



SUSINCHAIN
SUSTAINABLE INSECT CHAIN

KU LEUVEN

Research Group for Insect
Production & Processing

WP 6: Safety along the insect value chain

HORIZONTAL TRANSFER OF FOOD PATHOGENS FROM SUBSTRATE TO INSECTS DURING REARING

DRIES VANDEWEYER

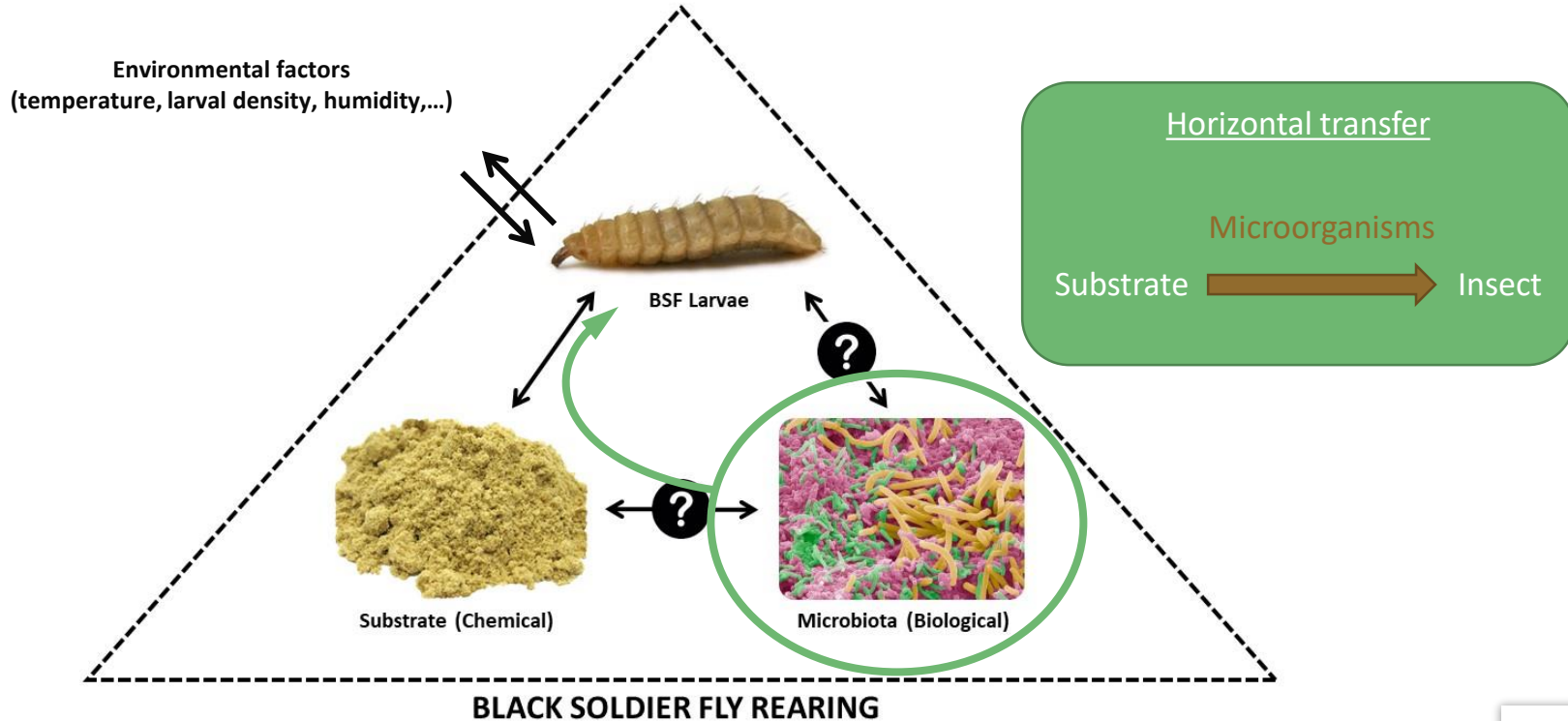
DARIO LACHI, LAURENCE VAN MOLL, ELLEN GORRENS, JEROEN DE SMET, LEEN VAN CAMPENHOUT



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Insect-microbe-substrate interactions



Relevant foodborne pathogens in insect rearing

- Literature study: microbiological safety evaluation
 - Substrate ingredients
 - Insects as food and feed
- Most relevant pathogens:

Pathogen

Salmonella spp.

