Epidemiological aspects of *Pseudomonas syringae* pv. *actinidiae*

Pseudomonas syringae pv. *actinidiae* (Psa) is the causal agent of bacterial canker of kiwifruit. This pathogen affects *Actinidia* species (*Actinidia deliciosa* and *A. chinensis*) worldwide. The disease is a serious threat for kiwifruit production, due to high tree mortality and reduced production. The recent severe outbreaks of bacterial canker of kiwifruit in the EPPO region and in New Zealand have been related to the appearance of a local, very aggressive aplotype of Psa called Psa biovar 3. Possible pathways of pathogen introduction and disease spread into new territories are *Actinidia* spp. plants for planting, which represent the main pathway for long distance dissemination of Psa. However, positive pollen samples were recovered in New Zealand and in Italy. The possibility that infected pollen could be a pathway for Psa introduction and disease spread was investigated and confirmed (EPPO, 2012; Tontou *et al*., 2014). Therefore, it has been suggested that pollen, as a pathway, should be certified free from Psa (Zespri, 2012; EPPO, 2016). Other dissemination ways are wind and wind driven rain, spring frost, equipment and tools.

One of the objectives of the project was the improvement of the knowledge on the life cycle and epidemiological behaviour of *Pseudomonas syringae* pv. *actinidiae* in different areas of Europe.

In northern Italy, a commercial orchard was planted in 2011, in order to confirm the epidemiological role of contaminated pollen in the introduction and survival of Psa in the field and its possible association to a disease outbreak. Such experimental orchard was located no less than 90 km far away from the nearest kiwifruit area, to ensure that no natural Psa inoculum could contaminate the experimental area through wind and rain driven cells, possibly present in infected kiwifruit orchards.
During the growing season 2014 the experimental orchard (4-year old trees) was artificially pollinated, according to the common application procedures, and disease development monitored. As a control, a neighbouring kiwifruit orchard was planted at the same time as a negative control (same age, same cultivar), and was pollinated with pollen certified free from Psa. During the five months after pollination, the experimental orchards were fortnightly inspected, in order to monitor disease development.

Disease symptoms (leaf spots) developed during 8-10 weeks after pollination with contaminated pollen; no canker developed. Meanwhile, no symptom was ever detected in the control orchard. In spring 2015, typical bleeding cankers started to develop in the orchard pollinated with Psa-contaminated pollen. Until September 2015, the disease progressed dramatically: a few trees died and several showed cankers on wines, cordons and trunks. No symptom related to Psa was ever detected in the control orchard during 2015. Therefore, according to our experimental results, it is confirmed that pollen is an efficient pathway for Psa dissemination into new areas and might be the cause of a severe disease outbreak through artificial pollination. Researchers also observed that the highest disease intensity may appear not during the months following pollination, but the following year, when a sufficiently high population of Psa may have built in the pollinated orchard.

In 2010, Psa was first detected in the North region of Portugal in *Actinidia deliciosa* orchards, as well as in propagation material used to plant new orchards. Since then, yearly national surveys have been performed by the Portuguese Phytosanitary Authority and samples analysed at INIAV phytobacteriology laboratory. Disease incidence and progression was also assessed in the Northern and Central regions, where kiwifruit orchards are economically important. Between 2010 and 2013, more than 100 bacterial isolates were collected along the country from infected plants of different cultivars and ages. The use of two conventional PCR protocols allowed identifying all known Psa biovars. Further, characterization allowed excluding the presence of biovar 2 among the Portuguese strains. Additionally, BOX-PCR fingerprinting profiles and the phylogenetic tree generated by rpoD were characteristic of biovar 3 for most of the strains tested. The lack of avrD1 amplification indicated the presence of a small population of biovar 4 strains recently allocated to *Pseudomonas syringae* pv. *actinidifoliorum*. Conclusions were drawn on the presence of two different pathovars of *Pseudomonas syringae* affecting Portuguese kiwi orchards. *P. syringae* pv. *actinidifoliorum* might have been introduced more than 10 years ago, while the main population, highly aggressive, is present in the infected plants within the major production areas, leading to relevant yield losses (Cruz *et al*., 2014).

Pseudomonas syringae pv. *actinidifoliorum* pv. *nov.*, described by Cunty *et al*. (2015), differed mostly from *Psa* at pathogenic level, as this pathovar cannot induce canker on wood.

Pseudomonas syringae pv. *actinidifoliorum* seems to be present in France before the detection of recent outbreaks of Psa.

In Spain, the presence of Psa was first discovered in Pontevedra, Galicia region, the main kiwi fruit producing region of Spain. The isolates were identified as similar to the virulent Psa biovar 3 reported in other countries (Abelleira *et al*. 2013). In the course of surveys performed in the following years, strains of *P. syringae* pv. *actinidifoliorum* were found in the close region of A Coruña (Abelleira *et al*. 2015). Other *P. syringae* strains close but not identical to this new pathovar are under study for their accurate taxonomic classification. As the asymptomatic plant material has been considered responsible of the introduction of the disease in new areas, regional surveys were performed since then and plant material analysed following the EPPO protocol, with small modifications. In general, a good correlation was observed among the results obtained with different techniques. Results from Galicia, where around 851 ha of kiwi are currently cultivated, indicate that in 2012-2014, 31 asymptomatic samples from nurseries (out of 164) and 47 from orchards (out of 165) were Psa positive. In Asturias, where around 168 ha of kiwi are currently cultivated, in 2012-2014, 5 samples from nurseries (out of 104) were Psa positive. In Comunidad Valenciana, where the estimated area of kiwi has increased from 23 ha in 2011 to 262 ha in 2013, in 2012-2014, 276 samples from nurseries and 36 from orchards were negative for the target. In Cantabria, where around 40 ha of kiwi are currently cultivated, in 2013-2014, 39 samples from orchards were negative for the target. In País Vasco, where around 75 ha of kiwi are currently cultivated, in 2013-2014, 5 samples from orchards analysed were also negative. These results confirm the frequent dissemination of the bacterium with asymptomatic plant material.

Project ID: *Pseudomonas syringae* pv. *actinidiae* (PSA): diagnosis, detection, identification and study of epidemiological aspects (PSADID).

References:

Abelleira A, Ares A, Aguin O, Peñalver J, Morente MC, López MM, Sainz MJ, Mansilla JP, 2015. J Appl Microbiol.,119(6):1659-71

Cunty A, Poliakoff F, Rivoal C, Cesbron S, Saux FL, Lemaire C, Jacques M, Manceau C, Vanneste J. 2015. Plant Pathology 64:582-596

Cruz L, Cruz J, Fernandes C, Chicau G, Tenreiro R. 2014. http://simposio.spf.scap.pt/images/Livro%20Resumos_SCAP-SPF_WEB_20nov2014.pdf

EPPO, 2012. Pest risk analysis for Pseudomonas syringae pv. actinidiae. EPPO, Paris, France

EPPO, 2016. Pseudomonas syringae pv. actidididiae. EPPO/OEPP Bulletin (in press)

Tontou R, Giovanardi D, Stefani E, 2014. *Phytopathologia Mediterranea* 53, 333-339

Zespri, 2012. Zespri Innovation Project V11285 report of February 2012, pp. 28