# DESCRIPTION AND PRELIMINARY VALIDATION OF RIMpro-Erwinia A NEW MODEL FOR FIRE BLIGHT FORECAST

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**Abstract**

**Fire blight caused by *Erwinia amylovora* is a sporadic disease that can cause major damage and important tree loss in many apple and pear growing regions. Many infections occur when suitable weather conditions are met during bloom period. Timely applications of antibiotics or biocontrol agents during this period can dramatically reduce disease severity. Consequently, a number of forecasting systems have been developed to help predict disease outbreaks based on these criteria. Unfortunately, these systems can generate false positive warnings because either the inoculum pressure in orchards was not sufficient to cause disease, because the models can overestimate pathogen growth, or the cultivar is less susceptible. Conversely, models can also generate false negative prognosis under conditions considered marginal for bacterial growth or when localised wetness events cannot be recorded adequately. The RIMpro-erwinia model addresses part of these problems by including recent findings on bacterial growth and infection through a simulation approach. The software calculates bacterial growth and the possibility of infection on each individual daily flower cohorts. Epiphytic bacterial growth calculations are based on a nonlinear model that accounts for low temperature growth. Flower infection is predicted based on population size during wetness events. Flower cohorts not meeting the colonization and infection criteria are discarded from calculations as they age. Preliminary data collected since 2007 suggest that using this approach improves blight prediction as compared to Cougarblight and Maryblyt.**

## INTRODUCTION

In most apple and pear growing regions around the world, fire blight caused by *Erwinia amylovora* is a sporadic disease that can cause major damage and important tree loss. Many infections occur when suitable weather conditions are met during bloom. Timely applications of antibiotics or biocontrol agents during this period can dramatically reduce disease severity. Consequently, a number of forecasting systems have been developed to help predict disease outbreaks (Billing, 2000) and are described on the UC Davis IPM web page (Anonymous, 2010).

Unfortunately, current systems in use are not very reliable (Dewdney et al., 2007). The models can generate false positive warnings (Type I error) because the inoculum pressure

in orchards was not sufficient to cause disease (Johnson and Stockwell, 1998), the cultivar is less susceptible, and possibly because the models tend to overestimate pathogen growth under certain conditions. Conversely, the models can also generate false negative prognosis (Type II error). Part of the errors are not due to the models per say, but as a consequence of regional weather networks that can be inadequate to reflect localized events. Nonetheless, models may also fail to predict outbreaks because conditions currently considered marginal for bacterial growth could in fact be sufficient to cause disease. Both types of errors can affect grower adoption of models for disease management. Since a single false negative prognosis can have a huge financial impact, growers can become over cautious for a number of years after the event, despite conditions unfavorable for disease. Consultants also tend to artificially increase Type I errors by relaxing model assumptions in favor of disease predictions. For instance, to avoid the risk of missing localized events, they may forego the requirement for a wetness event even if this plays a crucial role in fire blight outbreaks. Conversely, a high false discovery rate results in growers not following recommendations after a few years of unnecessary sprays and eventually putting the orchard again at risk. Antibiotics applied when conditions are not favorable for disease development are not very likely to impact pathogen resistance, but are prone to raise public concerns over health and environmental issues. Despite limitations in what can be achieved with disease forecasts, an improved model is likely to increase grower adoption and improve risk management.

The RIMpro-erwinia software was developed as an attempt to reduce model inadequacies by simulating from individual processes both the bacterial growth and the possibility of infection on individual daily flower cohorts. The complexity of calculations required to do so is in sharp contrast with empirical models such as Billings, Maryblyt and Cougarblight that are based around a few simple rules, but this is not a limiting factor for current computing technology. On the other hand, it does allow the flexibility to test the impact of different components of the biology on disease epidemiology. This is not the first attempt to simulate fire blight and follows some similarity to the approach published by Timmerman (1989) albeit with a different technique. Similarly to other RIMpro models (Trapman, 1994; Trapman, 2004; Trapman et al., 2008; Philion et al., 2009), the simulation is designed to follow in time each individual processes from flower opening, colonization, infection and disease expression.

