

# **Atti del "Workshop": II Incontro Nazionale sulle Malattie da Fitoplasmi**

3 - 4 Ottobre 2002, Roma, Italia

## **Comitato Scientifico**

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## **Organizzazione e Segreteria**

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## **Presentazione**

Il "II Incontro Nazionale sulle Malattie da Fitoplasmi", svoltosi in Roma a distanza di tre anni dal precedente tenutosi ad Udine, testimonia come sia particolarmente sentita la necessità di aggiornare periodicamente lo stato delle conoscenze su questi importanti patogeni vegetali. I fitoplasmi, agenti causali di gravi malattie, continuano, infatti, ad avere una grande importanza economica nei Paesi di tutto il mondo. I danni che in Italia le fitoplasmosi inducono su colture ad ampia diffusione quali la vite, i fruttiferi, le piante orticole e ornamentali, sono rilevanti. A testimonianza di ciò si ricorda che, nell'arco di tempo intercorso tra i due incontri, è stato necessario avviare specifici programmi di lotta sia a livello nazionale che regionale per controllare, rispettivamente, la diffusione della Flavescenza Dorata della vite nei vigneti del Nord Italia (D.M. n. 32442 del 31.5.2000) e degli scopazzi del melo in Valle D'Aosta e Trentino (programmi speciali regionali). L'Incontro, a cui hanno partecipato ricercatori e tecnici provenienti da tutte le Regioni Italiane, ha consentito di riunire competenze scientifiche afferenti a settori diversi (Patologi, Entomologi e Zoologi, Forestali, Fisiologi Vegetali, Frutticoltori e Viticoltori) e di illustrare le più recenti acquisizioni raggiunte negli specifici settori di ricerca. L'interesse suscitato ha confermato l'opportunità di proseguire questi Incontri, da tenersi a scadenza triennale, al fine di approfondire le conoscenze indispensabili per l'attivazione di sistemi di controllo e di lotta necessari al contenimento di gravi sindromi fitoplasmali che, sempre più spesso, inficiano la qualità delle produzioni vegetali. Spero, pertanto, che la pubblicazione di questi Atti, contenenti i contributi scientifici del II Incontro, serva di supporto informativo a tutte le forze attivamente impegnate nello studio delle fitoplasmosi.

## **Marina Barba**

Istituto Sperimentale per la Patologia Vegetale, ROMA

## ***Proceedings of the Workshop: 2nd National meeting on phytoplasma diseases***

*October 3 - 4, 2002, Rome, Italy*

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## **Forewords**

*The "2nd National Meeting on Phytoplasma Diseases", organized in Rome three years after the previous one held in Udine, showed the need to periodically up to date knowledges on this important plant pathogen group. Phytoplasma diseases, in fact, are still taking up an important economic role all over the world. In Italy they represent a real problem for important crops such as grapevine, fruit trees, vegetable and floricultural plants. In the last three years, national and regional programs have been set up to control natural spreading, respectively, of Flavescence Dorée in vineyards of Northern Italy (D.M. 32442 of May 5, 2000) and Apple proliferation in pome fruit orchards in Val D'Aosta and Trentino Regions. The Meeting, attended by scientists and technicians coming from different Italian Regions, has allowed to put together the most recent knowledges deriving from different research fields, i.e. plant pathology, applied entomology and zoology, plant physiology, pomology, viticulture. The interest aroused by the Meeting confirmed the opportunity to organize future triennial Meetings in order to discuss periodically the main findings on etiology, epidemiology, characterization and diagnosis of phytoplasmas and stimulate finalized programs aimed at controlling and containing economic losses caused by them. I hope these Proceedings will be a good informative support to the scientists actively involved in studies on phytoplasma diseases.*

### **Marina Barba**

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Petria 12(3), 325-343, (2002) Atti del "Workshop"/Proceedings of the "Workshop"

## **Malattie da fitoplasmi: stato dell'arte**

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Sono descritte le più recenti acquisizioni nel settore della fitoplasmologia da quelle ottenute mediante tecnologie molecolari a quelle più strettamente biologiche relative alle alterazioni fisiologiche delle piante infette ed alle modalità di trasmissione. In particolare mediante studi dei geni ribosomici si può accedere ad una classificazione molecolare che distingue almeno 15 gruppi diversi; l'utilizzo di altre porzioni di acido nucleico conservate quali quelli codificanti la proteina ribosomica s3, il fattore di allungamento tuf ed altri permettono inoltre una tipizzazione più accurata dei fitoplasmi ed un loro studio anche a livello ecologico-epidemiologico. Importanti sono inoltre le recenti identificazioni dei vettori di malattie importanti dal punto di vista economico quali lo scopazzo del melo e il giallume delle drupacee, la conferma della trasmissibilità transovarica di alcuni fitoplasmi negli insetti e la loro individuazione anche nel seme di erba medica. Ancora in fase preliminare sono le ricerche per studiare al livello molecolare la risposta delle piante alla presenza di questi procarioti.

**Parole chiave:** Fitoplasm, Identificazione molecolare, Epidemiologia, Trasmissione, Interazione pianta-patogeno.

### **Phytoplasma diseases: state of the art**

Recent scientific acquisitions about phytoplasmas and phytoplasma associated diseases are described considering molecular biology, physiological and biological aspects. The study of ribosomal genes allows to obtain a molecular classification of phytoplasmas describing at least 15 different groups; the study of different genes such as those coding ribosomal protein s3, elongation factor *tuf* and others allows to finer discrimination among phytoplasmas and opens the possibility for environmental and epidemiological studies. Recently insect vectors of important phytoplasma diseases such as apple proliferation and stone fruit yellows were identified; transovarial transmission in insects has also been confirmed and phytoplasma presence in alfalfa seeds was reported. At preliminary steps are however researches about molecular-plant interactions when these prokaryotes are infecting plants.

**Key words:** Phytoplasmas, Molecular identification, Epidemiology, Transmission, Plant-pathogen interaction.

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Petria **12**(3), 345-346, (2002) Atti del "Workshop"/*Proceedings of the "Workshop"*

#### **Analysis of genomic diversity of phytoplasmas using AFLP**

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The AFLP (amplified fragment length polymorphism) fingerprinting technique has been used as a tool to analyse genomic variations/diversity between phytoplasmas. Given the different GC% molar content between the plant and the phytoplasma DNA, the total DNA has been digested using enzymes with restriction site composed only of A or T nucleotides. After ligation with oligonucleotide adapters, selective amplification of sets of restriction fragment has been performed by the use of primers that extend into the restriction site in up to 3 A or T bases. Depending on the length of the extension, patterns of different complexity could be visualized by gel analysis. The use of primers with no base extension produced patterns with 30 or more bands, depending on the gel visualization method used (ethidium bromide, silver staining, fluorescence); amplification occurred also from the healthy sample DNA, although the number of bands produced was lower. Conversely, using primers with extension of 3 bases, each sample produced 4 -10 bands (depending on both the strain and the enzyme used), while no bands were detected in the healthy control. The analysis of the amplified fragment allowed a good discrimination between phytoplasmas belonging to different groups (Apple proliferation, Western X disease, Stolbur phytoplasmas). Excellent differentiation could also be obtained within groups, even between strains 100% identical 16S rDNA sequence.

**Key words:** Phytoplasmas, Genomic diversity, AFLP.

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#### **Molecular classification of elm yellows group (16SrV) phytoplasmas based on 16S rRNA and ribosomal protein genes**

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Elm yellows (EY) group (1GSrV) phytoplasmas were previously categorized based on RFLP analysis of 16S rRNA gene sequences. Phylogenetic analysis using 16S rDNA sequences revealed that they form a discrete phylogenetic group (subclade) within the phytoplasma clade. The EY group now represents the third most different phytoplasma cluster, next to aster yellows (group I6SrI) and X-disease (group I6SrIII) phytoplasma groups (Lee *et al.*, 1998). The American elm yellows phytoplasma strain EY1 is the type strain representing the group. The EY phytoplasma strains cause decline of American elms and Eurasian elm species and hybrids. Other EY group phytoplasmas are associated with devastating diseases in grapevine (Flavescence Dorée, FD), blackberry (rubus stunt, RuS), alder (Alder Yellows, ALY), jujube (jujube witches' -broom, JWB), cherry (cherry lethal yellows, CLY), peach (peach yellows, PY) and other plant species in America, Europe and Asia. For epidemiological studies and for quarantine purposes, it is important to have a classification system that allows for rapid and reliable differentiation among these diverse phytoplasma strains. The aim of the present study was to investigate phylogenetic interrelationships among members of the expanded EY phytoplasma group based on combined analyses of 16S rRNA and ribosomal protein (rp) gene sequences, and to develop and evaluate a new means for finer strain differentiation. Five subgroups were identified by RFLP analysis of 16S rRNA gene, while 12 subgroups were resolved by analysis of rp genes. Based on phylogenetic analyses of rp genes or 16S rRNA and rp genes combined, 12 different lineages were resolved consistent with rp subgroups defined by RFLP patterns. Subgroup differentiation based on rp gene sequences appeared to reflect the specific ecological niches (host plant, vector, or geographical regions) of phytoplasmas in the EY group. Thus, this approach represents a more efficient classification system.

