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Transmission of Sugarcane White Leaf Phytoplasma by *Yamatotettix flavovittatus*, a New Leafhopper Vector

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ABSTRACT Sugarcane white leaf disease is caused by plant pathogenic phytoplasmas that are transmitted to the plant by the leafhopper *Matsumuratettix hiroglyphic*us (Matsumura). To determine whether there are other insect vectors that transmit this disease pathogen, leafhopper species in sugarcane, *Saccharum officinarum* L., fields in northeastern Thailand were monitored by using light traps. Sixty-nine leafhopper species from family Cicadellidae were found. Using nested polymerase chain reaction (PCR) with specific primers, a 210-bp amplified DNA fragment corresponding to phytoplasma associated with sugarcane white leaf disease was detected from 12 species of leafhoppers [*Balclutha rubrostriata* (Melichar), *Balclutha* sp., *Bhatia olivacea* (Melichar), *Exitianus indicus* Distant, *Macrosteles striifrons* Anufriew, *Matsumuratettix hiroglyphicus* (Matsumura), *Recilia distincta* (Motschulsky), *Recilia dorsalis* (Motschulsky), *Recilia* sp., *Thaia oryzivora* Ghauri, *Yamatotettix flavovittatus* Matsumura, and *Xestocephalus* sp.]. The percentage of individual infection with phytoplasma varied from 5% in *B. olivacea* to 35% in *Xestocephalus* sp. The most abundant leafhopper species, i.e., *E. indicus*, *Y. flavovittatus*, and *M. hiroglyphicus* were used in transmission tests to determine their vector status for the sugarcane white leaf phytoplasma transmission. Infected insects were reared on healthy plants and specific PCR followed by sequencing of the amplicons was used to determine whether the phytoplasma was transmitted to the plants. The results showed that both *Y. flavovittatus* and *M. hiroglyphicus*, but not *E. indicus*, can transmit sugarcane white leaf phytoplasma to healthy sugarcane plants. The transmission efficiency of *M. hiroglyphicus* (55%) was higher than that of *Y. flavovittatus* (45%). We conclude that *Y. flavovittatus* is a newly discovered vector for sugarcane white leaf disease, in addition to *M. hiroglyphicus*. These two species peak at different times of the year and therefore complement each other in the transmission of the phytoplasma. Because there are no known alternative host plants for the sugarcane white leaf, management of the disease will necessarily require the control of both *Y. flavovittatus* and *M. hiroglyphicus*.

KEY WORDS *Matsumuratettix hiroglyphicus*, *Yamatotettix flavovittatus*, phytoplasma, sugarcane white leaf disease, transmission

Sugarcane white leaf (SCWL) disease is one of the most destructive diseases of sugarcane, *Saccharum officinarum* L., in Thailand. This disease is caused by a phytoplasma, which produces typical symptoms of total leaf chlorosis and tiller proliferation (Nakashima et al. 1994, Wongkaew et al. 1997). Sequences obtained from the intergenic spacer region between the 16S and the 23S rDNA showed that the SCWL pathogen belongs to the 16SrXI-B genetic group (Cronje et al. 1998, Lee et al. 1998). This phytoplasma cannot be cultured and is an obligate parasite of the host cell. Apart from disease management by eradication of infected plants to decrease the amount of inoculum, no efficient method of disease control has yet succeeded. Attempts to find alternative host plants were made, and it was thought for many years that the gramineous weeds such as Bermuda grass, *Cynodon dactylon* (L.); crowfoot grass, *Dactyloctenium aegyptium* (L.) Willd.; and brachiaria grass, *Brachiaria distachya* (L.) Stapf., also were reservoirs for the phytoplasma. However, sequencing data showed that the phytoplasma that infect these weeds were different from those infecting sugarcane (Hanboonsong et al. 2002). It is known that phytoplasmas that cause plant diseases are transmitted and spread by phloem-feeding insects, most commonly leafhoppers (Membracoidea) but also some planthoppers (Fulgoroidea) and psyllids (Psylloidea) (Ploaie 1981, Hill and Sinclair 2000). In sugarcane disease, the leafhopper *Matsumuratettix hiroglyphicus* (Matsumura) in the suborder Auchenorrhyncha of the Deltocephalinae is the only insect known to transmit SCWL phytoplasma in Taiwan and Thailand (Matsumoto et al. 1968, Chen 1974, Hanboonsong et al. 2002). It was reported that the leafhopper vector also plays an important role as

