Faculty of Science

Insect diseases in production systems: prevention and management

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Innovation Fund Denmark

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Diseases in insects produced for food and feed

J. Eilenberg[1*], J.M. Vlak[2], C. Nielsen-LeRoux[3], S. Cappellozza[4] and A.B. Jensen[1]

Journal of Insects as Food and Feed, 2015; 1(2): 87-102

Natural enemies in insect production systems

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In Huis, A. (ed) 2017: Insects as Food and Feed from Production to Consumption. Wageningen University Press, 201-222.

Diseases of insects in European production systems: Diagnosis, prevention and management

Krankheiten von Insekten in Europäischen Produktionssystemen: Diagnose, Prävention und Management

Antoine Lecocq, Annette Bruun Jensen, Jørgen Eilenberg

Berl Münch Tierärztl Wochenschr
DOI 10.2376/0005-9366-18061

Towards a coordination of European activities to diagnose and manage insect diseases in production facilities

J. Eilenberg, M.M. van Oers, A.B. Jensen, A. Lecocq, G. Maciel-Vergara, L.P.A. Santacoloma1, J.J.A. van Loon and H. Hesketh

Journal of Insects as Food and Feed, 2018; 4(3): 157-166
SPECIAL ISSUE: *Insects in European feed and food chains*

14

Prevention and Management of Diseases in Terrestrial Invertebrates

Jørgen Eilenberg and Annette Bruun Jensen

Department of Plant and Environmental Sciences, University of Copenhagen, Frederiksberg, Denmark

In Hajek, A.E. & Shapiro-Ilan, D. (2018): Ecology of Invertebrate Diseases, John Wiley and Sons

insects

MDPI

Article

Cannibalism as a Possible Entry Route for Opportunistic Pathogenic Bacteria to Insect Hosts, Exemplified by *Pseudomonas aeruginosa*, a Pathogen of the Giant Mealworm *Zophobas morio*

Gabriela Maciel-Vergara [1,2,3,*], Annette Bruun Jensen [1] and Jørgen Eilenberg [1]

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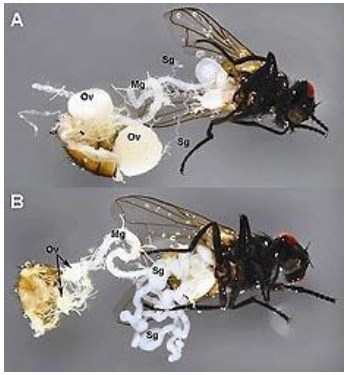
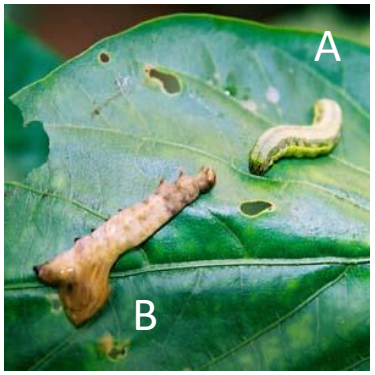
Virus infections in insects

RNA Virus: Honey Bee Deformed Wing Virus DWV

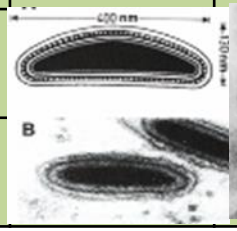
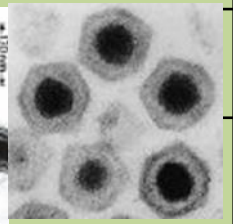
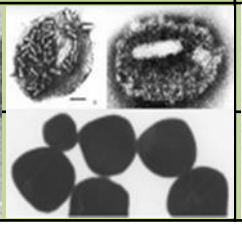
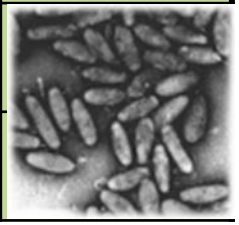


