Diaporthe perijuncta Does Not Cause Phomopsis Cane and Leaf Spot Disease of Grapevine in Australia

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ABSTRACT

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In Australia, *Diaporthe perijuncta* (formerly known as *Phomopsis* taxon 1) and *Phomopsis viticola* (*Phomopsis* taxon 2) have been associated with Phomopsis cane and leaf spot of grapevine. Although *P. viticola* causes distinct leaf spots, as well as lesions on shoots and canes, the pathogenicity of *D. perijuncta* is relatively unknown. The pathogenicity of *D. perijuncta* and *P. viticola* was studied in relation to symptom expression and bud loss. Only *P. viticola* caused brown-black, longitudinal, necrotic lesions on stem tissue and leaf spots characteristic of the disease, whereas both *D. perijuncta* and *P. viticola* induced bleaching of dormant canes. Inoculation of dormant buds with *D. perijuncta* did not cause bud death. *D. perijuncta* and *P. viticola* were re-isolated from inoculated tissue and into pure culture. *D. perijuncta* colonized the epidermis and cortex of the grapevine shoot but not the vascular tissue. *D. perijuncta* appears to be an endophyte, rather than a pathogen of grapevine.

Diaporthe perijuncta (formerly known as *Phomopsis* taxon 1) and *Phomopsis viticola* (*Phomopsis* taxon 2) have been associated with the disease Phomopsis cane and leaf spot of grapevine in Australia (16). The species are distinguished primarily on the basis of α -conidia morphology and the presence of a teleomorph. Scheper et al. (25) identified *D. viticola* as the teleomorph of *Phomopsis* taxon 1 in Australia. However, Phillips (21) examined *Diaporthe* isolates from grapevines grown in Australia and Portugal and determined that they should be assigned to *D. perijuncta* rather than *D. viticola*, but he did not propose a name for the anamorph. The teleomorph of *P. viticola* is not known. Mostert et al. (19) characterized several taxa of *Phomopsis* associated with Phomopsis cane and leaf spot of grapevine and determined that delimitation of the species on the basis of morphology was unreliable. Molecular analysis of the internal transcribed spacer (ITS) regions confirmed *Phomopsis* taxon 2 as *Phomopsis viticola* and that it was genetically distinct from *D. perijuncta* (19).

Historically, the earliest described symptoms of Phomopsis cane and leaf spot included failure of shoots to develop, dwarfed foliage, stunted shoots, and very small leaves on one arm of the vine (24). These observations were not truly characteristic of the disease because symptoms caused by *P. viticola* were confused with those due to other pathogens, such as *Eutypa lata*, in the same vine (17). It is now recognized that symptoms caused by *P. viticola* include distinct dark brown or black leaf spots with a yellow halo, and black longitudinal lesions on petioles and shoots leading to cracking of canes (9,11,13,22,29). Elongated, black lesions also develop on the rachis, resulting in girdling that can cause cluster fall (23). *P. viticola* causes yield loss through weakening canes, fruit rot, and reduction in bunch set (13,23), and has been associated with yield loss of up to 30% in Macedonia (2) and the United States of America (10,23). In Australia, there are no records of yield loss, although fungicides can reduce the incidence of disease (4,26).

Although the pathogenicity of *P. viticola* to grapevines has been demonstrated (10), the role of *D. perijuncta* as a pathogen is poorly understood. Infection by *D. perijuncta* is assumed to be less damaging than *P. viticola*, although Melanson et al. (15) suggested that *D. perijuncta* may be responsible for bud loss. There are few reports of *D. perijuncta* causing distinct visual symptoms on grapevine. Mostert et al. (18) observed light brown, small lesions on green excised shoots inoculated with *D. perijuncta*, but could not distinguish between *P. viticola* and *D. perijuncta* based

on lesion length. *D. perijuncta* has not been isolated from leaf spots and rarely is encountered on diseased material from vineyards affected by Phomopsis cane and leaf spot (19). Scheper et al. (25) described symptoms associated with the teleomorph as zone lines on bleached spurs. Based on stem inoculations, Mostert et al. (19) suggested that *D. perijuncta* was nonpathogenic to grapevine. However, only one isolate of *D. perijuncta* was tested and further tests are warranted to confirm the nonpathogenicity of the fungus.

