Special Lecture

MOLLICUTES AND MYCOPLASMA-LIKE AGENTS OF PLANT AND ANIMAL DISEASES*

by KARL MARAMOROSCH, *Waksman Institute of Microbiology, Rutgers University, New Brunswich, N. J. 08903, U.S.A.*

There are only a few animal diseases of known mycoplasma etiology. Human "atypical virus pneumonia" and the mycoplasma diseases of domestic animals are characterized by low pathogenicity of their causative mycoplasmas and are effectively controlled by tetracyclines and tylosine. In contrast, nearly 70 different, often highly destructive, plant diseases caused by mycoplasma-like agents have been reported since 1967. The morphological resemblance with mycoplasmas of the microorganisms, detected in cross sections of phloem elements, as well as in insect vector tissues, their disappearance upon tetracycline treatment, temporary recovery of treated plants, and interruption of insect transmission provided evidence for a mycoplasma-like etiology. The various groups of the Mollicutes cannot be distinguished morphologically since the genera *Mycoplasma*, *Acholeplasma* and *Thermoplasma* require culture techniques and serology for proper characterization. It now appears that some of the plant disease agents belong to a distinct group within the Mollicutes, the *Spiroplasma*. So far only one member of this group has been established in culture and characterized serologically, deposited in a type culture collection, and made available to animal mycoplasma workers for comparison with known Mollicutes. The economic importance of plant mycoplasma-like agents is evident from the list of diseases affecting rice and other cereals, maize, potato, coconut palm, sandal, citrus, willow, and many others. Temporary recovery of diseased plants treated with tetracyclines provides hope for developing chemical treatments that will result in an effective cure. Future possible cures might employ heat therapy, already proven effective in some diseases, and the use of mycoplasma-destroying viruses.

INTRODUCTION

There are only a few animal diseases of known mycoplasma etiology. Human "atypical virus pneumonia" and the mycoplasma diseases of domestic animals are characterized by low pathogenicity of their causative mycoplasmas and are effectively controlled by tetracyclines and tylosine. In contrast, nearly 70 different, often highly destructive plant diseases, caused by mycoplasma-like organisms (MLO) have been reported since 1967. I would like to focus attention upon the difficulties in defining these MLO agents of plants and insects.

HISTORICAL

Before 1967 most MLO were grouped together with viruses and known as yellows-type and witches' broom viruses. Although the original report of Doi *et al.* (1967)

*Supported in part by NSF research grant No. GB 29280 and a travel grant from the National Science Foundation, Washington, D.C.

left the identification open and specified that the MLO might belong to either of several groups, such as the Mycoplasmataceae, the Bedsonia, or the Chlamydia, subsequent descriptions from other laboratories sometimes used the term "plant mycoplasma," or "mycoplasma-like agents" indiscriminately for all MLO. The confusion was supported by several reports on the cultivation of MLO on cholesterol-containing media. However, these reports could not be verified (Maramorosch 1972*a*). It was established that cultured species of Mycoplasma and Acholeplasma from non-plant origin, taken up by roots, can be isolated from leaves of living plants (McIntosh and Maramorosch 1973). Negative (phosphotungstic acid) staining was occasionally used to characterize MLO in extracts from diseased plants and insect vectors but the distinction between artifacts and MLO was recognized as a serious pitfall of this method (Wolanski and Maramorosch 1970).

Antibiotic Remission

Temporary remission of MLO-caused plant diseases by application of antibiotics, first reported by Ishiie *et al.* (1967), has been confirmed in many laboratories. It was demonstrated that tetracycline antibiotics applied to the roots or wounded leaves of plants are taken up and retained in healthy as well as in diseased plants (Frederick *et al.* 1971; Sinha and Peterson 1972; Raychaudhuri *et al.* 1972; Nariani *et al.* 1971). In a few instances the disappearance of MLO, following antibiotic application, was studied by electron microscopy (Ishiie *et al.* 1967; Maramorosch *et al.* 1972; Sinha and Peterson 1972). Morphologically MLO destruction by antibiotics can be distinguished from natural degeneration (Hirumi and Maramorosch 1972). No permanent cure of a yellows disease has yet been achieved by tetracycline application, probably because these antibiotics are inhibitory rather than lethal. Heat treatments known to affect several yellows-type diseases may destroy the MLO permanently. Tetracyclines were found most effective when applied via plant roots in hydroponic cultures, and least effective when sprayed onto leaves or poured on the soil (Klein *et al.* 1970, 1972).

The Nature of MLO

The MLO share with viruses many features, such as interference of related strains in plants and in insect vectors, graft transmissibility, dodder tansmissibility, vectors belonging to the same families and sometimes even the same species, and to a certain extent cytopathic effects on vectors. The disease expression or, as plant pathologists term it, disease symptoms caused by MLO and by viruses are often similar, though not identical. Electron microscopy and antibiotic response help in distinguishing between MLO and viruses.

