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A Decision Aid to Support the Use of Curative Late Blight Fungicides

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Declaration

This thesis has been authored by myself, and is substantially my own work. The work has not been previously submitted as part of any other degree of professional qualification.

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Abstract

Late blight, caused by the oomycete pathogen *Phytophthora infestans* (Mont.) de Bary remains a severe threat to potato production in temperate regions and necessitates a high volume of fungicide inputs. A recent focus of research has been to improve the application of Integrated Pest Management (IPM) principles to the control of late blight. Extensive monitoring programmes and warning systems are currently delivered via online platforms, and growers also have access to information on fungicide properties and the relative resistance of different cultivars to the pathogen. Growers and agronomists would benefit from additional tools which aid the decision-making process and allow improved integration of available control strategies.

A strong example of this is the use of curative fungicides. Fungicides which can act curatively (within the incubation period of pathogen development) are an increasingly important component of late blight control, and there is scope to improve their deployment. The aim of this study was to produce a simple decision aid that can be used by growers and agronomists to inform their decision to use a fungicide with curative properties following weather conditions associated with a high risk of infection. Guidance available before the development of this decision aid was somewhat subjective, and did not take into account factors that may modify the efficacy of curative fungicides for which there is very little published information.

Several contemporary *P. infestans* isolates were characterised in this study for their growth rates, both visually and sub-clinically using a qPCR assay. These data were then used to test a range of potential pathogen growth models which have been used by previous authors to model temperature-dependent growth in other biological systems. Many of these models provided good descriptions, and the best performing was used to predict pathogen development with the decision aid.

Characterisations of the curative effect for a representative curative fungicide (propamocarb-HCl + fluopicolide) were generated for selected isolates in both laboratory assays and under field conditions. Within the laboratory bioassay, more frequent sampling (4 hour intervals) than is usually reported in experiments of this nature was used to assess the nature of the curative effect over a time frame of up to 72 hours post inoculation. Curative control declined rapidly with increasing pathogen development, with the relationship best described by a logistic function. This function, and the parameters generated from the bioassays, were used within the decision aid to predict the likely outcome of curative treatments.

Lay summary

Potato crops in temperate climates such as the UK are at high risk from the late blight pathogen *Phytophthora infestans*. Late blight of which *P. infestans* is the causal agent is one of the most destructive plant diseases known. It can rapidly destroy the foliage and stems of potato crops and is also capable of infecting potato tubers. The crops can be protected from this disease, but, in order to achieve this, they must be treated with fungicide many times (on average 12 in the UK) within the same season. This makes potato a challenging crop for the application of Integrated Pest Management (IPM). IPM is a set of principles and practices aimed at reducing the use of chemical control interventions to a level that is economically and environmentally justifiable by careful consideration of the available control options in conjunction with regular monitoring.

This project focuses on late blight fungicides which can act curatively; they can move into plant tissue after they have been applied and prevent very early infections from developing. This is not the chief way that they are used, as all fungicides are applied to prevent infection, but it is an important component of late blight control. Currently, there is sparse information on the 'curative control window', *i.e.* the period of time over which curative control is effective. The experiments in this project set out to gather information about this window: how long it lasts, and how different factors such as temperature or the variety of potato affect it.

A mix of experiments using controlled conditions in the laboratory, and field trials, was used to gather data. The results of the laboratory experiments were used to build two models: one of how rapidly the pathogen grows depending on the temperature; and a second which describes how the control provided by a curative fungicide declines with increasing time. As well as using classical plant pathology, methods such as estimating the size of visible disease symptoms, the molecular technique quantitative polymerase chain reaction (qPCR) was used to estimate

pathogen growth before any visible symptoms had developed. This is the period (the incubation period) in which curative control is possible and which had not been explored in detail before this study.

The project has generated useful datasets for late blight development in the incubation period in greater detail than is typical and also includes descriptions of how the disease control given by a representative curative fungicide declines with time at higher resolution than was previously available. The experiments also demonstrated that the degree of disease resistance in the host plant could help to extend the curative window in some circumstances.

Finally, a decision aid to help growers make choices around using curative products in their spray programmes is specified and then validated against data from the field. The decision aid is designed to complement existing disease forecasting and monitoring programmes which are used for potato late blight IPM, and suggestions are made on how the aid could be further improved and implemented.

Chapter 1 General introduction, background and literature review

1.1 Introduction

Sustainable control of pests and diseases which damage agricultural crops presents a number of challenges, and the use of a single method without regard to local conditions or the biology of the target organism (and its host) may lead to disappointing results. Several studies have demonstrated both theoretically and practically (Carolan *et al.*, 2017; Fernandez-Cornejo, 1998; Tang *et al.*, 2005) that Integrated Pest Management (IPM) can lead to improvements in disease control when compared to less subtle strategies – for example, an IPM strategy may involve only treating a crop with a pesticide when a disease warning is issued or when the problem organism's population reaches a threshold amount. Treatments before this may be wasteful in economic terms, and misused or excessive pesticide applications have the potential to cause harm to human health and non-target organisms (Margni *et al.*, 2002). Conventional pest and disease management is also beset with issues arising from resistance to pesticides in target organisms; and poor stewardship of the available control options only increases the probability of resistance developing (Alyokhin *et al.*, 2008).

The term IPM has its origins in entomological research (Stern *et al.*, 1959), but many of the tactics it prescribes were present in the phytopathological literature before references to IPM became widespread (Jacobsen, 1997). IPM has several definitions (Bajwa and Kogan, 1996), but it is generally taken to mean an approach that uses a diverse range of control measures (cultural, biological, chemical, etc.) in concert, with the ultimate aim of reducing the reliance on conventional pesticides, whilst maintaining economically sustainable levels of crop protection (Ehler, 2006). It should be noted that conventional crop protection products such as fungicides are an integral part of IPM, but a well formulated programme should contribute to the amelioration of some of their well-documented (Wilson and Tisdell, 2001) issues.

IPM is promoted by the regulatory authorities of several nations and intergovernmental bodies (Brewer and Goodell, 2012), for example, the European

Union mandates in Council Directive 2009/128/EC (2009) that its member states develop action plans which promote the use of IPM. The Sustainable Use of Pesticides directive sets out general principles of IPM which should be adopted, including the use of non-chemical methods where appropriate, the monitoring of harmful organisms, and the reduction of pesticide applications to levels that are economically and environmentally justified. The formulation and implementation of IPM programmes can be challenging and uptake has been, at best, variable, as growers can view novel strategies with scepticism (Lefebvre *et al.*, 2015); many of these difficulties are exemplified by the late blight-potato pathosystem. Late blight is caused by the oomycete phytopathogen *Phytophthora infestans* (Mont.) de Bary which is infamous for its capacity to cause explosive and severely damaging epidemics over short time frames (Kromann *et al.*, 2014). *P. infestans* was one of the first plant pathogens formally studied and explicitly targeted with biocidal compounds on an industrial scale (Johnson, 1935), but control remains both a challenge and a significant economic investment (Cooke and Andersson, 2013), particularly in areas with extensive potato production. Recent reviews rank late blight as amongst the most important potato diseases extant in the United Kingdom, with losses (excluding costs of control measures) per annum in the region of £55 M (Twinin *et al.*, 2009).

1.2 Pathogen biology and infection cycle

P. infestans belongs to the class Oomycota, whose members have a superficially similar filamentous appearance and analogous life history (heterotrophic nutrition, wind-dispersed spores, possession of an asexual reproductive pathway, etc.) to many true fungi. Indeed, they are often referred to as fungi for pragmatic and historical reasons (Barr, 1992). However, the oomycetes have a distinct evolutionary history from the true fungi, which is evident from molecular evidence and the presence of several distinguishing cytological characteristics such as: cell walls lacking significant quantities of chitin and which are instead composed primarily of β-glucans and cellulose (Mélida *et al.*, 2013); motile biflagellate asexual zoospores (Beakes *et al.*, 2011); and a typically diploid vegetative state (Judelson, 1997).

Modern taxonomies place oomycetes with the Stramenopiles, a group which also consists of diatoms (Bacillariophyceae) and brown algae (Phaeophyceae) (Baldauf, 2008).

Oomycetes occupy a range of ecological niches (Marano *et al.*, 2016) and several are important plant pathogens. They span the full range of pathogenic strategies with some species such as *Phytophthora cinnamomi* acting mainly necrotrophically (Delgado *et al.*, 2012, but see Crone *et al.*, 2013 for a discussion of the evidence for hemibiotrophy under some circumstances) through to obligate biotrophs such as *Bremia lactucae* the causal agent of downy mildew of lettuce (Judelson *et al.*, 1990). *P. infestans* is classified as a hemibiotroph as it displays phased behaviour whereby the initial interaction with the host is asymptomatic biotrophy, followed by a transition to necrotrophy. This secondary necrotrophic phase is particularly damaging, and is often lethal to the host plant (Mingora *et al.*, 2014). While some phytopathogenic oomycetes have a wide host range, such as *Phytophthora ramorum*, with over a hundred host species observed to date (Grünwald *et al.*, 2008), *P. infestans* is limited to a small number of Solanaceous plants, although it has been recently demonstrated that co-infection of *Arabidopsis thaliana* in the presence of the oomycete *Albugo laibachii* is also possible (Belhaj *et al.*, 2017).

In common with several oomycetes, *P. infestans* is capable of both sexual and asexual reproductive cycles and produces both sexual and asexual spores (Judelson and Blanco, 2005). In potato production systems within the United Kingdom, primary infection is typically via wind-borne, asexually produced sporangia (Warren and Colhoun, 1975). Sporangia are multinucleate (Maltese *et al.*, 1995) and have two potential germination pathways – either directly via the formation of a germ tube, or indirectly through the release of a variable number of zoospores (generally six, (Marks, 1965)). Zoospores are motile and possess two morphologically distinct flagella: an anterior flagellum adorned with mastigonemes, and a posterior flagella which is unadorned, typical of Stramenopiles (Riisberg *et al.*, 2009). There is a well-established relationship between temperature and

germination pathway: lower temperature (< 14 °C) favouring indirect germination, which is the release of zoospores (Bain and Convery, 2011). Zoospores display chemotaxis to host exudates such as glutamic acid (Latijnhouwers *et al.*, 2004), and those which locate host tissue then encyst; forming a cell wall which they initially lack, produce a germ tube and subsequently an appresorium, which is used to breach the host epidermis (Grenville-Briggs *et al.*, 2005). In both cases, spores require free water in order for infection to take place, making high humidity a critical risk factor for infection (Hartill *et al.*, 1990). Several potato host tissues, including foliage, stems, and tubers, are viable sites for the pathogen to gain entry (Hirst and Stedman, 1960), and the existence of the indirect germination pathway allows infection to occur at some distance from loci where sporangia settle (Appiah *et al.*, 2005) and is of epidemiological significance, as the presence of zoospores within soil has the potential to translate to tuber infection (Zan, 1962).

Amongst the most remarkable of *P. infestans* characteristics is the rapidity of its asexual life cycle; infection can take place in a matter of hours (Kandel, 2014) and, once within the apoplast, hyphae proliferate and ramify. During the initial biotrophic phase (which is asymptomatic), finger-like haustoria are formed within host cells and are thought to play a role in the delivery of effectors (Birch *et al.*, 2006) and/or the extraction of nutrients (Judelson *et al.*, 2009). There is considerable interest in the mechanisms by which these effectors establish a compatible interaction, which, as yet, is poorly characterised, but effectors are thought to suppress or circumvent the standard plant immune response to the perception of a threat (the so called PAMP (pathogen associated molecular pattern) triggered immunity). As the infection front progresses through the ground tissue, the region of initial colonization becomes necrotic; this development is responsible for the appearance of visible lesions. This shift is associated with a change in the pattern of gene expression in both the pathogen (Zuluaga *et al.*, 2016a) and the host (Zuluaga *et al.*, 2016b); it has been hypothesised that this may correspond to a transition from early suppression and evasion of plant defences to a focus on nutrient acquisition (Ah-Fong *et al.*, 2017).

At the interface between necrotic and living tissue, asexual sporangia are produced on specialized hyphal structures termed sporangiophores. Under optimal conditions, this asexual life cycle proceeds very rapidly and the production of sporangia (sporulation) can occur as little as 4 or 5 days following infection. The fecundity of *P. infestans* is also notable; estimates of sporulation capacity range from 50 to 500 sporangia per mm^2 of infected tissue (Knapova and Gisi, 2002). There is, however, considerable variation in these life history traits between (and potentially within) *P. infestans* populations (Mariette *et al.*, 2016), and they are strongly influenced by the genetic background of the host (Clément *et al.*, 2010) as well as the climatic conditions during the life cycle (Mizubuti and Fry, 1998).

Although the asexual reproductive pathway is the most important driver of the explosive epidemics caused by *P. infestans* in the field, the pathogen also possesses the capacity to reproduce sexually. Two mating variants exist (i.e. the species is heterothallic), designated A1 and A2. These mating types appear to be under the control of a single locus, and each mating type induces the formation of gametangia in its counterpart through the mating type specific phytol-derived hormones α1 and α2 (Ojika *et al.*, 2011). If both complementary mating forms are present within the same infected tissue (or artificial culture media), recombinant structures termed oospores may then be formed via the fusion of oogonia and antheridia (Frinking *et al.*, 1987). The potential for oospore production within a geographical region is a cause for concern, not only due to the generation of genetic diversity (and thus the possible emergence of a more virulent, aggressive or fungicide insensitive strain), but also because oospores are well adapted as resting structures, capable of surviving for extended durations under challenging conditions and in the absence of the host. Oospores may remain viable for 34 to 48 months, contingent on soil type and environmental conditions (Turkensteen *et al.*, 2000).

1.3 Population structure

The first characterisations of *P. infestans* isolates were based on phenotypic traits, in particular their compatibility with a set of known *R* genes introgressed into the potato host from *Solanum demissum* to produce an international standard set of differentials for the designation of 'races' of *P. infestans* (Malcolmson and Black, 1966). Mating type (Tantius *et al.*, 1986) and sensitivity to phenylamide fungicides (Dowley *et al.*, 2002) are also in widespread use as identifiers. Such classification schemes are very informative; for example, the presence of both mating types in sufficiently close proximity may highlight the potential for oospore formation, however there are also a number of weaknesses: procedures for phenotyping are usually time-consuming, cannot unambiguously distinguish between different lineages (Cooke and Lees, 2004) and can be inconsistent between different laboratories (Fry *et al.*, 1992).

Advances in molecular marker technology and high throughput analysis have facilitated detailed studies of the population dynamics of *P. infestans* (Fry *et al.*, 2008), such studies are important not only from a theoretical standpoint, but also because improved understanding in this area opens the possibility of tailoring control strategies to dominant local genotypes; for example, avoiding a particular active ingredient if a resistant lineage is present in local outbreaks. Any approach will be limited by the intensity and geographic spread of sampling, and all inferences must be made with these limitations in mind.

Many of the issues associated with classification through phenotypic traits are overcome via the use of genetic markers. Historically population studies have utilized several methods: isozyme assays based on glucose phosphate isomerase and peptidase (Tooley *et al.*, 1985); genetic fingerprinting using restriction fragment length polymorphism (RFLP) analysis based on probe RG57 (Goodwin *et al.*, 1992); and mitochondrial DNA (mtDNA) haplotype (Griffith and Shaw, 1998). More recently, several research groups have generated multilocus genotype designations using simple sequence repeat (SSR = microsatellite) markers (Knapova and Gisi,

2002; Lees *et al.*, 2006; Li *et al.*, 2010), and some of the most informative of these have been combined within a 12-plex multilocus assay (Li *et al.*, 2013a). SSR characterization has a number of advantages, including the ability to track within-lineage variation and to distinguish between clones and recombinants (Lees *et al.*, 2006). There has been extensive international collaboration using SSR assays (Cooke *et al.*, 2011a), and there are now several published national and regional studies (Akino *et al.*, 2013; Brurberg *et al.*, 2011; Chmielarz *et al.*, 2014; Chowdappa *et al.*, 2013; Cooke *et al.*, 2014b; Danies *et al.*, 2013; Deahl *et al.*, 2009; Delgado *et al.*, 2013; Lebecka *et al.*, 2007; Li *et al.*, 2012b, 2013a; Lucca and Huarte, 2011; Mariette *et al.*, 2015; Njoroge *et al.*, 2016; Peters *et al.*, 2014; Pule *et al.*, 2013; Runno-Paurson *et al.*, 2013; Savazzini and Galletti, 2015; Statsyuk *et al.*, 2014; Utami and Ambarwati, 2017) or on-going monitoring projects (César *et al.*, 2017) which make use of them. An alternative approach is to make use of the single nucleotide polymorphism (SNP) class of molecular markers, which have greater discriminatory power due to their abundance and lower mutation rates (Brumfield *et al.*, 2003). These have been used to examine within-lineage variation in North American isolates (Hansen *et al.*, 2016), but are not as well suited to detect new variant alleles as SSR assays (Hansen *et al.*, 2016).

Away from the pathogen's centre of origin in central Mexico (Goss *et al.*, 2014), *P. infestans* populations are typified by ephemeral outbreaks, regular migrations and displacements of lineages (Fry *et al.*, 1992; Goodwin *et al.*, 1998). The widespread global cultivation of potatoes preceded migration of the pathogen. *P. infestans* arrived in Europe circa 1843 (Andrivon, 1996), probably via a shipment that contained infected seed tubers (Lamb and Bourke, 1993). Analysis of mtDNA from herbarium specimens indicates that a single clonal lineage (designated HERB-1) was responsible for the subsequent infamous epidemic of 1845 (Yoshida *et al.*, 2013). At some point in the 1900s, this lineage was displaced by a genotype designated US-1, which was of mating type A1 (Forbes *et al.*, 1998). US-1 was then globally dominant for much of the twentieth century (Goodwin *et al.*, 1998). From the 1980s, isolates displaying A2 mating type behaviour were found in Europe

(Hohl and Iselin, 1984; Tantius *et al.*, 1986) and beyond (Singh *et al.*, 1994), probably due to the importation of infected material from Mexico (Niederhauser, 1991). The diversity of isolates obtained from the field has expanded since these events (Sujkowski *et al.*, 1994), and it is clear that in many regions sexual reproduction and the generation of oospores occur in the field. In Great Britain, a more recent population change occurred from the mid-2000s. Two genotypes now account for a large proportion of samples submitted to the national monitoring scheme by registered scouts. The clonal lineages 13_A2 (Cooke *et al.*, 2012) and 6_A1 (Kildea *et al.*, 2012) are currently the most commonly encountered lineages. These newer lineages are certainly more invasive and, in the case of 13_A2, show greater virulence (i.e. 13_A2 is compatible with a larger number of *R* genes) than older genotypes, but there is equivocal evidence on their aggressiveness compared with the populations they displaced (Mariette *et al.*, 2015).

In countries such as Great Britain where clonal lineages are the norm, their distribution is often uneven, and which lineages are dominant shifts periodically (Fry *et al.*, 2015). The dynamics by which these displacements take place are not yet well understood. With the exception of fungicide insensitive strains (Shattock, 2002), it is not clear which fitness components are the most important. Each season a large area of highly susceptible host tissue becomes available for colonization (Cooke *et al.*, 2011b), and, because of the short latent period of *P. infestans,* numerous infection cycles can be completed within an outbreak (Suassuna *et al.*, 2004). This is followed by an overwintering period in which the pathogen is obliged to survive on tuber tissue in suboptimal conditions (Kirk, 2003a). These two distinct phases apply selective pressures for different traits, and create the potential for trade-offs (Montarry *et al.*, 2007). The bottlenecking overwinter also increases the importance of stochastic factors, and it seems likely that founder effects have a large influence on each season's population (Drenth *et al.*, 1994).

It was initially proposed that aggressiveness, the extent of disease within a given host-pathogen interaction (Andrivon, 1993), explained much of the displacement by

successive clonal lineages, with strains that were most aggressive when interacting with common host cultivars being the most competitive (Day and Shattock, 1997), but this has recently been challenged with evidence suggesting that dominant lineages are not always the most aggressive (Mariette *et al.*, 2015). Exceptionally, aggressive lineages may also be maladapted to overwintering, rapidly destroying the tubers required for their survival (Andrivon *et al.*, 2013). Aggressiveness is a complex trait, which is contingent on the interaction of host, pathogen and environment (Pariaud *et al.*, 2009). This makes comparisons across studies difficult; relatively subtle phenotypic differences may confer a comparative advantage, and this may be influenced by climate or host traits (Yang *et al.*, 2016). For example there is evidence that some isolates may be temperature-adapted (Mariette *et al.*, 2016) and so it is likely that aggressiveness plays a variable role in the fitness of a lineage, dependent on these other factors.

P. infestans shows remarkable variability for a species that reproduces predominantly via an asexual pathway, particularly in its ability to defeat novel *R* genes (Fry, 2008)(see also Section 1.5, page 15), and some authors have reported *within* lineage phenotypic variation for aggressiveness associated traits (Goodwin *et al.*, 1995), sometimes comparable to the between-lineage differences (Chapman, 2012). It has recently been demonstrated than *P. infestans* isolates with the same genotype classification can differ markedly in gene expression, which translated into virulence on an otherwise resistant host (Pais *et al.*, 2017). This is a key set of observations that potentially undermines efforts to reliably predict phenotype from genotype. The dynamic nature of the *P. infestans* population, as well as the wealth of information generated through surveillance and SSR genotyping, has spurred the ongoing 'IPMBlight2.0' project, which seeks to establish the strength of genotype-phenotype links and to explore the possibility of incorporating data on population structure within decision support systems (Andrivon *et al.*, 2017). Another important goal of this project is to update the *R* gene differential set to include newly introduced resistance sources.

1.4 Epidemiology and risk prediction

The characteristics of late blight outbreaks vary between potato-growing regions due to differences in climate (Hijmans *et al.*, 2000), agronomic practices (Brylińska *et al.*, 2016; Forbes, 2004), and the specific traits of the local *P. infestans* populations (Mariette *et al.*, 2016) and *S. tuberosum* cultivars (Cooke *et al.*, 2011). In Great Britain, clonal lineages dominate (Cooke *et al.*, 2014) within the population. Recombinants, while encountered in some regions (Cooke *et al.*, 2014b), have had limited impact to date. As a consequence of this, primary inoculum is generally carried over from the previous season on host tissue in the form of non-harvested tubers (Pittis and Shattock, 1994), inadequately managed discard piles (Twinin *et al.*, 2009), or contaminated seed stocks (Boyd, 1974). An important feature of late blight epidemics is the polycyclic nature of the disease; multiple infection cycles occur within a single field within a season (Young *et al.*, 2018) and it is this, coupled with the rapidity and fecundity of *P. infestans,* which produces the potential for uncontrolled epidemics to completely defoliate a crop (Möller *et al.*, 2006).

It has long been recognized that environmental conditions are critical for successful infection and development of *P. infestans* (Beaumont, 1947), with temperature (Danies *et al.*, 2013; Mizubuti and Fry, 1998), leaf wetness (Hartill *et al.*, 1990), relative humidity (Minogue *et al.*, 1981) and solar radiation (Mizubuti *et al.*, 2000) being the most important factors. There is a rich literature relating to epidemiological modelling (Oijen, 1995) and risk warnings (Taylor *et al.*, 2003) for late blight, dating back almost a century. The definition of risk used within this study is discussed at the end of this section, page 13. Early studies in Great Britain sought to establish correlations between meteorological events and late blight outbreaks. These were followed by a chain of studies where modifications were made to the 'blight criteria' to improve performance (Table 1.1, page 11). It is important to emphasise that any biological interpretations of these risk criteria are *post hoc*, but are nonetheless helpful. Beaumont (1947) implies that the Beaumont criteria (which were themselves a modification of a set of criteria developed in the Netherlands, the "Dutch Rules") applied to an outbreak arising from infected seed

within a crop and this encompassed the following sequence of events: (i) sufficient tissue colonization at primary infection foci, (ii) sporulation, (iii) dispersal of sporangia to secondary infection sites, and (iv) secondary infection. Smith (1956) further modified these criteria by increasing the relative humidity threshold to 90 %.

Table 1.1 Risk criteria for outbreaks of late blight in potato crops, which have (or have historically had) widespread use in the United Kingdom.

Criteria	Temperature		Humidity	
	Threshold (°C)	Duration (hrs)	Threshold (RH)	Duration (hrs)
Beaumont (Beaumont, 1947)	≥ 10	48	≥ 75 %	48
Smith (Smith, 1956)	≥ 10	48	≥ 90 %	11 in each 24 hour period
Hutton (Dancey *et al.*, 2017)	≥10	48	≥ 90 %	6 in each 24 hour period

Researchers often produced risk criteria that were specific to their region of interest (Hansen, 1997) or modified criteria that had been developed elsewhere to better fit the local climate and potato production systems. An alternative approach often used is to construct models from empirically-determined pathogen traits and survival thresholds (Dowley *et al.*, 2001), which can then be used to determine probable pathogen risk for a given set of meteorological conditions.

There is a large spread in the complexity of these criteria, ranging from the use of a single predictor variable such as the accumulated exposure of spores to solar radiation (Skelsey *et al.*, 2018) through to more complex schemes that can encompass multiple weather parameters, as well as end-user actions such as the level of irrigation (Bouma and Hansen, 1999) or resistance of the host crop (Small *et al.*, 2015). Several models are available publicly as decision support systems (DSSs), which can be used by growers and agronomists to aid their late blight spray programmes. Within Great Britain, blight forecasting has been based on Smith Periods (Smith, 1956) determined for Meteorological Office monitoring. These have

recently been updated to the Hutton Criteria (Dancey *et al.*, 2017), and, in conjunction with a national network of disease monitoring and outbreak warnings, are an invaluable disease management tool.

Potato late blight has historical importance both socially and scientifically. Uncontrolled late blight outbreaks are dramatic examples of botanical epidemics (Dyer *et al.*, 1993) and late blight is one of the few plant disease which the general public have some awareness of (Kamoun *et al.*, 2015). Discussion of late blight often centres around risk, though the term is not always well defined. An appreciation of how risk is perceived, evaluated and understood is an important consideration; particularly when discussions between representatives of different backgrounds or disciplines take place (McRoberts *et al.*, 2011). One can easily imagine the mutual misunderstanding that could be caused if interlocutors use related, but different, meanings for risk. Informally, the word "risk" often carries connotations of both harm and uncertainty: something bad *could* happen (Bostrom, 1997). A straightforward analytical interpretation is to consider risk to be the probability of a harmful outcome (Burt, 2001; Preisler *et al.*, 2004), but definitions of risk also frequently encompass the magnitude of the harmful event (i.e. the greater the harm, the greater the risk) (Kasperson *et al.*, 1988).

Under some formulations, risk is the product of an event's probability and its impact (Boholm, 2003); the impact component could be measured in terms of economic consequences (Davidson *et al.*, 2015), mortality or morbidity (Silver, 2012), or some other 'cost' (Pollard *et al.*, 2004), depending on the field of interest. However, psychological factors are also an important consideration when defining risk, and some argue that risk is a concept inextricably linked to cultural and subjective perception, which are difficult (or impossible) to measure (Slovic *et al.*, 1980). For example, events are sometimes considered to carry risk if they are thought to have a highly *variable* outcome (Nauta, 2000). Perception of a risk may differ from the actual risk, both in terms of the likelihood of the negative event and of its impact. There may be tension between the two views of risk: risk defined as a

metric arrived at through analysis and risk as a feeling shaped by experience, intuition and culture (Dake, 1992). Concretely, a decision-maker may perceive the probability of a harmful event as being more (or less) likely than if it were measured objectively. This subjective perception of probability is called credence (Mellor, 2000). Credence often acts as a chief driver of behaviour, even in cases where the decision-maker has access to more objective estimates of an event's probability (Gold, 1989).

A further psychological factor connected to risk perception is fear. This is sometimes described by risk analysts as dread (Gregory and Mendelsohn, 1993). The anxiety produced by a risk is a very important factor in decision making, as it may push decision-makers to be more cautions than is warranted by a formal appraisal (Jagiello and Hills, 2018). Potato late blight carries high dread for several reasons: it was the proximate cause of a multi-year mass starvation event (Donnelly, 2007), and has thus accrued cultural significance (Fry and Goodwin, 1997); the disease can cause damage both in field and in store (Bjor and Mulelid, 1991); if uncontrolled it could potentially lead to complete crop failure; and the causal agent has remarkable fecundity (Fry and Smart, 1999). Late blight is a risk that is seen as very likely, very destructive and highly fear inducing (Turner, 2005).

Within this study, the straightforward definition of risk as the probability of a negative outcome will be used. This does not imply that the impact dimension of risk is unimportant, nor that psychological and cultural aspects of risk perception are irrelevant. Rather, that for this particular case they are not of primary concern; the impact of late blight is almost always assumed to be very severe (De Buck *et al.*, 2001), and so including an impact dimension to the definition of risk would not, in this instance, be very productive. Nor would it be useful to account for the perceptions of variability or of fear, as *P. infestans* inoculum is generally thought of as being ubiquitous (Skelsey *et al.*, 2017), and its potential arrival in a crop almost always inspires dread (Wustman, 2007).

The risk warnings discussed above (page 11) specify conditions under which the probability of infection is high (Singh *et al.*, 2013). This study focuses on curative fungicide treatments, which are, by definition, applied after infection has taken place. A more complete discussion of curative activity in relation to late blight is given in Section 1.7, page 24. Therefore, the specific 'risk' under consideration is not the risk that infections will occur, but the risk that infections will occur *and* will progress to produce visible lesions that will then (theoretically) produce further sporangia. Our scope is confined to early stages of an epidemic[1], either the initial loci of infection or secondary infections very early within an epidemic's progression. This is when fungicides with curative activity (Section 1.7, page 24) are thought to be most important in limiting pathogen spread, and it follows that another, perhaps more helpful, view of the definition of risk used here is that it represents the probability that curative control fails.

This time frame for this early or potential outbreak is difficult to specify precisely, because weather and variety will impact on the progression of a late blight epidemic within an affected crop (Andrivon *et al.*, 2003), but up to 14 days can be used as a very crude guide. Curative activity is not typically used to slow the progress of established epidemics but rather to attempt containment (even partial) where infection is suspected but has not developed to a stage where lesions are visible (Stein and Kirk, 2002). In practice, growers will often destroy the foliage of blighted crops (Miller *et al.*, 2006), either across the whole field or immediately surrounding an affected patch. At this point, recommendations on fungicide use and choice shift focus to protecting tubers within the soil from infection (Evenhuis *et al.*, 2016).

1. Note, epidemic in the wide sense of a change in disease incidence (number of plants of plant parts infected) and severity (plant area affected by disease), not necessarily only an increase (Madden *et al.*, 2007).

1.5 Host resistance

That variation in susceptibility to pathogens exists within and between plant species has long been appreciated, studied and deployed as a control strategy (albeit with varying degrees of success). The term resistance is used in a few different contexts within the literature, and potential ambiguities exist as resistance is also used to describe individuals that do not respond to the expected degree to chemical control measures (in order to distinguish clearly between the two the terms, *host* resistance and *fungicide* resistance will be used). Host resistance, in general, refers to a reduction in severity, or an absence of a disease phenotype, and it is instructive to consider plant resistance to pest and pathogen species as a hierarchy of the various different phenomena that the term is often used to describe (Niks *et al.*, 2015). It should be noted from the outset, however, that there is significant overlap in the plant immunity mechanisms underpinning these different categories of resistance (Gill *et al.*, 2015; Poland *et al.*, 2009).

The first useful distinction to make is between host and non-host resistance. Non-host resistance refers to the inability, notwithstanding exceptional or contrived conditions (see Belhaj *et al.* (2017)), of a given pathogen species to establish an interaction between it and the non-host species. Such resistance is often conferred when PAMP triggered immunity (PTI) systems (Zipfel, 2009), effector triggered immunity (ETI) systems (Nishimura and Dangl, 2010), preformed (Osbourn, 1996) or induced defences (such as callose deposition (Luna *et al.*, 2011) are sufficient to frustrate infection attempts by the pathogen. Non-host resistance does not concern us further here, but it is worth noting that, over evolutionary time scales, a pathogen may adapt to overcome non-host resistance, or a host may move to non-host status if a pathogen's effector repertoire or other pathogenicity factors lag behind the plant's ability to suppress them (Schulze-Lefert and Panstruga, 2011). In contrast, host resistance refers to resistance displayed by individuals, cultivars or accessions of a species for which a typical disease phenotype is usually observed. Classically, host resistance has been further subdivided into two forms: quantitative and qualitative resistance, with a number of synonyms for both. Qualitative has also

been termed vertical (Parlevliet and Zadoks, 1977), complete (Ballini *et al.*, 2008), major gene (Song *et al.*, 1995) and *R* gene (Ballvora *et al.*, 2002) resistance. Quantitative has been referred to also as horizontal (Nelson, 1978), partial (Hodgson, 1961), minor gene (Mundt, 2014), field (Kaniewski *et al.*, 1990), and basal (Niks and Marcel, 2009). This dichotomy is probably too simplistic, and it is likely that a continuum exists between the two forms of resistance, and that they share at least some molecular mechanisms (see page 18). Qualitative resistance is used to describe the observation that some pathogen-host genotype combinations are incompatible (i.e. disease symptoms are not or very rarely seen, even in conditions when they would be anticipated (Poland *et al.*, 2009)). This type of resistance is now understood as ETI, where the host carries specific *R* genes that enable recognition of pathogen effectors or their products, which in turn triggers cascades that in most cases result in a hypersensitive response (HR) in the host cells and an arrest of pathogen development. In contrast, quantitative resistance refers to a situation where the resistance is only partial.

A number of *R* genes that are active against *P. infestans* exist within the germplasm of *Solanum* species and work is ongoing to identify new candidates. Several genes have been mapped and/or cloned and a number have also been introgressed into cultivated potato varieties: initially the genes *R1*, *R2*, *R3a*, *R3b*, *R4* and *R10* from *Solanum demissum* (Rodewald and Trognitz, 2013). This strategy has not proved durable, as these *R* genes were rapidly defeated. For example, the cultivar Pentland Dell which carries *R1*, *R2*, *R3a* and *R3b* (Bozkurt *et al.*, 2012) became susceptible to late blight infection within only four years of its deployment (Stewart *et al.*, 2003). A single *R* gene presents a strong selection pressure on pathogen evolution (Bergelson *et al.*, 2001), and, once broken, offers limited protection in the field. Strains of *P. infestans* virulent to *R* genes *R5 – R11* were found in the field in Great Britain, although these genes had, at the time of sampling, yet to be utilized on a wide scale by breeders (Malcolmson, 1969). Many of the *P. infestans* effectors which *R* genes recognize belong to a class referred to as RxLR (as they possess the motif: arginine – variable – leucine – arginine). These

effectors are believed to gain entry to host cells through the use of a signal peptide region, and from there dampen the host's immune response (Whisson *et al.*, 2016). These effectors are strongly expressed in the early stages of infection (Judelson *et al.*, 2008), which has led to the proposition that they are associated with the maintenance of the initial biotrophic phase (Du *et al.*, 2015). Interestingly, the majority of RxLR genes within the *P. infestans* genome lie in dynamic regions rich in mobile elements (Haas *et al.*, 2009), which may be a possible explanation for the pathogen's resistance breaking propensity.

The second form of host resistance, quantitative resistance, manifests as individuals which are susceptible to infection but which display reduced symptoms and slower disease development than is typical. Quantitative resistance is currently not well understood, but it is likely more complex than qualitative resistance (which fundamentally involves a single pathogen-host gene pair (Flor, 1971)) and is generally viewed as a polygenetic trait (Solomon-Blackburn *et al.*, 2007). Quantitative resistance probably represents a range of related and distinct mechanisms that slow disease progression, and clearly it only makes sense if discussed in relative terms; a susceptible cultivar could be described as resistant when it is contrasted with an exceptionally susceptible cultivar (Niks *et al.*, 2015). Quantitative resistance is predicted as being more durable than fully functioning *R* gene based resistance (although there is evidence that in some circumstances it can be eroded, (see Andrivon *et al.*, 2007)). For this reason, and because *P. infestans* is recalcitrant to major-gene based resistance, breeding efforts have been directed more towards quantitative resistance over the past few decades.

It has long been appreciated that quantitative resistance (frequently referred to as 'field resistance' in the original studies) in *Solanum* against *P. infestans* has a number of components. Black (1970) classified these as: resistance to infection, resistance of tissue to hyphal colonization, delay in the initiation of sporulation, and reduction in the number of sporangia produced. This specification roughly corresponds to the aggressiveness components which are often assigned to isolates

in laboratory based studies. Other authors have focused on functional distinctions, partitioning field resistance into morphological features on the one hand and biochemical features on the other (Evers *et al.*, 2003). There are also several studies which explore specific mechanisms of resistance in detail. For example, Vleeshouwers *et al.* (2000a) observed delayed HR (hypersensitive response, i.e. rapid cell death in the vicinity of infection, see Lam *et al.* (2001)) in 'field resistant' and 'weak *R* gene' (*R10*) carrying cultivars, hyphae often escaped from necrotic areas and went on to form a biotrophic association typical of early infection stages. There is also evidence that defeated *R* genes contribute to quantitative resistance, although there are equivocal reports on the size of these 'residual' effects (Ordoñez *et al.*, 1998; Stewart *et al.*, 2003). There is also evidence of linkage between major *R* genes and 'minor' resistance genes, making it difficult to partition the respective contribution of each (Gebhardt and Valkonen, 2001).

There is evidence that the level of quantitative resistance varies amongst plant parts with plant age and can be influenced by a large number of additional factors, such as nutritional stress status of the crop (Thurston, 1971). Some of these effects appear to apply to a species generally and are not cultivar specific, for example: *S. tuberosum* leaves from more apical nodes are more resistant to *P. infestans*, irrespective of the age of said leaves (Visker *et al.*, 2003).

These interpretations of resistance are potentially confounded by the existence of susceptibility genes (*S* genes). These are host genes which the pathogen requires in order to establish a compatible interaction, for example, for the recognition of host cell wall characteristics or for critical points of interface with host metabolism (van Schie and Takken, 2014). The effects of *S* genes are recessive and manifest as reduced susceptibility to pathogen attack; they are also generally accompanied by some loss of function within the host plant cell an effect which may or may not be detrimental in itself). It can become a matter of semantics what is referred to as a resistance or a susceptibility gene (for example the gene *mlo* in barley, the discovery of which pre-dates the widespread use of the term *S* gene (Pavan *et al.*, 2010)). *S*

gene based resistance may be more durable than that based on *R* genes, but, because of their relative novelty, evidence on this is lacking. Functionally, *S* genes are encompassed by the above definitions of qualitative and quantitative resistance (i.e. the presence and nature of the disease phenotype), and a recent knock-down study of putative potato *S* genes generated five cases that would be classified as qualitative and one case of reduced susceptibility that would be classified as quantitative (Sun *et al.*, 2016). It is not known if some of the naturally occurring quantitative resistance found within potato cultivars is attributable to *S* genes.

1.6 Chemical control

The ubiquity of and the severity of infections caused by *P. infestans* necessitate routine chemical control. This is particularly the case in regions with prevailing cool temperatures and high relative humidity (which are very conducive to infection) such as northern Europe. Potato growers have access to a range of fungicides for the control of late blight, with active ingredients (a.i.s) representing several chemical classes, modes of action, and with different physical properties. This diversity is useful for late blight management programmes as products can be selected to complement criteria such as the growth stage of the crop, prevailing weather conditions, or local disease pressure (Evenhuis *et al.*, 2009). Potato production in temperate regions requires a high input of crop protection products, chiefly due to the pressure from late blight (Haverkort *et al.*, 2008). There are also legal restrictions on fungicide use, e.g. the number of applications and/or the total amount of a fungicide product which can be applied to a given crop (Schepers and Cooke, 2015). Such restrictions provide an additional need for a variety of products in order to conduct a compliant and effective management programme. The restrictions may be in place for environmental reasons or as a precautionary measure against the development of fungicide resistance. The United Kingdom-based Fungicide Resistance Action Group (FRAG-UK) publishes regular technical advice relating to fungicide resistance management (Burnett, 2011), including recommendations use for the various classes of fungicide which are available for late blight management. Access to multiple different modes of action is vital for fungicide resistance

stewardship. Several characteristics of *P. infestans'* life cycle and current management practices[2] lead to a moderate risk of fungicide resistance developing[3] (Qin *et al.*, 2016). These include a rapid life-cycle (Montarry *et al.*, 2007), a large population size when outbreaks occur (Fry *et al.*, 1992), and a high selection pressure exerted by the quantity of fungicide applied within a season (Doster and Fry, 1991). Major resistance problems exist for the phenylamide class of fungicides: control failures were reported in the 1980s (Gisi and Cohen, 1996), and several common contemporary strains such as 13_A2 are resistant to fungicides of this class (Cooke *et al.*, 2012). More recently, genotypes insensitive to fluazinam have been found, including a highly aggressive lineage designated 37_A2 (Andrivon *et al.*, 2017). FRAG guidance is specific to each mode of action, but generally single-site a.i.s are recommended for use in mixtures, and in some cases there are restrictions on the number of treatments a crop can receive (FRAG-UK, 2018).

The nature of the a.i.s used to control late blight in the field has changed considerably over time as newer actives have supplanted older compounds which have lost regulatory approval due to more stringent health and environmental safety requirements (Williams, 2012). The first class of fungicides which were used extensively were copper based, such as the copper sulphate/calcium hydroxide 'Bordeaux' mixture, which is toxic to *P. infestans* spores (Leach, 1966). These formulations had a number of drawbacks, including limited efficacy, phytotoxicity, and concerns over residue persistence (Edwards-Jones and Howells, 2001).

Routine treatment of potato crops with blight fungicides became common in the mid-20th century, initially with organometallic compounds such as fentin acetate (introduced in 1954), an ATP synthesis inhibitor (Fait *et al.*, 1994), and the dithocarbamates such as nabam and zineb introduced in 1942 (Schwinn and Staub, 1995). Several of these compounds are no longer approved for use in many

2. These are a response to the pathogen's life-cycle traits. Frequent fungicide treatments are necessitated by *P. infestans'* high reproductive capacity.

3. Though, clearly, resistance risk depends on properties of both the a.i. *and* the pathogen in combination.

countries due to human health and environmental concerns (López-Fernández *et al.*, 2012), but others such as mancozeb remain a core component of several late blight fungicide preparations (Gullino *et al.*, 2010). From the 1970s onwards, innovations in organic chemistry led to the development of new compounds with lower non-target toxicity, which were active at much lower concentrations than their predecessors (Russell, 2005). Several of these compounds had the additional benefit of mobility within plant tissues which opened up the possibility of curative control.

Currently, there is a well-developed market for late blight fungicides within the UK. Several a.i.s are available in a number of formulations. Commercial products frequently consist of two partnered a.i.s as a fungicide resistance management strategy (Burnett, 2011). Several different biochemical modes of action (MOA, this refers to the primary mechanism[4], if known, through which the a.i. exerts biocidal effects upon the target organism) are represented by the available compounds, which is fortunate from the perspective of fungicide resistance management. There is also significant diversity in physical properties and mobility *in planta* of the available compounds, and a thorough understanding of these features can add efficacy and flexibility to a late blight management programme. It is possible for an agronomist or grower to match an appropriate fungicide to the developmental stage of the crop, to the pressure exerted by the pathogen, and to local weather conditions. For example, if tuber blight is thought to be a significant risk (in a susceptible variety, late in the season) a product containing, for example, fluopicolide, which reduces zoospore motility and encystment, could be selected (Tafforeau *et al.*, 2006).

Late blight fungicides (and indeed, chemical species used for plant protection in general) can be classified in several ways using various criteria, such as their

4. If several mechanisms are important, the a.i. is described as *multi-site.* These compounds have particular utility in the management of fungicide resistance as they have an inherent 'low resistance risk', see: http://www.frac.info/docs/default-source/publications/frac-recommendations-for-multisites/frac-statement-on-multisite-fungicides-2018.pdf (accessed: May, 2019).

chemical structure or properties. Different classification schemes are appropriate in different situations. For example, because of the possibility of pathogens developing resistance to a.i.s, the Fungicide Resistance Action Committee (FRAC)[5] maintains a list of commercial fungicides distinguished by their MOA. A summary of these as related to curative late blight fungicides can be found in Table 1.2 (page 29). The fungicidal and fungistatic properties of the a.i.s used to target *P. infestans* vary between different developmental stages (i.e. sporangia, zoospores, mycelium *in planta* etc.) of the pathogen (Bruck *et al.*, 1981; Cohen and Gisi, 2007; Samoucha and Cohen, 1990). This is generally a function of both the compound's MOA and its mobility within the plant. From a practical perspective, mobility (also called systemicity) is a key characteristic informing fungicide choice (Cooke *et al.*, 2011) and refers to the propensity of a.i.s to redistribute or not within plant tissue after application (Klittich, 2014). Fungicide mobility is a complex trait (Satchivi *et al.*, 2006) which is influenced by a variety of factors including: the physical and chemical properties of the a.i. molecule, e.g. melting point (Stevens *et al.*, 1988), molecular weight (Bauer and Schönherr, 1992), water solubility (Baker *et al.*, 1992), lipo-phobicity/philicity (Riederer and Friedmann, 2008): the anatomical properties of the treated plant, e.g. leaf surface structure (Boize *et al.*, 1976) and canopy architecture (Sharpe *et al.*, 2018); as well as the environmental conditions prevalent at, or immediately after, application, e.g. temperature (Cabras *et al.*, 1999). Additionally, it is common for fungicide manufacturers to produce formulations that seek to optimise a.i. uptake by including humectants (Steurbaut, 1993), spreading solvents (Zabkiewicz, 2007), etc., or for agronomists to recommend adjuvants to tank mix with the fungicide (Heremans *et al.*, 2018).

Although fungicide mobility is best understood as a continuum (Klittich and Ray, 2013), compounds are frequently grouped into categories for convenience. For example the EuroBlight fungicide table (Evenhuis *et al.*, 2016) classifies products as: (i) contact fungicides, which are not appreciably taken up into plant tissues; or (ii)

5. FRAC is a grouping of technical experts under the auspices of CropLife international, an international trade association of agrochemical producers. FRAC regularly publishes guidelines and recommendations concerning 'at risk' fungicides. See: http://www.frac.info/about-frac (accessed: May, 2019).

translaminar fungicides, which can be redistributed within leaf tissue but do not move into new plant growth not treated at the time of application; or (iii) systemic fungicides, which can be redistributed via xylem to younger leaves (acropetal movement) and sometimes to progeny tubers through basipetal movement. These three definitions will be adopted here to ensure consistency with the general literature (Buchholz and Nauen, 2001; Hinds, 2000; Kromann *et al.*, 2008), but they should not be considered as absolute categories.

Generally, a fungicide with low or no systemicity (i.e. a contact fungicide) can only be deployed as a protective treatment (Dias, 2012); meaning that any applications should be prior to the pathogen challenge. Fungicide molecules will then be encountered by the pathogen at the plant (generally leaf) surface and exert their effects there. In contrast to this, systemic and translaminar compounds offer greater flexibility; as with contact-only fungicides, they are almost universally used as protectants (Johnson *et al.*, 2000; Serey *et al.*, 2007), but several additional advantages arise from the fact that they are taken up by, and are mobile within, plant tissues. Systemic fungicides may be less susceptible to erosion by environmental factors (Evans, 1971), providing extended residual activity after application (although see (Vicent *et al.*, 2007). Their redistributive properties may compensate if there has been poor spray coverage (Vincelli and Dixon, 2007), and, after application, they may move into and accumulate within regions of active plant growth (Sicbaldi *et al.*, 1997). This last property is particularly useful for management of the potato-late blight pathosytem, specifically within the period of rapid haulm growth (Evenhuis *et al.*, 2006) during which a large area of foliage can be produced between spray applications. In addition to the advantages of systemic and translaminar fungicides listed above, mobility within plant tissues also opens the possibility of their controlling the pathogen early post-infection. This post-infection activity is usually described as curative (see Section 1.7, page 24) as it acts upon pathogens which are already established within plant tissues.

Although the positive aspects of systemic fungicides have been highlighted here, there are a number of cautionary points which must also be considered. It is worth

noting that only a relatively small proportion of any applied systemic a.i.s will move into plant tissues (Skylakakis, 1983), making the formulation critical. Curative treatments may increase the risk of the target pathogen gaining resistance (Kable, 1987) especially if the formulation includes a contact-only partner, which will have little effect on established infections, thus effectively undermining the partnering strategy (Staub, 1991).

1.7 Curative activity and its evaluation

All late blight fungicides are applied as prophylactics and the majority are contact-active, meaning their activity is due to pathogen sporangia, zoospores and/ or germ tubes encountering fungicide deposits at the leaf surface. To ensure that a crop is adequately protected, there must be regular reapplications because fungicide at the phylloplane erodes over time with exposure, and rapid growth of new plant tissue effectively dilutes the quantity of a.i. present. The timing of fungicide application in relation to weather-based high risk periods can be critical. Late blight fungicides that are contact-acting have greater efficacy if timed shortly before a high risk period (R. Bain, SRUC, Auchincruive, UK, personal communication).

A subset of late blight fungicides also have some mobility within plant tissues. One, metalaxyl-M, is truly systemic and two, oxathiapiprolin and propamocarb, are acropetally systemic (i.e. movement is towards shoot apex from site of absorption). Several others display translaminar activity. The uptake of these compounds into plant tissue opens the possibility that post-infection pathogen structures will come into contact with a.i.s. Any toxicity toward the pathogen at this stage is termed a *curative* effect to distinguish it from the protectant effect mentioned above (Bain, 2016). Most authors further restrict curative activity to control that occurs post-infection but before the appearance of symptoms, i.e. during the incubation period. A further category, *eradicant* effect, is used for chemical control that is effective after the onset of symptoms. This is less frequently encountered in practice and there is sometimes confusion between the terms curative and eradicant.

Curative fungicides are an important component of late blight control programmes (Tafforeau *et al.*, 2006). There are surprisingly few studies that focus on the curative activity of late blight fungicides, and fewer still that explore factors which may influence how well a given curative fungicide performs. There are a handful of comparative studies (Bugiani *et al.*, 2010; Johnson *et al.*, 2000; Pirondi *et al.*, 2017), and rankings are provided in the EuroBlight table[6]; but these are subjective, being based on expert opinion and disparate sources rather than data generated from a harmoized protocol. There also seems to be some diversity of opinion; Schepers (1998) found little agreement on the scale of curative active for various a.i.s in responses to a questionnaire sent to agrochemical company representatives and advisors from university extension services. Several results from small commercial trials are available, but it is difficult to make comparisons as some methodologies differ. Most studies confirm that regardless of a.i., curative activity is time-limited and that it compares unfavourably with protectant control (Stein and Kirk, 2002). Nevertheless a short 'curative window' exists in which useful control is possible.

Curative activity may be dependent on the developmental state of the pathogen and the a.i.s currently available are only effective in early stages of infection development. Curative activity may also be influenced by other factors, such as the strain of the pathogen or the state of the host (age, cultivar etc.). For example, there are two economically important hosts for *P. infestans*: *S. tuberosum* and *S. lycopersicum* (Flier *et al.*, 2003a), and studies on curative treatments exist for both. It is not clear if any curative a.i.'s behaviour differs between the two hosts, as no comparative studies exist, but it is worth noting that *P. infestans* has been reported as having an extended biotrophic period when infecting *S. lycopersicum* (Zuluaga *et al.*, 2016a).

A further difficulty arises from the definition of curative activity. Curative activity is sometimes quantified differently by different authors, and is often

6. http://agro.au.dk/forskning/internationale-platforme/euroblight/control-strategies/late-blight-fungicide-table/ (accessed Sept. 2019)

contingent on the methods used. For example, if whole plants are bulk-inoculated and then subsequently treated with a curative fungicide, curative control may be assessed by the proportional reduction in diseased leaf area (Komyoji *et al.*, 1995). In other cases where point inoculation is used, counts can be made of sites that do or do not go on to develop lesions (Johnson *et al.*, 2000). It is also common for studies to consider the effect of curative treatments on later spore production as this is an important component of late blight's epidemiology (Bashi *et al.*, 1982; Harrison, 1992). The issue is illustrated by a study (Bugiani *et al.*, 2010) which quantifies curative activity in terms of both disease *severity* (area covered by lesions) and disease *incidence* (counts of lesions per plant); the magnitude of curative control appeared larger with the former than the latter.

In some instances, a developer or distributor may not wish to claim systemicity or any degree of curative activity for an a.i., even when data exist suggesting it possesses these characteristics. This could be because the compound's curative activity is very weak or time-limited in comparison to other a.i.s, and a company may feel that its impact would be negligible in a field situation. It is possible that they may suffer reputational damage or litigation if a product does not meet end-users' expectations. Even if a product contains a 'curative' a.i., literature aimed at the user (such as the product label or accompanying advice sheets) will likely avoid stressing this property, as potato late blight is best controlled with a protectant approach. This complicates the task of listing curative compounds, particularly if one uses only fungicide labels as the information source. In this study, any fungicide for which there is published information indicating above-negligible post-infection activity is considered to have curative activity. This definition is used for completeness and clearly, in some cases, the duration of the curative window may be so short or magnitude of the effect so small that using the a.i. curatively in the field will likely make no appreciable contribution to disease control.

These points notwithstanding, there is some consistency in the literature in the duration of curative activity and the size of the effect from several commonly used

a.i.s.. Two studies, Bugiani *et al.* (2010) and Pirondi *et al.* (2017), have assessed a large range of fungicide formulations (9 and 22 respectively) against *P. infestans*-infected *S. lycopersicum*. The rank order of fungicide effectiveness was broadly the same as the curative ratings in the EuroBlight table (although different formulations and dose rates may be important) with significant reductions in disease severity provided at 24 hours post infection for the majority of formulations tested. The phenylamides performed particularly well, as did formulations containing two a.i.s considered to have curative activity (i.e. propamocarb + cymoxanil). Formulations which performed less well tended to be those that included only a single translaminar a.i. (i.e. propamocarb at lower doses, dimethomorph, etc.) or those which the EuroBlight table classifies as contact only (i.e. cyazofamid, etc.).

A single time-point (24 hours post inoculation) was assessed by Pirondi *et al.* (2017), but Bugiani *et al.* (2010) tested the same fungicides at 48 and 72 hours post inoculation. No curative activity was reported at 72 hours, but many fungicide formulations reduced disease levels significantly at 48 hours post inoculation, although to a lesser degree than at 24 hours; with one formulation (fluopicolide + propamocarb) showing the greatest reduction in effectiveness between the two time-points. This pattern was repeated in many of the studies which rated curative activity based on reductions in disease severity. Those that included later (72+ hours) time-points generally reported no effect, although one (Mayton *et al.*, 2001) found very minor (~ 10 %) reductions when cymoxanil was applied at 96 hours post inoculation. Studies that included intermediate time-points (36 - 48 hours) gave very variable results and differences between a.i.s became clearer: Johnson (2000) reported a low curative control at 36 hours (*S. tuberosum*, detached leaves) from a dimethomorph + mancozeb formulation, but very good control from propamocarb + chlorothalonil as well as cymoxanil + mancozeb.

Most studies which sought to quantify curative control of late blight were laboratory-based (this was also the case for other plant pathogens, see: Reuveni *et al.* (2003), Schwabe *et al.* (1984), and Usall *et al.* (2008)), which was probably due to

the logistical difficulties involved in plot-scale or field-scale experiments. Nevertheless, it is relatively common for reports of field trials to mention curative activity in passing (Anwar *et al.*, 2015; Kankwatsa *et al.*, 2003) and there are examples where it has been assessed directly. Stein and Kirk (2002) demonstrated that for several fungicides, curative treatments beginning 72 hours following an artificial inoculation event significantly reduced epidemic development to such an extent that the authors classified them as contained, but these treatments did not fully prevent disease spread. Other studies support the observation that spray programmes can offer effective control post infection if begun within a 'critical curative window' (see next section) following inoculation or high-risk weather (Cohen *et al.*, 2007; Engelbrecht, 2003; Kessel *et al.*, 2018; Siddique *et al.*, 2016). Perhaps surprisingly, the rank order of fungicide curative activity was not always the same in these field trials as in laboratory studies (Bødker and Nielsen, 2000; Griend and Bosch, 2007). A single study (Nielsen and Bødker, 2002) compared curative control in the field across seasons, which gave relatively consistent results if treatment time was expressed as a proportion of the incubation period. Formulations containing contact a.i.s provided 'curative' control no later than the first 20 % of the incubation period, two products (mancozeb + dimethomorph and famoxate + cymoxanil) provided control up to 50 % of the incubation period, and a further two (mancozeb + propamocarb, mancozeb + metalaxyl) gave good control up to 40 to 66 % of the incubation period.

There are several possible factors that could influence the curative efficacy of a fungicide a.i.: perhaps most obviously dosage, spray coverage or weather conditions at the time of application, but also several aspects of the plant-pathogen interaction may have indirect effects. It seems likely that the time-limited nature of curative activity is governed by the stage of the infection, with control decreasing as the pathogen increases its biomass. Any factors that increase the rate at which the pathogen colonizes tissue will, as a consequence, reduce the time period over which a curative a.i. shows activity, and it follows that the converse is also true.

Table 1.2. Compounds used as fungicides against *P. infestans* which have some degree of curative activity. Compound common names, chemical classes, FRAC codes and biochemical modes of action are taken from Fungicide Resistance Action Committee definitions. Curative ratings and mobility classifications are those listed in the Euroblight table: ● 'reasonable effect', ●● 'good effect', ●●● 'very good effect'. Half filled circles ◐ represent intermediate cases, thus: ●◐ indicates an effect between 'reasonable' and 'good'. Usage data refers to registered potato cropped area in Scotland, as reported by the SASA pesticide usage survey for 2016 (Monie *et al.*, 2016). The EuroBlight table is reproduced in Appendix 3.

Active ingredient common name	FRAC group name	FRAC code	Curative rating	Mobility in plant	Biochemical mode of action	Usage in Scotland, 2016 (treated ha) [a]
Benalaxyl-M	Phenylamide	4	●●◐	Systemic	Interferes with rRNA synthesis	-
Metalaxyl-M			●●◐	Systemic	Interferes with rRNA synthesis	74
Propamocarb-HCl	Carbamate	28	●●	Systemic	Undetermined, likely interferes with fatty acid synthesis.	25,086
Cymoxanil	Cyanoacetamide-oxime	27	●●	Translaminar	Unknown	136,637
Oxathiapiprolin	Piperidinyl thiazole isoxazoline	49	●●	Systemic	Targets an oxysterol binding protein	approved in 2018
Benthiavalicarb	Valinamide carbamate	40	●◐	Translaminar	Targets cellulose synthase (CesA3)	9,039

Table 1.2. (continued from previous page)

Active ingredient common name	FRAC group name	FRAC code	Curative rating	Mobility in plant	Biochemical mode of action	Usage in Scotland, 2016 (treated ha)[a]
Mandipropamid	Mandelic acid amide	40	●	Translaminar	Targets cellulose synthase (CesA3)	39,997
Dimethomorph	Cinnamic acid amide		●	Translaminar	Targets cellulose synthase (CesA3)	32,379
Cyazofamid	Quinone inside inhibitor	21	- [b]	Contact [c]	Interferes with oomycete cellular respiration	62,104
Fluopicolide	Benzamides	43	- [b]	Contact [c]	Delocaoluzation of spectrin-like proteins	11,718

[a] Conventionally-managed commercial potato crops typically receive multiple fungicide applications for control of late blight within a growing season, which accounts for the treated area exceeding the planted area. In 2016, total estimated area of potato cultivation for Scotland was 27,525 ha (https://www2.gov.scot/Topics/Statistics/Browse/Agriculture-Fisheries/PubAbstract, accessed May, 2019).

[b] Not considered curative by EuroBlight table. http://agro.au.dk/forskning/internationale-platforme/euroblight/control-strategies/late-blight-fungicide-table/ (accessed, Oct. 2019)

[c] As classified by the EuroBlight table.

1.7.1 Carboxylic acid amides (CAAs)

CAA fungicides that are commonly used against *P. infestans* include dimethomorph (first reported in 1988 (Kuhn *et al.*, 1991)), mandipropamid (first introduced in 2005 (Zhang *et al.*, 2014)) and benthiavalicarb (introduced in 2003 (Reuveni, 2003)). These compounds are structurally diverse but are grouped together due to evidence of cross-resistance in target organisms (Kuhn *et al.*, 1991). They act on the oomycete specific cellulose synthase enzyme PiCesA3 (Blum *et al.*, 2010). Early asexual structures are the most sensitive stages to CAAs (Sun *et al.*, 2010), and thus CAAs are particularly effective when applied preventively, but their translaminar mobility, which varies between a.i.s, also confers some curative activity (Cohen and Gisi, 2007; Toffolatti *et al.*, 2011).

1.7.2 Cyazofamid

Cyazofamid, which was introduced in 2001, is listed as a contact-acting fungicide by the Euroblight table and is marketed as a protectant only in products marked for the control of *P. infestans* (Mitani *et al.*, 2005). However, several studies report some degree of translaminar mobility (Mitani *et al.*, 2001) and curative activity (Mitani *et al.*, 2002), although often with less efficacy than some other curatively applied a.i.s (Pirondi *et al.*, 2017). Cyazofamid interferes with oomycete respiration by binding to mitochondrial complex III within the electron transport chain (Mitani *et al.*, 2001). Cyazofamid is one of the more commonly applied fungicides (see Table 1.2, page 30) and this is probably due in part to its activity against zoospores, which makes it useful for the control of tuber blight (Desnouck *et al.*, 2012; Ebersold, 2002).

1.7.3 Cymoxanil

Cymoxanil was developed by DuPont in the early 1970s (Cohen and Coffey, 1986). To date, its mode of action has not been resolved, but it is thought to act in conjunction with plant metabolism (Ziogas and Davidse, 1987). Due in large part to its curative activity, cymoxanil is one of the most widely used curative fungicides, particularly since the emergence of phenylamide-insensitive strains. Cymoxanil has a very short half-life *in planta* (2 - 3 days), and there is strong evidence that it can

act synergistically with other a.i.s (Wang *et al.*, 2002). Cymoxanil is one of the few curative fungicides for which an external factor has been shown to modify the curative effect – higher temperatures appear to lead to a shorter curative window (Genet *et al.*, 2001; Mayton *et al.*, 2001) The mechanism behind this is not fully clear but could be due to different rates of pathogen biomass accumulation.

1.7.4 Oxathiapiprolin

Oxathiapiprolin is the most recently introduced fungicide which has curative activity against *P. infestans* and other oomycetes. Developed by DuPont, it gained authorization for use in the United Kingdom in 2018. The compound targets an oxysterol-binding protein, disrupting lipid metabolism (Pasteris *et al.*, 2016). It is active against several life cycle stages of the pathogen: inhibiting the release of zoospores, reducing lesion formation, and suppressing sporulation (Cohen, 2015). the compound shows translaminar movement after its rapid uptake into the epicuticle (Cohen, 2015), and its curative properties against oomycetes (including *P. infestans*) have been demonstrated in several studies (Cohen *et al.*, 2018a, 2018b; Miao *et al.*, 2016).

1.7.5 Propamocarb

Propamocarb belongs to the carbamate class of fungicides and was first used commercially in 1978 (Cohen and Coffey, 1986). Its mode of action has not been fully determined, but it is suspected to be linked to lipid metabolism (Gisi and Sierotzki, 2008); cell membrane permeability is greatly increased by propamocarb in target organisms, but this can be reversed by the addition of sterols to the culture media (Papavizas *et al.*, 1978), suggesting propamocarb interferes with sterols synthesis or transport. Propamocarb is translocated acropetally, and this property has led to its use as a soil drench to combat plant pathogens in propagation nurseries (Hu *et al.*, 2007; Meyer and Hausbeck, 2013; Moorman and Kim, 2004). Propamocarb has some curative activity (Samoucha and Cohen, 1990) which appears to be enhanced when it is applied with an appropriate partner a.i. (Bugiani *et al.*, 2010). Mayton *et al.* (2001) observed a suppressive effect on an established field epidemic: a formulation containing propamocarb + chlorothalonil caused

lesion growth rates to decrease, though this falls outside the definition of curativity adopted in this study.

