

SCIENTIFIC REPORT ON: GENETICS AND PRELIMINARY BIOLOGICAL EVIDENCES ON THE *Xylella* *fastidiosa* strain CoDiRO

The molecular data developed by the research group of the Institute of Sustainable Plant Protection (former Institute of Plant Virology), CNR and of the University of Bari, clearly indicated that the isolate of *Xylella fastidiosa* recovered, in the Salento peninsula, from olives affected by the Olive Quick Decline Syndrome (OQDS) is related to the subspecies *pauca* (Fig. 1). Furthermore, specific comparative analysis among the strains belonging to the subsp. *pauca*, provides strong evidence that the Apulian olive isolate is distinct from the *pauca* strains so far characterized and available in the public databases (see the position of the olive strain in the phylogenetic tree reported in figure 1). Moreover, the genotypic profile assigned to the Apulian strain, based on the MLST approach, is a new profile (ID “53”, <http://pubmlst.org/xfastidiosa/>), never described before, except for a finding currently under investigation in Costa Rica (see our previous communication).

X. fastidiosa subsp. *pauca* is known to be restricted to Central and South America. A brief review of the host range of this subspecies in these areas is reported in Annex I. As emerge from the literature, the major crops affected by *X. fastidiosa* subsp. *pauca* are *Citrus* spp. (mainly sweet orange varieties, while most mandarins and rootstocks are either tolerant or resistant) and *Coffea* spp. The diseases induced on these susceptible species are Citrus Variegated Chlorosis (CVC) and Coffee Leaf Scorch (CLS).

Recent studies on the genetics and the biology of the strains associated to CVC and CLS, clearly showed they are genetically and biologically distinct (Almeida et al., 2008). As shown in figure 2, they cluster in 2 distinct subgroups, and as shown by

experimental cross-inoculation the strains associated to coffee LS are not able to infect citrus plants and *viceversa* (Almeida et al., 2008).

The surveys and the investigations carried out in the contaminated area of Apulia, allowed to determine that olive, oleander, almond and vinca sp. are susceptible hosts under natural field infections. An in depth survey was done to test the presence of the bacterial infections in mature grapevines and adult citrus trees grown in the heavily contaminated area. Both group of hosts were found always free from infections (tested negative in diagnostic assays) as well free from any symptom resembling those associated to Pierce’s disease and CVC (Fig. 3), respectively (see our previous communications).

In conclusion, based on the sequences of 7 housekeeping genes analysed by using the MLST approach, widely recognized as the most efficient typing method for bacteria, the Apulian isolate can be related to the subspecies *pauca*. Its distinctiveness from the known *pauca* strains, strongly supports that it represents a novel strain, for which the name *Xylella fastidiosa* strain CoDiRO has been proposed. *Citrus* spp. are amongst the principal hosts for *X. fastidiosa* subsp. *pauca* in south America. Our biological data, even if not yet supported by the pathogenicity tests, show that under natural infection conditions, the strain CoDiRO is not able to infect citrus. This evidence is not something unexpected if we consider the high host-specificity within the *pauca* subspecies. As mentioned before, the coffee LS strains although genetically close related to the citrus CVC strains, even closer than the CoDiRO strain (Fig.2), fail to infect citrus and *viceversa*. Accordingly, it could be expected that experimental inoculations of the CoDiRO strain on citrus plants will result in the failure to reproduce successful infections, confirming the evidences so far recovered from the field surveys.

ALMEIDA R. P. P., NASCIMENTO F. E., CHAU J., PRADO S.S., TSAI C.W., LOPES S.A., J.R. S. LOPES, 2008. Genetic Structure and Biology of *Xylella fastidiosa* Strains Causing Disease in Citrus and Coffee in Brazil. *Appl Environ Microbiol.* Jun 2008; 74(12): 3690–3701.

Figure 1. Differentiation of the *X. fastidiosa* (*Xf*) subspecies based on MLST analysis.

