Spatiotemporal Spread of Cacao Swollen Shoot Virus Severe Strain 1A in Mixed Hybrid Cacao Pre-inoculated With Mild Strain N1

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Abstract

The spatiotemporal spread of cacao swollen shoot virus disease (CSSVD), which is caused by cacao swollen shoot virus (CSSV) severe strain 1A in mixed hybrid cacao pre-inoculated with CSSV mild strain N1 (CSSV-N1), was investigated during a field experiment from 2006 to 2017, at the Cocoa Research Institute of Ghana. The development of disease epidemics has been described by the use of statistical modeling. Protecting all cacao plants with CSSV-N1 reduced the rate of CSSV-1A symptom appearance by 43% ($P = 0.05$) compared with the nonprotected control and by 33% compared with plots where cacao plants in the outer three or five rows were protected with CSSV-N1. Similarly, creating the protective outer rings three or five rows deep reduced the rate of CSSV-1A symptoms by 14% ($P = 0.05$) compared with the nonprotected

control. CSSV-1A epidemics increased approximately 18% faster ($P = 0.05$) in transects oriented from the north and east compared with those oriented from the south and west. During the last 2 years of the study, CSSVD spread decreased significantly ($P = 0.05$) faster in plots where all test cacao plants were inoculated with CSSV-N1 compared with other treatments. The growth of cacao did not differ significantly among the treatments over the 9-year assessment period. Similarly, differences in the cumulative yield among the treatments over the 8-year assessment period were not significant.

Keywords: disease gradients, epidemiology, mild strain cross-protection, modeling, severe strain

Cocoa swollen shoot virus disease (CSSVD) is caused by a mealybug-transmitted double-stranded DNA virus (family: *Caulimoviridae*; genus: *Badnavirus*) possessing nonenveloped bacilliform particles (Lockhart 1990; Lot et al. 1991). Even though approximately 16 species of mealybugs (*Pseudococcidae*) are known to transmit the swollen shoot virus, *Formicococcus njalensis* (Laing) and *Planococcus citri* (Risso) are widely believed to be most important (Bigger 1981; Campbell 1983; Roivainen 1980). Symptoms of CSSVD include foliar chlorosis, root and stem swellings, shoot dieback, and death of plants (Posnette 1947, 1951). Swollen shoot disease has been a major constraint for cacao production in West Africa, particularly Ghana, where the disease has caused the destruction of more than 254 million cacao trees through the cutting-out campaign schemes initiated in 1946 (Cocoa Health and Extension Division of Ghana Cocoa Board, *unpublished*). In addition, millions of cacao trees have died naturally from the disease. Swollen shoot disease continues to spread to new areas in Ghana, Cote d'Ivoire, and Togo despite numerous interventions (Andres et al. 2017, 2018; Oro et al. 2012).

Mild strain cross-protection (MSCP) is an alternative control method considered for CSSVD in Ghana (Crowdy and Posnette 1947; Ollennu and Owusu 2002, 2003; Posnette and Todd 1955). Research of the possibility of using MSCP against severe cacao swollen shoot virus (CSSV) strains started in the 1940s at the Cocoa Research Institute of Ghana (Posnette 1947). It is predicted that if MSCP is recommended as an alternative CSSVD control measure, then mild strain-inoculated cacao seedlings would be supplied to farmers for planting in areas of mass infection, where control by eradication may no longer be a plausible option. One major constraint to the

smooth implementation of MSCP is the lack of rapid, cost-effective, and less laborious procedures for inoculating cacao beans or seedlings with mild strains on a commercial scale. In addition to a suitable inoculation method, any approach capable of limiting the number of cacao planting material required to be inoculated with mild strains without compromising protection against severe strains would be vital because of the aforementioned challenges.

