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Sugarcane yellow leaf virus introduction and spread in Hawaiian sugarcane industry: Retrospective epidemiological study of an unnoticed, mostly asymptomatic plant disease

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Abstract Yellow leaf (YL) caused by *Sugarcane yellow leaf virus* (SCYLV) was first reported as a sugarcane disease in the 1990s, when it had already spread over many parts of the world. The time of introduction into the plantations is unknown. A worldwide screening identified only a few places isolated from cultivar exchange for more than 20 years which appeared SCYLV-free. Control tests with infected cultivars propagated for 12–16 generations by cuttings remained SCYLV-infected, proving that SCYLV is not eliminated by vegetative propagation. *De novo* infection by SCYLV-vectors in Hawaii occurred only over short distances. To reveal the period when SCYLV was introduced to Hawaii, volunteer sugarcane plants from closed Hawaiian plantations and from previous sites of the Hawaiian Sugarcane Planters´ Association breeding station were tested. The results suggest that SCYLV appeared in the breeding station between 1960 and 1970, whereas the plantations became infested after 1980. Imports in the 1960s obviously introduced the virus to the Hawaiian breeding station from where it spread to susceptible cultivars. Eighty percent of the cultivars, developed between 1973 and 1995, acquired the virus at the breeding station, in some cases within 4 years, indicating the rapid spread of SCYLV in the breeding station. The strain of SCYLV found in a Réunion cultivar in Hawaii, and the differing SCYLV-infection of CP-cultivars which were exported more than 20 years ago, suggested that also Réunion and Florida may still have been SCYLV-free in the 1970s. The study showed that retrospective epidemiology can be conducted on a disease which was unnoticed for more than 20 years.

Keywords Sugarcane *Saccharum spec.* · Volunteer plants · Yellow leaf (YL)

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Introduction

The sugarcane disease yellow leaf (YL) was first detected in the early 1990s in Hawaii (Schenck 1990). Later, similar symptoms were reported from mainland United States (Comstock et al. 1994), Brazil (Vega et al. 1997), Mauritius (Moutia and Saumtally 1999) and many other sugarcane countries (Bailey et al. 1996; Victoria et al. 1998; Lockhart and Cronje 2000). The polerovirus *Sugarcane yellow leaf virus* (SCYLV) was identified as a possible causal agent (Vega et al. 1997). The virus arose as a recombination product of *Barley yellow dwarf virus* and *Potato leaf roll virus*

(Smith et al. 2000; Moonan et al. 2000) and 3–4 clusters of strains could be grouped, whereby a Colombian strain was proposed as the original population, which diverged out of the polerovirus group (Moonan and Mirkov 2002; Abu Ahmad et al. 2006). The fact that the disease was detected not earlier than in the 1990s points to a more recent worldwide distribution, even though a report in 1968 about yellow wilt in sugarcane in Eastern Africa is attributed as, possibly, a first account of yellow leaf (Ricaud 1968; Bailey et al. 1996). On the other hand, since YL and SCYLV respectively were detected at the same time in many countries in the late 1990s, a worldwide distribution of infected sugarcane must have occurred some time before the first recognition of the disease. The time of SCYLV introduction into the plantations is unknown, because the infection remains mostly asymptomatic and therefore unnoticed. Specific tests for SCYLV have been available only since the late 1990s (Schenck et al. 1997; Comstock et al. 1998).

The sugar industry in Hawaii was founded in the mid-1850s and had expanded up to World War II with more than 50 sugarcane estates on five islands. By merger and closure the number was reduced to 10 in the 1990s and currently (2009) to two. Although many former sugarcane fields were transferred to other crops, still some sugarcane plants survived over many years as volunteers due to the favourable, tropical climate and the fact that sugarcane is a perennial plant. These volunteer plants might represent a testimony of the SCYLV-infestation state of the plantations at the time when they were no longer used for sugarcane cultivation. The idea of the following study was to search and test these volunteer plants from the former plantations for SCYLV with the aim to identify a time range in which the Hawaiian plantations became infested by SCYLV.

Material and methods

Growth of plants

Plants grown in the field in Hawaii were planted and cultivated following general cultural practices. Seed pieces of approximately 50 cm length (containing 3 nodes) were cut from a virus-free seed plot in Laie, Oahu. They were hot water treated at 50°C for 30 min and then dipped in fungicide propiconazole at 25 g a.i./l (Tilt, Novartis).

Plants in pots were grown in the greenhouse at Bayreuth University from single-node seed pieces. The seed pieces were treated the same way as described above. They were first germinated on wet paper towels and, when the shoots and roots had developed, the seedlings were planted in 20 l pots with commercial potting soil. The potted cultivars were routinely replanted twice a year with stem cuttings.

Virus-free bait plants of the highly susceptible cultivar H87-4094 were grown in the greenhouse as bait plants to prove that the greenhouse is free of SCYLV insect vectors.

