BRIEF HISTORICAL ACCOUNT OF OLIVE LEAF SCORCH ("BRUSCA") IN THE SALENTO PENINSULA OF ITALY AND STATE-OF-THE-ART OF THE OLIVE QUICK DECLINE SYNDROME

S. Frisullo[1], I. Camele[2], G.E. Agosteo[3], D. Boscia[4] and G.P. Martelli[5]

[1] *Dipartimento di Scienze Agrarie, degli Alimenti e dell'Ambiente, Università degli Studi, Via Napoli 25, 71100 Foggia, Italy*
[2] *Scuola di Scienze Agrarie, Forestali, Alimentari ed Ambientali, Università degli Studi della Basilicata, Viale dell'Ateneo Lucano 10, 85100 Potenza, Italy*
[3] *Dipartimento di Agraria, Università Mediterranea, Località Feo di Vito, 89122 Reggio Calabria, Italy*
[4] *Istituto di Protezione Sostenibile delle Piante del CNR, sezione di Virologia Bari, Via Amendola 165/A, 70126 Bari, Italy*
[5] *Dipartimento di Scienze del Suolo della Pianta e degli Alimenti, Università degli Studi Aldo Moro, Via Amendola 165/A, 70126, Bari, Italy*

SUMMARY

"Brusca" (leaf scorch or marginal leaf burn) is a disease of olive (*Olea europaea*) typically characterized by the desiccation and death of tissues at the tip and/or along the edge of the leaf blade, which can be followed by defoliation. Since the end of the 18th century this disorder has reappeared periodically, after long periods of quiescence, in the same olive-growing areas of the province of Lecce (Apulia, Salento peninsula, southern Italy). Over time, this disease has been the object of repeated investigations, first by a couple of local physician, who were the authors of its description, then, since the beginning of the 20th century, by professional plant pathologists. These studies have established that "brusca" may have multiple origins as determined by abiotic ("brusca non parassitaria" = non parasitic scorching) or biotic ("brusca parassitaria" = parasitic scorching) causes. "Brusca non parassitaria" can be induced by any physical cause that affects water supply to the leaf margin cells (e.g. insufficient moisture in the soil, water is lost too quickly from the leaves to be replaced adequately, damaged roots), or by hot dry winds, salty winds, nutrient deficiency/toxicity. By contrast, weak foliar pathogens (i.e. the discomycete fungus Stictis panizzei) or xylem-invading fungi or bacteria that plug the water conducting vessels can, in principle, be the agents of "brusca parassitaria". These agents may include *Xylella fastidiosa*, a xylem-limited bacterium, which has recently been found associated with an olive disease denoted "quick decline syndrome". This disease occurs in some areas of the Lecce province where severe cases of "brusca" have repeatedly been observed in the past. This coincidence, and the type of symptoms (extensive scorching and desiccation of the canopy) that somewhat recall those described in the early literature, may lead one to speculate that *X. fastidiosa* has been sitting in the area for about 250 years, rather than being a pathogen of recent introduction. This is not the case, as discussed in the present paper.

Key words: Stictis panizzei, brusca, leaf scorch, *Xylella fastidiosa*, olive quick decline.

Olive leaf scorch, the first outbreak recorded in the 18th century. Cosimo Moschettini, a physician from Martano (province of Lecce), was the first to describe at the end of the 18th century (Moschettini, 1777, 1789) a disease called "brusca" (leaf scorch, marginal leaf burn), affecting the leaves of olive trees in the groves of some locations of the province of Lecce, the southernmost part of the Salento peninsula (Apulia region, south-east Italy) (Fig. 1). The disease appeared suddenly in the autumn and spring, mainly on the lower branches of the trees and was particularly severe on "Ogliarola di Lecce", the olive cultivar most widely grown in the area, whereas "Cellina di Nardò", the other major local cultivar, appeared to be resistant.

Shortly afterwards, Giovanni Presta, a physician from Gallipoli, another small town in the province of Lecce, described the disease as follows: "*We here say that olive plants are "bruscate" (= scorched, burned), when their vigorous, luxuriant and green canopy suddenly desiccates here and there, as if the leaves were burned by fire in the apical half of the blade or longitudinally along the margins from the apex to the petiole, as if they were invested by the flame of oil dregs burning under the tree. Scorched leaves are shed, leaving the twigs naked, or remain attached to the plants, dried and shriveled, in which case "brusca" affects also the twig's wood, which desiccates and is unable to sprout again. Instead, defoliated twigs can vegetate in the following spring, producing new leaves*" (Presta, 1794).

As to the cause of the disease, Moschettini (1789), attributed its origin either to the action of sun rays
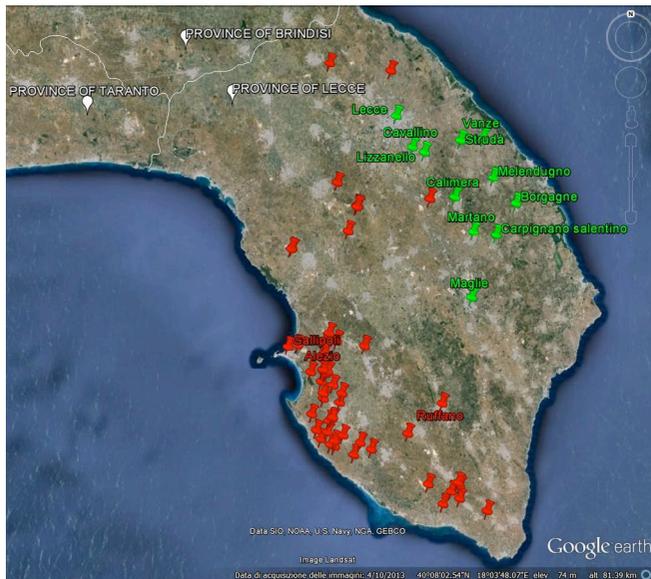
Corresponding author: S. Frisullo
Fax: +39.0881.589324
E-mail: salvatore.frisullo@unifg.it

Fig. 1. Map of the Lecce province showing the position of all villages mentioned in the text and the distribution of "brusca" disease, as reported by the 18th-20th century records (green marks) and of the current olive quick decline syndrome (red marks).

following foggy mornings or, more likely, to the quick alternation of cold and hot periods, conditions that he defined as "brusca days". He also noticed that "brusca" did not appear recurrently. In fact, he was unable to draw conclusions from field experiments with which he intended to investigate the effect of fertilization and cultural practices, because: "*for the last 12 years "brusca" has almost totally disappeared*".

