MYCOPLASMA OR PLT LIKE MICRO ORGANISMS DETECTED IN LEAVES OF SUGARCANE PLANTS INFECTED WITH WHITE-LEAF DISEASE AND THE SUPPRESSION OF THE DISEASE SYMPTOMS BY THE ANTIBIOTICS OF TETRACYCLINE GROUP

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The sugarcane white-leaf disease was found in Pingtong district in Taiwan in 1958, and named by LING and CHUNG-YANG in 1962. Since then, the etiologic agent has been presumed to be a virus because of 1): no visible organisms have been found in the affected plant tissues, 2): symptoms of the disease have been reproduced from affected stalks, 3): the causal agent could be suppressed by hot water treatment, although it could not be transmitted either by mechanical inoculation or by aphids. Recently, MATSUMOTO, LEE and TENG (1968) found a leafhopper, *Epittetix hiroglyphicus* MATSUMURA to be the vector.

In 1967, a co-operative study on the sugarcane white-leaf disease was started to investigate the cuasal agent of the disease by means of electron microscopy, between the senior author and the Sugar Experiment Station, Tainan, Taiwan, The Republic of China, where at that time late Dr. Takashi MATSUMOTO was a consultant.

In July of 1967, the presence of mycoplasma or PLT like microorganisms in the phloem elements of the diseased leaves of mulberry dwarf, potato witches' broom, aster yellows and Paulownia witches' broom diseased plants was reported in Japan (DOI *et al.* 1967). In addition, ISHIE *et al.* (1967) reported the recovery of the mulberry dwarf diseased plants by antibiotics of the teracycline group. They concluded that the etiologic agent of the disease could be mycoplasma or PLT like microorganisms.

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Shortly after their findings, the senior author found similar organisms resembling mycoplasma or PLT like structures, which were accumulated in the phloem cells of the diseased leaves of sugarcane plants. Then further experiments on the effect of some teracycline group antibiotics were planned in August 1967, and the tests started in September of 1967 in the Sugar Experiment Station in Taiwan, under the direction of late Dr. Takashi MATSUMOTO. Some of the experiments were conducted in the laboratory of plant virology, Department of Botany, Faculty of Agriculture, Hokkaido University, Sapporo, Japan. This paper deals with the finding of mycoplasma or PLT like organisms in the phloem cells of the diseased leaves of sugarcane plants and the results obtained with treatments of Terramycin, Achromycin and Aureomycin on the development of the disease.

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Electron microscopic studies

Meterials and methods. Small pieces (1 mm × 6 mm) of the diseased sugarcane leaves at various stages of infection were cut and fixed in 5% glutaraldehyde in 1/10 M phosphate buffer for 90 min., postfixed in 2% osmium tetroxide in dist. water for 120 min., dehydrated in graded ethanol and then embedded in epon. Ultrathin sections were cut by the Porter-Blum ultramicrotome equipped with glass knives. The thin sections were doubly stained by uranyl acetate and lead hydroxide, and examined by an electron microscope, JEM-5Y.

Results. Hundreds of thin sections of the diseased leaves at various stages of infections such as yellow stripe, pale white leaves, entirely white leaves have been examined. No uniform particles that would be the virus have been found either in the vascular bundle or parenchymatous cells or in any other tissues of the leaves. However, some large microorganisms which sometimes plugged the cells of the phloem were detected under the electron microscope. They are limited to the phloem of the diseased leaves but never been found in the healthy leaves (Fig. 1). The approximate size of these microorganisms is 100 to 900 mμ, almost round or ellipsoidal in shape and sometimes elongates (Fig. 2, 3, 4). High magnification of electron micrographs of such micro organisms has shown that they are surrounded by two layered thin membranes,

probably unit membranes, of approximately 8 to 10 mμ thick without cell walls. Inner structures of these microorganisms consists of small ribosomal granules of 12 to 15 mμ in diameter, and net-like strands of nuclear substances. Large bodies are round in shape with central vacuoles and accumulation of ribosomal particles at the periphery of the bodies. Characateristic polymorphic structures are seen in Fig. 2, showing the elongate bodies of approximately 60 × 300 mμ and the smaller dense bodies of 80 × 200 mμ in diameter. Sometimes the elongate bodies accumulated around the sieve plates. They seem to pass through the sieve plates. The round bodies that seem budding the small dense bodies and separate into two are shown in Fig. 3. Vacuolated large bodies of irregular shape packed in a cell are presented in Fig. 4. Sometimes, electron dense granular structures are intermingled with those cells. Characteristic degenerations of some of the cells filled with dense granular bodies are observed occasionally. The morphology and structures of these bodies entirely correspond with the mycoplasma or PLT like microorganisms reported by Doi *et al.* (1967).

Effect of the tetracycline group antibiotics

Although the mycoplasma or PLT like microorganisms were apparently found in the phloem of the sugarcane white leaf diseased plants, there was no experimental evidence available to demonstrate that these micro organisms were the etiologic agent of this disease. The only available test is the application of some antibiotics that can not control virus diseases, but cure mycoplasma or PLT like microorganisms affected plants, such as mulberry dwarf disease.

