Development of a counting protocol for predatory mites

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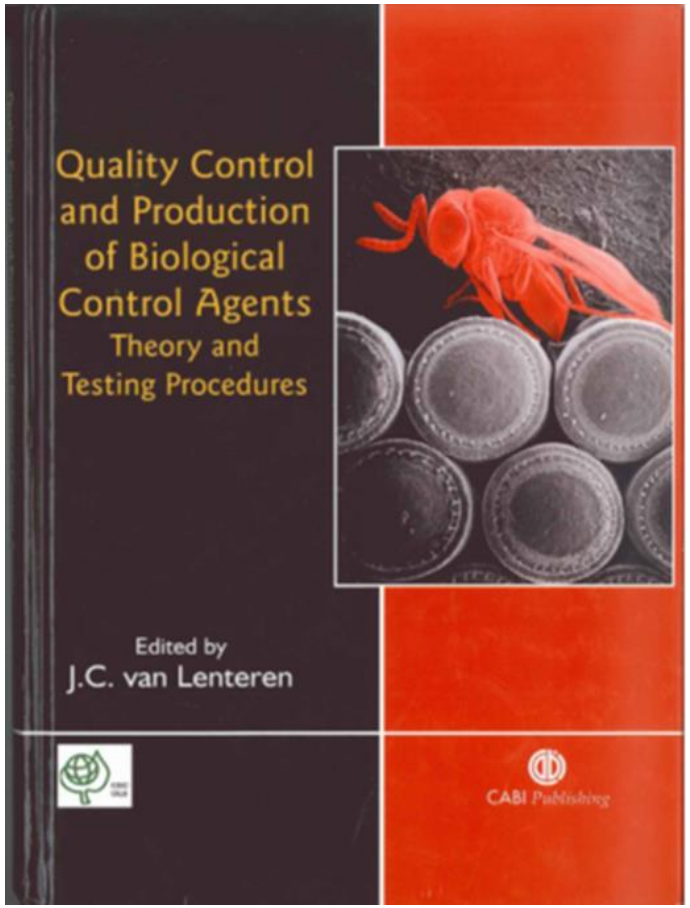
Setting the scene: QC

- @ Producers gate (longevity and fecundity, #)
- Distrubion centre
- End user (grower) → 3rd party contract labs

Setting the scene: background



- IOBC guidelines QC IBCAs (30 species) 2003 are outdated:
- Often the predatory mite numbers that our under discussion-> need for update

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Setting the scene: concrete case

- LTO- Dutch Chrysanthemum growers April 2018:
  - High variability (10x) in third party counting results for predatory mites
  - Need for a robust, reliable, reproducible third party counting method for predatory mite counting supported by industry.

Setting the scene: background

- Joint Meeting in Merida (MEX)
- (SMCB, ANBP, IOBC (MRQA) and IBMA)
- Current IOBC **Quality control guidelines** (ed. Joop van Lenteren) need update:
  - Missing products (eg predatory mites)
  - Protocol for 3rd party testing and practical, simple end user testing
  - Set up a SC with IOBC/ANBP/IBMA members to compile lists and review existing guidelines.
  - Define project and secure funding

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Adress grower’s need in the NL

- Transferred of draft third party counting protocol to external accredited party in collaboration with Artemis/IBMA NL

Groen Agro Control
LABORATORIUMONDERZOEK & ADVIES

Artemis
BELANGENVERENIGING

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Example of possible role for BPG

BioProtection Global (BPG) is a worldwide federation of biocontrol and biopesticides industry associations. These associations are comprised primarily of manufacturers of biocontrol and biopesticide products for professional use in agriculture, public health, forestry, animal health and other non-crop uses.

