# Final Report to the Viticulture Consortium-Eastern Grants Program
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## Water and nitrogen management to reduce atypical aging of wine

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### Introduction

Wines with atypical aging (ATA) defect lose their varietal flavors very quickly - sometimes before the wine is one year old before they are 3 years old. With the disappearance of the varietal flavor, atypical flavors appear, which are described as "dish cloth", "floor polish", "linden blossom", "furniture varnish" etc.

ATA primarily occurs in white wines, such as Riesling, Chardonnay, Pinot Gris, Cayuga White. This defect was first identified and reported in Germany in 1980's. Since then it has been reported in many European countries and other wine growing regions of the world. This problem has been recognized in the Northeast, Midwestern, and Western USA. Based on the notes taken at large wine competitions, we estimate that the percentage of wines affected with ATA might be as high as 20%. It can potentially cause serious economic loss to the grape and wine industry.

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Very little is known about the exact cause(s) of ATA on the viticulture side. The general understanding is that ATA is induced by stresses on the vine. The critical time appears to be before and during veraison. ATA occurs more frequently on dry vineyard sites and in dry years. Nitrogen deficiency may play an important role in ATA as the identification of ATA in Europe coincided with dramatic reduction of nitrogen use in vineyards. Generally, under ATA-inducing conditions, berries do not reach their full maturity, and therefore lack intense varietal flavor or a sufficient pool of fruit flavor precursors. In the Northeastern USA, most of the winegrape vineyards do not have irrigation and rely totally upon natural rainfall. Although use of nitrogen fertilizers varies widely among vineyards, a significant proportion of the vineyards uses little nitrogen fertilizers. The soil nitrate content in these vineyards can be very low around veraison (Martison 1999 unpublished data). In drought years, the situation gets worse because root uptake of nitrogen from soil may be further reduced by water stress. It is not known if nitrogen deficiency alone would trigger ATA. Anecdotal reports from growers indicate that foliar N application at veraison helped control ATA, but no experiment has been done to provide proof. If N deficiency alone induces ATA, foliar application of nitrogen around veraison may help reduce ATA even under drought conditions.

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## Objectives

To determine if nitrogen deficiency, alone or in combination with water stress, causes ATA, and under what conditions (water stress or adequate water supply) nitrogen application via foliage or soil at veraison improve vine nitrogen status, fruit quality and consequently reduce occurrence of ATA under Northeast cool climate conditions.

## Procedures

***Field trial 2001 - 2003***

1. Treatments and field setup

A field experiment was initiated using mature Riesling vines on Couderc 3309 rootstock at Prejean Winery in the Finger Lakes area of New York in 2001. Two factors were considered in this experiment: nitrogen fertilization and water supply before and during veraison. There were two regimens of water supply (no irrigation or irrigation) and three variants of N fertilization (No N, foliar N, or soil N), resulting in a total of 6 treatment combinations: (1) No irrigation nor N application; (2) No irrigation with foliar N application; (3) No irrigation with soil N application; (4) Irrigation without N application; (5) Irrigation with foliar N application; and (6) Irrigation with soil N application. All the treatments were replicated 5 times with two panels of vines (6 vines) in each plot in a completely randomized design. There was one buffer panel between any two adjacent plots and a buffer row between any two adjacent rows.

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Drip irrigation was provided once a week at 15,000 gal/acre/week from July 18 through September 13 in 2001 and from July 16 through September 4 in 2002. No supplemental irrigation was provided during 2003 season due to abundant rainfall. For soil N treatment, urea was applied to soil surface at a rate of 30 lb actual N per acre on July 31 in all 3 years. Foliar N sprays began on July 31 with a 0.8% urea solution (6.5 lb of urea/100 gal water at 200 gallons/acre) at weekly intervals for a total of 5 applications (equivalent to 30 lb actual N).

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2. Vine monitoring and harvesting

Vine responses to the treatments were monitored from veraison to harvest.

Leaf nitrogen content: Recent fully expanded leaves were collected every two weeks for N measurement. Leaves were washed thoroughly before drying. Leaf N was determined by the Kjeldahl method.

Stem water potential: The water potential (i.e. tension on the water in the tissue) in stems, an indicator of vine water stress, was estimated by enclosing mature mid-canopy leaves in foil-covered plastic bags to stop leaf water loss. After a minimum of 30 minutes the leaf equilibrates with the stem, which also integrates the water status of many leaves of the canopy. The stem potential also is particularly relevant as it is the baseline water status that affects shoot tip and fruit water status. On each of six dates from just before veraison to harvest, stem potentials were determined under sunny conditions for 3 of the 5 replicate plots in the study.

Leaf photosynthesis: To determine leaf function as affected by treatment, we monitored photosynthesis of exposed, mature leaves with a portable gas exchange system on sunny afternoons for uniform conditions. The same replicate plots that were monitored for stem water potentials were used although the measurements were not always on the same days.

Berry development. Fifty-berry samples were taken from each of 3 of the 5 replicate plots at 4 pre-harvest intervals as well as from all the replicates at harvest in 2002. Mean berry weights were determined.

Fruit from all vines in the experimental units was hand harvested on October 16 in 2001, October 15 in 2002 and October 30 in 2003. For each vine, the number of clusters per vine was recorded. Harvested fruit was weighed. A total of 50 to 200 berries was randomly collected from each experimental unit and weighed in the laboratory to determine berry weight. Approximately 100 ml of juice was expressed from each sample, and % sugar (Brix) was measured with a hand refractometer.

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3. Juice analysis and wine making

Juice samples were analyzed for Brix, pH, titratable acidity (TA), yeast available nitrogen (ammonia + amino nitrogen) and free and bound monoterpenes.

For both 2001 and 2003 harvests, fruit from six different vineyard treatments were each divided into two fermentation lots (2 replicates each treatment). After settling and racking, musts with less than 22 Brix were chaptalized to 22 Brix, and were inoculated with AMH yeast for fermentation. For 2002 harvest, due to high incidence of bunch rot there was insufficient quantity of fruit and no replicate fermentations were possible. Also, because bunch rot affected so much of the fruit, at least one lot from each treatment was pasteurized. Musts were inoculated with yeast EC1118 for fermentation. Each lot yielded 3 to 5 gallons of wine. After cold stabilization, acids were adjusted where necessary. Finished wines were analyzed for residual sugar, alcohol, pH, titratable acidity, organic acids, and ATA indicator flavors.

4. Sensory evaluation of wine

Twelve wines were made from six vineyard treatments with two fermentation reps for each treatment. In the spring of 2002 when the wines were 6 months old, a blind tasting was held where tasters evaluated each of the twelve wines twice. Participants rated the wines based on seven perceived flavor characteristics: varietal fruit intensity, vegetativeness, linden blossom/black locust/acacia, furniture varnish/floor polish, dish cloth, body/mouthfeel, and bitterness. These characteristics were chosen by the taste panelists based on a preliminary evaluation of the wines and because their presence or absence may be indicative of ATA.

