

I: Vitality (Winter Mortality)

II: Varroa (Winter Bees and varroabestrijding)

III: Nutrition (Pollen (replacement))

Sjef van der Steen



**Good
Laboratory
Practice**



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Contentpresentation

□ **winter Mortality**

• **winter Bees**

- arise
 - winter Bees and protein hemolymph
- vitellogenin and Varroa destructor
- pollen Gestation and Varroa destructor
- Springerwinter bees and Varroa destructor
- Varroabestrijding
- Pollen (replacement)





winter Mortality

- multifactorial
 1. Varroa destructor
 2. Nosemaspp.
 3. environment
 1. pesticides
 2. surroundings
 4. beekeeper
- Degree of impact question from exposure



Varroa destructor



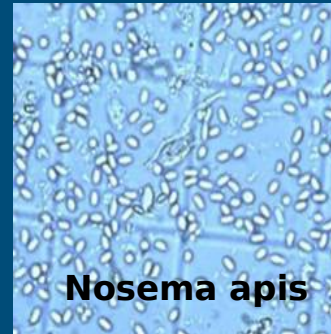
- Varroa destructor is large culprit.
- Continuous exposure, impact on physiology / resistance / Varroabestrijding
 - how this sits, is since treated slide 15.



Nosema apis, Nosema ceranae



- notifications bee mortality since first half previous century (April and October)
- Nosema apis Zander 1909
 - Food / Stress protein
- Nosema ceranae 1996 Apis mellifera
- Pollen
- Hygiene



Nosema apis



Nosema ceranae



environmental pesticides



- Neonicotinen
 - Imidacloprid
 - thiacloprid
 - acetamiprid
- synergy pesticides
 - Pyrethroids / fungicides
- sublethal effects
 - systemic drugs
 - Learning Behavior
 - lifetime
 - Orientation
 - communications
- effect certain by exposure:
 - $PEC: PNEC > 1 = \text{RISK}$



environmental



□ decreased gestation



- scale magnification Agriculture
- strongly exchanged pollen transfer
- urbanization



beekeeper

□ year round care

□ Varroabestrijding

- year round

□ Hygiene

- honeycombs

□ food

- varied pollen nutrition
- worry in front of enough sugar / honey buffer)

□ Housing

□ seeks Ring

- On time and choice make in front of gestation)

□ schooling





Results survey winter mortality PO Bees 2006-2007

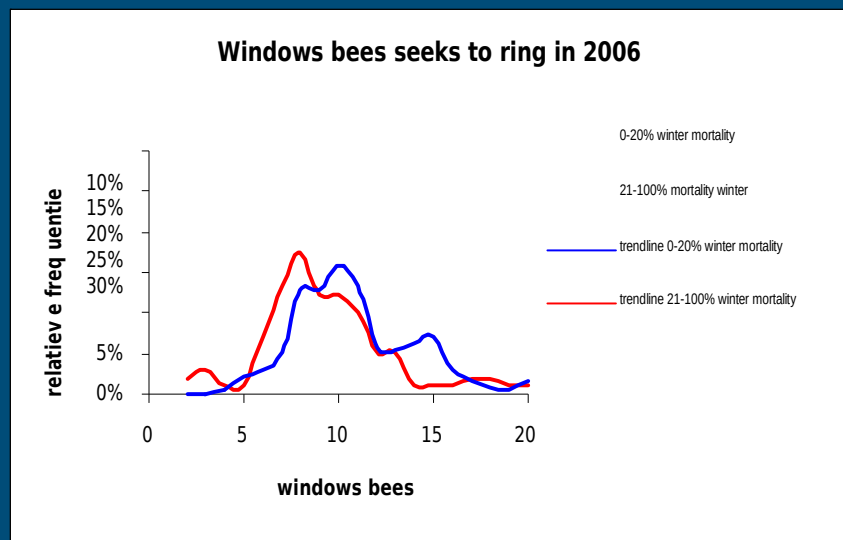
Winter Mortality > 20%

number beekeepers with winter mortality = 46
 number beekeepers with winter mortality = 174

• demonstrable link between
 Varroa bestrijding and winter mortality

• continuous fight and alertness
 beekeepers reduce Varroa the
 opportunity for winter mortality and leads until
 more nations

• late combat can winter mortality
 not prevent difference is in
 spring / summer created.