DNA virus: House fly *Musca domestica* infected (B) with SGHV



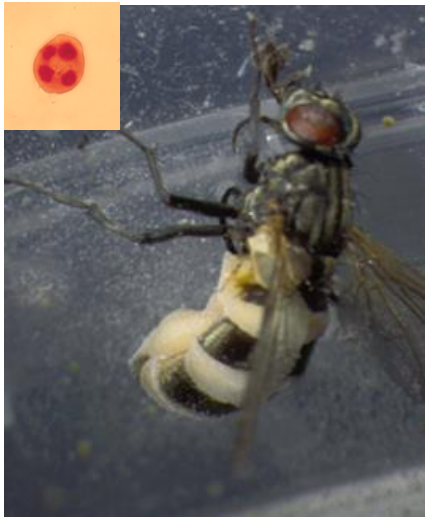
DNA Virus: Caterpillar (B) infected with Baculouvirus

Examples of large invertebrate DNA viruses

Virus	Asco-virus	Irido-virus	Entomo-poxvirus	Baculo-virus	Nima-virus
Virion					

Photos: Just Vlak, http://en.wikipedia.org/wiki/Deformed_wing_virus
http://www.ars.usda.gov/is/graphics/photos/apr13/d2679-1.htm;
http://afrsweb.usda.gov/News/News.htm?modecode=36-22-10-00&docid=3797&page=1

Insect pathogenic fungi: specialists/generalists



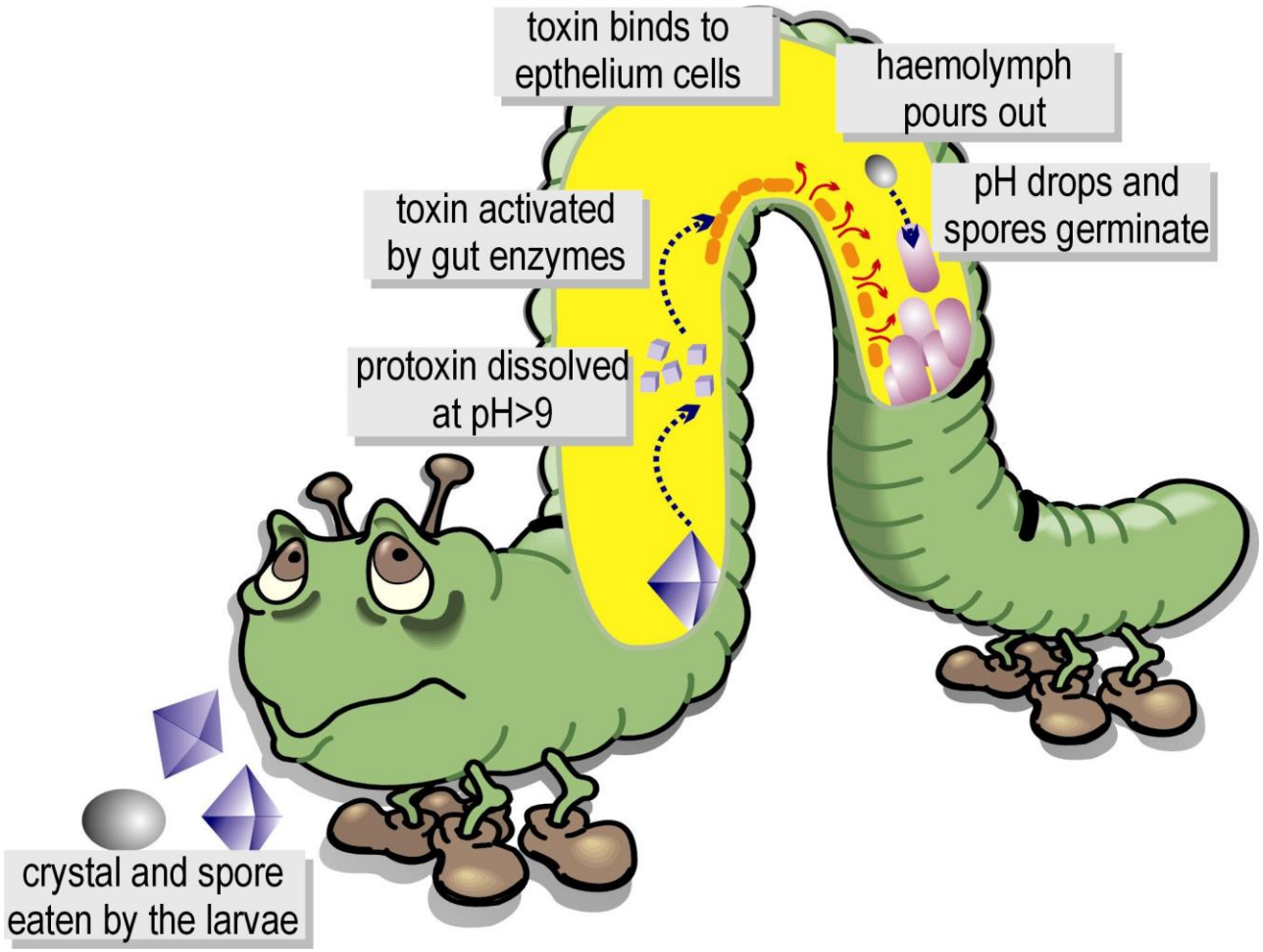
House fly *Musca domestica* infected with *Entomophthora schizophorae*

