Field evaluation of the protective capability of CSSV mild strain N1 against severe strain New Juaben (1A) isolate

L. A. A. OLLENNU & G. K. OWUSU
Cocoa Research Institute of Ghana, P. O. Box 8, New Tafo, Ghana

ABSTRACT

A completely randomised block design trial replicated five times was established at the Cocoa Research Institute of Ghana from 1987 to 2000 to evaluate the protective capability of cocoa swollen shoot badnavirus (CSSV) mild strain N1. Although 6 300 cocoa plants were inoculated with CSSV mild strain N1 on three different occasions, only 2 791 (44.3 %) showed visible symptoms. However, subsequent virobacterial agglutination (VBA) tests on the symptomless plants indicated that 835 (70.0 %) of them contained the mild virus. Forty months after superinoculation with CSSV severe strain 1A, the production of typical symptoms of severe 1A was suppressed in 1 154 (70.7 %) of the plants which showed visible symptoms of the mild strain. Furthermore, 840 (70.4 %) of the plants which remained symptomless after the repeated N1 inoculations also did not produce the severe symptoms of severe 1A. This suggests that these plants were infected with the mild strain but remained symptomless. Mild strain N1 reduced yield by 446 kg (8.9 %) while the combination of mild strain N1 and severe strain 1A caused 28.7 per cent (1 443 kg) yield loss within a 4-year period. The yield losses were significant among the treatments. These results suggest that mild strain N1 can protect a high proportion of cocoa trees against the devastating effects of CSSV severe 1A. The results are discussed with the control of swollen shoot disease in the West African sub-region with particular reference to Ghana.

Original scientific paper. Received 18 Jan 02; revised 20 Jan 03.

RÉSUMÉ

Ollennu, L. A. A. & Owusu, G. K.: *Evaluation au champs de la capabilité protectrice de CSSV souche bénigne N1 contre la souche grave isolat New Juaben (1A).* Un essai du dessin de bloc complètement choisi au hasard réparti cinq fois était établi à l'Institut de Recherche du Cacao au Ghana de 1987 à 2000 pour évaluer la capabilité protectrice de CSSV souche bénigne N1. Malgré l'inoculation de 6,300 plantes de cacao avec CSSV souche bénigne N1 à trois différentes occasions, seulement 2,791 (44.3 %) montraient les symptômes visibles. Toutefois, les essais d'agglutination virobactérienne (AVB) suivants sur les plantes sans symptômes indiquaient que 835 (70.0 %) d'elles contenaient les souches bénignes. Quarante mois après la superinoculation avec CSSV grave1A, la production de symptômes typiques du grave 1A comme réprimée en 1154 (70.7 %) de plantes qui montraient des symptômes visibles de la souche bénigne. De plus, 840 (70.4 %) des plantes qui restaient sans symptômes après les inoculations de N1 répété aussi n'ont pas produit les symptômes sévères du grave 1A. Ceci suggère que ces plantes étaient infectées avec la souche bénigne mais restaient sans symptômes. La souche bénigne N1 reduisait le rendement par 446 kg (8.9 %) alors que la combinaison de la souche bénigne N1 et la souche grave 1A provoquaient 28.7 % (1,443 kg) de perte du rendement en une période de quatre ans. Les pertes du rendement étaient considérables parmi les traitements. Ces résultats suggèrent que la souche bénigne N1 pourrait conférer une protection à unè proportion élevée de cacaoyers contre les effets ravageurs de CSSV grave 1A. Les résultats sont discutés en rapport avec le contrôle de la maladie de virose du cacaoyer dans la sous-région de l'Afrique de l'Ouest en faisant référence au Ghana en particulier.

Introduction

The concept of mild strain protection was first discovered by McKinney (1929). The phenomenon occurs when a plant virus strain that has first infected a host plant inhibits multiplication of or symptom induction by another strain of the same or related virus which later invades the plant. After these earlier discoveries on the concept of mild strain protection, Crowdy & Posnette (1947) and Posnette & Todd (1951, 1955) showed that mild strains of CSSV could protect cocoa trees against severe strains. However, general opinion in the scientific world and official policy on CSSV control at the time did not permit further investigations on the practical implications of the subject (Ollennu, Owusu & Thresh, 1989).

