INTEGRATED MANAGEMENT OF BACTERIAL SPOT DISEASE OF STONE FRUITS CAUSED BY XANTHOMONAS ARBORICOLA PV. PRUNI: DEVELOPMENT OF A DISEASE FORECASTING SYSTEM

Gerard Morales Nicolàs

Universitat de Girona

DOCTORAL THESIS

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DECLAREM:

Que el treball titulat "*Integrated management of bacterial spot disease of stone fruits caused by* Xanthomonas arboricola *pv.* pruni*: development of a disease forecasting system",* que presenta **Gerard Morales Nicolàs** per a l'obtenció del títol de doctor, ha estat realitzat sota la nostra direcció.

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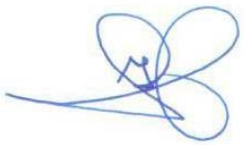

Dr. Isidre Llorente Cabratosa Dra. Concepció Moragrega Garcia

Girona, 7 de desembre de 2017

Universitat de Girona

El **Dr. Isidre Llorente Cabratosa**, la **Dra. Concepció Moragrega Garcia** i el **Dr. Emilio Montesinos Seguí**, com a coautors dels articles següents:

Morales G, Llorente I, Montesinos E, Moragrega C (2017) A model for predicting *Xanthomonas arboricola* pv. *pruni* growth as a function of temperature. *PLoS ONE*, 12(5): e0177583. https://doi.org/10.1371/journal.pone.0177583

Morales G, Moragrega C, Montesinos E, Llorente I. Effects of leaf wetness duration and temperature on the infection of *Prunus* by *Xanthomonas arboricola* pv*. pruni*. *PLoS ONE*. Under Revision.

Morales G, Llorente I, Montesinos E, Moragrega C. Environmental and inoculum effects on epidemiology of bacterial spot disease of stone fruits. Submitted to *European Journal of Plant Pathology*.

Acceptem que el Sr. Gerard Morales Nicolàs presenti els articles esmentats com a autor principal i com a part de la seva tesi doctoral, i que aquests articles no puguin, per tant, formar part de cap altra tesi doctoral.

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List of abbreviations

A.D.	After death
B.C.	Before Christ
BIR	Blossom infection risk
BIS	Billing's Integrated System
BOS	Billing's Original System
BRS	Billing's Revised System
CDD	Cumulative Degree-day
CFBP	Collection Française de Bactéries associées aux Plantes
CFU	Colony-forming unit
DD	Degree-day
DH	Degree-hour
DSS	Decision support system
EIP	Epiphytic infection potential
EPPO	European and Mediterranean Plant Protection Organization
EU	European Union
GC	guanine-cytosine
hrp	Hypersensitive response and pathogenicity
IP	Inoculum potential
IPM	Integrated Pest Management
IR	Infection risk
LB	Luria-Bertrani medium
Peps	Plant elicitor peptides
Psa	*Pseudomonas syringae* pv. *actinidiae*
Pss	*Pseudomonas syringae* pv. *syringae*
Psm	*Pseudomonas syringae* pv. *morsprunorum*
RH	Relative humidity
rpf	Regulation of pathogenicity factors
SAR	Systemic acquired resistance
T2SS	Type II secretion system
T3SS	Type III secretion system
T_{max}	Daily maximum temperature

T_{min}	Daily minimum temperature
Xcv	*Xanthomonas campestris* pv. *vesicatoria*
Xap	*Xanthomonas arboricola* pv. *pruni*
YPGA	Yeast-peptone-glucose agar

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List of publications

This PhD thesis is presented as a compendium of three publications:

Morales G, Llorente I, Montesinos E, Moragrega C (2017). A model for predicting *Xanthomonas arboricola* pv. *pruni* growth as a function of temperature. *PLoS ONE*, *12*(5): e0177583. https://doi.org/10.1371/journal.pone.0177583.

Morales G, Moragrega C, Montesinos E, Llorente I. Effects of leaf wetness duration and temperature on the infection of *Prunus* by *Xanthomonas arboricola* pv*. pruni*. *PLoS ONE*. Under Revision.

PLoS ONE has an impact factor of 2.806 and it was situated in the first quartile (Q1) in the multidisciplinary sciences category in 2016 (© Journal Citation Reports Science Edition, published by Thompson Reuters).

Morales G, Llorente I, Montesinos E, Moragrega C. Environmental and inoculum effects on epidemiology of bacterial spot disease of stone fruits. Submitted to *European Journal of Plant Pathology*.

European Journal of Plant Pathology has an impact factor of 1.478 and it was situated in the second quartile (Q2) in the Agronomy, Plant Sciences and Horticulture categories in 2016 (© Journal Citation Reports Science Edition, published by Thompson Reuters).

Summary

Bacterial spot of stone fruits is a plant disease caused by *Xanthomonas arboricola* pv. *pruni*, which affects a wide range of *Prunus* species, including fruit crops and ornamental species of great economic interest. The disease mainly causes lesions on leaves and fruits, but also cankers on twigs. The economic impact of the disease consists of a reduced quality and marketability of fruits, reduced orchard productivity, and increased costs of nursery productions.

Xanthomonas arboricola pv. *pruni* is considered a harmful quarantine organism in the EU (Council Directive 2000/29/EC), regulation that is aimed to restrict the introduction and dissemination of the pathogen. However, several European countries have reported local outbreaks, which represent a long-term threat to other EU countries since it means that the disease is spreading out of restricted areas (Italy and France).

Disease control is difficult and mainly based on preventive copper spray applications. The moderate efficacy of copper compounds in disease control together with the potential for their accumulation in soil, the selection for pathogen resistance and the phytotoxicity in some stone-fruit crops are limiting factors for the use of copper-based compounds in the control of this disease. Plant disease forecasting systems may help to improve the efficacy of disease control, since they give information on the risk of infections and disease development on the basis of disease epidemiology and pathogen biology. Plant disease forecasting models are key elements of the decision support systems (DSS) for the management of plant diseases, since they provide guidelines for an efficient and rational use of pesticides in a wide diversity of hazard-disease situations.

The present thesis is focused on developing a mechanistic forecasting system for bacterial spot of stone fruits. The forecasting system was conceptualized considering three separate processes, identified as essential steps on the disease cycle: the multiplication of epiphytic bacteria to produce inoculum, the infection process, and the development of disease symptoms. As a result, the general scheme of the forecasting system comprises three components which operate in sequence, corresponding to these three essential processes: i) the epiphytic inoculum potential, ii) the infection model, and iii) the disease symptom development model.

The first component of the model is aimed at predicting the epiphytic inoculum potential of *X. arboricola* pv*. pruni* according to the environmental conditions and determined by bacterial multiplication. With this purpose, the pathogen growth was analyzed *in vitro* and modeled as a function of temperature using the modified Ratkowsky equation. Similarly, the effect of temperature on epiphytic growth of *X. arboricola* pv*. pruni* was analyzed under wetness or under low RH conditions, in detached leaf assays with *Prunus* leaves that simulate natural conditions. The range of temperatures for *X. arboricola* pv*. pruni* growth *in vitro* was 5-35ºC, with an optimum between 20 and 33ºC. The optimum temperature for *X. arboricola* pv*. pruni* epiphytic growth on host leaves was 25ºC, and no growth was observed at temperatures below 15ºC. Although bacterial multiplication is a temperature-dependent process, the presence of wetness is also required for growth. In absence of wetness, a rapid decline of the bacterial population on leaf surfaces during the first 6 h of dryness was observed followed by a slow and continuous inactivation of bacterial cells, which was modeled using the Cerf model. The modified Ratkowsky model for predicting *X. arboricola* pv*. pruni* growth as a function of temperature could be the basis to estimate the inoculum potential. However, the dynamics of epiphytic populations may be predicted by the combination of the growth and the inactivation models depending on wetness and RH conditions. The effect of inoculum density on infection of *Prunus* by *X. arboricola* pv*. pruni* was evaluated in detached leaf assays with three different inoculation methods. The population density of 10^6 CFU/ml was established as the minimum infective concentration, and could be used as the inoculum threshold to link the epiphytic inoculum potential and the infection model.

The goal of the second component of the forecasting system for bacterial spot disease of stone fruits is to assess the conduciveness of the weather conditions to infection occurrences. The quantitative effects of wetness and temperature during the wetness period on the infection of *Prunus* by *X. arboricola* pv. *pruni* were evaluated under controlled enviroment conditions and modeled using a modification of the Weibull equation. Optimal temperatures for infection ranged from 20 to 30ºC, in agreement with the optimal temperatures for bacterial growth. Leaf wetness was necessary for pathogen infection, and from 3 to 6 h of wetness were enough to cause high disease severity ($S' \geq 0.5$) at optimal temperatures. Disease severity was significantly lower ($S' < 0.4$) at temperatures below 20ºC under periods of wetness up to 24 h. The value of daily infection risk index $S' = 0.5$ predicted by the infection

model was proposed as the infection risk threshold. The minimum temperature to reach this threshold was 17.2ºC under 24 h of wetness, whereas only 9.8 h leaf wetness at 20ºC and 4-5 h of leaf wetness at temperatures over 25ºC were necessary to achieve the infection risk threshold. These results provided further evidence for the importance of the combined effect of wetness and temperature on the infection of *Prunus* by *X. arboricola* pv. *pruni*, and demonstrated that short periods of leaf wetness are enough to trigger the infection process at temperatures above 20ºC.

Finally, the third component of the forecasting system predicts symptom development once infections occurred. Disease progress was monitored over time in *Prunus* detached leaves inoculated with suspensions of *X. arboricola* pv. *pruni* at three inoculum densities (10^4, 10^6 and 10^8 CFU/ml), and incubated at constant temperatures from 10 to 35ºC. Temperature and inoculum population size were demonstrated to be important factors on disease development. High final disease severity and short incubation periods were observed at optimal temperatures (20-30ºC) and high bacterial population size (10^6 and 10^8 CFU/ml). Cumulative degree-days (CDD) with a base temperature of 0ºC were calculated, and the CDD-disease progress curves obtained at optimal conditions were used to elaborate the model for predicting disease symptom development. This model was succesfully validated in whole plant assays performed under greenhouse conditions. According to the symptom development model 150, 175 and 280 CDD are required for obtaining disease severities of 5, 10 and 50%, respectively. The biofix date, moment to initiate the computation of CDD, would be each day with risk of infection determined by the infection model. The symptom model enables both the percentage of the symptom development and the calculation of the day on which first symptoms are expected to appear.

This study provides new knowledge on the epidemiology of the bacterial spot disease of stone fruits and offers new possibilities in the management of the disease. However, evaluation and validation under field conditions are needed before the forecasting system can be used as part of a decision support system in the disease management.

Resum

La taca bacteriana dels fruiters de pinyol és una malaltia vegetal causada per *Xanthomonas arboricola* pv. *pruni* que afecta a un ampli rang d'espècies del gènere *Prunus*, inclosos tant cultius de fruiters com espècies ornamentals de gran interès econòmic. La malaltia causa principalment lesions sobre fulles i fruits, però també poden aparèixer xancres sobre les branques. L'impacte econòmic de la malaltia ve donat per una reducció de la qualitat dels fruits i la impossibilitat de la seva comercialització; una reducció de la productivitat dels arbres i l'augment dels costos dels materials de propagació provinents de vivers.

El bacteri *X. arboricola* pv. *pruni* es considera un organisme de quarantena a la UE segons la Directiva 2000/29/CE del Consell, regulació que té com a objectiu restringir la introducció i difusió del patogen. Tot i això, la seva presència s'ha detectat en diversos països europeus, fet que representa una amenaça a llarg termini per a altres països de la UE perquè significa que la malaltia s'està dispersant des de les zones restringides (Itàlia i França).

El control de la malaltia és difícil i es basa fonamentalment en tractaments fitosanitaris preventius amb compostos cúprics. L'eficàcia moderada d'aquests compostos en el control de la malaltia, juntament amb el potencial de la seva acumulació al sòl, la selecció de resistència dels patògens i la fitotoxicitat que presenten alguns cultius són factors limitants en l'ús dels compostos derivats del coure en el control de la malaltia. Els sistemes de predicció de malalties vegetals poden ajudar a millorar l'eficàcia en el control de la malaltia, ja que proporcionen informació sobre el risc d'infeccions i el seu desenvolupament a partir del coneixement de l'epidemiologia de la malaltia i de la biologia dels patògens. Els models de predicció de malalties vegetals són elements clau dels sistemes de suport a la presa de decisions (DSS) en el seu maneig, ja que proporcionen pautes per a un ús eficient i racional dels pesticides en diferents situacions de risc de desenvolupament de la malaltia.

Aquesta tesi es centra en el desenvolupament d'un sistema de predicció de la taca bacteriana dels fruiters de pinyol. El sistema de predicció es va conceptualitzar tenint en compte tres processos diferents, identificats com a passos clau en el cicle de la malaltia: la multiplicació de bacteris epífits per a la producció d'inòcul, el procés d'infecció i el desenvolupament de símptomes de malaltia. Com a

resultat, l'esquema general del sistema de predicció consta de tres components que operen en seqüència, corresponents a aquests tres processos clau: i) el potencial epífit d'inòcul, ii) el model d'infecció, i iii) el model de desenvolupament de símptomes de la malaltia.

La finalitat del primer component del model és predir el potencial epífit d'inòcul de *X. arboricola* pv*. pruni* en funció de les condicions ambientals i determinades a partir de la multiplicació del bacteri. Amb aquest propòsit, el creixement del patogen es va estudiar *in vitro* i es va modelar en funció de la temperatura utilitzant l'equació modificada de Ratkowsky. De forma similar, l'efecte de la temperatura en el creixement epifídic de *X. arboricola* pv*. pruni* es va estudiar en condicions d'humectació o de baixa humitat relativa en assaigs realitzats amb fulles de *Prunus* per simular condicions naturals. El rang de temperatures en el qual es va observar creixement de *X. arboricola* pv*. pruni* va ser de 5-35ºC, amb un creixement òptim entre 20 i 33ºC. La temperatura òptima per al creixement epífit de *X. arboricola* pv*. pruni* sobre les fulles de *Prunus* va ser de 25ºC, i no es va observar creixement a temperatures inferiors a 15ºC. Tot i que la multiplicació bacteriana és un procés dependent de la temperatura, la presència d'humectació també és necessària per al seu creixement. En absència d'humectació, es va observar una disminució ràpida de la població bacteriana sobre la superfície de les fulles durant les primeres 6 hores seguida d'una inactivació lenta i contínua de cèl·lules bacterianes, modelada amb el model de Cerf. El model modificat de Ratkowsky utilitzat per predir el creixement de *X. arboricola* pv*. pruni* en funció de la temperatura podria ser la base per estimar el potencial de l'inòcul. No obstant això, la dinàmica de les poblacions epífites podria predir-se mitjançant la combinació dels models de creixement i d'inactivació del bacteri segons les condicions d'humectació o d'humitat relativa. L'efecte de la quantitat d'inòcul en la infecció de *Prunus* es va avaluar en assaigs de fulles utilitzant tres mètodes d'inoculació diferents. La densitat de població de 10^6 CFU/ml es va establir com la concentració mínima infectiva, que es podria utilitzar com el llindar de la quantitat inòcul que relaciona el potencial epífit d'inòcul i el model d'infecció.

L'objectiu del segon component del sistema de predicció de la taca bacteriana dels fruiters de pinyol és avaluar quan les condicions meteorològiques són favorables per a la infecció del bacteri. Els efectes quantitatius de la humectació i la temperatura durant el període d'humectació en la infecció de *Prunus* per part de

X. arboricola pv*. pruni* van ser avaluats en condicions controlades i es van modelar utilitzant una modificació de l'equació de Weibull. Les temperatures òptimes per a la infecció van des de 20 a 30ºC, coincidint amb les temperatures òptimes per al creixement bacterià. La humectació de la fulla va ser necessària per a la infecció del patogen, i de 3 a 6 hores d'humectació van ser suficients per provocar una severitat alta de la malaltia ($S' \geq 0.5$) a temperatures òptimes. La severitat de la malaltia va ser significativament menor ($S' < 0.4$) a temperatures inferiors a 20ºC en períodes de d'humectació de fins a 24 hores. El valor del risc d'infecció diari $S' = 0.5$ predit pel model d'infecció es va proposar com el llindar de risc d'infecció. La temperatura mínima per arribar al llindar d'infecció va ser de 17.2ºC amb 24 hores d'humectació; mentre que només 9.8 hores d'humectació a 20ºC i 4-5 hores d'humectació a temperatures superiors als 25ºC van ser necessàries per assolir-lo. Aquests resultats han proporcionat més evidències sobre la importància de l'efecte combinat de la humectació i de la temperatura en el procés d'infecció de *Prunus* per part de *X. arboricola* pv*. pruni*, i s'ha demostrat que els períodes curts d'humectació són suficients per infectar els teixits vegetals a temperatures superiors als 20ºC.

Finalment, el tercer component del sistema de predicció mostra el desenvolupament dels símptomes una vegada s'han produït les infeccions. El progrés de la malaltia es va observar en assaigs realitzats amb fulles de *Prunus* inoculades amb suspensions de *X. arboricola* pv. *pruni* a tres densitats d'inòcul (10^4, 10^6 i 10^8 CFU/ml), i incubades a temperatures constants de 10 a 35ºC. S'ha demostrat que la temperatura i la densitat d'inòcul són factors importants en el desenvolupament de la malaltia. Es va observar una severitat final alta de la malaltia i períodes curts d'incubació a temperatures òptimes (20-30ºC) i amb una elevada densitat d'inòcul (10^6 i 10^8 CFU/ml). Es van calcular els graus-dia acumulats (CDD) amb una temperatura base de 0ºC, i les corbes del progrés de la malaltia respecte als graus-dia acumulats obtingudes en condicions òptimes van utilitzar-se per elaborar el model de desenvolupament de símptomes de malaltia. Aquest model va ser validat amb èxit amb assaigs realitzats amb plantes incubades en un hivernacle de seguretat. Segons el model de desenvolupament de símptomes 150, 175 i 280 CDD són necessaris per obtenir severitats de la malaltia del 5, 10 i 50%, respectivament. El biofix, moment per iniciar la computació de CDD, correspondria a cada un dels dies amb risc d'infecció determinat pel model d'infecció. El model de

símptomes permet predir tant el percentatge del desenvolupament dels símptomes com el càlcul del dia en què s'espera que apareguin els primers símptomes.

Aquest estudi proporciona nous coneixements sobre l'epidemiologia de la taca bacteriana dels fruiters de pinyol i ofereix noves possibilitats en la gestió de la malaltia. Tanmateix, cal avaluar i validar el sistema de predicció en condicions de camp abans que aquest pugui utilitzar-se com a part d'un sistema de suport a la presa de decisions en la gestió de la malaltia.

Resumen

La mancha bacteriana de los frutales de hueso es una enfermedad vegetal causada por *X. arboricola* pv*. pruni* que afecta a un amplio rango de especies del género *Prunus*, incluidos tanto cultivos de frutales como especies ornamentales de gran interés económico. La enfermedad causa principalmente lesiones sobre hojas y frutos, pero también pueden aparecer cancros sobre las ramas. El impacto económico de la enfermedad viene dado por una reducción de la calidad de los frutos y la imposibilidad de su comercialización; una reducción de la productividad de los árboles y el aumento de los costes de los materiales de propagación provenientes de viveros.

La bacteria *X. arboricola* pv*. pruni* se considera un organismo de cuarentena en la UE según la Directiva 2000/29/CE del Consejo, regulación que tiene como objetivo restringir la introducción y difusión del patógeno. Sin embargo, su presencia se ha detectado en varios países europeos, lo que representa una amenaza a largo plazo para otros países de la UE ya que significa que la enfermedad se está dispersando des de las zonas restringidas (Italia y Francia).

El control de la enfermedad es difícil y se basa fundamentalmente en tratamientos fitosanitarios preventivos con compuestos cúpricos. La eficacia moderada de estos compuestos en el control de la enfermedad, junto con el potencial de su acumulación en el suelo, la selección de resistencia de los patógenos y la fitotoxicidad que presentan algunos cultivos son factores limitantes en el uso de los compuestos derivados del cobre en el control de la enfermedad. Los sistemas de predicción de enfermedades vegetales pueden ayudar a mejorar la eficacia en su control, ya que proporcionan información sobre el riesgo de infecciones y su desarrollo a partir del conocimiento de la epidemiología de la enfermedad y de la biología los patógenos. Los modelos de predicción de enfermedades vegetales son elementos clave de los sistemas de apoyo a la toma de decisiones (DSS) en su manejo, ya que proporcionan pautas para un uso eficiente y racional de los pesticidas en diferentes situaciones de riesgo de desarrollo de la enfermedad.

Esta tesis se centra en el desarrollo de un sistema de predicción de la mancha bacteriana de los frutales de hueso. El sistema de predicción se conceptualizó teniendo en cuenta tres procesos diferentes, identificados como

pasos clave en el ciclo de la enfermedad: la multiplicación de las bacterias epífitas para la producción de inóculo, el proceso de infección y el desarrollo de los síntomas de enfermedad. Como resultado, el esquema general del sistema de predicción consta de tres componentes que operan de forma secuencial, correspondientes a estos tres procesos clave: i) el potencial epífito de inóculo, ii) el modelo de infección, y iii) el modelo de desarrollo de los síntomas de la enfermedad.

La finalidad del primer componente del modelo es predecir el potencial epífito de inóculo de *X. arboricola* pv*. pruni* en función de las condiciones ambientales y determinadas mediante la multiplicación de las poblaciones bacterianas. Con este propósito, el crecimiento del patógeno se estudió *in vitro* y se modeló en función de la temperatura utilizando la ecuación modificada de Ratkowsky. De forma similar, el efecto de la temperatura sobre el crecimiento epífito de las poblaciones de *X. arboricola pv. pruni* se estudió en condiciones de humectación o con baja humedad relativa en ensayos con hojas de *Prunus* para simular condiciones naturales. El rango de temperaturas en el que se observó crecimiento del patógeno fue de 5-35ºC, con un crecimiento óptimo entre 20 y 33ºC. La temperatura óptima para el crecimiento epífito de *X. arboricola* pv*. pruni* sobre las hojas de *Prunus* fue de 25ºC, y no se observó crecimiento a temperaturas inferiores a 15ºC. Aunque la multiplicación bacteriana es un proceso dependiente de la temperatura, la presencia de humectación también es necesaria para su crecimiento. En ausencia de humectación, se observó una disminución rápida de la población bacteriana sobre la superficie de las hojas durante las primeras 6 horas seguida de una inactivación lenta y continua de las células bacterianas, moldeada con el modelo de Cerf. El modelo modificado de Ratkowsky utilizado para predecir el crecimiento de *X. arboricola* pv*. pruni* en función de la temperatura podría ser la base para estimar el potencial del inóculo. Sin embargo, la dinámica de las poblaciones epifitas se puede predecir mediante la combinación de los modelos de crecimiento y de inactivación según la presencia de humectación o del valor de la humedad relativa ambiental. El efecto de la cantidad de inóculo en la infección de *Prunus* se evaluó en ensayos con hojas utilizando tres métodos de inoculación diferentes. La densidad de población de 10^6 CFU/ml se estableció como la concentración mínima infectiva, y se podría utilizar como el umbral de la cantidad de inóculo que relaciona el potencial epífito de inóculo y el modelo de infección.

El objetivo del segundo componente del sistema de predicción de la mancha bacteriana de los frutales de hueso es evaluar cuando las condiciones meteorológicas son favorables para la infección de la bacteria. Los efectos cuantitativos de la humectación y la temperatura durante el periodo de humectación en la infección de *Prunus* por parte de *X. arboricola* pv*. pruni* fueron evaluados en condiciones controladas y se moldearon utilizando una modificación de la ecuación de Weibull. Las temperaturas óptimas para la infección van desde 20 a 30ºC, coincidiendo con las temperaturas óptimas para el crecimiento bacteriano. La humectación de la hoja fue necesaria para la infección del patógeno, y de 3 a 6 horas de humectación fueron suficientes para provocar una alta severidad de la enfermedad ($S' \geq 0.5$) a temperaturas óptimas. La severidad de la enfermedad fue significativamente menor ($S' < 0.4$) a temperaturas inferiores a 20ºC en periodos de humectación de hasta 24 horas. El valor de riesgo de infección diario $S' = 0.5$ predicho por el modelo de infección se propuso como umbral de riesgo de infección. La temperatura mínima para llegar al umbral de infección fue de 17.2ºC con 24 horas de humectación; mientras que sólo con 9.8 horas de humectación a 20ºC y 4-5 horas de humectación a temperaturas superiores a los 25ºC fueron necesarias para alcanzarlo. Estos resultados han proporcionado más evidencias sobre la importancia del efecto combinado de la humectación y la temperatura en el proceso de infección de *Prunus* por parte de *X. arboricola* pv*. pruni*, y se ha demostrado que períodos cortos de humectación son suficientes para infectar los tejidos vegetales a temperaturas superiores a los 20ºC.

Finalmente, el tercer componente del sistema de predicción muestra el desarrollo de los síntomas una vez se han producido las infecciones. El progreso de la enfermedad se observó en ensayos realizados con hojas de *Prunus* inoculadas con suspensiones de *X. arboricola* pv*. pruni* a tres densidades de inóculo (10^4, 10^6 y 10^8 CFU/ml), e incubadas a temperaturas constantes de 10 a 35ºC. Se demostró que la temperatura y la densidad de inóculo son factores importantes en el desarrollo de la enfermedad. Se observó una severidad final alta de la enfermedad y periodos cortos de incubación a temperaturas óptimas (20-30ºC) y con una elevada densidad de inóculo (10^6 y 10^8 CFU / ml). Se calcularon los grados-día acumulados (CDD) con una temperatura base de 0ºC, y las curvas de progreso de la enfermedad respecto a los CDD obtenidos en condiciones óptimas fueron utilizadas para elaborar el modelo de desarrollo de síntomas de enfermedad. Este modelo fue

validado con éxito con ensayos realizados con plantas incubadas en un invernadero de seguridad. Según el modelo de desarrollo de síntomas 150, 175 y 280 CDD son necesarios para obtener severidades de la enfermedad del 5, 10 y 50%, respectivamente. El biofix, momento para iniciar la computación de CDD, correspondería a cada uno de los días con riesgo de infección determinado por el modelo de infección. El modelo de síntomas permite predecir tanto el porcentaje del desarrollo de los síntomas de la enfermedad como el cálculo del día en que se espera que aparezcan los primeros síntomas.

Este estudio proporciona nuevos conocimientos sobre la epidemiología de la mancha bacteriana de los frutales de hueso y ofrece nuevas posibilidades en la gestión de la enfermedad. Sin embargo, hay que evaluar y validar el sistema de predicción en condiciones de campo antes de que éste pueda utilizarse como parte de un sistema de apoyo a la toma de decisiones en la gestión de la enfermedad.

Chapter 1:

General Introduction

Fruit crops are a major part of agricultural production. In 2014, the most popular fresh fruits worldwide were bananas (114.1 million tons), watermelons (111.0 million tons), apples (84.6 million tons), grapes (74.5 million tons) and oranges (72.3 million tons); and the countries with major fruit production were China, India, Brazil, United States of America, Mexico and Spain (FAOSTAT, 2017).

The stone fruits belong to the cosmopolitan genus *Prunus*, which is classified in the large and diverse family Rosaceae. Peaches, nectarines, apricots, almonds, plums and cherries are referred as stone fruit because of the highly lignified seed capsule contained in drupe fruits (Kole and Abbott, 2012). Peaches and nectarines are the most economically important stone fruits with a production of 22.8 million tons throughout the World (FAOSTAT, 2017). Currently, the main producers in decreasing order are China, Spain, Italy, Greece, the United States and Turkey (Figure 1). Although the production is forecast to decline in the European Union, Turkey and the United States in 2017; world production is predicted to be constant by gains in China (USDA, 2016). The global production of the rest of stone fruit species are shown in Table 1. Asia plays an important role in the stone fruit production with China and Turkey as the main productive countries; whereas Spain, Italy and Greece are leading stone fruit production in Europe.

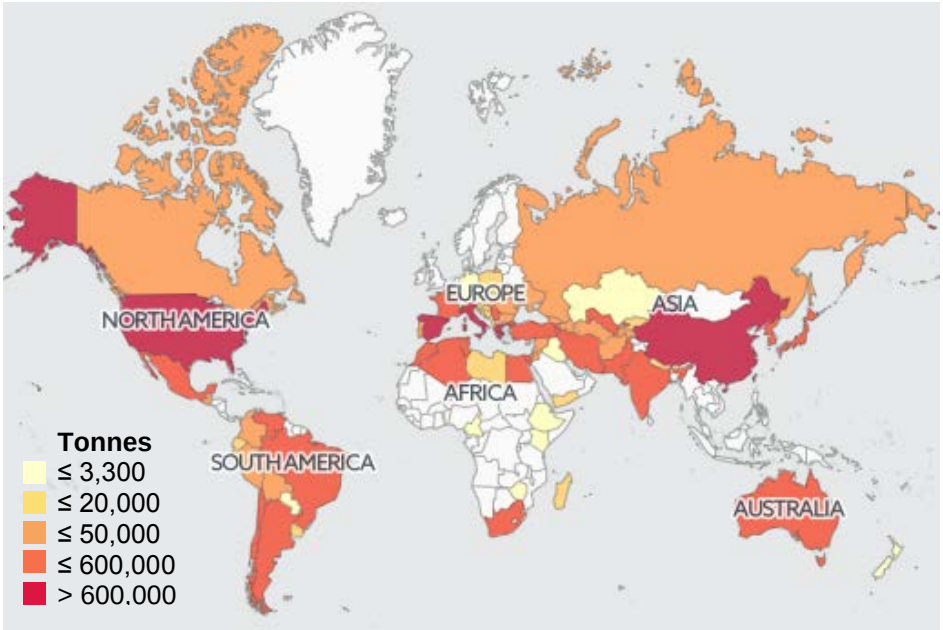

Figure 1. Production (in tonnes) of peach and nectarine throughout the world in 2014 (FAOSTAT, 2017).

Table 1. World production of stone fruits (in million tons) in 2014 and percentage of the global production in brackets (FAOSTAT, 2017).

	Almonds	Apricots	Cherries	Peaches and nectarines	Plums and sloes
World	2.70	3.37	2.25	22.80	11.28
Asia	0.39 (14.4%)	1.80 (53.4%)	0.88 (39.4%)	15.07 (66.1%)	7.61 (67.4%)
Europe	0.30 (11.2%)	0.96 (28.5%)	0.88 (39.1%)	4.51 (19.8%)	2.47 (21.9%)
America	1.58 (58.4%)	0.10 (3.0%)	0.44 (19.6%)	2.27 (10.0%)	0.82 (7.2%)
Northern America	1.55	0.06	0.35	0.99	0.24
South America	0.03	0.04	0.09	1.08	0.51
Africa	0.27 (10.0%)	0.50 (14.7%)	0.03 (1.3%)	0.87 (3.8%)	0.37 (3.2%)
Oceania	0.16 (5.9%)	0.01 (0.4%)	0.02 (0.7%)	0.07 (0.3%)	0.02 (0.2%)

1. History of stone fruits

Stone fruits are soft-fleshed temperate fruits cultivated primary for their mesocarps, with the exception of almond which is cultivated for its seed. Although stone fruits are distributed worldwide, the genus *Prunus* originated in Central Asia, with secondary centers in Eastern Asia, Europe, and North America (Janick, 2005).

Prunus persica, the scientific name of peach, was given because the Romans acquired peaches from Persia at the beginning of the Christian and believed that peaches originated there. However, China is the actual native area of peaches, where they were cultivated since 3300-2500 B.C. (Faust et al., 2011). From China peaches were exported to Eurasia (Kashmir, Bukhara and Persia), later expanded to Europe and, finally, peaches arrived in America brought by Spanish settlers. From Mexico peach spread to New Mexico, Arizona and California during the next centuries.

Similarly, the name of *Prunus armeniaca* was given to apricot assuming that its origin was in Armenia (Near-Eastern Center). Apricot was introduced in Greece

by Alexander the Great (356 – 323 B.C.) and in Italy by General Lucullus (118-56 B.C.), where became common in Roman food 200 A.D. However, according to Nikolai Vavilov (1992) there are two more possible origins for cultivated apricot: Chinese Center and Central Asian Center (India) (Socquet-Juglard, 2012). In the seventh century, the Arabs introduced apricot in Spain and in Southern France prior to 1000, although it was not until the 17th Century that apricot arrived in England and Russia. Apricot dissemination throughout the world continued with Spanish settlers who brought apricot to Virginia and California in the 18th century, and British settlers to Australia (Faust et al., 2011).

Otherwise, the scientific name of almond is *Prunus dulcis*, from Latin *dulcis* for sweet. Although the genetically similarities between almond and peach, they were originated on opposite sides of Asia and were independently domesticated approximately 5000 years ago (Velasco et al., 2016). Widespread dispersal of cultivated almonds occurred in four stages: Asiatic, Mediterranean, Californian, and Southern Hemisphere (Faust et al., 2011; Gradziel, 2009). The Asiatic stage included the initial domestication and spread throughout central and southwestern Asia; from the current Iran to China, India, Pakistan, Turkey, Israel and Syria. Then, Greek and Persian civilizations had an important role in the Mediterranean dissemination of almonds, which were known as the sweet almond or the Greek nut.

Plums have been domesticated independently on three continents originating different species: *P. domestica* with origin in Europe, *P. cerasifera* in western and central Asia, *P. salicina* in China, and the species of the section Prunocerasus such as *P. americana* in North America (Badenes and Byrne, 2012).

The origin of sweet cherry (*Prunus avium*) has been suggested that was in an area south of the Caucasian mountains with a secondary dissemination into Europe, while sour cherry (*P. cerasum*) is native from the middle and south Europe to north India, Iran, and Kurdistan, and its center of origin extends from the south border of the Black Sea along Anatolia and the south Caucasus to Iran (Badenes and Byrne, 2012). Cherry growing started in America with the first settlers and became widespread throughout the land (Faust et al., 2011).

Throughout history, the stone fruit crops are subject to many pests and diseases that limited its production and produce significant economic losses. Pests are caused not only by insects, such as the Oriental fruit moth (*Cydia molesta*), the peach twig borer (*Anarsia lineatella*), the Mediterranean fruit fly (*Ceratitis capitata*),

San Jose scale (*Comstockaspis perniciosa*), aphids (*Myzus persicae* and *Brachycaudus swchartzi*), and thrips (*Frankliniella occidentalis*) (D'Aquino et al., 2011; Il'ichev et al., 2004; Sciarretta and Trematerra, 2006); but also by mites, like the European red mite (*Panonychus ulmi*), the two-spotted spider mite (*Tetranychus urticae*) and the peach silver mite (*Aculus fockeui*) (Blaauw et al., 2017; DAAM, 2016). The main fungal diseases affecting stone fruit are peach leaf curl (*Taphrina deformans*), brown rot (*Monilinia fructicola, Monilinia laxa* and *M. fructigena*), powdery mildew (*Sphaerotheca pannosa* and *Podosphaera tridactyla*), and shot hole disease (*Wilsonomyces carpophilus*) (Ogawa et al., 1995; Sholberg and Kappel, 2008). Moreover, fruit rots caused by *Monilinia* spp., *Botrytis cinerea*, and *Rhizopus* spp. are always important problems during post-harvest storage and transport of stone fruit (Adaskaveg et al., 2005; Bonaterra et al., 2003; Sholberg and Kappel, 2008). The major bacterial diseases of stone fruit species are the bacterial canker, caused by *P. syringae* pv. *syringae* (*Pss*) and *P. syringae* pv. *morsprunorum* (*Psm*); and bacterial spot of stone fruit caused by *Xanthomonas arboricola* pv. *pruni* (*Xap*) (Scortichini, 2010). An appropriate management of these pests and diseases is important to reduce yield losses, thus many recommendations and guidelines for pesticide rates are included in integrated pest management guides from different countries (Blaauw et al., 2017; DAAM, 2016). Another facet of integrated management systems is the use of genetic resistance to various disease and pest problems to reduce the amount of pesticides and fungicides used in stone fruit production, which is an important trend in breeding programs (Byrne, 2005).

2. Bacterial spot disease of stone fruits

Bacterial spot is one of the most serious diseases in *Prunus* orchards all over the world (Garcin et al., 2011b; Kawaguchi, 2014; Lamichhane, 2014; Linvill, 2002; Medeiros et al., 2011; Pagani, 2004). The disease was first described on Japanese plum in North America in 1903, caused by *X. arboricola* pv. *pruni* (Smith, 1903; Vauterin et al., 1995). Since then, the disease has been reported in the major stone-fruit-producing areas throughout the five continents (Figure 2): South Africa and Zimbabwe in Africa; Argentina, Bermuda, Brazil, Canada, Mexico, USA, and Uruguay in America; China, India, Iran, Japan, North and South Korea, Lebanon, Pakistan, Saudi Arabia, Taiwan and Tajikistan in Asia; Bulgaria, France, Italy,

Moldova, Montenegro, the Netherlands, Romania, Russia, Slovenia, Spain, Switzerland and Ukraine in Europe; and Australia and New Zealand in Oceania (EPPO, 2017). The hypothesis that the pathogen originated in the United States and subsequently has been disseminated to other stone-fruit-growing regions of the world by human-aided migration is supported by a population genetics study (Boudon et al., 2005).

