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Phenotyping grapevine cultivars for resistance to Eutypa dieback

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Summary. Eutypa dieback of grapevine is a trunk disease that affects vineyard productivity. Wood symptoms of this disease develop consistently in greenhouse-grown plants, after inoculation of woody stems with the causal fungus *Eutypa lata*. Wood symptoms are a common measure of host cultivar resistance and *E. lata* isolate virulence. Leaf symptoms of the disease also develop in the greenhouse, although reports of low correlations between severity of wood and leaf symptoms (for some cultivars and isolates) indicate that a definitive procedure is required for evaluating cultivar resistance. Three 'phenotyping assays', replicated with two *E. lata* isolates (BX1-10 and M14), were assessed for quantifying resistance of a set of *Vitis vinifera* cultivars ('Black Corinth', 'Carignane', 'Husseine', 'Merlot', 'Muscat Hamburg', 'Palomino', 'Peloursin', 'Primitivo', and 'Thompson Seedless'). The methods were: Assay 1 (leaf and woody-stem symptoms measured 1 year post-inoculation on plants propagated from rooted, dormant cuttings); Assay 2 (green stem symptoms measured 4 months post-inoculation on plants propagated from rooted, green cuttings); and Assay 3 (leaf symptoms measured 6 weeks post-inoculation on plants propagated from rooted, dormant cuttings). High rates of mortality among some cultivars ('Merlot') in Assay 3 confounded results based on leaf symptoms. Results from Assays 1 and 2 were more consistent with each other, especially for the most resistant cultivars ['Merlot' and 'Primitivo' (aka 'Zinfandel')]', than they were for these cultivars in Assay 3. Compared to resistant cultivars, there was more variation in the most susceptible cultivar, including 'Black Corinth', 'Carignane', 'Husseine', and 'Thompson Seedless', regardless of the assay. Assay 1 with isolate BX1-10 was the most repeatable and provided data on wood and leaf symptoms for cultivar comparisons. Assay 2 was the most rapid, and gave results similar to those from Assay 1. Assay 2 also accommodated germplasm that can only be propagated from green cuttings.

Keywords. Grapevine Trunk Disease, disease resistance, *Vitis vinifera*.

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INTRODUCTION

Eutypa dieback of grapevine (*Vitis vinifera*), caused by the fungus *Eutypa lata* (Pers: Fr.) Tul and C. Tul. (syn. *E. armeniacae* Hansf. and M.V. Carter), is a chronic disease, which negatively impacts crop yield and vineyard longevity (Munkvold *et al.*, 1994; Creaser and Wicks 2000; Siebert

2001; Kaplan *et al.,* 2016). Wind-dispersed ascospores of *E. lata* infect grapevines through pruning wounds (Carter 1991). After infection, internal wood cankers form near the infected wounds. *Eutypa lata* colonizes all host xylem tissue types, utilizing structural glucose, xylose of hemicellulose, and starch as nutrition sources (Rolshausen *et al*., 2008; Rudelle *et al*., 2005). Similar to several *Diatrypaceae* [e.g., *Eutypella parasitica*, the canker pathogen of maple (Worrall *et al.,* 1997)], the type of wood decay caused by *E. lata* has been classified as a 'soft rot' (English and Davis 1978), a term currently applied to all forms of decay caused by Ascomycota (Goodell *et al.,* 2008).

Leaf symptoms of Eutypa dieback typically appear 3 to 8 years after infection, on shoots growing from fruiting positions near wood infections (Carter 1991). Most apparent between budbreak and bloom, stunted shoots have dwarfed, deformed leaves ('cup-shaped' or flattened, with veins growing in parallel, rather than the typical fan-shape vein orientations), and leaves have brown, necrotic margins. Symptomatic shoots may die late in the growing season, and then the entire fruiting position may die during the dormant season, with no shoot growth the following growing season. This is the 'dieback' symptom. The few flower inflorescences that form on symptomatic shoots frequently become scorched and fail to develop into fruit clusters (Moller and Kasimatis 1978). Over years, symptomatic vines accumulate dead fruiting positions and produce less fruit, which is how Eutypa dieback impacts vineyard longevity. In addition to wood degradation, the metabolites produced by *E. lata* (acetylenic phenols, such as eutypinol, eulatichromene, and eutypine) (Mauro *et al.,* 1988; Tey-Rulh *et al.,* 1991; Molyneux *et al.,* 2002; Mahoney *et al.,* 2003; Lardner *et al.,* 2006) are also probably important in the infection processes, as some are phytotoxic (Mahoney *et al.,* 2003; Rudelle *et al.,* 2005b). Further characterization of compounds produced *in vitro* by *E. lata* identified polypeptide compounds, including hydrolytic enzymes (Schmidt *et al.,* 1999; Rolshausen *et al.,* 2008). Leaf symptoms probably result, in part, from translocation of *E. lata* metabolites and polypeptide compounds via host vascular systems, from mycelium (in infected wood) to shoots, as indicated by detection of some of these compounds in shoots of symptomatic grapevines (Octave *et al.,* 2006a; Octave *et al.,* 2009). Although *E. lata* causes chronic wood infections, and consistent leaf symptoms would be expected, there are annual variations in symptom presence/severity (Sosnowski *et al.,* 2007b).

Vitis species and grapevine cultivars vary in susceptibility to Eutypa dieback (Dubos 1987; Péros and Berger 1994; Sosnowski *et al.,* 2007a; Travadon *et al.,* 2013; Moisy *et al.,* 2017; Sosnowski *et al.,* 2022). The traditional method for evaluating cultivar resistance and/or isolate virulence is based on measurements of internal wood symptoms or extent of wood colonization by the pathogen (Sosnowski *et al.,* 2007a; Travadon *et al.,* 2013). This method is repeatable, but can take up to 2 years for obtaining results, and is only applicable to host germplasm that roots from dormant cuttings. More rapid methods (also for host germplasm propagated from dormant cuttings) quantify fungal biomass in inoculated stems by quantitative real-time PCR (qPCR) (Moisy *et al.,* 2017), or simply rate leaf symptom severity (Péros and Berger 1994). However, leaf symptoms are not always correlated with the extent of wood colonization, but may be well-correlated with lengths of wood symptoms (Sosnowski *et al.,* 2007a) and/or pathogen biomass in the wood (Moisy *et al.,* 2017).

The objectives of the present study were to: (i) compare three previously used methods to evaluate grapevine cultivar resistance, as greenhouse 'phenotyping assays' for plants rooted from dormant and green cuttings, and (ii) evaluate relationships between leaf and stem symptoms for individual cultivars.

MATERIALS AND METHODS

Plant material

Nine grapevine cultivars were used, that represent the genetic diversity of *Vitis vinifera*, which was previously characterized based on a total of 366 accessions of the USDA-ARS National Clonal Germplasm Repository (Aradhya *et al.,* 2003). The cultivars 'Carignane', 'Primitivo', and 'Muscat Hamburg' represented Central European grapes, 'Thompson seedless', 'Husseine', and 'Black Corinth' represented Mediterranean table grapes, and 'Palomino', 'Merlot', and 'Peloursin' represented Western European wine grapes (Table 1, Supplementary Figure 1). These three groups correspond to eco-geographical groups (Negroul 1946) and morphological groups (Troshin *et al.,* 1990), which were previously defined, respectively, as *Pontica*, *Orientalis*, and *Occidentalis*. 'Merlot' is considered to be resistant, based on leaf symptoms of Eutypa dieback (Dubos 1987; Péros and Berger 1994), so was included as a resistant control. The cultivars with black fruit were 'Black Corinth', 'Carignane', 'Merlot', 'Muscat Hamburg', 'Peloursin', and 'Primitivo' (also known as 'Zinfandel'), while those with white fruit were 'Husseine', 'Palomino', and 'Thompson Seedless'.

