Ann. appl. Biol. (1963), 52, 217-224 Printed in Great Britain

Field trials on the effect of a Nigerian swollen-shoot virus on the growth of different cacao types

By J. F. LONGWORTH AND J. M. THRESH* West African Cocoa Research Institute, Ibadan, Nigeria

(Received 2 March 1963)

SUMMARY

Two brief field trials in Western Nigeria compared the effects of infection by a virulent strain of swollen-shoot virus on growth of West African Amelonado cacao with those on other types. These included hybrid progenies from crosses with Upper Amazon cacao. Within each progeny, severity of leaf symptoms was correlated with growth rate, but the Amelonado showed more severe symptoms than the Amazon progenies, although infection decreased its growth rate proportionally less. However, most of the Amazon progenies are so much more vigorous that some infected ones grew more rapidly than the uninfected Amelonado. Three progenies were exceptionally tolerant and their growth rate was little affected by infection: such types may prove valuable in areas where attempts to eradicate swollen-shoot disease have been abandoned.

INTRODUCTION

The types of cacao in West Africa react differently to infection with swollen-shoot virus. They differ in their resistance to infection by mealybug vectors, in the duration of the latent period and in the type and severity of the symptoms produced (Posnette & Todd, 1951; Dale, 1957; Blencowe, 1961; Longworth, 1961a; Longworth & Thresh, 1963). These observations suggest that losses from swollen-shoot might be decreased were the existing populations of highly susceptible and sensitive Amelonado trees replaced by types that resist or tolerate infection.

There is no known immunity to cacao viruses (Posnette & Todd, 1951) and laboratory tests of resistance are slow, laborious and difficult to standardize. Graft tests to detect tolerant types are much more practicable and Longworth & Thresh (1963) found clones which showed only slight symptoms when infected with virulent isolates of Nigerian virus. However, there is no information from Nigeria or elsewhere demonstrating that the severity of the leaf symptoms developed by different cacao progenies is related to their subsequent growth. This paper reports the results of two brief field tests of the effects of infection on the growth of several different progenies.

MATERIALS AND METHODS

The experiments were on land cleared from thicket near Alakia, 8 miles east of Ibadan, Western Nigeria. They were intended to continue for only 2 or 3 years and

* Now at East Malling Research Station, East Malling, Maidstone, Kent.

217

G.L.

J. F. Longworth and J. M. Thresh

218

the spacing of 4 ft. by 4 ft. was adopted to include many trees and increase precision. The soil was mulched with Guinea grass and, during the first dry season, shade was provided by cut palm fronds supported on bamboo poles.

The first experiment began in July 1959 and ended in December 1961. Seedlings of fifteen different progenies were grown in pots for 6 months and planted in twelve randomized blocks. Progenies not available in sufficient quantity were incompletely replicated. Five of the ten seedlings in each plot were graft-infected with a virulent isolate of virus from Egbeda (Thresh, 1961) in February 1960.

The second experiment began in April 1960 and ended in December 1961. Potgrown seedlings of six different progenies were compared with one of two standard types in each of six randomized blocks. Fifteen of the thirty seedlings of each progeny in each plot were graft-infected with the Egbeda isolate of virus in August 1960.

Planting material

The field trials included all the types that Mr D. A. Glendinning, Plant Breeder, W.A.C.R.I., Ghana, could supply. The progenies were of the I, II and Nanay series (Glendinning, 1962).