Mild strain protection has been used to control citrus tristeza virus (CTV) in Brazil (Muller & Costa, 1977; Costa & Muller, 1980) and elsewhere (Fulton, 1986). Cross protection against tomato mosaic virus has been used in glasshouse crops in several countries including the UK and The Netherlands (Fletcher, 1978; Brunt, 1986) and Japan (Oshima, 1975). Cross protection has also been used to control papaya ring spot potyvirus (Yeh *et al.*, 1988) and Zucchini yellow mosaic virus (Walkey *et al.*, 1992).

These developments in the use of mild strain protection in disease control in commercial crops, especially citrus tristeza in citrus (Muller & Costa, 1977), have rekindled interest in the earlier studies on cocoa. Consequently, a programme was started in 1985 to re-appraise cross protection by CSSV mild strains (Owusu & Ollennu, 1987; Ollennu & Owusu, 1989; Ollennu, Hughes & Owusu, 1996).

This paper reports on recent field evaluation (1987-2000) of CSSV mild strain N1 and discusses the implications of the result for controlling swollen shoot disease.

Materials and methods

Virus strains

CSSV mild strain N1 was isolated from symptomless trees within severe CSSV outbreaks (Ollennu *et al.*, 1996). It induces mild symptoms which disappear within 3 to 5 days. It is believed to be a mild isolate of the severe New Juaben isolate (1A).

The severe New Juaben isolate (1A) induces severe leaf symptoms, stem/root swellings, drastic yield losses, and eventually kills the plant (Posnette, 1941). CSSV severe 1A is the standard virulent isolate used for all CSSV studies at the Cocoa Research Institute of Ghana (CRIG).

Four female parents from the seed gardens supplying farmers with planting materials in the Eastern Region were selected for the field evaluation of CSSV mild strain N1 (Table 1). Each female parent was crossed with the pollen of Amelonado and with one of several possible Upper Amazon pollen parents to produce Inter-Amazon progenies likely to be more resistant to virus infection than the Series II hybrids (Lockwood, 1981). Seeds produced by hand pollination were sown in fresh top soil in individual poly bags in a nursery and kept up to 6 months before planting in the field. The seedlings were

TABLE 1

Source of the Female Parents of the Cocoa Progenies Planted in the Trial

T85/799 (Apedwa seed garden)	*T85/799 (Apedwa seed garden)*	*T85/799 (Apedwa seed garden)*	*T85/799 (Apedwa seed garden)*
× T65/326	× T17/524	× IMC 60	× T17/524
× T79/501	× IMC 60	× T65/238	× T87/1312
× Pa7	× T60/887	× Amelonado	× IMC 60
× T65/238	× T65/238		× Amelonado
× S84; E 104/90	× Amelonado		
× Amelonado			

transplanted into the field at CRIG at the onset of the main rains in May/June 1987. The trees were planted out in a completely randomised block design with five replications. Each plot consisted of 35 trees spaced at 1.5 m triangular. The cocoa was grown under heavy shade of mixed forest trees.

The plot was surrounded by two-row perimeter guard trees and one-row internal guard between plots. Missing cocoa trees were replaced for up to 2 years after planting. Insect damage was controlled with insecticides, but neither fungicides nor fertilizers were applied.

The treatments applied to the seedlings were as follows: mild-strain inoculated; mild-strain inoculated and later superinoculated with the CSSV severe 1A isolate; and healthy control. A total of 350 seedlings of each of the progenies were inoculated with the CSSV mild strain N1. Two years after the seedlings were planted in the field (seedlings were well established by then), they were inoculated by patch grafts with the CSSV mild strain N1. The hybrid seedlings were given a total of three separate inoculations over a period of 3 years to establish the mild strain. To assess the level of infection, the cocoa trees were coppiced after the third mild strain inoculation to induce symptom expression in the regrowth as described by Posnette (1951) and Legg *et al.* (1984). The trees were superinoculated with CSSV severe strain 1A 6 years after establishment. One hundred and seventy-five healthy trees were also inoculated with severe 1A from the same source at the time of the superinoculation. Samples of symptomless trees as well as trees which had produced symptoms were indexed by using the VBA test (Hughes & Ollennu, 1993) before superinoculation with CSSV severe 1A.

The experimental trees were physically examined every month for symptom expression. However, 40 months after the superinoculation, the test trees had grown so tall due to the heavy shade and close planting of 5 m triangular. This was discontinued because symptom recording was very difficult. The population of the experimental trees was reduced to the recommended cocoa planting distance of 3 m × 3 m spacing, and some of the shade trees were killed by poisoning with gallon two (a tree poison). Yield data for 4 years were then recorded after reducing the plant population to 3 m × 3 m planting space distance.