Staphylococcus aureus

Bacillus cereus s.l.

Clostridium perfringens



- Most relevant insect species:

Insect

Mealworm

Black soldier fly larva (BSFL)

House cricket/Grasshopper

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SPECIAL ISSUE: Advancement of insects as food and feed in a circular economy



Biological contaminants in insects as food and feed

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Pathogen x insect matrix

| Pathogen \ Insect | Mealworm | Black soldier fly larva | House cricket Grasshopper |
|--------------------------------|----------|-------------------------|------------------------------|
| <i>Salmonella</i> spp. | | | |
| <i>Staphylococcus aureus</i> | | | |
| <i>Bacillus cereus</i> s.l. | | | |
| <i>Clostridium perfringens</i> | | | |

Experiments “Horizontal transfer of food pathogens from substrate to insects during rearing”

Food Control 100 (2019) 227–234

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Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont



Risks related to the presence of *Salmonella* sp. during rearing of mealworms (*Tenebrio molitor*) for food or feed: Survival in the substrate and transmission to the larvae

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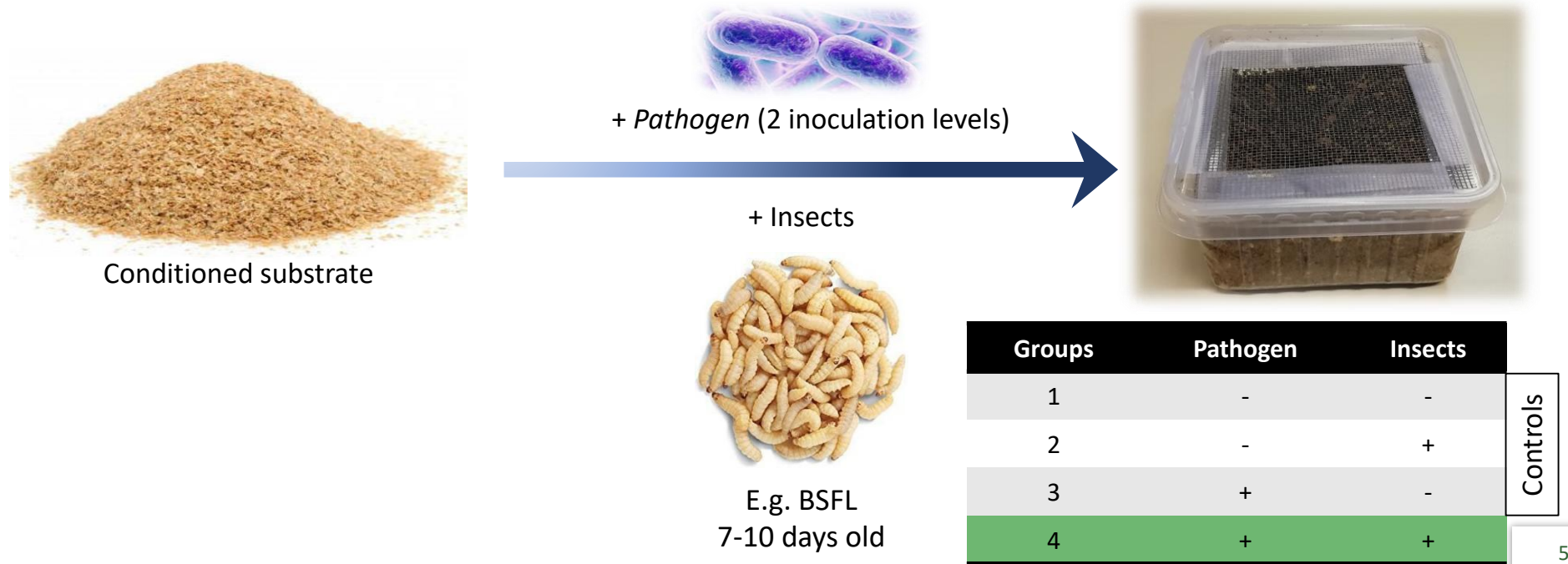
Dynamics of *Salmonella* inoculated during rearing of black soldier fly larvae (*Hermetia illucens*) on chicken feed

J. De Smet,  D. Vandeweyer, L. Van Moll, D. Lachi,  L. Van Campenhout

doi: <https://doi.org/10.1101/2021.04.13.439665>

This article is a preprint and has not been certified by peer review [what does this mean?].

