

# Seasonal abundance of *Maiestas banda* (Hemiptera: Cicadellidae), a vector of phytoplasma, and other leafhoppers and planthoppers (Hemiptera: Delphacidae) associated with Napier grass (*Pennisetum purpureum*) in Kenya

Shinsaku Koji · Satoshi Fujinuma · Charles A. O. Midega · Hassan M. Mohamed ·  
Tadashi Ishikawa · Michael R. Wilson · Manfred Asche · Simon Degelo ·  
Tarô Adati · John A. Pickett · Zeyaur R. Khan

Received: 7 March 2011 / Accepted: 12 July 2011 / Published online: 26 July 2011  
© Springer-Verlag 2011

**Abstract** Napier grass (*Pennisetum purpureum* Schumacher), the main fodder crop in eastern Africa, has recently been severely affected by Napier stunt disease, which is caused by phytoplasma. We have recently confirmed that a leafhopper, *Maiestas banda* (Kramer) transmits phytoplasma in Napier grass in Kenya. Several other species of leafhoppers (Cicadellidae) and planthoppers (Delphacidae) are also associated with Napier grass, and some could be potential vector(s) of phytoplasma, but little is known about phenology and movement activity of these insect groups. We monitored the populations of *M. banda* and other leafhoppers and planthoppers in Napier grass fields at two sites in western Kenya for 18 months. High population densities were observed for *M. banda*, suggesting that it plays a major role in the disease epidemics in the region. Sampling with Malaise traps and a vacuum-suction sampler revealed that *M. banda* and five other species (*Cicadulina mbila* (Naudé), *Cofana polaris* Young, *Cofana unimaculata* (Signoret),

*Leptodelphax dymas* Fennah, *Maiestas* sp.) exhibited a high proportion of males in the trap catches, implying higher mobility in males than females. A suction sampler collected more individuals of three species (*M. banda*, *Maiestas* sp., and *Thriambus strenuus* Van Stalle) from phytoplasma-infected than healthy plants at both sites. The populations of *M. banda* and other five species (*C. unimaculata*, *Maiestas* sp., *L. dymas*, *T. strenuus*, and *Rhinotettix* sp. 1) showed a conspicuous peak at the end of the short rainy season (Sep to Dec), implying that common regulatory factors such as rainfall may affect the leafhopper and planthopper complex.

**Keywords** Delphacidae · Cicadellidae · Population dynamics · Napier stunt disease · Phytoplasma

## Introduction

Napier grass *Pennisetum purpureum* Schumacher is the main fodder crop in the zero-grazing dairy cattle production

Communicated by T. Haye

S. Koji (✉)  
Center for Regional Collaboration, Kanazawa University,  
Kakuma, Kanazawa 920-1192, Japan  
e-mail: shinsak1@hotmail.com

S. Fujinuma · T. Ishikawa  
Faculty of Agriculture, Tokyo University of Agriculture,  
Funako 1737, Atsugi, Kanagawa 243-0034, Japan

C. A. O. Midega · H. M. Mohamed · Z. R. Khan  
International Centre of Insect Physiology and Ecology (ICIPE),  
P. O. Box 30772, Nairobi, Kenya

M. R. Wilson  
Department of Biodiversity and Systematic Biology,  
National Museum of Wales,  
Cardiff CF10 3NP, UK

M. Asche  
Hemiptera Research Group, Museum für Naturkunde, Leibniz  
Institute for Research on Evolution and Biodiversity  
at the Humboldt University Berlin, Invalidenstr. 43,  
Berlin 10115, Germany

S. Degelo  
Department of Environmental Sciences, Swiss Federal Institute  
of Technology, Zurich, Switzerland

T. Adati  
Faculty of International Agriculture and Food Studies, Tokyo  
University of Agriculture, Sakuragaoka 1-1-1, Setagaya-ku,  
Tokyo 156-8502, Japan

J. A. Pickett  
Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK

systems in East Africa, constituting between 40 and 80% of the forage used in the region (Arocha et al. 2009). The grass has also been identified as a trap plant for cereal stemborers (Khan et al. 2006, 2007), and has been used in a novel “push–pull” management system for these pests in Africa (Cook et al. 2007). In recent years, Napier grass production in East Africa has suffered from epidemics of a disease called “Napier stunt disease” (Ns-disease), which is caused by a phytoplasma. Characteristic symptoms of the disease include foliar yellowing, little leaves, tiller proliferation, and shortening of internodes, to the extent that clumps appear severely stunted (Jones et al. 2004). Often the whole stool is affected with a complete loss in yield and eventual death. The Ns-disease has been reported in Kenya, Uganda, and Ethiopia, posing a serious negative effect on both dairy and cereal production in the predominantly mixed crop-livestock farming systems in the region (Jones et al. 2004, 2007; Nielsen et al. 2007; Arocha et al. 2009; Obura et al. 2009). According to phylogenetic analysis of 16S rDNA sequences, the phytoplasmas associated with Ns-disease in Kenya and Uganda are classified into the 16SrXI group (*Candidatus* Phytoplasma *oryzae* or rice yellow dwarf) (Jones et al. 2004; Nielsen et al. 2007), while those occurring in Ethiopia are classified into the 16SrIII group (X-disease) (Jones et al. 2007).

Phytoplasmas are phloem-restricted and are transmitted through (1) vegetative propagation or grafting of infected plant material; (2) vascular connections made between infected and uninfected host plants by parasitic plants; and/or (3) phloem-feeding insects, most commonly leafhoppers (Cicadellidae) and planthoppers (Delphacidae) (Weintraub and Beanland 2006). Since there is no parasitic plant associated with Napier grass, the primary means of Ns-disease spread are the introduction of infected plant material (i.e., cane cuttings or clump splits) by farmers and/or insect vectors carrying the phytoplasma. Recently, Obura et al. (2009) tested the Ns-phytoplasma transmission ability of five leafhopper and three planthopper species and identified the leafhopper *Maiestas banda* (Kramer) [recently transferred from genus *Recilia*, Webb and Viraktamath (2009)] as a vector of Ns-phytoplasma in Kenya. However, despite that several different insect species are able to transmit the same kind of phytoplasma (Weintraub and Beanland 2006), little is known about fauna of leafhoppers/planthoppers on Napier grass in Africa. Therefore, it is important to identify the composition of leafhoppers and planthoppers found in the disease-affected Napier grass fields by the season-long monitoring.