Materials and methods. The antibiotics used in this experiments were: tetracycline hydrochloride (Achromycin), Oxytetracycline hydrochloride (Terramycin), Chlortetracycline hydrochloride (Aureomycin) and Agrimycin 100 (15% Streptomycin and 1.5% Terramycin, Pfeizer Taito Co.). The antibiotics were dissolved in distilled water at 10 ppm, 50 ppm, 100 ppm, 200 ppm and 400 ppm.

The diseased stalks of sugarcane (No. 56–2080), showing typical symptoms, were collected in the field of Yuching district, Tainan, Taiwan. Each diseased stalk was divided into four seed-cuttings, using 10 stalks for treatments. The seed-cuttings were numbered 1, 2, 3, and 4 from the top to the bottom of the stalk respectively, and then distributed in an experimenatal design as shown in Table I.

To asure the effect of the antibiotics, two experiments were carried out.

Experiment A. Diseased stalks, cut into four seed-cuttings, were planted in 19 cm diam. pots. After ten days to two weeks, the plants showing typical symptoms were taken from the soil, washed the roots and then immersed in

TABLE I. Experimental design of the antibictic treatments in relation to the diseased stalks and the seed-cuttings.

Number of diseased stalks used	Seed-cuttings number treated with			
	Untreated controls	10 ppm	50 ppm	100 ppm
1	1*	2	3	4
2	4	3	2	1
3	1	2	3	4
4	4	3	2	1
5	1	2	3	4
6	4	3	2	1
7	1	2	3	4
8	4	3	2	1
9	1	2	3	4
10	4	3	2	1

* Seed-cutting are numbered from the top to the bottom of the diseased stalks.

TABLE II. Effect of oxytetracycline on the development of sugarcane white leaf disease. The roots of the diseased sugarcane plants were immersed in the oxytetracycline solution for 24 hours. The diseased seed-cuttings were plnated on Nov. 11, 1967, and the treatment of the roots was carried out on Nov. 22 to 23. (Terramycin-A).

Conc. of oxytetra-cycline	Stalk number used	Seed cutting number	Symptoms on Nov. 29	Symptoms on the new leaves emerged after the treatments							
				29/I	7/XI	14/XII	23/XII	30/XII	8/I	13/I	22/XII
100 ppm	1	4	YSt	± *	±	± ± **	±—	——	+++ ***	+++	+++
	2	1	YSt	±	## +	+++ ±	+++ ±	+++ ±	+++	+++	+++
	3	4	PYW	##	## ##	###	+##	+##	+++ ##	++	++
	4	1	PYW	—	——	——	—— ——	—— ——	—— ——	—— ——	###
	5	4	PYW	##	## ##	###	###	###	###	###	###
	6	1	PYW	+	## ##	##					
	7	4	YSt	—	—+	—## ±	—## —	—## —	± ## ##	+## ##	## ##
	8	1	YSt	±	——	——	——	——	——	## ##	
	9	4	PYW	—	—±	—±	—± ±	—± ±	± ± ±	+++	+++
	10	1	YSt	±	——	——	—— ——	—— ——	+++	+++	
50 ppm	1	3	YSt	+	± ±	± ±	± ±	± ±	± + ±		
	2	2	YSt	+	## ##	## ##	—## ±	—## —	+ ## ##		
	3	3	PYW	##	± ±	± ±	± ±	± ±	± ++		###
	4	2	PYW	±	——	——	——	——	—— ——		
	5	3	PYW	##	## ##	## ##	## ## ±	## ## ±	## ## ##		
	6	2	PYW	##	## ##	## ##	###	###	###		
	7	3	PYW	##	——	——	—— ——	—— ——	—— ——		
	8	2	YSt	##	## ##	+++ ##	+——	+——	+++		
	9	3	PYW	±	##	## ±	## ±				
	10	2	YWSt	##	## ##	++	++	++	+++		+

10 ppm	1	2	YSt	+	+	++					
	2	3	YSt	##	+ ±	++ ±	—+ ±	—+ ±	—++		
	3	2	PYW	##	##	##	##	##	###	###	
	4	3	PYW	##	## ±	##	## ±	## ±	###		
	5	2	PYW	##	##	##	##	##	###		
	6	3	PYW	##	##	##	##	##	##		
	7	2	PYW	##	—+	++	—+	—+	++		
	8	3	PYW		##	##	##	##	##		##
	9	2	PYW	##	##	##	##	##	##		##
	10	3	YSt	—	——	—±	——	——	——		
Dist. water	1	1	YSt	(+)	(+)	(+)	(+)	(+)	(+)	(+)	
	2	4	SYt	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
	3	1	PYW	(##)	(##)	(##)	(##)	(##)	(##)	(##)	(##)
	4	4	PYW	(##)	(##)	(##)	(##)	(##)	(##)	(##)	(##)
	5	1	PYW	(##)	(##)	(##)	(##)	(##)	(##)	(##)	
	6	4	PYW	(##)	(##)	(##)	(##)	(##)	(##)	(##)	(##)
	7	1	YSt	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
	8	4	PYW	(##)	(##)	(##)	(##)	(##)	(##)	(##)	(##)
	9	1	PYW	(##)	(##)	(##)	(##)	(##)	(##)	(##)	(##)
	10	4	YSt	(+)	(+)	(+)	(+)	(+)	(+)	(+)	

Abbreviations: ## represents the symptoms on the new leaves of sugarcane plants emerged after the treatments, showing yellow leaves (YL), yellowish white leaves (YW) pale yellowish white leaves (PYW) and entirely white leaves (EW).

