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INFLUENCE OF ALTERNATIVE PLANT HABITATS ON CASSAVA WHITEFLY COLONISATION IN MALAWI

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ABSTRACT

Cassava (*Manihot esculenta* Crantz) is an important food security and income generating crop in Malawi; whose productivity is constrained by the whitefly (*Bemisia* spp.) pest complex and associated viral diseases. Current cassava tuber yields in the country are estimated at barely 30% of the potential yield of 91 tonnes per hectare. The objective of this study was to investigate the effect of alternative whitefly host plants within habitats, on whitefly infestation in cassava fields in Malawi. A field study was conducted at Lilongwe and Nkhata Bay in Malawi, in 2017. Treatments included (i) target cassava (<2 months old, and old cassava (8 - 13 months after planting); (ii) natural vegetation; (iii) pumpkin; (iv) sweet potato; (v) beans; (vi) groundnuts; (vii) Irish potato; (viii) soybean; (ix) tobacco and (xi) tomato. Clean sentinel cassava plants were placed in each habitat (three pots per habitat), at the beginning of the growing season. They were collected after four weeks to assess the relative colonisation rates of whitefly, by determining the number of nymphs. Plants placed in the cassava habitats presented the greatest number of nymphs (39.3); followed by natural vegetation (17.8) and old cassava (14.1); compared to other habitats. There were no significant differences ($P>0.05$) in the amount of parasitism of nymphs between habitats. This study suggests that in diverse landscapes alternative habitats can substantially influence whitefly population dynamics in cassava fields.

Key Words: *Bemisia tabaci*, *Manihot esculenta*, sentinel cassava

RÉSUMÉ

Le manioc (*Manihot esculenta* Crantz) est une culture importante pour la sécurité alimentaire et la génération de revenus au Malawi. Sa productivité est limitée par le complexe de ravageurs de la mouche blanche (*Bemisia* spp.) et les maladies virales associées. Les rendements actuels de tubercules de manioc dans le pays sont estimés à peine à 30 % du rendement potentiel de 91 tonnes par hectare. L'objectif de cette étude était d'examiner l'effet de plantes hôtes alternatives de la mouche blanche dans les habitats, sur l'infestation de la mouche blanche dans les champs de manioc au Malawi. Une étude de terrain a été menée à Lilongwe et à Nkhata Bay au Malawi, en 2017. Les traitements comprenaient (i) le manioc cible (< 2 mois et le vieux manioc (8 à 13 mois après la plantation); (ii) la végétation naturelle; (iii) la citrouille; (iv) la patate douce; (v) les haricots; (vi) les arachides; (vii) la pomme de terre; (viii) le soja; (ix) le tabac et (xi) la tomate. Des plants de manioc sentinelles propres ont été placés dans chaque habitat (trois pots par habitat), au début de la saison de croissance. Ils ont été collectés après quatre semaines pour évaluer les taux de colonisation relatifs de l'aleurode, en déterminant le nombre de nymphes. Les plants placés dans les habitats de manioc présentaient le plus grand nombre de nymphes (39,3); suivis de la végétation naturelle (17,8) et du vieux manioc (14,1); par rapport aux autres habitats. Il n'y avait pas de différences significatives ($P > 0,05$) dans la quantité de parasitisme des nymphes entre les habitats. Cette étude suggère que dans des paysages variés, des habitats alternatifs peuvent influencer considérablement la dynamique des populations d'aleurodes dans les champs de manioc.

Mots Clés: *Bemisia tabaci*, *Manihot esculenta*, manioc sentinelle

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is an important food security and income generating crop in Malawi (Mwebaze *et al.*, 2022). However, its productivity is heavily constrained by diseases and pests; particularly the cassava whiteflies (*Bemisia* spp.) (Campo *et al.*, 2011; Chikoti and Tembo, 2022). Cassava has a potential yield of 91 tonnes per hectare, but owing to biophysical constraints, including whitefly infestations, current statistics reveal national yields of less than 30 tonnes per hectare (Adiele *et al.*, 2021). In a study conducted in Tanzania during 2014 - 2016, yield losses as high as 60% were reported to be prevalent due to CMD, CBSD and whitefly infestation (Mkamilo *et al.*, 2024).

Whiteflies are a major pest due to their ability to spread at least two cassava viral diseases, namely, cassava mosaic and cassava brown streak diseases (CMD and CBSD, respectively) (Maruthi *et al.*, 2005; Gamarra *et al.*, 2016). Cassava mosaic disease is caused by *Begomoviruses* of the family

Geminiviridae; while CBSD is caused by *Ipomoviruses* of the family Potyviridae. The whitefly species prevalent on cassava in Malawi include the *Bemisia afer* (Priesner and Hosny) and the pest complex of *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae) (Tay *et al.*, 2022). However, the role of *B. afer* in transmission of *Ipomoviruses* is not fully understood. These diseases are also spread *via* the use of infected cassava cuttings by farmers. Further damage from whiteflies is through sucking plant sap, leading to shoot chlorosis, leaf distortion and low root yield (Shirima *et al.*, 2019). Moreover, honeydew secreted by nymphs can lead to the growth of sooty mould on leaf surfaces, which then reduces photosynthetic area.

