Exploring opportunities to induce epizootics in greenhouse aphid populations

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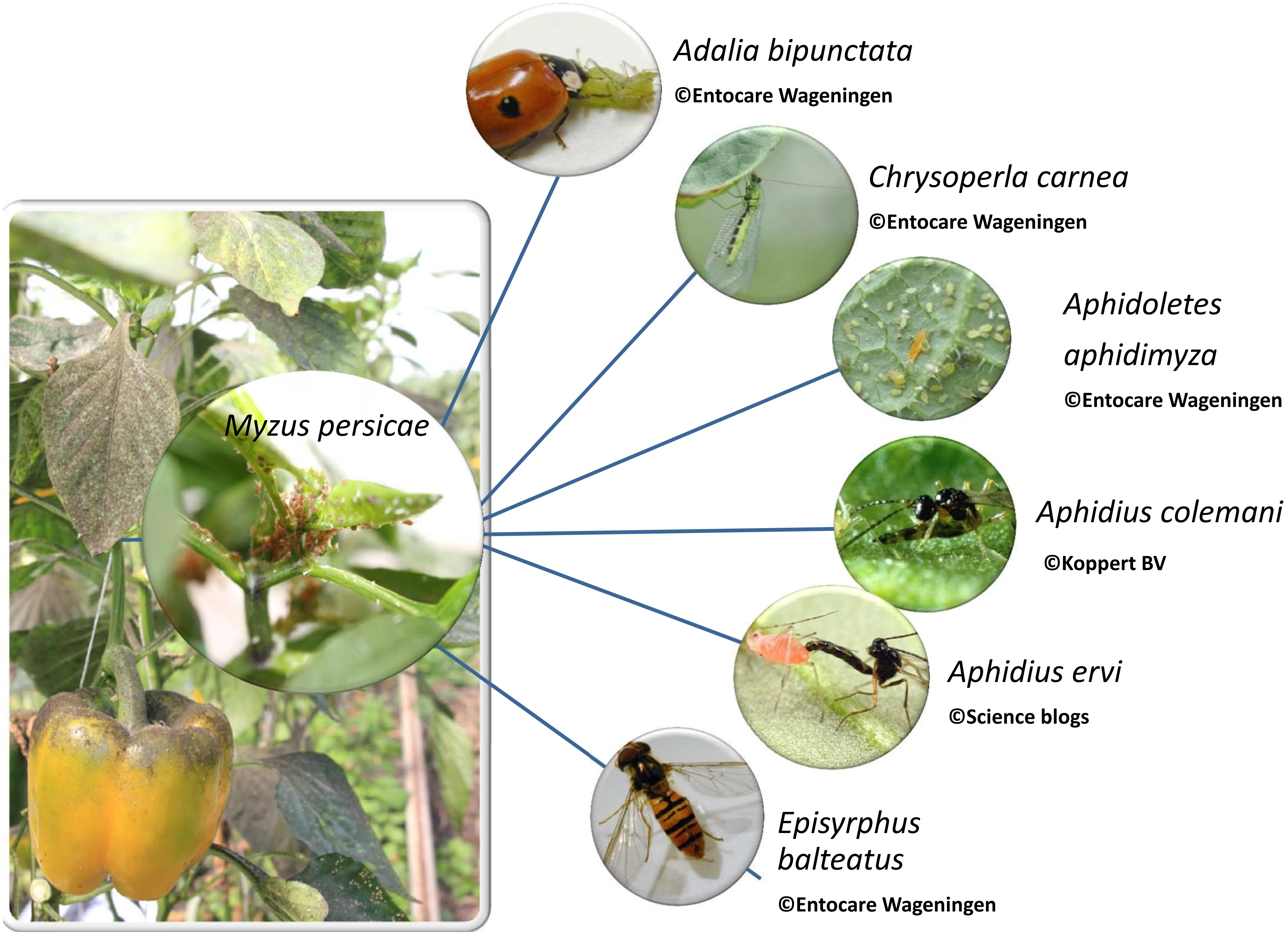
Augmentative biological control with parasitoids and predators



