The heritability of methyl anthranilate and total volatile esters in *Vitis* spp. hybrids

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S u m m a r y : Two grapevine seedlings, V. 72181 and V. 72182, selected for extremely high methyl anthranilate (MA) and total volatile esters (TVE) content, were selfed to create families 8020 and 8021 respectively, to test the inheritance of these two components of labrusca flavour character. Reynolds *et al.* (1982) had postulated a three-gene, dominant and complementary system (M, A, F) for MA and a two-gene dominant and complementary system (T, V) for TVE. Families 8020 and 8021 segregated 3 : 1 for MA, indicating only one heterozygous locus for MA in the parents. This would question Reynolds' assignment of genotypes for the grandparents of these two families and would suggest a more complex environmentally influenced system. The TVE segregation patterns followed Reynolds' hypothesis and segregated 3 : 1 for one heterozygous locus.

K e y w o r d : genetics, heritability, flavour, methyl anthranilate, volatile esters, biometry, sensory rating, breeding.

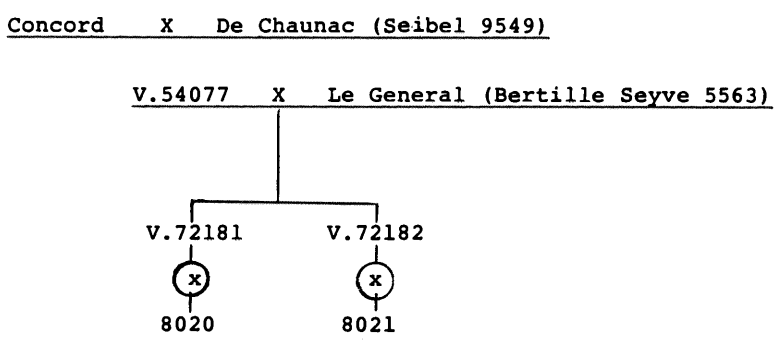
Introduction

Since 1913, the grape breeding programme at the Horticultural Research Institute of Ontario (HRIO) has been striving to produce wine grapes of suitable hardiness for the climatic conditions in southern Ontario, Canada. Flavour and colour requirements have changed markedly since that time and the breeding programme has been altered to meet the new requirements. With more recent demands for non-labrusca flavoured varieties, the heritability of labrusca flavour components has become quite critical.

Since 1970, Dr. T. Fuleki of this Institute has been surveying the HRIO variety collection as well as the seedlings resulting from the HRIO breeding programme for compounds thought to be instrumental in the perception of labrusca flavour, i. e. methyl and ethyl anthranilate (MA) and total volatile esters (TVE). He later used these components to develop the Vineland Grape Flavour Index (VGFI) that became the objective flavour selection criterion for the grape seedling populations at the Institute (Fuleki 1982).

The analysis of grape seedling selections for the above compounds became routine procedure, but perhaps more important was the impact on the choice of parents in the breeding programme for subsequent generations. The MA and TVE survey values were examined to assess the potential labrusca flavour of seedlings according to the MA/TVE/VGFI values of the parents. Based on the hypothesis that heritability could be predicted from these values, Reynolds studied three seedling families, 7216, 7218 and 7219, which had high and low labrusca flavoured parents. The seedling populations of these three families were examined in 1977 and 1978 and Reynolds postulated genotypes for MA and TVE synthesis for their parents. He also identified two seedlings, V. 72181 and V. 72182, with extraordinarily high MA and TVE values (Reynolds 1980). These two seedlings were maintained in the HRIO vineyard and were selfed in 1980 to create families 8020 and 8021, respectively (Fig. 1).

Reynolds postulated that MA synthesis was governed by three dominant and complementary genes (M, A and F), with the F locus necessary in the dominant condition for MA synthesis to proceed and M and A loci both dominant for excessive MA production. The presence of M and/or A in the recessive condition would result in lower amounts of MA, below the organoleptic threshold of labrusca flavour influence, and the presence of the F locus in the recessive



[a] Fisher, Ann. Rept. HRIO, 18-20, 1981.

