PROJECT TITLE : The Effects of Selected Vineyard Management Practices on the Nutritional Status of Grape Juice

INTERIM REPORT

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PROJECT SUBJECT AREA: Viticulture

Proposed Research Objectives:

A multi-campus interdisciplinary team of researchers is in the process of conducting a broad study relating grape juice nutritional (nitrogen) status, as determined by several analytical methodologies, to vineyard practices and wine quality. This research continues efforts to develop and optimize the Formol method for determining assimilable nitrogen levels and funded studies building a database of grape nitrogen levels related to vineyard location and management practices.

The objectives of this proposed research were to –

- Evaluate the relationship between vineyard management and total assimilable nitrogen levels in grapes
- Evaluate the relationship between vineyard management and proline/arginine ratios
- Evaluate the relationship between proline/arginine ratios and the results from various analytical methodologies (especially the Formol titration)
- Evaluate the relationship between total nitrogen levels and the ratio of fusel oils to esters
- Evaluate the relationship between total nitrogen levels and wine quality

Experimental Procedures to Accomplish Objectives:

Experimental procedures involve the following –

- Characterize juice samples using Formol method
- Evaluate spectrophotometric methods on juice samples
- Optimize HPLC analytical method and evaluate juice samples
- Select cooperative vineyards
- Assess canopy microclimate
- Evaluate yield components at harvest
- Conduct small lot fermentations and evaluate fermentation volatiles (esters and fusel oils)

Current Status of Research and Results:

- The research team has established relationships with seven vineyards, Virginia, as well as the central coast, central and southern San Joaquin Valley and the Lodi area of California, with respect to vineyard trials, sampling and small lot wine production as described in the proposal (Experimental Procedures).
- A total of 528 berry samples (excluding duplicates) corresponding to crop load and canopy orientation and multiple sample periods from veraison to harvest have been collected and analyzed by Formol, OPA and ArOPA procedures for FAN and NH_3. Comparison of the analytical methods by crop load level and canopy orientation indicated no statistically significant differences (T-test, $p<0.05$) between high and low crop load levels for that season. There were significant

differences in OPA amino acids and OPA arginine values between east and west sides of the canopy. Higher levels were observed on the west side. Initial results are presented in Appendices A and B.
A preliminary comparison of Formol values with summed OPA and ammonia from 1999 Virginia Cabernet Sauvignon samples indicated that the Formol method produced significantly higher values for assimilable nitrogen than did OPA + ammonia for the same sample ($p < 0.05$).

- Initially, two methods were evaluated for expression of juice from collected fruit . A comparison of blender and stomacher processing methods was performed to evaluate possible effects of seed breakage or analyte stratification on analytical results. Early season and post-harvest samples of 1999 Virginia Cabernet Sauvignon fruit were tested. No significant differences ($p < 0.05$) in Formol values were observed. For the OPA measurements, no significant differences were observed in the early season sample set, but the stomacher method was found to produce higher OPA nitrogen values for the late season samples. Tables 1 and 2 in Appendix C compare OPA results with those of Formol.
- In conjunction with Formol titrations, spectrometric (OPA) analyses for general total amounts of the alpha amino acids, arginine specific spectrometric (ARGOPA) analyses, and ammonia, we are attempting to also conduct HPLC analyses for all the amino acids present. The purpose for the latter analyses is to allow us to look at proline/arginine ratios, and specific amounts of cysteine, methionine, and other specific amino acids, as a function of vineyard or winemaking study.
- Currently we are investigating the use of phenylisothiocyanate, dimethylamino-azobenzene sulfonyl, and dimethylaminoazobenzene thiohydantoin amino acid derivatizations to determine which of these procedures will work best with our juice and wine samples. A Hewlett-Packard model 1050 HPLC with diode array detection is being used in this work. To date the instrument operational conditions have been established for the derivatives being run and chromatograms of some of the derivatized amino acids run.
- Analysis of fermentation volatiles (fusel alcohols and esters) has begun but, at present, we have insufficient analytical data to report.
- Analyses of remaining samples is continuing. Upon completion, selected samples will be exchanged between laboratories as an estimate of reproducibility.