Results

Inoculation with CSSV mild strain N1

By January 1991, only 2 010 out of the 6 300 trees inoculated with CSSV mild strain N1 had shown symptoms after the three inoculations. The inoculated trees which were still symptomless were therefore coppiced in February 1991 to enhance the detection of missed infections. Consequently, 781 more infections were detected after the cut back in 1991 (Table 2). The overall infection (assessed by symptom expression) recorded was 44.3 per cent of the total number of trees inoculated. Infection among the different progenies ranged from 21.7 to 84.0 per cent (Fig. 1). The highest infection rates were recorded in hybrids from crosses between Amelonado and Upper Amazon, while crosses with IMC60 had the least. With the exception of four progenies involving Amelonado, percentage infection in the remaining 14 progenies was below 50 per cent (Table 2).

Serological detection of CSSV mild strain N1

Despite the three inoculations with CSSV mild strain N1, 55.7 per cent of the trees remained symptomless, indicating that the plants were either not infected or symptoms were missed during the visual inspections. Samples of leaves were taken randomly from inoculated, symptomless trees as well as from trees that had produced symptoms. These were indexed by using enzyme-linked immunosorbent assay (ELISA) and the VBA test.

ELISA (both direct and indirect) did not detect CSSV mild strain N1 in symptomless plants and in plants which had previously shown symptoms. Table 3 shows the results of the VBA test carried out on the healthy and CSSV N1-inoculated plants.

TABLE 2

Number of Trees Showing Symptoms after Each Inoculation with CSSV Mild Strain N1

	Number of trees showing symptoms				
Cocoa hybrid	*1989*	*1990*	*1991**	*1992*	*Total (percent)*
T85/799 × Pa7/808	49	2	86	4	141 (40.3)**
T85/799 × T65/238	44	10	69	8	131 (37.4)
T85/799 × T65/326	32	12	86	2	132 (37.8)
T85/799 × T79/501	31	7	52	4	94 (26.9)
T63/967 × T17/524	117	22	30	0	169 (48.3)
T63/967 × T65/238	96	19	47	6	168 (48.0)
T63/967 × Amelonado	98	16	29	4	147 (42.9)
T85/799 × Amelonado	228	35	2	1	266 (76.0)
T79/467 × T17/524	47	7	62	4	119 (34.0)
T85/799 × S85/E104/90***	235	46	8	5	294 (84.0)
T79/467 × T87/1312	41	9	66	4	120 (34.3)
T79/467 × IMC60/112	29	9	44	5	87 (24.9)
T79/467 × Amelonado	47	1	29	5	82 (23.4)
T63/967 × T69/887	110	15	13	2	140 (40.0)
T63/971 × T65/238	44	9	44	3	100 (28.6)
T63/971 × IMC60/112	32	8	30	6	76 (21.7)
T63/971 × Amelonado	200	49	2	4	255 (72.9)
T85/799 × S84/E104/90	231	23	11	4	269 (76.9)
Total	1711	299	710	71	2791 (44.3)

*Trees coppied to induce new shoots on which the symptoms were easily observed.
**Figures in parenthesis represent percentage of inoculated trees showing symptoms.
*** S84/E104/90 is an Amelonado cocoa. Total number of seedlings inoculated/progeny = 350