Table 1. The nine *Vitis vinifera* cultivars phenotyped for resistance to Eutypa dieback, representing three genetic groups (Aradhya *et al.,* 2003). Accessions of each cultivar originated from the National Clonal Germplasm Repository, United States Department of Agriculture, Agricultural Research Service [Germplasm Resources Information Network (http://www.ars-grin.gov/)]. Plants for Assays 1 and 3 (see Materials and Methods) were propagated by the Baumgartner Laboratory. Plants for Assay 2 were propagated by Foundation Plant Services, University of California, Davis.

Genetic group	Cultivar	Clone	Accession number	Country of origin
Central European grapes	Carignane	3	DVIT 1064	Spain
	Muscat Hamburg	3	DVIT 1059	Germany
	Primitivo[a]	3	DVIT 1342	Croatia
Mediterranean table grapes	Black Corinth	2	DVIT 0354	Greece
	Husseine	2	PI 171099	Afghanistan
	Thompson Seedless	02A	DVIT 0535	Turkey
Western European wine grapes	Merlot	15	DVIT 0826	France
	Palomino	01A	DVIT 0882	Spain
	Peloursin	1	DVIT 0710	France

[a] Also known as 'Zinfandel'.

Phenotyping Assay 1 – Leaf symptoms (severity, incidence), shoot lengths, and woody-stem symptoms (lesion lengths) measured 1 year post-inoculation on plants propagated from rooted, dormant cuttings

Two replicate experiments began 2 weeks apart. In each experiment, plants were arranged in a randomized complete block design (RCBD) with two blocks, located in two separate greenhouses (Armstrong Plant Pathology Field Station, Davis, California, United States of America). Greenhouse temperatures were 25 ± 1°C (day), 18 ± 3°C (night), with natural photoperiod, unless noted otherwise. Plants were each watered daily for 15 min using a drip-irrigation system (0.5 L h^{-1}). Each block consisted of ten replicate plants per cultivar per each of three inoculation treatments (ten plants per cultivar × nine cultivars × three inoculation treatments × two blocks × two experiments = 1,080 total plants). The three inoculation treatments were as follows:

1. *Eutypa lata* isolate BX1-10. This is a virulent isolate, which has previously been used in studies in France (Péros and Berger 1994, 1999; Camps *et al.,* 2010; Moisy *et al.,* 2017; Cardot *et al.,* 2019). It originates from perithecia on dead wood of 'Cabernet-Sauvignon' from Bordeaux, France (Péros and Berger 1994). Inoculum consisted of mycelium fragments from liquid Potato Dextrose Broth (PDB; Difco) cultures (Travadon *et al.,* 2013).
2. *Eutypa lata* isolate M14. This is an isolate shown to be virulent in greenhouse studies (Travadon *et al.,* 2013). It is a mass-hyphal isolate from symptomatic wood of 'Merlot' from Napa, California, United States of America (Travadon *et al.,* 2012). Inoculum consisted of mycelium fragments from liquid PDB cultures (Travadon *et al.,* 2013).
3. Non-inoculated control. These plants were 'mock-inoculated' with sterile PDB).

Plants were each propagated from dormant cuttings, the woody stem of which was inoculated after callusing and at the time of planting. One-year-old dormant canes were cut into dormant cuttings of uniform length (≈ 30 cm) containing three nodes. The cuttings were surface-sterilized in 1% sodium hypochlorite (Clorox®) solution for 15 min and then rinsed in water overnight. The cuttings were then callused for 3 weeks in a mixture of perlite and vermiculite (1:1, v/v), at 30°C and 85% relative humidity. Once root and shoot initials emerged from the callus tissues, a power drill was used to wound each woody stem (2 mm width × 3 mm depth) at approx. 3 cm below the top node. Each cutting was then inoculated by pipetting 20 µL of liquid inoculum (1×10^6 mycelium fragments mL^{-1}) into the wound, which was then sealed with Vaseline® (Unilever) and Parafilm® (American National Can). Non-inoculated experimental controls were each 'mock-inoculated' with 20 µL of PDB. After inoculation, the cuttings were submerged in melted paraffin wax (Gulf Wax®, Royal Oak Enterprises) within 4 cm of the roots and were then potted in sterile potting mix ['UC mix' (Baker 1957)], amended with slow-release fertilizer (Osmocote® Pro 24-4-9, Scotts).

In a previously published assay that compared leaf symptoms and wood symptoms among *V. vinifera* cultivars (Sosnowski *et al.,* 2007a), inoculated plants were kept in an outdoor shadehouse (under more natural climate conditions than in a greenhouse) for 2 years. Leaf symptoms were visible on the new shoots that grew in spring, after a normal winter period of dormancy and winter pruning (i.e., at the start of the second 'growing season' for the potted grapevines). This assay was modified for the greenhouse and for 1 year, as follows:

1. Inoculated plants were grown for 7 months (from May to November, with natural light and at summer greenhouse temperatures of 24 to 27°C during the day and 16 to 22°C at night). Plants were watered twice per week for 15 min using a drip-irrigation system (0.5 L h^{-1}).
2. Shoots were pruned to two buds, and plants were forced into a winter period of dormancy for 3 months (from December to February, with natural light and at winter greenhouse temperatures of 10 to 13°C during the day and 3 to 6°C at night).

3. Plants were brought out of dormancy by returning to summer greenhouse temperatures (as 1., above). Leaf symptoms were rated on the new shoots that grew during the final 2 months of the assay (from February to April).

Following budbreak in February, the plants were monitored for the presence of leaf symptoms, and final assessments were made in April, at 1 year post-inoculation (after approx. 6 to 8 weeks of shoot growth). Severity of leaf symptoms was rated visually on a scale of 0 to 5, using an ordinal scale adapted from that of Péros and Berger (1994) (Figure 1). The lengths of the green shoots were measured at 1 year post-inoculation. Also at this time, internal lesions were measured in the woody stems. Plants were removed from the soil, roots and shoots were excised, and bark was scraped from their woody stems. The stems were surface sterilized in 1% sodium hypochlorite solution for 2 min, and then rinsed with tap water. The lengths of the woody stems were measured, and each stem was then cut longitudinally and the length of internal wood discolouration extending above and below the inoculation site (i.e., lesion length) was measured with an electronic caliper.

To evaluate the extent of wood colonization by mycelium of each isolate, attempts were made to recover the pathogen from a subset of plants in each treatment. Four small pieces of wood (each approx. 5 × 2 mm) were cut from the woody stem with a flame-sterilized scalpel at 0, 2, 4, and 6 cm below each inoculation site, and at the lower margin of the lesion if present at > 60 mm below the inoculation site. Wood pieces were then surface-sterilized for 1 min in 0.6% sodium hypochlorite solution (pH 7.2), rinsed twice (1 min each) in sterile distilled water, and then incubated on PDA amended with 0.01% tetracycline hydrochloride for 2 weeks.

