Resveratrol Content of Muscadine Berries is Affected by Disease Control Spray Program

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Abstract. Control of muscadine diseases is necessary to minimize yield loss and is especially important for highest quality fresh-market berries. In a systematic disease control spray program, four fungicides registered for grapes were applied sequentially at 10- to 20-day intervals from early bloom until just before harvest to five muscadine cultivars. Objectives of the study were to: 1) determine the effects of the spray schedule on foliage and berry diseases; and 2) study the relationship between disease incidence and resveratrol content of the berries. Resveratrol, a phytoalexin, has shown potential value in prevention and treatment of cardiovascular disease and certain cancer processes. Foliar diseases were rated visually twice during the season. Berry disease ratings were made at harvest. All fungal foliage and berry diseases were significantly reduced by fungicide treatments. Resveratrol was determined separately on berry skins, seed and pulp/juice by gas chromatography/mass spectrometry (GC/MS). Overall, resveratrol levels in berry skins from unsprayed vines were much higher than those of sprayed vines. Concentrations varied by cultivar and within cultivar by treatment. The relationship between resveratrol concentration in skins and total disease score or scores of specific diseases was not established. Seed resveratrol concentrations differed by cultivar but were not affected by the fungicide treatments. Resveratrol concentration of seed was lower than that of skins. Accumulation of resveratrol in juice/pulp was much lower than in skins and seeds.

Muscadine grapes (Vitis rotundifolia Michx.) are native to the southeastern United States and have been cultivated in this area for more than 400 years (Olien, 1990). They are enjoying a resurgent of consumer interest because recent research has shown that muscadines and muscadine products contain resveratrol (Ector et al., 1996; McMurtry and Watkins, 1997; Threlfall et al., 1999), a phenoic compound which may prove beneficial in nutritional and chemopreventative treatment of cardiovascular disease and cancer. Resveratrol (trans-3,5,4'-trihydroxystilbene) has favorably affected processes implicated in cardiovascular disease; it lowers serum lipids (Kimura et al., 1985; Pace–Asciak et al., 1995) and inhibits oxidation of human low-density lipoprotein (Frankel et al., 1995). The potential value of resveratrol in prevention and treatment of cancer has been suggested by various laboratory assays in which resveratrol has inhibited cellular events associated with tumor initiation, promotion and progression, the three major stages of carcinogenesis (Jang et al., 1997; Jang and Pezzuto, 1999; Mbimonye et al., 1998).

Stilbenes, including resveratrol, are found in a number of plant families and generally are considered to be phytoalexins, i.e., compounds produced by the plant as a defense response to disease challenge or other stress. Resveratrol production by Vitis sp. in response to fungal infection was first reported by Langcake and Pryce (1976); they also noted that ultraviolet (UV) irradiation and mechanical injury could induce resveratrol production in leaves and fruit of Vitis sp. Dai et al. (1995) demonstrated that resveratrol was involved in the response of 'Carlos' muscadine leaves to infection by the downy mildew fungus Plasmopara viticola (Berk. & Curt.). Berl & de Ton. The association between stilbene phytoalexins and disease resistance in Vitis has been well-documented by Dercks et al. (1995).

While muscadines generally are less susceptible to diseases than are bunch grapes (Clayton, 1975), the warm humid weather of the southeastern United States favors development of fungi that cause fruit and foliage diseases. Foliar diseases such as bitter rot leaf spot [caused by Greenaria uvicola (Berk. & Curt.) Punithalingam (syn. Melanconium fuliginem (Scribner & Via) Cav.) and angular leaf spot [caused by Mycosphaerella angulata Jenkins (anamorph Cercospora brachypus Ell. & Ev.)] (Pearson and Goheen, 1988) can significantly reduce vigor of susceptible cultivars. Bitter rot is the most important berry disease of muscadines in south Mississippi (Kummuang et al., 1996). Macrophomina [caused by Macrophomina phaseolina (Moug. ex Fr.) Ces. & de Not. (syn. B. ribis Gross. & Duggar, anamorph Macrophomma sp.)] and ripe rot [caused by Colletotrichum gloeosporioides (Penz.) Penz. & Sacc. (teleomorph Glomerella cingulata (Stonom.) Spauld. & Schrenk), and C. acutatum Simmonds ex Simmonds] may cause significant losses of some cultivars. Black rot [caused by Guignardiabidwellii (Ellis) Viala & Ravaz f. muscadinii Luttrell (anamorph Phyllosticta amelicipida (Engleman) Van der Aa)], and russet (etiology unknown) are both detract from the appearance of the fruit but do not affect fruit quality (Kummuang et al., 1996; Pearson and Goheen, 1988).