Existing virus-infected cacao trees bordering newly planted cacao farms usually serve as sources of inoculum from which CSSV spreads gradually to new farms. Consequently, the incidence of CSSVD tends to be high around the periphery of existing outbreaks as mealybugs move and feed among interlocking canopy branches and spread the virus from infected to healthy cacao plants (Posnette 1943; Strickland 1950; Thresh et al. 1988a). Thresh (1958) recommended an investigation of the extent to which the spread of virulent strains could be checked by inoculating the apparently healthy cacao trees around virus outbreaks with closely related mild virus strains. In this way, the mild strain-inoculated cacao trees in the outer rows would serve as a barrier to prevent or at least delay the spread of the severe virus to the inner rows.

The CSSV mild strain N1 (CSSV-N1) has been shown during many gauze house and field experiments in Ghana to be the most promising candidate for cross-protection because of its protective ability and mild effects on the growth and yield of cacao (Ameyaw et al. 2016; Domfeh et al. 2018; Ollennu et al. 1999; Ollennu and Owusu, 2002, 2003). Therefore, this mild strain has been used for many MSCP experiments at Cocoa Research Institute of Ghana.

The current experiment was designed to characterize the temporal and spatial spread of CSSV-1A epidemics in plots treated with the mild strain CSSV-N1. To provide further information regarding the suitability of this method of CSSVD control, growth and yield performances of test cacao plants were recorded.

Materials and Methods

Mild strain inoculation. Beans of mixed hybrid cacao without testae were soaked in water overnight and exposed to mealybugs [*Formicococcus njalensis* (Laing)] previously fed on CSSV-N1-infected plants (Posnette and Strickland 1948) at a rate of 10 nymphs per bean. Resulting seedlings that showed clear symptoms

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of CSSV-N1 (transient red vein banding) were selected for field planting. The symptom of red vein banding is unique to CSSV and has been used in previous studies to indicate successful infection by CSSV-N1 (Ollennu et al. 1999; Ollennu and Owusu 2002; Owusu et al. 1996).

Field experiment. The experiment was established at Cocoa Research Institute of Ghana, Plot A24, New Tafo, Ghana (N 6° 12' 51.4", W 0° 22' 34.6") in June 2006 (Domfeh et al. 2007). A randomized complete block design with three replicates and four treatments was used. Each experimental plot had 361 cacao trees planted every

Fig. 1. Diagrammatic representation of the experimental layout of a single replication of the study conducted to determine the effects of different numbers of rows of plants inoculated with the N1 strain of cacao swollen shoot virus (CSSV) on the spread of epidemics caused by CSSV-1A strain. Treatments were as follows: T1, first five rows inoculated with CSSV-N1; T2, first three rows inoculated with CSSV-N1; T3, no CSSV-N1-inoculated plants; T4, all rows inoculated with CSSV-N1.

2.4 m in 10 concentric rows spaced every 2.4 m (Fig. 1). *Gliricidia sepium* and plantain (*Musa* spp.) were used to provide temporary shade, and *Terminalia ivorensis* and other shade tree species provided permanent shade. In 2010, 4 years after transplanting, the two outermost rows of each plot were inoculated with the New Juabeng strain of CSSV (CSSV-1A) through patch grafting (Legg et al. 1980) to serve as source plants. The inner eight rows of each plot were identified as 1 through 8, with row 1 being the closest to the source plants and row 8 being at the center of the plot. Four treatments were applied to plants in the inner rows as follows: treatment 1 (T1), first five rows inoculated with CSSV-N1; treatment 2 (T2), first three rows inoculated with CSSV-N1; treatment 3 (T3), no CSSV mild strain (noninoculated); and treatment 4 (T4), all inner rows (1 to 8) inoculated with CSSV-N1.

