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YELLOW LEAF SYNDROME

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Summary

Sugarcane yellow leaf virus (SCYLV) has been shown to be transmitted by several aphid species and to infect a number of host plants other than sugarcane. Some sugarcane cultivars are now known to be resistant to SCYLV and this resistance is inherited. Virus-free plants of the susceptible H87-4094 were produced and have been planted in field trials to compare growth and yield with infected plants of the same cultivar. There is evidence that SCYLV infection reduces cane tonnage, but not sugar content of cane juice, whether or not there are any evident yellowing symptoms. Symptom expression is associated with cultivar, cold temperatures or other stress factors and infected plants in the field usually remain symptomless. In greenhouse studies and field plots no single factor could be correlated with symptom appearance in all instances.

Introduction

Yellow leaf syndrome is now known to be caused by a virus, Sugarcane Yellowleaf Virus (SCYLV) (Scagliusi and Lockhart 2000). It is a luteovirus and like others in this group, is phloem-limited, not mechanically transmitted, has aphid vectors and produces yellowing symptoms in host plants. The development of a diagnostic technique allowed us to study the transmission, spread and effects on yield of the virus (Schenck *et al.* 1997). As reported previously (Pathology Report 67), the virus is widespread in Hawaiian sugarcane fields although infected plants do not usually show the yellowing symptoms. It was also found that some cultivars are universally infected while others always remain virus-free. Several aphids common on sugarcane were tested for their ability to transmit the virus and the sugarcane aphid and corn leaf aphid were shown to be vectors while the yellow sugarcane aphid is not (Schenck and Lehrer 2000). Since the virus is not transmitted in true seed (fuzz) or by mechanical methods, the only means by which it can spread in fields is by planting infected seed cane or by the aphid vectors. The virus cannot be eliminated from seed cane by heat treatment or by any chemical treatments. Infected plants can be made virus-free only by a meristem tissue culture technique (unpublished data).

Plants of the sugarcane cultivars that remained virus-free in the field were inoculated using viruliferous aphid vectors. Cultivars H78-4153, H78-3567, H78-7750, and H87-4319 did not become infected in these greenhouse studies and are therefore assumed to be resistant to the virus (Schenck and Lehrer 2000). Cultivars H65-7052, H73-6110, H77-4643, and H87-4094 are susceptible. Crossing experiments are underway to determine whether resistance is inherited. Since results so far indicate that it is, breeding for resistance may be possible. Research is also continuing on the effects of SCYLV infection and symptom expression on yield and juice purity.

Methods

Testing for infection. A serological method was developed that allows us to detect the virus in infected plants (Schenck *et al*. 1997). Midribs of sample leaves are cut transversely and the cut end is pressed against a nitrocellulose membrane.

Virus in the vascular bundles adheres to the membrane and can be detected as purple spots when the membranes are treated with conjugated antibodies and a dye substrate. Uninfected leaves do not produce the purple spots. This test can be used to rapidly screen large numbers of plants in a relatively short time.

Aphid vectors. Several different aphid species were tested for their ability to transmit the virus by collecting them in the field and maintaining colonies on virus-infected sugarcane plants in pots. Virus-free plants of H87-4094, a susceptible cultivar, were produced by meristem tip culture. The aphids were transferred from the infected plants to the virus-free plants with a small brush. After a few days the aphids were removed and if the plants became infected, the virus could be detected in the leaves within three to four weeks.

Meristem tip culture for generation of virus-free plants. Meristem tips of the shoot apical meristem and the lateral buds, 0.3 to 0.7 mm in diameter, were excised, dipped for 20 seconds in 20% commercial hypochlorite bleach solution, rinsed in sterile water, and plated on Murashige-Skoog medium (Murashige and Skoog, 1962), enriched with 100 mg/L myo-inositol, 4 mg/L thiamine-HCl, 3% sucrose, and 3 g/L Phytagel (Sigma Chemical Co., St. Louis, MO), 3 mg/L 2,4-dichlorophenoxyacetic acid and 0.2 mg/L benzylaminopurine. The plated meristems were stored under reduced lights at 28ºC and examined weekly for growth of embryogenic calli. Calli of about 10 mm diameter were regenerated on growth regulator-free medium. Green plants were subcultured monthly until they were large enough to be planted in a peat-based commercial potting soil. Potted plants were drenched with the recommended doses of the fungicides Tilt (Syngenta) and Ridomil Gold (Syngenta) to prevent fungal growth. The plants were acclimatized slowly to laboratory conditions under fluorescent lights by initially covering them with plastic bags that were slowly vented. Regenerated plants were in soil and of sufficient size to be tested for SCYLV 4 to 5 months after excision of the meristem tips.