**Key words:** Phytoplasma, Elm yellows group, Ribosomal protein genes, RFLP.

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### **H<sub>2</sub>O<sub>2</sub> localization in apple trees infected with phytoplasmas**

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The localization of H<sub>2</sub>O<sub>2</sub> in apple plants, healthy, AP-infected and recovered is here discussed. Peroxidase activity valuation, glutathione and malondialdehyde content were also carried out as attempt to better know the "recovery" phenomena.

**Key words:** H<sub>2</sub>O<sub>2</sub>, Apple, AP phytoplasma, Recovery, Electron microscopy, Peroxidase, Glutathione, Malondialdehyde.

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### **Phytoplasmas and insect vectors**

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The phytoplasma-transmitting insects belong to the order Hemiptera and most frequently to the Families *Cixiidae*, *Cicadellidae* (*Auchenorrhyncha*), and *Psyllidae* (*Sternorrhyncha*). They are all plant suckers, provided with piercing mouthparts, and feed into the plant phloem. The nutritional relations with plants, to which infective insects may transmit phytoplasmas, can be of three types:

obligated, facultative or occasional. The occasional vector species, whose adults feed on various herbaceous, shrubby and woody plants, often behave in a very unpredictable way and, under particular environmental conditions, they can turn into extremely dangerous pests. By feeding on infected plants, the insects can acquire phytoplasmas and become infected. Once infected, they can transmit phytoplasmas to other plants, even belonging to species different from that on which the acquisition has been accomplished. The propagative transmission process is characterized by three different phases, namely: acquisition, latent period and inoculation. The acquisition, lasting some hours/days, is followed by a latent period of 2-4 weeks. The ability to acquire phytoplasmas by feeding on infected plants is higher for nymphs than adults. The retention of infectivity is not affected by moulting and the insect remains infected for its whole life. The most studied vector species in the *Auchenorrhyncha* group belong to *Cicadellidae*, particularly of the subfamily *Deltocephalinae*. Among these, the following species should be mentioned: *Euscelidius variegatus* (Kirschbaum), *Euscelis incisus* (Kirschbaum) and a few species of the genus *Macrosteles*, that live on either mono- or dicots and transmit phytoplasmas causing yellows diseases of weeds, fruit trees, vegetables and ornamental plants; *Fieberiella florii* (Stål), a vector of phytoplasmas to broadleaf and fruit trees; *Scaphoideus luteolus* van Duzee and *S. titanus* Ball, both of which are of nearctic origin. The former, not occurring in Europe, lives on elm trees to which it can transmit yellows diseases while the latter, which has been introduced in the Palaearctic Region more than 40 years ago, is monophagous on grapevine and transmits the phytoplasma agent of Flavescence Dorée. The subfamily Macropsinae, including the species *Macropsis fuscata* (Zetterstedt) and *Rhytidodus decimusquartus* (Schrank), vectors of phytoplasmas to *Rubus* spp. and to *Populus nigra* L. respectively, has recently increased in importance due to the experimental demonstration that also *Oncopsis alni* (Schrank) can transmit phytoplasmas to alder - its natural host - and to grapevine in Germany (Arzone e Alma, 2000; Cousin *et al.*, 1999; Maixner *et al.*, 1995; Maixner e Reinert, 1999). The family *Cixiidae* includes several vector species whose adults feed on trees and shrubs. Many species are polyphagous, while a few are oligophagous or monophagous and feed on plants characterising the habitat they live in. Their nymphs live underground, often associated with ants, and feed on the roots of herbaceous plants. The most economically important species are those infesting palm trees such as *Cocos nucifera* L., to which they transmit phytoplasmas that may bring plants to death in 3-6 months. In both Americas, the vector of this serious disease is *Haplaxius crudus* (van Duzee) (= *Myndus crudus*), whereas in tropical Africa the vector is *Myndodus adiopodoumensis* (Synave) (= *Myndus adiopodoumensis*). In Europe, the most important species is *Hyalesthes obsoletus* Signoret, which is a vector of Stolbur phytoplasmas to Solanaceae such as potato and tomato. In both Germany and France, this insect has recently been found involved in the transmission of phytoplasmas causing in grapevine a disease known as Vergilbungskrankheit or Bois noir (Maixner *et al.*, 1995; Sforza *et al.*, 1998). The following vector species should also be mentioned: *H. mlokosiewiczzi* Signoret, widespread in the Caucasic region, *Pentastiridius beieri* Wagner, which transmits Stolbur phytoplasmas to sugar beet in France, and *Oliarus atkinsoni* Myers, monophagous on flax to which it transmits a disease causing leaf yellowing in New Zealand (Gatineau *et al.*, 2001; Holzinger *et al.*, 2002). Among the *Sternorrhyncha*, an important role in the transmission of phytoplasmas belonging to the Apple proliferation group is played by some species of the genus *Cacopsylla*. The psyllae complete one or more generations per year on wild and cultivated Rosaceae (*Pomaceae* and *Drupaceae*) and overwinter as adult forms, usually on conifers. The following species are known as vectors: *C. pyricola* (Förster) and *C. pyri* (L.), transmitting the pear decline agent; *C. pruni* (Scopoli), vector of European stone fruit yellows to *Prunus* spp.; *C. costalis* (Flor) in north-eastern and *C. melanoneura* (Förster) in north-western Italy, both known as vectors of Apple proliferation (Osler *et al.*, 1999; Tedeschi *et al.*, 2002). The epidemiology of phytoplasma diseases is greatly influenced by their persistent-propagative mode of transmission by either leafhopper or psyllid vectors. Some peculiar aspects of the phytoplasma -vector relationships and of their effects on both the survival and spread of these plant pathogens in field conditions are

discussed in details, namely: the transmission of phytoplasmas to long distance by leafhoppers; the possibility for them to survive in insect vectors that overwinter as either nymphal/adult forms or as eggs (transovarial transmission); the transmissibility of phytoplasmas through the seeds of their hostplants and their possible harmful effects on insect vectors; the duration of the incubation period in plants infected at various stages of growth. More accurate information on this last point would be much useful to prevent the long distance spread of phytoplasma diseases as well as their introduction into new agricultural areas.

**Key words:** Phytoplasmas, Transmission, Epidemiology, Leafhoppers, Psyllas.

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### **Natural infectivity of *Cacopsylla pruni*, vector of European stone fruit yellows phytoplasma**

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*Cacopsylla pruni* transmits European stone fruit yellows (ESFY)-phytoplasma in a persistent manner and retention of infectivity in the vector lasts through the winter (Carraro *et al.*, 2001). This means that the overwintering insects are already infective when in spring they reach the stone fruit trees. The aim of the present work was to determine the natural infection (percentage of insects carrying ESFY-phytoplasma) and the natural infectivity (percentage of insects able to transmit the agent) of *C. pruni*. Groups of 3, 5, 10 and 20 insects were collected in ESFY-infected orchards. They were immediately analysed for the presence of ESFY-phytoplasma or used in transmission trials to infect *P. salicinate* plants. PCR analyses (Deng and Hiruki, 1991; Lorenz *et al.*, 1995) were carried out on groups of: (i) "first" reimmigrant *C. pruni*, that had acquired infectivity the previous year and possibly had retained infectivity during the overwintering on shelter plants; (ii) "old" reimmigrant, that had acquired infectivity the previous year and possibly during April and May of the following spring; (iii) new generation, that had acquired infectivity before abandoning the stone fruit trees as young adults. The transmission trials were carried out in the glasshouse using groups of "first" reimmigrant and new generation of *C. pruni*. Considering the results obtained by PCR of groups of 3 and 5 insects, the minimum expected percentage of *C. pruni* carrying ESFY-phytoplasma is 9.0% for the "first" reimmigrant, 17.6% for the "old" reimmigrant and 9.5% for the new generation. Regarding the infectivity of the same groups, a minimum of 7.6% for the "first" reimmigrant and 0% for the new generation of *C. pruni* were able to transmit the ESFY-agent to test plants. In fact only groups of 10 or 20 individuals infected test plants when *C. pruni* of the new generation was used. The results obtained show that: (i) the "first" reimmigrant *C. pruni*, when they reach the stone fruit trees are highly infected and infective; (ii) the reimmigrant can increase their infection rate by feeding on ESFY-infected stone fruit trees; (iii) the new generation acquire ESFY-phytoplasma on stone fruit trees but not all the infected *C. pruni* are able to transmit the agent before abandoning the primary hosts; this is probably because the latency period has not yet been completed.

