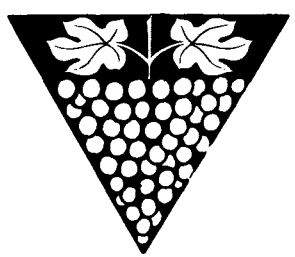
Proceedings
of the
THIRD INTERNATIONAL SYMPOSIUM
ON GRAPE BREEDING



JUNE 15-18, 1980

In conjunction with
Grape and Wine Centennial Symposium 1980
Department of Viticulture and Enology
University of California, Davis

FOREWORD

The First International Symposium on Grape Breeding was convened in 1973 in West Germany at the renowned institution of Geilweilerhof (Pfalz), continuing the tradition of Erwin Baur and his successors, Bernard Husfeld and G. Alleweldt. Some of the papers presented were published at intervals in Vitis and other journals.

The Second Symposium was hosted in Bordeaux, June 14-18, 1977 under the auspices of the Institut National de la Recherche Agronomique, Station de Recherches de Viticulture, Centre de Recherches de Bordeaux, Pont-de-la-Maye, France. The Proceedings were published by INRA, 149 Rue de Grenelle 75007, Paris, 1978.

The Third International Symposium on Grape Breeding was hosted by the University of California at Davis, June 15-18, 1980. This was an auspicious occasion, since it was held in conjunction with the Centennial Symposium of the Department of Viticulture and Enology.

This publication comprises most of the papers presented during the Grape Breeding Symposium. A considerable amount of editing and translation has been necessary. To avoid further delay, no proofs were sent to authors in foreign countries.

I wish to thank the Department of Viticulture and Enology and Mrs. Peggy Howarth for assisting me with the preliminary editing. The final editing, format arrangement and typing of these *Proceedings* are the result of the patience and expertise of Mrs. Evelyn Zielesch, to whom I am grateful. We also wish to acknowledge the assistance of Mr. Stephen Andrews, University of California, Printing Department, Berkeley.

H. P. Olmo
Professor of Viticulture, Emeritus
University of California, Davis
and Program Chairman

CONTENTS

SECTION IV

BREEDING FOR DISEASE

AND INSECT RESISTANCE

CLONAL SELECTION AND GENE POOL PRESERVATION OF TRADITIONAL GRAPE CULTIVARS

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ABSTRACT

Grape cultivars which have been grown for centuries in traditional wine regions are being replaced more and more by a few clones. These varieties often show high genetic variability which is important to conserve.

A large research program was undertaken in some very old vineyards in the northeast of France in order to select about 500 genotypes of each principal variety. The objective was to explore the heterogeneity within populations and to provide basic information for clonal selection programs.

The first investigations were done on Savagnin (Traminer), Riesling, and Pinot varieties.

Vine cultiavars often show a very high genetic variability resulting from the accumulation of mutations. This results from vegetative propagation over many centuries. This variability is particularly clear in traditional vine growing areas where a single cultivar has often been grown for centuries, if not millenia.

As a result of clonal selection, many vine cultivars would be constituted into a few clones which may be of interest in modern viticulture. At the same time, however, the variability of the population of cultigens rapidly diminishes.

In order to conserve this variability, in 1971 we started a large safeguard operation for the cultivars planted in the northeast of France. This work concerned: 1) the Savagnins group composed of the highly aromatic Gewürztraminer, the non-aromatic pink Savagnin, and the white Jura Savagnin. All of these have similar foliage,

2) the Riesling, and

3) the Pinot group including Pinot blanc, Pinot gris, and Pinot noir.

METHODS AND MATERIALS

For each operation about 40 plots planted before 1930 were choosen, i.e., at a time when a systematic mass selection did not play a big role. Each of these plots represents a kind of "mini-population" where many vines, in fact, have the same

origin, i.e., they belong to the same clone; and it is, therefore, very important to use the largest possible basis.

Controls were made for four years on three different dates: in spring, in fall before the harvest, and in winter. For each cultivar group, about 15 to 17,000 vines have been checked 12 times for yield, vigor, and virus symptoms according to a 5-point visual evaluation scale and for the appearance of particular ampelographic characters.

At the end of these four years' observations in the vineyard, we made a choice of about 650 clones from all the different plots. For three quarters of the vines chosen, the main criteria were the variability of yield and vigor, and for the remaining quarter it was the ampelographic characters: color of the herbaceous shoots, canes, foliage, berries, hairiness and leaf shape, Of course, clones which showed virus symptoms were eliminated.

For each clone, 30 grafts on *Riparia-berlandieri* 504 were made and put in a nursery. One year in the nursery allowed further control, mainly for detecting leafroll virus.

In the spring of the sixth year (1976), five vines of each clone were planted in an homogeneous fumigated plot. The trellising system was the traditional one used in Alsace.

RESULTS AND DISCUSSION

Objectives of the genetic variability preservation plots: These plots represent a store of intra-cultivar variability. Many observations have to be made and collected for biometrical calculation on computer. It will, therefore, be possible to study the intra-cultivar genetic variability. This variability will allow future clonal selection.

The first of these plots concerning the Savagnin group was planted in 1976, but the first observations were not made until 1979. We have observed differences among Gewürztraminer, non-aromatic pink Savagnin, and white Savagnin in yield, in sugar content, acidity, and pruning wood weights (Figs. 1 - 4).

We hope that these first results will provide the basis for future clonal selection. The main interest of this work is to conserve variability reserves and to describe intra-cultivar variations in major grapevine varieties.

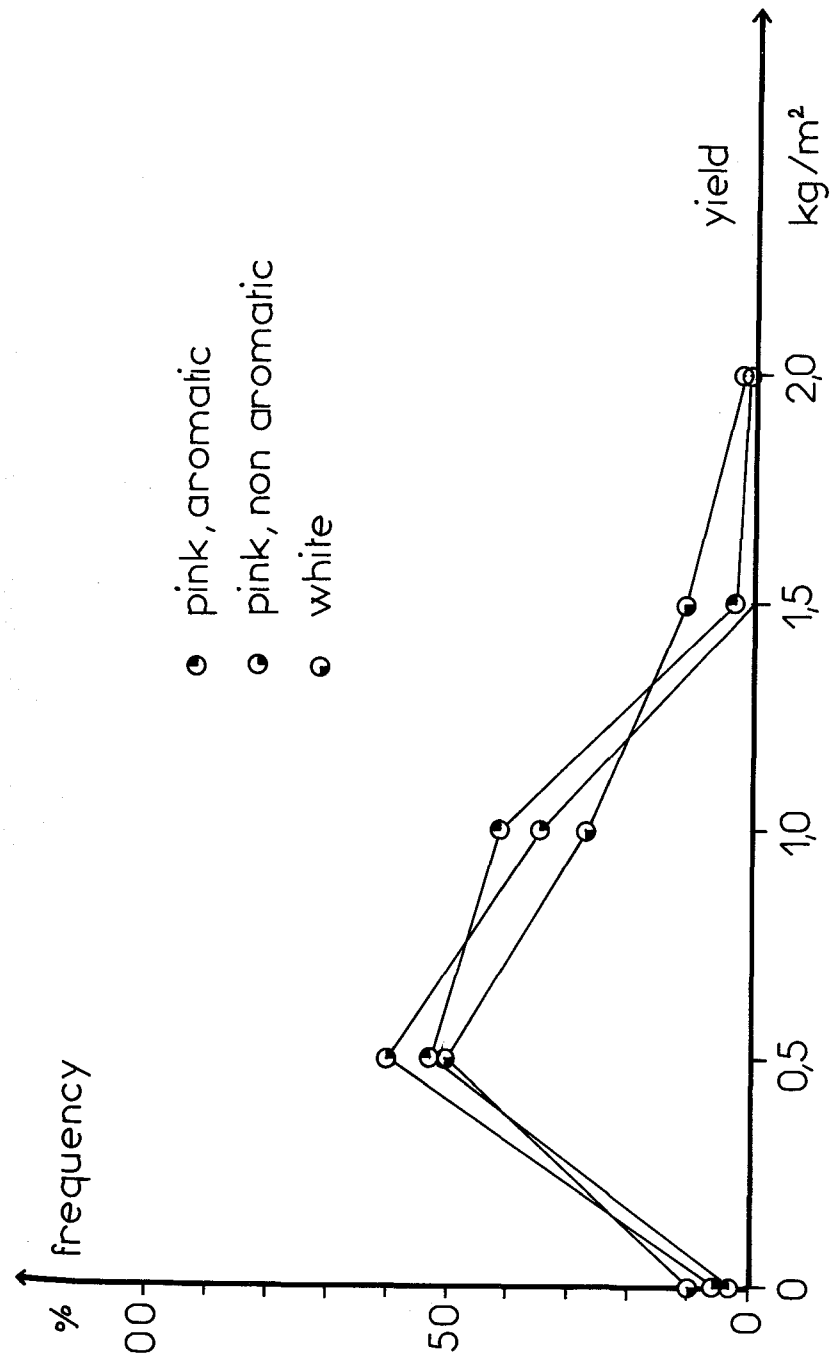

Fig. 1. Yield of Savagnin clones, 1979.

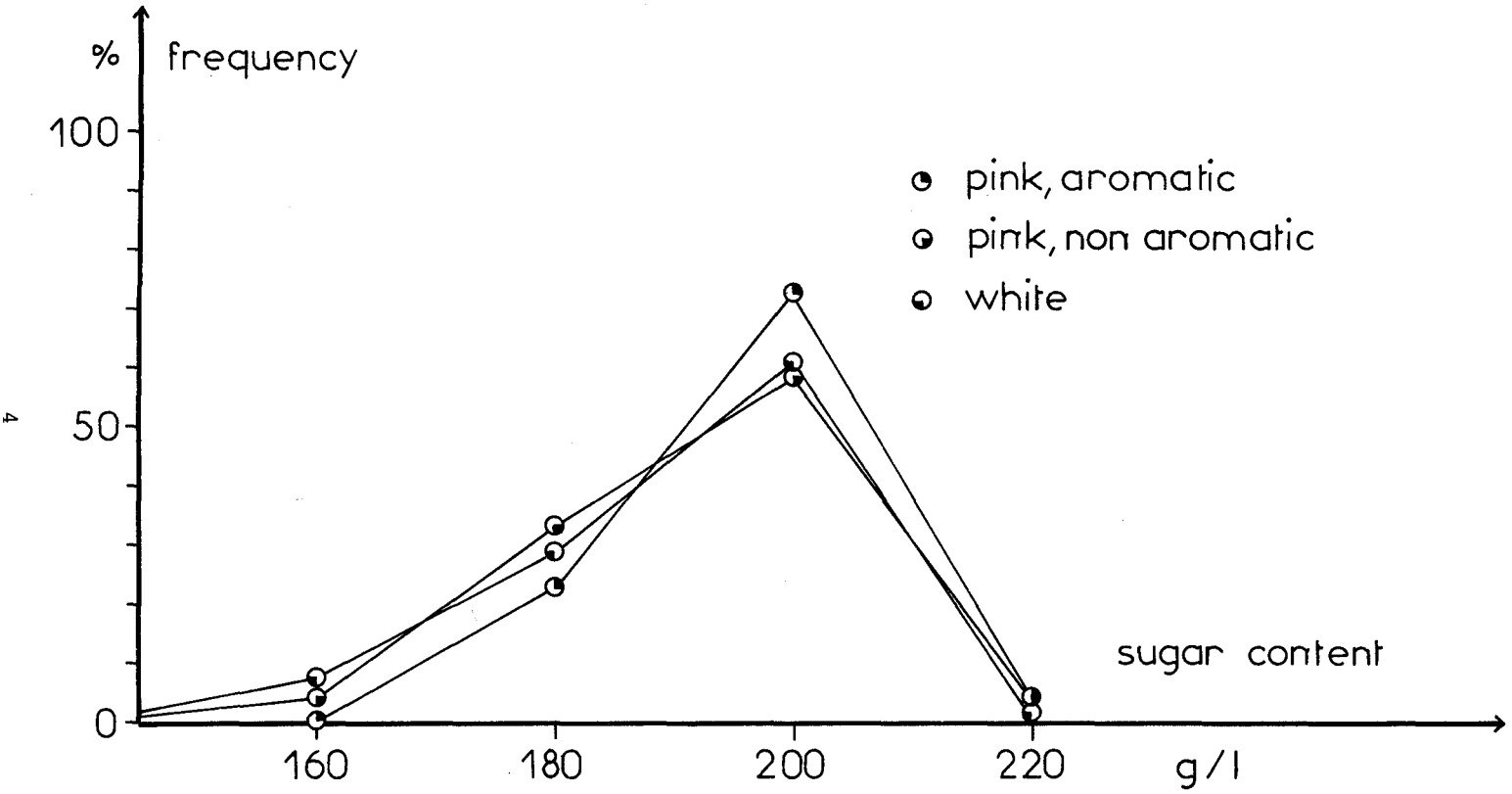

Fig. 2. Sugar content of Savagnin clones, 1979.

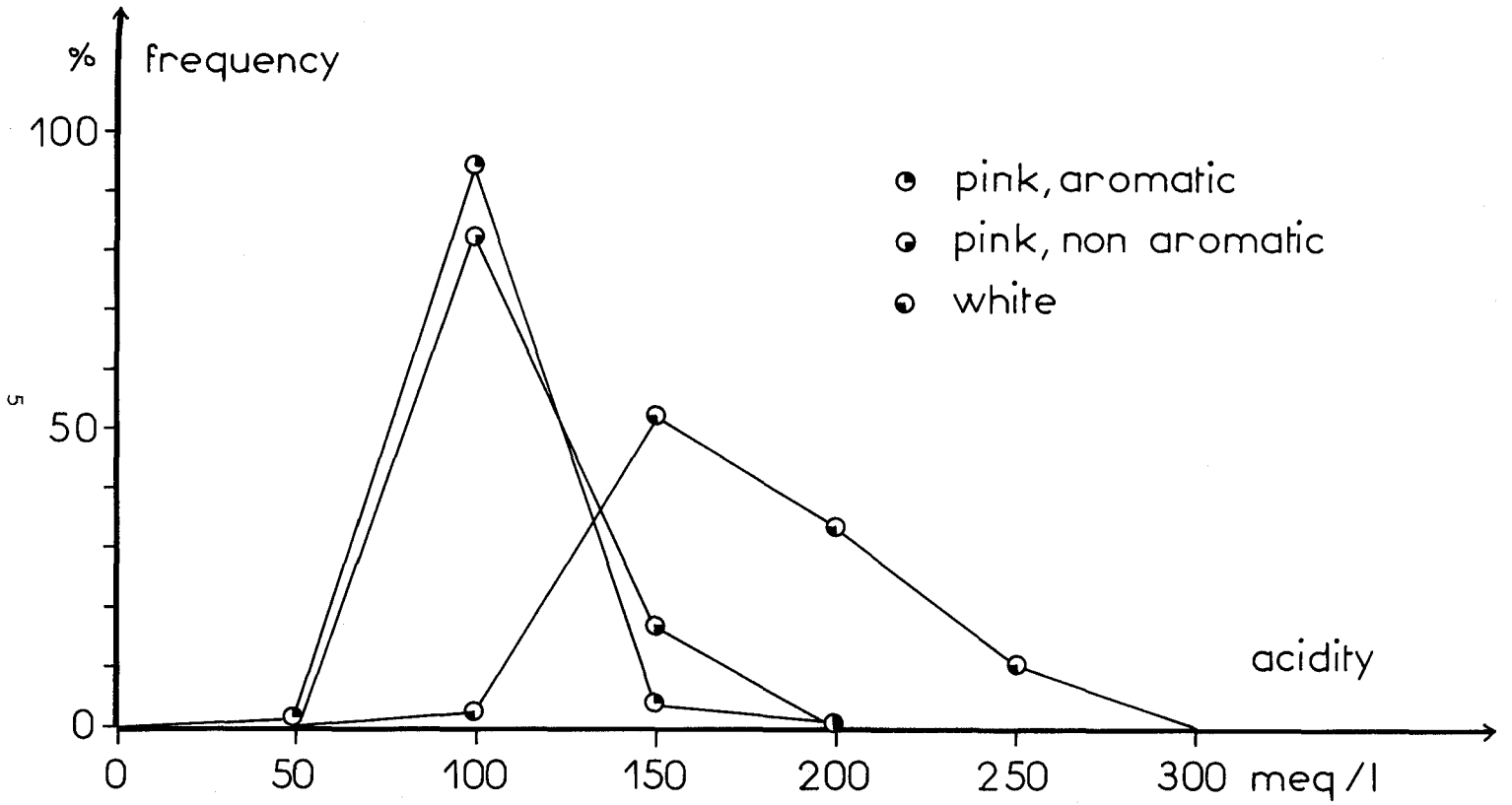

Fig. 3. Savagnin clones in 1979.

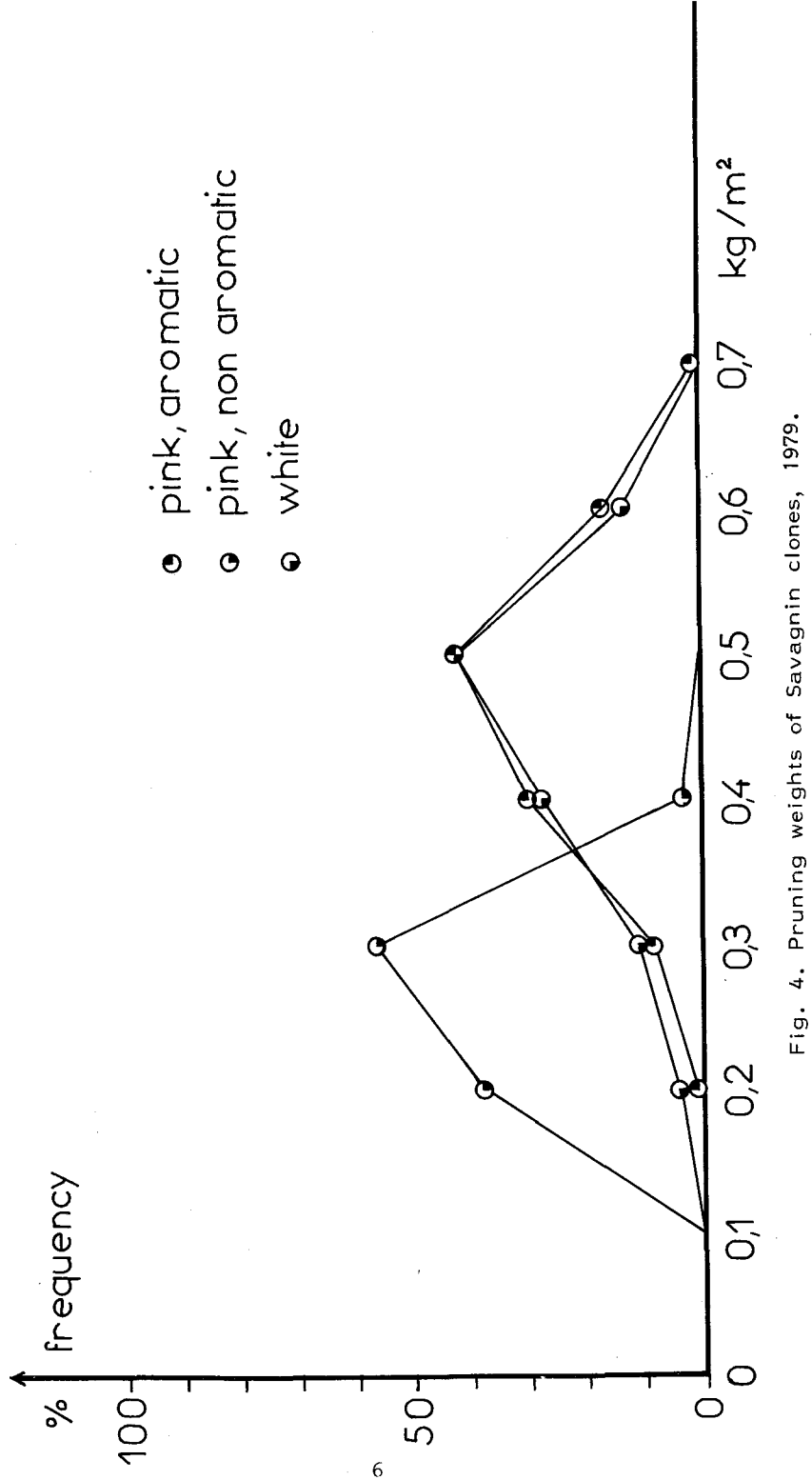

Fig. 4. Pruning weights of Savagnin clones, 1979.

GERM PLASM OF VITIS VINIFERA IN YUGOSLAVIA

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ABSTRACT

The ampelographic collection in SFR Yugoslavia numbers about 1500 wine and table varieties and rootstocks. Included are several autochthonic varieties which are interesting in several agrobiological and technological properties, especially in fruiting potential, resistance to *Botrytis cinerea*, etc. Within autochthonic and some introduced varieties, many clones with high agrobiolobical and technological properties were observed. Also there exists a stock of 50,000 seedlings which are a result of inter- and intraspecific hybridization as well as 20 newly created table and wine varieties for commercial introduction.

Climatic and soil conditions are very variable in Yugoslavia. The country is divided into seven climatic zones, including Mediterranean and continental climates. Added to the climatic diversity is the ebb and flow of the history of the Balkan peninsula, astride important sea and land routes. The country is in a central position in relation to the distribution of geographical races of cultivated grapevines, as outlined by Negrul, occupying the bridging area between the western European wine grape group "occidentalis" and the middle eastern table grape group or "orientalis".

Until the devastation of vineyards caused by the introduction of phylloxera, Yugoslav viticulture relied entirely on the assortment of ancient and indigenous domesticated varieties, types fulfilling local needs for both table and wine use. Many of these varieties have female flowers.

With reconstitution of vineyards, many wine grapes of high quality were introduced from western Europe for the first time, including many that proved their merit and became widely planted. These included Sémillon, Sauvignon, Italian Riesling, Pinots, Sylvaner, Gamay, Cabernet sauvignon, Cabernet franc and Merlot.

MATERIALS AND METHODS

Populations of *Vitis vinifera* subspecies *sylvestris* Gmel. in Yugoslavia: Natural populations of wild *vinifera* have been discovered in many river valleys and are particularly well represented along the Dunav, Neretva and Vardar Rivers. Different vine types have been described according to leaf indentation, either lobed or entire; sexual type of the flower, either functionally male or female; black fruit color is variable in expression but is classified into two groups: alba and nigra.

The fruit clusters are very small in size and usually have straggly clusters. The vines are not resistant to low winter temperatures and their reaction to downy mildew *(Plasmopara viticola)* in their natural environment appears to differ from the cultivated varieties. More detailed studies of the indigenous vines of Yugoslavia are in the planning stage.

Ampelographic collection in Yugoslavia: Special attention has been directed to the collection of domestic and indigenous cultivars. Introductions from other viticultural countries, including wine and table varieties of vinifera, have increased the total number of cultivar accessions to approximately 1,500.

Resistance to low winter temperature is an important consideration in making collections of native North American and Asiatic species. *Vitis amurensis* from eastern Asia is well represented. Introductions have been classified on the basis of winter hardiness into three main groups:

1) Very resistant, buds surviving temperatures of -23 to -27° C.

2) Resistant, buds surviving temperatures of -18 to -21°C.

3) Slightly resistant, buds surviving temperatures of -14 to -17°C.

It has been necessary to test our collections in the different viticultural zones of the country, giving special attention to the following problems:

1) High and regular productivity

2) High quality of the product

3) Resistance to fungus diseases

4) Resistance to virus diseases

5) Resistance to low temperatures.

Resistance to fruit spoilage caused by *Botrytis* infection is considered a prime objective, because of the rainfall experienced during harvest. Varieties are classified in three groups: resistant, moderately resistant and susceptible. The vegetative vigor of the vine plays a role, and rootstocks must also be evaluated as a contribution to the reaction of the fruiting cultivar.

Populations of hybrid grape seedlings: The grape breeding program has been given increased emphasis during the past three years. Over 50,000 seedlings have been grown, representing more than 300 parental combinations. Both inter- and intraspecific hybridization has been employed. The objectives are the production of new varieties of wine and table grapes that are highly productive, have multiple resistance to the common fungus diseases, principally *Plasmopara, Oidium, and Botrytis* and that are resistant to low temperature. New rootstocks are also being

selected. The season of maturity will be extended in table grapes by selecting for earlier and later types than the current varieties now used.

Clonal selection of indigenous and imported varieties: Many of the cultivars introduced more recently from foreign countries such as France, Germany, Italy, Russia and other eastern countries are represented in our collection by a number of clones. These include the following:

Afus-Ali	6 clones	Pinot blanc	5 clones
Cabernet franc	4 "	Pinot noir	8 "
Cabernet sauvignon	6 "	Red Traminer	15 "
Franconie	3 "	Rkasiteli	3 "
Gamay	4 "	Saperavi	4 "
Italian Riesling	6 "	Sémillon	5 "
Merlot	16 "		

These clones are characterized generally by high yield, some resistance to low temperatures, good sugar content and aroma in the must, and some resistance to *Botryis cinerea.*

Clonal selection has also been applied to 20 important local and indigenous varieties; the number of clones retained are given in Table 1.

TABLE 1. Clones of local and indigenous cultivars.

Variety	Type	No. clones	Variety	Type	No. clones
Bagrina	RW	2	Plavac mali	RW	4
Belo zimsko	T	3	Plovdina	RW	8
Bogdanusa	WW	4	Prc	WW	2
Kadarun	RW	3	Prokupac	RW	34
Kraski teran	RW	4	Smederevka	WW	2
Kratosija	RW	0	Stanusina	RW	4
Malvazija istarska	WW	5	Valandovski red drenak	T	0
Marastina	WW	4			
Nincusa	RW	4	Vranac	RW	0
Okatac	RW	2	Zacinak	RW	4
			Zilavka	WW	2

*RW = Red wine, WW = White wine, T = Table

In addition to the principal varieties, clonal selection has not proceeded on some 50 additional indigens.

CONCLUSIONS

Yugoslavia is endowed with a very rich and diversified germ plasm of *Vitis vinifera*. The wild *vinifera* subspp. *sylvestris* Gmel. still exists in some of the river valleys in the dioecious form but has small clusters and is relatively unfruitful. More detailed study of its genotype, comparative morphology and physiology, vis-à-vis the indigenous cultivars, is necessary to establish some proof of evolutionary descent.

Added to the large number of indigenous cultivars were the high quality wine varieties of Western Europe that were imported after the decimation of the Yugoslav vineyards by phylloxera. Collections were enriched further by introduction of North American and Asiatic species.

Both historically and geographically, Yugoslavia occupies a favored position for the mingling of *vinifera* germ plasm.

Extensive collections are put to use in a breeding project to improve yield and quality. Emphasis is on introducing resistance to fungus diseases and greater cold hardiness.

Introduction of improved germ plasm by clonal selection has been accomplished in both indigenous and foreign cultivars.

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INTERSPECIFIC HYBRIDS USED IN BREEDING WINE GRAPES FOR SOUTHERN ONTARIO, CANADA (43°N LATITUDE)

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ABSTRACT

The use of native *V. riparia* as a direct parent is less useful than complex, interspecific hybrids in improving quality and hardiness of cultivars developed for southern Ontario. Interspecific hybrids used prior to 1949 came from T. V. Munson (Texas) as combinations of *V. linceumii, V. rupestris, V. bourquiniana, V. labrusca, V. champini,* and *V. vinifera,* and from E. S. Rogers (Massachusetts) as *V. labrusca* and *V. vinifera* crosses. After 1949, French direct-producer hybrids were used with greater success in developing cultivars for the wine industry. These hybrids were based on *V. rupestris, V. linecumii,* and *V. vinifera,* with only very slight reference to *V. labrusca* and *V. riparia.* The present program involves Vineland hybrids derived from the above, backcrossed to *V. vinifera* and some more promising French hybrids.

There are two major districts for grape growing in Canada, one approximately 2000 hectares in British Columbia in the western sector of the country and the other approximately 10,400 hectares in southern Ontario, in the eastern sector of the country. The larger area in southern Ontario has a climate moderated by the presence of the Great Lakes, particularly Lake Ontario. This large body of water remains unfrozen most winters, mitigating the temperature of the prevailing west winds and providing moisture for a protective snow cover. The most favored district is situated on the south shore of Lake Ontario between Hamilton and Niagara Falls. Within this area, winter minima and spring frost risk can differ considerably as a result of an escarpment of 110 m running roughly parallel to the lake. Air drainage due to this slope and/or distance from the lake are critical in determining the suitability of the individual site to the growing of labrusca, American hybrid, French producer hybrid or pure vinifera cultivars.

Winter minima average -7.0°C in midwinter with extremes to -26°C. Winter maxima average -0.5°C with extremes to +20.0°C. Summer minima average +13.0 to +16.0°C with extremes to +4.0 to +6.0°C. Summer maxima average 26.0°C with extremes to +39.0°C. The seasons in general suffer from fluctuating temperatures with an average frost free season of approximately 177 days (air temperatures, 50-year average, Vineland Station, Ontario). Winter hardiness is a problem in that hardy varieties with low chilling requirements will break bud early during a January thaw and be damaged by later cold weather. Varieties with longer chilling requirements but less absolute hardiness are killed by the minima extremes.

Precipitation is generally even throughout the season with an average of 6 cm per month but 8 cm in August. This tends to aggravate *Botrytis* in thin-skinned varieties and mildew in susceptible varieties.

As a wild vine in its native state, *Vitis riparia* Michx. grows to profusion in the area described above, being a procumbent vine, frequent in the hedgerows and deciduous woods and having considerable longevity. The winter minima and the fluctuation temperatures over the whole winter season do not seem to affect the hardiness of this species. As a result, it has been used in the wine grape breeding program of the Horticultural Research Institute of Ontario.

The tables show some of the results of using *V. riparia* Michx. in various forms, as a direct parent, clonal selections of *V. riparia* Michx. as a direct parent, a grandparent, and possible great-grandparent.

The use of *V. riparia* Michx. as a direct parent has not proven very useful in a program designed to produce new varieties rather than lines carrying certain traits. Table 1 shows that only one selection from each of three families was deemed suitable for retention in the breeding stock. This first generation was very characteristic of a pure *riparia,* having small shiny leaves, small berries, small straggly bunches, breaking bud very early and exhibiting early leaf fall. Nearly all fruit was blue. Perhaps if these selections are selfed, a broader segregation will be seen.

TABLE 1. Breeding families using *V. riparia* Michx. as a direct parent.

Family No.	Parents	No. seeds produced	No. vines planted	No. of selections
6414	Elvira x *V. riparia*	68	12	1
6706	S.13047 x *V. riparia*	741	488	1
6716	V.49404 x *V. riparia*	900	684	4
6719	Vincent x *V. riparia*	614	413	1

In Table 2, a similar collection of crosses are shown. The South Dakota clones, although very hardy in their own right, imparted little of this in the progeny and many seedlings were lost over the first winter in the nursery.

TABLE 2. Breeding families using clonal selections of *V. riparia* Michx. as a direct parent.

Family No.	Parents	No. seeds produced	No. vines planted	No. of selections
7106	Foch x S.D.9-135	45	16	0
7107	Foch x S.D.11-42	65	21	0
7108	Foch x S.D.12-101	34	0	0
7205	Foch x S.D. 9-135	59	22	0
7206	Foch x S.D.10-77	37	5	0
7207	Foch x S.D.11-42	86	26	0
7208	Foch x S.D.12-101	82	42	0

In Table 3, crosses with *V. riparia* Michx. at the grandparent level are shown. Elvira as a male parent proved very successful in seed set but, unfortunately, the strongest inheritance in the F_1 was for the *riparia* characteristics--small bunches, small berries subject to cracking and wild rangy growth.

In Table 4, *V. riparia* Michx. as a great-grandparent is illustrated. Ventura has not been used in later crosses because of its susceptibility to Eutypa dying-arm, but its hardiness qualities have been excellent. Foch is of good quality and it was hoped this cross would result in some hardier, better quality wine selections. Castel 19637 seemed to impart high sugar potential and earliness to its progeny. The 7518 family looks promising at present for survival but has yet to be fruited and assessed for quality.

Some of the more successful crosses have been named. Their genealogies are attached. Vincent was a cross made in 1949 when the direct producer hybrids were first introduced to Ontario. The industry required better colored varieties than the blue labruscas available at the time. Chelois (Seibel 10878) carried improved quality and Vineland 370628 carried heavy color from Lomanto, a Munson hybrid from Texas. Vincent has been used as a high quality teinturier but is somewhat subject to powdery mildew and occasional winter injury.

Ventura is a hardy, white variety grown in the coldest districts of the Niagara peninsula. The riparia qualities are a little too evident for use in other than white wine blends. It has shown some dying-arm symptoms *(Eutypa armeniacae)*, the susceptibility presumably inherited through Chelois.

TABLE 3. Breeding families using *V. riparia* Michx. as a possible grandparent.

Family No.	Parents	No. seeds produced	No. vines planted	No. of selections
5106	Chelois x Elvira	126	44	1
5401	Baco 1 x S.V.14287	7	1	0
5402	Baco 1 x V.35081	222	25	0
6422	Vincent x Elvira	547	59	1
6535	V.35122 x Elvira	186	16	0
6626	V.51013 x Elvira	1060	398	0
6704	S.13047 x Elvira	303	128	0

TABLE 4. Breeding families using *V. riparia* Michx. as a possible great-grandparent.

Family No.	Parents	No. seeds produced	No. vines planted	No. of selections
7209	Foch x Ventura	1393	519	1
7214	Ventura x Foch	164	88	*
7508	Castel 19637 x Foch	804	66	*
7518	Veeblanc x Ventura	520	120	*

*Incomplete selection period.

Veeblanc has perhaps the most complex genealogy because it was derived from two French direct producers. Wine quality has been a considerable improvement over any existing local white varieties. There are problems with powdery mildew in some seasons.

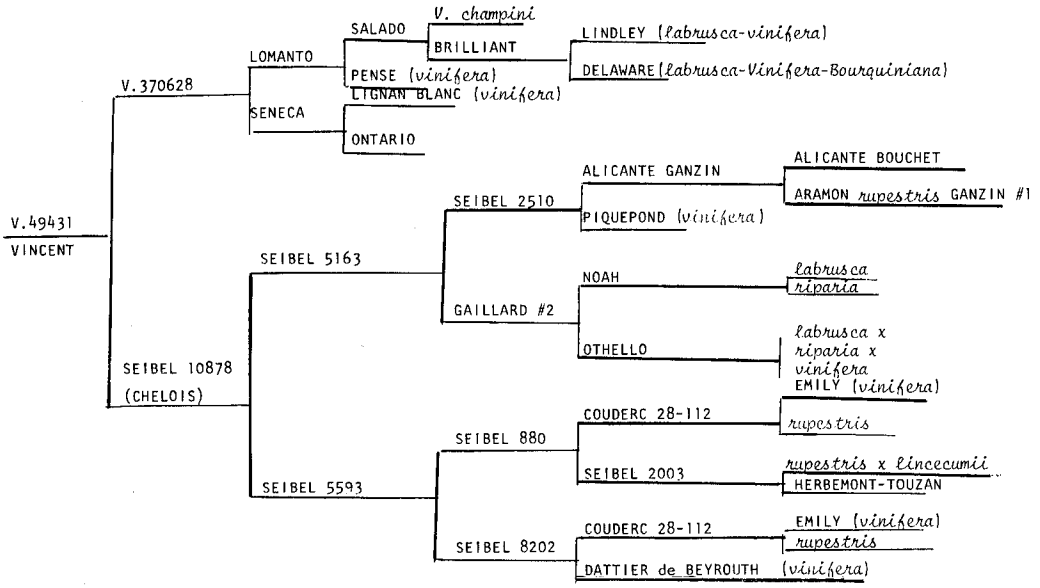

Fig. 1. Genealogy of Vineland 49431 (Vincent).

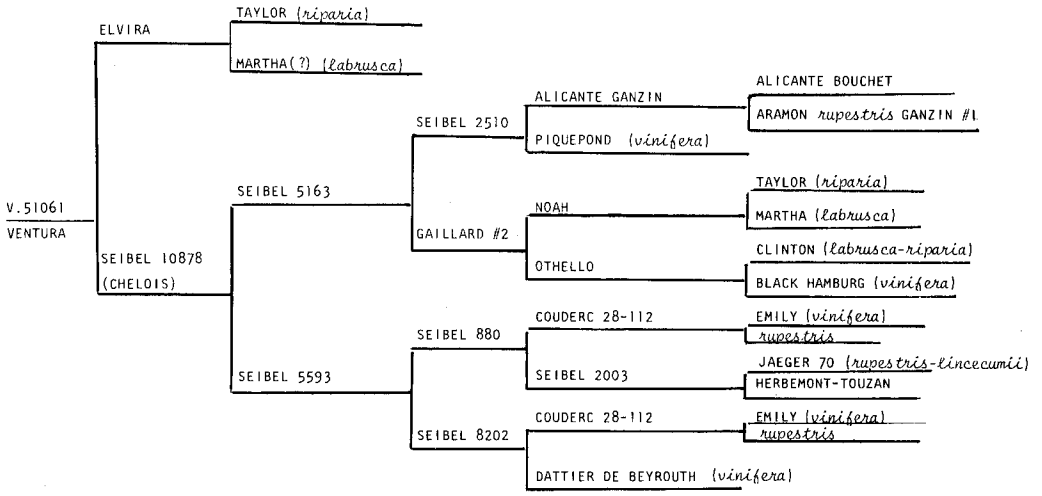

Fig. 2. Genealogy of Vineland 51061 (Ventura).

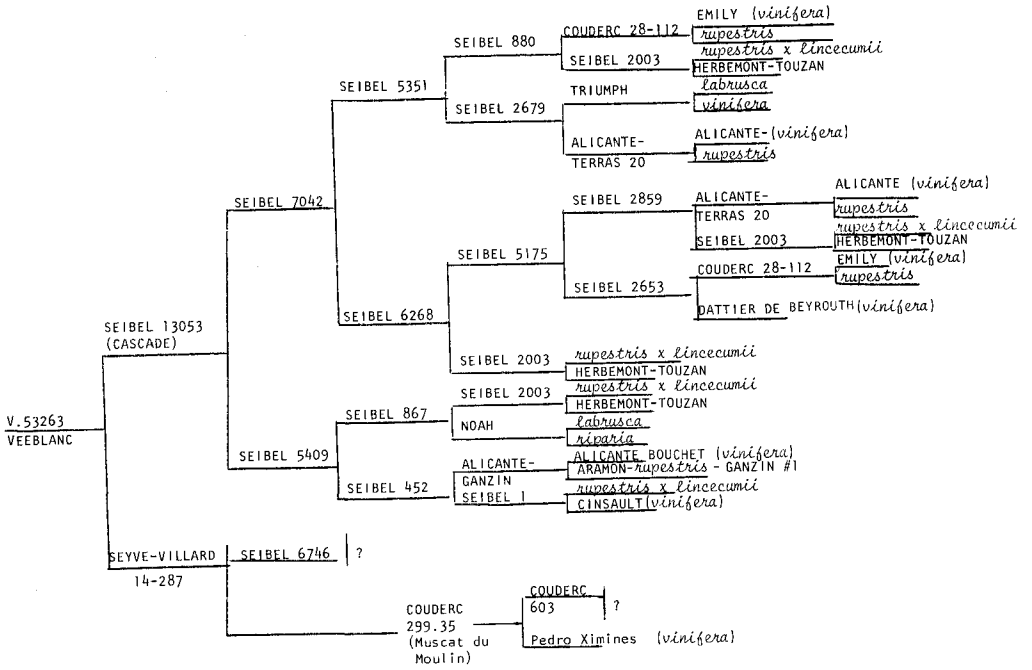

Fig. 3. Genealogy of Vineland 53263 (Veeblanc).

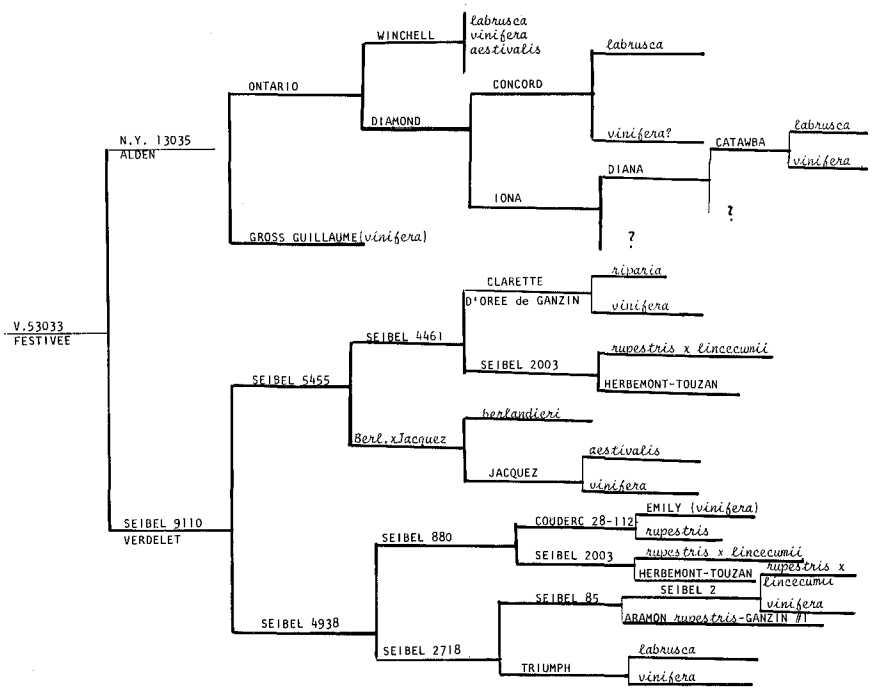

Fig. 4. Genealogy of Vineland 53033 (Festivee).

Festivee is a dessert variety and suffers some winter injury in most years due to its *vinifera* grandparent and tender French hybrid parent. Eating quality is excellent. Bunches sometimes reach 1 kilogram in weight and the berries are large.

The breeding program at HR10 will continue to use *V. riparia* Michx. and interspecific hybrids in the hopes of improving the hardiness of the new wine varieties. The dessert industry has considerable potential due to the population base in the Niagara-Hamilton-Toronto area. Quality such as Festivee sells very well, but lack of hardiness is a serious drawback. Several more generations of breeding are required before truly satisfactory results will be obtained.

A PRELIMINARY EVALUATION OF CLIMATIC REGIONS FOR GRAPES IN NORTH CHINA

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ABSTRACT

The growing wine industry, though still small, is pushing forward in the P.R.C. Wine making, as an industry, has a history of about 80 years. It was negligible in the pre-liberation years and has increased several hundred fold. Grape production, however, increased only 2-3 fold, with fluctuation in acreage in the past 30 years. Measures are being taken to minimize this gap.

The best grape areas are located mainly to the north of the marginal line for vineyards existing without winter protection. This complicates the improvement of the existing management system. An evaluation of climatic regions for grapes on a scientific basis is essential. Introduction and breeding of high quality grape cvs. as well as cold-hardy ones has been carried on and has given some positive results. *V. amurensis*, a cold-hardy native species, has been used fairly successfully in breeding.

A division and evaluation of climatic regions for grapes is essential and pressing for China where a new rise in grape and wine industries is now taking place. Division of climatic regions for grapes is a matter of strategic significance. Provided this work is done on a scientific basis, blindness in the future development may be lessened to a minimum.

As regards winemaking, cultivars have their decisive effects on wines, but only under favorable circumstances can a cultivar fully display its inherent merits. Of all external factors affecting wine quality, climate has been proved to be of primary significance.

Since the territories to the south of Changjiang (Yantze) River, generally speaking, are too humid to grow the *vinifera* grapes with satisfactory quality, only the northern parts of China will be dealt with in this paper, with major emphasis on wine grape production. The author has attempted this work with reference to the researches mainly by M. A. Amerine and A. J. Winkler in the USA and by F. F. Davitaja in the USSR.

According to the experience and research in advanced grape producing countries, the production of good quality table wines requires a cool and relatively dry summer. Grapes grown under such conditions may acquire moderate sugar content, relatively high degree of acidity, and other qualitative features that may endow the resulting wines with delicacy, mildness, and harmony.

Usually, heat summation (heat summation used here means the sum of mean daily temperature from which 10°C has been subtracted) in these regions may not exceed 2000°C, average temperature in the warmest month, 16 to 24°C, and rainfall less than 150 mm during the month prior to harvest. The production of good quality dessert wines requires even a warmer and drier summer. Grapes grown under such conditions may acquire high sugar content, relatively low degree of acidity, and other qualitative features that may endow the dessert wines with good quality. Heat summation above 10°C in these regions is about 1800 to 2300°C; average temperature in the warmest month 20 to 28°C; and rainfall less than 100 mm during the month prior to harvest.

Although cultivars of various ripening dates require different heat summation for their maturation, in the regions with excessive heat the ripening changes may proceed with great rapidity; the coloring and aromatic substances may develop poorly, and the wines made of such grapes may be harsh and coarse.

F. F. Davitaja (1) suggested the reliability of evaluating the water regime by using water-heat coefficient on the basis of her extensive investigations and analyses of renowned wine producing areas throughout the world. She concluded that under K < 1.5 during one to two months prior to harvest wines of good quality can be produced, and under K < 2.5 only wines of moderate quality may be obtained. She also stated that K < 0.5 in the early season of growth is a limiting factor for grape culture without irrigation.

$$K = \frac{P \times 10}{\Sigma t}$$

where : K - water-heat coefficient;

P - amount of rainfall in a given period; mm.

Σt - total heat summation used here means the sum of mean daily temperatures above 10° C in the same period.

To produce table grapes with good shipping and keeping quality, greater heat summation (over 1800°C) and less rainfall are needed. Wide diurnal range of temperatures may foster the accumulation of sugar in grape juice. For raisin production, both a very dry and warm summer and autumn are indispensable.

A low temperature of about -18°C may cause serious injury to the bud eyes of *vinifera* grapes. Also, according to Davitaja, the isotherm of absolute minimum temperature of -15°C averaged for many years may serve as a north limit for *vinifera* vineyards existing without winter protection. On the basis of our investigations, however, the present most northerly distribution of vineyards without winter protection in China is delimited in the identity with the isotherm of absolute minimum temperatures averaged for many years of about -14°C as shown in Fig. 1.

The climate in China, being continental and influenced by the monsoon from the Pacific Ocean, is characterised by the abundance of solar radiation in the majority of her territories and

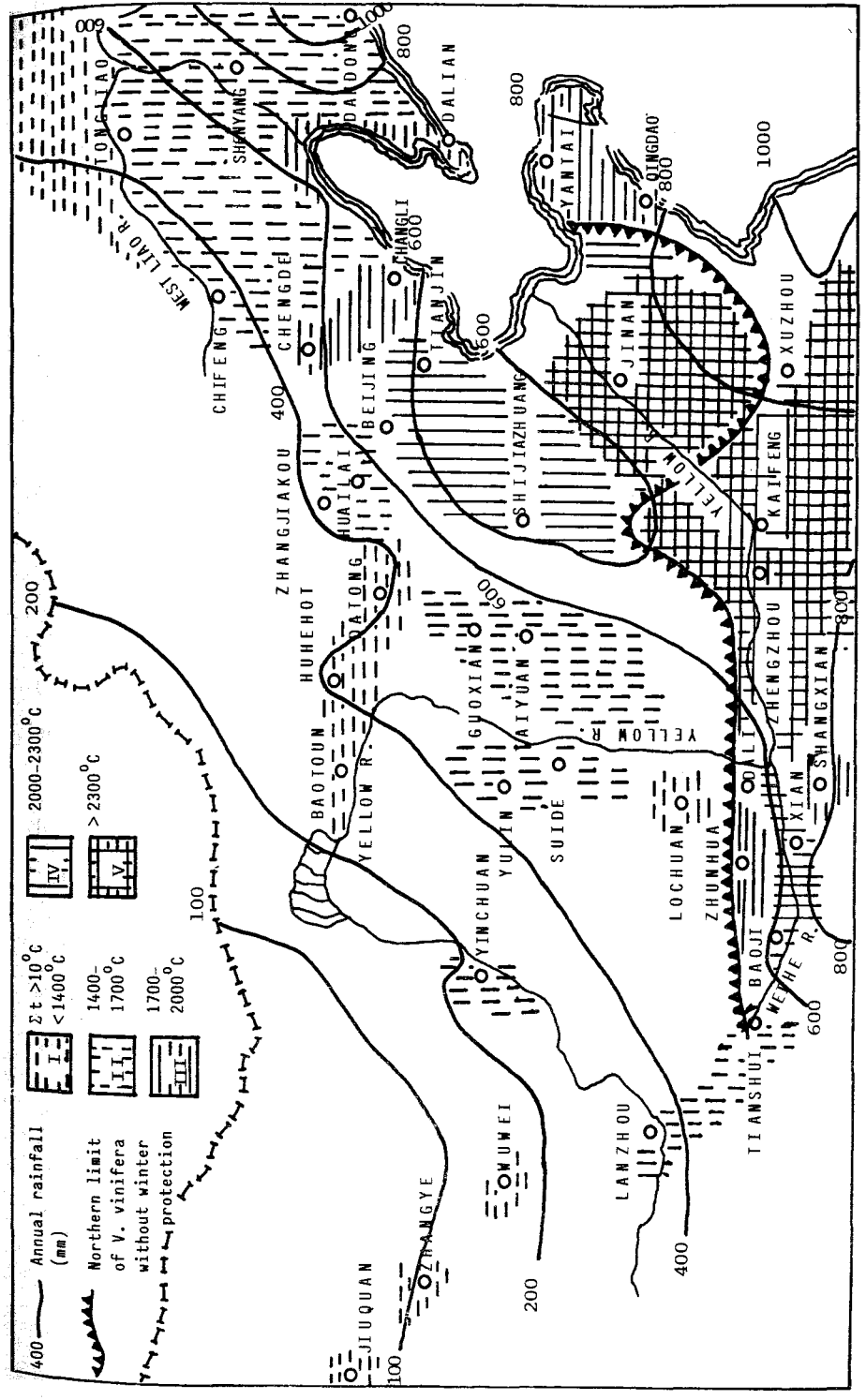

Fig. 1. The climatic regions for grapes in North China.

of a rather hot but, in varying degrees, humid summer and a cold dry winter. Such a climate is in sharp contrast with that of the renowned Mediterranean wine producing countries which generally have a mild winter but a dry and warm summer. In view of this, a judicious and reasonable division and evaluation of climatic regions seems to be of even greater importance to China.

According to Amerine and Winkler (2), heat summation above 10°C (50°F) from April through October may be used as a basis for the division of climatic regions for grapes. Taking heat summation above 10°C, as a main figure, we have made an attempt to segregate the grape growing areas in northern parts of China into five types of climatic regions: I) Coolest region (less than 1400°C); II) Cool region (1400 to 1700°C); III) Moderately warm region (1700 to 2000°C); IV) Warm region (2000 to 2300°C), and V) Hot region (more than 2300°C). The following is the evaluation of these climatic regions, taking into consideration other relevant climatic factors (Figs. 1 and 2 and Table 1).

I. Coolest regions (Heat summation above 10°C less than 1400 °C):

1. Datong (Tatung) Prefecture of Shanxi (Shansi) Province and Huhehot-Baotou (Paotow) Prefecture of Inner Mongolia: Annual precipitation is about 300 to 450 mm. Rainfall in the ripening period is not in excess. K value is less than 1.5 in July to September except in a few cases. Average temperature in the warmest month is about 21 to 22°C. Early or medium maturing cultivars for table wines or local fresh market may be grown here; it is advisable to use hardier cultivars. Attention may well be given to adoption of cold hardy rootstocks, for the winter here is severe. Irrigation is beneficial in dry, early summer.

2. Central and northern parts of Manchuria: Annual precipitation increases from north (approx. 450 mm in Qiqihaer /Tsitsihar/) to south (approx. 900 mm in Tonghua). K value in the ripening period is high or very high. Average temperature in the warmest month is about 21 to 23°C. Grapes collected from a wild species *V. amurensis* is used for making sweet wines. The domestication of this species is almost at the trial stage. Hardier early or medium maturing cultivars of vinifera grapes and hybrids between *vinifera* and *labrusca* grapes grafted on cold resistant rootstocks, such as *amurensis* grape or 'Beta', are grown fairly successfully for local fresh market.

3. Central parts of Gansu (Kansu) Province (Zhangye and Jiuquan Prefecture): Annual precipitation is very low (about 100 mm). Average temperature in the warmest month is about 21 to 22°C. The diurnal range of temperature is wide. Sunshine is abundant. Good quality table wines may be produced here. Irrigation is indispensable.

II. Cool regions (1400 to 1700°C):

1. Zhangjiakou (Kalgan) Prefecture of Hebei (Hopei) Province (including north-western part of Peking): Annual precipitation is about 350 to 450 mm. Rainfall in the ripening period is not in excess. K value is favorable in July to September. Average

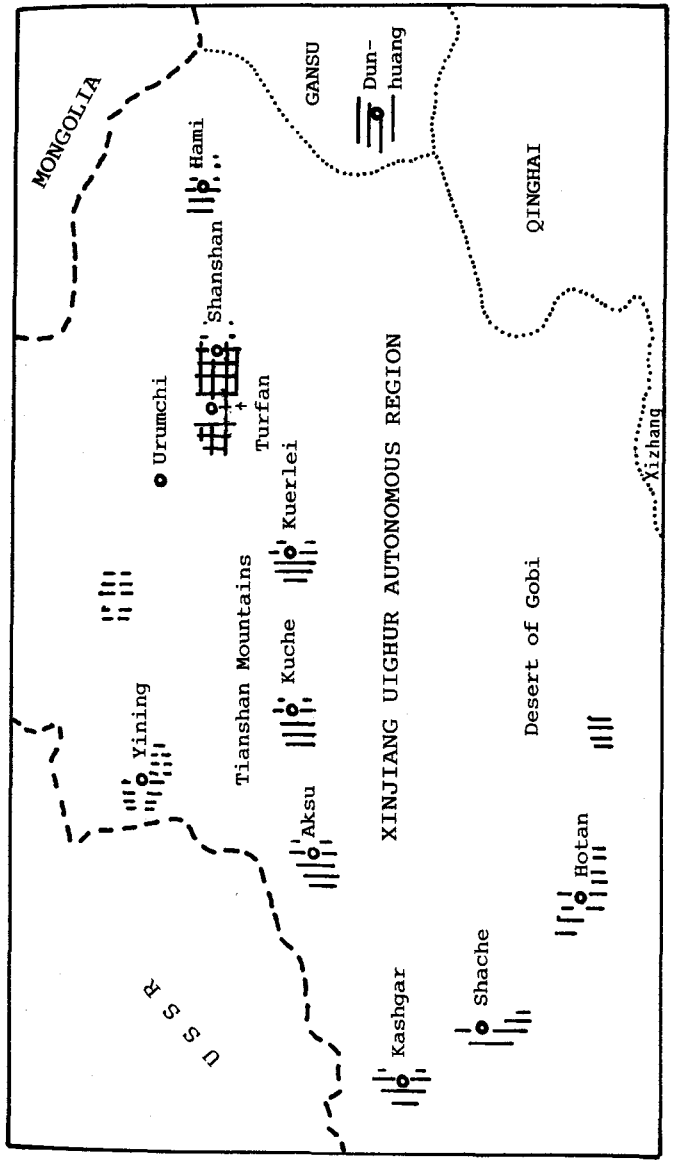

Fig. 2. The climatic regions for grapes of Xinjiang (Sinkiang).

TABLE 1. Water-heat coefficient (K) of major localities in north China.

No. of climatic regions	Localities	K value				
		April-June	July	August	Sept.	Oct.
I - 1	Datong	0.7[z]	1.5	1.5	1.2	-
	Huhehot	0.7[z]	1.5	2.2	0.8	-
I - 2	Jiuquan	0.2[z]	0.3	0.3	0.2	-
I - 3	Qiqihaer	0.9[z]	2.0	1.7	1.4	-
	Tonghua	1.9[z]	3.2	3.2	2.1	-
II - 1	Huailai	0.6	1.6	1.8	0.9	-
	Zhuolu	0.6	1.4	1.7	0.8	-
II - 2	Taiyuan	0.7	1.7	1.5	1.4	-
II - 3	Suide	0.8	1.4	1.7	1.6	1.2
	Yulin	0.6	1.3	1.9	1.3	-
II - 4	Yinchuan	0.4[z]	0.5	0.8	0.6	-
	Lanzhou	0.6	0.9	1.3	1.1	-
	Tianshui	1.1	1.5	1.4	2.0	1.4
II - 5	Yining	0.5	0.4	0.2	0.3	-
II - 6	Tongliao	0.8[z]	1.9	1.4	0.9	-
	Chifeng	0.8[z]	1.4	1.4	0.8	-
II - 7	Shenyang	1.2[z]	2.9	2.4	1.7	-
	Dandong	1.5[z]	4.9	3.8	1.9	1.6
III - 1	Yantai (Chefoo)	0.9	2.0	1.9	1.0	0.6
	Qingdao (Tsingtao)	1.2	2.7	1.9	1.8	1.0
III - 2	Dalian (Dairen)	1.2[z]	2.7	1.9	1.1	0.8
	Yingkou (Yingkow)	1.0[z]	2.3	2.5	1.5	1.1
III - 3	Chengde	1.0	2.1	2.1	1.1	0.7
	Changli	1.0	3.0	2.7	1.0	0.6
III - 5	Shangxian	1.3	2.0	1.6	1.9	1.6
III - 6	Dunhuang	0.05	0.1	0.06	0.04	-
IV - 1	Hotan	0.1	0.04	0.05	0.06	0.003
IV - 2	Baoji	1.2	1.6	1.3	2.4	1.7
	Xian (Sian)	1.0	1.3	1.0	1.7	1.5
IV - 3	Beijing (Peking)	0.7	2.4	3.2	1.1	0.5
	Tianjin (Tientsin)	0.7	2.3	2.0	0.7	0.5
V - 1	Turfan	0.02	0.02	0.04	0.01	0.01
V - 2	Changwei	1.0	2.5	1.7	1.0	0.8
	Heze	0.9	2.5	1.6	1.3	0.7
V - 3	Zhengzhou (Chengchow)	0.9	1.6	1.7	1.1	0.9
	Xuzhou	1.2	3.1	1.8	1.4	0.7

[z]K value of May-June

temperature in the warmest month is about 23 to 24°C. Grapes produced in this region usually have moderate or high sugar content and relatively high degree of acidity with good coloration. Table wines of good or excellent quality can be produced here. This area is considered very promising as one of the wine producing bases in China.

2. Central part of Shanxi (Shansi) Province: Annual precipitation is 400 to 500 mm. Rainfall in the ripening period is not in excess. Average temperature in the warmest month is about 22 to 24°C. The climatic conditions here resemble those of the Zhangjiakou prefecture.

3. Northern parts of Shaanxi (Shensi) Province: Annual precipitation varies from about 400 mm in Yulin to about 600 mm in Lochuan. Rainfall in the ripening period is not or only slightly excessive. K value in July to October is favorable in Yulin, Mizhi, and Suide and relatively high to the south of Yanan (Yenan). Average temperature in the warmest month is about 22 to 24°C. The diurnal range of temperature is fairly wide. There is abundance of ultra-violet rays due to the high altitude (about 800 to 1000 m) of the loess plateau. It favors coloration of various fruits including grapes. This area possesses, on the whole, desirable climatic conditions for the production of good quality table wines.

4. Yinchuan Prefecture of Ningxia (Ninghsia) and Lanzhou (Lanchow) - Tianshui (Tienshui) Prefectures of Gansu Province: Annual precipitation varies from 205 mm in Yinchuan and 330 mm in Lanzhou to 550 mm in Tianshui. K value is ideal except in September in Tianshui. Average temperature in the warmest month is about 22 to 24°C. Best quality table wine may be produced here. These Prefectures must be considered as promising table wine producing bases in China. Irrigation should be developed owing to the dry, early season.

5. Yining (Kuldja) Prefecture of Xinjiang (Sinkiang) Uighur Autonomous Region: Annual precipitation is about 200 to 300 mm. Rainfall is low in the ripening period. Average temperature in the warmest month is 22 to 23°C. The climatic conditions here are suitable for table wine production. Irrigation is needed.

6. West Liaohe River basin (Chifeng - Tongliao Prefectures): Annual precipitation is around 400 mm. Rainfall in the ripening period is low. K value is favorable in July to September. The climatic conditions here are suitable for table wine production. Cold hardy rootstocks may be of benefit here.

7. Southern part of Manchuria (Shenyang Prefecture and northern part of Liaodong peninsula): Annual precipitation varies from 750 mm in Shenyang (Mukden) to nearly 1100 mm in Dandong (Tantong). Rainfall in the ripening period is excessive and K value is high. Average temperature in the warmest month is 23 to 25°C. Cold hardy rootstocks are used to simplify the covering of vines during winter. Table grapes of American hybrids are grown for local fresh market.

III. Moderately warm regions (1700 to 2000°C):

1. Shandong (Shantung) peninsula: Annual precipitation is 600 to 800 mm. Rainfall in the ripening period is relatively excessive and K value in July to August is relatively high but is more favorable in September to October. The climate is distinctly maritime with mild summer and narrow diurnal range of temperature, which favors the color and flavor development. Both table and dessert wines are produced. Medium and late maturing cultivars are more suited here. The mild winter makes possible overwintering of *vinifera* grapes without protection.

2. Southern part of Liaoning peninsula (mainly the Lüda/Port Arthur and Dairen/Prefecture): The climatic conditions here resemble that of the Shandong peninsula, except winter protection is needed here.

3. Hilly areas in the central and lower reaches of Luanhe River (Changli and Chengde/Chengteh/Prefectures): Annual precipitation is about 600 to 700 mm. Rainfall in the ripening period is relatively high, but K value turns more favorable in September to October. Average temperature in the warmest month is 24 to 25°C. Better quality grapes are produced on hillslopes. Medium or late maturing cultivars for both table use and wines are suited for this region.

4. Highland region to the north of the upper reaches of Weihe River in central Shaanxi (Shensi) Province: Annual precipitation is about 500 to 600 mm. The rainfall in the ripening period is slightly excessive. Average temperature in the warmest month is 24 to 26°C. The winter is mild so that grapevines need no protection during winter. Medium or late maturing cultivars for ordinary wines may be suited here.

5. Shangxian Prefecture of Shaanxi (Shensi) Province: Annual precipitation is about 700 to 800 mm. Rainfall in the ripening period is relatively high, while K value in August to October is fair. Average temperature in the warmest month is about 25°C. Grapevines need no protection during winter. Medium or late maturing cultivar for ordinary wines may be suited here.

6. Dunhuang (Tunhuang) Prefecture of West Gausu (Kansu): Annual precipitation is less than 50 mm. Summer is almost rainless. Sunshine is abundant. The diurnal range of temperature is wide. Table grapes and dessert wine grapes of good quality may be produced here in irrigated vineyards.

IV. Warm regions (2000 to 2300°C):

1. Southern part of Xinjiang (Sinkiang): Annual precipitation is only 30 to 60 mm. Summer is almost rainless. Average temperature in the warmest month is 25 to 26°C. Sunshine is abundant. The climate is dry and irrigation is absolutely needed. Hotan Prefecture is one of the most important grape producing areas in China. Good keeping and shipping table grapes and fine dessert wine grapes can also be produced here, provided the transport conditions are improved markedly.

2. Guanzhong basin in central Shaanxi (Shensi) - Baoji (Paoki), Xian and Dali Prefectures: Annual precipitation increases

from 540 mm in Dali to 700 mm in Baoji. Rainfall in the ripening period is not excessive. K value in July to October is favorable except in Baoji, where it is high in September. Average temperature in the warmest month is 25 to 27°C. Grapevines need no protection in winter. Dessert wine may be produced in this region.

3. Central and southern parts of Hebei (Hopei) Province: Annual precipitation decreases from 600 to 700 mm in the inland areas to 450 to 550 mm in the coastal areas. Rainfall in the ripening period is excessive, but K value turns fair in September to October. Average temperature in the warmest month is about 25 to 27°C. High humidity and high temperature in summer favor a number of fungus diseases, notably the white rot (*Charrima diplodiella*), which at times causes serious crop damage. Late-maturing and disease-resistant cultivars for table use or ordinary wines show promise in this area.

V. Hot regions (more than 2300°C):

1. Turfan - Shanshan Prefecture of Xinjiang (Sinkiang): There is abundance of heat and sunshine in this area. Heat summation above 10°C in Turfan reaches 3200°C or more. Average temperature in the warmest month is 33°C in Turfan and 29.3°C in Shanshan. Annual precipitation is very low and both summer and autumn are almost rainless. All this has made Turfan a famous China raisin-producing base. Good keeping and shipping table grapes and dessert wine grapes may also be produced here. Irrigation is absolutely needed.

2. The inland areas of Shandong (Shantung) Province: Annual precipitation increases from about 600 mm in the north to about 900 mm in the south. Average temperature in the warmest month is about 27°C. Summer is hot and humid, but K value is fair in September to October. In most parts, except the southwestern tip of this area, *vinifera* grapevines are usually covered with soil to prevent freezing in winter. Late-maturing and disease-resistant cultivars for table use or ordinary wines may be grown.

3. The old course of the Huanghe (Yellow) River (including the eastern part of Henan/Honan/ Province and the northern parts of Anhui/Anhwei/ Province and Jiangsu/Kiangsu/ Province): Annual precipitation increases from 600 to 700 mm in the west to 800 to 900 mm in the east. Rainfall in the ripening period is excessive, but K value in September to October is fair. Average temperature in the warmest month is 27 to 28°C. High temperature with high humidity in summer fosters grape fungus diseases and is also detrimental to sugar accumulation and coloration. It is a matter of no little interest that there is a vast area of reclaimable land and that *vinifera* grapevines generally need no winter protection. Table or wine grape cultivars resistant to fungus diseases may be recommended for this area.

In the light of the preliminary evaluation of climatic regions stated above, localities relatively ideal for table wine production may be found readily in the cool regions and possibly in some areas in the moderately warm regions, where K value in the ripening period is low. As for the dessert wines, there is little

possibility to assign ideal localities except in Xinjiang owing to the wet summer conditions in warm or hot regions in most parts of China. It is a misfortune that areas that are ideal or favorable for growing wine grapes of fine quality are mostly located to the north of the isotherm which serves as a northern limit to vineyards existing without winter protection. This fact has made our grape and wine production more labor intensive and, hence, more costly and has posed to us a pressing but difficult task to breed cold-resistant cultivars of desirable quality.

It is necessary to further delimit subregions within the regions stated above. This may be done on the basis of refinements of evaluation for each locality using climatological theories and by carrying out local systematic observations in combination with extensive investigations. In the meantime, responses of cultivars in productivity, disease resistance and adaptation to local conditions including topography, soil and various deleterious factors, should also be observed seriously and taken into account.

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NATURAL HYBRIDIZATION OF INDIGENOUS VITIS CALIFORNICA AND V. GIRDIANA WITH CULTIVATED VINIFERA IN CALIFORNIA.

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ABSTRACT

There have been many contrasting opinions on the role of hybridization in modifying the native grape flora of the United States. In areas where many species are sympatric, objective evidence for supposed hybridity is difficult to verify. The two relatively simple and well-defined species of *californica* and *girdiana* in the state has made it possible to study the introgression of cultivated *vinifera*, whose time of introduction is well documented. In southern California, the so-called "old Mission" vines that reached enormous proportions are actually *girdiana* x *vinifera* hybrids and not the *vinifera* cultivar Mission as has long been accepted.

The role of hybridity: There is a great difference of opinion as to whether hybridization occurs between grapevine species under natural conditions in the United States. Obviously, the variation that would follow creates difficult problems in resolving the identity of the usual taxonomic units. The breeding of new varieties would be much facilitated if we knew more of the range of natural variation and its origin.

Engelmann (3), considered the most learned American botanist of his time and the most knowledgeable on grapevine classification, summarized his views: "Hybridization is an abnormal, I may say, an unnatural process, which is usually prevented by countless obstacles. If it were not so, we would meet with more hybrids in our woods and prairies than with genuine species: but how rare are they and what a find it is to discover one!"

Viala served as head of a mission delegated by the French government to study the native vines, particularly to seek any that were highly resistant to calcareous soils (9). On hybridization he recounted: "It suffices to traverse the woods of the United States to be well convinced that the hybridization between vine species is a common thing and not an exception, or an impossibility, as some botanists have affirmed." "In some cases the wild hybrids are more frequent than the pure species."

Bailey (1) in the most recent monograph on the North American species, remained skeptical: "No one is ready to deny hybridity of *Vitis* in feral conditions but its record must be based on evidence rather than on assumed standards, that is, approached objectively."

M. Millardet (7) was one of the first to call attention to the

hybrid vines of America. Hermann Jaeger of Neosho, Missouri and T. V. Munson of Denison, Texas described numerous hybrids in their field studies of native grapevines. Although most natural hybrids may be of little interest, some have been of great viticultural value because they may combine desirable features of the pure species and often display great hybrid vigor, thus surpassing the parental species and have a greater range of adaptation. In retrospect, the most famous natural hybrid is the *rupestris* x *lincecumii* vine discovered by Jaeger, forming the initial breeding stock of most of the French hybrids.

Endemic species of California: The native grape flora of California is relatively uncomplicated. There is general acceptance among botanists that a uniform species occupies the Great Central Valley and surrounding foothills, confined to the borders of live streams or perennial springs. This is *Vitis californica,* first described by Bentham in 1844 from the Sacramento River, where it reaches its greatest development. It seldom ascends into the mountains above 2500 feet elevation.

In the arid southern part of the San Joaquin Valley, it intergrades into the species *girdiana* of southern California, abundant along the tributaries arising from the coastal ranges of San Rafael, Santa Ana and Laguna. *Girdiana* is adapted to a milder winter climate, where stream flow is sporadic from flash floods and the vines inhabit deep beds of coarse sand and gravel, sending roots deeply for moisture to survive the long summer drought.

The distinguishing morphological characters usually considered in separating the species are summarized in Table 1. The most conspicuous difference relates to the leaves; *californica* is orbicular or even somewhat reniform, with serrations reduced to scallops, and the teeth are mucronate. The leaf of *girdiana* is often shouldered, tending to be three lobed, the central one triangular and pointed. The teeth are serrate and more prominent than *californica.*

The relatively simple and confined species distribution offers an ideal locale for the study of natural hybridization with vinifera cultivars, whose time of introduction is a matter of historic record. This leads to some interesting speculation on the time span involved in the modification of the grape flora.

Introduction of cultivated vinifera. The first actual record of planting the *vinifera* grape in Alta California appears in a letter from Father Pablo de Mugártegui to President Serra written at Mission San Juan Capistrano on March 15, 1779 (12). The translation that is of interest follows: "Now that the severe cold has moderated and the floods subsided the grape cuttings have all been planted, the cuttings which on your recommendation were sent to us from the lower countries." Lower countries would infer Baja California, Mexico, where a chain of Jesuit missions already existed and the 'Mission' vine cultivated. It is likely that cuttings would have been taken from nearby San Diego Mission, if vines had been established there. Thus, it appears that the vine was planted about the same time in both missions, taking us back two centuries.

TABLE 1. Contrasting characters of the two California grape species.

	californica	*girdiana*
leaf:	orbicular to slightly reniform; entire, shallow sinuses; tufted arachnoid hairiness; margin scalloped, teeth mucronate.	cordate, shouldered, triangular apex; three lobed; sinuses medium depth, tomentose, very hairy; teeth serrate, acute.
shoot:	tomentose, becoming arachnoid hairy.	heavily tomentose, hairiness persistent on canes.
cluster:	small to medium; compact; pedicels short, warty.	medium to large, compound, loose; pedicels smooth, slender, long.
berry:	small; heavy bloom; skin not pungent; pulp sweet, seedy, lacks juice.	very small; no bloom; skin pungent; pulp acid, juicy.

The chain of 21 Franciscan missions along the coast extended from San Diego (founded July 16, 1769) in the south to San Francisco Solano in Sonoma (founded August 25, 1823). Grapes were planted at all of the missions and vineyards expanded where the climate was suitable and where water was available for some irrigation. It was at San Gabriel Mission, terminus for the first overland expeditions from Sonora, Mexico, where expansion of vineyards was most important, becoming known as the "Viña Madre." At the time of expropriation in 1834, the vineyard had about 163,000 vines or approximately 200 acres (2).

By the late 1870's, the most extensively planted wine grape was the Zinfandel, distributed widely from Sonoma after its introduction there in 1857 and 1858 by Agostin Haraszthy (4). Hybrid vines of the Sierra foothill region often show segregation for the distinctive leaf lobing of Zinfandel.

Along the tributary streams entering the flat Central Valley from the Sierra Nevada near Lodi, extensive vineyards of Flame Tokay (Ahmeur bou Ahmeur) were planted, beginning about 1890, often contiguous to the native vines. Natural hybridization with this cultivar has occurred in many areas within a century. This cultivar was also a favorite table grape in the Sierra foothill mining communities after the gold rush in 1849, and hybrids have also been found in these areas.

Californica - *vinifera* hybrids: From time to time, plant collectors have sent us leaf or fruit specimens of unusual vines for identification from natural woodlands, specimens that did not conform to the description of *Vitis californica*, a distinct and easily identified species. The unusual vines appeared to be hybrids of *californica* with the cultivated *vinifera*. Several surveys were made in the northern part of the state and additional tentative hybrids were located in many areas. However, more objective evidence was needed.

Progeny studies: Fruit was gathered from individual open-pollinated vines thought to be typical of the wild species or of supposed hybrids. The localities are given on the outline map (Fig. 1). The seedling progenies were grown to the fruiting stage and classified for flower sexual type, berry size, fruit color and other characters of secondary interest.

The black-fruited hybrid vines of Colfax, Modesto and Hilton segregate for recessive white fruit color, indicating the parental hybrid as an F_1 resulting from the recessive gene originally contributed by a *vinifera* cultivar. We have never observed white-fruited forms in isolated populations of *californica*. In some natural hybrid populations, white-fruited forms have been found, suggesting that a second generation from F_1 hybrid vines has arisen already. Such vines have been observed only in localities where hybridization has been extensive. The Blue Lakes collecting area is mountainous and far removed from cultivated vineyards, completely encircled by forest and natural vegetation. Progenies from two fruiting vines segregate only males and females and are homozygous for black fruit color, typical of *californica*.

The berry of *californica* is spherical and weighs less than

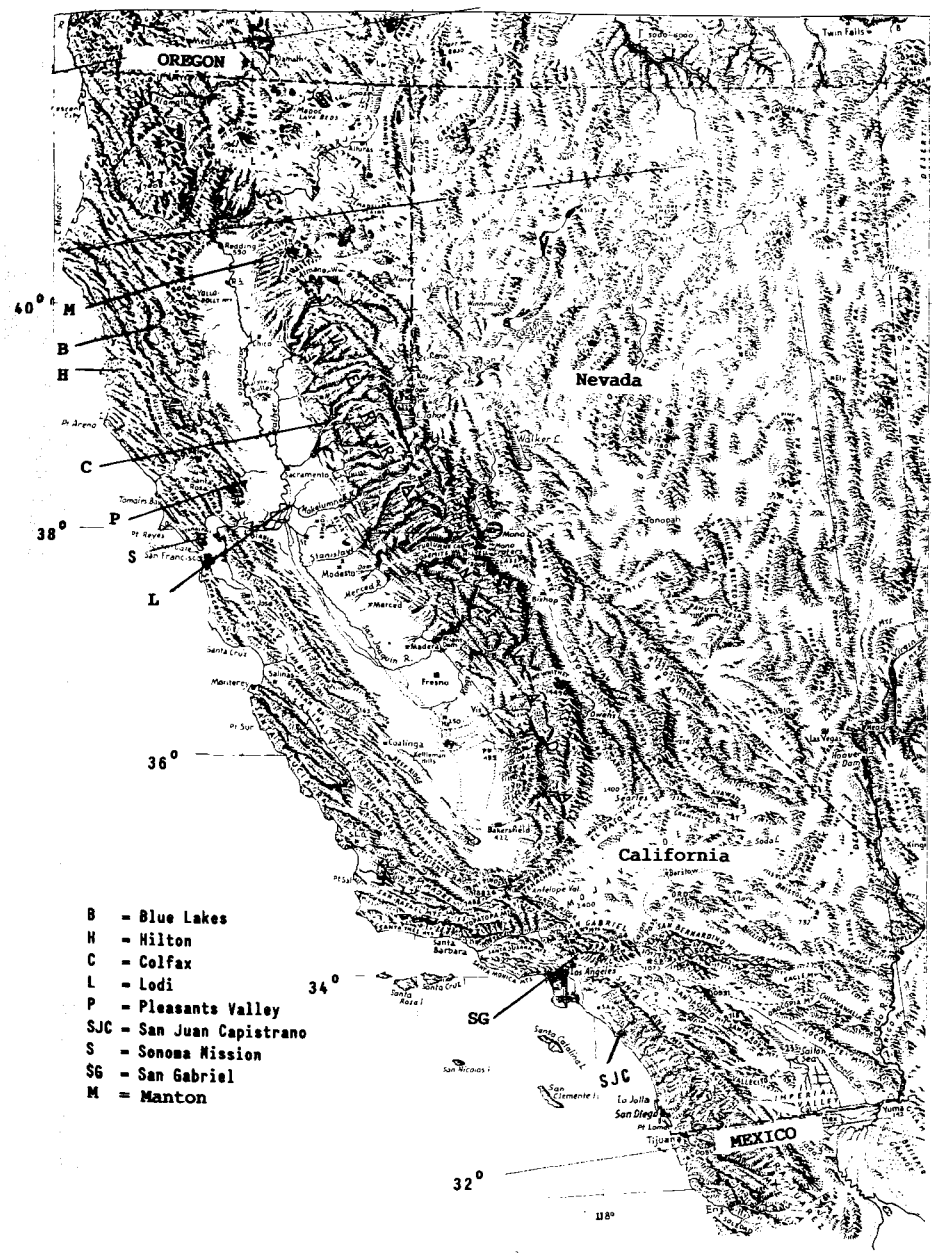

Fig. 1. Localities mentioned in text.