Possible factors that may influence the abundance of whitefly populations in cassava fields were reviewed by Macfadyen *et al.* (2018); and alternative non-cassava host plants were among the factors identified as potentially having an influence on the whitefly populations on cassava (Sequeira *et al.*, 2009). Although many plants may be suitable for whitefly adults

to alight upon, the choice to oviposit is not guaranteed, and survivorship may be different on each plant type.

In Uganda, non-cassava crops were found to be reproductive hosts of *B. tabaci* species complex (Kalyebi *et al.*, 2021). Nymphs collected from leaves of soybean, groundnut and sesame were identified as *B. tabaci* Sub-Saharan Africa 1 (SSA1); while other *B. tabaci* species nymphs were recorded on pumpkins, beans, sweet potato and *Euphorbia* weeds (Kalyebi *et al.*, 2021). In addition to cassava being grown year-round in agroecosystems in Malawi, the alternative host plants used by whiteflies may support high whitefly proliferation, and hence, cassava diseases occurrence. Such host plants in habitats found in cassava landscapes, could provide continuous survival and sources of the whitefly in cassava fields (Kumar *et al.*, 2020). Several plants, other than cassava, have been identified as hosts of whiteflies in Malawi and elsewhere (Macfadyen *et al.*, 2021), but the information on their influence on whitefly colonisation and abundance in cassava is still unavailable.

Natural enemies such as predators, parasitoids, and pathogens, can cause mortality and impact the population dynamics of whiteflies (Qureshi and Stansly, 2009; Sequeira *et al.*, 2009; Katono *et al.*, 2021). Parasitism by Aphelinids in Malawi has been reported in surveys on whiteflies in cassava and tomatoes (Theu and Sseruwagi, 2005; Thindwa and Khonje, 2005; Tay *et al.*, 2022). Knowing the presence of parasitoid at the beginning of the cassava growing season; and the alternative host plants that support them would help guide integrated pest management decisions. The objective of this study was to investigate the impact of alternative whitefly host plants within habitats, on whitefly infestation in cassava in Malawi.

METHODOLOGY

Site description. The study was conducted in Nkhata Bay and Lilongwe districts (Fig. 1); located at 400-600 and 1040-1220 meters

above sea level (m.a.s.l.), respectively. The major soil types in Nkhata Bay sites are Lixisol, Fluvisol and Cambisol; while those in Lilongwe are Luvisol and Gleysol (Omuto and Vargas, 2019). Nkhata Bay receives mean annual rainfall of 1,600 mm and a mean temperature of 23.2 °C; while Lilongwe receives 900 mm and a mean temperature of 19.3 °C.

Twelve fields were selected in each district and were spaced at least five kilometres apart. Cassava fields, irrespective of varieties, were those that had been planted at the beginning of rains in December of 2016.

Experimental design and treatments. This study was part of a twelve-month longitudinal study, of cassava whitefly populations in selected farmers' fields from 2016 to 2017. The study ran from when the target cassava field was one month old (January) to two months old (February). To quantify whitefly resource use in landscapes in Malawi, we placed and monitored sentinel cassava plants, free from pests, in a diversity of habitats as treatments. Treatments included (i) target cassava (<2 months old); (ii) old cassava (8 - 13 months after planting); (iii) natural vegetation (mainly grass (Poaceae), trees (Chrysobalanaceae, Fabaceae, Fabaceae subfamily- Caesalpiniaceae, Combretaceae, Annonaceae); annual herbs, (Asteraceae, Pedaliaceae, Malvaceae)); (iv) pumpkin; (v) sweet potato; (vi) beans; (vii) groundnuts; (viii) Irish potato; (ix) soybean; (x) tobacco; and (xi) tomato.

Treatments were laid out in factorial design and replicated 3 times. The study was repeated 3 times (3 pots in each treatment field patch) in plots of various sizes, ranging from 20-50. m length by 10-30 m width; separation paths of 2-50 m wide. These habitats were selected because they had the potential to contain plants that could host whiteflies and were present in the study regions (Table 1).

Sentinel cassava plants. Clean cassava sentinel plants of the Manykola variety were raised in a greenhouse in five litre plastic