ANOVA, or difference



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winter Bees



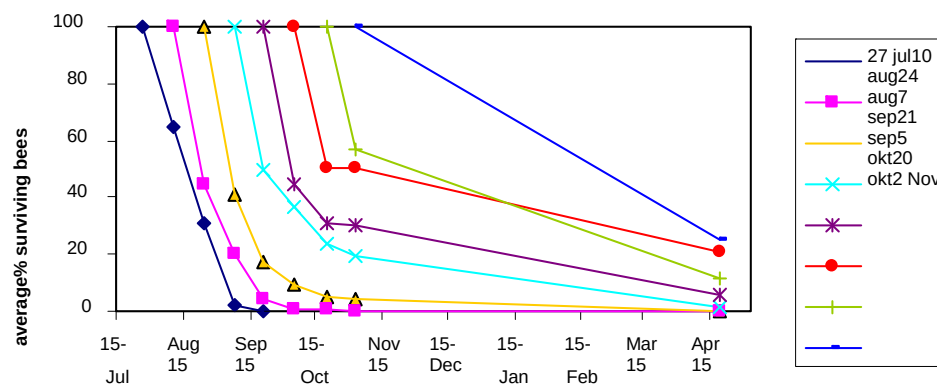
arise

research PRI Bees @ WUR

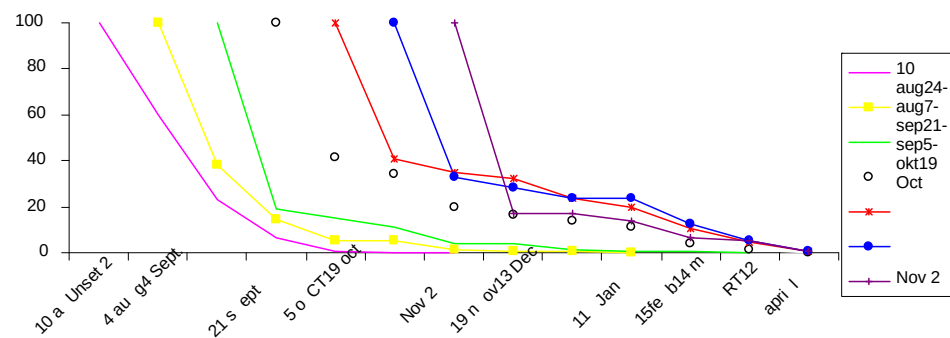
- winter Bees arise in **September, 2005 - 2007**



2005 - 2006



2006 - 2007





winter Bees (Genesis)

□ how do winter bees

- winter Bees his the bees in autumn no spawn
need to take care of/ Are in contact with spawn.

- The lifespan of a at depends on parental care

- Maurizio, A. 1950. The influence of pollen feeding and rearing bread on the length of life and physiological of the honeybee, Bee World 31: 9-12

- hereby is the protein stock and structure
voedersapklieren in at not addressed in front of
voedersapproductie >> deferred aging.

- so the emergence of winter bees has all too
make with brothers in autumn.





winter Bees (Genesis)

□ breeding Feed in autumn

□ dependent pollen supply

- Stop pollen supply >>> brakes brood rearing >>> winter bees
- As long as pollen comes, comes the spawn >> no whether later formation winter bees

- Mattila, J. H., Otis, G. W. 2007. dwindling resources pollen trigger the transition to much less populations of long-lived honeybees each autumn. Ecological Entomology 32: 496-505





winter Bees (Genesis)

□ breeding Feed in autumn

□ dependent queen

- in nations which do not a new queen got be previously winter bees created than in nations that do a new queen received have.