0.5 gm. With the introgression of the *vinifera*, the berry size of F_1 hybrids is increased considerably, the magnitude dependent principally on the berry size of the individual *vinifera* parent.

Flower type: Male and female vines of *californica* are found in about equal numbers. No hermaphroditic vines have been discovered, nor do they appear in progenies from controlled pollinations of wild vines far removed from cultivated vineyards.

Hermaphroditic vines have been found only in natural hybrid populations; they are often easily recognized by very heavy cropping and the larger berry size. The allele has introgressed from a *vinifera* cultivar. Both *californica* and *girdiana* have the same anthocyanins in the berry skin pigments and also have diglucosides present, which condition is dominant over the monoglucosides of our *vinifera* cultivars. F_1 hybrids (wild x *vinifera*) should, therefore, be heterozygous unlike the homozygous wild vine. Progeny analyzed from a few hybrid vines show such segregation (Table 2).

Spontaneous (*californica* x *vinifera*) F_1 hybrids can be identified most easily by the presence of hermaphroditic flowers, leaves that are more deeply lobed and less pubescent, with margins having large, serrate teeth, somewhat elongated berries larger than *californica* and skin pigments having anthocyan diglucosides typical of *californica*.

Further selfing or outcrossing of these vigorous and fruitful hybrid vines under natural conditions may lead to a wide range of segregation and introgression and modification of the existing feral forms. Segregation of vines with white fruit indicate that a second segregating population has been established in some natural environments.

Isozyme patterns: Seed samples from individual vines of one large population of hybrid vines near Manton, Wolfe (unpublished) has found certain bands in zymograms typical of *vinifera* cultivars, but these are present in a *californica* background.

The study of sponteneous grape hybrids in northern California turned our attention to certain individual vines in the southern coastal area that became famous because of their extremely large size and longevity. These have always been referred to as unusually fine specimens of the 'Mission' grape. Evidence now indicates that these exceptional vines are, in fact, hybrids of *girdiana* and the cultivated Mission. Their exceptional development can be attributed to hybrid vigor. These vines appeared close to the first centers of grape culture in the mission gardens where *girdiana* was near at hand. The vineyard at the San Gabriel Mission was the largest and most successful *vinifera* planting.

Matthew Keller, a prominent grape grower of Los Angeles, published a review on the grapes and wines of Los Angeles for the year 1858 (4), noting the county had 1,510,000 bearing and 875,000 non-bearing vines, with preparations under way to plant a million cuttings. "The grape in cultivation is a variety introduced by the early mission priests...." More significant, at this stage, is his

TABLE 2. SEGREGATION FROM SELECTED VINES ASSUMED TO BE NATURAL *CALIFORNICA* (N) OR HYBRID *CALIFORNICA* x *VINIFERA* (H).

Type	Locality	Progeny vines	Fruit color ♂	Fruit color ♀	Fruit color ♂♀	Fruit color White	Fruit color Black	Range* Berry size
N	Blue Lakes 1	17	5	11	0	0	11	0.3-0.8
N	Blue Lakes 2	3	1	2	0	0	2	-
N	Pleasants Valley 1	20	9	9	0	0	9	0.3-0.6
H	Pleasants Valley 2	19	8	6	2	0	7	0.5-0.8
H	Colfax 1	16	1	10	3	4	7	0.5-2.6
H	Modesto 10	17	7	8	1	1	8	0.7-2.3
H	Hilton 1	5	1	3	1	1	3	0.5-1.0

*Avg. of 10 largest berries per cluster, g.

astute observations on the wild vines of the countryside.

"The wild grape abounds in all parts of this county, and there appear to be three varieties--one a rambling kind, producing little or no fruit; another less rambling, but still raising itself from the ground some distance and furnishing heavy crops of well shaped bunches, fruit large and thin-skined, and juice saccharine with well-developed vinous flavor; and a third, which climbs to the top of the tallest trees, bearing light bunches of small fruits. It is replete with coloring matter, and I have no doubt that, if properly tested, it will prove an invaluable acquisition to the state. Our cultivated grape has not equally good qualities for making wines of the red as of the white class, it is deficient in color."

These three types of wild vine can be defined as follows: the first unfruitful type represents the male vine; the second type is the spontaneous hybrid between the "wild" vine and the cultivated 'Mission,' is hermaphrodite and highly fruitful, and the third type is the female vine of the wild type. Keller also recognized the principal deficiency of the 'Mission' as a red wine grape, a lack of color.

In 1889 Munson visited California to study the native grapes. He recognized the old "Mission Grape" as a *vinifera* × *girdiana* hybrid. Quoting Munson (7): "The old Mission Grape of California clearly shows characteristics of *V. girdiana* and I am fully of the opinion that it is an accidental hybrid of *V. girdiana and V. vinifera*. Here (Denison, Texas) it is far more subject to mildew, rot and cold than most of the vinifera varieties. Its long stringy compound cluster with delicate yellowish-white raches and berries with pungent skin are some of the points indicating *V. girdiana* blood." In discussing the *girdiana* he also says:

"This species is frequently found hybridizing with *V. vinifera* near the old vineyards. One such, grown in grounds of Mr. A. Scott Chapman near S. Gabriel sent ripe clusters in 1886 that were a foot in length, berries of medium size and quite a good grape. When I saw the vine, it was nearly dead, having been attacked by the Anaheim grape disease. Other similar hybrids were seen on Mr. Chapman's place, in 1889."

The first ampelography (3) published in California in 1877 states, "Precedence is naturally given to the Mission variety, as it is the only one of the transplants to the Pacific slope whose European lineage has been apparently lost or effaced in its American naturalization. Now regarded as indigenous to our soil, it is familiarly called "The California." Though the early Spanish and Mexican settlers found growing wild in the state a vine of the species Labrusca* from which a tolerable good wine and brandy were made, this was soon abandoned for the Mission vine which they found growing in the vineyards of the Catholic missions along the coast--hence the name." The description of this variety and the beautiful colored plate exemplifies the Mission as the commerical variety we grow to this day.

*Author's note: at this period, the term "labrusca" was often used to signify a wild form of grapevine and had no species significance.

Why did they retain hybrid vines in the missions and elsewhere when the cultivated vine was well known and widely distributed? First of all, the hybrids were much more vigorous than 'Mission' and easy to establish and maintain. They were often trellised overhead and provided shade over patios and walks in the gardens around the mission buildings. They were much more tolerant of *Oidium* (powdery mildew). But most important of all, they were much more tolerant of Pierce's disease (Anaheim disease), which was concentrated and epidemic in the area. Note Munson's remark about Anaheim disease in 1879. The *vinifera* 'Mission', on the other hand, is very susceptible and did not survive more than a few years, as is still the story today. The cultivated vineyards were grown on shifting sand with a minimum of weed cover, hence vectors of Pierce's disease were absent because of scarcity of host plants or adequate cover.

The hybrid vines were often left unpruned, yet they did not overbear. Fruit production was much reduced because they were often derived from male vines that crossed with hermaphrodite Mission, thus showing partial fertility of the flowers and setting straggly clusters. The hybrid vine left in the mission gardens soon became the alias for the original *vinifera*. The story of many of these giant or mammoth vines in Southern California is an intriguing one, typified by the "Carpinteria vine" unwittingly used as an example of the vigor, hardiness, enormous size and longevity of the *vinifera* 'Mission.'

Further selfing or outcrossing of these vigorous and fruitful hybrid vines under natural conditions may lead to a wide range of segregation and introgression and modification of the existing feral forms.

DISCUSSION

The evidence for natural hybridization has been mostly circumstantial rather than scientific. It is based on the phenotypic resemblance of the supposed hybrid (usually assumed intermediate) with two or more sympatric species. Since hybrid populations are so variable and seldom exhibit any barrier to gene exchange, it is no wonder that there has seldom been unanimity in identifying the ancestral species. A case in point is x *V. champini* of J. E. Planchon, the authoritative monographer of the genus, who named this Texas species after M. A. Champin of the Drome, who received it in a collection of *rupestris* sent by Hermann Jaeger of Missouri. It was soon labelled as a *candicans-rupestris* hybrid. Pierre Viala, whom we mentioned previously, traced back the area in Texas from which the "*champini*" had been collected. *Champini* he recognized, but it was in a region where no *rupestris* could be found. However, since both *berlandieri* and *monticola* were there, he concluded that it was difficult to decide which of the three species might have hybridized with *candicans*. Despite the questionable origin of *champini* or whether it represents a "good" species, it is used in California as an important rootstock, a vine that is extremely vigorous even in thin sandy soils of very low fertility. Other vines of its type might be bred, but what species do we turn to? This is but a single example of the necessity of

knowing more about hybrid origins.

It is evident that much of the information obtained and published concerning the wild species in California is in essence describing the hybrid vines which are, as expected, more akin to the *vinifera* grape. Thus, Rives (10) has established a parallel in the resemblance and disease response of the hybrid vis-à-vis *vinifera*, rather than the true wild vine. An equivalent case is the erroneous acceptance of the hybrid Concord as a pure descendent of feral *labrusca*.

Introgression of *vinifera* cultivars has occurred spontaneously in both *californica* and *girdiana* over a very wide range of territory and in many different habitats in California.

As expected, the most extensive hybridization has occurred in areas where both the cultivated and native species coexist and where there are or have been extensive vineyards. Another prerequisite is a satisfactory ecological niche for the hybrid seedlings to develop and reach maturity. These areas are usually in silted overflow areas or sinks along live streams where a continuous source of moisture is present and some shade and humus is provided by overhanging deciduous trees; thick undergrowth of brush protects the plants from grazing animals. Such areas are also attractive to birds which appear to be an important factor in dissemination of seeds. However, since we have not observed the cultivated vine become spontaneous even in these favored localities, the hybrid seed or plant must have a better survival advantage and allows them to compete more successfully with the wild vines.

Natural hybridity in the grapevine (*Vitis* spp.) would be favored by the sympatric distribution of species. In parts of central Texas, as many as eight species overlap in their distribution. It is from this area that many of the polemics about hybridity have arisen. Wild vines are predominantly dioecious, enforcing cross-pollination by wind or insects. Numerous artificial hybrids have been produced within the genus *Vitis* without any report of sexual sterility or breakdown in the first or succeeding generations. Controlled crosses of many species have produced only vigorous and fertile offspring in the first generation (9). Thus, there are no troublesome barriers to gene transfer, as in the *Vitis* x *Muscadinia* hybrids (9). Geographical isolation has played little, if any part, in species differentiation.

Hybridity and its natural consequences, in spawning variation in succeeding generations, may best explain the difficulties that continue to plague grape taxonomists in their attempts to identify and compartmentalize species. Rather, we must continue widespread field studies to understand the extent of variation and collect and make use of the broad complex of germ plasm existing in nature's own vineyard. It would be a serious mistake to assume that a grape species is a uniform and definable group of plants instead of a swarm of different genotypes. Finally, only genetic analysis can provide an answer to the evolution of the important taxons and their relationships.

Although it has been relatively easy to demonstrate the occurrence of natural hybrids between each of the two California species and introduced cultivars of *vinifera*, one must remember that the introgression of *vinifera* germ plasm was provided in massive doses, at first, under very favorable circumstances in the San Gabriel Valley where large expanses of 'Mission' vineyards coexisted with the native *girdiana*. On an evoluationary time scale, the period involving change in the native grape flora is relatively short, two centuries in Southern California and mostly a century and a half in northern California. Although there are now being collected some hybrid vines filed in herbaria as *Vitis californica*, it is difficult to predict the long-term effect of hybridization in future generations of the native grape flora. It must also be pointed out that the role of hybridity needs verification in a completely natural environment where gene introgression can be demonstrated in resident species, not an exogenously introduced *vinifera*. Nevertheless, the advantages of hybridization are evident in this case, where new vines can establish themselves under natural conditions in which the cultivated ones (*vinifera*) have never succeeded in establishing themselves spontaneously.

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VITIS x MUSCADINIA HYBRIDIZATION : A NEW WAY IN GRAPE BREEDING FOR DISEASE RESISTANCE IN FRANCE

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ABSTRACT

A breeding program based on *V. vinifera* x *V. rotundifolia* hybridization was initiated in 1974 in order to transfer desirable genetic factors for disease resistance from the muscadine grape into new varieties well-adapted to French conditions. The aim was two-fold: 1) selection of rootstock varieties with combined resistance to phylloxera, root-knot and dagger nematodes, and 2) selection of table and wine grape varieties with combined resistance to downy and powdery mildew and high organoleptic qualities.

This work will contribute to our knowledge of the genetical relationship between *Vitis* and *Muscadinia*. We can consider them as distinct genera in view of their morphological, anatomical and caryological characteristics.

Success of hybridization depends mainly on the *vinifera* genotypes used as females. Chromosomal pairing is incomplete in the F_1 plants and under genetical control. When pollinated by *V. vinifera*, hybrids show a large variation, from complete sterility to almost complete fertility. Selection of F_1 genotypes with high ovule fertility makes possible back-crosses at the diploid level with a good probability of effective crossing-over. First observations on the vigor, disease resistance and quality of some back-cross seedlings are very promising.

Only two pure species of grapes are cultivated for fruiting in the world. *Vitis vinifera*, the old world grape or European grape, is extensively grown on more than 25 million acres on all the continents. Contrariwise, the muscadine grape, native of the South Eastern United States, is cultivated on a few thousand acres in a restricted area corresponding practically to the Cotton Belt of the U.S.A. (1). The American grapes commonly referred to as *Vitis labrusca* varieties, especially the variety Concord, are likely natural hybrids between this wild species and *V. vinifera* varieties introduced in the XVIII^{e} or XIX^{e} century (15).

According to the taxonomic classification presented by Planchon (14), *V. vinifera* and the muscadine grape belong to different sections or subgenera of the genus *Vitis*, namely, section *Euvitis* (the true grapes or bunch grapes) and section *Muscadinia*.

These two sections are distinguished by morphological, anatomical and caryological characteristics (Table 1).

These two sections are so distantly related that we agree completely with Small (16) who raised the section *Muscadinia* to generic rank and reserved the genus *Vitis* for the bunch grapes. Actually, only three species of *Muscadinia* have been identified : *Muscadinia rotundifolia*, the so-called Muscadine grape, *M. munsoniana*, native of Florida and *M. popenoei*, native of Mexico.

We think that *Muscadinia* is a relictual genus which was widely distributed before the Ice Age as demonstrated by the presence of numerous fossilized seeds of the *Muscadinia* type in the tertiary sediments of Northern Europe (9). The genus *Muscadinia* makes probably the transition between the genus *Vitis*, adapted to temperate climates, and the genus *Ampelocissus*, adapted to tropical climates, whose species present some similarities with *Muscadinia*. Attempts to hybridize *Muscadinia* with species of *Ampelocissus*, native of Central or South America, may corroborate or weaken this hypothesis.

Muscadinia rotundifolia possesses the most remarkable combination of desirable characters to counterbalance the main deficiency of *Vitis vinifera*, namely, its great susceptibility to pest and fungus diseases, a factor which adds heavily to the cost of production (Table 2).

Despite this great potential, no hybridization between *Vitis vinifera* and *Muscadinia rotundifolia* has been done in France, because of the lateness and lack of winter hardiness in the muscadine grape, the lack of rooting and grafting ability and the sterility problems. Work on *Vitis* x *Muscadinia* hybridization in the United States, initiated in 1859 by Wylie (17), was fragmentary and inconclusive up to a decade ago (4,5,11). But the results recently obtained at the University of California (6,7,8,12) were sufficiently promising for reconsideration of the problem.

A breeding program based on *Vitis* x *Muscadinia* hybridization was initiated in 1974 at the viticultural research station of Bordeaux in order to transfer desirable genetic factors for disease resistance from the muscadine grape into new varieties better adapted to cultural and economical conditions of French vineyards.

MATERIALS AND METHODS

Breeding goals: The first challenge is to obtain new rootstock varieties with complete resistance to phylloxera and root-knot nematodes, contrary to most usual rootstocks which are only tolerant to these pests. But the main objective is to incorporate in these new varieties a high field resistance to the transmission of grape fanleaf virus by its vector, the dagger nematode *Xiphinema index* (2).

The second challenge is to obtain table and wine grape varieties with combined resistance to downy and powdery mildew, high palatability of the fruit and organoleptic quality of the wine. In this perspective, the good resistance of *M. rotundifolia* to anthracnose is of some importance. Anthracnose is now considered

TABLE 1. Characteristic differences between the genera *Vitis* and *Muscadinia* (16).

	Vitis	*Muscadinia*
Lenticels	Absent	Present
Tendrils	Forked	Simple
Seeds	Ovoid-shaped smooth chalaza	Oblong-shaped wrinkled chalaza
Pith	Discontinuous	Continuous
Phellogen	Deep seated	Subepidermal
Phloem fibers	Tangential	Radial
Specific gravity of the wood	< 1	> 1
Chromosome number	2n = 38	2n = 40
Number of species identified	56[a]	3

[a]Galet

TABLE 2. Potentialities of *Muscadinia rotundifolia* in grape breeding (*Vitis* × *Muscadinia* hybridization).

Qualities	Deficiencies
Disease and pest resistances	
Downy mildew (*Plasmopara viticola*)	Lateness of the growth cycle
Anthracnose (*Gleosporium ampelophagum*)	Lack of winter hardiness
Powdery mildew (*Uncinula necator*)	Lack of rooting ability
Black-rot (*Guignardia bidwellii f. euvitis*)	Lack of grafting ability
Phylloxera (*Dactylosphaera vitifoliae*)	Poor palatability and organoleptic quality of the fruit
Root-knot nematodes (*Meloidogyne* sp.)	
Dagger nematode (*Xiphinema index*)	Poor crossability with *Vitis vinifera*
Resistance to transmission of grape fan leaf virus	Sterility of the hybrids

in France as a minor fungus disease because it is well controlled when spraying against downy mildew with Bordeaux mixture or organic-copper fungicides. The fungus is often very damaging on rootstocks and hybrids tolerant to downy mildew and grown without chemical spraying, so it is absolutely essential that new varieties selected for resistance to downy mildew possess some genes for resistance to anthracnose.

Cultivated varieties and wild genotypes of *M. rotundifolia* that we have in collection in Bordeaux are completely resistant to powdery mildew. However, some of these varieties have been reported to be rather susceptible in their native area. We then presume the existence of two or more races of powdery mildew, differing by their pathogenicity against *M. rotundifolia*. These observations are quite similar to those made by (10) who reported existence of two races of Blackrot differing by their pathogenicity against *M. rotundifolia, Vitis vinifera* and other bunch grapes.

RESULTS AND DISCUSSION

First results obtained: F_1 crosses *Vitis vinifera* x *Muscadinia rotundifolia:* All F_1 crosses which succeeded were made using *V. vinifera* as female and *M. rotundifolia* as male. Reciprocal crosses were a complete failure as observed by Wylie (17) and confirmed by Patel and Olmo (13). To avoid tedious emasculation, *V. vinifera* seedlings with reflexed stamens were used systematically. Genetic origin of the *V. vinifera* seedlings used is very wide. Ten varieties of *M. rotundifolia*, male or hermaphroditic, were used as pollen sources. Most pollen samples were sent from the North Carolina University, Raleigh, by W. B. Nesbitt.

Table 3 gives the results of hybridization during six years. Despite the number of F_1 hybrids obtained, it clearly points out the difficulty of F_1 crosses under the climatic conditions of Bordeaux. Only 33% of the female seedlings pollinated have produced berry set, and the number of seeds obtained per cluster pollinated was generally very low. Better results were obtained in 1976 and 1979. In 1976 the blossoming period was exceptionally hot and dry, while in 1979 pollinations were made under plastic greenhouses. Good results were also obtained in 1978 under the warmer climatic conditions of Montpellier.

Success of the crosses depends mainly on the female genotype. Best results were obtained when we used seedlings originating from the crosses Cabernet Sauvignon x Alicante Bouschet, Cabernet Sauvignon x Grenache, Carignan x Cabernet Sauvignon and Ugni blanc x Cabernet Sauvignon. Good results were also obtained when we used directly the variety Cabernet Sauvignon as female after emasculation. Even in the best conditions, the berry and ovule sets of *V. vinifera* seedlings are much lower when they are pollinated with *M. rotundifolia* than in the control crosses with *V. vinifera* pollen (Table 4).

Influence of the female genotype on the crossability between *V. vinifera* and *M. rotundifolia* is clearly demonstrated in Table 5 by the comparison of the two seedlings 29/30 and 29/31, originating from the same cross Ugni blanc x Cabernet Sauvignon. Ovule set varies practically from 1 to 12 when pollinated with

TABLE 3. Results of hybridization between *Vitis vinifera* and *Muscadinia rotundifolia* (1974 to 1979).

Year	No. of *Vinifera* seedlings used as females	No. of clusters pollinated	No. of females with berry set	No. of seeds obtained	No. of seeds by cluster pollinated	% of germination	% of inviable and abnormal seedlings	No. of viable F_1 seedlings
1974	18	56	5	85	1.5	54.1	97.8[b]	1
1975	42	204	9	366	1.8	29.2	84.1[b]	17
1976	10	102	6	1296	12.7	49.8	38.2	398
1977	18	189	14	359	1.9	49.6	51.1	87
1978	67	258	26	542	2.1	54.6	35.8	190
1978[a]	8	21	8	546	26.0	50.4	31.6	188
1979	18	96	12	1579	16.4	29.7	42.2	270
6 Years	124	926	41	4773	5.1	42.3	43.0	1151

[a]Crosses made at the Viticultural Research Station at Montpellier

[b]Including pure *Vitis vinifera* seedlings.

TABLE 4. Effect of the female genotype on the crossability between *Vitis vinifera* and *Muscadinia rotundifolia* (crosses 1979).

V. vinifera seedlings used as females	Pollinated with	Pollinated		Set (%)				Seeds	
		Clusters	Flowers	Berry	Ovule	Avg/berry	Total	Floaters (%)	Germination (%)
5 seedlings from the cross Cabernet Sauvignon x Alicante Bouschet	*V. vinifera*	11	2094	43.8	23.2	2.1	1941	9.1	35.1
	M. rotundifolia cv. Carlos	22	3438	21.1	7.4	1.4	1016	21.5	34.3
	M. rotundifolia cv. Noble	22	3953	4.8	1.2	1.1	220	20.4	44.0
4 seedlings from the cross Ugni Blanc x Cabernet Sauvignon	*V. vinifera*	10	2130	51.0	22.1	1.7	1887	10.1	30.5
	M. rotundifolia cv. Carlos	9	2290	15.1	3.2	0.9	307	2.3	19.3
	M. rotundifolia cv. Noble	12	2851	8.9	2.0	0.9	233	3.8	16.5

TABLE 5. Effect of the female genotype on the crossability between *Vitis vinifera and Muscadinia rotundifolia* (Crosses 1979).

V. vinifera seedlings used as females	Pollinated with: *Vitis vinifera*		*M. rotundifolia* cv. Carlos		*M. rotundifolia* cv. Noble	
	Ovule set (%)	Floaters (%)	Ovule set (%)	Floaters (%)	Ovule set (%)	Floaters (%)
Cabernet Sauvignon x Alicante Bouschet						
42/28	19.2	9.0	3.8	21.2	1.8	41.6
42/32	26.3	2.6	9.9	0.6	0.8	0.0
43/28	41.1	13.1	14.2	53.5	2.5	44.0
43/31	17.9	10.0	6.7	0.0	1.7	3.8
43/33	32.8	17.1	9.6	48.9	1.1	37.5
Ugni Blanc x Cabernet Sauvignon						
29/29	12.4	3.6	1.8	6.1	0.7	20.0
29/30	23.2	4.4	0.7	4.3	0.3	0.0
29/31	15.3	0.0	8.2	0.0	5.1	0.0
29/33	35.4	16.4	4.7	2.8	3.5	2.5

M. rotundifolia cv. Carlos and from 1 to 17 when pollinated with *M. rotundifolia* cv. Noble. Another example of the influence of the female genotype is given in Table 6. On three seedlings originating from the same cross Cabernet Sauvignon x Grenache, the number of seeds obtained per cluster pollinated varies from 1 to 10. We can conclude that the crossability between *V. vinifera* and *M. rotundifolia* is improved by genetic factors, which are likely present in the variety Cabernet Sauvignon and are segregating in the progenies.

Fertility of the hybrids: Table 7 gives the results of back-crossing the F_1 hybrids by *V. vinifera* during five years. More than 70% of the hybrids pollinated did not produce berry set and can be classified as totally sterile. The others are classified as highly sterile if their ovule set is lower than 0.1% and as partially fertile if the ovule set is higher than 0.1%. Contrary to, as expected, the germination of the seeds and the percentage of viable seedlings are not significantly lower in the back-cross progenies than in the F_1 progenies. In fact, presence of numerous genotypes genetically unbalanced in the BC1 progenies is more evident after one year's growth.

Table 8 gives the results of pollination of 12 partially fertile hybrids with different *V. vinifera* varieties. Ovule set varies from 0.6 to 2.7% with a mean of 1.7% while percentage of floaters varies from 0.0 to 64.4. On three hybrids of the T6 series, Jelenkovic and Olmo (6) reported ovule set varying from 0.6 to 5.9% with a mean of 3.0% and percentage of floaters varying from 3.1 to 71.4.

Influence of the pollen parent on the ovule fertility of the F_1 hybrids is not clearly evident, as shown in Table 9. Good results have been obtained with Cabernet Sauvignon, Syrah and Mourvedre, which give on every hybrid a higher berry set than the mean of all the pollens used. In fact, influence of the pollen parent is much more evident on the frequency of unbalanced genotypes in the BC1 progenies.

Chromosomal analysis of the F_1 hybrids (Fig. 1): All hybrids are diploid and have 39 somatic chromosomes, which is a cytological proof of the hybridity of these plants. Meiotic pairing has been analyzed on six hybrids and the data are summarized in Table 10. Mean number of univalents vary from 2.1 to 9.4. The wide range of variation in the chromosome configurations observed on every hybrid can be partly explained by loose pairing, as a result of cryptic structural differences between the genomes. Mean number of univalents observed in the highly sterile hybrid NC 6-15 is practically the same as the one observed by Patel and Olmo (13). But mean numbers of univalents observed in the three partially fertile hybrids of the T6 series are considerably higher than those observed by Jelenkovic and Olmo (7,8). Causes of these differences are unknown. Mean number of quadrivalents and other multivalents are not significant because observations of these meiotic configurations are very difficult in grapes. Improved cytological techniques are needed before concluding in favor of the presence of translocations and other structural changes in the chromosome complements of *V. vinifera* and *M. rotundifolia*.

TABLE 6. Effect of the female genotype on the crossability between *Vitis vinifera and Muscadinia rotundifolia* (Crosses 1978).

Vitis vinifera seedlings used as females	Pollinated with *M. rotundifolia* cv. Carlos	No. of clusters Pollinated	No. of seeds obtained	% Of germination
Cabernet Sauvignon x Grenache	cv. Carlos	4	350	56.0
2434-5-33	cv. Carlos	4	33	63.6
2434-6-1	cv. Carlos	3	71	35.2
2373-14-34				

Crosses made at the Viticultural Research Station of Montpellier.

TABLE 7. Results of the back-crosses of the F_1 *Vitis* × *Muscadinia* hybrids by *Vitis vinifera* (Crosses 1975 to 1979).

Year	No. of F_1 hybrids pollinated	No. of F_1 hybrids with berry set	No of seeds obtained	% Of Germination	% Of unviable BC_1 seedlings	No. of viable BC_1 seedlings
1975	2[a]	2	18	38.9	28.5	5
1976	2[a]	2	66	31.8	23.8	16
1977	6 (2[a])	4	140	61.4	47.7	45
1978	50 (3[a])	14	485	53.2	46.9	137
1979	65 (5[a])	19	1815	28.9	45.0	288
5 Years	78 (5[a])	22	2524	35.3	45.2	491

(a) F_1 hybrids introduced from the USA.

TABLE 8. Ovule fertility of the F_1 *Vitis* × *Muscadinia* hybrids pollinated with *Vitis vinifera* varieties (Crosses 1979).

F_1 Hybrids	Pollinated		Set (%)		Seeds			
	Clusters	Flowers	Berry	Ovule	Avg./berry	Total	Floaters (%)	Germination (%)
T 6-38[a]	64	7222	10.1	2.7	1.05	769	10.4	26.3
T 6-42[a]	15	1457	3.6	0.9	1.06	55	20.0	15.9
T 6-44[a]	15	3347	9.3	2.4	1.04	325	17.2	30.2
VRH 8625	20	7519	3.2	0.8	1.02	247	64.4	45.5
VRH 8724	1	193	1.6	0.4	1.00	3	0.0	33.3
VRH 8733	2	320	8.1	2.3	1.12	29	10.3	17.8
VRH 8736	1	154	3.9	1.0	1.00	6	16.7	20.0
VRH 8742	1	294	2.7	0.7	1.00	8	0.0	25.0
VRH 8744	3	582	2.4	0.6	1.00	14	7.1	7.7
VRH 8771	6	1238	7.1	1.9	1.06	93	8.6	41.2
VRH 8773	2	563	3.0	0.7	1.00	17	11.8	46.7
VRH 8778	4	626	9.3	2.2	0.95	55	21.9	36.4
All hybrids	134	23515	6.6	1.7	1.04	1621	20.5	28.5

[a]Hybrids introduced from the University of California, Davis.

TABLE 9. Berry set (%) of the F_1 *Vitis* x *Muscadinia* hybrids pollinated with different *V. vinifera* varieties (Crosses 1979).

F_1 Hybrids	*Vitis vinifera* varieties used as pollinators					
	Cabernet Sauvignon	Syrah	Pinot Noir	Grenache Noir	Other pollens	All pollens
T 6-38	15.2 (1386)[a]	-	6.4 (659)	9.2 (5513)	9.3 (4664)	10.1 (7222)
T 6-42	4.8 (250)	5.5 (109)	3.0 (525)	2.6 (232)	3.5 (341)	3.6 (1457)
T 6-44	-	-	-	9.3 (3347)	-	9.3 (3347)
VRH 8625	3.0 (1625)	3.6 (3591)	-	-	2.7 (2303)	3.2 (7519)
VRH 8724	1.6 (193)	-	-	-	-	1.6 (193)
VRH 8733	-	8.3 (120)	8.0 (200)	-	-	8.1 (320)
VRH 8736	-	-	3.9 (154)	-	-	3.9 (154)
VRH 8742	2.7 (294)	-	-	-	-	2.7 (294)
VRH 8744	-	-	-	0.5 (209)	3.5 (373)	2.4 (582)
VRH 8771	8.1 (406)	10.6 (358)	2.8 (105)	-	3.8 (369)	7.1 (1238)
VRH 8773	3.0 (563)	-	-	-	-	3.0 (563)
VRH 8778	-	10.4 (338)	-	8.7 (138)	7.3 (150)	9.3 (626)
All hybrids	7.0 (4717)	4.9 (4516)	5.0 (1643)	8.5 (4439)	6.7 (8200)	6.6 (23515)

[a]Number of flowers pollinated.

Fig. 1. Chromosomal analysis of *Vitis vinifera*, *Muscadinia rotundifolia* and F_1 hybrids.

2 a : mitotic metaphase of *Vitis vinifera* (2 n = 38)

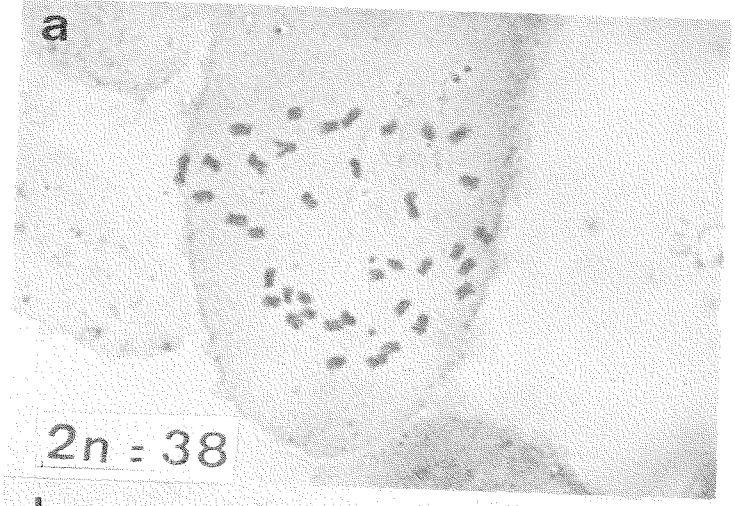
2 b : mitotic metaphase of *Muscadinia rotundifolia* (2 n = 40)

2 c : mitotic metaphase of F_1 hybrid (2 n = 39)

2 d : meiotic metaphase of F_1 hybrid (19 II + 1 I)

2 e : meiotic metaphase of F_1 hybrid (15 II + 1V + 5 I

2 f : meiotic metaphase of F_1 hybrid (15 II + 9 I)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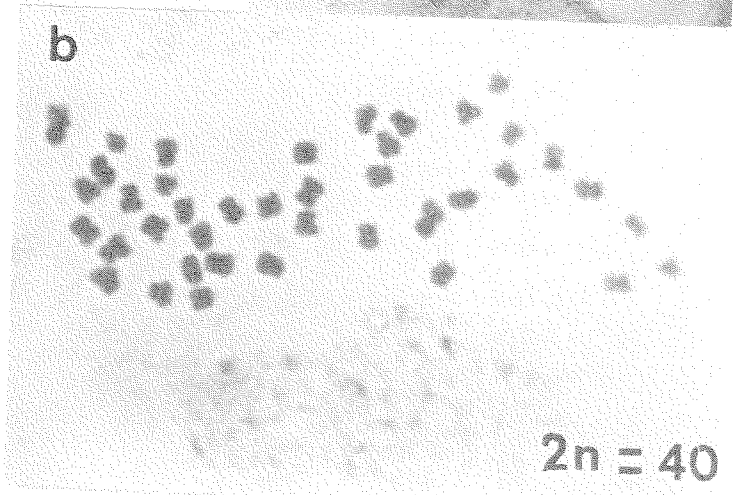
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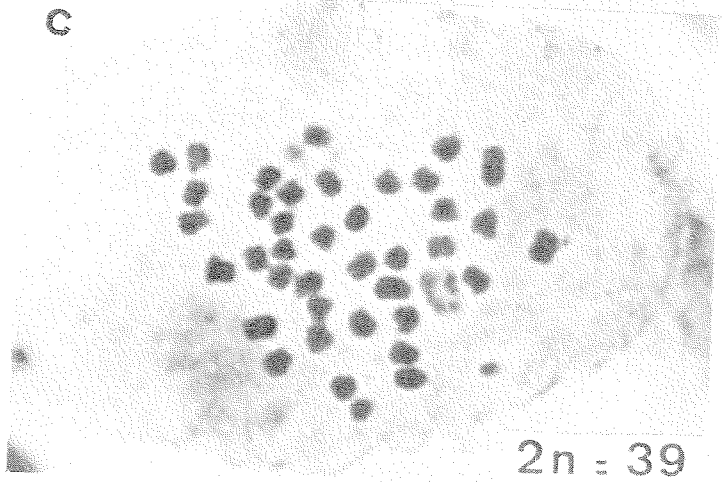
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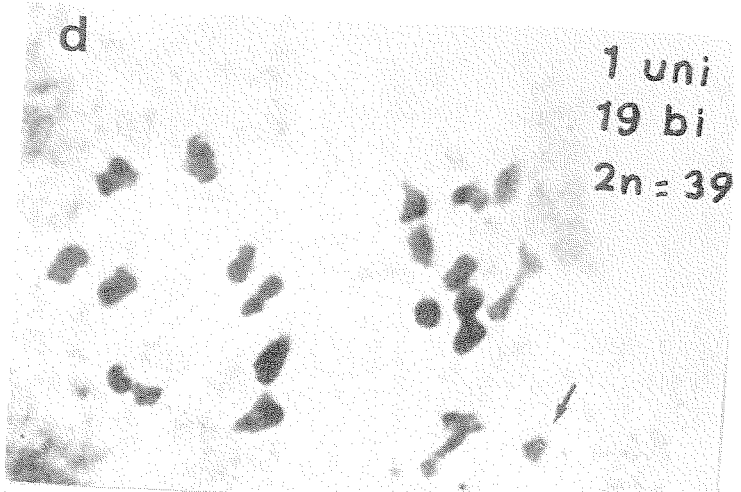
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19 bi
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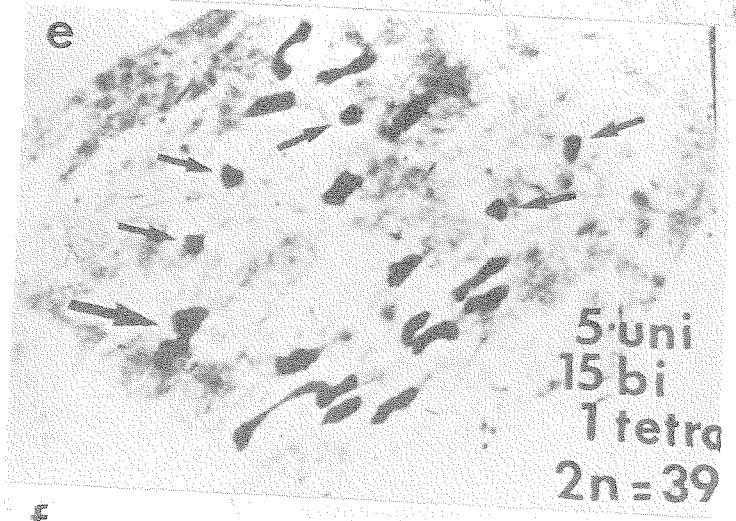
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15 bi
1 tetra
2n = 39

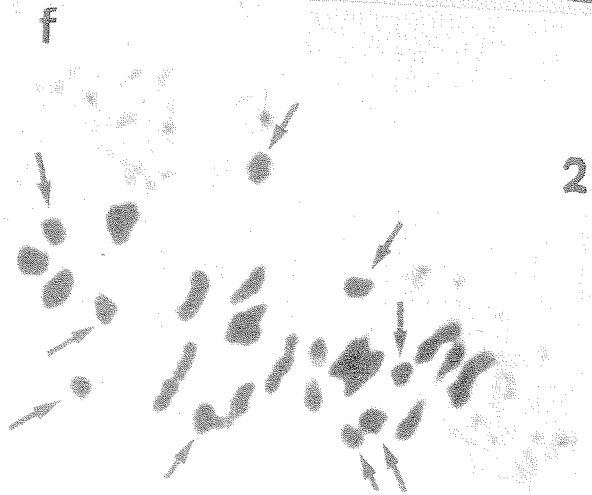
f
9 uni
15 bi
2n = 39

TABLE 10. Chromosome associations at M I of diploid F_1 *Vitis* x *Muscadinia* hybrids.

F_1 Hybrids	PMC analyzed	Univalents I	Bivalents II	Trivalents III	Quadrivalents IV	Other Multivalents V and VI
N C 6-15	90	9.4[a] (0-18)[b]	14.4 (9-18)	0.22 (0-2)	0.04 (0-1)	0
N C 6-16	60	3.7 (0-11)	16.8 (12-19)	0.31 (0-1)	0.13 (0-2)	0.016 (0-1)
T 6-38	179	7.0 (0-18)	13.9 (5-19)	0.85 (0-3)	0.33 (0-3)	0.06 (0-1)
T 6-42	50	7.6 (1-15)	14.4 (9-19)	0.78 (0-2)	0.06 (0-1)	0
T 6-44	23	4.5 (1-9)	16.9 (14-19)	0.21 (0-1)	0.04 (0-1)	0
VRH 8625	81	2.1 (1-7)	17.9 (16-19)	0.30 (0-1)	0.05 (0-1)	0

[a] Mean.
[b] Range.

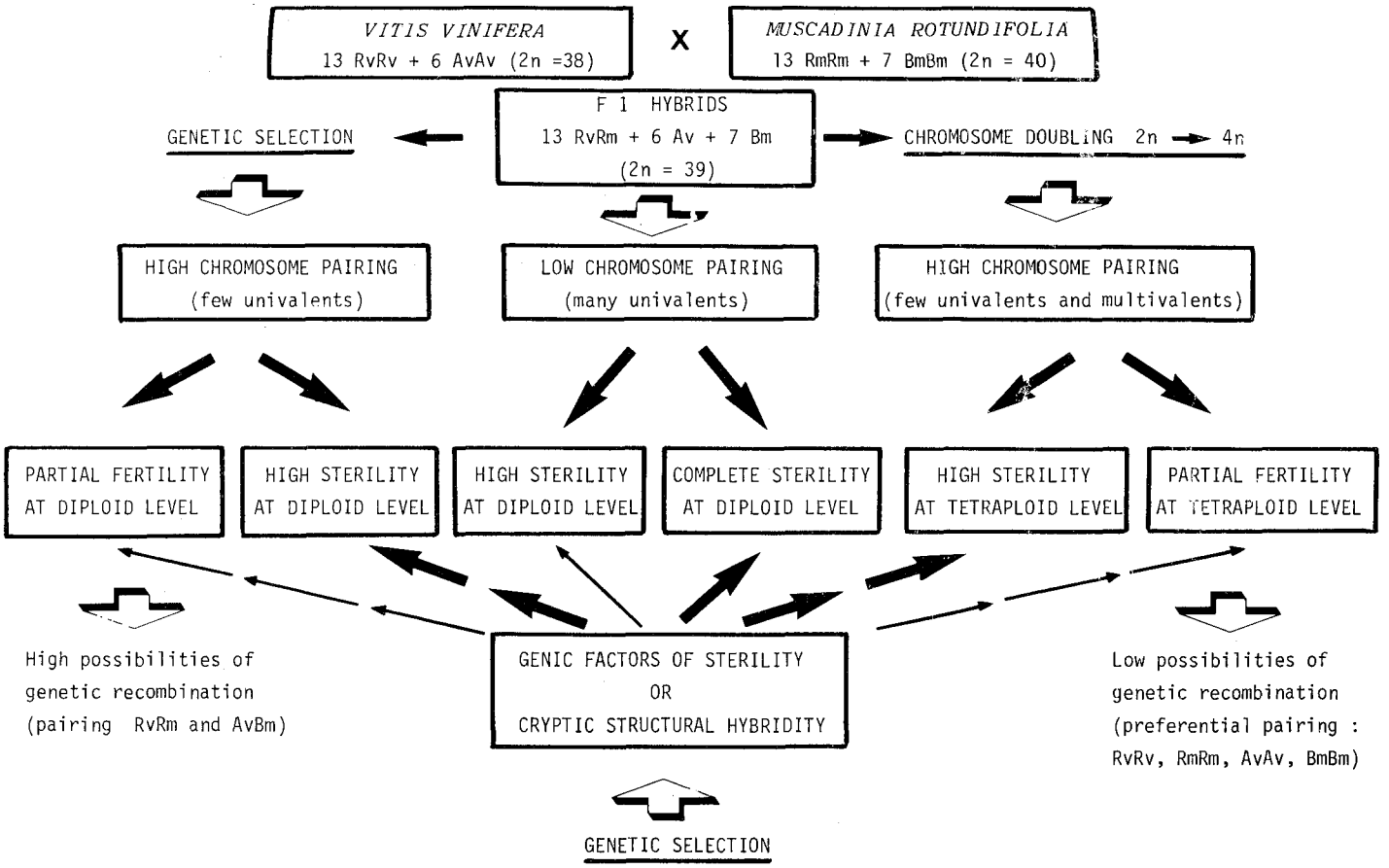

Fig. 2. Chromosome and genic sterilities in the *Vitis* x *Muscadinia* hybrids. (Genome constitution from Olmo, 1955).

CONCLUSION

Absence of positive correlation between chromosome pairing and ovule set indicates that the sterility of the hybrids is both chromosomal and genic in nature. Lack of homology between the chromosome complements of *V. vinifera* and *M. rotundifolia* is evident. Patel and Olmo (13) suggested existence of two sets of chromosomes in each genome, one set of 13 homologous chromosomes, and one set of 6 or 7 non-homologous chromosomes. In this hypothesis, *Vitis* and *Muscadinia* can be defined as ancient secondary allohexaploids. Observation of partially fertile hybrids with low number of univalents indicates that chromosome pairing between non-homologous sets is likely genetically controlled. However, we need more information to conclude on the origin of these genetic factors: *V. vinifera, M. rotundifolia* or both species?

Some researchers have used the classical method of doubling the chromosome number to restore fertility of the hybrids at the tetraploid level (3,7,8). In fact, sterility is still a problem, in spite of a relatively low number of univalents and multivalents observed in meiotic configurations. We think that tetraploid hybrids are of less interest than diploid hybrids, because preferential pairing occurrring within the doubled sets of chromosomes reduces strongly the probability of effective cross overs between chromosomes of *V. vinifera* and chromosomes of *M. rotundifolia*. (Fig. 2).

Selection of diploid hybrid genotypes with high chromosome pairing and high ovule fertility is quite possible. These hybrids will allow obtaining back-cross progenies with a sufficient number of seedlings to secure the selection of well-balanced and recombined genotypes. First observations on the vigor, fruitfulness, disease resistance and quality of some back-cross seedlings are very promising and corroborate these hopes.

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VITIS ARMATA A NEW SOURCE OF GERM PLASM IN GRAPE BREEDING

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ABSTRACT

Vitis armata is a native species from the mountainous regions of southern China. For several years a male strain showed field resistance against the common fungus diseases in German vineyards. From this point of view, crosses have been carried out with Müller-Thurgau. Of 213 F_1-plants 100 flowered and resulted in 43 hermaphrodites, 25 females and 32 males. As the chromosome number of *armata* is 2n = 38, fertility of the hybrids was normal, 84% on the average. Berries varied in size from small to medium; they were of dark-blue color and differed from *Vitis vinifera* by a high content of malvidin 3,5-diglucoside. Berries showed a high degree of resistance against *botrytis* rot.

Vitis armata is a wild growing species from the mountainous regions of Central and Southern China. It was first described by Diels and Gilg from the province of Hupeh in Central China in 1901. For many years a male strain of this species has been cultivated in several institutions in Europe. *Vitis armata* is distinguished from all other species by its prickles on the stems and petioles. The latter character has lead to its name *armata,* which means armed in this case, by prickles. The leaves are heart-shaped, quite thick, dark green and shiny.

During several years' observations, we found the strain of *Vitis armata* under field conditions resistant to the common diseases in German vineyards, i.e., downy mildew *(Plasmopara viticola)* and powdery mildew (Oidium). Resistance against downy mildew occurs only in the field. Under laboratory conditions the leaves showed a high degree of susceptibility after artifical inoculations.

From the point of view of field resistance, crosses have been carried out between the cultivar Müller-Thurgau as female and *Vitis armata* as male. 213 F_1-plants have been obtained of these crosses which resulted in 100 normal growing plants. They consisted of 25 females, 32 males and 43 hermaphrodites. This ratio is in accordance with the hypothesis of heterogamety of the male sex. The offspring fits well a segregation of 1 male : 1 female : 2 hermaphrodites. This leads to the conclusion that sex determination in *Vitis* is not controlled by a simple XY mechanism, but a series of three alleles with dominance of the allele for hermaphroditism over maleness; the female type is double recessive.

The chromosome number of *Vitis armata* is 2n=38, found also in the hybrids. Pollen fertility in the males and hermaphrodites

varied from 60 to 98% with an average of 84% normal looking pollen grains. From that and the segregation for sex types, it may be assumed that meiosis is normal and, therefore, homology exists between the genomes of *Vitis armata* and *Vitis vinifera*. The female fertility, that means seed set per berry, was also normal. The number of berries per cluster in the females and hermaphrodites was, however, quite variable from plant to plant. There occurred, for example, some plants which produced only 2 to 3 berries; others produced many inflorescences on each vine. These clusters were produced in most cases toward the apex of the canes.

A great variation was observed in the F_1 concerning morphological and physiological characters, as well. Bud burst of most of the F_1 plants was, as in *Vitis armata*, nearly one week earlier than in Müller-Thurgau. Besides a certain number of dwarfs and weak plants, most of the hybrids were very vigorous and showed heterosis for length of shoots and leaf size. The leaves were lobed, approaching Müller-Thurgau. The prickles showed a somewhat intermediate expression; there were small excrescences on the stems and petioles of all F_1-plants.

Concerning the resistance to downy mildew, no definitive observations could be made because of lack of natural infections in the last two years. In case of powdery mildew, different degrees of resistance have been observed. Some plants showed a reasonable degree of resistance. The expression of the character may lead to the conclusion that it has a polygenic basis of inheritance.

Although there were no applications of botryticides, we never have observed gray-mold rot *(Botrytis cinerea)* attack the berries. This was also the case in late season, i.e., in 1979 when harvest was 31 October with 95°Oechsle (22.5°Brix) and 10.1 g/l acidity.

Among the 68 females and hermaphrodites, only one plant showed signs of "Stielfäule," that is a peduncle rot caused by physiological disharmonies in the content of minerals. All the other plants were not affected by that disease.

The size and form of cluster varied from plant to plant. There occurred rather straggly clusters, intermediate ones and also compact ones. The berries were spherical and varied in size between small to medium and were of dark blue-red color with tough skins. As the blue-red berry color usually behaves as a dominant, *Vitis armata* can be assumed as a species homozygous for colored berries. The berries were neutral in flavor; no pronounced aroma could be detected. The quality was reasonable, about 90°Oechsle = 21.5°Brix in average. But the relatively small yield must be considered.

Although the hybrids have originated from a cross of a white wine cultivar, the wines had a fine red wine character with a deep red color when fermented on the skins. No odd taste could be observed in the young wines, as one would expect from a hybrid with a wild growing species.

Like the Eastern asiatic *Vitis amurensis*, *armata* is also dis-

tinctly separated from *vinifera* by its high content of malvidin 3,-5-diglucoside.

From our investigations with *Vitis armata*, it may be demonstrated that valuable characters are still to be found in the wild growing *Vitis* species. All efforts, therefore, should be made to collect, preserve and investigate the sources of germ plasm to advance the grape-breeders' work.

A STUDY OF SEXUAL PROGENIES OF BICANE X SULTANINA (VITIS VINIFERA L.). EVIDENCE FOR GENETIC DIFFERENCES BETWEEN SULTANA CLONES IN BERRY WEIGHT

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ABSTRACT

The sexual progenies of three Sultana clones are significantly different from each other in their average berry weight. Their relative ranks for this character are the same from one year to the other.

The phenotype of Sultana clones may be used to forecast the average berry size in their progeny from crosses with another variety, provided these clones are free of leaf-roll virus.

Clonal selection exploits both genetic differences and differences in health existing within a variety (5). For the variety Sultana (*Vitis vinifera* L. cv. Sultanina, syn. Thompson Seedless) in Australia, Woodham and Alexander (8) showed that there was a variation between clones, notably in yield, even though the origin of the population on which selection was made could be traced to only eight plants, the only survivors of an introduction to the Adelaide Botanic Gardens in 1867 (4). Work at Merbein also revealed clones with large differences in mean berry weight. To study whether these differences were genetic in origin, a reciprocal grafting trial using two clones very different in this respect was carried out (1). No effect on berry weight could be shown to be transmitted by grafting and thus the simplest explanation appeared to be that the difference was genetically determined. To confirm this in a simpler situation, which should separate the genetic and health effects, sexual progeny of the clones were compared. It is well known that in *Vitis vinifera* plants raised from seed have an excellent state of health; therefore, only genetic effects should be responsible for any significant differences revealed between progenies.

MATERIALS AND METHODS

The material studied comprised, on the one hand, the various clones used as parents (a clone of Bicane obtained under the name of Raisin de Dame and Sultana clones H23, H5 and G2) and, on the other hand, their progenies obtained by sexual crossing.

All this material was planted on its own roots in the experimental vineyard of the Merbein Station. The three progenies of Bicane x Sultana were set out on level ground in randomized blocks with 120 replications of three treatments (the progenies of

each Sultana clone). The basic plot was a single vine, i.e., one genotype of one progeny. Mean berry weight per vine was estimated for three seasons.

In 1979 more complete measurements were made. In addition to mean berry weight, it was possible to record the other variables shown in Table 1.

Sampling of berries comprised three steps:

1. *Choice of bunches:* This took place after the vine was harvested. All bunches were used in the sampling unless the yield of the vine exceeded 9 kg (about 30% of the vines) when only half of the yield was used.

2. *Choice of the portions of bunches which made up the sample:* The bunch was divided into 4 or 5 equal parts according to its shape, each to be represented in the final sample. The size of the portion actually taken was such that a sample of about 100 to 200 berries was obtained.

3. *Division into sub-samples:* The effect of number of seeds on berry weight is so decisive (Fig. 1) that the sample must be straitified into two or three classes of berry size according to whether a bimodal or trimodal distribution is found.

4. *Estimation of mean berry weight:* As the hypothesis of normal distribution was not tenable at genotype level, the arithmetic mean is no longer the best estimate (2). Therefore, in 1979 the results given by two different estimates were compared: the arithmetic mean and the mean weighted for the weight of fruit in each sub sample. As no alterations to distributions or classifications were observed at progeny level, only the arithmetic means were retained. This allowed the results obtained in 1979 to be combined with those already taken in 1971 and 1972.

Estimation of mean seed weight: A preliminary study on the variety Bicane, on berries having only one seed per berry, showed the importance of this variable in accounting for berry size.

Given the small weight of seeds in the progenies studied, above all in the genotypes close to "sultana-type" seedlessness (seeds aborted at more or less early stages of development), only seeds coming from the category "large berries" have been sampled. The other categories of berries generally have seeds too small to be worth weighing. Consequently, this datum is not representative of all berries; however, it does permit some indication of the order of magnitude of the variations between genotypes.

RESULTS AND DISCUSSION

Mean berry weight of the clones of which the sexual progenies were studied: *Sultana (Table 2):* As there were no data for direct comparison of all three clones G2, H5 and H23, an indirect comparison was made, combining the results of three trials by a non-parametric method (problem of the leader) expounded by Tomassone (7). The calculation of "iterated powers" always gives the same ordering of the three clones with respect to one another,

TABLE 1. Comparison of progeny mean for several characters (results obtained in 1979).

Progenies (number of vines)	Berry weight g.	Number of seeds	Weight of 10 seeds g.	Harvest Date	Yield kg/vine	Brix	Trunk circumfer. inches
Bicane x Sult. Cl.H5	2.95	1.32	0.61	07/03	4.33	19.8	6.20
Bicane x Sult. Cl.62	2.70	1.24	0.56	08/03	4.25	19.6	6.07
Bicane x Sult. Cl.H23	2.58	1.33	0.56	09/03	4.35	19.3	5.23
P	0.05	NS	NS	NS	NS	NS	0.01

P : Significance level of t test.

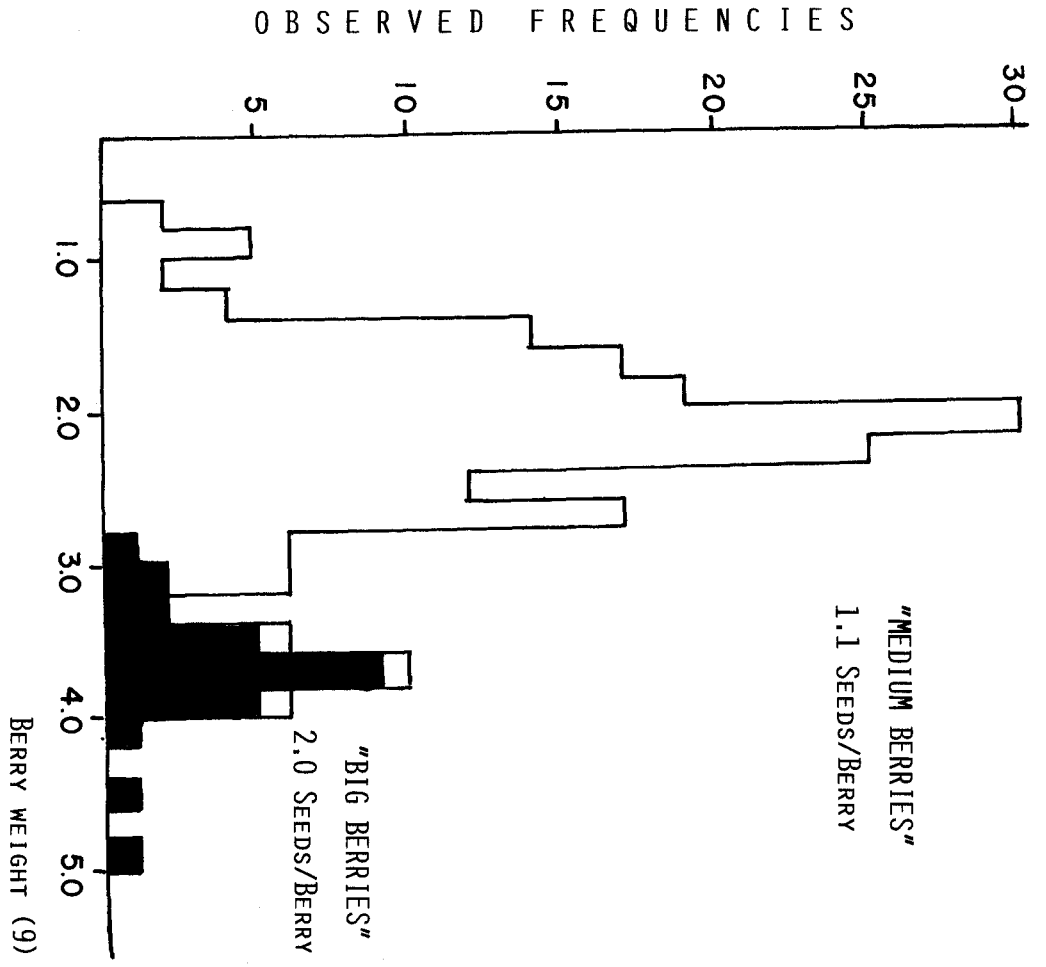

Fig. 1. Berry weight distribution of one vine from one progeny. Biomodal distribution explained by the influence of number of seeds on berry weight.

TABLE 2. Mean berry weight of the clones of which the sexual progenies were studied. Ranking of these clones.

Clones	Result of the trials			"Powers" After K Iterations			RANKING OF THE CLONES
	1	2	3	K = 1	K = 10	K = 50	
G2	1.87	1.67	-	.555	.909	.908	1
H5	1.77	-	1.56	.333	.086	.019	2
H23	-	1.29	1.11	.111	.004	.000	3
P	0.05	0.005	0.001				

whatever the number of iterations performed. The results shown in Table 2 give the "powers" found for each clone after 1, 10 or 50 iterations.

Bicane: Only two vines could be sampled, the mean berry weight for this clone of Bicane in 1979 was 4.7 g, while the mean of the clones of Sultana (H23 and H5) was about 1.5 g in that year.

Study of progenies: results obtained in 1979. Table 1 shows the results for all data collected in 1979. Only two variables show a significant difference between progenies: mean berry weight and trunk circumference.

The difference for mean berry weight confirms a result already found in 1972 on somewhat fewer data. But the higher significane of trunk circumference of the vines was not expected. In studying Table 1, it should also be noted that the order of the progenies for vigor (trunk circumference) and berry weight is identical, although a cause- and-effect relation should not be suspected since no correlation can be detected between these two variables within any of the three progenies.

In no case could the number or size of the seeds, the yield or the state of maturity explain the difference in mean berry weight found between progenies.

Within a progeny the only significant correlation found related to the weight of seeds of a berry. This correlation also appeared at the level of berries for a genotype.

Distribution of berry weight in the progenies: Only the progeny Bicane x Sultana cl H23 gave a histogram not differing significantly from a normal distribution. The other two progenies deviated significantly from it both in 1972 and 1979. On Fig. 2 the shift of means between progenies shows clearly, as well as a difference between standard deviations.

4. Comparison of progenies over several years in berry weight (Fig. 3) : The effect "progeny" and "year" are very marked; moreover, the order of the levels of each of these two factors in relation to the levels of the other remains identical, suggesting that the progeny x year interaction is unimportant in relation to the two principal effects.

In order to be assured of the validity of the conclusions drawn from the analysis of the whole of the data concerning mean berry weight, we have used both the analysis of variance (Table 4) and a non-parametric method (6) (Table 5).

The two analyses give identical results: effects of progenies and years are very highly significant with absence of interaction. Thus, there is indeed a genetic difference between the three clones of Sultana of which the progenies have been studied. The differences between years could not be related to the meteorological data collected at Merbein and, as well, it has been verified that the genotypes which were the latest to reach the fruiting stage do not show, on average, smaller berries than the rest of the

TABLE 3. Significance of Spearman rank correlation within each of the three studied progenies.

	Seed Weight	Harvest Date	Yield	Brix	Trunk circumfer.
Mean berry weight	***	NS	NS	NS	NS

*** : Significance level 0.01
NS : Not significant

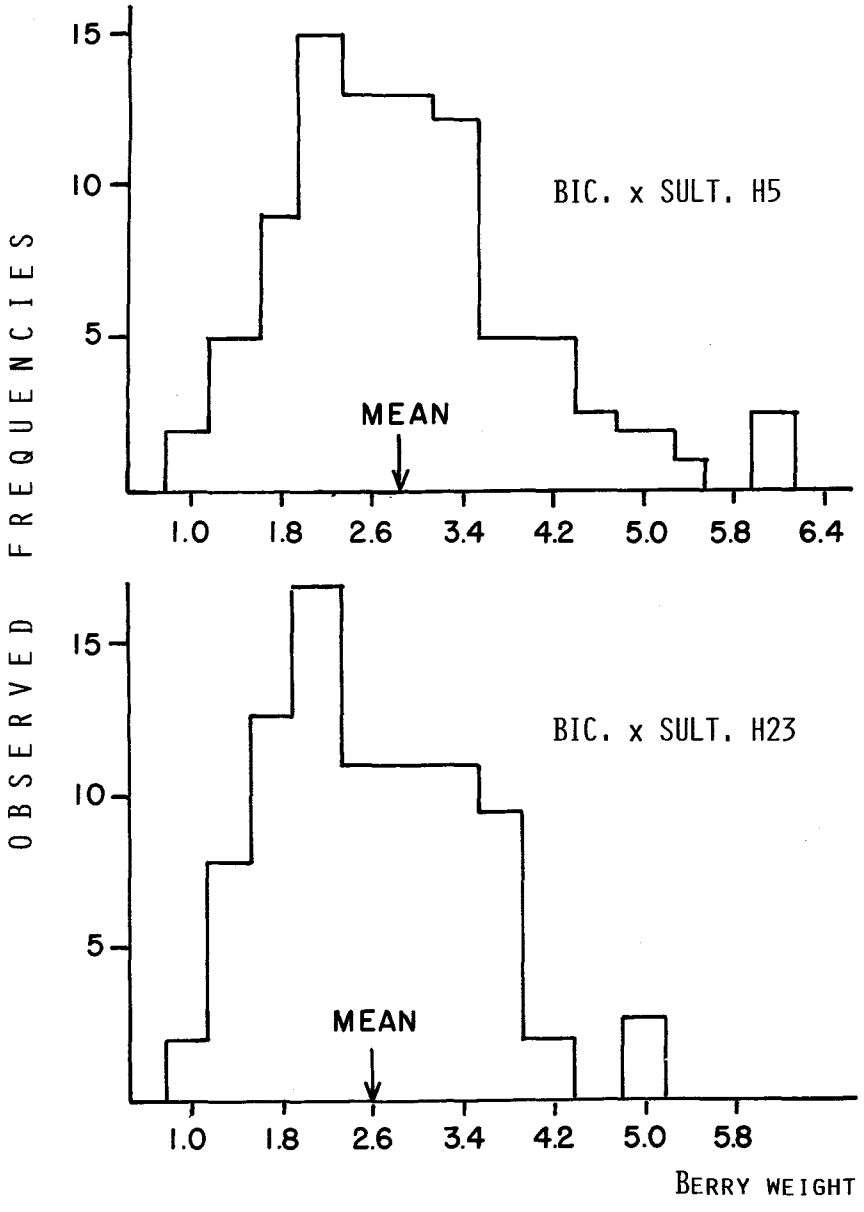

Fig. 2. Distribution of berry weight in the progenies (1979). Shifting of means between progenies. Difference between standard deviations.

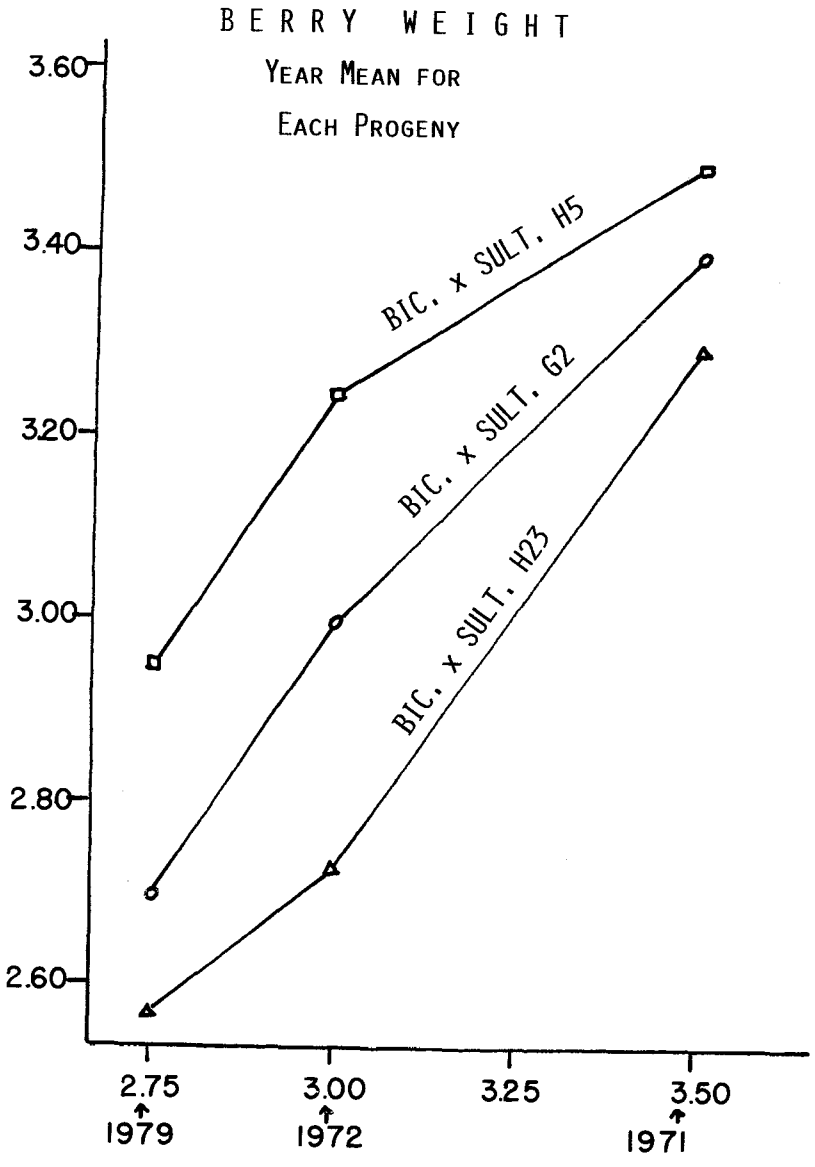

Fig. 3. Comparison of progenies for several years.

TABLE 4. Comparison of progenies for mean berry weight : 2 way analysis of variance with non-orthogonal data.

Fitting progenies before years				Fitting years before progenies			
	D.F.	F	P	P	F	D.F.	
Progenies	2	-	-	-	-	2	Years
Interaction	4	1.	NS	NS	1.	4	Interaction
Years	2	11.5	0.001	0.005	6.0	2	Progenies
Within cells	8	-	-	-	-	8	Within cells
Error	483	-	-	-	-	483	Error
Total	491	-	-	-	-	491	Total

TABLE 5. Comparison of progenies for mean berry weight : procedure of Schreier, et. al (extension of Kruskal-Wallis test).

Fitting progenies before years				Fitting years before progenies			
	D.F.	H_m	P	P	H_m	D.F.	
Progenies	2	-	-	-	-	2	Years
Interaction	4	6.6	NS	NS	6.6	4	Interaction
Years	2	119.4	<0.005	<0.005	106.6	2	Progenies
Within cells	8					8	Within cells

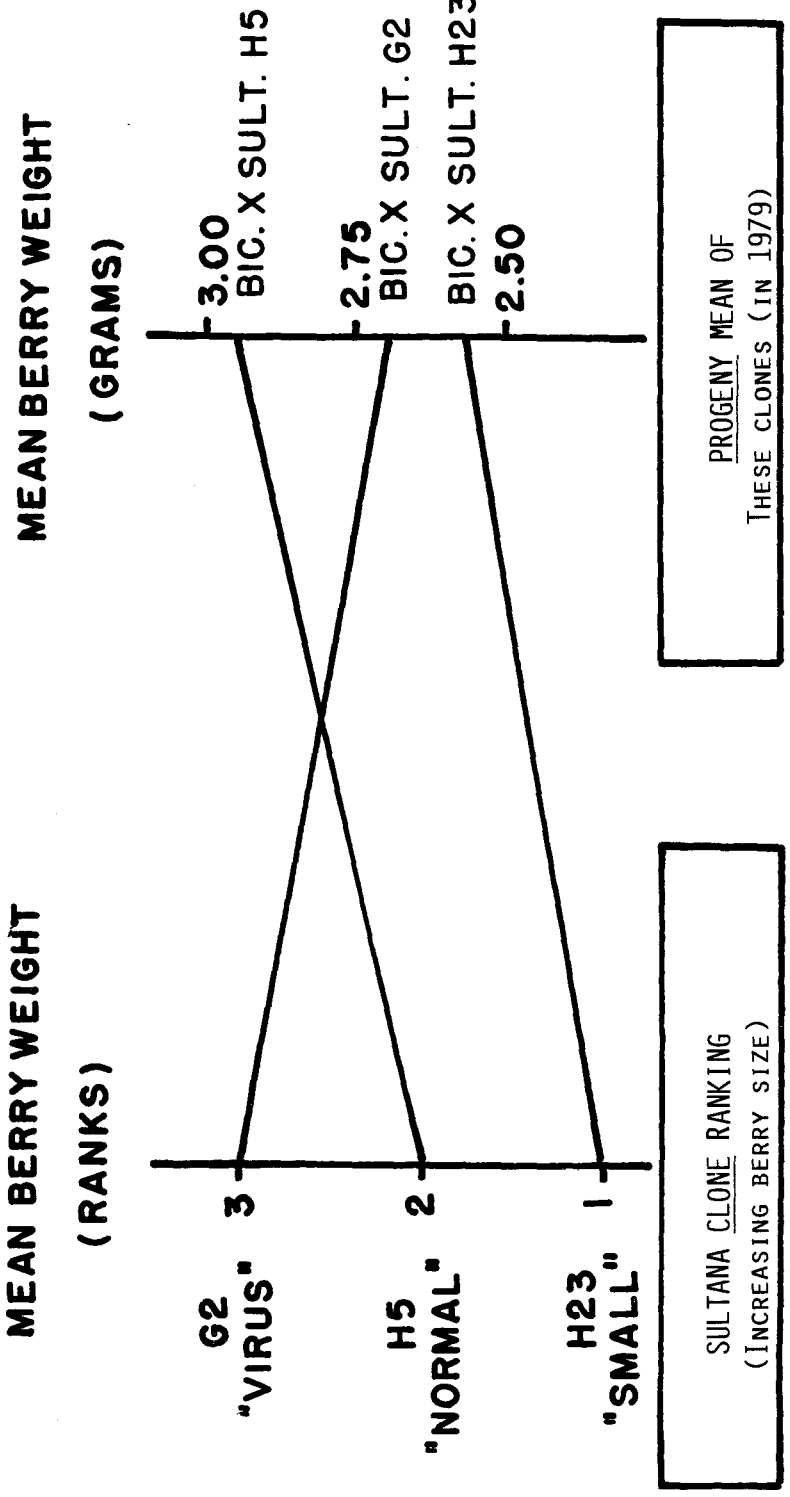

Fig. 4. Comparison: Parent not in common-progeny. (Prediction of the behavior of the sexual progeny of a clone seems possible only for clones not infected with leafroll).

progeny.

Comparison: parent not in common--progeny for mean berry weight: The clone infected with leafroll behaves differently from the two clones with a better state of health: prediction of the behavior of the sexual progeny of a clone seems possible only for clones not (or slightly) infected with leafroll.

The important point is that it is not immaterial for the breeder to choose any particular clone within a variety to obtain a progeny. In the case of Fig. 4, it can be seen, in fact, that the percentage of plants with a berry weight greater than 4 g is very different, according to whether one chooses clone H5 (16%) or clone H23 (6%). A priori one might have supposed that the small variation revealed between the clones of Sultana would be buried in the large variation found in all progenies from crosses between varieties of *Vitis vinifera* for the majority of morphological characters and particularly for berry size.

It should be stated, however, that it is difficult to gauge the generality of this result because, for obvious practical reasons, only one (the variety Bicane) was used while several would be necessary to estimate the ability of each of the three clones of Sultana to transmit to its progeny the character "berry size" (Aptitude génerale à la combinaison : Demarly 1977).

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IDENTIFICATIONS IN COLLECTIONS OF GRAPEVINES

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ABSTRACT

It can be roughly estimated that the number of *Vitis vinifera* varieties of natural origin registered in the world is at least 5000. These varieties are often given several different names as, for example, in the *Ampelographie* of Vialla and Vermorel: 24,000 names for only 5200 varieties.

Varieties play a large part in wine production, and knowledge of them is indispensable if regulations are to be observed in order to maintain or improve quality. In most vine-growing countries, grapevine collections have been established in order for specialists to study and identify different varieties. However, the number of varieties one person can identify is necessarily limited, and a system has been sought to extend the possibilities of identification.

PROCESS OF IDENTIFICATION

The identification of a variety can be divided into two operations: 1) the observer, using his knowledge, experience and observation, first makes one or several hypotheses about the identity of the unknown sample, and 2) then, having narrowed down the identity to a small number, he can turn to a comparison with references grown in a collection. If his knowledge is at fault, the identification is impossible, but the descriptions of the varieties established in a collection may remedy this deficiency. This alternative is being studied now.

Description of varieties: The varieties established in the collection of I.N.R.A. at Vassal (near Montpellier) have been described along the lines of a system drawn up in 1951 by the International Office of Vines and Wines, using the table of characteristics fixed by U.P.O.V. (International Union for the Protection of New Varieties of Plants) in 1977. Descriptions of 5500 clones have been indexed, with one punch-card for each clone.

Use of punch-cards: The transcriptions of descriptions onto punch-cards raised some problems. Indeed, it is frequently the case that, on one vine and even more on the five vines belonging to the same clone, characteristics can show variations. So it has been decided that, when a state is evidently the most frequent, it should be the only one recorded in the description. However, when two or more states appear on a single characteristic, with frequencies rather similar, they should be registered together. The process used allows a record to be kept of the fluctuation encountered. To identify an unknown variety, the punch-cards are subjected to successive sortings among all the recorded varieties, in order to find the ones which have the same characteristics. Then the identification leads to a comparison between the unknown vine and the varieties so listed.

Choice of characteristics: It is necessary to eliminate, during the sortings, a sufficient number of cards in order to reduce the number of comparisons. It is also very important to keep relevant cards. The characteristics used for the descriptions must be selective, to allow an important elimination, and reliable enough to avoid the loss of the identical variety. At first 96 characteristics were noted, but then only 27 were used in the course of sortings. A study made upon 2402 clones of white varieties of *Vitis vinifera* allows an estimation to be made on the reliability and selectivity of those characteristics.

Fluctuation and reliability: An examination of the tables of distribution shows that one can distinguish several kinds of characteristics--ones which show no fluctuation, as in the case of seedlessness and sex of flowers. Their states are formally fixed and there is practically no chance of error using them; consequently, they are very reliable. Unfortunately, these characteristics can be used only rarely, since, in the considered population, more than 98% of varieties have berries with seeds, and more than 95% have hermaphrodite flowers. It is possible to connect to this group the flavor of berries.

If there is fluctuation, the total of percentages is above 100. The other characteristics show a fluctuation whose origin arises, most frequently, from the presence of different states with similar frequency of the same characteristic. This occurs, for instance, for berry size, when it is very near the limit between two classes.

The fluctuation may also arise from the variation in the state of a characteristic, for instance the number of lobes on the whole leaves taken from the same clone, or from the nonconformity of the character over a period of time.

The most reliable characters are obviously the ones which fluctuate least.

Selectivity: The distribution of the varieties into the different states of a characteristic provides information about its selectivity, which depends on the number of classes. In fact, the state of a characteristic is often so selective that its frequency is lower. So we can see the point of using, for descriptions, a high enough number of characteristiscs in order to choose the most favorable in different cases.

TEST OF THE PROCEDURE : SIMULATIONS

In practice, when an unknown variety is to be identified in this way, a failure is due to: 1) the variety is different from all those described in the card index, or 2) a failure of the procedure itself.

