Thaumatin-like Proteins and Chitinases, the Haze-Forming Proteins of Wine, Accumulate during Ripening of Grape (*Vitis vinifera*) Berries and Drought Stress Does Not Affect the Final Levels per Berry at Maturity

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Thaumatin-like proteins and chitinases, which are pathogenesis-related (PR) proteins, were the major soluble protein components of grapes from five cultivars of *Vitis vinifera*. This dominance of PR proteins was apparent at berry softening (véraison) and then throughout berry development for the Muscat of Alexandria, Sultana, and Shiraz cultivars and in the berries of the Sauvignon Blanc and Pinot Noir cultivars examined at commercial maturity. The M_r of the major thaumatin-like protein from Muscat of Alexandria grapes was 21 272, and those of the three major chitinases from this cultivar, ChitB, ChitC, and ChitD, were 25 588, 25 410, and 25 457, respectively. The vines in the study were irrigated and showed no obvious signs of disease. Shiraz vines that had not been irrigated throughout the season were clearly water stressed, but had levels of PR proteins in the berry similar to vines that had been fully irrigated. It appears that the production of PR proteins that cause protein instability in wines by grapes may be little influenced by environmental conditions.

Keywords: *Grape; wine instability; pathogenesis-related protein; thaumatin-like; chitinase; irrigation; drought stress; Vitis vinifera*

INTRODUCTION

The quality of white wines can be lowered by the appearance of sediments and hazes after bottling. Such precipitates are commonly the result of denaturation of grape proteins in wine (Bayly and Berg, 1967; Hsu and Heatherbell, 1987; Waters et al., 1992). The proteins that cause haze in wine have been identified as pathogenesis-related (PR) proteins, specifically thaumatin-like proteins and chitinases, from the grape berry (Waters et al., 1996, 1998).

PR proteins were first observed in tobacco as a response to viral infection (Gianinazzi et al., 1970; Van Loon and Van Kammen, 1970). The expression of this class of proteins is often induced systemically in many plants as a response to stress, pathogenic attack, and wounding (Boller, 1987; Linthorst, 1991; Stintzi et al., 1993). It has become evident over the past few years, however, that PR proteins are expressed in some fruits in the absence of stress, pathogenic attack, or wounding and may be involved in fruit development (Clendennen and May, 1997; Diaz-Perales et al., 1998; Fils-Lycaon et al., 1996; Harpster, 1997; McCollum, 1997; Nairn et al., 1997; Pressey, 1997). These proteins have also been implicated as the cause of allergic responses in humans to avocado (Diaz-Perales et al., 1998) and cherry (Inschlag et al., 1998).

The two groups of grape PR proteins involved in wine haze, chitinases and thaumatin-like proteins, also appear to be expressed in berries in the absence of stress or pathogen attack. In *Vitis vinifera* L. Muscat of Alexandria, both the expression of the gene VVTL1 and protein levels of the thaumatin-like protein in berries coded by this gene dramatically increased at the onset of berry softening (véraison). VVTL1 continued to be expressed and protein continued to accumulate throughout berry development (Tattersall et al., 1997). The deduced protein sequence of VVTL1 is identical to that of the thaumatin-like protein originally identified as a major haze-forming protein in wine by Waters et al. (1996). A study on *Vitis labruscana* L. Concord using tobacco antibodies (Salzman et al., 1998) also showed that thaumatin-like proteins and chitinases accumulate during berry development. A number of other studies have shown that chitinase activity also increases during berry development of *V. vinifera* (Derckel et al., 1996, 1998; Robinson et al., 1997), and two genes have been identified in *V. vinifera* L. Shiraz that code for the chitinases expressed during development (Robinson et al., 1997). The protein sequences deduced from these genes had high homology to the chitinases identified as major haze-forming proteins (Waters et al., 1996, 1998).

Although at least some PR proteins appear to be expressed in grape berries irrespective of environmental conditions, this situation does not mean that the classical PR protein gene "inducers", stress and pathogenic attack, cannot also modulate the levels of PR proteins in grapes. Indeed it has been shown that chitinase gene expression increased in *V. vinifera* leaves when the leaves were infected with fungi (Busam et al., 1997). Wounding also increased chitinase activity in *V. vinifera* leaves (Derckel et al., 1996) and berries (Derckel et al., 1998). The effect of a commercial form of wounding,

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mechanical harvesting, on the PR protein levels in grapes (Pocock et al., 1998a) and the subsequent wines (Pocock et al., 1998b) has also been examined. Although mechanical harvesting coupled with prolonged transport of the wounded fruit resulted in higher PR protein levels in the juice and wine, this appeared to be due to extraction of protein from the skins rather than due to wounding eliciting a physiological response by the berry in the form of increased levels of protein synthesis.

