Lectures and papers... 2nd Slov. Conf. Pl. Prot., Radenci 21.-22. Febr.1995, p. 27-46

PRESENT KNOWLEDGES ON PHYTOPLASMA DISEASES OF FRUIT TREES AND GRAPEVINE

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IZVLEČEK

SEDANJE ZNANJE O FITOPLAZMATSKIH BOLEZNIH NA SADNEM DREVJU IN VINSKI TRTI

Mikoplazmam podobni organizmi (MLOs) so povzročitelji več kot 300 rastlinskih bolezni. Za te povzročitelje so v zadnjem času predlagali preimenovanje v "fitoplazme". Resnično ugotovitve o sekvencah DNA kažejo, da kažejo fitoplazme evolucijske razlike glede na mikoplazme.

V Evropi so med fitoplazmatskimi boleznimi na sadnem drevju najpomembnejše jablanova metličavost (Apple proliferation, AP), propadanje hrušk (Pear decline, PD), slivova leptonekroza (Plum leptonecrosis, PLN = Plum Decline = Japanese plum leptonecrosis = Japanese plum decline) in Flavescence dorée (FD) in sorodne rumenice vinske trte (Grape yellows, GY). Uporaba nekaterih tehnik kot sta PCR in RFLP analizi genskih sekvenc nakazuje genetske korelacije med agensi AP, PD in PLN. Na drugi strani pa so organizmi, ki povzročajo FD, GY in Western-X-disease razvrščeni v druge skupine.

Jablanova metličavost je nevarna bolezen, ki okužuje drevje jablan in v manjšem obsegu drevje hrušk. Najpomembnejše jablanove sorte so občutljive zanjo. Okužuje tudi *Malus floribunda* Sieb. in jablanove sejance, zlasti najbolj rastne. Povzročitelj jablanove metličavosti se prenaša s cepljenjem, zlasti če se uporabljajo veliki koreninski potaknjenci in s predenico. *Fieberiella flori* Stahl kaže, da je eden naravnih vektorjev.

Povzročitelj AP ni stalno zastopan v krošnji, remisija simptomov se lahko zgodi naravno ali zaradi aplikacije tetraciklinov. Krčenje drevja se zdi, da ne preprečuje naravnega širjenja bolezni. Danih je nekaj priporočil za zmanjšanje pojava bolezni v sadovnjakih.

Poleg jablanove metličavosti so opisani še PD, PLN, FD in GY s posebnim ozirom na etiologijo, epifitotiologijo, simptomatologijo in preprečevanje.

ABSTRACT

Mycoplasmalike organisms (MLOs) are the causal agents of more than 300 plant diseases. These agents were recently suggested to be renamed "Phytoplasmas". In fact the DNA sequence data indicate that phytoplasmas show evolutionary differences from the Mycoplasmas.

In Europe, among the fruit tree phytoplasma diseases, the most important are Apple proliferation (AP), Pear decline (PD), Plum leptonecrosis (PLN) (= Plum Decline = Japanese plum leptonecrosis = Japanese plum decline) and Flavescence dorée and related Grape yellows (GY). The use of molecular techniques such as PCR and RFLP analyses of gene sequences suggest genetic correlations among the agents of AP, PD and PLN. On the contrary, the organisms causing FD, GY and Western X-disease are assigned to different groups (clusters).

Apple proliferation (Apple witches-broom) is a serious disease that affects apple trees and to a lesser degree pear trees. The most important varieties of apple trees are susceptible to AP. Also *Malus floribunda* Sieb. and apple seedlings particularly the most vigorous are susceptible to AP. The agent of AP is transmissible by grafting, particularly if large root cuttings are used, and by dodder. *Fieberiella florii* Sthall is indicated as one of the natural vectors.

The presence of the agent of AP is not constant in the crown; remission of the symptoms can occur naturally or induced by tetracycline treatments. Roguing seems not to prevent the natural diffusion of the disease. Some recommendations are given to reduce the AP incidence in the field. The most reliable diagnostic techniques are also indicated.

Besides Apple Proliferation, PD, PLN, FD and GY are described, with special attention to the epidemiology, aethiology, symptomatology and prevention.

