University of North Carolina Asheville
Journal of Undergraduate Research
Asheville, North Carolina
December 2018

Effectiveness of Compost Tea Application in Decreasing Environmental and Biological Stressors of *Vitis vinifera*: Field Trial in Western North Carolina

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Abstract

The state of North Carolina, which boasted a thriving grape growing industry previous to prohibition, is moving back into the production of grapes for winemaking on a large scale. Despite the rise of opportunity for the southeastern viticulturist, the variable, high humidity climate of North Carolina poses a number of problems for the cultivation of *Vitis vinifera*, the european grapevine. Industry is currently dominated by synthetic chemical management, thus sustainable methods of managing vineyards in the climate of North Carolina are needed. Application of Aerated Compost Tea (ACT) in the form of both a foliar spray and soil drench has shown promise for managing diseases affecting *Vitis vinifera*, including powdery mildew, botrytis and a number of others. ACT application has further been shown to aid in nutrient uptake as well as the establishment of beneficial microbial communities in both soil and on foliage. In order to test the effect of ACT on a functional vineyard, four separate treatments were tested against a control group across a total of 199 *Vitis vinifera* vines. Treatments consisted of an ACT foliar spray, an ACT soil drench, a combination of ACT foliar and drench, a fish emulsion foliar and a control. The ultimate goal of the experiment was to ascertain which, if any of the treatments had an effect on vine health by quantifying leaf chlorophyll levels. During the time of the experiment, the region saw unseasonably high rainfall, leading to heightened disease levels. Pest pressures in combination with a deer intrusion during the experiment led to extreme defoliation across the vineyard. Due to the defoliation, very little data was able to be collected post-treatment as many of the vines did not sufficiently recover, severely impacting the intended sample size for each treatment. Statistical assessment of the treatments did not show any significant difference between treatment and control groups.

1. Introduction

The North Carolina wine grape industry is currently in its infancy and faces a number of challenges in becoming established. Despite the challenges faced, the North Carolina grape growing industry has incredible potential for growth in the long run; having already grown to a 30 millions dollar industry.[14] While native *Vitis* spp. varieties are well adapted and largely disease resistant in the North Carolina climate, more significant economic potential is sure to be found in cultivars of *Vitis vinifera*, the European grapevine, which are well proven in both viticulture and winemaking. Development of the grape growing industry has been swift in the last decade, with the addition of 32 wineries between 2000 and 2005 alone.[14] Thus far, viticulturalists have largely failed to adopt sustainable management practices in vineyards due to high disease pressure and abundance of both pests and weeds. The challenges which vineyard managers face in North Carolina are most often met with synthetic fertilizers, herbicides, pesticides and fungicides which may work in a number of ways to degrade soil, ecosystem and fruit quality. As of now, there are only two vineyards with a USDA Organic certification in the state, while there are 189 wineries registered with the North Carolina Alcoholic Beverage Control Commission.[11;16] As a substitute for synthetic chemical dominant management regimes, organic and biodynamic practices are becoming more popular among vineyard managers. In

contrast to the soil-depleting nature of synthetics, organic viticulture has demonstrated the ability to increase soil organic matter, micronutrients, abundance of soil microbes and both plant and fungal feeding nematode densities.[2]

Sustainable management practices offer numerous benefits to producers, and are also perceived as virtuous and healthy by consumers. Throughout the 20th century, foods and beverages produced with the aid of synthetic chemicals in agriculture became the norm. In recent time demand for organic food and beverage products has grown significantly, by $11.2 billion between 1990 and 2004 alone.[5] In the vineyards of France alone between 2001 and 2008, the area of organically managed vineyards increased 110% from 13,426 ha to 28,190 ha.[2] As the wine grape industry continues to expand in North Carolina, it is pertinent to find alternatives to the array of synthetic sprays which currently dominate vineyard management.