Grasshopper nymph *Schistocerca americana* infected with *Entomophaga grylli*

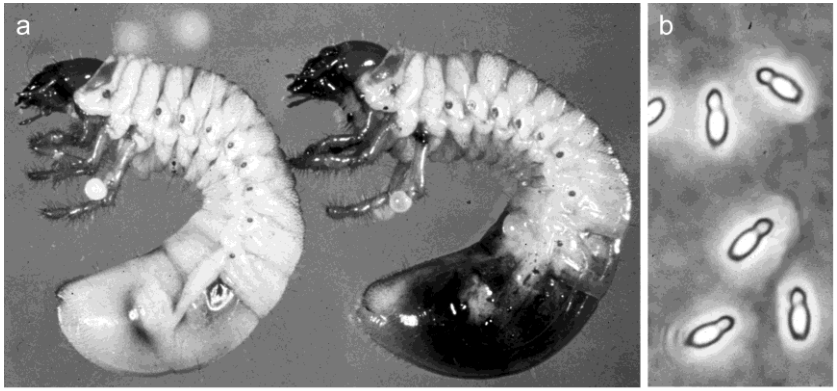
Adult weevil *Strophosoma* sp. infected with *Beauveria bassiana*

Photos: Henrik de Fine Licht, Univ Copenhagen ;
http://entnemdept.ifas.ufl.edu/creatures/field/american_grasshopper.htm

Bacillus thuringiensis



A specialist insect pathogenic bacterium



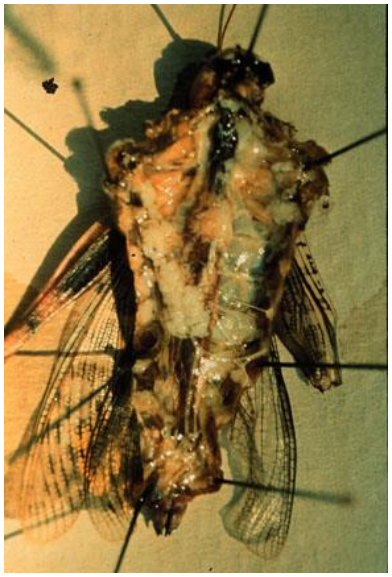
Bacillus popilliae, ‘Milky White Disease’, a pathogen om scarabs

