

Influence of Viticultural Practices on Juice Yeast Assimilable Nitrogen

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Outline

- Yeast assimilable nitrogen (YAN)
 - Importance of YAN
 - Fermentation success
 - Aroma/Flavor
 - Managing YAN in the vineyard
 - General thoughts
 - Specific research

Importance of YAN

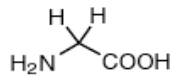
- Successful fermentations rely on adequate must nitrogen availability
 - Free amino nitrogen Proline is not assimilable
 - 150 mg/L considered to be accepted minimum
 - Maximum?

Role of Nitrogen for Yeast

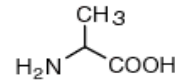
- Yeasts use nitrogen to form:

- Amino Acids
- Polypeptides
- Proteins

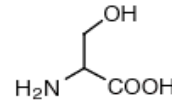
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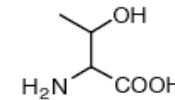
Glycine (Gly, G)
MW: 57.05



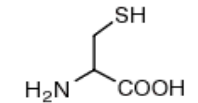
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MW: 71.09



Serine (Ser, S)
MW: 87.08, pK_a ~ 16

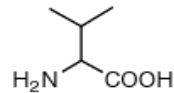


Threonine (Thr, T)
MW: 101.11, pK_a ~ 16

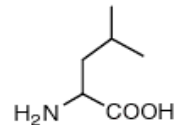


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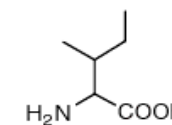
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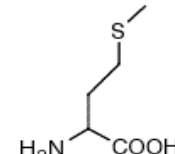
Valine (Val, V)
MW: 99.14



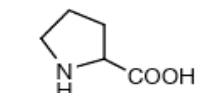
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MW: 113.16



Isoleucine (Ile, I)
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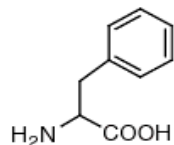


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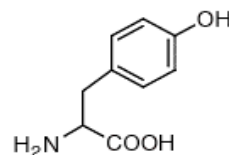


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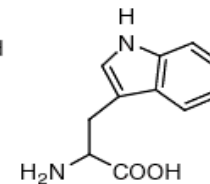
Aromatic



Phenylalanine (Phe, F)
MW: 147.18

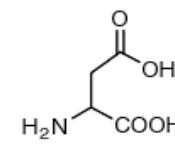


Tyrosine (Tyr, Y)
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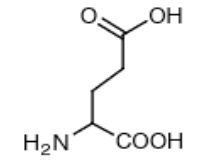


Tryptophan (Trp, W)
MW: 186.21

Acidic

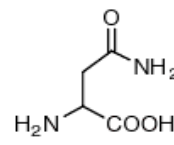


Aspartic Acid (Asp, D)
MW: 115.09, pK_a = 3.9

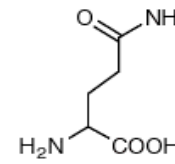


Glutamic Acid (Glu, E)
MW: 129.12, pK_a = 4.07

Amide

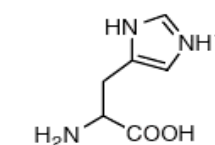


Asparagine (Asn, N)
MW: 114.11

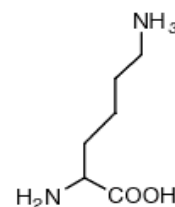


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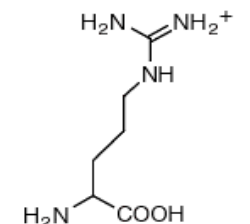
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Histidine (His, H)
MW: 137.14, pK_a = 6.04



Lysine (Lys, K)
MW: 128.17, pK_a = 10.79

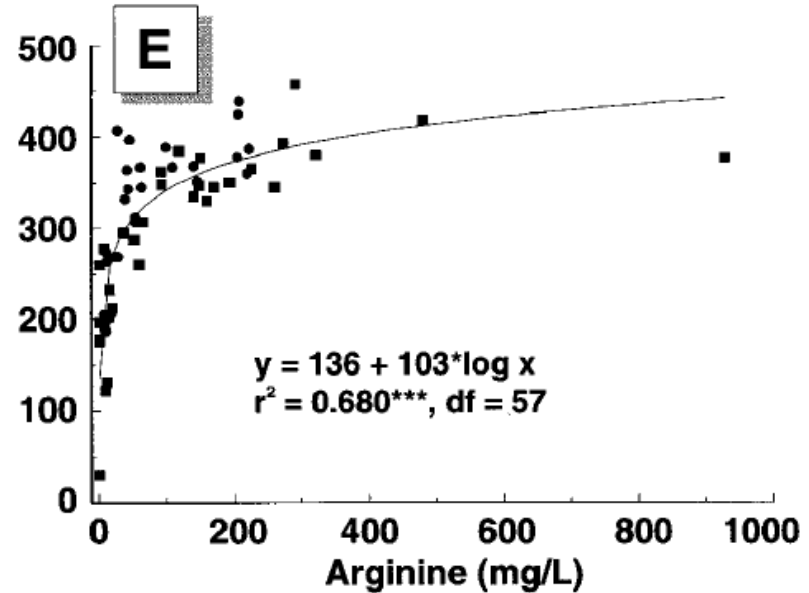
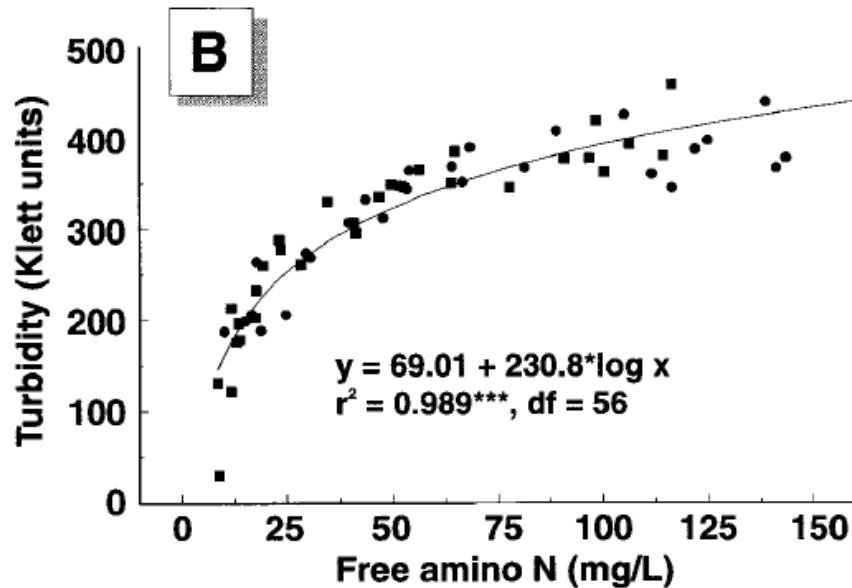
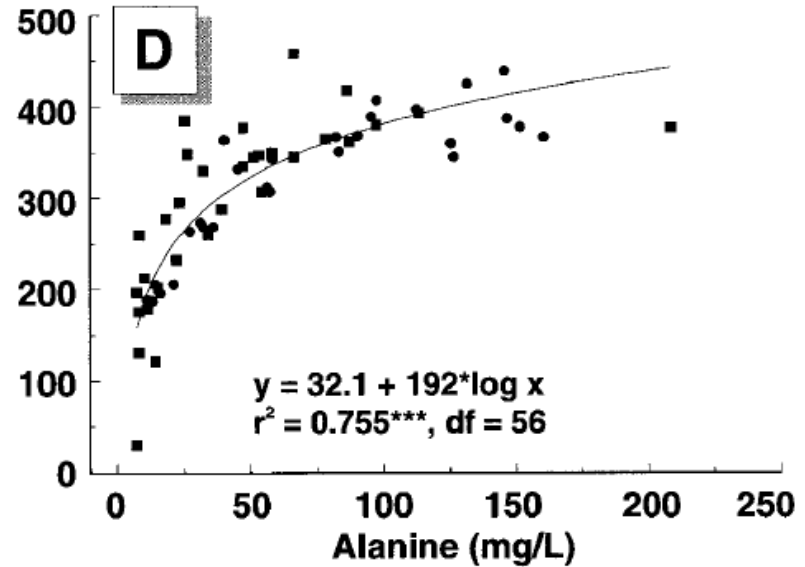
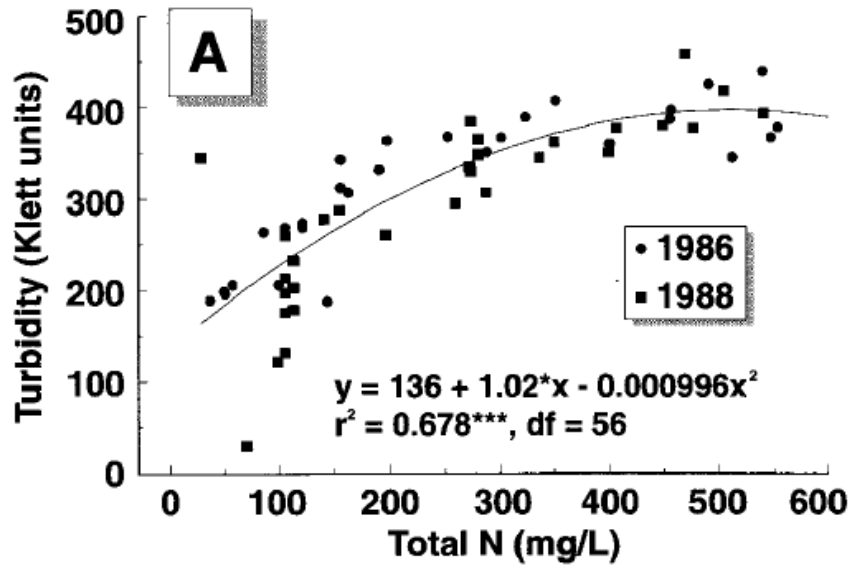