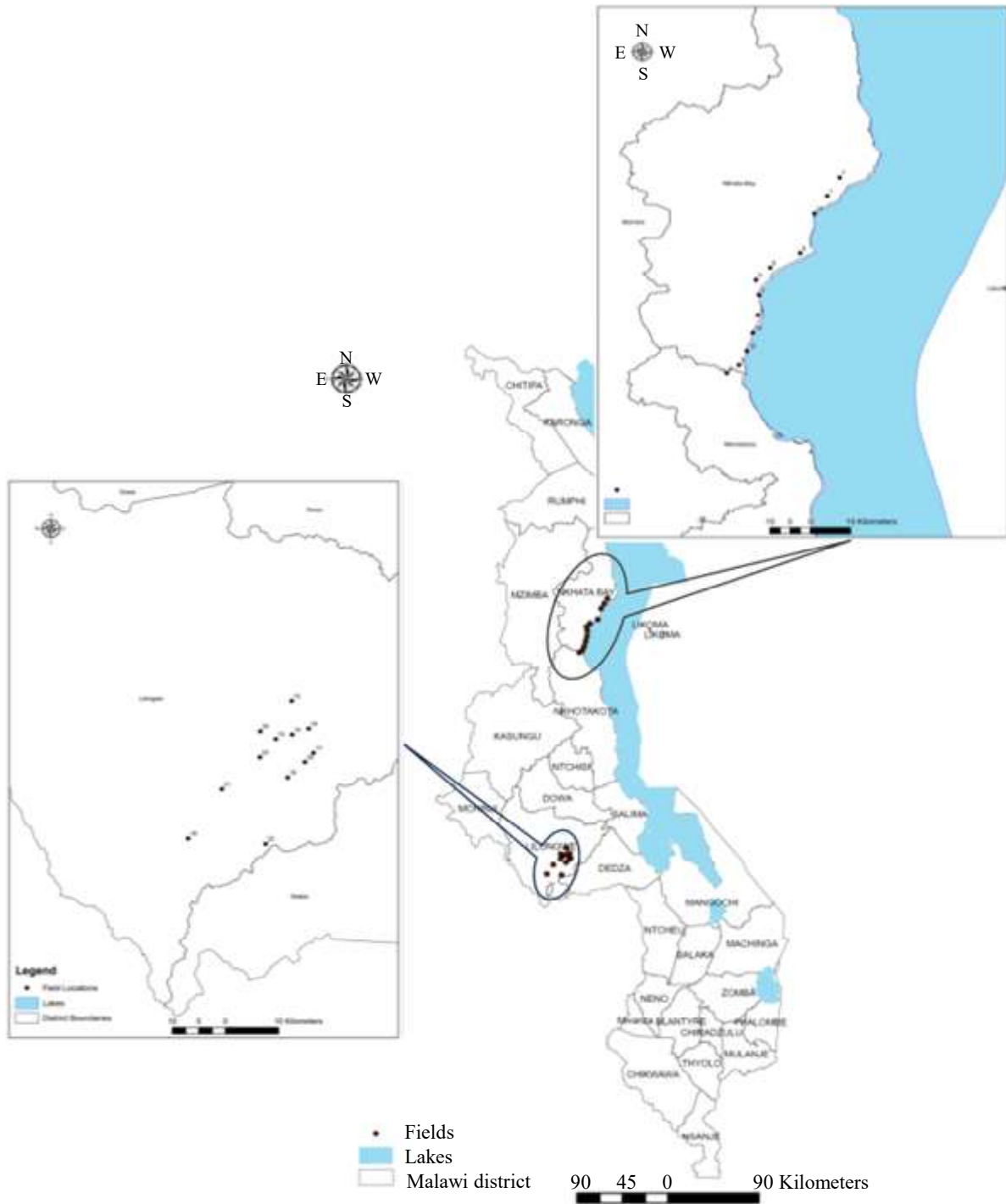


Figure 1. Map of Malawi showing target cassava field sites of Lilongwe and Nkhata Bay. The sites were targeted to be 5 kilometres apart; however, where a cassava field was lacking, the nearest cassava field was chosen.

TABLE 1. Habitats available and selected (+) and not available (-) for placement of sentinel plants around target cassava fields in Nkhata Bay and Lilongwe in January 2017

Habitat	Field ID																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
¹ Target cassava	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
² Old cassava	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
³ Natural vegetation	-	-	-	-	+	-	+	+	-	+	-	-	-	-	-	-	-	-	+	+	-	+	-	-
Sweet potato	+	-	-	-	-	-	+	+	-	-	+	-	+	-	-	-	-	-	+	+	+	-	-	-
Pumpkin	+	-	-	+	+	+	+	-	+	-	+	-	+	-	-	+	+	+	+	+	+	-	-	+
Beans	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Groundnuts	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	+	-	+
Irish potato	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Soybean	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-	+	-	-	-	-	+	+
Tobacco	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-
Tomato	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	+	-	-	-

¹ Target cassava was the chosen field of newly planted cassava; ² Old cassava was 8 to 13 months after planting; ³ Natural vegetation represent natural vegetation with mainly grass (Poaceae), trees (Chrysobalanaceae, Fabaceae, Fabaceae subfamily -Caesalpinaceae Combretaceae, Annonaceae,) and annual herbs, (Asteraceae, Pedaliaceae, Malvaceae)

buckets (pots) of 23 cm in height and 13 cm in diameter (manufactured by ArKay Plastics, Ltd., Blantyre, Malawi). Sentinel plants are a bioassay technique established for use in field insect monitoring at specific points in the growing season of the focal crop (Mansfield *et al.*, 2019). They can be either clean to monitor the presence of insect pests in the field or pre-infested with insect pest for monitoring natural enemies. Sentinel plants as tools in entomological studies are widely used (Wang *et al.*, 2004; Feng *et al.*, 2017; Kalyebi *et al.*, 2018; Mkenda *et al.*, 2019), but to address different research questions.

