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Budrot in ‘green’ kiwifruit (*Actinidia* sp.) varieties – Spring 2014

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CONTENTS

EXECUTIVE SUMMARY

Budrot in 'green' kiwifruit (*Actinidia* sp.) varieties - Spring 2014

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Bacterial blossom rot of kiwifruit in New Zealand was first recorded in 1973 and the plant-pathogenic bacterium *Pseudomonas viridiflava* was confirmed as the causal agent of the disease. 'Bacterial blight' symptoms were described as 'rot of floral buds and flowers, and spots on leaves'. Later, the bacterium causing blossom blight was re-examined and re-classified as *Pseudomonas* sp. LOPAT II (kiwifruit).

Since the incursion of *Pseudomonas syringae* pv. *actinidiae* (Psa) into New Zealand in 2010, kiwifruit budrot caused by Psa has also been observed.

Recently there has been some suggestion of varying symptoms associated with budrot in green varieties of kiwifruit (*Actinidia* spp.). This project aimed to identify the primary cause of budrot in varieties of green kiwifruit over the spring of 2014 and involved testing for Psa, *Pseudomonas* sp. (blossom blight) and fungi on buds of green varieties during spring 2014.

Almost all the buds, in all orchards and at both sampling dates, tested positive for Psa.
On average, 30% of the buds also tested positive for *Pseudomonas* sp. (blossom blight).
Phomopsis sp. was the most common fungal isolate, but is not considered to be the cause of the budrot symptoms.

It is likely that the majority of budrot symptoms seen in this study were the result of infection by Psa.

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1 INTRODUCTION

Bacterial blossom rot of kiwifruit in New Zealand was first recorded by Wilkie et al. (1973) and the plant-pathogenic bacterium *Pseudomonas viridiflava* was confirmed as the causal agent of the disease. Later, Young et al. (1988) described the 'bacterial blight' symptoms caused by *P. viridiflava* on *Actinidia deliciosa* as 'rot of floral buds and flowers, and spots on leaves'.

Pennycook & Triggs (1992) extensively surveyed the incidence of bacterial blossom blight (*Pseudomonas viridiflava*) over a five-year period and established the association of the disease with rainfall during the blossom period. During spring 1991, Everett & Henshall (1994) also studied the epidemiology and population ecology of kiwifruit blossom blight caused by *Pseudomonas viridiflava*.

Later, Young et al. (1997) examined the New Zealand kiwifruit blossom blight bacterium using genomic and phenotypic characterisation and concluded that it differed from previously characterised pseudomonads. Young et al. (1997) referred to the New Zealand kiwifruit blossom blight bacterium as *Pseudomonas* sp. LOPAT II (kiwifruit). In a later publication, Hu et al. (1999) continued to call the blossom blight pathogen *Pseudomonas* sp.

Since the incursion of *Pseudomonas syringae* pv. *actinidiae* (Psa) into New Zealand in 2010 (Everett et al. 2011), budrot caused by Psa has also been observed.

Recently there has been some suggestion of varying symptoms associated with budrot in green varieties of kiwifruit (*Actinidia* spp.; Figure 1). This project aimed to identify the primary cause of budrot in varieties of green kiwifruit over the spring of 2014.

Figure 1. Budrot symptoms on *Actinidia deliciosa* 'Hayward' in the Te Puke district, 2012.

2 METHODS

2.1 Samples

Kiwifruit flower buds with 'browning' were collected from five orchards at two sampling times (approximately ten days pre-blossom and immediately prior to flowering). Ten symptomatic buds were collected from each site (collections organised by Zespri; Figure 2). The details of the samples are shown in Table 1.

Whole buds were surface sterilised in 0.1% NaOCl for 20 min, followed by two consecutive rinses in sterile reverse osmosis (RO) water, then air-dried in a laminar flow cabinet. Each bud was then aseptically halved, and one half used for bacterial isolations, and the other half used for fungal isolations.

Table 1. Collection details of kiwifruit buds for rot sampling, 2014. G14 = *Actinidia chinensis x A. deliciosa* 'Zesh004' (commonly known as Green14).