The superficial resemblance of sectioned MLO to mycoplasmas, the response to tetracycline treatment *in vivo*, resulting in temporary remission of diseases, and the interruption of insect transmission by antibiotics strengthened the assumption that all MLO were either mycoplasmas, or that they were "mycoplasma-like" organisms. Although considerable progress has been achieved during the past five years and the methods of study have become more accurate, the MLO are still a poorly

defined, heterogenous group of disease agents, with but one exception. The difficulties encountered in their isolation and cultivation on artificial media have not yet been overcome. If some of the MLO should eventually prove to be *Chlamydia* or *Rickettsiae* as suggested by Davis and Whitcomb (1971), their failure to grow on artificial media could easily be explained.

Taxonomy of MLO

Members of the class Mollicutes, to which the family Mycoplasmataceae belongs, are organisms devoid of a cell wall and incapable of synthesizing the mucopeptide polymer and its precursors (Freundt 1973). At present, two families have been recognized in the class Mollicutes : Mycoplasmataceae and Acholeplasmataceae. The requirement of sterols by the Mycoplasmataceae, but not by the Acholeplasmataceae, distinguishes the two groups. Various species of Mycoplasma and Acholeplasma have been determined as causative agents of diseases of man and warm-blooded animals, but not as plant disease agents. Additional genera have been recognized in the class Mollicutes and given names *Thermoplasma* and *Spiroplasma* (Maramorosch 1972b). The latter is the first, and so far the only representative of the Mollicutes properly isolated from plants, characterized, cultured (Fudl-Allah *et al.* 1971, 1972; Saglio *et al.* 1972), and deposited in a type culture collection (Cole *et al.* 1973; Saglio *et al.* 1973). The MLO of citrus greening seems to differ morphologically from *Spiroplasma citri*, associated with citrus stubborn disease having a 20 Å to 25 Å cell wall and resisting attempts of cultivation in the medium that supports the growth of *S. citri* (Saglio *et al.* 1972). Phase contrast permits the observation of spiral forms of *S. citri* in culture. It is also a useful means of identifying spiral forms in extracts from corn stunt-infected *Zea mays* plants (Davis *et al.* 1972; Davis and Worley 1973; Davis 1973). However, it has not been demonstrated that the spiral forms seen in plant extracts from stunted corn plants, or the spiral forms isolated and cultured from stubborn diseased citrus are pathogenic to plants. It should be remembered that the identified and cultured *S. citri* and the presumptive spiroplasma of corn stunt have not been shown to belong to the same genus. Such a determination would only be possible if the spiral forms from corn were cultured, and this has not yet been achieved. Despite the apparent progress, the study of MLO is still in its infancy.

A presumptive *Spiroplasma* was cultured from citrus little-leaf affected plants (Daniels *et al.* 1973). The disease has since been identified as identical with stubborn. The authors reported that they were able to induce an experimental disease in clover (*Trifolium repens*) plants by injecting the cultured microorganisms into *Euscelis plebejus* leafhoppers and confining the inoculated insects on clover plants. If confirmed, this might represent the first instance of a cultured, plant-pathogenic *Spiroplasma*. The cultured microorganisms have not yet been made available for comparison with *S. citri* and have not been deposited in a type culture collection, so they could not be given a Latin name.

At first, the antibiotic sensitivity of MLO seemed to provide strong support of the contention that all MLO are similar to Mycoplasmataceae, but it was soon pointed out that some of the latter are resistant to tetracyclines and that tetracycline sensitivity should not be used as a definitive criterion (Maramorosch *et al.* 1970). Recently

some MLO were found to be susceptible to penicillin treatment in diseased plants *in vivo,* and subsequent electron microscopy revealed that these MLO possessed a cell wall, and not a unit membrane (Windsor and Black 1972, 1973). It is therefore apparent that the term MLO is ill-defined, most likely comprising members of the class Mollicutes, as well as microorganisms resembling *Chlamydia* and *Rickettsia*

