

Influence of Rootstocks on Changing the Pattern of Phenolic Compounds in Thompson Seedless Grapes and Its Relationship to the Incidence of Powdery Mildew

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Abstract: The total phenolic content and individual groups of phenols, such as flavonones, flavonoids, and flavon-3-ols, were estimated in the leaves and berries of Thompson Seedless grapes grafted onto different rootstocks and own-rooted vines during 3 stages of berry development. The leaves at all the stages of sampling contained more total phenol and individual phenolic groups than berries. The reduction in phenolic content was gradual in leaves from the first to third stage of sampling, but it was drastic in berries. Thompson Seedless grafted onto Dog ridge, 110 R, and 1103 P rootstocks had the highest phenolic content and individual phenolic groups in the third stage of sampling and the least on Thompson Seedless grafted onto 99 R, St. George rootstocks, and onto own-rooted vines. The number of bunches infested with powdery mildew was the highest on own-rooted vines, and on those grafted onto St. George and 99 R. A significant negative correlation was observed between the number of bunches infected by powdery mildew and phenolic content.

Key Words: Grapes, rootstocks, phenolic compounds, powdery mildew

Introduction

Grape (*Vitis vinifera*) is one of the most widely cultivated fruit crops in the world and is the most economically important. In contrast to other fruit crops, grapes are not only consumed as fresh fruit, but are also the basis for production of value added products like juices, raisins, wine, and spirits. In India, although the value addition to grapes has been gaining importance in recent years, the majority of grapes produced are used for fresh consumption. Thompson Seedless is the leading commercial variety in India and it is known for its good yield and quality parameters. However, this variety is highly susceptible to major fungal diseases, such as anthracnose, downy mildew, and powdery mildew under Indian climatic conditions. Powdery mildew is the second most important disease of grapes and is caused by the fungus, *Uncinula necator* Burr. It is an obligate parasite that develops superficial mycelium on the developing buds, which become dormant as buds mature. Immediately after buds burst, the fungus becomes active and grows on young shoots. Climatic factors, including

temperatures of 21-27 °C and relative humidity of more than 60%, lead to the spread of the disease. Infection on developing berries leads to death of affected epidermal cells and the subsequent growth of berries becomes uneven, leading to cracking of the berries. Formation of scars on the infected berries decreases their market value.

Plants have defense mechanisms, which are aided by some biochemical molecules. Compounds such as phenols, salicylic acid, chloroisonicotinic acid, and benzothiadiazole-7-carbothioic acid S-methyl ester (BTH) are able to induce systemic acquired resistance against a wide range of microbial pathogens in a variety of plants (Sticher et al., 1997). Polyphenols are a part of the complex immune system, which can be acquired in tissues under stress (Feucht, 1994). Contrary to animals, plants cannot synthesize antibodies for defense, but can produce numerous phenolic substances – phytoalexins. These are secondary metabolites, which inhibit and kill pathogenic organisms (Bennett and Wallsgrove, 1994). Phenolics are known to inhibit the feeding of many insects and have

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demonstrated toxicity (Grayer et al., 1992). The involvement of phenols in plant disease resistance is based, to a large extent, on their cytotoxicity, which is associated with their oxidation products (Aver'yonav and Lapikova, 1994). Phenolics consist of such compounds as condensed tannins, flavonoids, phenyl propyl, etc. Flavonoids are fairly well distributed in the plant kingdom (Hermann, 1988). They are known to possess insecticidal and antimicrobial activity (Barberan et al., 1988). It has been proposed that the first stage of the defense mechanism of plants involves a rapid accumulation of phenols at the infection site, which function to slow down the growth of the pathogens. Polyphenols play a vital role in the growth and propagation of plants and protect plant tissues from damage. A number of phenols are regarded as pre-infection inhibitors, providing plants with a certain degree of basic resistance against pathogenic microorganisms.

