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Bundesamt für Landwirtschaft

Proposal for a BLW Fire Blight Control Strategy Research Project

ACHILLES

Fire Blight Applied Genomics to Develop Innovative Control Products: Identify and exploit the pathogen Achilles' heel(s) as control targets

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For BLW to complete:

Project Nr. .

Submission Date:

Project Duration: 36 Months

Project Budget: 960'000 CHF

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1 Project Summary

Fire blight has been the **major threat to pome fruit production** globally since it was described in the late 1790s as the first plant disease caused by a bacterium. Fire blight has also been one of the **most intensively studied plant diseases over the past 100 years** in epidemiology, pathogen biology, host resistance and control strategies. Fire blight today **remains a bane to apple and pear growers worldwide**, despite considerable efforts by major international phytopathology laboratories, and spanning numerous eminent careers. **Identifying control compounds** (biocontrol, alternative chemicals) or plant resistance **has until now relied upon a hit-or-miss screening approach**.

Recently, **Swiss research delivered a major breakthrough in fire blight research** with the complete genome sequencing of the fire blight pathogen (Smits et al. 2010a). Building upon this success, ***E. amylovora* weaknesses can now be exposed and exploited** to develop novel control compounds to **directly target pathogen Achilles' heel**(s) by interfering with pathogen epidemiological survival and virulence genes. Swiss research also accomplished genome sequencing of the most promising fire blight biocontrol agents, *Pantoea agglomerans* and *P. vagans* (Smits et al. 2010b). This work is positioned to **identify and sharpen biocontrol 'arrows'** that can improve the efficacy and reliability of fire blight control as an **alternative to antibiotics**.

This project will **build upon prior BLW projects** and apply genomics **in order to deliver practical solutions to fire blight**. Three Modules will link pathogen and biocontrol agent genomics to identify innovative targets for control, streamline biocontrol screening and optimization, and integrate genomics-based tools into field epidemiology.

Module 1 will identify pathogen genes critical for infection, develop pathogen biosensors to rapidly identify conditions that suppress these genes, and then apply for **streamlined screening of new control compounds**.

Module 2 will develop biocontrol agent biosensors of anti-fire blight genes and apply these to **optimize application and formulation conditions that improve activity.**

Module 3 will link sensitive, genomics-based pathogen detection tools to epidemiological analysis of resistant host plants and **reveal significance of latent, asymptomatic infection** that will then enable us to **improve phytosanitary control measures** and ensure **durability of host resistance**.

In addition to long-term**, major advancement of fire blight research**, this **project will deliver short-term results with translation into streamlined discovery and optimization of commercial alternatives to antibiotics**. Epidemiology tools based on genomic biomarkers will be applied to **monitor the durability of resistant apple varieties developed in ZUEFOS II** under field conditions. This will provide **short-term results to tighten phytosanitary guidelines and prevent fire blight spread** and will answer unresolved questions of latent infection.

2 Research plan

Module 1: Pathogen [80% PD and 30% PhD]

In the previous 3 years we have obtained a solid platform of genome sequence data for the fire blight pathogen. This project will take us to the next level and illuminate which genes are actually expressed in flowers during the process of pathogen colonization, epiphytic growth and then invasion into the plant. Work of our group and others has provided clues to virulence genes active inside the host plant. However, currently nothing is known about the genes that are critical for pathogen *invasion* of host plants. Focusing on invasion determinants is critical for finding effective control products that are applied to the flower surface and must prevent invasion. Once the pathogen enters flower tissues, external products are no longer effective to suppress fire blight development.

a: RNAseq transcriptomics will be performed on apple flowers during the colonization and invasion stage just prior to infection. This is an efficient approach to identify all pathogen genes expressed in on step. This also ensures that unexpected genes critical to invasion will be able to be discovered.

b: Proteomics will be performed on the same apple and pear flower samples in order to identify which expressed genes from (a) are processed into functional proteins.

c: Genes identified to have a critical function in fire blight flower colonization and invasion (a-b) will be used to construct reporter-gene biosensors (i.e., *inaZ* ice-nucleation gene; GFP fluorescent protein). Biosensors will be validated as sensitive indicators of pathogen gene expression using quantitative PCR.