The soil used in the pots was collected from Bunda forest; it was rich in organic matter (9.7%). The soil was watered to field capacity. The plants were kept free of pests and diseases before exposure in the field to represent a uniform resource for whiteflies that enables comparisons between habitats. Three potted clean cassava plants were placed in each habitat for whitefly colonisation. At the time of placement in the field, the plants were roughly the same age (one month) as the target cassava field plants managed by the farmers.

Data on nymphs in sentinel plants. The sentinel plants were placed in habitats after four weeks of growth; and exposed in the habitats for four weeks. Figures 2 and 3 are examples target cassava field site maps, showing the placement of sentinel plants. The habitats were positioned from five meters to one hundred meters from the target cassava field. In each habitat, three sentinel pots were placed in a zigzag manner to spread them equally across each field unit (Figs. 2 and 3).

Four weeks later, the pots were collected and returned to the laboratory at Lilongwe University of Agriculture and Natural Resources, Bunda College. Nymphs from each plant were counted from every leaf, including the exuviae, when present; and recorded separately. Hand lenses and stereo-microscopes with 10X magnification, were used to view and count the nymphs on the undersides of cassava leaves.

Whitefly adults and nymphs. The number of whitefly adults and nymphs per plant was determined in the same habitats in January and at the collection of sentinel plants in February. The scouting was done by sampling ten plants haphazardly across the area covered by the habitat. The upper surface of the top five leaves of cassava were assessed for adults and the underside of three older leaves down the stem (leaves 8 to 12 from the top) were assessed for nymphs, only one prominent branch was assessed on branched plants (Legg *et al.*, 2006; Omongo *et al.*, 2012).

There was no species identification in the field survey, as it was challenging to identify whiteflies and nymphs down to species level under field conditions. For the trailing plants (sweet potatoes and pumpkins), the number of adults were also checked from the top five leaves of the main vine and later nymphs recorded from the next three leaves (Omongo *et al.*, 2012). Scouting in beans followed a similar trend, observing the top three bean leaves for adults before inspecting the lower next leaves for nymphs. All the leaflets of the trifoliate leaf were inspected. Similarly, non-crop plants (natural vegetation) were carefully checked for whitefly adults and nymphs by turning over leaves.

Parasitism of nymphs. The whitefly nymphs that were in the third and fourth instar from both sentinel plants and habitat plants, were reared by placing leaf sections, on which they fed, in emergence containers. These small containers (2.5 centimetre by 3 centimetre diameter) were made of black painted plastic, with a clear plastic 5 millimetre vial inserted onto the lid. Emerging adult whiteflies and wasps moved into the clear vial and were trapped for easy counting. Nymphs from different plants were incubated separately and the results recorded.

Data analysis. Counts of whitefly nymphs from every sentinel plant were entered individually into an Excel sheet, then the means were calculated using R software version