- young queens brood longer and others factors

» Mattila, J. H., Harris, J. L., Otis, G. W. 2001. Timing of the production of winter bees in honey bee colonies. *Insectes Soc.* 48: 88-93





Bees and protein hemolymph

□ building eitwitten / Vitellogenin in hemolymph

□ 6 days old bees (lab test 120 bees)

- bee Bread 27.57 ug protein / UI, 68.76% vitellogenin
- Soy / yeast 24.06 ug protein / UI, 47.40% vitellogenin
- Pollen 11.36 ug protein / UL, 26.85% vitellogenin
- corn flour 3.98 ug protein/ UI, 10.96% vitellogenin
- sugar 2.17 ug protein/ UL, 5.48% vitellogenin

- Cremonez, TM, De Jong, D., Bitondi, MM 1998. Quantification of hemolymph proteins as a fast method for the evaluation of protein diets for honey bees. J. Econ. Entomol. 91: 1284

1289





Winterbees and protein

- building eitwitten / Vitellogenin in hemolymph
 - In winter bees more protein in hemolymph than in summer bees.
 - In winter bees few juvenile hormone (determines transition huisbij - haalbij).
 - JH inhibits vitellogenin production, in house and winter bees few JH and many vitellogenin, in gathering bees the other way around
 - December 100 ug protein / ul
 - March 50 - 60 ug protein/ ul
 - June 4 - 49 ug protein/ ul

- Fluri, P., Wille, H., Gerig, L. Lüscher, M. 1977. Juvenile hormone, vitellogenin and haemocyte composition in winter worker honeybees. experienta 1240-1241





vitellogenin and Varroa destructor

□ Bees in the pupa stage parasitized his by
Varroa destructor can less vitellogenin
(Reserve protein) build

□ Bees 15 days

- Parasitized 50 vitellogenin ug / ul
- Not parasitized 80 - 90 vitellogenin ug / ul

- Amdan, GV, Heart Felder, K. Norberg, K. Hagen, A., Omholt, S.
2004. Altered physiology in worker honey bees infested with the mite
Varroa destructor . A factor in colony loss during hibernation.
J. Econ, Entomol 97: 741-747





vitellogenin and Varroa destructor

Own research 2008

- Correlation between vitellogenin in **people** (Mixed sample 25 bees) and varroa infestation

Results

- proven negative regression line>>> how more mites, the less vitellogenin

periode	Intercept ug ug vit./ hemolymph at 0 mites	P = probability on wrong conclusion
May	12:45 ug ug vit./	
July	16:10 ug ug vit./	30%
Sept.	31.43 ug ug vit./	3%
Nov	19.79 ug ug vit./	



vitellogenin and Varroa destructor

- Continuation of vitality research 2008 PRI bee
- longer exposure to varroa leads until it
 - **not** / too late arise of winter bees
 - too few winter bees
 - too weak winter bees
 - the natural compensatory mechanisms of a colony in front of protein deficiency (e.g. brood rearing, cannibalism) meet not at additional protein loss by the Varroa mite.



research 2009 vitality and power supply

□ Research question: relationship between

□ Degree of varroa infestation,

□ food (Continuous versus discontinuous).

- parameters

- Weekly number mites on bottom shelf (May - Nov)
- Number bees and open brood cells (Month colony May - Nov)
- Vitellogenin titer in September

» **research runs yet**





Varroa, birth weight, protein and fats of bees

- parasitized bees have less
 - proteins in the cap,
 - proteins in the abdomen.
 - fewer carbs (sugars and fats) in abdomen
- parasitized bees lighter
- A Varroa mite consumes per 24 h 0.67 ul hemolymph
 - A bee has 1 - 10 ul hemolymph
- A brings varroasis hemolymph from one at to the other >>> Conveying pathogens

- Bowen Walker, PL, Gunn, A. 2001. The effect of the ectoparasitic mite, Varroa destructor on adult worker honeybee, emergence weights, water, protein, carbohydrate and lipid levels. Entomologia Experimentalis et Applicata 101: 207-217





lab tests

birth Weight

- Not parasitized Bees: 118.9 mg (n = 391)
- parasitized 1 mite: 113.6
- parasitized 2 mites: 108.7 mg

bee Bread

- Well bee bread, not parasitized weight gain 1st week: 9.3 mg
- No bee bread, not parasitized weight loss 1st week: 11.5 mg
- All parasitized take bees downweight, regardless of well or no bee bread

- research 2006 PRI Bees @ wur. J. van der Steen, L. Gerritsen, J. Thunders. B. Cornelissen.



