Romanian Journal for Plant Protection, Vol. XI, 2018
ISSN 2248 – 129X; ISSN-L 2248 – 129X

THE FIRE BLIGHT (*ERWINIA AMYLOVORA*), A DANGEROUS DISEASE FOR FRUITING TREES (ROSACEAE) ORCHARDS

Sergiu Fendrihan[#], *Marian Lixandru*

Research Development Institute for Plant Protection, Romania

#correspondence address:
Research - Development Institute for Plant Protection
8 Ion Ionescu de la Brad 013813, Bucharest, Romania
Phone: + 40 21269 32 31;
Fax: + 40 21269 32 39
e-mail: ecologos23@yahoo.com

Abstract. The fire blight produced by the bacteria *Erwinia amylovora* infects many orchards of fruit trees from our country and from all over the world. Much effort are made for detection in time of the infection, and to find new solution for treatment and prevention, from chemical pesticides to biological control solutions, very required in the last times. The paper reviews all problems of fire blight biology and control, and measures to be taken to enhance research in this field.

Key words: *fire blight, chemical and biological control solutions*

INTRODUCTION

The fire blight is a bacterial pest (*Erwinia amylovora*) of the fruit trees which can destroy entire orchard producing important economic damages. The disease appears and attacked orchards of apple, quince and pear, and only in one county (Mures) spread very fast from one plantation in 1998 to many in 2002 (Porav-Hodade et al, 2008). Being very resistant, it is not easy to control. He stimulates a reaction similarly with hypersensitive reaction in host plants. Other related pathogen *E. pyrifolia* was discovered to produce the Bacterial shoot blight of pear (BSBP) in Japan (Geider et al., 2009). The genetics of apple and of the *E. amylovora* were analyzed in order to understand bacteria - host relations (Malnoy et al., 2012). Virulence factors are reviewed by Pique et al., (2015) as the EPS, amylovoran, biofilm formation, the Type III secretion system, motility and its complex system of regulatory network, and quorum sensing.

Experiments shown that the proteins which are forming the transcription regulation system are abundant when the strains are inoculated in planta. The genes related with RNA processing were up-regulated (Holtappels et al., 2018). The Hfq, a small RNA chaperone is involved in post translational regulation, acting as a virulence regulator in *E. amylovora* (Zeng et al., 2013).

Bacteria are able to survive in shoots overwinter (Crepel et al., 1996). Another possibility of survival is the so called very interesting VNBC - viable but non-culturable state, in fruit calices (Ordax et al., 2009). This state, in our opinion, must be study intensively, because many bacteria has this strange biological stage which pose questions of molecular biology and general biology problems (n.a.). The waaL mutants demonstrated changes in lipo-polysaccharides and have a reduced virulence and resistance to oxidative stress in exposure to hydrogen peroxide (Berry et al., 2009). They resist to starvation, in microcosms at 20°C, showing VNB responses (viable but not culturable), losing motility, reducing size, converted to roundish shape, and forming surface vesicles and retaining virulence (Santander et al., 2014).

COMMENTS

There are lots of possible solution for prevention and control of fire blight. In Switzerland, it consist in obtaining resistant cultivars, use of streptomycin, and chemical and biological control, with human health and environmental protection, but still with some gaps to be filled in near future (Guisberti et al., 2015).

Preventive solution. The plant materials must be selected and controlled to be pathogens free. In special in buying from abroad, the quarantine services must act responsibly, to not accept all infected material. Even inside a country the seedlings and young trees must be controlled when transported to the new orchards.

Diagnosis. Diagnosis is done generally after apparition of sign of the disease, but should be done earlier in order to destroy the infected biological material and avoid pathogens spreading. Some recommend the identification by various PCR methods (Guilford et al., 1996). Some immunoassay for detection of that pathogen can be used- the lateral flow immunoassay (Braun-Kiewnick et al., 2011). Important is to quantify the amount of bacteria in plants, which can be done by RT- PCR method based on chromosomal genes (Svircev et al., 2009).