Among the array of organic solutions available to address disease and soil nutrient management in North Carolina vineyards, Aerated Compost Tea (ACT) seems to have good potential. Aerated Compost Tea as used here is a concentrated, aerobic mixture of beneficial, aerobic microbes made by adding compost to dechlorinated water and aerating the mixture for twenty four to thirty six hours.[8] Used in the form of a soil drench, ACT works to increase abundance of soil organisms, offers an immediate nutrient influx in plants and improves soil structure, aiding in nutrient uptake to vines.[1;6] Used in the form of a foliar spray, ACT acts as biological disease control, increasing the number of culturable bacteria on leaves for up to 21 days and successfully preventing or significantly decreasing incidences of powdery mildew, botrytis and other destructive fungi.[4;8] ACT has been shown to reduce powdery mildew levels on vines to roughly equal levels as vines treated with conventional fungicides, while simultaneously increasing culturable bacteria, fungi and yeast on leaf surface 1 hour following treatment compared to samples taken 30 mins prior to application.[4]

It is the purpose of the experiment to determine if ACT as a viticultural application is effective at significantly altering chlorophyll levels in the foliage of *Vitis vinifera*. Chlorophyll is measured as a means of establishing relative health of vines. A small fish emulsion treatment will be included in the experimental design as a contrasting organic foliar treatment. Like ACT, fish emulsion offers disease suppression to crops and has been shown to have similar effects on crop yields to traditional synthetic fertilizers when equal amounts of N in treatments were equal.[9] As the two treatments have demonstrated similar properties and effects on crop, they will be an interesting additional contrast within the experiment. The primary measure of health used will be concentration of chlorophyll within grapevine leaves using a field chlorophyll meter. Non-destructive chlorophyll measurement via field chlorophyll meters is a common and effective means to monitor plant health in response to environmental conditions and adversity.[13] If ACT is indeed effective at suppressing otherwise significant disease and aiding in health, vines should have a significant response in the form of chlorophyll production which will serve as the primary measure of plant health in the study.

As the primary recognized action of ACT in viticulture is disease suppression, measure of plant stress is crucial in determining if ACT may be used as an effective viticultural prescription. If results indicate that there are significantly higher concentrations of chlorophyll in treated *V. vinifera* vines, further local field trials on ACT application will be incentivized. Further, there may appear significant differences in chlorophyll concentrations between vines treated with foliar and soil drench applications, pointing to which might offer the most efficient utilization of often limited resources.

2. Methods

2.1. Study Site

The vineyard being used for study is located in Alexander, North Carolina between 2,086-2,073 ft elevation with a southwest aspect and a gentle slope. Soils consist primarily of a red clay hardpan below a 1-1.5 ft layer of fertile topsoil. Soil pH prior to amendment with calcitic lime was 5.5. B, Ca and P levels were low and Mg was high. In line with the terroir-centric approach being taken by the owners, micronutrients were not adjusted. As an alternative to micronutrient amendments, ACT and future compost application will be relied upon to provide vines with essential micronutrients. Approximately 83 ft^3 of BioChar was incorporated into soil prior to planting in order to encourage establishment of beneficial mycorrhizal and bacterial cultures. 240 lbs of calcitic lime was applied across entire vineyard in order to adjust pH. The approximate size of the study area is 6,200 ft^2. Trellising is “high cordon”, consisting of line posts placed every 20 ft, and each vine spaced 4ft from the next. Each row in the vineyard is 100 ft long in the planted area and consists of 25 vines. Each row consists of a 3 ft wide cultivated area, while the 5 ft wide aisles were left to grass.

2.2. *Vitis vinifera* Cultivars, Planting and Experimental Design

The vineyard is planted to three varieties of *Vitis vinifera*, 'Cabernet Franc,' 'Chardonnay,' and 'Barbera.' Each *V. vinifera* vine is grafted to one of two North American rootstocks which are a cross between *Vitis riparia* and *Vitis rupestris* and are known as '3309-Couderc' and '101-14 Millardet et de Grasset.' Rootstock selection as well as the planting of the unproven Barbera were largely exploratory and were made previous to the synthesis of the experiment. Each varietal is listed below with a description.

