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Quantitative stock-scion relationships in vine Preliminary investigations by the analysis of reciprocal graftings

by

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Relations quantitatives entre porte-greffe et greffon chez la vigne Première approche par l'analyse des greffages réciproques

Résumé. — Chez la vigne, on observe que le comportement d'un même greffon peut varier lorsqu'on l'associe à différents porte-greffes; ceci a été souvent noté, particulièrement en ce qui concerne l'expression de la vigueur.

Cet effet simple n'est en fait qu'un des éléments de la décomposition que l'on peut faire à partir de toute caractéristique ou performance quantitative de la combinaison du greffon et du porte-greffe.

Par analogie avec l'étude des effets principaux et de leur interaction dans les associations binaires, l'analyse de greffages réciproques peut être effectuée par le modèle biométrique suivant:

$$y_{ij} = m + p_i + g_j + k_{ij}$$

où p_i et g_j sont respectivement ce que nous appelons les «fonctions» porte-greffe et greffon, et k_{ij} leur interaction.

Une autre décomposition possible où apparaissent des «effets génétiques» est la suivante:

$$y_{ij} = m + c_i + c_j + s_{ij} + q_{ij}$$

c_i et c_j étant les «Aptitudes Générales à l'Association» des génotypes i et j , s_{ij} leur «Aptitude Spécifique à l'Association» et q_{ij} l'effet réciproque de ces deux génotypes.

Des expériences préliminaires basées sur un système de greffages réciproques complet de quatre génotypes, dont nous avons mesuré la croissance au cours de trois cycles de végétation, chacun de 120 jours, nous ont permis:

1. de tester l'intérêt de ces deux modèles biométriques et celui des paramètres que nous proposons.
2. de calculer l'ordre de grandeur de ces paramètres à différents stades de la croissance.

Introduction

A vine is generally a rootstock-scion association; thus the quantitative characteristics or performances of this combination (RIVES 1971 a) may be theoretically divided into:

- two simple effects due to each of its parts, the upper and the lower one
- a complex effect due to their interaction; these two parts differing from one another, both and at least, in their functions in the combination and in their genotypes.

Grafting in perennials has been, and is even today, looked on as a way to modify the behaviour of the scion, by use of a given rootstock, without regard to the fact that in return the scion acts upon the rootstock.

If examples are numerous in annuals, as well as in perennials, of the ability of the stock to act on the scion, for example in soybean at the level of photosynthetic activity (SULLIVAN and BRUN 1975), a few experiments offer evidence for the scion's influence on root growth and development and on ion absorption by the roots (KLEEZE and SMITH 1970).

Little work has been done on stock-scion relationships, especially with respect to the expression of quantitative characters. MOORE (1975) concluded from an experiment on apple-trees that interaction effects were large for fruiting characters, nevertheless he did not quantify them. TUBBS (1973) in a general review of this problem underlined the necessity and the interest of taking into account the interaction in perennials.

In the vine, some physiological studies provided evidence, at a qualitative level, of the existence of interactions between the upper and the lower parts of the plant, particularly in relationship to iron metabolism (POUGET 1974); the carbohydrates elaborated in the leaves migrate to the root where they take part in citric acid synthesis, this acid migrating in turn to the foliage in an iron-citrate form.

From a quantitative point of view, especially concerning vigour, RIVES (1971 a and 1972) on the basis of rootstock experiments suggests that the idea of a pure and simple additivity in stock-scion relationships has to be rejected.

For our part, we proposed to quantify each of the three effects stock, scion and interaction, with respect to the single quantitative character of shoot growth. In other words, we tried to determine, at different stages of the grafted combination's growth, the amount of the total quantitative variation we may ascribe to each of these effects. With the experiments presented in this paper we designed to find a suitable methodology of investigation, and to specify the meaning and the interest of the proposed parameters.

