

マイコプラズマ様微生物(MLO)によるケケンポナシ(*Hovenia tomentosa*)てんぐ巣病(新称)

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 論文

Hovenia (*Hovenia tomentosa*) Yellows, Caused by Mycoplasma-like Organisms (MLOs) in Japan

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KUSUNOKI, Manabu, OKUDAIRA, Torao, KOBAYASHI, Motowo and HIRUKI, Chuji: **Hovenia (*Hovenia tomentosa*) yellows, caused by Mycoplasma-like organisms (MLOs) in Japan. J. Jpn. For. Soc. 76: 78~83, 1994** Hovenia yellows, a disease of *Hovenia tomentosa* (MAKINO) MAKINO caused by mycoplasma-like organisms (MLOs), was found to be widely distributed in north-eastern area of Aichi prefecture. The infected *Hovenia* trees showed a witches'-broom symptom with narrow yellow leaves and stunted internodes. Decline and dieback symptoms were also common. Abnormal fluorescence and pleomorphic MLOs were observed in phloem elements of diseased trees. Hovenia yellows MLOs were transmitted to healthy seedlings by grafting to reproduce symptoms identical with those of natural infection.

楠木 学・奥平虎雄・小林元男・比留木忠治：マイコプラズマ様微生物（MLO）によるケケンボナシ（*Hovenia tomentosa*）てんぐ巢病（新称）日林誌 76：78~83, 1994 愛知県の東北部に広く分布するケケンボナシの病気が見つかった。罹病したケケンボナシは節間が短縮し、黄化、小型化した葉を叢生し、典型的なてんぐ巢病徴を現した。罹病した樹はやがて衰弱枯死した。罹病木の葉の葉柄や葉脈では篩管内に異常な蛍光が観察され、また電子顕微鏡観察により篩管内に多数の MLO が観察された。MLO は接ぎ木により健全な実生苗に伝搬され、野外と同じ病徴が再現された。

I. Introduction

Hovenia (*Hovenia tomentosa* (MAKINO) MAKINO) a common deciduous broad-leaved tree belonging to Rhamnaceae, is widely distributed in Honshu and Shikoku islands in Japan. Although the species is not useful as a lumber tree, it is important as a major species together with other species, such as *Euptelea polyandra* SIEB. et ZUCC. and *Cornus controversa* HEMSLEY, in natural forests in the area. In the Houraiji prefectural forest park in Minamishitara-gun, Aichi prefecture, many *Hovenia* trees grow more than 15 m in height and 20~30 cm in diameter at breast height. Among these *Hovenia* trees, a new disease was found to show witches'-broom symptom of branch or twig and later resulted in dieback. Even mildly affected trees finally succumbed to MLO infection 3~4 years later.

In this paper, we report the symptoms, distribution and causal agent of Hovenia yellows, mycoplasma-like organisms (MLOs).

II. Materials and Methods

1. Plant materials

The *Hovenia* trees showing typical yellows symptoms were found for the first time in the Houraiji prefectural forest park in 1990. Plant materials for transmission electron microscopy (TEM) and light fluorescence microscopy were sampled from naturally infected trees or from transplanted diseased trees in Kyushu Research Center, Forestry and Forest Products Research Institute (FFPRI), Kumamoto, Japan.

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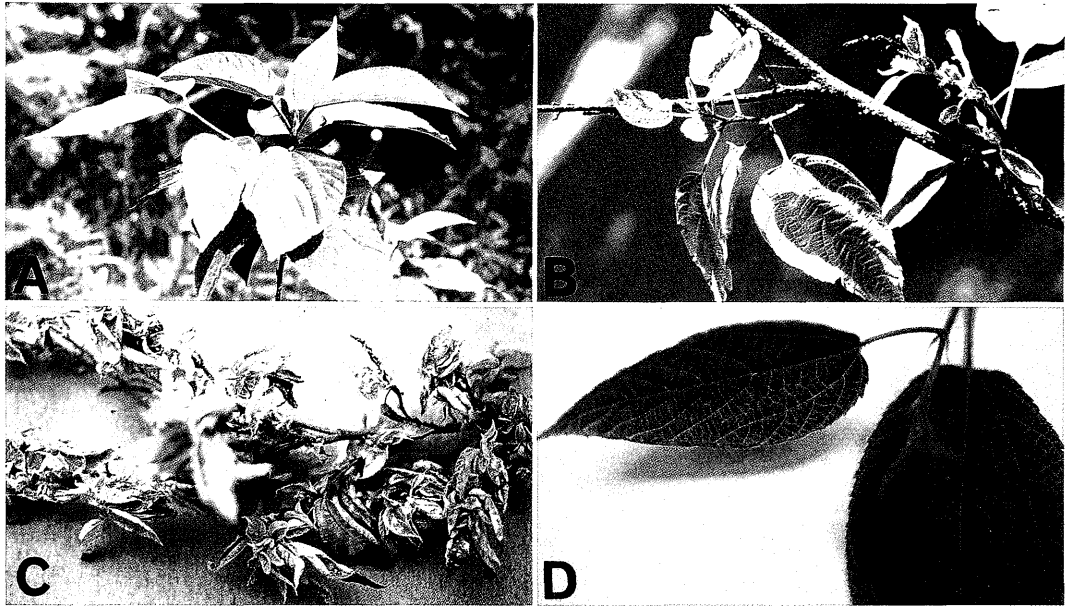