Control of muscadine fruit diseases is necessary to minimize yield loss and is especially important to ensure the highest quality fresh-market berries. In a 1997 study to select fungicides for a field study of berry disease control, four muscadine cultivars whose fruit were nearing ripeness (~2 weeks before harvest) were treated with single applications of several individual fungicides. Fruit composition data from this study (not shown) indicated the resveratrol content was affected by the incidence of berry diseases. The objectives of this study were to: 1) determine the effects of the spray program on muscadine foliage and berry diseases; and 2) study the relationship between the incidence of berry diseases and residual resveratrol content of field-grown berries at harvest.

Materials and Methods

Five muscadine cultivars in the U.S. Dept. of Agriculture, Agricultural Research Service (USDA–ARS) Small Fruit Research Station vineyards in McNeill, Miss., were selected on the bases of fruit color and estimated overall disease reaction to the five major berry diseases (S = susceptible, I = intermediate, and R = resistant). Light-fruited cultivars were 'Summit' (R), 'Higgins' (S), and 'Carlos' (I), Dark-fruited cultivars were 'Noble' (R), and 'Cowart' (S). The vines were part of a cultivar study planted in 1990 in a randomized complete-block design with four replications. Each plot consisted of a single vine trained to a Geneva double curtain trellis, 6-m arms. All vines were part of a cultivar study (Berk et al., 1998) and maintained under cultural practices recommended by the Mississippi State Univ. Cooperative Extension Service.

Throughout the 1998 growing season, vines of these cultivars were treated under a systematic fungicide spray program recommended by the Mississippi Cooperative Extension Service. The recommended spray program used sequential applications of four fungicides labeled for grapes (Table 1). Fungicides were applied nine times at 10- to 20-day intervals from early bloom until just before harvest with a tractor-mounted air blast mist sprayer. Con-
trol vines received no fungicides.

Foliar disease ratings were made twice during the season using a visual rating scale. Symptoms of bitter rot leaf spot, black rot leaf spot, angular leaf spot and Pierce’s disease were rated on a scale of 0 = no symptoms to 5 = severe symptoms.

Ripe berries were hand-harvested into field lugs. Two random 1-L samples were taken from each lug for disease ratings and chemical analyses. Using a system developed by Smith (Kummuang et al., 1996), two independent observers scored 25 berries individually for berry rot diseases and other blemishes on a scale of 0 = no symptoms, 1 = very mild symptoms, 2 = moderate symptoms, and 3 = severe symptoms. Berries rated 3 were inedible and considered as culls. The disease score assigned was a function of symptoms visible on the berries’ skins; only in advanced stages would the disease symptoms involve the underlying pulp. Berries for resveratrol analysis were cut in half longitudinally, seeds removed and pulp and free-flowing juice separated from the skins by finger pressure. Pulp/juice samples were rinsed with deionized water, dried at 35°C, and ground to a fine powder in a laboratory mill. All three dried powders in a laboratory mill. All three dried powders were dissolved in methanol and 50-µL aliquots were dried under nitrogen and treated with 100 µL of 1:1 dimethylformamide:N,O-bis(trimethylsilyl)trifluoroacetamide (Pierce, Rockford, Ill.). The solutions were heated at 70°C for 30 min prior to injection. Resveratrol was quantified on the tris-trimethylsilyl derivative m/z 444. Solutions of resveratrol (Sigma, St. Louis) were prepared for the calibration curve, and 50 µL of each was treated in the same fashion as the samples. For each sample, resveratrol peak area was measured and resveratrol concentration calculated from the standard curve. Concentration of resveratrol is reported in µg·g⁻¹ on a dry weight basis for each component.

The study was conducted in a randomized complete block design, and data were analyzed by the Statistical Analysis System general linear means procedure (SAS Institute, Cary, N.C.). Foliar and berry disease means were separated by Fisher’s least significant difference (LSD), P ≤ 0.05, calculated on square root transformed disease severity data (Tables 2 and 3).

Results and Discussion

Disease control. All fungal foliar disease scores were reduced by the full season fungicide treatment (Table 2). Scores of Pierce’s disease (caused by the bacterium, Xylella fastidiosa Wells et al.) on the foliage were not different among treatments. Fungicide treatments reduced the total disease severity of Pierce’s disease by 50%. Bitter rot scores of ‘Higgins’, ‘Carlos’, ‘Summit’, and ‘Cowart’ were lower on fruit from fungicide treated vines than on fruit from untreated vines. Ripe rot scores of the three light-colored cultivars were reduced by fungicide treatments, while no symptoms of ripe rot were seen on berries of the dark-fruited cultivars, Cowart and Noble. In scoring disease incidence on light vs. dark fruit, a possible bias could have been introduced because small disease lesions, typically black or dark, were more difficult to see on the darker-skinned fruit. ‘Noble’ fruit was particularly difficult to score because of its small size and black color. Fungicide treatments also reduced Macrophoma rot scores by about two-thirds for ‘Higgins’ and ‘Summit’. Regular fungicide applications during the growing season will reduce the incidence of fruit and foliar diseases.

