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The Panama Tall and the Maypan hybrid coconut in Jamaica: did genetic contamination cause a loss of resistance to Lethal Yellowing?

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Abstract We applied Bayesian population assignment methods to assess the trueness to type of four populations of the coconut cultivar Panama Tall (PNT) located in Jamaica and found that two of them presented a high percentage of off-types, while genetic contamination was low in the two others. The PNT is the pollen parent of the MAYPAN hybrid, which used to be planted in Jamaica to control an epidemic disease: Lethal Yellowing. The main source of contamination was the susceptible Jamaica Tall, thus increasing the susceptibility in the resulting MAYPAN progeny. The incidence of genetic contamination seems however to be insufficient to be the only cause of the latest outbreak of the disease. Neither the MAYPAN nor its parents can be said resistant in the present context of Jamaica.

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Abbreviations

SMD-CNRA	Station marc delorme, Centre National de la Recherche Agronomique (Côte d'Ivoire)
CIB	Coconut industry board (Jamaica)
CICY	Centro de investigation Cientifica de Yucatan
Fadcanic	Fundación para la Autonomía y el Desarollo de la Costa Atlántica de Nicaragua (Nicaragua)

Introduction

The coconut cultivar Panama Tall (PNT) is the pollen parent of the MAYPAN hybrid, which has been planted extensively in Jamaica during the last 25 years, as a control measure against an epidemic disease, the Lethal Yellowing (LY) caused by a phytoplasm. In the late 70s, the results of resistance trials (Been 1981) had lead to encourage plantation of the Malayan Yellow Dwarf (MYD) and of the MYD × PNT hybrid or MAYPAN as a control measure against the disease. The PNT itself was not resistant: irs mortality rate was 38–67% at 10 years according to the trial site (0–10% in the Malayan Dwarfs). The MAYPAN was the best hybrid with 4 and 21% mortality and was considered as a good compromise between resistance level, yield and product quality. Although it has fostered a revival of the Jamaican coconut industry for 20 years, this hybrid is now being massively destroyed by a new outbreak of the disease (Broschat et al. 2002).

A previous paper (Lebrun et al. 2007), demonstrated that the MYD was more variable in Jamaica than in the rest of the world, providing evidence of genetic contamination. However, this contamination appears to have existed since the introduction of the cultivar and thus is unlikely to be the main cause of the apparent loss of resistance since the 80s. We now consider the case of the other parent of the hybrid: the PNT.

The methods required to study the PNT differ from those used in the case of the MYD. Unlike the Dwarfs, Tall coconuts are predominantly cross-pollinating. As a result, all its individuals differ from each other although to a lesser extend than from individuals from other cultivars. Identifying members of cross-pollinating cultivars requires the use of probabilistic methods (Rannala and Mountain 1997). These methods have been developed for coconut as part of a microsatellite kit for coconut cultivar identification (Baudouin and Lebrun 2002) based on a set of 14 microsatellite markers. Diversity studies based on these markers and conducted on a large number of coconut cultivars made it possible to distinguish two main genetic groups: Indo-Atlantic and Pacific (Lebrun et al. 2003).

We show here how assignment methods can be used to assess the trueness-to-type of genetic material used in genetic trials and in seed production. All tested samples in the present study are supposed to be PNTs, but unwanted intercrossing may have occurred during rejuvenations. Apart from the PNT, which originated in the Pacific coast of Panama, two cultivars are widespread in Jamaica and are thus the most probable sources of genetic contamination. The local cultivar is the Jamaica Tall (JMT) and belongs to the Indo-Atlantic group. Quite similar coconut cultivars are widespread in the whole Caribbean region including the Atlantic coast of Mexico and Panama where they are known as Alto Atlantico. The Malayan Dwarfs (mainly MYDs) was introduced several times during the last century. It belongs to the Pacific group, like the PNT, but these cultivars are easy to distinguish, thank to their low diversity. As a result, it is often possible to identify the source of contaminating pollen and the type of cross and to assess its potential effect on LY resistance level. This paper presents the characterization of 86 PNT individuals from four populations in Jamaica. These populations have been used as pollen sources for seed production since the 70s.

Materials and methods

The Microsatellite kit

The molecular markers used in this study were 14 microsatellite (SSR) markers from the "microsatellite kit for coconut cultivar identification" (Baudouin and Lebrun 2002; Lebrun et al. 2005). The selection criteria for theses markers were that they should be easy to implement and to score. SSR analyses were performed on an automatic sequencer Li-Cor IR2 (Lincoln, Nebraska). All technical conditions are described in Baudouin et al. (2006).