\+ represents the symptoms on the new leaves of sugarcane plants emerged after the treatments, showing yellow stripe (YSt) and yellowish white streak (YWSt).

± represents the symptoms on the new leaves of sugarcane plants emerged after the treatments, showing light green colored leaves (LGL).

— represents the green leaves (GL).

* ** *** These three signs shown in the table indicate the symptoms of first newly emerged leaves, second emerged leaves and third emerged leaves respectively after the treatments.

(##) represents that whole plants used for the controls still remained severly diseased, initially started at symptoms of yellow leaves more or less pale yellowish white leaves and entirely white leaves.

(+) represents that whole plants used for the controls still remained severly diseased, initially started at symptoms of yellow streak leaves and yellowish streak leaves.

the antibiotic solutions for 24 hours. After washing the treated roots in distilled water, the plants were placed again in the pots. The untreated contols were immersed in distilled water in the same manner as the antibiotic treatments.

Experiment B. The diseased seed-cutting were immersed in the antibiotic solutions for 72 hours at room temperature before planting. The treated seed-cutting were washed and planted in the pots. Untreated controls were immersed in distilled water for 72 hours and then planted.

TABLE III. Effect of oxytetracycline on the development of sugarcane white leaf disease. The diseased stalks were immersed in the oxytetracycline solution for 72 hours before planting. The treatment of the diseased seed-cutting was carried out on Nov. 22 to 25, 1967 (Terramycin-B).

Conc. of oxytetra-cycline	Stalk number used	Seed cutting number	Symptoms on Nov. 29	Symptoms on the new leaves emerged after the treatments							
				29/XI	7/XII	14/XII	23/XII	30/XII	8/I	13/I	22/I
200 ppm	1	4	YWSt	+	+	+					
	2	1									
	3	4	YWSt	+	+	+ ++	+ ++	+	+	+	+
	4	1									
	5	4	PYW	++	++	++	++	++	++	++	++
	6	1									
	7	4	PYW	++	++	++					
	8	1	LGL	±	±	± ±	±				
	9	4	LGL	±	±	±	±	±	±	±	±
	10	1	GL	—							
100 ppm	1	3	PYW	++ ±	+ ++	+ ++	+ ++	+ ++	± ++	± ++	± ++
	2	2	YWSt	+	++	++	++	++	++	++	++
	3	3	YWSt	+	+ ±	+ ±	+	+	+	+	+
	4	2	GL	—	—	—	—	—	+	+	+
	5	3	PYW	++	++	++	++	++	++	++	++
	6	2	PYW	++	++	++	++	++	++	++	++
	7	3	PYW	++	++	++ ++	++				
	8	2	LGL	±							
	9	3	PYW	++	++	++	++				
	10	2	GL	—	—	—	—	—	—	—	— ±
50 ppm	1	2	PYW	++							
	2	3	PYW	++							
	3	2									
	4	3	LGL	±	——	——	——	——	+—	+—	+——
	5	2	PYW	++	++						
	6	3									
	7	2	PYW	++							
	8	3	PYW	++	++						
	9	2	PYW	++	++	++	++				
	10	3	LGL	±	±	±	±	++			
Dist. water	1	1	YWSt	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
	2	4	PYW	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)
	3	1	PYW	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)
	4	4	YWSt	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
	5	1	PYW	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)
	6	4									
	7	1	PYW	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)
	8	4	PYW	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)
	9	1	PYW	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)
	10	4	PYW	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)

Abbreviations: see Table II.

TABLE IV. Effect of tetracycline on the development of sugarcane white leaf disease. The diseased stalks were immersed in the tetracycline solution for 72 hours. The treatment of the diseased seed-cuttings was carried out on Nov. 2 to 5, 1967 (Achromycin-B).

Conc. of tetra-cycline	Stalk number used	Seed cutting number	Symptoms on Oct. 29	Symptoms on the new leaves emerged after the treatments							
				29/XI	7/XII	14/XII	23/XII	30/XII	8/I	13/I	22/I
100 ppm	1	4	LGL	±	—	—	—	——	+—		—
	2	1	YW	++	++	++	++				
	3	4	YSt	+	++	++	++	++	++		
	4	1	PYW	++	++	++					
	5	4							+		
	6	1							+		
	7	4							+		
	8	1	LGL	±	—	——	——	——	+		
	9	4	LGL	±	—	+—	+—	+—	+		
	10	1	LGL		±	±	++	++	++		
50 ppm	1	3							±		
	2	2	PYW	++	++	++	++	++	++		
	3	3	PYW	++	++	+	+	+			
	4	2	EW	++	++	++					
	5	3									±
	6	2	YSt	±	—	+—	+—	+—	+		
	7	3									
	8	2	YSt	+	+—	++	++	++	+		±
	9	3							+		
	10	2	GL	—	—	+	+	+	+		++
10 ppm	1	2	GL		—	—	—				
	2	3	YL		++						
	3	2	PYW		++						
	4	3	EW	++							
	5	2									
	6	3	PYW	++	++	++					
	7	2			±	±	±	±	—		
	8	3	PYW	++	++	++	++	++	++		++
	9	2			++						
	10	3	PYW	++	++	++	++	++	++		++
Dist. water	1	1									
	2	4									
	3	1	PYW	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)
	5	4	PYW	(++)	(++)	(++)	(++)	(++)	(++)	(++)	
	4	1									
	6	4	PYW	(++)	(++)	(++)	(++)	(++)	(++)	(++)	
	7	1									
	8	4	PYW	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)
	9	1	PYW	(++)	(++)	(++)	(++)	(++)			
	10	4	PYW	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)

Abbreviations: see Table II.

