Egytptian Virol. 5 (1): 1-19 (2008)

The discovery of phytoplasmas: A historical reminiscence of success and failure

Numerous plant diseases earlier

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described as virus diseases are now known to be caused by phytoplasmas and spiroplasmas. Earlier attempts to concentrate, purify and visualize by electron microscopy the presumptive viruses have failed. Causative agents of yellows-type plant diseases were classified as viruses because no fungi or bacteria could be detected in diseased plants. The inadequate characterization of viruses delayed the discovery of the pathogens of yellows-type diseases by forty years. In 1967 Japanese plant pathologists and entomologists announced the discovery of mycoplasma-resembling pathogens in diseased plants and insect vectors and the temporary recovery of diseased plants treated with tetracycline antibiotics. The recognition of mycoplasma-like structures was made accidentally by a veterinarian, Kaoru Koshimizu, but no credit was given to his crucial role. The simultaneous announcement of the detection of phytoplasmas in a leafhopper vector by Japanese entomologists was not mentioned by Tokyo plant pathologists. Attempts to culture the fastidious phytoplasmas failed, while spiroplasmas have been cultured and properly characterized and classified. Several careers were made by phytoplasma and spiroplasma researchers, but some were destroyed by erroneous reports and one tragically ended through political involvement. The striking progress in the study of phytoplasmas illustrates the benefits derived from collaboration between experts working in diverse fields of science and from participation in symposia and congresses.

Key words: phytoplasma, spiroplasma, tetracycline antibiotics, yellows-type plant diseases.

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(Received March 2008)
(Accepted May 2008)

INTRODUCTION

This historical recollection of the discovery of phytoplasmas and spiroplasmas spans five decades. I shall focus here not only on the published findings but also on the participating scientists involved in the discovery of phytoplasmas and spiroplasmas. Numerous workers in laboratories around the world tried to detect the presumptive viruses of yellows-type diseases but failed, because they searched for particles that morphologically resemble known viruses of plants, animals or bacteria.

Several errors were made before the discovery of the microbial agents that resembled mycoplasmas. The race to publish results and receive recognition for the discoveries affected a number of virologists, plant pathologists and entomologists.

I shall describe here my own failure to find the microbial pathogens of aster yellows and corn stunt diseases, failed attempts to culture phytoplasmas, the incident that led to the recognition of phytoplasmas in Japan, and the successful cultivation of spiroplasmas. Pitfalls and errors that occurred in my own laboratory and elsewhere illustrate the influence of preconceived ideas and the necessity to collaborate with diverse researchers. Until the end of 1967 scientists were not aware that certain vector-borne plant pathogens, described as viruses, were actually microorganisms resembling mycoplasmas. The criteria applied to viruses were inadequate to distinguish between viruses and other filterable agents that could not be cultured in cell-free media. When electron microscopy of thin sections of diseased plants and insect vectors came into use, no virus-like particles were detected in yellows-diseased plants and none were found in purified and concentrated plant extracts. Errors made before and after 1967 by me and others demonstrated how failure to collaborate with colleagues working in different fields resulted in missed opportunities. The conclusion of my historical presentation is the concept that virologists should participate in conferences and symposia of scientists working in other fields, and plant and bacteriophage workers should participate in meetings of animal/human virologists. Such conferences provide a unique opportunity to learn about new research findings, techniques and

approaches.

Missed opportunities

In 1924 L. O. Kunkel (Fig.1) solved the mystery of the aster yellows disease, when he found that the causative agent of this disease was transmitted to plants by a leafhopper vector **(Kunkel, 1926).** According to the prevailing criteria of viruses, the agent of aster yellows was a virus. No fungi or bacteria were found in diseased plants. Kunkel suspected that the aster yellows virus multiplied in leafhopper vectors and this assumption was confirmed by my serial passage technique, using needle inoculation (Fig.2) of the leafhopper vector *Macrosteles fascifrons* **(Maramorosch, 1952).**

