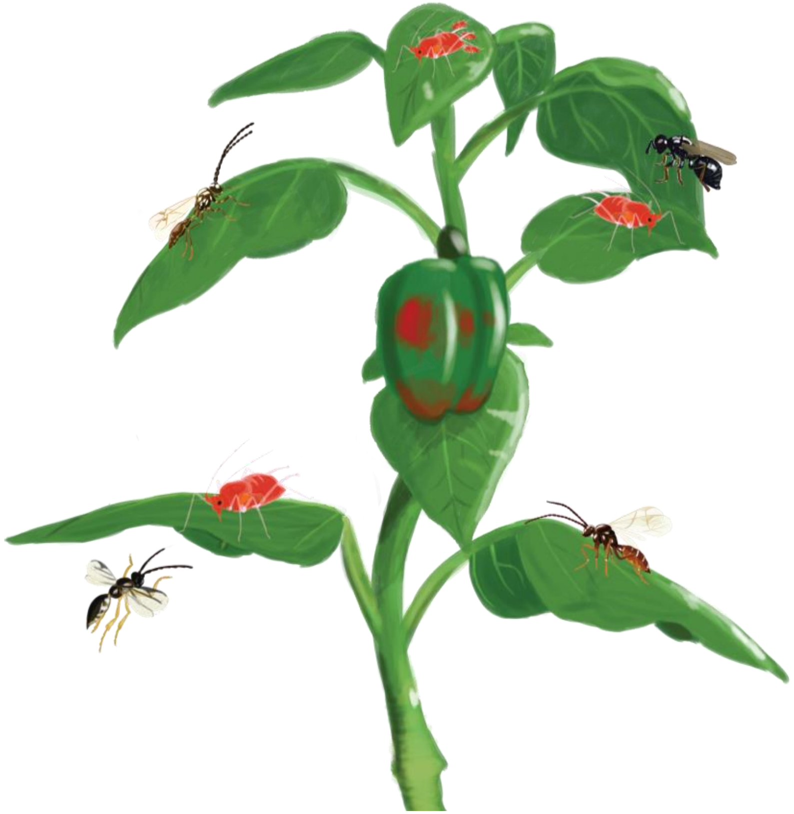
Innovative biological control of aphids in the protected cultivation of sweet pepper and small fruits (BIOTRACT)

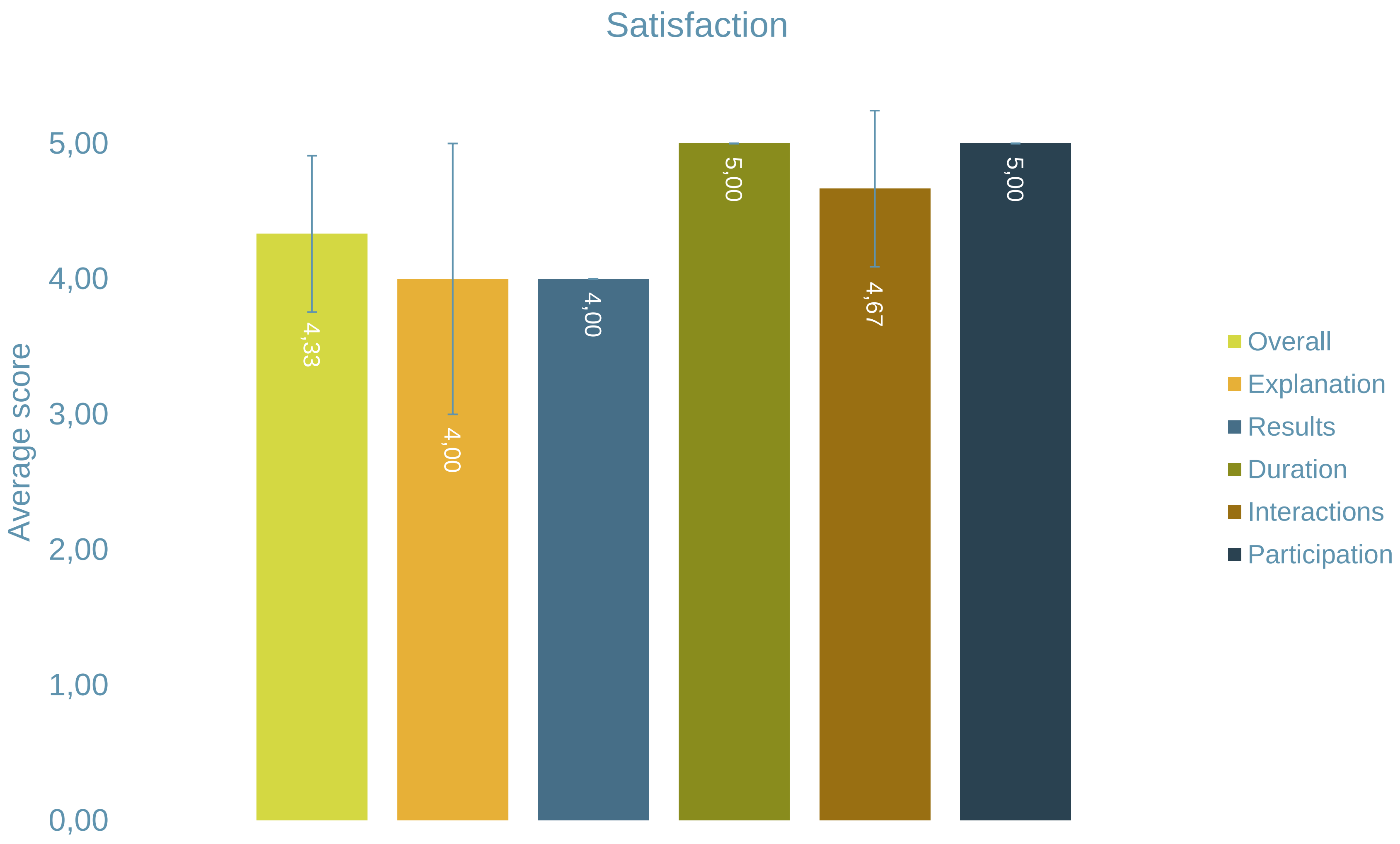
User committee meeting 04-12-2023

Program

1. Satisfaction analysis
2. Introduction
3. Project overview
4. Obtained results
5. Take home messages
6. Perspectives



Satisfaction analysis May 2023: Results



Aphids: among the most destructive insect pests on cultivated plants in temperate regions

Sap-sucking insects feeding on phloem

Ability to rapidly increase population size by asexual reproduction

Direct damage

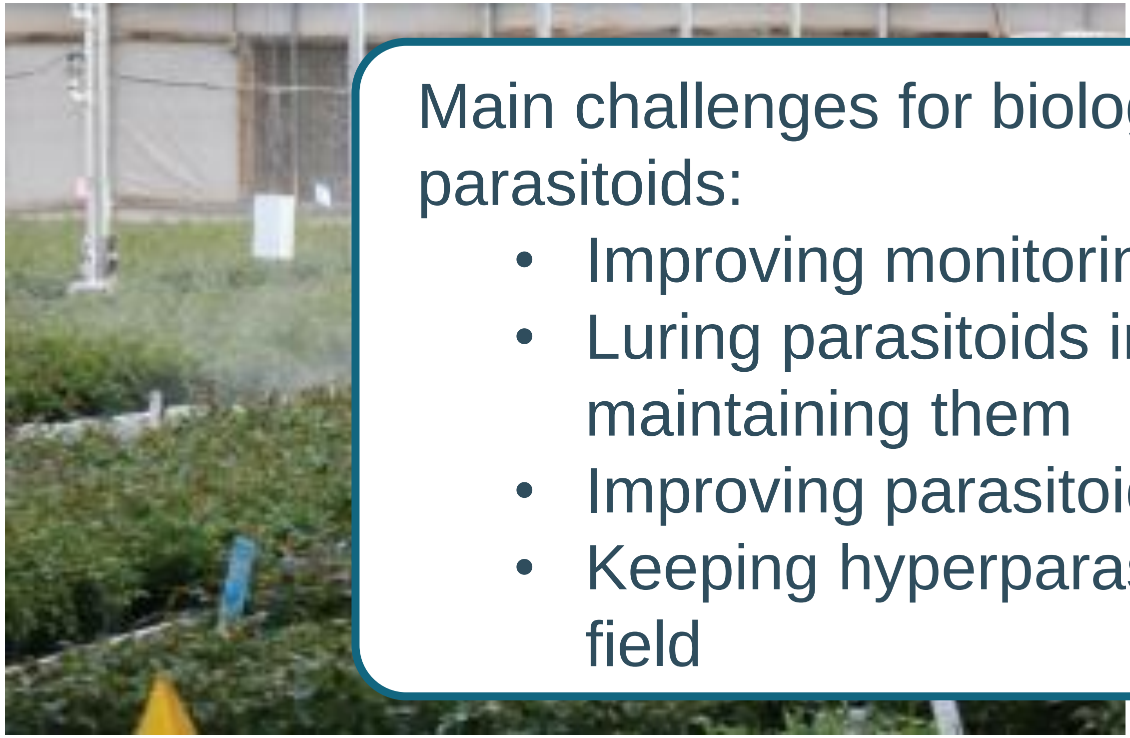
- Growth reduction
- Deformation or stunting of leaves

Indirect damage

- Transmission of plant viruses
- Production of honeydew

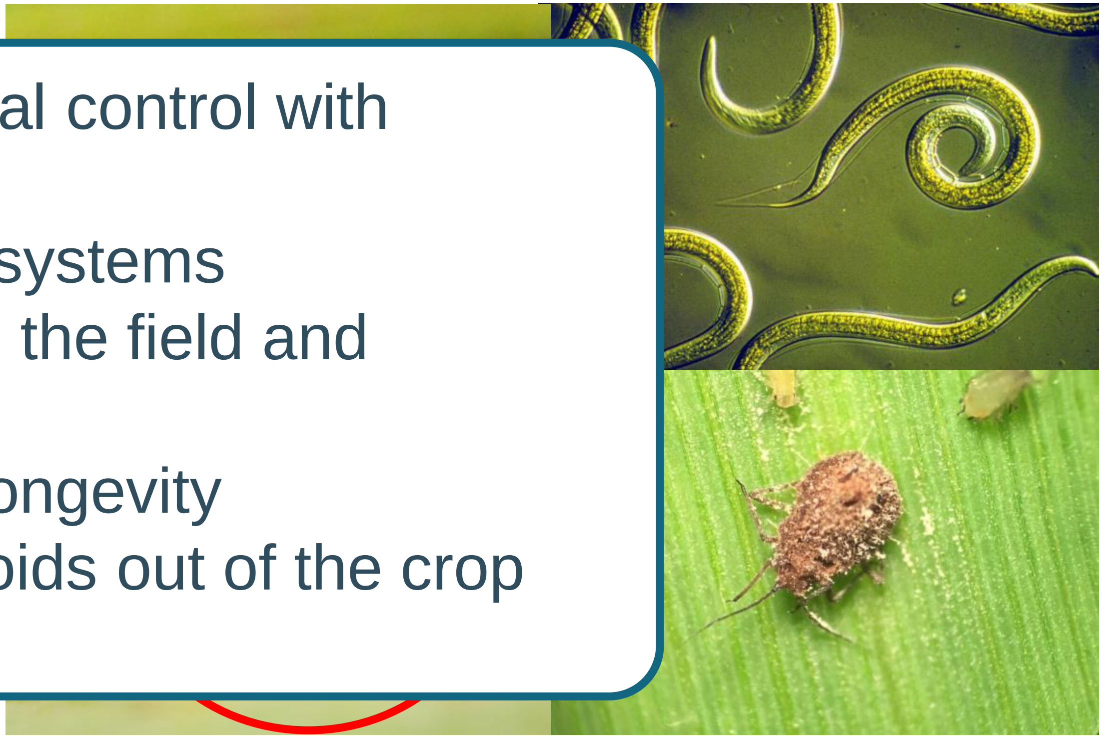
Sweet pepper and small fruits

Management of aphids



Main challenges for biological control with parasitoids:

- Improving monitoring systems
- Luring parasitoids into the field and maintaining them
- Improving parasitoid longevity
- Keeping hyperparasitoids out of the crop field



Hyperparasitoids: enemies of enemies…

Parasitoids of primary parasitoids

Potentially a large problem for aphid biocontrol

High percentage of aphid mummies may be hyperparasitised in the high season (April/May: 55-78% of all mummies)

Dendrocerus & *Asaphes* most common genera in literature from Canada, Japan, France, Germany

Attractants and repellents as auxiliaries

Attractive and repellent volatile compounds may be a useful tool:

- Lure insects towards monitoring systems
- Localization of feeding devices
- Push-pull management strategies
- Attract & kill hyperparasitoids

Microorganisms may be a promising source for these compounds!

