Genetic Parameters and Selection in a Multisite Wild Cherry Clonal Test

By F. SANTI, H. MURANTY, J. DUFOUR and L. E. PAQUES

Institut National de la Recherche Agronomique, Station d'Amélioration des Arbres Forestiers, 45 160 Ardon, France

(Received 1st August 1996)

Summary

A 7-year-old multisite clonal test with 33 clones provided the first estimations of genetic parameters and potential gains from clonal selection of wild cherry. Available traits included periodic stem height increments between years 0 and 2, and between years 2 and 7 after plantation (1st and 2nd stem HI), breast height (BH) girth, stem straightness and pruning notes, number, angle and thinness (stem diameter/branch diameter ratio) of branches, leaf dimensions and number of nectaries, susceptibility to aphid and anthracnosis.

Second stem HI, BH girth, branch angle and susceptibility to anthracnosis (or leaf spot) were the most heritable traits (0.56, 0.70, 0.57 and 0.83 at Sarrazac). The number and thinness of branches were also heritable, but only on agricultural sites (0.66 and 0.22 at Sarrazac). Second stem HI, BH girth and number of branches were genetically well correlated with each other, and also correlated with resistance to anthracnosis. The branch angle was unrelated with such traits. An ecovalence analysis of the site x clone interaction showed that for each trait a high proportion of clones and sites were weakly interactive.

We selected 8 clones after multisite index selection on 2nd stem HI, BH girth and, to a lesser degree, for thinness of branches. Two clones were eliminated for other reasons. With a weak selection intensity (1/4) we obtained a genetic gain of +11% for 2nd stem HI and +13% for BH girth. Six clones are now on the certification list. They are the first clonal varieties of wild cherry officially available in EU countries.

Key words: Prunus avium, heritability, correlation, genetic gain, clone, stability, spatial analysis, aphid, anthracnosis.

FDC: 232.11; 165.441; 165.3; 176.1 *Prunus avium.*

Introduction

Wild cherry (*Prunus avium* L.) is mainly a European species, occuring generally as isolated trees, clumps, rows at edges of forest, and only rarely in denser mixed stands. Natural suckering leads to clones covering up to at least 0.5 ha, reducing dramatically the number of different genotypes, especially in the most dense populations (FRASCARIA *et al.,* 1993).

Due to its valuable wood, which is used for panelling and cabinet-making, and its fast growth (rotation of 50 to 60 years), the species is now increasingly planted in Europe, both in afforestation of abandoned farm land and enrichment of forests. The use of wild cherry will be further encouraged by EU programmes (CAVAILHES and NORMANDIN, 1993). Preferred soils are deep, loamy, as well as quite rich and they must have a suitable water table (FRANC *et al.,* 1992). Intensive silviculture (large spacing, protection against rodents and game, weed and pest control, pruning) is currently practiced. Active research is conducted in almost all European countries on the silviculture on wild cherry (CATRY and POULAIN, 1991; BESSIERES, 1992) and breeding programmes are well-established (LEMOINE *et al.,* 1992).

Selected seed stands have been available in France since 1989, but this material is mostly insufficient because (i) variability inside selected seed stands is not large (FERNANDEZ *et al.,* 1994), and (ii) the level of genetic improvement is not suited to the well-spaced plantations currently used for wild cherry (around 400 trees/ha).

The breeding programme conducted at INRA, one of the oldest in Europe, was initiated in 1978 by plus-tree collection and their vegetative propagation by *in vitro* culture (RIFFAUD and CORNU, 1981) and soft wood cuttings (CAZET *et al.,* 1993). We provided material of known origin for clones while awaiting selection of seed stands. Concomitantly, the clones were field tested in appropriate designs, which now allow us to provide genetically improved clones. Twelve clones have been submitted for certification in 1994. Simultaneously, genetic information is becoming available. Initial results on genetic parameters and possible gains from clonal selection are presented, based on a multisite clonal test.

Material and Methods

Material and sites

Thirty-four plus-trees originating from the northern half of France were multiplied by *in vitro* culture in 1984 and provided 1-year-old plantlets at the end of 1984.

The clones were planted at 7 m x 3 m over 5 sites (*Table 1*), each totaling 495 plants. Sufficient space was lacking in one site, so that the experiment was divided into 2 parts: 9 blocks at Ecouvottes (297 plants) and 6 blocks at Vaux-les-prés (198 plants), 12 km apart. This trial was finally treated as two different sites, because stand potentials were quite different (*Table 1*). The design was unbalanced incomplete blocks with random composition. Six to 18 ramets per clone and per site were planted in 3-tree linear plots. Borders and unfavourable zones were also planted with the same set of clones to neutralize border effects.

Ideotype and measurements

Rapid stem height increment during the first years following plantation was important to reduce the length and the total cost of silvicultural interventions, but also to reduce the number of pseudo-whorls. The total height (cm) was measured at time of plantation and from the 1st to 7th year following plantation. Two periodic stem height increments (stem HI) were calculated: "1st stem HI" from the years 0 to 2 (after plantation), which includes the period of plantation establishment, and "2nd stem HI" from the years 2 to 7. Breast height girth, "BH girth" (mm) was measured at the end of the 7th year.

Stem straightness was an important factor to consider for the future use of the wood. 5 scores (1=perfect to 5=very poor) were noted for the year 7 at Saveuse and Neufchatel.