Enhancing host resistance. Many horticultural scientists try to obtain cultivars (apple, pear, others) with superior genetic qualities and in the same time resistant to phytopathogens inclusive to fire blight. A new cisgenic apple was obtained by Kost et al., (2015) who are more resistant to *E. amylovora* attack. The apple cultivar Gala was modified with a resistance gene from other cultivar of *Malus robusta*, obtaining a cultivar with superior resistance characteristics to fire blight (Broggini et al, 2014).

A **mechanical solution** is recommended by University of California Cooperative Extension- by pruning and even entire tree destruction.

Thermal treatment. The bacteria can resist in bud sticks of apples, and they must be treated by immersion in hot water, end they are able to survive about 50% 60 min at 48°C, in the same time, no live cells of *E. amylovora* were detected (Aldwinckle & Gustafson, 1993). Reduction of bacterial population at high temperature was obtained at 11 strains of the bacteria (Keck et al, 1990) and they were totally killed at 40°C.

Chemical solutions. Using solutions of copper sulphate is one of the classic treatments of fire blight**.** Anyway there are some strains of *E. amylovora* which are resistant to copper sulphate treatment, demonstrated by in vitro tests (Al-Daoude et al, 2009). The tests were performed on about 75 isolates, tested with about 1.2 mM copper sulphate - majority survives. At 2.4 mM no strain survived.

Other treatment is with BTH (S-methylbenzo-1,2,3-thiadiazole-7-carbothiate) which is an elicitor for resistance of apples to fire blight, involving the enhancement of activity of phenolic acids, flavonoids, ferrulic acid peroxidase, chlorogenic and coumarin acids (Sklodowska et al., 2011). Different fungicides and essential oils were tested against *E. amylovora* (Mikiciński et al., 2012). The pesticides were Ridomil MZ Gold 68 WG (with active substances 3.8% metalaxyl-M and 64%, mancozeb respectively), Miedzian 50 WG (active substance - 50% copper oxychloride), Euparen Multi 50 WG (active substance 50% tolylfluanid), Captan 80 WG [active 80% N-(captan)], Dithane Neotec 75 WG (active substance 75% mancozeb). After tests performed in vitro (*Erwinia amylovora, Xantomonas arboricola, Agrobacterium tumefaciens)* and in cultivated lands, the best against fire blight were metalaxyl M, copper oxychloride (which inhibited all bacterial strains), and oils from clover, thyme and sage were effective.

Preventive treatment with Fosethyl AL (Alliette) produces a reduction of susceptibility and decrease of the disease development, a reduction of bacteria ooze formation (Deckers et al., 2010). A treatment with substances of natural origin, as plant extracts, can be use (Arafat et al., 2015) on reduced surfaces. Short chain Fatty acids, used in food preservation, can inhibit growth of *E. amylovora*, and reduce symptoms of the disease (Konecki et al., 2013). Plants extract were used too against fire blight, for example extracts of *Viscum album* and *Hedera helix*, the last having an efficiency close to streptomycin (Mosh et al., 1996).

Antibiotic uses. Generally speaking, antibiotics treatment used in agriculture is only 0.12 of the used in animal breeding (Stockwell & Duffy, 2012) and applied in spring for infection prevention in orchards, of broad use is streptomycin, oxytetracycline, oxolinic acid and gentamycine. Streptomycin is currently used against this disease, and showed no effects on soil bacterial communities in orchards (Shade et al., 2013).

Antimicrobial peptides showed to be effective, but are very costly, the use of them together with lysosyme, can reduce the quantity of BP100 necessary for control, and enhance the effect (Cabrefiga et al., 2017). *Polybacillus polymyxa* produces Polymixin B which looks like effective against fire blight (Niu et al, 2013).

Biological control. Antagonistic bacteria, present on plants of inside (endophytes) can release antimicrobials. For example, *Bacillus amyloliquefaciens* produces antimicrobials like difficidin and bacilysin actives against *E amylovora* (Chen et al., 2009). The bacteria *Pseudomonas fluorescens* strain EPS62e are used in the control of *E. amylovora*, and its efficiency can be enhanced with osmo-adaptation and nutritional enhancement in special culture media (Cabrefiga et al., 2011).

Streptomyces sp. was found to suppress the *E. amylovora* attack - at leaves level it was reduced with 70% (Doolotkeldieva & Bobusheva, 2016). A strain of *Pseudomonas aeruginosa* is releasing L-2-amino-4-methoxy-*trans*-3-butenoic acid acts as antimetabolites against *E. amylovora* cells (Lee et al., 2013).