**Key words:** Stone fruit species, Phytoplasma, Psyllids.

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### **Detection of European Stone Fruit Yellow Phytoplasma (ESFY) in Homoptera insects captured in peach orchards in northern Italy**

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In the three year period 2000-2002, to obtain greater information about the spreading of European Stone Fruit Yellow Phytoplasma (ESFY) in peach orchards, leafhoppers and psyllids were captured in peach orchards of the Ravenna and Verona provinces. The insects were captured using the "frappage" method or chromotropic traps placed on cultivated and wild stone fruit trees, and, during the winter, on conifers and other wild plants surrounding the areas where an increase had been noted in the disease in recent years. Tests were also made on other wild rosaceous plants (*Prunus cerasifera*, *P. spinosa*, *Rosa canina*, *Rubus fruticosus*), generally asymptomatic, in the vicinity of the affected orchards. Total DNA was extracted from groups of insects (2-5), after classification, or from individual plants and ESFY was identified with PCR-ELISA as described previously (Poggi Pollini *et al.*, 2001). The results obtained showed that:

- numerous groups of *C. pruni* (Scopoli), captured in both provinces on different stone fruit trees (*P. cerasifera*, *P. persica* and *P. spinosa*) were infected by ESFY;
- various *P. cerasifera* and *P. spinosa* trees were found to be infected although asymptomatic. The largest number of infected psyllids were captured precisely on these trees;
- in the winter traps placed on conifers, only one group of *C. pyrisuga* (Forster), collected from a cypress tree in the Verona province in 2002, was positive for ESFY;
- out of the numerous leafhoppers tested, there were only two positive groups of *Macrostelus cristatus* (Ribaut), collected from peach trees in the summer of 2000 in the province of Verona.

Further tests on these leafhoppers in subsequent years did not however confirm these results. The occasional finding of ESFY in leafhoppers has already been reported (Jarausch *et al.*, 2001). It can be concluded that individual infected *C. pruni* were present in the peach orchards of the two provinces examined and that wild stone fruit trees certainly have a role in the epidemiology of the disease as these can, as already indicated in the past, represent good hosts for ESFY, without showing symptoms, as well as for the insect vector (Carraro *et al.*, 2001; Jarausch *et al.*, 2001). This indicates the importance of including these plants in a control program to eliminate the vector psyllid.

**Key words:** Peach tree, Homoptera insects, ESFY phytoplasma, PCR-ELISA.

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## **Vector and graft transmission of European stone fruit yellows phytoplasma to *Prunus* species**

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Several *Prunus* species in Europe are affected by diseases associated with phytoplasmas. Lorenz *et al.*, (1994) determined the common aetiology of these diseases and proposed the single name "European Stone Fruit Yellows" (ESFY). Occurrence of ESFY, widespread in apricot and Japanese plum, has been reported also in both cultivated and wild *Prunus* species (Carraro *et al.*, 2002). In some - i.e. *P. domestica* and *P. spinosa* - the presence of the causal agent was found to be common and frequent; in other species - i.e. *P. avium* - sporadic. The objectives of the present study were to determine, under controlled conditions, the susceptibility of various *Prunus* spp. to ESFY-phytoplasma, by using vector and graft-transmission methods. Seedlings of *P. armeniaca*, *P. persica*, peach GF 305, *P. cerasifera*, *P. spinosa*, *P. tomentosa*, *P. padus*, *P. mahaleb* and micropropagated plants of *P. salicina* were exposed (10 plants/species) to 50 individuals of the vector *Cacopsylla pruni*, collected at the end of winter in plum orchards. Longevity of the insects as

well as oviposition and the presence of the new generation were checked. Similarly, 10 plants/species were graft-inoculated, using as sources of inoculum *P. salicina* and *P. cerasifera* ESFY-infected plants. All the test plants, a representative number of negative controls and groups of *C. pruni*, were analysed for the presence of ESFY-phytoplasma by nested-PCR using the primer pair P1/P7 (Deng and Hiruki, 1991) and f01/r01 (Lorenz *et al.*, 1995). The results obtained showed different susceptibility and sensitiveness of the examined *Prunus* species to ESFY-phytoplasma. *P. salicina* and *P. armeniaca* were highly susceptible and sensitive; *P. persica*, GF 305 and *P. tomentosa* highly susceptible and less sensitive; *P. cerasifera* and *P. spinosa* highly susceptible and tolerant; *P. padus* and *P. mahaleb* highly resistant and tolerant.

**Key words:** Stone fruit species, Phytoplasma, Psyllids.

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### **Natural diffusion of apple proliferation on autochthonous genotypes of apple trees**

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In order to preserve the biodiversity of autochthonous fruit trees, the regional service for agricultural development (ERSA) of Friuli Venezia Giulia Region established, in 1991, a collection-orchard in Enemonzo (Udine). The orchard contains 123 autochthonous genotypes of apple trees from different areas of the Region; 83 of them are represented by 3 plants and the others by 1 or 2 plants. Most of the cultivars were described and classified on the bases of their pomological characteristics (Youssef *et al.*, 2000). The aim of this work was to identify apple varieties that are rustic, adapted and suitable for an organic regime, and to evaluate the susceptibility to the most relevant diseases, such as apple scab, powdery mildew and apple proliferation (AP). Concerning AP, since 1994, the year infected trees first became evident, the orchard has been constantly monitored for the presence of symptomatic trees; severity of symptoms was also recorded. The presence and infectivity of the natural vector *Cacopsylla costalis* (Frisinghelli *et al.*, 2000) was also investigated. Molecular and serological methods were used (Lorenz *et al.*, 1995; Loi *et al.*, 2002) to detect AP. 61% of the plants became symptomatic for at least 1 year; during the last four years the percentage of symptomatic plants ranged from 31,3% to 33%. In 13 out of the 83 cvs represented by 3 trees, not one of the plants has shown AP-symptoms since 1994; serological and molecular biology analyses were performed on roots and branches of these plants. AP-phytoplasma was detected in over 90% of the roots tested. The results obtained show a great difference among apple-tree cvs in response to AP-phytoplasma infection. None of the analysed cvs was completely resistant, but some cvs made a lasting recovery (Osler *et al.*, 2000).

**Key words:** Apple tree, Apple proliferation, Biodiversity.

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### **Experimental transmission of apple proliferation-phytoplasma from both recovered and symptomatic apple trees**

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Recovery” is the spontaneous remission of symptoms in plants previously symptomatically affected by a pathogen (Schmid, 1965). The causes of this phenomenon are so far unknown. Recovery is common in plants affected by phytoplasmas; in particular it is very important for apple proliferation

(AP) (Osler *et al.*, 2000). The purpose of the present work was to clarify some aspects of recovery, such as the presence, localisation and transmissibility of AP-phytoplasma in apple trees. In an orchard of cv Florina, checked yearly since 1990 for AP symptom expression, we chose three groups of plants: symptomatic for at least two years; recovered for at least two years; asymptomatic from the first year of plantation. Roots, stems and leaves from 20 plants/group, were analysed for the presence of AP-phytoplasma by using both serological (Loi *et al.*, 2002) and molecular methods (Lorenz *et al.*, 1995). The results of the analyses showed that all the symptomatic trees were infected in the canopy and in the roots; almost all the trees that had recovered were infected only in the roots (19/20) and more than half of the plants that had never shown AP-symptoms were infected only in the roots (12/20). These results were reinforced by transmission trials carried out by grafting roots and buds, from both symptomatic and recovered apple trees, onto one-year old Florina test plants. The transmission rate of AP-phytoplasma from symptomatic and recovered plants by root-grafting was respectively 98% and 97%; by chip-budding 52% and 0%. We can conclude that there is a correlation between symptom expression and AP-phytoplasma presence in the upper part of the apple trees and consequently the recovery phenomenon depends on the disappearance of the pathogen from the canopy. The phytoplasma in recovered plants is present only in the roots as is the case in some apple trees that have never shown AP-symptoms.

**Key words:** Apple trees, Phytoplasma, Recovery.

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### **Spread of Apple proliferation by root bridges**

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The presence of AP in trees located in the same row or in patches in affected orchards has supposed the existence of natural grafts in the roots of neighbouring trees and the spread of AP by this bridge. In medium-aged or old orchards in Trentino this phenomenon seems to play an important role. Trials applying a systemic herbicide exclusively on the cut of infected trees have caused herbicide symptoms in neighbouring trees presumably by compound translocation through a root bridge. The removal of infected trees often revealed root bridges between neighbouring trees. Histological observations confirmed the tissue connection. We have already been able to evidence the spread of Apple Chlorotic Leaf Spot Virus (ACLSV) by these root bridges. Trials are under way to prove AP transmission.

