—whether they occur during meiosis, fertilization, or the preimplantation cleavage stages which follow—a study of them in the hamster should produce greater success than in other small animal models.

MASAHARU YAMAMOTO*
Epidemiology Study Center, Framingham, Massachusetts 01701
THEODORE H. INGALLS
Department of Community Medicine, Boston University School of Medicine, Boston, Massachusetts 02118, and *Epidemiology Study Center, Framingham, Massachusetts 01701*

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* Present address: Department of Hygiene and Preventive Medicine, Niigata University Medical School, Niigata City, Japan.

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Helical Filaments Produced by a Mycoplasma-Like Organism Associated with Corn Stunt Disease

Abstract. *Mycoplasma-like bodies with helical filaments were seen by phase contrast microscopy in juice expressed from tissues of plants infected with corn stunt agent. Each filament is bounded by a "unit membrane," and no cell wall, sheath, envelope, or second membrane has yet been discerned by electron microscopy. The association of these filaments with development of disease, their occurrence in phloem cells as seen by both freeze-etching and thin-section electron microscopy, the diagnosis of infection based on their presence in plants without symptoms, and their absence in noninfected corn are consistent with the hypothesis that these unusual filaments are formed by the mycoplasma-like organism presumed to be the corn stunt agent.*

The causal agents of the plant yellows diseases had been thought to be viruses for over 40 years prior to 1967, but now evidence indicates that these agents may be mycoplasmas (*1*, *2*). Among the morphological forms so far described for the presumed mycoplasma-like yellows agents, none are unique to these organisms. The mycoplasma-like bodies in yellows-diseased hosts are morphologically and ultrastructurally similar in every previously reported respect to members of the class Mollicutes (*3*, *4*). The present report, however, describes helical filaments produced by a mycoplasma-like organism in plants with corn stunt (CS) disease. Filaments with this morphology are previously undescribed for the organisms in plant yellows infections, are previously unreported for the described mycoplasmas, and to our knowledge are unknown among cell wall–deficient organisms of any type. A preliminary announcement of these findings has been made (*5*).

The mycoplasma-like helical filaments were first found in 1970 during the course of examining, by phase contrast microscopy under oil immersion (×1000), extracts from stems of corn (*Zea mays* L.) plants infected with the Rio Grande strain of corn stunt agent. In subsequent work, the filaments were found in juice expressed from leaves, tassels, or roots of more than 200 infected plants but have not been found in any of over 150 CS-free plants so far examined.

Under phase contrast, the helical filaments could readily be seen, although their very small width and Brownian motion made photography very difficult. By the constant motion of the filaments, however, and the consequent presentation of different views of the same filament to the eye, the helical morphology of the filaments was readily apparent. The filaments (Fig. 1A) typically were about 3 to 12 μm in length and roughly 250 nm in diameter. The amplitude of the spirals was usually about 0.4 μm. Spherical bodies, 400 to 600 nm in diameter, were often attached to the filaments.

In several tests, we examined juice expressed from various plant parts to determine the association of the helical filaments with CS disease development. Helical filaments first appeared in roots and later in the youngest leaves, where first symptoms of CS also appeared, and could be found in juice from some plants as early as 1 week before symptoms appeared. In some plants containing helical filaments, however, symptoms of CS failed to appear by 8 to 10 weeks after inoculation. By this time the plants had developed tassels and ears. The evidence that the helical filaments are diagnostic for CS infection suggests that such plants were subclinically infected. In plants with symptoms, numbers of helical filaments in leaves were directly correlated with age of infection and increasing severity of CS symptoms.

To gain information on the ultrastructure of the helical filaments, we turned to electron microscopy. Negative contrast [phosphotungstic acid (PTA) 2 percent, *p*H 7.0] of juice expressed from CS-infected plants characteristically revealed filamentous structures of even caliber and often attached