5. Aroma chemistry

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Defining the chemistry of ATA is the first step towards developing a standard chemical assay to detect onset of the defect.

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*Experiment 1: Measuring concentrations of O-AP in ATA Finger Lakes wines*

Five NYS Finger Lakes Region wines were selected from a group of over 50 by an expert panel of winemakers from Europe familiar with the ATA defect. The ATA defective wines were ranked 1-5 in order of increasing degrees of the defect. Concentrations of ortho-aminoacetophenone (O-AP) would be compared to scores of ATA defect to determine correlation between O-AP concentration and the ATA defect. GC-MS scanning for skatole and indole as possible agents of ATA would also be performed.

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Samples were prepared for GC-MS analysis utilizing a special stir-bar extraction process called the Twister™ (Gerstel, USA) which extracts volatile components from aqueous media into a Poly Dimethyl Siloxane (PDMS) polymer coating on the exterior of the stir bar. The bar was then thermally desorbed by a Gerstel TDS-CIS4 thermal desorption injection system onto a DB-5 chromatographic column in an HP5973 Mass Selective Detector (GC/MS). Selected ion monitoring of key ions of O-AP was done to increase sensitivity to < 0.2 ppb for O-AP in a wine matrix. Standard O-AP was prepared at calibration standards of 0.1, 0.3 and 0.5 ppb and spiked into wine previously screened and found to have no O-AP. The spiked wines were extracted with the Twister™ for 2h prior to desorption and injection into the GCMS. Samples were desorbed at 250ºC and temperature ramped on the column at 4ºC/min. Peak areas for O-AP were recorded and entered in a calibration table for external standard quantitation. The wine samples were then extracted under the same conditions and in duplicate and area counts for O-AP calculated from the calibration table. In addition to O-AP, separate runs under selected ion monitoring mode were made on each wine in order to detect skatole and indole, two additional candidates as identified in Germany as possible agents of ATA.

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*Experiment 2. Identify compounds, other than or in addition to O-AP, that may be responsible for the ATA defect in US wines.*

Two samples of Finger Lakes Riesling were analyzed in triplicate by CharmAnalysis and GC-MS. One was a 1999 Finger Lakes Riesling considered by the expert tasters to exhibit a high degree of ATA defect. The other was a wine from the same vineyard and winery but was a 2001 vintage. Taste panels agreed the 2001 was an excellent example of a well made Riesling with representative varietal character and would act as a control in the experiment. Both wines were assayed via Gas Chromatography Olfactometry (GC-O) to determine the retention indices, odor character and potencies of the individual odor components in the wines. By comparing the results of the GC-O chromatograms, it was hoped that differences would pinpoint compounds responsible for the ATA defect.

*Experiment 3. Assay and compare concentration of terpenoid compounds in ATA defect wine vs. control wine via GC-MS analysis.*

Many researchers believe the key varietal components of Riesling lies in the terpenoid compounds which provide a citrus-like character to the wine. As part of our initial studies into the nature of ATA, an analysis of an ATA wine focusing on a possible reduction in varietal character was deemed important.

Terpenoid compounds were assayed via scanning GC-MS in two Finger Lakes wines: a 1999 Riesling showing strong ATA defect and a 2001 Riesling (control) from the same vineyard and winery showing no signs of ATA.

## Results and discussions

1. Vine N status, water status, and leaf photosynthesis

**Vine N status:** In both 2001 and 2002, leaf N content in the foliar N treatments tended to be higher than no nitrogen controls over the sampling period, with occasional statistical difference detected (such as samples collected on August 29, 2001) (Fig. 1 and 2). Soil N application did not affect vine N status. Irrigation did not affect leaf N in

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2001, but significantly increased N content of leaves collected on September 18, 2002. In 2003, foliar or soil N application did not significantly increase leaf N (Data not shown).

**Vine water status and leaf photosynthesis:** Over the three years of the experiment, 2001 – 2003, two years (2001 and 2002) had significant drought stress as measured by stem water potential and reductions in leaf photosynthesis. In 2001, non-irrigated vines had significantly lower stem water potential than the irrigated vines in August and September. The non-irrigated vines reached the threshold stem water potential (-1.0 MPa) for loss of photosynthesis just about veraison in mid-August, and likely had impaired canopy function for about one month during the post veraison period. In 2002, the stress developed about a month earlier than in 2001. The non-irrigated vines showed significant reductions of stem water potential and leaf photosynthesis compared to the irrigated vines as early as 24 July (Fig. 2). By early August and until early September stem water potentials of the non-irrigated vines were much below the previously determined critical value of -1.0 MPa. It appears that the leaf function was markedly limited during August, with partial recovery in September. By early October the rates of irrigated and non-irrigated vines were comparable although the effect was primarily due to the rates of the irrigated vines gradually declining. So, in 2002 there was clearly reduced leaf function for almost the entire ripening period. In contrast, in 2003, there was so much rain that no significant effects on water status or leaf function were seen and no irrigation was needed.

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**Berry development**. Berry development was monitored in 2002. When the berry sampling began in mid-August the berries on the non-irrigated vine were already about 35% smaller than the irrigated (Fig. 4). The difference of about 0.4-0.5 grams/berry persisted until harvest even though the vine water status recovered in mid-September. This suggests that the mid-season stress effects on berry size were not reversible by better water status later in the season.

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2. Fruit yield and juice soluble solids

Because vine size was quite variable, N application or irrigation did not significantly affect total fruit yield per vine in 2001 or 2002 (Table 1 and 2). However, some of the yield components did respond to irrigation. In 2001, berry weight and cluster weight were increased by irrigation from 1.59g to 1.74g and from 88.1g to 97.7g, respectively (Table 1). In 2002, berry weight was increased by irrigation from 1.33g to 1.75g (Table 2). The increase in berry weight by irrigation was much larger in 2002 compared with 2001. In 2002, berry number per cluster was lower in the irrigated vines than in non-irrigated vines, however the apparent reduction in berries per cluster in the irrigated treatments was almost certainly due to higher levels of bunch rot in the irrigated treatments rather than true differences in berry number per cluster. Cluster number per vine was not significantly affected by irrigation in 2001 or 2002. Juice soluble solids was increased significantly by irrigation from 20.3 to 21.9 in 2001 from 18.6% to 21.5% in 2002. N applications did not significantly affect any of the yield components or juice soluble solids.

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In 2003, vines that were irrigated in 2002 had a significantly higher fruit yield than those not irrigated (Table 3). The higher yield was due to the increased cluster number per vine as irrigation in 2002 significantly increased vine size (Table 2). Juice soluble solids was slightly lower in the vines that received irrigation in 2002, Due to

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much higher crop level in irrigated vines. This clearly demonstrates that irrigation not only benefits fruit quality in the current year but also fruit yield in the following year via improving vine size.

