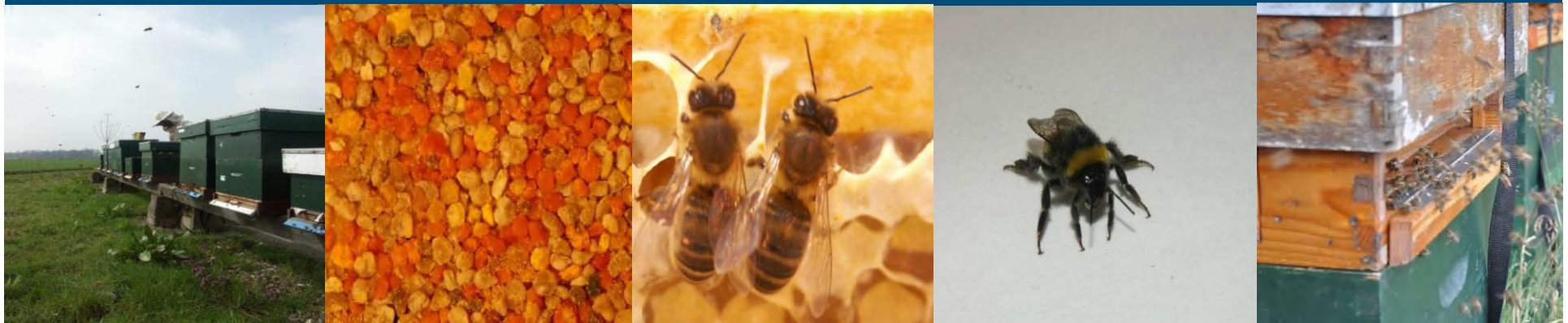


Vitellogenin as a parameter of honey bee colony vitality

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24 Sept. 2011



Vitality individual bees / colony

Individual bees / hoarding cages

- Age related physiological condition
- Early death first week 20-50%
- Queen less stress
- Number of bees

Colony

- Super organism
- Trophallaxis
- Feed back mechanisms
- Age related physiological condition



Parameters vitality colony

- Hemolymph protein
 - Vitellogenin
 - HSP
 - Immune related proteins
 - Defensin-1
 - Hymenoptaecin
 - abaecin
- # worker bees
- # cells sealed brood
- # cells bee bread
- Food gland development
- Fat-protein body
- others



Why vitellogenin as parameter of the vitality of a honeybee colony?

- Main storage protein (91% protein, 7% fat and 2% sugar).
- Larvae, queen, workers and drones feed.
- Conversion of yolk protein to larval food is compatible with the physiological condition of the nurse bee.
- Increase in vitellogenin reverses immunosenescence
- The vitellogenin titer in winter bees is **relatively high**.
- The winter bees of European honey bees, *Apis mellifera*, have a relatively high vitellogenin titer. The feature to build up vitellogenin is an evolutionary adaptation and an important condition in order to live long and survive the winter.



Vitellogenin



Vitellogenin, bees, sealed brood cells are related to each other via feed back systems

