ISSN: 0191-2917
e-ISSN: 1943-7692

plant disease

Editor-in-Chief: Alison E. Robertson
Published by The American Phytopathological Society

Posted online on 6 Nov 2017.
https://doi.org/10.1094/PDIS-04-17-0510-PDN

DISEASE NOTES

First Report of *Pseudomonas syringae* pv. *actinidiae* on Kiwifruit Pollen from Argentina

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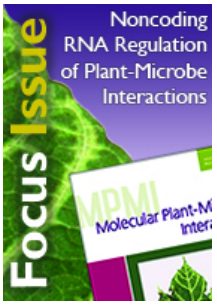
In recent years, in the Mar del Plata area of Argentina, many kiwifruit orchards composed of only male plants (*Actinidia deliciosa* 'Chieftain') have been established for pollen production and commercialization, since the country was considered unaffected by *Pseudomonas syringae* pv. *actinidiae* (Psa). When mature, the anthers are detached and dried, and grains of pollen are collected with a portable cyclonic pollen collector. Then, routine controls aimed to assess the risk of infection by Psa, the causal agent of kiwifruit bacterial canker (Takikawa et al. 1989), are carried out by plating 1-g aliquots of pollen on semiselective medium (modified KB) and incubating plates for 48 to 72 h at 27°C. In February 2015, during one of these controls, some bacterial colonies with morphological features similar to Psa were detected. After purification, four of them (labeled as Arg1.1 to Arg2.3) were found to be gram-negative, nonfluorescent, positive for levan production, and caused hypersensitive response on tobacco. The isolates were further characterized and tested negative for cytochrome C oxidase, oxidative in glucose metabolism, negative for aesculine hydrolysis, and negative for nitrate reduction. Genomic DNA was purified from each isolate, quantified, and diluted to a final concentration of 20 ng μl^{-1}. Pathovar specific PCR assays (Gallelli et al. 2011; Rees-George et al. 2010) generated amplicons of the expected sizes, confirming the identity of all the isolates as Psa. Moreover, the sequences of housekeeping genes *gapA*, *gltA*, *gyrB*, and *rpoD* were obtained and found identical between the isolates. When BLASTed in GenBank, they showed 100% identity to analog sequences of Psa biovar 3 (Chapman et al. 2012). The housekeeping gene sequences of the isolate Arg2.1 were deposited (accessions KY327791–94). Pathogenicity was confirmed by artificial inoculation of *A. deliciosa* 'Hayward' and *A. chinensis* plantlets. Ten 3-month-old plantlets, each 30 cm high, were singularly dipped for 1 min in a bacterial cell suspension (8×10^6 CFU/ml in 0.01 M $MgSO_4$) of Arg2.1 isolate and then incubated at

Article History

Published: 6 Nov 2017
First Look: 5 Sep 2017
Accepted: 30 Aug 2017

high RH (80%), with 14 h daylight and 24/21°C day/night temperature cycle. Positive and negative controls were likewise inoculated with Psa virulent strain CFBP 7286 and 0.01 M $MgSO_4$, respectively. After 7 days, brown spots with pale green halos were observed on inoculated plants. According to Koch's postulates, bacteria were reisolated from diseased tissues and have been found identical to the original strains in morphology, biochemical tests, and pathovar-specific PCR products. Negative control plants did not develop any symptoms. To our knowledge, this is the first scientific proof of the presence of Psa in Argentina, and specifically on kiwifruit pollen but not on plants, at the pollen harvest time. This result requires great attention to the potential risk of pathogen spread in the future. Extensive monitoring and laboratory analyses are in progress.



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The research has received funding from the European Union's Seventh Framework program for research, technological development and demonstration under grant agreement number 613678 (DROPSA).

Citation