Site	Area	KPIN	Variety	Bud collection 1	Bud collection 2
A	Allport Rd, Paengaroa	8281	G14	31 Oct. 2014	4 Nov. 2014
B	Orchard Rd, Edgecumbe	-	*A. deliciosa* 'Hayward'	4 Nov. 2014	21 Nov. 2014
C	Kelly Rd, Paengaroa	5903	'Hayward'	4 Nov. 2014	21 Nov. 2014
D	River View Orchard, Katikati	-	G14	10 Nov. 2014 ("2nd batch")	24 Nov. 2014
E	Pa Orchard, Pyes Pa, Tauranga	1362	'Hayward'	24 Nov. 2014	9 Dec. 2014

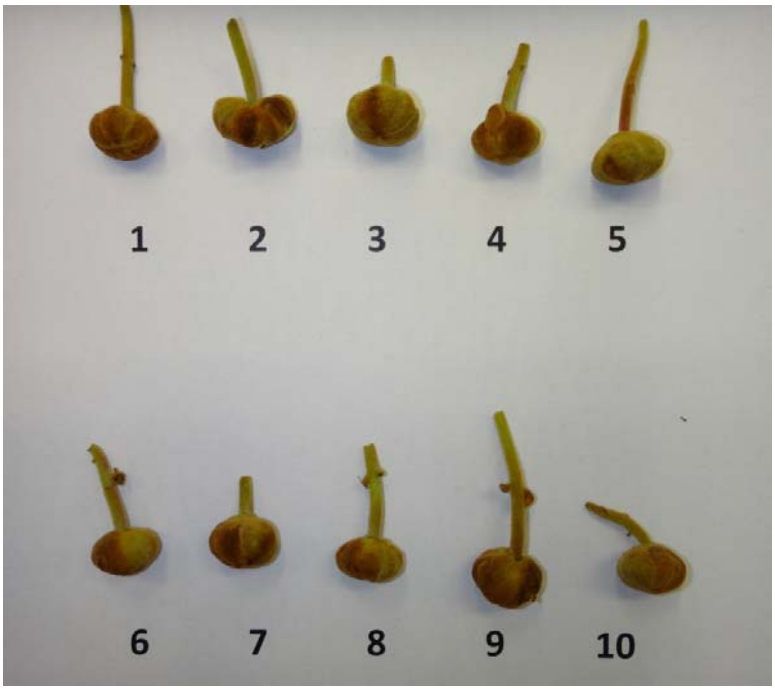

Figure 2. Kiwifruit flower buds with 'browning' from site B, collection 1 (*Actinidia deliciosa* 'Hayward'), 2014.

2.2 Bacterial isolation and identification

Surface-sterilised 'half-buds' were macerated in 200 µL bacteriological saline (0.85% NaCl in sterile RO water) and left for 5 min, after which 100 µL of the resulting suspension was spread across KBC, an agar medium semi-selective for *Pseudomonas syringae* (Mohan & Schaad 1987). The isolation plates were incubated at 20°C for 72 h and then assessed for bacterial growth. DNA extraction from the mixed-colony plates, and qPCR conditions and analysis, were done as described by Tyson et al. (2012).

The Psa-specific primers PsaF3 and PsaR4, developed by Rees-George et al. (2010), were used to detect Psa, and the primers BB1 and BB2, also developed by Rees-George (unpubl.), were used to detect blossom blight (*Pseudomonas* sp.). The blossom blight primers have not yet been rigorously tested and as a consequence this should not be regarded as a definitive test. However, it will give an idea of the frequency of *Pseudomonas* sp. (blossom blight) in/on the symptomatic buds.

2.3 Fungal isolation and identification

Surface-sterilised 'half-buds' were aseptically quartered and the pieces placed on Potato Dextrose Agar (PDA) amended with the antibiotics rifampicin and ampicillin to inhibit bacterial growth. Isolation plates were incubated at ~20°C under near-UV light banks (12 h light – 12 h dark) and the resultant fungi were identified using morphological characteristics.

3 RESULTS

3.1 Bacterial isolation and identification

Almost all the buds, from all orchards and at both sampling dates tested positive for Psa (Table 2). The exception was the first collection at site D, where only 60% of the buds were Psa-positive (six of the 10 buds). On average, 30% of the buds also tested positive for *Pseudomonas* sp. (blossom blight).

