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Occurrence of *Pseudomonas syringae* pv. *actinidiae* in Jin Tao kiwi plants in Italy

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Summary. During 2007–2008 bacterial canker caused damage in Jin Tao cv. kiwi (*Actinidia chinensis*) plants grown in northern and central Italy. A bacterial population was repeatedly isolated from these plants. Based on morphological, physiological, biochemical and molecular tests, the causal agent was identified as *Pseudomonas syringae* pv. *actinidiae* (epidemiology and control strategies are discussed).

Key words: bacterial canker.

Italy is the main kiwi producer in the world with a surface area of 27,000 ha and an annual production of 518,000 tons. The most important kiwifruit areas are Lazio, Piemonte, Emilia Romagna and Veneto. Among the *Actinidia* spp. cultivated in Italy during the last ten years, *A. chinensis* cultivars (such as Jin Tao and Hort 16) have substantially increased both their acreage and their production, to 927 ha and 9,910 tons respectively (Testolin and Ferguson, 2009).

During 2007 and 2008, different kiwi orchards with suspected bacterial disease symptoms were recorded in central Italy (Latina province, Lazio) and in northern Italy (Treviso province, Veneto). Symptoms similar to those caused by *Pseudomonas syringae* pv. *actinidiae* (Serizawa *et al*., 1989) were seen on different kiwi plant organs of *A. chinensis* cv. Jin Tao. The symptoms included browning of the buds and flowers, brown angular spots surrounded by yellow haloes on the leaves, and cankers with reddish exudates on the twigs, leaders and trunks (Fig. 1, 2, 3).

Pseudomonas syringae pv. *actinidiae* has recently been reported to be the causal agent of bacterial canker on *A. chinensis* cv. Hort 16A (Balestra *et al*., 2008). The aim of the present paper is to report on a two-year investigation on the occurrence of *P. s.* pv. *actinidiae* on Jin Tao kiwi plants in central and northern Italy.

Diseased samples were collected to verify bacterial infection. Isolates were purified and identified using standard bacteriological techniques as described by Takikawa *et al*. (1989). Suspected bacterial colonies were white, convex, with smooth to ondulate margins. According to Lelliott and Stead (1988), ten isolates were Gram negative, produced fluorescent pigment on KB medium, caused hypersensitivity on tobacco leaves (var. Virginia Bright), and were levan and urease positive. The isolates produced a negative reaction for oxidase, potato soft rot, arginine dehydrolase, tyrosinase, urease, and nitrate.

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Fig. 1. Bacterial canker on Jin Tao kiwi plants; symptoms on the leaves showing angular brown spots surrounded by yellow halos caused by severe infection of *Pseudomonas syringae* pv. *actinidiae*.

Fig. 2. Cankers and red exudates produced by *Pseudomonas syringae* pv. *actinidiae* on a Jin Tao kiwi twig.

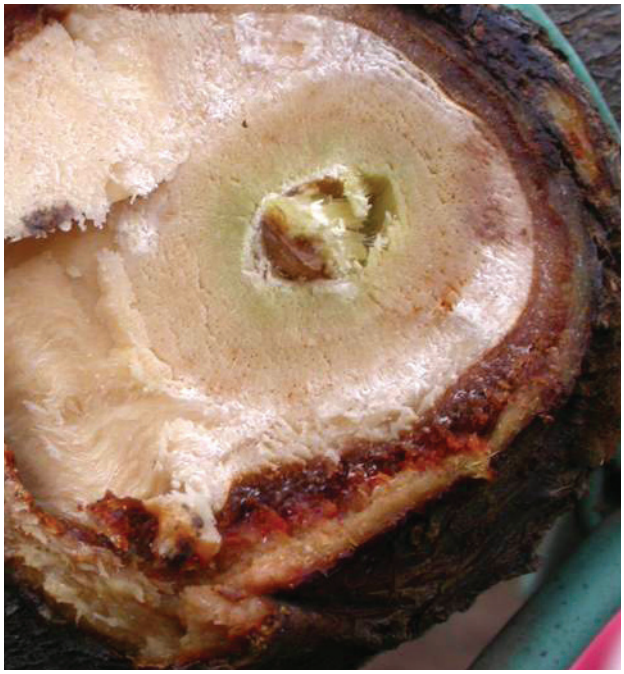

Fig. 3. Section of a Jin Tao leader kiwi heavily infected with *Pseudomonas syringae* pv. *actinidiae*.