A high number of branches, fastigated or with a high diameter, leads to more difficult pruning. Branches with large diameter or very fastigated result in bigger wounds and necessitate rapid and severe pruning. A corrective pruning score with 5 grades (1=no defect, 2 and 3=one and several

Table 1. – Characteristics of sites.

TRIAL	Lat	Long	Annual rainfall (mm)	Mean temperature (°c)	soil pH	Characteristics
Sarrazac	45°26'	1°01'	1050	10	6.5	divided in 2 zones, one was a meadow, the other was cultivated before. Soil very favourable, well-cared-for by owner.
Neufchatel	49°44'	1°29'	760	10	4.7	forest site. Numerous stones reducing the water content capacity.
Saveuse	49°54'	2°14'	650	10	5.5	divided in 2 zones, one was afforested, the other was a meadow before.
Vaux-les-prés	47°15'	5°56'	1070	10	-	clam before, deep favourable soil.
Ecouvottes	47°10'	6°02'	"	"		forest soil, very heterogeneous.
Benon	46°14'	1°14'	830	12	-	shallow and clayrion forest soil.

defects rectifiable by pruning, 4=important pruning necessary with unknown result on straightness, 5=impossible to correct form by pruning) was made at Neufchatel and Saveuse in year 7. Number of branches formed the second year, angles and diameters of the 3 biggest branches and diameter of the stem just above the pseudo-whorl (formed the previous year) were measured in year 3 at Neufchatel, Sarrazac and Vaux-les-prés. Mean angles were calculated, and also stem diameter/mean branch diameter ratio ("stem D/branch D"), which is a measure of branch thinness, a more interesting trait than the diameter of branches itself.

Leaf length and width, as well as length of petiole and number of nectaries were observed at Neufchatel on a sample of 203 plants (1 to 6 ramets/clone, mean of 4.8). The mean measurements of the 5th, 6th and 7th leaves down from the apex were calculated for each plant.

Aphids bend apical stems and affect stem form and straightness. An aphid (*Myzus cerasi*) attack was scored (1=few to 3=severe) at Sarrazac. Susceptibility to anthracnosis, a fungal (*Blumeriella jaapii*) disease of leaves is the worst sanitary problem of wild cherry in France. It was observed and scored at Sarrazac from years 5 and 9 and at Neufchatel in year 9 (1=almost no attack, 2=less than 10 spots per leaf, 3=10 to 100 spots per leaf, few fallen leaves, 4=more than 100 spots per leaf, many fallen leaves, 5=almost all leaves fallen. Interval notes 1.5, 2.5… were taken in year 9).

Selection procedure

The multitrait selection with the HAZLE-SMITH Index includes heritable enough traits, and economical weighting, both of which were optimized after simulation of several possible choices. We balanced the multisite selection index with half forest sites and half agricultural sites, because wild cherry clones are intended to be planted on both forest soils and agricultural soils.

Elimination of plant material

Individual growth curves for height data were examined and aberrant data were discarded.

Errors in clone identification were suspected for clones 218 and 153, based on form and susceptibility to anthracnosis. Buds from twigs collected at Sarrazac were analysed by protein electrophoresis (method detailed in SANTI and LEMOINE, 1990). Isoenzymatic multiloci genotypes confirmed that "clone 153" was in fact clone 106, already present in the field test, and that clone 218 was mixed with another clone. The total number of clones was then 33. The plants mixed with clone 218, unidentified, were discarded from the analysis. In other tests, where twigs were not collected, clone 218 was systematically discarded.

Data analysis

Blocks were designed at time of planting. However, to optimize results from the heterogeneous field tests and the large plantation spacing we choose to use the iterative Papadakis method, which is a spatial analysis of site effects which uses performances of neighbours (here we used a 3x3 grid, that is 8 neighbours) to estimate microsite effect on each plant in absence of competition between plants. The model for the adjusted data is then:

Y for each plant = mean + clone effect + error

This method is more efficient as it minimizes residual variance (PAPADAKIS, 1937; PICHOT, 1993). The degrees of freedom for this variance are higher since none is required for another effect like as a traditional analysis with blocks. With our data, we verified that the residual variance was always lower with the PAPADAKIS method, when compared with the traditional one.

The 4 postulates of analysis of variance were graphically verified, except for 1) "The expectation of residual error is null", which is impossible to verify.

2) "The variance of residual is independant of the level of factors" is verified with graphs of residus, function of estimated values for each level.

3) "The residues for each level of factor are independant" is verified with a graph of residues in the order of measure.

4) "The residues are normally distributed" is verified with a graph of reduced residues function of quantiles of standard normal (Henry line).

Multisite analysis of variance (6 sites for growth traits, 3 sites for branch number and branch angle) with adjusted data was done according to the model:

Y for each plant = mean + clone effect + site effect + clone · site interaction effect + error

The clone effect is random, whereas the site effect is fixed.

Ecovalences of sites and clones were calculated (WRICKLE, 1965).

The *Splus* package (1988) was used for basic statistical analysis and graphs. The *Select* package (MANGIN, 1992) was used for the estimations of genetic and residual variances-covariances by the restricted maximum likelihood method with an interactive method (and variances of these estimations) and of genetic and index values (BLUPs=Best Linear Unbiaised Predictor). Correlations and their estimation variances were then estimated. Comments on genetic correlations will take into account that, most often, several sites are analysed, which provide several estimations. Broad-sense heritabilities were estimated with the assumption of no or negligible C-effect (defined by BURDON and SHELBOURNE, 1974):

Table 2. – Percentage of non measurable trees at each site and each trait (%T), residual coefficient of variation (%CV) and F-test for clone (F).