Drought and salt stress have been implicated in PR protein induction in other plants, and these potential PR protein gene "inducers" are of relevance to commercial wine grape growing conditions. In tobacco plants, thaumatin-like proteins are accumulated as a response to salt stress (Singh et al., 1987), and because of this, thaumatin-like proteins are sometimes called "osmotins" in the literature. A gene for a putative osmotin in *V. vinifera* L. Sultanina has been identified and its expression observed in immature berries (Loulakakis, 1997). The deduced protein sequence of this gene has homology to VVTL1, the genes coding for one of the major haze-forming proteins in wine. In tomatoes, Yu et al. (1998) showed that drought induced the expression of chitinase genes in fruit and caused an increase in chitinase activity in the fruit. The effect of drought and salt stress on the PR protein levels in grape berries is not known, although other effects of these stresses on the berries and vines are well documented (Smart and Coombe, 1983).

The study presented in this paper was initiated with two aims. One aim was to confirm that the major haze-forming proteins in wines, thaumatin-like proteins and chitinases, accumulated in grape berries during ripening of a number of *V. vinifera* cultivars grown commercially in the absence of environmental stress. The second aim was to examine the effect of drought stress on the level of thaumatin-like proteins and chitinases in the fruit of *V. vinifera* L. Shiraz to determine whether this stress increased the expression of PR proteins that would subsequently cause haze in wine.

MATERIALS AND METHODS

Fruit Samples. All fruit samples except those from *V. vinifera* L. Shiraz were from the 1996 growing season, and all vines were drip irrigated.

V. vinifera L. Muscat of Alexandria and Sultana (synonym Thompson Seedless) fruit was obtained from the vineyard of the Waite campus of the University of Adelaide at Urrbrae, South Australia. Small clusters of berries were taken at random from the middle of the bunches. All the sound berries were cut from the clusters, and a 50-berry subsample was taken from the sound berry sample for processing.

V. vinifera L. Sauvignon Blanc and Pinot Noir fruit was hand-picked from commercial vineyards at Padthaway, South Australia. Entire bunches (40) were taken at random from the vineyard. Samples were held on ice during transport to the laboratory and until the next day when they were processed. Berries from the top (2), the middle (2), and the bottom (1) of the bunches of fruit were taken at random to give 200 berries in total. From these 200 berries, a 50-berry subsample was selected for processing.

V. vinifera L. Shiraz fruit was obtained from both the 1995 and 1996 seasons from an irrigation trial at the Department of Primary Industries, South Australia at Waikerie, South Australia. The irrigation trial had been established for five years, and the samples discussed in this work were taken in the fourth and fifth years. The trial consisted of eight irrigation treatments with nine replicates in a randomized block design. Treatments included an unirrigated and a fully irrigated treatment together with six others in which irrigation was withheld during defined growth periods (McCarthy, 1997). For this study, only samples from the unirrigated and fully irrigated treatment were taken. Irrigation was applied after the first 30 mm of soil water had been depleted from a total water content of 130 mm in 1.2 m soil depth. Unirrigated vines were drought stressed by the end of the growing season; for example the fourth year was characterized by an average growing season temperature of 19.3 °C and total rainfall during the growing season of 130 mm. Full details of the trial are given in McCarthy (1997). Berries from the top (2), the middle (2), and the bottom (1) of bunches of fruit on three vines were taken at random to give 150 berries in total from each of nine replicate plots. Samples were held on ice during transport to the laboratory and until the next day when a 50-berry subsample was selected at random and processed.

Identification and Quantification of Soluble Protein from Grapes. The soluble protein fraction of the berries was the "free run" juice. Free run juice was extracted by gently pressing 50 berries by hand in a plastic bag, coarse filtration of the pressed berries through 2 mm mesh, and then centrifugation (10000*g*, 15 min).

The fraction containing the total protein content of the berries was prepared by homogenizing the pulp and skins of berries in an equivalent weight of a solution approximating grape juice (model grape juice) and containing the following absorbents and antioxidants: poly(ethylene glycol) (2% w/v), polyvinylpyrollidone (2% w/v), polyvinylpolypyrollidone (10% w/w of skins and pulp), and sulfur dioxide (500 mg/L), as described by Pocock et al. (1998a).