Determination of distances for de novo infection with SCYLV

The short-distance spread of SCYLV-infection between plants was determined with virus-free, susceptible plants of cultivar H87-4094. They had been generated by meristem tip culture as previously described (Fitch et al. 2001). The seedcane plot for these virus-free plants was at Brigham Young University garden at Laie, Oahu, remote from active sugarcane research or plantation fields.

For the determination of very short-distance spread (1–3 m), virus-free plants were planted in test plots in fields, next to SCYLV-infected plants. The seed pieces were planted in parallel rows at 1 m spacing with eight seed pieces per row. Fertilization and irrigation was made according to plantation practice. For determination of infection spread over longer distances, virus-free plants were planted in small plots with at least 5 seed pieces at selected places distant from infected, operating sugarcane plantations. In three cases virus-free, susceptible plants from former plantations were collected in previous, now abandoned sugarcane fields.

Collection of leaf samples

The fields of former sugarcane plantations were surveyed for volunteers in the years 2001 and 2003. Leaf samples were collected from the sugarcane plants and preserved for the day in plastic bags. On the same day tissue prints of the leaf midribs were made on nitrocellulose membranes. The leaf samples from plants in the breeding station at Maunawili were collected in the year 2000 and processed the same way.

The seed pieces for PCR and northern Blot were obtained from plants in the greenhouse of Bayreuth

University. The detection of SCYLV in newly emerged seedlings was performed from freshly cut seed pieces germinated in an insect-tight cage for 3 weeks.

Tissue prints were also produced mostly in the years 2000–2002 by researchers in India (G.P. Rao), Phillippines (R. Cu and F. dela Cueva), Guadeloupe (J.-H. Daugrois), Morocco (J. Enahari) and Spain (H. Richter) and sent to Hawaii Agriculture Research Center (HARC), Aiea, for further development, which was performed by Dr. S. Schenck. Leaf material and/or stem pieces of sugarcane were collected near Termez, Uzbekistan, from the Sugar Crops Research Institute, Giza, Egypt, and from private fields near Baniyas, Syria (Z. Soufi). This material was tested for SCYLV by tissue blot immunoassay (TBIA) and by RT-PCR.

Test for SCYLV by TBIA, RT-PCR and northern Blot

The membranes were kept in sealed Petri dishes until development for TBIA. They were incubated with antibody against SCYLV (a gift from Dr. Lockhart, University of Minnesota), then incubated with goat anti-rabbit alkaline phosphatase conjugate (Fitch et al. 2001). The colour development was observed under the microscope.

RNA was extracted from leaf samples and tested for SCYLV by RT-PCR and northern Blot (Comstock et al. 1998; Sambrock and Russell 2001). The freshly emerged leaves (about 10 cm high) of the germinated bud were homogenized and the RNA was purified from the soluble extract with phenol-chloroform. Twenty µg of RNA was separated on a 1.2% formaldehyde agarose gel, and RNA was transferred to a positively charged nylon membrane (0.45-µm pores, Hybond N^+, Amersham) by capillary transfer. The RNA probe to SCYLV was produced by using PCR generated templates for *in vitro* transcription. The DNA fragments were amplified by RT-PCR with gene-specific primers (FW: 5′-CTTTCAAGGTTCGCTCGTTC-3′, RV: 5′-TGAGCTGGTTGACTGGAGTG-3′) and cloned into pGEM®-T, creating a 165 bp fragment. The orientation of the insert fragment was determined by sequencing. The transcription of RNA anti-sense probe and the hybridization were performed as described in the DIG System Users Guide (Roche Diagnostics, Switzerland).

The test for SCYLV in different cultivars by RT-PCR was performed with RNA extracted from source leaves. The primers for PCR were the same as described above, producing a 165 bp fragment of SCYLV.

Plantation records

The business history of the Hawaiian sugarcane estates was investigated in the library of HARC Experiment Station, Aiea, (former Hawaiian Sugar Planters′ Association (HSPA)) and the Kauai Museum, Lihue. The sugarcane cultivars, which had been planted in the different plantations during their operation, were traced in the records of the breeding department of former HSPA.

Results

Worldwide distribution of SCYLV

The worldwide distribution of yellow leaf syndrome (YLS) was first reported by Lockhart and Cronje (2000) as a “disease of uncertain etiology”. We collected samples from many sugarcane plantations around the world in the early 2000s and most of them proved to be infected by SCYLV (Fig. 1). When these results are complemented by reports from other research groups (references in Fig. 1), the worldwide dissemination of SCYLV is obvious. SCYLV-free samples were obtained from a few places only, namely Termez, Uzbekistan, where sugarcane cultivation had been terminated in 1988, Salobreña, Spain, where production ceased in 2002, farmer fields in Baniyas, Syria, where no new cultivars were introduced in the past 50 years, and cultivars from Giza, Egypt. Differing results were obtained for some old cultivars from Hawaii, Florida and Louisiana (Table 1); in some places these cultivars contained SCYLV, in others they were SCYLV-free. The fact that these cultivars were infected in some places indicated that they were susceptible to the virus, but were probably SCYLV-free at the time of export. In summary it appeared that sugarcane plantations, which were isolated from cultivar exchange in the past 20–30 years, and some old cultivars exported more than 20 years ago, were SCYLV-free.