Presta (1794) observed that "brusca" affected mainly the olive canopy sectors exposed to south or west and that its appearance in autumn (October, November) and spring (March) was favoured by quiet and cold nights followed by hot and sunny days in the absence of wind. Since this author had initially attributed the disease to the action of sea winds, he finally concluded that "*the real origin of "brusca" disease is unknown*". In any case, Presta suggested, as a remedy, to top-graft the resistant cv. Cellina di Nardò on the susceptible cv. Ogliarola di Lecce.

Interestingly, Presta (1794) reported also the common occurrence in olive orchards attacked by "brusca", of a large larva that drilled galleries in the trunk and branches, particularly in trees under water stress. Presta described this insect as a lepidopteron but was unable to determine its species for he did not observe the adult stage. However, from his drawings, it is possible to recognize the larval stage of the leopard moth (*Zeuzera pyrina*). This insect, which is endemic and still widespread in the Salento peninsula, was not mentioned in a review on the harmful olive pests in the Kingdom of the Two Sicilies (Costa, 1857) over sixty years after Presta's first report, escaping the attention of the local entomologists.

The second severe outbreak at the beginning of the 20th century. "Brusca" reappeared unexpectedly in the Salento peninsula in 1899. It was particularly severe in 1901 and 1902, causing heavy yield losses. As described earlier by Moschettini (1789) and Presta (1794), the disease popped up suddenly on extensive areas, causing in one or two weeks the vegetation of affected plants to turn from a healthy and luxuriant condition to a suffering and "burned" aspect. The damages to the crop and the concern of the growers were such that the Italian Ministry of Agriculture took action, entrusting the most prominent plant pathologists of that time, namely Orazio Comes, Giuseppe Cuboni, Ugo Brizi and Lionello Petri, with the task of studying the disease.

Comes (1900, 1901) was the first to visit olive orchards in the "agro" (countryside) of Lizzanello and Melendugno (province of Lecce), coming to the conclusion that "brusca" was consequent to injuries to large roots that resulted in localized rotting. In a second report he attributed the disease to sudden temperature changes favouring the production of gums that invaded roots, trunks and branches.

Brizi (1903) found the consistent association of a discomycete fungus, *Stictis panizzei* De Not. [Syn. *Martamyces panizzei* (De Not.) Minter], with typically scorched leaves, on whose necrotic areas, primarily at the tip of the blade, it produced many apothecia clearly visible with the naked eye (Fig. 2B). The same author tested the pathogenecity of the fungus by transmission trials in which infected leaves with apothecia were placed in contact with leaves of healthy young plants maintained under a hood, coming to the conclusion that *S. panizzei* had a primary role in the genesis of "brusca". This was strongly criticized by O. Comes (see Petri, 1911) who considered this mycete a simple saprophyte of negligible importance, that colonizes necrotic foliar tissues.

Cuboni (see Petri, 1911) shared Brizi's views on the pathogenic aetiology of the Apulian "brusca" which, as he pointed out, was favoured by the coexistence of predisposing environmental factors that influenced the vegetative vigour of the plants. Cuboni's conclusion had found support in its own discovery in the vicinity of Sassari (Sardinia, insular Italy) of very severe cases of a disease with the same outward appearance of the "brusca" prevailing in the province of Lecce, to which the presence of *S. panizzei* was consistently associated (Cuboni, 1905).

Petri's (1911) attempts to reproduce the symptoms of "brusca" by inoculating healthy olive plants with *S. panizzei* failed. Thus, notwhistanding the authoritative claims by Brizi and Cuboni, he criticized Brizi's pathogenicity tests, whose results he regarded as being consequent to methodological errors.

Petri pushed to a much greater extent the studies on "brusca" and, as specified further ahead, defined the lifecycle of *S. panizzei*. He visited the infected olive orchards starting from 1905, a year in which "brusca" attacks in

the province of Lecce were less severe but more wide-spread than in the preceding years, and continued the observations from 1907 to 1910, during the period of activity of the "Osservatorio per lo studio delle malattie dell'olivo" (Observatory for the study of olive diseases) of Lecce. He so described the "brusca" situation in the province of Lecce in 1905: (i) affected olive orchards are primarily concentrated in the area between Lecce and Maglie, a land that does not exceed an altitude of 40 meters above sea level, being the lowest in the province; (ii) in the "agro" of Cavallino e Lizzanello, 99% of the plants of cv. Ogliarola di Lecce are diseased, 10 to 40% of their leaves being scorched. In this area, the majority of the symptomatic leaves host *S. panizzei* apothecia in their necrotic tissues. The disease shows also, though less severely, on the resistant cv. Cellina di Nardò scions grafted on cv. Ogliarola di Lecce; (iii) in the "agro" of Vanze and Strudà, all plants of cv. Ogliarola di Lecce are diseased while those of cv. Cellina di Nardò look normal; (iv) in the "agro" of Martano, Borgagne, Melendugno,

Calimera e Carpignano the attacks are very heavy, but fungal apothecia are seldom visible on scorched leaves. Here and then, the disease is so severe that also cv. Cellina di Nardò is affected, the same as cv. Ogliarola di Lecce.

