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Genetic variation in resistance to vascular-streak dieback in cocoa (*Theobroma cacao*)

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Summary. Genetic variation in disease resistance to vascular-streak dieback (Vsd) was studied in 54 cocoa hybrid progenies from Trinitario × Amazonian crosses. The disease was assessed by the number of Vsd infected branches per tree, total length of infected branches, average depth of disease penetration within branches and percentages of infected and dead plants. Highly significant differences due to general combining ability for both Trinitario female parents and Amazonian male parents were obtained for all characters. Percent of dead plants was the only character where specific combining ability was also important. Gene effects were predominantly additive for most characters, indicating that selection for progenies resistant to Vsd is effective in the present breeding population. There is strong evidence that resistance to Vsd is in the form of horizontal resistance. It is polygenic and largely inherited as additive genes. Therefore, breeding for resistance is an effective means of controlling Vsd in Papua New Guinea.

Key words: Cocoa – Vascular-streak dieback – Disease resistance – General combining ability – Specific combining ability

Introduction

Vascular-streak dieback (Vsd) of cocoa, caused by *Oncobasidium theobromae* Talbot and Keane (1971), was first reported in Papua New Guinea in the early 1960s (Anon 1963, 1964; Bridgland et al. 1966a, b, 1967) and reached epidemic levels in several areas. It has since become one of the most destructive diseases of cocoa in Malaysia, particularly in Sabah where cocoa is one of the most acceptable crops in agricultural development. It has recently spread widely in Indonesia. In Papua New Guinea, although Vsd is no longer a serious threat to mature trees with a degree of resistance, it still causes serious damage to young seedlings.

No fungicide is effective for the control of Vsd on a commercial scale. Since the early 1960's the heterogeneous cocoa population in PNG includes types with a high level of resistance to the disease. Several resistant Trinitario clones were identified at the Lowlands Agricultural Experiment Station during the early stages of the initial epidemic (Anon 1963). During the 1960's and 1970's, the main avenue of disease control was to encourage propagation of resistant clones and hybrids derived from resistant clones. This resistance lowers the proportion of newly infected branches and also reduces the rate of invasion and subsequent sporulation of the fungus within infected branches (Keane 1981). Resistance of particular clones is durable, having been maintained since 1963 (Anon 1963). The presence of variation in field resistance among hybrid progenies (Tan 1981) has made selection for resistance to Vsd one of the major objectives in our cocoa breeding programme.

This paper reports the relative importance of additive and non-additive effects in genetic control of resistance to Vsd in a hybrid progeny trial. Disease severity was assessed by the number of infected branches per tree, total length of the infected branches, average depth of disease penetration within branches and percentages of infected and dead plants. The associations between these characters and plant vigour, and their implications in the selection programme, are discussed.

Materials and methods

Six Trinitario female parents were crossed with nine Amazonian male parents in all combinations. The resulting 54 F_1

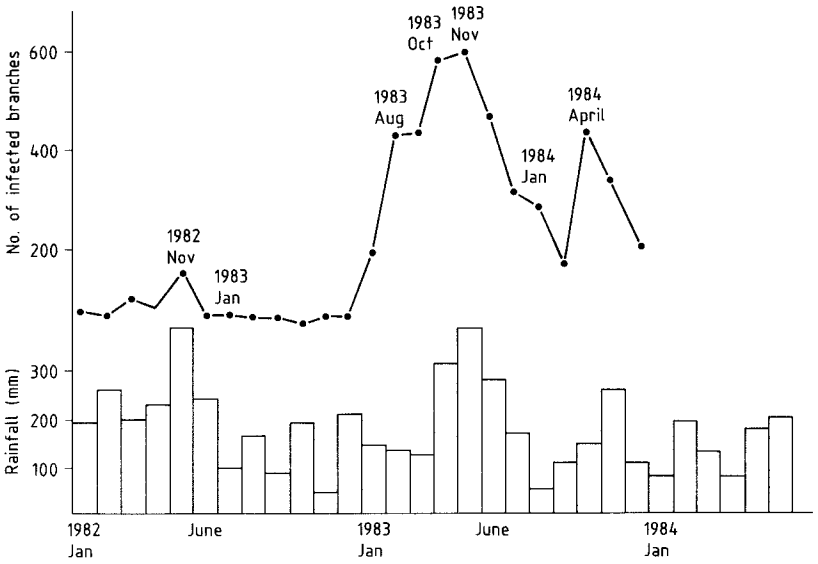

Fig. 1. Monthly rainfall from January 1982 to June 1984 and number of infected branches in hybrid progeny trial recorded monthly from July 1982 to June 1984

hybrid progenies were planted in the field in a random complete block design with four replications. Each plot consisted of 16 plants spaced in 4 m squares, giving a density of 625 trees per hectare.

