Pathogenicity of *Phoma betae* isolates from red beet (*Beta vulgaris*) at seed farms in Canterbury, New Zealand

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Abstract *Phoma betae* is an economically important pathogen of red beet causing pre-emergence seedling damping, leaf spot and root rot. However, the pathogenicity of *P. betae* is unknown in New Zealand despite the economic importance of this pathogen. Twenty-five isolates were collected from a survey of red beet seed farms in Canterbury, New Zealand during 2016/2017 and three of these PB101 (from seeds), PB103 (from roots) and PB106 (from leaves) were used for pathogenicity testing of two red-beet cultivars. Isolate PB106 was further used to investigate its effects on spinach and fodder beet as well as red beet under greenhouse conditions. All three *P. betae* isolates were pathogenic on both red-beet cultivars tested, causing leaf-spot symptoms. Isolates PB101 and PB106 produced significantly larger leaf-spot lesions ($P<0.001$) compared with PB103. *Phoma betae* isolate PB106 was pathogenic to both red-beet cultivars, spinach and fodder beet but fodder beet was less susceptible than the other species tested. Regardless of cultivar, *P. betae* is an important pathogen of beets and is capable of causing leaf spots.

Keywords *Phoma betae*, pathogenicity, *Beta vulgaris*, seed farms, red beet, spinach, fodder beet, New Zealand.

INTRODUCTION

The fungus *Phoma betae* is an important plant pathogen for red beet (*Beta vulgaris* subsp. *vulgaris*), causing damage at multiple growth stages. The pathogen has been recently reclassified as *Neocamarosporium betae* within order the *Neocamarosporiaceae* (Ariyawansa et al. 2015; Vaghefi et al. 2019), but the name *Phoma betae* is still widely recognised by researchers and industry. Therefore, the name *Phoma betae* will be used here. It is known to cause substantial damping-off disease in both pre-emergent and post-emergent seedlings (Harveson 2006). The pathogen can also cause root rot, leaf spot, and rotting of beets during storage (Keskin 1964; Herr 1971; Harveson 2006; Gilardi et al. 2011). *Phoma betae* is a widely distributed pathogen and commonly detected on red-beet seed lots produced throughout the world (Byford & Gambogi 1985).

Phoma betae is a haploid ascomycete (De Gruyter et al. 2009; Koenick et al. 2018) capable of asexual and sexual reproduction. Asexual reproduction contributes to the rapid spread of the disease. In high humidity, especially during cool rainy seasons (winter and spring), pycnidia of the fungus ooze conidia in large masses. These are rapidly spread by splashing rain or overhead sprinklers. The sexual structure, pseudothecia, produce ascospores that are aerially dispersed over long distances. *Phoma betae* is also seed borne; therefore, conidia and ascospores contact

with developing floral parts can result in seed infection (Nyvall 1989; Shoemaker & Bissett 1998; Pethybridge et al. 2018).

Brown-to-black lesions appear on young seedlings, which leads to the seedings to damp off (Gilardi et al. 2011; Pethybridge et al. 2018). Leaf spots appear as brown, round to oval shaped lesions containing dark concentric rings. As the disease progresses, leaf spots can coalesce rapidly to cause die off and defoliation of the leaves. Infection spreads from dead leaves attached to the crown into the crown where it causes crown rot, then spreads into the taproot causing root rot with dark, sunken spots that become soft and watery (Byford & Gambogi 1985; Harveson 2006; Harveson et al. 2009).

The pathogenicity of *P. betae* has been reported by various authors (Byford & Gambogi 1985; Monte & Garcia-Acha 1988; Avasthi et al. 2013; Pethybridge et al. 2018) with *P. betae* the only species affecting beets in the USA. *Phoma betae* has also been reported to cause symptoms of leaf spot and root rots in eastern Europe, Canada and the UK (Hanson et al. 2012; Vaghefi et al. 2019).

