Omics, epidemiology and integrated approach for the coexistence with bacterial canker of kiwifruit, caused by *Pseudomonas syringae* pv. *actinidiae*

Marco Scortichini,[1,2] Patrizia Ferrante,[1] Simone Marcelletti,[1] Milena Petriccione[2]

[1]Centro di ricerca per la Frutticoltura, Consiglio per la Ricerca e Sperimentazione in Agricoltura, Roma; [2]Unità di ricerca per la Frutticoltura, Consiglio per la Ricerca e Sperimentazione in Agricoltura, Caserta, Italy

Abstract

Bacterial canker of kiwifruit, caused by *Pseudomonas syringae* pv. *actinidiae*, is a destructive disease found in all major areas of production of green-fleshed (*Actinidia deliciosa*) and yellow-fleshed (*A. chinensis*) kiwifruit of the world (*i.e*, Europe, China, New Zealand and Chile). A series of studies and field trials concerning epidemiology, agronomical techniques, new bactericides effectiveness as well as molecular typing analysis, genomic and proteomic, allowed us to elucidate the cycle of disease of the pathogen, to dissect its main genomic features, to point out the plant proteins involved in resistance/tolerance to the bacterium, to modify some basic agronomical techniques and to propose new compounds that currently, at least in the province of Latina and Rome, Italy, allow the farmers to coexist with the pathogen by reaching the full yield and quality of the crop as before the appearance of the disease.

Introduction

In the provinces of Latina and Roma (Latium region, central Italy), during spring 2008, the first occurrence of symptoms resembling those induced by *Pseudomonas syringae* pv. *actinidiae* (Psa) were observed in kiwifruit orchards cultivated with the high-prized, yellow-fleshed *Actinidia chinensis* cvs Hort16A and JinTao (Ferrante and Scortichini, 2009, 2010). The outbreak continued during summer, autumn and winter causing severe damages and economic losses. Main symptoms were leaf spotting, twig wilting, reddening of the lenticels, canker along the branches and trunk, oozing from twig, branches and trunk. In Latium, during the following two-three years, the disease was observed also on the green-fleshed *A. deliciosa* cv. Hayward and destroyed almost totally about 900 ha of *A. chinensis*. During 2009-2011, the bacterium was also recorded, both on *A. chinensis* and *A. deliciosa* and their pollinators, in the other main areas of kiwifruit cultivation in northern (Emilia-Romagna, Piemonte, Veneto, Friuli-Venezia Giulia) and southern Italy (Calabria, Campania). In any area the damages caused by Psa were very relevant, so that some regions devoted money to partly compensate part of the farmers. The absence of registered agrochemicals to control the pathogen augmented both the severity of the disease and the alarmism of the farmers. Contemporaneously, bacterial canker of kiwifruit affecting either *A. chinensis* or *A. deliciosa* was also found in all main countries where the crop is cultivated, namely New Zealand, Chile, China, France, Portugal and Spain (Scortichini *et al*., 2012).

Correspondence: Marco Scortichini, Centro di ricerca per la Frutticoltura, Consiglio per la Ricerca e Sperimentazione in Agricoltura (C.R.A.), via di Fioranello 52, 00134 Roma, Italy. Fax: +39.0679340158.
E-mail: marco.scortichini@entecra.it

Key words: bacterial canker of kiwifruit, chitosan, cycle of disease, genomic, proteomic.

Conference presentation: Meeting on *Environmental Sustainability and Food Security*, Potenza, Italy, 2014.

Received for publication: 24 June 2014.
Revision received: 18 November 2014.
Accepted for publication: 22 November 2014.

Italian Journal of Agronomy 2014; 9:606
doi:10.4081/ija.2014.606

Materials and methods

The economic importance of the crop and the severity of the epidemics prompted us to start studies aimed at dissecting both some basic molecular features of the pathogen and its field behaviour. So, some investigations for elucidating the population structure of the pathogen, the genomic features of such populations as well as to point out proteins involved both in the necrothrophic and biothrophic phase of the bacterium were performed. Currently, three Psa populations are recognised: Psa 1 including strains of past epidemics in Japan and Italy (1984-1992); Psa 2 including only strains isolated in South Korea during 1990's, and Psa 3, the current, pandemic populations spread in all major areas of kiwifruit cultivation of the world (Ferrante and Scortichini, 2014). The geographic origin of the pandemic population is, most probably, China (Mazzaglia *et al*., 2012; Butler *et al*., 2013). The genomic assessment revealed that all Psa strains share the genetic potential for copper resistance, antibiotic detoxification, high affinity iron acquisition and detoxification of nitric oxide of plant origin (Marcelletti *et al*., 2011). A virulence factor like the phaseolotoxin has been lost by the current, highly aggressive population of the pathogen without decreasing the relative virulence of the bacterium. It has been also shown that the mobile arsenal of phytopathogenic bacteria (*i.e*. plasmids and prophages) can be lost and gained by different popula-