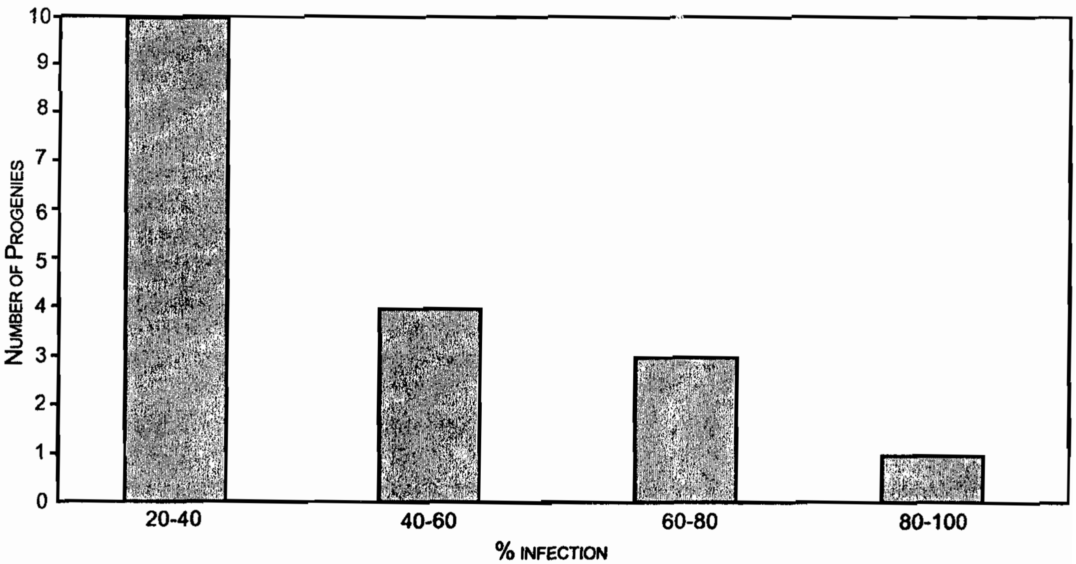

Fig. 1. Histogram showing the percentage of infected trees after three mild strain N1 inoculations.

The VBA results for all the healthy control trees were negative while all the trees which had shown symptoms recorded positive results. Seventy per cent of the symptomless inoculated trees which were tested also had positive VBA results, suggesting that these symptomless trees contained the virus.

Protection by mild strain N1

Table 4 shows the reaction of plants previously inoculated with mild strain N1 to superinoculation with CSSV severe 1A 40 months after the superinoculation. Cocoa swollen shoot virus mild strain N1 apparently protected 70.7 per cent (58.9 - 81.3 %) of the plants which previously produced mild symptoms. The reaction of the remaining

TABLE 3

Detection of CSSV Mild Strain N1 in Inoculated Symptomless Trees and those which had Produced Symptoms Using Virobacterial Agglutination (VBA) Test

Cocoa trees	*No. of trees tested*	*No. positive by VBA*	*Mean time of agglutination (s)*
Healthy (uninoculated control)	52	0	220
N1-inoculated (symptomless)	155	109	28
N1-inoculated (symptoms exhibited)	123	123	31

TABLE 4

Reaction of CSSV Mild Strain N1-Protected Cocoa Trees 40 Months After Superinoculation with CSSV Severe 1A

Cocoa hybrid	*Plants showing mild symptoms before superinoculation*	*Apparent protection (%)*	*Plants with no symptoms before superinoculation*	*Apparent protection (%)*	*Total protection (%)*
T85/799 × Pa7/808	39/60*	65.0	56/100	56.0	59.4
T85/799 × T65/238	47/69	68.1	65/93	69.9	67.9
T985/799 × T65/326	79/100	79.0	42/67	62.7	72.5
T85/799 × T79/501	48/73	65.8	47/76	61.8	63.8
T63/967 × T17/524	78/96	81.3	48/66	72.7	77.8
T63/967 × T65/238	71/90	78.9	52/79	65.8	72.3
T63/967 × Amelonado	63/92	68.5	26/52	50.0	61.8
T85/799 × Amelonado	128/164	78.0	NA	NA	78.0
T79/467 × T17/524	50/75	66.7	73/89	82.0	75.0
T85/799 × S84/E104/90	113/168	67.3	NA	NA	67.3
T79/467 × T87/1312	38/60	63.3	46/77	59.7	61.3
T79/467 × IMC60/112	29/50	58.0	65/98	66.3	63.5
T79/467 × Amelonado	29/48	60.4	79/111	71.2	67.9
T63/967 × T60/887	53/82	64.6	58/73	79.5	71.6
T63/971 × T65/238	36/54	66.7	85/102	83.3	77.6
T63/971 × IMC60/112	30/38	78.9	98/111	83.1	85.9
T63/971 × Amelonado	105/152	69.1	NA	NA	69.1
T85/799 × S84/E104/90	118/162	72.8	NA	NA	72.8
Total	1154/1633	70.7	840/1194	70.4	70.5

* Numerator – Number of plants protected
Denominator – Number of plants superinoculated
NA - No plants available

N1-inoculated plants which were symptomless but were also superinoculated was similar. About 70 per cent (56.0 and 83.3 %) of these plants were symptomless 40 months after the superinoculation (Table 4). Of the 175 seedlings inoculated with only severe 1A strain, 98.3 per cent produced typical symptoms of CSSV severe 1A, indicating that the CSSV severe 1A used for the superinoculation was highly infectious. The apparent protection afforded by CSSV mild strain N1 against the virulent effects of CSSV severe 1A amongst the plants superinoculated ranged from 59.4 per cent in progeny T85/799 × Pa7/808 to 85.9 per cent in progeny T63/971 × IMC60/112 (Table 4).

