MINIREVIEW

BACTERIAL DISEASES OF WALNUT AND HAZELNUT AND GENETIC RESOURCES

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SUMMARY

In 2008, a global production rounding 1,724 and 1,053 million metric tonnes respectively for walnut and hazelnut has been estimated. Bacterial diseases are threatening these nut crops all over the World. Xanthomonas arboricola pv. juglandis (Xaj) is the agent of walnut bacteriosis, and has been associated also to brown apical necrosis. Several walnut genotypes have shown a range of tolerance to this disease in diverse countries. Deep bark canker (Brenneria rubifaciens) and Shallow bark canker (Brenneria nigrifluens) are the other bacterial diseases affecting walnut. Being polyphenols involved in tolerance to bacterial diseases, it has been shown that the gene jrPPO1 is the sole polyphenol oxidase PPO gene in walnut able to encode a jrPPO enzyme that is expressed in the leaves, hulls and flowers of walnut trees. It can also happen that some modifications in the anatomical traits of the epidermis of walnut progenies, such as hair density or wax thickness could act as shields against Xaj infection. These changes could explain segregation of tolerance in walnut progenies. A range of tolerance to hazelnut blight, induced by Xanthomonas campestris pv. corylina, has been observed in France in diverse nursery plants. Pseudomonas syringae pv. avellanae, observed first in northern Greece, is affecting hazelnuts also in Italy, where this disease it is known as ‘moria’ (‘death’). No resistance or tolerance against ‘moria’ has been found.

INTRODUCTION

Walnut (Juglans regia L.) world production has been estimated around 1,052 mMT: Asia (873) [Turkey (800), China (151), Europe (145) [Italy (111), Spain (18), France (5), Greece (2.5)], and USA (33) (Faostat, 2008). The main bacterial diseases that threaten both crops and possibilities to control them by using genetic resources are discussed below.

BACTERIAL DISEASES OF WALNUT

Walnut blight. Xanthomonas arboricola pv. juglandis (Xaj) is the causal agent of walnut blight, the most important bacterial disease of Juglans regia and other Juglans species, which affects a high percentage of pistilate flowers and fruits, but does not kill bearing trees. This bacterium has been also isolated from tissues affected by bacterial apical necrosis (Belisario et al., 2002). Blight has been recorded from Europe (Austria, Bulgaria, Denmark, France, Germany, Greece, Italy, Moldova, Netherlands, Poland, Portugal, Romania, Russia, southern Russia, Slovenia, Spain, Switzerland, UK, Ukraine and former Yugoslavia) Asia, [Azerbaijan, China, (Hebei, Henan, Jiangsu, Shaanxi, Shandong), Georgia, India, (Himachal Pradesh, Uttar Pradesh), Iran (Qazvin, Hamadan, Tehran, Markazi, Aradabil, Guilan, Mazendaran and Golestan provinces (Golmohammadi et al., 2002)], Iraq, Israel, Lebanon and Uzbekistan], Africa (South Africa and Zimbabwe), North America (Canada and Mexico), USA (Alabama, Arkansas, California, Connecticut, Delaware, Georgia, Indiana, Kansas, Louisiana, Maine, Maryland, Michigan, Minnesota, Missouri, New Jersey, New York, Ohio, Oregon, Pennsylvania, Texas, Virginia and Washington), Caribbees (Bermuda), South America (Argentina, Chile, Uruguay), and Oceania [Australia (New South Wales, Queensland, South Australia, Tasmania, Victoria, Western Australia) and New Zealand] (CABI-EPPO, 2001).

J. ailantifolia, J. ailantifolia var. cordiformis, J. Californica, J. Cinerea, J. Windsii and J. nigra have been also recorded as Xaj hosts (Miller and Bollen, 1946; Bradbury, 1967). The disease was reproduced on tender shoots and leaves of J.nigra, J. Californica, J. Windsii, J. Cinerea, J. Ailantifolia, J. Ailantifolia var. cordiformis, and ‘Paradox’ (J. Windsii x J. Regia) and ‘Royal’ (J. nigra x J. Regia) hybrids.
Symptoms of the disease consist of dark brown to black spots on new leaves, stems and nuts. Many nuts fall prematurely; others reach full size, but their kernel become blackened, dried and wrinkled. Rain is the most important dispersal agent (Miller and Bollen, 1946; Piccirillo, 2006). The presence of aphids (Chromaphis juglandicola) did not increase the percentage of affected fruits, but it favored a higher incidence of Xaj on the leaves (Arquero et al., 2006). Pollen from infected catkins can contain Xaj. Miller and Bollen (1946) did not consider pollen transmission to be important in Oregon (USA) although several experiments suggest that such transmission occurs (Ercolani, 1962, cited by Bradl, 1967). Main infection avenue is through stomata or wounds. The bacterium overwinters in diseased buds and twigs. Xaj severity increases with earliness of flowering (Aletà, 2004; Piccirillo and Petriccione, 2006).

