EVALUATION OF THE PATHOGENICITY OF *PHOMA EXIGUA* VAR. *EXIGUA* ON CALIFORNIAN THISTLE

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ABSTRACT

Pathogenicity of isolates of *Phoma exigua* var. *exigua* Desm. on detached, wounded Californian thistle (*Cirsium arvense* (L.) Scop.) leaves was determined by measuring lesion development after inoculation with conidial solutions (1×10^5 spores/ml). Results showed significant lesion development by all six test isolates compared to control treatments, but inconsistent lesion development was observed between isolates and between assays. The six isolates also gave greater ($P < 0.05$) lesion development than the control treatment 18 days after inoculation of wounded Californian thistle leaves on whole plants, with no significant differences in lesion development between isolates. For whole plants and detached leaves, lesion development correlated with the recovery of *P. exigua* var. *exigua* from lesion sites.

Keywords: - *Phoma exigua* var. *exigua*, pathogenicity, Californian thistle, *Cirsium arvense*.

INTRODUCTION

This study was initiated after *Phoma exigua* var. *exigua* Desm. was isolated from diseased Californian thistles (A.G. Spiers, pers. comm.). Microbial agents have been evaluated for control of Californian thistle in New Zealand, as this is an economically important weed (Popay & Cheah 1990; Bourdôt et al. 1993). A *Phoma* sp. has previously been found to be a weak leaf spot pathogen of Californian thistle (Waipara et al. 1997). This paper describes the pathogenicity of six *P. exigua* var. *exigua* isolates towards Californian thistle using detached leaf and whole plant assays. Since wounding of plant tissue has been reported to facilitate infection on some plant species (e.g. soya bean (*Glycine max* L.) (Marcinkowska 1984)), *P. exigua* var. *exigua* infection of wounded and non-wounded leaves was evaluated. Assessments of the root system and foliage were included in the whole plant assay to determine the capacity of *P. exigua* var. *exigua* to deplete root reserves and impact on the population dynamics of Californian thistle.

METHODS

Detached leaf assay (assay 1)

Six isolates were evaluated in this study: isolate HW from Bulls in the Manawatu; DSA, DSB and DSC from Eketahuna, and HA and HB from Hamilton. Cultures were maintained on potato dextrose agar (PDA) incubated at 22°C under a 12:12 h light:dark cycle.

Sterile distilled water (SDW) (5 ml) was pipetted onto 4-week old cultures of each isolate and the surface scraped with a sterile scalpel. The resulting solution was filtered through a single layer of muslin cloth and polyester mesh (100-120 µm mesh size) and centrifuged at 500 x g for 15 min. The pellet was dissolved in 5 ml of 1% Tween 80 (polyoxyethylene (20) sorbitan mono-oleate), and the spore concentration adjusted to 1×10^5 spore/ml using a haemocytometer.

Small (44-68 mm long/18-32 mm wide) Californian thistle leaves were collected from a farm site, washed under water and sterilised by dipping into 1% sodium hypochlorite solution for 20 sec, then rinsed with SDW. Leaves received 12 wounds with a sterile

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needle. All inoculations were made on wounded leaves with the exception of HB-2 (unwounded treatment). After wounding, spore solutions were applied to the leaves as a fine spray from a pump atomiser until run-off. Twenty leaves were inoculated for each fungal treatment and the control (1% Tween). Pairs of leaves were then placed in deep (22 mm) Petri dishes lined with moist filter paper. Each dish comprised a single replicate, with ten replicates for each treatment.

Leaves were assessed after 48, 96 and 144 h, and lesion area calculated (as a percentage of the total leaf area). After the last assessment, tissue samples from lesion margins were surface sterilised in 1% sodium hypochlorite and rinsed twice in SDW before being placed onto water agar and incubated at 22°C under a 12:12 h light:dark cycle. Agar blocks of mycelial growth on the water agar were transferred onto PDA. Colony growth and spore dimensions (Sutton 1980) were used to confirm *P. exigua* var. *exigua* identity. An ANOVA was performed for mean percentage lesion area for all isolates, and Tukeys - Honestly significant difference test was used to calculate LSD values.

