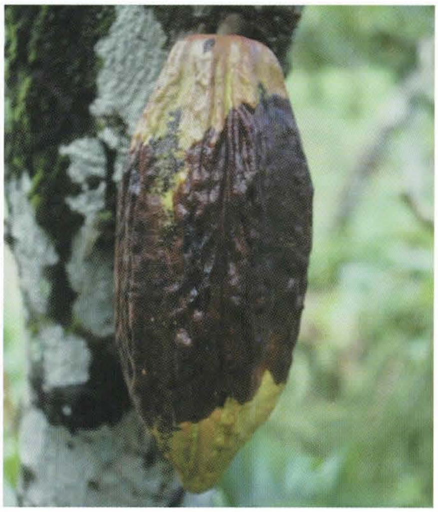
Working for better resistance to cocoa pod rot

Production losses caused by *Phytophthora* rot diseases (photo) are estimated at 15-20% worldwide; the selection of cocoa trees that are less susceptible to pod rot has therefore become a top priority. Despite a great deal of work, the search for cocoa trees with total resistance to this disease has drawn a blank. However, studies based on observing attacks in field trials have revealed the existence of genetically transmissible partial resistance. Transmission of this trait was primarily additive. An international project entitled Genetic bases of cocoa tree resistance to *Phytophthora* diseases was implemented between 1995 and 2000, with a view to proposing techniques for the sustainable control of *Phytophthora* diseases causing pod rot. The project received financial backing from European chocolate makers through CAOBISCO and involved several research centres: CRU, CNRA, IRAD and CIRAD. Its main objectives were to:

- improve knowledge of the structure and distribution of pathogen populations,
- identify the different factors involved in resistance,
- develop and validate resistance tests,
- locate areas of the genome involved in the resistance trait, by searching for QTLs,
- launch a process of genetic improvement for this trait.

The results obtained were released in publications and conference papers, and are to be compiled in a final book currently being drafted. This article sets out to highlight a few salient facts in our knowledge of resistance mechanisms and their genetic determinism.



C. Cilas

Photo. *Phytophthora* pod rot symptoms.

Resistance measured by the rotten pod rate in the field

The results shown here came from several mating designs and a clonal trial set up in zones affected by the disease in Cameroon and Côte d'Ivoire. They are compared with data obtained in Togo by ITRA's forest zone agricultural research station.

Losses caused by pod rot were estimated from potential production (minus rodent damaged pods) using a formula giving the rot rate per tree:

$$\text{Rotr} = \frac{\sum \text{rotten pods}}{\sum \text{rotten pods} + \sum \text{ripe pods} + \sum \text{healthy pods at last count}}$$

Analyses of variance carried out on the mating designs indicated that general combining abilities (GCA) were preponderant, confirming that transmission of the resistance trait was primarily additive.

The parents were classed according to the rot rate observed in their progenies. This involved a multiple comparison of GCAs estimated per parent (table 1). All in all, the parent classifications tallied well between the three countries despite different pathogen species. For instance, the selection carried out in Côte d'Ivoire for resistance to *P. palmivora* will be useful should the species *P. megakarya* invade that country.

Genetic and environmental correlations were calculated between the rot rate and potential production, estimated from the total number of pods produced per tree. Genetic correlations between potential production and rotten pod rates were favourable (negative). It was therefore possible to proceed with combined selection for these two traits. Combined "individual-family" selection based on an index combining high yields and good resistance to black pod was proposed for Cameroon and Côte d'Ivoire, in order to select individuals within worthwhile families, which were suitable for use as clones or as parents for new crosses. On the other hand, environmental correlations were systematically positive between rot rate and potential production. This correlation might have been due to secondary infections, from pod to pod, which would increase in line with the density of fruits on the trees.

Narrow sense and broad sense heritabilities were estimated for rot rate (table 2). The larger the number of years taken into account, the greater were the heritability values. Indeed, genetic values increased in accuracy in line with the number of years' observations. For instance, the data for the design in Côte d'Ivoire should be considered the most reliable, whilst additional observations are required in Togo.

Trinitario parents were generally the most susceptible to the disease. The length of the fruiting cycle in this material may have contributed to its poor performance in the field. Amelonado type Lower-Amazon parents and some Upper-Amazons, such as Sca 6, P 7, Pa 150 or T85/799 should help in creating less susceptible varieties, notably in Cameroon, where these parents are not used.

This classification, obtained with GCAs, only had one inversion compared to that based on the actual clone values estimated in a clonal trial located at the same experimental station in Cameroon.

Tests on leaves and pods

Methods

The leaf test is a method of artificial inoculation for early assessment of resistance in genotypes. This test was first developed on whole leaves, then it was applied to leaf discs with a diameter of 15 mm, with a view to reducing the space necessary for comparing genotypes.