While *O. theobromae* has been cultured on agar medium (Varghese et al. 1981) and in 50% coconut water (Musa 1983), it has not been possible to induce sporulation in axenic culture. Sporulation has been induced only in dual culture with cocoa callus (Prior 1982), but the technique was too difficult to use in routine screening for Vsd resistance (Prior 1985). Currently, use of natural infection in the field is the only way to assess resistance to Vsd. To ensure an adequate source of inoculum during the period of disease assessment, the progeny trial described adjoined two blocks heavily infected by Vsd that was deliberately left untreated.

Records of Vsd were carried out bi-weekly on each plant, starting 7 months after field planting for two years. This growth period is considered the most crucial for disease development and damage to plants. Vsd can easily be detected on plants by the characteristic yellowing of leaves on the second or third flush behind the growing tip (Keane et al. 1972). Measurement of the extent of Vsd infection was made by splitting infected shoots. Browning of the cambium and the streaked discolouration of woody vascular tissue is typical of Vsd and is a good diagnostic character. It has been shown that the pathogen grows in the xylem vessels for a few centimetres beyond the region of discoloured xylem (Keane et al. 1972). The extent of each infection and the total number of infected branches were recorded for each plant. The average extent of disease penetration was calculated from the total length of infection in branches divided by the total number of infected branches. The numbers of infected and dead plants for each plot were expressed as a percentage of the total.

Results were analysed for combining abilities on plot mean basis according to the methods of Comstock and Robinson (1952) and Simmonds (1979). According to Kempthorne (1957), σ_f^2 and σ_m^2 (variances due to female and male) both estimate ¼ $\sigma_A{}^2$ (additive genetic variance) and σ_{fxm}^2 estimates ¼ σ_D^2 (dominance genetic variance), assuming that epistatic variance is negligible.

Results and discussion

Figure 1 shows the relationship between rainfall data and the incidence of Vsd recorded in the trial. There was a highly positive correlation (r) ($r=0.60$, $P<0.01$) between monthly rainfall for the period July 1982 to December 1983 and the number of Vsd infected branches recorded monthly, starting 6 months later, i.e., from January 1983 to June 1984. This supports previous evidence (Keane 1981) that infection severity is associated with monthly rainfall and that the disease has a 3–6 month incubation period (Keane et al. 1972; Keane 1981). According to Keane et al. (1972), basidiospores of *O. theobromae* are released at night only after fruiting bodies have been moistened by rain in the late afternoon or early evening. Also, free water is required for spore germination and infection on leaves.

Highly significant correlation coefficients ($r=0.50$–0.70, $P<0.01$) were obtained for the number of infected branches of the progeny means among four replications. This indicated that not only was inoculum of *O. theobromae* uniformly distributed over the area of the trial, but also that the resistant progenies were consistently differentiated from susceptible progenies over all replications. Mean values of the five characters used for assessing resistance to Vsd are given in Table 1. Over 70% of plants were infected in the most susceptible progenies (Fig. 2 d). The results provided further evidence that adequate inoculum of *O. theobromae* was present in the trial area.

