Euphytica (2008) 164:1–11
DOI 10.1007/s10681-008-9691-8

REVIEW

Genetic diversity among coconut varieties for susceptibility to Cape St Paul Wilt Disease

S. K. Dery · R. Philippe · L. Baudouin · R. N. Quaicoe · J. Nkansah-Poku · J. Owusu-Nipah · R. Arthur · D. Dare · N. Yankey · M. Dollet

Received: 21 November 2006 / Accepted: 8 April 2008 / Published online: 24 April 2008

Abstract The CSPWD is the Ghanaian form of lethal yellowing disease (LYD) of coconut, caused by a phytoplasma and has been active in Ghana since 1932. The paper updates the results of screening trials conducted with 38 pure and hybrid coconut varieties since 1981. Although no variety was found totally resistant, a wide range of susceptibility level was observed: almost all SGD were still unaffected, while the local WAT had almost totally disappeared, additive values were calculated for the parental varieties and it was shown that, in the average, hybrids are slightly more susceptible than predicted by a purely additive model. According to this genetic model, the SGD × VTT hybrid will be appreciably less susceptible than the MYD × VTT currently being used for replanting devastated farms in Ghana. Our results tend to confirm the general trend that cultivars from the Pacific group (especially the Dwarfs) are less susceptible than the Indo-Atlantic cultivars. Proposals are made to adapt planting material to the risk level. Genetic control can only be efficient if it is considered as a link in a chain of control measures involving the choice of a proper planting site, good management and early eradication of diseased trees.

S. K. Dery · R. N. Quaicoe · J. Nkansah-Poku · J. Owusu-Nipah · D. Dare · N. Yankey
OPRI, P.O. Box 245, Sekondi, Ghana
e-mail: cocopri@africaonline.com

R. Philippe (✉) · L. Baudouin · M. Dollet
CIRAD, Campus Int. de Baillarguet, TA80/A, 34398 Montpellier Cedex 5, France
e-mail: rene.philippe@cirad.fr

R. Arthur
CSDP, P.O. Box 245, Sekondi, Ghana

Keywords Coconut · Ghana · Lethal yellowing · Screening · Varieties

Abbreviations

ADOT	Andaman Tall
CATD	Catigan Green Dwarf
CRD	Cameroon Red Dwarf
EGD	Equatorial Guinean Green Dwarf (synonym to the Brazilian Green Dwarf)
LCT	Laccadive Tall
MRD	Malayan Red Dwarf
MYD	Malayan Yellow Dwarf
MLT	Malayan Tall
PNT	Panama Tall
RIT	Rennell Island Tall
SGD	Sri Lanka Green Dwarf
TACD	Tacunan Green Dwarf
TAGT	Tagnanan Tall
TAT	Tahiti Tall (synonym to the Polynesian Tall)
VTT	Vanuatu Tall
WAT	West African Tall

Introduction

The coconut palm, *Cocos nucifera* L., is of significant importance in the economies of the coastal areas of

Ghana. About 4.2% of the population of Ghana depends on coconut for their livelihood, and in the Western Region, where about 80% of coconut plantings in the country occurs, it is estimated that 20% of the rural folks depend on coconut for sustenance (Adams et al. 1996; Dery and Arthur 1996; Ofori and Nkansah-Poku 1995). This situation has arisen because on the coastal soils, coconut is the only suitable cash crop. The coconut industry is, however, threatened by a phytoplasma mediated disease known locally as Cape St Paul Wilt disease (CSPWD). The disease believed to be transmitted by an insect vector (Dery et al. 1995), has already collapsed the coconut industry in the Volta Region and devastated about 5,500 ha of coconut plantings in the Western and Central Regions of the country. It is still spreading (Ofori and Nkansah-Poku 1995; Dery and Arthur 1996).

The CSPWD is the Ghanaian form of a group of diseases collectively known as lethal yellowing disease (LYD) and found in West Africa, East Africa and the Caribbean. Its symptoms are: pre mature nut drop with or without yellowing of fronds. This is followed by progressive yellowing or, in some instance, browning of the crown from the older leaves upwards. Eventually, the whole crown turns yellow, dries up and then falls off, leaving only the stipe standing (telegraph pole condition). The symptoms may vary according to the variety: in some of them, including the MYD × VTT hybrid, the leaves may take a bronze colour instead of the usual yellowing.

Attempts to find a lasting solution to the CSPWD has long been going on. In 1942 the Crop Research Institute started preliminary investigations into the nature, causes and control of the disease. No headway was made at that time (Chona and Andoh 1970). The use of resistant coconut varieties has long been identified as the best way of controlling CSPWD and LYD in general (Dery et al. 1995, 2000, 2005; Dery and Arthur 1996; Harries 1995; Mariau et al. 1996). Already in 1956, the Crop Research Institute had set up a CSPWD resistance screening trial with Malayan Dwarf varieties (red, yellow and green) which all succumbed to the disease (Chona and Andoh 1970; Addison 1974). Following from here much more impetus was given to the search for disease resistant germplasm through resistance screening. The Crops Research Institute established yet another trial at Cape Three Points using red, yellow and green Malayan dwarfs and the local WAT as a susceptible control in 1977. In addition a few *Veitchia* sp. and *Phoenix* sp. species were planted. With the exception of *Veitchia* and *Phoenix* species, all the coconut varieties succumbed to the disease (Addison 1980).