Methodology

The problem posed by the study of principal additive effects and their interaction in binary association is formally analogous to the study of General and Specific Combining Ability (GCA and SCA) in quantitative genetics (GRIFFING 1956) and to the study of plant competition in pair associated crops (GALLAIS 1970). From this analogy we were led in our search for an experimental methodology to a bi-factorial design, generalizing to more than two genotypes, the reciprocal grafting method developed by POUGET and OTTENWALTER (1973) for more qualitative investigations.

1. The biometric model

According to the meaning one gives to the parameters, the biometric model of a diallel-type bi-factorial system may be written in different equivalent forms. It is easy to go from one to another using some formulae (G. LEFORT 1976).

According to the classic factorial break-down, one obtains at the level of a root-stock-scion association the following model:

$$y_{ij} = m + p_i + g_j + k_{ij}$$

where:

- m is the overall mean of the compared combinations,
- p_i is the deviation from m of the i th genotype's mean effect, i taken to be the stock, i.e. the mean of all combinations where i is the rootstock,

- g_j is the deviation from m of the j th genotype's mean effect, j taken to be the scion, i.e. the mean of all combinations where j is the scion,
 k_{ij} is the deviation from m of the effect (positive or negative) due to the interaction between the i th stock and the j th scion.

Thus, this first model breaks down the variation on the basis of the rootstock's or scion's function in the combination considered.

Another possible formulation of break-down presents parameters similar to those used in quantitative genetics (GCA, SCA and reciprocal effects). It allows us to study the combination not from the point of view of the function fulfilled, but from that of the mean genetic effects observed for both functions.

By analogy to the terminology used in quantitative genetics, these parameters may be defined as follows:

- General Associating Ability (GAA) of the i th genotype,

$$c_i = \frac{P_i + G_i}{2}$$

- Specific Associating Ability (SAA) of the i th and j th genotypes,

$$s_{ij} = \frac{k_{ij} - k_{ji}}{2}$$

- General Reciprocal Effect (GRE) of the i th genotype,

$$m_i = \frac{P_i - G_i}{2}$$

- Specific Reciprocal Effect (SRE) of the i th and j th genotypes,

$$r_{ij} = \frac{k_{ij} + k_{ji}}{2}$$

Hence, the model becomes:

$$y_{ij} = m + c_i + c_j + m_i - m_j + s_{ij} + r_{ij}$$

or, more simply:

$$y_{ij} = m + c_i + c_j + s_{ij} + q_{ij}$$

if one considers that the Reciprocal Effect (RE) of the i th and j th genotypes is:

$$q_{ij} = r_{ij} + m_i - m_j$$

Thus, the experimental system of reciprocal graftings and the biometric models by which one can analyse it, allow the study of:

- firstly, the effects due to the functions,
- secondly, the effects due to the genotypes.

2. Biological meaning of the parameters

The parameters described above (which can be computed for any quantitative character) represent deviations from the overall mean, nevertheless we shall define them in terms of means.

The biological meaning of the effects due to the function in the combination is straight forward:

— Mean rootstock effect: the mean performance of a given rootstock in combination with all scions. In the case of vigour measurements, this parameter would exactly cover the notions of "conferred vigour".

— Mean scion effects: the mean performance of a scion in combination with all rootstocks.

— Interaction effect between rootstock and scion: measures the relative "affinity" between the two partners for a determined function. The i th rootstock's

and the j th scion's interaction will be, *a priori*, different from that of the j th rootstock and the i th scion.

— GAA: a mean (additive) genetic effect relating to a genotype, independent of the function performed in the combination.

— SAA: a genetic effect due to the presence of two genotypes in a particular arrangement, whatever the combination.

— RE: the effect due to the difference between the performances of the i/j combination and the j/i reciprocal one. It measures the more or less high ability of the genotypes to behave differently according to the function in the association, i.e. roughly something one can call "functional specialization".

Materials and methods

As we have already pointed out, the experiments were designed to perfect the methodology and to specify the interest and the approximate values of the different parameters. Thus, we tried to work on a representative sample of the existing vigour-scale of genotypes. However, taking into consideration the complexity of the experimental design (n genotypes producing n^2 combinations) we initially retained only four genotypes:

- two of them are habitually used as rootstocks:
Vitis riparia var. "Gloire de Montpellier"
Vitis rupestris var. "du Lor"
- two of them are habitually used as scions:
Vitis vinifera var. "Cabernet Sauvignon"
Vitis vinifera var. "Ugni blanc".

