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Identification of potential vectors of the coconut lethal disease phytoplasma

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Lethal disease (LD) is a lethal yellowing-type disease of coconuts associated with phytoplasmas in Tanzania, but the insect vector for it has not yet been identified. In this study, the auchenorrynchous insects in LD-infected coconut fields were surveyed to determine potential vectors for the disease. No significant correlation was found between disease incidence and numbers of insects collected from the field, possibly reflecting the unknown incubation period for the disease. However, analysis of more than 5000 individual insects by the polymerase chain reaction (PCR), using LD-specific primers derived from the phytoplasma 16S rRNA gene, revealed PCR products of the correct size from eight individuals of *Diastrombus mkurangai* and four of *Meenoplus* spp. When digested with restriction endonucleases, fragments of the same size as the LD phytoplasma were obtained. No PCR products were detected in any of the other insect species tested. These results implicate *D. mkurangai* and *Meenoplus* spp. as probable vectors of the LD phytoplasma.

Keywords: coconut, *Diastrombus mkurangai*, LD phytoplasmas, *Meenoplus* spp., PCR products, vectors

Introduction

Coconut palm (*Cocos nucifera*) is an important perennial oil crop of Tanzania, providing food, building materials and also conservation of the environment. However, a destructive lethal yellowing-like disease known as Lethal Disease (LD) has caused extensive damage to plantations on the mainland for more than 30 years, and is now present on the island of Mafia. Symptoms of LD are premature nutfall, bronzing of successively younger leaves, blackening of young emergent inflorescences, drying of older inflorescences, rot and collapse of the spear leaf, and decay of the root system, with subsequent sudden death (Schuiling *et al*., 1992). Similar symptoms have been reported for other yellowing diseases of coconut associated with phytoplasmas, including lethal yellowing (LY), Cape St Paul Wilt and Awka disease (Eden-Green, 1993). All these yellowing diseases, including LD, are collectively referred to as lethal yellowing-type diseases (LYD). Despite symptomatological similarities with LY, LD differs in its epidemiology, susceptibility of coconut cultivars and insect vectors. Whereas LY is characterized by a 'rapid jump spread' pattern in the Caribbean (McCoy, 1976), this pattern of spread is rare with LD (Schuiling *et al*., 1992). Where the vector of a phytoplasma disease has been identified, it is an insect in the order Homoptera and especially suborder Auchenorryncha (Nielson, 1979; Nienhaus & Sikora, 1979; Wilson, 1988). Of these, the true leafhoppers (Cicadellidae) have been considered the most important vector group (Nielson, 1979). These insects, however, are not the predominant group on coconut palms. In Jamaica, during a search for vectors of LY, leafhoppers were found to predominate in the undergrowth, while planthoppers (Fulgoridae) were the most prevalent group on palms (Dabek, 1981). The cixiid, *Myndus crudus*, was the most abundant planthopper, and remained as the prime suspect vector of LY in Jamaica although extensive transmission trials failed to confirm this possibility (Schuiling *et al*., 1976; Eden-Green, 1978; Eden-Green & Schuiling, 1978; Dabek, 1981). Subsequently, this planthopper was shown, in transmission trials, to be a vector of LY in Florida (Howard *et al*., 1983). Studies on the homopterans associated with palms in Tanzania revealed findings similar to those in Jamaica, although the planthopper *Myndus crudus* has not been observed in Tanzania. The auchenorrynchous insects occurring on palms in Tanzania were predominantly planthoppers, with species in the family Derbidae being the most abundant (Kaiza, 1987). Similar studies in Ghana showed the planthoppers *Myndus adiopodoumeensis* (Cixiidae) and *Nzinga palmivora* (Typhlocibinae) to predominate, but *M. crudus* was not found (Dery *et al*.,