Use of rootstocks is gaining importance in Indian viticulture and the majority of grape vineyards are established on rootstocks to overcome the adverse effects of abiotic stresses, such as drought and salinity. In addition, rootstocks are known to influence the growth pattern of scions after grafting and some may even induce resistance to diseases by various physiological and biochemical changes in the grafted vines (Sobhana, 1988). As rootstocks provide a root system to the scion variety grafted onto them, and influence various physiological and biochemical changes in the scion leaves (Satisha et al., 2005), it is necessary to observe the influence of rootstocks in the accumulation pattern of phenolics and other important secondary metabolites in the scion leaves grafted onto them. This will be helpful in classifying rootstocks into those that impart tolerance to grape fungal diseases by the accumulation of phenols in scion leaves. Hence, the purpose of the current study was to analyze the changes in the phenolic composition of berries and leaves at different stages of development, and the correlated incidence of powdery mildew in the berries of Thompson Seedless grapes grafted onto different rootstocks.

Materials and Methods

The present study was conducted at the experimental plots of the National Research Centre for Grapes, Pune, during the fruiting season of 2005-2006. Pune is situated in mid-west Maharashtra, at an altitude of 559

m; it lies on lat 18.32°N, long 73.51°E. The soil of this region is black, having a slightly alkaline pH. Five-year-old Thompson Seedless vines grafted onto 5 different rootstocks, viz. Dog ridge, St. George, 110 R, 99 R, and 1103 P were selected, while ungrafted Thompson Seedless vines served as the control. The vines were planted at a spacing of 3 × 1.8 m and drip irrigated as per the irrigation schedule developed for this region at various phenological stages. The representative leaf and berry samples were collected during 3 different stages; the pre-veraison stage (8-10 mm berry size, stage I), veraison stage (14-15 mm berry size, stage II), and harvesting stage (18-19 mm berry size, stage III). The details of the sampling and berry characters are as follows:

Sample No.	Days after pruning	Berry size (mm)	TSS (°B)
1	70-75	8-10	11
2	90-95	14-15	14
3	125-130	19-20	20

Observation of Bunches Affected by Powdery Mildew

After pruning and the emergence of bunches, excess bunches were thinned out, depending on the canopy size, and the total number of bunches retained on each vine were counted. During stage II of sampling, when the incidence of disease was severe, every bunch on every vine was physically inspected for the incidence of disease and infected bunches were removed; only healthy bunches were retained until harvest. Finally, the percentage of bunches infected were calculated for each rootstock.

Chemicals

The standard reference chemicals, viz., (+) catechol (98% purity), gallic acid, and quercetin (98% purity), were obtained from Sigma (St. Louis, MO, USA). All other solvents and chemicals used in this study were of HPLC grade and obtained from Merck (Mumbai, India).

Sampling Method

Leaf and berry samples were collected randomly from 6 vines grafted onto different rootstocks. After harvesting, the samples were washed thoroughly with distilled water, air-dried, and stored at $-20\text{ }^{\circ}\text{C}$. Each sample was subsequently lyophilized using a freeze drier (Benchtop 4 K VIRTIS) at $-78\text{ }^{\circ}\text{C}$. Lyophilized samples were blended thoroughly and sieved through a 40-mesh sieve and stored at $-20\text{ }^{\circ}\text{C}$ until processed further.

Extraction of Samples

One gram of each sample lyophilized in each of the 3 replications was extracted by overnight shaking at room temperature (RT) on a mechanical shaker in the dark. The solvent used was 80% aqueous methanol, reported to be a good solvent for polyphenol extraction (Bonilla et al., 2003). The mixture was centrifuged at 12,000 rpm for 15 min at $4\text{ }^{\circ}\text{C}$. The residue was re-extracted (3 times, 3 hours each) in similar conditions. Complete extraction for the berries and leaves was ensured by a qualitative Folin-Ciocalteu negative test on filter paper. The filtrates were pooled and concentrated to one-third volume using a TurboVap concentrator under a gentle stream of nitrogen. The leaf sample extracts were treated with chloroform to remove chlorophyll and residual aqueous extracts were washed with ethyl acetate (Park and Cha, 2003). The extracts were filtered through $0.45\text{ }\mu\text{m}$ filters and stored at $0\text{ }^{\circ}\text{C}$ until analyzed further (Ju and Howard, 2003).