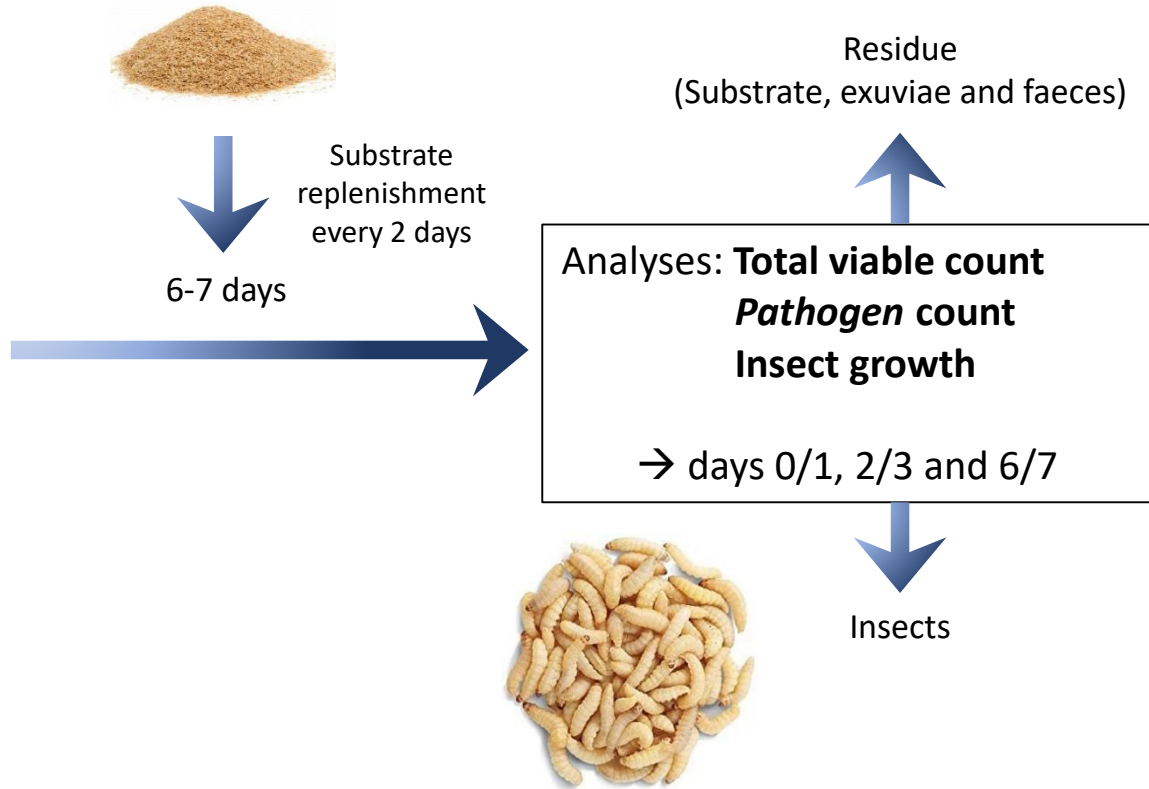
Experimental set-up



Experimental set-up (2)



Suitable rearing conditions
(E.g. BSFL: 28 °C, 65% RH)



Challenges and insights through experiments

- Background microbiota
 - Selective agar plates are not always selective enough
 - Use of antibiotic-resistant pathogen strain
 - *Salmonella* Typhimurium KAN^R, *Salmonella* Infantis KAN^R
 - *Staphylococcus aureus* KAN^R

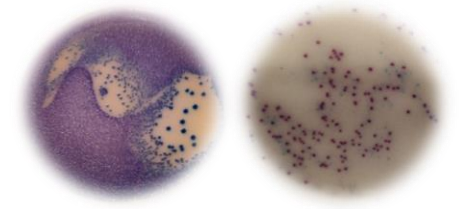


Figure: RAPID[®]Salmonella agar without (left) and with (right) kanamycin. High numbers of background microbiota (blue colonies) impede correct *Salmonella* (purple colonies) counting. By adding an antibiotic, inoculated antibiotic-resistant *Salmonella* cells can easily be counted.

- Contamination of controls can occur ← Airborne pathogen
 - Separated incubation per test group
 - Proper disinfection!