Arginine (Arg, R)
MW: 156.19, pK_a = 12.48

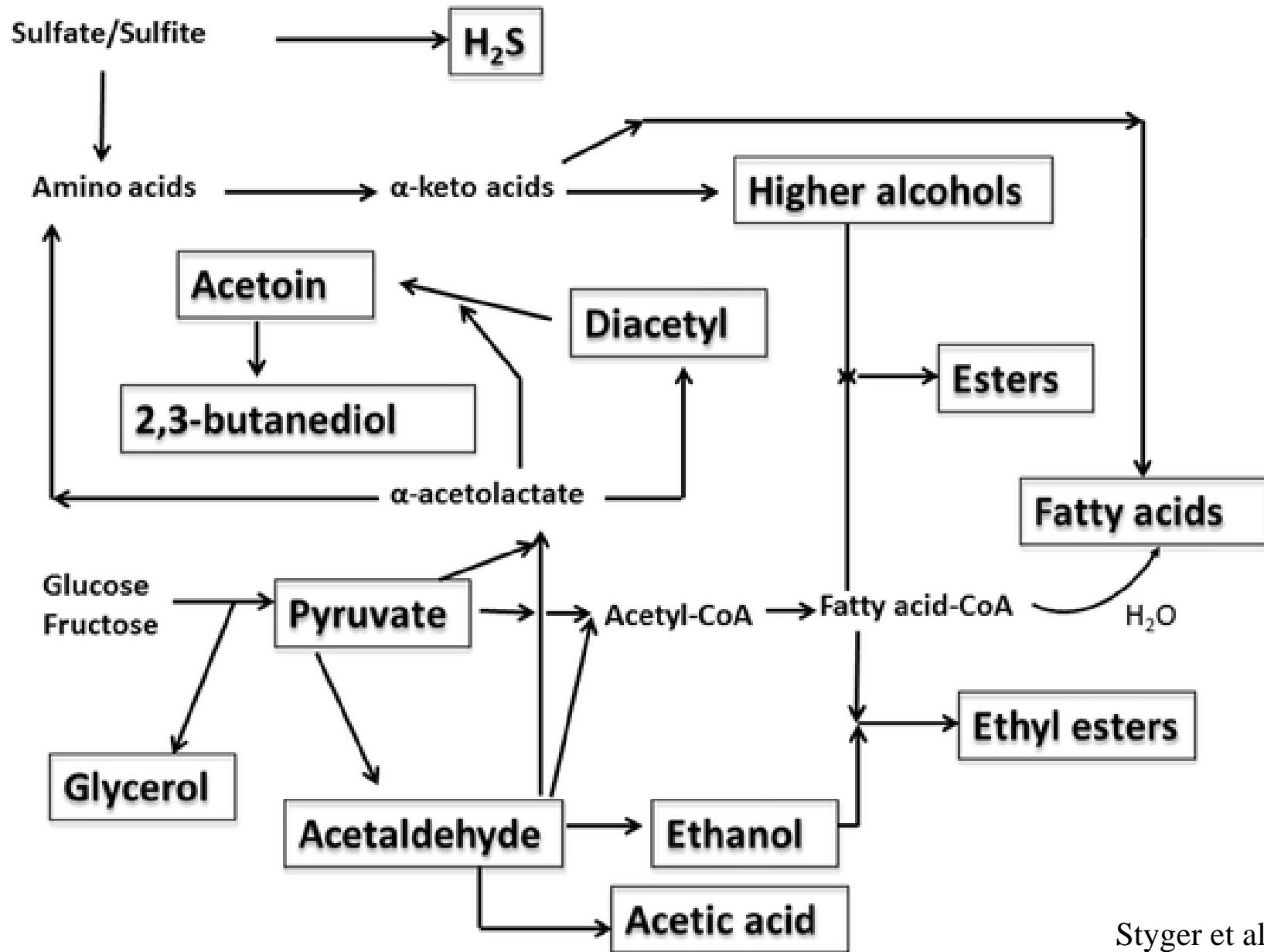
Yeast Nitrogen Uptake

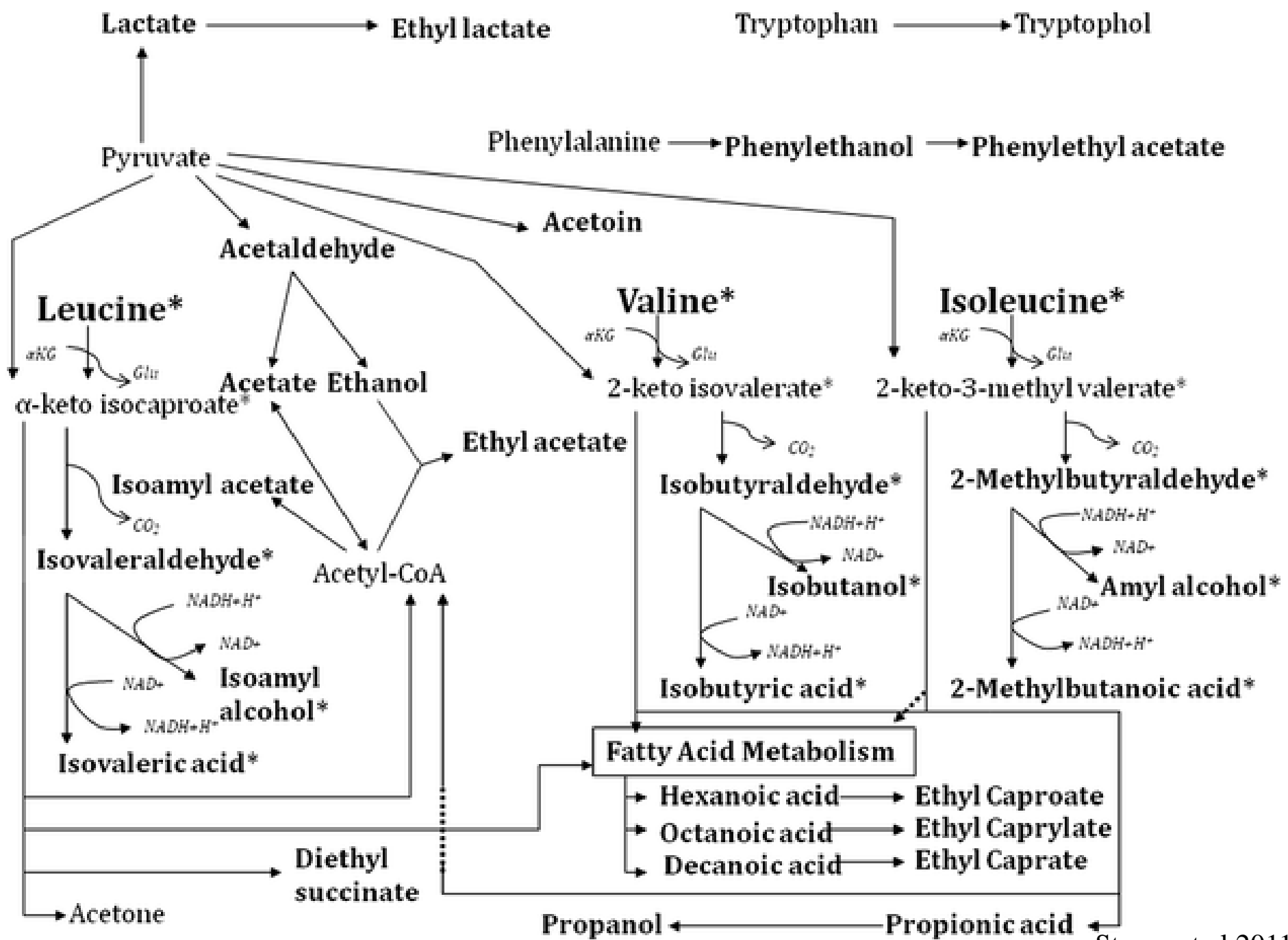
- Yeast use a mechanism called nitrogen catabolite repression (NCR).
- NCR mediates the selection of good nitrogen sources
- Good (N) sources ammonium, glutamine and asparagine.
- Less preferred arginine, alanine, aspartate, glycine and glutamate.
- Poorer nitrogen sources: **urea and proline.**

Turbidity vs Must Nitrogen



Aroma/Flavor

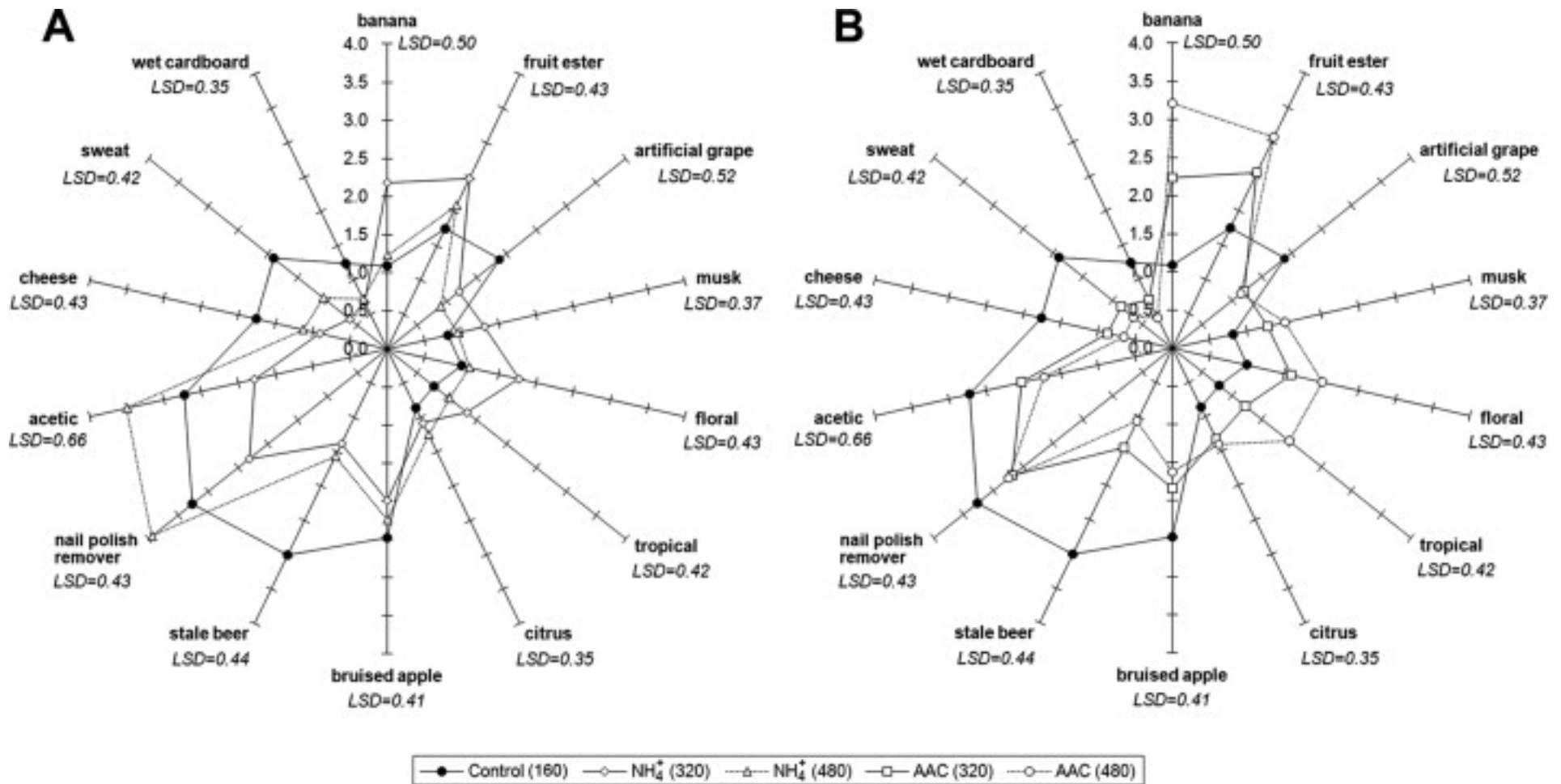




Aroma/Flavor

Compound	Amino acid	Concentration in wine (mg/l)	Odor
Isovaleraldehyde	Leucine	Traces	Fruity, nut-like
Isobutyraldehyde	Valine	Traces	Slightly apple-like
2-Methylbutyraldehyde	Isoleucine	NR	Green (herbaceous), malty
Isobutyric acid	Valine	Traces	Sweet, apple-like
Isovaleric acid	Leucine	<3	Rancid, cheese, rotten fruit
2-Methylbutanoic acid	Isoleucine	NR	Fruity, waxy, sweaty fatty acid
Isoamyl alcohol	Leucine	45–490	Alcohol
Isobutanol	Valine	40–140	Fruity, alcohol, solvent-like
Amyl alcohol (active)	Isoleucine	15–150	Marzipan (almond)
Isoamyl acetate	Leucine	0.03–8.1	Banana, pear
2-Phenyl acetate	Phenylalanine	0.01–4.5	Rose, honey, flowery
Ethyl isovalerate	Leucine	0–0.7	Apple, fruity
Isobutyl acetate	Valine	0.01–0.8	Banana
Ethyl 2-methylbutanoate	Isoleucine	0–0.9	Strawberry, pineapple

Nitrogen Source Makes a Difference



Low Must YAN

- Results
 - Low yeast population
 - Increased risk of stuck fermentation
 - Increased hydrogen sulfide
 - Decreased production of aroma compounds

Can Inorganic Nitrogen Additions Suffice?