The spread of plant pathogens by insect vector(s) depends on their abundance and inter-plant movements (Irwin and Ruesink 1986; Power 1987). The abundance and movement activity of sap-sucking insects is affected by temperature, precipitation, host plant traits such

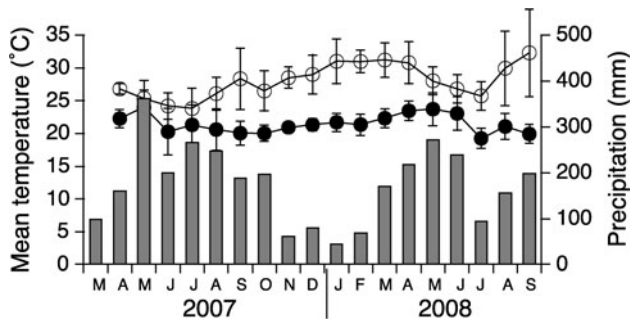
as age (Chiykowski 1981; Atakan 2011) and disease infection status (McElhany et al. 1995; Sisterson 2008), host plant density (Power 1987), and the vector's gender (Hunt et al. 1993; Beanland et al. 1999). Detailed knowledge of the factors affecting population dynamics and the dispersal of vectors is a prerequisite not only for understanding Ns-disease epidemiology but also for developing an integrated pest management program against the disease. Despite the importance of *M. banda* as a vector of Ns-disease in Kenya, little information exists on its seasonal distribution in the Napier grass fields.

In this study, we sampled leafhoppers and planthoppers in Napier grass fields in western Kenya to (1) identify the components of leafhopper/planthopper species complex, (2) understand the seasonal phenology of the dominant species, and (3) evaluate whether the insect's gender and/or disease infection of host plants influenced their density. Leafhoppers and planthoppers were sampled by means of a vacuum-suction sampler that collected foliage-dwelling insects regardless of their flight ability and Malaise traps, which mainly collected active fliers. Hence, by comparing the sex ratio in the trap catches with those obtained from suction samples, we were able to compare the flight activity of males and females of each species. Furthermore, we compared the abundance of the species on phytoplasma-infected and uninfected plants by suction sampling to test whether disease infection status of the host plant affects insect abundance on the plants.

## Materials and methods

### Field sites

Leafhoppers and planthoppers were sampled at Kabula (0°29'N, 34°31'E) and Sangalo (0°31'N, 34°35'E), Bungoma district of western Kenya. The sites are characterized by a bimodal rainfall distribution (Mugalavai et al. 2008), with peaks in May and October and annual precipitation of approximately 1,500 mm (Fig. 1). Bungoma district is one of the most severely affected areas by Ns-disease in Kenya (Orodho 2006). At the onset of the study, the disease infection rate (percentage of infected plants) in Sangalo was relatively moderate (ca. 30–40%), while that in Kabula, which is located 5 km from Sangalo, was high (ca. 80–90%, unpublished data). A sampling plot (approximately 50 × 50 m) was established in the center of Napier grass mono-crop fields in Kabula and Sangalo (0.5 and 8.0 ha, respectively). In each field, Napier grass (bana variety) was planted with a spacing of 1 m between and 40–50 cm within rows. The normal agronomic practices, such as fertilization and irrigation, for growing Napier



**Fig. 1** Seasonal change in monthly precipitation (bars) and monthly mean ( $\pm$ standard error) of daily maximum (open circles) and minimum (closed circles) temperature in Bungoma district, western Kenya

grass were followed during the course of the experiment (Nyaata et al. 2000).

### Sampling protocol

Regular sampling of planthoppers and leafhoppers was conducted using a modified suction sampler (hand blower THB-2510N, Tanaka Co. Ltd., Chiba, Japan) and Malaise traps. For the suction sampling, five healthy (asymptomatic) and five infected (symptomatic) Napier grass plants were selected randomly per site. Each plant was sampled from bottom to top by moving the suction sampler head (11 cm diameter) onto the plant surface for 40 s. Sampling was carried out biweekly between 10:00 and 17:00 when the vegetation was dry. One Malaise trap (2 m long, 1.1 m width, 1.7 m height) was placed in the center of each monitoring plot. The ground within a radius of ca. 2.5 m of the trap was kept cleared during the monitoring period. The insects captured in a head vial (containing 250 ml of 70% ethanol) were collected weekly. The planthoppers and leafhoppers in the samples were sorted and insect species identified whenever possible, and adult sex determined. Only adults were identified, and immature individuals were included along with the unidentifiable specimens and reported as “unidentified”. All voucher specimens were deposited at the biosystematics unit of the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya. Suction and Malaise samplings were conducted 41 and 65 times, respectively, from March 2007 to September 2008. Malaise sampling was not performed from February 2008 to May 2008 because of the unavailability of insect preservative (ethanol) as a consequence of political tension in Kenya.

### Data analysis

The sex ratio (i.e., the total number of males divided by the total number of females) was calculated for species with

ten or more individuals. The ratios obtained from suction sampling and Malaise trapping were compared by a *G* test (Sokal and Rohlf 1995). For 12 most abundant species with ten or more occurrences, insect abundances (pooled per five plants) between healthy and diseased plants were compared throughout the entire sampling period, using the Wilcoxon matched pairs test (Sokal and Rohlf 1995). Seasonal variation in population densities was examined for six predominant species (see “Results”). Two-way analysis of variance (ANOVA) was conducted to determine the influence of season (sampling week) and Ns-disease infection status of host plants (healthy or infected) on the number of insects per plant. Density data were  $\log_{10}(x + 0.5)$ -transformed before analysis. Differences between healthy and infected plants were further investigated for each sampling period using Wilcoxon test. All statistical tests were carried out using JMP version 5.1.1 for Mac (SAS Institute 2004).

## Results

### Identified leafhoppers and planthoppers

Table 1 is a list of the identified species collected with suction sampler and Malaise traps. A total of 4,714 leafhoppers and 2,207 planthoppers were collected with the suction sampler over an 18 month monitoring period, of which 2,466 leafhoppers and 1,824 planthoppers were identified to species/morphospecies or to genus levels. The Malaise traps collected 6,944 leafhoppers and 1,058 planthoppers, of which 2,275 and 668, respectively, were identified. The identified leafhopper species were dominated by *M. banda*, *Cofana unimaculata* (Signoret), *Cicadulina mbila* (Naudé), *Exitianus attenuatus* Ross, and *Maiestas* sp. The predominant planthoppers were *Leptodelphax dymas* Fennah, *Thriambus strenuus* Van Stalle, *Rhinotettix* sp. 1, *Sogatella manetho*, and *Leptodelphax* sp. These species together comprised 96.4 and 67.8% of all leafhoppers and planthoppers identified, respectively. Due to identification difficulties, females of the genus *Sogatella* were pooled as *Sogatella* spp. and excluded from further analysis.