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Member Associations

ABCBio · Asobiocol · ANBP · bpia · IBMA · JBCA · PMFAI · SABO

Counting predatory mites

- Small (0,5 – 1 mm)
- Mixed with prey mites -> recognition
- In carrier material (bran, vermiculite, sawdust)

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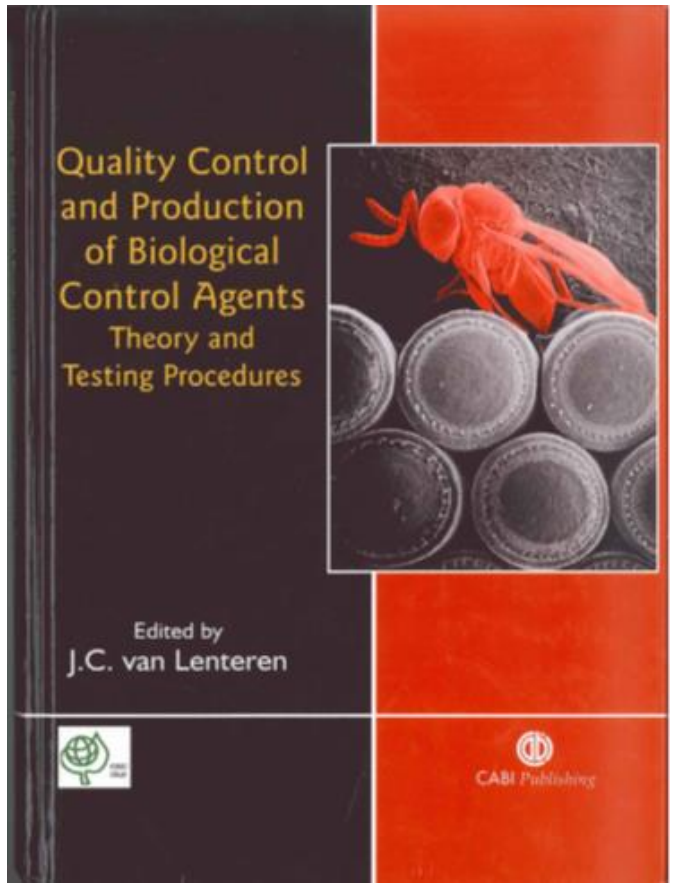
IOBC Berlese method 2003



- Accurate
- Equipment not standard available
- Sensitive

Description of testing methods

Quantity

Neoseiulus cucumeris is normally sold as a mix of bran, bran mites (as a food source) and the predatory mites themselves. Both the ratio of the two mite species and the concentration can vary considerably, depending on the product and the producer.

Mites can be washed out of the material with (hot) water, though counting is not easy because of reflection and difficult identification of the mite species. A more accurate method is to use a 'Berlese technique', as described below. Mites are driven out of the material with the heat of a lamp. The advantage is that the mite species are clearly visible and dead mites will remain behind in the sieve. Make sure that a 'warming-up time' is allowed for. This gives the small bran mites the chance to walk downwards before getting burned. Full heat is needed to drive the predatory mites out of the sieve.

Use material from one container or four sachets, depending on the product. Empty the contents into a bucket and weigh the content. Mix thoroughly with a spoon to get a homogeneous mixture. According to the density, take the following samples:

Density of *N. cucumeris*	Sample size
1000 5 g^{-1}	0.5 g
500 5 g^{-1}	0.5 g
250 5 g^{-1}	1.0 g
100 5 g^{-1}	1.0 g

Put the material directly in a sieve of 6 cm diameter, 2.5 cm height, mesh width 333 µm, 42% open. Spread the material as evenly as possible. Place the sieve at a distance of 4 cm under a lamp of 150 W. The warming-up time should take 5 min. Full power for an extra 10 min (see Figs 19.9 and 19.10). Put a piece of black sticky tape under the sieve to trap the falling mites.

The number of mites can be counted directly with a grid or if the mites can still walk over the glue, they can be killed in the freezer (20 min). Use fibre light to prevent melting of the glue. The stickiness of the glue is very important. When the tape is not sticky many mites will walk off the tape.

An alternative for the black sticky tape is to use a black plate with a ring of pure detergent as a barrier. Mites must be killed in the freezer immediately after extracting them. The humidity of the material is also very important. Within the range of 16.5–19% there does not seem to be a difference in the counting. At a higher humidity of the material, there may be a different total heating time, because mites stay longer in the material.