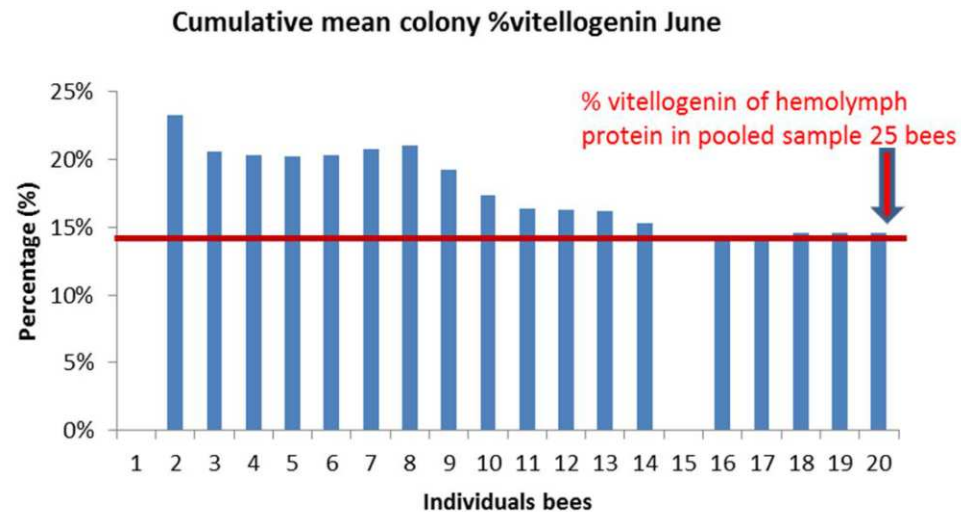
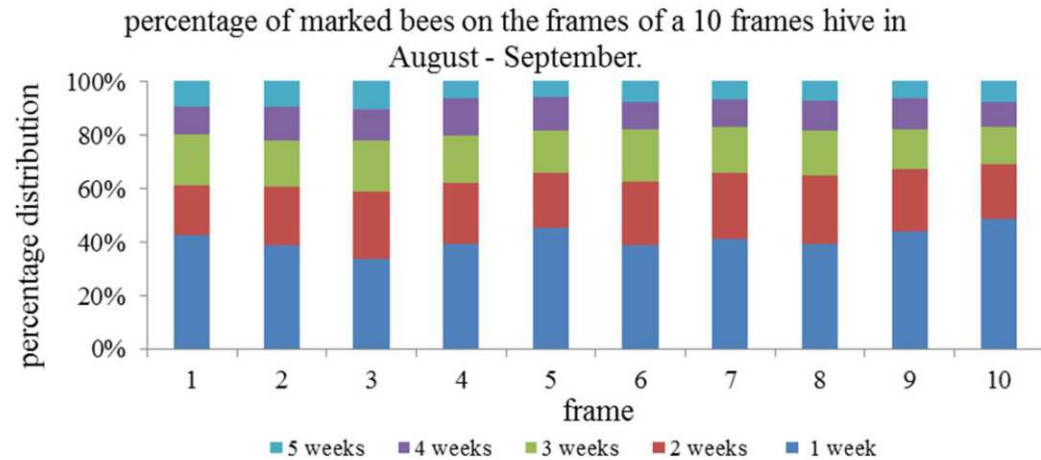
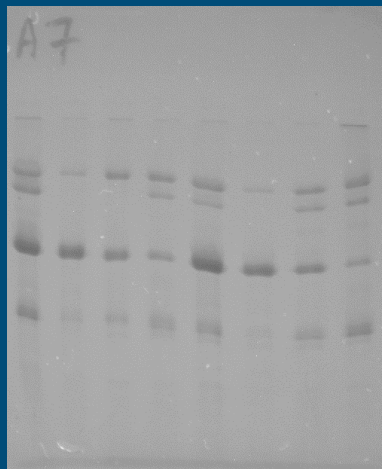
Therefore study of vitellogenin, in combination with # bees, # brood on colony level, to establish whether disrupting factors affect vitellogenin synthesis.