Starting from 1906, "brusca" attacks became progressively less intense and widespread and consistently decreased in the years that followed so that, by 1910, it was very difficult to find symptomatic trees in the area (Petri, 1911). The rapid disappearance of the disease forced Petri to interrupt his studies, the same as it had occurred to C. Moschettini in the 18th century.

Petri (1911) was the first to observe on scorched leaves the presence of pycnidia which were produced in autumn in the lower leaf blade, at the first appearance of foliar symptoms. These fruiting bodies were immersed in the spongy tissue and were not visible with the naked eyes. Later, Petri (1915) referred these pycnidia to the genus *Phyllosticta*, the anamorph of *S. panizei*, and named the fungal species *Phyllosticta panizzei* (Fig. 2C, D). Infections
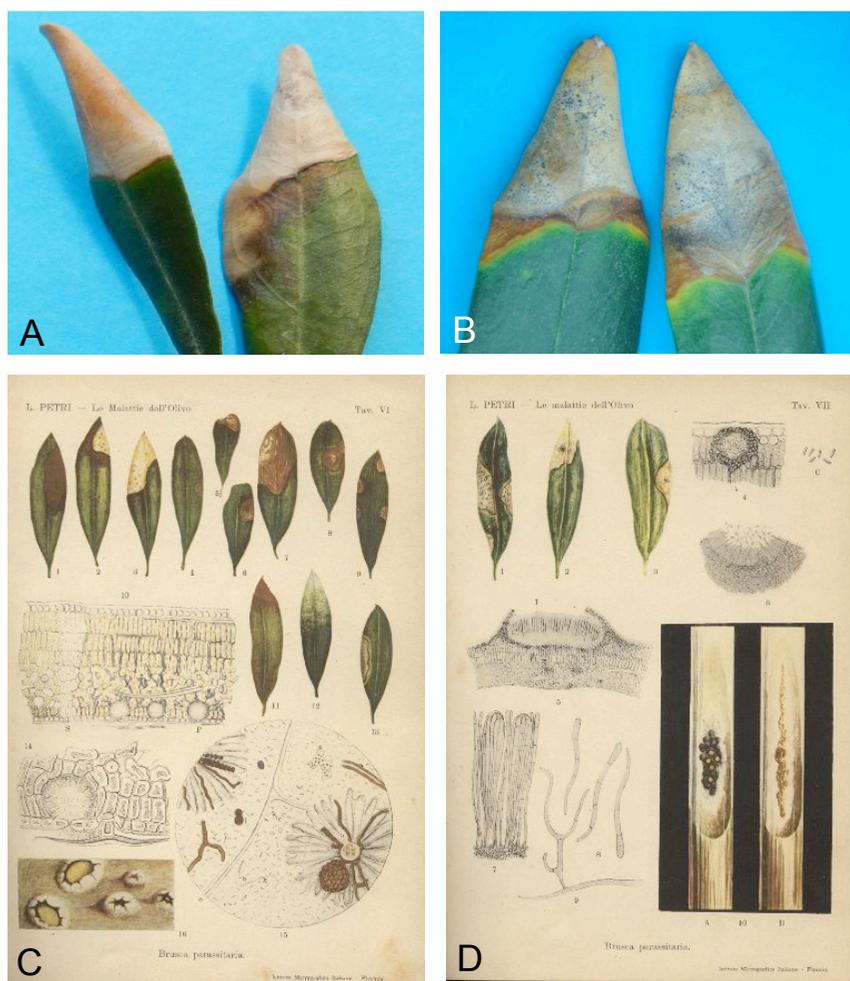

Fig. 2. A. "Brusca non parassitaria" (non parasitic leaf scorch). There is no sign of fungal infection on the necrotic leaf tips. B. "Brusca parassitaria" (parasatic leaf scorch). Necrotic leaf tips show plenty of *Stictis panizzei* apothecia. C and D. Petri's (1915) drawings of leaves affected by "brusca parassitaria" and of the life cycle of the associated pathogen: *Phyllosticta panizzei* (C), the anamorph of *S. panizzei* (D).

start in autumn by conidia released from pycnidia produced by the mycelium that oversummers in the infected leaves of the previous year. Conidial germ tubes enter the leaf tissues through the stomata, then the mycelium colonizes the mesophyll forming apothecia in winter and spring on the adaxial leaf surface. Ascospores are abundantly released in spring but, rather than causing new foliar infections, they colonize organic substrates.

Petri (1915) was also the first to define this type of scorching as "brusca parassitaria" ("parasitic leaf scorch") (Fig. 2B) to distinguish it from "brusca non parassitaria" ("non parasitic leaf scorch") (Fig. 2A) caused by abiotic factors, such as any physical cause that affects water supply to the leaf margin cells (e.g. insufficient moisture in the soil, water is lost too quickly from the leaves to be replaced adequately, damaged roots), or by hot dry winds, salty winds, nutrient deficiency/toxicity. In the case of "brusca parassitaria" the necrotic areas may interest equally well the apex, the margin and/or the internal portions of the leaf blade with a clear separation between the necrotic and the healthy, normally green, tissues, often with the interposition of a cork layer. Moreover, in winter and spring the necrotic tissues show plenty of fungal apothecia (Fig. 2B). The disease never involves young leaf tissues and appears in autumn, initially as a barely perceptible discoloration of tip and/or leaf margin. The leaves that were initially attached to the plant, unless the necrosis had reached the petiole, are shed in the spring, giving rise to extensive defoliations.

