RESISTANCE TO CSSV

The must-have ingredient of a successful disease management strategy

by

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- Plant Breeder in the British Research Team at Tafo, Ghana 1969-78
- Search for resistant material described as the biggest ever attempt at breeding for resistance to a tree crop virus
- Programme was successful, but this was not proven until material had been out on farms exposed to severe strains of CSSV for up to 25 years

Definitions in Cocoa

RESISTANCE refers to the rate at which the virus spreads through a particular genotype. It ranges from great susceptibility when the virus spreads rapidly, to strong resistance when the virus spreads relatively slowly. Immunity to CSSV, meaning can't be infected, is not known in *Theobroma cacao*

TOLERANCE refers to the reaction of the plant once infected. If the plant reacts badly, it is said to be sensitive to infection; if it shows few symptoms it is said to be tolerant of infection

Why Resistance?

There is strong evidence that effective resistance to the spread of severe strains of CSSV can be incorporated in acceptable varieties, as this paper will show

A proven, if technically demanding, predictive test is available for use in seedling breeding

The inheritance of resistance is understood. It appears to be effective against several virus strains

Tolerance proved to be unstable in Ghana. No proven predictive test is available for use in seedling breeding

The Epidemiology of CSSV

- Most spread of CSSV depends on viruliferous mealybugs crawling from tree-to-tree through the inter-locking canopies of cocoa plantings
- It follows that if the land for a new planting is free of cocoa and alternative hosts of CSSV and there is no canopy contact between the new planting and surrounding infected cocoa, disease invasion will be slow

CSSV Resistance in Practice

- From 1973 to 1979, in areas of Ghana with mass infection with severe strains of CSSV, 128 farms 0.1 – 4.3 ha were replanted after CSSV control by cutting out infected trees.
- 23 of these farms were re-examined in 1997, all except one was planted with inter-Amazon crosses

Disease Incidence 19-26 Years after Planting

- 70% of farms had fewer than 5% of the trees infected
- 17% of farms had 5 – 11% infection
- 13% of farms had over 20% infection and so were coming to the end of their useful lives

Replanting with resistant material is effective in an area of mass infection even when CSSV strains are severe

Formal Rate of Virus Spread Trials

Comparative virus rate of spread trials can be done by patch graft infecting a line of trees to provide a source of virus to spread into adjacent uninoculated trees, with plots separated by internal guard trees, as the Figure shows. Valid comparison rests on uniform canopy contact between the entries in the trial, which can be relied upon with broadly similar seedling varieties, but not with clones

Layout of Virus Rate of Spread Trials

	G		S2		G				Triangle
T4		T2		T2		T7			spacing
	G		S2		G				1.6m
G		G		G		G			
	G		S1		G				S =source
T5		T1		T1		T8			
	G		S1		G				T = test
T5		T1		T1		T8			
	G		S1		G				G = guard
T5		T1		T1		T8			
	G		S1		G				1, 2 , 3….
T5		T1		T1		T8			genotypes
	G		S1		G				
T5		T1		T1		T8			
	G		S1		G				
G		G		G		G			
	G		S3		G				
T6		T3		T3		T9			
	G		S3		G				
T6		T3		T3		T9			
	G		S3		G				

Rate of Spread Trials – Field Practice

- Six years from planting to results
- Staff readily trained in symptom recognition on the test trees
- Large experimental errors mean that ten replications are needed and preferably more
- 20 crosses recorded for 42 months generate 336,000 observations. Computers help
- Fast epidemic to minimise “noise” in the trial
- Analysis by regression of incidence of visible infection on time, so latent period accounted for

Resistant Crosses - some Results

Progeny	Rate virus spread Logit proportion infected/day x10^4	Yield kg/ha	% Black Pod as angle
T85/799 x Amelonado	55.0	5,947	18.3
T85/799 x T79/501	24.7	6,756	14.7
T85/799 x T17/524	13.2	4,236	18.1
T85/799 x Pa7	30.7	8,344	14.0
T85/799 x Sca6	29.9	7,480	13.8
SED	7.79	889.9	1.27

Rapid Test for Resistance

- Peeled seeds can be infected with virus after soaking overnight in water. They can be brushed with purified virus or infested with viruliferous first instar nymphs of *Formicoccus njalensis*

- The mealybug method is preferred because of the difficulty of preparing high quality inoculum on the scale required by a breeding programme

Rapid Tests - Success Factors

- Seed pods ripen simultaneously and are true to type. Up to 56 crosses tested simultaneously
- Efficient system for rearing first instar nymphs and their virus acquisition feed
- Trained operators to transfer nymphs to pre-soaked seeds (typically three operators, 20 seeds of each cross inoculated each day, day = replicate)
- space for growing and observing the seedlings.

Rapid Tests – Biometry Rules

The experimental design and statistical analysis should account for all controllable sources of variation, including genotypes (and any mating design), replications (days on which tests are done, typically five) and operators (the individuals who transferred the nymphs, typically three).

Interactions involving operators are uninterpretable and almost always the experiment has to be discarded

Validation of Predictive Test

When predictive tests are free of anomalous interactions and there are statistically significant differences in rates of virus spread, the estimates of resistance based on first flush data are correlated

Product moment correlations were 0.87 (field trial 1, n=8), 0.70, 0.87 and 0.83 (field trial 2, n=16) and 0.87 (field trial 3, n = 7)

Resistance: Conclusions

- The IMC and Nanay collections provided the strongest resistance, there is limited evidence of resistance in the Rio Branco collection
- The Moronas, Parinaris and Scavinas show less resistance
- The resistance to virus infection in the IMCs and Nanays is associated with resistance to the mealybug vector, which amplifies the effect of resistance to virus on rate of spread in the field
- There is some evidence that the resistance is effective across CSSV strains

Resistant Clones?

- Consideration of resistant clones implies acceptance of the very high cost of establishing clonal plantings
- No validated rapid test for resistance is available. Use of general combining ability and challenging budded plants with viruliferous mealybugs can be considered
- Confirmatory rate of spread trials would be difficult to standardise because of great differences in vigour, growth habit and so canopy contact between clones
- Very large numbers of candidates would require evaluation because of the low success rate in clone selection in cocoa

Auchinlech 19 May 1937
Letter to the Governor of the Gold Coast

"I have no wish to be alarmist or to act prematurely, but an outbreak of any disease of cocoa, under the conditions on which the Gold Coast industry is carried out, must be disturbing. If the present trouble proves to be due to an infectious disease we may quite possibly be faced with a long and uphill fight"

Conclusion: Resistant Seedling Varieties can be Developed in C d'I.

- Ghana's experience from 1969 – 1999 shows that agronomically acceptable seedling planting material with resistance that is effective if deployed appropriately can be made available to farmers. This is an immense step forward
- In Côte d'Ivoire it could be deployed in the field within twelve years
- It is possible because of the development of seed production by mass manual pollination