1.7.6 Phenylamides

Phenylamides are a class of fungicides including metalaxyl-M, oxadixyl and benalaxyl (Gisi, 2002). These compounds were heavily used in blight spray programmes due in part to their high mobility and specificity to oomycetes. Phenylamides act on oomycete RNA polymerase I, showing little activity against zoospores, sporangia or the formation of primary haustoria because these structures are well supplied with ribosomes, but development following these stages is well controlled by the accumulation of RNA precursors due to the disrupted metabolism (Sukul *et al.*, 2000). In some countries, phenylamides were marketed as single a.i. products, and this was followed by the rapid emergence of insensitive strains of several oomycete pathogens. Metalaxyl was suspended from use during the early 1980s in both the Netherlands and the Republic of Ireland (Staub, 1991). The hiatus led to a fall in the proportions of insensitive isolates appearing in population surveys and phenylamides were reintroduced as co-formulations, with cautious use advised. Phenylamide use in contemporary late blight spray programmes within Great Britain is very low and generally advised against as genotypes such as 13_A2 have been shown to be insensitive to metalaxyl (Cooke *et al.*, 2012).

1.7.7 Fluopicolide

Fluopicolide belongs to the benzamides group of fungicides (Toquin *et al.*, 2007) and induces the delocalization of spectrin-like proteins, disrupting the cytoskeleton (Toquin *et al.*, 2010). It was introduced in 2006 and is currently available as a co-formulation with propamocarb (Tafforeau *et al.*, 2006). Fluopicolide is described as having translaminar mobility (Cooke and Little, 2002), via xylem redistribution (Jiang *et al.*, 2015), and there is evidence that a greater proportion of the a.i. moves into leaf tissue in the presence of propamocarb-HCl (Tafforeau *et al.*, 2006). Mixtures of fluopicolide and pyraclostrobin provided greater curative activity against *P. infestans* (reported as EC_{50} values after 24 hours) than the a.i.s applied singly (Wang *et al.*, 2014).

1.8 Diagnostics

Traditionally, plant pathologists have relied on the scoring of visual symptoms to assess the extent and severity of infections, and this can yield a great deal of information if practised by a skilled assessor. There are, however, several limitations to visual assessments: scores may vary between practitioners; the relationship between outward symptoms and *in planta* pathogen growth may be complex; and no information can be obtained before symptoms appear, which prevents the study of initial stages of infection. These limitations can be overcome to a certain extent by the use of molecular diagnostics, which allow enhanced exploration of plant-pathogen interactions (Atkins and Clark, 2004) as well as providing a tool to address applied epidemiological questions.

Initial general diagnostic techniques were non-specific, such as the measurement of compounds synthesised by the pathogen and not the host, i.e. chitin, ergosterol, etc. (Plassard *et al.*, 1982). Later, enzyme-linked immunosorbent assays (ELISAs) were used extensively to study plant pathogens including *P. infestans* (Harrison *et al.*, 1990), which had the advantage of being species or genus specific but had a number of drawbacks including cross-reactivity. These have been superseded by DNA/RNA probe and PCR amplification technology, which offer rapid, high-throughput and specific quantification and detection. End-point PCR assays specific to *P. infestans* have been developed (Judelson and Tooley, 2000) and used for the detection of contamination in seed stocks (Keil *et al.*, 2010a), to assess if infections were initiated from the mother tuber of plants in the field (Lehtinen and Hannukkala, 2008), to detect the presence of sporangia, zoospores or oospores (Wangsomboondee and Ristaino, 2002) and to explore historical outbreaks (Ristaino *et al.*, 2001). Molecular diagnostics can also be used to answer fundamental epidemiological questions. For example, Appel *et al.* (2001) artificially inoculated tubers and later used a PCR assay to confirm the presence of *P. infestans* DNA within stems originating from the tuber, before the appearance of lesions or sporulation – demonstrating that infections originating in the tuber can spread to other plant parts through mycelial growth.

Quantitative resistance is not solely an attribute of a cultivar but arises from the interaction of specific pathogen genotypes with specific host genotypes (Andrivon, 1993). There is a substantial body of descriptive research related to host-pathogen genotype interactions for *P. infestans* (Latin *et al.*, 1981). It has been established cytologically that in some resistant cultivars, hyphal proliferation and the rate of tissue colonization (Berggren *et al.*, 1988) are slowed. There are previous studies that have examined rates of tissue colonization using ELISA (Harrison *et al.*, 1990), but these used a limited range of cultivars and a single *P. infestans* isolate. The modifying effects of temperature, radiation (both photoperiod and light intensity) on quantitative resistance have also been explored (Harrison *et al.*, 1994), with authors reporting marked genotype-environmental interaction effects and a high degree of variability across experiments, highlighting the large number of factors that can potentially influence quantitative resistance.

Recently, quantitative PCR (qPCR) (McCartney *et al.*, 2003) has come into widespread use by plant pathologists. qPCR makes use of fluorescence (either via intercalating dyes or labelled probes) to monitor the accumulation of PCR products in real time (Bustin *et al.*, 2009). This circumvents several disadvantages of end-point PCR such as the need for time consuming post-reaction electrophoresis and staining (Taylor *et al.*, 2010). Assays are available for *P. infestans*, and this project will make use of the primers and probes developed by Lees *et al.* (2012).

1.9 Decision aids and support systems

Successful crop management can be a choice-intensive, high-stakes process (Moschini and Hennessy, 1999) and it is common for growers to make use of various sources of information to guide their decision making (Aubry *et al.*, 1998). These issues are particularly acute when managing crop pests and diseases (Norton, 1976) and when formulating crop protection programmes. Potato late blight in particular is a challenge for decision-makers, as control failure can lead to complete crop loss (Rotem *et al.*, 1983) , in contrast to many other plant pathogens which may

only confer a yield penalty, albeit a very large one in some circumstances (Figueroa *et al.*, 2018). A number of considerations (sometimes competing) need to be taken into account, with the ultimate aim of adequate suppression of the problem organism in a cost-effective manner (Kogan, 1998), with minimal negative consequences for human health (Hernández *et al.*, 2013), and for the wider environment (Casida, 2012). When using pesticides, unnecessary or mistimed treatments are wasteful (Shtienberg, 2013), as is the use of an inappropriate product or formulation (Kleinhenz and Jörg, 1999). Thorough knowledge of the pest, crop, treatment, local environment and likely weather conditions is needed (Madden and Ellis, 1988), and these features can sometimes interact in unexpected ways (Bustos-Korts *et al.*, 2016).

Access to good information is therefore critical and generally takes the form of expert knowledge; either from the grower themselves (Ortiz *et al.*, 2004), or from an external advisor (Guenthner *et al.*, 2001). This situation is made more challenging by the dynamic nature of many crop-disease systems; new strains of pathogens emerge (Anderson *et al.*, 2004), crop protection products can be lost due to insensitivity (Judelson and Roberts, 1999) or market factors, and regulatory frameworks can impose additional requirements on their use (Gullino and Kuijpers, 1994). In addition to this, new insights from the research community are not always transferred to growers in a timely or user friendly way (Srivastava, 2003). With many authorities now recommending or mandating the use of Integrated Pest Management (IPM), a very active research area has been the development of tools and systems aimed at assisting growers in the choices that they make during the crop management process (Andrivon, 2018; Barzman *et al.*, 2015; Knight, 1997; Lagos-Ortiz *et al.*, 2019; Lamichhane *et al.*, 2018; Lefebvre *et al.*, 2015).

The complexity of these support aids varies, from simple empirically-derived rules (Fabre *et al.*, 2007) to complex models integrating simulations of numerous processes (van Maanen and Xu, 2003). A strong research theme has been the use of electronic computers (Bouma, 2007) as platforms for such aids, although the

development of decision support aids, particularly in the area of disease forecasting pre-dates the widespread use of consumer information technology (Gent *et al.*, 2013), and they are often described as Decision Support Systems (DSSs). A DSS can be defined very broadly, as a system which uses some combination of empirical data, expert knowledge, models, databases and geographic information systems to assist in the problem solving process (Shtienberg, 2013). A DSS will often present relevant information to end-users in an accessible format, to inform or to act as a basis for ongoing choices (Magarey *et al.*, 2005). In many cases a DSS will involve a software application (Bajwa *et al.*, 2003), but a printed look-up table, flow chart or similar diagrammatic representation are just as valid formats. A representation as prosaic as a checklist could be considered a decision support system (Algaze *et al.*, 2016), and can be of greater use than a complex computer based application. A DSS which does not rely on information technology may be more accessible to end-users in a variety of situations (for example, if they lack the resources to acquire and maintain the necessary technological platforms), will likely be more portable, and possibly more straightforward to use.

The domain of a DSS may be as specific as a single decision, or as complex as a single cropping cycle (Kleinhenz and Rossberg, 2000). Initially, development of DSSs which used computer based applications was, for the most part ,theoretical, but barriers to adoption have progressively lessened in the developed world with the widespread adoption of personal computing devices (Antonopoulou *et al.*, 2010). Hundreds of DSSs exist for many disparate cropping systems (Hayman, 2004), but uptake has been uneven (Lindblom *et al.*, 2017), and this disappointing trend has been the subject of some debate (Matthews *et al.*, 2008). The most important issue (or, at least the issue with most relevance to this study) is the perception of risk (distinct from the definition of risk given on page 13) by growers and agronomists. There are two aspects to this, the first is the way decision-makers view the risk associated with disease outbreaks (Hanson *et al.*, 2004), which may be seen as too damaging or too likely to alter established approaches to crop protection. The second, related aspect is that growers may view adopting a DSS as

'risky', because the benefits of adoption may be uncertain (McRoberts *et al.*, 2011). Shtienberg (2013) identified input-intensive cropping systems as those where widespread use of DSS has been relatively poor, with the late blight-potato system a particularly pertinent example (Wharton *et al.*, 2008). The costs associated with control failure in these systems are high (Haverkort *et al.*, 2008), principally because of the tuber phase of the disease (R. Bain, SRUC, Auchincruive, UK, personal communication). *P. infestans* is capable of sporulating on and infecting potato tubers (Lambert *et al.*, 1998), and is thus able to spread in-store (Dowley and O'Sullivan, 1991). Blighted tubers can also become colonized by bacterial species, which increases the rate of tissue decay (Powelson *et al.*, 2002), and entire stocks can be lost. Additionally, blighted tubers within stocks intended as seed potatoes can act as a sources of inoculum within the subsequent crop (Boyd, 1980). As well as the economic losses that these events imply, a grower could suffer serious reputational damage if blight related issues become apparent after a stock has been sold (Shepard and Claflin, 1975). Growers, therefore, generally see it as more prudent to stick to regularly scheduled treatments as an insurance policy (Shtienberg *et al.*, 1989), and may view it as 'risky' to change their behaviour, because they may view the outcome as unpredictable; using the decision aid itself could be seen as a risk.

In addition to their role in supporting growers during the decision-making process, DSSs can have useful theoretical applications (Thorburn *et al.*, 2011), both to academics and to end-users. A DSS is essentially a model of the system of interest, and it can therefore be interrogated to explore likely outcomes of different conditions by altering its parameters. This could take the form of exploring the risks associated with different treatment strategies, well before the actual decisions need to be made, generating a range of probable outcomes under different scenarios. The potential for disruption due to biotic factors can be explored: for example, one could evaluate the probable impact of the emergence of a new strain with resistance to key a.i.s, or to assess if fungicide inputs can be reduced if a novel disease resistant variety is grown. An area of current interest is what impact climate change will have on the potato late blight (Gaucher *et al.*, 2018; Litschmann *et al.*, 2018; Pacilly

et al., 2018; Skelsey *et al.*, 2016; Sparks *et al.*, 2014) and other (Moretti *et al.*, 2019; Tang *et al.*, 2017) cropping systems, which may lower risk in some areas, whilst raising them in others. A particular powerful set of DSSs are those that incorporate a GIS, and these have been used to ask fundamental questions regarding the global scale of late blight risk (Hijmans *et al.*, 2000).

The control of *P. infestans* is well represented in the literature on DSSs (Baker and Kirk, 2007; Batista *et al.*, 2006; Bruhn and Fry, 1981; Hadders, 1997; Small *et al.*, 2015): there are a number of systems in use, developed by both public and commercial institutions. The EuroBlight website[7] lists thirteen systems from ten nations, which, in general, focus on disease forecasting, fungicide choice and spray scheduling. Some of these systems also integrate active monitoring of seasonal outbreaks such as the GB-wide AHDB Fight against Blight mapping system[8]. In fact, decision rules for the prediction of late blight outbreaks (and thus, indirectly the scheduling of fungicide treatments) were some of the earliest to be formally developed (Smith, 1956), and some of these are incorporated within modern systems (Leonard *et al.*, 2001). In Great Britain, risk criteria for the occurrence of late blight outbreaks based on relative humidity and temperature have been available since the 1950s and have recently been updated (Dancey *et al.*, 2017). There are also analogous criteria for other nations (Dowley and Burke, 2004). As well as the systems which are used, or are intended for use on a wide scale by potato growers, there are examples of decision rules or systems which have been published as proof-of-concept, or which are in intermediate stages of development (Skelsey *et al.*, 2018). A particularly common example is the validation of an existing DSSs to a new location (Batista *et al.*, 2006; Eremeev *et al.*, 2006; Filippov *et al.*, 2009; Kleinhenz and Jörg, 1999; Koppel *et al.*, 2003), as DSSs are generally designed for use in a specific geographic area, and because there can be marked differences in climate (as well as the make up of the local *P. infestans* population) between regions, such validation

7. http://euroblight.net/control-strategies/dss-overview/ (accessed: Aug., 2019)

8. https://blightwatch.co.uk/ (accessed Aug., 2019)

is a necessary step before deployment of a DSS outside of its original geographic remit.

Decision rules and support systems can be of arbitrary complexity, often depending on their intended use or on the specific interest of their designers. At one extreme, very simple heuristics are decision rules (Marewski and Gigerenzer, 2012). At the other, DSSs can be very sophisticated; accounting for the geospatial aspect of plant disease spread, and modelling complex processes such as spore dispersal (Skelsey *et al.*, 2009). Complex models are not necessarily superior. For example, models containing even a single predictor variable can provide robust results (Skelsey *et al.*, 2018), and much depends on how the system is intended to be used, as well as the scale (from within a single field through to a large geographic region) over which it is designed to apply (Xue *et al.*, 2013). The processes which are most frequently modelled and incorporated within DSSs pertaining to potato late blight are infection risk (Ritchie *et al.*, 2018a), disease progression (Andrade-Piedra *et al.*, 2005), and development of fungicide resistance (Doster and Fry, 1991). In many cases these are given descriptions via simulation models (Oijen, 1995), which may include stochastic factors to model features such as climate (Bruhn, 1980) or mutation rates (Milgroom and Fry, 1988). Although some DSSs do include references to curative activity, these are most often included as recommendations for action, rather than as explicitly modelled processes (Spits and Wander, 2001).

1.10 Structure of project

The brief of this project is: to explore the feasibility of producing a simple decision aid for the use of curative fungicides in crop protection programmes against potato late blight; to establish if some key modifying factors can influence curative activity; and if possible, to produce a prototype of the aid. The exact form which the aid will take is not specified, and additional information and resources outside the scope of this investigation, such as input from stakeholders within the potato industry, will be needed before a full realization can be achieved. The main focus of the project will be to gather data from experiments which can be used to

formulate the decision aid. Currently, the curative activity of fungicides used against late blight is an area with many open questions and lack of detail (see Section 1.7, page 24). Data are needed on how curative control declines with increasing pathogen development and whether factors other than the chronological time which has elapsed are important. This approach should mean that even if the prototype decision aid is incomplete or shown to be inadequate, the data generated will provide others with a useful starting point in designing decision tools for the use of curative fungicides.

Notwithstanding the focus on experimentation, an outline conception of the decision aid was formulated early within the project time line. Of key importance was that the aid be usable in a practical crop management situation, and this idea guided much of the following laboratory and fieldwork. As mentioned in Section 1.7, fungicides with curative activity form an important part of late blight management in potato crops. It is standard practice for non-organic growers to treat their potato crops at regular intervals, generally every 7 days. The choice of fungicide products at any given treatment time is dependent on economic cost (Schepers *et al.*, 2009), advice from agronomists or other experts (Hinds, 2001), resistance management practices (i.e. alternating a.i.s from different chemical groups (Kromann *et al.*, 2008)), and which aspects of the pathogen's life cycle will be most affected by a particular a.i..

The potato-late blight pathosystem is a challenging area for the development of IPM approaches and tools. Decision-maker credence in the likelihood of disease occurrence is (arguably justifiably) high, and the costs of a false negative from any decision tool (i.e. when the recommendation is to not treat a crop, but disease does occur, meaning the correct decision was to apply treatment) is very high. Growers are unlikely to deviate from their set intervals, aside from perhaps reducing them to 6 or even 5 days when disease pressure is perceived to be very high (Hansen *et al.*, 2016a). Furthermore, weather conditions sometimes interfere with spray programmes, meaning that intervals between treatments are unavoidably increased

against the wishes of decision-makers. Compounding the logistical difficulties are the number of treatments needed per season (an average of 12 applications in England and Wales in 2014 (Schepers *et al.*, 2017)). Taken together, these factors make it unlikely that recommendations to further alter treatment times will be well received or followed.

Over the past two decades there has been a dramatic increase in the use of fungicides with curative properties in the management of potato late blight, as can be seen in Figure 1.1 (page 43). Anecdotally, the curative properties of these fungicides are a key reasons for their selection over alternative a.i.s (R. Bain, SRUC, Auchincruive, UK, personal communication). The curative effect of these a.i.s is poorly defined and it is not clear that inclusion of a curative product will always provide a benefit; for example, if the time-window for curative activity has elapsed by the time a 'curative' treatment is applied, little disease control may occur (Griend and Bosch, 2007).

Growers and agronomists may choose to prioritise fungicides and adjuvants with properties other than curative activity if they are aware that the window for good curative control had elapsed. The decision aid then will not provide a recommendation as to *when* to treat or *if* to treat, but will instead feed information to the decision-maker which can be used to select the most appropriate tactic to fit the specific circumstances. It is beyond the scope of this project to set out these alternatives, but in some cases there could be savings in terms of cost of product or amount of fungicide applied (R. Bain, SRUC, Auchincruive, UK, personal communication).

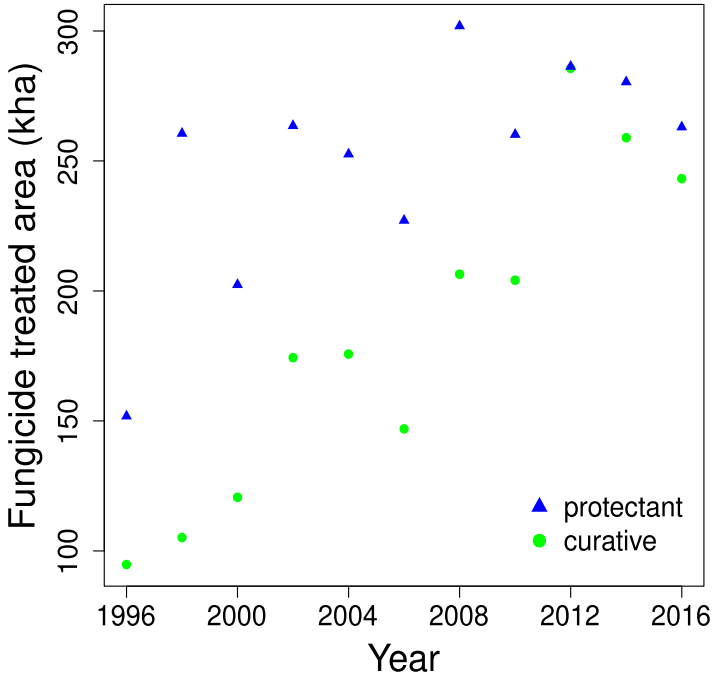

Figure 1.1. Estimated use of late blight fungicides with different properties in Scotland between the years 1996 and 2016. The data are taken from SASA's biannual pesticide survey, which gives estimates of area treated with each active ingredient (a.i.s). These a.i.s were classified as *curative* if they were described as such by the EuroBlight table, or as *protectant* otherwise. This classification scheme is imperfect, because fungicides with curative properties are primarily used in protective treatments, but is nevertheless useful for illustrative purposes. Note that fungicide-treated area per year exceeds the potato planted area because most crops receive multiple treatments within a season.

A standalone DSS for curative fungicide use would not be appropriate, for several reasons. A spray programmes which puts a strong emphasis on curative control is inadvisable for this pathosystem, as attempting to control sub-clinical infections routinely with curative sprays will be much less effective than a robust protectant regime, and may increase the probability of insensitive *P. infestans* strains emerging. Additionally, well-used and freely available DSSs for late blight control already exist for Great Britain: the meteorological-based warning systems 'BlightWatch' and 'BlightCAST', as well as the 'Fight against Blight' disease surveillance service. The most sensible approach is to design a decision aid which complements, and could be incorporated as a component of these existing systems. BlightWatch produces notifications when the Hutton Risk criteria are met, and could potentially also forecast their occurrence (though this has not been implemented to date). The Hutton Criteria consist of 2 consecutive days where the minimum temperature is 10 °C or above and there are ≥ 6 hours with a relative humidity ≥ 90 %. This is essentially an updated version of the Smith Period criteria, which, in turn, are derived from the Beaumont criteria. As initially conceived (see Section 1.4, page 11) these criteria cover the period from sporulation on the inoculum source (i.e a lesion present on a plant within or adjacent to the crop) through to successful infection at secondary loci. The proposed decision aid could be used in the immediate aftermath of a Hutton Period, providing the user with a guide to the likely efficacy of a curative treatment given the time elapsed and other potential factors such as the local air temperature since the Hutton Period warning, which cultivar composes the crop to be treated etc.

An outline overview of the decision aid components is shown in Figure 1.2 (page 46). This schema acted as a guide for areas of investigation, as it was not known at the outset which factors would be informative, nor the form that the prototype decision aid would take. The diagram includes references to the thesis chapters concerned with each component. Pathogen development was the most intuitive candidate for influencing the duration of the curative window, as pathogen development defines the time boundaries for the curative effect (treatments after the

incubation period cannot, by definition, be curative). The development of organisms is usually strongly influenced by temperature, and it was considered critical that the decision aid take temperature during the pathogen's incubation period into account. This is discussed in Chapter 3. The nature of the curative effect and how it changes with time (or pathogen development) was not well characterised before this study. An accurate characterisation of the anticipated level of curative control at a given time point is the most important component of the proposed decision aid, and this is provided in Chapter 4. Chapter 5 provides details on host resistance and how this influences pathogen growth *in planta* and thus the potential role it plays within the decision aid. Finally, a sketch of the potential model output and proposals for assessing its performance are made in Chapter 6. It must be stressed that the specified aid is provisional. The form of output will likely require input from end-users within the potato industry to ensure it provides useful information. It will also require a full validation and, potentially, modification with data sets representing a range of conditions. Some proposals for further work are made in Chapter 7.

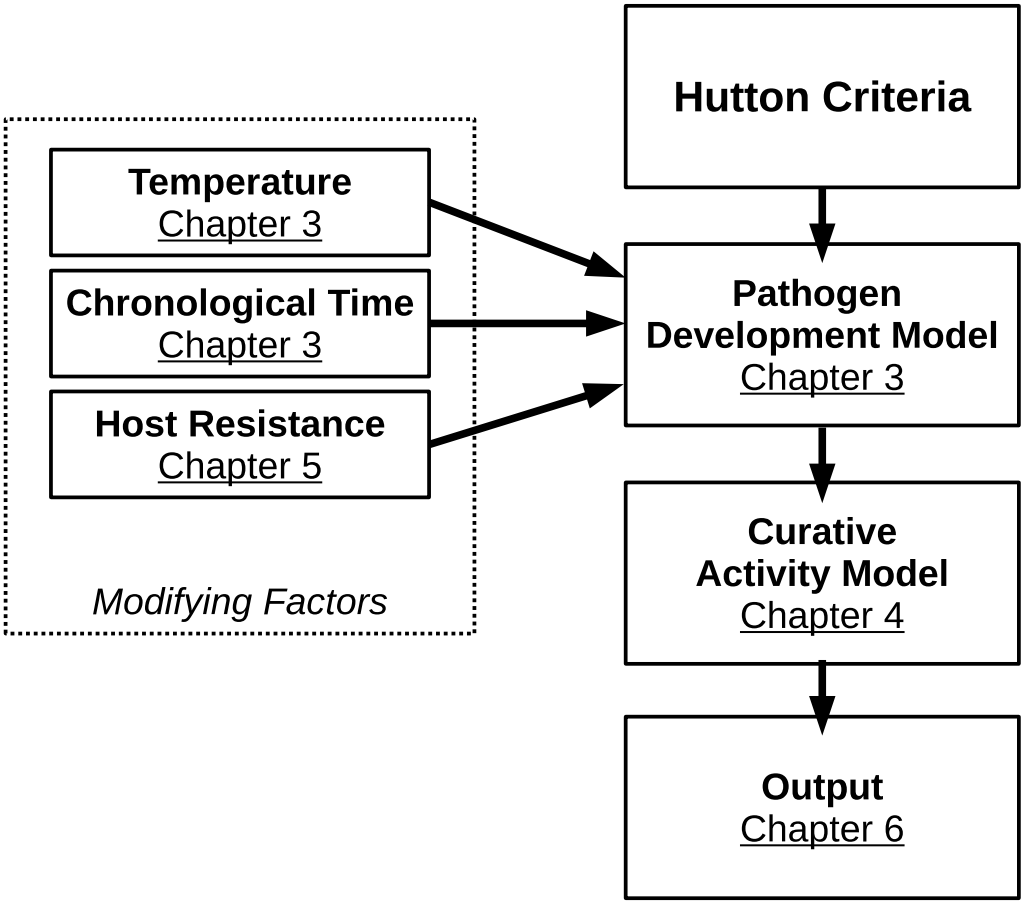

Figure 1.2. Schematic overview of the proposed decision aid. Each box contains components of the aid: sub-models, input variables or the final output. Where relevant, the boxes also include chapter numbers where the relevant experiments and discussion can be found. Other modifying factors were considered in the planning stage, such as the effect of previous fungicide treatment, but the schema includes only those that were considered in detail and where implementation seemed plausible.

1.11 Project objectives

Appropriate timing of curative fungicides is crucial, but users currently have limited information on which to base their decisions. Spray programmes can easily be disrupted due to adverse weather conditions, and with the presence of more aggressive pathogen lineages necessitating tight intervals, the relative importance of curative fungicides is increasing. Ratings for curative blight fungicides are available, and, although these are evidence based, they lack objectivity and specific detail.

The ultimate aim of this project is the construction and verification of a simple aid to help growers decide whether the inclusion of a curative active ingredient, as part of the planned fungicide application, is justified or not. It is anticipated that the decision aid will contribute to the integrated control of potato late blight by eliminating unnecessary use of curative a.i.s. Subsidiary objectives are:

1. Establish thresholds, in terms of *P. infestans* biomass, for curative activity for a representative late blight fungicide.
2. Evaluate the rate at which foliar tissue is colonized[9] for a range of *P. infestans* isolates obtained from the United Kingdom, by quantifying DNA levels via qPCR.
3. Assess the modifying effects of air temperature, cultivar foliar resistance and previous fungicide treatment on the rate of tissue colonization.
4. Develop a decision aid for the application of curative fungicides, parameterized from the results of Objectives 1 – 3.
5. Validate and evaluate the decision aid in the field.

9. Tuber and stem infections will not be evaluated. The former are not relevant, as late blight fungicides are not used as tuber treatments. The later, while difficult lesions to control (Schepers, 2000), probably represent a minority of late blight infections (Keil *et al*., 2010b), and would require an additional suite of experiments to evaluate.

Chapter 2 General methods

2.1 Maintenance of *P. infestans* cultures

Isolates of *P. infestans* were obtained from the culture collection at the James Hutton Institute (JHI), isolates were selected based on the following criteria: (i) 'contemporary' i.e. isolated within the past decade, (ii) 'representative' belong to clonal lineages which have accounted for a significant proportion of the Fight Against Blight monitoring programme in recent years, and (iii) judged to differ in their aggressiveness or other fitness components. Mating type and multi-locus genotype had previously been assigned (Li *et al.*, 2013b). Cultures were maintained on 'Rye A Agar' (Caten and Jinks, 1968) (2 % w/v sucrose (Sigma-Aldrich, Dorset, UK), 1.5 % w/v Oxoid Technical Agar No. 3 (ThermoFisher Scientific, Leicestershire, UK), and rye extract 'method A' (see below)) in sealed Petri dishes (90 mm diameter). Agar was sterilized by autoclaving at 121 °C for 15 minutes (this applies to all media or equipment listed as sterilised in this chapter). These were sub-cultured every 6 weeks. Rye extract for the Rye A agar was prepared by soaking 60 g of organically produced rye grains (BHFO, Kent, UK) in sterile distilled water for approximately 24 hours. The supernatant was decanted and set aside. The rye grains were then blended with 200 ml sterile distilled water for 2 minutes, this mixture was heated in a water bath at 68 °C for an hour, after which the mixture was filtered through several layers of muslin, and the filtrate was then combined with the original supernatant. Each isolate is referred to within this text with a unique code listed in Table 2.1 (page 50).

Table 2.1 Isolates used within this study. Genotype, PCL number (an arbitrary identifier assigned at isolation) and information on the collection of the isolate were obtained from JHI. The study identifier is a code assigned to each isolate to ensure that their genotype and unique identify can be unambiguously determined by the reader.

Genotype	PCL no.	Study identifier	Date	Location	Cropping where infection found	Variety	Outbreak size
13_A2_1	9922C	2012_13A2-1	18 July 2012	Norfolk, England	Conventional	Unknown	Scattered throughout field
13_A2_5	10014D	2012_13A2-2	20 July 2012	Lancashire, England	Conventional	Unknown	Scattered throughout field
6_A1	6090A	2008_6A-1	12 June 2008	Shropshire, England	Unknown	Maris Piper	Unknown
7_A1	10290A	2012_7A1-1	30 July 2012	Essex, England	Conventional	Maris Piper	Patch
8_A1	10702B	2012_8A1-1	06 July 2013	Ceredigion, Wales	Volunteer	King Edward	Several patches

2.2 Growth and maintenance of plants

The majority of laboratory based experiments and inoculum preparations made use of glasshouse propagated *S. tuberosum* plants and took place at SRUC's King's Buildings Site (City of Edinburgh, UK); a small subset (which will be indicated when relevant within the text) were conducted at a separate site (SRUC, Auchincruive, Ayrshire, UK) at which plants were propagated within a poly-tunnel. As experiments were conduced throughout the year, over three consecutive years a natural photoperiod was used during periods that coincided with the growing season, outside of this artificial illumination was used to ensure no less than a 16 hour photoperiod was achieved. The glasshouses were set to 20 °C, but temperatures ranged from (10 – 35 °C) depending on the season.

Seed tubers of *S. tuberosum* used in all experiments were SPCS (Seed Potato Classification Scheme) certified and were obtained in the case of cultivars King Edward, Cara, and Sarpo Mira from WCF Horticulture (Perth, UK); from McCain Potatoes (Angus, UK) in the case of Cara, Pentland Dell, and Innovator; and from a range of horticultural suppliers for other cultivars which were used in smaller quantities (Suttons Seeds, Devon, UK; Marshall Seeds, Cambridgeshire, UK; Thompson & Morgan, Suffolk; Pennard Plants, Somerset, UK; Skea Organics, Angus, UK). Tubers were stored at 4 °C until use. When used within glasshouse experiments, tubers were chitted for ~ 7 days, and were then sown in 6 l plastic pots (two tubers per pot) using Levington M3 – pot/bedding high nutrient compost. Plants were grown for ~ 6 weeks before material was harvested.

2.3 Growth and maintenance of plants in the field

Field trial sites were managed using standard agronomic practice. Typically the ground was cultivated with 100 : 50 : 50 NPK fertilizer, before being mechanically formed into ridges. Plots were marked out using fibreglass canes. The dimensions of the plots varied between experiments and will be given, where appropriate, in the following text. In all cases potato seed tubers were hand-planted with 0.3 m spacing within rows. Plots were treated with pre-emergence herbicide to reduce competition from weeds, this consisted of linuron (as Afalon, Adama, suspension concentrate,

450 g linuron l^{-1}, applied at 1.35 l ha^{-1}), diquat (as Retro, Syngenta, 200 g diquat l^{-1}, applied at 2 l ha^{-1}), and rimsulfuron (as Titus, Adama, dispersible granules, 250 g rimsulfuron kg^{-1}, applied at 50 g ha^{-1}) in 250 l water ha^{-1}. An assessment of plant health (presence of other disease symptoms such as black leg, virus induced mosaic or natural late blight infection etc.) and cultivar was made around two days before the commencement of any fieldwork and any plants suspected of carrying infection (of any plant pathogen) or of being an incorrect cultivar were removed. This ensured that only plants which appeared disease-free and true to planted cultivar were included in the trial. This occurred only only a single occasion with a King Edward plant showing mild mosaic symptoms in the field trial at Boghall in 2015 during the plot scale varieties trial (see Chapter 5, Section 5.4, page 159), it was removed before inoculations with *P. infestans* took place.

2.4 Production of inoculum

A uniform protocol for inoculum production was used for all experiments and field trials involving artificial inoculations, as source of inoculum has been show to influence the phenotype of *P. infestans* infections (Fry, 2016). Isolates were transferred from Rye A agar plates (Section 2.1, page 49) to Rye B agar plates (2 % w/v sucrose (Sigma-Aldrich, Dorset, UK), 1.5 % w/v Oxoid Technical Agar (ThermoFisher Scientific, Leicestershire, UK), $5 \cdot 10^{-5}$ w/v β-sitosterol (Sigma-Aldrich, Dorset, UK) and rye extract 'method B', see below). Rye extraction 'method B' consisted of soaking 60 g of organically produced rye grains (BHFO, Kent, UK) in sterile distilled water for approximately 24 hours, after which the supernatant was set aside, the rye grains were then placed within 200 ml boiling sterile distilled water on a hot plate for one hour (the water volume was monitored and amended at regular intervals). This mixture was then filtered through several layers of pre-sterilised muslin, and the grains discarded. Finally, the filtrate was combined with the original supernatant.

After ~ 14 days growth on Rye B agar, sporangial suspensions were prepared by flooding the plates with 2 ml sterile distilled water, the surface of the culture was scraped with a sterile glass rod, and the suspension transferred through several

layers of muslin (to remove large mycelial fragments) to 50 ml plastic tubes. The volume of suspension prepared was varied depending on the quantity of inoculum required. The density of sporangia within the suspension was quantified microscopically using a haemocytometer (Improved Neubauer; NanoEnTek Inc., Korea), and the suspension adjusted to 10^5 sporangia ml^{-1}. This suspension was used to inoculate detached potato leaflets (cv. King Edward), which were then sealed (using transparent plastic bags) within transparent plastic trays (465 · 270 · 85 mm) which had been lined with damp paper towels. These trays were then placed within a growth cabinet, set at a constant 18 °C with an 18 hour photoperiod. After between 7 – 12 days leaves were inspected for visible sporulation, on leaflets where sporulation was evidant an ethanol sterilized soft paintbrush was used to transfer sporangia to a 50 ml plastic tube containing fresh oxygenated sterile distilled water, and this suspension was again adjusted to 10^5 sporangia ml^{-1}. This process was repeated using newly harvested leaflets, with the inoculum resulting from this passage used as the experimental inoculum.

2.5 Bioassay incubation conditions

Two methods were used for the incubation of infected plant material within the bioassays: the first method, used during 2014 - 15, involved sealing leaflets individually within Petri dishes and their stacking within LED lined boxes, the second of placing several leaflets within larger plastic trays and was used during the final two years of the project. The change was made for logistic reasons, as the number of dishes necessitated by the size of the experiments became cumbersome; which method was used will be made explicit within the text.

Within the dish method, individual leaflets which had been inoculated and/or treated were placed within Petri dishes which were lined with a piece of damp filter paper, these were then sealed using parafilm and stacked within large opaque plastic crates (780 · 350 · 395 mm), lined with LED light-strips (Biard LED, UK; IP20 5050; output: 900 lumens m^{-1}), the LEDs were wired to a single timer set to a 18

hours on / 6 hours off cycle. The crates were then placed within a growth room with conditions specific to the bioassay in question.

The second method ('tray' method) utilized transparent plastic trays (465 · 270 · 85 mm), which were lined with damp filter paper folded into ridges to minimize contact with plant tissue. Experimental plant material was placed within these trays, which were then sealed in turn within transparent plastic bags. These trays were then placed within a growth cabinet programmed with conditions corresponding to the experiment in question, most often this was a photoperiod of 16 hours and a constant 18 °C, but exceptions will be noted within the text.

2.6 Temperature monitoring

In the majority of experiments temperature logging was achieved using iButtons (Maxim Integrated, USA). The iButtons internal clocks were synchronised using the manufacturer's software (OneWire Viewer, Maxim Integrated, USA) ~ 30 minutes before each experiment, and were programmed to record temperature readings at 10 minute intervals. The iButtons were placed within Petri dishes (60 mm diameter), the lids of which were shaded using a small circular piece of cardboard. These were then in turn placed amongst Petri dishes containing the experimental plant material or within the transparent plastic trays depending on which was used. Two iButtons were used in each bioassay, and the mean temperature at each time point was used as the temperature reading. iButtons were calibrated at 6 month intervals during the period of data collection by comparison with a reference thermometer, and a correction applied to any discrepancy.

2.7 Visual assessment of disease symptoms

Within laboratory based bioassays, visible symptoms of late blight were assessed using a standardised protocol, as disease symptoms can be variable, and their assessment can sometimes be subjective (Bock *et al.*, 2010). Assessment involved either a categorical scale, a presence/absence assessment, or as diseased area (i.e. area occupied by lesions). The categorical scale is given in Table 2.2 (page 55).

Table 2.2. Late blight symptoms key, used in assessments both in the field and in laboratory based experiments within this project.

Score	Infection interpretation	Description
0	Infection failed	No visible symptoms, healthy tissue
1	Infection failed	Small necrotic patches of tissue, no larger than 25 mm^2
2	Infection successful	Lesion. Large (> 25 mm^2) region of necrotic tissue, but without any visible sporulation.
3	Infection successful	Sporulating lesion. Visible areas of tissue with sporangiophores, with or without necrotic tissue.

Where disease area was quantified as a continuous variable, digital images were taken of leaves or leaflets using a digital camera (Motorola, 8 MP $3264 \cdot 2448$) which was affixed 200 mm above the sample using a retort stand. In all images care was taken to ensure that both a graduated rule for scale, and a label with experiment details (treatment, replication number etc.) were visible in frame. Images were stored in .jpeg format and analysed using the ImageJ application (Schneider *et al.*, 2012). The `polygon` function was used to manually construct a shape layer corresponding to the edge of the lesion(s) present, with *edge of lesion* defined as either the boundary between necrotic and healthy tissue if no sporulation was present, *or* the boundary between tissue regions where sporulation was clear and healthy tissue. Scale was set using the gradations on the ruler present in frame; this was validated by including an image of a $100 \cdot 100$ mm piece of card along with each set of leaflet images.

2.8 Biomass estimation using qPCR

The threshold for curative control for most curative fungicides most likely lies within the pathogens incubation period, where pathogen load cannot be assessed using visual methods. Primers and probes have previously been developed by Lees *et al.* (2012), which are specific to *P. infestans*, and these are give in Table 2.3 on page 56. These were used to estimate the quantity of *P. infestans* DNA present within samples in situations where an index of pre-symptomatic pathogen biomass

was required. Details of the hydrolysis probe, as well as forward and reverse primers can be found in Table 2.3. DNA standards were prepared from cultures grown on Pea Broth (modified from Hollomon, 1966). Pea Broth was prepared by boiling 120 g of garden peas in 1 l of distilled water for an hour, this was then strained through sterile muslin. The resulting filtrate was autoclaved before use. 20 ml of Pea Broth was poured into 90 mm diameter Petri Dishes, and a small piece of *P. infestans* mycelium from a Rye A (see Section 2.1, page 49) culture was inoculated on to this. After 14 days, mycelium from these cultures was transferred to 1.5 ml micro-centrifuge tubes and freeze dried for 16 hours (Alpha 1-2 Freeze Drier, Christ, Germany).

Table 2.3. Probe and primer sequences used to quantify *P. infestans* genomic DNA.

Oligo name	Type	Sequence
PinfTQF	Forward primer	5′-AAC CCA ATA GTT GGG GGT CTT AC-3′
PinfTQR	Reverse primer	5′-TCG TCC CCA CAG TAT AAT CAG TAT TAA-3′
PinfTQPR	Hydrolysis probe	5′-AAG CTA CTA GCT CAG ACC GAA GTC CAA ACG CT-3′

Genomic DNA was then extracted using the Nucleon PhytoPure Kit (GE Healthcare, USA) following the manufacturer's instructions. Purity was assessed using a spectrophotometer (DS-11 Fx$^+$, DeNovix, USA), by inspecting the ratio of absorbance at 260 / 280 nm, and at 260 / 230 nm wavelengths. When purity was insufficient (*i.e.* ratio 260/280 below 1.7 and/or ratio 260/230 below 1.8), a second 'clean-up' purification step was carried out. A volume of chloroform : phenol : 3-methylbutan-1-ol (in the ratio 25 : 24 : 1) equal to the sample was added, vortex mixed and then centrifuged at $2 \cdot 10^4$ *g* for 15 minutes. The upper aqueous phase was then transferred to a new micro-centrifuge tube, and an equal volume of isopropanol was added to this, and left to stand at room temperature (20 °C) to allow for DNA precipitation. The sample was centrifuged at $2 \cdot 10^4$ *g* for 10 minutes, and the supernatant was discarded. The resultant DNA pellet was then washed with 70 % ethanol, before re-suspension in sterile distilled water.

Plant material for DNA extractions consisted of individual leaflets placed in 2 ml Eppendorf tubes and freeze dried as above. A small sterile steel ball bearing was placed in each tube, and samples were then ground within a ball mill at 50 cycles per second, for 2 minutes. The steel balls were then removed and 1.8 ml of DNA extraction buffer consisting of tris-base 0.06 % w/v, NaCl 0.027 % w/v, EDTA 0.023 % w/v, phenanthroline 0.0008 % w/v, polyvinylpyrrolidone 0.02 % w/v, 0.048 % β-mercaptoethanol v/v was added. Samples were then incubated at 70 °C for 20 minutes prior to being centrifuged at $2 \cdot 10^3$ *g*. The supernatant was transferred to a new microcentrifuge tube containing 0.9 ml of 7.5 M ammonium acetate solution, and then stored at −20 °C for 12 hours. After centrifugation at $2 \cdot 10^4$ *g* for 15 minutes, the supernatant was transferred to 1.5 ml microcentrifuge tubes containing 0.8 ml isopropanol and left to stand for 15 minutes to allow DNA precipitation. A final wash was carried out with 0.4 ml of 70 % ethanol, before centrifugation at $2 \cdot 10^4$ *g* for 5 minutes. The obtained DNA was allowed to air dry for 15 minutes before being resuspended in sterile distilled water, and stored at −20 °C. Total genomic DNA quantification and purification when necessary were carried out as described above prior to being diluted to 20 ng μl^{-1}.

Quantitative PCR amplification of samples followed similar methods to Cullen *et al.* (2001) and Lees *et al.* (2012). The assay was performed in 96-well plates using an AriaMx Real-Time PCR System (Agilent Technologies, USA) All wells were prepared in duplicate. Primers and probes listed in Table 2.3 (page 56), as well as other reaction components (TakyonTM MasterMix) were obtained from Eurogentec (UK). Reaction volume within each well was 25 μl, consisting of forward primer and reverse primer at 0.3 μM, hydrolysis probe at 0.1 μM and 20 ng μl^{-1} of the sample DNA. A two stage thermal profile was used: denaturation at 95 °C for 2 minutes, followed by 35 amplification cycles of 95 °C for 15 seconds and 1 minute at an annealing temperature of 61 °C. Within each plate, a serial dilution of *P. infestans* DNA from concentrations of 20 ng μl^{-1} to $2 \cdot 10^{-5}$ ng μl^{-1} (seven dilutions in total) were included in order to generate a standard curve. Log (base 10) DNA concentration of standards was plotted against the obtained C_q values (Bustin *et al.*,

2009). In all cases R^2 values of this relationship were between 0.99 and 1. comparisons of sample C_q values to this relationship facilitated estimation of the quantity of *P. infestans* DNA in each sample. Within each plate, 2 wells were allocated to non-template controls, and contained a volume of sterile distilled water in place of a sample.

2.9 Statistical analysis

Unless otherwise specified, all statistical tests and data plots were performed using the R software package (R Core Team, 2013).

Chapter 3 Selection of a temperature-dependent development model for describing growth of *P. infestans in planta*

Abstract

The rate at which pathogens develop within a host is influenced by temperature. Investigators often consider 'thermal time' (a function of time and temperature) rather than chronological time alone when describing growth and development, but there are many approaches which can be used to achieve this. This chapter provides a discussion of the arguments and approaches used in the calculation of thermal time and how this relates to the development of *P. infestans* within its incubation period. A set of laboratory based experiments with fixed temperatures (6 – 30 °C) and four *P. infestans* isolates were conducted to generate data from which parameters of a relationship between thermal time and pathogen growth could be estimated. Pathogen growth was measured using both visible symptoms over several time points (8 through to 72 hours), and by estimating quantities of *P. infestans* DNA in detached leaflets using a qPCR assay. Estimated DNA quantity acted as an index for pathogen biomass. A susceptible cultivar (King Edward) was used in all experiments. A literature review of thermal development models was conducted, and a range of candidate relationships were tested for goodness-of-fit with both the visible symptom development (lesion growth) and pre-symptomatic growth rates (biomass index) established in the laboratory based experiments. The most aggressive isolate was used in the fitting of these relationships, as it represents a hypothetical 'worst case scenario' for an infected crop. One relationship, the Kontodimas function was judged to provide the best description, based on the goodness-of-fit measurements, and other considerations such as the shape of the relationship. This function, using the parameters estimated for the pre-symptomatic growth will form a component of the prototype decision aid detailed in Chapter 6.

3.1 Introduction

The growth of an organism, or its progression through developmental phases, is generally a mutable process, with factors such as the availability of resources (Meletiadis *et al.*, 2001), or the presence of competitors (Mille-Lindblom *et al.*, 2006) directly or indirectly affecting developmental rates. One of the most important influences on development is environmental temperature; this is particularly true of ectothermic organisms which do not invest heavily in thermal homeostasis. Differing temperature profiles during the development of these organisms can have a profound impact on the speed at which they grow (i.e. biomass is accumulated) (Meier *et al.*, 2010) or the chronological time which must elapse before they reach specific life cycle stages (Rossi *et al.*, 2008).

It has long been recognized that a reliance on chronological time alone is inadequate for the prediction or modelling of biological processes (McMaster and Wilhelm, 1997). In agricultural systems, both the crop plants themselves (Hatfield and Prueger, 2015), and their pests and diseases (Bergot *et al.*, 2004) are often highly sensitive to temperature. Consequently, events such as the optimum harvest date (Werner, 1942) or the probable onset of a particular pest or disease (and thus the most appropriate time for the use of a crop protection measure) may vary between seasons (Yang *et al.*, 1990). A careful consideration of environmental conditions is therefore a prerequisite for successful pest and disease management.

The sensitivity of many organisms to temperature is due to the influence it exerts on metabolism: temperature affects rates of diffusion (Sarcina *et al.*, 2001); membrane fluidity and permeability (Quinn, 1988); the solubility of some biologically relevant compounds (Farquhar *et al.*, 1980); and most critically the speed of many enzyme catalysed reactions (Somero, 2004). These factors ultimately contribute to rates of development, as well as defining the thermal niche to which an organism is adapted (Tracy and Christian, 1986). For most ectotherms, developmental rates initially increase with increasing temperatures, and are maximal at an organism's 'optimum temperature', once this optimum is exceeded

developmental rates decline (Shi *et al.*, 2017). The range of temperatures over which an organism is metabolically active is generally bounded by both an upper and lower limit (Thomas and Blanford, 2003), and exposure to temperature outside of this range yield no development and may potentially be lethal.

Temperature is an important factor for plant pathology studies, and authors often account for the effects of temperature on pathogen development (Grove *et al.*, 1985; Magarey *et al.*, 2005; Weir *et al.*, 2015). As the overall objective of this project is the development of a simple decision aid, which can be used by practitioners in different locations, across different seasons, and at different occasions within a season, it is important that temperature and its influence on early establishment of the host-pathogen interaction are adequately accounted for.

3.2 Modelling temperature dependence in biological processes

An understanding of the relationship between temperature and development for a given biological system opens several useful applications. For example, the timing of future developmental events under different temperature schedules (such as successive seasons) can be estimated (Streck *et al.*, 2007); potential geospatial distributions of a species can be determined (Manrique and Hodges, 1989); and the suitability, or lack thereof, of a crop to a novel climate can be assessed (Keulen and Stol, 1995). There is a rich literature exploring both methods and applications for incorporating temperature as a factor in developmental models; much of this work focuses on simple model systems (Corkrey *et al.*, 2014; Ratkowsky *et al.*, 1982), and most applied studies relate to entomology (Rebaudo *et al.*, 2017), but the general framework and conclusions seem to apply to the majority of organisms.

3.2.1 Heat units and accumulated thermal time

The temperature dependence of biological processes can be modelled in several ways, but many of these methods are inter-convertible, and a mathematical generalization is given by Moore and Remais (2014). The most widespread and conceptually straightforward method is to consider development as a function of accumulated heat units or 'thermal-time', which is defined as the product of time

elapsed and temperature. These products can then be summed to arrive at the accumulated thermal-time. Theoretically thermal-time could be defined as a continuous function:

$$^{\circ}D_a = \int_{t_i}^{t_n} T(t)\,dt \qquad (1)$$

Where $^{\circ}D_a$ is the accumulated thermal-time (the units of which depend on the scales used to measure time and temperature), T is temperature (usually in °C) as a function of time, and t is chronological time (days, hours , seconds etc.). t_i and t_n refers to the lower and upper time boundaries respectively. However, in practice it is usually calculated using discrete time intervals:

$$^{\circ}D_a = \sum_{i=0}^{n} \left(\frac{T_i + T_{i+1}}{2} \right) \Delta t \qquad (2)$$

Where $^{\circ}D_a$ is the accumulated thermal-time, T is temperature (usually in °C) as a function of time, and t is chronological time (days, hours , seconds etc.). Δt is the difference in time between the upper and lower time intervals. i is the index of observations T_i is the temperature at the ith observation, and n is the number of observations. In this project, unless otherwise stated thermal time will be calculated in terms of degree centigrade-hours (°C · hr).

For most applications the completion of a developmental event (such as dormancy break, anthesis, a biomass threshold, etc.) can be associated with a thermal constant (sometimes represented as K), which is generally empirically determined or estimated. In most cases, K has units in thermal-time so that it can be directly compared to the accumulated degree-time within a given application, but it is sometimes expressed as a value between 0 and 1, which corresponds to initiation and completion of the developmental event respectively (Moore, 2009); in this case a coefficient (K^{-1}) is used to convert the right hand sides of equations 1 and 2 to a proportion. There are several classical phenological studies which make use of this or related approaches (Allen, 1976; Baskerville and Emin, 1969; Burke, 1968), and it

is commonplace for agronomic advice to be given in terms of 'degree-days' or 'growing degree-days'; some organisations regularly publish estimates of thermal time for a given region[10].

Although very useful, there are a number of limitations that result from the assumptions of the thermal-time concept, some of which can be overcome using modifications. Firstly, as thermal time is a simple product, the relationship between temperature (and indeed, time) and the calculated heat units will be linear. This is generally a reasonable assumption, particularly in situations where the temperature profile is anticipated to remain well within a organism's developmental limits (Trudgill, 1995), and is advantageous because models can be constructed using simple linear regression techniques, but the assumption will be violated as temperatures reach an organism's developmental extremes (Yin *et al.*, 1995). In practice negative temperatures are never summed, as this would imply, implausibly, that reverse development is taking place (this is avoided if temperature is expressed in °K), but it is common for organisms to cease growth (though not necessarily incur mortality) well above 0 °C (Moot *et al.*, 2000; Slafer and Savin, 1991). Similarly, growth as well as development generally has an optimum temperature value, above which developmental rate declines, often sharply, until it ceases (Wang, 1960). Note that the optimum temperatures for growth and development need not be the same; for many plant species the optimum temperatures for the development of some reproductive structures are less than for vegetative growth (Hatfield and Prueger, 2015).

This issue can be resolved by incorporating thresholds within the degree-day calculations, so that degree-time units are only accumulated above the estimated developmental minimum, generally called the 'base' temperature (T_b), and below the developmental maximum (T_m). If both of these thresholds are included the temperature response function is undefined outside of the range $T_b < T < T_m$.

10. Some examples can be found at: http://www.cpc.ncep.noaa.gov/products/analysis_monitoring/cdus/degree_days/ (accessed: Aug 2019), http://www.farmwest.com/climate/pest (accessed: Aug 2019), and http://pnwpest.org/wea/weaexp.html (accessed: Aug 2019).

Occasionally a third parameter, the developmental optimum temperature (T_o) is also included to describe the decline in developmental rates which generally occurs between an organism's optimum and maximum temperatures. It is important to note that a $°D_a$ figure is meaningless without reference to these thresholds (particularly the base temperature).

An estimate for T_b is generally obtained from a linear regression of observed development over a range of temperatures, with T_b corresponding to the horizontal axis intercept (i.e. the temperature where development equals zero). Zero development at this temperature is frequently not biologically true, but any error is often negligible for practical applications (Sharratt *et al.*, 1989). It is not always possible to precisely identify T_b experimentally. For example, if the pathogen isolate is a sample, rather than a pure clone, low temperatures will select for the most cold-tolerant individuals which may lead to an underestimation. In any case it can only be stated that T_b is located within the interval between the lowest experimental temperature showing growth, and the next lower experimental temperature.

High temperature responses are more difficult to account for, and may be more variable (Leach and Cowen, 2013), leading to a number of different approaches to their modelling. The simplest, mentioned above, is to leave the thermal-time equation as undefined as $T > T_m$, which is usually described as a vertical cut-off method (Roltsch *et al.*, 1999). Alternatively, thermal-time accumulation above T_m can be set as equal to that at T_m , described as a horizontal cut-off method due to its appearance when plotted (Grigorieva *et al.*, 2010). Both of these methods are poor representations of the response of most organisms to high temperature, and a range of so-called intermediate cut-off methods are also sometimes used where T_o is introduced and thermal-time accumulation is set to decrease above T_o. It is also possible to treat T_m as T_o within a linear model, to avoid adding additional parameters. In many situations, the different threshold methods yield only marginally different values for accumulated thermal-time (Moore and Remais, 2014); in others, particularly where the temperature is often outside of the

thresholds, which method performs best depends on the organism and the local climate (Moore, 2009).

3.2.2 Non-linear models of temperature-dependent development

The limitations of linear models of temperature-dependent development are well known, and have led several researchers to develop mathematical descriptions which more accurately describe the temperature development relationship (Damos and Savopoulou-Soultani, 2012). As these models are non-linear, it is not possible to calculate a thermal constant, and they generally provide estimates of developmental rates at different temperatures rather than thermal-time *per se* (Ratkowsky *et al.*, 1982). Some authors, particularly those interested in the more theoretical aspect of thermal biology advocate describing temperature-dependent development in terms of 'developmental accumulation' (Blanford *et al.*, 2013; Xu, 2003), or 'physiological time' (which is sometimes denoted as Γ)(van Straalen, 1983). This adds conceptual clarity, and also allows the incorporation of additional rate-influencing factors (humidity, solar radiation, etc.) within the function.

The chief goal of these non-linear descriptions is to capture the two distinct responses organisms show to high and low temperature, as the temperature-development relationship is frequently not symmetrical about T_o. There are several approaches to solving this problem. For example, Sharpe and DeMichele (1977) constructed an equation based on a hypothetical rate-limiting enzyme, which is denatured as high and low temperature thresholds are approached. An attractive feature of this model is that it has parameters which are biologically meaningful, but there is a large number of them which complicates fitting, although the authors later provided a modified form to address this issue (Schoolfield *et al.*, 1981). A more common approach is to produce a mathematical model which generates curves similar to the observed temperature-development response (Bayoh and Lindsay, 2003; Briere *et al.*, 1999). Although these sometimes have parameters without a biological or physical interpretation, their fitting is often more straightforward, even in cases where only a few observed values are available.

No one scheme describing temperature-dependent development is accepted as a universal description, each has strengths and weaknesses. Some, for example, approach zero development asymptotically and therefore cannot estimate T_b (Logan *et al.*, 1976); others may provide a good fit for some organisms or developmental stages, but a poor fit for others. It is therefore necessary to select a non-linear model based on the intended application, and to evaluate it with data for the relevant organism, life-history stage and temperature range (Gilbert *et al.*, 2004). It is also important to balance model fit with the number of parameters: simpler models are likely to be more robust and easier to handle with statistical software packages, but more complex models may provide a better description of the relationship. In several entomological studies (Aghdam *et al.*, 2009; Kontodimas *et al.*, 2004; Marchioro *et al.*, 2017; Özgökçe *et al.*, 2016; Roy *et al.*, 2002) models have been evaluated by fitting candidates to the same data set and then comparing them using multiple different measures of goodness-of-fit. For example, Marchioro *et al.* (2017) used R^2 (the coefficient of determination), the residual sum of squares (RSS) and the Corrected Akaike Information Criterion (AIC_c)[11] when evaluating 14 growth models for three life cycle stages of *Tuta absoluta* (Lepidoptera, tomato leafminer. An invasive moth which is a pest of tomato crops). R^2 and RSS were used used to assess individual model goodness-of-fit, while the AICc was used as a criteria for selection between models. Roy *et al.* (2002) used the RSS and adj-R^2 (adjusted R^2, a variant of R^2 which includes a penalty based on the number of parameters used in the explanatory model (Leach and Henson, 2007)) from fits of 11 different models to temperature-growth data gathered for *Tetranychus mcdanieli* (Trombidiformes, McDaniel spider mite, an apple orchard pest) and *Stethorus punctillum* (Coleoptera, lesser mite destroyer, a biocontrol agent). In this case, the authors sought selected models which were 'better' than others based on the R^2 and RSS values they produced. Aghdam *et al.* (2009) report RSS, R^2, AIC (Akaike Information Criterion), and adj-R^2 values for fits of 14 temperature-development models to data from *Cydia pomonella* (Lepidoptera, codling moth, an apple

11. Some of these goodness-of-fit measures are discussed in Section 3.5.2, page 82.

orchard pest), and state that only the latter two goodness-of-fit measures are useful for comparison between models. In some causes, authors give consideration to features other than measures of fit, for example Roy *et al.* (2002) favour models that provide parameters with biological interpretations.

3.3 Temperature-dependent development in *P. infestans*

In common with most plant pathogens, *P. infestans* is highly sensitive to temperature, and there are several studies which explore the relevance of temperature to late blight epidemiology (Batista *et al.*, 2006; Bruhn and Fry, 1981; Harrison *et al.*, 1994; Iglesias *et al.*, 2009; Oijen, 1995). As the establishment of infection is a critical stage, the response and survival of spores when exposed to different temperatures has been explored in detail (Fay and Fry, 1997; Mizubuti and Fry, 1998; Sunseri *et al.*, 2002), with temperature frequently considered in conjunction with relative humidity (Minogue *et al.*, 1981). There is a well-established relationship between temperature and the mode of sporangia germination (Judelson and Blanco, 2005), with cooler temperatures favouring indirect germination (the release of zoospores), and warmer temperatures direct germination (the formation of a germ tube directly from the sporangia). A representative sample of relatively contemporary isolates from Great Britain has demonstrated highest rates of indirect germination within the range 4 – 10 °C, with no major differences between isolates (Bain and Convery, 2011).

As well as infection, the effect temperature exerts on several developmental stages has also been explored, and several controlled studies of incubation period (Andersen and Ospina-Giraldo, 2011; Lees *et al.*, 2009; Maziero *et al.*, 2009), sporulation capacity (Seidl Johnson *et al.*, 2015; Sujkowski, 1987), and measures of growth such as lesion size (Chapman, 2012) have been published. Some authors report discrepancies between growth rates on artificial media and plant material (Seidl Johnson *et al.*, 2015), which brings into question the applicability of pathogen characteristics (e.g. sporulation capacity, growth rates) measured *in vitro* to a field situation. Much recent interest has focused on differences between genotypes

(Mabon *et al.*, 2015), or on comparisons of isolates from different climatic regions (Mariette *et al.*, 2015). The methodologies employed in these studies are often different, making comparisons difficult, but several general trends are clear. The physiologically relevant temperature range for *P. infestans* appears to be ~ 4 – 30 °C, most authors report no growth at 30 °C using cultures on agar plates or infected plant tissue (Harbaoui *et al.*, 2013). The lower end of the temperature scale is less well resolved; but development, albeit very slow, has been reported as low as 5 °C (Chapman, 2012). Prolonged sub-zero (°C) temperatures are lethal to *P. infestans* mycelium (Kirk, 2003b). Of the studies which produced temperature growth curves for *P. infestans*, many do not have a descriptive model fitted to the data. Those that do generally use a polynomial (usually of degree 2) which usually provides a good description (Becktell *et al.*, 2005; Hartill *et al.*, 1990; Olanya *et al.*, 2009; Seidl Johnson *et al.*, 2015). It appears that only one laboratory-based study has made use of a non-linear model (Shakya *et al.*, 2015) to describe *P. infestans* behaviour - a modified, four parameter, Sharpe DeMichele equation (see Table 3.2, page 89) which indicated that lesion growth rates and duration of incubation were negatively skewed, but that other epidemiologically important traits such as infection efficiency and sporulation capacity were positively skewed with respect to temperature.

When isolates representing different clonal lineages are compared, differences in response to temperature are often found, both between and within genotypes (Chapman, 2012). The rank order of isolates showing the most growth sometimes changes at different temperatures (Belkhiter *et al.*, 2017), and different isolates may have different optimal temperatures. There is some evidence that local adaptation to temperature takes place. Mariette *et al.* (2016) reported that isolates within the same clonal lineage from Western Europe have faster lesion growth on detached leaves than those of Mediterranean origin at low temperatures (10 & 14 °C), but that the pattern was reversed at a higher temperature (18 °C).

3.4 Estimating *P. infestans* biomass accumulation at different temperatures

Curative fungicides act within a pathogen's incubation period, when direct observation of pathogen growth is not possible. Environmental temperature is very likely to influence the rate at which these early infections develop, and it is plausible that temperature may act as a modifying factor on curative activity. In fact, this effect has been demonstrated by Genet *et al.* (2001), although it is also possible that temperature may be affecting the rate at which active compounds are metabolised, or the rate at which they are redistributed *in planta*. It is therefore desirable that the final decision aid is underpinned by a temperature-developmental model which satisfies the following criteria: (1) accurately describes *P. infestans* growth within the incubation period over different temperatures; (2) is able to describe this behaviour over a range of temperatures likely to be encountered in the field; and (3) generates predictions that reflect the behaviour of the contemporary *P. infestans* population.

The required data were not available from the literature. Although there are several studies that give relatively recent information on *in vitro* growth or progression of symptoms on detached leaves (Belkhiter *et al.*, 2017; Chapman, 2012; Seidl Johnson *et al.*, 2015), there was little information on pre-symptomatic growth at different temperatures. We were also unaware of any studies which evaluated the substantial number of published temperature-dependent growth models for describing *P. infestans* growth. A reliable and accurate temperature-development model is crucial for the final decision aid, as it will allow an estimation of pathogen development given appropriate time and temperature inputs. It may be possible to relate this estimate of development to an expected level of curative control, which will be explored in Chapter 4. The following sections describe temperature assays which were conducted to obtain temperature-dependent growth data for a small number of *P. infestans* isolates. Pathogen growth will be measured using both traditional symptom measurement techniques and a qPCR assay which will allow the pre-symptomatic pathogen biomass *in planta* to be estimated. The following aspects will be investigated using the assays: that *P. infestans* growth is dependent

on temperature and can be assessed at both a visual level and using a biomolecular assay, that there are differences in growth between different *P. infestans* isolates and that there is a correlation between visible symptoms and sub-clinical growth (i.e growth in the incubation period). Once growth estimates have been obtained, models from the literature can be evaluated to see which provides the most useful description, the evaluation will use criteria described in Section 3.5.2, page 82. This model, and its fitted parameters can then be incorporated into the final decision aid (Chapter 6, Section 6.4, page 185).