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Project goals

Enhancing the biological control of aphids in sweet pepper and small fruits (raspberry, blackberry) by employing microbe-derived volatile compounds

- Optimization of existing monitoring techniques
- Development of an attractive feeding device to support aphid parasitoids with sugars
- Development and application of a VOC-based management strategy for aphids and hyperparasitoids

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Work plan

WP0 Identifying the community of hyperparasitoids in sweet pepper and raspberry

WP1 Screening and identification of microbial semiochemicals to attract or repel selected target insects

WP2 Application of microbial semiochemicals to enhance biological control of aphids

WP3 Evaluation of developed strategies to enhance biological control of aphids

WP4 Dissemination of project results

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WP 0: Hyperparasitoid survey

Survey at sweet pepper and raspberry growers

Sweet pepper: 5 growers, 10 locations
Raspberry: 5 growers, 10 locations
All growers are followed up monthly between March & October

Sampling using bankerplants

Bankerplants + ~500 *Sitobion avenae* aphids
Parasitized for 72 hours by *A. ervi*

Placed in greenhouse for 7 days

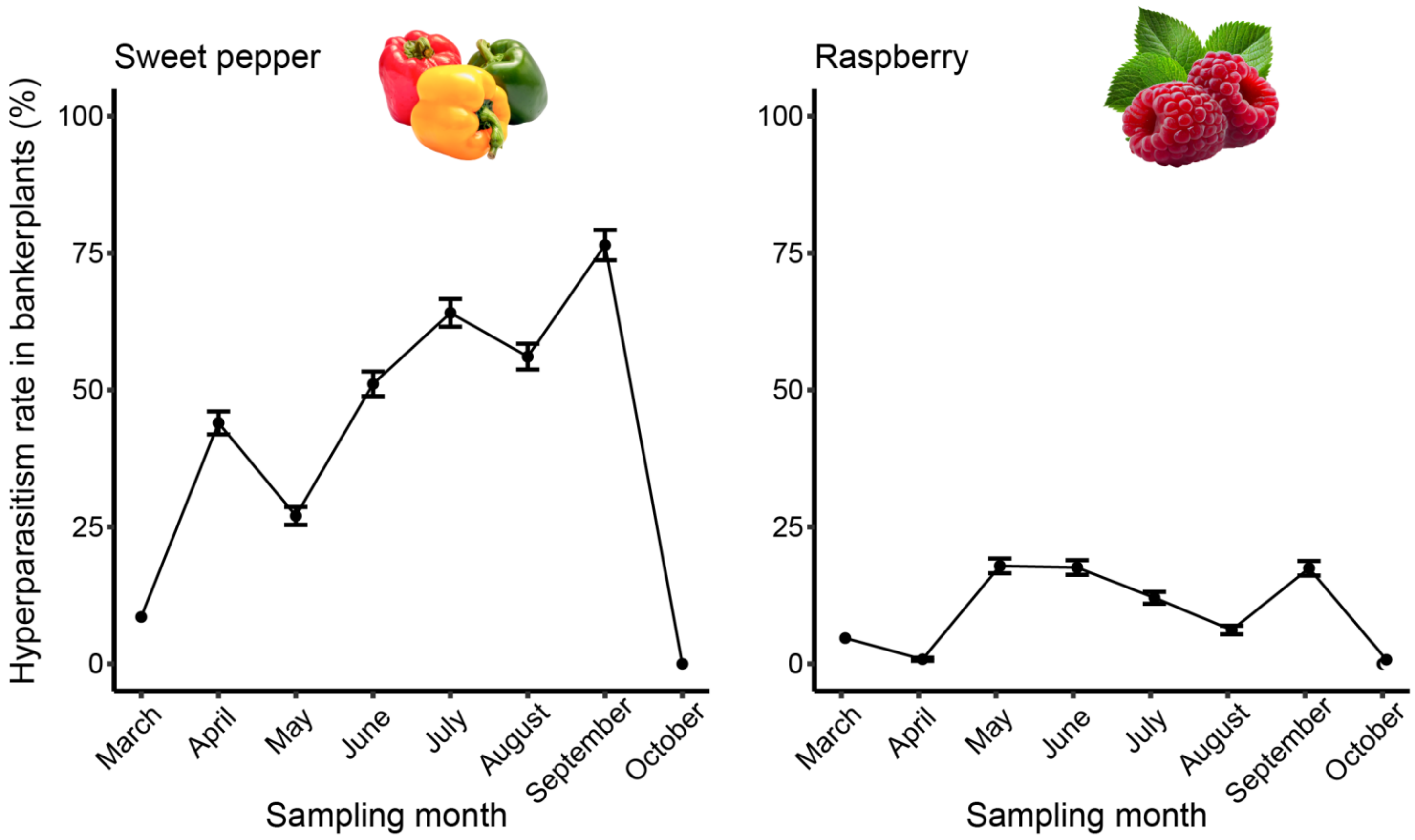
Incubate ± 20 shoots with mummies at 23°C until emergence

Emerged individuals are identified and counted

WP 0: Hyperparasitoid survey

Higher level of hyperparasitism in sweet pepper than in raspberry (42% vs 10%)

Hyperparasitism present early in the season and present throughout the year

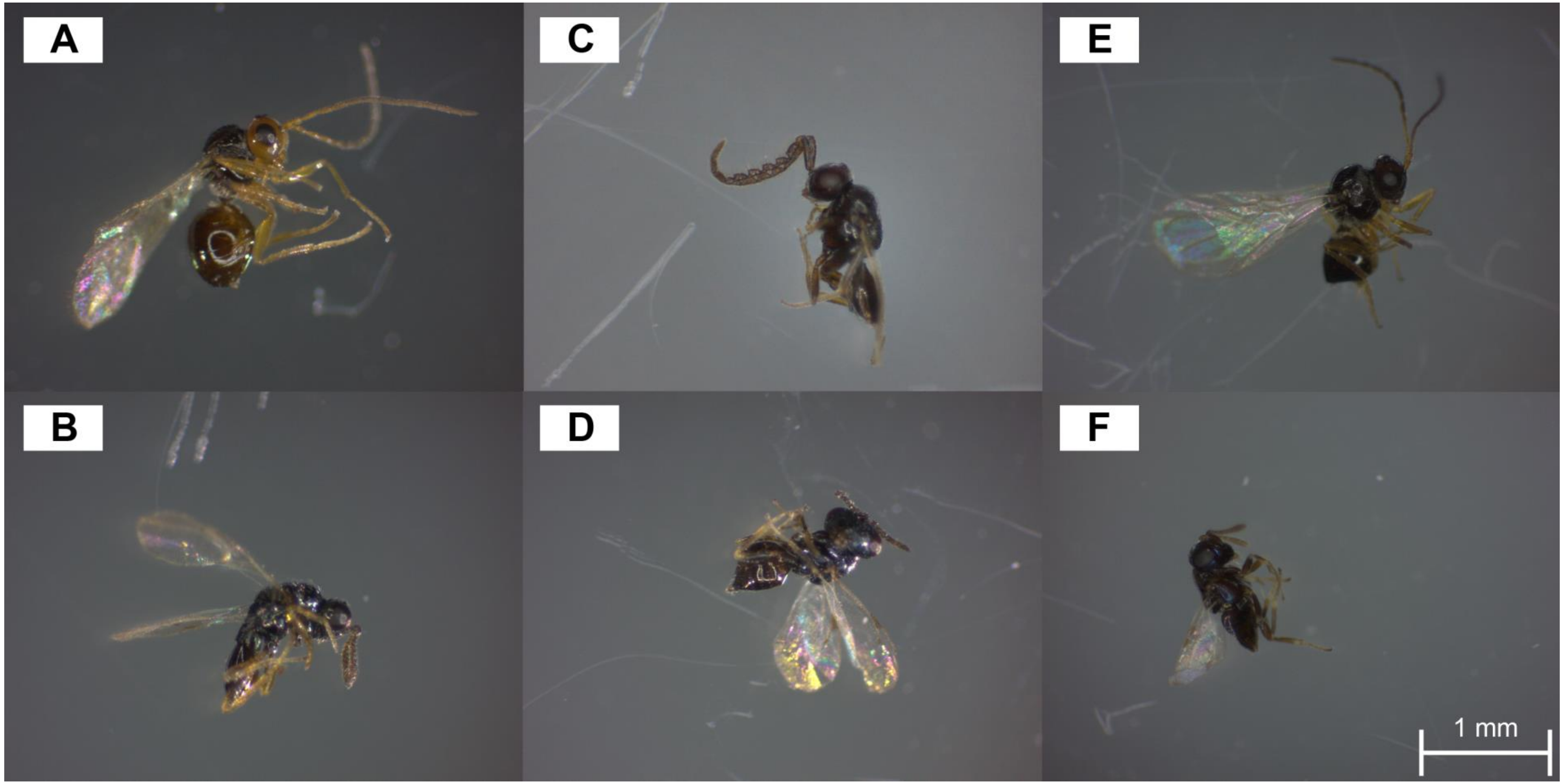


WP 0: Hyperparasitoid survey

	Sweet pepper								Raspberry							
	March	April	May	June	July	Aug.	Sept.	Oct.	March	April	May	June	July	Aug.	Sept.	Oct.
Alloxysta			1.7	68.9	12.0	28.7					9.9	9.1				
Asaphes				6.7		1.7			100.0	33.3	5.6					
Dendrocerus	100.0	100.0	73.3	20.0		39.1				33.3	84.5	90.9	78.1	80.0	93.2	
Pachyneuron			25.0	2.2	88.0	27.8	42.3			33.3				20.0	6.8	
Phaenoglyphis						0.9	57.7						21.9			100.0
Syrphophagus				2.2		1.7										

Dendrocerus hyperparasitoids was the most common genus identified and was present in most sampled locations.

WP 0: Hyperparasitoid survey



A: *Alloxysta*
B: *Asaphes*
C: *Dendrocerus*
D: *Pachyneuron*
E: *Phaenoglyphis*
F: *Syrphophagus*

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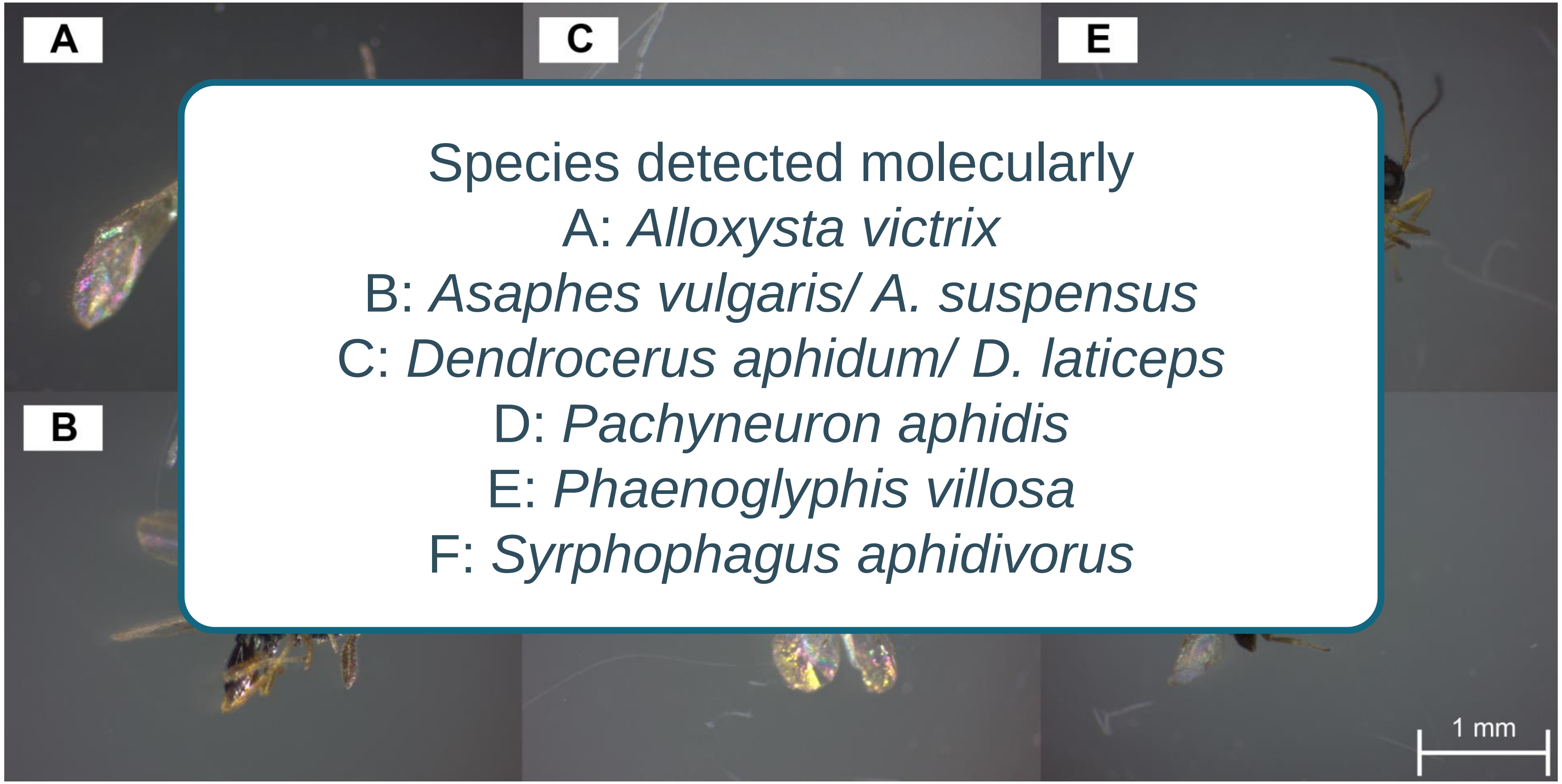
WP 0: Hyperparasitoid survey