Data collection. Visual symptoms typical of CSSV-1A such as red vein banding, vein clearing, diffused flecking (Fig. 2), and stem swelling were monitored closely and recorded twice per year for a period of 7 years (2011 to 2017). These symptoms were used as indicators of CSSV-1A spread. Symptom recording was performed by staff members with decades of experience with CSSV disease spotting. Plants that showed symptoms of CSSV-1A were not removed from the field. Yield data (number of pods per tree for each plot) were obtained from 2010 to 2017, and the cumulative amounts were expressed as dry cacao yield (kilogram per hectare) using the formula $[dY = 1736/NT \times NP/28]$, where dY represents the estimated dry bean yield per hectare, NT represents the number of cacao trees per plot, and NP represents the number of fresh pods harvested per plot; the constant 28 represents the pod value (number of pods that provided 1 kg of dry cacao beans), and 1736 represents cacao plant density per hectare. Growth was assessed by measuring the trunk circumference at a height of 15 cm from the ground at 6-month intervals from 2009 to 2017.

Data analyses. To model the temporal and spatial development of the epidemics, data were divided into transects oriented perpendicular to each other. Each transect was five plants wide (12 m) and six rows deep (14.4 m), running from the outer edge of the plots inoculated with CSSV-1A toward its center (Fig. 3). Transects running from north to south were identified as north, from east to west were identified as east, from south to north were identified as south, and from west to east were identified as west (Fig. 3).

Temporal models. For each experimental plot, the cumulative yearly mean percentage of disease incidence was calculated for transects and across treatments. The linearized form of five temporal models: linear

$$y = y_0 + rt$$

exponential;

$$y = y_0 e^{rt}$$

monomolecular;

$$y = 1 - (1 - y_0)e^{-rt}$$

logistic;

$$y = \frac{1}{1 + e^{\left(-\ln\left(\frac{y_0}{1-y_0}\right) + rt\right)}}$$

gompertz;

$$y = e^{\ln(y_0)e^{-rt}}$$

were fit to each data set using the linear regression procedure (PROC REG) of Statistical Analysis System version 9.4 (SAS, Cary, NC) as described by Madden et al. (2007). The fitness of each model was evaluated considering the representativeness of the data (R^2 ; highest was better), presence of bias (plots of residuals at random were better), and its predictive power (residual mean square error of the back-transformed models; smaller values were considered better). To compare mean square errors, the predicted disease incidences (y) for each model were back-transformed using the following equations:

$$Y = y$$

for the linear model;

$$Y = e^y$$

for the linearized exponential model;

$$Y = 1 - e^{\ln\left(\frac{1}{1-y}\right)}$$

Fig. 3. Illustration of transects running from north to south (N), east to west (E), south to north (S), and west to east (W). Each transect was 12 m wide and 14.4 m long, running from the edge of the areas inoculated with a virulent strain of cacao swollen shoot virus (red) to the center of the plot.

Fig. 2. Foliar symptoms of infection of cacao plants by the severe strain 1A of the cacao swollen shoot virus (CSSV). **A and B,** Red vein banding on young cacao leaves. **C,** Red vein banding turning into vein clearing in maturing cacao leaves. **D,** Chlorotic vein clearing on mature cacao leaves. Images were taken from different cacao varieties.

for the linearized monomolecular model;

$$Y = \frac{1}{1 + e^{-\left[\ln\left(\frac{y}{1-y}\right)\right]}}$$

for the linearized logistic model; and

$$Y = e^{-e^{-[\ln(-\ln(y))]}}$$

for the Gompertz model. The mean square errors were calculated using PROC REG. The slope parameters from the selected model were analyzed using the General Linear Model procedure (PROC GLM) of SAS to determine whether significant differences existed among treatments, and their means were discriminated using the least significant difference (LSD) test at $\alpha = 0.05$.

