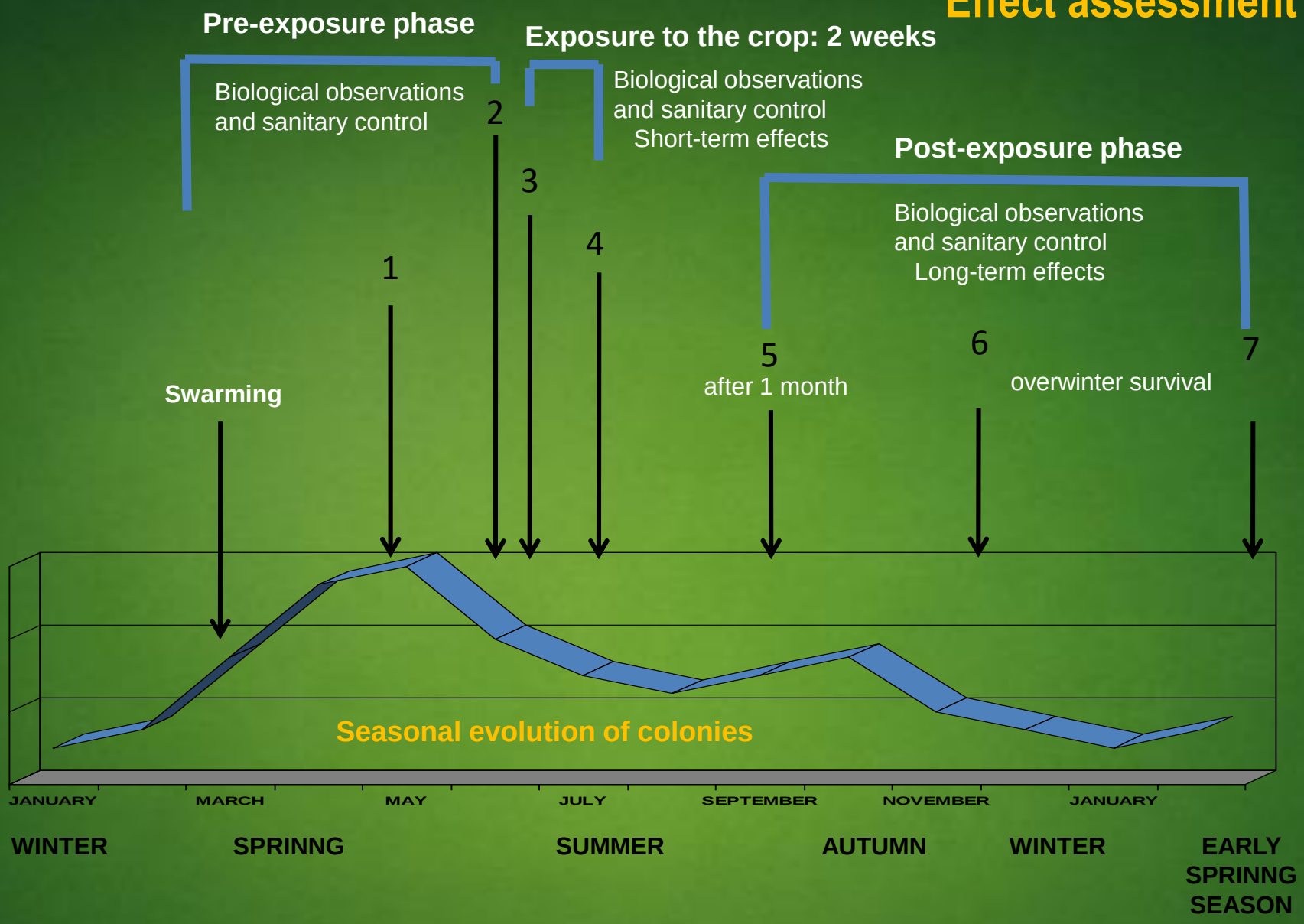
Clothianidin, 5 ppb.