d: Pathogen biosensors will then be applied to develop a streamlined screening process for rapid, high-throughput discovery of new fire blight control products. Current methods are based on in vitro plate assays that are generally poor indicators of efficacy during later scale-up tests (in plants, orchards). The new biosensors developed here will economize the evaluation of potential chemicals and antagonists – increasing chances of finding products that will perform after scale-up.

e: Applied genomics (a-b) will be analyzed to identify plant defense determinants that can suppress pathogen flower colonization and invasion. Discovery of plant factors that interfere with pathogen invasion can be exploited for smarter breeding. This is an innovative alternative to current QTL-based approaches. When a plant factor is identified, we will design molecular markers to screen breeding material. *Link to ZUEFOS II*

Module 2: Biocontrol [80% PD and 40% PhD]

In the past 3 years we have also obtained a solid genome sequence data set for *Pantoea*, which are the most effective fire blight commercial strains. This project will apply our genomics to filter out those specific genes that are expressed in flowers and are critical for in planta biocontrol activity. This will then be directly used to develop biosensor tools that will enable efficient optimization of biocontrol compound production during the formulation stage and during-after application in orchards.

a: RNAseq transcriptomics and proteomics will be used to identify genes that are actually expressed and functional in flowers.

b: Molecular markers will be designed for genes that are found to be critical for biocontrol performance.

c: Molecular markers will then be applied to streamline the isolation of Swiss biocontrol agents. Currently, this is a hit-or-miss process that requires immense effort to find a few promising antagonists, most of which then fail to perform in scale-up screening on plants and in orchards. This new approach will increase our chances by pre-screening isolates and selecting those that posses known biocontrol mechanisms. This will enable Swiss companies to economically pursue discovery of Swiss biocontrol agents.

d: Reporter-gene biosensors will be constructed for biocontrol agents to monitor expression of critical genes under any condition.

This will enable companies to optimize formulation of antagonists by rapidly identifying fermentation conditions that increase synthesis of the active ingredient(s) such as natural antibacterial compounds. This will give biocontrol a ‘head-start’ in orchards and provide the most promising alternative to streptomycin.

This will also enable us to efficiently identify application conditions that optimize performance in orchards, and will enable us to link biocontrol more wisely with fire blight forecasting models.

Module 3: Systems Biology – Epidemiology [40% PD and 30% PhD]

a: Results from Modules 1 and 2 will be further integrated into epidemiology study of the pathogen and biocontrol agents in orchards in order to adapt application to forecasting to improve control approaches.

b: Molecular tools for the pathogen will be adapted and applied to monitor pathogen behavior directly in orchards. For example, these will enable us to efficiently monitor asymptomatic

pathogen colonization of flowers during the invasion process. This will shed light on the long-standing controversy of latent infections that may arise when flower infection occurs after low-dose invasion and/or late in the flowering season. It is hypothesized that this may explain epidemics that occur in areas where no previous disease was reported, however, this topic has not been well-studied. The new tools developed in this project will facilitate such an investigation.

c: Molecular tools for pathogen monitoring will also be applied in surveys of orchards where resistant apple developed in ZUEFOS II will be planted. This will enable us to evaluate the durability of resistance, the mechanisms of resistance in terms of plant defense reaction to pathogen (Module 1). It will also enable us to determine whether resistant plants suppress fire blight, but do not 'mask' asymptomatic pathogen colonization. If resistant plants still support latent pathogen infections, this would have devastating epidemiological consequences.

This *link to ZUEFOS II* is a key component in the field selection of new fire blight resistant apple varieties.

d: Molecular tools for biocontrol agents from Module 2 will be applied in orchards to monitor the environmental fate, antagonist gene expression in orchards, and evaluate environmental impact. This monitoring is a critical component for the registration of new fire blight products.