**Key words:** Apple, Apple proliferation, Transmission, Root bridge.

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### **The identification of the crucial period for the transmission of Apple proliferation by *Cacopsylla melanoneura* (Förster) (Homoptera Psyllidae)**

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*Cacopsylla melanoneura* (Förster) was described as a vector of the Apple Proliferation (AP) in northwestern Italy (Tedeschi *et al.*, 2002). The psyllids lay the eggs on apple trees, where they develop until the emergence, then they move to other hosts, probably conifers. The seasonal abundance of the vector was measured using yellow sticky traps with weekly changes from the end of January until the end of May. *C. melanoneura* accomplishes one generation per year: the overwintered adults start to colonise apple orchards at the end of January and reach the peak

between mid-February and mid-March; from the beginning of May the new adults start to emerge and remain on apple trees until the end of the month, then they reach alternative hosts where they spend the summer and overwinter. The overwintered adults were present in the apple orchards with a higher population level and for a longer period comparing with the newly emerged adults (14 versus 6 weeks). The presence of AP in the adults was detected by PCR. Total DNA was extracted from batches of 5 insects following the procedure described by Marzachi *et al.*, (1998). A first assay with the universal primers P1/P7 (Schneider *et al.*, 1995) was followed by a second amplification with the primers f01/r01, specific for the AP-group (Lorenz *et al.*, 1995). The estimated percentage of AP-positive adults was higher for the overwintered insects comparing with the springtime generation (3.5% versus 0.8% in 2000 and 2.9% versus 0% in 2001). The higher population level, the longer permanence on apple trees and the higher proportion of AP-positive specimens suggest the crucial role of the overwintered adults in the diffusion of the AP phytoplasma. The most critical period for AP transmission is between mid- February and mid-March, when the population level reaches the peak. Earlier transmissions, due to the first overwintered immigrant adults (already infected), feeding on the buds before the development of the leaves, cannot be excluded.

**Key words:** Phytoplasma, AP, Population dynamics, Transmission, Molecular diagnosis.

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Petria **12**(3), 381-386, (2002) Atti del "Workshop"/*Proceedings of the "Workshop"*

### **Phytoplasma diseases of forest trees, landscape trees and shrubs in Europe**

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In Europe, severe yellows, witches'- broom and decline diseases affect elm, alder, poplar, eucalypt, buckthorn, Spanish broom, broom (*Sarothamnus scoparius*, Judas tree, brambles, blueberry, ornamental apples and flowering cherry. These diseases are more widespread than previously known and are of considerable economic and environmental importance. They are caused by distinctly different phytoplasmas of which the identity has accurately been determined. Phytoplasmas have also been reported to occur in oak, hornbeam, ash, European hackberry, cypress, magnolia, willow, rose, forsythia, spirea, myrtle, lilac and red dogwood. Their detection is only based on highly sensitive nested PCR assays, with no data from pathological studies. However, in these cases, the etiological role of the low-number infections needs to be confirmed by graft transmission experiments. Phytoplasma diseases of black locust, hazel (*Corylus maxima* e *C. colurna*), hawthorn, black elder, European red elder and weeping-willow have been described on the basis of symptomatology and microscopical examination. However, the identity of phytoplasmas infecting these species has never been determined. Recently, an hitherto unknown type of phytoplasma has been identified in pine (*Pinus sylvestris*).

**Key words:** Phytoplasmas, Elm yellows, Alder yellows, Decline diseases.

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### **Identification of a phytoplasma associated with Flavescence Dorée in clematis (*Clematis vitalba*)**

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*Flavescence Dorée* is a serious grapevine disease, spreading out mainly in Italy, France and Spain, where it causes heavy economical damages. FD etiological agents are some phytoplasmas very similar to each other, belonging to ribosomal group 16SrV (Lee *et al.*, 1998), transmitted by the

ampelophagus Cicadellidae *Scaphoideus titanus*. Five FD isolates were identified so far: FD70 and FD2000, detected only in France, FD-D, also named FD88 or FD92, recovered in France, Italy and Spain, and two strains FD-C, detected in Italy, one in Veneto region and the other in North-West Italy. (Daire *et al.*, 1997; Martini *et al.*, 1999; Angelini *et al.*, 2001; Martini *et al.*, 2002; Angelini *et al.*, in preparation). None of the FD *sensu stricto* isolates have been ever detected in nature in other plant species but grapevine. The aim of this study was to identify plant species that in nature can harbour phytoplasmas causing FD. During summer 2001, about 40 different woody or herbaceous plant species were collected in the thicket close to some vineyards with declared presence of Flavescence Dorée (FD) since many years, in Treviso district. PCR/RFLP assays were carried out on DNA extracted from leaves of each species, in order to detect possible phytoplasmas presence. Universal primer pairs for phytoplasmas and primer pairs specific for ribosomal group 16SrV were used, in direct and nested PCR experiments. The DNA fragment coding for phytoplasmal 16S ribosome was amplified using primer pairs P1/P7, followed by R16F2n/R2, by 16r758f/M23Sr or by R16F1/R1(V). The extra-ribosomal DNA FD9 portion was amplified using primer pairs FD9f/r, followed by FD9f3/r2. Samples showing amplification bands after nested PCR with primer pairs R16F2n/R2, 16r758f/M23Sr and FD9f3/r2 were digested with restriction endonucleases *TaqI* and *MseI*. Only samples from *Clematis vitalba* were found to be infected with phytoplasmas. RFLP patterns were always identical to those from FD-C Italian isolates. Sequencing was carried out on ribosomal fragment 16r758f/M23Sr and extra-ribosomal fragment FD9f/r2 from clematis phytoplasma. Both sequences were identical to those obtained from FD-C phytoplasma recovered in symptomatic grapevines grown in the close vineyards. This work pointed out the possibility that other wild plants can harbour FD-C phytoplasma, which is present in Treviso district on grapevine since '90. The discovery of clematis infected with phytoplasmas states the problem of the role of wild plants growing close to vineyards. In fact those plants, not sprayed, could have a role in epidemics and/or reinfections of surrounding vineyards, even in presence of pest control against phytoplasmas vectors. Also the particular sanitary situation of grapevines located in the border of vineyard, close to thickets, could be correlated to phytoplasmal infections of wild plants.

**Key words:** *Flavescence Dorée*, Grapevine, Phytoplasma, PCR/RFLP, Sequencing.

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### **Detection and molecular characterization of group V phytoplasmas in several herbaceous hosts**

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During 2000, one *Euphorbia characias* L. with phytoplasma-like symptoms was collected in the Mediterranean bush near Varigotti and a tomato (*Lycopersicon esculentum* Mill.) with symptoms was sampled in a field in the Albenga area. During 2001 two cultivated marguerite daisies (*Argyranthemum frutescens* L.) without clear symptoms, but showing lack of flowering were also collected in the same region. Total DNA was extracted from each sample and phytoplasma infection was determined by PCR with specific primers. Group-V phytoplasmas were detected in each sample and further sub-group classification was achieved by RFLP analysis of ribosomal as well as other genomic sequences. Two sub-groups V phytoplasmas, -A and -C/-E, were detected in the two daisy samples. RFLP characterization of the group-V phytoplasma detected in tomato showed that digests with 2 restriction endonucleases were identical to those obtained from V-C and V-E reference isolates, while digests with the remaining 3 endonucleases were unique and different from those observed with all the available group-V reference isolates. RFLP patterns of the ribosomal group-V specific amplicon obtained from *E. characias* were identical to those obtained

from V-C and V-E reference isolates, but a distinctive and unique combination of RFLP patterns resulted from analyses of another genomic sequence. This is the first report of group-V phytoplasma infection in *A. frutescens* and *E. characias*.

**Key words:** Phytoplasmas, Detection, Molecular characterization, Herbaceous hosts.

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Petria 12(3), 393-394, (2002) Atti del "Workshop"/*Proceedings of the "Workshop"*

### **Detection of phytoplasmas in ruscus with decline and yellows symptoms**

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Since spring 2001, 10 samples of ruscus (*Danae racemosa* L.) from Western Liguria were tested to verify the presence of phytoplasmas and/or viruses associated to symptoms such as declines, yellows or loss of apical dominancy (witches' brooms); some of the samples tested showed also green spots on the leaves. Together with symptomatic samples, two asymptomatic plants from the same fields, were tested. Virological assays (mechanical inoculation on indicator plants, PAS-ELISA, electron microscopy) allowed to verify the presence on plant showing green leaf spots of AMV (*alfalfa mosaic virus*), already reported to infect *Ruscus hypoglossum* L. in Emilia-Romagna (Bellardi *et al.*, 1994) and *D. racemosa* in Tuscany (Garibaldi *et al.*, 2000). To verify the possible phytoplasma infection different nested-PCR assays were performed. After a direct PCR with universal primers P1/P7 (Deng and Hiruki, 1991; Schneider *et al.*, 1995) two nested PCR pathways were compared: the first one using primers R16F2/R2 (Lee *et al.*, 1995) followed by primers 16R758f/16R1232r (=M1/M2) (Gibb *et al.*, 1995), and the second one using primers F1/B6 (Lee *et al.*, 1995; Padovan *et al.*, 1995). Six positive samples were detected following both PCR pathways. RFLP analyses on the nested-PCR products obtained from the two PCR pathways, using the restriction enzymes *TruI* and *Tsp509I* allowed to preliminarily classify the phytoplasmas detected. The positive samples tested in Spring were found to be infected by phytoplasmas belonging to ribosomal group 16SrIX and 16SrV, while one of the samples tested in summer was found to be infected by a phytoplasma of 16SrXII-A subgroup. The samples infected by phytoplasmas belonging to ribosomal groups 16SrV and 16SrXII-A were the two asymptomatic plants. A correlation between symptom and detected agent was possible only for the plants showing yellows that were found to be all infected by phytoplasmas belonging to group 16SrIX. No correlations have been found between phytoplasma infections and symptoms such as witches' brooms or bushings.

