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Variation between and within grapevine families in reaction to leaf inoculation with downy mildew sporangia under controlled conditions

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Summary

Vine reaction to downy mildew [*Plasmopara viticola* (Berk. & Curt.) Berl. & de Toni] inoculation was investigated between and within seven full-sib grapevine families under controlled culture conditions. Families were generated by crossing resistant x susceptible and susceptible x susceptible genotypes. Leaf infection following downy mildew inoculation was assessed using cultured leafed single node cuttings under controlled conditions. The severity of disease infection 7 days after inoculation was based on the expression of leaf chlorosis and sporulation symptoms using a 1 to 9 scale where a score of 1 meant there was no visible sign of infection and 9 meant > 80 % of the leaf area was infected. A measure of the hypersensitive response (discrete necrotic spots) was also used to rank vine reaction to inoculation. Hybrids within families varied widely in their reaction to inoculation. Any hybrid that received a mean symptom expression score ≤ 3, which was equivalent to or less than that assigned to 'Chambourcin', was classified as downy mildew resistant. The proportion of vines within families scored as resistant ranged from 4.6 to 22.5 % and from 4.6 to 47.4 % for leaf chlorosis and sporulation, respectively, between crosses. There was a strong correlation between leaf chlorosis and sporulation expression within each family (r^2 ranged from 0.6 to 0.8). The number of resistant hybrids selected within families by the combined symptoms of leaf chlorosis and sporulation ranged from 3 to 7. Depending on family, segregation of resistant to susceptible phenotypes based on symptoms of leaf chlorosis and sporulation fitted 1:15, 1:7, 1:3 or 1:1 ratios. Segregation for hypersensitive reaction (HR) to non-HR fitted 1:1, 1:2 or 1:3 ratios within families. Hybrids that displayed the HR had mean scores for leaf chlorosis and sporulation less than those not displaying the necrotic spots characteristic of the reaction in four of the families investigated. The results are discussed in terms of the inheritance of resistance and the development of a strategy for future breeding.

K e y w o r d s : *Plasmopara viticola,* disease resistance/susceptibility, breeding, segregation, *Vitis* spp.

Introduction

Downy mildew, caused by *Plasmopara viticola* (Berk. & Curt.) Berl. & de Toni, is an important viticultural disease in Australia (Magarey *et al*. 1994). The disease is present year round and overwinters in the vineyard mainly as oospores within infected leaf debris. Mature oospores produce sporangia or zoospores during rainy periods in spring when minimum temperature reaches 11 °C. Zoospores are disseminated by wind and/or rain splashes, wetting leaves where primary infections occur (Lafon and Clerjeau 1988). Infection spreads rapidly under warm wet conditions, which favour sporangiophore formation, sporangium dissemination and germination, leading to new infections and, ultimately, epidemics (Lafon and Clerjeau 1988). Once leaf infections are advanced they in turn provide inoculum to infect actively growing shoots, flower clusters and young, developing berries. Infected grapevines have reduced photosynthetic capacity, stunted shoots, necrotic flower clusters and shrivelled berries, whereupon vines become less productive with reduced yields of poor quality fruits. In severely infected vineyards, detrimental effects can persist for several seasons (Emmett *et al*. 1992). Although dry, warm climates in most Australian grape growing regions do not favour the occurrence of downy mildew, yield reductions and poor fruit quality associated with the disease and the use of preventative chemicals add significantly to production costs each season (Magarey *et al.* 1994).

In order to reduce costs and minimise chemical inputs, efforts have been made to select downy mildew resistant grapevines worldwide. Resistance has been reported in American and Asian *Vitis* species (Staudt and Kassemeyer 1995, Brown *et al.* 1999 a, Patil *et al.* 1989) and in other genera within the *Vitaceae* (Patil *et al.* 1989). Within *Vitis vinifera* there is much genotypic variability in vine reaction to the disease, although most varieties are susceptible (Sohi and Sridhar 1970, Patil *et al.* 1989, Brown *et al.* 1999 a). Intercrossing resistant *Vitis* species and commercial *vinifera* varieties, followed by several generations of backcrossing to *vinifera*, has led to the release of resistant wine grape varieties with satisfactory fruit quality in many national improvement programs (Becker and Zimmermann 1978, Alleweldt 1980, Borgo *et al.* 1990, Eibach and Töpfer 2003). Resistant vines have also been reported from clonal selection (Coutinho and Corte 1980), following x-or γ-ray irradiation (Coutinho and Martins 1990), and through recurrent selection strategies (Filippenko and Shtin 1978).

Grapevine resistance to downy mildew, expressed in terms of leaf chlorosis and sporulation, has been reported as being inherited quantitatively with narrow sense heritabilities estimated from 0.26 to 0.88 (Brown *et al.* 1999 b,

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Eibach *et al.* 1989, Eibach 2000). The frequency of resistant hybrids within progenies appears to be affected both by the magnitude of resistance shown by parents and their combining abilities (Borgo *et al.* 1990, Brown *et al.* 1999 b, Kozma 2000). Suggested maternal influences on the transmission of resistance through breeding have been inconclusive (Becker and Zimmermann 1978, Brown *et al.* 1999 b, Alleweldt 1980).