This study was undertaken to evaluate the pathogenicity of isolates of *D. perijuncta* from grapevine and to compare the susceptibility of a range of cultivars to infection by *D. perijuncta* and *P. viticola* using excised grapevine shoots. The role of *D. perijuncta* in bud loss also was investigated.

MATERIALS AND METHODS

Fungal isolation. Bleached canes with pycnidia were collected in autumn and winter from a number of vineyards in South Australia naturally infected with *D. perijuncta* and *P. viticola*. Canes were incubated in dark, moist conditions at 15°C for at least 1 week to induce sporulation. Conidia, exuded from pycnidia, were diluted in sterile, double-distilled H₂O, then transferred aseptically to potato dextrose agar (PDA) and incubated for 7 days in a cycle of 12 h of light (Philips TLD 18W/33 3F, 380 to 780 nm and near ultraviolet) at 22°C and 12 h of darkness at 16°C. Pure cultures were obtained by transferring mycelium from germinating conidia to new PDA plates. Cultures sporulated after 7 and 21 days for *P. viticola* and *D. perijuncta*, respectively. Isolates were identified as *D. perijuncta* or *P. viticola* on the basis of conidial morphology (16). *P. viticola* taxon 3 (DAR69458) was provided by M. Priest, Agricultural Scientific Collections Unit, NSW Agriculture, Orange, Australia.

Preparation of spore suspension. Isolates of *D. perijuncta* and *P. viticola* (Table 1) were chosen based on geographical origin and the presence of adequate quantities of conidia and mycelium needed for inoculations. Carnation leaf agar (CLA) was used to produce pycnidia of *D. perijuncta* and *P. viticola* in vitro (25). Pieces of carnation leaf were surface sterilized in 0.5 M sodium hypochlorite (NaOCl) for

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30 s, rinsed in sterile water, then autoclaved at 121°C for 20 min. Leaf pieces (10 by 5 mm) were placed on the surface of 1.2% water agar (WA) in petri dishes. Mycelial plugs from cultures on PDA (10 by 10 mm) were placed onto CLA and incubated as above. For each isolate, several cirrhi were suspended in sterile, double-distilled water (28) and the concentration adjusted to 10⁴ to 10⁶ conidia/ml using a haemocytometer.

To assess conidial germination, five 10-µl droplets of spore suspension of each isolate were placed on 1.2% WA and incubated overnight as described. Percent germination was examined after 10 to 12 h.

Assessment of pathogenicity of *D. perijuncta*. To compare symptoms caused by *D. perijuncta* and *P. viticola*, excised shoots were inoculated with mycelium in the glasshouse. Green, 6-month-old terminal shoots of *Vitis vinifera* (cv. Sultana), 0.5 to 1.2 cm in diameter and cut to 30 cm long, were pruned from healthy vines in a vineyard at the Waite Agricultural Research Institute, South Australia. All leaves and tendrils were removed from the shoots prior to spraying with 70% ethanol. Shoots were placed in 500-ml plastic jars containing 200 ml of tap water, so that the lower half of the shoot was submerged. Eight replicate shoots per treatment were wounded 10 cm from the top of the shoot by removing the cortex with a 4-mm-diameter cork borer. Shoots were inoculated with 4-mm-diameter plugs from 14-day-old cultures on PDA: *D. perijuncta* isolates A223.1, H307, L406, and J5, and *P. viticola* isolates C603, P712, 902.4, and

M851. Control shoots received an uncolonized PDA plug. Each wound was sealed with parafilm and shoots were maintained in a glasshouse at 25°C with natural light. The water was replaced every second day. Lesion length (minus diameter of initial wound) was measured 14 days after inoculation. Pieces of tissue, approximately 5 by 5 mm, were excised from the margin of lesions and surface sterilized with 0.5% NaOCl for 30 s, followed by 1-min rinses in sterile water. Sections then were plated on PDA and incubated for 3 days at 16 and 22°C in the dark and light, respectively, as described. Re-isolation was attempted from four shoots per treatment.

