



| Algemene gegevens | |
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| PPS-nummer | ALWGR.2017.006- TU 17010 |
| Titel | Aphids out of control? The role of symbiont-driven resistance to parasitoids in greenhouse biocontrol success |
| Thema | TKI T&U |
| Uitvoerende kennisinstelling(en) | Wageningen University & Research |
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| Contactpersoon overheid (indien relevant) | nvt |
| Adres projectwebsite | nvt |
| Startdatum | 01-08-2018 |
| Einddatum | 31-07-2023 |

| Goedkeuring penvoerder/consortium | |
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| De jaarrapportage dient te worden besproken met de penvoerder/het consortium. De TKI's nemen graag kennis van eventuele opmerkingen over de jaarrapportage. | |
| De penvoerder heeft namens het consortium de jaarrapportage | <input type="checkbox"/> goedgekeurd <input type="checkbox"/> niet goedgekeurd |
| Eventuele opmerkingen over de jaarrapportage: | Deze jaarrapportage is nog niet besproken in het consortium, dat wordt bij de volgende consortium meeting gedaan (April 2021). Deze jaarrapportage voor TKI is echter gebaseerd op de goedgekeurde jaarrapportage voor ENW voor 2020-2021, welke is goedgekeurd door het consortium. |

| Inhoudelijke samenvatting van het project | |
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| Probleemomschrijving | <p>Aphids are major crop pests, both in greenhouses and in the field, and are usually controlled using pesticides. Chemical control of aphid outbreaks is not environmentally friendly, nor sustainable because of the evolution of aphid insecticide resistance. Furthermore, key chemicals for aphid control have been banned, further increasing the urgency of implementing alternatives such as biocontrol.</p> <p>Aphid biocontrol is most effective through the use of parasitoids that prevents early-stage aphid population expansion. Unfortunately, the effectiveness of biocontrol is sometimes failing. Endosymbionts associated with aphids can confer resistance against parasitoid attacks thus playing a role in this failure. However, information is scarce for endosymbiont variation and research about its functionality and the specificity of aphid-endosymbiont associations, and the role that the symbiont community plays in resistance is mostly lacking. In this project, we will provide this essential information through, (i) monitoring and establishing the extent of aphid-endosymbiont associations, aphid resistance, and parasitoid virulence, (ii) unravelling the aphid-symbiont interactions and mechanisms of defence, (iii) determining the aphid-parasitoid interactions and variation in virulence, in order to (iv) deliver advice on biocontrol resource development and biocontrol application strategies.</p> <p>Our project will provide key insights into the role of aphid-associated endosymbionts in the interactions between aphids and parasitoids at</p> |

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| | greenhouse levels, which, in close-collaboration with world-leading biocontrol experts from Koppert, can be implemented in the production of many important greenhouse crops. Our programme thus contributes to increasing the resilience and sustainability of crop production and contributes to technology development in the field of biocontrol. |
| Doelen van het project | <p>Our research program has the following objectives:</p> <ol style="list-style-type: none"> 1. To monitor and establish the extent of aphid-endosymbiont associations, aphid resistance, and parasitoid virulence; 2. To unravel aphid-symbiont interactions and mechanisms of defence; 3. To determine aphid-parasitoid interactions and variation in virulence; 4. To deliver advice on biocontrol resource development and application strategies. <p>These objectives are addressed in two separate PhD projects, one focusing on aphids (Beekman) and another on parasitoids (Donner).</p> |