## MODEL DESCRIPTION

### Inoculum potential and prebloom buildup

The idea of integrating recent outbreak history in the vicinity of a grower's orchard (Jacquart-Romon and Paulin, 1991; Smith, 1998) and/or to include weather conditions prior to bloom (Powell, 1965; Thomson et al., 1982), as risk factors that can contribute to inoculum dissemination once flowers open, was recognized as an important aspect of disease epidemiology that was discussed by Billing (2000). Early season climatic conditions favoring pre bloom inoculum development was also suggested as an explanation for recent outbreaks of fireblight in Southern Germany, Switzerland and Austria which prompted RIMpro-erwinia development. Unfortunately, this is not easily quantifiable and remains mostly empirical and thus was not included in our proposed framework (Table 1). This may be included in a future version of RIMpro, possibly as

part of a method to account for overall inoculum availability. In the current version of RIMpro, inoculum availability is considered non limiting.

**Opening of flowers and colonization by *Erwinia amylovora*.**

Flowers are generally considered devoid of bacteria before the petals open and are colonized only as bees or other insect vectors visit them, or from rain splashed from surrounding bacteria sources. Bacteria are mostly deposited on flower stigmas though physical contact of insects, which usually don't touch the lower hypanthium tissue (Thomson, 1986). Because of host defense mechanisms, the low number of bacteria deposited is not likely to cause direct infection, so population buildup is generally required before infection can take place. The main exception to this rule is probably rain that can carry large amounts of bacteria from surrounding sources that can directly infect the flower (Thomson, 1986). In Maryblyt (Steiner, 1990), the proportion of open flowers colonized by the pathogen gave origin to the criteria of accumulation of degree- hours (DH) >18,3C following research done on pears (Zoller and Sisevich, 1979). In the current version of RIMpro, the software assumes initial inoculation of the whole daily flower cohort to occur as soon as daytime temperature reaches at least 15C for 2 hours, based on the temperature required for pollinating insect flight. The software does not attempt to determine what proportion of the cohort is colonized and this is beyond the current version. Lindow and Suslow (2003) and Stockwell et al. (1999) both report flowers devoid of bacteria over a week after opening because colonization was limited by local inoculum availabitility. As more data becomes available, the RIMpro model assumption may change to include adjustments for the availability of local inoculum source, or the rapid redistribution of bacteria due to rain. Currently, once the daily the colonization criteria are met, a submodel for epiphytic bacterial development on stigma is triggered for the flower cohort of that day with a starting inoculum default of 10 CFU.

**Epiphytic growth**

Stigmas are known to be the site where most bacterial development occurs prior to infection (Thomson, 1986). Data reviewed by Billing (2000) clearly shows that epiphytic populations can reach levels sufficient for infection under natural conditions, despite average temperatures of only 14C and daily high temperatures below 16C. Additionally, about 15% of fire blight cases recorded from around the world that were analyzed by Dewdney et al. (2007) occurred under temperatures below the thresholds defined in Maryblyt. Furthermore, some models use a temperature function which is linearly proportional to temperature, wheras bacteria growth is not. In consequence, both RIMpro and Cougarblight calculate epiphytic bacterial growth based on a nonlinear growth curve. In RIMpro, data adapted from a few studies (Billing, 1974; Schouten, 1987; Pusey and Curry, 2004) were compared and the equation of Schouten was selected in a preliminary version, with a correction factor to calibrate the model for field conditions. Bacteria doubling time (in hours) is modeled with: $((24/20)/\sin(4.2*10^{-4} * T^{2.46}))/0.55$ where T is equal to hourly air temperature (in Celcius) and negative outcomes are reset to zero. Further refinements based on recent data from (Pusey and Smith, 2010) and a different modeling approach (Arauz et al., 2010) are planned in a future version. It follows that at lower temperatures both Cougarblight and RIMpro do not include a provision for a reduction in bacterial colonization as suggested in Maryblyt, but rather a reduction in population growth rate (Table 1).