TABLE 1

DISTRIBUTION OF WHITE VARIETIES
(2402 clones)

Flower sex	clones	%	Total
Hermaphrodite	2,264	95.6	
Female	103	4.4	100
Not noted	35		
Seed presence			
Seeds present	2,296	98.2	
Seedless	43	1.8	100
Not noted	63		
Berry particular flavor			
None	2,141	91.5	
Muscat	206	8.8	
Aromatic	24	1.0	
Special	6	0.2	101.5
Not noted	25		

TABLE 2

DISTRIBUTION OF WHITE VARIETIES
(2402 clones)

Berry size	clones	%	total
Very small	18	0.8	
Small	327	14.0	
Medium	1,698	72.7	
Large	896	38.4	
Very large	207	3.0	128.9
Mature leaf : number of lobes			
Undivided	137	5.7	
Three	604	25.1	
Five	1,957	81.5	
Seven	871	36.3	
More than seven	371	15.6	164.2

In order to assess the confidence we can have in the system, we proceeded to simulations of identifications, starting from clones of the collection treated in the same way as an unknown variety.

TABLE 3

SIMULATION OF IDENTIFICATION

LISTAN = PALOMINO

2,402 cards of white varieties
18 clones of Listan

Sortings on characteristics	cards kept
Berry shape	1,021
Berry size	794
Angle between main veins	386
Number of lobes	287
Shape of petiolar sinus	209
Shape of teeth	89
Length of teeth relative to their width at the base	34
Villosity of the mature leaf	34

Out of 34 cards there are 9 clones of Listan.

Our investigations covered 40 varieties represented in the collection by at least 5 clones, which were searched for among 2402 clones belonging to 1031 white varieties of *Vitis vinifera*. On an average, using ten sortings, each sorting based on one characteristic, 32 clones can be listed. Fifty percent of the clones belonging to the varieties looked for are kept.

In the case of an identification of an unknown variety, some clones can be immediately discarded if they are well enough known by the person who makes the sorting, or after a fast comparison with leaf references in the herbarium, using photographs and descriptions.

Finally, there remain only a few comparisons to make during the growing period.

TABLE 4

Overall result for 40 varieties:

Cards sorted	96,080
Cards kept after the last sorting	1,287 (1.3% of sorted cards)
Clones belonging to the 40 varieties looked for	482
Clones of varieties looked for after the last sorting	252 (52.3% of clones)
Total number of sortings	397 (on average 10 for each variety)

TABLE 5

AVERAGE RESULT OF SORTINGS
(40 Tests)

Cards sorted	2,402
Cards kept after sortings	32 (1.3%)
Clones belonging to variety looked for	12
Clones of this variety kept after sortings	6 (50%)

Control of characteristics' selectivity and reliability: It is interesting to note what characteristics have been used most often in the tests and their reliability and selectivity. Out of the 27 characteristics described, 19 were used. The eight characteristics not used were:

1) characteristics very selective for a particular state but which did not show that state in any instance, and

2) characteristics in strict relation with others which have already been used.

Characteristics most often used: The most often used characteristics were:

1) on the berry: shape and size (40), and

2) on the leaf: angle between the main veins (40), number of lobes (39), shape of petiole sinus (39), shape of teeth (39), length of teeth relative to their width at the base (39), density of hairs on blade at lower side (39).

Selectivity: The selectivity of a characteristic can range largely according to its state. On the 10 most used characteristics, the variation of the selectivity is:

the lowest on the berry shape: from 42.5% to 54.4%
and highest on the number of lobes: from 23.8% to 88.3%
and the shape of petiole sinus: from 23.4% to 97.9%.

TABLE 6

SELECTIVITY

VARIETY	STATE	SELECTIVITY
Berry shape		
Listan	roundish	42.5%
Clairette	elliptic	52.7%
Sauvignon	roundish-elliptic	54.4%
Shape of petiole sinus		
Romorantin	like V	23.5%
Viognier	like V-U	50.7%
Furmint	like lyre	72.5%
Macabeo	like lyre - lyre closed	82.4%
Gros Bourgogne	like U-lyre	97.9%
Number of lobes		
Furmint	undivided-three	23.8%
Calitor	seven and more	41.1%
Marsanne	five	81.5%
Gros Bourgogne	three-five	88.3%

Generally, the low selectivity of the shape of petiole sinus is due to the fact that this characteristic has been used, in sortings, after the angle between the main veins, with which it is correlated.

The characteristics, like shape of berry, whose selectivity ranges little, can be used in every instance.

Other characteristics, whose selectivity ranges very much, can only be used in some favorable instances: selective states.

Reliability: Reliability is assessed through the percentage of clones belonging to the varieties to be identified, which have been eliminated.

TABLE 7

RELIABILITY

Characteristic	% clones eliminated
Berry shape	6.1
Berry size	3.5
Angle of veins	6.5
Number of lobes	9.9
Shape of petiole sinus	4.7
Shape of teeth	6.0
Teeth : length/width	5.8
Angle of term. lobe	6.4
Villosity of mat. leaf	2.8
Color of main veins	7.5
Total percentage of clones eliminated	59.2%

Among the 10 most used characteristics, the most reliable (which has eliminated 2.8% of clones) is the villosity of the mature leaf. Less reliable is the number of lobes (9.9%).

Particular cases: Simulations were carried out on three particular varieties : Gibi, Chardonnay, and Muscat of Alexandria.

Each of these varieties has a characteristic which is very selective and, at the same time, very reliable in terms of identification.

Gibi : female flowers
Chardonnay : petiole sinus often limited by veins at petiole end.

Muscat of Alexandria: Muscat flavor.

In these three cases, the number of sortings is lower and the percentage of cards retained is higher. These instances show clearly the importance of the choice of characteristics and the order in which they should be used.

TABLE 8

Results

Cards sorted	7,206
Cards kept after the last sorting	115 (1.6% of sorted cards)
Clones belonging to varieties looked for	88
Clones of varieties looked for after last sorting	69 (78.4% of clones)
Total number of sortings	16 (5 or 6 for each variety)

CONCLUSIONS

The conclusion that can be drawn from the results obtained in the simulations is that the procedure can be valuable when identifying plants studied in a collection. On the other hand, it has little validity when identifying samples from a vineyard. Its efficiency depends on the standard of the reference-card index and the way the sorting is programmed.

Card index: In the simulations, more than one clone out of three, of the variety to be identified, was kept after the last sorting. It may be deduced from this that the reference-card index must be established with the descriptions of at least three clones or vines of the same variety. In the case of cultivars showing a large variation, as Pinot or Chenin, the number of described clones must be higher. It does not seem necessary to describe many characteristics, but the plant descriptions require a high degree of accuracy.

Sorting program: The description of the unknown variety must be as accurate as those of the references. The sorting program should be established using visual examination of the sample itself, rather than descriptions which do not take into account the extent of the variation of some characteristics, hence, one of the reasons why this procedure can only be used with plants grown in a collection.

Using this procedure, we have made searches upon 13 varieties in order to know if they had synonyms in the collection. In every instance we obtain a list of varieties which have the same characteristics. A comparison with references in herbarium has shown the necessity to make 14 comparisons during the growing period. Out of the 14 comparisons, 6 allowed ascertaining the following synonyms:

Brachet blanc, formerly in Provence, synonymous with Valensi blanc or Panse.

Boal rosado, Aseitão, Portugal, synonymous with Jampal, Torres Vedras, Portugal.

Alarije, Extramadura, Spain, synonymous with Malvasia de Rioja, Spain.

Oul b' Ouzgueur, table grape, Algeria, synonymous with Ladari, Algeria.

Bariadorgia, Alghero, Sardinia, synonymous with Carcajolo blanc, Corsica.

Sarfeher and Tuskespupu zamatos, table grape, Hungary, synonymous with Coarna alba, Rumania.

These synonyms have to be confirmed during the whole vegetative cycle.

SECTION II

BREEDING METHODS

CLONAL SELECTION

TISSUE CULTURE

IN VITRO TECHNIQUES FOR STUDYING OBLIGATE PATHOGENS OF VITIS

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ABSTRACT

Twelve grape cultivars (*Vitis vinifera, V. labruscana,* and French hybrids) were cultured *in vitro* on modified Jona-Webb medium. Explants from nodal stem segments were transferred to media with decreasing concentrations of benzyladenine to induce sequentially shoot proliferation, elongation, and rooting. Water droplets containing *Plasmopara viticola* zoosporangia from surface-sterilized infected leaves were placed on the under surfaces of leaves of plantlets *in vitro*. Symptoms developed in 10 days at 19°C on seven cultivars. Dual cultures of *Uncinula necator* with seven cultivars were obtained by placing conidia on the upper surfaces of leaves of plantlets and transferring conidia sequentially to axenic plantlets.

The objective of this research was to obtain dual cultures *in vitro* of diverse cultivars of *Vitis* spp. with the biotrophic (obligate) pathogens, *Uncinula necator* (powdery mildew, Oidium) and *Plasmopara viticola* (downy mildew) in order to determine physiologic races of the pathogens and possibly new sources of resistance to them.

During the past 20 years, there have been several reports of *in vitro* culture and propagation (micropropagation) of grapevine. Originally, this technique was investigated as a means of eliminating viruses from propagating material, rather than for propagation *per se*. The number of cultivars successfully micropropagated has been limited and the rate of multiplication has been low. Jona and Webb (9) reported good multiplication of Sylvaner Riesling from nodal cuttings, and Barlass and Skene (3) propagated Cabernet Sauvignon from fragmented apices.

Potted plants have been inoculatd with *Uncinula necator* by dusting the leaves with heavily infected leaves of source plants and maintaining the dusted plants in a humid greenhouse (6). Conidia also can be suspended in water with the aid of a surfactant and misted onto leaves with an atomizer (2,7). These methods have been used to inoculate seedlings, rooted cuttings, and excised leaves in petri dishes. Aldwinckle, et al., (2) showed that the reaction of artificial inoculation in the greenhouse was correlated with natural infection in the field although there were some exceptions, possibly due to different races in the field.

Boubals (6) used Morel's method (11) to inoculate callus cultures of *V. riparia, V. rupestris,* and several cvs. of *V. vinifera* with contaminant-free inoculum of *U. necator*. He reported that

reaction of the calli was correlated with reaction of whole plants.

Husfeld (8) inoculated grape seedlings in the greenhouse with *Plasmopara viticola* by spraying the lower surfaces of the leaves with a suspension of conidia and maintaining the leaves wet for 24 to 48 hours. Boubals (4) inoculated excised leaves by placing droplets of water containing conidia on the abaxial surfaces of leaves floating upside down in petri plates.

Morel (10, 11) obtained contaminant-free inoculum of *P. viticola* by, first, surface sterilizing infected grapevine leaves and then allowing the fungus to grow through the leaf surface and sporulate. Sporangia were removed from the leaves with sterile, distilled water and allowed to form zoospores, which Morel used to inoculate grapevine callus. Boubals (5) inoculated callus of various *Vitis* species by a similar technique and obtained reactions that were correlated with the reaction of whole plants.

METHODS AND RESULTS

Nodal shoot segments, ca. 1 cm in length, were surface sterilized with dilute NaOCl and placed on semi-solid medium containing mineral salts (9), thiamin, myo-inositol, and 2 X 10 M benzyladenine (BAP) at 27°C with a 16-hour photoperiod. Cultures were maintained for two 3-week cycles on this medium, one 3-week cycle on 1 X 10 M BAP, and two 4-week cycles on 6.6 X 10 M BAP. Shoot proliferation and elongation of three *V. vinifera* cultivars (Cabernet Sauvignon, Riesling, and Thompson Seedless), seven *V. labruscana* cultivars (Buffalo, Catawba, Delaware, Glenora, Niagara, Seneca, and Urbana), and two *Vitis* sp. cultivars (Cayuga White and DeChaunac) (Fig. 1).

Shoots excised from proliferated cultures of Riesling, Buffalo, Catawba, Glenora, Seneca, Cayuga White, and DeChaunac and placed on medium with 1 X 10 M BAP produced roots at the rate of 15 to 83% after 25 days. Several rooted plantlets were transferred to pots in the greenhouse and grew normally.

Delaware grape seedlings were inoculated by spraying the young leaves with a suspension of *Plasmopara viticola* sporangia and placing the plants into a chamber with 100% relative humidity at 24°C. When oily chlorotic lesions appeared, leaves were excised, surface sterilized with dilute NaOCl and placed in sterile plastic boxes containing moistened paper towel. Sporangia formed on the leaves after 24 to 48 hours and were removed aseptically into distilled water using a Pasteur pipette. Plantlets *in vitro* were inoculated with this axenic inoculum by placing droplets on the undersides of their leaves. Typical symptoms of downy mildew appeared after 10 days at 19°C (Fig. 2). Using this procedure, dual cultures of *P. viticola* with grape cultivars Riesling, Buffalo, Catawba, Glenora, Seneca, Cayuga White, and DeChaunac were obtained.

Axenic cultures of *U. necator* were obtained by transferring conidia from infected Delaware seedlings in a growth chamber of plantlets *in vitro* and then through several cycles on axenic plantlets. Successful dual cultures with grape cultivars Riesling, Buffalo, Catawba, Glenora, Seneca, Cayuga White, and DeChaunac

Fig. 1. Proliferated shoot culture of *Vitis labruscana* cv. Glenora after 17 weeks' growth *in vitro* on artificial media. Culture was removed from culture vessel for photograph.

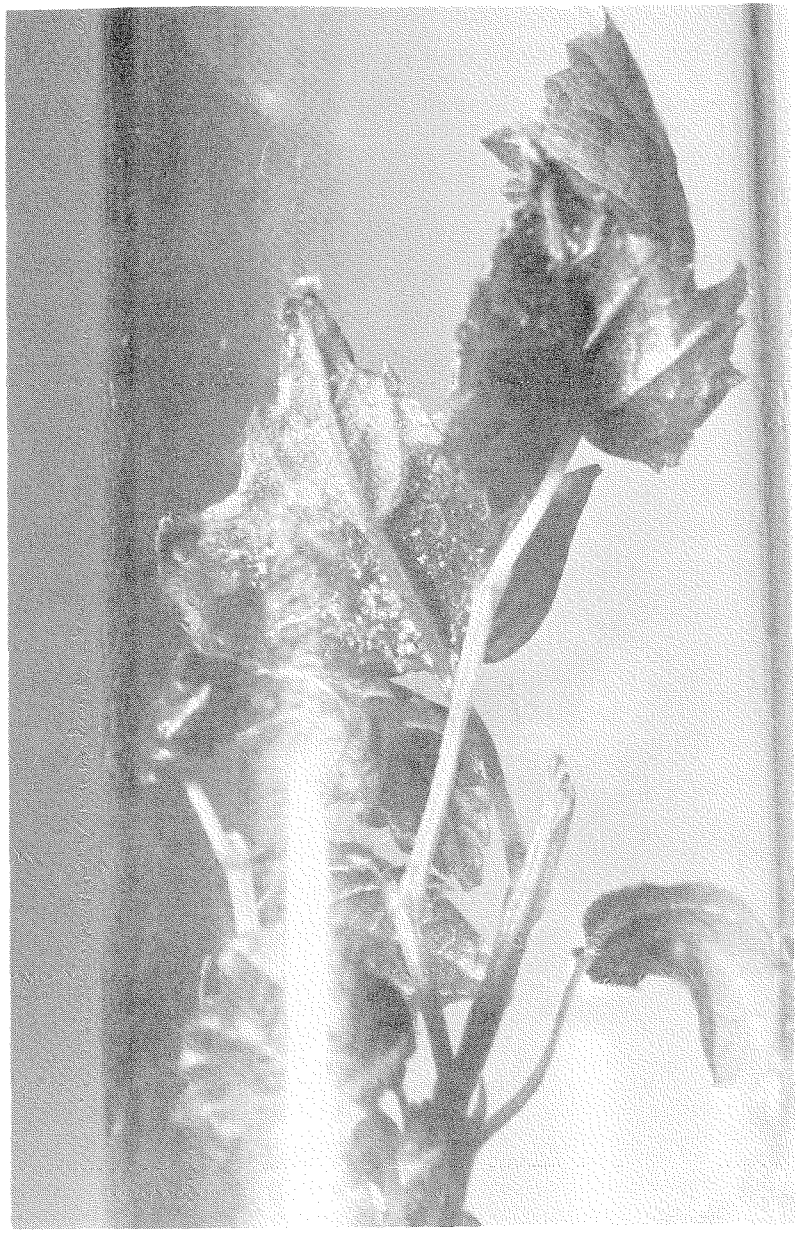

Fig. 2. Leaves of *in vitro* plant of *Vitis labruscana* cv. Glenora infected with downy mildew *(Plasmopara viticola)* 10 days after inoculation.

were obtained.

DISCUSSION

The success with *in vitro* culture of several grape cultivars and with dual cultures of the biotrophic fungi causing downy and powdery mildews pave the way for studying these fungi more critically. In particular, it is hoped to define the pathogenicity patterns within each fungus to cultivars of *Vitis* spp. It may be possible in the future to utilize these techniques for development of new disease resistant clones of existing cultivars by *in vitro* selection of variants.

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INHERITANCE OF BERRY MATURITY TIME IN VITIS VINIFERA

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ABSTRACT

The time of berry maturity in *Vitis vinifera* is an important character in grape breeding research. Earliness is often the target for new table grapes and for wine grape varieties, too, since early berries ripen well. Consequently, the wine produced is often better. The literature cited shows that the inheritance of maturity time is influenced by several genes with partial dominance of earliness (1) and of lateness.

This is an attempt to determine clearly the inheritance of maturity time. It is based upon 30 years' of observations carried out in the grape breeding research station of Vassal on 16,262 seedlings.

MATERIAL AND METHODS

Material: Maturity time has been observed, in particular, on seedlings derived from: the selfings of five table grape varieties, the selfings of 21 wine grape varieties, and 207 F_1 crosses of 68 varieties of *Vitis vinifera* (table grape and wine grape varieties).

Definition of the characteristic time of berry maturity: In Vassal, the time of berry maturity is obtained by tasting weekly all the seedlings of the same progeny. Maturity time is stated in terms of weeks before or after the average maturity time of Chasselas, which is itself represented by a number of clones scattered over the Vassal Station vineyard. Maturity time for each seedling is carefully recorded during two or three years in succession.

RESULTS

The characteristic time of berry maturity in the progeny is distributed according to an histogram reminding one of polygenic systems. Each progeny can be characterized by the mean value of the observations made on seedlings and its standard deviation.

Case of selfings: When selfing a *Vitis vinifera* variety, the distribution of the characteristic maturity time does not completely cover the species' variation on the character scale, even when the number of seedlings per progeny is high.

Table 2 shows how the characteristic maturity time is distributed in the progeny of some of the 26 selfings made in Vassal.

TABLE 1. Rating scale of *Vitis vinifera*.

Number of weeks in relation to Chasselas -3-2-1	Maturity of Chasselas 0	+ 1 + 2 + 3 + 4 + 5 + 6 + 7 + 8
Classes of the scale 1 2 3	4	5 6 7 8 9 10 11 12

TABLE 2. Seedling maturity time of some selfings in *Vitis vinifera*.

Selfing of	Time of maturity	Distribution of maturity time in the progeny												Mean time of maturity of the progeny	Standard deviation
		1	2	3	4	5	6	7	8	9	10	11	12		
Aleatico	6.0					2	13	16	20	8				7.32	1.07
Chasselas	4.0	1	1	5	19	32	10	4	1					4.79	1.15
Chenin	7.0					1	4	10	38	31	45	4	1	8.87	1.22
Jaoumet	3.5			9	46	121	12	4	5					4.85	0.88
Madeleine Royale	2.5	1	1	3	1	17	4		1					4.75	1.35
Muscat of Alexandria	8.0					1	1	5	5	16	19			8.94	1.22
Muscat of Frontignan	6.0				2	10	5	3	5	1				6.08	1.41

Generally the standard deviations of the frequency distributions have the same range no matter when the maturity time of selfed parent occurs.

The examination of the distribution frequencies proves that the seedlings of a selfed variety ripen more generally after this variety.

Case of F_1 progenies derived from crossings: Observations have been made on the seedlings of 207 crosses of *Vitis vinifera* varieties. Refer to Table 3 for a few examples.

The examination of the distribution of characteristics of the F_1 progenies supports the hypothesis of the polygenic inheritance of the characteristic maturity time of the berries. The cumulative effects of the genes of the system are of arithmetic nature.

As in the case of selfings, the mean value of the character maturity time of the berry falls later in comparison with the arithmetic mean of the phenotypic value of the character in both crossbreeding parents: 1) In 168 crossings, the F_1 mean value is higher, the difference with the mean value of both parents being sometimes very wide, 2) In two crossings, mean values are equal, and 3) In 37 crossings, the F_1 mean value is lower, the difference with the mean value of both parents generally being very small.

Therefore, it seems easier with crosses of *Vitis vinifera* varieties to obtain seedlings maturing after the arithmetic mean of the characteristic values than to obtain earlier seedlings before the same value (Table 3). At first sight, we might say that an heterosis effect in crossbred seedlings accounts for these results; but since the same phenomenon occurs in selfings that are subject to phenomenon contrary to hybrid vigor, it seems better to put forward the hypothesis of partial gene dominance conditioning "lateness."

When two varieties of *Vitis vinifera* are crossed, a point that could serve as a reference date for maturity time is the arithmetic mean of the phenotypic value of the character in both parents. The percentage of seedlings ripening one, two, three, four and five weeks before or after this arithmetic mean time has been determined. Calculations were based on 33 crosses whose total number of seedlings generally exceded 100, or 50 for a few of them.

Results are shown in Table 4 on which groups of progeny have been classified according to their differences in maturity time. The application of the "Chi square" test to the results obtained in Table 4 points out the homogeneity of percentages in the first four groups. The last two groups, however, show an increasing dispersion. It is the final consideration of these percentages that induces a calculated approach towards a solution to the following problem in grape breeding: When two varieties having clearly determined maturity times (and differences stated in weeks) are crossed, how many F_1 seedlings are necessary, to be sure to obtain at least one seedling having a clearly determined maturity time (refer in Table 4 to the figures in brackets).

The Vassal station is now in a position to assess, for

TABLE 3. Seedlings maturity time of some crossings in *Vitis vinifera*.

Crossing between	Time of maturity	Arithmetic mean	Distribution of maturity time in the progeny												Mean time of maturity progeny	Standard deviation
			1	2	3	4	5	6	7	8	9	10	11	12		
Olivette b. x Madeleine Angevine Oberlin	9 2	5.5		2	3	27	21	28	20	32	30	25	10	2	7.22	2.28
Gros Colman x Sultanine	8.5 6.5	7.5				2	7	13	31	47	52	9	1		7.92	1.30
Ribier x Perle de Csaba	6.5 1	3.7	4	23	48	43	37	42	10	3					4.27	1.55
Grenache x Aramon	8 8	8				1	12	48	172	510	450	104	13		8.30	1.02
Carignan x Aramon	8.5 8	8.2					1	16	62	247	412	241	80		8.71	0.97
Aramon x Syrah	8 6.5	7.2				6	14	38	90	143	73	6	4		7.64	1.21
Cinsaut x Carignan	7 8.5	7.7					1	12	89	235	277	190	31		8.76	1.08
Listan x Chardonnay	6 5	5.5				4	36	40	60	33	10				6.61	1.21
Jaen b. x Chardonnay	8 5	6.5							6	20	23	9	1		8.64	0.92
Jaen b. x Clairette	8 8	8							7	53	99	64	9		9.06	0.88
Folle noire x Portugais bleu	6.5 4.5	5.5				17	40	51	16	3	3				5.67	1.07
Mondeuse x Gamay	6.5 5.5	6				1	12	26	99	53	15	1			7.16	0.98
Ohanes x Grenache b.	11 8	9.5						1		5	10	15	3	1	9.47	1.16
Jaen b. x Jacquère	8 7.5	7.7							11	97	160	83	5		8.93	0.83

TABLE 4.

Range of maturity between the cross-bred parents (in weeks)	Number of progenies	Percentage of seedlings ripening								
		before			same	after				
		the arithmetic mean of the parents' maturity time								
		- 4	- 3	- 2	- 1	0	+ 1	+ 2	+ 3	+ 4	+ 5
0 or 0.5	11	0.18% (556)	0.49% (205)	2.12% (48)	9,45% (11)	25.94% (4)	34.71% (3)	16,80% (6)	7,94% (13)	0.92% (109)	0.38% (264)
1.5	8	0.25% (400)	0.61% (164)	3.45% (29)	9.93% (11)	19.92% (6)	37.08% (3)	21.92% (5)	5.13% (20)	0.83% (121)	0.07% (1429)
2	4		0.57% (176)	2.60% (39)	10.47% (10)	27.10% (4)	29.37% (4)	20.45% (5)	10.42% (10)	0.40% (250)	
2.5	4		0.20% (500)	3.45% (29)	13.67% (8)	21.77% (5)	25.97% (4)	23.07% (5)	9.85% (11)	1.72% (59)	
3,5 to 4,5	3		1.80% (56)	7.56% (14)	15.80% (7)	24.03% (5)	19.16% (6)	21.66% (5)	9.10% (11)	1.40% (72)	
5,5 to 6	3		0.60% (167)	6.56% (16)	18.90% (6)	20.40% (5)	20.10% (5)	17.63% (6)	7.66% (14)	5.53% (19)	2.30% (44)

The numbers in brackets are the total seedlings to observe in a cross between two varieties of *Vitis vinifera* to be sure to obtain a seedling ripening at the time indicated in the table and corresponding with the range of maturity of both parents.

many *Vitis vinifera* varieties in the world, the time of berry maturity in relation to Chasselas.

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A NUMERICAL TAXONOMIC APPROACH TO THE AMPELOGRAPHY OF VINIFERA WINE GRAPES

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ABSTRACT

Eight factors of the 28 variables have been retained in this analysis. Cluster weight, lobe number, shoot tip color, berry diameter, pH, etc. present the highest loadings; and, therefore, they give the highest contribution to the Factors. The grouping of wine varieties into nine clusters, based on eight variables, shows the same order of amalgamation as that based on 28 variables; the only difference is in the clusters' composition due to the inter-cluster distances.

During the last hundred years, various classifications of vine varieties, which are reviewed by Eynard (3), have been proposed. These classifications are based on the methods of traditional taxonomy, but they don't describe relationships among varieties. The grouping of varieties into "natural" classes by a coefficient of similarity has been applied in different species (2,4,5). Several ampelographers have employed the description of numerous morphological characters. The simple description of characters lacks objectivity, decreases the utility of classification and increases the difficulty of taking measurements.

Other writers have confined the ampelographical description of grape varieties to a few characters which are considered fairly constant and that might differentiate individual varieties.

The description of a genotype by noting a few phenotypic characters is rather vague since most characters are correlated and they affect one another. This study has been conducted to reduce, through a factor analysis, p correlated variables in n $<$ p uncorrelated variables and to determine by cluster analysis the degree of congruence between a classification based on few variables and that based on several variables.

MATERIALS AND METHODS

The research has been carried out on 24 wine-grape varieties grown in Foggia and Bari areas (Apulia) and overhead arbor trained. Twenty-eight characters (Table 1) were measured from 10 random plants (8) in each locality and in two successive years, 1976 and 1977. Only leaves beyond the sixth node of growing shoots were sampled.

Second crop clusters were discarded. Qualitative multistate characters (petiolar sinus, shoot tip color, etc.) were coded and scaled for computation to preserve useful information.

TABLE 1. Results of factor analysis in wine grapes (*Vitis vinifera*).

TRAITS	FACTORS								COMMUNALIITY
	I	II	III	IV	V	VI	VII	VIII	
100 Seed weight	-0.51	-0.04	0.25	0.30	-0.06	0.06	-0.43	-0.29	0.53
10 Berry weight	-0.90	-0.09	0.03	-0.27	-0.61	0.14	-0.28	-0.42	0.86
Cluster weight	-0.92	-0.13	-0.09	0.06	-0.09	-0.06	-0.02	-0.16	0.92
Stem length	-0.84	-0.02	0.02	0.02	-0.32	0.10	0.06	-0.28	0.84
Lobe number	-0.25	0.83	-0.28	-0.01	-0.03	-0.02	-0.33	-0.05	0.76
Petiolar sinus	0.33	-0.79	-0.27	-0.21	-0.19	-0.41	-0.27	-0.17	0.77
Upper sinus depth	0.08	0.74	0.09	0.06	-0.07	-0.13	-0.11	-0.16	0.84
Width/length leaf	0.07	0.69	-0.76	0.03	-0.13	0.12	-0.01	-0.03	0.76
Width of petiolar sinus	0.04	-0.24	0.61	0.09	0.23	0.01	0.19	0.09	0.56
Leaf surface	0.10	0.17	0.20	0.81	0.06	0.05	0.11	0.01	0.69
Teeth series	-0.13	0.03	-0.82	0.07	0.07	0.05	0.05	0.12	0.83
Distance first branch of cluster	-0.07	-0.01	-0.03	-0.05	-0.81	-0.08	-0.19	-0.12	0.54
Distance second branch of cluster	0.10	0.18	-0.02	-0.13	-0.86	-0.02	0.07	-0.03	0.56
Shoot tip color	-0.03	0.23	0.01	-0.89	-0.01	0.04	0.17	0.37	0.78
Distance third branch of cluster	-0.33	0.02	0.01	0.19	-0.84	-0.01	-0.01	-0.11	0.87
Wing length	-0.80	-0.12	-0.03	-0.02	-0.57	0.83	0.07	-0.03	0.80
Distance fourth branch of cluster	-0.25	0.23	-0.22	0.40	-0.83	-0.16	-0.05	0.16	0.71
Berry diameter	-0.78	0.05	0.05	-0.08	-0.87	-0.81	-0.55	0.28	0.55
Berry color	0.31	-0.11	0.14	-0.31	-0.20	0.01	-0.38	-0.68	0.46
°Balling	0.19	0.06	0.03	-0.04	0.09	0.04	0.01	0.88	0.72
Total acidity	0.32	0.20	-0.14	0.27	-0.06	-0.16	-0.73	-0.14	0.83
pH	-0.19	-0.24	0.01	0.01	-0.04	0.08	0.81	0.24	0.87
Bud break time	-0.25	0.18	0.09	0.12	-0.02	0.11	-0.09	-0.73	0.67
Flowering time	-0.19	0.30	0.10	0.02	-0.09	0.12	-0.07	-0.79	0.70
Length first branch of cluster	-0.61	-0.15	-0.03	-0.02	-0.50	0.80	0.02	-0.10	0.78
Length second branch of cluster	-0.51	0.02	0.01	-0.01	-0.52	0.86	0.14	-0.12	0.87
Length third branch of cluster	-0.50	0.05	-0.04	-0.05	-0.80	0.67	0.08	-0.02	0.73
Length fourth branch of cluster	-0.47	0.04	-0.03	-0.01	0.90	0.63	0.10	-0.14	0.76
Factor variance	5.69	4.84	4.27	3.72	3.08	2.34	1.72	1.03	
% of factor variance	21	18	16	13	11	8	6	3	100%
% of total variance	20	17	15	13	11	8	6	3	93%

The factor analysis model and most of its mathematical procedures are described by Harmon (6). The estimate of loading coefficients were obtained according to the Principal Factor Procedure (6), and the rotation of factor matrix to simple structure was made by the varimax method (7).

For cluster analysis, the similarity measures were the Euclidian distances. The mathematical calculations for factor and cluster analysis were done by a computer program (1).

RESULTS AND DISCUSSION

Table 1 reports the loading and the communality values, which explain respectively the relative contribution of an individual variable to each factor and the variance of each variable in terms of common factors. Eight factors of the 28 variables have been retained in this analysis. Together they explain 93% of the variance of the original variable while the remaining variance (7%) is of no value since it expresses random variation.

Factor 1 includes several characters (cluster weight, 10 berry weight, stem length, etc.), and it may be interpreted as a productivity factor. The most important character of the Factor I is represented by cluster weight, which has the highest loading (-0.92) and the highest communality (0.92). Factor II has the highest loading for lobe number and petiolar sinus and Factor III for teeth series; they may be related to leaf size and leaf shape.

Factor IV includes shoot tip color and leaf surface; it seems a factor of the plant's appearance.

Factor V represents an association among berry diameter and the distance from the peduncle of the fourth branch of the cluster; it may interpret the compactness of the cluster.

Factor VI includes the length of the cluster wing and the second branch of the cluster, and it may interpret the cluster shape.

Factor VII might be defined as a "quality" factor because it includes pH and total acidity.

Factor VIII includes °Balling, flowering time and bud break, and it may represent the factor of vegetative-reproductive cycle. The amount of variance of the original data, explained by Factor I, IV, V, VI, and VII, suggests the dominant role of grape clusters. The reduction of several variables in a few factors seems attractive to make a classification of wine varieties based on few variables and to compare it with that based on many characters.

The grouping of 24 wine varieties in nine clusters, based on eight variables, one for each factor and with the highest loading coefficients, is reported in Table 2 and Fig. 1. The diagram of Fig. 1 reveals that amalgamation of varieties into clusters follows the same order as that based on several variables. The only difference is represented by the cluster composition (Table 2), and

TABLE 2. Cluster analysis of wine grapes based on 8 and 28 variables.

Cluster	Cluster Composition on 28 variables	Cluster composition on 8 variables
I	Barbera, Negro A.	Barbera, Negro A., Nebbiolo, Malvasia n., Primitivo, Lambrusco, Pinot b., Riesling.
II	Nebbiolo, Malvasia n. Lambrusco, Primitivo	Chenin b., Colombard
III	Pinot b., Riesling, Chenin b. Colombard	Ruby Cabernet
IV	Ruby Cabernet	Sangiovese, Sangiovese C., Montepulciano, Montepulciano A.
V	Sangiovese, Sangiovese C., Montepulciano A., Negrara	Negrara, Ottavianello, Ciliegiolo
VI	Ottavianello, Ciliegiolo	Barberone
VII	Barberone, Piedilunga	Piedilunga
VIII	Malvasia di Candia, Malvasia b.	Malvasia di Candia, Malvasia b.
IX	Peverella, Verdeca	Peverella, Verdeca

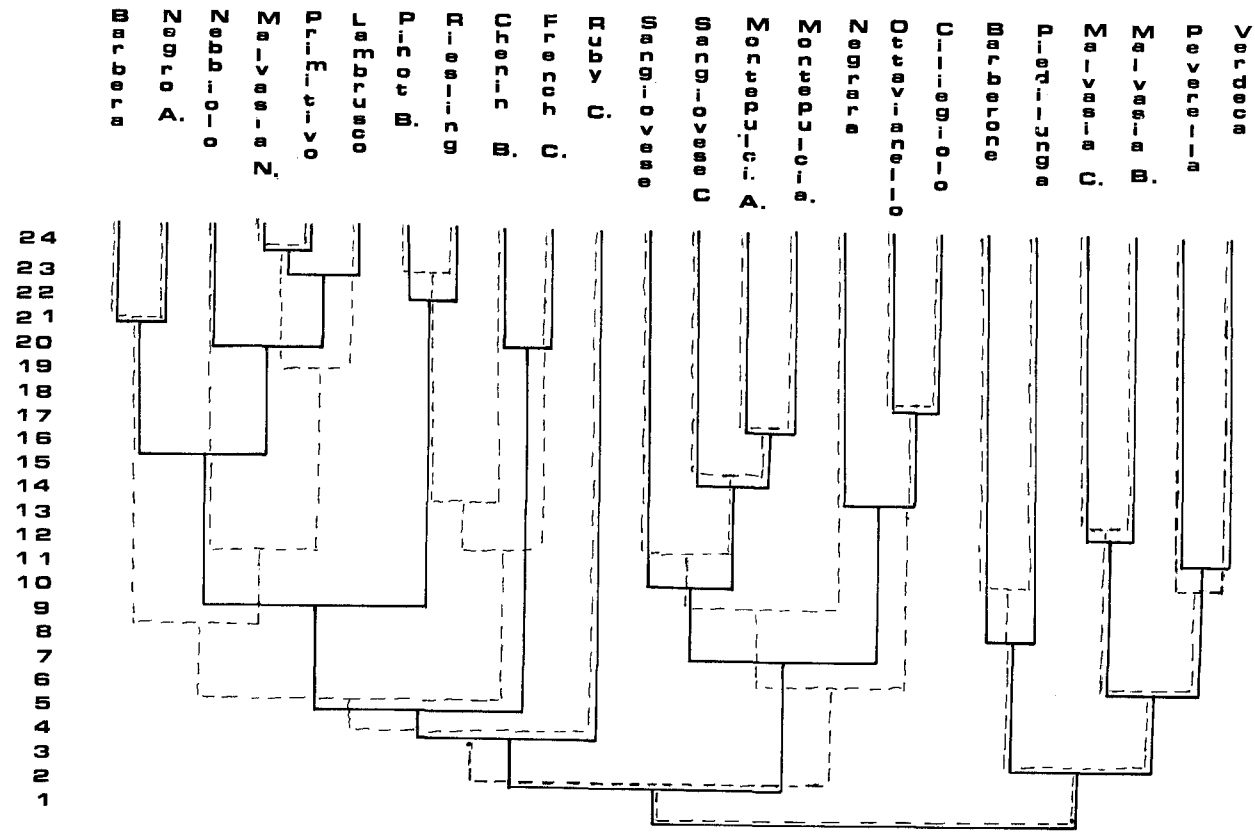

Fig. 1. Cluster maps of wine-grapes (*Vitis vinifera*) based on 28 (dotted line) and 8 (straight line) variables.

it is due to inter-cluster distances.

The classification of wine varieties, based on eight characters, shows a good degree of correspondence with that based on 28 characters; therefore, the factor analysis seems an attractive procedure to reduce the phenetic dimension even if there is no simple solution to the problem of the choice of characters and their weight, which might make a change in the cluster diagram.

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MULTIVARIATE ANALYSIS TO ESTIMATE THE GENETIC DIVERSITY OF WINE GRAPES (VITIS VINIFERA) FOR CROSS BREEDING IN SOUTHERN ITALY

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ABSTRACT

A cluster analysis to discover natural classes of varieties has been employed. The 24 wine grape varieties fall into 9 clusters. This analysis reveals the absence of relationship between geographical regions and phenetic similarity; similar varieties very often are grown in different localities under different names. The intra and inter-cluster distances are also discussed for cross-breeding purposes.

The importance of divergent parents for a successful cross-breeding program has been recognized in various species. Molls et al. (6) reported that genetic diversity of the parents gave rise to superior progenies.

The introduction of germ plasm from different geographic regions is a traditional practice in most breeding programs. Similar varieties are very often grown in different localities under different names (3), and this provides very little genetic variability.

A study of genetic diversity of local and introduced wine grape varieties grown in Apulia (Southern Italy) was explored by cluster analysis in order to discover natural classes of varieties and to make crosses between varieties of different groups.

MATERIALS AND METHODS

Measurements of 28 characters were taken on 24 vine varieties (Vitis vinifera), grown in the Foggia and Bari areas (Apulia) and overhead arbor trained, from 10 random plants (8) in two successive years, 1976 and 1977.

The following parameters from various parts of the vine have been measured: 100 seed weight, 10 berry weight, cluster weight, stem length, lobe number, petiolar sinus, upper sinus length, width/length leaf, width of petiolar sinus, leaf surface, teeth series, distance of first, second, third and fourth branch of the cluster, shoot tip color, cluster wing length, berry diameter, berry color, °Balling, pH, total acidity, length of first, second, third and fourth branch of cluster, bud break time and flowering time. Only leaves beyond the sixth node of each growing shoot were sampled. Second-crop clusters were discarded.

The similarity measures for the cluster analysis, were the Euclidian distances.

$$d\ (X_{i\cdot}, X_{j\cdot}) = \sum_{K=1}^{P} (X_{ik} - X_{jk})^2$$

X_{ik} represents the K^{th} observation. A special cluster program released by the Statistical Analysis System (SAS 76) of North Carolina State University was used.

RESULTS AND DISCUSSION

The grouping of operational taxonomic units into clusters has been defined by Sneath and Sokal (9) and Sokal (10).

In this study an hierarchic and agglomerative algorithm (1,5) has been used. The analysis begins forming one cluster for each genotype. Next, the two closest clusters are combined into one and it proceeds to form a final cluster containing all genotypes. The grouping must not be considered as an evolutionary tree.

The results of this analysis are represented graphically in Fig. 1, and the genotype composition for each cluster, if nine clusters have been arbitrarily chosen, is reported in Table 1.

The first consideration to make is that cluster V presents the lowest intra-cluster distance, which suggests the presence of homogeneity among varieties. It did, in fact, include varieties such as Sangiovese, Sangiovese compatto, Montepulciano, and Montepulciano d'Abruzzi, which are quite similar even though they are grown in different localities or regions. The absence of relationship between geographical regions and phenetic similarity (2,3,7) is also shown by clusters I, II, VI, IX. Each of these clusters contains similar varieties even though they are grown in very different regions (Negro A., Verdeca, Ottavianello, etc., in Southern Italy, and Barbera, Peverella, Ciliegiolo, etc., in Northern Italy). The grouping of varieties with different climatic conditions into the same cluster could be explained by their common heritage and by their responses to environments, where the cumulative force of evolution (selection, mutation, mode of pollination, etc.) had played an important role. Table 1 shows also that genotypes from the same regions such as Primitivo, Negro Amaro, Ottavianello, etc. (from Apulia) and Ciliegiolo, Lambrusco Maestri, etc. (from Northern Italy) have been grouped into different clusters. This might be due to the polygenic nature of most characters that makes the local populations sensitive to environmental changes and results in different locally adapted varieties (4). The different values of the inter-cluster distances (Table 2) suggest a dissimilarity among the nine clusters. Only clusters I and II have the lowest values and this suggests a certain homogeneity among these two clusters.

CONCLUSION

In conclusion, the multivariate analysis, applied in the present study, provides useful information of the genetic diversity of the local and introduced genotypes before starting a wine-grape

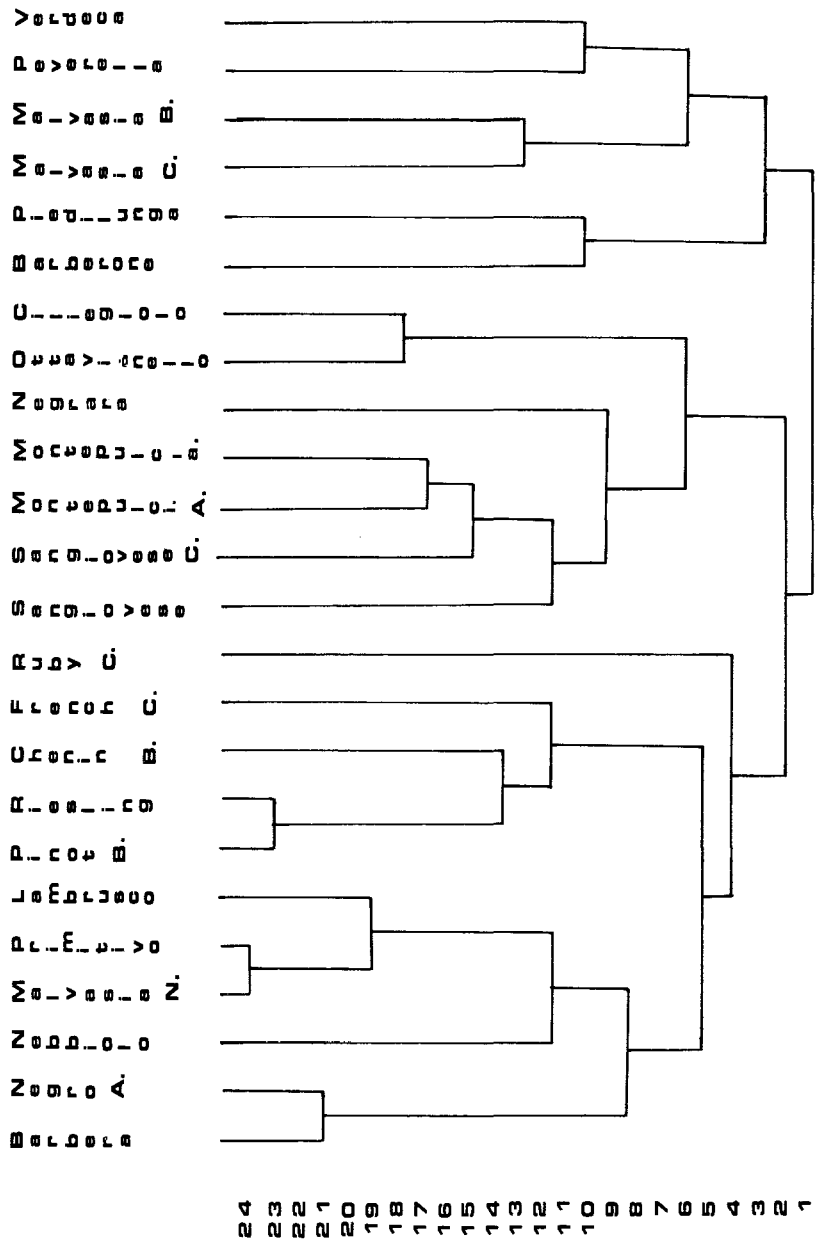

Fig. 1. Cluster map of wine grapes (*Vitis vinifera*).

TABLE 1. Clustering pattern of 24 varieties showing intra-cluster (the diagonal) and inter-cluster distances (x 0.1).

VARIETIES		CLUSTERS								
		I	II	III	IV	V	VI	VII	VIII	IX
I.	Negro A., Barbera	5.2	7.1	30.4	28.2	24.1	23.1	38.2	19.6	21.3
II.	Primitivo, Nebbiolo Malvasia n., Labrusco m.		8.2	25.2	45.3	18.8	16.7	30.2	31.3	26.1
III.	Pinot B., Riesling It., Chenin, Colombard			10.1	27.5	40.5	35.6	40.5	23.9	17.9
IV.	Ruby Cabernet				0.0	25.1	41.2	32.3	35.7	21.8
V.	Sangiovese, Sangiovese C., Montepulciano, Montepulciano A., Negrara					4.6	15.2	28.5	30.8	35.5
VI.	Ciliegiolo, Ottavianello						8.4	26.7	32.1	30.7
VII.	Piedilunga, Barberone							5.2	51.3	66.1
VIII.	Malvasia di Candia, Malvasia b.								6.7	18.2
IX.	Peverella, Verdeca									12.9

TABLE 2. Cluster analysis of wine grapes based on 8 and 28 variables.

Cluster	Cluster Composition on 28 variables	Cluster composition on 8 variables
I	Barbera, Negro A.	Barbera, Negro A., Nebbiolo, Malvasia n., Primitivo, Lambrusco, Pinot b., Riesling.
II	Nebbiolo, Malvasia n. Lambrusco, Primitivo	Chenin b., Colombard
III	Pinot b., Riesling, Chenin b. Colombard	Ruby Cabernet
IV	Ruby Cabernet	Sangiovese, Sangiovese C., Montepulciano, Montepulciano A. Negrara, Ottavianello, Ciliegiolo
V	Sangiovese, Sangiovese C., Montepulciano A., Negrara	
VI	Ottavianello, Ciliegiolo	Barberone
VII	Barberone, Piedilunga	Piedilunga
VIII	Malvasia di Candia, Malvasia b.	Malvasia di Candia, Malvasia b.
IX	Peverella, Verdeca	Peverella, Verdeca

breeding program. In addition, it should be noted that the clustering of wine-grape varieties could be improved by including more variables.

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PLANTLETS FROM CULTURED ANTHERS OF VITIS SPECIES AND HYBRIDS

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ABSTRACT

Embryos and plantlets have been produced *in vitro* from callus formed by isolated anthers of several species, hybrids and cultivars of grapevines. Anthers with uninucleate microspores are chilled (4°C, 72 h) before culture with Nitsch medium containing 2,4-D (5 µM) and benzyladenine (1 µM). For production of normal plantlets, embryos require chilling (4°C) for up to six weeks. Ability to form callus and embryos from anthers varies with genotype and is heritable. Maleness seems to be a predisposing factor. Callus derived from anthers contains haploid and diploid cells, but all plantlets produced so far are diploid. The genetic constitution of plants produced from anther-derived callus is being investigated. Present indications are that plants are derived from somatic cells of the anther rather than from pollen.

There have been many reviews on the significance of haploid plants for crop improvement (13,16,17,23,24 and others). In woody perennial fruits, where breeding is made difficult by long generation times and by the highly heterozygous nature of most fruit species, the availability of haploids, and of the homozygous diploids derived from them, is highly desirable both for vine breeding and for research in vine genetics.

Haploid grapevines have not been recorded in nature (14), but cytochimeric (mixoploid) seedlings (2n, n) have been observed by Bouquet (2) in some polyembryonic cultivars. Research into the possibility of producing haploid grapevines by cultivation *in vitro* of isolated anthers and isolated pollen was first reported in 1971. This work was based on techniques developed originally for induction of androgenesis in *Solanaceae* (4,11,19) and was confined to commercially important grape cultivars (8). A few multinucleate pollen grains were found in cultured anthers, but neither callus nor embryoids were produced. Later, Gresshoff and Doy (3) were able to induce formation of callus in cultured anthers of certain *vinifera* grapes and in the interspecific hybrid JS23-416. This callus was found to contain metaphases with the haploid number of chromosomes (n = 19), and it was assumed that cells were of pollen origin. Attempts to induce differentiation of embryos or adventitious organs were unsuccessful.

Shoot-like organs were formed in callus from isolated anthers of *Vitis thunbergii* (5). Finally, techniques were developed for production of very large numbers of embryos and plantlets from cultured anthers of a *V. vinifera* x *V. rupestris* hybrid (15). These techniques, which were based on use of agitated liquid

media (9), have since been refined and extended to a wide range of *vinifera* cultivars, *Vitis* species and interspecific hybrids.

In the present paper a summary will be given of our earlier work (15) and of our recent findings, further details of which are being published elsewhere. As will become clear, the major problem in all of this work has been, and remains, the question of the origin of the plantlets produced by anthers, i.e., whether they are derived from cells of pollen origin or from somatic cells of the anther wall.

MATERIALS AND METHODS

Embryos and plantlets from cultured anthers of "Gloryvine": Anthers of a hybrid male (ornamental) grapevine, known locally as Gloryvine *(V. vinifera* x *V. rupestris)*, were excised at the uninucleate stage of microspore development and chilled (4°C) for 72 h before culture with Nitsch medium (12) containing 2,4-dichlorophenoxyacetic acid (2,4-D; 5 µM) and benzyladenine (1 µM). After culture in darkness for 10 to 20 days, the anthers ruptured; and a yellow-white callus was extruded (Fig. 1). This callus was later dispersed into a suspension containing single cells and cell aggregates, and the suspension was transferred to basal medium. During the next 20 days, very large numbers of adventive embryos were formed (> 2500/25 ml of liquid medium) (Fig. 2).

For production of normal plantlets, the embryos required chilling (4°C) for two weeks; and the chilling treatment was effective in breaking dormancy when applied at any stage of embryogeny. Plantlets were established, first, under sterile conditions on agar-based media (Fig. 3) and then in the glasshouse.

In this investigation it was found that the ability of vine anthers to produce callus in vitro was affected by genotype and by sex expression. Gloryvine, a *vinifera* x *rupestris* hybrid (♂), gave callus much more readily than *vinifera* (♀) cultivars. Gloryvines in the Sydney region are variable with respect to fruiting. Some vines are strictly male and have no known history of fruit production. Others are seasonally fertile and have a history of intermittent cropping. Anthers from unfruitful and from fruitful Gloryvines had markedly different behavior *in vitro*, and maleness was associated with the ability to form callus and embryos.

The callus produced by anthers was found to contain cells with chromosome numbers consistent with both the haploid (n = 19) and diploid (2n = 38) conditions. In the case of plantlets, however, an extensive investigation revealed only chromosome numbers of 38. This result raised two possibilities: 1) that diploid plants arise from spontaneously doubled haploid cells of pollen origin, and 2) that diploid plants arise from diploid somatic cells of the anther wall or connective. The resolution of these possibilities has been the subject of later research.

Embryos and plantlets from anthers of *Vitis* species and hybrids: *Effects of genotype and maleness:* The importance of genotype and maleness as factors affecting the ability of anthers to

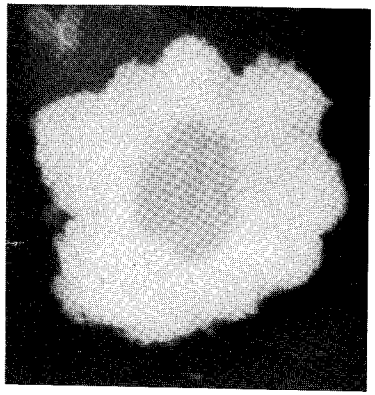

Fig. 1. Callus production by an anther. Photographed after culture for 20 d (x 40).

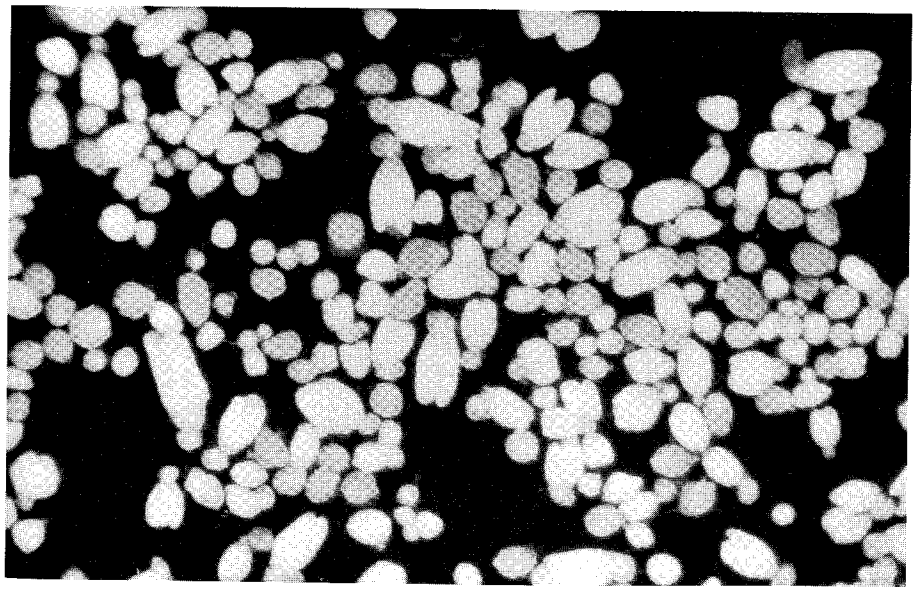

Fig. 2. High frequency production of embryos (x 7.5).

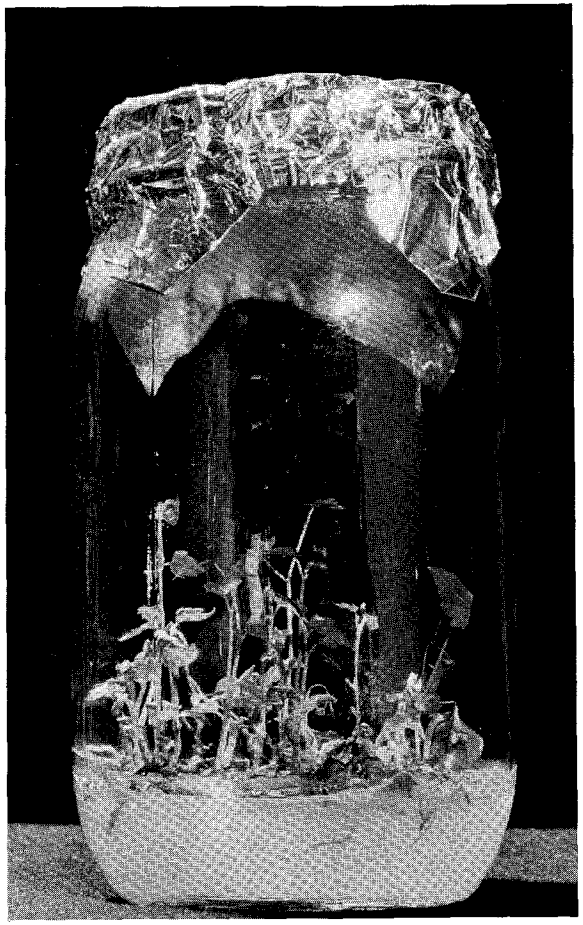

Fig. 3. Plantlets grown from chilled (4°C) embryos. Photographed after 15 d of planting (actual size).

produce callus and plantlets *in vitro* has been confirmed. Among the *Vitis* species which have been investigated, *V. rupestris* and *V. longii* have the greatest propensity to embryo formation. This character seems to be heritable, and regenerative competence of anther-derived tissues is exhibited by most hybrids in which *rupestris* is a parent. Included are highly complex hybrids such as JS23-416, Villard noir and Villard blanc in which the contribution of *V. rupestris* is very dilute. In the *vinifera* grapes, Grenache is the only cultivar which has been induced to form large numbers of embryos. These embryos require extensive chilling to break dormancy (> 6 weeks at 4°C). Hybrids of Grenache x Cabernet Sauvignon do not form embryos. A number of other intra-specific hybrids have been tested, but Sumull x Cabernet Sauvignon is the only cross in which callus has been produced by anthers. No embryos were formed.

With regard to sex expression, Grenache is the only hermaphroditic grapevine which has been found to produce embryos. When male inflorescences of a *vinifera* x *rupestris* hybrid were converted into hermaphrodites by treatment with the cytokinin 6-(benzylamino)-9-(2-tetrahydropyranyl)-9H-purine (PBA), according to the method of Negi and Olmo (10), the ability of anthers to form callus *in vitro* was lost. Similarly, the conversion of tendrils of male vines into inflorescences by use of PBA (18) gives hermaphrodite flowers in which the anthers are lacking in ability to form callus and embryos.

Origin of plantlets: Cytological evidence: Pollen grains were studied in squashes of cultured anthers or were collected from the liquid culture media by use of a millipore filter. The pollen grains were stained with Lacto-propiono-DMSO-carmine (1) or Belling's iron acetocarmine. There was much variation in the size of pollen grains from cultured anthers. About 10% of grains had a diameter of 60 µM, i.e., twice the normal size and were staining darkly with Toluidine Blue. The significance of these observations is not yet clear. Multinucleate pollen grains were observed with a relatively high frequency (Fig. 5); 3% of grains had four or more nuclei. Rarely, pollen grains were seen with up to seven nuclei. However, no direct evidence has been obtained that the contents of these multinucleate pollen grains give rise to, or contribute to, the callus which is formed by cultured anthers. Several hundred anthers have been studied, but pollen grains from which the multinucleate contents are emerging or to which callus is attached have never been observed.

By contrast, there is strong evidence of callus production from somatic cells of the anther. The grape anther is composed of two lobes, each of which contain two locules (microsporangia). The pattern of callus formation in cultured anthers was of two types. In the first, cells were produced from the connective which joins the two lobes. In anthers of Grenache, in particular, there was prolific growth of callus from the scar of the filament. In the second type, callus arose from cells of the connective situated between the loculi. In some anthers this callus emerged as a tongue of tissue which, on cursory examination, appeared to come from within the locule (Fig. 4).

Further studies have been made on the cytology of the callus

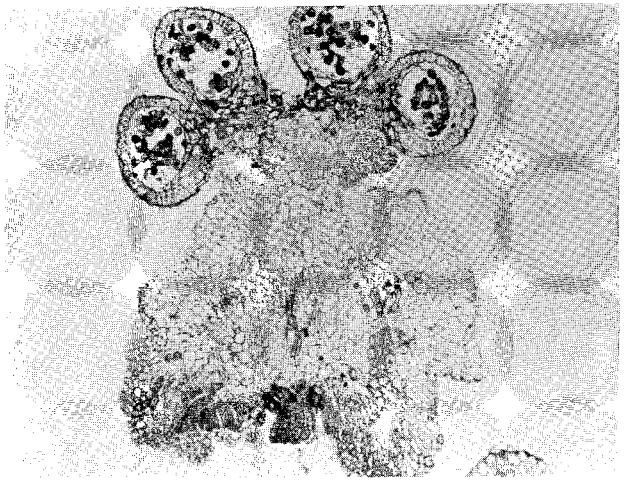

Fig. 4. T.S. anther showing the origin of the callus from the connective tissue (x 40).

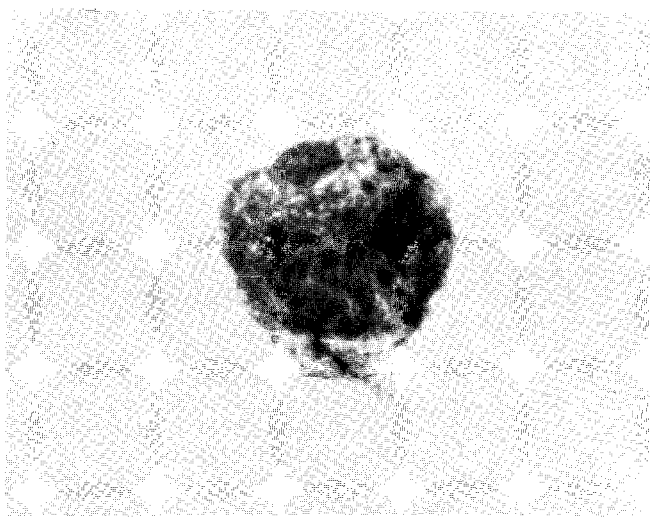

Fig. 5. A multinucleate pollen grain stained with Belling's Iron acetocarmine. Photographed after 10 d of culture (x 1200).

formed by isolated anthers. After 10 days of culture, up to 5% of metaphases in the newly formed callus of Gloryvine and JS23-416 had chromosome numbers consistent with the haploid condition (n = 19). After 20 days of culture, haploid figures accounted for less than 1% of the total. As in the original research on Gloryvine, all plantlets that have been established *in vitro* from anther-derived callus are diploids.

Origins of plantlets: Genetic inference:

Initial observations on populations of plantlets from anther-derived callus suggested that the plants were of pollen origin. First, there was a high incidence of mortality and abnormality including albinism and delayed albinism. This is in accord with the fact that the vine carries a heavy load of deleterious recessive genes (14) and that, accordingly, few of the haploid sets from meiosis are likely to be lethal-free. Second, the experimental conditions for callus production from grape anthers, including the stage of microsporogenesis at which anthers must be excised and the necessity for low-temperature pre-treatment, are similar to the conditions for pollen-plant production in species in which the phenomenon of androgenesis is well established (21).

Plants from anther culture were established in the field and were made to flower in their first year by conversion of tendrils into inflorescences with PBA (18). With this technique, the calyptrae fail to dehisce and self-pollination (cleistogamy) is assured. Subsequently, these anther-derived plants set seeded fruits and a progeny was raised from each of them. The character of these progenies provides a definitive answer to the question of the origin of the parent. The occurrence of segregation in the progeny of a selfed plant is evidence of heterozygosity and of somatic origin. Uniformity in the progeny of selfed plants indicates homozygosity and pollen origin. This work is still in progress; but, so far, all the progenies produced from selfing anther-derived plants have been highly variable with respect to vigor and leaf character. It must be concluded, therefore, that none of these parents is a homozygous diploid which arose from the doubling of a haploid nucleus, either by fusion of the generative and vegetative nuclei within a pollen grain or by endoreduplication in haploid callus (22).

CONCLUSIONS

Adventive embryos can be produced with high frequency from callus from cultured anthers of a range of *Vitis* species and hybrids (Fig. 2), but no conclusions can yet be made concerning the origin of the embryos. Evidence has been obtained which both supports and opposes the hypothesis that the callus is derived from the pollen.

One of the most significant missing pieces in this puzzle is the lack of direct evidence that the multinucleate pollen grains discharge their contents into the locule and that cells derived from pollen contribute to the callus formed by the anther. It is clear that the diploid vegetative tissues of the connective are a major source of the callus produced by anthers (Fig. 4). How, then, does one account for the occurrence of haploid metaphases in this

tissue? One possibility is that the culture conditions favor somatic reduction (6), but the existence of this process is discounted by many plant cytologists.

There is clearly a need for further information on the quantitative cytology of callus formation in cultured anthers. At present, such information is scanty, even in species which are favorable subjects for cytological work. The acquisition of information on karyotype dynamics in difficult material such as grape callus is a challenging task.

It is well known that callus and suspension cultures are susceptible to gross chromosomal variability (20) and the mutagenicity of 2,4-D has been established, particularly in regard to production of albino mutants (7). These factors may have accounted for the variability and abnormality of the grapevines produced by anther-derived callus.

Results, so far, indicate that production of homozygous diploid grapevines from isolated anthers is unlikely, but this possibility cannot be dismissed until a much larger population of plants has been subjected to genetic analysis, either by studies on the progenies of selfed plants as before, or by studies of enzyme polymorhism.

Acknowledgement

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BIOMETRICAL ANALYSIS OF MUST AROMAGRAMS: APPLICATION TO GRAPE BREEDING

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ABSTRACT

Volatile aromatic components of musts were measured by gas chromatography. Three variable factors were considered by pairs in several experiments: genotypes (7 varieties), maturity (6 dates of harvest), years (3). Biometrical analysis of these quantitative data show that it is possible to reduce them to a number of key data which allows one to find some stable structures. The application of this kind of analysis in breeding for quality is discussed.

Viticulture is a field where quality of the product has always been the main endeavor and is becoming today more and more important. In grape breeding work, however, some difficulties arise in testing and then in improving quality traits:

1) because the characters taken into consideration in the early stages don't cover quality in all its bearings. I mean the classical determination of sugar content, acidity and, in some cases, polyphenols for red grapes, and 2) secondly because evaluation by tasting cannot occur before the second stage of selection, when growing 10 replicates of each genotype, and depends on contingencies which give it some random aspects. The possible effects of microvinification and the subjectivity of tasting panels complicate the selection process.

Therefore, the search for an early test of qualitative rank of new vine genotypes, allowing us to improve the classical measures, led us to investigate the aroma composition of grape juice. In 1977 we carried out the quantitative variation of aromatic volatiles detected by gas chromatography (GC) among seven white vine varieties, usually grown in the northeast of France. We also took into account the variations due to the maturity stages of the berries. This study was set up on a field experimental design with replicates permitting estimation of error variances.

MATERIALS AND METHODS

Plant material and sampling: Each of the seven vine varieties belongs to the *vinifera* species. Two of them are strongly aromatic: Muscat Ottonel (M) and Gewürztraminer (G). Riesling (R) is rather fruity. The remaining four, Pinot blanc (P), Auxerrois (A), Sylvaner (S), and Chasselas (C) are more or less neutral.

The field experimental design (six-year-old plants in 1977) was a complete randomized block (seven varieties x six blocks,

i.e., 42 plots). In each plot, 10 particular clusters were collected according to a method complying with the maturity variations from plant to plant (1).

The effects due to the maturity stage of the berries were studied by sampling once a week, for three weeks before and three weeks after, the optimum harvesting date. When collected, the samples were immediately stabilized by SO_2.

Volatiles extraction and GC analysis: The method followed has been perfected in our laboratory (3). One kg of berries per sample is crushed and the juice filtered. The aromatic volatiles are extracted with and concentrated in Freon-11 (trichlorofluoromethane) as proposed by RAPP et al. (2). GC analysis itself is performed in a GIRDEL 3000 (FID) connected with a LTT ICAP-10 integrator.

The extract (0.5×10^{-6} l) is injected in a glass capillary column (50 m x 0.35 mm) of carbowax 20M. The temperature increases regularly from 85°C at the beginning to 180°C about two hours later. We must note that the injection system used doesn't allow the detection of the lightest volatiles, including, in our case, less than 13 carbon atoms.

Biometrical analysis: in consequence of fluctuations in experimental conditions ascribable, in part to the progressive obsolescence of the glass capillary column, it appeared that the retention times were not available as reference marks for the successive components. A manual adjustment of the aromagrams was necessary, using the terpenes whose identity and peak shape were well establised.

The first step of the biometrical analysis was the detection of aromatic volatiles showing, among varieties or/and maturity stages, statistically significant variation at defined levels of probability. This was obtained by performing Univariable Analysis of Variance (according to varieties, maturity stages and replications) on the data set relative to each peak.

The next steps concerned the only significant selected variables; they were: 1) studying the similarity (or distances) between "aromatic profiles" taken as a whole, by mean of parametric (we used Log. transformation to get normal distributions) and nonparametric correlation coefficients, 2) searching for a description of the observed variation and trying to determine the substances which were mainly responsible for this variation by Principal Component Analysis (PCA), 3) estimating the degree of association between the volatiles group and the varieties group by Canonical Correlation Analysis, and 4) searching an axes system discriminating varieties and trying to determine substances that best separate the varieties by Factor Discriminant Analysis (FDA) or Canonical Analysis.

The last three analyses were carried out by Elisabeth Bienaime on the Phillips P. 880 computer of the INRA Laboratory of Biometry in Nancy, France. All other computations were performed in our laboratory with a Hewlett-Packard 9825 microcomputer.

RESULTS AND DISCUSSION

Data from GC analysis confirmed that all volatiles do exist in all varieties and that the differences between them are of a quantitative nature. This justified the adequacy of using statistical tools, at least for a first investigation. Fig. 1 represents the 1977 "mean aromagram" over varieties and maturity stages, showing with which kind of aromatic substances our analysis is concerned. This aromagram essentially covers the terpenes zone.

First selection of data: GC analysis furnished for each sample a sequence of 180 peaks; these were reduced to 130 after the necessary adjustment of retention times. Among them, 49 show significant variances for the variety factor and 13 for the maturity factor (10 of them for both factors).

Thus, we retained, as basis for our further analysis, 52 components whose variation was statistically significant at least at the 0.05 probability level. Thirty-nine of them, i.e., 75% of the "significant" volatiles are localized between hexanol and phenylethanol, giving evidence of the particular importance of this aromagram space according to varietal and maturity effects. We must note that, if substances having the highest peak areas generally exhibit significant variation, other components, each present in lower quantity, play an important role in the total variation.

Varietal and maturity mean effects: Fig. 2 represents the sum of peak area evolution for each variety according to maturity stage. The total amount of substances appears to increase greatly with maturity. Note that the total amount of volatiles, i.e., the aroma content, may show 1 to 20 differences according to variety.

Correlation analysis: This analysis on the "aromatic profiles," each taken as a whole, joined to clustering methods (4) permitted us to establish a dendrogram (Fig. 3) representing distances between varieties, taking into consideration all significant substances.

This dendrogram shows first a group of three weakly aromatic varieties with rather similar profiles: Auxerrois, Pinot blanc, and Sylvaner; then follow by increasing distances: Riesling, Chasselas, Gewürztraminer, and finally Muscat.

Note that Chasselas, despite its weakly aromatic character, is rather distant from the first group. In fact, the detailed analysis of its aromatic profile seems to show that its volatile composition is very unique and different from the other three.

The main interest of this dendrogram is that it shows that the quantitative analysis of GC data illustrates the same relationships between varieties that we could establish by wine tasting. This result justifies the quantitative approach in aroma research by demonstrating that, at least in terms of probability, the GC data do account for the major part of sensorial ratings.

Description of the variation: In the following analysis we

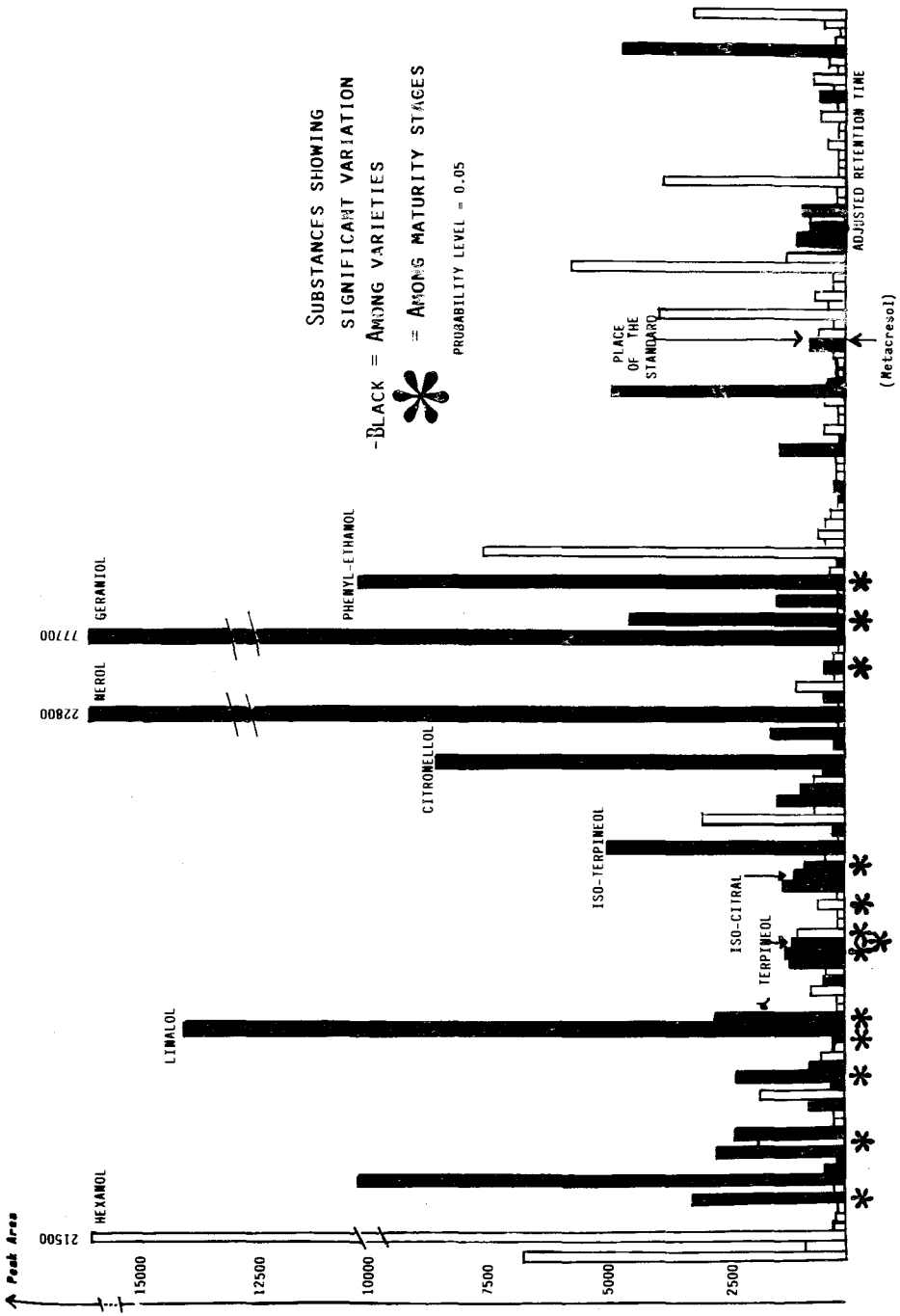

Fig. 1. 1977 Peak area means, over 6 varieties (Muscat excluded) and 6 maturity stages.

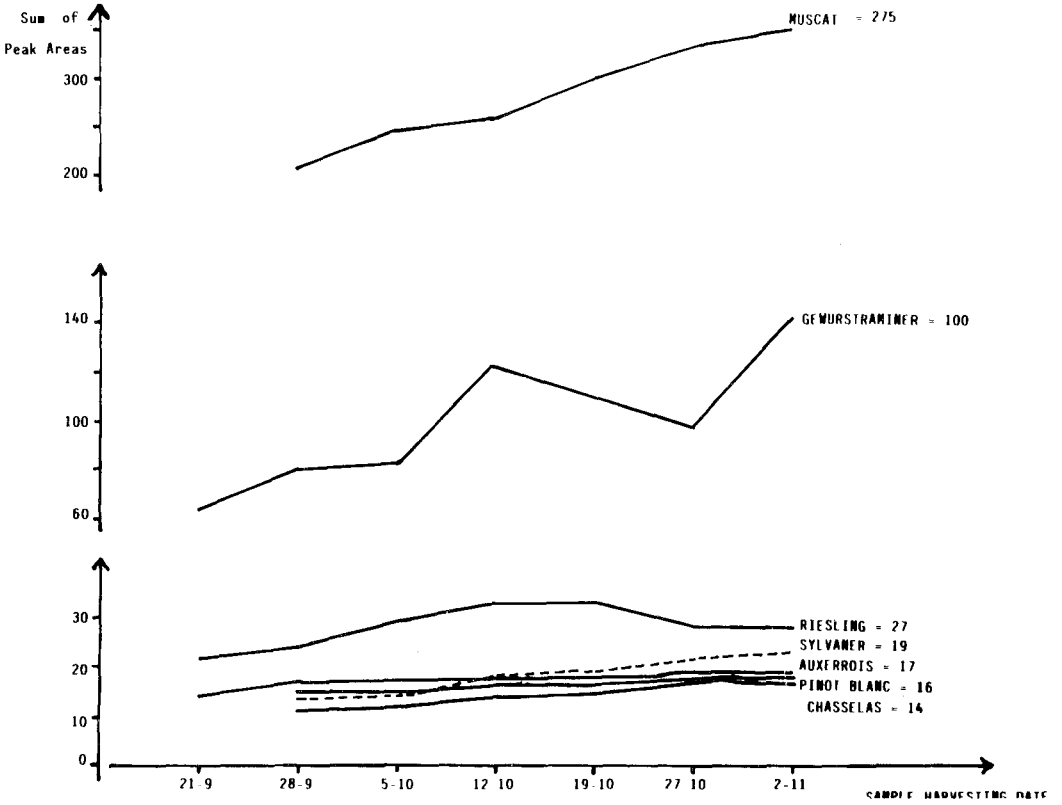

Fig. 2. 1977 Sum of peak areas for each variety according maturity stage.