In addition, the pathogenicity of 11 *D. perijuncta* isolates (A223.1, A223.2, H307, L406, J4, J5, L427, L424, T100, T101, and M860), 1 *P. viticola* isolate (M831.1), and 1 *Phomopsis* taxon 3 isolate (DAR69458) was compared by inoculating eight replicate excised shoots (cv. Sultana) in a separate experiment. Shoots were wounded as described above and inoculated with 7-day-old mycelium. Controls were inoculated with uncolonized PDA plugs.

The susceptibility of selected grapevine cultivars was assessed by inoculating green, 6-month-old shoots of four red grape cultivars (Shiraz, Grenache, Merlot, and Cabernet Sauvignon) and three white grape cultivars (Semillon, Chardonnay, and Sauvignon Blanc) as described above with *D. perijuncta*, *P. viticola*, and sterile PDA plugs (Table 1). Four excised shoots of Semillon were selected for re-isolation of isolate A223.1 from both necrotic tissue and from asymptomatic tissue 20 to 40 mm distant from the point of inoculation.

Selected shoots were examined microscopically to investigate colonization of host tissue by *D. perijuncta* and *P. viticola*. Tissue samples were taken 2 weeks after inoculation at the site of wounding and at intervals of 10 mm above and below the point of inoculation from three replicate excised shoots (cv. Sultana) inoculated with *D. perijuncta* isolates A223.2, J5, and T101, *P. viticola* isolate M831.1, or PDA. In addition, shoots (cv. Grenache) inoculated with *D. perijuncta* isolate A223.1 or *P. viticola* isolate M851 were examined. Sections up to 40 mm from the margin of the lesion were assessed. Fresh shoot tissue was cut into 10-mm sections and submerged in 25 ml of 1 M KOH in a McCartney bottle for a minimum of 30 min at room temperature, followed by three rinses in sterile, double-distilled H₂O for approximately 5 min each (14). The stain solution, 0.05% aniline blue (dye CI #42755, BDH) in 0.067 M K₂HPO₄, pH 9.0, was prepared at least 2 h before use. Tissue was hand sectioned, either as a cross-section of the shoot or a longitudinal section of the epidermis, and mounted on glass slides containing several drops of stain solution. Tissue was examined immediately with a UV microscope (BP495 exciter: barrier fluorescence filters) at ×100 magnification.

Colonization of dormant buds. To examine the effect of *D. perijuncta* on budburst, two inoculation methods were used to facilitate rapid colonization of the bud by either penetration through a wound site or penetration through a prematurely created leaf scar. Dormant grapevine cuttings

Table 1. Isolates of *Diaporthe perijuncta*, *Phomopsis viticola*, and *Phomopsis* taxon 3 used in pathogenicity experiments, cultivars inoculated (Inoc.), and corresponding inoculation method

Isolate	Species	Site ^w	Experiment number, cultivar, method						Microscopy ^y
			1, Sultana, shoot mycelium	2, Sultana, shoot mycelium	3, various cvs., shoot mycelium ^x	4, Shiraz, bud mycelium	5, Chardonnay, bud & leaf conidia	6, Shiraz, bud conidia	
A223.1	<i>D. perijuncta</i>	Ashton, SA	Inoc.	Inoc.	Inoc.	Inoc.	...	...	Inoc.
A223.2	<i>D. perijuncta</i>	Ashton, SA	...	...	...	...	...	...	Inoc. ^z
H307	<i>D. perijuncta</i>	Summertown, SA	Inoc.	Inoc.	...	...	Inoc.	...	...
L406	<i>D. perijuncta</i>	Carey Gully, SA	Inoc.	Inoc.	...	...	...	Inoc.	...
J4	<i>D. perijuncta</i>	Mount Compass, SA	...	Inoc.	...	...	...	...	...
J5	<i>D. perijuncta</i>	Mount Compass, SA	Inoc.	Inoc.	...	...	...	...	Inoc.
L427	<i>D. perijuncta</i>	Lenswood, SA	...	Inoc.	...	...	...	...	...
L424	<i>D. perijuncta</i>	Lenswood, SA	...	Inoc.	...	...	...	...	...
T100	<i>D. perijuncta</i>	McLaren Vale, SA	...	Inoc.	...	...	...	...	...
T101	<i>D. perijuncta</i>	Langhorne Creek, SA	...	Inoc.	...	...	...	...	Inoc.
M860	<i>D. perijuncta</i>	Margaret River, WA	...	Inoc.	...	...	...	...	...
51C.1	<i>D. perijuncta</i>	Coonawarra, SA	...	...	...	...	Inoc.	...	...
M831.1	<i>P. viticola</i>	Wagga Wagga, NSW	...	Inoc.	...	...	...	...	Inoc.
M851	<i>P. viticola</i>	McLaren Vale, SA	Inoc.	...	Inoc.	...	...	...	Inoc.
LR10T6	<i>P. viticola</i>	Lenswood, SA	...	...	...	...	Inoc.	Inoc.	...
C603	<i>P. viticola</i>	Coonawarra, SA	Inoc.	...	...	...	...	...	...
P712	<i>P. viticola</i>	Padthaway, SA	Inoc.	...	...	...	...	...	...
902.4	<i>P. viticola</i>	Robinvale, Vic	Inoc.	...	...	Inoc.	...	...	...
DAR69458	<i>Phomopsis</i> taxon 3	Yarra Valley, NSW	...	Inoc.	...	...	...	...	...