Total Phenolic Content

Total polyphenol content of the extract was determined by the Folin-Ciocalteu method (Singleton and Rossi, 1965), using gallic acid as the standard. The concentration of total phenolics was expressed as the gallic acid equivalent (GAE mg g^{-1}) of the lyophilized sample.

Total Flavonoids

Total flavonoids were determined colorimetrically, following the procedures proposed by Kim et al. (2003). The amount of total flavonoids was expressed as the catechin equivalent (CE, mg g^{-1}) of the lyophilized sample.

Flavon-3-ols

Total flavon-3-ol content was estimated using the DMACA method (Arnous et al., 2001). The concentration of total flavon-3-ols was expressed as the CE (mg g^{-1}) of the lyophilized sample.

Total Flavonols

Flavonol content was estimated by measuring the absorbance of the extract at 360 nm after the addition of 2% HCl (Mazza, 1999) and was expressed as the quercetin equivalent (QE mg g^{-1}) of the lyophilized sample.

Statistical Analysis

The experiment used a randomized block design with 3 replications. Each replication consisted of 6 vines. The results were analyzed with by Student's unpaired t-test using M STAT software v.11.0; a P value of 0.05 was considered to be significant.

Results

Significant differences were observed in the total phenolic content of leaves during stage I of sampling; Thompson Seedless grafted onto St. George had the most phenols (58.99 mg g^{-1} GAE), flavonoids (26.03 mg g^{-1} CE), and flavonols (25.16 mg g^{-1} QE), while ungrafted Thompson Seedless had the most total flavon-3-ols (2.69 mg g^{-1} CE). The lowest total phenolic content and total flavonoids were recorded in cv. Thompson Seedless grafted on 110 R rootstock (Table 1). There was a gradual reduction in total phenolic content and individual phenolic groups, though the rate of reduction was greater during harvesting from stage I to III of sampling. There was a slight reduction in these phenolic compounds among the rootstocks from stage I to III (Figure 1). The greatest reduction was in the leaves of ungrafted Thompson Seedless, and in those grafted onto St. George and 99 R. A slight reduction was observed in the leaves of Thompson Seedless grafted onto 110 R, Dog ridge, and 1103 P. The same trend was observed for the changes in total flavonoid content in the leaves of Thompson Seedless grafted onto different rootstocks from stage I-III; however, a consistent change was not observed for the contents of flavonols and flavon-3-ols.

The total phenolic content and individual phenolic compounds in the berries of ungrafted Thompson Seedless and those grafted onto different rootstocks are shown in Table 2. Total phenolic content and individual phenolic groups during stage I of berry sampling were the highest in Thompson Seedless grafted onto Dog ridge, 1103 P, and 110 R rootstocks. Among these, Thompson Seedless grafted onto 110 R rootstock recorded the highest phenols (36.43 mg g^{-1}), total flavonoids (15.66

Table 1. Total phenolic content of Thompson Seedless leaves grafted onto different rootstocks during different stages of growth.