3.4.1 Temperature bioassay: methods and materials

P. infestans growth data were obtained from a sequence of assays using detached leaves incubated at different temperatures, which were conducted in 2017. The data which these assays generated were then used to assess potential models. A single growth cabinet (Chapter 2, Section 2.5, page 54) was used for all assay runs, with the order of temperatures assessed randomized. Lateral leaflets were detached from King Edward potato plants, approximately 6 weeks old and which had been propagated as described in Chapter 2 (page 51), planting was staggered to ensure that plants were approximately the same age ($\pm$3 days) when leaflets were sampled. For each experimental run, leaflets were harvested from each node beginning at the 4th through to the 11th ascending from the base of the stem. One leaflet was taken from each leaf and a small sticker was affixed to its petiole, denoting plant and node. Leaflets were then placed within plastic trays lined with damp paper towels corrugated into ridges.

Two distinct types of tray were prepared: 'symptom measurement' and 'biomass estimation' trays. Each symptom measurement tray contained 34 leaflets: four sequential sets of leaflets representing positions from the 4th to 11th node (8 leaflets in each) from randomized plants, and two leaflets of random position (between nodes 4 to 11) from a randomized plant which were designated as controls. Each set was assigned to an inoculation category (one of four *P. infestans* isolates, or sterile distilled water), which was marked on the label. The position of the leaflets

within each tray was then randomized. The two control leaflets were assigned to a sterile distilled water inoculation. Two symptom measurement trays were included in each run, giving a total of 16 leaflets per isolate per run. The second category of tray, biomass estimation, consisted of four sequential runs of six leaflets from node positions 4 though to 9, i.e. 24 leaves in total per tray. Each leaflet in a run was harvested from a different plant. Each set of sequential leaflets was then assigned to an inoculation category (see above) and this was marked on the label. Six biomass estimation trays were included in each experimental run, a total of 36 leaflets per isolate per run.

Four separate sporangial suspensions from lesions on detached leaves (cv. King Edward) were prepared as described in Chapter 2, Section 2.4 (page 52) from the following isolates: 2012_13A2-1, 2008_6A1-1, 2012_7A1-1 and 2012_8A1-1. Each suspension was adjusted to a concentration of 10^5 sporangia ml^{-1} and kept at room temperature before use. The intention was to favour direct over indirect germination. The assigned leaflets were then inoculated with a 20 µl droplet, placed on the adaxial surface, away from any main veins. Once all leaflets within a tray were inoculated, trays were sealed within transparent plastic bags and placed within growth cabinets. The trays were then partially shaded by placing paper sheets over them.

The growth cabinet was programmed with a two-phase schedule: the initial phase consisted of a constant temperature of 18 °C with all illumination off for 12 hours; the second phase consisted of 16 hours light : 8 hour dark cycle, with temperature held constant at one of seven temperatures (6, 10, 14, 18, 22, 26, or 30 °C). The initial phase of 18 °C and darkness was used to maximize the probability of infection, as differences in infection efficiency at different temperatures (Shakya *et al.*, 2015) would likely confound later measurements of pathogen growth (Lapwood and McKee, 1966). The positions of the trays within the chamber were randomized. Two iButton temperature loggers, enclosed within small plastic pots and shaded with a piece of cardboard, were placed within the growth chamber during each run.

These were programmed to collect temperature readings at 10 minute intervals. The readings from the two data loggers were then averaged, and used to calculate a degree-minute sum for the experimental run. Comparison of these values with the expected degree-minute sum for each temperature did not differ by more than ± 3% for the infection phase (12 hours, 18 °C), or ± 5% for the constant experimental temperature phase.

Twelve hours after inoculation the biomass estimation trays were opened and one leaflet per inoculation category was removed from each tray (6 leaflets per isolate, from random positions). Each leaflet was then placed in a micro-centrifuge tube (2 ml), frozen and then stored at −20 °C. This procedure was repeated at 24, 36, 48, 60, and 72 hours post inoculation. After 120 hours of incubation, leaflets were removed from the symptom measurement trays, and digital images of each leaflet were taken as described in Chapter 2, Section 2.7 (page 55). The leaflets were then returned to their trays, which were re-sealed and placed back within the growth chamber in the same position. This procedure was repeated at 144 and 168 hours post inoculation, so that in total three images of each leaflet were taken per experimental run. Once the experiment had been performed for all seven temperatures, the cycle was repeated, but within the second run of each temperature the biomass estimation trays were omitted due to time constraints. Thus growth at each temperature was assessed twice, on different occasions using different plant material.

The digital images were inspected, and those which showed the presence of disease symptoms (necrotic tissue or sporulation) had the lesion area quantified using `polygon` function in ImageJ (Schneider *et al.*, 2012) as described in Chapter 2 (page 55). The square roots of these areas were obtained, and a simple linear regression against time performed with the equation: $\sqrt{LA} = \beta_{lg0} + \beta_{lg1} \cdot t$, where LA is the lesion area in mm^2, β_{lg0} is the intercept parameter with units mm, β_{lg1} is the slope parameter in mm hr^{-1}, and t is time elapsed from inoculation in hours for each infected leaflet. The slope coefficient β_{lg1}, is a measure of the linear lesion

growth rate ($mm \cdot hr^{-1}$) (Visker *et al.*, 2003). Images of leaflets without lesions at any of the observation time points were assigned a growth rate of zero.

Genomic DNA was extracted from the frozen leaflets as described in Chapter 2, Section 2.8 (page 56). The quality and quantity of total DNA within each sample was assessed using spectrophotonic analysis (DS-11 FX+ Spectrophotometer, DeNovix). Samples were then diluted to 20 ng μl^{-1} using sterile distilled water and loaded in duplicate onto 96-well plates – details of primers, reaction mix, etc. can be found within Chapter 2, Section 2.8 (page 56). A serial dilution from 20 ng μl^{-1} to $2 \cdot 10^{-5}$ ng μl^{-1} of *P. infestans* genomic DNA was used to generate a standard curve. This was matched to the isolate inoculated on to the specific leaflet to avoid biases due to differing ploidy or gene copy number between isolates.

Target DNA was detected in all samples which had been inoculated with *P. infestans* sporangia, and no C_q values were obtained from water controls. Estimates of initial target template quantity (which is an index of *P. infestans* biomass) were plotted against time for each isolate and temperature combination. For almost all combinations for which there appeared to be a positive relationship between target concentration and time the increase was exponential. Simple linear regression of the natural logarithm of initial target template quantity against time was performed. The regression equation was: $\ln(Pi_{DNA}) = \beta_{ba0} + \beta_{ba1} \cdot t$, where Pi_{DNA} is initial target template quantity of *P. infestans* DNA with units pg, β_{ba0} is the intercept parameter with units ln(pg), β_{ba1} is the slope parameter in ln(pg) hr^{-1}, and t is time elapsed from inoculation in hours. As with the visible symptom measurement, the coefficient β_{ba1} gives a measure of the rate of growth, with units ln(pg) hr^{-1}, which we interpret as an index of the rate at which biomass is accumulated, and which will be referred to subsequently as the 'biomass accumulation coefficient'. In instances where there was no statistically significant positive relationship, a coefficient of zero was assigned. However, it is important to note that this does *not* in all cases denote no growth, rather that the rate of biomass increase was not detectable by the methodology used within this assay.

3.4.2 Temperature bioassay: statistical analysis

To assess the influence of the different factors included within the bioassay on visible symptom development, data were analysed via an analysis of variance (ANOVA). The statistical model included linear lesion growth rate as the response variable, and temperature, leaf position, isolate, experimental tray, and experimental run as factors. Leaflets with zero late blight lesion growth were excluded. The assumptions for the analysis were assessed by inspecting residual plots: natural logarithms of growth rate were taken to ensure homogeneity of variance. Temperature was included as a factor, as at this stage no assumptions about the shape of the relationship between temperature and lesion growth were made.

The biomass accumulation trays used a destructive sampling method, and so it was not possible to calculate growth indexes for each leaflet individually. Additionally, unlike the lesion growth data each temperature was only assessed on a single occasion. However all four isolates were included at each temperature, and the linear regression model (see Section 3.4.1, page 73) could be fitted to the data. An analysis of covariance (ANCOVA) was performed with $\ln(pi_{DNA})$ as the response variable, inoculation time (t) as a covariate, and isolate as a factor. The feature of most interest here was whether the regression lines for each isolate (considered individually) were parallel, because the slope coefficient β_{ba1} provides a measure of the growth rate (in terms of DNA accumulation, and it is inferred, biomass). In other words non-parallel regression lines indicate differences in growth rates between isolates at the assessed temperatures. A significant interaction between incubation time and isolate was interpreted as a difference in growth rates.

3.4.3 Temperature bioassay: symptom measurement results

All four isolates showed a similar pattern of response to temperature, the data are summarized in Figure 3.1 (page 75). At the lowest experimental temperature (6 °C) very small lesions (0.17 – 11.38 mm^2, after 168 hours) and slow growth were observed for three of the isolates: 2012_13A2-1, 2008_6A1-1 and 2012_8A1-1, but no

symptoms were present on leaflets inoculated with isolate 2012_7A1-1 at any of the three time points. No lesions developed on leaflets incubated at 30 °C for any of the isolates tested. No lesions developed on control leaflets.

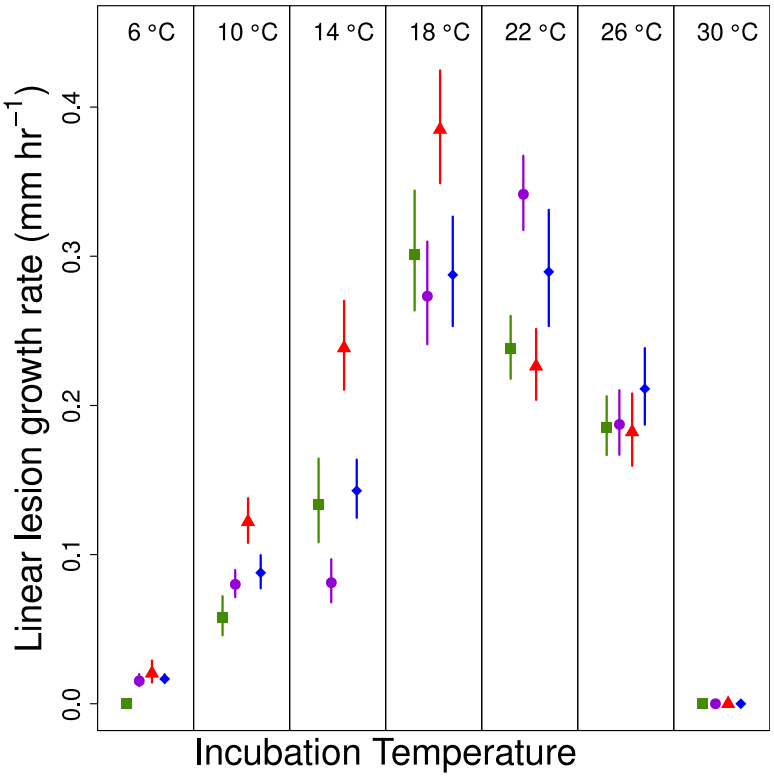

Figure 3.1. Graphical representation of mean linear lesion growth rates (mm hr^{-1}) for four *P. infestans* isolates infecting detached King Edward leaflets. The different isolates are indicated by different colours and symbols (■ green squares = 2012_7A1-1, ● purple circles = 2012_8A1-1, ▲ red triangles = 2008_6A1-1 , and ◆ blue 'diamonds' = 2012_13A2-1). Leaflets were incubated at a constant temperature which is noted at the top of each division of the plot. Means are shown, with lines indicating the 95% confidence intervals.

Lesion growth across the two sets of experimental runs was not significantly different ($p = 0.24$), so data from both runs were pooled. Including leaf position as a covariate yielded a significant relationship ($p < 0.01$), leaves from higher positions were associated with smaller lesions as has been reported elsewhere (Visker *et al.*, 2003) but this did not alter the interpretation of any of the other factors as no statistically significant interactions between leaf position and temperature or isolate were present. A large proportion of the variance was due to temperature ($p < 0.01$),

but both isolate ($p < 0.01$), and the interaction between isolate and temperature ($p < 0.01$) were significant. The rank order of isolates varied across the different temperatures (as indicated by the significant interaction term temperature · isolate from the ANOVA): isolate 2008_6A1-1 displayed the fastest lesion growth rates within the temperature range 6 – 18 °C; but at 22 °C, 2012_8A1-1 showed faster lesion growth; and at 26 °C, 2012_13A2-1 had more rapid growth than the other isolates.

3.4.4 Temperature bioassay: biomass estimation results

Statistically significant positive linear relationships (see Figure 3.2, page 77, for a representative example) between initial template and disease development time could be detected for temperatures 6 – 26 °C for isolate 2012_13A2-1, between 10 – 22 °C for isolate 2008_6A1-1, between 18 – 26 °C for isolate 2012_7A1-1 and at temperatures 10, 18, and 22 °C for isolate 2012_8A1-1. Generally speaking temperature and isolate combinations that had more rapid growth rates (i.e. larger β_{ba1} values) were associated with higher R^2 values for the relationship between $\ln(Pi_{DNA})$ and incubation time, in other words a greater proportion of the variation was explained when growth was faster. Temperatures close to the developmental extremes were the most likely to be assigned a growth coefficient of zero.

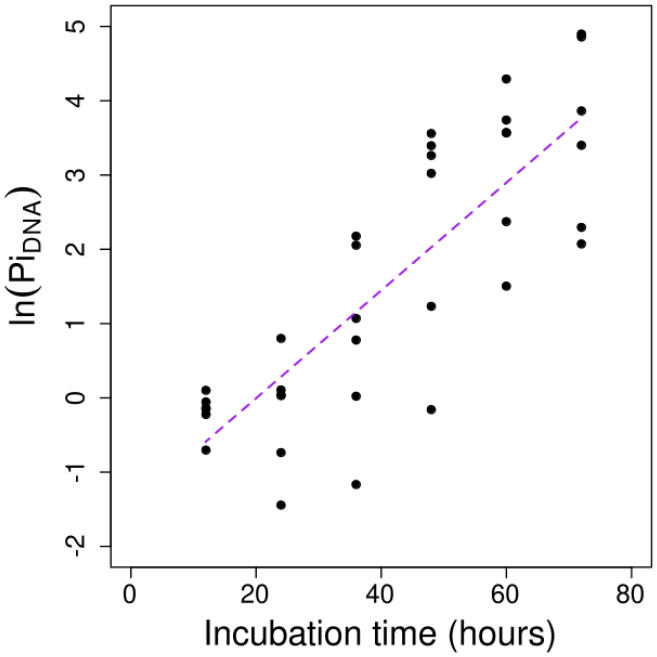

Figure 3.2. Relationship between initial *P. infestans* DNA quantity in a sample (pg) and time elapsed (hr) between inoculation and sampling. Data shown are from one isolate and temperature combination (2012_8A1-1, 22 °C) to illustrate the method by which incubation growth coefficients were obtained. Each point (●) represents an extraction and quantification from a single inoculated leaflet (n = 6, per time point). The data are described by a simple linear relationship (shown on the diagram by the dashed line) with the formula: $\ln(Pi_{DNA}) = \beta_{ba0} + \beta_{ba1} \cdot t$ ($p < 0.01$, $R^2 = 0.67$, $\beta_{ba0} = -1.458$ ln(pg), $\beta_{ba1} = 0.073$ ln(pg) · hr^{-1}) where Pi_{DNA} is initial target template quantity of *P. infestans* DNA, β_{ba0} is the intercept parameter with units ln(pg), β_{ba1} is the slope parameter in ln(pg) hr^{-1}, and t is time elapsed from inoculation in hours. R^2 refers to the coefficient of determination.

Table 3.1. Incubation biomass accumulation coefficients (obtained by linear regression of the natural logarithm of initial template quantity against incubation time) from pre-symptomatic King Edward leaflets which were sampled between 12 and 72 hours post inoculation. The biomass accumulation coefficients correspond to β_{ba1} within the equation $\ln(Pi_{DNA}) = \beta_{ba0} + \beta_{ba1} \cdot t$, where Pi_{DNA} is initial target template quantity of *P. infestans* DNA, β_{ba0} is the intercept parameter with units ln(pg), β_{ba1} is the slope parameter in ln(pg) hr^{-1}, and t is time elapsed from inoculation in hours. Quoted significance values are those of the coefficient rather than the regression line. In instances where the coefficient β_{ba1} was not significant, a value of zero was assigned (this is denoted as 'ns'). R^2 refers to the coefficient of determination about each regression lines.

Isolate	Temperature (°C)	Biomass accumulation coefficient ($\ln(pg) \cdot hr^{-1} \pm 95$ % ci)	Significance	R^2
2012_13A2-1	6	0.0105 (± 0.0085)	p = 0.02	0.15
	10	0.0611 (± 0.0098)	p < 0.01	0.83
	14	0.0623 (± 0.0167)	p < 0.01	0.62
	18	0.0678 (± 0.0167)	p < 0.01	0.68
	22	0.0839 (± 0.0262)	p < 0.01	0.58
	26	0.0879 (± 0.0177)	p < 0.01	0.75
	30	0		ns
2008_6A1-1	6	0		ns
	10	0.0666 (± 0.0110)	p < 0.01	0.82
	14	0.0481 (± 0.0230)	p < 0.01	0.35
	18	0.0515 (± 0.0201)	p < 0.01	0.44
	22	0.0824 (± 0.0467)	p = 0.01	0.27
	26	0		ns
	30	0		ns
2012_7A1-1	6	0		ns
	10	0		ns
	14	0		ns
	18	0.0196 (± 0.0106)	P < 0.01	0.31
	22	0.0779 (± 0.0136)	p < 0.01	0.80
	26	0.0345 (± 0.0150)	p < 0.01	0.39
	30	0		ns

Table 3.1. (continued from previous page)

Isolate	Temperature (°C)	Biomass accumulation coefficient ($\ln(pg)\cdot hr^{-1} \pm 95\ \%$ ci)	Significance	R^2
2012_8A1-1	6	0		ns
	10	0.0273 ($\pm$ 0.0183)	$p < 0.01$	0.21
	14	0		ns
	18	0.0384 ($\pm$ 0.0175)	$p < 0.01$	0.37
	22	0.0726 ($\pm$ 0.0179)	$p < 0.01$	0.67
	26	0		ns
	30	0		ns

When differences between isolates at each temperature (see Section 3.4.2, page 74) were assessed via ANCOVA, the interaction term time · isolate was significant at 10 °C ($p < 0.01$), 18 °C ($p < 0.01$), and at 26 °C ($p < 0.01$), but not at 14 °C ($p = 0.32$) or 22 °C ($p = 0.95$).

3.4.5 Correlation between biomass and symptom measurement

Current methods for estimating biomass accumulation, such as the qPCR assay used within this project, are relatively time-consuming and require access to specialized facilities. If visible symptom development is highly correlated with sub-clinical biomass accumulation, the former could be a useful proxy for the latter. The estimated values for linear lesion growth rate and biomass accumulation growth coefficient are plotted against each other in Figure 3.3 (page 80). There was a significant positive relationship between the two ($p < 0.01$), but the R^2 value of 0.43 indicates that less than half of the variation was explained by the biomass coefficient. Removal of combinations where one of the growth rates was allocated a value of zero did not improve the relationship. Fitting a linear relationship to each isolate individually yielded R^2 values of 0.76 ($p = 0.01$) for isolate 2012_13A2-1 and 0.66 ($p = 0.03$) for 2012_8A1-1. The relationship was not significant for isolate 2012_7A1-1 ($p = 0.26$) or 2008_6A1-1 ($p = 0.17$). This implies that, for some isolates at least observed lesion growth rates may not be useful for estimation of pre-symptomatic growth.

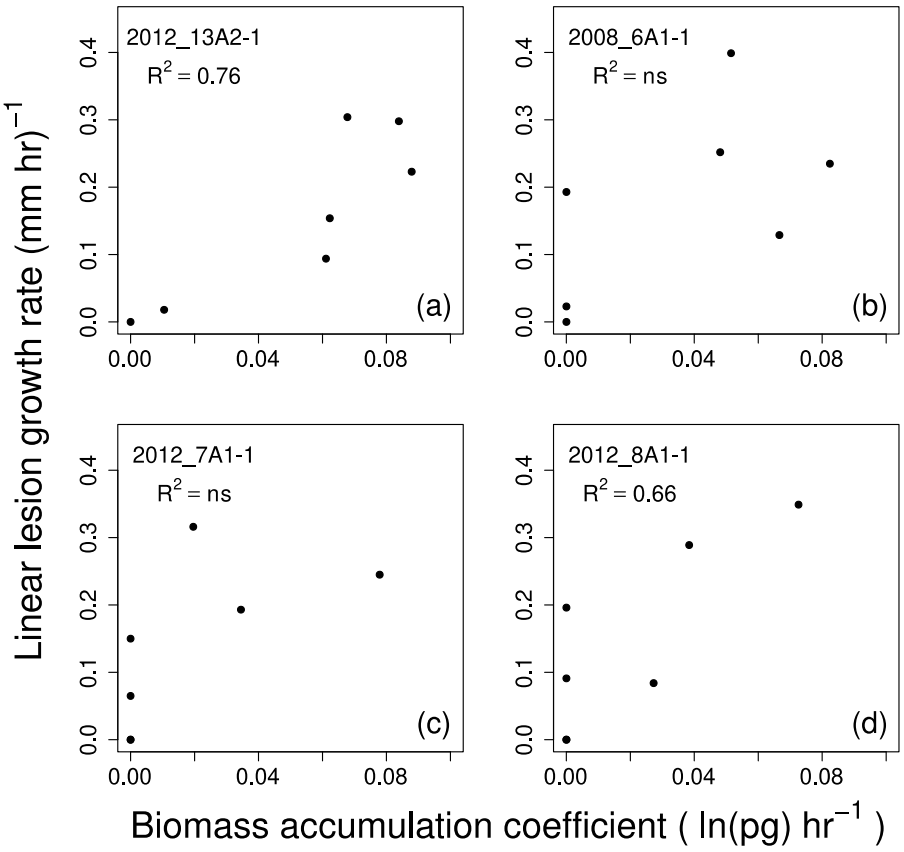

Figure 3.3. Linear lesion growth rates for *P. infestans* isolates 2012_13A2-1 (a), 2008_6A1-1 (b), 2012_7A1-1 (c), and 2012_8A1-1 (d) plotted against their corresponding incubation biomass accumulation coefficients obtained using infected detached leaflets over the temperature range 6 – 30 °C. The R^2 values for each relationship are shown, with ns indicating that there was no significant relationship.

3.5 Model selection

3.5.1 Candidate models of temperature-dependent development

A large number of published temperature-development models are available, and a useful summary and software toolkit is provided by Rebaudo *et al.* (2017). This toolkit covers a range of organisms, chiefly arthropods, but does include some models derived from studies with micro-organisms. A review of the literature did not return any studies where a range of mathematical models was compared using a single data set for plant pathogen growth post-infection. However, there are a handful of theoretical descriptions, and several studies of this type for arthropod development (see Section 3.2.2, page 66). Linear models and polynomials are commonly used to describe relationships between disease development/growth of plant pathogens and temperature. The most commonly used non-linear model appears to be the Sharpe-DeMichele equation, or a modification (Bernat *et al.*, 2017; Carisse *et al.*, 2000; Davidson *et al.*, 2003; Xu, 2003, 1999), which has been used in at least one study to model *P. infestans* (Shakya *et al.*, 2015). A second model which is also sometimes used (Legler *et al.*, 2011) is the Analytis (1977) equation which, unlike many other models, was developed specifically for plant pathogens. Many of the studies that model temperature-dependent development are interested in the infection phase, for example Lehsten *et al.* (2017) use a Beta growth function[12] for the accumulation of 'blight units' which explain the first late blight outbreaks of a season. There is some limited evidence to suggest that early phases of pathogen development may have a different temperature-growth profile than later development (Shakya *et al.*, 2015).

Fifteen models of temperature-dependent development were selected and assessed for their ability to describe separately the symptom development and biomass accumulation relationships obtained in the temperature bioassay. These were chosen to span a range of complexities and a number of different approaches,

12. The Beta growth function is sometimes used to describe plant growth (Archontoulis and Miguez, 2015; Mao *et al.*, 2018; Yin and Struik, 2010) and was derived from the Beta distribution function (Yin *et al.*, 2003). It is useful for modelling plant growth as it has endpoints which can be fixed, a single mode, and is capable of generating asymmetrical curves.

with preference given to models which have previously been used in empirical plant pathology studies. Mathematical definitions of the models can be found in Table 3.2 (page 86).

3.5.2 Model fitting

Non-linear equations were fitted to each data set using the `nlsLM` (Nonlinear Least Squares Levenberg-Marquardt) function in R from the 'minpack.lm' package (Elzhov *et al.*, 2016), which uses the Levenberg–Marquardt algorithm as a fitting method. This algorithm uses an iterative procedure to minimize the the residual sum of squares (Lourakis, 2005) of a fitted equation to a specified data set. Fitting non-linear least-squares estimates of parameters for the equations requires a set of reasonable start values or the fitting algorithm will not converge. Appropriate starting values were obtained from either: the literature; by following the recommendations of the model authors; or by visually inspecting several arbitrary test curves. With fitting algorithms there is a danger that a local (rather than global) minimum is found, so several different sets of start values were used for each temperature-growth model, as recommended by Schabenberger and Pierce (2002). The precedent set in the literature on arthropods of using several goodness-of-fit measures to assess temperature-development models was followed here. For the linear (in their parameters) models, the R^2 (of predicted against actual values) and RSS were calculated. The residual sum of squares (RSS) provides a measure of the closeness of the fit to the data (Ritz and Streibig, 2008). For linear models one interpretation of R^2 is as the proportion of the total variation explained by the regression curve (Mead, 2017). For non-linear models the use of R^2 is often discouraged (Helland, 1987), and for this reason additional goodness-of-fit metrics were included. The first was the pseudo-R^2 value as specified by Schabenberger and Pierce (2002); this is an R^2 variant which like R^2 cannot take values greater than one, and is not interpretable as the proportion of variation explained by the model. The second was the corrected Akaike information criterion (AIC_c). The classical AIC is a criterion from information theory (Burnham and Anderson, 2004; Symonds and Moussalli, 2011), which estimates the amount of information lost by representing

the process which generated the original data as a model[13]. In the corrected AIC (AIC_c) a penalty term is applied to the AIC value to reduce the danger of overfitting when sample size is small (Brewer *et al.*, 2016), as is the case here.

Linear models (eq. 3 – 7, and 18 – 22) were fitted by linear regression using the `lm` function (in combination with the `poly` function for eq. 6, 7, 21, and 22) in R (R Core Team, 2013). The thermal-time models 4, 5, 19, and 20, include temperature cut-offs at an upper temperature threshold. This can be interpreted as T_m for the vertical cuff-off as no development takes place above it. These cut-offs were set as the lowest temperature at which the fastest growth was obtained in the developmental assay. In the case of isolate 2012_13A2-1 this was at 18 °C for linear lesion growth and at 26 °C for biomass accumulation index. A special procedure was used for the linear models 3 – 5, and 18 – 20: only the data up to T_m/T_o were used in the fitting, but goodness-of-fit measures were calculated from the whole dataset (predicted vs. observed growth rates from 6 – 30 °C). This is artificial, but the motivation was to represent a situation where a linear relationship has been established over part of the temperature range before the optimum growth temperature (the temperature development relationship is often well approximated by a linear relationship up until T_o (Montagnes *et al.*, 2003)), and is then either extrapolated to higher temperatures, or has a cut-off applied.

The intention was to produce a deterministic model for inclusion in the prototype decision aid. With this in mind, for the symptom measurement data, mean lesion growth for each constant temperature served as the observations to which the model was fitted, whereas for the incubation period biomass accumulation dataset the growth coefficients (Table 3.3, page 92) were used. In both cases the data for isolate 2012_13A2-1 solely were used. This isolate was the only one with a positive relationship for biomass accumulation and visible lesion growth at each constant temperature up to and including 26 °C (i.e. where each non-zero lesion growth value had a corresponding biomass accumulation

13. Note that this is an estimate, as the true process that generates the data is almost always unknown.

coefficient). Additionally, as this appeared to be the most aggressive isolate it represents a 'worst case' scenario for *P. infestans* challenge in the field and so is appropriate for use as the basis of the final decision aid.

3.5.3 Model assessment for linear lesion growth rates

All three linear models provided a poor fit to the observed lesion growth rate data set, although the R^2 values were improved by the inclusion of a cut-off. This was chiefly due to the lack of fit at temperatures exceeding T_m. It should also be noted that at intermediate temperatures (i.e. 6 °C – 18 °C), a linear fit provided a very good description of the rates of symptom development ($R^2 = 0.96$).

The linear polynomial models, as well as all of the non-linear models assessed provided much better descriptions of the data, but the goodness-of-fit of each varied, with R^2 or pseudo-R^2 values ranging between 0.83 – 0.98. The models which deviated the greatest from observed values and had relatively high AIC_c values (more negative values indicate more informative models) were Bayoh (eq. 15), Lactin (eq. 11) , Logan (eq. 9), and the quadratic function 6. The Analytis function (eq. 8) had a very large AIC_c value (33.37), which was due to the high penalty incurred from the number of parameters it requires. Equations 9, 14 and 15 adequately represented the asymmetry of the temperature-growth relationship, but significantly underestimated development near optimum temperatures; the quadratic function (6) generates a symmetrical curve which does not match the data's negative skew. The models most commonly used for plant pathogen growth curves (eq. 16 and 17), which are both based on the Sharpe-De Michele model (Sharpe and DeMichele, 1977) performed relatively well (pseduo-$R^2 = 0.93$ and 0.91, respectively), but model 17 had an unfavourable AIC_c value (2.47). The number of parameters for model 16 was so large that a AIC_c value could not be calculated.

Several models (eq. 7, eq. 8, eq. 10, eq. 12, and eq. 13) described the data very well, with only marginal differences between them. The highest R^2 or pseudo-R^2 between predicted and observed growth rates was obtained for the cubic (eq. 7),

Analytis (eq. 8) and Kontodimas (eq. 12) models, which is reassuring as (eq. 8) was developed specifically for descriptions of plant pathogens. The most informative model based on AIC_c value was the Kontodimas (eq. 12) function (-20.83). This model is attractive as only three parameters need to be estimated, two of which have biological interpretations which readily lend themselves to applied situations (T_b and T_m). Functions eq. 8 and eq. 12 are in fact related, with eq. 12 being a simplified form of eq. 8, where two of the parameters (β_{vAY2} and β_{vAY3}) are replaced with constants (2 and 1 respectively). These constants are relatively close to the estimated values for β_{vAY2} (3.22) and β_{vAY3} (1.32) from the fit of equation eq. 8, and the discrepancy between AIC_c values between the two models is attributable to the extra parameters needed for eq. 8. A very strong fit of the data is provided by eq. 7, the cubic equation ($R^2 = 0.98$) which compares favourably with many of the more complex non-linear models. However, eq. 7 has a local minimum at ≈ 4.8 °C which would lead to overestimates of development at temperatures lower than this if an appropriate base temperature is not also incorporated.

Several of the models include parameters which have concrete biological meanings, or are able to estimate parameters commonly used in physiological-time applications (T_b, T_o and/or T_m). For the most part the estimates they provide here are plausible, notwithstanding the inherent uncertainty in their estimation. The main exception to this is eq. 14, which gives a point estimate of T_b as -4.3 °C, which does not seem physiologically reasonable; although its associated standard error is high ($\pm$ 8.72 °C).

Table 3.2. Fitted coefficients and goodness-of-fit metrics for a selection of linear and non-linear models. Models were fitted to linear lesion growth rates for blight-infected (isolate 2012_13A2-1) detached leaves. Equations (3) through (7) are linear in their parameters, and (8) through (16) are non-linear. For the fitted parameters, the standard error (se) is given in brackets. RSS = residual sum of squares, R^2 is the coefficient of determination determined from actual vs. predicted values, pseduo-R^2 is a variant R^2 recommended for non-linear regression, and AIC_c is the corrected Akaike information criterion.

Model	Equation[a]		Fitted parameters[a] (± se)		RSS	R^2	pseduo-R^2	AIC_c
Linear, no cut-offs	$f(T) = \beta_{vL0} + \beta_{vL1}T$	(3)	$\beta_{vL0} = -0.13$ $\beta_{vL1} = 2.30 \cdot 10^{-2}$	$(\pm 4.56 \cdot 10^{-2})$ $(\pm 3.48 \cdot 10^{-3})$	0.385	0.05	-	-
Linear, vertical cut-off	$f(T) = \begin{cases} \beta_{vL0} + \beta_{vL1}T & if\ T < T_m \\ 0 & else \end{cases}$	(4)	$\beta_{vL0} = -0.13$ $\beta_{vL1} = 2.30 \cdot 10^{-2}$ $T_m = 18$	$(\pm 4.56 \cdot 10^{-2})$ $(\pm 3.48 \cdot 10^{-3})$	0.140	0.14	-	-
Linear, horizontal cut-off	$f(T) = \begin{cases} \beta_{vL0} + \beta_{vL1}T & if\ x < T_o \\ \beta_{vL0} + \beta_{vL1}T_o & else \end{cases}$	(5)	$\beta_{vL0} = -0.13$ $\beta_{vL1} = 2.30 \cdot 10^{-2}$ $T_o = 18$	$(\pm 4.56 \cdot 10^{-2})$ $(\pm 3.48 \cdot 10^{-3})$	0.084	0.35	-	-
Quadratic	$f(T) = \beta_{vL0} + \beta_{vL1}T + \beta_{vL2}T^2$	(6)	$\beta_{vL0} = -0.38$ $\beta_{vL1} = 6.95 \cdot 10^{-2}$ $\beta_{vL2} = -1.85 \cdot 10^{-2}$	(± 0.13) $(\pm 1.59 \cdot 10^{-2})$ $(\pm 4.33 \cdot 10^{-4})$	0.016	0.83	-	-

[a] Where T is the temperature in °C, T_b is the minimum (base) developmental temperature constant (°C), T_m is the maximum developmental temperature (°C), and To is the optimum developmental temperature constant (°C). Other parameters which are model specific are denoted by β and an accompanying subscript. References for their derivation, interpretations and units are provided.

Table 3.2. (continued from previous page)

Model	Equation[a]		Fitted parameters[a] (± se)	RSS	R^2	pseduo-R^2	AIC_c
Cubic	$f(T) = \beta_{vL0}+\beta_{vL1}T+\beta_{vL2}T^2+\beta_{vL3}T^3$	(7)	$\beta_{vL0} = 0.10$ (± 0.12) $\beta_{vL1} = -3.90 \cdot 10^{-2}$ ($\pm 2.46 \cdot 10^{-2}$) $\beta_{vL2} = 4.97 \cdot 10^{-3}$ ($\pm 1.50 \cdot 10^{-3}$) $\beta_{vL3} = -1.26 \cdot 10^{-4}$ ($\pm 2.76 \cdot 10^{-5}$)	0.002	0.98	-	-
Analytis (Analytis, 1977)	$f(T) = \beta_{vAY1}\left(T-T_b\right)^{\beta_{vAY2}}\left(T_m-T\right)^{\beta_{vAY3}}$	(8)	$\beta_{vAY1} = 9.57$ ($\pm 1.31 \cdot 10^{-5}$) $\beta_{vAY2} = 3.22$ (± 3.42) $\beta_{vAY3} = 1.31$ (± 0.72) $T_b = 2.71 \cdot 10^{-2}$ (± 12.36) $T_m = 30.00$ (± 1.06)	0.002	0.98	0.98	33.37
Logan (Logan *et al.*, 1976)	$f(T) = \beta_{vLG1}\left(e^{\beta_{vLG2}T}-e^{\beta_{vLG2}T_m-\frac{T_m-T}{\beta_{vLG3}}}\right)$	(9)	$\beta_{vLG1} = 2.86$ ($\pm 7.47 \cdot 10^5$) $\beta_{vLG2} = 0.15$ (± 55.91) $\beta_{vLG3} = 6.46$ ($\pm 2.34 \cdot 10^3$) $T_m = 29.91$ (± 0.46)	0.011	0.90	0.89	4.37
Taylor (Taylor, 1981)	$f(T) = \beta_{vTY1}e^{\left(-0.5\left(\frac{T-T_m}{\beta_{vTY2}}\right)^2\right)}$	(10)	$\beta_{vTY1} = 0.33$ ($\pm 3.42 \cdot 10^{-2}$) $\beta_{vTY2} = 5.40$ (± 0.67) $T_m = 20.10$ (± 0.65)	0.007	0.92	0.92	−12.06

[a] Where T is the temperature in °C, T_b is the minimum (base) developmental temperature constant (°C), T_m is the maximum developmental temperature (°C), and T_o is the optimum developmental temperature constant (°C). Other parameters which are model specific are denoted by β and an accompanying subscript. References for their derivation, interpretations and units can be found in the cited papers.

Table 3.2. (continued from previous page)

Model	Equation[a]		Fitted parameters[a] (± se)	RSS	R^2	pseduo-R^2	AIC_c
Lactin (Lactin *et al.*, 1995)	$f(T) = e^{\beta_{vLN1}T} - e^{\beta_{vLN1}T_m - \left(\frac{T_m - T}{\beta_{vLN2}}\right)}$	(11)	$\beta_{vLN1} = 0.15$ (± 2.11 · 10^{-2}) $\beta_{vLN2} = 6.44$ (± 0.85) $T_m = 29.91$ (± 0.40)	0.011	0.90	0.89	−9.64
Kontodimas (Kontodimas *et al.*, 2004)	$f(T) = \beta_{vKD1}(T - T_b)^2(T_m - T)$	(12)	$\beta_{vKD1} = 1.16 \cdot 10^{-4}$ (± 1.20 · 10^{-5}) $T_b = 5.09$ (± 0.91) $T_m = 29.98$ (± 0.27)	0.002	0.98	0.98	−20.86
Ratowsky (Ratkowsky *et al.*, 1982)	$f(T) = \left(\beta_{vRT1}(T - T_b)\left(1 - e^{\beta_{vRT2}(T - T_m)}\right)\right)^2$	(13)	$\beta_{vRT1} = 3.95 \cdot 10^{-2}$ (± 1.20 · 10^{-2}) $\beta_{vRT2} = 0.15$ (± 7.50 · 10^{-2}) $T_b = 2.38$ (± 2.47) $T_m = 30.52$ (± 1.03)	0.002	0.98	0.98	−7.25
Briere (Briere *et al.*, 1999)	$f(T) = \beta_{vBR1}T(T - T_b)(T_m - T)^{\frac{1}{2}}$	(14)	$\beta_{vBR1} = 1.68 \cdot 10^{-4}$ (± 6.39 · 10^{-5}) $T_b = -4.30$ (± 8.72) $T_m = 30$ (± 1.81 · 10^{-4})	0.009	0.92	0.91	−10.90

[a] Where T is the temperature in °C, T_b is the minimum (base) developmental temperature constant (°C), T_m is the maximum developmental temperature (°C), and T_o is the optimum developmental temperature constant (°C). Other parameters which are model specific are denoted by β and an accompanying subscript. References for their derivation, interpretations and units can be found in the cited papers.

Table 3.2. (continued from previous page)

Model	Equation[a]		Fitted parameters[a] (± se)	RSS	R^2	pseduo-R^2	AIC_c
Bayoh (Bayoh and Lindsay, 2003)	$f(T)=\beta_{vBY1}+\beta_{vBY2}T+\beta_{vBY3}e^{T}+\beta_{vBY4}e^{-T}$	(15)	$\beta_{vBY1} = 3.00\cdot10^{-2}$ ($\pm$ 0.11) $\beta_{vBY2} = 1.04\cdot10^{-2}$ ($\pm$ $5.91\cdot10^{-3}$) $\beta_{vBY3} = -3.20\cdot10^{-14}$ ($\pm$ $1.01\cdot10^{-14}$) $\beta_{vBY4} = -30.27$ ($\pm$ 43.48)	0.016	0.83	0.83	7.31
Schoolfield (= modified Sharpe-De Michele) (Schoolfield *et al.*, 1981)	$f(T)=\frac{\beta_{vSdM1}\left(\frac{T+273.16}{298}\right)e^{\frac{\beta_{vSdM2}}{R}\left(\frac{1}{298}-\frac{1}{T+273.16}\right)}}{1+e^{\frac{\beta_{vSdM3}}{R}\left(\frac{1}{\beta_{vSdM4}}-\frac{1}{T+273.16}\right)}+e^{\frac{\beta_{vSdM5}}{R}\left(\frac{1}{\beta_{vSdM6}}-\frac{1}{T+273.16}\right)}}$	(16)	$\beta_{vSdM1} = 0.88$ ($\pm$ 27.20) $\beta_{vSdM2} = -8.64\cdot10^{4}$ ($\pm$ $6.75\cdot10^{5}$) $\beta_{vSdM3} = -5.74\cdot10^{4}$ ($\pm$ $2.05\cdot10^{5}$) $\beta_{vSdM4} = 3.00\cdot10^{2}$ ($\pm$ $1.35\cdot10^{2}$) $\beta_{vSdM5} = -1.16\cdot10^{5}$ ($\pm$ $6.22\cdot10^{5}$) $\beta_{vSdM6} = 2.98\cdot10^{2}$ ($\pm$ 57.88)	0.006	0.94	0.93	- [b]
Schoolfield, four parameter (Schoolfield *et al.*, 1981)	$y=\frac{\beta_{vSF1}\left(\frac{T+273.16}{298}\right)e^{\frac{\beta_{vSF2}}{R}\left(\frac{1}{298}-\frac{1}{T}\right)}}{1+e^{\frac{\beta_{vSF3}}{R}\left(\frac{1}{\beta_{vSF4}}-\frac{1}{T+273.16}\right)}}$	(17)	$\beta_{vSF1} = 0.86$ ($\pm$ 0.59) $\beta_{vSF2} = 2.54\cdot10^{4}$ ($\pm$ $1.12\cdot10^{4}$) $\beta_{vSF3} = 8.23\cdot10^{4}$ ($\pm$ $2.36\cdot10^{4}$) $\beta_{vSF4} = 2.96\cdot10^{2}$ ($\pm$ 2.64)	0.008	0.92	0.91	2.47

[a] Where T is the temperature in °C, T_b is the minimum (base) developmental temperature constant (°C), T_m is the maximum developmental temperature (°C), and T_o is the optimum developmental temperature constant (°C). Other parameters which are model specific are denoted by β and an accompanying subscript. References for their derivation, interpretations and units can be found in the cited papers.

[b] Calculation not possible due to number of parameters.

3.5.4 Model assessment for incubation period biomass accumulation

A single isolate, 2012_13A2-1, provided the observed biomass accumulation rates used to parametrize the incubation period models which can be found in Table 3.3 (page 92), and thus the results should be interpreted with caution. It is useful to consider the response of the other isolates included in the temperature bioassay, the observed values and model fits for symptom measurement, as well as the wider literature on early *P. infestans* development when considering which description will offer the greatest utility.

As with the symptom development models, the simple linear (eq. 18) description of the incubation biomass-temperature relationship left almost all variation unexplained (R^2 = 0.01); however, both threshold models (eq. 19 and 20) were better descriptions of the growth curve (R^2 = 0.89 and 0.58 respectively) in this instance . The estimated optimum temperature was higher than might be expected (26 °C), however, inspecting the standard errors of the estimated growth coefficients illustrates the low confidence that growth rates at 26 °C were truly greater than at 22 °C. Reducing the optimum temperature to 22 °C lowered the R^2 values substantially (0.23 for vertical cut-off and 0.12 for horizontal cut-off, data not shown). Both polynomial functions provided reasonable descriptions, with the asymmetrical cubic (eq. 21, R^2 = 0.81) outperforming the quadratic (eq. 22, R^2 = 0.73).

Goodness-of-fit for the non-linear models was variable. The Taylor function (eq. 25) generated a curve which was close to symmetrical, overestimating rates in the central temperature range and generally underestimating more extreme values, and thus was the least descriptive non-linear model. Functions 24, 26, 27, 28, and 32 all provided reasonable descriptions of the growth rate-temperature relationship; all generated negatively skewed curves. Models 24, 26, 28, and 32 were least accurate at low temperatures 6 – 10 °C and were probably overestimating growth as T_b is approached. Two models, Analytis (eq. 23) and Briere (eq. 29) produced similar curves which were very good descriptions of the relationship, but both of which

underestimated the observed development at 14 °C. Two models (eq. 23 and 27) also provided a reasonable point estimates of T_b : 1.2 °C and 0.45 °C, respectively.

Excellent descriptions of the data were provided by functions 30 and 31, chiefly due to the shape of curve that they yielded: a central linear phase between 10 and 26 °C, with very sharp declines in predicted growth rates either side of this. Although goodness of fit measurements were very favourable (model 30: $R^2 = 0.99$, $AIC_c = -31.16$ and model 31: $R^2 = 0.99$) the models are possibly overfitted to the data set: both produced high estimates of T_b, and the general shape of the relationship is unusual when compared to published pathogen temperature development curves (Rebaudo et al., 2017). Model 31 also has a very large number of parameters, and so an AIC_c could not be calculated for it. Similarly, the Analytis model (eq. 23) has a very high AIC_c value (27.32) due to the number of parameters required.

Table 3.3. Fitted coefficients and goodness-of-fit metrics for a selection of linear and non-linear models. Models were fitted to pre-symptomatic growth measures at different temperature obtained via qPCR from blight-infected (isolate 2012_13A2-1) detached leaves. Equations (18) through (22) are linear in their parameters, and (23) through (32) are non-linear. For the fitted parameters, the standard error (se) is given in brackets. RSS = residual sum of squares, R^2 is the coefficient of determination determined from actual vs. predicted values, pseudo-R^2 is a variant R^2 recommended for non-linear regression, and AIC_c is the corrected Akaike information citerion.

Model	Equation[a]	Fitted parameters[a] (± se)	RSS	R^2	pseduo-R^2	AIC_c
Linear, no cut-offs	$f(T) = \beta_{bL0} + \beta_{bL1}T$ (18)	$\beta_{bL0} = 9.58 \cdot 10^{-3}$ $(\pm 1.47 \cdot 10^{-2})$ $\beta_{bL1} = 3.29 \cdot 10^{-3}$ $(\pm 8.46 \cdot 10^{-4})$	$1.25 \cdot 10^{-2}$	0.01	-	-
Linear, vertical cut-off	$f(T) = \begin{cases} \beta_{bL0} + \beta_{bL1}T & \text{if } T<T_m \\ 0 & \text{else} \end{cases}$ (19)	$\beta_{bL0} = 9.58 \cdot 10^{-3}$ $(\pm 1.47 \cdot 10^{-2})$ $\beta_{bL1} = 3.29 \cdot 10^{-3}$ $(\pm 8.46 \cdot 10^{-4})$ $T_m = 26$	$8.02 \cdot 10^{-2}$	0.89	-	-
Linear, horizontal cut-off	$f(T) = \begin{cases} \beta_{bL0} + \beta_{bL1}T & \text{if } T<T_o \\ \beta_{bL0} + \beta_{bL1}T_o & \text{else} \end{cases}$ (20)	$\beta_{bL0} = 9.58 \cdot 10^{-3}$ $(\pm 1.47 \cdot 10^{-2})$ $\beta_{bL1} = 3.29 \cdot 10^{-3}$ $(\pm 8.46 \cdot 10^{-4})$ $T_m = 26$	$9.86 \cdot 10^{-3}$	0.58	-	-
Quadratic	$f(T) = \beta_{bL0} + \beta_{bL1}T + \beta_{bL2}T^2$ (21)	$\beta_{bL0} = -8.08 \cdot 10^{-2}$ $(\pm 4.44 \cdot 10^{-2})$ $\beta_{bL1} = 1.80 \cdot 10^{-2}$ $(\pm 5.52 \cdot 10^{-3})$ $\beta_{bL2} = -4.89 \cdot 10^{-4}$ $(\pm 1.50 \cdot 10^{-4})$	$1.95 \cdot 10^{-3}$	0.73	-	-

[a] Where T is the temperature in °C, T_b is the minimum (base) developmental temperature constant (°C), T_m is the maximum developmental temperature (°C), and T_o is the optimum developmental temperature constant (°C). Other parameters which are model specific are denoted by β and an accompanying subscript. References for their derivation, interpretations and units are provided.

Table 3.3 (continued from previous page)

Model	Equation[a]		Fitted parameters[a] (± se)	RSS	R^2	pseduo-R^2	AIC_c
Cubic	$f(T) = \beta_{bL0}+\beta_{bL1}T+\beta_{bL2}T^2+\beta_{bL3}T^3$	(22)	$\beta_{bL0} = 1.67 \cdot 10^{-2}$ ($\pm$ $9.66 \cdot 10^{-2}$) $\beta_{bL1} = -3.99 \cdot 10^{-3}$ ($\pm$ $2.02 \cdot 10^{-2}$) $\beta_{bL2} = 8.91 \cdot 10^{-4}$ ($\pm$ $1.24 \cdot 10^{-3}$) $\beta_{bL3} = -2.56 \cdot 10^{-5}$ ($\pm$ $2.27 \cdot 10^{-5}$)	$1.37 \cdot 10^{-3}$	0.81	-	-
Analytis (Analytis, 1977)	$f(T) = \beta_{bAY1}\left(T-T_b\right)^{\beta_{bAY2}}\left(T_m-T\right)^{\beta_{bAY3}}$	(23)	$\beta_{bAY1} = 1.21 \cdot 10^{-3}$ ($\pm$ $1.34 \cdot 10^{-2}$) $\beta_{bAY2} = 1.19$ ($\pm$ 2.87) $\beta_{bAY3} = 0.32$ ($\pm$ 0.83) $T_b = 1.20$ ($\pm$ 18.34) $T_m = 30$ ($\pm$ $1.80 \cdot 10^{-5}$)	$6.92 \cdot 10^{-4}$	0.92	0.90	27.32
Logan (Logan *et al.*, 1976)	$f(T) = \beta_{bLG1}\left(e^{\beta_{bLG2}T}-e^{\beta_{bLG2}T_m-\frac{\left\|T_m-T\right\|}{\beta_{bLG3}}}\right)$	(24)	$\beta_{bLG1} = 1.89 \cdot 10^{-2}$ ($\pm$ $1.37 \cdot 10^{-2}$) $\beta_{bLG2} = 8.10 \cdot 10^{-2}$ ($\pm$ $6.70 \cdot 10^{-2}$) $\beta_{bLG3} = 3.62$ $\pm$ 3.78) $T_m = 30$ ($\pm$ 0.41)	$8.33 \cdot 10^{-4}$	0.88	0.88	−12.98
Taylor (Taylor, 1981)	$f(T) = \beta_{bTY1}e^{\left(-0.5\left(\frac{T-T_m}{\beta_{bTY2}}\right)^2\right)}$	(25)	$\beta_{bTY1} = 8.50 \cdot 10^{-2}$ ($\pm$0.18) $\beta_{bTY2} = 7.60$ ($\pm$2.11) $T_m = 19.24$ ($\pm$1.86)	$2.3 \cdot 10^{-3}$	0.63	0.62	−19.16

[a] Where T is the temperature in °C, T_b is the minimum (base) developmental temperature constant (°C), T_m is the maximum developmental temperature (°C), and To is the optimum developmental temperature constant (°C). Other parameters which are model specific are denoted by β and an accompanying subscript. References for their derivation, interpretations and units are provided.

Table 3.3 (continued from previous page)

Model	Equation[a]		Fitted parameters[a] (± se)	RSS	R^2	pseduo-R^2	AIC_c
Lactin (Lactin *et al.*, 1995)	$f(T) = e^{\beta_{bLN1}T} - e^{\beta_{bLN1}T_m - \left(\frac{T_m - T}{\beta_{bLN2}}\right)}$	(26)	$\beta_{bLN1} = 0.14$ ($\pm 1.9 \cdot 10^{-2}$) $\beta_{bLN2} = 6.97$ (± 0.90) $T_m = 30.05$ (± 0.43)	$9.07 \cdot 10^{-4}$	0.87	0.87	−26.79
Kontodimas (Kontodimas *et al.*, 2004)	$f(T) = \beta_{bKD1}(T - T_b)^2(T_m - T)$	(27)	$\beta_{bKD1} = 2.21 \cdot 10^{-5}$ ($\pm 9.1 \cdot 10^{-6}$) $T_b = 0.45$ (± 2.92) $T_m = 30.51$ (± 0.98)	$1.38 \cdot 10^{-3}$	0.81	0.81	−23.85
Ratowsky (Ratkowsky *et al.*, 1982)	$f(T) = \left(\beta_{bRT1}(T - T_b)\left(1 - e^{\beta_{bRT2}(T - T_m)}\right)\right)^2$	(28)	$\beta_{bRT1} = 2.02 \cdot 10^{-3}$ ($\pm 3.41 \cdot 10^{-3}$) $\beta_{bRT2} = 0.52$ (± 0.41) $T_b = -15.53$ (± 30.14) $T_m = 30.14$ (± 1.85)	$8 \cdot 10^{-4}$	0.89	0.89	−13.52
Briere (Briere *et al.*, 1999)	$f(T) = \beta_{bBR1}T(T - T_b)(T_m - T)^{\frac{1}{2}}$	(29)	$\beta_{bBR1} = 4.84 \cdot 10^{-5}$ ($\pm 1.88 \cdot 10^{-5}$) $T_b = -7.25$ (± 10.03) $T_m = 30$ ($\pm 1.72 \cdot 10^{-4}$)	$7.62 \cdot 10^{-4}$	0.90	0.89	−28.77

[a] Where T is the temperature in °C, T_b is the minimum (base) developmental temperature constant (°C), T_m is the maximum developmental temperature (°C), and To is the optimum developmental temperature constant (°C). Other parameters which are model specific are denoted by β and an accompanying subscript. References for their derivation, interpretations and units are provided.

Table 3.3 (continued from previous page)

Model	Equation[a]		Fitted parameters[a] (± se)	RSS	R^2	pseduo-R^2	AIC_c
Bayoh (Bayoh and Lindsay, 2003)	$f(T)=\beta_{bBY1}+\beta_{bBY2}x+\beta_{bBY3}e^{T}+\beta_{vbY4}e^{-T}$	(30)	$\beta_{bBY1} = 3.82 \cdot 10^{-2}$ ($\pm 7.13 \cdot 10^{-3}$) $\beta_{bBY2} = 1.94 \cdot 10^{-3}$ ($\pm 3.79 \cdot 10^{-4}$) $\beta_{bBY3} = -9.01 \cdot 10^{-15}$ ($\pm 6.46 \cdot 10^{-16}$) $\beta_{bBY4} = -1.58$ (± 2.79)	$6 \cdot 10^{-5}$	0.99	0.99	−31.16
Schoolfield (= modified Sharpe-De Michele) (Schoolfield *et al.*, 1981)	$f(T)=\frac{\beta_{bSdM1}\left(\frac{T+273.16}{298}\right)e^{\frac{\beta_{bSdM2}}{R}\left(\frac{1}{298}-\frac{1}{T+273.16}\right)}}{1+e^{\frac{\beta_{bSdM3}}{R}\left(\frac{1}{\beta_{bSdM4}}-\frac{1}{T+273.16}\right)}+e^{\frac{\beta_{bSdM5}}{R}\left(\frac{1}{\beta_{bSdM6}}-\frac{1}{T+273.16}\right)}}$	(31)	$\beta_{bSdM1} = 8.96 \cdot 10^{-2}$ ($\pm 1.03 \cdot 10^{-2}$) $\beta_{bSdM2} = 4.07 \cdot 10^{3}$ ($\pm 2.89 \cdot 10^{3}$) $\beta_{bSdM3} = 7.62 \cdot 10^{5}$ ($\pm 1.36 \cdot 10^{9}$) $\beta_{bSdM4} = 3.00 \cdot 10^{2}$ ($\pm 1.65 \cdot 10^{3}$) $\beta_{bSdM5} = -8.32 \cdot 10^{5}$ $\pm 2.45 \cdot 10^{12}$) $\beta_{bSdM6} = 2.79 \cdot 10^{2}$ ($\pm 7.46 \cdot 10^{5}$)	$4.85 \cdot 10^{-5}$	0.99	0.99	- [b]
Schoolfield, four parameter (Schoolfield *et al.*, 1981)	$y=\frac{\beta_{bSF1}\left(\frac{T+273.16}{298}\right)e^{\frac{\beta_{bSF2}}{R}\left(\frac{1}{298}-\frac{1}{T+273.16}\right)}}{1+e^{\frac{\beta_{bSF3}}{R}\left(\frac{1}{\beta_{bSF4}}-\frac{1}{T+273.16}\right)}}$	(32)	$\beta_{bSF1} = 0.10$ ($\pm 2.58 \cdot 10^{-2}$) $\beta_{bSF2} = 9.36 \cdot 10^{3}$ ($\pm 4.37 \cdot 10^{3}$) $\beta_{bSF3} = 3.78 \cdot 10^{5}$ ($\pm 6.34 \cdot 10^{6}$) $\beta_{bSF4} = 3.00 \cdot 10^{2}$ (± 10.96)	$9.27 \cdot 10^{-4}$	0.87	0.87	−29.30

[a] Where T is the temperature in °C, T_b is the minimum (base) developmental temperature constant (°C), T_m is the maximum developmental temperature (°C), and To is the optimum developmental temperature constant (°C). Other parameters which are model specific are denoted by β and an accompanying subscript. References for their derivation, interpretations and units are provided.

[b] Calculation not possible due to number of parameters.

3.6 Discussion and final physiological-time model selection

The observed temperature-development relationships for *P. infestans* lesions presented in this chapter are in agreement with those in previous investigations (Chapman, 2012; Shakya *et al.*, 2015). A wide range of models have been assessed for their descriptive power whereas authors typically use a single function without explicit consideration of its merits (Hartill *et al.*, 1990; Mizubuti and Fry, 1998). Additionally, a growth index within the incubation period, i.e. the increase in the proportion of *P. infestans* genomic DNA present in infected host material, was obtained directly. The usual practice is to infer sub-clinical development from incubation periods, but this approach will be sensitive to the frequency of observation, as well as to the method used for assessment; i.e. either microscopic (Shakya *et al.*, 2015) or unaided (Lebecka and Sobkowiak, 2012; Maziero *et al.*, 2009).

Although only four isolates were included in the temperature bioassay, they were explicitly selected as representative of the relatively contemporary *P. infestans* population extant in Great Britain, and were thought to differ in aggressiveness. However, as each genotype was only represented by a single isolate due to logistic constraints, no assessment of intra-clonal variation was made (Dey *et al.*, 2018). The rates of visible lesion expansion were of the same order of magnitude as those reported by others (Mariette *et al.*, 2016), but comparisons are difficult as lesion growth is contingent on several other factors (host cultivar, inoculum density, etc.). The rank order of isolates for growth rate differed across the assessed temperatures: within the range 6 – 18 °C isolate 2008_6A1-1 had the fastest average linear lesion growth rate, but at 22 °C was replaced as the fastest growing isolate by 2012_8A1-1, which in turn was supplanted by 2012_13A2-1 at 26 °C.

When growth models were fitted to the isolates individually, slightly different estimates of T_o were obtained (data not shown). This is consistent with several other studies in which differences between isolates were found (Belkhiter *et al.*, 2017; Chapman, 2012; Michalska *et al.*, 2016). More data are needed before any

attributions of temperature adaptation are made. Other authors have reported variation between inoculum batches obtained from the same isolate (Chapman, 2012). Although no significant differences were obtained between the repeat runs at the same temperature this possibility cannot be excluded, and it may act as an additional source of variation between the different temperatures tested. This should also be kept in mind when interpreting the biomass accumulation results, as it was only possible to test each constant temperature a single time. It is also possible that isolates of the same genotype designation differ in phenotypic traits (Hansen *et al.*, 2016b), such as response to temperature (Marano *et al.*, 2016). Ideally this within-genotype variability would have been assessed by including several isolates of the same genotype within the bioassays, but this was not possible due to resource and time constraints. The results presented here should not be extrapolated to genotype groups without further evidence.

Several of the tested models gave descriptions of the relationship between temperature and the rate of *P. infestans* tissue colonization that were a good fit to the observed values across the tested temperature range. Surprisingly, the two growth indices used (linear lesion growth and incubation biomass accumulation) had only a moderately strong correlation. This in particular was due to the two isolates (2008_6A1-1 and 2012_7A1-1) showing no apparent correlation between the two variables. Linked to this was the observation that the performance of almost all of the growth models differed between the two growth indices. For example, the Bayoh function performed relatively poorly in describing lesion growth (eq. 15), but was the most informative model when applied to the biomass accumulation dataset (eq. 30). The converse was, in essence, true of the Taylor function (eqs 10 and 25). It is not possible to determine if this is a robust finding and whether biomass accumulation within the incubation period and visible symptom development require different descriptions or whether this is an artefact of the methodology used to generate the two measurement types.

The most appropriate measurement on which to base the final physiological-time model is the incubation biomass accumulation, as it is within the incubation

period that curative fungicides are active, and the threshold for curative control will almost certainly lie in the time period included in the temperature bioassays detailed in Section 3.4 (page 69). Although the coefficients generated from the qPCR estimation of biomass increase within the incubation period are useful, there are caveats to consider before any inferences are made. Firstly, not all temperature-isolate combinations could be assigned a growth coefficient, which indicates that either (i) no growth occurred, (ii) very slow growth occurred which could not be detected by the assay or, (iii) the data were too variable to fit a statistically significant linear relationship. In instances where no lesions developed in the corresponding symptom development observations, we can be reasonably confident of (i). However, there were also several combinations where no incubation coefficient could be assigned, but where lesion growth was observed. It is difficult to see how this is possible if (i) applied. These cases can probably be attributed to (ii) and (iii), or possibly a combination of both as it was often the case that the biomass data appeared to give a truncated range of physiologically relevant temperatures. Isolate 2012_13A2-1 represents a lineage thought to be more aggressive (Cooke *et al.*, 2012), more aggressive isolates colonize host tissue at a more rapid rate and this could be a factor in why growth could be detected at most temperatures. However this does not offer a complete explanation as isolate 2008_6A1-1 is also considered to be an aggressive lineage (Stroud *et al.*, 2016).

The above being the case, the best approach is in fact to select a model which is flexible enough to accurately describe growth curves for both pre- and post-symptomatic development (naturally, the fitted parameters will be different for each). If necessary it will be straightforward to update the model used at a later date, if new data are produced which give more accurate information on the temperature-growth relationship. If more resources had been available, the biomass accumulation measurements could have been repeated over a longer time-scale (up to 96 or 120 hours) to capture a larger proportion of the exponential growth curve. This curve is likely to shift to the right for temperatures approaching T_b and T_m. More could also have been done to improve the biomass estimates at each time-

point, by taking a larger number of samples, and/or by ensuring greater standardisation. Although leaflets of a similar size were selected, there was some natural variation which will have increased the variance of *P. infestans* DNA quantity estimates. A recommendation for future work is to fix the mass of host tissue across experiments of this nature, but in some situations this could introduce further errors in that sub-clinical lesions could be missed when a standardised area of leaflet is sampled.

The selection of a final model is difficult as many of the tested models provided reasonable descriptions of the relationship between pathogen development and temperature (based on their goodness-of-fit measures). A very strong candidate is the Analytis function (eq. 23). It provided appropriate curve shapes, plausible estimates of base and maximum temperatures, and was informative for both types of growth measurement. However, it also has a number of weakness, which are mainly due to the small sample size of the data set. The equation has a relatively large number of parameters, and this resulted in a very large AIC_c value (as the penalty term was large). There was also a large standard error for the T_b , which resulted in a large interval estimate. The Bayoh (eq. 30) and Schoolfield models (eq. 31) are probably not appropriate choices as the shape of curve they generated for pre-symptomatic development is unusual for temperature-dependent biological growth. The four parameter Schoolfield model (eq. 32) would be a reasonable choice, but many of its parameter have interpretations based enzyme kinetics (Schoolfield *et al.*, 1981), which are probably not intuitive to users of a plant disease forecasting application such as the planned decision aid. The Ratowsky (eq. 28) and Briere (eq. 29) models had favourable goodness-of-fit measures, but underestimated T_b to levels that are not biologically plausible (−15.53 °C and −7.25 °C). The Logan (eq. 24) and Lactin (eq. 26) both provided good descriptions, but do not provide estimates for T_b.