In 1957 I was spending the summer at the Cold Spring Harbor Laboratory on Long Island, New York. There the plant geneticist, Barbara McClintock (Fig.3), who 30 years later received the Nobel Prize for her discovery of "jumping genes", permitted me to use her greenhouses for keeping leafhopper vectors of aster yellows and corn stunt. The leafhoppers were already known as alternate hosts of the causative agents of the two plant diseases. A few years earlier, at the Brooklyn Botanic Garden and at Rockefeller University, I perfected the needle inoculation of leafhoppers with tiny amounts of plant or vector extracts, rendering the insects infective after an intrinsic incubation period **(Maramorosch, 1951 and Maramorosch, 1956).** Now I prepared extracts from diseased plants and from vector leafhoppers, adding measured amounts of penicillin, streptomycin, and tetracycline. It was well known that these antibiotics had no effect on viruses and I was convinced, therefore, that the results of my tests would confirm the well-known principle.

Insect vectors that received small doses of penicillin or streptomycin transmitted the infectious agents just as the control insects that received no antibiotics. However, leafhoppers injected with extracts containing tetracycline failed to transmit corn stunt and aster yellows. I was convinced that these results were meaningless. Everybody knew that tetracyclines had no effect on viruses and I concluded that the failure to transmit was, most likely, caused by the high temperature in the greenhouse. Instead of repeating the tests during the cooler fall, when I returned to the Rockefeller

University greenhouses, I published the results of the Cold Spring Harbor tests together with my wrong conclusion **(Maramorosch, 1958)**. Had I repeated the tests, perhaps the correct conclusion could have been reached and I would have made the discovery of phytoplasmas 10 years before my Japanese colleagues announced their findings in Tokyo. I missed the boat because I believed the generally accepted and well documented statements of animal and plant virologists that tetracyclines did not affect viruses, and I had no doubts about the viral nature of the two plant diseases.

In 1966 I assembled a large group of postdoctoral associates at the Boyce Thompson Institute in Yonkers, New York. One of my associates, Hiroyuki Hirumi, who worked with me for 10 years and who became a naturalized U.S. citizen, was perfecting leafhopper cell culture and studying thin sections of leafhopper organs by electron microscopy. Hirumi traveled with me to Philadelphia, where we visited the virus laboratory of Werner Henle at the University of Pennsylvania School of Medicine. One of. Henle's associates, Hummeler, looked at electron micrographs made by Hirumi and remarked: I see that you have mycoplasma contamination in your cell culture. Neither Hirumi nor I had ever heard the word mycoplasma. Instead of inquiring what the meaning of this word was, I said that the electron micrograph was not of a cell culture, but of a thin section of a leafhopper salivary gland **(Hirumi and Maramorosch, 1969).** This was a fatal mistake on my part. I was not familiar with the work carried out at that time by Leonard Hayflick, Michael Barile and Robert L. Chanock at the US National Institutes of Health in Bethesda , Maryland **(Chanock *et al.*, 1962).** They studied the infectious agent of "atypical virus pneumonia" and were able to culture the causative disease agent in a cell-free medium. Hayflick called the agent a mycoplasma, *Mycoplasma pneumoniae*. Electron microscopy revealed that human pneumonia, was not caused by a virus, but by a microorganism that contained both RNA and DNA. Viruses contain only one type of nucleic acid, either DNA or RNA. The discovery and cultivation of mycoplasmas made Hayflick famous. It explained why

tetracyclines could cure the "atypical viral pneumonia" because this type of pneumonia was not caused by a virus.

A disease of turkeys, caused by *Mycoplasma gallisepticum*, was studied in several laboratories and tetracyclines were used to cure infected birds. Unfortunately neither I nor other plant virologists were familiar with the work carried out by veterinary and human virologists at that time.

The 1967 breakthrough

In 1967 members of the Japanese Plant Pathology Society were holding their annual meeting in Sapporo. The secretary of the Society, Prof. Eishiro Shikata, had worked during 1963 and 1964 with me as a postdoctoral associate at the Boyce Thompson Institute (Fig.4). He was one of the hardest workers among my more than 40 postdoctorals. Using electron microscopy, Shikata was the first who detected a plant virus in both insect vectors and diseased plants. He left in my laboratory several hundred electron micrograph negatives, after publishing 16 refereed papers as a senior author. A letter arrived from Shikata in the fall of 1967, requesting 4 specific negatives from the several hundred left in my office. Shikata wrote that the negatives contained pictures of the aster yellows pathogen. When I found and checked the requested negatives, I saw only images of sectioned plant cells but no virus particles. I concluded that Shikata probably had too much sake when he wrote his letter and, believing that he was drunk, I did not send him the requested negatives.

