# Final Report

| Project title (Acronym): |
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| Role of weed hosts as pathogen reservoirs of insect vectored diseases (WEEDVECT) |

**Project duration:**

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# 1. Research consortium partners

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# 2. Short project report

## 2.1. Short executive summary

'*Candidatus* Liberibacter solanacearum' (Lso) is a phloem-limited phytopathogenic bacterium vectored by psyllids. Lso has caused major damage in Solanaceous and Apiaceous crops worldwide where the psyllid vectors use cultivated crops as their main host or food plant. Psyllids often have a limited range of plant hosts but may feed on plants closely related to their host. The diversity and availability of plant hosts surrounding crops plants can significantly influence the epidemiology of Lso because of vector feeding behaviour and migration (Cooper et al. 2016). In many regions the presence and diversity of Lso and its vectors is poorly understood, especially for weed hosts surrounding cultivated crops. The WeedVect project aimed to develop a better understanding of Lso, in terms of its crop hosts, vectors and native/weed hosts. Knowledge on potential vectors and natural reservoirs of Lso may help to anticipate or prevent outbreaks in new areas and mitigate the impact on crops in existing areas. Liberibacters have been found in several psyllid species and while some seem to be benign, others are known as the causative agents of plant diseases with significant impacts on crop production worldwide. For Lso several new plant and psyllid vectors are being described and different Lso haplotypes are being characterised. Discovery of these alternative insect vectors and plant hosts will improve our understanding of Lso interactions with plants and insects, evolution, and epidemiology and will inevitably increase knowledge on new haplotypes of Lso, thus broadening current knowledge on the bacterium taxonomy.

WeedVect is a consortium of 3 institutions working to better understand the role of weed plants in the epidemiology of Lso. The consortium has also explored the potential role of aphids and other hemiptera as vectors of Lso. The project aimed:

- To improve the understanding of Lso diversity and presence in weed hosts including findings of Lso in previously unreported non-crop host plants
- To improve the understanding of Lso diversity, including the characterization of new Lso haplotypes, and presence of Lso in psyllids,
- To identify Lso within non-psyllid plant-sucking insects, including the characterization of new Lso haplotypes
- To assess the potential for using DNA barcoding to identify food plants of psyllids and other plant-sucking insects

## 2.2. Project aims

The main objectives of the project were:

- To survey psyllid populations to determine the presence or absence of known Lso vector species
- To test psyllid populations for the presence of Lso to investigate the prevalence of the pathogen in the environment and to identify possible new vector species
- To sample potential Apiaceae weed and crop hosts across carrot growing areas and test them for the presence of Lso.
- To investigate the host plant range of psyllid species by barcoding their gut contents
- To investigate the prevalence of Lso in the environment by developing a robust protocol for testing aphid species for the presence of Lso.

## 2.3. Description of the main activities and main results

### 2.3.1. Monitoring Lso in psyllid and plant reservoirs in carrot fields in Europe (AGES, SASA)

Lso diversity and presence in psyllids and weed plants surrounding carrot fields was monitored in Scotland and Austria. Apiaceous and other weeds of importance (psyllid hosts) were collected from field margins and boundaries in major carrot growing regions along with the psyllids present on these plants. Psyllids were collected in the field using a sweep net and collected by an aspirator. DNA was extracted from plants using a DNeasy Plant Mini Kit (Austria) or Biosprint Plant kit (Scotland). DNA was extracted from psyllids using the DNeasy blood and tissue kit. Psyllid and plant extracts were tested for the presence of Lso using real-time PCR (Li *et al*. 2009). Lso haplotypes of positive samples were examined using DNA barcoding techniques examining 16S, 16-23S and 50S ribosomal genes and an outer membrane protein gene.

