A bacterial disease of hellebore caused by *Pseudomonas viridiflava* in New Zealand

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Abstract *Pseudomonas viridiflava* is reported causing a disease of hellebore (*Helleborus* × *hybridus*) in New Zealand. The bacterium was isolated from diseased plants exhibiting distinct symptoms consisting of black leaf spots, necrotic petal and stem lesions. *P. viridiflava* was identified on the basis of biochemical and molecular tests and confirmed as the causal agent by fulfilling Koch's postulates.

Keywords *Pseudomonas viridiflava* · Hellebore

Hellebores are grown in New Zealand as an ornamental plant and as a commercial crop to supply cut flowers to local and export markets. The most popular hellebores are *Helleborus orientalis* and its hybrids (*H.* × *hybridus*). Hellebores are mainly propagated in shade houses and watered by drip irrigation.

In August 2010, diseased hellebore plants (*H.* × *hybridus* Double White) were observed in a nursery in Tauranga, New Zealand. Symptoms developed after several days of moderate rainfall and more than 90% of the plants were affected, resulting in unsuitability for sale. Symptoms consisted of circular, black leaf spots which were 1.5–2 mm in diameter, black stem lesions and dry, grey to brown lesions with distinct margins on the flower petals (Fig. 1). The symptoms were different from those caused by other leaf-spotting pathogens of hellebore such as the fungus *Coniothyrium* sp. and the bacterium *Xanthomonas* sp. (Gleason et al. 2009).

Saline suspensions of macerated diseased leaf, petal and stem tissues were streaked onto King's B medium (King et al. 1954) and pale yellow, fluorescent bacterial colonies were consistently growing following 48 h incubation at 25°C. All isolates were Gram negative and were levan, oxidase and arginine dehydrolase negative. They all produced a hypersensitive reaction on tobacco leaves after 24–36 h and showed pectolytic activity on potato tuber slices. The bacterium was identified as *P. viridiflava* on the basis of these biochemical tests and is in group II of the LOPAT determinative scheme of Lelliott et al. (1966). The house-keeping gene *gyrB* (Sarkar and Guttman 2004) was amplified from two isolates (ICMP 18563, 18564), producing a 600 bp amplicon. BLASTn analysis of the *gyrB* amplicons showed that the region was 99.0–99.8% homologous to *P. viridiflava* sequences in GenBank, with highest homology to accessions AY606756 and AY606738. *Pseudomonas viridiflava* was isolated consistently from leaf, petal and stem tissue adjacent to leaf spots, petal and stem lesions. Pathogenicity tests with two selected isolates of *P. viridiflava* (ICMP 18563, 18564) were performed on hellebore (*H.* × *hybridus*) cut flowers, young detached leaves and petals. Each isolate was inoculated onto three detached petals and leaves by placing 20 µl of bacterial suspension (10^7 cfu/mL) on the surface and pricking through the drops using sterile needles. After inoculation, the detached petals and leaves were kept in Petri dishes lined with moist filter paper, incubated at 25°C and checked daily for symptoms. Three healthy cut flowers with stems submerged in flasks of water were inoculated per isolate in a similar manner, enclosed in clear polyethylene bags and incubated at 25°C. Controls were inoculated in the same way using sterile physiological saline (0.85% NaCl). Necrosis developed on the cut flowers, leaf and petal tissues 3 days after inoculation. The symptoms induced on cut flowers,

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Fig. 1 Hellebore flowers with symptoms caused by *Pseudomonas viridiflava*: **a** necrotic petal lesions; **b** leaf black spot and stem lesions

detached petals and leaves were similar to those caused by natural infections. Bacterial colonies were re-isolated from petal and leaf lesions and identified as *P. viridiflava*, fulfilling Koch's postulates. No disease symptoms developed on the control plants. The pathogenicity tests were performed twice.

The climatic conditions in the Tauranga region at the time disease symptoms were observed were of mild temperatures (12.9–15.9°C), high humidity (>90%) and moderate rainfall (>24 mm per day). *P. viridiflava* has been found to survive epiphytically on plants for various periods and these conditions are ideal for the bacterium to cause significant plant disease (Young et al. 1988).

P. viridiflava is a cosmopolitan phytopathogenic bacterium found on a wide variety of hosts that span at least 31 different species in 14 different families (Bradbury 1987).

Predominantly a leaf spotting pathogen, the bacterium has also been reported to cause leaf rot, leaf blotch, stem necrosis and blossom blight on a number of hosts (Wilkie et al. 1973). In New Zealand, *Pseudomonas viridiflava* was first found on peas in 1971 (Watson and Dye 1971).

This is the first report of *P. viridiflava* causing a disease of hellebore in New Zealand. Reference cultures have been deposited in the International Collection of Microorganisms from Plants, Landcare Research, Auckland, New Zealand (ICMP 18563, 18564). Worldwide, there are no other reports of *P. viridiflava* on hellebores, with the exception of one website record. The database of plant diseases in Japan (NIAS Genebank 2010) records *P. viridiflava* as associated with the genus *Helleborus* in Japan. However, extensive literature searches have not revealed any further details of this association.

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