The series I progenies have been produced since 1953 for field trials in Ghana, Sierra Leone and Nigeria. The parents are vigorous, high-yielding Amazon trees in the Introductions Area at Tafo, which was established with seed collected in Trinidad in 1943 (Posnette, 1951; Knight & Rogers, 1955). The parents of the series I and Nanay types were selected trees from the following progenies:

	Trinidad parents			
Progeny	Female	Male Unknown		
T 12	Scavina 12			
T16	Iquitos 24	Unknown		
T60	Parinari 7	Nanay 32		
T61	Nanay 33	Nanay 32		
T62	Nanay 33	Nanay 34		
T63	Parinari 35	Nanay 32		
T79	Nanay 32	Parinari 7		
T81	Nanay 32	Nanay 31		
T85	Iquitos 60	Nanay 34		

The series II progenies were first produced in 1953 and exploit the hybrid vigour resulting from crosses between distinct cacao types. Selected Amazons from the Introductions Area were crossed with outstanding West African Amelonado trees or with Amelonado × Trinitario selections (Posnette, 1943).

The Nanay progenies resulted from crosses between trees in the Introductions Area that had shown some evidence of resistance to a swollen-shoot virus in Ghana (Dale, 1957). Table I shows detailed parentage.

Growth and symptom records

Stem diameters between marked points 6 in. from ground level were recorded regularly with a vernier caliper. Although the progenies had been grown in uniform nursery conditions, they differed slightly in size when transplanted and when grafted.

It was conve of the graft was found in

Τ

* During T16 parents.

The gra appeared a October 10 second. Th 0 no Ι slig slig 2 inte 3 con 4 ver 5 The two o analyses.

Most o Amelonad between a ficantly m The vir in each pr

Effects of swollen-shoot virus on cacao types

It was convenient, therefore, to use the increment in stem diameter between the date of the grafting and the final observations for all statistical analyses. No advantage was found in using a covariance analysis which allowed for initial differences in size.

Table 1.	The parentage	of the	progenies	planted	in eac.	h field	trial
----------	---------------	--------	-----------	---------	---------	---------	-------

- Contraction of the second seco		
Test progenies	Parents	Experiment
W.A.C.R.I. IA	$T_{12/26} \times T_{12/151}$	1, 2
W.A.C.R.I. IG	$T_{60}/885 \times T_{60}/888$.	I Na32 Pa7
W.A.C.R.I. IM	$T_{79/1137} \times T_{79/1138}$	I Na 32 191
W.A.C.R.I. IX	Unselected F ₃ Amazon	I
W.A.C.R.I. IY	Trinitario \times T(N) 38	I
W.A.C.R.I. IIA	P_4 Amelonado $\times T_{63/971}$	I, 2 Pass Nasa
W.A.C.R.I. IIC	E1 Trinitario × T63/971	2 2 1924012 1,2 Na 32 Pa 7
W.A.C.R.I. IID*	E 1 Trinitario \times T 16/613	2 1924012
W.A.C.R.I. IIE	$T_{79/416} \times E_{1}$ Trinitario	1,2 Na 32 Pa/
W.A.C.R.I. IIF	$T_{79/501} \times T_{85/799}$	I
W.A.C.R.I. IIK	W_{41} Trinitario \times T63/967	2
Nanay a Na	$T81/1880 \times T81/1792$	I Wer 32 × Ner 31
Nanay b N b	$T81/1992 \times T81/1880$	I
Nanay c N c	$T62/977 \times T62/891$	I Wa33x34
Nanay d N d	$T62/958 \times T62/1407$	1 1 Na 33 × 32
Nanay e Ne	T61/1313×T61/1403	I NO(33 × 35
Nanay f N f	$T61/1239 \times T61/1664$	I
Standard types		
Amelonado Ad	Unselected	I, 2
W.A.C.R.I. IQ	$T(N)_{38} \times T(N)_{38}$	2

* During the experiment it was established that some of these trees were from open-pollinated T16 parents.

The grafted seedlings were inspected frequently to see when symptoms first appeared and the severity of the symptoms was recorded monthly from June to October 1960 for the first experiment, and in January and November 1961 for the second. The scoring used was:

- o no symptoms.
- 1 slight leaf symptoms.
- 2 slight leaf symptoms and/or slight swellings.
- 3 intermediate or conspicuous leaf symptoms and/or swellings.
- 4 conspicuous leaf symptoms, large swellings, stunted growth.
- 5 very conspicuous leaf symptoms, swellings and die-back.