Resistance may also be observed as a hypersensitive response (HR) (Matthews 1981) and vines exhibiting this have reduced symptoms of leaf chlorosis and sporulation (Brown *et al*. 1999 a and b, Liu *et al*. 2003). In addition, vine reaction to the disease may be expressed differently according to the growth stage of the vine at disease colonisation. For example, the entrance of geminated tubes of encysted zoospores into the sub-stomatal cavity can be blocked at a certain leaf age, which curtails haustoria formation or limits hyphae growth (Denzer *et al*. 1995, Langcake and Lovall 1980). This resistance at the stomatal level was governed by a single dominant gene and restriction of hyphae growth inside leaf tissues was polygenic (Boubals 1959).

Breeding for resistance to mildew diseases is an objective in CSIRO's grape improvement program. So far the only variety released with downy mildew resistance under vineyard conditions has been 'Marroo Seedless', which is a dual-purpose table and dried fruit variety (Clingeleffer and Possingham 1988). Continued efforts in this direction have generated many breeding progenies aimed at increasing yield, improving quality and incorporating mildew resistance. Here we report variability for infection characteristics between and within seven full-sib families when leafed single node cuttings were cultured and inoculated with downy mildew sporangiophores. The results have led to further discussions concerning the inheritance for resistance in these families and thus strategies to breed for downy mildew resistance in future crosses.

Material and Methods

Controlled crosses were made in 1993 and 1994 between a range of parents rated as either resistant or susceptible to downy mildew (Tab. 1). Resistant parents were 'Chambourcin' (Barlass *et al.* 1986) and 'Marroo Seedless' (Clingeleffer and Possingham 1988). 'Sunmuscat' (Okie 2000), 'Hunisa', 'Kishmishi' and breeding selections 23-06 ('Carolina Blackrose' x 'Ruby Seedless'), 46-32 ('Kishmishi' x ('Carolina Blackrose' x 'Flame Seedless')) and 23-80 (39.639 'Seyve Villard' x 'Sultana') from CSIRO's grapevine breeding program were used as susceptible parents.

Hybrids from the seven combinations were established during 1995-1996 in a Coomealla sandy loam soil (Penman *et al*. 1939) at CSIRO's research vineyard near Merbein in NW Victoria (142°2'E; 34°13'S) as family groups at 1.5m intra- and 2.5 inter-row spacings. Individuals were randomised within families. Vines were trained to single wire trellis, cane-pruned annually during dormancy and subjected to uniform vineyard management. Irrigation was via low-level sprinklers that delivered approximately 1000 mm rainfall-equivalent of water per annum. Vines of parents, approximately 30-years-old, were grown under the same viticultural conditions.

The reaction of hybrids within families to downy mildew inoculum was evaluated in a number of screening experiments using the leafed single node cutting (LSNC) method described by Liu *et al.* (2003). Experiments were conducted in a culture room at 25 ± 2 °C with a 16 hr pho-

Table 1

Crosses between resistant or susceptible parents that gave rise to families used to investigate the reaction of leafed single node cuttings to inoculation with downy mildew sporangia. The genetic percentage[a] of *vinifera* and non-*vinifera*[b] species in the parents and thus hybrids within families are presented

Cross	female parent		male parent	
	% *vinifera*	% non-*vinifera*	% *vinifera*	% non-*vinifera*
Hunisa x Chambourcin (SxR)[c]	100	0	60.13	39.88
Chambourcin x Sunmuscat (RxS)	60.13	39.88	100	0
23-06 x Chambourcin (RxS)	94.53	5.47	60.13	39.88
Kishmishi x Chambourcin (SxR)	100	0	60.13	39.88
46-32 x Marroo Seedless (SxR)	78.09	21.91	94.53	5.47
Hunisa x 23-80[d] (SxS)	100	0	x	100 - x
23-06 x 23-80 (SxS)	94.53	5.47	x	100 - x

[a] Percentages are based on known pedigrees of the parents used. Information was drawn from Striem (2000) and from CSIRO's breeding records and assumes each parent contributes 50 % to its offspring. [b] Non-*vinifera* parents include *V. labrusca*, *V. rupestris*, *V. berlandieri*, *V. riparia* and *V. lincecumii*. [c] Type of cross; eg. SxR = susceptible x resistant. [d] 23-80 was selected from a cross made by CSIRO between 39.639 'Seyve-Villard' x 'Sultana'. 39.639 'Seyve-Villard' was listed by Galet (1988) as an early maturing, disease resistant white berried interspecific hybrid of unknown origin. As efforts to seek out the parentage of 39.639 'Seyve-Villard' have been unsuccessful, the genetic percentage *vinifera* and non-*vinifera* in 23-80 is listed here as being x % *vinifera* and 100-x % non-*vinifera*, where x < 100.

toperiod supplied by cool-white fluorescent illumination (320 µmol·mol^{-2}·s^{-1}). Due to the limitations of space, families exceeding 50 hybrids were completed in two screening experiments. Hybrids were evaluated using cuttings collected from shoots of 'Hunisa' x 'Chambourcin', 'Chambourcin' x 'Sunmuscat', 'Kishmishi' x 'Chambourcin', and 46-32 x 'Marroo Seedless' families during spring 2001 and from 23-06 x 'Chambourcin', 'Hunisa' x 23-80, and 23-06 x 23-80 families during spring 2002.

Three shoots with at least 2 or 3 fully expanded healthy young leaves were collected from individual hybrids and parental genotypes. These were surface-cleaned in the laboratory by washing in soapy water for 1 min and rinsing thoroughly with tap water followed by distilled water. Shoots were then pruned carefully such that one LSNC from either the 4th to 5th node back from shoot tips was retained for culture and inoculation. 'Sultana', a susceptible genotype, was used as a standard control to monitor infection development between different screening experiments. Other than the two resistant parents used in the crosses, there were no resistant control genotypes included in experiments. LSNCs of 'Chambourcin' and 'Marroo Seedless' were tested in five and one screening experiments allowing 15 and 3 observations per genotype, respectively (Fig. 1).