**Key words:** Ruscus, Decline, Phytoplasma, PCR-RFLP.

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### **Molecular identification of phytoplasmas in hypericum plants showing virescence and witches' broom symptoms**

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*Hypericum perforatum* L. (*Hypericaceae* = *Guttiferae*) is an important medicinal and aromatic plant with several medical properties (digestive, immunostimulant, antidepressant, antispasmodic, anti-inflammatory, antiseptic, astringent, woundhealing, etc.). During spring and summer 2002 molecular tests to verify phytoplasma presence were carried out in plants belonging to an experimental field at the second year of cultivation and located in Ozzano (Bologna). In the cultivation were present 10 varieties and/or cultivars grown from seed produced in 2000 from farms located in Italy and abroad. Starting from April increasing percentages of plants showing dwarfing,

yellow leaves and lack of flower production were observed. The majority of the symptoms (more than 50%) was observed in cv Godet Deborance, that is normally dwarf but that in this case showed plant only 10-15 cm high. Specific direct and nested PCR as well as RFLP analyses were performed on symptomatic and asymptomatic samples of “Godet Deborance”, of symptomatic “Zorzi” and “linea 24”. As further healthy control samples of asymptomatic hypericum were collected in the Herb Garden “Augusto Rinaldi-Ceroni” of Casola Valsenio (Ravenna). From the tests performed the majority of symptomatic samples showed the presence of phytoplasma specific bands in agarose gel after nested PCR with primers PA2f/r on P1/P7 amplicons (Deng and Hiruki, 1991; Schneider *et al.*, 1995; Heinrich *et al.*, 2001). RFLP analyses with *TruI* e *Tsp509I* showed that the phytoplasma present could be assigned to group 16SrVII (“ash yellows”). Samples from Casola Valsenio as well as asymptomatic samples were negative in direct and in nested PCR. This is the first report of phytoplasmas in hypericum and of group 16SrVII in Europe. The disease is severely affecting from economical point of view the hypericum cultivation since not only reduces the regular plant growth but also the inflorescence formations. This is the most important part of the plant used to extract of molecules required for farmaceutical market. Further studies are in progress to verify influence of phytoplasma presence on quantity and quality of hypericin and essential oils.

**Key words:** *Hypericum*, Virescence, Witches’ broom, Phytoplasma, PCR-RFLP.

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Petria 12(3), 399-404, (2002) Atti del "Workshop"/*Proceedings of the "Workshop"*

### **Grapevine yellows: evolution of the disease since its appearance in Italy**

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A grapevine disease, very similar to Flavescence Dorée (FD) in France, was observed in Italy at the end of ‘60 years in Oltrepò pavese (Lombardia region, northern Italy). Later, FD occurred in other regions including Veneto, Liguria, Friuli Venezia Giulia, Piemonte, Emilia and Trentino where severe damage to the plants and serious crop losses for the production were recorded: also, in the same areas, high populations of *Scaphoideus titanus*, the vector of the disease, were observed. The phytoplasmas detected in FD affected plants belong to elm yellows group (16SrV) and in particular to the subgroups 16SrV-C and 16SrV-D. Bois noir (BN), the less epidemic form of grapevine yellows, is widely distributed in Italy: the phytoplasma 16SrXII-A is constantly associated with diseased grapevines. Other phytoplasmas, like 16SrI-B, 16SrIII and 16SrX, were occasionally found in symptomatic and in symptomless vines but their role in the disease etiology should be investigated. Nowadays, in northern Italy, FD is present in almost all the areas where grapevine is cultivated: 16SrV-D and 16SrV-C phytoplasmas were identified in grapevine although the first one is prevalent in the northeastern area with the exception of Piemonte region where, up to now, only 16SrV-C phytoplasma has been found. Besides, after the first report in southern Italy, BN largely occurs in all the Italian regions even if its importance is seldom scarce; moreover, *Hyalestes obsoletus*, the vector of the disease, is present in the central and southern Italian regions. Recently, the finding of FD phytoplasma in vineyards of the central Italy (Marche region) indicates the necessity of a more strict application of the prevention measures for the control of such disease and the need of the use of healthy grapevine material for the new vineyards.

**Key words:** Yellows, Grapevine, Phytoplasmas

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### **Distribution of Flavescence Dorée and Bois noir in Lombardia region (northern Italy)**

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In Italy, after the first record in Oltrepò pavese, Flavescence Dorée (FD) occurred in Veneto, Friuli-Venezia Giulia, Piemonte, Lombardia and Emilia. Currently, the disease is widely distributed in northern Italy with the exception of Valtellina (Lombardia region). In 1999 the administration of Lombardia region supported a specific research project in order to acquire, also, detailed information about the distribution of FD and Bois noir (BN) in this region. Thus, specific investigations were conducted in 18 vineyards located in six zones: Oltrepò pavese, S. Colombano hills, Alto Mantovano, Oltrepò mantovano, Garda bresciano and Franciacorta. Each vineyard was examined for two years (2000 and 2001): after the observation of the symptoms, selected symptomatic and asymptomatic grapevine plants were sampled and tested for the identification of the phytoplasmas involved. DNA extracts from such grapevine tissues were analyzed by PCR. The primer pair 16SrF2n/16SrR2 (abbreviated 16SrF2n/R2), designed for universal amplification of 16SrDNA gene from all known phytoplasmas, was used for a first round of PCR tests. A second round of PCR test (nested-PCR) was conducted for all the examined samples, using primer pairs designed for specific amplification of aster yellows and stolbur (R16[I]F1/R1), and elm yellows (R16[V]F1/R1) phytoplasma groups. RFLP analyses using 10 different restriction enzymes were conducted by single enzyme digestions of the above mentioned amplicons. The obtained data indicate that FD is broadly distributed in all the vineyards: in particular the phytoplasma subgroup 16SrV-D was detected in all the areas above cited while 16SrV-C was found only in some vineyards of Oltrepò pavese. At the end, although, 16SrXII-A (BN) phytoplasma was found in several vineyards, its frequency was clearly low.

**Key words:** Flavescence Dorée, Bois noir, Lombardia Region.

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### **Possible reduction of grapevine Flavescence Dorée by a careful winter pruning**

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Tests were done in five vineyards located in Lombardia (northern Italy). 68 grapevine plants showing symptoms of Flavescence Dorée (FD) and tested positive for the FD phytoplasma were chosen during late summer 2000. In February 2001 the plants were pruned by leaving only the canes without symptoms. In July and September 2001 the 68 vines were checked for both FD symptoms and FD infection. Complete recovery (no symptom and no infection) was obtained in 17 vines with the following distribution among the different tested varieties: 7 out of 20 in Barbera, 3 out of 7 in Cabernet Sauvignon, 5 out of 22 in Lambrusco, 2 out of 19 in Chardonnay. Five more plants of Barbera did not show any symptom during summer 2001 and resulted negative in July and positive in September for FD-phytoplasma infection. This can be due to a new infection transmitted in summer 2001 by the insect vector *Scaphoideus titanus*. These results suggest the possibility of reducing FD incidence using a careful winter pruning supported by an efficient control of the insect vector.

**Key words:** Flavescence Dorée, Grapevine, Winter pruning efficacy.

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***English summary not available***

## Studio del deperimento vegeto-produttivo della vite dovuto a Flavescenza Dorata

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A partire dalla primavera-estate del 1999 sono stati riscontrati gravi sintomi di un deperimento vegeto-produttivo sulle viti dell'Oltrepò pavese. Le indagini di campo e le successive analisi biomolecolari hanno permesso di accertare la presenza della Flavescenza Dorata in vigneti dell'area suddetta, analogamente a quanto segnalato nelle confinanti aree viticole piemontesi. Immediatamente, la Regione Lombardia ha istituito un gruppo di lavoro formato da ricercatori, e tecnici appartenenti ad Enti diversi del settore vitivinicolo. È stato, quindi, allestito e finanziato un progetto di ricerca triennale (1999-2001) coordinato sotto l'aspetto scientifico dall'Istituto di Entomologia e Patologia Vegetale dell'Università Cattolica del Sacro Cuore di Piacenza e dall'Istituto di Patologia Vegetale dell'Università degli Studi di Milano. La presente comunicazione ha lo scopo di illustrare gli obiettivi del progetto di ricerca, le attività finanziate e i principali risultati raggiunti, mentre rimanda per il dettaglio dei lavori svolti agli specifici contributi curati dai due Istituti universitari.