Mycoplasmas are the smallest free-living prokaryotes up to now known. Mycoplasma like organisms (MLOs) are the causal agents of many plant diseases. More than 300 plant diseases are indicated as caused by MLOs or associated with them.

Very recently on the basis of phylogenetic investigations, it was proposed to change the term "mycoplasma" to "phytoplasma". Latin binomials were also proposed to distinguish the different phytoplasmas (Tenth Meeting of the International Organization for

Mycoplasmology, IOM, Bordeaux, 1994). DNA sequence analyses confirm the phylogenetic position of the "phytoplasmas" among Mollicutes. In fact the phytoplasmas are characterized by a low G+C content (25-30%), a small genome size (about 500-1000 Kb) and by a 16s rRNA sequence closely related to the members of the Mollicutes (rather than to the bacteria). The DNA sequence data indicate however that the phytoplasmas are evolutionarily different from the *Mycoplasmataceae* so as a consequence, the term "mycoplasma" is considered incorrect. However, the "MLOs" abbreviation is maintained in the present paper for practical convenience.

After the discovery of MLOs as the causal agent of the "yellows" plant disease, a large number of tree MLOs diseases were noticed. Among these the fruit tree diseases are of a considerable importance all over the world.

In north-central Europe the greatest effects occur in pomefruits, stonefruits and in grapevine plants.

Within the fruit trees *sensu lato* (*s. l.*) the most notable phytoplasma diseases are "Apple proliferation" (AP), "Pear decline" (PD), "Plum leptonecrosis" (PLN) (= Plum decline), "Flavescence doree" (FD) and related "Grape yellows" (GY).

Recently, a method has been introduced into plant mycoplasmology to amplify the 16S rDNA gene of the phytoplasmas by polymerase chain reaction (PCR) and then to digest the amplified sequences by restriction endonucleases (Restriction fragment length polymorphism analyses, RFLP). More recently, the phylogenetic relationships of different phytoplasmas was established by 16/23SrDNA spacer sequences (Kirkpatrick *et al.*, 1994). According to the sequence data obtained and to the results of the RFLP analyses the following relationships are now proposed for the most important MLOs infecting the fruit trees: AP, PD and PLN are related but not identical; PLN and ACLR (Apricot chlorotic leaf roll) are probably identical; FD is remotely related to EY (Elm yellows) but distinct from AP, PLN, PD; BN (Bois noir) VK (Vergilbungskrankheit) and other grape yellows (GY) are close to Stolbur; Western-X disease is in a distinct cluster (Lee *et al.*, 1993; Lee *et al.*, 1994; Namba *et al.*, 1993; Schneider *et al.*, 1993; Seemüller *et al.*, 1994).

Apple proliferation. Apple proliferation, also called Apple witches' broom is a serious disease that affects apple trees and, to a lesser extent pear trees. AP is present in Europe. The more important varieties of apple are susceptible to AP. Among the most sensitive are Bell de Boskoop, Canadian Renette, Golden Delicious, Goldparmane (Blattny and Blattny, 1960; Bovey, 1961; Marenaud *et al.*, 1978; Schmid, 1975; Seidl and Komarkova, 1977; Zawadzka, 1976). Unfortunatelly also *Malus floribunda* Sieb. and its progeny resistant to scab (i.e. Florina, Prima and Priscilla) are particularly susceptible to AP (Loi *et al.*, 1995). Apple rootstocks are sensitive too, particularly the most vigorous ones such as Frank and M16. The less vigorous M9 seems to reduce the sensitivity of the scion; Reine de Renette, Antonowka, Yellow transparent, Rose de Benejama and Welthy are considered tolerant (Pena-Iglesias, 1975; Schmid, 1975; Seidl and Komarkova, 1977; Zawadzka, 1976).

The most reliable symptoms of AP are witches' broom, small leaves with a short petiole and abnormal stipules; bronze, reddish and chlorotic leaves; phyllody, virescence and apostasis of the flowers; pale, flat fruits with a long petiole. The commercial value of the fruit decreases by 30% to practically nil.

The causal agent is a MLO. On the basis of the sequence analysis of the 16S rDNA gene, AP is a member of the apple proliferation strain cluster (that includes also PD and European stone fruit yellows strain).