Fig. 1: Genealogy of grape vine cultivars/seedlings used in MA/TVE inheritance studies [a].

Mm Aa Ff X MM Aa ff = (excess MA) 3:5 (low or no Ma)
Tt Vv X tt Vv = (excess TVE) 3:5 (low TVE)

[a] Reynolds et al, Am . J. Enol. Vitic., 33:1:14-19, 1982.

Fig. 2: Segregation ratios for MA and TVE for Family 7218 (V. 54077 x Le Général) [a]

condition would preclude any MA synthesis. Reynolds also postulated that TVE was governed by two dominant complementary genes (T and V) and that either in the recessive condition would constitute TVE production below the organoleptic level of labrusca flavour influence (Reynolds *et al.* 1982).

These hypotheses led to the genotypic assumptions presented in Fig. 2.

Since seedlings V. 72181 and V. 72182 were selected for their exceptionally high MA and TVE, it is assumed that they would have the genotypic make-up for excess MA and TVE production. Based on Reynolds' genotypic assignments for the parents, the following genetic combinations could be assigned to the above seedlings, as outlined in Table 1.

In order to confirm the ratios postulated in Table 1, the following study was initiated.

Method

Plant material

Parental lines were planted in the vineyard as individuals or as populations of vegetatively propagated clones. Their genealogy is illustrated in Fig. 1.

Selfed populations were created by bagging flower clusters of the appropriate parental vines prior to capfall. Seedling populations (8020, 8021) were planted as a group in the seedling block of the main vineyard.

Table 1: Proposed genotypes and segregation ratios for MA and TVE for F_2 populations derived from V. 72181 and V. 72182[a] crosses

Excess MA	Segregation Ratio		Excess TVE	Segregation Ratio	
MM AA Ff	L/NL[b]	3:1	Tt VV	L/NL	3:1
MM Aa Ff MM aA Ff mM AA Ff	L/NL	9:7	Tt Vv Tt vV	L/NL	9:7
mM Aa Ff mM aA Ff	L/NL	27:37			

[a] Reynolds et al, Am . J. Enol. Vitic., 33:1:14-19, 1982
[b] L = labrusca, NL = non-labrusca flavour character

Table 2: Threshold values for organoleptic detection of labrusca flavour characters

Methyl anthranilate	0.10 ppm	(Nelson et al, 1977)
Total volatile esters	12.0 ppm	(Fuleki, 1982)

Harvest

At maturity, the total crop was harvested from each seedling vine (total crop was sub-sampled from a parent vine). Grapes were washed, removed from the stems, frozen and maintained at -30 °C until analysis. Analyses for MA and TVE were carried out within 3 months of the harvest date. Data are presented for the harvests of 1986, 1987 and 1988 in Tables 3-6.

To obtain representative samples for analyses, frozen grape samples were ground without breaking the seeds. A household and a 5 HP commercial meat grinder (Butcher Boy, Model A 42.50, Laser Mfg. Co. Inc., Los Angeles, CA, USA) were used for smaller and larger lots respectively. The ground frozen material was thoroughly mixed and two 50 g aliquots were removed for steam distillation.

Steam distillation

Samples were placed in an all-glass distillation apparatus (Cat. No. JD-2115, SGA Sci., Bloomfield, N.J., USA). The distillation was carried out on duplicate 50 g samples, collecting 100 ml in 15 min. The distillate was used to determine both the MA and the TVE. Glass distilled water was steam distilled under the same conditions as the grapes to produce blanks for the MA and TVE analyses.