**Parole chiave:** Vite, Flavescenza Dorata, *Scaphoideus titanus*.

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Petria 12(3), 411-412, (2002) Atti del "Workshop"/*Proceedings of the "Workshop"*

## Role of *Hyalesthes obsoletus* Signoret (*Homoptera Cixidae*) in the transmission of grapevine Bois noir in Italy

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Phytoplasmas belonging to the taxonomic subgroup 16Sr-XII-A (stolbur), *sensu* Lee *et al.*, (1998), are known to cause yellows in grapevine. These diseases, widespread in different vine-growing areas in Europe, are characterised by the same symptomatology, but different epidemiology and are known with the names of: Bois noir (BN) in France and in many other countries, Vergilbungskrankheit (VK) in Germany and Legno Nero (LN) in Italy; BN and VK are transmitted by *Hyalesthes obsoletus* Signoret (Alma e Conti, 2002). The research carried out, to ascertain the role of the cixiidae, by field surveys in different vine-growing areas of Piedmont, laboratory rearings, transmission trials and molecular analysis of plants and insects, started in 2001 and is still in progress. Total DNA from symptomatic field-collected grapevines and wild plants, experimentally inoculated plants and *H. obsoletus* was analysed by PCR with the specific primers pair M1/P8 (Marzachì *et al.*, 2000). All plant and insect samples with negative result were analysed by molecular hybridisation with a labelled specific probe. In the laboratory, *H. obsoletus* was able to complete its biological cycle only on *Urtica dioica*, confirming the field results. Nettle proved to be the principle host plant and, with the 17% of stolbur-positive samples, a dangerous and new source of inoculum. The molecular analysis revealed that 40% of field collected *H. obsoletus* adults were infected with the stolbur phytoplasma. The grapevine seedlings and periwinkles experimentally inoculated with adults of the cixiidae collected in different grapevine-growing areas showed the first symptoms after one month and all the symptomatic ones tested positive for the presence of stolbur.

**Key words:** Legno Nero, Bois noir, *Hyalesthes obsoletus*, *Urtica dioica*, Transmission.

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## **Program report of three years of actions taken for the eradication of grapevine Flavescence Dorée in Friuli**

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Viticulture in the Friuli-Venezia Giulia (F-VG) region holds a leading role in agricultural business: a high quality grape and vine producing system is operative in this region, alongside vine nurseries which are famous world-wide and which traded over 50 million grafted young vines in 2001. The local Plant Protection Service's main goal is to take care of these important areas of activity. Among Grape Yellows (GY), Bois Noir (BN) in F-VG have been identified since the beginning of 80's, whereas first records of the presence of Grapevine Flavescence Dorée *sensu stricto* (FD) date back to 1996. The disease which had been detected as a result of a precise monitoring (which is still continuing) done in a phase prior to that of an economic loss, had remained confined to a small area up until 1998. Then, since 1999 FD has, on the other hand, begun to spread at an alarming rate. In 2000 the F-VG's authorities set up a special Program in order to eradicate FD from the region. The program's procedures which can be carried out thanks to a national decree regarding the compulsory control of FD (D.M. 2000.5.31 Th) includes both the control of the leaf hopper *Scaphoideus titanus* Ball, (vector of FD) and the removal of infection foci due to infected vines. The control of *S. titanus*, which has already been imposed on the vine nurserymen in Pordenone since 1990 and included in local Integrated Pest Management programs, has been made obligatory for all vineyards in the region, and is based on one or more seasonal spraying procedures, depending on epidemic risk. Since it is impossible to distinguish FD from other GY just through observation of the symptoms, removal of FD infected vines has been done by uprooting out all vines with GY symptoms. When the incidence of symptomatic vines rose above 25%, the entire vineyard had been uprooted. The singling out of symptomatic vines has been done during systematic inspections which were carried up in each vineyard included in Focus Area by specifically qualified inspectors. Since the program is of public interest, economical support has been provided to the grape growers who have undergone financial loss of a vineyard or of single vines as a result of uprootage; support also regards insecticide sprays. Financial procedures involved Co-operative Vineries and other relative associations. In the year 2000 Focus Area included 949 hectares of vineyard, divided up into a large number of proprietries, in 6 councils in the province of Pordenone, close to the borderline of the Veneto region. In the year 2001 the Focus Area included two other councils, amounting to 1.380 hectares of vineyard in all. Finally in the year 2002 the Focus Area involved 13 councils in the Pordenone province and 2 in Udine, extending across an area over 1.900 hectares of vineyard in all. In 2000, 15,01 hectares and other 14.987 single vines, which put together represent 2,58% of the vines in Focus Area, had been uprooted. In 2001 another 19,67 hectares and another 13.921 single vines (equivalent to 2,08% of the total) were uprooted. In 2002, in addition to the eradication program (mentioned previously) within the Focus Area, similar measures have been carried out on 1.900 hectares of european vineyards, within a very large area of nursery activity. Results within the Focus Area are very encouraging (0,66% of symptomatic vines) and confirm the efficacy of the measures that have been adopted. In the Nursery Area (0,16% of symptomatic vines mostly present in fields not used as Mother Plant vineyards) the disease seems to be under control. All symptomatic vines sampled in Nursery Area submitted to PCR testing resulted affected by BN. In all areas in which FD is known to be diffused, almost all grape cultivars showed GY symptoms. While as far as BN is concerned, mainly Chardonnay and the Pinot group proved to have symptomatic vines. Collected data confirms that vineyards planted recently, and well managed by skilled grape growers shows a lower frequency of GY affected vines compared to old vineyards managed with less professional skill. The Phytosanitary measures adopted since, made it possible to contain the damage in grape growing and wine making within a reasonable margin of economic loss and has also kept the area involved in nursery production free

from FD. Prospects of eradication of FD in F-VG seems now reasonably favourable, at least to easily maintain the condition of "Low Pest Prevalence" included in FAO glossary. As far as the economical cost is concerned (about 750.000 Euro in 3 years, including financial support to vine growers and inspection costs) it altogether represents less than 1 per thousand of the protected production's value. It seems that this figure has made up for any potential damage to the grape and wine business, as well as the fitosanitary department's guaranteed support to the local vine nursery sector.

**Key words:** Flavescence Dorée, Bois Noir, Grapevine Yellows, Monitoring, Eradication Program, Vine Nurseries.

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## POSTER SESSION

Petria **12**(3), 419, (2002) Atti del "Workshop"/*Proceedings of the "Workshop"*

### **Report of phytoplasma in arboreal cultivations in Lombardy**

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Besides the outbreak of the epidemic of Grapevine Flavescence Dorée, even the presence of other phytoplasmosis on some arboreal cultivations in the provinces of Brescia and Sondrio was verified. The presence of European Stone Fruit Yellows, Pear Decline and Apple Proliferation was pointed out on sensible species. Such diseases had already been present in Lombardy for some years, but their spreading within allotments had kept to low levels up to now, not causing considerable economical damages to cultivations for the time being. A more accurate analysis was done for the spreading of Apple Proliferation in the province of Sondrio, where the disease showed a strong growth during last years, affecting even young implantations and sometimes reaching infection levels higher than 20%. As for vectors, the presence of *Cacopsylla melanoneura* has been identified, while that of *Cacopsylla costalis* has not been verified, yet.

**Key word:** Phytoplasmas, Arboreal cultivation, Lombardia.

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Petria **12**(3), 421-422, (2002) Atti del "Workshop"/*Proceedings of the "Workshop"*

### **Preliminary results of a study performed in Campania and Latium to evaluate fruit tree phytoplasma transmission ability of *Empoasca decedens* Paoli**

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Leafhoppers were field collected to test their involvement in phytoplasma transmission to plum and apricot trees. Two plum and apricot orchards located in Rome (Latium) and in Caserta province (Campania) respectively, where symptomatic trees resulted infected by 16SrX-B phytoplasmas (ESFY = "European Stone Fruit Yellows"), were surveyed. The leafhoppers, collected in Campania in June and July 2001, resulted positive to molecular tests for phytoplasma detection and were identified as *Empoasca decedens* Paoli (*Homoptera - Typhlocybinae*). The *E. decedens* Paoli are green leafhoppers with adults 3-4 mm long, very frequent in Southern Italy, where develop several generations (also 4-5), wintering as adults on herbaceous plants; they induce alterations of parenchyma, particularly in potato and beet, afterwards they remove upon fruit trees (Tremblay,

1995). Nested-PCR/RFLP analyses on these insects revealed ESFY phytoplasma presence in some of the *E. decedens* collected in Casertan field. Laying adhesive snares in January and February 2002 on apricot and plum trees, numerous adults of *E. decedens* in Campanian field were found; these samples resulted positive to 16SrI, 16SrV and 16SrX-B phytoplasmas. In the Roman field, in the same period, no *E. decedens* was found on the snares. Many samples, prevalently young, of *E. decedens* were collected in March and April 2002, but resulted negative to molecular tests for phytoplasma detection. Nucleic acids of adult samples of *E. decedens*, collected in May and July, were extracted and the nested-PCR/RFLP analyses are in progress. *Empoasca decedens* Paoli was never reported before as phytoplasma vector.