In recognition of the complex nature of "brusca parassitaria" Petri (1911, 1915) admitted that "*in the expression of the disease have a great importance an infinite number of unknown events in which the fungus may have only a secondary role*". Moreover, *S. panizzei* was "*without doubt the direct cause of dryness of the leaf blade*" but it could exert a pathogenic action only in the presence of "*particular conditions of the leaves*". Thus, the fungus was to be considered somewhat like "*a specific marker of an inside alteration of the physiological processes of the leaf, alteration which in the absence of a parasite could remain without external visible symptoms*".

The same author attributed the localization of "brusca parassitaria" in the Salento peninsula "*to local climatic, soil and also biological conditions, which influenced both the fungus, increasing its pathogenic activity, and the olive plants, thus determining conditions very favorable to the development of the parasite*". In particular, Petri associated the periodicity with which the disease appears in a severe and destructive form with recurrent episodes of high autumnal humidity in concomitance with the consistent presence of the parasite in the area. The remarkable intensification of the attacks in 1901 and 1902, after more than a century of disease quiescence, "*was observed in the presence of an important increase of humidity during autumn and winter, together with humid and warm winds*".

With his basic studies, Petri (1911, 1915) estimated that the susceptibility of olive to fungal attacks, thus to "brusca parassitaria", could be determined by particular physiological alterations of the leaves consequent to root failure. He suggested that a predisposing factor for disease expression could be the poor growth of the absorbing rootlets which, as he observed, were shorter and profusely branched, with the cortical layer invaded by the mycelium of an endotrophic mycorrhiza. Diseased olive plants from the province of Lecce and Sassari were showing a very high incidence (75 to 100%) of mycotrophic rootlets, which were two to three times more frequent than in the non affected plants of the same areas. This condition was caused by unfavorable conditions for the rooting apparatus, such as dryness and low organic matter content in the soil, which reduced the absorbing capacity of the rootlets, hence the vegetative vigour of the plants. To aggravate the context, Petri observed in summer a degenerative processes of the mycorrhized roots caused by the invasion by the fungal parasite *Cryptoascus oligosporus*, that covered the root tips with a cap of brown filaments and perithecia, thus decreasing the water uptake ability of the plants.

Petri attributed the resistance of cv. Cellina di Nardò to "brusca" to a better physiological activity of its leaves as compared with that of the leaves of cv. Ogliarola di Lecce. He remarked that the disease was closely related to peculiar environmental conditions and only secondarily with the properties of the cultivar since, under highly unfavorable conditions, also cv. Cellina di Nardò could suffer for severe attacks. Petri suggested to control "brusca parassitaria" by Bordeaux mixture treatments against fungal infections and, in particular, by implementing agronomic practices aimed at restoring root functionality and restraining the formation of mycotrophic roots. He also suggested pruning and cutting of the rootlets during winter ("sbarbettatura"), to be done upon opening a circular trench under the canopy projection, in correspondence of the extreme ends of the root system, followed by a fertilization.

From 1910, "brusca" became quiescent, entering a phase of half-dormancy characterized by occasional reports from different Italian regions (Sardinia and Liguria) (Petri, 1942). In more recent times, the disease has also been observed sporadically in Calabria (southern Italy) in olive plants growing under poor agronomical conditions and in absence of any chemical treatment (Agosteo, 2000).

The sudden disappearance of "brusca" from the Lecce province in 1910 induced Petri to discontinue his studies. He was aware of not having identified "*all the causes that contribute to the disease*" and wrote that he was leaving for the future generations of researchers, in the event of disease recurrence, the task of resuming the studies, starting from the results that he had obtained (Petri, 1911).

Olive quick decline, an epidemic disorder first investigated in 2013. Towards the end of the first decade of 2000, a novel, serious disease of olives appeared in some orchards of the countryside of Alezio, near the city of Gallipoli (province of Lecce) (Fig. 1). Its presence was brought to the attention of the researchers of the National Research Council (CNR, Virology Unit of the Istituto per la Protezione Sostenibile delle Piante) and of the University of Bari (UNIBA, Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti) only in the autumn 2013, when it had already spread over a surface of about 8,000 ha. Disease symptoms consisted basically in the appearance of leaf scorching and desiccation in small peripheral branches distributed at random on the plant's canopy (Fig. 3A) which, within a short time, extended to the rest of it (Fig. 3B). Field surveys carried out from September 2013 onwards, primarily by researchers from CNR-UNIBA, disclosed that disease severity seemed to vary with the age of the trees.

Very old, centenarian plants are, in fact, the most heavily affected. The dieback of the canopy is so extensive and rapid that the growers prune the trees heavily in the hope to induce new sprouting. This rarely happens, and when new flushes of vegetation are produced, they wither and desiccate in a short while. These heavily compromised trees survive for some time, push abundant suckers from the crown (Fig. 3B) until the roots are viable, then they die (Fig. 3C) The described symptomatology has won the disease the name of "Olive quick decline syndrome" (OQDS).

The investigations carried out by CNR-UNIBA in the affected area soon disclosed that the OQDS shown by very aged tree is generally associated with poor agricultural management of the orchards and appears to be a complex disorder (Martelli, 2013) in whose aetiology the following agents seem to be involved: (i) the leopard moth (*Zeuzera pyrina*), the very same lepidopteron reported in the 18th century literature as a frequent pest in the area (Presta, 1794), whose galleries open the way to an extensive fungal colonization of the sapwood; (ii) a set of xylem-inhabiting fungi of the genus *Phaeoacremonium* in particular, with a prevalence of *P. parasiticum* (Nigro *et al.*, 2013). These fungi are frequent inhabitants of olive wood and the precursors of a common wood condition of old trees known as "carie del legno" (wood decay); (iii) the xylem-limited bacterium *Xylella fastidiosa* (Saponari *et al.*, 2013).

