



Combatting Cocoa Swollen Shoot Virus Disease: What do we know?



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ABSTRACT

Cocoa Swollen Shoot Virus Disease (CSSVD) is one of the major factors limiting cocoa (*Theobroma cacao* L.) productivity in West Africa. The only cure for CSSVD is to cut out visibly infected trees and the official eradication campaign in Ghana has cut out more than 200 million trees since 1946. 80 years of research on preventive control measures have mainly focused on resistance breeding, mild strain cross-protection (inoculation of cocoa seedlings with a mild strain of the virus to protect against the severe strain) and control of mealybug vectors. Meanwhile, diversification measures such as agroforestry (for shading) or barrier (strip) cropping have received less attention. Despite promising results, CSSVD is more prevalent in the field than ever before. The large body of knowledge on preventive control measures for CSSVD is fragmented and many publications are not easily accessible. Furthermore, the literature has never been systematically evaluated and quantitatively assessed. Hence, we consolidated this knowledge with an extensive literature review followed by meta-analysis to identify the pertinent research gaps. Out of 423 publications on CSSVD-related issues, we selected 34 studies, which contained 52 datasets on seven different preventive control measures. Results showed that resistance breeding and mild strain cross-protection may reduce CSSVD infection by 30 percent, while the potential of diversification measures (shading/agroforestry and barrier (strip) cropping) seems to be considerably higher (40 and 85 percent, respectively). However, there is a lack of evidence because of a low number of studies about diversification measures, indicating that our results have to be interpreted with care and calling for more research in this area. Future testing is needed to evaluate the efficacy of barrier (strip) cropping to reduce CSSVD, and address the effect of shade on CSSVD symptom severity. Furthermore, the practical relevance of different preventive control measures for farmers needs to be assessed, and shade should be considered in current breeding programs for CSSVD resistance.

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1. Introduction

Cocoa swollen shoot virus disease (CSSVD) has plagued cocoa (*Theobroma cacao* L.) production in West Africa for more than eight decades (Dzahini-Obiatey et al., 2010). CSSVD is caused by the cocoa swollen shoot virus (CSSV), belonging to the genus *Badnavirus* (Lot et al., 1991). CSSVD only occurs in West Africa, and has been reported in Côte d'Ivoire, Ghana, Nigeria, Sierra Leone and Togo (Fig. 1, (Muller, 2016)). The disease has been particularly severe in Ghana, where it was already observed in 1922 (Paine, 1945) and

described in 1936 (Steven, 1936).

Several institutes in Nigeria, Togo and particularly in Ghana have done extensive research on CSSVD in virology, epidemiology and genetic improvement of cocoa germplasm. CSSV is genetically diverse; different isolates/strains can cause different symptoms, including transient red veins and mottling in young leaves, different shades of chlorosis in mature leaves and pods, root atrophy and stunting, as well as root and stem swellings. Most of these symptoms are unique to different strains and symptom expression depends on host genotype. Highly pathogenic strains cause severe leaf chlorosis, which may result in rapid death of cocoa trees (Dzahini-Obiatey et al., 2010).

Different species of mealybugs (Pseudococcidae spp.), the most important ones being *Formicococcus njalensis* (Laing) and

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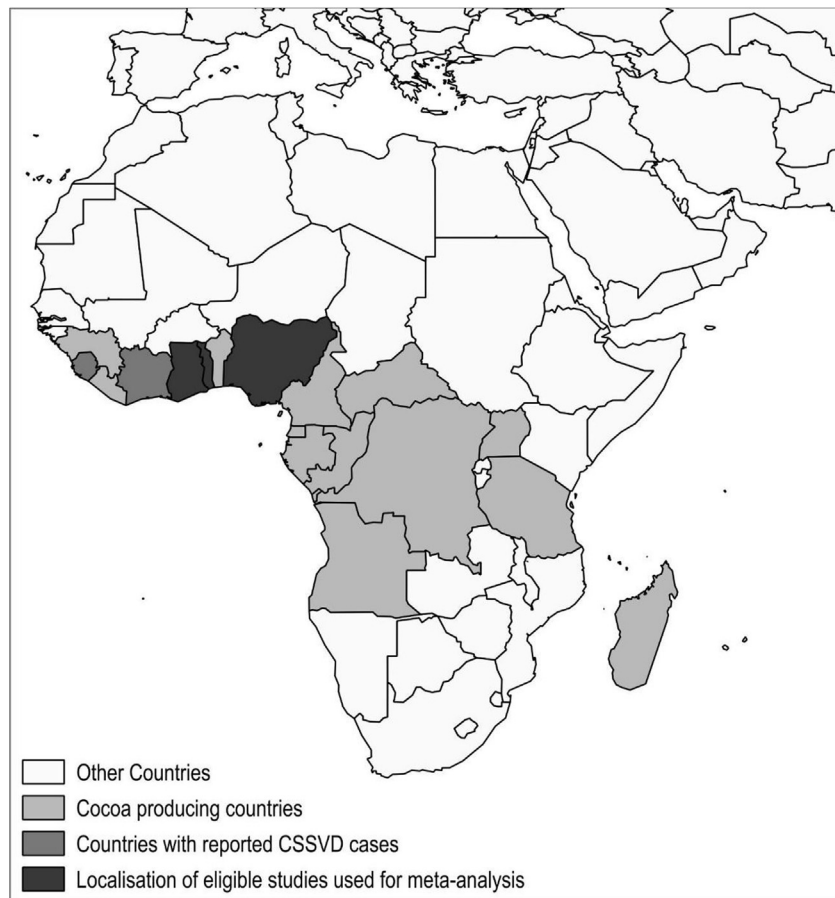