3 Project deliverables

Module 1 Applied Deliverables:

- Pathogen biosensors will be produced that efficiently report expression of genes involved in flower colonization and invasion – *unravel the mystery of pathogen flower invasion*
- Streamlined screening method will be developed and applied for rapid, high-throughput discovery of new fire blight control products (alternative chemicals, organics, biocontrol) – *efficient tool for discovery of new fire blight products*
- Molecular tools will be developed to identify plant defense determinants that for potentially improving resistance breeding

Module 2 Applied Deliverables:

- Biocontrol agent biosensors will be produced and applied to identify fermentation conditions that increase active ingredient in biocontrol formulations – *optimize biocontrol formulations*
- Biocontrol biosensors will be applied in greenhouse (and field conditions in the USA) to determine conditions that optimize performance during and after application in orchards
- Molecular tools will be developed to monitor biocontrol agents after application in orchards and determine environmental fate and impact – *determine environmental safety of biocontrol*
- Molecular tools will be developed to streamline screening of biocontrol isolates from Switzerland that have higher-chance efficacy against fire blight – *deliver a cost-effective method for discovery of Swiss biocontrol agents*

Module 3 Applied Deliverables:

- Pathogen-Biocontrol results and molecular tools from Modules 1 and 2 will be applied to harmonize application of control products with fire blight forecasting systems – *harmonize biocontrol with forecasting to improve application timing*
- Molecular tools from Modules 1 and 2 will be applied to improve epidemiological understanding of fire blight in orchards and improve implementation of phytosanitary measures
- Durability of fire blight resistant apples will be evaluated in orchards to eliminate new varieties that may suppress symptoms but still support latent infection – *improve selection of fire blight resistant apple varieties (link with ZUEFOS II)*

4 Investigator competence relevant to achieving goals

The Principal Investigator is an established leader in international fire blight research. B. Duffy has a solid record of high-impact publications, invited lectures, invited membership of commissions/journal editorial boards/meeting organizing committees, invited participation in international/national research collaborations/projects in fire blight research and including all aspects of the disease from pathogen genetics, epidemiology, and control to host resistance. Competence is also evidenced by election as host of the next international fire blight congress (*ISHS Zürich, 2013*) and invitation to co-author a major review on fire blight (*Annu. Rev. Phytopathology*). A CV is provided as Annex 1.

The Principal Investor has a wide-network of international and Swiss collaborators that can support this project. This includes collaboration with the co-Investigator that evaluated novel fire blight control compounds within a recent CTI/KTI Feasibility Project. The co-Investigator is an up-and-coming young scientist with strong competence in molecular biology and practical analysis of alternative chemicals.

5 Budget: 960'000 CHF

Salaries: 800'000 CHF [2 post-docs (PD) - 642'000 CHF; 1 PhD – 158'000 CHF]

Materials: 135'000 CHF

Basic supplies (microbiology, molecular, plants, orchards – 12'000/scientist) = 108'000 CHF
Applied genomics (RNAseq transcriptomics, proteomics, bioinformatics) = 27'000 CHF

This is far below standard calculations of 14'000 for material support per scientist, 12-20 CHF/tree for greenhouse trials, orchard maintenance, and highly expensive 'omics materials expenses. Additional funds will be arranged to supplement this support.

Travel: 15'000 CHF

ISHS Fire Blight, Zürich, 2013: registration fees for CoPI/PDs/PhD = 2'400 CHF
IS-MPMI XVI Intl Congress, *venue N. Am.*, 2013; registration-travel for PD = 3'200 CHF
10th Intl Congress of Plant Pathology, Beijing, 2013; registration-travel for PI/PD = 6'400 CHF
Exchange with international partners for PhD/PD (e.g., Italy, USA – part funding) = 3'000 CHF

International travel requests will only cover 60-80% of costs. These are in addition to at least 10 other trips for dissemination for which other funding will be arranged.

Dissemination publications: 10'000 CHF

Expected at least 10 papers, with 4 of these targeted in premier journals with fees

This is a conservative estimate based on record from prior BLW projects. This would only pay for 60-80% of the fees for just 4 papers. For example, just for the high-profile *Erwinia amylovora* genome publication fees were approximately 3'900 CHF.