Yield

Table 5 summarizes the yields for the different cocoa progenies. In the 4 years that yields were recorded, the healthy plants had significantly higher yield than the two virus treatments. The yield of the trees inoculated with mild strain only (M treatment) was also significantly higher than those inoculated with mild and severe strains (M + S treatment). The 4-year cumulative yield of the M-treatment was significantly less (8.9 %) than the control, but 21.8 per cent significantly higher than the M + S treatment. This means that mild strain N1 reduced yield by 8.9 per cent while the combination of N1 and severe 1A caused a yield loss of 28.7 per cent.

Table 6 shows the mean yield of dry cocoa beans of the different progenies for the 4 years. The differences among the progenies in the 1st and 3rd years were significant, and also the total yield for the 4 years. The progeny T85/799 × S84/E104/90 (Amelonado) had the highest yield followed by T85/799 × Pa7/808, while T79/467 × T87/1312 and T63/971 × IMC60/112 crosses were generally low yielding.

Discussion

Sequira (1984) stated that very efficient inoculation with mild strain is necessary for a high level of cross protection. In the gauzehouse, graft inoculation of seedlings with mild strain N1 was more efficient than mealybug transmission to seedlings (Ollennu *et al.*, 1996). However, three graft inoculations with mild strain N1 in this study induced symptom expression in less than 50 per cent of the seedlings inoculated. This low rate of transmission was therefore unexpected. However, the VBA test could detect mild strain N1 from 70.3 per cent of the 155 symptomless N1-inoculated plants tested. This indicated that many of the plants which remained symptomless after the inoculations were infected but no symptoms were induced, or that the transient symptom was missed by the recorders. The characteristic red-vein banding induced by CSSV mild strain N1 is transient and usually lasts between 3 and 5 days (Ollennu *et al.*, 1996). In addition, the symptoms may not occur again in the life span of some plants, depending on the CSSV mild strains involved (Posnette, 1947).

Visual examination for CSSV infection has such limitations even in its use to detect infection by CSSV severe isolates in outbreaks of swollen

TABLE 5

Mean Yield of Dry Cocoa Beans (kg ha^{-1}) of the Treatments

Virus treatment	Years				Cumulative
	1997/98	1998/99	1999/2000	2000/2001	1997/98-2000/01
Healthy	613.9	1123.7	1762.8	1550.8	5020.2
Mild only	522.3	904.8	1648.2	1498.4	4573.7
Mild and severe	464.9	751.6	1249.4	1110.8	3576.7
Sed (212 d.f)	8.27	15.51	21.31	21.76	50.70

TABLE 6

Mean Yield of Dry Cocoa Beans (kg ha^{-1}) of the 18 Progenies

Virus treatment	*Years* 1997/98	1998/99	1999/2000	2000/2001	*Cumulative* 1997/98-2000/01
T85/799 × Pa7/808	692.7	1087.3	2242.3	1616.7	5637.7
T85/799 × T65/238	512.7	984.0	1464.3	1479.7	4440.7
T85/799 × T65/326	652.7	972.7	1775.7	1513.0	4914.0
T85/799 × T79/501	524.3	874.0	1476.3	1468.0	4342.7
T63/967 × T17/524	580.0	774.7	1215.0	1361.0	3930.7
T63/967 × T65/238	594.3	926.7	1570.0	1389.0	4447.0
T63/967 × Amelonado	454.3	781.3	1107.7	1195.7	3554.0
T85/799 × Amelonado	709.7	1351.0	1764.3	1764.3	5589.0
T79/467 × T17/524	448.7	893.0	1673.0	1280.3	4295.0
T85/799 × S84/E104/90	571.0	1167.3	2258.3	1735.3	5730.7
T79/467 × T87/1312	319.0	664.3	1024.3	902.0	2909.7
T79/467 × IMC60/112	363.3	763.3	1168.7	1192.3	3487.7
T79/467 × Amelonado	506.7	939.3	1392.7	1345.0	4183.7
T63/967 × T60/887	487.7	784.0	1200.0	1218.3	3734.0
T63/971 × T65/238	639.7	927.7	1701.7	1303.0	4592.0
T63/971 × IMC60/112	460.7	617.0	1275.7	1276.3	3619.7
T63/971 × Amelonado	624.7	1089.3	1678.7	1396.3	4755.7
T85/799 × S84/E104/90	464.3	1083.3	1970.3	1524.0	5042.0
Sed (212 d.f)	20.24	NS	52.20	NS	124.20