**Carrying capacity of stigma**

The maximum bacterial population on the pistil in RIMpro was set at $1x10^7$ CFU following work on the carrying capacity discussed in Johnson and Stockwell (1998). Stigma may harbour bacteria for a long time, but the period of receptivity during which it can sustain population increases is limited (Lindow and Suslow, 2003). According to Thomson and Gouk (2003), the inability of *E. amylovora* to multiply on older stigma could be associated with papillae collapse. In RIMpro, the model for the maximum sustainable population during flower life (Figure 1) was built using data from Thomson and Gouk (2003).

The model relates the carrying capacity of the flower to the age of the flower when it is colonized and the time required for bacteria to reach the carrying capacity. According to this model, for flowers immediately colonized after opening (inoculation age = 0), the carrying capacity on apple is reduced to $10^{3.5}$ CFU after 113 DD (base 4C), in contrast to either Maryblyt and Cougarblight which limit bacterial growth to a maximum of 45DD base 4C and 4 days respectively (Table 1). A different model may be required for pear blossoms.

**Floral expansion model**

Because flowers start opening over a number of days, only a proportion of flower clusters can be colonized on any particular day, thus influencing the daily relative risk. The most striking example of this comes from "late" flowers (also refered to as rat tail bloom) that are less numerous but are likely to open under higher temperatures conducive for fire blight. However, since few flowers can also be found several weeks after normal bloom, the relative risk of these must be weighed. The current version of RIMpro doesn't include a correction factor for this and each flowering day is currently treated with equal weight and assumed at maximum bloom. In consequence, advisors need to adjust for local conditions, varieties and special cases such as the flowering dates of newly planted trees. Since fire blight dynamics is most likely heavily influenced by flowering dynamics, this aspect needs to be addressed, but inclusion in a general model is difficult and beyond the scope of the current project. None of the models currently in use include a correction for floral expansion.

**Movement of bacteria from stigma to hypanthium**

Bacteria present on flower stigma need to reach the hypanthium for infection. In RIMpro, this process was adapted from work published by Pusey (2000) and Pusey and Smith (2004). When flowers are wetted, the initial population on the hypanthium is set be 3 logs lower than that reached on the stigma of apple and only 1 log lower for pears. Any wetness event that can be recorded by the datalogger is deemed sufficient to carry the transfer process. Given the carrying capacity of pistils and the maximum number of bacteria transferred, $10^4$ bacteria is the maximum starting population on the wet hypanthium.

**Epiphytic growth on hypanthium**

Bacteria driven down from the stigma surfaces to the bottom of the flower cup need to enter the plant through nectarthodes which are the site of release of the nectar produced by the nectaries (Bubán and Orosz-Kovács, 2003). Normally, bacteria die in close proximity of the nectarthodes because the water activity of the nectar is very low. However, when the flowers are wet, the nectar is diluted and bacterial growth becomes

possible. Growth of bacteria on hypanthium tissue is most likely a key difference between fire blight epidemiology in wet climates (East coast of America and Northern Europe) as opposed to dryer climates (West cost of America and Southern Europe) (Thomson, 1986). Pusey (2000) published a model showing a sigmoidal relation between relative humidity and bacterial growth on both stigmas and hypanthia and showed that humidity >80% can play a significant role in bacterial growth on the hypanthium. Although these models were not meant to be used directly, it seems likely that a simulation model should include provision for bacterial growth on the hypanthium for ambient relative humidities higher than 80%. In RIMpro, as long as the flowers remain wet or the ambient relative humidity remains above 80%, bacterial growth on the hypanthium is modeled using the temperature equation used for epiphytic growth. The bacterial population at the surface of the nectaries is set to zero whenever the flowers are dry and relative humidity is below 80%.

**Infection of nectaries in relation to flower age**

Because hypanthium susceptibility decreases with flower age, the time available for infection is limited. From Pusey and Smith (2008), it was possible to derive a model for hypanthial susceptibility in vitro. Their data relating flower age and temperature to disease incidence showed a nearly perfect negative linear relation with disease incidence on the logit scale (Figure 2). According to this model, flowers remain at maximum susceptibility until about 45 DD (base 4C) and then decreased to 0 at 150DD. Field disease severity data from the same study (Figure 3) showed a less striking impact of flower age, but also overall reduced disease incidence. In this study, field infection following hypanthial inoculation occurred at a lower temperature and relative humidity than the laboratory experiment and this might have masked part of the hypanthial age effect. It is noteworthy that contrary to other forecaster assumptions, flowers remain susceptible for a number of days after petal fall which starts at about 45 DD (base 4C).