A minimum of three LSNCs were tested for every hybrid and parent; for some genotypes, e. g. 'Chambourcin' (see above) additional LSNCs were screened in more than one experiment. Cuttings were randomised within a 47 x 37 x 10.5 cm plastic tray. Four genotypes plus a 'Sultana' standard were accommodated in each tray giving 20 cuttings per tray. LSNCs were inoculated using a wetted camel hair brush to apply a 1 x 10^5 sporangia per ml suspension (Liu *et al.* 2003).

The inoculum initially came from diseased leaves of susceptible 'Sunmuscat' and 'Sultana' vines grown in pots within a glasshouse. Subsequently, sporangia were collected from highly infected leaves at the end of every screening experiment and used as the inoculum source for the next one.

Downy mildew infection severity was rated visually on the expression of leaf chlorosis and sporulation 7 d after inoculation. At this stage, 80 % of the lamina surface area of 'Sultana' leaves was chlorotic and covered by sporangiophores. Ratings were scored using a scale of 1-9 representing the proportion of the leaf surface showing symptoms of the disease. Thus, a score of 1 = no symptoms, 2 = 0 to 2.5 %, 3 = 2.5 to 10 %, 4-5 = 10 to 25 %, 6-7 = 25 to 50 %, 8 = 50 to 80 % and 9 = > 80 % of the leaf surface showing symptoms, respectively. Scores were used to assign hybrids within families into one of six classes from resistant to susceptible (Tabs 2 or 3). Hybrids allocated a mean score of 3 or less were considered resistant as they were equivalent to 'Chambourcin' in their reaction to the disease. Hybrids with mean score of 3.0 were not significantly different from 'Chambourcin' (t values < 1.47, P > 0.16, df = 14) while those having a mean score of 2.0 or less were rated significantly more resistant than this parent genotype (t values > 5.0, P < 0.01, df = 14). The incidence of the hypersensitive response (HR), seen as distinct necrotic spots, was also recorded.

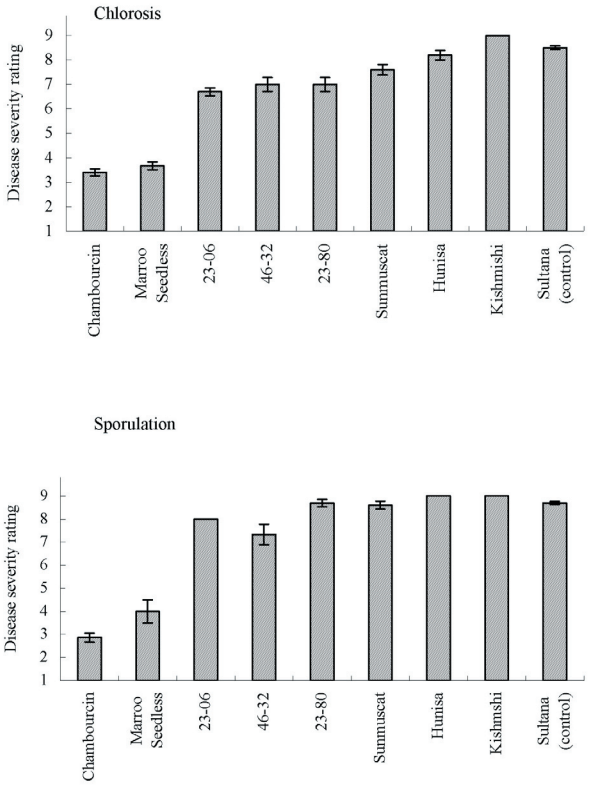

Fig. 1: Mean (n = 3 to 27) rankings assigned to parents and Sultana, which was used as a standard variety in all experiments, for the expression of leaf chlorosis (upper) and sporulation (below) symptoms on leaves 7 d after inoculation with downy mildew sporangia. Symptom expression was scored using a scale of 1-9 representing the proportion of the leaf surface showing symptoms of the disease. Thus, 1 = no symptoms, 2 = 0 to 2.5 %, 3 = 2.5 to 10 %, 4-5 = 10 to 25 %, 6-7 = 25 to 50 %, 8 = 50 to 80 % and 9 = > 80 % of the lamina surface showing symptoms, respectively. Vertical bars represent standard errors of means. The columns without the bar indicate zero standard error of the observed infection mean scores.

Segregation ratios for resistant (mean score ≤ 3.0) to susceptible (mean score > 3.0) were calculated for each family based on chlorosis and sporogenesis observations; χ^2 was used to test for goodness of fit for these against model ratios.

Results

As the standard genotype used in all experiments, 'Sultana' was assessed for its reaction to downy mildew inoculum using more cuttings than for other genotypes. Over all experiments, LSNCs of 'Sultana' had a consistently high score for symptom expression of both chlorosis and sporulation (Fig. 1). This indicated that conditions for infection were consistent and favourable across all screening experiments.