Table 1. Classification of the different parents for their susceptibility to black pod rot. Newman and Keuls test (5%).

	Cameroon *P. megakarya* (data over 3 years)		Togo *P. megakarya* (data over 1 year)		Côte d'Ivoire *P. palmivora* (data over 9 years)	
- susceptible						
			IFC 5	a	Pa 150	a
			SNK 64	ab	Sca 6	a
			T86/45	ab	P 7	a
			T85/799	ab	T85/799	b
			T60/887	ab	T60/887	bc
			Sca 6	ab	T79/416	bc
			ICS 100	ab	T79/501	bc
	UPA 134	a	UPA 134	abc	Pa 7	bcd
			ICS 40	abc	T63/967	bcde
	SNK 413	b	Na 32	abc	Na 32	bcde
	ICS 84	bc	UF 676	bc		
	IMC 67	bc	IMC 67	c	IMC 67	bcde
	ICS 95	bc			T63/971	bcde
	SNK 10	c			T79/467	bcde
					Na 79	cde
					IMC 78	de
					Pa 35	e
+ susceptible						

Table 2. Narrow sense heritability (h^2) and broad sense heritability (h^2,L) of the rotten pod rate.

Country	Pathogen species	Number of years' observations	h^2	h^2_L
Cameroon	*P. megakarya*	3	0.109	0.133
Togo	*P. megakarya*	1	0.061	0.061
Côte d'Ivoire	*P. palmivora*	9	0.681	0.681

Leaf discs were placed in trays, then inoculated with 10 µl drops of a *Phytophthora* sp. zoospore suspension. Observations were carried out after 5 and 7 days of incubation at 26°C.

The pod test consisted in measuring the diameter of a rot patch on fruits inoculated artificially with a calibrated zoospore suspension. Such inoculations can be carried out on fruits still on the tree, or on loose fruits, with or without wounding of the epidermis.

Correlations between leaf tests and pod tests

Data relative to leaf and pod tests carried out in a clone comparative trial were examined using a multivariate analysis of variance, in order to determine the different correlations.

"Clone" effects were significant for each of the traits studied. Phenotypic correlations between leaf tests and pod tests were positive and significant.

Based on the multivariate analysis of variance, it was possible to calculate genetic and environmental correlations between the different variables. Genetic correlations corresponded to correlations between the means per clone. They were systematically positive between the leaf tests and pod tests. However, they were not significant, as they were calculated on too small a number of clones (5 clones).

On the other hand, environmental correlations were negative for most of the variables considered. Test expression was therefore subject to environmental effects, and those effects differed depending on the organ tested.

Heritability of the resistance trait estimated with the leaf test

The heritability of the scores obtained by the leaf test was estimated in different trials in Côte d'Ivoire. The first trial was a diallel planted in the nursery at CNRA's Bingerville station. It was a 5 x 5 design including selfs (without the reciprocal crosses). The parents used were: P 7, PA 150, T60/887, IFC 1 and a hybrid tree UPA 402 x UF 676, identified for its resistance to *P. palmivora*, assessed on leaves (table 3). The assessment involved thirty plants of each progeny in two series of inoculations. Each series comprised two batches of 250 plants, and fifteen plants per family were tested in each batch.

The second trial was a 4 x 2 factorial mating design (table 4). Eight progenies, all made up of adult trees, were planted in an experimental plot at CNRA's Bingerville station in a randomized single tree plot design. The female parents were Upper-Amazons and Trinitarios, whilst the male parents were two Upper-Amazons. The assessment protocol used in the diallel trial was also used here.

In both the mating designs studied, the heritability of the scores obtained in the leaf test increased with the number of series taken into account, as the precision of the genetic values was better when the plants were assessed several times.

Correlations between resistance tests and rot rates in the field

As regards relations between the results of the leaf test and rot rates observed in the field, it was found that no strong phenotypic correlations (tree by tree) existed, be it in the clonal trials or in the hybrid trials. On the other hand, a significantly positive genetic correlation was often detected between the rot rates and the pod tests, and especially with the leaf tests. This indicates that the means per clone or per hybrid were correlated and that it is therefore possible to select a clone or cross on the basis of leaf tests carried out in an appropriate experimental design.

However, the selection of genotypes within a progeny is not reliable yet with leaf tests, and further trials need to be carried out before considering this method for early selection of individuals.

The correlations between leaf tests, pod tests and rot rates in the field were also studied on clones in Trindidad and Tobago (table 5).

Resistance tests on leaves or pods were significantly correlated to the rot rates observed in the study plots. They were correlations established on a clonal level, hence determined from the means of several trees for each of the clones. Unlike the leaf tests, the pod tests were better correlated to the rot rates in the field when they were carried out without wounding the epidermis.