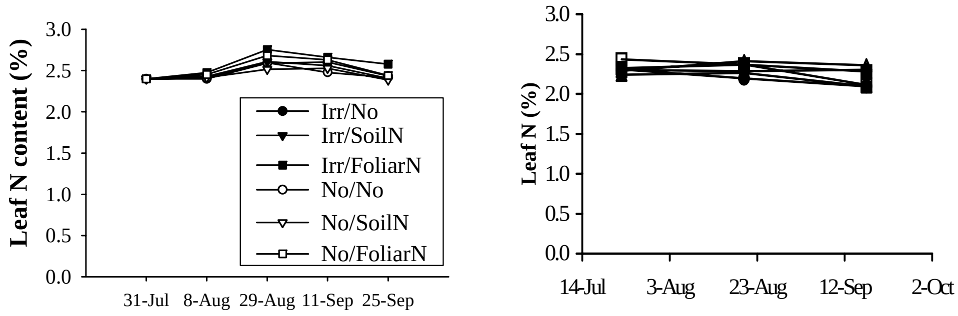


Fig. 1 Leaf N in response to irrigation and N treatments in 2001 (left) and 2002 (right)

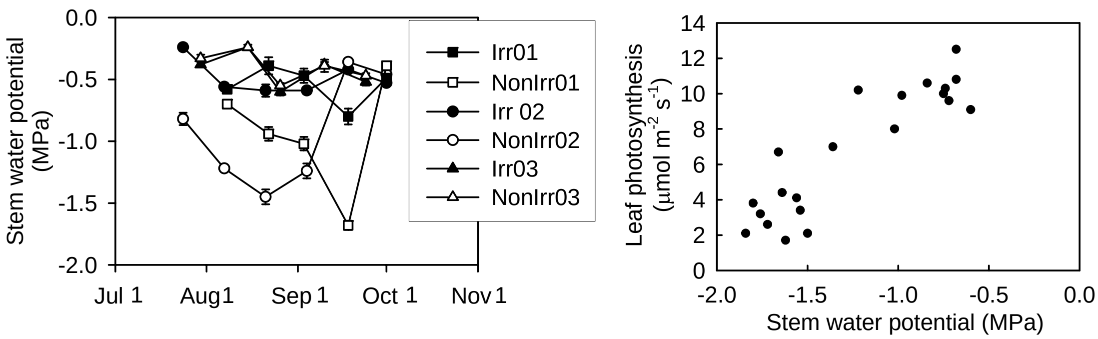


FIG. 2. SEASONAL TRENDS IN VINE WATER STRESS AS EXPRESSED AS VINE MID-DAY STEM WATER POTENTIAL. THE LOWER THE VALUE THE GREATER THE WATER STRESS. VALUES BELOW -1.0 MPA ARE ASSOCIATED WITH LOSS OF LEAF PHOTOSYNTHETIC FUNCTION.

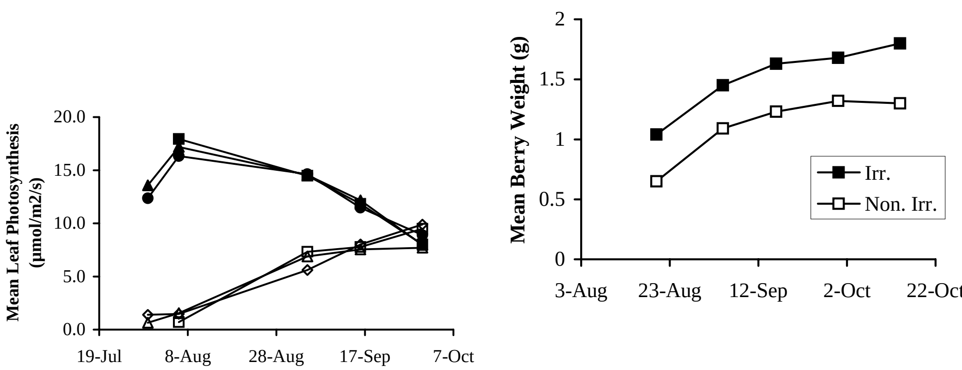

Fig. 3 (left) Leaf photosynthesis in response to irrigation and nitrogen treatments in 2002
Fig. 4 (right) Berry growth in response to irrigation and nitrogen treatments in 2002

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**Deleted:** not only benefits fruit quality in the current year but also fruit yield the following year via improving vine size

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Table 1. Effects of nitrogen applications and irrigation on Riesling yield in 2001

| *Treatments* *Irrigation* | *N* | *Brix (%)* | *Yield (lb/vine)* | *Berry Wt (g)* | *Berry# (#/cluster)* | *Cluster Wt (g)* | *Cluster # (#/vine)* |
|---|---|---|---|---|---|---|---|
| No | 0 | 20.3 | 10.6 | 1.58 | 55.7 | 87.7 | 54.5 |
| | Foliar N | 20.0 | 10.6 | 1.57 | 58.6 | 91.5 | 52.8 |
| | Soil N | 20.2 | 9.6 | 1.62 | 52.9 | 84.4 | 50.1 |
| Yes | 0 | 22.8 | 10.0 | 1.73 | 58.8 | 99.8 | 45.1 |
| | Foliar N | 22.1 | 8.3 | 1.74 | 54.9 | 95.2 | 38.7 |
| | Soil N | 22.7 | 11.5 | 1.75 | 56.5 | 98.2 | 53.1 |
| Significance | | | | | | | |
| Irrigation | | 0.0001 | ns | P<0.01 | ns | P<0.001 | ns |
| N | | ns | ns | ns | ns | ns | ns |

P values indicate the significance level. ns: non-significant.

Table 2. Effects of irrigation and nitrogen applications on Riesling yield and Brix (2002) and pruning weight

| *Treatments* *Irrigation* | *N* | *Brix (%)* | *Yield (kg/vine)* | *Berry Wt (g)* | *Berry# (#/cluster)* | *Cluster Wt (g)* | *Cluster # (#/vine)* | *Pruning Wt* (lb)* |
|---|---|---|---|---|---|---|---|---|
| No | 0 | 18.9 | 5.04 | 1.31 | 58.1 | 76.3 | 65.9 | 0.87 |
| | Foliar N | 18.5 | 5.42 | 1.36 | 56.5 | 77.0 | 70.1 | 1.17 |
| | Soil N | 18.4 | 5.78 | 1.33 | 56.5 | 74.9 | 76.5 | 1.10 |
| Yes | 0 | 21.7 | 5.57 | 1.82 | 42.8 | 77.9 | 72.1 | 1.89 |
| | Foliar N | 21.2 | 5.91 | 1.74 | 43.2 | 75.4 | 78.6 | 1.80 |
| | Soil N | 21.6 | 5.13 | 1.70 | 43.7 | 73.3 | 70.1 | 1.76 |
| Significance (P) | | | | | | | | |
| Irrigation | | 0.0001 | ns | 0.0001 | 0.0001 | ns | ns | 0.0001 |
| N | | ns | ns | ns | ns | ns | ns | ns |

P values indicate the significance level. ns: non-significant.
*Vines were balance-pruned in March 2003.