Disease gradient models. The linearized form of six spatial models; negative exponential

$$y = ae^{-bs}$$

power law;

$$y = as^{-b}$$

temporal monomolecular analog;

$$y = 1 - ae^{bs}$$

logistic;

$$y = \frac{1}{1 + ae^{bs}}$$

power law analog;

$$y = 1 - as^b$$

and Berger and Luke;

$$y = \frac{1}{1 + a(s + \lambda)^b}$$

were evaluated as described by Qandah and del Río Mendoza (2012) for their fitness using data sets created for each year, treatment, replication, and transect direction. Each transect was six rows deep and five plants wide. Model fitness was evaluated following the same procedure used for the temporal models. The back-transformed values of disease incidence (Y) for each model were calculated as

$$Y = e^y$$

for the negative exponential model and the power law model;

$$Y = 1 - e^{-y}$$

for the monomolecular analog and power law analog models; and as

$$Y = \frac{1}{1 + e^{-y}}$$

for the logistic and Berger and Luke models. The slope parameters of the model that provided the best fit were compared as described for temporal models.

Disease incidence, growth, and yield. Disease incidence data for the sixth and seventh years of the study were averaged for each plot and year. Treatments were compared using PROC GLM and the LDS test at $\alpha = 0.05$. Growth and yield data were analyzed as repeated measures using the Mixed Model approach (PROC MIXED) of SAS. Compound symmetry with heterogeneous variances and autoregressive covariance structures were selected for the growth and yield data, respectively, based on the Akaike Information Criterion (AIC; smaller was considered better). For growth and yield analyses, the entire data sets were used.

Results

Temporal development of epidemics. All cacao plants in the outer two rows, which were inoculated with CSSV-1A, showed visible symptoms of the disease by the eighth month after inoculation. The first appearance of CSSV-1A symptoms in non-CSSV-1A-inoculated plants was detected in T3, for which no plant was protected with CSSV-N1, and T4, for which all trees were protected with the CSSV-N1 strain. The following year, infected plants were detected in the other two treatment groups (T1 and T2). There was a large increase in disease incidence across all treatments during the third year after CSSV-1A inoculation, with T4 having almost 50% less occurrences than the other three treatments (Fig. 4). During subsequent years, disease incidence continued to increase for all treatments; by the end of the seventh year, treatments T1, T2, and T4 had significantly lower incidences ($P = 0.05$) than the nonprotected control (T3). However, the epidemic growth rate during the years after year 3 was not different among the treatments because the epidemic seemed to grow linearly rather than have a logarithmic phase (Fig. 4).

The linear and logistic models provided the best fit for the temporal disease data when transect directions were not considered. The Gompertz model also provided good fit. No significant interactions ($P = 0.798$) were detected between treatments and direction of

Fig. 4. Effects of inoculation of cacao swollen shoot virus (CSSV) mild strain N1 on temporal spread of CSSV disease caused by the severe strain 1A in cacao. Incidence values with the same letters within 1 year are not significantly different based on the least significance difference (LSD) test at $\alpha = 0.05$. Treatments were as follows: T1, first five rows inoculated with CSSV-N1; T2, first three rows inoculated with CSSV-N1; T3, no CSSV-N1-inoculated plants; T4, all rows inoculated with CSSV-N1.

Table 1. Impact of inoculating cacao plants with cacao swollen shoot virus mild strain N1 (CSSV-N1) on the temporal increase of CSSV disease caused by severe strain 1A (CSSV-A1), with and without transect orientation

Treatment	Temporal rates ^a
First five inner rows inoculated with CSSV-N1 (T1)	0.06
First three inner rows inoculated with CSSV-N1 (T2)	0.06
No CSSV-N1-inoculated plants (T3)	0.07
All inner rows (1–8) inoculated with CSSV-N1 (T4)	0.04
LSD ($\alpha = 0.05$)	0.01
Transect direction	
North to south (N)	0.058
East to west (E)	0.068
South to north (S)	0.055
West to east (W)	0.050
LSD ($\alpha = 0.05$)	0.010

^a Rates of disease increase and spread were discriminated using Fisher's protected least significant difference test ($\alpha = 0.05$).