Maintenance of SCYLV-infection in sugarcane stalks

Sugarcane is propagated vegetatively by cuttings. It was important to show whether SCYLV is

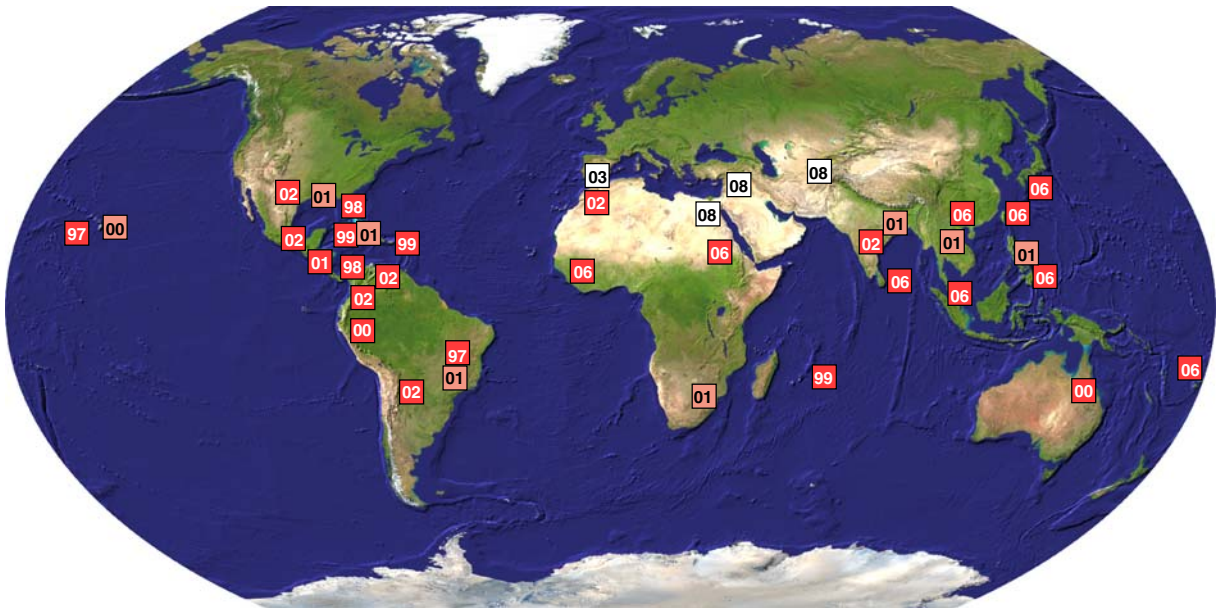

Fig. 1 *Proven presence of SCYLV in sugarcane regions of the world*. Squares in red indicate the presence of SCYLV and white squares the absence of SCYLV, light red according tests conducted by the authors, dark red according published reports (Schenck et al. 1997; Vega et al. 1997; Comstock et al. 1998; Victoria et al. 1998; Moutia and Saumtally 1999; Arocha et al. 1999; Alegria et al. 2000; Smith et al. 2000; Avila et al. 2001; Comstock et al. 2002; Nadif et al. 2002; Rassaby et al. 2004; Abu Ahmad et al. 2006). The numbers in the squares show the year, when the tests for SCYLV were made respectively were reported. (World map reproduced from www.uwec.edu and modified)

Table 1 SCYLV-infection state of some old cultivars in different areas of the world. The tests (TBIA) were conducted in 2000 and 2001

Cultivar	SCYLV-infection	Origin of collection
H39-3633	−	Thailand; Guadeloupe (France)
	+	Hawaii (USA)
CP61-39	−	Thailand, Guadeloupe (France)
	+	Florida (USA)
L62-96	−	Kenitra (Morocco)
	+	Louisiana (USA)
CP65-357	−	Spain
	+	Florida (USA)
CP66-346	−	Kenitra (Morocco)
	+	Gharb (Morocco)
CP70-321	−	Kenitra (Morocco)
	+	Gharb (Morocco)
CP84-1198	−	Phillippines
	+	Thailand, Guadeloupe (France)

propagated by seed pieces to successive generations. Rassaby et al. (2004) already reported that SCYLV-infection was maintained during regrowth after ratooning. Cultivars which were imported as one-node seed pieces from Hawaii in 2001, were grown in the greenhouse at the Bayreuth University. The plants were cut 1–2 times per year and each time regenerated from seed pieces. The cultivars, which were SCYLV-infected 8 years ago when collected in the field (Schenck and Lehrer 2000), still contained SCYLV after the 12–16 cycles of replanting in the greenhouse (Fig. 2). When seed pieces of infected plants were germinated in an insect-proof cage, the freshly emerged leaves already contained SCYLV (Fig. 3). Virus-free plants of the susceptible cultivar H87-4094 remained virus-free in the Bayreuth greenhouse even when held outside of insect-proof cages over several years (Fig. 2), which indicates that the greenhouse is free of SCYLV-vectors. The presence of SCYLV in the infected cultivars over so many replantings did not therefore originate from *de novo* infection in the greenhouse.