Species detected molecularly

A: *Alloxysta victrix*

B: *Asaphes vulgaris/ A. suspensus*

C: *Dendrocerus aphidum/ D. laticeps*

D: *Pachyneuron aphidis*

E: *Phaenoglyphis villosa*

F: *Syrphophagus aphidivorus*

WP 0: Conclusions

Hyperparasitoids were found throughout the sampling season

Six species, within six genera were found. All cosmopolitan

Most common species throughout season in both crops: *Dendrocerus aphidum*

Hyperparasitoids could be found from the start of the sampling season

Bankerplants could be used to monitor hyperparasitoids in your crop

Work plan: Study system

Hyperparasitoids

Dendrocerus aphidum

Asaphes suspensus

Parasitoids

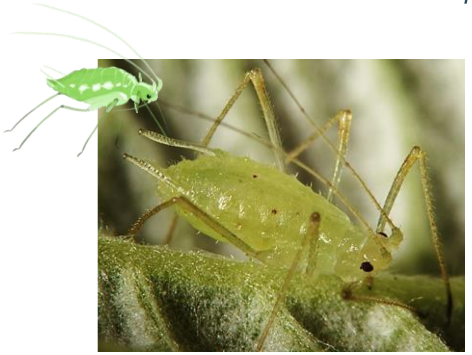
Aphidius colemani

Aphidius matricariae

Aphidius ervi

Aphids

Myzus persicae var. *nicotianae*



Amphorophora idaei

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Work plan: Study system

ID	Species	Origin
ST18.16/133	*Bacillus pumilus*	*Aphidius ervi*
ST18.16/085	*Curtobacterium* sp.	*Myzus persicae* var. *nicotianae*
ST18.16/160	*Staphylococcus saprophyticus*	*Macrosiphum euphorbiae*

WP1: Screening and identification of microbial semiochemicals

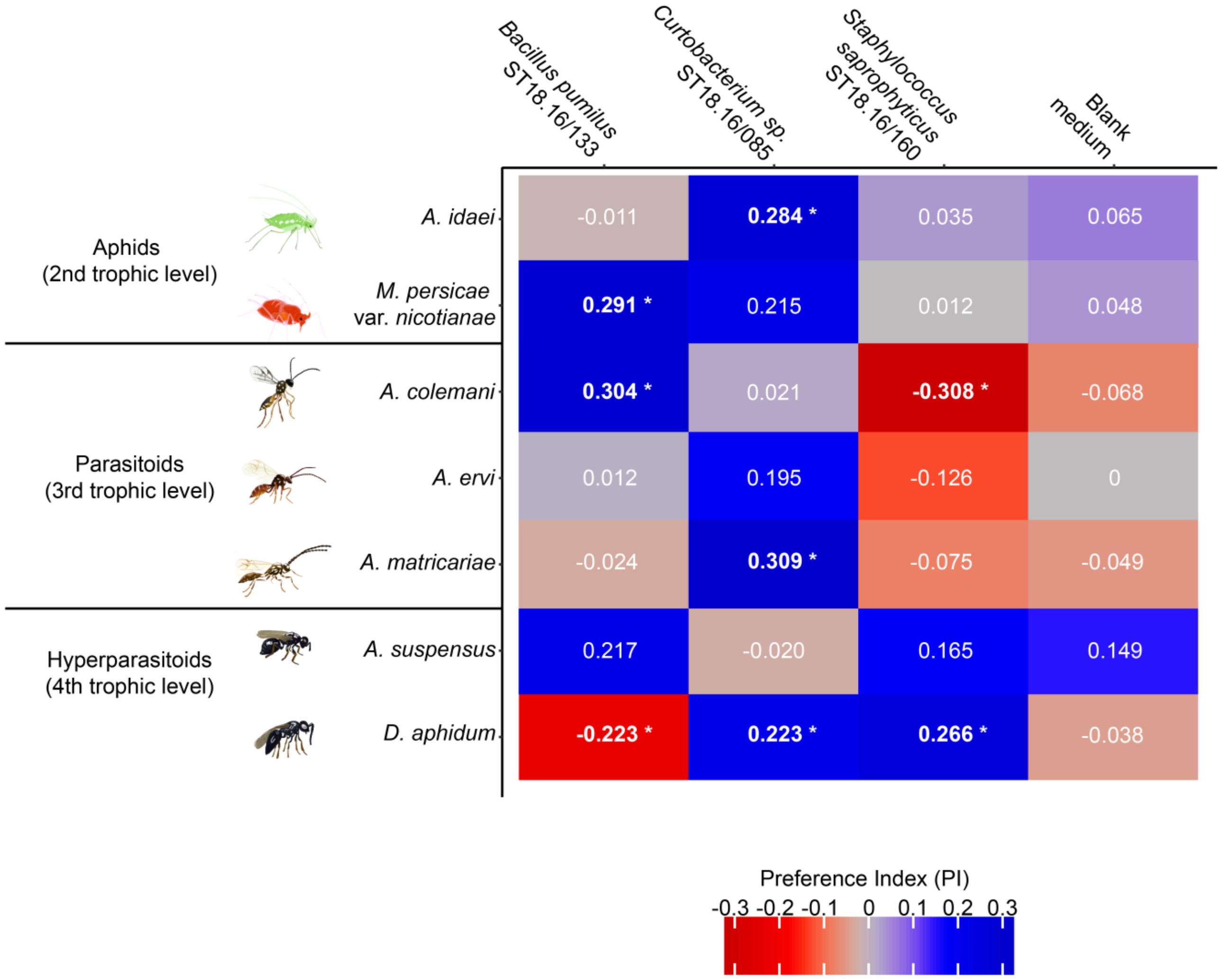
To screen microbial volatile blends in order to identify microbial semiochemicals

- **Task 1.1**: Screening of bacterial volatiles
- **Task 1.2**: Identification of VOCs
- **Task 1.3**: Development of synthetic volatile blends

Task 1.1: Screening bacterial volatiles

Bacterial volatiles induce differential responses in insects within and between trophic levels

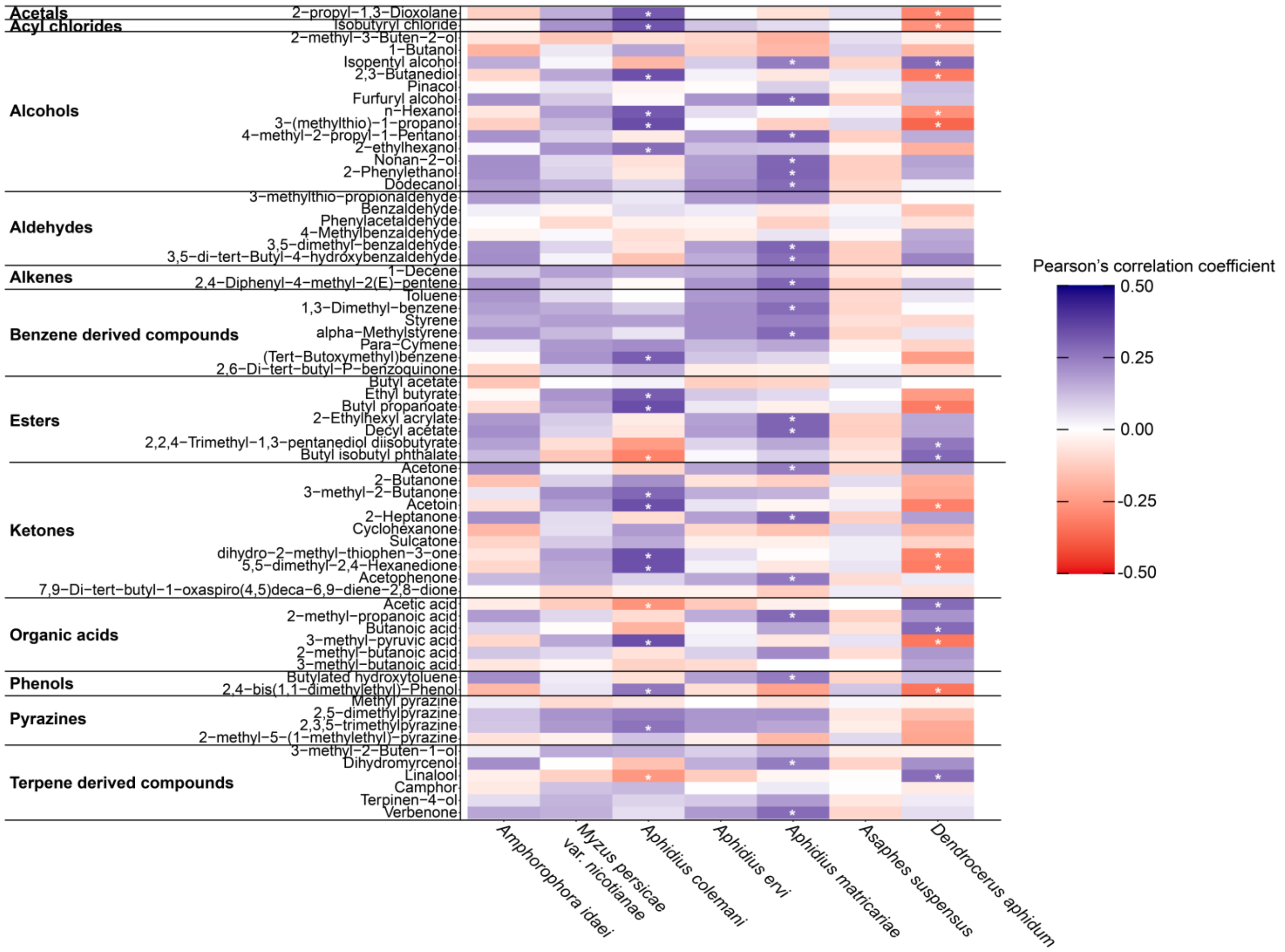
Parasitoids and hyperparasitoids show a different olfactory response to similar bVOCs



Task 1.2: Identification of VOCs

11 compounds were identified as possible candidates to attract parasitoids and repel hyperparasitoids using a correlation analysis

For example: linalool, acetic acid, 1-hexanol, styrene, and benzaldehyde.



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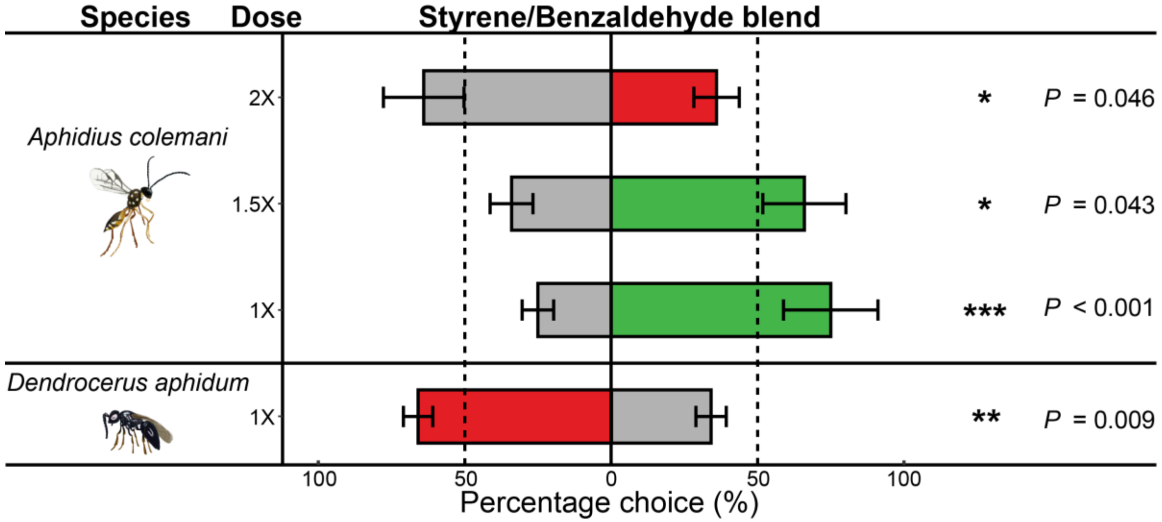
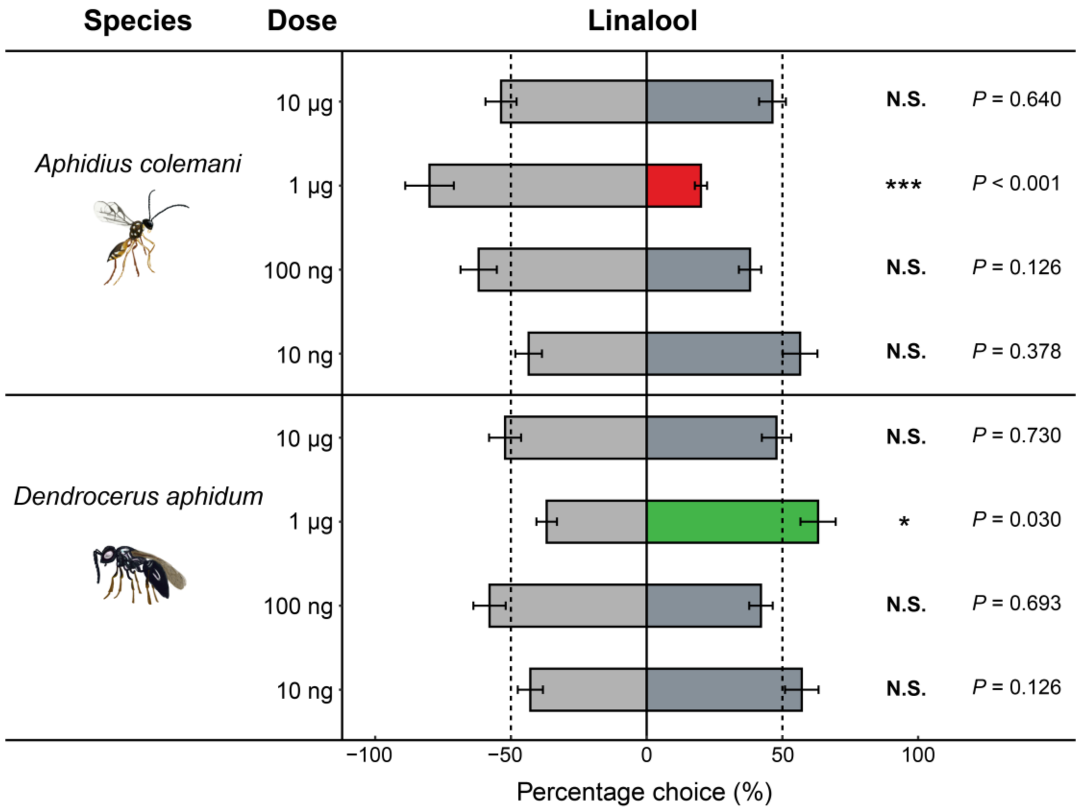
Task 1.3: Development of synthetic volatile blends