Analysis of variance showed that differences due to general combining abilities (GCA) for both Trinitario female parents and Amazonian male parents were sig-

Table 1. Combining ability analysis and experimental means for five characters measured on Vsd infected hybrid progenies, on plot mean basis

Source of variance	df	No. of infected branches	Total length of infected branches cm	Avg. length of disease penetration cm	% of infected trees	% of dead trees
General combining ability (female)	5	1,342.8**	2,294**	157.3**	1,854.1**	143.8*
General combining ability (male)	8	1,544.6**	3,059**	94.9*	1,555.1**	116.4*
Specific combining ability (F×M)	40	144.8	252	41.7	154.4	105.7**
Error	159	160.7	251	44.7	203.4	57.1
Expt. mean ± SE		24.0±8.9	951.7±354.6	39.6±4.7	45.7±10.0	8.8±5.3

*, ** Significant at the 5% and 1% levels of probability, respectively

Table 2. Variance component analysis for five characters measured on Vsd infected hybrid progenies

Variance components	No. of infected branches	Total length of infected branches	Avg. length of disease penetration	% infected plants	% dead plants
σ_f^2	33.27± 19.95[a]	56.72± 34.09	3.21±2.34	47.21± 27.54	1.05± 2.22
σ_m^2	58.32± 28.80[b]	116.95± 57.04[b]	2.21±1.80	58.36± 29.01[b]	0.44± 2.37
σ_{fxm}^2	0[c]	0.25± 15.42	0	0	12.15± 5.98[b]
σ_A^2	194.72±101.56	375.12±192.84	10.37±8.0	216.28±113.77	2.69± 9.24
σ_D^2	0	1.00± 61.68	0	0	48.60±23.92[b]

[a] SE
[b] Variance components are greater than twice the SE
[c] All negative estimates were treated as zero

nificant for all characters (Table 1). Specific combining ability (SCA) was highly significant only for the percentage of dead plants. Variance component analysis (Table 2) revealed that gene effects were predominantly additive for all characters used for assessing resistance to Vsd, the exception again being that of percentage of dead plants where a substantial non-additive gene effect was evident. Plots (Fig. 2) of the observed data of progenies against the predicted values from parental GCA for various characters support this conclusion. In such plots, a high correlation coefficient indicates that GCA accounts for a large portion of the differences between crosses, whereas a small r value and greater scatter about the line indicates the importance of SCA (Simmonds 1979). Although a moderate value of r was obtained for the average depth of disease penetration, the fact that only GCA mean square was significant indicated that GCA was more important than SCA. The presence of large additive gene effects for most of the characters associated with field resistance to Vsd indicates that selection for hybrid progenies resistant to the disease must be effective in the present breeding population. Furthermore, a highly positive Spearman's rank correlation coefficient ($r_s = 1.0$) for the number of infected branches was obtained between the six Trinitario parental clones in a previous clone trial (unpublished data from Lowlands Agricultural Experiment Station) and the estimates of GCA effects on the same set of clones were used as female parents in the present trial. These results provide further evidence that GCA is a major genetic component of field resistance to Vsd, and that resistance of parental material provides a reliable estimate of the relative resistance breeding values of the parents in hybrid progeny trials. In other words, hybrid progenies of resistant parents are also expected to be resistant.

However, Table 2 also shows that large standard errors were associated with the genetic variance components for most parameters. Comstock and Moll (1963) discussed the problem of poor precision of variance components, which was frequently encountered in studies of quantitative genetics. Nevertheless, it