transects for the lineal model. However, significant differences were found among treatments ($P < 0.001$) and among transect directions ($P < 0.0244$). Protecting all plants by inoculating them with CSSV-N1 reduced the rate of CSSV-1A symptomatic plants by 43% ($P = 0.05$) when compared with the treatment in which none of the plants was protected and by 33% when compared with plots including a ring of N1 protected plants three or five rows deep. Similarly, creating a protective outer ring three or five rows deep reduced the rate of CSSV-1A by an average of 14.3% ($P = 0.05$) compared with the treatment for which no test plant was protected. When gradient directions were considered, the temporal development of CSSVD was best described by the linear model. Using this model, CSSV-1A epidemics increased approximately 18% faster ($P = 0.05$) in transects oriented from the north and east compared with those oriented from the south and west (Table 1).

Table 2. Comparison of the rate of disease spread of cacao swollen shoot virus strain 1A (CSSV-1A) epidemics among treatments during a 7-year period using parameters from the linearized power law (Gregory) model

Treatment ^a	Year ^b						
	1	2	3	4	5	6	7
T1	-1.27	-1.27	-1.20	-1.21	-1.10	-0.81	-0.65
T2	-1.27	-1.30	-1.21	-1.11	-1.08	-0.76	-0.69
T3	-1.25	-1.24	-1.30	-1.21	-1.18	-0.78	-0.53
T4	-1.27	-1.30	-1.40	-1.25	-1.32	-1.32	-0.96
LSD ($\alpha = 0.05$)	0.03	0.09	0.18	0.25	0.28	0.28	0.23

^a T1 = first five rows inoculated with CSSV-N1; T2 = first three rows inoculated with CSSV-N1; T3 = no CSSV mild strain (noninoculated); T4 = all inner rows (1–8) inoculated with CSSV-N1.

^b Rates of spread were discriminated using Fisher's protected least significant difference test ($\alpha = 0.05$).

Table 3. Comparison of the rate of disease spread of cacao swollen shoot virus strain 1A (CSSV-1A) among transect directions over a 7-year period using parameters from the linearized power law (Gregory) model

Transect direction ^a	Year ^b						
	1	2	3	4	5	6	7
E	-1.27	-1.23	-1.34	-1.21	-1.19	-0.87	-0.59
N	-1.25	-1.33	-1.15	-1.06	-1.06	-0.80	-0.63
S	-1.27	-1.27	-1.19	-1.18	-1.10	-0.89	-0.68
W	-1.27	-1.27	-1.42	-1.33	-1.32	-1.12	-0.93
LSD ($\alpha = 0.05$)	0.03	0.09	0.18	0.25	0.28	0.28	0.23

^a Transects running from north to south were identified as north (N), from east to west as east (E), from south to north as south (S), and from west to east as west (W).

^b Slopes were discriminated using Fisher's protected least significant difference test ($\alpha = 0.05$).

Disease gradient models. The power law (Gregory) and Burger and Luke models provided equally good fit for the disease dispersal data. Data from the power law model are presented here. When transect directions were not considered, disease dispersal gradients for years 1 and 2 were almost identical for all treatments (Tables 2). Three years after inoculation of the source trees, plots where all trees were protected with CSSV-N1 had a steeper ($P = 0.05$) gradient compared with plots with three or five rows of plants inoculated with CSSV-N1. No differences in gradient steepness ($P > 0.05$) were detected among treatments or transects during year 4 and 5; however, for the last 2 years of the study, dispersal of CSSV-1A decreased significantly faster on plots where all plants had been inoculated with CSSV-N1 compared with other treatments (Table 2). CSSV-1A symptomatic plants were not observed in the center of plots (rows 6 to 8) where all plants had been protected with CSSV-N1 (T4) until the fourth year, whereas plots where no plants were protected (T3) had CSSV-1A symptomatic plants in the center of plots 1 year earlier. When transect directions were considered (Table 3), the more consistent difference was observed between disease gradients in the north and west transects, with the north having a statistically steeper slope during years 3, 4, 6, and 7 of the study.