Site map for field 13

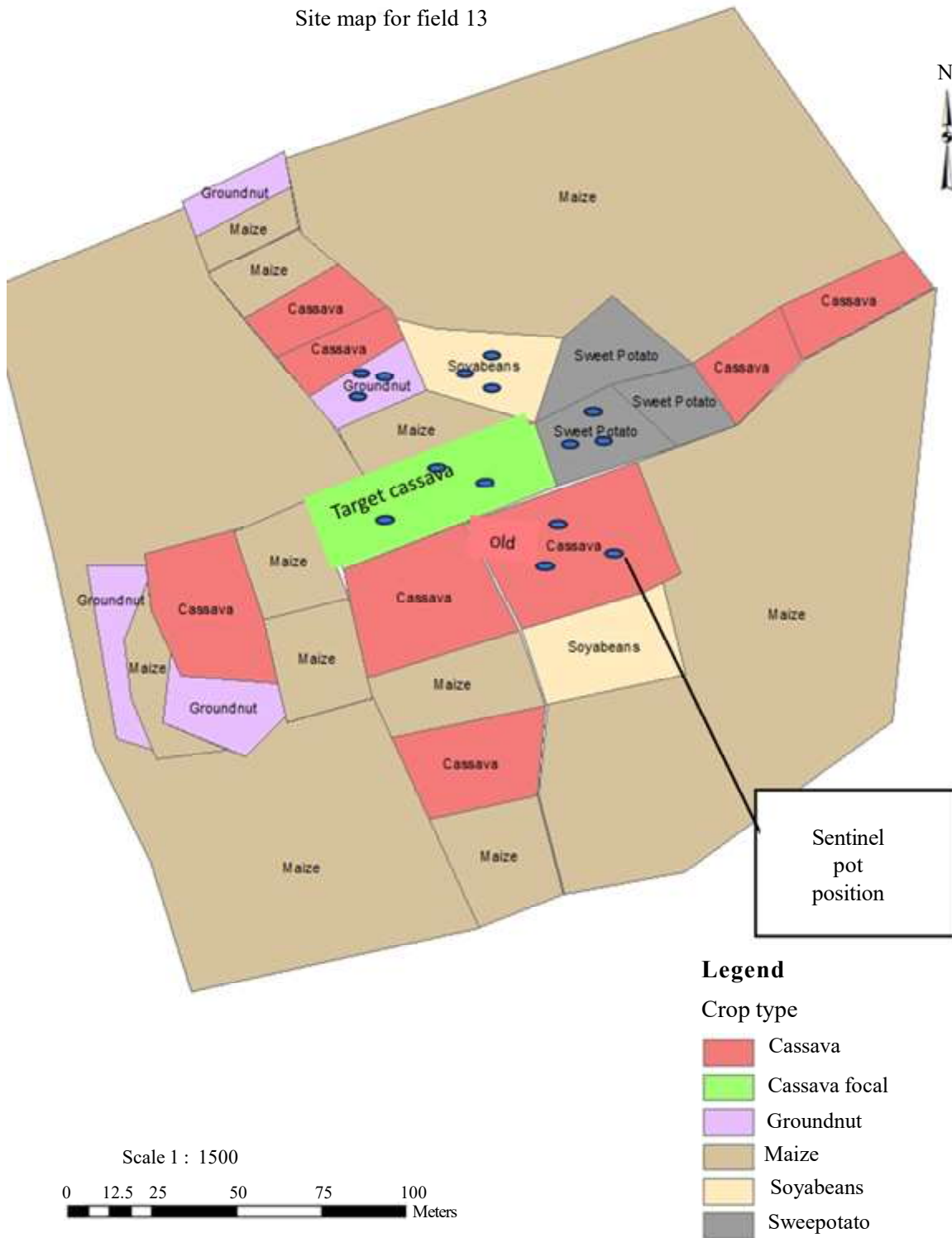


Figure 2. Landscape around one target cassava field 13 showing different forms of whitefly habitats where sentinel plant pots were placed in Lilongwe, Malawi.

Site map for field 5



Figure 3. Landscape around one target cassava field 5 showing different forms of whitefly habitats where sentinel plant pots were placed in Nkhata Bay.

4.3.1, library 'dplyr' in package 'tidyverse'. Field one of Nkhata Bay was not included in the analyses because cowboys removed all sentinel pots on that site, except one. An analysis of variance using a negative Binomial Generalised Linear model, was used to compare the mean nymphs between each habitat type. Analysis was completed using R software version 4.3.1, using library 'MASS' from package 'MASS' (Venables and Ripley, 2002). The formula used was:

```
glm.nb=whitefly nymphs ~ habitat
```

Where:

nb = 0.2548, link = log, AIC = 628.31; and residual deviance = 100.86 on 91 df (degrees of freedom).

Significant mean separation was by LSD Test function in R agricolae library. The number of nymphs on sentinel plants from Lilongwe were very few, most plants had zero nymphs; however, when we combined these with the Nkhata Bay data, the models could not be resolved. Therefore, the Lilongwe data were separated and no hypothesis testing was performed.

The numbers of nymphs and adults from the naturally occurring plants in each habitat were entered in Excel and subjected to computation of mean numbers per plant per habitat patch in R software version 4.3.1, library 'dplyr' which were used in the statistical analysis.

Descriptive means were calculated for the number of whitefly adults and nymphs in habitats in Lilongwe, using the 'dplyr' from package MASS in R software (Venables and Ripley, 2002). For counts in Nkhata Bay habitat plants, the 'ggplot2' was used to plot means with standard errors. To test the association between numbers of whitefly nymphs on plants in habitats and nymphs on sentinel plants, the Spearman's rank correlation test in 'corrplot' from package 'corrplot' was used. When a significant correlation was observed, a robust regression analysis from package

'robustbase' was used to understand how the presence of nymphs on habitat plants influenced sentinel plant colonisation.

The proportion of parasitised nymphs from sentinel plants were summarised per habitat since the numbers were few on plant basis. The data on parasitism did not meet the ANOVA assumptions; hence, the Kruskal Rank sum test was performed to assess for differences between habitats.

RESULTS

Whitefly nymphs in sentinel plants.

Sentinel plants placed in target cassava fields had the highest level of whitefly colonisation. This was followed by those in natural vegetation and old cassava fields; while the least colonisation was in plants placed in sweet potato, followed by those in pumpkin (Table 2).

This trend, however, was affected by location, with Lilongwe having colonisation being generally low in all habitats, although sentinel plants placed in target cassava were more colonised than those in other habitats (Table 3). In Nkhata Bay sentinel cassava plants placed in target cassava had a significantly higher population of nymphs (mean=39.3), than in natural vegetation, old cassava, pumpkin and sweet potato habitats (P=0.002). However, nymph counts on sentinel cassava placed in natural vegetation and old cassava, were similar; but significantly higher than those in pumpkin and sweet potato habitats (Table 2). Sentinel plants placed in the Lilongwe habitats (Table 3) had nymphs with a grand mean of 0.13 and were not significantly different among habitats (P =0.51).

When examining the naturally occurring plants in habitats in Nkhata Bay, certain habitats (natural vegetation, old cassava, pumpkin, and sweet potato) were frequently colonised by whitefly adults or nymphs (Table 4). While in Lilongwe there were very low populations although they were present in all habitats (Table 4).