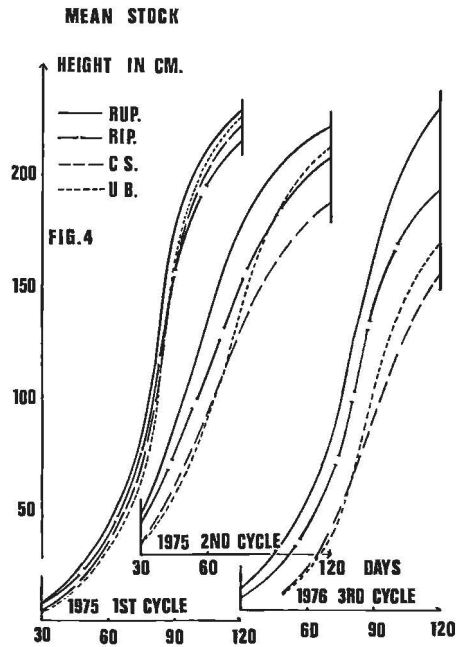
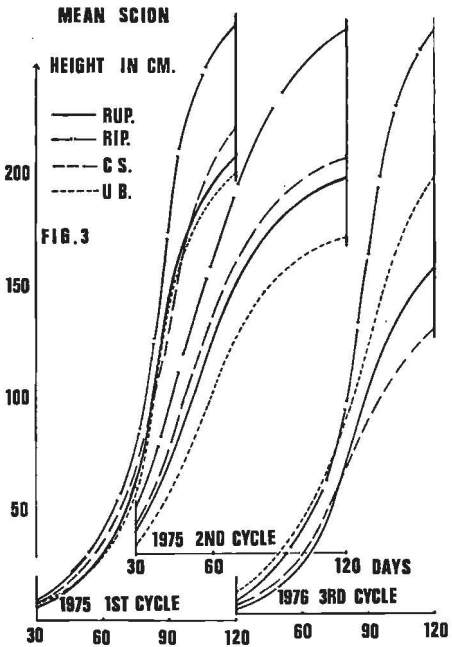
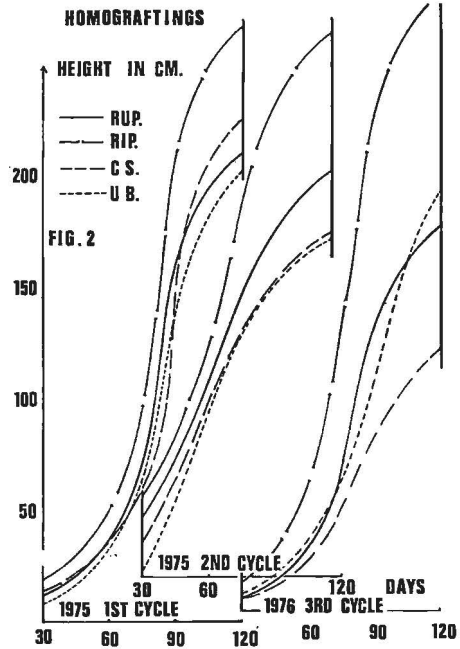
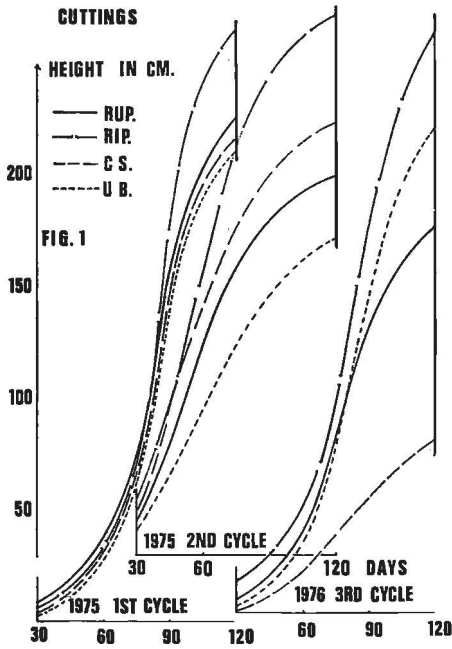
Starting from these four genotypes, we realized the two by two possible graftings, including the homograftings. We compared them in a randomized block design (2 blocks, 3 repetitions per block). The cuttings of each genotype were added. There were 16 combinations and 4 cuttings, i.e. in all 20 comparisons. The plants were grown during 3 cycles in hydroponic cases. Each cycle was 120 days long. The first one began in April 1975 in a greenhouse, each plant growing in a pot, the second cycle followed immediately under the same conditions after cutting down the green shoots. The third cycle took place in 1976, after pruning the mature shoots. The winter dormancy period occurred at this cycle, and the plants were grown outdoors in hydroponic cases. The third cycle was much closer to the natural environmental field conditions than the first two.