Red-beet seed production is a small industry in New Zealand; however, it is a very high-value crop. New Zealand is a major source of red-beet seed for overseas markets and currently supplies about 50% of the world's red-beet seeds yet the pathogenicity of *P. betae* isolates found on red-beet cultivars commonly grown for seed production in New Zealand is not known. Also, the pathogenicity of *P. betae* isolates derived from red beet has not been investigated on crops from other members of the Chenopodiaceae family that are grown in close proximity to red beets or commonly used as part of a crop-rotation cycle. The aims of this research were to determine the pathogenicity of *P. betae* isolates obtained from red-beet seed-growing regions in New Zealand and to assess the effects on other host plants from the Chenopodiaceae family, spinach (*Spinacia oleracea*), and fodder beet (*Beta vulgaris*), under greenhouse conditions.

METHODS

Fungal isolates

Twenty-five *P. betae* isolates were recovered from plant material collected as part of a survey of red-beet seed farms in Canterbury, New Zealand during 2016/2017 (Chand et al. 2018). Three of these isolates were randomly selected, PB101 (from seeds), PB103 (from roots) and PB106 (from leaves), and were used in this study. The three *P. betae* isolates were identified morphologically and with DNA analysis of the internal transcribed spacer (ITS) region (Byford & Gambogi 1985; Shoemaker & Bissett 1998). Each isolate was grown on one-fifth strength potato dextrose agar (1/5 PDA: 7.8 grams PDA (Difco®) and 7.5 g agar per litre of reverse osmosis water) for 10 days at 20°C (12 hours light and 12 hours dark). Conidial suspensions were prepared by pouring 10 mL of sterile distilled water (SDW) containing 1 drop of Tween 80 (BDH Chemicals Ltd, Poole England) onto each 10-day-old sporulating culture and rubbing the colony surfaces with a sterile glass rod to dislodge the conidia. The conidial suspensions were then filtered through sterile Miracloth™ (CALBIOCHEM®, Germany) into 15-mL tubes. Conidial concentration was adjusted to 1 x 10^6 conidia/mL and an aliquot of 70 µL was used for inoculation. Conidial viability was assessed by plating of the suspensions on 1/5 PDA.

Pathogenicity of *Phoma betae* on two open-pollinated red-beet cultivars

The pathogenicity of the three *P. betae* isolates was assessed on two open-pollinated red beet cultivars, 'OH34' (seed supplied by PGG Wrightsons from their Kimihia Research Station, Lincoln, New Zealand) and Smith's 'OP2016' (seed supplied from Smith Seeds, Ashburton, New Zealand). These cultivars are known to be susceptible to the major diseases affecting red-beet seed production and were also used for seed multiplication in the 2016/2017 season. Four seeds of each cultivar were sown in 500-mL pots containing autoclaved soil and placed in a greenhouse at 17–22°C (under natural day length) for 18 days. After this time, the seedlings

were thinned, leaving only one seedling per pot. After 28 days, two leaves per plant were wounded by pricking with a hypodermic needle prior to inoculation and a 6-mm sterile modelling clay ring was placed around the wounded area (Fig. 1A). The leaves were then inoculated by placing a 70-µL droplet of conidial suspension onto the wounded area within the ring. Control plants were inoculated with sterile water instead of a conidial suspension. After the inoculum had dried (approximately 2 hours), each seedling was covered with a clear plastic bag, which was sprayed with sterile water on the inside, to maintain 100% relative humidity for 72 h. Eight replicate seedlings were set up for each treatment and arranged in a completely randomised block design on a bench in a greenhouse.

Pathogenicity of *Phoma betae* isolate PB106 on other host plants

To determine the pathogenicity of *P. betae* on other hosts, isolate PB106 was used to inoculate both red-beet cultivars 'OH34' and 'OP2016', as well as one cultivar of spinach 'SPS9090' (seed supplied from South Pacific Seeds, Ashburton, New Zealand) and fodder beet 'Bangor' (seed supplied from Wholesale Seeds, Ashburton, New Zealand) under greenhouse conditions. Seedlings of each cultivar were grown in the greenhouse and 28-day-old seedlings were inoculated with a conidial suspension of *P. betae* following the same methods as described previously.

Assessments and data analysis

Disease symptoms were observed daily and recorded 6, 9 and 18 days post-inoculation (dpi). Disease assessment was conducted on the inoculated leaves only and the diameter (mm) of the leaf lesion was measured in two perpendicular directions using a digital calliper (Mitutoyo UK Ltd.). Data for the average leaf-lesion diameter were statistically analysed using ANOVA by GenStat 16th Edition (VSN International Ltd). All means were separated using Tukey's Protected Least Significant Difference (LSD) test at $P<0.05$.