Colony protein / vitellogenin assessment

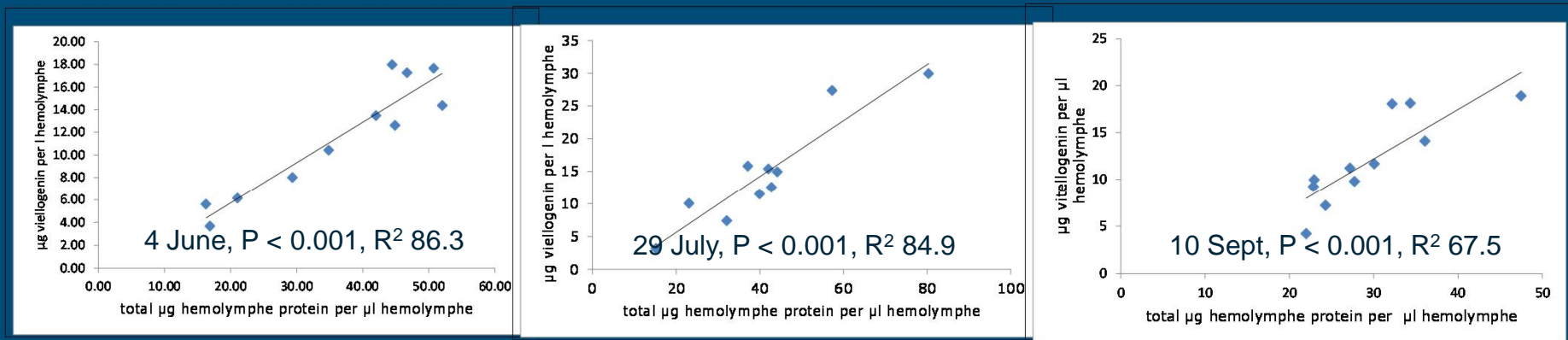
Sample size per colony: 25 bees

Analysis: Phast electrophoresis system



Vitellogenin can be expressed as

1. μg vitellogenin / μl hemolymph



2. Percentage vitellogenin of total hemolymph protein



Why the parameter: vitellogenin percentage?

Significant correlation between total hemolymph protein and vitellogenin titer.

However, hemolymph with a certain amount of protein can have relatively more or less vitellogenin (expressed as percentage).

- The result of better or worse capability to synthesize vitellogenin due to

1. Varroa? (individual proven, colony ?)

3. pollen amount and diversity? (individual proven, colony?)

4. pesticides (hormone analogues)

5. others

Due to natural variation (age class distribution, environment) only an overall reduction of the percentage vitellogenin can be detected on colony level



Vitellogenin percentage, brood and Varroa (2008 study)

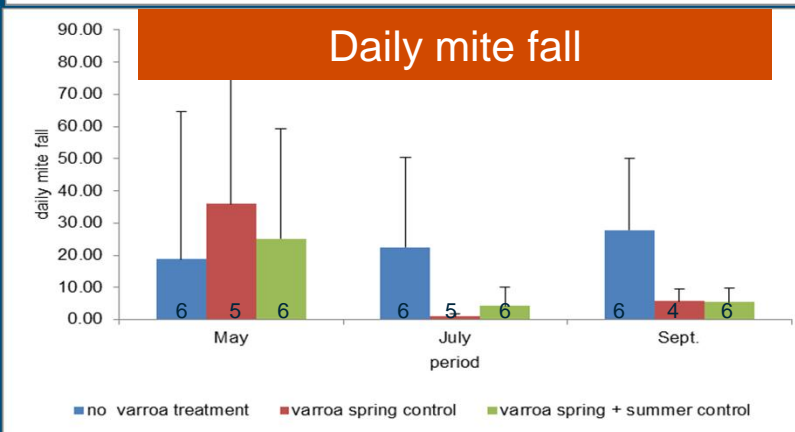
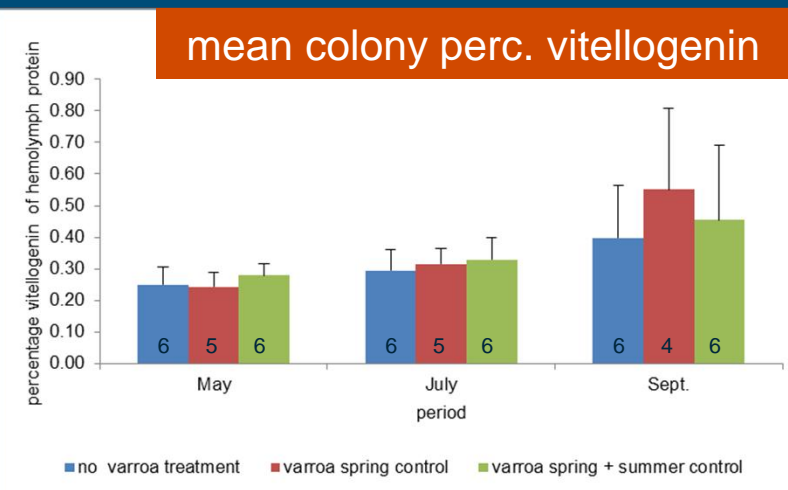
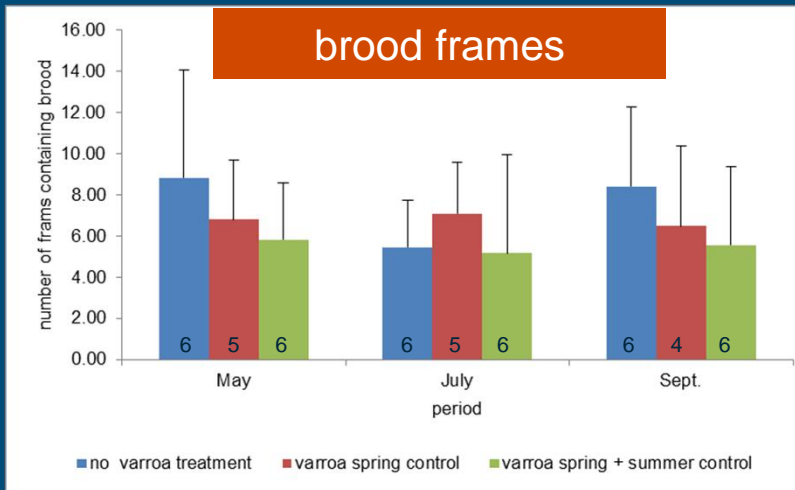