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1997). In both countries, however, transmission trials have so far failed to implicate any of these species as vectors (Schuiling *et al.*, 1992; Dery *et al.*, 1997). Transmission experiments for LD were conducted with insects collected directly from the field on diseased palms because the breeding habits of these insects are unknown (Anonymous, 1987; Kaiza, 1987). To date, insects used in transmission attempts have not been assayed to confirm whether they feed on infected palms and ingest phytoplasmas. Until recently, detection of phytoplasmas in their insect vectors has been difficult. It could be achieved only in a few cases by use of serological assays (Lin & Chen, 1985). However, the advent of DNA-based diagnostics has enabled sensitive detection of phytoplasma infections in both plant and insect hosts (Kirkpatrick *et al.*, 1987; Davis *et al.*, 1988; Rahardja *et al.*, 1992; Vega *et al.*, 1993; Harrison, 1996). By use of cloned random fragments of phytoplasma DNA as probes in DNA–DNA hybridization assays, the Western X-disease phytoplasma of stone fruits was detected in plants and insects, and demonstrated to multiply in the leafhopper vector, *Colladonus montanus*, as well as in celery, an experimental herbaceous host plant (Kirkpatrick *et al.*, 1987). Similarly, by use of the polymerase chain reaction (PCR), phytoplasma ribosomal DNA was amplified from both vector and nonvector insects alike after they fed upon phytoplasma-infected plants (Vega *et al.*, 1993; Harrison, 1996), implying that even nonvector insects can acquire the phytoplasma during feeding. However, by use of PCR and specific DNA probes to monitor insects, multiplication of the LY phytoplasma was demonstrated to occur only in the vector, planthopper *M. crudus* and not in the nonvector *Peregrinus maidis* (Harrison, 1996). In contrast, these techniques have been used to demonstrate that the severe strain aster yellows (SAY) phytoplasma multiplies in both its leafhopper vector *Macrosteles fascifrons* and the nonvector *Dalbulus maidis* (Vega *et al.*, 1993). These findings provided the stimulus for attempting similar studies on the LD phytoplasma. In previous work, PCR methods for the detection of LD in coconut palms were detailed (Tymon *et al.*, 1997; Mpunami *et al.*, 1999). In the present study these techniques were utilized on collections of insects to determine potential candidates for the LD vector. Although detection of the phytoplasma in an insect does not necessarily prove its vector status, such detections serve to narrow down the number of species to a few likely candidates that could be conveniently screened in biological assays, increasing the probability of identifying the real vector(s).

Materials and methods

Collection of insects

Variation in the populations of homopterans suspected as vectors of LD were studied at two variety trial sites, Chambezi and Kifumangao, representing moderate and high disease incidence areas, respectively (Mpunami *et al.*, 1999). Thirty palms were selected as source of insects at each site within the disease screening trials, to include tall (mature) and short (young) palms, and palms located at both the edge and the centre of the field. Insect traps were attached to each of the selected palms. A trap consisted of a plywood board, 10 × 15 cm, painted bright yellow with oil paint and having a hole in one corner. Both surfaces were coated with the sticky insect adhesive, Oecotac. The traps were enclosed with a wire mesh (chicken wire) to prevent leaves from getting stuck to them. Each trap was hoisted high up into the canopy of the selected palm, using a sisal string that formed a pulley system, and was tied to the lower part of the palm trunk. Once a week the traps were lowered, and all trapped insects removed into collection bottles containing kerosene to dissolve any adhering Oecotac. The insects from each palm were then transferred to fresh bottles containing 70% (v : v) ethanol, and transported to the laboratory for sorting, identification and enumeration.