The two or more values were averaged to give the symptom scores used in statistical analyses.

RESULTS

First Alakia experiment

Most of the fourteen progenies grew more vigorously when healthy than the Amelonado standard (Fig. 1). The experiment did not give a very precise comparison between all progenies, as some were inadequately represented, yet eight were significantly more vigorous than the Amelonado. Nanay b and e and IG were outstanding.

The virus infection invariably decreased growth, although not to the same extent in each progeny. Proportionally the slow-growing Amelonado standard was affected

J. F. Longworth and J. M. Thresh

less than all but one of the Amazons. However, most of the Amazons were very vigorous and the infected progenies IM and Nanay b grew faster than the uninfected Amelonado. Clearly, most of the Amazons were intolerant and IIA and Nanay f appeared particularly sensitive, as virus decreased their growth by 46 and 43%, respectively. The evidence for this is inconclusive as they were inadequately represented and IIA showed a less severe reaction in the second experiment.



Fig. 1. The effect of virus infection on the growth of the fifteen progenies in the first experiment at Alakia.

Fig. 2. The effect of virus infection on the growth of the eight progenies in the second experiment at Alakia. Progenies in each figure arranged according to their vigour when healthy. The data for the Amelonado standard is indicated by an asterisk*. \square , increment for healthy trees. \square , Increment for infected trees.

Second Alakia experiment

The healthy plants of the six Amazon progenies represented in the second Alakia experiment grew more rapidly than the Amelonado and Trinitario standards. However, the comparison between individual progenies was not sufficiently precise to show significant differences.

As in the first experiment infected trees grew more slowly than the uninfected ones (Fig. 2). The differences were not significant for the tolerant IID, but significant at P < 0.05 for all others. Again, although the growth of the Amazons was affected proportionally more by infection than that of the Amelonado, their intrinsic vigour meant that most infected trees still grew at a satisfactory rate, some exceeding the growth rate of uninfected Amelonado.





of the f Fig. 4. of the e

infection h difficult to rapid appe gerated im been assoc

The syn In the firs sensitive p

220

Vigour and effects of virus

In the first experiment, the mean stem diameter increments made by the uninfected plants of each progeny were positively correlated with the decrease in growth caused by virus (r = +0.583, P = 0.05). The correlation was also positive in the second experiment, but was significant only when the data for the particularly tolerant progenies IID and LM were omitted (r = +0.456 when n = 8 and r = +0.981, P = 0.001 when n = 60. This relationship between vigour and the effects of virus



Fig. 3. Relation between mean symptom score and the effects of virus on the growth of the fifteen progenies in the first Alakia experiment.

Fig. 4. Relation between mean symptom score and the effects of virus on the growth of the eight progenies in the second Alakia experiment.

infection has also been demonstrated previously (Longworth, 1961*b*) and makes it difficult to evaluate the unexpected results obtained with the Amelonado cacao. The rapid appearance of conspicuous symptoms in this material may have given an exaggerated impression of its sensitivity, or the slight effects of virus on growth may have been associated with the lack of vigour.

Symptom severity and growth rate

The symptoms shown by the seedlings of each progeny differed in type and severity. In the first experiment mean symptom scores ranged from 2.70 for the particularly sensitive progeny IIA, to 0.57 for Nanay b. Most progenies showed a smaller range,

which in the second experiment was from 1.87 for IIK to 0.89 for the tolerant IID (Figs. 3 and 4).

The conspicuous leaf necrosis described recently by Longworth & Thresh (1963) was again restricted to a proportion of the Scavina and Parinari material. Progenies IA, IG, IM, IIA, IIC, IIE, IIF and IIK were affected and the necrosis spread to the petioles, stems and growing points of the most severely affected trees. The different Nanay progenies developed swellings and similar leaf symptoms of reticulate patterns of cleared veins and chlorotic specks. Differences in the severity and extent of these symptoms were associated with different symptom scores and effects on growth (Figs. 3 and 4).