Fig. 1. Localisation of the eligible studies used for meta-analysis.

Planococcus citri (Box, 1945; Cornwell, 1953, 1958, 1960; Thresh and Tinsley, 1959; Roivainen, 1971; Bigger, 1981) spread CSSVD. The spread of CSSVD can happen in two ways: either through “radial spread”, caused by relatively immobile adult mealybugs that move along interlocking branches of adjacent trees, or through “jump spread”, caused by more mobile first instar nymphs transported by wind, which create new outbreaks (Jeger and Thresh, 1993).

The only treatment for CSSVD-infected cocoa is to cut out visibly infected trees (eradication). Thereby, symptomatic trees are removed together with all adjacent, apparently healthy trees. When large outbreaks occur, extensive areas are cleared and replanted (Thresh and Owusu, 1986). The official eradication campaign in Ghana, launched in 1946, has cut out more than 200 million trees by 2010, and has been called the costliest campaign of its kind anywhere in the world (Thresh et al., 1988; Ampofo, 1997). However, the eradication campaign failed due to various reasons, such as the campaign being disrupted and discontinued several times (Ollennu et al., 1989; Dzahini-Obiatey et al., 2006), and because of deficient eradication procedures (Thresh et al., 1988). Consequently, the disease continues to spread into new areas (Domfeh et al., 2011).

Past research has had some focus on the development of preventive control measures to be implemented along with eradication (Table 1). However, farmers have only to a limited extend implemented those measures, which may be due to their perspective and situation not sufficiently being taken into account. For example, farmers said they would not do barrier (strip) cropping with oil palm because it attracts rodents, which can damage cocoa pods (Wetten, pers. comm.). The selection of the suitable

trees that match farmers' preferences and expectations is most likely to be successful when done in a participatory manner together with farmers, so that their views and knowledge are taken into account (Vaast and Somarriba, 2014).

There is a lot of anecdotal knowledge about preventive control measures for CSSVD, especially in the library of African institutions such as the Cocoa Research Institute of Ghana (CRIG). However, this information is fragmented and has never been systematically evaluated and quantitatively assessed. In this study, we consolidated this knowledge in order to provide a basis for future research and development. We conducted an extensive literature review followed by meta-analysis to elaborate the relative effectiveness of different preventive control measures for CSSVD. We hypothesised that preventive control measures related to diversification (barrier (strip) cropping, shading/agroforestry) reduce CSSVD infection significantly more than preventive control measures related to breeding and mild strain cross protection.

2. Materials and methods

2.1. Data collection

To identify studies reporting preventive control measures for CSSVD we searched the databases of Web of Science, Scopus and Google Scholar using the following keywords: „Cocoa Swollen Shoot Virus Disease“, „Cocoa Swollen Shoot Virus“, „Swollen Shoot Virus“, „Swollen Shoot Disease“, „Swollen Shoot“, „CSSVD“ and „CSSV“. We gathered further articles from the reference sections of already collected articles, and from an Endnote library on CSSVD-

Table 1

Overview and explanation of preventive control measures for CSSVD and of improved practices (IP) presented in eligible studies (see section 2.1).