shoot disease (Legg *et al.*, 1984; Ollennu, 1989). Legg *et al.* (1984) showed that 20 per cent of all infections were detected by routine inspection due to differences in efficiency of the inspecting teams, and the effect of time of day and period of year for the inspections. Additionally, a significant proportion of these symptomless plants (70.4 %) was apparently protected. This percentage protection was similar to the 70.7 per cent protection afforded to those plants which showed the mild symptoms after the N1 inoculations before the superinoculation. Owusu *et al.* (1996) observed the same phenomenon when they worked with mild strains of CSSV.

ELISA did not detect mild strain N1 even in plants that showed red-vein banding. This agrees with the finding of Sagemann *et al.* (1985). They could only detect some CSSV mild strains with difficulty, while other mild strains were not detected at all, using an antiserum to CSSV severe 1A.

In general, the number of Upper Amazon × Amelonado hybrids which expressed the mild strain symptom of transient red-vein banding was higher than that for Inter-Amazon hybrids. Legg & Lockwood (1981) observed that the Inter-Amazon hybrids appeared to offer some tolerance to infection by CSSV. This may cause a delay in symptom expression in these hybrids (Ollennu, 1989). The apparent differences in the expression of symptoms may also be due to low concentration of the mild strain in the Inter-Amazon hybrids. The difference in the reaction of the two hybrid types to inoculation with CSSV mild strains may indicate the susceptibility of the cross-protected hybrids to super-infection by the severe isolates.

The CSSV mild strain N1 apparently protected 70.5 per cent of all the N1-inoculated plants superinoculated with CSSV severe 1A. Posnette & Todd (1955), in a 3-year cross-protection trial in the field using Amelonado cultivar, reported 92 per cent protection against CSSV severe 1A. The difference between their results and those for this study might be due to the method of superinoculation. Posnette & Todd (1955) planted out the mild strain-inoculated trees in the field for natural infection. In this study, every cocoa tree previously inoculated with mild strain N1 was superinoculated with CSSV severe 1A isolate by grafting. The method of superinoculation may have been too drastic or the protecting inoculum level was too low.

Frazer, Long & Cox (1968) and Costa & Muller (1980) observed that with mild isolates of citrus tristeza virus (CTV), protection broke down if budding was used for superinoculation. However, when citrus trees were protected with mild CTV isolates and exposed to natural infection in the field, protection did not break down. They concluded that a level of protection adequate for practical control might not be detected by an inoculation method such as grafting that may overwhelm the protective mechanism. Ollennu (2001) observed the same phenomenon when working with CSSV mild strain N1 in the gauzehouse. More plants were protected by mild strain N1 when superinoculation was by the mealybug vector than when superinoculation was by grafting. In this study, T63/971 × IMC60/112 was the best protected hybrid and this, perhaps, confirms existing knowledge that the IMCs offer a high tolerance to CSSV infection (Legg & Lockwood, 1981).

The effect of the CSSV mild strain N1 on the 4-year cumulative yield was 8.9 per cent compared with 28.7 per cent of the combination of the mild and severe strains. The T85/799 × Pa7/808 progeny had the highest yield. Adomako & Adu-Ampomah (2000), on evaluating hybrids between Upper Amazon cocoa selections in Ghana, recorded this hybrid among the hybrids which had high yields. However, in this study, crosses with IMCs were among the least-yielding hybrids. This suggests that it is imperative to improve the yield performance of the IMCs before they could be effectively used in CSSV control. The 28.7 per cent reduction in yield by the M + S treatment was similar to what Adomako *et al.* (2001) reported for cocoa hybrids infected by CSSV severe strains for a 12-year period. Clearly, once the protection broke down, the virulent effect of the severe strain was fully expressed without any hindrance from the mild strain.