Rootstocks	Stage I	Stage II	Stage III
Total Phenolics (mg g ⁻¹ GAE)			
110 R	43.65 ± 2.97c	44.32 ± 2.98a	39.94 ± 0.70a
Dog ridge	48.88 ± 4.80b	40.45 ± 2.69bc	35.32 ± 0.30b
1103 P	46.15 ± 1.40bc	41.71 ± 9.44b	39.04 ± 0.71a
T. Seedless	48.89 ± 1.67b	45.26 ± 3.89a	30.23 ± 1.84c
St. George	58.99 ± 5.93a	38.87 ± 0.50c	39.04 ± 0.26a
99 R	43.94 ± 0.68c	41.90 ± 1.38b	22.86 ± 0.21d
Total Flavonoids (mg g ⁻¹ CE)			
110 R	16.73 ± 1.69cd	22.54 ± 0.60a	15.39 ± 0.20b
Dog ridge	18.52 ± 1.38c	19.07 ± 1.56b	12.84 ± 0.65c
1103 P	18.81 ± 2.04c	16.96 ± 1.49c	15.86 ± 0.65ab
T. Seedless	21.09 ± 0.84b	20.03 ± 0.31b	16.36 ± 1.21 a
St. George	26.03 ± 1.41a	20.41 ± 1.30ab	15.85 ± 0.05ab
99 R	16.73 ± 1.69cd	12.90 ± 2.11d	11.23 ± 0.70cd
Flavonols (mg g ⁻¹ QE)			
110 R	22.48 ± 0.41b	23.08 ± 1.61a	17.69 ± 0.55ab
Dog ridge	16.28 ± 1.38c	18.47 ± 0.83bc	16.16 ± 0.56bc
1103 P	18.00 ± 0.64c	22.57 ± 4.18a	19.16 ± 0.20a
T. Seedless	24.87 ± 0.41 ab	23.03 ± 1.22a	19.02 ± 0.84
St. George	25.16 ± 1.97a	19.60 ± 1.57b	19.16 ± 0.20a
99 R	23.16 ± 0.80b	17.23 ± 1.60c	15.51 ± 0.25c
Flavon-3-ols (mg g ⁻¹ CE)			
110 R	2.09 ± 0.17b	2.31 ± 0.17a	1.40 ± 0.04b
Dog ridge	2.91 ± 0.18a	1.61 ± 0.81b	1.85 ± 0.11a
1103 P	1.89 ± 0.12b	1.46 ± 0.32b	1.83 ± 0.03a
T. Seedless	2.69 ± 0.03a	1.74 ± 0.16ab	0.89 ± 0.09 c
St. George	2.32 ± 0.15ab	2.01 ± 0.03a	1.70 ± 0.03ab
99 R	2.59 ± 0.34 a	1.24 ± 0.11bc	0.85 ± 0.02c

*Values are the mean ± SD of 3 replications and those marked with different letters in the same column are significantly different at P ≤ 0.05.

Table 2. Total phenolic content of Thompson Seedless berries grafted onto different rootstocks at different stages of growth.

Rootstocks	Stage I	Stage II	Stage III
Total Phenolics (mg g ⁻¹ GAE)			
110 R	36.43 ± 2.98a	4.88 ± 0.44cd	4.32 ± 0.25a
Dog ridge	27.53 ± 1.66b	4.96 ± 1.39c	4.12 ± 0.07a
1103 P	33.54 ± 1.29a	6.99 ± 1.08b	3.97 ± 0.52ab
T. Seedless	14.57 ± 2.39d	7.40 ± 1.17a	3.81 ± 0.23bc
St. George	16.04 ± 2.46c	4.16 ± 0.56d	3.24 ± 0.29c
99 R	16.02 ± 2.5cd	7.08 ± 0.47ab	3.57 ± 0.26c
Total Flavonoids (mg g ⁻¹ CE)			
110 R	15.66 ± 2.32a	1.78 ± 0.23d	1.54 ± 0.25ab
Dog ridge	12.96 ± 1.77b	2.01 ± 0.33cd	1.68 ± 0.22a
1103 P	15.61 ± 1.83a	3.12 ± 0.36a	1.37 ± 0.2bc
T. Seedless	12.23 ± 2.35bc	2.85 ± 0.36b	1.25 ± 0.14c
St. George	12.82 ± 0.41b	1.55 ± 0.18d	1.23 ± 0.12c
99 R	11.88 ± 1.33c	2.71 ± 0.28b	1.60 ± 0.06a
Flavonols (mg g ⁻¹ QE)			
110 R	3.19 ± 0.20a	0.51 ± 0.08bc	0.61 ± 0.03 a
Dog ridge	2.30 ± 0.09b	0.49 ± 0.009c	0.47 ± 0.04b
1103 P	2.97 ± 0.16a	0.61 ± 0.12a	0.55 ± 0.07a
T. Seedless	1.04 ± 0.11d	0.60 ± 0.13a	0.39 ± 0.09d
St. George	0.30 ± 0.10e	0.37 ± 0.01d	0.43 ± 0.02bc
99 R	1.20 ± 0.24c	0.39 ± 0.40d	0.40 ± 0.04 cd
Flavon-3-ols (mg g ⁻¹ CE)			
110 R	5.75 ± 0.55a	0.71 ± 0.07bc	0.52 ± 0.02a
Dog ridge	4.85 ± 0.68b	0.70 ± 0.16c	0.51 ± 0.09a
1103 P	5.85 ± 0.73a	0.97 ± 0.15ab	0.42 ± 0.06b
T. Seedless	3.25 ± 0.96d	1.04 ± 0.15a	0.44 ± 0.05b
St. George	4.13 ± 0.15c	0.80 ± 0.10b	0.37 ± 0.03c
99 R	4.01 ± 1.30c	0.93 ± 0.14ab	0.44 ± 0.06b