Statistical analysis. All statistical analyses were conducted using SAS v. 9.4 (SAS Institute). ANOVAs were carried out for the main and interactive effects of each experiment (1 or 2), block (1 or 2), inoculation treatment (control, BX1-10, or M14), and cultivar ('Black Corinth', 'Carignane', 'Husseine', 'Merlot', 'Muscat Hamburg', 'Palomino', 'Peloursin', 'Primitivo', or 'Thompson Seedless'), on lesion length, shoot length, and incidence of leaf symptoms. ANOVAs were carried out using the MIXED procedure, with all effects considered as fixed, except for the main and interaction effects of experiment and block (random effects). Normality was assessed using normal probability plots and homogeneity of variances was evaluated using Levene's test. Transformations of lesion lengths ($\log_{10}$) and shoot lengths (square root) were used to meet parametric assumptions. For statistical significance (F values with $P < 0.05$), means were compared using the LSMEANS procedure. P-values and 95% confidence limits for mean differences were adjusted for multiple comparisons using the Tukey-Kramer method ($\alpha = 0.05$).

Because the severity of leaf symptoms was rated on an ordinal scale, non-parametric analysis with PROC MIXED (Shah and Madden 2004) was used to determine the main and interaction effects on leaf-symptom ratings of experiment, block, inoculation treatment, and cultivar. Rather than comparing the mean for each cultivar (as is done for continuous data), the measure used for comparison of leaf symptom ratings in this non-parametric analysis was the relative treatment effect and its corresponding 95% confidence interval. Relative treatment effects were calculated from the PROC MIXED lsmeans, using the LD_CI macro, which uses rank

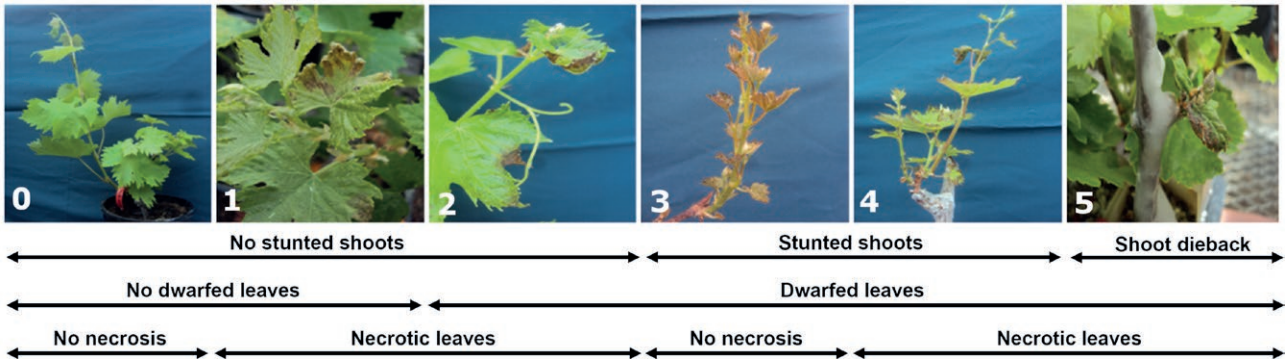

Figure 1. Leaf symptom severity scale used to assess grapevine shoots that were 6 to 8 weeks old, in Assays 1 and 3. 0 = no symptoms ('Muscat Hamburg'); 1 = normal-sized leaves and shoots, but some leaves have necrotic (brown) margins ('Muscat Hamburg'); 2 = normal shoot length, but some leaves dwarfed and/or with necrotic margins ('Thompson Seedless'); 3 = stunted shoots with dwarfed leaves, but no leaves with necrotic margins ('Black Corinth'); 4 = stunted shoots with dwarfed leaves, and some leaves dwarfed with necrotic margins ('Primitivo'); and 5 = no shoot growth or stunted shoot that grew and then died ('Merlot').

transformations of the medians as a basis for calculating the relative treatment effects (Brunner *et al.*, 2002). For statistically significant effects (ANOVA F values with $P < 0.05$), relative treatment effects without overlapping confidence intervals were considered significantly different ($\alpha = 0.05$). Correlations among the relative treatment effects of the leaf symptom ratings, the incidence of leaf symptoms, and lesion lengths were determined for each inoculation treatment × cultivar combination, using the CORR procedure in SAS based on the Spearman rank-order correlation (i.e., a non-parametric measure of association, based on the ranks of the data values).

Phenotyping Assay 2 – Shoot lengths and green-stem symptoms (lesion lengths, incidence of external cankers) measured 4 months post-inoculation on plants propagated from rooted, green cuttings

The experimental design and the three inoculation treatments were the same as used in Assay 1. In contrast to Assay 1, inoculum for Assay 2 was grown on solid medium (PDA) and 3 mm diam. agar plugs were used for inoculations. Plants were propagated from green cuttings, the green stems of which were inoculated after the cuttings were rooted. After fruit set in June, green shoots were cut from grapevines in the field, with each shoot of uniform length, containing two nodes (≈ 10 cm long × 0.8 cm diam.). The basal bud was removed, and one leaf at the top node was retained (trimmed to a leaf area ≈ 2 cm^2). Green cuttings were then rooted for 2 to 3 weeks in a mixture of perlite and vermiculite (1:1, v/v), with natural light, at greenhouse temperatures of 24 to 27°C during the day and 16 to 22°C at night, under a mist system with mist sprayed from the top of the greenhouse for 5 sec every 2 min (during daylight hours). After roots formed (during the 2 to 3 weeks in the mist system), plants were removed from the mist system, transplanted into a mixture of peat, sand, and perlite (1:1:1, v/v/v), and grown for 1 month in the greenhouse. Then, approx. 2 months after the green cuttings were made (August), a 3 mm diam. cork borer was used to wound each plant's green stem at approx. 3 cm below the uppermost node. To inoculate the green stems, a 3 mm agar plug from the margin of a 5 d PDA culture was inserted into each wound, and sealed with Vaseline® and Parafilm®. Non-inoculated controls were 'mock-inoculated' with sterile PDA.

At 4 months post-inoculation (December), shoot lengths and lesion lengths were measured, and the presence/absence of cankers was assessed. Each green shoot that grew over the 6 months of the experiment (2 months pre-inoculation to 4 months post-inoculation) from the top of the green stem was measured. Presence of an external canker was noted, visible as an area of the stem surface that was desiccated, dry, and brown. Plants were removed from the peat/sand/Perlite medium, and their roots and shoots were excised. Stems were surface sterilized in 1% sodium hypochlorite solution for 2 min, and rinsed with tap water. The length of each stem was measured, and the stem was cut longitudinally and the length of internal discolouration extending above and below the point of inoculation was measured with an electronic caliper. In order to confirm infection, attempts were made to recover the pathogen from each inoculation site of all the inoculated plants, using the methods described above.

Statistical analysis. All statistical analyses were conducted in SAS v. 9.4. ANOVAs were carried out for the main and interaction effects of experiment (1 or 2), block (1 or 2), inoculation treatment (control, BX1-10, or M14), and cultivar ('Black Corinth', 'Carignane', 'Husseine', 'Merlot', 'Muscat Hamburg', 'Palomino', 'Peloursin', 'Primitivo', or 'Thompson Seedless'), for lesion length, shoot length, and incidence of cankers. ANOVAs were carried out using the MIXED procedure, with all effects considered as fixed, except for the main and interaction effects of experiment and block (random effects). Normality of data was evaluated using normal probability plots, and homogeneity of variances was assessed using Levene's test. Transformation of lesion lengths (reciprocal square root) was used to meet parametric assumptions. For significant effects (F values with $P < 0.05$), means were compared using the LSMEANS procedure. P-values and 95% confidence limits for mean differences were adjusted for multiple comparisons using the Tukey-Kramer method ($\alpha = 0.05$). Correlations among shoot lengths, incidence of cankers, and lesion lengths were determined for each inoculation treatment × cultivar combination, using the CORR procedure in SAS, based on the Spearman rank-order correlation (i.e., a non-parametric measure of association, based on the ranks of the data values).