### Sex ratio

For five leafhopper species (*C. mbila*, *C. polaris*, *C. unimaculata*, *M. banda*, and *Maiestas* sp.) and one planthopper species (*L. dymas*), the sex ratios obtained from the Malaise traps were strongly male biased, and the male ratio was higher than that obtained via suction samplings (Table 2). On the other hand, there were no differences in the sex ratios between the sampling methods for one leafhopper species (*Exitianus distanti*) and four

**Table 1** Overall abundances of leafhoppers (Cicadellidae) and planthoppers (Delphacidae) collected by the suction sampler and Malaise traps at two sites (Kabula and Sangalo) in western Kenya

Family	Species	Kabula		Sangalo		
		Suction (41) <sup>a</sup>	Malaise (65) <sup>a</sup>	Suction (41) <sup>a</sup>	Malaise (65) <sup>a</sup>	
Cicadellidae	<i>Cicadulina mbila</i> (Naudé, 1924)	48	72	202	237	
	<i>Cofana polaris</i> Young, 1979	0	4	15	46	
	<i>Cofana spectra</i> (Distant, 1908)	3	0	1	2	
	<i>Cofana unimaculata</i> (Signoret, 1854)	160	734	203	512	
	<i>Exitianus attenuatus</i> Ross, 1968 <sup>b</sup>	69	20	51	20	
	<i>Exitianus distanti</i> Ross, 1968	30	12	15	4	
	<i>Glossocratus afzelii</i> (Stål, 1854)	5	22	3	7	
	<i>Maiestas banda</i> (Kramer, 1962)	361	79	570	328	
	<i>Maiestas</i> sp.	267	63	463	113	
	Unidentified Cicadellidae <sup>d</sup>	1290	1693	958	2976	
	Delphacidae	<i>Asiracina</i> sp.	56	1	22	2
		<i>Leptodelphax dymas</i> Fennah, 1961	315	78	217	73
		<i>Leptodelphax maculigera</i> (Stål, 1859)	1	0	0	0
		<i>Leptodelphax</i> sp.	56	3	102	15
<i>Oaristes snelli</i> (Muir, 1929)		1	0	1	0	
<i>Rhinotettix</i> sp. 1		130	38	140	57	
<i>Rhinotettix</i> sp. 2		6	3	0	0	
<i>Sogatella kolophon</i> (Kirkaldy, 1907) <sup>c</sup>		5	1	2	1	
<i>Sogatella manetho</i> Fennah, 1955 <sup>c</sup>		60	21	96	110	
<i>Sogatella nigrigenis</i> (Jacobi, 1917) <sup>c</sup>		14	9	23	13	
<i>Sogatella</i> spp. <sup>c</sup>		89	17	166	140	
<i>Tagosodes cubanus</i> (Crawford, 1914)		13	3	10	19	
<i>Thriambus levis</i> Van Stalle, 1984		7	0	0	0	
<i>Thriambus strenuus</i> Van Stalle, 1984		152	21	139	43	
<i>Thriambus vegetatus</i> (Melichar, 1912)	0	0	1	0		
Unidentified Delphacidae <sup>d</sup>	220	143	163	247		
Total individuals	3358	3037	3563	4965		

The total catch throughout the entire sampling period is shown

<sup>a</sup> Number of sampling times

<sup>b</sup> Only males were identified to the species level, and females were included in “Unidentified Cicadellidae”

<sup>c</sup> Only males were identified to the species level, and females were pooled as *Sogatella* spp

<sup>d</sup> Including unidentifiable females, immature individuals, and damaged specimens

planthopper species (*Leptodelphax* sp., *Rhinotettix* sp. 1, *T. cubanus*, and *T. strenuus*).

#### Species response to diseased and healthy host plants

In Kabula, higher population densities occurred in diseased plants for six species (*C. unimaculata*, *E. attenuatus*, *E. distanti*, *M. banda*, *Maiestas* sp., and *T. strenuus*), compared with two species (*C. mbila* and *S. manetho*) in healthy plants (Fig. 2). In Sangalo, of the 12 species tested by the Wilcoxon method, 6 species (*C. polaris*, *M. banda*, *Maiestas* sp., *L. dymas*, *Rhinotettix* sp. 1, and *T. strenuus*) showed a higher density in diseased plants compared with healthy plants, but another 6 species were not influenced by host plant infection (Fig. 2). Three species, *M. banda*, *Maiestas* sp., and *T. strenuus*, exhibited significantly higher population densities on infected plants in both Kabula and Sangalo.

#### Seasonal variation in population densities of predominant species

Seasonal phenology patterns were analyzed for six predominant species (*M. banda*, *Maiestas* sp., *C. unimaculata*, *L. dymas*, *T. strenuus*, and *Rhinotettix* sp. 1). When sampling was performed using suction samplers, higher abundance of *M. banda* occurred on diseased plants than on healthy plants (Table 3; Fig. 3). The Kabula population showed a noticeable peak in October 2007 with densities of 7.8 and 1.6 on diseased and healthy plants, respectively (Fig. 3). In Sangalo, the peak population densities of *M. banda* occurred in November and December 2007 with densities of 7.6 and 8.0 on diseased and healthy plants, respectively. The number of *M. banda* caught in the Malaise trap showed no clearly defined peak in Kabula (Fig. 4). The Sangalo population had two noticeable peaks in April 2007 and December 2007, and a weak peak around August 2007.