| Resultaten | |
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| Beoogde resultaten 2020 | <p>Monitoring of aphid communities in pepper greenhouses (Beekman & Donner)</p> <ul style="list-style-type: none"> • Start full profiling of the microbiomes (bacterial and fungal) of sweet pepper greenhouse aphids using the MinION nanopore sequencer. This will allow us to discover any unknown endosymbionts influencing the parasitoid success. • Test the endogenous resistance of <i>M. persicae</i> against the parasitoids <i>Aphidius matricariae</i> and <i>Aphidius colemani</i>. <i>Myzus persicae</i> clones were collected from eleven different pepper greenhouses. <i>A. solani</i> clones are currently being sampled from different greenhouses and will be tested against the parasitoids <i>Aphidius ervi</i> and <i>Aphidius colemani</i>. • Publish our findings on (1) the microbiome and genetic structure of sweet pepper greenhouse aphids and (2) the presence of endosymbionts in aphid field population in the Netherlands. <p>Monitoring of aphid communities on strawberry (Beekman & Donner)</p> <ul style="list-style-type: none"> • Extend our sampling program of aphids in strawberry greenhouses to monitor aphid and endosymbiont diversity in time and space. • Start on the characterization of the virulence of parasitoids based on the tests of the endogenous resistance and on aphid-endosymbiont combinations sampled in the strawberry greenhouses. <p>Monitoring of aphid field populations in the Netherlands (Donner)</p> <ul style="list-style-type: none"> • Finish the study on endosymbionts in field aphids by finishing the molecular laboratory work (sequencing of COI, identification of endosymbiont presence) on the aphids sampled from Dutch field sites and around greenhouses. • Publish our findings on the presence of endosymbionts in aphid field population in the Netherlands. <p>Aphid population genetic structure (Beekman)</p> |

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| | <ul style="list-style-type: none"> • Continue to sample aphids in pepper greenhouses at low intensity, to increase our understanding of aphid population dynamics over consecutive growth seasons. • Finish the microsatellite analysis to determine the population genetic structure of <i>M. persicae</i> from sweet pepper greenhouses, and <i>M. persicae</i> collected throughout the Netherlands by the NAK, growth season 2019. • Perform next-generation genome sequencing, in silico microsatellite development and population genetic structure analysis for <i>A. solani</i> from pepper greenhouses and <i>A. solani</i> collected throughout the Netherlands by the NAK, growth season 2019. |
| Behaalde resultaten 2020 | <p>Monitoring of aphid communities in pepper greenhouses (Beekman & Donner) During the first half of 2020 we developed and optimized methods to use nanopore sequencing (MinION; Oxford Nanopore Technologies) of the 16s rRNA gene to study the aphid microbiomes in detail. Using mock communities containing different aphid endosymbionts we were able to proof the validity of our methods. During the second half of 2020, we used these methods to sequence and analyze the microbiomes of aphids from bell pepper greenhouses. None of the analyzed aphids contained any of the known secondary endosymbionts. Of the total reads; 96.2% belonged to the primary aphid endosymbiont <i>Buchnera aphidicola</i>, 2.2% belonged to the <i>Escherichia/Shigella</i> genus, 0.34% belonged to <i>Pseudomonas</i> and 0.17% belonged to <i>Bacillus</i>.</p> <p>We have tested the endogenous resistance of <i>M. persicae</i> lines gathered from bell pepper greenhouses against the parasitoids <i>Aphidius matricariae</i> and <i>Aphidius colemani</i>. No differences were found between the lines from different greenhouses or aphid control strategies. The wasps were generally successful in parasitizing all lines.</p> <p>Monitoring of aphid communities on strawberry (Beekman & Donner) Because we did not detect any endosymbionts in bell pepper aphids, we chose to redirect our research to strawberry, a crop in which our mixed-greenhouse sampling in May 2019 showed that both the bacterial endosymbionts <i>Regiella insecticola</i> and <i>Hamiltonella defensa</i> are often present. Strawberry is also host to many different aphid species, increasing our chances to find aphid-endosymbiont combinations that are protected against parasitoid wasps.</p> <p>In May 2020, we collected aphids from 16 different strawberry greenhouses located in different provinces of the Netherlands. Part of these companies grow strawberries in closed greenhouse compartments, but many other growers grow their strawberries in plastic tunnels or outside in (shielded) racks. At each location we collected aphids from five to eight locations that were furthest apart, to minimize the chance to sample the same clonal line multiple times. Aphids were first visually identified, following by molecular confirmation by sequencing the CO1 gene. The presence of known aphid endosymbionts was tested using diagnostic PCR using species specific primers.</p> <p>The most abundant aphid species that were found on strawberry were <i>Acyrtosiphon malvae</i> (16%), <i>Macrosiphum euphorbiae</i> (14%), <i>Aulacorthum solani</i> (12%), <i>Myzus ascalonicus</i> (10%) and <i>Chaetosiphon</i></p> |