^w SA = South Australia, WA = Western Australia, Vic = Victoria, NSW = New South Wales.

^x *Vitis vinifera* cultivars: Shiraz, Grenache, Merlot, Cabernet Sauvignon, Semillon, Chardonnay, and Sauvignon Blanc.

^y Tissue samples of excised grapevine shoots were hand sectioned, submerged in 1 M KOH for 30 min, and stained with 0.05% aniline blue dye in 0.067 M K₂HPO₄, pH 9.0, 2 weeks after inoculation with *D. perijuncta* or *P. viticola*.

^z Six isolates under microscopy.

were obtained from the Vine Improvement Centre, Monash, South Australia. Roots were induced on 30 grapevine cuttings approximately 40 cm long (cv. Shiraz) by submerging in water for 24 h at 4°C, then in water containing 10 ml of sodium hypochlorite for a further 24 h. Following additional soaking in clean water for 24 h, each cutting was dipped in indole-3-butyric acid (IBA; Sigma-Aldrich, St. Louis) at 2 g/liter for 40 s. Cuttings were transferred into water-soaked rock wool blocks (40 by 40 mm) and planted in 20-cm-diameter pots containing University of California (UC) potting mix (1) and 10 g of Osmocote slow-release granular fertilizer (Arthur Yates and Co. Ltd, Homebush, NSW, Australia).

After 10 months in the greenhouse, 0.7-mm-diameter lignified shoots on each vine were prepared for inoculation. Grapevine plants were at the stage of leaf fall. Green leaves were removed and dormant buds retained. Ten plants were inoculated with *D. perijuncta* isolate A223.1, *P. viticola* isolate 902.4, or a PDA plug (control).

Of the 10 plants receiving each treatment, 4 were wounded with a scalpel and 6 were wounded by leaf removal. For the first method, 16 dormant buds were inoculated among the four plants. The number of buds inoculated was not the same per plant. A wound (approximately 5 mm wide and 3 mm deep) was made with a scalpel on the lignified shoot below each dormant bud. A mycelial plug of the test isolate was taken from the margin of a 7-day-old colony on PDA, inserted into the wound, and sealed with parafilm. Controls were treated with a sterile PDA plug. For the second method, 20 buds among the six plants were inoculated. A premature leaf scar beneath the bud was created by detaching the petioles by hand (27) and inoculated as above. Plants were placed in a shade house and maintained until budburst for at least 8 weeks. The percentage of buds burst, bleaching, and development of pycnidia were recorded over 8 weeks, commencing at budburst. Four plants from each treatment were selected arbitrarily for re-isolation of the fungi.