Posnette & Todd (1955) estimated that if the loss of yield due to CSSV mild strains is up to 20 per cent, then the mild strain could be considered for cross protection. In this study, CSSV mild strain N1 protected about 70 per cent of the trees against the devastating effects of CSSV severe 1A and also reduced yield by about 9 per cent. CSSV N1 could, therefore, be used to control swollen shoot disease in Ghana based on the recommendations by Posnette & Todd (1955).

The results reported in this study and other recent findings on the subject indicate that mild strain N1 can protect a high proportion of cocoa trees against the devastating effects of CSSV severe 1A. Other suitable CSSV mild strains have also provided some protection in preliminary studies, although not as high as that of strain N1 (Ollennu *et al.*, 1996). Although cross protection does not always provide 100 per cent protection in crops, it may be preferred to previous control measures or where attempts at control have been unsuccessful, as in the use of cross protection for the control of tomato mosaic virus disease in glasshouse tomatoes in Europe (Fletcher, 1978), or in the control of papaya ringspot virus by attenuated mutants in Taiwan (Yeh *et al.*, 1988). Additional measures such as crop sanitation are then applied to enhance the protection provided by the mild strain.

In the endemic area in the Eastern Region where control of swollen shoot disease has virtually been abandoned, cross protection could be an alternative method of control. This could be combined with agronomic practices such as using CSSV-immune crops to isolate new cocoa plantings from old establishment. In addition, any protected plant that breaks down would have to be rouged immediately and replaced.

Conclusion

The results reported in this study suggest that mild strain cross protection can be used to control cocoa swollen shoot disease. However, it is unlikely that mild strain protection would be appropriate for use throughout Ghana. An overall small reduction in yield could be justified in the endemic areas where the risk of total yield loss is great. However, in areas such as Ashanti, Western and Brong Ahafo Regions where the risk of infection is small, there can be little justification in reducing yield through wide-spread dissemination of mild strains.

Acknowledgement

The technical assistance for the maintenance and recording of the trials is acknowledged as well as the statistical analysis by Dr F. K. Oppong. This paper is published with the permission of the Executive Director of CRIG.

REFERENCES

Adomako, B. & Adu-Ampomah, Y. (2000) Reflections on the yield of Upper Amazon cocoa with reference to breeding for cocoa swollen shoot virus resistant varieties. *Cocoa Growers' Bull.* **52,** 33-46.

Adomako, B., Adu-Ampomah, Y., Owusu, G. K. & Ollennu, L. A. A. (2001) Effect of cocoa swollen shoot virus on the yield of mature bearing trees of Upper Amazon cocoa hybrids in Ghana. *J. Ghana Sci. Ass.* **3,** 1-6.

Brunt, A. A. (1986) Tomato mosaic virus. In *The plant viruses* Vol. 2 (ed. M. H. V. van Genmortel and H. Frankael-Convot). Plenum.

Costa, A. S. & Muller, G. W. (1980) Tristeza control by cross protection. *Pl. Dis.* **64,** 538-541.

Crowdy, S. H. & Posnette, A. F. (1947) Virus diseases of cacao in West Africa II. Cross immunity experiments with viruses 1A, 1B and 1C. *Ann. appl. Biol.* **34,** 403-411.

Fletcher, J. T. (1978) The use of avirulent virus strain to protect plants against the effects of virulent strains. *Ann. appl. Biol.* **89,** 110-114.

Frazer, L. R., Long, K. & Cox, J. (1968) Stem pitting of grape fruit – Field protection by the use of mild virus strains. *Proc. 4th Conf. Int. Org. Citrus Virol.* pp. 27-21.

Fulton, W. R. (1986) Practices and precautions in the use of cross protection for plant virus control. *A. Rev. Phytopath.* **24,** 67-81.

Hughes, J.d'A. & Ollennu, L. A. A. (1993) The virobacterial agglutination test as a rapid means of detecting cocoa swollen shoot virus. *Ann. appl. Biol.* **22,** 299-310.

Legg, J. T. & Lockwood, G. (1981) Resistance of cocoa swollen shoot virus in Ghana. I. Field trials. *Ann. appl. Biol.* **97,** 75-89.

Legg, J. T., Lovi, N. K., Ollennu, L. A. A., Owusu, G. K. & Thresh, J. M. (1984) Coppicing experiments on the spread and control of cocoa swollen shoot virus in Ghana. *Crop Prot.* **3,** 289-304.