MLOs are restricted to the phloem cells, particularly in stipules, petioles, peduncles of fruits, but they are irregularly distributed in the plant tissues. During the winter the MLOs seems to be located exclusively in the roots, from where they reinvade the crown during the following spring (Seemüller *et al.*, 1994).

The AP agent is transmitted by grafting, particularly if large root cuttings are used as inoculum (Kunze, 1972; Refatti *et al.*, 1986), and by micropropagation.

The AP agent was also transmitted by dodder from apple to *Catharanthus roseus* L. (Carraro *et al.*, 1988; Marwitz *et al.*, 1974) and back to apple from *C. roseus* (Petzold and Marwitz, 1976). The hopper *Fieberiella florii* Stal. is indicated as one of the natural vectors (Krczal *et al.*, 1988). The disease spreads naturally, especially in orchards not treated with insecticides (Bliefernicht and Krczal, 1994). The results obtained in Friuli-Venezia Giulia during a seven year period of investigation indicate that the disease can involve up to 93% of the young Florina trees (Loi *et al.*, 1995).

The most reliable diagnostic techniques are: use of test plants; electron microscopy; fluorescence microscopy using DAPI staining; antibiotic treatement (tetracycline); nucleic acid hybridization, PCR and IC-PCR (immune capture - PCR) (Firrao *et al.*, 1994a, 1994b; Rajan and Clark, 1994; Seemüller, 1976)

In trees treated with tetracycline there is a temporary remission of the symptoms. The re-injections of such a trees is less effective. Heat treatments are successful. Roguing of affected trees is a practice that seems not to prevent the diffusion of the disease.

Some reccomendations can be made to reduce the AP incidence in the field: 1) avoid the most sensitive cultivars in areas where the disease is epidemic; 2) select the less vigorous rootstocks; 3) avoid severe pruning; 4) utilize tested materials when planting new orchards (Trifonov and K'nev, 1978); 5) use resistant rootstocks to AP such as certain selections of apomictic seedlings derived from crosses between *Malus sieboldii* Rehd. or *M. sargentii* Rehd. with cvs of *M. pumila* Mill. (Seemüller *et al.*, 1992).

Plum leptonecrosis. It is one of the most important stone-fruit disease caused by MLOs occuring in Europe. The original name "leptonecrosis" cames from the phloem necrosis induced in the infected trees. "Japanese plum decline" is a synonim of PLN. At present, "Apricot chlorotic leaf roll", "Peach chlorotic leaf roll" and "European stone-fruit yellows" are believed to be caused by organisms closely related to PLN. The above cited MLOs, on the bases of the level of sequence homology, are placed in the apple proliferation strain cluster. In contrast, the Western-X disease agent is placed in a separate cluster (Seemüller *et al.*, 1994).

PLN is a decline that occurs primarily on Japanese plum (*Prunus salicina* Lindl). The disease can attack also almond, apricot, peach and sweet cherry (Giunchedi *et al.*, 1982). The European plum trees are symptomless carriers. Trees of plum "Požegača" sometimes show symptoms of PLN. However the most typical ones on plum and apricot are: premature bud opening, sometimes in November-December; development of leaves before flowers in spring; upward rolled and small leaves with chlorotic or reddish, thick and brittle lamina; small flowers that blosson out of season; reduced fruit size; malformed and corky fruits that do not ripe regularly; necrosis of the phloem; branches are brittle and die 1-3 years after the initial symptoms; the entire plant can die but the rootstock (i.e. Myrabolan) survives even if it shows symptoms of the disease, such as small leaves (but not bark necrosis) (Carraro *et al.*, 1992; Giunchedi *et al.*, 1978 and 1982).

A similar disease on *P. salicina* grafted on Myrabolan was reported in Spain (Sanchez-Capuchino and Forner, 1973), Greece (Syrgianidis *et al.*, 1976) and Germany (Lederer and Seemüller, 1992). ACLR is reported in France (Morvan, 1956), Switzerland (Bovey, 1959), Yugoslavia (Paunović, 1968) and Spain (Llacer, 1972).