TABLE V. Effect of chlortetracycline on the developement of sugarcane white leaf disease. The roots of the diseased sugarcane plants were immersed in the chlortetracycline solution for 24 hours. The diseased seed-cuttings were planted on Oct. 31, 1967, and the treatment of the roots was carried out on Nov. 13 to 14 (Aureomycin-A).

Conc. of chlortetra-cycline	Stalk number used	Seed cutting number	Symptoms on Nov. 29	Symptoms on the new leaves emerged after the treatments							
				29/XI	7/XII	14/XII	23/XII	30/XII	8/I	15/I	22/I
100 ppm	1	4	PYW	++	+++	+++	+++	+++	+++		
	2	1	PYW	++	++	++	++	++	++		
	3	4	YW	++	+++	+++	+++	+++	+++		
	4	1	YSt	+	±	±—	+—	+—	++		
	5	4	LGL	±	—	——	——	——	±+	+	
	6	1	PYW	++	++	+++	+++	+++	+++		
	7	4	PYW	++	++	++	+++	+++	+++		
	8	1	PYW	—	++	+++	+++				
	9	4	PYW	++	+±	++	+++	+++	+++		
	10	1	PYW	++	+±	++	+++	++	++		
50 ppm	1	3	PYW	++	+++	+++	+++	+++	+++	+++	
	2	2	PYW	++	++	+++	+±	+++	+++	++	
	3	3	PYW	++	++	++	+++	+++			
	4	2	PYW	++	++	++	+++	+++	+++		
	5	3	YSt	+	+	++	+				
	6	2	PYW	+++	+++	+++	+++	+++	+++		
	7	3	PYW	++	++						
	8	2	PYW	++	++						
	9	3	PYW	++	++						
	10	2	PYW	++							
10 ppm	1	2	PYW	++	+++	+++	+++	+++	+++		
	2	3	PYW	++	++	++	++	+++			
	3	2	PYW	++	+++	+++	+++	+++	+++		
	4	3	PYW	++	++	++	++	+++	+++		
	5	2	YWSt	+	+—	+—	+—	+			
	6	3	PYW	++	+++	+++	+++	+++	+++		
	7	2	PYW	++	++	+++	+++				
	8	3	PYW	++	++	+++	+++	+++	+++		
	9	2	YW	++	++						
	10	3	PYW	++	++						
Dist. water	1	1	EW	(++)	(++)	(++)	(++)	(++)	(++)		
	2	4	EW	(++)	(++)	(++)	(++)	(++)	(++)		
	3	1	EW	(++)	(++)	(++)	(++)	(++)	(++)		
	4	4	PYW	(++)	(++)	(++)	(++)	(++)	(++)		
	5	1	YSt	(+)	(++)	(++)	(++)	(++)	(++)		
	6	4	PYW	(++)	(++)	(++)	(++)	(++)	(++)		
	7	1		(++)	(++)	(++)	(++)	(++)	(++)		
	8	4		(++)	(++)	(++)	(++)	(++)	(++)		
	9	1		(++)	(++)	(++)	(++)	(++)	(++)		
	10	4	EW	(++)	(++)	(++)	(++)	(++)	(++)		

Abbreviations: see Table II.

TABLE VI. Effect of chlortetracycline on the development of sugarcane white leaf disease. The diseased stalks were immersed in the chlortetracycline solution for 72 hours. The treatment of the diseased seed-cuttings wes carried out on Nov. 13 to 16, 1967 (Aureomycin-B).