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a reservoir of the SCWL phytoplasma. The phytoplasma is widely distributed throughout the body of the insect and showed transovarial transmission from generation to generation of the insect vector (Hanboonsong et al. 2002). Thus, the leafhopper *M. hiroglyphicus* is a key target for SCWL control, because it is both reservoir and vector for the phytoplasma. It is known that several species of leafhoppers can transmit the same kind of plant phytoplasma and that a particular leafhopper species is also able to transmit several phytoplasma pathogens (Fletcher et al. 1998, Lee et al. 2000, Palmano and Firrao 2000). In this study, we set out to determine whether there are other leafhopper species that transmit the sugarcane white leaf phytoplasma in addition to *M. hiroglyphicus.*

Materials and Methods

Plant Sources. White leaf disease and healthy sugarcane plants from 8 mo old were collected from sugarcane-growing areas in Udonthani Province, northeastern Thailand. The axillary buds from both ends of healthy sugarcane shoots were tested by polymerase chain reaction (PCR) with specific SCWL primers to confirm their disease-free status. Then, young healthy sugarcane plants were generated from the remaining axillary buds of the middle part of the shoot. The plants were grown in pots and kept in an insect-proof greenhouse. When the plants were 3 wk old, before using them for the transmission test, a few leafs were tested by PCR to confirm their disease-free status.

Monitoring and Collection of Leafhoppers. Leafhoppers from sugarcane fields in Udonthani Province were collected monthly by using light traps to attract the insects and an insect aspirator to catch them. The first insects captured were sent to a leafhopper taxonomist in Japan for species identification. Additional specimens were identified by comparison to the voucher specimen identified by the leafhopper taxonomist. The leafhoppers were counted and tested for SCWL phytoplasma by specific PCR. Occurrence of white leaf disease in the sugarcane field also was surveyed monthly from seedling (2 mo old) until harvesting stages (11–12 mo old). The disease survey was set by observing and counting plants showing disease symptoms from 130 plants per row. A total of 12 rows with a 10-plant interval between rows from 2.1 ha were monitored.

Transmission Test. Three leafhoppers species *Matsumuratettix hiroglyphicus* (Matsumura), *Yamatotettix flavovittatus* Matsumura, and *Exitianus indicus* Distant were collected from sugarcane fields and maintained separately on disease-free young sugarcane plants in nylon mesh insect-proof cages. Then, plants and insect colonies were kept in an insect-proof greenhouse. Insects were kept without food for 4 h before the transmission test. Then, thirty individuals of each insect species were placed on caged SCWL symptomatic plants for acquisition periods of 48 h. One hundred insects from each species were then transferred in groups of five on a total of 20 test plants (3-wk old disease-free sugarcane seedlings) for 5 d. After the inoculation access period, test plants were maintained in nylon mesh insect-proof cages and kept in an insect-proof greenhouse for 8 wk until PCR assays. Symptoms of disease development also were observed daily. Twenty individual plants with no contact with insects kept in the same condition as the test plants were used as control.

PCR Assays for SCWL Phytoplasma in Test Plants. DNA from each test plant and SCWL disease plants was extracted from two leaf midribs by using the cetyltrimethylammonium bromide method (Kollar et al. 1990, Nakashima et al. 1991). PCR amplification was performed in a 25-μl reaction mixture containing 20–25 ng of plant genomic DNA, 0.2 mM each dNTP, 0.25 μM each primer, 1 U of *Taq*DNA polymerase (Promega, Madison WI) in 1× PCR reaction buffer (supplied by the manufacturer) containing 1.5 mM $MgCl_2$. Two sets of oligonucleotide primers matching the 16S rRNA and 23S rRNA of phytoplasmas were used (Namba et al. 1993, Wongkaew et al. 1997). Primers MLO-X and MLO-Y were used in the first round PCR, and primers P1 and P2 were used in nested PCR (Wongkaew et al. 1997, Hanboonsong et al. 2002). PCR conditions were as follows: denaturation for 1 min (5 min for the first cycle) at 92°C, annealing temperature of 60°C for 30 s, and an extension time of 1.30 min (10 min for the last cycle) at 72°C for 35 cycles. The nested-PCR assays were carried out using 1 μl of the first PCR product (diluted 1:50 in sterile deionized water) as template. In total, 40 cycles were conducted for nested PCR under the same conditions of denaturation and extension as the first PCR, except that the annealing temperature was 68°C. Test plants without contact with insects and no DNA were used as negative controls. DNA from a SCWL plant was used as positive control. PCR products were analyzed by electrophoresis on a 1.5% agarose gel in Tris borate-EDTA buffer containing 0.5 μg/ml ethidium bromide.