Phenotyping Assay 3 – Leaf symptoms (severity, incidence) and shoot lengths measured 6 weeks post-inoculation on plants propagated from rooted, dormant cuttings

The experimental design and the three inoculation treatments were the same as those used in Assay 2 (above). Inoculum was grown on solid medium (PDA) and 3 mm diam. agar plugs were used for inoculations. Plants were propagated from dormant cuttings, but, in contrast to Assay 1, the cuttings were inoculated and immediately planted without first callusing or rooting. The plants for Assays 1 and 3 were propagated from the same vines. However, these assays were not carried out at the same time because there was not enough cane

wood on the vines for all the cuttings required of both assays and available greenhouse space was not sufficient for both assays together.

Cuttings (≈ 15 cm long) from 1-year-old dormant canes were harvested from dormant field-grown grapevines, and the basal buds were removed. The cuttings were then surface-sterilized in 1% sodium hypochlorite for 15 min and rinsed in water overnight. The cuttings were then stored in plastic bags at 4°C for 2 months. A 3 mm diam. cork borer was then used to wound each cutting at approx. 3 cm below the top node. A 3 mm diam. agar plug from the margin of a 5 d PDA culture of either *E. lata* isolate was then inserted into the wound, and sealed with Vaseline® and Parafilm®. Non-inoculated controls were 'mock-inoculated' with sterile PDA. After inoculation, cuttings were submerged in melted paraffin wax within 4 cm of the roots and the cuttings were potted in a mix of perlite and vermiculite (1:1, v/v) in plant bands (5 × 5 × 20 cm; Monarch Manufacturing Inc.), which were held in plastic trays (35 × 35 × 15 cm; 49 plants per tray) placed on top of rooting mats to promote root growth at 24°C. At 4 to 6 weeks post-inoculation, following budbreak, severity of leaf symptoms on the new shoots of each cutting was rated visually using the 0 to 5 scale (Figure 1). The length of the green shoot emerging from the node above the inoculation site was also measured.

Statistical analyses. All statistical analyses were conducted in SAS v. 9.4. ANOVAs were carried out on data of shoot length and incidence of leaf symptoms, for the main and interactive effects of experiment (1 or 2), block (1 or 2), inoculation treatment (control, BX1-10, or M14), and cultivar ('Black Corinth', 'Carignane', 'Husseine', 'Merlot', 'Muscat Hamburg', 'Palomino', 'Peloursin', 'Primitivo', or 'Thompson Seedless'). ANOVAs were carried out using the MIXED procedure, with all effects considered as fixed, except for the main and interaction effects of experiment and block (random effects). Normality of data was evaluated using normal probability plots, and homogeneity of variances were was evaluated using Levene's test. Transformation of shoot lengths (square root) was used to meet parametric assumptions. For significant effects (F values with $P < 0.05$), means were compared using the LSMEANS procedure. P-values and 95% confidence limits for mean differences were adjusted for multiple comparisons using the Tukey-Kramer method ($\alpha = 0.05$). The same methods described (above) for Assay 1 were used to calculate and analyze leaf symptom severity. Correlations among the relative treatment effects of the leaf symptom severity, incidence of leaf symptoms, and shoot lengths were determined for each inoculation treatment × cultivar combination using the CORR procedure in SAS, based on the Spearman rank-order correlation (i.e., a non-parametric measure of association, based on the ranks of the data values).

RESULTS

Phenotyping Assay 1 – Leaf symptoms (severity, incidence), shoot lengths, and woody-stem symptoms (lesion lengths) measured 1 year post-inoculation on plants propagated from rooted, dormant cuttings

Mean lesion lengths for inoculated plants varied among cultivars ($P < 0.0001$). The effect of isolate on either measure of lesion length was not significant ($P = 0.5$), nor was the interaction of cultivar × inoculation treatment ($P > 0.3$). For plants inoculated with either isolate, 'Primitivo' had the smallest lesions compared to 'Thompson Seedless', which had the largest lesions. All the other cultivars had intermediate mean lesion lengths (Table 2). In addition to 'Thompson Seedless' having the longest lesions, the pathogen colonized the stems of this cultivar far beyond the lesion margins, compared to all the other cultivars. Maximum recovery distances below the inoculation sites of 'Thompson Seedless' were 219 mm for plants inoculated with BX1-10, and 215 mm from M14 inoculations.

Comparing lesion lengths of plants inoculated with each isolate, relative differences among cultivars were consistent for those with the smallest lesions ('Primitivo', 'Merlot') and for those with the largest lesions ('Thompson Seedless', 'Husseine', 'Carignane', 'Black Corinth'; Table 2). Positive recovery of each isolate was similar for the two isolates, with recovery rates ranging from 43 to 82% for BX1-10 and 25 to 73% for M14 (Table 2). Mean lesion lengths of the non-inoculated controls were < 10 mm.

Incidence of leaf symptoms (% symptomatic plants) varied among the cultivars ($P = 0.0003$) and between the two isolates ($P = 0.009$), although the interaction effect of cultivar × inoculation treatment was not significant ($P = 0.2$). Based on the results of a non-parametric analysis of the relative treatment effects (RTEs), there was a significant interaction effect of cultivar × inoculation treatment for leaf symptom severity ratings ($P = 0.01$). 'Black Corinth' and 'Husseine' had consistently high RTEs and a high incidence of leaf symptoms, regardless of pathogen isolate (Table 3). Non-inoculated control plants showed no leaf symptoms (median = 0), which amounted to an RTE of 0.39. However, for cultivar × inoculation treatment combinations with the greatest incidence of leaf symptoms (cvs 'Black Corinth' and 'Husseine'), only 45 to 54% of plants had leaf symptoms. As such, the majority of cultivar × inoculation treatment combina-

Table 2. Assay 1. Mean internal woody stem lesion lengths of rooted, dormant cuttings, at 1 year post-inoculation, for plants inoculated with either *Eutypa lata* isolate BX1-10 or isolate M14. Each value is the mean of 17 to 37 observations, summed across two replicate experiments, with two blocks per experiment. Means for each inoculation treatment accompanied by different letters are significantly different (Tukey's test, $P > 0.01$, $\alpha = 0.05$).