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Grower Guide:
Quality Assurance of Biocontrol Products

Compiled by Rose Buitenhuis, PhD, Research Scientist, Biological Control,
Vineland Research and Innovation Centre, 2014

Purpose of Guide

Successful biocontrol programs are dependent on a number of factors, but good quality natural enemies are fundamental. However, as living organisms, biocontrol products are subject to variability caused by various factors, starting at the insectary where they are reared through to the crop where they are released. Production of biocontrol agents is a self-regulated industry and quality assessments by the end-users are important to provide producers with feedback and to maintain high quality products.

Biocontrol suppliers are facing the challenge of producing a constant and reliable supply of high quality natural enemies. Therefore, quality control (QC) checks are done at the supplier level to make sure the products meet certain standards before they are shipped to the customer. However, it often takes several days before the products arrive at the grower and are released into the greenhouse. During this time, uncontrolled packaging, transport and storage conditions may affect the quality of the product and therefore the performance in pest control. Shipping is probably the most critical period. Temperature extremes, condensation from ice packs, restricted oxygen supply, unnatural high population densities and long shipping and storage times are some of the factors that can adversely affect quality. Therefore, growers should open packages upon arrival to provide a better environment for the biocontrol agents and to detect any potential problems related to shipping conditions (too warm, too cold, wet, bad smell).

In an ideal situation, growers would perform quality checks on every biocontrol product they receive as quality will directly impact efficacy; a shipment of poor-quality can result in failure to control the target pest. If a quality issue is detected the grower can react proactively, adjusting release rates accordingly.

vineland
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Predatory mites

(Amblyseius degenerans, Amblyseius swirskii, Amblyseius andersoni, Neoseiulus californicus, Neoseiulus cucumeris, Neoseiulus fallacis, Phytoseiulus persimilis)

Packaging

All stages in tube, bag or bucket with vermiculite or bran

Quality assessment at arrival

Determine the total volume of the product. Mix the material well, immediately take a 5 ml sample. Spread the sample on a sheet of white paper under a warm light bulb inside a ring of detergent. Count the live predators (adults and nymphs) running out of the material and go through the material systematically to count predators hiding in the material. Squashing each predator as it is counted will prevent counting individuals double. Repeat for at least three samples. Calculate the mean number of predatory mites per sample and estimate the total quantity of predatory mites in the package (mean number of predatory mites in samples*(total volume of material/5 ml)). Note the difference between food mites (slow moving, milky colour or with long hairs) and predatory mites (fast moving, tan coloured, egg shape). *A. degenerans* is dark instead of tan, *P. persimilis* is red.

Packaging

Slow release sachet

Quality assessment at arrival

Weekly emergence: Suspend sachet from a wire hanger (or attach on a clip/cork) above a sticky trap surface. Keep the set-up in a shaded area at room temperature and 60-90% relative humidity (important!). Change the sticky card or liquid weekly and count the number of predators. Repeat for at least 3 sachets.

From left to right: Predatory mites in carrier material (IQDHO), counting set-up (Vineland Research and Innovation Centre), predatory mites and food mites (Vineland Research and Innovation Centre).

2011 workshop

- Demonstration of methods used by producers
- Counting different samples with each others methods (tape counting)