The length of the shoots was measured at 30, 60, 90 and 120 days after budburst.

The four genotypes retained for our experiments were not interesting in themselves but as representatives of the *Vitis* genus. Our aim was not to analyse and to compare these genotypes, but rather to try to draw general conclusions with respect to the population of genotypes of vine behaviour.

With such an outlook and although the random character of our sampling may be debatable in this first experiment, we carried out the statistical analysis of our data according to the mixed model of analysis of variance (RIVES 1971 b), with fixed effects on the variation due to the blocks and random effects on the variation due to genotypes or functions.

Similarly, we followed the analysis by calculating the variance components, that we expressed in our tables in percentages of the non-random variance, i.e. the overall variance minus the residual variance.



Figs. 1—4: Comparison of the 4 genotypes for each function.
 Comparaison des 4 génotypes pour chaque fonction.

Results

We present, firstly, the mean heights observed at each cycle and the corresponding growth curves, secondly the analysis of the variation and its break-down into components.

A. Growth curve analysis

The growth curves are shown in the Figs. 1 to 8. They allow us to compare:

— Firstly, the 4 genotypes for each of the 4 functions: cuttings (Fig. 1), homo-graftings (Fig. 2), mean scion effects (Fig. 3) and mean stock effects (Fig. 4).

— Secondly, the 4 functions among themselves for each genotype in turn: *Riparia* (Fig. 5), *Rupestris* (Fig. 6), *Cabernet-Sauvignon* (Fig. 7) and *Ugni-Blanc* (Fig. 8).

In all cases, the dispersion of the compared curves reaches its maximum at the 120th day (maximum value of the FISHER'S F statistic).

a) Comparison between the 4 genotypes for each function

The cuttings (Fig. 1): *Riparia* shows the strongest growth in each cycle. In the first cycle its growth is significantly superior to the other three genotypes which do not differ among themselves. The dispersion rises in the second and still more in the third cycle. At this stage, only *Cabernet-Sauvignon*, whose growth is very slow, differs significantly from the others. *Riparia* is significantly superior to *Rupestris*, *Ugni-Blanc* does not differ from either *Riparia* or *Rupestris*.

The homograftings (Fig. 2): The situation is the same as for the cuttings, except for the fact that at the end of the third cycle *Riparia* is significantly superior to the other three genotypes.

The mean scion effects (Fig. 3): One observes once more the same classification of the genotypes as in the previous cases. The peculiar behaviour of *Ugni-Blanc*, low growth during the first two cycles, much higher in the third, is again observed.

At the end of the third cycle, the genotypes are all statistically different from each other at the 0.05 probability level of significance, the decreasing order being as follows:

$$\underline{Riparia} > \underline{Ugni-Blanc} > \underline{Rupestris} > \underline{Cabernet-Sauvignon}$$

The mean rootstock effects (Fig. 4): The four mean growth curves for each genotype are closely clustered during the first cycle, the difference between the two extremes (*Riparia* and *Rupestris*), however, approaches the limit of significance. The dispersion increases during the following two cycles, although remaining smaller than in the three previous cases.