Measure category	Measure/IP	Explanation	Advantages	Disadvantages	Included in meta-analysis?	References
Breeding/mild strain cross protection	Breeding	Breeding for varietal resistance to CSSVD	Slows down CSSVD spread	Limited availability of seedlings	Yes	(Kenten and Lockwood, 1977; Lockwood, 1981a, b; Posnette, 1981; Firempong, 1984; Campbell, 1990; Dzahini-Obiatey et al., 2006; Dzahini-Obiatey et al., 2010; Padi et al., 2013; Ameyaw et al., 2014; Muller, 2016)
	Mild strain cross protection	Inoculating cocoa beans/seedlings with mild CSSV isolate/strain to protect from severe strain	Adverse effects of severe strain can be prevented for several years	Difficult to find appropriate mild isolates/strain for different severe strains due to high genetic diversity of CSSV, simultaneous protection against different severe strains impossible, high cost	Yes	(Ollennu et al., 1999; Dzahini-Obiatey et al., 2010; Muller, 2016)
Diversification	Barrier (strip) cropping	Lining newly planted cocoa with 10 m barrier of non-host crop (e.g. citrus, oil palm, coffee, rubber, etc.)	Non-host crops generate additional income	Additional income generated may be less than potential income generated by cocoa	Yes	(Ollennu et al., 2003; Ollennu et al., 2005; Dzahini-Obiatey et al., 2006; Dzahini-Obiatey et al., 2010)
	Shading/agroforestry	Shading of cocoa seedlings and plantations (agroforestry)	See Andres and Bhullar (2016)	Lower short-term cocoa yields	Yes	(Adegbola, no date)
Eliminating sources of inoculum	Cordon sanitaire	Leaving 10 m-wide cocoa-free zone around newly planted cocoa	Effective method	Less cocoa planted means less income for farmers, leaving land under fallow could lead to loss of user rights for land	No	(Vanderplank, 1947; Dzahini-Obiatey et al., 2006; Dzahini-Obiatey et al., 2010)
	Removing alternative host trees	Removing alternative host trees which naturally harbor the virus	Potential source of infection and habitat for vectors removed	Shade for young cocoa plants may be missing, and chance to generate additional income may be lost	No	(Dzahini-Obiatey et al., 2006; Dzahini-Obiatey et al., 2010; Ameyaw et al., 2014; Muller, 2016)
Screening method for resistance breeding	Antiviral substance	Cocoa leaf and embryo extracts as well as quercetin used to inhibit CSSV transmission in seeds (screening methods for resistance breeding)	n.a.	n.a.	Yes	(Adomako and Owusu, 1974; Dzahini-Obiatey et al., 2005)
	Somatic embryogenesis*	Tissue culture technique to generate disease-free clonal propagules from visibly infected trees (screening methods for resistance breeding)	n.a.	n.a.	Yes	(Quainoo et al., 2008)
Vector control	Insecticide	Controlling mealybug vectors with insecticides	Easy application	Inefficient due to mealybugs' water-repelling (waxy) body and behaviour (hiding), adverse effects on natural antagonists (disrupting functional biodiversity), no practically feasible method for farmers available	Yes	(Tscharntke et al., 2012; Forster et al., 2013; Ameyaw et al., 2014; Muller, 2016)

* Somatic embryogenesis is of no practical relevance for farmers, but may be engaged to support breeding efforts; n.a. = not applicable.

related literature, which was compiled by researchers from CRIG (Ameyaw, pers. comm.), and the University of Reading, UK (Cryer, pers. comm.). These were often technical or annual reports, which were only available as hardcopies in the CRIG library (grey literature). This procedure led to a total number of 423 articles related to CSSVD. In the following, preventive control measures will be referred to as “improved practices”.

We screened all articles and selected those for meta-analysis (in the following referred to as “eligible studies”) that met the following criteria: i) an improved practice was compared to a control under the same pedo-climatic conditions, ii) the data presented values of visible CSSVD symptoms (of seedlings/trees) in the improved practice and control treatment, respectively, and iii) the mean values of improved practice and control were reported together with a measure of variability (standard deviation, standard error of the mean or *p* value) and number of replications (*n*). This led to the selection of 34 studies with 52 sub-studies (Table 2) that reported data on seven different improved practices (Table 1). A full reference list of the papers included in the meta-analysis is provided in Appendix A. In this paper, the term “study/studies”

refers to individual papers/publications, while the term “sub-study/sub-studies” refers to different experiments/trials presented in the same study. Furthermore, we used visible CSSVD symptoms as a proxy for CSSVD infection. Actual CSSVD infection can only be confirmed by serology or nucleic acid-based testing. However, for ease of understanding we used the term “CSSVD infection” in the text.