It turned out that, as secretary of the forthcoming annual meeting, Shikata read two abstracts submitted by plant virologists from Tokyo University's Plant Pathology department, headed by Prof. H. Asuyama (Fig.5). In one of these abstracts, Y. Doi (Fig.6) and associates described mycoplasma resembling microorganisms in the phloem of plants infected with mulberry dwarf, potato witches broom, aster yellows, and Paulownia witches broom **(Doi *et al.*, 1967).** In the second, T. Ishiie and associates described the suppressive effects of tetracyclines on symptom development of mulberry dwarf disease **(Ishiie *et al.*, 1967).** The conclusion of the Japanese authors was that mulberry dwarf and the other yellows-type diseases were not caused by viruses but by

mycoplasma-like agents. Shikata immediately recalled that the described structures, detected by electron microscopy, resembled those that he had detected three years earlier in his thin sections of aster yellows diseased plants. He was honest and did not disclose to me the content of the two abstracts ahead of their presentation at the November 1967 meeting. He wanted to take part in the discussion and present his own electron micrographs. My failure to send the requested negatives prevented his participation in the discussion.

At the same meeting in Sapporo the entomologist S. Nasu (Fig.7) submitted an abstract on the etiologic agent of rice yellow dwarf disease **(Nasu *et al.*, 1967).** **Nasu *at al.* (1967)** found mycoplasma-like structures not only in diseased rice plants, but also in the leafhopper vector *Nephotettix apicalis.* Nasu's abstract was never mentioned in subsequent Japanese papers and reviews. Japanese plant pathologists and European reviews omitted the important contribution completely and this puzzled me. I decided to find out the reason for their silence and also the background of the Japanese recognition of phytoplasmas. How was this discovery made by plant virologists in Prof. Asuyama's department? Why were the Japanese contributions not mentioned by French workers in 1968? In part, the reason was that the 1967 abstracts were in Japanese and no translations were provided. But this could not explain the omission of Nasu's contribution. I decided to find out how my Japanese colleagues were able to solve the puzzle and wherefrom they got the idea that mycoplasma-like organisms, and not viruses, were the causes of the investigated diseases.

It took several years before I was able to solve the riddle. Someone mentioned to me that Doi was alerted by a veterinarian that the structures detected in electron micrographs were mycoplasmas. I wrote to Prof. Asuyama, asking who this veterinarian was, but I received no reply. This was unusual because Japanese scientists were known to be very polite and almost always were replying to letters. After a few weeks I mailed a copy of my first letter, asking Prof. Asuyama whether he received the earlier one and requesting a reply. Again, no reply arrived. I did not give up and after

3 months I send a third letter.

This time I received an answer. Asuyama stated that Doi had read all available literature about mycoplasmas and that he was very well familiar with this subject. There was no word about a veterinarian and Prof. Asuyama insisted that the discovery was solely the result of Doi's studies. Asuyama's reply did not satisfy me. Doi became familiar with everything that had been published about mycoplasmas, but he started reading mycoplasma papers only after he was tipped of by a veterinarian. Who was this mysterious person whose role my Japanese colleagues denied?

In 1974 I organized a US-Japan seminar in Tokyo, sponsored by the US National Science Foundation and the Japan Society for Promotion of Science. One of my former Japanese associates promised to find the veterinarian whom I was trying to discover in vain. At a coffee break I was finally able to meet Kaoru Koshimizu personally and confirm how the actual discovery of phytoplasmas was made. I was told that in 1967 only one electron microscope was available to scientists of diverse university departments in Tokyo. If a request to use the instrument was approved, permission to use the microscope for 4 hours each day/night was granted. Prof. Asuyama applied, and received permission to have the use of the microscope by one of his associates every fortnight for 4 hours, from midnight till 4:00 a.m. The virologist Doi performed the research but he looked for virus particles and could not find any. By the end of the fiscal year the electron microscope application had to be renewed. Prof. Asuyama requested Doi to provide a dozen electron micrographs to support the renewal application. When Doi stated that he was still unable to find any viruses in diseased plants, Asuyama assured him that this would not prevent the approval of the further use of the electron microscope. The following night Doi selected several negatives, enlarged the pictures, and proceeded to wash and dry them. He did not use the microscope, being very busy with the printing of selected pictures. At 4:00 a.m. the next user of the microscope entered the room. It was the veterinarian, Koshimizu, from the poultry department of the university. He greeted Doi and glanced at the 8x10 micrographs,