Thiamethoxam 5 ppb



Effect assessment at colony level



Biological observations and sanitary control

Primary assessment endpoints: forager mortality, colony strength (number of bees), over-wintering success

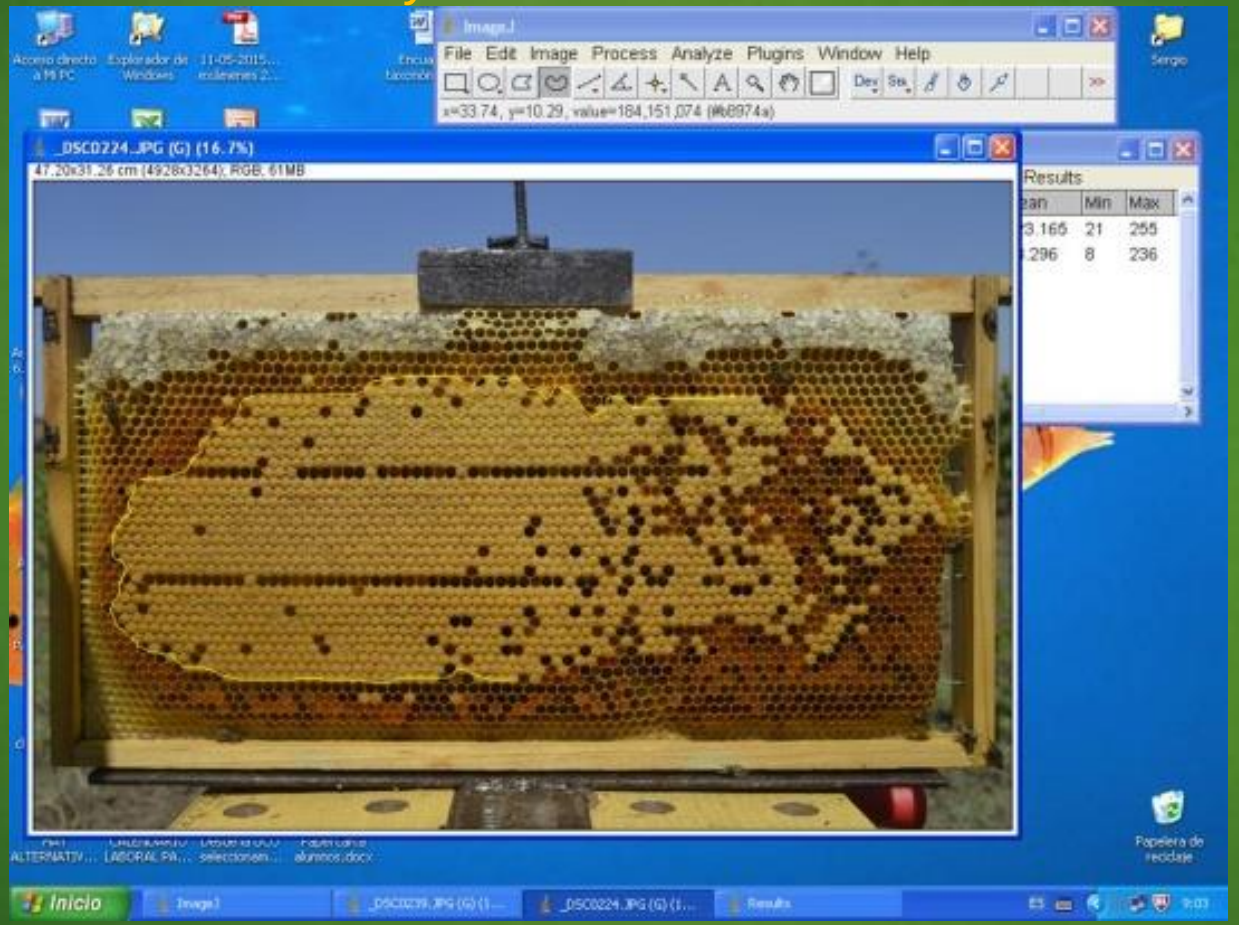
Secondary assessment endpoints: behavioural effects- behaviour of foragers on flowers and returning to the colony, behaviour of guard bees at the colony entrance)

Primary assessment endpoints

- Colony size and success assessment (hive weight) & adult bee population (Liebefeld method) .
- Area of brood (opened and sealed)
- Pollen stored inside hives
- Honey yield

Biological observations

Colony performance based observations such as brood, food stores (pollen and honey): Image processing and statistical analysis



72 beehives X 10 frame/hive X 2 sides/frame X 7 repetitions = 10.080 photographs

Thank you very much