The protein content of the free run juices and the homogenates was determined as described in Pocock et al. (1998a).

The analytical data for the Shiraz samples were subjected to one-way analysis of variance using SAS Inst. Inc. JMP 3.1.6 software (Cary, NC). Samples for statistical analysis were selected from all the available samples if their soluble solid level was within a defined range. Thus samples with soluble solids levels between 14 and 16 °Brix were not necessarily harvested on the same day.

Purification of Muscat of Alexandria PR Proteins. *V. vinifera* L. Muscat of Alexandria grapes from the 1997 season were harvested at commercial maturity from the vineyard of the Waite Campus of the University of Adelaide at Urrbrae, South Australia. Free run juice was extracted by gently pressing fruit in a bladder press, coarse filtration of the pressed berries through 2 mm mesh, centrifugation (17000*g*, 30 min), and then close filtration through a membrane of 0.45 μm. The juice was then concentrated 10-fold by ultrafiltration through a 10 000 Da nominal molecular weight cut-off membrane (YM-10, Amicon Corporation, Danvers, MA) and then desalted on an Econo-Pac 10DG column (BioRad Laboratories, Sydney, Australia) into water.

Desalted samples of total protein (1 mL) were loaded at 1 mL/min onto a semipreparative C18 column (10 × 250 mm, Vydac, Hesperia, CA) fitted with a C18 guard column (4.6 × 10 mm, Alltech, Sydney, Australia) equilibrated in a mixture of 71% (v/v) solvent A [0.05% (v/v) trifluoroacetic acid (TFA)] and 29% solvent B [70% acetonitrile, 0.035% (v/v) TFA] and held at 40 °C. Proteins were eluted by a gradient of solvent B from 29% solvent B to 61% solvent B in the first 7 min, 61% to 70% from 7 to 15 min, 70% to 79% from 15 to 16 min, 79% to 94% from 16 to 30 min, and then held at 94% for a further 5 min.

Peaks were detected at 280 nm, and those eluting between 11.5 and 12 min (named VVTL1), between 13.5 and 14 min (minor TL), between 16.5 and 17 min (ChitA), between 22.2 and 22.5 min (ChitB), and as a double peak between 22.5 and 23 min (ChitC and ChitD) were collected. The last fraction was diluted with solvent A and reinjected under the same chromatographic conditions, and the peaks eluting between 22.5 and 22.6 min (ChitC) and between 22.7 and 22.9 min (ChitD) were collected. Retention times vary from those observed during the separations on the analytical column because the gradient was optimized on the semipreparative column to purify the proteins under these conditions.

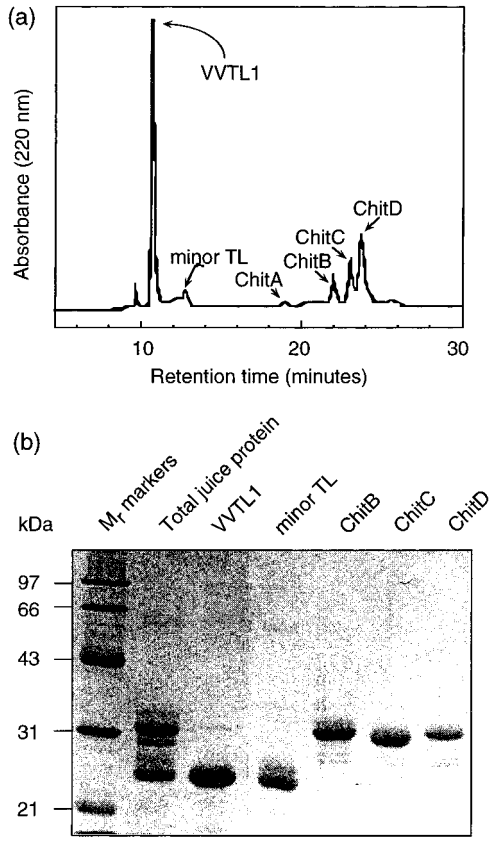

Figure 1. Protein composition of free run juice from *Vitis vinifera* L. Muscat of Alexandria berries by (a) reverse phase HPLC and (b) SDS PAGE. Proteins were identified by comparison of (a) their retention time and (b) position to that of the purified and sequenced proteins under the same chromatographic conditions. The position of purified and sequenced proteins is shown in (b). M_r: relative molecular mass. Approximately 4 μg of protein loaded per lane in (b).