After Lso symptomatic celery and carrot plants were reported in Austria in 2015[1], a survey of 20 fields was carried out between 2016-2019. 84 plants from Apiaceae and Solanaceae were collected from field margins and tested for Lso. 43% (n=72) of Apiaceous plants were positive for haplotype C and no positives were found from Solanaceous plants. Infected plants were carrot (*Daucus carota*), celery (*Apium graveolens*), parsnip (*Pastinaca sativum*), parsley (*Petroselinum crispum*) and common hogweed (*Heracleum sphondylium*). This is the first report of Lso in common hogweed.

Psyllids and plants were collected from 9 sites in Scotland; spread across the major carrot growing regions (Sumner-Kalkun *et al.* 2020). 535 psyllids from 19 different species were collect and tested for Lso. The most abundant psyllids were *Trioza urticae* (found at all sites), *Craspedolepta nebulosa* (two sites) and *Trioza anthrisci* (found at only one site). 587 plant specimens were tested and Lso positive material was found from 6 sites.

### 2.3.2. Lso diversity and presence in psyllids and plants (AGES, SASA)

Six different non-cultivated plant species were found positive for Lso with most species coming from the Apiaceae. Positive species included: *Anthriscus sylvestris, Aegopodium podagraria, Urticaria dioica* (stinging nettle), *Urticaria dioica* subsp. *galeopsifolia* (non-stinging nettle), *Galium* spp., and *Chenopodium album*. Haplotypes in cow parsley were haplotype C and stinging nettle harboured haplotype U; this is congruent with the haplotypes found in the psyllids for which these plants are the natural host. Haplotype C was also detected in a small number of cultivated carrot plants at one site in Scotland (9.34%, n=139). Interestingly, no psyllids known to carry haplotype C were found at this location, so the source of infection for these plants is unknown.

Lso U haplotype was found at all sites from the psyllid *Trioza urticae* and stinging nettle. This haplotype seems widely spread in weeds but it's impact on cultivated crops is not known, however it is not likely to represent a major threat.

*Trioza anthrisci* was found at only one site in the North of Scotland on cow parsley and on cultivated carrot plants in the field. All individuals (n=13) tested were harbouring Lso haplotype

[1] The first report of 'Candidatus Liberibacter solanacearum' in Austria is available from the EPPO Reporting Service https://gd.eppo.int/reporting/article-4455

C. Furthermore, the psyllid species *Craspedolepta subpunctata* and *Craspedolepta nebulosa* were found to harbour two Lso haplotypes that had not yet been characterised and were new to science. These haplotypes were named Cras1 and Cras2 and were found in both species. Other positive psyllid species were found but haplotyping was not successful.
The full reports of these sampling efforts can be found in the following publications: Lethmayer and Gottsberger (2020) and Sumner-Kalkun *et al*. (2020).
A comparative verification of the detection of Lso in plants was performed by AGES using the real-time PCR of Li *et al*. (2009) and compared to the conventional PCR of Ravindan *et al*. (2011). 18 *Apium graveolens* samples and positive control samples from the previous year's sampling campaigns were also included. Two different DNA extraction procedures were also compared: DNeasy Plant mini Kit (Qiagen) and innuPREP Plant DNA I Kit-IPC16, protocol 2 (Analytik Jena AG). No differences in qualitative results between the extraction procedures or the detection methods (real time and conventional PCR) could be recorded.
Field work efforts were severely disrupted by the pandemic and COVID-19 restrictions meaning no field work was carried out in 2020.

