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EUROPEAN STONE FRUIT YELLOWS: A DESTRUCTIVE DISEASE IN THE MEDITERRANEAN BASIN

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SUMMARY - Stone fruit species are affected by severe diseases associated with phytoplasmas. European stone fruit yellows (ESFY) is present in several countries of Europe and the Mediterranean. ESFY - phytoplasma induces economically important disorders in apricot, Japanese plum and peach and can infect several other *Prunus* spp. *Cacopsylla pruni* is the vector of the disease transmitting it in a persistent manner. The observation of symptoms in sensitive stone fruit species (apricot and Japanese plum) can indicate the presence of ESFY in a given area. However, several other methods are reliable for the detection of the pathogen: (i) greenhouse indexing using peach GF 305; (ii) DAPI method; and (iii) molecular methods (PCR or nested-PCR). Being a phytoplasma disease, it is in practice not curable. Therefore, control is necessarily based on prevention using healthy planting material, which is of basic importance. In areas with low infection pressure, where the disease is absent and the presence of the vector is very low (or absent), the use of healthy plants can be sufficient. In areas with medium or high infection pressure, where ESFY is endemic (present on wild plants) and the populations of *C. pruni* are abundant, the control of the vector is necessary. In areas with high natural pressure of ESFY the cultivation of tolerant species (i.e. European plum) instead of sensitive ones (i.e. Japanese plum) is often advisable. In addition plants with induced-resistance (cross protection) can be used especially in already infected areas

Key words: Mediterranean, stone fruits, phytoplasma, ESFY, diagnosis, disease control

RESUME - *Les espèces fruitières à noyau sont affectées par plusieurs maladies associées à des phytoplasmes. L'European stone fruit yellows (ESFY) est répandu dans de nombreux pays de l'Europe et du bassin méditerranéen. Le phytoplasme de l'ESFY induit des affections d'importance économique sur abricotier, prunier japonais et pêcher et il peut aussi infecter diverses autres espèces de Prunus. Cacopsylla pruni est le vecteur de cette maladie qu'il transmet sous mode persistant. L'observation des symptômes sur les espèces sensibles (abricotier e prunier japonais) peut révéler la présence de l'ESFY dans une région donnée. Par ailleurs, il existe d'autres méthodes fiables pour la détection du pathogène: l'indexage en serre utilisant le pêcher GF305, la technique DAPI et les outils moléculaires (PCR ou nested-PCR). S'agissant d'une maladie à phytoplasme, aucun traitement curatif n'est pratiquement pas possible. La lutte doit ainsi reposer sur la prévention et donc, sur l'utilisation de matériel de multiplication sain qui est d'importance fondamentale. Dans des zones avec une faible pression d'infection où la maladie est absente et la diffusion du vecteur très limitée, voire nulle, le recours au matériel végétal sain est suffisant. Dans des zones avec une pression d'infection moyenne ou élevée, où l'ESFY est endémique (présent sur les plantes sauvages) et les populations de C. pruni abondantes, la lutte contre le vecteur est un impératif. Dans les zones avec une pression naturelle de l'ESFY, la culture d'espèces tolérantes (prunier européen) au lieu des espèces sensibles (le prunier japonais), est souvent conseillée. En plus, des plantes portant une résistance induite (protection croisée) peuvent aussi être employées, en particulier dans des zones déjà infectées.*

Mots-clés: *Méditerranéen, espèces fruitières à noyau, phytoplasme, ESFY, diagnostic, lutte contre la maladie.*

INTRODUCTION

In Europe, and particularly in the Mediterranean basin, several stone fruit (*Prunus*) species are affected by severe diseases associated with phytoplasmas. These include apricot chlorotic leaf roll (Morvan, 1977), plum leptonecrosis (Giunchedi *et al*., 1978), peach yellows (Poggi Pollini *et al*., 1993) and peach decline (Ionica, 1985). Lorenz *et al*. (1994) determined the common aetiology of these diseases and proposed the single name 'European stone fruit yellows' (ESFY). On the basis of sequence and restriction fragment length polymorphism (RFLP) analysis of PCR-amplified 16S rDNA, the agent of

