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Host plants identification of mealybugs main species, vectors of the swollen shoot virus in the counties of Abengourou, Bouaflé and Divo.

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ABSTRACT
Objective: The aim of this study was to determine the abiotic and biotic factors that lead to the spread of swollen shoot disease in Côte d'Ivoire.
Methodology and results: The study was carried out in nine (9) plots in each of the departments of Abengourou, Bouaflé and Divo. In each plot, six 100m^2 plots were set up, three in disease foci and three in disease-free areas (at least 50 meters from the foci). All the plant species in each plot harboring mealybugs were inventoried and identified. The colonies of the mealybug species were also counted. The results showed the presence of four (4) species of scale insects, *Formicococcus njalensis, Ferrisia virgata, Planococcus citri and Pseudococcus longispinus* in the three departments. In addition to these species, *Dysmicoccus brevipes and Phenacoccus hargreavesi* were observed in Divo, making a total of six species. *Phenacoccus hargreavesi* was also observed in Abengourou, for a total of five species. With regard to host plants, 33 plant species were identified as hosts of mealybug species in the three departments. The species *Xanthosoma maffafa, Dioscorea cayennensis, Spondias mombin, Ficus exasperata, Euphorbia heterophylla, Chromolaena odorata* and *Manihot esculenta*, which are among the 33 plant species, are known to host the swollen shoot virus. Finally, the study identified 20 new host plants for one or more CSSV-vector mealybug species.
Conclusion and application of results: Six species of swollen shoot mealybug vectors out of the seven known in Côte d'Ivoire were identified. In addition, 33 plant species were identified as hosts of these mealybugs. Seven (7) of these plants are known to be hosts of the swollen shoot virus and constitute a threat to the spread of the swollen shoot disease because they are both hosts of the mealybugs and hosts of the swollen shoot virus. It is recommended that these plants be systematically eliminated from the plots and that an assessment be made of those whose status is not known with regard to the swollen shoot virus.
Key words: mealybugs; swollen shoot; host plants; cocoa, Côte d'Ivoire.

RESUME

Objectif: Cette étude a pour objectif de connaitre les facteurs abiotiques et biotiques qui entrainent la propagation de la maladie du swollen shoot en Côte d'Ivoire.

Méthodologie et résultats: Elle a été réalisée dans neuf (9) parcelles de chacun des départements d'Abengourou, de Bouaflé et de Divo. Ainsi, dans chaque parcelle, six placettes de 100m^2 dont trois dans les foyers de la maladie et trois dans les parties indemnes (distantes de 50 mètres minimum des foyers) ont été installées. Toutes les espèces végétales contenues dans chaque placette et hébergeant les cochenilles ont été inventoriées et identifiées. Par ailleurs, les colonies des espèces de cochenilles ont été dénombrées. Les résultats ont montré la présence de quatre (4) espèces de cochenilles, *Formicococcus njalensis*, *Ferrisia virgata*, *Planococcus citri* et *Pseudococcus longispinus* dans les 3 départements. En plus de ces espèces, *Dysmicoccus brevipes* et *Phenacoccus hargreavesi* ont été observées à Divo, soit 6 espèces au total. *Phenacoccus hargreavesi* a été également observé à Abengourou, soit au total 5 espèces. Concernant les plantes hôtes, 33 espèces végétales ont été identifiées comme hôtes des espèces de cochenilles farineuses dans les 3 départements. Les espèces *Xanthosoma maffafa*, *Dioscorea cayennensis*, *Spondias mombin, Ficus exasperata, Euphorbia heterophylla, Chromolaena odorata* et *Manihot esculenta* qui figurent parmi les 33 espèces végétales sont connues comme hôtes du virus du swollen shoot. Enfin, l'étude a mis en évidence 20 nouvelles plantes hôtes d'une ou plusieurs espèces de cochenilles vectrices du CSSV.

Conclusion et application des résultats: Six espèces de cochenilles vectrices du swollen shoot sur les sept connues en Côte d'Ivoire ont été mis en évidence. Par ailleurs 33 espèces végétales ont été identifiées comme hôtes de ces cochenilles. Parmi ces plantes, sept (7) sont connues comme hôtes du virus du swollen shoot et constitue une menace de propagation de la maladie du swollen shoot du fait qu'elles soient à la fois hôtes des cochenilles et hôtes du virus du swollen shoot. Il est donc recommandé d'eliminer systematiquement ces plantes des parcelles et faire une évaluation de celles dont les statuts ne sont pas connus vis à vis du virus du swollen shoot.