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2011- different methods

IOBC
Berlese extraction

alternative 1
extraction by sieving

alternative 2
direct sampling

alternative 3
wet extraction

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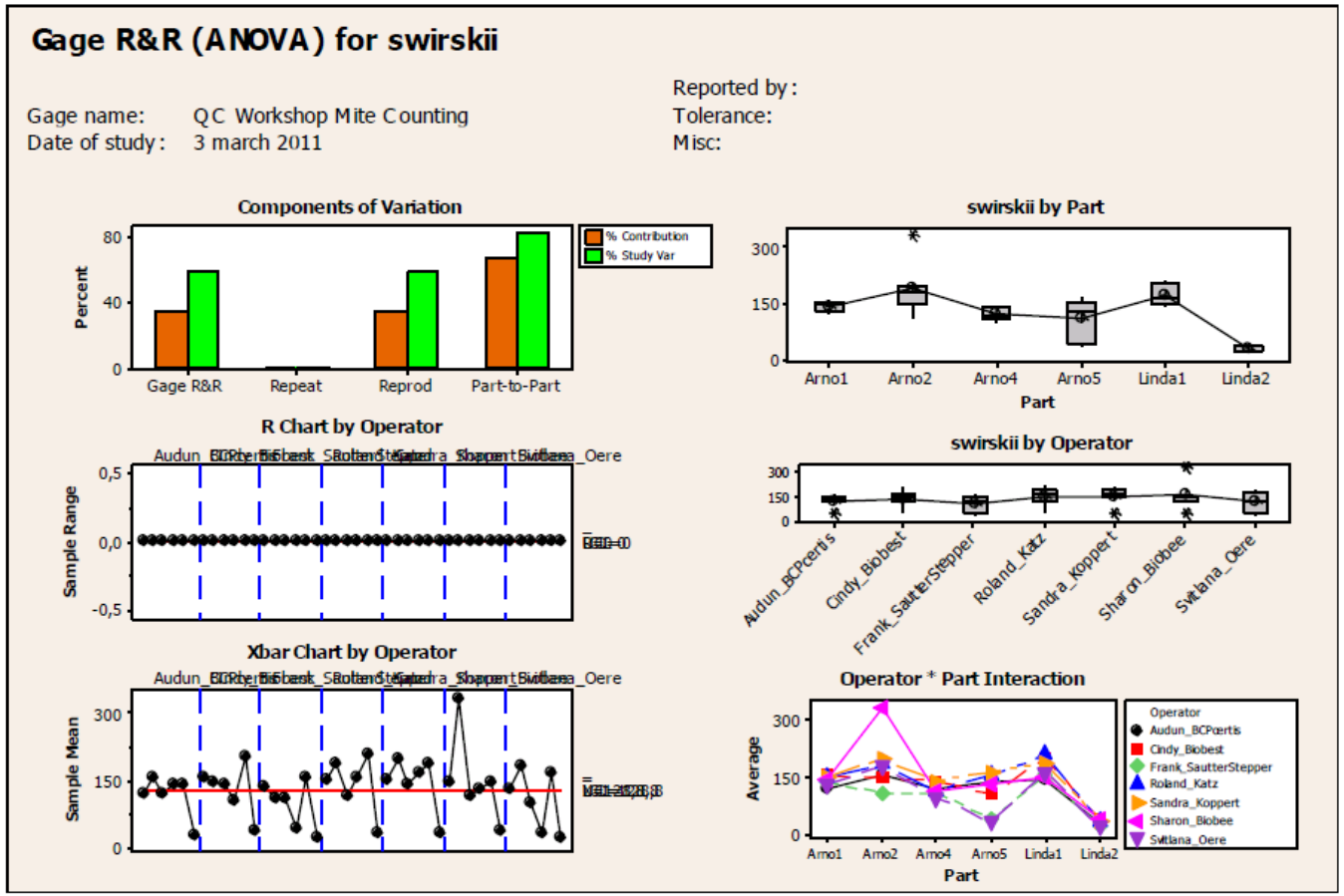
Statistical analysis



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Achievements 2011

- Understanding of each others methods
- Understanding of complexity of mite counting
- **Dry sieving method** looked most promising
- Was tried by the different producers ->
  - Not easy to count
  - not reliable enough

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2017 restart - mite counting workshop

New attempt, starting with comparison methods

Aim: find one common method for 3rd party counts of predatory mites

Demonstration and hands on counting of 3 x 3 concentrations of *A. swirskii* with different methods