2.2. Data analysis

We extracted the data from the 52 eligible sub-studies manually and compiled them in a common Microsoft Excel data matrix. We calculated the reduction of CSSVD infection as follows:

$$\text{Reduction of infection}[\%] = \left(1 - \left(\frac{\text{Infection}_{\text{IP}}}{\text{Infection}_{\text{control}}}\right)\right) * 100$$

where Infection_{IP} and Infection_{control} = absolute (number of seedlings/trees) or relative (percentage) values of CSSVD infection in improved practice and control treatment, respectively.

Table 2

Overview of the studies on preventive control measures for cocoa swollen shoot virus disease included in the meta-analysis (Appendix A).

Reference	Institution and location of study	Improved practice (IP)	Type of publication ^a	Scale of comparison	Number of sub-studies ^b	Age IP ^c	Duration of measurements ^d
Adomako and Owusu, 1974	CRIG, Tafo, Ghana	Antiviral substance	0	Greenhouse/lab	1	n.a.	1 yr
Dzahini-Obiatey et al., 2005	CRIG, Tafo, Ghana	Antiviral substance	1	Greenhouse/lab	1	n.a.	1 yr
Ollennu et al., 2005	CRIG, Bunso, Ghana	Barrier (strip) cropping	1	Plot	1	5 yr	11 yr
Adomako, 1977	CRIG, Tafo, Ghana	Breeding	1	Greenhouse/lab	1	n.a.	84 d
Adu-Ampomah et al., 1996	CRIG, Tafo, Ghana	Breeding	1	Plot	2	3.5 yr	3 yr
Cilas et al., 1988	IRCC, Kpalime, Togo	Breeding	0	Greenhouse/lab	1	n.a.	1 yr
Dale, 1957	CRIG, Tafo, Ghana	Breeding	1	Greenhouse/lab	1	n.a.	2 yr
Djiekpor et al., 1995	IRCC, Kpalime, Togo	Breeding	0	Greenhouse/lab	1	n.a.	1 yr
Kenten and Legg, 1970	CRIG, Tafo, Ghana	Breeding	0	Greenhouse/lab	1	n.a.	1 yr
Kenten and Lockwood, 1977	CRIG, Tafo, Ghana	Breeding	0	Greenhouse/lab	1	n.a.	1 yr
Legg and Kenten, 1970	CRIG, Tafo, Ghana	Breeding	0	Greenhouse/lab	1	n.a.	1 yr
Legg and Kenten, 1971a	CRIG, Tafo, Ghana	Breeding	0	Plot	1	3.5 yr	9 yr
Legg and Kenten, 1971b	CRIG, Tafo, Ghana	Breeding	1	Plot	1	4 yr	10 yr
Legg and Lockwood, 1977a	CRIG, Tafo, Ghana	Breeding	0	Plot	1	3 yr	9 yr
Legg and Lockwood, 1977b	CRIG, Tafo, Ghana	Breeding	1	Plot	1	n.a.	1 yr
Legg and Lockwood, 1981	CRIG, Tafo, Ghana	Breeding	0	Plot	7	3 yr	4 yr
Lockwood, 1981a	CRIG, Tafo, Ghana	Breeding	0	Greenhouse/lab	1	n.a.	1 yr
Lockwood, 1981b	CRIG, Tafo, Ghana	Breeding	0	Greenhouse/lab	1	n.a.	1 yr
Longworth et al., 1965	CRIG, Tafo, Ghana	Breeding	0	Greenhouse/lab	1	n.a.	1 yr
Paulin et al., 1994	IRCC, Kpalime, Togo	Breeding	1	Greenhouse/lab	3*	n.a.	1 yr
Posnette and Todd, 1951	CRIG, Tafo, Ghana	Breeding	0	Greenhouse/lab	4	n.a.	1 yr
Tsatsu and Bekou, 2003	IRCC, Kpalime, Togo	Breeding	1	Plot	3**	15 yr	15 yr
Hanna and Heatherington, 1957	Chesterford Research Park, Atukrum, Ghana	Insecticide (vector control)	0	Farm	1	2 yr	2 yr
Crowdy and Posnette, 1947	CRIG, Tafo, Ghana	Mild strain cross protection	0	Plot	1	6 yr	3 yr
Ollennu and Owusu, 1997	CRIG, Tafo, Ghana	Mild strain cross protection	1	Plot	1	6 yr	7 yr
Ollennu and Owusu, 2002	CRIG, Tafo, Ghana	Mild strain cross protection	1	Plot	1	0.5 yr	8 yr
Ollennu and Owusu, 2003	CRIG, Tafo, Ghana	Mild strain cross protection	0	Plot	1	4 yr	3.5 yr
Ollennu et al., 1996	CRIG, Tafo, Ghana	Mild strain cross protection	0	Plot	1	6 yr	7 yr
Ollennu, 1989	CRIG, Tafo, Ghana	Mild strain cross protection	1	Plot	1	1.5 yr	1 yr
Ollennu et al., 1999	CRIG, Tafo, Ghana	Mild strain cross protection	0	Plot	1	4 yr	1 yr
Owusu et al., 1999	CRIG, Tafo, Ghana	Mild strain cross protection	1	Greenhouse/lab	3*	n.a.	1 yr
Owusu, 1971	CRIG, Tafo, Ghana	Mild strain cross protection	1	Plot	2***	17 yr	1 yr
Adegbola, –	CRIN, Ibadan, Nigeria	Shading/agroforestry	1	Greenhouse/lab	1	n.a.	56 d
Quainoo et al., 2008	University for Development Studies, Tamale, Ghana	Somatic embryogenesis	0	Greenhouse/lab	2	n.a.	1 yr