Then, 7 variety trials with 27 varieties were planted in the Western Region in 1981, followed by two more in 1995, raising the number of tested varieties to 38. In the same time, a set of 33 adaptive trials were set up with the MYD × VTT hybrid in the three coconut cultivation regions (Western, Central and Volta). Finally a rehabilitation/replanting programme, the Coconut Sector Development programme (CSDP) covering 1,300 ha was launched in 1999 by the Ministry of Food & Agriculture (MOFA) with funding from Agence Française de Développement (AFD), involving the extension of the same variety, which was at that time the only promising hybrid that could be produced at a large scale.

The aim of this paper is to update the results of the 1981 trials in terms of CSPWD resistance, to present preliminary results for the 1995 trials and to discuss their significance for the fate of the coconut industry in Ghana.

Materials and method

In the absence of efficient artificial inoculation methods, the only way to test CSPWD resistance or tolerance of genotypes is to expose them to natural contamination. Visual assessment of the symptoms is the primary way of recognising diseased palms. Most of the observations referred to in the present paper were based on visual assessment according to the symptoms described in the introduction.

PCR assessment

In addition, a Polymerase Chain Reaction (PCR) diagnostic has been used to confirm the visual assessment after 1998. Nucleic acids were extracted according to Doyle and Doyle protocol (1990) modified by Daire et al. (1997) using cetyltrimethylammonium bromide and isopropanol precipitation. The primers P1/P7 (Deng and Hiruki 1991) know as “universal phytoplasmas primers”, and G813/AKSR (Tymon et al. 1998) claimed to be specific of CSPW in Ghana, were used for DNA amplifications with a thermocycle described by the respective authors.

Cloning of PCR products was obtained with the kit TOPO TA (Invitrogen). Plasmids were prepared using the QIAquick PCR Purification Kit (Qiagen) and sent for sequencing. Sequences were analysed with Vector NTI software.

The 1981–1982 set of coconut varieties trials

The 1981/1982 set of trials consisted of 27 ecotypes/hybrids at 7 locations; namely Cape Three Points, Akwidae, Dixcove, Agona Junction, Princess Town, Dadwen and Axim. Initially, the number of trees per variety was 24 (4 blocks with 6 trees per block) at the first 4 locations and 18 (3 blocks with 6 trees per block) at the 3 others. The tested varieties include self-pollinating Dwarf varieties, cross-pollinating Tall varieties as well as Dwarf × Tall and Tall × -hybrids (Table 1 and Appendix 1). These varieties were chosen in order to represent a panel of the global genetic diversity in coconut. The local West Africa Tall was used as susceptible control and additional plots of this cultivar were also planted near the trials. The status of the planting areas varied according to the site: Cape Three Points and Princess Town were already devastated by the disease; active disease foci were present at Akwidae and Dixcove;

Table 1 Cumulative record of disease at Akwidae, Agona Junction and Axim trial plots as at April 2006

Variety	Agona Junction			Akwidae			Axim		
	Total no. of palms	No. of diseased palm[a]	Level of disease (%)	Total no. of palms	No. of diseased palm	Level of disease (%)	Total no. of palms	No. of diseased palm	Level of disease (%)
CRD	24	12	50.0	13	3	23.1	–	–	–
CRD × MLT	11	7	63.6	18	8	44.4	7	2	28.6
CRD × TAT	8	4	50.0	–	–	–	4	1	25.0
CRD × RIT	8	0	0.0	–	–	–	5	3	60.0
CRD × VTT	7	6	85.7	–	–	–	3	3	100
CRD × WAT	24	16	66.7	24	23	95.8	2	1	50.0
EGD	18	13	72.2	5	0	0.0	3	0	0.0
EGD × VTT	9	6	66.7	–	–	–	6	5	83.3
EGD × WAT	11	7	63.6	–	–	–	8	8	100.0
MLT	7	3	42.9	–	–	–	4	2	50.0
MYD × MLT	10	6	60.0	–	–	–	2	2	100.0
VTT × MLT	6	5	83.3	–	–	–	6	5	83.3
MRD	18	2	11.1	5	0	0	1	0	0.0
MRD × TAT	17	7	41.2	18	7	38.9	7	4	57.1
MRD × WAT	22	19	86.4	24	15	62.5	1	0	0.0
MYD	24	17	70.8	16	5	31.3	–	–	–
MYD × TAT	3	1	33.3	–	–	–	5	4	80.0
MYD × RIT	5	0	0.0	–	–	–	3	2	66.7
MYD × VTT	9	4	44.4	–	–	–	9	6	66.7
MYD × WAT	23	22	95.7	23	23	100.0	7	5	71.4
TAT	3	2	66.7	–	–	–	1	0	0.0
RIT	9	5	55.5	–	–	–	8	5	62.5
RIT × WAT	24	20	83.3	19	18	94.7	10	2	20.0
SGD	16	0	0.0	15	0	0.0	4	1	25.0
SGD × WAT	24	21	87.5	23	23	100.0	–	–	–
VTT	8	0	0.0	–	–	–	5	5	100.0
WAT	64	63	98.4	55	45	81.8	11	11	100.0

[a] Diseased or killed by CSPWD

Agona Junction, Dadwen and Axim were disease free but near the advancing front of the disease.