While there were differences between the parents in leaf symptom scores after inoculation of LSNCs with the disease, none were completely resistant with a mean of 1 (Fig. 1). 'Chambourcin' and 'Marroo Seedless' had significantly lower symptom scores than the other parents. The

standard errors of mean leaf sporulation scores were greater than those for leaf chlorosis with 'Chambourcin' and 'Marroo Seedless'. All other parents had symptom scores greater than those assigned to 'Chambourcin' and 'Marroo Seedless'. Amongst parents, the hypersensitive response (HR) to inoculation was observed only on LSNCs of 'Chambourcin' and 'Marroo Seedless'. 'Chambourcin' and 'Marroo Seedless' were thus considered downy mildew resistant and the other parents susceptible, which supported documented vineyard observations (Barlass *et al.* 1986).

Of the crosses investigated, two were between susceptible x susceptible and five were between resistant x susceptible (or reciprocal) parents based on the observations recorded for the parents (Tabs 2 and 3). The response of hybrids to disease inoculation varied within each family. A proportion of all hybrids tested in each family were observed with symptom scores ≤ 3.0 and, depending on family, between 3-to-20 and 4-to-8 were classed as resistant based on leaf chlorosis and sporulation scores, respectively.

While scores for leaf chlorosis and sporulation symptoms were correlated within families with r^2 values ranging from 0.60 to 0.80 (Tab. 4), there were some hybrids that received contrasting scores for the two symptoms of infection. For example, in the family from 'Chambourcin' x 'Sunmuscat', 5 of 10 hybrids rated as resistant based on their score for leaf chlorosis were scored as susceptible on symptoms of leaf sporulation. Similarly, 3 of 8 hybrids rated resistant on leaf sporulation symptoms were scored as susceptible based on leaf chlorosis (Fig. 2). Nevertheless, within each family, there were between 3 and 7 individuals that had a mean score of ≤ 3.0 for both leaf chlorosis and sporulation symptoms (Tab. 6).

The proportions of hybrids within families classed as either resistant or susceptible varied between families. The percentage of resistant hybrids (mean score ≤ 3) ranged from 4.6 % in 23-06 x 'Chambourcin' to 47.4 % in 46-32 x 'Marroo Seedless', based on leaf chlorosis (Tab. 2), and from 6.1 % in 23-06 x 'Chambourcin' to 36.8 % in 46-32 x 'Marroo Seedless', based on sporulation (Tab. 3). Except

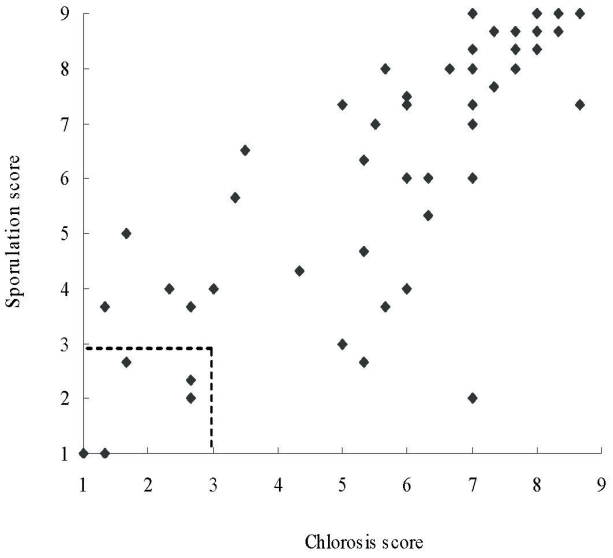

Fig. 2: Relationship of mean scores for leaf chlorosis and sporulation symptom expression on leaves 7 d after inoculation with downy mildew sporangia for 52 hybrid vines in a family generated from the cross of 'Chambourcin' x 'Sunmuscat'. Symptom expression was scored using a scale of 1-9 representing the proportion of the leaf surface showing symptoms of the disease. Thus, 1 = no symptoms, 2 = 0 to 2.5 %, 3 = 2.5 to 10 %, 4-5 = 10 to 25 %, 6-7 = 25 to 50 %, 8 = 50 to 80 % and 9 = > 80 % of the lamina surface showing symptoms, respectively. The dots enclosed in the dash lines at the score point of 3 represent resistant hybrids.

for the family derived from 46-32 x 'Marroo Seedless', over 65 % hybrids within each family were classed as susceptible (a mean score > 5.0). There were no significant relationships between mid-parent value and offspring mean for the expression of either chlorosis (r = 0.17) or sporulation (r = 0.22).

Five families produced individuals with a mean symptom score of 1 suggesting they were totally resistant to the inoculum used in the experiments (Tabs 2 and 3). These resistant hybrids occurred in families from crosses between resistant x susceptible and susceptible x susceptible parents. Thus, from scores for leaf chlorosis symptoms, six

Table 2

Percentage frequency of hybrids within families ranked on mean (n ≥ 3) expression of chlorosis symptoms 7 d after inoculation of leaves with downy mildew sporangia

Family	No. hybrids	% of hybrids assigned to different symptom expression rankings [a]						Ratio of resistant: susceptible hybrids [b]
		1	≤ 2	≤ 3	4-5	6-7	8-9	
Hunisa x Chambourcin	81	1.2	1.2	2.5	13.6	27.2	54.3	4:77 (1:15 $\chi^2 = 0.24^{ns}$)
Chambourcin x Sunmuscat	52	1.9	7.7	9.6	9.6	44.2	26.9	10:42 (1:3 $\chi^2 = 0.92^{ns}$)
23-06 x Chambourcin	65	1.5	0.0	3.1	15.4	23.1	56.9	3:62 (1:15 $\chi^2 = 0.29^{ns}$)
Kishmishi x Chambourcin	33	0.0	6.1	3.0	15.2	21.2	54.5	3:30 (1:7 $\chi^2 = 0.35^{ns}$)
46-32 x Marroo Seedless	19	15.8	10.5	21.1	21.1	10.5	21.1	9:10 (1:1 $\chi^2 = 0.05^{ns}$)
Hunisa x 23-80	89	4.5	4.5	13.5	12.4	22.5	42.7	20:69 (1:3 $\chi^2 = 0.30^{ns}$)
23-06 x 23-80	51	0.0	9.8	3.9	5.9	21.6	58.8	7:44 (1:7 $\chi^2 = 0.07^{ns}$)