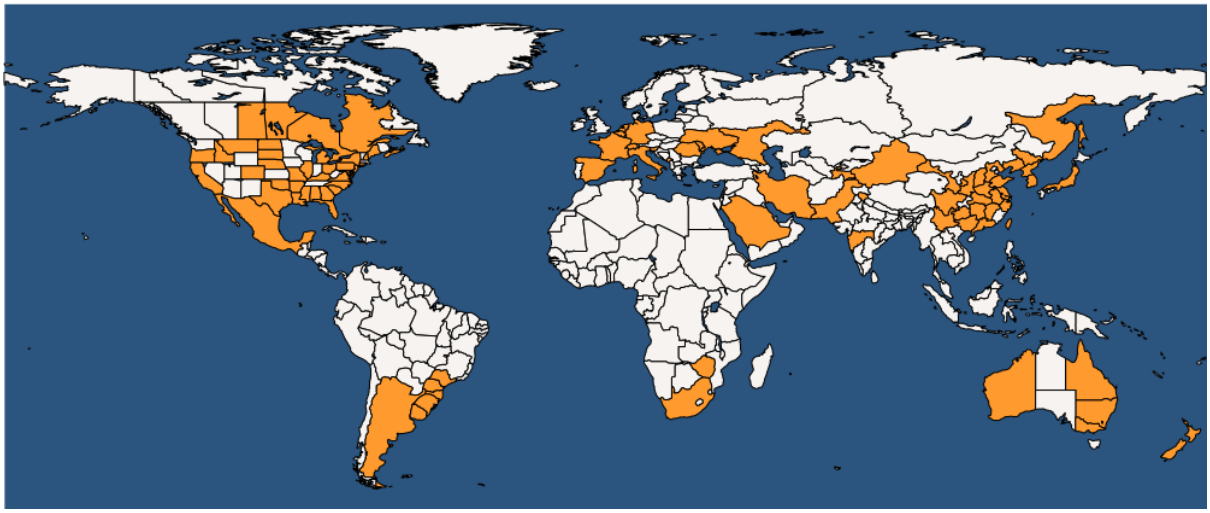

Figure 2. Map of world distribution of *Xanthomonas arboricola* pv. *pruni* (EPPO, 2017).

Xanthomonas arboricola pv. *pruni* is considered a quarantine organism by the phytosanitary legislation of the European Union (Council Directive 2000/29/EC) and the European and Mediterranean Plant Protection Organization (EPPO), who included the bacterium in the A2 list, as present in the EPPO region but not widely distributed (EPPO/CABI, 1997). The pathogen is absent from large regions of Europe, but there is a great potential for the pathogen to spread into areas where stone fruits are cultivated and weather conditions are conducive to disease, having a severe impact on commercial horticulture (EFSA, 2014). The disease has a restricted distribution in Italy, France, Spain, Slovenia and Romania, and few occurrences are reported in Germany, the Netherlands and Belgium (EFSA, 2014). In Italy, the disease was occasionally reported in the 1930s, and in the late 1970s a number of severe outbreaks were reported on plum and peach in the north-east of the country (Emilia Romagna, Veneto and Friuli Venezia Giulia regions), where the pathogen is now considered endemic (EPPO, 2017). The first documented observation of the disease in France was in 1995 in the departments of Drôme and

Gard (Garcin, 2000), and currently, it is locally present in Gard, Hérault, Drome, Ardèche, Lot et Garonne, and Bouches-du-Rhône mainly on peach and Japanese plum (EFSA, 2014). The emergence of the disease in France was due to an introduction of the most prevalent genotype of the bacterium found worldwide, probably introduced via Italy (Boudon et al., 2005). In Spain, the disease was first recorded in 2002 in Badajoz (Extremadura) in Japanese plum. Since then several outbreaks have been detected mainly in plum, peach and nectarine in the following regions: Andalucía, Aragón, Baleares, Catalunya, Comunidad Valenciana, and Navarra. The genetic diversity of different Spanish isolates suggests multiple introductions of this pathogen in Spain and confirms that imported plant propagation material is a major pathway for pathogen introduction and spread (López-Soriano et al., 2016). Moreover, for the first time in the EU, the pathogen was detected on almond in Spain between 2006 and 2009 (Palacio-Bielsa et al., 2010), and on cherry laurel in 2008 in the Netherlands (Tjou-Tam-Sin et al., 2012) and in 2010 in Italy (Marchi et al., 2011).

2.1 Causal agent and pathogenicity

Xanthomonas (from the Greek *xanthos*, meaning 'yellow', and *monas*, meaning 'entity') is a large genus of Gram-negative bacteria that are associated with plants, causing serious diseases in a wide variety of economically important crops, such as rice, citrus, banana, cabbage, tomato, pepper and bean (Ryan et al., 2011). Furthermore, many species among the genus *Xanthomonas*, specially *X. campestris* and *X. arboricola,* produce xanthan gum, an extracellular polysaccharide used in the food industry as additive and thickening agent (E415), and in oil and cosmetics industry (García-Ochoa et al., 1998; Mayer et al., 2011). Xanthan gum was the first biopolymer produced industrially (Rosalam & England, 2006), which is carried out inside bioreactors with nutrient supply and continuous aeration/agitation (Borges et al., 2008; García-Ochoa et al., 1998; Letisse et al., 2003).

The causal agent of bacterial spot disease of stone fruits was originally named *Xanthomonas pruni* after its first report by Smith in 1903. Later, the bacterium was named *X. campestris* pv. *pruni* by Dye, as a result of a new classification that included the term pathovar to distinguish species which were not distinguishable by routine with *in vitro* tests, but differed only in the range of host plants to which they

were pathogenic (Young et al., 1978). Finally, the genus *Xanthomonas* was reclassificated after a DNA-DNA hybridization study (Vauterin et al., 1995). Derived from this reclassification, the species *X. campestris* was restricted to pathovars obtained from cruciferous plants, *X. axonopodis* was emended to include other former *X. campestris* pathovars and new species name were proposed, such as *X. arboricola*.

Xanthomonas arboricola (from the Latin *arbor*, meaning 'tree', and *colere*, meaning 'to inhabit') was initially composed of six pathovars pathogenic on tree species that are distinguished on the basis of phytopathogenic specialization (Vauterin et al., 1995): *X. arboricola* pv. *populi* on poplar, *X. arboricola* pv. *poinsetticola* (type C strains of the former *X. campestris* pathovar) on poinsettia, *X. arboricola* pv. *celebensis* on banana, *X. arboricola* pv. *juglandis* on walnut, *X. arboricola* pv. *corylina* on hazelnut and *X. arboricola* pv. *pruni* on stone fruits. Therefore, the three names *X. pruni*, *X. campestris* pv. *pruni* and *X. arboricola* pv. *pruni* have been used in literature to refer to the causal agent of bacterial spot disease of stone fruits. The pathovar *populi* is responsible for bark necrosis of poplar (Kam, 1984), but later it was considered an "opportunistic pathogen" (Haworth and Spiers, 1992); whereas the International Code of Nomenclature of Bacteria and the Standards for Naming Pathovars considered that *X. arboricola* pv. *poinsetticola* was an invalid name and it had to be referred as *X. axonopodis pv. poinsettiicola* or *X. campestris pv. poinsettiicola* (Bull et al., 2010). In 2001, *X. arboricola* pv. *fragariae* was described causing leaf blight of strawberry (Janse et al., 2001), but the pathovar status of *X. arboricola* from strawberry was recently questioned (Vandroemme et al., 2013). However, in the EPPO database (EPPO, 2017) there are only five pathovars listed for the species *Xanthomonas arboricola*: *celebensis*, *corylina, fragariae, juglandis* and *pruni.* The three most economically important pathovars within the species are pathovars *pruni*, *corylina*, and *juglandis* (Fischer-Le Saux et al., 2012).

Xanthomonas arboricola pv. *pruni* is Gram-negative rod, motile by a single polar flagellum, measuring 0.2-0.8 x 0.8-1.7 µm and strict aerobic (Figure 3A) (EPPO/CABI, 1997). Colonies are wet shining, convex, of a slimy mucoid consistency and produce a yellow water-insoluble pigment (Figure 3B) (Hayward and Waterston, 1965).

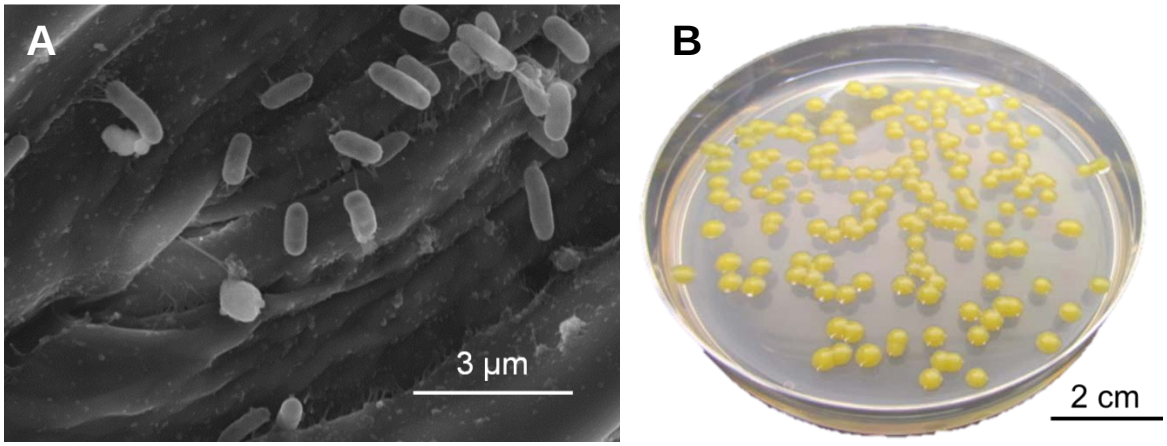

Figure 3. Scanning electron microscopy image of *X. arboricola* pv. *pruni* cells on peach leaf surface (**A**) and colony morphology after 48 h growth on YPGA medium at 27°C (**B**).

The complete genome of *X. arboricola* pv. *pruni* was first sequenced for a genotypic-representative strain from Europe (Italy, CFBP 5530) (Pothier et al., 2011a). The bacterium has a single circular chromosome of 4.85 Mb with a 65.6% content of GC. The genome contains several gene clusters associated to pathogenesis, common in all *Xanthomonas* species: *xps*, which encodes a type II secretion system (T2SS), a two-step secretion system that secretes proteins which are first translocated across the inner membrane by the general secretion pathway, generally associated with the breakdown of host tissues; the cluster regulation of pathogenicity factors (*rpf*), which regulates the synthesis of pathogenicity factors; the hypersensitive response and pathogenicity (*hrp*) genes, which encode a type III secretion system (T3SS), a multisubunit protein apparatus that is used to secrete or inject effector proteins which contribute to interactions with eukaryotic cells; and the *gum* genes, which encode synthases for the extracellular polysaccharide xanthan (Crossman and Dow, 2004; Hajri et al., 2012; He and Zhang, 2008; Pothier et al., 2011a; Ryan et al., 2011; von Bodman et al., 2003). Additionally, the genome of *X. arboricola* pv. *pruni* contains genes encoding for the synthesis of putative enzymes involved in the degradation of the plant cell wall, such as cellulase, polygalacturonase, xylanase and protease, which are secreted by T2SSs; and adhesins, which are involved in bacterial attachment to surfaces and contribute to virulence, such as the type IV pili.

Moreover, *X. arboricola* pv. *pruni* is characterized by the presence of the ubiquitous plasmid pXap41 (Pothier et al., 2011b), which has 41.2 kb with a 62.3%

content of GC. The plasmid is absent in other *X. arboricola* pathovars and it contains some putative virulence genes (Ryan et al., 2011). A comparative analysis of the genome for virulent and avirulent strains reveals differences in the presence of structural and regulatory components associated with motility (type IV pilus) and virulence (type III secretion system, type III effectors, variations in the number of the type IV secretion systems, and the presence of the plasmid pXap41 only in the virulent strain) (Garita-Cambronero et al., 2016).

2.2 Host range, disease symptoms and economic losses

Xanthomonas arboricola pv. *pruni* can affect a wide range of cultivated, ornamental and forest *Prunus* species and their hybrids. Bacterial spot disease of stone fruits has been reported on the cultivated species: *Prunus armeniaca, P. avium, P. buergeriana, P. cerasus P. crassipes, P. davidiana, P. domestica, P. donarium, P. dulcis, P. laurocesasus, P. mume, P. persica* and *P. salicina* (EFSA, 2014), although the most sensible and major hosts are the Sino-Japanese plum group (*P. salicina* and *P. japonica*), peach and nectarine (*P. persica*) and apricot (*P. armeniaca*) (EPPO, 2017; Ritchie, 1995).

Symptoms of bacterial spot disease are observed on leaves, fruit, twigs, branches and trunks. Main symptoms are leaf spot and shot-hole, leaf drop, twig and branch die-back, shoot, branch and trunk cankers, and sunken and necrotic fruit lesion (Scortichini, 2010), but differ throughout the affected species.

On leaves, symptoms first appear as small angular pale green to yellow spots (1 to 2 mm) that enlarge to 2 to 3 mm and become dark or purple in color and necrotic over time (Figure 4) (EPPO/CABI, 1997; Ritchie, 2005). These spots can drop out giving a shot-hole appearance to the leaf, usually leaving a dark ring on the diseased tissue. Affected tissues are often surrounded by a chlorotic halo, and leaves severely infected turn yellow and drop from the plant. Lesions are observed first near the mid-rib, but then spots are generally concentrated at the leaf tip where bacteria are accumulated by rain or dew. Also spots may appear along the leaf margin where the hydatodes secrete guttation fluid. On peach, affected leaves become chlorotic and leaf drop is common, whereas on plum, cherry and cherry laurel, affected leaves remain on the tree and the shot-hole effect is usually more pronounced (EFSA, 2014).

Small circular brown spots appear on fruit, which become sunken over time. The margins of the spots are often water-soaked with light green haloes, giving a mottled appearance to the fruit (Figure 4). Pitting and cracking occur in the vicinity of the spots as a result of the enlargement of the fruit. Gummosis is common on almond, although it may occur after rain in other species, which may easily be confused with insect damage. Although spots are often only superficial, the commercial quality standards by most stone fruit-producing countries do not allow the marketing of affected fruits, which suppose important economic losses (Stefani, 2010). On almond, besides the circular dark spots appear on the endocarp and may affect the nut, the affected fruits either fall prematurely or mummify, becoming a potential source of inoculum (Roselló and Santiago, 2012).

Figure 4. Symptoms of bacterial spot of stone fruits caused by *Xanthomonas arboricola* pv. *pruni* on peach fruit (**A** and **C**) and leaves (**B** and **C**).

On twigs, spring cankers can occur on the apex of overwintering twigs, frequently starting from a leaf scar. Symptoms start with small, water-soaked, slightly darkened, superficial blisters that extend up to 10 cm parallel to the long axis of the twig (Figure 4). The top of the twig dies when the canker encircles it, while the tissue immediately below the dead area, in which the bacteria are present, is characteristically dark, this is known "black tip" injury. Twig infections later in the season result in summer water-soaked cankers and dark-purple spots around the lenticels. Large developing cankers may result in the death of whole branches and, finally, in the death of the tree. On plum and almond, cankers are perennial and continue developing in twigs of 2 and 3 years old, whereas perennial cankers are not reported for peach (EFSA, 2014).

The economic impact of the disease consists of reduced quality and unmarketability of fruits, reduced productivity as a result of weakened trees after defoliation, and higher production costs (propagating materials) (Stefani, 2010). Although yield losses are variable depending on the susceptibility of the cultivars and the weather conditions affecting the development of the disease, these are some reports from years favorable to disease development: 33-76% of the peaches could show lesions in Georgia (USA) (Dunegan, 1932); an economic loss of 10% of the value of the whole Australian stone fruit production, which is equivalent to 3.1 million Australian dollars annually (Lamichhane, 2014; Stefani, 2010); yield losses that can exceed 10,000 €/ha in Japanese plum in Italy (Stefani, 2010); and decrease production in susceptible almond cultivars that may reach 47% in Spain (Palacio-Bielsa et al., 2015).

2.3 Biology and epidemiology

The disease cycle of bacterial spot disease of stone fruits include two alternating phases of *X. arboricola* pv. *pruni*: the parasitic phase, with many secondary cycles during a growing season; and the epiphytic or survival phase (Figure 5).

The sources of inoculum for primary infections are many, since *X. arboricola* pv. *pruni* can overwinter in woody cankers (present on trunks, branches or twigs), dormant buds, leaf scars and in infected leaf debris on the soil (Dhanvantari, 1971; Feliciano and Daines, 1970; Zaccardelli et al., 1998). The bacterium has an epiphytic phase whereby it can persist on symptomless twigs and buds at any time of the year with large populations (Shepard and Zehr, 1994; Zaccardelli et al., 1995). Moreover, survival and growth of *X. arboricola* pv. *pruni* has been reported on non-host plants under favorable conditions (Timmer et al., 1987).

In spring, when conditions are favorable, with warm temperatures and the presence of free water (EPPO/CABI, 1997; Garcin et al., 2011b; Linvill, 2002), the bacteria start to multiply. The bacteria in the intercellular spaces of leaf scars cause the spring canker, as a result of the rupture of the epidermis (Feliciano and Daines, 1970). The effects of temperature on *X. arboricola* pv. *pruni* growth have been studied *in vitro* (Young et al., 1977), in which the optimum temperature was estimated as 31ºC, while the bacteria did not grow at temperatures below 5ºC. Although temperature is the most important weather variable for bacterial growth, water also plays an important role on bacterial multiplication in plant tissues.

Epiphytic populations grow on host tissues when vegetation is wet, regulated by the temperature of the water film in which bacteria are living (Linvill, 2002; Young, 1974), and spread to emerging leaves by dripping dew, rain and wind (Ritchie, 2005). Once the conditions are suitable, bacteria can infect host plants through natural openings (leaf stomata and fruit and twig lenticels) or wounds (EPPO/CABI, 1997; Garcin et al., 2011a; Goodman, 1976), and migrate systemically inside the plant (du Plessis, 1986, 1987). Two different empirical studies concluded that temperature and wetting events are key factors in the infection by *X. arboricola* pv. *pruni*, since at least three successive rainy days with temperatures between 14 and 19°C were necessary for primary infections on peach trees in Italy (Battilani et al., 1999); and 4 hours of wetness with a mean temperature of 12ºC were sufficient to produce infections in France (Garcin et al., 2011b). Experiments performed under controlled-environment conditions at 24 and 30ºC, demonstrated that wetness and water congestion are required for host tissue infection by *X. arboricola* pv. *pruni* (Zehr et al., 1996). Another important factor in the infection process is the bacterial density population. Inoculum doses from 10^6 to 10^8 CFU/ml were reported to be necessary to cause necrosis on peach leaves under controlled conditions (Civerolo, 1975).

Leaf infections generally occur from mid to late spring and its symptoms can be observed after 6-26 days (Battilani et al., 1999) or around 250 degree-days after an episode of rain (Garcin et al., 2011b). Primary infections in both spring cankers and leaves, provide inoculum for new infections to newly emerging leaves, fruit and twigs (forming summer cankers) until late autumn. Also pruning may transmit the bacteria mechanically and create wounds for infection. The disease tends to appear and spread in the spring, makes little progress through the summer, but late infections may occur in the autumn.

The progress of both incidence and severity of bacterial spot disease of stone fruits is variable at the same orchard between years, conditioned by the environmental conditions (Battilani et al., 1999; Garcin and Bresson, 2009; Ritchie, 2004). Moderate temperatures from 19 to 28ºC combined with frequent rains, followed by wetness periods, and accompanied by heavy winds are the most favorable conditions for severe infections (EPPO/CABI, 1997; Linvill, 2002; Stefani, 2010).

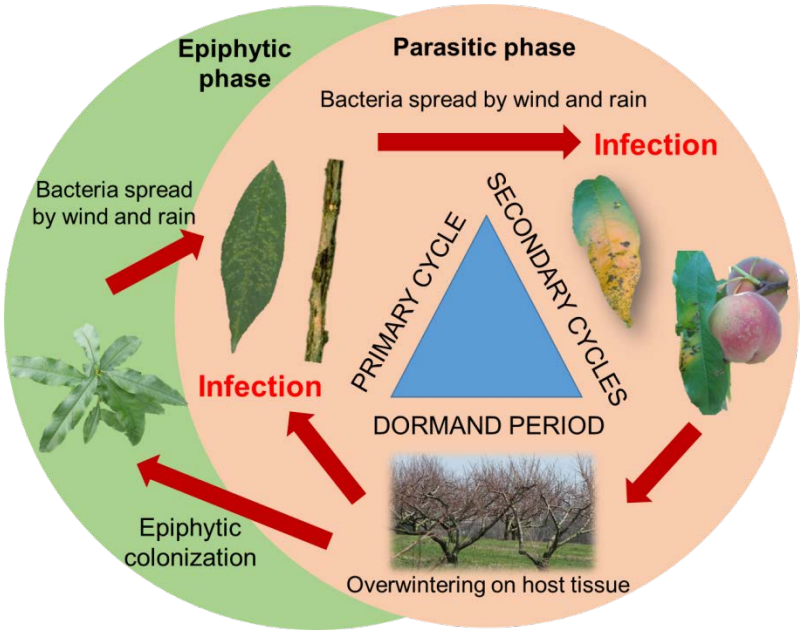

Figure 5. Disease cycle of bacterial spot of stone fruits caused by *Xanthomonas arboricola* pv. *pruni*. Adapted from Jones and Sutton (1996).

2.4 Disease management

The development and implementation of successful control strategies for bacterial spot disease of stone fruit should integrate multiple factors: (i) an adequate legislation; (ii) breeding for resistance; (iii) suitable agro-technical measures; and (iv) disease control in the orchard (Stefani, 2010).

An adequate legislation is the first step to ensure the use of healthy planting material and avoid the introduction and spread of the pathogen in its latent form from countries where it currently occurs. *X. arboricola* pv. *pruni* is considered a relevant harmful organism in the European Union, according to the Council Directive 2000/29/EC on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community. Moreover, EPPO included the pathogen in the A2 list for regulation of quarantine pests, which are present in the EPPO region but not widely distributed (EPPO/CABI, 1997). In the United States, nursery inspections and certification are a part of integrated pest management under the responsibility of the U.S. Department of Agriculture, Animal and Plant Health Inspection Service (USDA-APHIS) (Lamichhane, 2014). Rapid detection methods of the pathogen in plant materials, sensible enough to detect very low numbers of bacteria despite asymptomatic samples, have been developed to determine the presence of the pathogen for plant-

protection services' surveillance and avoid its spread (Ballard et al., 2011; Palacio-Bielsa et al., 2011).

Host resistance is an important component of integrated pest management (Lamichhane, 2014), since expenses for sprays and the addition of toxic chemicals to the environment may be reduced, as well as losses from disease (Agrios, 2005). However, *Prunus* species and cultivars vary in their susceptibility to bacterial spot (Dunegan, 1932; EFSA, 2014; EPPO, 2017). In order to select tolerant or resistant varieties, susceptibility of cultivars to bacterial spot has been evaluated (Table 2), although most peach, apricot and Japanese plum genotypes are either highly susceptible or susceptible (du Plessis, 1988; Garcin and Bresson, 2009; Garcin et al., 2007; Kole and Abbott, 2012; Medeiros et al., 2011; Nasrollahnejad and Mousavi, 2008; Suesada et al., 2013; Werner et al., 1986). Moreover, when environmental conditions are favorable for infection, all cultivars show at least some symptoms (Yang et al., 2013), and many of the cultivars rated as highly resistant became susceptible (Werner et al., 1986). Different breeding programs have focused on selecting tolerant varieties to bacterial spot as a preventive measure to control the disease at the same time of selecting varieties according to the market trends (Byrne, 2005). The breeding program in the USA, currently named RosBREED, was successful in developing a series of bacterial spot resistant cultivars with exceptional tolerance and/or resistance, particularly in "Clayton", although many resistant cultivars lacked specific desirable fruit and marketing characteristics (Okie, 1998). Currently, marker-assisted breeding, based on genetic studies that identified quantitative trait loci associated with resistance to bacterial spot (Socquet-Juglard et al., 2013; Yang et al., 2013), is being used to develop new varieties with an increased tolerance within the framework of the more recent projects RosBREED in the USA (www.rosbreed.org) (Bassil et al., 2011) and FruitBreedomics in the EU (www.fruitbreedomics.com) (Laurens et al., 2010).

Agrotechnical measures or cultural methods are actions that the grower can do to help plants avoid contact with the pathogen, creating environmental conditions unfavorable to the pathogen or avoiding favorable ones, and eradicating or reducing the amount of a pathogen in a plant, a field, or an area (Agrios, 2005). The role of the growers on the control of the bacterial spot start with choosing a suitable location for an orchard, planting resistant varieties and certified healthy plants (Stefani, 2010). Soil texture and plant nutrition have an effect on the disease development,

since heavy soils with high water potential and excess in nutrients favor disease development (Matthee and Daines, 1968, 1969). Additionally, the potential pathogen reservoir can be reduced with the elimination of weeds and orchard grasses where the pathogen can survive and grow (Timmer et al., 1987), mixing varieties or cultivars, as well as intercropping, since the genetic diversity of an orchard increases (Lamichhane, 2014).

Finally, the use of chemical compounds that are toxic to the pathogens is one of the most common means of controlling plant diseases in the field, with the aim at protecting plants preventively from pathogen infections or curing an infection that is already in progress (Agrios, 2005). Copper-based compounds are effective in the control of bacterial spot disease when applied preventive, not curative, representing a bacterial-killing barrier on the surface of the tree to epiphytic bacteria before they can multiply and infect (Bazzi and Mazzucchi, 1980; du Plessis, 1987; Garcin et al., 2005; Ritchie, 2011). The use of copper products is recommended to be applied in autumn after leaves have fallen and before flowering in spring to reduce the inoculum in the orchards; but during the growing season copper sprays are also recommended in the event of disease outbreaks, always prior to the occurrence of conditions for infection in order to reduce secondary inoculum and dissemination of the pathogen (Blaauw et al., 2017; EFSA, 2014; Regione Emilia Romagna, 2017). However, the use of copper is limited because not only its use may cause additional problems for the host plants, as they are very sensitive to phytotoxicity, especially peach and nectarine (Francini and Sebastiani, 2010; Lalancette and McFarland, 2007; Ritchie, 2004), but also non-target organisms and chemical processes in the environment are affected (Flemming and Trevors, 1989; Kunito et al., 1999) and selection for resistance in the pathogen has been reported (Vanneste et al., 2005). To reduce these effects, copper compounds are alternate with sprays of the antibiotic oxytetracycline in USA (Blaauw et al., 2017), but its use is not approved in EU (Directive 2009/128/EC). Different alternatives to copper have been tested, such as the use of phages (Civerolo, 1976), *Pseudomonas* spp. strains and acid lactic bacteria as biological control agents (Biondi et al., 2006; Roselló, 2015), antimicrobial peptides (Montesinos and Bardají, 2008), and plant elicitor peptides (Peps) as inducer of systemic acquired resistance (SAR) (Ruiz et al., 2017), but the efficacy of these compounds or biological agents is still unclear and/or need to be tested under field conditions.

Table 2. Categorization of *Prunus* species and cultivars based on the susceptibility to bacterial spot of stone fruits disease. Modified from Cambra (2014) and Garcin and Bresson (2009).

	Sensible		Moderate		Lowly susceptible or Resistant	
Apricot	Ajami	Paviot	Bergeron	Superb	Bergarouge®	
	Blenril	Precoce Cremonini	$Kioto_{cov}$	Viceroy	Búlida	
	Boccuccia	Reale d'Imola	Moorpark		Canino	
	Early Golden	Rouge de Roussillon	Newcastle		Early Blush®	
	Goldbar®	San Castrese	Orangered®		Moniqui	
	Goldrich	Tardif de Bordoneil	Pinkcot®		Sungiant	
	Hargrand	Veecot	Polonais		Tyrinthos	
	Palummella		Stella			
Plum	Angeleno	Frontier	Golden Japan	Laroda	Au Amber	Mirabelle de Nancy
	Au frontier	Laroda	Au Producer	Morris	Au Crimson	Président
	Au rosa	M1	Beauty	Nubiana	Bellerosa	Prune d'Ente
	Au rubrum	Marianna	Blackamber	Ozark	Bruce	Queensland
	Black beauty	Methley	Burbank	Premier	Earlisweet	Robusta
	Black diamond	Santa Rosa	Burmosa	Queen	Explorer	
	Black gold	Shiro	Byrongold	Redhaert	Fortune	
	Black star	Tc Sun	Eldorado	Rosa	Globe Sun	
	Calita		Friar	Rubysweet	Homeside	

Continued on next page

Table 2. *(continued)*.

	Sensible		Moderate		Lowly susceptible or Resistant	
Peach	Autum Lady	Monroe	Ambre	Jerseyqueen	Ambergem	Newhavean
	Autumglo	O'Henry	Allgold	Legend	Belbinette®	Plusplus®
	Baby Gold 5	Opale	Allstar	Loring	Belle of Georgia	Ranger
	Baby Gold 9	Parade	Autumn Star	Madison	*Benedicte*®	Redbird
	Big Sun	Royal May	Beaumont	Majestic	Biscoe	Redkist
	Blake	Ryan Suncov	Bellaire	Maygold	Blazing Star	Royal Gem
	Brackett	Scarletpearl	Bicentennial	Messina	Bounty	Rubired
	Brilliant	Snow Beauty	Blake	Norman	Candor	Salem
	Calred	Snow Brite®	Carolina Belle	Ouchita Gold	Cardinal	Sentinel
	Carnival	Snow King®	Contender	Raritan	Cherryred	Siberian C
	Cullinan	Spring Flame	Coralie$_{cov}$	Red Fair®	Clayton	Silver Late®
	Diamond Princess$_{cov}$	Spring Lady	Coraline®	Red Haven	Comanche	Southhaven
	Early East	Springold	Cresthaven	Redskin	Contender	*Spring White*®
	Elberta	Sullivan Elberta	Encore	Rich Lady$_{cov}$	Derby	Starfire
	Elegant Lady	Summer Ladycov	Ernie's Choice	Rio Oso Gem	Dixired	Sweethaven
	Fairtime	Summer Richcov	Flameglo®	Roseredglobe	Early-Free Red	Volupte®
	Fireprince	Summerglo	Gabriella	Ruston Red	Earlyglo	Zephir®
	Firered	Summerpearl	Garnet Beauty	Summergold	Emery	
	Flavorcrest	Sunbrite	Gaucho	Sunprince	Harbelle	

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Table 2. *(continued)*.

	Sensible		Moderate		Lowly susceptible or Resistant
Peach	Hale Harrison	Suncrest	Gladys®	Topaz	Harbrite
	Halehaven	Sunhigh	Glengo	Vermeil®	Harken
	Jerseyland	Surecrop	Grazano	Victoria	Harrow Beauty
	July Elberta	Sweet Sue	Harbrite	Winblo	Harrow Diamond
	Laurol	White Hale	Harvester	Zeeglo$_{cov}$	Jerseydawn
	Marsum	Yukon King	Ivoire®		Mayflower
	May Lady		Jayhaven		Nemaguard
Nectarine	Arctic Belle	Flavortop	Armking	Summer Beauty	Sweet Melody
	Arctic Gold	Honey Royale	Earlbird	Sunbright	Carolina Red
	Arctic Jay	Honeyblaze	Fantasia	Sunfre	Diamond Bright$_{cov}$
	Arctic Pride	Honeykist	Firebrite	Sunglo	Emeraude®
	Big Top	Jade®	Harblaze	Sungrand	Le Grand
	Cassiopia	June Glo	Hardired	Tasty Free	Nectared 3
	Crimson Gold	Lagold	Harflame		Nectared 6
	Crimson Snow	Late Gold	Jade		Royalkist
	Diamond Raycov	Red Gold	Roseprince		Topaze®
	Firebrite	Scarlet Pearl	Royal Giant		
	Flamekist	Zephyr	Snow Queen		

3. Epidemiology of plant diseases

Infectious plant diseases are caused by a pathogenic organism such as a fungus, bacterium, mycoplasma, virus, viroid, nematodes, or parasitic plant. The main factors involved in a plant disease are the host, the pathogen and the environment. Biological processes concerning to both the pathogen and the host are regulated by environmental factors, basically temperature and moisture on plant surface (Dickson and Holbert, 1928; Huber and Gillespie, 1992). Processes like pathogen growth (Billing, 1974; Feil and Purcell, 2001; Young et al., 1977), number of spores formed and released (King et al., 1997; Paul and Munkvold, 2005) and development of a disease after infection (Fukui et al., 1999; King et al., 1997; Koizumi and Kochinotsu, 1977; Lovell et al., 2004) are temperature-dependent, while moisture is indispensable for infection, germination of fungal spores, sporulation, distribution and spread of the pathogen, and also increases the succulence of host plants and, consequently, their susceptibility (Agrios, 2005; Huber and Gillespie, 1992; Magarey and Sutton, 2007). Moreover, soil nutrients, light, wind and soil pH can also influence on the growth and susceptibility of the host, on the multiplication of the pathogen, or on the host-pathogen interaction (Agrios, 2005).

The disease triangle is a conceptual model that shows the effects of the host, the pathogen and the environment on disease development, in which each of these components is placed in one vertex of a triangle. The interaction between the three factors is required for disease development, since the lack of only one factor results in no disease. The disease triangle can be modified to a pyramid or a tetrahedron including humans or vectors as additional agents (Agrios, 2005). Otherwise, the dimension of time has been added to reflect the duration when the other three factors are favorable for disease development, illustrated as a cone or a right angle prism shape rather than a tetrahedron (Francl, 2001).

3.1 Plant disease forecasting models

Plant disease forecasting models predict outbreaks or increases in intensity of a disease based on information about the weather, crop, and/or pathogen (the disease triangle) (Campbell and Madden, 1990). These predictions allow to determine whether, when, and where a particular management practice should be applied (Agrios, 2005; Campbell and Madden, 1990). A rational management of plant

diseases, both economically and environmentally, may result by using forecasting models, since risks, costs and benefits of each of numerous decisions are weighted according to model predictions to determine when control measures are warranted (Gent et al., 2011). Forecasting models are part of decision support systems (DSS) and they are used for scheduling management operations, including scouting and application of pesticide treatments (Damos, 2015; Isard et al., 2015; Magarey et al., 2002).

A successful forecaster is that one adopted and implemented by growers, and the attributes that will help its success are: reliability (based on biological and environmental data and adequately validated), simplicity, importance (the disease is of economic importance to the crop, but sporadic enough that the need for treatment is not a given), usefulness (the forecasting model should be applied when the disease and/or pathogen can be detected reliably), availability (necessary information about the components of the disease triangle), multipurpose applicability (ideally if monitoring and decision-making tools can be done simultaneously for several diseases), and cost effectiveness (cost affordable relative to available disease management tactics) (Campbell and Madden, 1990).

Plant disease forecasting models are usually disease specific because of the important differences in the biological cycle of the agents, especially between fungal and bacterial pathogens, which difficult the existence of a global model for all plant diseases. Although an abundant number of forecasting models of plant diseases have been developed, only a small proportion have been adopted and implemented as part of Integrated Pest Management (IPM) (Gent et al., 2011; Shtienberg, 2013). The main reasons for the limited acceptance by growers are technical constrains and grower's perception. Possible solutions to technical constrains concerning the environmental inputs, model construction and parameterization, validation, and implementation have been proposed to reduce forecast model failure (Magarey and Isard, 2017). However, grower's perception and attitude towards disease prediction have the greatest impact on the adoption and implementation of a forecasting model (Campbell and Madden, 1990; Gent et al., 2011). One of the most important obstacles to the use of plant disease forecasters has been the perception of financial risk from not spraying preventatively (Wearing, 1988). Grower's reactions in their pest management to risky situations can be different when forecasting models include subjective probabilities of disease risk, based on their knowledge, opinion or

intuition (Yuen and Hughes, 2002). Therefore, grower education on how to integrate the outputs of the forecasting model in their decision making is fundamental, as well as incorporating subjective probabilities into IPM recommendations, which may be one means to reduce grower uncertainty and improve trust of these systems (Gent et al., 2011).

Fusarium head blight of wheat (www.wheatscab.psu.edu) and Asian soybean rust (www.sbrusa.net) are two current examples of plant disease forecasters providing daily information on-line in USA, with background information on the disease and management recommendations (Esker, 2008). Similarly, the Plant Health Service of Catalunya (Spain) (http://ruralcat.gencat.cat/web/guest/agrometeo) provide daily forecasts on-line using meteorological data from the weather station network (Servei Meteorològic de Catalunya) for several plant diseases: apple scab (*Venturia inaequalis*, Mills model), brown spot of pear (*Stemphylium vesicarium*, BSPcast model), and vine downy mildew (*Plasmopara viticola*); and pests: codling moth (*Cydia pomonella*), peach moth (*Grapholita molesta*), *Anarsia lineatella* in peach, apple brown tortrix (*Pandemis heprana*), summer fruit tortix (*Adoxophyes orana*), and white peach scale (*Pseudaulacaspis pentagona*).