TABLE 2. Whitefly colonisation on clean sentinel cassava potted plants (two months old) placed in different host habitat patches in Nkhata Bay in Malawi

Habitat	*Mean	SE	N
Target cassava	39.3 c	14.0	33
Old cassava	17.8 bc	11.3	12
Natural vegetation	14.1 bc	4.9	27
Pumpkin	6.5 b	4.0	15
Sweet potato	1.2 a	4.0	9
Statistical tests			
GLM negative binomial (0.2548) log link			
AIC	628.31		
Null Deviance on 95 df	117.94		
Residual Deviance on 91 df	100.86		
P value	0.002		

* Different letters beside the mean indicate significant differences at 5% level of significance. P value = 0.002, LSD test of multiple comparison. SE is standard error of the mean, GLM stands for generalised linear model and AIC is Akaike's Information Criterion. Clean sentinel plants were left exposed to colonisation for four weeks and then returned to the laboratory to count the number of nymphs

TABLE 3. Whitefly colonisation on clean sentinel cassava potted plants (two months old) placed in different host habitat patches in Lilongwe, Malawi

Habitat /statistical test	Mean*	SE	N
Target cassava	0.42	0.18	36
Old cassava	0	0	3
Natural vegetation	0	0	9
Sweet potato	0.07	0.47	9
Pumpkin	0.04	0.04	24
Beans	0	0	3
Groundnuts	0.07	0.07	15
Irish potato	0	0	3
Tobacco	0	0	9
Tomato	0	0	15
Soybean	0	0	36
Kruskal-Wallis test			
Chi-square	9.1442		
Degrees of freedom	10		
P value	0.5185		

*Means are per plant per field. Kruskal Wallis test for significant differences between means was used. As the colonisation was very low with many zeros. SE is standard error of the mean. Clean sentinel plants were left exposed to colonisation for four weeks and then returned to the laboratory to count the number of nymphs

TABLE 4. Number of whitefly adults and nymphs on habitat plants in Nkhata Bay and Lilongwe, Malawi

	Nkhata Bay				Lilongwe			
	Whitefly adults		Whitefly nymphs		Whitefly adults		Whitefly nymphs	
	Mean ¹ (SE)	N	Mean (SE)	N	Mean (SE)	N	Mean (SE)	N
Target cassava	2.77(0.50)	220	10.60(1.16)	220	1.0(0.2)	240	2.4(0.4)	240
Natural vegetation	0.47(0.08)	79	0.91(0.13)	79	0.3(0.1)	40	0.3(0.1)	40
Old cassava	1.30(0.26)	120	3.32(0.55)	120	0.3(0.1)	10	1.2(0.7)	10
Pumpkin	0.36(0.06)	70	0.10(0.04)	70	0.3(0.1)	80	0.0(0.0)	80
Sweet potato	0.42(0.11)	60	0.72(0.12)	60	0.8(0.3)	20	0.0(0.0)	20
Groundnut	NA	NA	NA	NA	0.2(0.1)	60	0.1(0.0)	60
Irish potato	NA	NA	NA	NA	0.3(0.2)	10	0.3(0.2)	10
Soybean	NA	NA	NA	NA	0.4(0.1)	110	0.2(0.1)	110
Tobacco	NA	NA	NA	NA	0.3(0.1)	20	0.1(0.1)	20
Tomato	NA	NA	NA	NA	1.2(0.3)	40	0.3(0.1)	40

¹ Numbers in brackets are standard errors (SE) of the mean. Means are per plant per field. No statistical analyses were done for the various habitat plant types. NA stands for 'not available'. The plants in the habitats where the sentinel cassava was placed were inspected for whitefly adults and nymphs.

TABLE 5. Nymph parasitism on sentinel plants in Nkhata Bay and on habitat plants in Nkhata Bay and Lilongwe in Malawi

Habitat / statistical tests	Proportion of parasitised nymphs					
	'Sentinel plant nymphs			Habitat plant nymphs		
	Nkhata Bay		N	Nkhata Bay		Lilongwe
Natural vegetation	Means (² SE)	11	Means (SE)	14	Means (SE)	N
Old cassava	0.32(0.07)	23	0.38(0.09)	24	NA	NA
Pumpkin	0.12(0.05)	7	0.31(0.06)	NA	0.25(0.10)	4
Sweet potato	0.13(0.05)	2	NA	3	NA	NA
Target cassava	0	30	0.33(0.08)	68	NA	NA
Groundnuts	0.13(0.04)	NA	0.28(0.03)	NA	0.17(0.06)	19
Soybean	NA	NA	NA	NA	NA	NA
	NA	NA	NA	NA	0.27(0.08)	15
Kruskal-Wallis Rank sum tests						
Chi-squared	7.21		1.78		0.97	
Degrees of freedom	4		3		3	
P-value	0.13		0.62		0.62	

¹ Sentinel cassava nymphs were younger than habitat nymphs, hence fewer had been exposed to parasitism at the time of field collection. Lilongwe sentinel nymph parasitism omitted in analysis, due to only three nymphs in two fields were reared for parasitism due to low colonisation of sentinel cassava. ² Numbers in parentheses are standard errors (SE) of the mean. Habitats where nymphs were unavailable for rearing are designated 'NA' meaning 'not available'.