TRAITS		Sarrazac			Neufchatel			Saveuse			Vaux-les-prés			Ecouvottes			Benon		
measured the year :		***%T***	%CV	F	***%T***	%CV	F	***%T***	%CV	F	***%T***	%CV	F	***%T***	%CV	F	***%T***	%CV	F
1st stem HI	0-2	***1***	13	6.3**	***10***	21	3.4**	***10***	33	4.3**	***6***	23	1.3ns	***10***	27	2.7**	***4***	43	1.2ns
2nd stem HI	2-7	***1***	08	9.1**	***16***	21	5.1**	***7***	21	4.2**	***7***	21	4.2**	***55***	40	2**	***13***	27	2.4**
BH girth	7	***1***	10	16.3**	***4***	30	5.6**	***4***	28	5.3**	***4***	27	2.8**	***53***	46	1.2ns	***52***	25	3**
branch number	3	***2***	17	22**	***16***	37	4**				***5***	39	2**						
branch angle	3	***8***	12	17**	***13***	11	10**				***27***	12	3.8**						
stem D/branch D	3	***8***	18	5**	***13***	15	2.8**				***26***	28	1.8*						
stem pruning	7				***7***	57	3**	***7***	44	1.6**									
stem straigthness	7				***7***	50	2.1*	***7***	26	3**									
aphid attack	5	***1***	35	8.8**															
anthracnosis	5	***1***	27	13.5**															
anthracnosis	9	***3***	16	74**	***4***	11	136**												

**) significant at 1% level, *) significant at 5% level, ns not significant

$$h^2_{bs} = \frac{\sigma^2_G}{\sigma^2_G + \sigma^2_e}$$ for monosite heritabilities,

$$h^2_{bs} = \frac{\sigma^2_G}{\sigma^2_G + \sigma^2_{GxS} + \sigma^2_e}$$ for multisite heritabilities

with σ^2_G = genetic variance, σ^2_e = error variance, σ^2_{GxS} = clone · site interaction variance. $\sigma^2_{GxS}/\sigma^2_G$ was the relative importance of interaction. Their estimation variances are estimated with the variances of estimations of variances and covariances.

Results and Discussion

Principal general means per site

The percentage of dead plants in year 7 was negligible at all sites (0.4% to 5%), except at Ecouvottes (41%), due to a non-adapted herbicide treatment. No clonal variation was related to the percentage of death. The proportion of non-measurable trees varied a great deal (*Table 2*). It was never higher than 16% at Sarrazac, Neufchatel and Saveuse, but at Vaux-les-prés it reached 27% for branch measurements (for early pruning), and more than 50% at Ecouvottes (for high mortality) and Benon (for growth difficulties).

The mean annual stem height increment was very different between sites, reflecting fertility differences ranging from 23 cm/year at Benon to 100 cm/year at Sarrazac. Mean height increased quite regularly in all sites except at Sarrazac where trees grew slowly during the first year in comparison with following years (*Figure 1*). The late planting date (March, 21th, 1985) for this southern site probably explained this more severe effect during establishment. Breast height girth means followed this pattern, varying from 78 mm and 56 mm at Ecouvottes and Benon respectively, to 333 mm at Sarrazac. The mean branch numbers per pseudo-whorl were comparable at