Conc. of chlortetra-cycline	Stalk number used	Seed cutting number	Symptoms on Dec. 29	Symptoms on the new leaves emerged after the treatments						
				29/XI	6/XII	14/XII	23/XII	30/XII	8/I	13/I
100 ppm	1	4	PYW		++	++++	++++	++++		
	2	1	GL	—	——	——	———	———	——±	+++±
	3	4	PYW				++	++	++++	
	4	1	LGL	±	——	——	+++—	+++—	+++—	
	5	4	YW	+	+++	+++++	+++++	+++++	+++++	
	6	1	GL	—	——	——	——	——	+++	++++
	7	4	LGL	±	+++	+++	+++±	+++±	+++++	++++
	8	1	LGL	±	+—	+—	+++—	+++—	+++—	
	9	4	LGL	±	++++	++++	±++±	±++±	±++±	
	10	1	LGL	±	++	++++	+++++	+++++	+++++	
50 ppm	1	3	LGL	±	±	++++	++++	++++	++++	
	2	2	LGL	±	+++	+++++	+++++	+++++	+++++	
	3	3	PYW		++	++++	+++±±	+++±±	+++++	
	4	2	YSt		+	+—	+——	+——	+++++	
	5	3	PYW		++	++++	+++++	+++++		
	6	2	GL	—	+—	+—	+—	+—	++	
	7	3	YSt	+	+—	+—	+—	±—	+—+	
	8	2	GL	—	+++	+—	+—	+++		
	9	3	PYW	++	++	++	++	++	++++	
	10	2	PYW	++	++++	++++	+++	+++	+++++	
10 ppm	1	2	LGL	±	++±	++++	+++++	+++++	+++++	
	2	3	LGL	±	++	++++	+++++	+++++	+++++	
	3	2	YW	++	++	++++	+++++	+++++	+++++	
	4	3	GL		—	+—	+——	+——	+——	+++
	5	2	YW	++	+++	+++	+++	+++		
	6	3	GL	—	++++	+++++	+++++	+++++	+++++	
	7	2	PYW	++	+++	+++	+++	+++	+++	
	8	3			++	++	+++	+++	+++	
	9	2	LGL	±	+++	+++	+++	+++	+++	
	10	3	YW	++	++	+++	+++	+++	+++	
Dist. water	1	1	PYW	(++)	(++)	(++)	(++)	(++)	(++)	(++)
	2	4	PYW	(++)	(++)	(++)	(++)	(++)	(++)	(++)
	3	1	PYW	(++)	(++)	(++)	(++)	(++)	(++)	(++)
	4	4	YSt	(+)	(+)	(+)	(+)	(+)	(+)	(+)
	5	1	PYW	(++)	(++)	(++)	(++)	(++)	(++)	(++)
	6	4	PYW	(++)	(++)	(++)	(++)	(++)	(++)	(++)
	7	1	YSt	(+)	(+)	(+)	(+)	(+)	(+)	(+)
	8	4	EW	(++)	(++)	(++)	(++)	(++)	(++)	(++)
	9	1	PYW	(++)	(++)	(++)	(++)	(++)	(++)	(++)
	10	4	EW	(++)	(++)	(++)	(++)	(++)	(++)	(++)

Abbrevistions: see Table II.

Results. The results of Experiment A and B are shown in Table II to VI. In experiment A, some of the test plants recovered the healthy green or light green color appearance by treatment of the roots in Terramycin, Achromycin and Aureomycin at 50 ppm and 100 ppm. However, these leaves showed again yellow stripe or white color after 1 to 5 weeks following of the treatments. It means that the effect of these antibiotics at these doses was suppressive, and not curative. Sometimes, the first leaves were normal after the treatments, but the next emerged leaves showod typical symptoms of the white leaf. In experimant B, a long delay of the germination took place. Nevertheless, the disease was cured by immersion of the seed-cutting before planting in the antibiotics of Terramycin, Achromycin and Aureomycin. Usually, newly growing leaves after the treatments of experiment B. showed increased green area and color as compared with untreated controls. The effect of the antibiotics seemed to be more effective to plants showing somewhat slight symptoms such as stripe.

Further tests were carried out in the green house of the Department of Botany, Faculty of Agriculture, Hokkaido University, Sapporo, Japan, and revealed a completely recovery of the plants by the immersion of seed-cutting in Terramycin at 400 ppm for 72 hours before planting. However, the germination of the plants was considerably delayed and most of the treated plants suffered in their germination. The recovered plants were still growing healthy after 3 months as shown in Fig. 5. Neither mycoplasma nor PLT like microorganisms were found in the leaf phloem of the healthy plant shown in Fig. 5. Agrimycin inhibited germination at all doses, and did not show any effect on the disease.

Discussion and conclusion

Mycoplasma or PLT like microorganisms were always found in the phloem cells of white leaf diseased sugarcane plants by examination with an electron microscope. However, no virus like particles were observed in the diseased plants. Their structures are very similar to those found by DOI *et al.* (1967) in some of the witches' broom and yellow type diseases in Japan. However, up to date, there is no definite eveidence demonstrating that these mycoplasma or PLT like microorganisms are actually the causal agents of these disease, because of unsuccessful growth of these microorganisms on culture media. The detection of these charactersitic microorganisms in the diseased leaves by the electron microscope, and the suppression of the effects of the disease in the plants by use of antibiotics that do not cure virus diseases are the only available evidence as shown by DOI *et al.* (1967), and ISHIE *et al.* (1967). Accordingly, once the mycoplasma or PLT like microorganisms were detected

in the sugarcane white leaf plants, further experiments were performed in the Taiwan Sugar Experiment Station and the Department of Botany, Faculty of Agriculture, Hokkaido Univeristy, Sapporo in 1967 with the antibiotics of Terramycin, Achromycin and Aureomycin.

The differences in symptom expression on leaves of individual seed-cuttings from the same stalk, and the masking of the symptoms occuring mostly in the stripe-type leaves, made the tests rather difficult to account for the antibiotic effect. Therefore, an arrangement of the treatments for the individual seed-cuttings from the same diseased stalk was carefully designed in Table I. Unfortunately, the experiments were carried out from October to December in 1967, when the condition for the growth of the sugarcane plants was not suitable. Thus it seems likely that the effect of the antibiotics considerablly differed in each treated plant; some were cured after a week and some were slowly cured as the effect were seen in the next leaves emerged after the treatments. Nevertheless, the suppressive effect of Terramycin, Achromycin and Aureomycin on the sugarcane white leaf disease was shown by the treatments of roots immersion and seed-cutting immersion.

Some distinct appearance in the phloem cells, mostly sieve tubes was pointed out by LING (1963) by anatomical studies under a light microscope. In this regards, it is interesting to mention that mycoplasma or PLT like microorganisms present in the diseased sugarcane leaves sometimes appeared accumulated and seemed to plug some of the phloem cells, as shown in Fig. 1.