PCR Assays for SCWL Phytoplasma in Leafhoppers. Adults leafhoppers collected from sugarcane fields were ground singly in Eppendorf tubes with DNA extraction buffer (200 mM Tris, pH 8.0, 250 mM NaCl, 25 mM EDTA, 0.5% SDS, and 0.1 mg/ml proteinase K). Insect DNA was extracted by the phenol-chloroform method (Hanboonsong et al. 2002). PCR assays for SCWL phytoplasma in insect leafhoppers were carried out as described for the test plants.

Sequencing Analysis. The 210-bp products of the nested PCR amplification of test plants and two leafhoppers species (*M. hiroglyphicus* and *Y. flavovittatus*) were purified using a commercial kit (QIAquick PCR purification kit, QIAGEN, Valencia, CA) and sequenced using an ABI Prism automated sequencer (Applied Biosystems, Foster City, CA). The sequencing data from both sugarcane plants and leafhoppers were aligned and analyzed by using SeqMan, version 5.08 (DNASTAR Inc., Madison, WI).

Table 1. Leafhopper species of family Cicadellidae captured from sugarcane fields

Subfamily	Species names	No. captured insects in 2002–2003
Cicadellinae	*Cicadella* spp.	25
	Cofana spectra (Distant)	22
	Cofana subvirescens (Stal.)	12
Deltocephalinae	*Balclutha* spp.	294
	Balclutha rubrostriata (Melichar)	33
	Deltocephalus spp.	10
	Exitianus indicus Distant	942
	Exitianus spp.	35
	Hecalus arcuatus (Motschulsky)	4
	Hecalus prasinus Matsumura	31
	Hecalus porrectus (Walker)	7
	Macrosteles striifrons Anufriew.	105
	Matsumuratettix hiroglyphicus (Matsumura)	1,362
	Nephotettix nigropictus (Stal.)	13
	Nephotettix pravus Matsumura	33
	Nephotettix virescens (Distant)	26
	Paralimnus sp.	6
	Recilia spp.	419
	Recilia distincta (Motschulsky)	299
	Recilia dorsalis (Motschulsky)	148
	Scaphoideus festivus Matsumura	16
	Scaphoideus spp.	13
	Yamatotettix flavovittatus Matsumura	431
Selenocephalinae	*Bhatia olivacea* (Melichar)	156
Typhlocybinae	*Empoascanara* spp.	76
	Thaia oryzivora Ghauri	72
Xestocephalinae	*Xestocephalus* spp.	170

Results

Distribution of Leafhoppers in Sugarcane-Growing Area. Leafhopper insects from the sugarcane-growing areas were trapped monthly by using light traps as described. Insects from 16 genera of family Cicadellidae were captured and identified (Table 1). The leafhoppers *M. hiroglyphicus*, *E. indicus*, and *Y. flavovittatus* were the most abundant species recorded. Population dynamics studies of leafhoppers showed high populations during the rainy season from late April to August (Fig. 1). However, the highest peak period varied with species. For example, the peaks for *M. hiroglyphicus* and *E. indicus* were during April—May, whereas those for *Y. flavovittatus* were July–August. These population dynamics results were obtained in 2002–2003 and are consistent with those obtained at the same location in 1997–1999 (Wongkaew 1999). A correlation between abundance of leafhoppers trapped and disease prevalence was not always present. The highest prevalence of SCWL disease was in August–September, which corresponds to the population peak of *Y. flavovittatus* but is several months later than the peak of *M. hiroglyphicus.* The highest disease prevalence was when the sugarcane was at the elongation phase in August to September rather than in the tillering and harvesting phases (Fig. 1).

Testing Insects for Infection with SCWL Phytoplasma. Nested PCR with primers specific for SCWL phytoplasma was used to screen field-collected leafhoppers for potential vectors of the disease. A PCR product of 210 bp specific for phytoplasma associated with SCWL was detected in 12 of 69 field-collected leafhopper species (Table 2). The percentage of individual insects infected with the phytoplasma varied from 5% [*Bhatia olivacea* (Melichar)] to 35% (*Xestocephalus* sp.). In comparison, the known leafhopper vector *M. hiroglyphicus* showed 25% of phytoplasma infection.