Ultimately, the Kontodimas function (eq. 27) was chosen for the following reasons: it has a small number of parameters, it generates plausibly shaped curves for both liner lesion growth and biomass accumulation, it provides estimates of both

T_b and T_m which are plausible, and it is closely related to the Analytis function (eq. 23) which was disqualified on the basis of its high AIC_c value but was otherwise a strong candidate. The Kontodimas function did have slightly lower RSS and pseduo-R^2 values ($1.38 \cdot 10^{-3}$ and 0.81, respectively) than some of the other models when used to describe the biomass accumulation data, and it may mean that the model underestimates biomass accumulation towards the high temperature developmental extreme (see Figure 3.4, page 101). This was judged an acceptable trade-off with the other criteria (number of parameters, estimates of T_b and T_m, fit to linear lesion growth data, etc.). This is a point which could be revisited if a larger set of pre-symptomatic growth observations become available. Fits of the function to the linear lesion growth rate and biomass accumulation data for isolate 2012_13A2-1 are shown in Figure 3.4.

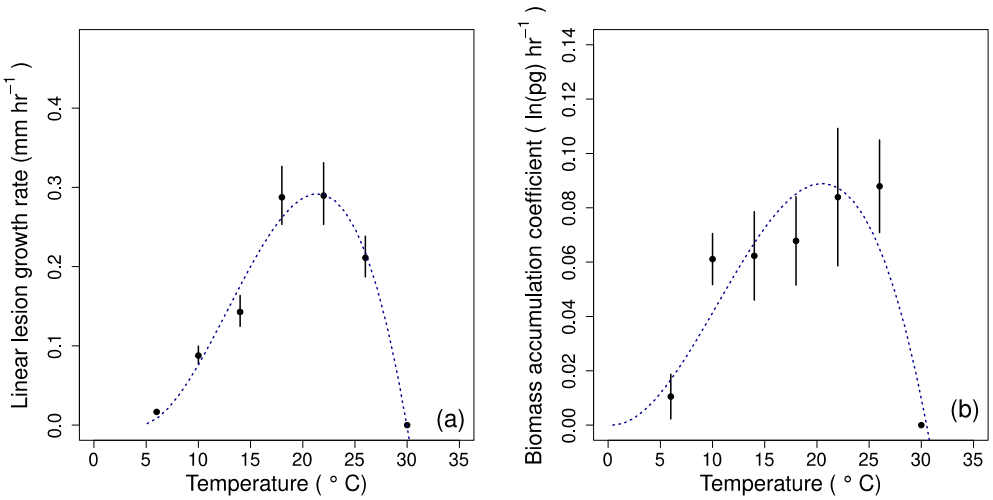

Figure 3.4. Temperature-dependent development of the *P. infestans* isolate 2012_13A2-1 on detached leaves when incubated at a range of constant temperatures for (a) visible symptom development measured by the linear lesion growth rate (mm hr^{-1}), and (b) biomass accumulation within the latent period measured by the quantity of pathogen DNA present(estimated via qPCR). Points in (a) represent mean values from 32 infected detached leaflets and lines represent the 95% confidence intervals, points in (b) are the biomass accumulation coefficients estimated by linear regression of the natural logarithm of *P. infestans* DNA (ln(pg)) against time (six leaflets per time point at 12 hour intervals between 12 and 72 hours), and lines represent the 95% confidence intervals. The blue dashed curves are the nonlinear least squares fits of the Kontodimas function to each of the data sets, for (a) this fitted relationship was: linear lesion growth (mm hr^{-1}) = $1.16 \cdot 10^{-4}$ $(T - 5.09)^2$ $(29.98 - T)$, and for (b): *P. infestans* DNA accumulation (ln(pg) hr^{-1}) = $2.21 \cdot 10^{-5}$ $(T - 0.45)^2$ $(30.51 - T)$. In both cases T was the incubation temperature in °C. Note that because the model includes a base (T_b) and a maximum (T_m) developmental temperature, only predicated development in the range between T_b and T_m is shown.

The final model which will be used to express temperature in terms of physiological time is therefore defined as:

$$f(T) = \begin{cases} 0 & T < 0.45 \\ 2.21 \cdot 10^{-5}(T - 0.45)^2(30.51 - T) & 0.45 \leq T \leq 30.51 \\ 0 & T > 30.51 \end{cases} \quad \text{(27-A)}$$

where f(T) is a rate function of the observed temperature T (°C), with the units ln(pg) hr $^{-1}$. As the function includes a base (T_b = 0.45 °C) and a maximum (T_m = 30.51 °C) developmental temperature, no development accumulates outside of the range T_b to T_m. Temperature will generally be available in the form of readings at fixed intervals, which will allow the calculation of the estimated development of *P. infestans* biomass for each interval. Subsequent chapters of this thesis will link this expected development to the level of curative control using laboratory based experiments (Chapter 4) and will validate the model on datasets not used in the construction of the model (Chapter 6). The units of the selected model can alternatively be expressed as a relative of development using a conversion constant (k = 1 / ln(pg)), or as physiological-time (Γ) with the constant (k = hr^2 / ln(pg)).

Several assumptions have been made in the construction of this model, and several factors which may be important have not been considered. Firstly, no account has been taken of the 'Kaufmann effect' which describes inaccuracies in thermal-time models under oscillating temperatures compared with constant ones (Worner, 1992). This effect is likely to be a significant issue over relatively long time-scales, and where temperature observations are infrequent or are estimated (i.e. daily highs and lows). For the decision aid it is envisaged that hourly (or perhaps even more frequent) temperature readings will be available. Additionally, the relevant time windows are in the region of 3 – 6 days (i.e. within the incubation period of *P. infestans*), and the fact that the selected temperature model is non-linear should limit any discrepancies due to temperature oscillations.

The model implicitly assumes that temperature is the *only* factor which influences the rate of development of *P. infestans*, which is clearly untrue. This and other studies have observed subtle differences between isolates which may impinge on development rates (Chapman, 2012). In future, if very rapid genotyping and rigorous population monitoring are in place, it may be useful to include an aggressiveness term within the model. Currently however, it seems prudent to assume that a crop is under threat from one of the most aggressive isolates currently present as natural inoculum. This is the chief reason isolate 2012_13A2-1 was used to parametrize the temperature-biomass accumulation model. Although genotypes which appear to be more aggressive have since emerged (Ritchie *et al.*, 2018a), this post-dates the experimentation detailed within this study.

Several other factors may also modify the rate at which *P. infestans* colonizes potato leaf tissue, such as the density of the challenging inoculum (Lapwood and McKee, 1966) and the host cultivar (Bengtsson et al., 2014; Carlisle et al., 2002; Young et al., 2018). A susceptible cultivar (foliar resistance rating 3) was used in the temperature bioassay because the majority of potato varieties of commercial importance in Great Britain have low foliar resistance to late blight (foliar resistance ratings of 2 to 5, see Chapter 5, Section 5.1, page 145). The impact cultivar resistance has on tissue colonization will be explored in a subsequent chapter (Chapter 5), but the experiments included in this project will not establish the presence or absence of a temperature · cultivar effect. This remains an open and interesting research question. A relatively high inoculum density was used in the temperature bioassay (~ 2,000 sporangia per inoculation site). Biomass accumulation rates may be slower if the initial number of spores is low (Kroll and Eide, 1981). However, it is possible that inter-spore competition at the inoculation site could also lower the measured growth rate (Shakya *et al.*, 2015; Staves and Knell, 2010). In future studies it would be useful to establish growth curves for different inoculum concentrations. This could potentially lead to the inclusion of an 'inoculum density' term in rate-development models. In practice, local spore traps could be used to estimate this density, but further (challenging) work would be needed to establish the

relationship between sporangial numbers detected in the traps with spore density at the leaf surface.

Chapter 4 Determining the effect of pathogen development on the level of curative control

Abstract

Fungicides with a curative effect are used extensively in controlling potato late blight, and although they are chiefly applied as protectants, curative activity is recognized as an important part of late blight control programmes. It is well documented that curative control declines as *P. infestans* infections develop (i.e. as the amount of pathogen biomass present in host tissue increases). However, most published data to date has assessed this decline with large (usually 24 hour) time intervals. A series of bioassays were conducted using detached leaves, as well as a novel leaf-disc method, to assess the decline in curative control over time for two *P. infestans* isolates. Staggered inoculation and curative treatment times were used to achieve a range of disease development times from 8 to 72 hours. A field trial was conducted to establish if a similar relationship could be found in the field. A representative curative fungicide formulation (propamocarb + fluopicolide) was used in all experiments, as well as a susceptible cultivar (King Edward). Thermal time was calculated in all experiments, and used as the explanatory variable in subsequent analysis. The nature of the relationship was deemed to be analogous to a dose-response curve, and a classic logistic model was fitted to the leaf disc data, as this was the most detailed set of measurements. This relationship which uses expected *P. infestans* development after a given thermal time value (determined previously in Chapter 3) as an input and provides a probability of curative control as an output, was used as the central component of the prototype decision, details of which are provided in Chapter 6.

4.1 Introduction

Many of the fungicide formulations which are routinely used for control of the late blight causal pathogen *P. infestans* within potato cropping systems have curative properties. A fungicide which acts curatively is capable (under some circumstances) of arresting infections which have occurred, but that have not yet progressed to the point where visible symptoms are apparent (van den Bosch *et al.*, 2014). Although a strategy which relies solely on curative treatments is inadvisable (Beckerman *et al.*, 2015; Keinath and DuBose, 2004), the curative properties of some systemic fungicides can be an important component of disease control programmes (Nielsen *et al.*, 2014). In practice, a grower may know when conditions of high risk for infection have occurred or are predicted to occur (Olanya *et al.*, 2012), or if there are potential sources of inoculum in the local area (Bradshaw *et al.*, 2004), but they will not know with precise certainty when infection will occur (Rossing *et al.*, 2006). Relatedly, fungicide treatment is often disrupted by blight high-risk weather conditions (Dorn *et al.*, 2007), so that in practice it may not be possible to apply protectant treatments at an optimal time. In these situations, early infections may be adequately controlled by a fungicide with curative properties (Kessel *et al.*, 2004); provided that application is timely. Additionally, there are some situations where curative fungicides can lessen the severity of epidemics (Fry *et al.*, 1979), as infections at various different stages will be present at the time of application. It is also worth noting that some curative fungicides can have an impact short of full eradication (though some may still refer to this as 'eradicant effects', see (Hodgson, 1963)) on plant pathogens even once symptoms are present: slowing disease progress (Klopping and Delp, 1980), and in many cases acting as anti-sporulants (Genet *et al.*, 2001; Hu *et al.*, 2007; Töfoli *et al.*, 2012).

4.2 Curative activity and pathogen development

Curative control of *P. infestans* is by definition time-limited (i.e. it occurs within the incubation period). There is therefore a critical time period (or 'window of opportunity' (Xu and Butt, 1996)) for curative control, which seems to be appreciably shorter than the full incubation period (Bødker and Nielsen, 2000), and

which varies between the different fungicide a.i.s (Johnson *et al.*, 2000). Much of the advice on late blight management aimed at end users gives a window for curative action of ~ 24 hours. However, any decline in curative control is a function of pathogen development rather than simply time elapsed from infection, and it is therefore more appropriate to consider the curative opportunity window in terms of pathogen development rather than time.

There are data on the curative properties of some of the fungicide formulations used for management of late blight in both potato and tomato in the literature, although they do not for the most part include enough information on which to base a decision aid for the use of curative fungicides. Studies which are know to us at time of writing are listed, along with key features such as a.i.s used and application times in Appendix 1. All of the reviewed studies reported the incubation conditions for the curative experiments. The vast majority used controlled conditions with temperatures in the region 18 – 22 °C. This corresponds to the optimum temperature for development of *P. infestans in planta* reported by this study (see Chapter 3, Section 3.4.3, page 75) and previous ones (Belkhiter *et al.*, 2017; Chapman, 2012). From this we can cautiously infer that rates of development were relatively rapid, and perhaps represent a lower estimate for the critical curative time window. The influence of temperature on this critical period has been demonstrated in controlled conditions. Genet *et al.* (2001) obtained significantly better control with cymoxanil at 17 °C than 20 °C when treatments were applied curatively to infected leaf discs after 36 hours. Similar results were obtained using whole plants, for both a cymoxanil, and a propamocarb + chlorothalonil formulation. These observations, as well as evidence from field trials, illustrate that temperature is an important modifying factor for curative control of *P. infestans*.

4.3 Characterisation of curative opportunity window

Information currently available provides a useful guide to identify in general situations where curative action is likely to occur. However, the time intervals used in the experiments that generated the information have generally been large, often 24 or 12 hours, and data were obtained under conditions that were optimal for the pathogen. An improved characterisation of the relationship between *P. infestans* development and response to curative treatment would be very useful for IPM programmes and decision support systems (DSSs). This chapter aims to provide this description, based on empirical data from bioassays and field trials. The most appropriate methodology to use was not apparent at the outset of the investigation, and the following sections follow the progression of experimentation towards an assay which was judged to yield data usable for a decision aid. It was important than any relationship between disease development and curative control uncovered under controlled conditions could also be observed in the field, and a small field trial was also conducted to test the hypothesis that such a relationship could be found.

4.3.1 Fungicide selection

The screening of a large number of curative fungicides for efficacy was not an objective of this project. Although cymoxanil is the most widely used commercial a.i. with curative properties (Monie *et al.*, 2016), preliminary experiments indicated that it was not the best choice for the planned investigations. The formulation Infinito (propamocarb-HCl + fluopicolide; Bayer) was selected for the majority of experiments as (i) it is assigned a 'good effect' (●●) rating in the EuroBlight table, representing a middle ranking for curative activity, and (ii) previous studies identified a fall in curative efficacy for this fungicide between 24 and 48 hours under optimal conditions. Unless otherwise stated, fungicides were applied at the full label rate (for propamocarb-HCl + fluopicolide as Infinito, Bayer, suspension concentrate , 62.5 g l^{-1} propamocarb-Hcl + 62.5 g l^{-1} fluopicolide, this was 1.6 l ha^{-1} in 200 l H_2O) to ensure consistency with typical crop protection practice.

4.3.2 Detached leaf bioassays: methods and materials

It is common for the curative activity of a.i.s to be described as a binary variable, i.e. there is a critical threshold after which curative control is not achieved. It is unlikely that agronomists mean this literally when the curative effect is described as having a duration of ~24 hours, but rather that there is a gradual decline in curative control with increasing disease development time, and they have implicit control thresholds in mind. The rate or abruptness of the decline in control is therefore very important, particularly if modifying factors are also considered. Detached-leaf bioassays were conducted between autumn 2015 and summer 2016 to assess the decline in control with increased late blight subclinical lesion development. For each experimental run, potato plants (cv. King Edward) were cultivated as described in Chapter 2, Section 2.2 (page 51) for 6 – 7 weeks. Immediately preceding the experiment small plastic loop-lock labels were affixed to leaves on all stems; the leaf position from stem base and a code designating individual plants were recorded on these labels. Plants were assigned randomly to one of three groups: (a) *P. infestans* morning inoculation, (b) *P. infestans* evening inoculation and (c) mock inoculation (H_2O). This was necessary to avoid cross-contamination due to the inoculation method, and to facilitate the sampling method which is explained below.

A *P. infestans* sporangial suspension from single-isolate cultures was prepared for each experimental run (1st run: isolate 2012_13A2-2, 2nd run: isolate 2012_13A2-1, and 3rd run: isolate 2012_8A1-1) as described in Chapter 2, Section 2.4 (page 52). Sporangia were obtained by washing lesions on detached leaves (cv. King Edward) with sterile distilled water, and each inoculum batch was adjusted to a concentration of 10^5 sporangia ml^{-1} and stored at room temperature (< 1 hour) before use. Two *P. infestans* inoculations took place during each experimental run: a morning inoculation (a) and an evening inoculation (b) at (a + 12 hours). A third mock-inoculation (c) with sterile distilled water took place between these two timings. Inoculations were made with leaves attached to plants. Single 20 µl droplets were placed on the adaxial surface of the terminal leaflet, and two adjacent lateral leaflets on the designated plants, with main veins avoided. Healthy leaves

from between positions 5 to 16 were selected for inoculation. Once all assigned leaves had been inoculated, whole plants were enclosed within large transparent polyethylene bags, sealed around the base of the pot with string. These were in turn placed within a growth room set at a constant 20 °C. After 24 hours the polyethylene bags were removed.

The experiment was designed to generate a range of disease development times, which could be arranged chronologically with 3-hour intervals between 24 and 72 hours post inoculation. It was not possible to run all timings in series, therefore there were two parallel inoculation times (a) and (b) which resulted in the following fungicide treatment times (F) across 3 days: F_1 (a) 24 hours ; F_2 (a) 27 hours; F_3 (a) 30 hours; F_4 (a) 33 hours; F_5 (a) 48 hours and (b) 36 hours; F_6 (a) 51 hours and (b) 39 hours; F_7 (a) 54 hours and (b) 42 hours; F_8 (a) 57 hours and (b) 45 hours; F_9 (a) 72 hours and (b) 60 hours; F_{10} (b) 63 hours; F_{11}, (b) 66 hours; F_{12}, (b) 69 hours. Thus, four fungicide treatments took place each day between the hours 08:00 and 17:00. At each fungicide treatment time 10 leaves (= 30 inoculated leaflets) were selected at random from the relevant inoculation category (a) and/or (b) and detached, as well as 10 leaves (= 30 leaflets) from the mock inoculated (c) plants. Five inoculated leaves (= 15 leaflets) and five mock-inoculated controls (= 15 leaflets) were then placed, adaxial surface upwards in a 3 · 5 arrangement with ~ 5 cm between adjacent leaves. The leaf petioles were covered with separate pieces of paper towel to avoid contact between fungicide and the cut surface. Fungicide was then applied via an AZO compressed air sprayer in a single pass, with walking speed and flow rate calibrated to apply the field dose. The fungicide was applied at 350 kPa through Lurmark F03-110 nozzles. The procedure was then repeated in a separate area with the remaining leaves, but with the treatment consisting of a water control, delivered via a CP 5 l hand compressed sprayer. Only one AZO sprayer was available, and there was insufficient time to alternate the application of fungicide and water control from a single sprayer.

After treatment, individual leaflets were detached and placed in Petri dishes lined with damp paper towels (cut to size), which were then sealed with plastic

paraffin film. The dishes were then placed within LED boxes (see Chapter 2, Section 2.5, page 53) with a 16-hour photo-period. The boxes were in turn placed within a growth room set at a constant 20 °C. At each fungicide treatment time, new dishes were distributed amongst the LED boxes so that position within each box was randomized. For each experimental run temperature was recorded between inoculation and fungicide treatment using two iButtons (see Chapter 2, Section 2.6, page 54), placed in shaded plastic tubs amongst the foliage of the inoculated plants. After 7 days, leaflets were removed from the light chambers and their symptoms assessed visually using Table 2.2 (Chapter 2, page 55). The size of any lesion present was also determined by taking a digital image of the leaflet as described in Chapter 2, Section 2.7, page 55. Diseased area was then quantified using the `polygon` function in ImageJ, as in Section 3.4.1 (page 70).

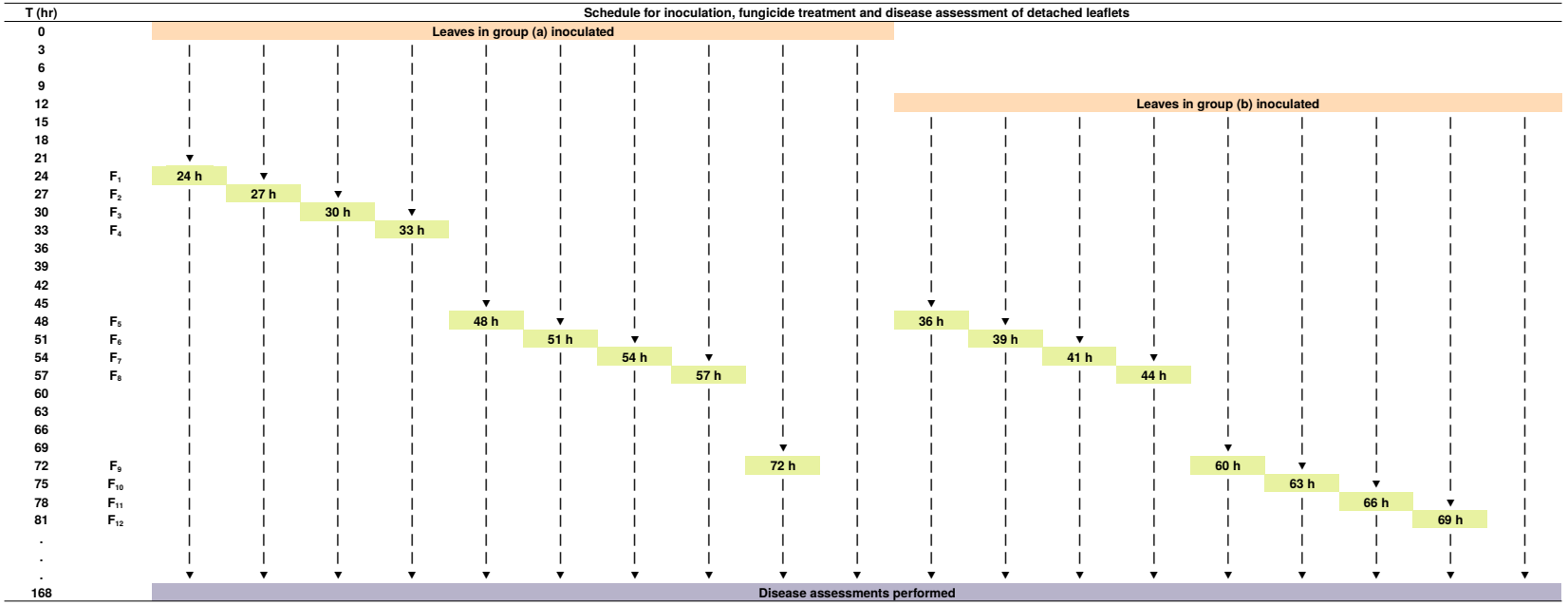

Figure 4.1. Overview of the inoculation, fungicide treatment and disease assessment timings for the detached leaf experiment described in Section 4.3.2 (page 10). Inoculations were made with leaves attached to plants at either time (a) or (b), the left most column headed T (hr) represents the time elapsed in hours from the start of the experiment. Fungicide treatment times are denoted F_i, where i is an index of the treatment sequence number. Batches of 15 leaflets were treated curatively at the times shown in the diagram. The vertical arrows show the sequence of events for each batch of leaflets, and the inoculation categories and fungicide sprays to which they correspond. Note that the control batches were untreated, and so do not have an associated fungicide treatment time.

4.3.3 Detached leaf bioassays: data handling and statistical analysis

To establish a relationship between *P. infestans* development and disease control, symptoms were grouped into two categories: (1) curative fungicide effective, which corresponded to either no symptoms or small necrotic flecks; and (2) no curative control, which was represented by large lesions with or without sporulation. This definition follows from Miyake *et al.* (2005), who observed similar results with benthiavalicarb-isopropyl when treating *P. infestans*-infected tomato plants at 24 hours post infection. A mean lesion count for the inoculated but untreated controls was calculated for the two inoculum batches in each experimental run, and values for fungicide treatment at each time-point were scaled using the level of infection in the corresponding control (i.e. scaled lesion count = lesion count in treated observation / lesion count in control) to correct for differences in infection rates between inoculum batches.

Thermal-time values for the different intervals between inoculation and fungicide treatment were calculated using the discrete time function[14] equation 2 from Chapter 3 (Section 3.2.1, page 62), and are expressed as degree-hours. Inoculations had a duration of ~ 1 hour, and fungicide treatment of leaves one of ~ 30 minutes. When calculating the disease development time, the mid points of these durations were used. The mean of the two values from the two iButtons included in each run was used as the temperature to calculate thermal-time.

Lesion sizes were analysed using ANOVA, with treatment type as a factor and thermal time as a covariate. The null hypotheses was that lesion size was independent of thermal time, and that there were no differences in mean lesion size between treated and untreated leaflets. Inspection of residual plots indicated that

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14 $^{\circ}D_a = \sum_{i=0}^{n} \left(\frac{T_i + T_{i+1}}{2} \right) \Delta t,$

Where $^{\circ}D_a$ is the accumulated degree-time unit (in this case with units °C · hr), T is temperature (°C) as a function of time and t is chronological time (in hours). Δt is the difference in time between the upper and lower time intervals. i is the index of observations T_i is the temperature at the ith observation, and n is the number of observations.

the data required square root transformation to meet the assumptions of the analysis.

4.3.4 Detached leaf bioassays: results

In all three experiments infection was high, but not uniform, in the inoculated but untreated leaves (mean values: 2012_13A2-2, am inoculation = 57 %, pm inoculation = 83 %; 2012_13A2-1, am = 93 %, pm = 69 %; 2012_8A1-1, am = 64 %, pm = 87 %). As these values were < 100% this resulted in scaled values for treated leaves sometimes exceeding 1.0. No lesions developed on any of the mock-inoculated (H_2O) leaves. Accumulated thermal time values did not deviate more than 6 % from those expected, leading to roughly comparable treatment intervals across the three experimental runs.

Relationships between thermal-time and the number of leaflets in a sample which went on to develop lesions are shown in Figure 4.2 (page 115). In general the count of leaves with lesions increased with thermal time. For isolates 2012_13A2-2 and 2012_8A1-1 early thermal time points (465 – 645 °D_a) showed a low amount of lesion development (0.0 – 0.2), but for points greater than 700 °D_a the scaled number of infected leaflets ranged from between 0.4 – 1.1 for isolate 2012_13A2-2, and 0.6 – 1.2 for isolate 2012_8A1-1. In contrast the run which included isolate 2012_13A2-1 contained no treated leaf batches which fell below 50 % of leaves developing lesions. A statistically significant relationship between the scaled number of infected leaflets and thermal time existed for isolates 2012_13A2-1 and 2012_8A1-1. For 2012_13A2-1 a quadratic equation ($LI = 0.63 + 0.34\ °D_a - 0.57\ °D_a^2$, $p < 0.01$, where LI is the scaled number of infected leaflets, and °D_a is the accumulated thermal time with units °C · hr) was the better fit than a simple linear relationship (adj-R^2 of 0.55 compared with 0.41 for the simple linear equation). For isolate 2012_8A-1 there was little difference in fit between quadratic and simple linear functions (the adj-R^2 was 0.70 for a quadratic function with the formula $LI = 0.68 + 1.33\ °D_a - 0.43\ °D_a^2$, and 0.76 for a linear equation with the formula $LI = \beta_{li1}\ °D_a + \beta_{li0}$, where β_{li1} is the slope coefficient with units $°C^{-1} \cdot hr^{-1}$, and β_{li0} is the intercept parameter with the same

units as LI). For isolate 2012_13A2-2 there was no statistically significant relationship between infection and time, either from fitting a linear equation ($p = 0.12$) or a quadratic one ($p = 0.22$).

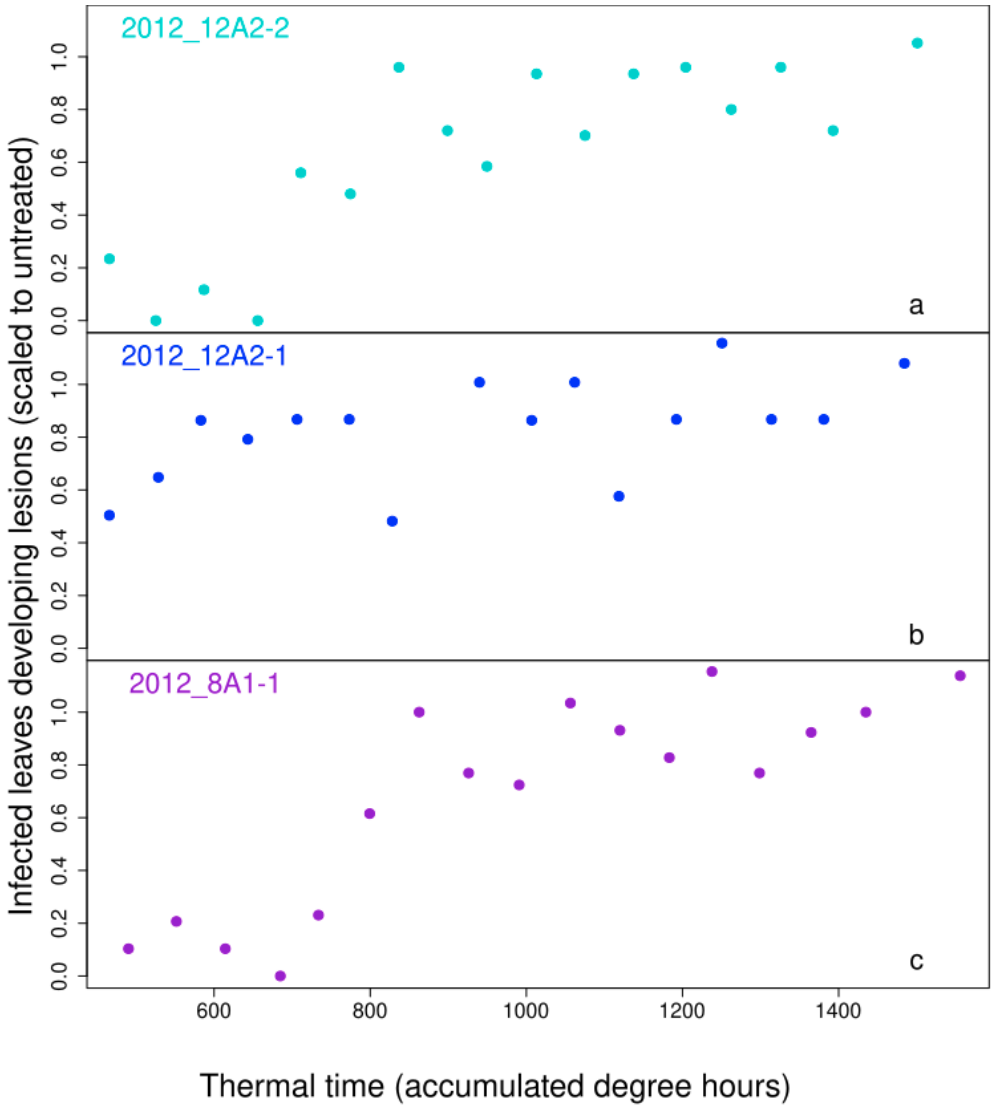

Figure 4.2. Scaled count of detached leaflets (n = 15, scaling is to untreated) which developed active lesions after point inoculation with *P. infestans* and subsequent curative treatment with propamocarb-HCl + fluopicolide at a range of disease development times between 24 and 72 hours. Disease development time (interval between inoculation and treatment) is expressed as thermal time (accumulated degree hours). Different isolates were included in each experimental run, which are displayed in separate panels: (a) 2012_13A2-2 (light blue points), (b) 2012_13A2-1 (blue points), and (c) 2012_8A1-1 (purple points).

When lesion areas were analysed, in all experimental runs the mean square root of diseased area was significantly different for untreated leaflets than for those that had received a fungicide treatment (2012_13A2-2: $p < 0.01$, 2012_13A2-1: $p < 0.01$, and 2012_8A1-1: $p < 0.01$), and lesions were larger in the untreated leaflets (data not shown). Thermal time was a significant covariate for 2012_13A2-2 ($p = 0.04$) and 2012_8A1-1 ($p < 0.01$), but not for 2012_13A2-1 ($p = 0.25$). The interaction between thermal time and treatment was significant for all three isolates: 2012_13A2-2 ($p < 0.01$), 2012_13A2-1 ($p < 0.01$), and 2012_8A1-1 ($p = 0.01$). For treated leaflets, lesion size increased with higher accumulated thermal time values for fungicide-treated leaflets (data not shown). Although there may have been differences between inoculum batches, these were not included as a factor in the ANOVA, as the treatment structure was not orthogonal.

4.3.5 Leaf disc bioassay: methods and materials

The data produced by the detached leaf bioassay were judged insufficiently robust for use in the final decision aid. It appeared that the experimental design did not in all cases cover the critical period for curative control, and the number of inoculation sites per sampling time ($n = 15$) was too low considering the variability in infection and lesion development. To address these issues, an alternative methodology using cut leaf-discs was used; this allowed for an increased number of inoculation sites, both in total and per sampling time, traded-off against similarity to a field situation. With the new system curative activity was tested from 8 hours after inoculation rather than from the 24 hours from inoculation used in the detached leaf methodology.

Acrylic tiles (170 mm · 170 mm Perspex frame) were obtained from a commercial supplier, and 64 holes (12 mm diameter) were drilled into each in an 8 · 8 equidistant pattern (Figure. 4.3, page 117). A second, intact, tile was then affixed to the base in order to form a frame into which cut leaf discs could be loaded. All frames were washed and surface sterilized with ethanol before use. On the day preceding an experimental run, leaf discs of corresponding size to the spaces in the frame were cut using a sterilised cork borer from 6 to 7 week-old *S. tuberosum*

leaves, which had been cultivated as described in Chapter 2, Section 2.2 (page 51). The discs were then loaded into the acrylic frames. Disc edges were covered using horizontally and vertically arranged strips of paraffin film, which left a central area ≈ 100 mm^2 of each disc exposed. Frames were wrapped in new plastic film to prevent dessication and stored overnight at 4 °C in darkness.

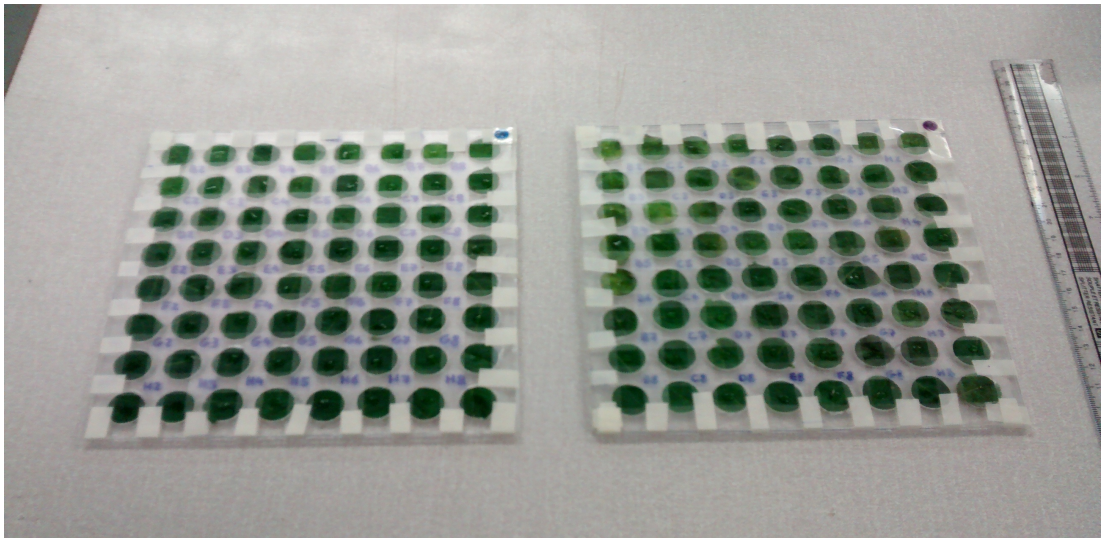

Figure 4.3. Potato leaf discs in 64-hole inoculation frames. Each frame was constructed from acrylic tiles. The drilled holes were 12 mm in diameter and held cut leaf discs in place during fungicide treatment. To prevent fungicide a.i. uptake at cut edges, parafilm strips were used to cover disc edged; tape which secures the film in place can be seen at the edges of the frames in the above image. Once each frame was fully constructed and loaded, each potato leaf disc had an approximate area of 100 mm^2 left exposed to fungicide treatment.

Experiments were conducted between winter 2016 and autumn 2017. A time sequence similar to that described in Section 4.3.2 (page 110) was obtained through staggered inoculations and fungicide treatments. A number of modifications were made to the method used in the detached leaf bioassay: time steps were spaced at 4 hour intervals, and the treatment timings were scheduled to yield a range from 8 – 72 hours. A total of 23 frames were used in each experimental run: ten were inoculated in the morning (08:00, referred to as the (a) inoculation) and eleven were inoculated in the evening of the same day (20:00, i.e. am + 12 hours, referred to as the (b) inoculation). Each leaf disc in a frame was inoculated with a 20 µl droplet of *P. infestans* sporangial suspension (10^5 sporangia ml^{-1}) derived from a single

isolate. One frame per inoculation timing was set aside as an untreated control, and two frames per timing for *P. infestans* biomass estimation. An additional fully loaded frame was mock-inoculated with sterile distilled water on the same day, as a control. On all trays, the inoculation category was recorded on the base. Inoculated frames were sealed within plastic trays (two per tray, randomly allocated) lined with damp paper towels to ensure high relative humidity. The trays were then shaded using paper sheets, enclosed within transparent polyethylene bags and placed within a single growth chamber, with the position of the trays randomized.

The growth chamber was programmed to maintain a constant 18 °C with illumination at a 16 hour photoperiod. This was scheduled so that the first 8 hours of incubation were in darkness. At the relevant timings frames selected randomly from the appropriate inoculation group were removed from trays and treated curatively with fungicide as described in Section 4.3.2 (page 110). The following treatment timings were used: F_1 (a) 8 hours, F_2 (b) 12 hours and (a) 24 hours, F_3 (b) 16 hours and (a) 28 hours, F_4 (b) 20 hours & (a) 32 hours, F_5 (b) 36 hours and (a) 48 hours, F_6 (b) 40 hours and (a) 52 hours, F_7 (b) 44 hours and (a) 56 hours, F_8 (b) 60 hours and (a) 72 hours, F_9 (b) 64 hours, and finally F_{10} (b) 68 hours. Treated frames were then placed within fresh, damp paper towel lined trays to avoid any vapour-phase fungicide activity influencing untreated frames; these were sealed as above and returned to the growth chamber. Temperature was recorded throughout each experimental run using two iButtons (see Chapter 2, Section 2.6, page 54), placed in randomly selected trays. In addition to the symptom measurement some leaf discs were sampled for pre-symptomatic biomass estimation. At each fungicide timing six leaf-discs were randomly selected from the untreated frame assigned to biomass estimation. These were placed individually within micro-centrifuge tubes (2 ml), frozen and stored at −20 °C.

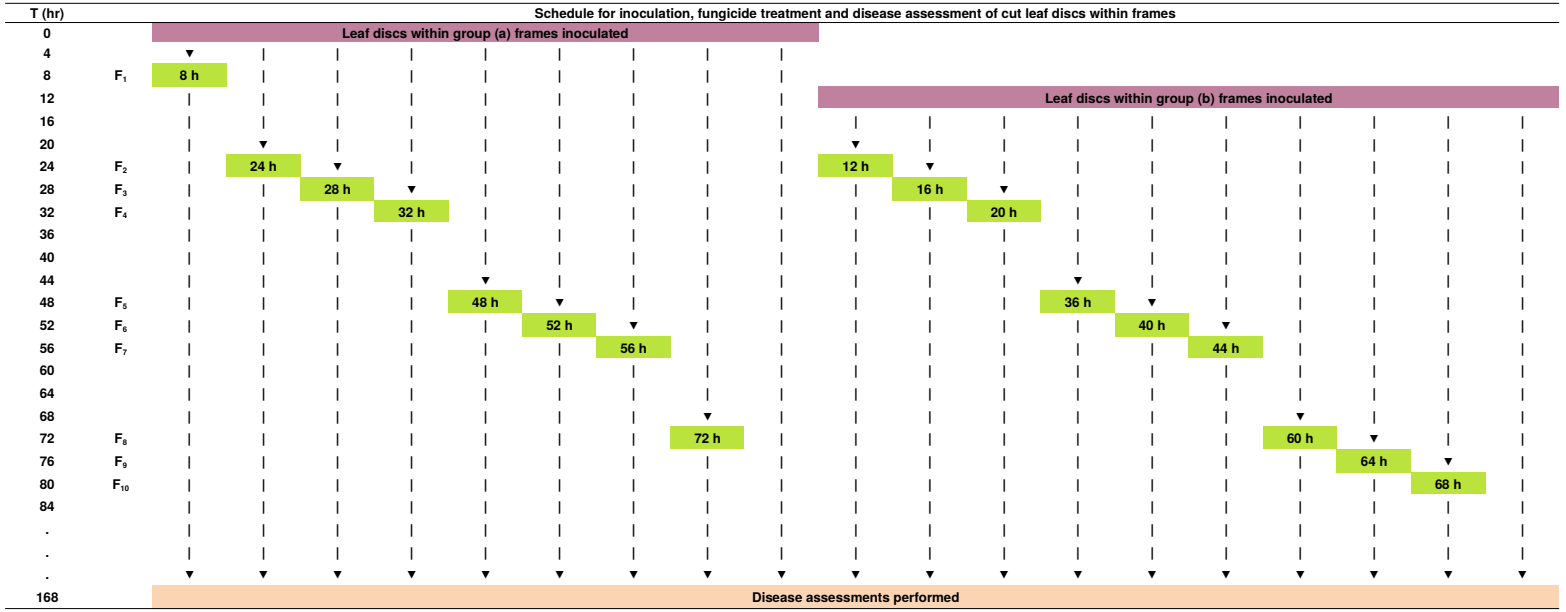

Figure 4.4. Overview of the inoculation, fungicide treatment and disease assessment timings for the cut leaf disc leaf experiment described in Section 4.3.5 (page 116). Inoculations were made on to cut leaf discs at either time (a) or (b). The left most column headed T (hr) represents the time elapsed in hours from the start of the experiment. Fungicide treatment times are denoted F_i, where i is an index of the treatment sequence number. Each frame consisted of 64 inoculated leaf discs that were treated curatively at the times shown in the diagram. The vertical arrows show the sequence of events for each frame, and the inoculation categories and fungicide sprays to which they correspond. Note that the control frames were untreated, and so do not have an associated fungicide treatment time.

After 7 days, all frames were removed from their incubation trays and symptoms of late blight were quantified and recorded. Discs which were necrotic or which showed signs of sporulation were classified as unsuccessful control, while those which showed only healthy tissue or which displayed only small arrested lesions were classified as successful curative activity (i.e. the lesion is very unlikely progress further and produce viable sporangia). Six successful runs of the experiment were conducted, three using isolate 2012_13_A2-1, and three using isolate 2012_7A1-1. These two isolates were selected as representatives of extremes in aggressiveness from the available clones (more and less aggressive respectively).

Leaf discs which were sampled from the frames assigned to biomass estimation were analysed using the qPCR assay described in Chapter 2, Section 2.8 (page 55) and in Chapter 3, Section 3.4.1 (page 73). This provided a quantification of pathogen DNA present within leaf discs which had been incubated for time durations corresponding to the curative fungicide treatment times. Total genomic DNA was extracted from frozen discs, and analysed as described in Chapter 2, Section 2.8 (page 55). As in Chapter 3 (page 73), DNA standards were matched to the isolate used within each experimental run.

4.3.6 Leaf disc bioassay: data handling and statistical analysis

To facilitate comparisons between runs and also inoculum batches, the discs in each frame where infections were deemed uncontrolled were expressed as a scaled count of the untreated control frames for the corresponding batch. Temperature data were used to calculate thermal-time as described in Section 4.3.3 (page 113). Data from runs using the same isolate were aggregated, and the infected lesion-disc counts were arcsine-transformed and regressed against thermal-time sums. The arcsine function is undefined for values ≥ 1, and two values (2012_13A2-1, 2nd run, 800 and 1314 $^{o}D_{a,}$) exceeded 1 due to the correction procedure described above, so the data were renormalized using min-max scaling (Patro and Sahu, 2015). To test for differences between isolates, ANOVA was used with the arcsine-transformed data, with infected lesion-disc count as a covariate and isolate as a factor.

Estimates of the quantity of *P. infestans* DNA within the biomass samples were plotted against accumulated thermal time (see Figure 4.6, page 124). Runs with the same isolate were combined, but because of time constraints only samples from the 2nd and 3rd runs with each isolate were quantified. To assess if the relationship was exponential, *P. infestans* DNA quantity was natural log transformed and regressed against the calculated accumulated thermal time for the combined inoculations for the corresponding experimental run.

4.3.7 Leaf disc bioassay: results

The frames containing discs that were inoculated but untreated generally had a high number of leaf discs developing lesions (2012_13A2-1: run 1 (a) 97 %, (b) 100 %; run 2 (a) 84 %, (b) 44 %; run 3 (a) 95 %, (b) 100 %. 2012_7A1-1: 100 % all runs). Calculated thermal-time sums did not deviate more than 5 % from those expected, making sample times closely comparable across experimental runs. The counts of infected discs (scaled to the inoculated but untreated frames) plotted against thermal-time can be found in Figure 4.5 (page 122). For both isolates the efficacy of curative treatment was reduced with increasing disease development thermal-time. For isolate 2012_7A1-1, the scaled count of infected leaf discs did not exceed 0.3 until thermal-time sums were > 600 $°D_a$, and were in the region of 0.4 – 1.0 for thermal-sums exceeding 1000 $°D_a$. In contrast, for isolate 2012_13A2-1 the scaled count of infected leaf discs ranged from 0 – 0.6 for thermal-time sums under 600 $°D_a$ and, aside from two outliers , rarely fell below 0.6 at thermal sums greater than 600 $°D_a$. The relatively low infection rate (44 %) in the inoculated, but untreated discs inoculated with isolate 2012_13A2-1 in run 2 inoculation time (b), cannot be readily explained. However, as all frames in this category received the same inoculum, and as the results were scaled, they were included in the final analysis.

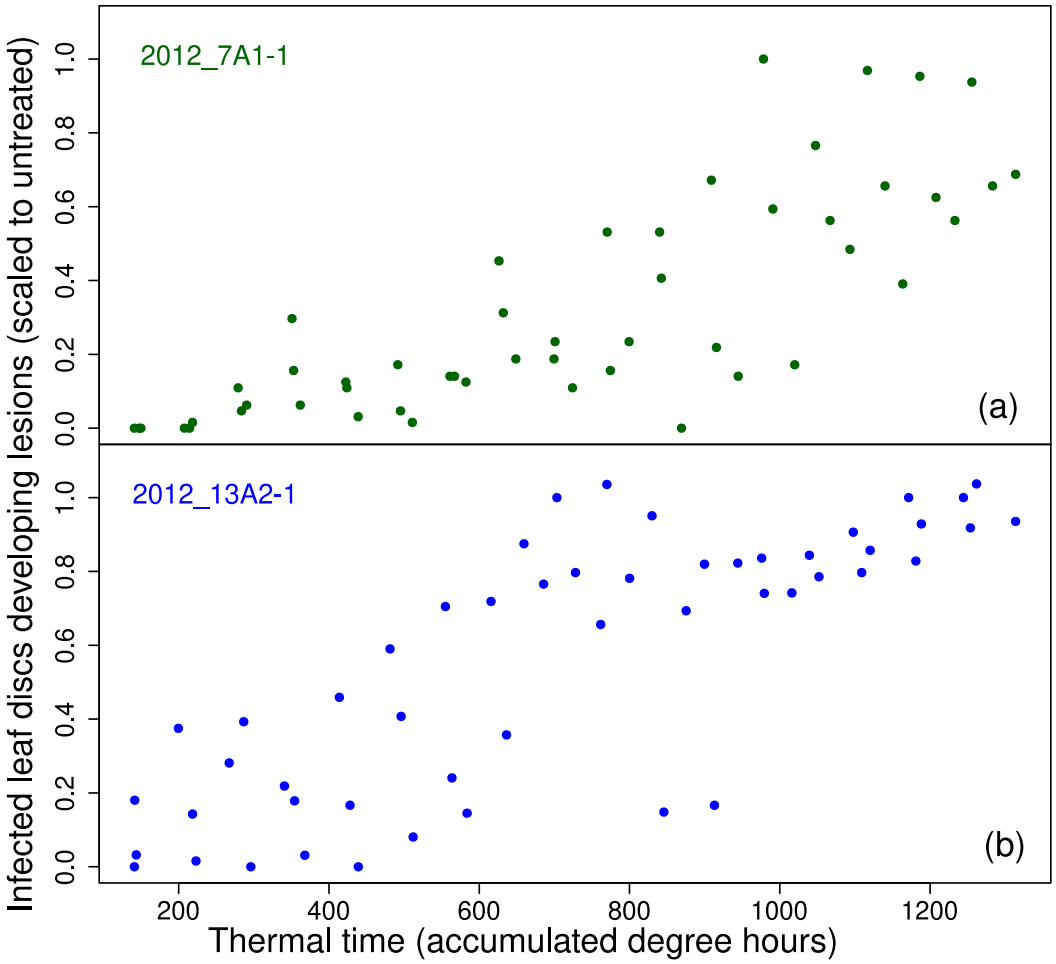

Figure 4.5. Scaled count of inoculated leaf discs displaying late blight symptoms (n = 64) in relation to curative fungicide (propamocarb-HCl + fluopicolide) treatment at different pathogen development timings. The counts of infected leaf discs are expressed in relation to untreated controls. Six experimental runs are shown, with three runs each using the same isolate in each panel: (a) isolate 2012_7A1-1 (●), and (b) isolate 2012_13A2-1 (●). Thermal time is a composite term calculated from the incubation temperature and the time between inoculation and treatment with the units °C · hr.

There was a significant linear increase in the arcsine transformed (performed to meet assumptions of the analysis) scaled count of infected leaf discs with increasing thermal time for both isolates (2012_7A1-1: $p < 0.01$, $R^2 = 0.64$, $DI = 3.38 \cdot 10^{-4}\,°D_a - 8.26 \cdot 10^{-2}$; 2012_13A2-1: $p < 0.01$ $R^2 = 0.62$, $DI = 1.02 \cdot 10^{-3}\,°D_a + 0.13$. Where DI is the scaled number of infected leaf discs, and $°D_a$ is the accumulated thermal time with units °C · hr). The intercept term was non-significant in both cases (2012_7A1-1: $p = 0.28$; 2012_13A2-1: $p = 0.17$) confirming zero disease development where curative fungicide treatment and inoculation occurred at the same time. There was a significant difference between the two isolates when infected disc scaled count (arcsine transformed) was analysed using ANOVA ($p < 0.01$).

Target *P. infestans* DNA was detected in all samples which had been inoculated with sporangia, and no C_q values were obtained from water inoculated controls. After a lag phase there was an increase in *P. infestans* DNA with increasing accumulated thermal-time, with the largest increase at $°D_a$ values > 1000. The relationship was exponential for both isolates, and the DNA quantities were natural log transformed for analysis (2012_13A2-1: $p < 0.01$, $\ln(Pi_{DNA}) = 2.44 \cdot 10^{-3}\,°D_a - 4.38$; 2012_7A1-1: $p < 0.01$, $\ln(Pi_{DNA}) = 1.72 \cdot 10^{-3}\,°D_a - 5.40$. See Figure 4.6 page 124). An ANOVA was performed where amount of DNA was the response variable, isolate was a factor and thermal time was included as a covariant. The null hypothesis was that there were no differences between the amount of *P. infestans* DNA present between the two isolates. This analysis indicated that there was a significant difference ($p < 0.01$) in DNA quantity between isolate 2012_13A2-1 and 2012_7A1-1 when thermal-time was included as a covariate.

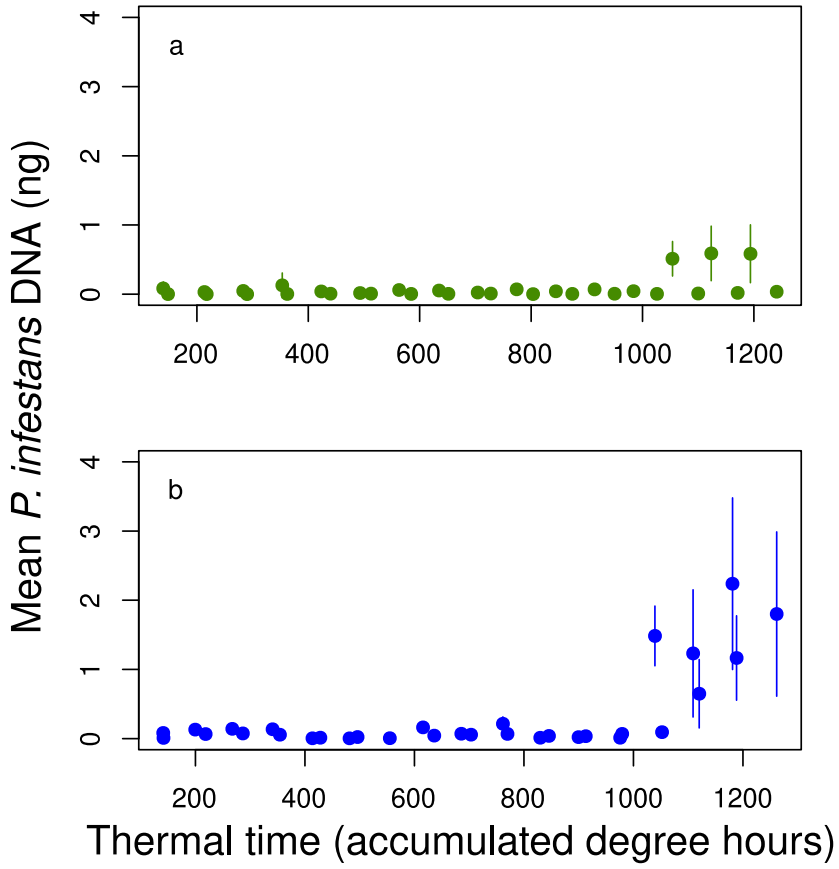

Figure 4.6. Mean (n = 6) *P. infestans* DNA template quantity (± 95% confidence intervals) present within inoculated potato leaf discs, determined via a qPCR assay, against accumulated thermal-time (°C · hr). Two experimental runs per isolate are shown: panel (a) 2012_7A1-1 (●), and (b) isolate 2012_13A2-1 (●).

4.3.8 Curative field trial: methods and materials

It can sometimes be the case that behaviours observed for plant pathogens in the laboratory differ from those observed in the field (Fry *et al.*, 2019). To address this, data were obtained for curative activity of the representative fungicide in a field trial. The field trial took place over the summer of 2016, at SRUC's Auchincruive site. Similar trials which included cultivars with different foliar resistance ratings against late blight, and trials which were used to assess the performance of the final decision aid, are detailed in Chapters 5 and 6 respectively.

The trial consisted of 28 plots (each 4.95 m long · 1.7 m wide) encompassing two ridges. Seed tubers of the late blight-susceptible variety King Edward (foliar resistance rating = 3) were hand planted, with 0.33 m spacings within rows. The treatments were designed to generate a series of disease development times broadly similar to those in the glasshouse bioassays and were allocated to plots in a randomized block design consisting of 6 blocks. Each block had four plots, to which were allocated four treatments: F_1, F_2, F_3, or an untreated control F_u. Additionally, four plots not within blocks were left uninoculated and untreated to assess the level of natural background late blight infection (F_0). Two artificial inoculation timings: (i) early morning and (ii) mid-afternoon, were applied to each plot, i.e. plants of the first ridge within a plot were inoculated at (i) and those in the second ridge at (ii). Thus there were 36 possible disease development time intervals, the product of two inoculation times, three curative treatment times and six plots per treatment time.

Plants for inoculation were selected one day before the start of the experiment. Each inoculation site consisted of two stems which could be bunched together easily and these were tagged with loop-lock labels, of separate colours for the different inoculation timings. A total of four inoculation sites (= 8 stems) per plot per timing were selected. Inoculum was prepared approximately an hour before the start of inoculations, with two separate batches, from samples of the same infected plant material, prepared for the different inoculation times.

Due to unavoidable contingencies, inoculum was prepared by washing lesions collected from untreated field-grown plants (cv. Cara). It was not possible to determine the isolate (or isolate mix) with complete certainty, but it was highly likely that a clone belonging to genotype 6_A1 was predominant (C. Kennedy, SRUC, Auchincruive, UK, personal communication). Inoculum was prepared as described in Chapter 2, Section 2.4 (page 52), and adjusted to $3.8 \cdot 10^4$ sporangia ml^{-1}. The inoculation method involved bunching the two stems together and applying ~ 2.6 ml of inoculum from a plastic spray bottle, i.e. ~ 10^5 sporangia per site. Inoculated haulm was then sealed within transparent plastic bags, with a small hole in one upper corner to moderate temperature. The bags ensured a high humidity environment to increase the probability of infection and were removed after 24 hours. Each inoculation took ~ 3 hours to complete. Plot inoculation times were recorded, together with the inoculation order of plots.

Assigned plots were treated curatively at one day (F_1), two days (F_2), and three days (F_3) from the start of inoculations, with precise spray times recorded. The treatment of plots took ~ 20 minutes for each of the timings. Plots received the curative treatment propamocarb-HCl + fluopiclode at 1.6 l ha^{-1} in a 200 litre water volume. At F_1 plots were sprayed using an AZO backpack sprayer (350 kPa spray pressure and Lurmark F03-110 nozzles), and at F_2 and F_3 a tractor-mounted sprayer (350 kPa spray pressure and Lurmark F03-110 nozzles). The different application methods reflected availability of equipment. Symptoms were assessed seven days post inoculation. Inoculated stems were identified via the loop-lock tags and a record was made of the number of late blight lesions per leaf. Leaves between the first fully unfurled leaf below the stem apex (leaf one) to leaf eight were assessed as these were the most likely to have intercepted both inoculum and fungicide spray.

4.3.9 Curative field trial: data handling and statistical analysis

Temperature data from the Met Office station at Auchincruive were used in conjunction with the recorded inoculation mid-point times and treatment times to calculate an accumulated thermal time for each plot. The average time to inoculate each plot was ~ 10 minutes. Six lesions were found in total in non-inoculated plots, indicating that natural late blight inoculum was present. However this count was an order of magnitude smaller than the number of lesions found in inoculated plots. Lesion number per compound leaf was recorded and then regressed against accumulated thermal time values to test for the existence of a relationship. Variance increased with increasing thermal time sum. A significant proportion of the compound leaves returned a lesion count of zero, therefore + 1 was added to each value prior to natural log transformation.

4.3.10 Curative field trial: results

The relationship between increasing accumulated thermal time and the mean lesion count per compound leaf can be found in Figure 4.7 (page 128). Lesions were found at all inoculation sites indicating that complete control was not achieved, even at lower accumulated degree hours. In inoculated but untreated plots, the mean lesion count per leaf was 2.59 ($\pm$ 0.32, 95% c.i.) for the morning inoculation and 2.57 ($\pm$ 0.34, 95% c.i.) for the evening inoculation. A t-test on natural log + 1 transformed values (see previous section) indicated no significant difference between the two inoculum batches ($p = 0.53$). There was a statistically significant exponential relationship between mean lesion count and thermal time ($p < 0.01$, $\ln(LC + 1) = 1.06 \cdot 10^{-3} + 0.06$, where LC is the lesion count).

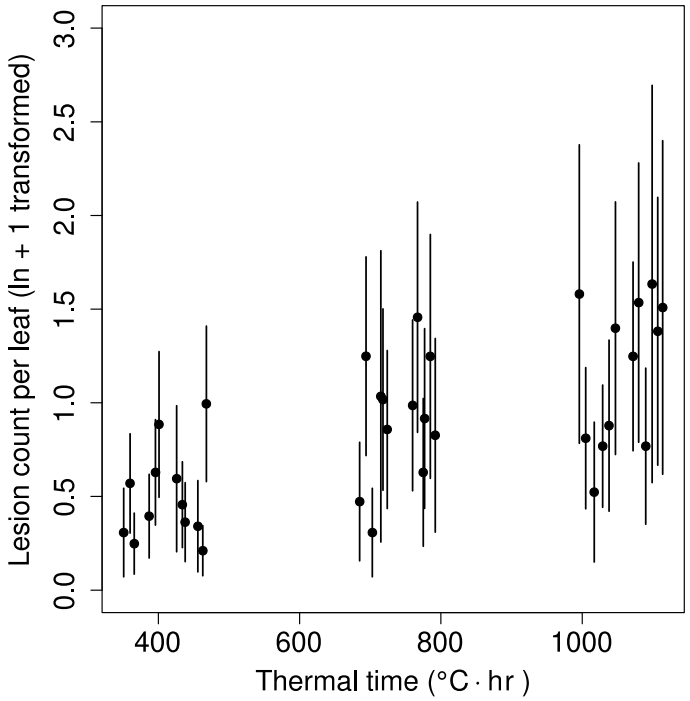

Figure 4.7. Natural log + 1 transformed lesion count per compound leaf with increasing thermal time between inoculation and curative treatment (propamocarb-HCl + fluopiclode). Each point represents the mean from four inoculation sites (= 64 leaves), and lines represent the 95 % confidence intervals. Data are from a field experiment (see Section 4.3.8, page 125); Thermal time was calculated for the interval from inoculation to time of treatment and has units °C · hr.

4.4 Relationship between curative control and pathogen development

The results from experiments reported in this chapter have been presented in terms of thermal-time in order to account for the effect of temperature on pathogen development, and to allow comparisons to be drawn across experiments. However, the final decision aid will use a developmental rate function which was set out in the previous chapter (Chapter 3). It was not appropriate to use this model for all the data-sets presented above, as it was parametrized only for isolate 2012_13A2-1. This isolate generated the shortest curative window in both detached leaf (Section 4.3.4, page 115) and leaf disc assays (Section 4.3.7, page 122), and can be used to represent a 'worst case' scenario for the duration of any curative effect.

The leaf disc assay provided a description of the relationship between disease control and thermal-time that could, as a rough overview be divided into three phases: good control, declining control, and little curative control. The situation is analogous to studies which determine EC_{50} or ED_{50} values from fitting control relationships to dose-response datasets (Ritz *et al.*, 2015), however in this case control falls with increasing biomass accumulation rather than with decreasing compound concentration. Dose-response relationships are commonly explored in toxicology (Clewell and Andersen, 2016; Cox, 1987), weed science (Kim *et al.*, 2002; Knezevic *et al.*, 2007), and in plant pathology (van Bosch *et al.*, 2011; Cohen *et al.*, 1991).

Within the leaf disc bioassay both temperature and time elapsed were monitored, and the results are displayed with thermal-time as the explanatory variable. In Chapter 3 (Section 3.6, page 96) a model for conversion of thermal-time to expected development was specified, and it is hypothesised that curative control declines with pathogen development (rather than thermal time *per se*). With this in mind, expected development given the temperature and time data was calculated using equation 27-A, which is reproduced below:

$$f(T) = \begin{cases} 0 & T < 0.45 \\ 2.21 \cdot 10^{-5}(T - 0.45)^2(30.51 - T) & 0.45 \leq T \leq 30.51 \\ 0 & T > 30.51 \end{cases} \quad \text{(27-A)}$$

where f(T) is a rate function of the observed temperature T (°C), with the units ln(pg) hr^{-1}. Expected development was calculated hourly, and this was summed for the disease development time between inoculation and treatment of each leaf disc to produce a value for expected accumulated development. It was desirable to use expected development as an explanatory variable because given eq. 27-A expected development could be calculated from any suitable set of temperature observations. Note that although pathogen biomass was quantified with the leaf disc assay (and was demonstrated to increase), these results are not directly comparable with

temperature-development model specified in Chapter 3 (Section 3.6, page 102) as DNA was extracted from complete leaflets rather than cut leaf dics.