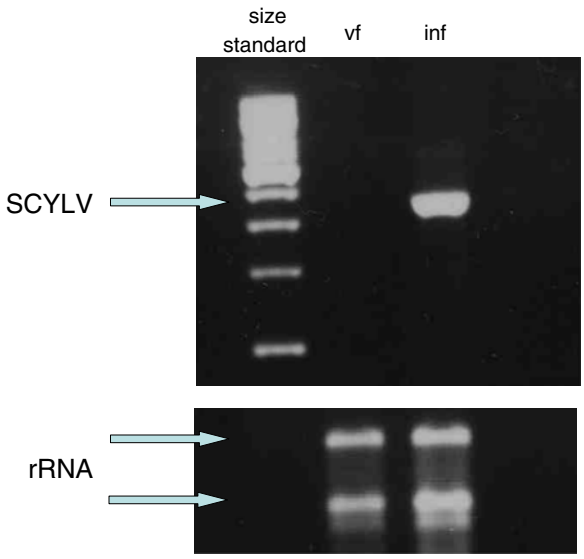

Fig. 2 RT-PCR for SCYLV in virus-free and infected cv. H87-4094 after 12–16 cycles of replanting, with rRNA as loading control. Virus-free (vf) and infected (inf) plants of H87-4094 were grown in the greenhouse outside of insect-proof cages. RNA from leaf samples was extracted and amplified with SCYLV-specific primers by RT-PCR. The reaction products were separated on gels and stained with ethidium bromide. Length of amplified SCYLV was 165 bp, the size standard was a 50 bp DNA-ruler from Fermentas (St. Leon Rot, Germany)

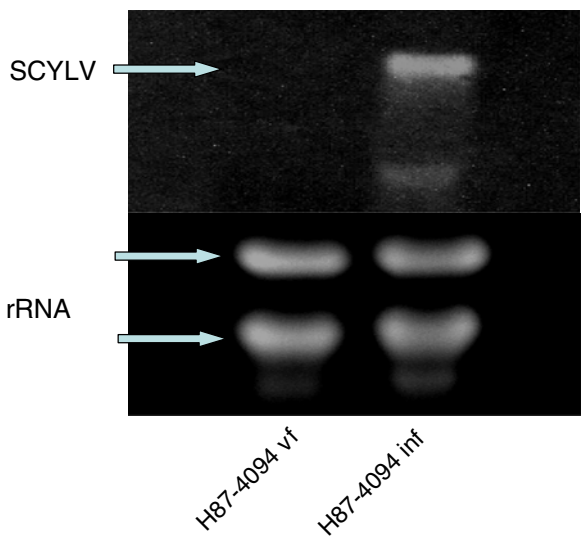

Fig. 3 Northern blot of RNA from freshly germinated seed pieces. Seed pieces of cv. H87-4094 were germinated for 3 weeks in insect-proof cages and RNA was extracted from the freshly emerged leaves. H87-4094 inf = infected cv. H87-4094, H87-4094 vf = virus-free plants of H87-4094. The RNA of SCYLV and, as a loading control, of rRNA is indicated by arrows

