Mycoplasma-Like Organism, Causal Agent of Mulberry Dwarf Disease

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The mulberry dwarf disease is one of the most important diseases attacking mulberry trees at present in Japan. It not only causes dwarf symptoms of mulberry trees (Fig. 1),

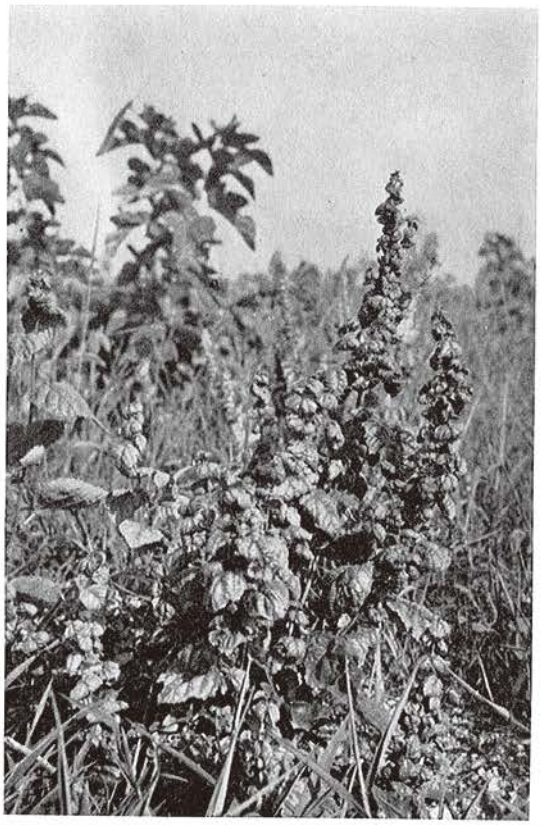

Fig. 1. Dwarf diseased mulberry tree with typical symptoms.

decreasing the yield of leaves, but also devastates a mulberry plantation in some years by its strong infectivity.

This disease is known to occur to some extent in Korea and China, but little is known about its attack in other countries. Therefore, great care must be taken about this disease concerning the promotion of sericulture in Southeast Asia in future.

Changes of etiological explanations

The study of this disease has a long history and can be traced to its origin in the eighteenth century. Formerly this disease was thought to be due to the overcutting of shoots and overpicking of leaves.

An opinion that the mulberry dwarf disease is caused by a kind of viruses was presented in 1935 when the disease was proved to be transmitted by grafting and by insect. And numbers of studies have been carried out on the basis of the virus theory since then without succeeding in the isolation and observation of any pathogenic virus.

Finally, in 1967, Doi *et al.* found that microorganism-like particles, which were different from viruses in size and shape, were present in the leaves and stems of dwarf-diseased mulberry trees and thought them to be the pathogenic agents of the disease.

Observation of the tissues of dwarf-diseased mulberry trees

When ultrathin sections of the tissues of dwarf-diseased mulberry trees were observed electron microscopically, many microorganism-like particles were found only in the phloem

Fig. 2. Mycoplasma-like organisms revealed by electron microscopy in the sections of phloem tissues of leaves from dwarf diseased mulberry (× 20.000).

(Fig. 2). These particles are irregular in size (80~800 mμ) and pleomorphic, being globular or irregular in shape. Small particles are nearly globular in shape and full of ribosome-like granules, occasionally having nucleus-like net-strands.

Most of the large ones are irregularly elliptic in shape and contain ribosome-like granules in the periphery but not in the central part, in which a few net-strands are found.

These large and small particles are mostly present in mixture in sieve tubes or in phloem-parenchyma cells, and small ones are supposed to grow into large ones. These particles have no clear cell wall, but they are enclosed with a two-layered unit membrane (about 8 mμ thick), respectively, and different from other plant cells in structure.

Such particles have never been observed in healthy mulberry leaves and are located only in the phloem tissue even in diseased leaves, being supposed to be organisms closely related to Mycoplasma found in animal and human bodies on the basis that they have pleomorphic shape, no clear cell wall and characteristic inner structure. Their presence in the phloem tissue of roots also has been confirmed.

Observation of the particles in viruliferous vectors

This disease is transmitted by insects, and two kinds of leafhoppers, Hishimon-yokobai (*Hishimonus sellatus* Uhler) and Hishimon-modoki (*Hishimonoides sellatiformis* Ishihara) are known as its insect vectors (Fig. 3).