Inoculation treatment	Cultivar	Total plants	Mean lesion length (mm)	Recovery attempts (No. plants)	Farthest recovery distance below inoculation site[a] (mm)									No. positive plants (% recovery attempts)
					0	10	20	30	40	50	60	>60	Max	
BX1-10	Primitivo	31	15.0a	11	1	1	2	0	0	0	2	0	60	6 (55%)
	Merlot	31	20.1ab	10	0	0	2	0	2	0	1	0	60	5 (50%)
	Muscat Hamburg	32	22.7ab	11	1	0	3	0	3	0	1	1	117	9 (81%)
	Peloursin	29	23.8ab	12	0	0	1	1	3	0	1	2	76	8 (67%)
	Palomino	26	31.0ab	15	0	0	2	2	3	0	1	0	60	8 (53%)
	Black Corinth	32	31.8ab	17	0	0	1	2	1	0	2	4	121	10 (59%)
	Carignane	22	37.3ab	13	0	0	0	0	5	0	1	1	80	7 (54%)
	Husseine	26	38.3ab	17	2	0	4	3	1	2	1	1	110	14 (82%)
	Thompson Seedless	28	67.9b	23	0	0	1	1	1	1	1	5	219	10 (43%)
M14	Primitivo	31	17.8a	12	2	1	1	0	2	0	1	0	60	7 (58%)
	Merlot	28	18.4ab	10	2	0	2	1	1	0	0	0	40	6 (60%)
	Peloursin	38	20.6ab	10	1	0	2	0	0	0	1	0	60	4 (40%)
	Palomino	23	23.5ab	11	0	0	1	0	2	0	0	2	74	5 (45%)
	Muscat Hamburg	29	25.3ab	9	0	0	1	1	3	0	1	0	60	6 (67%)
	Black Corinth	34	25.9ab	15	0	0	5	1	3	0	2	0	60	11 (73%)
	Carignane	29	31.1ab	19	0	0	1	1	3	0	2	0	60	7 (37%)
	Husseine	28	39.7ab	17	1	0	3	0	0	2	1	1	76	8 (47%)
	Thompson Seedless	22	84.9b	20	0	0	1	0	0	0	1	3	215	5 (25%)

[a] Means for each distance are numbers of plants from which *E. lata* was recovered from the inoculation site (0 cm) or below.

tions gave median leaf symptom values of 0; hence the utility of RTE for statistical comparisons. Plants inoculated with isolate BX1-10 had a greater incidence than those inoculated with M14, for seven of nine evaluated cultivars (Table 3).

The cultivars 'Black Corinth' and 'Husseine' had the greatest incidence and RTEs for leaf symptoms, regardless of pathogen isolate, but this was the only consistent trend in relative resistance among cultivars between the isolates (Table 3). In contrast, cultivars with the least incidences and RTEs of leaf symptoms varied between the two isolates ('Merlot' for BX1-10 and 'Primitivo' for M14). Strength of the association between mean lesion lengths and RTEs of leaf symptoms was significant for plants inoculated with BX1-10 (Spearman correlation coefficient of $r = 0.83$, $P = 0.006$), but not for those inoculated with M14 (Spearman correlation coefficient of $r = 0.55$, $P = 0.1$; Figure 2). In spite of the consistently large lesions on plants inoculated with either isolate, 'Thompson Seedless' had among the greatest RTEs (0.65) when inoculated with isolate BX1-10, but among the least (0.49) when inoculated with isolate M14 (Table 2).

Mean shoot lengths were significantly different ($P > 0.0001$) among the cultivars (Table 3). This was the only significant effect of the treatments on shoot length. There was a trend, though not statistically significant, for shorter shoot lengths of inoculated plants compared to the non-inoculated controls for all cultivars except 'Muscat Hamburg' and 'Palomino'.

Phenotyping Assay 2 – Shoot lengths and green-stem symptoms (lesion lengths, incidence of external cankers) measured 4 months post-inoculation on plants propagated from rooted, green cuttings

Mean lesion lengths for inoculated plants varied among the cultivars and between the two isolates (interaction effect of cultivar × inoculation treatment, $P = 0.04$). 'Merlot', 'Muscat Hamburg', and 'Primitivo' had the smallest lesions, from both isolates (Table 4). Consistently intermediate in mean lesion lengths, regardless of isolate, were 'Black Corinth' and 'Palomino'. Depending on the isolate, 'Husseine' or 'Carignane' had larger lesions than 'Merlot'. 'Peloursin' and 'Thompson Seed-

Table 3. Assay 1. Mean incidence of leaf symptoms (% symptomatic plants of total inoculated), leaf symptom severity (median, relative treatment effects), and mean lengths of shoots from rooted, dormant cuttings, at 1 year post-inoculation, for grapevine plants inoculated with either *Eutypa lata* isolate BX1-10 or isolate M14. Each value is the mean of 17 to 37 observations, summed across two replicate experiments, with two blocks per experiment. Means for each inoculation treatment accompanied by different letters are significantly different [based on Tukey's test ($P > 0.01$, $\alpha = 0.05$) for mean incidences of leaf symptoms and mean shoot lengths; based on no overlap of 95% confidence intervals for relative treatment effects].

Inoculation treatment	Cultivar	Mean incidence of leaf symptoms (% symptomatic plants)	Leaf symptom severity		Mean shoot length (mm)[b]
			Median[a]	Relative treatment effect (RTE)	
BX1-10	Merlot	5.6 a	0	0.43 a	353.0 bc
	Muscat Hamburg	23.7 ab	0	0.49 ab	278.7 ab
	Primitivo	28.4 ab	0	0.54 ab	404.9 bc
	Palomino	41.1 b	0	0.61 b	367.8abc
	Carignane	46.5 b	0	0.63 b	216.1 a
	Peloursin	47.3 b	0	0.62 b	494.5 c
	Thompson Seedless	52.5 b	1.5	0.65 b	283.0 abc
	Husseine	53.6 b	1	0.65 b	303.9 abc
	Black Corinth	54.2 b	1	0.66 b	246.4 ab
M14	Primitivo	11.9 a	0	0.45 a	447.0 cd
	Thompson Seedless	14.4 ab	0	0.49 ab	291.1 abc
	Palomino	19.0 ab	0	0.50 ab	423.2 bcd
	Merlot	19.4 ab	0	0.47 ab	294.8 abc
	Muscat Hamburg	21.9 ab	0	0.51 ab	287.7 abc
	Peloursin	30.0 ab	0	0.53 ab	559.2 d
	Carignane	36.5 ab	0	0.56 ab	239.7 ab
	Husseine	45.0 ab	0.5	0.64 b	248.1 ab
	Black Corinth	53.6 b	0.5	0.65 b	193.5 a

[a] Leaf symptom severity was assessed visually using a 0 to 5 scale, where 0 = no symptoms and 5 = no shoot growth or stunted dead shoot (See Figure 1).

[b] Mean lengths of the new green shoots that grew from the top of rooted, dormant cuttings were measured 1 year post-inoculation, although each shoot grew for 2 months.

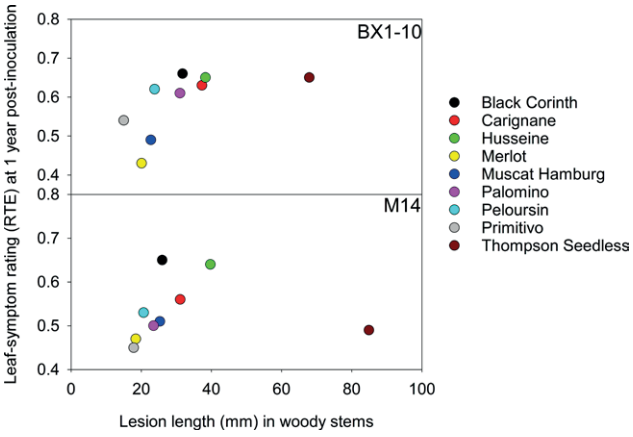

Figure 2. Assay 1. Relationships between mean lengths of internal lesions in woody stems of nine grapevine cultivars and mean leaf symptom severity ratings [relative treatment effect (RTE)], at 1 year post-inoculation, from plants inoculated with either *Eutypa lata* isolate BX1-10 or isolate M14.

less' had different mean lesion lengths between the two isolates, with both cultivars having lesion lengths either intermediate or large (relative to 'Merlot'), depending on the isolate. For plants inoculated with isolate BX1-10, all cultivars had larger lesions than those inoculated with M14, except for cvs 'Peloursin' and 'Husseine'. Mean lesion lengths of non-inoculated controls were < 10 mm. Cultivars with the smallest lesions in Assays 1 and 2 were 'Primitivo', 'Merlot', and 'Muscat Hamburg' (Figure 3). Mean lesion lengths were similar in both assays, in spite of the tripled incubation period for Assay 1, for all the cultivars, except 'Thompson Seedless', which developed much smaller lesions in Assay 2 (mean lesion lengths of 22.1 to 31.9 mm, depending on the isolate; Table 4), than in Assay 1 (mean lesion lengths of 67.9 to 84.9 mm, depending on the isolate; Table 2).