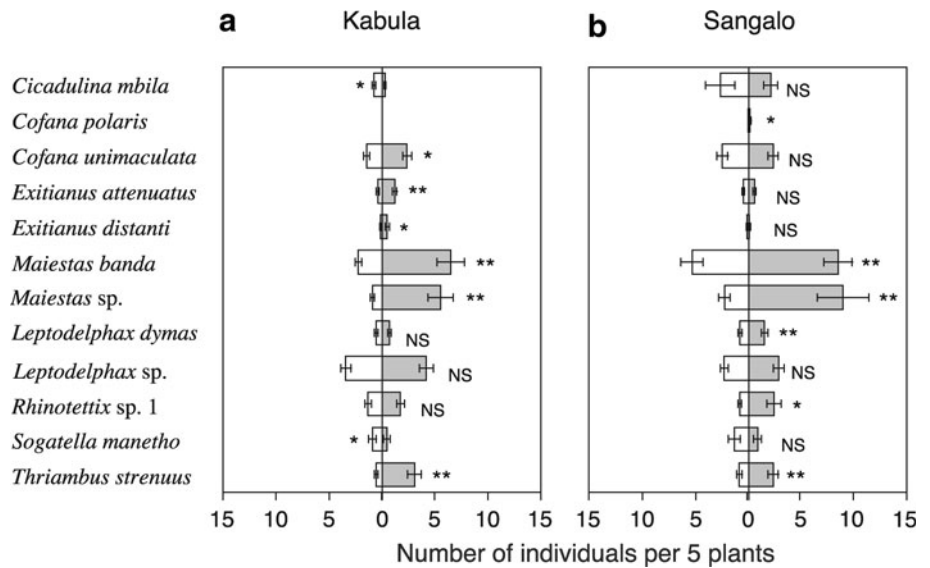
**Table 2** Sex ratio (total number of males divided by total number of females) in leafhoppers (Cicadellidae) and planthoppers (Delphacidae) collected by the suction sampler and Malaise traps at two study sites (Kabula and Sangalo) in western Kenya

Family	Species	Kabula		Sangalo	
		Suction	Malaise	Suction	Malaise
Cicadellidae	<i>Cicadulina mbila</i>	0.7	7.0*	0.3	4.4*
	<i>Cofana polaris</i>	–	–	0.7	14.3*
	<i>Cofana unimaculata</i>	1.4	27.2*	0.9	27.4*
	<i>Exitianus distanti</i>	1.1	2.0	1.1	–
	<i>Glossocratus afzelii</i>	–	3.4	–	–
	<i>Maiestas banda</i>	0.8	3.4*	0.6	5.6*
	<i>Maiestas</i> sp.	0.9	1.9*	0.7	1.8*
Delphacidae	<i>Leptodelphax dymas</i>	1.0	2.9*	0.8	4.6*
	<i>Leptodelphax</i> sp.	1.1	–	1.0	0.4
	<i>Rhinotettix</i> sp. 1	0.7	0.4	0.6	0.6
	<i>Tagosodes cubanus</i>	3.3	–	2.3	1.7
	<i>Thriambus strenuus</i>	3.5	2.5	3.0	1.5

Sex ratios are shown for species with ten or more individuals

\* Significantly different between the sampling methods (*G* test;  $P < 0.05$ )

**Fig. 2** Seasonal mean ( $\pm$ standard error) densities of leafhoppers and planthoppers collected by the suction sampler from healthy (*open bars*) and diseased (*gray bars*) Napier grass plants at **a** Kabula and **b** Sangalo, western Kenya (values pooled per five plants). Wilcoxon matched pairs tests between healthy and diseased plants: \* $P < 0.05$ ; \*\* $P < 0.01$ ; NS no significant differences



A similar temporal pattern was observed for the other three species, *Maiestas* sp., *T. strenuus*, and *Rhinotettix* sp. 1. The population densities of these species, sampled by the suction sampler, increased from July 2007 and peaked in November 2007, before gradually decreasing until March 2008 (Fig. 3). The population level remained low as the season advanced, but increased again in July 2008. The densities of *Maiestas* sp. and *T. strenuus* were generally greater in the diseased than in the healthy plants (Table 3; Fig. 3). The densities of *Rhinotettix* sp. 1 on diseased and healthy plants differed in Sangalo, but the difference was not statistically significant in Kabula (Table 3). The seasonal phenology patterns of these species collected by the Malaise traps were largely consistent with those collected by suction samplers in that the population peak occurred around November–December 2007 (Fig. 4).

The results for *C. unimaculata* collected by the suction sampler revealed greater population densities in the diseased than in the healthy plants in Kabula, but the difference was insignificant in Sangalo (Table 3). In Kabula, there were three noticeable peaks in July 2007, November 2007, and April 2008 (Fig. 3). In Sangalo, the peak population density occurred in November 2007 and March 2008, but no peaks were detected in July 2007. Both populations increased from July 2008 toward the end of the census period in September 2008. In the Kabula population, the results from both sampling methods were consistent in that the population peak densities were seen in July and November 2007 (Fig. 4). In Sangalo, there was a distinct peak in the *C. unimaculata* population in July 2007 from the Malaise samples, which was not detected in the suction samples.

**Table 3** Two-way analysis of variance table of sampling week (Season) and host plant disease infection (Disease) effects on the density of common leafhopper (Cicadellidae) and planthopper (Delphacidae) species collected by suction samplers