It is in addition to trade and stakeholder forum publications, promotion articles and stakeholder meeting organization.

Place and Date:	Signature of Project Leader:
Wädenswil, 08.11.2010	Dr. Brion Duffy

Annex 1: Dr. Brion Duffy - Principal Investigator Curriculum Vitae

Research Subjects

Bacterial plant diseases: We have projects dedicated to proactive understanding of several regulated, invasive bacterial pathogens of stone fruit/nut trees and vegetables. Specific projects involve *Xanthomonas arboricola* pv. *pruni* genomics, proteomics and diagnostics; *Xylella* diagnostics and biodiversity; and proteomics/diagnostics of *Clavibacter, Ralstonia, Dickeya,* and other vegetable bacterial pathogens.

Fire blight caused by *Erwinia amylovora* is the main focus of our group. This research is aimed at applying a holistic research approach to design integrated management strategies in diverse agro/forest ecosystems . Specific research projects involve: innovative diagnostics development; phytosanitary monitoring and forecasting modelling; host plant resistance germplasm screening and marker-assisted breeding; biological control environmental impact assessment and genomics-mediated performance improvement; and genomics-based elucidation of pathogen virulence, biology and disease epidemiology.

Genomics and other techniques: We integrate a wide-range of new biotechnology with classical tools to bridge the gap between basic and applied research for ultimate practical implementation by end-users (e.g., plant inspectors, growers). Our lab is equipped to perform: whole-genome sequencing, transcriptomics, and molecular analysis to elucidate bacterial virulence, biocontrol, and ecological fitness determinants; evolutionary and forensic genomics; diagnostics based on proteomic, serological, PCR and microarray approaches; host resistance QTL identification and high-throughput validation of Marker-Assisted Selection breeding tools; plant assays in quarantine-greenhouse and research/commercial orchards; ring-trials and technology transfer involving plant inspectors and stakeholders on-site.

Training Activities

Training schools: Our lab has an active program organizing/co-organizing training schools for international students and end-users (i.e., plant inspectors, growers) focused on bacterial pathogens/diseases or technology. Recent examples include European training schools for specific pathogens (2007, 2008, 2009, 2010), functional genomics (2009), and plant inspectors (2009, 2010).

Hosting international scientist exchanges: Our lab frequently hosts students, post-docs and faculty for training within the framework of collaborative research projects. Since 2005, we have hosted over 15 visiting scientists for periods ranging from a few weeks to a year.

Training and career-development of MS, PhD and Post-docs: B. Duffy has supervised/co-supervised 24 Apprentice/MS/PhD students and 13 Post-docs.

Education and Distinctions

1999	PhD, Natural Sciences, Phytopathology, ETH, Zürich, Switzerland (http://e-collection.ethbib.ethz.ch/view/eth:22947)
1992	MS, Plant Pathology, Washington State University, Pullman, USA
1988	BS, Tropical Crop Protection, University of Hawai'i, Hilo, USA
2002	OECD Fellowship (USA-NL, w/J. Raaijmakers)
1999	ETH Silver Medal – highest honour for dissertation
1985-1988	Univ. of Hawai'i, Dean's List

Employment History

2002-now	Research Leader Bacteriology, Agroscope Changins-Wädenswil ACW
2000-2002	Research Microbiologist, USDA, Food Safety Unit, Albany, California
1999-2000	Post-doctorate, ETH Zürich, Switzerland (w/ G. Défago)
1992-1993	Research Plant Pathologist, Hawaii Volcanoes National Park, University of Hawai'i
1992-1993	Lecturer Plant Pathology, University of Hawai'i at Hilo
1988-1989	Visiting Researcher, EMBRAPA Soil Microbiology Center, Brasil (w/ J. Döbereiner)

Publications (over 144 as author/co-author)

Scientific publications — 64 papers, reviews, volumes
Trade publications in technical and trade journals, popular press, edited volumes — over 80 articles/volumes

Invited Lectures (over 150 in 26 countries since 1996)

Competitive Research Funding

Principal Investigator (PI) or Co-PI (total 36 grants, ≈ 5'400'000 €; since 2003, ≈ 3'100'000 €)