No leaf symptoms developed in this assay. However, external cankers were visible on the surfaces of the green stems, with mean incidence of cankers of ≥ 50%

Table 4. Assay 2. Mean internal green stem lesion lengths, mean incidence of external cankers on the surfaces of green stems, and mean lengths of shoots of rooted, green cuttings at 4 months post-inoculation, for grapevine plants inoculated with either *Eutypa lata* isolate BX1-10 or isolate M14. Each value is the mean of 18 to 31 observations, summed across two replicate experiments, with two blocks per experiment. Means for each inoculation treatment, in each column, accompanied by different letters are significantly different (Tukey's test, $P > 0.01$, $\alpha = 0.05$).

Inoculation treatment	Cultivar	Mean lesion length (mm)	Mean incidence of cankers (% plants)	Mean shoot length (mm)[a]
BX1-10	Merlot	11.9 a	0 a	234.9 a
	Muscat Hamburg	16.4 ab	21 ab	265.2 ab
	Primitivo	22.1 bc	43 bc	344.4 bc
	Peloursin	22.4 bc	37 bc	374.4 bc
	Black Corinth	26.7 bc	69 c	318.3 b
	Palomino	28.5 bc	70 c	291.9 ab
	Husseine	29.7 c	53 bc	330.2 bc
	Thompson Seedless	31.9 c	59 bc	407.3 c
	Carignane	36.4 c	76 c	291.5 ab
M14	Merlot	11.0 a	0 a	273.1 ab
	Muscat Hamburg	15.3 ab	17 ab	288.6 ab
	Primitivo	19.4 bc	27 bc	343.1 ab
	Thompson Seedless	22.1 bc	43 bc	481.0 c
	Black Corinth	22.6 bc	41 bc	311.0 ab
	Palomino	23.9 bc	43 bc	266.6 a
	Carignane	24.6 bc	50 bc	357.7 b
	Peloursin	29.4 c	61 c	316.1 ab
	Husseine	35.1 c	60 c	283.9 ab

[a] Mean lengths of the new green shoots that grew from the top of rooted, green cuttings were measured after 6 months (2 months pre-inoculation plus 4 months post-inoculation).

for five of the nine cultivars inoculated with isolate BX1-10 and three of the cultivars inoculated with M14 (Table 4). There was an interaction cultivar × inoculation effect ($P = 0.04$) on incidence of cankers (% plants). Plants inoculated with isolate BX1-10 had greater incidence of cankers than those inoculated with M14, except for cvs 'Peloursin' and 'Husseine' (Table 4). Regardless of isolate, 'Merlot' had no cankers on inoculated plants, and 'Muscat Hamburg' had the lowest incidence of cankers, but this was the only consistent trend in relative resistance among cultivars between isolates. For plants inoculated with isolate BX1-10, 'Carignane', 'Palomino', and 'Black Corinth' had greater incidence of cankers than 'Merlot'. For plants inoculated with M14, 'Peloursin', 'Husseine'', and 'Carignane' had greater incidence of cankers than 'Merlot'. The relationships between lesion lengths (mm) and incidence of cankers was significant and positive for

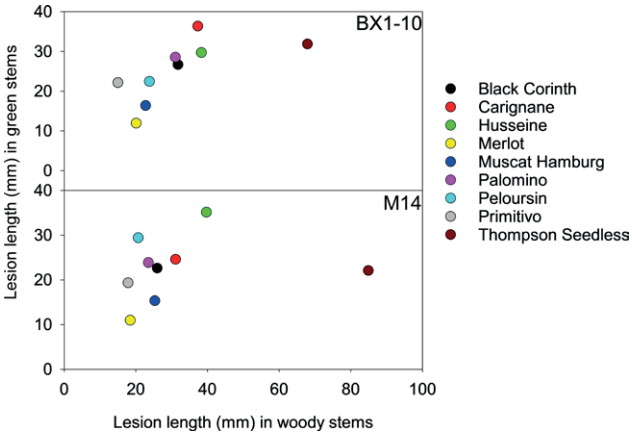

Figure 3. Relationships for nine grapevine cultivars, between mean lengths of internal lesions in the woody stems at 1 year post-inoculation (Assay 1), versus mean lengths of internal lesions in their green stems, at 6 weeks post-inoculation (Assay 2). Plants in both assays were inoculated with either *Eutypa lata* isolate BX1-10 or isolate M14.

plants inoculated with BX1-10 (Spearman correlation coefficient of $r = 0.85$, $P = 0.004$) or M14 (Spearman correlation coefficient of $r = 0.93$, $P = 0.0002$), so there was a trend for cultivars with long lesions to also have a high incidence of cankers.

There was a significant interaction cultivar × inoculation treatment effect ($P = 0.007$) on shoot lengths. In comparison to shoot lengths of the non-inoculated controls, the only consistency between isolates was for 'Muscat Hamburg', which had shorter shoots (though not statistically significant) than those of the non-inoculated controls. There was no significant association between shoot lengths and lesion lengths (for both pathogen isolates; Spearman correlation coefficients of $r > 0.1$, $P > 0.3$) or incidence of cankers (for both isolates, Spearman correlation coefficients of $r > 0.2$, $P > 0.4$).

Phenotyping Assay 3 – Leaf symptoms (severity, incidence) and shoot lengths measured 6 weeks post-inoculation on plants propagated from rooted, dormant cuttings

Many of the plants in this Assay did not develop shoots or roots (and were dead) before the 6-week post-inoculation period was reached, so disease data could not be determined for these plants. This contributed to low sample sizes of 13 to 32 across replicate experiments. There were high levels of mortality among all three inoculation treatments for 'Merlot' (49%), 'Black Corinth' (39%), and 'Muscat Hamburg' (17%).

Incidence of leaf symptoms (% symptomatic plants) varied among cultivars ($P = 0.0003$) and between isolates

Table 5. Assay 3. Mean incidence of leaf symptoms (% symptomatic plants of total inoculated), leaf symptom severity (median, relative treatment effects), and mean lengths of shoots from dormant cuttings, at 6 weeks post-inoculation, for grapevine plants inoculated with either *Eutypa lata* isolate BX1-10 or isolate M14. Each value is the mean of 13 to 32 observations, summed across two replicate experiments, with two blocks per experiment. Means for each inoculation treatment, in each column, accompanied by different letters are significantly different [based on Tukey's test ($P > 0.01$, $\alpha = 0.05$) for mean incidences of leaf symptoms and mean shoot lengths; based on no overlap of 95% confidence intervals for relative treatment effects].