*Values are the mean ± SD of 3 replications and those marked with different letters in the same column are significantly different at $P \leq 0.05$.

mg g⁻¹), flavonols (3.19 mg g⁻¹), and flavon-3-ols (5.75 mg g⁻¹). All of these compounds were lowest either on ungrafted Thompson Seedless or those grafted onto 99R and St. George rootstocks. Compared to the reduction in the phenolic content of leaves during stage I-III of sampling, there was a greater reduction in these compounds in the berries of ungrafted and grafted Thompson Seedless (Figure 2).

The disease incidence rates in bunches of ungrafted and grafted Thompson Seedless are shown in Table 3. The percentage of diseased bunches was lowest on Thompson Seedless grafted onto 110 R (14.0 %), followed by those grafted onto Dog ridge (14.3); however, the bunch infection rate was the highest on ungrafted Thompson Seedless (30.3 %), followed by Thompson Seedless grafted onto St. George (23.7%).

A strong negative relationship was observed between the disease incidence rate of bunches and total phenolic

content during stage I of sampling (Figure 3). As the phenolic content in the berries increased, the incidence of disease decreased (e.g., Thompson Seedless grafted onto 110 R and Dog ridge rootstocks), and vice versa (ungrafted Thompson Seedless and those grafted onto St. George).

Discussion

Total phenolic and individual phenolic derivatives content in leaves were higher at all the stages of sampling compared to those of berries. Flavonols found in the leaves of some *Vitis vinifera* cultivars include quercetin-3-glucuronoside, quercetin-3-glucoside, quercetin-3-rhamnoside, quercetin-3-rutinoside, apigenin-3-glucoside, luteolin-7-glucoside, quercetin-3-rhamnogalicoside, myricetin, quercetin, kaempferol, and caffeic acid (Hmamouchi et al., 1996). Although the only phenolic derivatives we could determine were the concentration of

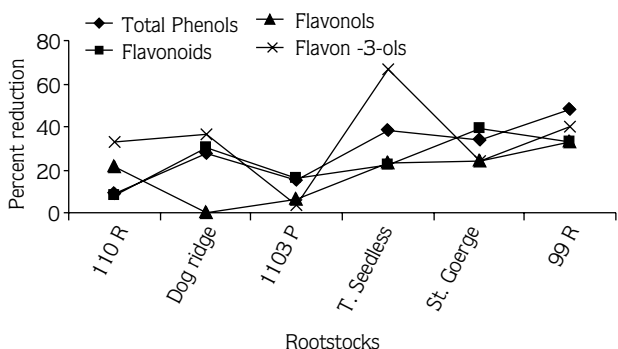


Figure 1. Percent reduction in phenolic content in the leaves of Thompson Seedless grafted onto rootstocks during the final stage.