**Key words:** Leafhoppers, *Empoasca decedens* Paoli, Detection, Phytoplasma, ESFY, PCR-RFLP.

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Petria 12(3), 423-425, (2002) Atti del "Workshop"/*Proceedings of the "Workshop"*

### **Detection of European stone fruit yellows and pear decline in Calabria and Basilicata (Italy)**

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In the neighbouring regions Calabria and Basilicata of southern Italy, diseased trees of peach, apricot and Japanese plum, showing typical symptoms of European stone fruit yellows and pear trees with pear decline symptoms were examined for phytoplasmal infections using PCR technology. From diseased apricot, Japanese plum and pear trees as well as from 8 out of 70 symptomatic peach trees, the target DNA could be amplified by one-round PCR using both universal phytoplasma primers and specific primers for fruit tree phytoplasmas of the apple proliferation group, all derived from 16S rDNA. RFLP analysis revealed that stone fruit and pear trees were infected with the European stone fruit yellows and pear decline phytoplasmas, respectively. This is the first report on the occurrence of these phytoplasmas in Calabria and of ESFY phytoplasma infecting peach and apricot in Basilicata. Within the ESFY phytoplasma, genetic polymorphism was observed when PCR-amplified Tuf gene sequences were digested with *TaqI* restriction enzyme. Two distinct restriction profiles were identified among the ESFY isolates examined.

**Key words:** Stone fruits, European stone fruit yellows, Pear decline, PCR.

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### **Identification of strains of Apple Proliferation in psyllids at several development stages in Trentino's orchards**

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Apple Proliferation (AP) is a disease caused by phytoplasmas in a close phylogenetic relationship with Pear Decline (PD) and European Stone Fruit Yellows (ESFY) phytoplasmas, which are classified in the same AP cluster. In Trentino the disease is known since 1950 but recently it has increased mostly in Val di Non and Val di Sole. The psyllid *C. costalis* was demonstrated one of the possible vectors of the disease in this area so AP is currently checked by molecular analysis on the insects. We aim to better define the acquisition period of the phytoplasma, the capacity of the vector transmission and to verify the possible involving of other psyllid species and other insects in the transmission of the disease. In the 2001 about 5500 psyllids were picked up periodically in orchards and uncultivated in different areas of val di Non. To detect AP, PCR analysis was performed using

the DNA extracted from groups composed by 15 insects split up by species and development stages. Amplification products were subsequently digested with restriction enzymes in order to distinguish different strains of the phytoplasma. Forty samples showed positive signal. In particular *C. costalis* overwintered was more infected than *C. melanoneura*. The youth stages resulted less infected than adult stages.

**Key words:** AP phytoplasma, Vectors, Psyllids, PCR, Monitoring.

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Petria 12(3), 429, (2002) Atti del "Workshop"/*Proceedings of the "Workshop"*

### **Detection of European Stone Fruit Yellows phytoplasma in Slovenia**

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Phytoplasmas are small wall-less prokaryotes that live exclusively in sieve tubes of their plant hosts and are transmitted by insect vectors and vegetative propagation. In Europe, stone fruits are severely affected by European Stone Fruit Yellows (ESFY) caused by phytoplasmas belonging to apple proliferation group (16SrX). Recently, progress of ESFY disease in Slovenia was reported based on visual assessments of symptoms. For the routine detection of ESFY sensitive and specific molecular methods were introduced. The presence of ESFY phytoplasmas was confirmed by polymerase chain reaction (PCR), nested PCR and restriction fragment length polymorphism (RFLP) analyses in apricot, peach and plum trees from different regions in Slovenia.

**Key words:** Detection, ESFY, PCR, Phytoplasma, RFLP, Stone fruit, Slovenia

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Petria 12(3), 433-435, (2002) Atti del "Workshop"/*Proceedings of the "Workshop"*

### **Identification and geographic distribution of grapevine yellows in Trentino**

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Grapevine yellows are diseases of *Vitis vinifera* caused by phytoplasmas. They were detected since 1986 in Trentino but strictly belonging to Bois Noir (Mescalchin *et al.*, 1986). Although the insect *Scaphoideus titanus*, the specific vector of Flavescence Dorée (FD), was discovered in Valsugana (Trentino) already in 1990, probably imported as overwintered egg with nursery material, Flavescence Dorée was not detected in Trentino until the 2001. As a consequence, starting from 2001 a broad monitoring campaign to detect the presence of FD in a large area near to the border of the Verona Province was initiated by the Istituto Agrario of San Michele all'Adige in collaboration with the Ufficio Fitosanitario. A molecular approach based on the amplification by PCR with primers specific to the 16 S ribosomal RNA of FD and BN was used. This DNA analysis allows to distinguish among differences in groups and subgroups of phytoplasmas in leaf and insect samples. In total 57 cases of FD and 100 of BN were found over the 160 farms that were monitored. This correspond to 12% of farms where was found at least a sample with FD infection in comparison of 36,4% of those in which was found at least a positive sample to BN and to 13,2% of those that presented a mixed infection. The parallel sampling of *S. titanus* in the same area showed an increase in the population with respect to the year 2000 but a low number of infected individual. For the actual year the aim is to monitoring the movement and the rate of diffusion of the phytoplasmatic diseases in plants and insects in the Province.

**Key words:** PCR detection, Yellows grapevine, Flavescence Dorée, Bois noir, Vectors.

## **Epidemiological studies on Central Tuscany vineyards affected by bois noir phytoplasmas**

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Beginning in 2000 epidemiological studies on two Chardonnay and one Sangiovese vineyards from Central Tuscany, affected by "bois noir" phytoplasmas, show an epidemic of the disease. From 2000 to 2002 Chardonnay symptomatic plants ranged from 15% to 31.32%; from 2001 to 2002 Sangiovese symptomatic grapevines ranged from 0,57% to 3,66%. Also vineyard environment can be supposed to influence "bois noir" spreading and grapevine possible recovery.

**Key words:** Phytoplasma, Grapevine, Bois noir.

## **Epidemiological studies on the grapevine bois noir disease in Emilia-Romagna**

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Research has been started into the epidemiology (insect vectors and alternate host range) of bois noir (BN) disease in the Emilia-Romagna region of Northern Italy. BN is an economically important grapevine yellows induced by a phytoplasma belonging to the 16SrXII group (Stolbur), subgroup A (Lee *et al.*, 1998). Populations of the planthopper *Hyalesthes obsoletus* Sign., reported to be a vector of the disease agent (Maixner *et al.*, 1995; Sforza *et al.*, 1998), were monitored during the 2001 growing season in two vineyards where BN was known to occur. Insects were captured at weekly intervals from June to August using yellow sticky traps, counted and tested by PCR for the presence of BN phytoplasma. Insects were also collected from nearby self-sown vegetation using a sweep net, counted and tested in the same way. The P1/P7 oligonucleotide universal primers were used for the initial amplification of a DNA fragment consisting of the complete 16S rRNA gene together with the 16S-23S spacer region and the 5' terminal region of the 23S rRNA gene. A second pair of nested primers, fStol/rStol, were subsequently used to amplify a specific target sequence from the stolbur phytoplasma associated with BN (Maixner *et al.*, 1995). Results from the survey showed that the population of *H. obsoletus* peaked around the middle of July. In total, 340 insects were collected and 218 assayed by nested PCR for the BN phytoplasma. Adults of *H. obsoletus*, tested either individually or in groups of three insects, were positive for stolbur at levels of 40.7% and 13.7%, respectively. Wild plant species were collected from within the vicinity of the vineyards and analyzed using the same PCR method. The wild plant species, *Cirsium arvense* (L.) Scop., *Convolvulus arvensis* L., *Picris echioides* L., *Plantago lanceolata* L., *Potentilla reptans* L., *Setaria viridis* (L.) Beauv., *Silene alba* (Miller) Krause., *Sonchus arvensis* L. and *Urtica dioica* L. have so far been shown to be infected with the BN phytoplasma. These results represent an important step towards a better understanding of the epidemiology of BN in our viticultural regions.