still floating in the water. "I see that you are also studying mycoplasmas" he said. "No, I am working with plant viruses ", replied Doi. Koshimizu opened his briefcase and removed one of his 8x10 micrographs. Doi looked at it and wondered aloud, wherefrom Koshimizu got Doi's picture. Koshimizu turned his picture around and pointed to the red "Incan", the Japanese printed sign of the name Koshimizu. "This is not your, but my electron micrograph". Doi remarked that it looked exactly like his pictures floating in the water. "Of course – this is what I am trying to tell you all the time – you are photographing mycoplasmas, not viruses". Then Koshimizu asked Doi whether tetracycline antibiotics have been tried to cure the plants. "No – antibiotics have no effect on viruses" replied Doi. "That is correct – but they affect mycoplasmas".

In the morning Doi described the event to Prof. Asuyama, repeating verbatim the conversation with Koshimizu. Asuyama was very interested and asked Doi to continue his electron microscopy research. Then he called the poultry department and asked his colleague what kind of antibiotics were used to cure poultry from mycoplasmas. He requested small samples of tetracyclines from the veterinary department and decided to send his associate T. Ishiie to fetch the samples. Asuyama instructed Ishiie to make dilutions of 1:100 and 1:1000, place roots of diseased seedlings in the solutions, and also spray the leaves and the soil of potted plants with the tetracycline solutions. Proper controls, treated with distilled water, were to be added and daily observations of the plants made by Ishiie. After several days Ishiie came to Asuyama's office to report that some of the tetracycline treated plants were recovering. This clinched the story. Asuyama prepared two abstracts **(Doi *et al.*, 1967 and Ishiie *et al.*, 1967)**, placing himself as the last coauthor of each. These were the abstracts noticed by Shikata, when he requested his electron micrograph negatives from me.

The abstracts were distributed a few days before the meeting. Dr. Nasu, working in Tsukuba at the Entomology Department, read the two abstracts . Immediately he decided to check his yellow dwarf diseased rice plants and insect vectors, to find whether similar mycoplasma-like

organisms occurred in his material. He worked 20 hours every day and discovered in both, the diseased rice plants and the leafhopper vectors, mycoplasma resembling structures. He was able to deliver his abstract to Prof. Shikata in time before the deadline for the printed abstract booklet. Nasu's abstract was enclosed with all others and distributed at the November 1967 meeting.

During the following years the Japanese plant pathologists did not mention Nasu's contribution. They kept complete silence about his work because they felt that he should not get any credit, even though he was the first who found phytoplasmas not only in the diseased plants but also in the leafhopper vectors. The Tokyo plant virologists wanted to get all the credit for their discovery and they actually received it, They never mentioned Koshimizu, without whom they would not have recognized the mycoplasma resembling structures. Nasu was very frustrated by the omission of his contribution from all reviews and papers of his Tokyo colleagues. He accepted a consulting position with the Food and Agriculture Organization of the United Nations (FAO) in Indonesia and retired after several years. My Japanese colleagues were rather unhappy that I found out about Koshimizu's role and that I credited Nasu with his simultaneous discovery of phytoplasmas.