CRIG = Cocoa Research Institute of Ghana, IRCC = Institut de Recherches du Café et du Cacao, CRIN = Cocoa Research Institute of Nigeria; n.a. = not applicable (in greenhouse/lab studies improved practice is implemented directly); *two greenhouse/lab experiments, one plot comparison; **two plot comparisons, one greenhouse/lab experiment; ***one greenhouse/lab experiment, one plot comparison.

^a 0 = scientific journal article, 1 = books chapter/proceeding/technical report.

^b Sub-studies are different experiments/trials presented in the same study.

^c Time period since adoption of improved practice in years (yr).

^d Time period of measurements in days (d)/years (yr).

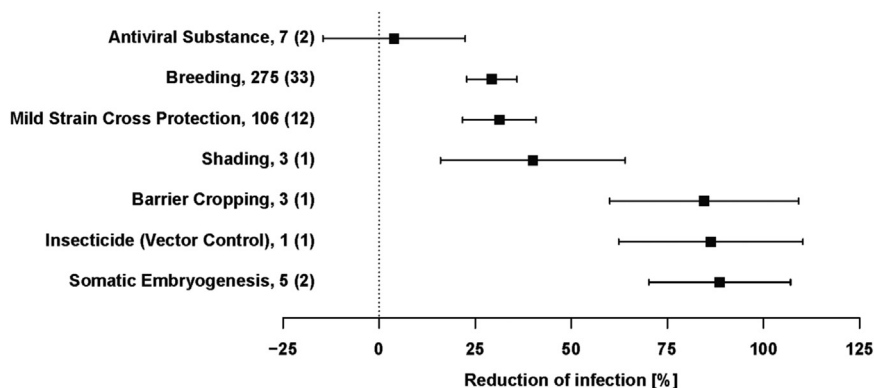


Fig. 2. Mean difference in reduction of infection with CSSVD of different improved practices vs. control (see section 2.1). Horizontal bars show 95% confidence intervals. The numbers displayed before and in brackets (after the name of the improved practices) represent the number of comparative data pairs and number of sub-studies, respectively.

Thereby, each data pair corresponded to one comparison of an improved practice with the control. For example, if one study presented data for five partially resistant varieties versus a susceptible control, this resulted in five data pairs (comparison of each variety with the control). This led to a total number of 400 data pairs (see legend in Fig. 2).

In some cases, there were multiple comparisons of different levels of improved practices with the same control in one study (e.g. five partially resistant varieties each time versus the same susceptible control). Since these data are not independent from each other, creating an individual data pair from each level of the improved practice with the same control led to an overestimation of the control value's confidence through artificial repetitions in the dataset. This overestimation was avoided by aggregating the data pairs to sub-study level at the cost of losing some information from the individual values of the different levels of the improved practices. We chose this procedure because the number of replications (n) was equal among different levels of the improved practices and the control in most studies. Therefore, we did not risk to lose information coming from the weighing of the mean values of the different levels of the improved practices (which depends on the value of n).