And whether the vectors contain any Mycoplasma-like organisms in their bodies or not

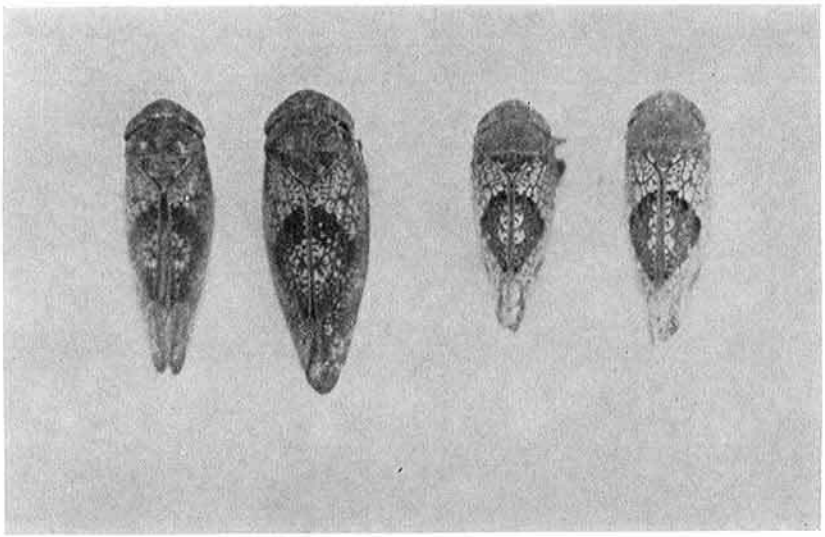

Fig. 3. Vector leafhoppers of mulberry dwarf disease. From right to left: ♀ and ♂ of Hishimonus sellatus, ♀ and ♂ of Hishimonoides sellatiformis.