- Must YAN's
 - Increase stuck fermentations below 140-150 mg/L
 - Higher YAN's not associated with stuck fermentations
- Why not prophylactically add DAP or equivalent?

High Must YAN

- Results
 - Increased ethyl acetate, acetic acid and volatile acidity
 - Increased haze-causing proteins, urea, ethyl carbamate and biogenic amines
 - Increased risk of microbial instability and atypical ageing characters

Nitrogen Cycle

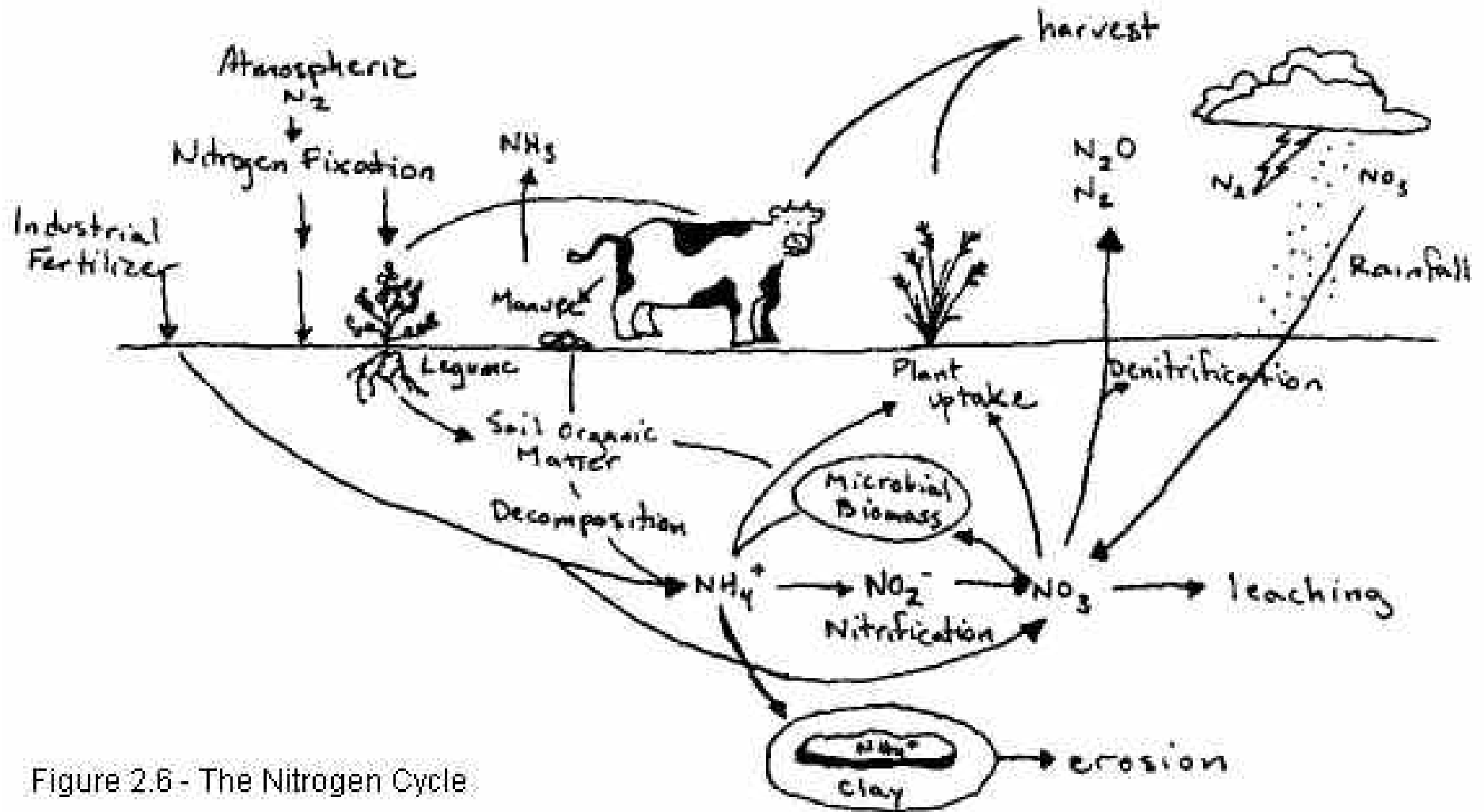


Figure 2.6 - The Nitrogen Cycle

Fertilization Impact on Musts

Good

- Must nitrogen constituents strongly correlate with applied fertilizer
 - Total nitrogen and amino acids
- Potential for yield increases

Bad

- Alter source:sink relationships....in a bad direction
- Increases in yield may be accompanied by decrease in fruit maturity and sugars
- Must urea, pH, aldehydes and VA can increase.
- Phenolics and grape-derived aroma compounds can be impacted due to altered fruit exposure

Fertilization Impact on YAN

Table 2. Must arginine (mg/L) for various fertilization treatments.

Replications	Treatments (Kg/ha)						
	Sod		Clean cultivation			Crop-thinned ^a	
	0	112	0	112	224	448	448
1	292	880	440	1720	1884	1656	1440
2	268	980	380	1532	1860	1720	1420
3	420	980	600	1408	1532	1160	1440
4	300	544	380	1200	1732	1372	1200
5	368	1140	420	980	1704	1780	772
6	320	1248	468	1372	1508	1720	1468
7	252	944	300	1304	1392	1820	1348
8	280	1050	472	1542	1720	1508	1360
Means	312	1050	432	1382	1667	1592	1360

F value = 680.1***
LSD @ 0.1% = 326.9

^a Half the clusters removed at véraison.

*** Significant at 0.001 significance level.

Fertilization Impact on Fermentation Time

Table 3. Overall fermentation time (days) for juices to ferment to dryness as affected by treatments.

Replications	Treatments (Kg/ha)						
	Sod		Clean cultivation			Crop-thinned ^a	
	0	112	0	112	224	448	448
1	23.0	12.0	16.9	9.0	9.0	9.0	13.0
2	22.0	12.0	18.0	9.0	9.0	9.0	13.0
3	16.0	15.0	14.0	9.0	9.0	9.0	13.0
4	14.0	15.0	18.0	9.0	12.0	9.0	13.0
5	19.0	12.0	18.0	12.0	9.0	9.0	13.0
6	16.0	12.0	19.0	9.0	9.0	9.0	13.0
7	22.0	12.0	19.0	9.0	9.0	9.0	13.0
8	25.0	9.0	16.0	9.0	9.0	9.0	12.0
Means	19.6	12.4	17.3	9.4	9.4	9.0	12.9
	F value = 44.8***						
	LSD @ 0.1% = 3.09						

^a Half the clusters removed at véraison.

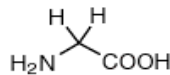
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Role of Nitrogen in Plants

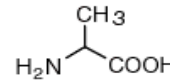
- Plants use nitrogen to form

- Amino Acids
- Polypeptides
- Proteins
- Chlorophyll
- Pyrazines
- Lecithins
 - Plant fat

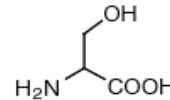
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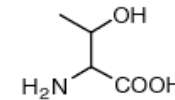
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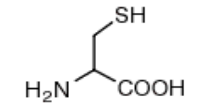
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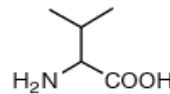


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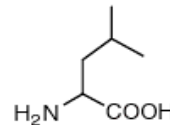


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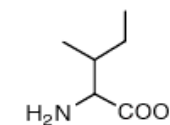
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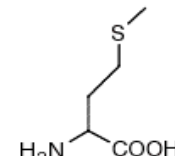
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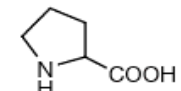
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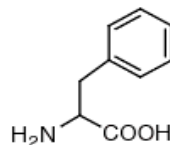


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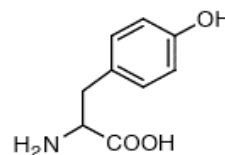


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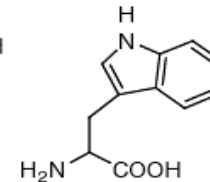
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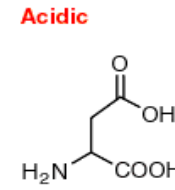
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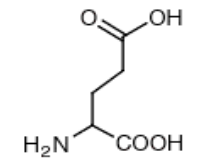
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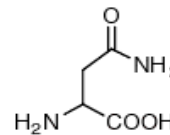


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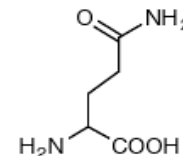


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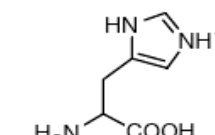


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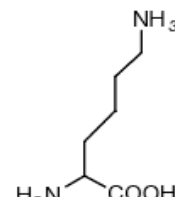