An opportunistic insect pathogenic bacterium

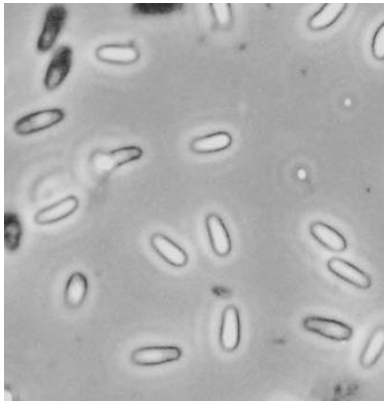
Probably genus *Pseudomonas*, infecting mealworm *Tenebrio molitor*

Photos: Society for Invertebrate Pathology & Univ Copenhagen

Microsporidia

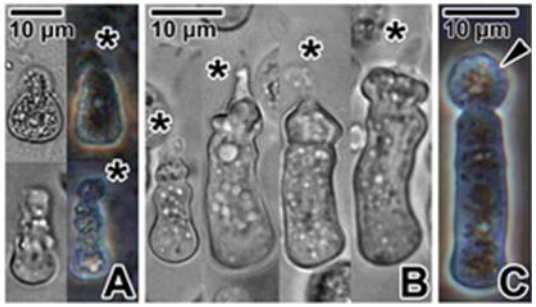


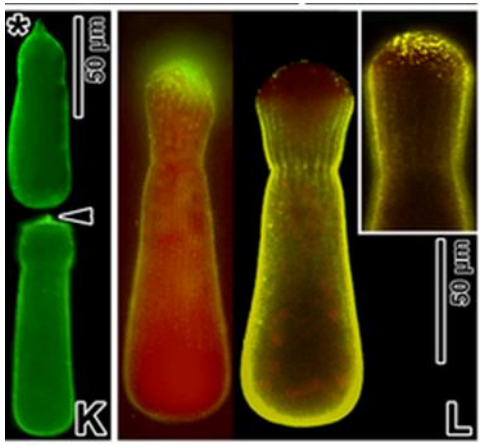
Grasshoppers infected with *Nosema locusta*



Spores of *Cystosporogenes deliaradicae* from infected fly *Delia radicum*

Neogregarines





Gregarina cuneata in infected Mealworm *Tenebrio molitor*

http://wwx.inhs.illinois.edu/research/biocontrol/pathogens/typesofpathogens/photos/6m-nlocustae/
http://www.diark.org/diark/species_list/Antonospora_locustae, Univ. Copenhagen
http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0042606

Some characteristics of important groups of insect pathogens

Type	Infection route	Specialists or generalists
Virus	Oral	Specialists
Bacteria	Oral	Generalists/specialists
Fungi	Through cuticle/oral	Generalists/specialists
Microsporidia	Oral	Specialists
Nematodes	Through body openings	Generalists