A blend of styrene and benzaldehyde is attractive for the parasitoid *A. colemani* but repellent for the hyperparasitoid *D. aphidum*



Task 1.3: Development of synthetic volatile blends

Linalool is attractive for the hyperparasitoid *D. aphidum* but repellent for the parasitoid *A. colemani*

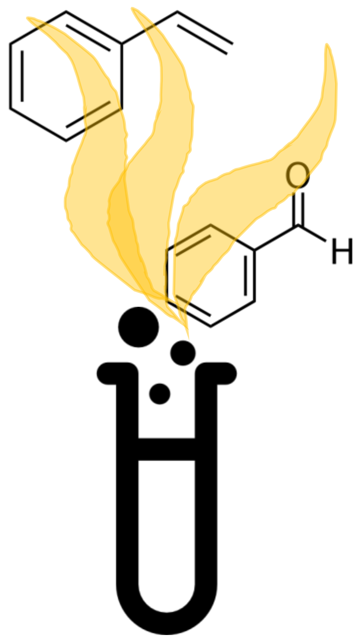


WP1: Conclusions

Bacterial volatiles can be used to manipulate behavior of insects important in agriculture (pest insects and natural enemies) in laboratory conditions

A small list of compounds from bacterial volatiles are responsible for this behavior

These volatiles and blends of these volatiles can be used to attract parasitoids and repel hyperparasitoids



WP2: Application of microbial semiochemicals to enhance biological control of aphids

Enhancing the biological control of aphids by applying the developed microbial semiochemicals

- **Task 2.1**: Evaluation of VOC blends for attracting parasitoids
- **Task 2.2**: Monitoring techniques
- **Task 2.3**: Attractive feeding device
- **Task 2.4:** Management of hyperparasitoids

Task 2.1: Evaluation of VOC dispensers

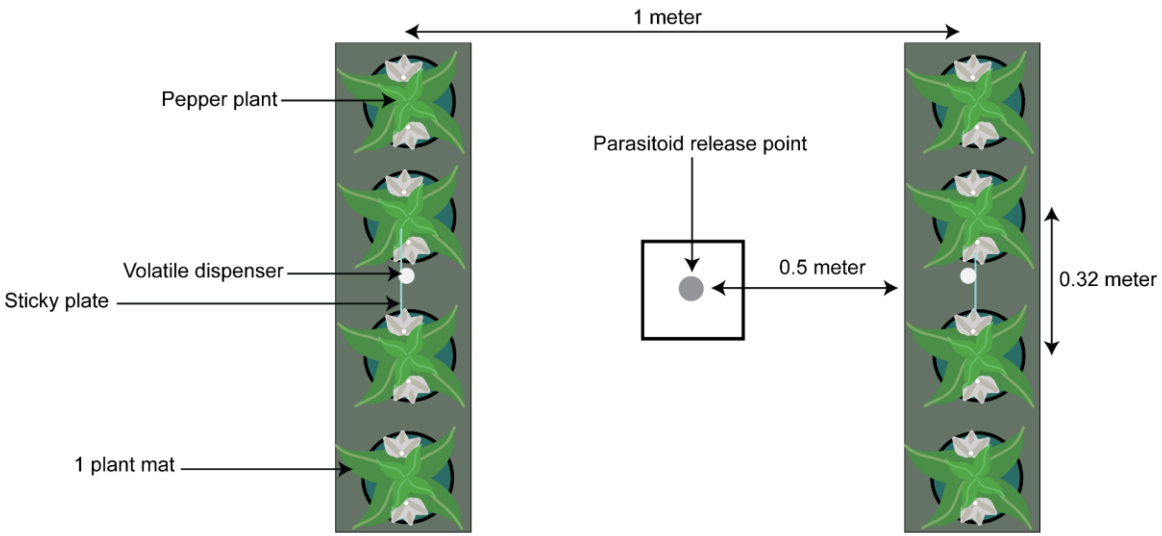
Evalueren van verschillende VOC dispensers om sluipwespen aan te trekken

Proeven in serre omstandigheden (PCH) (N=12)

Dispensers: Omnilure® and LDPE zakjes

Styreen/benzaldehyde: 1000×, 10.000×, 100.000×

Omnilure® dispensers met een 10.000× SB mengsel trokken de meeste *A. colemani* aan in serreomstandigheden



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Task 2.1: Evaluating the effect of distance

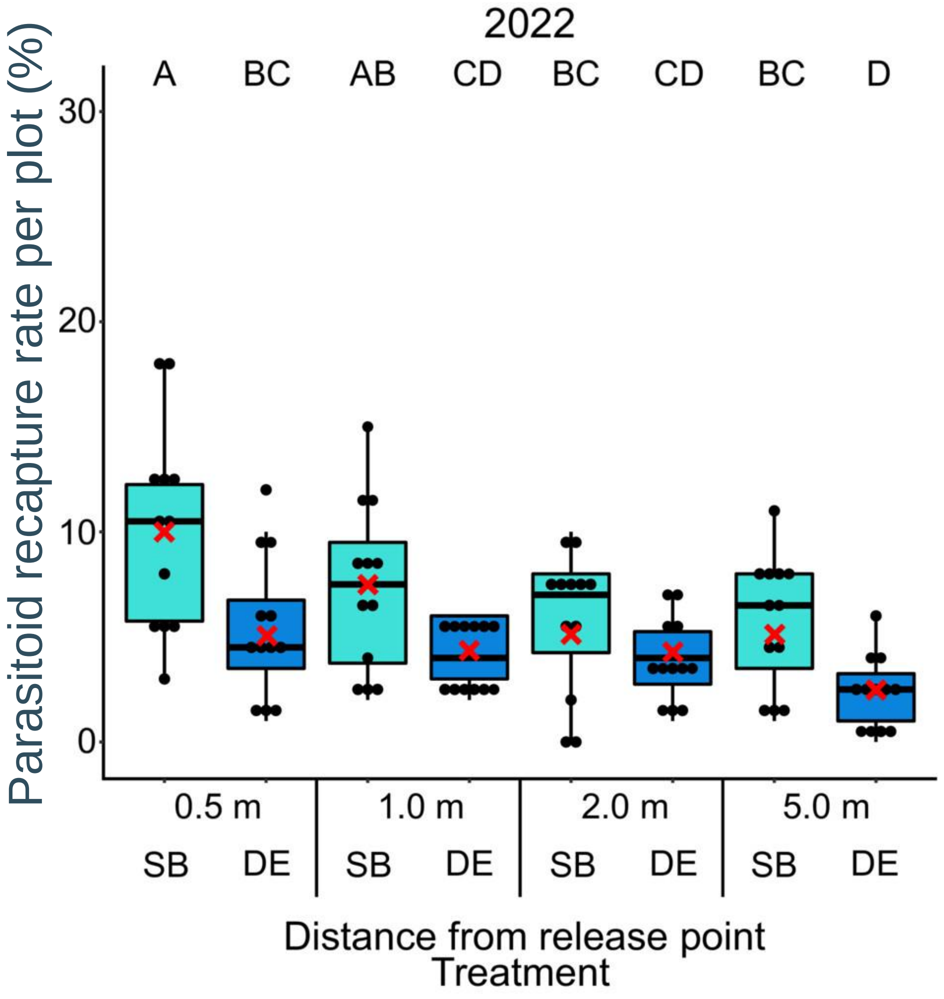
Over welke afstand is het 10.000× styreen/benzaldehyde mengsel in Omnilures aantrekkelijk?

Proeven in serre omstandighede (PCH)

5 verschillende afstanden
100 *A. colemani* vrouwtjes

0.5, 1, 2, 5m (2021 & 2022; N=12) & 7.5 m (2022; N=6)

A. colemani **kan aangetrokken worden over minstens 5 meter, maar niet tot 7.5 m**



Task 2.2: Enhancing parasitoid monitoring efficacy

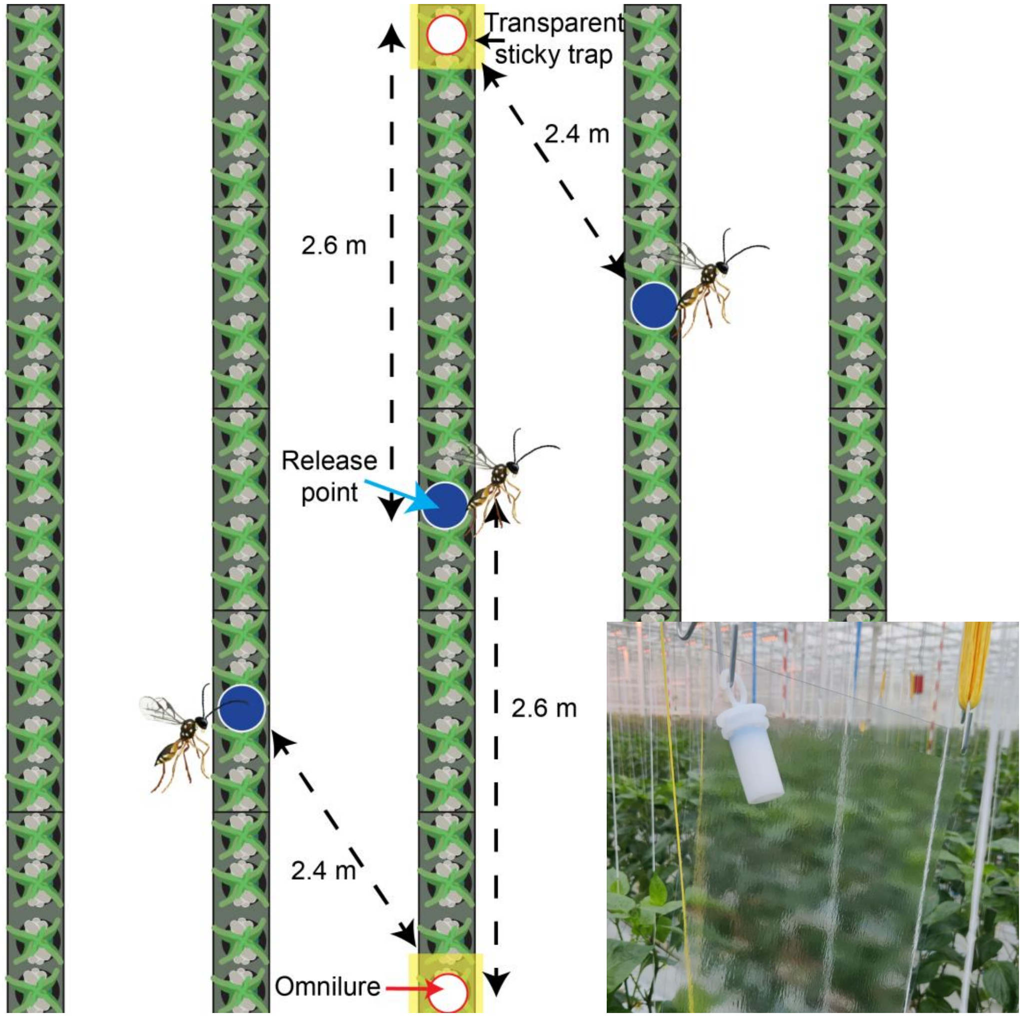
Monitoring the presence of parasitoids using sticky traps with 10.000× SB (Omnilure)

Two species of *Aphidius* released in the greenhouse (PSKW)

100 *A. colemani* per release point (N=4)
100 *A. ervi* per release point (N=4)

Only 9 parasitoids were recaptured after 7 days

Monitoring parasitoids using sticky traps and the 10.000× SB blend did not yield the expected results