The electron microscopic observations, first of all, revealed apparent mycoplasma or PLT like microorganisms associated with the white leaf disease of sugarcane, and no virus like particles. Secondly, the disease development was suppressed by the use of Terramycin, Achromycin and Aureomycin. These evidences, therfore, strongly suggest that the causal agent of the white leaf of sugarcane are mycoplasms or PLT like microorganisms rather than viruses.

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Explanations of Figures

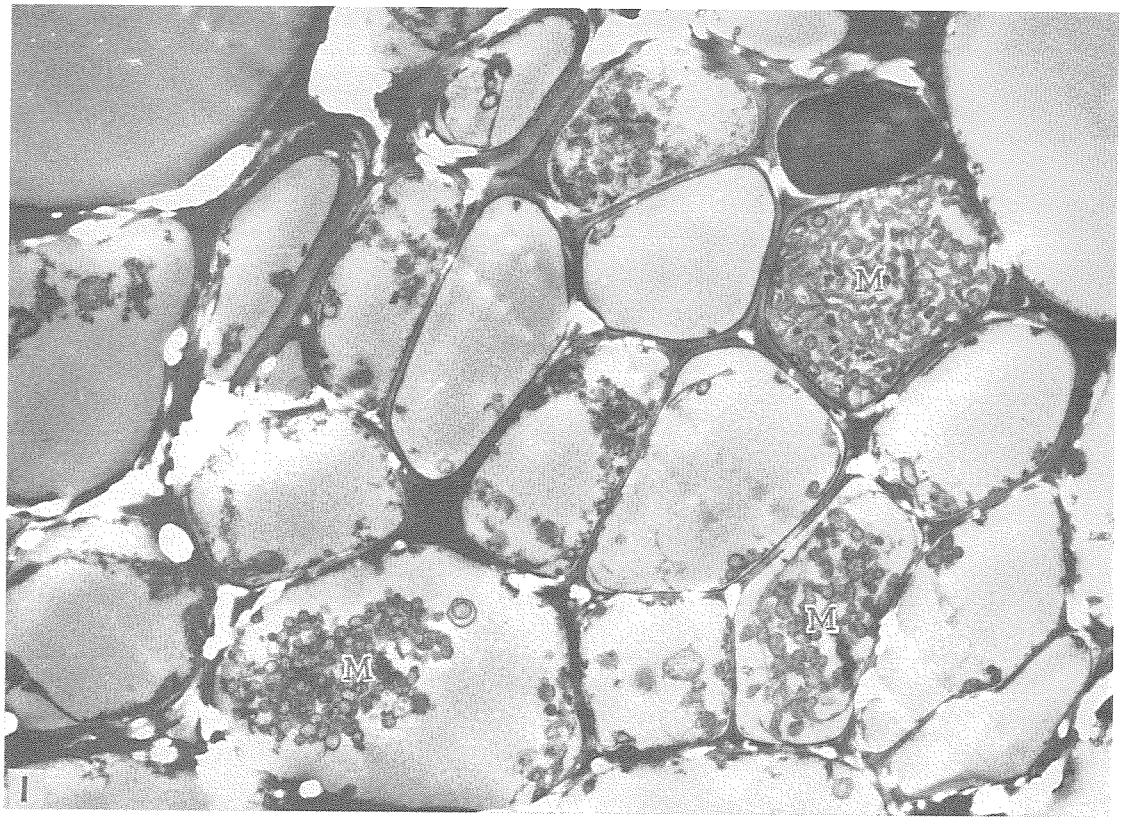
Fig. 1. An ultrathin section of a leaf of sugarcane showing white leaf disease symptom. Accumulations of mycoplasms or PLT like organisms (M) are shown in some of the phloem cells. × 6,000

Fig. 2. High magnification of a cell shown in Fig. 1, upper wright. Note that the cell is plugged with a mass of elongated or round shaped bodies. × 30,000

Fig. 3. Round bodies found in an ultrathin section of a cell of a diseased leaf of sugarcane plant. Note that some of the bodies seems budding the dense smaller bodies. × 40,000

Fig. 4. An accumulation of large vacuolated bodies present in a cell of a sugarcane white leaf infected leaf. Electron dense granular structures are sometimes intermingled in the infected cells. × 40,000

Fig. 5. A completely recovered sugarcane plant after the treatment with a 400 ppm Terramycin solution for 72 hours using the seed-cutting immersion method. Plant at wright: treated plant. Plant at left: untreated control. This picture was taken three months after the treatment.