In Côte d'Ivoire, fifteen clones compared in a trial set up in a totally randomized "single tree" plot design were assessed for their reaction to *Phytophthora palmivora*. These clones, planted in a trial at the Bingerville station (BL7), were assessed for several reasons:

- compare leaf tests and pod tests (attached pods and loose pods),
- assess tree effects within clones (environment),
- compare inoculation tests and resistance in the field,
- assess the influence of morphological traits (cortex thickness, pod size) and environmental factors on pod resistance.

The clonal values estimated by the different tests were then correlated to each other. It appeared that the rot rates were quite well correlated to the pod inoculation tests, and to a lesser degree with the leaf tests.

Mapping areas of the genome involved in resistance

In work to map areas of the genome involved in resistance to *P. palmivora*, several significant QTLs of resistance, assessed from the percentages of rotten pods, were identified. One QTL was located on chromosome 1 of clone UF 676, another on chromosome 10 of T 60/887, and another on chromosome 4 of IMC 78. Other putative QTLs, with lower *lodscore* values were also identified. An improvement in the experimental design, adapted to QTL analyses, notably with a larger number of individuals, would make it possible to increase the power of QTL detection. In progenies UPA 402 x UF 676 and T 60/887 x Amelonado, QTLs of resistance assessed by leaf tests on adult trees were identified on

Table 3. Individual heritability values for resistance to *Phytophthora palmivora* measured on leaves in two series of inoculations.

Heritability	Series 1	Series 2	Series 1 + Series2
Broad sense	0.490	0.427	0.662
Narrow sense	0.294	0.203	0.320

Table 4. Individual heritability values for resistance to *Phytophthora palmivora* measured on cocoa tree leaves in the field in three series of inoculations.

Heritability	S1	S2	S3	S1 + S2	S1 + S3	S2 + S3	S1 + S2 + S3
Broad sense	0.103	0.325	0.581	0.248	0.451	0.666	0.685
Narrow sense	0.103	0.292	0.565	0.248	0.451	0.657	0.662

Table 5. Correlations between resistance tests and rot rates in the field (Trinidad and Tobago).

	Leaf test		Pod test		Field observations	
	WW	NW	WW	NW	% BPTP	% BPRP
Leaf test						
WW		0,469 **	0,410 **	0,862 **	0,496 **	0,471 **
NW			0,106 ns	0,438 **	0,3734 *	0,415 *
Pod test						
WW				0,543 **	0,536 **	0,586 **
NW					0,660 **	0,642 **

WW: with wounding; NW: no wounding
% BPTP: percentage of black pods / total pods
% BPRP: percentage of black pods / ripe pods
* significant at 5%; ** significant at 1%; ns: not significant

several chromosomes, but the reproducibility of the results between experiments was found to be poor. Nevertheless, a few QTLs were detected on the trial mean. These trials were highly sensitive to environmental effects, which might explain the few significant effects of the QTLs and the weak correlations between the different resistance assessment methods applied to old trees. Possible interaction with other agronomic traits was also studied in two progenies in Côte d'Ivoire. In IMC 78, co-location was found between the QTL linked to resistance in the field, that linked to vigour (trunk circumference) and pod weight. On the other hand, in the progeny derived from T 60/887, the most important QTL for resistance in the field, found on chromosome 10, was not co-located with the QTLs associated with the vigour and yield traits. The regions identified in resistance to *P. palmivora* were located in different parts of the genome; a cumulation of different resistance genes therefore seems possible, with a view to increasing levels of resistance to this disease. Similar results were obtained for resistance to *P. megakarya*.

Discussion and conclusion

Resistance tests on leaves and pods, carried out in an appropriate experimental design, can be used to select less susceptible clones or hybrid families. However, these assessment techniques are not reliable, at this stage, for selecting resistant individuals within hybrid families. In this case, the unicity of the genotypes tested prevented these tests being carried out with robust experimental designs. Likewise, rot rates estimated individually on trees in a trial cannot be used to precisely determine the level of susceptibility in those trees. Indeed, the observed rot rate may depend on the immediate environment of the tree, its pod load, the layout of the pods, etc. In fact, field observations depend on many environmental factors that are difficult to control by conventional experimental designs.

In order to estimate the reliability of leaf tests, it is suggested that they be applied in the nursery and validated by observing the performance in the field of material tested in that way at an early stage. In fact, it is now a matter of validating these assessment techniques on a true scale, under actual breeding conditions, and of estimating the genetic gains obtained in rot rate for different selection rates determined from leaf tests.

Leaf testing was also assessed using molecular tools, notably with a view to identifying QTLs for this trait. Several resistance QTLs have been identified, and it is planned to cumulate resistance genes with a view to increasing levels of cocoa tree resistance to *Phytophthora* diseases. Some of these QTLs need to be confirmed by appropriate experimental designs and could be used for marker-assisted selection. ■

List of publications

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