The progenies with the most conspicuous leaf symptoms were usually those in which growth was most severely affected by virus. In the first experiment (Fig. 3), mean symptom score for each variety and percentage decrease in growth were correlated (r = +0.574, P = 0.05). The Amelonado standard was exceptional in that a large symptom score was associated with a small proportional decrease in growth and omitting the results with this variety increased the correlation coefficient to +0.746 (P = 0.01). Similar but more highly significant correlations were obtained in the second experiment (Fig. 4), with values of +0.964 (P = 0.001) using the complete data and +0.978 (P = 0.001) after omitting data for the Amelonado. These correlations were little affected when the effects of differences in the latent period were eliminated by partial correlation. This suggests that differences in latent period have no direct association with subsequent symptom expression and effects on growth.

The seedlings of each progeny did not all react to infection similarly, but the relationship between symptom expression and effect on growth held for the individual trees of each progeny as well as for the mean response of the entire populations. This was shown by the significant negative correlation coefficients between symptom score and growth increment following infection, for seven of the eight progenies in the second experiment. The correlation coefficients ranged from -0.322 (P = 0.01) for variety II D to -0.708 (P = 0.001) for the Amelonado standard. Progeny II D showed a relatively uniform and slight reaction to infection so that the effect of virus was small in relation to environmental factors influencing growth.

Symptom score and latent period

Leaf symptoms usually appeared before swellings; they were conspicuous and developed rapidly in the Amelonado standard, with a mean latent period of 85 days in the first experiment and 99 in the second. The latent periods in the test progenies varied and most were longer, with great differences within each type. Mean latent periods in the first experiment ranged from 97 days for the sensitive Nanay f, to 203 for the tolerant IM. Comparable values for the Amazons in the second experiment ranged from 123 to 199 days.

Mean symptom score was negatively correlated with length of latent period, both including the Amelonado standard (r = -0.732, P = 0.01) and excluding it (r = -0.676, P = 0.01). However, the partial correlation between these factors after eliminating the variation from differences in growth was not significant, suggesting that a long latent period reflects a tolerant reaction.

There was progenies wit by virus (r =differences fro between laten

The most s trees, but a s be impractica symptom pro (1963) infecte expression be symptoms we lation with ef (1958 *a*, *b*) fou Trinidad clor

Our result venient meth atypical mate tions on sym types require IA and Nana and the ren within the fe trials. Furthe types and the with observa of selecting Nanay, Iquit dinning in ()

Obviously types to infect large, long-te by graft-infe field trials or material coul term yield tr areas of Nige being plante show a sever

222

Latent period and growth

There was a tendency in the first experiment, though not in the second, for the progenies with a short latent period to be also those whose growth was most affected by virus (r = -0.559, P = 0.05). This correlation became negligible after eliminating differences from severity of symptoms, emphasizing that there is no direct association between latent period and growth reduction from infection.

DISCUSSION

The most satisfactory and useful criterion of tolerance is the yield of virus-infected trees, but a selection process based on yield would be expensive and so slow as to be impracticable. As an alternative, Dale (1957) recorded the severity and type of symptom produced after infecting beans by mealybugs and Longworth & Thresh (1963) infected seedlings by grafts. These tests showed great differences in symptom expression between progenies and within some, but they did not show whether leaf symptoms were correlated with effects on growth. We have now done this, but correlation with effects on yield has yet to be established. However, Jones & Maliphant (1958 a, b) found a close relationship between the early girths and yield of an uninfected Trinidad clone, as also did Glendinning (1960) with seedling cacao in West Africa.