Morphology of MLO

The MLO observed by electron microscopy in thin sections of diseased tissues of higher plants and of insect vectors comprise many diverse forms (Maramorosch *et al.* 1968, 1970; Davis and Whitcomb 1971). Thin sections have been interpreted to indicate the presence of RNA in the form of ribosomes, and DNA in the form of strands in the nuclear area. Many, but not all, MLO are bounded by a unit membrane. This membrane has been described as approximately 8 to 10 Å in thickness, but in a few instances as approximately twice that thick (Saglio *et al.* 1972; Holmes *et al.* 19712). The MLO with thicker membranes might represent *Chlamydia*, rather than Molicutes. Evidence is accumulating that the 20 to 25 Å "membranes" are actually cell walls. In addition to MLO observed in phloem tissues of diseased plants, xylem-invading microorganisms have also been discovered (Plavsic-Banjac and Maramorosch 1972; Maramorosch *et al.* 1973; Goheen *et al.* 1973; Hopkins and Mollenhauer 1973; Hopkins *et al.* 1973). Here I would like to call attention to the original observation of Giannotti *et al.* (1970) on walled microorganisms in the phloem of certain diseased plants, probably the first instance of a rickettsia-like plant pathogen. The authors of that early report did not mention rickettsiae, but their description of the microorganisms with rippled cell walls, rather than with unit membranes, seems to be characteristic of bacteria or rickettsiae. The description was overlooked when the possibility of plant diseases caused by rickettsiae was reviewed (Davis and Whitcomb 1971).

Conclusions

Considerable opportunities exist for the study of MLO of plant diseases. The field offers a challenge to team and individuals with wide biological interests. In addition to fundamental aspects of the nature of the disease agents and the practical aspects of plant and insect diseases, future work might be directed to aspects of possible toxic effects of the MLO on warm-blooded animals, including possible effects on man. It will be of interest to ascertain whether eating infected plant material, such as yellow-infected carrots, spinach, lettuce, celery, or potatoes, can cause chronic disorders in man.

References

Cole, R. M., Tully, J. G., Popkin, T. J., and Bové, J. M. (1973). Morphology, ultrastructure, and bacteriophage infection of the helical mycoplasma-like organism (*Spiroplasma citri* gen. nov., sp. nov.) cultured from "stubborn" disease of citrus. *J. Bact.*, **115**, 367–386.

Daniels, M. J., Markham, P. G., Meddins, B. M., Plaskitt, A. K., Townsend, R., and Bar-Joseph, M. (1973). Axenic culture of a plant pathogenic spiroplasma. *Nature, Lond.*, **244**, 523–524.

Davis, R. E. (1973). Occurrence of a spiroplasma in corn stunt-infected plants in Mexico. *Pl. Dis. Rep.*, **57**, 333–337.

Davis, R. E., and Whitcomb, R. F. (1971). *Mycoplasmas, rickettsiae,* and *chlamydiae*; possible relation to yellows diseases and other disorders of plants and insects. *A. Rev. Phytopath.*, **9,** 119–154.

Davis, R. E., and Worley, J. F. (1973). Spiroplasma: motile, helical microorganism associated with corn stunt disease. *Phytopathology,* **63,** 403–408.

Davis, R. E., Worley, J. F., Whitcomb, R. F., Ishijima, T., and Steere, R. L. (1972). Helical filaments produced by a mycoplasma-like organism associated with corn stunt disease. *Science,* **176,** 521–523.

Doi, Y., Teranaka, M., Yora, K., and Asuyama, H. (1967). Mycoplasma-or PLT group-like microorganisms found in the phloem elements of plants infected with mulberry dwarf, potato witches' broom, aster yellows, or paulownia witches' broom. (*In Japanese,* Engl. abstr.) *Ann. phytopath. Soc. Jap.,* **33,** 259–266.

Frederick, R. J., Klein, M., and Maramorosch, K. (1971). Acquisition and retention of tetracycline-hydrochloride by plants. *Pl. Dis. Rep.,* **55,** 223–226.

Freundt, E. A. (1973). Principles of mycoplasma classification. *Ann. N. Y. Acad. Sci.,* **225,** 7-13.

Fudl-Allah, A. E.-S., Calavan, E. C., and Igwegbe, E. C. K. (1971). Culture of a mycoplasma-like organism associated with stubborn disease of citrus. *Phytopathology,* **61,** 1321. (*Abstr.*).

Fudl-Allah, A. E.-S., Calavan, E. C., and Igwegbe, E. C. K. (1972). Culture of a mycoplasma-like organism associated with stubborn disease of citrus. *Phytopathology,* **62,** 729–731.

Giannotti, J., Vago, C., Marchoux, G., Devauchelle, G., and Duthoit, J. L. (1970). Infection de plante par un type inhabituel de microorganisme intracellulaire, *C. r. hebd. Séanc. Acad. Sci., Paris* Ser. D, **271,** 2118–2119.

Goheen, A. C., Nyland, G., and Lowe, S. K. (1973). Association of a rickettsia-like organism with Pierce's disease of grapevines and alfalfa dwarf and heat therapy of the disease in grapevines. *Phytopathology,* **63,** 341–345.

Hirumi, H., and Maramorosch, K. (1972). Natural degeneration of mycoplasma-like bodies in an aster yellows infected host plant. *Phytopathol. Z.,* **75,** 9–26.