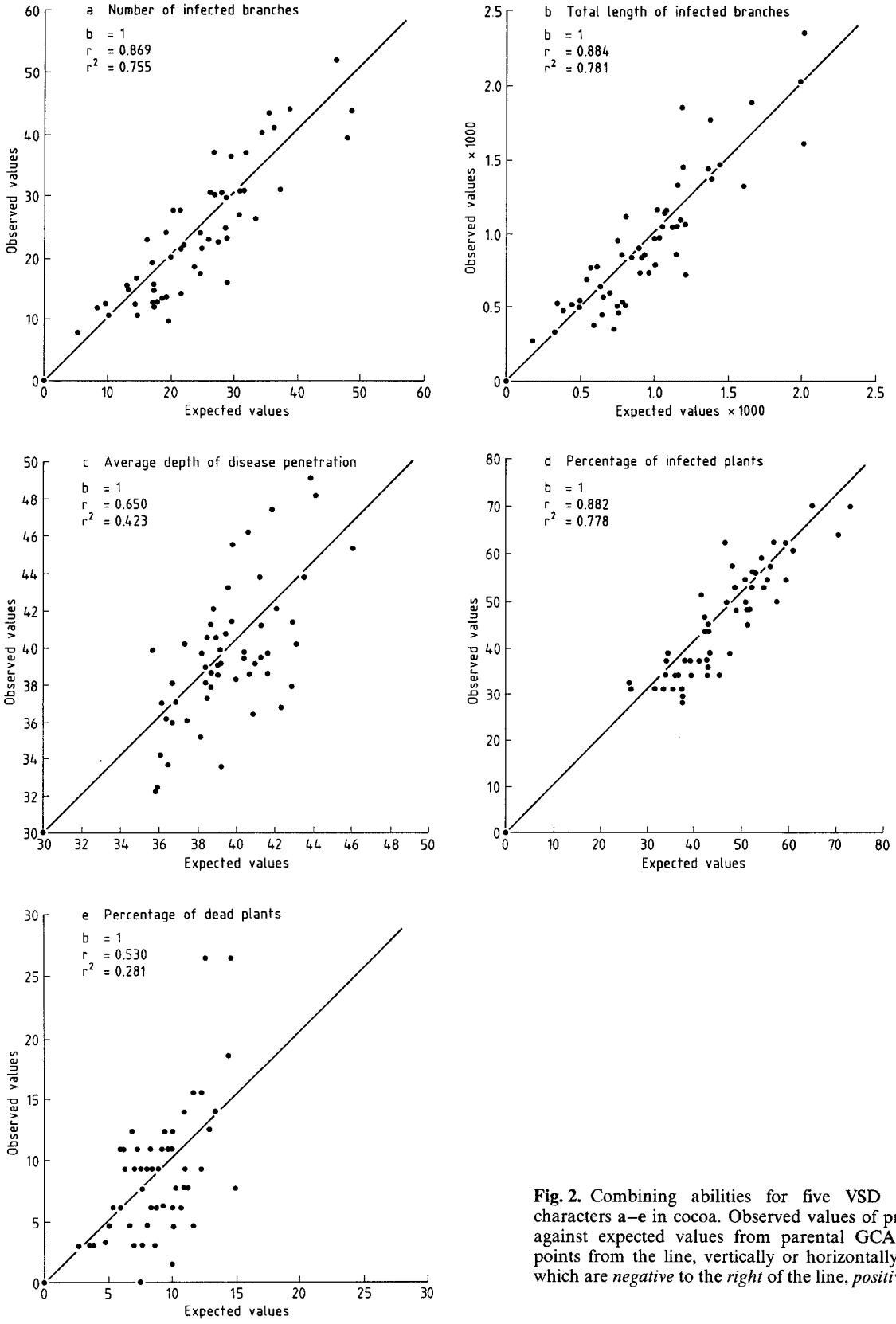

Fig. 2. Combining abilities for five VSD disease related characters **a–e** in cocoa. Observed values of progenies plotted against expected values from parental GCA. Deviations of points from the line, vertically or horizontally measure SCA, which are *negative* to the *right* of the line, *positive* to the *left*

does indicate that the estimates of variance components for male parents were more precise than those for female parents in the present experiment.

The number of infected branches and the total length of disease penetration in infected branches were highly correlated ($r=0.97$, $P<0.01$), suggesting that either character could be used as a parameter to determine disease resistance. Counting the number of infected branches requires less time and is an easier method than measuring both the length of infected branches and the average depth of disease penetration. However, in certain circumstances the number of infected branches could be misleading in disease assessment. Field observations revealed that a single infection in some susceptible progenies could penetrate rapidly to the main stem and consequently kill the entire plant. On the other hand, some progenies tolerated infections that were confined to the branch tips. Moreover, the number of infected branches can be confounded by plant vigour, as shown by the close association between the two characters (Table 3). Some vigorous plants with an abundance of new flush leaves are more prone to infection, but the vigorous growth habit of the plant could also rapidly replace the branches lost to Vsd. Average depth of penetration of Vsd was shown to be independent of plant vigour (Table 3) and appeared to be a reliable parameter for assessing resistance.

Possible mechanisms of resistance to Vsd have been investigated by Prior (1979). These have included the inhibitory effects of leaf exudates on spore germination, the reaction of epidermal cells to fungal penetration and some anatomical features of resistant plants, but none of these factors were correlated with resistance. Different depths of penetration obtained among hybrid progenies in the present study indicated that resistance is caused by inhibition of fungal growth through the vascular system of the host plants. It is possible that anti-microbial compounds in the xylem may contribute to resistance. If so, the identification and quantitative analysis of these compounds might provide an efficient method of predicting resistance to Vsd.