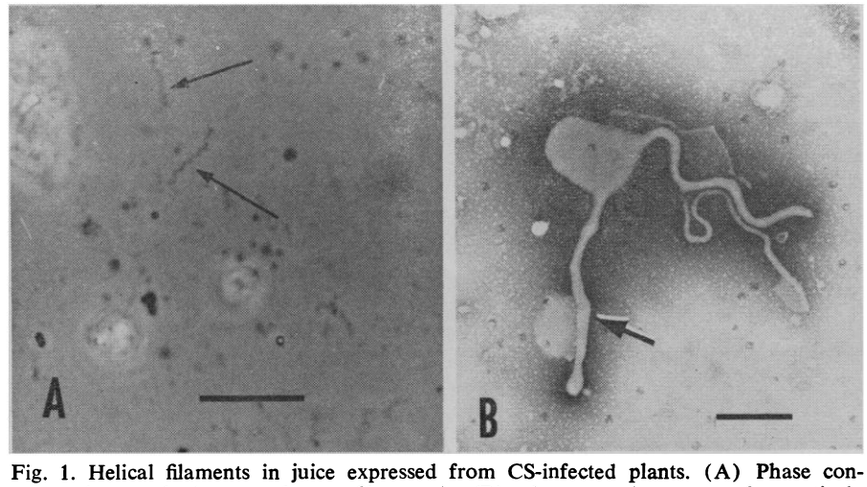

Fig. 1. Helical filaments in juice expressed from CS-infected plants. (A) Phase contrast micrograph (arrows denote filaments); (B) electron microgram of negatively contrasted mycoplasma-like body and attached filaments. Arrow denotes a filament lacking suggestion of helical shape. Bar equals 5 μm in (A) and 2 μm in (B).

to spherical bodies (Fig. 1B). Except for indications of helical morphology of many filaments, they resemble negatively stained cells (*6*) of the Mollicutes. Filaments lacking definite spiral shape (for example, arrow in Fig. 1B) may have been distorted by the negative contrast procedure.

Although the filaments we describe were easily recognizable by phase contrast and by negative staining, it seemed necessary to eliminate the possibility that the helical morphology seen by these methods was artifactual. For this reason, we employed thin-sectioning and freeze-etching methods.

In ultrathin sections of CS-infected plants, only short curved portions of filaments could be seen (Fig. 2, A and B). We propose that many of these curved bodies may be small portions of longer filaments with helical morphology. Sections interpreted as possibly revealing several turns of a filament helix (for example, arrow in Fig. 2A) strengthen this view. The filaments were predominantly about 200 nm in width, contained granules interpreted to be ribosomes and strands presumed to be nucleic acid, and were bounded only by a single triple-layered unit membrane about 100 Å thick. We are continuing to search, but our data so far give no suggestion of a second membrane, wall, envelope, or sheath, in addition to the limiting membrane, that might account for the helical shape.

Freeze-etching (*7*) revealed the three-dimensional and surface aspects of the helical filaments in situ (Fig. 2, C and D). The spiral morphology of the filaments is apparent and branching, of a type seen in mycoplasmas but unknown among the spiral bacteria, is evident close to the attached round body (Fig. 2C). Particles on the surface of the membrane resemble