Electrospray Mass Spectrometry. Purified proteins were subjected to mass spectrometric analysis using a PE Sciex API 300 with ion spray ionization (PE Sciex, Thornhill, Ontario, Canada) at the Mass Spectrometry Facility of the Waite Campus, University of Adelaide, as described previously (Peng et al., 1997).

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS PAGE). The protein composition of free run juice and isolated protein fractions was assessed by SDS PAGE analysis, according to Laemmli (1970).

RESULTS

Thaumatin-like Proteins and Chitinases Are Major Components of Ripe Muscat of Alexandria Berries. As described previously (Waters et al., 1998), and as shown here by HPLC and SDS PAGE analyses (see Figure 1a and 1b, respectively), the most abundant protein components of the free run juice from ripe *V. vinifera* L. Muscat of Alexandria grapes were thaumatin-like proteins and chitinases. There were 2 thaumatin-like proteins identifiable by HPLC (VVTL1 and minor TL, eluting at 10.9 and 12.9 min, respectively). There were four chitinases separated by HPLC in the free run juice from the Muscat of Alexandria grapes (ChitA, ChitB, ChitC, and ChitD eluting at 18.9, 22.1, 23.1, and 23.8 min, respectively).

The relative molecular masses (M_r's) of the PR proteins from Muscat of Alexandria grapes were estimated by SDS PAGE (Figure 1b except ChitA, data not shown) and electrospray ionization−mass spectrometry (ESI-MS). Both values are given in Table 1.

Table 1. Relative Molecular Masses (M_r's) of the PR Proteins from *V. vinifera* L. Muscat of Alexandria Grapes

protein	M_r by SDS PAGE[a]	M_r by ESI-MS[b]
VVTL1	24 000	21 272[c]
minor TL	25 000	21 260
ChitA	32 000	25 942
ChitB	33 000	25 588
ChitC	32 000	25 410
ChitD	34 000	25 457

[a] Estimated from comparison to standards, ±2000 Da. [b] ±5 Da. [c] Ion at 21 250 also detected in all samples of VVTL1 and under all conditions of mass spectrometry.

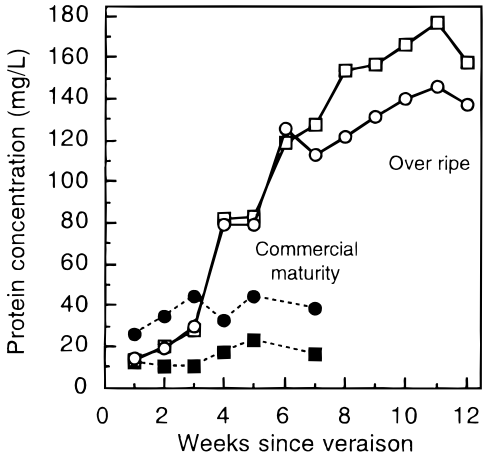

Figure 2. Effect of berry development on the concentration of thaumatin-like proteins (□, ■) and chitinases (○, ●) in the free run juice from *V. vinifera* L. Muscat of Alexandria and *V. vinifera* L. Sultana berries, respectively. For Muscat of Alexandria berries, 6, 7, and 8 weeks after véraison were designated as "commercial maturity" because sugar levels of free run juice were 20.6, 23.4, and 25.3 °Brix, respectively. For Sultana berries, 5 and 7 weeks after véraison were designated as "commercial maturity" because sugar levels of free run juice were 21.0 and 22.8 °Brix, respectively.

Thaumatin-like Proteins and Chitinases Are Also Major Components of Muscat of Alexandria and Sultana Grapes throughout Ripening. The concentration of soluble proteins in berries from two grape cultivars (Muscat of Alexandria and Sultana) was examined throughout berry development. The major soluble proteins in the grapes were thaumatin-like proteins and chitinases in all samples examined. Figure 2 shows how the concentration of these PR proteins in the free run juice changes from the onset of berry softening (véraison) past commercial maturity to overripeness.

Thaumatin-like Proteins and Chitinases Are Major Components of Ripe Grapes from Other *V. vinifera* Cultivars. The soluble protein content of a selected number of other cultivars of *V. vinifera* grapes picked at commercial maturity was also examined by HPLC (Table 2). In all cases, thaumatin-like proteins and chitinases were the major soluble protein components. The identity of proteins in these other cultivars was deduced from comparison to retention times of known proteins from Muscat of Alexandria. It is noteworthy that the retention times of the thaumatin-like proteins were identical in all samples examined, whereas the retention times of the chitinases tended to vary slightly (data not shown).