Spiroplasmas

The discovery of phytoplasmas focuses attention at the important role played by the exchange of information and collaboration of scientists working in different fields. Collaboration between scientists working in diverse areas very often results in unexpected scientific progress. The discovery of spiroplasmas in plants and in animals was originally made by plant virologists. At first, several spiroplasma diseases were believed to be caused by fastidious phytoplasmas. At the University of California in Riverside stubborn disease of citrus was studied **(Fudl-Allah *et al.*, 1972)** and in France, in the laboratory of Jose Bove in Bordeaux, the citrus greening was investigated. When **Saglio *et al.*, (1971)** announced that the causative agent of citrus greening was maintained in a cell-free medium, I became interested in finding out how the culture medium was proven free of

mycoplasma contamination. Among the co-authors of Saglio's report was the name of C. Bonissol. I attended a mycoplasma symposium in Glasgow, Scotland, and took a bus tour to the north of Scotland. Next to me on the bus was a French lady, with a name tag Bonissol. I asked her whether she was the wife of C. Bonissol and was stunned when she replied in flawless American English that it was she who's name was C. Bonissol. Seeing my surprise, she explained that she worked for 10 years in the United States where she was trained in mycoplasma cultivation. She was married to an American of French descent by the name Bonissol and thanks to her training and expertise the *Spiroplasma citri* of citrus greening was successfully grown in Bove's laboratory.

In 1972 the Eastern Branch of the American Phytopathological Society was meeting at the Boyce Thompson Institute (BTI) in Yonkers, New York. At that time I was Program Director of Virology and Insect Physiology at BTI. Among the papers presented at the meeting was a report by Robert E. Davis (Fig.8) of the U.S .Department of Agriculture on his discovery of the spiral form of the corn stunt pathogen (**Davis *et al*., 1972 and Davis and Worley, 1973**), earlier described by Granados et al. in my laboratory as a mycoplasma-like agent (**Granados *et al.*, 1968**). We were using electron microscopy for the localization of phytoplasmas but Davis was not able to use electron microscopy himself. Russell L. Steere, who headed the department where Davis worked, was the sole user of the electron microscope and he did not permit anyone else to use the instrument. Therefore Davis decided to use dark field and phase contrast microscopy, even though it was generally believed that phytoplasmas could not be seen without much higher magnification. Davis was not only able to observe the corn stunt pathogens but he detected that the mollicutes were moving and forming spiral structures in the phloem cells of *Zea mays*. At first I did not believe the observation and I criticized Davis, assuming that the described movements were due to Brownian movement. When I returned to my laboratory and tried to repeat Davis' work, I found the same kind of movement of the spiral forms and I apologized for my unfounded criticism. We became close friends during the

following years. Davis coined the name spiroplasmas for the novel forms. After the corn stunt spiroplasma was cultured and Koch's postulates confirmed that it was the causative agent of the disease **(Williamson and Whitcomb, 1975)**, it was named *Spiroplasma kunkelii*, in honor of the late Louis O. Kunkel who was the first to describe the disease and discover its leafhopper vector two decades earlier **(Kunkel, 1946).**

Davis coined the term spiroplasma because of the helical, motile morphology of cells grown in liquid media. After their recognition in diseased plants they have also been recognized as pathogens of warm blooded animals and insects **(Maramorosch, 1981).** In nature spiroplasmas and phytoplasmas are transmitted to plants by insect vectors, belonging to leafhoppers, plant hoppers, and psyllids. Their multiplication in their specific invertebrate vectors demonstrates that they are not merely plant disease agents. Their low pathogenicity to their vectors might indicate a long evolutionary period of adaptation to invertebrate hosts. I have speculated that spiroplasmas and phytoplasmas may have originated as insect pathogens and gradually became less harmful to their original hosts. Their ability to infect plants, in which they cause severe and sometimes fatal diseases, might be of more recent origin. *Spiroplasma kunkelii* hardly affects the leafhopper vector *Dalbulus maidis*, but the lifespan of *D. elimatus* is drastically shortened by the same spiroplasma **(Granados and Meehan, 1975).**

The first decade of phytoplasma recognition

After the 1967 Japanese announcements about phytoplasmas in mulberry dwarf, potato witches broom , aster yellows , Paulownia witches broom , and rice yellow dwarf disease, I rushed my abstract to the January 1968 program announcement of the New York Academy of Sciences of my paper, to be presented in January 1968, on "Structures resembling mycoplasma in diseased plants and insects". The paper appeared in the Transactions of the Academy a few weeks later **(Maramorosch *et al.*, 1968).** The program announcements were distributed to approximately 20,000 Academy members around the world. Among the recipients was Prof. C. Vago (Fig.9), Director

