The presence of *Xylella fastidiosa* in Apulia region (Southern Italy) poses a serious threat to the whole Euro-Mediterranean region

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Background

That is now a fact! The main causal agent which is devastating the olive groves of Apulia is a strain of the bacterium *Xylella fastidiosa* (Xf) subspecies *pauca,* named "Codiro" (acronym of "Complesso del Disseccamento Rapido dell'Olivo" that means "olive quick decline syndrome", OQDS). Koch's postulates are expected to be fulfilled soon and their completion will dispel any doubt about the causal relationship between the bacterium and OQDS. However, the close association of *X. fastidiosa* with symptomatic olive trees seems to leave little doubt about the involvement of this pathogen in the disease aetiology. The severe damage caused by this bacterium on the olive crop represents an unprecedented exceptional situation which must be faced adopting a different approach compared to the strategies already applied in other countries. However, other infectious agents, in particular some fungi of the genera *Phaeoacremonium* and *Phaeomoniella*, commonly found together with *X. fastidiosa* in the OQDS-infected trees of Apulia, are supposed to play an important synergistic role in the severity of symptoms.

At present, the disease is ravaging an olive growing area of *ca.* 90,000 hectares in the Salento peninsula (the province of Lecce), the boot-heel of Italy bordering the Ionian and the Adriatic seas, but concern remains that it could spread further through its natural vectors across Apulia, the most important Italy's olive producing region, with a total surface area of about 380,000 hectares (32% of the area under olive trees in Italy).

Following the application of traditional serological (ELISA) and molecular (PCR) detection techniques, *X. fastidiosa* was identified as a possible agent of the disease in September 2013 (Saponari *et al.*, 2013), although OQDS-like symptoms had already been reported some years earlier. Genome sequencing allowed then for the final and detailed characterization of the pathogen. At first, only a few characterizing genes of the bacterium were sequenced through Multilocus sequence typing (MLST) (Elbeaino *et al.*, 2014); subsequently, the entire genome was determined by "deep sequencing" (Giampetruzzi *et al.*, 2015). As a result, the Apulian isolate came to be one of the few *X. fastidiosa* isolates fully sequenced in the world. Molecular characterization provided a step forward in determining more clearly the pathogen identity, exploring its biological characteristics and suggesting hypotheses on its geographical origin.

Origins and Consequences

As indicated before, the Apulian strain of *X. fastidiosa* displays some genetic traits which relate it to the isolates of subspecies *pauca*, also including the causal agent of "Citrus variegated chlorosis" in South America. More precisely, it exhibits a very high level of genetic identity with an isolate of *X. fastidiosa* from Costa Rica, known to affect oleander, mango, coffee and other ornamental species, but not the olive tree which is not grown in this country. It is highly probable that the Apulian isolate originates from Central America because the European countries annually import from Costa Rica millions of ornamental plants belonging to several species, which rarely undergo appropriate phytosanitary inspections at the point of entry into the EU. The recent interceptions of *X. fastidiosa*, by the Dutch, French, German and Italian Customs and in a number of nurseries, on ornamental coffee plants imported from Costa Rica and Honduras provide further evidence in support of this hypothesis.

The very first records indicated that the olive tree was the preferred host of *Xf*-Codiro strain; furthermore, numerous other plant species were found to be vulnerable to this polyphagous bacterium. The list of the susceptible hosts, still provisional, already includes a number of cultivated, ornamental and wild species, i.e. almond (*Prunus dulcis*), cherry (*P. avium*), oleander (*Nerium oleander*), myrtle (*Myrtus communis*), rosemary (*Rosmarinus officinalis*), September bush (*Polygala myrtifolia*), coastal rosemary (*Westringia fruticosa*), periwinkle (*Vinca minor* and *Chatarantus roseus*), genista (*Spartium junceum*), golden wattle (*Acacia saligna*), Mediterranean buckthorn (*Rhamnus alaternus*), etc. Hopefully, the susceptibility of grapevine and *Citrus* spp. to *Xf*-Codiro strain in nature might be excluded, because not a single plant in these species has tested positive to the bacterium in severely infected areas. However, a final conclusion can only be reached once the results of the infectivity tests are made available.

Expansion and eradication strategy

The rapid spread of the bacterium in Apulia is undoubtedly facilitated by the abundance of its vector, the meadow spittlebug *Philaenus spumarius,* in the infected areas where research evidence immediately demonstrated high transmission efficiency (Saponari *et al*., 2014). At least two more species, *Neophilaenus campestris* and *Euscelis lineolatus,* proved to be capable of harbouring the bacterium, although there is no conclusive data so far that they can transmit the infection (Elbeaino *et a*l., 2014).

In a very short time the pathogen has invaded several thousands of hectares of olive trees in Apulia, expanding its range throughout an area that includes almost the whole province of Lecce and some isolated outbreaks in the province of Brindisi.

The presence of the pathogen in infected trees can be detected by different diagnostic tools. Technical protocols based on ELISA and conventional PCR, already utilized for the detection of *X. fastidiosa* in other plant species and countries, were applied to olive samples and compared and validated via an interlaboratory ring-test, during which both procedures proved to be equally effective. Considering its lower cost, in addition to its simplicity, ELISA was the preferred method for a large-scale monitoring programme in Apulia, which preliminarily involved about 20,000 plants, mainly olives but also other host species. The more sensitive PCR technique was instead used exclusively for confirmation tests and for doubtful cases.