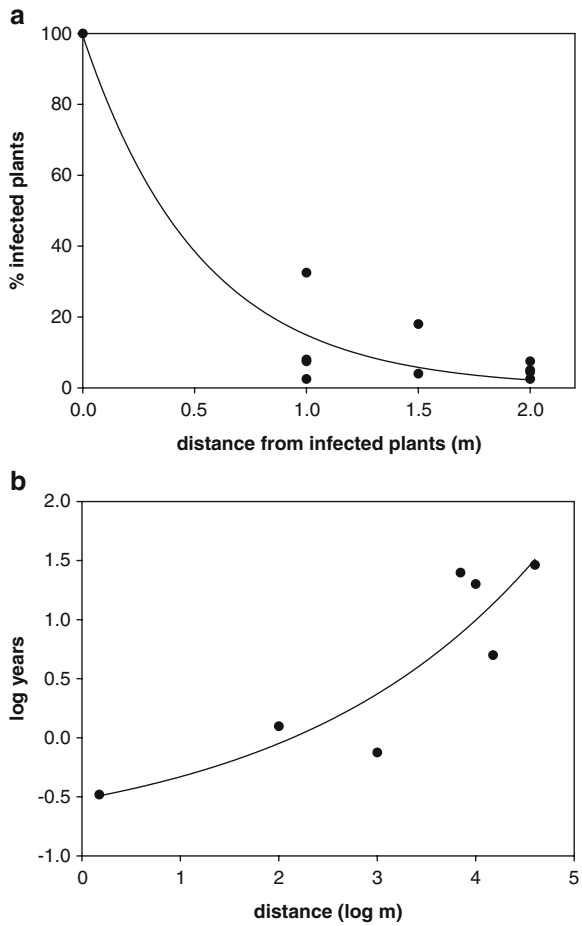

Fig. 4 De novo infection frequency of sugarcane plants placed at different distances. Virus-free, susceptible plants of cv. H87-4094 were grown at different distances from rows or fields of SCYLV-infected plants. The cultivar which remained virus-free for more than 20 years was H50-7209. a): Infection frequency at short distance after 11 months (r^2=0.93). b): Minimum time after which virus-free plants placed at different large distances were still virus-free. It represents the minimum time, because the plants might have stayed virus-free for longer (r^2=0.79)

Spread of SCYLV by de novo infection

The *de novo* infection in fields in Hawaii and in Louisiana by viruliferous aphids such as *Melanaphis sacchari* is relatively slow (Lehrer et al. 2007; McAllister et al. 2008). In controlled tests virus-free plants of the susceptible cultivar were planted in plantation fields at different distances from SCYLV-infected plants and tested for SCYLV after 11 months. On average only 5% of plants had acquired SCYLV at a distance of 2 m (Fig. 4a). Virus-free plants distant between 100 m and 25 km from infected plants were

not infected at the time of testing, which varied between 1 year and 30 years after planting (Fig. 4b). Apparently such dispersal distances are not likely for aphids in Hawaii.

SCYLV in sugarcane plants in fields of present and former plantations

Hundreds of samples had been collected from operating plantations in the late 1990s by Dr. Schenck, which led to the discovery of YL (Schenck 1990) and SCYLV (Schenck et al. 1997; Schenck and Lehrer 2000). This survey was extended in 2001 and 2003 to all plantations, which existed in Hawaii in the 1990s, and to fields of former plantations and estates, which went out of operation between 1945 and 1990. Leaf samples were collected, with each sample taken from a different stool. The number of samples was high from the operating plantations and from those which had closed recently (Table 2). In contrast only a few sugarcane clones were found in plantation fields that were abandoned more than 30 years ago, except the former estates in Kohala and in Kahuku. The sampled leaf pieces were tested for SCYLV by TBIA. Whenever one leaf sample reacted positively with the SCYLV-antibody, the plantation and site of collection were considered to be infested by SCYLV. The tests showed that all plantations which were still under operation or which were under operation up to recently, were infested, including plants from fields that were abandoned already in the 1980s (Fig. 5). In contrast, plantations from estates which went out of business before 1980 were SCYLV-free. This picture held true for each of the four "sugar islands" of Hawaii. As a reminder, the first yellowing symptoms were recorded in fields of two plantations (Hamakua and Oahu Sugar) in the late 1980s which led to the discovery of YL (Schenck 1990).

SCYLV in past breeding and quarantine station sites

The breeding station of HARC (former HSPA) was tested for SCYLV, including former sites of the breeding station and former quarantine station sites (Table 2). The breeding station had changed place several times. First it was placed what is now Lyon Arboretum, Honolulu. Volunteer plants at Lyon Arboretum were SCYLV-free. In 1960 the breeding station changed to Maunawili next to Pali highway. Volunteers from this site, which was closed 1972, were SCYLV-infected, which indicates that the breeding station had acquired SCYLV in the time between 1960 and 1972. A few clones were detected at the former quarantine station sites at Waianae (closed 1960) and Kaaawa (closed 1975). Whereas the plants from the former were virus-free, the plants of the latter were infected. All these findings narrow the time of SCYLV-introduction into the breeding station to the period of 1960–1972 (Fig. 5). Two further facts corroborate that conclusion: *Saccharum spontaneum* hybrid plants had been provided as windbreaks to papaya growers in 1975. These plants turned out to be infected with SCYLV. In 1982, seed pieces of cultivars H32-8560 and H50-7209 were exported to Peru and plants of these cultivars were later reported to be infected by SCYLV (Alegria et al. 2000). The cultivars may have acquired SCYLV in Peru, however, the authors reported (in 2000) that the plants exhibited unusual, severe leaf yellowing in the past 18 years, i. e. since 1982, when imported from Hawaii.

SCYLV spread in the present breeding station

Evidence from the former locations of the breeding station indicated that the breeding station had SCYLV at least 10 years ahead of the plantations. A survey was conducted, in which all newly-bred clones at the breeding station were tested for SCYLV to reveal whether infection of virus-free plants proceeds in the environment of the breeding station. On average 80% of the clones were infected, including those which had been derived from crossings just 4 years before (Fig. 6). Considering that all these clones originated from virus-free, true seeds, the plants must have acquired SCYLV in the breeding station through *de novo* infection by viruliferous aphids. Although the infection spread by aphids is relatively slow (Fig. 4a) it is obviously fast enough to infect the susceptible clones within a few years in the breeding station, where plants are standing at 1 m distances within and between rows. In the experiment 20% of plants at a distance of 1 m became infected within 11 months (Fig. 4a).