Reference populations

We used six representative samples (Table 1) from populations already characterized in previous studies (Lebrun et al. 2005) as reference populations for the three main cultivars found in Jamaica:

- In order to represent the diversity of the PNT, we considered three sub-populations of this cultivar: the first one corresponds to what can be considered as the "typical" Panama Tall (PNTty): although the sample is made up of individuals collected at two different places, it is very homogeneous. The second one comes from two fields planted with the same population "Aguadulce" (PNTagu). About 10% of its genes result from introgression from "Alto Atlantico". The third one (PNTcr, from Costa Rica) can only be distinguished from the "typical" PNTs by the presence of a small number of specific alleles.
- We represented the MYD by 69 individuals, representative of the local population and already studied in Lebrun et al. (2007). The local population is more variable than the MYD found elsewhere in the world, which is usually monomorphic.
- Finally, we represented the JMT by two samples, one originating from the JMT itself and one made up of the closely related Mexican Atlantic Tall (MXAT).

Table 1 Origin of the reference samples

Cultivar	Reference sample	Size	Origin	Provided by
Panama Tall: PNT (group A7) cross-pollinating	"Typical" PNT (PNTty)	26	22 from Monagre (Panama)	SMD-CNRA
			4 from Peru	CIB
	PNT Aguadulce (PNTagu)	27	14 from Aguadulce (Panama)	SMD-CNRA
			13 from Nicaragua	Fadcanic
	PNT Costa Rica (PNTcr)	21	21 from Costa Rica	Fadcanic
Malayan Yellow Dwarf: MYD (group A1a) self-pollinating	Local MYD (MYDjam)	69	5 from Fair prospects	CIB
			43 from Barton Isle	
			11 from Hemitage	
			10 from Balllard Valley	
Jamaica Tall (JMT) (group B1) cross-pollinating	Jamaica Tall (JMT)	5	Jamaica	CIB
	Mexican Atlantic Tall (MXAT)	9	Campeche (Mexico)	CICY

Tested material

The tested material consisted in 86 genotypes collected at four locations. Ten genotypes (noted BD) came from Bowden. The samples from Green Castle and were divided into four batches based on the colour of their inflorescences: three groups of ten trees each with green (GCa), bronze (GCc) and intermediate (GCb) inflorescence. The colour was not specified for the last batch (GC). Twenty-six individuals came from Plantain Gardens: 15 of them (PGa) had green inflorescences and 11 (PGc) had bronze inflorescences. Finally, 11 samples from Agualta Vale were noted "AV".

Statistical analyses

We used software *Geneclass* 2 (Piry et al. 2004) to calculate the assignment scores of the reference populations for all PNT individuals. This score is equal to $-\log_{10} \boldsymbol{L}_{ij}$, where $\boldsymbol{L}_{ij}$ is the likelihood of population i for individual j. The more a population is likely to be the source of an individual, the lower is its score for this individual. Absolute values are almost meaningless because they depend on the number of loci and of their discriminating values. However, comparison between populations for the same individual is meaningful: the most probable origin of the individual is the population with the lowest score (Baudouin et al. 2004). Since the score is logarithmic, a difference of, e.g. 3, 6 or 9 means that likelihood ratio are, respectively, 1,000, 10^6 or 10^9.

Likewise, comparisons between individuals can help detecting possible hybrids: the presence of genes from population B in a member of population A is expected to increase the score of population A and to reduce that of population B. It will also increase its heterozygosity. Although none of these criteria is sufficient by itself to consider an individual as an outlier, their combination amounts to a strong suspicion of hybridization. We were thus able to identify probable outliers and, in some cases, to formulate hypotheses about their true origin (e.g. a F_1 hybrid between PNT and another cultivar). Finally, we confirmed these hypotheses by direct examination of their genotypes.

To validate our findings, we also ran *Structure* 2.1 software (Falush et al. 2003) using population information for the reference populations only and allowing for admixture. A 10,000 iteration period was followed by 50,000 iterations.