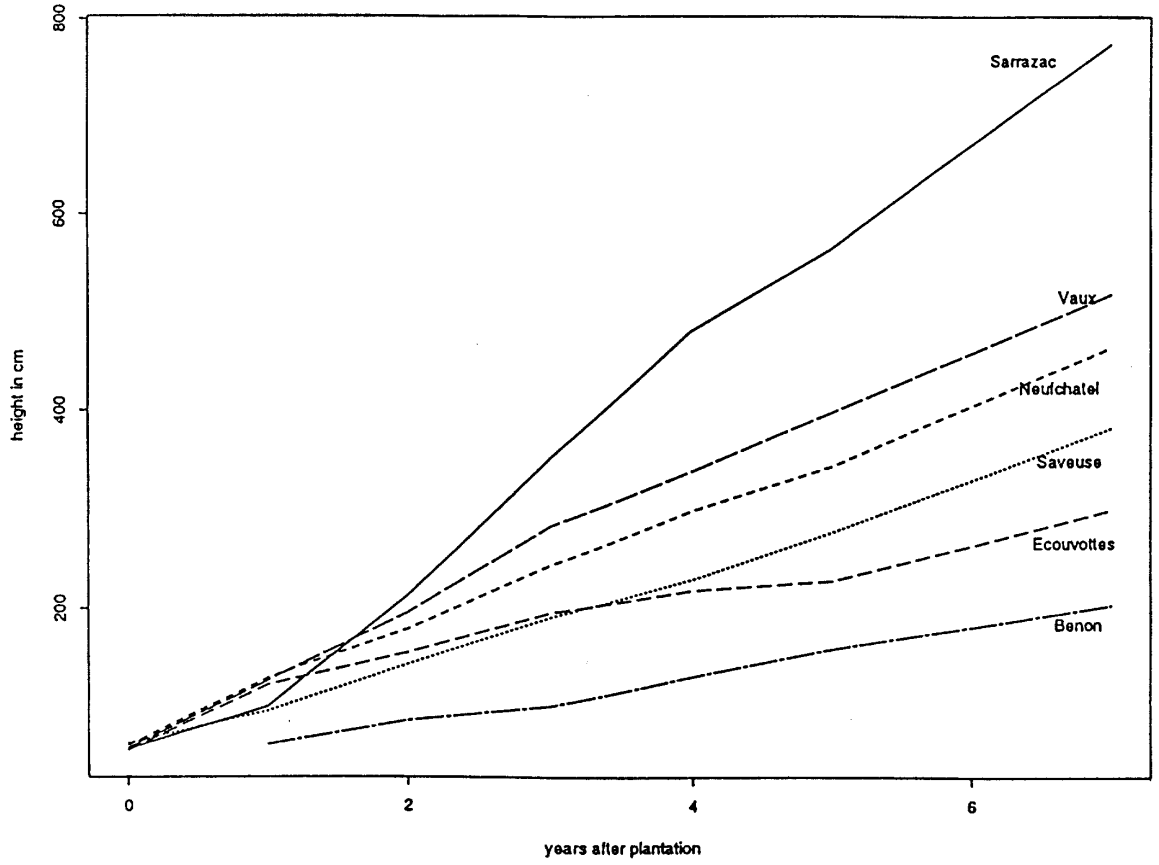

Figure 1. – Development of mean height from year 0 to 7 in the 6 sites.

Sarrazac, Vaux-les-pres and Neufchatel (5.1, 4.7 and 5.8, respectively). At Sarrazac and Vaux-les-prés, the mean branch angles were both of the same magnitude (43° and 46°), whereas it was higher at Neufchatel (52°). The mean stem D/branch D was worst at Sarrazac (1.31) when compared to Neufchatel (1.50) and Vaux-les-prés (1.65).

Analysis of variance site per site

For almost all traits, the clone effect was significant (*Table 2*). In Benon, Ecouvottes and Vaux-les-prés differences between clones were less well expressed (weak F-values). Moreover, clone effects were undetectable for 1st stem HI at Benon and Vaux-les-prés and for girth at Ecouvottes. This might be explained by less data being available at these sites, and Benon and Ecouvottes were very heterogenous. They had the most important residual coefficients of variation. By comparison, the residual variation coefficients for growth traits were smallest at Sarrazac, due to the excellent growing conditions and the homogeneity of the site.

For stem pruning and stem straightness values, the residues of weakest estimated values are more often negative, the residues of biggest estimated values are more often positive, and the distribution of residues is not normal. Care must to be taken in the further analysis these data.

Broad-sense heritabilities for individual sites (Table 3)

The heritability of 1st stem HI was always smaller than 2nd stem HI heritability, corresponding to the crisis associated with plant establishment. The Saveuse site planted earlier in winter did not show such a crisis. Weak heritabilities were also a function of weak genetic variances: σ^2_G=1056 at Ecouvottes compared to 1990 at Sarrazac for 2nd stem HI.

The heritability for breast height girth was high at Sarrazac but lower at other sites. The heterogeneities due to competing tree vegetation which could be expected in forest sites may provide an explanation for lower heritability at Benon, Neufchatel and Saveuse. For Vaux-les-prés, which was planted on an agricultural site, like Sarrazac, perturbation due to severe pruning could explain the results.

The number of branches had very different heritabilities at the 3 sites. Expression of the trait was less favoured at Neufchatel (h^2=0.20), a forest site possessing a variety of competition microenvironments. The heritability of branch angle was higher at Sarrazac (0.57). The thinness of branches had a low heritability (0.13) at Neufchatel, the forest site, compared to 0.22 and 0.32 at Sarrazac and Vaux-les-prés.

Stem straightness and stem pruning scores had very low heritabilities. Adverse incidents during the growth could account for these scores. For example at Saveuse, we noticed an attack by insects (cecidomyiids), bending and arresting the growth of the leader. Low heritabilities could also be explained by a little genetic variance: at Saveuse, the difference between the best and the worst clone scores was 1 point on a 5-point theoretical scale. In general, stem form was quite good. Moreover, since problems of estimations had already been detected when looking at residues, these traits were discarded from further analysis.

Length of petiole and number of nectaries showed good heritabilities: 0.49 (SE=0.085) and 0.48 (SE=0.085) whereas length x width of the leaf heritability was 0.23 (SE=0.082).

Aphid attack susceptibility was less heritable (0.40) than anthracnosis susceptibility. The last score done for anthracnosis, in 1993 showed greater heritability (0.83 at Sarrazac or 0.91 at Neufchatel instead of 0.56 scored in 1989 at Sarrazac) as the attack was more severe allowing better scoring (1 to 5 scale instead of a 1 to 3 scale).

Major intra-site genetic correlations (Table 4)

First and 2nd stem HI (Sarrazac, Neufchatel, Saveuse, Ecouvottes) were well correlated at Neufchatel (0.60) and at Saveuse (0.70), with an environmental correlation in the same sense (0.26 and 0.15) whereas at Sarrazac, the genetic correlation was only 0.25. The interpretation of these results could be that: during these two growing periods, roughly the same genes were expressed, except at Sarrazac where the plantation crisis was comparatively severe.

The genetic correlations between BH girth and stem HI (all sites except Ecouvottes) were high (0.32 to 0.75 with 1st stem HI and 0.46 to 0.85 with 2nd stem HI) and environmental correlations were positive (0.32 to 0.63 for 1st stem HI, and 0.52 to 0.85 for 2nd stem HI), which was expected. The correlation of susceptibility to anthracnosis and 2nd stem HI was found to be negative (-0.56 at Sarrazac, -0.58 at Neufchatel). This correlation is even more evident with girth growth (-0.77 and -0.76). Attacks on leaves reduced photosynthetic capacity, and the effects were consequently more severe on girth. Stem height growth stoped in July, before major attacks occurred, while trees continued to grow laterally.