Growth and yield. Growth of cacao plants (assessed by trunk circumference measurements) did not differ significantly among the treatments over the 9-year period ($p = 0.38$) (Table 4). Trunk circumferences (averaged across years) ranged from a mean of 29.3 cm among cacao trees that were not inoculated with CSSV-N1 to 30.1 cm for the treatment with all trees inoculated with CSSV-N1 (Table 4). Cacao trees that had the first five or first three outer rows inoculated with CSSV-N1 had mean growth of 29.4 cm and 29.3 cm, respectively (Table 4). Cacao yield was not significantly different among the treatments ($p = 0.27$) (Table 4). The yield ranged from a mean of 530 kg/ha for the treatment group in which no test cacao trees were inoculated with CSSV-N1 to 698 kg/ha for the treatment group in which the first three outer rows of cacao trees received CSSV-N1 protection (Table 4).

Discussion

To the best of our knowledge, this is the first study that investigated the extent to which the spread of a virulent strain of CSSV could be checked by protecting the outer rows of cacao with a mild strain of the virus. Inoculating all plants in a field with protective strains is the default procedure used by most cross-protection strategies for the management of plant virus diseases (Crowdy and Posnette 1947; Fulton 1986; Hughes and Ollennu 1994; McKinney 1929; Posnette and Todd 1955; Ollennu and Owusu 2002, 2003; Yeh and Gonsalves 1994). In some cases, repeated mild strain inoculations of plants in the field are required for continuous protection against the effects of the severe strain (Hughes and Ollennu 1994).

Even though the temporal rates of disease increase between ring-protected (three or five rows deep) and nonprotected plots differed

Table 4. The effects of cacao swollen shoot virus strain 1A (CSSV-1A) epidemics on growth and yield of cacao inoculated with CSSV mild strain N1 (CSSV-N1)

Treatment ^a	Growth estimates (cm) ^b	Standard error	Yield estimates (Kg/ha) ^c	Standard error
T1	29.4	0.65	565	56.23
T2	29.3	0.65	698	56.23
T3	28.4	0.65	530	56.23
T4	30.1	0.65	635	56.23

ANOVA						
Effects	Growth		Pr > F	Yield		Pr > F
	Numerator DF	Denominator DF		Numerator DF	Denominator DF	
Treatment	3	7.78	0.3793	3	14.1	0.2080
Year	8	19.30	<0.0001	7	53.3	<0.0001
Treatment × year	24	22.10	0.9999	21	50.8	0.8554

^a T1 = first five rows inoculated with CSSV-N1; T2 = first three rows inoculated with CSSV-N1; T3 = no CSSV-N1-inoculated plants; T4 = all plants inoculated with CSSV-N1.

^b Estimates and test of fixed effects for growth were averaged across 9 years.

^c Estimates and test of fixed effects for yield were averaged across 8 years.

statistically, the difference was not large enough to create a significant separation of incidences until the seventh year after CSSV-1A inoculation. The protection offered by the five-row and three-row rings were not as strong ($P = 0.05$) as that observed when all test cacao trees were inoculated with CSSV-N1 (default procedure).

Among other things, this study sought to rekindle interest in the use of MSCP as a management strategy for CSSVD in Ghana by providing information that could potentially lead to considerable savings in the time and labor and other logistics needed for the mass inoculation of plants with mild strains. Presently, mild strain inoculation of cacao beans or seedlings used for cross-protection studies is accomplished by mealybug transmission or grafting from infected “source” plants; until a more efficient inoculation method becomes available, we predict that this would be the same way that mass inoculation of cacao would be performed if and when MSCP is recommended for managing CSSVD in Ghana (Hughes and Ollennu 1994). Moreover, because it is a virus strain, inoculating all cacao trees with N1 could lead to some amount of yield loss (Domfeh et al. 2018; Ollennu and Owusu 2003). Consequently, any procedure that could lead to a reduction in the number of cacao plants needing inoculation with this mild strain without compromising protection of the crop against effects of the severe strain would be of great interest to all stakeholders. Our results from both the temporal and spatial models, however, support the default procedure. Notwithstanding, this study provides useful baseline information that future studies could use to determine the optimum number of outer rows of cacao that should be inoculated with the CSSV-N1 for successful cross-protection.