of the Experiment Station at St. Christol les Ales, France, and professor at Montpellier University. After reading the abstract, Vago summoned his staff member J. Giannotti and requested him to purify and isolate mycoplasma-like microorganisms from plants affected by "Flavescence doree". My published paper listed the 1967 papers of Doi et al, Ishiie et al, and Nasu et al, but the short abstract published in the program and distributed in December 1967 did not mention the Japanese contributions. A short paper by Giannotti et al was submitted to Compte Rendu and published in May 1968 (**Giannotti *et al.*, 1968**). Neither the Japanese nor our contributions were mentioned and the French workers gave the impression that they were the first, sole discoverers of the new group of plant pathogens. A whole series of papers followed and in all the French contributions were hailed as an original French discovery of as great an importance as Pasteur's work in the XIX century.

I complained to Vago, who earlier was very helpful in my own attempts to grow insect cells in vitro, and with whom I organized jointly the first insect cell culture conference in 1962. I pointed out that phytoplasmas were first discovered in Japan, and then confirmed in my laboratory, and I was surprised by the omission of the work that preceded the French papers. Vago, who claimed complete credit for phytoplasma discovery, explained his failure to credit the original discoveries by unrests at Montpellier University. Despite my complains, Giannotti continued to claim that the original discovery was made at St. Christol les Ales and in subsequent papers never mentioned the Japanese contributions. Moreover, he claimed that he was able to culture phytoplasmas in cell-free media. Since this could not be repeated by others, Giannotti was invited to the laboratory of Jose Bove in Bordeaux to demonstrate his technique. Robert E. Davis was spending a few weeks in Bove's laboratory at that time. Giannotti brought his plant material and his media to Bordeaux. As requested, he performed the experiments and in a few days the inoculated media showed mycoplasma growth. Was it mycoplasma contamination of Giannotti's media or did he succeed and culture phytoplasmas? On the day when Giannotti was to leave for the airport to return t

Montpellier, he wanted to retrieve his plants and media. They were in a locked greenhouse and Giannotti was told that the gardener who had the key was ill and could not come to open the greenhouse. Giannotti was desperate. He wanted to break the glass to fetch his material but was not permitted to do this and he departed, leaving his material in Bordeaux. During the following weeks Bove and Davis tried to repeat Giannotti's experiments, using his plants and media, but were unable to do this and notified Giannotti that his claims of successful phytoplasma cultivation could not be verified .Unfortunately, Giannotti continued his claims. A few others, who believed him, tried to confirm his claims. One, after visiting him in France, published positive results, not realizing that the observed growth in ther culture medium was not of plant phytoplasma but of contaminating mycoplasmas.

At the First International Plant Pathology Congress in London, in the summer of 1968, Prof. Asuyama presented the Japanese plant pathology findings. My first mentor, Lindsay M. Black, and I took part in the discussion and both of us congratulated Asuyama and his co-workers for their very important discovery. During subsequent years nearly 80 plant diseases, earlier classified as virus diseases, were found to be caused by phytoplasmas **(Maramorosch *et al.*, 1970)** and at present approximately 800 phytoplasma diseases have been reported **(Bertaccini and Maini, 2007).**

Cultivation attempts

Attempts to culture phytoplasmas were also made in my laboratory. One of my postdoctoral associates, Biljana Plavsic (Fig 10), used horse serum in her media and after a few days she observed what appeared like colony growth (Fig.11). The presumptive colonies continued to grow and we thought that a breakthrough had been achieved. My associate Hiroyuki Hirumi prepared a poster and he was very anxious to claim credit for the successful phytoplasma cultivation. Biljana objected, because this was her project, but Hirumi argued that Biljana was only a Fulbright scholar who would soon depart while the breakthrough would greatly help Hirumi's career. Fortunately, before rushing to submit the description of the

colonies to a scientific journal, I mailed photographs of the "colonies" to Ruth G. Wittler a mycoplasma expert at Walter Reed Army Institute of Research in Washington, D. C. Wittler called my attention to the article published in Vol. IV of Methods in Virology by A. Brown and J. E. Officer. The authors described "pseudo colonies" that were often mistaken for mycoplasma colonies (**Brown and Officer, 1968**). These were mineral deposits that were "growing" when high concentrations of horse or rabbit sera were used in the culture media. Wittler's reply saved me the embarrassment of publishing the presumptive successful cultivation of phytoplasmas, but it was still embarrassing, because I myself, as an editor of Methods in Virology, have edited the article of Brown and Officer in 1968, but forgot completely the description of the pseudo colonies. Afterwards I published a short abstract about "pseudo colonies (**Maramorosch *et al.*, 1971**). Although cultivation of phytoplasmas has not yet been achieved, I hope that collaboration between phytoplasma researchers and microbiologists will eventually result in the cultivation of the fastidious microorganisms.