3.2 Forecasting model development

The maximum amount of information about a disease (or each of the causal agents) is useful to develop a model to predict plant disease epidemics. The information used in developing a forecasting system can be fundamental or empirical. Fundamental forecasters, also referred as mechanistic, are those developed in the laboratory, controlled-environment chambers, greenhouse, or field, which describe one or more aspects of the host-pathogen relation as influenced by environment (Campbell and Madden, 1990). On the other hand, empirical forecasters use a statistical approach to quantify the relationship between current and historical data on disease levels and weather data, although they lack the explanatory capability of fundamental systems (Campbell and Madden, 1990; Magarey and Isard, 2017).

A plant disease is a complex system influenced by many factors, but not all of them have an equitable role on the disease development (Del Ponte et al., 2006). Therefore, forecasting models can predict the behavior of a disease simplifying the system, considering only the main factors or the critical steps on disease

development, similarly to hazard analysis and critical control point (HACCP) in food industry (Notermans et al., 1995). Consequently, the knowledge of one or two factors can be sufficient for the formulation of a reasonably accurate forecast (Agrios, 2005). Wetness duration and temperature are the key weather parameters that control the infection process for fungal pathogens (spore germination and infection) (Duthie, 1997; Magarey and Sutton, 2007; Magarey et al., 2005b), and many forecasting models have been developed using only this concept (Arauz et al., 2010; Carisse et al.2000; Christiano et al., 2009; Montesinos et al., 1995). However, infection mechanisms are different for bacterial diseases and, consequently, the basis for bacterial forecasters may be different than for fungal diseases. The amount of initial inoculum (Baker, 1971; Burdon and Chilvers, 1982; Latorre et al., 2002c; Trapero-Casas and Kaiser, 1992) and the weather conditions favorable to infections are both important parameters to forecast bacterial disease development (Beattie and Lindow, 1999; Rouse, 1985). Many bacterial forecasters, such as Maryblyt (Lightner and Steiner, 1992), CougarBlight (Smith, 1993), and Billing's integrated system (BIS95) (Billing, 1996) for fire blight of apple and pear, caused by *Erwinia amylovora*; the risk model for bacterial canker of kiwifruit (Beresford et al., 2017); or bacterial spot on hot pepper caused by *Xanthomonas campestris* pv. *vesicatoria* (Kim et al., 2014) are based in two separated processes (Table 3). The first one consists in the bacterial multiplication to provide inoculum, which is a temperature-dependent process and requires the presence of moisture (surface wetness or high RH) (Billing, 1974; Feil and Purcell, 2001; Hirano et al., 1996; Moh et al., 2011; Rouse, 1985; Shrestha et al., 2005). The second process is related to the dispersion of bacterial inoculum and to favorable conditions for infection occurrences, which depends basically on temperature, wetness (rainfall or irrigation), wind and host phenology (Huang, 1986; Jones, 1984; Kushalappa and Zulfiquar, 2001; Latorre et al., 2002b; Lightner and Steiner, 1992). Although all the models presented in Table 3 use practically the same input variables, they differ in how these variables are used to do their predictions. The bases of each forecasting model are presented in the following sections.

Table 3. Examples of forecasting models for plant diseases caused by bacteria.

Disease (pathogen)	Model	Input variables[Y]	Output variables[Z]	Reference
Fire blight (*Erwinia amylovora*)	Maryblyt	Daily T_{max} and T_{min} (ºC or ºK) Rainfall (mm) Phenology Trauma	EIP HWTR BBS CBS SBS TBS	(Lightner and Steiner, 1992; Steiner, 1990)
Fire blight (*Erwinia amylovora*)	CougarBlight	Daily T_{max} (ºC or ºK) Rainfall (mm) Presence of blossoms Blight history in the neighbourhood	Infection risk on flower blossom	(Smith, 1993)
Fire blight (*Erwinia amylovora*)	BIS95	Daily T_{max} and T_{min} (ºC or ºK) Rainfall (mm) Phenology Insect activity Disease incidence	Infection risk on flower blossom BBS	(Billing, 1996)
Bacterial spot on hot pepper (*Xanthomonas campestris* pv. *vesicatoria*)	*Xcv* infection model	Hourly temperature (ºC or ºK) Rainfall (mm) Wetness duration (h) Wind	Infection risk	(Kim et al., 2014)
Bacterial canker of kiwifruit (*Pseudomonas syringae* pv. *actinidiae*)	*Psa* risk model	Hourly Temperature (ºC or ºK) Wetness duration (h) RH (%)	Infection risk	(Beresford et al., 2017)

[Y] T_{max} and T_{min}: maximum and minimum temperatures.

[Z] BBS: Blossom blight symptoms; BHWTR: Daily infection risk level; CBS: Canker blight symptoms; EIP: Epiphytic Potential Inoculum; RH: relative humidity; SBS: Shoot blight symptoms; TBS: Trauma blight symptoms.

3.2.1 Maryblyt

This is the most used model for fire blight prediction, because it was the first one to be commercially available and completely computerized (Lightner and Steiner, 1992; Steiner, 1990) and validated (Jones, 1992; Shtienberg et al., 2003; van der Zwet et al., 1994). Maryblyt monitors the development of the host, pathogen populations (*E. amylovora*), insect vector availability and symptom development with multiple cumulative heat unit "clocks" using maximum and minimum temperature (Biggs and Turechek, 2010):

- Cumulative degree-days (DD) > 4.4ºC are used to monitor the age of flowers and the appearance of insect vectors.
- Cumulative degree-hour (DH) > 18.3ºC are used to establish the epiphytic infection potential (EIP), which is an index for infection risk. As the threshold for infection is 110 DH > 18.3ºC, the EIP is expressed as the percentatge of cumulative heat of this value. For example, if EIP is below 100 then few infections will occur, if EIP is 100-150 this is sufficient to support a blossom blight epidemic, and if EIP exceeds 200-250, then a large number of infections can be expected with any wetting event.
- Cumulative DD > 12.7ºC are used to predict symptom development, once infection has occurred.

The model, assuming the presence of fire blight bacteria and susceptibility of the host plant, predicts specific infection events and the appearance of four distinct types of fire blight symptoms: blossom, canker, shoot and trauma blight. For blossom blight four conditions must be met in sequence:

1. flowers must be open with stigmas and petals intact.
2. accumulation of at least 110 DH > 18.3ºC
3. occurrence of either dew, a rain of 0.25 mm or greater during the current day, or 2.5mm during the previous day.
4. daily average temperature greater than or equal to 15.6ºC.

When all of these conditions are met in sequence, infection occurs and first blossom blight symptoms are expected after an accumulation of additional 57 DD > 12.7ºC (Figure 6). DH are reduced by one-third, one-half or reset to zero if the temperature does not surpass a threshold of 17.8ºC during one, two or three days, respectively. However, once DH exceeds 400 (EIP = 200), no negative adjustments are made.

Otherwise, canker blight is predicted with heat accumulation after green tip, shoot blight develops after the first blossoms or canker blight symptoms, and trauma blight symptoms are expected when the EIP reaches 100, but trauma blight are generally more severe when the EIP exceeds 200-250.

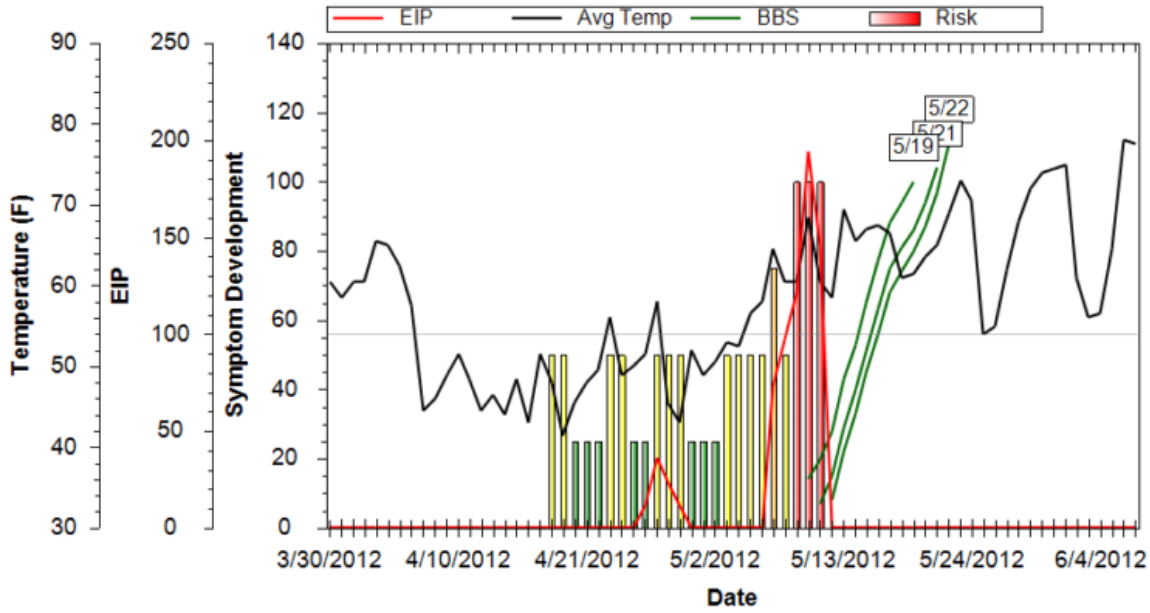

Figure 6. Graphical presentation of *Maryblyt*™ output example. Variables shown are: average temperature, EIP and EIP = 100 (reference line), blossom blight symptoms (BBS) (shown for three infection events), and infection risk (Biggs et al., 2014).

3.2.2 CougarBlight

The CougarBlight model (Smith, 1993) predicts blossom infections using temperature data to estimate the growth rate of fire blight bacteria (*E. amylovora*) on the stigma during over the approximately 96 hours prior to a wetting period, which is required for flower infections (Figure 7). Two factors determine infection risk: bacterial potential growth predicted from temperature data; and potential for pathogen presence, which determines different thresholds of infection risk depending on different scenarios of blight history in the neighborhood (Table 4).

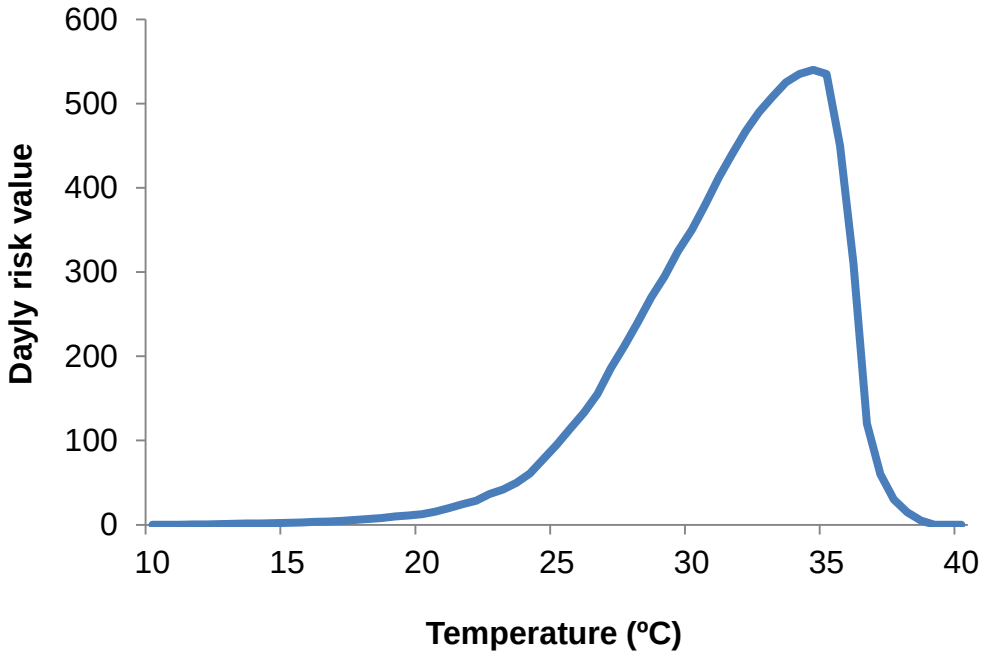

Figure 7. Average daily risk values related to the daily high temperature used by CougarBlight, derived from *Erwinia amylovora* population growth on stigmas of detached crab apple flowers held at various temperatures for 24 h (Smith and Pusey, 2011).

Table 4. Risk value thresholds of CougarBlight for different orchard blight history scenarios (Smith and Pusey, 2011).

	Low	Caution	High	Extreme	Exceptional
1. No fire blight in the neighborhood last year	0-300	300-500	500-800	> 800	-
2. Fire blight occurred in the neighborhood last year	0-100	100-200	200-350	350-500	> 500
3. Fire blight is now active in the neighborhood.	-	0-100	100-200	200-300	> 300

3.2.3 Billing's Integrated System (BIS95)

The model was designated as a substitute for Billing's original and revised systems for fire blight (BRS and BOS), which were based on *E. amylovora* potential doublings derived from *in vitro* growth rates (Billing, 1980, 1992), in order to make it simpler and clearer. The model uses two types of degree-day (DD) calculations to help assess the risk of fire blight (Billing, 1996, 1999):

- DD18 = the sum of daily values above 18ºC for the maximum temperature. DD18 calculations begin on the first day of bloom, and continue throughout the bloom period. If the maximum temperature falls to 16-17ºC for two days or to 15ºC or lower for one day, the DD18 sum is reset to zero.
- DD13 = the sum of daily values of 0.5ºC or more above a 13ºC mean. DD13 calculations begin on the day after each infection risk (IR) day. DD13 is used to time orchard scouting for signs of new disease.

Infection risk (IR) depends on inoculum potential (IP), host susceptibility which is increased by tissue damage, and warmth and wetness at the time of infection. A day is classified as IR if:

1. The IP is principally coming from ooze and is spread by rain during pre-bloom, blossom, young shoot and fruit growth stages.
2. Rain is ≥ 3 mm and mean temperature on the wet day or the day before is ≥ 13ºC.

Blossom infection risk (BIR) occurs in situations where IP levels depend on flower colonization by the pathogen and spread by insects. A day is classified as a BIR day if:

1. The DD18 sum is ≥ 17, the open flowers are wet by heavy dew, mist, or rain, and the mean temperature is ≥ 15ºC on the day of wetting.
2. Rain is ≥ 3 mm and mean temperature on the wet day or the day before is ≥ 13ºC.
3. There is no wetting event but the maximum temperature for the day is ≥ 27ºC and/or the mean temperature for the day is ≥ 20ºC.

In addition to degree-day and infection risk calculations based on weather data, the model also involves intensive orchard scouting and systematic recording of host growth stages, insect activity, and disease incidence to determine risk of new disease.

3.2.4 Infection model of bacterial spot on hot pepper

The model for bacterial spot caused by *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) on hot pepper predicts the primary disease infection date (Kim et al., 2014). The model is based on population densities of the pathogen on a host, which are calculated from one bacterium at the beginning on a leaf. Hourly temperature, relative humidity, and precipitation during the growing season are the input variables for the model simulation. Epiphytic populations of *Xcv* increase or decrease depending on wetness condition. In order to successfully infect, three requirements must be met: pathogens densities have to be above 5×10^3 cells/ml, more than 4 mm/h of rain and wind speed greater than 3 m/s. The pathogen population inside the leaf starts to colonize from 1/100 times of the epiphytic population at the time of entering and its growth inside the leaf depends on temperature. When population of *Xcv* inside the host reaches 10^{15} cells/ml, the model warns the timing of the first infection date (Figure 8).

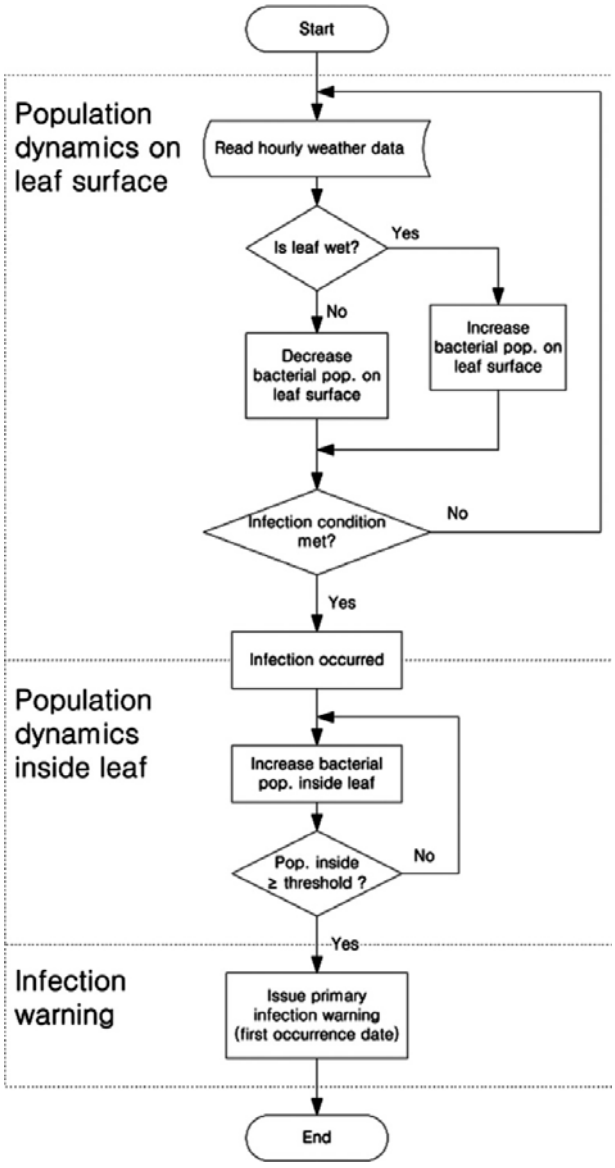

Figure 8. Scheme of the forecasting model of bacterial spot caused by *Xanthomonas campestris* pv. *vesicatoria* on hot pepper (Kim et al., 2014).

3.2.5 Infection risk model for bacterial canker of kiwifruit

The prediction model for bacterial canker of kiwifruit, caused by *Pseudomonas syringae* pv. *actinidiae* (*Psa*), was developed using the mechanistic scheme composed of the multiplication and the dispersal concepts (Beresford et al., 2017). Bacterial multiplication is estimated from a temperature function, the *M* index, accumulated from hourly air temperature over 3 days for hours when the leaf canopy is wet. Rainfall provides free water to move inoculum to infection sites, and the daily risk indicator, the *R* index, is the 3-day accumulation of the *M* index output on days with total rainfall > 1 mm; otherwise, *R* is zero (Figure 9).

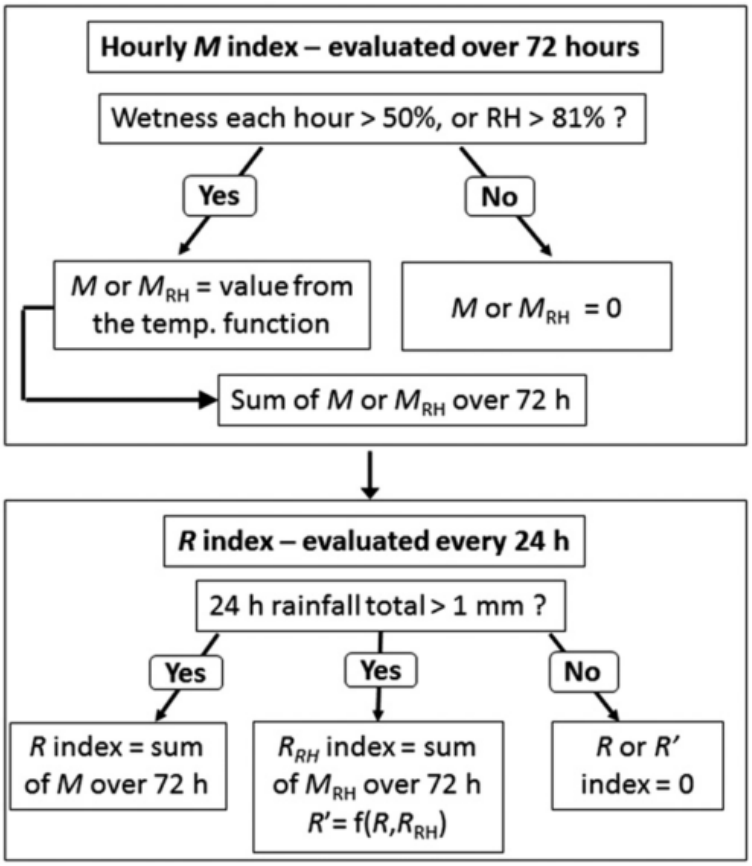

Figure 9. Scheme for the *Pseudomonas syringae* pv. *actinidiae* risk model on kiwifruit. Calculation of the *M* index from hourly temperature and surface wetness data over 3 days and the *R* index from daily rainfall data. The M_{RH} index and *R'* indexes are calculated using hourly relative humidity (RH) and were used for implementing the model using weather forecasts (Beresford et al., 2017).

4. Context and approach of this PhD thesis

The current trends of crop protection have been reoriented to a rational use of pesticides and to a reduction of the number of registered active ingredients to those certainly unavoidable, more selective, less toxic and with a lower negative environmental impact (Montesinos, 2003; Montesinos and Bonaterra, 2009). In this context, the interest in accurate timing of pesticide applications for optimizing their effectiveness and reducing the number of applications has increased. The use of plant disease forecasting models as part of DSS has an important role for a rational use of pesticides, since risks, costs and benefits of each of numerous decisions are weighted according to model predictions to determine when control measures are warranted (Gent et al., 2011). As Campbell and Madden (1990) pointed out, forecasting models would become more valuable to producers as the cost of pesticides and regulatory constrains increase.

The research of the Plant Pathology group of the University of Girona has been focused on sustainable management of plant diseases since 1993 by the selection or development of biological control agents, design and development of new compounds such as antimicrobial peptides, and development and evaluation of forecasting models for different pathogens. An infection model for brown spot of pear caused by the fungus *Stemphylium vesicarium* (BSPcast) was developed based on temperature and wetness duration (Montesinos et al., 1995), which was evaluated in reduced fungicide programs for the management of the disease (Llorente et al., 2000). Currently, BSPcast is used by the Plant Health Service of Catalunya (Spain) (https://www.ruralcat.net/) and Emilia-Romagna Region (Italy) (http://agricoltura.regione.emilia-romagna.it/) to provide daily forecasts on-line using meteorological data from the national weather station network (Servei Meteorològic de Catalunya and Servizio Idro-Meteo-Clima di l'Agenzia regionale per la prevenzione e l'ambiente dell'Emilia-Romagna, respectively). Moreover, PAMcast model (*Pleospora allii* maturation forecast) was developed to establish the relationship between the environmental conditions and the maturity of pseudothecia of *P. allii* (teleomorph of *Stemphylium vesicarium*) (Llorente and Montesinos, 2004). On the other hand, an infection model for the bacterium *X. arboricola* pv. *juglandis* was developed for the Mediterranean basin and tested under field conditions in collaboration with IRTA (Catalunya) and CTIFL (France) (Ninot et al., 2002).

Recently, the forecasting model for bacterial blight of walnut has been implemented in a fruit grower platform (Hespèrides, Click S.C.C.L.) as part of a DSS for the management of the disease. Furthermore, the experience of the group in forecasting models also includes working with existing models. For example, epidemiological studies of fire blight in Spain were performed, producing weather-based risk maps using Billing's Revised System (BRS), Billing's Integrated System (BIS95), Powell Model, Maryblyt and CougarBlight (Llorente et al., 2002, 2017; Ruz, 2005); and a control strategy of apple scab in Catalonia (Spain) has been evaluated using Mills and RIMpro models to predict the release of ascospores of *Venturia inaequalis* (Vilardell et al., 2012).

According to the background in forecasting models of the Plant Pathology research group, the present thesis is focused on developing a forecasting system for *X. arboricola* pv*. pruni*.

Firstly, the essential steps/processes on the disease cycle for disease development were identified and modeled (when possible): the multiplication of epiphytic bacteria to produce inoculum, the infection process and symptom development. The study of the pathogen-host-environment interaction in each process of the disease cycle was studied separately and organized in different chapters in this thesis. The operational scheme of the forecasting system was conceptualized as being based on these processes, resulting in three components included in a global system (Figure 10). Moreover, it is important to define how these components will be linked to make predictions when working together.

Since the *X. arboricola* pv. *pruni* is a quarantine pathogen in the EU (Council Directive 2000/29/EC), the applications of the disease forecaster are twofold: guidance of scouting tasks in disease surveillance and scheduling pesticide treatments. Risk maps can be a very useful tool in disease surveillance to locate geographically areas most at risk by the invasion of the disease, where the prospections of symptoms may be intensified (Magarey et al., 2007; Sparks, 2009). Thus, disease outbreaks may be detected rapidly and protective measures can be adopted to avoid the spread of the disease. On the other hand, the forecasting system can be used as part of a DSS to determine when pesticide applications are warranted according to the model predictions.

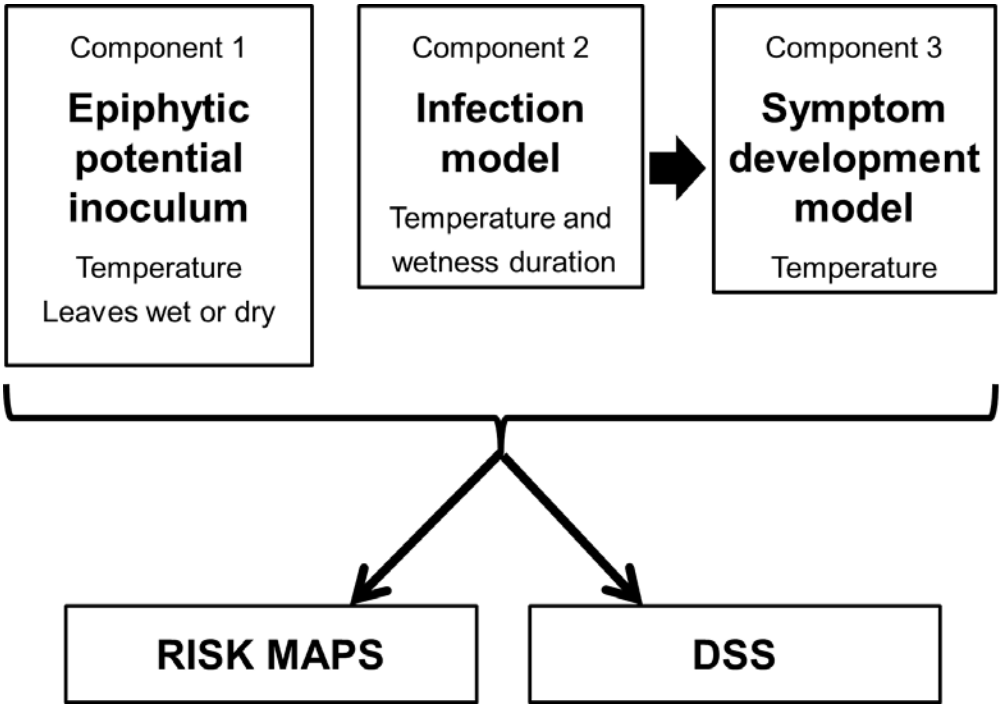

Figure 10. Conceptualization of the forecasting system for bacterial spot of stone fruit caused by *Xanthomonas arboricola* pv. *pruni*.

Chapter 2:

Objectives

The main objective of this PhD thesis was to develop a forecasting system for bacterial spot disease of stone fruits. The specific objectives were:

1. To develop a mathematical model for predicting *X. arboricola* pv. *pruni* growth *in vitro* as a function of temperature.
2. To quantify the effects of temperature and leaf wetness duration on the infection of *Prunus* by *X. arboricola* pv. *pruni*; develop a model describing these effects; and to evaluate the capacity of this model to forecast bacterial infection risk.
3. To determine the effect of temperature and relative humidity on *X. arboricola* pv. *pruni* growth on *Prunus* leaf surface; to evaluate the effect of inoculum density of *X. arboricola* pv. *pruni* on infection on *Prunus* leaves; and to analyze the effect of temperature and inoculum density on disease progress and symptom development.

Following the same order as the objectives, the research developed in this thesis is organized into the following chapters:

Chapter 3. A model for predicting *Xanthomonas arboricola* pv. *pruni* growth as a function of temperature

Chapter 4. Effects of leaf wetness duration and temperature on the infection of *Prunus* by *Xanthomonas arboricola* pv. *pruni*

Chapter 5. Environmental and inoculum effects on epidemiology of bacterial spot disease of stone fruits

Chapter 3:

A model for predicting *Xanthomonas arboricola* pv. *pruni* growth as a function of temperature

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RESEARCH ARTICLE

A model for predicting *Xanthomonas arboricola* pv. *pruni* growth as a function of temperature

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Abstract

A two-step modeling approach was used for predicting the effect of temperature on the growth of *Xanthomonas arboricola* pv. *pruni*, causal agent of bacterial spot disease of stone fruit. The *in vitro* growth of seven strains was monitored at temperatures from 5 to 35˚C with a Bioscreen C system, and a calibrating equation was generated for converting optical densities to viable counts. In primary modeling, Baranyi, Buchanan, and modified Gompertz equations were fitted to viable count growth curves over the entire temperature range. The modified Gompertz model showed the best fit to the data, and it was selected to estimate the bacterial growth parameters at each temperature. Secondary modeling of maximum specific growth rate as a function of temperature was performed by using the Ratkowsky model and its variations. The modified Ratkowsky model showed the best goodness of fit to maximum specific growth rate estimates, and it was validated successfully for the seven strains at four additional temperatures. The model generated in this work will be used for predicting temperature-based *Xanthomonas arboricola* pv. *pruni* growth rate and derived potential daily doublings, and included as the inoculum potential component of a bacterial spot of stone fruit disease forecaster.

Introduction

Xanthomonas arboricola pv. *pruni* [1, 2] is the causal agent of bacterial spot disease of stone fruit [3, 4], which is one of the most important diseases of *Prunus* species and their hybrids. Although it can affect all cultivated *Prunus*, the most severely affected hosts are peach, nectarine, apricot, and plum [5]. Recently, the disease has been reported on almond [6] and on cherry laurel [7, 8] in Europe. Disease symptoms include lesions on leaves, twigs, fruit, and stem cankers [9, 10]. Affected fruits are unmarketable, and infected trees may show severe defoliation with significant yield and quality losses [11].

The disease was first described in North America in 1903 [1], and currently, it is distributed throughout the major stone-fruit-producing areas worldwide [9]. The bacterium, considered a quarantine organism by the phytosanitary legislation of the European Union [12] and the European and Mediterranean Plant Protection Organization [3], has become locally established in

from MECD (FPU13/04123; http://www.mecd.gob.es) from Spain.

Competing interests: The authors have declared that no competing interests exist.

several European countries, and it is currently spreading in many others, where local outbreaks have been reported [13, 14].

Disease control is based on preventive applications of copper-based bactericides and antibiotics [15]. Copper sprays applied early in the growing season have moderate efficacy [16] and have been associated with plant phytotoxicity [17]. Information on the most effective system for their application is limited, and copper sprays are not always applied at the appropriate time, when environmental conditions are favorable for bacterial infections. The disease is strongly influenced by the weather variables, mainly temperature, rain, and wetness [18, 19]. A disease forecasting model based on weather conditions could be used as a support system to guide pesticide applications [5] and increase disease control efficacy.

The pathogen population density is considered a critical parameter to forecast bacterial plant disease development [20–22]. Most forecasters of bacterial diseases are based on temperature-dependent bacterial multiplication in the presence of moisture to provide inoculum for infections, and on occurrence of weather conditions suitable for infections. Potential daily doublings of the associated pathogen are estimated from *in vitro* specific growth rates at different temperatures under optimal relative humidity conditions [20, 23, 24]. The optimum temperature and doubling times of *X. arboricola* pv. *pruni* were determined in a previous work [25], but no empirical models have been developed for predicting its growth as a function of temperature.

Predictive modeling has been widely used to estimate the growth of foodborne bacteria under different physical and chemical conditions [26–29] and to optimize the design and operation of bioreactors [30]. Primary and secondary models are used for predicting bacterial growth [28, 31]. Primary models describe the changes in population size over time under isothermal conditions [32]. Sigmoidal models, such as the modified Gompertz model [33], Baranyi model [30], and Buchanan model [34], can be used to fit bacterial growth to the three kinetic phases: the lag phase, exponential growth phase, and stationary phase. Secondary models are constructed to describe the growth parameters obtained from primary models as a function of independent variables, such as temperature [32].

In vitro bacterial growth can be expressed in terms of microbial density or optical density as an indirect measurement. Optical density measurements are less time consuming and allow for monitoring of bacterial population growth in real time. There are some limitations to directly fitting primary growth models to the optical density measurements [35, 36]. The use of calibration factors [24, 36] and calibration curves to recalculate the optical density data to viable count data [37, 38] have been proposed to correct the non-linearity of absorbance measurements.

The aim of this work was to develop a mathematical model for predicting *X. arboricola* pv. *pruni* growth *in vitro* as a function of temperature. Prior to growth curve modeling, the relationship between optical density and viable count data was analyzed, and a calibration curve was developed. Primary models were used to estimate the growth kinetic parameters, namely the maximum specific growth rate (μ_{max}) and the lag time, under isothermal conditions within a temperature range from 5 to 35˚C. Afterwards, aiming at the description of the estimated growth rate as a function of temperature, secondary models were developed and validated.

Materials and methods

Bacterial strains

Seven strains of *X. arboricola* pv. *pruni* selected from different host plant species and geographic region were used (Table 1). The strains were routinely grown at 27˚C on Yeast-

Table 1. *Xanthomonas arboricola* pv. *pruni* strains used in the study.

Strain [y]	Host	Geographic region
CFBP 3894 [z]	*Prunus salicina*	New Zealand
CFBP 3903	*Prunus domestica*	Italy
CFBP 5530	*Prunus persica*	Italy
CFBP 5563	*Prunus persica*	France
CFBP 5725	*Prunus persica*	EUA
IVIA 33	*Prunus amygdalus*	Spain
IVIA 3162–1	*Prunus amygdalus*	Spain

[y] CFBP: Collection Fraînçaise de Bactéries Phytopatogènes (Angers, France); IVIA: Instituto Valenciano de Investigaciones Agrarias (Moncada-Valencia, Spain).

[z] Pathotype strain

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Peptone-Glucose Agar (YPGA) [16] and maintained at -70˚C in YPG broth [16] supplemented with glycerol (20% wt/vol).

Relationship between optical density and viable cell count

The *in vitro* growth of *X. arboricola* pv. *pruni* was modeled from continuous absorbance measurements taken with an automated turbidimetric system (Bioscreen C, Labsystem, Helsinki, Finland). A calibration curve was generated to recalculate the optical density data to viable count data. The seven strains of *X. arboricola* pv. *pruni* listed in Table 1 were used to obtain the calibration curve. Bacterial suspensions in sterile distilled water were prepared individually for each strain from cultures grown on Luria-Bertani (LB) agar [39] for two days at 27˚C. Suspensions were adjusted to 1–5 x 10^8 CFU/ml, corresponding to an optical density at 600 nm of 0.5 [40]. LB was used instead of YPGA to reduce xanthan production. In order to evaluate a wider range of optical densities and bacterial concentrations, the bacterial suspensions were 10- and 100-fold diluted in sterile distilled water, and the initial concentrations of 10^6, 10^7, and 10^8 CFU/ml were used. Honeycomb 100-well microplates were filled with 180 μl LB broth and inoculated with 20 μl of the corresponding bacterial suspension. Negative controls were inoculated with 20 μl sterile distilled water. Six wells were used per strain and concentration. The inoculated multiwell plates were incubated in the Bioscreen C system at 25˚C for 60 h. Optical density measurements at 600 nm were performed every 60 min with 10 s of shaking prior to reading. At 1, 12, 24, 36, 48, and 60 h of incubation, after the optical density reading, 100 μl of the bacterial suspensions were removed from the corresponding wells for each strain and initial concentration, and appropriate 10-fold serial dilutions were plated in duplicate onto YPGA medium and incubated for three days at 27˚C to determine the viable count. After removing the bacterial suspension, the wells were excluded from further analysis. The optical densities, corrected with the values from the negative controls, and corresponding $\log_{10}$-transformed data of the viable counts were used to fit the regression curves. The Beer-Lambert, quadratic [41], cubic [42], and logarithmic [43] equations were fitted to the data (equations 1, 2, 3, and 4, Table 2) using SPSS v. 23.0 software (IBM Corp., Armonk, NY). The *R*-squared, adjusted *R*-squared, and the number of parameters in the model were the criteria used to select the equation for the calibration curve.

Bacterial growth

Bacterial suspensions (1–5 x 10^8 CFU/ml) of seven *X. arboricola* pv. *pruni* strains (Table 1) were prepared as described previously and 10-fold diluted in sterile distilled water. The wells

Table 2. Equations of models used in the study.