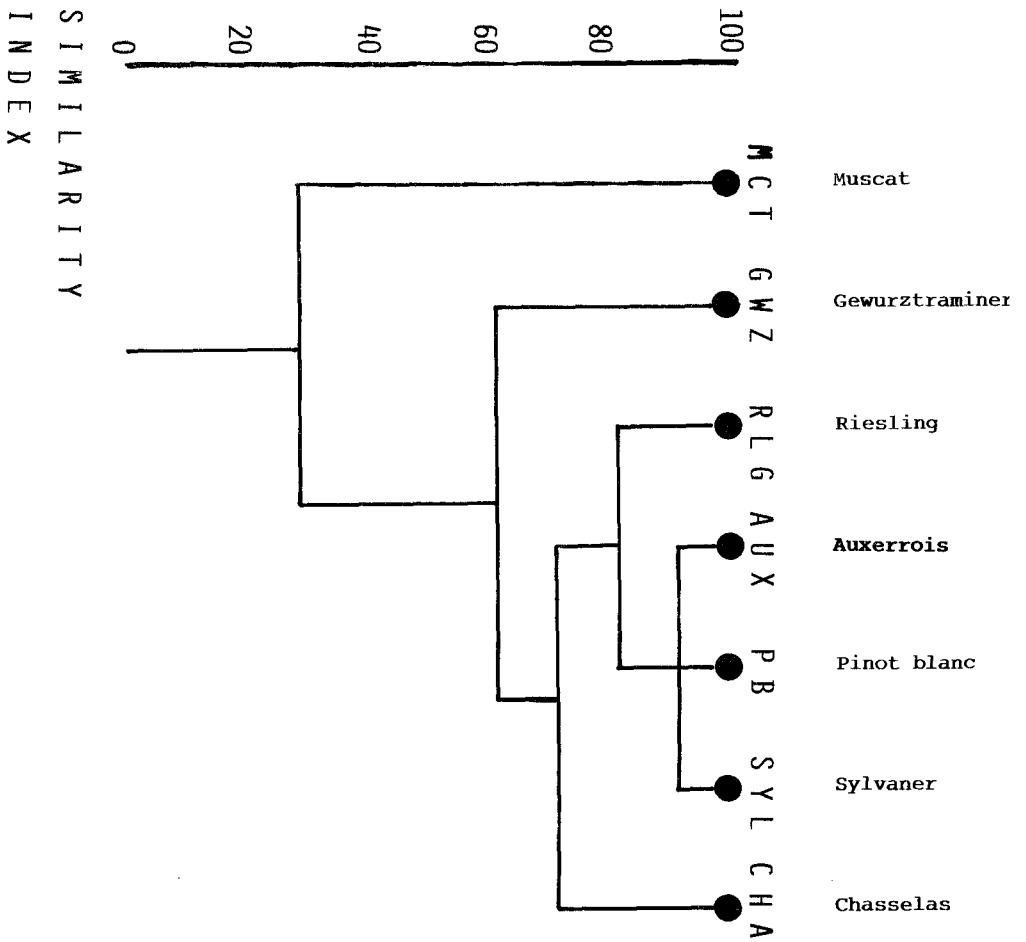

Fig. 3. Similarity description between varieties on aromatic profile taken as a whole.

excluded Muscat because of its very high scale of variation, overshadowing the variation of the other six varieties.

The first Principal Component accounts for 28% of the variation, the second for 14% and the third for 8%. So the first three Principal Components describe only 50% of the total variation (Fig. 4). Along the first axis, it can be seen that the main part of the variation is due to differences between Gewürztraminer, on the one hand, and the remaining varieties, on the other. Among this last group, Riesling is nevertheless separated clearly. This axis is highly correlated (r=0.85) with 13 substances among which are seven identified terpenes (benzylic-alcohol and phenyl-ethanol not included) and six other substances. The scond Principal Component gives a description of the variation among the less aromatic varieties. This second axis doesn't exhibit high correlation coefficients with any particular volatile. Note that Chasselas, as in the dendrogram, is well specified on this axis.

Canonical Correlation Analysis leads to a similar description but reveals, in addition, three high correlations each between a variety and a volatile: 1) between Pinot blanc and a component localized just before geraniol, we call provisionally geraniol-1 (r=0.85), 2) between Chasselas and a component localized 2 peaks before citronellol, we call provisionally citronellol-2 (r=0.91), and 3) between Auxerrois and a component localized just after linalool, we call provisionally linalool + 1 (r=0.83).

Discrimination of varieties: Fig. 5 shows that it is possible to obtain by Factor Discriminant Analysis a very good inter-varietal discrimination.

Eleven of the most discriminant volatiles belong to the previous group of most variable volatiles.

CONCLUSIONS

In spite of some particular aspect of the varietal material we were concerned with, some general conclusions may be drawn from our preliminary results: 1) the GC quantitative data of grape juice allowed us to obtain a correct evaluation of vine variety quality and type. 2) Although a few substances vary between maturity stages, these differences are unimportant compared to varietal differences. 3) The number of volatiles measured by GC analysis may be reduced by convenient statistical techniques to some fifteen, which determine the variation and the discrimination of the varieties considerd in this study. 4) Our data were gathered for only one year. It will be important to verify that the variation is explained by the same substances over successive years. Some additional experiments, conducted in 1978, lead us to believe it will be so.

In conclusion, it seems that methods combining GC and Multivariable Statistical Analysis may permit us to perfect classical evaluation for quality trials in the vine and give the basis of early tests of potential quality of new vine genotypes. The perfection of such tests appears today particularly critical when considering the development of new breeding programs using interspecific hybridization, in which quality criteria must be of

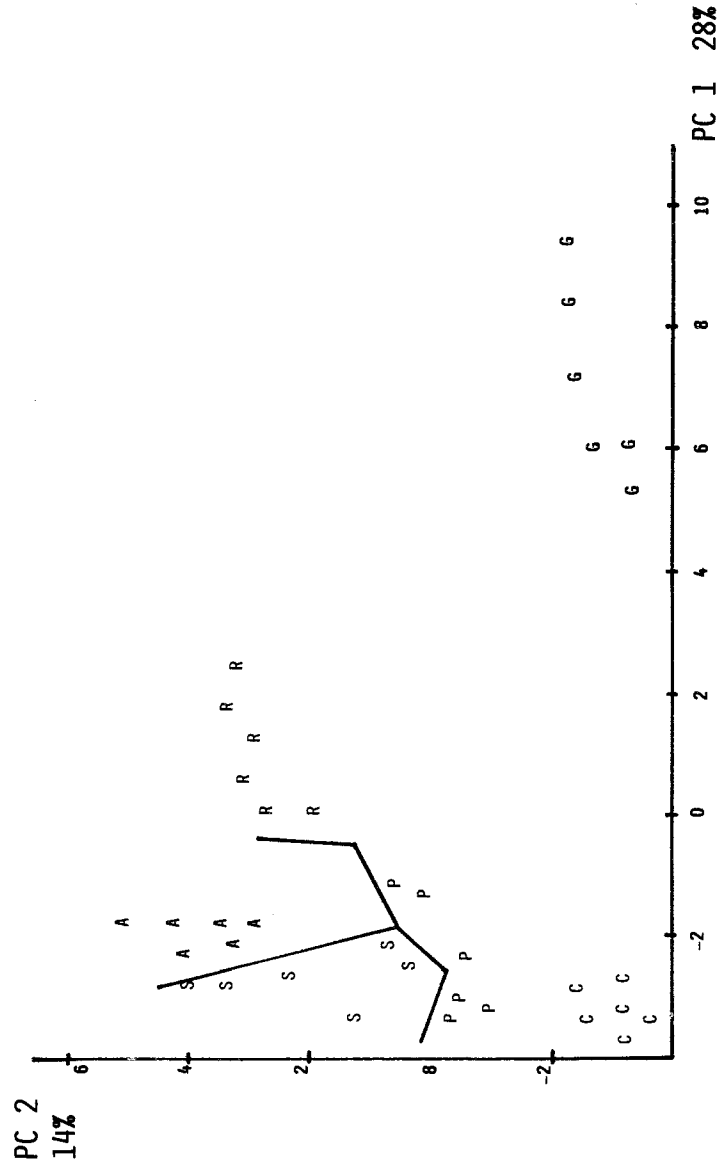

Fig. 4. Dispersion of the varieties upon 1st and 2nd principal components gathering 42% of total variation.

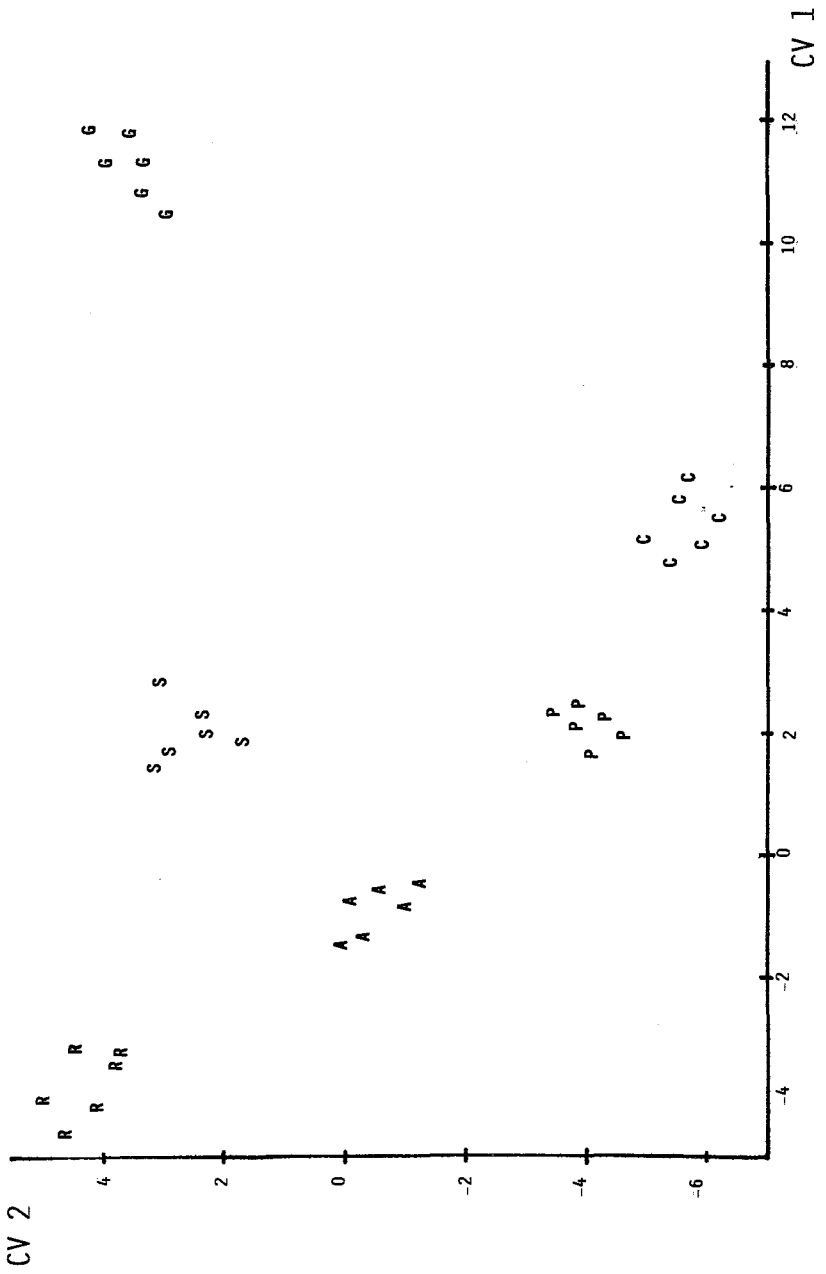

Fig. 5. Inter-varietal discrimination upon 1st and 2nd canonical variables.

first importance.

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QUALITY OF DIFFERENT CATEGORIES OF GRAPE SEEDS

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ABSTRACT

Seven seed categories from more than 10,000 berries were distinguished. The mineral content of the seeds was determined (K, Ca, Mg, Fe, Mn, Cu, Zn). In addition, the seeds were analyzed for total soluble phenolic compounds, proanthocyanins, and for composition of common fatty acids (palmitic, stearic, oleic, linoleic and linolenic acids) of the three lipid classes: glyco-, phospho- and neutral lipids. The amounts of all these substances and thus the seed quality varies according to both the number of seeds per berry and their localization in one or both of the two locules of the ovary.

We know that the number of seeds per grape berry is variable and that the percentage of ovule abortions differs from grape variety to grape variety. Within the same variety, there are also differences which depend on the placement of the grape bunch on the vine shoot. When several seeds develop in a single berry their inductive capacities are partially inhibited, because the more seeds a berry contains the lower the berry weight each is likely to initiate.

It was thus of interest to study whether there might be a relationship between the differences already demonstrated (1) and the chemical composition of the different categories of seeds in terms of their number and of whether they were localized in only one or in both of the ovary locules. The results presented here concern the dry weight of the seeds and the mineral elements as well as the amounts of phenolic compounds and lipids which they contain.

MATERIALS AND METHODS

The grape varieties studied were Ugni blanc and Cabernet Sauvignon. The sample grape bunches were taken from vines at Grande Ferrade (INRA, Bordeaux). More than 10,000 berries were used. The seeds extracted from these berries were classified into seven categories (Fig. 1) based on the number of seeds per berry and per locule and also on the size of the seeds when there were two seeds per locule. The seeds were oven dried at 105°C and then incinerated. The ashes thus obtained were analyzed by atomic absorption spectroscopy, except potassium, for which flame emission was used.

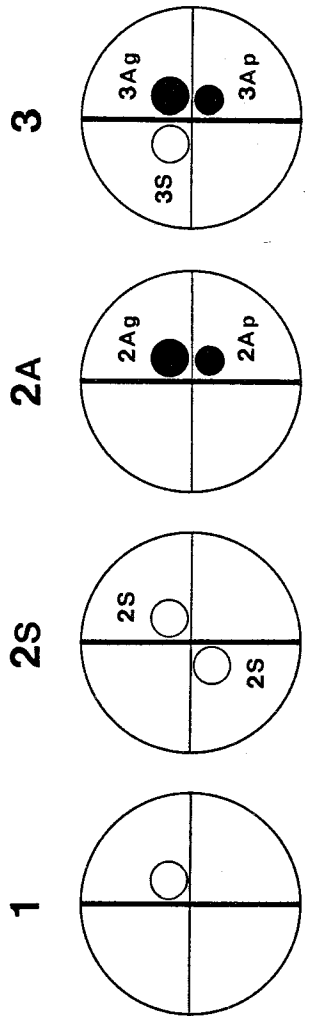

Fig. 1. Diagram showing locations in the grape berries of the 7 seed categories defined in the text.

The extraction of phenolic compounds and lipids was simultaneously carried out on the same seeds (3). At the end of this process, the two types of constituents had separated because of their difference in solubility.

The quantitative analysis of phenolic compounds and lipids was done by means of spectrocolorimetric measurement using the Folin-Ciocalteu reagent for the total phenolic compounds and Bate-Smith's reaction for the proanthocyanidins.

The neutral lipids were separated from the polar lipids using silicic acid chromatography according to the technique described by Vorbeck and Marinetti, adapted by Chavant and Sancholle (2). The fatty acids were analyzed using gas liquid chromatography.

RESULTS

Dry weight of seeds: This was determined in 1977 using seeds of the Ugni blanc variety (rank I and II grape bunches). Table 1 shows that the dry weight of the different categories of seeds is different and that the categories can be placed in the following order:

$$1 > 2Ag > 2S = 3S > 3Ag > 2Ap > 3Ap$$

This classification applies to bunches I and II, which shows that the variations observed do not occur at random, and this allows us to show the following two facts.

Importance of the number of seeds per berry: A characteristic of type 1 seeds is that they always have the greatest dry weight. Thus the berry seeds' capacity for synthesis is limited by the presence of a second seed, whether this second seed be localized in the same locule as the first seed or in the other locule. We may thus conclude that the weight of type 1 seeds is very close to the maximum weight that a seed is likely to attain.

The comparison of type 2A and 3A seeds shows that 3Ag > 2AG and 3AP > 2AP is always true, which can only be explained by the existence of the third seed (3S) present in the other locule of those berries containing three seeds.

Importance of whether the seeds are localized in a single locule or in both locules: The differences between types 2S and 2A show that whether the seeds are localized in only one locule or in both locules has an effect on their development.

Both type 2S seeds weigh the same and everything would seem to indicate that the nutritious substances which reach them are evenly distributed between the two locules, probably at the level of the bourrelet (first center of distribution). On the contrary, type 2A seeds are of different weights, which allows a very exact distinction to be made between a large one (2Ag) and a smaller one (2Ap), but the mean weight of these seeds (2Ag+Ap/2) is identical to the weight of type 2S seeds. In other words, the total synthesis which is possible is the same. The difference is due to the fact that type 2A seeds compete with each other, since

TABLE 1. Dry weight (in mg/seed) and mineral element content (in µg/seed) of the different seed categories (rank I and II grape bunches).

Elements	Bunches	1	2S	2Ag	2Ap	3S	3Ag	3Ap
Dry weight	I	27.3	24.6	26.5	22.9	24.7	23.4	22.5
	II	27.2	24.2	26.9	23.6	24.1	24.6	22.7
K	I	262	241	326	215	262	248	205
	II	256	211	274	149	219	229	207
Ca	I	1.5	1.0	1.2	0.7	1.4	1.3	1.0
	II	1.9	1.4	1.7	0.8	1.7	1.5	1.3
Mg	I	50	44	37	24	47	42	37
	II	50	43	48	28	45	44	41
K + Ca + Mg	I	313	286	364	240	310	291	243
	II	308	255	324	178	266	274	249
K/Ca + Mg	I	5.1	5.3	8.5	8.8	5.4	5.7	5.4
	II	4.9	4.7	5.5	5.2	4.7	5.0	4.9
Fe	I	1.2	1.3	1.0	1.1	1.1	1.0	0.7
	II	1.3	1.0	1.1	0.6	1.0	1.3	0.9
Mn	I	0.7	0.5	0.5	0.3	0.6	0.6	0.5
	II	0.8	0.6	0.7	0.4	0.6	0.6	0.6
Zn	I	0.6	0.4	0.5	0.3	0.5	0.3	0.3
	II	0.6	0.4	0.4	0.3	0.4	0.4	0.4
Cu	I	0.5	0.4	0.4	0.2	0.4	0.4	0.3
	II	0.4	0.3	0.4	0.2	0.4	0.4	0.4
Fe + Mn + Zn + Cu	I	3.0	2.6	2.4	1.9	2.5	2.3	1.8
	II	3.1	2.3	2.6	1.5	2.4	2.7	2.3

the distribution of nutritious substances between them depends on a second center, in such a way that one of them (2Ag) becomes preponderant and develops more than 2S, but always less than 1.

The fact that 2S = 3S shows that, in berries with three seeds, the development of the seed which is alone in a locule is not hindered by the fact that there are two seeds in the other locule anymore than it would be if there were only one seed in the other locule. A first competition does occur, however, at the level of the first distribution center, but it takes place to the detriment of type 3A seeds (3Ag + 3Ap $<$ 2Ag + 2Ap). Afterward there is a second competition at the level of the second distribution center between 3Ag and 3Ap, but this is less intense than the one involving 2A, since 3Ag $<$ 2Ag.

Mineral elements: These were determined using the same samples as above, and the results (Table 1) are expresed in terms of 1 seed.

Macro-elements : K, Ca and Mg: In all seed categories, the most abundant element is K. Mg is five times less abundant and Ca is only present in very small amounts. Contents in these three elements are on the same order of magnitude in the seeds of both bunches I and bunches II. However, it is to be noted that all categories of seeds from bunch I are slightly higher in macro-elements than equivalent categories from bunch II, although their dry weights are the same. This is due to K, for, on the contrary, there is a bit less Ca in bunch I and the Mg contents are identical. The classification of samples in terms of the amounts of macro-elements they contain is as follows:

$$2Ag > 1 > 3S > 2S > 3Ag > 3Ap > 2Ap$$

This classification is exactly the same for bunch I and bunch II and follows rather closely the classification based on dry weight.

The differences observed are essentially due to contents found in 2Ag and 2Ap and which, in the series under study, seem abnormal as the K/Ca + Mg relationship shows. They are explained by the fact that the competition between the two seeds has resulted in a heavy accumulation of macro-elements in 2Ag to the detriment of the 2Ap seeds. What is more curious and has not been explained, is that this accumulation occurs in the type 2A seed of both bunches I and II but in a less intense manner in bunch II.

Oligo-elements: Fe, Mn, Zn, Cu: In all tyeps of seeds, the most abundant element is iron, followed by manganese, zinc and copper. There are no significant differences between bunch I and bunch II. The classification of samples based on their oligo-element content is as follows:

$$1 > 2S \simeq 3S > 2Ag \simeq 3Ag > 2Ap = 3Ap$$

This, too, can be superposed rather well on the dry weight classification, and it seems, here again, that the values concerning 2Ag and 2Ap present an anomaly which is especially marked in the case of bunch II. The seeds which are alone in a locule seem capable of accumulating more oligo-elements than the other seeds.

Finally, for macro-elements as for oligo-elements, seeds 1 have an advantage and seeds 3Ag, 2Ap and 3Ap are always at a disadvantage. This is the same as in the case of the dry weight.

Phenolic compounds: The results obtained on Cabernet Sauvignon in 1978 (Table 2) allow us to draw the following conclusions. The different seed categories do not contain the same amounts of phenolic compounds. Type 2S seeds are considerably richer in total phenolic compounds and in proanthocyanidins than the other seeds. Type 3Ap seeds are those which contain the least: their level reaches only 25% of that of the other type 3 seeds and 15% of type 2S seeds. Type 3Ap and 2Ap seeds contain very different amounts; type 1 and type 2Ag have very similar contents and this is also true of type 3S and 3Ag seeds. The classification of the seven seed categories is the following:

Total phenolic compounds : $2S > 1 > 3S \equiv 3Ag > 2Ap > 2Ag > 2Ap$

Proanthocyanidins : $2S > 1 \equiv 2Ag > 3S \equiv 3Ag > 2Ap > 3Ap$

When a locule contains a single seed, the amount of phenolic compounds which that seed contains is not constant but depends on whether or not there are also other seeds in the berry (2S > 1 > 3S). When there are two seeds in a single locule, one of them always contains larger amounts of phenolic compounds than the other, but the difference in amount depends on the number of seeds per berry. The difference is sizable when the berry contains three seeds and much less great, though still marked, if the berry only contains two seeds. Type 2Ag seeds, as in the case of mineral elements, seem to display a special behavior. In berries with three seeds, there are always two seeds which are rich in phenolic compounds, while the third seed (3Ap) only contains a small amount. The two rich seeds (3S and 3Ag) are situated in a different locule.

The result of these facts is that the extremes in content are very different: 2.43 mg for type 2S seeds and 0.44 mg for type 3Ap seeds, in the case of proanthocyanidins. We may consider the content of type 2S seeds as corresponding to the maximum amount that a seed in our samples was likely to accumulate. The different characteristics described, and especially the fact that type 1 seeds are not the richest, lead one to think that the synthesis of phenolic compounds might be linked to the power of attraction of the locules. This attraction would be more efficient for the synthesis of phenolic compounds when there are two seeds than when there is only one seed, and it would be more efficient when two seeds are each situated in a locule than when they are both situated in the same locule. But there is a limit to the capacity for attraction, and when there are three seeds, one of them (3Ap) is unable to become rich in phenolic compounds.

Lipids: The results obtained refer to Cabernet Sauvignon. In respect of the total amounts of fatty acids they contain (Table 3), the seven categories of seeds can be classified in two groups:

$$1 \equiv 2S \equiv 3S \equiv 2Ag > 3Ag \equiv 2Ap \equiv 3Ap$$

TABLE 2. Phenolic compound content of seeds.

Phenolic compounds	1	2S	2Ag	2Ap	3S	3Ag	3Ap
Total phenolics (10 OD - 725 nm)	5.40	5.88	5.08	5.28	5.34	5.31	1.56
Proanthocyanidins (per seed)	1.73	2.43	1.73	1.36	1.55	1.57	0.44

TABLE 3. Distribution (%) of unsaturated (UFA) and saturated (SFA) fatty acids in the three lipid classes.

Fatty acids	NL	GL	PL
UFA	84	75	49
SFA	16	25	51

The first group consists of seeds which are alone in a locule and also type 2Ag seeds; the second group consists of the other seeds. Whether a locule contains only one seed or several seeds thus has an effect upon the seeds' ability to accumulate fatty acids.

The above classification applies to neutral lipids, NL. The fatty acids in these lipids add up to 2400 mg for the seeds in the first group but only 2000 mg for the second group. When there are three seeds in a locule, the diminution of content affects 3Ag and 3Ap but not 3S. Thus we find here something analogous to the phenolic compounds. Despite the difference in amounts contained, the relative proportions of NL remain quite similar in the different types of seeds (Table 4).

The three lipid classes contain the same fatty acids: palmitic acid, stearic acid, oleic acid and linoleic acid, but in different proportaions (Table 5). The most abundant fatty acid is C18:2 in the case of neutral lipids, NL (70%) and the glycolipids GL (63%), C16:0 in the case of phospholipids, PL (44%); the least abundant is C18:0 (5%) in the case of the NL, the GL (7%) and the PL (8%).

The quantitative importance of these three lipid classes is not related to the difference in seed type. No matter what the seed type, the NL are most abundant (96%) then the GL (4%) and the PL (1%). In the three lipid classes, the AGI and the AGS are distributed as shown in Table 3. This distribution is valid for all

TABLE 4. Amounts of fatty acids (in µg/seed), neutral lipids (NL), glycolipids (GL) and phospholipids (PL) in the different seed types, and relative proportions of the different lipid classes.

LIPIDS		1	2S	2Ag	2Ap	3S	3Ag	3Ap
Amounts	NL	2399	2391	2454	2011	2439	2091	1972
	GL	100	121	80	79	92	88	71
	PL	33	30	22	19	22	25	17
	Total	2532	2542	2556	2109	2553	2204	2060
	UFA	2157	2207	2165	1774	2051	1886	1680
	SFA	375	335	391	335	502	318	380
	UFA/SFA	5.9	6.6	5.5	5.3	4.1	5.8	4.4
%	NL	94.8	94.0	95.9	95.3	95.5	94.9	95.6
	GL	3.9	4.8	3.1	3.7	3.6	4.0	3.4
	PL	1.3	1.2	1.0	1.0	0.9	1.1	1.0
	UFA	85.2	86.8	84.7	84.1	80.3	85.6	81.5
	SFA	14.8	13.2	15.3	15.9	19.7	14.4	18.5

TABLE 5. Relative proportions of fatty acids in the different lipid classes (NL, GL and PL) and proportions of saturated and unsaturated fatty acids (SFA and UFA).

Lipids		1	2S	2Ag	2Ap	3S	3Ag	3Ap
NL	C16:0	8.0	7.6	8.8	8.7	11.7	10.0	10.4
	C18:0	5.7	4.6	5.8	6.4	7.4	8.0	7.5
	C18:1	12.7	14.4	6.1	6.8	13.5	18.0	17.4
	C18:2	73.6	73.4	79.3	78.1	67.4	64.0	64.7
	UFA	86.3	87.8	85.4	84.9	80.9	82.0	82.1
	SFA	13.7	12.2	14.6	15.1	19.1	18.0	17.9
GL	C16:0	18.0	16.2	18.8	20.3	19.1	17.6	17.6
	C18:0	6.2	6.2	7.2	7.8	6.9	6.5	7.1
	C18:1	11.6	12.4	12.0	12.5	12.3	11.8	12.5
	C18:2	64.2	65.2	62.0	59.4	61.7	64.1	62.8
	UFA	75.8	77.6	74.0	71.9	74.0	75.9	75.3
	SFA	24.2	22.4	26.0	28.1	26.0	24.1	24.7
PL	C16:0	43.2	43.1	41.8	46.3	43.8	41.5	44.6
	C18:0	8.1	9.1	8.0	9.1	8.7	6.8	7.1
	C18:1	12.1	9.7	10.1	9.7	11.1	11.6	12.9
	C18:2	36.6	38.1	40.1	34.9	36.4	40.1	35.4
	UFA	48.7	47.8	50.2	44.6	47.5	51.7	48.3
	SFA	51.3	52.2	49.8	55.4	52.5	48.3	51.7

seed types. Type 1 and 2S seeds differ from the others in the higher amounts of PL and GL which they contain, which also has repercussions on the relative proportions of these lipid classes, especially as concerns the PL. Type 2Ag and 2Ap seeds contain more C16:0 and an amount of C18:1 which is very slight compared to that in the other seeds. This is not the case of the PL and the GL, and the slight C18:1 content is accompanied by a larger amount of C18:2 (Table 5). Everything would seem to indicate that the desaturation process had been activated.

Seeds of types 3Ag and 3Ap contain a great deal more C18:1 and less C18:2. In their case it would therefore seem that, on the contrary, desaturation was partially inhibited. Thus we also observe a difference in behavior between seeds 2Ag and 2Ap, on one hand, and 3Ag and 3Ap, on the other hand, although these are all seeds which are localized in a single locule. The other types of seeds (1, 2S, 3S) contain the same amount of C18:1. The presence of two seeds in a single locule thus appears likely to bring about a change in the functioning of desaturases, which is inhibited when the berries contain three seeds and activated when they contain two seeds.

CONCLUSION

Type 2Ap and 3Ap seeds are less well developed than the others, their dry weight is less, and we note that type 3Ag seeds resemble them. These three types of seeds contain less of the macro- and oligo-elements, less total phenolic compounds, less proanthocyanidins and total fatty acids. The seeds which are alone in a locule (1, 2S and 3S), which type 2Ag seeds resemble, are markedly different from these categories. The amounts observed, of whatever constituent we may choose to consider, are thus indeed linked to the seven categories of seeds, which proves the distinctions we have made between them to be justified, and that they do in actual fact correspond to differences in seed quality.

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AN APPLICATION OF RECURRENT SELECTION TO GRAPE BREEDING

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ABSTRACT

The need to preserve genetic variability leads to formulating long term breeding programs in which the selection of new cultivars would parallel the improvement of the germ plasm which is the basis of this selection. Accordingly, a recurrent selection program was initiated in 1977 at Montpellier. Its aim is the improvement of a *Vitis vinifera* set of genotypes which will be used, after each selection process, as germ plasm for breeding new varieties able to produce wine of good quality and color and which would be well adapted to southern French vineyards.

Interest in a recurrent selection scheme for breeding work in grapes: Up to now, two different ways have been followed for breeding grapes. The first one favored the immediate genetic improvement at the expense of the amount of the genetic diversity which is available in following generations. It is the method which was used by French hybridizers during the first half of the 20th century (1).

The other one has been used by (2) for the improvement of *Vitis vinifera* varieties. They chose two parents among a population Po (variety collection) to get progeny in which individual selections are made to find vines which conform to a given selection goal (Fig. 1). This scheme can be followed for a long time, as there are lots of different combinations which can be used. But, as there is no improvement of the parents, it is necessary to start from the beginning (Po) each time. The goal is, therefore, to gain some genetic improvement from one generation to the other without losing too much genetic variability.

This goal can be achieved by adopting a recurrent selection scheme, which was pointed out by (4) and which may be useful for most plant species. Gallais stressed that the improvement of the source material must be done completely independent of selecting new varieties. In the former case (improvement of the source material), the goal is to analyze and to concentrate the germ plasm which is at hand. In the latter (new varieties), the goal is to make a synthesis in taking advantage of this superior germ plasm.

It is named a recurrent scheme because it can be carried out several times. At each cycle, the source material population undergoes at the same time: 1) a recombination of genes by an

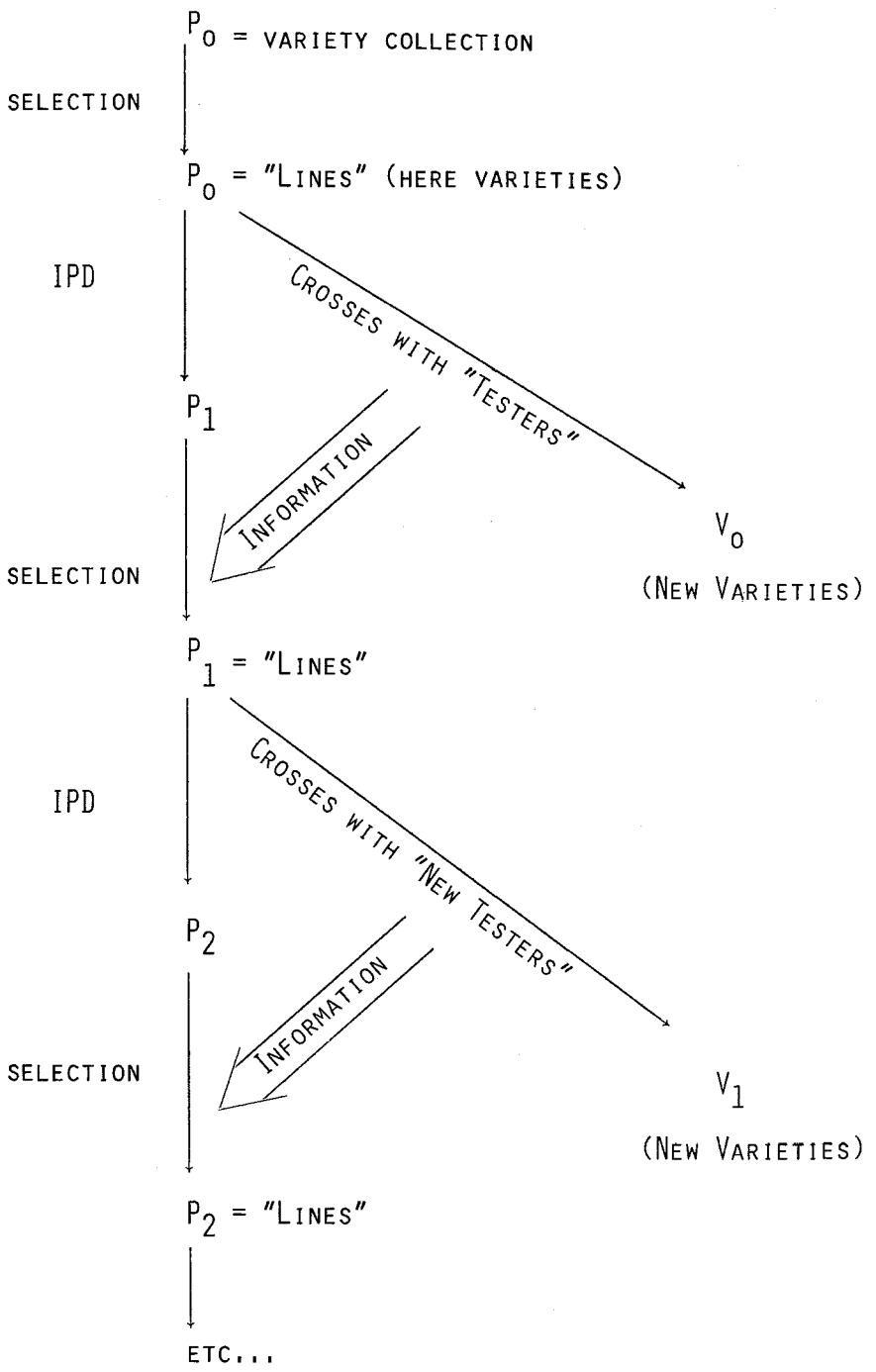


Fig. 1. Recurrent selection scheme.

adequate reproduction scheme through seed and 2) an improvement through selection.

Utilization of recurrent selection in a grape breeding program. *The selection goal:* We will consider only the case of an important economic character which can be measured objectively. As an example, we will take the program which started in 1977 at the Viticultural Research Station, INRA, Montpellier, for selection on the ability to give good colored wines. It is out of the question to use interspecific hybrids or red juice varieties which give in their progenies an excessive percentage of plants yielding a poor wine.

Choice of the source material: Two criteria were used to make this choice: 1) all the primordial main distribution sites of *Vitis vinifera* should be represented, and 2) the selected lines [lines or varieties (only one clone stands for a variety)] should be as numerous as possible.

Among the 781 black varieties grown (only one clone stands for a variety) in the variety collection at Vassal (close to Montpellier), the choice is made by taking into account only their phenotype: the ability to give wine with intense color. The 35 varieties chosen, according to their origins, are put in four separate geographical groups (Table 1). Within each group, only the best four have been selected. Thus the source material includes only 16 varieties (or lines); but, for the reason explained below, this restricted number of parents, nonetheless, involves the making of numerous controlled crosses.

Improving the source material: 1) Procedure for mixing the gene pools: In a small population, where male and female gametes combine at random (Panmictic population), the smaller the population, the higher is the rate of inbreeding (3). In our case, there are few varieties (16 only), and we cannot expect to carry out a reproductive system as effective as random mating which avoids inbreeding. So we must minimize, as much as possible, the genetic relationship between varieties which will be crossed to give the next generation. Taking into account practical advantages, the only handy reproduction system which fulfills this requirement of minimizing inbreeding is the Incomplete Partial Diallel (I.P.D.) (5), namely, each line is taken 3 times as a male and 3 times as a female, and it is important that lines crossed are not of the same geographical group (Table 2).

The crosses are made at a time when the breeding value of the 16 lines is not known, so all are to be taken into account for the IPD crossing procedure.

How to choose the best lines for the next generation: This selection has to be managed so as to reduce to 16 the number of lines which will give the next generation of the improvement program.

a) Selection procedure between half sib progenies: the selection criterion is the ability of the lines (varieties here) to give to their progenies an intense wine color. We used two "tester" varieties (Grenache and Cabernet Sauvignon) which were both

TABLE I

LISTING OF THE HIGHLY COLORED VARIETIES FOR THE FOUR DIFFERENT GEOGRAPHICAL SITES CONSIDERED.

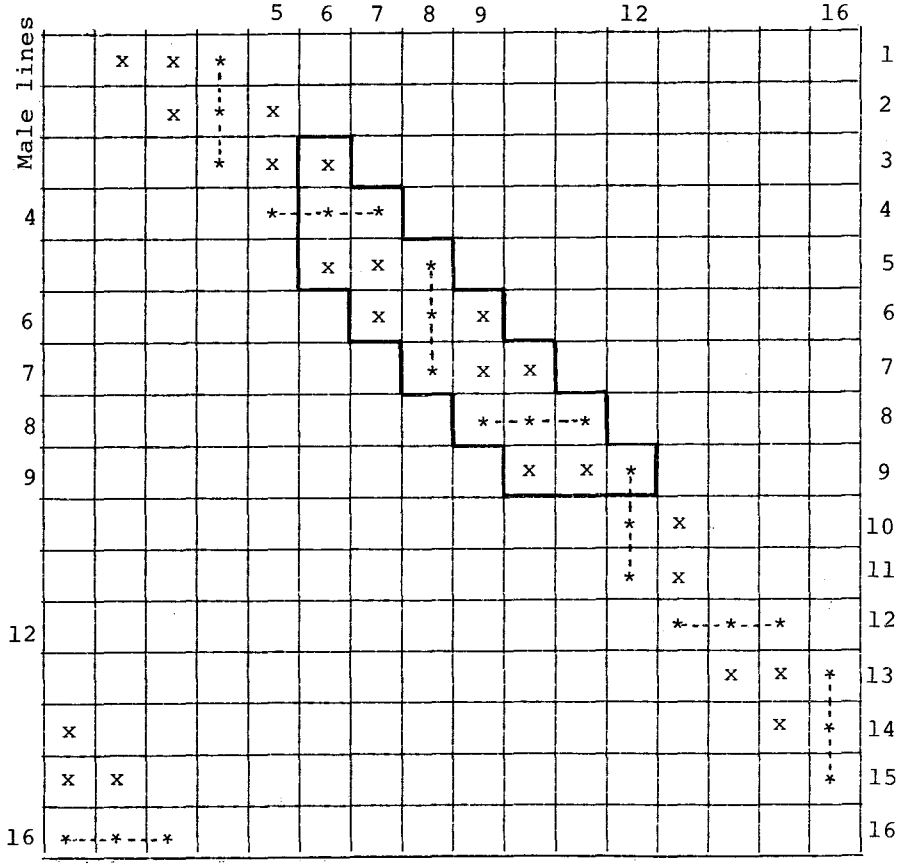
(The color intensity is evaluated in comparison with the color intensity of a Cinsaut wine).

1. ITALY and SAVOIE		2. OCEANIC REGIONS:	
Calabrese	5.9	Cabernet Sauvignon	3.6
Castiglione	3.0	Carmenère	4.5
Ciliegiulo	4.0	Manseng	10.0
Colorino	6.6	Mureto	4.0
Croetto	5.0	Petit Verdot	3.6
Dolcetto	4.0	Ruby Cabernet	3.3
Fogarina	10.0	Saint Macaire	3.1
Fumin	3.3	Souzao	5.5
Joubertin	6.4	Touriga	6.3
Lambrusco Maestri	6.6		
Malvasia di Casorzo	3.3		
Perricone	4.0		
3. NORTHERN or SUB-CONTINENTAL REGIONS:		**4. ADRIATIC and BLACK SEA REGIONS:**	
Abouriou	5.6	Refoscone	6.6
Argant	5.0	Terrano	10.0
Gelbholzer Blau	3.3	Soinouri	6.6
Goron	3.3	Saperavi	(>3.0)
Robin	4.5		
Limberger	3.0		
Saint Laurent	4.0		

TABLE 2. 1st case: Lines selected, within dark outline, 18 progenies, minimum.

2nd case: Lines selected = * -- 24 progenies, maximum.

Female lines



crossed together with each of the 16 lines. It must be stressed that Grenache and Cabernet Sauvignon have been frequently used as parents for breeding new varieties.

Only 4 lines of the 16 are expected to be selected. Those which are left are intended to transmit genes to the next generation, as the selection procedure acts at the level of half sib progenies. For each selected line, there will be retained 6 full sib families from crosses between this line and other lines, either discarded or not (this can be seen from Table 2); so the total number of full sib families retained will be 24 at maximum and 18 at minimum, of 48 which have been bred.

b) Selection procedure between full sib progenies: In the first selection cycle, the crosses made between the 16 lines, together with the two tester varieties, will serve several goals:

1) to test the ability of the lines to transmit to the progeny an intense wine color.

2) to raise new varieties, because it is likely that several interesting vines will be detected in these progenies, and

3) to obtain new tester lines for the next selection cycle: it is obviously desirable to manage at the same time improvement of the lines and improvement of the tester lines, which will be used to judge them.

If we adhere to these goals for another selection cycle, we have to accept a constraint. The degree of genetic relationship between future lines and future test lines must be minimized. These considerations will serve to select 16 full sib families among the 18 to 24 families which remain.

c) Selection procedure between vines among a full sib progeny: One vine per progeny must be selected so as to have only 16 lines for the next generation. At this selection stage, all the phenotypic characters can be used for discarding vines because it is necessary to take into account all the characters which make a line suitable for use as a parent (enough bunches, good set, medium earliness... etc.).

Raising new varieties: Obviously, new varieties can be produced in crosses other than those of line x tester-varieties. The progenies will be grown with the goal of taking advantage of the genetic progress achieved at the level of the lines selected for several different characters (for instance, good-colored wine and regular cropping). But this will be possible only in the future.

Interest of the selected lines for other vine breeding stations: The lines will be tested for their ability to transmit a given character to their offspring. If they are crossed with vines, other than those which have been used as testers, it is not sure that their general combining ability (7) will be maintained at the same level. Nevertheless, it can be expected that often their performance will be better than those of lines which would have been selected only on phenotype or on the basis of data taken from

a few progenies not judged in the same trail.

In additon to the color of the wine, several other characteristics important for the efficiency or the quality of the grape production can be studied to obtain very useful lines.

It is obvious that for each specific goal there is a lot of work to be done so that a given research station can only hope to achieve one goal at a time, but there are several on which it should work.

Accordingly, an international distribution of the main selection objectives should be undertaken. Each station should then undertake such a program as described to create lines of grape germ plasm which could be more easily exchanged than are new varieties. With cooperation, each research station could make better progress in creating new varieties. Such lines would have considerable economic value for the future and better knowledge of grape germ plasm could also be achieved.

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EARLY PHYSIOLOGICAL TESTS OF SELECTION: A KEY FOR BREEDING PROGRAMS

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ABSTRACT

Stomatal density and resistance is very variable among genotypes, both under natural and controlled conditions. The most interesting fact seems to be the relationship between stoma-density and relative variation of stomatal resistance, when environmental conditions are changing rapidly. The widest stomatal regulation amplitudes occur for genotypes possessing the highest stomatal density.

Rough Photosynthesis: It is possible to compare genotypes under controlled conditions at early developmental stages of cuttings.

Rootstocks: A drip irrigation design in a greenhouse, using small pots and including 3 water regimes (maximum transpiration, moderate dryness, water-stress) allows an adequate drought resistance evaluation.

The breeding programs for the grapevine involve so much criteria and characters that the selection strategy must optimize the general recombination level. In this way, an interesting scheme of recurrent selection was pointed out (4,6). But this selection work is very onerous, in so far as a perennial plant needs many years per selection cycle. But if one establishes some early tests of selection, it is useful to use recurrent selection with short cycles (2 years, for example). It is possible to evaluate early some disease resistances, some aspects of vigor and fertility. In order to complete this selection power, it is necessary to evaluate early some physiological parameters genetically correlated to the vigor, the yield and the maturation of the adult plant in different environments.

Stomatal parameters and gross photosynthesis: Since 1977, different measurements were made both under natural conditions and on plants cultivated in pots which possessed a single shoot bearing only 6 leaves. In the last case, the plants were grown in a greenhouse, and the measurements reported here were done in a controlled chamber (giving 1200 µE P.A.R.).

Transpiration and photosynthesis are among the most fundamental parameters of the whole plant biology. Our experiments on training systems (2) have shown, first, the good correlation between photosynthesis and yield or berry sugar content, and

second, the interest to insure a good photosynthesis for some extreme microclimates stimulating the accumulation of polyphenols and aromas which is also related to berry skin sugar content according to Pirie and Mullins (8).

Hence, we have noted (stomatal density (d/mm^2) by using the Schoch's technique (9), stomatal resistance (s/cm) and gross photosynthesis (mg $CO_2/dm^2/h$) by using a ventilated diffusion porometer, the chamber of which allowed the assimilation of $^{14}CO_2$ (2). The total leaf water potential was measured by a pressure chamber.

In the first experiment, the leaf temperature has varied. Fig. 1 shows that there are significant differences in stomatal resistance between varieties at 30°C and 37°C where stomatal resistance is the iowest. The correlation coefficient with similar field conditions is good: + 0.88. In this case the mean leaf water potential is - 5 bars. Also, stomatal density is greatly modified by the genotype and the correlation with field conditions equal + 0.79.

An interesting observation concerns the significant relationship between the absolute variation of stomatal resistance per unit of leaf temperature (Δ /°C) and the stomatal density. If within the same variety or among different genotypes, a leaf possesses a high number of stomata, the variation of their apertures is great when the ambient conditions (temperature) are changing. This is perhaps due to a "competition" between stomata, if their number is high. In such conditions, the mean individual aperture is reduced and often below the maximum. This could allow greater possibilities of variation than if stomata are near their maximum aperture plateau (3).

These results can explain some ecological adaptations or rusticity, for example, Cabernet Sauvignon possesses a low stomatal resistance, few stomata and a slight stomatal regulation. Its physiology could tolerate, more than other varieties, relatively great environmental variations. Merlot and Sauvignon are similar: Grenache noir and Pinot noir are more susceptible.

Fig. 2 indicates also that the variety influences both stomatal resistance and stomatal density. An increasing leaf water potential (measured with a pressure chamber) bound to an increasing dryness enhances stomatal resistance differently in relation to the variety. Cabernet Sauvignon and Sauvignon are more tolerant than Ugni blanc and above all Pinot noir, Syrah and Grenache noir. In this case, the mean leaf temperature is 30°C.

The absolute variation of stomatal resistance per unit of leaf water potential (Δ / bar) is significantly correlated to stomatal density between -5 and -8 bars but not between -8 and -13 bars. In fact, for the level of -13 bars, it seems that other factors occur, probably deep modifications of the leaf tissues and interaction with the previous relationship.

Fig. 3 indicates the discrimination of the varieties according to their mean and their mean square (with the corresponding significance intervals and ellipses at the 5 percent level). It

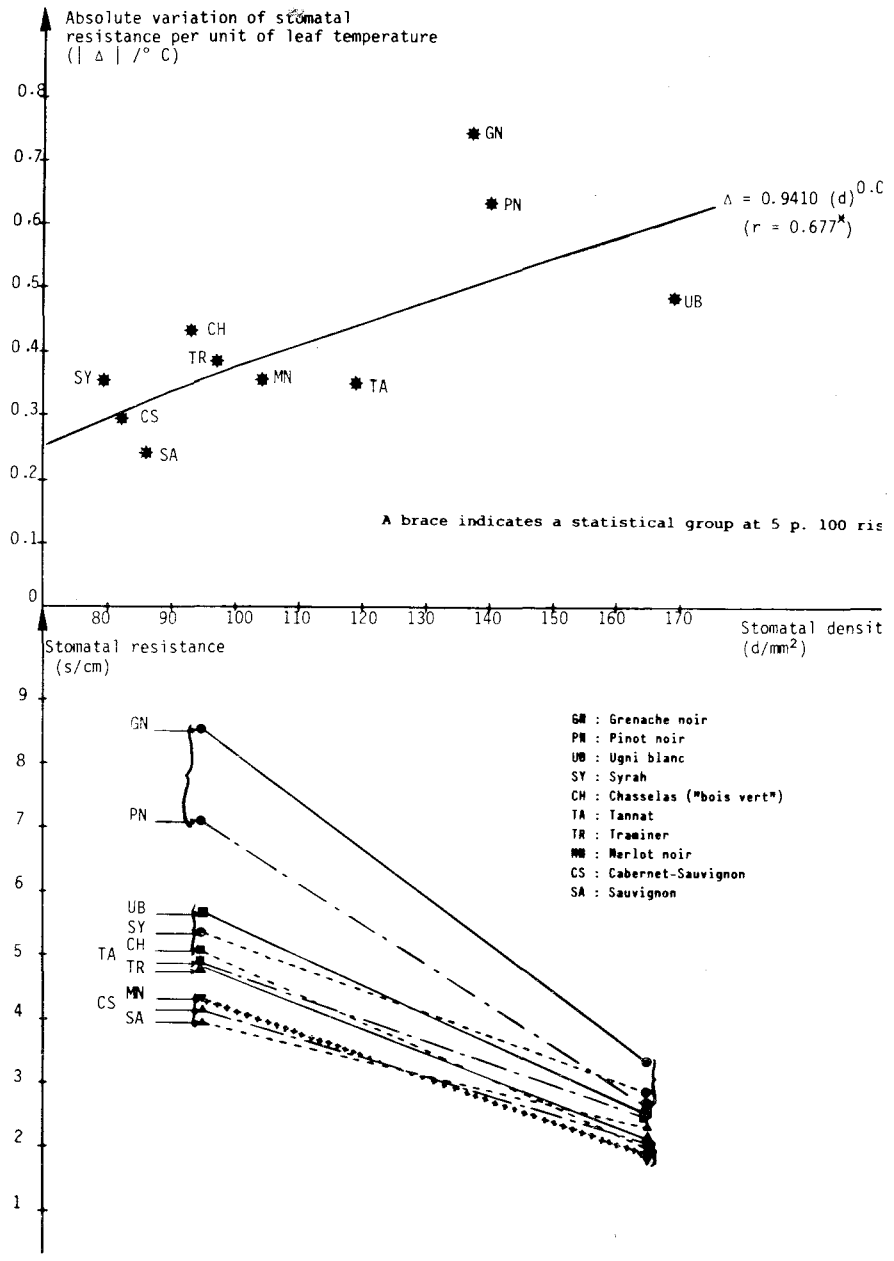

Fig. 1. Influence of the variety and the leaf temperature on stomatal resistance and on the relationship between stomatal density and the absolute variation of stomatal resistance per unit of leaf temperature.

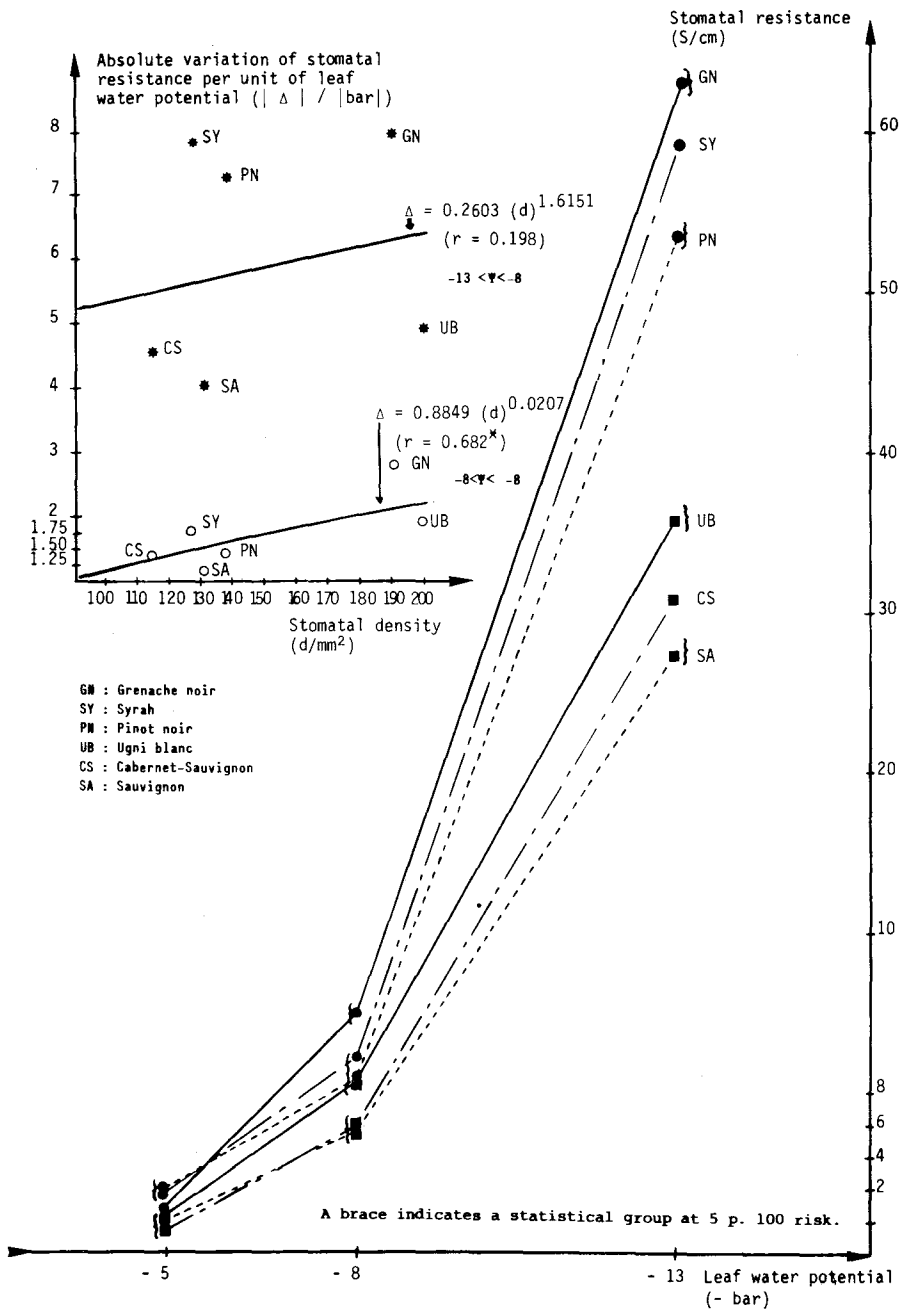

Fig. 2. Influence of the variety and the leaf water potential on stomatal resistance and on the relationships between stomatal density and the absolute variation of stomatal resistance per unit of leaf water potential (■ = between -5 and -8 bars; ✱ = between -13 and -8 bars).

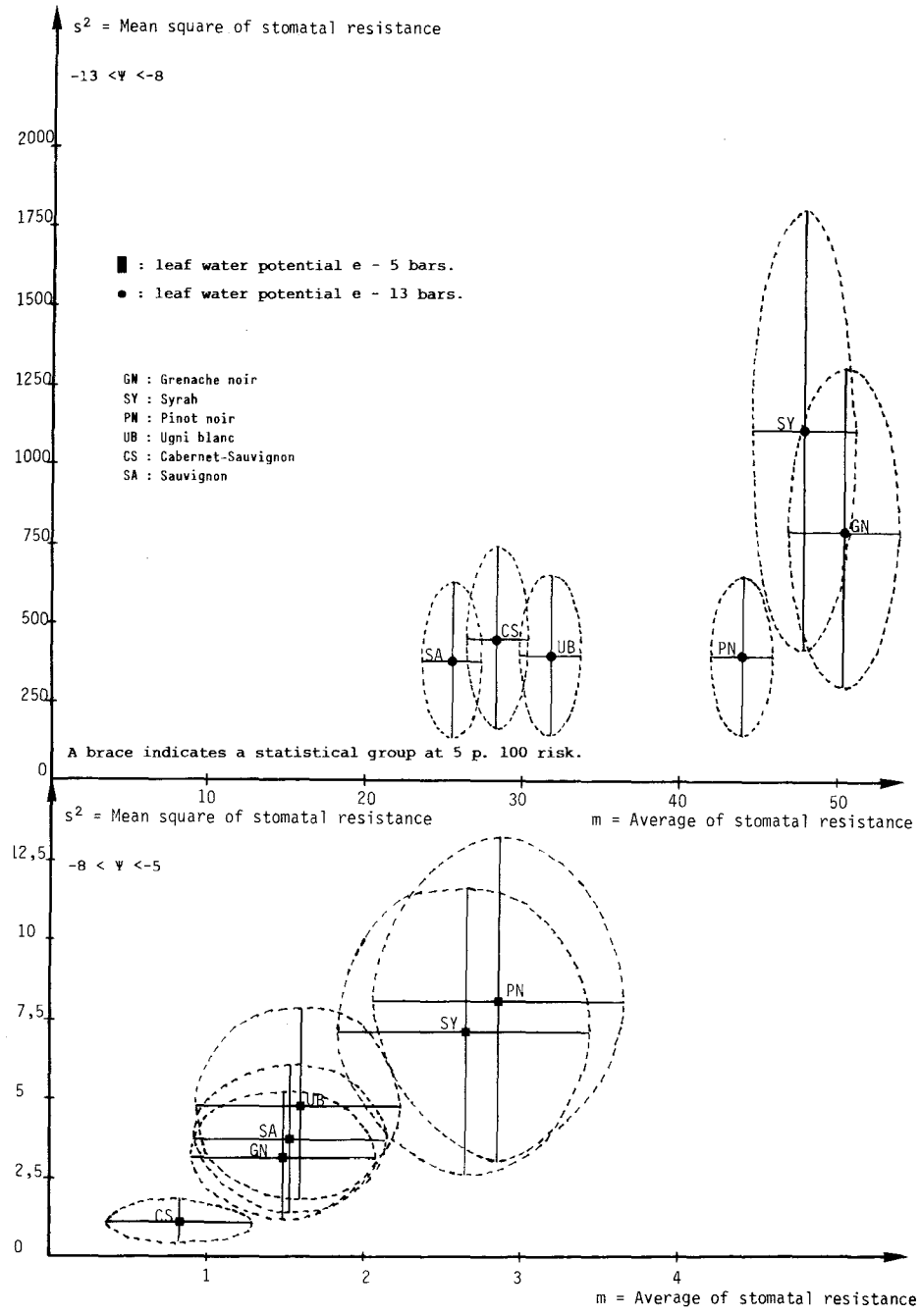

Fig. 3. Discrimination of the varieties on stomatal resistance by using both the mean (m) and the mean square (s^2):

appears that it is possible to discriminate more varieties if one considers the mean and the variation around the mean. This is also of physiological importance. Fig. 3 shows also that between -5 and -8 bars of leaf water potential the mean square increases parallel to the mean. This signifies that stomatal resistance is deeply bound to the environmental conditions and not stabilized. Between -8 and -13 bars of leaf water potential, the mean square is quite stabilized in relation to the mean but increases strongly after a given threshold. This signifies that, after this level, other consequences of dryness begins for genotypes which have first reached this level. This can explain that the stomatal regulation changes.

Gross photosynthesis per unit of leaf area is also significantly influenced by the variety. The correlation with field conditions is good: + 0.88. The best values are obtained by Cabernet-Sauvignon, Sauvignon, and Ugni blanc, the lowest ones by Syrah and Chasselas, the ambient temperature being 30°C. Nonsignificant values occur in the middle of the ranking for Traminer, Pinot noir, Merlot noir, Tannat, and Grenache noir.

These observations suggest that breeders should determine if stomatal density, which varies much according to the variety but keeps similar classifications if the environment changes, can be used to control the variability in a recurrent selection program. Besides, genotypes possessing a low stomatal density tend to support well the climatic fluctuations.

Stomatal resistance, absolute variations of stomatal resistance when the environment changes, gross photosynthesis, can be utilized in selecting genotypes which are able, for different ambient conditions (leaf temperature and leaf water potential) and in relation to leaf area, to insure good and stable yield and maturation. Some good physiological correlations exist. It is now necessary to analyze the genetical ones in a breeding program.

The use of the mean and the mean square of these parameters, in the area of maximum variability for a pure phenomenon in controlled conditions, can improve the discrimination between genotypes.

Polyphenol accumulation in leaf disks: We have utilized, since 1978, the Pirie and Mullins' technique (7) and grown in a controlled chamber leaf disk cultures *in vitro* (Petri dishes) on a saccharose medium (100 g/l). The total polyphenols and the anthocyanins were analyzed after two weeks. The coloring was shown to be due to the sugar itself and not to an osmotic effect, by using a mannitol medium at the same osmotic level.

The aim is to note berry coloring before having seen the berries, hence, several weeks after sowing. The condition is that the correlation between berry coloring and leaf coloring is good. This assumption is realistic mainly for *Vitis vinifera*.

The varieties are ranked according to the increase in berry coloring:

1) Pinot blanc, Grenache blanc,

2) Pinot gris, Grenache gris,

3) Gamay, Pinot noir, Grenache noir, Cabernet Franc, Cabernet Sauvignon, Merlot noir, and

4) Alicante Bouschet and Gamay teinturier.

Fig. 4 shows significant differences among genotypes when the ambient temperature is changing:

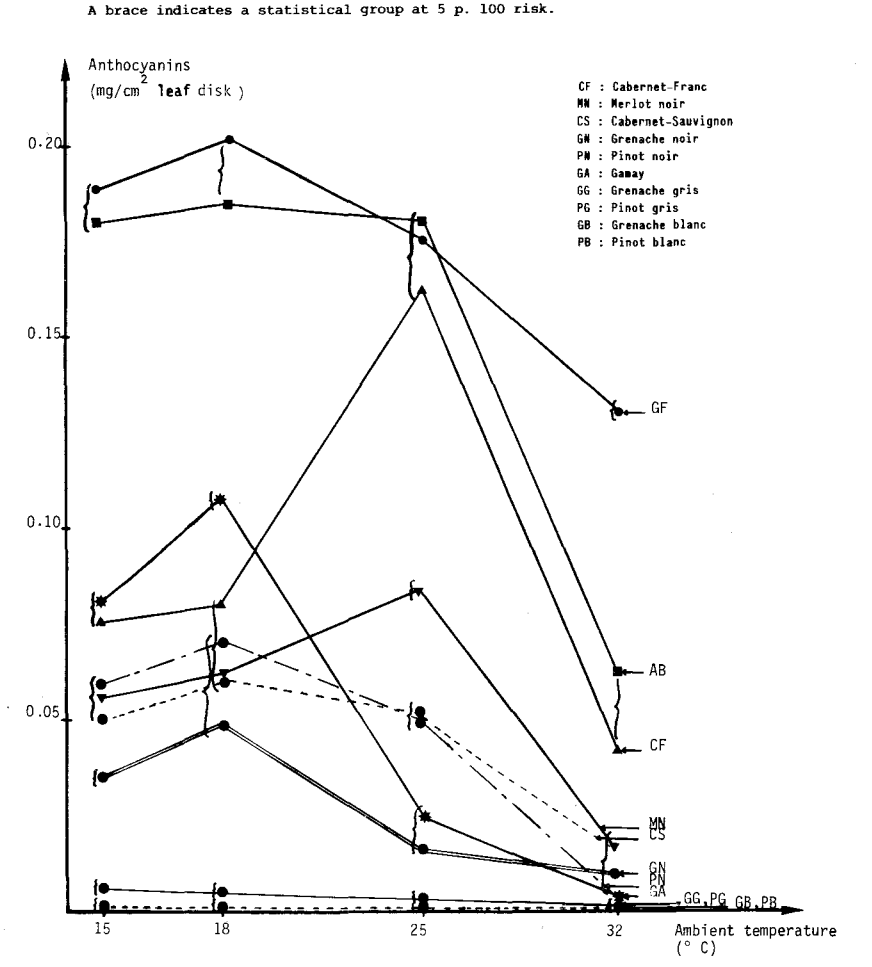

Fig. 4. Influence of the variety and the temperature on anthocyanins content of leaf disks grown *in vitro* on a saccharose medium.

1) white varieties give no colored disk,

2) grey or pink varieties give a very light coloration (sometimes quite brown as for Ugni blanc in similar experiments, and

3) red varieties are located at a mean level, the optimum temperatures being from 18° to 25°C. Gamay, Pinot noir and Grenache noir have this optimum near 18°C; Cabernet Sauvignon is quite stable in this zone; Merlot noir and Cabernet Franc have this optimum near 25°C. Cabernet Franc gives very colored disks at 25°C, and

4) "red-juice" varieties always give very colored disks. But Cabernet Franc, mainly at 25°C, curiously shows similar responses, more so than do Alicante Bouschet or Gamay Freau.

Hence, in such conditions, it seems possible to appreciate the kind of berry coloring (white, pink, red or very dark) and some relationship between ambient temperature and polyphenol accumulation, but not slight differences in the same group. Regardless, this very early selection test can present some interest.

Rootstock response to water regime: We have installed in a greenhouse, since 1978, a pot culture (1 each of 8 varieties) with a controlled drip irrigation on a sandy soil, in order to study the rootstock response to three water regimes: maximum evapotranspiration (MET) and at the end of the vegetative phase, 0.3 MET and 0.15 MET. The scion possessing a single shoot, summer pruned at the beginning was Cabernet-Sauvignon which has a good transpiration rate.

Measurements were made of stomatal resistance, leaf water potential, shoot leaf area, mature shoot and root fresh weight.

The stock variety sample covers the different species: Berlandieri Resseguier No. 2, Riparia Gloire, Rupestris du Lot, 140 Ruggeri, 110 Richter, Fercal, and *rotundifolia* 'Yuga' used by green grafting.

Fig. 5 indicates the significant differences between these varieties, 1 h and 3 h, after watering according to a transpiration index: which is the ratio "shoot leaf area on stomatal resistance" (cm^3/s) and expresses the adaptation of the plant to water stress.

Few differences occur for maximum evapotranspiration. The moderate drought indicates 2 groups 3 h after watering:

1) Well adapted genotypes : Riparia Gloire (which gives the best transpiration 1 h after watering), 110 Richter and 140 Ruggeri.

2) Less adapted genotypes : Rupestris du Lot and Fercal.

The most intense drought indicates 3 groups 3 h after watering :

1) Well adapted genotypes : 110 Richter, 140 Ruggeri and *rotundifolia* 'Yuga' (which curiously was not so evident 1 h after watering).

2) More susceptible genotypes, mainly at extreme dryness : *Berlandieri* Resseguier No. 2 and *Riparia* 'Gloire'.

A brace indicates a statistical group at 5 p. 100 risk.

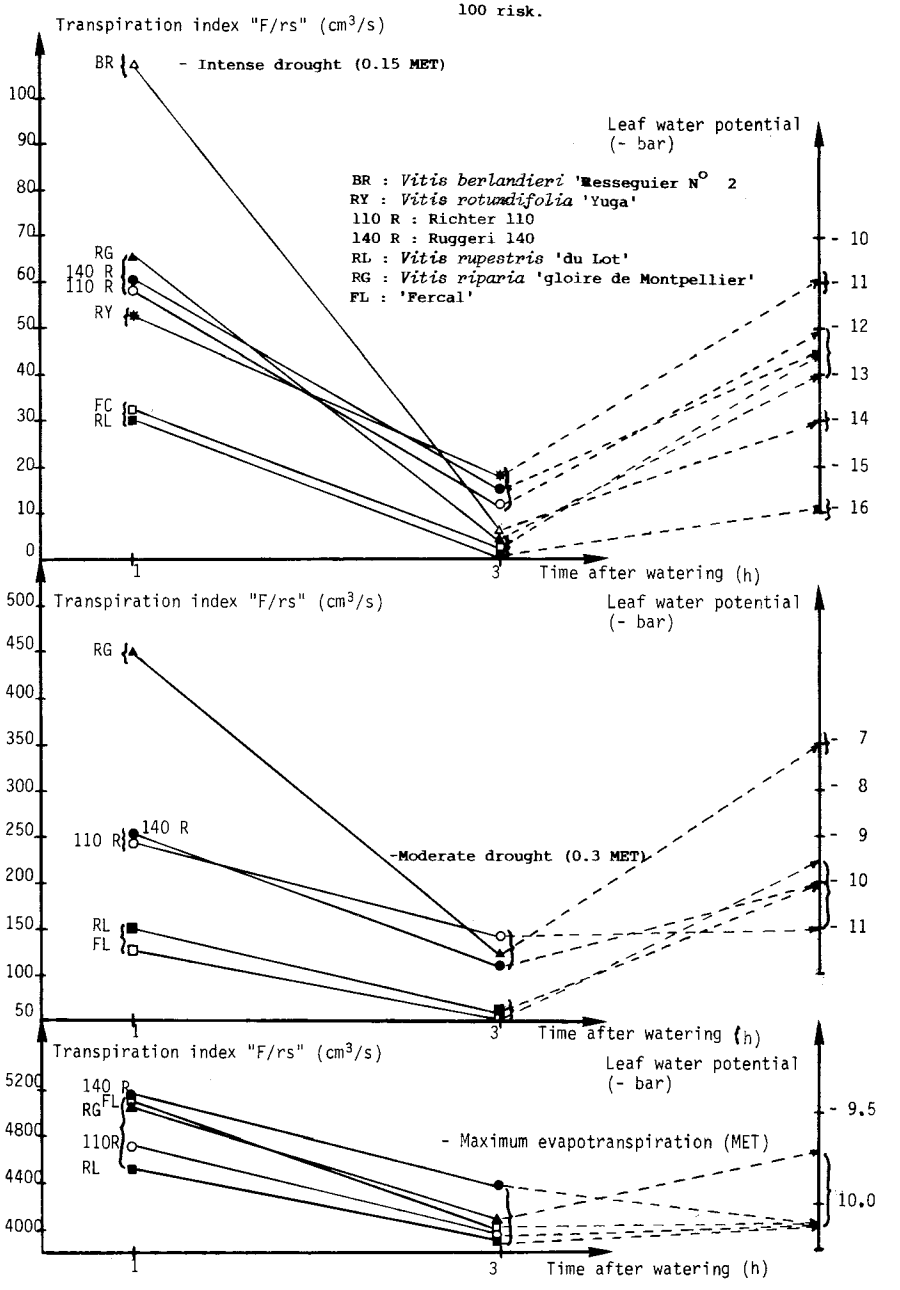

Fig. 5. Influence of the rootstock variety on the transpiration index and the leaf potential for different water regimes and times after watering.

TABLE 1. Influence of the rootstock and the water regime on leaf area (dm) mature shoot weight (g) and root fresh weight (g). A letter bound to a value indicates a statistical group at 5 p. 100 risk.

	Maximum évapotranspiration (MET)					0.30 MET					0.15 MET						
	RG	RL	FL	110R	140R	RG	RL	FL	110R	140R	RG	RL	FL	110R	140R	BR	RY
Leaf area (dm)	48.a	45.a	45.a	46.a	48.a	23.a	24.a	16.b	23.a	17.b	3.6a	3.3a	2.9a	3.2a	3.1a	8.5b	7.8b
Mature shoot weight (g)	87.5a	100.5a	95.5a	97.5a	86.5a	23.8a	25.0a	15.8b	26.8a	15.3b	1.6a	1.4a	1.7a	1.4a	2.1	5.3b	3.9b
Root fresh weight (g)	14.6a		22.4a	14.1a	14.5a	19.9a		26.8a	16.8a	25.0b	13.5a		15.6a	11.7a	14.6a	6.3b	

RG : *Vitis riparia* cv. 'Gloire de Montpellier'
RL : *Vitis rupestris* cv. 'du Lot'
FL : 'Fercal'

110 R : 110 Richter
140 R : 140 Ruggeri
BR : *Vitis berlandieri* cv. 'Resseguier No. 2'
RY : *Vitis rotundifolia* cv. 'Yuga'

3) Most susceptible genotypes : Fercal and *Rupestris* du Lot.

The correlation between leaf water potential (absolute value) and transpiration index equals - 0.74 for intense drought: some physiological differences occur between the transpiration flow and the remaining water. Hence, the leaf water potential presents some interest to complete the information provided by the transpiration index.

The analysis of the mature shoot weight and the fresh root weight indicates a negative correlation between these 2 parameters, the extreme situations being : Fercal with the best root weight and the lowest shoot weight and *Berlandieri* Resseguier No. 2 with the reverse results (Table 1).

These first observations suit, quite well, some classical field observations and seem promising in selecting rootstocks adapted to a given water regime, particularly to water stress.

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STATISTICAL ANALYSIS OF A WINE EVALUATION TEST WITH NEW VARIETIES IN THE UPPER MOSELLE WINE-GROWING DISTRICT

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ABSTRACT

Since 1974 trials have been conducted with "protected" varieties in the Upper Moselle vine growing area. Data on field performance were collected and the vinifications followed. Consumers and experts evaluated 15 protected varieties in comparison with the check variety White Elbling. The "Scoring Method" used was the DLG-20 Point Scale. The influences of blending, vintage year, location and winemaker were also analyzed statistically.

Since 1974 a Federal Research Project has been in progress in the Upper Moselle wine-growing district with the purpose of changing from the old White Elbling cultivar to new and qualitatively improved varieties. The main problems involved are, on the one hand, the testing for frost resistance and, on the other, the judging of the wine. This paper will comment on the findings of Wine Tasting Test III (WET) within Research Phase I. The results are intended to help determine the varieties for Research Phase II (1,3-7).

MATERIALS AND METHODS

In the wine evaluation, we have included wines from 16 varieties, 3 cellarmasters, 3 vintages, 4 sites and 6 blends. Details can be found in Tables 1 and 2.

The vinification took place based on the demands of the market according to the German wine laws. The must was fermented with selected yeast in 30 l glass-lined tanks. Where necessary, acidity was reduced and the wine improved with sugar. The residual sugar was adjusted by using the must of the particular variety.

A total of 200 testers participated in the wine tasting test, yet only 141 questionnaires could be interpreted. In the end 80 tasters were selected on the basis of dispersion as being reliable. Forty of these were experts and 40 consumers.

The wine evaluation was carried out by the scoring method according to the DLG-20 point system in Table 3. This scale is prescribed in the Federal Republic of Germany as the official judging score card for control of quality.

TABLE 1: Description of the sites, training methods and variety type

Characteristics	SITE I Oberbillig	II Wehr	III Wasserliesch	IV Nittel
Type of soil	Sandy loam	Sandy loam	Sandy loam	Sandy loam
Elevation	220 m	185 m	130 m	150 m
Slope	5%	0%	0%	8%
Exposition	N-W	Flat	Flat	S-W
Water supply	Medium	Medium	Medium poor	Good
Year of planting	1971	1972	1972	1971
Method of trial	Blocks (N = 4)	Blocks (N = 4)	Blocks (N = 4)	Blocks (N = 4)
Varieties	N = 10	N = 10	N = 10	N = 10
Rootstock	SO4	SO4	SO4	SO4
Row width	1.80 m	2.40 m	1.60 m	1.80 m
Vine distance	1.40 m_2	1.20 m_2	1.35 m_2	1.40 m_2
Spacing per vine	2.52 m^2	2.88 m^2	2.16 m^2	2.52 m^2

Neutral wines	Flowery wines	Aromatic wines
Faber	Albalonga	Comtessa
Forta	Fontanara	Kanzler
Fr 523-52	Freisamer	Ortega
Kerner	Gutenborner	Perle
White Elbling	Optima	Schönburger
	Regner	

TABLE 2 Grape and wine analyses of the tested wines.