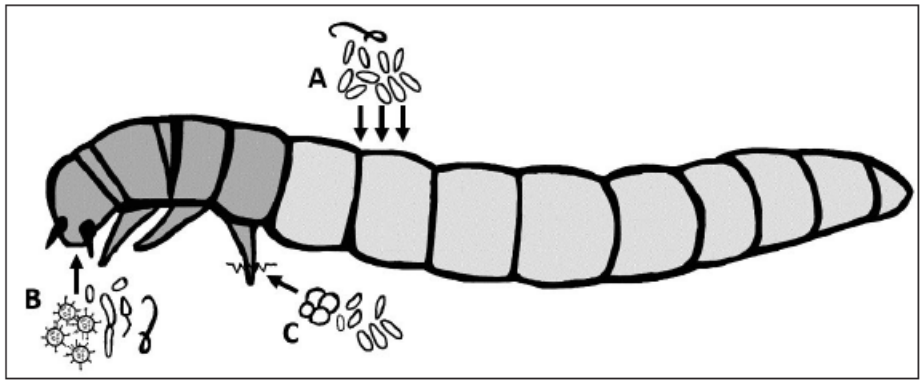


FIGURE 1: *Infection routes for insect pathogens. (A) Entomopathogenic fungi and nematodes can enter the insect's body cavity through the cuticle. (B) Pathogens such as viruses and bacteria, but also microsporidia, protozoa and nematodes, infect their host upon ingestion, alongside feed or faeces. (C) Injury to an insect's limb or body, often as a result of aggression among conspecifics, can allow opportunistic pathogens like certain bacteria and fungi, to enter the body and replicate. (source: Antoine Lecocq)*

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Lecocq et al 2019

Table 14.2 Selected results from a survey (2014–16) of insect diseases found by employees in production facilities for insects used as food and feed.

Production insect	Disease	Symptoms	Action
Acheta domesticus	Bacterium sp.	Increased mortality, red appearance	Cleaning of cages
Acheta domesticus	*Metarhizium* sp. and *Beauveria bassiana*	Some mortality in population	Quarantine, new breeding stock
Acheta domesticus	Cricket paralysis virus (CrPV)	Collapse of cricket population: the virus seems to spread globally	Switching to new breeding stock or even new cricket species
Gryllus bimaculatus	*Gryllus bimaculatus* iridovirus	Swollen abdomen, strikingly sluggish, mortality close to 100%	Occurs occasionally
Tenebrio molitor	*Beauveria bassiana*	Some mortality in population	Cleaning, removal of dead larvae, quarantine
Zophobas morio	*Pseudomonas* sp.	Increased mortality: recurrent problem	Removal of dead larvae
Musca domestica	*Entomophthora muscae*	Dead adult flies with spores, epidemic	Cleaning, removal of dead flies, quarantine
Hermetia illucens	Unknown (bacterium?)	Elongated, rounded mature larvae, moving slowly before dying	Quarantine

Eilenberg and Jensen 2018

A typical situation:

What is wrong with my *Tenebrio molitor* in culture ?

Some get discolored, some die

Is it an insect disease or some saprophytic microorganisms?

Any action needed?

Photo credits: E. Erin Morris, Univ. Copenhagen

Diagnosis needed

Bacterium, unknown species

Fungus (*Beauveria* sp.) infected

Nematode (*Steinermena feltiae*) infected

Uninfected

Photo credits: E. Erin Morris, Univ. Copenhagen

Common insect diseases in mealworm, *Tenebrio molitor*

A) Healthy larvaof mealworm

B) First outgrowth of fungus from larva. Typical fungus infections are caused by genera *Beauveria* and *Metarhizium*

C) Extended outgrowth and sporulation, all whitish. The fungus is *Beauveria bassiana*, having white spores

D) In this case, the causal agent is the fungus *Metarhizium brunneum*, having green spores in dense layers

E) A larva (dark) killed by a bacterium. Bacterial infections are caused by spore forming or non-sporeforming species

F) Pupa (pinkish) killed by the bacterium *Serratia marcescens*

G) Adult with swollen abdomen, possibly infectedby a bacterium or by another microorganism. Symptoms can also be due to other reasons than infection

Tenebrio molitor can be affected by lethal insect pathogens, both in larval, pupal and adult stages. Commonly found are fungi from the genera *Beauveria* and *Metarhizium*, and bacteria (genus *Serratia*and others). To diagnose fungal infections, allow dead larvae with early symptoms (B) to stay in humid chamber (moist filter paper added) up to two weeks. If infected by the *Beauveria* or *Metarhizium*, extensive outgrowth will be observed (C and D). First diagnosis is based on color of fungus and other visible symptoms, later to be accompanied by spore morphology to determine genus name. If species name is needed, PCR can be added. For bacteria, it might be difficult to distinguish between true infections caused by an insect pathogen and growth caused by opportunistic bacteria. Due to few prominent morphological characters like colorand swelling of host (E, F and G), often PCR is needed to determine to genus or species level of bacteria.Other microorganisms like insect virus and protozoa can also infect mealworms, although little is known about their effects on host. Fungal growth on dead insects can be due to mold fungi. Malfunctions, discolorationand death of *Tenebrio* may also be due to other reasons than infections.