Infection of buds and leaves. To determine if infection by *D. perijuncta* conidia caused bud death and to clarify symptom expression, eight replicate mature grapevine plants were inoculated in the glasshouse with one of the following four treatments: *D. perijuncta* isolate H307 at 10^4 α -conidia/ml, *D. perijuncta* isolate 51C.1 at 10^6 α -conidia/ml, *P. viticola* isolate LR10T6 at 10^6 conidia/ml (50% α - and β -conidia), and sterile, double-distilled H₂O as a control. Plants were pruned 4 weeks prior to inoculation and then to two green shoots 1 day before treatment. The average length was 26.2 and 17.7 cm for bud and leaf-inoculated shoots, respectively. On one shoot, the bud at the basal node was inoculated with a 10- μ l droplet of spore

suspension or sterile water. On the other shoot, conidial suspension was applied to the leaf at the first node using a Jet-Pak spray power unit (Wattyl Australia Pty Ltd, Blacktown, NSW, Australia). Clear plastic bags were placed over the shoots for 48 h and the temperature in the bags measured during this time with a datalogger (Tinytalk II; Hastings, NSW, Australia). Buds, leaves, and internodes of inoculated shoots were rated for disease severity weekly for 8 weeks. A 0-to-4 scale was used based on the Barratt and Horsfall rating system, modified from Phillips (20), where 0 = no sign of disease, 1 = few lesions covering no more than 25% of leaf or internode, 2 = many lesions covering 25 to 50% of leaf or internode, 3 = over 50% leaf or internodal area severely scarred or necrotic, and 4 = death of shoot or bud (no shoots emerged). Re-isolations were made from dissected buds and leaves 8 weeks after inoculation as described. The woody canes were placed on moist paper towel in a sealed container at 15°C in darkness to induce production of pycnidia.

Dormant buds were inoculated to determine if *D. perijuncta* causes delayed budburst, or bud death, and to assess symptoms on developing shoots. Eight dormant cuttings of grapevine (cv. Shiraz; Vine Improvement Centre) comprising three buds each were inoculated with a 50- μ l droplet of 10^6 α -conidia/ml. The surface of the bud was inoculated with *D. perijuncta* isolate L406, *P. viticola* isolate LR10T6, or sterile, double-distilled H₂O. In addition, the entire epidermis of eight dormant cuttings was sprayed with a suspension of *D. perijuncta* isolate L406 (10^6 α -conidia/ml) as before. The cuttings were incubated in a growth room at 19 and 14°C in the light and dark, respectively, and enclosed in

clear plastic bags with a datalogger, as above. Each bud was assessed for stage of development, shoot length, and disease severity weekly over 8 weeks. Disease severity was assessed (20) and the Modified Eichhorn-Lorenz system was used to determine stage of development, such that 0 = death of a dormant or developing bud; 1 = winter bud (dormant); 2 = bud swell; 3 = woolly bud, brown bud visible; 4 = green tip, first leaf tissue visible (budburst); and thereafter, 5 = shoot (14).

Statistical analyses. Data were transformed to give a normal sampling distribution and log transformation values were subjected to analysis of variance (ANOVA) using the Statistix statistical package (version 7; Analytical Software, Tallahassee, FL). Means were separated by the least significant difference test at $P = 0.05$.

RESULTS

Pathogenicity of *D. perijuncta*. Shoots inoculated with *P. viticola* generally were weaker and paler in color than controls and those inoculated with *D. perijuncta*. Three days after inoculation with *P. viticola*, browning or discoloration of tissue at the wound site was observed in most excised shoots. Shoots inoculated with *P. viticola* developed dark brown or black, longitudinal, dry lesions that resembled symptoms characteristic of Phomopsis cane and leaf spot in the vineyard. *P. viticola* caused lesions up to 41 mm long (mean, 11 mm), but the length of lesions produced by the four isolates was highly variable (Fig. 1). Typically, bleaching was observed on shoots approximately 12 days after inoculation.

Two weeks after inoculation, most shoots inoculated with *D. perijuncta* remained green and turgid with slight, dark

Fig. 1. Average lesion length (mm) produced on excised, green shoots (*Vitis Vinifera* cv. Sultana) 2 weeks after inoculation with *Diaporthe perijuncta*, *Phomopsis viticola*, or potato dextrose agar (control). Vertical bars represent standard error of the means.

green discoloration and minor lesions around the wound site. Pycnidia and distinct narrow lines similar in appearance to that of a black marker pen (zone lines) were observed on two shoots inoculated with *D. perijuncta*. Lesion length did not differ among isolates of *D. perijuncta* ($P = 0.565$). Control shoots remained green and turgid and there was little or no discoloration on around the wound site. *D. perijuncta* and *P. viticola* were re-isolated from tissue taken from the margin of the brown areas on four replicate shoots. Isolation from the control shoots yielded various fungi, including *Aspergillus* spp., *Penicillium* spp., and yeasts. Neither *Pho-*

mopsis viticola nor *D. perijuncta* was detected.