tions of this pathogen. A first proteomic study aimed at investigate which protein were differentially expressed during the infection of the shoot was carried out. The categories of plant defence proteins differentially expressed in the infected shoots were: i) basal defence; ii) pathogenesis-related; iii) oxidative stress; iv) heat-shock; v) transport and plant signal (Petriccione *et al*., 2013). Concerning the leaf apoplast colonisation, it has been pointed out a concerted action of different classes of proteins belonging to the plant defence category, which possibly intervened at different times and actively participated in reducing Psa population size. Peroxidase and heat shock proteins were found as mainly expressed in the first week of bacterial colonisation of the apoplast, whereas chitinases and pathogen-related proteins were over-represented afterwards. Similarly, metabolic enzyme β-galactosidase was consistently up regulated during the last week of Psa colonisation, suggesting an active role of this protein in enhancing the cell leaf defence. In addition, bacterial outer membrane bacterial proteins and chaperones were highly represented during the first week of the leaf apoplast colonisation (Petriccione *et al.*, 2014). Basic, hydrophilic, low-molecular weight and hydrophilic, high-molecular weight compounds were proven as phytotoxic metabolites produced by a Psa strain grown *in vitro* in a minimal medium (Andolfi *et al*., 2014). In addition, Psa apparently exhibits a differently regulated quorum sensing system (Patel *et al*., 2014).

Results

In parallel, epidemiological studies allowed to ascertain that autumn (*i.e*., 2007) and spring (*i.e*., 2008) frosts together with an increase of 30-35% in the rainfall precipitation during 2008, largely contributed to promote the disease and spread the inoculum in central Italy (Ferrante and Scortichini, 2013), and that the pathogen can colonise all the main organ of the plant almost all-year-round (Ferrante *et al*., 2012). In addition, similar to other fruit tree species, it has been demonstrated that during thawing, Psa, in case of colonisation through wounds, can systemically migrates throughout the twig vascular system within few minutes after penetration. This process occurs several times during winter, so that Psa could effectively colonise internal portions of one-year-old kiwifruit twigs and, subsequently, migrate to the leader and main trunk during the following season. The endophytic phase can occurs also through migration from leaf veins to petiole and twigs (Petriccione *et al*., 2013). Such epidemiological approach allowed also to define the Psa cycle of disease and pointing out that spring and autumn-winter are very conducive for the pathogen spread within and between the orchards, whereas the Mediterranean summer (*i.e*., temperature higher than 35°C) largely reduced the possibility of the bacterium multiplication within the plant. Within this context, some agronomical techniques (*i.e*., pruning, tying of young twigs) causing wounds to the tree greatly contribute to increase the possibility of colonisation of the plant (Scortichini *et al.,* 2012). Antifreeze protection using irrigation sprinklers did not influence the short-term period of Psa multiplication in both *A. deliciosa* and *A. chinensis* twigs. However, there is some concern that the extensive supply of water could favour the dispersal of the pathogen in the case of exudates. These information provided a reliable knowledge to apply for timely spray treatments having the aim to reduce the possibility of pathogen colonisation and spreading within and between the orchards. Main periods for protecting the crop are: leaf sprouting, blossoming, fruit set, after the harvest, leaf fall as well as before and after frost, hail and pruning. The risk for the pathogen spreading through latently infected pollen (Tontou *et al*., 2014), stress the utilisation of certified pollen for the artificial pollination.

Discussion and conclusions

Due to an excessive use of copper sprays, previous outbreaks of bacterial canker of kiwifruit in Japan induced the occurrence of copper-resistant strains causing the failure of control programmes (Goto *et al.*, 1994; Nakajima *et al.*, 2002). To possibly avoid this problem, with compounds showing an *in vitro* bactericidal efficacy, field trials were performed to further verify if some product could be added to protect the vines from Psa during the whole season. Compounds containing derivatives of chitin (*i.e*., chitosan) are retained quite promising for substituting copper in protecting the plant from the colonisation of Psa (Scortichini, 2014). The traditional *pergola* training system has been changed into *vaso* to increase the air circulation in the canopy and to reduce the proportion of woody tissue to diminish the possibility of plant colonisation by Psa (Scortichini *et al*., 2014). Some agronomical techniques has changed as well: nitrogen supply should not exceed 120 kg/ha to avoid risk of vegetative vigour, the irrigation should reduce both the volume and duration of water in order to be more regular during the season, wounds induced by the operators are immediately protected to reduce the possibility of pathogen colonisation, diseased plant parts are immediately removed. The farmers who are applying such an integrated approach are obtaining yields of very good quality and quantity as before the occurrence of the epidemic of bacterial canker. Finally, area strategies are strongly recommended to obtaining protection on a larger scale.