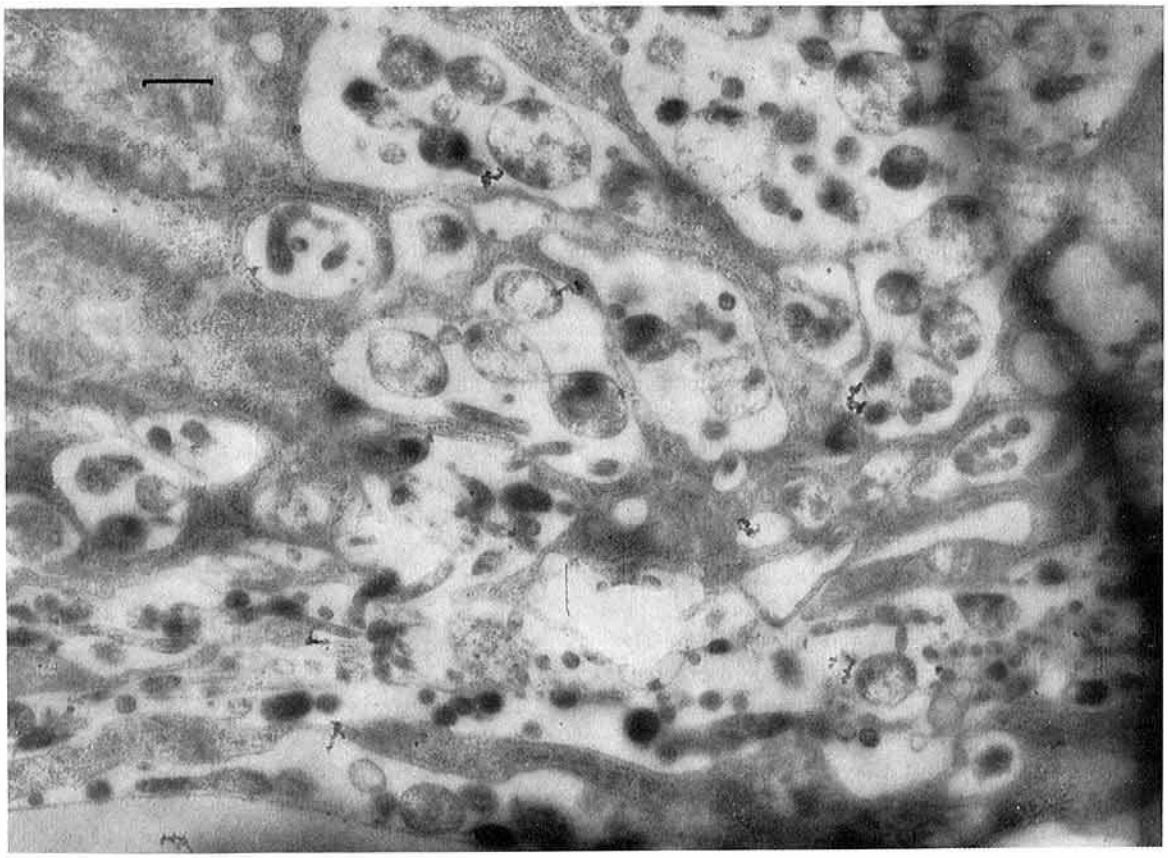

Fig. 4. Mycoplasma-like organisms revealed in the section of a salivary gland of viruliferous vector, Hishimonus sellatus Uhler. (× 18.000).

was examined with the result that the presence of these organisms was confirmed in the salivary gland of the vectors (Fig. 4).[3)]

Many pleomorphic particles of various sizes were observed to be contained locally in the peripheral part of each gland cell in nearly the same way in both kinds of insect vectors. This finding that the viruliferous vector contains Mycoplasma-like organisms similar to those observed in diseased leaves seems to be an evidence supporting the pathogenicity of the organisms.

Inhibitory effect of the tetracycline group of antibiotics on the occurrence of the mulberry dwarf disease

The organisms of the group of Mycoplasma have a common characteristic to be susceptible to tetracycline antibiotics. So if the pathogen of this disease belongs to the group, tetracyclines are expected to be effective for inhibiting the multiplication of the pathogen and for preventing the occurrence of the disease. From such a viewpoint, the authors have carried out experiments on the treatment of diseased mulberry saplings with tetracycline drugs.

The author's results showed that Kanamycin, which is a non-tetracycline antibiotics, was ineffective for inhibiting the occurrence of the disease, while Achromycin (tetracycline) and Aureomycin (chlortetracycline) were clearly effective (Fig. 5).

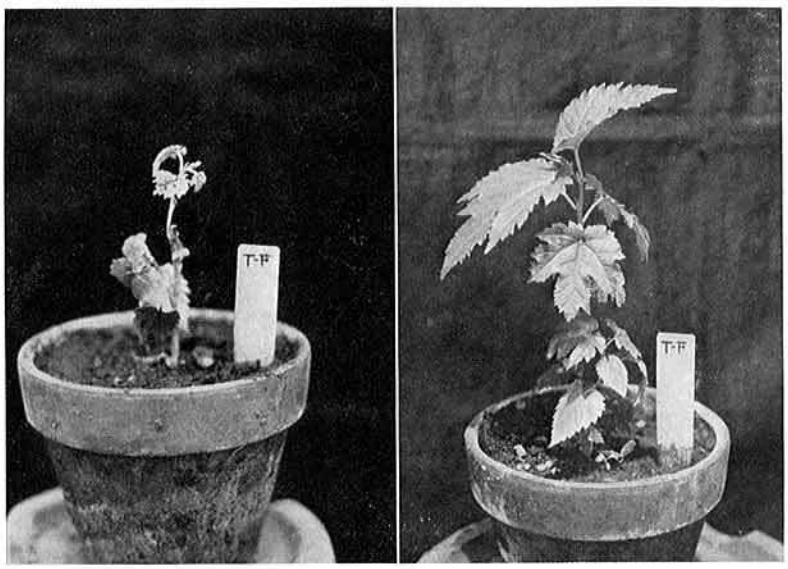

Fig. 5. Suppressive effect of root dipping in Achromycine 100 ppm solution on symptoms.
Left: Prior to treatment.
Right: 7 days after treatment.

The efficacy of these drugs differs with the method of application. Root dipping was the most effective method (Table 1), being followed by foliage spraying (Table 2), and soil drenching was ineffective.

When the treatment with these drugs was interrupted after a continuation, the symptoms which had once disappeared during the treatment tended to reappear (Tables 1 and 2), and the period until the reappearance of symptoms was apt to be different according to the size of saplings or the method of treatment.