Holmes, F. O., Hirumi, H. and Maramorosch, K., (1972). Witches-broom of willows : Salix yellows. *Phytopathology,* **62,** 826–828.

Hopkins, D. L., French, W. J., and Mollenhauer, H. H. (1973). Association of a rickettsia-like bacterium with phony peach disease. *Phytopathology,* **63,** 443, (*Abstr.*).

Hopkins, D. L., and Mollenhauer, H. H. (1973). Rickettsia-like bacterium associated with Pierce's disease of grapes. *Science,* **179,** 298–300.

Ishiie, T., Doi, Y., Yora, K., and Asuyama, H. (1967). Suppressive effects of antibiotics of tetracycline group of symptom development of mulberry dwarf disease. (*in Japanese, Engl. abstr*). *Ann. phytopath. Soc. Jap.,* **33,** 267–275.

Klein, M., Frederick, R. J., and Maramorosch, K. (1970). Buffers affecting tetracycline chemotherapy. *Phytopathology,* **60,** 130, (*Abstr.*).

————(1972). Chemotherapy of aster yellows: tetracycline-hydrochloride uptake by healthy and diseased plants. *Phytopathology,* **62,** 111–115.

Maramorosch, K (1972*a*) The enigma of mycoplasma in plants and insects. *Phytopathology,* **62,** 1230–1231

Maramorosch, K. (1972 *b*) Mycoplasma and mycoplasma-like agents of plant and animal diseases. Abstracts of Papers, Symposium on Mycoplasmal Diseases, Chandigarh, India. *p.* 4 (*Abstr.*)

Maramorosch, K., Granados, R. R., and Hirumi, H. (1970) Mycoplasma diseases of plants and insects. *Adv. Virus Res.,* **16,** 135-193.

Maramorosch, K., Klein, M., and Wolanski, B. S. (1972). Beitrag zur Ätiologie der Hexenbesenkrankheit der Kaktee *Opuntia tuna* (=*Runa monstrosa*). *Experientia,* **28,** 362–363.

Maramorosch, K., Plavsic-Banjac, Biljana, Bird, J., and Liu, L. J. (1973). Electron microscopy of ratoon-stunted sugar cane: microorganisms in xylem. *Phytopathol. Z.,* **77,** 270–273.

Maramorosch, K., Shikata, E., and Granados, R. R. (1968). Structures resembling mycoplasma in diseased plants and in insect vectors. *Trans. N. Y. Acad. Sci.,* **30,** 841–855.

McIntosh, A H., and Maramorosch, K. (1973). Mycoplasma and Acholeplasma in plants. *Ann. N. Y. Acad. Sci., (In press).*

Nariani, T. K., Raychaudhuri, S. P., and Viswanath, S M. (1971) Response of greening pathogen. of citrus to certain tetracycline antibiotics. *Curr. Sci.*, **40**, 552

Plavsic-Banjac, B., and Maramorosch, K. (1972). Electron microscopy of the xylem of ratoon stunted sugarcane. *Phytopathology*, **62**, 498–499.

Raychaudhuri, S. P., Chenulu, V. V., Ghosh, S. K., Varma, Anupam, Rao, P. S., Srimathi, R. A., and Nag, K. C (1972) Chemical control of spike disease of sandal. *Curr. Sci.*, **41**, 72-73.

Saglio, P., Laflèche, D., L'Hospital, M. Dupont, G., and Bové, J. M. (1972). Isolation and growth of citrus mycoplasmas, p. 187–203. *In* : Pathogenic Mycoplasmas. Ciba Foundation Symposium. Associated Scientific Publishers, Amsterdam.

Saglio, P., L'Hospital, M., Laflèche, D., Dupont, G., Bové, J. M., Tully, J. G., and Freundt, E. A. (1973). Characterization of *Spiroplasma citri* gen. and sp. nov. : a new mycoplasma-like organism associated with a "stubborn" disease of citrus. *Int. J. Syst. Bact.*, **23**, 191-204.

Sinha, R. C., and Peterson, E. A. (1972). Uptake and persistence of oxytetracycline in aster plants and vector leafhoppers in relation to inhibition of clover phyllody agent. *Phytopathology*, **62**, 377–383.

Windsor, I. M., and Black, L. M. (1972). Clover club-leaf : a possible rickettsial disease of plants. *Phytopathology*, **62**, 1112. (*Abstr.*)

Windsor, I. M., and Black, L. M. (1973). Remission of symptoms of clover club leaf following treatment with penicillin. *Phytopathology*, **63**, 44–46.

Wolanski, B., and Maramorosch, K. (1970). Negatively stained mycoplasmas: fact or artifact? *Virology*, **42**, 319-327.