Diagnosis, detection and identification 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. Recently, an EPPO standard has been published as formal guidance on procedures for the detection of Psa (EPPO, 2014). Screening and identification methods are mainly based on conventional PCR (single and duplex PCR's) (Rees-George *et al*., 2010, Gallelli *et al*., 2011a) and of rep-PCR. Considering the worldwide high impact of this pathogen on kiwifruit, during the last few years several authors have developed new molecular methods (Biondi *et al*., 2013; Balestra *et al*., 2013; Gallelli *et al*., 2014). However, these latter methods need to be validated for their inclusion in the procedure for detection of Psa as screening and/or identification tests.

The main objective of the project was to organise a test performance study of relevant detection methods to be used for the detection and identification of Psa from symptomatic and symptomless kiwifruit materials.

National Reference Laboratories (NRL) need to verify the performance of detection and identification methods developed in-house or chosen from those available. By this way, the most reliable methods should be taken into consideration during the development of official diagnostic procedures. In order to meet these needs, a test performance study (TPS) was performed as collaborative studies among ten European (France, Spain, Greece, Austria, Portugal, Italy), two New Zealanders and one Turkish laboratories by comparing the available Psa detection methods: isolation on selective NSA and KB media, single, duplex, nested, multiplex, real-time PCR based methods (Rees-George *et al*., 2010, Gallelli *et al*., 2011a, Biondi *et al*., 2013; Balestra *et al*., 2013; Gallelli *et al*., 2014). Their ability to detect Psa was assessed by testing blind samples consisting of 13 woody extract and 11 pollen samples of kiwifruit spiked with Psa bacterial suspensions at different concentrations (from 10^7 up to

10 CFU/mL of plant extract). The evaluation of these methods followed the performance criteria defined in the EPPO pest management standards PM7/98 (2), and PM7/72 (2) (EPPO 2014, 2010).

Results on the detection and isolation of Psa from pollen samples were unexpected (little success in isolating the pathogen or to detect its DNA from experimentally infected samples), probably because Psa cells died during sample transport. The hypothesis taken into consideration was that the artificial inoculation of pollen makes Psa more vulnerable to external conditions, with respect to the natural colonization, and Psa was degraded and eventually died.

Results from woody samples responded to the necessity of the NRL concerning Psa detection and identification. These results, currently in press, allowed the participating laboratories to assess all available methods, thus obtaining an overview of the performance criteria for all tested protocols.

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

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