The 1995 set of coconut varieties trials

The 1995 variety trials consisted of 11 ecotypes at 2 locations (Tumentu and Cape Three Points). The intended experimental design was a randomized complete block design of 5 replicates, 7 plots per replicate and 12 palms per plot. However, some varieties were not replicated at both locations because of insufficient numbers. The local West African Tall was used as the control. The Cape Three Points site was the same as for the 1981 trial, where all trees had eventually died except for a few SGD. Tumentu was located on an active disease front.

Statistical analysis

Since the available data amount at each site was insufficient to allow reliable ranking of the varieties, we had to group the trials from Akwidae, Agona Junction and Axim with those from the 1981 trials at Dixcove and Cape Three Points (Mariau et al. 1996). The data of those two trials are given in Appendix 1. We thus considered each trial as a replication of a larger, unbalanced trial and analyzed this trial under the generalized linear model (GLM: McCullagh and Nelder 1989): we considered a hidden variable Y_{ijk} representing the intensity of the disease. Its value for a variety V_{ij} at site S_k is assumed to be the sum of the intercept, of the genetic effect and of a local effect:

$$Y_{ijk} = I + G_{ij} + L_k \qquad (1)$$

Indices i and j represent the parents of the tested varieties (in the case of pure varieties, $i = j$); k stands for the site. A low value for G_{ij} denotes a high level of resistance while a high value for L_k corresponds to a high infestation rate. We cannot observe directly Y_{ijk} but only its consequences on the coconut survival. More precisely, the logarithm of the odds for a tree to die from LY is considered equal to Y_{ijk}. As a result, the link function is logistic:

$$\Pr(\mathrm{LY}) = e^{Yijk} / \left(1 + e^{Yijk}\right) \qquad (2)$$

In an attempt to predict the behaviour of untested hybrids, we also tested an additive genetic model where each parent populations of a treatment are assigned a coefficient ½, corresponding to the proportion of the genes coming from this parents. We also explored the effect of the hybrid condition on mortality. This led us to treat the genetic value of a variety as a sum of 4 terms:

$$G_{ij} = \frac{1}{2}P_i + \frac{1}{2}P_j + H\left(V_{ij}\right) + D_{ij} \qquad (3)$$

P_i and P_j represent the additive contributions of the parents, $H\left(V_{ij}\right)$ corresponds to the "hybrid effect" and is null for pure varieties. The deviation D_{ij} is the part of genetic variation that is left unaccounted for by the model.

We made this analysis using the *glm* function of R language (R Development Core Team 2004) and obtained estimates for the model parameters, the predicted losses and analysis of deviance tables (the equivalent for "analysis of variance" in GLM).

Results

Evolution of the disease

To date, the Dadwen 1982 and Tumentu 1995 trials were never affected. In the Princess Town trial, only one palm died in 1996 and the trial has since been disease free. This situation contrasts sharply with what was observed in Dixcove and particularly in Cape Three Points: there, the disease first reached this village in 1964 and a first trial was planted in 1977 by the Crops Research Institute. All coconuts (Malayan Dwarfs and West African Tall) died but other palm species *Veitchia* sp. and *Phoenix* sp. survived. In the second trial planted in 1981, the disease resurfaced in the early 90s and devastated it rapidly, except for the SGD palms. The third generation was planted in 1995; the disease re-infected the plot as early as 2004 and is still active.

In the remaining trials, the evolution was slower: although the first cases in the Akwidae plot appeared 7 years after planting, only three palms (CRD × TAT, RIT × WAT and MRD × TAT) were attacked between 1988 and 1990, after which the disease remained dormant till 1996 when there was a resurgence and rapid spread up to 2002, and after that has remained dormant to date.

The first palm to be infected at Agona Junction was a West African Tall in 1995 (15 years after planting), and this also happened to be the tallest

Fig. 1 23 Years old SGD at Axim (R. Philippe)

palm on the plot. The disease then spread rapidly, reaching a peak in 2002, then stopped suddenly.

At Axim, the first palm to be attacked by the disease in 2000 was a VTT and the disease has been spreading rapidly. All the VTT were killed. The SGD (Fig. 1) and VTT palms in this plot are abnormal. They have no fruits and are severely stunted.

Genetic variation of resistance level

The losses due to CSPWD at Akwidae, Agona Junction and Axim trials as at October 2005 are presented in Table 1. Due to shortage of seedlings at the planting time and to subsequent losses due to other causes (drought, bush fire etc.), the total number of trees is lower than planted. On the other hand, additional WAT plots planted near the trials were included into the results. None of the tested varieties was found fully resistant to the disease. However, important variations in the level of susceptibility were observed as shown by the genetic analysis.