4 x



Results: *Salmonella* x mealworm

| Experiment details | |
|--------------------|---|
| Substrate | Wheat bran |
| Pathogen strains | S. Infantis + S. Typhimurium + S. Enteritidis |
| Incubation | Not separated, 7 days, 28 °C, 65% RH |

Number of samples testing positive for *Salmonella* sp.

| Sample | Target <i>Salmonella</i> sp. contamination level in substrate (log cfu/g) | Number of <i>Salmonella</i> sp. positive samples per batch (6 replicates each) | | | | | |
|--------------------------|---|--|---------|---------|---------|---------|---------|
| | | Day 1 | | | Day 7 | | |
| | | Batch 1 | Batch 2 | Batch 3 | Batch 1 | Batch 2 | Batch 3 |
| Substrate without larvae | Control (0) | | | | | | |
| | 2 | | | | | | |
| | 4 | | | | | | == |
| Substrate with larvae | Control (0) | | | | | | |
| | 2 | | | | | | > |
| | 4 | | | | | | == |
| Larvae, not disinfected | Control (0) | | | | | | |
| | 2 | | | | | | > |
| | 4 | | | | | | > |

- *Salmonella* sp. appears to be reduced in the presence of mealworms
- Lowest contamination level → larvae negative for *Salmonella* sp. after 7 days

BUT: only for the circumstances investigated!

Results: *Salmonella* x BSFL

| Experiment details | |
|--------------------|--|
| Substrate | Chicken feed |
| Pathogen strains | <i>S. Infantis</i> KAN ^R + <i>S. Typhimurium</i> KAN ^R |
| Incubation | Separated, 6 days, 28 °C, 65% RH |

| Sample | Target <i>Salmonella</i> sp. contamination level in substrate (log cfu/g) | Number of <i>Salmonella</i> sp. positive samples per batch (2 replicates each) | | | | | |
|--------------------------|---|--|---------|---------|---------|---------|---------|
| | | Day 0 | | | Day 6 | | |
| | | Batch 1 | Batch 2 | Batch 3 | Batch 1 | Batch 2 | batch 3 |
| Substrate without larvae | Control (0) 3 | | | | = | | |
| Substrate with larvae | Control (0) 3 | | | | = | | |
| Larvae, disinfected | Control (0) 3 | | | | < | | |

- No reducing effect on *Salmonella* sp. is observed after 6 days
- Similar observations for higher contamination levels (data not shown)

BUT: only for the circumstances investigated!

Results: *S. aureus* x BSFL

| Experiment details | |
|--------------------|-----------------------------------|
| Substrate | Chicken feed |
| Pathogen strains | <i>S. aureus</i> KAN ^R |
| Incubation | Separated, 6 days, 27 °C, 60% RH |

| Sample | Target <i>S. aureus</i> contamination level in substrate (log cfu/g) | Number of <i>S. aureus</i> positive samples (6 replicates each) | |
|--------------------------|--|---|-------|
| | | Day 0 | Day 6 |
| Substrate without larvae | Control (0) | 3 | 7 |
| | 3 | | |
| Substrate with larvae | Control (0) | 3 | 7 |
| | 3 | | |
| Larvae, disinfected | Control (0) | 3 | 7 |
| | 3 | | |

But:

- Higher suppression at day 2 (data not shown)
- Lower detection limit!

- High suppression of *S. aureus* in presence of BSFL
- No *S. aureus* detected in the larvae after 6 days

BUT: only for the circumstances investigated!

Conclusions

- *Salmonella* x mealworm
 - Limited horizontal transfer to mealworms
 - Small reducing effect of mealworms on *Salmonella* presence in substrate
- *Salmonella* x BSFL
 - Horizontal transfer to BSFL
 - No effect of BSFL on *Salmonella* presence in substrate
- *S. aureus* x BSFL
 - No horizontal transfer to BSFL
 - High reducing effect of BSFL on *S. aureus* presence in substrate

Horizontal transfer of food pathogens from substrate to insects during rearing highly depends on circumstances:
Pathogen (species and contamination level) – insect species – rearing conditions – ...



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<https://doi.org/10.3920/JIFF2020.0060> (Biological contaminants in insects)
<https://doi.org/10.1016/j.foodcont.2019.01.026> (*Salmonella* x mealworm)
<https://doi.org/10.1101/2021.04.13.439665> (*Salmonella* x BSFL)