To complete Koch's postulates, small pieces (5 mm) of symptomatic tissue from the inoculated leaves were taken from three randomly selected plants for each of the isolate and control treatments. The re-isolation process was undertaken by surface-sterilising the leaves then air drying them inside a laminar-flow hood. Symptomatic plant tissue was dissected out and placed onto 1/5 PDA amended with streptomycin (5 mg/mL) and penicillin (5 mg/mL). Identification of the resulting colonies was based on colony morphology.

RESULTS

Pathogenicity on two open-pollinated red-beet cultivars

Conidial viability assessed by dilution plating of conidial suspensions was 99% for all *P. betae* isolates used in these experiments. Leaf lesions, characteristic of *P. betae*, developed on inoculated leaves by 6 days post-inoculation (dpi); however,

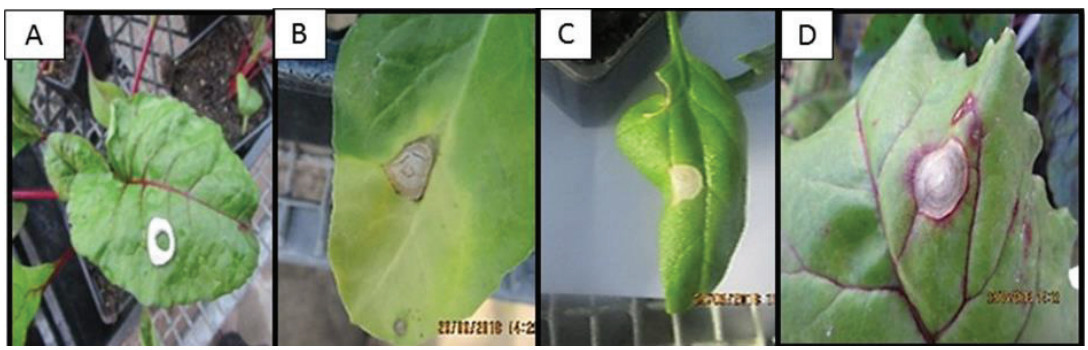

Figure 1 (A) modelling-clay ring on control plant with no lesion; (B) leaf lesions on fodder beet; (C) spinach; and (D) red-beet cultivar 'OH34' at 18 days after inoculation with *Phoma betae* isolate PB106.

lesions were very small. By 9 dpi, the lesions were more obvious, and pale-brown to greyish in colour. By 18 dpi, the disease had progressed and leaves had become chlorotic, with some dying and dropping off.

Leaf-lesion diameters assessed at 18 dpi are summarised in Table 1 and presented as the mean of two perpendicular diameters. All plants inoculated with one of the three *P. betae* isolates developed leaf lesions and this result differed significantly (P=0.001) from the control plants where no lesions formed. There was a significant interaction (P=0.001) between cultivar and isolate, with the relative pathogenicity of the isolates differing between cultivars. Cultivar alone had no significant effect (P=0.171) on leaf-lesion diameter, with the average mean diameter ranging from 6.3 to 6.8 mm.

Isolates PB101 and PB106 produced significantly larger lesions on the plants of cultivar 'OH34'compared with PB103. There was no significant difference (P>0.05) between the lesion diameter of the plants inoculated with isolate PB101 and PB106. Isolate PB106 produced significantly larger lesions on the plants of cultivar Smith's 'OP2016' compared with either PB101 or PB103. There was no significant difference (P>0.05) between the lesion diameter of plants inoculated with either isolate PB101 or PB103.

Pathogenicity of *Phoma betae* isolate PB106 on other host plants.

Leaf lesion diameters assessed at 18 dpi are summarised in Table 2 and presented as the mean of two perpendicular diameters. All plants inoculated with *P. betae* isolate PB106 produced leaf lesions (Figs. 1B, C & D) compared with all control plants (no lesion; P<0.001). There was a significant difference (P=0.029) between the diameter of the leaf lesion and the plant host. Significantly larger lesions occurred on plants of the red-beet cultivar Smith's 'OP2016' compared with fodder-beet plants. There was no significant difference (P>0.05) in the relative pathogenicity of *P. betae* to fodder beet, spinach and red-beet cultivar 'OH34', nor between spinach and the two open pollinated red-beet cultivars, 'OH34' and Smith's 'OP2016.