The percentages of losses varied from 63% to 73% according to the trial. It has to be kept in mind that the disease is in constant evolution and that these percentages don't represent the final stage of the disease. For example, the Cape Three Points trial was entirely wiped out in 1995, save for 3 SGD, which are still alive. Only a few palms remained alive at Dixcove when the trial was abandoned in 1997. The data analyzed represent rather "snapshots" taken at a moment, where it is still possible to reveal the genetic differences between varieties.

Table 2 synthesizes the analysis of deviance tables for mortality due to LY. The site effect was not significant, but the overall genetic effect was highly

Table 2 Analysis of deviance table for losses to CSPWD

	Df[a]	Deviance[b]	Resid. Df[c]	Resid. deviance[d]	F test[e]	Pr(>F)[f]	Significance[g]
"Null" model			102	584.73			
Site (*L*)	4	4.46	98	580.27	1.1	0.354	NS
Variety (*G*)	26	356.91	72	223.36	13.7	1.03×10^{-18}	***
Additive effects (*P*)	9	287.40	89	292.87	31.9	3.24×10^{-24}	***
Hybrid effect (*H*)	1	17.07	88	275.81	17.1	8.19×10^{-05}	***
Deviation (*D*)	16	52.44	72	223.37	3.3	2.82×10^{-04}	***

Italicized letters refer to model components. See relations (1) and (3)

All factors are introduced sequentially

[a] Df, Number of degrees of freedom of the factor

[b] Deviance, Reduction of the deviance attributed to the factor

[c] Residual Df, Number of degrees of freedom of the residual deviance

[d] Residual Deviance, deviance before introduction of the factor minus Deviance

[e] F test, Deviance/Df

[f] Pr(>F), Probability for a random variable with a F distribution with Df and Residual Df degrees of freedom to be superior to F test

[g] Significance, NS: Non significant at the level 0.05, *** significant at the level 0.001

significant. About 4/5 of the genetic deviance was accounted for by the additive effect of the parents. In addition, we found that hybrids were on the average more sensitive than anticipated based on the purely additive model: this represented a 7% mortality increase in average. About 15% (i.e. 52.44/356.91) of the genetic deviance was left unexplained by the genetic model and the corresponding effect was still highly significant. Table 3 shows the calculated coefficients of the GLM procedure.

Based on the full model—referring to relation (1)—the predicted losses across sites were highly correlated with the observations (coefficient of determination $R^2 = 0.99$), but at individual sites, the coefficient of determination was lower due to sampling errors. It remained high (between 0.64 and 0.87 at Agona Junction, Akwidae and Cape Three Point. At Dixcove it was 0.47 and only 0.13 at Axim.

Considering the purely additive model, the coefficient of determination R^2 was 0.65 for mortality across sites. It rose to 0.69 when introducing the effect of hybridization (Table 4). The adjustment was not perfect: the typical prediction error for the

Table 3 Results of the GLM procedure

Genetic effects	Coefficient	SD
Parent (*P*)		
Sri Lanka Green Dwarf (SGD)	−5.029	0.382
Malaysian Red Dwarf (MRD)	−4.348	0.324
Rennell Island Tall (RIT)	−3.991	0.454
Cameroon Red Dwarf (CRD)	−3.110	0.257
Malaysian Yellow Dwarf (MYD)	−3.050	0.276
Vanuatu Tall (VTT)	−3.010	0.368
Equatorial Guinean Green Dwarf (EGD)	−2.946	0.325
Malaysian Tall (MLT)	−2.876	0.308
Tahiti Tall (TAT)	−2.661	0.393
West African Tall (WAT) ex Ghana	0.000	0.260
Hybrid effect (*H*)		
Hybrids	0.681	0.163
Pure populations	0.000	
Site effects (*L*)		
Akwidae	−0.306	
Axim	−0.085	
Agona Junction	0.000	
Cape Three Points	0.253	
Dixcove	0.697	
Intercept (*I*)	2.828	

Italicized letters refer to model components. See relations (1) and (3)

Example: the linear predictor for VTT × MYD at Akwidae is obtained by summing (−3.010−3.050)/2, for the additive value $(P_i + P_j)/2$, 0.681 for H, −0.697 for L and 2.828 for I. The result is 1.176. Applying relation (2), this corresponds to 76% losses. The value(s) 0.000 for West African Tall (WAT), Pure populations and Agona Junction are arbitrary determined