There was considerable variation in lesion length on shoots inoculated with 11 isolates of *D. perijuncta* (Table 2). Most (73%) of the shoots inoculated with *D. perijuncta* remained healthy or developed small lesions (<5 mm) which consisted of pale brown tissue around the immediate wound area, indicative of physical tissue damage. The average lesion length on shoots inoculated with the isolates of *D. perijuncta* was 4.2 mm.

Lesions produced on shoots inoculated with the single *P. viticola* isolate were dark brown-black and more than twice as long

as the longest lesion caused by *D. perijuncta*. In addition, *P. viticola* produced lesions that were wider (approximately 5 mm wide) than those produced by isolates of *D. perijuncta* (approximately 1 to 2 mm wide). Shoots inoculated with *P. viticola* were unhealthy and pale in color. As above, lesions did not develop on control shoots or those inoculated with *Phomopsis* taxon 3. *D. perijuncta* and *P. viticola* were recovered from the margin of the lesions in the appropriate inoculated shoots, and also from asymptomatic tissue approximately 3 cm from the wound.

D. perijuncta caused significantly smaller lesions than did *P. viticola* ($P = 0.001$) on the various cultivars assessed. *P. viticola* caused dark brown or black, longitudinal lesions of similar length, severity, and width on cvs. Semillon, Sauvignon Blanc, and Chardonnay, whereas lesions on cvs. Shiraz, Merlot, and Cabernet Sauvignon were less severe (Fig. 2). ANOVA and comparison of means showed that lesions were significantly longer ($P = 0.011$ and 0.000) on Grenache than on the other cultivars tested when data for *D. perijuncta* and *P. viticola* was analyzed separately. In addition, pycnidia developed on bleached sections of shoots of cv. Grenache inoculated with *P. viticola*. *D. perijuncta* and *P. viticola* were re-isolated from all inoculated shoots tested.

Microscopic examination of tissue inoculated with *D. perijuncta* revealed hyphae in the intercellular spaces of the cortex but not in the xylem and phloem. Tissue of excised green shoots inoculated with *D. perijuncta* was darkened at the site of inoculation, although cells approximately 10 mm from the wound were healthy. In comparison, lesions caused by *P. viticola* M851 and M831.1 extended along the shoot, with the hyphae appearing as a subcuticular mass. However, the cortical cells were intact and hyphal growth was intercellular. At the site of inoculation, mycelial growth was abundant and cell arrangement was disrupted. Tissue that appeared healthy 40 mm away from the site of inoculation was examined and hyphae observed under the cuticle. Single hyphae were observed intercellularly, but were not associated with discoloration or cell death. No hyphae were observed in vascular cells or the pith. Hyphae were not observed in control shoots, but cells were disrupted at the wound and cortical cells darkened. Darkening of cells in the control shoots resembled that caused by *D. perijuncta* at the point of inoculation. Callose deposits were observed near the wound and in the epidermal layer in tissue inoculated with *P. viticola*, but were not evident in control shoots.

Colonization of dormant buds. Twelve weeks after inoculation with *D. perijuncta*, all lignified shoots were bleached and bore pycnidia, particularly around the nodes and sites of inoculation. Similarly, 91.5% of

Table 2. Mean lesion length on excised green shoots (*Vitis vinifera* cv. Sultana) 2 weeks after inoculation with mycelium of 11 isolates of *Diaporthe perijuncta*, 1 isolate each of *Phomopsis viticola* and *Phomopsis* taxon 3, and potato dextrose agar (control)^y