Determination of MA

A highly sensitive fluorometric method was used (Casimir *et al.* 1976). The measurements were carried out on a ZFM 4 fluorescence attachment of a Zeiss DMR21 recording spectrophotometer (C. Zeiss, Oberkochen, Germany). The exciting radiation was isolated with a

Table 3: Methyl anthranilate values and equivalent labrusca/non-labrusca codes for Family 8020 (1986 to 1988)

Seedling Number	Methyl anthranilate (ppm)					
	1986	Code[a]	1987	Code	1988	Code
1	1.776	L	7.446	L	2.776	L
2	0.136	L	-	-	-	-
3	-	-	5.096	L	4.596	L
4	0.618	L	0.560	L	-	-
5	-	-	17.218	L	14.746	L
6	0.290	L	0.482	L	0.767	L
7	0.244	L	0.676	L	2.164	L
8	-	-	-	-	8.546	L
9	-	-	-	-	0.037	NL
10	0.143	L	0.042	NL	0.052	NL
11	-	-	1.786	L	1.950	L
12	-	-	6.446	L	11.326	L
13	-	-	-	-	0.541	L
15	0.866	L	0.039	NL	1.654	L
16	4.076	L	4.076	L	5.386	L
17	5.436	L	5.556	L	-	-
19	-	-	0.022	NL	0.079	NL
20	0.163	L	0.239	L	0.261	L
21	0.358	L	0.565	L	0.882	L
23	0.093	NL	0.479	L	1.733	L
24	-	-	0.106	L	0.192	L
25	7.956	L	9.836	L	6.819	L
26	1.746	L	4.876	L	2.626	L
27	0.096	NL	0.301	L	0.354	L
28	-	-	-	-	2.167	L
29	0.006	NL	0.006	NL	0.014	NL
30	0.079	NL	0.471	L	-	-
31	3.436	L	-	-	-	-
32	0.366	L	1.356	L	1.119	L
33	4.166	L	12.606	L	11.796	L
34	-	-	0.065	NL	0.070	NL
35	2.936	L	2.826	L	6.071	L
37	0.000	NL	0.011	NL	0.031	NL
38	-	-	-	-	0.992	L
39	0.013	NL	0.032	NL	0.044	NL
40	0.032	NL	0.018	NL	0.015	NL
42	0.000	NL	0.006	NL	0.044	NL
43	0.036	NL	-	-	0.060	NL
44	-	-	3.046	L	2.583	L
45	10.096	L	15.776	L	7.826	L

[a] L = labrusca, NL = non-labrusca flavour character

365 nm monochromatic filter from the line spectrum of a mercury lamp. The 4MQIII monochromator of the spectrophotometer was set at 425 nm to measure the fluorescence emitted by the MA. The fluorometer reading was related to the MA concentration by using a standard curve. To eliminate interference from fluorescent volatile substances other than methyl and ethyl anthranilate, the reading taken on a *Vitis vinifera* L. cultivar corresponding to a few ppb MA was subtracted from each measurement.

Table 4: Methyl anthranilate values and equivalent labrusca/non-labrusca codes for Family 8021 (1986 to 1988)

Seedling Number	Methyl anthranilate (ppm)					
	1986	Code[a]	1987	Code	1988	Code
1	0.176	L	1.526	L	2.370	L
2	0.179	L	0.816	L	0.72	L
3	-	-	0.037	NL	0.087	NL
4	2.726	L	7.516	L	2.836	L
5	0.189	L	1.436	L	0.931	L
6	-	-	0.655	L	0.530	L
8	0.386	L	0.024	NL	0.097	NL
10	0.748	L	-	-	0.511	L
11	0.023	NL	0.361	L	1.346	L
12	0.336	L	-	-	1.137	L
13	0.082	NL	0.075	NL	0.066	NL
14	0.081	NL	0.121	L	0.172	L
15	3.696	L	15.676	L	11.776	L
17	-	-	17.696	L	13.966	L
18	3.976	L	8.216	L	2.832	L
19	0.077	NL	0.221	L	-	-
20	0.136	L	0.053	NL	0.202	L
21	0.471	L	1.112	L	2.206	L
23	0.452	L	0.630	L	0.571	L
24	0.020	NL	0.026	NL	0.029	NL
25	0.034	NL	0.137	L	0.140	L
27	0.251	L	0.683	L	0.436	L
28	0.627	L	1.416	L	1.715	L
29	1.236	L	2.776	L	4.055	L
30	0.730	L	1.876	L	-	-
31	-	-	-	-	2.919	L
32	0.028	NL	0.384	L	0.213	L
33	0.480	L	1.424	L	0.639	L
34	1.236	L	2.546	L	2.366	L
35	0.451	L	0.752	L	0.925	L
38	1.326	L	2.705	L	2.266	L
39	0.484	L	1.276	L	1.074	L
40	2.836	L	3.766	L	5.207	L
42	2.566	L	3.586	L	6.336	L
43	-	-	-	-	0.038	NL
44	-	-	0.018	NL	3.266	L
45	-	-	0.924	L	0.920	L
46	0.559	L	1.626	L	0.694	L
47	-	-	0.051	NL	0.081	NL
48	0.159	L	0.096	NL	0.193	L
50	0.010	NL	0.063	NL	0.043	NL
54	-	-	-	-	5.206	L