No.	Vintage	Variety	Site	GRAPE				WINE				
				Yield (kg/g)	Must density (°Oe)	Total acidity (g/I)	Grape rot (%)	Alcohol content (g/I)	Resid. sugar (g/I)	Total acidity (g/I)	Sugar-free extract (g/I)	pH-value
1H	1975	Albalonga	Wehr	134	71	14.2	62	70.3	34.6	7.1	23.0	3.20
1L	1975	Albalonga	Wehr	134	71	14.2	62	67.8	23.7	6.9	24.3	3.20
1W	1975	Albalonga	Wehr	134	71	14.2	62	71.0	24.2	6.8	21.6	3.20
2	1975	Comtessa	Nittel	81	88	8.9	31	77.4	30.6	6.0	21.8	3.30
3	1975	Faber	Nittel	163	80	13.0	34	71.2	30.2	7.0	25.3	3.40
4	1975	Fontanara	Nittel	175	79	10.8	17	71.0	25.6	6.2	19.7	3.00
5	1975	Forta	Nittel	130	79	13.3	25	72.2	26.0	7.7	24.9	3.30
6	1975	Freisamer	Nittel	148	89	12.9	12	76.7	34.0	6.9	22.1	3.10
7	1975	Gutenborner	Nittel	164	83	8.3	25	72.4	36.2	7.2	20.9	3.00
8	1975	W. Elbling	Nittel	253	63	10.9	20	75.6	25.2	7.5	20.3	3.00
9	1975	Regner	Nittel	157	80	8.7	32	74.0	35.0	6.6	21.6	3.10
10	1975	Schönburger	Nittel	205	80	7.1	9	71.0	31.0	5.1	18.6	3.20
11	1975	Kanzler	Nittel	110	96	7.9	30	77.0	32.8	6.8	22.2	3.10
12	1975	Perle	Nittel	72	78	4.8	30	65.0	37.6	5.6	21.2	3.30
13	1975	Fr.523-52	Nittel	169	84	11.2	70	74.4	31.2	6.8	20.3	3.30
14	1976	Kerner	Oberbillig	180	83	8.4	12	78.8	19.3	7.2	20.0	2.90
15	1976	Kerner	Wehr	168	82	5.9	19	68.7	17.5	7.9	23.3	2.90
16	1976	Optima	Oberbillig	137	92	7.4	37	90.5	20.4	6.5	24.8	3.20
17	1976	Optima	Wasserliesch	149	90	7.0	51	85.3	19.5	5.7	28.0	3.50
18	1976	Optima	Wehr	92	88	7.6	32	80.9	21.5	6.7	24.4	3.20
19	1976	Ortega	Oberbillig	185	80	5.2	14	65.0	20.4	5.0	14.2	3.20
20	1976	Ortega	Wasserliesch	220	76	5.7	6	72.2	19.5	5.4	22.6	3.50
21	1976	Ortega	Wehr	191	80	5.9	29	68.9	21.8	6.0	20.5	3.10
22	1976	Fr.523-52	Nittel	141	94	8.0	70	82.5	41.8	6.2	23.1	3.20
23	1976	Perle	Nittel	133	91	4.9	30	78.8	40.0	5.5	22.6	3.10
24	1976	Kanzler	Nittel	201	99	6.4	30	88.7	39.2	5.0	22.2	3.20
25	1977	W. Elbling	Nittel	337	52	15.2	3	57.2	18.0	9.5	23.9	3.10
26	1977	Freisamer	Nittel	150	68	15.0	0	65.7	18.0	7.4	26.6	3.50
27	1977	Faber	Nittel	312	57	16.1	0	61.6	18.3	8.6	22.8	3.20
28	1977	Ortega	Nittel	243	66	11.6	0	77.0	17.6	6.4	27.6	3.60

TABLE 3. DLG wine-judging score card.

Feature	Evaluation	Points	Highest number of points per feature
Color	Pale or dark	0	2
	Bright yellow	1	
	Typical	2	
Clarity	Cloudy	0	2
	Bright	1	
	Crystal clear	2	
Odor	Unsound	0	4
	Lacking character	1	
	Clean	2	
	Fragrant-flowery	3	
	Fruit and perfume	4	
Taste	Unsound	0	12
	Sound	1-3	
	Clean and vinous	4-6	
	Matured and harmonious	7-9	
	Well-matured and noble	10-12	
Maximum attainable points per wine			20

Analysis of Variance followed by the Duncan Test (2) was used in the statistical analysis of the results.

RESULTS

Variety comparison: As far as the comparison of varieties is concerned, the results of Table 4 indicate that of the eight new types tested all of these in the case of the experts and six in the case of the consumers are significantly superior in quality to the White Elbling, with which they were compared. The maximum difference is 1.4 and 2.6 points, respectively.

Cellar-master comparison: Since, however, the results of Table 5 indicates a significant additional influence on the quality of the wine by the cellarmaster, the vinification would have to be carried out by several cellarmasters in the future. Only in this way can conclusive evidence be given on the superiority of the new varieties.

TABLE 4. Sensorial evaluation of 1975 wines of eight varieties and the comparative variety White Elbling, with significance data.

Experts (N=40)			Site: NITTEL								
Variety		x	9	8	7	6	5	4	3	2	1
Gut.	14.6	1	+	+	-	-	-	-	-	-	-
Fre.	14.6	2	+	+	-	-	-	-	-	-	
Fab.	14.6	3	+	+	-	-	-	-	-		
Com.	14.5	4	+	+	-	-	-	-			
Reg.	14.5	5	+	-	-	-	-				
Sch.	14.1	6	+	-	-	-					
Fon.	14.1	7	+	-	-						
For.	13.9	8	+	-							
W.El.	13.2	9	-								
Sch.	12.1	1	+	+	+	+	+	+	-	-	-
Gut.	12.0	2	+	+	+	+	+	-	-	-	
Reg.	11.4	3	+	+	+	-	-	-	-		
Fre.	11.3	4	+	+	+	-	-	-			
Com.	10.9	5	+	+	-	-	-				
Fab.	10.7	6	+	+	-	-					
For.	10.3	7	-	-	-						
Fon.	9.7	8	-	-							
W.El.	9.5	9	-								

Interaction: Tester groups x varieties (+++).

TABLE 5. Sensorial evaluation of 1975 wines of the Albalonga variety vinified by three different cellarmasters with significance data.

Testers (N = 80)			Site: NITTEL
Variety	$\bar{x}$		3 2 1
Albalonga (Cellar W)	12.6	1	+ - -
Albalonga (Cellar L)	12.4	2	+ -
Albalonga (Cellar H)	11.5	3	-

Interaction: Tester groups x cellarmasters (-).

Vintage comparison: The individual vintages at our disposal for tasting do not include any possible mistake in this respect. As the result of Table 6 indicates, the range of the wines is the same in both years even though there is a significant difference in level. It is to be assumed, however, that in years with vintages inferior to those of 1975 and 1976 the quality pattern of these wines would be subject to varying influence. For this reason, it is planned to include more than two vintages in the evaluation of wines of different varieties.

TABLE 6. Sensorial evaluation of wines of two vintages (1975 and 1976) with three new varieties with significance data.

Testers (N = 80)			Site: NITTEL
Variety Year	$\bar{x}$		6 5 4 3 2 1
'76 Kanzler	16.0	1	+ + + + - -
'76 Perle	15.7	2	+ + + - -
'76 Fr 523-52	15.4	3	+ + + -
'75 Kanzler	14.5	4	+ - -
'75 Perle	14.3	5	+ -
'75 Fr 523-52	13.4	6	-

Interaction: Tester groups x vintages (-).
Interaction: Varieties x vintages (-).

Site comparison: In the variety experiments, wine quality

and aroma are also greatly dependent on the site. Thus we see from the results of Table 7 that the wine of the Ortega variety from the Wasserliesch site comes out significantly better than that from the Oberbillig site. Existing interactions will be discussed later.

TABLE 7. Sensorial evaluation of 1976 wines of new varieties on three sites with significance data.

Testers (N = 80) Site: N = 3

Variety	Site	$\bar{x}$		8	7	6	5	4	3	2	1
Ortega	Wasserliesch	12.2	1	+	+	+	-	-	-	-	-
Optima	Wehr	12.2	2	+	+	+	-	-	-	-	
Optima	Wasserliesch	12.1	3	+	+	-	-	-	-		
Optima	Oberbillig	11.9	4	+	+	-	-	-			
Ortega	Wehr	11.9	5	+	+	-	-				
Ortega	Oberbillig	11.6	6	+	+	-					
Kerner	Oberbillig	10.4	7	+	-						
Kerner	Wehr	9.8	8	-							

Interaction: Tester groups x sites (-).
Interaction: Varieties x sites (+).

Blend comparison: Owing to the poor frost resistance of the new varieties, which according to the site constitutes a risk for general cultivation, the wines were tested for their unblended vinification as well as for their blending potential. Two blending ratios of 15 and 35% were applied. From the results of Table 8, we may conclude that the blends with the Faber, Freisamer and Ortega varieties and with the White Elbling are significantly superior to the must-specific vinified Elbling wine in 33 out of 36 cases. This indicates that blending possibilities with other varieties and different blending ratios are also worth consideration.

Consumer-expert comparison: It is not without relevance to our further investigations to establish the degree to which the judgments of the experts differ from those of the consumers. From the statistical analyses it emerges that in the variety comparison, as well as in those of the cellarmasters, vintages, sites and blends, the judgments of the experts are in each case significantly different from those of the ordinary consumer. The higher average number of points was consistently awarded by the experts: in the variety comparison by 3.3 points, in the cellarmaster comparison by 2.1 points, in the vintage comparison by 1.5 points, in the site comparison by 4.7 points and in the blend comparison by 4.4 points. Thus it will be necessary in

TABLE 8. Sensoric evaluation of 1977 blending wines and the compared variety White Elbling with significance data.

Experts (N = 40)			Site: NITTEL								Consumers (N = 40)		Site: NITTEL							
Variant		x	9	8	7	6	5	4	3	2	1	Variant	x	9	8	7	6	5	4	3	2	1
W.EL + 15 % FRE		13.3	1	+	+	+	+	+	+	-	-	W.EL + 15 % FRE	9.2	1	+	+	+	+	+	+	-	-
W.EL + 35 % ORT		13.1	2	+	+	+	+	-	-			W.EL + 15 % ORT	9.2	2	+	+	+	+	+	-	-	
W.EL + 15 % FAB		12.8	3	+	+	+	-	-	-			W.EL + 35 % ORT	8.9	3	+	+	+	+	+	-		
W.EL + 15 % ORT		12.7	4	+	+	+	-	-	-			W.EL + 35 % FAB	8.1	4	+	+	+	+	-	-		
W.EL + 35 % FRE		12.5	5	+	+	+	-	-				W.EL + 15 % FAB	8.0	5	+	+	+	-	-			
W.EL + 35 % FAB		12.4	6	+	+	+	-					W.EL + 35 % FRE	7.6	6	-	-	-	-				
W.EL		11.7	7	-	-	-						W.EL	7.0	8	-	-						
W.EL		11.7	8	-	-							W.EL	7.0	8	-	-						
W.EL		11.6	9	-								W.EL	7.0	9	-							

Interaction: Tester-groups x Blends (+)
Interaction: Varieties x Blends (+++), Experts: (+) Consumers: (++)

further wine tasting tests to coordinate the number of experts and consumers in the variety comparisons in such a way as to preserve balance. This is equally important in the calculation of the interactions.

Interactions: The relation between varieties and tester groups as well as between blends and tester groups is significant. In contrast no interactions could be proven between cellarmasters, vintages and sites and tester groups. Since, as a rule, consumers tend to attach more importance to aroma and residual sugar content whereas experts seem to prefer the more neutral type and are not so greatly influenced by the residual sugar, it is not surprising that the two tester groups sometimes diverge in their judgment (Figs. 1 and 2). Schönburger is a typical example of an aromatic variety, Faber of a neutral one. The findings, therefore, make it necessary to include consumers, since these reflect the market trends. At the same time it is not advisable to exclude experts, as we wish to avoid decisive deviations from the regional type, which is neutral.

Yet the aforementioned positive reaction of consumers to aromatic wines must not be overestimated. When we examine the interaction between blends with experts and consumers, respectively, we perceive that still other unknown factors are at work.

More important for the choice of experimental methods is the fact that we found a significant interaction between varieties and sites as well as blending ratios. That could not be established with regard to vintages. This may well be due to the fact that we tested only two vintages, and both good ones. At any rate it is clear that each variety has one specific optimal site. Equally they are differentiated by their blending potential. This means that the blending ratios in the other different varieties result in varying degrees of improvement. This factor complicates and extends the entire research procedures.

Regression analysis: We refrained from subjecting our data to regression analysis, since in the case of earlier, extensive analyses, it was not possible to prove any relevant relations. For this reason we restricted ourselves only to the relation of most interest to us, namely, that between wine evaluation and residual sugar. This relation is linear and significant both in the case of the consumers and of the experts. Every increase of the residual sugar by 10 g/l leads to an addition of 1.9 points with consumers but only 0.9 with experts. If, therefore, the sensory evaluation is to give exact recommendations, it will be essential that all the wines be tested with the same residual sugar content. Where larger quantities of wine are available for tasting, the several vinification variants and vinification in several cellars ought to be involved. At any rate, these considerations will have to be taken into account in Research Phases II and III. In the case of only one experimental vinification variant being at our disposal, one ought to select that which comes nearest to the market requirements. Owing to insufficient quantities of must, we had to be guided by this consideration in Research Phase I.

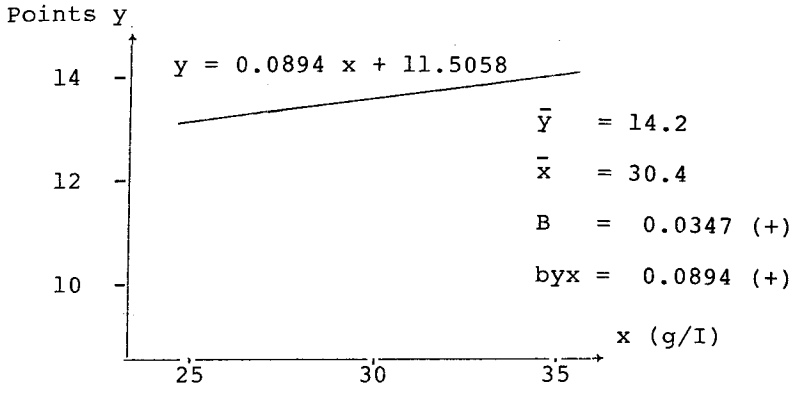

Fig. 1. Wine evaluation (y) as a function of the residual sugar content (x) by experts.

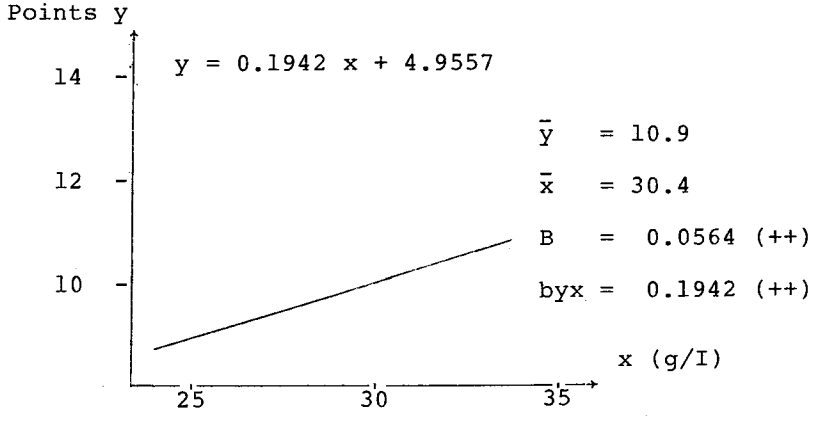

Fig. 2. Wine evaluation (y) as a function of the residual sugar content (x) by consumers.

CONCLUSION

In conclusion we may say that on the basis of the results of the sensorial wine tests using a scoring method there are significant differences between varieties, cellarmasters, vintages, sites and blends as well as taster groups. Futhermore, the latter do not evaluate the varieties and blending wines in the same way as the regression analyses would indicate, especially the residual sugar content. There are also interactions between varieties, sites and blending ratios. Since the commercial life of the vine may be 25 years or more, we cannot afford to make mistakes in recommendations; it is imperative to include additional factors in further research. This means that the tests must become more intensive which will necessitate an extension of the test areas and of vinification. Above all, more vinification variants are required. A larger number of participants should also be employed as the maturation and aging of the wine have to be tested. Sensory evaluations are indispensable in spite of all the difficulties. Owing to the great investment of money and work required for the vinification and testing of the wines, however, only worthwhile subjects should be included.

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SECTION III

BREEDING FOR ADAPTATION TO ENVIRONMENTAL STRESS

BREEDING FOR BERRY-SPLIT RESISTANCE IN A PURE VITIS VINIFERA CONTEXT UNDER SUMMER RAINFALL CONDITIONS

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ABSTRACT

In most countries where table grape breeding is undertaken in summer rainfall areas, *Vitis* species such as *Vitis labrusca, rotundifolia, simpsonii, smalliana,* etc., which usually possess disease and berry-split resistance genotypes, are mainly used in breeding programs.

In the Republic of South Africa, *vinifera* cultivars have been used as parents to develop crack-resistant cultivars for the summer rainfall area. Results of the breeding program undertaken at the Horticultural Research Institute near Pretoria have proved that 'Barlinka' is an excellent parent as a source of crack-resistance and fruit quality.

Table grapes are the second important deciduous fruit crop grown in South Africa. Only apples are more important. During the past decade the average annual production has been 101,100 tons (2). It is estimated that during the past few seasons, table grapes provided these growers with a yearly net income of R25 million. This figure includes grapes exported, sold on the local markets and used for drying purposes.

Of the more than 7,000 hectares planted in the Republic of South Africa, about nine tenths are planted in the Western Cape Province; and the rest is planted in the summer rainfall area of Barkly West, Groblersdal, Lydenburg, Warmbaths, Nylstroom and Pretoria.

The Mediterranean climate of the Western Cape Province with its wet, cold winters and hot, dry summers is ideally suited for the production of good quality table grapes. This favorable climate and expertise of grape growers has enabled them to export 28,401 tons (2) of grapes annually during the past decade. Although this export tonnage is a mere 0.5% of the world total production, this nevertheless represents 65% of the grapes exported to Europe and Great Britain from the Southern Hemisphere countries of Australia, Argentina, Chile and the Replublic of South Africa.

Until fairly recently 11 cultivars were grown in the Cape Province for export purposes. These were all *Vitis vinifera* derivatives and consisted of the following cultivars, viz., Alphonse Lavallée, Almeria, Barlinka, Golden Hill, New Cross, Olivette,

Prune de Cazouls, Queen of the Vineyards, Red Emperor, Salba and Waltham Cross. Although 11 cultivars are grown, it is interesting to note that approximately 85% of the volume exported consists of Barlinka (51%), Waltham Cross (22%) and Alphonse Lavallée (12%) (1). Most of the *Vitis vinifera* cultivars grown in the Western Cape are poorly adapted in the summer rainfall area. This is due to the fact that in this area most of the rain (this varies annually from 400 mm to 1,100 mm) occurs during the months of October to March.

Because rain occurs mainly during the growing season and in particular during the ripening period of the grapes, producers in the summer rainfall area face two major problems. These are: 1) Leaf and berry diseases caused by oidium *(Oidium tuckeri)*, anthracnose *(Sphaceloma ampelinum)* and downy mildew *(Plasmopara viticola)* and 2) Splitting of berries.

Although leaf and berry diseases can be controlled satisfactorily, splitting of berries remains a serious problem in the summer rainfall area. As a result of the latter problem, a breeding program was started at the Horticultural Research Insitute at Roodeplaat in 1956 to develop cultivars which would be more adapted to the adverse summer rainfall conditions. This paper deals with the results that have been achieved in overcoming this problem.

RESULTS AND DISCUSSION

In order to determine the adaptability of grape cultivars in the summer rainfall area, 69 *vinifera* and 14 labrusca-type cultivars were planted at Roodeplaat in 1949. The results of this evaluation trial proved that 10 of the 14 labrusca-type cultivars were highly resistant to diseases and berry split, while only 9 *vinifera* cultivars possessed good berry-split resistance. Of these 9 *vinifera* cultivars only Barlinka, Waltham Cross and Cereza possess good eating quality, but because earliness is an important factor in the summer rainfall area, these three cultivars are not extensively grown. The two cultivars generally grown are Queen of the Vineyard and Alphonse Lavallée. Both these cultivars are extremely susceptible to berry split when rains occur just prior to or during the harvesting period.

In most countries where table grapes are bred for summer rainfall conditions (e.g., in the USA) *Vitis* species such as *labrusca, rotundifolia, simpsonii, smalliana, inter alia* are used as parents. These species possess disease and berry-split resistance and are, therefore, well adapted to moist summer rainfall conditions. Likewise, four *Vitis labrusca* cultivars which are well adapted in the summer rainfall area were used in most of the crosses made at Roodeplaat. One of the *labrusca* cvs., viz., Isabella, which is grown in most gardens in the summer rainfall area, was hybridized with 28 other *Vitis vinifera* cultivars. After evaluating 2160 *vinifera* x labrusca-type seedlings, it was clear that most of the progeny were resistant to disease and berry split; but these seedlings seldom produced clusters larger than 300 g, and the average berry weight seldom exceeded 5 g. Most seedlings also possessed mucilagenous pulp, a labrusca or foxy-flavor (which is unacceptable for export purposes), a tough skin and

large seeds.

In 1964 it was decided to use *vinifera* cultivars as parents and to establish whether it would be possible to select seedlings possessing good fruit quality such as good cluster size, desirable compactness, large firm berries, good pedicel adherence as well as being more resistant to berry split than existing cultivars.

During the next few years 18 *vinifera* cultivars (Table 1) were used in 69 combinations and 10,913 seedlings were evaluated. The crack-tolerant cultivars Barlinka, Waltham Cross, Cereza, Raisin blanc, Olivette blanche, Muscat Hamburg and Muscat of Alexandria were mainly used as parents. Seedlings which produced small berries (4.5g) or small clusters (300g), lacked vigor or which were highly susceptible to the prevailing diseases and showed signs of cracking were immediately discarded. Of the 128 seedlings selected for a more critical evaluation, 27 selections were progeny of Barlinka.

TABLE 1

Vitis vinifera cultivars used as parents: Roodeplaat 1964-76.

Alphonse Lavallée	* Duc de Magenta	* Pirovano No.15
* Barlinka	* Keuka	Queen/Vineyard
* Barbarossa	* Muscat Hamburg	* Raisin blanc
Black Monukka	* Muscat of Alexandria	Sultanina
Black Spanish	Muscat Ottonel	* Waltham Cross
* Cereza	* Olivette blanche	
Crystal		

* = Good split-resistance.

To determine the split-resistance of selections and cultivars, the following procedure was used: Selections or cultivars are considered to be ripe when the total soluble solid content reaches 16°Brix. The clusters are then allowed to hang for another four weeks, and then the amount of pedicel cracking or splitting is determined. Two samples of 100 berries per sample are examined. The individual berries plus the pedicel or stem attachment are severed from the peduncle by means of a scissor and then examined for splitting.

During the past five years, the summers of 1975-76, 1977-78 and 1979-80 have been wetter than usual, thus enabling us to evaluate the selections under exceptionally adverse conditions. For example, the average annual rainfall for Roodeplaat is 715 mm, but during 1975-76, 1053 mm was recorded. During 1977-78, 261 mm was recorded in January alone. Notwithstanding the unfavorable climatic conditions, some selections proved to be very resistant to berry splitting. These results are presented in Table 2.

TABLE 2 : Berry-crack resistance of grape cultivars and selections at Roodeplaat (Summer Rainfall Area).

1	2	3	4				5				
		Berry					Rain (mm)				
Cultivar/selection	Ripening date (16°B)	Weight (g)	Crack (%) 1976/77	1977/78	1978/79	1979/80	1976/77	1977/78	1978/79	1979/80	Average (25 years)
a. Pirobella	Dec.14	3.6	0	0	0	2	Oct. 73	142	73	70	74
b. Bien Donne	24	4.2	1	5	0	1					
c. Queen/Vineyard	28	5.8	19	100	16	23	Nov.147	68	28	140	123
d. BAR/Q69-699	Jan. 3	6.5	1	3	1	7	Dec.124	75	23	65	115
e. CER/PIR 70-2201	10	9.6	-	5	1	9					
f. K/WC 70-4775	21	5.5	2	0	0	0	Jan. 67	261	79	203	135
g. BAR/BB 70-763	27	8.9	-	0	1	2					
h. Alphonse Lavallée	Feb.12	7.8	48	100	44	54	Feb. 74	189	49	212	89
i. AL/BAR 70-273	17	7.7	2	0	2	4					
j. Barlinka	Mar.13	6.9	2	0	8	0	Mar.125	67	124	49	67

Average

Latitude S. 25° 35'

Longitude E. 28° 21'

Altitude 1 164 m

Rainfall per annum, 715 mm

Monthly daily max. (Oct.-Mar.), 29°C

Monthly daily min. (June-July), 2°C

TABLE 3: Evaluation results of *Vitis vinifera* Grape Seedlings 1964-1976.

Cross	Cultivars	Combinations	Seedlings evaluated	Seedlings selected Initial	Final
V.V. x V.V.[a]	18	56	10,913	101 (0.9%)	3 (0.028%)
Bar x V.V.	13	13	1,837	27 (1.5%)	4 (0.217%)

[a]V.V. = *Vitis vinifera*

Bar = Barlinka

Our latest evaluation results indicate that most of the promising selections which have good fruit quality and split resistance usuaily have Barlinka as one parent. When comparing the success achieved, the results indicate (Table 3) that the chances of breeding quality and split-resistance cultivars in a pure *vinifera* context is nearly eight times greater if Barlinka is used as one of the parents. Although Barlinka is a late-ripening cultivar, it is interesting to note that many of the Barlinka progeny ripen in January even when hybridized with mid-season cultivars such as Waltham Cross.

In view of the encouraging results obtained when breeding in a pure *vinifera* context, a larger number of crosses involving berry-split tolerant cultivars as parents have now been made. During the following few years, six to eight thousand seedlings will be evaluated for fruit quality and berry-crack resistance. It is expected that even larger numbers of seedlings will be found that will be more adapted to the adverse climatic conditions of the summer rainfall area. Some of these will possibly be the new crack-resistant cultivars of tomorrow. Not only will they make an important contribution to the economy of the country, but they should also lay a sound foundation on which a healthy and vigorous grape industry in the summer rainfall area can be established. With the necessary expertise, grape producers in this area can even set their sights on the early export market and thus contribute towards the export effort of our country.

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BREEDING OF INTERSPECIFIC GRAPEVINE VARIETIES

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ABSTRACT

The aim is to create varieties of great productivity, good quality, high cold hardiness and fungus disease resistance. Asiatic *V. amurensis* and American species are used as donors of resistance. Up to now we have tested about 4000 seedlings. Methods to speed up seedling development have been used so that fruiting started in the second or third year. A few clones which possess the desired characteristics have been saved for trial.

It has been proven, with a great number of examples in the last 10 years, that the quality of grapes and the characteristics of resistance could be inherited independently (1,2,3,4,5,6,7,8,9).

The aim of our work is breeding varieties with high productivity, good quality, earlier maturity, high cold hardiness and resistance to fungus diseases. Besides wine-grape varieties we have been concerned with table grapes, juice grapes and grapes for wine distillate production.

METHODOLOGY

The breeding process included four stages: 1) Screening of varieties originated from interspecific hybridization, 2) Inter-crossing varieties selected from three parental groups, 3) Growing of seedlings, and 4) Evaluation and selection of seedlings.

Each of these stages has its specific methodology, partly adopted from other breeders who have a long tradition in vine selection (notably West Germany, Hungary, Soviet Union), and partly originally developed in our Institute. The major part of the investigation has been performed in the experimental station of the Institute in Sremski Karlovici.

RESULTS

Screening of varieties: In order to choose the best parent varieties, a survey was made to locate and collect the most promising resistant hybrids. It was possible to observe their performance in our collection, which had about 40 such hybrids established. From these, the best performers under local conditions were used as parents. In addition, it was possible to use the results of previous hybridizations in our project.

Hybridization: Since the varieties eventually selected fell into one of three groups, the crossing combinations were

systematized (Table 1).

TABLE 1. Crossing combinations.

Crossing type	Number of combination per year					Total
	1975	1976	1977	1978	1979	
A x C	8	5	9	2	3	27
B x C	-	3	9	-	1	13
(A x C) x (A x C) or (A x C) x A	-	-	-	1	3	4
B x B	-	-	-	5	3	8
A x B or (A x C) x B	-	-	-	3	3	6

A = varieties originated from *V. amurensis*.
B = varieties originated from American species.
C = pure *V. vinifera* varieties.

Group A varieties had resistance derived from *V. amurensis:*

Zarja severa, Saperavi severni (J. I. Potapenko), Negru de Jaloveni (N.I. Guzun)--created in the Soviet Union;

Kunbarát and Kunleány (I. Tamasi, I. Koleda)--created in Hungary;

Own selections: SK 76-3|3, SK 76-3|1, SK 76-1|1, SK 77-10|34, SK 77-4|27, SK 77-12|6, SK 77-2|28.

Group B included varieties that derived their resistance from native American species:

SV 12-375, SV 20-347, SV 20-473, SV 20-366 (Seyve-Villard), S 7053 (Seibel)--created in France;

Zalagyöngye (J. Csizmazia, L. Beleznai)--created in Hungary;

Moldova, Strashenski (M. S. Zsuravel), Ljana, Dojna, M. dnjestrovski, II 65-89, V 68-91 (D. D. Verderevski, K. A. Vojtovics), Vierul 59 (N. I. Guzun)--created in the Soviet Union.

Group C included only *vinifera* varieties, intended to contribute superior fruit quality.

The varieties or selections from each group used in the breeding program were the following:

White wine varieties

Pinot gris	Muscat Ottonel
Traminer	Zupljanka
Italian Riesling	Jubileum 75

Red wine varieties

Pinot noir	Blaufränkisch
Merlot	Portugieser
Cs 162 (P. Kozma)	

Table grape varieties

Muscat Hambourg	Drenak blue and red
Afuz ali	Cardinal
Irsai Oliver	Italia
Nimrang	Queen of Vineyards

Growing of seedlings: We developed a special treatment for growing seedlings which shortened the juvenile stage to half compared to the usual one. This procedure has been based on experiences obtained in West Germany (Institut für Rebenzüchtung und Rebenveredlung, Geisenheim) and Hungary (Eger and Kecskemét). Most seedlings have been bearing the first crop in the second year but almost all of them in the third year.

Germination of the seed was performed in an incubator at the beginning of February. Planting of germinated seeds was done in Jiffy 7 briquettes in a glasshouse. In the two to three leaf stage the seedlings were transplanted to Jiffy pots, with a mixture of peat and perlite. In the second half of May, the seedlings were planted in a permanent place in the field at a distance of 3 x 1 m and cultivated as a normal young vineyard. By the end of the first year, the seedlings had developed a few meters long. So far we have established about 4000 seedlings.

Evaluation and selection of seedlings: Although the first crop was relatively small (up to 2 kg per plant), it offered some important data which characterized the value of each seedling and even of the crossing combination, e.g., we could evaluate the type of the flower to get an impression of future fruit bearing, to see the time of grape ripening, the structure of cluster, color, size, shape and uniformity of the berries, to perform organoleptic tests, to determine the sugar and acid content, to estimate the sensitivity of grapes to *Botrytis*, and even produce some wine.

Because of the strong vegetative growth of the seedlings, their resistance to low temperature could be rated in the second year. In the third year, the pruning system could be formed to get a normal grape yield.

Thus far, we have selected a number of seedlings which have some promising characteristics.

SK 76 - 3/3 (Irsai Oliver x Kunleány): The preliminary test showed that the seedling SK 7/6 - 3/3 had higher sugar content,

higher yield, better resistance to *Botrytis* and low temperature, and it had earlier maturity than the check variety (Italian Riesling). It had large loose clusters (300 g) with golden-yellow berries. The wine was alcoholic, well balanced, neutral but *vinifera* type in taste and aroma.

SK 76 - 4/9 (Nimrang x Kunleány): This selection ripened by the end of September and was extremely high yielding. The must and wine quality proved to be comparable with those of Italian Riesling. The cluster was very large (580 g). The berries were large, greenish-pink and resistant to *Botrytis*. The vine was vigorous and had good tolerance to low temperature. Bud burst in spring was rather late.

CONCLUSION

The results obtained, thus far, have been promising so that in the near future some new varieties can be expected which will insure grape production under temperate continental climates and have the necessary resistance.

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DROUGHT RESISTANCE OF SOME VITIS SPECIES AND CULTIVARS

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ABSTRACT

A prerequisite for breeding drought resistant grapevines is to have a selection method which comprises as many as possible of the attributes which are responsible for the complex characteristic "drought resistance." In the first step some morphological and physiological features of drought resistance were analyzed using *Vitis* species and cultivars. Measurements of the shoot:root ratio, the number of stoma, the degree of succulence, the water conduction, the water storage and the diffusion resistance under normal conditions and under water stress were carried out with varieties whose degree of resistance is already known. The degree of succulence, the leaf water potential (using excised leaves) and the ratio Δ diffuse resistance to Δ water potential proved to be practical and useful methods, even to test a large number of seedlings whose resistance is unknown. But as drought resistance in viticulture entails resistance against losses of yield and quality, in the second step these cultivar-specific factors have to be taken into account.

One of the aims of grape breeding is to produce cultivars adapted to environmental changes. "Adapted" means the ability of cultivars (1) to survive and (2) to produce constant high yields and qualities even under suboptimal conditions (14). As water is the main factor limiting yield and quality in viticulture in arid zones and even in some humid areas in the world and as irrigation in many regions provokes economic, ecological and technical problems, the importance of breeding drought-resistant grapevines is generally accepted.

In a series of experiments Geisler (5,6) in Germany and Fregoni et al. (4) in Italy studied morphological and physiological properties of grapevines, especially of rootstocks, during and after water stress.

In our experiments we tried to find a selection method which takes into consideration as many parameters as possible of the complex attribute "drought resistance". Thus in the first step some aspects of drought resistance were analyzed using "a priori" resistant and sensitive cultivars. In the second step physiologically relevant and practical methods for predicting the degree of drought resistance of a newbred have to be chosen; the reliability of which has to be examined in a third step by tests under field conditions with regard to yield and quality.

In this paper we report on the first step, i.e., the analysis of drought-resistant parameters of ungrafted cultivars; the modifying effect of rootstocks will be examined later.

RESULTS

Degree of succulence: According to Larcher (10) the degree of succulence of leaves is given by the water content at saturation related to the surface area of the leaf; this quotient characterizes the water storage capacity of the leaves. Resistant cultivars like Riesling, Comtessa and Optima have a significantly higher degree of succulence than sensitive cultivars like SO4 or Müller-Thurgau (Table 1). By measurements of this morphological character, resistant and sensitive cultivars can be selected rapidly. Although measurements of the degree of succulence do not take into account the adaptive potential of a cultivar, this method may be useful as a pretest.

TABLE 1. Degree of succulence of fully expanded leaves of glasshouse-grown grapevines.

BFAR Dg	Degree of succulence	1980
Cultivar	Water content under conditions of saturation per leaf area ($g.dm^{-2}$)[a]	
Riesling	0.92 ($\pm$ 0.04)	
Comtessa	0.93 ($\pm$ 0.06)	
Optima	0.93 ($\pm$ 0.05)	
Müller-Thurgau	0.85 ($\pm$ 0.01)	
SO 4 (*berlandieri* x *riparia*)	0.82 ($\pm$ 0.03)	

[a]Mean values of 9 replicates

Stomatal frequency: Morphological adaptation of leaves is indicated by the fact that mesophytes under dry air and soil conditions develop a greater number of stomata per unit leaf area than under humid conditions, e.g., in a glasshouse (13). Table 2 indicates varietal differences of stomatal frequency within the groups of the field grown and the glasshouse grown vines. The modification of stomatal frequency by environmental conditions gives additional information about the ability of adaptation of a cultivar to dry and humid conditions. The results indicate that Riesling has the highest modification rate or ability for adaptation and Silvaner the lowest.

Stomatal behavior and water potential: While the importance

TABLE 2. Stomatal frequency of leaves from field-grown and glasshouse-grown grapevines.

BFAR Dg	Stomatal frequency			1980
Cultivar	Number of stomata per mm^2		difference	
	Field grown	Glasshouse grown	Abs.	Rel. (%)
Riesling	209.9	157.2	52.7	25.22
Müller-Thurgau	244.8	208.3	36.5	14.91
Silvaner	185.1	172.2	12.9	6.97
Bacchus	236.4	213.8	22.6	9.56

Mean values of 60 to 360 determinations, significant at the 1% level.

of stomatal frequency as an indicator for drought resistance and the modification rate of stomatal frequency are still under discussion (9,12), stomatal behavior is generally considered to be a useful parameter (7,8). Stomata that are sensitive to water stress are effective in reducing water loss and in maintaining high tissue water content (3). This positive effect conflicts with the negative effect that gas exchange and photosynthesis are reduced the more stomata are closed (11). Measurements of stomatal behavior during a short-term stress of 8 hours with a drop of the available soil water of 2.5% per hour led to a marked increase of stomatal resistance in Riesling and to a distinctly lower increase in Müller-Thurgau and *V. rupestris* (Fig. 1). This result indicates a higher stomatal sensitivity of Riesling compared to Müller-Thurgau and *V. rupestris*. During a prolonged stress of 72 hours with a drop of the available soil water of 0.27% per hour, stomatal resistance increased again in Riesling and Müller-Thurgau. It is suggested that in Riesling the smaller reduction in transpiration, which corresponds well to the higher changes of water potential, enables these plants to a prolonged gas exchange and photosynthesis under stress conditions. However, in resistant cultivars this prolonged gas exchange and transpiration must be accompanied by plant chracteristics which guarantee a sufficient water supply to the leaves. This will be discussed in the next chapter. The different behavior of both cultivars under water stress is expressed by the quotient of Δ stomatal resistance and water potential (Table 3). The relationship between stomatal resistance and CO_2-uptake for Riesling and Müller-Thurgau is shown in Fig. 2, indicating a higher CO_2-uptake at a given degree of stomatal resistance in Riesling. These results were obtained using unhardened plants; the modifying effect of hardening will be examined later.

Recovery from water stress: The resistance to water flow between root and leaf, the so-called "recovery resistance" (2) was studied leaving Bacchus, Riesling and Müller-Thurgau plants unirrigated for 6 days and selecting plants with a leaf water potential of -10 to -11 bars. After rewatering, the water potential increased rapidly in Bacchus and Riesling plants. Here a recovery was recorded after about 65 minutes, while Müller-Thurgau had recovered about 35 minutes later, i.e., after 100 minutes (Fig. 3). This result seems to indicate a rapid water uptake and a high hydraulic conductivity to the leaves of Riesling, a prerequisite for the above-mentioned prolonged transpiration under stress.

Transpiration coefficient and water use efficiency: In a test under controlled climatic conditions, the individual water consumption of cuttings from the *V. vinifera* cultivars Riesling, Bacchus, Forta, Müller-Thurgau and Silvaner was measured and related to the dry matter produced. This quotient, the so-called transpiration coefficient, differed greatly between the 5 cultivars confirming the water-saving dry matter production of Riesling compared to Müller-Thurgau and Silvaner. Corresponding to that the water use efficiency (WUE), i.e., dry matter production related to the water consumed (1) was high in Riesling and low in Müller-Thurgau and Silvaner (Table 4).

Water potential of excised leaves: When leaves of field grown vines were cut off, leaf water potential decreased, the rate of

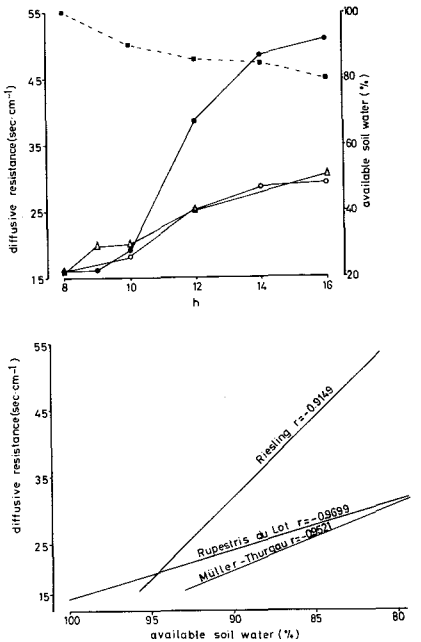

Fig. 1. Effects of a short-term water stress on the diffusive resistance of leaves of ● Riesling, o Müller-Thurgau and Δ Rupestris du Lot. ■ available soil water.

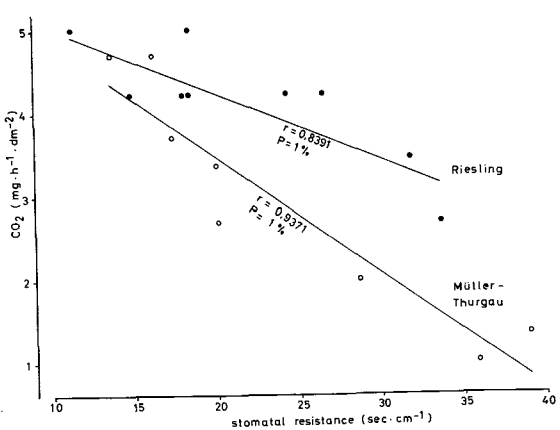

Fig. 2. Relationship between stomatal resistance and Co_2- during water stress.

TABLE 3. Relative changes of leaf water potential and stomatal resistance during a three day stress.

BFAR Dg	Changes of leaf water potential and stomatal resistance during stress			1980
Duration of stress (d)	Cultivar	water potential (bar)	stomatal resistance ($sec.cm^{-1}$)	stomatal resistance / water potential
1	Müller-Thurgau	0.52	0.5	0.96
	Riesling	1.33	0.4	0.30
2	Müller-Thurgau	0.50	3.1	6.14
	Riesling	0.83	1.4	1.69
3	Müller-Thurgau	0.70	8.0	11.43
	Riesling	1.95	4.0	2.05

TABLE 4. Transpiration coefficient and water use efficiency (WUE) of vine cuttings.

BFAR DG	Transpiration coefficient and water use efficiency[a]	1980
Cultivar	Transpiration coefficient ($ml \cdot g^{-1}$ dry matter)	Water use efficiency (mg dry matter $\cdot ml^{-1}$)
Riesling	87.7	11.7
Bacchus	94.9	10.7
Forta	118.9	8.7
Müller-Thurgau	132.9	7.7
Silvaner	137.9	7.9

[a]average of three assays with 12 cuttings each

decrease depending mainly on leaf water saturation deficit, temperature, air humidity and wind velocity. To avoid uncontrolled environmental effects and to standardize leaf water saturation deficit the leaves were stored 4 hours in chambers at 100% relative air humidity. Under controlled light, temperature and air humidity the leaves were then laid upside down on filter papers. After 10, 20 and 30 minutes the water potential of the leaves was measured as described earlier (3). The results show that the mean values obtained after 10, 20 and 30 minutes at 40% relative humidity differ over a wide range from -6.9 bars in *V. cinerea* to -14.7 bars in *V. rupestris* 'du Lot,' the resistant species having a smaller decrease of the mean water potential than the sensitive species. As expected, the mean water potential at 80% relative humidity, in general, decreased less than that at 40% (Table 5). By the measurements of the mean leaf water potential of 32 cultivars, a provisional and arbitrary classification in three categories was performed which indicates that besides some Italian cultivars Kerner and Riesling showed the highest resistance to water loss, while cultivars like Chasselas, Grenache blanc and Silvaner showed very low resistance to water loss (Table 6).

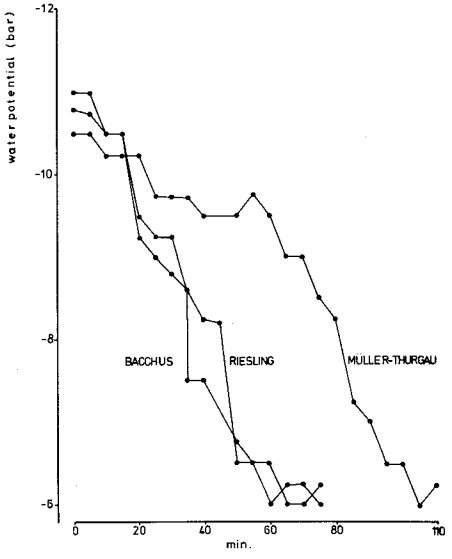

Fig. 3. Recovery resistance. Leaf water potential after irrigation (at 0 min.).

TABLE 5. Mean leaf water potential of *Vitis* species 10, 20, and 30 minutes after exposure of excised, water saturated leaves to 40 and 80% relative humidity.

BFAR Dg Mean leaf water potential of detached leaves (*Vitis* species) 1980

Species	Mean leaf water potential (-bar)[a] after 10, 20, 30 min. at				Ravaz (1902)	Galet (1956)
	40% rel.hum.	SD	80% rel.hum.	SD		
Vitis cinerea	6.9	2.71	-	-	Resistant	-
Vitis labrusca	10.6	0.69	7.2	0.52	-	-
Vitis berlandieri Reséguier	11.5	1.17	8.5	0.98	Resistant	-
Vitis amurensis	11.7	0.50	9.7	0.87	-	-
Vitis monticola	12.3	0.73	-	-	Resistant	-
Vitis riparia 'Gloire de Montpellier'	13.3	3.84	10.4	2.21	Sensitive	Sensitive
Vitis rupestris 'du Lot'	14.7	0.88	14.2	1.19	Sensitive	Sensitive

[a] average of 5 replicates

TABLE 6. Mean leaf water potential of *Vitis vinifera* cultivars 10, 20 and 30 minutes after exposure of excised, water saturated leaves to 40% relative humidity.

BFAR Dg	Mean leaf water potential of detached leaves (*V. vinifera* cultivars).		1980
Cultivar	Mean leaf water potential (-bar) at 40% rel. humidity after 10, 20, 30 min.[a]	SD	leaf drought resistance
Kerner	5.3	0.64	
Primitivo	5.3	0.67	
Negro Amaro	5.8	1.65	
Riesling	6.0	1.44	
Bombino	6.0	0.39	
Muscat blanc	6.0	1.02	
Comtessa	6.1	0.25	High
Barbera	6.4	2.38	
Prosecco	6.4	1.11	
Morio-Muskat	6.6	0.70	
Forta	6.7	0.17	
Uva di Troia	7.2	0.09	
Regner	7.3	0.58	
Bacchus	7.4	0.72	
Huxelrebe	8.0	0.60	
Ehrenfelser	8.1	1.39	
Optima	8.3	3.12	
Scheurebe	8.3	2.42	
Pinot noir	8.5	1.35	
Müller-Thurgau	8.5	2.60	Medium
Sauvignon	8.8	2.36	
Muscat d'Alexandrie	9.2	1.15	
Trollinger	9.6	2.11	
Reichensteiner	9.7	0.96	
Domina	9.7	1.93	
Rabaner	10.4	1.02	
Lagrein	10.5	1.33	
Chasselas	11.5	2.50	
Kanzler	12.4	2.26	Low
Flame Seedless	12.7	0.92	
Grenache blanc	12.9	2.50	
Silvaner	13.8	1.30	

[a]average of 5 replicates.

CONCLUSIONS

As breeders usually want rapid screening techniques to test a great number of selections in a breeding program, in most cases the actual drought resistance of grapevines will be determined by measuring the mean water potential of excised leaves--a method which characterizes the water storing or retaining capacity of leaves. But the degree of drought resistance in many species or cultivars depends also on the recovery resistance and especially on dynamic adaptation processes, e.g., osmotic adjustment (14). Thus, more information about the potential drought resistance can be obtained after a hardening process by which the genotypes reveal their individual ability for adaptation. Besides a standardization of the hardening process, especially stress duration and intensity, for these tests the plant material has to be standardized with respect to plant age and stress prehistory.

As drought resistance, depending on the genotype as well as on the type of drought, is a highly complex attribute, two or more tests may be necessary in a selection program. It is presumed that the results obtained by measuring the recovery resistance, the relation between leaf water potential and stomatal resistance, and the mean water potential of excised leaves are well correlated with the degree of drought resistance under field conditions.

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BREEDING GRAPEVINE ROOTSTOCKS FOR RESISTANCE TO IRON CHLOROSIS

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ABSTRACT

Lime induced chlorosis (iron chlorosis) is a serious physiological disease in French vineyards where the usual rootstocks present an unsatisfactory level of resistance. Twenty years of hybridization and selection in Bordeaux have been devoted to producing new rootstocks with satisfactory resistance. A new variety, called 'Fercal', shows in all calcareous soils, where it is tested, a higher level of resistance to iron chlorosis than all other rootstocks. Moreover, this variety has very good rooting ability and its affinity with cultivars of *Vitis vinifera* is also very high. The vigor conferred to the scion by this new variety is moderated so that the rate of sugar accumulation in berries is higher than in the case of other rootstocks like 41B. 'Fercal' is now officially authorized in France and its culture is recommended in all vineyards where iron chlorosis may be dangerous. This new rootstock should take the place of 41B in calcareous soils.

At the end of the last century, the introduction of grafting in France led to an increase of lime-induced chlorosis (iron chlorosis) symptoms in calcareous grape growing soils. Indeed, varieties of *Vitis vinifera*, which are relatively resistant when grown on their own roots, become susceptible when grafted upon insufficiently resistant rootstocks. Usual varieties such as 140Ru, 41B and 333EM, which are supposed to be the most resistant ones to iron chlorosis, often induce on *Vitis vinifera* varieties severe symptoms reducing the quantity and the quality of the production. In this case, vine cultivation needs treatments against iron chlorosis which are expensive and often unsuccessful.

In France, the vineyards established on calcareous soils represent about 200,000 ha. Among them, the grape growing areas of Cognac and Champagne are the most famous. On account of the important damage of iron chlorosis on grapes involved by the insufficient level of resistance of usual rootstocks, we carried out, since 1959, an important program of hybridization and selection in order to improve the resistance of rootstocks to lime-induced chlorosis.

BREEDING OBJECTIVES

Every new rootstock variety should combine, at the highest level, all physiological and agronomic characters required for a good rootstock.

Characters of the mother plant: The leaves of the mother plant must be absolutely resistant to fungus diseases (downy mildew, powdery mildew, etc.) and mineral deficiencies (K. Mg, Mn, etc...) which reduce the accumulation of starch in canes. Moreover, the vigor of the new genotype must be as high as possible in order to ensure a good production of cuttings.

Characters of the rootstock: The main quality of a rootstock is a very high resistance to phylloxera. Moreover, rooting and callusing ability, affinity with cultivars of *Vitis vinifera* must be taken into account. Resistance to mineral deficiencies (K, Mg, Mn, etc.) and to rootknot nematodes *(Meloidogyne)* are also required for new rootstocks.

The vigor of the scion induced by the rootstock is a physiological property which rules the relationship between yield and wine quality. Thus, the vigor of new genotypes needs to be known with precision in order to produce wine of a determined type.

METHODOLOGY OF BREEDING

Choice of parents: Parents used for hybridization were chosen among varieties of the two species *Vitis berlandieri* and *Vitis vinifera,* known for their resistance to iron chlorosis and also among their hybrids. Though these two species are the most resistant ones among the genus *Vitis,* an important variability of this character can be noted among the different varieties. Interspecific crosses and backcrosses carried out since 1959 have produced about 2,000 seedlings annually.

Tests of selection: In order to hasten the selection process, two early tests have been used for resistance to phylloxera (2,3) and resistance to iron chlorosis (4,5). The latter is founded on the reciprocal grafting method which reveals what new genotypes are more resistant to iron chlorosis than the usual rootstock 41B. Then, after this test, the behavior of the best varieties is observed in field trials established on soils with a high lime content. The ability of a soil to induce iron chlorosis is easily estimated with the I.P.C. (Indice de Pouvoir Chlorosant or "Chlorosing Power Index"), defined by Juste and Pouget (1) as follows:

$$\text{I.P.C.} = \frac{\text{Ca } CO_3}{(\text{Fe})^2} \times 10^4 \quad \text{in which}$$

Ca CO_3 = "active lime" (percent of soil)

Fe = easily extractible iron (ppm of soil)

This index, which gives a better evaluation of the ability of a soil for inducing iron chlorosis than the index based on the "active lime" alone, is now used in France for the choice of rootstocks before planting in calcareous soils.

In the field trails, established mainly in the Cognac area, symptoms of chlorosis were observed on every plant during 5 years

at least (6). Moreover, for every plant, the yield, the alcohol and acid contents of must, and the weight of wood removed by pruning were recorded.

EXPERIMENTAL RESULTS

Using results and observations made during 15 years in more than 50 plots distributed over the French vineyard areas, we have been able to distinguish a new variety which is everywhere more resistant to iron chlorosis than the most resistant usual rootstocks. This genotype, called 'Fercal', originates from the following cross:

BCI *(berlandieri* x Colombard) No.1 x 333EM (Cabernet Sauvignon x *berlandieri*).

We have chosen some physiological and agronomic characters in order to point out the superior behavior of the variety 'Fercal' compared with other usual varieties.

Rooting ability: In Table 1, we can see that, in nursery conditions, the rate of rooting noted on cuttings of 'Fercal' is significantly higher (P = .01) than the rate for 41B. Numerous field trials have given the same results.

TABLE 1. Percentages of rooting in nursery (1000 cuttings per variety).

Years	Rootstocks	
	'Fercal'	41B
1974	61.6[a]	31.2
1975	66.3[a]	43.1

[a]Significantly greater than 41B (P = .01).

Grafting and callusing ability: Numerous grafts were made between 'Fercal' and more than 30 cultivars of *Vitis vinifera.* The percentage of success, which depends upon years and cultivars, is always higher than with the 41B variety. Range of variation, between 50 and 80%, is similar to that noted on usual rootstocks which possess a good grafting and callusing ability *(Riparia glo-ire,* 3309, etc...).

Resistance to lime-induced chlorosis: Resistance to lime-induced chlorosis is the main character which distinguishes 'Fercal' from other rootstocks. In all our field experiments and for all cultivars, the symptoms of iron chlorosis observed on plants

grafted upon 'Fercal' are always either missing or much less severe than on plants grafted upon usual rootstocks used as controls (41B, 140Ru, 333EM). So we believe that this new variety reaches a level of resistance higher than all other rootstocks. Symptoms of chlorosis may be observed on plants grafted upon 'Fercal' only in calcareous soils where the value of I.P.C. is higher than 120. Table 2, drawn from our experimental results shows, on a scale of resistance to iron chlorosis, the place of the most resistant rootstocks in connection with the values of soil I.P.C. This scale is very useful for choosing the best adapted rootstocks for a calcareous soil.

TABLE 2. Scale of resistance to iron chlorosis based upon I.P.C. (Chlorosing Power Index)
Most resistant varieties.

Rootstocks	Value of I.P.C. above which there are risks of iron chlorosis.
'Fercal'	120
140Ru	90
333EM	70
41B	60
RS B1	50

Yield: Yield parameters (weight of bunches, alcohol and acid contents of must), which have been recorded for every plant during several years, show the performance of 'Fercal' compared with usual rootstocks.

We have calculated for every year the regression of the alcohol content on the yield per plant in every experimental plot. We can see in Figs. 1 and 2 the regression lines drawn in the confidence interval (t=05) of the yield mean. These data, collected from two areas (Cognac and Rhone Valley) and for two cultivars (Ugni blanc and Grenache), show that the alcohol degree decreases in a different way according to rootstocks when yield per plant rises (negative regression coefficient). It is noteworthy that the value of the regression coefficient is a good estimate of the reacting ability of a rootstock to an increase of yield. The comparison of annual values of regression coefficients points out the regularity and stability of the yield of rootstocks. We can note in Table 3 that two varieties ('Fercal' and 140 Ru) show a low mean value of their annual regression coefficient. They are therefore able to tolerate better than the others an increase of yield without a strong decrease of their alcohol degree.

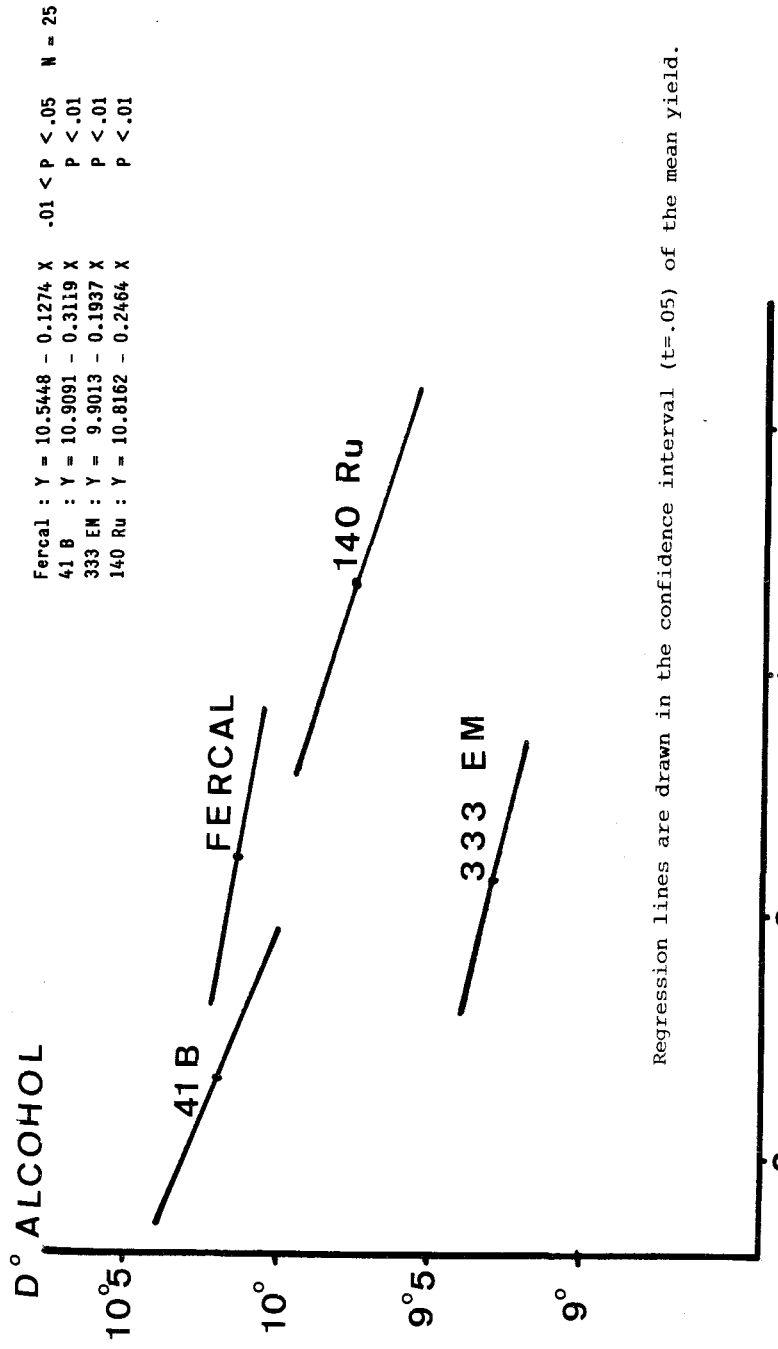

Fig. 1. Regression line of the alcohol content (degrees) on the yield (kg) per plant (cultivar: Ugni blanc; vintage 1979 in the Cognac area).

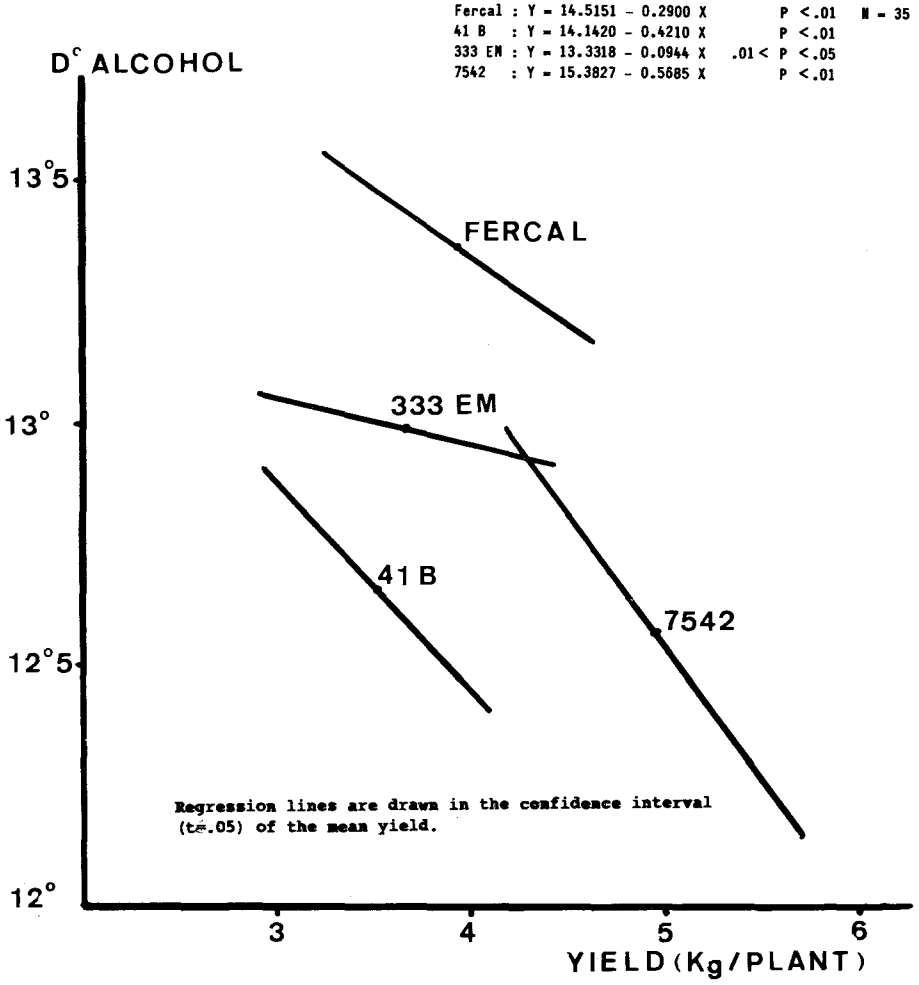

Fig. 2. Regression line of the alcohol content (degrees) on the yield (kg) per plant (cultivar: Grenache; vintage 1979; Rhône Valley area).

TABLE 3. Regression coefficient of alcohol content on yield per plant, mean of six years, cultivar Ugni blanc.

Rootstocks	'Fercal'	41B	333EM	140Ru
Mean of regression coefficient	-0.1636	-0.3215	-0.2222	-0.1813

The vigor of the scion induced by the new rootstock 'Fercal' is an average one. Furthermore, compared with 41B, 'Fercal' induces an earlier ripeness and a higher sugar content of berries when yield is the same.

CONCLUSION

Our breeding program has led to the selection of a new rootstock variety which combines interesting physiological and agronomic characters. This rootstock should take the place of 41B in calcareous soils where symptoms of iron chlorosis are severe.

'Fercal' is now patented by U.P.O.V. (International Union for the Protection of New Varieties of Plants). Its cultivation has been authorized in France since 1978, and in 1980 about 30 ha of mother plants have been established in order to insure its extension in French vineyards.

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FROST RESISTANCE OF VITIS VINIFERA VARIETIES IN THE UPPER MOSELLE VINE-GROWING AREA

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ABSTRACT

During the winter 1975-78, 11 "registered" vine varieties were compared with three standard ones for cold hardiness. An automatic cold-storage chamber was used at six different temperatures over three periods of time. Single bud cuttings were used to compare sprouting rate, shoot length and weight, callus and root development.

In a second analysis (winters 1978-79), 22 "registered" vine varieties were compared with three standard ones following natural frost. The temperature dropped from + 10°C to -20°C within 24 hours. Four locations were retained for the tests; damage was evaluated from longitudinal bud sections and growth and potential grape production of one-bud cuttings.

Since 1975 we have been working on a research project on behalf of the Government of the Federal Republic of Germany. The aim of this project is to find substitutes for the base variety Weißer Elbling growing in the Upper Moselle Area because of its quality failures. This assignment should include three phases: the first one covers five years' production and reduces the number of vine varieties planted from 20 down to 10. Difficulties have occurred mainly when we tried to determine the degree of frost resistance. As this characteristic is particularly important, this is clearly indicated in Table 1 showing damages and losses affecting sprouting and grape production after winter frost in 1978/79. We have been conducting artificial frost tests for many years.

MATERIALS AND METHODS

The three new varieties Kerner, Optima and Ortega, as well as the base variety Weißer Elbling, were tested. These were grafted on SO 4 and planted in the Oberbillig area in 1971. In 1975, canes were cut at random in three periods during winter time and underwent artificial frost. The four levels of temperature used for the test varied between -10° and 30°C. A temperature gradient of 6°C per hour helped to cool down and bring up to a warmer temperature again the 60 buds retained for each vine variety, period and temperature level. The actual operation in the six programed cold chambers of the Botanical Institute of the University of Würzburg, in each case according to the chosen degree of temperature, required two hours. The control was stored in a cool-storage chamber at +2°C. TTC tests on axial parts could

TABLE 1. Fruitful buds without sprouting after winter frost 1978/79 in four vineyards of the Upper Moselle Area.

Estimation: 25. - 31.5. 1979

Serial No.	Vineyard I (Oberbillig)		Vineyard II (Wasserliesch)		Vineyard III (Wehr)		Vineyard IV (Nittel)	
1	Ortega	65%	Weinsberg 373	69%	Ortega	72%	W. Elbling	89%
2	Kerner	68	W. Riesling	74	Kerner	73	Osiris	90
3	W. Elbling	79	Ortega	75	W. Elbling	80	Comtessa	91
4	Rabaner	82	W. Elbling	81	Albalonga	82	Gutenborner	92
5	Würzer	84	Bacchus	82	Schönburger	92	Schönburger	93
6	Jubiläumsrebe	88	M-Thurgau	88	M-Thurgau	93	Fontanara	94
7	Optima	89	Schönburger	90	Jubiläumsrebe	95	Faberrebe	94
8	Nobling	90	Markant	93	Optima	95	Forta	95
9	Reichensteiner	93	Optima	97	Nobling	98	Regner	97
10	Senator	100	Arnsburger	98	Regner	98	Freisamer	100
x		84%		85%		88%		94%

Grape production for all varieties kg/ha

1975	19490		16473		16183		15997	
1976	15203		11315		15508		19853	
1977	23380		24258		23858		23983	
1978	9628		9063		9485		6643	
1975 / 1978	16925 =	100%	15277 =	100%	16259 =	100%	16619 =	100%
1979	3322 =	20%	4098 =	27%	1385 =	9%	1503 =	9%

Temperatures and precipitations before, during and after main period of frost 1978/79

Day	Maximum(2-m high)	Minimum(2-m high)	Minimum(5-cm high)	Precipitations
25.12.1978	9.5°C	6.8°C	6.0°C	8.8 mm
26.12.	9.8	4.2	5.2	-
27.12.	7.6	2.1	-1.0	3.1
28.12.	11.0	7.4	5.0	11.7
29.12.	11.5	7.5	9.0	23.4
30.12.	8.5	2.0	5.9	2.0
31.12.	10.0(x= 9,7)	-10.6(x= 5.0)	-1.5(x= 4,1)	14.5(x=9.1)
01.01.1979	-3.7	-18.3	-19.0	-
02.01.	-3.1	-16.6	-19.9	-
03.01,	-3.1	-13.4	-14.6	-
04.01.	-3.7	-13.0	-14.0	-
05.01.	-6.8	-10.7	-13.0	-
06.01	-7.0	-15.5	-21.5	-
07.01.	-5.2(x= -4.7)	-16.6(x= -14.3)	-21.0(x= -17.6)	-

Information: Weather Service Trier
Weather Station: Trier-Berg

not be conducted after the frost test. In Trier a 4 to 5 weeks single-bud-cutting culture followed the experiment. The bud cuttings were first soaked in a half percent solution of chinosol for 12 to 15 hours and planted afterwards with 51 single-bud cuttings per vine variety in three replicates. Five parameters were included for the estimation later as shown in Table 2.

Because of the winter frost in 1978/79, it was possible to check the results obtained with artificial frost. For the first time since 1956 and 1963, the temperature dropped drastically to -21.5° C. The maximum drop occurred within 24 hours and showed a 28.3° C difference, the maximum temperature 18.3°C below zero. This corresponds to a temperature gradient of 1.2°C per hour and a maximum value of 3.2°C. Within this period of natural frost, it was possible to conduct three different experiments. First, three weeks after the frost had set in buds were cut at random and 51 longitudinal bud sections made for each vine variety in order to determine damage caused to primary and secondary buds. In a second test, 51 buds per variety were subjected to a single-bud (eye) cutting culture in three replicates. Third, data regarding sprouting in the field were collected. The results of these three experiments appear in Table 3.

The data obtained and calculated according to the variance analysis and subsequently the Duncan-Test allowed us to compare vine varieties as well as methods.

RESULTS

In order to give a general idea, considerable damage brought about through winter frost was noted. As shown in Table 1, an observation of sprouting in four locations shows in May 1979 average damage from 84 to 94% following the winter frost of 1978/79, at which time temperatures dropped to -21.5°C. The grape production therefore decreased between 27% and 9% as compared to the average production of the four previous years.

Artificial frost tests: In addition to the base variety Weißer Elbling, three new vine varieties with good viticultural characteristics were chosen from location I (Table 1) for the first artificial frost test. Four various degrees of temperature were retained for each of the three periods. An estimation of single-bud cuttings for all vine varieties listed in Table 2 confirms that a control at +2°C never brought about more significant damage than the variants treated at temperatures below zero. That speaks well for the quality of our experimentation just as well as the fact that a not so intensive level of frost never brought about significantly greater damage.

Table 3 compares the resistance of the vine varieties over all levels of temperature. In the first period, at the end of January, the variety Kerner always showed, with one exception, significantly more resistance to frost than the other varieties. On the contrary, the variety Optima performed poorly.

At the beginning of March, which is the second period, this variety, however, can stand temperatures below zero better than

TABLE 2. Estimation of single-bud cuttings over all vine varieties (N = 4) according to a specific degree of temperature with significant data.

Period I: 27.1.1976 Estimation: 24.2.1976		Period II: 8.3.1976 Estimation: 7.4.1976		Period III: 29.3.1976 Estimation: 11.5.1976	
Single-bud cuttings without sprouting (%)	5 4 3 2 1		5 4 3 2 1		5 4 3 2 1
1 -15.0°C	+ + + - -	1 Control	+ + + - -	1 Control	+ + + + -
2 Control	+ + + -	2 -10.0°C	+ + - -	2 -10.0°C	+ + + -
3 -20.0	+ + -	3 -12.5	+ + -	3 -12.5	+ + -
4 -25.0	- -	4 -15.0	+ -	4 -15.0	- -
5 -30.0	-	5 -17.5	-	5 -17.5	-
Length of shoots (mm)	5 4 3 2 1		5 4 3 2 1		5 4 3 2 1
1 Control	+ + + - -	1 Control	+ + + - -	1 Control	+ + + - -
2 -15.0°C	+ + + -	2 -12.5°C	+ + + -	2 -10.0°	+ + + -
3 -20.0	+ + -	3 -10.0	+ + -	3 -11.5	+ + -
4 -25.0	- -	4 -15.0	+ -	4 -15.0	- -
5 30.0	-	5 -17.5	-	5 -17.5	-
Weight of shoots (g)	5 4 3 2 1		5 4 3 2 1		5 4 3 2 1
1 Control	+ + + - -	1 Control	+ + + + -	1 Control	+ + + - -
2 -15.0°C	+ + + -	2 -12.5°C	+ + + -	2 -10.0°C	+ + + -
3 -20.0	+ + -	3 -10.0	+ + -	3 -12.5	+ + -
4 -25.0	- -	4 -15.0	+ -	4 -15.0	- -
5 30.0	-	5 -17.5	-	5 -17.5	-
Development of callus (Marks)	5 4 3 2 1		5 4 3 2 1		5 4 3 2 1
1 Control	+ + + + -	1 -15.0°C	+ - - - -	1 Control	+ + + + -
2 -15.0°C	+ + + -	2 -10.0	+ - - -	2 -10.0°C	+ + + -
3 -20.0	- - -	3 -12.5	+ - -	3 -12.5	+ + -
4 -25.0	- -	4 Control	+ -	4 -15.0	+ -
5 -30.0	-	5 -17.5	-	5 -17.5	-
Development of roots (Marks)	5 4 3 2 1		5 4 3 2 1		5 4 3 2 1
1 Control	+ + + + -	1 -12.5°C	+ + + - -	1 -10.0°C	+ + + - -
2 -15.°C	+ + + -	2 Control	+ + + -	2 Control	+ + + -
3 -20.0	+ + -	3 -10.0	+ + -	3 -12.5	+ + -
4 -25.0	- -	4 -15.0	+ -	4 -15.0	- -
5 -30.0	-	5 -17.5	-	5 -17.5	-

1 = most positive estimation.
5 = most negative estimation.

TABLE 3. Estimation of single-bud cuttings over all levels of temperature (N = 4) depending on vine variety with significant data.

Period I: 27.1.1976 Estimation: 24.2.1976		Period II: 8.3.1976 Estimation: 7.4.1976		Period III: 29.3.1976 Estimation: 11.5.1976	
Single-bud cuttings without sprouting (%)					
	4 3 2 1		4 3 2 1		4 3 2 1
1 Kerner	+ + + -	1 Optima	+ + - -	1 W.Elbling	+ + - -
2 Ortega	- - -	2 W.Elbling	+ - -	2 Optima	+ + -
3 W.Elbling	- -	3 Kerner	- -	3 Ortega	+ -
4 Optima	-	4 Ortega	-	4 Kerner	-
Length of shoots (mm)					
	4 3 2 1		4 3 2 1		4 3 2 1
1 Kerner	+ + + -	1 Optima	+ + + -	1 Optima	+ - - -
2 Ortega	+ + -	2 Ortega	+ - -	2 Ortega	+ - -
3 Optima	- -	3 W.Elbling	- -	3 W.Elbling	+ -
4 W.Elbling	-	4 Kerner	-	4 Kerner	-
Weight of shoots (g)					
	4 3 2 1		4 3 2 1		4 3 2 1
1 Kerner	+ + + -	1 Optima	+ + + -	1 W.Elbling	+ + - -
2 Ortega	+ + -	2 W.Elbling	+ + -	2 Optima	+ - -
3 W.Elbling	- -	3 Kerner	- -	3 Ortega	+ -
4 Optima	-	4 Ortega	-	4 Kerner	-
Development of callus (Marks)					
	4 3 2 1		4 3 2 1		4 3 2 1
1 W.Elbling	+ + - -	1 Kerner	+ + - -	1 W.Elbling	+ + + -
2 Kerner	+ + -	2 Ortega	+ + -	2 Ortega	+ + -
3 Ortega	- -	3 Optima	- -	3 Optima	+ -
4 Optima	-	4 W.Elbling	-	4 Kerner	-
Development of roots (Marks)					
	4 3 2 1		4 3 2 1		4 3 2 1
1 Kerner	+ + + -	1 W.Elbling	+ + - -	1 Optima	- - - -
2 Ortega	+ + -	2 Optima	+ + -	2 Ortega	- - -
3 Optima	+ -	3 Kerner	+ -	3 Kerner	- -
4 W.Elbling	-	4 Ortega	-	4 W.Elbling	-

1 = most positive estimation.
4 = most negative estimation.

Significant superiority		**Significant superiority**		**Significant superiority**	
Kerner	14 x	Optima	10 x	W.Elbling	8 x
Ortega	6 x	W.Elbling	5 x	Optima	5 x
W.Elbling	2 x	Kerner	3 x	Ortega	5 x
Optima	1 x	Ortega	3 x	Kerner	0 x

any other variety. In 10 out of 15 cases, it is superior to the other three varieties. The Kerner and the Ortega react quite negatively. In the third period, the Kerner appears very sensitive to frost while the Weißer Elbling is very resistant.

These results enable us to observe an interaction between varieties and stress periods, which is significant for all tested characteristics. More information is presented in Fig. 1. This observation renders tests more difficult. In order to record frost resistance more exactly, it would be necessary to include five periods in the program from December to April, but this means, of course, a great deal of extra work.

The same thing could be said about the interaction varieties x levels of temperature. This is what the varieties show particulary well in the second and third period, as can be seen in Fig. 2. The vine varieties do not react homogeneously when the temperature changes, i.e., sometimes worse, sometimes better. This is why it is not possible to obtain detailed information with less than five levels of temperature. As these should be distributed orthogonally in all periods, it would be necessary to increase the number of temperature possibilities in order to explore adequately the information provided in lower as well as higher temperature ranges.

Natural frost process: If we compare the degree of resistance of the four vine varieties after winter frost in 1978/79, the variety Kerner shows positive effects (Table 4). Because of the results obtained through an examination of buds, single-bud cutting cultures and sprouting in the vineyard, it resists in seven cases four times significantly better than the other three varieties. In the other three cases, it is twice superior to two varieties; in one case, it was not possible to record any difference.

The variety Optima, on the contrary, always ranks last; this explains why it may be considered as slightly resistant to frost, according to this analysis. On the other hand, the Ortega appears relatively resistant. It is not only more resistant to frost than the new variety Optima but also the base variety Weißer Elbling; but it is significantly inferior four times to the variety Kerner and shows identical results three times.