**Infection of nectaries in relation to inoculum concentration**

Contrary to other studies using whole flower inoculations, Pusey and Smith (2008) looked at the effect of inoculum concentration in the hypanthium in relation to disease severity. A model derived from their data (Figure 3) was used to determine the proportion of the daily flower cohort infected in relation to the hypanthial population and age. Using the age slope of the model in Figure 2 and substituting in the inoculum model gives the proposed model: Logit of incidence = -0.0875* DD (base 4C) + 1.12*log10(CFU) – 3.5. The intercept was arbitrarly set so that the threshold for cohort infection in the software is 2%.

**Infection of nectaries in relation to temperature**

On wet flowers, the diluted nectar originating from the nectarthodes creates a solution attractive to *Erwinia amylovora* which uses its flagella to follow the gradient to the nectarthodes. This process is temperature dependent (Steiner, 2000) and also depends on the presence of sufficient water to permit movement of bacteria. This was recognized in Maryblyt as a rule which inhibits infection at temperatures below 16C, which is a threshold temperature for coordinated flagellar movement towards the nectarthodes. Because infections are observed at lower temperatures, it seems this process is not a requirement for infection. In consequence, there is currently no provision for inhibition of infection at lower temperatures in RIMpro. As more data linking hypanthia inoculum levels and infection temperature become available, this may be included in a future

version. Currently, the population of bacteria on the wetted hypanthium in relation to its age are the only criteria used for infection.

## DISCUSSION

RIMpro is built as a chain of biological sub process that are modeled according to our present knowledge of the underlying relations with environmental factors. The state of the sub processes are updated in short time steps (30 min). This so called "boxcar" approach (Rabbinge and De Wit, 1989) facilitates integration of both nonlinear equations and dispersion of the population on environmental influences, and also better reflects conditions at each time step. Wheras values such as the EIP in Maryblyt were designed to encompass many risk factors such as availability of open flowers, bee activity, etc (Biggs and Turechek, 2010), the goal of RIMpro is to separate these into submodels. Even though conditions favorable for individual factors are often correlated, the use of submodels can reflect particular conditions that can be missed by an empirical approach.

The RIMpro-erwinia model is currently only in the very early stages of development and doesn't address the full complexity of blossom blight. Nonetheless, a preliminary version that did not include the impact of hypanthial age was released to consultants for evaluation purposes. It successfully identified infection events that were not predicted by either Cougarblight and Maryblyt. It is possible that the limited dataset currently available for testing and validation may prove inadequate or insufficient to warrant large scale field use at this point. Nonetheless, following the recent papers published on bacterial populations on stigma and hypanthium susceptibility we felt it was time to release at least a framework for a simulation model.

Still, current models including RIMpro will remain prone to generate false positive predictions when local inoculum levels are low. The inclusion of orchard fire blight history in Cougarblight helped improve the specificity of this model (Dewdney et al., 2007) so that less false positive cases are found. Similar observations were done using Firescreens (Lecomte et al., 1996). A similar indicator of inoculum pressure is also needed in RIMpro. It is conceivable that in a near future a cheap and rapid detection technique will make this possible (Temple et al., 2007). Until inoculum pressure is fully integrated in the disease forecast, there is a limit to the level of accuracy that can be achieved with any model.

The next step of this project will be to evaluate the accuracy of RIMpro-erwinia in comparison to other forecasters using ROC analysis and possibly other techniques which can assess whole season forecasts (Dewdney et al., 2007). A international database of fire blight epidemiology that would index cases (and controls) alongside inoculum pressure, cultivar, and weather files would be very useful to test RIMpro and other models.