Descriptive means of whitefly adults and nymphs. Table 4 presents the means of whitefly adults and nymphs on habitat plants in Nkhata Bay and Lilongwe in fields where the sentinel plants were placed. Whitefly adults were highest on target cassava (mean=2.77); while on old cassava (mean=1.3) was second in Nkhata Bay; natural vegetation, pumpkin and sweet potato, all had means less than 1 whitefly per plant (Table 4).

Nymphs followed a similar trend, with target cassava recording highest mean of 10.6 nymphs per plant and old cassava 3.3 nymphs per plant. In Lilongwe, the highest number of adults was in tomato (mean=1.2), seconded by target cassava (mean=1.0); while the natural vegetation, old cassava, pumpkin, sweet potato, groundnuts, Irish potato, soybean and tobacco had means less than 1 adult per plant.

The nymphs in Lilongwe were also higher in target cassava (mean=2.4) seconded by old cassava (mean=1.2); and the other habitats had nymphs less than 1. Sweet potato and pumpkin recorded zero nymphs per plant.

Association between number of whitefly nymphs on plants and nymphs on sentinel plants. The relative number of nymphs on sentinel plants placed in different habitats in Nkhata Bay was positively associated with nymphs on habitat plants (estimate =0.9, $P=0.04$, $R^2=0.28$).

Robust regression analysis. The robust regression analysis revealed the mean of sentinel plant nymphs (intercept) of 5.2, $SE=2$, $t=2.49$, $P=0.02$; and habitat whitefly nymph mean of 0.9, $SE=0.4$, $t=2.1$, $P=0.04$. Thus, sentinel plant nymphs are expected to increase by 0.9 when habitat whitefly nymphs increase by 1.

Parasitised nymphs. Parasitism of nymphs on sentinel cassava placed in Nkhata Bay habitats was higher in those placed in natural vegetation (32%), although not significantly different from those placed in old cassava,

pumpkin, sweet potato and target cassava habitats (<15%) (Table 5). Only three parasitised nymphs in Lilongwe were observed on sentinel cassava plants placed in sweet potato and target cassava habitats. Parasitism of nymphs reared from habitat plants in both Nkhata Bay and Lilongwe was not significantly different ($P>0.05$) between habitats, although there tended to be more parasitised nymphs from natural vegetation (38%) in Nkhata Bay than from old cassava (31%), sweet potato (33%) and target cassava (28%) habitats (Table 5). The commonest parasitoid identified was *Encarsia sophia*.

DISCUSSION

Whitefly nymphs in sentinel plants. This study has uncovered landscape patterns relevant to mixed whitefly pest species situations, which is always the case in real production landscapes (Theu and Sseruwagi, 2005; Macfadyen *et al.*, 2021), therefore, providing insights that might guide management strategies. Besides cassava itself, other non-crop habitats (especially native vegetation) significantly contributed to whitefly colonisation; while non-cassava crops such as pumpkin and sweet potato, had least influence on sentinel plant colonisation than cassava.

Sentinel plant infestation data, indicated from which habitats colonising whiteflies could potentially emigrate to infest new cassava plants. Our study showed that natural vegetation and old cassava have an impact on whitefly colonisation in the new cassava at the beginning of the growing season in Malawi. The non-cassava plants (alternative host plant habitats) present in our study included weed plants, trees and crops of the families, Fabaceae, Caesalpiniaceae, Asteraceae, Pedaliaceae, Malvaceae, Cucurbitaceae and Convolvulaceae (Table 1). These plant taxa have been found to host *B. tabaci* species complex and *B. afer* in several studies elsewhere (Kalyebi *et al.*, 2021; Mugerwa *et al.*, 2021; Namuddu *et al.*, 2023). The

whiteflies on alternative host plants act as sources of colonisation on the new cassava crop if they are present within the vicinity of new cassava crop being established.

The low colonisation rates of the sentinel plants in non-cassava crop habitats observed in this study, showed that these habitats were less colonised by the whiteflies infesting cassava in the studied regions in the four-week period that the sentinel plants were there. It is known that after rain, most plants may experience a flush in vegetative growth which may make them more attractive to whiteflies; however, such a scenario did not impact the high populations of whiteflies in the alternative host plants in our study. The present study was also constrained by the lumping of whitefly species together in the field survey approaches used. Using molecular techniques to further resolve which species use which host plants may provide greater clarity about when to remove or support certain plants in the landscape.

Descriptive means of whitefly adults and nymphs. Cassava habitat plants recorded more whitefly nymphs than other habitats, indicating that the former had the most potential to colonise the sentinel cassava plants. The non-cassava crops had much less whitefly populations at the beginning of the rainy season. The role of non-cassava crops as source of whitefly colonisation on cassava in the early season is, therefore, minimal. Togni *et al.* (2021), alluded to the fact that after host crop harvest, whiteflies use non-crop habitats, though the latter compromise habitat quality. Thus, the presence of old cassava meant that whiteflies had a cassava host prior to the planting of the new cassava crop. Hence, old cassava habitat nymph numbers following the numbers in target cassava habitat.