Sugarcane imports

It appears that the Hawaiian breeding station acquired SCYLV in the years between 1960 and 1970,

Table 2 Collection places and sample numbers of volunteer plants in recent and former Hawaiian sugarcane plantations. Only plantations which existed 1945 and later were considered. The company names listed here were those which existed at the time when the plantation was closed

Island, plantation name and place of sugar mill	year of plantation closure	number of collected samples
Kauai		
(1) Kilauea Sugar Co., Kilauea	1975	19
(2) McBride Sugar Co., Eleele	1986	11
(3) The Lihue Plantation Co., Anahola	1990	13
(4) Kekaha Sugar Co., Kekaha	1999	20
(5) The Lihue Plantation Co., Lihue	2000	>100
(6) Gay and Robinson, Kaumakani	in operation	>100
Oahu		
(1) Waianae Sugar Co., Waianae	1946	3
(2) Waimanalo Sugar Plantation	1947	3
(3) Honolulu Plantation Co., Honolulu	1950	3
(4) Laie Sugar Plantation, Laie	1953	6
(5) Kahuku Plantation Co., Kahuku	1972	32
(6) Oahu Sugar Co., Waipahu	1994	24
(7) Waialua Sugar Co., Waialua	1995	13
Maui		
(1) Kaeleku Sugar Co., Kaeleku	1946	7
(2) Olowalu Sugar Co. (some fields)	1982	3
(3) Pioneer Mill Co., Lahaina	1996	10
(4) Hawaiian Commercial and Sugar Co., Puunene and Paia	in operation	>100
Hawaii		
(1) Olaa Sugar Co., Kapoho	1960	23
(2) Kohala Sugar Co., Hawi	1973	48
(3) Puna Sugar Co., Olaa	1982	56
(4) Hamakua Sugar Co., Paauilo	1994	48
(5) Mauna Kea Sugar Co./Hilo Coast Processing Co., Wainaku, Pepeekeo	1994	58
(6) Kau Sugar Co. Pahala	1996	34
HSPA		
(1) Breeding station, Lyon Arboretum	1960	7
(2) Quarantine station, Waianae	1960	2
(3) Breeding station, Maunawili Pali Rd	1973	16
(4) Quarantine station, Kaaawa	1975	21
(5) Windbreaks for papaya farmers, windward Oahu	1975	3
(6) Export to Peru	1982	unknown
(7) Breeding station, Maunawili hill side	In operation	>100

probably by import of infected clones. The station imported 234 cultivars between 1955 and 1972, mostly from other sugarcane breeding stations worldwide, but also from a collection expedition to New Guinea. In the probably crucial period of 1960–1970 the majority of imports came from Asia and the Australo/Pacific area, but also some from North and South America (none from Colombia), and only few from Africa (Fig. 7). Thus no definitive hint as to the origin of an imported SCYLV-infected cultivar could be obtained from the import records.

