Pomace: Waste or Valuable Resource?

Progress Report
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Research Proposal

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INTRODUCTION

Pomace. In the U.S., wine is produced from one or more varieties of the European grape (*Vitis vinifera*) or from North American grapes (e.g., *Vitis aestivalis, V. labrusca*, *V. rupestris*, *V. rotundifolia* and *V. riparia* or hybrids of two species) (Anonymous, 2005). The muscadine grape (*V. rotundifolia*) is native to the southern U.S. and is relatively disease and pest resistant. Muscadine fruit contain approximately 40% skin and seeds (Morris and Brady, 2004). In contrast, *V. vinifera* contains about 18% skin and seeds (calculated from values presented in Anonymous, 2005). In wine-making, grapes are crushed, then either allowed to ferment with the skins (red wines) or immediately filtered, with the juice being allowed to ferment (white wines). **Pomace** is the material that remains after the grapes are pressed and consists of skins, seeds and a small percentage of stems. Most of the potentially useful natural compounds in grape remain in the pomace (Sovak, 2001)

Phenolic compounds in grapes- Chemistry and activity. Grapes and grape products contain high concentrations of simple phenolic and polyphenolic compounds (stilbenes, flavonoids, and anthocyanins). Polyphenolic compounds have potential for antioxidant, anti-inflammatory, and other anti-atherogenic therapies.

Resveratrol. Use of resveratrol, a compound found in wine and pomace, has captured public interest in recent years due to reports of positive human health effects. The amount of resveratrol depends upon the grape variety or species. Resveratrol is found in highest concentrations in grape skins and seeds. Muscadine grapes and their products contain more resveratrol and other antioxidants than any other type of grape (http://www.ncwine.org/muscadine/muscadineHealth.html). Resveratrol is a phytoalexin, i.e., it is produced in the plant in response to pathogen attack. It has a low toxicity in humans and is a naturally-occurring fungicide (Jung et al., 2005). Fungi reported to be sensitive to resveratrol include human pathogens *(Candida albicans, Saccharomyces cerevisiae* and *Trichosporon beigelii*) (Jung et al., 2005) and plant pathogens [*Phytophthora palmivora, P. capisci* (Zhu et al., 2004), *Aspergillus flavus* (Jung et al., 2005), *Phoma medicaginis*, *Colletotrichum trifolii*, *Leptosphaerulina briosiana, Fusarium* spp. and *Verticillium* spp. (Hipskind and Paiva, 2000)]. Transgenic papaya plants that contain the resveratrol synthesis gene from grape (gene product = stilbene synthase) had increased resistance to *Phytophthora palmivora* (Zhu et al., 2004). Constitutive expression of resveratrol-glucoside in transgenic alfalfa increased resistance to *Phoma medicaginis* (Hipskind and Paiva, 2000).

Other phenolics. Although resveratrol is the most studied by researches, and most well-known by the popular press, grapes produce an abundance of other natural compounds that have antioxidant and antifungal activity. Gallic acid is the standard for phenolic analysis in wines and

grape extracts. Gallic acid is active against *Fusarium semitectum*, *F. fusiformis* and *Alternaria alternate* (Dowd et al., 2002). The red and purple colors of grapes are due to the presence of anthocyanins, antioxidants (Williams and Grayer, 2004), that are localized primarily in the skin. Catechin, a flavanol, is well known as a potent antioxidant (Yilmaz and Toledo, 2004) but it is also a powerful antifungal agent.

Pomace – As a resource. Pomace contains relatively large quantities of natural products and has been used in the production of several commercial products – grape seed oil, fiber additive, pomace wine, and health food products (Morris and Brady, 2004). The California Sustainable Wine Alliance surveyed California grape/wine producers regarding views on pomace and most considered it a resource (Anonymous, 2004). However, we believe that the percentage of vintners who view pomace as a resource is very low in the southeast, although there have been some attempts to capitalize on this resource (Hampton, 2005; Anonymous, 2004; Morris and Brady 2004).