ESFY is placed within the apple proliferation (AP) group of phytoplasmas (Lee *et al*., 1998; Seemüller *et al*., 1998). This cluster also includes phytoplasmas associated with other perennial fruit tree diseases present in Europe such as AP and pear decline (PD). These phytoplasmas are closely related but distinguishable from each other (Carraro *et al*., 2001b; Jarausch *et al*., 1998; Lorenz *et al*., 1995). ESFY-phytoplasma is also distinctly different from the agents of X-disease and peach yellow leaf roll, major phytoplasma diseases of stone fruit trees in North America, which have not been detected in Europe (Kison *et al*., 1997). ESFY-phytoplasma induces economically important disorders in apricot (Desvignes and Cornaggia, 1982), Japanese plum (Dosba *et al*., 1991) and peach (Marcone *et al*., 1996) and can infect several other *Prunus* spp.

GEOGRAPHIC DISTRIBUTION

European stone fruit yellows is present in several countries of Europe. Occurrence of the disease has been reported in Albania (Myrta *et al*., 2003), Austria (Laimer da Camada Machado *et al*., 2001), Bulgaria (Topchiiska *et al*., 2000), Czech Republic (Navratil *et al*., 2001), England (Davies and Adams, 2000), France (Desvignes and Cornaggia, 1982), Germany (Lederer and Seemüller, 1992), Greece (Rumbos and Bosalidis, 1985), Hungary (Süle, 1999), Italy (Goidanich, 1933), Romania (Ionica, 1985), Slovenia (Brzin *et al*., 2001), Spain (Sanchez Capucino and Forner, 1973), Switzerland (Ramel *et al*., 2001). Recently the presence of ESFY was ascertained also in Turkey (G. Sertkaya, unpublished).

The incidence of the disease is different in each country, depending on various factors. In any case ESFY is a serious problem in the countries bordering on the Mediterranean sea (Spain, France, Italy, Balkans), where the cultivation of susceptible and sensitive *Prunus* species (apricot and Japanese plum) is widespread. Likewise, ESFY could become important in the future in other areas where stone fruit trees are cultivated, if control measures are not applied.

HOSTS AND SYMPTOMATOLOGY

ESFY-phytoplasma can infect several *Prunus* species. Among these, apricot and Japanese plum are the most susceptible and sensitive. Although symptom severity is fairly variable, infected *P.armeniaca* and *P.salicina* trees in general show typical yellows accompanied by leaf roll followed by leaf reddening, reduction or suppression of dormancy with the consequent risk of frost damage, severe and progressive necroses, decline and eventual death of the tree. Within the most sensitive cultivars, 100% of the infected plants can die. Production can be totally lost.

In peach the disease is not so devastating as in apricot and Japanese plum, although in north Italy Poggi Pollini *et al*. (2001) recently detected a disquieting diffusion.

European plum is susceptible but generally tolerant to ESFY (Carraro *et al*., 1998a). Some cvs. however, can show weak symptoms but low mortality (Jarausch *et al*., 2000).

Wild or cultivated *Prunus* species, such as *P. cerasifera, P. mahaleb, P. padus, P. spinosa, P. tomentosa*, are highly tolerant to the disease and the presence of specific symptoms is an exception (Ferrini *et al*., 2002); however *P. cerasifera, P. spinosa* and *P. tomentosa* were fairly susceptible (Carraro *et al*., 2002a). *P.avium* in contrast demonstrated a high level of resistance to ESFY (Jarausch *et al*., 1999). The presence of the agent was reported also in *P. serrulata, P. amygdalus, P. insititia* and *P. cerasus.*

The susceptibility and sensibility of the rootstocks to ESFY varies according to the different genotypes: some are highly sensitive, i.e. apricot seedlings and Rubira peach; others, such as Bromptom, are tolerant (Kison and Seemüller, 2001).

EPIDEMIOLOGY

European stone fruit yellows is an epidemic disease, characterised by rapid and widespread diffusion especially when the conditions are favourable for host-plants and vectors.

Cacopsylla pruni (Fig.6) is the vector of the disease (Carraro *et al*., 1998b); the insect completes one generation per year and overwinters as adult on shelter plants (conifers are reported). At the end of winter, *C.pruni* moves from shelter plants to stone fruit trees for oviposition. From May till the beginning of July,

the new generation feeds on the primary host; as soon as adult, *C.pruni* abandons the stone fruit trees (Conci *et al*., 1992). The insect is strictly oligophagous on *Prunus* spp and has a Centralasiatic-European distribution.