Fig. 1. Photographs of healthy and yellows-infected *Hovenia tomentosa*

A, Healthy *Hovenia tomentosa*; B, Yellows symptoms of *Hovenia tomentosa*; C, Witches'-broom symptoms of a severely affected *Hovenia tomentosa*; D, Vein-clearing symptoms of *Hovenia* which developed after grafting.

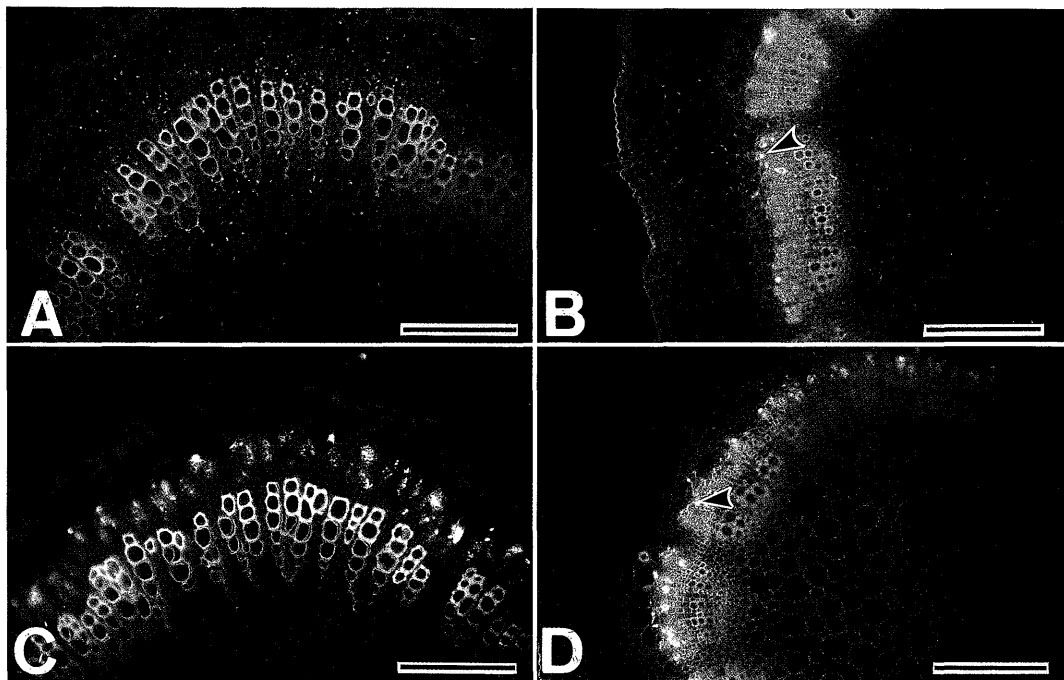


Fig. 2. Fluorescence micrographs showing normal and abnormal fluorescence

A, A healthy stem section stained with DAPI. Bar represents 200 μm ; B, A *Hovenia* yellows-infected stem section, stained with DAPI; C, A healthy stem section stained with aniline blue; D, A *Hovenia* yellows-infected stem section, stained with aniline blue.