Treatment	Isolate	Lesion length (mm) ^z	
		Mean	±SE
<i>D. perijuncta</i>	T101	10.4 b	2.8
<i>D. perijuncta</i>	L427	6.6 bc	3.3
<i>D. perijuncta</i>	A223.2	6.1 bcd	2.7
<i>D. perijuncta</i>	J5	5.6 bcde	3.1
<i>D. perijuncta</i>	T100	4.3 cde	1.1
<i>D. perijuncta</i>	L406	3.3 cde	1.3
<i>D. perijuncta</i>	L424	3.1 cde	1.5
<i>D. perijuncta</i>	A223.1	3.0 cde	0.7
<i>D. perijuncta</i>	H307	2.0 cde	0.9
<i>D. perijuncta</i>	J4	0.7 de	0.4
<i>D. perijuncta</i>	M860	0.6 de	0.5
<i>P. viticola</i>	M831.1	23.0 a	4.4
<i>Phomopsis</i> taxon 3	DAR69458	0.1 e	0.1
Control	...	0.9 cde	0.4

^y Mean lesion length based on eight replicate excised green shoots per treatment.

^z SE = standard error. Means within a column followed by different letters are significantly different ($P = 0.05$) based on least significant difference.

Fig. 2. Lesion length on excised green shoots of seven grapevine cultivars, Semillon (SEM), Sauvignon Blanc (SAVBL), Chardonnay (CHARD), Shiraz (SHIRAZ), Cabernet Sauvignon (CABS AV), Merlot (MERLOT), and Grenache (GREN) 2 weeks after inoculation with *Diaporthe perijuncta* isolate A223.1 or *Phomopsis viticola* isolate M851. Values are means of eight replicates per treatment. Shoots inoculated with potato dextrose agar (control) did not produce lesions. Vertical bars represent standard error of the means.

spurs inoculated with *P. viticola* were bleached and 90% bore pycnidia. The bleaching symptoms were typical of those observed in the field, and bleaching alone could not be used to visually differentiate between *P. viticola* and *D. perijuncta*. One lignified shoot inoculated with PDA was slightly bleached, but no pycnidia were present. In general, budburst percentage was poor regardless of treatment. *D. perijuncta* and *P. viticola* were isolated readily from pycnidia on inoculated grapevine plants.

Infection of buds and leaves. There was no evidence that *D. perijuncta* or *P. viticola* caused bud death, because most buds remained healthy or burst and internal tissue of dissected buds was intact and green. All shoots that developed on inoculated and control plants were healthy.

Small necrotic leaf spots (approximately 1 mm in diameter) surrounded by a distinct yellow halo developed 3 weeks after leaves were inoculated with *P. viticola*. The leaf spots developed on less than 25% of the leaf area. After 4 to 5 weeks, dark-brown, longitudinal lesions developed on the corresponding petioles. *P. viticola* was re-isolated from the margin of several leaf spots but not from asymptomatic tissue. Control vines and those inoculated with *D. perijuncta* remained symptomless. *D. perijuncta* was re-isolated from inoculated grapevine tissue.

Following inoculation of dormant buds with conidia, neither *D. perijuncta* nor *P. viticola* had a significant effect on bud development ($P = 0.07$). By week 2, 100% of terminal buds (bud three) burst while most basal buds (bud one) remained dormant throughout the assessment period, regardless of treatment. Shoots from bud three generally were longer and healthier than those from other buds. Shoots developing from buds inoculated with *D. perijuncta* were similar in appearance and length to those inoculated with *P. viticola* (data not shown). Three weeks after inoculation, brown spots approximately 1 mm in diameter with a characteristic yellow halo were observed on leaves on plants inoculated with *P. viticola*. By week 5, six dormant buds on plants inoculated with *P. viticola* turned brown and died. *P. viticola* was isolated from five of these dead buds. ANOVA indicated that disease severity on developing shoots did not differ significantly ($P = 0.73$) between replicates or treatments.