Appendix A. Data for 1999 Cabernet Sauvignon Crop Load Versus FAN, Ammonia and Arginine Levels

Table 1. Formol Titration Versus Vineyard Treatments

	Formol	(mg/L N)			
Sample	8/30/99	10/4/99	10/11/99	10/18/99	10/27/99
LE1		203	202	197.0	
LE2	180	225	221	215.0	210
LE3		195	199	193.0	
LE4	186	205	202	195.0	191
LE5		199	212	199.0	
LE6	193	205	199	206.0	193
LW1		191	195	191.0	
LW2	191	218	212	226.0	207
LW3		212	235	213.0	
LW4	205	212	213	215.0	190
LW5		214	201	211.0	
LW6	197	212	224	213.0	180
HE1		210	221	222.0	
HE2	175	248	221	235.0	225
HE3		205	201	201.0	
HE4	161	203	182	184.0	167
HE5		210	217	195.0	
HE6	186	246	233	242.0	214
HW1		212	217	213.0	
HW2	188	220	222	215.0	221
HW3		201	212	202.0	
HW4	190	218	199	202.0	214
HW5		206	201	221.0	
HW6	184	233	233	235.0	171

L = Low Crop Load; H = High Crop Load; E = East Side Canopy; W = West Side Canopy

Table 2. Spectrophotometric OPA Versus Vineyard Treatments

	OPA	(mg/L N)			
Sample	8/30/99	10/4/99	10/11/99	10/18/99	10/27/99
LE1		130	157	138	
LE2	124	153	160	164	176
LE3		128	146	141	
LE4	114	133	137	140	129
LE5		133	146	154	
LE6	123	155	143	152	131
LW1		137	148	148	
LW2	122	149	165	184	103
LW3		150	152	163	
LW4	137	146	155	172	123
LW5		151	151	162	
LW6	135	152	165	171	162
HE1		136	157	163	
HE2	119	172	165	178	138
HE3		124	149	150	
HE4	102	143	139	132	146
HE5		142	160	146	
HE6	114	165	199	197	140

HW1		137	162	165	
HW2	115	167	171	176	195
HW3		144	170	167	
HW4	117	158	150	161	130
HW5		146	153	168	
HW6	114	168	192	190	139

Table 3. Enzymatic Ammonia Levels Versus Vineyard Treatments

			Ammonia (mg/L)		
Sample	8/30/99	10/4/99	10/11/99	10/18/99	10/27/99
LE1		13.2	22.4	18.6	
LE2	22.5	13.5	22.3	22.5	37.2
LE3		13.5	22.2	22.8	
LE4	22.4	13.6	22.4	20.7	41.2
LE5		13.6	22.4	21.5	
LE6	22.4	12.9	22.3	21.8	40.9
LW1		13.8	22.1	19.0	
LW2	21.6	14.2	22.2	23.7	40.9
LW3		14.1	22.2	23.7	
LW4	22.1	13.7	22.3	23.3	40.8
LW5		14.3	22.2	22.4	
LW6	22.4	13.8	21.7	22.5	41.4
HE1		21.8	22.6	21.7	
HE2	22.2	21.9	not det	21.7	39.7
HE3		22.1	22.6	22.1	
HE4	22.3	22.1	22.6	22.1	42.6
HE5		22.0	22.5	21.7	
HE6	22.0	21.8	22.5	21.7	39.6
HW1		22.2	22.4	22.2	
HW2	22.1	22.1	22.1	22.2	42.4
HW3		22.2	22.3	22.2	
HW4	22.2	19.7	23.0	22.3	42.3
HW5		22.2	22.1	22.2	
HW6	22.2	22.2	22.2	18.6	42.8