[a] Symptom expression was scored using a scale of 1-to-9 representing the proportion of the leaf surface showing symptoms of the disease. Thus, 1 = no symptoms, 2 = 0 to 2.5 %, 3 = 2.5 to 10 %, 4-5 = 10 to 25 %, 6-7 = 25 to 50 %, 8 = 50 to 80 % and 9 = > 80 % of the leaf surface showing symptoms, respectively. [b] Hybrids receiving a mean score of ≤ 3 were resistant to downy mildew infection. Actual and best fit phenotypic segregation ratios are given (ns = not significant at P = 0.05).

Table 3

Percentage frequency of hybrids within families ranked on mean (n ≥ 3) expression of sporulation symptoms 7 d after inoculation of leaves with downy mildew sporangia

Family	No. hybrids	% of hybrids assigned to different symptom expression rankings [a]						Ratio of resistant:susceptible hybrids [b]
		1	≤ 2	≤ 3	4-5	6-7	8-9	
Hunisa x Chambourcin	81	3.7	1.2	3.7	3.7	12.3	75.3	7: 74 (1:15 $\chi^2 = 0.79^{ns}$)
Chambourcin x Sunmuscat	52	3.8	3.8	7.7	17.3	23.1	44.2	8:44 (1:7 $\chi^2 = 0.40^{ns}$)
23-06 x Chambourcin	65	4.6	1.5	0.0	3.1	10.8	80.0	4:61 (1:15 $\chi^2 = 0.00^{ns}$)
Kishmishi x Chambourcin	33	0.0	3.0	9.1	9.1	15.2	63.6	4:29 (1:7 $\chi^2 = 0.00^{ns}$)
46-32 x Marroo Seedless	19	10.5	10.5	15.8	26.3	10.5	26.3	7:12 (1:2 $\chi^2 = 0.11^{ns}$, 1:1 $\chi^2 = 1.31^{ns}$)
Hunisa x 23-80	89	2.2	3.4	2.2	5.6	19.1	67.4	7:82 (1:15 $\chi^2 = 0.40^{ns}$)
23-06 x 23-80	51	0.0	3.9	5.9	9.8	11.8	68.6	5:46 (1:7 $\chi^2 = 0.34^{ns}$)

[a] Symptom expression was scored using a scale of 1 to 9 representing the proportion of the leaf surface showing symptoms of the disease. Thus, 1 = no symptoms, 2 = 0 to 2.5 %, 3 = 2.5 to 10 %, 4-5 = 10 to 25 %, 6-7 = 25 to 50 %, 8 = 50 to 80 % and 9 = > 80 % of the leaf surface showing symptoms, respectively. [b] Hybrids receiving a mean score of ≤3 were resistant to downy mildew infection. Actual and best fit phenotypic segregation ratios are given (ns = not significant at P = 0.05).

Table 4

The relationship between individual hybrid rankings for leaf chlorosis and sporulation symptoms 7 d after inoculation of leaves with downy mildew sporangia within seven families from crosses between resistant x susceptible and susceptible x susceptible parents. The relationship is expressed as the correlation coefficient

Family	Combination	Correlation coefficient
Hunisa x Chambourcin	S x R	0.79**
Chambourcin x Sunmuscat	R x S	0.79**
23-06 x Chambourcin	S x R	0.78**
Kishmishi x Chambourcin	S x R	0.90**
46-32 x Marroo Seedless	S x R	0.90**
Hunisa x 23-80	S x S	0.80**
23-06 x 23-80	S x S	0.87**

** indicates significance at the 0.01 probability level.

hybrids were assigned a rating of 1 from four of the resistant x susceptible crosses and 4 hybrids were similarly identified from one of the susceptible x susceptible crosses. Ten and 2 hybrids from resistant x susceptible and susceptible x susceptible crosses, respectively, were identified with a score of 1 based on sporulation symptoms.

Segregation ratios of resistant to susceptible hybrids in families are given Tabs 2 and 3 where hybrids with a score of 3.0 or less were considered resistant. Based on leaf chlorosis scores, segregation for resistant:susceptible showed no significant deviation from 1:15 in 'Hunisa' x 'Chambourcin' and 23-06 x 'Chambourcin', 1:7 in 'Kishmishi' x 'Chambourcin' and 23-06 x 23-80, 1:3 in 'Chambourcin' x 'Sunmuscat' and 'Hunisa' x 23-80, and 1:1 in 46-32 x 'Marroo Seedless'. Based on leaf sporulation scores, these same ratios fitted the data obtained for 'Hunisa' x 'Chambourcin' and 23-06 x 'Chambourcin' (1:15), 'Kishmishi' x 'Chambourcin' and 23-06 x 23-80 (1:7), and 46-32 x 'Marroo Seedless' (1:1). Segregation ratios for 'Chambourcin' x 'Sunmuscat' (1:7) and 'Hunisa' x 23-80 (1:15) based on sporulation scores differed from those obtained for chlorosis.