Comparison of the methods of research: The results obtained here show clearly that a relation exists between the degree of resistance to frost of the vine varieties and the onset of the frost period. But this does not seem to influence the method used in any particular way. In Table 5 the results of the first period coincide with those of the three tests used as natural frost onset. The variety Kerner ranks first every time, the Optima last every time. Frost onset is namely in January, i.e., at a time when the vines had a maximum sugar content--after they had reached their maximum starch content in November. Later as the temperature rises again, toward the end of February (Table 6), the sugar will be changed into starch again. The results for the second and third period, with the aritificial frost process in March (the relations starch/sugar are then different), vary greatly from the result mentioned before. The good results obtained here with the variety Optima indicate that some vine varieties are able, even in

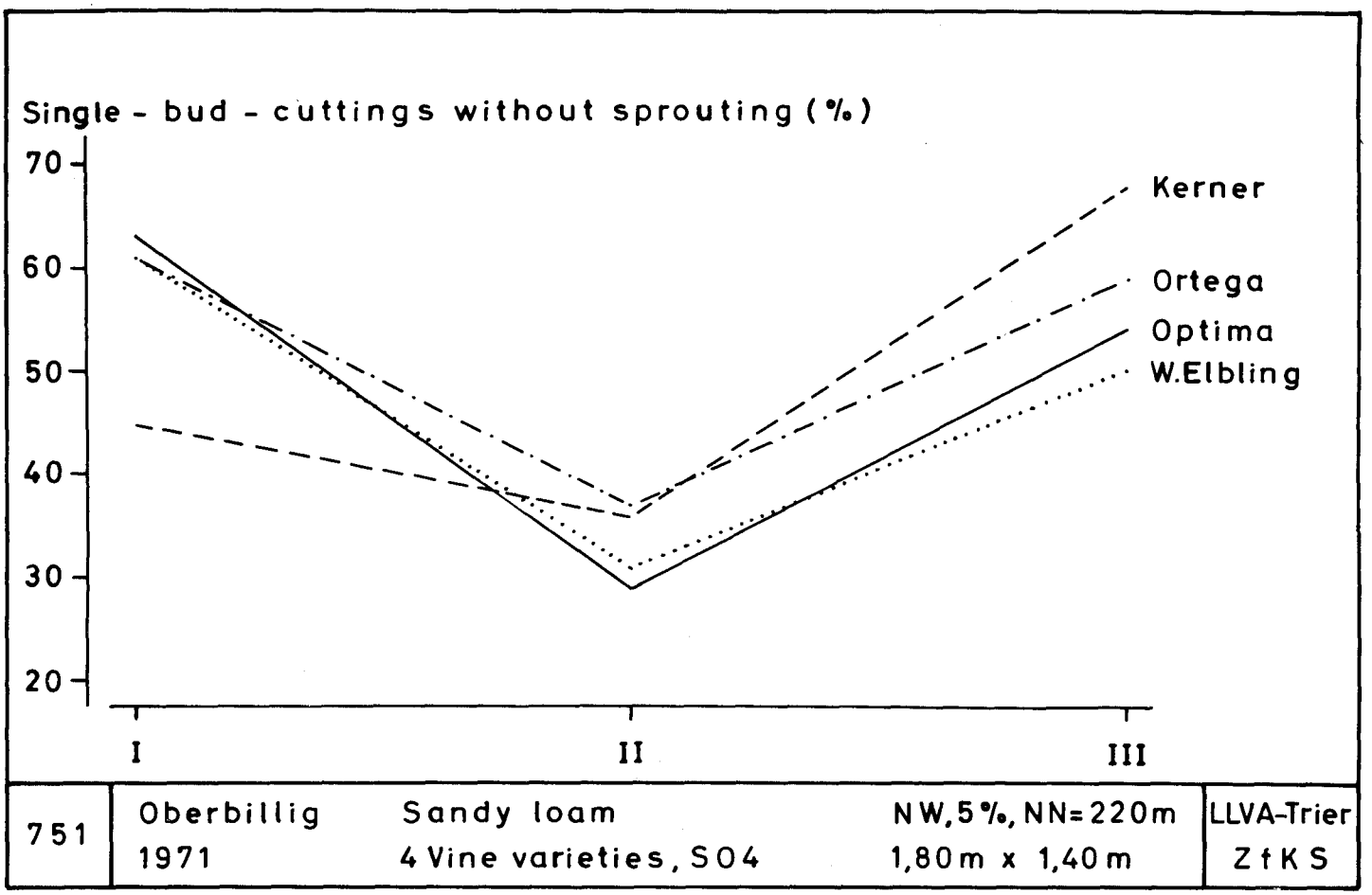

Fig. 1. Interaction vine varieties x periods.

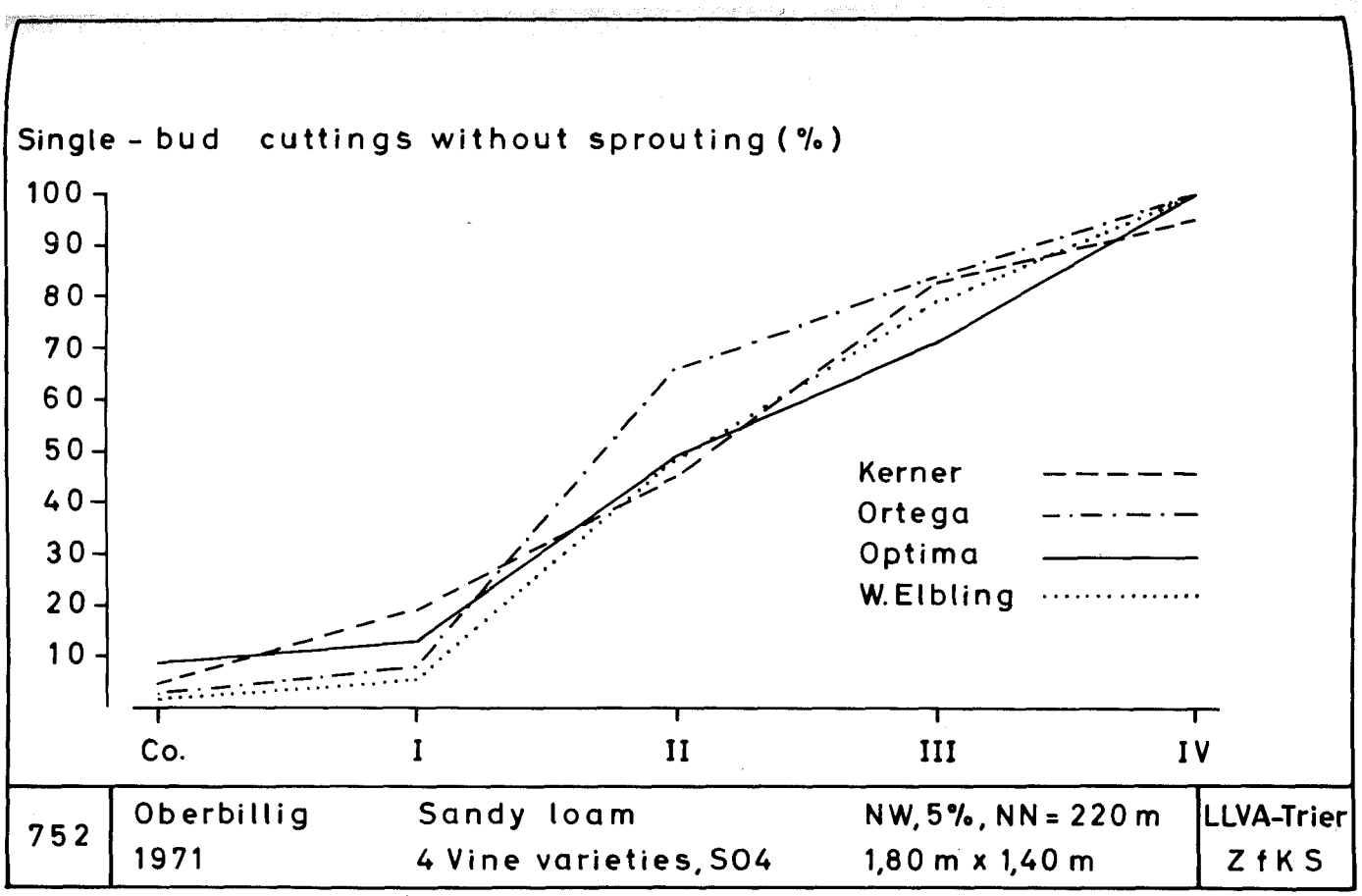

Fig. 2. Interaction vine varieties x levels of temperature.

TABLE 4. Estimation regarding frost resistance for four vine varieties, based on longitudinal bud sections, single-bud cutting cultures and field observations after winter frost 1978/79.

Longitudinal bud sections

Wood samples: 17.1.79
Estimation : 22.1.79

Healthy buds (N)

	4	3	2	1
1 Kerner	+	+	+	-
2 Ortega	+	+	-	
3 W.Elbling	-	-		
4 Optima	-			

Single-bud-cutting cultures

Wood samples: 17.1.79
Estimation : 12.2.79

Sprouting (%)

	4	3	2	1
1 Kerner	+	+	+	1
2 Ortega	+	+	-	
3 W.Elbling	+	-		
4 Optima	-			

Length of shoots (mm)

	4	3	2	1
1 Kerner	+	+	-	-
2 Ortega	+	+	-	
3 W.Elbling	-	-		
4 Optima	-			

Weight of shoots (g)

	4	3	2	1
1 Kerner	+	+	+	-
2 Ortega	+	+	-	
3 W.Elbling	-	-		
4 Optima	-			

Development of callus (Marks)

	4	3	2	1
1 Ortega	-	-	-	-
2 W.Elbling	-	-	-	
3 Kerner	-	-		
4 Optima	-			

Development of roots (Marks)

	4	3	2	1
1 Kerner	+	+	+	-
2 Ortega	-	-	-	
3 W.Elbling	-	-		
4 Optima	-			

Sprouting in field

Pruning : 5.3.79
Estimation: 31.5.79

Sprouting (%)

	4	3	2	1
L Ortega	+	+	-	-
2 Kerner	+	+	-	
3 W.Elbling	+	-		
4 Optima	-			

1 = most positive estimation.
4 = most negative estimation.

TABLE 5. Frost resistance for four vine varieties depending on research methods.

Artificial frost			Natural frost		
Period I	Period II	Period III	L. bud sections	Cutting culture	Sprouting
Frost:	Frost: 8.3.1976	Frost: 29.3.1976		Frost sets in: 31.12.1978	
Kerner	Optima	Optima	Kerner	Kerner	Kerner
Optima	Kerner	Kerner	Optima	Optima	Optima

TABLE 6. Weather conditions 10 days before pruning of test vines as noted for each period of time.

Day	Temperature(2 m high)		Precipitation	Rel. humidity(2 m high)	
	Maximum(°C)	Minimum(°C)		Minimum(%)	Maximum(%)
17.1.1976	6.0	3.3	0.1 mm	64	88
18.1.	6.5	0.5	0.1	66	95
19.1.	3.7	-0.4	2.3	89	95
20.1.	8.0	2.9	2.5	79	95
21.1.	8.1	3.1	2.5	76	91
22.1.	7.8	3.3	8.8	92	94
23.1.	7.7	1.4	0.6	76	87
24.1.	2.0	-0.9	0.3	72	91
25.1.	1.0	-2.3	0.5	91	95
26.1.	0.4	-3.9	2.1	63	97
27.1.		-4.6			97
27.1.1976: Pruning of test-vines for artificial frost process in period I.					
27.2.1976	11.5	-1.1	-	56	98
28.2.	9.4	-0.7	0	72	98
29.2.	13.8	-0.7	0.2	60	98
1.3.	11.2	4.1	-	38	73
2.3.	10.5	-2.5	-	46	96
3.3.	10.8	-0.4	-	26	91
4.3.	10.1	-0.8	-	28	64
5.3.	4.6	-3.0	-	43	73
6.3.	2.0	-4.6	-	51	73
7.3.	1.3	-5.7	-	53	88
8.3.		-7.4			91
8.3.1976: Pruning of test-vines for artificial frost process in period II.					
19.3.1976	6.7	1.1	- mm	49	90
20.3.	7.0	-3.4	-	31	80
21.3.	6.1	-3.2	-	31	73
22.3.	5.0	-5.6	0.5	34	67
23.3.	5.1	-2.2	0	58	92
24.3.	10.8	-0.9	0.3	30	78
25.3.	7.2	1.8	6.7	92	96
26.3.	10.3	5.1	7.3	64	92
27.3.	8.4	0.7	0	65	98
28.3.	12.7	5.0	-	69	90
29.3.		-0.5			98
29.3.1976:Pruning of test-vines for artificial frost process in period III.					
Average I :	5.1	0.2	2.0	77	93
II :	8.5	-2.1	0	47	86
III:	7.9	-0.2	1.5	52	87

Information: Weather Service, Trier Weather Station: Trier-Berg

March, to react against a temperature drop with a new sugar concentration. The variety Kerner is hardly able to show any resistance in March. Its ability to produce rapidly more sugar out of starch becomes apparent in January, even though the temperature drops drastically. The variety Ortega shows a similar attitude.

CONCLUSION

The varieties Kerner and Ortega resist frost better than the base variety Weißer Elbling in December and January. At the beginning of March, it is the vine variety Optima that is superior to the base variety. The threshold of the general frost compatibility is at 20°C below zero.

On account of the existing interaction varieties x periods, the vine varieties do not always react homogeneously against frost. The same could be said about the relations between varieties and levels of temperature. This is why it is absolutely necessary to include 4 to 6 periods and levels of temperature in the program if we wish to record exact data about the resistance of a particular variety against frost. To recognize the specific threshold of frost resistance, the temperature intervals should not cover more than 3°C.

The artificial as well as the natural frost processes show similar results as long as they take place during the same period. It is then possible to use various methods to come to the same results. The method concerning longitudinal bud sections requires less work and equipment than others.

It would be good if we could, within the scope of breeding, develop vine varieties which allow economical quality and can stand temperatures colder than -20°C. Moreover, these varieties should also be able to react during the whole winter against sudden periods of frost through a transformation of starch into sugar.

INHERITANCE OF SOME CHARACTERS IN PROGENIES OF VITIS VINIFERA FROM CROSSES WITH DABOUKI AND ALPHONSE LAVALLÉE

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ABSTRACT

In an analysis of 18 progenies (12 derived from crosses with Alphonse Lavallée, six with Dabouki), highly significant parent-progeny correlations were found for days to fruit ripening (r=0.87), berry weight (r=0.72), cluster size (r=0.65), cluster compactness (r=0.61), and berry firmness (r=0.67). Correlation between berry weight and days to ripening was (r=0.74). Progenies from crosses between red and white-skinned varieties yielded a 1:1 color ratio. Crosses between Alphonse (black) and red or white varieties segregated either 1 red: 1 black or approximately 1 red: 3 black. Progenies between round and oval-berried varieties showed a partial dominance of elongated berry, with many deviations. One selection from a Dabouki x Cardinal cross and one selection from a Dabouki x Alphonse cross have been named.

A table grape breeding program was initiated in 1969 for the development of early and late ripening new cultivars. Queen of the Vineyards, Cardinal, Alphonse Lavallée and Dabouki, all cultivars with large berries, figured prominently in the program. Emphasis was laid on crosses between local cultivars (such as Dabouki, Zeni Balouti, and Tufahi) and established introduced cultivars (e.g., Alphonse Lavallée and Cardinal). This report constitutes an attempt to analyze the mode of inheritance of some fruit characters and the date of ripening in 20 progenies from crosses performed between 1969 and 1972. The analysis has been confined to 12 progenies with Alphonse as one parent, six progenies with Dabouki as one parent, as well as to the first selfed generations of both cultivars. As Alphonse and Dabouki have also been crossed reciprocally, actually 13 Alphonse and 7 Dabouki crosses are involved apart from the selfed progenies. Characters studied included days to ripening, berry weight, cluster size, cluster compactness, berry firmness, berry form, and berry color. In all, about 1500 progeny from 20 combinations were analyzed. Four hybrids from the crosses contained in this analysis have been selected and two have been named.

MATERIALS AND METHODS

The populations studied were grown on their own roots, closely planted (3 m x 0.40 m) at Bet Dagan, in a trellised V-shaped cordon, under standard irrigation conditions. Most, though not all, parent cultivars used for crossing were grown in the same plot as the hybrids or in an adjacent collection plot.

Evaluations were made for a period over two to six years from 1973 to 1979. Values for parents and progenies were usually based on a three-year average. Correlations between consecutive years were determined for ripe date (r=0.61), berry weight (r=0.52), and cluster size (r=0.35).

Days to ripening were adjusted, for statistical treatment to number of days elapsed, from 1 May to ripe date. Berry weight was determined on a sample of 20 berries. Cluster size was determined on a scale of 1 to 5 (5-very large) clusters. Cluster compactness was rated on scale of 1 to 4 (4-very firm). Progeny means were regressed against mid-parent values to determine the extent to which progeny performance could be predicted from that of the parent. Segregations for berry color (rated either white or black) as well as berry form (rated either round, elliptical or other) were also determined.

RESULTS AND DISCUSSION

Regression of progeny values on mid-parent values showed high heritability of berry weight (r=0.72), (Table 1) and essentially polygenic mode of inheritance. High heritability of this trait has been found also by Avramov et al. (1) and by Golodriga and Trochine (7).

TABLE 1. Regression of progeny means on mid-parent values for berry traits and date of ripening in the grape.

	Correlation coefficient	Regression coefficient slope
Berry weight	0.72[a]	0.99
Cluster size	0.65[a]	0.65
Cluster compactness	0.61[a]	0.30
Berry firmness	0.67[a]	0.47
Days to ripening	0.87[a]	1.13

[a] Highly significant (p = 0.001).

Average berry weight for nearly all progenies tended to be lower than mid-parent values (Table 2). Berry size has been subject to selection pressure for larger berries. In such cases there is a tendency for the progeny mean to have lower values than the parental mean. The largest relative depression of berry weight was found with selfed Dabouki, but also in the following progenies: Dabouki x Queen of the Vineyards, Dabouki x Perlette, and Alphonse x Queen of the Vineyards. While Queen of the Vineyards seems to transmit lower berry weight when crossed, this

TABLE 2. Berry weights of parents and progeny.

Cross	Mid-parent	Progeny mean	S.D.	Progeny range (g)
Dabouki x Alphonse	6.00	5.77	1.04	2.1 - 13.0
Dabouki x Dattier	5.90	5.21	1.22	2.0 - 8.3
Dabouki x Cardinal	5.95	5.81	1.62	2.0 - 11.1
Dabouki x Exotic	6.10	6.14	1.51	4.2 - 8.4
Dabouki x Queen of the Vineyards	5.70	4.59	1.68	2.0 - 11.9
Dabouki x Perlette	4.15	3.51	0.91	1.9 - 5.3
Alphonse x Dabouki	6.00	5.68	1.50	1.7 - 11.4
Alphonse x Dattier	5.50	5.42	0.94	3.6 - 8.2
Alphonse x Cardinal	5.55	4.20	1.27	1.0 - 8.0
Alphonse x Exotic	5.70	4.07	0.99	2.4 - 7.5
Alphonse x Queen of the Vineyards	5.30	3.68	1.01	1.9 - 5.9
Alphonse x Muscat Hamburg	4.45	4.47	1.38	2.7 - 8.0
Alphonse x Calmeria	5.80	4.97	1.10	3.0 - 7.4
Alphonse x Zeni	5.95	5.24	1.20	1.7 - 9.1
Alphonse x Balouti	5.95	4.96	1.30	2.6 - 7.9
Alphonse x Toufahi	6.05	5.26	1.39	3.0 - 8.4
Alphonse x Bitouni	6.05	5.95	1.62	3.6 - 10.4
Alphonse x Einonu	5.55	5.13	1.47	3.1 - 7.4
Dabouki, selfed	6.40	4.05	1.60	2.1 - 8.3
Alphonse, selfed	5.60	4.86	1.43	2.5 - 8.6

is not always the case with Cardinal. Dabouki x Cardinal gave rise to very vigorous progeny, excelling also in high berry weight.

An important transgression in berry weight of hybrids occurred in certain progenies, especially with Dabouki x Alphonse (and the reciprocal cross), Dabouki x Cardinal, Dabouki x Queen of the Vineyards, and Alphonse x Bitouni. Days to fruit maturity were adjusted to number of days from 1 May to date of ripening. Results are given in Table 3. This trait was found to be highly heritable (r=0.87). A rather limited range in time of maturity was evident in the selfed progenies of Dabouki and Alphonse. The limited variation in time of ripening of selfed progenies was noted also by Huglin et al. (9).

A considerable range in ripening dates was obtained in the progeny of the Dabouki x Queen of the Vineyards cross (88 days) and a more limited one with the Dabouki x Cardinal cross (74 days). The Alphonse x Dabouki progeny deviated considerably in average days required for ripening from the mid-parent mean, progeny mean exceeding the latter by 19 days. With the size of populations in our work, it was not possible to exceed the range for late ripening already attained in the Dabouki x Alphonse and Dabouki x Dattier progenies, even when later ripening cultivars, such as Calmeria, were used with Alphonse or Dabouki (the latter progeny was not included in Table 2).

A highly significant correlation was found between berry weight and date of ripening (r=0.74). Thus confirming the tendency for larger berry weight with later ripening.

Determination of inheritance of cluster size was less accurate, based on five categories only (Table 4). Moreover, most cultivars used in our crosses had long clusters (4); only two had very long (5) clusters, and one had medium clusters (3).

While correlation of progeny means with mid-parent values was significant (r=0.65), all 18 progenies analyzed, as well as the two selfed progenies, showed a decrease in cluster size compared with the mid-parent. An especially significant decrease was noted in selfed Alphonse Lavallée progeny.

The largest proportion of long and well-shaped clusters was found in the Dabouki x Exotic progeny and in selfed Dabouki. It seems that both cultivars contribute to their progeny large cluster size as well as favorable cluster shape. Degree of cluster compactness is of considerable importance in table grapes and one of the most important criteria for selection (4). Unfortunately, our rating in the past was limited to four classes. A highly significant correlation between progeny and mid-parent mean was found (r=0.61). The progeny of Dabouki x Queen of the Vineyards and of Dabouki x Perlette had, on the average, the most compact clusters.

Surprisingly, the selfed progeny of Dabouki had a better average rating (less compact clusters) than the parent cultivars. This may have been due in part to straggly clusters and lower ovule fertility in a sizable portion of the selfed Dabouki progeny.

TABLE 3. Days to fruit maturity of parents and progeny. (Expressed as number of days from 1 May).

Cross	No. of progeny	Mid-parent	Progeny mean	S.D.	Progeny range
Dabouki x Alphonse Lavallée	183	95	101.2	9.9	69 - 134
Dabouki x Dattier	71	98.5	107.5	19.0	71 - 134
Dabouki x Cardinal	99	82.5	90.4	20.7	58 - 132
Dabouki x Exotic	22	87	96.2	12.8	72 - 115
Dabouki x Queen of the Vineyards	126	82.5	79.3	16.1	45 - 133
Dabouki x Perlette	21	78.5	74.9	12.7	60 - 110
Alphonse x Dabouki	88	95	114.2	8.5	82 - 136
Alphonse x Dattier	63	93.5	109.5	16.2	78 - 134
Alphonse x Cardinal	131	77.5	77.6	13.4	54 - 127
Alphonse x Exotic	41	82	86.5	11.7	72 - 118
Alphonse x Queen of the Vineyards	100	77.5	77.3	15.2	54 - 122
Alphonse x Muscat Hamburg	19	88	88.4	14.7	72 - 132
Alphonse x Calmeria	40	108	104.6	12.4	75 - 134
Alphonse x Zeni	169	98.5	101.7	9.9	82 - 127
Alphonse x Balouti	45	102	104.2	10.7	89 - 131
Alphonse x Toufahi	54	100	107.5	10.9	90 - 134
Alphonse x Bitouni	29	102	104.7	20.6	94 - 126
Alphonse x Einonu	31	101	104.7	12.6	90 - 132
Dabouki, selfed	28	100	103.3	11.1	75 - 127
Alphonse, selfed	42	90	91.5	15.1	74 - 126

TABLE 4. Cluster size of parents and progeny.[a]

Cross	Mid-parent	Progeny mean
Dabouki x Alphonse Lavallée	4.5	3.36
Dabouki x Dattier	4.5	3.60
Dabouki x Cardinal	4.5	3.92
Dabouki x Exotic	5.	4.33
Dabouki x Queen of the Vineyards	4.5	3.91
Dabouki x Perlette	4.5	3.39
Alphonse x Dabouki	4.5	3.33
Alphonse x Dattier	4.	3.30
Alphonse x Cardinal	4.	3.41
Alphonse x Exotic	4.5	3.78
Alphonse x Queen of the Vineyards	4.	3.15
Alphonse x Muscat Hamburg	3.5	2.86
Alphonse x Calmeria	4.	3.48
Alphonse x Zeni	4.5	3.96
Alphonse x Balouti	4.	3.75
Alphonde x Toufahi	4.	3.85
Alphonse x Bitouni	4.5	3.79
Alphonse x Einonu	4.	3.78
Dabouki, selfed	5.	4.59
Alphonse, selfed	4.	2.53

[a]Ratings for cluster size: 1 = very small, 2 = small, 3 = medium, 4 = large, 5 = very large.

Another important characteristic in table grapes is berry firmness. Here again, ratings were confined to our classes only, with only Tufahi rated as 4 (very firm). Correlation between progeny and the mid-parent was highly significant (r=0.67). A decrease in berry firmness was noted in most progenies with few exceptions (Dabouki x Queen of the Vineyards, Alphonse x Einonu). All traits mentioned so far seem to be highly heritable, with an essentially additive gene action.

Other parameters studied included berry shape and berry skin color. All selfed Dabouki progeny gave elongated (elliptical) berry shape, similar to the parent cultivar. Nearly all selfed Alphonse gave the characteristic round shaped berry of the parent. In some crosses between cultivars with elongated berries (e.g., Dabouki) and cultivars with round berries (e.g., Alphonse Lavallée, Exotic, Cardinal), a ratio close to 3 elliptical:1 round was obtained. However, many segregations often deviated considerably from this ratio, and no definite conclusion considering inheritance of this trait can be drawn. Berry skin color is considered to be under monogenic (3,6) or oligogenic (2,12) control with black and red dominant over white.

Alphonse Lavallée is often considered homozygous for black. Results from our crosses with Alphonse and with Dabouki are summarized in Table 5. All crosses between different whites as well as selfed Dabouki yielded whites only. Progeny from crosses between white and red also confirmed the 1 white : 1 red segregation obtained in similar cases (Barrit) (2,4). The Exotic cultivar behaved as a red genotype. Selfed Alphonse Lavallée segregated about 90% black, 10% red. Crosses of Alphonse with certain whites, such as Dabouki (when the combined results of the two reciprocal cross are considered), Zeni, and Dattier, yielded a segregation of 1 red : 1 black, pointing to an Alphonse genotype of BbRR (with B epistatic to R). However, in progeny from crosses of Alphonse with other whites (Queen of the Vineyards, Calmeria), segregations were 1 red : 2 black.

Ratios deviating from 1:1 and rather close to 1 red: 3 black have been found in crosses of Alphonse with Tufahi (red), Muscat Hamburg (heterozygous, weak black), and Cardinal (red). Moreover, selfed Alphonse has been found to consist of 90% black-skinned progeny. While major gene control, with colored skin dominant over white, has thus been fully confirmed this may well be superimposed on a quantitative pattern--affecting only distribution between red and black in the progeny. Ribéreau-Gayon et al. (10) have already pointed out that differences in berry skin color have been due to different proportions of monoglucosides of delphinidin, cyanidin and paeonidin.

Our findings on the inheritance of certain characters in the selfed progeny and that of various crosses made with two important table grape cultivars, Alphonse Lavallèe and Dabouki often used in breeding (5,8,11), have shown that the traits evaluated (time of ripening, berry weight, cluster size, cluster compactness, berry firmness) are highly heritable, with mostly additive gene action. Some trend toward smaller berry weight as well as smaller cluster size was also evident.

TABLE 5. Segregation of berry skin color.

Cross	Progeny		
	White	Red	Black
Dabouki(W) x Alphonse Lavallée(B)	0	63	105
Dabouki(W) x Dattier(W)	50	0	0
Dabouki(W) x Cardinal(R)	24	21	3
Dabouki(W) x Exotic(B)[1]	6	10	0
Dabouki(W) x Queen of the Vineyards(W)	80	0	0
Dabouki(W) x Perlette(W)	22	0	0
Alphonse(B) x Dabouki(W)	0	48	31
Alphonse(B) x Dattier(W)	0	29	30
Alphonse(B) x Cardinal(R)	0	24	103
Alphonse(B) x Exotic(B)	1	8	29
Alphonse(B) x Queen of the Vineyards(W)	0	28	65
Alphonse(B) x Muscat Hamburg(B)	0	2	13
Alphonse(B) x Calmeria(W)	0	15	32
Alphonse(B) x Zeni(W)	0	52	53
Alphonse(B) x Balouti(B)	0	8	9
Alphonse(B) x Toufahi(R)	0	9	38
Alphonse(B) x Bitouni(B)	0	7	14
Alphonse(B) x Einonu(B)	0	4	23
Dabouki(W), selfed	29	0	0
Alphonse(B), selfed	0	3	32

[1]Fruit of this cultivar did not attain black color under our conditions.

Selfed Alphonse progeny showed a large decrease in cluster size, while selfed Dabouki had a significantly reduced berry weight compared with the parent. Correlation was especially high (r=0.87) for time of ripening (days to maturity after 1 May). A marked correlation was found to exist between berry weight and date of ripening. The Dabouki x Cardinal cross resulted in progeny with a relatively wide variation in date of maturity. There was practically no decrease in the average berry weight of this progeny compared with the mid-parent. This cross also gave rise to the largest number of valuable selections. Further information was gained on inheritance of berry skin color. While, generally, results are in agreement with previous studies and conclusions concerning mono or aligogenic control and the dominance of colored over white berried skin, the breeding performance of Alphonse Lavallée, generally considered homozygous for black (3), suggests a BbRR genotype for this cultivar superimposed on a quantitative pattern of inheritance. This quantitative pattern would affect only the relative distribution between red- and black-skinned progeny, thus increasing the proportion of black-skinned hybrids in certain cross combinations with Alphonse Lavallée as a parent.

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RELATIONS BETWEEN BERRY GROWTH STAGES AND BERRY REMOVAL FORCE IN GRAPEVINES

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ABSTRACT

This experiment was conducted to determine the relationships between berry growth stages and berry removal forces in Hafizali, Razaki, and Chaouch grape varieties. The force required to remove a grape berry from its pedicel by extension has been correltated with the growth stages of the berry. The force required to pluck the berry was measured with a specially designed balance. Highest berry removal forces were determined at the end of the first rapid berry growth stage in all three varieties. The results of the experiment are discussed in this paper.

The growth curve of the grape berry is double sigmoid and has two distinct periods of active growth (Stage I or pre-lag phase and Stage III or post-lag phase). These two classic growth periods are separated by a slow growth period, Stage II or lag phase (3,10,12,16,18). In some research reports, there was mention of four growth stages of grape berries (2,6,8,19). Numerous factors play different roles in controlling the various phases of berry growth.

Berry drop due to the low berry removal force at the maturity period in table grapes are a significant problem for transport and storage. There are numerous studies on physiological, anatomical, morphological and histological reasons of this phenomenon (4,7,11). In recent years, studies on this subject were centralized in solving this problem using some hormonal chemicals (4,5,7,11,9,13,14,17,20,21). We found only one research report (15) on the amount of tensile force required to pull the *rotundifolia* berries from the pedicel with the ripeness of the berry, but there is no study on the correlation between berry removal force and berry growth stages in grapes.

MATERIALS AND METHODS

Mature grapevines, *Vitis vinifera* L. cv. Hafizali, Razaki, and Chaouch, growing in the Department of Viticulture and Vegetable Crops vineyard, were used for this experiment. To improve uniformity, berry samples were collected from vigorous, normally pruned vines. At weekly intervals, from anthesis to maturity, one hundred berries were used to measure berry length, width and berry removal force for each cultivar. Berry removal force measurements began two weeks after anthesis. A self-designed

balance was used to measure berry removal force (1). This balance had a range of 0 to 1000 g. Complete bunches of berries were removed from vines and taken to the laboratory where they were separated using special scissors. Thumb and forefinger were used to hold the pedicel of the berry, and the balance was extended and read when the berry separated from the pedicel. The length and width of the ovaries of the berry samples taken at full bloom were measured with binoculars using a micrometer.

After véraison, total soluble solid contents of berry samples were determined by hand refractometer.

In this experiment we try to correlate the amount of tensile force required to pull the berry from the pedicel with the growth stages of the berry.

RESULTS AND DISCUSSION

The berries of Hafizali, Razaki, and Chaouch grape cultivars gave double sigmoid growth curves that are similar to those recorded by other researchers (Figs. 1,2,3). Changes of berry length showed a characteristic sigmoid curve as compared to berry width. For complete development from anthesis to full maturity of the fruit, 84 days were required for Razaki and Chaouch, but it took 98 days for Hafizali. First rapid growth stages (I-II) lasted 35 days in Hafizali and Chaouch and 28 days in Razaki (Figs. 1,2,3). The degree or the time of the lag phase varies with the environmental factors, particularly temperature (12), and the time of flowering (10). It was observed that duration of the lag phase in wine grape cultivars was correlated with the period required to reach full maturity (6). In the same experiment, lag phases of earlier varieties were shorter than the later ones. The various phases of berry growth may also be affected by the rapid development of the seed in the seeded cultivars. This in turn may prolong the slow growth period of seeded berries (16,22). Our data on the degree of the lag phase (III) are similar to the results above. Lag phases of Chaouch (mid-early cv.), Razaki (mid-season cv.), and Hafizali (late cv.) lasted 17, 28 and 42 days, respectively. On the contrary, the second rapid growth phase (IV) of Hafizali was shorter compared to Razaki and Chaouch (Figs. 1,2,3). Véraison was observed five days later than the beginning of the phase for Chaouch, mid-time of lag phase for Hafizali, and 10 days before the beginning of second rapid growth phase for Razaki. The lag phase of berry width is shorter than berry length in all cultivars.

Berry removal forces of the berries reached maximum just at the end of the first rapid growth phase in all cultivars, then decreased suddenly, showed a short lag phase and continued decreasing rapidly. A clear stability of berry removal force near maturity, varying with cultivars, was observed.

In this experiment, total soluble solids content of the berries increased regularly from véraison to maturity. Inverse correlation between berry removal force and the amount of total soluble solids of the berry is not evident in this period.

We also did not observe any correlation between the peak

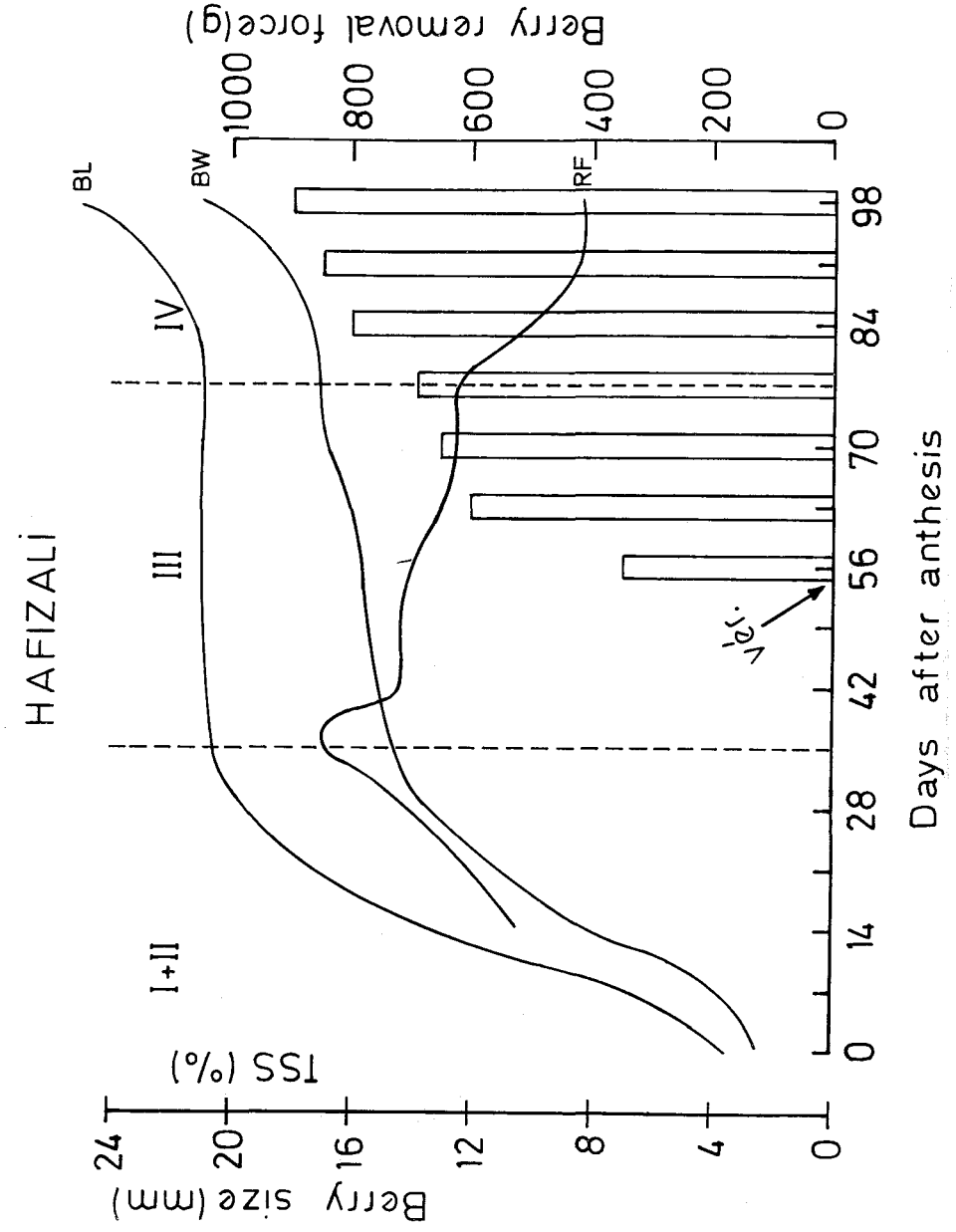

Fig. 1. Relationships between berry growth stages and berry removal force in Hafizali grape variety.

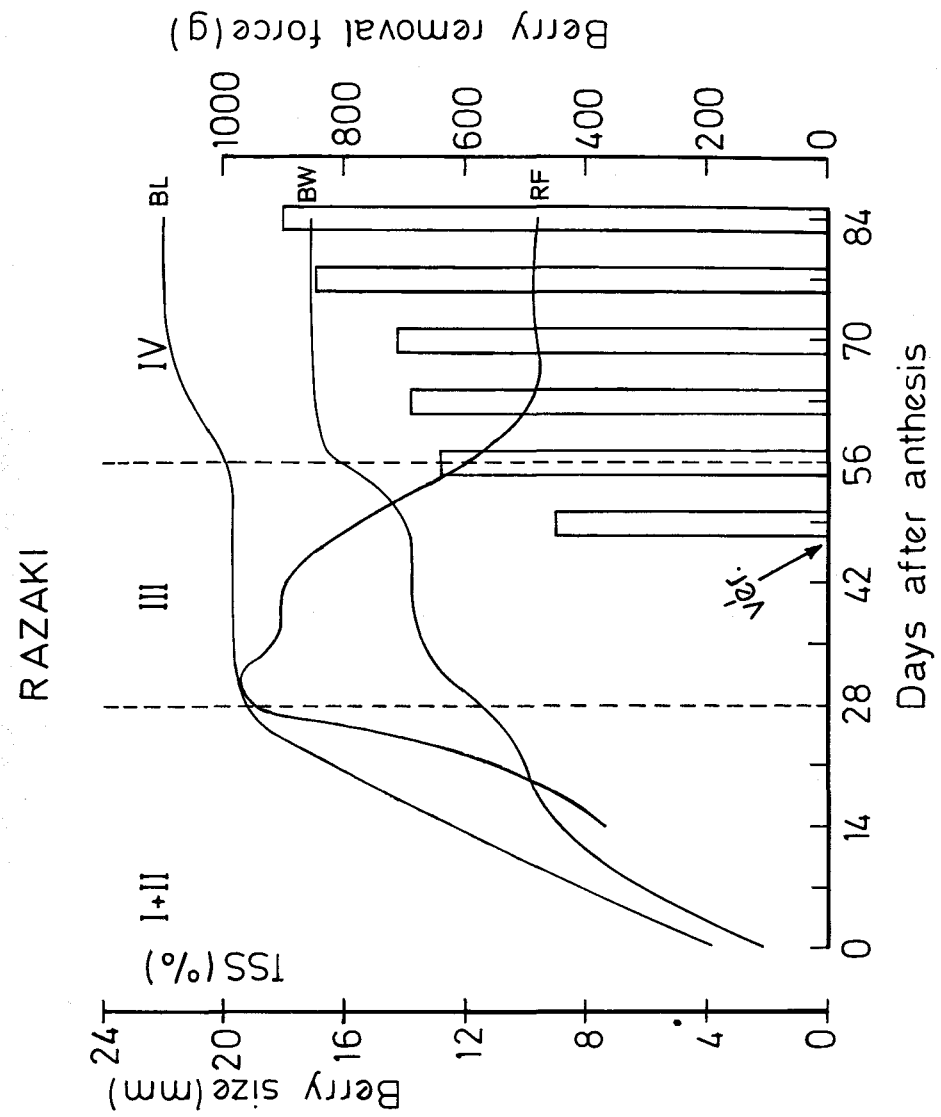

Fig. 2. Relationships between berry growth stages and berry removal force in Razaki grape.

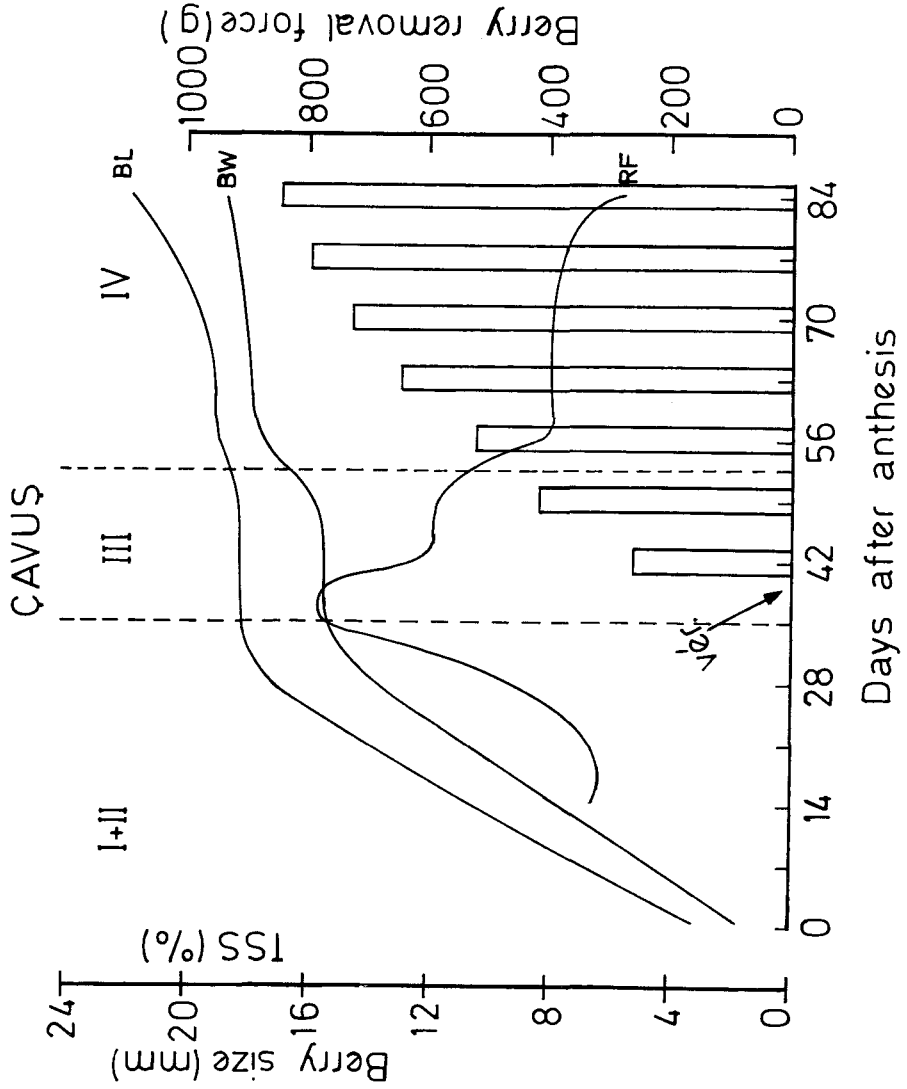

Fig. 3. Relationships between berry growth stages and berry removal force in Chaouch (Cavus) grape.

time of berry removal force and the time of véraison in all cultivars.

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HEREDITY OF EARLINESS OF FRUIT RIPENING IN VITIS VINIFERA L.

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ABSTRACT

Starting in 1968, a series of crosses using Italia as female parent and the early cultivars Volta, Pearl of Csaba and Primus as pollen parents was realized by the Istituto Sperimentale per la Viticoltura in Conegliano. The purpose was to study the inheritance of early ripening.

The results are related to the phenological stages of the growth and reproductive cycle. To obtain early progeny, the possible parents must be examined case by case, considering the existence of at least two mechanisms of heredity in the cycle length (referred to as type Volta and type Pearl of Csaba). Several traits may modify the cycle, sometimes acting independently and at other times are correlated with each other.

Obtaining early-ripening varieties represents one of the goals often proposed in the breeding of grapevines and of table grapes in particular.

The probability of success in a program of variety improvement essentially depends on the intuition and experience applied by the breeder in the choice of parents. This is likewise the case in breeding for early fruit maturity, because of the lack of understanding of the hereditary basis.

Considering the necessity that the crosses are planned with a higher degree of confidence in forecasting the results, we thought it useful to start a study on the genetic behavior of the characteristics connected with earliness even if forced to use rather limited material.

MATERIALS AND METHODS

In 1968 a series of crosses was performed using 'Italia,' a late variety characterized by excellent quality, as seed parent. The early ripening 'Volta' (Pirovano 105), Pearl of Csaba and Primus were used as pollen parents. The reciprocal crosses have not been carried out, even if the effects of the crossing direction cannot be underestimated (5). The parent plants have been selfed

in order to assess in the progenies the extent of segregation for the characters under consideration.

The seedlings obtained have been grown in hydroponic culture for the first year. After grafting on a common rootstock, the plants (80 selfs and 212 hybrids) were planted in the same environment as the parents in the vineyard of the Istituto Sperimentale per la Viticoltura in Conegliano, Veneto.

Beginning in 1975, when all the progenies were in full fruiting phase, the dates of the phenological stages bud burst, blossom, veraison and ripening were recorded.

Data were collected in five consecutive years (1975-1979) and submitted to analysis of variance. The coefficients of heritability and correlation between pairs of characters were calculated also.

RESULTS

The correlations between different phenological stages and the time periods between them have been controlled to isolate possible relationships and to recognize the characteristics deserving most attention.

In Table 1, the coefficients of correlation are shown distinctly for all the progenies from selfing and from crossing. As can be observed, the total length of the cycle and the stage of ripening are closely correlated to the length of the periods blossom-veraison and veraison-ripening. No relationship has been revealed between length of cycle and date of bud burst and date of blossom and length of the period bud burst-blossom.

In the analyses on selfings and crossing within each family, a particular behavior has been revealed in the case of selfings of Pearl of Csaba and crosses of Italia x Pearl of Csaba, where the total length of cycle correlates positively with the date of bud burst ($r = + 0.726$ for the crosses). It seems, therefore, that early bud burst and short cycle, independent in the other varieties, are related in Pearl of Csaba and in its progeny.

Concerning the stage of ripening, it is ascertained that in the selfings this is independent of the date of bud burst, while there is a close correlation between date of veraison and periods blossom-veraison and veraison-ripening.

In the crosses, moreover, a relationship between date of bud burst and date of ripening is noted, a relationship which is also influenced by the existing high correlation coefficient ($r = + 0.814$) observed in the cross Italia x Pearl of Csaba. The heritability coefficients of the characters considered relative to the selfings and the crosses are shown in Table 2.

From this point of view, it is evident that all the phenological stages have a sound genetic basis and that this is particularly important for veraison and ripening dates.

TABLE 1. Correlation coefficients between phenological epochs and their duration.

Epoch and period	F	G-F	I	F-I	M	I-M	T
			Selfings				
G	0.480	-0.755	0.210	0.150	0.366	0.151	0.259
F		0.081	0.483	0.281	0.133	0.183	0.241
G-F			-0.176	-0.159	-0.187	-0.115	0.177
I				0.731	0.712	-0.218	0.588
F-I					0.863	0.206	0.725
M						0.450	0.761
I-M							0.441
			Crosses				
G	0.546	0.182	0.435	0.295	0.581	0.180	0.258
F		0.418	0.485	0.412	0.526	0.389	0.352
G-F			0.028	0.093	0.218	0.180	0.290
I				0.895	0.781	0.864	0.951
F-I					0.679	0.941	0.783
M						0.859	0.918
I-M							0.842

Underlined values are significant at $P = 0.05$.

G = bud burst epoch.
F = blossom epoch.
I = veraison epoch.
M = ripening epoch.
G-F = period bud burst-blossom.
F-I = period blossom-veraison.
I-M = period veraison-ripening.
T = total cycle length.

TABLE 2. Hereditability (h^2) of phenological epoch and their duration.

Epoch and periods	Selfings	Cross
Bud burst	0.51	0.31
Blossom	0.37	0.37
Veraison	0.74	0.91
Ripening	0.73	0.85
Period bud burst-blossom	0.13	0.09
Period blossom-veraison	0.57	0.76
Period veraison-ripening	0.31	0.40
Total cycle	0.48	0.69

As to length of the periods, the highest heritability has been observed for blossom-veraison and total length of the cycle, followed by veraison-ripening.

On the basis of the above-mentioned statistical parameters, it can be concluded that the most interesting stages to consider, in order to select early varieties, are represented by the periods blossom-veraison and veraison-ripening.

Taking into consideration the total length of the cycle, it is important to observe how the progeny are distributed within families, with particular attention to Italia and Primus, for which the greatest number of offspring is available (Table 3). Here this trend is noted: Italia selfed gives progeny equal to Italia; Primus selfed gives progeny equal to Primus; the Italia x Primus crosses are intermediate.

TABLE 3. Distribution analysis within families for cycle length.
(In percent of the total number of individuals).

Families	Primus	=Primus	Inter= mediate	=Italia	Italia
Italia self			17.6	70.6	11.8
Primus self		62.5	37.5		
Italia x Primus		27.2	9.1	45.5	18.2

Individuals were classed based on "t" test.

From the selfings it appears that, as expected, the varieties are not homozygous for the characters considered, even if the prevailing behavior of the progeny is similar to that of the mother plants. As a consequence, a rather wide variability between the plants obtained by crossing has resulted, which compels attention to be turned to the averages for a more synthetic view of the phenomenon (Table 4).

TABLE 4. Mean values, ± standard error and minimal values of cycle length in parents and progenies.

Parents and progenie	Average length of cycle (in days)	
	Mean values	Minimal values
Italia	160.0 ± 7.7	
Italia self.	159.0 ± 8.6	145.6
Primus	134.2 ± 7.8	
Primus self.	141.6 ± 5.6	129.6
Italia x Primus	152.4 ± 6.7	127.8
Pearl of Csaba	114.8 ± 5.4	
Pearl of Csaba self.	154.2 ± 7.2	147.2
Italia x Pearl of Csaba	142.2 ± 9.6	124.4
Volta	124.0 ± 5.3	
Italia x Volta	136.3 ± 7.7	115.2

Comparing the different crosses it doesn't seem that homogeneous conclusions can be drawn; in fact, Italia x Pearl of Csaba and Italia x Primus give offspring with intermediate values tending to late, while Italia x Volta gives intermediate values tending to early. This happens even though Volta is less early than Pearl of Csaba.

In order to examine the phenomenon in more detail, the periods blossom-veraison and veraison-ripening are of interest. Their averages, referring to the progenies, are shown in Table 5 along with the minimum values found.

From this analysis it is noted that even at the level of sub-periods a uniform behavior does not exist in the crosses.

In particular for the period blossom-veraison and, in a less evident way, for the veraison-ripening, the crosses Italia x Pearl of Csaba and Italia x Primus show a behavior different from the crosses Italia x Volta. In these last mentioned, a marked average

TABLE 5. Mean values, ± standard error and minimal values of bud burst epoch and periods blossom-veraison and veraison-ripening in parents and progenies.

Parents and Progeny	Bud burst epoch [a]		Period blossom-veraison (days)		Period veraison-ripening (days)	
	Mean values	Minimal values	Mean values	Minimal values	Mean values	Minimal values
Italia	54.8 ± 4.2		75.2 ± 6.6		35.6 ± 4.9	
Italia self.	48.9 ± 6.3	41.8	71.2 ± 8.2	59.8	33.4 ± 4.4	27.8
Primus	42.8 ± 3.7		46.4 ± 5.1		31.8 ± 4.2	
Primus self.	41.7 ± 4.1	36.6	51.9 ± 5.2	42.2	29.0 ± 3.3	23.6
Italia x Primus	46.8 ± 3.2	44.6	63.4 ± 5.4	44.4	32.6 ± 3.6	27.2
Pearl of Csaba	41.6 ± 2.9		42.0 ± 3.5		19.4 ± 2.5	
Pearl of Csaba self.	48.5 ± 3.8	44.2	68.0 ± 5.4	63.6	32.0 ± 2.6	26.4
Italia x Pearl of Csaba	46.7 ± 5.1	43.2	60.5 ± 7.2	44.2	30.0 ± 3.2	24.2
Volta	43.0 ± 4.6		45.0 ± 4.3		24.0 ± 2.4	
Italia x Volta	44.6 ± 5.5	39.6	53.0 ± 6.7	40.8	28.1 ± 3.0	18.8

[a] Number of days from 10 March.

shortening of the two periods is manifested, compared with the later parent: 28% and 21% in Italia x Volta, versus 19% and 15% in Italia x Pearl of Csaba and 15% and 8% in Italia x Primus.

Presumably, therefore, in the materials under observation, two different systems of control exist for the length of the vegetative cycle: type Volta, giving early crosses (in some cases even earlier than Volta itself) and type Pearl of Csaba, which gives crosses intermediate tending to late, likely because of existing relationships with vigor.

The observation that in the selfings of Pearl of Csaba the cycle is much longer compared to the mean of the variety helps to clarify this interpretation. We verified, in these progenies, a correlation between date of bud burst and total length of the cycle. The lack of viable selfs, with similar cycle to the mother plant and equal or earlier bud burst, makes us suppose an earliness related to low vigor. Possibly, this is ascribable to a difficult repeatable combination of different genetic factors in this variety. In fact, the average length of the cycle of Pearl of Csaba (about 115 days under the conditions of the experiment) was equaled only by a cross Italia x Volta.

DISCUSSION

The duration of the vegetative cycle in the vines demonstrates, after genetical analyses, to be regulated by numerous factors with prevailing additive action (2). Consequently, the products of intervarietal crosses tend to assume an intermediate behavior to that of their parents. Cases exist, however, in which the progeny obtained by crossing early types with medium or late ones presents a cycle rather close to the early parent (3). It has been several times ascertained, on the other hand, that it is suitable to use parents with a short cycle like Pearl of Csaba in breeding for earliness by crossing (1,4,5).

In the crosses studied here, it appeared difficult to obtain earliness related to early bud burst, typical of this variety. A higher probability of success in genetical improvement programs should be attained, using as parents the varieties endowed with earliness based on the shortening of the last phases of the cycle. Proposing this, Hactrjan (3) emphasizes that the type "Northern early" tends to transmit to its crosses a short duration of the phase veraison, suggesting a dominant action of the genes involved. In our material this seems confirmed particularly by the cross Italia x Volta, in which the phase blossom-veraison also demonstrates a prevalence of earliness.

If we consider that the period blossom-veraison represents an index of the speed of growth of the berries and buds (phase of vegetative development) and that the period veraison-ripening represents the speed of accumulation in the grapes only, it can be understood which are the physiological processes subjected to this genetic control.

CONCLUSIONS

It is ascertained, from a practical point of view, that to

obtain early progeny the possible parents must be examined case by case considering the existence of at least two mechanisms of heredity of earliness (here referred to as type Volta and type Pearl of Csaba) and of several traits influencing it, sometimes independent and at other times correlated to each other.

Finally, it is to be pointed out that owing to the variability inherent in the cultivated varieties it would be convenient, in order to increase the probability of isolating interesting forms from the programed crosses, to select the parents among the products of a selfed generation (6).

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TABLE GRAPE BREEDING IN ROMANIA

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ABSTRACT

In Romania, grapevine breeding for producing new table grape varieties is carried out according to special annual and prospective programs at the research stations of the Research Institute for Viticulture and Enology, Valea Călugăreasca and at the viticulture departments of the Agricultural Colleges in Bucharest, Cluj and Craiova.

Hybridization is the common breeding method; clonal selection is also applied to older, nonhomogeneous varieties.

Among the new, valuable table grape varieties registered during the last few years are "Victoria" with large, attractive bunches and berries, firm pulp, good shipping quality and long shelf life. "Muscat Timpuriu de Bucuresti," "Triumf," "Chaselas de Baneasa," and "Timpuriu de Cluj" with early to mid-season ripening, large, attractive bunches and berries, and pleasing taste.

The use of polyploidy, mutations and selection for resistance to pests and diseases is going to be expanded in the breeding program.

Cultivated, since time immemorial on the territory of Romania, the grapevine has always been paid careful attention, as it represents an important sector of the national economy. Romania ranks seventh in the world's wine areas, while the grape products meet the most exacting requirements of the present consumer due to their superior quality. Higher and higher production, both as to quantity and quality, are the main objects of Romanian viticulture. The old vineyards, enjoying natural environmental conditions similar to those of the prestigious wine regions in Central and Western Europe, were expanded with modern plantations over large areas. New technologies particular to industrialized viticulture were adopted.

The integration of modern conceptions in the classical tradition of the Romanian table grape varieties is reflected both in the technology and in the present range of varieties which is continuously being renewed, enlarged and diversified.

Thus, of utmost importance is Chasselas doré (40%) followed in decreasing order by Afuz Ali (22%), Cardinal (11%), Muscat

Hamburg (9%), Italia, Coarnă neagră, etc. (16). These percentages express, very briefly, the diversity of soil and climatic factors in Romania. The above-mentioned varieties ensure for 105 to 180 days-in-the-year fresh or preserved grapes under controlled conditions.

In order to extend the grape season, the ripening period should be very carefully considered. The creation of new varieties by breeding is necessary. Grapes ripening earlier than Pearl of Csaba, between Cardinal and Chasselas doré, and later than Coarnă neagră and Italia are needed (1). The results of grape breeding also contribute to improving and diversifying the present range of grape varieties in the northern vineyards where the climate is rather cold.

The main objects of grape breeding are: creation by self-pollination of table grape varieties superior to the native ones, more productive crops, early ripening, good palatability, large berry size, small number of seeds, thin skin, and firm pulp (13). Higher biological resistance to unfavorable conditions of the environment, pests and diseases is also an important target of the grape breeding program (9). Breeding studies are being carried out to produce dwarf or semi-dwarf varieties to enable the use of small areas, the optimum use of solar energy, as well as to get a higher efficiency per unit area. Methods using cell cultures and non-differentiated merismatic tissues for creating grapes resistant to pests and diseases are going to be applied to a higher extent. Mutations and polyploidy are expected to be more intensively used (11).

These studies are organized according to annual or prospective programs coordinated by the Ministry of Agriculture and Food Industry through the Academy of Agricultural and Forest Sciences. They are carried on by the Research Institute of Viticulture and Enology, eight wine research stations and the teaching staff of the agricultural colleges in Bucharest, Cluj and Craiova.

The native grape varieties Coarnă albă, Coarnă neagră, Coarnă roşie, Braghină and Crîmpoşie were initially used for breeding. These are phylogenetically old varieties adapted to the environmental conditions of Romania. They taste very good but also have certain shortcomings such as pollen sterility and average-sized berries. Foreign old and new varieties, well known for their valuable features are: Pearl of Csaba, Cardinal, Bicane, Muscat Hamburg, Muscat d'Adda, Afuz Ali, Italia, Blackrose, etc., are used as parents.

Special attention is given to sexual hybridization, which became a common method, being the source of great variability and permits the selection of new and valuable varieties. The variability is more obvious as the hybridization is performed between originally different varieties, spontaneous mutants and cultivated varieties, new varieties and old cultivated forms, etc. Clonal selection is still a necessary method whose efficiency increases when associated with hybridization.

F_1 and sometimes F_2 offspring are produced by hybridization with about 300 to 700 plants in each intra- or interspecific hybrid

combination, within which studies are performed and genotypes are chosen according to the previously mentioned objectives determined for each breeding institute. The heterosis phenomenon and the occurrence of transgressive forms offering significant breeding advantages are carefully diagnosed (4,5,7).

Producing new table grape varieties presumes knowledge of the biological and economical value of the initial material as well as the way of transmitting the main characters and features (1). Therefore, a great many hybrids are obtained and studied both for theoretical and practical reasons. A careful study on the 11,736 hybrids resulted from direct and mutual crossing of 57 different genitors enabling us to know the inheritance of characters and features as: flower sex; size, shape, color and aroma of the berry, precocity, capacity of sugar accumulation and seedlessness (14,15).

Crosses between the Bicane, Coarnă neagră, Crîmpoşia, etc., varieties as mothers and other varieties belonging to the Orientalis, Occidentalis and Pontica groups permitted the study of the combining ability of these varieties (2,10). The large variation of characters and the productivity heterosis, size of bunch and berries, etc., enabled a selection of outstanding individuals in view of registration for commercial use.

The outstanding individuals selected are vegetatively propagated and rigorously checked in testing and control plantations, according to the selection schemes. Here they are followed up by the State Commission for Variety Testing and Registration, which promotes the valuable genotypes for viticulture (3).

This breeding acitivity has enriched the viticultural patrimony with 9 new table grape varieties and 3 clones, which are now going to be propagated in new vine plantations (Tables 1 and 2).

All 9 of the approved varieties have morphologically and functionally hermaphrodite flowers and can ensure high to very high crops ranging between 12 and 21 metric tons per hectare.

Their genetical origin is the directed and natural intraspecific sexual hybridization. Parental combinations were chosen according to the breeding objectives. In most cases, the maternal parent was one of the native self-sterile varieties with functionally female flowers such as: Coarnă albă, Coarnă neagră, Crîmpoşie. New varieties, where the pollen parent is Afuz Ali, transmits to a great extent the shape, size and color of berries, as well as the size and shape of bunches, are also very valuable (12,14).

Among the new varieties, which are the object of this report, is the complex hybrid 'Victoria,' which resulted from crossing Cardinal and Afuz Ali. It is remarkable due to its precocity, its ripening period being similar to that of the mother, Cardinal, or two to three days later. It is attractive on the market, with large to very large, amber-yellow berries, firm pulp, ships well and has good keeping qualities. A very short period elapses (15 to 18 days) between the time the grapes begin to ripen and their complete maturation. A long marketing period is possible, since the

TABLE 1. VARIETY ORIGIN, RIPENING PERIOD, BERRY CHARACTERISTICS.

Name and genetical origin	Breeders	Year		Ripening period	Berry characteristics	
		Hybridi-zation	Homolog-ation		Shape	Color
Muscat timpuriu de Bucuresti Coarnă albă x Regina viilor	Gh. Constantinescu Elena Negreanu	1956	1969	Very early July 20-Aug.10	Oval	Gold yellow
Victoria Cardinal x Afuz Ali	Victoria Lepadatu	1964	1978	Early Aug.8-Aug.25	Cylindrical oval	Amber yellow
Triumf Lignan x Afuz Ali	V. Dvornic	1957	1969	Middle early Aug.26-Sept.8	Oval	Gold yellow
Timpuriu de Cluj Crimposie x Frumoasă de Ghioroc	St. Oprea	1962	1979	Middle early Aug.25-Sept.8	Ovoidal round	Greenish-yellow
Chasselas de Băneasa Chasselas doré natural hybridization	V. Dvornic	1959	1978	Middle Sept.1-Sept.15	Round-slightly ovoidal	Greenish-yellow
Coarnă neagră tamîioasa Coarnă neagră natural hybridization	Gh. Constantinescu Elena Negreanu	1948	1969	Late Sept.27-Oct.10	Oval	Dark red
Select Bicane x Chasselas doré	V. Dvornic	1957	1969	Late Sept.29-Oct12	Oval	Greenish-yellow
Greaca Bicane x Afuz Ali	Gr. Gorodea	1963	1979	Late Sept.28-Oct.15	Oval	Gold yellow
Roz romanesc Bicane x Afuz Ali rez	Elena Negreanu	1959	1978	Late Sept.25-Oct.10	Round	Reddish-pink

Table 2. Fertility and quality features of new grape varieties.

Name of new grape variety	Absolute fertility coefficient	Bunch weight (g)	Weight of 100 berries (g)	Sugar (g/L)	Acidity H_2SO_4 (g/L)	Crop (tons/ha)
Muscat Timpuriu de Bucuresti	1.6	250-380	360-450	147	4.7	12.3-15.0
Victoria	1.8	425-1230	632-1160	162	4.1	14.7-17.5
Triumf	1.6	350-700	512-605	139	6.2	13.5-15.0
Timpuriu de Cluj	1.7	182-208	251-326	145	3.8	14.0-16.1
Chasselas de Baneasa	1.9	340-520	320-391	175	5.1	13.6-14.3
Coarna neagra tamîioasa	1.8	350-425	302-340	180	4.2	12.7-18.0
Select	1.7	315-426	414-562	144	4.5	13.1-16.6
Greaca	2.0	407-572	610-963	168	4.9	19.2-21.0
Roz românesc	2.0	250-300	368-603	150	4.8	15.8-17.2

fruit holds in good condition on the vine. This grape can be stored for 5 to 6 months with minimum losses.

The Muscat Timpuriu de Bucureşti, Triumf, Chasellas de Baneasa and Timpuriu de Cluj varieties are characterized by early to mid-season ripening, large berry size and fine aspect of the bunch. The Greaca, Select, Coarnă neagră tămîioasă, and Roz românesc have a harmonious flavor and nice bunches which ripen rather late. These grapes extend the fruit consumption period, produce high crops and can be stored.

Vegetative breeding by means of clonal selection also offers great possibilities of improving some native and foreign varieties, valuable for certain characteristics.

Clonal selection was successful as it produced valuable commercial clones such as clones 4 and 424 of Muscat Hamburg and clones 93 of Afuz Ali, which were registered for use.

In Romania grape breeding has used both sexual crosses and clonal selection. Breeding for resistance to pests and diseases, which should be intensified, and genetical engineering were less applied. Interspecific hybridization, mutagenesis and polyploidy are expected to be used as efficient methods for these vegetatively propagated plants, since it is well known that the phenotypical stability has a hereditary background and really expresses the morphological and physiological characteristics peculiar to each genotype.

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SECTION IV

BREEDING FOR DISEASE AND INSECT RESISTANCE

THE BREEDING OF FUNGUS- AND PHYLLOXERA-RESISTANT GRAPEVINE VARIETIES

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ABSTRACT

Breeding of grapevines with resistance to *Oidium*, *Plasmopara* and *Botrytis* is characterized by the fact that all known hybrids are more or less resistant to them but do not possess desirable wine quality of *Vitis vinifera*. Continuous back-crossing with *vinifera* varieties, however, has led to resistant vines with the necessary wine quality. Meanwhile interesting volatile components, which are not present in *Vitis vinifera* berries were identified, thus making it possible to discard these vines from further breeding. Furthermore a phytoalexin against *Botrytis* which is synthesized in vine organs was found and identified chemically.

HISTORICAL SURVEY

Since *Plasmopara viticola* Berl. spread rapidly over all European vine growing areas in the last century, breeding efforts were initiated in order to achieve *Plasmopara*-resistant varieties capable of producing an acceptable wine. It is well known, however, that the introduction and planting of fungus resistant cultivars into European vineyards was, as far as wine quality is concerned, not very successful. Consequently, disappointment and a restrictive legislation led in many countries, for instance in Germany, to a general prohibition of all fungus-resistant grape varieties.

With this general view in mind, E. Baur, a German geneticist and plant breeder, convinced by the idea that no genetic barrier excludes the possible combination of fungus resistance and wine quality, put Husfeld in charge in 1926 of breeding a so-called "Idealrebe." This grapevine ought to be a plant which possesses all desirable characteristics of *V. vinifera* and genes for resistance from American species.

Husfeld's breeding program for fungus-resistant grapevine varieties was based mainly upon *Plasmopara*-resistant seedlings which had been selected by selfing the variety Oberlin 595 (=Oberlin noir), an offspring from the cross *V. riparia* x Gamay. The resistance genes against downy mildew as well as powdery mildew and *Botrytis* originated from *V. riparia*. To improve wine quality, the *vinifera* variety Riesling was chosen. The results of his first breeding efforts, which had been terminated about 1940, were both disappointing and optimistic. Although Husfeld did not succeed in this first breeding period in developing a grapevine variety of viticultural interest, he could prove with the variety Aris, for the first time, the combining ability of high fungus and

phylloxera resistance with a superior wine quality, not distinguishable from *V. vinifera*.

During the second breeding period, large-berried *vinifera* table grapes, such as Foster's White Seedling, were used to improve the yield parameters of his breeding strains. The result of these investigations shall be presented in part 3 of this report.

The third breeding period of Husfeld's program was characterized by crossing newly developed hybrids from France, for instance, those from Seibel 7053 or Seyve-Villard (12-375, 5-276) with newly developed *V. vinifera* cultivars. This breeding period began about 1955. Using this route, remarkable results could be rather quickly achieved. I shall refer to this part later in my report.

GENETIC AND BREEDING ASPECTS

In analyzing the extensive breeding efforts of Husfeld, there is evidence that selfing or repeated back crossing with one and the same genotype leads to inbreeding depression. The seedlings show, on an average, a reduced growth and a lower yield capacity. Obviously, selfing or repeated back crossing with the same individual leads to homozygosity. This is probably based upon the occurrence of recessive alleles in a homozygous status. An interpretation of this phenomenon leads to the hypothesis of overdominance. Basing upon this hypothesis a heterozygous combination of alleles of a definite locus, for instance Aa, compared with their homozygous combination AA or aa, leads to an increased growth. That means that the effect of heterosis increases with an increasing number of heterozygous alleles in a genotype (6). All investigations within grape breeding point to the validity of the overdominance hypothesis, not excluding, however, the possibility that even in self-populations or after back crossing, seedlings of viticultural value can be found. But, from the practical standpoint, it is, however, of importance that the percentage of improved seedlings can evidently be raised by avoiding homozygosis. This leads to an improved efficiency considering the time-consuming breeding work involved.

A special problem within breeding procedures, in general, and within breeding for resistance, in particular, is the selection of parents for crossing purposes and the heredity of useful characters to the progeny. In addition to those refelections which coincide with the overdominance hypothesis, it was rapidly proved in grape breeding that a specific combining ability exists. The only way to determine combining ability is via diallelic crosses. From Table 1 it is to be seen that the percentage of positively assessed seedlings within the populations of B-7-2 times Forta, Gloria, Gf. 31-17-115 or Sieger, respectively, is somewhat between 0.5 and 7.5%. The cross B-6-18 times Diana, Optima or Sieger (Table 2) brought similar results.

The specific combining ability can be found by a set-up of populations with about 200 individuals. The results of these diallele crossings are used in our institute to establish extensive populations with at least 1500 to 2500 seedlings. This series of

diallele crossings and selected crossings for breeding purposes is extremely effective as can be stated up to now.

TABLE 1. The specific combining ability of the cross B-7-2 x *V. vinifera*.

B-7-2	*V. vinifera*			
	Forta	Gloria	GF.31-17-115	Sieger
Seedlings (n)	571	987	385	707
Selections (n)	3	27	14	53
Selections (%)	0.5	2.7	3.6	7.5

TABLE 2. The specific combining ability of the cross B-6-18 x *V. vinifera*.

B-6-18	*V. vinifera*		
	Diana	Optima	Sieger
Seedling (n)	1010	1547	254
Selections (n)	9	23	18
Selections (%)	0.9	1.5	7.1

Another aspect, most important from the genetic point of view, is the maintenance of *Plasmopara* resistance even after repeated crossing with genotypes susceptible to *Plasmopara*. As can be seen from Table 3, the percentage of *Plasmopara*-resistant seedlings within the first filial generation (F_1) is between 3.5 and 14.5%. The higher percentage of resistant individuals in F_1 from those crosses, in which the genotypes conferring resistance are used as mother plant, seems to be due more to unintentional selfings than to plasmatic heredity (1). Although the latter cannot be excluded, it has to be stated that even after repeated crossing with *V. vinifera* varieties the percentage of *Plasmopara*-resistant seedlings within the cross population is still between 2.5 and 9.5%. In other words, this percentage is high enough to secure the

breeding aim, i.e., resistance and wine quality.

To an increasing degree, not only is resistance to downy mildew and powdery mildew of importance but also to *Botrytis cinerea*. Resistance genes exist not only in American cultivars or species but in *V. vinifera* as well. Furthermore, when selecting for resistance to *Plasmopara*, it was found in many cases that these genotypes also possess a high resistance to *Botrytis*. There is, however, sufficient evidence that the combination of resistance to *Plasmopara* and *Botrytis* is not due to a linkage of genes.

TABLE 3. Percentage of seedlings resistant to downy mildew (*Plasmopara viticola*).

Crossing	No. of seedlings	Resistant seedlings	
		No.	%
R x V	108,255	15,643	14.5
(R x V)V	15,022	1,550	10.3
(R x V)V_2	63,760	6,081	9.5
V x R	72,934	2,526	3.5
(V x R)V	3,146	304	9.7
(V x R)V_2	60,565	1,520	2.5

V = *V. vinifera*, R = genotypes resistant to downy mildew. Hybrid populations Geilweilerhof 1951 - 1969.

The breeding selection Ga-58-30 (further characteristics shall be presented later) possesses high resistance to *Plasmopara* and powdery mildew, combined with an inferior resistance to *Botrytis*. With the increasing use of *V. vinifera* for crossing with the aim of improving wine quality, the percentage of seedlings susceptible to *Botrytis* increased within the progeny. This observation led to the necessity of developing a method for early diagnosis of *Botrytis* resistance. Langcake and Price (5) were able to prove that vines are capable of producing phytoalexins, for instance resveratrol and its oxidizing products--the viniferins, which are effective against *Botrytis*. With this knowledge, investigations are now carried out to determine the ability of a genotype to produce viniferin and to utilize this knowledge in grape breeding. For this purpose, sections of leaves are arranged on filter-paper and pretreated with substances inducing the formation of phytoalexins, such as mucic acid. About 24 hours later, resveratrol was exuded from the wounded margins of the

leaf sections. The extent of this area is a measurement of the degree of resistance of a grapevine variety to *Botrytis* (Fig. 1).

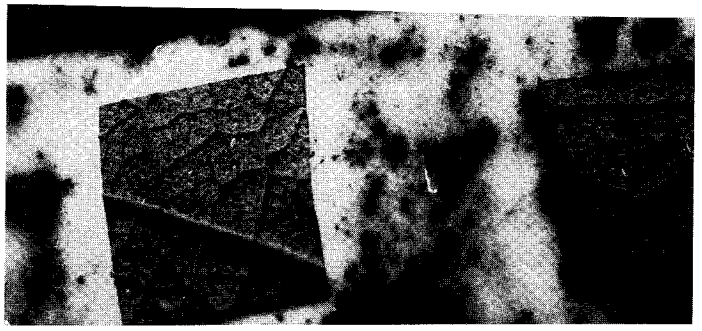

Fig. 1. Leaf sections of two *Vitis* species on filter paper, which were pretreated with a nutrient suspension and spores of *Botrytis*. Strong inhibition of spore germination around the left leaf section; a slight inhibition on the left side of the right leaf section [From Blaich and Bachmann (2)].

The deficient wine quality of the American and French hybrids, which led to many doubts about a successful *Plasmopara*-resistance breeding program, is due to their odd and strange taste, caused by the synthesis of aromatic compounds obviously not existing in the *vinifera* species. Within their extensive efforts to separate and identify the volatile substances in must and wine, Rapp et al. (7) succeeded in identifying two components, causing the so-called "strawberry taste," present in *V. labrusca* and in some offspring between *V. labrusca* and *V. vinifera*. Two furanon derivates (Fig. 2.) are responsible for this flavor. Based upon this experimental evidence, an early diagnosis should now make it possible to identify the seedlings with a strawberry flavor at a very early stage of breeding in order to exclude them from a further breeding program. Further research, however, is still needed to identify those flavor components responsible for the crude, phenolic taste occurring in the wines of many fungus-resistant varieties.

The American wild species are considered in Europe as bearers of resistance genes only. Husfeld (4) noticed rather early that American species, besides carrying genes for resistance, carry a lot of other genes of viticultural interest, for instance the high winter hardiness of *V. riparia*. In order to utilize this huge resource in our breeding program, we discarded the so-called "Plasmopara sieve," once introduced into the breeding program by Husfeld (3) prior to the planting of seedlings in the testing blocks, in order to obtain *Plasmopara*-resistant seedlings only.

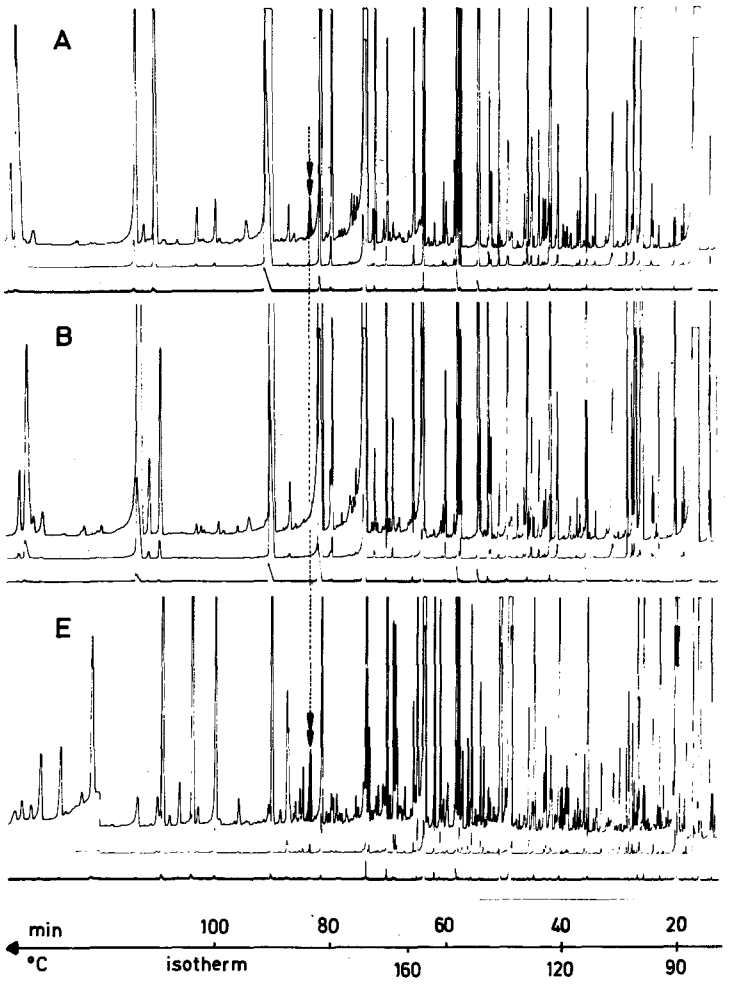

Fig. 2. The gas chromatographic identification of 2,5-dimethy-1-4-hydroxy-2,3-dihydro-3-furanon. Wines of Castor (A), Riesling (B) and of fresh strawberries (E). The arrow marks the peak which is responsible for the strawberry-like flavor.