Among the eligible studies, there were two groups of studies: studies, which were carried out at the greenhouse-/lab-scale, and studies, which were carried out at the plot/farm-scale. To account for the compilation of all studies in one common data matrix, we run two separate meta-analyses for the two subgroups. This allowed us to check whether their outcomes would differ from the outcome of the single meta-analysis performed on the pooled dataset. Since the outcomes did not differ, we pursued the single meta-analysis of the pooled dataset at sub-study level.

We calculated the magnitude of effects ("effect sizes") as the raw mean difference (MD) of improved practices over the control using a random effects meta-analysis model as described by Borenstein et al. (2010). The raw mean difference (MD) was calculated as follows:

$$\text{Mean difference (MD)} = \text{mean}_{\text{improved}} - \text{mean}_{\text{control}}$$

MD is used when the scale of the outcomes is equal among all the studies, which was true in our case. We represented the precision of the effect sizes by weighing the means of each sub-study using their respective standard deviations and numbers of replications (n). We chose the random-effects model because it assumes that single effect sizes depend on the study context (differences in methods and sampling), which was very much the case in our heterogeneous dataset. We estimated the average true effect and

variance τ^2 with the restricted maximum-likelihood estimator (REML), and used the Knapp-Hartung adjustment to account for uncertainties when estimating (residual) heterogeneity. We found no outliers in our dataset by DFBETAS value analysis (Viechtbauer, 2010). We defined the weighted MDs of the improved practices to be significantly different from zero or from each other when the 95% confidence interval (CI) did not overlap zero or the 95% CI of another improved practice. We conducted all analyses with the "metafor" package of the R statistical software, version 3.3.1 (Viechtbauer, 2010).

3. Results

From the five countries with reported CSSVD cases, only three had eligible studies that we included in the meta-analysis (Fig. 1), and the vast majority of the eligible studies were conducted in Ghana (Table 2). Most of the studies focused on the two improved practices breeding and mild strain cross protection (56% and 26%, respectively), both of which showed a potential to reduce CSSVD by about 30% (Fig. 2). There were only two studies concerning diversification measures (shading/agroforestry and barrier (strip) cropping). Both improved practices showed a higher potential to reduce CSSVD by 40% (shading/agroforestry) and 85% (barrier (strip) cropping), respectively (Table 2).

Further, we only found one study about the effect of insecticide (vector control) on the number of visibly infected trees, which was also the only farm-scale study we included in the meta-analysis. The mean value of 86% (of the 11 consecutive field observations conducted on a monthly interval), shows a trend towards a high potential of this improved practice (Fig. 2). Regarding screening methods for resistance breeding, we had only one eligible study for somatic embryogenesis and two for antiviral substance. With a mean value of 89%, the potential of somatic embryogenesis was clearly superior to antiviral substance (4%).

4. Discussion

We first discuss our results' implications and knowledge gaps for each improved practice, then outline the limitations of our dataset and finally provide an outlook on solving the CSSVD problem.

4.1. Best practices and knowledge gaps in the combat of CSSVD

Our results show that all preventive measures included in the meta-analysis significantly reduced CSSVD infection under

researcher managed conditions, except for antiviral substance (Fig. 2). Furthermore, they depict that the focus of past research activities has been on breeding and mild strain cross protection, while the other improved practices, which could be very much of practical relevance for farmers (i.e., barrier (strip) cropping, shading/agroforestry and insecticide (vector control)), are only represented by a single study each. According to Posnette (1981), no other feasible measures would be as effective in reducing the losses caused by CSSVD as an increase in varietal resistance, which may decrease the rate of spread by up to 20%. Our results for breeding confirm this, and even show a higher potential for this improved practice (30%) than claimed by the author. Many viral diseases in other crops such as potato, tobacco and tomato have effectively been managed by increasing varietal resistance (Swaminathan, 1993; Moury et al., 2010). However, a recent publication in this field of research by Padi et al. (2013) shows no significant differences in resistance between existing and new varieties, underlying the limitations inherent in recycling the minimal resistance available within a narrow genetic base, and calls for a re-appraisal of variety recommendations.

Our results suggest that the potential of mild strain cross protection is just as high as breeding. Preventing the adverse effects of severe virus strains by inoculating cocoa beans or seedlings with mild strains is certainly promising (Ollennu et al., 1999; Dzahini-Obiatey et al., 2010; Muller, 2016). However, implementing this improved practice on a large scale is very laborious and costly, and entails several technical and logistical challenges such as inoculating and distributing several millions of cocoa seedlings to farmers every year (Domfeh, pers. comm.). Furthermore, the high genetic diversity of CSSV makes it difficult to find appropriate mild strains for different severe strains, and simultaneous protection against several different severe strains is practically not feasible (Ollennu et al., 1999; Dzahini-Obiatey et al., 2010; Muller, 2016).