Some researchers tried to find antagonists on apples, they found a lots of epiphytic bacteria in apple blossoms, mainly from *Pseudomonas* genus and other genera (*Pantotea*), and yeasts as *Cryptococcus,* some of them being good antagonists for *E. amylovora* (Pusey et al., 2009).

Not only bacteria, but viruses- bacteriophages of *E. amylovora* were discovered and sequenced (Esplin et al., 2017) and were proposed to be used in biological control (Nagy et al., 2012). Phage population in apples with *Erwinia* attack contains polyvalent and specific viruses (Tovkach et al., 2013). An EPS depolymerase, virus encoded, can be use against *Erwinia* bacteria (Kim & Geider, 2000). A commercial virus product was obtained in Hungary from virus Phi EaH1 and H2, which is used as biopesticide (Meczker et al., 2014). The solution is good, with the condition to survey the possible virus mutation (n.a.).

CONCLUSIONS

Considering the presented facts, *Erwinia amylovora* represent a main threat for orchards with trees from Rosaceae family, having a resistance to environmental factors and to different chemical pesticides use for its control. The biological control looks like to be a better solution in special for bio commercial production, but we must look further to the best solutions even combined solution, in order to control the pathogen and to assure the safety of man and environment with appropriate legislation in this field.

REFERENCES

AL-DAOUDE, A., ARABI, M.I.E., AMMOUNEH, H. (2009). Studying *Erwinia amylovora* isolates from Syria for copper resistance and streptomycin sensitivity. *Journal of Plant Pathology*, 91(1), 201-203.

ALDWINCKLE, H., GUSTAFSON, H. (1993). Hot water treatment for eradication of *Erwinia amylovora* from propagating wood. *Acta Horticulture*, 338, 309-310

ARAFAT, K.H., HANAN, S.A., RABAB, A.E.A.M. (2015). Antibacterial activity of antagonistic bacteria and plant extract on Erwinia *amylovora* the pathogen of fire blight disease in Egypt. *International Journal of Phytopathology*, 04 (02), 73-79.

BERRY, M.C., MCGHEE, G.C., ZHAO, Y., SUNDIN, G.W. (2009). Effect of a waaL mutation on lipopolysaccharide composition, oxidative stress survival, and virulence in *Erwinia amylovora*. *Microbiology Letter*, 291, 80–87.

BRAUN-KIEWNICK, A., ALTENBACH, D., OBERHÄNSLI, T., BITTERLIN, W., DUFFY, B. (2011). A rapid lateral-flow immunoassay for phytosanitary detection of *Erwinia amylovora* and on-site fire blight diagnosis. *Journal of Microbiological Methods*, 87, 1-9.

BROGGINI, GAL., WOHNER, T., FAHREBTRAPP, J., KOST, T.D., FLACHOWSKY, H., PEIL, A., HSANKE, M.V., RICHTER, K., PATOCCHI, A., GESSLER, C. (2014). Engineering fire blight resistance into the apple cultivar 'Gala' using the FB_MR5 CC-NBS-LRR resistance gene of *Malus* 3 *robusta* 5. *Plant Biotechnology Journal*, 12, 728-733.

CABREFIGA, J., MONTESINOS, E., (2017). Lysozyme enhances the bactericidal effect of BP100 peptide against *Erwinia amylovora*, the causal agent of fire blight of rosaceous plants. *BMC Microbiology*, 17, 39. DOI 10.1186/s12866-017-0957-y.

CHEN, X.H., SCHOLZ, R., BORRISS, M., JUNGE, H., MÖGEL, G., KUNZ, S., BORRISS, R. (2009). Difficidin and bacilysin produced by plant-associated *Bacillus amyloliquefaciens* are efficient in controlling fire blight disease. *Journal of Biotechnology*, 140, 38-44.

CREPEL, C., GEENEN, J., MAES, M. (1996). The latent survival of *Erwinia amylovora* in hibernating shoots. *Acta Horticulture*, 411, 21-26.