Transmission of SCWL Phytoplasma from Leafhoppers to Plants. The three most abundant leafhopper species that were positive for SCWL phytoplasma (*M. hiroglyphicus*, *E. indicus*, and *Y. flavovittatus*) were used for the transmission trial. After 8 wk of incubation, none of the sugarcane plants fed on by the three leafhopper species showed any white leaf symptoms. However, when the nested PCR with specific SCWL phytoplasma primers was used on these plants, a 210-bp SCWL phytoplasma-specific band was obtained in 55 and 45% of those plants that were used to feed *M. hiroglyphicus* and *Y. flavovittatus*, respectively (Table 3). Thus, *M. hiroglyphicus* and *Y. flavovittatus* can transmit sugarcane white leaf phytoplasma to healthy sugarcane plants. No phytoplasma was detected in the sugarcane plants fed to *E. indicus* (Fig. 2) and in control plants that were not exposed to leafhoppers.

Sequencing Data. To confirm that the phytoplasma amplified from the plants infected in the laboratory was the same as the phytoplasma that infects the field-collected plants, we sequenced the PCR amplicons with the primers that were used for the nested PCR. We found that the sequences of hypervariable 16S/23S DNA spacer region obtained from *Y. flavovittatus* infected in the laboratory, *M. hiroglyphicus* infected in the laboratory, sugarcane collected in the field, sugarcane infected in the laboratory with *M. hiroglyphicus*, and sugarcane infected in the laboratory with *Y. flavovittatus* were identical (Fig. 3). In contrast, there were several differences between these sequences and those obtained from sugarcane with grassy shoot disease (another sugarcane disease caused by phytoplasma) or from the weed Bermuda grass, *Cynodon dactylon* (L.), with white leaf disease.

Discussion

SCWL disease is a major agricultural problem, causing severe losses to the sugarcane industry. Effective control of the disease requires a good understanding of the vector and reservoir of the phytoplasma. It has been known for many years that the leafhopper *M. hiroglyphicus* is a vector of the disease, and we have shown in recently published work that this insect is also the reservoir, and not the weeds that grow in sugarcane fields, as previously thought. We therefore set out to determine whether other species of leafhopper also were implicated in the transmission of SCWL phytoplasma to the sugarcane, based on the following criteria: 1) the insects must be abundant in the sugarcane-growing areas; 2) their populations dynamics must match the cycle of the SCWL disease, i.e., they must peak when the disease is most prevalent;

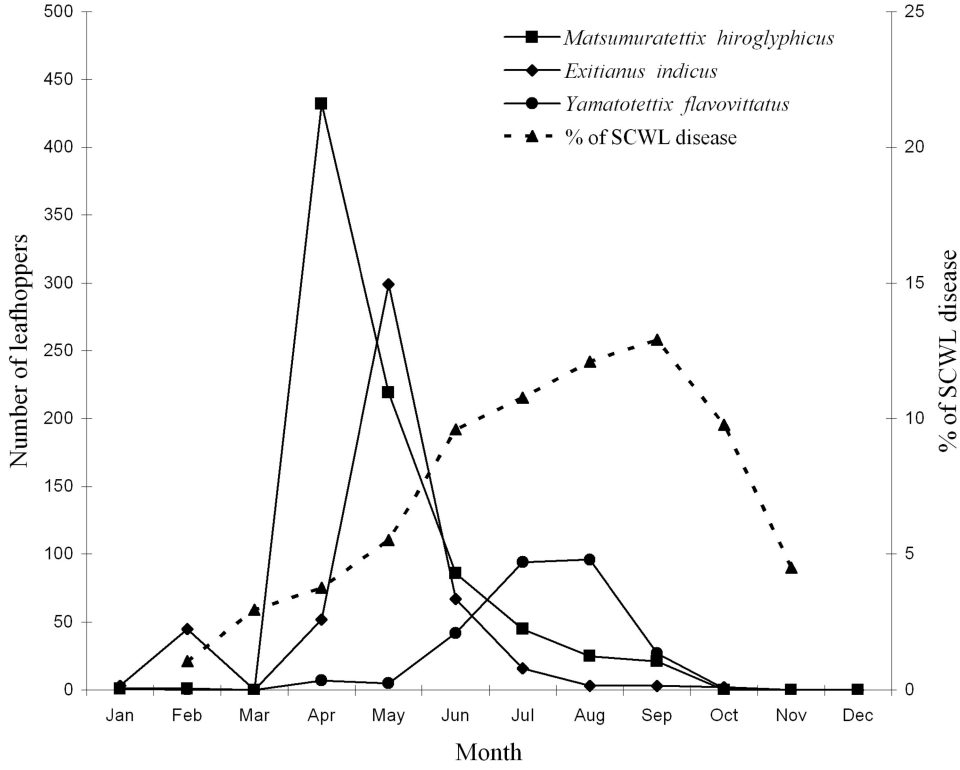

Fig. 1. Abundance and population dynamics of three leafhoppers species (*M. hiroglyphicus*, *E. indicus*, and *Y. flavovittatus*) and percentage of symptomatic sugarcane with white leaf disease

3) They must carry the phytoplasma; and 4) they must infect healthy plants in the transmission test. Although other criteria also could have been used instead or in addition to criteria 1 and 2 (e.g., a low-abundance, highly active vector may be very effective in spreading a disease), our criteria were easily measurable and useful for an initial screen.