Hybrids either did or did not display a hypersensitive response (HR) and, within each family, HR and non-HR hybrids segregated to fit 1:1, 1:2 or 1:3 ratios (Tab. 5). Hybrids showing a HR had lower mean disease symptom scores for both leaf chlorosis and sporulation than non-HR hybrids in four families, which were 'Hunisa' x 'Chambourcin' (t = 3.5 to 4.3, P <0.01), 23-06 x 'Chambourcin' (t = 6.2 to 11.0, P <0.01), 'Hunisa' x 23-80 (t = 4.6 to 8.0, P < 0.01) and 23-06 x 23-80 (t = 6.5 to 7.7, P < 0.01) (Figs 3 and 4). In the family from 'Kishmishi' x 'Chambourcin', HR hybrids exhibited lower mean scores than non-HR hybrids for leaf chlorosis (t value = 2.4, p = 0.03) but not for sporulation. A similar difference was not evident in the two families from 'Chambourcin' x 'Sunmuscat' and 46-32 x 'Marroo Seedless'. Large standard errors, however, were associated with the HR groups across families. In addition, a HR was recorded in 24 of the 32 resistant hybrids identified by sequential selection for the low expression of leaf chlorosis and sporulation and in all families but one (*viz*. 46-32 x 'Marroo Seedless') the majority of resistant hybrids exhibited the HR (Tab. 6).

Discussion

Under the conditions in which hybrids were tested, within family variability in leaf downy mildew symptoms after inoculation with sporangia was similar for the resistant x susceptible and susceptible x susceptible combinations examined. Resistant and highly susceptible hybrids were observed in all families. Some families included hybrids that appeared to be completely resistant to the disease in that symptoms of chlorosis or sporulation were absent. In this respect, these hybrids displayed greater resistance than the resistant parents used for the crosses. Resistant hybrids with scores of 1 were observed in 4 families from susceptible x resistant crosses and one from a susceptible x susceptible cross. These observations suggest that reaction to inocula by the parents used in this investigation was governed by additive and/or recessive alleles, and that

Table 5

Segregation ratios of hybrids with and without hypersensitive response (HR) to downy mildew infection in seven families from crosses between resistant x susceptible and susceptible x susceptible parents

Family	Combination	Number of hybrids		Best fit segregation ratio for HR:non-HR hybrids [b]
		HR	No HR	
Hunisa x Chambourcin	S x R	28	53	1:2 ($\chi^2 = 0.07^{ns}$)
Chambourcin x Sunmuscat	R x S	31	21	1:1 ($\chi^2 = 1.92^{ns}$)
23-06 x Chambourcin	S x R	15	50	1:3 ($\chi^2 = 0.13^{ns}$)
Kishmishi x Chambourcin	S x R	18	15	1:1 ($\chi^2 = 0.27^{ns}$)
46-32 x Marroo Seedless	S x R	6	13	1:2 ($\chi^2 = 0.03^{ns}$)
Hunisa x 23-80	S x S	42	47	1:1 ($\chi^2 = 0.28^{ns}$)
23-06 x 23-80	S x S	25	26	1:1 ($\chi^2 = 0.02^{ns}$)

ns = not significant at P = 0.05.

Table 6

Number of resistant hybrids identified by being assigned a score of ≤ 3 for leaf chlorosis and sporulation symptom expressions and the number of these displaying a hypersensitive response to downy mildew infection within families from crosses between resistant x susceptible (RxS) and susceptible x susceptible (SxS) parents

Family	Combination	Number of hybrids assigned a score of ≤ 3 for leaf chlorosis and sporulation symptoms	Number of hybrids displaying the HR
Hunisa x Chambourcin	S x R	3	2
Chambourcin x Sunmuscat	R x S	5	5
23-06 x Chambourcin	S x R	3	3
Kishmishi x Chambourcin	S x R	3	3
46-32 x Marroo Seedless	S x R	7	2
Hunisa x 23-80	S x S	7	7
23-06 x 23-80	S x S	4	4

transgressive segregation occurred for resistance. The absence of any relationships between mid-parent values and family means for leaf chlorosis and sporulation symptoms of the disease, however, probably discounts simple additive gene effects.

The observations reported here were similar to those for families from crosses between resistant French hybrids and susceptible *V. vinifera* varieties (Becker and Zimmermann 1978) and for intraspecific crosses of *V. amurensis* (Song *et al.* 1998). It was interesting that for every family tested in the current investigation, at least one parent had a complex pedigree in which one or more non-*vinifera* parents had been used (Tab. 1). The observations reported here, however, contradict those reported for table (Brown *et al.* 1999 b) and wine grape crosses (Kozma 2000) in which resistant hybrids were more frequent in families from crosses where at least one parent was resistant than for susceptible x susceptible crosses. Both of these investigations were carried out under vineyard conditions and vines would have been subjected to a variable disease pressure. When or if inoculum levels are low and/or variable in the vineyard, the chances for individual vines to escape infection could be high leading to incorrect assignment of resistance or susceptibility. All vines within families in this study were tested using the LSNC method (Liu *et al.* 2003) under controlled conditions. The variability of the results presented for a susceptible control, 'Sultana', and a resistant parent, 'Chambourcin', (Fig. 1) suggested that LSNCs were tested under uniform conditions conducive to high infection rates. Thus, hybrids identified either as resistant or susceptible under such conditions would be expected to behave similarly when tested under vineyard conditions.

