Manipulating Soil Moisture and Nitrogen Availability Part II: Effects on Pinot noir Must and Wine Composition

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INTRODUCTION

The objectives of this study are to evaluate the effects of manipulating soil moisture and nitrogen availability using different approaches, including supplemental irrigation, nitrogen addition to the soil or to the leaves, and the elimination of competition for water and nutrients between the ground cover and the grapevines by tilling. The effects of these viticultural practices on juice and must composition, fermentable nitrogen content, fermentation behavior, wine composition, aroma, flavor, and wine quality are being conducted over several vintages at a mature commercial Pinot noir vineyard in the southern Willamette Valley of Oregon.

Drought conditions or conditions of low soil nitrogen availability may affect fruit maturation and grape and wine composition. Fruit maturity affects soluble solids content, acidity, pH, and aroma and flavor development. Anthocyanin and phenolic content of the skins are known to be affected by viticulture practices, environmental factors, and grape maturation (Mullins 1992, Price, 1994 and 1995, Reynolds, 1994). The composition of phenols in seeds is also known to change significantly with ripening (Kennedy, 2000). Fermentable nitrogen levels in the fruit may also be affected by viticulture practices and environmental factors.

If assimilable nitrogen levels at harvest are too low, fermentations may be slow and may 'stick', producing wines with undesirable levels of residual sugar (Ingledew and Kunkee, 1985). Problem fermentations are also sometimes accompanied by production of hydrogen sulfide and other 'reduced' sulfur odors (Kunkee 1991; Jiranek, Langridge, and Henshcke 1995). Other undesirable flavors have been described in wines produced under drought and stressed conditions, including those related to atypical aging (UTA) syndrome in white wines (Sponholz, 2000). Winemakers often add supplements to juice and fermenting wines to balance perceived nutritional deficiencies (Montiero and Bisson, 1992; Bisson, 1999). It is also thought that variations in climate, soil type, and cultivation, soil moisture, and fertilizer practices may have an impact of juice and must composition and nutrition (Butzke, 1998; Ingledew, 1985).

MATERIALS AND METHODS

Vineyard Experimental Design

This trial is being conducted at a mature commercial Pinot noir vineyard in the southern Willamette Valley of Oregon in collaboration with the OSU Department of Horticulture. The experimental design of this study is a factorial of irrigation, nitrogen soil

and foliar applications, and soil cultivation in a randomized block design. (see ***Manipulating Soil Moisture and Nitrogen Availability Part 1: Vine physiological performance, yield components, ripening dynamics, and fruit compostion*** , Jessica Howe and Carmo Vasconcelos). Water was applied using drip irrigation during lag phase at a rate of 0.5gal/hr for 200 hours. Nitrogen treatments included an unfertilized control, 35 lbs N/acre supplied to the soil in the spring, and 2.66 lbs N/acre supplied foliarly during veraison. Tilling was done in early spring to encourage nitrogen utilization and reduce nutrient and water competition. Each of 12 treatments was replicated five times in groups of eleven vines each. Treatments included Zero Nitrogen (0N), Foliar Nitrogen (FN), Soil Nitrogen (SN) applications with and without Irrigation (Irr and Dry) and with and without Tilling of alternate rows (Till and NoTill).

Yeast assimilable nitrogen content

Cluster samples taken at two-week intervals from veraison to harvest were crushed, pressed and the juice analyzed for the yeast fermentable nitrogen content. Berry weights were determined from the weight of 100 berries from each sample. Ammonia content was determined using a Sigma enzymatic diagnostic kit and the alpha amino acid content was determined using the NOPA spectrophotometric assay (Dukes and Butzke, 1998) with isoleucine (ile) as the standard. The yeast assimilable nitrogen content (YANC) is expressed as mg (N)/L as the sum of the assimilable nitrogen from ammonia plus the assimilable nitrogen from alpha amino acids.