From the above-mentioned results, it is concluded that the pathogen of the mulberry

Table 1. Change of the symptoms by foliage spraying with antibiotics

	③ \ ①	Treatment period						After treatment				
		6/III	13/III	17/III	21/III	29/III	2/IV	12/IV	22/IV	30/IV	8/V	22/V
	③ \ ②	—	7	11	15	23	26	10	20	28	36	50
T—100 ppm	1	++	++	+	±	−	−	−	−	−	+	+++
	2	++	+++	+	±	−	−	−	−	+	++	+++
	3	++	++	+	−	−	−	−	±	+++	+++	+++
	4	++	++	+	−	−	−	−	−	−	−	+
	5	++	++	+	−	−	−	−	−	−	−	−
T—10 ppm	6	++	++	++	++	++	++					
	7	++	+++	++	+	++	++					
	8	++	++	+	+	±	±					
	9	++	+++	++	+	++	++					
	10	++	++	+	±	±	×					
K—100 ppm	11	++	+++	+++	++	+++	+++					
	12	++	+++	+++	+++	+++	+++					
	13	++	+++	++	++	−	±					
	14	++	+++	+++	+++	+++	+++					
	15	++	+++	++	+	±	+++					
K—10 ppm	16	++	+++	+++	+++	+++	+++					
	17	++	+++	+++	+++	+++	+++					
	18	++	+++	+++	+++	+++	+++					
	19	++	++	++	+	++	++					
	20	++	++	++	+	+	+					

T: Achromycin
K: Kanamycin
Sign:
+++ severe symptoms
++ moderate symptoms
+ slight symptoms
± doubtful symptoms
× death
Treatment: Spraying made to foliage at intervals of 2–3 days from March 6 to April 2.

① Date of observation ② Days after either beginning or ending of treatment ③ Seedling No.

Table 2. Change of the symptoms by root dipping in antibiotics 10 ppm solution

	③ \ ①	11/III	13/III	17/III	19/III	21/III	24/III	26/III	29/III	31/III	2/IV
	③ \ ②	—	2	6	8	10	13	15	18	20	22
K—a		+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
K—b		+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
K—c		+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
T—a		+++	++	++	±	−	−	−	±	+	++
T—b		+++	++	+	−	−	−	−	+	+	++
T—c		+++	++	+	−	−	−	−	−	±	+

K: Kanamycin T: Achromycin ① Date of observation ② Days after treatment ③ Seedling No.

dwarf disease is susceptible to tetracycline antibiotics, and this phenomenon seems to be a strong evidence for the new theory that Mycoplasma-like organisms are the causal agents of the disease.

Mycoplasma-like organisms discovered in other cases of plant diseases

Being encouraged by the above-mentioned discovery in dwarf-diseased mulberry trees,

Doi *et al.* went on with their studies on the yellows and witches' broom group of plant diseases, which had been thought to be virus diseases but their pathogenic viruses had not yet been found, and discovered the presence of Mycoplasma-like organisms in such cases as potato witches' broom, aster yellows and paulownia witches' broom.[1]

Active studies have been carried out since for various plant diseases not only in Japan but also in foreign countries, and these microorganisms have been successively found for nearly 40 kinds of plant diseases, as the rice yellow dwarf disease in Japan and Philippines, the sugarcane white leaf disease in Formosa,[6] the corn stunt in America and the phyllody in France.[5]

These diseases are all transmitted by leafhoppers with a few exceptions, and Mycoplasma-like organisms have been confirmed to be present in *Nephotettix cincticeps*, the vector of the yellow dwarf disease, and in *Macrosteles fascifrons*,[4] the vector of the corn stunt, etc.

The plant diseases for which Mycoplasma-like organisms have been discovered are noticed in common to show the symptom of yellows and witches' broom, which can be improved by the application of tetracycline antibiotics.

Problems requiring solution

Whether the microorganism-like particles found in dwarf-diseased mulberry saplings and in the vectors are the ones belonging to the group of Mycoplasma or not is a problem to be solved finally by the confirmation of their pathogenicity. Though the culture of these particles in an artificial medium has not yet been successful to prove that this disease is caused by Mycoplasma, it may safely be said that the pathogen is a kind of microorganisms closely related to Mycoplasma on the basis of the evidences obtained until now.

The preventive measures for the mulberry dwarf disease now in practice are the elimination of diseased trees in the early stage and the extermination of the insect vectors. And whether or not the application of antibiotics is useful as a practical measure for the control of this disease is an important question to be settled by further studies.

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