Looking for entomopathogens / natural outbreaks

Entomopathogenic fungi are considered to be an important factor regulating pest insect populations and one of the best candidates for **biological control of aphids**

Verticillium longisporum
Beauveria bassiana
Erynia neoaphidis
Pandora neoaphidis
Entomophthora aphidis
Entomophthora planchoniana

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Fungi of the **Order Entomophthorales** are the **major fungal pathogens of aphids**

- 33 entomophthoralean species organized into 9 genera have been recorded in aphid hosts

- none of the species of this order have been developed as commercial biological control products

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Entomophthoralean fungi are characterized by:

- **FAST DISEASE DISPERSAL** in dense colonies
- **HIGH REPRODUCTIVE RATE** which enable them to spread fast (short infection cycle, conidia are forcibly discharged from conidiophores
- effectiveness at **LOW TEMPERATURES**
- possibility to enhance **DISPERSAL OF CONIDIA THROUGH NON-TARGET INSECTS**

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Entomophthoralean fungi are characterized by:

- high **HOST SPECIFICITY**

  **augmentative biological control*

- surviving and **PERSISTING IN THE ENVIRONMENT** as resting spores

  **conservational biological control*

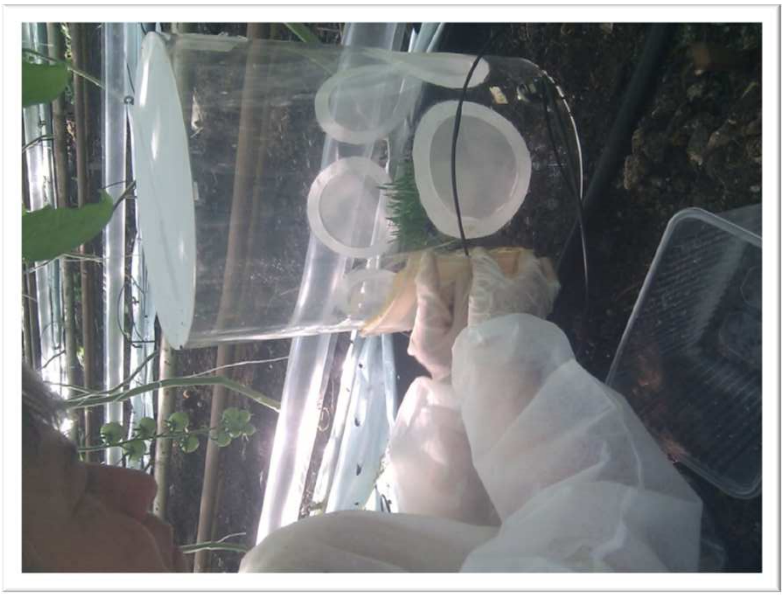
Lab trials






Lab trials

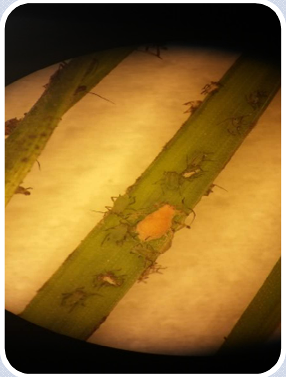
Greenhouse trials



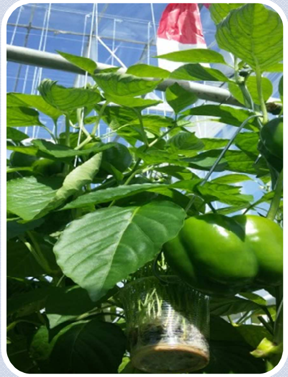
Greenhouse - plant cage trials

Inducing epizootics by introducing inoculum of infected aphids into the crop

Banker plants



Artificial infection



Introducing in crops

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Banker plants

The grain aphid ***Sitobion avenae*** was reared on seedlings of **winter wheat**.