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Table 3 . Effects of irrigation and nitrogen applications on Riesling yield and Brix (2003)

| *Treatments Irrigation* | *N* | *Brix (%)* | *Yield (kg/vine)* | *Berry Wt (g)* | *Berry# (#/cluster)* | *Cluster Wt (g)* | *Cluster # (#/vine)* |
|---|---|---|---|---|---|---|---|
| No | 0 | 18.5 | 4.82 | 1.86 | 67.4 | 125.7 | 38.0 |
| | Foliar N | 18.3 | 6.45 | 1.87 | 68.9 | 128.6 | 50.0 |
| | Soil N | 19.0 | 5.60 | 2.04 | 63.4 | 129.1 | 43.2 |
| Yes | 0 | 17.4 | 11.63 | 1.92 | 66.4 | 127.4 | 92.4 |
| | Foliar N | 17.6 | 9.74 | 1.95 | 61.2 | 118.9 | 82.8 |
| | Soil N | 17.4 | 9.79 | 1.90 | 64.7 | 123.0 | 79.7 |
| Significance (P) | | | | | | | |
| Irrigation | | 0.010 | 0.0001 | ns | ns | ns | 0.0001 |
| N | | ns | ns | ns | ns | ns | ns |

*3. Effect of irrigation and vineyard nitrogen application on juice composition*

In both 2001 and 2002, foliar N application increased yeast available nitrogen, including both ammonium N and amino N (Table 4 and 5). In contrast, soil N application at the same rate was not effective at all whether or not irrigation was provided. Irrigation also increased yeast available nitrogen in both 2001 and 2002. The effects of foliar N application and irrigation on yeast available nitrogen in 2001 were additive as there was no interaction between N application and irrigation. pH was slightly lower in the non-irrigated treatments and the titratable acidity was slightly higher in the irrigated treatments in both 2001 and 2002. This may indicate higher physiological ripeness in the irrigated fruit. In 2003, the yeast available nitrogen level was much higher than that in 2001 and 2002, but there was no significant difference among treatments (Table 6). No difference was observed in titratable acidity or pH. Thus these juice analytical parameters suggest no difference in fruit maturity in 2003 fruit.

Table 4. Effects of irrigation and N applications on juice composition in 2001

| *Treatments* Irrig | Nitrogen | $NH_4^+$-N (mg/l) | Amino-N (mg/l) | YAN (mg/l) | TA (g/l) | pH | Phenolics |
|---|---|---|---|---|---|---|---|
| No | 0 | 81.5 | 99.5 | 180.9 | 8.6 | 3.18 | 2.01 |
| | Foliar N | 111.5 | 165.6 | 277.1 | 8.9 | 3.21 | 2.92 |
| | Soil N | 78.8 | 90.1 | 168.9 | 8.7 | 3.15 | 4.49 |
| Yes | 0 | 95.6 | 149.1 | 244.7 | 10.1 | 3.24 | 2.41 |
| | Foliar N | 119.1 | 210.1 | 329.2 | 9.8 | 3.26 | 3.33 |
| | Soil N | 90.3 | 154.5 | 244.8 | 9.5 | 3.21 | 4.28 |
| Significance (P) | | | | | | | |
| Irrigation | | 0.01 | 0.001 | 0.001 | 0.0001 | 0.0001 | ns |
| N | | 0.067 | 0.001 | 0.001 | ns | 0.002 | ns |

Note- table 4 is split between two pages.

Table 5. Effects of irrigation and N applications on juice composition in 2002

| *Treatments* Irrig | Nitrogen | $NH_4^+$-N (mg/l) | Amino-N (mg/l) | YAN (mg/l) | TA (g/l) | pH |
|---|---|---|---|---|---|---|
| No | 0 | 81.9 | 71 | 152.9 | 7.5 | 2.94 |
| | Foliar N | 136.9 | 116 | 252.9 | 8.1 | 3.03 |
| | Soil N | 53.6 | 53 | 106.6 | 8.2 | 2.91 |
| Yes | 0 | 67.3 | 99 | 166.3 | 9.5 | 3.03 |
| | Foliar N | 97 | 138 | 235 | 10.0 | 3.03 |
| | Soil N | 72.6 | 96 | 168.6 | 9.1 | 2.99 |

P values indicate the significance level. ns: non-significant.

Table 6. Effects of irrigation and N applications on juice composition in 2003

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| *Treatments* *Irrig* | *Nitrogen* | *$NH_4^+$-N (mg/l)* | *Amino-N (mg/l)* | *YAN (mg/l)* | *TA (g/l)* | *pH* |
|---|---|---|---|---|---|---|
| No | 0 | 220 | 185 | 405 | 13.1 | 2.96 |
| | Foliar N | 258.3 | 190 | 448.3 | 14.1 | 2.95 |
| | Soil N | 191.2 | 147 | 338.2 | 13.2 | 2.94 |
| Yes | 0 | 255.8 | 205.5 | 461.3 | 13.4 | 2.98 |
| | Foliar N | 241.7 | 197 | 438.7 | 13.8 | 2.96 |
| | Soil N | 203 | 196 | 399 | 13.2 | 2.97 |
| Significance (P) | | | | | | |
| Irrigation | | ns | ns | ns | ns | ns |
| N | | ns | ns | ns | ns | ns |

P values indicate the significance level. ns: non-significant.

*4. Sensory evaluation of 2001 wines*

In the first sensory evaluation of the 2001 wines (at 6 months of age) there were significant differences in perceived varietal flavor, bitterness, body, dish cloth, furniture varnish, and linden blossom; no perceived difference in vegetativeness (Table 7, Fig. 5). The highest rating in varietal flavor was given to the wine from irrigated fruit without nitrogen vineyard applications, followed with non-irrigated and irrigated with 30 lbs N in four pre-veraison applications. Lowest fruit intensity was in wines from non-irrigated fruit without N or with one N application pre-veraison. These wines also had the highest dish cloth and furniture varnish ratings and bitterness. These are typical ATA flavors. We will continue to follow these wines to see how varietal fruit and ATA flavors develop.

Table 7. Effects of irrigation and N application on the perceived flavors of 2001 wine

| *Treatments* *Irrig* | *N* | *Varietal flavor* | *Body* | *Furniture varnish* | *Dish cloth* | *Linden* | *Bitterness* | *Vegetative* |
|---|---|---|---|---|---|---|---|---|
| No | 0 | 3.54c | 3.57ab | 2.19a | 1.57ab | 1.35b | 2.49ab | 1.45a |
| | Foliar N | 4.40b | 4.00a | 1.01b | 1.47b | 1.44b | 2.55ab | 1.56a |
| | Soil N | 2.93d | 3.31b | 1.99a | 2.17a | 1.43b | 2.58ab | 1.69a |
| Yes | 0 | 5.62a | 4.03a | 1.03b | 0.73c | 1.38b | 2.23ab | 1.31a |
| | Foliar N | 3.86bc | 3.87ab | 1.38b | 1.46b | 2.13a | 2.14b | 1.55a |
| | Soil N | 4.22b | 3.85ab | 1.25b | 1.40bc | 1.23b | 2.72a | 1.42a |

Different letters indicate significant difference at 0.05%
Within each column, the higher the number the more intensive the perceived flavor.