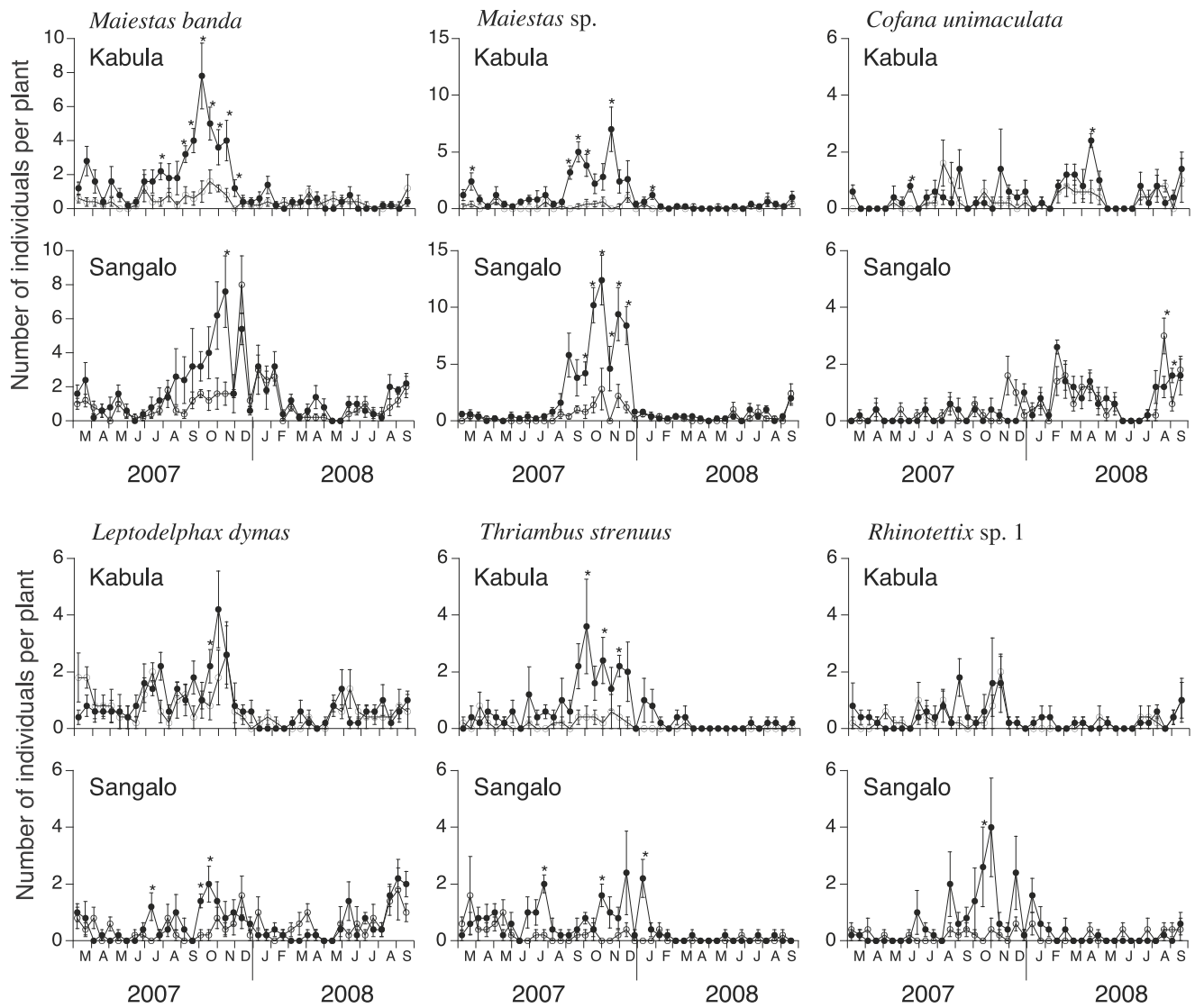
Species	Source	df	Kabula		Sangalo	
			F	P	F	P
<b>Cicadellidae</b>						
<i>Maiestas banda</i>	Season	40	5.77	<0.001	6.50	<0.001
	Disease	1	43.80	<0.001	13.69	<0.001
	Season × disease	40	2.37	<0.001	0.96	0.539
	Error	328				
<i>Maiestas</i> sp.	Season	40	5.29	<0.001	11.73	<0.001
	Disease	1	98.29	<0.001	68.88	<0.001
	Season × disease	40	3.56	<0.001	3.06	<0.001
	Error	328				
<i>Cofana unimaculata</i>	Season	40	3.38	<0.001	7.50	<0.001
	Disease	1	7.53	<0.001	0.04	0.845
	Season × disease	40	1.24	0.164	1.53	0.026
	Error	328				
<b>Delphacidae</b>						
<i>Leptodelphax dymas</i>	Season	40	3.73	<0.001	3.87	<0.001
	Disease	1	2.89	0.090	4.19	0.042
	Season × disease	40	1.25	0.152	1.75	0.005
	Error	328				
<i>Thriambus strenuus</i>	Season	40	3.65	<0.001	3.22	<0.001
	Disease	1	46.84	<0.001	28.67	<0.001
	Season × disease	40	1.74	0.005	2.18	<0.001
	Error	328				
<i>Rhinotettix</i> sp. 1	Season	40	2.37	<0.001	2.94	<0.001
	Disease	1	0.45	0.503	10.54	0.001
	Season × disease	40	0.78	0.829	1.70	0.007
	Error	328				

When sampling was performed using suction samplers, the density of *L. dymas* was greater in the diseased than in the healthy plants in Sangalo, but the difference was insignificant in Kabula (Table 3). The *L. dymas* population in Kabula and Sangalo showed a similar pattern of seasonal occurrence. The population densities had six peaks in March 2007, July 2007, November 2007, March 2008, June 2008, and September 2008 (Fig. 3). Malaise trap samples from both study sites indicated noticeable population peaks in March–April 2007, July 2007, November 2007, and September 2008, which was consistent with suction samples (Fig. 4).

## Discussion

Sampling with Malaise traps and a vacuum-suction sampler revealed that *M. banda* was abundant in both Kabula and Sangalo. Considering its already known vectoring capability for Ns-disease (Obura et al. 2009) and high

population density, *M. banda* is likely to play a major role in the Napier stunt epidemic currently affecting the region. Leafhoppers in the genus *Maiestas* are mostly grass feeders (Webb and Viraktamath 2009), and some species have been reported to transmit phytoplasmas to gramineous crops. For example, the zigzag leafhopper *Maiestas dorsalis* (Motschulsky) is a vector of rice orange leaf phytoplasma in Asia (Rivera et al. 1963). *Maiestas distinctus* (Motschulsky) and *M. dorsalis* have been shown to be associated with sugarcane white leaf phytoplasma in polymerase chain reaction (PCR) assays designed to detect phytoplasma from field-collected specimens (Hanboonsong et al. 2006). Rice orange leaf, sugarcane white leaf, and the Kenyan strain of Napier stunt phytoplasma are all members of the 16SrXI rice yellow dwarf group of phytoplasmas (i.e., *Candidatus Phytoplasma oryzae*), suggesting complex interactions between this group and leafhoppers of the genus *Maiestas*. It is worth noting that the results of this study showed the presence of at least one other species of the genus *Maiestas* (*Maiestas* sp.), which was common in