DECKERS, T., SCHOOFS, H., VERJANS, W., DE MAEYER, L. (2010). Reduction in bacterial ooze formation on immature fruitlets after preventive treatments of Fosethyl-Al against fire blight *Erwinia amylovora*. *Communications in Agricultural and Applied Biological Sciences*, 75(4), 569-76.

DOOLOTKELDIEVA, T., BOBUSHEVA, S. (2016). Fire Blight Disease Caused by *Erwinia amylovora* on *Rosaceae* Plants in Kyrgyzstan and Biological Agents to Control This Disease. *Advances in Microbiology*, 6, 831-851.

ESPLIN, I.N.D., BERG, J.A., SHARMA, R. (2017). Genome Sequences of 19 Novel *Erwinia amylovora* Bacteriophages. *Genome Announcements*, 5(47), 5, e00931-17. https://doi.org/10.1128/genomeA.00931-17.

GEIDER, K., AULING, G., JAKOVLJEVIC, V., VOLLKSCH, B. (2009). A polyphasic approach assigns the pathogenic *Erwinia* strains from diseased pear trees in Japan to *Erwinia pyrifoliae*. Letters in Applied Microbiology, 48(3), 324-330.

GUSBERTI, M., KLEMM, U., MEIER, M.S., MAURHOFER, M., HUNGER-GLASER I. (2015). Fire Blight Control: The Struggle Goes On. A Comparison of Different Fire Blight Control Methods in Switzerland with Respect to Biosafety, Efficacy and Durability. *International Journal of Environmental Research and Public Health*, 12, 11422-11447.

GUILFORD, P.J., TAYLOR, R.K., CLARK, R.G., HALE, C.N., FORSTER, R.L.S. (1996). PCR-based techniques for the detection of *Erwinia amylovora*. *Acta Horticulture*, 411, 53-56.

HOLTAPPELS, M., NOBEN, JP, VAN DIJCK, P., VALCKE, R. (2018). Fire blight host-pathogen interaction: proteome profiles of *Erwinia amylovora* infecting apple rootstocks. *Scientific reports*, 8:11689, DOI: 10.1038/s41598-018-30064-x

KECK, M., CHARTIER, R., ZISLAVSKY, W., PAULIN, J.P. (1990). Sensitivity of *Erwinia amylovora* to high temperatures-possible use of heat treatment for plant propagation material. *Acta Horticulturae*, 273: V International Workshop on Fire Blight.

KIM, W.S., GEIDER, K. (2000). Characterization of a viral EPS depolymerase, a potential tool for control of fire blight. *Phytopathology*, 90, 1263-1268.

KOST, T.D., GESSLER, C., JÄNSCH, M., FLACHOWSKY, H., PATOCCHI, A., BROGGINI, G.A.L. (2015). Development of the First Cisgenic Apple with Increased Resistance to Fire Blight. *PLoS ONE*, 10(12), e0143980. doi:10.1371/journal.pone.0143980.

KONECKI, K., GERNOLD, M., WENSING, A., GEIDER, K. (2013) Growth inhibition of *Erwinia amylovora* and related *Erwinia* species by neutralized short‐chain fatty acids. *Archives of* Microbiology, 195, 759–764.

LEE, X., AZEVEDO, M.D., ARMSTRONG, D.J., BANOWETZ, G.M, REIMMANN, C. (2013). The *Pseudomonas aeruginosa* antimetabolite L-2-amino-4-methoxy-*trans*-3-butenoic acid inhibits growth of *Erwinia amylovora* and acts as a seed germination-arrest factor. *Environmental Microbiology Reports*, 5(1), 83-89.

MALNOY, M, MARTENS, S., NORELLI, J.L., BARNY, M.A., SUNDIN, G.W., SMITS, T.H., DUFFY, B. (2012). Fire blight: applied genomic insights of the pathogen and host. Annual Review of Phytopathology, 50, 475-94.

MECZKER, K., DÖMÖTÖR, D., VASS, J., RÁKHELY, G., SCHNEIDER, G., KOVÁCS, T. (2014). The genome of the *Erwinia amylovora* phage PhiEaH1 reveals greater diversity and broadens the applicability of phages for the treatment of fire blight. *FEMS Microbiology Letters,* 350(1), 25-27. DOI: 10.1111/1574-6968.12319.