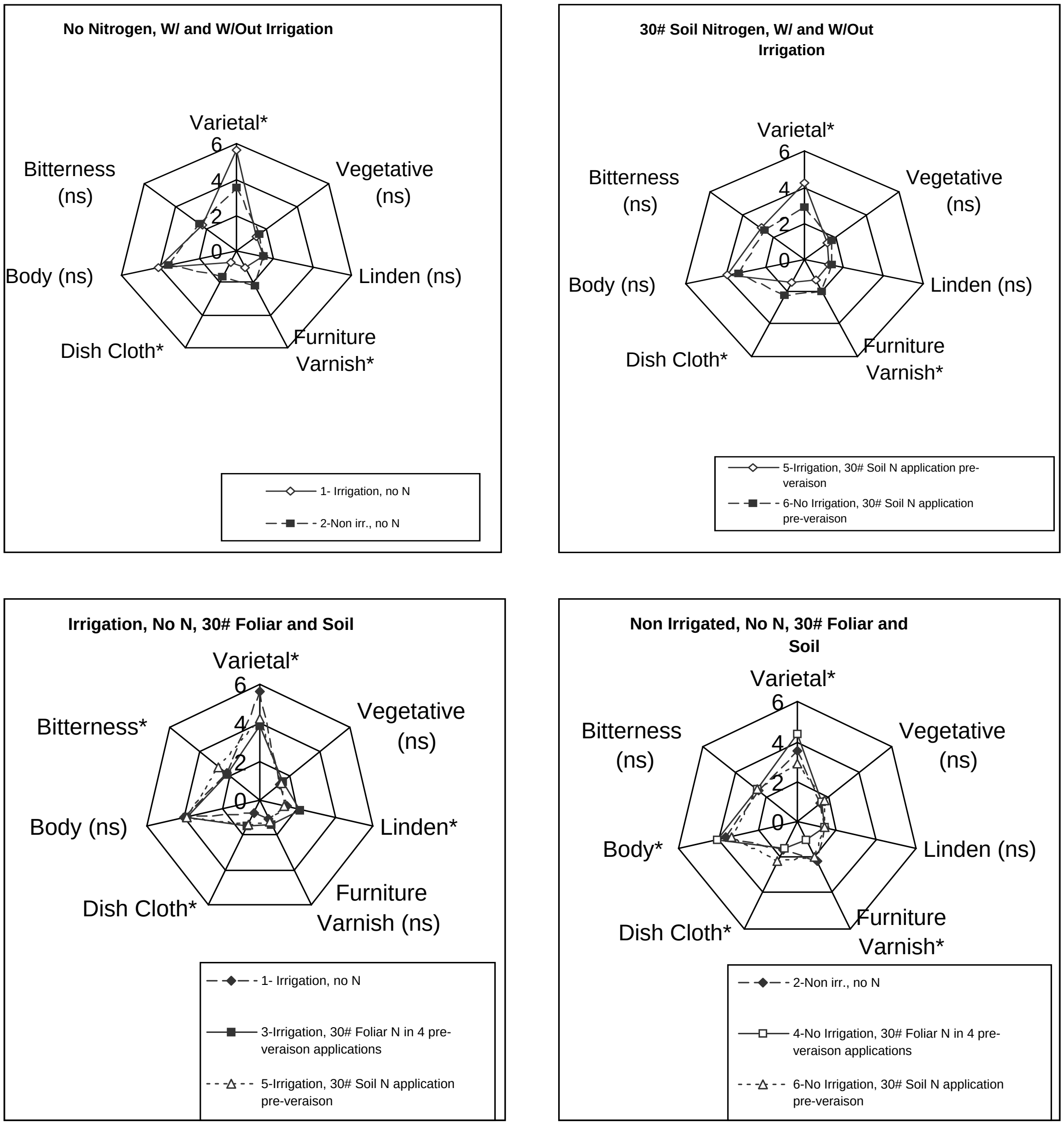


Fig. 5: Effect of vineyard nitrogen and irrigation treatments on the perceived flavor characteristics of Riesling wine at 6 months of age.

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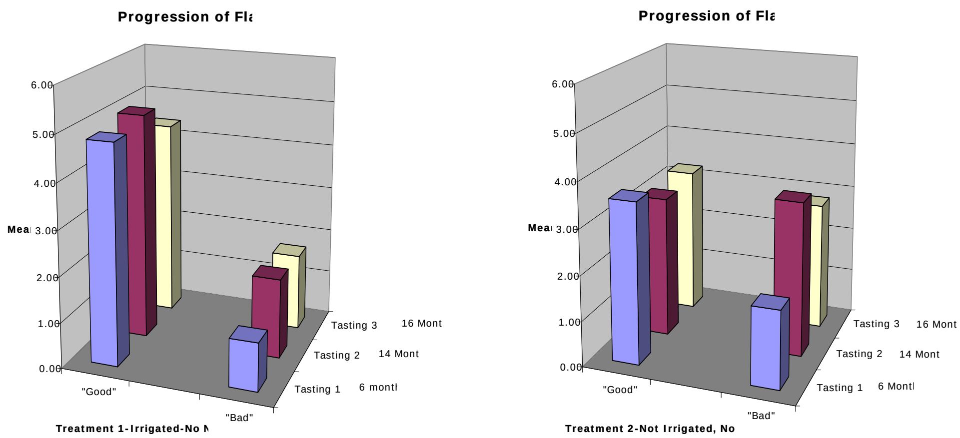


Fig. 6: Examples of progression of fruit varietal flavors ("good") and ATA ("bad") flavor defect in two wines, irrigated and no nitrogen application and not irrigated and no nitrogen application. Flavor intensity was rated by trained taste panels. Each wine was tasted when it was 6, 14 and 16 months old.