Inheritance of resistance. Crosses were made during the 1998, 1999, and 2000 breeding seasons and fuzz was planted in flats. After plants reached the size for separating into individual plants, they were inoculated with viruliferous aphids. They were subsequently tested for SCYLV infection and then planted in field plots at the HARC breeding station. Virus infection occurs naturally at the station and plants continue to be monitored for infection.

Effect of virus on yield. Yield studies under commercial field conditions have been difficult to carry out because large amounts of virus-free seed of susceptible cultivars are needed. Virus-free plants of H65-7052, H73-6110, and H87-4094 have been produced and a seed field of virus-free H87-4094 was established in Laie in an isolated area. This has been maintained virus-free for over two years and enough seed has been harvested to plant three field trials comparing virus-free and infected H87-4094. One of these field trials is now over one year old and has had a sample harvest taken. Growth, yield and other physiological characteristics are being measured in all three trials.

Results

Aphid vectors. The aphid species tested for their ability to transmit SCYLV were: the sugarcane aphid, *Melanaphis sacchari;* the yellow sugarcane aphid, *Sipha flava;* the corn leaf aphid, *Rhopalosiphum maidis;* the rice root aphid, *Rhopalosiphum rufiabdominalis;* and the rusty plum aphid, *Hysteroneura setariae*. In each of two trials, 9 sugarcane plants were inoculated with only 10 *M. sacchari* individuals each. In both trials, 8 of

the plants became infected with SCYLV (89% transmission). In another test, a single *M. sacchari* was placed on each of 34 wheat seedlings. After 4 weeks, 25 of the 34 seedlings (73.5%) tested positive for SCYLV.

The corn leaf aphid is common on corn in Hawaii and will occasionally infest sugarcane in the field. In our trials, *R. maidis* transmitted SCYLV to a low percentage of sugarcane plants even though the aphids fed and multiplied on sugarcane. When 14 virus-free sugarcane plants were inoculated with 100 *R. maidis* each, only one of the plants eventually tested positive for the virus. The rice root aphid transmitted SCYLV from infected wheat seedlings to uninfected wheat seedlings, but did not transmit the virus from sugarcane to sugarcane. This was probably due to the fact that the aphids do not survive well on sugarcane. Attempts to raise the aphids on infected wheat seedlings and transmit it to sugarcane also failed. The yellow sugarcane aphid and the rusty plum aphid did not transmit SCYLV.

Alternate hosts. A number of weeds have been screened for SCYLV infection but, so far, none has been found to be infected. More work with aphid inoculations of weed species needs to be done. In addition, corn, rice, oats, barley and wheat were tested as possible hosts. These crop plants were planted from seed in pots and inoculated with viruliferous sugarcane aphids. The percentage infection is shown in Table 1. All of these can be infected with SCYLV but only a low percentage of corn and rice plants tested positive. Some sugarcane relatives can also become infected with SCYLV. The noble canes, *Saccharum officinarum,* are quite susceptible as is *S. robustum*. *Saccharum spontaneum, S. sinensis* and *Erianthus* sp. are usually virus-free in Maunawili field plots and are assumed to be relatively resistant.

Infection and symptom expression in Hawaiian cultivars. Ten Hawaiian sugarcane cultivars planted in field plots in several locations were infected with SCYLV in a pattern consistent with prior plantation field surveys (Table 2). Cultivars H78-4153, H78-7750, H87-4319, and H82-3569 remained virus-free for the duration of the experiment. Cultivar H78-3567, which has always tested negative in plantation fields, gave rare positive reactions in this test. This agrees with results obtained by BSES in Australia. They tested H78-3567 using PCR and got very weak positive or negative reactions. Therefore, H78-3567 may sometimes be infected with very low concentrations of SCYLV. Cultivars H73-6110, H87-4094, H78-3606 were infected. Cultivars H65-7052 and H77-4643 gave variable reactions. These latter two cultivars continued to be infected with SCYLV, but the serological diagnostic tests were sometimes positive and sometimes negative. This may have been because the virus concentration in H65-7052 and H77-4643 is low and often below the level of detection with the tissue blot procedure. Symptom expression in the infected plants was more pronounced during the cooler winter months at all locations. Symptoms also often appeared as plants aged or when they suffered from drought stress. However, there was no single environmental factor that could be correlated with YLS symptom expression.