Mots clés: cochenilles; swollen shoot; plantes hôtes; cacaoyer, Côte d'Ivoire.

INTRODUCTION

The cocoa tree (*Theobroma cacao* L., 1759) is a tropical perennial plant of the family Malvaceae (Motamayor *et al.*, 2002) formerly classified in the family Sterculiaceae (Metcalfe and Chalk, 1950). It is native to South America, mainly the Amazon basin (Motamayor *et al.*, 2002). Cocoa cultivation is important worldwide and employs 14 million people in West Africa (Oro, 2011). Ghana, Togo, Nigeria and Côte d'Ivoire alone produce 70% of world production. Côte d'Ivoire is the world's leading cocoa producing country with a production of about 2, 248,000 tones in the 2020-2021 season (ICCO, 2022). This large cocoa production contributes to more than 14% of its Gross Domestic Product (CCC, 2017) and is the main source of livelihood for 800,000 smallholder farmers in rural areas. Currently, cocoa accounts for about 38% of Côte d'Ivoire's export earnings and thus provides important economic support to the country (CCC, 2017). Despite this socio-economic importance, cocoa production in Côte d'Ivoire is faced with many constraints that cause a significant decrease in production. Among these constraints, biotic ones occupy an important place. Mirids and cocoa stem borers are the main pests of cocoa in Côte d'Ivoire. These pests can cause 30 to 40% of production losses (N'Guessan and Coulibaly, 2001). Diseases are also a source of concern. Among them, black pod disease, which was once considered to be of minor importance, is becoming more prevalent every year. In Côte

d'Ivoire, this disease caused by *Phytophthora* spp. causes production losses from 10% to 60% (Coulibaly et *al.,* 2018). Parasitic plants weaken cocoa trees and require increased vigilance (Aka *et al.*, 2017). Today, the resurgence of swollen shoot in a new threat for cocoa cultivation (Kébé et al., 2006). Swollen shoot of cocoa is one of the most economically important plant viral diseases in the world (Dzahini-Obiatey *et al.,* 2010). The disease was first discovered in Ghana in 1922 (Steven, 1936). Subsequently, swollen shoot was observed in several other West African countries including Côte d'Ivoire in 1943 (Alibert and Mallamaire, 1946). Cocoa swollen shoot is a disease transmitted by mealybugs of the family *Pseudococcidae* (Thresh, 1986). Several species of mealybugs are capable of transmitting the virus. The most important are *Formicococcus njalensis, Planococcus citri* and *Ferrisia virgata* (Posnette, 1950; Dufour, 1988; Dufour *et al*., 1993). The mealybugs acquire the Swollen Shoot virus by feeding on infected cocoa trees and later transmit it to healthy cocoa trees (Kébé *et al.*, 2016). However, some plant species (shade plants, weeds, food crops, etc.) can also host the virus (Kébé *et al*., 2016). These potential hosts can also participate in the spread of the disease (Tinsley, 1971). In addition, other plant species can host the mealybug vectors of the disease. These plant species may serve as mealybug propagation media or as mealybug refuges, or they may be both mealybug and virus hosts. In order to understand the spread of the disease in the orchard and to develop an effective control method against swollen shoot, knowledge of the mealybug host plants is necessary. This study was based on the main hypothesis that biotic and abiotic factors are involved in the spread of swollen shoot disease in Côte d'Ivoire. The following specific hypotheses follow from this hypothesis:

- some plants species host the main species of mealybug vector of the swollen shoot virus in the main cocoa-producing areas of Côte d'Ivoire,
- some plants species host both the mealybug vectors and the pathogen of the swollen shoot disease in infected areas.