Springerwinter bees and Varroa destructor



nations with much mites brood longer

Group 1: Thymovar July 1

Group 2: Thymovar September 1

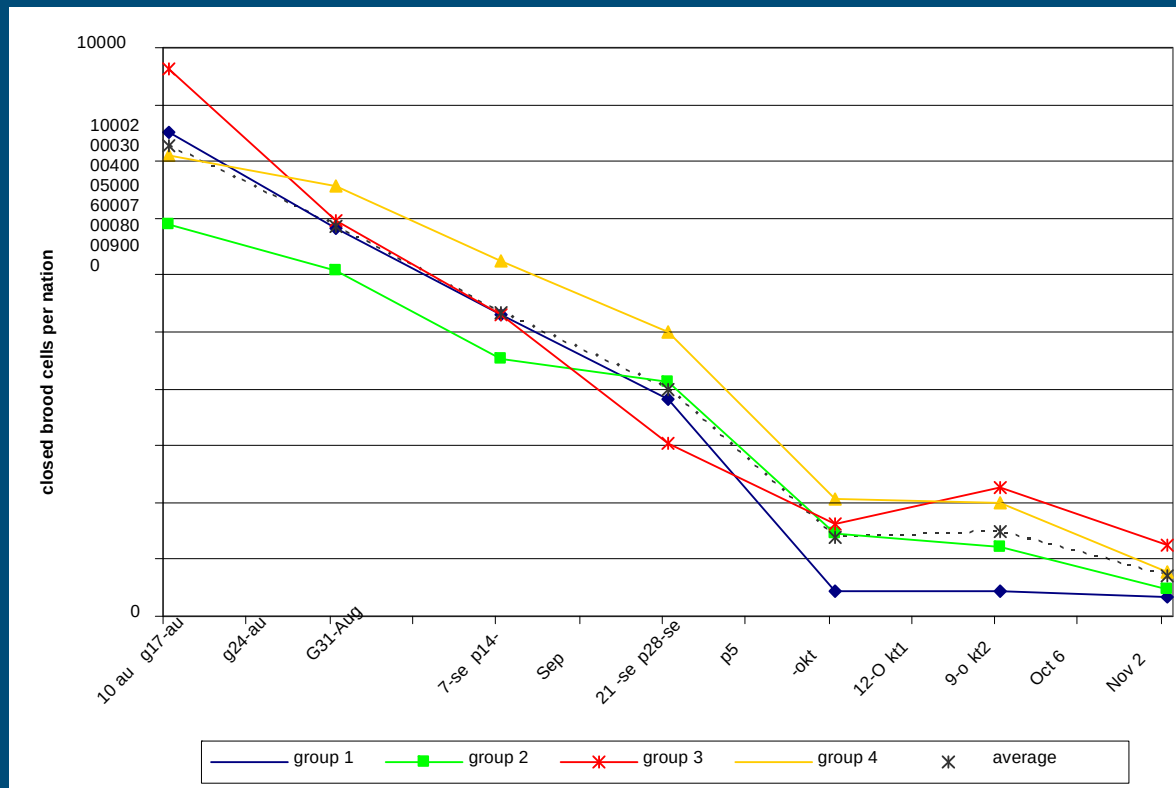
Group 3: Thymovar October 1

Group 4: no Varroa-behandeling
after-treatment all nations

13 Dec OA

per treatment 6 nations

research PRI Bees @ wur 2005
Lonne Gerritsen



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Varroabestrijding

- Beekeeper Practice oriented: good and healthy bees require a year round approach Varroa destructor
 - feed Winter bees: september >>> strong their attendants in August >>> not parasitized spawn July.