Hybrids displaying a hypersensitive reaction (HR) were observed in all families segregating as 1:1, 1:2 or 1:3 ratios for HR to non-HR individuals. These ratios also suggested that expression of the HR after inoculation with sporangia was governed by recessive alleles, at least for the parents used in this investigation. This, however, differs from the conclusion that HR related resistance was determined by a single dominant gene in *Tetrastigma* and *Cissus*, both related genera in the *Vitaceae* (Matthews 1981) and as a dominant characteristic in a range of wine grape crosses (Kozma 2000).

Within families tested, hybrids that displayed the HR had lower mean scores for either leaf chlorosis or sporulation symptoms, although large standard errors meant the HR displayed by a hybrid did not prevent it from showing other symptoms of the disease (Figs 3 and 4). Also, the HR

was not observed for some of the resistant vines (Tab. 6). This raises a question as to how the HR is recognised and scored. Brown *et al*. (1999 b) reported the HR as small necrotic flecks. Widely accepted descriptors for *Vitis* spp. (IPGRI UPOV OIV 1997; OIV 2001), however, suggest that the HR may take two forms as either tiny necrotic spots, which may be the flecks reported by Brown *et al*. (1999 b), or larger necrotic spots or patches < 1 cm in diameter. Both these types of necroses are associated with little or no sporulation. This suggests perhaps that the HR to downy mildew infection can be classified into two types; one having small necrotic flecks and the other small, but more defined localised necrotic lesions or spots. For the families investigated here, all necrotic spots regardless of size were classed as HR and grouped together. This may account for the large variation in each family for expression of other symptoms between vines assigned as showing a hypersensitive type of reaction to initial infection.

In the vineyard, where conditions conducive to the development of the disease may change quickly, the HR

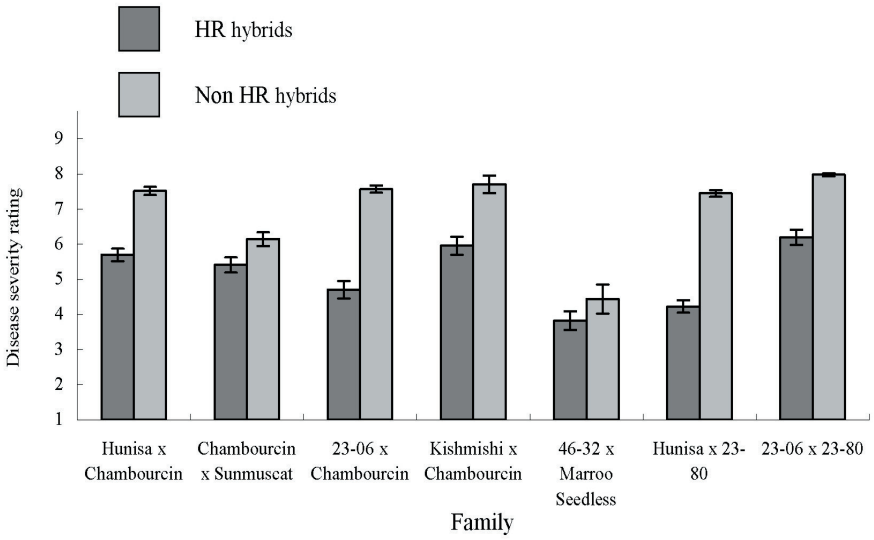

Fig. 3: Mean expression of leaf chlorosis symptoms by hybrids grouped within families based on whether they did (HR) or did not (non HR) display the hypersensitive response to leaf inoculation with downy mildew sporangiophores. Symptom expression was scored using a scale of 1-9 representing the proportion of the leaf surface showing symptoms of the disease. Thus, 1 = no symptoms, 2 = 0 to 2.5 %, 3 = 2.5 to 10 %, 4-5 = 10 to 25 %, 6-7 = 25 to 50 %, 8 = 50 to 80 % and 9 = > 80 % of the lamina surface showing symptoms, respectively. Vertical bars represent standard errors of the means.