International Project Leadership and Commissions

European Union Projects: Chair COST Action 873, Vice-chair COST 864, WP Leader in 4 projects
COST Action 873 webmaster: www.cost873.ch
Swiss National Delegate: EPPO Bacteriology Panel; COST Actions 830, 864, 873, 924

European SAFE Consortium, Microbiology Committee
International Society of Horticultural Science ISHS, Fire Blight Committee
American Phytopathological Soc.: Committee Chair (Soil Microbiology 1999, Biotechnology 1997-01)

Organiser/co-organiser of over 40 international meetings and symposia

Professional Memberships and Service

American Phytopathological Society APS; American Society for Microbiology ASM
International Society of Plant Pathology ISPP; International Society for Horticultural Sciences ISHS
International Society for Molecular Plant-Microbe Interactions IS-MPMI
German Phytiatry Society DPG; Swiss Microbiology Society SGM; Swiss Phytiatry Society SPG

Editorial Service

Editorial Boards (7):

Senior Editor Bacteriology:	Phytopathology (2008-present)
Associate Editor:	Biocontrol Science & Technology (Assoc. Ed. 2006-present)
	Journal of Microbiological Methods (Assoc. Ed. 2010-present)
	Plant Pathology BSPP, New Disease Reports (2008-present)
	Biotechnology (2007-present); Plant Pathol. ANSI Net (2007-present)

Ad hoc reviewer (≈250 reviews since 2007)

Journals including: Nature, Appl. Environ. Microbiol., Plant Disease, BMC Genomics, FEMS Microbiol. Ecol., Environ. Microbiol., Biol. Control, HortScience, Eur. J. Plant Pathol., Plant Soil, Mol. Plant-Microbe Interact., Eur. J. Soil Biol., Eur. J. Hort. Sci., J. Phytopathol., J. Appl. Microbiol., Comp. Sci. Utiliz.

Grants including: EU FP7 KBBE, COST European Sci. Res., EU ESF Res. Networking, EU PLANT-KBBE, German GABI-Future, French ANR Prog. Génomique, Austrian Science Fund, Dutch Science Fund, South African Natl. Sci. Foundation, US-Israel BARD, USDA Small Business Innovative Research

Peer-reviewed journal publication list (1994-2010)