Wine production

In 1999, 2000, and 2001 Pinot noir grapes were harvested from Benton Lane Vineyards from 3 of 5 field replications of the 12 treatments for a total of 36 lots for each vintage. The grapes were crushed, destemmed, and 50 mg/L sulfur dioxide was added. Musts were inoculated with 1 g/L of Lalvin RC 212 Bourgorouge yeast and the fermenting wines were punched down twice daily. Fermentation temperatures reached a maximum of 32 ^{0}C for 48 hours and the wines were pressed from the skins at dryness after seven days. Fermentation rates were monitored by hydrometer readings taken at crushing and every 24 hours until pressing. The new wines were settled, racked from the primary yeast lees, and inoculated with 0.025g/gallon with OSU 1-Step (Lalvin) freeze-dried malolactic bacteria. After completion of malolactic fermentation the wines were cold stabilized at 4 ^{0}C, racked and bottled with the addition of 25 mg/L of sulfur dioxide.

Must and wine analysis

Must samples were taken after processing before the onset of fermentation and were analyzed for degrees Brix, titratable acidity (TA), pH, and malic acid content. New wines were analyzed for per cent alcohol content, TA, pH, per cent residual fermentable sugar (RS), volatile acidity (VA), color intensity (CI), and for total anthocyanin and phenolic content. Color intensity was determined as the absorbance at 520 + 420 nm at wine pH (1mm cuvette with no dilution). Total anthocyanin content was measured by the absorbance at 520 nm at a pH<1 using a 1% extinction coefficient of 380 (Singleton, 1982). Total phenolic content was measured by the Folin ciocalteau spectrophotometric assay and expressed as gallic acid equivalents (GAE) (Ough and Amerine, 1988). Sulfides analysis of selected wines from each of the three vintages was done at ETS Laboratories, ST. Helena, CA by GC/SCD headspace analysis and included hydrogen sulfide, ethyl and methyl mercaptan, and ethyl and methyl polymercaptan (diethyl and dimethyl sulfide).

RESULTS AND DISCUSSION

Slow and stuck fermentations are common in Oregon and winemakers often report fermentation problems with fruit from specific vineyard blocks over several vintages. An approximation of the total yeast fermentable nitrogen content in juice or must is taken as the sum of the nitrogen available from ammonia and the alpha-amino acids present (Bisson 1991; Dukes and Butzke 1998; Jiranek, Langridge, and Henshcke 1995). Recommended levels of fermentable nitrogen needed by yeast for 'healthy' fermentations are reported to vary from as low as 140 mg (N)/L to as high as 500 mg (N)/L or more (Butzke, 1998; Spayd, 1998).

An earlier study of petiole analysis of Oregon vineyards indicated that a high percentage of the vines were consistently deficient in nitrogen, based upon bloom-time (California) and veraison standards (Oregon). Commercial Oregon juice/must samples (207) were also taken at harvest and analyzed for fermentable nitrogen content. The yeast assimilable nitrogen content (YANC) ranged from as low as 38 to as high as 500 mg (N)/L. A significant percentage of Oregon juice and must samples were found to be lower than the minimal recommended level of 140 mg(N)/L and both varietal differences and vintage differences were observed. The percentage of Chardonnay juice samples with less than 140 mg (N)/L was 80% in 1997, 79% in 1998, and 17% in 1999. The percentage of Pinot noir must samples with less than 140 mg (N)/L was 37% in 1997, 34% in 1998, and 8.6% in 1999 (Watson, Hellman, Specht, and Chen, 2000).

Yeast assimilable nitrogen content and fermentation behavior of Pinot noir

The changes in juice ammonia and juice alpha amino acid content during ripening in the Pinot noir trial at Benton Lane Vineyard in 2001 are shown is Figures 1and 2. The juice ammonia content decreased in all treatments. Ammonia levels were significantly greater in Till compared to NoTill treatments during ripening but not at harvest. Juice alpha amino acid content increased in all treatments. Levels were significantly greater for Dry compared to Irr treatments and for Till compared to NoTill treatments during ripening and at harvest. Levels for SN and FN were significantly greater during ripening but not at harvest. The YANC taken as the sum of the N from ammonia and the N from alpha amino acids is shown in Figure 3. YANC of Dry treatments was significantly higher than Irr during ripening but not at harvest. YANC was significantly higher during ripening and at harvest for Till compared to NoTill treatments and the YANC of SN and FN treatments tended to be greater during ripening and at harvest than 0N treatments though not significantly. The same pattern of decreasing juice ammonia and increasing juice alpha amino acid content was also observed in the 1999 and the 2000 vintages. The net effect in all three vintages was an increase in YANC during the later stages of grape ripening. This suggests that wine grape maturity is an important parameter with respect the YANC in the juice/must at harvest.