Lockwood, G. (1981) Genetic aspect of resistance to cocoa swollen shoot virus in Ghana. *Ann. appl. Biol.* **98,** 131-141.

McKinney, H. N. (1929) Mosaic disease in the Canary Island, West Africa and Gibraltar. *J. agric. Res.* **39,** 557-578.

Muller, G. W. & Costa, A. S. (1977) Tristeza control in Brazil by preimmunization with mild strains. *Proc. int. Soc. citriculture* **3,** 289-304.

Ollennu, L. A. A. (1989) Physical methods for the detection of cocoa swollen shoot virus. *Proc. Cocoa Swollen Shoot Virus Wkshop* (ed. M. Manu and E. K. Tetteh) CRIG. pp. 22-23.

Ollennu, L. A. A. (2001) Some recent studies with mild strains of cocoa swollen shoot badnavirus (CSSV) in Ghana. An invited paper presented at the *XXXIV Congress Brasileiro de Fitopatologia e XI Congresso Latino-Americano de Fitopatologia, Estado de Sao Paulo, Brazil no periodo de 05 a 10 de agosto de* 2001.

Ollennu, L. A. A. & Owusu, G. K. (1989) Isolation and study of mild strains of cocoa swollen shoot virus for possible cross protection. *Proc. 4th int. Pl. Virus Epidemiol. Wkshop, Montpellier*, 1989, pp. 119-122.

Ollennu, L. A. A., Owusu, G. K. & Thresh, J. M. (1989) The control of cocoa swollen shoot disease in Ghana. *Cocoa Growers Bull.* **42,** 26-36.

Ollennu, L. A. A., Hughes, J.d'A. & Owusu, G. K. (1996) Mild strain cross-protection of cocoa against cocoa swollen shoot badnavirus. *Trop. Sci.* **36,** 116-128.

Oshima, N. (1975) The control of tomato mosaic disease with attenuated virus of tomato strain of TMV. *Rev. Pl. Prot. Res.* **8,** 126-135.

Owusu, G. K. & Ollennu, L. A. A. (1987) Cocoa swollen shoot disease: studies of mild virus strain protection. *Rep. Cocoa Res. Inst. Ghana.* 1985/86, pp. 79-80.

Owusu, G K., Ollennu, L. A. A. & Dzahini-Obitey, H. (1996) The prospects of mild strain cross-protection to control cocoa swollen shoot disease in Ghana. *Proc. 12th int. Cocoa Res. Conf., Salvador-Bahia, Brazil,* 17th – 23rd November, 1996. pp. 121-127.

Posnette, A. F. (1941) Swollen shoot virus disease of cacao. (Review of research work to November, 1940). *Trop. agric., Trinidad.* **18,** 87-90.

Posnette, A. F. (1947) Virus diseases of cacao in West Africa. I Cacao viruses 1A, 1B, 1C and 1D. *Ann. appl. Biol.,* **34,** 388-402.

Posnette, A. F. (1951) Virus research at the West African Cocoa Research Institute, Tafo, Gold Coast. *Trop. agric., Trinidad.* **28,** 133-142.

Posnette, A. F. & Todd, McA. (1951) Virus diseases of cacao in West Africa VIII. The search for virus resistant cacao. *Ann. appl. Biol.* **38,** 785-800.

Posnette, A. F. & Todd, McA. (1955) Virus diseases of cacao in West Africa IX. Strain variation and interference in virus 1A. *Ann. appl. Biol.* **43,** 433-453.

Sagemann, W., Lesemann, D-E., Paul, H. L., Adomako, D. & Owusu, G. K. (1985) Detection and comparison of some Ghanaian isolates of cacao swollen shoot virus (CSSV) by enzyme-linked immunosorbent assay (ELISA) and immunoelectron microscopy (IEM) using an antiserum to CSSV strain 1A. *Phytopath. Z.* **114**, 78-89.

Sequira, L. (1984) Cross protection and induced resistance: their potential for plant disease control. *Trends Biotech.* **2,** 25-29.

Walkey, D. G A., Lecoq, H., Collier, R. & Dobason, S. (1992) Studies on the control of zucchini yellow mosaic virus in courgettes by mild strain protection. *Pl. Path.* **41,** 762-771.

Yeh, S. D., Gonsalves, D., Wang, H. L., Namba, R. & Chiu, R. J. (1988) Control of papaya ring spot virus by cross protection. *Pl. Dis.* **72,** 375-380.