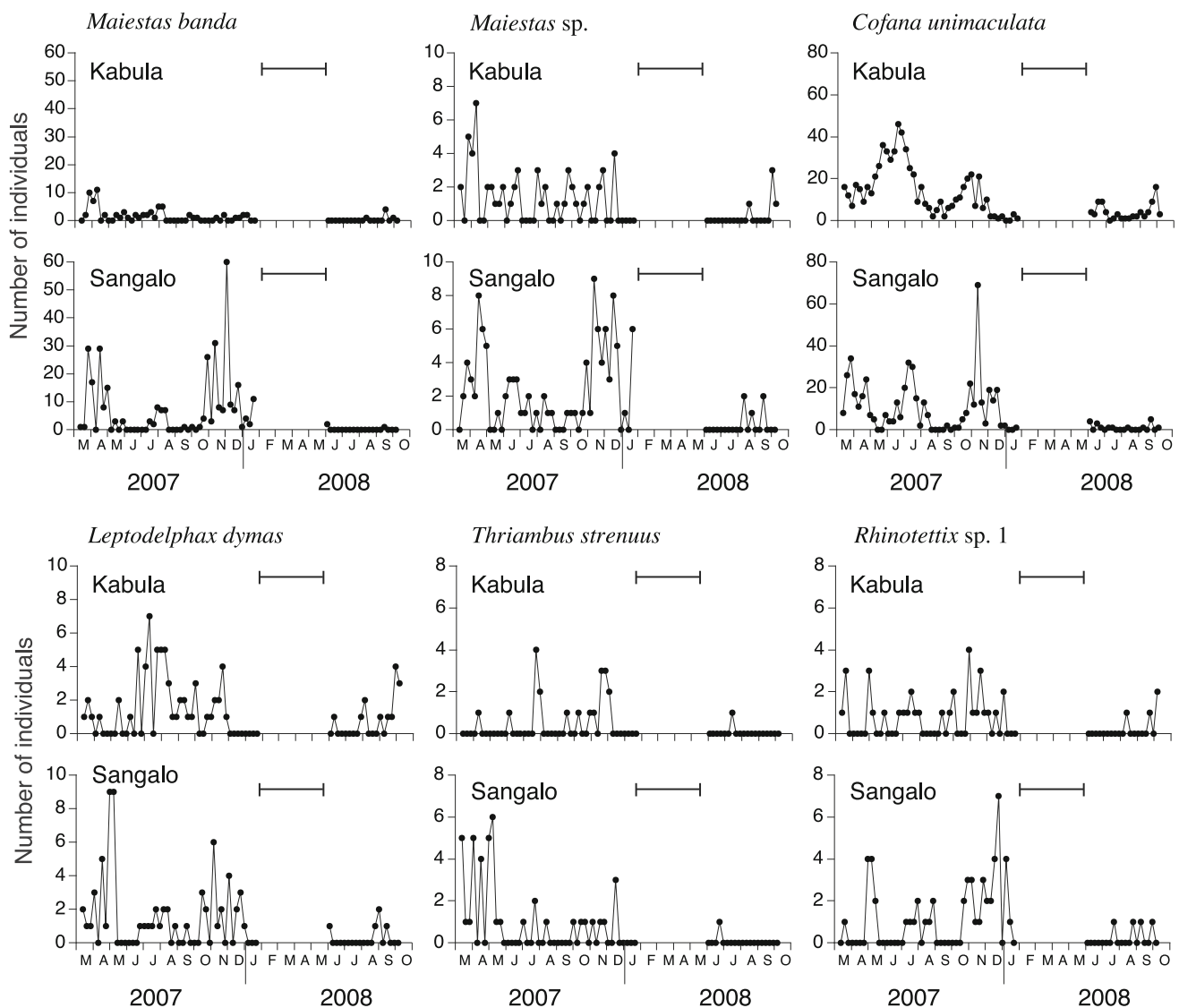
**Fig. 3** Mean ( $\pm$ standard error) densities of leafhopper species (*Maiestas banda*, *Maiestas* sp., *Cofana unimaculata*) and planthopper species (*Leptodelphax dymas*, *Thriambus strenuus*, *Rhinotettix* sp. 1) collected by the suction sampler at two study sites (Kabula and

Sangalo). For periods with an asterisk, a significant difference was detected between infected (closed circles) and healthy (open circles) Napier grass plants (Wilcoxon rank sum test,  $P < 0.05$ )

the study fields. Further investigation is required on its taxonomic identity and its capability to transmit Ns-phytoplasma.

In addition to *M. banda*, there could be other leafhoppers or planthoppers associated with Ns-phytoplasma transmission. Several species identified in this study belong to the genera *Exitianus* and *Leptodelphax*, the members of which have been reported as possible vectors of phytoplasma. *Exitianus capicola* (Stål) has been reported as a vector of phytoplasma in *Limonium* hybrids in Israel (Weintraub et al. 2004). Arocha et al. (2009) demonstrated that field-collected *Leptodelphax dymas* carried a 16Sr III-A phytoplasma, a pathogen responsible for Ns-disease in Ethiopia, and suggested this planthopper as a candidate

vector for this phytoplasma. On the other hand, in pathogen transmission experiments, *L. dymas* was unable to transmit a Kenyan phytoplasma strain (i.e., 16Sr XI phytoplasma) to healthy Napier grass plants, and insects reared on diseased Napier grass for 30 days were phytoplasma negative (Obura et al. 2009). These results indicate that *L. dymas* is not currently a vector of Ns-phytoplasma in Kenya, but its capability to acquire 16Sr III-A phytoplasma suggests the possibility of this planthopper acting as a vector if the Ethiopian phytoplasma strain spreads to the region. Further studies would elucidate other vectors of Ns-phytoplasma among the species that we have collected, especially among species of the genera *Maiestas*, *Exitianus*, and *Leptodelphax*.



**Fig. 4** Seasonal abundance of leafhopper species (*Maiestas banda*, *Maiestas sp.*, *Cofana unimaculata*) and planthopper species (*Leptodelphax dymas*, *Thriambus strenuus*, *Rhinotettix sp. 1*) collected by

the Malaise trap at Kabula and Sangalo. Data was not available for the period shown with a horizontal bar

For the other species identified in this study, four leafhopper species (*Glossocratus afzelii*, *C. polaris*, *C. spectra*, and *C. mbila*) and two planthopper species (*T. strenuus* and *S. manetho*) were observed to feed and survive on Napier grass under caged conditions (Obura et al. 2009). Using Safranine dye technique developed by Khan and Saxena (1984), we demonstrated that all insects, even leafhoppers of the genus *Cofana* that are generally considered to be xylem feeders, fed on the phloem sap and could potentially transmit Ns-phytoplasma (Khan, unpublished data). However, results from transmission experiments showed that all six species were unable to transmit Ns-phytoplasma in Kenya (Obura et al. 2009).

In five leafhopper species (*C. mbila*, *C. polaris*, *C. unimaculata*, *M. banda*, and *Maiestas sp.*), more males

than females were collected with Malaise traps, while the sex ratio was more balanced in the vacuum-suction samples. Such a male-biased sex ratio on a trap catch basis, compared to a sex ratio based on sweeping or suction sampling has been reported for many species of leafhoppers (Rodriguez et al. 1992; Hoy et al. 1999; Lessio et al. 2009). While the vacuum-suction method can collect any insects residing on the surfaces of plants regardless of their flight ability, Malaise traps usually collect active fliers. Hence, a higher proportion of males in trap catches indicates a higher mobility of males. A high male proportion in trap catches was also observed in the planthopper *L. dymas*. As with many species of planthoppers, *L. dymas* exhibited wing-dimorphism, and the macroptery rate was higher for males than for females (Fujinuma, unpublished data).