Based on the large number of samples tested in Apulia, the distribution map of *X. fastidiosa* within the region was designed with a certain level of precision. For this purpose and to improve the planning of monitoring activities, the support of multimedia systems and tools was crucial. The whole regional area was subdivided into grids of about 10 km^2, the OQDS-suspected trees were identified by photointerpretation of high resolution aerial images, sampled plants were geo-referenced, the collected data were acquired through a field application (XylApp) and sent to a central server (XylWeb) for their rapid storage and analysis. At present, the area of expansion of the bacterium seems to be restricted to the southern part of the Salento peninsula. Given the current state of affairs, efforts are mainly aimed at protecting the area to prevent further spread of the disease northwards.

After completing the monitoring activities in the whole region of Apulia, which represented the first step taken when the pathogen was identified, a multidisciplinary strategic approach was adopted to contain the spread of *X. fastidiosa*, including a set of regulatory measures (at regional and subsequently, at national level) which led to the enactment of a law for the mandatory control of the bacterium (D.M. n. 2777 of 26/09/2014), the appointment of a Commissioner to deal with "*Xylella fastidiosa* emergency", the establishment of a national scientific committee to oversee all control initiatives and research activities, the earmarking of special funds to cover the cost of control.

Salento, Target area of the Action Plan

Salento is the target area of the action plan supervised by the Commissioner and has been divided into different zones, according to whether the pathogen has been found or not. The action plan encompasses the mandatory measures aimed at strengthening surveillance, removing the infection sources and controlling the vector. Considering the extent of this plague, which is now inflicting damages to a very wide area of Apulia, attempts at the total eradication are no more a feasible option. Indeed, there is no record of successful eradication anywhere in the world. As a result, to stop the spread of this invasive disease, a coast-to-coast "eradication zone" (a strip about 30 Km large and 50 km long), running from the Adriatic Sea to the Ionian Sea, has been set up in the northernmost part of the outbreak area.

At the same time, to prevent the bacterium from expanding northwards, a surrounding 2-4 Km wide "buffer zone" has been carved and a further 30 Km "prevention zone", next to the buffer zone, has also been established. In these areas (eradication, buffer and prevention zones), up-rooting concerned not only the infected olive trees and plants around in a radius of 100 meters, but also all ornamental and wild plants known to host *X. fastidiosa*. Vector control has become mandatory and it is based on an integrated management approach. Cultural practices (ploughing, harrowing, removal of shrubs and bushes), physical methods (weed flaming in places which are otherwise inaccessible), biological and chemical control measures are combined to fight against all life stages of the vector while always trying and preserving the environment. In addition, other actions are to be carried out such as cleaning canals, ditches, roads and public areas.

Furthermore, plant nurseries are submitted to regulatory restrictions concerning the production of *Xf*-host plants and also of potentially susceptible hosts, for prevention purposes. These restrictions include the total prohibition of plant movement outside the outbreak area, the production and trade of plant material in compliance with specific plant health requirements and even, plant growing under the screenhouse, if necessary, laboratory testing of plant material intended for export and chemical treatments of plant material.

However, drastic action would not be enough unless information campaigns are launched targeting practically the whole civil society to increase education and awareness about eradication measures, prohibition of infected plant movement and risk of disease spread by human assistance.

Apart from containment measures, only scientific research can provide effective solutions to this emergency, which represents an exceptional case worldwide. Hence, much research effort is required not only at local level, but also at national and EU levels, because *X. fastidiosa* is likely to become a global threat. Despite the severity of this problem, to date no concrete financial support has been provided to research on this important issue, except for few funds allocated by the Apulian authorities. In fact, current research is carried out thanks to the commitment of local highly motivated researchers who are aware of the serious situation in Apulia and are putting their expertise at the service the community. Today, a number of avenues are being explored to fight against the bacterium survival or limit its ability to reproduce inside the plant, identify and/or develop plant resistance/tolerance (especially, genetic), develop advanced techniques for quick and effective detection of the pathogen in a given area, improve control of insect vectors, possibly through environmentally friendly methods.

Concluding remarks

Once again, the *X. fastidiosa* crisis in Apulia has shown the fragility of agro-ecological systems, when plant invasive harmful organisms are brought into new vulnerable regions. Unfortunately, in the recent years undesirable organisms have been more and more frequently introduced into the EU countries like the red palm weevil (*Rhynchophorus ferrugineus*), the spotted wing drosophila (*Drosophyla suzukii*), the Asian chestnut gall wasp (*Dryocosmus kuriphilus*), the tomato leaf miner (*Tuta absoluta*), the citrus longhorned beetle (*Anoplophora chinensis*), the bacterial canker of kiwifruit (*Pseudomonas syringae pv. actinidiae*), etc.

As a result, we urgently need a more effective quarantine system, due to the increasing movement of commodities and people across boundaries, which is leading to the globalization of plant disease problems. Strengthening the inspection services at points of entry/exit would be desirable, in terms of both personnel and equipment, especially as regards the use of appropriate diagnostic tools for early detection. Of utmost importance is also focusing on the main pathways through which these organisms can be brought into new areas. As it has occurred with the introduction of *Xylella fastidiosa* into the EU, ornamental plant species, more often not regulated by the law, are the main carriers of most undesirable pests. However, apart from improved knowledge of stakeholders, the civil society as a whole should gain a better understanding of the phytosanitary risks posed by the transport of plant products when travelling.

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