Association between numbers of whitefly nymphs on plants in habitats and nymphs on sentinel plants. The alternative host plant habitats had nymphs, indicating that some whiteflies were able to feed and reproduce

on the plants, thus acting as true host plants. Cassava whiteflies are polyphagous, hence, knowing the implications of the presence of alternative host plants on whiteflies on cassava, could help the development of more efficacious management strategies for this pest. There was a positive correlation between the abundance of the nymphs in habitats and the abundance of nymphs on the sentinel plant, suggesting that our bioassay technique reflected real ecological relationships. The plants in the target cassava, old cassava and natural vegetation habitats had more nymphs than those in the other habitats, and the nymphs on sentinel plants followed a similar pattern as those on habitat plants. The other non -cassava crop hosts, although were at an active early growing stage, had not many whiteflies to be good sources of infestation on the newly planted cassava; thus, being the reason for very low nymphs observed on sentinel plants placed in the non-cassava crops hosts.

In Uganda, Kalyebi *et al.* (2021) reported a negative correlation between the amount of potential non-cassava host crops (beans, soybeans and sweet potato) in the landscape and the abundance of *B. tabaci* Sub-Saharan Africa 1 (SSA1) in target cassava. This corroborates the finding of the present study that sentinel plants in non-cassava crops were scarcely infested by whiteflies. Understanding the influence of host plants in native vegetation habitats on the dynamics of whitefly pest populations is important for designing cropping systems that lower the risk of pest outbreaks (Togni *et al.*, 2021). Plants in the Pedaliaceae (e.g. wild sesame) and Malvaceae (e.g. *Cida acuta*) tended to be perennial in the natural vegetation areas, producing more new shoots at the slight onset of rains prior to crop planting. Such plants can support whiteflies which can later migrate to crops.

Our data show that the primary source for whitefly infestation of cassava in the early stages of growth is mainly old cassava and natural vegetation. Thus, alternative whitefly host plants in the natural vegetation play a significant role in contributing to whitefly

colonisation in cassava at the onset of the rainy season. Non-cassava crops showed no significant role in colonisation of sentinel cassava plants. This indicates that such crops have little impact on new cassava field colonisation at the beginning of the growing season. They may play a role in cassava that is planted three months later in the season, since some whitefly nymphs were recorded on them (Table 4). These short-lived crops would be senescing, then whiteflies would migrate to the more attractive new cassava crop.

Robust regression analysis. The robust regression revealed that an increase in whitefly populations in habitats, where sentinel cassava was placed, would lead to a proportional increase in sentinel colonisation. These results suggest that alternative habitats near new cassava fields, if they are infested with whiteflies, they would enhance whitefly colonisation in the new cassava fields. Furthermore, this supports the phenomena of spillover effects of herbivores across habitats due to their polyphagous nature (Blitzer *et al.*, 2012).

Parasitised nymphs. Parasitism rates in both the sentinel plants and habitat plants were variable and non significant. The trend can be attributed to the uncontrolled number of nymphs reared from the host plants (and in some cases the low numbers of nymphs reared). The variation between habitats was large. However, parasitoids could come from multiple habitats in the landscape and could reduce the number of whiteflies in multiple habitats, given that they are highly mobile. The high parasitism rate in natural vegetation (32%), indicates a potential source habitat for parasitoids. Some plants like *S. indicum* were shown to support *Encarsia* spp. with flower nectar (Liu *et al.*, 2017). Parasitism rates over 17% by *Encarsia* spp. on *B. tabaci* spp. found on weeds was observed in Florida (Smith *et al.*, 2014). High parasitism rates (75%) on soybean plants infested with *B. tabaci* were

observed in Turkey (Malik and Karut, 2012); while Mansaray and Sundufu (2010) reported higher emergence rates of *Encarsia bimaculata* Heraty and Polaszek (Hymenoptera: Aphelinidae) on soybean than other crops. Host plants mediate interactions between pests and their natural enemies (Inbar and Gerling, 2008). Given that soybeans are harvested within three months while cassava is left much longer in the field it could be a source of parasitoids in Lilongwe, however further research would be required to confirm this. Also, there were more natural vegetation patches in Nkhata Bay than in Lilongwe, and those in latter site were mostly bare ground just before the planting rains. The parasitism rate observed in natural vegetation did not seem to negatively impact on whitefly colonisation of the sentinel plants. If more nymphs in the natural vegetation were parasitised one would expect less whitefly colonisation on newly planted cassava in the colonisation source of natural vegetation.

CONCLUSION

This study has shown that in diverse landscapes, which contain more than just cassava fields, alternative habitats can influence the population dynamics of whitefly in cassava fields. Cassava and natural vegetation are important in whitefly colonisation; although non-cassava crops tend to have less influence. An ecological relationship between sentinel cassava nymphs and habitat nymphs has been established. Whitefly and its associated CMD management in cassava production systems, must consider monitoring of natural vegetation and non-cassava crops, all of which possess potential for acting as alternative habitats for this pest, or part of its lifecycle.

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