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Tables

Table 1. Outline of major differences between RIMpro and most often used fire blight models.

| *Model assumptions* | *Maryblyt* | *Cougarblight* | *Rimpro* |
|---|---|---|---|
| Inoculum availability | Abundant | Grower input | Abundant |
| Prebloom inoculum buildup | Not included in bloom predictions | None | None |
| Relation between epiphytic growth and temperature | linear (Degree-hours above 18.3C) | Nonlinear | Nonlinear |
| Bacterial death | 33,50,100% when max. T < 18,3C for 1,2,3 days | None | None |
| Flower death | 100% if min T < -4.4C | None | None |
| Sustainability of bacterial growth on flower stigma | 44.4 DD (>4.4C) | 4 days | Carrying capacity based on flower age. |
| Temperature and time required for infection | 110DH > 18.3C (EIP) | Variable risk index | Flower age dependent |
| Exemple : | NA @ 16C | NA @ 16C | 4 days @16C |
| | 65hrs @ 20C | 4 days @ 20C | 2,5 days @20C |
| | 11hrs @ 28C | 1 day @ 28C | 1,5 days @28C |
| Infection criteria following population threshold. | >3hrs wetness or 0.25mm rain or >2.5mm rain in last 24hrs before EIP reached and T > 15.6C | Rain and High risk index | Measured wetness |