Males would have higher flight activity and be more likely to be trapped than females.

The vector preference for disease-infected plants could influence vector dispersal activity and hence affect disease epidemiology (McElhany et al. 1995; Sisterson 2008). Many studies have therefore examined the preference of insect vectors for infected plants, but the results varied [reviewed in Sisterson (2008)]. In this study, the diseased Napier grass supported larger populations of *M. banda* than did healthy plants. This might be attributed to the yellowing of infected plants, which become more visually attractive to such insects (Todd et al. 1990; Marucci et al. 2005). Another explanation may involve leafhopper behavioral responses to disease-induced changes in plant nutritional quality (Hammond and Hardy 1988) or in plant odor composition (Eigenbrode et al. 2002; Mayer et al. 2008). The effects of plant color, nutritional quality, and volatiles of phytoplasma-infected Napier grass on the host selection behavior of *M. banda* are not understood and warrant further investigation.

This study demonstrated that populations of four species (*M. banda*, *Maiestas* sp., *T. strenuus*, and *Rhinotettix* sp. 1) exhibited similar cyclical annual fluctuations, with a conspicuous peak at the end of the short rainy season (October–December) in 2007. The population dynamics of leafhoppers and planthoppers may be explained in part by weather conditions (e.g., rainfall and temperature), availability and quality of food resources, and impact of natural enemies (Denno and Roderick 1990; Bosque-Pérez 2000; Bi et al. 2005). In this study, Napier grass was regularly cut and collected from only a proportion of the cultivated field at a time, and all growth stages of the plant were always present in the study field. Therefore, host plant availability can be ruled out as a possible regulatory factor of the population fluctuations observed in this study. As the temperature in western Kenya was relatively stable, with no particular seasonal pattern, rainfall might be an environmental factor affecting the population dynamics of the species examined in this study. In humid forest locations in southern Nigeria, Asanzi et al. (1994) found that the population levels of leafhoppers of the genus *Cicadulina* were consistently low during most of the year, but after the rains ended in October, the leafhopper population density rapidly increased, producing a sharp peak. They suggested heavy rainfall as the responsible factor for this pattern as it can cause insect mortality. Studies in Zimbabwe (Rose 1972) and northern Nigeria (Alegbejo and Banwo 2005) also demonstrated that rainfall consistently affected the abundance of *Cicadulina* leafhoppers. In this study, a conspicuous population peak was observed in the same season for six species, implying that common regulatory factors such as rainfall might affect the leafhopper and planthopper complex. However, since this conclusion is based on an 18 month

observation that covered only one cycle of the population fluctuation, further long-term studies are necessary to address the relationships between weather conditions and leafhopper/planthopper population dynamics in more detail.

In conclusion, this study revealed the components of the leafhopper and planthopper species complex on Napier grass and showed that *M. banda* was one of the abundant species. Considering its vectoring capability and high population density, *M. banda* may play a major role in the Ns-disease epidemiology in western Kenya. This study elucidated some of the population characteristics of *M. banda*, such as the higher mobility of males compared to females, the high abundance on disease-infected plants, and cyclical population fluctuation with a peak at the end of the short rainy season. In addition, this study provided information on the influence of disease infection of host plants on the abundance of non-vector species, which is important for estimating the positive and negative effects of the Ns-disease epidemic on the insect herbivore community present on Napier grass. The biological characteristics of the insect vectors are certainly of epidemiological relevance and are being studied further with experimental methodologies. Since the alternative/reservoir plants harboring the Ns-phytoplasma are unknown, understanding of the host range of *M. banda* as well as other potential vector insects is also desirable.

**Acknowledgments** The authors thank Eshmail Kidiavai, Romanus Odhiambo, Washington Omondi and staff at *ICRPE* Thomas Odhiambo Campus, Mbita Point for their field and laboratory assistance, and Masaya Matsumura for his helpful comments on earlier drafts of the manuscript. We are indebted to the Lake Basin livestock multiplication centre and Kabula catholic parish for allowing us to work in their fields. This study was partly funded by the Japan Society for the Promotion of Science via a Grant-in-Aid for Scientific Research (no. 21405018) and the McKnight Foundation.

## References

- Alegbejo MD, Banwo OO (2005) Relationship between some weather factors, maize streak virus genus *Mastrevirus* incidence and vector populations in northern Nigeria. *J Plant Prot Res* 45:99–105
- Arocha Y, Zerfy T, Abebe G, Proud J, Hanson J, Wilson M, Jones P, Lucas J (2009) Identification of potential vectors and alternative plant hosts for the phytoplasma associated with Napier grass stunt disease in Ethiopia. *J Phytopathol* 157:126–132
- Asanzi CM, Bosque-Pérez NA, Buddenhagen IW, Gordon DT, Nault LR (1994) Interactions among maize streak virus disease, leafhopper vector populations and maize cultivars in forest and savanna zones of Nigeria. *Plant Pathol* 43:145–157
- Atakan E (2011) Development of a sampling strategy for the leafhopper complex [*Asymmetrasca decedens* (Paoli) and *Empoasca decipiens* Paoli] (Hemiptera: Cicadellidae) in cotton. *J Pest Sci* 84:143–152
- Beanland L, Hoy CW, Miller SA, Nault LR (1999) Leafhopper (Homoptera: Cicadellidae) transmission of aster yellows phytoplasma: does gender matter? *Environ Entomol* 28:1101–1106