One banker plant unit consisted of a small transparent 200 ml plastic cup with approximately 200 winter wheat seedlings (9 g) grown on a small layer of peat.

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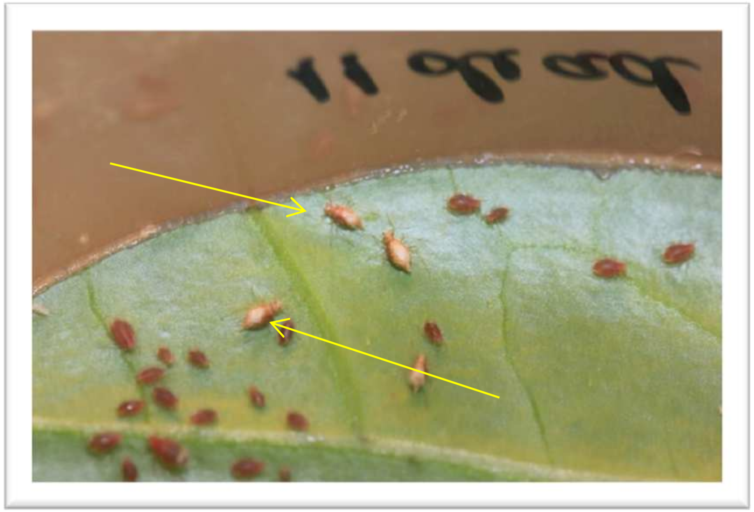
Artificial infection

Detached leaf bioassay

Pandora neoaphidis was recovered from a natural outbreak in an organic greenhouse from The Netherlands in 2014 and it was preserved in laboratory by successive infection of healthy peach aphids (*Myzus persicae* - red phenotype) from Wageningen rearing.



Artificial infection

The grain aphids from banker plants were exposed directly to “conidia showers“ by putting 6-10 *P. neoaphidis* infected and unsporulated peach aphids on wet tissue above the banker plants infested with healthy grain aphids. The methods used for infection of aphids by *P. neoaphidis* were based on the detached leaf bioassay and “shower conidia” method as described by Keller (2007).

Keller, S. (2007) Entomophthorales: Biology, Ecology, Identification. COST Action 842. EUR 22829.

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Artificial infection

Second protocol: putting *P. neoaphidis* infected and unsporulated peach aphids on cut leaves between the healthy grain aphids ...

...and covering the plants in a plastic bag for 24 hours at 18 ^{0}C, after misting all aphids with water.

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Artificial infection

The mean time for infection is 4.5 hours at 100%RH, 20 ^{0}C and sporulation begins within 2 hours of aphid death (Keller, 2007).

The lethal time is 3-5 days at incubation temperatures above 20 ^{0}C, whereas it is much higher at lower temperatures (Nielsen et al., 2001).

Nielsen, C., Eilenberg, J., and Drumm, K. (2001). Entomophthorales on cereal aphids: characterisation, growth, virulence, epizootiology and potential for microbial control. København: Frontlinien, Miljøministeriet. (Bekæmpelsesmiddelforskning fra Miljøstyrelsen; No. nr. 53).

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Introducing in crops

Five days after artificial infection, each banker plant contained 30-50 infected aphids. Growers were provided with 30 of those Pandora banker plant units per aphid outbreak.

The system of introducing "Pandora bankers" has been applied by 8 organic greenhouse growers in The Netherlands with various results.

The banker plant cups were pierced with an s-shaped iron wire for hanging them in the right position in the top of a pepper plant (this position enabled *P. neoaphidis* to shoot the conidia on the target aphids in the pepper plants below the banker plants).

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Results

The introduction of Pandora banker plants resulted only in one of all 8 greenhouses in an epizootic of *P. neoaphidis*. The infection of aphids was in this case very high with aphids colonies on every plant and serious damage to the crop.