Figures

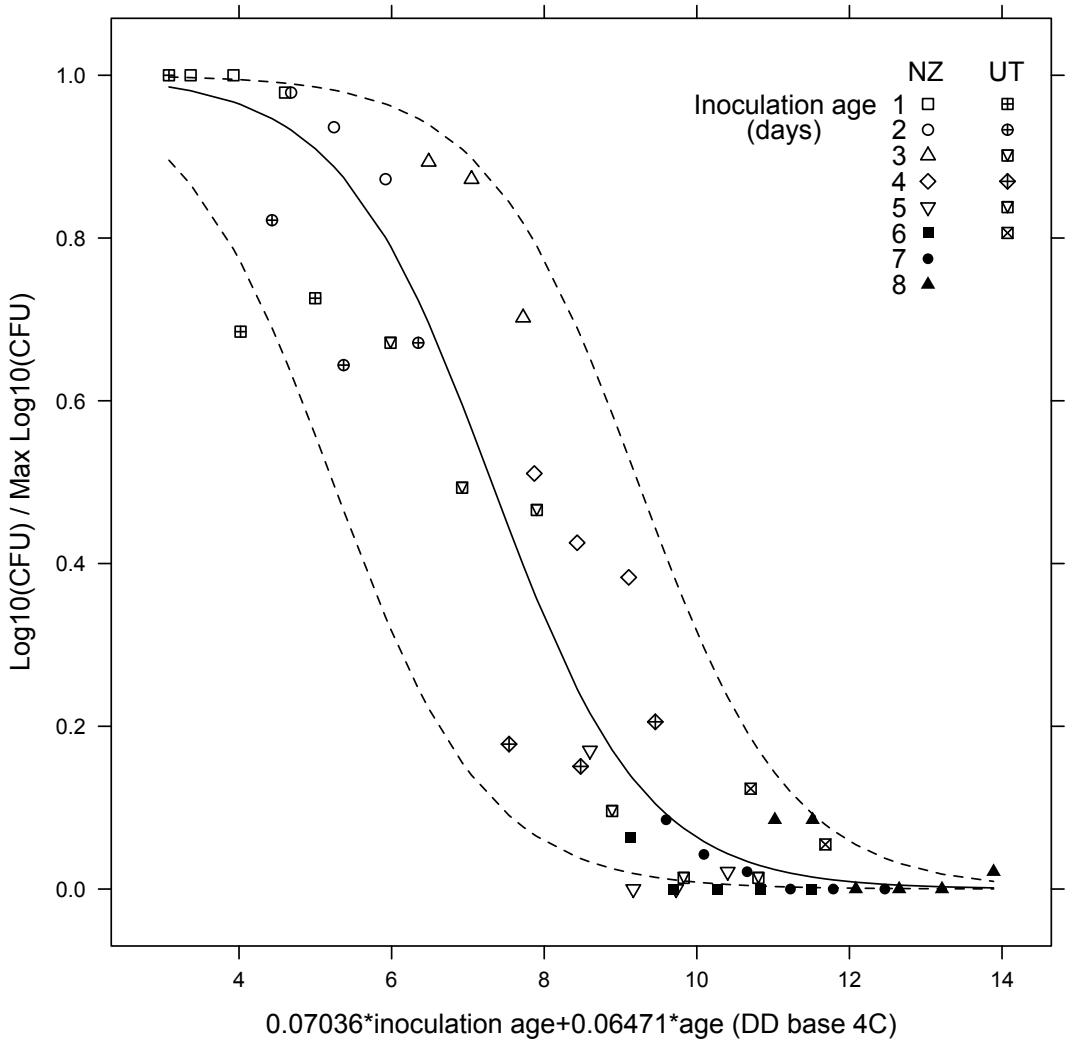


Fig. 1. Effect of flower age at colonization time and during epiphytic growth on the carrying capacity for *Erwinia amylovora.* Data adapted from Thomson and Gouk (Bates and Maechler, 2010). For each flower inoculation age, the daily population reached after the initial maximum was assumed to be the carrying capacity of the flower on that day. Missing climate data were retrived from online sources using the Ruakura station for New Zealand (http://cliflo.niwa.co.nz/) and Hill air force base near Ogden for Utah (http://www.ncdc.noaa.gov). The model was fitted using logistic regression in a mixed effect linear model (GLMM) using the lmer function of R (Bates & Maechler 2010). Model selection was based on AIC and residual analysis. Inoculation age and sampling day within site were included as random intercepts. The fixed effects of the model were: Logit (log10(CFU) / max log10(CFU)) = 7.31073 - 0.07036 * inoculation age - 0.06471* sampling age. The fitted equation (filled line) and extremes of the random effects (dotted line) are represented on the graph.