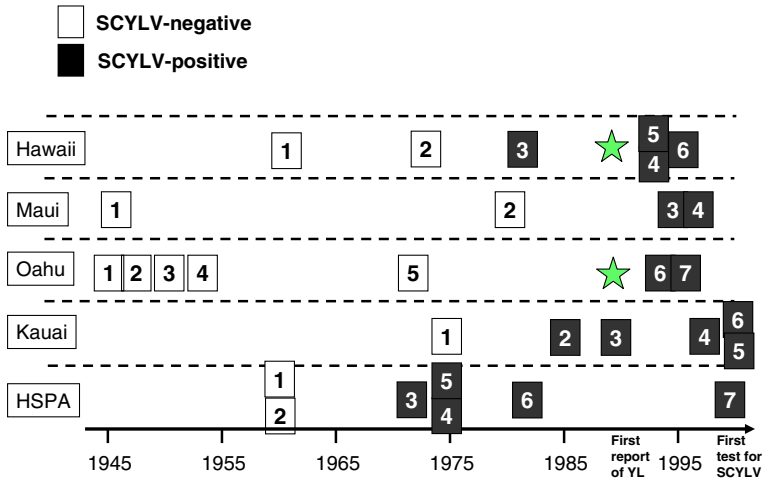

Fig. 5 SCYLV-infestation of past and present sugarcane plantations in Hawaii. Sugarcane plants from operating and from closed plantations were tested in 2001 and 2003 for SCYLV by TBIA. Open squares (□): no SCYLV was found, dark squares (■): SCYLV was detected at least in one sample. The star (☆) shows when YLS symptom outbreak was noticed for the first time. The plantations are ordered according to the island in which they are/were situated, the HSPA breeding station sites are plotted separately. The numbers in the squares denote the names (in short form) of the plantations (see also Table 1). *Hawaii*: 1 Kapoho, 2 Kohala, 3 Puna, 4 Hamakua, 5 Mauna Kea, 6 Kau. *Maui*: 1 Kaeleku, 2 Olowalu. 3 Lahaina, 4 HC & S (Puunene). *Oahu*: 1 Waianae, 2 Waimanalo, 3 Honolulu, 4 Laie, 5 Kahuku, 6 Oahu (Waipahu), 7 Waialua. *Kauai*: 1 Kilauea, 2 McBryde, 3 Anahola, 4 Kekaha, 5 Lihue, 6 G & R (Makawele). *HSPA breeding station*: 1 Lyon Arboretum, 2 Waianae quarantine, 3 Maunawili Pali Rd., 4 Kaaawa quarantine, 5 windbreaks, 6 export to Peru, 7 Maunawili hill side

SCYLV-types in sugarcane cultivars from Hawaii and clone R570 from Réunion

Four types of SCYLV had been identified so far (Moonan and Mirkov 2002; Abu Ahmad et al. 2006) and one plant had been analysed from the Hawaii collection, namely R570, which is a cultivar from Réunion. This plant contained the BRA-PER strain of SCYLV, not the REU-strain, which is typical for Réunion and which was found in R570 on Reúnion, Guadeloupe and Mauritius (Table 3). SCYLV of H87-4094 and H73-6110 was sequenced to analyse SCYLV of a Hawaiian cultivar grown in Hawaii. The sequences indicated that these two Hawaiian cultivars contain the PER type of SCYLV (data not shown).

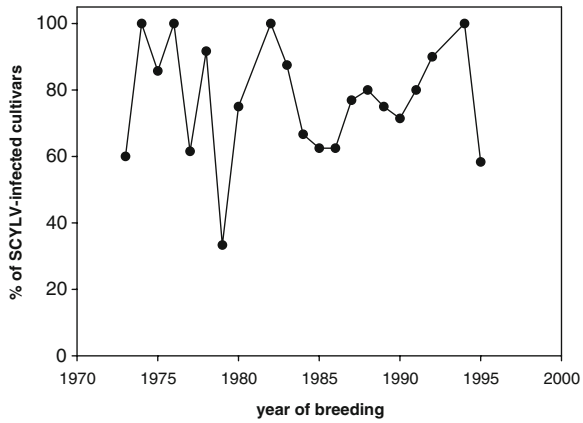

Fig. 6 Percent of SCYLV-infected Hawaiian cultivars from crossings in the years 1972–1986, tested in 2000

Discussion

The following picture emerged from this study: The HSPA breeding station became infested by SCYLV between 1960 and 1970 at a time when the plantations were still free of SCYLV. No hint as to which import(s) may have carried SCYLV into the breeding station was obtained. The aphid vector *Melanaphis sacchari*, which is present throughout Hawaii, will have disseminated the virus among the plants in the breeding station, infecting the cultivar collection and the newly-bred clones. Although the speed of infection propagation from plant to plant is only a few metres per year (Lehrer et al. 2007, Fig. 4), it is sufficiently fast to infect susceptible varieties within a few years. Four years was enough to infect 80% of the newly

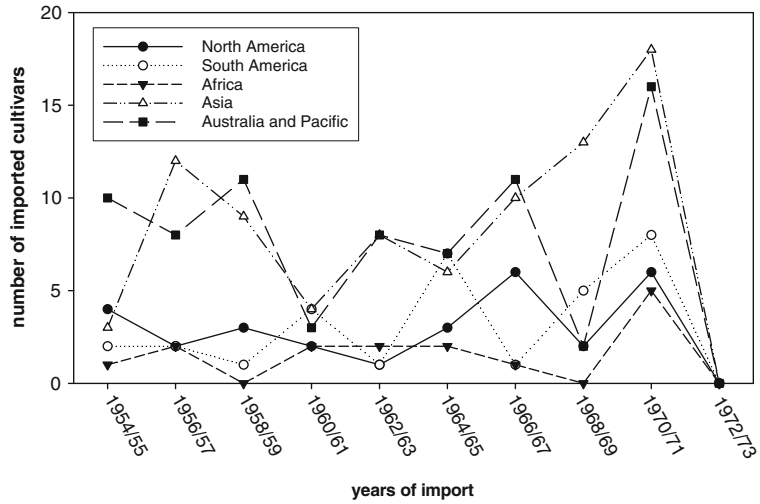

Fig. 7 Number of imported cultivars to the Hawaiian breeding station in the years 1954–1972 and their provenience

developed crossings at the Maunawili breeding station. Similarly a screening of clones at the breeding station Canal Point, Florida, had revealed that the SCYLV incidence increased during the CP sugarcane cultivar development program from 30% to 55% within 3 years (Comstock and Miller 2003). As a result of the many clones which were imported from around the world in the 1960s, several infected clones may have arrived in the breeding station, thus facilitating the uniform infestation of the entire station within a few years. The infestation may then have been carried over to the plantations by the field trials of new cultivars. The first reports of YL outbreak date to the late 1980s, also indicating that the plantations had not been fully SCYLV-infested in the years before.