[a] L = labrusca, NL = non-labrusca flavour character

Determination of TVE

A modification of THOMPSON's method (1950) was used. The reaction of esters with hydroxylamine in aqueous alkaline solution forms hydroxamic acids, which react with the ferric ion to form red ferric hydroxamate complexes.

Table 5: Total volatile esters values and equivalent labrusca/non-labrusca codes for Family 8020 (1986 to 1988)

Seedling Number	Total volatile esters (ppm)					
	1986	Code[a]	1987	Code	1988	Code
1	56	L	66	L	59	L
2	128	L	-	-	-	-
3	-	-	90	L	456	L
4	170	L	28	L	-	-
5	-	-	81	L	197	L
6	233	L	223	L	126	L
7	3	NL	5	NL	9	NL
8	-	-	-	-	20	L
9	-	-	-	-	2	NL
10	212	L	6	NL	162	L
11	-	-	19	L	15	L
12	-	-	22	L	61	L
13	-	-	-	-	266	L
15	92	L	70	L	70	L
16	150	L	141	L	208	L
17	288	L	58	L	-	-
19	-	-	2	NL	3	NL
20	235	L	20	L	42	L
21	5	NL	8	NL	21	L
23	2	NL	2	NL	4	NL
24	-	-	62	L	297	L
25	127	L	32	L	48	L
26	239	L	75	L	134	L
27	-	-	144	L	255	L
28	-	-	-	-	36	L
29	20	L	22	L	21	L
30	3	NL	2	NL	-	-
31	116	L	-	-	-	-
32	89	L	58	L	95	L
33	52	L	51	L	181	L
34	-	-	20	L	166	L
35	45	L	39	L	66	L
37	1	NL	1	NL	1	NL
38	-	-	-	-	3	NL
39	80	L	36	L	72	L
40	4	NL	62	L	186	L
42	1	NL	2	NL	2	NL
43	4	NL	-	-	2	NL
44	-	-	40	L	152	L
45	194	L	154	L	119	L

[a] L = labrusca, NL = non-labrusca flavour character

Fresh alkaline hydroxylamine solution was prepared by mixing equal volumes of 6 M hydroxylamine hydrochloride and 10.5 N sodium hydroxide. 2 ml of this solution and 20 ml of the distillate were added to a 25 ml volumetric flask. These were mixed thoroughly and allowed to stand for 5 min. Then, 1 ml of concentrated hydrochloric acid was added, followed by 1 ml of 1.11 M ferric chloride. The flask was filled to volume with 0.046 M ferric chloride solution and the colour measured on a Zeiss DMR21 spectrophotometer at 500 nm. A standard curve, prepared by

Table 6: Total volatile esters values and equivalent labrusca / non-labrusca codes for Family 8021 (1986 to 1988)