Trees aged 50 to 70 years or younger, show a somewhat milder syndrome. Leaf desiccation and dieback of twigs and small branches are still outstanding, but leopard moth and fungal attacks are much less frequent, whereas the presence of *X. fastidiosa* is consistent. These trees undergo pruning, but not as heavily as the bigger centenarian trees, and they may not necessarily die, at least in the light of the observations made so far (CNR-UNIBA).

Fig. 3. Various stages of the Olive quick decline syndrome. A. Beginning of symptom appearance. B. Extensive desiccation of the canopy. C. Heavily pruned trees with "burned" vegetation but pushing green symptomless suckers (arrows). D. Dead trees.
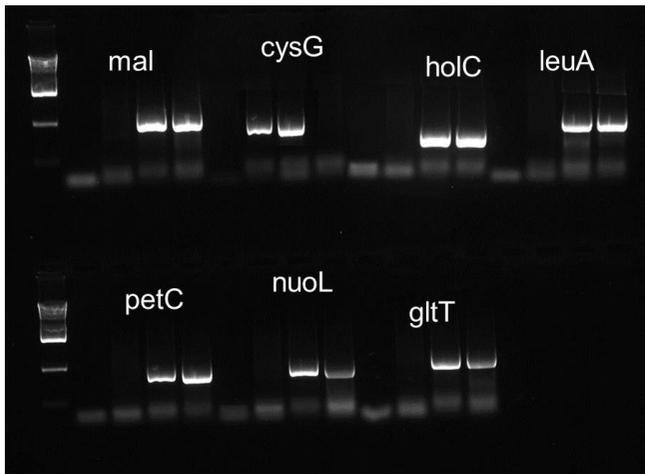
Fig. 4. Multilocus sequence typing based on the amplification of seven different genes (courtesy of M. Saponari and G. Loconsole). A phylogenetic tree constructed with the concatenated sequences of the seven genes showed that the strain CoDiRO belongs to the *Xylella fastidiosa* subs. *pauca* (M. Saponari and G. Loconsole, personal communication).

X. fastidiosa is a Gram-negative, xylem-restricted bacterium, and a quarantinable pathogen for the European Union (EU) included in the EPPO A list, whose putative presence in Europe and the Mediterranean basin was based on two unconfirmed reports, i.e grapevines in Kosovo (Berisha *et al*., 1998) and almonds in Turkey (Güldur *et al*., 2005). Thus, its discovery in olive trees in southern Italy (Saponari *et al*., 2013), represents the first substantiated record in the EU. Following detection, the olive strain of *X. fastidiosa*, denoted CoDiRO (abbreviation from the Italian name "Complesso del Disseccamento Rapido dell'Olivo"), has been the object of intensive investigations, carried out primarily by the CNR-UNIBA research team.

The main results obtained so far can be summarized as follows: (i) a number of infected symptomatic hosts other than olive have been identified, among which almond (*Prunus dulcis*), oleander (*Nerium oleander*) (Saponari *et al*., 2013) and, more recently, cherry (*Prunus avium*), myrtle-leaf milkwort (*Polygala myrtifolia*) and coastal rosemary (*Westringia fruticosa*) (Saponari *et al*., 2014a). The latter two species are new *X. fastidiosa* hosts thus, they add up to the long list of already known susceptible plants (http//www.cnr.berkeley.edu/xylella/control/hosts.htm, see also Janse and Obradovic, 2010); (ii) pure cultures of the strain CoDiRO were obtained first from periwinkle (*Vinca rosea*) plants which had been infected by insect vectors carrying the bacterium and from symptomatic oleanders (Cariddi *et al*., 2014), then from olive, almond, cherry and *P. myrtifolia* plants (M. Saponari, personal communication); (iii) molecular characterization by multilocus sequence typing (MLST) (Fig. 4; M. Saponari and G. Loconsole, personal communication) and by comparison of the partial nucleotide

sequence of the DNA *gyrase* subunit B, has ascertained that strain CoDiRO is a variant of *X. fastidiosa* subsp. *pauca* (Cariddi *et al*., 2014), a subspecies of South American origin. Thus, it differs from the strain that infects olive in California, which belongs to *X. fastidiosa* subsp. *multiplex* (Hernandez-Martinez *et al*., 2007; Krugner, 2010; Krugner *et al*., 2014); (iv) strain CoDiRO has been identified in adults of the meadow spittlebug (*Philaenus spumarius*), a leafhopper that thrives also on olive trees (Fig. 5A, B), and was able to transmit the bacterium to periwinkle seedlings (Saponari *et al*., 2014b).