Fungicide treatments on fruit also reduced symptoms to 5 = severe.

Those of untreated vines, probably because the sequential fungicide applications reduced the amount of inoculum available for new infections. While ‘Noble’ fruit is resistant, ‘Noble’ vines received the highest total foliar disease score, while ‘Cowart’ and ‘Summit’ vines received the lowest. The only significant interaction between cultivar and treatment on foliar diseases was for angular leaf spot in which ‘Noble’ unsprayed vines received a higher disease score than the other cultivars. Fungicide-treated vines of all cultivars had very low disease scores for angular leaf spot.

The full season fungicide treatment reduced all fungal fruit disease scores (Table 3), again probably because of reduced inoculum pressure. Scores for russet on berries were not reduced.Treated vines had more fruit without visible disease symptoms and fewer inedible berries than untreated vines. ‘Higgins’ fruit (bronze) from untreated vines had the highest scores for bitter rot, ripe rot, Macrophoma rot, and total berry rots, affirming its inclusion in the study as a susceptible cultivar. Fruit from untreated vines of ‘Noble’ (selected as resistant) had the lowest total berry rots score. The other three cultivars were rated intermediate in their resistance. Fungicide treatments reduced the total berry disease score of ‘Higgins’ by almost 50%. Bitter rot scores of ‘Higgins’, ‘Carlos’, ‘Summit’, and ‘Cowart’ were lower on fruit from fungicide treated vines than on fruit from untreated vines. Ripe rot scores of the three light-colored cultivars were reduced by fungicide treatments, while no symptoms of ripe rot were seen on berries of the dark-fruited cultivars, Cowart and Noble. In scoring disease incidence on light vs. dark fruit, a possible bias could have been introduced because small disease lesions, typically black or dark, were more difficult to see on the darker-skinned fruit. ‘Noble’ fruit was particularly difficult to score because of its small size and black color. Fungicide treatments also reduced Macrophoma rot scores by about two-thirds for ‘Higgins’ and ‘Summit’. Regular fungicide applications during the growing season will reduce the incidence of fruit and foliar diseases.

Table 1. Fungicide spray schedule for muscadines, McNeill, Miss., 1998

| Date      | Fungicide | Formulation   | Manufacturer | Kg·ha⁻¹ |
|-----------|-----------|---------------|--------------|---------|
| 8 May     | Myclobutanil | Nova 40W     | Rhom & Haas  | 0.28    |
| 8 June    | Captan    | Captan 50-WP | Micro Flo   | 4.48    |
| 19 June   | Azoxystrobin | Abound       | Zeneca      | 0.84    |
| 29 June   | Myclobutanil | Nova 40W     | Rhom & Haas  | 0.28    |
| 10 Aug.   | Benomyl   | Benlate 50-WP | Du Pont    | 1.12    |
| 20 July   | Azoxystrobin | Abound       | Zeneca      | 0.84    |
| 29 July   | Captan    | Captan 50-WP | Micro Flo   | 4.48    |
| 10 Aug.   | Azoxystrobin | Abound       | Zeneca      | 0.84    |
| 20 Aug.   | Captan    | Captan 50-WP | Micro Flo   | 4.48    |

Table 2. Cultivar and fungicide treatment effects on foliar disease scores of muscadines.

| Cultivar/Treatment | Bitter rot | Black rot | Angular leaf spot | Pierce’s disease |
|--------------------|------------|-----------|-------------------|------------------|
| Noble              | 3.3 a      | 0.6 ab    | 2.1               | 0.9 a            |
| Higgins            | 2.4 bc     | 1.4 a     | 0.6               | 0.0 b            |
| Carlos             | 2.6 ab     | 0.6 ab    | 0.0               | 0.2 b            |
| Cowart             | 1.8 c      | 0.4 b     | 0.1               | 0.0 b            |
| Summit             | 1.0 d      | 0.3 b     | 0.2               | 0.0 b            |

| Control            | 2.9 a      | 1.0 a     | 1.0 a             | 0.2 b            |
| Fungicide          | 1.6 b      | 0.4 b     | 0.3 b             | 0.3              |