PLN-ACLR is one of the limiting factors in developing the orchards of Japanese plum and apricot in many European countries. In Italy PLN occurs mainly in Japanese plum, to a lesser extent in apricot and rarely in peach. In France ACLR seems to be more important in apricot. Five to 30 percent of the infected trees can die each year depending on the plant cultivar. In Friuli-Venezia Giulia on the bases of the surveys conducted in experimental fields it appears that 50 to 70% of the originally healthy Ozark Premier plum trees gradually become infected within the first three or four years after planting. Similarly, around 2-5% of the Ozark Premier bite-plants in various localities of the region showed PLN symptoms within the first year and about 20-25% during the second year.

The quick spread of the disease when sensitive plum varieties are newly introduced into affected orchards, indicate that sources of inoculum and efficient vectors are already present in such areas. Still up to now, in spite of the evident natural spread of the disease, no positive result was obtained in the transmission trials using various candidate vectors, mainly leafhoppers (Carraro *et al.*, 1992).

Repeatedly MLOs have been found in plum and peach trees with PLN by using e.m. (Carraro *et al.*, 1992; Giunchedi *et al.*, 1978; Musetti *et al.*, 1994; Poggi Pollini *et al.*, 1993) and many alterations were observed in the diseased leaf tissues (Musetti *et al.*, 1994). MLOs have been also detected in plum, apricot, peach and in *C. roseus* by PCR using primers and RFLP analyses (Ahrens and Seemüller, 1992; Marcone *et al.*, 1994; Poggi Pollini *et al.*, 1993).

The peach "GF 305", the apricot "Tilton" and the plum "Ozark Premier" are among the most suitable indicators of PLN. The plant to plant transmission of PLN by grafting, is much easier than AP. MLOs were transferred from apricot affected by ACLR (Morvan *et al.*, 1973) to *C. roseus* using dodder.

In Italy, two different MLOs were transmitted to *C. roseus* by *Cuscuta campestris* Younk from plum trees with PLN symptoms (Loi *et al.*, 1994). The results indicate that plum can host different MLOs perhaps also in mixed infections but the precise aetiological involvement of the two MLOs is not yet clear.

Some control measures: avoid Japanese cvs in infected areas; use tolerant apricot cvs and European plums in infected areas. The possibility of controlling ACLR has been studied in France by cross-protection using MLOs variants with reduced pathogenicity (Morvan *et al.*, 1986).

Pear decline. Pear decline is a destructive disease of *Pyrus.*; it has been known for more than 50 years in Italy (Catoni, 1934). In 1948 it was observed in British Columbia and in Central Washington (McLarti, 1948). In 1953 PD was found in Oregon and in California (Nikols *et al.*, 1960; Woodbridge *et al.*, 1957).

In Europe the disease is present in Italy (Firrao *et al.*, 1994b; Giunchedi *et al.*, 1994; Refatti, 1967), Greece (Agrios, 1972; Plakidas, 1962), Germany (Kegler and Klinkowski, 1967; Schaper and Seemüller 1982; Seemüller *et al.*, 1984a, b; Seemüller, 1988), Spain (Avinent and Llacer, 1994; Rallo, 1973), France (Lansac and Dosba, 1993; Lemoine, 1975), Czechoslowakia (Blattny and Vana, 1974), Switzerland (Schmid, 1974), Yugoslavia (Grbić, 1974) and England (Davies *et al.*, 1992).

MLOs are associated with infected pear trees (Avinent and Llacer, 1994; Behnke *et al.*, 1980; Davies *et al.*, 1994; Firrao *et al.*, 1994b; Giunchedi *et al.*, 1994; Hibino and Schneider, 1970; Kirkpatrick *et al.*, 1994; Schaper and Seemüller, 1982).

An American strain of PD and a European one are believed to be distinct (Desvignes, 1990). The disease affects cultivars and rootstocks of French pear (*Pyrus communis* L.), Japanese pear (*P. pyrifolia* Nakai), Chinese pear (*P. ussuriensis* Maxim.) and other species as *P. betulaefolia* Bunge and *P. serotina* Rehd. and quince (*Cydonia oblonga* Mill.) (Poggi Pollini *et al.*, 1994a).

PD is most severe on pears grafted on oriental rootstock such as *P. ussuriensis*, *P. pyrifolia* and *P. serotina* (Blodgett *et al..*, 1962) and it is less severe in trees growing on *P. betulaefolia, P. calleriana* Decne and *C. oblonga*. PD occurs also on trees with *P. communis* rootstocks, but the severity of the disease is influenced also by the scion. Different *P. communis* rootstocks possess variable degrees of tolerance to PD.