To characterize the bacterial isolates, pathogenicity tests were carried out (Serizawa and Ichikawa, 1993). Ten 2-year-old healthy plants of *A. chinensis* cv. Jin Tao, *A. deliciosa* cv. Hayward, and pear, liliac and lemon were inoculated with bacterial suspensions of about 1×10^7 CFU ml^{-1} as described by Takikawa *et al*. (1989). Only isolates from kiwi produced symptoms similar to those naturally observed on the buds, flowers and leaves after one week, and on the branches, after 2 weeks. The original bacterial strains were reisolated from all the developed symptoms. Known strains of *P. viridiflava* (NCPPB 1252), *P. s.* pv. *syringae* (NCPPB 1087) and *P. s.* pv. *actinidiae* (NCPPB 3739) were used as controls during the tests.

The 16S rDNA region (Moore *et al*., 1996) of two strains (PSA7T08-PSA8L08) was sequenced and compared with the corresponding sequences in INSD (GenBank, EMBL and DDBJ). The genomic DNA of the 16S rDNA and 16S-23S rDNA intergenic spacer regions of 2 randomly chosen isolates was extracted and sequenced for molecular characterization. The sequences were then compared to the databases available at the National Centre for Biotechnology Information (NCBI) using their Blast search software (Altschul *et al*., 1990). The isolates were also tested with *P. s.* pv. *actinidiae* specific primers (Koh and Nou, 2002). Both sequences showed complete identity of the two isolates with those of the *P. s.* pv. *actinidiae* strains in the databases. This record is the first occurrence of *P. s.* pv. *actinidiae* on *Actinidia chinensis* cv. Jin Tao in Italy.

Disease symptoms in the field were found mainly on *A. chinensis* plants cv. Jin Tao and occasionally on *A. deliciosa* plants cv. Hayward in adjacent orchards. Disease incidence in the field ranged from 30 to 50%, with a mean of 40%, and the symptoms (as compared with those recorded on cv. Hort 16 A, Balestra *et al*., 2008), were most common and evident on the leaves, buds and flowers, and were less severe on the twigs, leaders and trunks.

Due to the irregular distribution of Jin Tao orchards in Italy, the epidemiology of this disease should be studied and control strategies devised to prevent any further spread of the disease.

Considering the success of the Jin Tao kiwi cultivar in Italy, it is important to determine the main sources of infection. Adequate cultural practices and copper treatment have so far been highly effective in reducing disease damages. An epidemiological study of bacterial canker on different *Actinidia* spp./cv. in Italy and of new strategies to control *P. s.* pv. *actinidiae* has been initiated.

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Literature cited

Altschul S.F., W.Gish, W.Miller, E.W.Myers and D.J. Lipman, 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215(3) 403–10.

Balestra G.M., A. Mazzaglia, R. Spinelli, S. Graziani, A. Quattrucci, A. Rossetti, 2008. Cancro batterico su *Actinidia chinensis*. *L'Informatore Agrario* 38, 75–76.

Koh J.K., I.S. Nou, 2002. DNA markers for identification of *Pseudomonas syringae* pv. *actinidiae*. *Molecules and Cells* 13(2) 309–314.

Lelliott R.A. and D.E. Stead, 1988. *Methods for the Diagnosis of Bacterial Diseases of Plants*. Blackwell Scientific, Oxford, UK.

Moore E.R.B., M. Mau, A. Arnscheidt, E.C. Böttger, R.A. Hutson, M.D.Collins, Y. Van De Peer, R. De Wachter and K.N. Timmis, 1996. The determination and comparison of the 16S rRNA gene sequences of species of the genus *Pseudomonas* (*sensu stricto*) and estimation of the natural intrageneric relationships. *Systematic and Applied Microbiology* 19, 478–492

Serizawa S. and T. Ichikawa, 1993. Epidemiology of bacterial canker of kiwifruit. 1. Infection and bacterial movement in tissue of new canes. *Annals of Phytopathological Society of Japan* 59, 452–459.

Serizawa S., T. Ichikawa, Y. Takikawa, S. Tsuyumu and M. Goto, 1989. Occurrence of bacterial canker of kiwifruit in Japan: description of symptoms, isolation of the pathogen and screening of bactericides. *Annals of Phytopathological Society of Japan* 55, 427–436.

Takikawa Y., S. Serizawa and T. Ichikawa, 1989. *Pseudomonas syringae* pv. *actinidiae* pv. nov.: the causal bacterium of canker of kiwifruit in Japan. *Annals of Phytopathological Society of Japan* 55, 437–444.

Testolin R. and A.R. Ferguson, 2009. Kiwifruit (*Actinidia* spp.) production and marketing in Italy. *New Zealand Journal of Crop and Horticultural Science* 37, 1–32.

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