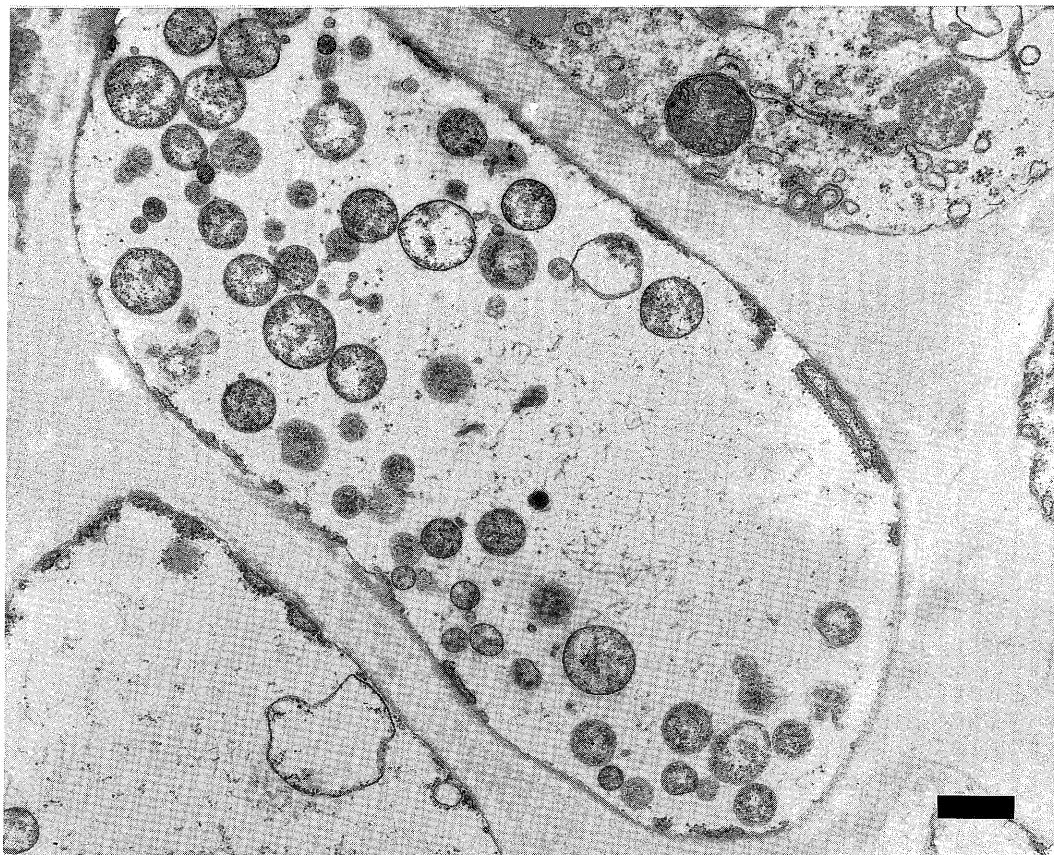


Fig. 3. Electron micrograph showing mycoplasma-like organisms in phloem elements
Bar represents 500 nm.

2. Histochemistry

Transverse sections of stems, petioles and mid-veins of leaves were cut about 50 μm thick using razor blades. Sections were fixed in 5% glutaraldehyde for 2 h (in 0.1 M phosphate buffer pH 7.0). After rinsing, these sections were stained in a solution (1 $\mu\text{g}/\text{ml}$) of 4',6-diamino-2-phenylindole 2HCl (DAPI) or in a 0.01% aniline blue solution (in 1/15 M K_2HPO_4) (3). Sections were mounted in a drop of DAPI or aniline blue solution and observed under an Olympus reflected fluorescence microscope.

3. Electron microscopy

Plant materials for a TEM observation, small pieces (about. 2×15 mm) of leaf materials including a main vein and side veins were cut from diseased trees showing the yellows symptoms. The tissue samples were pre-fixed with 5% glutaraldehyde in 0.1 M phosphate buffer pH 7.0 and followed by post-fixation with 1% osmic acid in Dalton's solution pH 7.2 at 0°C for 2 h. After dehydration in a graded ethanol series, these pieces were embedded in Quetol resin. Ultrathin sections were cut with a diamond knife on a Reichert ultramicrotome (Ultra E). Sections were stained with uranyl acetate and lead citrate and examined under an electron microscope (Hitachi H7000) at 75 kV.

III. Results

1. Symptomatology

In the early stage of infection, MLO-affected *Hovenia* showed vein clearing symptoms on young expanded leaves (Fig. 1D). Infected trees continued to produce abnormal branching and finally showed typical yellows