Inoculation of leaves on whole Californian thistle plants (assay 2 and 3)

Ten week old Californian thistle plants were grown individually in standard potting mix in 1.5 litre containers. Six young leaves on each plant were wounded 12 times with a sterile needle. Spore suspensions of each fungal isolate were prepared and applied as previously described for the detached leaf assay. Control plants (wounded in the same way) were sprayed with 1% Tween. Five replicate plants were prepared for each treatment. Following inoculation, each plant was covered with a plastic bag to create a humid environment. In a repeat assay (assay 3), before bagging, two marked leaves (of six) were removed and placed in a deep Petri dish, as described for the detached leaf assay. Two leaves from each plant constituted a replicate, with five replicates for each treatment prepared. Each plant was placed in a randomised block design of five blocks in a glasshouse. Each block comprised seven plants, one each of the six isolates and one control. Plants were watered from the base and plastic bags were kept over the plants except during some inspections.

Lesion area as a percentage of the total leaf area was recorded. Plants were assessed after 6, 8, 10, 16 and 22 d for assay 2 and after 6, 8, 10, 12 and 18 d for assay 3. Thirty-four days after inoculation, plants from assay 2 were harvested, the wet weights of the foliage and roots were recorded, and then foliage and roots dried (65°C for 48 h and 85°C for 12 h, respectively in a forced air oven) and weighed. Statistical analysis was conducted as described for the detached leaf assay.

RESULTS

Detached leaf assay

At 96 h, two isolates had significantly ($P < 0.001$) greater mean lesion coverage than the control and other isolates (control 5.8%, DSC 71.0%, HA 43.2%) (Table 1). At 144 h, mean lesion coverage for the control treatment had increased to 17.1%. All fungal treatments, apart from the unwounded HB-2 treatment, gave significantly greater mean lesion coverage than the control. DSC had significantly ($P < 0.001$) greater lesion development than DSA, HB and HW. Recovery of *P. exigua* var. *exigua* from tissue samples was variable across the different isolate treatments (6-68%).

Whole plants – assay 2

Average temperatures recorded inside the plastic bags covering the Californian thistle plants was 20.8°C (range 10.0 to 38.5°C). All isolates produced lesions on wounded leaves, with DSB and DSC showing significantly ($P < 0.05$) larger lesions than the control from day 8 to the end of the assay (Table 2). By day 16, all isolates produced lesions significantly ($P < 0.05$) greater than the control. At no time point were there any significant differences between isolates. Root and foliage dry weights did not differ significantly between the fungal and control treatments or between the different isolates (data not presented). Isolations of *P. exigua* var. *exigua* were made from five of six isolates tested (range of recovery 50-100%).

TABLE 1: Mean¹ percentage lesion coverage on detached Californian thistle leaves after inoculation with *P. exigua* var. *exigua* isolates and mean percentage recovery of *P. exigua* var. *exigua* from diseased tissue in assay 1.

Isolate	96 h	144 h	% Recovery
Control	5.8 c ²	17.1 e	4
DSA	4.3 c	52.0 cd	68
DSB	27.1 bc	76.8 abc	50
DSC	71.0 a	97.5 a	41
HA	43.2 b	86.0 ab	51
HB	7.7 c	54.0 bcd	42
HB-2	2.3 c	32.8 de	52
HW	19.8 bc	56.8 bcd	6
LSD	25.8	33.5	
P-value ³	P<0.001	P<0.001	

¹Mean of 10 replicates. ²Means followed by the same letter within columns are not significantly different. ³ANOVA between all isolates excluding control.

Whole plants – assay 3

Significant differences in lesion development on whole plants relative to the control were observed between isolates from day 6 to day 18 (Table 3). Lesion development over time was greatest with isolate DSA. At day 18, all isolates were significantly different (P<0.05) to the control and isolate DSA had greater lesion development than isolate HB. *P. exigua* var. *exigua* was recovered from lesions produced by all isolates (range 25-100%).