Estimated number of mites in a nation at 0, 1, 5 and 13 mites in 100 bee sample



mites OP100 bees	mites on 10,000 bees	mites spawn (Estimated)	Total maximum (No hatching)	Total maximum (or breeding)
0	0-100	0-200	100	300
1	100-200	200-400	200	600
5	500-600	1000-1200	600	1800
13	1300-1400	2600-2800	1400	4200



Varroabestrijding

Spring

- Darrenraat remove
- In broodless period May (Swarm prevention) oxalic acid

Summer

- after pregnancy (July August) formic acid or thymovar

Winter

- oxalic acid clean beginning in spring



Varroabestrijding

April	Thymol, Formic acid, Oxalic acid spray swarm prevention, breeding of charge)	(combi
May	Thymol, Formic acid, Oxalic acid spray swarm prevention broodless)	(combi
June	Thymol, Formic acid, Oxalic acid spray swarm prevention, breeding of charge)	(combi
July	Thymol, Formic acid, Oxalic acid spray swarm prevention broodless)	(combi
Aug	Thymol, Formic acid, Oxalic acid spray swarm prevention broodless)	(combi
winter	Oxaalzuur spray, evaporate, drip	



FOOD



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Average composition pollen

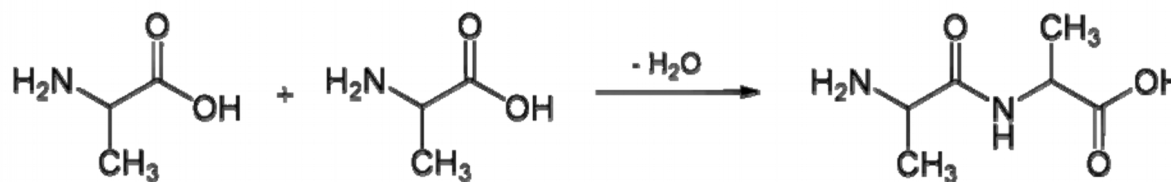
- 23% of sugars
- 24% protein
- 24% water
- 5% fat
- Rest: fibers, enzymes, vitamins. flavoring agents, coloring agents





Pollen (replacement)

- at pollen substitutes takes to the proteins
 - Protein: material it constructed is off **amino acids** that with peptide bonds to each other (compound between carboxyl (COOH) amino group and NH_2).
 - there is **20** amino acids (organic compounds with both a carboxyl as a amino group)
 - The food Honeybee must therefore minimal 10 specific amino acids contain because the bee not that self can make

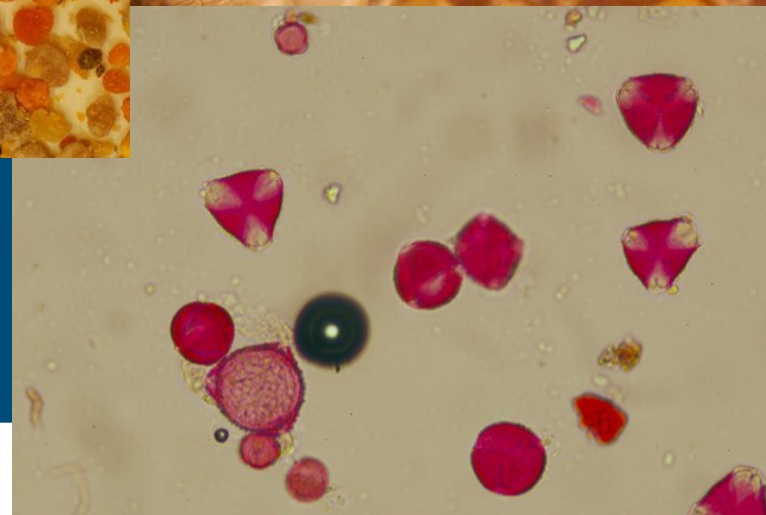
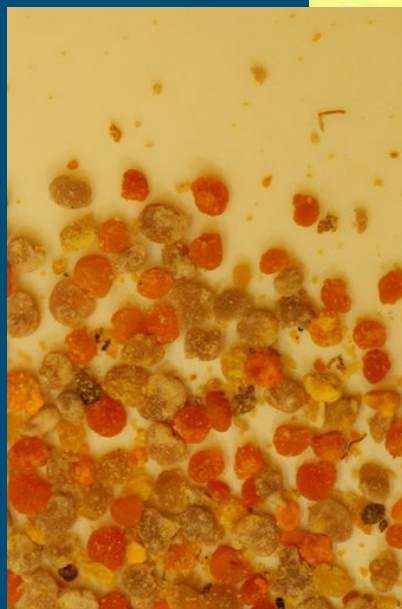


Pollen (replacement)



□ The 10 amino acids that a bee requires (de Groot 1953)

- threonine
- valine
- methionine
- leucine
- Iso-leucine
- phenylalanine
- lysine
- histidine
- arginine
- tryptophan





Pollen (replacement)

□ contains pollen always all amino acids ?