2.2.1 vitis vinifera

'Cabernet Franc:'A cultivar well suited to cooler, inland climates, ripens more reliably than it's offspring Cabernet Sauvignon, and less susceptible to inclement weather following veraison than Cabernet Sauvignon. In North America, Cabernet Franc has become the *V. vinifera* variety of choice in much of the east, including states as close as Virginia.[14] Further, there is currently high demand for Cabernet Franc fruit in North Carolina.[14]

2.2.2 vitis vinifera

'Chardonnay:' Thrives in a variety of climates, exhibiting high vigor and producing naturally high yields making the vines malleable to the desires of the viticulturist and winemaker.[15] Vines are highly susceptible to disease, frost damage and cold injury making careful site selection essential.[14] Varying ripeness levels may be used to produce a wide stylistic range of wines.[15]

2.2.3 vitis vinifera

'Barbera:' Known in Italy as 'the people's wine', Barbera is highly productive and versatile varietal in both vineyard and wine production. The cultivar is well suited to medium to hot climates as there is a high level of natural acidity within berries, enabling the vines to produce a balanced wine when left to ripen in medium climates or when picked on time in hot climates.[15]

2.2.4 vitis riparia x vitis rupestri

s '101-14 Millardet et de Grasset' (101-14 MG) and '3309-Couderc' (3309C): Both cultivars are adapted to North Carolina soils and do not offer excess vigor to vine, thus they work well with already highly vigorous varietals.[14]

Rows were planted as listed below:

Row 1:	Cabernet Franc grafted to 3309C.
Row 2-3:	Cabernet Franc grafted to 101-14MG.
Row 4-5:	Chardonnay grafted to 101-14MG.
Row 6:	Chardonnay grafted to 3309C.
Row 7-8:	Barbera grafted to 3309C.

Experimental design was optimized to provide the greatest distribution of the three ACT treatments and control among the different varieties of vinifera as well as rootstocks. Fish emulsion was incorporated into the design as a minor component, only taking up 8 vines. In the vineyard, each continuous row of a given treatment, as seen in Fig. 1, was flagged on either side with the designated color for the respective treatment. The first treatment was applied on Aug. 16, 2018 and the final data was collected Sept. 20, 2018.

Aisle /Row	Grape	1-5	6-9	10-13	14-17	18-21	22-25
A0							
R1	Cab						
A1							
R2	Cab						
A2							
R3	Cab						
A3							
R4	Char						
A4							
R5	Char						
A5							
R6	Char						
A6							
R7	Bar						
A7							
R8	Bar						
A8							

Aisle
ACT - Foliar
ACT - Drench and Foliar
ACT - Drench
Fish Emulsion

Figure 1. Experimental Design

Figure 1 Experimental design is visualized through the use of cells to represent each block of treatment. Cells are organized by columns which represent number of vines treated in each block, while rows represent trellised vineyard rows. Treatments were spread as evenly as possible amongst each Vitis vinifera variety and treatments were grouped closely whenever possible to reduce risk of overlap with conflicting treatments.

2.3. Preparation and Application of Treatments

Two different methods were used to prepare ACT as drenches were only necessary on a biweekly basis, requiring a far higher volume of ACT than foliar sprays, which were necessary on a weekly basis. On weeks where both soil drench and foliar application were necessary, a 60 gal ACT was prepared. On weeks when only foliar application was necessary, a single 5 gal ACT was prepared. Fish emulsion was prepared in a consistent manner week to week. Compost was sourced locally and ACT was fed a proprietary mixture of compounds to enhance fungal growth. The proprietary microbial food was obtained from the company GEOTEA and is a mixture of micronized, soluble powders which encourage fungal growth. The fungal dominance of the source compost was essential as it may work symbiotically below ground with woody plants to help the plant to absorb more nutrients as well as fight off diseases above ground.[8]

On weeks in which both foliar and soil drench applications were performed, ACT was prepared using a 120 gal hard plastic tank with a single protruding air diffuser and a powerful air pump. Sixty gallon batches of ACT were prepared for soil drenches and foliar sprays on a biweekly basis in the 120 gal ACT brewer using 9.13lbs fungal dominant compost, 1.2lbs microbial food and 60 gal of non-chlorinated well water. The brewer was first filled with 60 gal of water, then the compost was added via a hanging mesh bag which protruded approximately six inches into the water, allowing the compost to be entirely submerged. After aerating for thirty minutes, 1.2 lbs of microbial food was added to the brew to promote fungal growth. The brew was then allowed to aerate for between 24 and 48 hours. Twenty four hours was the ideal aeration time for teas; however, aeration time was extended up to the 48 hour mark if inclement weather prevented spraying.