fragaefoli/thomasi (8%). Of *A. malvae*, 33% of our collected aphids contained *H. defensa*, 6% *R. insecticola*, and 50% were co-infected with both *H.defensa* and *R.insecticola*. Of *M. euphorbiae*, 13% were infected with *H. defensa* and 27% with *R. insecticola*. *Aulacorthum solani* was infected in 22% with *H. defensa* and in 11% with *R. insecticola*. This is remarkable since we never found secondary endosymbionts present in *A. solani* in bell pepper. These results seem to suggest there is an effect of hostplant or cropping method on endosymbiont infection status of *A. solani*. *Myzus ascalonicus* was never infected with any secondary endosymbionts. Finally, fifty percent of our *C. fragaefoli/thomasi* samples were infected with *Fukatsuia symbiotica*.

Many different aphid lines were also collected alive and are currently kept in culture in our laboratory for future experiments studying the effects of symbiont infections on aphid fitness and resistance against parasitoid wasps.

In September/October 2020 we sampled the strawberry greenhouses for a second time. The sampling was successful, and we have gathered living aphid lines to use in future experiments. We also collected aphids from each greenhouse in ethanol. We are still in the process of analyzing these samples, which will be in the 'beoogde resultaten' section of 2021.

Monitoring of aphid field populations in the Netherlands (Donner)

In 2019, we had sampled aphids from natural populations spread out over the Netherlands. In addition to our sampling of field aphids in 2019, we have expanded the monitoring of field aphid populations for endosymbionts into the 2020 sampling season. We have sampled an additional 150 colonies, that contain a at least 3 new species and at least 10 new aphid-endosymbiont combinations compared to last year. We finished the lab work on the samples from 2019 and on the new samples from 2020. We have tested about 450 colonies from 100 aphid species, of which 17% carried the endosymbiont *H. defensa*, 14% carried the endosymbiont *R. insecticola*, 14% carried the endosymbiont *F. symbiotica*, 10% carried an *Arsenophonus* species, 6% carried the endosymbiont *S. symbiotica*, and *Rickettsiella* and *Rickettsia* species were both found in 3% of the species.

Aphid population genetic structure (Beekman)

Myzus persicae

For *M. persicae*, we used already existing microsatellite markers. One of these markers, *myz3*, resulted in null-alleles. Further investigation showed an insertion of approximately 70bp in the original primer target site in some alleles. Therefore, we developed a new forward primer for this marker to make sure we are still able to use this marker for population genetic structure analyses.

Our current results, using data from five microsatellite markers, show that *M. persicae* populations from organic greenhouses have much higher genetic variation than *M. persicae* populations from conventional greenhouses. Of the total 32 multi locus genotypes (MLG) observed, 7 came from conventional greenhouses with one genotype accounting for 75% of all sampled conventional-greenhouse aphids. In organic greenhouses, this genotype was also found but only made up 16% of the samples. The most abundant organic-greenhouse aphid genotype made up 26% of the organic-greenhouse samples. This genotype was not found in conventional greenhouses. Our data shows significant linkage-disequilibrium among markers, even after clone-correcting our data, meaning that our population is truly clonal. Also, there was no