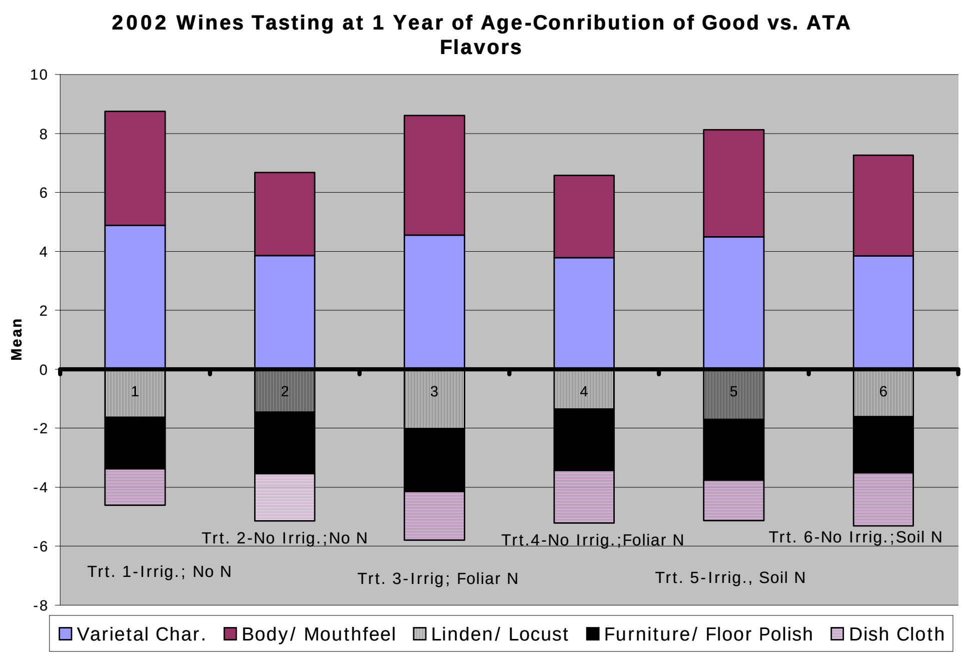


Fig. 7: Sensory evaluation of the wines from 2002. Positive sensory descriptors varietal character and body/mouthfeel are shown about the zero line, negative sensory characters below. Sensory evaluation was carried out by trained tasters when the wines were one year old.

The comparison of values in Fig 6 shows clearly how the flavor intensity of the ATA defect increases with age. The two wines selected as examples are (on the left) the wine from irrigated fruit, no nitrogen application and (on the right) from non-irrigated fruit with soil nitrogen application. Over time, the perceived intensity of fruit varietal flavors increases and the intensity of ATA off-flavors increases. The big difference between the wines from the irrigated and the non-irrigated fruit is the wine from the irrigated fruit started with a much higher perceived fruit flavor intensity and ATA off-flavors were already high in the wine from the non-irrigated fruit at 6 months of age. As the wines from the non-irrigated fruit aged, the ATA off-flavors increased dramatically; the varietal fruit flavors decreased. It is very interesting that in the sensory perception of our tasters, some ATA off-flavors are in all wines, even if a wine is not rated as defective. A wine is rated as defective when the varietal fruit flavors are low and the ATA flavors are high. This indicates a presence of both aroma complexes at the same time and some very important interactions, or matrix effects of the ATA flavors with the varietal fruit flavors. This also shows that flavor maturity in the drought stressed fruit was lower than in the irrigated fruit! And, apparently, less ATA flavors were formed in the irrigated fruit and these ATA flavors did not reach the same intensity as in the wine from the non-irrigated (drought stressed fruit).

The results of the first tasting of the 2002 wines show the same trends as seen in 2001. Wines made from fruit from irrigated vines (non-drought stressed) have more ripe fruit flavors and less ATA. Fruit flavor intensity (=flavor ripeness) is very clearly linked to irrigation (=avoidance of drought stress). ATA off-flavors are found to varying degrees in all wines. This is an indication that irrigation was not set up optimally and/or that other factors such as nitrogen availability are also affecting flavor maturity and formation of ATA off-flavors. More work needs to be done to optimize irrigation for flavor maturity and flavor intensity and to lower potential ATA flavors. More work needs to be done to understand the role of other factors such as nitrogen availability of the development of ATA in wines.

**2003**

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Riesling grapes from Prejean Winery were picked and crushed on 28 October 03. Two five-gallon fermentations were made for each of the six different vineyard treatments – irrigated and non-irrigated each with no nitrogen application, with soil nitrogen application, and with foliar nitrogen application. Grape musts were inoculated with yeast EC1118, and all had finished fermentation by 26 November 03. Each of the 12 lots yielded 3 gallons of wine. Wines are currently undergoing cold stabilization, after which acids will be adjusted. Juice samples have been analyzed for pH, titratable acidity, organic acids, and yeast available nitrogen. Finished wines will be analyzed for pH, titratable acidity, organic acids, grape varietal flavors and ATA indicator compounds. Wines will be evaluated for sensory characteristics by trained taste panelists after 6, 12, and 24 months of aging.

Analysis of the juice samples from the 2003 harvest showed no significant differences in pH, titratable acidity, tartaric, malic, and acetic acid. Yeast available nitrogen in the grape musts was again increased by foliar application of nitrogen. Juice samples from plots with soil applied nitrogen tended to have lower ammonia content than samples from plots with no nitrogen applied. Samples from the irrigated blocks (there was not irrigation applied in 2003 but there was in 2002 and in 2001) still tended to have higher yeast available nitrogen content than samples from the non-irrigated blocks. Due to large sample variation between replicates and since only 2 samples were available for each treatment the differences noted are not statistically significant.

In the dry years of 2002 and 2001, the juice composition showed effects of availability (or lack) of water and nitrogen to the grape vines. The non-irrigated fruit had was less ripe having lower sugar content, lower pH, less yeast available nitrogen (ammonia and free amino acids). Further, irrigation and nitrogen fertilization together also improved fruit ripeness.
In the wet and cloudy growing season of 2003, there was never a water stress and irrigation was not used. The fruit composition showed no ripeness differences between the different vineyard treatments. It will be very interesting to find out whether the water stress and nitrogen limitation experienced during the preceding seasons affect the formation of ATA in the wines of 2003. It is entirely possible that the metabolism of the vine was affected by the nutrient and water availability of the previous year.

**Summary of sensory effects of irrigation and nitrogen fertilization**

The sensory character of all wines is dominated by volatile esters produced during alcoholic fermentation. The wines loose these fermentation aromas as they age. After the fermentation (ester-) aromas are gone the wine flavor is characterized by grape varietal and by regional (vineyard site) flavors.

Our experience has shown that wines younger than 6 months do not show any flavor differences which might be due to different vineyard practices or different vinification practices. Also the atypical aging defect is almost never recognized in wines younger than 6 months. The fermentation esters and varietal fruit flavors which are dominant in very young wines apparently mask the atypical aging flavors or the ATA flavors are formed only later as the wine ages. Usually a wine is 1 to 2 years old before it shows ATA. Only in extreme cases have we found ATA flavors in wines only 6 months old.

Sensory evaluation of the 2001 wines have been carried out at 6, 14, and 16 months of age. The sensory results show clearly that drought stress strongly affects development of ATA in the wines. Wines from non-irrigated fruit and no soil nitrogen or with soil nitrogen had the lowest varietal fruit character and had the highest perceived intensity of furniture varnish and dish cloth flavors (= ATA flavors). Foliar application of nitrogen without irrigation improved the intensity of varietal fruit flavors to about the same intensity as irrigated fruit with foliar or with soil nitrogen application.

In an initial tasting, the wines produced in 2002 showed the same trends as seen in the 2001 wines. Drought stress again lowered varietal fruit flavor intensity and increased ATA flavors. Further sensory evaluation on these wines is needed as these wines age in order to more fully explore the flavor effects. Further tastings are planned at 18 and 24 months of wine age.

The wines produced from the 2003 fruit are being stabilized. Tastings of these wines are planned when they will be 6, 12, 18 and 24 months old.