The YANC of the 12 vineyard treatments in 2001 is shown in Table 1 as the average of the three field replications harvested for wine production. The YANC ranged from an average of 54 to 142 mg(N)/L averaging 93 mg(N)/L. On average 71% of the YANC at harvest was from alpha amino acids and 29% from ammonia. The largest treatment differences were between Till and NoTill treatments. The Till treatments averaged 51% higher YANC at harvest than No Till treatments averaging 112 and 74 mg(N)/L, respectively. The only exception was the Irr NT SN compared to the Irr Till SN treatments which had similar YANC at harvest. The fermentation rates for the Till and the NoTill

treatments is shown in Figure 4. The data represents the mean of the Brix readings taken daily for 18 fermenting lots of wines from Till treatments and 18 lots of wines from NoTill treatments. The wines were fermented on the skins and pressed after 7 days. On average, the Till treatments fermented faster than the NoTill treatments. In 2000 the YANC of the Till treatments was also greater than the NoTill treatments at harvest averaging 136 and 98 mg(N)/L, respectively. All the NoTill treatments averaged less than 140mg(N)/L and the wines from the Till treatments tended to ferment more rapidly than the wines from the NoTill treatments (Watson, 2000). By contrast in 1999, the first year of the trial, the YANC at harvest was similar for both Till and NoTill treatments averaging 187 and 183 mg(N)/L, respectively, and no differences were observed in the fermentation rates.

Tilling of alternate rows suppreses competitive ground cover and appears to allow vines to take up greater amounts of nitrogen from the soil than in the non tilled treatments. This effect was particularly noticeable in 2000 and 2001 the second and third years of this trial. These two vintages also had considerably lower YANC at harvest than in 1999. In 1999 the YANC at harvest averaged 185mg(N)/L compared to 117 mg(N)/L in 2000 and 93 mg(N)/L in 2001. Overall, the YANC in 2001 averaged 21% less than in 2000 and 50% less than in 1999 indicating that there is also a strong vintage effect on the levels of fermentable nitrogen in juice and must at harvest.

Wines from the Dry NoTill 0N, Irr NoTill 0N, Dry Till 0N, and Irr Till 0N treatments from all three vintages were selected for analysis for sulfides profiles based upon the relatively low YANC in the NoTill treatments compared to the Till treatments at harvest. A total of 12 samples were analyzed by ETS Laboratories in St. Helena, CA. Hydrogen sulfide, ethyl mercaptan, methyl mercaptan, and ethyl polymercaptan (diethyl sulfide) were at or below detectable limits of about 0.5 ng/ml (5ppb) and below reported sensory threshold levels for all samples. Methyl polymercaptan (dimethyl sulfide) levels ranged from 7.1 to 19.3 ng/ml and averaged 13.6 ng/ml. The NoTill treatments and the Till treatments had comparable levels of methyl polymercaptan, averaging 12 and 15 ng/ml, respectively. Reported sensory threshold levels for dimethyl sulfide range from 17-25 ng/ml. Sensory descriptors for dimethyl sulfide include canned corn, truffels, "sulfidy", and vegetal. Sub threshold and low threshold levels may contribute to musty, mushroom, and vegetal odors in some samples in these trials.

Must and Wine Analysis

Few differences were observed in must analysis in 2001 (Table 2). SN treatments tended to have lower TA, higher pH, and lower malate than FN and 0N, similar to the results obtained in 1999 and 2000. Till treatments had higher Brix at harvest in 1999 and 2000 but not in 2001. New wine analysis for the 2001 wines is shown in Tables 3, 4, and 5. The SN treatments tended to have lower TA and pH in new wines than FN and 0N treatments. Dry treatment wines tended to have higher anthocyanin content than Irr treatments (averaging 219 and 199 mg/L, respectively) and greater color intensity (averaging 5.73 and 4.97, respectively), similar to the results obtained in 1999 and 2000. Wines from No Till treatments in 2001 had greater color intensity (but similar anthocyanin content) and higher total phenolic content than wines from the Till treatments. In 1999 and 2000 no differences were observed in color intensity between Till and NoTill treatments (Helms, 2000; Watson, 2000). In all three vintages the wines produced from the Dry Till SN treatments had high anthocyanin content, high color intensity, high total phenolic content, and the low average berry weights.