Approximately 2 gal were extracted from the brewer and transferred to a backpack sprayer where it was diluted with 2 gal of non-chlorinated well water. The remaining 55-57 gal were transferred into a holding tank in the back of a

truck and diluted with around 90 gal of non-chlorinated well water. A 1" Honda GX25, WX10TA water transfer pump was used in conjunction with a garden hose to apply the drench. Between 0.70 and 0.66 gal was applied to each of the designated vines for two of the five weeks. One week previous to the trial, approximately 115 gal of concentrated ACT, diluted to 300 gal was applied to all vines.

On weeks in which only a foliar application was performed, five gallons of ACT was prepared using a five gallon bucket in combination with a fish tank air pump and ceramic air diffusers. A five gallon bucket with four air diffusers adhered to the bottom was filled within two to three inches of the top and 2.5 lbs of fungal dominant compost was added. After aerating for thirty minutes, 0.32 oz of microbial food was added and the brew was allowed to aerate for between 24 and 48 hours depending upon weather conditions. Approximately 2 gal were extracted from the brewer and transferred to a 4 gal backpack sprayer where it was diluted with 2 gal of non-chlorinated well water. Using the backpack sprayer, approximately 2.9 fl oz was applied to each each of the designated vines for each of the five weeks.

Fish emulsion solution was prepared by diluting 1 tbsp of commonly available, Organic Materials Review Institute (OMRI) Certified Neptune's Harvest Fish-Seaweed Blend to 1 gal of well water and shaking the spray container thoroughly. Following preparation, the handheld sprayer was used to apply approximately 3.20 fl oz of fish emulsion to each of the designated vines for each of the five weeks.

A potential source of error exists within the dilution scheme used. The concentration of ACT in each separate application is variable due to limitations of equipment used in the experiment to measure exact quantities. Further, the larger preparation of ACT was increased by 5 gal after two weeks to aid in efficiency. In a laboratory setting, dilutions ranging between 1:1 and 1:100, H_2O:ACT offered the same level of protection from *Botrytis cinerea*.[3] Variation of dilution falls well within the range that the above mentioned study establishes as effective, and thus variations likely had little effect on efficacy of the treatment. Very little literature seems to exist on acceptable dilution rates of ACT and would be a worthy study in itself.

2.5. Collection of Samples and Measurement of Leaf Chlorophyll

Pre-application leaf samples were collected by cutting a minimum of two specimens from each vine. Samples collected were facing roughly southwest to match the aspect of the vineyard and insure leaves collected were collecting maximum sunlight. Exceptions were made when no appropriate facing, healthy leaves were readily available. Samples were generally below half way up the height of the vine, at approximately one foot on most vines; however, exceptions were made in cases where foliage below one foot was not present or severely damaged. Generally, foliage with severe damage from japanese beetles was avoided as gaps in the leaf may cause the absorbance device to report false measurements. Likewise, foliage with severe damage from mildew was avoided as chlorophyll may not be accurately measured in dead surfaces. Once two leaves were collected, they were placed into a bag labelled for the respective vine and packed onto ice shortly afterward. In some cases, two leaves were not able to be collected and thus a single leaf was used as the representative sample. Leaf samples were taken to the University of North Carolina at Asheville for measurement of chlorophyll, where two chlorophyll readings were taken on each parallel side of each leaf and later averaged as the representative value for each vine. Where readings were not possible on adjacent lobes, a reading was taken on the least obstructed part of the leaf, usually directly above or below the first reading. If two leaves were not present, the readings from a single leaf were averaged as the representative sample.

Unfortunately, in post-application collection of samples, defoliation had become severe in the majority of the vineyard and only a single leaf was able to be collected from vines which were able to provide samples at all; providing a significantly less robust data set. The majority of leaves collected were only able to provide a single chlorophyll reading reliably which was used to represent that vine.