Results

Reference populations

In order to calibrate the hybridization criteria, we determined the normal variation range of heterozygosity and of assignment scores among PNT of known origin. Figure 1 represents the results for the "typical" PNTs. We ordered individuals by increasing score for their population of origin. For all but the last one, the score associated to the "typical" PNT varied smoothly from 3 to 10. Scores for the Aguadulce and

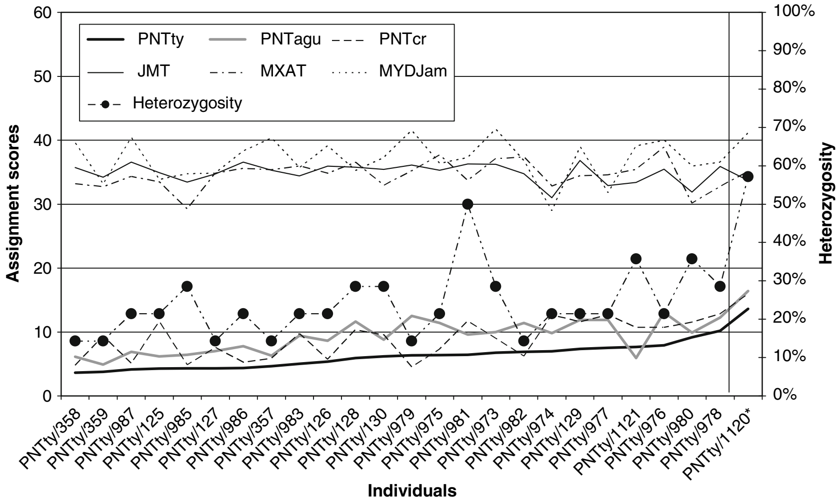

Fig. 1 Assignment scores and heterozygosity in the true Panama Tall. Individuals were ordered according to the score for the typical Panama Talls. The trueness-to-type of individual PNTty1120 (to the right) is dubious. It was excluded from subsequent analyses

Costa Rica populations were in average three units above the "typical" sub-population, which is just enough to ensure that most "typical" PNTs are assigned to their true origin, rather than to one of the other sub-populations of the PNT. Contrastingly, the scores of the JMT, MXAT and MYD cultivars were between 30 and 40 preventing any confusion with these cultivars. Finally, heterozygosity was generally comprised between 0.15 and 0.35. Only individual PNTty/1120 could be seen as suspect: its genotype was 22,000 times less probable than the next least probable and its heterozygosity was unusually high. We removed this individual from subsequent analyses, although its influence on the results is negligible.

The results for the members of the other PNT sub-populations (Aguadulce and Costa Rica—not shown) were quite similar and the scores of their respective origins were also comprised between 3 and 10 and only slightly higher for the other Panamean populations. The scores of JMT and MXAT were comprised between 30 and 45. Heterozygosity was more variable and appreciably larger in the Aguadulce population (0.38 in average instead of 0.25 and 0.29 for PNTty and PNTcr, respectively).

To summarize this section devoted to the reference samples, a PNT has a score ranging between 3 and 10 for its own source sub-population. Differences among PNT origins result in a slight increase of the scores of the other sub-populations. On the other hand, confusion with the Indo-Atlantic Talls or the MYD is impossible with scores systematically exceeding 25. In addition, a heterozygosity rate above 0.25 is unusual, except for the Aguadulce origin. We could thus set the following thresholds for the present study: if an individual had a score below 12 for the PNTty, we considered it as true-to-type. If its score was above 20 for the PNTty and below 25 for one of the possible source of contamination, we considered it as an outlier. In between, its trueness to type was dubious, especially if its heterozygosity was high.

Tested populations

Figure 2 represents the results of the PNT planted at Green Castle and Bowden. They behaved very much like the Panama reference populations, except for individuals GC/1313 and GCb/2187, which we identified as F_1 hybrids between PNT and JMT: they both combine a high score for the PNT and the MYD with a low score for the JMT and high heterozygosity. Although the colour of the inflorescence is of genetic origin and is potentially a diagnostic criterion, we did not find differences between colours: the mean scores were, respectively, 8.9, 8.1 and 8.7 for categories GCa, GCb and GCc.