The time range (1960–1970) where SCYLV is suspected to have arrived and spread in Hawaii is corroborated by a few other facts: the infected windbreak plants in the papaya plantations, the BRA-PER strain infection of the Hawaiian cultivars, which were exported to Peru in 1982, and finally the BRA-PER-strain of the variety R570 in Hawaii. That cultivar was imported from Réunion in 1981 and contains the BRA-PER strain (Abu Ahmad et al. 2006), although the same cultivar in Réunion harbors the REU strain and, in Mauritius, both REU and BRA-PER. Thus, Réunion was apparently SCYLV-free at 1981 when this cultivar was imported to Hawaii. Recent sequence analysis of SCYLV from Hawaiian sugarcane plants indicated that the strains group together with the PER-strain and are distinctly different from the South American BRA-strain, which is evidence that the Hawaiian cultivars grown now in Peru contained already SCYLV, when they were exported to Peru (ElSayed, pers. communication).

Table 3 SCYLV-strain in Hawaiian cultivars and in cultivar R570 from different sugarcane regions

Cultivar	Origin of tested material	Date of introduction	SCYLV-type
H87-4094	Hawaii	bred 1987	PER
H73-6110	Hawaii	bred 1973	PER
H50-7209	Peru	introduced 1982[b]	BRA-PER[a]
H32-8560	Peru	introduced 1982[b]	BRA-PER[a]
R570	Hawaii	introduced 1981	BRA-PER[a]
R570	Réunion	–	REU[a]
R570	Guadeloupe	introduced early 1980[c]	REU[a]
R570	Brazil	?	BRA-PER and REU[a]
R570	Florida	introduced before 1999[d]	BRA-PER
R570	Mauritius	introduced 1975	BRA-PER and REU[a]

[a] from Abu Ahmad et al. 2006

[b] from Alegria et al. 2000

[c] pers. communication J.-H. Daugrois

[d] pers. communication J. Comstock

[e] pers. communication S. Saumtally and K. Ramdoyal

There is no indication that the virus is eliminated by vegetative propagation of sugarcane. The infection is maintained through ratooning (Rassaby et al. 2004) and plants of several tested cultivars, for example cv. H87-4094, have kept the SCYLV-infection for at least 12–16 generations of successive seed piece plantings (Fig. 2). Therefore volunteer plants from closed plantations, which were found to be virus-free, were most likely already virus-free when the plantation was operating and had not lost a previous SCYLV-infection. Although the number of collected clones from the plantations and estates, which were closed in the 1940s and 1950s, was small so that by chance a resistant clone might have been among them, the large numbers of SCYLV-free clones of susceptible cultivars from Kahuku and from Kohala, both closed in 1970s, affirm that before 1970 the plantations were SCYLV-free.

An infection of plants after the time when the plantation was closed is very unlikely because a distance of only 100 m was already sufficient to prevent infestation from infected cane for at least 1 year (Lehrer et al. 2007) and a distance of several km was not bridged by viruliferous aphids for 30 years (Fig. 4). Most Hawaiian plantation estates were several km apart, except those at the Eastern Hawaii coast. In these, however, YL was already detected in the early 1990s (Schenck 1990), thus they were obviously already infected at that time. Therefore SCYLV-positive volunteer plants detected in fields which were given up in the late 1980s, indicate that the fields were indeed already infested at the time of closure.

The disease was not recognized for at least 30 years till it became obvious in the 1990s, it stayed unnoticed until it had spread through the plantations throughout the world. So far no suggestion existed as to when SCYLV infested the sugarcane plantations. The study presented here, using the fact that many sugarcane plantations and estates in Hawaii were closed in the past 50 years, gives a first hint that SCYLV entered the Hawaiian sugar industry in the 1960s through the breeding station and from there, in the 1980s, into the plantations. The SCYLV-infestation of Hawaii seemed to precede SCYLV-infestation of Réunion by more than 10 years. Also the Florida breeding station (Canal Point) may have been at least partly SCYLV-free in the 1970s, because cultivars, which were developed in and before that time and exported to Morocco, Spain or Thailand, were virus-free (Table 1). Samples from a Louisiana cultivar had thylakoid structures which were typical for SCYLV-infected plants (Robinson-Beers and Evert 1991; Yan et al. 2009), indicating that the virus was already present at the Louisiana breeding station at Houma in the 1980s. Thus it appears that all these important breeding stations acquired SCYLV in the 1970s or 1980s. It would also explain why sugarcane fields which had been isolated from cultivar exchange over the past 30 years (Uzbekistan, Syria) were SCYLV-free (Fig. 1); and why some old cultivars which had been exported 30 years ago to different places in the world were SCYLV-free (Table 1), unless they were infected later by more recent cultivar introductions.

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