P. neoaphidis was introduced twice in ten days. Three weeks after the second introduction, all aphids in the greenhouse were dead through an infection by *P. neoaphids*.

High humidity levels were achieved with mist foggers during two days from 22:00 till 2:00. In this period the temperature was reduced to 16 ^{0}C.

Results

In all other cases, *P. neoaphidis* did not succeed, or only a limited number of aphids were infected.

The reasons for this are:

- Aphids were **controlled by other natural enemies**, like *Aphidoletes aphidimyza*
- The **aphid densities** at the time of application were **too low**

- The temperatures during the day were probably too high
- The high humidity levels were not achieved

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Integrated Control in Protected Crops, Temperate Climate
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pp. 259-264

Efficacy of commercial and non-commercial strains of entomopathogenic fungi against the peach aphid *Myzus persicae* (Sulz.)

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Abstract: The efficacy of twenty-two strains of entomopatogenic fungi, isolated from natural outbreaks and also from commercial products (*Lecanicillium (Verticillium) lecanii* (Zimm.) Zare & Gams, *Lecanicillium longisporum* (Petch) Zare & W. Gams, *Beauveria bassiana* (Bals.-Criv.) Vuill, *Metarhizium brunneum* (*anisopliae*) (Metschn.) Sorokin, *Isaria fumosorosea* Wize, *Paecilomyces lilacinus* (Thom) Samson, *Hirsutella kirchneri* (O. Rostr.) Minter, B. L. Brady & R. A. Hall and *Hirsutella thompsonii* F. E. Fisher), was tested for the control of the peach aphid *Myzus persicae* (Sulz.). Pathogenicity of the selected isolates was first evaluated through a quick screening test: aphids were exposed to conidia for 5 minutes, by gently transferring them to sporulated cultures (on PDA), and then transferred to plastic boxes (detached leaf bioassay) and kept at 20 °C, 80% RH for 10 days. Signs of infections were assessed daily. The most virulent strains were selected for the next step, the laboratory trial. For effectiveness of strains in the laboratory trial, conidiospores in three different doses (10^5, 10^7, 10^9 conidia/ml) were sprayed on aphids. Treated aphids were kept to detached leaves of sweet pepper on 1% water agar for pathogen incubation and pathogenicity of most fungal species was confirmed at all the three doses. The most virulent strains were tested in greenhouse, on aphids reared on potted plants. All the tested strains gave significant control of aphid in the laboratory trial, but only *Lecanicillium longisporum* (Vertalec® isolate) showed a reasonable control of aphids in greenhouse trial with infection rates up to 40%. An increasing trend in mortality of aphids (epizootic) was observed in all the treatments with an increase in aphid population.

Key words: aphids, entomopathogens, greenhouse, biocontrol

Introduction

Aphids represent one of the world's major insect pests causing serious economic damage in field and greenhouse crops. Hypocreales fungi have been widely explored as biological control agents for aphids, but most of the commercialised strains of entomopathogens are not effective against aphids (Jandricic *et al.*, 2014). Yet, there is as strong need for the use of effective entomopathogens, particularly in organically grown sweet pepper crops where augmentative biological control with parasitoids and predators is expensive and often not successful.

In this study, we wanted to explore the pathogenicity of some fungal strains, isolated from natural outbreaks, and to compare them with those used in commercial products.