**Key words:** Grapevine, Bois Noir, *Hyalesthes obsoletus*, Phytoplasma, Plant hosts, PCR.

## Observations on “Bois noir” of grapevine in Sardinia

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The presence in Sardinia of yellows symptoms was confirmed in grapevine cvs Cannonau, Nieddera, Vermentino, Malvasia, Moscato, Redagliadu, Chardonnay, Trebbiano, Bianca madura, Mona bianca, Taloppo, and in cv. Cinsault from Corsica. Field observations, conducted from May to October, showed that symptomatological reactions were erratic, differing according to the variety and the year. ‘Vermentino’ was the object of a three-year investigation for assessing the distribution of the disease and defining its symptomatological and etiological aspects. About 600 vines from three different vineyard in the Alghero area were monitored. The peak of symptomatological expression, consisting of typical yellow vein banding was between September and October. Symptoms were often limited to some leaves and were sporadically associated with irregular maturation of the wood, leaf rolling, and withering of the bunches. The highest percentage of vines with typical leaf symptoms (20%) was recorded in 1999, while the highest incidence of uncertain, non typical symptoms (33%) was observed in 2001. PCR tests showed that phytoplasmas of the 16SrXII-A subgroups were present in 18% of the asymptomatic vines, 36% of the vines with uncertain symptoms, and 26% of the vines that had shown typical symptoms at least once. The latter result suggests that some of these vines may contain phytoplasmas which do not belong in the stolbur group, but cause similar symptoms. Electrophoretic profiles of DNA restriction fragments amplified with primers R16(I)F1/R1 and digested with MseI, showed the presence, in some vines, of mixed infection by phytoplasmas belonging in 16SrXII (Stolbur) and 16SrI (Aster yellow) groups.

**Key words:** Grapevine, Phytoplasmas, Bois Noir, Sardinia.

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## Identification of the phytoplasma infecting “Falanghina” grapevines in Campania and other vine varieties in Basilicata

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In Southern Italy, “Falanghina” grapevines showing grapevine yellows (GY) symptoms have been observed in the Campania region, whereas GY-affected grapevine plants of cvs. Chardonnay and Barbera have been observed in the neighbouring Basilicata. Using PCR amplification with phytoplasma-universal primers, all symptomatic plants resulted to harbour phytoplasmal infections. RFLP analysis of PCR-amplified ribosomal DNA indicated that phytoplasmas infecting grapevine in Campania and Basilicata were genetically uniform and indistinguishable from the reference strain STOL of the stolbur phytoplasma. “Falanghina” which is a local and very important grapevine cultivar in the Campania region, was not previously known to be affected by GY-diseases.

**Key words:** Grapevine yellows, Stolbur, Falanghina cultivar, 16S rDNA.

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## Diagnosis of Flavescence Dorée (FD) in grapevine: results from the second year of comparative analyses

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In 2000, the Italian Ministry of Agricultural and Forestry Policies coordinated a working group of experts to deal with the diagnosis of FD in grapevine. The outputs of that ring test clearly indicated that a quick protocol, although less efficient, might be used for detecting FD related phytoplasma in grapevines during large FD surveys. Nevertheless, other more expensive and time consuming total DNA extraction protocols were strongly advised when higher sensitivity was needed in the diagnosis of the disease. The working group also pointed out that other aspects of FD diagnosis yet needed to be addressed. It therefore continued its research activity focusing on two topics:

- better evaluation of the quicker total DNA extraction protocol previously proposed
- collection of evidences to determine the most effective sampling period for FD diagnosis. The laboratories of the Istituto di Virologia Vegetale, CNR - Torino and of the University of Bologna (DiSTA, Patologia Vegetale) were then assigned to these tasks and their results are discussed here. The quicker total DNA extraction protocol proved to be very useful for FD diagnosis although for better efficiency with very important materials any of the more time consuming extraction protocols were still needed. The results also showed that the titre of FD varies during the vegetative season sometimes being so low to interfere with diagnosis. This fluctuation in the titre of FD does not follow the same seasonal pattern in the two viticultural areas.

**Key words:** Flavescence Dorée, Diagnosis, Comparative analyses.

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### **Vegetative and productive withering of grapevine affected by Flavescence Dorée: experiments for vector control**

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Following the communications that, starting from spring and summer 1999, reported in Oltrepò pavese vineyards the presence of severe symptoms of a vegetative and productive withering that the biomolecular analysis had confirmed to be linked to the phytoplasma of the "Golden Flavescence", several experimental activities have been started to evaluate control strategies to be adopted against the vector: *Scaphoideus titanus*. In summer 2000 and in the following year, to evaluate the best control strategies but with a reduced impact against mites populations, some experimental test were setup in several grape growing area of Lombardia. Two different approaches have been adopted: efficacy trials in randomized block (3-4 products compared) and on whole vineyards (2 products compared). In 2000 2 treatments were done while in 2001 in randomized block test and in some vineyards only 1 treatment was done. Results are quite satisfactory: generally a good efficacy was achieved without any negative effect on mites populations.

**Key words:** Grape, Golden Flavescence, *Scaphoideus titanus*, Mites, Control.

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*English summary not available*

## **Flavescenza Dorata nelle aree viticole lombarde: monitoraggio, fitomappe e controllo del materiale di propagazione**

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Il Servizio Fitosanitario Regionale ha avviato nell'estate del 1999 un monitoraggio sistematico delle aree viticole, che ha permesso di individuare la diffusione sul territorio lombardo del fitoplasma della Flavescenza Dorata della vite. Le indagini svolte hanno accertato la presenza dell'infezione in quasi tutte le aree viticole della regione. In particolare la malattia risulta presente nella quasi totalità dei comuni viticoli dell'Oltrepò pavese, dove si concentra più della metà della viticoltura regionale. Per quanto riguarda le altre zone viticole della Lombardia, il fitoplasma è presente anche nelle provincie di Bergamo, Brescia, Como, Cremona, Lecco, Mantova, Milano, Lodi, e Varese. Unica zona viticola indenne, al momento attuale, risulta essere la Valtellina (provincia di Sondrio). Il monitoraggio, effettuato su tutto il territorio regionale, ha riguardato anche la presenza del vettore del fitoplasma, la cicalina *Scaphoideus titanus*. Per quanto riguarda il controllo del materiale vivaistico, la legislazione prevede che non possa circolare materiale di propagazione affetto da Flavescenza Dorata, in quanto tale fitoplasma è considerato un organismo da quarantena. Per questo motivo vengono annualmente effettuate, direttamente dagli ispettori fitosanitari, visite sistematiche in tutti i campi di prelievo di marze e di portinnesti regolarmente denunciati. Là dove viene riscontrata la presenza della malattia, viene bloccato per due anni il prelievo di materiale da propagazione e vengono fatte estirpare le piante sintomatiche. I risultati delle attività di monitoraggio e di controllo vivai hanno permesso l'elaborazione delle fitomappe di distribuzione della Flavescenza Dorata sul territorio lombardo. Esse sono state elaborate con ArcView, un programma che permette la georeferenziazione dei dati che risultano fondamentali per la programmazione delle attività di profilassi e la cura della malattia.

**Parole chiave:** Vite, Flavescenza Dorata, *Scaphoideus titanus*.

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## **Symptom expression of grapevine yellows in varieties cultivated in Lombardia region (Northern Italy)**

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Several papers describe the symptoms caused by grapevine yellows (GY) and in particular by Flavescence Dorée (FD) disease, but they often are related to the main grapevine cultivars like Chardonnay, Pinot bianco, Prosecco etc., or Cabernet, Pinot nero, Merlot. So far, scarce information are available concerning the behavior, toward FD, of several cultivars that are important for the production of typical wines in northern Italy and in particular in Lombardia region. Therefore, specific investigations were conducted in selected vineyards located in different areas of Lombardia region, like Oltrepò pavese, Franciacorta, Garda bresciano, Alto mantovano and Oltrepò mantovano, during 1999, 2000 and 2001 years. Each inspected grapevine plant was also sampled: leaf tissues were collected and then examined by molecular tests for the phytoplasma detection and identification. Among the black berries varieties, Barbera, Sangiovese and Cabernet franc were the more sensitive to FD. Quite severe symptoms were observed on Coatina, Pinot nero, Marzemino and Lambrusco Marani varieties. Merlot, Groppello, Rondinella, Fortana, Ancellotta e Molinara cultivars showed low levels of symptom intensity. Moreover, among the white berries varieties,

Chardonnay, Trebbiano and Verdea showed very severe symptoms, while lower level of symptom expression showed Riesling italico. At the end, faint symptoms were observed on Moscato and Cortese.

**Key words:** Grapevine yellows, Flavescence dorée, Symptomatology.

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