Statistical analysis: Gage R&R

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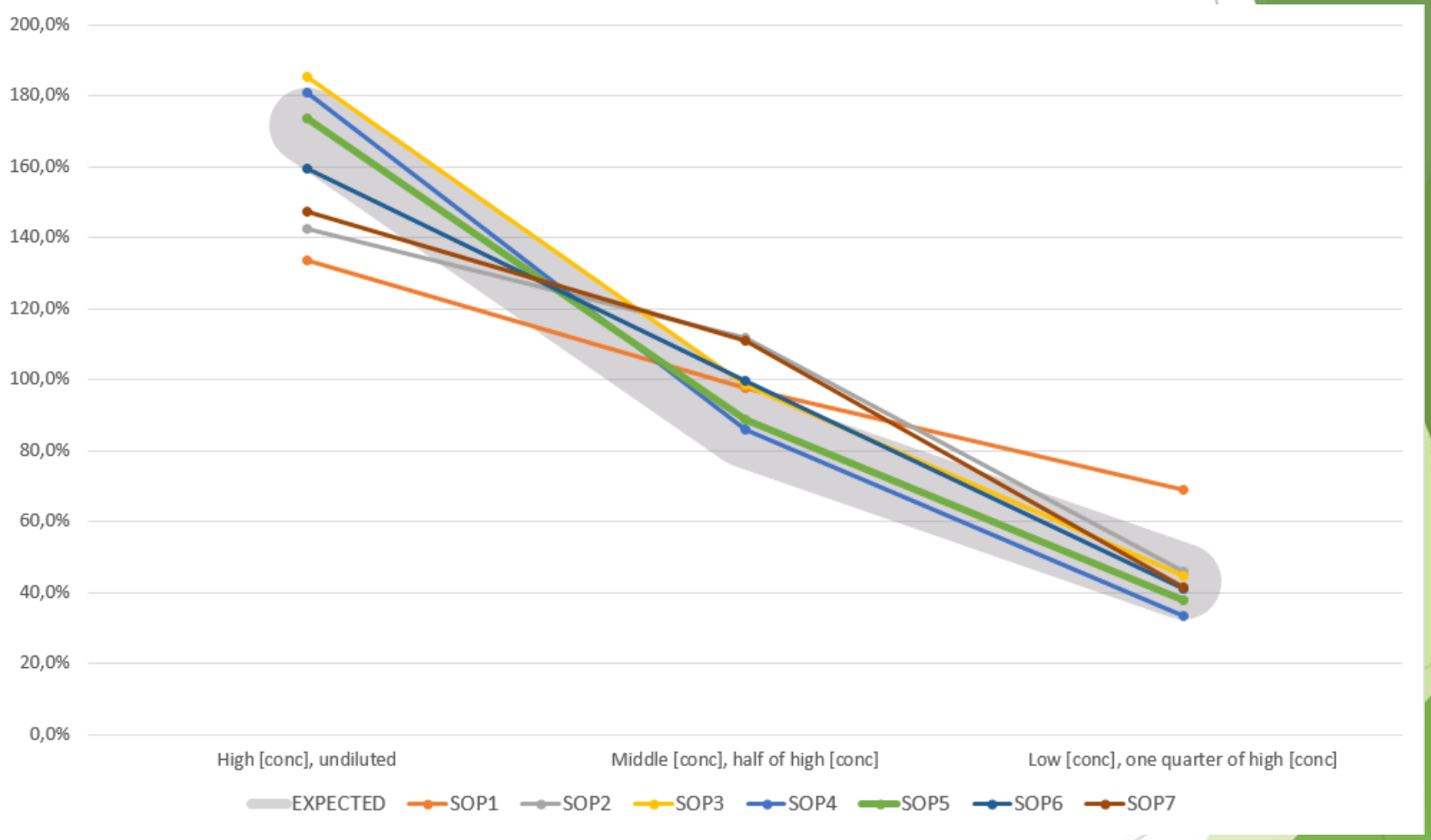
Mite counting workshop Nov 28-29th 2017

Results

- SOP 1: dry sieving
- SOP 2: Berlese
- SOP 3: no extraction
- SOP 4: wet extraction
- SOP 5: Berlese (IOBC)
- SOP 6: wet extraction 2
- SOP 7; dry counting



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Conclusions workshop

- Results rather comparable, esp. at product concentration
- SOP3 and SOP4 give comparable results to SOP5 (=IOBC method)
- SOP3, SOP4 and SOP5 follow the expected curve
- SOP3 method seems a good candidate
  - No complex equipment required
- Further testing required:
  - Number of subsamples required
  - Practical issues
  - Effect of operator skills