Inoculation treatment	Cultivar	Mean incidence of leaf symptoms (% symptomatic plants)[a]	Leaf symptom severity		Mean shoot length (mm)[b]
			Median[a]	Relative treatment effect (RTE)	
BX1-10	Primitivo	9.4 a	0	0.35 a	74.1 bc
	Carignane	26.8 ab	0	0.43 ab	53.7 abc
	Husseine	28.6 ab	0	0.44 ab	86.9 c
	Thompson Seedless	35.0 ab	0	0.46 ab	89.0 c
	Palomino	37.5 ab	0	0.49 bc	49.0 ab
	Peloursin	55.2 bc	2.5	0.62 bc	70.9 bc
	Muscat Hamburg	67.9 bc	2	0.64 c	54.9 abc
	Black Corinth	73.3 bc	2.5	0.68 c	29.9 a
	Merlot	79.2 c	3	0.66 c	47.2 ab
M14	Primitivo	18.8 a	0	0.40 a	64.6 bc
	Thompson Seedless	24.6 a	0	0.43 a	62.9 bc
	Palomino	25.9 a	0	0.43 a	48.4 ab
	Carignane	32.7 a	0	0.48 ab	53.7 ab
	Husseine	33.5 a	0	0.45 a	91.6 c
	Peloursin	40.6 ab	0	0.52 ab	77.6 bc
	Merlot	44.9 ab	0	0.53 ab	62.0 abc
	Muscat Hamburg	47.2 ab	0	0.56 ab	56.7 abc
	Black Corinth	71.3 b	4	0.71 bc	26.3 a

[a] Leaf symptom severity was assessed visually using a 0 to 5 scale, where 0 = no symptoms and 5 = no shoot growth or stunted dead shoot (See Figure 1).
[b] Mean lengths of the new green shoots that grew from the top of rooted, dormant cuttings were measured after 6 weeks.

($P = 0.04$), although the cultivar × inoculation treatment interaction was not significant ($P = 0.2$). There was a significant effect of cultivar on RTE ($P > 0.0001$), with no effects of inoculation treatment ($P = 0.2$) or cultivar × inoculation treatment ($P = 0.5$). 'Primitivo' had the least RTE and incidence of leaf symptoms, regardless of isolate (Table 5). 'Black Corinth' and 'Muscat Hamburg' had the greatest RTEs and incidences of leaf symptoms, regardless of isolate, and similarly for 'Merlot' plants inoculated with BX1-10. Plants inoculated with BX1-10 had greater incidences than those inoculated with M14 for six of nine assessed cultivars.

Comparing RTEs measured 1 year post-inoculation in Assay 1 (from shoots that were 8 weeks old) with those measured at 6 weeks post-inoculation in Assay 3 (Figure 4), these values were similar for 'Primitivo' and 'Black Corinth'. These two cultivars also ranked similarly in both assays, although at opposite extremes. 'Primitivo' had the lowest RTE from isolate M14 in Assay 1 (Table 3) and the lowest RTEs, regardless of isolate, in

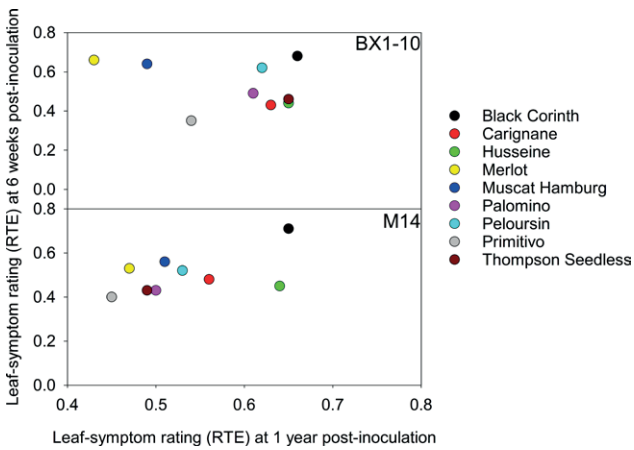

Figure 4. Relationships for nine grapevine cultivars, between mean leaf symptom ratings [relative treatment effect (RTE)], at 1 year post-inoculation (Assay 1), versus mean leaf symptom ratings, 6 weeks post-inoculation (Assay 3). Plants in both assays were inoculated with either *Eutypa lata* isolate BX1-10 or isolate M14.

Assay 3 (Table 5). 'Black Corinth' had the greatest RTEs, regardless of isolate, in Assay 1 (Table 3), and the greatest RTE from M14 in Assay 3 (Table 5). In contrast, 'Merlot' and 'Muscat Hamburg' were ranked very differently in the two assays (Figure 4). 'Merlot' had the least RTE from BX1-10 in Assay 1 (Table 3), but the greatest RTE from BX1-10 in Assay 3 (Table 5). 'Muscat Hamburg' had low to intermediate RTEs, depending on isolate, in Assay 1 (Table 3), but high RTEs, regardless of isolate, in Assay 3 (Table 5).

Mean shoot lengths were different (P = 0.001) among the cultivars, but not between inoculation treatments (P = 0.7), and the cultivar × inoculation treatment interaction was not significant (P = 0.4). There was a trend (though not statistically significant) for shorter shoot lengths of inoculated plants compared to those of the non-inoculated controls for all cultivars except 'Primitivo'. 'Black Corinth' had the shortest shoot lengths, regardless of pathogen isolate, and this corresponded with the greatest RTEs (Table 5). However, the strength of the association between mean shoot lengths and the RTEs, when considered among all the cultivars, was not statistically significant for plants inoculated with either isolate.

DISCUSSION

The grapevine cultivar 'Primitivo' was consistently the most resistant of the nine grapevine culivars assessed, to Eutypa dieback, or among the most resistant cultivars, depending on *E. lata* isolate. This was true for results from Assay 1 (short lesion lengths, low RTE, low incidence of leaf symptoms), from Assay 2 (short lesion lengths, low incidence of cankers), and from Assay 3 (low RTE, low incidence of leaf symptoms). These results of minimal symptoms on different plant organs (stems and leaves), for different tissue inoculations (green stems and woody stems), between two isolates (BX1-10 and M14), for different incubation periods (6 weeks, 4 months, or 1 year), and for plants propagated from different types of cuttings (green or dormant), indicate that 'Primitivo' is resistant to Eutypa dieback. The resistance of 'Primitivo' (aka 'Zinfandel') to Eutypa dieback possibly explains why this cultivar is surviving in Northern California, where vineyards planted decades ago are still in production.

The cultivar 'Merlot' ranked differently between Assays 1 and 3. 'Merlot' was most resistant, or among the most resistant depending on the isolate, in Assay 1 (short lesion lengths, low RTE, low incidence of leaf symptoms) and in Assay 2 (short lesion lengths, low incidence of cankers), but was very susceptible, regardless of isolate, in Assay 3 (high RTE, high incidence of leaf symptoms). 'Merlot' has been reported as resistant to Eutypa dieback, based on little to no leaf symptoms in separate field surveys conducted in Europe and Australia (Dubos 1987; Sosnowski *et al.,* 2022). Although the differences were not as great as for 'Merlot', 'Muscat Hamburg' also ranked differently between Assays 1 and 3. Consistently for the two *E. lata* isolates, 'Muscat Hamburg' was moderately resistant, based on the results from Assays 1 and 2 (short lesion lengths in woody and green stems), and based on the results from Assay 2 (intermediate RTE, intermediate incidence of leaf symptoms), but was susceptible, based on the results from Assay 3 (high RTEs, high incidence of leaf symptoms). Although Assay 3 was convenient, with no plant rooting necessary and a short (4 to 6 weeks) incubation period, the very different results for 'Merlot' (and to a lesser extent for 'Muscat Hamburg') for leaf symptoms compared to Assay 1 make it difficult to rely on the results obtained from Assay 3. Cuttings are not callused or rooted before inoculation in Assay 3, and the mortality rate of especially 'Merlot' was high, with no roots developing among a high proportion (49%) of cuttings. With low sample sizes among the non-inoculated and inoculated plants, it was difficult to evaluate whether the incidence of leaf symptoms and RTEs were representative of the host responses to infection of 'Merlot' and 'Muscat Hamburg'. Because cuttings for Assays 1 and 3 were obtained in different years, it is possible the low viability of 'Merlot' and 'Muscat Hamburg' cuttings for Assay 3 was specific to the field conditions in the year the cuttings were collected, possibly caused by low carbohydrate reserves. Regardless, Assay 3 did not provide results as definitive as obtained from Assays 1 and 2.