- Bi JL, Castle SJ, Byrne FJ, Tuan SJ, Toscano NC (2005) Influence of seasonal nitrogen nutrition fluctuations in orange and lemon trees on population dynamics of the grassy-winged sharpshooter (*Homalodisca coagulata*). *J Chem Ecol* 31:2289–2308
- Bosque-Pérez NA (2000) Eight decades of maize streak virus research. *Virus Res* 71:107–121
- Chiykowski LN (1981) Epidemiology of diseases caused by leafhopper-bone pathogens. In: Maramorosch K, Harris KF (eds) *Plant disease and vectors*. Academic Press, New York, pp 106–159
- Cook SM, Khan ZR, Pickett JA (2007) The use of ‘push-pull’ strategies in integrated pest management. *Annu Rev Entomol* 52:375–400
- Denno RF, Roderick GK (1990) Population biology of planthoppers. *Annu Rev Entomol* 35:489–520
- Eigenbrode SD, Ding H, Shiel P, Berger PH (2002) Volatiles from potato plants infected with potato leafroll virus attract and arrest the virus vector, *Myzus persicae* (Homoptera: Aphididae). *Proc R Soc Lond B* 269:455–460
- Hammond AM, Hardy TN (1988) Quality of diseased plants as hosts for insects. In: Heinrichs EA (ed) *Plant stress–insect interactions*. Wiley, New York, pp 381–432
- Hanboonsong Y, Ritthison W, Choosai C, Sirithorn P (2006) Transmission of sugarcane white leaf phytoplasma by *Yamatotettix flavovittatus*, a new leafhopper vector. *J Econ Entomol* 99:1531–1537
- Hoy CW, Zhou X, Nault LR, Miller SA, Styer J (1999) Host plant, phytoplasma, and reproductive status effects on flight behavior of aster leafhopper (Homoptera: Cicadellidae). *Ann Entomol Soc Am* 92:523–528
- Hunt RE, Parr JC, Haynes KF (1993) Influence of leafhopper (Homoptera: Cicadellidae) gender and female mating status on plant disease dynamics within a simple habitat. *Environ Entomol* 22:109–115
- Irwin ME, Ruesink WG (1986) Vector intensity: a product of propensity and activity. In: McLean GD, Garrett RG, Ruesink WG (eds) *Plant virus epidemics: monitoring, modelling and predicting outbreaks*. Academic Press, New York, pp 13–33
- Jones PB, Devonshire J, Holman TJ, Ajanga S (2004) Napier grass stunt: a new disease associated with a 16SrXI group phytoplasma in Kenya. *Plant Pathol* 53:519
- Jones P, Arocha Y, Sheriff T, Proud J, Abebe G, Hanson J (2007) A stunting syndrome of Napier grass in Ethiopia is associated with a 16SrIII group phytoplasma. *Plant Pathol* 56:345
- Khan ZR, Saxena RC (1984) Technique for demonstrating phloem or xylem feeding by leafhoppers (Homoptera: Cicadellidae) and plant hoppers (Homoptera: Delphacidae) in rice plant. *J Econ Entomol* 77:550–552
- Khan ZR, Midega CAO, Hutter NJ, Wilkins RM, Wadhams LJ (2006) Assessment of the potential of Napier grass (*Pennisetum purpureum* Schumacher) varieties as trap plants for management of *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae). *Entomol Exp Appl* 119:15–22
- Khan ZR, Midega CAO, Wadhams LJ, Pickett JA, Mumuni A (2007) Evaluation of Napier grass (*Pennisetum purpureum*) varieties for use as trap plants for the management of African stem borer (*Busseola fusca*) in a push-pull strategy. *Entomol Exp Appl* 124:201–211
- Lessio F, Tedeschi R, Pajoro M, Alma A (2009) Seasonal progression of sex ratio and phytoplasma infection in *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae). *Bull Entomol Res* 99:377–383
- Marucci RC, Lopes JRS, Vendramim JD, Corrente JE (2005) Influence of *Xylella fastidiosa* infection of citrus on host selection by leafhopper vectors. *Entomol Exp Appl* 117:95–103
- Mayer CJ, Vilcinskis A, Gross J (2008) Phytopathogen lures its insect vector by altering host plant odor. *J Chem Ecol* 34:1045–1049
- McElhany P, Real LA, Power AG (1995) Vector preference and disease dynamics: a study of barley yellow dwarf virus. *Ecology* 76:444–457
- Mugalavai EM, Kipkorir EC, Raes D, Rao MS (2008) Analysis of rainfall onset, cessation and length of growing season for western Kenya. *Agric For Meteorol* 148:1123–1135
- Nielsen SL, Ebong C, Kabirizi J, Nicolaisen M (2007) First report of a 16SrXI group phytoplasma (*Candidatus Phytoplasma oryzae*) associated with Napier grass stunt disease in Uganda. *Plant Pathol* 56:1039
- Nyaata OZ, Dorward PT, Keatinge JDH, O’Neill MK (2000) Availability and use of dry season feed resources on smallholder dairy farms in central Kenya. *Agrofor Syst* 50:315–331
- Obura E, Midega CAO, Masiga D, Pickett JA, Hassan M, Koji S, Khan ZR (2009) *Recilia banda* Kramer (Hemiptera: Cicadellidae), a vector of Napier stunt phytoplasma in Kenya. *Naturwissenschaften* 96:1169–1176
- Orodho AB (2006) The role and importance of Napier grass in the smallholder dairy industry in Kenya. [http://www.fao.org/ag/AGP/AGPC/doc/Newpub/napier/napier\\_kenya.htm](http://www.fao.org/ag/AGP/AGPC/doc/Newpub/napier/napier_kenya.htm). Accessed 5 March 2011
- Power AG (1987) Plant community diversity, herbivore movement, and an insect-transmitted disease of maize. *Ecology* 68:1658–1669
- Rivera CT, Ou SH, Pathak MD (1963) Transmission studies of the orange-leaf disease of rice. *Plant Dis Rep* 47:1045–1048
- Rodriguez CM, Madden LV, Nault LR (1992) Diel flight periodicity of *Graminella nigrifrons* (Homoptera: Cicadellidae). *Ann Entomol Soc Am* 85:792–798
- Rose DJW (1972) Times and sizes of dispersal flight by *Cicadulina* spp. vectors of maize streak disease. *J Anim Ecol* 41:494–506
- SAS Institute (2004) JMP user’s manual, version 5th ed. SAS Institute, Cary
- Sisterson MS (2008) Effects of insect-vector preference for healthy or infected plants on pathogen spread: insights from a model. *J Econ Entomol* 101:1–8
- Sokal RR, Rohlf FJ (1995) *Biometry*. Freeman, New York
- Todd JL, Harris HO, Nault LR (1990) Importance of color stimuli in host-finding by *Dalbulus* leafhoppers. *Entomol Exp Appl* 54:245–255
- Webb MD, Viraktamath CA (2009) Annotated check-list, generic key and new species of Old World Deltocephalini leafhoppers with nomenclatorial changes in the *Deltocephalus* group and other Deltocephalinae (Hemiptera, Auchenorrhyncha, Cicadellidae). *Zootaxa* 2163:1–64
- Weintraub PG, Beanland L (2006) Insect vectors of phytoplasmas. *Annu Rev Entomol* 51:91–111
- Weintraub PG, Pivonia S, Rosner A, Gera A (2004) A new disease in *Limonium latifolium* hybrids. II. Investigating insect vectors. *HortScience* 39:1060–1061