On detached leaves at 96 h, isolate HA had significantly (P<0.01) greater lesion coverage (30.5%) than the control (0.6%) (Table 3). At 144 h, four isolates had significantly (P<0.05) greater mean lesion coverage than the control treatment. Of these, HA (93%) was significantly greater than DSC (67%) and HW (65%), but not DSA (86.8%).

TABLE 2: Mean¹ percentage lesion coverage on wounded Californian thistle leaves on whole plants after inoculation with isolates of *P. exigua* var. *exigua* in assay 2.

Isolate	Days after inoculation				
	6	8	10	16	22
Control	0.0 a ²	0.0 b	0.0 b	0.0 b	6.0 b
DSA	12.9 a	18.3 ab	70.1 a	99.0 a	100.0 a
DSB	11.1 a	45.5 a	68.2 a	97.7 a	100.0 a
DSC	16.1 a	54.3 a	82.3 a	98.4 a	100.0 a
HA	3.4 a	23.0 ab	47.1 ab	89.2 a	97.6 a
HB	6.4 a	21.2 ab	49.3 ab	87.0 a	97.5 a
HW	3.6 a	22.0 ab	54.5 ab	88.7 a	100.0 a
LSD	16.2	38.5	55.7	28.8	10.0
P-value ³	ns	ns	ns	ns	ns

¹Mean of five replicates. ²Figures followed by the same letter within columns are not significantly different. ³ANOVA between all isolates excluding control.

TABLE 3: Mean¹ percentage lesion coverage on wounded Californian thistle leaves on whole plants and detached leaves after inoculation with isolates of *P. exigua* var. *exigua* in assay 3.

Isolate	Whole plants			Detached leaves			
	6 d	8 d	10 d	12 d	18 d	96 h	144 h
Control	0.0 b	0.0 b	0.0 c	0.5 d	0.5 c	0.6 b	3.2 c
DSA	1.1 a	16.4 a	77.5 a	97.5 a	100.0 a	17.1 ab	86.8 ab
DSB	0.3 ab	3.9 b	28.6 bc	69.7 abc	97.3 a	0.3 b	22.0 c
DSC	0.3 ab	3.5 b	43.4 ab	70.1 abc	97.5 a	19.0 ab	67.0 b
HA	0.5 ab	6.6 ab	36.4 bc	80.1 ab	99.5 a	30.5 a	93.3 a
HB	0.1 b	2.4 b	23.3 bc	28.8 cd	67.0 b	0.3 b	26.1 c
HW	0.2 b	2.2 b	20.1 bc	48.5 bcd	91.3 ab	14.0 ab	65.0 b
LSD	0.8	10.7	38.5	48.0	29.4	26.8	2.0
P-value ³	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.01	P<0.001

¹Mean of five replicates. ²Figures followed by the same letter in columns are not significantly different. ³ANOVA between all isolates excluding control.

DISCUSSION

The results of the detached leaf and whole plant assays showed that *P. exigua* var. *exigua* was pathogenic to Californian thistle. This was further supported by the isolation of *P. exigua* var. *exigua* from leaf tissue samples, a fulfilment of Koch's postulates. Wounding has been shown to enhance lesion development by *P. exigua* var. *exigua* for a number of plant species including soya bean (Marcinkowska 1984). Limited results from this study (HB isolate – wounded/unwounded) suggested that wounding was not necessary for successful disease progression on Californian thistle.

Consistent evidence for differences in pathogenicity between isolates was not found in this study. However, screening of a larger number of isolates may reveal isolates that are consistently more pathogenic. Comparison between detached and attached leaves in assay 3 indicated a trend whereby isolates exhibiting strong pathogenicity on detached leaves also performed well on leaves of whole plants, although some isolates gave inconsistent results. Rate of disease development was much greater on detached leaves than whole plants with high lesion coverage occurring within 6 days compared to 10–12 days for whole plants. Thus, the detached leaf assay has potential for use in large scale screening of isolates for differences in pathogenicity. The finding that there was no significant difference between root weights for the treatments was not unexpected given the limited number of leaves inoculated and the short period of the assay. Future work should focus on whole plant inoculations with sufficient experimental time to allow for complete expression of disease and subsequent differential effects on plant biomass to be detected.

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