Seedling Number	Total volatile esters (ppm)					
	1986	Code[a]	1987	Code	1988	Code
1	139	L	63	L	38	L
2	6	NL	55	L	2	NL
3	-	-	1	NL	4	NL
4	1	NL	3	NL	3	NL
5	89	L	24	L	59	L
6	-	L	59	L	61	L
8	91	L	56	L	55	L
10	1	L	-	-	4	NL
11	56	L	55	L	79	L
12	120	L	-	-	26	L
13	168	L	15	L	30	L
14	4	NL	1	NL	1	NL
15	183	L	147	L	261	L
17	-	-	56	L	93	L
18	105	L	100	L	58	L
19	1	NL	1	NL	-	-
20	118	L	28	L	100	L
21	108	L	54	L	51	L
23	174	L	82	L	154	L
24	44	L	17	L	25	L
25	1	NL	0	NL	1	NL
27	204	L	95	L	187	L
28	1	NL	1	NL	3	NL
29	2	NL	2	NL	2	NL
30	83	L	40	L	-	-
31	-	-	-	-	3	NL
32	54	L	16	L	128	L
33	165	L	59	L	238	L
34	51	L	12	NL	66	L
35	35	L	15	L	22	L
38	63	L	18	L	52	L
39	175	L	42	L	79	L
40	80	L	72	L	81	L
42	88	L	28	L	35	L
43	-	-	-	-	1	NL
44	-	-	0	NL	33	L
45	-	-	4	NL	23	L
46	184	L	36	L	170	L
47	-	-	0	NL	5	NL
48	56	L	28	L	52	L
50	1	NL	0	NL	2	NL
54	-	-	-	-	73	L

[a] L = labrusca, NL = non-labrusca flavour character

using various concentrations of ethyl acetate, was used to determine the concentration of the TVE in the sample.

Statistical analysis

Chi square analyses were performed on the observed ratios of labrusca/non-labrusca flavour components as they were identified by the MA and TVE analyses.

Results

The seedling families 8020 and 8021 (selfed populations of V. 72181 and V. 72182, respectively) displayed transgressive segregation in both MA and TVE distribution (Tables 3-6). All four distributions were highly skewed to the lower values of MA and TVE. To simplify the data, all MA and TVE values were coded as to labrusca or non-labrusca in flavour contribution, according to the threshold values in Table 2, and presented in Tables 3-6. These threshold values were also used by REYNOLDS *et al.* (1982).

The ratios postulated by the authors using genotypic assumptions of REYNOLDS *et al.* (1982) were tested and are presented in Tables 7-10.

Table 7: Chi square tests for MA segregation of Family 8020 (1986 to 1988)

Year	Predicted[a]	Observed	Statistic		
			X^2	P value	Significance[b]
1986	L/NL 3:1	18:9	0.50	$0.50<p<0.30$	ns
1987	L/NL 3:1	24:8	0.031	$0.90<p<0.70$	ns
1988	L/NL 3:1	25:10	0.08	$0.90<p<0.70$	ns
1986	L/NL 9:7	18:9	0.80	$0.50<p<0.30$	ns
1987	L/NL 9:7	24:8	3.84	$p = 0.05$	**
1988	L/NL 9:7	25:10	2.67	$0.10<p<0.05$	*

[a] L/NL = labrusca/non-labrusca flavour character

[b] significance levels for rejection: p=0.10=*, p=0.05=**, p=0.001=***

Table 8: Chi square tests for MA segregation of Family 8021 (1986 to 1988)

Year	Predicted[a]	Observed	Statistic		
			X^2	P value	Significance[b]
1986	L/NL 3:1	26:7	0.06	$0.90<p<0.70$	ns
1987	L/NL 3:1	28:9	0.013	$0.95<p<0.90$	ns
1988	L/NL 3:1	33:7	0.833	$0.50<p<0.30$	ns
1986	L/NL 9:7	26:7	5.87	$0.05<p<0.01$	**
1987	L/NL 9:7	28:9	4.92	$0.05<p<0.01$	**
1988	L/NL 9:7	33:7	10.15	$0.01<p<0.001$	***