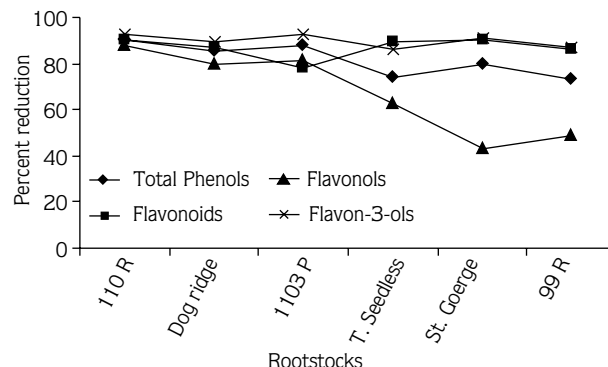


Figure 2. Percent reduction of phenolic content in the berries of Thompson Seedless grapes grafted on different rootstocks during the final stage.

Table 3. Powdery mildew incidence rate in Thompson Seedless grafted onto different rootstocks.

Rootstocks	Total Bunches retained*	Harvested bunches	Bunches infected by powdery mildew (%)
110 R	73	63	14.0
Dog ridge	68	58	14.3
1103 P	61	50	17.8
St. George	50	38	23.7
99 R	63	49	21.4
T. Seedless (Own rooted)	43	34	30.3

*Values are from 3 replications with 6 vines per replication (18 vines in each rootstock).

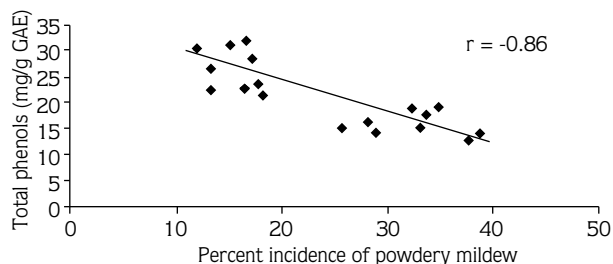


Figure 3. The relationship between total phenolic content and powdery mildew-infected bunches in Thompson Seedless grapes.

flavonoids, flavonols, and flavon-3-ols, the total phenol content must have been comprised of all the other individual flavonols, which might have been the cause for higher phenol content in leaves than in berries.

The reduction in total phenolic and individual phenolic derivatives content in berries from the first to third sampling stage might be attributable to the accumulation of water in berries during maturation, which enhances the hydrolysis of higher molecular weight phenolic compounds. This finding is similar to the data of Cimato et al. (1990). Similarly, the reduction in total phenolic content during maturation of olive fruits was observed by Amiot et al. (1989), which was attributed to accumulation of hydrolyzed products, some of which were even reported to be non-phenolic compounds. During the initial stages of berry development, flavonol content in berries was the highest, which suggests a role in the protection of tissues during the early stages of berry development (Winkel-Shirley, 2002). Flavon-3-ols content, measured as catechin, was very low during all the 3 stages of sampling, which is in agreement with the findings of Marquettes (1999) who reported that the sum of (+)-catechin and (-)-epicatechin decreases in general during grape ripening.

Pooja Doshi et al. (2006) also reported the most total phenolics, flavonoids, flavonols, flavon-3-ols, and antioxidant activity in berries during the initial stages of maturation; however, there was a drastic reduction in various phenolic contents at the completion of berry maturation in Sharad Seedless (Syn: Kishmish chernyi) grapes. The results of the present investigation are in accordance with this finding, wherein variations in total phenolic content and other individual groups in leaves were greater than in berries during different stages of berry development. Synthesis of various types of phenolic compounds varied during different stages of berry

growth, which was indicated in the present study by the variation in the accumulation of phenolic compounds during different stages of berry development.