Model	Equation [z]	
Beer-Lambert	$\log_{10} N = a + b \cdot OD$	(1)
Quadratic	$\log_{10} N = a + b \cdot OD + c \cdot OD^2$	(2)
Cubic	$\log_{10} N = a + b \cdot OD + c \cdot OD^2 + d \cdot OD^3$	(3)
Logarithmic	$\log_{10} N = a + b \cdot \ln OD$	(4)
Baranyi	$\log_{10}N_t = \log_{10}N_{max} + \log_{10}\left(\frac{-1+\exp(\mu_{max}\cdot lag)+\exp(\mu_{max}\cdot t)}{\exp(\mu_{max}\cdot t)}\right) - 1 + \exp(\mu_{max}\cdot lag)\cdot 10^{A}$	(5)
Buchanan	Lag phase: for $t \leq t_{lag}$, $N_t = N_0$ Exponential growth phase: for $t_{lag} < t < t_{max}$, $N_t = N_0 + \mu(t - t_{lag})$ Stationary phase: for $t \geq t_{max}$, $N_t = N_{max}$	(6)
Modified Gompertz	$\log_{10}N_t = \log_{10}N_0 + A\cdot\exp\{-\exp[\frac{\mu_{max}\cdot e}{A}\cdot(lag - t) + 1]\}$	(7)
Ratkowsky	$\mu_{max} = [b\,(T - T_{min})]^2$	(8)
Modified Ratkowsky	$\mu_{max} = (b\,(T - T_{min})\cdot\{1 - \exp[c\,(T - T_{max})]\})^2$	(9)
Modified Ratkowsky	$\mu_{max} = [b\,(T - T_{min})]^2\cdot\{1 - \exp[c\,(T - T_{max})]\}$	(10)

[z] A: logarithmic increase of bacterial population $\log_{10}$ (CFU/ml); e: exp(1); lag: lag time (h); N: cell concentration; N_0 and N_{max}: initial and final population densities, respectively (CFU/ml); N_t: population density at time t (CFU/ml); OD: optical density; t: time (h) in logistic models; t_{lag}: time to the end of lag phase (h); t_{max}: time when the maximum population density is reached (h); T: temperature (˚C); T_{min} and T_{max}: minimum and maximum temperatures, respectively, at which the specific growth rate is zero; μ: specific growth rate in Buchanan model (h^{-1}); μ_{max}: maximum specific growth rate (h^{-1}).

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of 100-well Honeycomb plates were filled with 180 μl LB broth and inoculated with 20 μl of a 1–5 x 10^7 CFU/ml bacterial suspension, so that the initial bacterial concentration in each well was 1–5 x 10^6 CFU/ml. Water activity (a_w) of initial bacterial suspensions in LB broth ranged from 0.974 to 0.976, similar to LB broth (a_w = 0.975). Measurements of a_w were performed for all strains using a Novasina LabMaster-aw device (Novasina AG, Lachen, Switzerland). For each incubation temperature, three replicates of three wells were inoculated with each strain separately. Wells inoculated with 20 μl distilled water were used as blanks. The inoculated plates were incubated in the Bioscreen C system (Labsystem, Helsinki, Finland) at 15, 20, 25, 30, 33, 34 and 35˚C for five days. Measurements were performed every 60 min with 10 s of shaking prior to the optical density reading at 600 nm. Incubations at 5 and 10˚C were conducted in a growth chamber (MLR-350 Growth Cabinet, SANYO, Japan) for ten days and optical density measurements were performed twice a day by placing plates in the Bioscreen C system maintained at 15˚C in a cold room to avoid problems with establishing temperature and optical density stability. The experiment was repeated twice.

The optical density values at each time point of inoculated wells were corrected with the optical densities of blanks. OD data at each time point of three wells in a replicate were averaged prior to data analysis. The calibration curve obtained previously was used to recalculate the optical density data to viable count data. Growth curves were obtained by plotting $\log_{10}$ (viable count) against the incubation time. Three growth curves were generated per strain and temperature in each of two independent experiments.

Primary modeling of *X. arboricola* pv. *pruni* growth

X. arboricola pv. *pruni* growth *in vitro* at each temperature was modeled using primary models. Six viable cell count growth curves were used per strain to estimate the growth parameters at

each temperature. A total of forty two growth curves were modelled per temperature. The Baranyi [30], Buchanan [34], and modified Gompertz [33] models (equations 5, 6, and 7, Table 2) were fitted to the growth curves under isothermal conditions by nonlinear regression using R (R Development Core Team 2015) package nlstools [44]. The model with the lowest residual sum of squares (RSS) was selected to estimate the maximum specific growth rate (μ_{max}) and lag time for *X. arboricola* pv. *pruni* at each temperature. The doubling time (DT) was calculated as DT = ln 2 / μ_{max}. The effects of strain, experiment, and temperature on the specific growth rate were determined using the general linear models (GLM) procedure after confirmation of normality and homoscedasticity, and mean comparison was performed with Tukey's HSD test. The non-parametric test procedure for independent samples was used for determining the effects of experiment and strain on the lag time using the Mann-Whitney U and Kruskal-Wallis test, respectively. Arrhenius plot on the logarithm of the specific growth rate and reciprocal temperature was drawn and regions with significant linearity were detected by linear regression.

Secondary modeling of *X. arboricola* pv. *pruni* growth

Secondary models were used to describe changes in the maximum specific growth rate as a function of temperature. The square root model Ratkowsky and its variations (equations 8, 9 and 10, Table 2) [28, 45, 46], were fitted to the averaged maximum specific growth rates of the *X. arboricola* pv. *pruni* strains derived from the primary models by nonlinear regression. The average of maximum specific growth rates over experiments and strains was used for secondary modeling to reduce data variability and improve curve fitting [47], since no differences were observed among strains and between experiments at any temperature ($P > 0.057$). The criteria for choosing the best model were the minimum RSS and the lowest number of parameters in the model. Furthermore, doubling times for *X. arboricola* pv. *pruni* at different temperatures reported in the literature [25] were back transformed to specific growth rates and the modified Ratkowsky equation 10 (Table 2) was fitted to the data as described previously. The optimum temperature for *X. arboricola* pv. *pruni* growth (at which the growth rate is maximum) was determined by equaling the first derivative of modified Ratkowsky model to zero.

Validation of *X. arboricola* pv. *pruni* growth models

The model obtained for predicting the maximum specific growth rate of *X. arboricola* pv. *pruni* as a function of temperature was validated using data derived from four additional experiments at temperatures of 17, 22, 27, and 31˚C for the seven strains separately (Table 1). Assays were performed as described previously. Briefly, three replicates of three wells per replicate filled with 180 ml LB were inoculated with 20 **μ**l 1–5 x 10^7 CFU/ml suspensions of each strain and incubated at the corresponding temperature for three to five days in the Bioscreen C system (Labsystem, Helsinki, Finland). Optical density (600 nm) measurements were performed hourly and transformed to viable count values using the calibration curve obtained earlier. Averaged data of three wells in a replicate at each reading time were used in data analysis. A total of twenty-one growth curves were generated, three per strain and temperature. The modified Gompertz model (equation 7, Table 2) was used to estimate the observed maximum specific growth rate for each growth curve. The predicted specific growth rates at the new tested temperatures were calculated with the modified Ratkowsky model developed earlier. The correlation between observed and predicted data, and the performance indices of bias factor (Bf) and accuracy factor (Af) [48], were used for evaluating the performance of the constructed predictive models. A perfect consistency in a predictive model would have Bf = Af = 1. Bf values ranging from 0.9 to 1.05 are considered good, the range of 0.7–0.9 or 1.06–1.15 is

considered acceptable, and either < 0.7 or > 1.15 is considered unacceptable [31]. Otherwise, the Af will increase by 0.1 to 0.15 for each predictive variable. In this study, the acceptable range for Af was less than 1.15 because only one environmental factor was employed.

Results

Relationship between optical density and viable cell count

Individual data pairs were pooled to analyze the relationship between optical density and viable count in *X. arboricola* pv. *pruni*. In total, 126 data points were used to generate a unique calibration curve. Optical densities corrected with blank values ranged from 0.001 to 0.711 for bacterial densities from 5.40 to 9.36 $\log_{10}$ (CFU/ml) (Fig 1). A large increase in viable counts but low variation in optical density was observed at optical densities below 0.07, whereas increases in optical densities from 0.2 to 0.8 were related to viable count increases from 8 to 9.36 $\log_{10}$ CFU/ml (Fig 1). Beer-Lambert (linear), quadratic, cubic, and logarithmic models (equations 1, 2, 3 and 4, respectively, Table 2) were fitted to the data for the entire data range. The regression analysis results and parameter estimates are summarized in Table 3. The linear (equation 1) and quadratic (equation 2) models gave the poorest fits to the data, whereas the best fits to the data were achieved with the cubic (equation 3) and logarithmic (equation 4) models (Table 3). A model accepted statistically with as few parameters as possible was the

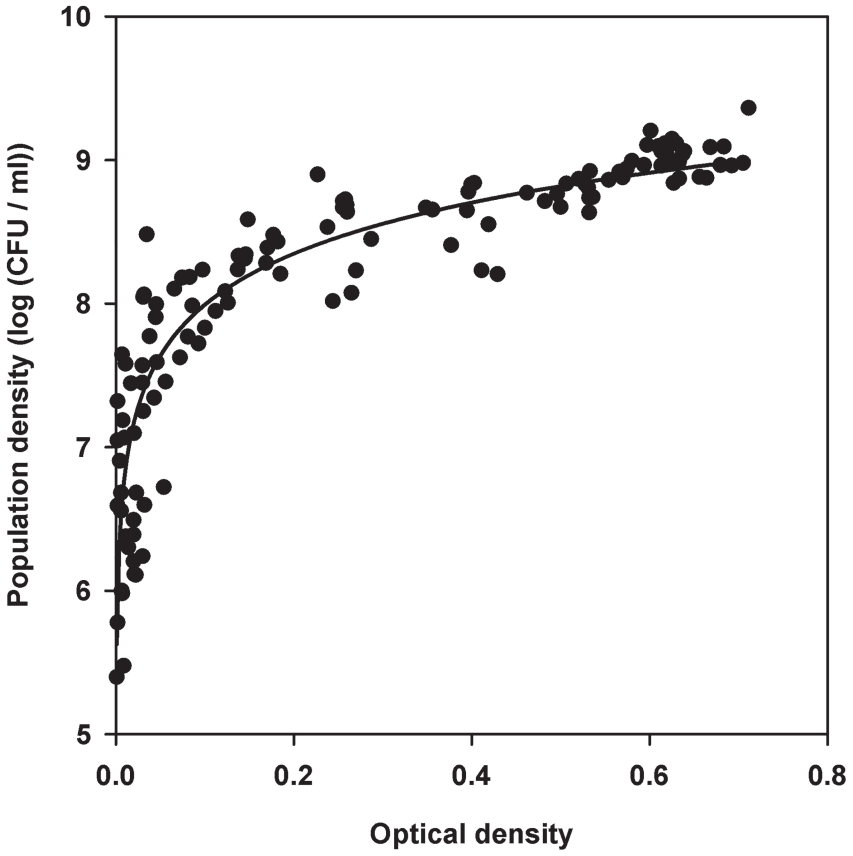

Fig 1. Relationship between population density and optical density at 600 nm for *X. arboricola* pv. *pruni*. Data from suspensions of seven strains incubated at 25˚C were used. The curve generated by the logarithmic model is shown.

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Table 3. Regression analysis between optical density at 600 nm and viable count for *X. arboricola* pv. *pruni* with different models.

Model [x]	Model Summary [y]					Parameter Estimate [z]			
	R^2	R^2_{adj}	*F*	df1	df2	a	b	c	d
Beer-Lambert (1)	0.660	0.657	240.57	1	124	7.20 (0.08)	3.14 (0.20)		
Logarithmic (2)	0.812	0.810	534.02	1	124	9.17 (0.06)	0.51 (0.02)		
Quadratic (3)	0.757	0.753	191.53	2	123	6.88 (0.08)	8.27 (0.75)	-7.95 (1.13)	
Cubic (4)	0.811	0.807	174.44	3	122	6.63 (0.08)	16.13 (1.49)	-40.01 (5.52)	32.17 (5.45)

[x] Model equations are displayed in Table 2.

[y] All model *F* values were highly significant ($P < 0.0001$). df1: regression effective degrees of freedom; df2: residual effective degrees of freedom.

[z] Models were fitted to 126 data points using linear regression analysis. Standard errors are reported in parentheses.

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criterion for selecting the logarithmic model for generating the calibration curve, for which the equation was $log_{10} N = 9.17 + 0.51\ ln\ OD$ (Fig 1), where *OD* is the optical density at 600 nm and *N* is the population density (CFU/ml). This equation was further used to transform optical densities to viable counts.

Modeling bacterial growth

Optical densities obtained from the Bioscreen C system were transformed to viable counts using the calibration curve generated in this study, as described above. In total, 189 viable count growth curves were obtained per experiment, corresponding to three replicates of seven strains per temperature. All *X. arboricola* pv. *pruni* strains were able to grow *in vitro* at temperatures from above 5 to 34˚C. No growth was observed for the first five days of incubation at 5˚C and longer incubation durations were needed to detect bacterial growth at this temperature, which could be considered the minimum for *X. arboricola* pv. *pruni* multiplication. Growth at 35˚C was variable, depending on the strain. The type strain CFBP 3894 was unable to grow at 35˚C, and only 24 out of 42 growth curves for the other strains showed growth at 35˚C and were included in the analysis.

The Baranyi, Buchanan, and modified Gompertz models (equations 5, 6, and 7, Table 2) were fitted to the 189 log_{10}-transformed viable count growth curves of each experiment by nonlinear regression to estimate the growth parameters for each *X. arboricola* pv. *pruni* strain and temperature replicate. Model fitting to one growth curve of *X. arboricola* pv. *pruni* strain CFBP 5530 at 25˚C is shown in Fig 2, where the RSS values of the Baranyi, modified Gompertz and Buchanan models are shown and representative of all datasets. The modified Gompertz model (equation 7, Table 2) showed the best fit to the data in all data sets, with the lowest RSS (data not shown), and was selected to estimate the maximum specific growth rate and lag time of *X. arboricola* pv. *pruni* strains at each temperature. The *F*-test results indicated no significant differences in the maximum specific growth rates between the two independent experiments ($P = 0.1848$). Similarly, the maximum specific growth rates were not significantly different among strains ($P > 0.057$) at any temperature. Therefore, the maximum specific growth rates were pooled over experiments and strains and averaged data were used in secondary modeling.

The means of the maximum specific growth rate and doubling time at each temperature are presented in Table 4. A significant effect of temperature on the maximum specific growth rate ($P < 0.001$) was observed, and differences among temperatures were detected according to

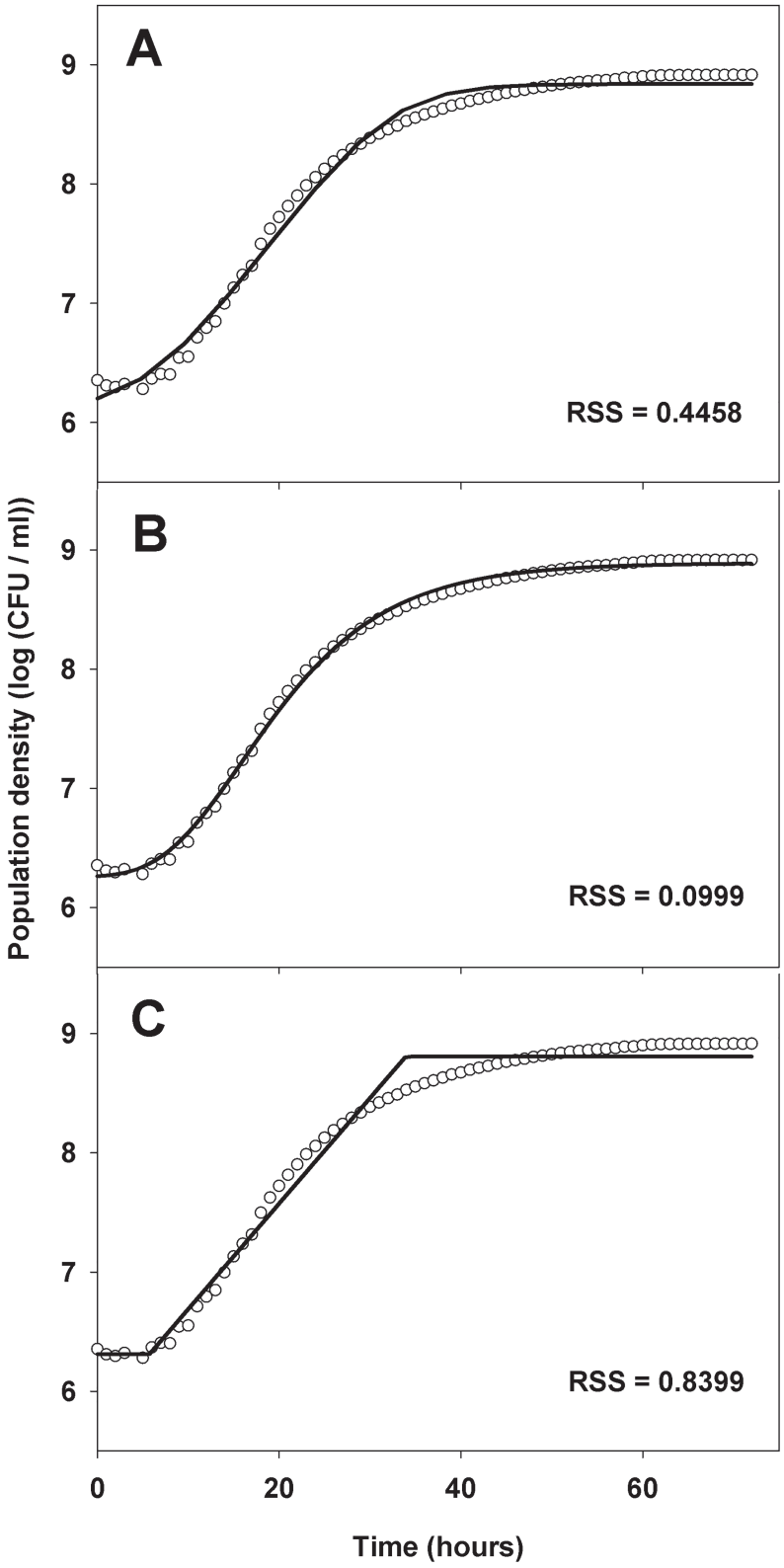

Fig 2. Primary model fitting to one of the six experimental growth curves for *X. arboricola* pv. *pruni* strain CFBP 5530 at 25˚C. (A) Baranyi, (B) Gompertz modified, and (C) Buchanan models were fitted to experimental data. The residual sum of squares (RSS) for each model is reported.

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Table 4. Growth parameters and corresponding standard error for *X. arboricola* pv. *pruni* at different temperatures (T) estimated by the modified Gompertz model.

T (˚C)	Maximum specific growth rate (h^{-1}) [x]		Doubling time (h)	Lag time (h) [x]
5	0.033 ± 0.002	d [y]	20.69 ± 1.20	92.27 ± 4.33
10	0.059 ± 0.004	d	11.74 ± 0.76	37.22 ± 2.19
15	0.128 ± 0.006	c	5.41 ± 0.26	26.29 ± 1.57
20	0.162 ± 0.006	c	4.28 ± 0.16	5.67 ± 0.62
25	0.228 ± 0.010	ab	3.03 ± 0.14	6.58 ± 0.67
30	0.252 ± 0.009	a	2.75 ± 0.10	2.49 ± 0.34
33	0.225 ± 0.006	ab	2.86 ± 0.08	2.38 ± 0.37
34	0.209 ± 0.007	b	3.08 ± 0.11	7.72 ± 1.29
35 [z]	0.138 ± 0.006	c	3.31 ± 0.24	33.68 ± 4.98

[x] Values are the mean of parameter estimates from the modified Gompertz equations obtained for 42 growth curves at each temperature, corresponding to seven strains and three replicates per strain in two independent experiments.

[y] Means within the same column followed by the same letter do not differ significantly ($P = 0.05$) according to the Tukey's HDS mean comparison test.

[z] Growth at 35˚C was variable. Only data from strains that were able to grow at 35˚C were included.

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Tukey's HSD test (Table 4). The maximum specific growth rate of *X. arboricola* pv. *pruni* increased progressively when the temperature increased from 5 to 30˚C, and decreased at temperatures above 30˚C. The highest growth rates were obtained at temperatures from 25 to 33˚C, whereas the significantly lowest growth rates were observed at 5 and 10˚C (Table 4). The Arrhenius plot in Fig 3 shows the relationship between the logarithm of the maximum specific growth rate and temperature. The curve is characterized by a continuously changing slope between the minimum (5 to 15˚C), medium (15 to 30˚C), and maximum (30 to 35˚C) temperatures. Regions of linearity can be observed corresponding to these three intervals of temperature, but not over the entire range. Linear equations obtained for each region are displayed (Fig 3).

The negative lag times estimated from the modified Gompertz model were assumed to be 0. No significant differences ($P = 0.072$) were observed in lag times between experiments at all temperatures, except for 5, 15, and 25˚C, according to the Mann-Whitney U test. The results from the Kruskal-Wallis test indicated no significant differences in lag time among strains at all temperatures ($P = 0.079$), except for 35˚C ($P = 0.017$). Mean lag time values of seven strains at each temperature are presented in Table 4. The mean of the lag time decreased when temperature increased from 5˚C to 33˚C. Above 33˚C the lag time increased to a maximum at 35˚C (Table 4).

In the second step of modeling, the Ratkowsky equation and its modifications (equations 8, 9 and 10, Table 2), were fitted to modified Gompertz estimates of the specific growth rates at the tested temperatures. Non-linear regressions were based on averaged data for the specific growth rate for each temperature presented in Table 4. The parameter estimates and statistical analysis are presented in Table 5. The Ratkowsky model (equation 8) did not fit well to the data (Table 5 and Fig 4). The modified Ratkowsky equations 9 and 10 described bacterial growth over the entire temperature range and were found to be more appropriate (Table 5). The minimum and maximum temperatures estimated from each model are presented in Table 5. The maximum temperatures from the modified Ratkowsky models were close to 35˚C, the highest temperature at which some strains of *X. arboricola* pv. *pruni* were able to growth, whereas the minimum temperature estimates were lower than the minimal temperature tested in this work and do not have biological meaning (Table 5). The modified Ratkowsky equation 10 had the lowest RSS and the highest adjusted *R*-squared (Table 5). Therefore, this model was chosen for

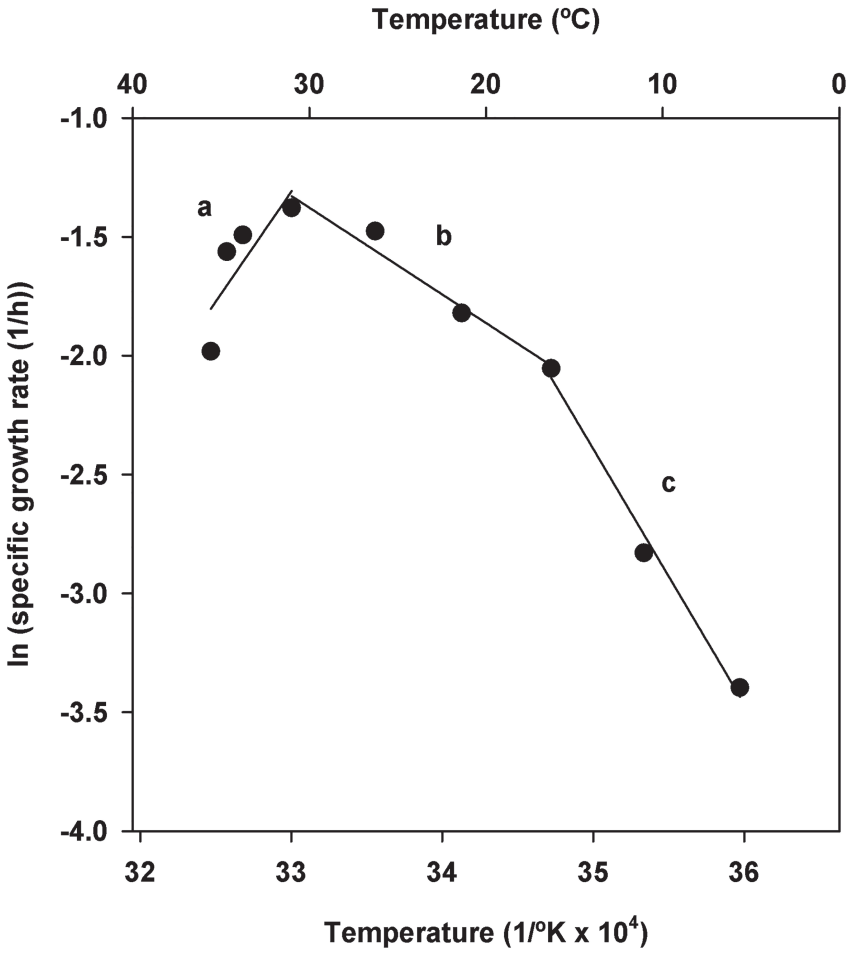

Fig 3. Arrhenius plot of the maximum specific growth rates for *X. arboricola* pv. *pruni*. Lines show three linear regions: (a) $ln(\mu_{max}) = 0.92T - 31.81$ ($R^2 = 0.66$); (b) $ln(\mu_{max}) = -0.41T + 12.35$ ($R^2 = 0.97$); and (c) $ln(\mu_{max}) = -1.08T + 35.18$ ($R^2 = 0.99$). Where T is 1/°K x 10^4.

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predicting the *in vitro* specific growth rate of *X. arboricola* pv. *pruni* as a function of temperature. The optimal temperature for *X. arboricola* pv. *pruni* growth *in vitro* derived from this equation was 29.97˚C, with a specific growth rate of 0.26 h^{-1} and a doubling time of 2.66 h. These values agreed with experimental data (Table 4).

In addition, the modified Ratkowsky equation 10 (Table 2) fitted well to specific growth rates at different temperatures (T) reported for *X. arboricola* pv. *pruni* in the literature [25] (Fig 4). The equation obtained was: $\mu_{max} = (0.0233 * (T—(-0.8714)))^2 * (1—exp(0.2801 * (T—36.8614)))$ (RSS = 0.0093 and adjusted R-squared = 0.9717). The optimal temperature for *X. arboricola* pv. *pruni* growth estimated from this equation was 30.82˚C.

Model validation

The predictive model based on the modified Ratkowsky equation 10 (Table 5) was evaluated at four new temperatures (17, 22, 27 and 31˚C) for the seven strains. No significant differences ($P < 0.001$) were observed among strains in the maximum specific growth rate derived from the modified Gompertz model at each temperature. Table 6 shows the averaged maximum

Table 5. Parameter estimation and statistical evaluation for the secondary models describing the maximum specific growth rate for *X. arboricola* pv. *pruni* as a function of temperature.

Model parameter and statistics	Maximum specific growth rate models [y]		
	Ratkowsky (8)	Modified Ratkowsky (9)	Modified Ratkowsky (10)
b	0.007 (0.002) [z]	0.014 (0.001)	0.015 (0.002)
c	-	0.332 (0.087)	0.270 (0.075)
Tmin	-35.15 (21.54)	-8.27 (2.78)	-7.77 (2.64)
Tmax	-	37.86 (0.71)	36.67 (0.41)
RSS	0.0187	0.0008	0.0007
R^2_{adj}	0.5512	0.9727	0.9766

[y] Equations of Ratkowsky model and its variations are listed in Table 2. Equation number is shown in parentheses.

[z] Standard error of estimates are reported in parentheses.

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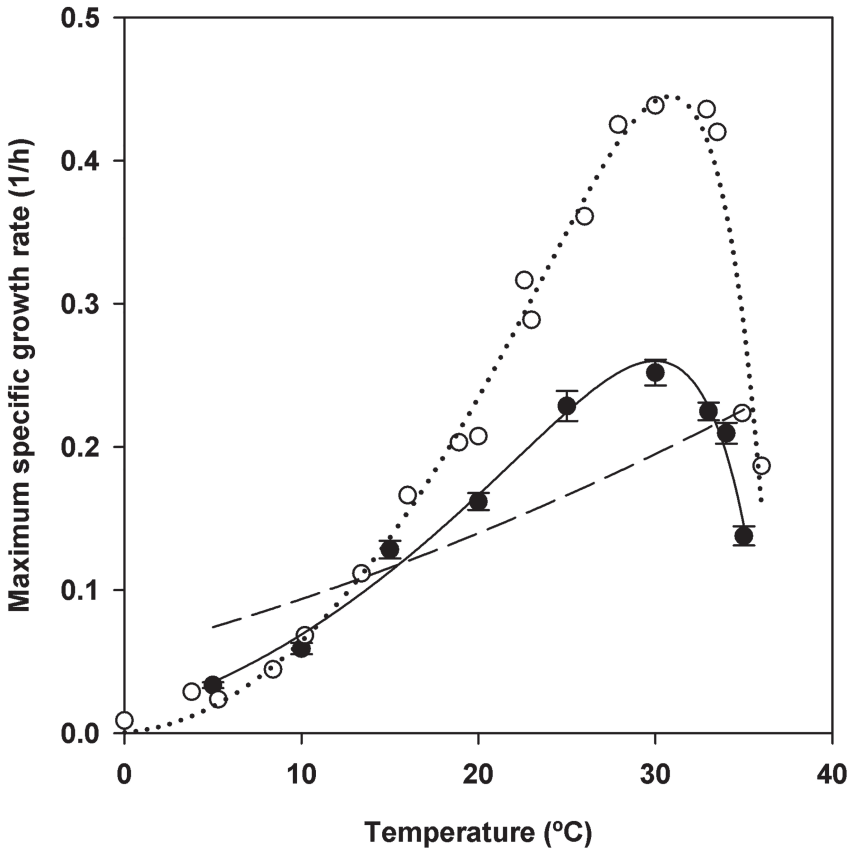

Fig 4. Model fitting to the maximum specific growth rate for *X. arboricola* pv. *pruni* as a function of temperature. Values of the maximum specific growth rate (black symbols) are the mean of two experiments, seven strains and three replicates per strain. Error bars are the standard errors. Modified Ratkowsky models are coincident and represented with continuous line; dashed line represents the Ratkowsky model. The modified Ratkowsky model (equation 10) fitting to the growth rate data from the literature (white symbols) [25] is shown in dotted line.

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Table 6. Observed and predicted maximum specific growth rate for *X. arboricola* pv. *pruni* at temperatures tested in model validation.

Temperature (˚C)	Maximum specific growth rate (h^{-1})	
	Observed [y]	Predicted [z]
17	0.137 ± 0.009	0.133
22	0.198 ± 0.008	0.190
27	0.257 ± 0.011	0.245
31	0.313 ± 0.015	0.257

[y] Values are the mean of parameter estimates from the modified Gompertz equations obtained for 21 growth curves at each temperature, corresponding to seven strains and three replicates per strain.

[z] Maximum specific growth rate predicted by the modified Ratkowsky equation: $\mu_{max} = (0.015 * (T—(-7.77)))^2 * (1—exp(0.270 * (T—36.67)))$.

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specific growth rate of seven strains and three replicates per strain and those predicted with the secondary model equations generated in this work. The correlation between the predicted and observed maximum specific growth rates, as well as the indices of bias and accuracy were determined. A significant correlation between the predicted and observed specific growth rates was obtained for the modified Ratkowsky model ($P = 0.026$), with a Pearson coefficient $r = 0.974$. Bias (0.93) and accuracy factors (1.08) were also in the good range.

Discussion

Understanding the growth parameters of *X. arboricola pv. pruni*, as well as their variation with temperature, is essential and the first step in the development of a model for predicting infections on host plants. Doubling times and daily potential doublings, calculated from specific growth rates at different temperatures, are often included in bacterial plant disease forecasting models [21, 23, 24]. Growth studies on plant pathogenic bacteria are generally based on the manual quantification of viable counts or optical density measurements at discrete time intervals, and parameter estimation is performed directly on either partly or fully experimental growth curves [20, 21, 25]. Predictive modeling of bacterial growth is an area in food microbiology where the effect of environmental factors on bacterial growth are quantified and modeled with mathematical equations [31, 48]. In our work, predictive microbiology was used to describe the response of *X. arboricola pv. pruni* to temperature. The results demonstrated that the *in vitro* growth of *X. arboricola pv. pruni* can be modeled and that primary and secondary models can be used for predicting the specific growth rate as a function of temperature. To our knowledge this is the first time that predictive microbiology is used for modeling the growth of plant pathogenic bacteria, and a similar approach could be used in other bacterial species.

The development of models in predictive microbiology requires large quantities of data obtained from growth curves. The classical method for constructing bacterial growth curves, based on plate counting of culture samples taken at several time intervals, is laborious and time consuming, whereas optical density measurements are rapid and nondestructive and, if automated, provide real-time growth curves. Bioscreen C, an automated turbidimetric system, is an adequate system for obtaining data for predictive microbiology, and made it possible to generate and model a large number of growth curves for *X. arboricola* pv. *pruni* incubated at a wide range of temperatures. However, when modeling optical density growth curves, the fitted parameters are different from the parameters derived from viable counts [36, 37], and calibration factors are needed [26]. In the present work, a calibration curve to convert optical densities to viable counts was obtained for *X. arboricola* pv. *pruni* in order to overcome the

limitations of the direct modeling of optical density growth curves [36]. Among the four equations evaluated, the Beer–Lambert equation showed a low goodness of fit to the entire data range, from 10^5 to 10^9 CFU/ml, as expected, because there is only a narrow range where the relationship between turbidity values and bacterial concentrations is linear [49]. The logarithmic transformation of the optical density and viable count data, which resulted in a linear relationship, produced the best fit to the data. This equation was used to recalculate the optical density data to viable counts, prior to the primary modeling of *X. arboricola* pv. *pruni* growth. A similar calibrating relation was used modeling *Bacillus cereus* growth [50].

Primary models describe the evolution of the bacterial population over time. The modified Gompertz equation [33], the model of Baranyi and Roberts [30], and the Buchanan model [34] are among the most widely used and were chosen for fitting to the *X. arboricola* pv. *pruni* growth curves. At all temperatures, the modified Gompertz model showed the best goodness of fit, with the lowest RSS. Consequently, it was selected to estimate the kinetic parameters of *X. arboricola* pv. *pruni* at each temperature. The selection of an adequate equation is especially important when practical applications are derived from the model estimates, because their values may differ depending on the predictive model used [33]. A significant effect of temperature on the maximum specific growth rate of *X. arboricola* pv. *pruni* was observed. The highest specific growth rates and the lowest doubling times were obtained at temperatures from 25 to 33˚C, with a maximum at 30˚C. The specific growth rates were lower below and above this range and minimal at 5 and 10˚C. Therefore, the suboptimal temperatures for *X. arboricola* pv. *pruni* growth can be established as from 15 to 20˚C. The specific growth rate at 34˚C was higher than at 20˚C, but only 50% of replicates for six out of the seven strains were able to grow at 35˚C. The *in vitro* growth of *X. arboricola* pv. *pruni* had been previously reported at 35 and 36˚C [25], with doubling times similar to those obtained at 35˚C in our study. The optimal temperatures for the growth of *X. arboricola* pv. *pruni* are similar in both studies and similar to that of *X. campestris* pv. *vesicatoria* [21], but they are higher than those determined for other plant pathogenic bacteria, such as *Erwinia amylovora* [20] and *Pseudomonas syringae* [25].

In secondary modeling, the Ratkowsky model only modeled the maximum specific growth rate below the optimal growth temperature and it was dismissed. The modified Ratkowsky models fitted the data well and were proved to be reliable in predicting the effect of temperature on the specific growth rate over the entire temperature range. Finally, the modified Ratkowsky equation 10 was selected because when extrapolating at temperatures above the maximum for growth, no positive specific growth rates are predicted [28]. This secondary model was successfully validated at four new temperatures for the seven bacterial strains. Additionally, the modified Ratkowsky model also fitted well to the specific growth rate data derived from a previous study [25], and similar estimates were obtained for a, b and Tmax parameters although higher specific growth rates were reported. Differences in growth kinetics have been reported among strains of foodborne pathogens such as *E. coli* [51]. Similarly, the differences in the growth rate obtained in the present work and those reported by Young et al. [25] may be attributed to strain biological variability. Young et al. [25] studies were based on a group of strains recovered from infected *Prunus sp*. samples in New Zealand (NZ), whereas our work was performed with strains recovered from host species in Europe (five strains), USA (one strain) and NZ (one strain). The strain CFBP 3894 used in our work, recovered from *Prunus salicina* in NZ, had not been tested in the previous work. On the other hand, composition of growth media can also affect the bacterial growth, since bacteria adapt their growth to nutrient availability. Not only differences in growth rate of *X. campestris* pv. *campestris* were observed depending on the growth medium [52], but also biofilm formation and bacterial aggregation was related to the media composition and particularly to the calcium content in the medium. Similarly, the differences in the growth rate of *X. arboricola* pv. *pruni* found in our work could

be partially attributed to differences in the growth medium composition. Under the restricted growth conditions used in the two experiments, no nutrients were added during the incubation period, the type of nutrients and their concentration would limit the bacterial growth. Sezonov et al. [53] found that the steady-state growth of *E. coli* in LB broth ended early, followed by an extended period during which the growth rate decreased gradually, due to the lack of utilizable carbon sources. Finally, experimental and methodological aspects concerning starting bacterial densities or the physiological state of initial cells, could also explain in part the above mentioned differences in *X. arboricola* pv. *pruni* growth rates found in the two studies. However, the optimal temperature for *X. arboricola* pv. *pruni* growth was estimated to be 30˚C by both equations.