Isolation of DNA from insect specimens

It was originally intended to use insects collected from traps for DNA isolation and PCR. However, the percentage of insects recovered intact after washes in kerosene and alcohol was small, and many were bruised and presumed to be releasing inhibitors that might interfere with the screening process. Extraction of DNA from trapped insects was therefore abandoned. Instead, insects of selected species were collected manually from the underside of leaves on palms showing typical LD symptoms, using large conical flasks to minimize damage. More than 15 000 insects were collected, and DNA extracted according to the procedure of Harrison *et al.* (1996). Insects used for DNA extraction were either freshly collected from the field, or frozen at −20°C immediately after collection. Single insects, or groups of three, were crushed in microfuge tubes in 300 μL of prewarmed (65°C) CTAB buffer (2% (w : v) CTAB, 100 mM Tris-HCl, pH 8·0, 20 mM EDTA pH 8·0, 1·4 M NaCl, 1% (w : v) PVP-40, 1% (w : v) 2-mercaptoethanol) using disposable pestles made from blue Eppendorf pipette tips. Ground samples were incubated for 15 min at 65°C, cooled to room temperature, and extracted with an equal volume of chloroform: isoamyl alcohol (24 : 1). The mixture was centrifuged at 12 000 *g* for 15 min at room temperature (20°C) to separate the phases, and nucleic acids were precipitated from the aqueous phase with 0·6 volume of room temperature isopropanol for 30 min. Nucleic acids were pelleted by centrifugation for 15 min at 12 000 *g*, washed in 70% (v : v) ethanol, air dried, and dissolved in 50 μL of TE, pH 8·0, then stored at 4°C until required.

Screening putative insect vectors for phytoplasma DNA by PCR

DNA extracted from single insects or batches of three insects was screened by PCR for presence of phytoplasma

Table 1 Numbers of insects trapped at Chambezi (moderate disease incidence) and Kifumangao (high disease incidence) between August 1995 and July 1996

	Chambezi												Kifumangao											
	1995					1996							1995					1996						
Sp.[a]	a	s	o	n	d	j	f	m	a	m	j	j	a	s	o	n	d	j	f	m	a	m	j	j
A	0	3	23	3	0	6	2	11	9	4	1	0	28	18	25	11	4	2	0	4	3	3	0	0
B	3	7	6	4	2	1	4	10	2	6	3	4	3	9	9	13	5	3	5	7	2	7	3	4
C	0	0	0	0	0	0	2	0	0	0	0	0	0	0	1	1	0	0	3	0	1	1	0	0
D	0	0	0	0	0	0	0	0	1	0	0	0	0	4	1	6	5	0	0	1	0	0	0	0
E	1	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
G	24	14	10	4	26	5	1	13	7	3	6	2	40	23	27	7	8	1	11	40	15	8	11	6
H	22	11	10	13	10	7	4	0	15	4	15	2	32	5	5	11	92	60	15	0	22	15	13	6
I	4	1	0	3	1	0	1	0	1	2	3	0	21	0	0	4	0	0	0	0	1	1	2	0
J	0	0	0	2	0	0	1	1	0	2	1	0	0	4	1	5	5	5	1	6	4	2	5	1
K	1	1	5	0	0	1	1	0	0	1	0	0	1	5	2	4	1	2	2	3	0	2	1	2
L	0	1	0	0	0	0	1	0	1	1	4	0	2	2	2	1	0	1	0	2	1	3	3	1
M	1	3	3	0	0	0	1	1	0	0	0	0	11	4	3	3	2	1	0	2	0	0	1	0
N	1	1	0	1	0	0	0	0	1	0	1	0	0	0	0	2	0	2	1	0	0	0	0	0
O	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2	5	2	7	1	0	1
P	53	62	49	12	38	17	12	16	14	16	24	7	33	19	34	22	14	15	18	54	36	29	10	21
Q	1	4	6	2	2	10	6	18	11	6	7	6	25	21	10	7	8	20	20	6	33	8	12	11
Total	111	108	114	44	79	47	36	70	62	45	64	21	198	115	120	97	144	114	81	127	125	80	62	53

[a] Family Derbidae: A, *Diastrombus abdominalis*; B, *Diastrombus mkurangai*; C, *Phenice pongwei*/*Paraphenice mawae*; D, *Robigus magawae*; E, *Diastrombus schuilingi*; F, *Zoraida fuligipennis*; G, unidentified grass-green Cicadellidae; H, *Amania angustifrons*/*Nesodryas antiope*; I, *Kamendaka kordofana*; J, *Lydda woodi*; K, *Zorabana* spp. Family Cercopidae: L, *Bandusia erythrostena*; Family Lophodidae: M, *Elasmosceles cimicoides*; Family Meenoplidae: N, *Meenoplus* spp.; Family Nogodinidae: O, *Diazanus* spp.; P, unidentified planthoppers; Q, other insects.