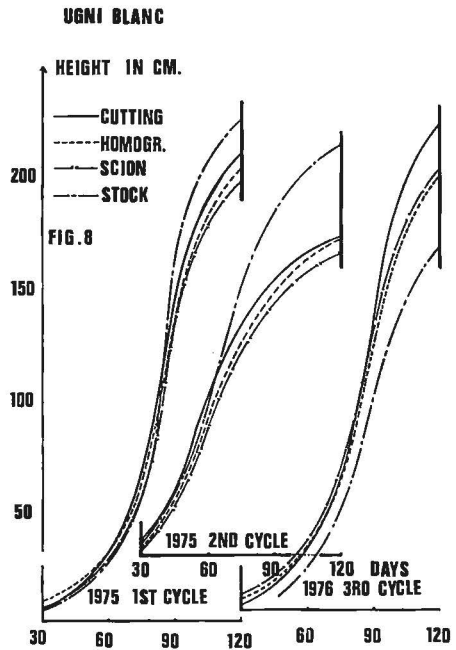
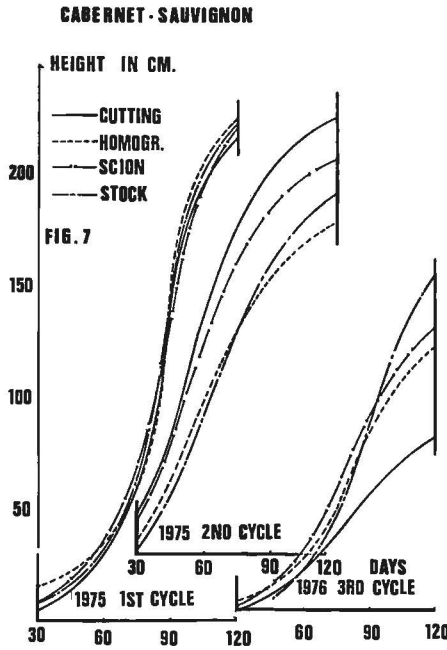
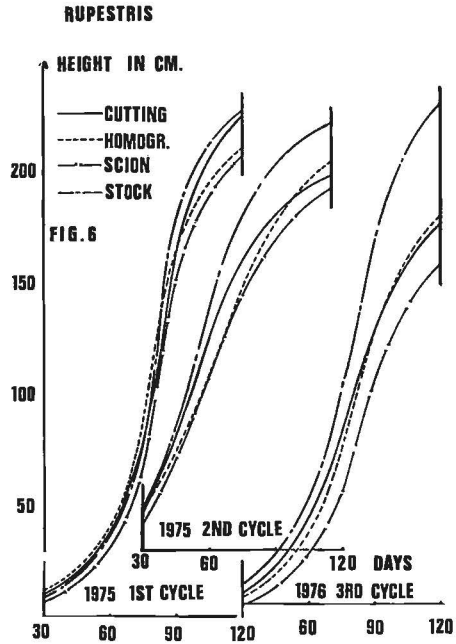
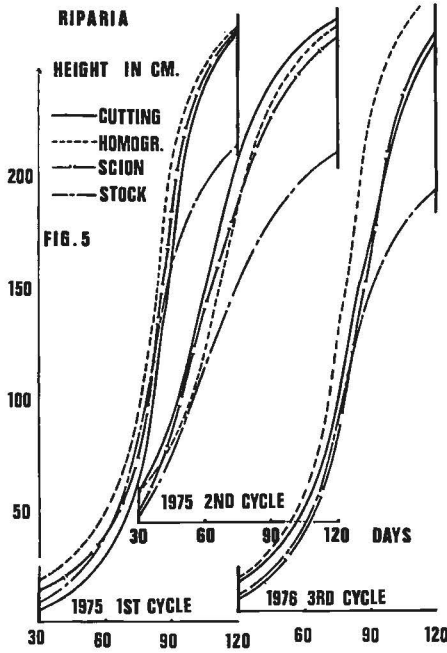
The mean performances conferred by the two "Vinifera rootstocks" decrease from the first to the third cycle. At the end of this last cycle, *Rupestris* is significantly superior to the three other genotypes and particularly to *Riparia*; *Cabernet-Sauvignon* is significantly inferior to *Riparia*. The decreasing order is as follows:

$$\underline{Rupestris} > \underline{Riparia} > \underline{Ugni-Blanc} > \underline{Cabernet-Sauvignon}$$

b) Comparison between the 4 functions for each genotype

Riparia (Fig. 5): Its rootstock function is significantly inferior to that of the other three, which do not differ from one another during the three cycles.

Rupestris (Fig. 6): There is no dispersion during the first cycle, in the second cycle its rootstock function becomes significantly greater than its scion function,



Figs. 5—8: Comparison of the 4 functions for each genotype.
 Comparaison des 4 fonctions pour chaque génotype.

and finally than all other functions during the third cycle. These latter functions do not differ among themselves.

Cabernet-Sauvignon (Fig. 7): Very closely grouped during the first cycle, its functions disperse during the second cycle, although no comparison is significantly different. During the third cycle, the growth is very slow, whatever the function. This genotype seems to show only weak functions, at least under the growth conditions of the third cycle.

Ugni-Blanc (Fig. 8): Rootstock and scion are significantly different as early as in the first cycle, the difference becomes evident in favour of the rootstock during the second cycle, the mean rootstock effect being very superior to the three other functions. This is reversed during the third cycle.

c) Conclusions

The dispersion of the curves increases from the first to the third cycle. During the last cycle, in particular, the tested sample of genotypes allows us, with respect to our experimental conditions, to demonstrate clear statistical differences. From that point of view, this sampling seems to be suitable for the measuring of the phenomena we wish to study.

Furthermore, especially for *Riparia* and *Rupestris* this material presents the classical behaviour expected, which is for the first an important "self growth" (as shown by cutting and homografting) and a weaker "conferred growth" (mean stock effect), the opposite holding for *Rupestris*. This indicates that the experimental conditions induced normal reactions.

Among the two *Vinifera* genotypes, *Cabernet-Sauvignon* seems to be weak for all functions and *Ugni-Blanc* to be average, at least during the third cycle. This cycle differs from the other two, for example with regard to the occurrence of competition relations between the neighbouring root-systems.

The two *Riparia* and *Rupestris* genotypes each present a good stability of the ordering of their functions during the three cycles, so that the order observed during the first cycle can be extrapolated to the following cycles. The two *Vinifera* genotypes are much less stable; their behaviour during the third cycle is somewhat different from that shown during the other two. To be easily and clearly interpreted, this situation requires measurements over a longer period; the three cycles of observation do not appear to be long enough.

Finally, with respect to the phenomena themselves and not the method, one can say that the three functions cutting, homografting and mean scion effect are generally very close to one another and that the fourth function, mean rootstock effect, is generally very different from the previous three.

This strengthens firstly the well known fact for vine of a total independence between the "self behaviour" of a genotype and the behaviour it confers as rootstock, and secondly shows that the mean behaviour of a scion (in combination with several other rootstocks) is close to that of the cutting.

B. Parameters, variance components

a) Grafting effect

The analysis of variance of the four genotypes used as cuttings or grafted on themselves allows the break-down of the total variance, at each stage of each cycle, and to assign to each source of variation (grafting, genotypes and grafting \times genotypes interaction) a percentage of the non-random variance (Table 1).

Thus, the grafting effect is an important source of variation during the first two months of the first growth cycle; it disappears during the following months to the benefit of the genotypes' effect. During the second year (1976), the variation due to the grafting (comparison between grafted and non-grafted plants) is equal to zero, even at the beginning of the growth.