Table 4. Arginine Levels Versus Vineyard Treatments

		Arginine	(mg/L)		
Sample	8/30/99	10/4/99	10/11/99	10/18/99	10/27/99
LE1		204	404	272	
LE2	271	348	487	398	563
LE3		233	385	342	
LE4	269	264	370	302	598
LE5		281	402	327	
LE6	278	257	398	370	436
LW1		264	402	312	
LW2	301	368	506	494	458
LW3		356	457	441	
LW4	348	351	468	374	317
LW5		344	410	419	
LW6	322	361	502	505	475
HE1		177	456	394	
HE2	224	469	479	470	436
HE3		279	376	370	
HE4	146	257	347	283	431
HE5		206	399	304	
HE6	261	527	618	611	411

HW1		336	443	413	
HW2	269	380	416	488	396
HW3		370	528	483	
HW4	312	459	422	451	290
HW5		363	366	428	
HW6	291	447	608	560	356

Table 5. Averages for All Analytical Methods X Treatments

Treatment	Side	Date	Formol	OPA	NH3	OPA+NH3	Arginine
Low	East	8/30/99	186	121	22.4	139	273
Low	West	8/30/99	198	131	22.0	150	324
High	East	8/30/99	174	112	22.2	130	210
High	West	8/30/99	187	115	22.1	133	291
Low	East	10/4/99	205	139	13.4	150	264
Low	West	10/4/99	210	148	14.0	159	340
High	East	10/4/99	220	147	21.9	165	319
High	West	10/4/99	215	153	21.8	171	393
Low	East	10/11/99	206	148	22.3	167	408
Low	West	10/11/99	213	156	22.1	174	457
High	East	10/11/99	213	161	22.6	177	446
High	West	10/11/99	214	166	22.4	185	464
Low	East	10/18/99	201	148	21.3	166	335
Low	West	10/18/99	212	167	22.4	185	424
High	East	10/18/99	213	161	21.8	179	405
High	West	10/18/99	215	171	21.6	189	470
Low	East	10/27/99	198	145	39.8	178	532
Low	West	10/27/99	192	129	41.0	163	417
High	East	10/27/99	202	141	40.7	175	426
High	West	10/27/99	202	155	42.5	190	347

Appendix B. Data for 1999 Chardonnay Crop Load Versus FAN, Ammonia and Arginine Levels

Table 1. Summary of Analytical Methods for All Crop-Level Treatments

Treatment	Side	Rep	lbs Yield	Date	mg/L N Formol N	mg/L N OPA N	Mg/L NH3	mg/L OPA+NH3	mg/L Arginine
Low	East	1	0	9/23/99		174	18.7	192	559
Low	East	3	0	9/23/99		189	19.0	208	633
Low	East	5	0	9/23/99	273	195	18.9	214	647
Low	West	1	0	9/23/99	224	164	19.0	183	487
Low	West	3	0	9/23/99	262	192	19.0	211	642
Low	West	5	0	9/23/99	268	192	10.8	203	649
High	East	1	0	9/23/99		188	18.6	206	568
High	East	3	0	9/23/99	235	153	19.4	172	494
High	East	5	0	9/23/99	275	175	19.2	194	587
High	West	1	0	9/23/99	244	179	19.0	198	525
High	West	3	0	9/23/99	228	159	18.9	178	497
High	West	5	0	9/23/99	259	197	19.0	216	561

Appendix C. Comparison of Blender and Stomacher Processing for Cabernet Sauvignon Grape Juice Samples.

Table 1. OPA Amino acid values (mg/L)

Sample	Blender	Stomacher	Difference
HW1,2	176	177	-1
HW3,4	129	172	-43
HW5,6	131	181	-50
HE1,2	103	175	-72
HE3,4	123	156	-33
HE5,6	162	167	-6
LW1,2	138	166	-28
LW3,4	146	147	-1
LW5,6	140	160	-20
LE1,2	195	164	31
LE3,4	130	145	-15
LE5,6	139	161	-21

Table 2. Formol Titration (mg/L)

Sample	Blender	Stomacher	Difference
HW1,2	221	212	9
HW3,4	214	205	9
HW5,6	171	210	-39
HE1,2	225	223	2
HE3,4	167	191	-24
HE5,6	214	205	9
LW1,2	207	195	12
LW3,4	190	178	12
LW5,6	180	186	-6
LE1,2	210	205	5
LE3,4	191	182	9
LE5,6	193	205	-12