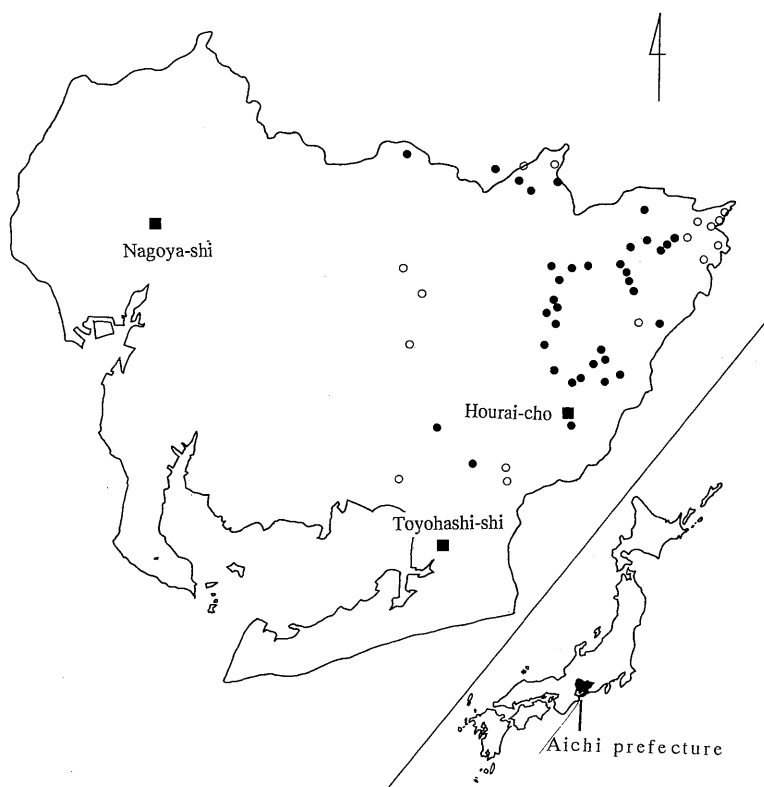


Fig. 4. Distribution of *Hovenia* yellows in Aichi prefecture

■, City or town; ●, The location of the MLO-diseased trees; ○, The location of healthy trees.

symptoms with stunted internodes and narrow leaves (Fig. 1B, C). Branches showing witches'-broom symptom died within a few months. Infected trees showed systemic symptoms 1~2 years after the primary infection and finally died about 3~4 years later. The occurrence of the yellows disease was found in 1990 for the first time, among dead trees and severely affected dying trees.

2. Histochemistry

After staining with DAPI, stem sections taken from healthy *Hovenia* showed autofluorescence of the xylem wall, but no abnormal fluorescence was observed in phloem elements (Fig. 2A). Samples taken from MLO-infected stems showed bright blue fluorescence in the external phloem elements (Fig. 2B). After staining with aniline blue, stem sections taken from both healthy and infected *Hovenia* showed bright yellow fluorescence in outer layer of xylem, but the intensity of fluorescence of the infected stem (Fig. 2D) was much brighter than that of healthy stems (Fig. 2C). When the results of fluorescence observation were compared with those of TEM observation using fourteen healthy and diseased samples, MLOs were observed in each sample showing abnormal fluorescence.

With regard to the distribution of fluorescence in the stem tissues after grafting of a MLO-infected *Hovenia* twig to a healthy stocks, samples from the grafted seedling showed abnormal fluorescence in the newly developed shoots about one month after the grafting.

3. Electron microscopy

Numerous MLOs were found in the sieve elements of the sections prepared from trees with yellows symptoms (Fig. 3), but not from healthy trees. The MLOs observed were devoid of cell wall, enclosed by a unit membrane and had thread-like nucleic acid inside the pleomorphic body measuring 200 to 800 nm in diameter.

4. Distribution

Distribution of the yellows disease of *Hovenia* in Aichi prefecture was investigated. As shown in Fig. 4, the disease is widely distributed in north-eastern area of the prefecture.

5. Graft transmission

Symptomless *Hovenia* seedlings were collected at Hourai-cho, transplanted into pots and kept in a glass house at Kyushu Research Centre of FFPRI. After an observation period of about six months, symptomless seedlings were used as stocks to be grafted with MLO-infected twigs. About one month after grafting, vein-clearing symptoms were observed on newly developed leaves on the stocks. Typical witches'-broom symptoms developed two to three months after grafting, in association with abnormal twigs with yellow narrow leaves. When young *Hovenia* seedlings with a stem diameter of about 1 cm were used, all the graft-inoculated seedlings died by the autumn of the year. Since this disease was newly recognized in Japan, it was named *Hovenia* yellows.