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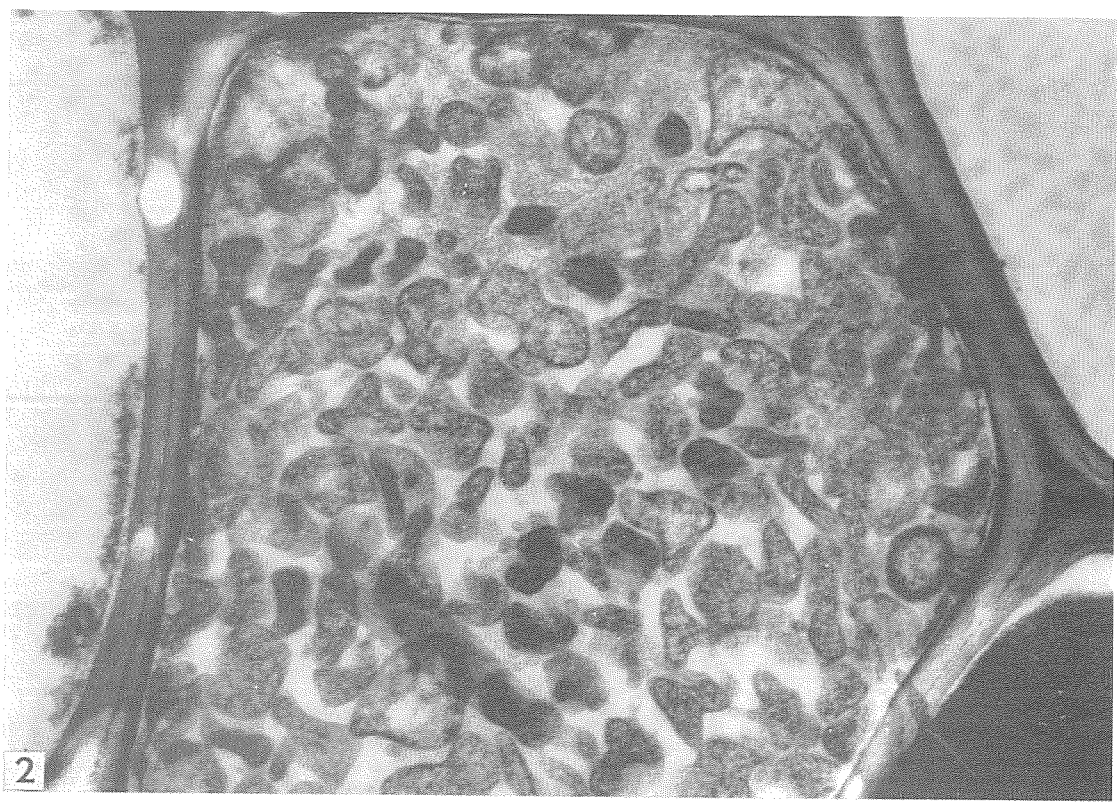