It is possible to obtain plants tolerant to Xaj when two susceptible genitors are crossed (Rovira et al., 2007). On the other hand, the evaluation in the open in an ex situ collection showed a segregation of Xaj tolerance in J. regia progenies (Frutos et al., 2008, 2009, 2010; Ruiz-García et al., 2010). These segregations found in natural conditions suggest that anatomic changes in the epidermis of young twigs, leaves and flowers could act as barriers against Xaj infection.

Table 1 summarizes the information from different countries on tolerance/resistance/low susceptibility to Xaj in J. regia.

**Polyphenols and Xaj.** It is known that polyphenol oxidase (PPO) catalyzes the oxidation of polyphenols to quinones, which are very reactive against disease agents. The PPO-encoding gene jrPPO1 from a walnut pistillate flower cDNA library has been cloned and shown to be the sole gene of walnut encoding a jrPPO enzyme expressed in the leaves hulls and flowers of the host (Escarb et al., 2008). When a bacterial infection occurs, a necrosis develops in nuts or leaves resulting in their abscission. In this mechanism of defense phenolic compounds as juglone and their glucosides are involved, which are induced even when a very low Xaj contaminant occurs (Garcin and Duchesne, 2001). Matias et al. (2009) found that cv. Franquette had lower contents of gallic acid, vanillic acid and juglone than cv. Hartley, but blight symptoms were significantly stronger in the latter. On the other hand, no correlation between damages elicited by artificial infections and percentage of contaminated fruits in the open field was found for cvs. Fernet, Erjavec, Cisco, Zdole, Seiferdorfski and Sampioni, although, after infection, an accumulation of different phenolic compounds was detected in both natural and artificial inoculation. There is some evidence of a protective function of some phenolic compounds as juglone, ellagic acid and hyperin (Solar et al., 2007, 2009). Except for the very sensitive cultivars, Xaj damages are not extensive in old J. regia wood. Taking into account this fact, it would be interesting to evaluate if such old wood contains higher levels of phenolics than leaves or young twigs or nuts. This information may lead to a better understanding of the role of polyphenols in Xaj control.

**Deep bark canker.** This disease (DBC) induced by Brenneria rubifaciens is characterized by cracks in scaffold branches and trunks, where infection is first established. The disease advances slowly upward, affecting more and more branches which are weakened, while the crop decreases progressively. The symptoms do not extend into the rootstock. Cankers ooze a characteristic reddish to dark-brown substance from late spring through early fall. ‘Hartley’ has been reported as the most susceptible cultivar to DBC. High temperatures favour the development of DBC, which makes this disease to prevail in the Central Valley rather than in the coastal areas of California (Anonymous, 2007a). Brenneria rubifaciens has been also isolated from cv. Hartley in Badajoz (Spain) (Gonzalez, 2002). A specific test for the early detection of DBC has been developed in mutants deficient in rubifacine (pig), the pigment oozed from cankers. This test can be useful to investigate B. rubifaciens ecology under both nursery and orchard conditions (McClean et al., 2008).