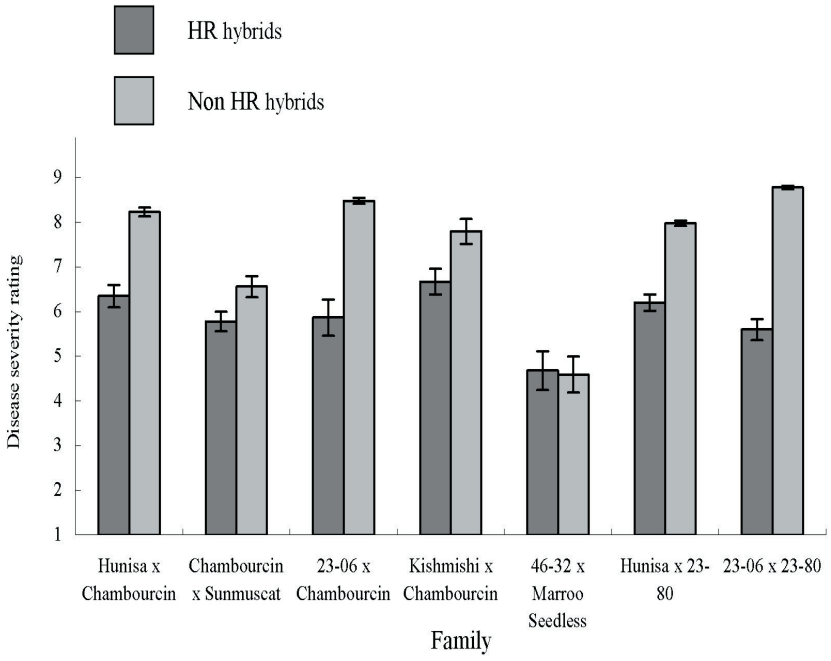

Fig. 4: Mean expression of sporulation symptoms by hybrids grouped within families based on whether they did (HR) or did not (non HR) display the hypersensitive response to leaf inoculation with downy mildew sporangiophores. Symptom expression was scored using a scale of 1-9 representing the proportion of the leaf surface showing symptoms of the disease. Thus, 1 = no symptoms, 2 = 0 to 2.5 %, 3 = 2.5 to 10 %, 4-5 = 10 to 25 %, 6-7 = 25 to 50 %, 8 = 50 to 80 % and 9 = > 80 % of the lamina surface showing symptoms, respectively. Vertical bars represent standard errors of means.

could act in suppressing other symptoms giving a resistant phenotype. This would support the observations of Brown *et al*. (1999 b). The conditions employed for evaluating LSNC are conducive to the development of the disease and so even vines displaying a HR may still develop chlorosis and sporulation symptoms. This suggests that further research is needed to investigate whether different forms of a HR affect a vine's resistance to downy mildew infection under different environmental conditions. Gindro *et al.* (2003) reported the synthesis of callose in infected stomata of a resistant variety associated with necrosis of surrounding cells was indicative of the HR to downy mildew infection. Such a HR was not evident for a susceptible variety in which necrosis only appeared after the emergence of *P. viticola* sporangiophores. Similar microscopic examination of hybrids described in the present study may have helped to classify the types of resistance observed.

The association of the HR to both low leaf chlorosis and sporulation in most of the families tested here supports our previous finding that the HR can be used as a selection indicator of resistance (Liu *et al.* 2003). Caution, however, should be exercised since its expression may not necessarily suppress leaf chlorosis and sporulation. This varied between hybrids within families (Figs 3 and 4) and suggested that the conditions and methods to screen vines for resistance are important. From a practical breeding point of view, when vines are selected as resistant under conditions of natural infection in the vineyard it would be prudent to test selections based solely on HR using a laboratory-based test such as the LSNC method as well as across seasons and/or locations to ensure reliable selections have been made.

Grapevines are considered highly heterozygous (Alleweldt and Possingham 1988) and many recessive alleles are carried for which expression is suppressed. The appearance of resistant hybrids based on symptoms of chlorosis and sporulation in all families investigated in the current study, regardless of combination, suggested that susceptible parents possessed recessive alleles conferring resistance. A score of 3 or less for these symptoms meant some hybrids displayed a greater level of resistance than 'Chambourcin' and 'Marroo Seedless', which are downy mildew resistant under vineyard conditions. Using this score to separate resistant and susceptible hybrids, segregation within families fitted ratios (Tabs 2 and 3) that suggested the action of four independent genes with recessive alleles conferring resistance and dominant alleles epistatic such that their presence at any locus results in a susceptible phenotype. From this, 'Chambourcin' and 'Marroo Seedless' would be homozygous recessive across all loci and when crossed with susceptible parents carrying varying numbers of dominant alleles in the heterozygous state, segregation for resistant to susceptible phenotypes would occur in ratios of 1:15, 1:7, 1:3 or 1:1 (Tabs 2 and 3). Similarly, when heterozygous susceptible genotypes were inter-crossed their families segregated for resistant and susceptible phenotypes. To a degree, this supports Matthews (1981), who reported that downy mildew resistance was controlled by 1 to 4 genetic factors in interspecific crosses, and suggests that by scoring hybrids using the LSNC method, the inheritance of downy mildew resistance for the families listed in Tab. 1 could be described in relatively simple terms unlike for crosses reported elsewhere that indicated a more complicated polygenic inheritance (Brown *et al.* 1999 b, Song *et al.* 1998, Eibach *et al.* 1989, Eibach 2000).

However, while observed segregation ratios for resistance may be accommodated by a model of 4 recessive genes, when genotypes were assigned to the parents used, it failed in a couple of instances, which suggested that the inheritance of downy mildew resistance in these crosses was more complex or family size was too small to explore segregation patterns completely. As families were relatively small, it would be interesting to expand the investigation by incorporating additional and larger families plus resistant x resistant crosses to explore this model for the inheritance of resistance in terms of chlorosis and sporulation.

The observed segregation ratios for the expression of HR are also difficult to explain via a simple model and perhaps warrant further investigation with additional and larger progenies including resistant x resistant crosses in which segregation for different types of necrotic lesion that have been associated with the HR could be investigated.

Regardless of being able to explain adequately the inheritance of the downy mildew resistant phenotype investigated here, it was possible to identify and select new hybrids more resistant to downy mildew than any of the parents used in the crosses, and this is important for the Australian grape growing industry. The occurrence of new segregants with greater resistance to downy mildew than any of the parents used is important for future resistance breeding. Improved disease resistance is a sought after characteristic to reduce inputs and improve consumer acceptance due to less reliance on preventative agrichemical sprays. The results presented here have indicated that continued breeding efforts based on pair-crosses involving parents with complex pedigrees incorporating non-*vinifera* germplasm will result in improved downy mildew resistant varieties, and the inclusion of at least one resistant parent in crosses should increase the proportion of resistant progeny in successive generations. The resistance is likely to be recessive and controlled by a few genes. By mapping these genes and identifying perfect or close linked markers improved breeding efficiencies and cost savings should eventuate (see Eibach *et al.* 2007).

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