Lag phase has been estimated by applying the Gompertz equation to OD growth curves with starting inoculum level sufficiently high to produce an initial OD above the detection threshold of the turbidimeters used [37]. However, lag phase modeling based on a narrow range of initial high bacterial concentrations, can lead to poor estimates when using empirical models such as the modified Gompertz. The modified Gompertz model may produce negative lag times that have no biological meaning [36] at temperatures close to the optimal temperature, when bacterial cultures grow without a lag phase. High variability of lag time was observed in our work at temperatures 5, 15 and 25˚C. Variability in lag phase has been consistently reported in the literature; thus, poor estimates are often obtained for this parameter [54]. In fact, many parameters can affect the lag phase including, temperature history and the physiological state of initial cells, as well as the starting bacterial density. The lag phase allows time for the adaptation measures required for bacterial cells to begin to exploit new environmental conditions, and genes involved in translation, protein synthesis, cell polarity, cell division, and cell cycle control are induced [55]. Various factors may influence the lag phase apart from temperature [54]. A better understanding of the lag phase and the physiological factors that affect it is needed in order to use this parameter for epidemiological or disease forecasting purposes.

Previous studies reported that at least three successive rainy days with temperatures between 14 and 19˚C were necessary for primary infections of *X. arboricola* pv. *pruni* on peach trees in Italy [18] and that a mean temperature of 12˚C was sufficient to produce infections in France [56]. These temperatures are suboptimal for *X. arboricola* pv. *pruni* growth. In fact, daily mean temperatures do not reflect the real temperature over the entire day and short periods of temperatures above 15˚C may be sufficient for the bacterial population to increase to potentially infective concentrations. Additionally, the leaf temperature of many plants are 5 to 10˚C above the air temperature in direct sunlight [25], so temperatures on and in leaves may be more favorable for pathogen multiplication. Otherwise, primary infections on peach are observed from May to mid-July in Italy [18], when daily fluctuating temperatures may reach the optimal range for *X. arboricola* pv. *pruni* multiplication. The disease incubation period has been reported to be between 7 and 25 days in warm and cold weather, respectively; suggesting similar effects of temperature on *X. arboricola* pv. *pruni* multiplication in host plant tissues.

X. arboricola pv. *pruni* is an aerial plant pathogenic bacterium that can survive and multiply on the surface of host organs as epiphyte. Population densities of 1.3×10^2 to 2.1×10^4 CFU/g in peach and 3.2×10^3 to 1.5×10^6 CFU/g in plum were recovered during January, February and March [57], which may act as the inoculum reservoir for spring infections. Studies on pathogen epidemiology revealed that the relative humidity and especially water status of host plants are key parameters, as well as temperature, for *X. arboricola* pv. *pruni* multiplication and infections [58, 59]. Wetness is also required for *X. arboricola* pv. *pruni* infections on host tissues [56, 58, 59]. Preliminary studies on *X. arboricola* pv. *pruni* growth on the surface of peach leaves at optimal temperature and under different relative humidity conditions

conducted in our laboratory demonstrated that epiphytic pathogen populations under low relative humidity rapidly declined, whereas they were able to colonize the leaf surface under wetness conditions (unpublished data).

The temperature-based model for predicting *X. arboricola* pv. *pruni* growth rate developed in this manuscript will be used for determining the potential bacterial multiplication as a first component of a bacterial spot of stone fruit disease forecasting model, in a similar approach as Maryblight [22], Cougarblight [24], and Billing's integrated system [60] forecasting models for fire blight of apple and pear caused by *Erwinia amylovora*. Further studies on the effect of temperature on *X. arboricola* pv. *pruni* growth *in vivo* and on the effects of weather conditions on infections and disease development will be undertaken to develop infection models to be included in the disease forecaster.

X. arboricola pv *pruni* is a quarantine pathogen in the EU, so the practical applications of the disease forecaster are twofold: i) field assessment of disease spread and outbreaks based on infection risk prediction (specially for disease surveillance in regions were the disease is not stablished but some outbreaks have been detected), and ii) rational timing of products for disease control (bactericides), applied only when conditions are favorable for bacterial multiplication and infection.

Conclusions

Predictive modeling of bacterial growth based on optical density measurements, widely used in food microbiology area, has been successfully applied for the plant pathogenic bacteria *X. arboricola* pv. *pruni*.

This work demonstrates that the effects of temperature on *X. arboricola* pv. *pruni* growth can be predicted. The model generated in this work has been successfully validated and will be used for predicting temperature-based *Xanthomonas arboricola* pv. *pruni* growth rate and derived potential daily doublings, and included as the inoculum potential component of a bacterial spot of stone fruit disease forecaster.

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References

1. Smith EF. Observation on a hitherto unreported bacterial disease, the cause of which enters the plant through ordinary stomata. Science. 1903; 17:456–457.
2. Vauterin L, Hoste B, Kersters K, Swings J. Reclassification of *Xanthomonas*. Int. J. Syst. Bcteriology. 1995; 45:472–489.
3. EPPO/CABI. *Xanthomonas arboricola* pv. *pruni*. In: Smith IM, McNamara DG, Scott PR, Holderness M editors. Quarantine Pests for Europe. 2nd ed. CAB International, Wallingford, UK; 1997. pp. 1096–1100.
4. Ritchie D. Bacterial spot. In: Ogawa JM, Zehr EI, Bird GW, Ritchie DF, Uriu K and Uyemoto JK, editors. Compendium of stone fruit diseases. The APS Press, St. Paul, MN; 1995. pp. 50–52
5. Stefani E. Economic significance and control of bacterial spot/canker of stone fruits caused by *Xanthomonas arboricola* pv. *pruni*. J. Plant Pathol. 2010; 92:99–104.
6. Palacio-Bielsa A, Roselló M, Cambra MA, López MM. First report on almond in Europe of bacterial spot disease of stone fruits caused by *Xanthomonas arboricola* pv. *pruni*. Plant Dis. 2010; 94:786.
7. Marchi G, Cinelli T, Surico G. Bacterial leaf spot caused by the quarantine pathogen *Xanthomonas arboricola* pv. *pruni* on cherry laurel in central Italy. Plant Dis. 2011; 95:74–74.
8. Tjou-Tam-Sin NNA, van de Bilt JLJ, Bergsma-Vlami M, Koenraadt H, Naktuinbouw JW, van Doorn J, et al. First report of *Xanthomonas arboricola* pv. *pruni* in ornamental *Prunus laurocerasus* in the Netherlands. Plant Dis. 2012; 96:759.
9. EPPO. Xanthomonas arboricola pv. pruni (XANTPR). EPPO Global Database. 2016. Available from: https://gd.eppo.int.
10. Ritchie D. Bacterial spot. In: Horton D, Johnson D, editors. Southeastern peach growers' handbook. Cooperative Extension Service, University of Georgia, College of Agriculture and Environmental Sciences, Athens; 2005.
11. Janse JD. Bacterial diseases that may or do emerge, with (possible) economic damage for Europe and the Mediterranean basin: Notes on epidemiology, risks, prevention and management on first occurrence. J. Plant Pathol. 2012; 94:S4.5–S4.29.
12. Anonymous. Council Directive 2000/29/EC of 8 May 2000 on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community. Official Journal of the European Communities. 2000; L 169:1–146.
13. EPPO. Diagnostics *Xanthomonas arboricola* pv. *pruni*. EPPO Bulletin. 2006; 36:129–133
14. Scortichini M. Epidemiology and predisposing factors of some major bacterial diseases of stone and nut fruit trees species. J. Plant Pathol. 2010; 92:S1.73–S1.78.
15. Ritchie D. Sprays for control of bacterial spot of peach cultivars having different levels of disease susceptibility, 1998. Fungicide and Nematicide Tests. 1999; 54:63–64.
16. Boudon S, Manceau C, Nottéghem J-L. Structure and origin of *Xanthomonas arboricola* pv. *pruni* populations causing bacterial spot of stone fruit trees in western Europe. Phytopathol. 2005; 95:1081–1088.
17. Lalancette N, McFarland K. Phytotoxicity of copper-based bactericides to peach and nectarine. Plant Dis. 2007; 91:1122–1130.
18. Battilani P, Rossi V, Saccardi A. Development of *Xanthomonas arboricola* pv. *pruni* epidemics on peaches. J. Plant Pathol. 1999; 81:161–171.
19. Bugiani R, Giosuè S, Gianni C, Rossi V. Prediction of *Xanthomonas arboricola* pv. *pruni* infection on peaches. IOBC/WPRS Bulletin. 2008; 54:565–569.
20. Billing E. The effect of temperature on the growth of the fireblight pathogen, *Erwinia amylovora*. J. Appl. Microbiol. 1974; 37:643–648.
21. Kim J, Kang W, Yun S. Development of a model to predict the primary infection date of bacterial spot (*Xanthomonas campestris* pv. *vesicatoria*) on hot pepper. Plant Pathol. J. 2014; 30:125–135. https://doi.org/10.5423/PPJ.OA.09.2013.0090 PMID: 25288995
22. Lightner GW, Steiner PW. Maryblyt™: A computer model for predicting of fire blight disease in apples and pears. Comput. Electron. Agric. 1992; 7:249–260.
23. Billing E. Weather and fireblight in England. Ann. Appl. Biol. 1976; 82:259–266.
24. Smith T. A predictive model for forecasting fire blight of pear and apple in Washington state. Acta Hortic. 1993; 338:153–160.
25. Young J, Luketina R, Marshall A. The effects on temperature on growth in vitro of *Pseudomonas syringae* and *Xanthomonas pruni*. J. Appl. Bacteriol. 1977; 42:345–354. PMID: 885818

26. Dalgaard P, Ross T, Kamperman L, Neumeyer K, McMeekin TA. Estimation of bacterial growth rates from turbidimetric and viable count data. Int. J. Food Microbiol. 1994; 23:391–404. PMID: 7873339
27. McKellar R. A heterogeneous population model for the analysis of bacterial growth kinetics. Int. J. Food Microbiol. 1997; 36:179–186. PMID: 9217107
28. Zwietering MH, de Koos JT, Hasenack BE, de Witt JC, van't Riet K. Modeling of bacterial growth as a function of temperature. Appl. Envir. Microbiol. 1991; 57:1094–1101.
29. Zwietering MH, de Wit JD, Cuppers HGAM, van't Riet K. Modeling of bacterial growth with shifts in temperature. Appl. Environ. Microbiol. 1994; 60:204–213. PMID: 16349151
30. Baranyi J, Roberts TA. A dynamic approach to predicting bacterial growth in food. Int. J. Food Microbiol. 1994; 23:277–294. PMID: 7873331
31. Ross T. Predictive modelling of the growth and survival of *Listeria* in fishery products. Int. J. Food Microbiol. 2000; 62:231–245. PMID: 11156267
32. Oscar TP. Development and validation of primary, secondary, and tertiary models for growth of *Salmonella* Typhimurium on sterile chicken. J. Food Prot. 2005; 68:2606–13. PMID: 16355832
33. Zwietering MH, Jongenburger I, Rombouts FM, van't Riet K. Modeling of the bacterial growth curve. Appl. Environ. Microbiol. 1990; 56:1875–1881. PMID: 16348228
34. Buchanan R, Whiting R, Damert W. When is simple good enough: a comparison of the Gompertz, Baranyi, and three-phase linear models for fitting bacterial growth curves. Food Microbiol. 1997; 14:313–326.
35. Begot C, Desnier I, Daudin J. Recommendations for calculating growth parameters by optical density measurements. J. Microbiol. Methods. 1996; 25:225–232.
36. Dalgaard P, Koutsoumanis K. Comparison of maximum specific growth rates and lag times estimated from absorbance and viable count data by different mathematical models. J. Microbiol. Methods. 2001; 43:183–96. PMID: 11118653
37. Augustin J-C, Rosso L, Carlier V. Estimation of temperature dependent growth rate and lag time of Listeria monocytogenes by optical density measurements. J. Microbiol. Methods. 1999; 38:137–146. PMID: 10520594
38. Biesta-Peters EG, Reij MW, Joosten H, Gorris LGM, Zwietering MH. Comparison of two optical-density-based methods and a plate count method for estimation of growth parameters of *Bacillus cereus*. Appl. Environ. Microbiol. 2010; 76:1399–405. https://doi.org/10.1128/AEM.02336-09 PMID: 20081006
39. Maniatis T, Fritsch EF, Sambrook J. Molecular cloning: a laboratory manual. 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; 1982.
40. Socquet-Juglard D, Patocchi A, Pothier JF, Christen D, Duffy B. Evaluation of *Xanthomonas arboricola* pv. *pruni* inoculation techniques to screen for bacterial spot resistance in peach and apricot. J. Plant Pathol. 2012; 94:S1.91–S1.96.
41. McClure P, Cole M, Davies K, Anderson W. The use of automated tubidimetric data for the construction of kinetic models. J. Ind. Microbiol. 1993; 12:277–285.
42. Stephens P, Joynson J, Davies K, Holbrook R, Lappin-Scott H, Humphrey T. The use of an automated growth analyser to measure recovery times of single heat-injured *Salmonella* cells. J. Appl. Microbiol. 1997; 83:445–455. PMID: 9351226
43. Hudson JA, Mott SJ. Comparison of lag times obtained from optical density and viable count data for a strain of *Pseudomonas fragi*. J. Food Saf. 1994; 14:329–339.
44. Baty F, Ritz C, Charles S, Brutsche M, Flandrois J-P, Delignette-Muller M-L. A Toolbox for Nonlinear Regression in R: The Package nlstools. Journal of Statistical Software. 2015; 66(5):1–21.
45. Ratkowsky DA, Olley J, McMeekin TA, Ball A. Relationship between temperature and growth rate of bacterial cultures. J. Bacteriol. 1982; 149:1–5. PMID: 7054139
46. Ratkowsky DA, Lowry RK. Model for bacterial culture growth rate throughout the entire biokinetic temperature range. J. Bacteriol. 1983; 154:1222–1226. PMID: 6853443
47. Sant'Ana AS, Franco BD, Schaffner DW. Modeling the growth rate and lag time of different strains of *Salmonella enterica* and *Listeria monocytogenes* in ready-to-eat lettuce. Food Microbiol. 2012; 30:267–273. https://doi.org/10.1016/j.fm.2011.11.003 PMID: 22265311
48. Ross T. Indices for performance evaluation of predictive models in food microbiology. J. Appl. Bacteriol. 1996; 81:501–508. PMID: 8939028
49. Begot C, Lebert I, Lebert A. Variability of the response of 66 *Listeria monocytogenes* and *Listeria innocua* strains to different growth conditions. Food Microbiol. 1997; 14:403–412.
50. Chorin E, Thuault D, Cléret JJ, Bourgeois CM. Modelling *Bacillus cereus* growth. Int. J. Food Microbiol. 1997; 38:229–234. PMID: 9506288

51. Kim J, Ro E, Yoon K. Comparison of growth kinetics of various pathogenic *E. coli* on fresh perilla leaf. Foods. 2013; 2:364–373. https://doi.org/10.3390/foods2030364 PMID: 28239122

52. Dugé de Bernonville T, Noël LD, SanCristobal M, Danoun S, Becker A, Soreau P, et al. Transcriptional reprogramming and phenotypical changes associated with growth of *Xanthomonas campestris* pv. *campestris* in cabbage xylem sap. FEMS Microbiol Ecol. 2014; 89:527–541. https://doi.org/10.1111/1574-6941.12345 PMID: 24784488

53. Sezonov G, Joseleau-Petit D, D'Ari R. *Escherichia coli* physiology in Luria-Bertani broth. J Bacteriol. 2007; 189:8746–8749. https://doi.org/10.1128/JB.01368-07 PMID: 17905994

54. Swinnen IAM, Bernaerts K, Dens EJJ, Geeraerd AH, Van Impe JF. Predictive modelling of the microbial lag phase: A review. Int. J. Food Microbiol. 2004; 94:137–159. https://doi.org/10.1016/j.ijfoodmicro.2004.01.006 PMID: 15193801

55. Rolfe MD, Rice CJ, Lucchini S, Pin C, Thompson A, Cameron ADS, et al. Lag phase is a distinct growth phase that prepares bacteria for exponential growth and involves transient metal accumulation. J. Bacteriol. 2012; 194:686–701. https://doi.org/10.1128/JB.06112-11 PMID: 22139505

56. Garcin A, Vibert J, Leclerc A. *Xanthomonas* sur pêcher. étude des conditions d'infection. Développement de l'outil (1re partie). Infos CTIFL. 2011; 268:26–39.

57. Shepard DP, Zehr EI. Epiphytic persistence of *Xanthomonas campestris* pv. *pruni* on peach and plum. Plant Dis. 1994; 78:627–629.

58. Zehr EI, Shepard DP, Bridges WC Jr. Bacterial spot of peach as influenced by water congestion, leaf wetness duration, and temperature. Plant Dis. 1996; 80:339–341.

59. Morales G, Llorente I, Montesinos E, Moragrega C. Basis for a predictive model of *Xanthomonas arboricola* pv. *pruni* growth and infections in host plants. Acta Hortic. 2016; 1–8.

60. Billing E. Fire blight risk assessment: Billing's integrated system (BIS) and its evaluation. Acta Hortic. 1999; 399–406.

Chapter 4:

Effects of leaf wetness duration and temperature on the infection of *Prunus* by *Xanthomonas arboricola* pv. *pruni*

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Effects of Leaf Wetness Duration and Temperature on Xanthomonas arboricola pv. pruni Infections on Prunus sp.

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Corresponding Author:	Isidre Llorente Universitat de Girona Girona, SPAIN
Keywords:	Xanthomonas arboricola pv. pruni; bacterial spot disease of stone fruits; forecasting model; leaf wetness; temperature
Abstract:	Xanthomonas arboricola pv. pruni is the causal agent of the bacterial spot disease of stone fruits and almond. The bacterium is distributed throughout the major stone-fruit-producing areas of the world and is considered a quarantine organism by the European and Mediterranean Plant Protection Organization. The effect of leaf wetness duration and temperature on X. arboricola pv. pruni infections on Prunus sp. was determined in controlled-environment experiments. Potted plants of the peach-almond hybrid GF-677 were inoculated with bacterial suspensions and exposed to combinations of six leaf wetness durations (from 0 to 24 h) and seven fixed temperatures (from 5 to 35ºC). Disease severity was influenced by wetness duration and temperature. Leaf wetness was required for bacterial infection and three to six hour of wetness at temperatures from 20 to 35ºC were enough to reach high disease severity. The effect of leaf wetness duration and temperature on disease severity was quantified using a modification of the Weibull equation proposed by Duthie. The reduced-form of Duthie's model obtained by nonlinear regression analysis was selected for further analysis. The correlation coefficient and adjusted coefficient of determination for this model were R = 0.87 and R2adj = 0.85, respectively, and all parameters were significantly different from 0. The estimated optimal temperature for X. arboricola pv. pruni infection was 28.9ºC. The predictive capacity of the obtained model was evaluated using an additional set of data obtained from new wetness duration-temperature combinations. In 92% of the events the observed severity agreed with the predicted level of infection risk. The risk chart derived from the reduced form of Duthie's model can be used to estimate the potential risk for X. arboricola pv. pruni infection on Prunus sp. based on observed or forecasted temperature and wetness duration.
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Competing Interests You are responsible for recognizing and disclosing on behalf of all authors any competing interest that could be perceived to bias their work, acknowledging all financial support and any other relevant financial or non-financial competing interests. Do any authors of this manuscript have competing interests (as described in the PLOS Policy on Declaration and Evaluation of Competing Interests)? **If yes**, please provide details about any and all competing interests in the box below. Your response should begin with this statement: *I have read the journal's policy and the authors of this manuscript have the following competing interests:* **If no** authors have any competing interests to declare, please enter this statement in the box: "*The authors have declared that no competing interests exist.*"	The authors have declared that no competing interests exist.

Effects of Leaf Wetness Duration and Temperature on the Infection of *Prunus* by *Xanthomonas arboricola* pv. *pruni*

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Abstract

Xanthomonas arboricola pv. *pruni* is the causal agent of bacterial spot disease of stone fruits and almond. The bacterium is distributed throughout the major stone-fruit-producing areas of the world and is considered a quarantine organism in the European Union according to the Council Directive 2000/29/EC, and by the European and Mediterranean Plant Protection Organization. The effect of leaf wetness duration and temperature on infection of *Prunus* by *X. arboricola* pv. *pruni* was determined in controlled environment experiments. Potted plants of the peach-almond hybrid GF-677 were inoculated with bacterial suspensions and exposed to combinations of six leaf wetness durations (from 0 to 24 h) and seven fixed temperatures (from 5 to 35ºC) during the infection period. Then, plants were transferred to a biosafety greenhouse, removed from bags, and incubated at optimal conditions for disease development. Although leaf wetness was required for infection of *Prunus* by *X. arboricola* pv. *pruni*, temperature had a greater effect than leaf wetness duration on disease severity. The combined effect of wetness duration and temperature on disease severity was quantified using a modification of the Weibull equation proposed by Duthie. The reduced-form of Duthie's model obtained by nonlinear regression analysis fitted well to data (R = 0.87 and R^2_{adj} = 0.85), and all parameters were significantly different from 0. The estimated optimal temperature for infection by *X. arboricola* pv. *pruni* was 28.9ºC. Wetness periods longer than 10 h at temperatures close to 20ºC, or 5 h at temperatures between 25 and 35ºC were necessary to cause high disease severity. The predictive capacity of the model was evaluated using an additional set of data obtained from new wetness duration-temperature combinations. In 92% of the events the observed severity agreed with the predicted level of infection risk. The risk chart derived from the reduced form of

Duthie's model can be used to estimate the potential risk for infection of *Prunus* by *X. arboricola* pv. *pruni* based on observed or forecasted temperature and wetness duration.

Introduction

Xanthomonas arboricola pv. *pruni* is the causal agent of bacterial spot disease of stone fruits and almond [1,2], which are some of the most economically important tree crops worldwide [3]. The disease was first described in the USA in 1903 [4] and today it is distributed throughout the major stone-fruit-producing areas of the world [5]. Although the bacterium is considered a quarantine organism in the European Union, according to the Council Directive 2000/29/EC, and by the European and Mediterranean Plant Protection Organization (EPPO) [1], the pathogen is currently spreading in many European countries, which have reported local outbreaks [6,7].

Xanthomonas arboricola pv. *pruni* affects a wide range of *Prunus* species, including fruit crops, such as plum, nectarine, peach, apricot, cherry, almond, their hybrids, and ornamental species, as cherry laurel [8,9]. The pathogen mainly causes lesions on leaves and fruits, but twig infections, which can result in perennial cankers in some host species, are also observed in spring or summer. The economic impact of the disease depends on reduced quality and marketability of fruits, reduced orchard productivity, and increased costs of nursery productions [10].

Quarantine regulation of *X. arboricola* pv. *pruni* by the EU and EPPO is aimed to restrict the introduction and dissemination of this pathogen [1]. Disease control is currently limited to preventive copper spray applications in areas already affected by the disease [8,9,11]. Limitations on copper usage have been defined by the potential for its accumulation in soil, the selection for pathogen resistance [12] and the phytotoxicity in some stone-fruit crops, such as peach and nectarine [13]. In order to minimize the negative effects of copper and to optimize the control effectiveness, accurate timing of copper applications can guided by disease forecasting models according to specific levels of infection risk. Plant disease forecasting models help growers in the decision-making process regarding disease management as an alternative to a fixed spray schedule, and they have been successfully implemented in the management of many plant diseases, in which the number of pesticide sprays is reduced in comparison to a fixed spray schedule, but with similar efficacy of disease control [14–16].

Most forecasting models of bacterial plant diseases are based on two processes: i) a temperature-dependent multiplication process of epiphytic populations to provide inoculum followed by, ii) the occurrence of favorable weather conditions that allow infections [17–20]. A similar approach can be used in the development of a forecasting model of bacterial spot disease of stone fruits. The effect of temperature on the growth of *X. arboricola* pv. *pruni* was determined *in vitro* [21,22]. A model for predicting *X. arboricola* pv. *pruni* growth as a function of temperature was developed [22], which can be used to estimate the epiphytic inoculum potential of the pathogen. The study revealed that *X. arboricola* pv. *pruni* is able to grow within the temperature range from 5 to 35ºC and that optimal temperatures for bacterial multiplication are between 25 and 33ºC. Regarding the weather conditions conducive to bacterial infections and disease development, different studies agree on the importance of warm temperatures and wetness periods (rainfall, irrigation or dew) for the infection of *Prunus* by *X. arboricola* pv. *pruni* [1,9,23,24,25]. The need of wetness for infection is also supported by experiments performed under controlled environment conditions at optimal temperatures for *X. arboricola* pv. *pruni* growth (20-30ºC) [26,27]. Variability in the range of temperature and wetness period duration conducive to infection and disease development has been detected in studies under field conditions [24,25], probably due to the complex interaction of diverse factors affecting the disease development, such as inoculum populations [28], host susceptibility [7,29] and orchard management practices [9].

Quantification of the effect of environmental factors on the processes of infection and colonization by leaf pathogens is usually carried out in experiments conducted in growth chambers, thus allowing isolation of the effects of specific environmental factors. Therefore, to develop a prediction model of *Prunus* infection by *X. arboricola* pv. *pruni,* the combined effects of temperature and wetness period duration should be quantified under controlled environment conditions for the entire range of temperature at which *X. arboricola* pv. *pruni* is able to grow.

Polynomial equations are widely used to quantify the combined effects of wetness and temperature on infection of plants by pathogens for a wide variety of diseases [30], primarily fungal diseases [31,32]. However, various parameters estimated in polynomial equations lack a clear biological significance and some variables may be transformed (e.g. using logarithmic or square root transformations)

before fitting the equation. To overcome the weaknesses of polynomial equations, nonlinear models based on modified forms of the Weibull function have been proposed [33], in which all parameters can be interpreted to provide information on the mechanisms of disease response. Weibull based models have been used to quantify temperature and wetness requirements for infection in several diseases [34–37]. A similar approach is proposed in the present work to determine the combined effect of temperature and wetness duration on the infection of *Prunus* by *X. arboricola* pv. *pruni*.

The objectives of this study were to (i) quantify the effects of temperature and leaf wetness duration on the infection of *Prunus* by *X. arboricola* pv. *pruni*; (ii) develop a model describing these effects; and (iii) evaluate the capacity of this model to forecast bacterial infection risk.

Materials and methods

Plant material

Potted plants of the peach-almond hybrid rootstock GF-677 (*Prunus amygdalus* × *P. persica*) obtained by micropropagation (Agromillora Catalana, Subirats, Spain) were selected based on its susceptibility to bacterial spot disease. Plants were grown in 0.5 L pots filled with a commercial peat moss/vermiculite/perlite potting mix (type BVU, Prodeasa, Girona, Spain) in a greenhouse and fertilized once a week with a solution of 200 ppm N-P-K (20-10-20). Twenty-centimeter-high plants with 10 to 15 young expanded leaves were used.

Inoculum production

Xanthomonas arboricola pv. *pruni* strain CFBP 5563 isolated from peach in France obtained from CIRM-CFBP (International Center for Microbial Resources - French Collection for Plant-associated Bacteria, Beaucouzé Cedex, France) was used in this study. Bacteria were stored in stock tubes containing yeast-peptone-glucose broth (YPG) [38] supplemented with glycerol (20% wt/vol) at -70ºC. The inoculum was prepared from 24-h-old cultures grown on Luria-Bertrani (LB) [39] plates at 27ºC. Bacterial colonies were scraped from the cultures, resuspended in sterile distilled water and adjusted to an optical density of 0.3 at 600 nm, which corresponds to 5 x 10^8 CFU/ml. A viable count of the inoculum suspension was also determined by

spreading 0.1 ml of appropriate 10-fold serial dilutions on yeast-peptone-glucose agar (YPGA) [38] plates and incubation for 72 h at 27ºC.

Pathogen inoculation, incubation and disease assessment

Potted plants of the peach-almond hybrid GF-677 were inoculated by spraying 5 x 10^8 CFU/ml bacterial suspensions supplemented with diatomaceous earth (1 mg/ml) on plant leaves using an airbrush (model Junior Hobby; Sagola, Vitoria-Gasteiz, Spain) operated at 100 kPa. Adaxial and abaxial leaf surfaces were sprayed until runoff.

Inoculated plants were introduced into plastic bags (moist chambers) with the inner side sprayed with distilled water to maintain leaf wetness and transferred immediately to controlled environment chambers (model MLR-350; Sanyo, Gunma, Japan) at constant temperatures of 5, 10, 15, 20, 25, 30 or 35ºC in darkness, with a maximum variation of ±1ºC for all temperatures. The temperature inside the growth cabinets was monitored and recorded using HOBO Pendant® temperature/light data logger (Onset Computer Corp, Pocasset, MA, USA). At 3, 6, 12, 18 or 24 h time intervals, plants were removed from growth chambers and transferred to a biosafety greenhouse maintained at 15 to 25ºC with 70 to 80% relative humidity and natural photoperiod for 21 days for disease development. Weather parameters inside the biosafety greenhouse were monitored with a datalogger (CR10X, Campbell Scientific Ltd., UK) connected to combined temperature-relative humidity (model HMP35C) and leaf wetness (model 237) sensors. Plants sprayed with sterile distilled water and incubated at 25ºC during 24 h of leaf wetness were used as negative controls. When plants were introduced into the greenhouse, the plastic bags were removed and plants were exposed to a smooth airflow supplied by an electric fan until the leaf surface was dry (30 min). Plants corresponding to 0 h of wetness duration were inoculated directly in the greenhouse and the leaf surface was dried immediately after inoculation.

Disease severity was assessed 21 days after inoculation on the five youngest completely formed leaves at the moment of inoculation. The new leaves formed after the inoculation, during the incubation in the greenhouse, were not considered. A 0-to-5 scale severity index was used, corresponding to a leaf area affected by 0, 1, 3, 6, 12 and ≥ 24%, respectively [24]. Disease severity (*S*) was calculated for each plant according to the formula: $S = [(\sum_{n=1}^{N} I_n)/N \times 5] \times 100$, where I_n is the severity

index for each leaf, *N* is the number of leaves per plant, and *5* is the maximum severity index value in the scale.

A completely randomized experimental design with subsampling was used. The treatment layout was a factorial arrangement with seven temperatures (5, 10, 15, 20, 25, 30 and 35ºC) and six wetness periods (0, 3, 6, 12, 18 and 24 h) for a total of thirty six treatments. Each treatment consisted of five plants and five inoculated leaves were evaluated in a plant. The experiment was repeated twice.

Data analysis and model development

Averaged values of disease severity over the five plants per temperature-wetness combination were standardized using the maximum value observed in each experiment to compare both repetitions. Therefore, the relative disease severity (*S*') ranged from 0 to 1. The effect of experiment replicate was determined by analysis of variance using the general linear models (GLM) procedure of SPSS v.23 (IBM Corp., Armonk, NY). Previously the homogeneity of variance and normality were tested. As there were no significant differences between the two runs of the experiment for relative disease severity, the following analysis were performed with averaged data over the two runs.

The combined effects of leaf wetness duration (*w*) and temperature during the wet period (*t*) on relative disease severity were evaluated using the nonlinear model proposed by Duthie [33], which is based on a modified form of a Weibull function and it is described by the nonlinear equation of the form:

$$S' = f(w,t) = f(t) \times (1 - \exp\{-[B(w - C)]^D\}) \quad (1)$$

where *w* is the leaf wetness duration (h) and *t* is the temperature (ºC) during the wetness period. The upper asymptote is defined by the expression *f(t*), which characterizes the upper limit on the responses as wetness duration is extended, and it has the following equation:

$$f(t) = E' \times \left\{exp\left[\frac{(t-F)G}{H+1}\right]\right\} / \{1 + exp[(t - F)G]\} \quad (1.1)$$

in which

$$E' = E \times \left[\frac{H+1}{H}\right] H^{1/(H+1)} \quad (1.2)$$

f(t) characterizes the upper limit of the response when *w* is large. Each parameter in equations 1, 1.1 and 1.2 has an epidemiological meaning summarized in Table 1. The optimum temperature is given by:

$$t_{opt} = F - (1/G)\ln(H) \quad (1.3)$$

Table 1. Summary of epidemiological significance of parameters in the model proposed by Duthie [33] (equations 1, 1.1, and 1.2), which describes the response of foliar parasites to the combined effects of temperature and duration of wetness.

Parameter	Epidemiological significance
B	Intrinsic rate of increase in response with respect to wetness duration ($0 < B < 1$)
C	Length of delay before the start of the processes that drive the response
D	Period of wetness duration in which the response decelerates ($D > 0$)
E	Maximum response that occurs at the optimum temperature ($E > 0$)
F	Directly proportional to the optimum temperature
G	Intrinsic rate of decline from the maximum as temperature deviates from the optimum
H	Difference in the rate of acceleration in the decline from the maximum as temperature increases or decreases from the optimum

However, the iterative regression procedure failed to converge on a solution when the model included too many parameters. Overparameterization was assessed by the magnitudes of standard errors and correlation coefficients between estimated parameters. The model was simplified by fixing parameter *C* in equation 1 to a value of 0, assuming that bacteria respond immediately to the increase in leaf wetness duration; and parameter *E* in equation 1.2 was fixed to a value of 1, because the disease was measured on a scale of 0 to 1. Moreover, the asymmetry in the temperature response on infection, characterized by parameter *H*, may not be measured when under field conditions at high temperatures wetness is rare [31], and in this case asymmetry may be disregarded. It follows that $H = 1$, $t_{opt} = F$, $E' = 2E$. Equation 1 can be rewritten as [36]:

$$f(w,t) = [1 - \exp(-B \times w)^D]/\cosh[(t - F)G/2] \quad (2)$$

Duthie's model and its simplification (equations 1 and 2) were fitted to data. Regressions were based on mean infection data for each combination of

temperature and wetness duration rather than on pooled data to reduce data variability and improve curve fitting [40]. Parameters of Duthie's models (equations 1 and 2) were estimated using the sequential quadratic programming method of the nonlinear regression in SPSS v.23 (IBM Corp., Armonk, NY).

From the results obtained, the reduced form of Duthie (equation 2) was selected for further analysis. Several criteria were used to evaluate this model: (i) randomness and normality of residuals; (ii) goodness of fit between estimated and observed values; and (iii) standard deviation around the regression lines. The nonlinear regression obtained with the equation 2 was evaluated by performing a linear regression analysis between predicted (y) and observed (x) values as paired observations. The linear regression was analyzed by the coefficient of determination (R^2) and adjusted coefficient of determination (R^2_{adj}), and testing the significance of the difference in the intercept from 0 and the slope from 1.

The capacity of the model for predicting the infection risk was determined using data derived from an additional experiment with a new set of temperature and wetness duration combinations, different from those used for model development. The new set of incubation temperatures were 7.5, 12.5, 17.5, 22.5, 27.5 and 32.5ºC for wetness periods of 2, 5, 10, 20 or 24 h. The experiment was performed as described previously, with a total of 24 temperature-wetness duration combinations. Each treatment consisted of five replicates (plants) and five inoculated leaves were evaluated in a plant. The experiment was performed once. The predictions of the model at each temperature-wetness combination were compared with the observed disease severity values using the Pearson's correlation coefficient and an analysis of frequency was performed for the two categories proposed for model prediction values and for observed disease severity values, respectively.

Results

No lesions were observed on non-inoculated leaves and few infections occurred on inoculated plants incubated without leaf wetness. The disease severity values corresponding to 0 h of wetness duration (5.5 and 5.6% in experiments 1 and 2, respectively) were subtracted from the disease severity observed for each temperature-wetness combination, as it was probably due to the effect of the residual wetting times during the drying process. The highest disease severity was

observed at 30ºC for 24 h of wetness in both experiments, although maximum disease severity values were significantly different (76 and 40% in experiments 1 and 2, respectively). Disease severity values were standardized using the maximum severity observed in each experiment to make the repetitions comparable. No differences in relative disease severity were observed between the two independent experiments (P = 0.603), allowing the data from two experiments to be pooled for model development.

Relative disease severity was affected by temperature during the wetness period. The curves for the different wetness periods evaluated from 5 to 35ºC showed similar shapes, although disease severity values differed between them (Fig 1A). The optimal range of temperature for bacterial infection was from 20 to 35ºC, with a maximum at 30ºC. Below 30ºC, an increase of temperature resulted in a gradual increment of disease severity; while above 30ºC, there was a slight decrease in the relative disease severity.