The study years 2001, 2002, and 2003 have produced most valuable juice and wine samples to help us understand what causes atypical aging in wines - how water and nitrogen availability in the vineyard affect ATA in the resulting wines and identifying the chemistry of these damaging off-flavors. We also have developed some new approaches to let us identify the chemical nature of the ATA defect. To allow full analysis of the samples produced we ask for a one year extension of the project.

This initial study clearly shows that irrigation (or lack of water) and some nitrogen additions in the vineyard affect the fruit flavor development (fruit maturity) in the grapes. The effects of irrigation and vineyard fertilization on the effect of fruit flavor maturation should be further explored in separate new proposals in which different irrigation management is evaluated on different soils and different fertilization practices are evaluated in combination with irrigation.

**Budget for wine sensory analysis in 2004**
First tasting of the wine samples from 2003, two more tastings of the 2002 wines, and one tasting of the 2001 wines. Total 4 tastings of 12 wines each. 16 Days at $500 per day=$8,000

### *3. Aroma Chemistry*

In 2002 work was begun to study wines exhibiting ATA focusing on detection and identification of trace level odorants that could be the cause of an odor defect in ATA wines. The chemical source of the ATA sensory defect is thought to arise from two possible chemical means. One involves the possible loss of varietal aroma compounds in the wine as it ages, and the second related to the formation of an off-aroma by a specific chemical agent(s). It is also possible that the ATA is the combination of these two phenomena. The initial study in 2002 was designed to address both of these possibilities. Two Riesling wines were selected for study, one exhibiting an ATA like defect and a defect-free control that was a more recent vintage from the same commercial winery . Since terpenoids compounds are considered important to the varietal character of Rieslings, relative levels of terpenoids in the two wines were measured via Gas Chromatography Mass Spectography (GCMS) to determine relative levels of these compounds in the wines. Results showed levels of terpenoids in the control non-ATA wines to be an average of 2-3x higher than those found in the ATA wine, giving evidence of possible loss of aroma character in ATA wines. Also, GCMS Selected Ion Monitoring was employed to screen the ATA samples for compounds identified in Germany as agents of what they call UTA or Untypical Aging. Both control and the ATA wines were extensively screened for detection of O-aminoacetophenone, Indole, and Skatole to determine if these compounds were: present in the wines; whether they were increasing or decreasing; and whether they were odor-active in the wine flavor system. Results showed there were approximately equal trace levels of O-aminoacetophenone in both control and ATA wines and no indole or skatole present in either wine. These results pointed to ATA being a different phenomenon that UTA.

Gas Chromatography-Olfatometry (GCO) studies were also performed on the wines in an attempt to isolate and identify trace chemical odorants responsible for ATA as well as to screen for odor activity by the UTA suspect compounds. GCO confirmed the absence of indole and skatole as agents of the ATA and found equal odor activity of O-aminoactophenone in both sets of samples. GCO results of the overall spectrum of odorants in the two wines were inconclusive in determining a specific trace off-odorant specific to the ATA defect but two chemical indexes were identified which were more potent odor producers in the suspected ATA wine when compared to the control. Odor descriptor of “animal” and “urine” were somewhat consistent with sensory descriptions of the ATA defect. However GCMS studies of the odorant region of the chromatography did not yield sufficient material to make an identification of the compounds involved. Identification efforts of these compounds are ongoing.

The 2002 study was valuable in terms of developing a strategy and methods for investigating ATA in Finger Lakes Wines. Initial results point to ATA not being exactly the same defect being studied in Germany as UTA (Untypical Aging) where O-aminoacetophenone, indole and skatole are the suspected agents. Based on the 2002 ATA study, these compounds do not appear to be involved with the defect.

2002 Study --- Critical Evaluation

While useful in providing a chemical analysis methods for studying the ATA problem as well as producing results that point to the ATA problem as not being caused by o-aminoacetophenone and the other suspect compounds from the German studies, the 2002 study could not make definitive cause and effect correlations. This is due chiefly to the fact that strict sample and control experimental wines would not be available for study until late in 2003. The 2002 study wines analyzed were from different years and there was no possible means to document the exact source of the grapes, types of yeasts used, , and conditions etc. that existed in the commercial wines used in the study. While the data is useful in it’s own right, the aforementioned variables limit the scope of conclusions that can be made regarding the chemical differences in the wines. E.g. the levels of terpenoids in the wines, though significantly different as we reported, may be a function different viticultural conditions for the years the wines were made. The measurements therefore can only be viewed as a possible trend, not as definitive atypical aging processes per se.

The GCO results have similar limitations with differences found not definitively a function of ATA but possible indications of agents that may be responsible for the sensory difference between the wines. Whether these differences were ATA induced or the result of winemaking and viticultural practices can not be definitively stated without the strictly designed viticultural and vinification protocols that came to be available for study in 2003.

2003 ATA

With analytical methods in place the 2003 Study was designed to take advantage of a well controlled experiment using grapes grown and wines vinified under controlled conditions.. Lakso and Henick-Kling designed experiments using N2 and irrigation restrictions to either induce or restrict ATA formation in wines made with the grapes grown under these controlled conditions. With the grapes and wines now being from the same year ,vineyard, and winemaking protocols, meaningful chemical measurements could be made. Sensory evaluations by wine panels of wines from the Lakso-Kling experiment identified wines exhibiting formation of ATA as well as defect-free controls. These wines were then analyzed chemically by GCMS and GCO to track levels of varietal impact compounds as well as to identify agents of off-odor that could be associated with ATA.

Experiment 1 – GCMS-SIM for O-AAP, Indole, Skatole

Wines identified via taste panels as showing early signs of ATA were solvent extracted for chemical analysis via GCMS. Control wines showing no signs of ATA were similarly selected and analyzed. The three suspect compounds from German UTA studies , O-aminoacetophenone (O-AAP), Indole and Skatole were targeted for analysis via selected ion monitoring. Relative quantitations were made in the two wines. Table 1 shows relative amts. of the suspect UTA compounds found in both the control and ATA-induced Finger Lakes Riesling. O-AAP was detected in both wines at barely trace level, below 0.1ppb in the samples. Slightly more O-AAP did was present in the ATA sample but at these trace levels it's significance and impact on the aroma of the sample is questionable and will have to be assessed in the GCO experiment (see below). Selected ion mass spec scanning for indole and skatole turned up no detectable levels of either compound in either sample. These results are in agreement with 2002 findings showing similar levels of o-AAP and undetectable levels of indole and skatole. Based on these figures, we continue to question whether these compounds are causative agents in Finger Lakes wine ATA.

