

## Oral presentation

### Laboratory and field investigations into vertical transmission of CaLsol in parsnips, and practical application in seed production

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**Abstract:** Detection of *Candidatus Liberibacter solanacearum* (CaLsol) in *Pastinaca sativa* (parsnip) seeds and plants has impacted production and distribution of parsnips seeds. Vertical transmission of CaLsol has been investigated in Apiaceous crops but not within parsnip. Using the DNA extraction and real-time PCR protocol described by Bertolini et al (2014), including using propidium monoazide (PMA) to assess presence of living bacterium, parsnip seeds infected with CaLsol were identified and subsequently used to investigate vertical transmission. Laboratory experiments investigated detection of CaLsol from infected seed through early stages of growth (seed, imbibed seeds, radicles present, and cotyledons present). Preliminary results suggest detection at dry seeds stage only, with initiation of imbibing resulting in CaLsol either absent or below the detectable threshold of the real-time PCR. Covered polytunnel crops grown under insect proof mesh investigated movement of CaLsol from infected seeds to plants. Fifty plots of contaminated seeds were planted, along with 5 plots of uncontaminated seeds under insect proof netting. Leaf samples taken of the cotyledons, first true leaves and subsequently monthly were analyzed. Living or total CaLsol DNA via real-time PCR was not consistently detectable, with retesting producing negative results. Uncovered field trial of 140 plots that included 2 carrot varieties and 3 parsnip varieties were also undertaken. DNA extraction and real-time PCR was carried out on the cotyledons, first true leaves and after 11 months. Initial results detected CaLsol present, but retesting and verification of initial results with further dilution resulted in zero presence of CaLsol for all samples tested. Two trials looked at different disinfection treatments of parsnip seeds, in 2018 and 2019. During 2018, all treatments produced detectable levels of CaLsol. Whilst 2019 had shown possible reduction, eradication of living CaLsol was not effectively achieved. Testing of seeds for the presence of CaLsol has found certain requirements for production of CaLsol-free parsnip seeds in Europe. During 2017, 2018, and 2019 parsnip seeds (14, 13, and 13 samples respectively) were tested for the presence of CaLsol and only those produced under cover were found to be CaLsol-Free.

#### *Bibliography*

Bertolini, E., Teresani, G. R., Loiseau, M., Tanaka, F. A., Barbé, S., Martínez, C., Gentit, P., López, M. M. and Cambra, M. (2015), Transmission of '*Candidatus Liberibacter solanacearum*' in carrot seeds. *Plant Pathol*, 64: 276-285. doi:10.1111/ppa.12245.