Using criteria 1–3, we limited the number of candidate species to three, down from 69 species. These species were *M. hiroglyphicus*, the known vector of SCWL disease, which demonstrated the validity of our criteria. The two other species were *Y. flavovittatus* and *E. indicus*, which were subjected to the transmission test. Transmission of the SCWL phytoplasma from infected plant to insect to healthy plant was demonstrated only with *Y. flavovittatus*. This insect is fairly abundant, and its population shows a peak in July–August, closely matching the period of highest incidence of SCWL disease (August–September).

In the transmission test, we demonstrated infection by PCR amplification of the phytoplasma from the sugarcane, but we did not observe any symptoms of disease. This is consistent with previous studies of infection by phytoplasma of sugarcane and other plants where it was found that disease symptoms rarely occur in the laboratory (Tran-Nguyen et al. 2000, Pilkington et al. 2004). Besides, the rates of phytoplasma disease symptom expression in test plants are often reported to be low. Jarausch et al. (2001) reported 2% (one of 50 test plants) symptom transmission success between psyllid *Cacopsylla pruni* (Scopoli) and apricot seedlings infected with European stone fruit yellow phytoplasma. A 3.5% (three of 88 test plants) rate of

Table 2. Percentage of individuals infected with SCWL phytoplasma from 12 leafhopper species detected by nested PCR

Leafhopper species	% infection
Xestocephalus sp.	34.62
B. rubrostriata (Melichar)	30.76
Thaia oryzivora Ghauri	30.00
M. hiroglyphicus	25.35
Balclutha sp.	23.73
Y. flavovittatus	15.68
Recilia distincta (Motschulsky)	13.21
Recilia dorsalis (Motschulsky)	12.96
E. indicus	12.31
Recilia sp.	10.00
M. striifrons Anufriew	9.52
B. olivacea	5.41

Table 3. Percentage of transmission of SCWL phytoplasma by three leafhoppers species to healthy sugarcane plants

Leafhopper species	No. infected plants/ no. of test plants	% transmission
M. hiroglyphicus	11/20	55
Y. flavovittatus	9/20	45
E. indicus	0/20	0
Control (no insect)	0/20	0

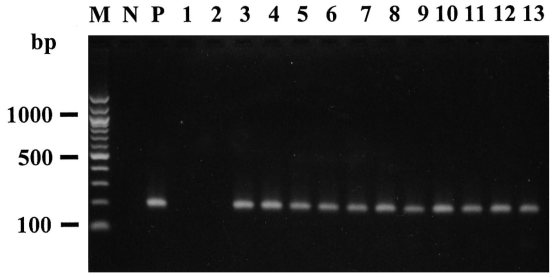

Fig. 2. Agarose gel electrophoresis of test plant DNA amplified by nested PCR with primers MLO-X/MLO-Y followed by primers P1 and P2. N is no DNA sample (negative control), and P is sugarcane white leaf DNA (positive control). Samples in lanes 1 and 2 are DNA from sugarcane healthy plant, lanes 3–5 are DNA from a sugarcane plant with white leaf disease symptoms collected from the field, lanes 6–9 are DNA from plants infected with leafhopper *M. hiroglyphicus*, lanes 10–13 are DNA from plants infected with leafhopper *Y. flavovittatus*, and M is the 100-bp ladder (sizes are shown in base pairs)

successful transmission of symptom was reported in trials between leafhopper *Oncopsis alni* (Schrank) and grapevine yellow symptoms (Maixner et al. 2000). However, a high transmission rate of 66% (24 of 36 test plants) also was reported in transmission tests with sugarcane yellow leaf phytoplasma by planthopper *Saccharosydne saccharivora* (Westwood) (Arocha et al. 2005). In our studies, the test plants did not show any disease symptoms. This could be due to a low phytoplasma titer in the relatively short period in which we observed the plants (8 wk). Growing of the test plants used in the transmission test in pots may have effected the normal development of symptoms of the disease. The sugarcane cultivar used in the transmission test also is known to be one of the major factors influencing symptom appearance (Cronje et al. 1998, Arocha et al. 2005). Furthermore, it has been suggested that symptoms of the disease occur only in certain environmental conditions, such as water shortage or high numbers of defoliating insects (Bertaccini et al. 1996, Tran-Nguyen et al. 2000). It is noteworthy that when we conducted the SCWL phytoplasma transmission test with *M. hiroglyphicus*, the plants also did not show any symptoms of the disease (data not shown).