Susceptibility to aphid attack and to anthracnosis were negatively correlated (-0.50): the largely defoliated trees were less susceptible to aphids. Aphid colonies scored in the spring originated from eggs laid near petioles during the previous autumn. We can suppose that females laying eggs on wild cherry trees choose ones with leaves, those least infested by anthracnosis. Moreover, an experiment in a clone bank with the same clones showed no clonal difference on colony growth

Table 3. – Individual and multi-site broad-sense heritabilities and $\sigma^2_{GxS}/\sigma^2_G$ estimations (standard error).

TRAITS *measured the year :*		Sarrazac	Neufchatel	Saveuse	Vaux-les-prés	Ecouvottes	Benon	Multisite h^2	Multisite $\sigma^2_{GxS}/\sigma^2_G$
1st stem HI	0-2	0.37 (0.07)*	0.15 (0.05)	0.24 (0.06)	–	0.08 (0.05)	–	0.10	1.20
2nd stem HI	2-7	0.56 (0.07)	0.32 (0.07)	0.25 (0.06)	0.50 (0.09)	0.22 (0.09)	0.34 (0.06)	0.20	0.90
BH girth	7	0.70 (0.06)	0.38 (0.07)	0.33 (0.07)	0.42 (0.09)	–	0.31 (0.06)	0.23	0.77
branch number	3	0.66 (0.06)	0.20 (0.06)		0.37 (0.09)			0.21	0.94
branch angle	3	0.57 (0.07)	0.45 (0.07)		0.46 (0.09)			0.38	0.31
stem D/branch D	3	0.22 (0.06)	0.13 (0.05)		0.32 (0.09)			0.04	3.92
stem pruning	7		0.16 (0.05)	0.05 (0.03)					
stem straigthness	7		0.14 (0.05)	0.13 (0.04)					
aphid attack	5	0.40 (0.07)							
anthracnosis	5	0.56 (0.07)							
anthracnosis	9	0.83 (0.04)	0.91 (0.02)						

Table 4. – Major intrasite genetic correlations (standard error).

TRAITS	Sarrazac	Neufchatel	Saveuse	Vaux-les-prés	Ecouvottes	Benon
1st stem HI - 2nd stem HI	0.25 (0.19)	0.60 (0.16)	0.70 (0.13)	-	0.50 (>1)	-
BH girth - 1st stem HI	0.32 (0.17)	0.46 (0.18)	0.75 (0.10)	-	-	-
BH girth - 2nd stem HI	0.46 (0.15)	0.85 (0.06)	0.77 (0.09)	0.85 (0.07)	-	0.68 (0.13)
anthracnosis - 2nd stem HI	-0.56 (0.13)	-0.58 (0.14)	-	-	-	-
anthracnosis - BH girth	-0.77 (0.08)	-0.76 (0.10)	-	-	-	-
anthracnosis - aphid attack	-0.50 (0.15)		-	-	-	-
branch number - 1st stem HI	0.35 (0.17)	0.24 (0.23)	-	-	-	-
branch number - 2nd stem HI	-0.32 (0.17)	-0.13 (0.22)	-	0.24 (0.22)	-	-
stem D/branch D - 1st stem HI	0.17 (0.21)	-0.57 (0.22)	-	-	-	-
stem D/branch D - 2nd stem HI	0.22 (0.20)	-0.71 (0.16)	-	-0.13 (0.25)	-	-
stem D/branch D - BH girth	0.01 (0.20)	-0.59 (0.17)	-	-0.23 (0.25)	-	-
leaf length*width - 2nd stem HI	-	0.70 (0.17)	-	-	-	-
petiole length - 2nd stem HI	-	0.60 (0.15)	-	-	-	-

after artificial infestation (i.e. no choice were offered to aphids) in the spring (10 adult aphids on 6 apical homogeneous wrapped twigs per clone).

The number of branches (Sarrazac, Neufchatel, Vaux-les-prés) was unsurprisingly better correlated with 1st stem HI (0.24 and 0.35), than with 2nd stem HI (-0.13 and -0.32) at Neufchatel and Sarrazac, as the counted branches appeared during year 2.

Branch thinness (Sarrazac, Neufchatel, Vaux-les-prés) was negatively correlated with 1st stem HI (-0.57), with 2nd stem HI (-0.71), and with BH girth (-0.59) at Neufchatel (the more vigourous trees had thicker branches). At Sarrazac and Vaux-les-prés, all the correlations were near zero.

Leaf length · width, and petiole length were well correlated with 2nd stem HI (0.7 and 0.6).

Clone x site interaction analysis

The F-tests for clone, site and clone x site values of multisite analysis were all significant at the 0.01 level.

The lowest interaction variance σ^2_{GxS} was noted for branch angle ($\sigma^2_{GxS}/\sigma^2_G$ = 0.3, *Table 3*) and the biggest was shown for the thinness of branches ($\sigma^2_{GxS}/\sigma^2_G$ = 3.9). For the other traits, the interaction variance was similar to the genetic variance. Vaux-les-prés was the most interactive site for all traits (37% to 77%, *Table 5*) except for 1st stem HI (Les Ecouvottes represented 44% of the interaction). The most interactive clones for growth traits are clones 255 and 113 (7% to 14% of the interaction), followed by 203, 109, 104 and 155 (4% to 6%). For branch thinness, the most interactive clones were 184, 139, 155, 132 and 255 (7% to 13%). Clone 139, very spreading, was by far the most interactive for branch angle (22%).