MATERIAL AND METHODS

Location of the study: The study was conducted in the counties of Abengourou, Bouaflé and Divo. These three **counties**, which constitute the study area, were selected based on their different agro-ecological characteristics and are host cocoa farms infected by the swollen shoot virus. The county of Abengourou is located in the eastern part of Côte d'Ivoire between latitudes 5°45 and 7°10 North and longitudes 3°10 and 3°50 West. It represents the old main cocoa production region characterized by a senescent orchard and also the first county where swollen disease was detected in the years 1943. The second county, Bouaflé is located in the central-west of Côte d'Ivoire and is characterized by wooded savannahs and secondary forests, which makes Bouaflé an essentially agricultural region. However, the resurgence of swollen shoot disease in 2003 caused huge losses in cocoa production in this county (Kébé and N'Guessan, 2003). Finally, the county of Divo is located in the southwest of Côte d'Ivoire and is marked by the cessation of cocoa production, the ageing of the orchard and the decline in soil fertility (Aka *et al*., 2013; Yao *et al*., 2014). This region is influenced by the humid sub-equatorial climate with an average annual rainfall of 1,400 mm unevenly distributed over an average temperature of 26˚C. Atmospheric humidity is around 86% (Kouamé *et al.*, 2017). The average temperature is 27 °C and varies annually between 19 °C and 33 °C, which allows for good cocoa yields.

Experimental design : The study set-up consisted of nine (9) plots distributed along three axes with three plots per axis in each of the counties of Abengourou, Bouaflé and Divo. For each plot, six plots of 10 m sides, three of which were inside the focus area and three outside the focus area, were set up (Figure 1).

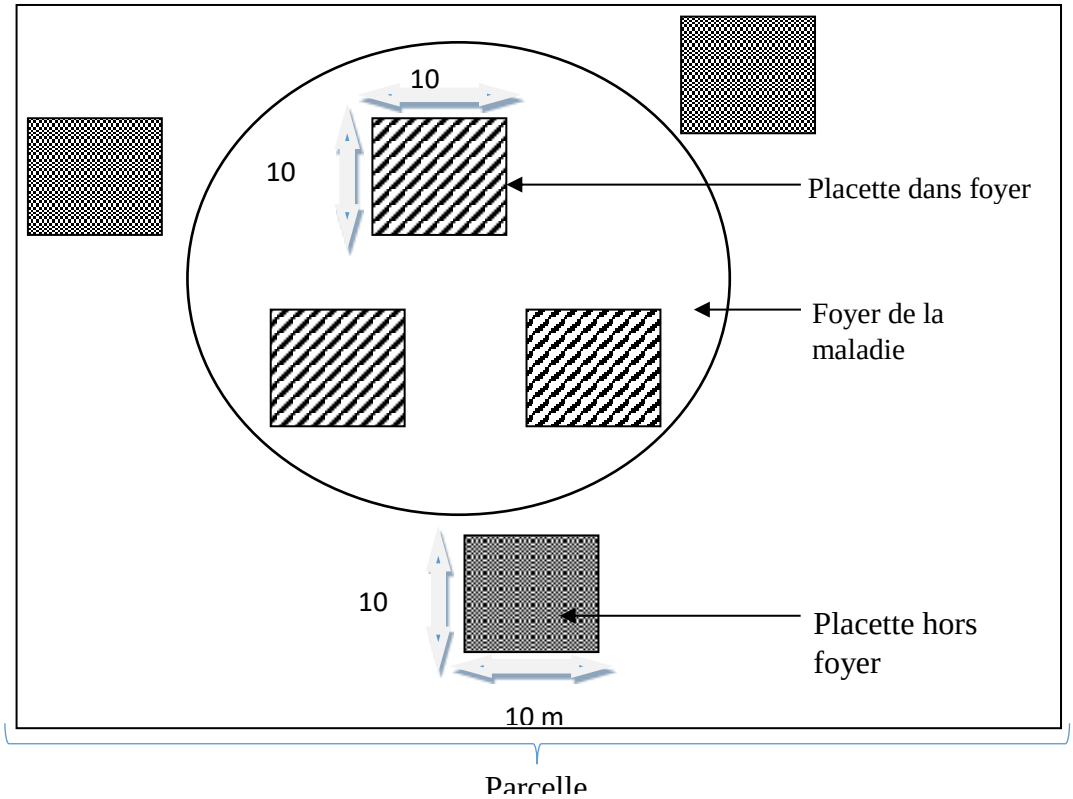

Figure 1: Schematic of the experimental setup

Data collection and analysis: All plant species in each plot were observed individually and those hosting mealybugs were inventoried, identified and recorded on a bloc note. The total number of host plants either was determined by direct counting or by estimation based on a rule of three, which extrapolates the number of four m^2 plants over 100 m^2, the area of a plot. The rule of three is applied when the plant to be identified is very abundant in the plot. The plants were identified in the field or taken with pruning shears and identified in the laboratory. In addition, the mealybugs species and the number of colonies were determined. The mealybugs were collected in the field and identified in the laboratory after slide-mounting using the technique of Sirisena *et al.* (2013). The collected data were subjected to analysis of variance using SAS software. The classes obtained were separated using the Student-Newman-Kheuls test at the 5% threshold.