RESEARCH OBJECTIVES

1. Determine antifungal activity of grape pomace
2. Determine impact of grape pomace on seed germination and plant growth
3. Determine total phenols and antioxidant activity in pomace

JUSTIFICATION

In Tennessee there are 400 acres in wine grape production with an average of 3 tons of fruit per acre. Pomace represents between 18 and 40% of the total weight or 300 tons, on average. North Carolina, which has 1350 acres in wine grape production, produces an estimated 1300 tons of pomace per year (www.ncwine.org). Based on conversations with Tennessee vintners and extension personnel, wineries in Tennessee either do not use or under-utilize this resource. Often it is deposited in piles at or near the winery and is of no further use. It has been suggested that the use of muscadine pomace could increase the market value per ton of fruit and reduce or eliminate waste disposal problems (Ector, 2001). In addition, greenhouse vegetable and bedding plant producers are in need of pesticide alternatives for managing soil- and water-borne diseases. It is our hypothesis that grape pomace, which contains high concentrations of antimicrobial compounds, can be used as an additive for plant growth media for control of plant diseases.

METHODOLOGIES

Research Objective #1. Determine antifungal activity of grape pomace.

Control of soilborne pathogens using grape pomace is possible only after careful screening for antifungal activity. Our *working hypothesis* is that the components of the grape pomace will restrict growth of a recalcitrant pathogen, *Sclerotinia* sp. The *rationale* behind this objective is that known antifungal compounds are present in the pomace and that they are released in the presence of water during degradation.

Samples of pomace obtained from Mountain Valley Vineyards (Sevierville, TN) and identified as 'Steuben' were used in 5 experiments.

Experiment 1. Pomace samples were tested using methods developed for screening antifungal activity in *Monarda* (Gwinn et al., 2003). The purpose of this experiment was to determine the impact of pomace mixed with greenhouse germination mix on germination of sclerotia of *Sclerotinia* sp. Pomace was first lyophilized then frozen with liquid nitrogen and

ground. The ground pomace was mixed with sterile germination mix (Berger BM-2, Berger Peat Moss, Sainte Modeste, Quebec) at rates of 5 and 10 percent v/v (BM-2/pomace) placed into small Petri dishes (60 × 15 mm). Each dish was moistened with 3ml of sterile water. Three sclerotia of *Sclerotinia* are placed on pre-moistened BM-2/pomace samples or on non-amended greenhouse plant growth medium (control). After incubating for 1 week at 25ºC, sclerotia were examined; if sclerotia germinated and produced mycelia, the sample was classified as no antifungal activity. If sclerotia did not produce visible mycelia, they were transferred to acidified potato dextrose agar (a-PDA). If sclerotia germinated and produced hyphae on a-PDA, the sample was classified as fungistatic; if no growth occurred, the sample was classified as fungicidal. Each sample was replicated five times.

Experiment 2. The purpose of this experiment was to determine if the initial screening method could be improved, i.e., growth of nonspecific fungi could be eliminated, by incorporating autoclaved pomace into water agar. Samples of autoclaved 'Steuben' pomace were added to water agar (2 grams pomace per 100ml agar). Following solidification of the agar, one sclerotium was placed in the center of plates containing pomace/agar mix or water agar only (control). Each sample was replicated five times. After incubating for 1 week at 25ºC, sclerotia were examined and impact on germination and growth was determined as described in Experiment 1.

Experiment 3. The purpose of this experiment was to determine if the initial screening method could be improved, i.e., growth of nonspecific fungi could be eliminated, by incorporating propylene oxide-treated pomace into water agar. Samples of propylene oxide-treated 'Steuben' pomace were added to water agar (2 grams pomace per 100ml agar). Following solidification of the agar, three sclerotia were placed in the center of plates containing pomace/agar mix or water agar only (control). After incubating for 1 week at 25ºC, sclerotia were examined and impact on germination and growth was determined as described in Experiment 1. Each sample was replicated five times and the experiment was repeated once, however in the repeated experiment one sclerotium was placed in each plate.

Experiment 4. Similarities in color and texture between sclerotia and ground pomace made visually locating sclerotia difficult. To facilitate location, a small amount of water soluble craft paint was applied to each sclerotia. The effect of paint on germination and growth of sclerotia was first tested. Painted and non-painted (control) sclerotia were placed on a-PDA . After incubating for 1 week at 25ºC, sclerotia were examined and impact on germination and growth was determined as described in Experiment 1. Each sample was replicated six times.