C.pruni transmits ESFY-phytoplasma in a persistent manner: the minimum acquisition period is 2-4 days; the minimum latent period 2-3 weeks; the minimum inoculation period 1-2 days. The retention of infectivity in *C.pruni* lasts through the winter and the following spring, when the overwintering insects reach the stone fruit trees, they are already infected and infective (Carraro *et al*., 2001a). Important similarities exist among pear decline, apple proliferation and ESFY: the agents of the three diseases are phytoplasmas all belonging to AP group and all transmitted by psyllids.

In areas with high infection pressure, the natural infectivity of *C.pruni* reaches levels greater than 10% (mean individual transmission potency) (Carraro *et al*., 2002b) and the annual rate of newly infected plants the 20% (Carraro *et al*., 1992). Carraro *et al*. (2002a) demonstrated the important role played by wild *Prunus* species - such as *P.spinosa* and *P.cerasifera*, both hosts for the vector and the agent of ESFY in the epidemic cycle of the disease; the phytoplasma can survive and persist in nature independently of the presence of cultivated susceptible plants. It should also be noted that some cultivated *Prunus* spp are completely tolerant; therefore these plants can act as sources of inoculum for the spread of ESFY.

Recently in France, Jarausch *et al*. (2001) detected ESFY-phytoplasma in *Celtis australis, Fraxinus excelsior* and *Rosa canina* growing in the surroundings of infected apricot orchards. The exact role played by these non-*Prunus* species in the epidemiology of the disease is not yet clear; probably they are end-hosts of the phytoplasma.

DETECTION

The simple observation of symptoms is not sufficient for an exact diagnosis of the disease because several *Prunus* spp. are tolerant to ESFY; but the observation of sensitive stone fruit species (apricot and Japanese plum) can indicate the presence of ESFY in a given area. Several other methods are reliable for the detection of the pathogen.

Greenhouse indexing, using peach GF 305 as a test plant (Desvignes *et al*., 1999) is a useful method. For large scale diagnosis, the DAPI method (Seemüller, 1976) can be used, although the percentage of false negatives can reach high levels; this generally occurs when phytoplasma colonisation of plants is poor or uneven.

Molecular methods (PCR or nested-PCR) are currently applied for the detection of ESFY-phytoplasma both in plants and in insects: they provide an exact and precise diagnosis. Different primer pair sequences are known and available for the amplification of portions of ESFY-phytoplasma DNA. Some of them, such as ECA1/ECA2 (Jarausch *et al*., 1998), are highly specific and do not need RFLP analyses; others, such as P1/P7 (Schneider *et al*., 1995), are 'universal' for phytoplasmas and can be used for the first amplification in nested-PCR followed by the second amplification using AP-group specific primers, as R16(X)F1/R1 (Lee *et al*., 1995) or f01/r01 (Lorenz *et al*., 1995). After the nested-PCR, the RFLP analyses of the PCR products is necessary for the exact identification of the ESFY-phytoplasma. It should be mentioned that the possibilities and the primer 'combinations' are numerous and all efficacious; in our lab, as a routine, we apply the nested-PCR technique using the primers P1/P7 followed by f01/r01 and RFLP analyses (Carraro *et al*., 2002a).

CONTROL

European stone fruit yellows, being a phytoplasma disease, is in practice not curable; therefore control is by necessity based on prevention. The use of tested-healthy plant material is of basic importance. It is clear therefore that precise knowledge of the epidemiology of the disease is necessary for its control.

In areas with low infection pressure, where the disease is absent and the presence of the vector is very low (or absent), the use of healthy plants can be sufficient. In areas with medium or high infection pressure, where ESFY is endemic (present on wild plants) and the populations of *C.pruni* are abundant, the control of the vector is necessary. Vector control has been made easier, since we know the period when the overwintering adults reimmigrate to *Prunus* species and when the new generation abandons the primary hosts. Besides, the vector has only one generation per year, this further facilitates its control.

In areas with high natural pressure of ESFY the cultivation of tolerant species (ex. *P.domestica*) instead of sensitive ones (ex. *P.salicina*) is often advisable. In addition plants with induced-resistance (cross protection) can be used especially in already infected areas (Morvan *et al*., 1991).

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