Severe symptoms of PD are reported in Italy on *P. pyrifolia* cv Hosui and Kosui grafted on *P. communis* seedling rootstocks (Poggi Pollini *et al.*, 1994b).

The most common symptoms of PD are either quick decline or slow decline and leaf curl. Quick decline is often associated with oriental rootstocks and is characterized by wilting and death of the tree within a few days. Slow decline can occur on trees with any kind of rootstocks , depending on the scion, the season and relative environmental conditions. Leaf curl occurs on the more tolerant rootstocks and it is usually followed by slow decline. Common symptoms connected with PD are: reduced size of the plant and of the fruits, few and small leaves with uprolled margins that become reddish and leathery in autumn, a brown line in the bark at the union of scion and roostock; additional phloem. Other factors such as incompatibility girdling, drought and malnutrition can induce symptoms similar to those of PD.

In nature PD is transmitted by *Cacopsylla pyricola* Förster in USA (Jensen and Erwin, 1963). In Europe *C. pyri* L. and *C. pyrisuga* Förster are believed to transmit PD. It is transmitted in a persistent way. After a few hours of aquisition feeding the vectors remain infective for a long time or for life. *C. pyricola* overwinters as an adult and has 3-5 generations/year.

The disease is easily transmitted by grafting of scions and to a lesser extent by budding. The incubation period ranges from a few months to 1-3 years.

The PD organism is eliminated from the crown during winter; it passes this season in suitable rootstocks; in spring the MLOs reinvade the stem (Schaper and Semüller, 1984; Seemüller *et al.*, 1984a).

William's, Comice and Precocious pears grafted on *P. communis* or oriental rootstocks were used as test plants (Schneider, 1977).

Some control measures: use of decline resistant or tolerant rootstocks (*P. betulaefolia* and quince); use of healthy plants; control of the vectors.

<u>Flavescence doree and other Grapevine Yellows</u>. This is a complex group of grapevine diseases caused by different phytoplasmas that induce a practically indistinguishable syndrome on susceptible plants. Flavescence doree (FD) *sensu stricto* (*s.s.*) was first described in Southwest France (Caudwell, 1957) where it was accurately studied for many years. The FD *sensu stricto* is specifically transmitted by the vector *Scaphoideus titanus* Ball (formerly *S. littoralis* Ball) (Schvester *et al.*, 1961).

The FD-similar disease was found in Northern Italy (Belli *et al.*, 1973, 1983; Refatti, 1993). Later on FD *sensu stricto* was detected in Northeastern Italy (Bianco *et al.*, 1993; Daire *et al.*, 1993).

Two other grape diseases have been studied in Europe, both characterized by symptoms indistinguishable from those of FD: Bois noir (BN) in Burgundy and Vergilbungskrankheit (VK) in West Germany (Caudwell, 1961; Gärtel, 1959). The agents of these diseases are not transmitted by *S. titanus* (Caudwell *et al.*, 1971; Maixner and Ahrens, 1993).

At the beginning of the eighties, a *S. titanus* apparently not associated GYdisease was noticed in Italy, where its diffusion was sometimes considerable and the damages were heavy (Belli *et al.*, 1983; Carraro *et al.*, 1986; Conti, 1986; Credi and Babini, 1984; Di Terlizzi *et al.*, 1993; Egger and Borgo, 1983; Granata, 1982; Mescalchin *et al.*, 1986; Vidano *et al.*, 1987), in Greece (Rumbos and Avgelis, 1985), in Switzerland (Cazelles *et al.*, 1992), in Israel (Tanne and Nitzany, 1973), in USA (Pearson *et al.*, 1985), in Australia (Magarey and Wachtel, 1985), in Moldavia and in Slovenia.

The most important and typical symptoms of FD and other GY are: downward rolling of the leaves; sectorial yellowing or reddening of leaf blade; necrosis of the leaf veins; epinasty; incomplete ripening and presence of black pustules of the canes; flexible shoots and drooping plants; abortion; shrivelling or necrosis of the fruit clusters.