Table 4 Comparison of observed and predicted losses across trials

Variety	Number	Observed (%)	Predicted (%)	Deviation (%)
CRD	59	53	44	8
CRD × MLT	54	52	63	−11
CRD × TAT	21	62	71	−9
CRD × RIT	19	47	54	−6
CRD × VTT	19	89	68	22
CRD × WAT	75	81	88	−6
EGD	45	49	51	−2
EGD × VTT	24	79	68	11
EGD × WAT	28	86	89	−3
MLT	17	65	54	10
MLT × MYD	22	55	70	−15
MLT × VTT	19	79	68	11
MRD	43	23	20	3
MRD × TAT	69	54	51	2
MRD × WAT	74	74	79	−5
MYD	51	45	44	1
MYD × TAT	13	69	70	−1
MYD × RIT	13	54	56	−2
MYD × VTT	22	59	64	−4
MYD × WAT	79	91	88	3
TAT	12	67	65	2
RIT	20	65	25	40
RIT × WAT	91	80	83	−2
SGD	60	2	11	−9
SGD × WAT	53	92	71	21
VTT	18	28	50	−22
WAT	155	93	94	−1
Total	1,175			12[a]
SGD × VTT			37[b]	

The predicted values are weighted averages of the predicted values at each site (see Table 3). The weights correspond to the number of useful palms of the variety at each site

[a] Square root of the mean squared error. [b] Estimated

observed varieties was ±12%. It exceeded this value for a few varieties, mainly those which were represented by a small number of individuals (Except for SGD × VTT, which had 21% more diseased trees than predicted by the model). The largest deviation was noted in the RIT (40%) but it remained below 12% for 22 out of the 27 tested varieties.

We are particularly interested in three varieties: the WAT is the local variety and its known susceptibility was confirmed in the trials: 93% of the trees died (predicted loss 94%). Its hybrids also had a poor performance. The MYD × VTT has been planted in large quantities since 1999 and predicted losses for the trials are about 64% in average (54% at Akwidae and 76% at Dixcove). Although only 22 individuals were tested, the predicted value agreed fairly well with the observations. Following favourable preliminary results in the SGD, a seed garden was planted and the hybrid between the best pure varieties, namely the SGD × VTT can now be extended. Although it was not tested in the trial, the linear predictor anticipates a mortality of about 37% in the same conditions.

Cape Three Points 1995 trial

The number of useful trees per cultivar (Table 5) in the Cape Three Points 1995 trial is variable and low due to losses after planting (notably to bush fire). Already 22% of the palms and 5 of the 8 varieties under screening had been affected at the census date. It has to be noted that 42% of the WAT ex Benin were killed. This rules out a hypothesis suggesting that the absence of the disease in Benin (contrarily to the neighbouring Togo and Nigeria) was a sign of resistance. Cultivars of the Indo-Atlantic genetic group (Lebrun et al. 2005) i.e. the WAT, LCT and ADOT had between 30% and 50% dead trees. Talls from the Pacific group i.e. the PNT01, PNT02 and TAGT lost between 5% and 9% to the disease. No Dwarf palms had died at the census date. This trial is yet another piece of evidence that probably no coconut variety would be able to withstand high inoculum pressure: at present, all varieties, including the Dwarfs, are severely affected by the disease. This trial is replicated at Tumentu which is still disease free.

PCR confirmation

The presence of phytoplasma was confirmed for the first time in the VTT and in the SGD at Axim. Two VTT palms, which were abnormally growing but devoid of typical symptoms, were tested in February 2004 with PCR using the primers P1/P7 and G813/AKSR primer pairs and gave positive results. Four months later, these palms were dying from CSPWD. One of them was still positive and another one (most certainly asymptomatic in February) was already dead. The three SGD present in February were tested negative with the same primers but one of them was positive in May and died a few months later. In May, the PCR product of the positive VTT and SGD were cloned and sequenced, completing the demonstration that these cultivars could be infected by the phytoplasma and eventually die from it.

Discussion

Two factors contribute to making research on CSPWD resistance difficult. First, as a tree crop,

Table 5 Record of the disease at Cape Three Points (1995 planting) as at April 2006

Variety	No. of palms	No. affected palms	% Affected palms
Catigan Green Dwarf (CATD)	9	0	0.0
Laccadive Tall (LCT)	49	18	36.7
Andaman Tall (ADOT)	43	12	27.9
Panama tall Monagre (PNTO2)	35	2	5.7
Panama Tall Aguadulce (PNT01)	45	4	8.9
Tagnanan Tall (TAGT)	32	2	6.3
Tacunan Green Dwarf (TACD)	10	0	0.0
WAT Benin Type (WAT)—ex Benin	56	24	42.9
Total/average	279	62	22.2

coconut is bulky and has a long life cycle, limiting the number of genotypes that can be tested in a given area. Second, in the absence of an efficient inoculation method, one is forced to rely on natural contamination, which tends to occur in an unpredictable way: although all trials were set up in areas where the disease was present, 3 of the 9 trials had no (or only one) affected tree, while at Cape Three Point, the diseased reappeared systematically. The first cases were observed 7–15 years after planting. In most cases an explosion occurred soon after the first case, but the delay could reach 8 years. Finally, the disease could leave only a handful of palms (Cape Three Points 1981) but some 25% of the trees at Agona Junction and Akwidae.

This sudden interruption of the contamination could have altered the results of this trial: some of the varieties could have remained unaffected by pure chance. This seems however not to be the case, since there is a good agreement between the results obtained at Agona Junction, Akwidae and Cape Three Points.