table xx. Correlation between perc. vitellogenin (response variate) and mean daily mite fall 3-week prior to vitellogenin analysis. GLM . $P \leq 0.05$

| period 2008 | P | R |
|-------------|------|------|
| May | 0.33 | 0 |
| July | 0.13 | 0.09 |
| Sept | 0.05 | 0.2 |

No different # brood, different mite fall → diverging vitellogenin perc.
Correlation mite fall and vitellogenin perc.



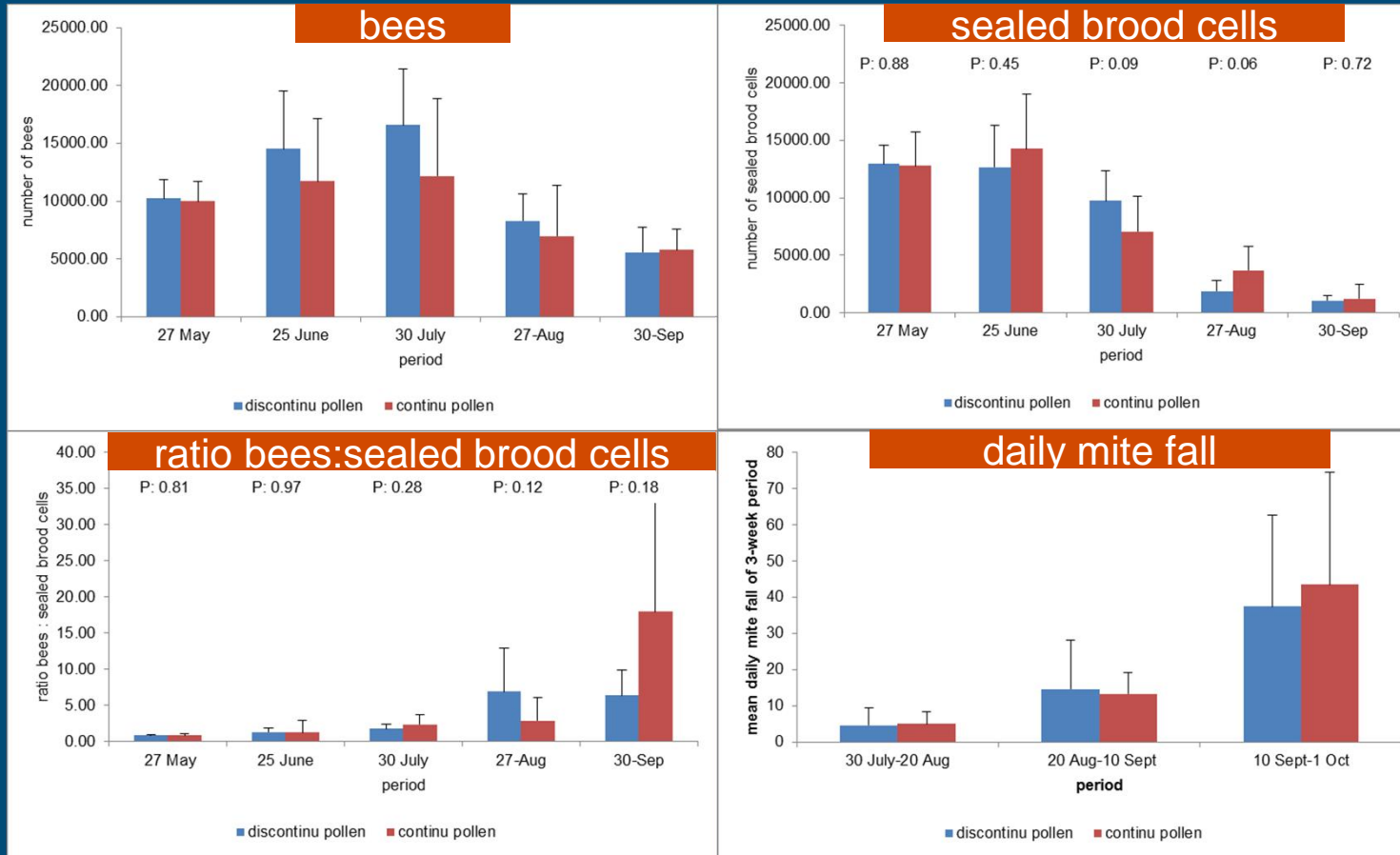
Hemolymph protein, vitellogenin and protein feed