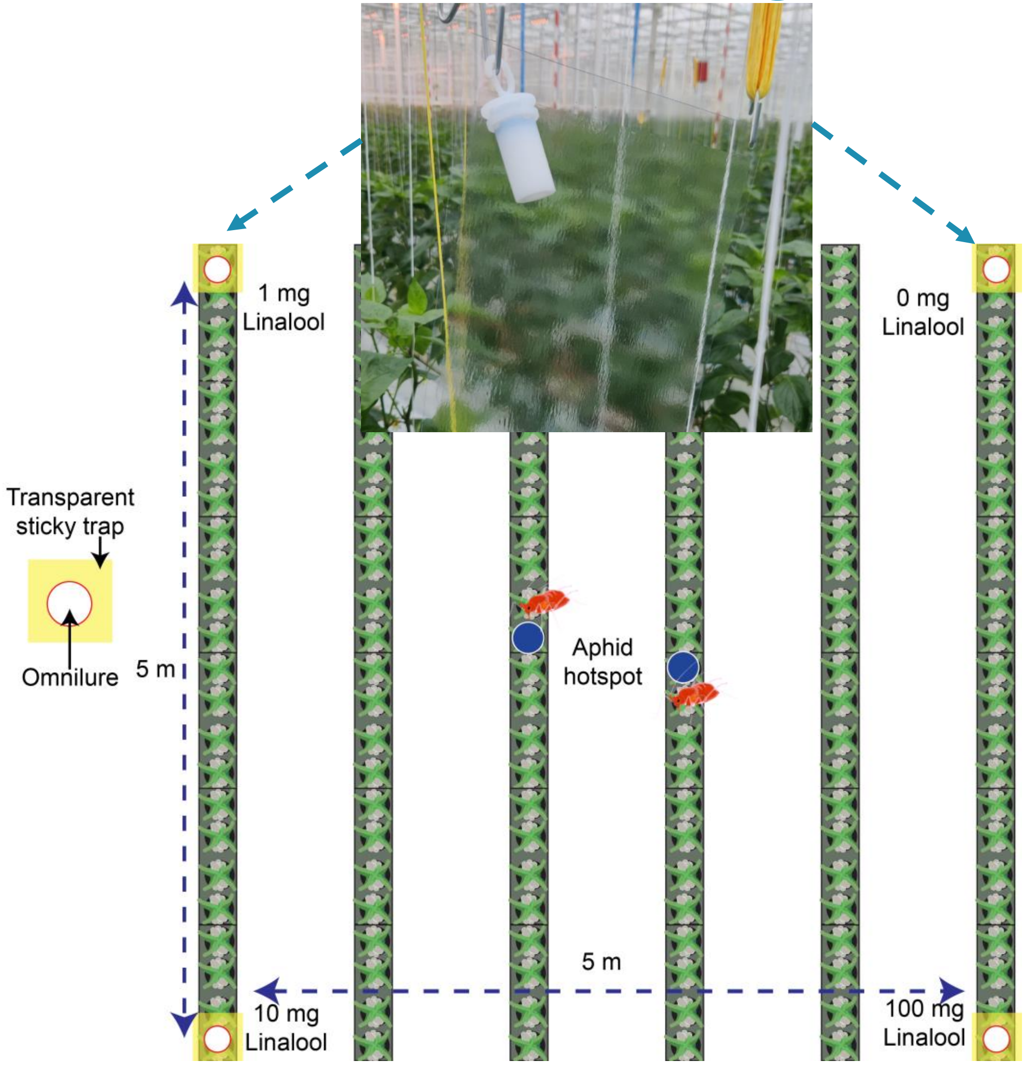
Task 2.2: Enhancing hyperparasitoid monitoring efficacy

Monitoring the presence of hyperparasitoids in greenhouses using sticky traps with linalool (Omnilure)

Linalool: 0 mg, 1 mg, 10 mg and 100 mg (N=6)

Asaphes spp. and *Pachyneuron* spp. were more attracted to traps with linalool. Unfortunately, so were aphids, *Aphidius* parasitoids, and *Macrolophus*

Can bankerplants be used to monitor hyperparasitoids?



Task 2.3: Development of a feeding device

To develop a method for providing sugars to *Aphidius* parasitoids

Cage experiment (PSKW)
6 feeding treatments (N=10)

Negative control [cotton ball + water]
Positive control [cotton ball + Glucose]
Wide dispenser with white cap [Glucose]
Narrow dispenser with white cap [Gluc.]
Wide dispenser with yellow cap [Gluc.]
Narrow dispenser with yellow cap [Gluc.]

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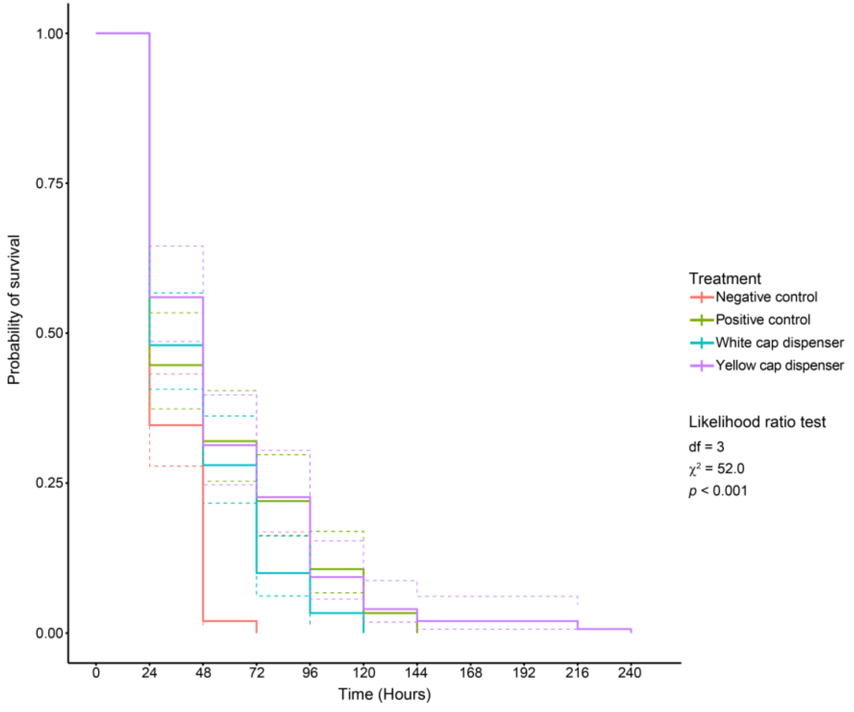
Task 2.3: Development of a feeding device

To develop a method for providing sugars to *Aphidius* parasitoids

1. **Sugar feeding enhanced longevity**
2. **Size and colour had no effect on longevity**
3. **Dispensers were as good as positive control**

Potentially could be upgraded with VOCs

Survival probability of *A. colemani* for the 4 tested treatments, plus water



Number at risk: n (%)

Treatment	0	24	48	72	96	120	144	168	192	216	240	
Negative control	150 (100)	150 (100)	52 (35)	3 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	c
Positive control	150 (100)	150 (100)	67 (45)	48 (32)	33 (22)	16 (11)	5 (3)	0 (0)	0 (0)	0 (0)	0 (0)	a
White cap dispenser	150 (100)	150 (100)	72 (48)	42 (28)	15 (10)	5 (3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	b
Yellow cap dispenser	150 (100)	150 (100)	84 (56)	47 (31)	34 (23)	14 (9)	6 (4)	3 (2)	3 (2)	3 (2)	1 (1)	a

Time (Hours)

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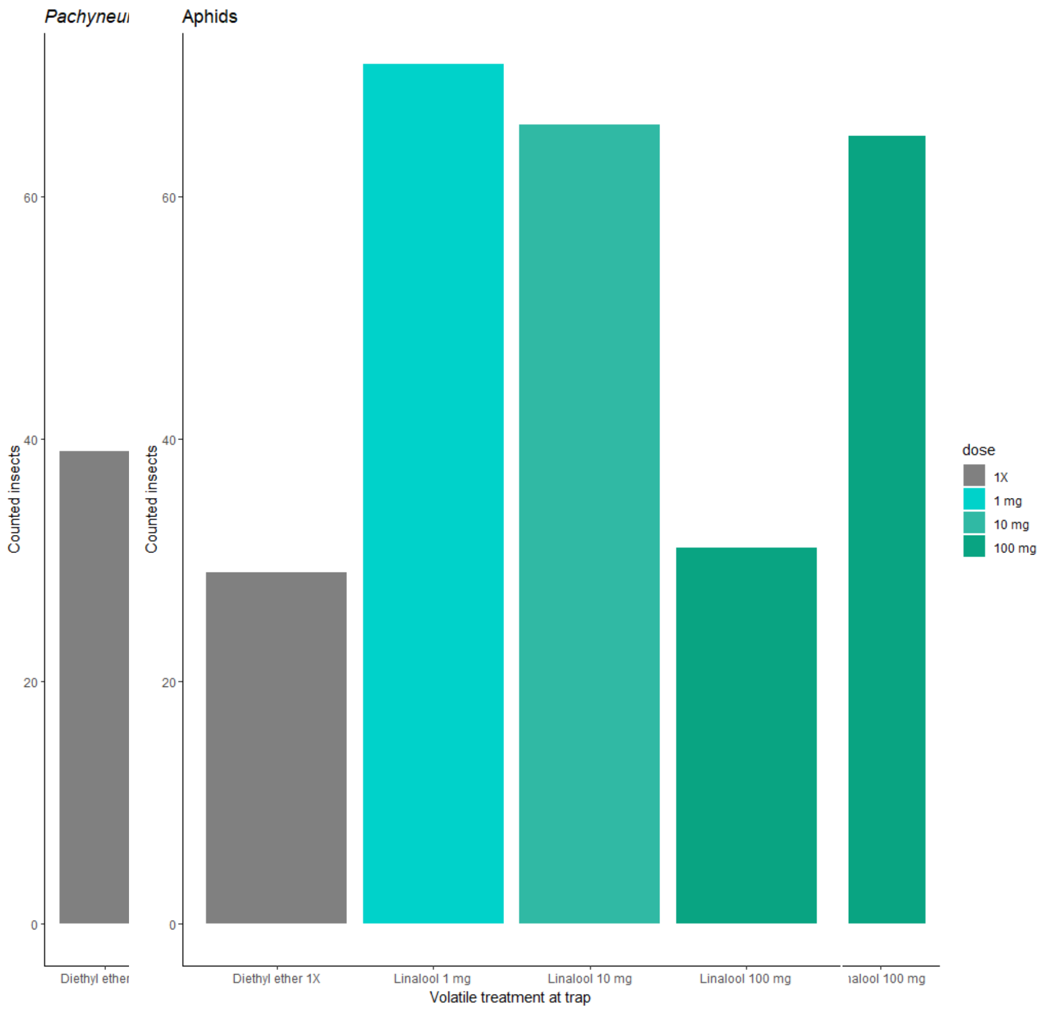
Task 2.4: Management of hyperparasitoids

To attract & kill hyperparasitoids through using sticky traps & volatiles

1. 1 mg linalool could be used to capture the hyperparasitoid *Asaphes* & *Pachyneuron*
2. 1 mg linalool also resulted in an increased capture of *Aphidius* & aphids

Using sticky traps & VOCs may not be ideal to attract & kill hyperparasitoids

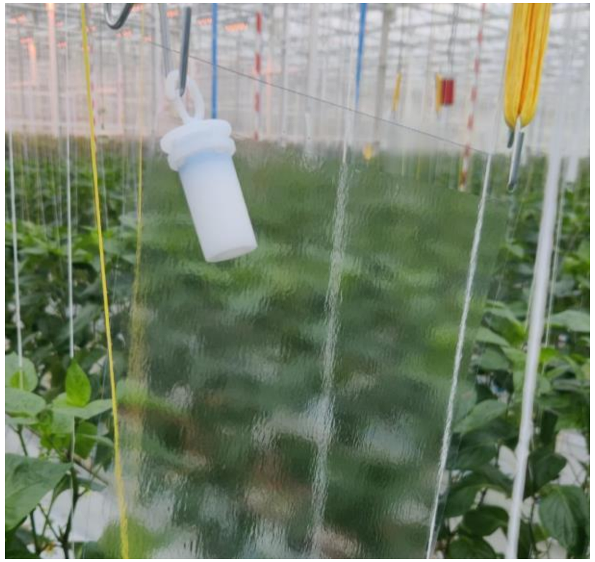


WP2: Conclusions

A blend of styrene and benzaldehyde can attract the parasitoid *A. colemani* over a distance of 5 m

Sugar feeding can enhance longevity

Linalool can be used to attract & kill hyperparasitoids, but they also attract parasitoids



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WP 3: Evaluation of the developed strategies

Evaluating the developed strategies under real-world conditions for their effectiveness in the biological control of aphids

- **Task 3.1:** Evaluation of attractants on biocontrol efficacy
- **Task 3.2:** Evaluation of sugar feeding on biocontrol efficacy