### 2.3.3. Detection of plant DNA from psyllid and aphid gut contents (SASA)

Psyllids have a narrow host range however some psyllid species such as *Bactericera cockerelli* can move onto a range of different host species some of which are commonly found in wild environments. These wild host plant may act as a bridge or reservoir for Lso and other plant pathogens when the psyllids main host or crop plant is not available. During the project, psyllid and aphid guts were screened for the presence of plant DNA to assess whether this is a useful method for understanding food plants on which the psyllids have been feeding. These techniques will be especially useful in cases where no plant host data is collected e.g. suction trap or sticky trap caught specimens. The approach has been demonstrated to be a useful tool for identifying host plants in certain psyllid species (Cooper *et al*. 2016).Suction trap caught aphids (*C. aegopodii* and *R. padi*) and lab reared psylids (*Trioza urticae*) were tested for the presence of plant DNA using conventional PCR of the chloroplast rubisco large subunit coding genes (Kress and Erickson 2007; Kress and Erickson 2009). Different techniques for dissection of psyllid gut samples were trialled. These included crushing the abdomen of the insect with a cover slip to extrude internal contents and using a fine pin and tweezers to physically pull the internal organs out. The latter technique was favoured. Results of sequencing PCR products obtained from plant barcoding of psyllid and aphid extracts were difficult to interpret and yielded no expected natural plant hosts. Difficulties arose in isolating and amplifying plant DNA from the psyllid host and sequencing results were low quality so that molecular barcoding of plants was difficult. Even lab reared control specimens of *Trioza urticae* reared on *Urtica dioica* for their entire life cycle did not give its host plant as a result. Further work on this should focus on NGS methods such as nanopore metabarcoding.

### 2.3.4. Testing of aphid species associated with carrot fields for their potential to carry Lso (SASA, VATZUM, AGES)

Other hemiptera were tested for the presence of Lso to build up a picture of which insects may also play a role in Lso transmission. Sap sucking insects which share a host plant with the main psyllid vectors of Lso could also be responsible for carrying and transmitting Lso to other plants. For the first steps to determining which hemipterans might be potential vectors of Lso, several aphid species were tested for the presence of Lso. Methods used were the same as

those used for testing psyllids for Lso, DNA extraction with the DNeasy Qiagen Blood and Tissue kit (SASA & VATZUM) and DNA extraction with QIAamp DNA Micro Kit (Qiagen) (AGES). Testing for Lso was performed with the real-time PCR (Li *et al*. 2009). In 2019 SASA tested three aphid species for Lso (Tab. 1) from suction trap samples collected in three different regions in 2018.

Table 1. Number of positive samples for Lso from three different aphid species tested

| | **Ayr** | **Dundee** | **Gogarbank** |
|---|---|---|---|
| | % +ve (n) | | |
| ***Cavariella archangelicae*** | 0% (n=12) | 0% (n=2) | 0% (n=17) |
| ***Cavariella aegopodii*** | 5% (n=20) | 23.1% (n=26) | 0% (n=14) |
| ***Rhopalosiphum padi*** | 22.7% (n =44) | 1.9% (n=54) | 32. 8% (n=67) |

| | | | |
|---|---|---|---|
| **TOTAL** | 14.5 % (n=76) | 8.5 % (n=82) | 22.4% (n=98) |

Low percentages of aphids infected with Lso were found in suction trap samples and only two of the three species tested positive (Table. 1). Although Lso acquisition was observed, it is still unknown whether feeding by infected aphids can lead to subsequent infection of plants. Furthermore, this result could not be repeated when specimens of *Rhopalosiphum padi* and *Cavariella aegopodii* from suction traps, caught in 2019 were tested by conventional PCR (Ravindran et al. 2011) at the VATZUM laboratories (Tab.2). Additionally, AGES tested field collected aphids from carrot and hogweed for the presence of Lso. Specimens of the family Aphididae such as *C. aegopodii* and *Myzus persicae* were found on carrot and *Cavariella theobaldi* and *Cavariella pastinacae* were found on common hogweed (*H. sphondylium*). All aphids tested were negative.

| Location | # of C. aegopodii | Postives | # of R. padi | Positives |
|---|---|---|---|---|
| Dundee | 35 | 0 | 37 | 0 |
| Ayr | 32 | 0 | 34 | 0 |
| Edinburgh | 36 | 0 | 34 | 0 |
| Inverness | 29 | 0 | 34 | 0 |

Table 2. Number of aphid samples tested for Lso from four suction trap location in Scotland.

4 out of 22 positives (18%) found in the suction trap samples from the UK in 2018 were successfully sequenced from haplotyping. These showed infection of *R. padi* with Lso haplotype U and the recently described Cras1 and Cras2. The results are particularly confusing as plant species with which these Lso haplotypes (U, Cras1 and Cras2) are associated are not know hosts of *R. padi* so it is not clear how it could acquire these haplotypes. These results will require verification to understand the role of aphids in the epidemiology of Lso and their interaction with this bacterium.