DNA. Extracts from 5000 of the total of 15 000 insects collected were screened using the primer pair Rohde forward (5′-GAG-TAC-TAA-GTG-TCG-GGG-CAA-3′) with Rohde reverse (5′-AAA-AAC-TCG-CGT-TTC-AGC-TAC-3′) (Rohde *et al*., 1993).

For each PCR a 25-μL reaction mixture contained about 50 ng template, 150 μM mixed deoxynucleotide triphosphates (dNTPs), 50 ng of each primer, 1 unit of Taq Polymerase (Promega Corporation), and PCR buffer (100 mM Tris-HCl pH 8·3, 500 mM KCl, 15 mM $MgCl_2$, 0·01% gelatin, 0·5% (v : v) Nonidet P40, 0·5% (v : v) Tween 20). The mixture was overlaid with 25 μL of mineral oil, and subjected to 36 cycles in an automated thermocycler (Biometra, UNO Thermoblock) using the following parameters: 1 min (2 min for the first cycle) denaturation at 94°C, 1 min 20 s annealing at 57°C, and 2 min 10 s (5 min for last cycle) extension at 72°C. PCR products were analysed by electrophoresis through 1% (w/v) agarose gels and visualized in the gel by UV transillumination after staining with ethidium bromide.

Results

Distribution and incidence of potential vectors

Insects were continuously trapped at two sites as previously described for a period of one year, and the numbers and diversity of insects trapped in each month are summarized in Table 1. The following genera and species were identified: *Diastrombus abdominalis, D. mkurangai, D. schulingi, Meenoplus* spp., *Phenice* spp., *Paraphenice* spp., *Elasmosceles cimicoides, Lydda woodi, Robigus* spp., *Amania angustifrons, Bandusia erythrostena, Zoraida fuligipennis, Zorabana* spp., *Diazanus* spp. and *Kamendaka* spp.

At Chambezi, peak insect populations were trapped during the drier months of August to October 1995. This was followed by a sharp decline in November (short wet season), but the numbers then increased slightly during the short dry season before declining once again in February and remaining low until the end of the wet season in June 1996. A similar pattern was obtained at Kifumangao, although overall more insects were usually collected at this site.

The insect species captured at both sites were predominantly planthoppers in the family Derbidae, except for four species, one each for families Cercopidae, Nogodinidae, Lophodidae, and Meenoplidae. Despite the low numbers, there were clear differences in the populations of several species on palms at different times of the year. The two morphologically similar derbids, *Nesodryas antiope* and *Amania angustifrons* (species H), which were previously suspected to breed on palms (Kaiza, 1987), were more abundant than the other species. The other abundant insect species were represented by an undescribed grass-green-coloured leafhopper (species G), and an undescribed planthopper (species P). The insect species suspected to be vectors of LD, *Diastrombus abdominalis*

Table 2 Association between numbers of insects collected and incidence of disease