IV. Discussion

While more than fifty MLO diseases of crops and ornamental flowers have been described in Japan (2, 4, 11, 12), only six tree-MLO diseases have been reported (2, 6). Furthermore, for the former, host range and insect-vector have been investigated (4, 11, 12), but with the latter, vector relationship have been investigated only for mulberry dwarf (9), paulownia witches'-broom (13) and jujube witches'-broom (10). Many host trees may possibly serve as an overwintering host for the pathogen. In order to understand the transmission ecology of MLOs, a study of tree MLO diseases should not be neglected. Since we have started a study on vectors and host range of *Hovenia* yellows, it is of great interest to relate our results to other currently important MLO diseases of agricultural and horticultural crops in the area (5). In MLO disease research, because of current inability to culture MLO on artificial media, new techniques of antiserum (1, 8) and RNA (DNA) probes (7) have been adopted in addition to morphological comparison and host range studies. In Japan, several MLO diseases were separated into three groups based on PCR amplification of 16 S rRNA of MLO (7). If these studies were completed to include many other MLO diseases, our understanding of the relationship among plant pathogenic MLOs would be greatly improved.

Although the involvement of plant pathogens is little known in "decline" of pioneer trees, MLOs are associated with at least two pioneer trees, *Rhus javanica* L. (KUSUNOKI, unpublished data) and *Hovenia tomentosa* (MAKINO) MAKINO. During the development of climax forest, it is conceivable that many pioneer trees die due to the impact of attack by insects, decay fungi and other organisms. In this investigation, decline of *Hovenia* due to MLO infection was demonstrated. Our study indicates that more attention should be focused on MLO as an important pathogenic agent in order to understand mechanisms of forest tree succession.

Literature cited

- (1) da ROCHA, A., OHKI, S. T. and HIRUKI, C. (1986) Detection of mycoplasma-like organisms *in situ* by indirect immunofluorescence microscopy. *Phytopathology* **76**: 864~868.
- (2) DOI, Y., TERANAKA, M., YORA, K. and ASUYAMA, H. (1967) Mycoplasma- or PLT-group-like microorganisms found in the phloem elements infected with mulberry dwarf, potato witches'-broom, aster yellows, or paulownia witches'-broom. *Ann. Phytopathol. Soc. Jpn.* **33**: 256~266.
- (3) HIRUKI, C. (1986) Little-leaf disease of *Brugamansia candida*, a new disease associated with Mycoplasma-like organisms. *Ann. Phytopathol. Soc. Jpn.* **52**: 675~682.
- (4) KATO, S. (1993) Recent occurrence of mycoplasma diseases of flowers. *Plant Protect. (Tokyo)* **47**: 59~63.
- (5) KATO, S. and IWANAMI, S. (1988) Eggplant stunt, a newly occurred disease in Aichi Prefecture and its' host range. *Ann. Phytopathol. Soc. Jpn.* **54**: 212. (Abstract)
- (6) LEE, J. T., YAMASHITA, S., DOI, Y. and YORA, K. (1983) Virus-disease survey of medicinal plants. 3. Virus diseases of *Aquilegia flabellata*, *Adonis amurensis* and MLO diseases of *Rhus javanica*, *Aralia elata* and *Cnidium officinale*. *Ann. Phytopathol. Soc. Jpn.* **49**: 83. (Abstract)
- (7) NAMBA, S. (1993) Detection and differentiation of plant-pathogenic mycoplasma-like organisms (MLO) using PCR amplification of 16 S rRNA genes. *Plant Protect. (Tokyo)* **47**: 86~93.

- (8) NAYAR, R. (1981) Inter-relations between MLO in spiked sandal and infected collateral hosts. *Eur. J. For. Pathol.* **11**: 29~33.
- (9) SAKAI, T. (1937) Leafhopper (*Eutettix discigutius* WALKER) transmission of mulberry dwarf disease. *Bull. Nagano Seri. Exp. Stan.* **39**: 1~14.
- (10) SENBOKU, T. (1983) Leafhopper (*Hishimonus sellatus* UHLER) transmission of mulberry dwarf-MLO to jujube plants. *Ann. Phytopathol. Soc. Jpn.* **49**: 425~426.
- (11) SHIOMI, T. and SUGIURA, M. (1984) Grouping of mycoplasma-like organisms transmitted by the leafhopper, *Macrostes orientalis* VIRVASTE, based on host range. *Ann. Phytopathol. Soc. Jpn.* **50**: 149~157.
- (12) SHIOMI, T. and SUGIURA, M. (1984) Difference among *Macrostes orientalis*-transmitted MLO, potato purple-top wilt in Japan and aster yellows MLO from USA. *Ann. Phytopathol. Soc. Jpn.* **50**: 455~460.
- (13) SHIOZAWA, H. (1986) Transmission of paulownia witches' broom by stink bugs, *Halyomorpha mista* UHLER. *Bull. Inst. Jpn. Plant Prot. Assoc.* **4**: 45~50.

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