Table 2. Bacterial isolations. Percentage of buds from kiwifruit at each site and sampling date (in 2014) positive for *Pseudomonas syringae* pv. *actinidiae* (Psa) and *Pseudomonas* sp. G14 = *Actinidia chinensis x A. deliciosa* 'Zesh004' (commonly known as Green14).

		Bud collection 1		Bud collection 2	
Site	Variety	Psa	*Pseudomonas* sp.	Psa	*Pseudomonas* sp.
A	G14	100%	20%	100%	0%
B	*A. deliciosa* 'Hayward'	100%	0%	100%	40%
C	'Hayward'	100%	50%	100%	40%
D	G14	60%	30%	100%	40%
E	'Hayward'	100%	50%	100%	50%

3.2 Fungal isolation and identification

The most common fungus isolated from the kiwifruit buds was *Phomopsis* sp. (Table 3). *Phomopsis* is commonly isolated from all parts of kiwifruit vines, and usually causes no symptoms until 'ripe rots' develop late in fruit storage. The most common fungi isolated from the buds are shown in Table 4.

Although many other fungal species were isolated from the buds, most of these are either known saprophytes and are unlikely to cause disease, or were potential pathogens that were isolated inconsistently within and between orchards. If the budrot symptoms were caused by a fungus, it would be expected to be isolated consistently from symptomatic tissue. Other fungi isolated from the buds included:

- *Acremonium* sp.
- *Botryosphaeria* sp.
- *Botrytis* sp.
- *Cladosporium* sp.
- *Epicoccum* sp.
- *Fusarium* sp.
- *Nigrospora* sp.
- *Penicillium* sp.
- *Pestalotiopsis* sp.
- *Phoma* spp.
- *Phomopsis* sp.
- *Rhizopus/Mucor* sp.
- *Stemphylium* sp.
- *Trichoderma* sp.

Table 3. Fungal isolations. Percentage of kiwifruit buds at each site and sampling date (in 2014) yielding *Phomopsis* sp. G14 = *Actinidia chinensis x A. deliciosa* 'Zesh004' (commonly known as Green14).

Site	Variety	Bud collection 1 *Phomopsis* sp.	Bud collection 2 *Phomopsis* sp.
A	G14	80%	40%
B	*A. deliciosa* 'Hayward'	70%	20%
C	'Hayward'	90%	80%
D	G14	100%	90%
E	'Hayward'	70%	60%

Table 4. Fungal isolations. Common fungal species isolated from kiwifruit buds at each site and sampling date (in 2014).

Site	COLLECTION 1 Most common species	COLLECTION 1 Other common species	COLLECTION 2 Most common species	COLLECTION 2 Other common species
A	*Phomopsis* sp.	*Fusarium* sp. *Phoma* sp.	*Phomopsis* sp.	*Alternaria* sp.
B	*Phomopsis* sp.	*Alternaria* sp.	*Alternaria* sp.	*Phomopsis* sp. *Phoma* sp.
C	*Phomopsis* sp.	*Alternaria* sp. *Phoma* sp.	*Phomopsis* sp.	*Fusarium* sp. *Phoma* sp.
D	*Phomopsis* sp.	*Alternaria* sp. *Phoma* sp.	*Phomopsis* sp.	*Alternaria* sp. *Botryosphaeria* *Phoma* sp.
E	*Phomopsis* sp.	*Alternaria* sp. *Botryosphaeria* sp. *Phoma* sp.	*Phomopsis* sp.	-

4 DISCUSSION

This study aimed to achieve a greater understanding of the primary cause of kiwifruit budrot in 'green' varieties in different areas. This involved testing for Psa, *Pseudomonas* sp. (blossom blight) and fungi on green variety buds during spring 2014.

There was very little difference in bacterial and fungal populations between sites and sampling times. Almost all the buds, in all orchards and at both sampling dates, tested positive for Psa. On average, 30% of the buds also tested positive for *Pseudomonas* sp. (blossom blight). *Phomopsis* sp. was the most common fungal isolate, but is not considered to be the cause of the budrot symptoms.

It is concluded that the majority of budrot symptoms seen in this study were the result of infection by Psa.

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