Location/			Disease incidence												
month	Total	Di + Me[a]	M	+1	+2	+3	+4	+5	+6	+7	+8	+9	+10	+11	+12
Chambezia															
a (1995)	111[b]	4	1[c]	1	1	3	3	7	4	3	9	1	5	4	4
s	108	8	1	1	3	3	7	4	3	9	1	5	4	4	5
o	114	6	1	3	3	7	4	3	9	1	5	4	4	5	0
n	44	5	3	3	7	4	3	9	1	5	4	4	5	0	2
d	79	2	3	7	4	3	9	1	5	4	4	5	0	2	2
j (1996)	47	1	7	4	3	9	1	5	4	4	5	0	2	2	4
f	36	4	4	3	9	1	5	4	4	5	0	2	2	4	5
m	70	10	3	9	1	5	4	4	5	0	2	2	4	5	1
a	62	3	9	1	5	4	4	5	0	2	2	4	5	1	0
m	45	6	1	5	4	4	5	0	2	2	4	5	1	0	
j	64	4	5	4	4	5	0	2	2	4	5	1	0		
j	21	4	4	4	5	0	2	2	4	5	1	0			
Kifumangaoa															
a (1995)	198	3	0	4	0	8	3	0	4	4	5	5	12	7	2
s	115	9	4	0	8	3	0	4	4	5	5	12	7	2	5
o	120	9	0	8	3	0	4	4	5	5	12	7	2	5	2
n	97	15	8	3	0	4	4	5	5	12	7	2	5	2	3
d	144	5	3	0	4	4	5	5	12	7	2	5	2	3	1
j (1996)	114	5	0	4	4	5	5	12	7	2	5	2	3	1	7
f	81	6	4	4	5	5	12	7	2	5	2	3	1	7	12
m	127	7	4	5	5	12	7	2	5	2	3	1	7	12	4
a	125	2	5	5	12	7	2	5	2	3	1	7	12	4	5
m	80	7	5	12	7	2	5	2	3	1	7	12	4	5	
j	62	3	12	7	2	5	2	3	1	7	12	4	5		
j	53	4	7	2	5	2	3	1	7	12	4	5			

[a] *Diastrombus mkurangai* and *Meenoplus* spp. (Di + Me)[b] Data are the total number of insects collected in the month (Total).[c] Disease incidence is recorded as the number of new trees that showed visible symptoms in the month, 1 month later (+1), 2 months later (+2) etc., up to 12 months later (+12).

Table 3 The use of PCR to screen leaf sucking insects (homoptera) for phytoplasma rDNA

Insect species	Total collected	Total screened	DNA band (+)
Diastrombus abdominalis	7529	2191	0
Diastrombus mkurangai	3415	1270	8
Diastrombus schuilingi	776	339	0
Phenice pongwei/Paraphenice mawae	31	23	0
Robigus magawae	2	0	0
Zoraida fuligipennis	40	0	0
Amania angustifrons/Nesodryas antiope	222	222	0
Kamendaka kordofana	12	3	0
Lydda woodi	3521	684	0
Zorabana spp. = *Pamendanga* spp.	160	87	0
Unidentified planthoppers	95	82	0
Family Cercopidae			
Bandusia erythrostena	6	0	0
Family Lophodidae			
Elasmosceles cimicoides	236	163	0
Family Meenoplidae			
Meenoplus Sensu latu	29	14	4
Diazanus spp.	249	47	0
Family Nogodinidae			
Others (unclassified)	58	20	0

(A) and *D. mkurangai* (B) (Kaiza, 1987), were not trapped in large numbers, although they were common and present throughout the year.

Correlation of insects trapped with disease incidence

The incidence of disease at each site was recorded monthly as the number of new palms that had become diseased. Table 2 shows these results alongside numbers of trapped insects and numbers of trapped *D. mkurangai* and *Meenoplus* spp. Because of the low numbers, there are no statistically significant results from these data. However, there is no apparent correlation between disease incidence and the insects present in the field at the same time, with the lowest incidence of disease occurring when most insects are present. By contrast, the incidence of disease showed a stronger linkage to the numbers of insects that were trapped 6–9 months previously. This linkage appeared particularly strong with the numbers of trapped *D. mkurangai* and *Meenoplus* spp., which may indicate that the incubation time between infection and symptom development is at least 6 months.

PCR detection of phytoplasma DNA in insect samples

The primer pair, Rohde forward/reverse, was routinely used to screen field-collected insects suspected to be potential vectors of LD. The possibility of inhibitors in insect DNA was always monitored by including control reactions in which insect DNA was spiked with palm-derived LD DNA for each PCR reaction. PCR products of the same size as those from infected plants were detected in only 12 out of more than 5000 insects screened by PCR (Table 3, Fig. 1a). These insects were identified as *Diastrombus mkurangai* (8) and *Meenoplus* (4) spp. PCR products from the insects and LD-infected coconut were digested with the restriction enzyme *Tru*91, and the restriction pattern from both sources was indistinguishable (Fig. 1b). Digestion with *Alu*I and *Taq*I also yielded a similar restriction pattern from palms and insects (results not shown). These results suggest that the PCR product from insects was the same as that detected in palms.