Although the precise mechanism of resistance is not understood and there have been no detailed studies of the virulence of a wide range of isolates of *O. theobromae*, it is suggested that this resistance is "horizontal resistance", in the terminology of Vanderplank (1963). It has not been overcome by virulence changes in the pathogen population despite wide exposure to the pathogen since 1963. It is a form of partial resistance which inhibits at least two aspects of the infection cycle, establishment of infection and rate of internal colonization of the plant. It reduces the rate of development of epidemics. And finally, it is largely inherited in an additive fashion and so is polygenic. In Papua New Guinea it has proved to be a very effective means of controlling vascular-streak dieback of cocoa.

Table 3. Correlation coefficients between the five Vsd characters and growth characters (df = 52)

Vsd characters	Growth characters		
	Girth diameter	Jorquette height	Vigor score
No. of infected branches	0.58**	−0.03	0.58**
Total length of infected branches	0.55**	−0.01	0.55**
Average length of disease penetration	0.13	0.12	0.11
Percentage of infected trees	0.61**	0.11	0.60**
Percentage of dead trees	0.21	0.40**	0.21

*, ** Significant at the 5% and 1% levels of probability, respectively

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References

Anon (1963) Annual report, Department of Agriculture Stock and Fisheries, Papua New Guinea, 1961–1963, p 49

Anon (1964) Annual report, Department of Agriculture Stock and Fisheries, Papua New Guinea, 1963–1964, p 41

Bridgland LA, Richardson JM, Edward LL (1966a) Dieback disease of cacao. Part 1. South Pac Plant 1:13–20, 28

Bridgland LA, Richardson JM, Edward IL (1966b) Dieback disease of cacao. Part 2. South Pac Plant 1:3–6, 22

Bridgland LA, Richardson JM, Edward IL (1967) Dieback disease of cacao. Part 3. South Pac Plant 1:9–11, 16–17

Comstock RE, Moll RH (1963) Genotype × environment interactions. In: Hanson WD, Robinson HF (eds) Statistical genetics and plant breeding. Nat Acad Sci. Nat Res Council 982, pp 164–194

Comstock RE, Robinson H (1952) Estimation of the average degree of dominance of genes. In: Gowan JW (ed) Heterosis. Iowa State College Press, Ames, pp 495–516

Keane PJ (1981) Epidemiology of vascular-streak dieback of cocoa. Ann Appl Biol 98:227–241

Keane PJ, Flentje NT, Lamb KP (1972) Investigation of vascular-streak dieback of cocoa in Papua New Guinea. Aust J Biol Sci 25:553–564

Kempthorne O (1957) An introduction to genetic statistics. Wiley and Sons, New York

Musa MJ (1983) Coconut water as culture medium for *Oncobasidium theobromae*. Malay Agric Res Dev Inst Res Bull 11:107–110

Prior C (1979) Resistance of cocoa to vascular-streak dieback disease. Ann Appl Biol 92:369–376

Prior C (1982) Basidiospore production by *Oncobasidium theobromae* in dual culture with cocoa callus culture. Tr Br Mycol Soc 78:571–574

Prior C (1985) Approaches to the control of disease of cocoa in Papua New Guinea. J Plant Prot Top 1:39–46

Simmonds NW (1979) Principles of crop improvement. Longman, New York

Talbot PHB, Keane PJ (1971) *Oncobasidium:* A new genus of tulasnelloid fungi. Aust J Bot 19:203–206

Tan GY (1981) Breeding for disease resistance to vascular-streak dieback, canker and black pod in hybrid cocoa. Proc 8th Int Cocoa Res Conf, Cartagena, Columbia, pp 731–734

Vanderplank JE (1963) Plant disease: epidemics and control. Academic Press, London New York

Varghese G, Zainae Abidin MA, Mainstone BJ (1981) Vascular-streak dieback of cocoa in Malaysia. II. Isolation and culture techniques of causal pathogen. Planter 57:576–580