A main reason for insect pathogens to occur and spread is a lack of cleaning and insufficient removal of dead insects. Also, a stressed population may be more susceptible to opportunistic bacterial infections. Management: Check carefully for dead insects, remove cadavers immediately. Note external symptoms, eventually also symptoms on living individuals. Immediate cleaning using standard washing and sterilizing liquids of all equipment is needed, potentially UV light as well (Eilenberg *et al.*, 2015). Smaller cohorts of insects should be kept in quarantine to avoid further spread. Contact an INSECTPATH laboratory for further assistance and advice.

Professor Jørgen Eilenberg, University of Copenhagen, Denmark. Jei@plen.ku.dk, phone + 45 23 30 20 24.

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Reference: Eilenberg *et al.* 2015, Journal of Insects as Food and Feed, 2: 87-102

inVALUABLE deliverable prepared by University of Copenhagen, December 2017.
Universityof Copenhagen: all photos, except F and G (Delphine Calas-List, Entomo Farm)

Fact sheet: *Tenebrio molitor*

For use in production facilities

Freely available jei@plen.ku.dk

Feedback from users is important

We plan to prepare similar sheets for other important insects in production

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Closed production facilities

A) **Closed production facilities** for lesser mealworm (*Alphitobius diaperinus*). Larvae are reared in plastic boxes on separate shelves. The Netherlands.

B) **Automatic separation** of *Tenebrio molitor* larvae from substrate. The Netherlands.

C) **Closed production facilities** for black soldier fly (*Hermetia illucens*). Larvae are reared in plastic boxes stacked at several meters height. South Africa.

Eilenberg & Jensen, 2018

Photo credits. A, B: Proti-Farm, The Netherlands. C: Agri-Protein, South Africa.

Semi-open production facilities

D), E) **Semi-open production facilities** for house cricket (*Acheta domesticus)* and black cricket *Gryllus bimaculatus.* New cohorts are started in the different sections at time intervals. Thailand.

Eilenberg & Jensen, 2018

Photo credits: Afton Halloran, Univ. Copenhagen, Denmark.

Outdoor production facilities

F) **Outdoor hives** for honeybee (*Apis mellifera*) in almond plantation with low biological diversity. California, USA

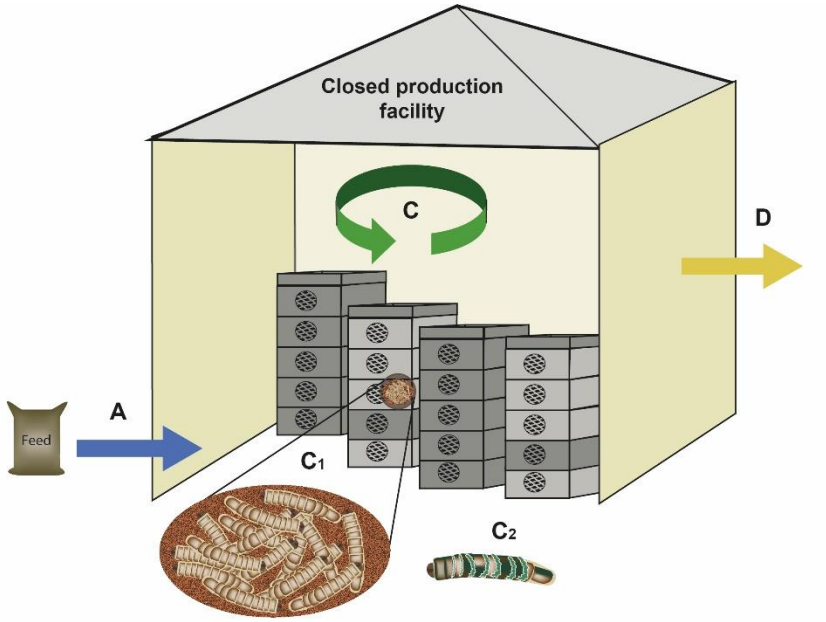
G) **Outdoor hives** for *A. mellifera* in an agricultural landscape with medium biological diversity. Denmark