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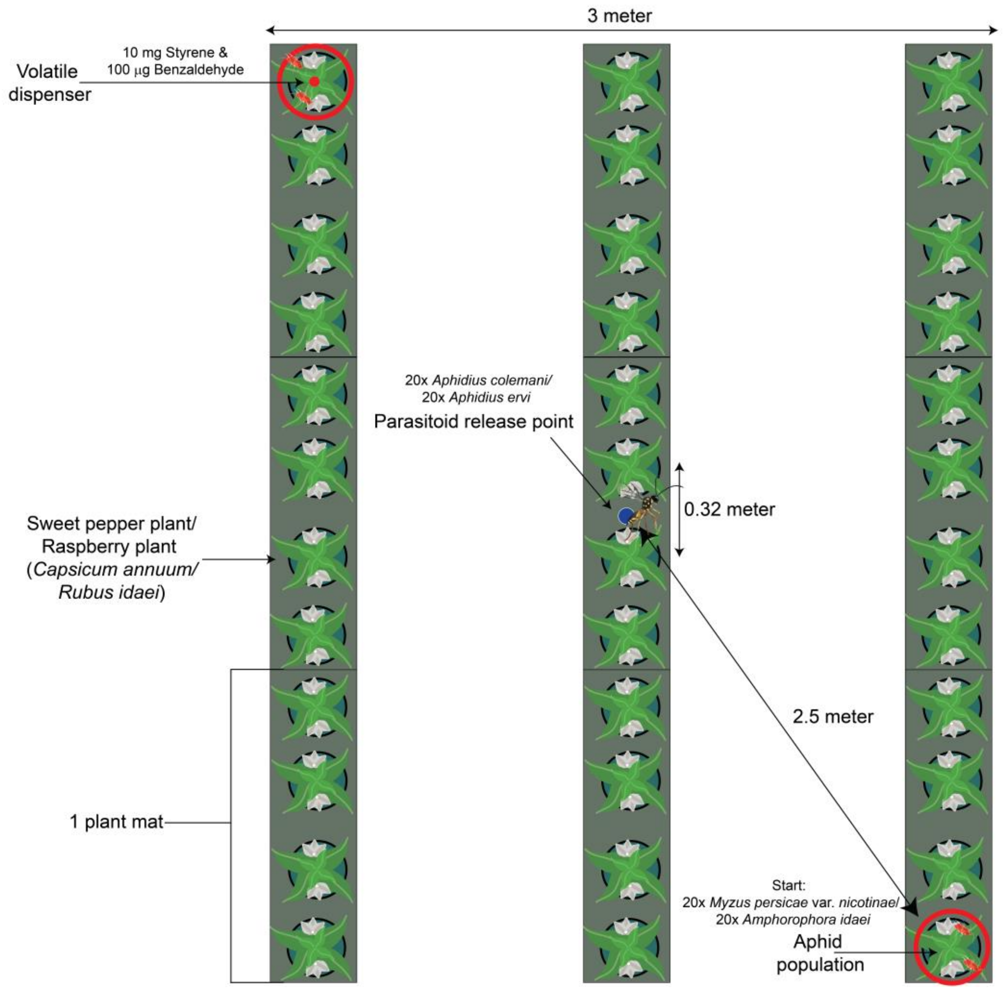
Task 3.1: Evaluation of attractants on biocontrol efficacy

Kan het toepassen van het 10.000× SB mengsel bij bladluishaarden de biocontrole verbeteren?

Proeven in serre omstandigheden met kunstmatige infectie

2 combinaties (N=10)

1. Geen geurstoffen vs. Geen geurstoffen
2. 10.000× SB mengsel vs geen geurstoffen



Task 3.1: Evaluation of attractants on biocontrol efficacy

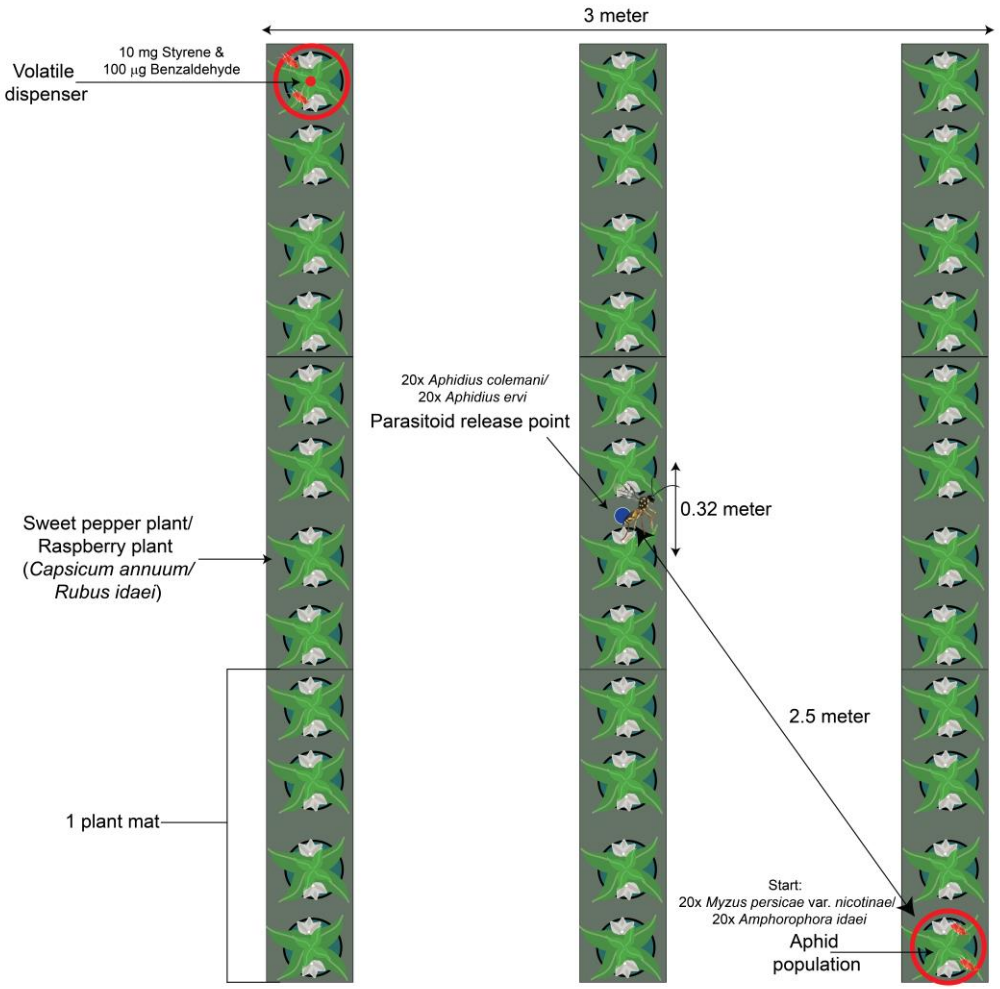
2 teelten
2 trofische interacties

1. Paprika (PCH)
2. Framboos (PCFruit)

1. *Aphidius colemani* & *Myzus persicae* var. *nicotianae* (PCH)
2. *A. ervi* & *Amphorophora idaei* (PCFruit)

Sluipwespen kregen 48u de tijd om bladluizen te parasiteren

Omnilure dispensers werden elke 24u vervangen



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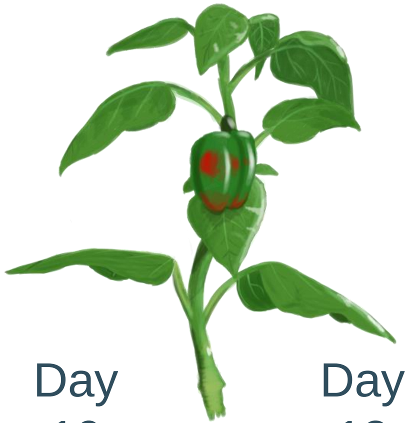
Task 3.1: Evaluation of attractants on biocontrol efficacy



Day 1

Day 10

Day 12

Day 17

Day 21

Uitzet van bladluizen
20/plant

Parasitering (48u)
(20 sluipwespen/plot)

Ontwikkeling van mummies
23°C & 70% R.H.

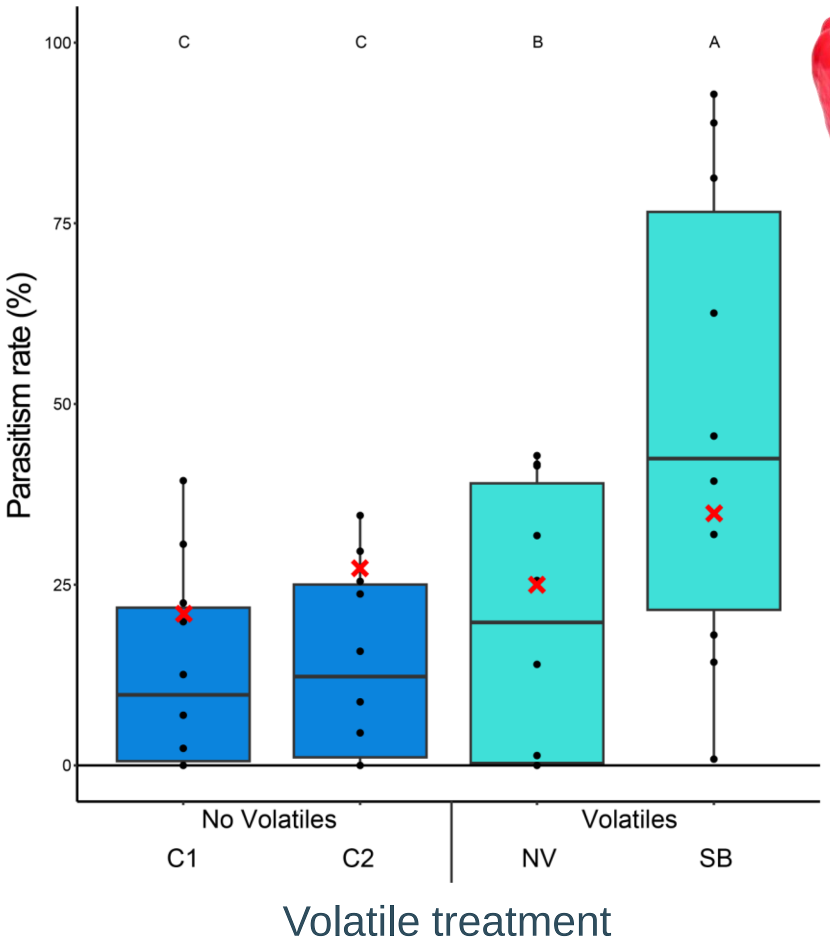
Task 3.1: Evaluation of attractants on biocontrol efficacy

Paprika (PCH)

Myzus persicae var *nicotianae*
Aphidius colemani

Geen verschil in referentie behandeling

1.5 maal meer parasitering in haarden met lokstoffen t.o.v. haarden zonder lokstoffen



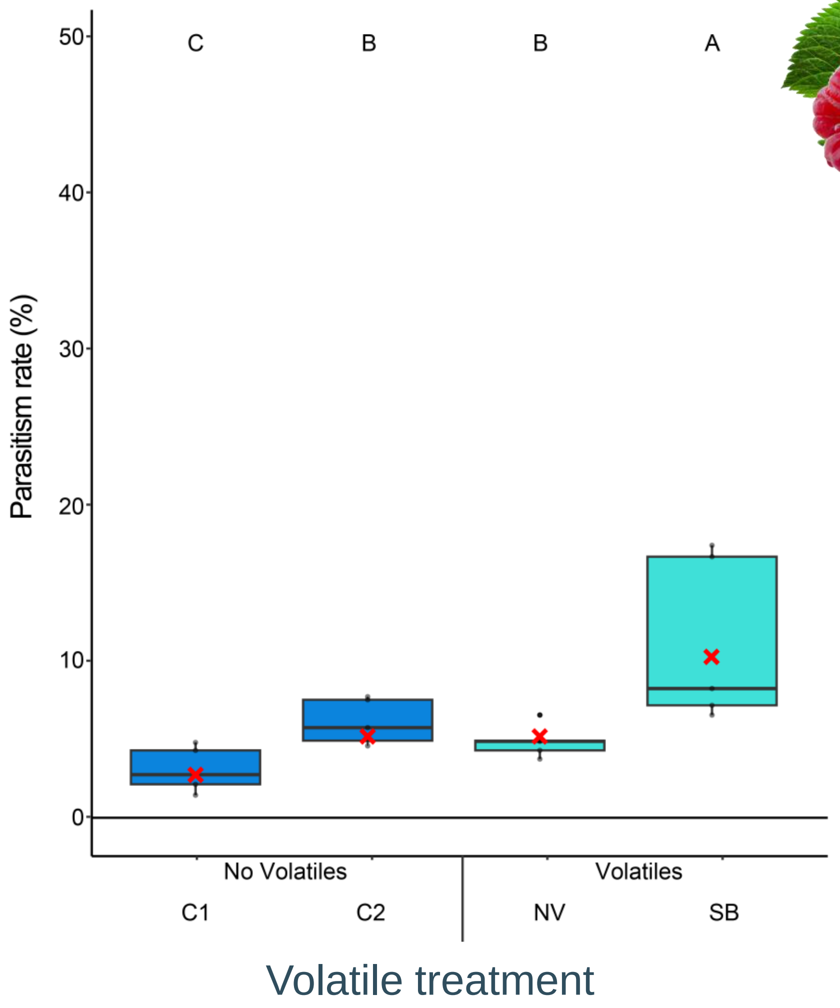
Task 3.1: Evaluation of attractants on biocontrol efficacy

Framboos (PCFruit)

Amphorophora idaei
Aphidius ervi

Laag parasiteringsniveau (≤20%)

1.8 maal meer parasitering in haarden met lokstoffen t.o.v. haarden zonder lokstoffen



