PPP Annual Report 2019
PPP projects which are under supervision of the "Topsectoren" must report annually on the scientific content and financial progress. This form is used to report the progress of the content of the project. PPP projects that finish in 2019 should make use of a different form: "PPP-final report."
The annual report will be published on the TKI / topsector website. Therefore, please ensure that there is no confidential information in the annual report.
The PPP-annual report must be sent, at the latest, by the 1st of March 2020 to the "TKI's": info@tkitu.nl or info@tki-agrifood.nl. For Wageningen Research, the report has to be sent to the "Topsector secretary" of your respective institute.

General information	
PPP-number	TU-18086
Title	Novel tools to breed for resistance against tuber diseases caused by obligate biotrophic pathogens
Theme	Duurzame Plantaardige Productie
Implementing institute	Wageningen University and Research
Project leader research (name + e-mail address)	Jack Vossen; jack.vossen@wur.nl
Coordinator (on behalf of private partners)	Stan Oome, HZPC
Project-website address	https://www.wur.nl/nl/Onderzoek-Resultaten/Onderzoeksprojecten-LNV/Expertisegebieden/kennisonline/Novel-tools-to-breed-for-resistance-against-tuber-diseases-caused-by-obligate-biotrophic-pathogens.htm
Start date	1-7-2019
Final date	31-12-2023

Approval by the coordinator of the consortium The annual report must be discussed with the coordinator of the consortium. The "TKI's" appreciate additional comments concerning the annual report.	
Assessment of the report by the coordinator on behalf of the consortium:	x Approved ☐ Not approved
Additional comments concerning the annual report:	

Summary of the project	
Problem definition	Potato wart disease, caused by *Synchytrium endobioticum* results in severe yield losses. Also the resting spores of this *Chytridiomycete* are hard, if not impossible, to eradicate from the soil. Therefore, wart disease is subject to world-wide quarantine regulations. Host resistance is a major component of these quarantine regulations, making wart resistance a "licence to grow" in affected areas. Despite strict control measures, outbreaks and unexpected appearances of wart disease have been reported (Denmark 2014, 2016; Emsland, Germany 2018). Also new pathotypes keep emerging in eastern Europe and neighbouring Asian countries. Despite the urgent necessity for wart resistant varieties, the characterisation of available resistance and identification of novel genetic resources for resistance breeding remains troublesome, mainly because *S. endobioticum* is an of

	obligate biotrophic pathogen. Bioassays are difficult due to the lack of pure inoculum. Also, current assays are based on tubers, which may vary in quality and are not available for related *Solanum* species. This impairs screens for novel sources of broad spectrum resistance from wild *Solanum* species. Apart from, the identification of novel resistance sources, also impairments to accurately map the causal resistance genes are encountered due to poorly reproducible, and time-consuming resistance assays.
Project goals	Due to the difficulties described above, wart disease has been intensively studied. Four years ago a TKI project (KV 1406-056) was started by the same consortium as the current project, to provide an –omics impulse for wart resistance management and breeding. KV 1406-056 has resulted in a number of crucial novel tools that facilitate the study of wart disease and wart disease resistance. Literally, resistance tests have been dug up from the soil and now see the light in above ground plant parts resulting unambiguous and reproducible phenotyping data. This allowed to formulate four main goals for the TU-18086 project: 1. Identification of novel effectors and Avr genes through differential gene expression studies. 2. Selection of effector signatures to identify "orphan" effectors for which no cognate *R* gene is known yet. 3. Screening for novel resistance sources. The WUR wild *Solanum* collection will be screened, using both the novel above ground wart assay and the "orphan" effectors 4. Fine-mapping of wart resistance genes can now be pursued using the foliar AVR response which allows to accurately phenotype recombinant populations

Results	
Planned results 2019	- Novel *S. endobioticum* candidate effectors. - Avr/effector signatures - Fine-mapping of wart disease resistance genes
Achieved results 2019	Novel *S. endobioticum* candidate effectors. In previous research we used presence absence information in the genomes of different pathotypes, in order to identify Avr candidates. It is, however, also known that effectors are upregulated upon plant colonistation by the pathogen. So, as a novel approach to mine candidate effectors from S. endobioticum, a comparative expression analysis was pursued. Available RNA-seq data have been used to select secreted proteins that show enhanced expression in fresh wart material, compared to resting spores. In order to select ubiquitous effectors that are potentially recognised by broad spectrum wart resistance genes among the in planta induced effectors, we only selected effectors that were present in all pathotypes. This yielded 15 candidate broad spectrum effectors. Avr/effector signatures. Currently, we have only one validated Avr (avrSen1). The protein has been analysed for repeated and conserved motifs. Several of such motifs were identified. Novel insights have been gained in the biological functions of these domains through transient expression assays in resistant (sen1 containing) and susceptible (not Sen1 containing) plants. Mainly, motifs have been identified required for recognition by Sen1. Involvement of other motifs in virulence induction are currently investigated.

	Fine-mapping. In the previous project we performed a recombinant screen to fine map the Sen5 resistance gene. We intended to phenotype these recombinants using Avr responses. Since the Avrsen5 has not been identified yet, we sought to phenotype the recombinants using a tuber based assay, in order to produce markers in a timely fashion. Tubers have been produced that will be tested in 2020.
Planned results 2020	Validation of broad spectrum Avrs: The 15 Avr candidates will be cloned and expressed in selected resistant plants harbouring the broad spectrum Sen 2, 3 and 4. Co-segregation of hypersensitive response with resistance will validate the candidate effectors as Avr genes. In addition, newly mined broad spectrum resistance from the germplasm screen (see below) will be tested for HR responses to the broad spectrum Avrs. Avr/effector signature The studies on AvrSen1 will be continued. Cloning and tagging tools that have been developed in 2019 will facilitate the functional analysis of newly identified Avrs, to identify common Avr signatures. Novel *S. endobioticum* candidate effectors: So far, we mined Avr genes from pathotype 1 genome sequences. We now, however, that some resistance genes that are active against higher pathotypes (like Sen5) are not providing resistance to pathotype 1. Therefore, we will use the genome of a pathotype 6 isolate to mine for Avrsen5. Fine-mapping: The phenotypic data from the Sen5 recombinants will be used to design markers closely flanking the resistance gene. This will yield markers for breeding, but also provide tools to validate potential Avrsen5 candidates. Germplasm screen The above ground inoculation assay will be used to identify novel broad spectrum resistance in 15 selected wild solanum accessions.

Deliverables/products in 2019 (provide the titles and /or a brief description of the products/deliverables or a link to a website.
Scientific articles: Not applicable
External reports: Not applicable
Articles in professional journals/magazines: Not applicable
(Poster) presentations at workshops, seminars, or symposia. Not applicable
TV/ radio / social media / newspaper: Not applicable
Remaining deliverables (techniques, devices, methods, etc.): Not applicable