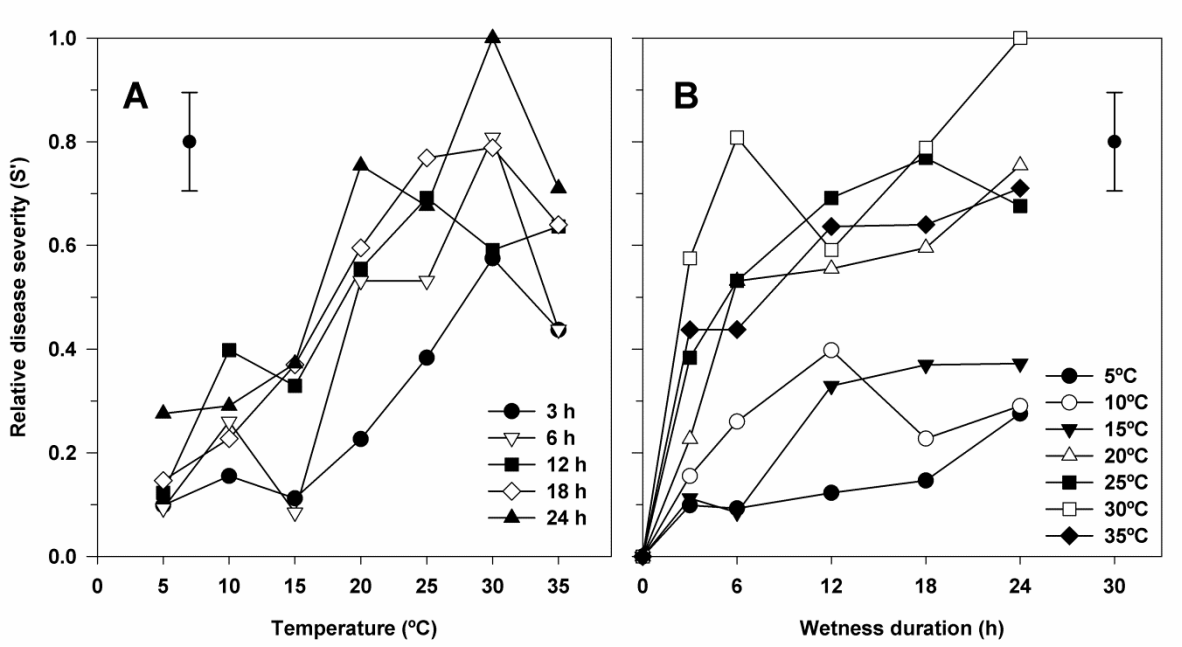

Fig 1. Effect of temperature (A) and leaf wetness duration (B) on relative disease severity caused by *Xanthomonas arboricola* pv. *pruni* on peach-almond hybrid GF-677 plants 21 days after inoculation. Symbols represent the mean relative disease severity of two experiments and five plants per experiment. Error bars in the upper left (A) or right (B) corner represent the mean standard error.

Wetness was required for infection of *Prunus* by the pathogen. In general, increasing wetness period duration from 0 to 12 h increased the relative disease severity (Fig 1B). Longer wetness periods, from 12 to 24 h resulted in no or slight increase in relative disease severity. At low temperatures (5, 10, and 15ºC) the disease severity increased progressively from 0 without wetness to 0.28, 0.29, and 0.37 under 12 to 24-h-wetness period, respectively. At temperatures from 20 to 35ºC, the disease severity also increased with wetness duration increase up to 12-24 h, but high values of disease severity (above 0.4) obtained under short wetness periods (3 or 6 h), which were higher than the maximum disease severity observed at low temperatures under longer wetness periods.

Estimated parameters of the model proposed by Duthie (equation 1) are presented in Table 2. Parameters *B*, *D, F* and *G* were significantly different from zero at $P < 0.05$, whereas parameter *H* presented high standard error and did not differ from zero. Moreover, parameter *H* was highly correlated with parameter *G* and *F* according to Pearson correlation coefficient ($R = 0.963$ and 0.948, respectively). The optimum temperature for the infection of peach-almond hybrid GF-677 plants by *X. arboricola* pv. *pruni* calculated with equation 1.3 was 30.1ºC.

Table 2. Estimated parameter values of Duthie's model (equation 1) describing the relative disease severity (*S'*) caused by *Xanthomonas arboricola* pv. *pruni* on peach-almond hybrid GF-677 plants based on combined effects of temperature (*t*) and duration of leaf wetness (*w*). In the model $S' = f(w,t) = f(t) \times (1 - \exp\{-[B \times w]^D\})$, where $f(t) = E'\{\exp[(t - F)G/(H+1)]\}/\{1 + \exp[(t - F)G]\}$, in which $E' = [(H+1)/H]H^{1/(H+1)}$.

Parameter[z]	Estimate	Standard Error	Lower Bound	Upper Bound	*t*-test	*P > t*
B	0.167	0.032	0.101	0.234	5.149	< 0.0001
D	0.582	0.111	0.356	0.808	5.265	< 0.0001
F	33.053	2.230	28.499	37.607	14.824	< 0.0001
G	0.266	0.080	0.103	0.429	3.339	0.0023
H	2.514	1.628	-0.811	5.838	1.544	0.1330

[z] Parameters as defined in the main text (equation 1).

The reduced four-parameter version of the model (equation 2) was obtained disregarding parameter *H* (*H* = 1). Estimated values are presented in Table 3, in which all parameters (*B, D, F* and *G*) were estimated precisely, significantly different from zero at $P < 0.05$, and correlation coefficients between parameters were low ($R < 0.605$). The optimum temperature is represented by parameter *F*, which was 28.9ºC. This model, corresponding to equation 2, was selected as all parameters were significant, the standard errors obtained were lower than those obtained using equation 1, high correlations between parameters were absent, and the number of parameters were fewer than the model obtained with equation 1.

Table 3. Estimated parameter values of the reduced form of Duthie's model (equation 2) describing the relative disease severity (*S*') caused by *Xanthomonas arboricola* pv. *pruni* on peach-almond hybrid GF-677 plants based on combined effects of temperature (*t*) and duration of leaf wetness (*w*) on. In the model $S' = f(w,t) = [1 - \exp(-B \times w)^D]/\cosh[(t - F)G/2]$.

Parameter[z]	Estimate	Standard Error	Lower Bound	Upper Bound	*t*-test	*P* > *t*
B	0.145	0.023	0.098	0.193	6.272	< 0.0001
D	0.553	0.097	0.354	0.752	5.670	< 0.0001
F	28.919	0.850	27.184	30.653	34.008	< 0.0001
G	0.195	0.018	0.159	0.231	11.116	< 0.0001

[z] Parameters as defined in the main text (equation 2).

Two- and three-dimensional representations of the response of the reduced form of Duthie's model (equation 2) are given in Fig 2. Relative disease severity increased sigmoidally with increasing wetness duration, while the temperature response was unimodal, with the optimal temperature at 28.9ºC. The model provided good prediction for all wetness duration-temperature combinations. The Pearson correlation coefficient (*R*) between observed and predicted values was 0.93, and the coefficients of determination R^2 and R^2 adjusted for the nonlinear regressions were 0.88 and 0.85, respectively.

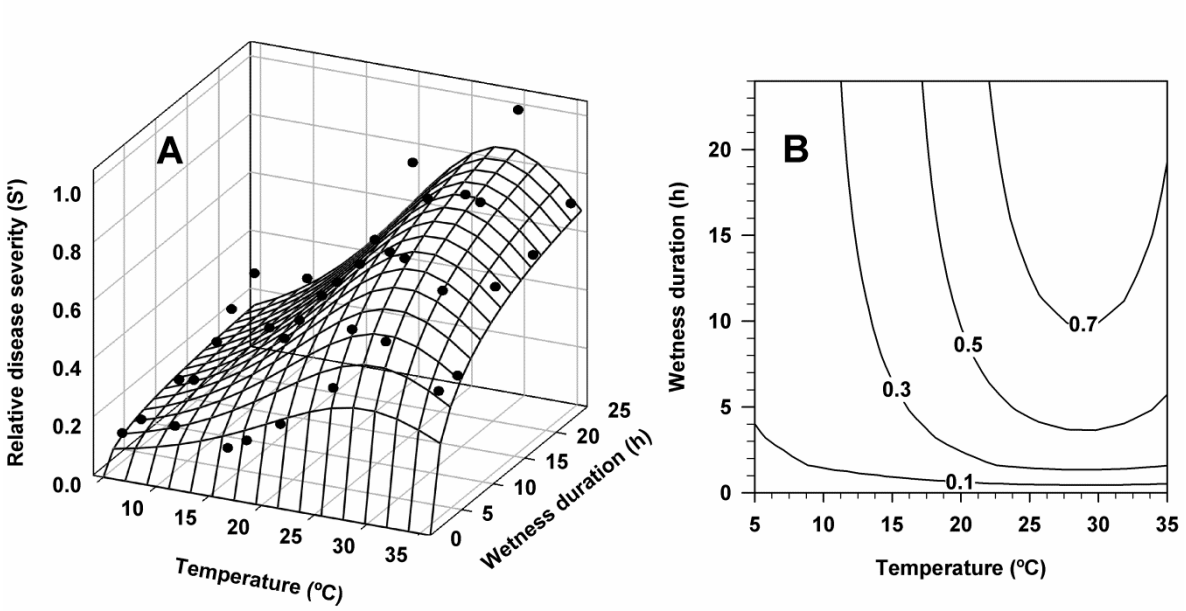

Fig 2. Three-dimensional response (A) and contour plot (B) of the combined effects of temperature and leaf wetness duration on relative disease severity caused by *Xanthomonas arboricola* pv. *pruni* on peach-almond hybrid GF-677 plants. The predicted values were calculated using the reduced form of Duthie's model, corresponding to equation 2. (A) The black points correspond to mean relative disease severity of two independent experiments and five plants per experiment observed 21 days after bacterial inoculation. (B) An area represents relative disease severity values lower than or equal to the label on the contour line on the right of the area.

The linear regression of the predicted values against the observed relative disease severity also showed the good relationship between observed and predicted values (Fig 3). The coefficients of determination R^2 and R^2 adjusted for the linear regressions were both 0.88. Although the intercept was not significantly different from 0 (P = 0.0823), the slope was slightly different from 1 (P = 0.0407). A slight underprediction of the model at high disease levels and a slight overprediction at low values were observed.

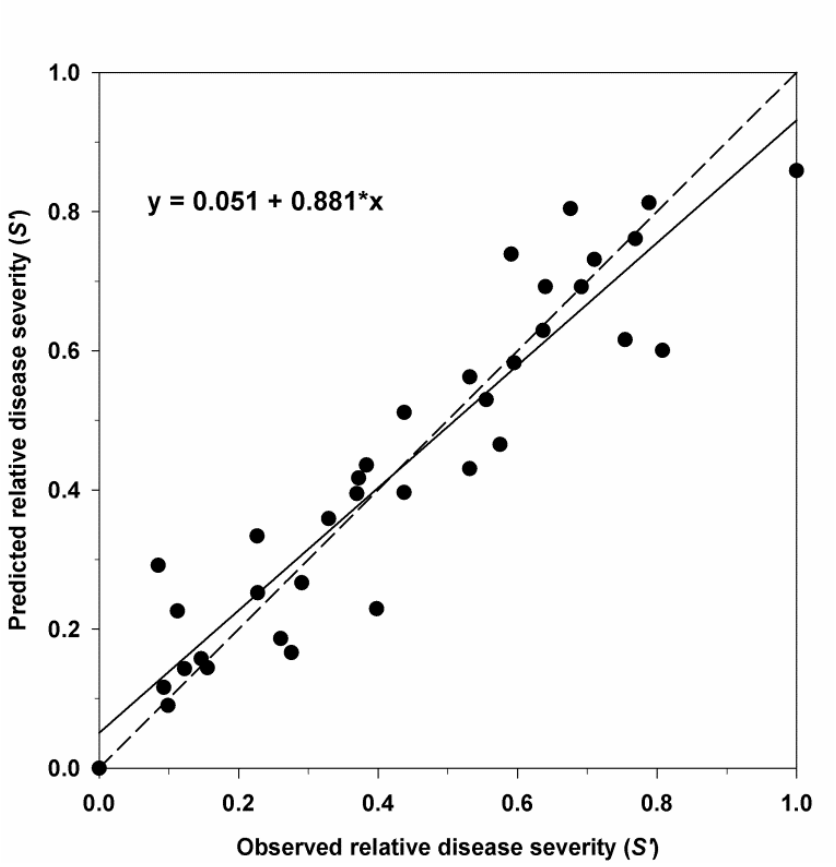

Fig 3. Observed relative disease severity versus estimated values with the reduced form of Duthie's model (equation 2). The regression line (solid) is slightly different from the dashed line, which corresponds to a fitted line with an intercept of 0 and a slope of 1.

The predictive capacity of the infection model was evaluated in a new experiment using an independent set of data with different temperature-wetness duration combinations. Observed relative disease severity (*S'*) was compared with model predictions (Fig 4). A positive and high correlation resulted between these two variables (R = 0.761). The relationship between predicted relative disease severity and observed severity was further analyzed in order to define a risk threshold for infection. Disease severity up to 25% was considered low since symptoms corresponded, on average, to leaves with an index up to 1.25 in a scale from 0 to 5. There was a significant relationship between observed disease severity lower than 25% and predicted relative severity lower than 0.5; and also between observed disease severity higher than 25% and predicted relative severities from 0.5 to 1. Consequently, the value 0.5 of relative severity predicted by the model (*S'*) was proposed as the infection risk threshold. Accordingly, two levels of infection risk were

established: low (*S'* < 0.5) and high (*S'* ≥ 0.5). On the basis of the infection risk levels, model predictions agreed on 22 of 24 cases (92%), 11 for low and 11 for high risk predictions. Only 2 mismatches were observed, corresponding to temperature-wetness combinations predicted as low infection risk that expressed medium severity values.

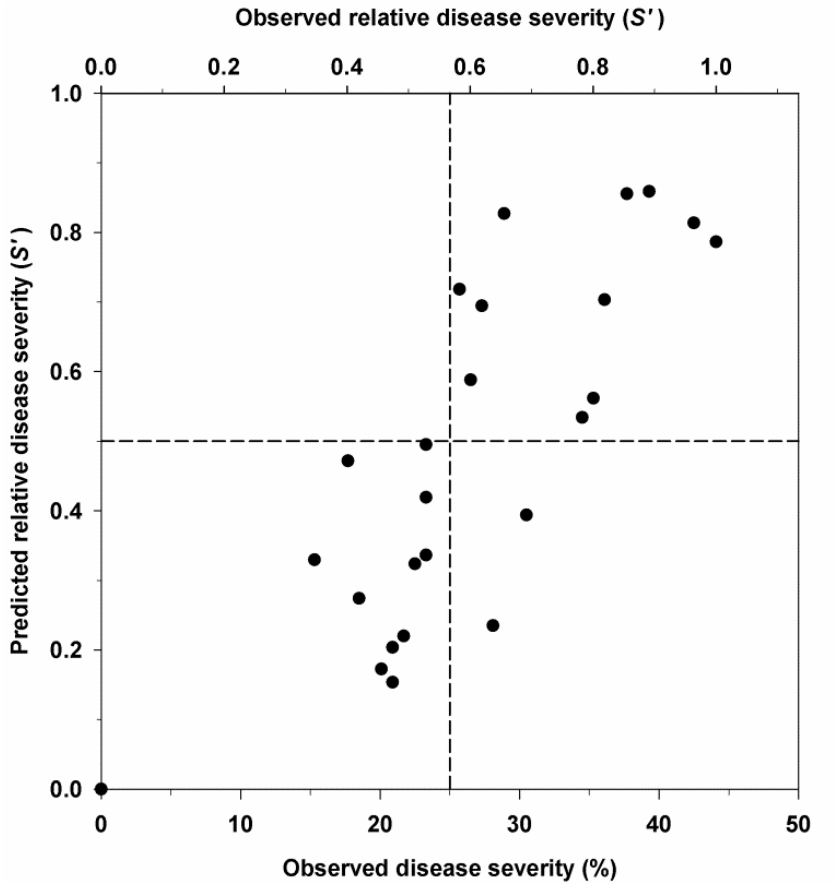

Fig 4. Evaluation of the prediction capacity of the infection model for *Xanthomonas arboricola* pv. *pruni* describing the combined effects of leaf wetness and temperature under greenhouse conditions. Comparison between the observed disease severity and observed relative severity versus the relative disease severity predicted by the reduced form of Duthie's model, corresponding to equation 2 on GF-677 peach-almond hybrid plants 21 days after inoculation.

Discussion

The combined effects of leaf wetness duration and temperature on the infection of *Prunus* by *X. arboricola* pv. *pruni* were determined under controlled environment conditions for the entire range of temperatures at which the bacterium is able to grow [21,22]. Optimal temperatures for infection by *X. arboricola* pv. *pruni* conducive to severe bacterial spot disease (*S'* > 0.5) ranged from 20 to 35ºC, with a maximum at 30ºC. Low temperatures (10-15ºC) resulted in low relative disease severity (*S'* < 0.5). Plants incubated at 5ºC developed few lesions which could be related to the effect of the residual free water on the leaf surface and the water congestion in leaf tissues during the drying process in the greenhouse. Optimal temperatures for infection on *Prunus* agreed with optimal temperatures for *X. arboricola* pv. *pruni* growth, with maximum bacterial growth rates at temperatures from 20 to 30ºC [21,22].

Leaf wetness durations from 3 to 6 hour were sufficient for infection and cause high disease severity at optimal temperatures (20-35ºC). Longer wetting periods up to 12 h resulted in increased disease severity, and wetness periods from 12 to 24 h had a similar effect on infection by the bacterium. The minimum leaf wetness period required for infection by *X. arboricola* pv. *pruni* depended on temperature, being short (3 h) at optimal temperatures and longer (6 to 12 h) at temperatures below 20ºC. These results confirm that wetness is required for the infection of *X. arboricola* pv. *pruni* on *Prunus* [25-27]. At 0 h wetness duration no disease symptoms were observed in the majority of plants. The few infections that did occur with 0 h wetness can likely be explained by residual water from the inoculation of the pathogen suspension that remains into the stomata tissues or on the leaf surface during the drying process in the greenhouse. Some disease symptoms were also observed in plants let to dry immediately after inoculation in previous studies [26]. Similarly, only 5 min of leaf wetness were sufficient for *X. arboricola* pv. *juglandis* to infect water-congested young walnut fruits [41].

The results obtained in this study under controlled environment conditions are consistent with field observations, which concluded that warm temperatures accompanied by frequent rains or heavy dews play an important role in disease development [1, 9, 24]. In fact, temperature, rainfall and wetness are considered key factors for infection by *X. arboricola* pv. *pruni* on peach orchards [24,25,42].

The combined effects of temperature and wetness duration on the infection by *X. arboricola* pv. *pruni* were evaluated and quantified using nonlinear equations proposed by Duthie [33], which are a variation of the Weibull equation. Nonlinear models proposed by Duthie [33] provide a more parsimonious description of empirical data than models that comprise polynomial equations and all parameters have an epidemiological significance. Many infection models use regression equations based on polynomials [31,43], but model parameters have no biological meaning and thus, poorly describe the response of a biological process [33]. Moreover, polynomial models are only reliable in the range of temperatures and wetness used to construct them; otherwise, unrealistic results are obtained if the equations are applied outside this range [36]. The model obtained in this work accurately described the relationship between wetness duration and temperature on disease severity, according to predicted and observed comparisons for disease severity and levels of infection risk. The model had a unimodal response, but some simplifications were performed to avoid overparameterization in the nonlinear regression. The parameter *C* (period before the start of the response) was fixed to 0 assuming that bacteria respond immediately to the increase in leaf wetness duration, and parameter *E* (upper limit of the response) was fixed to 1, because the disease was measured on a scale of 0 to 1. All parameters in the equation were significant, except parameter *H*, which represents the degree of asymmetry in the temperature response. *X. arboricola* pv. *pruni* is able to grow *in vitro* at temperatures from 5 to 34ºC, but not above 35ºC [21,22]. The optimal temperature for its multiplication *in vitro* was 31ºC [21,22], which means that the growth curve is skewed negatively. If the response of *Prunus* infection by *X. arboricola* pv. *pruni* to temperature were similar to the growth response, the parameter *H* might be >1. However, only one temperature (35ºC) was tested above the optimal temperature for infection; therefore, it was difficult to determine the grade of asymmetry of the curves. At least one more temperature above 35ºC should be tested (e.g., 40ºC) to assure the asymmetry of the response, but leaf wetness and temperatures above 35ºC rarely occur under field conditions in temperate regions where *Prunus* species are grown. Consequently, the regression was performed disregarding the asymmetry of the curve (parameter *H* was fixed to 1) [33].

The output of the model developed under controlled environment conditions is based on a quantitative relationship between leaf wetness duration and the

temperature during wetness periods on the infection by *X. arboricola* pv. *pruni*. As a result, the periods of risk for infection can be predicted. The evaluation of the accuracy of the model to determine the levels of disease risk, showed that *S'* = 0.5 could be used as a threshold, above which medium or high disease severity (S > 25%) was reached and consequently, days with risk of infection by *X. arboricola* pv. *pruni* could be identified based on weather parameters. *Xanthomonas arboricola* pv. *pruni* infections can occur with low wetness periods (3 to 6 h) at optimal temperatures, but according to the infection model, at least 10 h wetness at temperatures close to 20ºC, or 5 h of wetness at 25ºC or higher are necessary to reach the infection risk threshold of *S'* = 0.5. These weather conditions are not frequent in temperate regions where species of *Prunus* are grown. Therefore, days with favorable weather conditions to trigger bacterial spot infections may be related to rainfall events and/or long wetting periods in spring and summer. Epidemiological studies performed in Italian peach orchards [24,42] support our results. Primary infections by *X. arboricola* pv. *pruni* were observed in peach orchards when at least 3 successive rainy days occurred, with a mean temperature between 14 and 19°C, and the progress of disease severity on leaves was closely correlated with the number of rainy days after disease onset [24]. Consequently, wetness periods over 24 h could be considered by the additive effect of daily infection risk index, obtaining a cumulative infection risk index. The use of cumulative risk indices in plant disease-warning systems gives more accurate predictions and helps to explain field disease epidemics [30,44].

The model evaluation was performed under controlled greenhouse conditions, but field evaluation and validation are needed before the model can be used as part of a DSS in the management of the bacterial spot disease of stone fruits. The proposed model should be evaluated and validated in areas where the disease is present. The model was developed on the hybrid rootstock GF677 (*Prunus amygdalus* x *P. persica*), susceptible to the bacterial spot disease of stone fruits in nurseries, as representative of the *Prunus s*pecies which are hosts of *X. arboricola* pv. *pruni*. However, field validation trials should be performed on grafted plants of different *Prunus* species. Variability in cultivar susceptibility within each host species [7,29] could also be taken into account in the forecasting model.

The model presented in this manuscript determines the suitability of weather conditions for the infection of *Prunus* by *X. arboricola* pv. *pruni*. Upon the evaluation

and validation of the model under field conditions, it could be included as the second component of a bacterial spot of stone fruit disease forecasting model, in a similar approach as Maryblight [19], CougarBlight [18], and Billing's integrated system [20] forecasting models for fire blight of apple and pear caused by *Erwinia amylovora*. The first component of the forecasting model, corresponding to the inoculum potential, is based on the model for predicting *Xanthomonas arboricola* pv. *pruni* growth as a function of temperature [22], which can be used to predict the epiphytic potential inoculum. The integration of both components is fundamental to accurately predict bacterial infections, since both inoculum and favorable environmental conditions are required to come together at the same time for successful infection occurrences.

The forecasting model developed in this study could be used in warning systems for a rational timing of copper sprays for bacterial spot disease of stone fruits control in regions where the disease is established, but also in quarantine and surveillance strategies for early detection of disease outbreaks and elaboration of disease risk maps to define the areas with major risk of infection by *X. arboricola* pv. *pruni*.

Conclusions

The work presented here quantifies the effects of leaf wetness duration and temperature on infections of *Prunus* by *X. arboricola* pv. *pruni*. Leaf wetness was required for infection of *Prunus* by *X. arboricola* pv. *pruni*. However, the temperature had a greater effect than leaf wetness duration on the disease severity. Disease severity increased with increasing in temperature until the optimal at 28.9ºC, and with increasing leaf wetness duration up to 12 h. The combined effects of leaf wetness duration and temperature on disease severity have been described using the reduced-form of Duthie's model. An infection risk threshold of *S'* = 0.5 was established for the model. Accordingly wetness periods longer than 10 h at temperatures close to 20ºC, or 5 h at temperatures between 25 and 35ºC were necessary to cause high disease severity. The obtained model should be evaluated and validated under field conditions to be used as a forecasting model of the potential risk for infections of *Prunus* by *X. arboricola* pv. *pruni*.

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References

1. EPPO/CABI. *Xanthomonas arboricola* pv. *pruni*. In: Smith IM, McNamara DG, Scott PR, Holderness M editors. Quarantine Pests for Europe. 2nd ed. CAB International, Wallingford, UK; 1997. pp. 1096-1100.
2. Palacio-Bielsa A, Roselló M, Cambra MA, López MM. First report on almond in Europe of bacterial spot disease of stone fruits caused by *Xanthomonas arboricola* pv. *pruni*. Plant Dis. 2010; 94:786.
3. Lamichhane JR. *Xanthomonas arboricola* diseases of stone fruit, almond, and walnut trees: progress toward understanding and management. Plant Dis. 2014; 98:1600–1610.
4. Smith EF. Observation on a hitherto unreported bacterial disease, the cause of which enters the plant through ordinary stomata. Science. 1903; 17:456-457.
5. EPPO. *Xanthomonas arboricola* pv. *pruni* (XANTPR). EPPO Global Database. 2017. Available from: https://gd.eppo.int.
6. EPPO. Diagnostics *Xanthomonas arboricola* pv. *pruni*. EPPO Bulletin. 2006; 36:129-133
7. Scortichini M. Epidemiology and predisposing factors of some major bacterial diseases of stone and nut fruit trees species. J. Plant Pathol. 2010; 92:S1.73-S1.78.

8. EFSA Panel on Plant Health. Scientific opinion on pest categorisation of *Xanthomonas arboricola* pv. *pruni* (Smith, 1903). EFSA Journal. 2014; 12:3857
9. Stefani E. Economic significance and control of bacterial spot/canker of stone fruits caused by *Xanthomonas arboricola* pv. *pruni*. J. Plant Pathol. 2010; 92:99-104.
10. Janse JD. Bacterial diseases that may or do emerge, with (possible) economic damage for Europe and the Mediterranean basin: Notes on epidemiology, risks, prevention and management on first occurrence. J. Plant Pathol. 2012; 94:S4.5-S4.29.
11. Garcin A, Rouzet J, Notteghem J-L. *Xanthomonas* des arbres fruitiers à noyau. Edition CTFL; 2005.
12. Vanneste J, McLaren G, Yu J. Copper and streptomycin resistance in bacterial strains isolated from stone fruit orchards in New Zealand. N. Z. Plant Prot. 2005; 58:101-105.
13. Lalancette N, McFarland K. Phytotoxicity of copper-based bactericides to peach and nectarine. Plant Dis. 2007; 91:1122-1130.
14. Madden LV, Ellis MA, Lalancette N, Hughes G, Wilson LL. Evaluation of a disease warning system for downy mildew of grapes. Plant Dis. 2000; 84:549-554.
15. Llorente I, Vilardell P, Bugiani R, Gherardi I, Montesinos E. Evaluation of BSPcast disease warning system in reduced fungicide use programs for management of brown spot of pear. Plant Dis. 2000; 84:631-637.
16. Thomson SV, Schroth MN, Moller WJ, Reil WO, Beutel JA, Davis CS. Pesticide applications can be reduced by forecasting the occurrence of fireblight bacteria. Calif Agr. 1977; 31:12-14.
17. Beresford RM, Tyson JL, Henshall WR. development and validation of an infection risk model for bacterial canker of kiwifruit, using a multiplication and dispersal concept for forecasting bacterial diseases. Phytopathology. 2017; 107:184-191.
18. Smith T. A predictive model for forecasting fire blight of pear and apple in Washington state. Acta Hortic. 1993; 338:153-160.

19. Lightner GW, Steiner PW. Maryblyt^{TM}: A computer model for predicting of fire blight disease in apples and pears. Comput. Electron. Agric. 1992; 7:249-260.
20. Billing E. Fire blight risk assessment: Billing's integrated system (BIS) and its evaluation. Acta Hortic. 1999; 399-406.
21. Young J, Luketina R, Marshall A. The effects on temperature on growth in vitro of *Pseudomonas syringae* and *Xanthomonas pruni*. J. Appl. Bacteriol. 1977; 42:345-354.
22. Morales G, Llorente I, Montesinos E, Moragrega C. A model for predicting *Xanthomonas arboricola* pv. *pruni* growth as a function of temperature. PLoS One. 2017; 12(5): e0177583.
23. Linvill DE. Use the proper environmental temperature to describe disease. Acta Hortic. 2002; 592:689-694.
24. Battilani P, Rossi V, Saccardi A. Development of *Xanthomonas arboricola* pv. *pruni* epidemics on peaches. J. Plant Pathol. 1999; 81:161-171.
25. Garcin A, Vibert J, Leclerc A. *Xanthomonas* sur pêcher. étude des conditions d'infection. Développement de l'outil (1re partie). Infos CTIFL. 2011; 268:26-39.
26. Zehr EI, Shepard DP, Bridges Jr WC. Bacterial spot of peach as influenced by water congestion, leaf wetness duration, and temperature. Plant Dis. 1996; 80:339-341.
27. Morales G, Llorente I, Montesinos E, Moragrega C. Basis for a predictive model of *Xanthomonas arboricola* pv. *pruni* growth and infections in host plants. Acta Hortic. 2016; 1-8.
28. Randhawa PS, Civerolo E. A detached-leaf bioassay for *Xanthomonas campestris* pv. *pruni*. Phytopathology. 1985; 75:1060-1063.
29. Garcin A, Bresson J. Sensibilité des arbres à noyau au *Xanthomonas* - Bilan de 8 ans d'expérimentation. Infos CTIFL. 2009; 254:30-35.
30. Magarey RD, Sutton TB. How to create and deploy infection models for plant pathogens. In: Ciancio A, Mukerji KG, editors. General concepts in integrated pest and disease management. Dordrecht: Springer Netherlands; 2007. pp. 3-25.
31. Montesinos E, Moragrega C, Llorente I, Vilardell P, Bonaterra A, Ponti I, et al. Development and evaluation of an infection model for *Stemphylium*

vesicarium on pear based on temperature and wetness duration. Phytopathology. 1995; 85:586-592.

32. Gross MK, Santini JB, Tikhonova I, Latin R. The influence of temperature and leaf wetness duration on infection of perennial ryegrass by *Rhizoctonia solani*. Plant Dis. 1998; 82:1012-1016.
33. Duthie JA. Models of the response of foliar parasites to the combined effects of temperature and duration of wetness. Phytopathol. 1997; 87:1088-1095.
34. Carisse O, Bourgeois G, Duthie JA. Influence of temperature and leaf wetness duration on infection of strawberry leaves by *Mycosphaerella fragariae*. Phytopathology. 2000; 90:1120-1125.
35. Wu L, Damicone JP, Duthie JA, Melouk HA. Effects of temperature and wetness duration on infection of peanut cultivars by *Cercospora arachidicola*. Phytopathology. 1999; 89:653-659.
36. Arauz LF, Neufeld KN, Lloyd AL, Ojiambo PS. Quantitative models for germination and infection of *Pseudoperonospora cubensis* in response to temperature and duration of leaf wetness. Phytopathol. 2010; 100:959-67.
37. Furuya H, Takanashi H, Fuji S, Nagai Y, Naito H. Modeling infection of spring onion by *Puccinia allii* in response to temperature and leaf wetness. Phytopathology. 2009; 99:951-956.
38. Boudon S, Manceau C, Nottéghem J-L. Structure and origin of *Xanthomonas arboricola* pv. *pruni* populations causing bacterial spot of stone fruit trees in western Europe. Phytopathology. 2005; 95:1081-1088.
39. Maniatis T, Fritsch EF, Sambrook J. Molecular cloning: a laboratory manual. 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; 1982.
40. Sant'Ana AS, Franco BD, Schaffner DW. Modeling the growth rate and lag time of different strains of *Salmonella enterica* and *Listeria monocytogenes* in ready-to-eat lettuce. Food Microbiol. 2012; 30:267-273.
41. Adaskaveg JE, Förster H, Thompson D, Driever G, Connell J, Buchner R. Epidemiology and management of walnut blight. California Walnut Board, Walnut Research Report. 2005; 281-295.
42. Bugiani R, Giosuè S, Gianni C, Rossi V. Prediction of *Xanthomonas arboricola* pv. *pruni* infection on peaches. IOBC/WPRS Bulletin. 2008; 54:565-569.
43. Uddin W, Serlemitsos K, Viji G. A temperature and leaf wetness duration-

based model for prediction of gray leaf spot of perennial ryegrass turf. Phytopathology. 2003; 93:336-343.

44. Kim KS, Gleason ML, Taylor SE. Forecasting site-specific leaf wetness duration for input to disease-warning systems. Plant Dis. 2006; 90:650-656.

Chapter 5:

Environmental and inoculum effects on epidemiology of bacterial spot disease of stone fruits

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Abstract:	Bacterial spot disease of stone fruits, caused by Xanthomonas arboricola pv. pruni, is of high economic importance in the major stone-fruit-producing areas worldwide. A better understanding of disease epidemiology can be valuable in developing disease management strategies. The effects of weather variables (temperature and wet/dry period) on epiphytic growth of X. arboricola pv. pruni on Prunus leaves were analyzed, and the relationship between inoculum density and temperature on disease development was determined and modeled. The information generated in this study, performed under controlled environment conditions, will be useful to develop a forecasting system for X. arboricola pv. pruni. The density of epiphytic populations under wetness increased from 20 to 30ºC, with a maximum at 25ºC. In contrast, multiplication of epiphytic population was not only

	interrupted under low RH (<40%) at 25ºC, but also resulted in cell inactivation, with only 0.001 % initial cells recovered after 72 h incubation. A significant effect of inoculum density on disease severity was observed and 10^6 CFU/ml was determined as the minimal infective dose for X. arboricola pv. pruni on Prunus. Infections occurred at temperatures from 15 to 35ºC, but incubation at 25 and 30ºC gave the shortest incubation periods (7.7 and 5.9 days respectively). A model for predicting disease symptom development was generated and successfully evaluated, based on the relationship between disease severity and the accumulated heat expressed in cumulative degree day (CDD). Incubation periods of 150, 175 and 280 CDD were required for 5, 10 and 50% of disease severity, respectively.
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Environmental and inoculum effects on epidemiology of bacterial spot disease of stone fruits

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Abstract

Bacterial spot disease of stone fruits, caused by *Xanthomonas arboricola* pv. *pruni*, is of high economic importance in the major stone-fruit-producing areas worldwide. A better understanding of disease epidemiology can be valuable in developing disease management strategies. The effects of weather variables (temperature and wet/dry period) on epiphytic growth of *X. arboricola* pv. *pruni* on *Prunus* leaves were analyzed, and the relationship between inoculum density and temperature on disease development was determined and modeled. The information generated in this study, performed under controlled environment conditions, will be useful to develop a forecasting system for *X. arboricola* pv. *pruni*.

The density of epiphytic populations under wetness increased from 20 to 30ºC, with a maximum at 25ºC. In contrast, multiplication of epiphytic population was not only interrupted under low RH (< 40%) at 25ºC, but also resulted in cell inactivation, with only 0.001 % initial cells recovered after 72 h incubation. A significant effect of inoculum density on disease severity was observed and 10^6 CFU/ml was determined as the minimal infective dose for *X. arboricola* pv. *pruni* on *Prunus*. Infections occurred at temperatures from 15 to 35ºC, but incubation at 25 and 30ºC gave the shortest incubation periods (7.7 and 5.9 days respectively). A model for predicting disease symptom development was generated and successfully evaluated, based on the relationship between disease severity and the accumulated heat expressed in cumulative degree day (CDD). Incubation periods of 150, 175 and 280 CDD were required for 5, 10 and 50% of disease severity, respectively.

Chapter 6:

General discussion

Bacterial spot disease of stone fruits is one of the most serious diseases of *Prunus* species and it is distributed throughout the major stone-fruit-producing areas worldwide (EPPO, 2017). Although the causal agent, *Xanthomonas arboricola* pv. *pruni,* is a quarantine organism in the EU (Council Directive 2000/29/EC; EPPO/CABI, 1997), it is currently spreading in many European countries, which have reported local outbreaks (EPPO, 2017; Scortichini, 2010). A better understanding of the epidemiology of this disease is needed for the development of alternative management strategies, since no effective chemical control is available.

Trends in crop protection are oriented towards a rational use of pesticides and a reduction in the number of registered active ingredients used. The EU set rules for the sustainable use of pesticides to reduce their risks and impacts on people's health and environment, and promoting the use of integrated pest management (IPM) (Directive 2009/128/EC). IPM is a decision-based procedure, which considers all the available crop information and involves the coordinated use of multiple tactics for optimizing the control of whole categories of pests in an ecologically and economically sound manner, such as a reduction of pesticides use combined with non-chemical alternatives and practices (Agrios, 2005; Damos, 2015). Plant disease forecasting models have an important role in the IPM, since they support plant health services or farmers in the decision making for strategic and tactical disease control. These forecasting models improve the timing of pesticide applications and, consequently, the number of treatments can be reduced (Isard et al., 2015; Magarey and Sutton, 2007; Magarey et al., 2002). Moreover, forecasting models are the basis to construct weather-based risk maps, which enhance scouting and monitoring tasks (Magarey et al., 2007; Sparks, 2009). This doctoral thesis contributes to increase the knowledge on biological and epidemiological aspects of bacterial spot disease of stone fruits and provides new information that is the basis for the development of a forecasting system for this disease. The main objective is to integrate the forecasting system in a decision support system (DSS) to guide copper applications and disease surveillance tasks for early detection of new outbreaks or the spread of the disease.