The detection and differentiation of the GY diseases *s.l.* have been primarily carried out by hybridization with cloned probes, PCR, RFLP, sequence analyses and serology. The results of these studies indicate that various phytoplasmas are correlated with the grapevine yellows *s.l.* in different viticultural areas of the world: FD specifically transmitted by *S. titanus*, present in France and in Northern Italy, is related to Elm yellows but the two agents are not identical (Bianco *et al.*, 1994; Daire *et al.*, 1994); BN, VK and other grape yellows, occuring in France, Germany, Italy and Israel are grouped within the Aster Yellows cluster or more precisely in the AY stolbur subgroup. A phytoplasma isolated from grape to *C. roseus* in Northeastern Italy and one detected in grapevine in New York State and in Virginia were associated with peach X-disease (Chen *et al.*, 1993; Chen *et al.*, 1994; Prince *et al.*, 1993). Kuszala *et al.* (1993) demonstrated that no serological relationship exists between FD and other GY.

The pathogen of GY *s.l.* was detected in the phloem cells of infected grapevines by using transmission electron microscopy (Caudwell, 1993) and scanning electron microscopy (Quaroni *et al.*, 1988). This pathogen is irregularly distributed in the grapevine tissues.

Many varieties are sensitive to GY *s.l.* in the various viticultural areas of the world. In Northeastern Italy the most susceptible cvs to FD are Garganega, Perera, Chardonnay, Trebbiano toscano, Trebbiano di Soave, Pinot, Malvasia, Prosecco; in the case of the *S. titanus* not related GY, the most affected grapevines are Chardonnay and Pinot (Refatti, 1993). Also the rootstocks varieties can be infected by GY *s.l.* even if they are generally symptomless (Caudwell, 1993). In France the reaction of the plants to the FD infection was studied. Basically the behaviour of the grape depends on the cultivar: in the case of Ugni Blanc and Baco 22A, the plant shows heavy symptoms the year following the inoculation and then it tends to recover; on the contrary the cv Nieluccio after infection does not recover from the symptoms but the disease worsens till death (Caudwell, 1981). In Italy the behaviour of the GY infected grapevines cv Chardonnay was demontrated to be quite different: there are plants that remain constantly symptomatic for more than 6-7 years; other plants recover 1-5 years after the season of the first symptom expression; in some plants the recovery is stable, in other is temporaneous. This, happens both to grapevines exposed to natural inoculation (reinfection) after the first infection or protected inside an insect-proof screenhouse (Osler *et al.*, 1993).

In nature, the disease spreads mainly accordingly to the infection pressure and is influenced by the susceptibility of the grape cvs. The annual increment in affected plants depends on the number of newly infected grapevine over the number of recovered ones. When the ratio is above one the disease is increasing. In Chardonnay vineyards of F-VG during the last 10 years the incidence of the disease varied from 1 to 49% (Osler *et al.*, 1993). During the apex of the epidemics the annual rate of newly symptomatic grapes on the total can be about 20%. The production decrease in infected plants varies greatly; it can be 100%. The disease is spread by insect-vectors and by grafting. In Italy it was seen that GY is poorly transmitted by grafting (Credi *et al.,* 1990) but efficiently by natural vectors (Carraro *et al.*, 1994). The distribution of the disease in the vineyard is not by chance. Both in Germany (Maixner, 1993) and in Italy (Credi and Santucci, 1991) on the bases of indices of dispersion and of the spatial distribution significant aggregation of infected grapevines were detected. This indicates the activity of vectors and a particular presence of natural sources of inoculum. Unfortunately up to now, no natural vectors of the no FD diseases are known, even if numerous species of potential vectors have been put forward (Vidano *et al.*, 1987).

Control measures: use tested material; avoid the most susceptible cvs in infected areas; pollarding; treatements against the natural vectors. There is evidence suggesting that pollarding is advisable to hasten the recovery of the affected vines (Osler *et al.*, 1993). The effect of roguing is not demonstrated. In France 3-4 treatements against the vector *S. titanus* are recommended to reduce the spread of the disease (Planas, 1987). In Italy analogous results have been obtained in areas where the FD sensu stricto and its vector are present (Fortusini *et al.*, 1988). In Friuli-Venezia Giulia the control of the GY disease was not achieved through insecticidal treatements (Girolami and Egger, 1993).

More informationis needed on the presence of the natural vectors and on their biology in order to develop appropriate control strategies.

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