Thus all seedlings with a medium to weak resistance to *Plasmopara* but with positive viticultural characteristics were discarded. Taking into account that breeding efforts were undertaken simultaneously to improve *V. vinifera* cultivars, it seemed to make no sense to discard semi-resistant or even fungus susceptible seedlings within the breeding program. Since 1970, all seedlings have been planted into the field plots and selected with regard to their viticultural value. Thereafter, all of them were tested for *Plasmopara* resistance. The resistant strains are added to our *Plasmopara* resistance breeding program; whereas, the susceptible seedlings are used in the *vinifera* program.

The aim of this breeding activity is the isolation of a high number of productive breeding strains for further crosses considering the hypotheses of over-dominance and specific combining ability. We are, furthermore, convinced that even a partial resistance to pests and insects, combined with a high wine quality, is of viticultural interest and that some day, in the future, we may obtain an "Idealrebe" which was once envisioned by Baur and Husfeld.

Some results of the breeding program to Plasmopara resistance: The viticultural areas situated at the northern boundary of grape growing demand very special grapevine cultivars which are well adapted to the cool climate. These grapevines have to produce satisfactory wines even in years with unfavorable climatic conditions. Five promising breeding selections are presented in Table 4, which meet the requirements of resistance and wine quality, and are being tested in several viticultural areas of Germany. Their cropping potential, on an average of four years, is about 18 to 73% over Riesling; the sugar degree corresponds with Riesling or exceeds Riesling from 10 to 14%; acid content lies about 12 to 52% below Riesling. When comparing the yield of these *Plasmopara*-resistant breeding selection with that of the variety Müller-Thurgau, only the breeding selection B-6-18 is more productive. It can thus be concluded that yield, sugar degree and acid content of must range within the variability of *V. vinifera* cultivars.

TABLE 4. Yield and must quality of some fungus-and phylloxera-resistant breeding strains. Geilweilerhof 1977-1979.

Variety	Yield		Sugar (Must)		Acidity (Must)	
	kg/a	rel.	°Oe	rel.	%O	rel.
B-6-18	226	173	74	106	11.5	77
C 97-45	190	145	67	96	11.8	79
Ga 58-30	186	142	77	110	7.1	48
B-7-2	161	123	74	106	13.1	88
Ga 49-22	154	118	80	114	10.6	71
Müller-Thurgau	194	148	72	103	7.3	49
Riesling 90	131	100	70	100	14.9	100

B-6-18 (Pollux): Parentage: (Oberlin 595)F_1 x Foster's White Seedling. A vigorous, good yielding strain. Foliage and berries are highly resistant to powdery mildew, downy mildew and *Botrytis*, as well; occasionally, a late autumn infection can occur without, however, causing any damage. Its frost resistance is higher than that of all *vinifera* varieties. Berries are medium sized, the clusters compact. It is a late-maturing variety, producing wines with a neutral flavor. Due to its late maturity and high yield, Pollux ought to be planted in warmer sites.

B-7-2 (Castor): Parentage: (Oberlin 595)F_1 x Foster's White Seedling. Not as vigorous as B-6-18, but with a more pronounced fungus resistance. Occasional patches of *Plasmopara* occur which are sealed off by the plant. Its winter hardiness is high; up to now, no winter injuries have been recorded. The bunches are large and loose; the berries mature late. More favorable sites are required. Wines are aromatic. Those aroma components being responsible for the so-called strawberry taste were detected in must and wine. The acid content of the wine is lower than in B-6-18. Although this cultivar is superior to B-6-18, from the viticultural point of view, its particular fruit flavor will probably not be accepted by the traditional wine consumers.

C-97-45. Parentage [(Gamay noir x *V. riparia*)F_2 x Riesling] x Foster's White Seedling. This breeding strain is vigorous; in its habit, it can hardly be distinguished from *V. vinifera*. The resistance to powdery mildew, downy mildew and *Botrytis* is high. Its winter hardiness is excellent. The long bunches are not compact; the berries are medium sized and medium to late maturing. The wines are of neutral flavor comparable with Silvaner. Attention should be paid to C-97-45, inasmuch, as its

wine every year ranges within the variability of *V. vinifera* wines. It cannot be classified as a hybrid wine or distinguished from *vinifera* wines even by critical tasters.

Ga-58-30: Parentage: [(Silvaner x Riesling) x Müller-Thurgau] x S.V. 12-375. This breeding selection belongs to the third breeding phase. It yields crops at least as heavy as those of Müller-Thurgau. The resistance to downy mildew and powdery mildew is high, but the berries are susceptible to *Botrytis*. The medium-sized berries mature early to medium late. Most impressive is the neutral flavored, full-bodied wine with a soft acidity. It is well accepted as a wine of *V. vinifera* quality.

Ga-49-22: Parentage: [(Silvaner x Riesling) x Müller-Thurgau] x S.V. 12-375. This breeding selection resembles that of Ga-58-30. It can, however, be differentiated by its slight muscat flavor of the wine, together with a fresh, balanced acidity. The berries are susceptible to *Botrytis*, although foliage and berries show a high resistance to powdery mildew and downy mildew.

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RESVERATROL AND THE VINIFERINS, THEIR APPLICATION TO SCREENING FOR DISEASE RESISTANCE IN GRAPE BREEDING PROGRAMS

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ABSTRACT

Attempts to identify disease susceptible progeny by inoculating young vines with fungal spores can fail for several reasons, including the existence of fungal races with differing pathogenicities, failure to maintain conditions which optimize infection and the lack of congruity of susceptibility of juvenile and adult grape tissues.

Another approach is to identify the physical properties responsible for resistance. Phytoalexins, compounds which are inhibitory to the development of fungi, are reportedly produced by grapevines. To investigate the role of phytoalexins and their precursor, resveratrol, in powdery mildew resistance, we developed analytical techniques and studied their production following induction. We also report resveratrol distribution among grape species.

In breeding for resistance to any organism, the fundamental task is to develop a methodology which will identify susceptible and resistant individuals. The most frequent method of identifying disease susceptible progeny is to inoculate them with the disease organism in question and to monitor the development of the disease. The environment may or may not be modified in order to maximize the development (4). In breeding programs the most common practice is to inoculate young seedling populations while they are growing in a greenhouse, in order that susceptible progeny may be identified and eliminated at an early stage. In our efforts to breed for increased levels of resistance to *Uncinula necator* (powdery mildew, *Oidium*) at the New York State Agricultural Experiment Station, this procedure has been used (1) and has been reported to predict the incidence of disease in seedling populations after transplanting to the field (2). The latter study, however, involved young vines growing in a nursery, an environment much different than that found in normal vineyard situations.

To further test the efficacy of the greenhouse inoculation screening procedure, the field incidence of powdery mildew in progenies from several crosses which had been previously screened in the greenhouse was rated for two consecutive growing seasons.

During these two years, the only fungicide used in the vineyard was Captan, which has little, if any, effect on *Uncinula necator*. The incidence of powdery mildew on the fruit and foliage was rated three times during the growing season, using a five-point scale with a rating of 1 indicating no infection and 5 severe infection (1).

In the initial screening, 530 (39%) of 1357 seedlings had been rated as resistant (Table 1). When the field incidence of powdery mildew in these 530 vines was rated, only 274 (52%) were free of the disease. In the entire population, 40% of the vines were rated as field resistant so the screening procedure resulted in only a 10% enrichment in resistant progeny. The benefit of eliminating the greenhouse susceptible progeny would have been to eliminate 827 progeny from the test and would have increased the apparent resistant population by only 10%. Thus the technique, as presently used, does not seem to significantly contribute to our breeding program and is not currently used.

TABLE 1. Field incidence of powdery mildew in progeny from 30 grape crosses previously rated for powdery mildew susceptibility in the greenhouse.

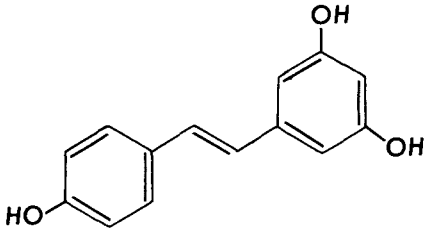
Field rating	Greenhouse rating		Total
	Resistant n(%)	Susceptible n(%)	n(%)
Resistant, n(%)	274(20)	270(20)	544(40)
Susceptible, n(%)	256(19)	557(41)	813(60)
Total, n (%)	530(39)	827(61)	1357(100)

The failure of the screening technique to adequately identify susceptible progeny leaves us with two options: 1) develop new inoculation procedures or identify incubation conditions which will improve our ability to identify susceptible materials or 2) search for another basis upon which susceptible progeny may be identified. In New York both of these options are being pursued, the first by H. Aldwinckle and the second, our efforts which form the basis for this paper.

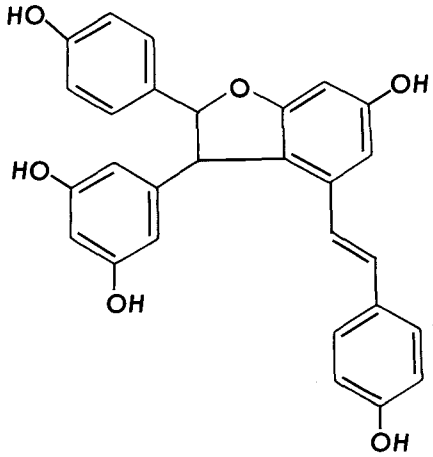
In 1976 Langcake and Pryce reported that a fluorescent compound was formed by grape leaves following challenge by *Botrytis cinerea* (7). This compound was isolated and shown to be the stilbene, trans-resveratrol (Fig. 1). They also reported that, while resveratrol was found only in infected leaves, it was constituitive in grape canes. Resveratrol was tested for its ability to suppress germination, growth or zoospore motility of *Botrytis cinerea, Cladosporium cucumerinum, Piricularia oryzae, Fusarium oxysporum* and *Plasmopara viticola*. It did not prove to be a very potent microstat (7).

In a second paper, the same authors extended this work and

showed that, in addition to resveratrol, three other fluorescent compounds were formed in response to *Botrytis* infection. These were isolated and two of them were shown to suppress growth or germination of the organisms tested above. Because of this activity, it was proposed that they constituted a new class of phytoalexins, the viniferins. The two were named ∝ and ε-viniferin. The structure of epsilon viniferin was proposed (Fig. 1) as being a dimer (8) and alpha-viniferin a trimer (10) of resveratrol. A separate paper reported that viniferins were induced in response to irradiation by short wave UV radiation (9).



TRANS RESVERATROL



∈ - VINIFERIN

Fig. 1. Structures of trans-resveratrol (upper) and ε-viniferin (lower).

Because these data suggested a possible mechanism for resistance of grape leaves to fungus attack, we began to study the relationship between resveratrol and its associated compounds in relation to disease resistance in grapevines. Since previous work has been almost exclusively on *V. vinifera* and because any program with the goal of producing disease tolerant clones must involve other grape species, we have concentrated our efforts on non-vinifera species. These appear to have significantly different resistance interaction mechanisms than *V. vinifera* (3). The thrust of our efforts has been to investigate the potential use of these compounds in a screening program to identify disease resistant progeny.

MATERIALS AND METHODS

Extraction, isolation and identification of resveratrol and ε-viniferin: Plant tissue (fresh leaves or freeze dried xylem from canes) was ground and extracted with 70% methanol. Methanol was distilled off *in vacuo*, the residue taken up in water, and partitioned into ethyl acetate. For gas chromatography (GC), the trimethylsilyll (TMS) derivatives were made. Octacosane was added before partitioning and served as an internal quantitative standard for GC. In addition to GC, LH-20 column, high pressure liquid (HPLC) and thin-layer (TLC) chromatography were used to separate resveratrol and ε-viniferin. The validity of the standard methodology was verified using resveratrol purified from grape canes. Following partition, the cane extract was purified by LH-20 chromatography, HPLC and TLC (Table 2). UV absorption spectra matched that reported (9) and GC:mass spectroscopy of the TMS derivative confirmed resveratrol. Similarly, ε-viniferin was confirmed by LH-20 chromatography, HPLC, TLC, UV-spectroscopy and direct inlet mass spectroscopy. Quantification was by UV absorption or by peak area determination (GC).

Resveratrol concentration in canes (xylem) of grape species: To investigate the potential relationship between constituitive resveratrol concentration in wood and the disease resistance of grape foliage in diverse species, internode pieces from the mid-cane region of the following vines growing at the New York State Agricultural Experiment Station were collected in January: *V. andersonii, V. argentifolia, V. berlandieri, V. champini, V. cinerea, V. cordifolia, V. labrusca, V. longii, V. riparia, V. rubra, V. rupestris, V. treleasei, V. vinifera* cv. Sultanina. The *V. vinifera* wood was taken from dormant vines growing in a ground bed in a cool greenhouse; other wood was collected from eight-year-old vines growing in the field. All vines except *V. vinifera* received no sprays which would control powdery mildew, and mature foliage infection was rated in September using a five-point scale (1, no infection to 5, severe infection).

Resveratrol induction by short wave UV radiation: UV radiation was reported to induce resveratrol production by vine leaves, but only when the lower (abaxial) surface was exposed (9). This was tested by taking 14-mm leaf disks from mature leaves of a vine growing in the greenhouse. Disks were floated on water in petri dishes with either their adaxial or abaxial surfaces uppermost. Four dishes were continuously incubated in the dark,

and four each of ad- and abaxial exposed surface dishes were exposed to short wave UV radiation (0.6 m w/cm^2) for 10 minutes and then incubated in the dark. After 48 hours, the disks were frozen, ground and resveratrol was extracted. The resveratrol content was measured by GC.

TABLE 2. Thin layer chromatography R_f values of t-Resveratrol and ε-viniferin.

Adsorbant	Solvent	R_f Resveratrol	R_f ε-viniferin
Silica gel	Teffa[a] (5:4:1)	0.36	0.22
Silica gel	$MeCL_2$/MeOH(4:1)	0.80	0.80
Cellulose	50% MeOH	0.40	0.80

[a]Teffa, toluene: ethylformate: formic acid.

Long and short term resveratrol production following exposure to UV radiation was determined by incubating 14-mm disks taken from mature, greenhouse grown *V. rupestris* leaves following a 10 minute exposure of the abaxial surface to UV radiation. Resveratrol was extracted at 0, 1.5, 3, 4.5, 6, 9 and 12 hours after exposure or, in a separate experiment, at 0, 1, 2, 3, 4, 5, 6 and 8 days after exposure. All extractions were performed in duplicate and resveratrol was quantified by GC.

Resveratrol and ε-viniferin production following inoculation with a spore suspension of *Botrytis cinerea*: The adaxial surface of leaves of a *V. riparia* vine in a growth chamber were sprayed with *Botrytis cinerea* spores suspended in dilute malt extract broth. Disks were taken daily for 6 days following inoculation. Resveratrol and ε-viniferin were measured by UV absorption after extraction and separation by TLC.

The effect of leaf position on the shoot on resveratrol production following leaf exposure to UV light was investigated by taking disks from successively older leaves on shoots of *V. riparia* and *V. rupestris* growing in the greenhouse. The youngest leaf selected was the smallest that would still provide five disks. The disks were floated on 0.1 M sucrose, given a 10 minute exposure to UV radiation and incubated in the dark. Following extraction and derivatization, resveratrol concentration was determined by GC.

Resveratrol production following inoculation with *Botrytis cinerea* spores in leaves of different ages and in plants of differing susceptibility was investigated using two different vines growing in the greenhouse. One vine was a clone of *V. cinerea* and the other was a seedling from the cross Chelois x Ives, which had previously been field rated as disease susceptible. The potted vines had two shoots. All leaves on one shoot/vine were sprayed

with *Botrytis cinerea* spores suspended in malt extract broth, the other shoot sprayed with M.E.B. without spores. The vines were inoculated on 24 May 1978, and necrosis began to appear two days later. Symptoms were most severe on younger leaves and were considerably lighter on *V. cinerea* than on the susceptible progeny. At two and five days after inoculation disks were removed from one-half of selected leaves. On the smallest leaves, whole or entire halves were sampled. Resveratrol was determined by GC.

RESULTS AND DISCUSSION

Resveratrol concentration in xylem: Although the variation among samples was large, there was a relationship between resveratrol concentration and field powdery mildew infection (Table 3). However, the hypothesis that constituitive resveratrol in xylem

TABLE 3. Resveratrol concentrations of mature grape internode xylem.

Species	Powdery mildew[a] infection rating	Resveratrol (mg/g)
V. champini	1	0.040
V. cinerea	1	0.030
V. riparia	1	0.028
V. rupestris	1	0.040
Mean		0.048
V. andersonii	2	0.061
V. argentifolia	2	0.030
V. berlandieri	2	0.101
V. cordifolia	2	0.043
V. cordifolia	2	0.022
V. labrusca	2	0.030
V. longii	2	0.080
V. rubra	2	0.137
V. treleasei	2	0.113
Mean		0.0684
V. cordifolia	3	0.098
V. vinifera	3-5	0.191
Mean		0.145

[a]1 = most resistant, 5 = least resistant

might be positively correlated with the ability of leaves to produce resveratrol or related compounds and that this in turn might be

related to disease resistance was not confirmed. More tolerant species had less rather than more resveratrol in the wood. It may be that resveratrol in the xylem reflects accumulation in response to disease infection sites on the shoots or leaves. Langcake and McCarthy (6) indicated that resveratrol or resveratrol-like compounds are to some extent translocatable within leaves.

Resveratrol induction by UV light: Resveratrol concentration increased significantly only when the abaxial surface was irradiated (Table 4). This confirms the findings of Langcake and Pryce (9). The apparent but nonsignificant increase in resveratrol following illumination of the adaxial surface conceivably may have been due to transmission through the leaf to the lower surface or due to reflection of UV light to the lower surface.

The lack of induction by upper surface irradiation raises questions about the localization of resveratrol synthesis and accumulation within grape leaves. Several important fungi primarily attack the upper leaf surface (as *Uncinula necator).* Evidence suggests that unknown factors in Concord (*V. labruscana)* tend to restrict powdery mildew infection to the upper epidermal cells (5). We intend to utilize these leaves to determine the site of synthesis as well as the site of induction.

TABLE 4. Leaf disk resveratrol concentration 48 hours after exposure of the upper (adaxial) or lower (abaxial) leaf surface to short wave ultra violet light.

Disk side exposed to UV light	Resveratrol (g/g)	
	Thin-layer* chromatography	GLC
No exposure	2.86^{b}	0^{b}
Adaxial	5.59^{b}	6.9^{b}
Abaxial	45.7^{a}	55.2^{a}

*Within a column means with the same superscript do not differ (P = .05).

Long and short term resveratrol production following exposure to UV radiation: Resveratrol was not formed for the first seven hours following irradiation. After that time, the concentration increased linearly for two days (Figs. 2 and 3). After two days there was a decline in resveratrol concentration. At six days resveratrol concentration had reached the level found before irradiation. The grossly different levels found in the two experiments are typical of our findings and those of others (9). Although the same clone was used for both experiments, there may have been differences in leaf age or other factors that were

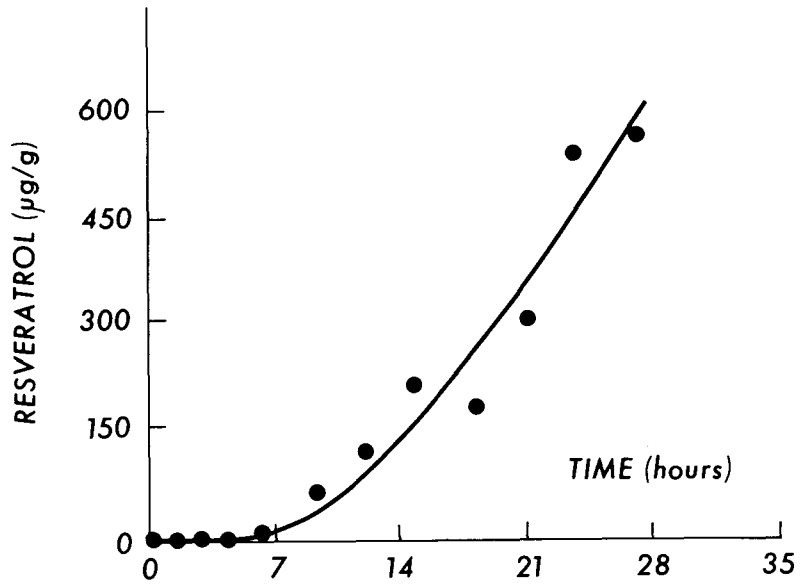


Fig. 2. Resveratrol production by *V. rupestris* leaf disks following UV irradiation.

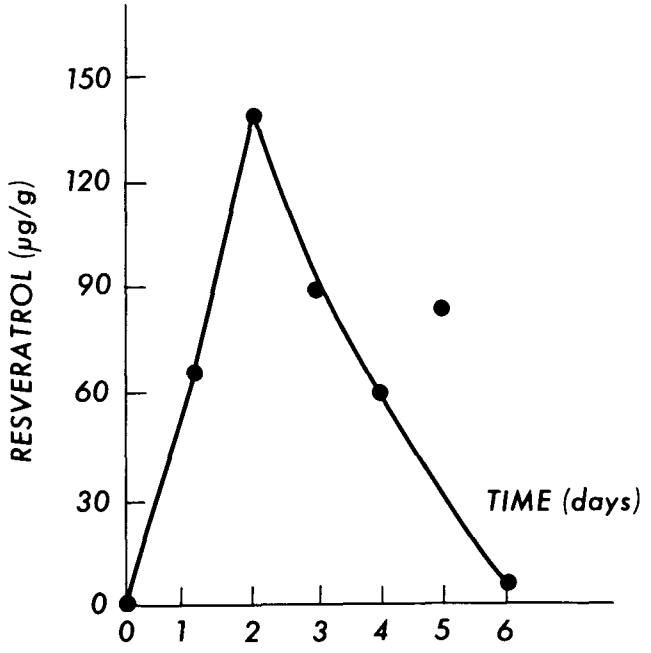


Fig. 3. Resveratrol production by *V. rupestris* leaf disks following UV irradiation.

responsible for the different levels found. Regardless of absolute amount found, the time course was similar in these and other experiments.

Time course of resveratrol and ε-viniferin production in response to *Botrytis:* Resveratrol and ε-viniferin concentration increased one day after inoculation and continued to increase for several days (Fig. 4). Unlike the response to UV radiation, the concentrations did not decline, probably because, in contrast to the brief irradiation, the inducing principle(s) remained present in the leaves.

The time course experiments indicate that both the parent compound, resveratrol, and its more biologically active condensation product, ε-viniferin, are produced in a time scale consistent with disease establishment and/or spread.

Resveratrol production by leaves of differing ages in response to UV irradiation: Similiar results were obtained on both *V. rupestris* (Fig. 5) and *V. riparia* (Fig. 6) vines. Younger leaves produced less resveratrol than recently mature leaves, and older leaves, in turn, produced less resveratrol. This pattern is consistent with the infection pattern we find when Concord or White Riesling leaves are inoculated with *Uncinula necator.*

Resveratrol production by leaves of different ages and susceptibilities in response to inoculation with *Botrytis cinerea:* The pattern of production was similar between the two species tested (Fig. 7) and resembled that found in response to UV radiation (Figs. 5 and 6). Younger and older leaves produced less resveratrol than mid-shoot leaves. With leaves near the apex or at mid-shoot, resveratrol production was inversely related to disease symptom development. That is, symptoms were greatest for young leaves and less for older leaves. Similarly symptom development was much greater and resveratrol production was much less on the susceptible seedling clone as compared to the *V. cinerea* clone.

CONCLUSION

In summary, we have confirmed the findings of Langcake and Price and extended them to include species which have significant resistance to disease. Using these species we have observed differential production of resveratrol both within and among clones in relation to the incidence of disease. The techniques we have used fit the requirements of a screening technique. Unlike the analytic technique most recently used by Langcake and his colleagues (6) resveratrol and the viniferins are separated in our procedures. Minimal amounts of tissue are required, and TLC combined with either UV or flourescence spectrophotometry give rapid quantitative results.

The use of UV to induce the production of these "stress metabolites" would free the breeder from the necessity of maintaining fungus cultures for inoculation tests. Culture maintenance is expensive when obligate fungi are to be tested. Of course, further study must be done to elaborate the role of these compounds in disease resistance and the efficacy of their role in predicting

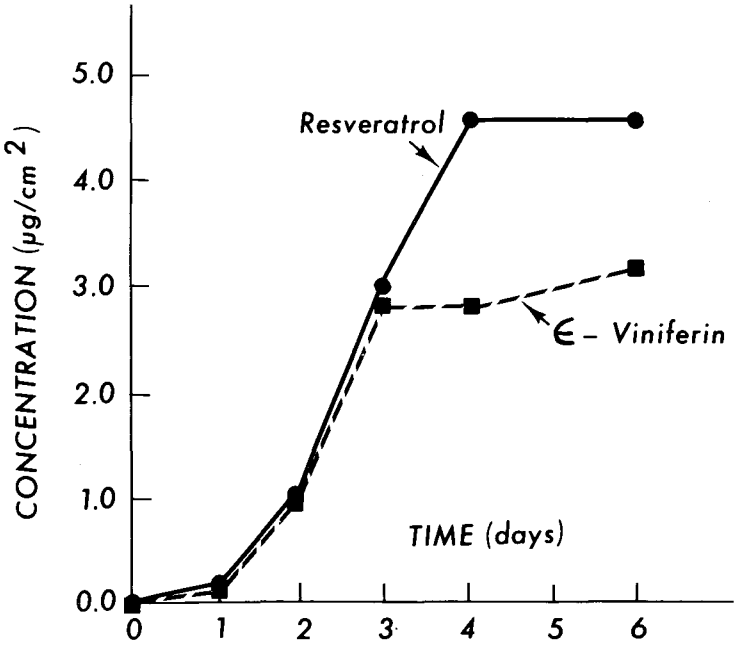


Fig. 4. Resveratrol and ε-viniferin concentration in *V. riparia* leaves following inoculation with *Botrytis cineria.*

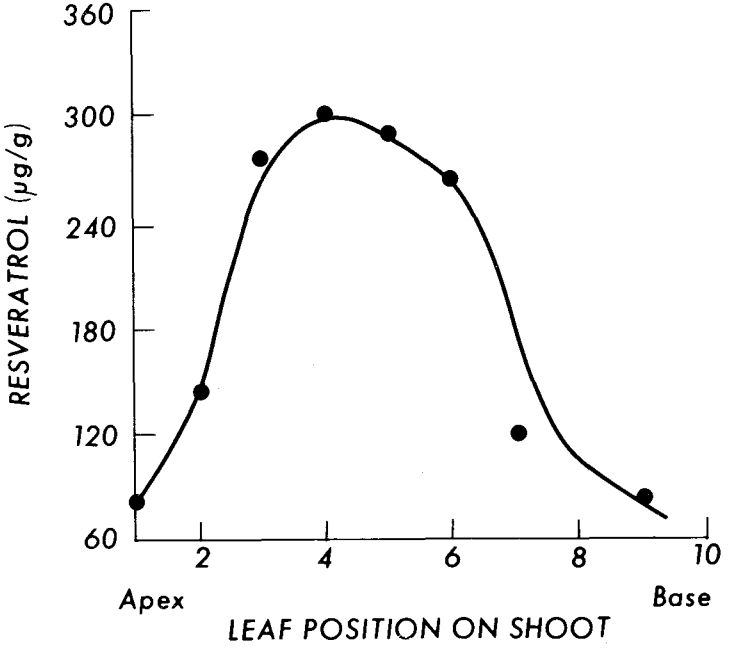


Fig. 5. Effect of leaf position on the shoot on resveratrol concentration in *V. rupestris* leaves following irradiation with UV light.

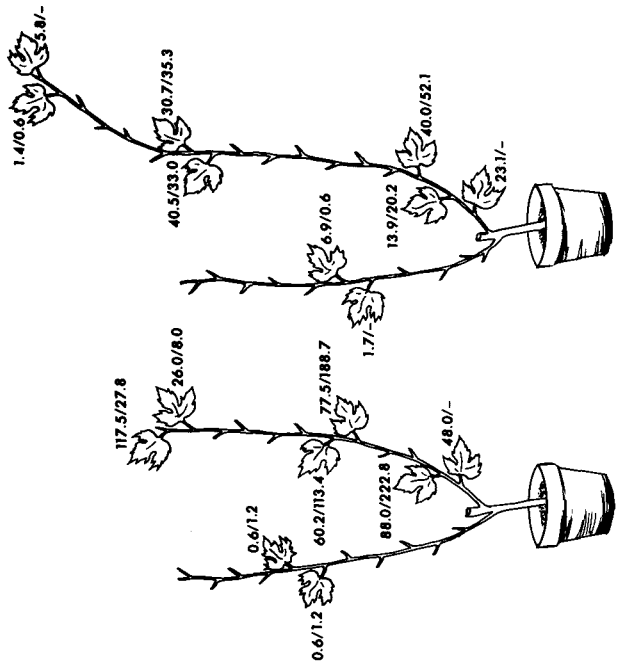


Fig. 7. Resveratrol concentration in different leaves of *V. cinerea* (more resistant) and a seedling from the cross, Chelois x Ives (less resistant following inoculation with *Botrytis cinerea*. The first number is the micrograms/cm 2 days following inoculation and the second number is the concentration 5 days after inoculation.

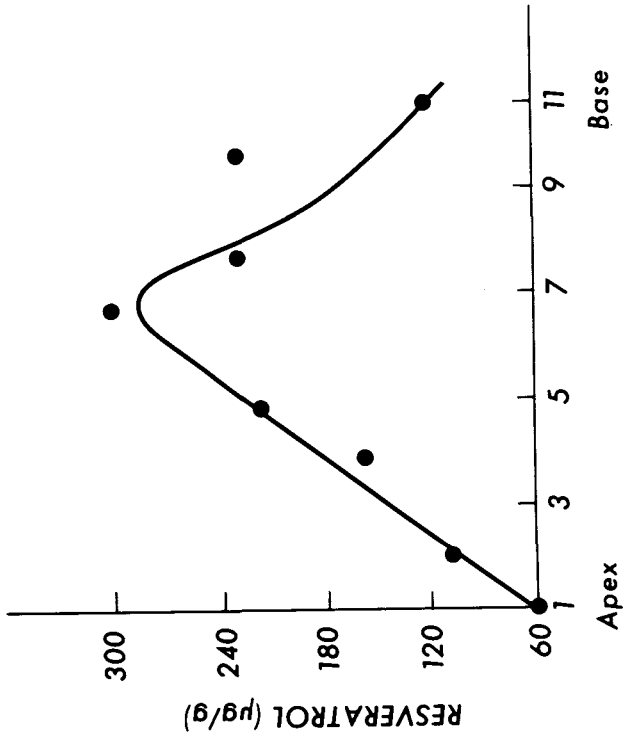


Fig. 6. Effect of leaf position on the shoot on resveratrol concentration of *V. riparia* leaves following exposure to UV light.

resistance.

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SOURCES AND INHERITANCE OF RESISTANCE TO ANTHRACNOSE (ELSINÖE AMPELINA (D BY) SHEAR) IN VITIS [1]

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ABSTRACT

A method of artificial inoculation of young grape seedlings with anthracnose was tested and found less reliable than several years' of vineyard observations during the warm, humid summer months. Individual seedlings from crosses and selfs were rated for severity of anthracnose and compared with those of parents and grandparents. A trigenic hypothesis is proposed that explains the ratios of resistant to susceptible seedlings observed in segregating progenies. It involves two dominant genes for susceptibility (An_1 and An_2) and a single dominant gene conditioning resistance (An_3), with independent inheritance of the 3 genes. A classification of cultivars according to level of resistance is presented, with genotypes proposed for each of those used as parents. Sources of resistance are primarily native species from humid areas of USA or derivatives from them.

Anthracnose is especially damaging to grapevines grown in warm, humid climates. In Florida, frequent fungicidal applications are necessary to control the disease (7). Resistance is needed to reduce the labor and expense of spraying and to assure marketable crops of grapes. Species of *Vitis* native to the humid Eastern United States provide resistant germplasm with which to breed (5).

Elsinöe ampelina, the causal fungus, grows best at 28°C in culture, but growth stops at 35°C (8). Wetness periods necessary for infection are 7-10h at 12°C to 3-4h at 21°C (1). Infection is initiated by conidia from over-wintered cane lesions; and after flowering, it spreads to young berries and pedicels (1). Gurme and Kore (6) tested 57 *Vitis* cultivars under conditions of natural and artificial infection. Three cultivars were highly resistant under both conditions: Bangalore Blue, Black Cornichon and Gulabi. Evans developed at least five new cultivars with resistance to anthracnose (2,3,4). Fennell (5), based on his own crosses and segregations observed among progenies, reported resistance to be conditioned by multiple factors. The majority of his crosses indicated that no more than 2 or possibly 3 major genes condition susceptibility. *Vitis vinifera* L. susceptibility was transmitted as a dominant to the F_1 progeny in all cases. Resistance was usually recessive, segregating in the second and later generations (5).

[1] Journal of Heredity 72:423-6 1981.

The purpose of this paper is to indicate sources of resistance and to present a workable hypothesis for the inheritance of resistance.

MATERIALS AND METHODS

Two categories of materials were involved: Seedlings from crosses between parents with different levels of resistance and seedlings from self-pollinated parents that arose from resistant x susceptible combinations (F_2 generation).

Young seedlings were spray-inoculated 6 weeks after seed sowing with spore suspensions of anthracnose obtained from either (a) infected grape shoots growing in the vineyard, or (b) from pure culture mycelium growing on test tube slants of yeast dextrose agar. The inoculated seedlings growing in wooden flats of sterilized soil were enclosed in moist chambers for at least 36 hours following spray-inoculation to encourage spore germination. Shading was provided to prevent excessive heat buildup in moist chambers.

Vineyard observations for anthracnose symptoms were most active during rainy periods from July to September. Since anthracnose symptoms on the fruit are more sporadic than those on the foliage, this paper deals only with stem and leaf symptoms. Severity of symptom development was recorded each year for 4 to 6 years on all the seedlings and their parents. Each seedling or parent was then classified on a scale of 1 to 7 (high to nil resistance, respectively). Since seasonal variation generally occurred in the symptom expression of a given plant, the period when symptoms were most severe was considered indicative of its level of resistance to anthracnose.

Data were arranged in tables and segregations of resistant vs. susceptible seedlings were tested for goodness-of-fit to expected genetic ratios under various schemes of inheritance. Once a hypothesis which fit the data was obtained, each parent was assigned hypothetical genotypes based on their breeding behavior in different combinations.

RESULTS AND DISCUSSION

Symptoms of anthracnose were evident within 2 weeks of the date of artificial inoculation. Clearly infected seedlings were cut off with a small pair of scissors and counted 3 weeks after inoculation. Unfortunately, the proportion of resistant seedlings for a given parental combination varied according to date of inoculation (Table 1). The increase in susceptibles for the April 30 and May 2 inoculations may be explained by increased succulence of seedling growth following fertilizer application on April 20. Results are not presented here since genetic analysis of segregations would be invalid. Spore suspensions derived from infected stems and berries had higher spore counts and were more infectious to seedlings than those from YDA cultures. Vineyard-produced spores were abundant and useful at times but not in April and May when spores are needed for seedling inoculation. Since sporulation in YDA culture is inadequate and no reliable source of spores has been developed for artificial screening of

TABLE 1. Percentage of seedlings resistant to anthracnose as affected by date of inoculation.

Date of	Pedigree							All
inoculation	73-11	73-9	73-37	73-26	73-29	73-40	73-17	progeny
Apr. 20	61.0	51.3						61.9
Apr. 23			84.0	66.7	74.5			51.6
Apr. 25	78.4					74.4		66.7
Apr. 27		47.7				69.0	55.4	68.2
Apr. 30			33.8	11.1	10.8			18.6
May 2	____	____	____	____	____	____	14.3	18.2
Difference	17.4	3.6	50.2	55.6	63.7	5.4	41.1	

young seedlings in the spring, artificial screening has been discontinued.

Results of several years' of pooled vineyard records on susceptibility to anthracnose have been fruitful in classifying various clones at seven different levels of resistance (Table 2). Moreover, the segregating progenies of seedlings so classified were bulked into groups as resistant (classes 1, 2, and 3) and susceptible (classes 4, 5, 6 and 7) for genetic analysis. After many hypotheses were tested and found inadequate to explain the segregations, a trigenic hypothesis with two dominant genes for susceptibility (An_1 and An_2) and a single dominant gene conditioning resistance (An_3) appeared workable. Independent inheritance of the 3 genes is assumed. When both An_1 and An_2 are present, the phenotype is susceptible regardless of whether An_3 is present or not. If either An_1 or An_2 are not present, An_3 conditions resistance and an_3 susceptibility (Table 3).

From Table 3 the ratios expected, if both parents were heterozygous at all three loci, would be 27:9:9:3:9:3:3:1 for the 8 respective possible genotypes, or 21 resistant (9+9+3): 43 susceptible progeny (27+9+3+3+1). Segregations from S_1 progenies of moderately susceptible Lake Emerald, Mantey, and W987 were reasonably close to the 21:43 ratio (Table 4). S_1 progenies from resistant clone W382, Blue Lake and Fla. A4-23 segregated 3 resistant: 1 susceptible, reflecting the segregation of An_3an_3 in these clones.

In resistant x susceptible combinations (Table 5), the progeny were either all susceptible or segregated 5:11 resistant to susceptible seedlings, depending on the susceptible parent. A ratio of 3:3:1:1:3:3:1:1 for the 8 respective genotypes in Table 3 explains the 5:11 ratio observed.

In susceptible x resistant combinations, the ratios obtained were 1:3 or 1:7, depending on the genotype of the resistant parent (Table 5). In resistant x resistant combinations, the segregations were 9:7 or 3:1, depending on whether An_1 or An_2 were heterozygous in the parent crossed with Concord.

Susceptible x susceptible combinations produced all susceptible, 1:7, 7:25, and 21:43, depending on the genetic makeup of the two susceptible parents. The 7:25 ratio is derived from the 9:9:3:3:3:3:1:1 ratio of the 8 possible genotypes expected in the proposed parental combination (Table 5).

Parental phenotypes and genotypes are summarized in Table 6. If the parents used in Fennell's inheritance study (5) are each assigned their respective genotypes (Table 6), the same hypothesis applies in explaining his progeny segregations (Table 7). Since Fennell had no susceptible x susceptible combinations there were no 21:43, 1:3, 1:7, or 7:25 ratios as occurred in my results.

Sources of resistance (Class 1 in Table 2 include 9 different *Vitis* species, of which the following 5 native species have been especially valuable for breeding: *V. aestivalis* ssp. *simpsoni*, *V. aestivalis* ssp. *smalliana*, *V. shuttleworthii*, *V. rotundifolia*, and

TABLE 2. Classification of 66 clones of *Vitis* for severity of anthracnose over several seasons.

Class 1 - No symptoms at all

V. aestivalis ssp. *simpsoni* cv. 'Pixiola'
V. aestivalis ssp. *smalliana* cv. 'Fla. 43-47'
V. caribaea DC (Syn. *V. tiliafolia* Humb. & Bonpl.)
V. champini Planch cv. 'LaPryor'
V. munsoniana Simps. (all cvs.)
V. rotundifolia Michx. (all cvs.)
V. rupestris Scheele cv. 'St. George'
V. shuttleworthii House cv. 'Haines City'
V. vulpina L. cv. 'B. Floyd'

Class 2 - Very slight symptoms

V. aestivalis ssp. *simpsoni* cv. 'Duval'
V. champini cv. 'Dog Ridge'
Blue Lake (Fla. 43-47 x Caco)
Champanel (*V. champini* x Worden)
Marguerite (*V. lincecumi* cv. Herbemont)
Ontario (Winchell x Diamond)
Urbana (Gov. Ross x Mills)
Fla. 13C-12 (*V. schuttleworthii* x Alden)
Fla. 48-1-26 (Fla. 43-47 x Niagara)
Fla. W382 (Pixiola x Golden Muscat)
Fla. W1521 (Fla. 449 x Lake Emerald)

Class 3 - Slight symptoms

Caco (Catawba x Concord)
Concord (*V. labrusca* cv. 'Carter' o.p.)
Delaware (Elsinburgh o.p.)
Extra (*V. lincecumi* x Triumph)
Leverkuhn (*V. candicans* o. p.)
Liberty (Fla. W716 x Buffalo)
Portland (Champion x Lutie)
Fla. A1-13 (Fla. 399 x Extra)
Fla. A4-23 (Fla. 449 x W907)
Fla. 399 (*V. aestivalis* ssp. *smalliana* o.p.)
Fla. 499 (*V. aestivalis* ssp. *smalliana* o.p.)
Fla. W716 (Fla. 43-47 x Golden Muscat)

Class 4 - Slight to moderate symptoms

Lake Emerald (Pixiola x Golden Muscat)
Mantey (*V. shuttleworthii* derivative)
Schuyler (Zinfandel x Ontario)

Class 5 - Moderate symptoms

Aurelia (Chaouch x Villard Blanc)
Buffalo (Herbert x Watkins)
Carman (*V. lincecumi* x Triumph)
Carolina Blackrose (Aurelia x Blackrose)
Golden Muscat (Muscat Hamburg x Diamond)
Herbemont (*V. aestivalis* derivative)
Lucile (Wyoming o.p.)
Niagara (Concord x Cassady)
Stover (Mantey x Roucaneuf)
Villard Blanc (S. V. 12-375)
Fla. A4-37 (Fla. 449 x W907)
Fla. B3-83 (W1001 x Villard Blanc)
Fla. W987 (Fla. 449 x Cardinal)

Class 6 - Severe Symptoms

Cabernet Sauvignon
Cardinal
Carignane
Exotic
Feher Szagos
Gros Colman
Lenoir (*V. aestivalis* derivative)
Malaga
Muscat of Alexandria
Norris (W987 x Lake Emerald)
Ribier (Alphonse Lavallée)
Roucaneuf (S.V. 12-309)
Tamiami (Fennell 6 x Malaga)
Fla. A3-47 (Lake Emerald x W987)

Class 7 - Very severe symptoms

Black Monukka
Golden Chasselas
Perlette
Thompson Seedless

TABLE 3. Trigenic interaction of An_1 and An_2 for susceptibility and An_3 for resistance to anthracnose in conditioning resistant or susceptible phenotypes.

8 Possible genotypes	Phenotype	Remarks
An_1--- An_2--- An_3---	Susceptible	Always susceptible
An_1--- An_2--- an_3an_3	Susceptible	with both An_1 and An_2
An_1--- an_2an_2 An_3---	Resistant	
An_1--- an_2an_2 an_3an_3	Susceptible	In absence of
an_1an_1 An_2--- An_3---	Resistant	either An_1 or
an_1an_1 An_2--- an_3an_3	Susceptible	An_2, resistance
an_1an_1 an_2an_2 An_3---	Resistant	conditioned by
an_1an_1 an_2an_2 an_3an_3	Susceptible	An_3

TABLE 4. S_1 progeny phenotypes and chi-square analysis for goodness-of-fit to expected genetic ratios.

Self-pollinated clone and its proposed genotype	Progeny observed R	Progeny observed S	Expected ratio	Chi-square	Probability
Lake Emerald (susc.) An_1an_1 An_2an_2 An_3an_3	44	81	21:43	0.33	.50-.70
Mantey (susc.) An_1an_1 An_2an_2 An_3an_3	44	81	21:43	0.33	.50-.70
Fla. W987 (susc.) An_1an_1 An_2an_2 An_3an_3	12	21	21:43	0.20	.50-.70
Fla. W382 (res.) an_1an_1 an_2an_2 An_3an_3	81	36	3:1	2.04	.10-.20
Blue Lake (res.) an_1an_1 An_2an_2 An_3an_3	107	44	3:1	1.36	.20-.30
Fla. A4-23 (res.) an_1an_1 an_2an_2 An_3an_3	52	14	3:1	0.51	.30-.50

TABLE 5. Segregations observed from crosses between clones with different levels of resistance.

Parental clones and their proposed genotypes		Progeny observed		Expected ratio	Chi-square	Probability
Female parent	Male parent	R	S			
(Resistant x Susceptible)						
W716	Thompson Seedless	0	133	all S	0.00	1.00
an_1an_1 An_2an_2 An_3an_3	An_1An_1 An_2An_2 an_3an_3					
W716	Buffalo	14	22	5:11	0.94	.30-.50
an_1an_1 An_2an_2 An_3an_3	An_1an_1 An_2an_2 an_3an_3					
(Susceptible x Resistant)						
Norris	Fla. A1-13	15	40	1:3	0.14	.70-.80
An_1An_1 An_2an_2 an_3an_3	An_1an_1 an_2an_2 An_3an_3					
Norris	Fla. W382	4	9	1:3	0.23	.50-.70
An_1An_1 An_2an_2 an_3an_3	an_1an_1 an_2an_2 An_3an_3					
Norris	Blue Lake	14	69	1:7	1:43	.20-.30
An_1An_1 An_2an_2 an_3an_3	an_1an_1 An_2an_2 An_3an_3					
Norris	Concord	1	10	1:7	0.13	.70-.80
An_1An_1 An_2an_2 an_3an_3	an_1an_1 An_2an_2 An_3an_3					
(Resistant x Resistant)						
Fla. 399	Concord	31	34	9:7	1.96	.10-.20
an_1an_1 an_2an_2 An_3an_3	an_1an_1 An_2an_2 An_3an_3					
Blue Lake	Concord	12	4	3:1	0.00	1.00
an_1an_1 An_2an_2 An_3an_3	an_1an_1 An_2an_2 An_3an_3					
(Susceptible x Susceptible)						
Lake Emerald	Fla. W987	75	126	21.43	1.83	.10-.20
An_1an_1 An_2an_2 An_3an_3	An_1an_1 An_2an_2 An_3an_3					
Fla. B3-83	Mantey	6	17	21.43	0.45	.50-.70
An_1an_1 An_2an_2 An_3an_3	An_1an_1 An_2an_2 An_3an_3					
Fla. A3-47	Stover	4	6	21.43	0.22	.50-.70
An_1an_1 An_2an_2 An_3an_3	An_1an_1 An_2an_2 An_3an_3					
Fla. A4-37	Stover	6	14	21.43	0.08	.70-.80
An_1an_1 An_2an_2 An_3an_3	An_1an_1 An_2an_2 An_3an_3					
Norris	Schuyler	8	88	1:7	1.52	.20-.30
An_1An_1 An_2an_2 an_3an_3	An_1an_1 An_2an_2 An_3an_3					
Norris	Stover	0	15	1:7	2.18	.10-.20
An_1An_1 An_2an_2 an_3an_3	An_1an_1 An_2an_2 An_3an_3					
Norris	Buffalo	0	12	all S	0.00	1.00
An_1An_1 An_2an_2 an_3an_3	An_1an_1 An_2an_2 an_3an_3					
Mantey	Aurelia	3	15	7:25	0.27	.50-.70
An_1an_1 An_2an_2 An_3an_3	An_1an_1 An_2an_2 an_3an_3					

TABLE 6. Phenotypes and probable genotypes of parents used in this paper and in Fennell's paper.

Cultivar	Phenotype	Probable genotype
Alphonse Lavallée	Susceptible	An_2An_2 an_3an_3
Aurelia	Susceptible	An_2an_2 an_3an_3
Blue Lake	Resistant	An_2an_2 An_3an_3
Buffalo	Susceptible	An_2An_2 an_3an_3
Cabernet Sauvignon	Susceptible	An_2An_2 An_3an_3
Concord	Resistant	An_2an_2 An_3an_3
Extra	Resistant	An_2an_2 An_3an_3
Gros Colman	Susceptible	An_2An_2 An_3an_3
Lake Emerald	Susceptible	An_2an_2 An_3an_3
Malaga	Susceptible	An_2An_2 an_3an_3
Mantey	Susceptible	An_2an_2 An_3an_3
Norris	Susceptible	An_2An_2 an_3an_3
Portland	Resistant	an_2an_2 An_3an_3
Schuyler	Susceptible	An_2an_2 An_3an_3
Stover	Susceptible	An_2an_2 An_3an_3
Thompson Seedless	Susceptible	An_2An_2 an_3an_3
Fenn. 5 (*shutt.*)	Resistant	an_2an_2 An_3An_3
Fenn. 6 (*rufo.* x *shutt.*)	Resistant	an_2an_2 An_3An_3
Fenn. 18 (*gigas*)	Resistant	an_2an_2 An_3An_3
Fenn. 23 (*shutt.* x *rufo.*)	Resistant	an_2an_2 An_3An_3
Fla. A1-13	Resistant	an_2an_2 An_3an_3
Fla. A3-47	Susceptible	An_2an_2 An_3an_3
Fla. A4-37	Susceptible	An_2an_2 An_3an_3
Fla. A4-23	Resistant	an_2an_2 An_3an_3
Fla. B3-83	Susceptible	An_2an_2 An_3an_3
Fla. 399	Resistant	an_2an_2 An_3An_3
V. tiliifolia	Resistant	an_2an_2 An_3an_3
Fla. W382	Resistant	An_2an_2 An_3an_3
Fla. W716	Resistant	An_2an_2 An_3an_3
Fla. W987	Susceptible	An_2an_2 An_3an_3

TABLE 7. Fennell's data (5) on anthracnose resistance supplied with assigned genotypes based on the trigenic hypothesis.

Parental clones and their proposed genotypes		Progeny observed		Expected	Chi-	Probability
Female parent	Male parent	R	S	ratio	square	
(Resistant x Susceptible)						
Fennell 9	Alphonse Lavallée	0	23	all S	0.0	1.00
an_1an_1 an_2an_2 An_3An_3	An_1An_1 An_2An_2 an_3an_3					
Fennell 9	Many *vinifera* parents	0	187	all S	0.0	1.00
an_1an_1 an_2an_2 An_3An_3	An_1An_1 An_2An_2 an_3an_3					
V. tiliafolia	Alphonse Lavallée	0	14	all S	0.0	1.00
an_1an_1 an_2an_2 An_3An_3	An_1An_1 An_2An_2 an_3an_3					
Fennell 5	Feher Szagos	0	13	all S	0.0	1.00
an_1an_1 an_2an_2 An_3An_3	An_1An_1 An_2An_2 an_3an_3					
Fennell 23	[Fennell 9 x Alphonse L.]	113	42	3:1	0.35	.50-.70
an_1an_1 an_2an_2 An_3An_3	An_1an_1 An_2an_2 An_3an_3					
Fennell 23	[*V. tiliafolia* x Alphonse L.]	8	2	3:1	0.32	.50-.70
an_1an_1 an_2an_2 An_3An_3	An_1an_1 An_2an_2 An_3an_3					
Fennell 23	Gros Colman	0	12	all S	0.0	1.00
an_1an_1 an_2an_2 An_3An_3	An_1An_1 An_2An_2 an_3an_3					
Fennell 6	Malaga	0	7	all S	0.0	1.00
an_1an_1 an_2an_2 An_3An_3	An_1An_1 An_2An_2 an_3an_3					
Fennell 18	Malaga	0	19	all S	0.0	1.00
an_1an_1 an_2an_2 An_3An_3	An_1An_1 An_2An_2 an_3an_3					
Fennell 18	[Fennell 9 x Lomanto]	4	6	1:1	0.40	.50-.70
an_1an_1 an_2 an_2 An_3An_3	An_1An_1 An_2an_2 An_3an_3					
Fennell 18	Cabernet Sauvignon	0	64	all S	0.0	1.00
an_1an_1 an_2an_2 An_3An_3	An_1An_1 An_2An_2 an_3an_3					
(Resistant x Resistant)						
Fennell 23	Portland	27	0	all R	0.0	1.00
an_1an_1 an_2an_2 An_3An_3	an_1an_1 an_2an_2 An_3an_3					
Fennell 18	Extra	7	0	all R	0.0	1.00
an_1an_1 an_2an_2 An_3An_3	An_1an_1 an_2an_2 An_3an_3					

V. munsoniana, the latter two with 2n=40. Named cultivars with *V. labrusca* parentage that are good sources of resistance are Blue Lake, Caco, Champanel, Concord, Delaware, Liberty, Ontario, and Urbana. None of the *V. vinifera* cultivars tested at Leesburg were resistant, but the following grape cultivars have been reported as resistant: Bangalore Blue, Black Cornichon, and Gulabi (6); Earlihane (4), Golden City and Jakaranda (both Queen of the Vineyard x Pearl of Csaba) (3), Muska and Pirobella (both Pirovano 15 x Isabella) (2). While none of these cultivars with reported resistance have been adequately tested for anthracnose resistance in Florida, plans to do so are now under way.

It is possible to obtain resistant segregants by crossing two susceptible cultivars provided that An_3 is present in at least one parent and that neither parent is homozygous for both An_1 and An_2. Unfortunately, *V. vinifera* is the principal source of fruit quality and most *vinifera* cultivars are homozygous for both An_1 and An_2. In order to use *V. vinifera* in crosses, we need to select the least susceptible F_1 segregants of resistant x *vinifera* for further use as parents because they are heterozygous at the An_1 and An_2 loci and are likely to carry gene An_3.

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RESISTANCE TO ROOT-KNOT NEMATODES IN EUVITIS X MUSCADINIA HYBRIDS

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ABSTRACT

Resistance of container-grown vines to root-knot nematodes was assessed. Shoot growth of a *Vitis vinifera* cultivar was equally suppressed after single inoculations with three *Meloidogyne* species (*M. incognita, M. arenaria,* or *M. javanica).* These nematode species galled roots and increased in numbers to different degrees. Parallel inoculations of three *Muscadinia* cultivars was unrelated to variability in shoot growth and roots remained free of galls. Nematodes were undetectable on *Muscadinia* cultivars four months after inoculation.

Muscadinia resistance to *M. incognita* was not found among several *Euvitis-Muscadinia* hybrid progenies derived from *Euvitis* backcrosses. On quasi-F_1 hybrids produced from matings between a susceptible *Euvitis* backcross selection and several *Muscadinia* cultivars, nematode populations declined from initial levels. Although some development of nematodes was observed on several of these quasi-F1s, others appeared equally resistant to the three *Meloidogyne* species as is *Muscadinia.*

The subgenus *Muscadinia* Planch. has long been recognized as a potential donor of valuable traits in hybridization with *Euvitis* Planch. (6,23). One such *Muscadinia* trait, historically inaccessible in graft combination with *Euvitis* scions due to incompatibility (5,31), is resistance to root knot caused by nematodes in the genus *Meloidogyne* (Goeldi) Chitwood. Though root knot is considered a principal limiting factor to viticulture in warm climates (15,18,24), especially in nurseries (29) and in replant situations (25,26,27), its effects on vine growth and production have yet to be clearly characterized experimentally (8). Root knot has never been reported on *Vitis rotundifolia* Michx. (*Muscadinia*) roots under vineyard conditions in North Carolina (3). In greenhouse inoculations with several common root-knot nematode species, *V. rotundifolia* consistently remained free of both galls and evidence of infection (3,14,22). In contrast, well characterized *Euvitis* forms exhibit a range of reactions varying from high susceptibility, in *V. vinifera* L. (28), to high resistance as found in the commerical rootstocks derived from *V. champini* Planch. and *V. solonis* Hort. Berol. (15,28). Even the most extensively used *Euvitis*-derived stocks, however, can serve as hosts to some *Meloidogyne* populations (9,15,32).

F_1 hybrids between *V. rotundifolia* and susceptible *Euvitis*

clones not only appear fully graft compatible with *Euvitis* scions (5,16,17), but also are vigorous and retain a high level of root-knot nematode resistance, so far indistinguishable, in some hybrids, from that of *V. rotundifolia* (22). Most of the F1s, though, possess certain flaws which may preclude their use as commercial rootstocks (5,19). The occurrence of partial fertility in some hybrid forms (7,11,12) makes possible genetic investigations into the feasibility of breeding desirable traits from both subgenera into improved rootstocks for *Euvitis* scions. The eventual combination, in *Euvitis-Muscadinia* hybrid cultivars, of high quality *V. vinifera* fruit characters with *Muscadinia* resistance, would contribute to the elimination of the costly grafting operation.

The objective of this investigation was to determine the level of resistance present in several advanced-generation *Euvitis-Muscadinia* progenies and certain other hybrids to *M. incognita* (Kofoid and White) Chitwood. Additionally, resistance to the three prevalent warm-climate *Meloidogyne* species, *M. incognita, M. arenaria* (Neal) Chitwood, and *M. javanica* (Treub) Chitwood, was studied in selected *Euvitis* and *Muscadinia* cultivars. The criteria for assessing resistance were nematode reproduction, root galling and plant growth response.

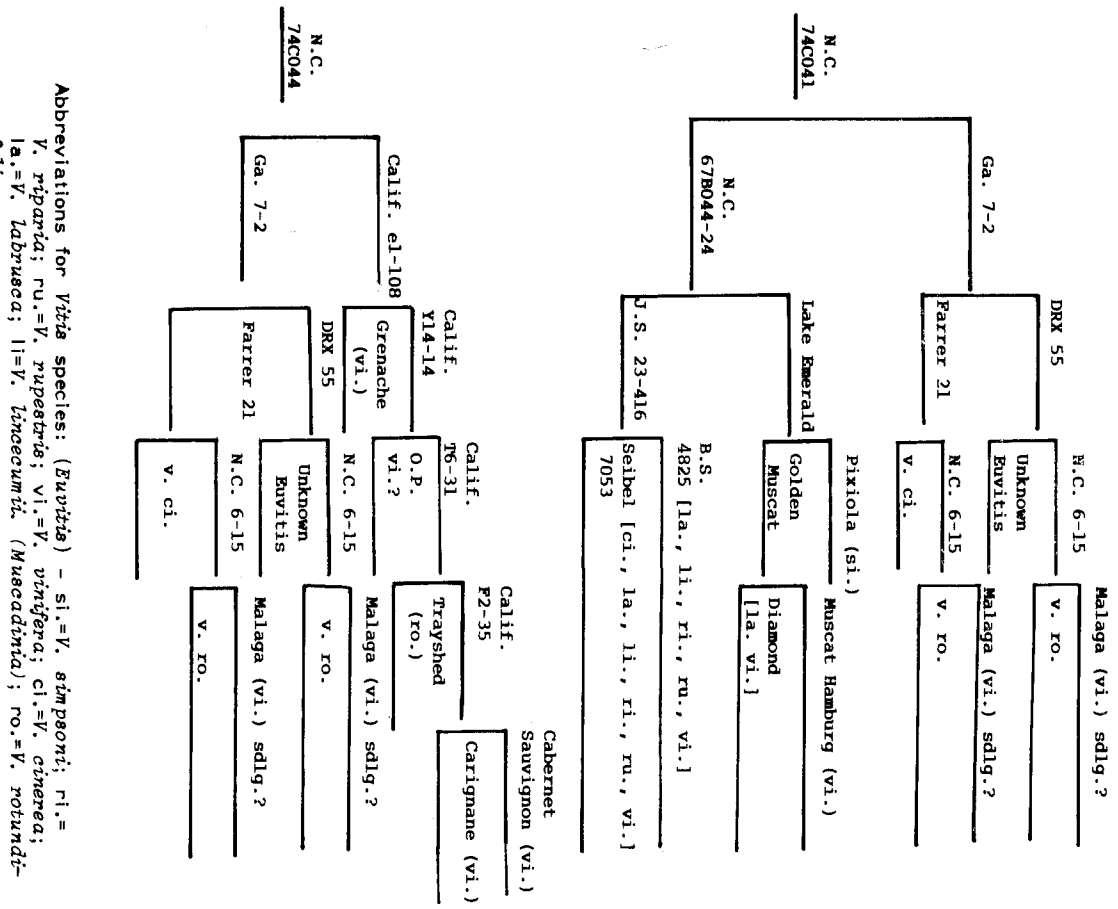
MATERIALS AND METHODS

Experiment No. 1: From each of two advanced-generation *Euvitis-Muscadinia* (E-M) hybrid progenies (Fig. 1), 25 seedlings were randomly selected for inoculation with *M. incognita.* These seedlings, already established in the field, were vigorous and fruitful, with no obvious morphological marks of *V. rotundifolia.* Additional cultivars and selections inoculated concomitantly (Table 1) were Alden and Couderc 1613, serving, respectively, as susceptible and resistant *Euvitis* standards, Sterling and Magnolia, both *V. rotundifolia* cultivars and, if available, the parents and grandparents of both E-M hybrid progenies. F_1 and first-generation backcross clones were not available. Included as substitutes for F1s were five seedlings derived from crossing Calif. e4-12, a second-generation *Euvitis* backcross seedling, with either Sterling or Magnolia (Table 1). These were termed "quasi-F1s" due to the wholly *Euvitis* aspect of Calif. e4-12 and to distinguish them from true E-M F_1 hybrids.

Vines were propagated from softwood cuttings and grown in 15-cm diameter pots containing a pasteurized 1:1 mixture of sandy loam soil and 60-mesh silica sand. Lateral growth was removed and a single shoot on each plant was trained to a 120-cm stake. Inocula, consisting of nematode eggs surface-sterilized with 0.53% NaOCl (10), were reared on greenhouse-grown tomato plants.

Two treatments were imposed across all entries: 1) check (no nematodes), replicated three times, and 2) inoculation with 20,000 *M. incognita* eggs, replicated five times. A day/night ambient temperature of 30/26°C was maintained throughout the experiment.

Three months after inoculation, data were taken on fresh weight of shoots and roots, nematode numbers, and galling severity. Juveniles (larvae) were extracted from a 500-cm^3 sample of each vines's growth medium by a combination of semi-automatic



Abbreviations for *Vitis* species: (*Euvitis*) - si.=*V. simpsoni*; ri.=*V. riparia*; ru.=*V. rupestris*; vi.=*V. vinifera*; ci.=*V. cinerea*; la.=*V. labrusca*; li=*V. lincecumii*. (*Muscadinia*); ro.=*V. rotundifolia*.

Fig. 1. Genealogies of *Euvitis-Muscadinia* hybrid progenies in Experiment No. 1.

TABLE 1. Additional *Vitis* clones in Experiment No. 1.

Cultivar or selection	Subgeneric[a] classification[a]	Parentage or species[b]
Alden	E	*V. labrusca, V. vinifera*
Couderc 1613	E	*V. solonis* x Othello (ri., la., vi.)
Lake Emerald	E	See Fig. 1
J.S. 23-416	E	See Fig. 1
Sterling	M	*V. rotundifolia*[c]
Magnolia	M	*V. rotundifolia*[c]
Ga. 7-2	E-M	See Fig. 1
Calif. e4-12	E-M	Calif. Y14-23[d] x Grenache (vi.)
N.C. 74C048-11	E-M	Calif. e4-12 x Sterling
N.C. 74C048-15	E-M	Calif. e4-12 x Sterling
N.C. 74C048-17	E-M	Caif. e4-12 x Sterling
N.C. 74C048-19	E-M	Calif. e4-12 x Sterling
N.C. 74C049-8	E-M	Calif. e4-12 x Magnolia

[a] E = *Euvitis*; M = *Muscadinia*.
[b] Abbreviations for *Vitis* species: vi. = *V. vinifera*; ri. = *V. riparia*; la. = *V. labrusca*.
[c] *V. munsoniana* also in ancestry.
[d] Calif. Y14-23 = Calif. T6-38 [Calif. F2-35 (vi.) x Trayshed (*V. rotundifolia*)] x O.P. (vi.?).

elutriation (2), centrifugal flotation (13) and sieving. Eggs were extracted from a representative 5-g sample of each plant's roots by dissolution of egg masses with 0.53% NaOCl and mechanical agitation, followed by sieving (1). Samples were then counted at 30X magnification whereupon an estimate of the total numbers of eggs and juveniles per plant were calculated.

Experiment No. 2: One *Euvitis* and three *Muscadinia* cultivars comprised the experimental entries in another study (Table 2). Except for check plants, each experimental entry was inoculated singly with three *Meloidogyne* species *(M. incognita, M. arenaria,* or *M. javanica)* at each of three levels (10^3, 10^4, or 10^5 eggs per plant). All treatment combinations were replicated four times.

Observational entries (Table 2), excluding checks, received 10^5 eggs per plant in single inoculations with the same three nematode species. Treatment combinations were unreplicated except as indicated in Results and Discussion. In this experiment, plant spacings were increased to lessen the risk of contamination between inoculated plants during watering. The experiment was terminated four months after inoculations at which time the harvest procedures described previously were repeated.

To reduce the heterogeneity of variance of nematode population and gall data, logarithmic transformations $[\log_{10}(X+1)]$ were used in statistical analyses.

RESULTS AND DISCUSSION

Experiment No. 1: On the basis of nematode population size at the termination of the experiment, two broad groups emerged: a susceptible group in which populations grew and a resistant group in which populations declined from the initial level (Fig. 2). This separation was corroborated by root gall means (Fig. 2). Unfortunately, segregation for the resistant *Muscadinia* phenotype did not occur within either advanced E-M hybrid progeny. Together with their represented ancestors, they were statistically no more resistant than Alden, the susceptible standard. One explanation for this result, given the high resistance exhibited in some F_1 hybrids (22), is that *Muscadinia* resistance is dominant and monogenic, but the essential factor was transmitted neither to the parental clones in question, namely Calif. e1-108 and Ga. 7-2, nor to Calif. e4-12. This is not unlikely among such a small sample of hybrids produced by backcrossing to *Euvitis* suscepts when selection for resistance was weak or absent (21). Alternatively, *Muscadinia* resistance may be complex. Under this hypothesis, some *Muscadinia* resistance factors may have been present in the advanced hybrids, but their effects were too small to confer a high level of resistance. Studies of first generation backcrosses to *V. vinifera* are needed to elucidate the mode of inheritance of *Muscadinia* resistance.

It appeared at first, from assay results, that *M. incognita* was capable of reproducing to some extent on all resistant entries including, unexpectedly, *V. rotundifolia* (Fig. 2). The eggs and juveniles in assays from resistant plants were not residual inocula. Only those nematode eggs produced on and adhering to roots are extracted by the egg assay, and juveniles were never

TABLE 2. *Vitis* clones in Experiment No. 2.

Cultivar or selection	Subgeneric classification[a]	Parentage[b] or species
Experimental entries		
Primiera	E	*V. vinifera*
Dixie	M	*V. rotundifolia*[c]
Magnolia	M	*V. rotundifolia*[c]
Sterling	M	*V. rotundifolia*[c]
Observational entries		
N.C. 66B158-19	E	*V. candicans*
Couderc 1613	E	See Table 1
Calif. T6-38	E-M	Calif. F2-35 (vi.) x Trayshed (ro.)
N.C. 74C048-12	E-M	Calif. e4-12 x Sterling
N.C. 74C048-16	E-M	Calif. e4-12 x Sterling
N.C. 74C048-18	E-M	Calif. e4-12 x Sterling
N.C. 74C048-20	E-M	Calif. e4-12 x Sterling
N.C. 74C049-10	E-M	Calif. e4-12 x Magnolia
N.C. 74C049-11	E-M	Calif. e4-12 x Magnolia

[a] E = *Euvitis*; M = *Muscadinia*.
[b] Abbreviations for *Vitis* species: vi. = *V. vinifera*; ro. = *V. rotundifolia*.
[c] *V. munsoniana* also in ancestry.

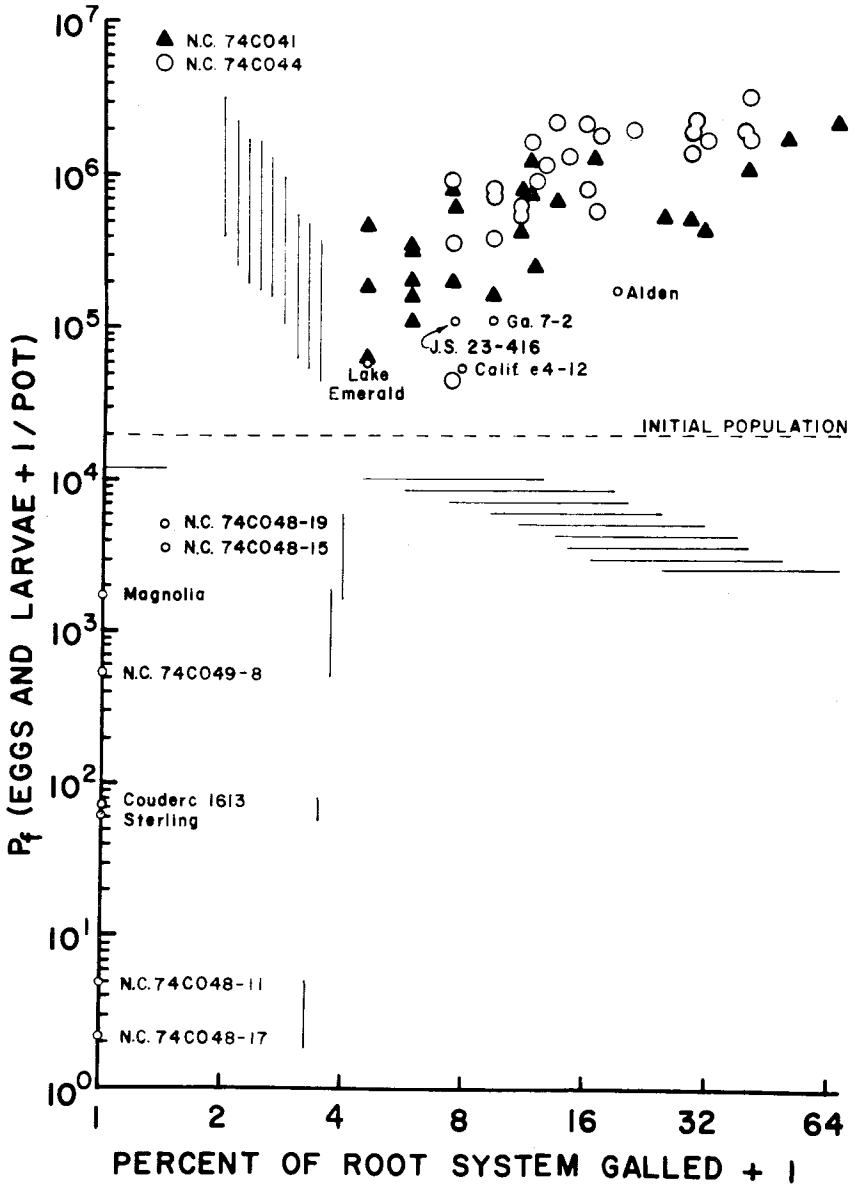


Fig. 2. Percent of root system galled and total final nematode population (P_f) of *Vitis* clones three months after inoculation with 20,000 *Meloidogyne incognita* eggs. Means of five replications. Values overlapped by the same horizontal or vertical line do not differ in percent of root system galled or total final population, respectively. (P = 0.05, Duncan's Multiple Range Test).

found in soil assays where they had been deprived of a host for the duration of an experiment. Subsequently, contamination was implicated in that 3% of assays from uninoculated check plants contained nematodes in numbers comparable to those in assays from the inoculated resistant plants. On only two resistant entries (N.C.74C048-15 and N.C.74C048-19), both quasi-F_1s, was there very strong evidence of successful parasitism as judged by consistent appearance of eggs in assays, large populations relative to other resistant entries, and macropscopic signs of nematodes on the root systems (Table 3). Despite the sporadic occurrence of nematode eggs and juveniles in assays from Couderc 1613, *V. rotundifolia*, and the remainder of the quasi-F_1s, on none of these clones were there macroscopically evident galls, females, or egg masses (Fig. 2, Table 3).

Inoculation of plants with nematodes had no significant effect on the mean fresh weight of any entry, probably because the vines never suffered from apparent moisture or nutrient stress (20) or because attempts to confine growth during training suppressed any potential differences.

Experiment No. 2: There were no detectable galls or populations of *Meloidogyne* species (Table 4) on *V. rotundifolia* cultivars. These results are in agreement with earlier reports and observations indicating that *V. rotundifolia* possessess high resistance, if not immunity, to *Meloidogyne* species (3,4,14, 22,23). In contrast, *V. vinifera* Primiera, apparently like all cultivars of this species (28), was a good host. Nematode populations increased on Primiera but to differing levels depending on quantity of inoculum and *Meloidogyne* species (Table 4, Fig. 3). *M. incognita*, at all initial numbers, multiplied at a greater rate than did *M. javanica*, whose rate of multiplication was greater than that of *M. arenaria*. The smaller final population of *M. incognita*, at the highest inoculum level in relation to the intermediate level, may have resulted from plant stunting or from sex reversal of potential females that occurs with crowding of this parasite in host roots (30).

Despite its relatively low reproductive potential on Primiera, *M. arenaria*, by far, induced the heaviest galling of the three nematode species. Regardless of inoculum level, it galled about 20% of the root system; whereas, both *M. incognita* and *M. javanica* galled only about 5% of roots.

Shoot growth of *V. rotundifolia* cultivars was highly variable and unrelated to inoculation with nematodes (Table 5). The fresh shoot weight of Primiera, however, was suppressed by about 2 g for every increase in the inocula by a power of 10, irrespective of *Meloidogyne* species (Table 5, Figs. 4,5). These results provide experimental support for the suspected pathogenicity of *Meloidogyne* species to *V. vinifera* (8,15,18,24).

When observational entries were assayed, no populations of *M. arenaria* nor juveniles of *M. incognita* and *M. javanica* were found. The observational *Euvitis* entries appeared as resistant to the *Meloidogyne* species as *V. rotundifolia* (Table 6). Earlier reports indicated that certain *Meloidogyne* populations parasitize Couderc 1613 (9,15,27,32) or can parasitize the *Euvitis* species, *V.*

TABLE 3. Final nematode populations as indicated by assays on resistant *Vitis* clones three months after inoculation with 20,000 *Meloidogyne incognita* eggs; the relation between occurrence of nematodes in assays and macroscopic detection of females and egg masses.

Cultivar or selection	No. of plants assayed	P_f mean[a]	No. of assays: With eggs	With juveniles	With eggs and juveniles	♀♀ or egg masses
			Euvitis			
Couderc 1613	5	1,022	3	2	2	No
			Muscadinia			
Sterling	3	2,710	1	1	0	No
Magnolia	2	2,113	1	1	0	No
			Quasi-F_1			
N.C. 74C048-11	5	729	1	0	0	No
N.C. 74C048-15	5	11,334	5	1	1	Yes
N.C. 74C048-17	5	14	0	1	0	No
N.C. 74C048-19	5	15,178	5	2	2	yes
N.C. 74C049-8	5	701	5	3	3	No

[a]Arithmetic mean of the estimated number of eggs and juveniles per plant; values differ from geometric mean antilogs shown in Fig. 2.

TABLE 4. Mean final nematode populations (eggs and juveniles/plant in thousands) on *Vitis* cultivars four months after single inoculations with *Meloidogyne incognita*, *M. arenaria* or *M. javanica* at each of three levels (10^3, 10^4 or 10^5 eggs/plant). (Means of four replications).

Cultivar	Check	*M. incognita*			*M. arenaria*			*M. javanica*		
		10^3	10^4	10^5	10^3	10^4	10^5	10^3	10^4	10^5
					Euvitis					
Primiera	0	140	594	445	54	111	156	110	190	335
					Muscadinia					
Dixie	0	0	0	0	0	0	0	0	0	0
Magnolia	0	0	0	0	0	0	0	0	0	0
Sterling	0	0	0	0	0	0	0	0	0	0

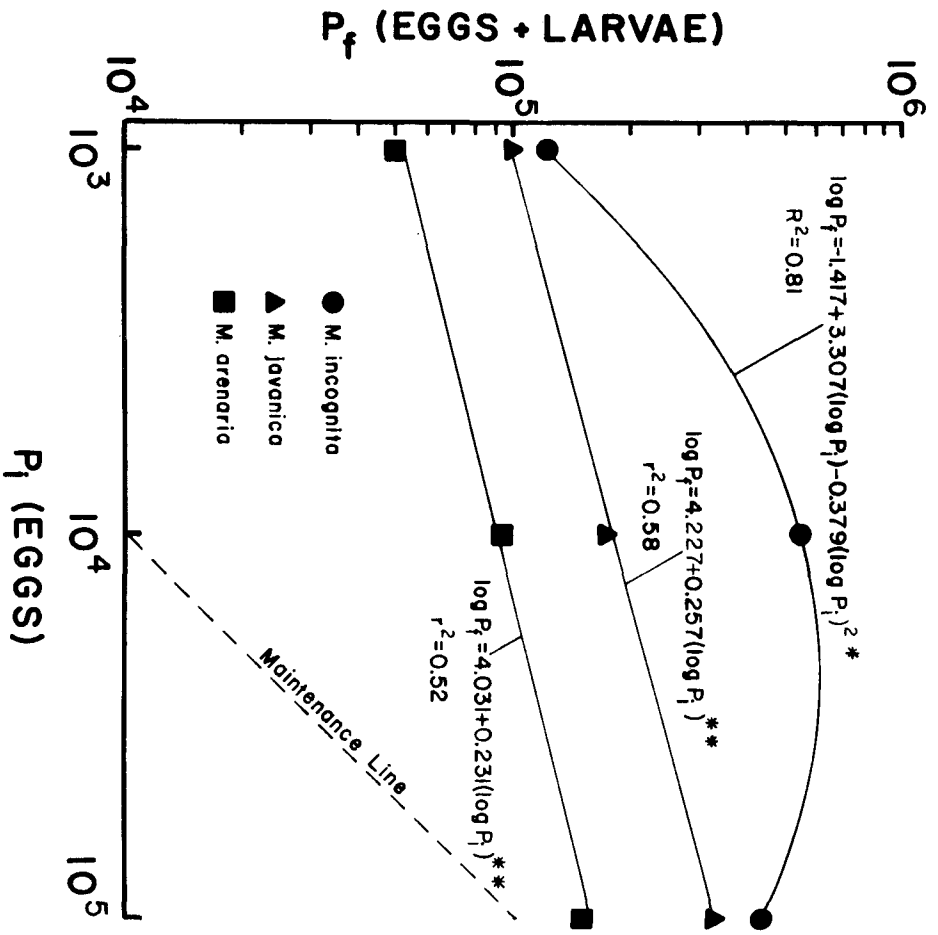


Fig. 3. Relation between initial population (P_i) and final population (P_f) per plant of *Meloidogyne* spp. on *V. vinifera* Primiera four months after inoculation. (means of four replications).