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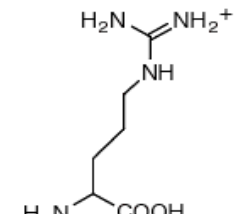
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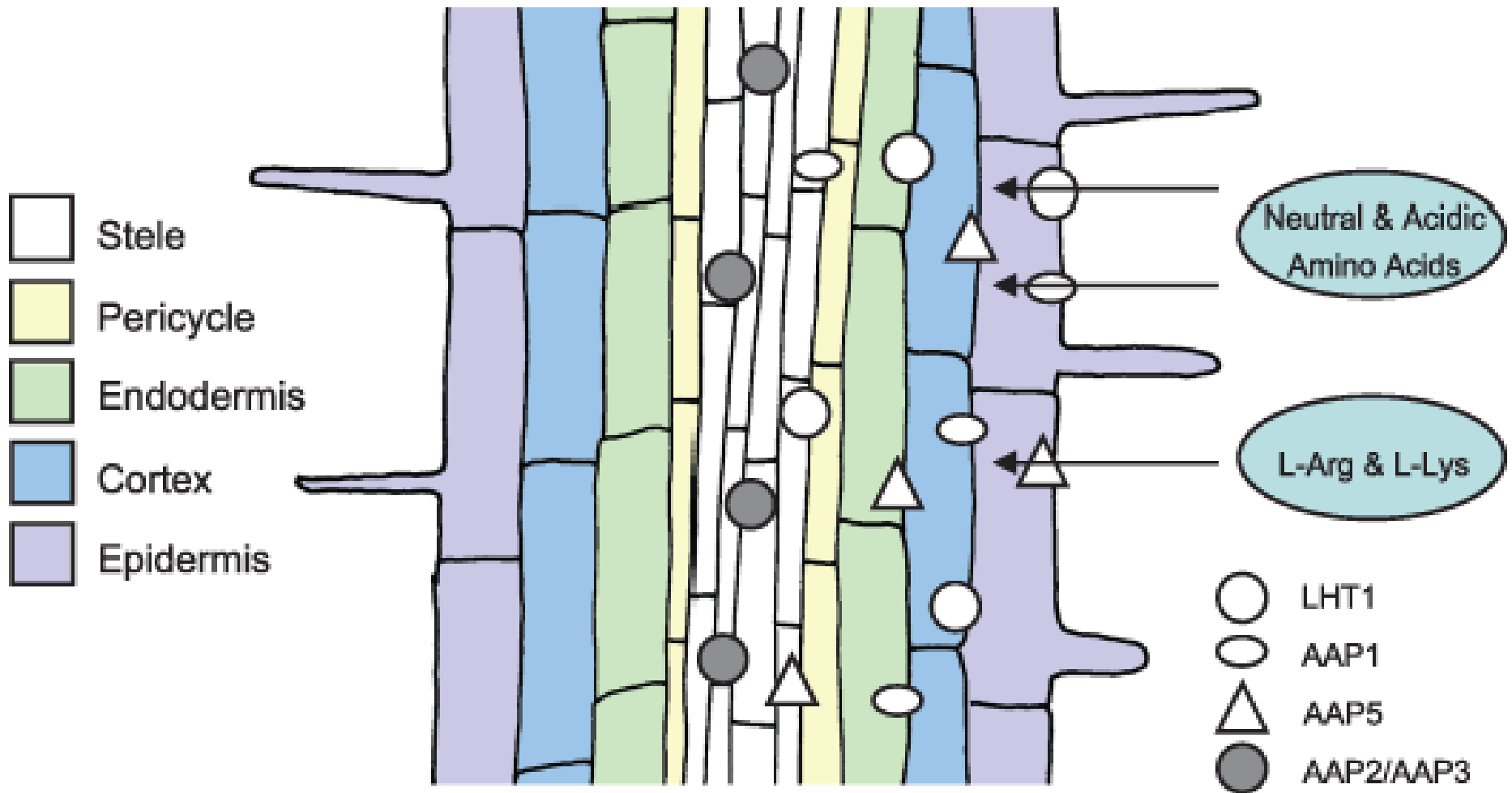


Lysine (Lys, K)
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Uptake of organic nitrogen by plants



New Phytologist

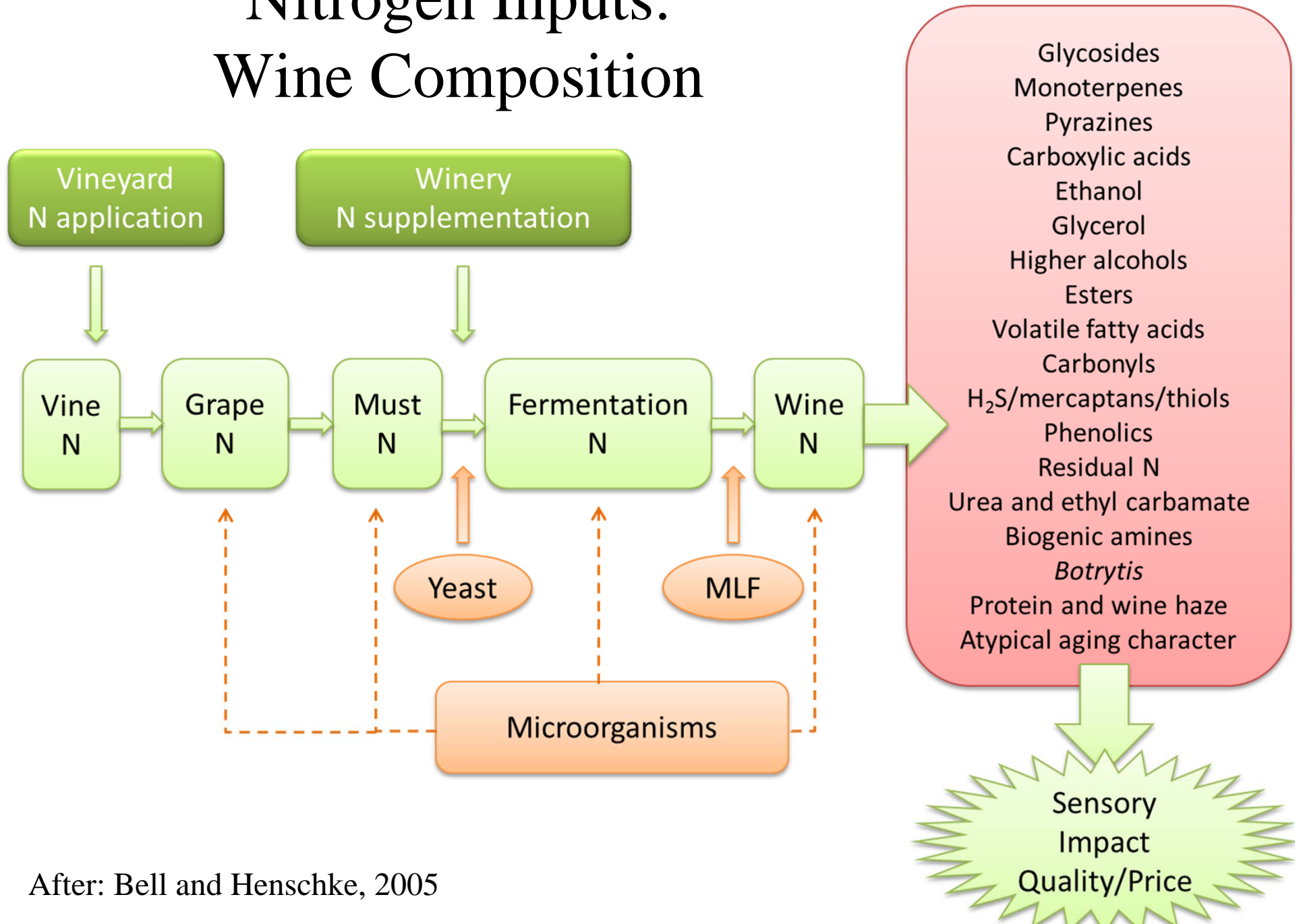
Volume 182, Issue 1, pages 31-48, 5 FEB 2009 DOI: 10.1111/j.1469-8137.2008.02751.x

<http://onlinelibrary.wiley.com/doi/10.1111/j.1469-8137.2008.02751.x/full#f3>

Sources of Nitrogen

- Decomposition of organic matter
- Applied fertilizer (urea, ammonium and nitrate)
 - Commercial fertilizer UAN 32
- Rain water
 - In polluted areas, rain water can be equivalent to applying 25 lbs N/acre
- Soil (N) compounds converted to nitrate
 - More soluble, easily absorbed when irrigated
 - *Nitrosomonas* and *Nitrobacter*