The are many potential models which are used to describe dose-response relationships, and these can become quite complex, for example involving switching functions if hormetic[15] effects are anticipated (Schabenberger and Pierce, 2002). Many of the functions generate sigmoid curves, but there are several such functions to choose from, a useful review of some of these is given by Ritz (2010) who recommends using a model with as few parameters as possible to improve parameter estimate and ease the fitting process. The classical logistic function is one of the simpler models used when fitting dose-response curves, and is defined as (Oliver, 1964):

$$f(x) = \frac{L}{1 + e^{\beta_1(x - \beta_2)}}$$

where $f(x)$ is the response, x is the predictor variable, L is the upper asymptote, β_1 is a constant which is sometimes called the logistic growth rate and controls the steepness of the curve (Noel *et al.*, 2017), and β_2 is a constant with the same units as the predictor variable. When $\beta_2 = x$, the response is half of L. In many case β_2 is the most important parameter for interpretation as the dose level at which a 50 % response occurs (EC_{50}, or LC_{50} when mortality occurs). A distinction can be made between continuous dose response curves where the response variable is some direct measurement (such as the observed growth of an organism), and quantal dose-response relationships where the response is expressed as a proportion or as a percentage. The leaf disc assay is a quantal case, which if considered as a proportion, can conceptually can range between zero (full curative control) and one (no curative control). This seemed a reasonable assumption from inspection of Figure 4.8 (page 133), and the logistic function with L fixed to one was used to describe the relationship between expected development and curative control. There

15. Hormesis refers to a situation where low doses of a toxicant have a stimulating effect on a biological system.

are good biological reasons for applying a simple sigmoid curve here: pathogen biomass increases exponentially in early development (Lees *et al.*, 2012) and the level of curative control should theoretically decrease accordingly, as pathogen development increases curative control will fail completely (i.e all infections will develop into sporulating lesions, notwithstanding factors other than fungicide application which may arrest pathogen growth) and it seems likely that this level of complete (or near complete) control failure will be approached asymptotically; see for example Reis *et al.*, 2016, where curative control of *Phakopsora pachyrhizi (*the causal agent of Asian soybean rust) by chlorothalonil declines in this way with increasing disease development time. Other descriptions could have been used to represent the decline of curative control with increasing pathogen development, and this is an area other investigators may wish to revisit, but for this particular application a simple sigmoid curve seemed the most appropriate for the biological reasons given, as well as for ease of implementation.

As the data were recorded as a successful/unsuccessful infection by *P. infestans* (i.e. a binary outcome), one approach could have been to use a generalized linear model with a logistic link function (Hothorn, 2014; Skelsey *et al.*, 2018) to describe the relationship between expected development and curative control. This method was not used because the response variable (infected leaf discs) was scaled to the untreated control. Instead, as in Chapter 3 (Section 3.5.2, page 82), nonlinear least squares analysis using the R function `nlsLM` was used to estimate the parameters that best fit the data. The equation for which parameters were estimated was:

$$DI_s(d_{ac}) = \frac{1}{1 + e^{\beta_{di1}(d_{ac} - \beta_{di2})}} \qquad 28$$

where d_{ac} is the expected development with units ln(pg), $DI_s(d_{ac})$ is the count of infected leaf discs scaled to the untreated control as a function of the expected development, β_{di1} is the slope parameter with units $\ln(pg)^{-1}$, and β_{di2} is the 50 % response parameter with units ln(pg). Inspection of a plot of standardized residuals against fitted values (data not shown) indicated that the scaled number of infected

leaf discs varied more at smaller values for accumulated development than at larger values of accumulated development, and so a weighted least squares non-linear analysis was applied by calling the weights argument in the function `nlsLM`. The weightings applied were the square root of the accumulated development value: `weights = sqrt(acc)`, in R syntax (were `acc` is a vector containing the expected accumulated development values) as these seemed reasonable from the aforementioned residuals fitted values plot. Parameter estimates are given in Table 4.1 (see below), applying weights changed the parameter estimates very slightly, but the lack of homogeneity of variance was improved. In general, when dose-response relationships are determined it is the mean function which is of central interest (Ritz, 2010), and with this in mind the parameter estimates will be carried forward to the final decision aid. This does not mean that the confidence measures of the parameter estimates are of no interest, and these are presented here. The level of curative control from the leaf disc bioassay was variable, particularly at early time-points, the implications of this are discussed in Section 4.5 (page 134).

Table 4.1. Estimates of parameters for the scaled leaf disc infection after curative treatment model. The model is given in equation 28 (page 131). β_{di1} is the slope parameter with units $\ln(pg)^{-1}$, and β_{di2} is the 50 % response parameter with units $\ln(pg)$. A plot of this relationship, along with the data used in the fitting process can be found in Figure 4.8 (page 133).

Parameter	Fitted estimate	Standard Error	95% confidence interval
β_{di1}	−0.865	0.149	−1.163, −0.566
β_{di2}	2.924	0.200	2.522, 3.326

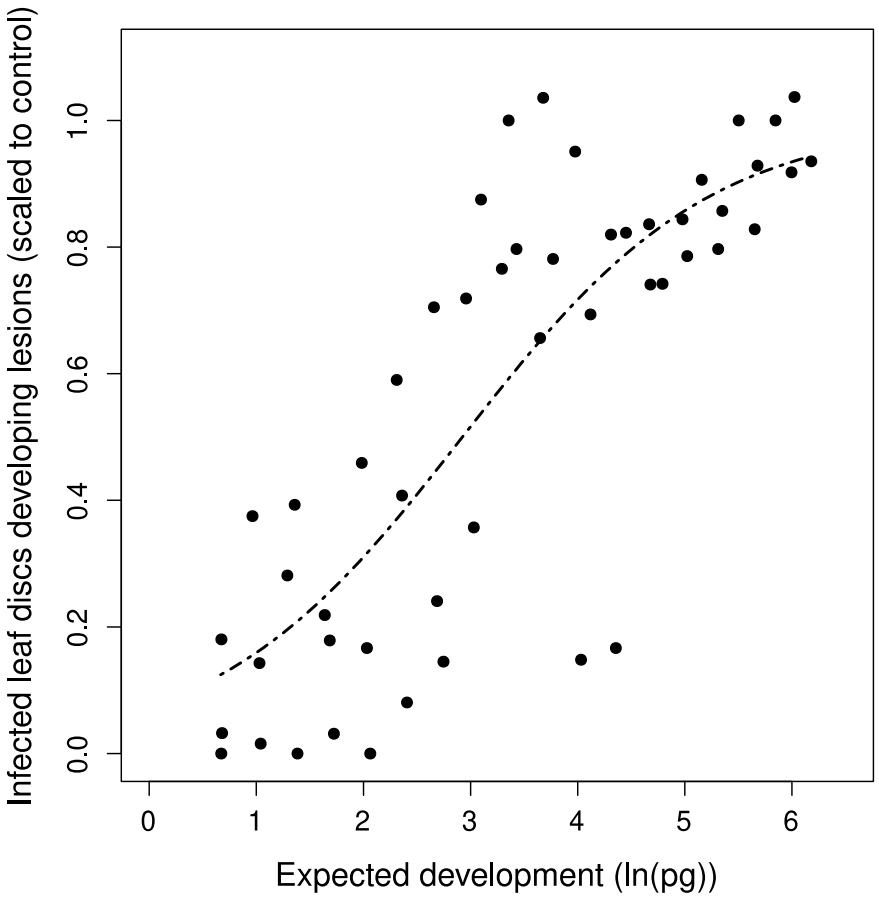

Figure 4.8. Scaled (to untreated control) count of leaf discs developing lesions after inoculation with isolate 2012_13A2-1 (see Section 4.3.5, page 116) and subsequent treatment with curative fungicide (propamocarb-HCl + fluopiclode) to generate a range of disease development times. Expected accumulated development is a function of both time and the incubation temperature and has the units ln(pg). Three runs of the same experiment are shown, incubation occurred under controlled conditions (18 °C) and fungicide treatment intervals were the same in each run, although there were slight differences in both between runs and hence expected development is not always exactly the same for the same treatment slot across runs. A logistic model (the fitted parameters of which can be found in Table 4.1, page 132

) describes the relationship and is shown as the dashed curve.

4.5 Discussion

The time frames for curative control demonstrated in this chapter were similar to those reported in other sources (Bugiani *et al.*, 2010; Genet *et al.*, 2001; Johnson *et al.*, 2000; Nielsen and Bødker, 2002; Pirondi *et al.*, 2017). Expressing control in terms of thermal-time, or as accumulated development, proved useful in interpreting results and highlights the importance of considering the influence of temperature on pathogen development when the timings of crop protection interventions are considered. The results from the three different approaches (field trial, detached leaf bioassay, and leaf disc bioassay) were broadly in agreement. Isolate 2012_13A2-1 showed similar responses to heat sums > 600 degree hours in both the detached leaf and leaf disc bioassays, which may not have been apparent if only chronological time had been considered. There were, however, several significant differences between the approaches, and between situations which may be encountered in the field. For example, fungicide coverage of infected tissue may have been better in the bioassays than in the field trial. This may lead to an overestimation of the duration of the curative window. However, this is unlikely to be a serious issue as other factors act against any error: in a commercial situation the crop will likely have received several previous treatments, providing a well protected crop, and curative fungicides' mobility will likely compensate for poor coverage within a crop canopy. (R. Bain, SRUC Auchincruive, UK personal communication). Additionally, the aggressiveness of the isolate(s) used in the field trial was not known definitively, so comparisons should be made cautiously.

The two isolates which were chosen to represent contrasting levels of aggressiveness showed different responses in the leaf disc bioassay, with the curative window shorter for the more aggressive isolate. This could have useful implications for sophisticated integrated management, e.g. genotype-specific late blight control. However, this study could not rule out the possible influence of differential isolate sensitivity to propamocarb-HCl and/or fluopicolide, and this may be a possible alternative explanation. This point notwithstanding, the biomass accumulation data for this bioassay support isolate phenotype strongly contributing

to the difference in the curative window. Different responses between isolates were also obtained in the detached leaf bioassay, and these broadly agreed with isolate aggressiveness. Interestingly, different responses were obtained from different isolates with the same multi-locus genotype designation: other studies have also reported within-lineage variation (Dey *et al.*, 2018). However, the results of the detached leaf bioassay should be interpreted with caution, as it was only possible to include each isolate in a single run. It would have been very useful to repeat these experiments with a wider range of isolates.

There are some caveats to bear in mind when considering the curative activity-expected development relationship represented by equation 28. Other studies have demonstrated the importance of developmental temperature on curative activity (Genet *et al.*, 2001), but only inferential evidence is presented here. It would be very useful to demonstrate directly the effect of temperature on the curative window, and to conduct a series of experiments to describe the relationship, and to assess the accuracy of the accumulated development model in predicting this. As time constraints did not permit this within the current study, the model should be interpreted with reference to this fact. Only a single fungicide formulation was tested here. This probably represents the mid-range response, and other a.i.s may offer longer or shorter critical windows. Finally, there was some variability in the level of curative control offered at similar time points (and therefore levels of expected development) between runs of the same experiment. The reason for this is not immediately obvious, and warrants further investigation. It is possible that curative control is inherently variable for this pathosystem; complete curative control was not always obtained even at the disease development times of shortest duration. This fact should be communicated to end-users, and re-enforces the point that curative treatment, while an important component of late blight management should in no circumstances be the central strategy within a spray programme. Alternatively, the variability could be due to the methodology used, and future experiments may produce more precise estimates of the curative control-disease development relationship.

In conclusion a scientifically acceptable model can only be generated by obtaining response curves for diverse genotypes of *P. infestans* using fungicides with different curative ratings, at different dose rates (a.i. rate is not standard across products), and with different levels of inoculum challenge. A programme of this nature was however beyond the scope of the resources available here, and the final development-response curve should be viewed as a pragmatic starting point. It should be noted that in practical terms the aim was to produce a decision aid that was sufficiently accurate to allow growers to distinguish between when the use of a curative fungicide was justifiable (economically and/or environmentally) and when it was not. Also, currently very few decision support systems for late blight control take account of *P. infestans* genotype, inoculum density or fungicide dose. However, some of these factors are included in present-day research (Andrivon *et al.*, 2017; Kessel *et al.*, 2018).

Chapter 5 Modifying effect of host resistance on fungicide curative activity

Abstract

Potato cultivars vary in the level of host resistance they display against late blight. Although it has not been investigated previously, it was hypothesised that the curative window may be extended in more resistant cultivars compared with more susceptible ones. A set of experiments was conducted using small plants of three cultivars with varying resistance levels (King Edward, foliar resistance rating 3; Cara, foliar resistance rating 5; and Sarpo Mira, foliar resistance rating 7. Low ratings indicate susceptibility, higher ratings resistance). There were exposed to natural inoculum, and subsequently treated with curative fungicides. A curative effect (measured by the number of lesions which developed after treatment) was detected at later time points in the resistant cultivars (Cara, Sarpo Mira), but not the susceptible cultivar (King Edward). An expedient method of incorporating this modifying effect into the decision aid was sought: categorical ratings for host resistance are published by AHDB potatoes, and if these ratings correspond to rates of tissue colonization, it may be possible to estimate from them the extent to which the curative effect is modified. Several experiments were conducted using detached leaves and a range of cultivars with different resistance ratings. Rates of host tissue colonization by *P. infestans* were measured using similar methods to the temperature assays in Chapter 3 (by visually scoring symptoms and by quantifying the amount of pathogen DNA using a qPCR assay). A strong relationship was found between rating and visual symptoms, but no such relationship was found for the resistance ratings and pre-symptomatic growth. This may be due to experimental factors, or may be the result of the resistance ratings capturing components of resistance unrelated to tissue colonization.

5.1 Introduction

The working hypothesis of this study is that slower rates of tissue colonization by a pathogen result in an extended window of opportunity for curative control. Logically, any factor which slows pathogen growth or spread should increase the

time period over which curatively-applied fungicides give good control (Richardson and Munnecke, 1964). Temperature, which will often be the most critical factor has been discussed in Chapter 3, and some other potentially important factors are discussed in Chapter 7. A factor which deserves specific attention is host resistance, as it is a key component of Integrated Pest Management (IPM) programmes (Elliott *et al.*, 1995). Host (or varietal) resistance refers to the propensity of cultivars with certain traits to display a lessened or absent disease phenotype (Poland *et al.*, 2009), and is a well utilised and effective method of crop protection (Oerke and Dehne, 2004). A central goal of IPM is to select crop protection interventions that can act synergistically (Barzman *et al.*, 2015), and there are a number of studies which explore the complementary use of host resistance and pesticide inputs (Landa *et al.*, 2004; Willyerd *et al.*, 2012; Wolfe, 1981); including many examples from the potato late blight pathosystem (Fry and Shtienberg, 1990; Fry *et al.*, 1983; Small *et al.*, 2015).

Fungicides and host resistance can sometimes exert a strong selective pressure on pathogen populations, and both of these control methods are therefore vulnerable: pesticides to the development of insensitivity (Judelson and Roberts, 1999), and host resistance to the emergence of resistance breaking strains (Fry, 2008). One suggested approach is to use combinations of fungicide treatments and host resistance as mutual guards against loss of efficacy (Carolan *et al.*, 2017). Other authors suggest that coordinating fungicide treatments and host resistance could be used to reduce fungicide inputs (Kessel *et al.*, 2004; Nærstad *et al.*, 2007), lessening economic and environmental burdens. There is also evidence that fungicide performance can be improved when used in conjunction with resistant cultivars (Ritchie *et al.*, 2017), for example, Ritchie *et al.* (2018b) report that many combinations of fungicide and host resistance performed better in a set of field trials than predicted when AUDPCs from field experiments were compared with those predicted by a multiplicative survival model (though the authors caution that this could be due to trial methodology).

Definitions and classification of host resistance have been discussed previously (Section 1.7, page 24). Broadly speaking, there are two forms which host resistance takes: qualitative (or complete) resistance (Thrall *et al.*, 2016), and quantitative (or partial) resistance (St.Clair, 2010). Qualitative resistance is frequently governed by the presence of major resistance genes (*R* genes) which recognize pathogen effectors and are generally monogenetic, segregating in classical Mendelian ratios (McDonald, 2010). This results in an incompatible interaction between the pathogen strain and the host (Song *et al.*, 2003). However, it is also conceivable that the sum effect of several 'minor' genes may be sufficient to block infection (Corwin and Kliebenstein, 2017). Several different *R* genes are frequently present in a host (Kim *et al.*, 2012), and they are often structurally related, sharing similar genetic sequences and protein motifs when expressed (Huang *et al.*, 2004); however one cannot always predict the target of an *R* gene from its genetic structure, as related *R* genes can sometimes have disparate target organisms (Vossen *et al.*, 2000). In contrast to qualitative resistance, plants with some degree of quantitative resistance generally display a disease phenotype when interacting with specific pathogens, but the extent of symptoms is reduced compared to other 'susceptible' varieties (Niks *et al.*, 2015). Quantitative resistance is usually thought of as polygenetic, with several 'minor genes' or QTLs contributing to a reduction in the expressed symptoms of disease (Mundt, 2014). The precise mechanisms by which this takes place have implications for this project, and are discussed below.

As a clonal crop, differences in host resistance against late blight between individual potato plants of the same variety with similar physiological age and nutritional status do not differ greatly; although different tissues within the same plants can differ, see Visker (2004). However, between cultivars there is a wide spectrum of disease-susceptibility (Kadish *et al.*, 1990). A central goal for breeders of both potato and tomato has been to produce new varieties with strong and durable resistance against late blight (Nowicki *et al.*, 2011), and this has stimulated searches for, and characterisation of resistance mechanisms. A number of closely related non-domesticated *Solanum* species have acted as useful genetic pools for

late blight resistance (Yang *et al.*, 2017), and many modern cultivars carry resistance genes which originated in these wild potato species (Jansky, 2000; Rodewald and Trognitz, 2013). Much of this resistance is directly attributable to classical *R* genes, and a helpful review is given by Rodewald and Trognitz (2013). Unfortunately, after introgression the resistance conferred by these *R* genes did not prove durable and was rapidly defeated in the field (Toxopeus, 1956).

After the failure of single *R* genes to provide durable resistance, alternative approaches have been proposed. *R* genes which have not been overcome by any know *P. infestans* strain have been characterised, but are not yet widely deployed (Chen *et al.*, 2018; Haesaert *et al.*, 2015; Roman *et al.*, 2017), and hope remains that some of these may prove more robust sources of resistance. Transgenic techniques have been used to transfer some of these genes, for example *Rpi-blb1* and *Rpi-blb2* into popular cultivars (Dixelius *et al.*, 2012), although both of theses genes have been broken by some (albeit rare) strains of the pathogen (Förch *et al.*, 2010). This approach has several advantages to classical breeding: it can be accomplished much more rapidly, resistance genes can be introduced into varieties which already possess desirable traits, and it avoids the introduction of linked but undesirable genes. However, in the current regulatory environment these techniques cannot achieve widespread use (Hou *et al.*, 2014). Current breeding efforts are focused on 'pyramiding' or 'stacking' *R* genes, i.e. introducing several known *R* genes against a late blight within a single cultivar (Haverkort *et al.*, 2009), with the rationale this will prove a more formidable barrier to pathogen adaptation (Zhu *et al.*, 2012).

In addition to major *R* genes, quantitative resistance has also been a recent focus of research interest; although it is much less well understood and more challenging to manipulate (Kou and Wang, 2010). Contemporary reviewers of quantitative resistance (Corwin and Kliebenstein, 2017; Niks *et al.*, 2015; Poland *et al.*, 2009; St.Clair, 2010) provide overview classifications and lists of potential mechanisms underlying this form of resistance. QTLs for quantitaive resistance are diverse in their effects and their potential mechanisms: some are isolate specific, others broad

spectrum (Pilet-Nayel *et al.*, 2017); they often manifest in a continuous range of phenotypes (Roux *et al.*, 2014); and there may sometimes be differences in the life-history stage of pathogen at which they impact (Gabriel *et al.*, 2007). This last point is particularly relevant for potential modification of the curative opportunity window, particularly in a polycyclic disease such as *P. infestans*. A reduction in observed disease level on a field scale could be due to a number of factors including: (i) a reduction in infection efficiency, (ii) slower plant tissue colonisation, (iii) extended duration of the latent period, (iv) lessened symptom intensity, or (v) a reduction in reproductive capacity. Several of these factors are linked, as they represent sequential stages in *P. infestans'* life history (Black, 1970). The most important factor when a single infection cycle is considered is slower plant tissue colonization. There is evidence from other pathosystems that biomass levels are lower at comparable time points in more resistant cultivars. Barley and wheat genotypes classified as resistant to Fusarium head blight caused by *Fusarium graminearum* showed a lower copy number of the pathogen gene *Tri6* determined via qPCR at six days post inoculation compared with susceptible genotypes (Kumar *et al.*, 2015). In contrast Bengtsson *et al.* (2014) found no significant difference in *P. infestans* DNA quantity 96 hours post inoculation between the susceptible potato cultivar Bintje and the more resistant cultivar Ovatio.

A recent hypothesis for the mechanism(s) underlying quantitative resistance in the potato-late blight pathosystem is that it results from defeated and/or 'weak' *R* genes failing to completely contain infection attempts via a hypersensitive response (Vleeshouwers *et al.*, 2000a). All known *R* genes active in the *Solanum-P. infestans* pathosystem encode for proteins belonging to the CC-NB-LRR class (Rodewald and Trognitz, 2013), and all recognized effectors with experimental confirmation belong to the RxLR class (Vleeshouwers *et al.*, 2011). Even functional *R* genes, with viable *Avr* targets do not always completely contain infections, for example late blight resistance governed by *R* genes has been shown to be variable in response to plant age and inoculum density (Shah *et al.*, 2015; Stewart, 1990). There is also evidence that the presence of defeated *R* genes contributes to quantitative resistance: Stewart

et al. (2003) report that populations of potato plants carrying the *R* genes *R1, R10,* or *R11* had a greater level of quantitative resistance in field trials over two consecutive years than plants in which these genes were absent. Microscopic observations of early *P. infestans* infections in a range of potato cultivars with differing levels of quantitative resistance showed at least some hypersensitive responses in all cases, but this was delayed in the more susceptible varieties and pathogen hyphae were able to 'escape' from the region of cell death (Vleeshouwers *et al.*, 2000b). Additionally several of the QTLs associated with quantitative resistance are located near or within clusters of known *R* genes (Oberhagemann *et al.*, 1999), and the gene designated *Rpi-Smira2* (which recognises an RxLR effector) associated with quantitative resistance is only detectable in the field (Rietman *et al.*, 2012). At least some of the quantitative resistance against *P. infestans* found within the *Solanum* germplasm shares a common mechanism with qualitative resistance, in contrast to several other pathosystems, and this may be due to the rapidity with which *P. infestans* invades new host tissues (Saubeau *et al.*, 2016).

These points notwithstanding, it is not clear that the overlap been quantitative and qualitative resistance in this case is complete. Plant defence against pathogen attack is a set of complex, incompletely understood processes (Miller *et al.*, 2017). Many of the reported mechanisms, such as the oxidative burst (Wojtaszek, 1997), up-regulation of possibly antimicrobial proteins (Ahuja *et al.*, 2012), and the reinforcement of cell walls (Bellincampi *et al.*, 2014), occur in *P. infestans*-potato interactions (Desender *et al.*, 2007) It is likely that the efficiency of these processes in different cultivars, as well as the vulnerability of them against different *P. infestans* strains (or ability of the strains to circumvent them) contributes to the level of observed resistance. Some comparative studies of gene expression (Draffehn *et al.*, 2013; Gyetvai *et al.*, 2012) and metabolite profiles (Abu-Nada *et al.*, 2007; Yogendra *et al.*, 2014) in susceptible and resistant potato varieties have been conducted. Potato cultivars with similar levels of quantitative resistance can display very different defence related gene expression patterns after exposure to *P. infestans* PAMPs (Saubeau *et al.*, 2016), so it is possible that diverse mechanisms may contribute to

the level of partial resistance which is ultimately observed. In some cases, there are marked differences between gene expression patterns and metabolites present in susceptible and resistant cultivars; both constitutive (Ali *et al.*, 2012) and induced upon pathogen attack (Eschen-Lippold *et al.*, 2012). For example, the resistant genotype F06025 displayed marked accumulation of hydroxycinnamic acids amides (HCAAs) at 72 hours after exposure to *P. infestans* sporangia (Yogendra *et al.*, 2014). These secondary metabolites possibly play a role in cell wall thickening (Macoy *et al.*, 2015). Interestingly, genetic background appears to be crucial for *R* gene function. A recent study has demonstrated that possession of the *Rpi-blb1* gene, introduced using transgenic techniques, was not a guarantee of a high level of resistance (Shandil *et al.*, 2017).

Cultivars with high levels of quantitative resistance do exist, and to date this resistance has proved durable (Hao *et al.*, 2018; Rietman *et al.*, 2012). However, these cultivars often carry unrelated undesirable traits which make them unsuitable for large scale cultivation. Chief amongst these is late maturity (Visker *et al.*, 2004), and it seems that there is strong coupling between some sources of quantitative resistance and delayed maturity in the *Solanum* germplasm (Bormann *et al.*, 2004) as QTL for the two traits co-localize (Collins *et al.*, 1999). Two possible hypotheses for this observation are (i) that distinct genes are responsible for later maturity and quantitative resistance, but that they lie in close proximity, or (ii) that genes with pleiotropic effects are responsible. Recent evidence (Danan *et al.*, 2011) has provided some tentative support for the former hypothesis. Additionally, some of these studies have reported linkage between known *R* gene loci and quantitative resistance QTLs (Danan *et al.*, 2011; Li *et al.*, 2012a); providing further evidence of their important role.

Information on the resistance of various potato cultivars to a variety of pest and pathogens is available from the AHBD potato varieties database. Cultivars are assessed on a 1 – 9 scale of increasing resistance, with 9 being fully resistant. The experiments used to obtain these values are referred to as the Independent Variety

Trials, and are specific to potato production in Great Britain. These trials include reference cultivars as well new varieties, and they involve testing against a range of disease that are deemed a priority (Campbell *et al.*, 2012, 2013, 2014, 2015, 2016, 2017, 2018, and 2019). These data for foliar blight resistance are combined with the estimated potato plantings in Great Britain in Figure 5.1 (page 145); the vast majority of cultivars lie in the more susceptible region of the scale (3 – 4), and it is striking that almost 13 % of plantings are of the same cultivar (Maris Piper) which has a foliar resistance rating of 4. Many of the potential mechanisms for quantitative resistance such as pathogen escape from host HR (Kombrink and Schmelzer, 2001), stress due to antimicrobial metabolites (Poloni *et al.*, 2014), or increased difficulty in forming haustoria due to cell wall reinforcement (Ellinger *et al.*, 2013) will all slow tissue colonization, and thus may extend the curative control window. It is possible however that the form of quantitative resistance found in some cultivars does not impact tissue colonization, and will then not modify curative activity. This will clearly be the case if the source of resistance is gross morphology or pathogen escape (Niks and Rubiales, 2002), but it is also conceivable that the resistance affects pathogen processes other than lesion growth, such as sporulation capacity. It is a frequent observation that *P. infestans* fecundity is reduced in some (but not all) resistant cultivars (Van Oijen, 1991); this will slow epidemics, where the polycyclic nature of the disease is critical (Grünwald and Flier, 2005), but will have less relevance to the timing of a curative treatment post some suspected infection event. Although the Independent Variety Trials are a very valuable resource, most of the assigned ratings are derived from field trials using small plots (two plants) of each variety, which are assessed for percentage infection over the course of an epidemic (Campbell *et al.*, 2017). This methodology will capture most possible sources of resistance, some of which may not be relevant to rates of tissue colonization.

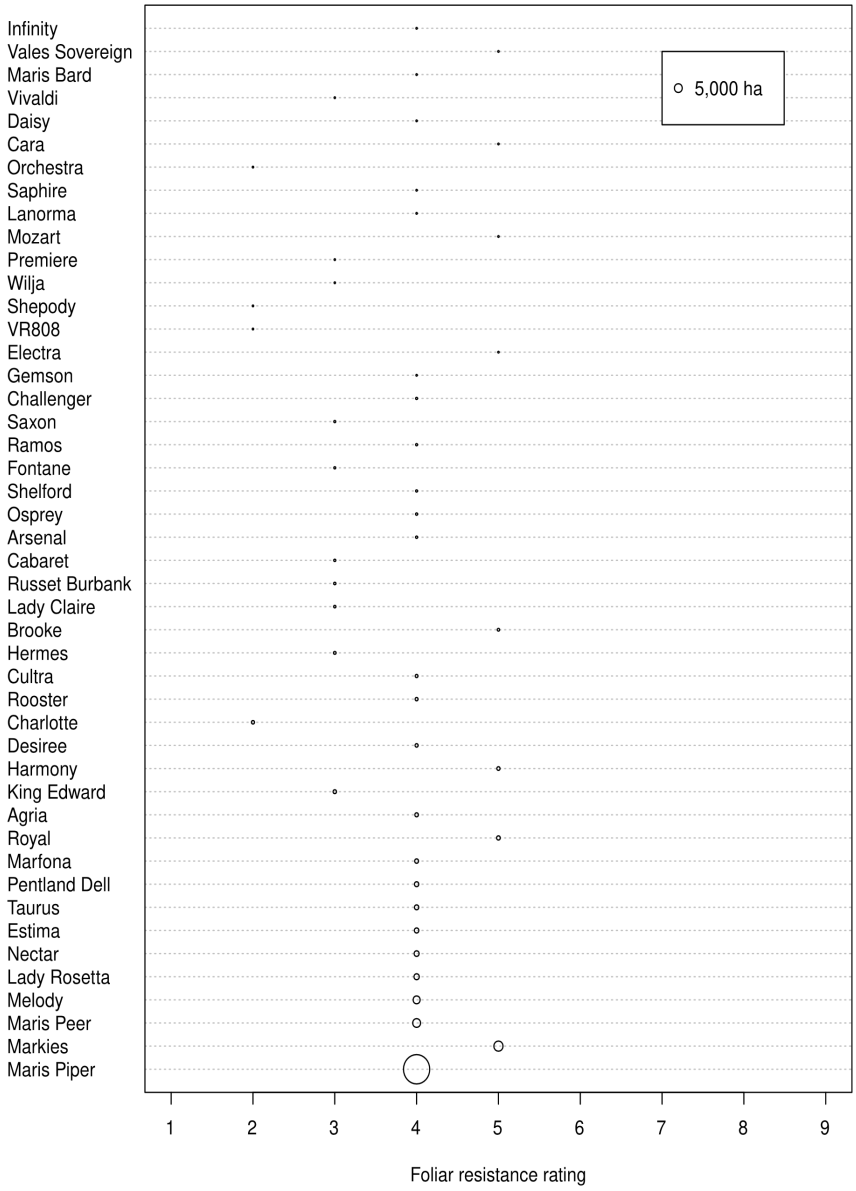

Figure 5.1. Commonly planted potato varieties which have assigned foliar resistance ratings in Great Britain. Common names are given along the vertical axis, and the foliar resistance rating on the horizontal axis. The size of each circle is scaled, based on the area planted of each variety. Data are from the AHDB planting estimates in Great Britain for 2017[16], cross-referenced against the AHDB potato varieties database[17]. Although the planting estimates list the top 50 varieties, four have been omitted as they were yet to receive a rating at time of writing. An additional area of $1.8 \cdot 10^4$ ha was planted in other varieties not listed individually within the AHDB planting estimate.

16. https://potatoes.ahdb.org.uk/publications/potato-plantings-variety-great-britain-2017 (accessed: May 2019)

17. http://varieties.ahdb.org.uk/ (accessed: May 2019)

5.2 Modifying effect of cultivar resistance field trials

5.2.1 Natural inoculum whole plant trial

Observed foliar resistance scores to late blight in potato are sometimes stable between field trials and laboratory experiments (Vleeshouwers *et al.*, 1999), and sometimes they differ (Rietman *et al.*, 2012). There are few published studies that consider modifying factors on curative fungicide performance, so an important initial goal was to establish if the combination of resistant cultivars and curative fungicide treatments improved curative control. The final decision aid will provide guidance on the likely progression of late blight infections and thus the anticipated level of curative control. There will always be some uncertainly as to when infections take place, but it is sensible to assume that inoculum is ubiquitous, and that therefore infection coincides with high risk weather conditions (see Chapter 1, Section 1.4, page 10). This risk-averse approach is often adopted by growers, and weather warnings may sometimes serve as justifications for the use of curatively active products.

5.2.2 Natural inoculum trial: materials and methods

A sequence of experiments was designed to mimic natural infection after conducive weather and subsequent treatment with curative fungicide. To ensure consistency with experiments used to generate the development-control curves in Chapter 4, unless otherwise specified the curative fungicide Infinito (Bayer CropScience, 62.5 g fluopicolid + 625 g propamocarb-HCl l^{-1}, suspension concentrate) was used at the recommended field dose of 1.6 l ha^{-1}, applications were made from either a tractor mounted or backpack AZO sprayer (350 kPa pressure through Lurmark F03-110 nozzles). Two methods of fungicide application were used for logistic reasons, as the tractor-mounted sprayer was sometimes in use servicing unrelated field trials. The experiment was repeated four times (designated as runs A – D), but because of logistic reasons the treatment structure differed slightly between each run, as explained below. Runs A – C were conducted over the late summer and autumn of 2015, and run D during late summer 2016.

Small potato plants were propagated from seed tubers in small pots (5 cm diameter) using John Innes potting compost No1, and were grown within a polytunnel for approximately 7 weeks. In runs A and C, two cultivars were used: King Edward (foliar resistance rating: 3) and Cara (foliar resistance rating: 5). In runs B and D, three cultivars were used: King Edward, Cara, and Sarpo Mira (foliar resistance rating: 7). Cultivars were selected to give a reasonable span of the available levels of resistance. Plants were propagated following a slightly different method in run D, as they were chitted for an extended period (8 weeks) before planting. Immediately prior to use the experiment plants were inspected for the presence of disease symptoms to minimize the possibility of established lesions confounding the results.

Late blight fungicide trials are conducted annually at SRUC's Auchincruvie site, involving several medium sized plots (typically 3.8 m · 8 m) with different varieties and fungicide treatment programmes, as well as untreated infecter rows. Once trials are sufficiently advanced, high inoculum pressure can be found within the trial fields, and as the initial artificial inoculations are usually conducted using an aggressive isolate (C. Convery, SRUC, Auchincruive, UK personal communication) this provides conditions that mirror a natural epidemic. Once the propagated plants were of a sufficient size (growth stages 107 – 112, assessed using BBCH key (Hack *et al.*, 1993)) weather forecasts were monitored until a Smith Period (Smith, 1956) or a 'near miss' was imminent (i.e. criteria met on one day, but not two consecutive days). When the experiments were conducted the more modern Hutton Criteria were still in development (Dancey *et al.*, 2017). The small plants were then transported to the field site, and left exposed to airborne inoculum for approximately 2 hours. The trays were placed across two ridges in approximately the centre of plots containing infected King Edward potato plants (as shown in Figure 5.2, page 149), when multiple trays were used each was placed 0.5 m apart. During this period leaf wetness was monitored visually and foliage was periodically misted with dechlorinated water to maximize the probability of infection. The pots containing plants were placed in doublets (runs A and C) or triplets (runs B and D)

containing one plant of each cultivar, these were held in place within larger plastic trays. After the allotted exposure time, trays containing plants were sealed within transparent plastic sheeting to ensure high relative humidity and were then placed in a growth room with conditions set to a 16 hour photoperiod and a constant 18 °C (temperature was monitored with iButtons as described in Chapter 2, Section 2.6, page 54).

Treatment categories differed with the different experimental runs, but in all cases each category contained a batch of 12 plants: the designated treatment was recorded using pot labels before their placement within the trays was randomized. In all experimental runs, one batch of plants per cultivar was exposed to natural inoculum, and then left untreated as a control. 12 additional plants per cultivar were not exposed to inoculum, but were otherwise incubated in the same manner as a control, to establish the level (if any) of disease development without exposure. Fungicide treatment timings were selected to cover the interval thought to be important in curative control (1 – 3 days). All plastic coverings were removed before treatment and the foliage was given time to dry. The small plants were supported with wooden canes during fungicide treatment. In run A, one batch of plants from each cultivar (King Edward, Cara) were removed from the growth room and treated with curative fungicide at 56 hours post exposure. In run B batches of all culitvars (King Edward, Cara, Sarpo Mira) were treated 43 hours post exposure. In run C, three cultivars (King Edward, Cara, Sarpo Mira) and three timings were used: 28 (F_1), 47 (F_2), and 69 hours (F_3). Finally, in run D two cultivars (King Edward, Cara) were treated at the following time points: 25 (F_1), 44 (F_2) and 65 hours (F_3) post exposure.

Figure 5.2. Small potato plants within plastic trays exposed to naturally produced *P. infestans* sporangia in the field during the natural inoculum field trial. Exposure time was approximately two hours, and experiments were conducted in weather conditions conducive to sporulation, at a site where plants with active blight lesions were numerous. Plants were subsequently incubated under controlled conditions and treated with curative fungicide.

In experimental runs A and D sample leaflets were detached at the fungicide treatment times (i.e. after plants had been exposed and incubated for the 1st period) and frozen at −20 °C. Petiole scars that remained on the plants were covered with a small patch of para-film to prevent fungicide absorption though the wound. These samples were later used to estimate *P. infestans* biomass present in the leaflets. Genomic DNA was extracted from frozen leaflets as described in Chapter 2 (Section 2.8, page 56), and the qPCR bioassay described in Chapter 2 (also Section 2.8, page 56) and in Section 3.4.1 (page 70) was used to determine the proportion which was *P. infestans*. DNA standards for this assay were extracted from a mycelial culture of genotype 2012_13A2-1, as described in Chapter 2 (Section 2.1, page 49).

After treatment all plants were returned to the growth room under the same conditions and allowed to incubate for a second period of 7 days . After this, plants were removed from the growth room and counts were made of the number of

visible late blight lesions. Size measurements of lesions were also taken, using a similar method to (Chapman, 2012): the longest length of each lesion (d_1) as well as its length at right angles (d_2) were recorded in mm, these measurements were then used to calculate an area, assuming the lesion was elliptic ($LA = 0.25 \pi d_1 d_2$, where LA is the lesion area in mm^2).

5.2.3 Natural inoculum trial: statistical analysis

The lesion count data for each experiment was assessed using ANOVA, data were transformed to meet the assumptions of the analysis (chiefly normal distribution and homogeneity of variance), this took the form of a natural log transformation in Run A, and a natural log + 1 transformation in all other experimental runs. This second transformation was used as in some cases lesion counts were zero. Area measurements were square root transformed before analysis, as inspection of residual plots revealed that they were not normally distributed, and this is often the most appropriate transformation for area measurements (Mead, 2017). In situations where ANOVA indicated significant factor effects, post-hoc comparisons of group levels of interest were conducted using Tukey's range test.

5.2.4 Natural inoculum trial: results

In the first run of the natural inoculum trial lesions developed on all plants exposed in the field, no lesion development was observed on the unexposed control plants. Mean counts for the four treatment combinations are shown in Figure 5.3 (page 151). There was a statistically significant difference between the natural log transformed lesion counts on fungicide-treated and untreated plants ($p < 0.01$), and between the two cultivars ($p < 0.01$), but the interaction term was not significant ($p = 0.78$). On average the number of lesions developing on fungicide-treated King Edward plants was reduced by 44 % when compared with the untreated control, and within the Cara plants the reduction attributable to fungicide treatment was 53 %. There was a significant difference in lesion size between fungicide-treated plants and those left untreated ($p < 0.01$), with smaller mean lesion size for the fungicide-treated plants, but there was no significant difference in lesion size between King

Edward and Cara plants (p = 0.84). *P. infestans* DNA was detected (C_q values were obtained) in all samples taken at the time of treatment from Cara plants, and in all but one of the King Edward samples. Mean values for *P. infestans* DNA (± 95% confidence intervals) were $9.33 \cdot 10^{-2}$ pg ($\pm$ $2.47 \cdot 10^{-2}$ pg) for Cara plants and 0.26 pg ($\pm$ 0.33 pg) for King Edward plants. A Welch's two-sample t-test (Delacre *et al.*, 2017) was conduced as the observed variance differed between the cultivars, and this indicated that there was no statistically significant difference between the cultivars (p = 0.33).

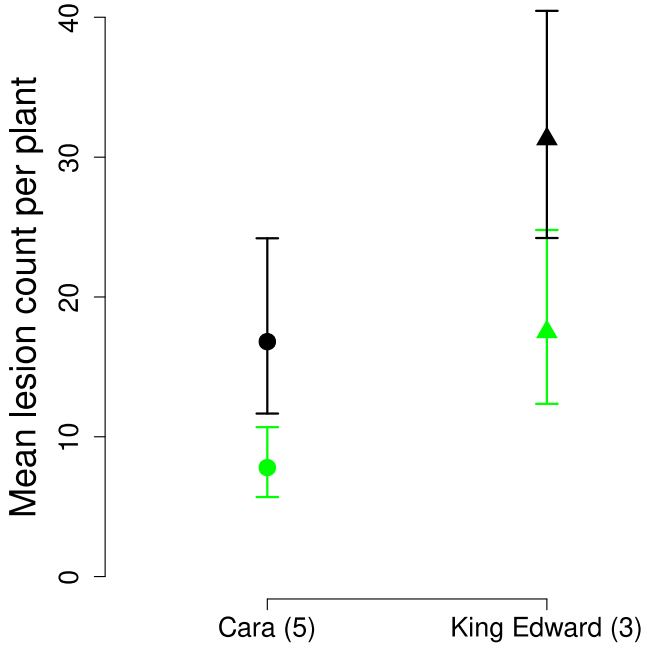

Figure 5.3. Natural inoculum experiment, run A. Small potato plants of the cultivars Cara (●) and King Edward (▲) were exposed to natural *P. infestans* inoculum in the field immediately following a period of high risk weather (Smith Period). Mean lesion counts (n = 12, ± 95 % confidence internals), 7 days from exposure are shown on the horizontal axis. Plants of each cultivar received one of two treatments: untreated (black shapes) or treatment with a curative fungicide (green shapes) with the a.i.s fluopicolid + propamocarb-HCl at 56 hours (1,008 $^{o}D_a$) post exposure.

Infection rates were lower in the second experimental run (B) than the first (data are shown in Figure 5.4, page 153), and on some of the inoculum-exposed plants no

lesions developed (two of the Cara, and three Sarpo Mira plants). ANOVA of natural log + 1 transformed lesion counts indicated that both curative treatment ($p < 0.01$) and cultivar ($p < 0.01$) were significant sources of variation. Additionally, the interaction term treatment · cultivar was significant ($p = 0.05$). In practice, this may mean that different responses to curative fungicide treatment may occur for infections present on cultivars with different resistance ratings. When groups were compared there was a significant treatment effect for Sarop Mira ($p = 0.03$), and for Cara ($p < 0.01$), but not for King Edward ($p = 0.99$). For the cultivar Cara, fungicide-treated plants had an average reduction in developing lesion count of 70% compared to the untreated control. This value was 84% for Sarpo Mira. Square root transformed lesion size was significantly different in fungicide-treated compared with untreated plants ($p < 0.01$); with smaller lesions present in the fungicide-treated plants. Unlike in run A, cultivar was a significant source of variation ($p = 0.03$), with the smaller lesions associated with the two more resistant cultivars (Cara and Sarpo Mira, data not shown). The interaction term was not significant ($p = 0.59$). Post-hoc comparisons of the square root lesion size means using Tukey's range test yielded a significant difference for the Sapro Mira-King Edward comparison ($p = 0.04$), approached significance for the Sapro Mira-Cara comparison ($p = 0.07$), and was not significant for the Cara-King Edward comparison ($p = 0.99$).

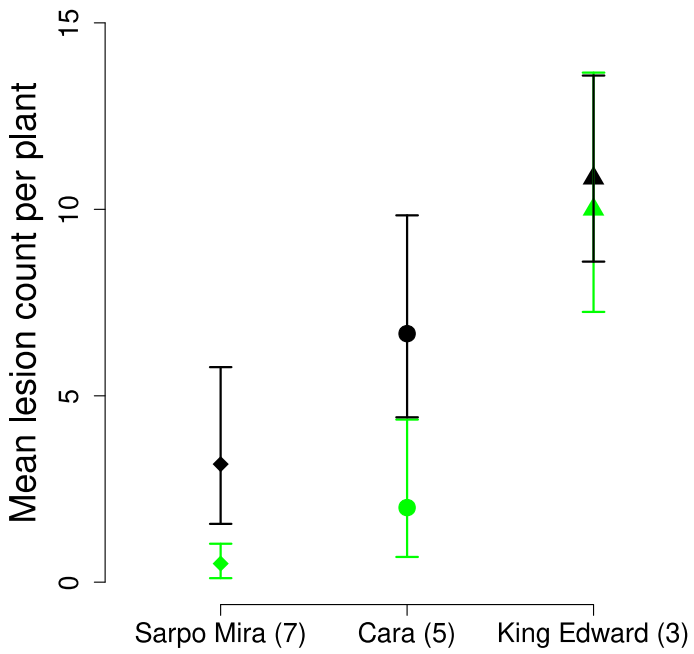

Figure 5.4. Natural inoculum experiment, run B. Small potato plants of the cultivars Sarpo Mira (♦), Cara (●) and King Edward (▲) were exposed to natural *P. infestans* inoculum immediately following a period of high risk weather (Smith Period). Mean lesion counts (n = 12, ± 95 % confidence internals), 7 days from exposure are shown on the y-axis. Plants of each cultivar received one of two treatments: untreated (black shapes) or treatment with a curative fungicide (green shapes) with the a.i.s fluopicolid + propamocarb-HCl at 43 hours (774 °D_a) post exposure.

In experimental run C a large proportion of the exposed plants did not develop lesions after the 7 day incubation period (31 % of the Kind Edward, 28 % of Cara, and 83 % of Sarpo Mira plants) and the majority of the plants which did show infection had only 1 – 2 lesions (data not shown graphically). There was no significant effect from fungicide treatment ($p = 0.41$), but cultivar was a significant source of variation. Comparisons of the natural log + 1 transformed lesion counts between Sarpo Mira and both Cara ($p < 0.01$) and King Edward ($p < 0.01$) were significantly different, with counts lower for the former than the later two, but there was not a significant difference between Cara and King Edward ($p = 0.20$).

Experimental run D incorporated two cultivars and three potential fungicide treatment timings, as well as an exposed but untreated control. Data from run D are summarised in Figure 5.5 (page 155). ANOVA of natural log + 1 transformed data indicated that both cultivar ($p < 0.01$) and curative fungicide treatment category ($p < 0.01$) were significant sources of variation, but their interaction was not significant at the 5% level ($p = 0.09$). Tukey's range test was used to obtain significance values for comparisons of interest. Fewer lesions were present on the Cara plants at F_1 ($p < 0.01$) and F_2 ($p = 0.04$), but not F_3 ($p = 0.18$) when the three treatment timings were compared with the untreated control. For the King Edward plants, only the comparison between F_1 and the untreated plants was significantly different ($p = 0.01$). Genomic DNA from *P. infestans* was present in all samples with the exception of the Cara T_3 samples where, C_q values could not be obtained for seven of the twelve samples. These data are summarised in Table 5.1 (page 155); the most abundant treatment category for initial template was King Edward at F_3. ANOVA of the transformed data (natural log + 1, as the variances were unequal) indicated statistically significant differences between the cultivars ($p = 0.01$), but that the treatment timing ($p = 0.09$) and the interaction term ($p = 0.58$) were not significant. If samples where no C_q was obtained are removed from the analysis (i.e. assuming no infection took place on these leaflets, and that they are not relevant to the developmental state of the pathogen at F_3), then on the log-transformed values both cultivar ($p < 0.01$) and treatment time ($p < 0.01$), but not their interaction ($p = 0.57$) were significant. Post hoc testing indicated that the King Edward plants in the F_1 category had significantly higher *P. infestans* DNA levels than Cara plants at timepoints F_1 ($p = 0.03$), and F_2 ($p = 0.02$) and that similarly King Edward plants within F_3 had higher levels than Cara plants at F_1 ($p < 0.01$) and F_2 ($p < 0.01$).

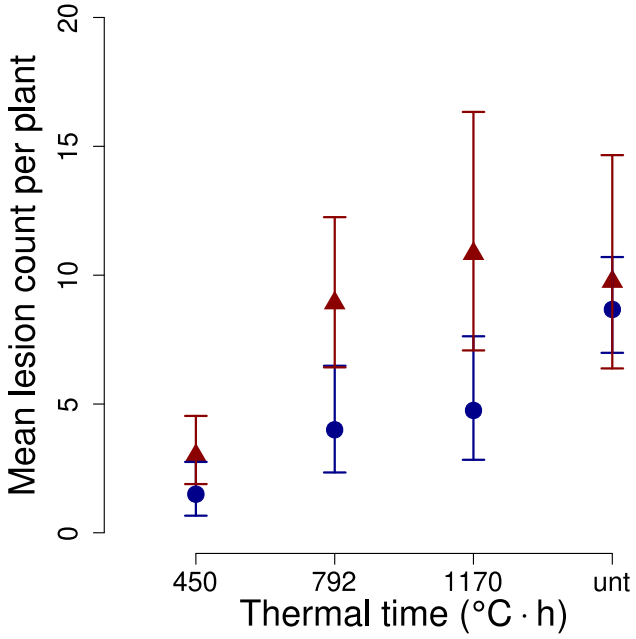

Figure 5.5. Natural inoculum experiment Run D. Small potato plants of the cultivars Cara (●) and King Edward (▲) were exposed to natural *P. infestans* inoculum immediately following a period of high risk weather (Smith Period). Mean lesion counts (n = 12, ± 95 % confidence intervals), 7 days from exposure are shown on the y-axis. Plants of each cultivar received one of four treatments: untreated (unt), or treatment with a curative fungicide with the a.i.s fluopicolid + propamocarb-HCl, at one of three timepoints (F_1: 25 hrs (450 °C·hr) ; F_2: 44 hrs (792 °C·hr); F_3: 65 hrs (1170 °C·hr)).

Table 5.1. Amount of *P. infestans* DNA obtained from potato leaflets within run D of the natural inoculum experiment. Small potato plants (Cara and King Edward) were exposed to naturally produced *P. infestans* sporangia in the field. These plants were then incubated in control conditions and treated with curative fungicide (fluopicolid + propamocarb-HCl) at one of three timings denoted as F_1, F_2, and F_3. Amount of pathogen DNA present was determined using qPCR. Disease development time is expressed as thermal time, the product of the chronological time elapsed and the temperature (°C·hr).

Thermal time between exposure and curative treatment (°C·hr)	Mean P.infestans DNA (pg) ± 95 % confidence intervals	
	Cara	King Edward
450 (F_1)	0.104 (± 0.027)	0.279 (± 0.086)
792 (F_2)	0.109 (± 0.011)	0.189 (± 0.019)
1170 (F_3)	0.195 (± 0.268)	0.443 (± 0.243)

5.3 Artificial inoculum trial

In addition to the natural inoculum trial, a trial using controlled inoculations was conducted in the late summer of 2017. Experiments with artificial inoculum allow greater control of the inoculum challenge (the isolate(s) used, the inoculum density applied etc.), and provide a useful complement to the experiments conducted in Section 5.2 (page 146).

5.3.1 Artificial inoculum trial: materials and methods

Two potato varieties with contrasting foliar resistance ratings were available for this trial: a susceptible variety, King Edward (foliar resistance rating: 3) and a moderately resistant variety, Mozart (foliar resistance rating: 5). Plots were cultivated following standard agronomic practice (see Chapter 2, Section 2.3, page 51). Cultivars were assessed in sequence: an experimental cycle was completed first with King Edward plants, and then the following week with the Mozart plants. Plots were inspected prior to leaf collection to ensure no natural infections were present. Leaves were harvested from the field on the day of inoculations, leaflet triplets (terminal and two lateral) were detached from plants in the field and transported to the laboratory. Two groups of leaflets were collected: one group from high in the canopy (node 1 – 5 from apex) and a second group from mid canopy (nodes 6 – 11). Leaflets were then distributed into transparent plastic trays lined with damp paper towels, 24 leaflets per tray, with two trays for each canopy-treatment combination (= 48 leaflets per category). Each individual leaflet was then inoculated on their adaxil surface (away from main veins) with a 20 μl droplet of inoculum containing ~ 2,000 *P. infestans* sporangia (isolate 2012_13A2-1), which had been prepared from sporulating lesions on detached King Edward leaflets as described in Chapter 2, Section 2.4 (page 52). Trays were then sealed within transparent plastic bags to ensure high humidity, and incubated within a growth room with the same conditions as the natural inoculum trial (16 hour photoperiod and a constant 18 °C), trays were shaded with paper towels during incubation. At three time points following inoculation (F_1: ≈ 1 day, F_2: ≈ 2 days, and F_3: ≈ 3 days; precise timings are given in results Section 5.3.3, page 157), two trays containing mid-canopy leaflets, and two trays containing high-canopy leaflets were removed

from the growth room. Leaflets were placed at random in an 8 by 12 grid with approximately 4 cm spacing between leaflets, small card markers were placed next to each leaflet to keep track of its canopy position. Small sections of paper towels were used to cover petioles to prevent fungicide uptake through wounded tissue. The leaflets were then treated curatively with fungicide applied from an AZO spayer (see page 146). Following treatment leaflets were resealed within the trays and returned to the growth room. After 7 days incubation, leaflets were inspected for disease development: non-symptomatic leaflets or leaflets displaying small arrested lesions were classed as curatively controlled infections, whereas leaflets with large necrotic lesions or lesions with abundant sporulation were classed as uncontrolled infections.

5.3.2 Artificial inoculum trial: statistical analysis

As the data are categorical (successful infection / no infection) a generalized linear model with a logit link function was fitted to the data, with infection as the response variable and fungicide treatment time, and canopy position as the explanatory variables. All levels of fungicide treatment were considered as categorical. Each run was analysed individually. Chi-squared tests were used to assess the importance of the factors canopy position and treatment timing.

5.3.3 Artificial inoculum trial: results

Summaries of both runs of the artificial inoculum trial are shown in Tables 5.2 and 5.3 (page 158). The highest count for successful infections was in untreated King Edward leaflets from the mid-canopy (94 %), the lowest in Mozart leaflets at the first treatment time (no infection); though counts in all F_1 categories were very low (0 – 2%). Within the King Edward experiment, there were significant differences between the curative treatment times ($p < 0.01$), but not between the two canopy positions ($p = 0.24$), or their interaction ($p = 0.94$). The proportion of leaflets infected increased with increasing disease development time, but did not reach the level of untreated leaflets even at F_3. In the Mozart run of the experiment, both treatment timing ($p < 0.01$) and canopy position ($p < 0.01$) were significant

factors. The interaction between treatment timing and canopy position was also significant ($p < 0.01$), indicating different patterns of response to treatment between the two canopy positions. The mid-canopy leaflets followed a similar pattern to King Edward, with low infection at F_1 progressively increasing with increasing time. In contrast, infection was low in untreated high-canopy leaves (26 % compared to mid canopy), and only the F_1 timing had an appreciably lower lesion count.

Table 5.2. Artificial inoculum field trial run A. Leaflets (n = 48 per category) from field grown King Edward plants (foliar resistance rating: 3) were inoculated with sporangial suspensions of isolate 2012_13A2-1, incubated at 18 °C and subsequently treated with curative fungicide fluopicolid + propamocarb, at one of three timepoints (F_1: 20 hrs (360 °C·hr) ; F_2: 43 hrs (774 °C·hr); F_3: 67 hrs (1206 °C·hr)). Counts are of the number of individual leaflets which went on to develop extensive late blight symptoms.

	Treatment timing (accumulated thermal time)			
	F_1 (360 °C·hr)	F_2 (774 °C·hr)	F_3 (1206 °C·hr)	Untreated
Canopy position	Infected leaflets count (n = 48)			
High	1	19	32	37
Mid	1	20	37	45

Table 5.3. Artificial inoculum field trial run B. Leaflets (n = 48 per category) from field grown Mozart plants (foliar resistance rating: 5) were inoculated with sporangial suspensions of isolate 2012_13A2-1, incubated at 18 °C and subsequently treated with curative fungicide fluopicolid + propamocarb, at one of three timepoints (F_1: 18 hrs (324 °C·hr) ; F_2: 42 hrs (756 °C·hr); F_3: 67 hrs (1206 °C·hr)). Counts are of the number of individual leaflets which went on to develop extensive late blight symptoms.

	Treatment timing (accumulated thermal time)			
	F_1 (°C·hr)	F_2 (°C·hr)	F_3 (°C·hr)	Untreated
Canopy position	Infected leaflets count (n = 48)			
High	1	8	12	8
Mid	0	10	25	31

5.4 Plot scale field trial

A plot scale field trial using potato varieties with contrasting resistances were conducted in the early summer of 2015. One purpose of this trial was to optimize the methodology used to parameterize and validate the final decision aid, and thus the methodology (particularly the inoculation method) was provisional, and was subsequently modified. It was difficult to interpret the size and scale of the curative effect in this trial, but the design did provide some useful information on a single disease cycle on field grown plants so a description is included here.

5.4.1 Plot scale field trial: materials and methods

Trials took place at SRUC's Boghall site in Midlothian and were conducted in early summer. Trials were scheduled for early in the season before late blight outbreaks became common, to reduce the risk of natural inoculum interfering with the results. The experiment was designed to incorporate three factors with contrasting levels: curatively treated/untreated plants, resistant/susceptible plants, and aggressive/less aggressive *P. infestans* isolates. The inoculations were also conducted in five distinct intervals to generate a range of disease development timings.

The trial area consisted of 8 plots (1.8 · 7 m) with 1.8 m gaps between plots, with each plot being the width of two planting ridges. Plots were split, with one half assigned for fungicide treatment and the other left untreated (which half received treatment was randomized). After cultivation, seed tubers of two varieties where hand planted within the plots: King Edward (susceptible, foliar resistance rating: 3) and Innovator (no official rating at time of experiment, but based on information from the breeder it was thought to be a resistant variety. This, however, was incorrect, as subsequently a published foliar resistance rating of 3 has been assigned[18]). In total, 60 tubers were planted in each plot (30 from each variety) with their position within each subplot randomized (so that both subplots within a plot were balanced) – these were pre-recorded to keep track of cultivar in later experimental stages, but the two varieties have very distinct phenotypes and are

18. http://varieties.ahdb.org.uk/varieties/view/Innovator (Accessed: Sept. 2019)

easily distinguished. Pre-emergence herbicides and fertilizers were applied following standard agronomic practice. Plants were then grown for 10 weeks.

The day prior to inoculations, plots were inspected for the presence of late blight lesions and to confirm placement of cultivars within each plot. At this stage, inoculation sites were also selected and six compound leaves per plant were tagged with loop lock labels. Leaves were selected that were undamaged and that were of a sufficient position within the canopy that they were likely to intercept fungicide spray at the time of treatment. The loop lock labels were colour-coded and labelled based on the isolate and inoculation timing they were allocated. There were 15 possible combinations per sub plot: five inoculation timings (I_1, I_2, I_3, I_4, or I_5) by three inoculum categories (2012_13A2-1, 2012_8A1-1, or sterilise distilled water. The positioning of these was randomized within each subplot.

Inoculum was prepared as described in Chapter 2 (Section 2.4, page 52) from cultures of the two experimental isolates on detached King Edward leaflets. Inoculum was prepared approximately 1.5 hours before the inoculation time, and was adjusted to $2 \cdot 10^4$ sporangia per ml before transport to the field. The inoculum was loaded into 100 ml hand pressurized spray bottles (Nalgene, Rochester, USA) to facilitate application. Bottles were calibrated before use; it was determined that a short spray delivered ~ 0.1 ml (~ 2,000 sporangia at the experimental density), and this was used as the inoculum dose at each inoculation site. Inoculations took place over the space of three days, I_1 on day one, I_2 and I_3 on day two, and I_4 and I_5 on day three. Inoculations took place in either the late afternoon or evening. Each inoculation took approximately 2.5 hours to complete. Once all inoculations were complete, plants were sealed within large transparent polythene bags with a small hole cut into the top corner to prevent overheating. Bags were left in place overnight, and were removed approximately 14 hours after each inoculation. Two iButtons in small shaded containers were placed amongst the crop to log temperature over the course of the experiment.

On the day following the I_4 and I_5 inoculations, one subplot per plot was treated with curative fungicide. In contrast to the other experiments detailed within this project the curative fungicide Option (Corveta, water dispersible granules, 600g cymoxanil kg^{-1}) was used at the full label rate of 0.15 kg in 200 l ha^{-1}. Fungicide was applied via an AZO backpack sprayer. Infections were left to incubate, and 1 week from the fungicide treatment date inspections were made of the inoculation sites. The initial protocol specified lesion counts be made, but this was modified when it became apparent that the vast majority of inoculation sites displayed large contiguous lesions. Lesion area at each site was therefore recorded following the method specified in Chapter 2, Section 2.7 (page 54).

5.4.2 Plot scale field trial: statistical analysis

An unexpected incompatible interaction was observed between isolate 2012_8A1-1 and Innovator, meaning that it would be inappropriate to analyse the experiment as a whole. Each isolate were therefore considered as a separate experiment, inoculations with 2012_13A2-1 incorporating both cultivars, and inoculations with 2012_8A1-1 only the different disease development timings and the fungicide treatments. ANOVA was performed on the two data sets with lesion area (square root transformed) as the response variable and treatment, disease development time, plot, and cultivar (if appropriate) as explanatory factors. Lesion areas were square root transformed to meet the assumptions of the analysis.

5.4.3 Plot scale field trial: results

Inoculation sites fell into one of two categories. In most cases large sporulating lesions were present at inoculation sites. However at Innovator sites which were inoculated with 2012_8A1-1 almost no lesions were observed (0.5% of sites) and this is probably attributable to an *R* gene based incompatibility. ANOVA of 2012_13A2-2 inoculated plants indicated a very large effect from timing category ($p < 0.01$), but this varied between timings without a distinctive pattern (data not shown). Treatment alone approached significance, but did not meet the 5 % threshold ($p = 0.07$), and there was no significant effect from cultivar ($p = 0.24$). However, the interaction terms treatment · cultivar ($p < 0.01$) and cultivar · disease development

time ($p < 0.01$) were significant. In the King Edward plants, no significant effect was present from treatment ($p = 0.32$), but similar to the Innovator plants there was a highly significant effect from inoculation time ($p < 0.01$) but this was not correlated with the increasing thermal time values (data not shown). The interaction term approached, but was not significant at the 5 % level ($p = 0.08$). The data are summarised in Tables 5.4 and 5.5

Table 5.4. Comparisons of mean diseased area at isolate 2012_13A2-2 inoculation sites within the plot scale field trial. Sections of foliage were inoculated with *P. infestans* sporangial suspension at several possible inoculation times. Area occupied by late blight lesions across leaflets and plots with the same treatment combination are displayed as scaled to the untreated control for their respective cultivars. The symbols $I_1 - I_5$ represent the inoculation time before curative fungicide treatment times, for which associated thermal time values are given.

	Disease development timing (accumulated thermal time)				
	I_1 (197 °C·hr)	I_2 (254 °C·hr)	I_3 (454 °C·hr)	I_4 (610 °C·hr)	I_5 (967 °C·hr)
Cultivar	Mean disease area as a proportion of untreated control				
King Edward	1.12	1.02	1.07	0.96	0.90
Innovator	0.75	0.71	0.72	0.98	1.00

Table 5.5. Comparisons of mean diseased area at isolate 2012_8A1-1 inoculation sites within the plot scale field trial. Sections of foliage were inoculated with *P. infestans* sporangial suspension at several possible inoculation times. Area occupied by late blight lesions across leaflets and plots with the same treatment combination are displayed as scaled to the untreated control for their respective cultivars. The symbols $F_1 - F_5$ represent the fungicide treatment times, for which associated thermal time values are given. The symbols $I_1 - I_5$ represent the inoculation time before curative fungicide treatment times, for which associated thermal time values are given.

	Disease development timing (accumulated thermal time)				
	I_1 (197 °C·hr)	I_2 (254 °C·hr)	I_3 (454 °C·hr)	I_4 (610 °C·hr)	I_5 (967 °C·hr)
Cultivar	Mean disease area as a proportion of untreated control				
King Edward	0.85	0.86	1.21	0.84	1.11

5.5 Host resistance and rates of tissue colonization

If the published foliar resistance ratings of potato against late blight from the independent variety trials (Campbell *et al.*, 2017) are a good index for rates of tissue colonization by *P. infestans*, then they could serve as a useful guide to growers and agronomists as to the period over which curative fungicide applications are likely to be effective. On the other hand, it is possible that there is a poor relationship, and that the ratings are chiefly capturing some other aspect of resistance. In order to evaluate this, a range of cultivars were tested in the laboratory under controlled conditions for their observed resistance to late blight.