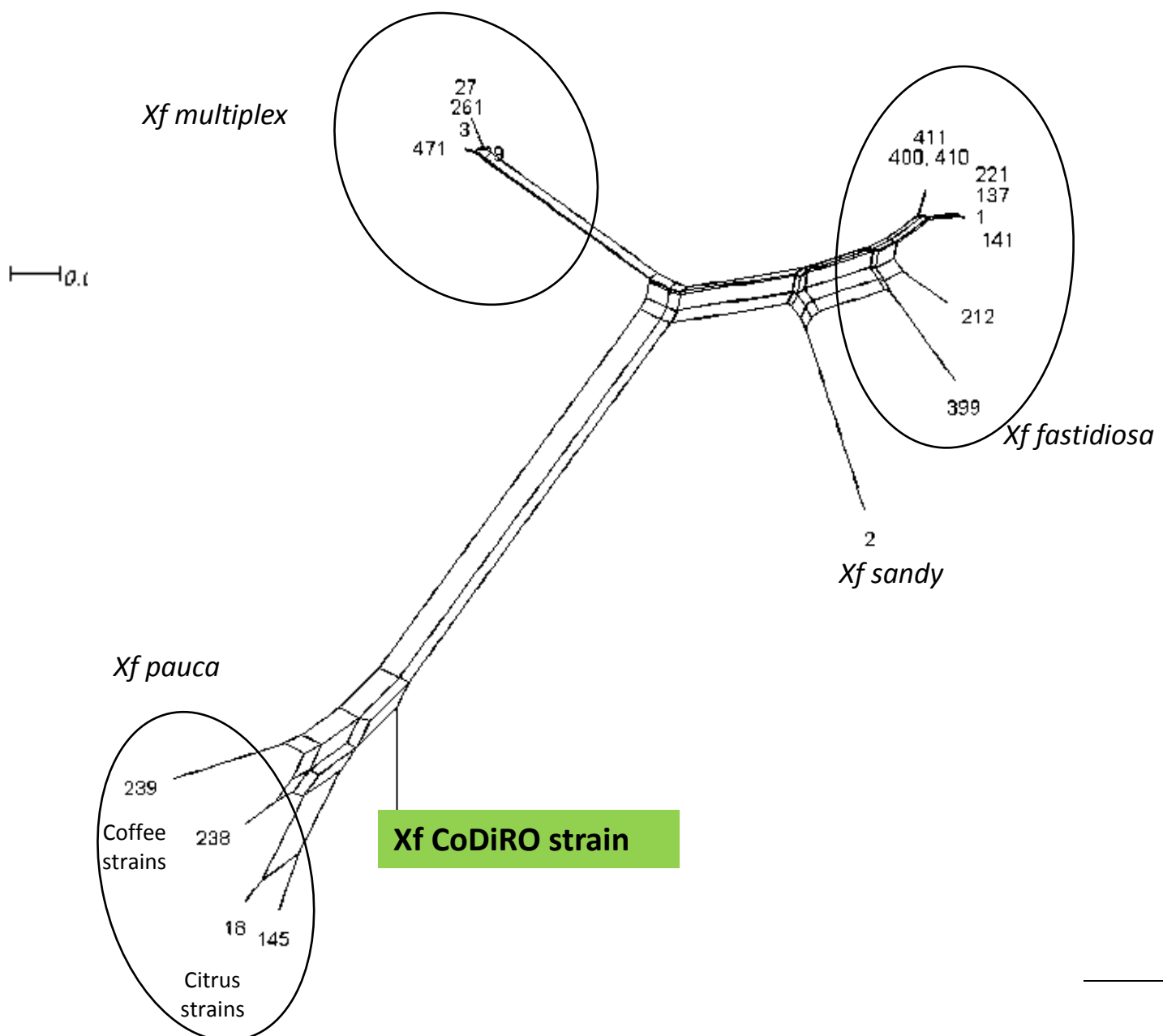


Figure 2. A close-up of the phylogenetic relationships amongst the strains belonging to the Xf subspecies *pauca*.