Among strains of the same virus, the mechanisms of cross-protection have been described as coat protein (CP)-mediated resistance, replicase-mediated protection, or RNA silencing (Beachy 1999; Goregaoker et al. 2000; Lu et al. 1998; Ziebell et al. 2007). These mechanisms eventually cause limited replication of the challenge virus strain, leading to suppression of symptoms of the challenge strain in host plants pre-inoculated with mild strains (Folimonova 2013; Wen et al. 2013). Much of the spread of CSSVD in the field occurs in a “radial” manner as virus-carrying mealybugs move and feed in interlocking branches of the cacao canopy (Cornwell 1960; Thresh et al. 1988a). Test plants that became infected with CSSV-1A were likely to have also served as sources of inoculum from which further spread by mealybugs occurred. In the absence of a protecting strain, unimpeded multiplication of the severe strain was expected, and this likely resulted in high availability of the severe virus in the host plants to the mealybug vector. In contrast, availability of the severe virus to mealybugs in test plants with CSSV-N1 protection was likely to be low because of limited replication. The scenarios described here are assumptions; however, if true, then they may, in part, explain why the increase in the CSSV-1A incidence among test trees was lowest for the treatment group in which all test trees were inoculated with CSSV-N1 and the direct opposite occurred for the treatment group in which no test plant was inoculated with CSSV-N1. Suppression of symptoms of CSSV-1A in CSSV-N1-protected cacao plants has been widely reported in previous studies (Ollennu and Owusu 2002, 2003; Owusu et al. 1996).

Protecting outer rows of cacao with a mild strain could be likened to planting a barrier crop around a farm to prevent the spread of a plant virus. The mild strain-protected outer rows of cacao are expected to intercept inoculum, which invariably comes from nearby infected farms through mealybugs feeding in interlocking branches as they move from one tree to another (Domfeh et al. 2016; Dzahini-Obiatye et al. 2006; Ollennu et al. 1989). Consequently, the spread of the virus to other trees on the farm could be checked as inoculum is intercepted by the protected outer rows of cacao plants and becomes less available to the vector. This implies that unless “jump” spread occurs, tree-by-tree spread of inoculum by mealybugs would presumably be slow. This may explain why inoculating the first five or three outer rows of test plants resulted in significantly higher reductions in CSSV-1A spread compared with the non-mild strain-inoculated treatment as well as the reduced incidence during the seventh year of the study. However, it appears that a protective ring more than five rows deep would be required to provide protection closer to that observed when all test plants were inoculated with the mild strain. This needs to be investigated further.

One major concern with limiting the mild strain inoculation to outer rows of cacao plants is the possibility of “jump” spread of the virus. This phenomenon of “jump” spread occurs when virus-carrying mealybugs are blown by the wind or carried by attendant ants (*Crematogaster* and *Camponotus* spp.) over and beyond the protected outer rows, thus starting an outbreak far from the original source of infection (Cornwell 1960; Ollennu et al. 1989; Strickland 1950). This mode of spread, however, has been reported to be relatively infrequent (Posnette 1943; Thresh 1958). In addition, frequent inspection of cacao trees for the presence of CSSVD symptoms and diligent removal of infected ones along with their neighboring “contacts” could probably provide a solution to the problem of “jump” spread when it occurs. Papaya ringspot virus (PRSV) is a major limiting factor for the wide-scale production of papaya (Yeh and Gonsalves 1994). During field tests conducted in Taiwan, the reduction of inoculum pressure through frequent roguing of infected papaya plants resulted in an 82% increase in yield in mild strain-protected papaya plants compared with unprotected trees (Wang et al. 1987).