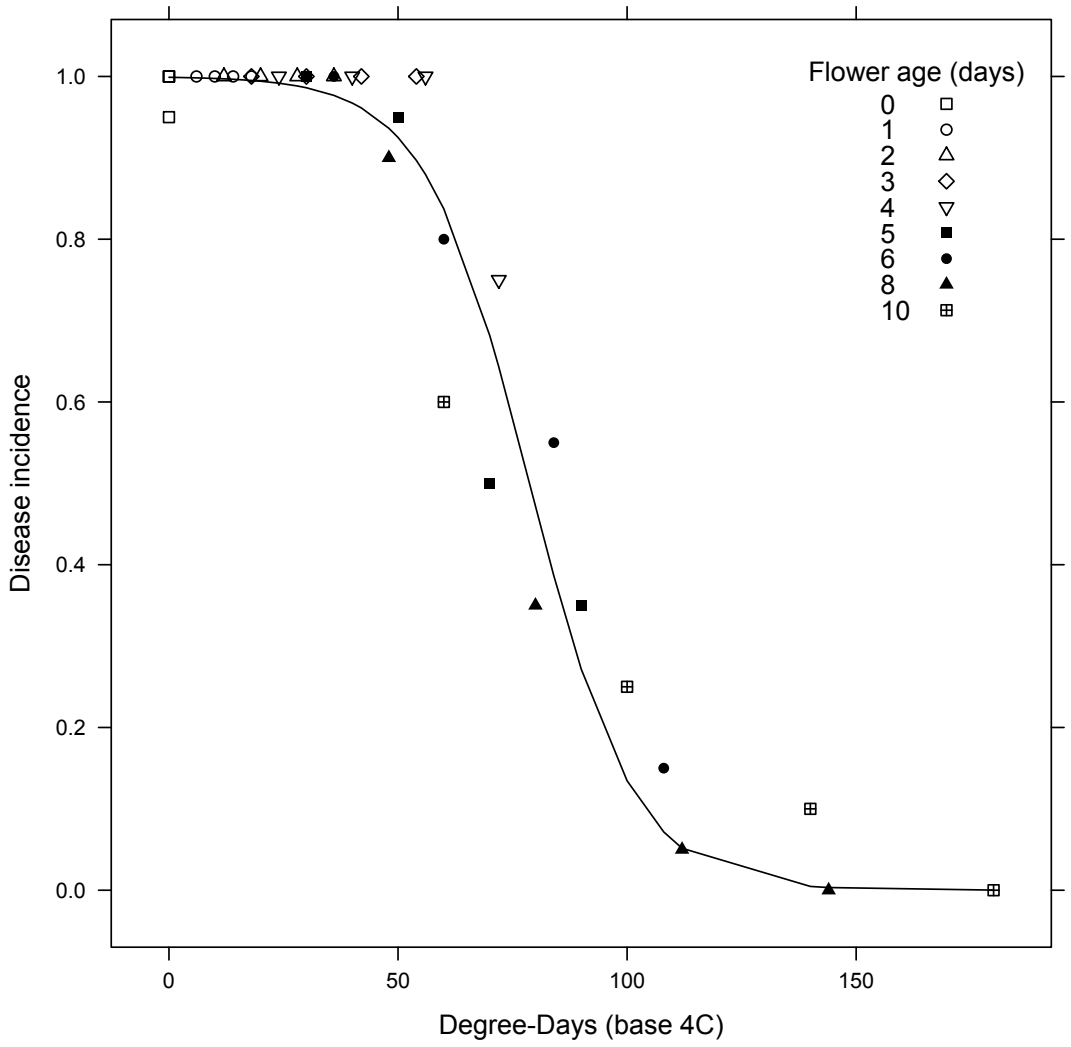


Fig. 2. Effect of flower age on fire blight incidence after direct inoculation of hypanthium with 2.5 x $10^6$ CFU. Data was adapted from Pusey and Smith (2008) by converting each temperature and flower age combinations to degree-days (base 4C). The model was fitted using logistic regression in a mixed effect linear model (GLMM) using the lmer function of R (Bates and Maechler, 2010). Model selection was based on AIC and residual analysis. Temperature was used as a random slope for each flower age as a random intercept. The fixed effects of the model were: Logit of incidence = -0.0875* flower age + 6.888. The fitted equation (filled line) is represented on the graph.

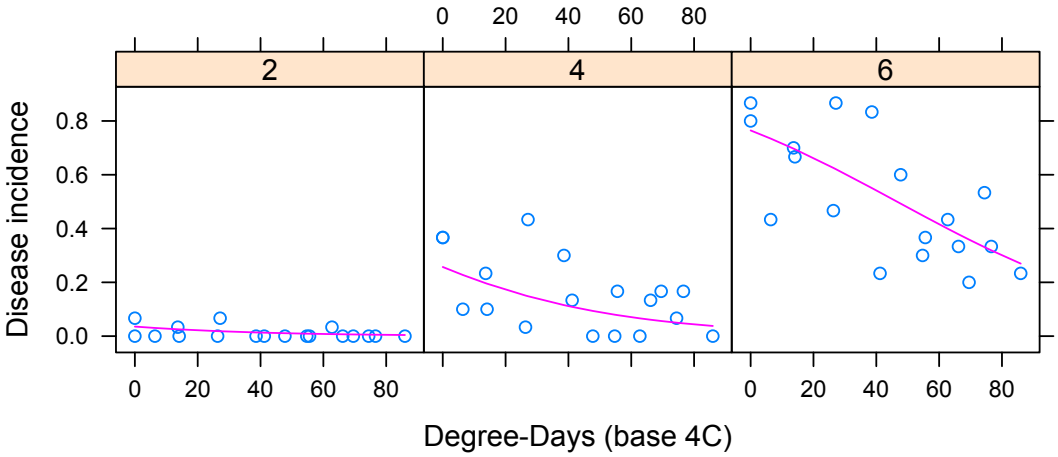


Fig. 3. Effect of inoculum concentration and flower age on disease incidence. Data was adapted from Pusey and Smith (2008) by converting each flower age in 2005 and 2006 to degree-days (base 4C). A different panel was used for hypanthia inoculated with $10^2$, $10^4$, and $10^6$ CFU respectively. The model was fitted using logistic regression in a mixed effect linear model (GLMM) using the lmer function of R . (Bates and Maechler, 2010). Model selection was based on AIC and residual analysis. Flower age within year was used as a random intercept. The fixed effects of the model were: Logit of incidence = -0.0253* flower age + 1.12*$\log_{10}$(CFU) - 5.541. The fitted equation (filled line) is represented on the graph.