Discussion

In this work, field studies on the behaviour of insects were combined with laboratory detection of phytoplasmas in insects to provide evidence suggesting that the insect species *D. mkurangai* and *Meenoplus* spp. are vectors of LD. Studies on the fluctuations in insect populations at selected sites have indicated that local environmental conditions may strongly affect flight and feeding behaviour of the insects. This has important consequences for pathogen acquisition and disease spread. The behaviour of *D. mkurangai* was particularly striking, because the distribution in the field varied with specific months. At Chambezi, for example, these

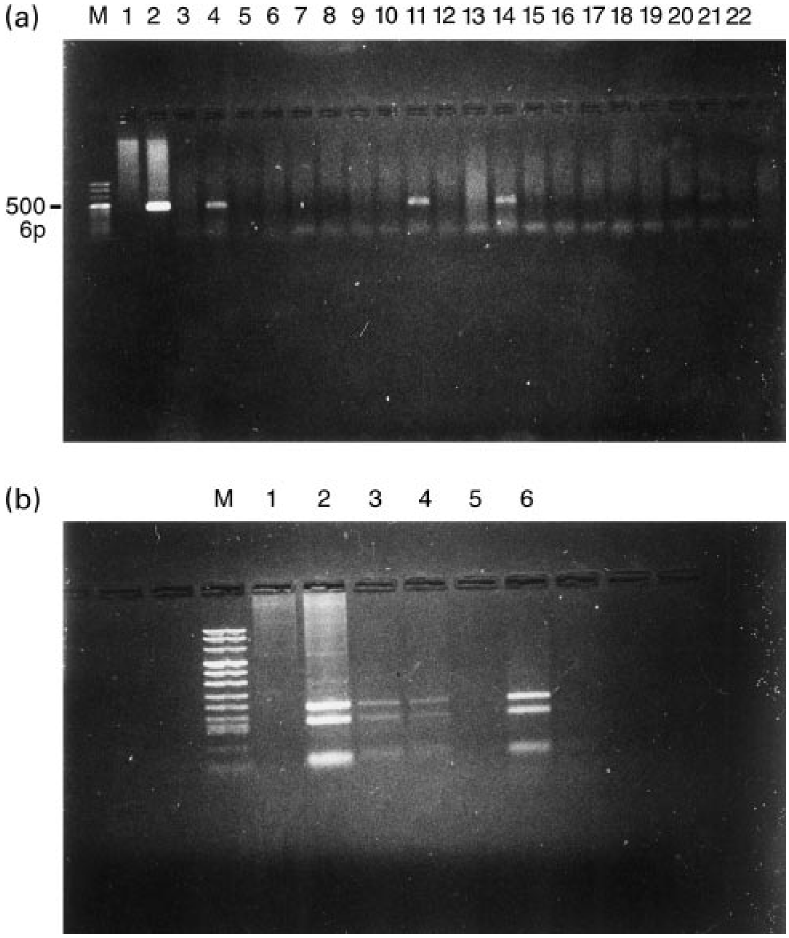

Figure 1 (a) Amplification of phytoplasma rDNA from putative insect vectors for LD. A 500-bp band was amplified from LD DNA and from several insects (*D. mkurangai* and *Meenoplus* spp.) using Rohde forward and reverse primers. Lanes: (M) 1 kb ladder; (1) healthy coconut DNA; (2) LD DNA; (3–22) samples of insect DNA, each sample containing the DNA extracted from 3 insects. (b) *Tru*91 restriction enzyme digestion of 16S rDNA amplified from LD-infected coconut DNA and DNA of putative insect vectors. Lanes: (M) 1 kb ladder; (1) healthy coconut DNA; (2) digested LD rDNA; (3–6) digested rDNA amplified from 4 different potential insect vectors.

insects were caught on palms near the edge of the field mainly during October, January, and March to April. In June and July they were caught only on tall palms, both at the edges and in the middle of the field. Their predominance during dry months would suggest that their flight into the field from outside was favoured during those months. For the rest of the year, they were caught in the middle of the field, and predominantly on short palms, which suggests breeding within the field.