Figure 3 represents the results at Agualta Vale and Plantain Gardens. Four groups can be distinguished: 24 individuals were true PNTs (left hand part of the graph). Putative hybrids (right hand part) were represented by a first generation back cross with JMT (AV/1324) and seven F_1 hybrids between PNT and JMT. Finally, five individuals were of dubious origin: with a score comprised between 12 and 20, they are proba-

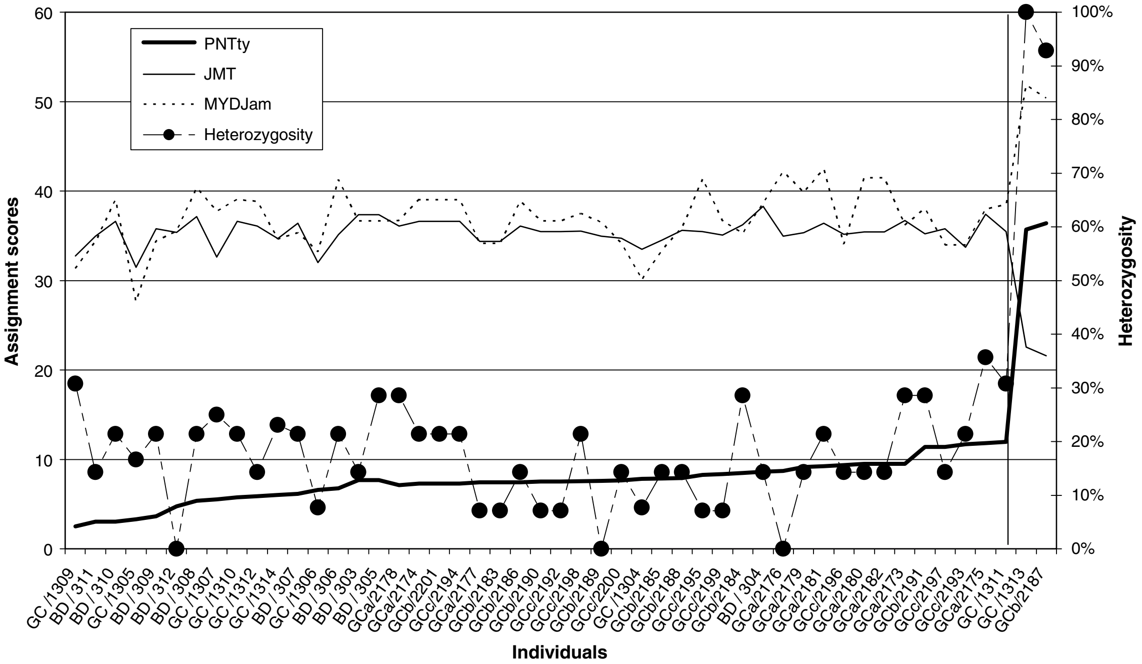

Fig. 2 Assignment scores and heterozygosity at Green Castle and Bowden. For clarity, score for Aguadulce, Costa Rica and MXAT are not shown. All individuals are conform except for GC /1313 and CCb/2167 (to the right) which are PNT × JMT F_1 hybrids

