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Short communication

Leaf necrosis and twig dieback of sweet persimmon (*Dyospiros kaki* L.) caused by *Pseudomonas syringae* pv. *syringae* in Italy

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Abstract

In spring 1996, extensive leaf necrosis and twig dieback were observed on young sweet persimmon (*Dyospiros kaki* L.) trees, cultivars O'Gosho, Hachija, Mercatelli and Kaki-tipo planted in the Abruzzo region (central Italy). Many trees were killed. When the dieback reached the trunk, in many cases, new vegetation was noticed above the graft point. The cultivar Jiro-C was not affected by the disease. During 1997, no symptoms were observed on any plant. The orchard was planted in a clay soil with a very low content of organic matter. Biochemical, nutritional and pathogenicity tests indicated *Pseudomonas syringae* pv. *syringae* van Hall as the causal agent of the disease. This is the first report of this bacterium as a pathogen of sweet persimmon in Europe.

In spring 1996, severe symptoms were observed in a three-year-old sweet persimmon (*Dyospiros kaki* L.) orchard located in the province of Chieti (Abruzzo region; central Italy). The orchard was planted with the cultivars O'Gosho, Hachija, Mercatelli, Kaki-tipo and Jiro C, grafted on sweet persimmon seedlings. Infected plants showed extensive leaf necrosis (Figure 1) and twig dieback that sometimes reached the trunk. When the cortical layer of the twig and the trunk was removed, blackening of the tissues below was observed (Figure 2). Longitudinal cracks along the trunk were also frequently observed. In many cases, re-sprouting of the plant was observed above the grafting point. Many plants were killed. Symptoms were present on 75 out of 125 trees (i.e. 60%). However, the cultivar Jiro C (i.e. other 60 plants) was not affected at all by the disease. During 1997, after pruning of the infected twigs and the removal of the dead plants, no symptoms were observed on the new foliage and twigs. Preliminary isolations from infected tissues consistently yielded fluorescent colonies on King's B medium (KB) (King et al., 1954). These isolates induced the hypersensitivity reaction on the tobacco leaves. Therefore, more detailed investigations were undertaken to identify the causal agent of the disease. Tissues from the margin of necrotic lesions on the leaves and beneath the bark were aseptically removed and crushed in sterile mortars containing sterile physiological saline (SPS) (0.85% of NaCl in distilled water). Serial ten-fold dilutions were also carried out. Aliquots (0,1 ml) of the suspensions were spread on petri dishes containing KB and incubated at 25 ± 1 °C for 48 h. Cultures were purified by three successive tranfers on nutrient agar (NA).

The determinative tests for the identification of fluorescent pseudomonads (i.e. LOPAT tests) (Lelliott et al., 1966) as well as the key tests for the identification of *P.s.* pv. *syringae* (Young, 1991) were carried out. On eight representative isolates (i.e. 4 from leaves and 4 from twigs for each cultivar) and on three *Pseudomonas syringae* pv. *syringae* van Hall reference strains, namely NCPPB 1087 isolated from *Prunus avium* L. in Hungary, NCPPB 1091 isolated from *Prunus persica* Batch in New Zealand and

Figure 1. Extensive necrosis on a sweet persimmon leaf caused by *Pseudomonas syringae* pv. *syringae.*

Figure 2. The removal of the cortical layer along the sweet persimmon twigs infected by *Pseudomonas syringae* pv. *syringae* shows out an extensive blackening of the external woody tissues.