Wines with higher color intensity tended to have higher polymeric anthocyanin content and polymeric phenolic content as measured by high performance liquid chromatography (Watson, 2000).

Overall, differences in juice composition including fermentable nitrogen content at harvest and differences in wine composition including anthocyanin content, color intensity, and phenolic content have been observed in these trials. Manipulating soil moisture and nitrogen availability can significantly effect grape and wine composition and wine quality.

Sensory evaluation

The 1999 and 2000 Pinot noir wines (36 wines consisting of 3 replications each from 12 field treatments for each vintage) underwent sensory evaluation for differences in color, aroma and flavor in the Sensory Sciences Laboratory of the Department of Food Science and Technology (see ***Manipulating soil moisture and nitrogen availability. Part III. Effects on wine color, aroma and flavor***, Heather Hjorth and Mina McDaniel). The 2001 wines are currently finishing malolactic fermentation and will undergo sensory evaluation during the summer of 2002.

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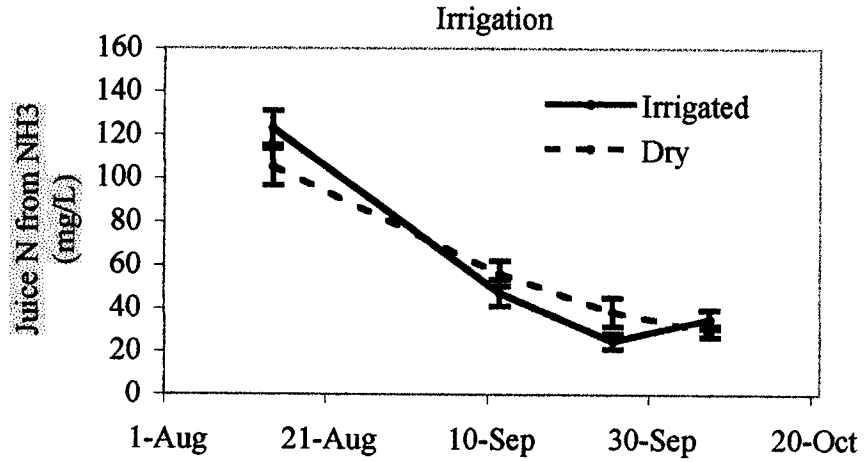
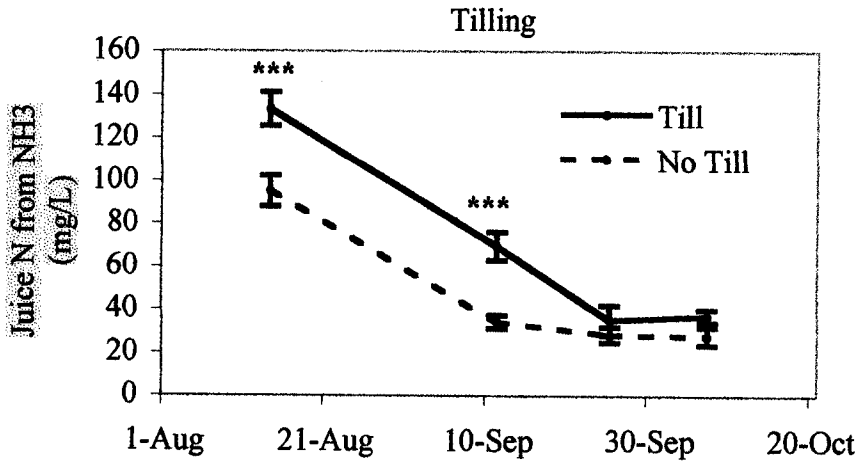
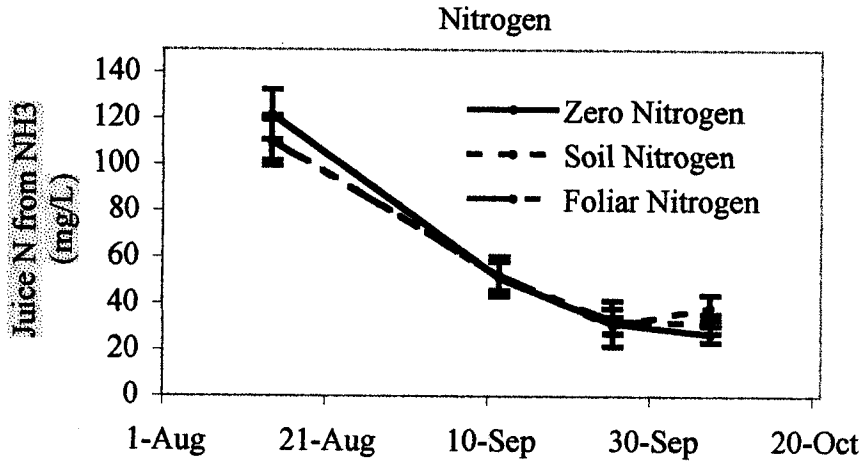
Figure 1. 2001 Juice Nitrogen from Ammonia during Ripening and Harvest