Table 5. – Relative ecovalences of sites (%).

TRAITS	Sar	Neu	Sav	Vaux	Eco	Benon
1st stem HI	21.3	16.3	18.6	-	**43.8**	-
2nd stem HI	10.1	13.1	7.8	**37.6**	20.1	11.3
BH girth	20.6	10.7	13.9	**36.9**	-	17.9
branch number	30.4	24.1		**45.5**		
branch angle	19.0	16.4		**64.6**		
stem D/branch D	11.7	11.3		**77**		

If we excluded Ecouvottes, Vaux-les-prés and Benon (interactive or unadapted sites), $\sigma^2_{GxE}/\sigma^2_G$ ratios were only 0.376 (1st stem HI), 0.356 (2nd stem HI) and 0.485 (BH girth). The clone 255 contributed towards a large part of the interaction terms (13%, 30% and 24% respectively) due to its bad ranking at Neufchatel where it had only one ramet.

The multisite heritabilities were of course lower than the best individual site heritabilities (*Table 3*). Genetic correlations between 2nd stem HI (or BH girth) at Sarrazac, Neufchatel, Saveuse and Vaux-les-prés were between 0.55 and 0.80 (data not shown) with the exception of 2nd stem HI at Neufchatel and Saveuse (0.38). The correlation between branch thinness at Sarrazac and Vaux-les-prés was only 0.18.

Selection of clones

The goal was to select 8 clones out of 33 and then to submit them for certification.

We included 2nd stem HI and BH girth with equal economical weighting. We also tried to include branch thinness in the index at Sarrazac and Vaux-les-prés where heritabilities were the highest and where no negative genetic correlation with growth traits has been noted. The relative balance of branch thinness in the index was fixed so that genetic gain was obtained inside each site for growth traits, with no loss of thinness of branches. Stem pruning and straightness scores had heritabilities too weak to be integrated in an index. Susceptibility to anthracnosis had already been highly correlated with growth parameters. The branch angle was not included in the selection as no clone were fastigiated enough to be excluded. Economical weights were thus 0.5 and 0.5 for 2nd stem HI and BH girth at Neufchatel and Saveuse, and were 0.375, 0.375 and 0.25 for 2nd stem HI, BH girth and stem diameter/branch diameter at Sarrazac and Vaux-les-prés. In the multisite index we eliminated Ecouvottes and Benon since these sites were obviously not good enough for wild cherry. Each of the 4 other sites (2 forest sites and 2 agricultural sites) received the same economical weighting for the multisite index.

In the resulting ranking we then eliminated the clone 184 which was revealed to be hooked by direct observation in tests. We excluded clone 164 as it was collected simultaneously with clone 165 in a forest stand. Depending on the surface to be planted, only 2 to 3 clones may, on occasion, be used. They must originate from different populations. The last selected clone was clone 255 instead of clone 132 (ranked just before), as we preferred not to take into account clone 255 at Neufchatel where it is only represented by one ramet, which is considered inadequate representation. The 8 clones (1/4 of the total) finally selected were clones 253, 227, 165, 141, 171, 108, 254 and 255.

Table 6. – Ranking of finally selected clones based on index selection in each site and their genetic values (differences from means) and means.

SELECTED CLONES		253	227	165	141	171	108	254	255	mean
Final selection ranking		1	2	3	4	5	6	7	8	
Multisite index		1.45	1.10	0.83	0.71	0.59	0.40	0.40	0.20	
Sarrazac	**local ranking**	**1**	**2**	**11**	**3**	**8**	**13**	**4**	**5**	
	2nd stem HI	*47*	*67*	*40*	*45*	*56*	*19*	*35*	*71*	*544 cm*
	BH girth	*78*	*43*	*23*	*45*	*11*	*29*	*51*	*-21*	*333 mm*
	anthracnosis	*-1.2*	*0.51*	*-1.3*	*-2*	*0.02*	*-1.1*	*-1.7*	*-0.26*	*2.7*
	stem D/branch D	*0.08*	*0.08*	*-0.09*	*0.14*	*0.02*	*-0.07*	*0.03*	*0.13*	*1.3*
Neufchatel	**local ranking**	**1**	**8**	**3**	**5**	**9**	**7**	**6**	**31**	
	2nd stem HI	*51*	*37*	*49*	*43*	*36*	*28*	*17*	*-59*	*301 cm*
	BH girth	*54*	*24*	*35*	*31*	*14*	*32*	*41*	*-60*	*161 mm*
	anthracnosis	*-1.8*	*0.6*	*-1.1*	*-2.3*	*0.4*	*-1.5*	*-2.3*	*-0.2*	*3.3*
	stem D/branch D	*-0.08*	*0.02*	*-0.13*	*-0.06*	*-0.05*	*-0.03*	*-0.09*	*0.07*	*1.5*
Saveuse	**local ranking**	**1**	**7**	**5**	**21**	**15**	**10**	**8**	**3**	
	2nd stem HI	*34*	*8*	*17*	*-9*	*20*	*10*	*15*	*44*	*238 cm*
	BH girth	*64*	*28*	*20*	*-5*	*-6*	*12*	*16*	*23*	*143 mm*
Vaux-les-prés	**local ranking**	**3**	**6**	**1**	**5**	**4**	**15**	**16**	**11**	
	2nd stem HI	*84*	*43*	*96*	*100*	*84*	*27*	*-40*	*55*	*324 cm*
	BH girth	*59*	*26*	*58*	*22*	*76*	*7*	*9*	*1*	*199 mm*
	stem D/branch D	*0*	*0.2*	*0*	*0*	*-0.2*	*-0.2*	*0*	*-0.2*	*1.6*