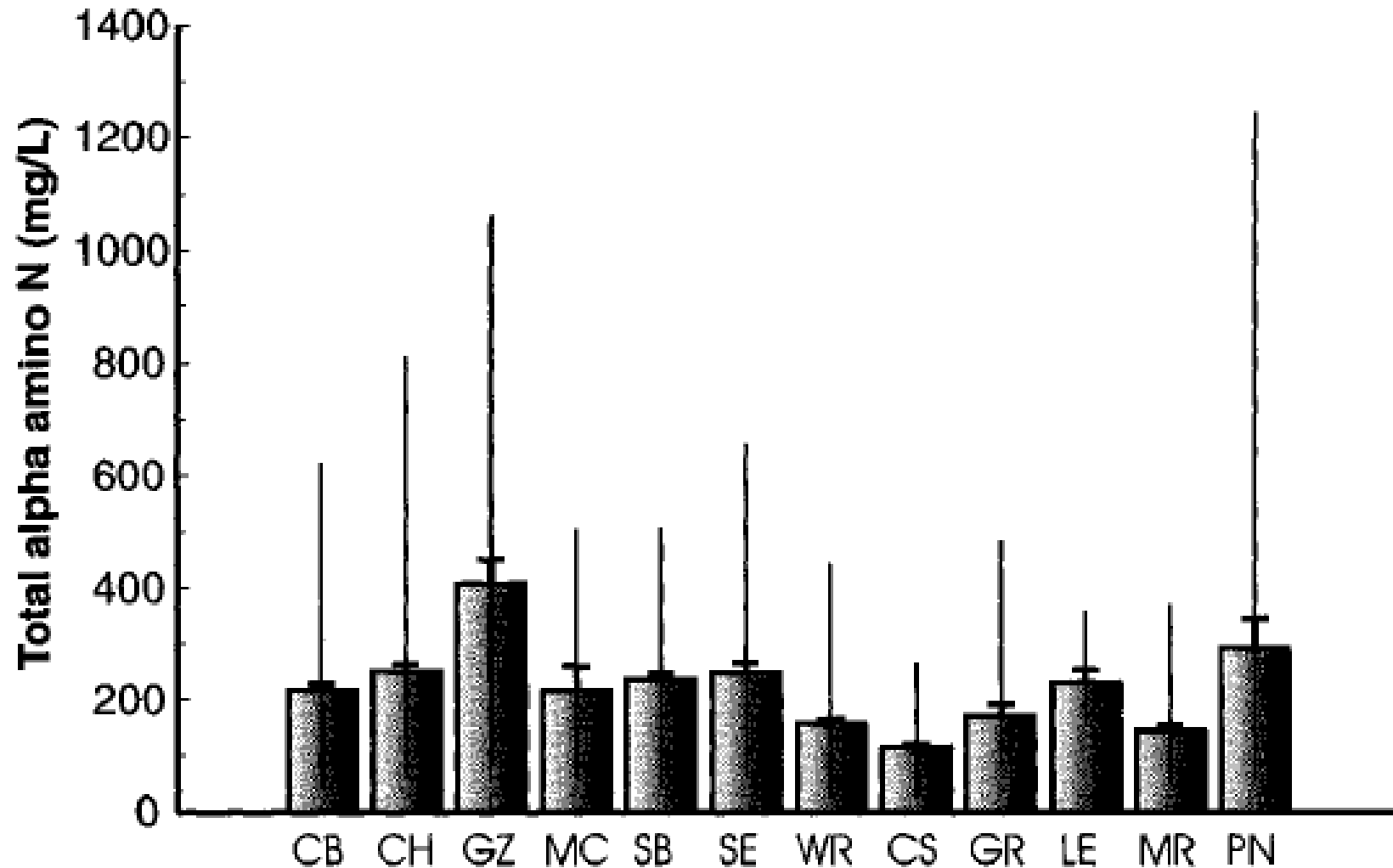
Nitrogen Inputs: Wine Composition



After: Bell and Henschke, 2005

Nitrogen Management in the Vineyard

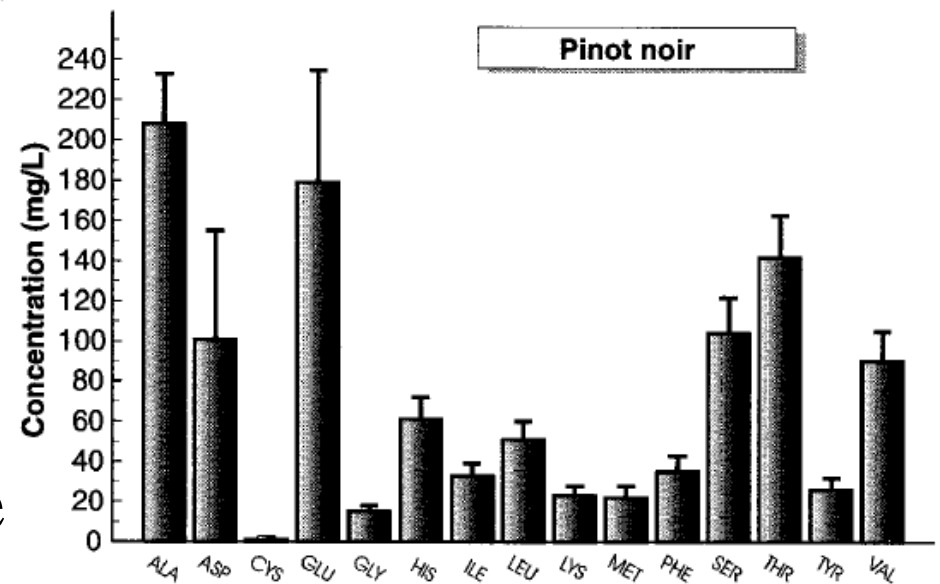
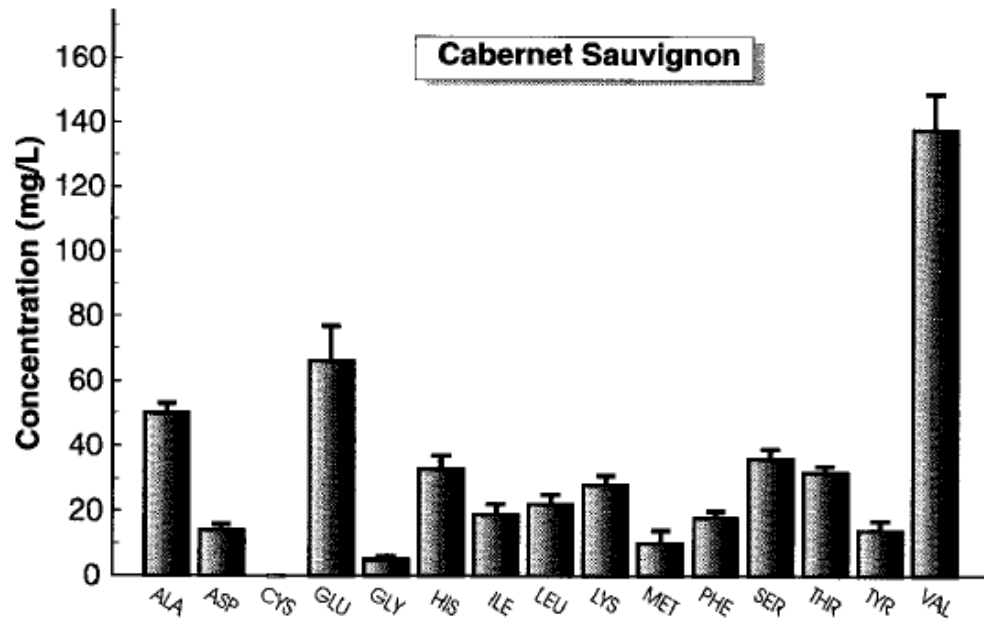
- Large seasonal variation



Study Period: 1986-1990

Spayd and Andersen-Bagge, 1996

Nitrogen Management in the Vineyard



- Ammonia, arginine, proline: largest variance
- Amino acid profiles have some varietal dependence

Study Period: 1986-1990

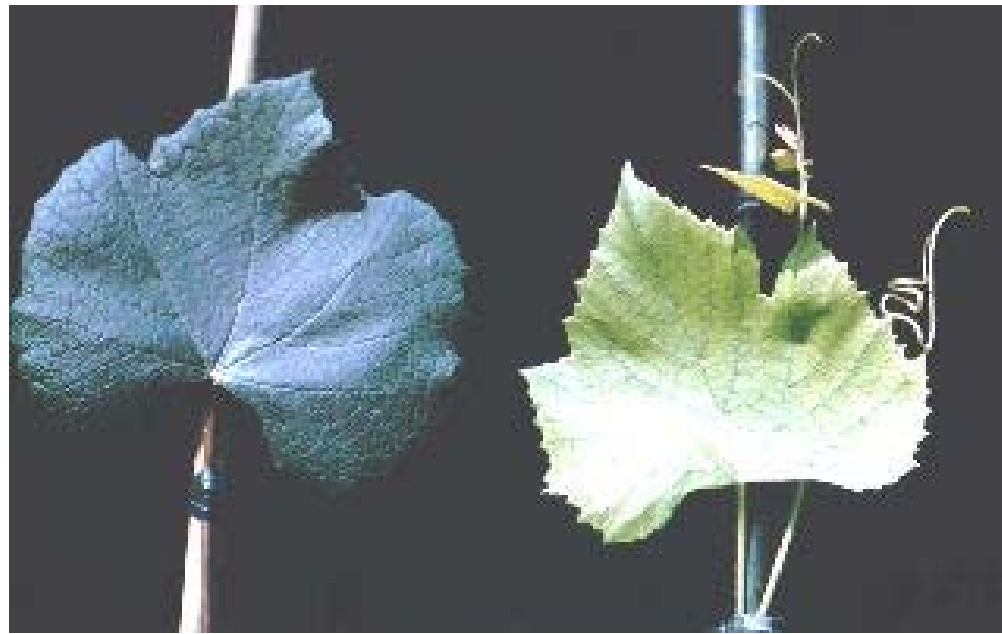
Spayd and Andersen-Bagge, 1996

Importance in Vineyard

- Nitrogen is the most widely needed vine nutrient in vineyards.
- Grapevines do not readily show symptoms of deficiency.
 - Growth and yields are reduced before symptoms arise.
- Problems arise more frequently from nitrogen toxicity, than deficiency
- Premium grape production tends to focus on vineyard de-vigoration

Nitrogen Deficiency

- Not easily detectable.
- Uniform pale green to yellowish color
- Possible decreases in yield and berry quality



Nitrogen Toxicity



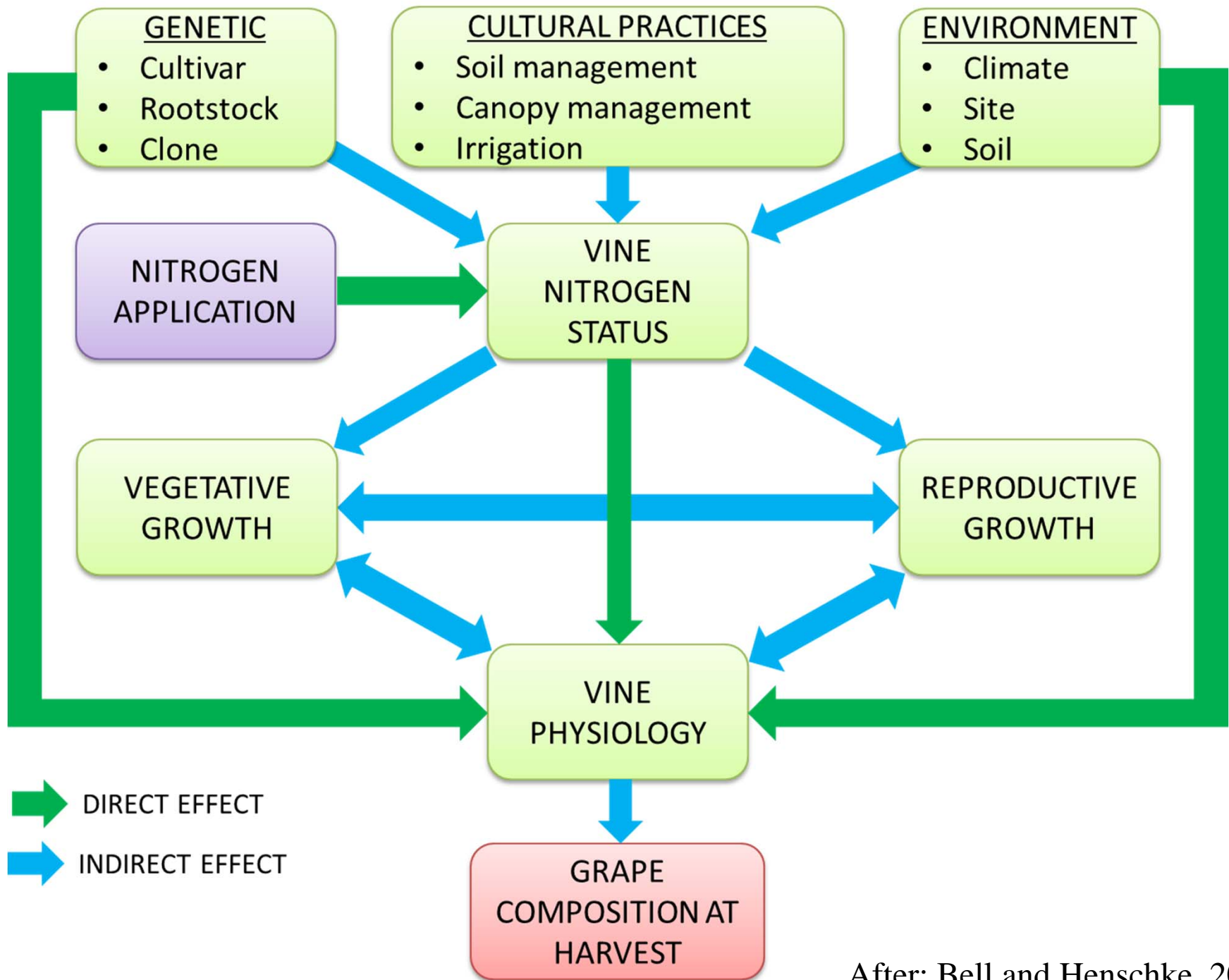
- Symptoms
 - Leaves turn dark green
 - Burn occurs if prolonged exposure
 - Poor fruit set
 - Occurs mostly in young vines
- Mechanism of Toxicity
 - Excess N leads to a build up of specific enzymes and NH_4^+ which can lead to Ca^+ deficiency.