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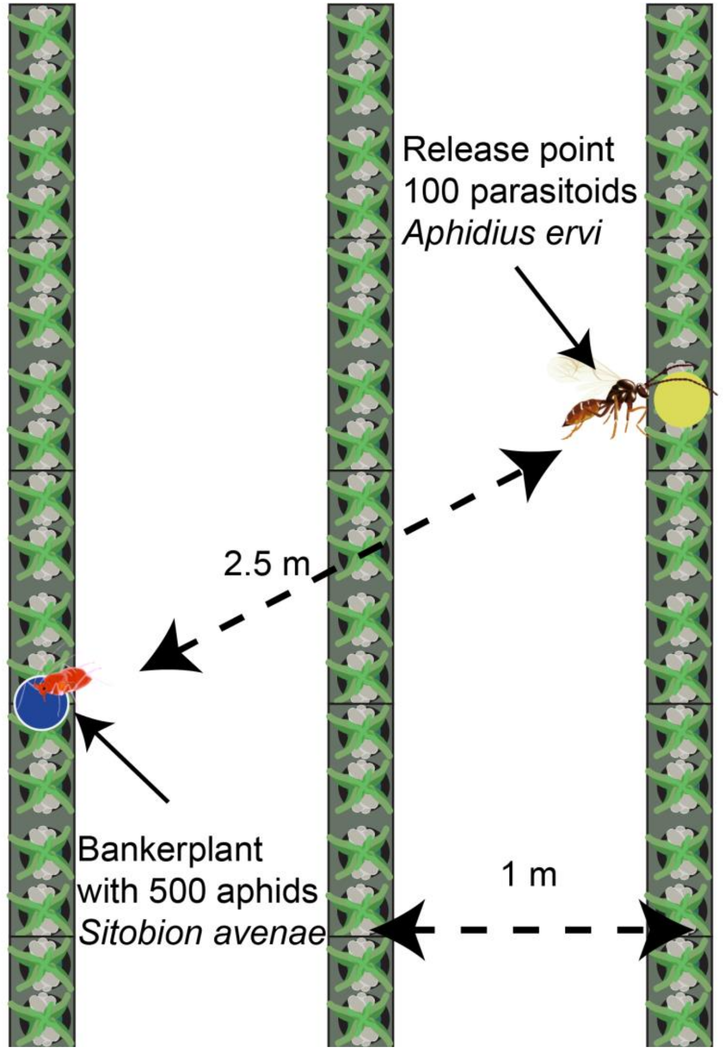
Task 3.2: Evaluation of sugar feeding on biocontrol efficacy

To evaluate the effect of feeding treatment on parasitoid biocontrol efficacy (PSKW)

Greenhouse trial (Bell pepper)

3 treatments (N=18)

1. Negative control
2. Feeding devices with 4% Attracker
3. Spraying the plants with 4% Attracker



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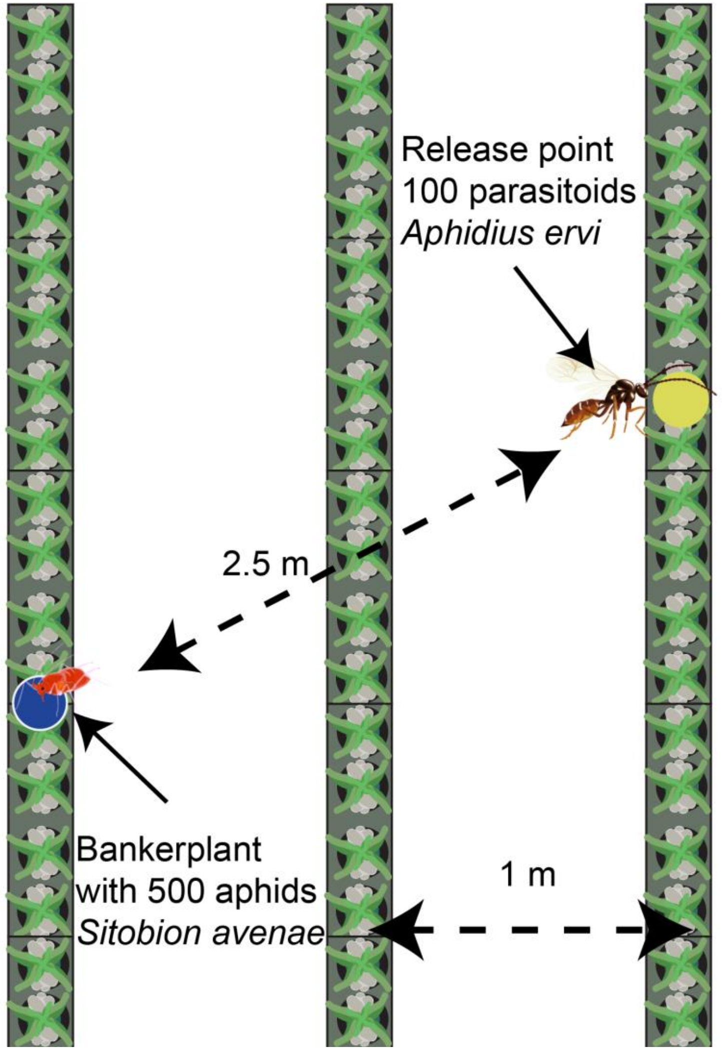
Task 3.2: Evaluation of sugar feeding on biocontrol efficacy

Attracker® product by Koppert (Glucose, Sucrose, Fructose)

Release of 100 *Aphidius ervi* mummies

Longevity is assessed by rate of parasitism in banker plants

1 banker plant/plot was used:
3 days after mummy release
6 days after mummy release
10 days after mummy release



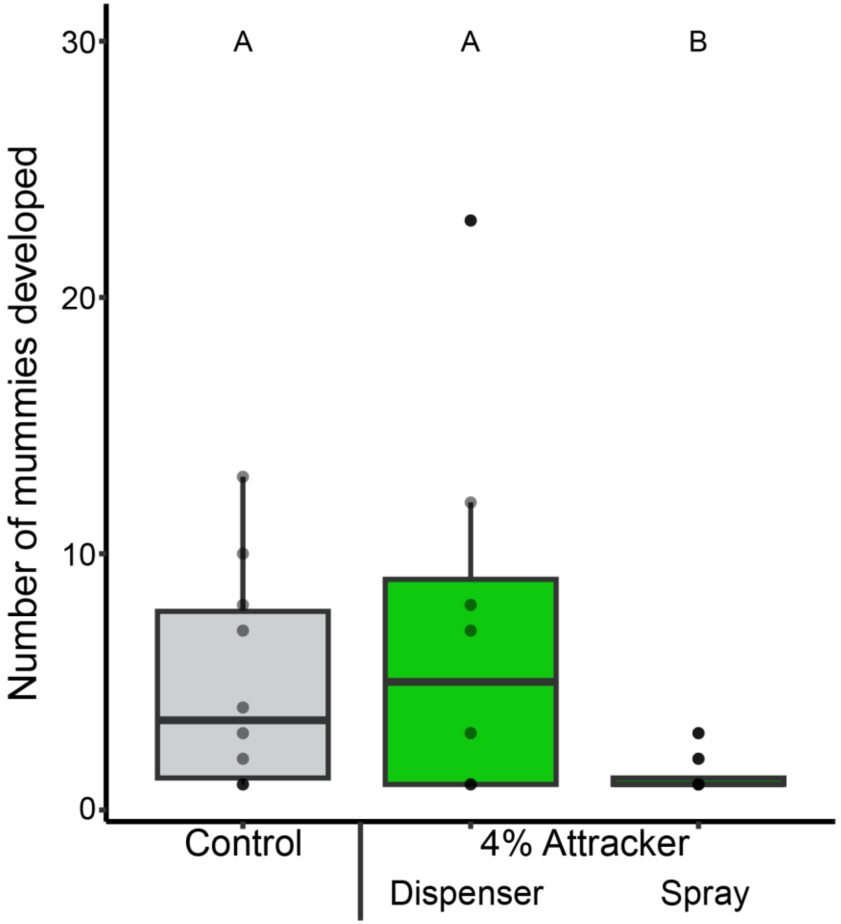
Task 3.2: Evaluation of sugar feeding on biocontrol efficacy

Leaves were sticky, but no development of sooty moulds

Best to apply sugars in dispenser

Does not improve biological control

But:
-Low concentration of sugars
-Parasitism only sampled for 10 days after mummy release. Longevity approx. 14 days after emergence



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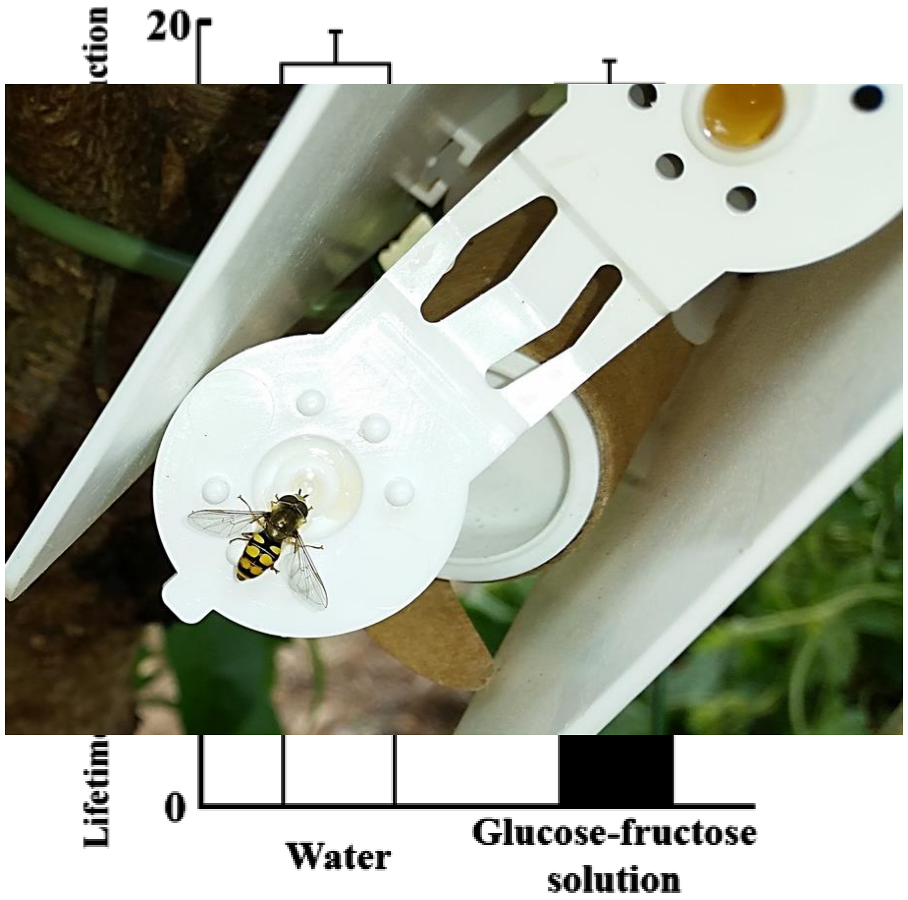
Task 3.2: Evaluation of sugar feeding on biocontrol efficacy (Literature)

Aphidius parasitoids are generally pro-ovogenic (Born with set number of eggs)
Longer life = More offspring = Better biological control

Aphidius parasitoids could live 2× longer when fed a simple solution of tap water and sugar

Feeding *Aphidius* parasitoids at the emergence site with sugar water may improve longevity & biocontrol



WP4: Dissemination of project results

To disseminate the project results to the sector and broader scientific community

WP4: Dissemination of project results

Peer reviewed journals

Identification and application of bacterial volatiles to attract a generalist aphid parasitoid: from laboratory to greenhouse assays (2020) T. Goelen, et al.

Bacterial volatiles elicit differential responses in insect species from the same and different trophic levels (2023) F.A.C. van Neerbos, et al.

Distance-dependent attraction of the generalist aphid parasitoid *Aphidius colemani* towards volatile organic compounds under greenhouse conditions (2023) F. A. C. van Neerbos, et al.

A comparison of flower borders and intercropping flowering plants for enhancing biological control of key pests in strawberry (2023). A. Alhmedi et al.

Seasonal changes in aphid hyperparasitoid abundance in sweet pepper and raspberry in Belgium (in prep) F. A. C. van Neerbos et al.

Posters

Presented at the Dutch entomological society day, Ede, December 13th 2019: Exploiting microbial volatiles to attract parasitic wasps. Goelen et al.

Presented at the post graduate course: Chemical communication, Wageningen University, February 2nd– 6th 2020: Of macro and micro: Assessing the specificity of microbial volatile organic compounds to improve biological pest control strategies. van Neerbos et al.

Presented at Fructura, May 2022: Innovatieve biologische bladluisbestrijding in de beschermde teelt van kleinfruit. Alhmedi et al.

Presented at International Strawberry Congress, September 2022: A comparison of flower borders and intercropping flowering plants for enhancing biological control of key pests in strawberry. Alhmedi et al.

Presented at IOBC, Brest, August 2023: Diversity and management of aphid hyperparasitoids in sweet pepper greenhouses in Belgium. De Vis et al.