RESULTS

List and frequency of plant species hosting mealybugs observed by county: The county of Abengourou hosted 23 host plants representing 15 families. Out of the 23 plant species observed, 17 were present in the foci and six were observed in the disease-free plot (Table 1). The county of Divo hosted 15 plant species included into nine families. Out of the 15 species observed in Divo, 13 were present in the foci and two were observed in the

disease-free area. Finally, in the county of Bouaflé, 12 plant species, also included into nine families were observed. Out of the 12 species observed, eight were present in the foci and four were observed in the disease-free plot.

Table 1: Plant species observed in foci and disease-free areas in Abengourou, Bouaflé and Divo

Families	Plant species	Abengourou		Bouaflé		Divo	
		foci	disease-free plot	foci	disease-free plot	foci	disease-free plot
Acantahceae	*Phaulopsis falcisepala*	1					
Anacardiaceae	*Spondias mombin*					1	
Apocynaceae	*Holarhena floribunda*		1				
Araceae	*Xanthosoma maffafa*	14		2		4	
Asteraceae	*Ageratum conizoides*	3		1			
	Chromolaena odorata			1		5	
Bromeliaceae	*Pineapple comosus*					1	
Combretaceae	*Combretum velutinum*	1					
Connaraceae	*Byrsocarpus coccineus*		1				
Cucurbitaceae	*Momordica charantia*				1		
Dioscoreaceae	*Dioscorea cayennensis*	1					
Euphorbiaceae	*Alchornea cordifolia*	2					
	Croton hirtus	5				5	
	Euphorbia heterophylla			1			
	Mallotus oppositifolius					1	
	Manihot esculenta	1		1		1	
Fabaceae	*Baphia nitida*	1					
	Centrosema pubescens		1				
	Glycyrrhiza glabra						1
	Milletia zechiana	4	2	2		1	
Lamiaceae	*Mint aquatica*					1	
Malvaceae	*Abelmochus esculentus*					1	
	Theobroma cacao	22	28	30	60	27	80
Mimosaceae	*Albisia sp*	1					
Moraceae	*Ficus exasperata*	2	1				
	Milicia regia						
Musaceae	*Musa paradisiaca*	4		4	2		
Solanaceae	*Solanum erianthum*	1		10		6	
	Solanum lycopersicum	1					
	Solanum melongena	1				3	
	Solanum rugosum	1		2		5	
	Solanum torvum	1					
Verbenaceae	*Clerodendrum umbellatum*	1					

Geographical distribution and species diversity of mealybugs within the counties: The mealybug species *F. njalensis*, *F. virgata*, *P. citri* and *P. longispinus* were present in all three counties. *D. brevipes* was only observed in Divo while *P. hargreavesi* was observed in Divo and Abengourou (Table 2).

Table 2: Geographical distribution and diversity of mealybug species observed by county

Counties	**Mealybug species**						
	F. njalensis	*F. virgata*	*P. citri*	*P. longispinus*	*D. brevipes*	*P. hargreavesi*	Not identified
Abengourou	X	X	X	X	-	X	X
Bouaflé	X	X	X	X	-	-	X
Divo	X	X	X	X	X	X	X

Comparison of the number of mealybug colonies between counties: The analysis of variance revealed a significant difference ($P < 0.05$) in the number of *Planococcus citri* colonies between counties. The county of Divo presented the highest number of colonies of this mealybug with 75 ± 17 colonies per 100 trees of host plant. With 20 ± 7 colonies per 100 trees of host plants, the county of Bouaflé had the lowest number of colonies of *Planococcus citri*. On the other hand, the analysis of variance did not show any significant difference ($P > 0.05$) in the number of colonies of the other mealybug species between the counties (Table 3). Nevertheless, *Formicococcus njalensis* was the wide spread among the mealybug species while *D. brevipes* and *P. hargreavesi* were rarely observed.