Petiole/Blade Analyses

	BLOOM		VERAISON		HARVEST	
	Deficiency	Excess	Deficiency	Excess	Deficiency	Excess
PETIOLES						
NO -N ppm	50-350	350-1000+	50-200	200-750+	50-200	200-750+
Total-N %	0.65-0.9	0.9-1.2+	0.65-0.85	0.85-1.2+	0.6-0.8	0.8-1.1+
BLADES						
Total-N %	2.6-3.4	2.8-3.5+	2.5-3.1	3.1-3.5+	1.8-3.0	2.8-3.4+

N application: 0-400 lbs/Ac; 4 year study

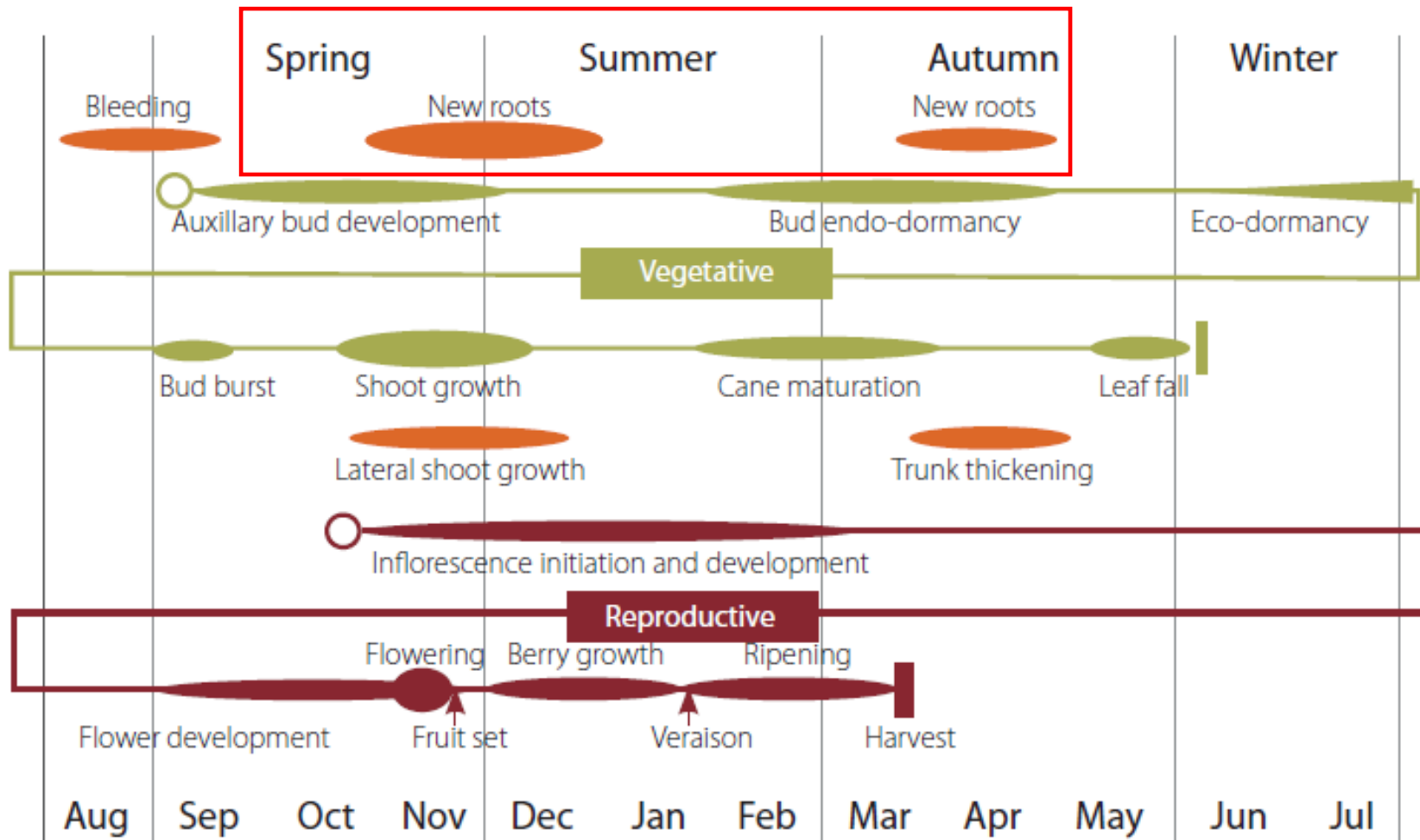
Christensen, 2002



After: Bell and Henschke, 2005

Timing of Nitrogen Application

Figure 4 Seasonal growth phases of the grapevine (adapted from Pearce and Coombe 2004)

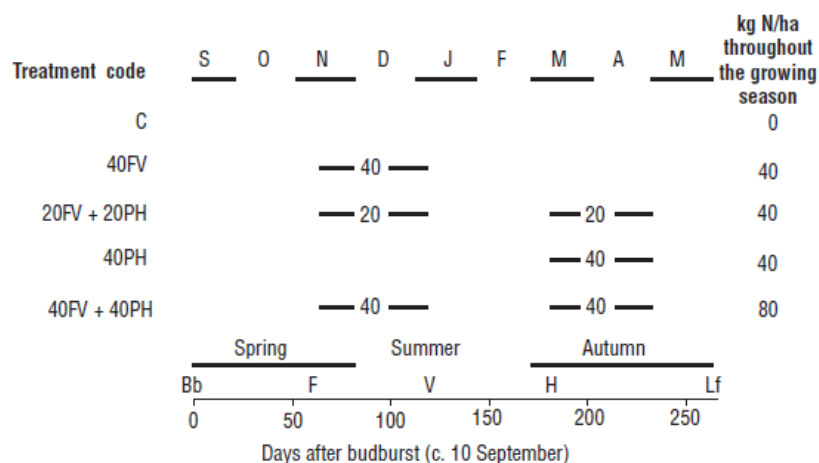


Rootstock Effect on Nitrogen Uptake

- Vigor roughly related to nitrogen uptake

	HIGH	MEDIUM	LOW
NITRATE- NITROGEN	039-16 Freedom St. George Ramsey	101-14Mgt 5BB 1103P 3309C Schwarzmann 44-53M 110R	Harmony 5C 1616C 420A

Rootstock and Fertigation Success

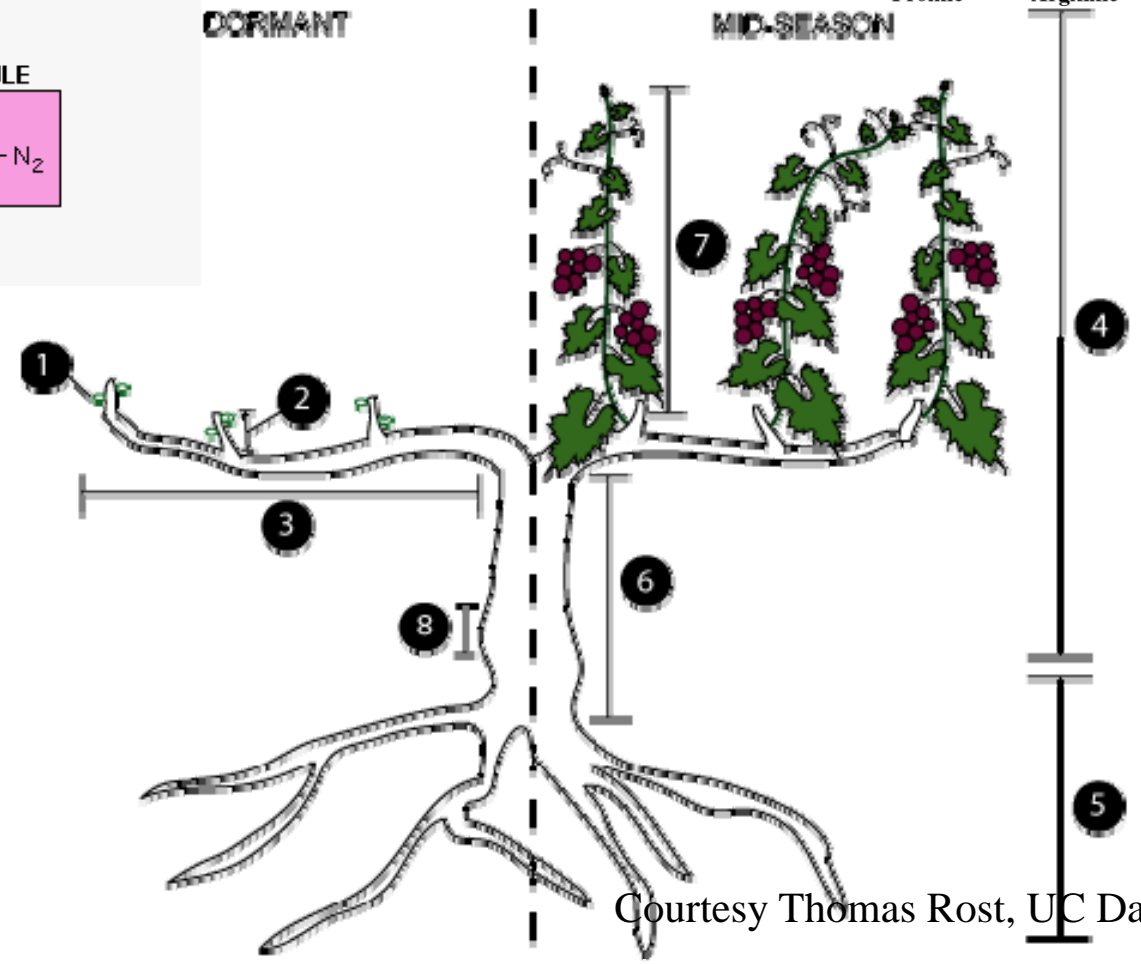
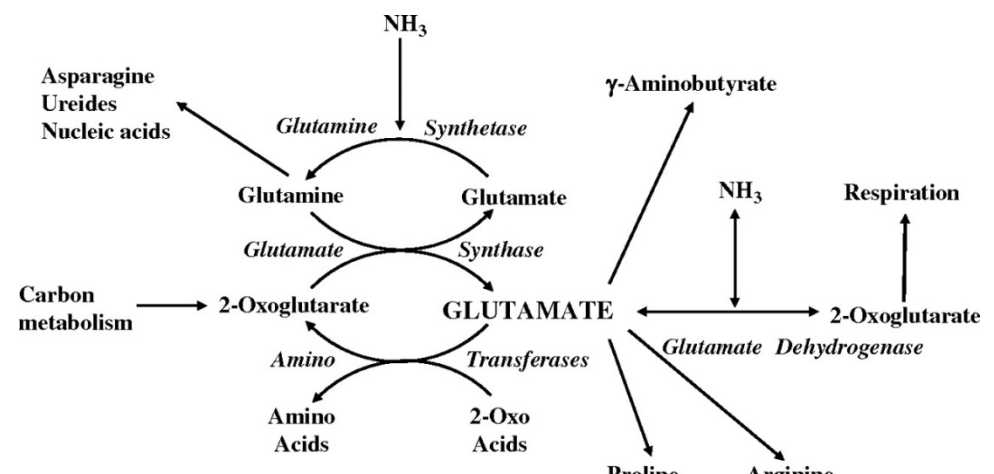
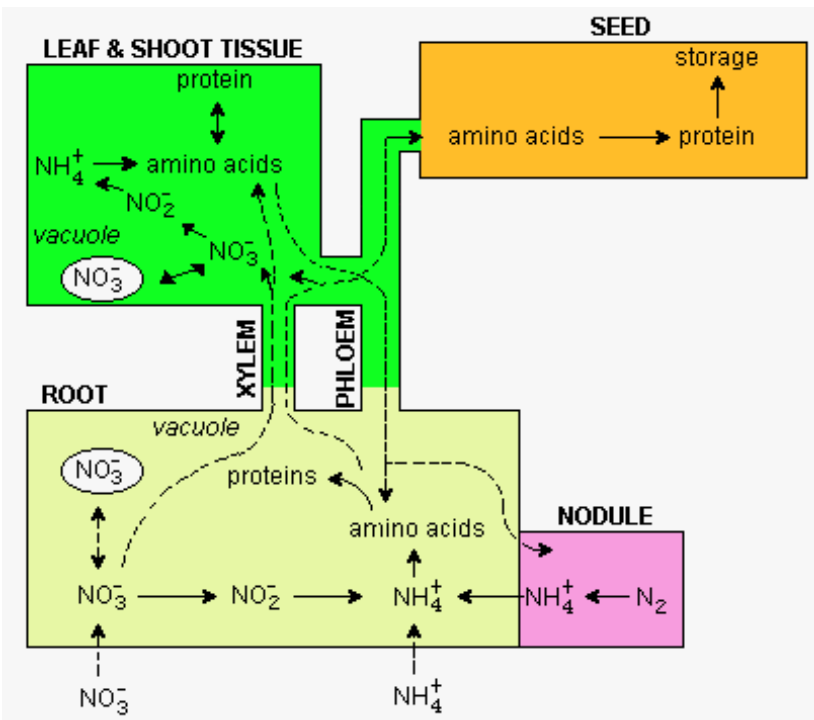