The selected clones had a good growth in height and girth (*Table 6*). The clone 255 was the most tapered and had the thinnest branches. Anthracnosis resistance was good, except for clones 227 and 171 which were slightly inferior or equivalent to the mean. These ought not to used in sites particularly susceptible to anthracnosis.

Table 7. – Expected genetic gains, expressed in percentage of each trait mean, obtained with the finally 8 clones selected.

TRAITS	Sar	Neu	Sav	Vaux	Multisite
2nd stem HI	9	8	7	17	11
BH girth	10	13	13	16	13
stem D/branch D	3	-3		-3	0
anthracnosis	33	31			

The superiority of the selected clones expressed in % of site means for 2nd stem HI and BH girth were satisfactory in all sites, varying from 7% to 17% (*Table 7*). A good improvement (31% to 33%) was also obtained on anthracnosis resistance.

General discussion

The only available results on wild cherry clonal tests come from Italy, where 7 clones were planted in another multisite trial (Ducci *et al.,* 1990). Components of variance are difficult to estimate with limited numbers of clones, but some results obtained by this group on the 3d year of growth after plantation are in agreement with ours. Typically, results are more reliable in fertile agricultural sites, the number of branches per pseudo-whorl is higher for fast-growing trees, and changes of ranking are generally limited between sites. A good relation between height at plantation and total height at 3 years was observed in all plantations in the Italian study, which we did not observe. However, our plantations were older and we choose to compare height at plantation with subsequent height increments instead of total height, which comprises the initial height itself (structurally correlated variables).

The optimization of selection of ramet number per clone and per site and number of sites is discussed elsewhere (Muranty *et al.,* 1996). Increasing the number of sites is very costly, so a proper choice of a few good sites is very important. Wild cherry, though able to survive on a great variety of soils, nevertheless produces the most valuable wood only in favourable conditions. Sites selected for tests must be a good sample of potential planting sites and must allow for good discrimination between clones. The most homogeneous and rich site: Sarrazac provided a far better discrimination of clones (higher heritabilities). Agricultural sites are probably the most suited to experimentation. However, since forest sites are also planted with wild cherry, relatively homogeneous forest sites, well-suited to wild cherry, could also be chosen for experimentation.

Weaker heritabilities for 1st stem HI confirmed the interest in the preferential use of 2nd stem HI when judging clones.

Early forks or terminal bud abortions which occurred by chance, would nevertheless require a corrective pruning. Stem straightness could be noted late and on the upper part of the stem.

Unfavourable correlations with growth traits affected the branch number per pseudo-whorl. However, vigorous trees have less pseudo-whorl per unit length of stem, so we could advantageously count branches per unit of stem length to estimate the total number of branches to prune.

The more vigorous a tree is, the bigger the branches are. Our interest is in selecting the most vigorous trees. As the trees have to be pruned anyway, the advantage of vigour lies in shortening the period when trees have to be pruned. When the desired length of pruned stem is obtained, the more vigourous trees would be expected to grow faster in girth. In our set of tests, the thinness of branches was uncorrelated between Sarrazac and Vaux-les-prés, which prevented a genetic gain for this trait in multisite selection. Only 3 branches of the 2nd pseudo-whorl were measured, which is perhaps not enough to reflect the mean thinness of branches along 6 meters of stem.

The high heritability of branch angle, and the lack of site · clone interaction suggest that phenotypic selection of plus-trees had probably some effect on this trait, provided its expression was the same in old trees. Moreover, no unfavourable correlation with stem HI was noted. Early selection in collection or in nursery stock against the most fastigated trees (< 30°) would probably be efficient.

The petiole length seems to have potential as a factor for early indirect selection of stem height growth (good heritability and positive correlation). But we must first test whether measurement in the nursery will give the same result.

Resistance to anthracnosis could also be selected for in collections or in nurseries, as heritability is very high.

Our measurements of susceptibility to aphid attacks did not reveal any opportunities for selection. This lack of variability is confirmed by studies on controlled aphid colony development on other clones preformed simultaneously to ours in Italy and in England (F. DUCCI and F. NICOLL, pers. comm).

In our set of sites, clone · site interaction for growth traits does not play a major role for three of the sites. The sites, though diverse (*Table 1*), do not represent all the potential sites for wild cherry. However, the selected clones have also been tested on some other sites with the same satisfactory results. These clones will be proposed as suitable for most French ecological conditions. Better estimation of adaptation with new plantations will nevertheless be necessary for final certification of clones (10 years later).

Final multisite selection did not bring any improvement of branch thinness. However, clones of interest for this trait (i.e. clone 139, 7th with Sarrazac index or clone 184, 2nd with Vaux-les-prés index) but unsuitable for certification due to crookedness or instability, will be included in future crossings.