Table 2. Concentration of PR Proteins in the Juice of Different Cultivars of *V. vinifera* at Commercial Maturity

		protein concentration (mg/L)		
cultivar	°Brix	thaumatin-like proteins	chitinases	total protein
Muscat of Alexandria	20.6	119	118	251
Sauvignon Blanc	21.5	119	76	191
Sultana	21.0	23	44	86
Pinot Noir	20.2	35	21	62
Shiraz	20.8	18	9	31

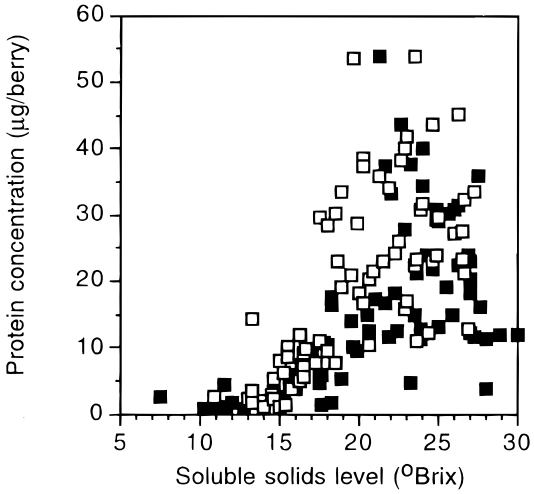

Figure 3. Effect of berry development on the total protein concentration in free run juice from irrigated (□) and nonirrigated (■) *V. vinifera* L. Shiraz fruit during 1996. Fruit was sampled weekly from 9 replicate plots of each treatment from véraison to overripeness, 9 sample times in total.

Effect of Water Stress on the PR Protein Concentration of Shiraz Berries. The soluble protein content of Shiraz berries from an irrigation trial conducted in a warm inland area of South Australia was examined. The protein concentration in the free run juice was determined for nine replicate lots of fruit from fully irrigated and nonirrigated vines sampled weekly from véraison (soluble solids level between 10 and 12 °Brix) through commercial maturity (from 20 to 26 °Brix) to over-ripeness (more than 26 °Brix). Once again, the major proteins in all samples were thaumatin-like proteins and chitinases (data not shown). The data for all the samples are shown on a per berry basis in Figure 3 and demonstrate that, while there was considerable variability among the samples, PR proteins also accumulated in *V. vinifera* L. Shiraz berries during fruit development.

During the early stages of ripening (up to about 17 °Brix), the free run juice of fruit from the irrigated vines tended to contain higher levels of protein than that of fruit from the nonirrigated vines (Figure 3). A statistical analysis of the data for selected samples from the two treatments with soluble solids levels from 14 to 16 °Brix is given in Table 3. This shows that the free run juice from irrigated berries at this ripeness level contained a significantly higher protein concentration on a per berry ($p < 0.0001$) and per gram basis ($p < 0.01$). The difference in protein concentration of the free run juice between the berries from the two treatments was not as large on a per volume or per gram basis as it was on a per berry basis because of the significantly ($p < 0.0001$) larger size of berries from the irrigated vines.

After the berries had reached a soluble solids content of more than 20 °Brix, the protein concentration in the free run juice from the irrigated fruit tended to be similar to that from the nonirrigated fruit, although the scatter in the data made this difficult to see in Figure 3. A statistical analysis of the data for selected berries from the two treatments with soluble solids levels from 19 to 21 °Brix, and from 24 to 26 °Brix, is given in Tables 4 and 5, respectively. These analyses show that there was no significant difference between the two treatments in the protein concentration of free run juice from berries at these two ripeness levels on a per berry or per volume basis. By the time the soluble solids level in berries had reached between 24 and 26 °Brix (Table 5), the difference in berry size between the treatments caused the protein concentration in the free run juice to be significantly lower for the irrigated vines on a per volume basis.