1. Pusey PL, Stockwell VO, Duffy J, Smits THM, Duffy B. 2010. Antibiosis activity of *Pantoea agglomerans* commercial biocontrol strain E325 against *Erwinia amylovora* on pome fruit flower stigmas. Phytopathology, *submitted*.
2. Bonaterra, A., Badosa, E., Duffy, B., Montesinos, E. 2010. Phenotypic comparison of clinical and plant-beneficial strains of *Pantoea agglomerans* in plant and animal models. FEMS Microbiol. Ecol., *submitted.*
3. Powney, R., Smits, T.H.M., Sawbridge, T., Frey, B., Blom, J., Frey, J.E., Plummer, K.M., Beer, S.V., Luck, J., Duffy, B., Rodoni, B. 2011. Genome sequence of an *Erwinia amylovora* strain with restricted pathogenicity to *Rubus* plants. J. Bacteriol., *submitted.*
4. Rezzonico F, Smits THM, Duffy B. 2010. Diversity, functionality, and evolution of clustered regularly interspaced short palindromic repeats (CRISPR) in the invasive fire blight phytopathogen, *Erwinia amylovora*. Appl. Environ. Microbiol., *submitted.*
5. Smits THM, Rezzonico F, Kamber T, Goesmann A, Ishimaru C, Stockwell VO, Frey JE, Duffy B. 2010. Complete genome sequence of *Pantoea vagans* plant-beneficial strain C9-1. J. Bacteriol., *in press.*
6. Smits THM, Rezzonico F, Duffy B. 2010. Applied genomics of *Erwinia amylovora*. J. Biotechnol., *in press.*
7. Duffy B. 2010. Graduate management skills are critical for young scientists. Nature Biotechnol., *in press.*
8. Janse J, Scorticini M, Duffy B. (eds.) 2010. *Special Issue :* Advances in Stone Fruit and Nut Bacterial Diseases and Management. J. Plant Pathol., *in press.*
9. Rezzonico F, Pflüger V, Vogel G, Duffy B, Tonolla M. 2010. Rapid identification and phylogenetic analysis of *Pantoea* spp. using intact cell MALDI-TOF mass spectrometry. Appl. Environ. Microbiol. 76:4497-4509.
10. Smits THM, Rezzonico F, Pelludat C, Goesmann A, Duffy B. 2010. Genomic and phenotypic characterization of a nonpigmented variant of *Pantoea vagans* biocontrol strain C9-1 lacking the 530-kb megaplasmid pPag3**.** FEMS Microbiol. Lett. 308:48-54.
11. LeRoux P-MF, Khan M, Broginni GAL, Duffy B, Gessler C, Patocchi A. 2010. Mapping of quantitative trait loci for fire blight resistance in the apple cultivars 'Florina' and 'Nova Easygro'. Genome 53:710-722.
12. Frey JE, Pasquer F, Pelludat C, Duffy B. 2010. Broad spectrum microarray for fingerprint-based bacterial species identification. BMC Biotechnology 10:13.
13. Smits THM, Rezzonico F, Kamber T, Blom J, Goesmann A, Frey JE, Duffy B. 2010. Complete genome sequence of the fire blight bacterium *Erwinia amylovora* CFBP 1430 and comparison to other *Erwinia* strains. Mol. Plant-Microbe Interact. 23: 384-393.
14. Smits THM, Jaenicke S, Rezzonico F, Kamber T, Goesmann A, Frey JE, Duffy B. 2010. Complete genome sequence of the fire blight pathogen *Erwinia pyrifoliae* DSM 12163^T and comparative genomic insights into plant pathogenicity. BMC Genomics, 11:2 (doi:10.1186/1471-2164-11-2).
15. Svercel M, Hamelin J, Duffy B, Moënne-Loccoz Y, Défago G. 2010. Distribution of *Pseudomonas* populations harboring *phlD* or *hcnAB* biocontrol genes is related to depth in vineyard soils. Soil Biol. Biochem. 42: 466-472.
16. Frey JE, Pasquer F, Pelludat C, Duffy B. 2010. A high-density random-oligonucleotide genome microarray for universal diagnostics. EPPO Bull. 40:40-45.
17. Pothier JF, Pelludat C, Genini M, Bünter M, Duffy B. 2010. First report of the quarantine pathogen *Xanthomonas arboricola* pv. *pruni* on apricot and plum in Switzerland. Plant Pathol. 59:404.
18. Pelludat C, Duffy B, Frey JE. 2009. Design and development of a DNA microarray for rapid identification of multiple European quarantine phytopathogenic bacteria. Eur. J. Plant Pathol. 125:413-423.
19. Paternoster T, Vrhovsek U, Pertot I, Duffy B, Gessler C, Mattivi F. 2009. Determination and confirmation of nicotinic acid and its analogs and derivates in apple and pear blossoms using high-performance liquid chromatography - diode array-electrospray ionization mass spectrometry. J. Food Agric. Chem. 57:10038-10043.