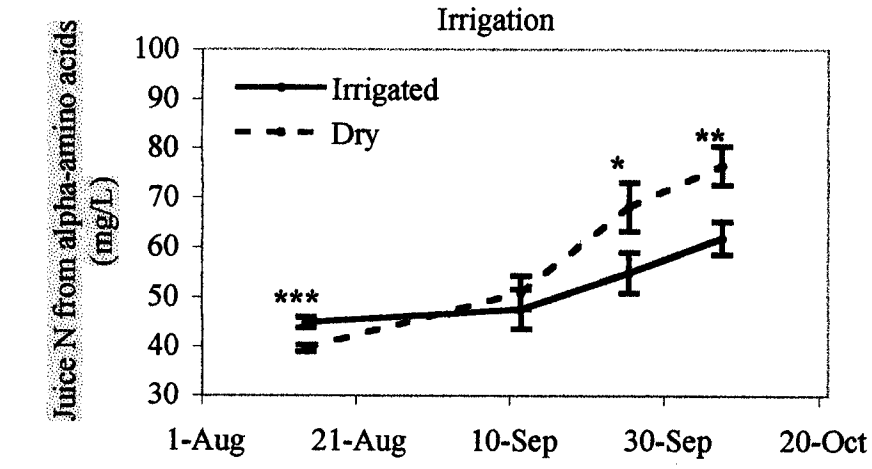


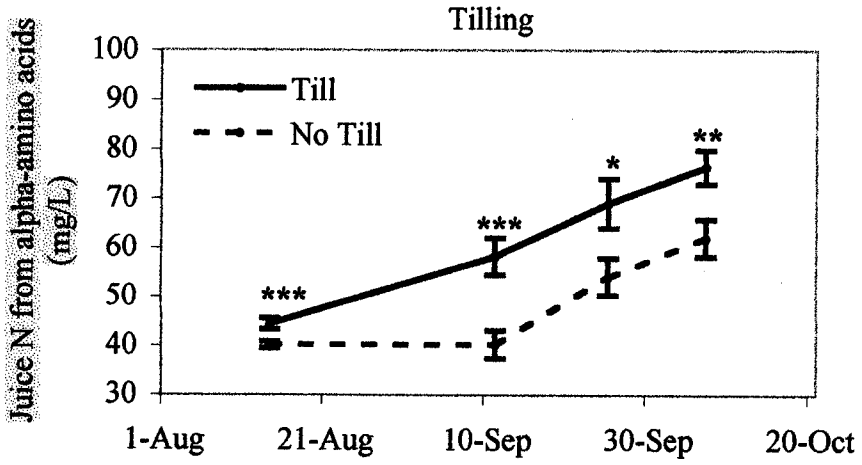


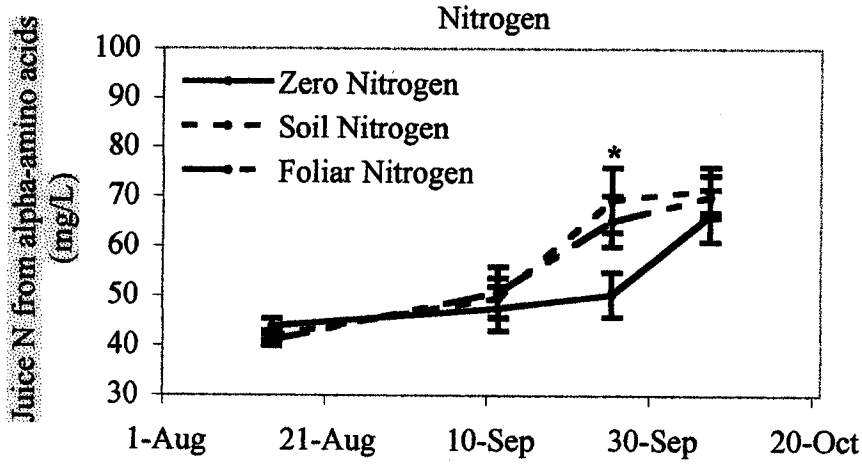


*, **, and *** indicate statistical significance at the 0.05, 0.01, and 0.001 levels of probability, respectively