The approach used in this thesis for the development of the forecasting for bacterial spot disease of stone fruits was based on three critical steps in the disease cycle, corresponding to the three components in the forecasting model: i) the epiphytic inoculum potential (EIP), ii) the infection model, and iii) the disease symptom development model. In most forecasting models of plant diseases caused

by fungi the presence of inoculum is assumed, considering that a large number of spores were produced the previous year and only the effects of wetness duration and temperature on the infection process are taken into account for predictions (Duthie, 1997; Magarey and Sutton, 2007; Magarey et al., 2005b). However, inoculum production is necessary prior to the infection process for sporeless bacterial pathogens, such as xanthomonads, since the number of survival cells during winter may be much lower compared to the number of latent fungal spores, which are structures specialized for the dispersion of microorganisms and adapted to carry them over long unfavorable periods (Agrios, 2005). For this reason, most forecasters of plant diseases caused by bacteria are based on two separate processes: the multiplication of epiphytic bacteria as a function of temperature, followed by favorable weather conditions for infection occurrences (Beresford et al., 2017). A similar approach was used in this thesis for the development of a forecasting system for bacterial spot disease of stone fruits, in which the infections of *Prunus* by *X. arboricola* pv*. pruni* can be predicted by a temperature-based growth model of epiphytic bacteria (EIP), and by the conduciveness of weather conditions for infections to occur. Additionally, a third component was included in the forecaster to predict the appearance of symptoms on the basis of temperature.

The environmental effects on the population dinamics of *X. arboricola* pv*. pruni* were analized in a similar way as done in the development of forecasters for other bacterial plant diseases (described in *Chapter 1*). The effects of temperture on the *in vitro* growth of *X. arboricola* pv*. pruni* and on the epiphytic growth under wetness conditions were determined, and complemented with the effect of low RH on epyphitic bacterial inactivation. As a result, a model for predicting bacterial growth as a funtion of temperature and a model for estimating the population survival as a funtion of the incubation period under low RH were developed. These models can be used in combination for estimating the EIP of *X. arboricola* pv*. pruni*.

The temperature range for *X. arboricola* pv*. pruni* growth *in vitro* was 5-35ºC and optimal between 20 and 33ºC, which classify the pathogen as a mesophilic bacterium. These results are not only comparable to previous studies performed with different strains of *X. arboricola* pv*. pruni* (Young et al., 1977), but also to the growth of *X. campestris* pv*. campestris* (Ruissen et al., 1993), *X. campestris* pv*. poae* (Imaizumi et al., 1999) and *X. campestris* pv*. vesicatoria* (Kim et al., 2014), confirming similar effects of temperature on growth *in vitro* of different *Xanthomonas*

species (Swings and Civerolo, 1993). In fact, plant diseases caused by *Xanthomonas*, such as bacterial leaf blight of rice caused by *X. oryzae* pv. *oryzae* or citrus canker caused by *X. citri*, are more commonly found in tropical, sub-tropical and temperate conditions (Young et al., 1977), since they are favored by warm temperatures (Swings and Civerolo, 1993). Otherwise, plant diseases caused by *Pseudomonas* species are typically endemic in temperate regions and, in some cases, favored by cold temperatures, such as bacterial shoot blight disease caused by *P. syringae* pv. *theae* (Tomihama et al., 2009) and blast of pear caused by *P. syringae* pv. *syringae* (Latorre et al., 2002b), or limited by high temperatures, as bacterial canker of kiwifruit caused by *P. syringae* pv. *actinidiae* (Beresford et al., 2017). While *Xanthomonas* species are not cold tolerant, *Pseudomonas* species have the ability to grow at low temperatures even though they have the optimum growth between 20 and 30°C (Fonseca et al., 2011; Purusharth et al., 2007; Young et al., 1977). This adaptive capacity to cold temperatures permits the development of the disease during periods in which the temperatures are high enough for the pathogen to grow but too low to allow optimum host development, being more susceptible because host defenses are not at their optimum (Agrios, 2005). In conclusion, the effects of temperature on the pathogen growth and its adaptive capacity to different conditions are important factors to determine the distribution of the disease throughout the world, and also the moment of the year when the disease may occur.

Ex vivo experiments were performed to add new information to the dynamics of epiphytic *X. arboricola* pv. *pruni* populations under different environmental conditions. The potential growth of the bacteria was determined by *in vitro* assays, in which there were no limiting factors for its growth; whereas *ex vivo* assays were performed on host tissues under environment controlled conditions, simulating the plant-pathogen interaction under field conditions. The dynamics of epiphytic populations of *X. arboricola* pv. *pruni* were monitored on a detached-leaf assay at different temperatures with the presence of leaf wetness to obtain *ex vivo* doubling times. Growth of epiphytic bacteria was observed only at 20, 25 and 30ºC, corresponding to the optimum growth range determined *in vitro*, although specific growth rates were lower than those derived from *in vitro* assays at the same temperatures. The maximum specific growth rates obtained *ex vivo* could be used to modify the optimum temperature of the growth model obtained *in vitro* from 30 to

25ºC, but keeping the same shape of the original temperature curve, as done in other forecasters (Beresford et al., 2017).

The growth model developed for *X. arboricola* pv*. pruni* (C*hapters 3 and 5*) may be used to predict the dinamics of bacterial populations using real or temperature forecasts. The effect of temperature on the pathogen growth is also the basis of the CougarBlight model (Smith, 1993), the *Xcv* infection model (Kim et al., 2014), the *Psa* risk model (Beresford et al., 2017) and the warning system for pear blossom blast caused by *Pseudomonas syringae* pv. *syringae* (Latorre et al., 2002a, 2002b), as well as the basis to construct the potential doublings table for fire blight prediction (Billing, 1974; Schouten, 1987).

Experiments *ex vivo* confirmed that although bacterial multiplication is a temperature-dependent process, the presence of wetness is also required (Linvill, 2002; Young, 1974). When epiphytic populations of *X. arboricola* pv*. pruni* were incubated under low RH at 25ºC, the population density was reduced in 3 log and the survival rate after 72 h of incubation was 0.001%. The inactivation curve showed two separate phases: a rapid decline of the bacterial population during the first 6 h of dryness followed by a slow and continuous inactivation of bacteria cells. A model for predicting bacterial inactivation as a function of the incubation period under low RH was developed, which was based on Cerf's equation (Cerf, 1977). This model may be combined with the growth model to estimate the dynamics of epiphytic populations depending on temperature and the presence of wetness or not. Thus, bacterial multiplication could be considered only when wetness is present (Linvill, 2002), whereas under dry periods the population density decrease according to the inactivation model. Similarly, a decrease of the pathogen population density on leaf surface is predicted by the *Xcv* infection model in absence of wetness (Kim et al., 2014), and bacterial growth is considered null by the *Psa* risk model without wetness or with low RH (Beresford et al., 2017).

Different inoculation methods were used to determine the effect of inoculum density on the infection of *Prunus* by *X. arboricola* pv*. pruni*, since the efficiency of artificially inoculation techniques had been reported variable (Socquet-Juglard et al., 2012). For the three methods evaluated, a significant increase of disease severity was observed in leaves inoculated with bacterial suspensions ≥ 10^6 CFU/ml, despite few symptoms were observed at low inoculum concentrations. These results agreed with previous studies, which reported that concentrations of *X. arboricola* pv. *pruni*

from 10^6 to 10^8 CFU/ml were necessary to cause necrosis on peach leaves in controlled conditions (Civerolo, 1975), and at least 10^6 CFU/ml were present in symptomatic leaves (Palacio-Bielsa et al., 2011). The high population concentration required for *X. arboricola* pv*. pruni* to cause infections in *Prunus* may be related to quorum sensing regulatory systems, which are often required for pathogenesis and colonization of host surfaces by plant-pathogenic bacteria (Dow et al., 2003; He & Zhang, 2008; von Bodman et al., 2003; Whitehead et al., 2001). Consequently, a population size of 10^6 CFU/ml may be considered as the inoculum dose threshold that epiphytic populations of *X. arboricola* pv*. pruni* need to reach to infect host tissues. Similar infection thresholds have been reported for other bacterial plant pathogens, like *Erwinia amylovora* (Billing, 1984)*, Pseudomonas syringae* pv. *syringae* (Lindemann, 1984), *X. campestris* pv. *vesicatoria* (Kim et al., 2014) and *X. axonopodis* pv. *citri* (Christiano et al., 2007).

In regions where the disease is absent, importations of infected plant material is a major pathway for pathogen introduction and spread (EFSA, 2014; Janse, 2012; López-Soriano et al., 2016); whereas in affected orchards, the primary sources of inoculum are located in buds, cankers or crop debris on the soil (Dhanvantari, 1971; Feliciano and Daines, 1970; Zaccardelli et al., 1998). The pathogen can survive from season to season by reducing its metabolism close to a standstill (Leben, 1981), with population densities from 10^2 to 10^4 CFU/g (Shepard and Zehr, 1994). Therefore, epiphytic populations of *X. arboricola* pv. *pruni* need to increase when host and weather conditions are favorable, especially in spring and summer, to reach the infection threshold of 10^6 CFU/ml, and probably to activate their virulence genes before disease onset. The dynamics of epiphytic populations are predicted by the first component of the forecasting system, combining the growth and the inactivation models depending on the environmental conditions.

Once the epiphytic inoculum potential reaches the infective concentration threshold, the infection model can be used to assess the conduciveness of the weather conditions to infection occurrences. This second component of the forecasting system for *X. arboricola* pv. *pruni* refers to the favorable conditions required for infection, closely related to temperature, rainfall, wetness and strong winds (*Chapter 4*). The quantitative effects of wetness and temperature during the wetness period on the infection process by *X. arboricola* pv. *pruni* were modeled using a modification of the Weibull equation (Duthie, 1997), differently from the

forecasting models described in *Chapter 1*, in which the occurrence of either dew or rain is sufficient to consider potential infections when inoculum is highly enough. The need of wetness for infection on *Prunus* by *X. arboricola* pv. *pruni* was previously reported and confirmed in this study (EPPO/CABI, 1997; Garcin et al, 2011a; Goodman, 1976; Morales et al., 2016; Zehr et al., 1996). Optimal temperatures for infection ranged from 20 to 35ºC, coinciding with optimal temperatures for bacterial growth, in which periods of 3 to 6 h of wetness were enough to cause high disease severity. On the other hand, disease severity was significantly lower under periods of wetness up to 24 h at temperatures below 20ºC. These results provide further evidence for the importance of the combined effect of wetness and temperature on the infection process by *X. arboricola* pv. *pruni*, and demonstrate that short periods of leaf wetness are enough to trigger the infection process at temperatures above 20ºC.

The infection model for *X. arboricola* pv. *pruni* developed in this thesis uses hourly weather data for wetness duration (h) and the temperature during the wetness period (ºC) of the previous 24 h to predict the daily risk of infection (*S'*). The value of *S'* ranges from 0 to 1, and *S'* = 0.5 was proposed as a threshold for high disease severity levels, based on the preliminary model evaluation assays performed under greenhouse conditions. However, further evaluation and validation under field conditions is still required. The minimum temperature to reach the threshold of *S'* = 0.5 was 17.2ºC under 24 h of wetness, whereas only 9.8 h of leaf wetness at 20ºC and 4-5 h of leaf wetness at temperatures over 25ºC were necessary to obtain a prediction of *S'* ≥ 0.5. The simultaneously presence of wetness and warm temperatures, necessary for bacterial infections and bacterial growth, is not frequent during spring and summer, conditioned by day-night fluctuations of temperature and relative humidity. Consequently, favorable weather conditions may be associated to rainfall and/or dew events occurring at night and early in the morning (Magarey et al., 2005a), which is consistent with previous observations and studies (Battilani et al., 1999; EPPO/CABI, 1997; Garcin et al., 2011b; Linvill, 2002; Stefani, 2010). Supporting the importance of dew events at night on bacterial infections, stomatal closure during periods of darkness is part of plant innate immune response to restrict bacterial invasion (Melotto et al., 2006), for which plant pathogenic bacteria have evolved specific virulence factors to effectively cause stomatal reopening as an important pathogenesis strategy. Some examples of these specific virulence factors

are DSF in *Xanthomonas campestris*, and syringolin A and coronatine in *Pseudomonas syringae* pv *syringae* (Melotto et al., 2006; Panchal et al., 2016). Moreover, the presence of wetness is essential for bacterial motility (Dechesne et al., 2010), another virulence factor of plant pathogenic bacteria, which could be regulated by light perception (Roden and Ingle, 2009). For this reason, the proper value of temperature used by the model is the temperature during the wetness period (Linvill, 2002), instead of daily maximum temperature or mean temperatures. Predictions of the infection risk would be overestimated using daily maximum temperature or daily mean temperature, since high temperatures without wetness at midday would be not discarded by the model even though they are not conducive to infections occurrences. Therefore, the predictions of the infection risk are the result of the combined effect of wetness and the temperature during the wetness period, with a null risk when wetness is absent.

Finally, symptom development model configures the third component of the forecasting system for bacterial spot disease of stone fruits. Detached-leaf assays were performed to evaluate the effect of temperature and inoculum dose on disease development. Symptoms of bacterial spot disease were observed at temperatures from 15 to 35ºC, although disease progress curves were variable for each temperature-inoculum density combination. The optimal temperature range for disease development (20-30ºC) coincided with the optimal temperature range for pathogen growth described above (Young et al., 1977). In accordance with these results, the incubation period for bacterial spot disease was reported to vary from 6 to 26 days in warm and cold weather in Italian peach orchards, respectively (Battilani et al., 1999). Regarding to inoculum dose, disease symptoms appeared in *Prunus* leaves at all inoculum doses evaluated (10^4, 10^6 and 10^8 CFU/ml); although longer incubation periods and lower final disease severity values were observed with lower inoculum doses. The results of the effect of inoculum concentration on symptom development are in conformity with previous studies performed with *X. arboricola* pv. *pruni* (Civerolo, 1975; Randhawa and Civerolo, 1985), but they are also comparable to studies with other plant pathogenic bacteria, such as *Xanthomonas citri* (Christiano et al., 2007), *Pseudomonas syringae* pv. *syringae* (Latorre et al., 2002a) and *Erwinia amylovora* (van der Zwet et al., 1994).

A model for predicting the disease incubation period and the progress of disease severity as a function of temperature, expressed as cumulative-degree days

(CDD), was elaborated (symptom development model). CDD had been previously used for predicting symptoms of bacterial blight of anthurium, caused by *X. campestris dieffenbachiae* (Fukui et al., 1999). In our model, the biofix date, moment to initiate the computation of CDD, would be a day with risk of infection determined by the infection model described above (developed in *Chapter 4*). According to the symptom development model 150, 175 and 280 CDD with a base temperature of 0ºC were required for a disease severity of 5, 10 and 50%, respectively. The symptom model enables both the percentage of the the progress of disease severity at a certain moment and the calculation of the day on which first symptoms are expected to appear. Previously, the incubation period was reported to be 250 CDD on basis 10.8ºC (Garcin et al., 2011b). Such discrepancies between studies may be due to the effect of inoculum density on disease development (Civerolo, 1975; Randhawa and Civerolo, 1985), since symptom development model was performed using a high dose of inoculum (10^8 CFU/ml), whereas results presented by Garcin et al. (2011b) were based on field studies with natural inoculum, probably around 10^5-10^6 CFU/ml (Randhawa and Civerolo, 1985). These experiments demonstrated that not only are temperature and inoculum density important factors related the infection process, but also in symptom development.

The result of this thesis is the development under controlled conditions, with experiments *in vitro*, *ex vivo* and in planta, of a mechanistic forecasting system for bacterial spot disease of stone fruits composed by three components: i) the epiphytic inoculum potential (EIP) (bacterial growth and inactivation models), ii) the infection model, and iii) the disease symptom development model. The proposed operational scheme consists of a collaborative and cooperative work between components, which are connected in sequence (Figure 11). At the beginning of the growing season, the inoculum production is assessed by the EIP, until the threshold of 10^6 CFU/ml is reached. From this moment onwards, it is assumed that there is enough inoculum to cause infections, which can be predicted by the infection model. Conducive weather conditions to infection occurrences may be considered when $S' \geq 0.5$. Finally, once the infection model has surpassed this infection threshold, the appearance of the first symptoms and the progress of disease severity are predicted using CDD by the third component.

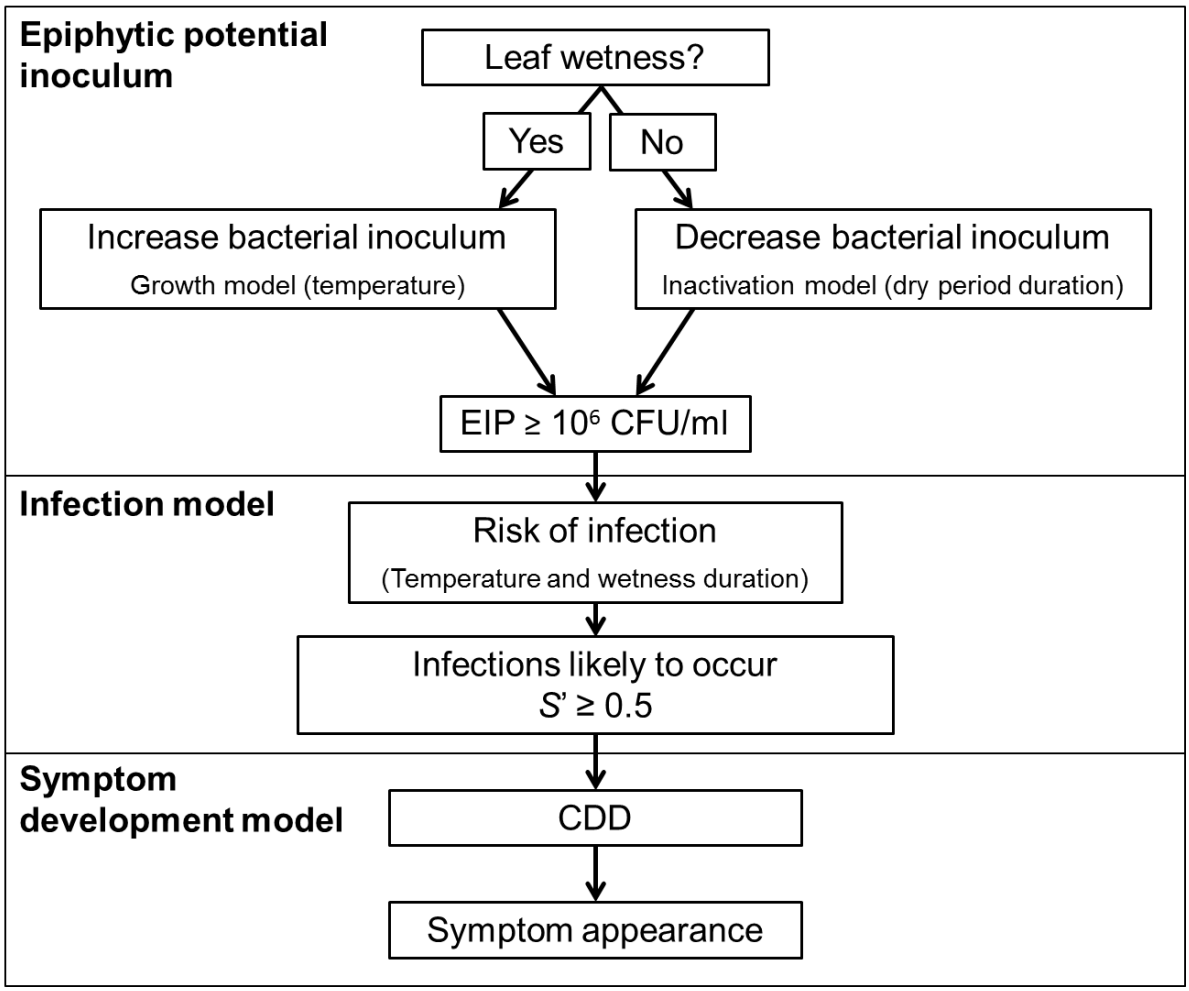

Figure 11. Operational scheme of the proposed forecasting system for bacterial spot of stone fruits caused by *Xanthomonas arboricola* pv. *pruni*.

However, field evaluation and validation are needed before the forecasting system may be used as part of a DSS in the management of the bacterial spot disease of stone fruits. The proposed system should be evaluated and validated in European regions where the disease is locally present, such as the region of Emilia Romagna in Italy or Drôme and Gard in France (EPPO, 2017). In the validation process, several adjustments can be done to improve its accuracy and simplicity of its use. First of all, the parameter related to the history of the disease in the neighborhood could be introduced in EIP predictions, since the quantity of inoculum reservoir may depend on the disease pressure of the previous year. Thus, the initial concentration could be adjusted to the actual density population present in the orchard. However, this measure would imply to take samples of twigs before the growing season and quantify the reservoir of epiphytic inoculum in the laboratory, which add complexity to the model and increases the probability to be rejected by

growers. Another possibility is to fix different initial concentrations of inoculum according to the history into the orchard. As a result, the same weather conditions during a period of time can result in different levels of risk depending on the initial inoculum considered. A similar approach is taken in the CougarBlight model for fireblight, in which different thresholds of EIP are fixed for different orchard blight history scenarios (Smith and Pusey, 2011).

The operational scheme is the second consideration to take into account in the validation process, since discarding one component would simplify its use. The accuracy of the predictions considering only the EIP or the infection model should be tested. In the case that only the EIP component was considered, the occurrence of dew or rain would be necessary for the infection process once the inoculum threshold of 10^6 CFU/ml was reached, which is the operational scheme used by the forecasters for bacterial diseases described in *Chapter 1*. In the case that only the infection model was considered, the presence of inoculum would be assumed, as most forecasters of fungal diseases do. Thus, the model should be used only during conducive periods to infections, for example in April and May. A similar criterion is used in Emilia Romagna Region for bacterial spot disease management: copper sprays are applied during April and May when three successive rainy days occurred, with temperatures between 14 and 19ºC. The evaluation of the forecasting system predictions under field conditions using components 1 and 2, working together or independently, is necessary to define its final operational scheme according to the accuracy of the predictions and to simplify its use as much as possible.

In the third place, the value predicted by infection model of $S' = 0.5$ was proposed as the threshold for medium or high disease severity levels, but different thresholds should be tested under field conditions for its use in the management of the disease. Moreover, cumulative risk indexes should be evaluated, since different epidemiological studies agree on the need of successive rainy days or consecutive days with wetness periods for disease development (Battilani et al., 1999; Garcin et al., 2011b). A cumulative infection risk gives the additive effect of daily infection risk during periods over 24 h (e.g. accumulation over 3 or 5 days), which provides more accurate predictions in plant disease-warning systems (Kim et al., 2006; Magarey and Sutton, 2007).

Finally, since the forecasting system was developed using two susceptible *Prunus* species and cultivars and the intensity of the disease response may depend

on the host susceptibility (Garcin et al., 2007; Ritchie, 1995), field trials should be performed on different varieties of *Prunus* species to adjust the threshold of the infection risk in accordance to varietal resistance (Berger et al., 1996).

Applications of the disease forecasting model are twofold: guidance in disease surveillance and scheduling pesticides treatments. Since *X. arboricola* pv *pruni* is a quarantine pathogen in the EU, risk maps may be a useful tool to locate geographically areas with major risk of infection for early detection of outbreaks and to avoid the spread of the disease. Risk maps have been elaborated based on the predictions of the infection model of the regions of Emilia Romagna (Italy), where the disease is locally established since the late 1970s, and Catalunya (Spain), where few outbreaks have been detected and eradicated since 2009 (EPPO, 2017); although they are not included in this PhD thesis.

The results obtained in this thesis contributed with new knowledge on the epidemiology of the bacterial spot disease of stone fruit and offer new possibilities in the management of the disease, which encourage us to continue working to validate the model under field conditions and to make the forecasting model part of a decision support system.

Chapter 7:

Conclusions

According to the studies enclosed in this thesis, the following conclusions can be drawn:

1. Temperature, wetness duration and inoculum density have a significant effect on different steps of the disease cycle of bacterial spot of stone fruits, such as *Xanthomonas arboricola* pv. *pruni* multiplication, infection of *Prunus* by the bacterium and disease symptom development. The effects of each factor can be quantified and modeled.

2. The effect of temperature on the growth of *X. arboricola* pv. *pruni* has been modeled using the modified Ratkowsky model (*growth model*), whereby the maximum specific growth rate and doubling time are predicted. The growth of *X. arboricola* pv. *pruni* is optimum in the range of temperatures from 20 to 34ºC, with a maximum at 30ºC *in vitro* (μ_{max} = 0.260 h^{-1}) and 25ºC *ex vivo* (μ_{max} = 0.141 h^{-1}).

3. Wetness is required for epiphytic multiplication of *X. arboricola* pv. *pruni* on *Prunus*. The inactivation of bacterial epiphytic cells under low RH (< 40%) follows the Cerf's model (*inactivation model*), which predicts a rapid decrease in bacterial population during the first 6 h at low RH followed by a slow and continuous inactivation of bacterial cells.

4. Inoculum density of epiphytic populations of *X. arboricola* pv. *pruni* affects the infection process (disease incubation period and disease severity). The minimum infective concentration for *X. arboricola* pv. *pruni* is 10^6 CFU/ml, which can be established as the *inoculum threshold* for infection.

5. The infection of *Prunus* by *X. arboricola* pv. *pruni* is conditioned by wetness period duration and temperature. Short periods of leaf wetness (< 10 h) are enough to trigger the infection process at temperatures above 20ºC.

6. The combined effects of wetness duration and temperature on the infection of *Prunus* by *Xanthomonas arboricola* pv. *pruni* have been quantified and modeled using a modification of the Weibull equation proposed by Duthie (*infection model*). The infection model predicts a daily infection risk index (*S'*) from temperature and leaf wetness duration, and *S'* = 0.5 is proposed as the *infection risk threshold*.

7. Development of disease symptoms, once the infections are initiated, is affected by temperature and inoculum density. Short disease incubation periods and high disease severity occur at optimal temperatures (25-30ºC) and high inoculum densities (≥ 10^6 CFU/ml).
8. The relationship between temperature, expressed as cumulative-degree days (CDD), and the disease severity has been modeled with the Gompertz model (*symptom development model*). According to the symptom development model, 150, 175 and 280 CDD with a temperature base of 0ºC are necessary for disease severity of 5, 10 and 50%, respectively.
9. The models developed and the thresholds established are the basis of a forecasting system for bacterial spot disease of stone fruits.

Chapter 8:

References

A

Adaskaveg, J. E., Förster, H., Gubler, W. D., Teviotdale, B. L., & Thompson, D. F. (2005). Reduced-risk fungicides help manage brown rot and other fungal diseases of stone fruit. *California Agriculture*, *59*(2), 109–114.

Agrios, G. N. (2005). *Plant pathology* (5th edition). San Diego, California: Elsevier Academic Press.

Arauz, L. F., Neufeld, K. N., Lloyd, A. L., & Ojiambo, P. S. (2010). Quantitative models for germination and infection of *Pseudoperonospora cubensis* in response to temperature and duration of leaf wetness. *Phytopathology*, *100*(9), 959–967.

B

Badenes, M. L., & Byrne, D. H. (2012). Fruit breeding. New York: Springer Science & Business Media.

Baker, R. (1971). Analyses involving inoculum density of soil-borne plant pathogens in epidemiology. *Phytopathology*, 61, 1280–1292.

Ballard, E., Dietzgen, R., Sly, L., Gouk, C., Horlock, C., & Fegan, M. (2011). Development of a Bio-PCR protocol for the detection of *Xanthomonas arboricola* pv. *pruni*. *Plant Disease*, *95*(9), 1109–1115.

Bassil, N. V., Peace, C. P., Main, D., Gilmore, B., Mockler, T., Wilhelm, L., Chagne, D., Gardiner, S.E., Crowhurst, R., Verde, I., Sosinski, B., Morgante, M., Scalabrin, S., Arus, P., Velasco, R., Troggio, M., Cestaro, A., Ficklin, S., Fazio, G., Norelli, J., Rees, J., Lawley, C., Hansen, M., Iezzoni, A. (2011). RosBREED deploys genome-wide scans in peach, apple, and cherry. *HortScience*, *46*(9 suppl.), S101.

Battilani, P., Rossi, V., & Saccardi, A. (1999). Development of *Xanthomonas arboricola* pv. *pruni* epidemics on peaches. Journal of Plant Pathology, *81*(3), 161–171.

Bazzi, C., & Mazzucchi, U. (1980). Epidemic of *Xanthomonas pruni* on plum. *Informatore Fitopatologico*, 30(5), 11–17.

Beattie, G. A., & Lindow, S. E. (1999). Bacterial colonization of leaves: a spectrum of strategies. *Phytopathology*, *89*(5), 353–359.

Beresford, R. M., Tyson, J. L., & Henshall, W. R. (2017). development and validation of an infection risk model for bacterial canker of kiwifruit, using a multiplication and dispersal concept for forecasting bacterial diseases. *Phytopathology*, *107*(2), 184–191.

Berger, F., Zeller, W., Gutsche, V., & Rossberg, D. (1996). A new fire blight forecasting system with first results in Southwest Germany. *Acta Horticulturae*, *411*, 155–162.

Biggs, A. R., & Turechek, W. W. (2010). Fire blight of apples and pears: Epidemiological concepts comprising the Maryblyt forecasting program. *Plant Health Progress*. doi:10.1094/PHP-2010-0315-01-RS

Biggs, A. R., Turechek, W. W., & Box, P. O. (2014). Maryblyt™ 7.1 (for Windows). A predictive program for forecasting fire blight disease in apples and pears. Retrieved from http://atl.agrometeo.org/help/maryblyt_user-manual.pdf

Billing, E. (1974). The effect of temperature on the growth of the fireblight pathogen, *Erwinia amylovora*. *Journal of Applied Microbiology*, *37*(4), 643–648.

Billing, E. (1980). Fireblight *Erwinia amylovora* and weather: a comparison of warning systems. *Annals of Applied Biology*, *95*(3), 365–377.

Billing, E. (1984). Principles and applications of fire blight risk assessment systems. *Acta Horticulturae*, *151*, 15–22.

Billing, E. (1992). Billing's revised system (BRS) for fireblight risk assessment. *Bulletin OEPP/EPPO Bulletin*, *22*, 1–102.

Billing, E. (1996). BIS95, An improved approach to fire blight risk assessment. *Acta Horticulturae*, *411*, 121–126.

Billing, E. (1999). Fire blight risk assessment: Billing's integrated system (BIS) and its evaluation. *Acta Horticulturae*, *489*, 399–406.

Biondi, E., Bini, F., Lancioni, P., Anacleiro, F., Brunelli, A., & Bazzi, C. (2006). Biological control agents as tools against some emerging bacterial plant diseases in Italy: a concrete perspective? *Mitteilungen aus der Biologischen Bundesanstalt für Land-und Forstwirtschaft Berlin-Dahlem*, *408*, 170–176.

Blaauw, B., Brannen, P., Bellinger, B., Lockwood, D., & Ritchie, D. (2017). Southeastern peach, nectarine and plum pest management and culture guide. Athens, GA: University of Georgia.

Bonaterra, A., Mari, M., Casalini, L., & Montesinos, E. (2003). Biological control of *Monilinia laxa* and *Rhizopus stolonifer* in postharvest of stone fruit by *Pantoea*

agglomerans EPS125 and putative mechanisms of antagonism. *International Journal of Food Microbiology*, *84*(1), 93–104.

Borges, C. D., Moreira, A. S., Vendruscolo, C. T., & Ayub, M. A. Z. (2008). Influence of agitation and aeration in xanthan production by *Xanthomonas campestris* pv. *pruni* strain 101. *Revista Argentina de Microbiología*, *40*(2), 81–5.

Boudon, S., Manceau, C., & Nottéghem, J.-L. (2005). Structure and origin of *Xanthomonas arboricola* pv. *pruni* populations causing bacterial spot of stone fruit trees in western Europe. *Phytopathology*, *95*(9), 1081–1088.

Bull, C. T., De Boer, S. H., Denny, T. P., Firrao, G., Fischer-Le Saux, M., Saddler, G. S., Scortichini, M., Stead, D. E., Takikawa, Y. (2010). Comprehensive list of names of plant pathogenic bacteria, 1980-2007. *Journal of Plant Pathology*, *92*(3), 551–592.

Burdon, J. J., & Chilvers, G. A. (1982). Host density as a factor in plant disease ecology. *Annual Review of Phytopathology*, *20*(1), 143–166.

Byrne, D. H. (2005). Trends in stone fruit cultivar development. *HortTechnology*, *15*(3), 494–500.

C

Cambra, M. A. (2014). La mancha bacteriana de los frutales de hueso y del almendro. Available online: http://www.agrocistus.com/jornadas/2014 [Accessed: May, 2015].

Campbell, C., & Madden, L. (1990). *Introduction to plant disease epidemiology*. New York: Wiley.

Carisse, O., Bourgeois, G., & Duthie, J. (2000). Influence of temperature and leaf wetness duration on infection of strawberry leaves by *Mycosphaerella fragariae*. *Phytopathology*, *90*(10), 1120–1125.

Cerf, O. (1977). Tailing of survival curves of bacterial spores. *Journal of Applied Bacteriology*, *42*(1), 1–19.

Christiano, R. S. C., Dalla Pria, M., Jesus Junior, W. C., Amorim, L., & Bergamin Filho, A. (2009). Modelling the progress of Asiatic citrus canker on Tahiti lime in relation to temperature and leaf wetness. *European Journal of Plant Pathology*, *124*(1), 1–7.

Christiano, R. S. C., Dalla Pria, M., Jesus Junior, W. C., Parra, J. R. P., Amorim, L., & Bergamin Filho, A. (2007). Effect of citrus leaf-miner damage, mechanical damage and inoculum concentration on severity of symptoms of Asiatic citrus canker in Tahiti lime. *Crop Protection*, *26*(2), 59–65.

Civerolo, E. (1975). Quantitative aspects of pathogenesis of *Xanthomonas pruni* in peach leaves. *Phytopathology*, *65*, 258–264.

Civerolo, E. (1976). Phage sensitivity and virulence relationships in *Xanthomonas pruni*. *Physiological Plant Pathology*, *9*, 67–75.

Council Directive 2000/29/EC of 8 May 2000 on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community. Official Journal of the European Communities L 169, 10.7.2000, 1–112.

Crossman, L., & Dow, J. M. (2004). Biofilm formation and dispersal in *Xanthomonas campestris*. *Microbes and Infection*, *6*, 623–9.

D

D'Aquino, S., Cocco, A., Ortu, S., & Schirra, M. (2011). Effects of kaolin-based particle film to control *Ceratitis capitata* (Diptera: Tephritidae) infestations and postharvest decay in citrus and stone fruit. *Crop Protection*, 30(8), 1079–1086.

Damos, P. (2015). Modular structure of web-based decision support systems for integrated pest management. A review. *Agronomy for Sustainable Development*, *35*(4), 1347–1372.

Dechesne, A., Wang, G., Gulez, G., Or, D., & Smets, B. F. (2010). Hydration-controlled bacterial motility and dispersal on surfaces. *Proceedings of the National Academy of Sciences, 107*(32), 14369–14372.

Del Ponte, E. M., Godoy, C. V, Li, X., & Yang, X. B. (2006). Predicting severity of Asian soybean rust epidemics with empirical rainfall models. *Phytopathology*, 96(7), 797–803.

Departament d'Agricultura, Ramaderia, Pesca, Alimentació i Medi Natural (DAAM) (2016). Norma tècnica per a la producció integrada de fruita de pinyol. Annexos de control integrat de plagues, malalties i de matèries actives (Revisió 02.06.2016). Generalitat de Catalunya. Available online: http://agricultura.gencat.cat [Accessed: March, 2017].

Dhanvantari, B. (1971). Overwintering sources of inoculum of bacterial spot of peach (*Xanthomonas pruni*) in southwestern Ontario. *Proceedings of the Canadian Phytopathological Society*, 37, 21–30.

Dickson, J., & Holbert, J. (1928). The relation of temperature to the development of disease in plants. *The American Naturalist*, *62*(681), 311–333.

Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009 establishing a framework for Community action to achieve the sustainable use of pesticides. Official Journal of the European Communities L 309, 24.11.2009, 71–86.

Dow, J. M., Crossman, L., Findlay, K., He, Y.-Q., Feng, J.-X., & Tang, J.-L. (2003). Biofilm dispersal in *Xanthomonas campestris* is controlled by cell-cell signaling and is required for full virulence to plants. *Proceedings of the National Academy of Sciences*, *100*(19), 10995–11000.

du Plessis, H. J. (1986). Systemic migration and establishment of *Xanthomonas campestris* pv*. pruni* in plum leaves and twigs. *Journal of Phytopathology*, *116*(3), 221–227.

du Plessis, H. J. (1987). Canker development on plum shoots following systemic movement of *Xanthomonas campestris* pv*. pruni* from inoculated leaves. *Plant Disease*, *71*(12), 1078–1080.

du Plessis, H. J. (1988). Differential virulence of *Xanthomonas campestris* pv*. pruni* to peach, plum, and apricot cultivars. *Phytopathology*, *78*, 1312–1315.