20. Svercel M, Christen D, Moënne-Loccoz Y, Duffy B, Défago G. 2009. Effect of long-term vineyard monoculture on rhizosphere populations of pseudomonads carrying the antimicrobial biosynthetic genes *phlD* and/or *hcnAB*. FEMS Microbiol. Ecol. 68:25-36.
21. Rezzonico F, Stockwell VO, Duffy B. 2009. Plant agricultural streptomycin formulations do not carry antibiotic resistance genes. Antimicrob. Agents Chemother. 53:3173-3177.
22. Rezzonico F, Smits THM, Montesinos E, Frey JE, Duffy B. 2009. Genotypic comparison of *Pantoea agglomerans* plant and clinical strains. BMC Microbiology 9:204.
23. Sehic J, Nybom H, Garkava-Gustavsson L, Patocchi A, Kellerhals M, Duffy B. 2009. Fire blight (*Erwinia amylovora*) resistance in apple varieties associated with molecular markers. Int. J. Hort. Sci. 15:1-5.
24. Elad Y, Maurhofer M, Keel C, Gessler C, Duffy B. 2009. Molecular Tools for Understanding and Improving Biocontrol (Editors), IOBC Bull. Volume 43.
25. Rezzonico F, Duffy B. 2008. Lack of genomic evidence of AI-2 receptors suggests a non-quorum sensing role for *luxS* in most bacteria. BMC Microbiology 8:154.
26. Duffy B, Ravva S, Stanker L. 2008. Canteloupe varietal differences as hosts for human pathogenic *Escherichia coli* O157:H7 and *Salmonella enterica*. Eur. J. Hort. Sci. 73:73-75.
27. Duffy B. 2007. Zinc and plant disease. *In*: Zinc. Mineral Nutrition and Plant Disease, ed. LE Datnoff, WH Elmer, DN Huber, APS Press, St. Paul, MN, USA.
28. Khan MA, Durel C-E, Duffy B, Drouet D, Kellerhals M, Gessler C, Patocchi A. 2007. Development of molecular markers linked to the `Fiesta' linkage group 7 major QTL for fire blight resistance and their application for marker-assisted selection. Genome 50:568-577.
29. Rezzonico F, Duffy B. 2007. The role of luxS in the fire blight pathogen *Erwinia amylovora* is limited to metabolism and does not involve quorum sensing. Mol. Plant-Microbe Interact. 20:1284-1297.
30. Svercel M, Duffy B, Défago G. 2007. PCR amplification of hydrogen cyanide biosynthetic locus *hcnAB* in *Pseudomonas* spp. J. Microb. Meth. 70:209-213.
31. Rezzonico F, Zala M, Keel C, Duffy B, Moenne-Loccoz Y, Défago G. 2007. Is the ability of biocontrol fluorescent Pseudomonads to produce the antifungal metabolite 2,4-diacetylphloroglucinol really synonymous with higher plant protection? New Phytol. 173:861-872.
32. Khan MA, Duffy B, Gessler C, Patocchi A. 2006. QTL mapping of fire blight resistance in apple. Mol. Breed. 17:299-306.
33. Ravva SV, Surreal CZ, Duffy B, Stanker L. 2006. Survival of *Escherichia coli* O157:H7 in wastewater from dairy lagoons. J. Appl. Microbiol. 101:891-902.
34. Bosshard E, Hilber-Bodmer M, Schärer H-J, Bünter M, Duffy B. 2006. First report of the quarantine brown rot pathogen *Monilinia fructicola* on imported stone fruits in Switzerland. Plant Dis. 90:1554.
35. Molina L, Rezzonico F, Duffy B, Défago G. 2005. Autoinduction in *Erwinia amylovora*: Evidence of an acyl-homoserine lactone signal in the fire blight pathogen. J. Bacteriol. 187:3206-3213.
36. Compant S, Duffy B, Nowak J, Clément C, Barka EA. 2005. Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl. Environ. Microbiol. 71: 4951-4959.
37. Lutz MP, Wenger S, Maurhofer M, Défago G, Duffy B. 2005. Signaling between bacterial and fungal biocontrol agents in a strain mixture. FEMS Microbiol. Ecol. 48:447-455.
38. Broggini GAL, Duffy B, Holliger E, Scharer HJ, Gessler C, Patocchi A. 2005. Detection of the fire blight biocontrol agent *Bacillus subtilis* BD170 (Biopro®) in a Swiss apple orchard. Eur. J. Plant Pathol. 111:93-100.
39. Duffy B, Schärer H-J, Vogelsanger J, Schoch B, Holliger E. 2005. Regulatory measures against *Erwinia amylovora* in Switzerland. EPPO Bull. 35:239-244.
40. Duffy B, Keel C, Défago G. 2004. Potential role of pathogen signaling in multitrophic plant-microbe interactions involved in disease protection. Appl. Environ. Microbiol. 70:1836–1842.
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