Figure 2. 2001 Juice Nitrogen from Alpha Amino Acids during Ripening and Harvest

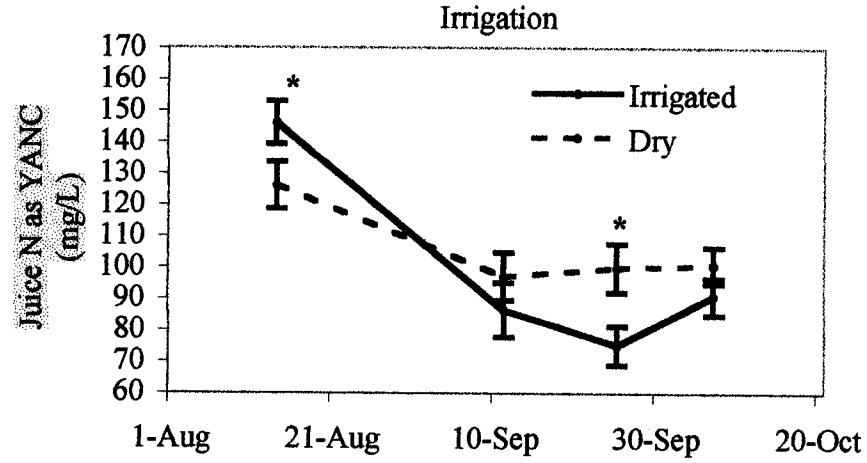


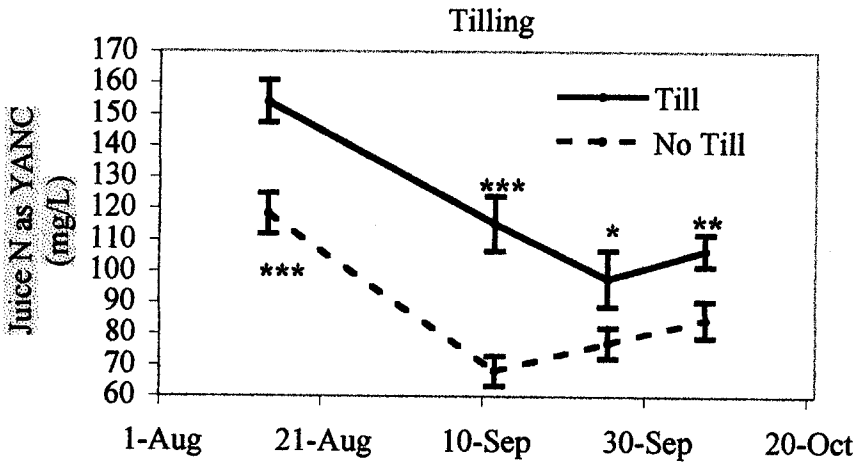


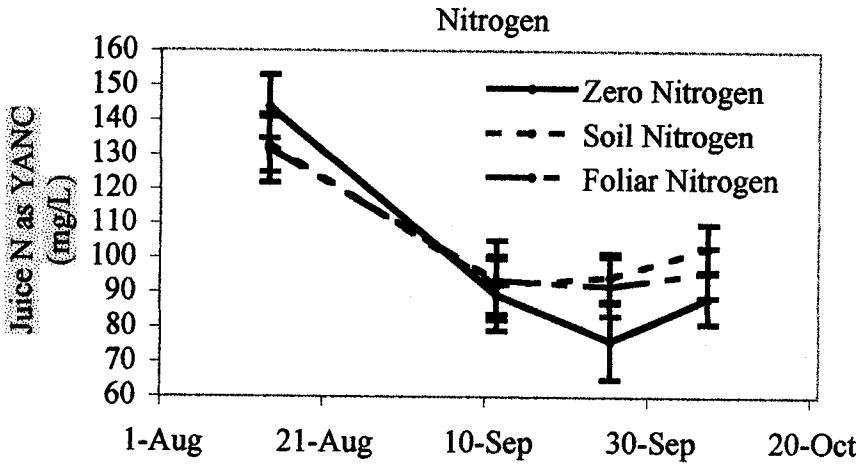


*, **, and *** indicate statistical significance at the 0.05, 0.01, and 0.001 levels of probability, respectively