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REVIEW

New opportunities for the integration of microorganisms into biological pest control systems in greenhouse crops

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Abstract Biological pest control with mass-produced arthropod natural enemies is well developed in greenhouse crops and has often resulted in the evolution of complex ecosystems with persistent populations of multiple arthropod natural enemy species. However, there are cases where arthropod natural enemies are either not effective enough, not available, or their use is rather costly. For these reasons, biological control based on microorganisms, also referred to as 'microbials', represents a complementary strategy for further development. Although commercially available microbials have been around for quite some time, research on and the applied use of combinations of arthropod natural enemies and microbials have remained relatively under explored. Here, we review current uses of entomopathogenic fungi, bacteria and viruses, and their possible direct and indirect effects on arthropod natural enemies in European greenhouses. We discuss how microbials might be combined with arthropod natural enemies in the light of new methodologies and technologies such as conservation biological control, greenhouse climate management, and formulation and delivery. Furthermore, we explore the possibilities of using other microorganisms for biological control, such as endophytes, and the need to understand the effect of insect-associated microorganisms, or symbionts, on the success of biological control. Finally, we suggest future research directions to optimize the combined use of microbials and arthropod natural enemies in greenhouse production.

Keywords Arthropod natural enemies · Microbials · Entomopathogens · Endophytes · Symbionts

Key message

- The application of microbials for pest control in greenhouse crops should be integrated with the use of arthropod natural enemies. Here we review the current uses of entomopathogenic fungi, bacteria and viruses, and their possible direct and indirect effects on arthropod natural enemies.
- New approaches in the use of conservation biological control, greenhouse climate management, formulation, delivery and endophytic microorganisms could increase the various ways in which microbials can interact with arthropod natural enemies, and these interactions can be both positive and negative for pest control.

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Plant health

Use of banker plants for Entomophthorales-caused epizootics in aphid populations

Fungi belonging to Order Entomophthorales are considered the major pathogen of aphids in natural ecosystems and also in agroecosystems. They are known to cause large scale epizootics, but none of the species of this order have been developed as commercial biological control products. They could be one of the best candidates for controlling aphids in greenhouses because entomophthoran fungi require shorter periods of high humidity for transmission than fungi belonging to the order Hypocreales (*Beauveria* sp., *Lecanicillium* sp., *Paecilomyces* sp. etc), are more effective at low temperatures and are also more host specific. More than 30 fungi of entomophthoralean species have been recorded as aphid entomopathogens. However, a major stumbling block to utilising these fungi as biological control agents has been the difficulties encountered in growing them in vitro. Another approach in utilising these fungi could be to try inducing epizootics by introducing inoculum of infected aphids into the crop by using banker plants.

What is a banker plant system?

In the banker plant method, long-lasting rearing units for beneficials are created in the crop by distributing plants infested with herbivores or carrying other food items, such as pollen. The method has been widely investigated over many years and used to aid establishment, development and dispersal of beneficial organisms employed in biological control. So far, they are mainly used to conserve and augment arthropod natural enemy populations. A widely applied system in greenhouse crops has been the use of monocotyledonous plants with cereal aphids that serve as alternative hosts for parasitoids of aphids that attack the crop. The advantage of this system is that the grain aphids are specific to monocotyledons and pose no threat to crops that are dicotyledons (or dicots) (Figure 1). The same cereal aphids could also be used as entomopathogenic spores reservoirs , a source of entomophthoralean inocula, to cause epizootics of aphids which attack greenhouses crops.

Figure 1. Banker plants - wheat seedlings infested with *Sitobion avenae*.

How to use banker plants for Entomophthorales-caused epizootics

One of the most effective entomophthoralean species to control aphids is *Pandora neoaphidis*. Banker plants with this fungus can be produced by raising the wheat aphid *Sitobion avenae* on monocots and infects them artificially with *P. neoaphidis*. The phases of fungal infection are: adherence of spores on the insect cuticle and germination, penetration of tegument by specialized cells coming from germ tubes, development of fungus in the insect s body as protoplasts or hyphal bodies, death of insect and initiation of the saprophyte phase of the fungus. Under favourable conditions of humidity and temperature, the conidiophores emerge from the cadavers 5 days post infection.

Figure 2. *Sitobion avenae* infected by entomophthoralean fungus.

The conidia of aphid-pathogenic entomophthorales are forcibly discharged from conidiophores (Figure 2) and they are able to cause epizootics quickly through spore dissemination.

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