WP4: Dissemination of project results

Publications in the sector

Nieuw project gaat de strijd aan met bladluizen in paprika en kleinfruit (2019, PTN14) B Lievens, H Jacquemyn, L Bosmans, T Beliën, L. Wittemans

Microbiële geurstoffen bieden perspectief bij biologische plaagbestrijding (2020, PTN16) F.A.C. van Neerbos, H. Jacquemyn, B. Lievens, A. Alhmedi, T. Beliën, L. Van Herck, L. Wittemans, R. Mertens, L. Bosmans

De grote frambozenluis *Amphorophora idaei*. Publication on the website of PCFruit for members (2020). A. Alhmedi

Innovatieve biologische bladluisbestrijding in de beschermde teelt van paprika en kleinfruit (BIOTRACT). Publication in NOBL (2020) B. Lievens, F.A.C. van Neerbos, H. Jacquemyn, T. Beliën, A. Alhmedi, L. Wittemans, L. Bosmans, R. Mertens

Sluipwespen in paprikagewas houden met synthetische lokstoffen (2021, PTN9) F.A.C. van Neerbos, T. Goelen, H. Jacquemyn, B. Lievens, R. Mertens, L. Bosmans, A. Alhmedi, T. Beliën, L. Van Herck, L. Wittemans

Synthetische lokstoffen trekken sluipwespen aan over 5 m afstand (2022, PTN 6) R. Mertens, L. Bosmans, F.A.C. van Neerbos, H. Jacquemyn B. Lievens, A. Alhmedi, T. Beliën, L. Van Herck, L. Wittemans, R. De Vis

IPM in kleinfruit: goed starten, monitoren en bijsturen. Publication in FRUIT 5 (2022). T. Beliën, A. Alhmedi, F. De Vis

Waarom moet je mieren in de boomgaard beheren? FRUIT 6 (2022). A. Alhmedi, T. Beliën

(Natuurlijke vijanden) De vijand van mijn vijand is mijn vriend FRUIT 9 (2022). A. Alhmedi, T. Beliën

Hyperparasitoïden, een grote uitdaging voor de biologische bestrijding tegen bladluizen, FRUIT (2022). A. Alhmedi, T. Beliën

Verschillende soorten hypersluipwespen wisselen elkaar af in paprika (2023, PTN19) R Clymans, E Van Erkel, L. Herman, A. Alhmedi, T. Beliën, F. van Neerbos, H. Jacquemyn, B. Lievens

Ongewenste sluipwespen in je frambozenteelt. Fruit 5 (2023) A. Alhmedi, T. Beliën

Belang van temperatuur voor effectiviteit van sluipwespen Fruit 11 (2023) A. Alhmedi, T. Beliën

Bladluizenbestrijding in de herfst Fruit 19-20 (2023) A. Alhmedi, T. Beliën

WP4: Dissemination of project results

Presentations

28/01/2020: Study meeting: Insect- and mite control in strawberries and small fruit (PCFruit)
03/03/2020: Online workgroup: Insect- and mite control in woody small fruit (PCFruit)
11/05/2020: BIOTRACT explanation (PCH)
14/09/2020: Online workgroup: Insect- and mite control in woody small fruit (PCFruit)
30/09/2020: Presentation: Technical committee paprika (PCH & PSKW)
26/08/2021: Rondgang paprikakring: BIOTRACT (PCH)
21/07/2022: International conference: International congress of Entomology (KULeuven)
15/12/2022: Rondgang: paprikakring: BIOTRACT (PSKW)
14/02/2023: Study-evening veiling BelOrta (PCFruit)
17/02/2023: Study-day REO-veiling (PCFruit)
20/03/2023: Study-evening ism Hermoo (PCFruit)
23/05/2023: International conference: International symposium on crop protection (KU Leuven)
26/06/2023: Agrolink Workshop Aphids (PCFruit)
26/09/2023: Open day PAH (PCFruit)
03/10/2023: Study-day PCFruit & Biobest: Strategieën voor geïntegreerde gewasbescherming in het zachtfruit (PCFruit)
13/11/2023: Telerswerkgroep PAH (PCFruit)
15/12/2023: International conference: Dutch Entomological Society Day (NEV) (KULeuven)

PROEFCENTRUM HOOGSTRATEN
proefstation VOOR DE GROENTETEELT
pcfruit PROEFCENTRUM FRUITTEELT VZW
KU LEUVEN

WP4: Dissemination of project results

User committee meetings

12/12/2019: First user committee meeting BIOTRACT
14/05/2020: Second user committee meeting BIOTRACT
05/02/2021: Third user committee meeting BIOTRACT
16/09/2021: Fourth user committee meeting BIOTRACT
05/10/2022: Fifth user committee meeting BIOTRACT
10/05/2023: Sixth user committee meeting BIOTRACT

Take home messages

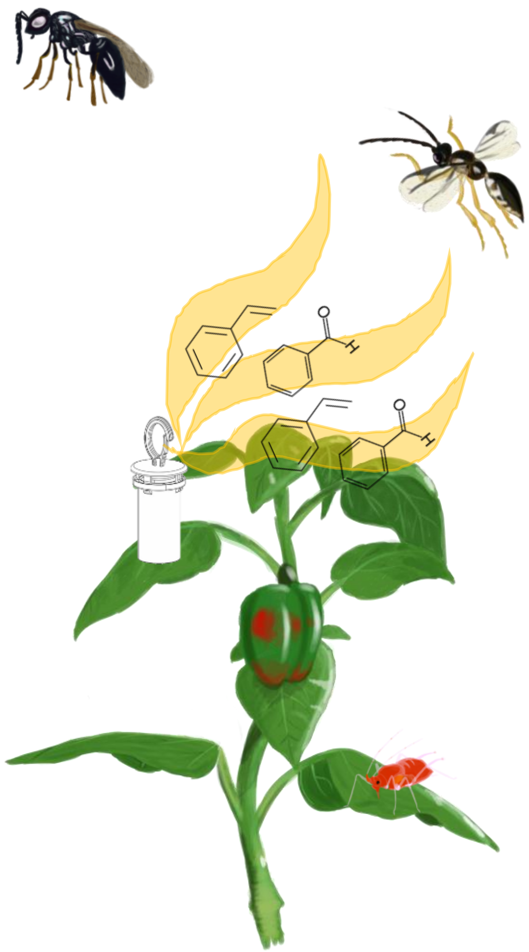
Hyperparasitoids are prevalent in Flanders
Limited number of species
Appear early in the season, but are still active late in the season

Hyperparasitoids may be monitored by the use of bankerplants

Switching to predators during late spring (May-June) may decrease hyperparasitism rates

Volatiles may improve biological aphid control, but effects are small

Sugar feeding enhances parasitoid longevity



Take home messages

Volatiles may improve biological aphid control, but effects are small

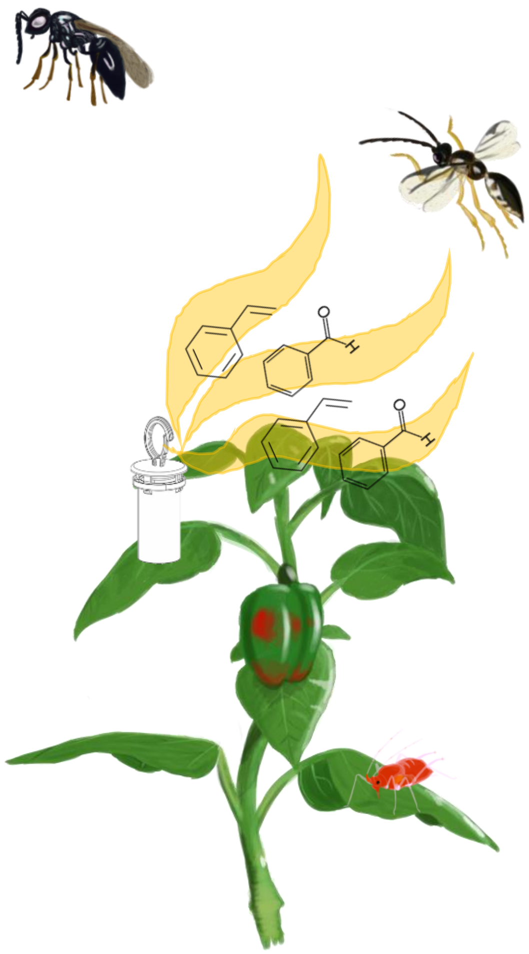
Parasitoids:

- Attraction limited to 5 m
- Biocontrol efficacy slightly enhanced when applied on aphid infested plants

Hyperparasitoids:

- Attract & kill trap also attracts other insects, including beneficials

Sugar feeding enhances parasitoid longevity



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Take home messages

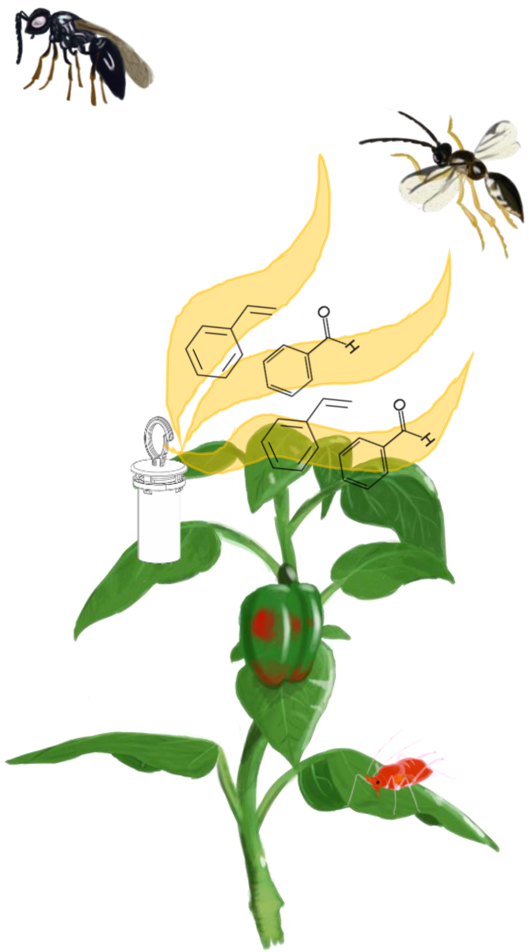
Sugar feeding enhances parasitoid longevity

Biocontrol efficacy

No effect on biocontrol efficacy, probably due to experimental set-up

Longevity

Providing sugar near the parasitoid release point may improve longevity, providing a longer window of biological control



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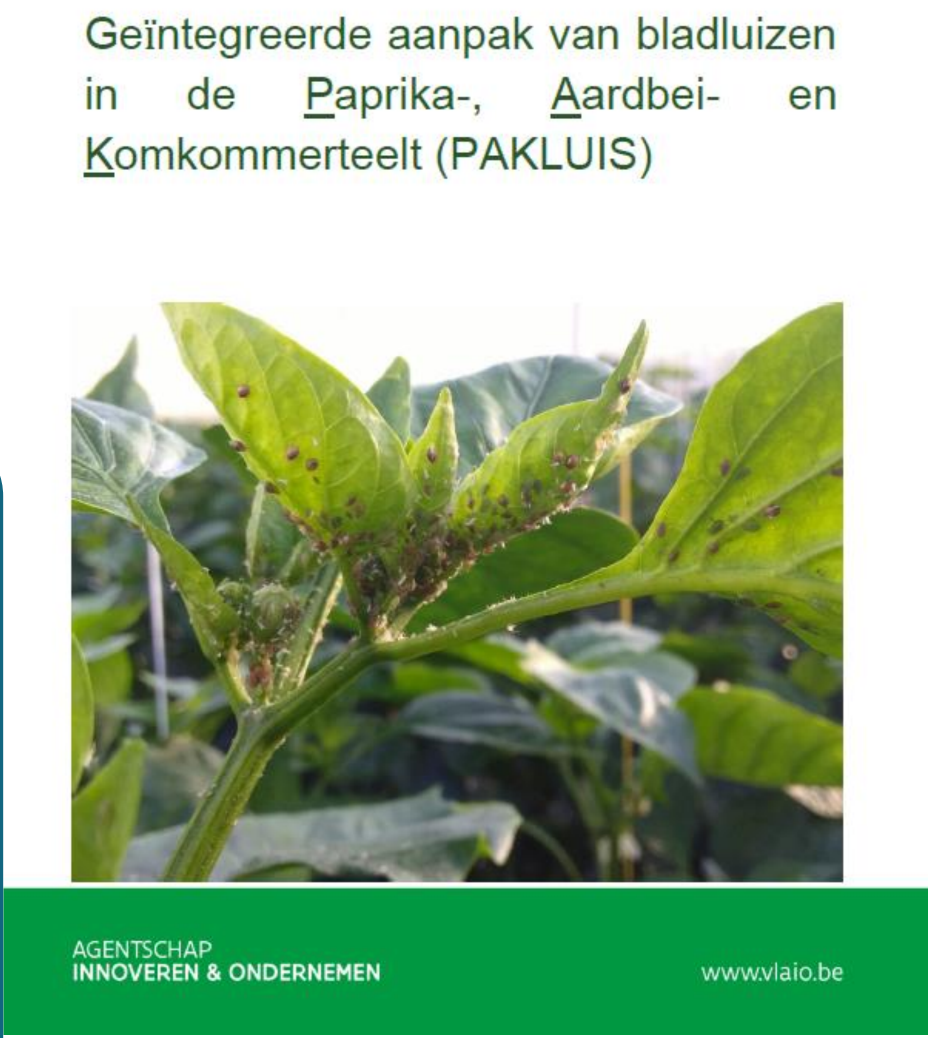
Perspectief

VLAIO landbouwtraject ingediend door projectpartners

"PAKLUIS: Geïntegreerde aanpak van bladluizen in de Paprika-, Aardbei- en komkommerteelt"

(1/11/2024 – 31/10/2028)

- Identificatie en monitoring (obv Biotract)
- Beheersingsstrategieën:
  - Preventief (afgazen, bloemenranden, plantdefensie elicitoren)
  - Biologisch (Preventief, bijvoederen)
  - Gewasbescherming (biologisch, fysisch, chemisch, bladluisdodende micro-organismen)
- Integratie in IPM strategie
- Waarschuwingssysteem



Satisfaction analysis December 2023

https://forms.office.com/e/RRbAUsuQYd

Link will be emailed to all of you

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Thank you for your attention!