Incompatibility of phytoplasma research and politics.

My Fulbright postdoctoral associate Biljana Plavsic worked in my laboratory for 18 months. In 1972 she made her most important discovery, examining inflorescences of lethal yellows infected coconut palms. Before 1972 the lethal yellows disease of palms was described as a virus disease and the devastation caused by it on several Caribbean islands and in the southern part of Florida was of great concern. Biljana found phytoplasmas in the diseased plant tissues (**Plavsic-Banjac*et al.*, 1972**) and her findings were soon confirmed in Great Britain and in Germany. She published 7 additional papers and continued her phytoplasma research after she returned to her university in Sarajevo, in former Yugoslavia. Had she remained at her successful plant virology and phytoplasma research, she would have become one of the best experts in these areas of research. However, when Yugoslavia fell apart into 7 republics, Biljana decided to become a politician. She was very successful at first, becoming the only woman elected president of the newly created Republic of

Bosnia. For two years she was hailed as the ablest politician in former Yugoslavia. She coped well with numerous problems but when war broke out between Croats, Serbs and Bosnian Muslims, Biljana became the supporter of the campaign of persecution, separating Croats and Muslims from Serbs in Bosnia. In 1992 tens of thousands of Bosnians were killed or fled and more than 400 camps were created and ethnic cleansing carried out. Biljana, who became vice-president under Radovan Karadzic, inspired the Serbs to take up arms against their Croat and Muslim neighbors and proclaimed Serbs' cultural and racial superiority over Muslims.

In 2002 Biljana traveled voluntarily to The Hague, to face the United Nations International War Tribunal. There she was promptly arrested and presented with the evidence of the horrendous war crimes. She confessed and expressed regret and her plea saved her from a life sentence. As the most senior official of the Serbs, she pleaded guilty to crimes against humanity during the Balkan conflict of the 1990's. Biljana was sentenced to 11 years in jail. If she survives, she would be freed from the Swedish jail at the age of 83. Had she remained a plant virologist instead of turning to politics, she would have been a very prominent scientist today. At present very few people know that the discoverer of the cause of lethal yellows disease is the same person who is now lingering in jail for – perhaps- the rest of her life.

Conclusions

Phytoplasma research has progressed greatly during the four decades since phytoplasmas have been identified by Japanese plant pathologists and entomologists in 1967. Collaboration between researchers from different countries and different disciplines accounted for the rapid progress achieved in recent years. Fortunately science recognizes no political, religious, ethnic, or geographic borders. As scientists, we speak the same language – the language of science, and we must collaborate with each other irrespective of background and political believes. At present plant pathology, entomology, and molecular biology researchers from different countries are collaborating, increasing and expanding knowledge of phytoplasma agents, phytoplasma

vectors, and phytoplasma diseases worldwide. More than 800 plant diseases and a large number of insect vectors have been reported. Recent research yielded new knowledge about phytoplasma ecology and phylogenetic relationships. New approaches to the control of phytoplasma diseases of crops and of phytoplasma vectors are being developed. Researchers are now able to study the whole spectrum of phytoplasma strains worldwide (**Lee *et al.*, 2007**). The historical events of the passed century provided the basis for the current molecular biology study of phytoplasmas.