Usable observations could thus be obtained only after a considerable amount of efforts and after a long period of observation. However, grouping 5 trials, with a total of 1,175 palms made it possible to demonstrate the existence of a wide range of variation among coconut varieties for susceptibility to CSPWD. Based on a genetic model involving the additive values of 10 pure cultivars and a penalty for hybrids, the WAT was ranked as highly susceptible, the SGD and the MRD were considered as the most resistant. The other 6 cultivars had an intermediate behaviour.

The above ranking doesn't always agree with the raw data of the corresponding pure varieties: the adopted genetic model only absorbed 85% of the genetic deviance but has two advantages over the full model: the estimated parameters were derived from a larger numbers of observations; they thus are less susceptible to sampling errors and, above all, it makes it possible to make inferences about hybrids that were not observed in the trials. This is the case of the SGD × VTT, which is a promising hybrid. However, like most models, it no doubt represents a somewhat oversimplified representation of genetic interactions and large deviations subsist for a few cultivars, such as RIT and VTT.

The general agreement between predictions and observations was however quite good at three sites. It was moderate at Dixcove and very low at Axim. These sites happen to have the lowest numbers of individuals per treatment and sampling error is partially responsible for these low values. However, the situation at Axim seems to differ from what was observed elsewhere, especially concerning the most resistant cultivars: it is the only place where all VTT died from CSPWD and the only affected SGD was also in Axim. Both cultivars had an abnormal phenotype there: the SGD palms were not growing at all; they were severely stunted and remained vegetative for more than 20 years. Similar condition is not infrequent (but generally at a lower degree) in other places in West Africa, including Akwidae and Cape Three points. The VTT palms remained vegetative throughout, and had spongy, "moisture-laden" stems.

This suggests some physiological malfunctioning or stress and could have compromised the inherent mechanism that conferred disease resistance to these ecotypes in the other trials. Significant genotype × interaction was found by Ashburner and Been (1995). Harries (1995) speculated that the poor CSPWD resistance of Malayan Dwarfs in South Eastern Ghana could be due to the drier conditions there. The Axim plot is located on very poor, coastal, sandy soil and the plot has not been fertilized for the past 10 years. A similar explanation might also account for the case of a VTT at Dixcove which succumbed to the disease after having been scorched by bush fire (after 1994). Likewise, of the 1300 ha of CSDP fields planted with MYD × VTT, the only one-hectare field that has suffered from a large percentage of losses to the disease so far was located in a wet area, unfavourable for coconut.

We observed earlier that the abnormal looking SGD found at various sites had necrotic inflorescences, and speculated that this could be a symptom of an attenuated form of CSPWD. These PCR test results add a further argument in favour of this speculation: the SGD would be partially tolerant. In any case, complete resistance can no longer be claimed for SGD and VTT.

Our results contrast with what had been observed for LY in Jamaica at the end of the 70s (Been 1981): the VTT is among the most sensitive cultivars and the MYD had virtually no losses (at that time). There are however common points: the Jamaican Tall is highly susceptible (it is genetically close to the WAT). It is also the case of all other Indo-Atlantic cultivars. Conversely, Dwarf cultivars tend to be less sensitive than the others. The situation has evolved in Jamaica: both the MYD and its hybrid with the Panama Tall, which used to be considered as resistant, are now severely affected (Broschat et al. 2002). Differences between Ghana and Jamaica are understandable since the pathogen strains differ (Tymon et al. 1998), as well as the vector: *Myndus crudus* doesn't exist in Ghana and extensive research failed to identify the congeneric *M. adiopodoumeensis* as a vector in Ghana (Philippe et al. 2007).

Conclusion and perspectives

To summarize, no cultivar has been found fully resistant to CSPWD, but large genetic differences exist for response to this disease. The SGD is the least sensitive cultivar; however, its agronomic characteristics are poorly adapted to the region. In addition to physiological disorders, it is highly sensitive to *Eriophyes* mites and it is difficult to use it except in hybrid combinations. Considering only the estimated breeding values (Table 3), the RIT would appear as the best Tall cultivar. However, its performance as a pure variety was poor, contrarily to the VTT, whose results were more consistent.

Although CSPWD is the main threat for coconut industry in Ghana, recommendations about planting material must take into account other factors including agronomic and technological performance as well as response to other biotic and abiotic stresses. In addition, establishing seed gardens takes several years and, although the MYD was not the most resistant Dwarf, extending the MYD × VTT was the only genetic option for reducing susceptibility at the beginning of the CSDP. Contamination was still possible, but its improved precocity would at least preserve a longer productive period for the benefit of the farmer. The first observations made in the adaptive trials and in the CSDP plots (see introduction) are thus not really surprising: the number of affected plots and of the severity of the attacks seems lower than in the case of the local Tall, but already about 3% of the plots have been affected. In a biased sample of 58 one-hectare plots (out of 1,300) where sanitary problem had been signalled, the presence of phytoplasma was confirmed by nested PCR 19 times. For the moment, the number of cases remains generally low but the situation is evolving and is being monitored carefully.