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### 2.4. Conclusions and recommendations to policy makers

The bacterium '*Candidatus* Liberibacter solanacearum' appears to be widespread and associated with a range of natural host plants and psyllid species. Work performed as part of this project has uncovered new psyllids and host plants for Lso but also genetic types of Lso that are new to science. The impact of these new psyllid species on cultivated crops is unknown and further research should include studies on the feeding behaviour and potential transmission of Lso by psyllids to important crop species. It is almost certain that other Lso-insect and Lso-plant combinations are yet to be discovered and further monitoring and surveillance is recommended to ensure associations are not overlooked. Given that haplotype Lso U has been found to be widespread and has not, yet, been reported to have caused negative impacts to crop yields it is considered to be of low concern. However, the presence of variants of Lso that do not cause damage to cultivated crops highlights the necessity to characterise Lso haplotypes to understand their role in disease outbreaks. Furthermore, regulation and quarantine of Lso infected material should also consider the presence of efficient vector species and the variety of Lso haplotypes present in a region. Newly discovered Lso haplotypes and psyllid vectors should be considered by policy makers and should be addressed in regulation once their impact on important host plants and Lso epidemiology is understood.

The role of other insect groups in the spread of Lso is still poorly understood. This project found a small amount of aphid and plant hopper species that were harbouring Lso but their role in transmission of Lso is not known. Transmission studies with those species found to carry Lso are the next step in understanding their role in Lso epidemiology and their risk to other crop species. While psyllids are the main vectors of Lso thus far, other hemipteran groups may share the ability to harbour and transmit the bacterium. The carrot psyllid *Trioza apicalis,* shares host plants with other hemipterans such as plant hoppers and aphids. It is unclear whether these insects play a part in Lso transmission or if it is only transmitted by psyllid vectors. Methods to detect the host plants on which psyllids and aphids are feeding could be

useful for resolving landscape- level ecology, locating potential overwintering sites, and the potential spread of Lso.

### 2.5. Benefits from trans-national cooperation

Given the different situations of countries regarding the Lso haplotypes present and the impact these have on cultivated crops in those regions. The use of consortia such as WeedVect enables countries to share expertise on surveillance, detection, and characterisation of Lso and its hosts. The ability to share knowledge on the different experiences and situations regarding Lso infection in different countries is invaluable in helping us understand why and when Lso becomes damaging to crops and the different levels of risk countries have. Understanding the impact of Lso in different climates and cultivation systems is improved when trans-national projects are put together and epidemiology can be reflected on in the context of geographic regions rather than discrete sampling efforts.

This project has also benefitted from increased capacity for testing and optimisation of techniques across laboratories, especially of testing plants and psyllid species, but also in testing alternative potential Lso carriers such as aphids.

# 3. Publications

## 3.1. Article(s) for publication in the EPPO Bulletin

None.

## 3.2. Article for publication in the EPPO Reporting Service

None.

## 3.3. Article(s) for publication in other journals

Two articles were published which include results obtained from the WeedVect project:

- Lethmayer C, Gottsberger RA (2020). First report of '*Candidatus* Liberibacter solanacearum' in common hogweed (Heracleum sphondylium) in Austria. *New Disease Reports* 42:17 https://doi.org/10.5197/j.2044-0588.2020.042.017
- Sumner-Kalkun JC, Highet F, Arnsdorf YM, Back E, Carnegie M, Madden S, Carboni S, Billaud W, Lawrence Z, Kenyon D (2020). '*Candidatus* Liberibacter solanacearum' distribution and diversity in Scotland and the characterisation of novel haplotypes from *Craspedolepta* spp. (Psyllidae: Aphalaridae). *Scientific Reports* 10:1–11 https://doi.org/10.1038/s41598-020-73382-9

# 4. Open Euphresco data

None.