- 6 days bees (lab test 120 bees)
 - beebread 27,57 μg protein / μl , 68,76 % vitellogenin
 - soya/yeast 24,06 μg protein / μl , 47,40 % vitellogenin
 - pollen 11,36 μg protein / μl , 26,85 % vitellogenin
 - corn 3,98 μg protein / μl , 10,96 % vitellogenin
 - sugar 2,17 μg protein / μl , 5,48 % vitellogenin
- Cremonez, T.M., De Jong, D., Bitondi, M.M. 1998.
Quantification of hemolymph proteins as a fast method for testing protein diets for honey bees. J. Econ. Entomol. 91: 1284-1289



Vitellogenin and pollen flow (study 2009)

discontinuous pollen = pollen trap in June and August



Vitellogenin perc and pollen flow (study 2009)

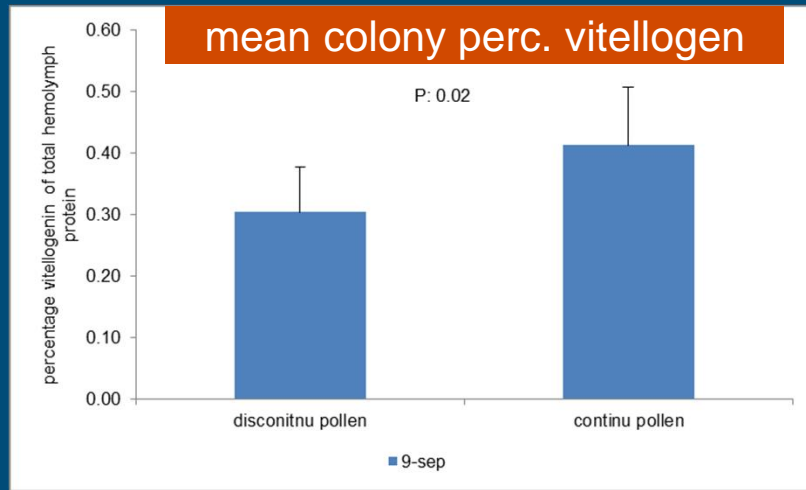



table xx. Correlation between perc. vitellogenin 9 Sept 09 (response variate) and ratio bees / brood. (GLM) $P \leq 0.05$

| date ratio bees / brood | P | R |
|-------------------------|------|------|
| 27-aug | 0.77 | 0 |
| 30-sep | 0.1 | 0.16 |

- Discontinuous pollen flow

- no significant impact on # bees, # brood and daily mite fall;
- diverging ratio bees / brood;
- trend correlation perc. vitellogenin – ratio bee / brood
- Significant different perc. Vitellogenin on colony level 