In contrast, diversification measures (barrier (strip) cropping and shading/agroforestry) showed a higher potential in our study. However, our results have to be interpreted with care due to the limited number of studies, which is also reflected in the wide confidence intervals of these improved practices, and explains why the positive error bar of barrier (strip) cropping goes beyond 100% reduction of infection (Fig. 2). Nevertheless, the most recent publication in the field of barrier (strip) cropping confirms our results (Domfeh et al., 2016). The promising trend we show here, coupled with the fact that the implementation of these improved practices by smallholders may be relatively easy (i.e., no need for costly inputs), calls for further research in this area. Furthermore, it would be important to consider shade as a factor in currently ongoing breeding programs for CSSVD resistance.

There is a knowledge gap about the potential of more diversified production systems such as agroforestry or strip crop systems to reduce the spread and/or severity of CSSVD. In general, the effects of shade trees on incidences of pests and diseases in cocoa production systems are complex and ambiguous (Beer et al., 1998; Staver et al., 2001; Bedimo et al., 2012). Many studies have shown that self-regulation, the natural control of pests and diseases, is enhanced in diversified cocoa production systems (Sperber et al., 2004; Isaac et al., 2007; Lin, 2011; Tschardt et al., 2011; Bieng et al., 2013; Gidoïn et al., 2014; Mbow et al., 2014; Vaast and Somarriba, 2014). However, the effects of different commonly used shade tree species on mealybug populations and CSSVD infection have not been investigated so far, and they may vary depending on individual shade tree species (Franzen and Mulder, 2007). Furthermore, it is difficult to identify adequate shade levels and tree species compositions that minimize mealybug populations and thus likelihood of CSSVD infection while ensuring favourable growing conditions for cocoa trees. This is because both

optimal shade levels for cocoa trees and mealybug populations vary in the course of the year. Therefore, more research on effective agroforestry designs to combat CSSVD is needed. In this respect, it might be worthwhile to facilitate exchange between CSSVD specialists and ongoing agroforestry systems research in the humid tropics such as the project “agroforestry for food security” (AFS4-Food) by CIRAD. AFS4Food assesses the performance of agroforestry systems to understand the compromises farmers have to strike between the products and the different services provided by such systems.

Interestingly, the result from the only study we included in the meta-analysis about vector control, suggests a high potential of a systemic insecticide to control the mealybug vectors and thus reduce CSSVD. This is particularly interesting, as it is the only farm-scale study (i.e., conducted under actual farmers' field conditions) we included in the meta-analysis. Systemic insecticides have also been found to be effective under researcher-managed conditions. However, a number of problems associated with their use have been reported, such as their high cost, toxicity to mammals and residues in cocoa beans (Domfeh, pers. comm.). Unfortunately, most studies on vector control (e.g. Hanna et al., 1952; Nicol, 1952) have only reported the effect of a particular insecticide on the abundance of mealybugs, and not on CSSVD infection. However, reporting CSSVD infection is crucial, as lower numbers of mealybugs do not necessarily translate into reduced CSSVD infection, especially due to the very patchy distribution of mealybugs in the field (Campbell, 1990). Therefore, such studies should be repeated under field conditions and in addition to the effects on mealybug populations, the number of symptomatic trees should also be reported.

Regarding screening methods for resistance breeding, our results suggest that somatic embryogenesis is superior to antiviral substances (quercetin, cocoa leaf and embryo extracts). However, care has to be taken in the interpretation of our results due to the limited number of studies, which also explains the high confidence intervals of these improved practices and the fact that the negative error bar of antiviral substance and the positive error bar of somatic embryogenesis are below 0% and beyond 100% reduction of infection, respectively (Fig. 2). This is because in a meta-analysis, the confidence intervals of each mean are weighted with the number of replications and the measure of variability presented in the original studies. Hence, the credible intervals may go below or beyond a certain meaningful value (like in our case) because of estimates done by the software.

Besides the effect of individual improved practices, the factor age (i.e., the period since adoption of an improved practice) may correlate with the reduction of infection. We checked this for the 16 studies where data on “Age IP” (Table 2) was available. The results showed that there is a slight trend towards lower reduction of infection with increasing time since adoption, but this was statistically not significant. However, other works suggest that both breeding and mild strain cross protection may be effective in delaying the spread of CSSVD, but not to completely prevent it (Posnette, 1981; Ameyaw et al., 2016).