DISCUSSION

This is the first study on the pathogenicity of *P. betae* on red beet in the Canterbury region of the South Island, New Zealand. Isolates used in the study were recovered from seeds, leaves or the roots of red-beet plants in a commercial crop area. The results of the pathogenicity testing in this study indicate that *P. betae* is one of the major causal agents of *Phoma* leaf spot in the red-beet seed farms surveyed. Previous studies by various researchers on *P. betae* have highlighted the

Table 1 Mean diameters (mm) of leaf lesions on two open-pollinated red-beet cultivars 'OH34' and Smith's 'OP2016' at 18 days post inoculation (dpi) with three *Phoma betae* isolates

	Leaf-lesion diameter (mm)		
Isolate	OH34	Smith's OP2016	Mean across cultivars
Control	0.0a	0.0a	0.0a
PB101	12.5de	10.2cd	11.3c
PB103	6.6b	8.2bc	7.3b
PB106	10.4cde	13.3e	11.8c
Mean across isolates	6.3a	6.8a	

*Mean values were separated according to Tukey's test using Genstat software (16th edition). Values within the rows or columns followed by the same letters are not significantly different.

Table 2 Mean diameter (mm) of leaf lesions on two open pollinated red-beet cultivars ('OH34' and Smith's 'OP2016'), spinach and fodder beet at 18 days after inoculation with *Phoma betae* isolate PB106.

Species and 'cultivar'	Leaf-lesion diameter (mm)	
	Control	PB106
Fodder beet 'Bangor'	0a	7.2b
Spinach 'SPS9090'	0a	8.1bc
Red beet 'OH34'	0a	10.3bc
Red beet 'Smith's OP2016'	0a	11.1c

*Mean values were separated according to Tukey's test using Genstat software (16th edition). Values within rows or columns followed by the same letters are not significantly different.

importance of this pathogen as a seed and soil-borne disease, causing pre- and post-emergence seedling disease, *Phoma* leaf spot and root rot, (Herr 1971; Harveson 2006; Harveson et al. 2009; Gilardi et al. 2011; Koenick et al. 2018). However, most of these studies were done on sugar beet. All three *P. betae* isolates (PB101, PB103 and PB 106) obtained from red-beet seed farms in Canterbury region of New Zealand in the current study are pathogenic.to the cultivars, cv 'OP34' and cv Smith's 'OP2016'. All plants inoculated with one of the three *P. betae* isolates caused leaf lesions. Leaf lesions of *P. betae* large enough to be visible had developed 6 days after inoculation but a minimum of 18 dpi was required before infected leaves underwent chlorosis and die off, while still attached to the stem and crown in some cases. Similar work undertaken in Italy indicated symptoms developed 5 days after inoculation of *P. betae* on 20-day-old potted plants when sprayed with an inoculum suspension (1×10^6 spores or mycelial fragments per mL) (Garibaldi et al. 2007; Avasthi et al. 2013). Pathogenicity studies on sugar-beet seedlings and roots showed 15.2% post-emergence damping off and 56.2% of root rot when inoculated with *P. betae* (Abada 1994).

Isolate PB106 was selected to further compare the pathogenicity of *P. betae* on two open-pollinated red-beet cultivars ('OH34' and cv. Smith's 'OP2016'), one cultivar of spinach and cultivar of fodder beet. All the plants inoculated with *P. betae* formed leaf spots. There was a significant difference between the leaf-lesion diameter on fodder beet and either red-beet cultivar Smiths 'OP2016' or spinach. In Spain, Bassimba et al. (2014) reported similar results where *P. betae* caused leaf spot of commercially produced spinach. Studies in other countries have also shown pathogenicity of *P. betae* in other crops. For example, in India, Avasthi et al. (2013) reported that *P. betae* caused leaf spots on *Aloe vera*. The results from current study indicate that *P. betae* isolated from red beet can be also cause leaf spots in fodder beets and spinach. Fodder beet and spinach are both commonly grown in the Canterbury region and may act as alternative hosts for *P. betae*.

CONCLUSIONS

Future studies are warranted to determine the effect of *P. betae* on seedling damping off and root rot on red beets in New Zealand.

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