Figure 3. 2001 Juice YANC from Ammonia and alpha Amino Acids during Ripening and Harvest







*,**,and*** indicate statistical significance at the 0.05,0.01,and 0.001 levels of probability, respectively

Table 1. 2001 Pinot noir Yeast Assimilable Nitrogen at Harvest

Treatment	Berry wt.	NH3	NH3	NOPA(ile)	YANC
	grams	mg/L	mg N/L	mg N/L	mg N/L
Dry NT 0N	1.34	12	10	54	64
Dry Till 0N	1.27	44	36	106	142
Dry NT FN	1.19	21	17	59	76
Dry Till FN	1.29	34	28	82	110
Dry NT SN	1.33	18	15	56	71
Dry Till SN	1.01	38	31	91	122
Irr NT 0N	1.26	16	13	41	54
Irr Till 0N	1.30	35	29	55	84
Irr NT FN	1.3	19	16	51	67
Irr Till FN	1.32	47	39	64	103
Irr NT SN	1.14	70	58	56	114
Irr Till SN	1.18	39	32	79	111
Average	1.24	33	27	66	93

Figure 4. 2001 Pinot noir Fermentation Rates, Till vs No Till Treatments

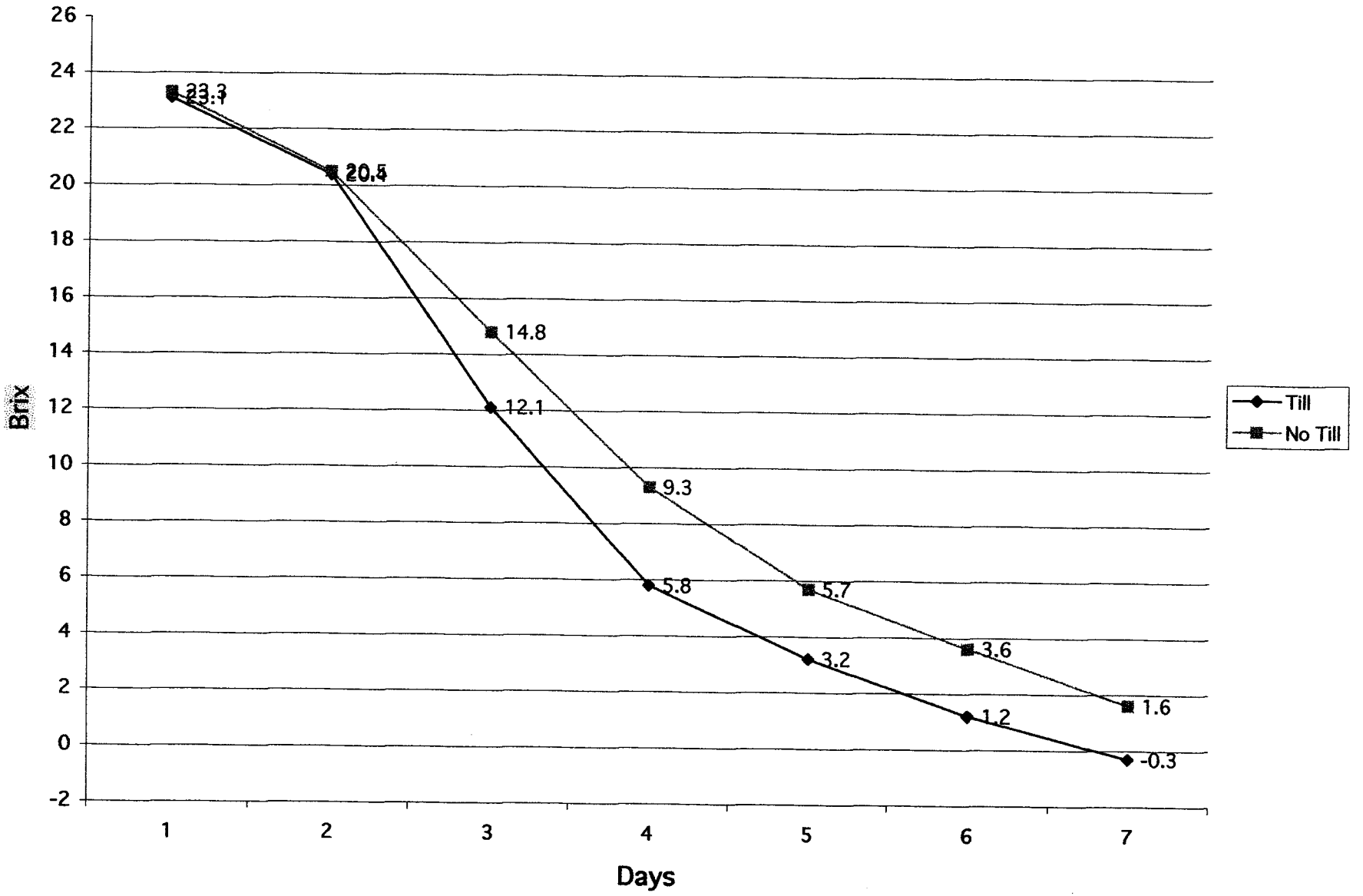


Table 2. 2001 BL Pinot noir Must Analysis at Harvest

		Brix g/100g	TA g/L	pH	Malate g/L
Irrigation					
	Irrigated	22.6	8.1	3.21	3.7
	Dry	23.0	8.2	3.22	3.6
Nitrogen					
	Zero Nitrogen	22.4	8.2	3.14	3.8
	Foliar Nitrogen	23.0	8.3	3.21	3.8
	Soil Nitrogen	22.9	7.9	3.30	3.4
Cultivation					
	Till	22.8	8.2	3.25	3.7
	No Till	22.8	8.1	3.19	3.6

Table 3. 2001 BL Pinot noir Wine Analysis

		Alcohol %	TA g/L *	pH*	Residual sugar %
Irrigation					