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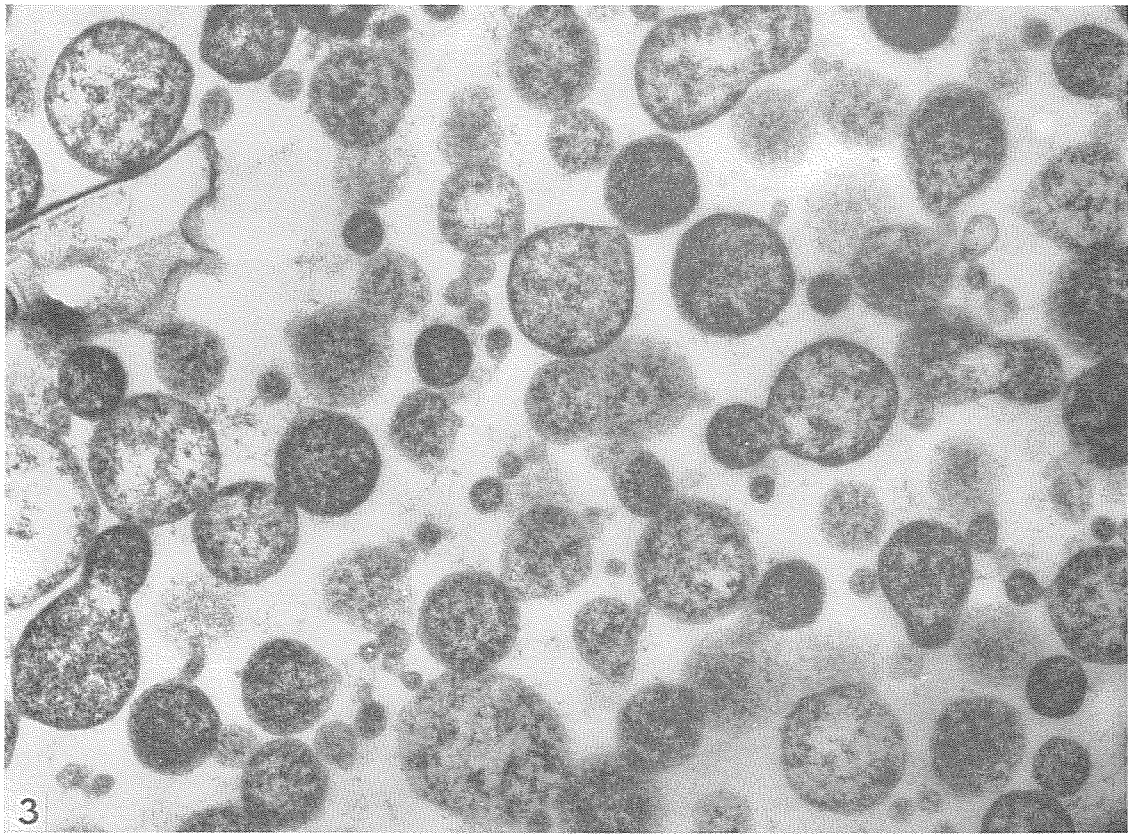
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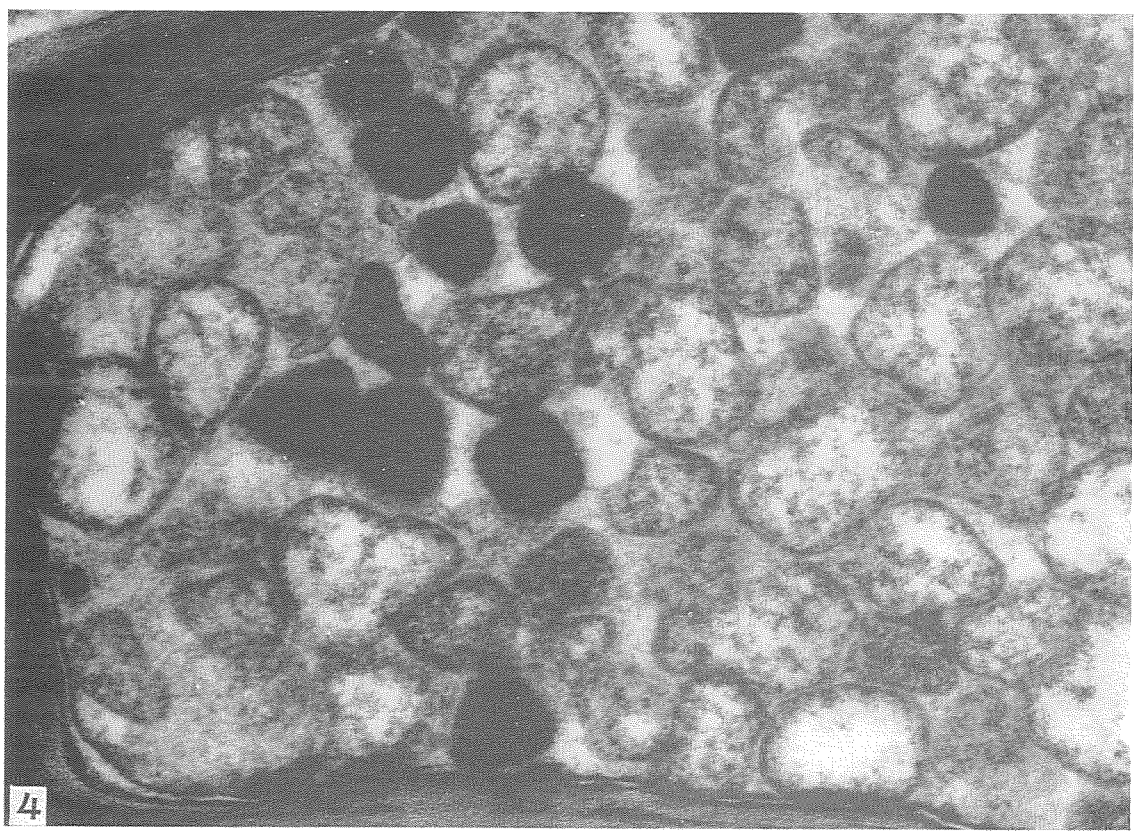
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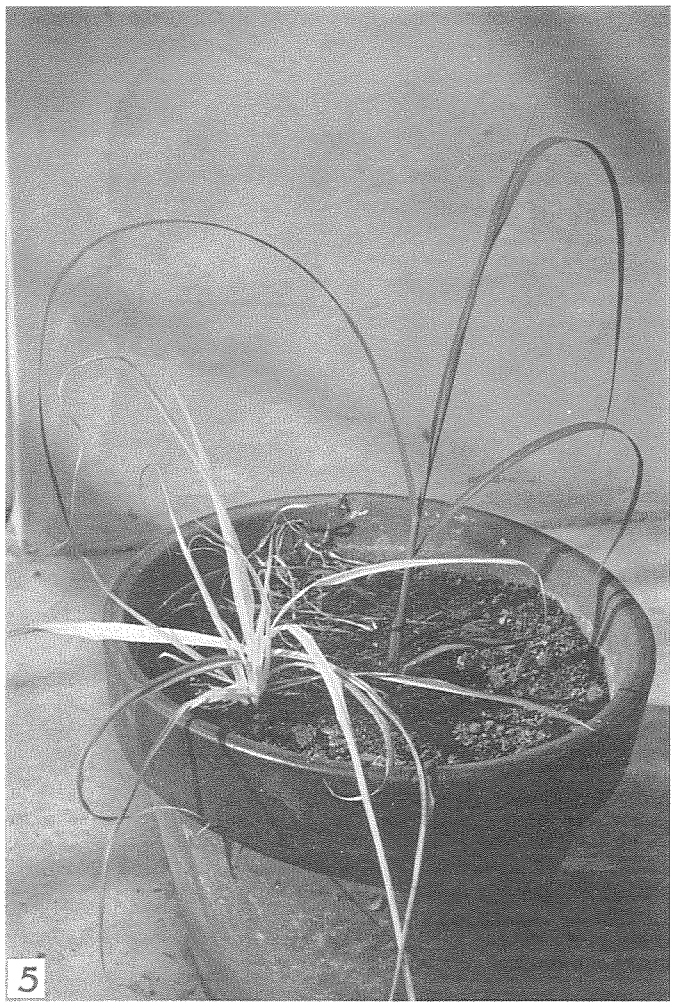
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