4.2. Limitations of the dataset

It is important to note that the effect size presented in this meta-analysis (reduction of infection as percentage) is a relative measure. As such, it may lead to an under- or overestimation of the potential of a particular improved practice to reduce CSSVD infection under field conditions. We accounted for this by aggregating the data pairs to sub-study level as explained in section 2.2. Finding an adequate effect size that would allow for comparison of the heterogeneous set of studies while accounting for the pooling of

comparisons at different scales (greenhouse/lab vs. plot/farm) was challenging, and the percentage reduction of infection we chose represented the best possible compromise to satisfy both. In this respect, we acknowledge that our meta-analysis is rather a descriptive analysis to identify overall patterns in the currently available dataset rather than an inferential statistical method delivering exact results. Another shortcoming of the dataset is that many of the studies are quite old (average year of publication was 1983). This is not to say that there are no recent publications on CSSVD. However, none of the more recent publications has looked at the effect size presented in this study, which is of practical relevance to farmers.

4.3. Solving the CSSVD problem: an outlook

80 years of disciplinary research and 70 years of official eradication campaign has been a very cost-intensive endeavour, which has not solved the CSSVD problem in Ghana (Dzahini-Obiatey et al., 2010). While the currently ongoing breeding efforts for CSSVD resistance and subsequent replacement of susceptible cocoa with resistant varieties may be part of the solution, there is certainly a long way to go until widespread implementation on the ground will be achieved, especially because resistance to the official eradication campaign by farmers remains strong.

However, the question about alternative, feasible options remains unresolved as of yet. As for research, the ongoing standardization of methodologies and protocols in breeding for CSSVD resistance is an important step towards resolution of the CSSVD problem (Padi et al., 2013). This is especially so, as we are becoming more aware of the major genetic variations in the virus across the West Africa regions (Muller and Sackey, 2005; Oro et al., 2012; Abrokwah et al., 2016). In addition, all CSSVD-related research should see to ensure that they report their results in a way that enables quantitative consolidation of several studies through meta-analytical tools.

In practice, on the other hand, an immediate strategy to deal with CSSVD infection is needed. As there are no short-term solutions to the problem, we need to find ways of living with CSSVD, especially in parts of the Eastern and Western Regions of Ghana, which were declared areas of mass infection where complete eradication and replanting is not feasible. Diversifying production systems and landscapes, which are not infected yet, while subsequently replacing diseased cocoa with more resistant varieties may be an interesting option for farmers.

Another interesting avenue for future research is biological control (Padi, 1997). After several initiatives in the 1950s had failed due to several reasons, such as the presence of secondary natural enemies, inefficient rearing and release methods, as well as introduction of ineffective host-specific parasitoids of other mealybug species, this topic was temporarily abandoned. However, efforts in biological control were recently revived at CRIG, and trials with the exotic predator *Cryptolaemus montrouzieri* and the pathogenic fungus *Beauveria bassiana* were conducted (Plantwise, 2016). Moreover, more attention should be given to the suggestion of farmers to control mealybugs by altering ant-complexes in the field, shifting the dominance from ant species, which are mealybug-tendering (*Crematogaster* spp.) to those who are not (*Oecophylla* spp.) (Ayenor et al., 2004).

5. Conclusion

This is the first time the effectiveness of different preventive control measures for CSSVD are summarized and weighed versus each other in a quantitative way. Since only a fraction of the large body of literature on CSSVD investigated the efficacy of preventive

measures, this meta-analysis is a stepping-stone for consolidating what we know about the combat of CSSVD, and for setting future research priorities. Our study clearly shows that there has been a strong focus on resistance breeding and mild strain cross protection. While these approaches certainly have their potential, they should be combined with other approaches to form an ensemble of solutions, which is needed to solve a problem as extensive as CSSVD. Barrier (strip) cropping and agroforestry systems (shading) might be well suited to complement breeding and mild strain cross protection, as they showed greatest potential to reduce CSSVD in our study. Moreover, these approaches could be implemented directly by farmers themselves. However, there is a severe knowledge gap about these approaches' potential to reduce the spread and/or severity of CSSVD, which has to be addressed by future research. Furthermore, shade should be considered in breeding programs for CSSVD resistance and the practical relevance of different preventive control measures for farmers needs to be studied.

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Appendix A: List of references included in the meta-analysis

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