In the meantime a new seed garden was set up and SGD × VTT hybrids can now be extended. It is thus possible to diversify the proposals:

- in the immediate vicinity of an active CSPWD focus, it is preferable to avoid planting coconut, since the risk of transmission of the disease is high, even for partially resistant cultivars,
- at some distance from these foci, the risk remains appreciable and the SGD × VTT hybrid is recommended,
- wherever the risk of contamination is low, the preference is given to the MYD × VTT, due to its favourable agronomic performances and to the fact that supplies of the other hybrid are limited.

A plot of SGD × VTT was also planted near the disease screening trial at Agona Junction and to date, 11 years after, no disease incidence has been observed. However, the CSPWD has not been active in Agona since 2003.

Even if we had identified a totally resistant cultivar, we should not take this solution for acquired forever, as demonstrated by the case of Jamaica. Diversification of the sources of (partial) resistance is probably the key to securing the situation of the coconut industry in Ghana. New Dwarf trials were planted in 2007 and further cultivars, especially Talls from the Pacific area should be tested. Finally, it has to be admitted that genetic resistance alone is insufficient to solve the problem of CSPWD. Well managed plantations, established in suitable areas seem to reduce the risks of contamination. Reliable seed production is a must (Lebrun et al. 2008; Baudouin et al. 2008). The evolution of the disease differed greatly according to the sites. More research is needed to better understand its initial transmission to a field and its eventual propagation to all (or to most of) the trees. Research on the vector is in progress in Ghana. Once a field is attacked, early eradication of diseased plants (Philippe et al. 2004) is the only control practice, which has given positive results so far, when applied thoroughly. It should thus be experimented further.

Acknowledgements The resistance screening programme has been funded at different times by French Embassy Project with Cote d'Ivoire and Ghana, The E.U under its STD 3 project, the World Bank under the National Agricultural Research Project (NARP) and Agricultural Services Sub Sector Investment Project (AGSSIP), the Agence Française de Développement and the Government of Ghana. The Support is gratefully acknowledged. In addition, we wish to acknowledge the great collaboration of Yane Kandassamy and Sandrine Fabre, technicians, for their work done in the laboratory of the Research Unit 29 of Cirad at Montpellier (France) and Dr. Laurence Rassaby who worked with the CIRAD-OPRI team in Montpellier and Takoradi between April 2003 and March 2004.

Appendix: Results of the Cape Three Points trials (after Mariau et al. 1996)

Variety	Cape Three Points			Dixcove		
	Total no. of palms	No. of diseased palm[a]	Level of disease (%)	Total no. of palms	No. of diseased palm[a]	Level of disease (%)
CRD	18	14	77.8	4	2	50.0
CRD × MLT	11	5	45.5	7	6	85.7
CRD × TAT	–	–	–	9	8	88.9
CRD × RIT	–	–	–	6	6	100.0
CRD × VTT	–	–	–	9	8	88.9
CRD × WAT	18	14	77.8	7	7	100.0
EGD	10	6	60.0	9	3	33.3
EGD × VTT	–	–	–	9	8	88.9
EGD × WAT	9	9	100.0	–	–	–
MLT	–	–	–	6	6	100.0
MLT × MYD	–	–	–	10	4	40.0
MLT × VTT	–	–	–	7	5	71.4
MRD	13	4	30.8	6	4	66.7
MRD × TAT	20	16	80.0	7	3	42.9
MRD × WAT	20	14	70.0	7	7	100.0
MYD	7	1	14.3	4	0	0.0
MYD × TAT	–	–	–	5	4	80.0
MYD × RIT	–	–	–	5	5	100.0
MYD × VTT	–	–	–	4	3	75.0
MYD × WAT	16	13	81.3	10	9	90.0
TAT	–	–	–	8	6	75.0
RIT	–	–	–	3	3	100.0
RIT × WAT	29	24	82.8	9	9	100.0
SGD	19	0	0.0	6	0	0.0
SGD × WAT	–	–	–	6	5	83.3
VTT	–	–	–	5	0	0.0
WAT	19	19	100.0	6	6	100.0

[a] Diseased or killed by CSPWD

References

Adams M, Arthur R, Duhamel G, Ghartey N, Overfield D, Willoughby N (1996) Analyses of natural resource utilization livelihood systems, coping strategies and natural resource research needs in coconut-growing areas of coastal zone ecosystems in Ghana (Phase 1), vol 1. NRI Document C0834, May, 1996, 93 pp

Addison EA (1974) Investigations on the Cape St. Paul Wilt Disease of coconut. In: Annual Report, 1973. Plant Pathology Section, Crops Research Institute, Kumasi, Ghana

Addison EA (1980) Varietal resistance trials with some dwarf coconut palms against the Cape St. Paul Wilt Disease in Ghana. Afr J Plant Prot 1(2):129–141