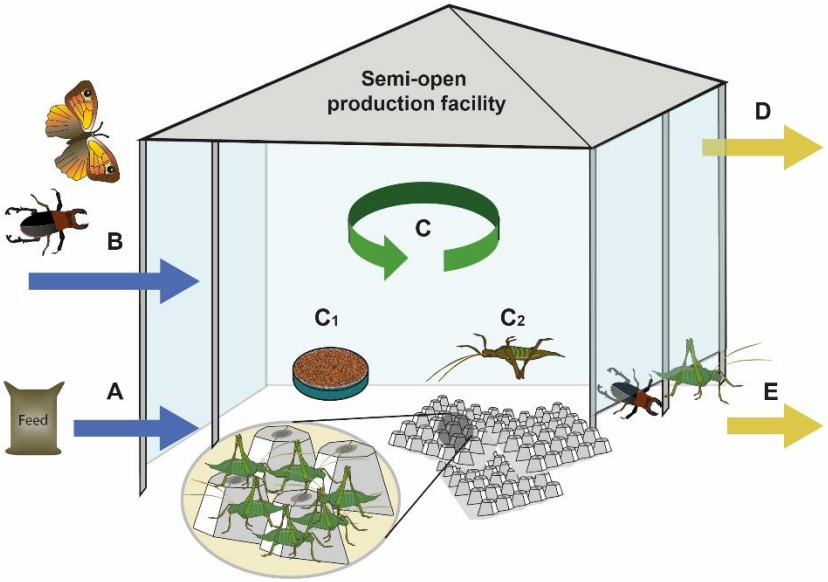
H) **Outdoor hives** for *A. mellifera* near tropical forest with high biological diversity. Tanzania.

I) **Outdoor production/foraging** of Mopane Moth *Gonimbrasia belina*, Zimbabwe

Eilenberg & Jensen, 2018

Photo credits: Annette Bruun Jensen, Univ. Copenhagen, Denmark. http://www.nydailynews.com/life-style/eats/zimbabwe-favorite-snack-mopane-worms-article-1.1247669





Main transportation routes of insect pathogens from the external environment and into facility, movement within facility and transportation from facility to the external environment

Eilenberg et al., 2018

Recommendation

- Check physical structure of production facilities. Do they prevent natural enemies to enter?
- Check substrates to enter the facilities. Any risk of microorganisms?
- Ensure optimal rearing conditions. Batches in separate units or continous production systems? Consider genetical variation in stock insects, inbreeding
- Frequent observations of production facilities and insects are needed
- Viruses, bacteria, fungi, microsporidia and protozoa all need different methods to be diagnosed , covert infections are tricky to discover
- Contact insect pathology laboratories: Jei@plen.ku.dk

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An EU supported IJD-ITN project. Fifteen PhD projects to be initiated in spring 2020, covering a range of basic and applied aspects.

Coordinator Prof. Monique van Oers, Wageningen University
Monique.vanoers@wur.nl

Beneficiaries

(= Universities hiring the students)

- Wageningen University*
- University of Copenhagen*
- Centre National di la Recherche Scientifique*
- University Valencia*
- National Institute for Agricultural Research
- University of Exeter*
- UK Centre for Ecology & Hydrology
- Julius Kühn Institute

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- Food and Agriculture Organisation (FAO)
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- Proti-Farm R&D BV
- YNSECT
- Entec Nutrition Ltd.
- Université Paris-Saclay

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