Influence of leaf age on infection of *Actinidia* species by *Pseudomonas syringae* pv. *actinidiae*

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Abstract The influence of leaf age on infection of *Actinidia* species by *Pseudomonas syringae* pv. *actinidiae* (Psa) was investigated using two approaches: (a) inoculation of potted 'Hayward' and 'Hort16A' kiwifruit plants and (b) inoculation of whole leaves in a detached leaf assay. Whole plants and detached leaves were spray inoculated with Psa biovar 3 (haplotype NZ-V; Psa-V) and maintained in a saturated environment. Flecking was evident on some of the leaves 8 days after inoculation. Many of the flecks later became necrotic spots with halos similar to the Psa leaf infections that have been observed in the field. With both methods, a higher percentage of leaves that were 1–3 weeks of age at inoculation had flecking and spotting than did leaves of other ages. Leaves that were 7 weeks or older did not show any symptoms of infection by Psa. Overall, leaves of 'Hort16A' showed slightly more flecking and spotting than 'Hayward'.

Keywords *Pseudomonas syringae* pv. *actinidiae*, Psa, bacterial canker, leaf age.

INTRODUCTION

The bacterial plant pathogen *Pseudomonas syringae* pv. *actinidiae* (Psa) is the causal agent of kiwifruit canker, a damaging disease of *Actinidia* species in a number of countries around the world. It was first discovered in New Zealand in 2010 (Everett et al. 2011) and continues to be a serious threat to the New Zealand kiwifruit industry.

The disease is characterised by leaf spots, budrot, cane dieback, cankers and the production of orange-red or milky-white exudates. The leaf spots may present as brown necrotic spots, with or without halos, or as dark brown angular ('blocky') spots.

In bacterial pathosystems, leaf age at inoculation may be positively or negatively correlated with infection. A study conducted on *Chrysanthemum morifolium* found that leaf age affected the number

and size of lesions caused by *Pseudomonas cichorii*, with older leaves being less susceptible to infection than younger, immature leaves (Jones et al. 1985). Conversely, Sanchez et al. (2003) found that in coffee (*Coffea arabica*), infection by *P. cichorii* was more frequent on older leaves. Young fruit and leaf tissues of *Citrus* are more susceptible to *Xanthomonas axonopodis* pv. *citri* (citrus canker) than mature tissues (Verniere et al. 2003).

Tissue age, particularly leaf age, could affect the susceptibility of kiwifruit to infection by Psa. In the field it has been observed that the younger leaves of kiwifruit vines are more susceptible to Psa. However, the influence of kiwifruit leaf age on Psa infection and symptom development has not been systematically studied.

The objective of this work was to determine the relative susceptibility of leaves of different ages to infection. These data can be used to better understand the relationship between crop phenology and subsequent disease development, and may provide important data for incorporation into disease risk models. This may aid in decisions on the management of vines and the optimisation of crop protection approaches.

MATERIALS AND METHODS

Inoculum preparation

Pseudomonas syringae pv. *actinidiae* isolate Psa3.2.3 (cc691) was used for all experiments in this study. This isolate belongs to Psa biovar 3 (haplotype NZ-V; Psa-V), and is known to be pathogenic in leaf disc assays and potted plant studies (Tyson et al. 2014).

For each experiment, the inoculum was grown on King's B medium (King et al. 1954) for 48 h, then suspended in bacteriological saline (BS, 0.85% NaCl in sterile distilled water) and the concentration adjusted to an optical density of ca 0.9 (600 nm) with an Agilent 8453 spectrophotometer. After three days, plate counts were performed to confirm the inoculum concentration (cfu/ml).

Potted plants

One-year-old potted *Actinidia deliciosa* 'Hayward' and *Actinidia chinensis* 'Hort16A', propagated from cuttings, were sourced from Kerikeri, an area that was at the time free of Psa. The leaves on each vine were numbered from the base to the growing tip. For 5 weeks before inoculation, leaf width and length were measured weekly, and new leaves recorded. At time of inoculation, leaves that were nearing full expansion at the first assessment were assumed to be 6 weeks old, and mature leaves assumed to be 7+ weeks of age. Leaves that emerged in the week prior to inoculation were labelled as '0 weeks'.

Both surfaces of leaves of six 'Hayward' and ten 'Hort16A' vines were spray-inoculated with Psa at a concentration of 6.9×10^8 cfu/ml in April 2012. Two further vines of each cultivar were inoculated with BS and served as controls.

Post-inoculation, the plants were maintained in a saturated environment in a plastic

greenhouse within the confines of a containment laboratory at Plant & Food Research, Mt Albert Research Centre, Auckland. The temperature within the plastic greenhouse was recorded over the timeframe of the experiment. The average daily temperature fluctuated between 19 and 25°C, with an average of 21.3°C.

Symptoms were assessed 8 days following inoculation and weekly thereafter. Leaves with spots and flecks were recorded and tracked over a 4-week period. At each assessment, changes in symptom expression on the leaves were recorded.

Bacterial isolations were made from representative leaf lesions (approximately 20) onto an agar medium semi-selective for *Pseudomonas* species, a slight modification of KBC (Mohan & Schaad 1987), using sucrose-nutrient medium as the base instead of King's B medium. Plates were incubated at 20°C for 48 h. Colonies were confirmed as Psa by quantitative Polymerase Chain Reaction (qPCR), using the primers PsaF3/R4 of Rees-George et al. (2010) and the conditions described in Tyson et al. (2012).