	Irrigated	13.8	7.7	3.60	0.11
	Dry	14.0	7.6	3.56	0.10
Nitrogen					
	Zero Nitrogen	13.7	8.1	3.53	0.10
	Foliar Nitrogen	13.0	7.6	3.58	0.12
	Soil Nitrogen	14.2	7.1	3.63	0.13
Cultivation					
	Till	14.0	7.6	3.61	0.14
	No Till	13.9	7.7	3.55	0.09

* pre malolactic fermentation

Table 4. 2001 BL Pinot noir Wine Analysis

		Anthocyanin mg/L	CI*	Hue**	Phenols mg/L
Irrigation					
	Irrigated	199	4.97	0.733	1865.0
	Dry	219	5.73	0.711	1826.0
Nitrogen					
	Zero Nitrogen	202	5.38	0.700	1823.0
	Foliar Nitrogen	204	5.28	0.700	1828.0
	Soil Nitrogen	221	5.39	0.748	1887.0
Cultivation					
	Till	208	5.08	0.700	1781.0
	No Till	210	5.63	0.700	1908.0

* Color intensity, 520+420nm
** 420/520nm

Table 5. 2001 BL Pinot noir Color and Phenols

Treatment	Berry wt.	Anthocyanin	Color Intensity	Hue	Total Phenols
	grams	mg/L	520+420nm	420/520nm	mg /L
Dry NT 0N	1.34	211	5.87	0.702	1807
Dry Till 0N	1.27	201	4.93	0.703	1600
Dry NT FN	1.19	215	6.24	0.70	1888
Dry Till FN	1.29	205	5.11	0.714	1693
Dry NT SN	1.33	229	5.96	0.72	1845
Dry Till SN	1.01	251	6.27	0.723	2012
Irr NT 0N	1.26	207	5.91	0.678	2110
Irr Till 0N	1.30	188	4.8	0.717	1662
Irr NT FN	1.3	192	4.87	0.717	1844
Irr Till FN	1.32	204	4.92	0.737	1887
Irr NT SN	1.14	207	4.92	0.748	1857
Irr Till SN	1.18	198	4.42	0.802	1833