- Arginine and Proline
- Cv. Shiraz
- Ammonium nitrate
- Weekly application

Rootstock	Treatment	mg free total amino N/L	mg free assimilable amino N/L	mg free non-assimilable amino N/L
Teleki 5C	C	199 ^a	103 ^a	96 ^a
	40FV	235 ^{ab}	117 ^a	118 ^b
	20FV + 20PH	207 ^a	107 ^a	101 ^{ab}
	40PH	157 ^a	80 ^a	78 ^a
	40FV + 40PH	314 ^b	182 ^b	132 ^b
Schwarzmann	C	191 ^a	86 ^a	105 ^a
	40FV	349 ^c	170 ^c	179 ^c
	20FV + 20PH	250 ^{ab}	124 ^{ab}	125 ^{ab}
	40PH	277 ^b	137 ^{bc}	139 ^b
	40FV + 40PH	424 ^d	230 ^d	194 ^c
Ramsey	C	244 ^{ab}	124 ^a	120 ^{ab}
	40FV	359 ^b	194 ^b	166 ^c
	20FV + 20PH	324 ^b	174 ^b	150 ^{bc}
	40PH	219 ^a	111 ^a	108 ^a
	40FV + 40PH	383 ^b	209 ^b	174 ^c

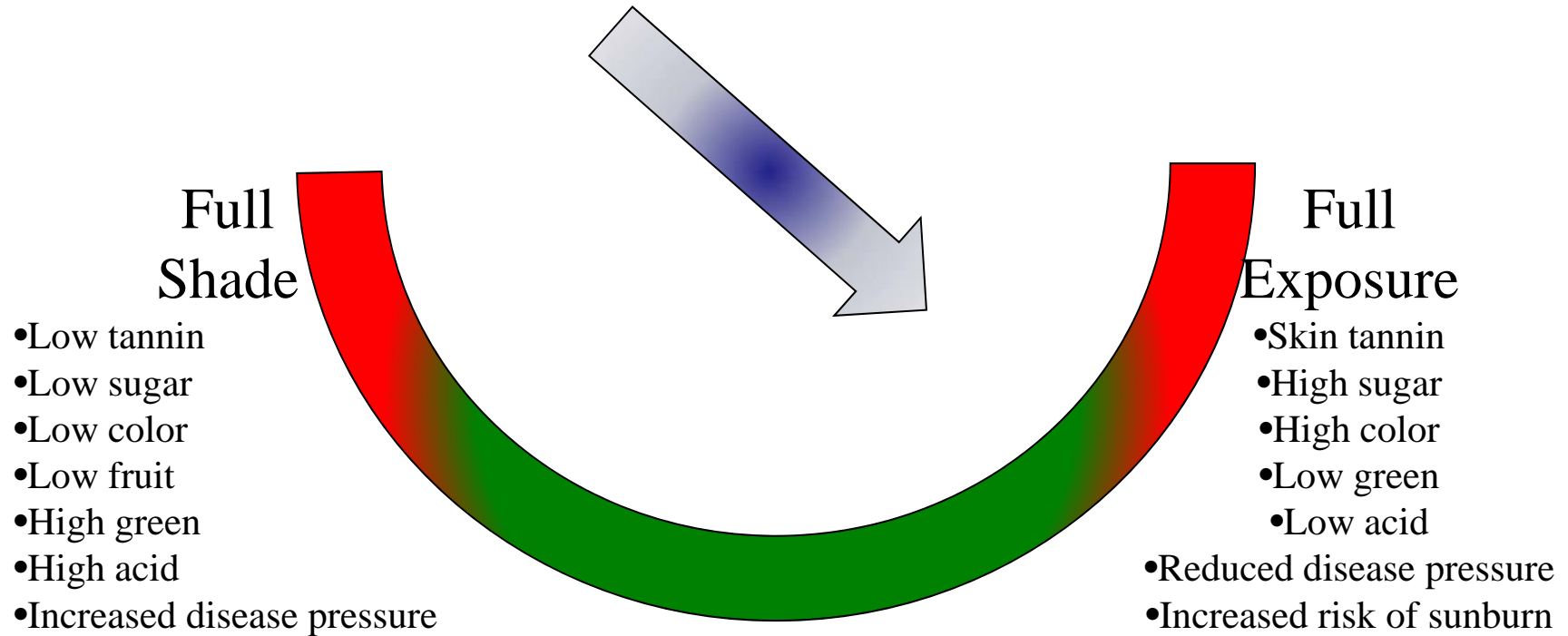
Cluster Exposure and Amino Acids

Amino acid (umol/L)†	Viticulture treatments					P-value‡
	Control	LR	ACRYLIC	PETG	POLY	
<i>α</i> -ketoglutarate						
Proline	1153 ^{a§}	610 ^b	510 ^b	711 ^b	673 ^b	0.002
Arginine	974 ^a	771 ^b	822 ^c	849 ^c	854 ^c	<.001
Glutamate	256 ^a	182 ^{bc}	168 ^b	225 ^{ac}	214 ^{abc}	0.038
Glutamine	853 ^{abc}	502 ^a	1286 ^{bc}	838 ^{ab}	1346 ^c	0.023
Histidine	170 ^a	109 ^b	107 ^b	146 ^{ac}	124 ^{bc}	0.003
Shikimate (aromatic)						
Phenylalanine	403 ^a	233 ^{bc}	220 ^b	304 ^c	280 ^{bc}	0.004
Tryptophan	153 ^a	89 ^{bc}	78 ^b	110 ^c	102 ^{bc}	0.002
Tyrosine	74 ^a	42 ^b	39 ^b	54 ^b	56 ^{ab}	0.025
Pyruvate						
Leucine	613 ^a	366 ^b	312 ^b	417 ^b	373 ^b	0.007
Valine	424 ^a	249 ^{bc}	205 ^b	298 ^c	249 ^{bc}	0.005
Alanine	422 ^a	288 ^b	230 ^b	275 ^b	265 ^b	0.002
Aspartate						
Aspartate	110 ^a	77 ^b	84 ^{bc}	111 ^a	93 ^c	<.001
Asparagine	28 ^a	10 ^{bc}	8 ^b	21 ^{ac}	13 ^{bc}	0.015
Threonine	817 ^a	465 ^b	466 ^b	571 ^b	542 ^b	0.001
Isoleucine	406 ^a	244 ^b	208 ^b	288 ^b	252 ^b	0.005
Methionine	145 ^a	76 ^{bc}	64 ^b	93 ^c	79 ^{bc}	0.001
Lysine	8	6	7	8	8	ns
3-phosphoglycerate						
Serine	304 ^a	170 ^b	147 ^b	210 ^b	204 ^b	0.004
Cysteine	6	3	0	12	0	ns
Glycine	21	13	21	22	23	ns



Courtesy Thomas Rost, UC Davis

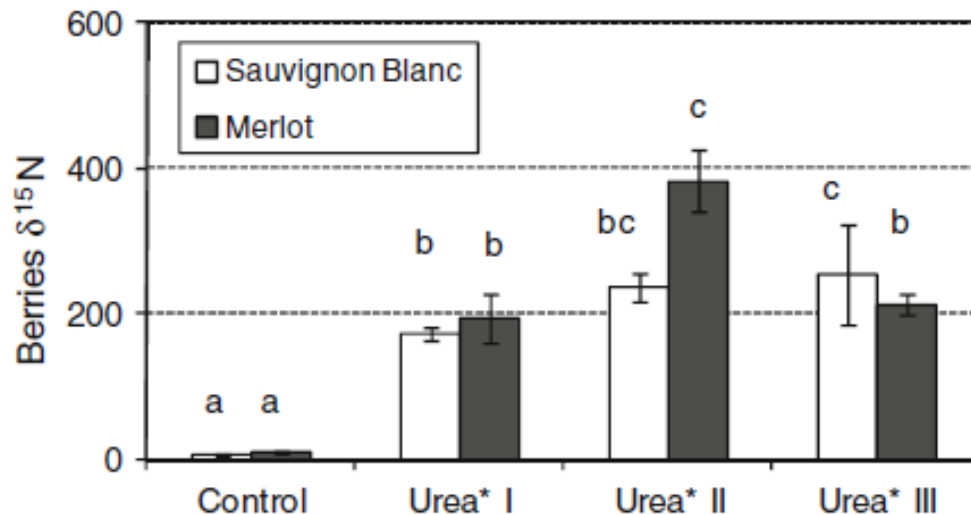
The Exposure Conundrum



Must Nitrogen?

Foliar Applications of Urea

- Foliar applications of urea can increase YAN in grapes
- Timing is important
- Absorbed urea is converted to other forms of N