Virus infection of Hawaiian breeding cultivars. Hawaii has had an extensive sugarcane breeding program for many years. Records of the cultivars and their offspring have been kept and many of the old parent cultivars still exist in plots at the Hawaii Agriculture Research Center breeding station. SCYLV occurs throughout the breeding station. The percentage of cultivars infected is unknown although less than 10% show symptoms. Susceptible new progeny clones planted there quickly become infected and it is very likely that any of the old cultivars not

yet infected are in fact resistant. The old cultivars were tested for SCYLV infection and this information was compared with the breeding records. The results of some of the crosses are shown below.

CO 213 (SCYLV) x POJ 2878 (SCYLV) → H32-8560 (SCYLV)

H32-8560 (SCYLV) x POJ 2878 (SCYLV) → H38-2915 (SCYLV)

H32-8560 (SCYLV) x ? → H44-3098 (SCYLV)

H32-8560 (SCYLV) x H34-1874 (**no** SCYLV) → H37-1933 (SCYLV)

H37-1933 (SCYLV) x ? → H50-2036 (**no** SCYLV)

H37-1933 (SCYLV) x H41-3340 (SCYLV) → H49-0005 (SCYLV)

H49-0005 (SCYLV) x H50-7209 (SCYLV) → H59-3775 (SCYLV)

H49-0005 (SCYLV) x ? → H57-5174 (**no** SCYLV)

H50-7209 (SCYLV) x ? → H65-7052 (SCYLV)

H50-7209 (SCYLV) x ? → H87-4094 (SCYLV)

H50-7209 (SCYLV) x ? → H73-6110 (SCYLV)

H73-6110 (SCYLV) x ? → H87-4319 (**no** SCYLV)

H57-5174 (**no** SCYLV) x ? → H74-6001 (SCYLV)

H57-5174 (**no** SCYLV) x ? H78-4153 (**no** SCYLV)

It can be seen that infected cultivars are more common than uninfected ones. In the cases where both parents were known, crosses between susceptible cultivars produced susceptible offspring. The male parents are unknown in many of the crosses and many of the old commercial cultivars have been lost so our information on inheritance of resistance is, as yet, incomplete.

Susceptibility of progeny of crosses. Inoculations with SCYLV of progeny among crosses of Hawaiian cultivars is ongoing, but some information has been obtained to date. A self of the resistant cultivar H78-4153 (H78-4153 x H78-4153) produced seed that yielded 17 progeny plants. When these were inoculated with aphids, only two of the plants eventually tested positive for SCYLV. The self of susceptible H73-6110 (H73-6110 x H73-6110) yielded 23 progeny plants. Although the parents were infected with SCYLV, none of the progeny were initially infected indicating that SCYLV is not seed transmitted. However, after inoculation, 22 of the 23 progeny became infected. Thus the progeny of a susceptible cultivar were mostly susceptible and those from the resistant cultivar were mostly resistant.

Subsequently, three susceptible cultivars were crossed with the resistant H78-4153 and the progeny seedlings were inoculated. Preliminary results of inoculations of these progeny showed 27% of them to be infected. These were planted in field plots where they were exposed to natural infection. After several weeks they were tested again with the following results:

H87-4094 (susceptible) as male or female parent = average of 87% infected

H73-6110 (susceptible) as male or female parent = average of 19% infected

H65-7052 (susceptible) as male or female parent = average of 27% infected

This study is still ongoing and more plants may become infected over time. Selfs of a number of susceptible and resistant cultivars were made in the 2000-2001 crossing season which should contribute more information on inheritance of resistance to SCYLV.