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Best results

SOP 1: dry sieving

SOP 2: Berlese 1

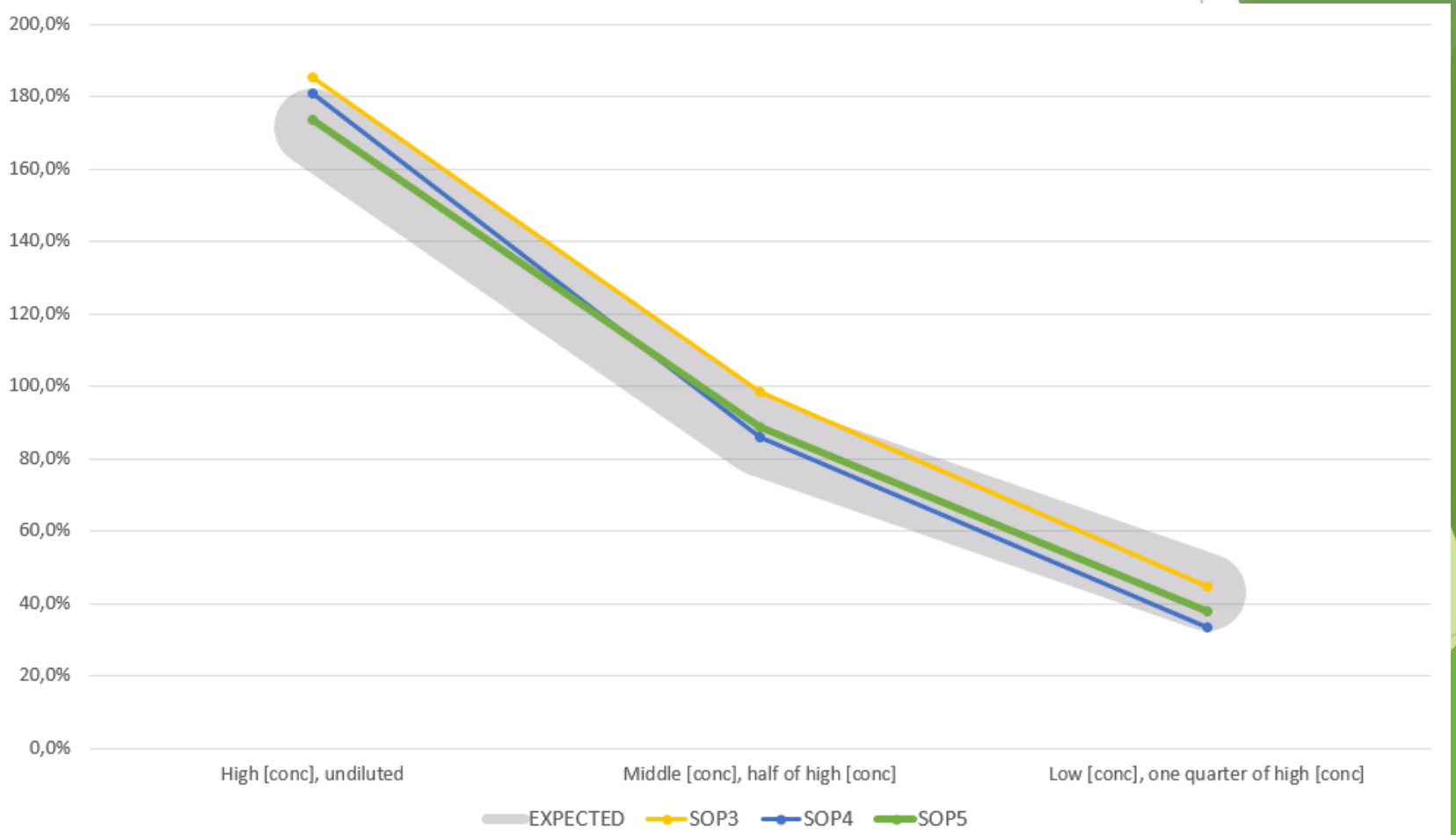
SOP 3: no extraction

SOP 4: wet extraction

SOP 5: Berlese (IOBC)