The species of genus *Vitis* that contain high polyphenols content (*V. rotundifolia*) are more resistant to infection caused by *Uncinula necator*, and when infected they produce larger amounts of polyphenols than varieties that are more susceptible (Dai et al., 1994). They also classified grape species *Vitis vinifera* as susceptible, *V. rupestris* as moderately susceptible, and *V. rotundifolia* as resistant. The resistant variety contained more gallic acid derivatives and catechin tannins as compared to the susceptible variety. The appearance of flavonoids during the early stage may play an important role in resistance. This suggests that the resistance of *Vitis* species against powdery mildew involves pre-existing and induced chemical barriers. Crude foliar extract of the resistant variety had greater antifungal activity than that of the moderately susceptible and susceptible variety. A positive correlation between the catechin content in grapes and resistance to gray mold has been reported (Goetz et al., 1999). In grapes infected by *Uncinula necator*, the synthesis of trans-resveratrol increased, especially due to the synthesis of anthocyanins (Piermattei et al., 1999).

The outcome of the experiment was in agreement with previous findings of Misirli et al. (1994), wherein susceptible and moderately susceptible almond hybrids had lower phenolic content than the hybrids that were more resistant to *Pseudomonas* infection. However, this is in contrast to the findings of Niederleitner et al. (1994), who reported that among cherry leaves infected with *Blumeriella jaapi*, the catechin content of susceptible varieties was higher, according to HPLC analysis of leaf extract. Similarly, sunflower varieties resistant to *Sclerotinia sclerotiorum* were found to accumulate more caffeoylquinic derivatives in healthy zones compared to susceptible groups. Phenolics and elevated activity of phenylalanine ammonia-lyase (PAL) have been associated with resistance in strawberry fruits to disease caused by pathogens (Jersch et al., 1989; Di Venere et al., 1998; Nigro et al., 1998).

The present investigation's results are in agreement with the findings of Piermattei et al. (1999); polyphenol content was lower in grapevines infected with *Uncinula necator* than in healthy vines. Both an increase and a decrease in the polyphenol content in resistant and

susceptible varieties/species has been observed by different researchers. Kaur et al. (1989), Kaur et al. (1991), and Baruah and Chowfla (1994) reported higher polyphenol content in healthy plant tissues. They concluded that higher secondary metabolites content in healthy plants protected the plants from infection; however, Kumar (1991), and Sharma and Chowfla (1991) reported higher amounts of total polyphenols in virus-infected plants. Guidoni et al. (1994) discovered lower polyphenol content in the skins of Nebbiolo grapes infected with GLRaV-3 and GVA than in those that were not infected. Similarly, the skin of Grignolino grapes infected with GLRAV-1 and GVA had lower polyphenol content than the skin of healthy grapes.

In the current study the incidence of powdery mildew was lower in Thompson Seedless grafted onto Dog ridge, 110 R, and 1103 P rootstocks, while it was higher in ungrafted Thompson Seedless and those grafted onto 99 R and St. George. Rootstock Dog ridge belongs to *Vitis champinii*, 110R, 1103 P, and 99 R belongs to *V. berlandieri* × *V. rupestris* group, St. George belongs to *Vitis rupestris*, and Thompson Seedless belongs to *Vitis vinifera*. Thus, rootstocks may influence the biochemical composition of the scion leaves grafted onto them, which in turn affects the degree of resistance or susceptibility

to disease. A strong correlation was found between the level of specific phenolic compounds in grape berries with that of grape leaves during different developmental stages (Jeandet et al., 1991). Mandavia et al. (1997) observed increased phenolic content in root and stem tissues of chickpeas plants that were both resistant and susceptible to wilt fungi infection, but in leaf tissues of the resistant variety they observed a decrease in phenolic content. This may explain why we observed less powdery mildew infection in vines grafted onto 110 R rootstocks. Though leaf phenolic content during the initial stages of sampling was lowest on this rootstock, higher phenol content in the berries might have contributed to reduced disease infection in the berries. Variation in the incidence of disease in Thompson Seedless grafted onto different rootstocks may have been due to the genetic makeup of the rootstocks, which may have indirectly contributed to the synthesis of various secondary metabolites due to differences in their uptake of nutrients and water from soil solution, as root development patterns vary with rootstock. However, these results need to be confirmed by systematic in vitro studies by spraying crude leaf extracts from different graft combinations on fungal colonies and observing their effects on antifungal activity.

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