REFERENCES

Bertaccini, A. and Maini, S. (2007). First International Phytoplasmologist Working Group Meeting, Bologna (Italy), Nov. 12-15, 2007. ***In: Bull. Insectology 60: 99-407.***

Brown, A. and Officer, J. F. (1968). Contamination of cell cultures by mycoplasma (PPLO). ***In: Methods in Virology IV. K. Maramorosch and H. Koprowski, eds. Academic Press, New York: 531-564.***

Chanock, R., Hayflick, L., Barile, M. F. (1962). Growth on artificial medium of an agent associated with atypical pneumonia and its identification as a PPLO. ***Proc. Nat. Acad. Sci. (US) 48: 41-49.***

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

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

Doi, Y., Terenaka, M., Yora, K., 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). ***Ann. Phytopathol. Soc. Japan 33: 259-266.***

Fudl-Allah, A. E. S., Calavan,

E. C., Igwegbe, C. K. (1972). Culture of a mycoplasma-like organism associated with stubborn disease of citrus. *Phytopathology 62: 729-731.*

Giannotti, J., Marchou, G., Vago, C. (1968). Micro-organisms de type mycoplasma dans les cellules liberiennes de *Malus sylvestris* L. atteinte de la maladie des proliferations. *C. R. Acad. Sci. D267:76-77.*

Granados, R. R. and Meehan, D. J. (1975). Patho9genicity of the corn stunt agent to an insect vector *Dalbulus elimatus*. *J. Invert. Pathol. 26: 313-320.*

Granados, R. R., Maramorosch, K., Shikata, E. (1968). Mycoplasma: suspected etiologic agent of corn stunt. *Proc. Nat. Acad. Sci. (US) 60:841-844.*

Hirumi, H. and Maramorosch, K. (1969). Mycoplasma-like bodies in the salivary glands of insect vectors carrying the aster yellows agent. *J. Virol. 3: 82-84.*

Ishiie, T., Doi, Y., Yora, K., Asuyama, H. (1967). Suppressive effects of antibiotics of tetracycline group on symptom development of mulberry dwarf disease (in Japanese). *Ann. Phytopathol. Soc. Japan 33: 267-275.*

Kunkel, L. O. (1926). Studies of aster yellows. *Am.Journal Bot. 13: 646-702.*

Kunkel, L. O. (1946). Leafhopper transmission of corn stunt. *Proc. Nat. Acad. Sci.(US) 32: 246-247.*

Lee, Ing-Ming, Davis, R. E., Wei, W., Martini, M. (2007). Prospects of DNA-based systems for differentiation and classification of phytoplasmas. *Bull. Insectology 60: 239-244.*

Maramorosch, K. (1951). A simple needle for microinjections. *Nature 167: 734.*

Maramorosch, K. (1952). Direct evidence for the multiplication of aster yellows virus in its insect vector. *Phytopathology 42: 59-64.*

Maramorosch, K. (1956).

Semiautomatic equipment for injecting insects with measured amounts of liquids containing viruses or toxic substances. *Phytopathology 46: 188-190.*

Maramorosch, K. (1958). Viruses that infect and multiply in both plants and animals. *Trans. N. Y. Acad. Sci. (Ser.II) 20: 383-393.*

Maramorosch, K. (1981). Spiroplasmas: agents of animal and plant diseases. *BioScience 31: 374-380.*

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

Maramorosch, K., Hirumi, H., Plavsic-Banjac, B. (1971). Artifact pseudocolonies on solid agar media inoculated with aster yellows or healthy plant material. *Phytopathology 61: 902.*

Maramorosch, K., Shikata, E., Granados, R. R. (1968). Structures resembling mycoplasmas in diseased plants and in insect vectors. *Proc. N. Y. Acad. Sci. 30: 841-855.*

Nasu, S., Sugiura, M., Wakimoto, T., Iida, T. T. (1967). On the etiologic agent of rice yellow dwarf disease (in Japanese). *Ann. Phytopathol. Soc. Japan 33: 343-344.*

Plavsic-Banjac, B., Hunt, P., Maramorosch, K. (1972). Mycoplasma-like bodies associated with lethal yellowing disease of coconut palms. *Phytopathology 62: 298-299.*

Saglio, P., Lafleche, D., Bonissol, C., Bove, J. M. (1971). Isolement, culture et observation au microscope electronique des structures de type mycoplasma associes a la maladie du stubborn des agrumes et leaur comparison avec les structgures observes dans le cas de la maladie des greening des agrumes. *Physiol. Veg. 9: 560-582.*

Williamson, D. L. and Whitcomb, R. F. (1975). A cultivable spiroplasma causes corn stunt disease. *Science 188: 1018-1020.*