5.5.1 Rates of tissue colonization: materials and methods

Two experimental cycles were conducted, one at intervals throughout 2016 and the second in 2017. It was not possible to screen a large range of cultivars in a single experiment because of time and resource constraints. In each experimental run three cultivars were tested, from a pool of available seed representing a range of resistance ratings, from amongst the most widely grown of important varieties within Great Britain where possible. A common cultivar, King Edward, was included as a standard reference in each run, so that approximate comparisons could be made even if there was an effect from different batches of inoculum at different times. In 2016, three experimental runs were completed: A (King Edward, Cara, and Sarpo Mira), B (King Edward, Pentland Dell, and Shepody), and C (King Edward, Harmony, and Markies). In 2017, six experimental runs were completed D (King Edward, Marfona, and Inca Bella), E (King Edward, Cara, and Chalotte), F (King Edward, Pentland Dell, and Sarpo Mira), G (King Edward, Maris Peer, and Sarpo Shona), H (King Edward, Premier, and Sharpo Una), and finally I (King Edward, Maris Piper, and Arran Pilot).

Methodology differed slightly between the two years, as improvements were made to the measurements taken in 2017 to better complement data gathered in Chapter 3 (Section 3.4.3, page 75) on rates of tissue colonization for different isolates and temperatures. Plants were propagated as described in Chapter 2 (Section 2.2, page 51), until they were approximately 6 weeks old. At this point leaflets were

harvested from between nodes 4 to 11 in sequential runs, small stickers were attached to the petioles of these leaflets denoting the position and plant from which they were obtained. In total, at each experimental run 32 leaflets per cultivar were harvested in this way. Additional leaflets were harvested from random positions, these were inoculated with sterile distilled water and included as controls.

Once leaflets were harvested and labelled they were distributed amongst transparent plastic trays lined with damp paper towels. Four trays were utilised in each experiment. Each tray was allocated 27 leaflets: three sequential runs (one run from each of the experimental cultivars) of leaflets representing positions 4 to 11 (8 leaflets in total per run) from randomized plants, as well as three of the control leaflets (one from each cultivar). The placement of the leaflets themselves within each tray was randomized. Leaflets were then inoculated with a 20 µl droplet containing ≈ 2,000 *P. infestans* sporangia (see Chapter 2, Section 2.4, page 52 for details of inoculum preparation), placed on the adaxil surface away from any main veins. Once inoculations were completed, trays were sealed within transparent plastic bags, shaded with paper sheets and placed with a growth cabinet set at a constant 18 °C and a 16 hour photoperiod. In the experimental runs conducted in 2017, four extra trays were included containing leaflets harvested and inoculated in exactly the same way but designated for biomass estimation rather than visual assessment.

In the experimental runs completed in 2016, trays were opened at 144 hours post inoculation and digital images of each leaflets were taken as described in Chapter 2, Section 2.7 (page 54). In the 2017 runs, trays were opened at three time points: 120, 144, and 168 hours and images were taken in the same way; this was done so that growth rates rather than simple lesion size could be determined. For the runs which included biomass estimation trays, these were opened at 12, 24, 36, 48, 60, and 72 hours post infection, and at each opening six leaflets were removed, placed into a micro-centrifuge tube (2 ml), frozen and stored at −20 °C until later DNA extraction and quantification.

Sizes of lesions visible in the digital images were quantified using the `polygon` function in ImageJ (Schneider *et al.*, 2012) as described in Chapter 2, Section 2.8 (page 55). In the 2017 runs, linear lesion growth rates (mm hr^{-1}) were obtained using a similar method to (Visker *et al.*, 2003): simple linear regression of square root lesion area was regressed against time for each individual leaflet. The slope coefficient of this regression is the linear lesion growth rate.

5.5.2 Rates of tissue colonization: statistical analysis

In order to compare lesion size or growth rate across all the cultivars within a run, a linear mixed model was used. Square root lesion size (runs in year 2016) or linear lesion growth rate (runs in year 2017) was included as the response variable, cultivar was included as a fixed effect, and experimental run, plant from which the leaflet was harvested, and the experimental tray were included as random effects. For the incubation period biomass accumulation data, the natural log of initial *P.infestans* DNA template was regressed against time, with the slope coefficient of this regression providing a rate of increase. Spearman's rank correlation coefficient (Dytham, 2011), denoted here as ρ, was used to assess the relationship between foliar late blight resistance rating and the measures of pathogen growth (lesion size after fixed time, linear lesion growth rate, or biomass accumulation coefficient). This measure was used as foliar resistance rating is an ordinal scale where only the rank order is meaningful.

5.5.3 Rates of tissue colonization: results

Within the 2016 run of experiments there was a statistically significant effect from cultivar ($p < 0.01$). Estimated means for the different cultivars are shown in Figure 5.6 (page 166), and were in very good accord with the published foliar late blight resistance ratings ($\rho = -0.96$); increasing foliar resistance ratings were associated with smaller values for square root lesion sizes.

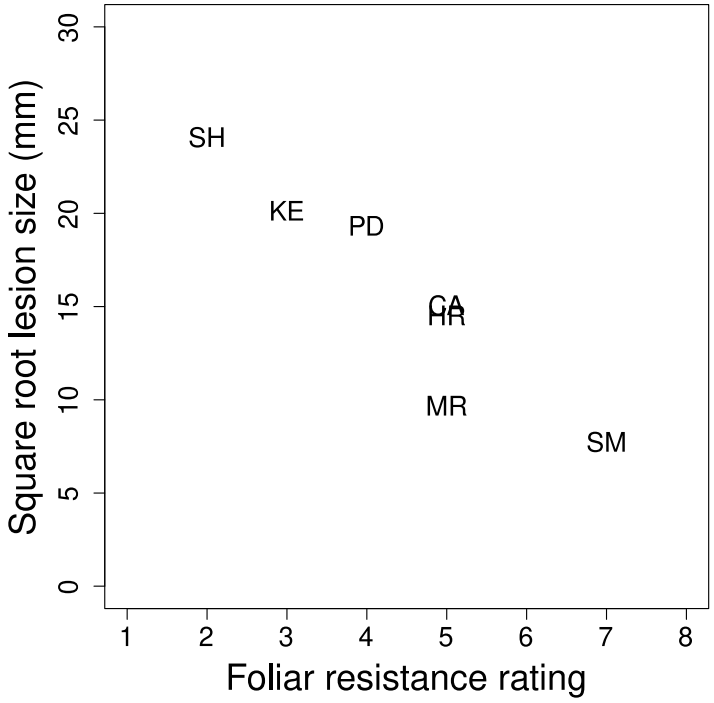

Figure 5.6. Mean lesion size after 144 hours in runs A – C of the tissue colonization assay. Square root of lesion size (mm) is shown on the vertical axis and the foliar resistance ratings according to the AHDB potatoes variety database are given on the horizontal axis. The two letter codes symbolise the cultivars for which the mean square roots of lesions sizes have been determined: SH = Shepody, KE = King Edward, PD = Pentland Dell, CA= Cara, HR = Harmony, MR = Markies, and SM = Sarpo Mira. Note that Cara and Harmony are almost coterminous within the graphic and their symbols may be difficult to read.

A larger range of cultivars were included within the 2017 cycle of experiments and a summary of these data can be found in Figure 5.6. There was a large and significant effect from cultivar within the mixed model ($p < 0.01$), and again there was a reasonable accord between the observed rates of tissue colonization and the published foliar resistance ratings. There were, however, some cultivars for which rank order was not as expected: Charlotte displayed lower rates of tissue colonization than would be anticipated from its foliar resistance rank, and Inca Bell significantly higher rates (comparable to the growth rates from cultivars with a resistance rating of 3). The correlation between expected foliar resistance rating and

observed mean lesion growth rates (see Figure 5.7 for a graphical representation) was $\rho = -0.68$.

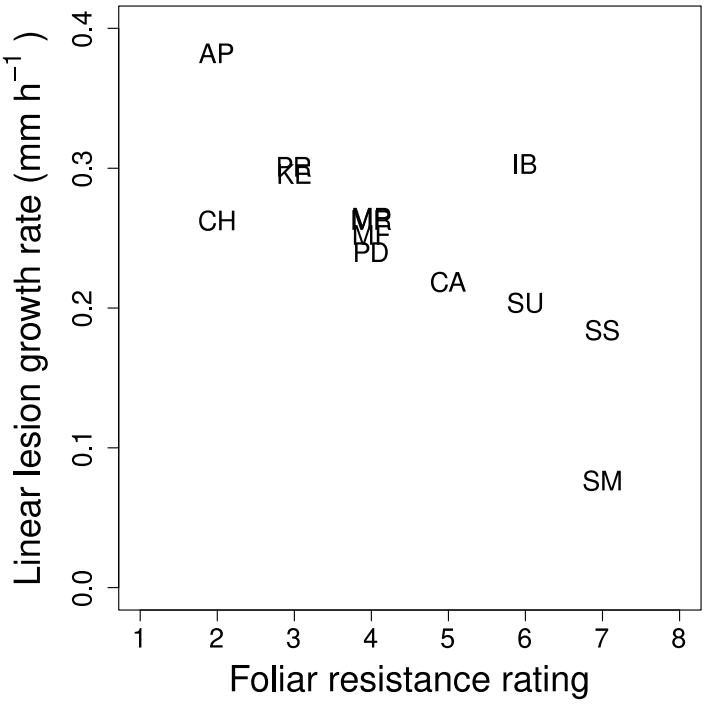

Figure 5.7. Rates of tissue colonization (mm hr^{-1}) in runs D – I of the tissue colonization assay plotted against the foliar resistance rating to late blight published within the AHDB potatoes variety database. Two letter codes refer to the cultivars included in the experiments (AP: Arran Piolt, CA: Cara, CH: Charlotte, IB: Inca Bella, KE: King Edward, MF: Marfona, MR: Maris Peer, MP: Maris Piper, PD: Pentland Dell, PR: Premier, SM: Sarpo Mira, SS: Sarpo Shona, and SU: Sarpo Una). Values for KE-PR and MR-MP-MF-PD were very close and may appear almost coterminous on the plot.

Biomass accumulate within samples taken during the latent period was determined using a similar method to Chapter 3 (Section 3.4, page 69); Figure 5.8 (page 168) shows the estimated DNA levels at the six timepoints for two contrasting cultivars: Sarpo Mira and Arran Pilot. C_q values were obtained for all samples which had been inoculated with *P. infestans* sporangia, whereas none were obtained from water inoculated controls, indicating no or very low (outside the range of detection) levels of *P. infestans* within the plant tissue.

Simple positive linear relationships between the natural log of *P. infestans* DNA and incubation time were significant for all cultivars, and these are summarised in Table 5.6 (page 169). A range of R^2 values were obtained from 0.75 for Arran Pilot to 0.18 for Pentland Dell, with lower values associated with slower growth estimates, indicating that there may be greater uncertainty at this end of the scale. Rank order of incubation coefficients did not match what would be expected from the foliar blight resistance ratings, nor from the lesion growth rates calculated from observed symptoms. The ranks of observed values are displayed graphically in Figure 5.9 (page 170); there is a weak association ($\rho = -0.38$) between higher foliar resistance ratings and slower growth rates.

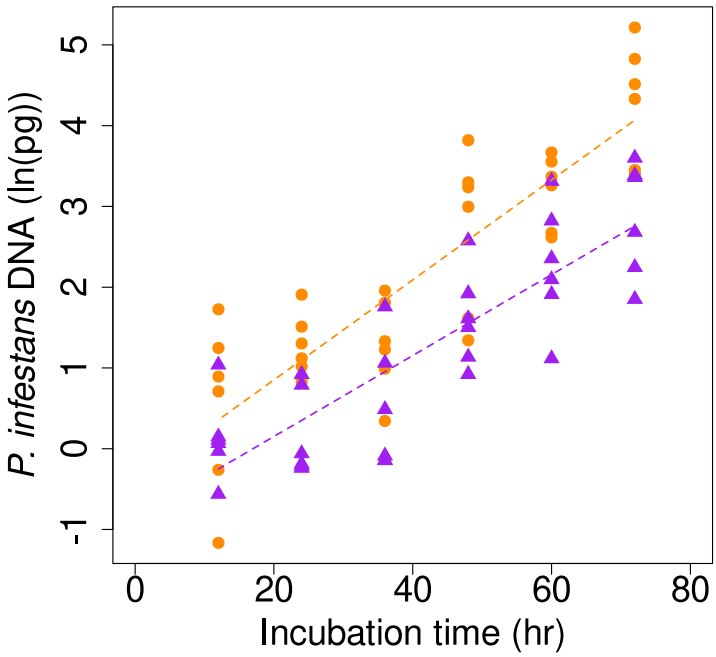

Figure 5.8. Relationship between initial *P. infestans* DNA quantity within a sample (ln(pg)) and time elapsed (hr) between inoculation and sampling. Data shown are from two contrasting isolates: Arran Piolt (●) and Sarpo Mira (▲), each point representing an extraction from a single inoculated leaflet (n = 6 per time point and cultivar combination). Simple linear relationships are fitted to each cultivar to obtain an index for biomass accumulation. For Arran Pilot this was $ln(Pi_{DNA}) = -7.29 + 0.062t$ ($p < 0.01$, $R^2 = 0.75$), and for Sarpo Mira $ln(Pi_{DNA}) = -7.76 + 0.050t$ ($p < 0.01$, $R^2 = 0.70$), where Pi_{DNA} is the quantity of *P. infestans* specific DNA in pg, and t is the time from inoculation to assessment in hours.

Table 5.6. Incubation biomass accumulation coefficients for 13 potato cultivars, obtained by linear regression of the natural logarithm of initial template quantity against incubation time. The accumulation coefficient corresponds to βba1 (with units ln(pg)hr^{-1}) in the fitted equation $\ln(Pi_{DNA}) = \beta_{ba0} + \beta_{ba1} \cdot t$. Quoted significance levels are for the parameter estimate for β_{ba1} rather than the regression line itself. The terms β_{ba0} are not shown here, these represent the natural logarithm of the amount of pathogen DNA present at the start of the assay (i.e. when t = 0).

Cultivar	Incubation coefficient (± 95% confidence intervals)	Significance of parameter estimate	R^2
Arran Pilot	0.06187 (± 0.01209)	$p < 0.01$	0.75
Cara	0.04208 (± 0.02217)	$p < 0.01$	0.29
Charlotte	0.08125 (± 0.02019)	$p < 0.01$	0.65
Inca Bella	0.05173 (± 0.01792)	$p < 0.01$	0.49
King Edward	0.06592 (± 0.01401)	$p < 0.01$	0.71
Marfona	0.05878 (± 0.01766)	$p < 0.01$	0.56
Maris Peer	0.07015 (± 0.02295)	$p < 0.01$	0.51
Maris Piper	0.04893 (± 0.01438)	$p < 0.01$	0.50
Pentland Dell	0.02280 (± 0.01626)	$p < 0.01$	0.18
Premier	0.04608 (± 0.02246)	$p < 0.01$	0.32
Sarpo Mira	0.05012 (± 0.01075)	$p < 0.01$	0.71
Sarpo Shona	0.05858 (± 0.03318)	$p < 0.01$	0.26
Sarpo Una	0.05508 (± 0.02103)	$p < 0.01$	0.44

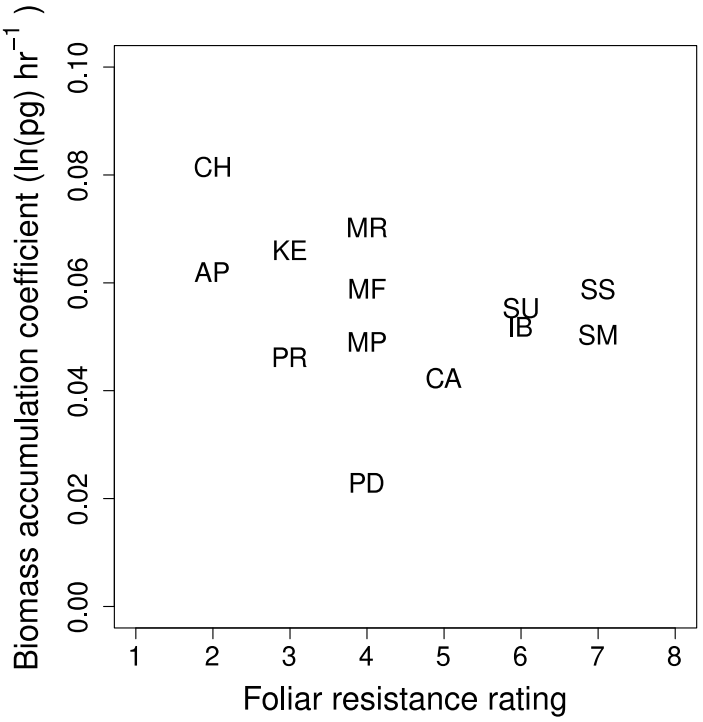

Figure 5.9. Rates of biomass accumulation ($\ln(pg)hr^{-1}$) in runs D – I of the tissue colonization assay plotted against the foliar resistance rating to late blight published from the independent variety trials (also available via AHDB potatoes' Varieties Database). Two letter codes refer to the cultivars included in the experiments (AP: Arron Piolt, CA: Cara, CH: Charlotte, IB: Inca Bella, KE: King Edward, MF: Marfona, MR: Maris Peer, MP: Maris Piper, PD: Pentland Dell, PR: Premier, SM: Sarpo Mira, SS: Sarpo Shona, and SU: Sarpo Una).

5.6 Discussion

Experiments within this chapter provide evidence that host resistance can act as a modifying factor on curative activity, but also that this effect may be variable and that further work is needed to give a full characterisation. Some indication of effect magnitude is provided, particularly by the natural inoculum trial (Section 5.2, page 146). Additionally, a range of cultivars were assessed for their rates of tissue colonization against an aggressive *P. infestans* clone on two separate measures. Implications from this and recommendations for the final decision aid are discussed briefly below, and in greater depth in Chapter 6.

Curative control was evident in runs A, B and D of the natural inoculum trial (see Section 5.2, page 146). Interestingly this differed between cultivars, with more resistant varieties showing larger proportional curative effects (runs A and B) and/ or extended curative control windows (run B and D). The control threshold in terms of degree hours did not appear to be constant across experimental runs. A curative effect was observed on King Edward plants in run A at 1008 °D_a, but not in runs B and D at 846 °D_a or 792 °D_a respectively. This may have been due to differing disease pressures at the exposure time, as the number of founder spores is very likely to also act as a modifying factor (Lapwood and McKee, 1966). One weakness of these experiments is that the isolate or isolate mix is not known. If quantitative resistance is pathogen and host genotype specific as suggested in previous work (Flier *et al.*, 2003b), then it would be unwise to assume a cultivar will always offer a fixed modifying effect in different locations and during different epidemics. This is one possible explanation for the variation seen between experimental runs.

The artificial inoculum trial (Section 5.3, page 156) and the plot scale field trial (Section 5.4, 159) generated more variable results. This may have been a function of the cultivars selected, the methodology used or simply due to the variability of field grown plants, as observed resistances can sometimes differ between the field and laboratory (Dorrance and Inglis, 1998). However, they both provided evidence that the curative effect declines with increasing pathogen development, and there were indications that this differed between cultivars. The low infection rates on high canopy Mozart leaves were interesting as leaf position is known to modify observed lesion growth rates (for example Carnegie and Colhoun (1980) report a linear relationship of increasing lesion size on more apical as compared to more basal leaves of King Edward plants from comparable inoculum concentrations), but as this was also reflected in the high-canopy untreated controls more data are needed before conclusions are drawn.

Results from the tissue colonization experiments demonstrated that the published foliar resistance ratings from the AHDB varieties trials are good general

guides to the rates of observed lesion growth. However, there were some varieties that deviated from their expected rank. There are three possible reasons for this (i) it is an artefact of the methodology used in these experiments, (ii) it is an artefact of methodology used in the independent variety trials or (iii) the form of quantitative resistance displayed by these varieties does not depend on slowing rates of tissue colonization or is only visible in the field. There is precedent for (iii) in the literature (Rietman *et al.*, 2012), and it is unfortunate that the time constraints did not allow the experimental cycle to be repeated to rule out (i), as (ii) seems unlikely.

The second measure of tissue growth used (genomic DNA quantification via qPCR) was less well correlated with the foliar resistance ratings, and indeed the two measures themselves were not well correlated. This could be due to the methodology used estimating biomass accumulation poorly, but the obtained growth coefficient for King Edward (0.066 $\ln(pg)hr^{-1}$) was very close to that obtained using the same experimental set up when the modifying effect of temperature in Chapter 3 (0.068 $\ln(pg)hr^{-1}$). An alternative explanation is that the two life cycle stages: tissue colonization within the incubation period and later symptom development progress at different rates. Life history trait of a pathogen can differ, and there may be unexpected interactions with host cultivars. However a much more extensive set of experiments would be needed to confirm this. The interaction between cultivar and genotype seen for Innovator is suspect to be due to the presence of an *R* gene to which one genotype was compatible, and the other incompatible. This has implications for curative control: one can imagine a situation where mix inoculum is present, and some spores are of incompatible genotypes with the cultivar grown. This would have the effect of reducing the founder population, and possibly leading to a larger than expected curative effect if a curative fungicide was applied. This is however quite a complex situation, and very difficult to determine in practice.

In terms of the final model, the inclusion of host resistance seems justified, with suitable caveats. It would be possible to use the obtained incubation coefficients to

modify the infection probability model developed in Chapter 4 on a cultivar-wise basis. This does not seem a sensible approach, as one would have to evaluate a very large range of cultivars (certainly the most commonly grown) and the final model would be cumbersome, probably requiring a large lookup table. As the link between foliar resistance rating does seem important, with the proviso that there are exceptions to this pattern (see above), and as the two measures differed, a pragmatic approach would be to use the foliar resistance ratings from the independent variety trials as a 'guide' factor with a suitable warning that results may vary in the field. The form that this will take is discussed in the next chapter, when the final decision aid is specified and validated against field data.

Chapter 6 Specification and validation of the decision aid

Abstract

This chapter specifies the requirements of the decision aid in more detail, and describes how it is intended to be used in terms of geographic scope, time frame, etc. Methods of validation are briefly summarized, and the index of separation (PSEP) selected as the most appropriate measure to use. A quantitative description of the decision aid is provided, as well as some simple implementation in R code. The final model takes time and temperature as inputs, and returns a probability of curative control failure. As a demonstration of potential outputs, this probability is then converted into one of four categories and reported to the end-user. While there is evidence that the cultivar can act as a modifying factor, it is not included in the final decision aid. Two field trials were conducted to generate data from which preliminary validation is carried out. The chapter concludes with recommendations for future validation programmes and potential revisions which could be made to the decision aid.

6.1 Introduction

The remit of this project was to investigate the feasibility of producing a decision aid to help guide the use of curative fungicides for the management of potato late blight, and to investigate factors which were thought to modify the curative effect. Over the previous chapters an appropriate model of temperature-dependent development has been selected (Chapter 3), and the relationship between pathogen development within the latent period and curative control has been described (Chapter 4). Information on the modifying factors developmental temperature (Chapter 3), *P. infestans* isolate (Chapter 4), and quantitative resistance of host cultivar (Chapter 5) has also been provided. These data provide a basis on which an outline of a decision aid can be constructed. It should be emphasised that the specifications here represent a starting point for further development and modification, suggestions for which are made in Section 6.6 (page 202). Nevertheless, even in its current form the decision aid may be informative.

6.2 Objective statement

The initial objectives of the investigations carried out in this project, as well as the potential decision aid were set out in Chapter 1 (Section 1.10, page 46 - 47), and are repeated in more detail here in the form of an objective statement (Haefner, 2005). The aid should be a simple *tactical* tool which provides growers and/or agronomists with guidelines for the appropriateness of curative fungicide treatments when managing potato late blight. The tool will be tactical and not strategic, because the choice to include a fungicide formulation with curative properties is a relatively short term decision made in response to local conditions: i.e. we suspect early latent infections of *P. infestans* are present within a crop, and we wish to arrest their development if possible. A strategic tool, in contrast might imply that curative treatments should be the main method used to control potato late blight, and that the aid provides criteria or thresholds that prescribe treatments. This is highly inadvisable, and an "only treat curatively" strategy would likely be counter-productive by increasing the probability of control failure, and potentially contributing to fungicide resistance issues (Beckerman *et al.*, 2015) as the number of usable a.i. would decrease (protectant only compounds would have limited usefulness).

It is envisaged that the curative decision aid therefore will sit as part of the wider system of potato late blight control. It should complement, as far as possible, existing aids and tools that are in widespread use. The most obvious of these is the BlightWatch warnings that use the Hutton Risk Critera to issue infection warnings (introduced in Chapter 1, Section 1.4, page 10), but the aid could be used with or within other systems, for example as a module to describe curative activity. Existing support systems tend to include curatively activity as a simple threshold which is based on chronological time: i.e. it is sometimes assumed that there is full curative activity before 24 hours post-infection, and there is no curative activity after this point (Spits and Wander, 2001). Replacing this with an expected level of control based on anticipated pathogen development may lead to better decisions on fungicide formulation choice and use.

The aid will use inputs based on the evidence gathered in Chapters 3 – 5. Disease development time, from exposure to inoculum under conditions conducive to infection (i.e. a Hutton Period) through to time of curative treatment and a temperature profile during this time (ideally with readings at hourly or sub-hourly intervals, in °C) are the most important inputs. The modifying effect of cultivar was provisionally included (see Chapter 1 section 1.10, page 46), but as is detailed in Section 6.4 (page 185), ultimately there was not enough evidence for its inclusion. While the experimental work in this project provided evidence that pathogen isolate (and perhaps genotype) can act as a modifying factor (see Chapter 4, Section 4.3.7, page 122) is not clear how, under the current epidemiological surveillance scheme for potato late blight such information can be ascertained in a timely fashion, nor estimated accurately. There is, at present, insufficient evidence to include other potentially modifying factors such as inoculum density or fungicide residues on the phylloplane.

The decision aid will be limited to the *P. infestans-S. tubersosum* pathosystem, and is intended for use in Great Britain (GB). However, much of the experimental work that underpins the model choice and parameter estimation in the aid has been laboratory or glasshouse based. The aid uses data derived from experiments using a single fungicide formulation, an aggressive *P. infestans* isolate, and (mostly) a single susceptible cultivar, as representative of late blight infections and control practices in potato crops grown across GB. Clearly, therefore, thorough validation under field conditions in a range of circumstances is required. Methods used for preliminary validation are given in Section 6.5 (page 192), and a specification for a wider validation on page 202. It is fully possible for others to test how informative the decision aid is under other climates, and with *P. infestans* population and potato cultivars common to other regions, though this is outside the scope of this study. It may also be possible to extend the aid to the *P. infestans-S. Lycopersicum* system, although very significant differences in the agronomic practices will likely necessitate modifications (Nick *et al.*, 2014).

The time-scale over which the aid will provide guidance is by definition prescribed due to the phenomena it describes (curative activity and the latent period of *P. infestans*). The duration of the latent period (and thus the curative control window) will vary with the air temperature and will thus be context dependent. A more abstract definition of the time-scale is that the decision aid is intended for use whenever a grower or agronomists is selecting a fungicide formulation after high infection risk conditions have occurred (Dancey *et al.*, 2017); if it turns out that this is substantially outside the curative control window, the aid will report this. The output which the aid provides, and the criteria for validation are inter-related and the following section (6.3) is given over to a discussion of this topic.

6.3 Decision aid output and criteria for validation

The form of output the aid produces is an important consideration, as is the criteria by which it will be validated. The term *validation* is used throughout here, rather than *verification* (or similar) as validation implies that the object in question has been judged acceptable (for example legally or logically) for a specific purpose. Verification implies truth, which it is clearly not possible to capture entirely in a simple model; it is entirely possible for a model to be untrue and to contain demonstrable inaccuracies, but still be useful for some purposes (Oreskes *et al.*, 1994).

In crop protection, decision aids and rules are typically concerned with prediction of disease or pest occurrence (Yuen, 2006), and under what circumstances decisions to withhold or apply a control measure are justified; i.e. a binary prediction of a true state which also takes one of two values (present/absent). A tool's prediction about the state of a system will either be correct, or it will be incorrect. There are well-developed methodologies to measure the performance of such predictive tools (Madden, 2006). Sometimes the potential outcomes of such a system are presented as a two by two contingency table (Skelsey

et al., 2018). From this table we may calculate the probability of disease given that disease was predicted (referred to as the positive predicted value, PPV) and the probability of no disease given that no disease was predicted (referred to as the negative predicted value, NPV). Both PPV and NPV vary with the prevalence of disease, so are not simple characteristics of a tool for making disease forecasts.

Also available from the two by two contingency table are sensitivity and specificity (Altman and Bland, 1994). Sensitivity is the true positive rate, and can be expressed as a proportion calculated from the number of situations where presence was *predicted* over the number of situations where the disease was *truly* present. Specificity, on the other hand is the true negative rate, and can be expressed as a proportion of *predicted* absence situations out of all situations where the disease was *truly* absent. Unlike PPV and NPV, sensitivity and specificity are independent of disease prevalence and so are characteristics of the prediction tool. From sensitivity and specificity, one can calculate a false positive rate, 1 − specificity, which refers to situations where disease is predicted but it in fact does not occur, and also the false negative rate, 1 − sensitivity, which refers to the inverse situation where no disease is predicted but disease does occur (Lalkhen and McCluskey, 2008). Sensitivity and specificity are linked, and can be changed by altering the threshold between 'presence' and 'absence'; often called a decision threshold, as it is the catalyst for implementing a control measure (Hughes, 1999; Yuen and Mila, 2004). A higher decision threshold will increase the specificity of a tool, but will at the same time lower the sensitivity. The inverse is also true; increasing sensitivity lowers specificity (Yuen and Hughes, 2002).

Decision tools can be evaluated graphically by plotting the true positive rate (sensitivity) against the false positive rate (1 − specificity) as the decision threshold is varied, in a procedure known as receiver operating characteristic (ROC) curve analysis which captures information about the trade-off between the sensitivity and specificity. A perfect system would score 1 for the true positive rate and 0 for false positive rate (Madden *et al.*, 2007). The worst possible systems would lie at some

point along a diagonal line (sometimes called the line of no-discrimination (Nguyen and Devarajan, 2008)) from co-ordinates (0, 0) to (1, 1). Predictions from a tool with such a profile are no better than those produced at random. Which specific cut-off value to use can be selected using statistics such as Youden's J[19] (Schisterman *et al.*, 2005), but can also be informed by the costs associated with each decision choice. If the costs of decisions are well understood, a decision threshold can be selected which minimizes these (Skaltsa *et al.*, 2010). Of course, a decision-maker's perception of risk (distinct from the definition given in Chapter 1, Section 1.4, page 13) is also an important consideration; if a decision-maker fears false negatives (disease is present, but it was predicted to be absent and treatment which should have been applied was withheld) then they may be inclined towards thresholds with higher sensitivity. The sensitivity and specificity of a tool can also be summarized using likelihood ratios, for example, the likelihood ratio of a positive prediction is given by sensitivity / (1 – specificity)[20]. This opens the possibility of Bayesian applications using odds and prior probabilities (Yuen and Hughes, 2002) which are not explored here.

As will be explained in Section 6.4 (page 185), the decision aid will output a metric, which can range between 0 and 1, and could be interpreted as the probability of curative control failure. It is possible that this metric could be presented to the end user in its raw form, but this is not thought appropriate. End-users (indeed, the public at large) sometimes lack an intuitive understanding of probabilities (Krebs, 2011) and as the aid is intended to add easily interpretable information, it should deliver this information in as accessible a format as is practical. Additionally, numbers presented without a thorough explanation of how they were produced tend to inspire more certainty than is justified, and therefore presenting probabilities or percentages may give an impression of greater accuracy than is warranted.

19. J = sensitivity + specificity – 1
20. This is more properly specified using conditional probabilities, which are not given here (Yuen and Mila, 2004).

Alternatively, a decision threshold could be used, and a binary prediction could be provided to the end user; i.e. curative control will likely succeed or it will likely fail. There are several attractive features to this approach. Firstly, it is very easy to interpret. Secondly, the methodology for validation and threshold selection via ROCs is well documented and has been applied to numerous other decision and assessment tools within plant pathology (Dancey *et al.*, 2017; Roscher *et al.*, 2016; Skelsey *et al.*, 2018; Tegg *et al.*, 2014). However, much information is lost when a continuous predictor is dichotomized (Beckstead and Beckie, 2011)[21], and part of the rationale behind using dose-response methodology (Ritz, 2010) to describe the relationship between pathogen development and expected curative control in Chapter 4, Section 4.4 (page 129) was to produce a relationship that could be interpreted rather than a simple threshold. A single decision threshold for curative control failure was therefore neither determined nor validated here, though it would be entirely possible for the data presented here to be used for this purpose.

Instead, as a demonstration of a possible output method, a multi-categorical system was used. In other words, the probability metric will be binned, and the labels of these bins presented to the end user. The nature of these bins, and the labels that they are assigned is presented in Section 6.4, page 185. A total of four bins were selected, but the choice was somewhat arbitrary. The decisions available to a grower or agronomist surrounding the use of a curative fungicide are not straightforward. They have many options, including, but not limited to: using curative a.i.(s) (but there are several to chose from), using protectant only a.i.(s) if the curative control window was thought to have elapsed, or using protectant a.i.(s) with an adjuvant to improve coverage (to focus on limiting new infections, rather than arresting the development of those that are already present). Breakpoints were set at 0.25, 0.50, and 0.75 (more formal definitions are given in Section 6.4, page 185) and were assigned labels indicating curative control is decreasingly likely to occur. It is anticipated that end-users will interpret this information in the context of their

21. Though note that information is not the same as meaning, so the 'information' that is lost many have no utility for our purposes, and thus the loss may not be as significant as it first appears.

own experience, and judgement of the specific situation (crop conditions, weather forecast, local disease pressure etc.) to make a control decision.

Multi-category predictors are not common in plant epidemiology, but there are examples of their use in the medical (Leonard *et al.*, 1991; Olsson *et al.*, 2004) and meteorological literature (Mason, 2008). Several approaches have been developed to assess the quality of these patient prognoses or weather forecasts (Benedetti, 2010). In a meteorological context, where simulation models are frequently used, probabilistic information from ensemble forecasting is sometimes presented. Ensemble forecasting refers to a process of generating a set of forecasts for a given situation using Monte Carlo analysis, and using uncertainty in initial conditions to generate the range of outcomes (Gneiting and Raftery, 2005). Statements can then be made about the spread of outcomes and their relative likelihoods. This approach cannot be used with the prototype decision aid because it is a deterministic model. An alternative validation approach sometimes used for probabilistic predictions in meteorology, and frequently used in medical epidemiology is the use of scoring rules (Moons *et al.*, 2002). There are several such rules (Benedetti, 2010), an example of which is the Brier score (Brier, 1950), which in its original specification ranged between zero (perfect forecast) and two (worst possible forecast). The Brier score is reduced (i.e. becomes better) as the correspondence between forecast probabilities and observed probabilities increases.

Even when a forecasting or prognostic tool meets a specified criterion for accuracy of prediction, it may still have little practical use if it is unable to designate situations (patients in a medical setting, individual plants or fields in a plant epidemiological one) into useful groups. Altman and Royston (2000) make a distinction between a statistically valid model and a clinically valid model. A statistically validated model meets standards set out in goodness of fit or bias assessments. A clinically validated model is one that is transportable (Royston and Sauerbrei, 2005) to a new 'case-mix' (Fetter *et al.*, 1980), that is a new set of patients at a different centre, and that still provides clinically useful classifications. A tool

can fail statistical validity measurements (it may for example produce biased predictions) but still separate patients into clinically useful groups. Altman and Royston (2000) give details of a measure termed the index of separation (PSEP) to determine then intrinsic prognostic information that a model provides. It can be used when a model assigns patients to categories which have associated probabilities for an event (survival to a set time-point, disease occurrence, etc.). The prognostic categories for best and worst outcomes (i.e. the groups with lowest and highest probabilities of mortality) are then examined, and the probabilities of the event for each group determined. P_{worst} is the predicted probability of the event occurring (death, disease etc.) for members assigned the worst prognosis, and P_{best} is the probability of the event (death, disease etc.) occurring for members assigned the best prognosis. The index of separation (PSEP) is calculated as:

$$PSEP = P_{worst} - P_{best}$$

For a predictor with two prognostic groups, PSEP = PPV + NPV − 1 (Altman and Royston, 2000). Calculation of PSEP can then be used to compare how well a prognostic tool separates patients with good or poor outcomes at different centres. Whether we consider the model valid based on this calculation depends on the aims of the model. Separating patients into groups will in most cases lead to different treatment decisions beings made for (at least some) of the groups, treatments may have associated costs (in economical terms, or in a medical setting they may be associated with risks for the patient), and we may only be able to justify treatment interventions for a subset of the assigned categories. This judgement will be context dependent.

The decision aid produced by this study, as set out in Section 6.4, page 185 is a prognostic model, with the chief predictor variable being the anticipated disease development time at the point of curative treatment. The prognostic groups are categorised pragmatically around the likelihood that the curative control treatment has failed, or in other words that the window for curative control has been missed.

It is a slightly unusual tool, as it is not making forecasts about disease occurrence as many other plant disease decision rules do (Yuen, 2006), but rather the prospects of a control measure (curative treatment) given that disease is assumed to have occurred. PSEP is therefore an appropriate measure to use here, as end-users will make a decision about which control measure to deploy. Absolute accuracy of predictions is perhaps less important than an ability to separate situations into those with a high probability that curative control will fail (worst prognosis) and those with a very low probability that curative will fail (best prognosis).

Measuring curative activity in the field is very challenging (how can we know that an outbreak is due to curative control failure if we do not know when initial infections occurred?), and it is not immediately obvious how data from which to validate the decision aid can be obtained. A tentative proposal is set out in Section 6.6 (page 202), and in addition two field trials with similar methodologies at different geographical locations were carried out to generate data-sets for preliminary validation.

6.4 Specification of the curative control decision aid

A schematic of the decision aid is set out in Figure 6.1:

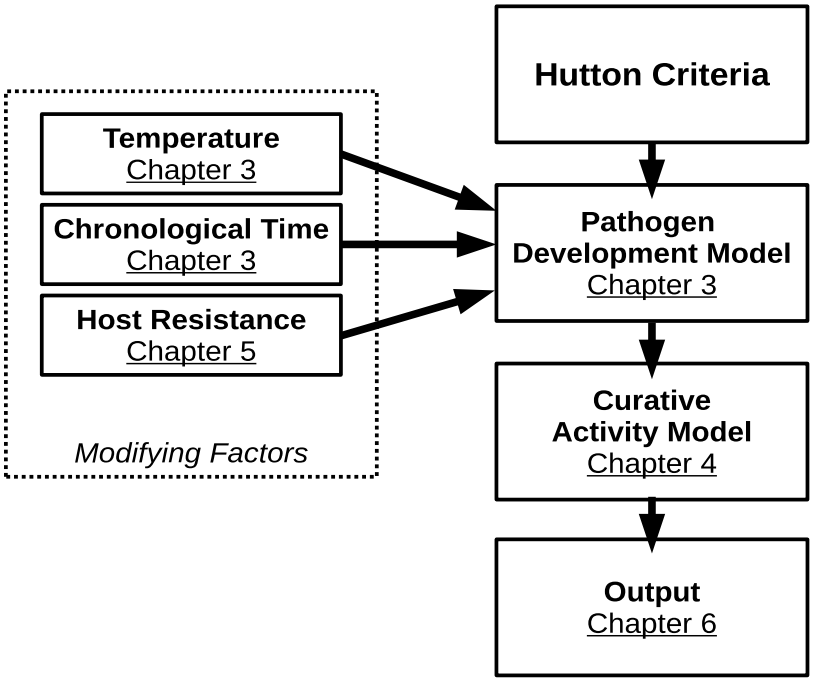

Figure 6.1. Schematic of decision aid, as set out in the planning stage of the project. Note that, although host resistance was explored within Chapter 5 the prototype decision aid set out in this chapter does not include a term for host resistance, but suggestions for its incorporation are made on page 188.

The first component of the decision is the temperature-dependent development model, which was derived in Chapter 3 (Section 3.6, page 102, Equation 27-A):

$$f(T) = \begin{cases} 0 & T < 0.45 \\ 2.21 \cdot 10^{-5}(T - 0.45)^2(30.51 - T) & 0.45 \leq T \leq 30.51 \\ 0 & T > 30.51 \end{cases}$$

where f(T) is a rate function of the observed temperature T (°C), with the units ln(pg)hr^{-1}. This gives the expected development *per hour* of *P. infestans* in planta given a specific temperature. It is anticipated that temperature data will be obtained

as regular readings for a given time step. The expected development for a set of temperature measurements and at given time-steps can then be summed to produce an accumulated expected development:

$$g(f(T),\ t_s) = \sum_{i=0}^{n} f(T)_i t_{si}$$

where $g(f(T), t_s)$ is the expected accumulated development with units lg(pg), $f(T_i)$ is the development per hour as a function of temperature defined above (page 185) for the ith temperature observation with units ln(pg) hr^{-1}, t_{si} is the time step in hours (i.e. if a daily average temperature was provided, $t_s = 24$) of the ith observation, and n is the number of temperature observations. From the expected accumulated development value ($g(f(T), t_s)$), the raw metric produced by the decision aid is produced. This is termed P_{cur}, and can be interpreted as the probability of curative control failure, given an expected pathogen development value:

$$P_{cur}(g(f(T,t_s))) = \frac{1}{1 + e^{-0.865(g(f(T),t_s) - 2.924)}}$$

where P_{cur} is the probability of curative control failure, $g(f(T), t_s)$ is the expected accumulated development given a set of temperature readings (with units °C) and a time step (in hours). For practical purposes P_{cur} can be binned into four categories shown in Table 6.1, these categories have been given labels which lend themselves to ease of interpretation.

Table 6.1. Ranges of P_{cur} and their associated prognostic categories. P_{cur} can be interpreted as the probability that curative control will fail, given an expected level of pathogen development.

P_{cur} range	Categorical output
$0 \leq P_{cur} < 0.25$	Curative control **very likely**
$0.25 \leq P_{cur} < 0.50$	Curative control **somewhat likely**
$0.50 \leq P_{cur} < 0.75$	Curative control **unlikely**
$0.75 \leq P_{cur} \leq 1.00$	Curative control **very unlikely**

For illustrative purposes a very simple implementation of the decision aid using R code will now be shown. Firstly, a function which returns the expected development per time step is defined:

```
R> ExpDev <- function ( T, delta_t = 1 )
+          { if ( T <=  0.45 )
+              { return( 0 ) }
+            if ( T >= 30.51 )
+              { return( 0 ) }
+            if ( T > 0.45 & T < 30.51 )
+             { return( 2.21e-05 *
+                      ( ( T - 0.45 )^2 ) * ( 30.51 - T ) *
+                      delta_t ) }
+           else { return( 0 ) } }
```

where `T` is the temperature with units °C, and `delta_t` is the time step in hours which defaults to 1 if not specified. Outside its defined range, `ExpDev` returns zero. For example, in a situation where three temperature readings of 18 °C , 17 °C and 16 °C are taken at ten minute intervals. the expected development would be:

```
R> temp.data <- c(18,17,16)
```

```
R> sapply( X       = temp.data,
+          FUN     = ExpDev,
+          delta_t = ( 1/6 ) )
```

```
[1] 0.01419229 0.01362989 0.01292317
```

where `temp.data` is a vector containing the temperature observations. `ExpDev` has provided us with expected developments of 0.01419229 ln(pg) for the first, 0.01362989 ln(pg) for the second and 0.01292317 ln(pg) for the third interval. Note that the time step `delta_t` *must* be specified or incorrect developmental values will be given. In this case, because temperature readings are taken every ten minutes, `delta_t` is set to 1/6. In the demonstration code `delta_t` defaults to 1 (= hourly intervals), but it may be desirable in future implementations to display a warning, or a check-point where `delta_t` must be manually assigned by the end user. Once

the expected development at each time-step is obtained, the accumulated development over a specific time period is calculated by summing the appropriate expected development outputs. In the demonstration code this is done manually, but it would be possible to automate the process. For example, if forecasts for air temperature over a future time period (for example 24 hours) are provided, then the expected curative control failure at set intervals (say 6, 12, 18 and 24 hours) could be displayed to the end-user. Below, the accumulated development for a simulated period of 24 hours where the air temperature was a constant 18 °C and readings were taken hourly, are shown:

```
R> temp.data <- rep( x     = 18,
+                    times = 24 )

R> hly.dev <- sapply( X       = temp.data,
+                     FUN     = ExpDev )


R> acc.dev.18 <- sum( hly.dev )

R> acc.dev.18

[1] 2.04369
```

where `temp.data` is a vector containing the temperature observations, `hly.dev` is a vector containing the expected development at each temperature observation and `acc.dev.18` is a single value obtained from summing the values contained in `hly.dev`. This accumulated development value can used as input for a function which calculates P_{cur}:

```
R> Pcur <- function ( acc.dev )
+    { return( 1 / ( 1 + exp( ( -0.8646 ) *
+                             ( acc.dev - 2.924 ) ) ) ) }

R> Pcur( acc.dev.18 )

[1] 0.3184041
```

where `acc.dev` is the input value for accumulated expected development. The returned value (0.3184041) can be interpreted as the probability of curative control failure. Using the categories specified in Table 6.1 (page 186), a projection of "curative control **somewhat likely**" would be given for this instance. The grower or agronomist can then make a decision, taken in conjunction with the other local factors (state of crop, time since last fungicide treatment, etc.), regarding the use of a fungicide product with curative activity.

In early iterations a term was included in the prototype model and demonstration code which slowed expected development if infections took place on a more resistant cultivar. However, what form this relationship should take was not obvious from the data generated in Chapter 4, and including an arbitrary scaling factor for resistant cultivars did not improve the decision aid's predictions (data not shown). Host resistance is clearly important as a modifying factor, and should more data become available it would be straightforward to incorporate it within the model. Indeed, conceptually many modifying factors could be included in the decision aid, provided their impact on pathogen development within the latent period is well described. Control failure predictions for a range of fixed temperatures are shown in Figure 6.2 (page 190). The relationship between the P_{cur} and accumulated development, and how this relates to the proposed prognostic categories are shown in Figure 6.3 (page 191). For example, if temperature readings after an infection event had remained at a constant 6 °C for 60 hours, a P_{cur} value of 0.16 would be obtained from the decision aid (the expected development given these conditions would be 1.00 ln(pg)). This P_{cur} value falls within the prognostic category of "curative control **very likely**", which corresponds to the bottom left section of Figure 6.3. It is expected that an agronomist would feel justified in including an active ingredient with curative properties within a blight spray given these circumstances.

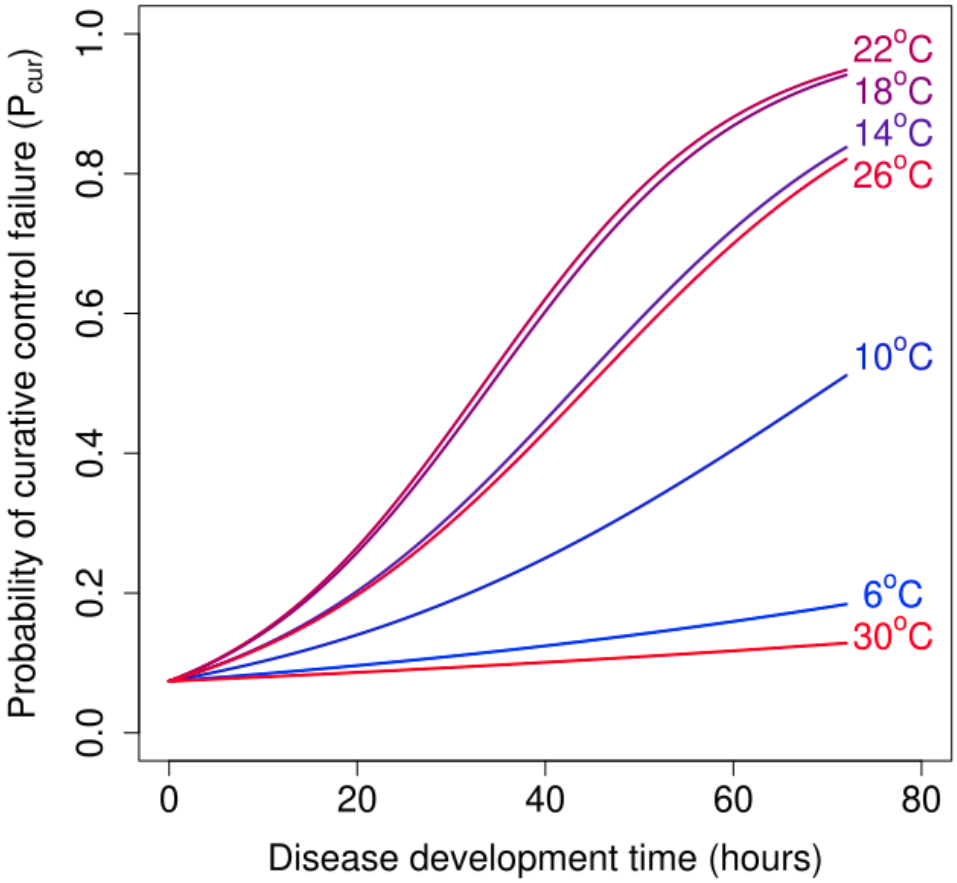

Figure 6.2. Output of the prototype decision aid when provided with simulated data containing constant temperature observations of 6, 10, 14, 18, 22, 26, and 30 °C. The coloured curves show the probability of control failure (P_{cur}) as disease development time increases. Note that these are simulated data, not derived directly from observations of curative control treatments in the laboratory or field.

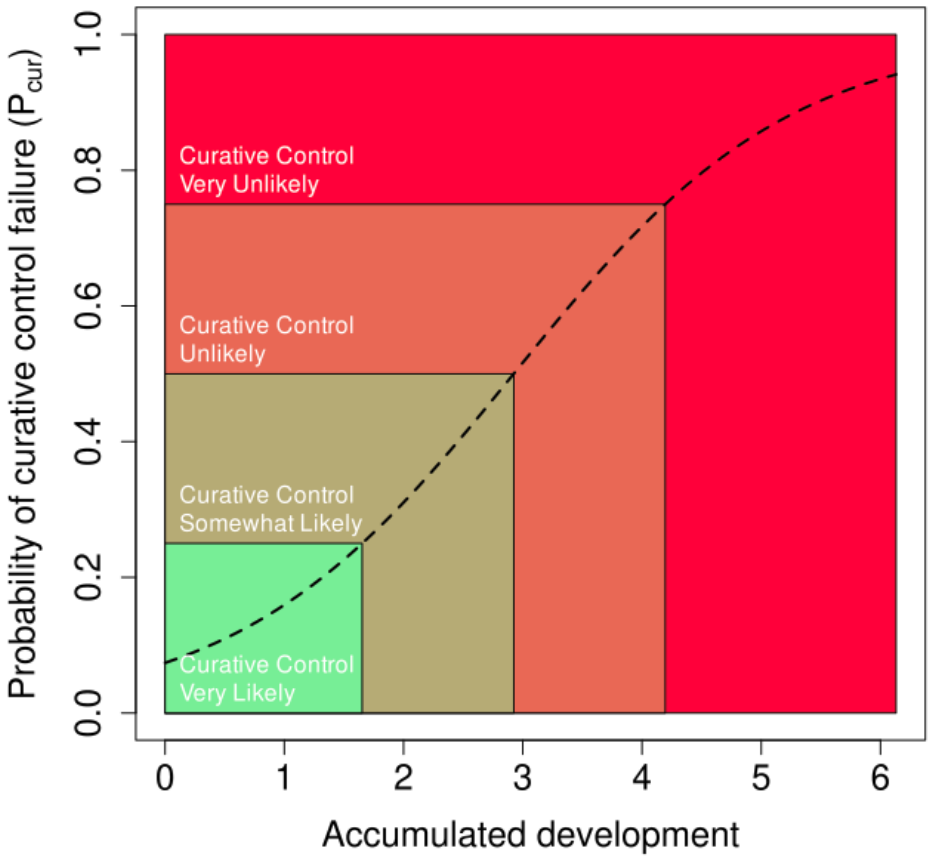

Figure 6.3. Plot representing the relationship between P_{cur} (the probability of curative control failure calculated by the decision aid), accumulated development (with units ln(pg)), and the four possible output categories of the decision aid. The black dashed line shows how the value of P_{cur} increases with increasing accumulated development. The greater the value of P_{cur}, the greater the predicted probability of curative control failure. the coloured sections of the plot represent which categorical output would be returned by the decision aid for a range of P_{cur} values.

6.5 Validation of the decision aid

The relationships that underpin both models (between thermal time and pathogen development, and between pathogen development and curative control) within the decision aid are based fundamentally on laboratory assays under controlled conditions, with some additional information from the field. However, to be useful to end-users the utility of the system should be validated in conditions similar to those in which it will be used. In order to accomplish this, field trials following a similar methodology to that laid out in Chapter 4 (Section 4.3.8, page 125) were conducted. The aims of this preliminary validation were to compare the predictions made by the decision aid to the control levels observed, and to assess the prognostic information that they contain (by calculation of PSEP). This will give an indication if the decision aid is providing useful separation of cases (plots in these instances), and if it is transportable to locations other than those in which it was constructed. The decision aid as specified is an unusual example of this type of tool, as it is specifying the risks of control *failure* within fungicide-treated units (field, plots, individual plants, etc.) which are anticipated to be infected. The methodology used here includes artificial inoculations, with the untreated plots serving to indicate the level of disease incidence without intervention. Control failure can then be assessed in relation to this level.

6.5.1 Validation field trials: materials and methods

Field trials were conducted in the summer of 2017. The same methodology was used at two sites approximately 80 miles apart; SRUC's Boghall site in Midlothian, and the Auchincruive site in South Ayrshire. Two aspects of the model were of particular interest: the influence of thermal-time on the observed curative effect, and the modifying role that varietal resistance plays. In order to assess these two features, field trials were designed to incorporate staggered inoculation times and curative treatments (to generate different disease development values at the curative treatment time) and two cultivars of contrasting foliar resistance ratings. The cultivars King Edward and Cara were selected, as these were the most heavily used in setting the models' parameter values.

At both sites the experimental structure was very similar: a total of 40 plots were arranged in four blocks, 10 plots per block. Plot dimension were 2.7 · 1.8 m at Boghall and 2.7 · 1.7 m at Auchincruive. The difference in width was due to the plots consisting of two planted ridges, with slightly different equipment available for cultivation at the two sites. 20 seed tubers were planted per plot, with 0.3 m spacing between plants within rows. A 'split-plot' structure was used, in that each pair of adjacent plots were allocated two varieties, Cara or King Edward. The position of these within a pair of plots was randomized. This resulted in five plot-pairs per block, which were allocated at random one of five categories: F_1, F_2, F_3, F_u, or I_0. $F_1 – F_3$ were treated 1, 2, or 3 days post inoculation and the F_u plots were left untreated, and I_0 plots did not receive an inoculation and were used as a negative control.

Artificial inoculations were used in both experiments, and the same isolate 2012_13A2-1 was used in both cases. Inoculum was prepared from sporulating lesions on detached King Edward leaves as described in Chapter 2, and was adjusted to ~ $3.85 \cdot 10^5$ sporangia per ml. Two batches of inoculum were used for two separate inoculations on the same day. Inoculation sites were pre-identified with loop-lock labels of distinct colours so that they could be easily tracked. Each inoculation site consisted of two stems which could easily be bunched together. The first ridge of each plot was assigned to the first set of inoculations, and the second ridge the second set of inoculations. The first set of inoculations took place in the morning, with the order, and time at which plots were inoculated recorded. The procedure was then repeated for the second set of inoculations beginning mid-afternoon. Each set of inoculations took approximately 5 hours, generating a range of development times when taken as a whole. The inoculation procedure itself was the same as that outlined in Chapter 4 (Section 4.3.8, page 125): at each pre-designated site the two stems were bunched together and ~ 2.6 ml of inoculum was delivered across the foliage from a misting bottle. This resulted in a scatter of droplets localized at the inoculation site, at some of these sites infection and

incubation occurred, and lesions developed. An example of an inoculation site not treated with fungicide, and a typical pattern of lesion scatter after 7 days, is shown in Figure 6.4. In total five sites (= 10 stems) were inoculated in each plot.

Figure 6.4. Blight lesions at an inoculation site (7 days post inoculation). Bunches of stems were artificially inoculated with a suspension of *P. infestans* sporangia applied from a spray bottle. This generated a number of inoculation sites (in at least some of locations where droplets settled), which then potentially developed into lesions. A typical pattern of lesion incidence at an inoculation site is shown in the image.

After inoculation, the foliage surrounding sites was bagged with transparent plastic bags to increase the relative humidity at the inoculation sites and maximize the probability of infections occurring. A small hole was made in the corner of each bag to limit the build up of heat in the bags. The bags were removed on the morning of the following day. Plots were treated curatively at ~ 24 hours (F_1), ~ 48 hours (F_2), or ~ 72 hours (F_3) from the commencement of inoculations, with propamocarb-HCl + fluopicolide at 1.6 l ha^{-1} in a 200 l water volume. At Boghall plots were sprayed using an AZO backpack sprayer, and at Auchincruive a tractor-mounted sprayer (350 kPa spray pressure and F110-03 nozzles) was used. Timing of treatments was recorded and used in conjunction with the plot inoculation times to calculate thermal time, and thus expected development. Symptoms were assessed 7 days post inoculation. Stems on which leaves had been exposed to inoculum were identified using the loop-lock tags, and a record was made of the number of late

blight lesions per leaf. Leaves between the first fully unfurled leaf below the stem apex (leaf one) to leaf eight were assessed as these were the most likely to have intercepted both inoculum and fungicide spray.

6.5.2 Validation field trials: data handling and statistical analysis

Temperature data from the Met Office stations at the two sites were used in conjunction with the recorded inoculation mid-point times and treatment times for each plot (the average time to inoculate each plot was approximately 10 minutes) to provide a temperature profile for each, from inoculation to treatment. The demonstration code presented Section 6.4 (page 185) was used to generate P_{cur} values and output categories. Physical and thermal time values were also calculated to aid with interpretation and comparisons. Based on experience with similar field trials (see Chapter 4, Section 4.3.8, Page 125), the raw lesion counts per compound leaf were recorded. Linear regressions of the natural log + 1 transformed counts against thermal time, with cultivar included as an additional factor were performed. As there were two inoculation times, and two distinct cultivars, an ANOVA was performed on the untreated plots only to see if data could be pooled, or alternatively if inoculation times needed to be treated separately. Natural log + 1 lesion count was included as the response variable, and with cultivar, inoculation time, and block as factors.

6.5.3 Validation field trials: results

At both sites there were large, significant differences in the mean lesion counts per compound leaf between the two inoculation times (Boghall: $p < 0.01$, Auchincruive: $p < 0.01$) and between the two cultivars (Boghall: $p < 0.01$, Auchincruive: $p = 0.04$), but in neither case was the interaction term significant (Boghall: $p = 0.11$, Auchincruive: $p = 0.59$). Each inoculum batch was treated as a distinct event and analysed separately. In both cases lesions were more abundant on King Edward than Cara plants, and more lesions developed at sites inoculated in the afternoon than the morning inoculations. Untreated lesion counts were two to six times greater in the untreated plots at Boghall compared with Auchincruive.

The results from the Boghall trial are summarized in Figure 6.5 (page 197), which is faceted to separate the two inoculation times. With the morning inoculations, inoculated but untreated Cara plants had a mean lesion count per leaf of 4.05 ($\pm$ 0.70, 95 % c.i.), and inoculated but untreated King Edward plants had a mean lesion count per leaf of 18.21 ($\pm$ 2.29, 95 % c.i.). The corresponding values were higher for the afternoon inoculations: 9.07 ($\pm$ 1.43, 95 % c.i.) for Cara, and 29.31 ($\pm$ 3.90, 95 % c.i.) for King Edward. The regression relationship between ln(lesion count + 1) and thermal time, with cultivar as a factor, provided a very good description of the data sets for both morning ($p < 0.01$, $R^2 = 0.87$) and afternoon ($p < 0.01$, $R^2 = 0.69$) inoculations. Parameter estimates for these relationships are given in Table 6.2, note that the R^2 values here refer to the linear relationships with the transformed data, and not the plots shown in Figure 6.5 (page 197). In both cases thermal time (morning $p < 0.01$, afternoon $p < 0.01$) and cultivar (morning $p < 0.01$, afternoon $p < 0.01$) were significant terms in the regression. Lesion count increased with time, and, in general, had reached values comparable to the untreated controls in the F_3 plots.

Table 6.2. Parameter estimates for the simple linear model: ln(lesion count + 1) = accumulated thermal time + cultivar (as a factor) of data from the Boghall curative field trial. Accumulated thermal time has the units °C · hr, and refers to the time between inoculation and treatment with a curative fungicide. Two batches of inoculations took place, one during the morning and one during the afternoon. Separate models were used to describe each in turn.

Term	Parameter Estimate	95% confidence interval
		Morning inoculations
Intercept	0.584	0.196, 0.973
Thermal time	$1.01 \cdot 10^{-3}$	$4.83 \cdot 10^{-4}$, $1.55 \cdot 10^{-3}$
Cultivar (King Edward)	1.461	1.194, 1.727
		Afternoon inoculations
Intercept	0.825	0.234, 1.147
Thermal time	$1.54 \cdot 10^{-3}$	$5.86 \cdot 10^{-4}$, $2.49 \cdot 10^{-3}$
Cultivar (King Edward)	1.381	0.904, 1.859

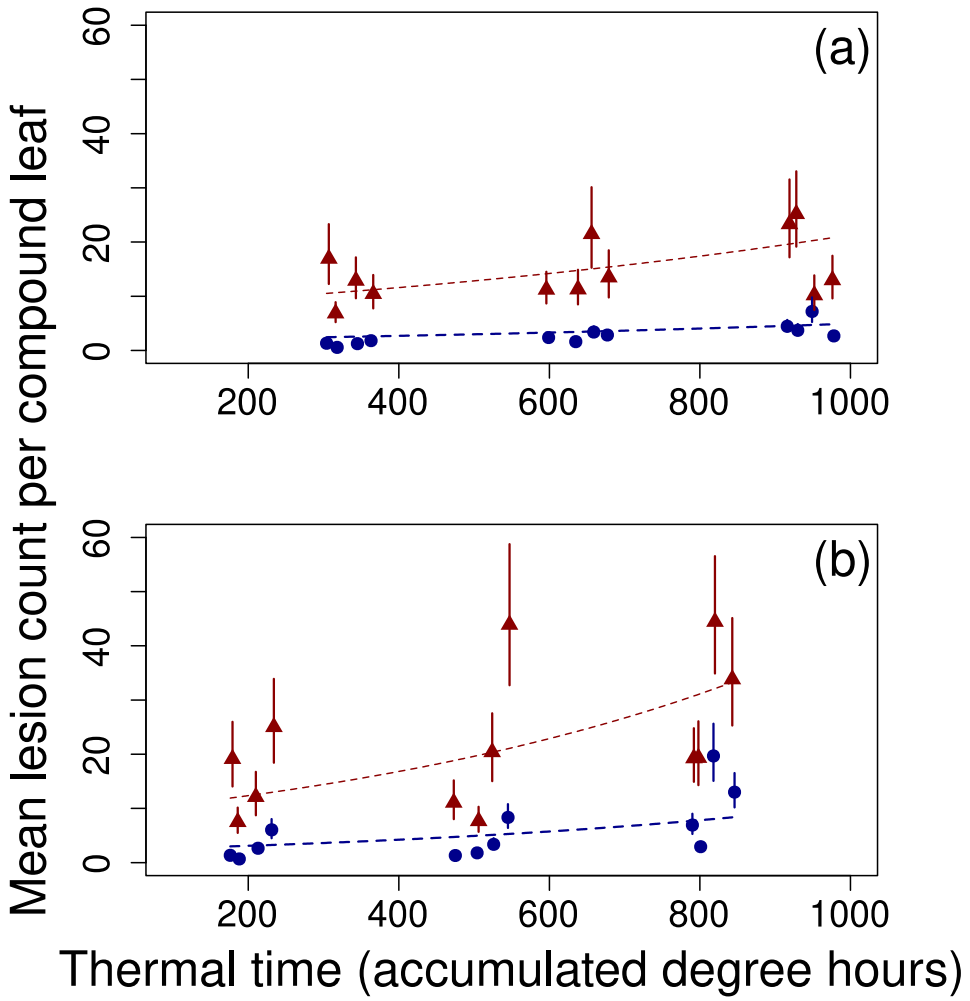

Figure 6.5. Mean lesion count (± 95% confidence interval) per compound leaf with increasing thermal time between inoculation and treatment at the Boghall site. Two cultivars were used in this experiment: Cara (●) and King Edward (▲). Morning inoculations are shown in (a), and afternoon in (b). See main text (page 195) and Table 6.2 (page 196) for description of the regression used to generate the trend-lines, which have been back-transformed in these plots.