Table 3: Number of colonies of each scale species observed per county

Counties	**Mealybug species**						
	F. njalensis	*F. virgata*	*P. citri*	*P. longispinus*	*D. brevipes*	*P. hargreavesi*	Not identified
Abengourou	212 ± 51a	70 ± 31a	51 ± 10ab	66 ± 31a	00 ± 0,00a	5,5 ± 0,55a	32,8 ± 24,8a
Bouaflé	269 ± 35a	58 ± 21a	20 ± 7b	5 ± 3a	00 ± 0,00a	00 ± 0,00a	12 ± 5a
Divo	290 ± 54a	50 ± 16a	75 ± 17a	50 ± 16a	1 ± 1a	3 ± 3a	20 ± 20a
Meaning (P)	**0,50**	**0,84**	**0,03**	**0,11**	**0,37**	**0,59**	**0,55**

Values in the same column followed by the same letter do not differ significantly from one another at P = 0.05 with the Student-Newman-Keuls tes

Host plants of the different mealybug species: In all counties, 33 plant species were identified as potential hosts of mealybug species. These plant species were marked by one cross (X) for associations known in the literature between mealybugs and host plants and by two crosses (XX) for associations newly observed through this study. The species *P. citri* was observed on 18 different plant species followed by *P. longispinus* with 16 plant species and *F. virgata* with 15 host plants. Conversely, the species *F. njalensis was* observed on seven host plants, *P. hargreavesi* on two host plants and *D. brevipes* on one host plant**.** Apart from these mealybug species, unidentified specimens were observed on seven plant species. In terms of host plants, the plant species *Millettia zechiana*, *Musa paradisiaca*, *Solanum erianthum*, *Solanum rugosum*, *Theobroma cacao* and *Xanthosoma maffafa* each hosted four different species of mealybug. As for the species *Crotton hirtus*, it hosted three different species of mealybug. (Table 4).

Table 4: Host plants associated with different mealybug species

Plant species	*Formicococcus njalensis*	*Planococcus citri*	*Pseudococcus longispinus*	*Ferrisia virgata*	*Phenacoccus hargreavesi*	*Dysmicoccus brevipes*	Not identified
Abelmochus esculentus			XX				
Ageratum conizoides							X
Albisia sp							X
Alchornea cordifolia	X						
Ananas comosus						X	
Baphia nitida		XX					
Byrsocarpus coccineus			XX				
Centrosema pubescens				XX			
Chromolaena odorata	XX		XX				
Clerodendrum umbellatum			X	XX			
Combretum velutinum		XX					
Croton hirtus		X	X	X			X
Dioscorea cayennensis				XX			
Euphorbia heterophylla				XX			
Ficus exasperata		X	X				
Glycyrrhiza glabra			XX				
Holarhena floribunda							X

Plant species	*Formicococcus njalensis*	*Planococcus citri*	*Pseudococcus longispinus*	*Ferrisia virgata*	*Phenacoccus hargreavesi*	*Dysmicoccus brevipes*	Not identified
Mallotus oppositifolius				XX			
Manihot esculenta		X	X				
Mentha aquatica			XX				
Milicia regia				XX			
Millettia zechiana	XX	XX	XX	XX			
Momordica charantia				X			
Musa paradisiaca	XX	X	X	X			
Phaulopsis falcisepala		XX					
Solanum erianthum	XX	X		XX	XX		
Solanum lycopersicum							X
Solanum melongena		X		X			
Solanum rugosum	XX	X	X	XX			
Solanum torvum			X				
Spondias mombin			XX				
Theobroma cacao	X	X	X	X			X
Xanthosoma maffafa		XX	X	XX	XX		