Concomitantly with the studies outlined above, a survey was initiated by the Apulian Regional Phytosanitary Service for assessing the distribution of *X. fastidiosa* in the olive groves of the whole province of Lecce and at the borders of the neighbouring provinces of Taranto and Brindisi (Fig.1). The analyses, performed by ELISA and PCR (Loconsole *et al*., 2014), were carried out by officially entrusted institutions, i.e. CNR-UNIBA, Istituto Agronomico Mediterraneo Bari, Centro di Ricerche Sperimentazione e Formazione in Agricoltura Locorotondo and the University of Foggia. Several new infection foci were discovered, among which a recent small one in an area comprising the countryside of Ruffano and some neighbouring villages. In this area 20 olive trees of either cv. Cellina di Nardò or Ogliarola di Lecce, 10 of which hosting *S. panizzei* apothecia on scorched leaves ("brusca parassitaria") and 10 without apothecia ("brusca non parassitaria") were tested by ELISA and PCR, for the presence of *X. fastidiosa*, followed by amplicon sequencing. Total nucleic acids were extracted from leaf petioles and midribs excised from scorched leaves using the DNeasy plant mini kit (Qiagen, Germany). DNA was amplified using primers targeting the 16S rDNA, i.e.. XF1-F/XF6-R (Firrao and Bazzi, 1994), or S-S-X.fas-0838-a-S-21/S-S-X.fas-1439-aA-19 (Rodrigues *et al*., 2003), which amplify products of 404 and 603 bp, respectively. Sterile distilled water and DNA extract from healthy olive leaves collected in an area far away from the OQDS site, were used as negative controls. Amplifications were performed with an automated thermal cycler in a 50 µl reaction volume. Aliquots (8 µl) of the amplified products were visualized using an UV transilluminator after electrophoresis in 1.2% agarose gel in TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8) and stained with ethidium bromide in the presence of a molecular weight marker (1-kbDNA ladder; Life Technologies, USA). After further electrophoresis in a 1.5% agarose gel, the same products were sampled, purified with the QIAquick Gel Extraction kit (Qiagen, Germany) and custom sequenced (BMR Genomics, Italy) The resulting sequences were compared with those available in GenBank using the BLAST software (Altschul *et al*., 1997). For serological investigations, a commercial ELISA kit was used (Loewe, Germany), following the manufacturer's instructions.

ELISA testing was consistently positive, and PCR amplification with both set of primers generated products of the expected size from symptomatic leaves (but not from the negative controls), whose sequence showed a 99% similarity with that of *X. fastidiosa* isolates from GenBank (accession Nos. EU560724; EU560721). Two sequences obtained using primers S-S-X.fas-0838-a-S-21/S-S-X.fas-1439-aA-19, were deposited in GenBank under the accession Nos. KF981190 and HG94160. These results further support the notion that *X. fastidiosa* has now spread to a proportion of some consequence of the olive groves of the Lecce province (approximately 8,000 ha comprised in an area of *ca*. 25,000 ha) and can be identified in scorched olive leaves, regardless of whether or not they are also infected by *S. panizzei.*

OQDS occurs in the Lecce province, the site of the very severe cases of "brusca" that were repeatedly observed and carefully described in the past. This coincidence, and the type of symptoms, characterized by extensive scorching, dieback and desiccation of the olive canopy, that recall those described in the early literature (Presta, 1794, in particular), may lead one to speculate that *X. fastidiosa* has been sitting in that area for about 250 years, rather than being a pathogen of recent introduction.

This, however, definitely does not seem to be the case, as it stems from the many discrepancies between the ancient descriptions and the extant situation.

(i) Symptomatology. Although, as stated above, a similarity may at first sight be found between the "ancient brusca" (AB) attacks and OQDS, this is more apparent that real. As described by C. Moschettini, G. Presta and L. Petri, AB affects primarily the leaves of the lower branches inducing apical and marginal scorching, followed by defoliation. By contrast, with OQDS the typical "brusca"-induced leaf scorch is not the most prominent symptom. Rather, are the upper branches to be preferentially symptomatic, bearing totally desiccated leaves that remain attached to the twigs, which, after leaf fall, do not vegetate again.

(ii) Timing of symptom appearance. AB was reported to show up in autumn and spring affecting mainly the olive canopy sectors exposed to south or west. With OQDS there is no preferential orientation of the symptoms, which become prominent and progressively more intensive at the onset of summer (June) when, with the raise of the temperatures, also the need of water uptake increases.

(iii) Varietal reaction. There is distinct differential behaviour in the reaction to AB of the two major olive cultivars grown in the province of Lecce, cv. Cellina di Nardò being unanimously reported as much less susceptible (in fact, "resistant") than cv. Ogliarola di Lecce. This is not the case with OQDS, for both these cultivars are equally susceptible and damaged.

(iv) Disease recurrence. All those who have investigated AB have unanimously remarked that the disease undergoes recurrent periods of quiescence or disappearance. Although it may appear too early to determine whether OQDS behaves in the same way, the involvement of *X. fastidiosa* in its aetiology makes it most unlikely that OQDS may act as AB. It is common knowledge that when *X. fastidiosa* enters an environment, thanks to its wide
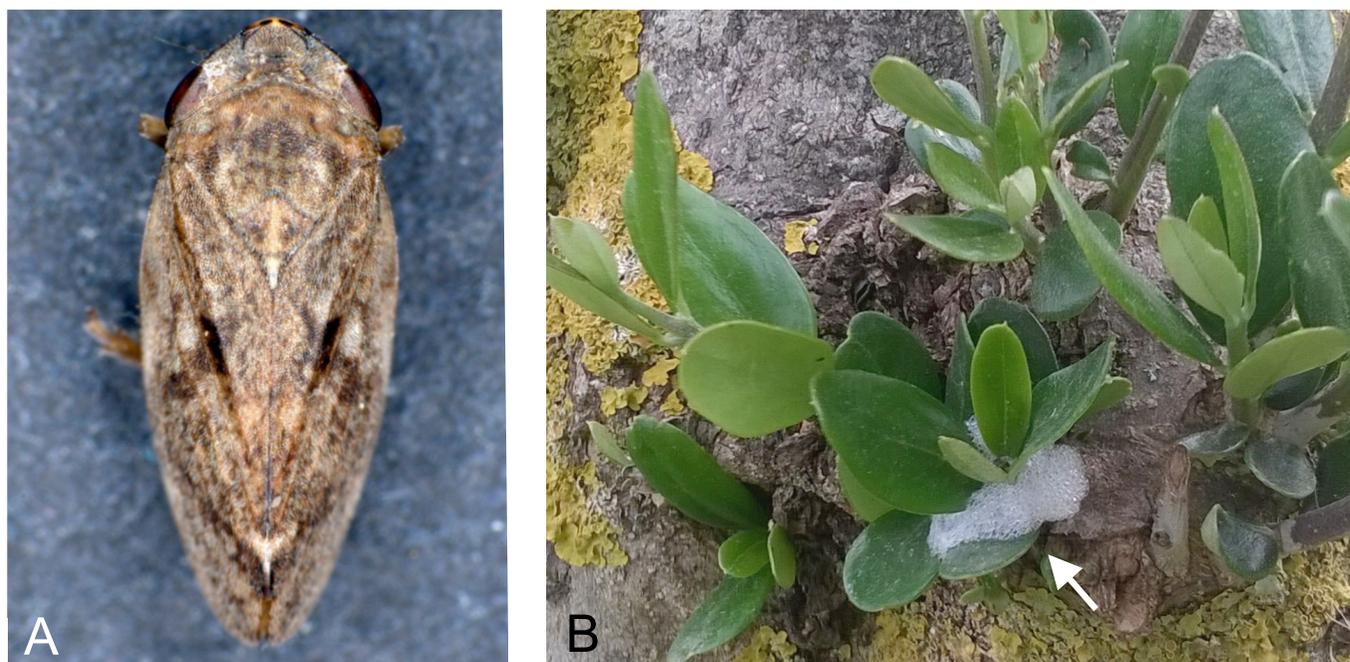
Fig. 5. A. Adult of *Philaenus spumarius* (courtesy of F. Porcelli). B. *P. spumarius* foam nest on a sprout of an olive tree from one of the OQDS foci.