Measurements of leaf chlorophyll levels were taken using an Opti-Sciences CCM-200 Plus Chlorophyll Content Meter, a device proven in use with *Vitis vinifera*.[12;10] The device measures optical absorbance in a 9.52 mm diameter circle using the 653 nm waveband.[12] The detector in the meter is a silicon photodiode which uses an integral amplifier for measurement of absorbance as well as a temperature compensator.[12] The absorbances measured by the device are converted into a Chlorophyll Concentration Index (CCI) value which is unitless and quantifies relative chlorophyll concentration.[10] Data was collected in single data points manually, rather than using the memory function of the device. The CCM-200 was chosen as it provided both ease of use for data collection and accurate data, negating the need to crush and incorporate between 216 samples into respective solutions and measure with a spectrophotometer. Further, the CCM-200 is proven in use with *Vitis vinifera*.[10]

Leaf samples were taken from each vine prior to installation of experimental design on July 26-27, 2018 and absorbances were read July 27-29, 2018. Samples from each vine were averaged into a single datapoint using a simple mean of the values. The second round of samples were taken and absorbances read on September 27, 2018 after five

weeks of treatment which were constituted of five foliar ACT treatments, five foliar fish emulsion treatments and three ACT drench treatments.

3. Data & Discussion

3.1. Environmental Considerations and Pest Pressures

A number of environmental and pest-related factors contributed to foliar decline in the vineyard being used for the experiment both before installation and during experimentation. Most dominant among the factors contributing to decline was the presence of *Popillia japonica*, the Japanese Beetle, which consumes leaf matter of grapevines at a rapid rate when populations are large. Previous to experimentation, 5 gal of diluted Spinosad were sprayed in an attempt to control the beetles with little effect. Mid-way through experimentation, hormone traps were installed which nearly immediately controlled the pest. By the time the beetles had been controlled, extreme defoliation had already taken place. Extreme defoliation by beetles was immediately followed by deer pressure which left the majority of vines nearly bare. Almost total defoliation was discovered in early August shortly after experimental installation. Following pest pressures, the majority of vines were successful in putting out a secondary set of leaves. Unfortunately, the three rows of Chardonnay were largely unsuccessful in recovering from the pressures and around 50% of the 75 vines were dead by the end of experimentation. Despite the pressures faced, around 95% of the Cabernet Franc and Barbera vines were still alive at the end of the experiment.

Exacerbating the foliar decline was an extremely rainy growing season, which increased the incidence of observed powdery mildew significantly from the previous year and led to mild incidence of downy mildew. Prior to experimentation, mildews were controlled with moderate success using multiple treatments of Potassium bicarbonate ($KHCO_3$) fungicide. Though fungicides were not used during experimentation, incidences of either mildew did not seem to increase in any block.

Despite moderate recovery of the majority of vines, many secondary leaves were both too small to be measured or were not abundant enough at the end of the experiment to be extracted. Chlorophyll levels increase significantly within the first 30 days after bud break in *Vitis vinifera* and therefore chlorophyll values observed before treatments are unable to be reliably compared to those taken on the young second growth leaves following treatments.[7] The abundance of pest and environmental pressures on the vineyard are unfortunate exogenous contributors to foliar decline that likely accounted for a greater degree of the difference in chlorophyll levels than the treatments being explored.

3.2. Statistical Analysis of Data

Unfortunately, only a single value was recorded for the post-experiment fish emulsion, therefore no dataset was available for the treatment. Three of the four post-treatment datasets were able to be considered statistically, specifically the ACT foliar, ACT drench and ACT foliar and drench combination. Datasets for individual treatments were smaller than expected and therefore the data collected did not allow for subsets large enough to compare chlorophyll values of individual cultivar groups within each treatment set. Though experimental design accounted for the differing species and rootstocks within the vineyard, post-experiment foliage abundance made comparison of levels between species impossible. The null hypothesis was that chlorophyll levels observed in individual treatments would be less than or equal to the chlorophyll levels observed in the control. The alternative hypothesis was that chlorophyll levels observed in individual treatments would be greater than the chlorophyll levels observed in the control.