Our results show that the selection of plants showing slight symptoms is a convenient method of distinguishing at an early stage those likely to be tolerant. However, atypical material of the Amelonado type would be overlooked in this way and observations on symptom expression alone are insufficient to identify the vigorous, tolerant types required. The growth records of the first experiment indicate that progenies IM, IA and Nanay d are tolerant, whereas IIA and Nanay f may be particularly sensitive and the remaining progenies show an intermediate reaction. These differences within the few progenies tested are encouraging and some warrant more extensive trials. Further selection from a greater range of material is likely to reveal other tolerant types and the budding technique described by Longworth & Thresh (1963), together with observations on symptoms and growth, provide a convenient and rapid method of selecting promising parents. Progenies from inter-crossing apparently tolerant Nanay, Iquitos, and Trinitario clones are now being tested in Nigeria, and Glendinning in Ghana is developing types which combine tolerance and vigour with resistance to black-pod disease.

Obviously it would be desirable to determine at an early stage the reaction of these types to infection with virus, if possible without having to establish many expensive, large, long-term field trials. Our results suggest that preliminary tests could be made by graft-infecting young seedlings of each progeny and growing on in short-term field trials only those that do not show conspicuous symptoms. In this way much material could be handled and the most promising types could be selected for long-term yield trials. Such trials would also indicate the value of tolerant types in the areas of Nigeria where control measures have been abandoned. These areas are now being planted with Amelonado, Upper Amazon and hybrid seedlings which often show a severe reaction to virus infection.

Very weak

J. F. Longworth and J. M. Thresh

We are grateful to Mr D. A. Glendinning of this Institute for providing seed and to Dr G. H. Freeman for assistance in analysing the data. Acknowledgements are made also to W. A. Soyele, G. Obaze and others for their assistance in planting and recording the field experiments.

REFERENCES

BLENCOWE, J. W. (1961). Future plans for cocoa breeding in Ghana. Proceedings of the Cocoa and Confectionery Alliance Conference, London, p. 141.

DALE, W. T. (1957). Studies on resistance and tolerance to cacao viruses. Proceedings of the Cacao Breeding Conference, 1956, p. 3 (W.A.C.R.I., Tafo, Ghana).

GLENDINNING, D. A. (1960). The relationship between growth and yield in cocoa varieties. *Euphytica*, 9, 351.

GLENDINNING, D. A. (1962). W.A.C.R.I. Cocoa varieties; explanatory notes. (Mimeographed.)
JONES, T. A. & MALIPHANT, G. K. (1958a). High yields in cacao field experiments and their significance in future cacao research. Trop. Agriculture, Trin., 35, 272.

JONES, T. A. & MALIPHANT, G. K. (1958b). Yield variations in tree crop experiments with specific reference to cacao. *Nature*, *Lond.*, 182, 1613.

KNIGHT, R. & ROGERS, H. H. (1955). Recent introductions to West Africa of *Theobroma cacao* and related species. I. A review of the first ten years. *Emp. J. exp. Agric.* 23, 113.

LONGWORTH, J. F. (1961a). Tolerance of cocoa to swollen shoot disease in Nigeria. Proceedings of the Cocoa and Confectionery Alliance Conference, London, p. 145.

LONGWORTH, J. F. (1961b). Virus Research. Ann. Rep. W. Afr. Cocoa Res. Inst. 1959-1960, 75.

LONGWORTH, J. F. & THRESH, J. M. (1963). The reaction of different cacao types to infection with a swollen shoot virus. Ann. appl. Biol. 52, 117.

POSNETTE, A. F. (1943). Cacao selection on the Gold Coast. Trop. Agriculture, Trin., 20, 49. POSNETTE, A. F. (1951). Progeny trials with cacao in the Gold Coast. Emp. J. exp. Agric. 19, 242.

POSNETTE, A. F. & TODD, J. MCA. (1951). Virus diseases of cacao in West Africa. VIII. The search for virus-resistant cacao. Ann. appl. Biol. 38, 785.

THRESH, J. M. (1961). Some isolates of virus causing swollen-shoot disease of cacao in Nigeria and their interrelationship. *Ann. appl. Biol.* 49, 340.

224