Ashburner GR, Been BO (1995) Characterization of resistance to lethal yellowing in Cocos nucifera and implication for genetic improvement of this species in the Caribbean Region. In: Eden-Green SJ, Ofori F (eds) Proceedings of an international workshop on lethal yellowing-like diseases of coconut, Elmina, Ghana, Nov 1995. NRI, Chatham, UK, pp 173–183

Baudouin L, Lebrun P, Berger A, Myrie W, Been B et al (2008) The Panama Tall and the Maypan hybrid coconut in Jamaica: did genetic contamination cause a loss of resistance to lethal yellowing? Euphytica (published online)

Been BO (1981) Observations on field resistance to lethal yellowing in coconut varieties and hybrids in Jamaica. Oléagineux 36(6):9–12

Broschat TK, Harrison NA, Donselman H (2002) Losses to lethal yellowing cast doubt on coconut cultivar resistance. Palms 46:185–189

Chona BL, Andoh PG (1970) Cape St. Paul Wilt—the present position. In: Chona BL, Adansi MA (eds) Coconut in Ghana. Bull No. 3. Crops Research Institute, CSIR, Ghana, 20 pp

Daire X, Clair D, Reinert W, Boudon-Padieu E (1997) Detection and differentiation of grapevine yellows Phytoplasma belonging to the elm yellows group and to the stolbur subgroup by PCR amplification of non-ribosomal DNA. Eur J Plant Pathol 103(6):507–514

Deng S, Hiruki C (1991) Amplification of 16S rRNA genes from cultural and nonculturable mollicutes. J Microbiol Methods 14:53–61

Dery SK, Arthur R (1996) Rehabilitation of the coconut industry in Ghana: a strategy. Report submitted to the Western Region Dep. Minister for Agric., September, 1996

Dery SK, N'Cho YP, Sangare A, Arkhurst ED (1995) Cape St Paul Wilt Disease: resistance screening and prospects for rehabilitating the coconut industry in Ghana. In: Eden-Green SJ, Ofori F (eds) Proceedings of an international workshop on lethal yellowing-like diseases of coconut, Elmina, Ghana, Nov 1995. NRI, Chatham, UK, pp 147–152

Dery SK, N'Cho YP, Quaicoe RN, Arkhurst ED, Philippe R (2000) Screening for resistance to lethal yellowing disease of coconut in Ghana: an update of trial results. Palm Diseases; Ch. 8. http://www.plantapalm.com/vpe/disease.cocnutly1.htm

Dery SK, Owusu-Nipah J, Andoh-Mensah E, Nuertey BN, Nkansah-Poku J, Arthur R, Philippe R (2005) On-farm evaluation of the coconut hybrid, Malayan Yellow Dwarf × Vanuatu Tall for tolerance to Lethal yellowing disease of coconut in Ghana. Cord 21(1):50–56

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus (Life Technology Inc.) 12:13–15

Harries HC (1995) Growing coconuts in Africa: resistance to lethal yellowing-like diseases. In: Eden-Green SJ, Ofori F (eds) (1997) Proceedings of an international workshop on lethal yellowing-like diseases of coconut, Elmina, Ghana, Nov 1995. NRI, Chatham, UK, pp 139–146

Lebrun PS, Berger A, Hodgkin T, Baudouin L (2005) Biochemical and molecular methods for characterizing coconut diversity. In: Batugal PA, Ramanatha Rao V, Oliver J (eds) Coconut genetic resources. International Plant Genetic Resources institute—Regional office for Asia, the Pacific and Oceania (IPGRI-APO), Serdang, pp 225–251

Lebrun P, Baudouin L, Myrie W, Berger A, Dollet M (2008) Recent Lethal Yellowing outbreak: why is the Malayan Yellow Dwarf Coconut no longer resistant in Jamaica? Tree Genet Genome 4:125–131

McCullagh P, Nelder JA (1989) Generalized linear models. Chapman & Hall, London

Mariau D, Dery SK, Sangaré A, N'Cho YP, Philippe R (1996) Le Jaunissement mortel du cocotier au Ghana et tolérance du matériel végétal. Plantations, Recherche, Developpement 3(2):105–112

Ofori F, Nkansah-Poku J (1995) The Cape St. Paul Wilt Disease of coconut in Ghana: history of its occurrence and spread. In Eden-Green S, Ofori F (eds) Proceedings of the international workshop on lethal yellowing-like diseases of coconut, Elmina, Ghana. NRI, Chatham, UK, pp 27–32

Philippe R, Dery SK, Nkansah-Poku J (2004) New data on a cultural control method against lethal yellowing in Ghana. Cord 20(2):21–27

Philippe R, Nkansah-Poku J, Fabre S, Quaicoe R, Pilet F, Dollet M (2007) Search for the vector of Cape Saint Paul Wilt (coconut lethal yellowing) in Ghana. First international phytoplasmologist working group meeting, Bologna, Italy 12–15 November 2007. Bull Insectology 60(2):179–180

R Development Core Team (2004) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org

Tymon AM, Jones P, Harrison N (1998) Phylogenetic relationships of coconut phytoplasmas and the development of specific oligonucleotide PCR primers. Ann Appl Biol 132(3):437–452