Because relying on disease symptoms in plants infected in the laboratory may lead to false negatives, we relied on the PCR test, and we sequenced the products of PCR amplification of sugarcane phytoplasma DNA obtained from the transmission tests and compared them to that of infected plants collected from fields. The 16S/26S spacer region sequenced is hypervariable (it is not under the same evolutionary pressure as the ribosomal RNA structural genes) and has been used previously to distinguish different phytoplasmas (Kirkpatrick and Smart 1995, Hanboonsong et al. 2002). We found a perfect match between the sequences obtained, demonstrating that the species of phytoplasma transmitted by *Y. flavovittatus* is the agent of SCWL disease.

The prevalence of *Y. flavovittatus* in the sugarcane fields is very similar to that of *M. hiroglyphicus* and its infection rate in the laboratory is somewhat lower (45 versus 55%). Furthermore, the population peaks of *M. hiroglyphicus* and of *Y. flavovittatus* follow each other, hardly overlap, and together cover the period of highest prevalence of the SCWL disease. The results of the population dynamics suggest that *M. hiroglyphicus* transmits the SCWL phytoplasma preferentially to the plants when they are at early elongation stage, whereas *Y. flavovittatus* transmits preferentially to late elongation stage plants. This might explain why the percentage of infectivity of *Y. flavovittatus* was somewhat low, because we used very young plants in the transmission test. Thus, it seems that *M. hiroglyphicus* and *Y. flavovittatus* are equally important in the transmission of SCWL phytoplasma.

There are many reports in the literature of multiple leafhopper species transmitting the same phytoplasma. For example, chrysanthemum yellows phytoplasma has been reported to be transmitted by three leafhopper species: *Euscelidius variegatus* Kirschbaum, *Macrosteles quadripunctulatus* (Kirschbaum), and *Euscelis incisus* (Kirschbaum) (Domenico et al. 1997). Aster yellows phytoplasma is transmitted by 24 leafhoppers species, and 15 leafhoppers species can transmit peach-X disease phytoplasma (Lee et al. 1998).

Despite evidence of being infected by phytoplasma, *E. indicus* did not transmit the SCWL phytoplasma to the plants. This is not surprising, because the presence of the phytoplasma in the insect body does not necessary mean that the insect is a vector for a disease (Vega et al. 1993). Our study identified 12 species of leafhopper present in sugarcane fields that carry SCWL phytoplasma, the most abundant three species of which were used for further tests. It is noteworthy that 3% of the sugarcane plants showed symptoms of SCWL disease in March, but no *M. hiroglyphicus* or *Y. flavovittatus* insects were collected from January to March. It is therefore possible that some of the less abundant nine species also are capable of transmitting the phytoplasma to the plants. For example, *Macrosteles striifrons* Anfriew. which is a polyphagous leafhopper species and one of the 12 PCR-positive species, has been previously shown to be a vector for 16 SrI-B phytoplasma group causing several phytoplasma diseases of eggplant dwarf and tomato and marguerite yellows (Lee et al. 1998). Future studies will determine whether there are other vectors of SCWL disease among the species that we have collected. Because the alternative host plants for the SCWL phytoplasma are unknown, understanding of the insect vectors' ecology is of prime importance. Our finding that *Y. flavovittatus* is also a vector for SCWL disease, in addition to previously known leafhopper vector *M. hiroglyphicus*, and that it peaks after *M. hiroglyphicus* explains the disease incidence after the seasonal decline of the *M. hiroglyphicus* population. Therefore, the disease can occur continuously over time. Further studies of this insect biology, ecology, and phytoplasma relationships can be used to