Experiment 5. This experiment modified the initial screening method by placing sclerotia directly onto propylene oxide-treated pomace. Painted and non-painted (control) sclerotia wree placed on samples of ground 'Steuben' pomace moistened with sterile water. After incubating for 1 week at 25ºC, sclerotia were examined and impact on germination and growth was determined as described in Experiment 1. Each sample was replicated five times.

Research Objective #2. Determine impact of grape pomace on seed germination and plant growth.
Many compounds, both natural and synthetic, that effectively control fungal growth, also inhibit plant growth. It is essential to the development of a greenhouse plant growth medium additive that the impact on plant growth and development be determined. Our *working hypothesis* is that some pomace samples that are identified in Research Objective #1 will not inhibit seed germination or plant growth. The *rationale* underlying this objective is that few of the compounds found in grape pomace are known to have phytotoxic effects and, therefore, the pomace should not affect plant growth and development.

Plant Growth and Development. Pomace samples were treated as described in Research Objective #1, Experiment 1, but pomace was treated with propylene oxide prior to being mixed with BM-2 germination mix. Tomato seeds ('Mountain Fresh') were planted (3 seeds/cell) in greenhouse germination medium (Berger BM-2) with 10 percent (v/v) dried ground grape pomace. Three pomace samples, 'Cynthiana,' muscadine 'Doreen' (both grown in Tennessee) and Merlot (grown in Georgia) are being tested. pH will be carefully monitored. Seedling emergence and plant quality will be recorded. This experiment will be conducted three times.

Research Objective #3. Determine total phenols and antioxidant activity.
Phenolic compounds in grapes are known to be antifungal. Our *working hypothesis* is that the total phenolic content or the antioxidant activity in grape pomace will be predictive of antifungal or phytotoxic activity. The rationale underlying this objective is that total activity may be more predictive than the concentration of a single compound.

We routinely use a Folin–Ciocalteau Micromethod developed to test phenolics in wine when testing plant responses to biocontrol agents (http://waterhouse.ucdavis.edu/phenol/folinmicro.htm). The only modification is the substitution of methanol for ethanol in reagents. Anitoxidant activity determination, like phenolic concentration, is a simple spectrophotometric assay based on the antioxidant capacity of a vitamin E analog (Pastrana-Bonilla et al., 2003).

RESULTS AND DISCUSSION

Research Objective 1
Research has been stalled due to problems outlined below with our attempts to adapt the screening methodology used for monarda herbage for use with pomace. Monarda herbage is relatively lacking in microflora and therefore fungicidal or fungistatic effects on sclerotia can more easily be determined. The abundance of microflora present in pomace contributes to non-specific fungal growth. This, combined with the additional growth-promoting carbon source it contains, has so far interfered with our ability to evaluate the antifungal properties of pomace.

Experiment 1. Sclerotia appeared to be sensitive to the presence of the pomace mixed with BM-2 (Table 1), but results were inconclusive due to contaminants. In addition, sclerotia were difficult to locate in the herbage due to the coloration of the pomace and to the presence of seeds.

Table 1. Impact of Pomace Mixed with BM-2 Germination Mix on Growth of Sclerotia

Variety	%	Rep	# of Sclerotia	# Germinated	(%) Germination
Steuben	5	1	3	1	33%
Steuben	5	2	3	0	0%
Steuben	5	3	3	0	0%
Steuben	5	4	3	1	33%
Steuben	5	5	3	1	33%
Total			15	3	20%
Steuben	10	1	3	0	0%
Steuben	10	2	3	1	33%
Steuben	10	3	3	1	33%
Steuben	10	4	3	0	0%
Steuben	10	5	3	1	33%
Total			15	3	20%

Experiment 2. Since contamination was problematic pomace was autoclaved to ascertain whether compounds responsible for antifungal activity are heat sensitive. The addition of pomace to water agar provided additional nutrients and growth in pomace-amended water agar was increased (Table 2)