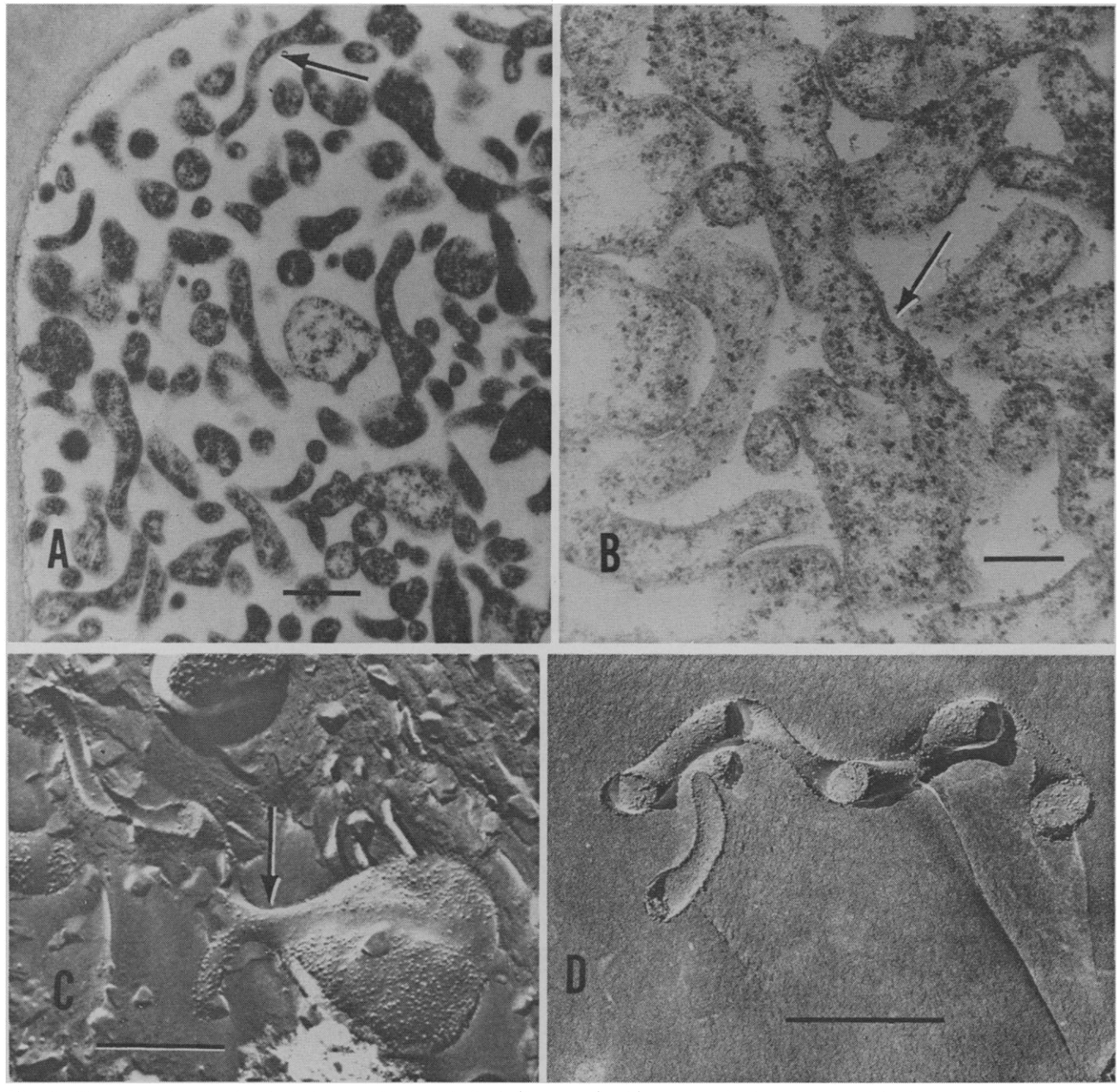

Fig. 2. Electron micrographs depicting portions of helical filaments in thin-sectioned (A and B) and in freeze-etched (C and D) phloem cells from CS-infected plants. (A) Possible suggestion of helical filament (arrow); (B) unit membrane (arrow); (C) point of branching (arrow) of helical filament. Bar equals 1 μm in (A) and 0.5 μm in (B), (C), and (D).

those seen on membranes of freeze-etched species of known mycoplasmas (*8*).

We do not yet know how widespread helical filaments are among organisms associated with other yellows diseases. Careful examination of published work, however, reveals a possible hint of helical filaments in thin-section electron micrographs of citrus with stubborn disease (*9*).

The findings so far obtained indicate that the helical filaments described here are associated with CS disease development in plants in a manner highly consistent with expectations for the CS agent itself. Preliminary data also suggest that tetracycline antibiotics interfere with development of CS symptoms and with formation of the helical filaments in inoculated plants. Moreover, the helical filaments coincide in internal ultrastructural detail and in distribution in CS-infected tissues with the mycoplasma-like bodies previously described by others (*10*) as the probable CS agent. We recognize that our findings are indirect and that alternative explanations for our data cannot be discarded, but the agreement of results from the several lines of investigation lead us to suggest that the helical filaments described here may derive from the CS agent itself.