The estimated gain on stem height increment and breast height girth is limited as we wanted to propose enough clones for certification (proportion selected: 25%), to retain enough genetic diversity. In future, additional clones will be selected while our younger tests are ageing and furnishing us with more pertinent genetic information. Final certification of clones will also limit the number of selected clones and thus allow better gains.

Acknowledgments

We thank the EEC which funded part of this work.

Literature

BESSIERES, F.: La conduite des peuplements de frêne (*Fraxinus excelsior* L.) et de merisier (*Prunus avium* L.). Rev. For. Fr. **XLIV** special, 115–120 (1992). — BURDON, R. D. and SHELBOURNE, C. J. A.: The use of vegetative propagules for obtaining genetic information. N. Z. J. For. Sci. **4**, 418–425 (1974). — CATRY, B. and POULAIN, G.: Le merisier en Nord – Pas de Calais – Picardie. Forêt Entreprise **91**, 19–24 (1991). — CAVAIHES, J. and NORMANDIN, D.: Déprise agricole et boisement: Etat des lieux, enjeux et perspectives dans le cadre de la réforme de la PAC. Rev. For. Fr. **XLV-4**, 465–482 (1993). — CAZET, M., DUFOUR, J. and VERGER, M.: Multiplication du merisier per bouturage herbacé. PHM Revue horticole **338**, 27–30 and **339**, 11–13 (1993). — DUCCI, F., VERACINI, A., TOCCI, A. and CANCIANI, L.: Primi resultati di una sperimentazione pilota di arboricoltura clonale da legno con *Prunus avium* L. Annali dell'istituto sperimentale per la selvicoltura, Arezzo, XXI (1990). — FERNANDEZ, R., SANTI, F. and DUFOUR, J.: Les matériels forestiers de reproduction sélectionnés de merisier (*Prunus avium* L.): classement, provenances et variabilité. Rev. For. Fr. **XLVI-6**, 629–638 (1994). — FRANC, A., BOLCHERT, C. and MARZOLF, G.: Les exigences stationnelles du merisier: revue bibliographique. Rev. For. Fr. **XLIV** special, 27–31 (1992). — FRASCARIA, N., SANTI, F. and GOUYON, P. H.: Genetic differentiation within and among populations of chesnut (*Castanea sativa* MILL.) and wild cherry (*Prunus avium* L.). Heredity **70**, 634–641 (1993). — LEMOINE, M., DUFOUR, J. and SANTI, F.: Le merisier. In: Amélioration des espèces végétales cultivées – objectifs et critères de sélection. Ed. GALLAIS, A. and BANNEROT, H.. INRA, Paris, France pp. (1992). — MANGIN, B.: SELECT: a program package for assisting in plant selection. XVth International Biometrics Conference, Hamilton, New Zealand (1992). — MURANTY, H., SANTI, F., PAQUES, L. and DUFOUR, J.: Optimisation du nombre de ramets par clone dans les tests clonaux. Ann. Sci. For. **6**, (1996). — PAPADAKIS, J.: Méthode statistique pour des expériences en champ. Thessalonique, Institut d'Amélioration des plantes, *Bull. Sci.* **23**, 30 p. (1937). — PICHOT, C.: Analyse de dispositifs par approches itératives prenant en compte les performances des plus proches voisins. Agronomie **13**, 109–119 (1993). — RIFFAUD, J. L. and CORNU, D.: Utilisation de la culture *in vitro* pour la multiplication de merisiers adultes (*Prunus avium* L.) sélectionnés en forêt. Agronomie **1** (8), 633–640 (1981). — SANTI, F. and LEMOINE, M.: Genetic markers for *Prunus avium* L.: 1. Inheritance and linkage of isozyme loci. Ann. Sci. For. **47**: 131–139 (1990). — Statistical Science Inc.: Splus reference manual. Seattle, Washington (1988). — WRIKCLE, G.: Die Erfassung der Wechselwirkungen zwischen Genotyp und Umwelt bei quantitativen Eigenschaften. Zeitschrift für Pflanzenzüchtung **53**, 266–343 (1965).

RAPD Markers for the Identification of *Populus* Species

By N. SANCHEZ, J. M. GRAU, J. A. MANZANERA[1]) and M. A. BUENO

CIFOR-INIA, Ctra. de la Coruña Km 7, 28040 Madrid, Spain

(Received 15th January 1997)

Abstract

Twenty five poplar clones, namely, 5 of *Populus nigra*, 5 of *P. deltoides*, 5 of *P. alba*, 5 of *P. tremula*, 1 of *P. trichocarpa*, 3 of *P. x canescens* and 1 of clone *"Platero"* (*P. tremula* x *P. alba "Bolleana"*) were screened for random amplified polymorphic DNA (RAPD) markers in order to evaluate the use of RAPD analysis for distinguishing the cited species. One of the markers revealed different banding patterns between species and similar banding patterns for clones of the same species. For hybrids such as *P. x canescens* and *"Platero"*, the banding pattern was the same as either *P. alba* or *P. tremula.* On the other hand, for other hybrids analysed, such as *P. x euroamericana* I-214 and *P. deltoides x P. alba* 7/32 B, the banding pattern differed from both parents. The mentioned marker showed characteristic bands for every species, and in the particular case of *P. alba* three different groups could be distinguished.

Key words: Populus, Random amplified polymorphic DNA (RAPD), fingerprinting, species identification.

FDC: 165.3; 165.5; 176.1 *Populus.*

[1]) E.T.S.I. Montes, UPM, Ciudad Universitaria s/n, Madrid, Spain