Table 2. Impact of Autoclaved Pomace on Growth of Sclerotia in Water Agar

Rep	Treatment	Colony Growth (cm)		Rep	Treatment	Colony Growth (cm)
1	Control	3.1		1	Pomace	5.4
2	Control	2.8		2	Pomace	7
3	Control	2.7		3	Pomace	6.6
4	Control	2.5		4	Pomace	5.6
5	Control	3.5		5	Pomace	5.2
	Average	2.92			Average	5.96

Experiment 3. Pomace was sterilized by propylene oxide and added to water agar in order to determine if antifungal activity could be determined. Addition of pomace resulted in the reduced growth after germination of sclerotia (Table 3a), but some growth appeared to be non-specific. In the repeated experiment (Table 3b), only one sclerotium was added per plate. The increased level of mycelial growth seen is believed to be as a result of the additional carbon source provided by the pomace.

Table 3a. Impact of Propylene Oxide-Treated Pomace on Growth of (3) Sclerotia in Water Agar

Rep	Treatment	Colony Growth (cm)			Avg.	Rep	Treatment	Colony Growth (cm)			Avg.
1	Control	0.0	2.8	2.3	1.7	1	Pomace	*	*	*	*
2	Control	0.0	3.2	0.0	1.1	2	Pomace	0.5	0.5	*	*
3	Control	2.7	2.5	2.3	2.5	3	Pomace	*	*	*	*
4	Control	3.0	1.6	0.5	1.7	4	Pomace	*	*	*	*
5	Control	1.0	1.9	3.0	2.0	5	Pomace	*	*	*	*
	Average				1.8		Average				*

*negligible growth

Table 3b. Impact of Propylene Oxide-Treated Pomace on Growth of (1) Sclerotia in Water Agar

Rep	Treatment	Colony Growth (cm)		Rep	Treatment	Colony Growth (cm)
1	Control	0.5		1	Pomace	4.5
2	Control	1.0		2	Pomace	4.3
3	Control	1		3	Pomace	3.0
4	Control	0.7		4	Pomace	3.5
5	Control	1.7		5	Pomace	3.3
	Average	0.98			Average	3.7

Experiment 4. After evaluating the results of Experiment 3, we decided to return to screening sclerotia for antifungal effects by placing them directly on the pomace, but this time painting the sclerotia so as to differentiate them from the ground pomace. Before using painted sclerotia in an experiment with pomace, we first determine that painting the sclerotia had no impact on germination and mycelial growth by testing them on PDA (Table 4).

Table 4. Effects of Painting on Growth of Sclerotia on PDA

Rep	Treatment	Colony Growth (cm)		Rep	Treatment	Colony Growth (cm)
1	Control	5.0		1	Pomace	5.0
2	Control	5.0		2	Pomace	5.0
3	Control	5.0		3	Pomace	5.0
4	Control	5.0		4	Pomace	5.0
5	Control	5.0		5	Pomace	5.0
	Average	5.0			Average	5.0

Experiment 5. Painted sclerotia were then tested on ground and propylene oxide-treated pomace. Although not seen in Experiment 4 with PDA, painting of sclerotia appeared to have an effect on pomace. Non-specific growth continued to be problematic in laboratory assays probably due to an insufficient exposure to the propylene oxide.

Table 5. Effects of Paint on Growth of Sclerotia in Pomace

Rep	Treatment	Colony Growth (cm)	Rep	Treatment	Colony Growth (cm)
1	Painted	0.2	1	Unpainted	5.0
2	Painted	0.4	2	Unpainted	5.0
3	Painted	NS	3	Unpainted	4.5
4	Painted	NS	4	Unpainted	5.0
5	Painted	*	5	Unpainted	5.0
				Average	4.9

NS = non-specific fungal growth
*negligible growth

Summary. Because of the difficulties with non-specific fungal growth, we have elected to proceed with the research outlined in Objective #2 as suggested by one reviewer of the grant. The greenhouse testing has recently begun. We also plan to add pathogen treatments once impact of pomace is determined.

Research Objective #2.

These experiments are in progress.

Research Objective #3.

These experiments are in progress.

ADDITIONAL OUTPUTS

As a result of this project, a grant proposal was submitted to the USDA Southern SARE On-Farm Research Project. The proposed research involves using a local East Tennessee source of pomace as a soil amendment/bioadditive to address a Phytophthora problem in pumpkin at an East Tennessee vegetable grower's operation.