The results from the Auchincruive trial can be found in Figure 6.6 (page 199), which are again faceted to separate the two inoculation times. Although lesion counts per compound leaf were much lower (note scale on vertical axis), there was a similar pattern of differences between untreated leaves of different categories, but with less marked difference between the cultivars. Untreated Cara plants in the morning inoculations had an average lesion count per compound leaf of 1.23 ($\pm$ 0.19, 95% c.i.), and for the afternoon inoculations 4.69 ($\pm$ 0.52, 95 % c.i.). The corresponding values for the King Edward plants were for the morning inoculated plants: 1.92 ($\pm$ 0.23, 95 % c.i.) lesions per compound leaf, and for the afternoon inoculated plants: 4.14 ($\pm$ 0.41, 95% c.i.) lesions per compound leaf. When the response to thermal time was explored with a simple: linear model (ln(lesion count + 1) = accumulated thermal time + cultivar (as a factor)), the influence of thermal time and cultivar resistance on curative activity was much more limited than at the Boghall site. In neither case was the thermal time term significant (morning, $p = 0.27$; afternoon, $p = 0.28$). However, cultivar was a significant factor in the morning inoculations ($p > 0.01$) with higher lesion counts on King Edward plants. There was no evidence of a significant differences between the cultivars in the afternoon inoculations ($p > 0.56$).

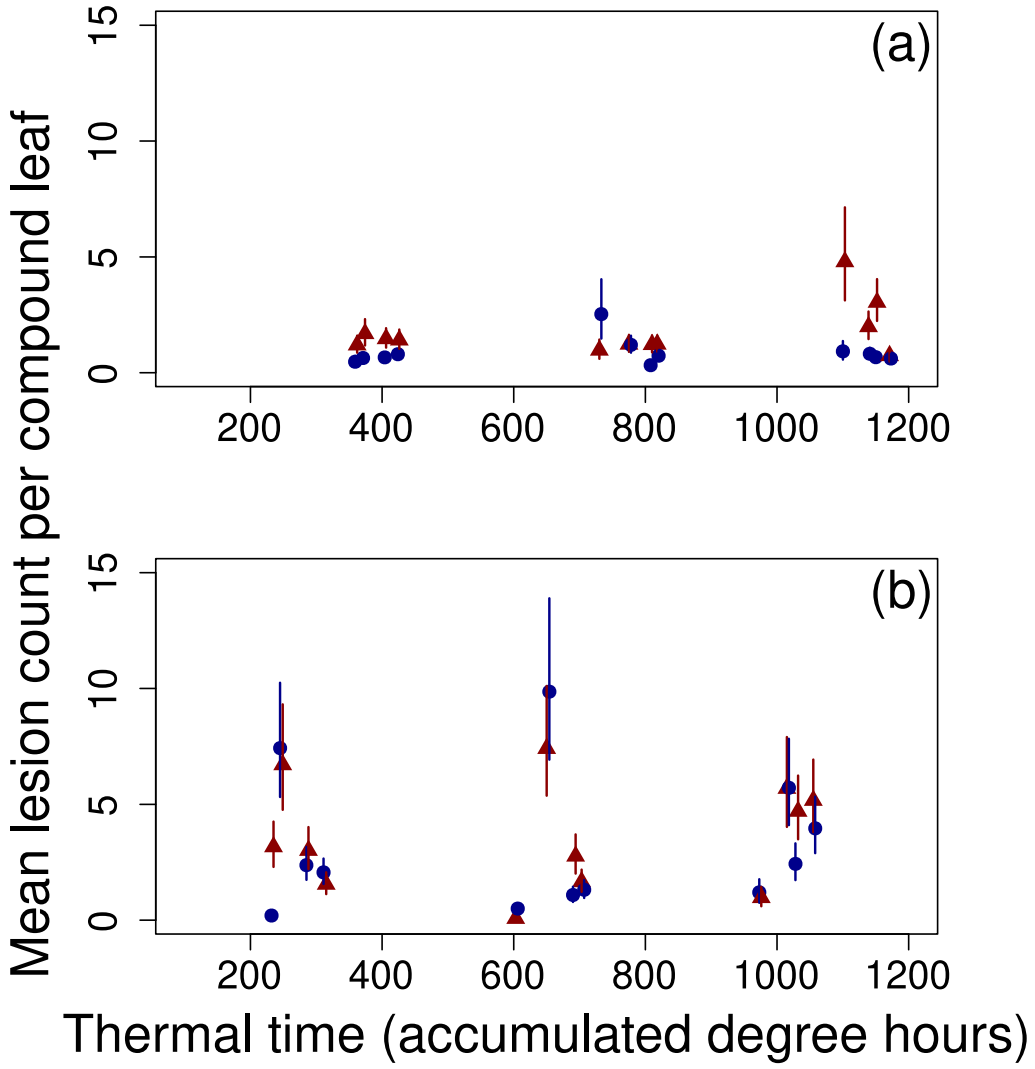

Figure 6.6. Mean lesion count (± 95% confidence interval) per compound leaf with increasing thermal time between inoculation and treatment at the Auchincruive site. Two cultivars were used in this experiment: Cara (●) and King Edward (▲). Morning inoculations are shown in (a), and afternoon in (b). The relationship between ln(lesion count) and thermal time was not significant for either .

6.5.4 Performance of decision aid

The data gathered in the validation field trials were used to assess the accuracy of the categories assigned by the decision aid, and to determine the prognostic information provided by the decision aid. Unfortunately, at both sites mean lesion counts within some of the treatment plots exceed those of the control plots, making it impossible for proportions to be calculated. The reason for this is not clear, it could be a result of the high background variability in lesion counts across plots at each trial site. Nevertheless, in cases where lesion counts exceeded the untreated controls, curative activity had clearly failed. It was therefore possible to assign each plot an 'observed P_{cur}' value by dividing the mean lesion count per compound leaf for the plot by the mean lesion count per compound for the untreated control plots. Where this value exceeded 1 (i.e. more lesions in the treatment plot than control) this was interpreted as complete control failure and assigned a value of 1. These 'observed P_{cur}' values were then assigned to 'observed categories' with reference to Table 6.1.

P_{cur} values and 'predicted outcome categories' were obtained using the demonstration code provided in Section 6.4 (page 185), and by using the relevant temperature profile for each plot as the input values (i.e. the temperature readings between inoculation and curative fungicide treatment). When observed and predicted categories were compared, the accuracy of the aid was poor: 23 % of calls were made correctly at the Boghall site and 36 % of calls were made correctly at the Auchincruive site. This indicates that there is scope to improve the model's predictions and a better model of curative activity can probably be constructed, but for reasons explained in Section 6.3 (page 178) it does not mean that the model has no use. The decision aid will be providing usable information to end users if it is able to separate plots where curative control has a strong prospect of success from those where there is very limited chance of successful curative control. With this in mind, indices of separation (PSEP) were calculated for the data from both sites, as well as from the original bioassay from which the development-curative control aspect of the decision aid was originally derived. P_{worst} and P_{best} were in each case

calculated from the 'observed P_{cur}' values of plots (or frames in the case of the bioassay) assigned to the relevant categories. The results of this exercise are summarised in Table 6.3.

Table 6.3. Values in table are curative control failure probabilities of treated subjects (plots or leaf-disc frames) based on the number of lesions occurring after both inoculation and treatment in comparison with untreated control plots. The number of observations within each category is shown in parentheses.

Curative Control Category	Original Bioassay (Glasshouse)	Field Trial 1 (Boghall)	Field Trial 2 (Auchincruvie)
Control Likely (best outcome)	0.16 (10)	0.46 (14)	0.66 (12)
Control Unlikely (worst outcome)	0.83 (18)	0.82 (6)	0.77 (24)
Total	0.59 (28)	0.57 (20)	0.73 (36)
PSEP	0.67	0.36	0.11

PSEP was 0.67 for the original bioassay, 0.36 for the trial at the Boghall site and 0.11 at the Auchincruvie site. This indicates that whilst the aid is providing prognostic information at all three 'centres', the value of PSEP is not stable and is much lower in the field trials than in the original glasshouse assay. There is also a large difference between the two trial sites; the decision aid provided much less prognostic information at Auchincruvie than Boghall. It appears that the model's estimated ability to separate groups is overstated. This is perhaps unsurprising, as the data from which the decision aid is derived were generated from carefully controlled laboratory assays, the levels of curative control may be greater, and the variability in curative control performance smaller in this setting than in the field. The difference between the trial sites may indicate that the decision aid is not transportable between locations, which is a very serious flaw. However, even in the worst performing situation (Auchincruvie site) the aid produced prognostic information which may have value to a decision-maker. It is also worth noting that little evidence of curative control could be detected when the Auchincruvie field trial data were analysed with a simple linear regression technique (Section 6.5.3,

page 198), and it may be that other factors, such as the UV intensity at inoculation times which is known to affect viability of inoculum (Mizubuti *et al.*, 2000) may have interfered the the curative effect. Mean lesion counts were much lower at the Auchincruvie site than at Boghall, and it may be that a critical number of lesions is needed before a curative effect can actually be detected. The 'case-mix' (Pettengill and Vertrees, 1982) was also different in the three situations. Total curative control failure probability was similar for the glasshouse bioasssay and the Boghall field trial, but was higher at the Auchincruvie site, providing additional evidence that the curative control in this particular instance was poor.

6.6 Discussion and recommendation for future validation

The decision aid, in its current form, has the ability to convey useful information to end users. There is currently no method, aside from expert judgement, of distinguishing between situations where curative control is likely to be effective and where it is likely to fail. The prognostic information which the aid produces will be of great utility when such judgements are being made. However, the accuracy of the aid is in need of improvement, and it seems likely that a more thorough investigation of factors which modify curative activity and their incorporation within the decision aid will improve its performance.

The validation carried out in this project was very limited due to time and resource constraints, and a more extensive program would be highly desirable. The cultivars from which the validation data are derived were the same as those used to obtain parameter estimates in the construction of the decision aid. Performance of the aid should ideally be assessed against a much wider range of cultivars, particularly given the relatively diversity of culitvars grown commercially in Great Britain. Additionally, it would be desirable to assess the aid under different climatic conditions, particularly given the importance of temperature to the categories which it assigns. It would be possible to out carry this validation as a set of extensive field trials using similar methodology used in this project. However, such trials are

logistically difficult and laborious, and it is not fully clear that they are the most effective way of assessing curative control.

The measurement of curative control in the field is very challenging, but if a method can be constructed to quantify levels of curative control retrospectively, perhaps through an investigation of fungicide treatment times, high risk weather conditions, and subsequent late blight outbreaks, then the decision aid could be validated on historical data sets (provided that temperature readings or estimates are also available).

Chapter 7 General discussion

7.1. Introduction

A large community of researchers is focused on the development of control strategies for the management of potato late blight, and our ability to monitor (Hansen *et al.*, 2016b; Schepers *et al.*, 2017) and profile (Andrivon *et al.*, 2017; Kessel *et al.*, 2018) the pathogen population is becoming ever more sophisticated. Growers have access to multiple control measures consistent with the principles of IPM and a number of DSSs to assist in their decision making. The range of fungicides available for late blight management is also well developed, although there are pressures from regulation (Williams, 2012), as well as the potential for rapid pathogen population change (Zhu *et al.*, 2015). These pressures highlight the importance of stewardship for sustainable control (Burnett, 2011). There has been recognition that some of the available information, tools and resources are underutilized (Lamichhane *et al.*, 2016; Lefebvre *et al.*, 2015), and that more work is needed to truly integrate them.

The aim of this study was to produce a simple decision aid to inform tactical choices in the use of fungicides with curative properties, an area which has not previously been explored in detail. There are relatively few studies characterising how curative effects diminish with increasing disease development time, or how the curative effect can be modified by factors such as temperature and host resistance. Additionally, although some DSSs do include reference to curative treatments (Hadders, 1997), they do not generally include a model of the likely efficacy of these treatments. A typical example is Apel *et al.* (2003), who included a fixed parameter for curative activity in their simulation, with the rationale that *P. infestans'* short incubation period will result in a uniform curative effect at all stages of pre-symptomatic development. Fungicides with curative activity are very widely used within late blight management in the UK, and can be an important component of spray programs (Hansen *et al.*, 2012) but application timing in relation to high-risk periods is not generally optimised. In field trials, more accurate explanations of the

control provided by a particular sequence of fungicides are obtained if the EuroBlight curative ratings are considered in conjunction with the decimal protectant ratings (assigned to products via a harmonised protocol, see Evenhuis (2006)) than if the decimal ratings alone are considered.

The nature of the contemporary *P. infestans* population in Europe presents several challenges for the designers of decision aids and support systems. The population is dynamic, rapidly overcoming host resistance (Du *et al.*, 2018), and adapting to local environmental conditions (Yang *et al.*, 2016). Its rapid life-cycle is also problematic (Leesutthiphonchai *et al.*, 2018). False negatives (a recommendation to withhold a control measure, when the correct decision was to treat the crop) have the potential to be very damaging and this could lead to a reluctance by growers to use such systems. Conversely, some of these characteristics open opportunities for novel DSSs developments. *P. infestans* populations are, in many countries including the UK, predominately clonal (Cooke *et al.*, 2016), and are well monitored (Cooke *et al.*, 2018). This opens the possibility of tailoring control tactics to match the local pathogen population. Although this has not been achieved to date, it is one of the explicit goals of the ongoing IPMBlight 2.0 project (Andrivon *et al.*, 2017). Preliminary results indicate that virulence profiles of local pathogen populations can be fed into existing decision support systems (Kessel *et al.*, 2018), reducing the number of unnecessary fungicide treatments.

The final decision aid produced by this project and the data generated to support its sub-models are complementary to these research efforts. Although a comprehensive DSS was not the goal, the framework laid out in Chapter 6 could be incorporated as a module within existing DSSs, or it could be used as a stand-alone tool where output of a DSS (for example risk factor from a web-based application) is used as part of its input. Additionally, the two sub-models within the decision aid, i.e. the temperature-pathogen growth model and the development-curative activity model may be helpful to others when specifying or updating DSSs which give recommendations for curative fungicide sprays. Surprisingly this is an area that has received little attention to date. DSSs for the management of late blight

often include models for weather related risk, varietal resistance, and for rates of fungicide erosion, but curative effects (if present at all) are often included as fixed or as a simple time dependent cut-off (Hadders, 1999).

7.2 Temperature models

As noted in Chapter 3, Section 6 (page 96), the temperature-development data gathered in this project are consistent with other studies (Chapman, 2012; Mariette *et al.*, 2016; Seidl Johnson *et al.*, 2015) both in terms of the rates of lesion growth (Maziero *et al.*, 2009) and the responses to high and low temperature (Belkhiter *et al.*, 2017). Although only one isolate per SSR genotype was assessed, there were subtle differences in the optimum and minimum temperatures for growth between the isolates, which again has been reported by other authors. It seems very likely that a range of temperature response phenotypes do exist within the *P. infestans* population, and although these differences are not large, there is the potential for temperature adaptation as suggested by Mariette *et al.* (2016). Other authors have reported within-genotype differences between isolates in response to temperature (Chapman, 2012) and therefore no generalizations can be made about genotypes and temperature from this project. However, it would be interesting to repeat the experiments with a larger number of isolates, and with several representatives from each genotype to better characterise between and within-lineage variation.

Estimates of in planta *P. infestans* biomass using a variety of methods, including qPCR, have been reported before (Halim *et al.*, 2007; Kamoun *et al.*, 1998), but the number of samples and span of time-points included in this project give a more detailed assessment of pre-symptomatic growth than is typically available. These data are particularly relevant to phenomena such as the effect of curative fungicides, which by definition occur within a pathogen's incubation period (Waard *et al.*, 1993). It is unsurprising that the growth curves within the incubation period were exponential, as this is often the case in other biological systems at analogous developmental points (Lejeune and Baron, 1998; Okada, 1967; Trinci, 1971). However, interpretation was complicated by the fact that a relationship could not

always be established between development time and oomycete DNA within samples. In some cases it was not possible to distinguish between zero and very slow growth; as noted in Chapter 3, there were some temperature-isolate combinations where visible lesion growth was observed but where an incubation growth coefficient could not be assigned. Additionally, for isolate 2012_8A1-1, incubation growth coefficients could be assigned for 10 °C and for 18 °C, but not for 14 °C, but it seems very unlikely that this is a true discontinuity. The most probable explanation is that stochastic factors led to growth within the sampled leaf discs falling below the range of detection statistically, and the discontinuity was an artefact of the experimental design. A related issue with the methodology is that there was no clear way to distinguish between successful and unsuccessful infections. The quantity of inoculum applied to a leaf surface would be sufficient to yield DNA values within the assay's range of detection (personal observation), and although the assay was designed to maximize the infection efficiency by having an initial 18 °C 'infection phase', it is possible that for the least optimal temperatures failed infections led to lower estimates of (or failure to estimate) growth rates. A possible solution to this would be the microscopic inspection of inoculation sites for infection-related structures (Schoina *et al.*, 2017); however this method may also be ambiguous without staining the leaflets or the use of a transformed fluorescent *P. infestans* strain to view internal plant interactions.

The number of models for pathogen growth in response to temperature assessed in this study was larger than is usually the case for plant pathogens, and the results and the methodology may be useful to others who are interested in modelling the growth dynamics of *P. infestans*. Several of the models gave robust descriptions of the temperature-growth relationship, so it is not a concern that different authors have used different models in the past. However, some of the simple linear models performed relatively poorly, highlighting the dangers of oversimplification. It is interesting that the models which provided the best descriptions differed between the visible symptom and biomass data sets, although this observation should be treated with caution. Only one isolate was used for model fitting in both cases

(2012_13A2-1), as this isolate yielded measurable biomass accumulation for all temperatures at which lesion growth was observed. It is possible that the methodology used to measure biomass accumulation within the incubation period gives poor growth estimates at temperature extremes, which may explain the discrepancy. The observation does merit further investigation however, as previously it has been demonstrated that 'internal' and 'external' life history stages have different profiles in response to temperature (Shakya *et al.*, 2015), and if different developmental stages have different optimum temperatures this could have epidemiological implications.

No direct demonstration of temperature extending or shortening the curative window was made because data were available in the literature which demonstrated the modifying effect that temperature during the incubation period can have on the activity of curative fungicides (Genet *et al.*, 2001), and also because development was directly linked to curative activity in the experiments in Chapter 4. This would have been a very useful experiment to perform, had more time and resources been available, both to confirm the role that temperature plays explicitly, and to provide a more direct estimate of the degree to which the curative window is modified. There was a clear benefit from expressing the results in thermal time or in relative development rather than physical time, with greater consistency between different classes of experiment in most cases. However, in the natural inoculum field trial described in Chapter 5 (Section 5.2, page 146) this was not the case, indicating that other factors such as inoculum pressure may be important.

7.3 The curative window

A core objective of this project was the characterisation of the curative control window. This was explored in both the laboratory and field (Chapter 5). The relationships between disease development time and curative control presented in this project may be more robust than in other studies as smaller time steps and larger sample sizes were used. The methodology used in the leaf disc bioassay, whilst a simplification of the situation in the field, may be useful to other

pathologists interested in curative control against *P. infestans* or other foliar pathogens. The final fitted relationship was a simple logistic curve, and the situation for declining curative control appears analogous to that of a dose-response relationship (Ritz, 2010), with increasing biomass substituting for decreasing fungicide dose. There is a well developed set of methods for exploration of dose-response curves, and it is relatively straightforward to apply these to the curative relationship as well. It is possible that a better fit to the data could have been achieved by using a more complex sigmoid function such as a Gompertz curve (Tjørve and Tjørve, 2017), but as curves such as these would introduce additional parameters they were not used for the sub-model within the decision aid.

Different response curves were obtained for the two isolates: 2012_13A2-1 and 2012_7A1-1. These isolates represented the extremes in aggressiveness from the isolates that were available, with 2012_13A2-1 the most and 2012_7A1-1 the least aggressive. Intuitively, aggressiveness could then be considered as a modifying factor for curative activity. Aggressiveness has several components (Flier and Turkensteen, 1999), and can depend on the scale at which it is measured (Pariaud *et al.*, 2009). However, if the rate of tissue colonization is an important determinant then this will have implications for the duration of the curative control window. This appears to be the case, at least for the isolates tested in this study. Pathogen DNA accumulated at a slower rate for isolate 2012_7A1-1 than for 2012_13A2-1. The qPCR assay provided a more direct estimate of tissue colonization than the observation of visible symptoms, which can be influenced by other factors (Bock *et al.*, 2010). Interestingly the rank order of isolate growth rates differed at different temperatures in the Chapter 3 experiments, so it is possible that a complex interaction between isolate aggressiveness phenotype and temperature exists for the curative window, but experimental data are needed to confirm this. It is possible that the different response curves generated for isolates 2012_13A2-1 and 2012_7A1-1 were not connected to aggressiveness, and were instead due to differing sensitivities to propamocarb-HCl of fluopicolide. Variation in sensitivity to propamocarb-HCl has been found in other *Phytophthora* species (Moorman and

Kim, 2004), but recent surveys of fungicide sensitivity in *P. infestans* isolates have not reported large variations between isolates for most a.i.s (Saville *et al.*, 2014). Nevertheless, it would be very useful to determine EC_{50} values (or a similar measure) to establish if the extended curative window is independent of fungicide sensitivity, because even subtle differences below the thresholds of concern may have an effect.

For logistic reasons, two staggered inoculations were used in the leaf disc bioassay, and staggered inoculations were also used in some of the field trials in Chapters 4 to 6. In several cases, the infection rates differed between the inoculation timings. When the results were analysed this was corrected by expressing values as a proportion of the untreated control, but it is possible that developmental rates differed between the two batches of inoculum. More rigorous experiments could be conducted in which a single inoculation was used, and all treatment intervals were performed within a continuous sequence, but this was beyond the resources available here. In the field trials, infection efficiency differences between batches were probably due to differences in environmental factors at the inoculation times. For example, morning inoculations will have received more solar radiation which very probably decreased infection efficiency by reducing the viability of sporangia at the leaf surface. Weather was monitored during the run-up to these experiments, and attempts were made to ensure high cloud cover on days of inoculation, but this was not always possible due to competing factors. It is possible that the inoculum batches themselves were intrinsically variable, leading to different infection rates. No account was made of circadian rhythms, of either the pathogen or of the host (Weyman *et al.*, 2006) as this was not within the scope of the project. Nevertheless, it is possible that this may have led to differences between inoculum batches when prepared and used for inoculation at different times of day (Roden and Ingle, 2009). Whilst the results should be interpreted with this in mind, our understanding of plant and plant-pathogen circadian rhythms is nascent (Lu *et al.*, 2017), and it is difficult to see how this could have been avoided.

7.4 Modifying factors

A large number of factors could potentially modify the duration of the curative control window, and it was only possible to conduct experiments on a subset of those deemed most important. As mentioned earlier, the modifying effect of temperature has been demonstrated by previous authors. Several experiments in this study indicated that more resistant cultivars could increase the duration of the curative window, but this was not always the case. Interestingly, the modifying effect of host resistance was most prominent when inoculum pressure was presumed to be low, such as in the trials where natural inoculation was used. The number of founder sporangia is known to influence the rate of pathogen development, with a greater number leading to shorter incubation periods (Lapwood and McKee, 1966). This may explain why a modifying effect from more resistant cultivars was not always detected, and why its size was variable when present. Data from the decision aid validation trials do suggest that it would be a mistake to omit host resistance from characterisations of the curative effect, but more data are probably needed before it can be modelled robustly.

Foliar resistance ratings from the AHDB Potato Variety Database agreed reasonably well with the observed rates of lesion growth for the majority of cultivars, which is reassuring. However, much more variation was evident when the measurements of growth were within the incubation period. The fact that there was no strong correlation between sub-clinical DNA accumulation rates and lesion growth rates suggests that caution should be exercised before using the former to forecast expected levels of curative control. Indeed, when the incubation coefficient calculated for Cara plants was used in the final decision aid it did not improve its predictive power. Whether the rate of *P. infestans* proliferation *in planta* during the incubation period is strongly affected by cultivar resistance remains an open question. Also it is possible that the variable results obtained from the qPCR assay with cultivars included in Chapter 5 was due to the methodology used. This is an important issue to explore further, however as the foliar resistance ratings were not

predictive of sub-clinical growth more detailed assessments will be required to accurately forecast the modifying effect of a given cultivar on curative activity.

A further limitation of many of the experiments conducted within this project is that assays on detached leaves, rather than on whole plants were used. This choice was based on the need to process a relatively large number of samples within controlled conditions, and is routinely performed by other pathologists in similar situations (Vleeshouwers *et al.*, 1999). However, detached leaves may differ physiologically from intact tissue, and it is possible that this could act as a modifying factor, particularly if expressed levels of quantitative resistance are altered. In at least one of the cultivars assessed (Sarpo Mira) other researchers have demonstrated that intact meristems can modulate the level of resistance (Orłowska *et al.*, 2012), indicating that an assessment of lesion growth and biomass accumulation rates using whole plants may be warranted. Similarly, some host resistance genes have been shown to be relevant only in the field situation (Rietman *et al.*, 2012), and quantitative resistance that manifests itself only over the course of an epidemic, rather than a single disease cycle, may not be relevant as a modifying factor for curative fungicides.

Two field trials were conducted which included a previous protectant fungicide spray were conducted (data not shown), but these did not provide evidence that the presence of previous fungicide acted as a modifying factor. In one case there was no detectable improvement between curative treatment with or without a previous fungicide treatment. In the other case, the previous fungicide treatment offered good control, leading to very low infection rates and it was thus not possible to evaluate any change in the curative effect. These results highlight the challenge of measuring such modifying factors, which may have subtle effects. In the case of both protectant and curative treatments timing is critical. Protectant fungicides weather after application (Fry *et al.*, 1983) and the protection that they offer declines with time. This process will depend on factors such as rainfall and temperature (Leonard *et al.*, 2001), and is sometimes modelled by DSSs. As the curative effect

itself is time-limited, and sensitive to other factors, it is likely that the interplay between these two processes is complex. Modern late blight control programmes take a prophylactic approach, with regular fungicide sprays. It is very likely that in a field situation a basal level of fungicide from previous treatments will be present when sprays which have a curative component are applied, making this a worthwhile factor for further investigation. Treatment of plants with either some fungicides or some host resistance elicitors can significantly reduce the area of late blight lesions (Bain and Walters, 2016). It is probably worth investigating this systematically under controlled conditions before exploring any effects in the field, but in this study other aspects of the curative effect and pathogen biology were judged to take priority in laboratory-based investigations.

It has been observed previously that leaf position on the stem has an effect on the level of quantitative resistance against late blight, with more apical leaves having greater levels of resistance than basal leaves (Visker *et al.*, 2003), for some potato cultivars at least. In several of the experiments in this project, this effect was detectable. There are also published reports of leaf age playing an important role in levels of quantitative resistance, with the youngest leaves being the most susceptible. The experimental design used in many assays in this project will have led to these two factors being confounded, although Visker *et al.* (2003) have demonstrated that they can be independent. The effect of leaf position was not included in the final decision aid because, as with quantitative resistance in general, more data are needed to give a full characterisation of how it interacts with curative treatments. It was also not clear which approach would be most appropriate for the inclusion of leaf position in the decision aid, or how this could be validated. It is surprising that, despite several authors acknowledging the importance of leaf position-dependent host resistance, there are no studies to date which explore its underlying mechanism. A better understanding of this phenomenon would be helpful in assessing if it is a likely modifying factor for curative activity.

There are several other factors which have the potential to modify the duration of the curative window. Any factor that alters the rate of pathogen development is a candidate for this, and it is also possible that there are other mechanisms for modification. For example, the metabolic profile of a particular potato cultivar may lead to altered rates of a.i. uptake and metabolism. Few data exist for this, but it may be a worthwhile area for investigation. Also, experiments in this project used sporangial suspensions, and were generally conducted at temperatures at which direct germination predominantly should be assumed. However, it is routine experimental practice to chill *P. infestans* inoculum before use to encourage the release of zoospores as this multiples the potential infection sites (Doke, 1975; Rohwer *et al.*, 1987; Stephan *et al.*, 2005). Surprisingly, there are few studies which directly compare infections arising from the different pathways: one study from the related species *Phytophthora ramorum* reported larger necrotic areas on host plants when zoospores, rather than sporangia (Widmer, 2009), were used as inoculum. This could be due to different rates of growth within host tissue between the two infection pathways, and if the same is true for *P. infestans* this could influence the duration of the curative window, though experimental confirmation of this would be needed.

7.5 Status of decision aid

The decision aids as currently formulated can provide useful prognostic information, but it is in need of wider validation. Modifications could also be made to it to improve the accuracy of its predictions. It is instructive to consider how the decision aid, as currently specified could be explained to individuals from different disciplines who would likely have some interaction with it should it be fully implemented. With this in mind, three such individuals are considered here: a late blight plant pathologist, a software engineer, and the end-user themselves (assumed to be an agronomist).

Plant Pathologist

The curative decision aid is a simple tool which can be used to project the expected control provided by curatively treating latent *P. infestans* infections. It makes use of models of pathogen growth as a function of time and temperature, and of the curative control expected as a function of the developmental state of the pathogen. The parameters it uses were derived from laboratory assays with plant cultivars and pathogen isolates relevant to current potato production in Great Britain. It is designed to complement existing infection risk models such as the Hutton Criteria, and it provides projects for results if a curative a.i. is used. It is intended to be tactical, rather than strategic and is intended to be used in conjunction with a local expert's (i.e. an agronomist's) judgement to help choose appropriate control measures for a given situation. Essentially, it answers the question 'are we too late?', given the temperature conditions and the time elapsed for a curative fungicide application to be effective.

The decision aid has the scope to be extended, and can easily be modified to include factors other than temperature and time which may modify the curative effect. For example, in the course of its development evidence was obtained to suggest quantitative host resistance can extend the time frame over which curative control is effective. This was not included in the final aid, but it would be straightforward to do so as new data become available. The two subcomponents of the aid have independent uses. The temperature development model is based on pathogen biomass in the latent period estimate by qPCR, and could be used to explore more other pathology and epidemiological questions, for example latent periods for *P. infestans* could be projected across different geographic regions of seasons for example. The relationship between pathogen development and expected curative control was similar to a fungicide dose-response curve, which opens the possibility of curative fungicides formulations being explored with methods extant in the relevant literature. For example, a developmental value analogous to an EC_{50} could be assigned to available products, updating the somewhat subjective ratings that are currently available.

Software engineer

The curative decision aid in its current form is the specification for a tool, the implementation of which is as yet undefined. The underlying models are simple, and can be readily implemented in computer code. The aid requires temperature data as an input, either recorded from the field or as a forecast. The end-user specifies the time periods of interest to them, and they will receive a simple categorical response which estimates the expected control level given the input conditions.

A typical use-case would involve a user accessing the system (application, web-page, or printed table), and supplying the conditions for the aid to work on. For example - "a suspected infection occurred in crop X , Y hours ago. The in-field temperature for this period is the set of observations Z. Is the use of a curative fungicide formulation justified?" If the answer is negative, other control options will be used. In its current form, the aid is fully deterministic, but the disease-crop system is very dynamic, so the aid should be implemented in such a way as to make modification of underlying parameters trivial.

Agronomist

The decision aid is a simple tool to help with fungicide product choice during a late blight spray programme. It does not recommend altering a fixed-interval, protectant approach to protecting potato crops from late blight, as the difficulties and potential costs associated with changing this approach are appreciated. Instead, it will provide an indication of the level of curative control expected, given the conditions which the crop is under. The decision aid will probably be attached to a system like BlightWatch; when a Hutton Period warning is issued, the decision aid will begin estimating development of blight lesions before symptoms can be seen. If the user is able to provide local temperature readings, perhaps from an in-field weather station or in-crop sensors this will improve the estimates the model provides. This estimate of pathogen development is then used to determine how well the curative effect will work. These estimates are based on probabilities, and

they should be interpreted with this in mind; many other factors that the decision aid does not take into account can also impact on curative activity. The output of the decision aid should be used along with the judgement of an agronomist who is familiar with the crop and has knowledge of the local conditions, to make decisions about control tactics.

The aid provides easily interpretable categories given a crop thought to be infected, a specified time for a hypothetical fungicide treatment, and temperature readings (either expected or recorded) between the time of infection and treatment. An agronomic decision can then be made with this in mind. For example, if the output is "curative control is **very likely**", the use of a product containing a curative a.i. is probably justified. However, this does not mean that control is certain. On the other hand an output of "curative control is **very unlikely**", indicates that the window of opportunity has probably passed (see Figure 6.3, page 191).

7.6 Future research

The methodology and datasets presented here may also be useful to researchers who wish to incorporate curative activity in other DSSs. The leaf disc bioassay used in Chapter 4 in particular produced reproducible and easily interpreted results and it may be possible to adapt the assay for other pathosystems, although this will be limited to situations where incubation periods are relatively short. As the curative activity sub-model was chiefly characterized from data produced using this method, it would be helpful to evaluate each of the modifying factors in the same fashion. This would generate a better characterisation of how the development-control curve was shifted (if at all) by the different conditions, and would provide a more direct measure of any modification of the curative window.

A single fungicide (propamocarb-HCl + fluopicolide) was used in the majority of experiments within this project, and was selected as a representative product with 'good' curative activity. There are currently eight a.i.s used in the management of late blight in the United Kingdom which are considered to have some degree of

curative activity. Ratings exist for the level of curative control that they offer, but they are not based on a harmonized protocol (Evenhuis *et al.*, 2016). It would be possible to evaluate these fungicides using the methodology developed within this project, and thus obtain a quantitative rating. This could be incorporated into the decision aid, but should also have value independent of this, as the ratings are likely to be better descriptors of curative effect in the field than those currently published in the EuroBlight table. Fungicide dose rates, formulations and the impact of adjuvants on the curative efficacy of fungicides were not evaluated by this project, but these could also act as modifying factors (Grayson *et al.*, 1996).

7.7 Key messages

- Air temperature is a vital consideration for predicting development of *P. infestans* infections at both pre-symptomatic and subsequent time-points, and is well described by a number of growth functions.

- The curative activity of a commonly used translaminar fungicide declined rapidly with increasing disease development time, with a relationship best described by a logistic curve.

- Varietal resistance can act as a modifying factor for curative activity, but this may vary at different disease pressure levels. Other factors such as the aggressiveness of the pathogen phenotype may be important, but more data are required.

- Foliar resistance ratings from the online AHDB Potato Variety Database offered a good guide to rates of *P. infestans* tissue colonization, but the resistance may be more complex in some cases. The relationship was less strong for development in the incubation period.

- Better curative control was obtained if treatments were made early within the incubation period, and use of the decision aid can act as a guide for this.

References

Abu-Nada, Y., Kushalappa, A.C., Marshall, W.D., Al-Mughrabi, K., and Murphy, A. (2007). Temporal dynamics of pathogenesis-related metabolites and their plausible pathways of induction in potato leaves following inoculation with *Phytophthora infestans*. European Journal of Plant Pathology 118, 375–391.

Aghdam, H.R., Fathipour, Y., Radjabi, G., and Rezapanah, M. (2009). Temperature-Dependent Development and Temperature Thresholds of Codling Moth (Lepidoptera: Tortricidae) in Iran. Environmental Entomology 38, 885–895.

Ah-Fong, A.M.V., Shrivastava, J., and Judelson, H.S. (2017). Lifestyle, gene gain and loss, and transcriptional remodelling cause divergence in the transcriptomes of *Phytophthora infestans* and *Pythium ultimum* during potato tuber colonization. BMC Genomics 18, 764.

Ahuja, I., Kissen, R., and Bones, A.M. (2012). Phytoalexins in defence against pathogens. Trends in Plant Science 17, 73–90.

Akino, S., Takemoto, D., and Hosaka, K. (2013). *Phytophthora infestans*: a review of past and current studies on potato late blight. Journal of General Plant Pathology 80, 24–37.

Algaze, C.A., Wood, M., Pageler, N.M., Sharek, P.J., Longhurst, C.A., and Shin, A.Y. (2016). Use of a Checklist and Clinical Decision Support Tool Reduces Laboratory Use and Improves Cost. Pediatrics 137, e20143019.

Ali, A., Moushib, L.I., Lenman, M., Levander, F., Olsson, K., Carlson-Nilson, U., Zoteyeva, N., Liljeroth, E., and Andreasson, E. (2012). Paranoid potato. Plant Signaling & Behavior 7, 400–408.

Allen, J.C. (1976). A Modified Sine Wave Method for Calculating Degree Days. Environmental Entomology 5, 388–396.

Altman, D.G., and Bland, J.M. (1994). Diagnostic tests. 1: Sensitivity and specificity. BMJ: British Medical Journal 308, 1552.

Altman, D.G., and Royston, P. (2000). What do we mean by validating a prognostic model? Statistics in Medicine 19, 453–473.

Alyokhin, A., Baker, M., Mota-Sanchez, D., Dively, G., and Grafius, E. (2008). Colorado Potato Beetle Resistance to Insecticides. American Journal of Potato Research 85, 395–413.

Analytis, S. (1977). Über die Relation zwischen biologischer Entwicklung und Temperatur bei phytopathogenen Pilzen. Journal of Phytopathology 90, 64–76.

Andersen, K., and Ospina-Giraldo, M.D. (2011). Assessment of the Effect of Temperature on the Late Blight Disease Cycle Using a Detached Leaf Assay. Journal of the Pennsylvania Academy of Science 85, 165–173.

Anderson, P.K., Cunningham, A.A., Patel, N.G., Morales, F.J., Epstein, P.R., and Daszak, P. (2004). Emerging infectious diseases of plants: pathogen pollution, climate change and agrotechnology drivers. Trends in Ecology & Evolution 19, 535–544.

Andrade-Piedra, J.L., Hijmans, R.J., Forbes, G.A., Fry, W.E., and Nelson, R.J. (2005). Simulation of potato late blight in the Andes. I: Modification and parameterization of the LATEBLIGHT model. Phytopathology 95, 1191–1199.

Andrivon, D. (1993). Nomenclature for pathogenicity and virulence: the need for precision. Phytopathology 83, 889–890.

Andrivon, D. (1996). The origin of *Phytophthora infestans* populations present in Europe in the 1840s: a critical review of historical and scientific evidence. Plant Pathology 45, 1027–1035.

Andrivon, D. (2018). IPMBlight2.0 - IPM2.0 for sustainable control of potato late blight - exploiting pathogen population data for optimized Decisions Support Systems. Impact 6, 61–63.

Andrivon, D., Lucas, J.M., and Ellissèche, D. (2003). Development of natural late blight epidemics in pure and mixed plots of potato cultivars with different levels of partial resistance. Plant Pathology 52, 586–594.

Andrivon, D., Pilet, F., Montarry, J., Hafidi, M., Corbière, R., Achbani, E.H., Pellé, R., and Ellissèche, D. (2007). Adaptation of *Phytophthora infestans* to Partial Resistance in Potato: Evidence from French and Moroccan Populations. Phytopathology 97, 338–343.

Andrivon, D., Montarry, J., Corbière, R., Pasco, C., Glais, I., Marquer, B., Clément, J. a. J., Castel, M., and Hamelin, F.M. (2013). The hard life of *Phytophthora infestans*: when trade-offs shape evolution in a biotrophic plant pathogen. Plant Pathology 62, 28–35.

Andrivon, D., Corbiere, R., Hansen, J.G., Eikemo, H., Puidet, B., Gaucher, D., Chatot, C., Aurousseau, F., Barbary, A., and Glorvigen, B. (2017). IPMBlight2.0: using pathogen population information to improve late blight control. In Proceedings of the Sixteenth Euroblight Workshop, (Aarhus, Denmark), 33–38.

Antonopoulou, E., Karetsos, S.T., Maliappis, M., and Sideridis, A.B. (2010). Web and mobile technologies in a prototype DSS for major field crops. Computers and Electronics in Agriculture 70, 292–301.

Anwar, M.M., Parveen, A., Hossain, M.M., Mahamud, N.U., and Roy, R.K. (2015). Efficacy of fungicides in controlling late blight of potato. Progressive Agriculture 26, 103–108.

Apel, H., Paudyal, M.S., and Richter, O. (2003). Evaluation of treatment strategies of the late blight *Phytophthora infestans* in Nepal by population dynamics modelling. Environmental Modelling & Software 18, 355–364.

Appel, R., Adler, N., and Habermeyer, J. (2001). A Method for the Artificial Inoculation of Potato Tubers with *Phytophthora infestans* and Polymerase Chain Reaction Assay of Latently Infected Sprouts and Stems. Journal of Phytopathology 149, 287–292.

Appiah, A.A., van West, P., Osborne, M.C., and Gow, N.A.R. (2005). Potassium homeostasis influences the locomotion and encystment of zoospores of plant pathogenic oomycetes. Fungal Genetics and Biology 42, 213–223.

Archontoulis, S.V., and Miguez, F.E. (2015). Nonlinear Regression Models and Applications in Agricultural Research. Agronomy Journal 107, 786–798.

Atkins, S.D., and Clark, I.M. (2004). Fungal molecular diagnostics: a mini review. Journal of Applied Genetics 45, 3–15.

Aubry, C., Papy, F., and Capillon, A. (1998). Modelling decision-making processes for annual crop management. Agricultural Systems 56, 45–65.

Bain, R.A. (2016). Report of the Control Strategies Subgroup meeting on 13 May 2015: Discussion and agreements reached. In Proceedings of the Fifteenth EuroBlight Workshop, (Brasov, Romania), 131–138.

Bain, R.A., and Convery, C. (2011). Zoospore production in relation to temperature for current *P. infestans* genotypes. In Proceedings of the thirteenth EuroBlight Workshop, (St. Petersburg, Russia), 193-194.

Bain, R.A., and Walters, D.R. (2016). The contribution of host resistance elicitors to the control of potato foliar blight in Scotland. In The Dundee Conference: Crop Protection in Northern Britain 2016, (Dundee, United Kingdom) , 205–210.

Bajwa, W.I., and Kogan, M. (1996). Compendium of IPM definitions (Corvallis, Oregon, USA: Integrated Plant Protection Center (IPPC), Oregon State University).

Bajwa, W.I., Kogan, M., and Center, I.P.P. (2003). Internet-based IPM informatics and decision support. (Corvallis, Oregon, USA: Integrated Plant Protection Center (IPPC), Oregon State University).

Baker, K.M., and Kirk, W.W. (2007). Comparative analysis of models integrating synoptic forecast data into potato late blight risk estimate systems. Computers and Electronics in Agriculture 57, 23–32.

Baker, E.A., Hayes, A.L., and Butler, R.C. (1992). Physicochemical properties of agrochemicals: Their effects on foliar penetration. Pesticide Science 34, 167–182.

Baldauf, S.L. (2008). An overview of the phylogeny and diversity of eukaryotes. Journal of Systematics and Evolution 46, 263–273.

Ballini, E., Morel, J.B., Droc, G., Price, A., Courtois, B., Notteghem, J.L., and Tharreau, D. (2008). A genome-wide meta-analysis of rice blast resistance genes and quantitative trait loci provides new insights into partial and complete resistance. Molecular Plant-Microbe Interactions 21, 859–868.

Ballvora, A., Ercolano, M.R., Weiß, J., Meksem, K., Bormann, C.A., Oberhagemann, P., Salamini, F., and Gebhardt, C. (2002). The R1 gene for potato resistance to late blight (*Phytophthora infestans*) belongs to the leucine zipper/NBS/LRR class of plant resistance genes. The Plant Journal 30, 361–371.

Barr, D.J.S. (1992). Evolution and Kingdoms of Organisms from the Perspective of a Mycologist. Mycologia 84, 1–11.

Barzman, M., Bàrberi, P., Birch, A.N.E., Boonekamp, P., Dachbrodt-Saaydeh, S., Graf, B., Hommel, B., Jensen, J.E., Kiss, J., Kudsk, P., Lamichhane, J., Messean, A., Moonen, A., Ratnadass, A., Ricci, P., Sarah, J., and Sattin, M. (2015). Eight principles of integrated pest management. Agronomy for Sustainable Development 35, 1199–1215.

Bashi, E., Ben-Joseph, Y., and Rotem, J. (1982). Inoculum potential of *Phytophthora infestans* and the development of potato late blight epidemics. Phytopathology 72, 1043–1047.

Baskerville G. L., and Emin P. (1969). Rapid Estimation of Heat Accumulation from Maximum and Minimum Temperatures. Ecology 50, 514–517.

Batista, D.C., Lima, M.A., Haddad, F., Maffia, L.A., and Mizubuti, E.S.G. (2006). Validation of decision support systems for tomato early blight and potato late blight, under Brazilian conditions. Crop Protection 25, 664–670.

Bauer, H., and Schönherr, J. (1992). Determination of mobilities of organic compounds in plant cuticles and correlation with molar volumes. Pesticide Science 35, 1–11.

Bayoh, M.N., and Lindsay, S.W. (2003). Effect of temperature on the development of the aquatic stages of *Anopheles gambiae sensu stricto* (Diptera: Culicidae). Bulletin of Entomological Research 93, 375–381.

Beakes, G.W., Glockling, S.L., and Sekimoto, S. (2011). The evolutionary phylogeny of the oomycete "fungi." Protoplasma 249, 3–19.

Beaumont, A. (1947). The dependence on the weather of the dates of outbreak of potato blight epidemics. Transactions of the British Mycological Society 31, 45–53.

Beckerman, J.L., Sundin, G.W., and Rosenberger, D.A. (2015). Do some IPM concepts contribute to the development of fungicide resistance? Lessons learned from the apple scab pathosystem in the United States. Pest Management Science 71, 331–342.

Beckstead, J.W., and Beckie, T.M. (2011). How Much Information Can Metabolic Syndrome Provide?: An Application of Information Theory. Medical Decision Making 31, 79–92.

Becktell, M.C., Daughtrey, M.L., and Fry, W.E. (2005). Temperature and Leaf Wetness Requirements for Pathogen Establishment, Incubation Period, and Sporulation of *Phytophthora infestans* on *Petunia* × *hybrida*. Plant Disease 89, 975–979.

Belhaj, K., Cano, L.M., Prince, D.C., Kemen, A., Yoshida, K., Dagdas, Y.F., Etherington, G.J., Schoonbeek, H., van Esse, H.P., Jones, J.D.G., Kamoun, S., and Schornack, S. (2017). *Arabidopsis* late blight: infection of a nonhost plant by *Albugo laibachii* enables full colonization by *Phytophthora infestans.* Cellular Microbiology 19, e12628.

Belkhiter, S., Khedidji, H., Krimi, A., Mabon, R., Corbiere, R., Andrivon, D., and Bouznad, Z. (2017). Variable temperature response in Algerian *Phytophthora infestans* EU_23_A1 isolates from tomato. In Proceedings of the 16th EuroBlight Workshop, (Aarhus, Denmark), 211–212.

Bellincampi, D., Cervone, F., and Lionetti, V. (2014). Plant cell wall dynamics and wall-related susceptibility in plant–pathogen interactions. Frontiers in Plant Science 5. 288.

Benedetti, R. (2010). Scoring Rules for Forecast Verification. Monthly Weather Review 138, 203–211.

Bengtsson, T., Holefors, A., Witzell, J., Andreasson, E., and Liljeroth, E. (2014). Activation of defence responses to *Phytophthora infestans* in potato by BABA. Plant Pathology 63, 193–202.

Bergelson, J., Kreitman, M., Stahl, E.A., and Tian, D. (2001). Evolutionary Dynamics of Plant R-Genes. Science 292, 2281–2285.

Berggren, B., Widmark, A.-K., and Umaerus, V. (1988). The expression of general resistance to late blight (*Phytophthora infestans*) in potato leaves. Potato Research 31, 611–616.

Bergot, M., Cloppet, E., Pérarnaud, V., Déqué, M., Marçais, B., and Desprez-Loustau, M.-L. (2004). Simulation of potential range expansion of oak disease caused by *Phytophthora cinnamomi* under climate change. Global Change Biology 10, 1539–1552.

Bernat, M., Segarra, J., Xu, X.M., Casals, C., and Usall, J. (2017). Influence of temperature on decay, mycelium development and sporodochia production caused by *Monilinia fructicola* and *M. laxa* on stone fruits. Food Microbiology 64, 112–118.

Birch, P.J., Rehmany, A.P., Pritchard, L., Kamoun, S., and Beynon, J.L. (2006). Trafficking arms: oomycete effectors enter host plant cells. Trends in Microbiology 14, 8–11.

Bjor, T., and Mulelid, K. (1991). Differential resistance to tuber late blight in potato cultivars without R-genes. Potato Research 34, 3–8.

Black, W. (1970). The nature and inheritance of field resistance to late blight (*Phytophthora infestans*) in potatoes. American Potato Journal 47, 279–288.

Blanford, J.I., Blanford, S., Crane, R.G., Mann, M.E., Paaijmans, K.P., Schreiber, K.V., and Thomas, M.B. (2013). Implications of temperature variation for malaria parasite development across Africa. Scientific Reports 3, 1300.

Blum, M., Boehler, M., Randall, E., Young, V., Csukai, M., Kraus, S., Moulin, F., Scalliet, G., Avrova, A.O., Whisson, S.C., and Fonne-Pfister, R. (2010). Mandipropamid targets the cellulose synthase-like PiCesA3 to inhibit cell wall biosynthesis in the oomycete plant pathogen, *Phytophthora infestans*. Molecular Plant Pathology 11, 227–243.

Bock, C., Poole, G., Parker, P., and Gottwald, T. (2010). Plant disease severity estimated visually, by digital photography and image analysis, and by hyperspectral imaging. Critical Reviews in Plant Sciences 29, 59–107.

Bødker, L., and Nielsen, B.J. (2000). Preventive and curative effect of fungicides against potato late blight under field conditions. In Proceedings of the fifth Workshop on the European Network for Development of an Integrated Control Strategy of Potato Late Blight. (Munich, Germany), 261–264.

Boholm, Å. (2003). The cultural nature of risk: Can there be an anthropology of uncertainty? Ethnos 68, 159–178.

Boize, L., Gudin, C., and Purdue, G. (1976). The influence of leaf surface roughness on the spreading of oil spray drops. Annals of Applied Biology 84, 205–211.

Bormann, C.A., Rickert, A.M., Castillo Ruiz, R.A., Paal, J., Lübeck, J., Strahwald, J., Buhr, K., and Gebhardt, C. (2004). Tagging quantitative trait loci for maturity-corrected late blight resistance in tetraploid potato with PCR-based candidate gene markers. Molecular Plant-Microbe Interactions 17, 1126–1138.

Bostrom, A. (1997). Risk Perceptions: Experts vs. Lay People Second Annual Cummings Colloquium on Environmental Law: Risk in the Republic: Comparative Risk Analysis and Public Policy: Conflicts between Expert and Public Risk Comparisons. Duke Environmental. Law & Policy Forum 8, 101–114.

Bouma, E. (2007). Computer aids for plant protection, historical perspective and future developments. EPPO Bulletin 37, 247–254.

Bouma, E., and Hansen, J.G. (1999). Overview of standard descriptions of *Phytophthora* decision support systems. In Proceedings of the Workshop on the European Network for Development of an Integrated Control Strategy of Potato Late Blight, (Uppsala, Sweden), 48–51.

Boyd, A.W. (1974). Sources of Potato Blight (*Phytophthora infestans*) in the East of Scotland. Plant Pathology 23, 30–36.

Boyd, A.W. (1980). Development of potato blight *Phytophthora infestans* after planting infected seed tubers. Annals of Applied Biology 95, 301–309.

Bozkurt, T.O., Schornack, S., Banfield, M.J., and Kamoun, S. (2012). Oomycetes, effectors, and all that jazz. Current Opinion in Plant Biology 15, 483–492.

Bradshaw, J.E., Bryan, G.J., Hackett, C.A., McLean, K., Pande, B., Stewart, H.E., and Waugh, R. (2004). Dissection and analysis of quantitative disease resistance in tetraploid potato. Euphytica 137, 13–18.

Brewer, M.J., and Goodell, P.B. (2012). Approaches and Incentives to Implement Integrated Pest Management that Addresses Regional and Environmental Issues. Annual Review of Entomology 57, 41–59.

Brewer, M.J., Butler, A., and Cooksley, S.L. (2016). The relative performance of AIC, AICC and BIC in the presence of unobserved heterogeneity. Methods in Ecology and Evolution 7, 679–692.

Brier, G.W. (1950). Verification of forecasts expressed in terms of probability. Monthly Weather Review 78, 1–3.

Briere, J.F., Pracros, P., Le Roux, A.-Y., and Pierre, J.-S. (1999). A Novel Rate Model of Temperature-Dependent Development for Arthropods. Environmental Entomology 28, 22–29.

Bruck, R.I., Fry, W.E., Apple, A.E., and Mundt, C.C. (1981). Effect of protectant fungicides on the developmental stages of *Phytophthora infestans* in potato foliage. Phytopathology 71, 164–166.

Bruhn, J.A. (1980). A stochastic model for the simulation of daily weather. Protection Ecology 2, 199–208.

Bruhn, J., and Fry, W. (1981). Analysis of potato late blight epidemiology by simulation modelling. Phytopathology 71, 612–616.

Brumfield, R.T., Beerli, P., Nickerson, D.A., and Edwards, S.V. (2003). The utility of single nucleotide polymorphisms in inferences of population history. Trends in Ecology & Evolution 18, 249–256.

Brurberg, M.B., Elameen, A., Le, V.H., Nærstad, R., Hermansen, A., Lehtinen, A., Hannukkala, A., Nielsen, B., Hansen, J., Andersson, B., and Yuen, J. (2011). Genetic analysis of *Phytophthora infestans* populations in the Nordic European countries reveals high genetic variability. Fungal Biology 115, 335–342.

Brylińska, M., Sobkowiak, S., Stefańczyk, E., and Śliwka, J. (2016). Potato cultivation system affects population structure of *Phytophthora infestans*. Fungal Ecology 20, 132–143.

Buchholz, A., and Nauen, R. (2001). Translocation and translaminar bioavailability of two neonicotinoid insecticides after foliar application to cabbage and cotton. Pest Management Science 58, 10–16.

Bugiani, R., Antoniacci, L., and Trevisan, I. (2010). Curative effect of fungicides against tomato late blight. In Proceedings of the Twelfth EuroBlight Workshop, (Arras, France), 311–312

Burke, W. (1968). Growing Degree-Days in Ireland. Irish Journal of Agricultural Research 7, 61–71.

Burnett, F. (2011). FRAG-UK (Fungicide Resistance Action Group–UK) A review of the context, work and aims of this UK resistance action group. Outlooks on Pest Management 22, 62–63.

Burnham, K.P., and Anderson, D.R. (2004). Multimodel Inference: Understanding AIC and BIC in Model Selection. Sociological Methods & Research 33, 261–304.

Burt, B.A. (2001). Definitions of risk. Journal of Dental Education 65, 1007–1008.

Bustin, S.A., Benes, V., Garson, J.A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M.W., Shipley, G.L., Vandesompele, J., and Wittwer, C.T. (2009). The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clinical Chemistry 55, 611–622.

Bustos-Korts, D., Malosetti, M., Chapman, S., and van Eeuwijk, F. (2016). Modelling of genotype by environment interaction and prediction of complex traits across multiple environments as a synthesis of crop growth modelling, genetics and statistics. In X. Yin, & P. C. Struik (Eds.), Crop Systems Biology, (Springer International), 55–82.

Cabras, P., Schirra, M., Pirisi, F.M., Garau, V.L., and Angioni, A. (1999). Factors Affecting Imazalil and Thiabendazole Uptake and Persistence in Citrus Fruits Following Dip Treatments. Journal of Agricultural and Food Chemistry 47, 3352–3354.

Campbell, H., A'Har, D., Kerr, J., Kiezebrink, D., Roberts, A., Kirkwood, M., Nevison, I., and Lees, A. (2012). Independent Variety Trials 2011 (Potato Council, United Kingdom).

Campbell, H., Kerr, J., Kiezebrink, D., Roberts, A., Kirkwood, M., Nevison, I., and Lees, A. (2013). Independent Variety Trials 2012 (Potato Council, United Kingdom).

Campbell, H., Kerr, J., Pickup, J., Kiezebrink, D., Wale, S., Roberts, A., Kirkwood, M., Nevison, I., and Lees, A. (2014). Independent Variety Trials 2013 (Potato Council, United Kingdom).

Campbell, H., Kerr, J., Wale, S., Fenton, B., Roberts, A., Kirkwood, M., Nevison, I., and Lees, A. (2015). Independent Variety Trials 2014 (AHDB Potatoes, United Kingdom).

Campbell, H., Hall, G., Strachan, H., Fenton, B., Roberts, A., Kirkwood, M., Nevison, I., and Lees, A. (2016). Independent Variety Trials 2015 (AHDB Potatoes, United Kingdom).

Campbell, H., Pickup, J., Fenton, B., Roberts, A., Kirkwood, M., Nevison, I., and Lees, A. (2017). Independent Variety Trials 2016 (AHDB Potatoes, United Kingdom).

Campbell, H., Fenton, B., Wale, S., Jessiman, I., Roberts, A., Vernon, T., Nevison, I., and Lees, A. (2018). Independent Variety Trials 2017 (AHDB Potatoes, United Kingdom).

Campbell, H., Wale, S., Jessiman, I., Nevison, I., Vernon, T., and Lees, A. (2019). Independent Variety Trials 2018 (AHDB Potatoes, United Kingdom).

Carisse, O., Bourgeois, G., and Duthie, J.A. (2000). Influence of Temperature and Leaf Wetness Duration on Infection of Strawberry Leaves by *Mycosphaerella fragariae*. Phytopathology 90, 1120–1125.

Carlisle, D.J., Cooke, L.R., Watson, S., and Brown, A.E. (2002). Foliar aggressiveness of Northern Ireland isolates of *Phytophthora infestans* on detached leaflets of three potato cultivars. Plant Pathology 51, 424–434.

Carnegie, S.F., and Colhoun, J. (1980). Differential leaf susceptibility to *Phytophthora infestans* on potato plants of cv. King Edward. Phytopathologische Zeitschrift 98, 108–117.

Carolan, K., Helps, J., Berg, F. van den, Bain, R., Paveley, N., and van den Bosch, F. (2017). Extending the durability of cultivar resistance by limiting epidemic growth rates. Proceedings of the Royal Society B 284, 20170828.

Casida, J.E. (2012). The Greening of Pesticide–Environment Interactions: Some Personal Observations. Environmental Health Perspectives 120, 487–493.

Caten, C.E., and Jinks, J.L. (1968). Spontaneous variability of single isolates of *Phytophthora infestans*. I. Cultural variation. Canadian Journal of Botany 46, 329–348.

César, V., Labbe, V., Mulot, E., Pirson, J., Laguesse, L., and Rolot, J.L. (2017). Phenotypic and genotypic characteristics of Belgian isolates of *Phytophthora infestans* in 2013-2016. In Proceedings of the Sixteenth EuroBlight Workshop, (Aarhus, Denmark), 207–208.

Chapman, A.C. (2012). The changing *Phytophthora infestans* population. PhD Thesis, University of Dundee, 296 pages.

Chen, X., Lewandowska, D., Armstrong, M.R., Baker, K., Lim, T., Bayer, M., Harrower, B., McLean, K., Jupe, F., Witek, K., Lees, A., Jones, J., Btyan, G., and Hein, I. (2018). Identification and rapid mapping of a gene conferring broad-spectrum late blight resistance in the diploid potato species *Solanum verrucosum* through DNA capture technologies. Theoretical and Applied Genetics 131, 1287–1297.

Chmielarz, M., Sobkowiak, S., Dębski, K., Cooke, D.E.L., Brurberg, M.B., and Śliwka, J. (2014). Diversity of *Phytophthora infestans* from Poland. Plant Pathology 63, 203–211.

Chowdappa, P., Kumar, N.B.J., Madhura, S., Kumar, M.S.P., Myers, K.L., Fry, W.E., Squires, J.N., and Cooke, D.E.L. (2013). Emergence of 13_A2 Blue Lineage of *Phytophthora infestans* was Responsible for Severe Outbreaks of Late Blight on Tomato in South-West India. Journal of Phytopathology 161, 49–58.

Clément, J. a. J., Magalon, H., Pellé, R., Marquer, B., and Andrivon, D. (2010). Alteration of pathogenicity-linked life-history traits by resistance of its host *Solanum tuberosum* impacts sexual reproduction of the plant pathogenic oomycete *Phytophthora infestans*. Journal of Evolutionary Biology 23, 2668–2676.

Clewell, R.A., and Andersen, M.E. (2016). Approaches for characterizing threshold dose–response relationships for DNA-damage pathways involved in carcinogenicity in vivo and micronuclei formation in vitro. Mutagenesis 31, 333–340.

Cohen, Y. (2015). The Novel Oomycide Oxathiapiprolin Inhibits All Stages in the Asexual Life Cycle of *Pseudoperonospora cubensis* - Causal Agent of Cucurbit Downy Mildew. PLoS One 10, e0140015.

Cohen, Y., and Coffey, M.D. (1986). Systemic Fungicides and the Control of Oomycetes. Annual Review of Phytopathology 24, 311–338.

Cohen, Y., and Gisi, U. (2007). Differential Activity of Carboxylic Acid Amide Fungicides Against Various Developmental Stages of *Phytophthora infestans*. Phytopathology 97, 1274–1283.

Cohen, Y., Gisi, U., and Mosinger, E. (1991). Systemic resistance of potato plants against *Phytophthora infestans* induced by unsaturated fatty acids. Physiological and Molecular Plant Pathology 38, 255–263.

Cohen, Y., Rubin, E., Hadad, T., Gotlieb, D., Sierotzki, H., and Gisi, U. (2007). Sensitivity of *Phytophthora infestans* to mandipropamid and the effect of enforced selection pressure in the field. Plant Pathology 56, 836–842.

Cohen, Y., Rubin, A.E., and Galperin, M. (2018a). Control of cucumber downy mildew with novel fungicidal mixtures of Oxathiapiprolin. Phytoparasitica 46, 689–704.

Cohen, Y., Rubin, A.E., and Galperin, M. (2018b). Oxathiapiprolin-based fungicides provide enhanced control of tomato late blight induced by mefenoxam-insensitive Phytophthora infestans. PLOS ONE 13, e0204523.

Collins, A., Milbourne, D., Ramsay, L., Meyer, R., Chatot-Balandras, C., Oberhagemann, P., Jong, W.D., Gebhardt, C., Bonnel, E., and Waugh, R. (1999). QTL for field resistance to late blight in potato are strongly correlated with maturity and vigour. Molecular Breeding 5, 387–398.

Cooke, D.E.L., and Andersson, B. (2013). *Phytophthora infestans* and potato late blight in Europe. In Lamour, K. (Ed.) *Phytophthora*: A Global Perspective, (CABI, United Kingdom), 59–67.

Cooke, D.E.L., and Lees, A.K. (2004). Markers, old and new, for examining *Phytophthora infestans* diversity. Plant Pathology 53, 692–704.

Cooke, L.R., and Little, G. (2002). The effect of foliar application of phosphonate formulations on the susceptibility of potato tubers to late blight. Pest Management Science 58, 17–25.

Cooke, D.E.L, Cano, L.M., Raffaele, S., Bain, R.A., Cooke, L.R., Etherington, G.J., Deahl, K.L., Farrer, R.A., Gilroy, E.M., Goss, E.M., Grünwald, N., Hein, I., MacLean, D., McNicol, J., Randall, E., Oliva, R., Pel, M., Shaw, D., Squires, J., Taylor, M., Vleeshouwers, V., Birch, P., Lees, A., and Kamoun, S. (2012). Genome Analyses of an Aggressive and Invasive Lineage of the Irish Potato Famine Pathogen. PLoS Pathogens 8, e1002940.

Cooke, D.E.L, Lees, A., Chapman, A., Cooke, L., and Bain, R.A. (2014a). R423, GB Late Blight Populations: Monitoring and Implications of Population Changes, (Potato Council, United Kingdom).