Dunegan, J. (1932). The bacterial spot disease of the peach and other stone fruits. *Technical Bulletin US Department of Agriculture*, *273*, 1–53.

Duthie, J. A. (1997). Models of the response of foliar parasites to the combined effects of temperature and duration of wetness. *Phytopathology*, *87*(11), 1088–1095.

E

EFSA Panel on Plant Health. (2014). Scientific Opinion on pest categorisation of *Xanthomonas arboricola* pv*. pruni* (Smith, 1903). *EFSA Journal*, *12*(10), 3857.

EPPO. (2017). *Xanthomonas arboricola* pv. pruni (XANTPR). EPPO Global Database. Available online: https://gd.eppo.int [Accessed: February, 2014].

EPPO/CABI. (1997). Xanthomonas arboricola pv. pruni. In: Smith I.M., McNamara D.G., Scott P.R., Holderness M. (eds). Quarantine Pests for Europe (2nd edition, pp. 1096–1100). Wallingford, UK: CAB International.

Esker, P. D., Sparks, A. H., Campbell, L., Guo, Z., Rouse, M., Silwal, S. D., Tolos, S., Van Allen, B., & Garrett, K. A. (2008). Ecology and epidemiology in R: Disease forecasting and validation. *The Plant Health Instructor*. doi: 10.1094/PHI-A-2008-0129-01.

F

Faust, M., Timon, B., Surányi, D., Nyujtó, F., & Gradziel, T. M. (2011). Origin and dissemination of *Prunus* crops: peach, cherry, apricot, plum and almond. Leuven, Belgium: International Society for Horticultural Science (ISHS).

Feil, H., & Purcell, A. H. (2001). Temperature-dependent growth and survival of *Xylella fastidiosa* in vitro and in potted grapevines. *Plant Disease, 85*, 1230–1234.

Feliciano, A., & Daines, R. (1970). Factors influencing ingress of *Xanthomonas pruni* through peach leaf scars and subsequent development of spring cankers. *Phytopathology*, *60*, 1720–1726.

Fischer-Le Saux, M., Bonneau, S., Essakhi, S., Manceau, C., & Jacques, M.-A. (2015). Aggressive emerging pathovars of *Xanthomonas arboricola* represent widespread epidemic clones distinct from poorly pathogenic strains, as revealed by multilocus sequence typing. *Applied and Environmental Microbiology*, *81*(14), 4651–68.

Flemming, C. A., & Trevors, J. T. (1989). Copper toxicity and chemistry in the environment: a review. *Water, Air, and Soil Pollution, 44*, 143–158.

Fonseca, P., Moreno, R., & Rojo, F. (2011). Growth of *Pseudomonas putida* at low temperature: global transcriptomic and proteomic analyses. *Environmental Microbiology Reports*, *3*(3), 329–339.

Food and Agriculture Organization of the United Nations (FAOSTAT), 2017. Statistics Division of the Food and Agriculture Organization of the United Nations. Available online: http://faostat.fao.org [Accessed: May, 2017].

Francini, A., & Sebastiani, L. (2010). Copper effects on *Prunus persica* in two different grafting combinations (*P. persica* × *P. amygdalus* and *P. cerasifera*). *Journal of Plant Nutrition*, *33*(9), 1338–1352.

Francl, L. J. (2001). The disease triangle: a plant pathological paradigm revisited. *The Plant Health Instructor*. doi: 10.1094/PHI-T-2001-0517-01.

Fukui, R., Fukui, H., & Alvarez, A. M. (1999). Effect of temperature on the incubation period and leaf colonization in bacterial blight of anthurium. *Phytopathology*, *89*(11), 1007–14.

G

Garcin, A. (2000). Maladie des taches bactériennes des arbres à noyau. Un nouveau pathogène en France. *Infos CTIFL*, *165*, 45–47.

Garcin, A., & Bresson, J. (2009). Sensibilité des arbres à noyau au *Xanthomonas*. Bilan de 8 ans d'expérimentation. *Infos CTIFL*, 254, 30–35.

Garcin, A., Neyrand, S., & Fabresse, M. (2007). Fruits á noyau: Sensibilité variétale au *Xanthomonas*. *L'arboriculture frutière*, *612*, 28–32.

Garcin, A., Rouzet, J., & Notteghem, J. (2005). *Xanthomonas* des arbres fruitiers à noyau. Paris, France: Edition CTFL.

Garcin, A., Vibert, J., & Cellier, M. (2011a). *Xanthomonas* sur pêcher: étude des conditions d'infection. Functionnement du modèle et résultats d'essais (2e partie). *Infos CTIFL*, *272*, 30–39.

Garcin, A., Vibert, J., & Leclerc, A. (2011b). *Xanthomonas* sur pêcher: étude des conditions d'infection. Développement de l'outil (1re partie). *Infos CTIFL*, *268*, 26–39.

García-Ochoa, F., Santos, V., & Alcon, A. (1998). Metabolic structured kinetic model for xanthan production. *Enzyme and Microbial Technology*, *23*, 75–82.

Garita-Cambronero, J., Palacio-Bielsa, A., López, M. M., & Cubero, J. (2016). Draft genome sequence for virulent and avirulent strains of *Xanthomonas arboricola* isolated from *Prunus* spp. in Spain. *Standards in Genomic Sciences*, *11*, 12.

Gent, D. H., De Wolf, E., & Pethybridge, S. J. (2011). Perceptions of risk, risk aversion, and barriers to adoption of decision support systems and integrated pest management: An Introduction. *Phytopathology*, *101*(6), 640–643.

Goodman, R. N. (1976). Physiological and cytological aspects of the bacterial infection process. In: Heitefuss, R., & Williams, P. H. (eds.). *Physiological Plant Pathology. Encyclopedia of Plant Physiology* (New Series), vol.4, pp. 172–196. Berlin: Springer.

Gradziel, T. M. (2009). Almond (*Prunus dulcis*) breeding. In: Priyadarshan, P.M., & Jain Mohan, S. (eds.) *Breeding plantation tree crops: Temperate species* (pp. 1–31). New York: Springer.

H

Hajri, A., Pothier, J. F., Fischer-Le Saux, M., Bonneau, S., Poussier, S., Boureau, T., Duffy, B., & Manceau, C. (2012). Type three effector gene distribution and sequence analysis provide new insights into the pathogenicity of plant-pathogenic *Xanthomonas arboricola*. *Applied and Environmental Microbiology*, *78*(2), 371–84.

Haworth, R. H., & Spiers, A. G. (1992). Isolation of *Xanthomonas campestris* pv*. populi* from stem lesions on *Salix matsudana* x *alba* 'Aokautere' in New Zealand. *European Journal of Forest Pathology*, *22*(4), 247–251.

Hayward, A., & Waterston, J. (1965). *Xanthomonas pruni*. In: *IMI Descriptions of Fungi and Bacteria*, No. 50. Wallingford: CAB International.

He, Y. W., & Zhang, L. H. (2008). Quorum sensing and virulence regulation in *Xanthomonas campestris*. *FEMS Microbiology Reviews*, *32*(5), 842–857.

Hirano, S. S., Baker, L. S., & Upper, C. D. (1996). Raindrop momentum triggers growth of leaf-associated populations of *Pseudomonas syringae* on field-grown snap bean plants. *Applied and Environmental Microbiology*, *62*(7), 2560–6.

Huang, J. (1986). Ultrastructure of bacterial penetration in plants. *Annual Review of Phytopathology, 24*(1), 141–157.

Huber, L., & Gillespie, T. J. (1992). modeling leaf wetness in relation to plant disease epidemiology. *Annual Review of Phytopathology, 30*(1), 553–577.

I

Il'ichev, A. L., Williams, D. G., & Milner, A. D. (2004). Mating disruption barriers in pome fruit for improved control of oriental fruit moth *Grapholita molesta* Busck

(Lep., Tortricidae) in stone fruit under mating disruption. *Journal of Applied Entomology*, *128*(2), 126–132.

Imaizumi, S., Honda, M., & Fujimori, T. (1999). Effect of temperature on the control of annual bluegrass (*Poa annua* L.) with *Xanthomonas campestris* pv. *poae* (JT-P482). *Biological Control*, *16*(1), 13–17.

Isard, S. A., Russo, J. M., Magarey, R. D., Golod, J., & VanKirk, J. R. (2015). Integrated Pest Information Platform for Extension and Education (iPiPE): Progress through sharing. *Journal of Integrated Pest Management*, *6*(1), 15. doi: 10.1093/jipm/pmv013

J

Janick, J. (2005). The origins of fruits, fruit growing, and fruit breeding. In: *Plant Breeding Reviews Vol. 25* (pp. 255–321). Oxford, UK: John Wiley & Sons.

Janse, J. D. (2012). Bacterial diseases that may or do emerge, with (possible) economic damage for Europe and the Mediterranean basin: Notes on epidemiology, risks, prevention and management on first occurrence. *Journal of Plant Pathology*, *94*(Supplement 4), S4.5-S4.29.

Janse, J. D., Rossi, M. P., Gorkink, R. F. J., Derks, J. H. J., Swings, J., Janssens, D., & Scortichini, M. (2001). Bacterial leaf blight of strawberry (*Fragaria (x) ananassa*) caused by a pathovar of *Xanthomonas arboricola*, not similar to *Xanthomonas fragariae* Kennedy & King. Description of the causal organism as *Xanthomonas arboricola* pv. *fragariae* (pv. nov., comb. nov.). *Plant Pathology*, *50*(6), 653–665.

Jones, A. L. (1992). Evaluation of the computer model MARYBLYT for predicting fire blight blossom infection on apple in Michigan. *Plant Disease, 76*(4), 344–347.

Jones, A. L., & Sutton, T. B. (1996). *Diseases of tree fruits in the East*. East Lansing, MI: Michigan State University, Cooperative Extension Service.

Jones, J. B. (1984). Effects of temperature and leaf wetness on development of bacterial spot of Geraniums and Chrysanthemums incited by *Pseudomonas cichorii*. *Plant Disease*, *68*(3), 248–251.

K

Kam, M. (1984). *Xanthomonas campestris* pv. *populi*, the causal agent of bark necrosis in poplar. *Netherlands Journal of Plant Pathology*, *90*(1), 13–22.

Kawaguchi, A. (2014). Risk factors for bacterial spot on peach in Okayama Prefecture, Japan. *Journal of General Plant Pathology, 80*(5), 435–442.

Kim, J., Kang, W., & Yun, S. (2014). Development of a model to predict the primary infection date of bacterial spot (*Xanthomonas campestris* pv*. vesicatoria*) on hot pepper. *The Plant Pathology Journal*, *30*(2), 125–135.

Kim, K. S., Gleason, M. L., & Taylor, S. E. (2006). Forecasting site-specific leaf wetness duration for input to disease-warning systems. *Plant Disease*, *90*(5), 650–656.

King, W. T., Madden, L. V., Ellis, M. A., & Wilson, L. L. (1997). Effects of temperature on sporulation and latent period of *Colletotrichum* spp. infecting strawberry fruit. *Plant Disease*, *81*(1), 77–84.

Koizumi, M., & Kochinotsu, B. (1977). Relation of temperature to the development of citrus canker lesions in the spring. *Proceedings of International Society of Citriculture*, *3*, 924–928.

Kole, C., & Abbott, A. G. (2012). *Genetics, genomics and breeding of stone fruits*. New York: CRC Press.

Kunito, T., Saeki, K., Oyaizu, H., & Matsumoto, S. (1999). Influences of copper forms on the toxicity to microorganisms in soils. *Ecotoxicology and Environmental Safety*, *44*, 174–181.

Kushalappa, A. C., & Zulfiquar, M. (2001). Effect of wet incubation time and temperature on infection, and of storage time and temperature on soft rot lesion expansion in potatoes inoculated with *Erwinia carotovora* ssp. c*arotovora*. *Potato Research, 44*, 233–242.

L

Lalancette, N., & McFarland, K. (2007). Phytotoxicity of copper-based bactericides to peach and nectarine. *Plant Disease*, *91*(9), 1122–1130.

Lamichhane, J. R. (2014). *Xanthomonas arboricola* diseases of stone fruit, almond, and walnut trees: progress toward understanding and management. *Plant Disease*, *98*(12), 1600–1610.

Latorre, B. A., Lillo, C., & Rioja, M. E. (2002a). Effects of temperature, free moisture duration and inoculum concentration on infection of sweet cherry by *Pseudomonas syringae* pv*. syringae. Phytoparasitica*, *30*(4), 410–419.

Latorre, B. A., Rioja, M. E., & Lillo, C. (2002b). The effect of temperature on infection and a warning system for pear blossom blast caused by *Pseudomonas syringae* pv*. syringae*. *Crop Protection*, *21*(1), 33–39.

Latorre, B. A., Rioja, M. E., Lillo, C., & Muñoz, M. (2002c). The effect of temperature and wetness duration on infection and a warning system for European canker (*Nectria galligena*) of apple in Chile. *Crop Protection*, *21*(4), 285–291.

Laurens, F., Durel, C. E., Patocchi, A., Peil, A., Salvi, S., Tartarini, S., Velasco, R., van de Weg, E. (2010). Review on apple genetics and breeding programmes and presentation of a new European initiative to increase fruit breeding efficiency. *Journal of Fruit Science*, *27*, 102–107.

Leben, C. (1981). How plant-pathogenic bacteria survive. *Plant Disease. 65*(8), 633–637.

Letisse, F., Lindley, N., & Roux, G. (2003). Development of a phenomenological modeling approach for prediction of growth and xanthan gum production using *Xanthomonas campestris*. *Biotechnology Progress*, *19*, 822–827.

Lightner, G. W., & Steiner, P. W. (1992). Maryblyt^{TM}: A computer model for predicting of fire blight disease in apples and pears. *Computers and Electronics in Agriculture*, *7*(3), 249–260.

Lindemann, J. (1984). Use of an apparent infection threshold population of *Pseudomonas syringae* to predict incidence and severity of brown spot of bean. *Phytopathology*, *74*(11), 1334–1339.

Linvill, D. (2002). Use the proper environmental temperature to describe disease. *Acta Horticulturae, 592*, 689–694.

Llorente, I., Badosa, A., Vilardell, P., & Montesinos, E. (2002). Epidemiological studies of fire blight (*Erwinia amylovora*) in Spain. *Acta Horticulturae*, *596*, 535–538.

Llorente, I., & Montesinos, E. (2004). Development and field evaluation of a model to estimate the maturity of pseudothecia of *Pleospora allii* on pear. *Plant Disease*, *88*, 215–219.

Llorente, I., Vilardell, P., Bugiani, R., Gherardi, I., & Montesinos, E. (2000). Evaluation of BSPcast disease warning system in reduced fungicide use

programs for management of brown spot of pear. *Plant Disease*, *84*(6), 631–637.

Llorente, I., Vilardell, P., Isern, M., Montesinos, E., & Moragrega, C. (2017). Fire blight risk assessment in Girona region (Catalonia): comparison of Maryblyt and CougarBlight models. *Journal of Plant Pathology*, *99*(Special issue), 75–80.

López-Soriano, P., Boyer, K., Cesbron, S., Morente, M. C., Peñalver, J., Palacio-Bielsa, A., Vernière, C., López, M. M., & Pruvost, O. (2016). Multilocus variable number of tandem repeat analysis reveals multiple introductions in Spain of *Xanthomonas arboricola* pv. *pruni*, the causal agent of bacterial spot disease of stone fruits and almond. *PLoS ONE*, 11(9), e0163729.

Lovell, D. J., Hunter, T., Powers, S. J., Parker, S. R., & Van den Bosch, F. (2004). Effect of temperature on latent period of septoria leaf blotch on winter wheat under outdoor conditions. *Plant Pathology*, *53*(2), 170–181.

M

Magarey, R. D., Fowler, G. A., Borchert, D. M., Sutton, T. B., Colunga-Garcia, M., & Simpson, J. A. (2007). NAPPFAST: An internet system for the weather-based mapping of plant pathogens. *Plant Disease, 91*(4), 336–345.

Magarey, R. D., & Isard, S. A. (2017). A troubleshooting guide for mechanistic plant pest forecast models. *Journal of Integrated Pest Management*, *8*(1), 1–7.

Magarey, R. D., Seem, R. C., Weiss, A., Gillespie, T., & Huber, L. (2005a). Estimating surface wetness on plants. In: *Micrometeorology in Agricultural Systems*, Agronomy Monograph no. 47. Lincoln, Nebraska: University of Nebraska.

Magarey, R. D., & Sutton, T. B. (2007). How to create and deploy infection models for plant pathogens. In: Ciancio, A., & Mukerji, K.G. (eds.). *Integrated Management of Plants Pests and Diseases* (Volume 1, pp. 3–25). Dordrecht: Springer Netherlands.

Magarey, R. D., Sutton, T. B., & Thayer, C. L. (2005b). A simple generic infection model for foliar fungal plant pathogens. *Phytopathology*, *95*(1), 92–100.

Magarey, R. D., Travis, J. W., Russo, J. M., Seem, R. C., & Magarey, P. A. (2002). Decision support systems: quenching the thirst. *Plant Disease, 86*(1), 4–14.

Marchi, G., Cinelli, T., & Surico, G. (2011). Bacterial leaf spot caused by the quarantine pathogen *Xanthomonas arboricola* pv*. pruni* on cherry laurel in Central Italy. *Plant Disease*, *95*(1), 74.

Matthee, F. N., & Daines, R. H. (1968). Effects of soil types and substrate aeration on stomatal activity, water diffusion pressure deficit, water congestion, and bacterial infection of peach and pepper foliage. *Phytopathology*, *58*, 1298–1301.

Matthee, F. N., & Daines, R. H (1969). The influence of nutrition on susceptibility of peach foliage to water congestion and infection by X*anthomonas pruni. Phytopathology*, *59*, 285–287.

Mayer, L., Vendruscolo, C. T., Silva, W. P., Vorhölter, F.-J., Becker, A., & Pühler, A. (2011). Insights into the genome of the xanthan-producing phytopathogen *Xanthomonas arboricola* pv. *pruni* 109 by comparative genomic hybridization. *Journal of Biotechnology*, *155*(1), 40–49.

Medeiros, J., Citadin, I., Santos, I., & Assmann, A. (2011). Reaction of peach tree genotypes to bacterial leaf spot caused by *Xanthomonas arboricola* pv*. pruni*. *Scientia Agricola*, *68*(1), 57–61.

Melotto, M., Underwood, W., Koczan, J., Nomura, K., & He, S. Y. (2006). Plant stomata function in innate immunity against bacterial invasion. *Cell, 126*(5), 969–980.

Moh, A., Massart, S., & Lahlali, R. (2011). Predictive modelling of the combined effect of temperature and water activity on the *in vitro* growth of *Erwinia* spp. infecting potato tubers in Belgium. *Biotechnology, Agronomy, Society and Environment*, *15*(3), 379–386.

Montesinos, E. (2003). Development, registration and commercialization of microbial pesticides for plant protection. *International Microbiology*, *6*(4), 245–252.

Montesinos, E., & Bardají, E. (2008). Synthetic antimicrobial peptides as agricultural pesticides for plant-disease control. *Chemistry and Biodiversity*, *5*(7), 1225–1237.

Montesinos, E., & Bonaterra, A. (2009). Microbial pesticides. In: Schaechter, M. (ed.) *Encyclopedia of Microbiology* (3rd edition, pp. 110–120). Elsevier Inc.

Montesinos, E., Moragrega, C., Llorente, I., Vilardell, P., Bonaterra, A., Ponti, I., Bugiani, R, Cavanni, P, & Brunelli, A. (1995). Development and evaluation of

an infection model for *Stemphylium vesicarium* on pear based on temperature and wetness duration. *Phytopathology*, *85*(5), 586–592.

Morales, G., Llorente, I., Montesinos, E., & Moragrega, C. (2016). Basis for a predictive model of *Xanthomonas arboricola* pv*. pruni* growth and infections in host plants. *Acta Horticulturae*, *1149*, 1–8.

N

Nasrollahnejad, S., & Mousavi, S. (2008). The investigation of resistance to *Prunus* bacterial spot among some cultivars of three *Prunus* species and chemical control of PBS in Golestan province of Iran. *Australian Journal of Basic and Applied Sciences*, *2*(4), 997–1002.

Ninot, A., Aletà, N., Moragrega, C., & Montesinos, E. (2002). Evaluation of a reduced copper spraying program to control bacterial blight of walnut. *Plant Disease*, *86*(6), 583–587.

Notermans, S., Gallhoff, G., Zwietering, M. H., & Mead, G. C. (1995). Identification of critical control points in the HACCP system with a quantitative effect on the safety of food products. *Food Microbiology*, *12*, 93–98.

O

Ogawa J. M., Zehr E. I., Bird G. W., Ritchie D. F., Uriu, K., & Uyemoto, J. K. (1995). *Compendium of stone fruit diseases*. St. Paul: APS.

Okie, W. (1998). *Handbook of peach and nectarine varieties. Performance in the Southeastern United States and index of names* (Agriculture Handbook No. 714, pp. 814). U.S. Department of Agriculture (USDA).

P

Pagani, M. (2004). An ABC transporter protein and molecular diagnoses of *Xanthomonas arbricola* pv. *pruni* causing bacterial spot of stone fruits. Ph.D. Thesis. University of North Carolina State, Raleigh, NC, USA. Available online: http://www.lib.ncsu.edu/resolver/1840.16/4540 [Accessed: December, 2013].

Palacio-Bielsa, A., Berruete, I. M., Lopez, M. M., Peñalver, J., Morente, C., Cubero, J., Garita-Cambronero, J., Sabuquillo, P., Redondo, C., Mitidieri, M., Gomes, C. B., Ueno, B., Suita de Castro, L. A., Leoni, C., & Silvera, E. (2015). La mancha

bacteriana de los frutales de hueso y del almendro (*Xanthomonas arboricola* pv. *pruni*) en España y Sudamérica. *Phytoma-España*, *271*, 21–28.

Palacio-Bielsa, A., Cubero, J., Cambra, M. A., Collados, R., Berruete, I. M., & Lopez, M. M. (2011). Development of an efficient real-time quantitative PCR protocol for detection of *Xanthomonas arboricola* pv*. pruni* in *Prunus* species. *Applied and Environmental Microbiology*, *77*(1), 89–97.

Palacio-Bielsa, A., Roselló, M., Cambra, M. A., & López, M. M. (2010). First report on almond in Europe of bacterial spot disease of stone fruits caused by *Xanthomonas arboricola* pv*. pruni. Plant Disease*, *94*(6), 786.

Panchal, S., Roy, D., Chitrakar, R., Price, L., Breitbach, Z. S., Armstrong, D. W., & Melotto, M. (2016). Coronatine facilitates *Pseudomonas syringae* infection of *Arabidopsis* leaves at night. *Frontiers in Plant Science, 7*, 880. doi: 10.3389/fpls.2016.00880

Paul, P., & Munkvold, G. (2005). Influence of temperature and relative humidity on sporulation of *Cercospora zeae-maydis* and expansion of gray leaf spot lesions on maize leaves. *Plant Disease*, *89*, 624–630.

Pothier, J. F., Smits, T. H. M., & Blom, J. (2011a). Complete genome sequence of the stone fruit pathogen *Xanthomonas arboricola* pv*. pruni*. *Phytopathology*, *101*, S144–S145.

Pothier, J. F., Vorhölter, F.-J., Blom, J., Goesmann, A., Pühler, A., Smits, T. H. M., & Duffy, B. (2011b). The ubiquitous plasmid pXap41 in the invasive phytopathogen *Xanthomonas arboricola* pv*. pruni*: complete sequence and comparative genomic analysis. *FEMS Microbiology Letters*, *323*(1), 52–60.

Purusharth, R. I., Madhuri, B., & Ray, M. K. (2007). Exoribonuclease R in *Pseudomonas syringae* is essential for growth at low temperature and plays a novel role in the 3′ end processing of 16 and 5 S ribosomal RNA. *Journal of Biological Chemistry*, *282*(22), 16267–16277.

R

Randhawa, P. S., & Civerolo, E. (1985). A detached-leaf bioassay for *Xanthomonas campestris* pv*. pruni. Phytopathology*, *75*(9), 1060–1063.

Ritchie, D. F. (1995). Bacterial spot. In: Ogawa J. M., Zehr E. I., Bird G. W., Ritchie D. F., Uriu, K., & Uyemoto, J. K (eds.) *Compendium of stone fruit diseases*. St. Paul: APS Press.

Ritchie, D. F. (2004). Copper-containing fungicides/bactericides and their use in management of bacterial spot on peaches. *Southeast Regional Newsletter*, *4*(1).

Ritchie, D. F. (2005). Bacterial spot. Available online: http://www.ent.uga.edu/peach/peachhbk/bacterial/bacterialspot.pdf [Accessed: December, 2013].

Ritchie, D. F. (2011). *Learning from peach bacterial spot epidemics: potential strategies for reducing fruit losses*. Presentation at the Great Lakes Fruit, Vegetable and Farm Market EXPO, Grand Rapids, MI. Available online: https://plantpathology.ces.ncsu.edu [Accessed: April, 2017].

Roden, L. C., & Ingle, R. A. (2009). Lights, rhythms, infection: the role of light and the circadian clock in determining the outcome of plant-pathogen interactions. *The Plant Cell, 21*(9), 2546–2552.

Regione Emilia Romagna (2017). Norme tecniche di coltura. Difesa integrata pesco. Available online: http://agricoltura.regione.emilia-romagna.it [Accessed: April, 2017].

Rosalam, S., & England, R. (2006). Review of xanthan gum production from unmodified starches by *Xanthomonas comprestris* sp. *Enzyme and Microbial Technology*, *39*, 197–207.

Roselló, G. (2015). Characterization and improvement of plant-associated *Lactobacillus plantarum*. Novel biocontrol agents for fire blight disease. Ph.D. Thesis. University of Girona, Spain. Available online: http://www.tdx.cat/handle/10803/403404 [Accessed: October, 2016].

Roselló, M., & Santiago, R. (2012). Current status of bacterial spot of stone fruits and almond caused by *Xanthomonas arboricola* pv*. pruni* in Spain. *Journal of Plant Pathology*, *94*(Supplement 1), S1.15–S1.21.

Rouse, D. I. (1985). A model relating the probability of foliar disease incidence to the population frequencies of bacterial plant pathogens. *Phytopathology*, *75*(5), 505–509.

Ruissen, M. A., Vossen, R. T. M., & Kocks, C. G. (1993). Growth of *Xanthomonas campestris* pv*. campestris* populations at constant and variable temperatures. *Netherlands Journal of Plant Pathology, 99*(Supplement 3), 173–179.

Ruiz, C., Nadal, A., Montesinos, E., & Pla, M. (2017). Novel Rosaceae plant elicitor peptides as sustainable tools to control *Xanthomonas arboricola* pv*. pruni* in *Prunus* spp. *Molecular Plant Pathology*. doi: 10.1111/mpp.12534

Ruz, L. (2005). Improvement of strategies for the management of fire blight (*Erwinia amylovora*). Evaluation and optimization of physical and chemical control methods, and use of decision support systems. Ph.D. Thesis. University of Girona, Spain. Available online: http://www.tdx.cat/handle/10803/7923 [Accessed: January, 2014].

Ryan, R. P., Vorhölter, F.-J., Potnis, N., Jones, J. B., Van Sluys, M.-A., Bogdanove, A. J., & Dow, J. M. (2011). Pathogenomics of *Xanthomonas*: understanding bacterium-plant interactions. *Nature Reviews Microbiology*, *9*(5), 344–355.

S

Schouten, H. (1987). A revision of Billing's potential doublings table for fire blight prediction. *Netherlands Journal of Plant Pathology*, *93*, 55–60.

Sciarretta, A., & Trematerra, P. (2006). Geostatistical characterization of the spatial distribution of *Grapholita molesta* and *Anarsia lineatella* males in an agricultural landscape. *Journal of Applied Entomology*, *130*(2), 73–83.

Scortichini, M. (2010). Epidemiology and predisposing factors of some major bacterial diseases of stone and nut fruit trees species. *Journal of Plant Pathology*, *92*(Supplement 1), S1.73-S1.78.

Shepard, D., & Zehr, E. (1994). Epiphytic persistence of *Xanthomonas campestris* pv*. pruni* on peach and plum. *Plant Disease*, *78*(6), 627–629.

Sholberg, A. P., & Kappel, F. (2008). Integrated management of stone fruit diseases. In: Ciancio, A., & Mukerji, K.G. (eds.). *Integrated Management of Plants Pests and Diseases* (Volume 3, pp. 3–25). Dordrecht: Springer Netherlands.

Shrestha, R., Lee, S., Hur, J., & Lim, C. (2005). The effects of temperature, pH, and bactericides on the growth of *Erwinia pyrifoliae* and *Erwinia amylovora*. *The Plant Pathology Journal*, *21*(2), 127–131.

Shtienberg, D. (2013). Will decision-support systems be widely used for the management of plant diseases? *Annual Review of Phytopathology*, *51*(1), 1–16.

Shtienberg, D., Shwartz, H., Oppenheim, D., Zilberstaine, M., Herzog, Z., Manulis, S., & Kritzman, G. (2003). Evaluation of local and imported fire blight warning systems in Israel. *Phytopathology*, *93*(3), 356–63.

Smith, E. (1903). Observation on a hitherto unreported bacterial disease the cause of which enters the plant through ordinary stomata. *Science*, *17*, 456–457.

Smith, T. J. (1993). A predictive model for forecasting fire blight of pear and apple in Washington State. *Acta Horticulturae*, *338*, 153–160.

Smith, T. J., & Pusey, P. L. (2011). CougarBlight 2010, a significant update of the CougarBlight fire blight infection risk model. *Acta Horticulturae*, *896*, 331–336.

Socquet-Juglard, D. (2012). Genetic determinism of *Xanthomonas arboricola* pv*. pruni* resistance and agronomic traits in apricot, and applications in marker-assisted selection. Ph.D. Thesis. Swiss Federal Institute of Technology (ETH), Zurich. Available online: https://doi.org/10.3929/ethz-a-007620646 [Accessed: February, 2014].

Socquet-Juglard, D., Duffy, B., Pothier, J. F., Christen, D., Gessler, C., & Patocchi, A. (2013). Identification of a major QTL for *Xanthomonas arboricola* pv*. pruni* resistance in apricot. *Tree Genetics & Genomes*, *9*(2), 409–421.

Socquet-Juglard, D., Patocchi, A., Pothier, J. F., Christen, D., & Duffy, B. (2012). Evaluation of *Xanthomonas arboricola* pv*. pruni* inoculation techniques to screen for bacterial spot resistance in peach and apricot. *Journal of Plant Pathology*, *94*(Supplement 1), S1.91-S1.96.

Sparks, A. H. (2009). Disease risk mapping with metamodels for coarse resolution predictors: global potato late blight risk now and under future climate conditions. Ph.D. Thesis. Kansas State University, Manhattan. Available online: https://krex.k-state.edu/dspace/handle/2097/2341?show=full [Accessed: June, 2017].

Stefani, E. (2010). Economic significance and control of bacterial spot/canker of stone fruits caused by *Xanthomonas arboricola* pv*. pruni*. *Journal of Plant Pathology*, *92*(Supplement 1), 99–104.

Steiner, P. (1990). Predicting apple blossom infections by *Erwinia amylovora* using the MARYBLYT model. *Acta Horticulturae*, 273, 139–148.

Suesada, Yuko, Yamada, Masahiko, Yamane, & Takayoshi. (2013). Varietal differences in susceptibility to bacterial spot (*Xanthomonas arboricola* pv*. pruni*) among 69 peach cultivars and selections as evaluated by artificial inoculation to shoots. *Journal of the Japanese Society for Horticultural Science*, *82*(4), 293–300.

Swings, J. G., & Civerolo, E. L. (1993). *Xanthomonas*. Dordrecht: Springer Netherlands.

T

Timmer, L., Marois, J., & Achor, D. (1987). Growth and survival of xanthomonads under conditions nonconducive to disease development. *Phytopathology*, *77*, 1341–1345.

Tjou-Tam-Sin, N. N. A., van de Bilt, J. L. J., Bergsma-Vlami, M., Koenraadt, H., Naktuinbouw, J. W., van Doorn, J., Pham, K. T. K., & Martin, W. S. (2012). First report of *Xanthomonas arboricola* pv*. pruni* in ornamental *Prunus laurocerasus* in the Netherlands. *Plant Disease*, *96*(5), 759.

Tomihama, T., Nonaka, T., Nishi, Y., & Arai, K. (2009). Environmental control in tea fields to reduce infection by *Pseudomonas syringae* pv*. theae. Phytopathology*, *99*(2), 209–216.

Trapero-Casas, A., & Kaiser, W. (1992). Influence of temperature, wetness period, plant age, and inoculum concentration on infection and development of Ascochyta blight of chickpea. *Phytopathology*, *82*, 589–596.

U

United States Department of Agriculture (USDA) (2016). Fresh Peaches and Cherries: World Markets and Trade. Available online: https://apps.fas.usda.gov/psdonline/circulars/StoneFruit.pdf [Accessed: December, 2016].

V

van der Zwet, T., Biggs, A. R., Heflebower, R., & Lightner, G. W. (1994). Evaluation of the MARYBLYT computer model for predicting blossom blight on apple in West Virginia and Maryland. *Plant Disease*, *78*(3), 225–230.

Vandroemme, J., Cottyn, B., Pothier, J. F., Pflüger, V., Duffy, B., & Maes, M. (2013). *Xanthomonas arboricola* pv*. fragariae*: what's in a name? *Plant Pathology*, *62*(5), 1123–1131.

Vanneste, J., McLaren, G., & Yu, J. (2005). Copper and streptomycin resistance in bacterial strains isolated from stone fruit orchards in New Zealand. *New Zealand Plant Protection*, *58*, 101–105.

Vauterin, L., Hoste, B., Kersters, K., & Swings, J. (1995). Reclassification of *Xanthomonas*. *International Journal of Systematic Bcteriology*, *45*(3), 472–489.

Velasco, D., Hough, J., Aradhya, M., & Ross-Ibarra, J. (2016). Evolutionary genomics of peach and almond domestication. *G3: Genes, Genomes, Genetics*, *6*(12), 3985–3993.

Vilardell, P., Vilajeliu, M., Batllori, L., & Llorente, I. (2012). Dynamic release of ascospores of *Venturia inaequalis* and apple scab disease control strategy based on the Mills and RIMpro models in Girona (Catalonia-Spain). *IOBC/wprs Bulletin*, *84*, 247–253.

von Bodman, S. B., Bauer, W. D., & Coplin, D. L. (2003). Quorum sensing in plant-pathogenic bacteria. *Annual Review of Phytopathology, 41*, 455–82.

W

Wearing, C. H. (1988). Evaluating the IPM implementation process. *Annual Review of Entomology*, *33*(1), 17–38.

Werner, D. J., Ritchie, D. F., Cain, D. W., & Zehr, E. I. (1986). Susceptibility of peaches and nectarines, plant introductions, and other *Prunus* species to bacterial spot. *HortScience*, *21*, 127–130.

Whitehead, N. A., Barnard, A. M. L., Slater, H., Simpson, N. J. L., & Salmond, G. P. C. (2001). Quorum-sensing in Gram-negative bacteria. *FEMS Microbiology Reviews*, *25*(4), 365–404.

Y

Yang, N., Reighard, G., Ritchie, D., Okie, W., & Gasic, K. (2013). Mapping quantitative trait loci associated with resistance to bacterial spot (*Xanthomonas arboricola* pv*. pruni*) in peach. *Tree Genetics & Genomes*, *9*(2), 573–586.

Young, J. M. (1974). Effect of water on bacterial multiplication in plant tissue. *New Zealand Journal of Agricultural Research*, *17*(1), 115–119.

Young, J. M., Dye, D. W., Bradbury, J. F., Panagopoulos, C. G., & Robbs, C. F. (1978). A proposed nomenclature and classification for plant pathogenic bacteria. *New Zealand Journal of Agricultural Research*, *21*(1), 153–177.

Young, J. M., Luketina, R. C., & Marshall, A. M. (1977). The effects on temperature on growth *in vitro* of *Pseudomonas syringae* and *Xanthomonas pruni. Journal of Applied Bacteriology*, *42*(3), 345–354.

Yuen, J. E., & Hughes, G. (2002). Bayesian analysis of plant disease prediction. *Plant Pathology*, *51*(4), 407–412.

Z

Zaccardelli, M., Consiglio, M., & Mazzucchi, U. (1995). Detection of *Xanthomonas campestris* pv*. pruni* in symptomless peach trees in winter. *Phytopathologia Mediterranea*, *34*(3), 199–203.

Zaccardelli, M., Malaguti, S., & Bazzi, C. (1998). Biological and epidemiological aspects of *Xanthomonas arboricola* pv*. pruni* on peach in Italy. *Journal of Plant Pathology*, *80*(2), 125–132.

Zehr, E. I., Shepard, D. P., & Bridges Jr, W. C. (1996). Bacterial spot of peach as influenced by water congestion, leaf wetness duration, and temperature. *Plant Disease, 80*(3), 339–341.