The protein concentration in the whole berry, rather than in free run juice, from the same sample set was analyzed at 2-week intervals (Figure 4). Trends similar to those seen with the free run juice were observed: fruit from the nonirrigated vines tended to contain less protein than that from irrigated vines at most ripeness levels examined. A statistical analysis of the data for selected berries from the two treatments with soluble

Table 3. Effect of Irrigation on the Berry Weight and Protein Concentration of the Free Run Juice from Selected *V. vinifera* L. Shiraz Berries with a Soluble Solids Level from 14 to 16 °Brix Grown during 1996

	not irrigated	irrigated	F^a
number of replicates	5	10	
°Brix	15.2	15.1	ns
berry weight (g)	0.904	1.363	****
protein concentration			
(µg/berry)	4	8	****
(µg/g)	4	6	**
(mg/L)	9	12	ns

[a] Significance of *F* value: ns = not significant ($p > 0.05$), *, **, and **** = $p < 0.05$, 0.01, and 0.0001, respectively.

Table 4. Effect of Irrigation on the Berry Weight and Protein Concentration of the Free Run Juice from Selected *V. vinifera* L. Shiraz Berries with a Soluble Solids Level from 19 to 21 °Brix Grown during 1996

	not irrigated	irrigated	F^a
number of replicates	9	11	
°Brix	20.6	20.3	ns
berry weight (g)	0.976	1.399	****
protein concentration			
(µg/berry)	18	26	ns
(µg/g)	18	19	ns
(mg/L)	35	36	ns

[a] Significance of *F* value: ns = not significant ($p > 0.05$), **** = $p < 0.0001$, respectively.

Table 5. Effect of Irrigation on the Berry Weight and Protein Concentration of the Free Run Juice from Selected *V. vinifera* L. Shiraz Berries with a Soluble Solids Level from 24 to 26 °Brix Grown during 1996

	not irrigated	irrigated	F^a
number of replicates	12	6	
°Brix	25.0	24.9	ns
berry weight (g)	0.976	1.383	****
protein concentration			
(µg/berry)	25	25	ns
(µg/g)	26	18	ns
(mg/L)	52	33	*

[a] Significance of *F* value: ns = not significant ($p > 0.05$), * and **** = $p < 0.05$ and 0.0001, respectively.

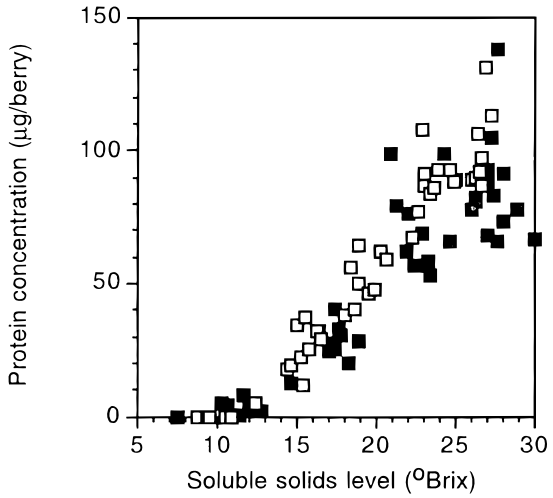

Figure 4. Effect of berry development on the total protein concentration in whole berries from irrigated (□) and nonirrigated (■) *V. vinifera* L. Shiraz fruit during 1996. Fruit was sampled every 2 weeks from 9 replicate plots of each treatment from véraison to overripeness, 5 sample times in total.

Table 6. Effect of Irrigation on the Berry Weight and Total Berry Protein Concentration of Selected *V. vinifera* L. Shiraz Berries with a Soluble Solids Level of Approximately 25 °Brix Grown during Two Years

	not irrigated	irrigated	F^a
1996 Berries			
number of replicates	6	8	
°Brix	25.4	25.2	ns
total protein concentration (µg/berry)	83	92	ns
1995 Berries			
number of replicates	8	7	
°Brix	25.3	24.7	ns
total protein concentration (µg/berry)	130	150	ns

[a] Significance of *F* value: ns = not significant.

solids levels from 23.5 to 26.5 °Brix is given in Table 6. This broader soluble solids range was chosen to increase the sample size. These analyses show that there was no significant difference between the two treatments in the protein content of whole berries. To confirm these data, the total protein content of whole berries with a soluble solids level between 24 and 26 °Brix from the previous year of the same irrigation trial was also examined (Table 6). There was no significant difference in the protein content of berries from the two treatments in this year either.