- Colonies, looking similar in Sept can have different vitality



Vitellogenin and pollen diversity (environment) (2010)

Two apiaries in a pollen divers and a simple pollen environment.

pollen diversity



| date | Grebbedijk (6 colonies) | Planken Wambuis (5 colonies) | P |
|--------|--------------------------------------|---------------------------------|------|
| | mean weight pollen flow in 24 h (sd) | | |
| 29-Jul | 15.89 (9.49) | 4.25 (2.46) | 0.03 |
| 13-Aug | 5.56 (3.69) | 11.54 (7.88) | 0.13 |
| 27-Aug | 0.16 (0.14) | 0 (0) | 0.04 |
| 10-Sep | 0.488 (0.91) | 9.516 (10.67) | 0.07 |

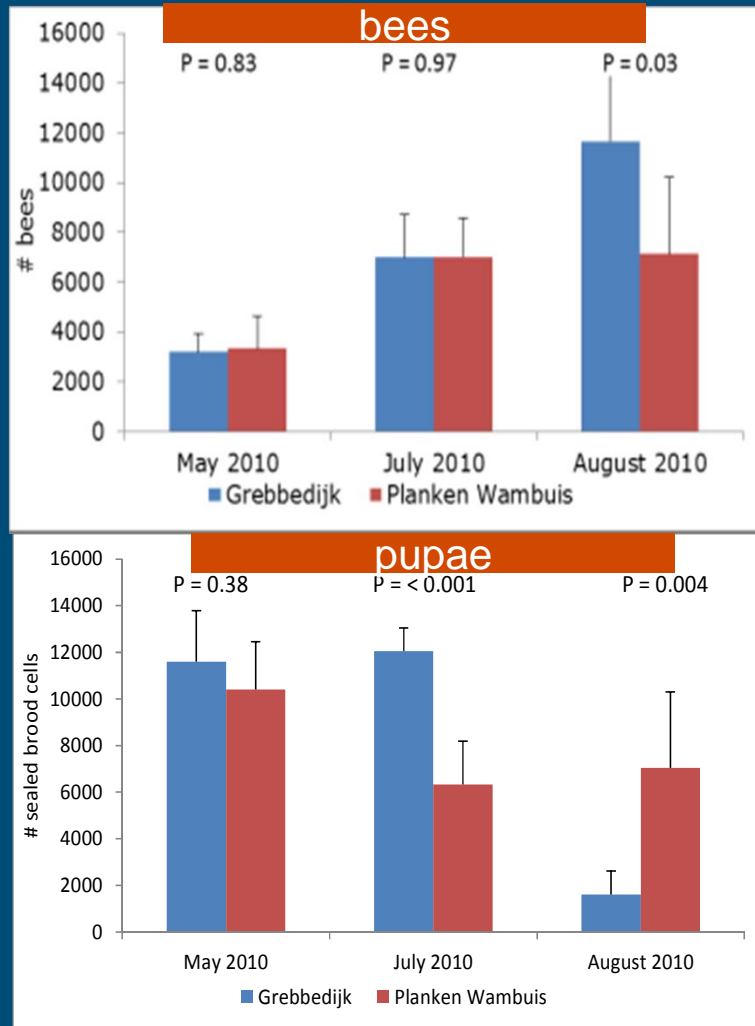
pollen diversity



In September pollen diversity at apiary PW is very low, almost unifloral (95%)

Pollen flow varied

Vitellogenin and pollen diversity (environment) (2010)