While an overarching decrease was observed in chlorophyll levels following treatment, this may again be attributable to deer and pest pressure. Despite general decline in chlorophyll levels between pre and post-treatment observations, it is notable that the mean chlorophyll level of the control post-treatment was lower than any of the ACT treatments. Multivariate Analysis of Variance (MANOVA) testing in Minitab 18 was used to establish statistical significance as three dependent variables with three different sample sizes had to be compared to a control group. MANOVA offered the most consideration in analyzing the three variables. The three dependent variables able to be tested against the control using MANOVA were the ACT foliar treatment, ACT drench treatment and the ACT drench and foliar combination treatment. As seen in Table 1, P-value returned was 0.08 and thus the null could not be rejected, in favor of no significant difference between the control and any of the three dependent variables. The results of the MANOVA

testing were not surprising as close inspection of the data would suggest little notable difference between the means of the three treatment groups and control.

Table 1. MANOVA Tests for ACT Foliar, ACT Drench and ACT Foliar and Drench Treatments

Test Statistic	Criterion	F	Num	Denom	P
Wilks'	0.83756	2.23	4	46	0.08
Lawley-Hotelling	0.19394	2.23	4	46	0.08
Pillai's	0.16244	2.23	4	46	0.08
Roy's	0.19394				
s = 1 m = 1.0 n = 22.0					

In addition to MANOVA, individual treatment means were tested against the control mean in two-tailed t-tests, the results of which are seen in Table 2. None of the resultant P-values were high enough to reject the null, thus supporting the null that chlorophyll in treated vines would be less than or equal to the chlorophyll values of the control. Though the standard t-tests are unable to account for the array of differences between datasets, they are useful in confirming the lack of ability to reject the null.

Table 2. Two-Tailed T-Tests for Individual Treatments

T-Test	Sample Size	Mean	Standard Deviation	T-Value	P-Value
ACT Foliar	16	5.540	1.730	1.846	0.085
ACT Drench	9	6.600	2.860	1.954	0.087
ACT Foliar & Drench	16	5.770	2.730	1.512	0.150

It was initially hypothesized that treatments would increase chlorophyll levels via increased nutrient uptake and less disease pressures during the growing season; therefore, pre-treatment chlorophyll levels would be lower than post experiment chlorophyll levels. The results of the above tests on individual treatment datasets favor the null hypothesis that chlorophyll levels would be the same or less after treatment than before. It is impossible to determine from the data available what factors caused the observed decline in chlorophyll levels and inability to confirm the alternative hypothesis above.

4. Conclusion

The aim of the experiment was to test if fish emulsion applied as a foliar spray or aerated compost tea applied in a drench or foliar format were effective, non-synthetic means of increasing plant chlorophyll via aiding in nutrient uptake and suppressing disease. Though sufficient statistical samples were set up for each respective treatment, foliar decline late in the growing season due to rampant pests and adverse climatic conditions limited the number of samples able to be taken from the population significantly. Past limiting sample size, pests and climate likely contributed to the observed effects on the plants to an unknown and unquantifiable extent. Statistical hypothesis testing indicates that none of the three post-treatment ACT treatment datasets were significantly different from the control. Chlorophyll values were on average substantially lower after experimentation than before due to external pressures, thus the potential for any statistical testing between pre and post treatment data was excluded. There are too many factors and potential sources of error present in the post-treatment data to say with certainty if any of the trial treatments contributed significantly to vine health

Further research, conducted on a well-managed, organically practiced vineyard during a season of average rainfall may have a greater potential of producing significant results than this experiment. Research on the efficacy of ACT is important to the development of sustainable viticultural practices in the southeast of the United States and is worthy of further exploration.

5. Acknowledgements

The author wishes to express his appreciation to CharGrow LLC for providing both equipment and exceptional advice for brewing of ACT, to Dr. Irene Rossell for providing the CCM-200 and to Living Web Farms who humbly provided compost. I wish to thank my friends and colleagues who both helped revise this work and spent many odd hours laboring in the vineyard. Lastly, I wish to thank Dr. Dee Eggers for her guidance and assistance through every step of this project.

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