XX = Mealybug species associated with new plant species

DISCUSSION

The study revealed six mealybug species vectors of CSSV out of the seven known in Côte d'Ivoire (N'Guessan *et al*., 2019) and other unidentified mealybug specimens in the three counties visited. These species are *Formicococcus njalensis*, *Planococcus citri*, *Pseudococcus longispinus*, *Ferrisia virgata*, *Dysmicoccus brevipes* and *Phenacoccus hargreavesi*. These results are similar to those of N'Guessan *et al.* (2019) in his study on the inventory of mealybug species in the cocoa orchard in Côte d'Ivoire. During his work, nine species of mealybugs were identified in several cocoa production areas in Côte d'Ivoire. These species include all the species that have been identified in this study. However, species such as *F. njalensis*, *F. virgata*, *P. citri* and *P. longispinus* were present in all three counties. In addition to these species, *D. brevipes* was observed in Divo and *P. hargreavesi* was observed in Divo and Abengourou. In sum, the results showed that the county of Abengourou recorded five species of mealybug while Bouaflé presented four. The greatest diversity was observed in the county of Divo with six mealybug species. Compared to the regions of Abengourou and Bouaflé, Divo seems to be the most watered area and would therefore be more favorable to sucking insects such as mealybugs (N'Guessan, 2021). In the three counties, the results also showed that the mealybug species *Formicococcus njalensis* was the most observed. The predominance of *F. njalensis* in the study of swollen shoot disease has been shown by several authors (Posnette, 1950; Andres, 2017; N'Guessan *et al*., 2019). Furthermore, the results showed a significant difference in the number of *Planococcus citri* colonies between the counties. The county of Divo had the highest number of colonies of this mealybug and the county of Bouaflé had the lowest. This particular difference in *Planococcus citri* species reflects a geographical distribution that varies according to host plant species and climatic conditions (Ouattara *et al*., 2016). It may be due to a differentiation of shade loss of cocoa trees in infected plots between counties. The plots that were visited in Divo would therefore be more advanced in the swollen shoot disease than those visited in the other two counties would. According to Dufour (1991), when there is a deterioration in the shade of cocoa trees and the appearance of clearance in the farms, conditions become more favorable for *Planococcus citri* rather than *Formicococcus njalensis*. The survey also identified potential host plants for these mealybug species in all three counties visited. Across the counties, 33 plant species were identified as potential hosts of mealybug species. Six (6) of these plant species (*Xanthosoma maffafa, Manihot esculenta, Milletia zechiana, Solanum erianthum, Solanum rugosum* and *Theobroma cacao*) were common to all three counties. On the other hand, the plants *Xanthosoma maffafa, Dioscorea cayennensis, Spondias mombin, Ficus exasperata, Euphorbia heterophylla, Chromolaena odorata* and *Manihot esculenta* are known to host the swollen shoot virus. These can cause the rapid spread of swollen shoot disease in a cocoa farm because they are both hosts of mealybugs and hosts of swollen shoot virus. Posnette *et al.* (1950), documented that the presence of wild alternative hosts of the virus in and around cocoa farms is a contributing factor to the spread and early reinfection of new cocoa plantations in Ghana. Regarding the relationships between mealybugs and plants, *P. citri* was observed on 18 different plant species and was the species with the highest number of host plants in this study. Unlike other mealybug species, *P. citri* would be able to adapt to several living environments such as cocoa farms infected with swollen shoot disease. This study identified new host plants of mealybugs, vectors of the swollen shoot virus. Indeed, among the mealybugs identified in this study,

some were observed for the first time on plants (Garcia *et al*., 2016). Future research should therefore take into account the detection of CSSV in these plants while increasing their identification range.

CONCLUSION AND APPLICATION OF RESULTS

This study was carried out with a view to identifying the host plants that harbor the mealybug vectors of the swollen virus in infected areas. It was carried out in three departments, namely Abengourou, Bouaflé and Divo. The results showed the presence of CSSV-vector mealybugs, other mealybug specimens and mealybug host plant species. Six of the seven mealybug vector species found in Côte d'Ivoire were identified in the departments. These were *Formicococcus njalensis*, *Planococcus citri*, *Pseudococccus longispinus*, *Ferrisia virgata*, *Phenacoccus hargreavesi* and *Dysmococcus brevipes*. The mealybug species *Formicoccus njalensis* was the most common in all departments. A total of 33 host plants were identified, 20 of which were the first to harbor the identified mealybugs. Seven of these host plants are also host plants for the swollen shoot virus: *Xanthosoma maffafa*, *Dioscorea cayennensis*, *Spondias mombin*, *Ficus exasperata*, *Euphorbia heterophylla*, *Chromolaena odorata* and *Manihot esculenta*. It is recommended that these plants be systematically eliminated from the plots and that an assessment be made of those whose status is not known with regard to the swollen shoot virus.

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