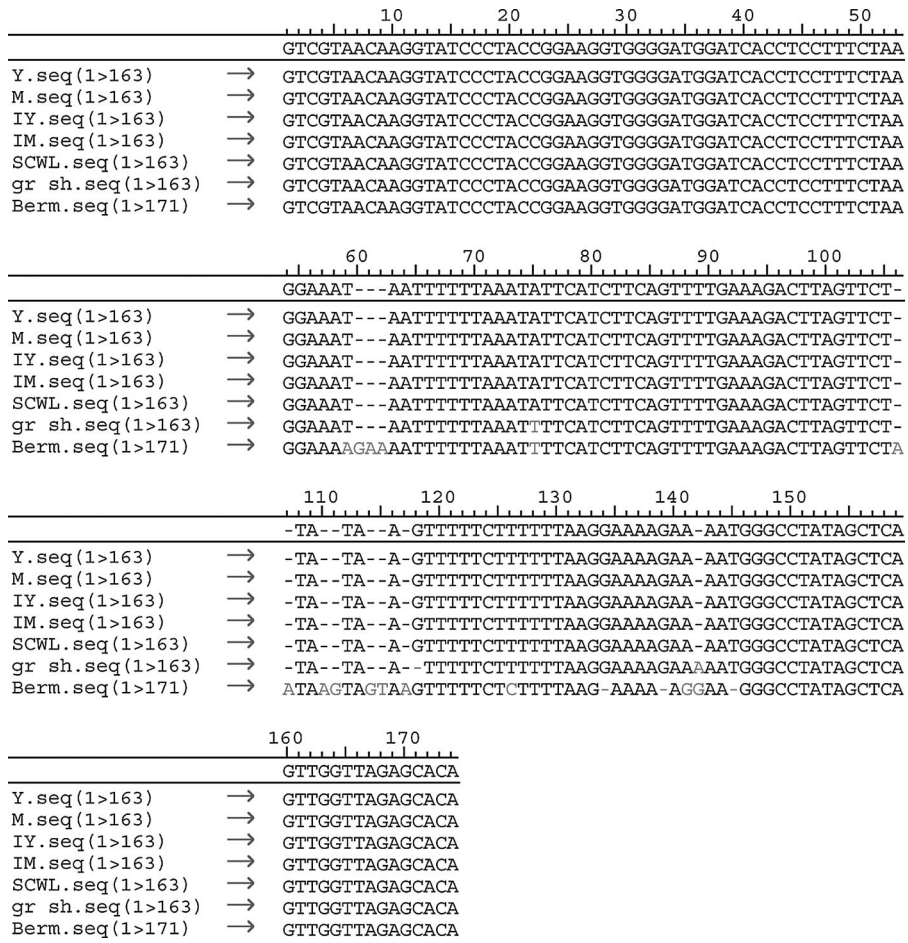

Fig. 3. Alignment of DNA sequences from the spacer region of phytoplasma DNA amplified from plants infected with leafhoppers *M. hiroglyphicus* (M), *Y. flavovittatus* (Y), and compared with the sequence of DNA amplified from sugarcane with white leaf disease (SCWL), insect leafhoppers *M. hiroglyphicus* (IM) and *Y. flavovittatus* (IY), sugarcane with grassy shoot disease (gr sh), and Bermuda grass with white leaf disease (Berm). The sequences of M, Y, SCWL, IM, and IY are identical but different from those of gr sh and Berm.

establish potential SCWL disease management strategies.

Acknowledgments

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References Cited

Arocha, Y., M. Lopez, M. Fernandez, B. Pinol, D. Horta, E. L. Peralta, R. Almeida, O. Carvajal, S. Picornell, M. R. Wilson, et al. 2005. Transmission of a sugarcane yellow leaf phytoplasma by the delphacid planthopper *Saccharosydne saccharivora*, a new vector of sugarcane yellow leaf syndrome. J. Plant Pathol. 54: 634–642.

Bertaccini, A., L. Mittempergher, and M. Vibio. 1996. Identification of phytoplasmas associated with a decline of European hackberry (*Celtis australis*). Ann. Appl. Biol. 128: 245–253.

Chen, C. T. 1974. Sugarcane white leaf disease in Thailand and Taiwan. Sugarcane Pathol. Newsl. 11/12: 23.

Cronje, C.P.R., A. M. Tymon, P. Jones, and R. A. Bailey. 1998. Association of a phytoplasma with a yellow leaf syndrome of sugarcane in Africa. Ann. Appl. Biol. 133: 177–186.

Domenico, B., M. Claudia, and B. Guido. 1997. Differential acquisition of chrysanthemum yellows phytoplasma by three leafhopper species. Entomol. Exp. Appl. 83: 219–224.

Fletcher, F., A. Wayadande, U. Melcher, and F. Ye. 1998. The phytopathogenic mollicute-insect vector interface: a closer look. Phytopathology 88: 1351–1358.

Hanboonsong, Y., C. Choosai, S. Panyim, and S. Damak. 2002. Transovarial transmission of sugarcane white leaf phytoplasma in the insect vector *Matsumuratettix hiroglyphicus* (Matsumura). Insect Mol. Biol. 11: 97–103.