a Disease severity rated on entire vine for each foliar disease on a visual scale of 0 = no symptoms to 5 = severe.
b Means followed by different letters within a column and within cultivar and treatment are significantly different (P = 0.05) based on Fisher’s LSD, calculated on square root +0.5 transformed disease severity data. Only the untransformed mean disease scores are shown.
from untreated vines; the differences were significant in ‘Noble’, ‘Summit’ and ‘Cowart’. Overall, resveratrol concentration of skins from fungicide-treated berries was lower than that of untreated plots. While enological variables can affect resveratrol levels of wines (Castellari, 1995). They noted that experimental wines from unpublished data cited by Dercks et al. (1998) in bronze- and dark-fruited muscadines, but were within the ranges reported by Jeandet et al. (1995) in seed of healthy ‘Pinot’, ‘Chardonnay’, and ‘Gamay’ grapes. Resveratrol, juice. Resveratrol was detected at very low concentrations (<0.03 μg·g⁻¹ dry weight) in the pulp/juice fraction of each cultivar and treatment except for unsprayed ‘Noble’ which was 0.167 μg·g⁻¹. We believe the higher concentration was the result of persistent. Symptoms of two or more diseases on the same berry were common. Date of infection was unknown, so resveratrol levels detected at harvest may not reflect the total amount produced in response to infection during the growing season. Resveratrol concentration in grapes increased after infection and then declined; concentration also declined as berries ripened (Bavareseco et al., 1997; Creasy and Coffee, 1988; Jeandet et al., 1991). Because cultivars differ in their capacity to produce and/or accumulate resveratrol, relationships of resveratrol concentration at harvest and scores of specific diseases were not apparent. While a cause-and-effect relationship was not established, skin resveratrol levels (Fig. 1) and total disease scores of unsprayed fruit (Table 3) were moderately positively correlated ($r = -0.77, P \leq 0.12$ by SAS PROC CORR). The correlation coefficient improved ($r = -0.96, P \leq 0.04$) when ‘Carlos’ was dropped from the calculation.

Resveratrol, seeds. Stilbenes are found in the woody tissues of many plant species, and resveratrol is a constituent of lignified grapevine stem tissue (Langcake and Pryce, 1976). Hardness of grape seed is a result of lignification of the integument. Resveratrol concentrations of seeds differed by cultivar (data not shown) but were not affected by the fungicidal spray program; seed resveratrol probably was not elicited by fungal challenge. Mean resveratrol concentration of seeds (0.83 μg·g⁻¹) was significantly lower than that of skins (2.84 μg·g⁻¹). Only in ‘Carlos’ was the seed concentration (0.69 μg·g⁻¹) greater than its skin concentration (0.33 μg·g⁻¹). Seed and skin concentrations were not highly correlated within cultivars; among the five cultivars, ‘Noble’ seed had the lowest concentration while ‘Noble’ skins had the highest. These seed concentrations were much lower than those reported by Ector et al. (1996) for bronze- and dark-fruited muscadines, but were within the ranges reported by Jeandet et al. (1995) in seed of healthy ‘Pinot’, ‘Chardonnay’, and ‘Gamay’ grapes.

**Table 3. Disease score $^a$, percentage culls (unmarketable or inedible) muscadine berries, and percentage with no visible symptom at harvest following season-long fungicide treatments.**

| Cultivar | Control | Fungicide |
|----------|---------|-----------|
| Carlos   | 2.1     | 1.7       |
|          | 0.7 $^a$ | 0.3 $^b$ |
|          | 0.5     | 0.2       |
|          | 0.6     | 0.4       |
|          | 0.1     | 0.4       |
|          | 1.1     | 0.1       |
|          | 3.1     | 4.4       |

$^a$Disease severity rated at harvest on 25 berries by two independent observers on a scale of 0 = no symptoms to 3 = severe symptoms, berry inedible.

$^b$Means followed by different letters within a column are significantly different ($P \leq 0.05$) based on Fisher’s LSD, calculated on square root +0.5 transformed disease severity data.

**Fig. 1.** Resveratrol concentrations (μg·g⁻¹) in skins of berries from fungicide-treated and control vines of muscadine cultivars Carlos, Cowart, Higgins, Noble, and Summit. Means separated by LSD, $P \leq 0.05$.
carry-over from the skins rather than from synthesis in the pulp/juice. ‘Noble’ berries are much smaller than those of the other four cultivars and the juice/skin contact when the berries were squeezed would have been greater. While Ector et al. (1996) reported higher juice levels, their muscadine juice was obtained by pressing which could have resulted in more resveratrol being expressed from the skins than we obtained from light finger pressure. We found no reports of significant resveratrol synthesis in the flesh of other Vitis sp.

In summary, full season treatment with a systematic fungicide spray program significantly reduced the incidence of foliar and fruit diseases of muscadine grapes. Treatment effects may have been more pronounced had the disease pressure not been lower than previous years because of very low rainfall. Fungicide-treated fruit were of higher visual quality because of lower disease incidence, but had lower resveratrol concentrations. Further work will include studying how horticultural practices (fungicides, foliar feeding, etc.) interact with muscadine fruit and foliar diseases to affect fruit yield, quality and content of phenolic phytochemicals.

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