The behaviour of these insects was different at Kifumangao. Although the general picture was that more insects were trapped on short palms in the middle of the field, these did not include *D. mkurangai*. For the entire year, this species was predominantly trapped on tall palms, especially near the edges of the field. In the few months that they were trapped on short palms, it was only on those palms located near the field edge. These results suggest that at this location, these insects were breeding outside, and continuously flying into the field. If they are the true vectors of LD, then this behaviour is in agreement with the strong edge effects that have been observed in the disease-affected fields (Schuiling *et al*., 1992), with higher disease incidence near the borders.

The other insect species highly suspected to be a putative vector of LD, *Meenoplus* spp., was found to be less abundant. At Chambezi they were trapped only during August, September, April and June and, except for August, they were trapped on palms in the middle of the field. At Kifumangao, they were trapped during November, January and February, and in all cases on tall palms, either in the middle of the field or near the edge. There is no evidence that these species breed on palms, and the data appear to suggest breeding both outside and within the field at Chambezi, and predominantly outside the field at Kifumangao. From the relative abundance of *Meenoplus* spp., it can be deduced that if they are vectors of LD, then their contribution to disease transmission would be restricted to short periods within a year. Furthermore, the correlation data between disease incidence and numbers of these species would suggest that there is an incubation period of at least 6 months between infection and symptoms of disease.

Reliable detection of phytoplasmas in insect vectors has been reported, both by use of DNA probes (Kirkpatrick *et al*., 1987; Rahardja *et al*., 1992; Nakashima *et al*., 1993) and PCR (Vega *et al*., 1993; Harrison *et al*., 1995). In this study, specific primers were used to screen potential insect vectors for the presence of the LD phytoplasma, and PCR products were amplified in eight individuals of *Diastrombus mkurangai* and four of *Meenoplus* spp., out of more than 5000 screened. This very low proportion of insects that tested positive for phytoplasma (0·16%) indicates that the LD phytoplasma is not readily acquired by the putative insect vectors. The low level of LD acquisition might be caused by low pathogen titre in infected plants, which in turn might be affected by weather conditions; however, although the numbers were small, more than 25% of *Meenoplus* spp. were positive for LD phytoplasma in the present experiments. In contrast, similar studies in Florida reported a comparatively high proportion (40%) of *Myndus crudus* captured on LY-infected palms that tested positive for phytoplasma by PCR (Harrison *et al*., 1994).

Davies *et al.* (1994) reported that the use of nested PCR improved detection of the pear decline phytoplasma in field-captured psyllid vectors to 10% positive, compared with previously inconsistent results. The PCR reaction involved a preliminary amplification of 15–20 cycles using phytoplasma group-specific primers (Deng & Hiruki, 1991), while the nested reaction used primers specific for pear decline. A similar approach will be adopted in future to attempt to overcome the problem of low levels of LD phytoplasma detection in insects. It will also be necessary to establish the extent by which weather conditions and the disease status of the palms affect the process of phytoplasma acquisition by insects. Since the initial tests have narrowed the number of suspects for potential LD vectors to two species, and confirmed that these insects can acquire the LD phytoplasma, trials will also be set up to determine whether they can transmit the disease to healthy palms. It may also be appropriate to test the unidentified leafhoppers (species G) for acquisition of LD. This was not performed in the current study because the planthoppers were assumed to be the most likely candidate vectors, based on the findings from previous studies on LY (Howard *et al*., 1983), and on their abundance on palms.

LD remains the most serious threat to coconut cultivation in Tanzania. The availability of rapid and sensitive methods for the detection of the LD phytoplasmas in their hosts should facilitate disease diagnosis, improve the understanding of the effect of the insect vectors and possible alternative hosts on disease spread, and enhance the breeding of resistant coconut varieties. The results presented here have paved the way for establishment of an integrated approach for effective control of the disease.

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