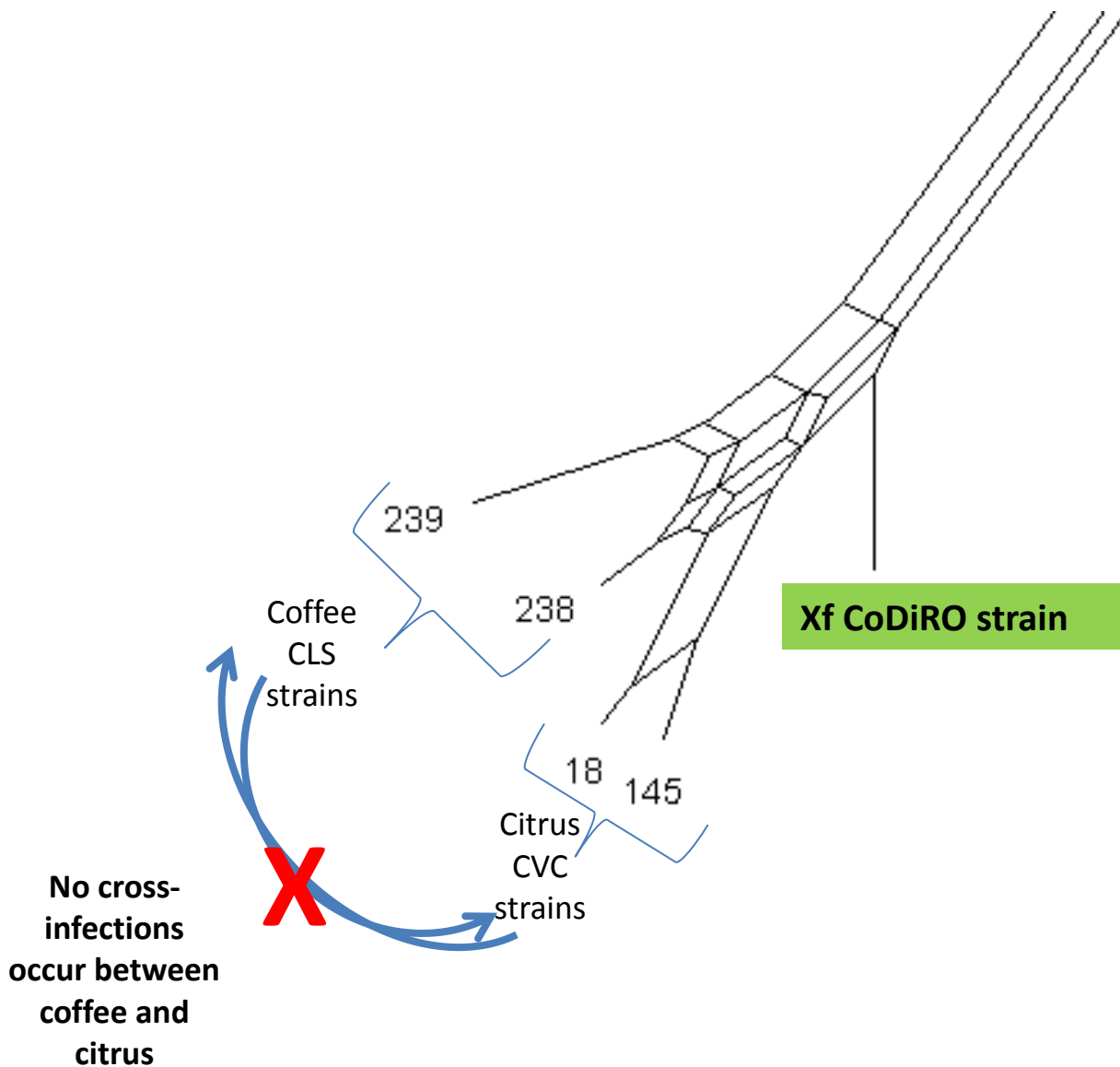


Figure 3. Symptomless citrus trees grown next to *Xylella fastidiosa*-infected olive trees severely affected by the Olive Quick Decline Syndrome. Laboratory tests confirmed citrus were free from *X. fastidiosa* infections.



ANNEX I. Plant species reported in literature as susceptible hosts of *Xylella fastidiosa* subsp. *pauca*

SUSCEPTIBLE HOST SPECIES	Country	Note (Reference)
<i>Prunus salicina</i>	Brazil, Paraguay	Natural and artificial infection (9)
<i>Citrus sinensis</i> and some <i>C. reticulata</i> varieties	Argentina, Brazil, Costa Rica and Paraguay	Natural and artificial infection (1,2, 3, 4,5,7,8,14,15,19)
<i>Coffea</i> spp	Brazil	Natural and artificial infection (14)
<i>Hibiscus schizopetalus</i>	Brazil	Natural infection (10)
<i>Alternanthera tenella</i>	Brazil	Natural infection (6,11)
<i>Brachiaria plantaginea</i> ,	Brazil	Artificial infection (18)
<i>Brachiaria decumbens</i>	Brazil	Natural infection (6,11,18, 20)
<i>Commelina benghalensis</i>	Brazil	Natural infection (18, 20)
<i>Digitaria horizontalis</i>	Brazil	Natural infection (6,11)
<i>Euphorbia hirta</i>	Brazil	Natural infection (18)
<i>Cenchrus echinatus</i>	Brazil	Natural infection (18)
<i>Digitaria horizontalis</i>	Brazil	Natural infection (18)
<i>Digitaria insularis</i>	Brazil	Natural infection (18)
<i>Spermacoce latifolia</i>	Brazil	Natural infection (18)
<i>Medicago sativa</i>	Brazil	Artificial infection (18)
<i>Echinochloa crus-galli</i>	Brazil	Artificial infection (18)
<i>Catharanthus roseus</i> L.	Brazil	Natural and artificial infection (16, 17)
<i>Nicotiana tabacum</i>	Brazil	Artificial infection (12)
<i>Bidens pilosa</i>	Brazil	Natural and artificial infection (13,18)
<i>Lepidium ruderale</i>	Brazil	Natural and artificial infection (13)
<i>Lolium multiplorum</i>	Brazil	Natural infection (13)
<i>Plantago major</i>	Brazil	Natural infection (13)
<i>Parthenium hysterophorus</i>	Brazil	Natural infection (13)
<i>Raphanus sativus</i>	Brazil	Natural and artificial infection (13)
<i>Rumex</i> sp.	Brazil	Natural infection (13)
<i>Solanum americanum</i>	Brazil	Natural and artificial infection (13,18)

<i>Vernonia</i> sp.	Brazil	Natural infection (13)
<i>Nerium oleander</i>	Costa Rica	Natural infection. Beatriz Ortiz, Centro de Investigación en Biología Celular y Molecular, Universidad de Costa Rica, personal communication.
<i>Macadamia</i> sp.	Costa Rica	
<i>Mangifera indica</i>	Costa Rica	

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