ROBERT E. DAVIS
JOSEPH F. WORLEY
ROBERT F. WHITCOMB
TAKASHI ISHIJIMA
RUSSELL L. STEERE

Plant Science Research Division, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland 20705

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Reactivated Triton-Extracted Models of Paramecium: Modification of Ciliary Movement by Calcium Ions

Abstract. *Triton-extracted models of* Paramecium *were reactivated to swim in solutions of adenosine triphosphate and magnesium ions. The cilia beat in the normal direction (toward the rear) when the calcium ion concentration was less than 10^{-6}M, and they beat in the "reversed" direction (toward the front) when calcium ion concentration was raised above 10^{-6}M. These results support the proposal that ciliary reversal, hence backward swimming, of live paramecia is mediated by an increased cytoplasmic calcium concentration around the ciliary system by calcium-dependent membrane responses to external stimuli.*

The locomotor behavior of *Paramecium* is primarily dependent on ciliary "reversal"—the change in direction of the ciliary power stroke—which causes a reversal of locomotion in response to external stimuli (*1*). *Paramecium* produces a graded, regenerative depolarization in response to an applied outward current or a mechanical or chemical stimulation of the anterior membrane. This is always followed closely in time by ciliary reversal (*2*). The regenerative response is mediated by an influx of Ca^{2+} caused by a temporary increase in the membrane conductance to Ca^{2+} (*3*). Ciliary reversal is also closely associated with the liberation of Ca^{2+} bound to the cell membrane (*4*). Therefore, it has been proposed that an increase of cytoplasmic concentration of Ca^{2+} around the cilia might be responsible for ciliary reversal (*4*, *5*).

To verify the hypothesis, we examined the effects of Ca^{2+}, and of Mg^{2+} and adenosine triphosphate (ATP), on the cilia of *Paramecium* specimens that had been extracted with detergent; in these models, the ciliary apparatus is expected to remain functional but the cell membrane is disrupted. When the model was in a mixture of ATP and Mg^{2+}, ciliary beating was in the normal direction (toward the rear), which caused the model to swim forward. When Ca^{2+} was added, the ciliary power stroke was reoriented to the front which caused the model to swim backward.

Specimens of *Paramecium caudatum* (mating type I, syngen 1) reared in hay infusion were washed in a solution of 2 m*M* $CaCl_2$ and 1 m*M* tris(hydroxymethyl)aminomethane (tris) hydrochloride (*p*H 7.2) and were then centrifuged to a loose pellet. The pellet was suspended in extraction medium (0° to 1°C), which contained 0.01 percent (by volume) Triton X-100 (*6*), 20 m*M* KCl, 10 m*M* of the tripotassium salt of ethylenediaminetetraacetic acid (EDTA) (Wako Pure Chemical Co., Tokyo), and 10 m*M* tris-maleate buffer adjusted to *p*H 7.0 with NaOH. After 30 minutes of extraction (*7*, *8*) the specimens were washed to remove Triton and EDTA with a solution (0° to 1°C) of 50 m*M* KCl and 10 m*M* tris-maleate buffer adjusted to *p*H 7.0 with NaOH. Specimens were kept in this solution for at least 30 minutes before being tested.

About 10^{-4} ml of the KCl solution, containing 100 to 500 models, was pipetted into about 1 ml of test solution, which contained test substances and 50 m*M* KCl, and was 19° to 21°C. A few seconds after the models were transferred into medium with ATP and

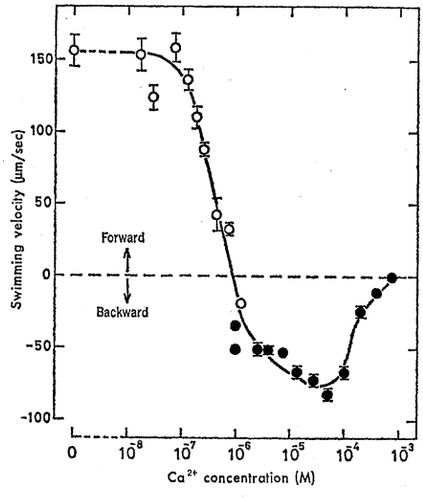

Fig. 1. Effect of Ca^{2+} concentration on both swimming velocity and direction of models of *Paramecium* reactivated by ATP and Mg^{2+}. The graph gives means and standard errors for measurements on 100 to 500 specimens. Concentrations of Ca^{2+} were adjusted by Ca^{2+} buffers in the range between 10^{-8} and 10^{-6}*M* (open circles) and by simple addition of $CaCl_2$ in the range above 10^{-6}*M* (solid circles). Although each Ca^{2+} concentration greater than 10^{-6}*M* is the sum of Ca^{2+} contaminants (*12*) plus Ca^{2+} added to the media, other factors (for instance, formation of complexes between Ca^{2+} and ATP) undoubtedly affect the free Ca^{2+} concentration. Therefore, the concentrations shown are approximate.