TABLE 5. Mean growth [fresh shoot wt. (g)] of *Vitis* clones four months after separate inoculations with *Meloidogyne incognita*, *M. arenaria* or *M. javanica* eggs at each of three levels (10^3, 10^4, or 10^5 eggs/plant). (Means of four replications).

Cultivar	Check	*M. incognita*			*M. arenaria*			*M. javanica*		
		10^3	10^4	10^5	10^3	10^4	10^5	10^3	10^4	10^5
					Euvitis					
Primiera	46.8	44.1	41.4	34.5	38.5	39.1	32.3	37.7	30.7	36.1
					Muscadinia					
Dixie	16.1	18.1	21.6	8.9	20.5	23.2	10.0	20.1	23.7	6.4
Magnolia	38.9	46.6	39.4	35.8	37.6	36.5	37.5	39.9	31.1	40.9
Sterling	45.0	43.4	50.5	-	34.8	41.8	35.8	34.5	47.7	36.9

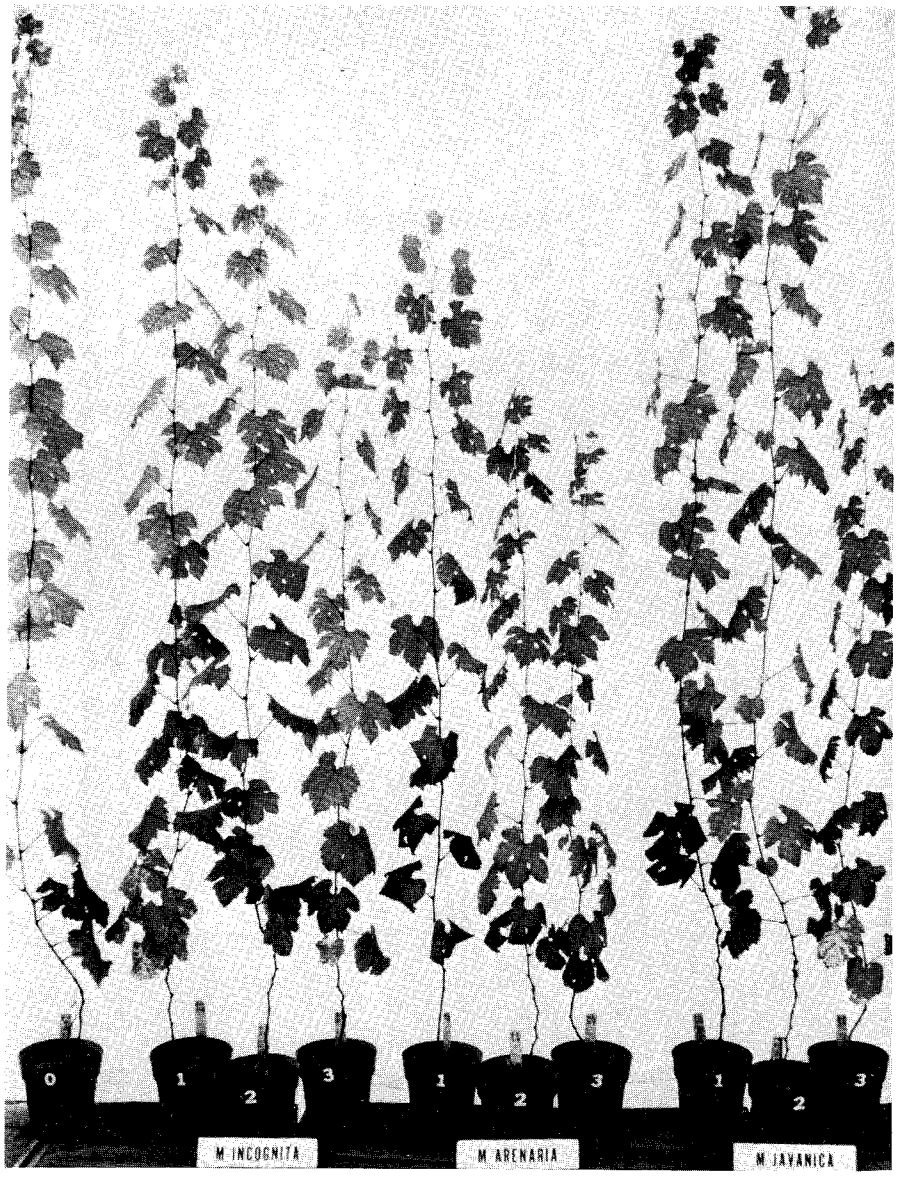


Fig. 4. Growth of *V. vinifera* Primiera four months after single inoculations with *Meloidogyne incognita*, *M. arenaria* or *M. javanica* at each of three levels, (0 = check, 1 = 10^3 eggs/plant, 2 = 10^4 eggs/plant, 3 = 10^5 eggs/plant).

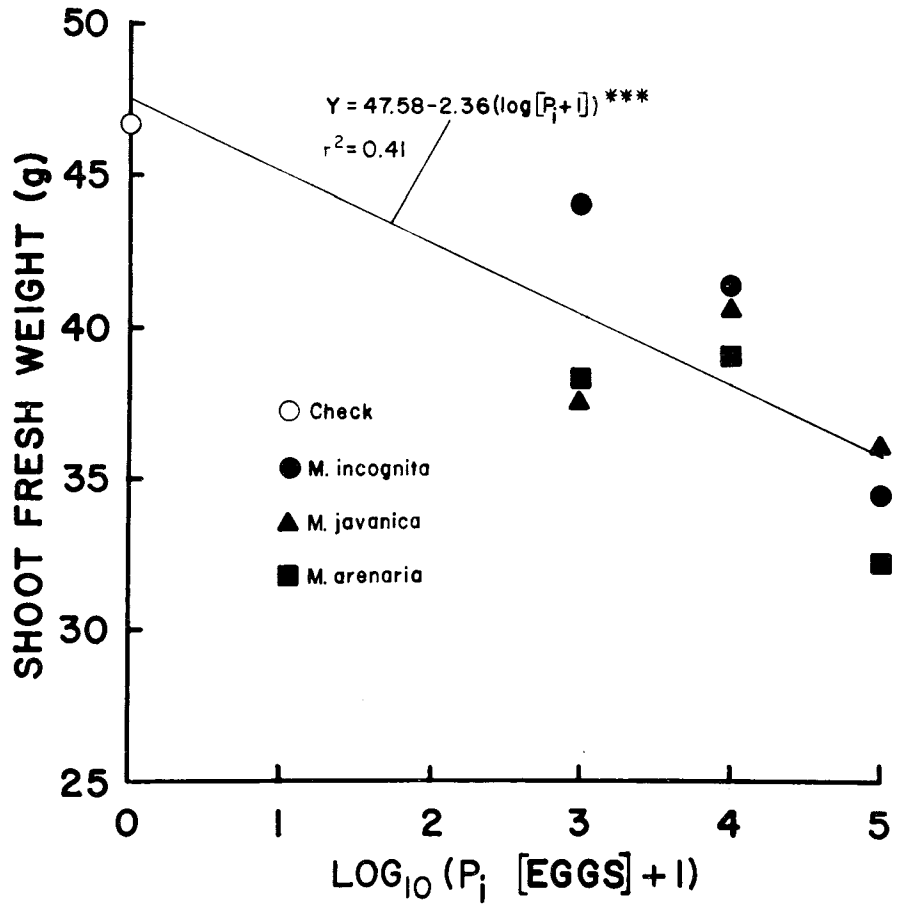


Fig. 5. Relation between single inoculations with three *Meloidogyne* spp. at three levels (P_i = 10^3, 10^4, or 10^5 eggs/plant) and fresh shoot weight (g) of *V. vinifera* Primiera four months after inoculation. (Means of four replications).

TABLE 6. Final nematode populations as indicated by assays on observational *Vitis* clones four months after inoculations with 10^5 *Meloidogyne incognita* or *M. javanica* eggs; the relation between occurrence of nematode eggs in assays and macroscopic detection of females and egg masses.

Cultivar or selection	*M. incognita*				*M. javanica*			
	No. of plants assayed	P_f mean[z]	No. of assays with eggs	♀ ♀ or egg masses seen	No. of plants assayed	P_f mean[a]	No. of assays with eggs	♀ ♀ or egg masses seen
Euvitis								
Couderc 1613	1	0	0	No	1	0	0	No
N.C. 66B158-19 (*V. candicans*).	1	0	0	No	1	0	0	No
Euvitis-Muscadinia								
F_1								
Calif. T6-38	1	0	0	No	1	0	0	No
Quasi-F_1								
N.C. 74C048-12	2	0	0	No	1	0	0	No
N.C. 74C048-16	2	28,075	2	Yes	1	0	0	No
N.C. 74C048-18	1	0	0	No	1	0	0	No
N.C. 74C048-20	1	0	0	No	1	0	0	No
N.C. 74C049-10	2	0	0	No	0	7,418	1	No
N.C. 74C049-11	1	0	0	No	1	0	0	No

[a]Final population: arithmetic mean of the estimated number of eggs per plant at the termination of the experiment.

champini and *V. solonis*, but not *V. rotundifolia* (14). Therefore, broad samples of *Meloidogyne* species are probably needed to make comparative evaluations of *Euvitis* and *Muscadania* resistances.

Egg assays indicated again that some quasi-F_1s were supporting low levels of nematode reproductions (Table 6). These assay results were confirmed by microscopic examination of preserved roots. The complete absence of nematodes, both in assays and in microscopic examination of other quasi-F_1s, strongly suggests the existence of genetic variability for resistance among quasi-F_1s. Similar variability of E-M F_1 hybrids was noted in single inoculations with *M. incognita* and *M. javanica* (22).

Although nematode populations on quasi-F_1s were always smaller than the initial inoculum level, their presence could represent selection of a virulent genotype in only the initial stages of increase. On at least one quasi-F_1, N.C. 74C049-10, it appeared, to the contrary, that populations were unstable and diminishing. Six weeks after inoculation, microscopically examined root samples from this hybrid revealed an egg mass of *M. incognita* and several developing females of *M. arenaria*. At the termination of the experiment, 12 weeks later, the very same plants were devoid of symptoms, signs, or populations of both *Meloidogyne* species (Table 6). Thus, had the experiment been prolonged, populations of *M. javanica* might eventually have disappeared (Table 6).

Muscadinia resistance to *Meloidogyne incognita*, in some quasi-F_1 hybrids, appears to be completely dominant, in that Calif. e4-12, parent to the quasi-F_1s, was susceptible (Fig. 2). Calif. T6-38, the *V. vinifera* x *V. rotundifolia* F_1 grandparent to Calif. e4-12, also appeared completely resistant (Table 6), indicating that an essential factor (or factors) for resistance was not transmitted to Calif. e4-12 in backcrossing to *V. vinifera*. Partially fertile E-M F_1 and quasi-F_1 hybrids are now being used in controlled pollinations to produce first-generation backcrosses to *Euvitis*. These hybrids should provide valuable information on the feasibility of transferring *Muscadinia* resistance into *Euvitis*.

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SELECTED VINE CLONES AS SOURCES OF RESISTANCE TO DOWNY MILDEW

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ABSTRACT

European vine resistance to downy mildew has been studied for a long time by one of the authors, with promising results obtained by intraspecific crossing and induced mutation. This paper reports the trial of some of these selected plants, not only in culture but also as a source of resistance to *Plasmopara*, in a breeding program for Madeira Islands. The behavior of the resistant plants was tested under the ecological conditions of the Islands and the results are presented. For the transfer of resistance, backcrosses with the traditional varieties Boal, Malvasia Candida and Sercial were employed. At present, the protoplastic fusion method is being explored. The importance of the old varieties in maintaining the quality of Madeira wine and the very favorable climatic conditions of the Islands for mildew infection justify this research.

The Agricultural Services of the Madeira Islands are highly interested in the replantation of the traditional varieties. At present, there are very few plants of these varieties; and it is, consequently, very important to search for these vines useful in breeding to contribute germ plasm. We found, for example, in Feja dos Padres two plants of the cultivar Malvasia Candida with very typical characteristics and in Porto Santo Island one plant of the old variety Verdelho Roxo that we thought had disappeared completely from Madeira.

A long-term future plan will also consider other aspects such as the downy mildew problem. This paper reports on some resistant *vinifera* clones selected by us particularly as a source of resistance in the vine breeding program. Mildew in Madeira is very important and the losses can be high, due to the very favorable climatic conditions for the development of the disease.

Some years ago the Agricultural Service of the region included in field trials near Lisbon some of our clones obtained by intraspecific crossing and induced mutation and selected as resistant to *Plasmopara*.

In spite of the great differences in ecological conditions, these plants have maintained resistance during many years in the Islands. Table 1 presents results obtained by technical workers of the region in four vineyards in different years.

Vinifera resistance to mildew is not diminished by changes in

TABLE 1. Behavior of the resistant clones to *Plasmopara*.

Crosses (*Vinifera* var.)	Clone No.	Field trials: Madeira Islands			
		Enxurros	Ribeira Brava	Bom Sucesso	Seixal
	4	R	R		NM
	9	R		R	NM
Jean	19	R	R	R	NM
x	26	R	R		
Azal branco	27	NM	-R		NM
	28	NM	-R	-R	
	34		-R	-R	NM
	35		NM		R
Souzao	48	R	NM	R	
x	59			R	
Azal de Correr	66	-R	NM		-R
	67		NM		-R

R = highly resistant, -R = resistant, and NM = no mildew.

TABLE 2. Classes of resistance.

VINES	Sources of inoculum.*						
	A	B	C	D	E	F	G
Rupestris	1	-2	+1		1	+1	
Bour. x R.93-5	2		2	-3	2		+2
C.19	+1	2	2	-2	+2		2
Dedo de Dama	5	+4		5	-5		
Vinhão	3		-4	3		-3	4
Azal	+2	3		-3	+3		
Assario	4		-5	4		+4	-4

*Sources of Inoculum: A., Aveiro; B., Cal Rainha; C., Evora; D., Faro; E. Mealhada; F., Santarém, and G. Torres Vedras.

TABLE 3. Covering of spores.

Varieties	Observ.	Leaf area (microscopic field)[a]			
		Infect.	Sporul.	No. Spor.	%Spor.
	I	400	360	40	90
Italia	II	400	381	19	95
(Susceptible)	III	349	259	90	74
	IV	344	305	39	89
	I	350	51	299	15
20-V:C.19	II	242	43	199	18
(Resistant)	III	183	45	138	25
	IV	258	42	216	16

[a]Unit = 0.03 mm^2.

TABLE 4. Crosses.

♀ Varieties as female parent	Origin	♂ Pollen parent
Boal	Madeira	Selected clone
Malvasia Candida		
Sercial		C.27
Verdelho		(Jaen x Azal branco)

environmental factors, since it is controlled by a polygenic system. From the practical point of view, we did not find any variation in resistance induced by different behavior of the fungus with sources of the inoculum from different hosts. The results are very similar (Table 2).

However, the degrees of this resistance are very much influenced by external conditions, thus justifying the interest of the heritability determination. This quantitative resistance is revealed by limited leaf spot infection and, as I showed at the last Symposium, usually with limited sporulation.

In Table 3 are presented recent results of quantitative differences in the density of conidia on the leaf spots of susceptible and resistant plants. We have also observed another aspect of resistance: the infection patches are limited by necrotic tissues. The necrotic tissues are marginal and not central as is usual in the susceptible plants. It is a curious aspect which resembles the "ringspot" type.

For a viticultural reorganization program, it is obviously important to use virus-free plants; but, unfortunately, many of the vines of the old varieties are infected by virus. Hence, for this purpose and considering the small quantity of the material, we use the culture *in vitro* with the Rose-Galzy medium. This material is very amenable to heat therapy, in which we use the carbon dioxide enrichment method, considered favorable for vine development in culture.

Concerning the problem of downy mildew, we believe it is important to have vines with some degree of resistance, because of the favorable climatic conditions for infection in the Islands. Vine breeders should now utilize these sources of resistance for improving traditional cultivars.

Two reasons justify the interest in the use of our selected clones: 1) the lack of "*Plasmopara* resistance" sources in the usual varieties of *Vitis vinifera*, and 2) the knowledge of the behavior of these plants in the Madeira Islands. The old varieties Boal, Malvasia Candida, Sercial and Verdelho were crossed with clone C.27 selected as resistant to downy mildew and obtained from the European cultivars Jaen and Azal branco. According to the opinion of some regional workers, this clone is also resistant to *Oidium* (powdery mildew) (Table 4).

This material will now be backcrossed with the old varieties. The present research includes protoplastic fusion and the use of resistant plants derived from induced mutations.

A GUIDE FOR SYSTEMATIC VIRUS-TOLERANCE SELECTION IN VITIS VINIFERA VARIETIES

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ABSTRACT

Virus tolerance may be of great economical importance because it is virtually impossible to eradicate virus reservoirs acting as sources of inoculum and the vectors (e.g., nematodes). The use of soil fumigants is already restricted in certain countries and is likely to become more so in the future because of their polluting abilities. A search for tolerant *Vitis vinifera* varieties and clones should be made because no source of genetic resistance or immunity has been found in *Vitis vinifera*. A guide for making a systematic virus-tolerance selection program is proposed.

There are plants which can tolerate an infectious disease better than others. This is also true in some grapevines which have become virus infected. So-called virus-tolerance has been described as the situation where the virus multiplies and spreads widely through the plant, but produces mild or negligible damage. Toleration of the virus resulting in less development of disease appears compatible with toleration of disease resulting in less damage (3); Matthews (2) noted that tolerance to certain viruses has been described for about 30 crops.

Virus-tolerance may be of great economical importance because it is quite impossible to exterminate virus sources and vectors (e.g., nematodes) completely. The use of liquid soil fumigants will be restricted more and more in consequence of environmental pollution. A search for tolerant *Vitis vinifera* varieties and clones should be made because no source of genetic resistance or immunity has been found to date in this species.

Experiences and observations: The question of tolerance of grape varieties and clones to grapevine viruses is not easily answered. There are several viruses and some of them have more than a single strain. In the course of some work, Hewitt (1), Vuittenez (4) and Goheen (personal communication) have made certain observations on varietal reactions, but they have little precise data. In general, varieties of *Vitis vinifera* are the only ones that express tolerance to the grape fanleaf virus. *Vitis* species from other parts of the world have reacted severely to the presence of the fanleaf virus strains.

Some new German grape selections of *Vitis vinifera* breeding lines don't show any reaction when infected with the arabis mosaic virus (Stellmach, unpublished). All *Vitis vinifera* varieties are

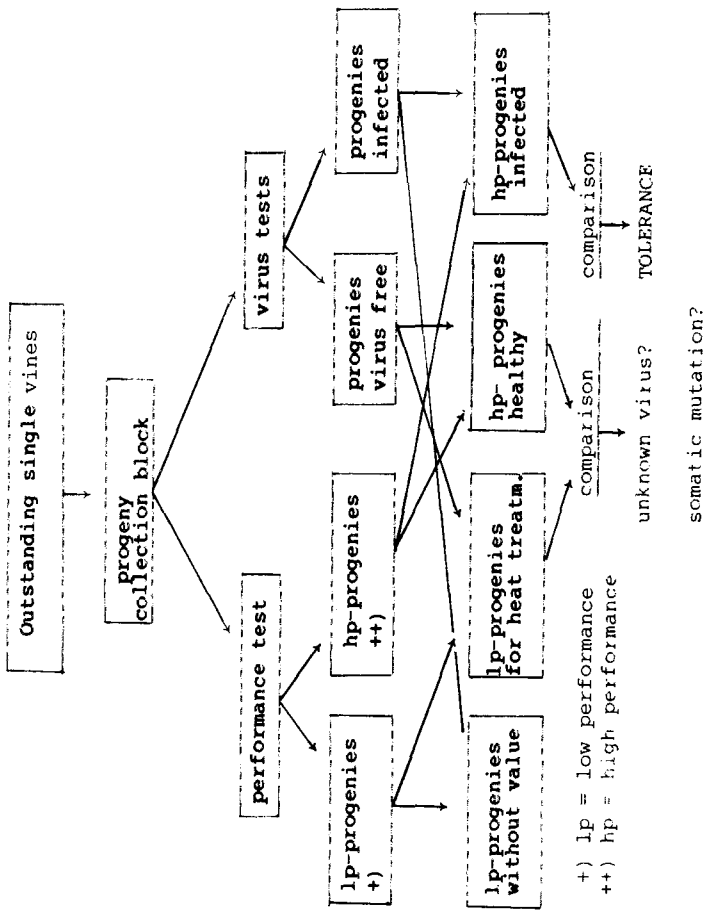

Fig. 1. Method of selecting virus tolerant varieties of high performance.

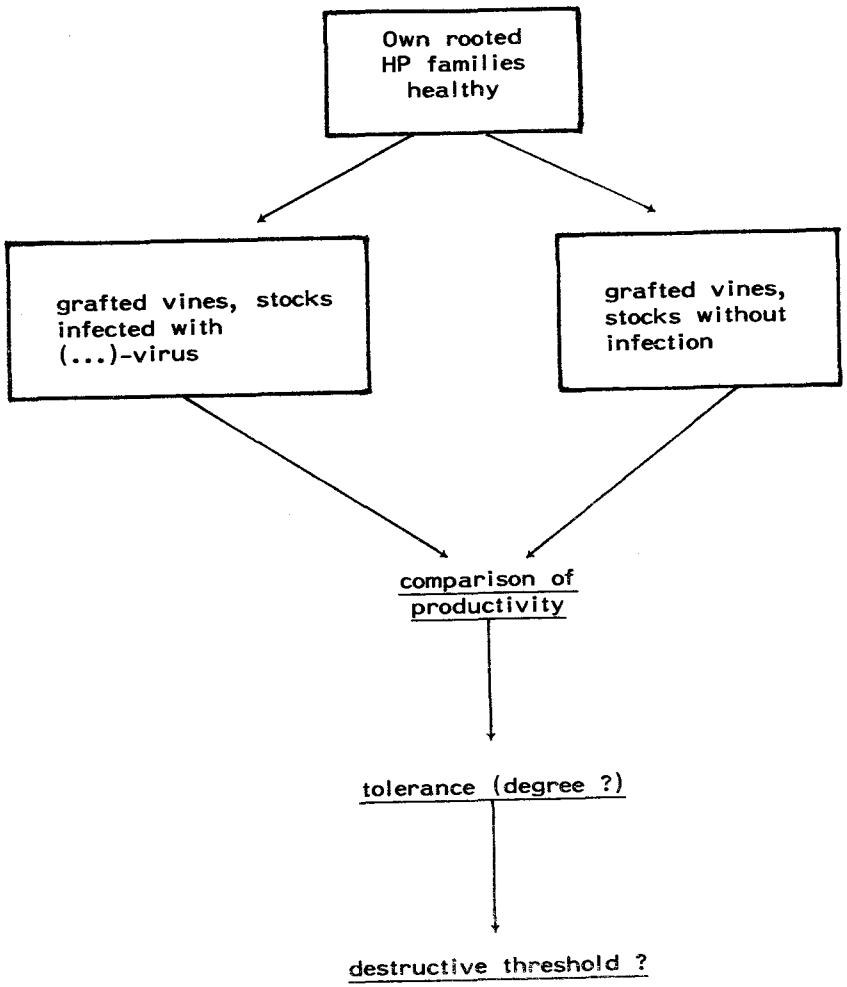

Fig. 2. Evaluation of virus tolerance by performance of infected and clean vines.

susceptible to leafroll, but it is much easier to see the symptoms in varieties that bear red or black fruit. The amount of rolling observed in leaves of infected vines varies. 'Pinot noir', 'Mission' and many others roll downwards very strongly while Riesling and another group show only a slight rolling of the leaves.

There are definitely mild and severe strains of leafroll that produce different symptom patterns in the same variety. The grape varieties most severely affected are useful indicators for indexing.

Proposed procedures: Starting with single grapevines with outstanding production (after three to five years of observation), "families" of own-rooted daughter plants should be made and planted in fumigated soil in a "Family collection." Each family consists of 40 or more individuals which are measured for oenological and technological qualities. Then the families may be divided into two groups. The first one is superior to the collection average (= high performance-, HP families, the second one performs below (low performance-, LP families).

A reasonably good estimate of virus tolerance could be made by comparing virus-infected HP families and virus-free HP families if they have been tested by means of indexing and serology and some positive results were found (Fig. 1).

Supposing that all tests on the HP families were negative, it would be possible to use them for making grafts from which stocks are definitively infected by the virus. Grafted grapes on healthy stocks should be planted for direct comparison (Fig. 2).

Supposing that all virus tests on LP families were negative, thermotherapy should be started hoping to find the reason for the differences (unknown virus?, somatic mutations?) (Fig. 1).

CONCLUSION

The use of virus - tolerant clones of *Vitis vinifera* varieties for cultivation would be an essential contribution for the "integrated grape protection" or "integrated grape disease control." However, hygienic measures must be added, qualified for reducing sources of virus infections. All measures that are able to reduce infection pressure following the burden of tolerance may lower the probability of overdrafting the destructive threshold of the pathogen. As a consequence, the production of virus-free planting material should continue as usual.

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BREEDING PLASMOPARA-RESISTANT VARIETIES IN VITIS

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ABSTRACT

F seedlings from the cross Kober 5 BB (*berlandieri* x *riparia*) x Gamay teinturier were classified for resistance to *Plasmopara viticola*.

On the basis of genetic analysis of the populations, seedlings were classified in three groups: 1) with absolute resistance, 2) with partial resistance, and 3) with no resistance.

The analysis has taken into account the inheritance of leaf color and other characters.

Plasmopara viticola is one of the most severe cryptogamic diseases in Yugoslavia. In some areas, the danger from this disease does not cease until the vintage is over. Therefore, apart from chemical plant protection measures (the most effective form of disease control today) we have also started, as have many other countries in the world, the creation of varieties resistant to this dangerous parasite of the grapevine. In doing so, special attention is also being paid to inheritance of individual characters in successive generations so that this problem can be solved most successfully from the point of plant genetics.

The primary reason of our orientation towards the creation of varieties resistant to *Plasmopara viticola* lies in the fact that recent results on the creation of these varieties in Federal Republic of Germany, USSR, Hungary and USA, as well as in other countries, show that the combining efficiencies in interspecific crossing are great; and, thereby, many shortcomings usually ascribed to resistant varieties can be overcome.

MATERIALS AND METHODS

Crossing and creation of interspecific hybrid populations began as early as 1958. The crossing followed the scheme:

A x E, or American species x European *vinifera* (Table 1)

E x A, or reciprocal of the above cross (Table 2).

TABLE 1. Parental combinations in the crosses (E x A).

Number of cross	*Vinifera* cultivars	Resistant rootstock varieties American species or hybrids.
1.	Bagrina red	Rupestris du Lot
2.	Burgundy black	Rupestris du Lot
3.	Drenak red	Rupestris du Lot
4.	Gamay black	Rupestris du Lot
5.	Italia	Rupestris du Lot
6.	Riesling Italico	Rupestris du Lot
7.	Pearl of Csaba	Rupestris du Lot
8.	Prokupaz black	Rupestris du Lot
9.	Oporto black	Rupestris du Lot
10.	Smederevka white	Rupestris du Lot
11.	Gamay Freaux	Rupestris du Lot
12.	Smederevka white	Rupestris du Lot
13.	Dattier de Beyrouth white	Rupestris du Lot
14.	Sémillon	Rupestris du Lot
15.	Muscat Hamburg	Rupestris du Lot
16.	Alicante Bouschet	Rupestris du Lot
17.	Drenak red	Riparia Portalis
18.	Dattier de Beyrouth white	Richter 110
19.	Burgundy black	Richter 110
20.	Italia	Richter 110
21.	Riesling Italico	Richter 110
22.	Pearl of Csaba	Richter 110
23.	Muscat Hamburg black	Richter 110
24.	Prokupaz black	Richter 110
25.	Semillon white	Richter 110
26.	Oporto black	Richter 110
27.	Smederevka white	Richter 110
28.	Dattier de Beyrouth white	Teleki 8 B
29.	Italia	Teleki 8 B
30.	Pearl of Csaba	Teleki 8 B
31.	Burgundy black	Kober 5 BB
32.	Gamay black	Kober 5 BB

TABLE 2. Parental combinations in the crosses (A x E).

Number	*Plasmopara* Resistant rootstock varieties	Susceptible varieties of *vinifera*
1.	Kober 5 BB	Dattier de Beyrouth white
2.	Kober 5 BB	Dattier de Beyrouth red
3.	Kober 5 BB	Pearl de Csaba
4.	Kober 5 BB	Cardinal
5.	Kober 5 BB	Muscat Queen of Vineyards
6.	Kober 5 BB	Muscat Hamburg
7.	Kober 5 BB	Muscat Ottonel
8.	Kober 5 BB	Prokupaz black
9.	Kober 5 BB	Burgundy black
10.	Kober 5 BB	Gamay black
11.	Kober 5 BB	Gamay Freaux (teinturier)
12.	Kober 5 BB	Vranaz black
13.	Kober 5 BB	Riesling I.
14.	Kober 5 BB	Semillon white
15.	Kober 5 BB	Smederevka white
16.	Kober 5 BB	Alicante Bouschet
17.	41 B	Pearl of Csaba
18.	41 B	Muscat Ottonel
19.	41 B	Gamay Freaux (teinturier)
20.	41 B	Madam Sauter

In making the crosses, pollinations were not controlled. The pollen of the male parent was dusted over the inflorescence of the variety serving as seed parent. Hybrid plants can be readily separated from those of self-pollinated origin.

Amongst the large number of F_1 populations of these interspecific hybrids, the most interesting one to be analyzed was Kober 5 BB x Gamay Freaux, a population of 313 vines grown on their own roots in the field and also under greenhouse conditions.

Ten vines of each seedling were propagated by rooting shoot tips. Natural infections of *Plasmopara viticola* took hold in the field plot. Infected leaves with sporulation were collected and kept 24 hours in plastic bags under moist conditions to produce fresh conidia. A camels-hair brush was used to collect the spores from the leaves and a suspension prepared in distilled water. The inoculum was applied as a mist spray to the greenhouse vines. The inoculated plants were covered with a plastic tent for 24 hours to maintain high humidity and encourage infection. Symptoms were noted daily beginning five days after inoculation. After 15 days a second inoculation was applied and symptoms noted again.

A genetic analysis of the population included the following characteristics:

Leaf resistance to *Plasmopara*.

Inheritance of leaf color and resistance.

Inheritance of skin and juice color.

Significance of the results were evaluated by x^2 (chi square test).

RESULTS AND DISCUSSION

Of 313 seedlings of the Kober 5 BB x Gamay Freaux, 160 remained uninfected and 153 were infected, corresponding to a 1:1 ratio. Of the infected plants, 68% were partially infected and the remainder showing very intense infection. The wide range in infection indicates a polygenic inheritance. Natural field infection and greenhouse tests gave similar results.

Resistance to *Plasmopara* in complex interspecific hybrids is itself very complex, due to the diverse genotypes involved and their interaction with each other and the parasite.

From the fact that about half the F_1 seedlings carry a block of genes from the American species, the species themselves are dominant and homozygous for resistance. One might expect in a backcross generation to *vinifera* to obtain superior fruit quality as well as resistance to *Plasmopara*.

Our research results confirm fully those of others, for example, Boubals (12), Voitović (8), Zuravelj (7), Negrulj (5), and Zankov (6).

Inheritance of leaf color in F_1 generation seedlings: Of 313 tested seedlings, 170 seedlings had green leaves; whereas, 143 seedlings had red leaves. The red coloration of the leaves is derived from the Gamay Freaux parent.

It may be concluded that the autumn red color of the leaf is heterozygous; whereas, the green or yellow one is homozygous. The presence of anthocyan in the leaves during the autumn is a dominant character and inherited according to a monofactorial scheme. Through genetic analysis of inheritance of this character, it may be concluded that there is a gene linkage which controls the characters of autumn color of the leaf and the skin color of the grape. Such conclusions were also reached earlier by Müller Thurgau, Kobel, Negrulj (5), and Golodriga et al. (3).

Inheritance of resistance to Plasmopora in seedlings with green leaves in the F_1 generation: Of 170 seedlings tested, 120 exhibited complete resistance to *Plasmopara*, while 50 of them showed susceptibility and were attacked by *Plasmopora* at different stages.

It is clear, from the results obtained, that all seedlings which inherited the green leaf color originating from Kober 5 BB also possess resistance to *Plasmopara viticola*. The resistance exhibited by the seedlings varies greatly in terms of its intensity. However, only two groups of seedlings distinguish themselves very

clearly--one group being completely resistant while the other shows tolerance in the ratio of 3:1. In this case, dominance was fully expressed in the inheritance of resistance to *Plasmopara viticola*. The large differences, in the degree of dominance in the character of resistance to *Plasmopara viticola*, are due to varied and complex nature of the final forms, by the occurrence of heterozygosity in *Vitis vinifera* and polygenic resistance to *Plasmopara*.

Inheritance of resistance to *Plasmopara* in seedlings with red leaves in the F_1 generation: The data obtained show that the progeny in the F_1 generation coincides with the hypothetical ratio of 3:1. However, as compared with the previous picture, in case of seedlings with red coloration, 40 of them exhibited full resistance to *Plasmopara* while 103 seedlings showed either partial or total susceptibility to this disease.

The results obtained from the analysis show that a large majority of the seedlings with red coloration is very susceptible to this disease. In this case, the dominant is the character of high susceptiblity to *Plasmopara* which controls alellic susceptibility, which is otherwise typical of each variety of *Vitis vinifera* L.

However, a small number of seedlings in which positive transgression was noted is of great significance for further selection. They are very similar to Gamay Freaux but, at the same time, are fully resistant to *Plasmopara*.

Inheritance of skin and juice color: Classification of vines in bearing for the red juice (teinturier) character, showed a 1:1 segregation. However, all of the seedling vines that fruited had black skin color, indicating both parents were homozygous for this gene. All of these F_1 seedlings also had red leaves (autumn coloration). Two groups of seedlings were clearly defined on the basis of juice color, half were similar to the Gamay parent and half had the colorless juice of the Kober 5 BB. The Gamay Freaux is thus heterozygous for the teinturier character. Juice color and skin color are inherited independently, agreeing with the conclusions reached by Negrulj (5) and Golodriga et al (3).

CONCLUSIONS

By studying the characters of resistance to *Plasmopara viticola* in the seedlings obtained through interspecific hybridization of Kober 5 BB x Gamay Freaux (teinturier), the following conclusions may be drawn:

1. The crossing results show that a large number of seedlings resistant to *Plasmopara viticola* were obtained in the first filial generation. In this case, inheritance of resistance is of dominant character; whereas, the genes controlling the resistance are of homozygous character.

2. Analysis of the results obtained show that most of the characters studied are inherited independently; and the recombinations of characters take place freely, which may probably be explained by the presence of a large number of chromosomes.

3. Inheritance of autumn red leaf color is heterozygous and dominant, while the inheritance of green-yellow leaf is homozygous and recessive.

4. All the seedlings in the fruit bearing stage had black skin color.

5. For further immunoselection a small number of seedlings were very significant, with which positive transgressions were recorded, because they possessed characters similar to Gamay Freaux variety and full resistance to *Plasmopara viticola*.

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WINE QUALITY OF NEWLY BRED GRAPE VARIETIES RESISTANT TO DOWNY MILDEW

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ABSTRACT

Forty-four wine consumers, interested in wine quality, tasted at home ten pairs of wine samples. Each of two new varieties, resistant to downy mildew and resulting from backcrosses of Seyve Villard-hybrids with *vinifera*, were represented by five wines. Each wine, from the new crosses, was paired with a wine of a traditional *vinifera* variety from the same vineyard and vintaged and processed in the same manner. The tasters knew nothing about the paired wines and gave their responses by completing questionnaires.

The two resistant varieties were rated better than the traditional *vinifera* varieties in more than 50 percent of all cases. The tasters found unusual or displeasing flavors more often in the *vinifera* varieties from poorer vintages and vineyards than in the new selections.

At the vine breeding symposium in Bordeaux, we reported on our efforts to breed vine varieties resistant to *Plasmopara viticol-a*. In 1951, J. Zimmermann began a program testing 100 newer French hybrids which had resulted from the breeding efforts of Seyve-Villard, Joannes Seyve, Bertille Seyve, Seibel, Burdin, Landot, Coulondre and Ravat. Twenty of these varieties, of which the wines did not present too much unusual flavor, were selected for further breeding after they had proved to be sufficiently resistant to downy and powdery mildew when grown under outdoor conditions.

Since 1954, these varieties have been backcrossed with *V. vinifera* varieties. The resulting seedlings were infected with *Plas-mopara* spores in the greenhouse for resistance selection. Only those seedlings which were completely resistant and had sharply delineated infection points were used for further tests. In the meantime, a second and third backcross was carried out. About 400 such cross combinations yielded approximately 400,000 seedlings which were tested under artificial inoculation. Only the resistant seedlings were planted and evaluated for growth, yield potential, cluster shape, maturity dates, etc. The juice yield of those vines which appear to be acceptable is fermented in 1 to 2-liter containers. Strict standards are applied to the organoleptic examinations of wines. A fair number of downy mildew resistant breeding lines are growing in several test vineyards.

Five of the downy mildew resistant varieties, derived from

this breeding program, are presently being tested in several wine producing areas of West Germany. They are outstanding for their robust growth, vitality and high yield. The leaves remain active until late autumn which ensures good cane hardening. These varieties are grafted on proven phylloxera-resistant rootstocks. It has not been our goal to breed direct producers. Reduced susceptibility to *Botrytis* is usually linked to downy mildew resistance. For the control of powdery mildew, one or sometimes two treatments are sufficient. In spite of the humid summer climate of our region, the resistant varieties receive no protective treatment against *Plasmopara* and *Botrytis*; whereas, the standard *V. vinifera* varieties require six to eight sprays.

In our report at Bordeaux, we discussed the results of a wine tasting at which we introduced wines of resistant selections for comparison to *vinifera* wines in order to have them graded by a group of experts. We could show that wines of these resistant varieties were often graded higher than wines of the *vinifera* varieties. By backcrossing and strict selection, it is also possible to overcome the strange flavor and the harsh astringent acidity of the old French hybrids while combining the resistance against *Plasmopara* with perfect quality in taste. This is certainly a gratifying success for our breeding efforts which have been going on for decades.

However, the grape varieties with the genotype of American species have a negative image in Europe. This is for several reasons: the old hybrids, which had been the result of a one-time cross between *vinifera* varieties and American wild species or between backcrosses of such selections and *vinifera* varieties, certainly produced wines of lower quality. On the other hand, however, these hybrids were robust, frost resistant, had a good yield and were resistant to fungus diseases. Therefore, at the beginning of this century, they were widely grown in European wine growing countries. Inasmuch as, on the one hand, the formulas and spraying machines to control fungus diseases were improved and, on the other hand, consumers expected higher quality, most European wine growing countries tried to reduce the cultivation of hybrids. In Germany, in 1935, the cultivation of the old hybrids was forbidden by law for phylloxera control because of the susceptibility of their foliage. The state officers for phylloxera control had to destroy all hybrids--if necessary, under police escort.

The prohibition of the cultivation of vines with the American genotype according to the phylloxera control law is still valid in Germany. However, according to our experience by means of various experiments we believe that gall phylloxera is no danger to our *Plasmopara*-resistant selections. In addition, nearly all vineyards in our country have been changed over to phylloxera-resistant grafted vines. Therefore, the phylloxera problem can be regarded as being solved for us.

Another reason for the bad image of varieties with the American genotype is the discussion on the health compatibility of hybrid wines among experts. In Germany, Breider and his colleagues (2-9), having performed experiments with hens, found toxic effects caused by hybrid wines. These results, however,

could not be confirmed by further experiments conducted by different authors (10,11,12,13,14,17). In the U.S., Stoewsand and Robinson (15) and Stoewsand et al. (16) also worked on this problem and could not confirm the results described by Breider and his co-workers. Finally, the cultivation of fruiting varieties with the American genotype is against the viticulture law of the European Community. According to article 3 of the EEC regulation No. 817-70 for the production of quality wines of delimited areas (French: Vin d'Appelation d' Origine controllée), only varieties of *V. vinifera* can be classified as recommended and approved vine varieties.

By means of the following experiment, we wanted to test whether the negative assessment of the quality of wines from interspecific crossbreeding which is embodied in the European winemarket regulation is still justified. Therefore, we tested whether wine consumers who regularly drink wine and have a taste for quality would find the wines of our *Plasmopara*-resistant varieties acceptable and how they would grade them.

MATERIALS AND METHODS

We delivered the wines, which had to be tested, in small 0.35-liter bottles to the homes of the tasters who participated in the wine test. In this way, the tasters could try and grade the wines in their normal surroundings and in a relaxed atmosphere. We asked the tasters to examine the wines alone and in peace, preferably for several evenings and not to judge too soon after the first sip. The wines were only labeled with numbers so that they would be introduced anonymously.

In the testing wines of two *Plasmopara*-resistant selections:

FR 993-60

Seyve Villard 5-276 x (Riesling x Pinot gris) and

Fr 946-60

(Seyve Villard 12-481 x (Pinot gris x Chasselas x (Riesling x Pinot gris)

were included. Five wines were taken from both selections which had been grown on different trial plots in 1977 and 1978. Altogether, the testing included 10 wines of the *Plasmopara*-resistant varieties. Each of these wines had to be compared to a wine of a *vinifera* variety. The 20 test wines and their dates of analysis are listed in Table 1.

By arranging the test, one had to be sure that the result would not be influenced by the way in which pairs the wines were combined. We tried to achieve this by comparing two wines which had been grown in the same year, on the same location (which means under the same environmental conditions) and which had been vinified by the same method. In 1978, the temperature summation and in 1977, also, the amount of sunshine hours during the vegetation period were below the average. Therefore, some of the wines had to be chaptalized. With some of them, must

TABLE 1. The ten pairs of wines.

Vintage and experimental vineyard	Wine No.	Variety or breeding	Wine No.	Variety or breeding
	Values of harvest / Wine analysis		Values of harvest / Wine analysis	
1977	1	Silvaner	2	Fr 993-60
Munzingen	59°Oe 9.8 g/I t.ac. 117 kg/a	84 g/I alc. 6.4 g/I t.ac. 9.8 g/I r. sug.	67°Oe 10.3 g/I t.ac. 159 kg/a	89 g/I alc. 6.4 g/I t.ac. 10.3 g/I r.sug.
1977	3	Fr 946-60	4	Nobling
Munzingen	73°Oe 11.0 g/I t.ac. 43 kg/a	89 g/I alc. 6.6 g/I t.ac. 10.3 g/I r.sug.	62°Oe 11.5 g/I t.ac. 134 kg/a	89 g/I alc. 6.4 g/I t.ac. 10.2 g/I r.sug.
1977	5	Fr 993-60	6	Chasselas
Freiburg Wonnhalde	76°Oe 10.8 g/I t.ac. 212 kg/a	85 g/I alc. 8.0 g/I t.ac. 11.3 g/I r.sug.	69°Oe 8.4 g/I t.ac. 93 kg/a	86 g/I alc. 7.0 g/I t.ac 8.7 g/I r.sug.
1977	7	FR 993-60	8	Müller-Thurgau
Munzingen	74°Oe 9.5 g/I t.ac. 104 kg/a	97 g/I alc. 6.3 g/I t.ac. 17.0 g/I r.sug.	70°Oe 8.5 g/I t.ac. 111 kg/a	98 g/I alc. 6.5 g/I t.ac. 17.0 g/I r.sug.
1978	9	Pinot gris	10	Fr 946-60
Munzingen	74°Oe 11.0 g/I t.ac. 44. kg/a	89 g/I alc. 6.5 g/I t.ac. 17.0 g/I r.sug.	75°e 12.3 g/I t.ac. 167 kg/a	94 g/I alc. 6.3 g/I t.ac. 17.0 g/I r.sug.
1978	11	Chasselas	12	Fr 993-60
Freiburg Wonnhalde	68°Oe 9.6 g/I t.ac. -- kg/a	83 g/I alc. 6.1 g/I t.ac. 10.9 g/I r.sug.	86°Oe 10.1 g/I t.ac. -- kg/a	91 g/I alc. 6.5 g/I t.ac. 10.7 g/I r.sug.
1978	13	Riesling	14	Fr 946-60
Hecklingen	77.°Oe 11.8 g/I t.ac. 107 kg/a	87 g/I alc. 6.7 g/I t.ac. 10.6 g/I r.sug.	84°Oe 11.9 g/I t.ac. 73 kg/a	91 g/I alc. 7.3 g/I t.ac. 9.8 g/I r.sug.
1978	15	Fr 993-60	16	Pinot gris
Hecklingen	87°Oe 8.4 g/I t.ac 80 kg/a	96 g/I alc. 7.1 g/I t.ac. 8.9 g/I r.sug.	82°Oe 9.6 g/I t.ac. 66 kg/a	89 g/I alc. 7.3 g/I t.ac. 10.7 g/I r.sug.
1978	17	Fr 946-60	18	Pinot gris
Müllheim	84°Oe 13.3 g/I t.ac. 100 kg/a	91 g/I alc. 7.1 g/I t.ac. 9.2 g/I r.sug.	84°Oe 11.0 g/I t.ac. 63 kg/a	88 g/I alc. 7.6 g/I t.ac. 10.8 g/I r.sug.
1978	19	Riesling x Pinot gris	20	Fr 946-60
Blankenhornsberg	86°Oe 10.2 g/I t.ac. 111 kg/a	78 g/I alc. 7.6 g/I t.ac. 21.7 g/I r.sug.	85°Oe 13.0 g/I t.ac. 125 kg/a	70 g/I alc. 7.3 g/I t.ac. 29.6 g/I r.sug.

Must density = degrees Oechsle; t.ac. = total titrable acidity

R.sug. = residual sugar; and yield = kg per ar (100 m^2).

deacidification was necessary. Therefore, the quality ranged from table wine (No. 1 of the test) to quality wines capable to be labeled as Kabinett which are not allowed to be chaptalized.

Table 1 shows that the *Plasmopara*-resistant selections in 8 of the 10 pairs of wines which were compared had a higher must density than the *vinifera* variety. The average must density of the 10 wines from the resistant selections was 79.1°Oe, while the comparable *vinifera* varieties reached only 73.1°Oe. In 6 of 9 pairs (as the yield dates are missing for one pair, because the grapes were used for crossbreeding), the resistant variety had a higher yield than the control variety. Since the respective wines were grown under the same environmental conditions, the higher yield and must denisty can be explained only by the greater capacity of the resistant varieties determined genetically.

In the testing pairs 3 and 4, the wine from selection Fr 956-60 came from the first vintage, while the variety chosen for comparison came from the main vintage. We tried to balance this by doing it the other way round in the pairs 9 and 10 where the selected variety came from the main vintage and the comparable *vinifera* came from the first vintage.

The tasters had to fill out a questionnaire for each pair of wines, as shown in Table 2.

RESULTS

Altogether, 44 persons filled out the questionnaires. The answers are evaluated in Tables 3 to 8. In Table 3, 4 and 6 there are not 44 answers, as the answers were either missing in the questionnaires or the answers could not be evaluated. First of all, it had to be decided for each pair which was the better one. The results can be seen in Table 3. Of 5 wines from the selection Fr 993-60, three are rated higher and attain at least 5 more points than the comparable wines. Looking at all the answers the wines from the selection Fr 993-60 are rated higher 115 times, while the comparable *vinifera* wines are graded higher 103 times. The result regarding the selection Fr 946-60 is quite similar. Three wines are rated clearly higher and two wines are rated lower than the comparable wines. Altogether, wines from the selection Fr 946-60 are graded higher 116 times, while the comparable *vinifera* varieties were chosen to be better only 103 times.

In the questionnaires, the question was asked whether the wines had an unusual character. Table 4 shows that in 5 wines more than 20 tasters could find such an unfamiliar and different character. Three of these 5 wines came from *Plasmopara*-resistant varieties, 2 from *vinifera* varieties. It is interesting to note that all 5 wines were grown at the experimental trial plot of Munzingen which has an adverse microclimate. Taking the sum of all answers the wines from the selection Fr 993-60 are felt to be "different" practically as often as the wines of the comparable *vinifera* varieties. For the selection Fr 946-60, the number of answers evaluating it as "different" is essentially higher than for the comparable *vinifera* varieties. This result is mainly due to wine No. 10 of the test.

TABLE 2. Questionaire to be filled out by the tasters for every pair of wines.

Name, profession, address:

	Left glass	Right glass
Please make a cross behind the number for the wine of your preference. Decide on one of the wines even if you find them almost equivalent.	No.	No.
Please answer the following questions <u>for both wines</u> by putting a cross into "yes" or "no" square.		
1. Has this wine for your personal taste a somewhat unusual, different character, which you have not yet experienced in the wines of our region?	Yes No	Yes No
2. Would you accept this wine for your personal taste and perhaps buy it?	Yes No	Yes No
(Only to be answered if for question 2 "no" was the answer).		
3. Has this wine something strange, unpleasant, irritating that would make you totally reject it?	Yes No	Yes No
If you wish to make further remarks, you may do so under "comments" or you will find space on the back of the page.	Comments:	Comments:

TABLE 3. Decision on the better wine.

Test pair wine No. a.	b.	Fr 993-60 superior number of answers a.	Compared varieties number of answers b.	Test pair wine No. a.	b.	Fr 946-60 better number of answers a.	Compared varieties number of answers b.
2	1	24	19	3	4	33	11
5	6	22	21	10	9	11	33
7	8	10	34	14	13	25	19
12	11	29	15	17	18	30	14
15	16	30	14	20	19	17	26
		Σ 115	103			Σ 116	103

TABLE 4. Answers to question No. 1: Has this wine for your personal taste a somewhat unusual, different character, which you have not yet experienced in the wines of our region?

Test pair wine No.		a.		b.		a. / b.					
		Fr 993-60 number of answers		compared varieties number of answers		Test pair wine No.		Fr 946-60 number of answers		Compared varieties number of answers	
a.	b.	Yes	No	Yes	No	a.	b.	Yes	No	Yes	No
2	1	12	30	15	27	3	4	6	37	8	33
5	6	8	33	9	33	10	9	16	26	3	39
7	8	18	25	13	28	14	13	10	32	7	35
12	11	10	32	10	32	17	18	5	36	8	34
15	16	2	40	4	38	20	19	9	33	8	34
	Σ	50	160	51	158		Σ	46	164	34	175

In Table 5, there is a summary of the answers according to the varieties used in the test. Most often the wines of the varieties Silvaner and Müller-Thurgau from the trial plot Munzingen are noted as being different. The Silvaner is a table wine of low quality. The wines Fr 993-60 are rated at an average of 23.3% of the possible choices as "unusual and different," the wines from Fr 946-60 in 21.4% of the possible choices. It is interesting to note that the variety Gutedel (Chasselas), which is well known in our region, rated by 22.1% was also experienced as being different.

In answering the question whether the taster would accept the wines for his personal taste and perhaps buy them, the high percentage of negative answers is surprising (Table 6). The wines were bottled with little or moderate residual sugar, as shown in Table 1. For some tasters, the wines were too sweet, for others too dry. Part of the disapproval is due to the question of personal taste which is documented by comments in the questionnaires. The decision - "not to accept" - "not to buy" - was especially often made for wines of a lower quality, tasted at the beginning of the test. All the wines which would not be "accepted" or "bought" by 20 or more tasters came from the trial plot Munzingen, which has an adverse microclimate. With the increasing quality of the wines tasted at the end of the test, there is a tendency to a smaller number of disapprovals. This proves that the tasters had the required sense of taste for the quality in wines. Summing up all the answers it can be concluded that wines of the *vinifera* varieties would be "not accepted" or "bought" as often as wines from *Plasmopara*-resistant selections.

From the answers of Questions 1 and 2, there are four possible combinations (Table 7). The Pinot gris and the crosses Riesling x Pinot gris attain the highest percentage of definitely positive answers (++). Both the resistant selections together with the Riesling and Chasselas take a middle place. The Silvaner, Nobling and Müller-Thurgau varieties attain the smallest percentage of definitely positive answers.

The Silvaner table wine attains the highest percentage of definitely negative answers (--). For the resistant selections, the answers of this kind are higher than for the rest of the *vinifera* varieties. The combination "unusual - yes", "buying - yes" is given in relatively few answers for all varieties. The percentage of answers is higher which grade the wines as "different" but, nevertheless, would opt for "not buying." Among this group are the answers of tasters who either find the wines too sweet or too dry or would just reject them because of low quality.

The tasters who would "not accept" or "buy" the given wine had to finally answer the question whether the wine had a strange, unpleasant and objectionable character resulting in their total refusal of this wine. As shown in Table 8, the strange, unpleasant, objectionable character is most often attributed to the Silvaner, which had the No. 1 in the test. Twenty-two out of 42 persons would "not accept' or "buy" this wine, among whom were 11 persons who would totally reject it as being strange and objectionable. Also, one wine of each resistant selection would not be bought by more than 20 people and, at the same time, was

TABLE 5. Evaluation of question No. 1 of the questionnaire by varieties.

Wine No. in the test	Variety or selection	Unusual or different character, percent of answers	
		Yes	No
1	Silvaner	34.9	65.1
8	Müller-Thurgau	30.2	69.8
2, 5, 7, 12, 15	Fr 993-60	$\bar{x}$ = 23.3	$\bar{x}$ = 76.7
6, 11	Chasselas	$\bar{x}$ = 22.1	$\bar{x}$ = 77.9
3, 10, 14, 17, 20	Fr 946-60	$\bar{x}$ = 21.4	$\bar{x}$ = 78.6
4	Nobling (Silvaner x Chasselas)	18.6	81.4
19	Riesling x Pinot gris	18.6	81.4
13	Riesling	16.3	83.7
9, 16, 18	Pinot gris	$\bar{x}$ = 11.6	$\bar{x}$ = 88.4

TABLE 6. Answers to question No. 2: Would you accept this wine for your personal taste and perhaps buy it?

		a.		b.				a.		b.	
Test pair wine No.		Fr 993-60 number of answers		Compared varieties number of answers		Test pair wine No.		Fr 946-60 number of answers		Compared varieties number of answers	
a.	b.	Yes	No	Yes	No	a.	b.	Yes	No	Yes	No
2	1	18	22	20	22	3	4	30	12	19	22
5	6	26	14	25	16	10	9	15	26	27	15
7	8	20	22	23	20	14	13	24	17	26	16
12	11	30	13	22	19	17	18	28	12	27	15
15	16	29	13	26	10	20	19	23	19	28	14
		123	84	116	87			120	86	127	82
Percent of answers		59.4	40.6	57.1	42.9	Percent of answers		58.3	41.7	60.8	39.2

Plasmopara-resistant varieties: buy "yes" : 59% buy "no" : 41"
Compared *vinifera* varieties buy "yes" : 59% buy "no: : 41%

TABLE 7. Combination of the answers of questions 1 and 2.

Wine No. in the testing	Variety or breeding	Unusual: No Buy: Yes	Unusual: Yes Buy: Yes	Unusual: No Buy: No	Unusual: Yes Buy: No
		+ +	+	−	− −
2, 5, 7. 12, 15	Fr 993-60	$\bar{x}$ = 52.4	$\bar{x}$ = 6.3	$\bar{x}$ = 24.3	$\bar{x}$ = 17.0
3, 10, 14 17, 20	Fr 946-60	$\bar{x}$ = 51.7	$\bar{x}$ = 6.8	$\bar{x}$ = 25.4	$\bar{x}$ = 16.1
1	Silvaner	42.9	4.8	21.4	30.9
4	Nobling	42.5	5.0	37.5	15.0
6, 11	Chasselas	$\bar{x}$ = 49.8	$\bar{x}$ = 7.6	$\bar{x}$ = 27.4	$\bar{x}$ = 15.2
8	Müller-Thurgau	34.1	17.2	34.1	14.6
9, 16, 18	Pinot gris	$\bar{x}$ = 66.0	$\bar{x}$ = 2.4	$\bar{x}$ = 21.9	$\bar{x}$ = 9.7
13	Riesling	53.7	7.3	29.3	9.7
19	Riesling x Pinot gris	61.9	4.8	19.0	14.3

TABLE 8. Answers to question No. 3: Has this wine something strange, unpleasant, irritating which causes you to totally reject it? Only to be answered if the answer for the special wine was: "not accepted, would not buy."

	a.	b.		a.	b.
Test pair wine No.	Fr 993-60 number of answers	Compared varieties number of answers	Test pair wine No.	Fr 946-60 number of answers	Compared varieties number of answers
a. b.	Yes No No answ.	Yes. No. No answ.	a. b.	Yes No. No answ.	Yes. No No answ.
2 1	6 14 2	11 9 2	3 4	4 7 1	6 10 6
5 6	6 5 3	4 11 1	10 9	10 13 3	3 10 2
7 8	10 6 6	4 11 5	14 13	8 6 3	3 11 2
12 11	6 7 0	5 11 3	17 18	1 7 4	6 6 3
15 16	2 10 1	4 5 1	20 19	7 10 2	6 7 1
Σ	30 42 12	28 47 12	Σ	30 43 13	24 44 14
Votes "not buy" out of Table 6	84	87		86	82

totally rejected by 10 people, i.e., a quarter of the tasters, for being strange and objectionable.

Summing up all the answers shows that a clear majority of persons who would "not accept" or "buy" either the wines of the resistant varieties or the *vinifera* varieties, nevertheless, do not find them unpleasant, strange or objectionable.

Out of the total number of votes, fluctuating between 82 and 87, which opted for "not accepting-not buying," the wines of the selection Fr 993-60 received only two more votes, refusing them for being strange and objectionable as compared with *vinifera* wines. However, the wines from the selection Fr 946-60 received 6 more votes "strange-objectionable - total refusal" than the comparable wines.

RESULTS AND DISCUSSION

To our knowledge, the test we have described here is the first attempt to have wines from newly bred varieties graded by consumers using questionnaires. There were methodological limits: first, concerning the number of tasters because of the limited amount of wine, and secondly considering the size of the test because there are limits for what may be expected from the tasters. The methodological difficulty, though, probably does not lie as much in the limited number of the 44 tasters as in the random choice of the pairs of wines; although the wines, which were introduced as a pair, came from the same vineyard plot and were of the same year. Our tasters were people chosen as having the necessary experience in detecting quality in wines. That we were right in our choice is proven by the fact that the tasters graded the wines of low quality as "different" (especially No. 1) and more often decided "not to accept or buy" these wines than the ones of higher quality.

Altogether, the results for the resistant varieties are relatively favorable. According to Table 3 they are getting better grades in a little more than half the votes for the control wines.

Table 6 shows that wines of the *vinifera* varieties are as often as in the resistant varieties "not accepted and not bought." In fact, the *vinifera* wines of low quality, according to Table 5, are more often experienced as being "unusual and different" than the resistant varieties. In the definitely positive answers combining Question 1 and 2 (see Table 7), the resistant varieties take the middle field.

On the other hand, it should not be overlooked that the percentage of definitely negative answers, from the combination of Question 1 and 2 (Table 7), was slightly higher for the resistant than for the *vinifera* varieties. Taking the sum of all the answers, there is a slightly higher percentage of the answer "unusual, different - yes" combined with "buying - no" for the resistant varieties than for the *vinifera* varieties, not taking the Silvaner into account. Table 8 points to this direction, also. Taking all the pairs for the selection Fr 946-60, in 6 more cases, there is something strange and unpleasant and objectionable to be

found. This result is determined by the one special wine No. 10 in the test. However, if one considers that the 44 tasters for the 5 wines of the selection Fr 946-60 could have given altogether 220 such votes, the majority of 6 votes seems minimal. The selection Fr 946-60 has a pronounced flavor which is also prevalent in some *vinifera* wines, for example, in the Scheurebe variety. This flavor is not appreciated by all consumers. It is possible that some of the tasters experience this flavor as being unpleasant and objectionable.

The total favorable gradings of the resistant varieties in the test, however, show that our selections can cope with the traditional *vinifera* varieties, as far as quality is concerned, and that they are not afflicted with a peculiar non-typical character. Therefore, the results of this test confirm the experiences which we received in many earlier tastings with groups of wine experts (1).

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THE SELECTION OF GRAPEVINE GENOTYPES RESISTANT TO FUNGUS DISEASES AND THEIR USE UNDER FIELD CONDITIONS

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ABSTRACT

Progenies were analyzed from interspecific crosses involving different *Vitis vinifera* cultivars as one of the parents. Many of these F_1's were also selfed and analyzed in the same manner.

In addition to the information provided on the genetic transmission of downy mildew resistance, it is shown that by applying a selection as strong and early as possible we hope to readily obtain some genotypes with good quality and enough resistance to be cultivated with fewer spray treatments. The advantages and limits of using such new cultivars are examined in relation to the new developments in vine protection.

Breeding for resistance to the numerous diseases and pests damaging the grapevine was only developed on a large scale after their arrival in Europe where they soon destroyed the native grapevine, *Vitis vinifera*. Such research was very successful in providing tolerant rootstocks, since most of those used today originated about a century ago and remain a valuable contribution to viticulture. On the contrary, the same approach with wine and table varieties had poor success: we know that the so-called "direct producers" are now almost entirely discarded from plantings in western Europe. It is evident that these varieties derived from interspecific crosses have never reached the high quality of the best *Vitis vinifera* varieties and their resistance was not sufficient to the rampant diseases. Thus, the decision of discarding the ancient "direct producers" seems entirely justified, even if some of them have been useful in the past, and this measure is considered beneficial for present viticulture. But it also has another effect which is detrimental: many people confused these ancient hybrids with interspecific hybrids, in general. What must be prohibited is the inferior material obtained up until now, but it would be an error to condemn interspecific hybridization itself as a long term solution.

In the past few years, the concept of resistance and ideas on the host-parasite relationship have evolved greatly and, at the same time, new chemicals against downy and powdery mildew have been released making their control easier and more efficient. Thus, genetic resistance may be conceived today much more like one of the contributive factors in this war between the plant and its

parasites than the only barrier to its development. Moreover, it appears that the previous breeding work did not succeed because the breeders failed to select for high tolerance to diseases along with high quality of the fruit.

Taking all these thoughts into account, we decided to breed some new genotypes of superior quality that showed enough tolerance to the main fungus diseases so that they could be cultivated with a minimum number (say two) of fungicide applications. The first disease to be considered was downy mildew because of its disastrous effect on the vine. We have to expect that the resistant plants selected will be more or less susceptible to other fungi. Therefore, a few polyvalent sprays will be needed particularly at blossoming time.

MATERIALS AND METHODS

In previous work (2) we described the main traits of the two resistant parents used: one is the new rootstock called 'Fercal' with good fruit quality, and the other (No. 7489) is an interspecific hybrid from the station but never released for commercial vineyards. Both were crossed with a range of *Vitis vinifera* varieties. The young seedlings at the 8 to 10 leaf stage are tested against *Plasmopara* by means of an artificial inoculation carried out in the greenhouse. Those seedlings classified as resistant are cultivated in the greenhouse to produce fruits by their second year of growth. Owing to the advanced growth of the vines under greenhouse conditions, it is possible to collect the pollen of these F_1 plants early enough to make crosses with varieties growing outdoors. At the same time, these F_1's can be selfed and also fruit by about July. We hoped that we could investigate which F_1's had the best capability to transmit its resistance in order to use only the most valuable ones for further backcrosses.

Finally, the few seedlings selected after two years of growth in the greenhouse are grafted and planted the following year in the vineyard for further experimentation and comparison with known varieties used as controls (Fig. 1).

RESULTS AND DISCUSSION

F_1 crosses: We no longer used 'Fercal' as a resistant parent because its offsprings are generally of poor quality with respect to fruit size and often have typical American-type leaves. They look like rootstocks more than fruiting varieties, so they would need several back-crosses to *vinifera* before having a more *vinifera*-like foliage. On the contrary, it seems much easier to select seedlings having fruits of good quality directly after the first cross between 7489 and *Vitis vinifera*. More crosses of this type were made and the progenies tested (Table 1). It can be seen that the cumulative percentage of plants belonging to class two and three varies between 0 and 10. These values are much less than those we previously reported (21.5 to 44% for the five progenies under test). Moreover, one can note that the cross (7489 x Chardonnay) was repeated from 1977 to 1979, and it gave the respective percentage of resistant plants: 21.5 and 1.3. At first sight, it seems that our evaluation became more and more critical

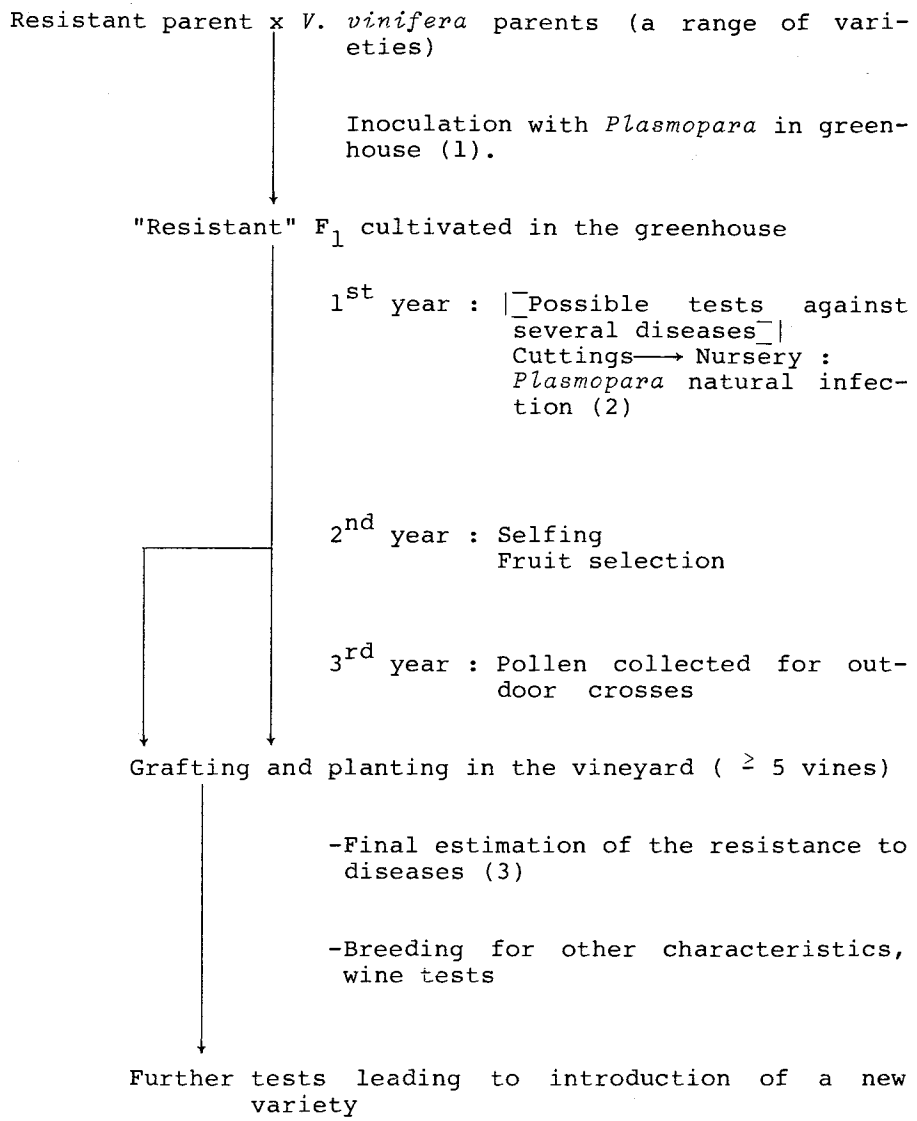

Fig. 1. Successive events in the breeding program for resistance to *Plasmopara*.

TABLE 1. Distribution of different progenies involving the same resistant parent (top-cross) among the different classes of resistance to downy mildew.

Crosses	Classes of resistance				Total	II + III p. 100
	V.	IV	III	II		
	Very susceptible	Susceptible	Resistant	Very resistant		
7489 x Cabernet Sauvignon	59	37	2	1	99	3.33
Cabernet Sauvignon x 7489	71	2	1	0	74	1.36
7489 x Chardonnay	121	33	2	0	156	1.28
7489 x Cot	82	28	3	0	113	2.65
7489 x Gamay	39	10	0	0	49	0
Mourvèdre x 7489	453	196	55	13	717	9.48
7489 x Pinot Meunier	119	17	0	0	136	0
7489 x Pinot noir	57	34	0	4	95	4.21
7489 x Syrah	916	238	19	10	1183	2.45

through the years; but other factors might be involved, as discussed later.

Selfing and back-crossing: A great number of the first F1's (7489 x *Vitis vinifera)* which had been ranked among classes two or three were selfed in order to estimate which were the best for further crosses. From the first results, it seems that no correlation exists between the class of resistance to which a plant belongs and the distribution of its progeny. This fact is not very surprising since the plants under study belong to classes 2 and 3, which are not very unlike. If we consider the 46 progenies comprising more than 30 seedlings each, subject to *Plasmopara* infection, only 15 gave more than 10% resistant plants. These best seedlings were again crossed with a *vinifera* variety but different from that involved in the initial cross. From the resistance point of view, these plants seemed to be no better than the resistant parent 7489. We again found among these plants great differences in the percentage of resistant seedlings they produced.

Estimating the resistance to *Plasmopara*: As already noted, we consider it very important to test the seedlings as soon as possible in order to avoid the cultivation of a great number of plants whose resistance is not yet known. So the young seedling having about 8 to 10 leaves (about two months old) are subjected to a first artificial contamination. After six to seven days of incubation, they are ranked according to a resistance scale derived from that of (1). Then the seedlings which show no symptoms or only mild ones (classes 2 and 3) are re-inoculated and, after a second ranking, are cultivated in the greenhouse with the most favorable growing conditions (hydroponic system of cultivation). When we examine the inoculated plants, we always take into account the leaf of each seedling which is the most damaged. The pathogen, *Plasmopara viticola*, is kept all year by successive transfers onto susceptible plants, for example, seedlings from open-pollinated *vinifera* varieties. Thus we have a reserve of inoculum at any time of the year.

Though we always tried to operate in the same way, we observed some discrepancies from year-to-year and even from one trial to another in the same year. This is quite unavoidable under our conditions where too many factors are not well controlled. For example, the temperature at the place where inoculations are made. The physiological status of the seedlings may vary and, consequently, their receptivity, which is known to be dependent upon their growth activity. Seedling progenies show a great heterogeneity for growth. We do not know precisely what sort of pathogen we have at hand. It was collected for the first time in the field and then repeatedly cultivated on more or less susceptible plants. Thus we cannot be certain that its pathogenicity has remained stable.

As a matter of fact, most of these critical considerations are partly counterbalanced by including in every trial some cuttings of varieties whose resistance is well known. But here again, one can object that these controls, vines grown from cuttings, are not comparable with the seedlings under test.

A second important fact to be pointed out is the comparative

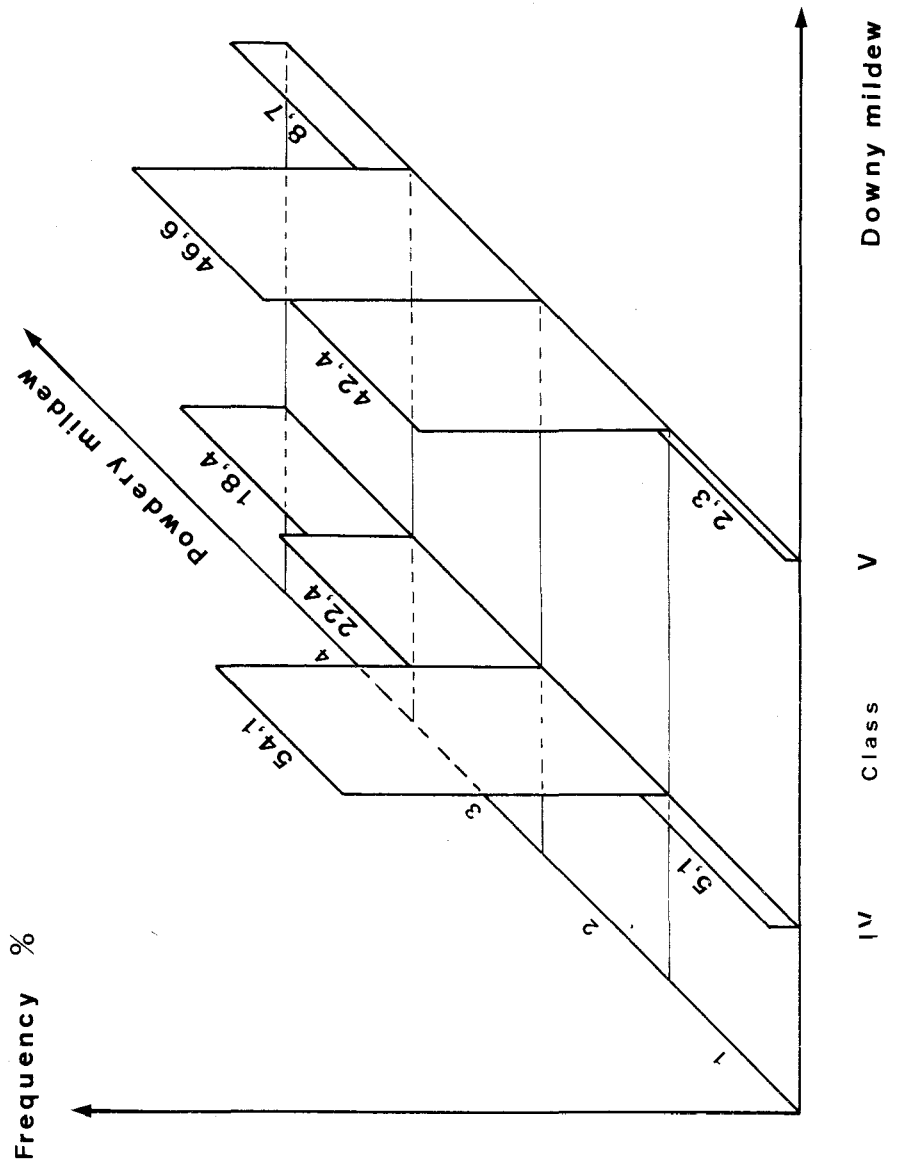

Fig. 2. Relationship between *Plasmopara* and *Uncinula* resistance : distribution of the *Plasmopara* - susceptible seedlings from a (Mourvèdre x 7489) cross among the four different classes of resistance to *Uncinula*.

(Total number of seedlings tested = 362)

behavior of plants exposed first to the early artificial test and then to the natural inoculation in the nursery. When comparing the two notations, we saw that, in the case of 'Fercal' progenies, most of the seedlings were attacked less in the nursery than in the greenhouse in spite of a more severe notation in the second place. Most of the 7489 offspring were in the reverse position. In fact, these differences can be explained by an additional event which happens only in the nursery: the leaf fall after attack by *Plasmopara*. This phenomenon appeared to be difficult to explain, and we noted that the seedlings originating from 'Fercal' are much more likely to retain their leaves alive than those of 7489 parentage, even if they are severely contaminated.

On account of these considerations, our temporary conclusion is that we must not be satisfied with doing only one test if we want to be sure that the selected genotypes are resistant enough. We are now developing, in cooperation with pathologists, new studies related to the pathogen itself and different aspects of the host-parasite relationship.

Behavior towards other diseases: The first genotypes selected for *Plasmopara* resistance (180 from five crosses) were planted in vineyard conditions in 1979, using five grafted vines each. This experiment is devoted to estimating the behavior of these genotypes not only towards downy mildew (for further confirmation) but also towards the other major fungus diseases. With this object in mind, only two fungicide applications a year are applied; so the possible susceptibility to different fungi will be quickly revealed. Then again a small trial related to *Oidium* resistance was carried out in 1979 on one progeny alone (Mourvèdre x 7489). In that case, the 362 *Plasmopara*-susceptible seedlings were not discarded but kept in the greenhouse and exposed to *Uncinula* inoculation.

The distribution of four classes of resistance to powdery mildew was drawn according to the two classes of susceptibility to downy mildew. It appears from Fig. 2 that some correlation may exist between both resistances.

CONCLUSION

Additional results of F_1 crosses involving our interspecific hybrid used as the resistant parent are reported. They apparently show the same pattern of distribution as previous data. Apart from that, we laid emphasis on the carefulness needed in the estimation of disease resistance which depends mainly upon the reliability of the tests used. Until now, *Plasmopara* only was taken into account; but, as the work proceeded, it appeared that some aspects are still questionable. For example, the possible variability of the fungus itself may condition the duration of plant resistance. The behavior towards other diseases of our first selected genotypes is now under investigation in a field experiment where only a minimum control of diseases is provided.

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