host range and epidemiological behaviour, it becomes stably entrenched in the local flora, i.e. susceptible native weeds, shrubs and trees, and agricultural crops. There is, therefore, an inoculum that persists all year round which, when the time comes (usually spring) is transferred by the vectors to new healthy hosts, that are infected and come down with symptoms.

(v) Disease distribution. As shown in Fig. 1, there is a clear-cut separation between the geographical distribution of AB (localized in the eastern areas of the province) and that of OQDS, which has started in the western part (Ionian coast) and is now spreading towards east (Adriatic coast).

(vi) Host range. From ancient reports it can be argued that AB is typically and strictly confined to olive, for no mention was made of the occurence of visible foliar symptoms (scorching, desiccation) in hosts other than *O. europaea*. By contrast, a number of symptomatic plants (e.g. oleander, almond, myrte-leaf milkwort, coastal rosemary) have now been identified in the OQDS-affected area, all of which are affected by strain CoDiRO. It is therefore conceivable that, if the presence of *X. fastidiosa* in the province of Lecce dated back to the 18th Century, this bacterium would not have spared also a number of hosts differing from olive. This would not have escaped the attention of keen observers like C. Moschetittini and G. Presta and, later, L. Petri and colleagues who would have noticed and recorded in their reports "brusca"-like symptoms in plants other than olive.

(vii) It is worth mentioning that *X. fastidiosa* evolves by adapting to different hosts and is naturally competent for recombination (Kung and Almeida, 2011; Nunney *et al.*, 2013). Its long presence (some 250 years, if this were the case) in a secluded area such as the southern part of the Salento peninsula and the exposure to a number of different hosts, some of which are new (Saponari *et al.*, 2014a), would have more than likely favoured the differentiation of a novel bacterial type differing from the extant ones. Instead, strain CoDiRO is not only a variant of X. *fastidiosa* subsp. *pauca*, one of the four recognized subspecies of this bacterium (Almeida *et al.*, 2014) thought to be of South American origin, but it was recently found to be molecularly identical to a strain from Costa Rica (M. Saponari, personal communication).

All this considered, it seems plausible to conclude that: (i) AB and OQDS are two quite different diseases; (ii) there are no reasons to believe that CoDiRO be not an exotic strain of *X. fastidiosa*, of recent and unfortunate introduction in southern Italy.

REFERENCES

Agosteo G.E., 2000. Le malattie fungine. In: Regione Calabria (ed.). Avversità e Difesa dell'Olivo in Calabria, pp. 13-88. Tipografica Lametina, Lamezia Terme, Italy.

Almeida R.P.P., Coletta-Filho H.D., Lopes J.R.S., 2014. *Xylella fastidiosa*. In: Liu D. (ed.). Manaul of Security Sensitive Microbes and Toxins, pp. 841-850. CRC Press, Boca Raton, FL, USA.

Altschul S., Madden T.L., Shäffer A.A., Zhang J,. Zhang Z., Miller W., Lipman D., 1997. Gapped BLAST and PST-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* **25**: 3389-3402

Berisha B., Chen Y.D., Zhang G.Y., Xu B.Y., Chen T.A., 1998. Isolation of Pierce's disease bacteria from grapevines in Europe. *European Journal of Plant Pathology* **104**: 427-433.

Brizi U., 1903. Sulla Malattia degli Olivi Denominata Brusca. Tipogafia Nazionale G. Bertero et Co., Roma, Italy.

Cariddi C., Saponari M., Boscia D., De Stadis A., Loconsole G., Nigro F., Porcelli F., Potere O., Martelli G.P., 2014. Isolation of a *Xylella fastidiosa* strain infecting olive and oleander in Apulia, Italy. *Journal of Plant Pathology* **96**: 425-429.

Comes O., 1900. Sul malanno degli olivi nel Leccese. *Bollettino di Notizie Agrarie, Direzione Generale dell'Agricoltura* **22**: 908.

Comes O., 1901. Sul malanno degli ulivi denominato brusca nel Leccese. *Bollettino di Notizie Agrarie* **23**: 4.

Cuboni G., 1905. La brusca dell'olivo nel territorio di Sassari. *Rendiconti della Regia Accademia Nazionale dei Lincei* **14**: 603-605.