**Shallow bark canker.** The agent of shallow bark canker (SBC) is Brenneria nigriiflans, whose symptoms consist of brownish to black round spots which appear on trunk or on lower scaffold limbs. The bark at the margins of these spots presents a water-soaked appearance with the centre showing a drop of black ooze that dries with time. These superficial cankers can be extensive, but they seem to cause little damage to the tree. Although SBC affects many commercial walnut cultivars, it is not considered a major disease in California. As it occurs with DBC, SBC can be severe in stressed trees (Anonymous, 2007b). SBC has been observed as much in nursery as in orchard in cvs Fernor, Chandler, Mayette, and Hartley, although Lara and Franquette could also be affected (Ménard et al., 2004). It has been reported that SBC induces abortion and fall of fruits in an ex situ collection located at Pignaturo Maggiore near Caserta (Italy), where affected trees can die in few years after infection (Piccirillo, 2003). Symptomatic trees were observed also in Veneto, Piedmont and Campania, Italy. In these cases, cankers were deeper and more se-
vere than those reported from USA, resembling those caused by B. rubrifaciens (Loreti et al., 2006). B. rugiceps has been also observed on walnuts in Iran (Yousefikopaei et al., 2007). Identification tests for early SBC identification have been developed (Loreti et al., 2008; Roshangar and Harighi, 2009).

**BACTERIAL DISEASES OF HAZELNUT**

**Blight.** In the 1970s Xantomonas campestris pv. corylina (Xcc) (= X. corylina) was identified as the causal agent of hazelnut (Corylus avellana) blight in south-western French orchards established with hazelnuts imported from Oregon, where the disease is endemic (Luisetti et al., 1975). Later, the presence of Xcc was confirmed on

<table>
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<tr>
<th>Country</th>
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<tr>
<td>China</td>
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<td>Kunnmeing, Yunnan</td>
<td>‘Yunxin Gaoyuan’, out of J. sigillata x J. regia ‘Yulin A7’, cross made in 1977</td>
<td>Resistant to Xaj in the open</td>
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<td>Kunnmeing, Yunnan</td>
<td>‘Yunxin 90303’ derived from J. sigillata cv. ‘Santai’ x J. regia cv. ‘Xiniao No. 13’</td>
<td>Diseases resistant</td>
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<td>Tai’an, Shandong</td>
<td>‘ Luguo 4’, open pollinated native genotype</td>
<td>Xaj resistant in the open</td>
<td>Zhang et al., 2009</td>
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<td>Ghengxian county, Gansu</td>
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<td>Xaj resistant in the open</td>
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<td>Italy</td>
<td>Pignataro Maggiore, area of Caserta</td>
<td>Ex situ collection</td>
<td>All Xaj susceptible</td>
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<td>Piccirillo and Petriccione, 2006</td>
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<td>Romania</td>
<td>Oltenia</td>
<td>550 genotypes out of native population</td>
<td>Several genotypes are Xaj tolerant</td>
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<td>Valcea</td>
<td>Valcor, Valmit and Valrex, out of native population</td>
<td>Texting in orchard for Xaj and other traits</td>
<td>Botu et al., 2007</td>
<td></td>
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<td>Valcea</td>
<td>‘Portval’ (syn. ‘VL 26 B’), seedling rootstock</td>
<td>Good resistance to diseases, Xaj not specified</td>
<td>Achim et al., 2007</td>
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<td>Spain</td>
<td>Galicia</td>
<td>&gt;550 natural genotype tolerant in the open were evaluated for Xaj, susceptibility</td>
<td>‘MBLu-20’, ‘MBLu-21’ and ‘MBC-45’, did show low Xaj susceptibility in lab.</td>
<td>Aleta et al., 2001</td>
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<td></td>
<td>Cantabria</td>
<td>Native seedlings aged over 15-years-old</td>
<td>28 were selected as Xaj free symptoms. In lab all were susceptible</td>
<td>Arrieta and Diaz, 2007; Lopez et al., 2007</td>
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<tr>
<td></td>
<td>Murcia</td>
<td>Ex situ collection</td>
<td>Segregation to Xaj tolerance were found in an ex situ collection</td>
<td>Frutos et al., 2008, 2009, 2010; Ruiz et al., 2010</td>
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<td>Slovenia</td>
<td>24 genotypes preselected</td>
<td>Most promising (Xaj resistant) are late leafing genotypes ‘Z-62’, ‘Krnc’ and ‘Z-60’</td>
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<td>Turkey</td>
<td>19 genotypes</td>
<td>All genotypes Xaj resistant</td>
<td>Akca and Ozongun, 2004</td>
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<td>Sen 2, C440, 77 H-1, Kaplan 86, Tokat 1, Kaman 1 and Bilecik</td>
<td>All selections Xaj low susceptible</td>
<td>Ozaktan et al., 2007</td>
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<td>Yugoslavia</td>
<td>Nbs. 10/88, 40/92, 30/93, 28/94, 9/96, selected from native population</td>
<td>Highly resistant to diseases</td>
<td>Mitrovic, 2003</td>
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<tr>
<td></td>
<td>Nbs. 10/88, 40/92, 30/93, 28/94, 9/96</td>
<td>All resistant to Xaj</td>
<td>Mitrovic et al., 2007</td>
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Table 1. *Juglans regia* accessions resistant/tolerant to *Xaj* found in native walnut population.
hazelnuts in several locations of Victoria (Australia), where blight is widespread, suggesting that it had been present for many years in the area before its discovery (Wimalajeewa and Washington, 1980). In 1985, Xcc-induced symptoms, i.e. death of buds and new shoots, cankers on branches and trunks, leaf spots, dark brown spots on nuts and bacterial exudates on necrotic lesions, were observed on 4-year-old trees of cv. Barcelona in Carillanca (Chile). Old hazelnut trees were less affected than the young ones (Guerrero and Lobos, 1987).