SOP 6: wet extraction 2

SOP 7; dry counting



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Conclusions workshop

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Conclusions workshop

- Scoop method seems a good candidate
- The results are following the expected outcome curve
- Results not different from Berlese method
- No complex equipment required

➢Homework: further testing scoop method

- Number of subsamples required
- Effect of operator skills

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Second candidate (SOP3): ‘scoop method’, adjusted from Vineland method

- Direct sampling, no extraction
- 10 small scoops
- Counting on black sticky tape

Conclusions
testing the scoop method

- Different experiences between producers
- Easy to perform but time consuming
- For swirski good results, for cucumeris less…..
- Variation between samples high
- For reliable result more samples required but lot of time
- Not enough value for time

➢Not suitable

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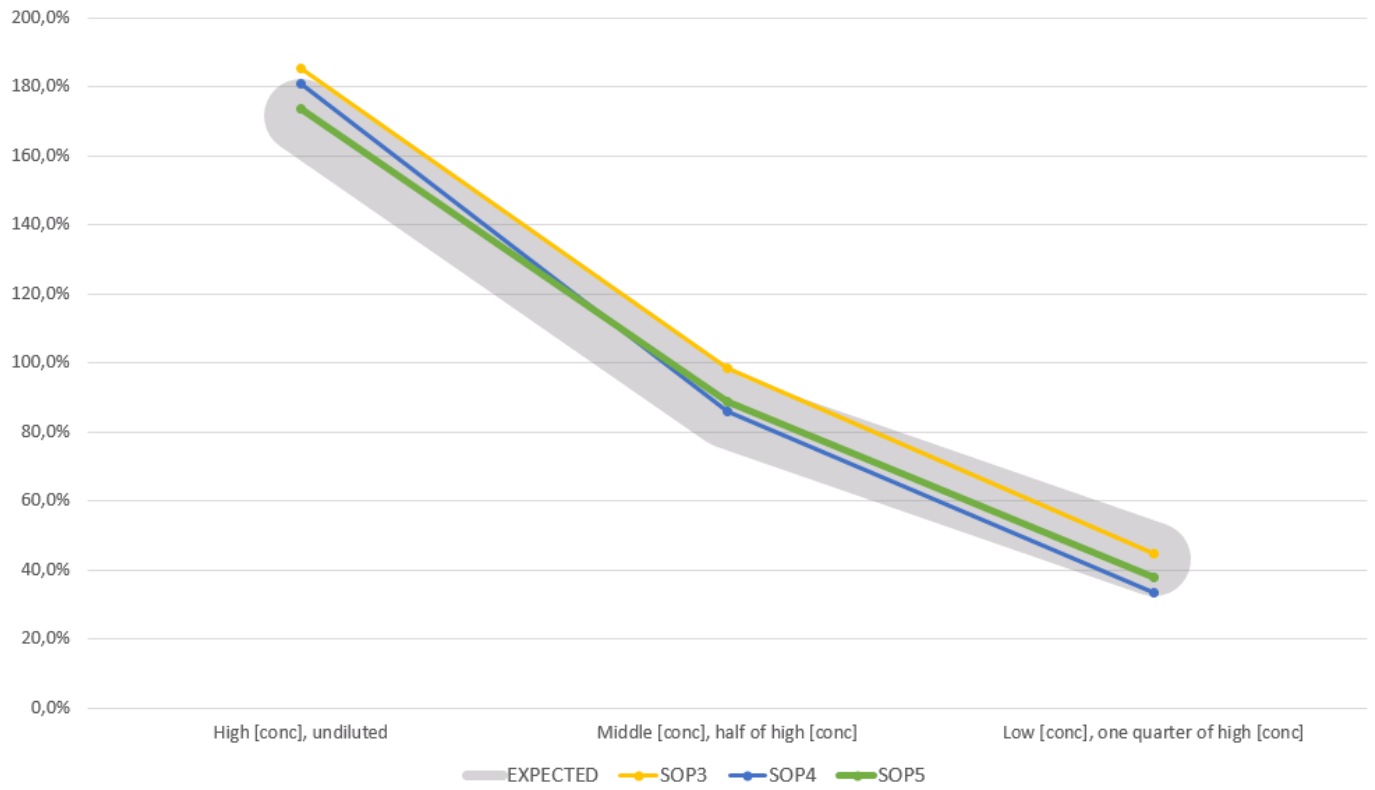
Third candidate: SOP 4
wet sieving/ wet count (adjusted Biobest method)