□ No

- therefore shall bees always of so much possible sources pollen gather
- example

- **dandelion Pollen** contains **tryptophan** and no **phenylalanine** and little **arginine**. with only dandelion pollen develop young bees less functional voedersapklieren and life shecurtail (Dafni et al., Pollen and pollination).

- lifetime bees raised with

- » Only sugar: 14 days
- » Sugar plus soy 19 days
- » Sugar plus dandelion pollen 20 days
- » Sugar plus Rubus pollen (braam, raspberry) 42 days

from Manning et al, 2007



amino acids in pollen, perc protein and relationship

amino acids

plant	Per protein	Thr	Val	Met	Leu	Iso	Ph	Lys	His	Arg	Try					
ideal		3.0	4.0	1.5	4.5	4.0	2.5	3.0	1.5	3.0	1.0					
rapeseed	23.84.95.1				2.3	7	4.6	4.3	8.2	2.1	5.1					
peer	26.24.45.42.46.94.14.26.42.64.8	Mais	14.95.15.91.66.84.83.85.61.94.7													
Lucerne	20	3.3	3.3	1.4	5	2.7	3.1	5.6	3.2	4.5	1.6					
white Clover	25.94.65.3				2.2	7	4.4	4.3	5.9	2.5	4.7					

From: Fat bees, bees skinny. D. Sommerville Australian Government



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pollen Replacement



□ Pollen Replacement Medium

- What must the well and not worry



well	Not or very few
Protein, 20 - 40% with the required amino acids	toxic sugars: lactose, stachyose, raffinose, galactose
granulated sugar, glucose and fructose	Starch Salt Oil
vitamins and minerals	contamination





pollen Replacement

□ consists there an ideal pollen substitute?

□ no, because

- The one replacement of the protein source for bees in the course of the evolution it evolved his.
- the attractiveness is always less than of pollen
- maximum two generations of bees with pollen substitute can be grown.

□ has the sentence with all limitations, yet a pollen replacement to offer

□ Yes because

- pollen Poor periods bridged can be with all benefits in front of vitality and nation building
- nations prepared can be in front of restoration or pollination work



pollen Replacement



protein Sources

soya (fromomerville, 2000)

- Cheap
- defatted because more than 15% of oil (giftg is for bees);
- It contains no tryptophan so only soya is insufficient
- It contains well SBTI = soyabean trypsin inhibitor
 - inhibits demolition of proteins in amino acids, cleavage nm of lysine and arginine
 - results in less functional voedersapklieren and shorter life
- Is not attractive in front of bees





pollen Replacement

protein Sources

Yeast: *Torulina*, bakers and brewer's yeast (from Somerville, 2000)

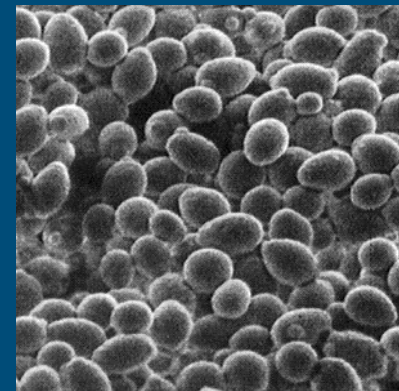
- contains well most amino acids but in insufficiently extent
- Is not attractive in front of bees

milk proteins (Stone 2007)

- No powdered milk because this contains lactose
- Well milk protein as
 - caseinate
 - Whey protein

in literature be more called

- pea flour (Pea flour)
- corn flour (starch!!)
- potato flour (starch!!)
- sunflower meal (oil)