Blight has been also reported from Turkey (Black Sea area), UK, Italy (Civilleri et al., 2007), former Yugoslavia, Russia (Bradbury, 1987; Anonymous, 1996), and Iran [Guilan province (Kazempour et al., 2006; Ali et al., 2006)]. In countries like Turkey, Italy and USA, which account for the bulk of hazelnut production (Viggiani, 1994), blight is regarded as the most important disease of this crop. The main problem of the Italian and Spanish hazelnut industry is the selection of varieties resistant to Xcc attacks (Avanzato et al., 2009).

Other hosts of Xcc are Corylus maxima, C. rostrata (= C. californica) and C. colurna, which proved susceptible when artificially inoculated (Bradbury, 1987).

Disease symptoms consist of buds and new shoots death, leaf spots, dark-brown spots on nuts, bacterial exudates on necrotic lesions, cankers on branches and trunks which may girdle these organs, including trunks of young trees, thus killing the parts of the tree above the lesions. Xcc overwinters in cankers on branches and buds, and spreads to other parts of the tree in wet weather. Limited spread from tree to tree takes place by water splash. Man is an important agent of bacterial dissemination, especially by pruning young trees with contaminated cutting tools and is also responsible for the introduction of the disease into new areas with infected planting material (Bradbury, 1987; Anonymous, 1996). The potential for natural spread can be considered as relatively low. However, seeds of fruits picked from infected trees can yield infected seedlings (Bogatzevska, 2007). The most important factor for reducing mortality from Xcc in newly established hazelnut plantings is an adequate irrigation in the first 2-3 years after planting (Moore et al., 1974).

As to reaction of hazelnut cultivars to Xcc attacks, preliminary observations in nursery showed that cvs. Fertile de Coutard is highly susceptible (Luisetti et al., 1975; Prunier et al., 1976), Ronde de Piemont shows an intermediate level of susceptibility, Imperatrice Eugenie is slightly susceptible and Negret, Merveille de Bollwiller and Segorbe are almost resistant (Prunier et al., 1976).

Bark canker and decline Pseudomonas syringae pv. avellanae, (Psa) [= P. avellanae (Scortichini, 2002)] is the causal agent of hazelnut bacterial canker and decline in Greece and Italy (Wang et al., 2007). This disease was first observed in northern Greece in 1976 (Psallidas and Panagopoulos, 1979) then in Italy, where is known under the name of "moria" (decline and death) and did spread rapidly, particularly in the province of Viterbo (Scortichini and Tropiano, 1994). Environmental factors play an important role in disease spread. For example, in a plantation with prevailing south-west winds, the disease moved progressively year after year to the south and west (Scortichini and Martins, 2000). Frost and hail injuries may also favor disease spread (Scortichini and Martins, 2000). In the course of surveys in forests of central Italy carried out in 1996-98 to verify the possible presence of bacterial canker in wild hazelnut trees, wilted twigs were found several times, especially in summer. Moreover, wild hazelnut trees growing near commercial orchards appeared completely wilted (Scortichini et al., 2000). Psaltria has been found in soils of orchards affected by "moria". Infections were favoured by high soil acidity, poor cultivation practices as planting very deep, use of acid fertilizers such as ammonium sulfate, high levels of aluminum in soils, and high levels of iron and manganese in the roots (Scortichini et al., 2001). Surveys carried out in the other main areas of hazelnut cultivation in Italy determined the presence of bacteria similar to Pseudomonas syringae pv. syringae van Hall (Psaltria), but severe cases of bacterial decline were not observed. Psaltria attacks, always of high virulence, are restricted to Corylus avellanae (Scortichini, 2002; Scortichini et al., 2002), as exemplified by the killing of more than 1800 adult hazelnut trees in a single orchard within 5 years (Scortichini and Tropiano, 1994). On a wider scale, the death of more than 40,000 trees on a surface of 20,000 ha planted with hazelnut in Latium (central Italy) has been attributed to "moria", which continues to damage trees on approximately 1000 ha (Scortichini, 2002).