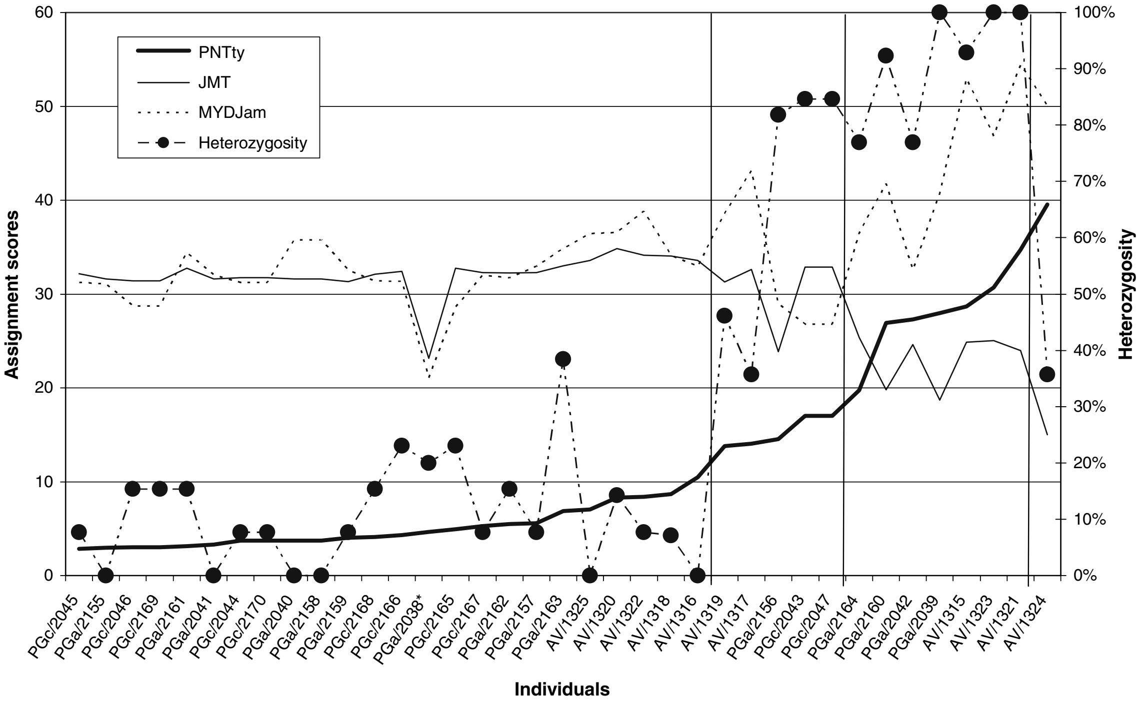

Fig. 3 Assignment scores and heterozygosity at Agualta Vale, and Plantain Gardens. For clarity, score for Aguadulce, Costa Rica and MXAT are not shown. From the left to the right: 24 truly Panama Talls (the abnormal scores for individual pga/2038 are due to missing values); five individuals of dubious origin; seven PNT × JMT F_1 hybrids and one (PNT × JMT) × JMT BC_1 hybrid

Table 2 Summary results of the tested material

	Number of palms	True Panama Tall		Dubious		Hybrids with JMT			
						F1		BC1	
Theoretical percentage of Panama Tall genes		100%		n. a.		50%		25%	
Bowden									
	10	10	100%						
Green castle									
Unspecified colour	10	9	90%			1	10%		
A (green)	10	10	100%						
B (intermediate)	10	9	90%			1	10%		
C (bronze)	9	9	100%						
Average	39	37	95%			2	5%		
Plantain garden									
A (green)	15	10	67%	3	20%	2	13%		
C (bronze)	11	9	82%	2	18%				
Average	26	19	73%	5	19%	2	8%		
Agualta Vale									
	11	5	45%	2	18%	3	27%	1	9
Grand total	86	71	83%	7	8%	7	6%	1	1

bly neither F1 hybrids, nor pure PNTs. These results are summarized in Table 2.

Finally, we note that, considering only the "true to type" individuals, observed heterozygosity was somewhat lower in the Jamaican populations (12–18%) than in the "typical" PNT (23%). As a result, nine individuals were totally homozygous: one in Bowden, two in Green Castle, four in Plantain Garden and two in Agualta Vale. This can be explained by several factors, including variation among the PNT populations collected initially, varying sample sizes and the fact that some of Jamaican populations represented the second or third generation since introduction, resulting in a lower genetic diversity, compared to the reference sample, which was a direct introduction.

Comparison with *Structure* software

We ran *Structure* 2.1 six times for each value of the assumed population number ranging from 3 to 6. A stable and minimal value of the log-likelihood was obtained only for five populations, which could be identified as the three PNT populations, the MYD from Jamaica and a last population grouping the JMT and the MXAT. The population parameters are given in Table 3. The estimated migration rate α_1 was low

Table 3 Summary results of *Structure*

Migration rate	$\alpha_1 = 0.047$				
	PNTty	PNTagu	PNTcr	MYDJam	JMT + MXAT
Population parameters					
F_{ST}	0.43	0.34	0.37	0.48	0.31
Kullback-Leibler "distance"					
PNTty	–	0.43	0.41	2.76	2.28
PNTagu	0.72	–	0.76	2.76	1.73
PNTcr	0.48	0.60	–	3.00	2.26
MYDJam	3.82	2.68	2.94	–	2.70
JMT + MXAT	4.61	2.55	3.36	3.86	–

and confirmed that the reference populations were clearly differentiated. However, the Kullback-Leibler “distances” among the three PNT sub-populations were much lower than the others (Table 3), confirming their close relationship. The estimated percentage of genes from JMT was 8% at Plantain Gardens and 26% at Agualta Vale.

Individuals identified as “true” PNTs based on the above criteria were confirmed by *Structure*, with an estimated percentage of Panamean genes above 97%. This percentage was comprised between 60 and 90% for the “dubious” genotypes and the “off-type” genotypes were also confirmed with a percentage of genes from the parental populations close to theoretical values for first or second generation hybrids. According to *Structure*, only three trees (PGc/ 2043, PGc/2047 and PGa/2042, see Fig. 3) inherited an appreciable part of their genes from the MYD. Minor differences of appreciation between the two approaches were noted in the case of GC/1313 and GCb/2187 (see Fig. 2): *Structure* suggest they are BC_1 towards JMT. Their high heterozygosity, among other things, indicate that they are actually F_1 hybrids.