DISCUSSION

D. perijuncta was not pathogenic to grapevine shoots or buds. Pathogenicity tests showed that failure of buds to burst, stunted shoots, and leaf or shoot symptoms associated with *Phomopsis* cane and leaf spot were not caused by *D. perijuncta*. In the present study, *D. perijuncta* isolates from various viticultural regions of Australia were consistently less virulent than the

isolates of *P. viticola* tested. Excised shoots inoculated with mycelium of *P. viticola* developed dark brown or black, longitudinal lesions similar to those produced by natural infection in the field. However, there was large variation among isolates of *P. viticola* tested. Koch's postulates were fulfilled by inoculation of grapevine plants and re-isolation of *D. perijuncta* and *P. viticola* from inoculated shoots. Mostert et al. (18) reported that *P. viticola* and *D. perijuncta* could not be distinguished on the basis of lesion length, but we demonstrated that colonization by *P. viticola* caused longer and more severe lesions on excised shoots than did *D. perijuncta*. There are limited reports on susceptibility of grape cultivars to *P. viticola* and *D. perijuncta*. Gubler and Leavitt (11) reported that cv. Grenache was highly susceptible to *Phomopsis* cane and leaf spot in years with wet springs. Furthermore, cv. Concord often is regarded as highly susceptible (5,6,24). The pathogenicity studies undertaken here suggest that inoculation of excised green shoots may be suitable for determining pathogen variability and cultivar susceptibility.

Both *D. perijuncta* and *P. viticola* induced bleaching on grapevine, and it was not possible to distinguish between the two fungi based on this symptom. Although bleached canes in winter often are associated with the disease, bleaching can be attributed to physical damage, extremes of weather, *Botrytis cinerea*, and saprophytic pycnidium-producing fungi (3). Nevertheless, the presence of bleaching and re-isolation of both fungi from pycnidia indicated a physiological change in grapevine tissue. This can be associated with fungal colonization of the epidermal cells and developing pycnidia, forcing the layer of epidermal cells away from the cortex (13).

D. perijuncta did not cause bud death, delay in bud burst, or stunted shoots. However, the inoculation methods used in the study were effective in facilitating fungal infection, as evidenced by bleaching and leaf symptoms. In general, bud burst percentage was low in all treatments, and studies of the effects of fungal colonization on bud development were inconclusive. Uddin and Stevenson (27) reported a *Phomopsis* sp. causing rapid development of peach shoot blight after inoculation of wounded dormant buds and breaking buds, and suggested that some *Phomopsis* spp. on woody plants may invade the shoot through the young, exposed tissue of the bud. *P. viticola* is known to overwinter in dormant buds (13), but it is unknown if natural colonization of buds can result in direct infection of new shoots. Perithecia of *D. perijuncta* have been observed protruding from naturally infected unburst buds in in vitro conditions, indicating that the fungus either may colonize buds directly (15) or simply overwinter in bud scales. It is possible that rapid develop-

ment of buds on newly propagated canes in the present study did not allow sufficient time for *D. perijuncta* or *P. viticola* to colonize and cause bud death under glasshouse conditions.

D. perijuncta colonized the epidermis of excised green grapevine shoots but not the vascular tissue. Likewise, *P. viticola* was restricted to the epidermal and cortical cells, with no growth into the vascular bundle. A large mass of subcuticular hyphae of *P. viticola* was evident beneath necrotic lesions. In addition, hyphal masses were concentrated on the proximal side of the inoculated point, with lesions formed longitudinally along the side of the shoot in either direction. Microscopic examination indicated that hyphal growth caused disruption by physically forcing the cells apart rather by intracellular growth. Therefore, eventual cracking of shoots infected with *P. viticola* may be attributed in part to the disruption of host cells. The findings support an earlier report that *P. viticola* does not invade healthy xylem tissue and is not associated with dieback of grapevine (17). In contrast, other *Phomopsis* spp., such as *P. vaccinii*, cause dieback of shoots resulting from necrosis and collapse of parenchyma cells and vascular tissue after invading the cortex (7).

The findings of this study clarify that *D. perijuncta* is not associated with *Phomopsis* cane and leaf spot in Australia. Rather, the limited pathogenicity of *D. perijuncta* on grapevine suggests that it is endophytic. *D. perijuncta* grew inconspicuously within the tissue of grapevine without causing symptoms or apparent injury. *D. perijuncta* was detected only by staining, or when pycnidia erupted through the epidermis. The degree of mutualism between the host and endophyte is dependent on the plant environment (12), and it is possible for endophytic fungi to become pathogenic when the host plant is weakened. Hence, further studies are needed to assess whether stress on the plant influences mycelial growth and the ability of the fungus to cause harm under a range of environmental conditions.

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