Pollen divers environment

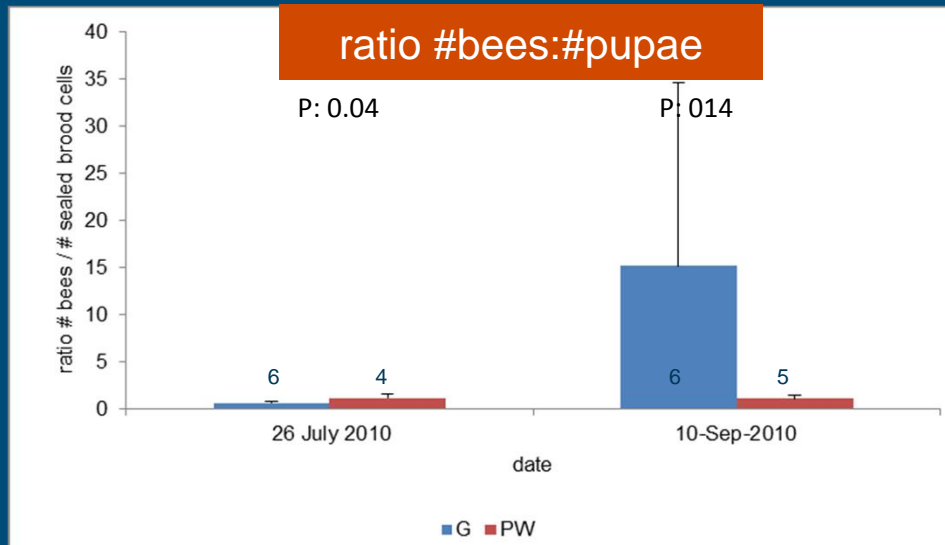
- Normal development colonies

Poor pollen divers environment

- stagnating # bees
- significant more brood in September



Vitellogenin and pollen diversity (environment) (2010)

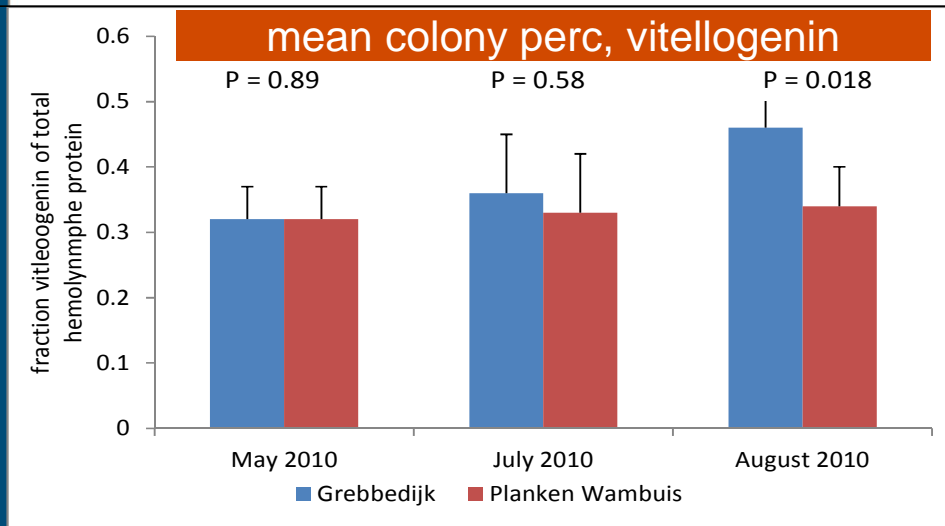


July.

Significant different ratio (more brood pollen rich environment), no significant difference perc vitellogenin

September.

Diverging ratio (more brood in poor pollen divers environment), significant different perc. Vitellogenin



Looks logic but

1. Colonies low perc. vitellogenin have not yet made transition to winterbees and the others have
2. Is low pollen diversity cause of low perc vitellogenin → continuous brood → **no transition to winterbees?**



Summary (1)

In September (in the Netherlands transition period summer to winterbees)

- negative correlation perc vitellogenin and daily mite fall.
- Continuous pollen flow → in significant higher perc vitellogenin
- Colonies, looking similar of bees and brood can have significant different perc. vitellogenin → less vital winter population
- Pollen divers environment results in significant higher perc vitellogenin
- Ratio # bees / # sealed brood cells in vital colonies = approximately **15**



Summary (2)

- In September the mean colony percentage vitellogenin is a feasible parameter to establish colony vitality as result of varroa infestation and pollen flow – diversity (environment).
- Impact of pesticides in study.
- Is relatively low mean colony perc vitellogenin in September (threshold?) the cause of continuous breeding and delayed transition to winter bees?