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| | <p>evidence for genotype clustering, meaning that our data supports random mating between aphids in our 'bell pepper greenhouses 2019' population. This sounds controversial to the data supporting that our population is truly clonal. This is probably due to the specific life-cycle of aphids where aphids reproduce sexually before winter, but reproduce clonally throughout the rest of the year.</p> <p><u><i>Aulacorthum solani</i></u> For <i>A. solani</i> no microsatellite markers are previously developed. Therefore, we tested markers designed in other aphid species for cross-species amplification and polymorphism in <i>A. solani</i>. Three out of 26 markers that were tested successfully amplified a polymorphic locus. Extra microsatellite markers were developed for <i>A. solani</i>. The <i>A. solani</i> genome was published on NCBI in September 2019. We scanned the sequences from this project for the presence of di- tri- and tetra-repeat motives with a minimum of eight repeats. Primers were designed against regions showing perfect microsatellites and a total of 43 primer pairs were tested. Of these, 17 showed correct amplification. Of these 17, four markers showed polymorphism in our population. Together with the cross-species amplifying markers we now have seven polymorph markers that can be used to distinguish different <i>A. solani</i> genotypes. These markers were not multiplexed and tested together yet. We are planning on doing this in the first half of 2021.</p> |
| Beoogde resultaten 2021 | <p>Expected delay caused by the Covid-19 situation</p> <p>Because of Covid-19 both PhD candidates were forced to work less hours in the lab and more hours from home. This has resulted in:</p> <ul style="list-style-type: none"> - Sampling of fewer strawberry greenhouses than planned - Suboptimal surveillance of living aphid lines in the laboratory. As a result, lines collected in the strawberry greenhouses were lost due to very low reproductive rates. - This caused a delay in the testing of strawberry-aphid resistance against parasitoid wasps. - None of the planned symposia and congresses happened in 2020. As a result, both PhD candidates were not able to disseminate their work to scientific peers. <p>All together we are expecting that the Covid-19 situation has, up until the end of 2020, caused this project a delay of approximately three months.</p> <p>Plans for 2021:</p> <p>Monitoring of aphid communities in pepper greenhouses (Beekman & Donner)</p> <ul style="list-style-type: none"> • Perform parasitism experiment with <i>Aphidius ervi</i> parasitoids on 6 <i>Aulacorthum solani</i> aphid lines from bell pepper greenhouses. • Test the DNA samples of the aphids gathered from bell pepper greenhouses in 2019 for parasitoid presence. We will use published parasitoid-specific primer sets for this. • Publish our work on aphid species and microbiomes of aphids sampled in bell pepper greenhouses in 2019 <p>Monitoring of aphid communities on strawberry (Beekman & Donner)</p> <ul style="list-style-type: none"> • Finish testing aphid samples from the second sampling point for endosymbionts. • Test resistance of most common strawberry aphid lines (<i>Macrosiphum euphorbiae</i> and <i>Acyrtosiphon malvae</i>) with different endosymbiont infections against <i>Aphidius</i> and <i>Praon</i> |

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| | <p>parasitoids to determine the effects of endosymbiont infections on resistance against biocontrol agents.</p> <ul style="list-style-type: none"> Nanopore sequencing of the strawberry aphid microbiomes to: i) check if no known endosymbionts are missed with the diagnostic PCR methods, ii) if there might be currently unknown new endosymbiotic bacteria present in strawberry aphids, and iii) if microbiome composition differs between aphids collected from greenhouses using different control strategies and between different species of aphids. <p>Monitoring of aphid field populations in the Netherlands (Donner)</p> <ul style="list-style-type: none"> Publish the dataset on aphid field populations. <p>Aphid population genetic structure (Beekman)</p> <ul style="list-style-type: none"> Multiplex the cross-species, and newly developed primers targeting microsatellites in <i>A. solani</i>, and use this set of microsatellites to analyze the population genetic structure of <i>A. solani</i> collected from bell pepper (2019) and strawberry (2020) greenhouses. Sample and analyze the population genetic structure of aphids from conventional bell pepper greenhouses in 2021, to add the population genetic data to the 2019/2020 dataset we collected so far. Deep sequencing of <i>M.persicae</i> lines from different bell pepper greenhouse, with same microsatellite-genotypes, to check if microsatellite analysis shows enough depth to be able distinguish between different aphid clonal lines. Make a start determining the population genetic structure of strawberry aphids, apart from <i>A. solani</i>. Most importantly <i>M.euphorbiae</i>-using pre-existing microsatellite markers, and <i>A.malvae</i>-using ddRAD-seq or SNP analysis. |
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| <p>Opgeleverde producten in 2020 (geef de titels en/of omschrijvingen van de producten / deliverables of een link naar de producten op de projectwebsite of andere openbare websites)</p> |
| <p><u>Wetenschappelijke artikelen:</u></p> <ul style="list-style-type: none"> N.v.t. |
| <p><u>Externe rapporten:</u></p> <ul style="list-style-type: none"> N.v.t. |
| <p><u>Artikelen in vakbladen:</u></p> <ul style="list-style-type: none"> N.v.t. |
| <p><u>Inleidingen/posters tijdens workshops, congressen en symposia:</u></p> |

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The presentations we were planning on giving at congresses during 2020 were canceled or postponed until 2021 or 2022 due to the Covid-19 situation.

TV/ Radio / Social Media / Krant:

- N.v.t.

Overig (Technieken, apparaten, methodes etc.):

- N.v.t.