References

Andolfi A, Ferrante P, Petriccione M, Cimmino A, Evidente A, Scortichini M, 2014. Production of phytotoxic metabolites by Pseudomonas syringae pv. actinidiae, the causal agent of bacterial canker of kiwifruit. J. Plant Pathol. 96:169-76.

Butler MI, Stockwell PA, Black MA, Day RC, Lamont TR, Poulter TM, 2013. Pseudomonas syringae pv. actinidiae from recent outbreaks of kiwifruit bacterial canker belong to different clones that originated in China. Plos One 8:e57464.

Ferrante P, Fiorillo E, Marcelletti S, Marocchi F, Mastroleo M, Simeoni S, Scortichini M, 2012. The importance of the main colonization and penetration sites of Pseudomonas syringae pv. actinidiae and prevailing weather conditions in the development of epidemics in yellow kiwifruit, recently observed in central Italy. J. Plant Pathol. 94:455-61.

Ferrante P, Scortichini M, 2009. Identification of Pseudomonas syringae pv. actinidiae as causal agent of bacterial canker of yellow kiwifruit (Actinidia chinensis Planchon) in central Italy. J. Phytopathol. 157:768-70.

Ferrante P, Scortichini M, 2010. Molecular and phenotypic features of Pseudomonas syringae pv. actinidiae isolated during recent epidemics of bacterial canker on yellow kiwifruit (Actinidia chinensis) in central Italy. Plant Pathol. 59:954-62.

Ferrante P, Scortichini M, 2013. Frost promotes the pathogenicity of Pseudomonas syringae pv. actinidiae in Actinidia chinensis and A. deliciosa plants. Plant Pathol. 63:12-9.

Ferrante P, Scortichini M, 2014. Redefining the global populations of Pseudomonas syringae pv. actinidiae based on pathogenic, molecular and phenotypic characteristics. Plant Pathol. [In press].

Goto M, Hikota T, Nakajima M, Takikawa Y, Tsuyumu S, 1994. Occurrence and properties of copper-resistance in plant pathogenic bacteria. Ann. Phytopathol. Soc. Jpn. 60:147-53.

Marcelletti S, Ferrante P, Petriccione M, Firrao G, Scortichini M, 2011.

Pseudomonas syringae pv. actinidiae draft genomes comparison reveal strain-specific features involved in adaptation and virulence to Actinidia species. Plos One 6:e27297.

Mazzaglia A, Studholme DJ, Taratufolo MC, Cai R, Almeida NF, Goodman T, Guttman DS, Vinatzer BA, Balestra GM, 2012. Pseudomonas syringae pv. actinidiae (PSA) isolates from recent bacterial canker of kiwifruit outbreaks belong to the same genetic lineage. Plos One 7:e36518.

Nakajima M, Goto M, Hibi T, 2002. Similarity between copper resistance genes from Pseudomonas syringae pv. actinidiae and P. syringae pv. tomato. J. Gen. Plant Pathol. 68:68-74.

Patel HK, Ferrante P, Covaceuszach S, Lamba D, Scortichini M, Venturi V, 2014. The kiwifruit emerging pathogen Pseudomonas syringae pv. actinidiae does not produce AHLs but possesses three LuxR solos. Plos One 9:e87862.

Petriccione M, Di Cecco I, Arena S, Scaloni A, Scortichini M, 2013. Proteomic changes in Actinidia chinensis shoot during systemic infection with a pandemic Pseudomonas syringae pv. actinidiae strain. J. Proteomics 78:461-73.

Petriccione M, Salzano AM, Di Cecco I, Scaloni A, Scortichini M, 2014. Proteomic analysis of the Actinidia deliciosa leaf apoplast during biotrophic colonization by Pseudomonas syringae pv. actinidiae. J. Proteomics 101:43-62.

Scortichini M, 2014. Field efficacy of chitosan to control Pseudomonas syringae pv. actinidiae, causal agent of kiwifruit bacterial canker. Eur. J. Plant Pathol. 140:887-92.

Scortichini M, Marcelletti S, Ferrante P, Petriccione M, Firrao G, 2012. Pseudomonas syringae pv. actinidiae: a re-emerging, multi-faceted, pandemic pathogen. Mol. Plant Pathol. 13:631-40.

Scortichini M, Marocchi F, Mastroleo M, 2014. Cancro batterico del kiwi: è possibile la convivenza. Inf. Agr. 17:42-4.

Tontou R, Giovanardi D, Stefani E, 2014. Pollen as a possible pathway for the dissemination of Pseudomonas syringae pv. actinidiae and bacterial canker of kiwifruit. Phytopath. Medit. 53:333-9.