[a] L/NL = labrusca/non-labrusca flavour character

[b] significance levels for rejection: 0=0.10=*, p=0.05=**, p=0.001=***

Discussion

The 3 : 1 segregation ratio for both MA and TVE in both families 8020 and 8021 had the most acceptable Chi square values. This would mean that the V. 72181 and V. 72182 genotype for both MA and TVE must be segregating for only one heterozygous locus each. For MA, if that locus is F, then the ff recessive must allow for some production of MA because no seedlings measured in family 8020 or 8021 had a zero level for MA with any consistency over the three harvest seasons. For it to be a locus other than F would preclude the grandparent Le Général and the great grandparent De Chaunac from having such low levels of MA. Perhaps the REYNOLDS hypothesis (REYNOLDS 1980) of an alternate MA synthesis pathway exists under the ff recessive condition as well as under the aa and/or mm recessive conditions.

The TVE inheritance pattern for both families 8020 and 8021 follows REYNOLDS' hypothesis (REYNOLDS 1980) since there was no condition completely precluding the synthesis of TVE.

Table 9: Chi square tests for TVE segregation of Family 8020 (1986 to 1988)

Year	Predicted[a]	Observed	Statistic		
			X^2	P value	Significance[b]
1986	L/NL 3:1	18:8	0.20	0.70<p<0.50	ns
1987	L/NL 3:1	24:8	0.0	p = 1	ns
1988	L/NL 3:1	27:8	0.013	0.90<p<0.70	ns
1986	L/NL 9:7	18:8	1.32	0.30<p<0.20	ns
1987	L/NL 9:7	24:8	3.84	p = 0.05	**
1988	L/NL 9:7	27:8	5.36	0.05<p<0.01	**

[a] L/NL = labrusca/non-labrusca flavour character

[b] significance levels for rejection: p=0.10=*, p=0.05=**, p=0.001=***

Table 10: Chi square tests for TVE segregation of Family 8021 (1986 to 1988)

Year	Predicted[a]	Observed	Statistic		
			X^2	P value	Significance[b]
1986	L/NL 3:1	24:9	0.009	0.95<p<0.90	ns
1987	L/NL 3:1	25:12	0.73	0.50<p<0.30	ns
1988	L/NL 3:1	28:12	0.31	0.70<p<0.50	ns
1986	L/NL 9:7	24:9	2.96	0.10<p<0.05	*
1987	L/NL 9:7	25:12	1.51	0.30<p<0.20	ns
1988	L/NL 9:7	28:12	2.54	0.20<p<0.10	ns

[a] L/NL = labrusca/non-labrusca flavour character

[b] significance levels for rejection: p=0.10=*, p=0.05=**, p=0.001=***

Acknowledgements

The assistance of R.B. PALABAY with the analytical determinations is gratefully acknowledged.

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Breeding grapes for basal fruitfulness

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A b s t r a c t : An attempt has been made to develop varieties with fruitful basal buds, as this character is of prime importance for any cultivar to be grown successfully on low-cost training systems like the head system. As a first step, the fruiting behaviour of several accessions from diverse geographic origin was made and certain genotypes were identified as promising sources for the desired character. Employing a microscopic bud analysis technique, bud fertility of these genotypes was measured from 2nd to 13th node and various biometric parameters were worked out to determine the extent of variability and inheritance of this character. The phenotypic and genotypic coefficients of variance were moderate to high and heritability estimate was convincing enough to begin hybridization.Extensive hybridization work was carried out for several years employing the identified parents and this has resulted in large number of hybrid progenies. The fertility analysis of these hybrids and their respective parents indicated that the varieties Bangalore Blue, Black Champa, Queen of the Vineyards and Convent Large Black are potential sources for basal fruitful buds. To incorporate this desirable character in the commercial cultivars like Anab-e-Shahi and Thompson Seedless, large number of crosses were made involving these cultivars and potential sources. From these progeny we were able to locate some promising hybrids which, besides possessing fruitful basal buds, are prolific bearers of high quality fruits.