The occurrence of interactions genotypes \times grafting indicates that all genotypes do not behave in a parallel manner to the grafting. The differences between cuttings and grafts are not the same for all genotypes.

b) Break-down of the variation for the functions

The variation observed for each function during the first and the third cycle are remarkably parallel (Table 2). The rootstock effects, each time pronounced at the start with reference to the growth determination, slowly make way to the scion effects, which play a leading part in the expression of the final height. However, the part of the variation due to the rootstocks remains important (19 %) at the end of the third cycle. The rootstock-scion interaction effects are higher in the first (fixing phase of the graft union) than in the third cycle and occur much more intensively at the beginning than at the end of each cycle. They may account for a very great part of the variation (65 % at the 30th day of the first cycle).

During the second cycle, specific in so far as it follows a green pruning, which implies no stop and restart of the root activity, the scion effects remain more or less the same. The two other sources of variation are progressively reversed.

These scales of the quantitative importance of the different parameters may be compared to those calculated by RIVES (1971 b) for vigour measurements made at the end of growth by the vine (data of SNYDER and HARMON 1948):

Rootstock effects	7 %
Scion effects	86 %
Interaction effects	7 %

c) Break-down of the variation for the genetic effects

— The SAA (Specific Associating Ability) accounts for nearly the total variation (82 %) at the beginning of the first cycle (Table 3). It reflects the importance of the associated partner's genetic affinity; the direction of the association (*i/j* or *j/i*) being at this stage immaterial in the growth determination. The importance of the SAA component then diminishes to the profit of the two other components:

— the GAA (General Associating Ability), i.e. the mean ability to grow as scion or to induce growth as rootstock,

— the RE (Reciprocal Effects) that we defined previously as the genotypes' ability to behave differently according to their function in the association. This component, weak at the beginning, increases regularly to over 62 % of the total variation at the end of the third cycle. At this stage the genotypes' behaviour as scions or as rootstocks is then very different.

It is interesting to note that at the end of this third cycle, if the variation due to the SAA is very weak, the variation due to the GAA is 34 % and represents the variation due to an "intrinsic genetic effect" of the genotypes in the determination of the final height. This is to be compared with the observations of BERNHARD and GERMAIN (1975) about peach rootstocks.

of different vine genotypes. The analysis of the principal components (the variables being the successive heights) showed that, if the first principal component, "final height" factor, accounts for 86 % of the inertia, the second one, "growth shape" factor, is still responsible for 10 % of the inertia.

Finally, these evaluations of the parameters show that the rootstock-scion interaction effect cannot be neglected in general and is even able, at some growth stages to account for the greater part of the variation. Thus, it appears necessary to take this interaction into consideration, not only in fine studies on the rootstock-scion association, but also at levels closer to practice like the classification of rootstocks for "conferred vigour". The great number of contradictory observations made in vineyards in relation to this feature can be, at least partially, attributed to the importance of that interaction and thus to the perturbation it introduces in this classification, based solely on the mean effects of each rootstock.

This interaction covers more or less the baldly defined, but classical term of "affinity" between rootstock and scion. It can now be considered in the light of the notion of genetic program recently developed by DEMARLY (1976). The grafting indeed deeply modifies the correlations between cells in each partner. At least around the grafting point, a change occurring in the cytoplasmic, and, hence, in the epigenetic situations provokes the genetic response to stop the "normal" program and to elaborate by means of producer genes (or structural genes) proteins directing the program towards what we might call a "morphogenesis of joining".

Is there at the level of this joining only confrontation of the genetic information arising from both rootstock and scion, or as recent works would tend to show (OTHA and CHUONG 1975, PANDEY 1975, 1976) crossing over these information at the union joint, particularly from the rootstock to the scion?

Summary

The behaviour variations of a scion in association with different rootstocks are well known, particularly in so far as the expression of vigour is concerned. This simple effect is only an element of one of the possible break-down of the rootstock-scion combination's quantitative characteristics.

We analyse experiments of complete reciprocal grafting by use of two biometric models, one breaking down the variation for the "functions" (rootstock, scion and interaction), the other breaking down the variation for "genetic effects".

Initial results allow us:

- 1 — to test the interest of these two models and the parameters we propose,
- 2 — to compute the quantitative importance of these parameters at different growth stages.

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