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Third candidate (SOP 4):
wet sieving/ wet count (adjusted Biobest method)

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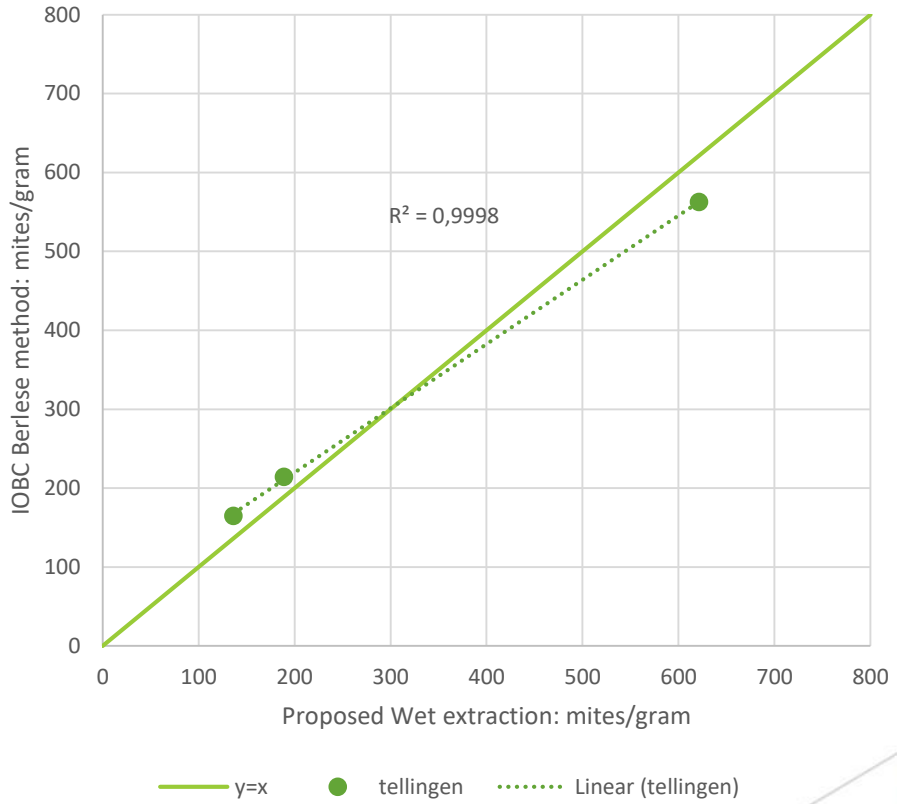
Comparison old and new method: perfect correlation

IOBC method

new method



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Conclusions
Counting method predatory mites:

- Wet extraction method seems reliable and feasible alternative for the IOBC Berlese method found for 3rd party counting of different species of predatory mites (swirskii, montdorensis a.o.)
- No faster method found that provides enough reliability
- Proper performance of method requires experience, especially in recognition of predatory mites between prey mites

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Future work (mite counting):

- Other carrier materials to be tested
- Ring testing
- Approval of method: IOBC?

- Idea: IBMA Workshops for interested 3rd parties
- Qualification of labs?

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		Lieve Vandendriessche
	SOP-Wet count Phytoseiid predatory mites	Revision:
		Date: 12-10-2018 JK
		Page 2

2		Put the first bucket on a scale and tare it to 0.0g
3		Pour the contents of the 2 packages/bottles into the first bucket (make sure to empty the packages completely, hitting it with a rod)
4		Note the exact total weight of the content of the 2 bottles

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Future work (guidelines general):

- Lining up with ANBP and IOBC-MRQA (Steering Cie)
- Project: Guidelines for all beneficials
- Important for counts by third parties:
  - Product handling
  - Number of samples
  - ➢Proper reporting

➢Certification system for external labs?

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Thank you for your attention

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