Costa A., 1857. Degl'insetti che attaccano l'albero ed il frutto dell'olivo, del ciliegio, del pero, del melo, del castagno e della vite e le semenze del pisello, della lenticchia, della fava, e del grano loro descrizione e biologia danni che arrecano e mezzi per distruggerli. Opera Coronata dalla Reale Accademia delle Scienze di Napoli. Stamperia e Calcografia Vico Freddo Pignasecca, Napoli, Italy.

Cuboni G., 1905. La "Brusca" dell'Olivo nel territorio di Sassari. *Rendiconti Regia Accademia dei Lincei* **14**: 603-605.

Firrao G., Bazzi C., 1994. Specific identification of *Xylella fastidiosa* using the polymerase chain reaction. *Phytopathologia Mediterranea* **33**: 90-92.

Güldür M.E., Çaglar B.K., Castellano M.A., Ünlü L., Güran S., Yilmaz M.A., Martelli G.P., 2005. First report of almond leaf scorch in Turkey. *Journal of Plant Pathology* **87**: 246.

Hernandez-Martinez R., de la Cerda K.A., Costa H.S., Cooksey D.A., Wong F.P., 2007. Phylogentic relationships of *Xylella fastidiosa* strains isolated from landscape ornamentals in Southern California. *Phytopathology* **97**: 857-864.

Janse J.D., Obradovic A., 2010. *Xylella fastidiosa*: its biology, diagnosis, control and risks. *Journal of Plant Pathology* **92**: S1.35-S.1.48.

Krugner R., 2010. Evaluation of pathogenicity and insect transmission of *Xylella fastidiosa* strains to olive plants. *California Olive Committee. Final Report 2010.*

Krugner R., Sisterson M.S., Chen J., Stenger D.C., Johnson M.W., 2014. Evaluation of olive as a host of *Xylella fastidiosa* and associated sharpshooter vectors. *Plant Disease* (http://dx.doi.org/10.1094/PDIS-01-14-0014-RE).

Kung S.H., Almeida R.P.P., 2011. Natural competence and recombination in the plant pathogen *Xylella fastidiosa. Applied and Enviornmental Microbiology* **77**: 5278-5284.

Loconsole G., Potere O., Boscia D., Altamura G., Djelouah K., Elbeaino T., Frasheri D., Lorusso D., Palmisano F., Pollastro P., Silletti M.R., Trisciuzzi N., Valentini F., Savino V., Saponari M., 2014. Detection of *Xylella fastidiosa* in olive trees by molecular and serological methods. *Journal of Plant Pathology* **96**: 7-14

Martelli G.P., 2013. Disseccamento rapido dell'olivo. *Georgofili INFO*, 30 October 2013.

Moschettini C., 1777. Della Brusca, Malattia Degli Ulivi di Terra d'Otranto sua Natura, Cagioni, Effetti. Mazzola-Vocola, Napoli, Italy.

Moschettini C., 1789. Della Brusca, Malattia degli Ulivi di Terra d'Otranto. 2a Edizione, Riordinata, Corretta ed Ampliata dall'Autore. Mazzola-Vocola, Napoli, Italy.

Nigro F., Boscia D., Antelmi I., Ippolito A., 2013. Fungal species associated with a severe decline of olive in southern Italy. *Journal of Plant Pathology* **95**: 688.

Nunney L., Vickerman D.B., Bromley R.E., Russel S.A., Hartman J.R., Morano L.D., Stouthamer R., 2013. Recent evolutionary radiation and host plant specialization in the *Xylella fastidiosa* subspecies native to the United States. *Applied and Environmental Microbiology* **79**: 2189-2200.

Petri L., 1911. Ricerche sulla brusca dell'olivo. In: Petri L. (ed.). Studi sulle malattie dell'olivo, pp. 13-147. Memorie della Regia Stazione di Patologia Vegetale. Tipografia Nazionale G. Bertero et Co., Roma, Italy.

Petri L., 1915. Le Malattie dell'Olivo. Manuale per l'Identificazione delle Malattie non Parassitarie e di Quelle Prodotte da Parassiti Vegetali con Speciale Riguardo ai Mezzi per Combatterle. Istituto Micrografico Italiano, Firenze, Italy.

Petri L., 1942. Rassegna dei casi fitopatologici osservati nel 1941. *Bollettino della Regia Stazione di Patologia Vegetale* **22**: 15-17.

Presta G., 1794. Degli Ulivi e delle Ulive, ed della Maniera di Cavar l'Olio. Stamperia Reale, Napoli, Italy.

Rodrigues L.M., Silva-Stenico M.E., Gomes J.E., Lopes J.R., Tsai S.M., 2003. Detection and diversity assessment of *Xylella fastidiosa* in field-collected plant and insect samples by using 16S rRNA and gyrB sequences. *Applied and Environmental Microbiology* **69**: 4249-4255.

Saponari M., Boscia D., Nigro F., Martelli G.P., 2013. Identification of DNA sequences related to *Xylella fastidiosa* in oleander, almond and olive trees exhibiting leaf scorch symptoms in Apulia (southern Italy). *Journal of Plant Pathology* **95**: 668.

Saponari M., Boscia D., Loconsole G., Palmisano F., Savino V., Potere O., Martelli G.P., 2014a. New hosts of *Xylella fastidiosa* strain CoDiRO in Apulia. *Journal of Plant Pathology* doi: 10.4454/JPP.V96I3.008

Saponari M., Loconsole G., Cornara D., Yokomi R.K., De Stradis A., Boscia D., Bosco D., Martelli G.P., Krugner R., Porcelli F., 2014b. Infectivity and transmission of *Xylella fastidiosa* Salento strain by *Philaenus spumarius* L. (Hemiptera: Cercopidae) in Apulia, Italy. *Journal of Economic Entomology* **107** (http://dx.doi.org/10.1603/EC14142).