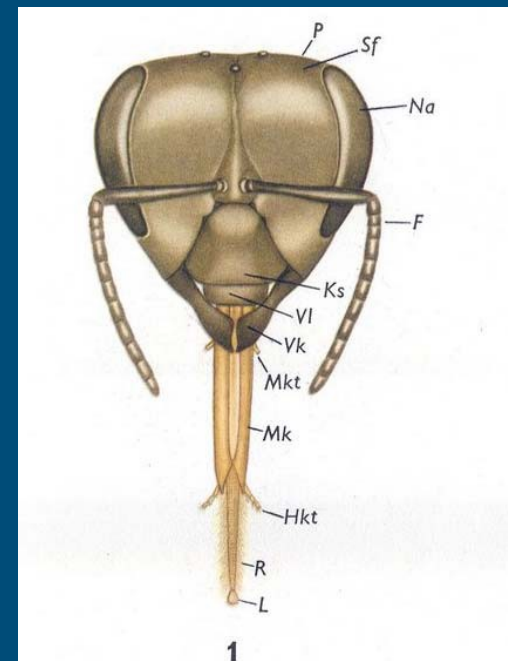
pollen Replacement

□ Attraction

- there consists no attractive Pollen Substitute
- Well appealing to make with
 - minimal 5 - 10% **pollen** The more, the more attractive
 - Fatty acids: linoleic acid (linseed oil)

□ Absorbing Powers

- bees can only wee grains to himselfake (Pollen size is about 25 microns (1/1000000 meters)



pollen Replacement



□ recipes

- each can to own insight a pollen substitute make
provided is to named conditions.



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pollen Replacement



□ recipes

(Stone, 2007)

□ soy flour	(Degreased)	3 parts by weight
□ Brewer's yeast		2 parts by weight
□ calcium caseinate		3.2 parts by weight
□ Whey protein		0.8 parts by weight
□ linseed oil		2 parts by weight
□ sugar Solution	50%	10 parts by weight



pollen Replacement

□ New on the market

□ feed Bee

- in literature positive posts about
 - well recording
 - No soya
 - Yet no literature over effects protein on mirror in hemolymph and lifetime)





pollen Replacement

□ Literature

- Barker, RJ 1977. Some carbohydrates found in pollen and pollen Substitutes are toxic to honeybees. J. Nutr. 107: 1859-1862
- Dastouri, MR, Maheri-Sis, N. 2007. The effect of replacement feeding or some protein sources with pollen on honey bee population and performance. Journal of Animal and Veterinary Advances 6: 1258-1261
- DeGrandi Hoffmann, G., et al, 2008. Comparisons or pollen substitute diets for honey bees.: consumption rates by colonies and effects on bread and adult Populations. J. Apic Res. 47: 265-270
- Manning, R., et al., 2007. Lipid enhanced pollen and lipid reduced flour- diets and Their impact on the longevity of honey bees. Astra Lian Journal of Entomology 46: 251-257
- Pernal, SF, Currie, R. 2000. Pollen quality of fresh and first-yeaqr old single pollen diets for worker honey bees. Apidolog 387-409
- Saffari, A. et al., 2004. A promising pollen substitute for honey bees. American Bee Journal March 2004.
- Sagili, RR et al., 2005 Effets or soybean trypsin inhibitor on hypopharyngeal gland protein content, total midgut protease activity and survival of the honey bee. Journal of Insect Physiology. 51: 953-957
- Stone, J. van der. 2007. Effects of a home made pollen substitute on honey bee colony development. J. Apic Res. 46: 114-119
- Somerville, D. 2000. Honey bee nutrition and supplementary feeding. NSW Agriculture, DAI 178, July 2000
- Somerville, D. 2005. Fat bees skinny bees, a manual on honey bee nutrition for beekeepers



Resume

- Vitality is by many factors certain
 - food
 - Varroa
 - bee diseases
 - Environment (gestation contaminants)
 - Beekeeper
- beekeeper plays hereby key role by
 - consistent Varroabestrijding
 - Hygiene / construction rate
 - food and gestation improvement (Bridging)



Warmly thanks for the interest



**Good
Laboratory
Practice**



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