NCPPB 3869 isolated from *Laurus nobilis* L in Italy. To test the pathogenicity of the isolates, two-year-old, pot-cultivated sweet persimmon cv. Mercatelli plants, grafted on *D. kaki* seedlings were utilized. Leaves were inoculated by pricking their abaxial surface with a needle and, subsequently, depositing 10 μl of bacterial suspension in SPS concentrated at 1–2 10^8 colony forming units/ml. Twigs were inoculated by depositing 10 μl of the suspensions into a T-cut made with a sterile scalpel, avoiding harming the wood. Inoculations were performed in spring under open-field conditions. Two twigs and four leaves per isolate were inoculated. Control plants were inoculated with SPS only. Symptoms were checked during the summer. Re-isolations were performed after the appearance of symptoms by utilizing the same technique. In addition, ripe lemon (*Citrus limon* Burm.) and orange (*C. sinensis* (L.) Osbeck) fruits were inoculated by pricking their surface and depositing 10 μl of the suspension. The fruits were incubated in a humid chamber for 10 days at 25 ± 1 °C. Leaves of lilac (*Syringa vulgaris* L,) were also inoculated as described for sweet persimmon (Young, 1991). KB consistently allowed the recovery of fluorescent colonies which, when streaked on NA, appeared flat, white-creamy coloured, with irregular margins. With respect to biochemical and nutritional tests, the eight representative isolates and the *P. s.* pv. *syringae* type-strains gave the response reported in Table 1 (i.e. group 1a of Lelliott et al., 1966). With regard to pathogenicity tests, on sweet persimmon and lilac leaves, dark necrosis developed after 4–6 days from the inoculation. No water-soaking was observed around the site of the inoculation. All of the inoculated twigs, during spring and early summer, showed necrosis around the site of inoculation. Subsequently, the necrosis tended to extend along the twig. In addition, all isolates induced necrosis on lemon and orange fruits. No differences in the virulence of isolates were observed. No symptoms were noticed on wounded but non inoculated control-plants. Re-isolations yielded fluorescent bacteria which gave the same LOPAT tests results as the inoculated isolates. On the basis of biochemical, nutritional and pathogenicity tests we conclude that the

Table 1. Biochemical and nutritional tests of 8 isolates from sweet persimmon trees and *Pseudomonas syringae* pv. *syringae* reference-strains

	Sweet persimmon isolates	*P.s.* pv. *syringae* reference-strains		
		1087	1091	3869
Levan production	+	+	+	+
Oxidase reaction	–	–	–	–
Potato soft rot	–	–	–	–
Arginine dihydrolase	–	–	–	–
Tobacco hypersensitivity	+	+	+	+
Gram staining	–	–	–	–
Metabolism of glucose	O	O	O	O
Presence of tyrosinase	–	–	–	–
Nitrate reduction	–	–	–	–
Arbutin hydrolisis	+	+	+	+
Esculin hydrolisis	+	+	+	+
Gelatin liquefaction	+	+	+	+
Utilization of:				
– inositol	+	+	+	+
– quinate	+	+	+	+
– sorbitol	+	+	+	+
– homoserine	–	–	–	–
– L(+)-tartrate	–	–	–	–

O: Mode of utilization: oxidative.

disease here reported on sweet persimmon was caused by *Pseudomonas syringae* pv. *syringae* van Hall. As far as we know, this is the first report of this bacterium as a pathogen of *D. kaki* in Europe. This pathogenic bacterium has previously been isolated from *D. kaki* only in New Zealand (Bradbury, 1986; Young, 1991) and in South Korea (Kim et al., 1987). However, as far as is known, the present study is the first one to ascertain by means of Koch's postulate the pathogenicity of this bacterium on sweet persimmon. It is interesting that the disease was quite severe in 1996 and that during the following year no infection was observed in the orchard. Different climatic conditions occurring in 1996 (i.e. temperature below 0 °C for some days at the end of winter) in the area during the period when the plant is susceptible to the pathogen might explain such an observation. Analysis of the soil revealed a high content of clay and silt (i.e. 47.2% and 32.0%, respectively) and a very low content of organic matter (i.e. 0.65%). These features are favorable for the aggressiveness of *P.s.* pv. *syringae* towards other plant species (Hattingh et al., 1989; Scortichini and Morone, 1997). Finally, the cultivar Jiro C that showed no symptoms of disease can be considered tolerant to the eight strains of the pathogen tested in Italy.

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