Table 1

| compound | RT | area-control | area-ATA | Con/ATA-induced |
|---|---|---|---|---|
| indole | 18.82 | *not found* | *not found* | |
| o-aap | 19.401 | 107233 | 154969 | **69.20%** |
| skatole | 21.12 | *not found* | *not found* | |
| terpenes | | | | |
| a-pinene | 9.058 | 3894 | 3631 | 107.24% |
| limonene | 11.891 | 231100 | 216548 | 106.72% |
| a-terpinene | 13.596 | 45466 | 43226 | 105.18% |
| linalool | 14.058 | 180073 | 95579 | **188.40%** |
| a-terpineol | 16.523 | 373856 | 442910 | 84.41% |
| ocimene | 16.746 | 26041 | 29347 | 88.73% |
| vitaspirane | 18.694 | 333621 | 464700 | 71.79% |
| ethyl esters | | | | |
| e. butyrate | 5.401 | 11529380 | 8813187 | **130.82%** |
| e. hex. | 11.187 | 61083900 | 51429718 | 118.77% |
| e. caprylate | 16.652 | 104296082 | 87708774 | 118.91% |
| e. caproate | 21.404 | 49209959 | 42175049 | 116.68% |
| e. laurate | 25.613 | 2488250 | 2323303 | 107.10% |
| alcohols | | | | |
| isoamyl | 4.206 | 1439001808 | 1178078097 | 122.15% |
| 1-hexenol | 7.504 | 276588801 | 250867732 | 110.25% |
| phenylethanol | 14.653 | 1335909427 | 1092403708 | 122.29% |
| TDN | 20.44 | 549345 | 1443410 | **38.06%** |
| 2002 Study | | | | |
| TDN | 26.18 | 443178 | 2956682 | **14.99%** |

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Experiment 2 – GCMS- Terpene analysis

In order to assess possible ATA effects on varietal compounds a range of terpenoids were bioassayed via GCMS. _Eight sometimes odor-active terpene species were identified in the samples. An internal standard, 2,6 dichloroaniline was used to ensure equal extraction of the samples and the samples were run in duplicate. Table 1 lists the compounds found and their relative concentrations in the control and ATA-induced samples. Results show no significant increase or decrease in the control vs. ATA-induce samples with the exception of linalool. By far the most important terpene from a flavor standpoint, linalool was present in the control sample at levels _90_% higher than in the ATA-induced sample. Limonene, terpinolene, beta Ocimene and alpha pinene were all at levels with differences statistically insignificant to sensory effects. Other trace level terpenes such were similarly found to be at equal amounts in both the control and ATA-induced sample. None of the remaining trace terpene species showed a difference in level greater than 40 %, again well below the sensory effect threshold.. Other possible varietal odor contributors such as the esters were also assayed. Of possible impact was ethyl butyrate was present at 30% higher in the control wine with all other esters showing no significant differences. However, this difference is below the Weber ratio that psychophysics predicts odor difference are detectable. Although the increase in TDN, a compound known to be produced by heat stress during low solar radiation, was also modulated less than the Weber ratio it showed an increase in every ATA comparison even in the 2002 experimental samples.

TDN could turn out to be an indicator of the ATA ageing process. Continued measurement of the samples developed in 2003 as they age should clarify this mystery at last.

With the exception of linalool, the results of this analysis in respect to the terpene content are at generally at odds with the results reported in 2002. This is almost certainly a result of the more controlled nature of the 2003 experiment where the grapes and wine were from the same vintage, vineyard etc. The difference in levels of terpenes reported last year thus may be the result of the two different vintages analyzed rather than an ATA effect. The new study provides evidence that with the possible exception of linalool and ethyl butyrate the varietal odor compounds in the ATA-induced wines have not been affected. However both ethyl butyrate and linalool are important odor components to the wines and reduced levels of these compounds may be having an impact on the varietal aroma of the wines.

## Summary and Conclusions

This experiment was the first to test the effect of defined vineyard treatments (irrigation and nitrogen fertilization) on development of ATA in finished wines and on vine physiology. Weather conditions were ideal for testing the hypothesis that drought-induced low N status and water deficits were related to the development of ATA sensory characteristics in wine. The first two years of the field experiment (2001 and 2002) had significant drought stress, as measured by vine water status and leaf photosynthesis levels. In both years, the stress occurred during the critical veraison and early fruit ripening phases. In 2002, water stress developed a month earlier, at the end of July. In both these years, drought stress led to sharply reduced photosynthesis levels (compared to irrigated vines) for four (2001) to six (2002) weeks during the warmest part of the growing season. As a result of this water stress, irrigated vines had larger berries (30-40% higher berry weight) with higher soluble solids (2 degrees higher brix in 2001; 4 degrees higher in 2002) than non-irrigated vines. Foliar nitrogen, applied around veraison, increased levels of yeast-available nitrogen (YAN), even in vines that were not irrigated. Irrigation also increased YAN, and vines with both foliar N applications and irrigation had the highest levels of YAN. Soil N application before veraison had no effect on vine N status or YAN. In 2001, irrigation had little effect on vegetative growth. However, in 2002, when irrigation started earlier, canopy growth continued, leading to larger vine size in the irrigated treatments.

The 2003 growing season had so much rainfall that no irrigation was applied, and no differences in water status or leaf photosynthesis were observed. However, there was a large difference in crop weight, with irrigated vines producing almost twice as much fruit as the non-irrigated treatments, and twice as many clusters per vine. We interpret this to be a carryover effect from the 2002 drought.

In the 2001 and 2002 wines, both the irrigation and the foliar N treatments resulted in readily apparent sensory differences. Trained sensory panels detected statistically significant differences in six of seven sensory characteristics, and irrigation or foliar N improved varietal character while reducing off-flavors associated with ATA. Sensory analysis of the 2002 and 2003 wines is ongoing.

Major progress was also made in characterizing the chemical nature of the ATA defect as it occurs in the Northeast and Finger Lakes. The ATA defect is not due to the loss or a deficiency of compounds that contribute to the fruity or varietal aroma of the wine. It very likely that ATA wines have less fruit character because of psychophysical suppression of varietal causing stimulants. Chemically, the ATA defect was determined not to be caused by O-aminoacetophenone, skatole or indole as has been speculated for UTA in Europe.

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**Practical Conclusions.** For wineries and vineyardists faced with ATA development, this research provides tools that may help them reduce or delay the ATA problem and economic losses associated with it. This study strongly supported the direct and indirect benefits of irrigation during the period around veraison. In drought years, irrigation resulted in riper, higher quality fruit, and delayed appearance of ATA symptoms in wine. Irrigation also provided protection from a loss in vine size and ½ of potential production during the 2003 growing season – a carryover effect of the 2002 drought. Foliar N in unirrigated plots, applied around veraison, increased yeast available nitrogen in harvested fruit, providing partial protection against ATA. Furthermore, N applied at veraison did not lead to increased vegetative vine growth, because much of the N absorbed appeared to be translocated to the fruit. Thus, foliar urea applications may be another management tool growers can use to minimize ATA. In addition, it may assist in producing musts in dry years that are less susceptible to stuck fermentations.

Characterization of the chemical components associated with ATA may allow development of rapid analytical methods to identify wines and juices prone to developing ATA. This will assist in identifying vineyard and winemaking practices that are associated with ATA. Wines produced by this experiment will be of continuing value in our analytical efforts to characterize the causes of ATA. Although no specific chemicals have yet been identified (it is still early in the ageing process) as the cause of ATA the formation of TDN as an indicator of the aging defect is compelling.

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