MIKICIŃSKI, A., SOBICZEWSKI, P., BERCZYŃSKI, S. (2012). Efficacy of fungicides and essential oils against bacterial diseases of fruit trees. *Journal of Plant Protection research,* 52(4), 467-471.

MOSCH, J., ZELLER, W., RIECK, M., ULLRICH, W. (1996). Further studies on plant extracts with a resistance induction effect against *Erwinia amylovora. Acta Horticulture*, 411, 361-366.

NAGY, J.K., KIRÁLY, L., SCHWARCZINGER, I. (2012). Phage therapy for plant disease control with a focus on fire blight. *Central European Journal of Experimental Biology,* 7(1), 1-12.

NIU, B., VATER, J., RUECKER, C., BLOM, J., LEHMANN, M., RU, J.J., CHEN, X.H., WANG, Q., BORRISS, R. (2013). Polymyxin P is the active principle in suppressing phytopathogenic *Erwinia* spp. by the biocontrol rhizobacterium *Paenibacillus polymyxa* M-1. *Microbiology*, 13, 137.

ORDAX, M., BIOSCA, E.G., WIMALAJEEWA, S.C., LOPEZ, M.M., MARCO-NOALES, E. (2009). Survival of *Erwinia amylovora* in mature apple fruit calyces through the viable but nonculturable (VBNC) state. *Journal of Applied Microbiology*, 107, 106-116.

PIQUÉ, N., MIÑANA-GALBIS, D., MERINO, S., TOMÁS, J.M. (2015). Virulence Factors of *Erwinia amylovora*: A Review. *International Journal* of *Molecular Sciences*, 16, 12836-12854.

PUSEY, P. L., STOCKWELL, V. O., MAZZOLA, M. (2009). Epiphytic bacteria and yeasts on apple blossoms and their potential as antagonists of *Erwinia amylovora*. *Phytopathology*, 99, 571-581.

SHADE, A., KLIMOWICZ, K., SPEAR, R.N., LINSKE, M., DONATO, J., HOGAN, C.S., MCMANUS, P.S., HANDELSMAN, J.O. (2013). Streptomycin Application Has No Detectable Effect on Bacterial Community Structure in Apple Orchard Soil. *Applied and Environmental Microbiology*, 79 (21), 6617-6625.

PORAV-HODADE, T., FLORIAN, V., CRISTEA, O. (2008). Evolution of the Rosaceae fire blight (E. *amylovora)* in Mures County. Revista Protectia Plantelor, 66-75.

SANTANDER, R.D., OLIVER, J.D., BIOSCA, E.G. (2014). Cellular, physiological, and molecular adaptive responses of *Erwinia amylovora* to starvation. *FEMS Microbiology Ecology*, 88, 258–271.

SKLODOWSKA, M., GAJEWSKA, E., KUZNIAK, E., WIELANEK, M., MIKICINSKI, A., SOBICZEWSKI, P. (2011). Phenolic profile and peroxidase activity in apple leaves after *Erwinia amylovora* infection and BTH treatment. *Journal of Phytopathology*, 159 (7-8), 495-504.

STOCKWELL, V.O., DUFFY, B. (2012). Use of antibiotics in plant agriculture. *Revue scientifique et technique*, 31(1), 199-210.

SVIRCEV, A.M., KIM, W.S., LEHMAN, S.M., CASTLE, A.J. (2009). *Erwinia amylovora:* modern methods for detection and differentiation. *Methods in Molecular Biology*, 508, 115-29.
doi: 10.1007/978-1-59745-062-1_10.

TOVKACH, F.I., MOROZ, S.N., KOROL, N.A., FAĬDIUK, I.U.V., KUSHKINA, A.I. (2013). Polyvalence of bacteriophages isolated from fruit trees, affected by bacterial fire blight. *Mikrobiology*, 75(2), 80-8.

ZENG, Q., RYAN, R., MCNALLY, SUNDIN G.W. (2013) Global Small RNA Chaperone Hfq and Regulatory Small RNAs Are Important Virulence Regulators in *Erwinia amylovora. Journal of Bacteriology*, 95 (8), 1706-171.