Conclusion

The assignment method implemented in *Geneclass* 2 was primarily devised to assign individuals to predetermined populations in cross-pollinating species. This paper illustrates its use to identify off-types among the members of a population. Both *Geneclass* 2 and *Structure* 2.1 have their own advantages: *Geneclass* 2 does not use the MCMC procedure and the result was obtained in a matter of seconds. *Structure* 2.1 was significantly more difficult to handle: we had to try several parameter combinations before obtaining stable and reliable results, but directly estimated the proportion of genes coming from the parent populations, confirming most of our hypotheses on the hybrids. Finally, the benefit of using two very different algorithms was to gives us more confidence in our results.

We demonstrated the presence of alleles from other populations in all the studied PNT populations, except for Bowden. At Green Castle, only two palms were F_1 hybrids, while in Agualta Vale and Plantain Gardens, 30–55% were not pure PNTs. This means that the controlled pollination techniques used for producing the last two accessions were not always perfect and that care should be taken in the future to maintain the genuine PNT. This implies to use palms known to be true to type and to apply an efficient controlled pollination method, such as those recommended in Santos et al. (1996).

The main source of non-PNT alleles was the JMT and seed production using pollen from Agualta Vale and Plantain Gardens is likely to result in an increased susceptibility to LY. Actually, these seed gardens contributed little to seed production in Jamaica: in the 80s, pollen came from St. James and White Hall Estates. In the early 90s, it came from Bowden and later on, Green Castle represented the main source, with an approximate 1% contribution from Plantain Gardens. Since St. James and White Hall Estates have been wiped out by the disease, their status is unknown. One may however suspect that, due to their age, they had less chance to be contaminated by unwanted pollen than more recent ones. Bowden was also destroyed since the sample collection.

Despite uncertainties about the status of some of the pollen sources, genetic contamination in the PNT was probably not the main reason for the increased mortality in the MAYPAN: firstly, this cultivar has never been fully resistant: its mortality rate ranged from 4 to 21% in Been (1981). Secondly, the most contaminated pollen sources do not appear to have been used in large quantities. The presence of up to 26% of JMT genes in a number of pollen batches was certainly sufficient to increase substantially mortality in a few plantations, but not to cause the “massive losses” that have been noted by Broschat et al. (2002). We have thus to conclude that neither the MAYPAN, nor its parents can be said truly and permanently resistant.

True or apparent variations of resistance level in these cultivars may have occurred for several reasons: mutation and selection in the phytoplasma may have favoured virulence against the MYD and the MAYPAN. On the other hand, the behaviour of the vector may also have changed: in the resistance trials, the MYD and the MAYPAN were planted together with many different cultivars. The vectors may have preferentially fed on other cultivars, such as the JMT, which were thus destroyed more rapidly. But, once the MAYPAN and the MYD represented the majority of the commercial plantations, they had no other choice than feeding on these cultivars. In the first hypothesis, the MAYPAN had (incomplete) resistance factors, mainly inherited from the MYD and these resistance factors were progressively overcome during the last 25 years. In the second one, neither the MYD nor the MAYPAN were resistant, but they initially escaped the disease. In both hypotheses, the new outbreak was favoured by the extensive planting of two closely related cultivars, with a narrow genetic basis.

In the present state of knowledge, no truly and permanently resistant cultivar to the Jamaican form of LY is known. As a result, a strategy to control LY should probably rely on an increased diversity of the planting material, rather than on a small number of highly uniform varieties. In Been (1981), several cultivars had a reduced mortality rate (although higher than for the MYD). They generally came from South-East Asia. Planting several cultivars from this region would probably reduce the rate of evolution of the pathogen and/or the vector, owing to their increased diversity. It would also probably make the effects of this evolution less dramatic. Such measures could probably contribute to increasing the economic life of coconut plantations in Jamaica, and thus to restoring their profitability. It would however not make them free of LY: all treatments of the trials mentioned in Been (1981) are now severely affected by LY (B. Been, W. Myrie, not published data). More research is needed for a better understanding of the conditions that affect the transmission of the disease.

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