Consistently between the two *E. lata* isolates, 'Black Corinth' was moderately susceptible (intermediate lesion lengths in woody and green stems), based on the results from Assays 1 and 2, but was highly susceptible (high RTEs, high incidence of leaf symptoms), based on Assays 1 and 3 results. Wood symptoms probably result, in part, from pathogen enzymatic activities at infections (Rolshausen *et al.,* 2008; Blanco-Ulate *et al.,* 2013; Morales-Cruz *et al.,* 2015). Leaf symptoms may be affected by translocation of pathogen metabolites and polypeptides from the infected wood to shoots (Mahoney *et al.,* 2003; Smith *et al.,* 2003; Octave *et al.,* 2006a). These compounds modify mitochondrial, plastid and plasma membranes of grapevine cells (Deswarte *et al.,* 1996; Amborabé *et al.,* 2001; Octave *et al.,* 2006b), affect chloroplast structure (Deswarte *et al.,* 1994), alter cell nutrient uptake by inhibiting proton flux at the

plasma membranes (Octave *et al.,* 2006b), and inhibit photosynthesis and respiration in leaf tissues by decreasing energy charge (Amborabé *et al.,* 2001; Octave *et al.,* 2006b). Therefore, the mechanisms of host resistance to wood and leaf symptoms may differ. Resistance to wood colonization may be associated with high lignin and suberin deposition in wood of some cultivars (Munkvold and Marois 1995). Resistance to leaf symptoms may be associated with tolerance/detoxification of fungal secondary metabolites *in planta* (Guillén *et al.,* 1998). Detoxification of eutypine, one of many secondary metabolites produced by *E. lata* (Mahoney *et al.,* 2005), into its corresponding alcohol eutypinol, by transgenic grapevines genetically engineered to do so, has been associated with reduced severity of leaf symptoms (Guillén *et al.,* 1998; Legrand *et al.,* 2003).

Assay 1 was the most time-consuming of the three assays, but lesion length in woody stems was a repeatable measure between the two *E. lata* isolates, as has been previously demonstrated (Sosnowski *et al.,* 2007a). Given the repeatability of the results for isolate BX1-10 and the strong correlation between wood and leaf symptoms, Assay 1 was the most robust of the three assays for differentiating the nine grapevine cultivars evaluated. Propagation from green cuttings, which is the only way to root some grapevine germplasm, allowed the development of lesions in Assay 2 that were comparable in size to those of Assay 1, but in a shorter 4 month timeframe. Assay 2 may therefore be suited for preliminary screening of large germplasm collections (e.g., progeny from crosses, different *Vitis* species, or hybrids with resistance to other grapevine diseases). Assay 2 could then be followed by Assay 1 for secondary screening of subsets of the most resistant germplasm. Plants in Assay 2 also developed visible, external stem cankers, the incidence of which was positively correlated with lengths of internal stem lesions. A practical disadvantage of Assay 2, however, was the need for a greenhouse mist system, to propagate plants from green cuttings. Although Assay 2 was more rapid than Assay 1, *E. lata* ascospores do not directly infect green host stems in the field, so measuring lesions in green stems may not measure resistance to Eutypa dieback. Nonetheless, based on lesion lengths in woody or green stems, both Assays 1 and 2 identified the same resistant cultivars as 'Merlot', 'Primitivo', and 'Muscat Hamburg', and some of the same susceptible cultivars as 'Husseine' and 'Carignane'.

Previous authors have emphasized the importance of evaluating lesion lengths and the points at which, beyond the lesion margins, *E. lata* is undetectable (Moisy *et al.,* 2017; Sosnowski *et al.,* 2022). Results from Assay 1 of greater distance of detection beyond the visible lesion margins (e.g., 15 cm beyond the margin in the very susceptible 'Thompson Seedless') are consistent with this pattern. Measuring only lesion lengths may not reflect a pathogen's ability to colonize apparently healthy wood (Sosnowski *et al.,* 2007a). Assuming the pathogen will eventually rot asymptomatic wood from which it is recovered beyond the lesion margin, the extent of wood colonization may therefore be an appropriate (though more time-consuming) measure of host susceptibility.

Phenotyping assays that use the same isolates and pre- and post-inoculation growing conditions may help to standardize screening protocols that can accommodate the diversity of grapevine germplasm. The present study used two 'reference' isolates from previous research (Péros and Berger 1999; Camps *et al.,* 2010; Travadon *et al.,* 2013; Moisy *et al.,* 2017; Cardot *et al.,* 2019). Isolate BX1-10 has previously been characterized as virulent (Péros and Berger 1994; Moisy *et al.,* 2017), whereas isolate M14 was associated with repeatable wood symptoms in a previous study (Travadon *et al.,* 2013). For the majority of cultivars inoculated with BX1-10 compared to M14, the present results of lesion sizes in Assays 1 and 2, and the RTEs and incidences of leaf symptoms in Assays 1 and 3, indicate that isolate BX1-10 was more virulent that isolate M14. Differences in virulence among *E. lata* isolates inoculated onto replicate plants of the same cultivar have been previously reported (Péros *et al.,* 1997; Péros and Berger 2003; Sosnowski *et al.,* 2007a; Travadon and Baumgartner 2015), and these may correspond to high genetic variation in *E. lata* populations (Péros and Berger 1999; Travadon *et al.,* 2012; Onetto *et al.,* 2022), and/or to differences in secondary metabolite production (Mahoney *et al.,* 2003; Lardner *et al.,* 2006). Previous studies have shown that severity of leaf symptoms in some cultivars correlates to severity of wood symptoms (Sosnowski *et al.,* 2007a), or to *E. lata* biomass as quantified by qPCR (Moisy *et al.,* 2017). Inconsistencies have been reported between isolates, with no relationships between leaf and wood symptoms (Sosnowski *et al.,* 2007a). Similar results were recorded here from Assay 1, with positive correlation between lesion length and RTE for all nine cultivars inoculated with BX1-10, but not for M14. When inoculated with M14, 'Thompson Seedless' did not have severe leaf symptoms, despite large wood lesions. For future host germplasm studies that include any of the three Assays, isolate BX1-10 is probably more suitable than M14.

Knowledge of grapevine cultivar resistance to *E. lata* informs fundamental research on molecular mechanisms of this resistance (Rotter *et al.,* 2009; Camps *et al.,* 2010). For example, high expression of gene families associated with host defense responses (e.g., enzymes of

the jasmonic acid, salicylic acid and phenylpropanoid pathways) among resistant 'Merlot' and 'Cabernet-Sauvignon', compared to highly susceptible 'Ugni blanc', indicates similar genetic determinants of resistance, even among different cultivars (Cardot *et al*., 2019). The development of phenotyping methods for measuring grapevine resistance to Eutypa dieback, as those outlined in the present study, is important for future genetics studies, which may identify genomic regions associated with resistance to this economically important grapevine disease (Dry *et al.,* 2019).

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