I: Preveraison, II: veraison, III: postveraison

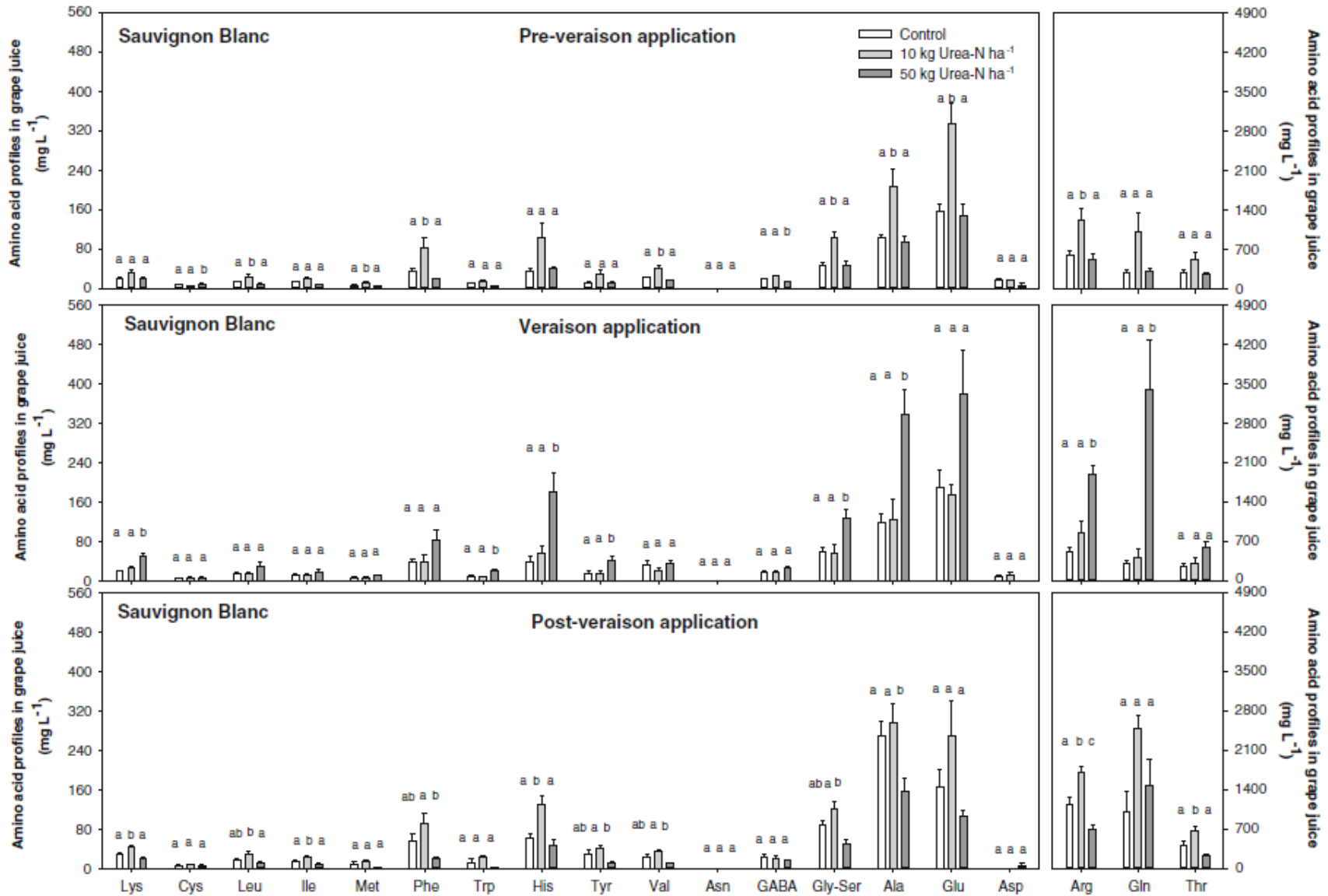
Foliar Applications of Urea

	Yeast assimilable N in grape juice (mg L ⁻¹)	Total amino acids in grape juice (mg L ⁻¹)	Proline in grape juice (mg l pro L ⁻¹)
1st year			
Sauvignon Blanc			
Control	292 a	2783 a	380 a
Urea II (10 kg N ha ⁻¹)	280 a	3095 a	276 b
Merlot			
Control	156 a	1182 a	1990 a
Urea II (10 kg N ha ⁻¹)	194 a	1144 a	2139 a
2nd year			
Sauvignon Blanc			
Control	353	2226	264
Urea I (10 kg N ha ⁻¹)	371	3795	471
Urea I (50 kg N ha ⁻¹)	465	1567	161
Urea II (10 kg N ha ⁻¹)	492	2133	264
Urea II (50 kg N ha ⁻¹)	491	7187	414
Urea III (10 kg N ha ⁻¹)	634	5967	437
Urea III (50 kg N ha ⁻¹)	514	2847	218
Merlot			
Control	187	628	1242
Urea I (10 kg N ha ⁻¹)	126	661	1345
Urea I (50 kg N ha ⁻¹)	242	797	1380
Urea II (10 kg N ha ⁻¹)	298	968	1886
Urea II (50 kg N ha ⁻¹)	232	2757	2369
Urea III (10 kg N ha ⁻¹)	428	875	1506
Urea III (50 kg N ha ⁻¹)	318	1070	1656

I: Preveraison, II: veraison, III: postveraison

Lasa et al., 2012

Foliar Applications of Urea



Vineyard (N) Effect on Fusel Oils

Higher alcohol compound	Nitrogen application in the vineyard	References
Total higher alcohols	Increase	Giorgessi et al. 2001
1-Propanol	Increase	Ough and Bell 1980
1-Butanol	Increase	Webster et al. 1993
Isobutanol (2-methyl propanol)	Increase	Webster et al. 1993, Giorgessi et al. 2001
Amyl alcohols (2,3-methyl-1-butanol)	Increase	Webster et al. 1993
1-Hexanol	Increase	Giorgessi et al. 2001
<i>Trans</i> -3-hexan-1-ol	Increase	Webster et al. 1993
<i>Cis</i> -3-hexan-1-ol	Increase	Webster et al. 1993
Benzyl alcohol	Increase	Webster et al. 1993
Amyl alcohols (2,3-methyl-1-butanol)	No effect	Spring 2002
Total higher alcohols	Decrease	Webster et al. 1993
Isobutanol (2-methyl propanol)	Decrease	Ough et al. 1968b, Ough and Bell 1980, Webster et al. 1993
Amyl alcohols (2,3-methyl-1-butanol)	Decrease	Ough et al. 1968b, Webster et al. 1993, Maigre 2002, Spring 2002
Active amyl alcohol (2-methyl-1-butanol)	Decrease	Ough and Bell 1980
Isoamyl alcohol (3-methyl-1-butanol)	Decrease	Ough and Bell 1980
2-Phenylethanol	Decrease	Webster et al. 1993, Maigre 2002, Spring 2002

Vineyard (N)Effect on Esters

Ester compound	Nitrogen application in the vineyard	References
Total volatile esters	Increase	Ough et al. 1968 b, Giorgessi et al. 2001
Ethyl acetate	Increase	Webster et al. 1993
Ethyl butyrate	Increase	Webster et al. 1993
Ethyl 4-hydroxy butyrate	Increase	Giorgessi et al. 2001
Ethyl hexanoate (caproate)	Increase	Ough and Lee 1981, Webster et al. 1993, Giorgessi et al. 2001
Ethyl octanoate (caprylate)	Increase	Webster et al. 1993, Giorgessi et al. 2001
Ethyl decanoate	Increase	Ough and Lee 1981, Webster et al. 1993
Isobutyl acetate	Increase	Webster et al. 1993
Isoamyl acetate	Increase	Ough and Lee 1981, Webster et al. 1993
Hexyl acetate	Increase	Giorgessi et al. 2001
Phenylethyl acetate	Increase	Webster et al. 1993, Giorgessi et al. 2001
Butyrolactone	Increase	Giorgessi et al. 2001
Diethyl malate	Increase	Giorgessi et al. 2001
Ethyl octanoate	No effect	Ough and Lee 1981
Hexyl acetate	No effect	Ough and Lee 1981
Phenylethyl acetate	No effect	Ough and Lee 1981

Indirect Effects?

- Can YAN be influenced indirectly?
- Can nitrogen application indirectly influence other grape components?

Irrigation – Indirect?

	2006	2007	2008	2009	2010
<i>Yeast assimilable nitrogen (mg N L⁻¹)</i>					
<i>Irrigation</i>					
Daily	83.1a	164.7	105.3	164.6	82.8a
Reduced	130.2b	199.8	120.0	170.8	132.9b
<i>N fertilization</i>					
0 g urea-N/vine	95.0a	182.7ab	107.3	132.0a	83.4a
5 g urea-N/vine	96.1a	171.4ab	109.4	156.1ab	90.0a
10 g urea-N/vine	102.8a	166.9a	109.5	192.1b	99.7a
20 g urea-N/vine	132.8b	208.0b	124.7	188.1b	158.3b
<i>Pr > F^z</i>					
Irrigation	**	NS	NS	NS	**
N Fertilization	****	*	NS	**	****
<i>Petiole N_{bloom} (%)</i>					
<i>Irrigation</i>					
Daily	0.79a	1.00	1.29	1.05	0.86
Reduced	0.93b	0.92	1.21	1.03	0.84
<i>N fertilization</i>					
0 g urea-N/vine	0.78a	0.91a	1.13a	0.92a	0.78a
5 g urea-N/vine	0.79a	0.93ab	1.14a	1.00a	0.81a
10 g urea-N/vine	0.87a	0.98ab	1.27ab	1.05ab	0.83a
20 g urea-N/vine	1.01b	1.02b	1.46b	1.19b	0.97b
<i>Pr > F^z</i>					
Irrigation	*	NS	NS	NS	NS
N Fertilization	****	*	**	***	****
Pearson's correlation coefficients	0.64	0.35	0.47	0.85	0.62
<i>r</i> ² =	****	*	***	****	****
<i>n</i> ² =	48	48	47	39	48

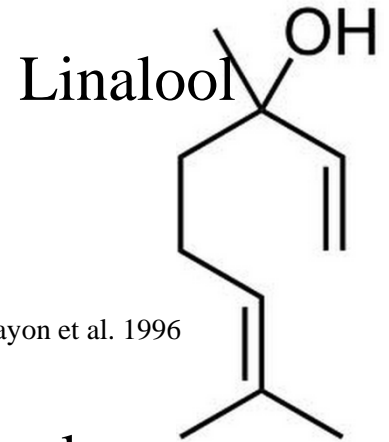
	2006 ^z	2007	2008	2009	2010
<i>Average berry weight (g)</i>					
<i>Irrigation</i>					
Daily	1.94a	1.23	0.87	1.42	0.90a
Reduced	1.51b	1.20	0.86	1.35	0.82b
<i>N fertilization</i>					
0 g urea-N/vine	1.73	1.20	0.90a	1.43	0.85
5 g urea-N/vine	1.73	1.22	0.91a	1.36	0.87
10 g urea-N/vine	1.72	1.25	0.86ab	1.37	0.89
20 g urea-N/vine	1.72	1.17	0.79b	1.36	0.83
<i>Pr > F^z</i>					
Irrigation	***	NS	NS	NS	*
N Fertilization	NS	NS	**	NS	NS
<i>Yield (kg vine⁻¹)</i>					
<i>Irrigation</i>					
Daily	4.5a	5.5	2.3	2.6	4.5
Reduced	2.9b	4.6	2.6	3.2	4.0
<i>N fertilization</i>					
0 g urea-N/vine	3.8	4.9	2.4	3.2	4.0
5 g urea-N/vine	3.1	5.2	2.7	3.1	3.9
10 g urea-N/vine	3.9	5.4	2.9	2.8	4.5
20 g urea-N/vine	4.0	4.8	1.8	2.5	4.5
<i>Pr > F^z</i>					
Irrigation	***	NS	NS	NS	NS
N Fertilization	NS	NS	NS	NS	NS

cv. Merlot, 5-year study, Okanagan Valley

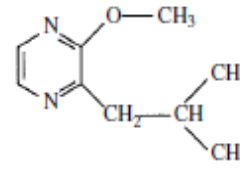
Hannam et al., 2013

Monoterpenes

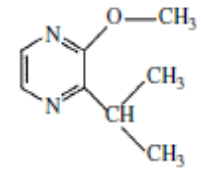
- Responsible for **floral aroma**
- Major Varietals-Muscat of Alexandria, Riesling and Gewürztraminer
- Compounds-monoterpene diols, linalool, geraniol, nerol, citronellol and etc. which are liberated during fermentation
- Studies link canopy microclimate to total monoterpene development
- **Nitrogen** application can increase vegetative growth and canopy density, consequently decreasing monoterpene development



Methoxypyrazines



Bell pepper



Earthy, moldy, 'cellar' flavor
PT = 0.002 ppb

- Responsible for **herbaceous, vegetative and bell pepper aromas/flavors**
- Major varieties: Merlot, Cabernet Sauvignon, Sauvignon Blanc
- Compounds: IBMP, IPMP, SBMP
- Indirect effect of increased leaf layers and fruit shading increasing levels of pyrazines.

Take Home Messages

- Excessive nitrogen can affect canopy microclimate and sun exposure to grapes
 - Budburst-Bloom N applications associated with increase in vegetative growth
 - Decreases beneficial compounds and increases unwanted characteristics
- An adequate amount of fertilizer is needed to ensure YAN levels are sufficient for yeast processes.
- DAP can help early to mid fermentation problems; prophylactic DAP use can lead to negative character (i.e.: EtAc, VA)
- Must nitrogen has a direct effect on flavor and aroma compounds
- Growers and winemakers need to find optimal nitrogen applications to avoid negative effects.
- Moderation is key!