The study of thirty-eight bacterial strains isolated from cv. Tonda Gentile delle Langhe showing twig dieback in Piedmont and Sardinia (Italy), justified the creation of a new pathovar denoted P. syringae pv. coryli (Psc) (Scortichini et al., 2005). Field surveys for evaluating the sanitary status of hazelnuts orchards in the provinces of Catania and Messina (sicily), disclosed the frequent occurrence of twig and branch dieback connected with the presence of Psc (Civilleri et al., 2007).

**Discussion**

The notion that Xaj can infect a very wide range of *Juglans* species and their interspecific hybrids (Smith et al., 1912; Smith, 1914; Miller and Bollen, 1946; Bradbury, 1967; Burokienie, 2009), prompted the search for *J. regia* genotypes showing high tolerance to this bacterium. Crossing susceptible-to-Xaj walnut materials yielded segregation of genotypes (Frutos et al., 2009, 2010; Ruiz et al., 2010), which suggested that the presence of anatomical differences in epidermal tissue, such as hairs
density and/or wax layer thickness, acted as a shield against \textit{Xaj} entry into walnut tissues.

DBC disease is less important than \textit{Xaj}-induced apical necrosis, although \textit{B. rubifaciens} can produce important damages to cv. Hartley, the most sensitive cultivar, which is becoming progressively less popular.

DBC appears more virulent in some European countries (Loreti et al., 2006; Piccirillo, 2003) than in California, where it is a disease of minor importance (Anonymous, 2007b).

\textit{Xcc} could be to hazelnut what \textit{Xaj} is to walnut. Some hazelnut cultivars have a low susceptibility to \textit{Xcc} (Prunier et al., 1976) thus could be used as parents for new genotypes highly tolerant to it. But for bark canker and decline caused by \textit{Ps}, no resistant/tolerant cultivars have been found in \textit{C. avellana}.

More tolerant genotypes to bacterial diseases seem to exist in walnut than in hazelnut. This fact may be connected with the propagation procedure. Whereas seeds have been traditionally used for propagating walnuts, vegetative propagation represents the norm in hazelnut. It ensues that more gene recombination and segregation occur in walnut.

Advances in knowledge of phenolic compounds in walnut led to think that the higher is phenolic content, the higher is tolerance to \textit{Xaj}. However, the opposite has been also pointed out, i.e., the more phenolics, the lesser tolerance (Matias et al., 2009). Laboratory testing for phenolic content were unable to find any walnut genotype resistant to \textit{Xaj}, whereas a number of tolerant genotypes free of blight in the open field have been observed in several countries. This is likely dependent on anatomical changes in the epidermis commented above, i.e hairs and wax acting as shields against \textit{Xaj}. However, when the bacterium breaks this barrier, its progress cannot be stopped, but can only be slowed down in cultivars with higher phenolic content.

CONCLUSIONS

The most important bacterial diseases in walnut and hazelnut seem to be due to \textit{Xaj} and \textit{Xcc}, respectively. Both pathogens have a worldwide distribution.

Hazelblnt blight and decline disease (\textit{Ps}) has produced important losses to hazelnut orchards and forests in Italy.

DBC (\textit{Brennaria rubifaciens}) and SBC (\textit{B. nigrifluens}) seem to be of lesser importance, although \textit{B. nigrifluens} has produced more damages in some European countries than in California.

Whereas laboratory tests for phenolic content have not disclosed any correlation with resistance to \textit{Xaj}, many blight-free walnut genotypes symptoms have been observed in the open field, possibly consequent to anatomical changes in epidermis of walnut progenies.

Concerning hazelnut blight disease, some cultivars seem to be more tolerant than others, but no real resistance has been found.

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