Detached leaf assay

Tissue-cultured plantlets of 'Hayward' and 'Hort16A' were potted into 12-cm diameter polythene bags and grown for 12 months in a glasshouse in a Psa-free area. New leaves were recorded for 7 weeks before inoculation. No '0 week' leaves were included in the detached leaf assay.

Leaves were detached from the plants and floated in sterile distilled water until needed, then patted dry and spray-inoculated with Psa at a concentration of ca 1×10^9 cfu/ml in March 2014. Leaves were not injured before inoculation. Leaves of each age class were inoculated with BS and served as controls. The base of the petiole was re-cut and the leaf immediately placed upright in a glass jar of sterile water and moved to a small plastic greenhouse. A humidifier, set on a timer to run for 20 min twice a day, allowed the leaves to remain damp but not wet for the duration of the experiment.

Leaf-spot symptoms were assessed 8 days following inoculation and weekly thereafter. Bacterial isolations and identifications were made from representative leaf lesions, as outlined previously.

RESULTS

In both the potted plants and the detached leaf assays, small, necrotic, angular spots were found 8 days after inoculation on some of the *Psa*-inoculated leaves. Further symptoms developed over the 4-week period, ranging from small brown flecks, with or without halos, to larger brown spots. All isolations from representative spots were confirmed as *Psa* by qPCR. Control leaves treated with only BS remained asymptomatic until at least 22 days after inoculation.

In both cultivars, a higher percentage of leaves that were 1–3 weeks of age at inoculation had flecking and spotting than leaves of other ages (Figure 1). Thereafter, symptom development declined with increasing leaf age.

In the potted plants, leaf width and length were measured prior to inoculation. The leaf ages with the greatest percentage of spotted leaves corresponded to the period when leaves were rapidly expanding (Table 1).

DISCUSSION

The objective of this work was to determine the relative susceptibility of kiwifruit leaves of different ages to infection by *Pseudomonas syringae* pv. *actinidiae*. The cultivars 'Hayward' and 'Hort16A' were investigated using two approaches: (a) inoculation of potted plants and (b) inoculation

Figure 1 Percentage of kiwifruit leaves of each age at inoculation showing leaf spot symptoms of *Pseudomonas syringae* pv. *actinidiae* (*Psa*) infection, assessed 1 to 5 weeks after inoculation.

of whole leaves in a detached leaf assay. With both methods and both cultivars, a higher percentage of leaves that were 1–3 weeks of age at inoculation had flecking and spotting than did leaves of other ages. Although 'Hort16A' is more susceptible to *Psa* than 'Hayward' in field situations, there was very little difference in the susceptibility to infection of leaves of comparable age.

In New Zealand kiwifruit orchards it has been observed that the younger leaves of kiwifruit

Table 1 For potted plants, the percentage of kiwifruit leaves of each age at inoculation showing symptoms of *Pseudomonas syringae* pv. *actinidiae* (*Psa*) infection (flecks, spots) and average leaf area for each age class at the time of inoculation.

Cultivar	Leaf age at inoculation (weeks)	% leaves with <i>Psa</i> symptoms	No. leaves assessed	Average leaf size (cm ²)
'Hort16A'	0	33.3	90	0.09
'Hort16A'	1	94.4	18	6.38
'Hort16A'	2	94.1	17	20.4
'Hort16A'	3	100.0	8	33.7
'Hort16A'	4	50.0	54	113.6
'Hort16A'	6	14.5	62	89.1
'Hort16A'	7+	6.3	32	90.4
'Hayward'	0	0.0	17	0.07
'Hayward'	1	75.0	4	8.96
'Hayward'	2	100.0	12	21.0
'Hayward'	3	100.0	5	84.0
'Hayward'	4	100.0	1	204.9
'Hayward'	6	42.9	14	124.7
'Hayward'	7+	0.0	16	49.0

vines are more susceptible to Psa. Although many authors have stated that the disease cycle of Psa includes the infection of young leaves in the spring, only Serizawa & Ichikawa (1993) studied the effect of leaf maturity in detail. They found that the susceptibility of new leaves of 'Hayward' kiwifruit to Psa increased when the leaf blades reached ca 2 cm in length, and then decreased as the leaves matured. The present work focused on leaf age rather than size and phenological stage of the canes, and confirmed that result. It also showed that, despite differences in field susceptibility of whole vines, the pattern of susceptibility of leaves of different ages of 'Hort16A' was similar to that of 'Hayward'.

Leaf production and emergence was variable during this trial, resulting in different numbers of leaves of each age group being inoculated. Leaves were particularly sparse on the 'Hayward' vines. In addition to this variability, a substantial number of the 7-week-old leaves, in both cultivars, abscised several days after inoculation. These leaves were not included in the analysis. Abscission occurred at a similar rate in inoculated and non-inoculated plants and it is likely that it was the result of the change of growing conditions between the greenhouse in Kerikeri and the laboratory in Mt Albert, Auckland.

In this study, leaf age has been shown to have a significant effect on infection and symptom development by Psa. Leaves from 1–3 weeks of age were particularly susceptible. In both cultivars, this corresponds to the period when leaves are rapidly expanding. This information on the relationship between leaf age and susceptibility to Psa not only aids management of Psa 'on orchard' but also is valuable in the development of disease risk models.

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