DISCUSSION

Unlike other tissues of other plants, the expression of PR proteins in grape berries examined here appeared to be developmentally regulated. This result is in accord with work by others on grape berries (Robinson et al., 1997; Tattersall et al., 1997) and adds to the growing body of data showing that some PR proteins accumulate in fruit and may be involved in fruit development and ripening (Clendennen and May, 1997; Diaz-Perales et al., 1998; Fils-Lycaon et al., 1996; Harpster, 1997; McCollum, 1997; Nairn et al., 1997; Pressey, 1997). The expression of PR proteins in fruit in the absence of pathogen attack does not, however, exclude a role in defense for these proteins. Other authors (Derckel et al., 1996; Esaka et al., 1993) have commented on the need for storage tissues with high sugar concentrations, e.g., fruit tissues, to have greater protection than non-carbohydrate-rich tissues due to the susceptibility to pathogenic attack of the former. While PR proteins in general are believed to have antifungal properties, chitinases are specifically implicated in the protection of plant tissues against fungal pathogens due to the ability of these enzymes to hydrolyze chitin, a component of fungal cell walls.

The dominance of these two groups of PR proteins, thaumatin-like proteins and chitinases, was evident at all stages of berry development and began early in the ripening phase. Despite their ubiquitous presence, there was an 8-fold difference in the overall concentrations of PR proteins among the five cultivars of *V. vinifera* examined here (Table 2). There were also differences in the relative concentrations of the thaumatin-like proteins and the chitinases among the five cultivars (Table 2). Both the major (VVTL1) and minor thaumatin-like proteins were present in free run juice from all cultivars examined here, and in similar proportions (data not shown). For the chitinases, however, the pattern of these enzymes present in the free run juice varied (data not shown). The soluble protein fraction of the fruit from Pinot Noir contained only one chitinase, whereas that from Sultana and Sauvignon Blanc contained two chitinases, that from Shiraz fruit contained three, and there were four chitinases present in the free run juice from Muscat of Alexandria berries. Preliminary data also indicate that the molecular masses (M_r's) of the chitinases from different cultivars varied (data not shown).

The M_r's of the PR proteins from Muscat of Alexandria grapes were estimated here by both SDS PAGE and electrospray ionization−mass spectrometry (ESI-MS, Table 1). These data indicate that all of the grape PR proteins migrated in SDS PAGE gels to positions corresponding to proteins that were several thousands of daltons higher than the M_r of the grape proteins determined by mass spectrometry. The anomalous migration of PR proteins in SDS PAGE has been previously reported (Cusask and Pierpoint, 1988; Fils-Lycaon et al., 1996). The M_r of 21 272 Da for VVTL1 from Muscat of Alexandria berries estimated by ESI-MS is identical to that reported by Tattersall et al. (1997) from berries of the same cultivar and similar to that reported for the major protein in Muscat of Alexandria and Sauvignon Blanc wine by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry by Weiss et al. (1998, M_r 21.3 kDa). The M_r's for the chitinases from Muscat of Alexandria berries of 25 .4−25 .9 kDa estimated by ESI-MS and 32 −34 kDa estimated by SDS PAGE are also similar to those described by others. Robinson et al. (1997) estimated a M_r of 25 330 from the deduced protein sequence of a chitinase gene from *V. vinifera* L. Shiraz berries, and Derckel et al. (1998) used SDS PAGE to estimate the M_r of a chitinase from *V. vinifera* L. Pinot Noir berries at 31 kDa.

The effect of water stress on the "baseline" expression of haze-forming proteins was examined in this study by analyzing the PR protein concentration of Shiraz berries from an irrigation trial. Contrary to expectations that lack of irrigation would induce a stress response in the form of increased PR proteins, it appears that water stress either had no effect or inhibited PR protein production by the berries. At comparable soluble solids

levels, grapes from the irrigated vines tended to contain either the same or more protein per berry than that from drought-stressed nonirrigated vines. Nevertheless, the protein concentration in the free run juice on a per volume basis from water-stressed berries can indeed be higher than that from irrigated berries because berries from irrigated vines are larger (Smart and Coombe, 1983). This phenomenon was seen here for Shiraz at maturity (soluble solids level of 24−26 °Brix). Thus the anecdotal reports in the wine industry that protein instability problems are greater in drought years are probably due to changes in berry sizes in these years rather than a direct physiological response of the berries to water stress on PR protein production.

ABBREVIATIONS USED

ESI-MS, electrosray ionization-mass spectrometry; M_r, relative molecular mass; PR, pathogenesis-related; SDS PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; TFA, trifluoroacetic acid.

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