Yield measurements. Preliminary results showed that virus infection reduced cane tonnage of H87-4094 compared to that of uninfected plants (see Table 3). However, the sucrose content in cane juice of infected and uninfected cane was not significantly

different. More data will be collected from tests installed at different locations. In addition, possible methods for maintenance of commercial virus-free seed fields are under investigation.

Discussion

We have confirmed the existence of SCYLV-resistant Hawaiian cultivars, and preliminary research indicates that resistance is inherited. Resistance may be due to total lack of infection or to the virus being unable to multiply to significant titre in the plants. But in either case, breeding for resistance and production of SCYLV-resistant cultivars may prove to be possible. Preventing virus spread by controlling the aphids would be very difficult and is not economically feasible. It is also possible to produce virus-free plants of susceptible sugarcane cultivars and to multiply them rapidly by means of micropropagation. However, this is costly and under ordinary conditions, fields would soon become reinfected by the sugarcane aphids.

The effects of SCYLV on growth and yield have not yet been fully quantified, although consistent growth reduction has been observed in infected cultivars. Studies are still underway comparing yields of virus-free and infected plots of susceptible cultivars. Currently, no control measures for SCYLV are practiced in Hawaii. This research has identified possible virus sources and vectors in plantations and has demonstrated the existence of resistant sugarcane cultivars. Continuing work to evaluate yield reduction and the effects of stress on infected cultivars will indicate whether breeding for resistance or planting of virus-free, susceptible cultivars will be worth the costs involved.

References

Scagliusi, S.M. and Lockhart, B.E.L. (2000). Transmission, characterization, and serology of a luteovirus associated with yellow leaf syndrome of sugarcane. Phytopathology 90:120-124.

Schenck, S., Hu, J.S., and Lockhart, B.E. (1997). Use of a tissue blot immunoassay to determine the distribution of sugarcane yellowleaf virus in Hawaii. Sugar Cane 1997(4):5-8.

Schenck, S. and Lehrer, A.T. (2000). Factors affecting the transmission and spread of sugarcane yellowleaf virus. Plant Disease 84:1085-1088.

Table 1. Infection of gramineous seedlings with Sugarcane Yellow Leaf Virus by inoculation with *Melanaphis sacchari* aphids.

Test plant	Seedlings tested	Aphids per plant	% plants infected
wheat	57	18	96.5
wheat (check)	20	0	0
oats	37	18	94.6
oats (check)	8	0	0
barley	63	18	93.7
barley (check)	19	0	0
rice	59	8	8.5
corn	19	30	10.5

Table 2. Sugarcane Yellowleaf Virus infection of 9 commercial cultivars in field plots over a 1-year period.[a]

Plant age (weeks)	78-3567	78-4153	78-7750	65-7052	73-6110	87-4094	78-3606	87-4319	82-3569
0	3	0 [b]	0	0	100	100	100	0	0
6	7	0	0	0	100	79	92	0	0
13	13	0	0	0	96	88	88	0	0
19	8	0	0	21	100	100	100	0	0
26	4	0	0	25	96	96	96	0	0
32	4	0	0	8	88	79	88	0	0
39	4	0	0	38	88	79	91	0	0
45	0	0	0	8	100	92	100	0	0
52	0	0	0	0	93	93	87	0	0
58	0	0	0	13	100	77	93	0	0
65	0	0	0	0	93	93	87	0	0
Total[c]	11/91	0/189	0/178	30/189	181/190	167/189	171/186	0/189	0/190
% positive	6	0	0	16	95	88	92	0	0

[a] Data presented are an average of eight field trials with three replicate plots of each cultivar in each trial.

[b] Percentage of plants tested giving positive reactions with tissue blot immunoassay.

[c] Number of positive reactions per total number of tested plants.

Table 3. Comparison of yield of SCYLV-infected (X) and virus-free (A) stalks of cultivar H 87-4094 in field plots.

	TCA		% refsol		% POL		purity		% fiber		POL/cane		TSA	
	A	X	A	X	A	X	A	X	A	X	A	X	A	X
mean	80.1	56.9	15.2	15.5	12.5	13.1	82.2	83.7	10.3	10.6	11.2	11.7	9.0	6.7
p-value	0.0532		0.304		0.217		0.230		0.279		0.230		0.0473	
	*		ns		ns		ns		ns		ns		*	

p-value = the probability of pairwised one-tail t-test. * = significant difference.