Hill, G. T., and W. A. Sinclair. 2000. Taxa of leafhoppers carrying phytoplamsmas at sites of ash yellows occurrence in New York state. Plant Dis. 84: 134–138.

Jarausch, W., J. L. Danet, G. Labonne, F. Dosba, J. M. Broquaire, C. Saillard, and M. Garnier. 2001. Mapping the spread of apricot chlorotic leaf roll (ACLR) in southern France and implication of *Cacopsylla pruni* as a vector of European stone fruit yellows (ESFY) phytoplasmas. J. Plant Pathol. 50: 782–790.

Kirkpatrick, B., and C. Smart. 1995. Phytoplasmas: can phylogeny provide the means to understand pathogenicity?. Adv. Bot. Res. 21: 188–206.

Kollar, A., E. Seemuller, F. Bonnet, C. Saillard, and J. M. Bove. 1990. Isolation of the DNA of various plant pathogenic mycoplasma like phytoplasmas from infected plants. Phytopathology 80: 233–237.

Lee, I. M., R. E. Davis, and D. E. Gundersen-Rindal. 2000. Phytoplasma: phytopathogenic mollicutes. Annu. Rev. Microbiol. 54: 221–255.

Lee, I. M., D. E. Gundersen-Rindal, and A. Bertaccini. 1998. Phytoplasma: ecology and genomic diversity. Phytopathology 88: 1359–1366.

Maixner, M., W. Reinert, and H. Darimont. 2000. Transmission of grapevine yellows by *Oncopsis alni* (Schrank) (Auchenorrhyncha: Macropsinae). Vitis 39: 83–84.

Matsumoto, T., C. S. Lee, and W. S. Teng. 1968. Studies on sugarcane white leaf disease of Taiwan with special reference to transmission by a leafhopper, *Epitettix hiroglyphicus* Mats. Proc. Soc. Sugarcane Technol. 13: 1090–1099.

Nakashima, K., S. Kato, S. Iwanami, and N. Murata. 1991. Cloning and detection of chromosomal DNA from mycoplasma like organisms that cause yellow dwarf disease of rice. Appl. Environ. Microbiol. 57: 3570–3575.

Namba, S., S. Kato, S. Iwanami, H. Oyaizu, H. Shiozawa, and T. Tsuchizaki. 1993. Detection and differentiation of plant-pathogenic mycoplasm like organisms using polymerase chain reaction. Phytopathology 83: 786–791.

Nakashima, K., W. Chaleeprom, P. Wongkaew, and P. Sirithorn. 1994. Detection of mycoplasma-like organisms associated with white leaf disease of sugarcane in Thailand using DNA probes. JIRCAS J. 1: 57–67.

Palmano, S., and G. Firrao. 2000. Diversity of phytoplasmas isolated from insects, determined by a DNA heteroduplex mobility assay and a length polymorphism of the 16S–23S rDNA spacer region analysis. J. Appl. Microbiol. 89: 744–750.

Pilkington, L. J., G. M. Gurr, M. J. Fletcher, A. Nikandrow, and E. Elliott. 2004. Vector status of three leafhopper species for Australian Lucerne yellows phytoplasma. Aust. J. Entomol. 43: 366–373.

Ploaie, P. G. 1981. Mycoplasma-like organisms and plant diseases in Europe, pp. 61–104. *In* K. Maramorosch and K. F. Harris [eds.], Plant disease and vectors: ecology and epidemiology. Academic, New York.

Tran-Nguyen, L., K. R. Blanche, B. Egan, and K. S. Gibb. 2000. Diversity of phytoplasmas in northern Australian sugarcane and other grasses. J. Plant Pathol. 49: 666–679.

Vega, F. E., R. E. Davis, P. Barbosa, E. L. Dally, A. H. Purcell, and I. M. Lee. 1993. Detection of a plant pathogen in a nonvector insect species by the polymerase chain reaction. Phytopathology 83: 621–624.

Wongkaew, P. 1999. Sugarcane white leaf disease management. Thailand Research Fund, Khon Kaen Pim Patana, Thailand.

Wongkaew, P., Y. Hanboonsong, P. Sirithorn, C. Choosai, S. Boonkrong, T. Tinnangwattana, R. Kitchareonpanya, and S. Damak. 1997. Differentiation of phytoplasmas associated with sugarcane and gramineous weed white leaf disease and sugarcane grassy shoot disease by RFLP and sequencing. Theor. Appl. Genet. 95: 660–663.

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