It is important to note that the results obtained during this study were based on visual assessment of CSSV-1A symptoms; possible asymptomatic infections of this strain were not considered. Symptomless infections have been widely reported to occur during many plant-virus interactions (Carnegie et al. 2012; Hughes and Ollennu 1994; Latvala-Kilby et al. 2009; Muller et al. 2001; Sokmen et al. 1998; Thresh and Owusu 1986), which implies that the actual incidence of the severe strain among tests plants could be higher than that reported in this study.

The differential rate of spread of CSSV-1A across treatments has not yet had a significant corresponding impact on growth and yield of the cacao plants involved. This assertion is supported by absence of significant differences in growth and yield among treatments during the assessment period. However, it is noteworthy that growth and yield were lowest for the treatment group in which none of the test cacao trees was CSSV-N1-protected. This was expected because of higher incidence of CSSV-1A in the nonprotected treatment group; we speculate that the yield and growth among cacao trees in this treatment would eventually become significantly lower as the inoculum pressure continues to increase and affected cacao trees succumb to infection, as observed previously (Ameyaw et al. 2016). The results imply that CSSV-N1 did not have a detectable negative effect on the growth and yield of cacao plants, either alone or in combination with CSSV-1A, despite it being a virus strain. This finding partly agrees with the results of Domfeh et al. (2018), who reported that mild strains of CSSV-N1 and SS356B did not have adverse effects on the growth of cacao during a 7-year field experiment.

The transect approach was selected because it provided an equal number of plants at each distance from the source as opposed to counting infected plants in each row. With the latter, as the center of the plot is approached, each plant accounts for increasingly higher percentage points (e.g., a single diseased plant in row 1 equated to a 1.8% incidence, whereas the same single plant equated to a 6.2% incidence in row 6 and a 12.5% incidence in row 7). Furthermore, the chance of each plant being infected is also reduced by different rates as the center is approached. However, the transect approach is not exempt from disadvantages. A decision was made to use transects that were five plants wide because that would encompass the width of the noninoculated plots and provide enough plants to allow for reliable readings (each diseased plant in a row accounted for a 20% incidence). Transects wider than five plants might facilitate interference between transects at shorter distances compared with the five-plant transects. Although using three-plant-wide transects would reduce the interference of other transects longer than the five-plant transects, each diseased plant in a row would account for a 33% incidence, thus making the incidence data less representative. Therefore, the transect approach helped to measure disease spread over time more accurately and aided in determining the direction where disease spread was fastest. Our results provide evidence that CSSVD epidemics developed approximately 18% faster in transects oriented from north and east compared with those oriented from south and west. Even though the observed differences are likely a function of mealybug movement and feeding patterns,

we do not have data that would enable detailed explanation of this finding; therefore, further investigation is required.

Conclusion

This study sought to determine the extent to which the spread of severe strain-1A of CSSV could be minimized by inoculating test cacao with mild-strain CSSV-N1. The results presented here support previous findings that protecting cacao with CSSV-N1 can lead to the suppression of CSSV-1A symptoms. However, protecting only the first five or three outer rows of cacao with N1 was not sufficient to reduce the disease incidence when compared with the treatment for which none of the test trees was protected until 7 years after CSSV-1A inoculation. By increasing the number of outer rows that require inoculation with the mild strain, an optimum number that would ensure adequate protection could be attained. The results also demonstrated that CSSV-N1 did not cause undue harm to cocoa plants in terms of growth and yield. Our results were based on the visual assessment of test plants to determine CSSV-1A symptoms; therefore, it must be interpreted with caution because of the possibility of symptomless infections.

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