Cooke, D.E.L, Lees, A.K., Lassen, P., and Grønbech-Hansen, J. (2011a). Making Sense of *Phytophthora infestans* diversity at national and international scales. In Proceedings of the Thirteenth EuroBlight Workshop, (St. Petersburg, Russia), 37–45.

Cooke, D.E.L., Skelsey, P., Dancey, S., Randall, E., Sullivan, L., and Lees, A.K. (2016). Potato late blight research update; populations, primary inoculum and predictions. The Dundee Conference: Crop Protection in Northern Britain 2016, (Dundee, United Kingdom), 211–216.

Cooke, D.E.L., Randall, E., Sullivan, L., and Lees, A.K. (2018). The origins and implications of a novel population of *Phytophthora infestans* on potato crops in Scotland. The Dundee Conference. Crop Production in Northern Britain 2018, (Dundee, United Kingdom), 203–208.

Cooke, L.R., Quinn, L., Nugent, P., and Walker, E. (2014b). The potato blight population in Northern Ireland in 2012: ongoing changes and fungicide performance. In Proceedings of the Fourteenth EuroBlight Workshop, (Limassol, Cyprus), 145–152.

Corkrey, R., McMeekin, T.A., Bowman, J.P., Ratkowsky, D.A., Olley, J., and Ross, T. (2014). Protein Thermodynamics Can Be Predicted Directly from Biological Growth Rates. PLOS ONE 9, e96100.

Corwin, J.A., and Kliebenstein, D.J. (2017). Quantitative Resistance: More Than Just Perception of a Pathogen. The Plant Cell 29, 655–665.

Cox, C. (1987). Threshold Dose-Response Models in Toxicology. Biometrics 43, 511–523.

Crone, M., McComb, J.A., O'Brien, P.A., and Hardy, G.E.S.J. (2013). Survival of *Phytophthora cinnamomi* as oospores, stromata, and thick-walled chlamydospores in roots of symptomatic and asymptomatic annual and herbaceous perennial plant species. Fungal Biology 117, 112–123.

Cullen, D.W., Lees, A.K., Toth, I.K., and Duncan, J.M. (2001). Conventional PCR and Real-time Quantitative PCR Detection of *Helminthosporium Solani* in Soil and on Potato Tubers. European Journal of Plant Pathology 107, 387–398.

Dake, K. (1992). Myths of Nature: Culture and the Social Construction of Risk. Journal of Social Issues 48, 21–37.

Damos, P., and Savopoulou-Soultani, M. (2012). Temperature-Driven Models for Insect Development and Vital Thermal Requirements. Psyche: A Journal of Entomology 2012, 1–13.

Danan, S., Veyrieras, J.B., and Lefebvre, V. (2011). Construction of a potato consensus map and QTL meta-analysis offer new insights into the genetic architecture of late blight resistance and plant maturity traits. BMC Plant Biology 11, 16.

Dancey, S., Skelsey, P., and Cooke, D. (2017). The Hutton Criteria: a classification tool for identifying high risk periods for potato late blight disease development in Great Britain. In Proceedings of the Sixteenth EuroBlight Workshop, (Aarhus, Denmark), 53–58.

Danies, G., Small, I.M., Myers, K., Childers, R., and Fry, W.E. (2013). Phenotypic Characterization of Recent Clonal Lineages of *Phytophthora infestans* in the United States. Plant Disease 97, 873–881.

Davidson, A.D., Hewitt, C.L., and Kashian, D.R. (2015). Understanding Acceptable Level of Risk: Incorporating the Economic Cost of Under-Managing Invasive Species. PLoS ONE 10, e0141958.

Davidson, G., Phelps, K., Sunderland, K.D., Pell, J.K., Ball, B.V., Shaw, K.E., and Chandler, D. (2003). Study of temperature-growth interactions of entomopathogenic fungi with potential for control of *Varroa destructor* (Acari: Mesostigmata) using a nonlinear model of poikilotherm development. Journal of Applied Microbiology 94, 816–825.

Day, J.P., and Shattock, R.C. (1997). Aggressiveness and other factors relating to displacement of populations of *Phytophthora infestans* in England and Wales. European Journal of Plant Pathology 103, 379–391.

De Buck, A., Van Rijn, I., Roling, N., and Wossink, G. (2001). Farmers' reasons for changing or not changing to more sustainable practices: an exploratory study of arable farming in the Netherlands. The Journal of Agricultural Education and Extension 7, 153–166.

Deahl, K.L., Perez, F.M., Thompson, J.M., Fleming-Archibald, C., Thompson, S., Collier, R., Kildea, S., and Cooke, L.R. (2009). Characterization of *Phytophthora infestans* Isolates from Jersey, Channel Islands. Potato Research 52, 341–354.

Delacre, M., Lakens, D., and Leys, C. (2017). Why Psychologists Should by Default Use Welch's t-test Instead of Student's t-test. International Review of Social Psychology 30, 92–101.

Delgado, M., Sánchez-Rodríguez, C., Escudero, V., Miedes, E., Fernandez, P., Jordá, L., Hernández-Blanco, C., Sanchez Vallet, A., Bednarek, P., Schulze-Lefert, P., Somerville, S., Estevez, J.M., Persson, S., and Molina, A. (2012). *Arabidopsis* Heterotrimeric G-protein Regulates Cell Wall Defense and Resistance to Necrotrophic Fungi. Molecular Plant 5, 98–114.

Delgado, R.A., Monteros-Altamirano, A.R., Li, Y., Visser, R.G.F., van der Lee, T. a. J., and Vosman, B. (2013). Large subclonal variation in *Phytophthora infestans* populations associated with Ecuadorian potato landraces. Plant Pathology 62, 1081–1088.

Desender, S., Andrivon, D., and Val, F. (2007). Activation of defence reactions in *Solanaceae*: where is the specificity? Cellular Microbiology 9, 21–30.

Desnouck, J., Testers, J., and Versmissen, C. (2012). Ranman Top, again a step forwards in late blight control. In Proceedings of the Thirteenth EuroBlight Workshop (St. Petersburg, Russia) 155–117.

Dey, T., Saville, A., Myers, K., Tewari, S., Cooke, D.E.L., Tripathy, S., Fry, W.E., Ristaino, J.B., and Roy, S.G. (2018). Large sub-clonal variation in *Phytophthora infestans* from recent severe late blight epidemics in India. Scientific Reports 8, 4429.

Dias, M.C. (2012). Phytotoxicity: An overview of the physiological responses of plants exposed to fungicides. Journal of Botany 2012, 1–4.

Dixelius, C., Fagerström, T., and Sundström, J.F. (2012). European agricultural policy goes down the tubers. Nature Biotechnology 30, 492–493.

Doke, N. (1975). Prevention of the hypersensitive reaction of potato cells to infection with an incompatible race of *Phytophthora infestans* by constituents of the zoospores. Physiological Plant Pathology 7, 1–7.

Donnelly, J.S. (2007). The great Irish potato famine (The History Press, United Kingdom).

Dorn, B., Musa, T., Krebs, H., Fried, P.M., and Forrer, H.R. (2007). Control of late blight in organic potato production: evaluation of copper-free preparations under field, growth chamber and laboratory conditions. European Journal of Plant Pathology 119, 217–240.

Dorrance, A.E., and Inglis, D.A. (1998). Assessment of laboratory methods for evaluating potato tubers for resistance to late blight. Plant Disease 82, 442–446.

Doster, M.A., and Fry, W.E. (1991). Evaluation by computer simulation of strategies to time metalaxyl applications for improved control of potato late blight. Crop Protection 10, 209–214.

Dowley, L.J., and Burke, J.J. (2004). Field validation of four decision support systems for the control of potato late blight in Ireland. In Proceedings of the Eighth Workshop of an European Network for Development of an Integrated Control Strategy of Potato Late Blight, (Jersey, United Kingdom), 45–60.

Dowley, L.J., and O'Sullivan, E. (1991). Sporulation of *Phytophthora infestans* (Mont.) de Bary on the surface of diseased potatoes and tuber to tuber spread of infection during handling. Potato Research 34, 295–296.

Dowley, L., Leonard, R., Rice, B., and Ward, S. (2001). Efficacy of the NegFry decision support system in the control of potato late blight in Ireland. In Proceedings of the Sixth Workshop of an European Network for Development of an Integrated Control Strategy of Potato Late Blight, (Edinburgh, Scotland), 81–92.

Dowley, L.J., Griffin, D., and O'sullivan, E. (2002). Two decades of monitoring Irish populations of *Phytophthora infestans* for phenylamide resistance. Potato Research 45, 79–84.

Draffehn, A.M., Li, L., Krezdorn, N., Ding, J., Lübeck, J., Strahwald, J., Muktar, M.S., Walkemeier, B., Rotter, B., and Gebhardt, C. (2013). Comparative transcript profiling by SuperSAGE identifies novel candidate genes for controlling potato quantitative resistance to late blight not compromised by late maturity. Frontiers in Plant Science 4, 423.

Drenth, A., Tas, I.C.Q., and Govers, F. (1994). DNA fingerprinting uncovers a new sexually reproducing population of *Phytophthora infestans* in the Netherlands. European Journal of Plant Pathology 100, 97–107.

Du, Y., Mpina, M.H., Birch, P.R.J., Bouwmeester, K., and Govers, F. (2015). *Phytophthora infestans* RXLR Effector AVR1 Interacts with Exocyst Component Sec5 to Manipulate Plant Immunity. Plant Physiology 169, 1975–1990.

Du, Y., Weide, R., Zhao, Z., Msimuko, P., Govers, F., and Bouwmeester, K. (2018). RXLR effector diversity in *Phytophthora infestans* isolates determines recognition by potato resistance proteins; the case study AVR1 and R1. Studies in Mycology 89, 85–93.

Dyer, A., Matusralt, M., Drenth, A., Cohen, A., and Splelman, L. (1993). Historical and recent migrations of *Phytophthora infestans*: chronology, pathways, and implications. Plant Disease 77, 653–661.

Dytham, C. (2011). Choosing and using statistics: a biologist's guide (Wiley-Blackwell, United Kindom).

Ebersold, D. (2002). Ranman, the new marathon fungicide. In Proceedings of the Sixth Workshop of an European Network for Development of an Integrated Control Strategy of Potato Late Blight, (Edinburgh, Scotland), 225–230

Edwards-Jones, G., and Howells, O. (2001). The origin and hazard of inputs to crop protection in organic farming systems: are they sustainable? Agricultural Systems 67, 31–47.

Ehler, L.E. (2006). Integrated pest management (IPM): definition, historical development and implementation, and the other IPM. Pest Management Science 62, 787–789.

Ellinger, D., Naumann, M., Falter, C., Zwikowics, C., Jamrow, T., Manisseri, C., Somerville, S.C., and Voigt, C.A. (2013). Elevated early callose deposition results in complete penetration resistance to powdery mildew in *Arabidopsis*. Plant Physiology 161, 1433–1444.

Elliott, N., Farrell, J., Gutierrez, A., van Lenteren, J.C., Walton, M., and Wratten, S. (1995). Integrated pest management (Springer, United States).

Elzhov, T.V., Mullen, K.M., Spiess, A.-N., Bolker, B., and Mullen, M.K.M. (2016). Package 'minpack. lm.' (CRAN).

Engelbrecht, S. (2003). Performance of a phosphite formulation against late blight in potatoes. C.H.I.P.S 40.

Eremeev, V., Lõhmus, A., and Jõudu, J. (2006). NegFry–DSS for the chemical control of potato late blight–results of validation trials in Tartu. Agronomy Research 4, 167–170.

Eschen-Lippold, L., Landgraf, R., Smolka, U., Schulze, S., Heilmann, M., Heilmann, I., Hause, G., and Rosahl, S. (2012). Activation of defense against *Phytophthora infestans* in potato by down-regulation of syntaxin gene expression. The New Phytologist 193, 985–996.

Evans, E. (1971). Systemic fungicides in practice. Pesticide Science 2, 192–196.

Evenhuis, A., Spits, H.G., and Schepers, H. (2006). Efficacy of fungicidal protection of newly developing potato leaves against *Phytophthora infestans*. Crop Protection 25, 562–568.

Evenhuis, A., Bain, R., Hausladen, H., Nielsen, B., van den Berg, W., and Schepers, H. (2016). Fungicide evaluation to rate efficacy to control leaf late blight for the EuroBlight table Results 2006-2015 (Wageningen UR, The Netherlands).

Evenhuis, B., Spruijt, J., Kalkdijk, R., Spits, H., Schepers, H. (2009). Evaluation of spray strategies to control potato late blight with respect to efficacy, economics and the environment. In Proceedings of the Eleventh EuroBlight workshop, (Hamar, Norway), 83–90.

Evers, D., Welschbillig, N., Dommes, J., and Hausman, J.F. (2003). Biochemical and morphological characterization of potato clones differing in their resistance to late blight. Potato Research 46, 105–115.

Fabre, F., Plantegenest, M., and Yuen, J. (2007). Financial benefit of using crop protection decision rules over systematic spraying strategies. Phytopathology 97, 1484–1490.

Fait, A., Ferioli, A., and Barbieri, F. (1994). Chapter 11 Organotin compounds. Toxicology 91, 77–82.

Farquhar, G.D., Caemmerer, S. von, and Berry, J.A. (1980). A biochemical model of photosynthetic CO_2 assimilation in leaves of C3 species. Planta 149, 78–90.

Fay, J.C., and Fry, W.E. (1997). Effects of hot and cold temperatures on the survival of oospores produced by United States strains of *Phytophthora infestans*. American Potato Journal 74, 315–323.

Fernandez-Cornejo, J. (1998). Environmental and economic consequences of technology adoption: IPM in viticulture. Agricultural Economics 18, 145–155.

Fetter, R.B., Shin, Y., Freeman, J.L., Averill, R.F., and Thompson, J.D. (1980). Case Mix Definition by Diagnosis-Related Groups. Medical Care 18, 1–53.

Figueroa, M., Hammond-Kosack, K.E., and Solomon, P.S. (2018). A review of wheat diseases —a field perspective. Molecular Plant Pathology 19, 1523–1536.

Filippov, A.V., Kuznetsova, M.A., Rogozhin, A.N., Spiglazova, S.Y., Smetanina, T.I., Derenko, T.A., and Statsyuk, N.V. (2009). Efficacy of the VNIIFBlight decision support system in the control of potato late blight in Russia. In Proceedings of the Eleventh EuroBlight workshop, (Hamar, Norway), 243–250.

Flier, W.G., and Turkensteen, L.J. (1999). Foliar Aggressiveness of *Phytophthora infestans* in Three Potato Growing Regions in the Netherlands. European Journal of Plant Pathology 105, 381–388.

Flier, W.G., Grünwald, N.J., Kroon, L.P., Sturbaum, A.K., van den Bosch, T.B., Garay-Serrano, E., Lozoya-Saldaña, H., Fry, W.E., and Turkensteen, L.J. (2003a). The population structure of *Phytophthora infestans* from the Toluca Valley of Central Mexico suggests genetic differentiation between populations from cultivated potato and wild *Solanum spp*. Phytopathology 93, 382–390.

Flier, W.G., Van Den Bosch, G.B.M., and Turkensteen, L.J. (2003b). Stability of partial resistance in potato cultivars exposed to aggressive strains of *Phytophthora infestans*. Plant Pathology 52, 326–337.

Flor, H.H. (1971). Current Status of the Gene-For-Gene Concept. Annual Review of Phytopathology 9, 275–296.

Forbes, G. (2004). Global overview of late blight. In Proceedings of the Regional Workshop on Potato Late Blight East and Southeast Asia and the Pacific. C. Lizarraga, Ed. (Yezin, Myanmar), 3–10.

Forbes, G.A., Goodwin, S.B., Drenth, A., Oyarzun, P., Ordoñez, M.E., and Fry, W.E. (1998). A Global Marker Database for Phytophthora infestans. Plant Disease 82, 811–818.

Förch, M., van den Bosch, G., van Bekkum, P., Evenhuis, A., and Vossen, J. (2010). Monitoring the Dutch *Phytophtora infestans* population for virulence against new R-genes. In Proceedings of the Twelfth Euroblight Workshop, (Arras, France), 45–49.

FRAG-UK (2018). Fungicide Resistance Management in Potato Late Blight (AHDB, United Kingdom).

Frinking, H.D., Davidse, L.C., and Limburg, H. (1987). Oospore formation by *Phytophthora infestans* in host tissue after inoculation with isolates of opposite mating type found in the Netherlands. Netherlands Journal of Plant Pathology 93, 147–149.

Fry, W. (2008). *Phytophthora infestans*: the plant (and R gene) destroyer. Molecular Plant Pathology 9, 385–402.

Fry, W.E. (2016). *Phytophthora infestans*: New Tools (and Old Ones) Lead to New Understanding and Precision Management. Annual Review of Phytopathology 54, 529–547.

Fry, W., and Shtienberg, D. (1990). Integration of host resistance and fungicide to manage potato diseases. Canadian Journal of Plant Pathology 12, 111–116.

Fry, W.E., and Goodwin, S.B. (1997). Resurgence of the Irish potato famine fungus. Bioscience 47, 363–371.

Fry, W.E., and Smart, C.D. (1999). The return of *Phytophthora infestans*, a potato pathogen that just won't quit. Potato Research 42, 279–282.

Fry, W., Apple, A., and Bruhn, J. (1983). Evaluation of potato late blight forecasts modified to incorporate host resistance and fungicide weathering. Phytopathology 73, 1054–1059.

Fry, W., Birch, P., Judelson, H., Grünwald, N.J., Danies, G., Everts, K.L., Gevens, A.J., Gugino, B., Johnson, D.A., Johnson, S.B., McGrath, M.T., Myers, K.L., Ristanio, J.B., Roberts, P.D., Secor, G., and Smart C.D. (2015). Five Reasons to consider *Phytophthora infestans* a re-emerging pathogen. Phytopathology. 105, 966–981.

Fry, W.E., Bruck, R.I., and Mundt, C.C. (1979). Retardation of potato late blight epidemics by fungicides with eradicant and protectant properties. Plant Disease Reporter 63, 970–974.

Fry, W.E., Goodwin, S.B., Matuszak, J.M., Spielman, L.J., Milgroom, M.G., and Drenth, A. (1992). Population Genetics and Intercontinental Migrations of *Phytophthora infestans*. Annual Review of Phytopathology 30, 107–130.

Fry, W.E., Cooke, D.E., McLeod, A., Forbes, G.A., Cao, K. (2008). Population genetics and population diversity of *Phytophthora infestans*. Oomycete Genetics and Genomics: Diversity, Interactions, and Research Tools 139–164.

Fry, W.E., Patev, S.P., Myers, K.L., Bao, K., and Fei, Z. (2019). *Phytophthora infestans* Sporangia Produced in Culture and on Tomato Leaflet Lesions Show Marked Differences in Indirect Germination Rates, Aggressiveness, and Global Transcription Profiles. Molecular Plant Microbe Interactions 32, 515–526.

Gabriel, J., Coca, A., Plata, G., and Parlevliet, J. (2007). Characterization of the resistance to *Phytophthora infestans* in local potato cultivars in Bolivia. Euphytica 153, 321–328.

Gaucher, D., Deudon, O., Huard, F., and Launay, M. (2018). Effects of climate change on potato late blight risk and adaptation strategies. 12e Conférence Internationale sur les Maladies des Plantes, 11 et 12 décembre 2018, (Tours, France), 19–28.

Gebhardt, C., and Valkonen, J.P.T. (2001). Organization of Genes Controlling Disease Resistance in the Potato Genome. Annual Review of Phytopathology 39, 79–102.

Genet, J.L., Jaworska, G., Geddens, R., Shepherd, C., and Bain, R.A. (2001). Effect of temperature on the curative and anti-sporulant action of cymoxanil for control of *Phytophthora infestans*. In Proceedings of the Workshop on the European Network for Development of an Integrated Control Strategy of Potato Late Blight (Munich, Germany), 107–117.

Gent, D.H., Mahaffee, W.F., McRoberts, N., and Pfender, W.F. (2013). The Use and Role of Predictive Systems in Disease Management. Annual Review of Phytopathology 51, 267–289.

Gilbert, E., Powell, J.A., Logan, J.A., and Bentz, B.J. (2004). Comparison of three models predicting developmental milestones given environmental and individual variation. Bulletin of Mathematical Biology 66, 1821–1950.

Gill, U.S., Lee, S., and Mysore, K.S. (2015). Host Versus Nonhost Resistance: Distinct Wars with Similar Arsenals. Phytopathology 105, 580–587.

Gisi, U. (2002). Chemical Control of Downy Mildews. In Advances in Downy Mildew Research, P.T.N. Spencer-Phillips, U. Gisi, and A. Lebeda, eds. (Springer, Netherlands), 119–159.

Gisi, U., and Cohen, Y. (1996). Resistance to phenylamide fungicides: a case study with *Phytophthora infestans* involving mating type and race structure. Annual Review of Phytopathology 34, 549–572.

Gisi, U., and Sierotzki, H. (2008). Fungicide modes of action and resistance in downy mildews. European Journal of Plant Pathology 122, 157–167.

Gneiting, T., and Raftery, A.E. (2005). Weather Forecasting with Ensemble Methods. Science 310, 248–249.

Gold, H.J. (1989). Decision analytic modeling for plant disease control. Plant Disease Epidemiology 2, 84–124.

Goodwin, S., Drenth, A., and Fry, W. (1992). Cloning and genetic analyses of two highly polymorphic, moderately repetitive nuclear DNAs from *Phytophthora infestans*. Current Genetics 22, 107–115

Goodwin, S.B., Sujkowski, L.S., and Fry, W.E. (1995). Rapid evolution of pathogenicity within clonal lineages of the potato late blight disease fungus. Phytopathology 85, 669–676.

Goodwin, S.B., Smart, C.D., Sandrock, R.W., Deahl, K.L., Punja, Z.K., and Fry, W.E. (1998). Genetic Change Within Populations of *Phytophthora infestans* in the United States and Canada During 1994 to 1996: Role of Migration and Recombination. Phytopathology 88, 939–949.

Goss, E.M., Tabima, J.F., Cooke, D.E.L., Restrepo, S., Fry, W.E., Forbes, G.A., Fieland, V.J., Cardenas, M., and Grünwald, N.J. (2014). The Irish potato famine pathogen *Phytophthora infestans* originated in central Mexico rather than the Andes. PNAS 111, 8791–8796.

Grayson, T., Batten, D., and Walter, D. (1996). Adjuvant Effects on the Therapeutic Control of Potato Late Blight by Dimethomorph Wettable Powder Formulations. Pesticide Science 46, 355–359.

Gregory, R., and Mendelsohn, R. (1993). Perceived Risk, Dread, and Benefits. Risk Analysis 13, 259–264.

Grenville-Briggs, L.J., Avrova, A.O., Bruce, C.R., Williams, A., Whisson, S.C., Birch, P.R.J., and van West, P. (2005). Elevated amino acid biosynthesis in *Phytophthora infestans* during appressorium formation and potato infection. Fungal Genetics and Biology 42, 244–256.

Griend, P., and Bosch, R.A. (2007). Curative control of *P. infestans* in potatoes. In Proceedings of the Tenth Workshop of an European Network for Development of an Integrated Control Strategy of Potato Late Blight, (Bologna, Italy), 365–368.

Griffith, G.W., and Shaw, D.S. (1998). Polymorphisms in *Phytophthora infestans*: Four Mitochondrial Haplotypes Are Detected after PCR Amplification of DNA from Pure Cultures or from Host Lesions. Applied and Environmental Microbiology 64, 4007–4014.

Grigorieva, E.A., Matzarakis, A., and de Freitas, C.R. (2010). Analysis of growing degree-days as a climate impact indicator in a region with extreme annual air temperature amplitude. Climate Research 42, 143–154.

Grove, G., Madden, L., Ellis, M., and Schmitthenner, A. (1985). Influence of temperature and wetness duration on infection of immature strawberry fruit by *Phytophthora cactorum*. Phytopathology 75, 165–169.

Grünwald, N.J., and Flier, W.G. (2005). The Biology of *Phytophthora infestans* at Its Center of Origin. Annual Review of Phytopathology 43, 171–190.

Grünwald, N.J., Goss, E.M., and Press, C.M. (2008). *Phytophthora ramorum*: a pathogen with a remarkably wide host range causing sudden oak death on oaks and ramorum blight on woody ornamentals. Molecular Plant Pathology 9, 729–740.

Guenthner, J., Michael, K., and Nolte, P. (2001). The economic impact of potato late blight on US growers. Potato Research 44, 121–125.

Gullino, M.L., and Kuijpers, L.A. (1994). Social and political implications of managing plant diseases with restricted fungicides in Europe. Annual Review of Phytopathology 32, 559–581.

Gullino, M.L., Tinivella, F., Garibaldi, A., Kemmitt, G.M., Bacci, L., and Sheppard, B. (2010). Mancozeb: Past, Present, and Future. Plant Disease 94, 1076–1087.

Gyetvai, G., Sønderkær, M., Göbel, U., Basekow, R., Ballvora, A., Imhoff, M., Kersten, B., Nielsen, K.-L., and Gebhardt, C. (2012). The Transcriptome of Compatible and Incompatible Interactions of Potato (*Solanum tuberosum*) with *Phytophthora infestans* Revealed by DeepSAGE Analysis. PLoS ONE 7, e31526.

Haas, B.J., Kamoun, S., Zody, M.C., Jiang, R.H.Y., Handsaker, R.E., Cano, L.M., Grabherr, M., Kodira, C.D., Raffaele, S., Torto-Alalibo, T., Bozkurtz, T., Ah-Fong, A., Alvarado, L., Anderson, V., Armstrong, M., Avrova, A., Baxter, L., Beyon, J., Boevink, P., Bollmann, S., Bos, J., Bulone, V., Cai, G., Cakir, C., Carrington, J.., Chawner, M., Conti, L., Costanzo, S., Ewan, R., Fahlgren, N., Fischbach, M., Fugelstad, J., Gilroy, E., Gnerre, S., Green, P., Grenville-Briggs, L., Griffith, J., Grünwald, N., Horn, K., Horner, N., Hu, C., Huitema, E., Jeong, D., Jones A., Jones J., Jones, R., Karlsson, E., Kunjeti, S., Lamour, K., Liu, K., Ma, L., MacLean, D., Chibucos, M., McDonald, H., McWalters, J., Meijer, H., Morgan, W., Morris, P., Munro, C., O'Neill, K., Ospina-Giraldo, M., Pinzón, A., Pritchard, L., Ramsahoye, B., Ren, Q., REstrepo,

S., Roy, S., Sadanandom, A., Savodor, A., Schornack, S., Schwartz, D., Schumann, U., Schwessinger, B., Seyer, L., Sharpe, T., Silvar, C., Song, J., Studholme, D., Sykes, S., Thines, M., van de Vondervoort, P., Vipaporn, P., Wawra, S., Weide, R., Win, J., Young., C., Zhou, S., Fry, W., Meyers, B., van West, P., Ristaino, J., Govers, F., Birch, P., Whisson, S., Judelson, H., and Nusbaum, C. (2009). Genome sequence and analysis of the Irish potato famine pathogen *Phytophthora infestans*. Nature 461, 393–398.

Hack, H., Gall, H., Klemke, T., Klose, R., Meier, U., Stauss, R., and Witzenberger, A. (1993). The BBCH scale for phenological growth stages of potato (*Solanum tuberosum* L.). In Proceedings of the Twelfth Annual Congress of the European Association for Potato Research, (Paris, France), 153–154.

Hadders, J. (1997). Experience with a Late Blight DSS (Plant Plus) in starch potato area of the Netherlands in 1995 and 1996. In Proceedings of the Workshop on the European Network for the Development of an Integrated Control Strategy for Potato Late Blight, (Lelystad, The Netherlands), 117–122.

Hadders, J. (1999). Experiences with Plant Plus in 1998. PAV–Special Report 5, 194–200.

Haefner, J.W. (2005). Modeling Biological Systems: Principles and Applications (Springer Science & Business Media, United States).

Haesaert, G., Vossen, J.H., Custers, R., De Loose, M., Haverkort, A., Heremans, B., Hutten, R., Kessel, G., Landschoot, S., van Droogenbroeck, B., Visser, R., Gheysen, G. (2015). Transformation of the potato variety Desiree with single or multiple resistance genes increases resistance to late blight under field conditions. Crop Protection 77, 163–175.

Halim, V.A., Eschen-Lippold, L., Altmann, S., Birschwilks, M., Scheel, D., and Rosahl, S. (2007). Salicylic Acid Is Important for Basal Defense of *Solanum tuberosum* Against *Phytophthora infestans*. Molecular Plant-Microbe Interactions 20, 1346–1352.

Hansen, J.G. (1997). Field specific and regional warning for development of potato late blight. In Proceedings from Workshop: European Network for Development of an Integrated Control Strategy of Potato Late Blight, (Lelystad, The Netherlands), 127–134.

Hansen, J.G., Andersson, B., Bain, R., Ritchie, F., Gulbis, G., Kildea, S., Cooke, L., Dubois, L., Chatot, C., and Filippov, A.(2012). The development and control of Late Blight (*Phytophthora infestans*) in Europe in 2010 and 2011. In Proceedings of the Thirteenth EuroBlight Workshop, (St. Petersburg, Russia), 11–31.

Hansen, J.G., Andersson, B., Sjoholm, L., Liljeroth, E., Edin, E., Bain, R., Lees, A., Ritchie, F., Kildea, S., and Cooke, L. (2016a). Epidemics and control of early & late blight, 2013 & 2014 in Europe. In Proceedings of the Fifteenth Euroblight Workshop, (Brasov, Romania), 11–30.

Hansen, Z.R., Everts, K.L., Fry, W.E., Gevens, A.J., Grünwald, N.J., Gugino, B.K., Johnson, D.A., Johnson, S.B., Judelson, H.S., and Knaus, B.J. (2016b). Genetic Variation within Clonal Lineages of *Phytophthora infestans* Revealed through Genotyping-By-Sequencing, and Implications for Late Blight Epidemiology. PLOS ONE 11, e0165690.

Hansen, Z.R., Knaus, B.J., Tabima, J.F., Press, C.M., Judelson, H.S., Grünwald, N.J., and Smart, C.D. (2016c). SNP-Based Differentiation of *Phytophthora infestans* Clonal Lineages

Using Locked Nucleic Acid Probes and High-Resolution Melt Analysis. Plant Disease 100, 1297–1306.

Hanson, J., Dismukes, R., Chambers, W., Greene, C., and Kremen, A. (2004). Risk and risk management in organic agriculture: Views of organic farmers. Renewable Agriculture and Food Systems 19, 218–227.

Hao, D., Yang, J., Long, W., Yi, J., VanderZaag, P., and Li, C. (2018). Multiple R genes and phenolic compounds synthesis involved in the durable resistance to *Phytophthora infestans* in potato cv. Cooperation 88. Agri Gene 8, 28–36.

Harbaoui, K., Lee, T. van der, Vleeshouwers, V.G.A.A., Khammassy, N., Harrabi, M., and Hamada, W. (2013). Characterisation of *Phytophthora infestans* Isolates Collected from Potato and Tomato Crops in Tunisia During 2006–2008. Potato Research 56, 11–29.

Harrison, J. (1992). Effects of the aerial environment on late blight of potato foliage–a review. Plant Pathology 41, 384–416.

Harrison, J.G., Barker, H., Lowe, R., and Rees, E.A. (1990). Estimation of amounts of *Phytophthora infestans* mycelium in leaf tissue by enzyme-linked immunosorbent assay. Plant Pathology 39, 274–277.

Harrison, J.G., Lowe, R., and Williams, N.A. (1994). Effects of temperature and light on non-race-specific resistance of potato leaflets to late blight. Plant Pathology 43, 733–739.

Hartill, W.F.T., Young, K., Allan, D.J., and Henshall, W.R. (1990). Effects of temperature and leaf wetness on the potato late blight. New Zealand Journal of Crop and Horticultural Science 18, 181–184.

Hatfield, J.L., and Prueger, J.H. (2015). Temperature extremes: Effect on plant growth and development. Weather and Climate Extremes 10, 4–10.

Haverkort, A.J., Boonekamp, P.M., Hutten, R., Jacobsen, E., Lotz, L.P., Kessel, G.J.T., Visser, R.G.F., and Vossen, E.A.G. van der (2008). Societal Costs of Late Blight in Potato and Prospects of Durable Resistance Through Cisgenic Modification. Potato Research 51, 47–57.

Haverkort, A.J., Struik, P.C., Visser, R.G.F., and Jacobsen, E. (2009). Applied Biotechnology to Combat Late Blight in Potato Caused by *Phytophthora Infestans*. Potato Research 52, 249–264.

Hayman, P. (2004). Decision support systems in Australian dryland farming: A promising past, a disappointing present and uncertain future. In Proceedings of 4th International Crop Science Congress'.(Brisbane, Australia).

Helland, I.S. (1987). On the Interpretation and Use of R^2 in Regression Analysis. Biometrics 43, 61–69.

Heremans, B., Dewitte, K., Pycke, B., De Schampeleire, M., Spanoghe, P., Steurbaut, W., and Haesaert, G. (2018). Effect of adjuvants on the efficiency of benthiavalicarb plus mancozeb (Valbon 1.6 kg/ ha) on the control of late blight in potato. In Proceedings of the Eleventh EuroBlight Workshop, (Hamar, Norway), 261–267.

Hernández, A.F., Parrón, T., Tsatsakis, A.M., Requena, M., Alarcón, R., and López-Guarnido, O. (2013). Toxic effects of pesticide mixtures at a molecular level: Their relevance to human health. Toxicology 307, 136–145.

Hijmans, R.J., Forbes, G.A., and Walker, T.S. (2000). Estimating the global severity of potato late blight with GIS-linked disease forecast models. Plant Pathology 49, 697–705.

Hinds, H. (2000). Using disease forecasting to reduce fungicide input for potato blight in the UK. PAV-Special Report No. 6 February 2000 82.

Hinds, H. (2001). Can blight forecasting work on large potato farms? PAV-Special Report No. 7 February 2001 99.

Hirst, J.M., and Stedman, O.J. (1960). The Epidemiology of *Phytophthora infestans*. Annals of Applied Biology 48, 489–517.

Hodgson, W. (1961). Laboratory testing of the potato for partial resistance to *Phytophthora infestans*. American Journal of Potato Research 38, 259–264.

Hodgson, W.A. (1963). The eradicant effect of some fungicides on potato late blight. American Potato Journal 40, 143–148.

Hohl, H.R., and Iselin, K. (1984). Strains of *Phytophthora infestans* from Switzerland with A2 mating type behaviour. Transactions of the British Mycological Society 83, 529–530.

Hollomon, D.W. (1966). Reducing Compounds and the Growth of *Phytophthora infestans* Microbiology, 45, 315–324.

Hothorn, L. (2014). Statistical evaluation of toxicological bioassays – a review. Toxicology Research 3, 418–432.

Hou, H., Atlihan, N., and Lu, Z.-X. (2014). New biotechnology enhances the application of cisgenesis in plant breeding. Frontiers in Plant Science 5, 389.

Hu, J., Hong, C., Stromberg, E.L., and Moorman, G.W. (2007). Effects of Propamocarb Hydrochloride on Mycelial Growth, Sporulation, and Infection by *Phytophthora nicotianae* Isolates from Virginia Nurseries. Plant Disease 91, 414–420.

Huang, S., Vleeshouwers, V.G.A.A., Werij, J.S., Hutten, R.C.B., van Eck, H.J., Visser, R.G.F., and Jacobsen, E. (2004). The R3 Resistance to *Phytophthora infestans* in Potato is Conferred by Two Closely Linked R Genes with Distinct Specificities. Molecular Plant-Microbe Interactions 17, 428–435.

Hughes, G. (1999). Sampling for decision making in crop loss assessment and pest management: introduction. Phytopathology 89, 1080–1083.

Iglesias, I., Escuredo, O., Seijo, C., and Méndez, J. (2009). *Phytophthora infestans* Prediction for a Potato Crop. American Journal of Potato Research 87, 32–40.

Jacobsen, B.J. (1997). Role of plant pathology in integrated pest management. Annual Review of Phytopathology 35, 373–391.

Jagiello, R.D., and Hills, T.T. (2018). Bad News Has Wings: Dread Risk Mediates Social Amplification in Risk Communication. Risk Analysis 38, 2193–2207.

Jansky, S. (2000). Breeding for disease resistance in potato. Plant Breeding Reviews 19, 69–156.

Jiang, L., Wang, H., Xu, H., Qiao, K., Xia, X., and Wang, K. (2015). Transportation behaviour of fluopicolide and its control effect against *Phytophthora capsici* in greenhouse tomatoes after soil application. Pest Management Science 71, 1008–1014.

Johnson, G.F. (1935). The Early History of Copper Fungicides. Agricultural History 9, 67–79.

Johnson, D.A., Cummings, T.F., and Geary, B. (2000). Postinfection Activity of Selected Late Blight Fungicides. Plant Disease 84, 1116–1120.

Judelson, H.S. (1997). The Genetics and Biology of *Phytophthora infestans*: Modern Approaches to a Historical Challenge. Fungal Genetics and Biology 22, 65–76.

Judelson, H.S., and Blanco, F.A. (2005). The spores of *Phytophthora*: weapons of the plant destroyer. Nature Reviews Microbiology 3, 47–58.

Judelson, H.S., and Roberts, S. (1999). Multiple loci determining insensitivity to phenylamide fungicides in *Phytophthora infestans*. Phytopathology 89, 754–760.

Judelson, H.S., and Tooley, P.W. (2000). Enhanced Polymerase Chain Reaction Methods for Detecting and Quantifying *Phytophthora infestans* in Plants. Phytopathology 90, 1112–1119.

Judelson, H.S., Michelmore, R.W. (1990). Highly abundant and stage-specific mRNAs in the obligate pathogen *Bremia lactucae*. Molecular Plant-Microbe Interactions 3, 225–232.

Judelson, H.S., Narayan, R.D., Ah-Fong, A.M.V., and Kim, K.S. (2008). Gene expression changes during asexual sporulation by the late blight agent *Phytophthora infestans* occur in discrete temporal stages. Molecular Genetics and Genomics 281, 193–206.

Judelson, H.S., Tani, S., and Narayan, R.D. (2009). Metabolic adaptation of *Phytophthora infestans* during growth on leaves, tubers and artificial media. Molecular Plant Pathology 10, 843–855.

Kable, P.F. (1987). Strategies for combating fungicide resistance—avoidance of curative and eradicative applications. EPPO Bulletin 17, 341–343.

Kadish, D., Grinberger, M., and Cohen, Y. (1990). Fitness of metalaxyl-sensitive and metalaxyl-resistant isolates of *Phytophthora infestans* on susceptible and resistant potato cultivars. Phytopathology 80, 200–205.

Kamoun, S., van West, P., and Govers, F. (1998). Quantification of late blight resistance of potato using transgenic *Phytophthora infestans* expressing β-glucuronidase. European Journal of Plant Pathology 104, 521–525.

Kamoun, S., Furzer, O., Jones, J.D.G., Judelson, H.S., Ali, G.S., Dalio, R.J.D., Roy, S.G., Schena, L., Zambounis, A., and Panabières, F. (2015). The Top 10 oomycete pathogens in molecular plant pathology. Molecular Plant Pathology 16, 413–434.

Kandel, K.P. (2014). Transcriptomic studies of the early stages of potato infection by Phytophthora infestans. Ph.D Thesis, University of Dundee, 246 pages.

Kaniewski, W., Lawson, C., Sammons, B., Haley, L., Hart, J., Delannay, X., and Tumer, N.E. (1990). Field resistance of transgenic russeet burbank potato to effects of infection by potato virus X and potato virus Y. Nature Biotechnology 8, 750–754.

Kankwatsa, P., Hakiza, J.J., Olanya, M., Kidenamariam, H.M., and Adipala, E. (2003). Efficacy of different fungicide spray schedules for control of potato late blight in Southwestern Uganda. Crop Protection 22, 545–552.

Kasperson, R.E., Renn, O., Slovic, P., Brown, H.S., Emel, J., Goble, R., Kasperson, J.X., and Ratick, S. (1988). The social amplification of risk: A conceptual framework. Risk Analysis 8, 177–187.

Keil, S., Benker, M., Lauer, F., Zellner, M. (2010a). Infection rate of potato seed tubers with *Phytophthora infestans* (Mont.) de Bary. Journal of Horticulture, Forestry & Biotechnology 14, 145–8.

Keil, S.B., Benker, M., and Zellner, M. (2010b). Double Setting of Potato Seed Tubers as a New Approach to Research Primary Stem Blight (*Phytophthora infestans* (Mont.) de Bary). American Journal of Potato Research 87, 27–31.

Keinath, A.P., and DuBose, V.B. (2004). Evaluation of fungicides for prevention and management of powdery mildew on watermelon. Crop Protection 23, 35–42.

Kessel, G., Flier, W., Spits, H., Wander, J., and Schepers, H. (2004). Exploitation of cultivar resistance using reduced fungicide dose rates, the Wageningen UR approach. In Proceedings of the Eighth Workshop of an European Network for Development of an Integrated Control Strategy of Potato Late Blight, (Jersey, United Kingdom), 211–214.

Kessel, G.J.T., Mullins, E., Evenhuis, A., Stellingwerf, J., Cortes, V.O., Phelan, S., van den Bosch, T., Förch, M.G., Goedhart, P., van der Voet, H., and Lotz, L. (2018). Development and validation of IPM strategies for the cultivation of cisgenically modified late blight resistant potato. European Journal of Agronomy 96, 146–155.

Keulen, H.V., and Stol, W. (1995). Agro-ecological zonation for potato production. In: Potato Ecology And Modelling of Crops under Conditions Limiting Growth, (Springer, Dordrecht), 357–371.

Kildea, S., Quinn, L., Mehenni-Ciz, J., Cooke, D.E.L., Perez, F.M., Deahl, K.L., Griffin, D., and Cooke, L.R. (2012). Re-emergence of the Ib mitochondrial haplotype within the British and Irish *Phytophthora infestans* populations. European Journal of Plant Pathology 135, 237–242.

Kim, D.S., Brain, P., Marshall, E.J.P., and Caseley, J.C. (2002). Modelling herbicide dose and weed density effects on crop: weed competition. Weed Research 42, 1–13.

Kim, H.J., Lee, H.R., Jo, K.-R., Mortazavian, S.M.M., Huigen, D.J., Evenhuis, B., Kessel, G., Visser, R.G.F., Jacobsen, E., and Vossen, J.H. (2012). Broad spectrum late blight resistance in potato differential set plants MaR8 and MaR9 is conferred by multiple stacked R genes. Theoretical and Applied Genetics 124, 923–935.

Kirk, W.W. (2003a). Thermal properties of overwintered piles of cull potatoes. American Journal of Potato Research 80, 145–149.

Kirk, W.W. (2003b). Tolerance of Mycelium of Different Genotypes of *Phytophthora infestans* to Freezing Temperatures for Extended Periods. Phytopathology 93, 1400–1406.

Kleinhenz, B., and Jörg, E. (1999). Validation of SIMPHYT I/II–a decision support system for Late Blight control in Germany. PAV–Special Report 5, 115–128.

Kleinhenz, B., and Rossberg, D. (2000). Structure and development of decision-support systems and their use by the State Plant Protection Services in Germany. EPPO Bulletin 30, 93–97.

Klittich, C.J.R. (2014). Fungicide Mobility and the Influence of Physical Properties. In Myung, K., Satchivi, N., and Kingston, C. (Eds) Retention, Uptake, and Translocation of Agrochemicals in Plants, (American Chemical Society, United States), 95–109.

Klittich, C.J.R., and Ray, S.L. (2013). Effects of physical properties on the translaminar activity of fungicides. Pesticide Biochemistry and Physiology 107, 351–359.

Klopping, H.L., and Delp, C.J. (1980). 2-Cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino)acetamide, a new fungicide. Journal of Agricultural and Food Chemistry 28, 467–468.

Knapova, G., and Gisi, U. (2002). Phenotypic and genotypic structure of *Phytophthora infestans* populations on potato and tomato in France and Switzerland. Plant Pathology 51, 641–653.

Knezevic, S.Z., Streibig, J.C., and Ritz, C. (2007). Utilizing R Software Package for Dose-Response Studies: The Concept and Data Analysis. Weed Technology 21, 840–848.

Knight, J.D. (1997). The role of decision support systems in integrated crop protection. Agriculture, Ecosystems & Environment 64, 157–163.

Kogan, M. (1998). Integrated Pest Management: Historical Perspectives and Contemporary Developments. Annual Review of Entomology 43, 243–270.

Kombrink, E., and Schmelzer, E. (2001). The Hypersensitive Response and its Role in Local and Systemic Disease Resistance. European Journal of Plant Pathology 107, 69–78.

Komyoji, T., Sugimoto, K., and Suzuki, K. (1995). Effect of Fluazinam, a New Fungicide, on Infection Processes of Several Plant Pathogenic Fungi. Japanese Journal of Phytopathology 61, 145–149.

Kontodimas, D.C., Eliopoulos, P.A., Stathas, G.J., and Economou, L.P. (2004). Comparative Temperature-Dependent Development of *Nephus includens* (Kirsch) and *Nephus bisignatus*

(Boheman) (Coleoptera: Coccinellidae) Preying on *Planococcus citri* (Risso) (Homoptera: Pseudococcidae): Evaluation of a Linear and Various Nonlinear Models Using Specific Criteria. Environmental Entomology 33, 1–11.

Koppel, M., Hansen, J.G., Lassen, P., Turka, I., Bimsteine, G., and Valskyte, A. (2003). Implementation of the NegFry decision support system in the Baltic countries in 1999-2002. In Proceedings of the Seventh Workshop of an European Network for Development of an Integrated Control Strategy of Potato Late Blight. PPO-Special Report, 47–57.

Kou, Y., and Wang, S. (2010). Broad-spectrum and durability: understanding of quantitative disease resistance. Current Opinion in Plant Biology 13, 181–185.

Krebs, J.R. (2011). Risk, uncertainty and regulation. Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences 369, 4842–4852.

Kroll, R.E., and Eide, C.J. (1981). Effect of inoculum concentration of *Phytophthora infestans* on potato late blight. American Potato Journal 58, 153.

Kromann, P., Leon, D., Taipe, A., Andrade-Piedra, J.L., and Forbes, G.A. (2008). Comparison of two strategies for use of translaminar and contact fungicide in the control of potato late blight in the highland tropics of Ecuador. Crop Protection 27, 1098–1104.

Kromann, P., Miethbauer, T., Ortiz, O., and Forbes, G.A. (2014). Review of Potato Biotic Constraints and Experiences with Integrated Pest Management Interventions. In: D. Pimentel, and R. Peshin, (Eds.) Integrated Pest Management, (Springer, Netherlands), 245–268.

Kuhn, P.J., Pitt, D., Lee, S.A., Wakley, G., and Sheppard, A.N. (1991). Effects of dimethomorph on the morphology and ultrastructure of *Phytophthora*. Mycological Research 95, 333–340.

Kumar, A., Karre, S., Dhokane, D., Kage, U., Hukkeri, S., and Kushalappa, A.C. (2015). Real-time quantitative PCR based method for the quantification of fungal biomass to discriminate quantitative resistance in barley and wheat genotypes to *Fusarium* head blight. Journal of Cereal Science 64, 16–22.

Lactin, D.J., Holliday, N.J., Johnson, D.L., and Craigen, R. (1995). Improved Rate Model of Temperature-Dependent Development by Arthropods. Environmental Entomology 24, 68–75.

Lagos-Ortiz, K., Medina-Moreira, J., Alarcón-Salvatierra, A., Morán, M.F., del Cioppo-Morstadt, J., and Valencia-García, R. (2019). Decision Support System for the Control and Monitoring of Crops. In R. Valencia-García, G. Alcaraz-Mármol, J. del Cioppo-Morstadt, N. Vera-Lucio, and M. Bucaram-Leverone, (Eds.) ICT for Agriculture and Environment, (Springer, International Publishing), 20–28.

Lalkhen, A.G., and McCluskey, A. (2008). Clinical tests: sensitivity and specificity. Continuing Education in Anaesthesia, Critical Care and Pain 8, 221–223.

Lam, E., Kato, N., and Lawton, M. (2001). Programmed cell death, mitochondria and the plant hypersensitive response. Nature 411, 848.

Lamb, H., and Bourke, A. (1993). The spread of potato blight in Europe in 1845-6 and the accompanying wind and weather patterns (Met Éireann, Dublin).

Lambert, D.H., Currier, A.I., and Olanya, M.O. (1998). Transmission of *Phytophthora infestans* in cut potato seed. American Journal of Potato Research 75, 257–263.

Lamichhane, J.R., Aubertot, J.-N., Begg, G., Birch, A.N.E., Boonekamp, P., Dachbrodt-Saaydeh, S., Hansen, J.G., Hovmøller, M.S., Jensen, J.E., and Jørgensen, L.N. (2016). Networking of integrated pest management: A powerful approach to address common challenges in agriculture. Crop Protection 89, 139–151.

Lamichhane, J.R., Akbas, B., Andreasen, C.B., Arendse, W., Bluemel, S., Dachbrodt-Saaydeh, S., Fuchs, A., Jansen, J.P., Kiss, J., and Kudsk, P. (2018). A call for stakeholders to boost integrated pest management in Europe: a vision based on the three-year European research area network project. International Journal of Pest Management 64, 352–358.

Landa, B.B., Navas-Cortés, J.A., and Jiménez-Díaz, R.M. (2004). Integrated management of *Fusarium* wilt of chickpea with sowing date, host resistance, and biological control. Phytopathology 94, 946–960.

Lapwood, D.H., and McKee, R.K. (1966). Dose-response relationships for infection of potato leaves by zoospores of *Phytophthora infestans*. Transactions of the British Mycological Society 49, 679–686.

Latijnhouwers, M., Ligterink, W., Vleeshouwers, V.G.A.A., van West, P., and Govers, F. (2004). A Gα subunit controls zoospore motility and virulence in the potato late blight pathogen *Phytophthora infestans.* Molecular Microbiology 51, 925–936.

Latin, R., MacKenzie, D., and Cole Jr, H. (1981). The influence of host and pathogen genotypes on the apparent infection rates of potato late blight epidemics. Phytopathology 71, 82–85.

Leach, S.S. (1966). Effects of copper and copper fungicide soil residues on *Phytophthora infestans*. American Potato Journal 43, 431–438.

Leach, L.F., and Henson, R.K. (2007). The use and impact of adjusted R^2 effects in published regression research. Multiple Linear Regression Viewpoints 33, 1–11.

Leach, M.D., and Cowen, L.E. (2013). Surviving the Heat of the Moment: A Fungal Pathogens Perspective. PLoS Pathogens 9, e1003163.

Lebecka, R., and Sobkowiak, S. (2012). Host–pathogen interaction between *Phytophthora infestans* and *Solanum tuberosum* following exposure to short and long daylight hours. Acta Physiologiae Plantarum 35, 1131–1139.

Lebecka, R., Sliwka, J., Sobkowiak, S., and Zimnoch-Guzowska, E. (2007). *Phytophthora infestans* population in Poland. PPO-Special Report No. 12 155.

Lees, A., Cooke, D., Stewart, J., Sullivan, L., Williams, N., Carnegie, S. (2009). *Phytophthora infestans* population changes: implications. PPO Special Report 13, 55–60.

Lees, A.K., Wattier, R., Shaw, D.S., Sullivan, L., Williams, N.A., and Cooke, D.E.L. (2006). Novel microsatellite markers for the analysis of *Phytophthora infestans* populations. Plant Pathology 55, 311–319.

Lees, A.K., Sullivan, L., Lynott, J.S., and Cullen, D.W. (2012). Development of a quantitative real-time PCR assay for *Phytophthora infestans* and its applicability to leaf, tuber and soil samples. Plant Pathology 61, 867–876.

Leesutthiphonchai, W., Vu, A.L., Ah-Fong, A.M., and Judelson, H.S. (2018). How Does *Phytophthora infestans* Evade Control Efforts? Modern Insight Into the Late Blight Disease. Phytopathology 108, 916–924.

Lefebvre, M., Langrell, S.R.H., and Gomez-y-Paloma, S. (2015). Incentives and policies for integrated pest management in Europe: a review. Agronomy for Sustainable Development 35, 27–45.

Legler, S., Caffi T., and Rossi V. (2011). A nonlinear model for temperature-dependent development of *Erysiphe necator chasmothecia* on grapevine leaves. Plant Pathology 61, 96–105.

Lehsten, V., Wiik, L., Hannukkala, A., Andreasson, E., Chen, D., O, T., Liljeroth, E., Lankinen, A, and Grenville-Briggs, L. (2017). Earlier occurrence and increased explanatory power of climate for the first incidence of potato late blight caused by *Phytophthora infestans* in Fennoscandia. PloS One 12, e0177580.

Lehtinen, A., and Hannukkala, A. (2008). Oospores of *Phytphthora infestans* in soil provide an important new source of primary inoculum in Finland. Agricultural and Food Science 13, 399–410.

Lejeune, R., and Baron, G.V. (1998). Modelling the exponential growth of filamentous fungi during batch cultivation. Biotechnology and Bioengineering 60, 169–179.

Leonard, R., Dowley, L.J., Rice, B., and Ward, S. (2001). Comparison of the NegFry decision suport system with routine fungicide application for the control of potato late blight in Ireland. Potato Research 44, 327–336.

Leonard, R.C.F., Hayward, R.L., Prescott, R.J., and Wang, J.X. (1991). Original article: The identification of discrete prognostic groups in low grade non-Hodgkin's lymphoma. Annals of Oncology 2, 655–662.

Li, J., Lindqvist-Kreuze, H., Tian, Z., Liu, J., Song, B., Landeo, J., Portal, L., Gastelo, M., Frisancho, J., Sanchez, L., Meijer, D., Xie, C., and Bonierbale, M.(2012a). Conditional QTL underlying resistance to late blight in a diploid potato population. TAG. Theoretical and Applied Genetics. Theoretische Und Angewandte Genetik 124, 1339–1350.

Li, Y., Govers, F., Mendes, O., Testa, A., Jacobsen, E., Huang, S., and Lee, T.A.J. (2010). A new set of highly informative SSR markers for *Phytophthora infestans* population analysis assembled into an efficient multiplex. Molecular Ecology Resources 10, 1098−1105.

Li, Y., Lee, T.A.J. van der, Evenhuis, A., Bosch, G.B.M. van den, Bekkum, P.J. van, Förch, M.G., Gent-Pelzer, M.P.E. van, Raaij, H.M.G. van, Jacobsen, E., Huang, S.W., Govers, F., Vleeshouwers, and Kessel, G. (2012b). Population Dynamics of *Phytophthora infestans* in the

Netherlands Reveals Expansion and Spread of Dominant Clonal Lineages and Virulence in Sexual Offspring. G3: Genes, Genomes, Genetics 2, 1529–1540.

Li, Y., van der Lee, T., Zhu, J.H., Jin, G.H., Lan, C.Z., Zhu, S.X., Zhang, R.F., Liu, B.W., Zhao, Z.J., Kessel, G., Huang, S., and Jacobsen, E. (2013a). Population structure of *Phytophthora infestans* in China – geographic clusters and presence of the EU genotype Blue_13. Plant Pathology 62, 932–942.

Li, Y., Cooke, D.E.L., Jacobsen, E., and van der Lee, T. (2013b). Efficient multiplex simple sequence repeat genotyping of the oomycete plant pathogen *Phytophthora infestans*. Journal of Microbiological Methods 92, 316–322.

Lindblom, J., Lundström, C., Ljung, M., and Jonsson, A. (2017). Promoting sustainable intensification in precision agriculture: review of decision support systems development and strategies. Precision Agriculture 18, 309–331.

Litschmann, T., Hausvater, E., Dolezal, P., and Bastova, P. (2018). Climate Change and its Impact on the Conditions of Late Blight Occurrence. Scientia Agriculturae Bohemica 49, 173–180.

Logan, J., Wollkind, D., Hoyt, S., and Tanigoshi, L. (1976). An analytic model for description of temperature dependent rate phenomena in arthropods. Environmental Entomology 5, 1133–1140.

López-Fernández, O., Rial-Otero, R., González-Barreiro, C., and Simal-Gándara, J. (2012). Surveillance of fungicidal dithiocarbamate residues in fruits and vegetables. Food Chemistry 134, 366–374.

Lourakis, M.I. (2005). A brief description of the Levenberg-Marquardt algorithm implemented by levmar. Foundation of Research and Technology 4, 1–6.

Lu, H., McClung, C.R., and Zhang, C. (2017). Tick Tock: Circadian Regulation of Plant Innate Immunity. Annual Review of Phytopathology 55, 287–311.

Lucca, F., and Huarte, M. (2011). Genotypic variation of *Phytophthora infestans* populations in Argentina. Special Report No. 15 201.

Luna, E., Pastor, V., Robert, J., Flors, V., Mauch-Mani, B., and Ton, J. (2011). Callose deposition: a multifaceted plant defense response. Molecular Plant-Microbe Interactions 24, 183–193.

Mabon, R., Mariette, N., Corbiere, R., Marquer, B., Montarry, J., and Andrivon, D. (2015). Genotypic and phenotypic characterization of *Phytophthora infestans* isolates sampled in Brittany in two consecutive years (2013 -2014),. In Proceedings of the Fifteenth EuroBlight Workshop (Brasov, Romania), 187.

Macoy, D.M., Kim, W.-Y., Lee, S.Y., and Kim, M.G. (2015). Biotic stress related functions of hydroxycinnamic acid amide in plants. Journal of Plant Biology 58, 156–163.

Madden, L.V. (2006). Botanical Epidemiology: Some Key Advances and its Continuing Role in Disease Management. European Journal of Plant Pathology 115, 3–23.

Madden, L.V., and Ellis, M.A. (1988). How to Develop Plant Disease Forecasters. In J. Kranz, and J. Rotem, (Eds.) Experimental Techniques in Plant Disease Epidemiology, (Springer Berlin, Germany), 191–208.

Madden, L.V., Hughes, G., and Bosch, F. van den (2007). The Study of Plant Disease Epidemics (American Phytopathological Society, United States).

Magarey, R., Sutton, T., and Thayer, C. (2005). A simple generic infection model for foliar fungal plant pathogens. Phytopathology 95, 92–100.

Malcolmson, J.F. (1969). Races of P*hytophthora infestans* occurring in Great Britain. Transactions of the British Mycological Society 53, 417−423.

Malcolmson, J.F., and Black, W. (1966). New R genes in *Solanum demissum* lindl. And their complementary races of *Phytophthora infestans* (Mont.) de bary. Euphytica 15, 199–203.

Maltese, C.E., Conigliaro, G., and Shaw, D.S. (1995). The development of sporangia of *Phytophthora infestans*. Mycological Research 99, 1175–1181.

Manrique, L.A., and Hodges, T. (1989). Estimation of tuber initiation in potatoes grown in tropical environments based on different methods of computing thermal time. American Potato Journal 66, 425–436.

Mao, L., Zhang, L., Sun, X., van der Werf, W., Evers, J.B., Zhao, X., Zhang, S., Song, X., and Li, Z. (2018). Use of the beta growth function to quantitatively characterize the effects of plant density and a growth regulator on growth and biomass partitioning in cotton. Field Crops Research 224, 28–36.

Marano, A.V., Jesus, A.L., Souza, J.I. de, Jerônimo, G.H., Gonçalves, D.R., Boro, M.C., Rocha, S.C.O., and Pires-Zottarelli, C.L.A. (2016). Ecological roles of saprotrophic Peronosporales (Oomycetes, Straminipila) in natural environments. Fungal Ecology 19, 77–88.

Marchioro, C.A., Krechemer, F.S., and Foerster, L.A. (2017). Estimating the development rate of the tomato leaf miner, *Tuta absoluta* (Lepidoptera: Gelechiidae), using linear and non-linear models. Pest Management Science 73, 1486–1493.

Marewski, J.N., and Gigerenzer, G. (2012). Heuristic decision making in medicine. Dialogues in Clinical Neuroscience 14, 77–89.

Margni, M., Rossier, D., Crettaz, P., and Jolliet, O. (2002). Life cycle impact assessment of pesticides on human health and ecosystems. Agriculture, Ecosystems & Environment 93, 379–392.

Mariette, N., Mabon, R., Corbière, R., Boulard, F., Glais, I., Marquer, B., Pasco, C., Montarry, J., and Andrivon, D. (2015). Phenotypic and genotypic changes in French populations of *Phytophthora infestans*: are invasive clones the most aggressive? Plant Pathology 65, 577−586.

Mariette, N., Androdias, A., Mabon, R., Corbière, R., Marquer, B., Montarry, J., and Andrivon, D. (2016). Local adaptation to temperature in populations and clonal lineages of the Irish potato famine pathogen *Phytophthora infestans*. Ecology and Evolution 6, 6320–6331.

Marks, G.E. (1965). The cytology of *Phytophthora infestans*. Chromosoma 16, 681–692.

Mason, S.J. (2008). Understanding forecast verification statistics. Meteorological Applications 15, 31–40.

Matthews, K.B., Schwarz, G., Buchan, K., Rivington, M., and Miller, D. (2008). Wither agricultural DSS? Computers and Electronics in Agriculture 61, 149–159.

Mayton, H., Forbes, G.A., Mizubuti, E.S.G., and Fry, W.E. (2001). The Roles of Three Fungicides in the Epidemiology of Potato Late Blight. Plant Disease 85, 1006–1012.

Maziero, J.M.N., Maffia, L.A., and Mizubuti, E.S.G. (2009). Effects of Temperature on Events in the Infection Cycle of Two Clonal Lineages of *Phytophthora infestans* Causing Late Blight on Tomato and Potato in Brazil. Plant Disease 93, 459–466.

McCartney, H.A., Foster, S.J., Fraaije, B.A., and Ward, E. (2003). Molecular diagnostics for fungal plant pathogens. Pest Management Science 59, 129–142.

McDonald, B. (2010). How can we achieve durable disease resistance in agricultural ecosystems? New Phytologist 185, 3–5.

McMaster, G.S., and Wilhelm, W.W. (1997). Growing degree-days: one equation, two interpretations. Agricultural and Forest Meteorology 87, 291–300.

McRoberts, N., Hall, C., Madden, L.V., and Hughes, G. (2011). Perceptions of Disease Risk: From Social Construction of Subjective Judgements to Rational Decision Making. Phytopathology 101, 654–665.

Mead, R. (2017). Statistical Methods in Agriculture and Experimental Biology (Chpaman & Hall/CRC, United States).

Meier, C.L., Rapp, J., Bowers, R.M., Silman, M., and Fierer, N. (2010). Fungal growth on a common wood substrate across a tropical elevation gradient: Temperature sensitivity, community composition, and potential for above-ground decomposition. Soil Biology and Biochemistry 42, 1083–1090.

Meletiadis, J., Meis, J.F.G.M., Mouton, J.W., and Verweij, P.E. (2001). Analysis of Growth Characteristics of Filamentous Fungi in Different Nutrient Media. Journal of Clinical Microbiology 39, 478–484.

Mélida, H., Sandoval-Sierra, J.V., Diéguez-Uribeondo, J., and Bulone, V. (2013). Analyses of Extracellular Carbohydrates in Oomycetes Unveil the Existence of Three Different Cell Wall Types. Eukaryotic Cell 12, 194–203.

Mellor, D.H. (2000). Possibility, chance and necessity. Australasian Journal of Philosophy 78, 16–27.

Meyer, M., and Hausbeck, M. (2013). Using soil-applied fungicides to manage *Phytophthora* crown and root rot on summer squash. Plant Disease 97, 107–112.

Miao, J., Dong, X., Lin, D., Wang, Q., Liu, P., Chen, F., Du, Y., and Liu, X. (2016). Activity of the novel fungicide oxathiapiprolin against plant-pathogenic oomycetes. Pest Management Science 72, 1572–1577.

Michalska, A.M., Sobkowiak, S., Flis, B., and Zimnoch-Guzowska, E. (2016). Virulence and aggressiveness of *Phytophthora infestans* isolates collected in Poland from potato and tomato plants identified no strong specificity. European Journal of Plant Pathology 144, 325–336.

Milgroom, M., and Fry, W. (1988). A simulation analysis of the epidemiological principles for fungicide resistance management in pathogen populations. Phytopathology 78, 565–570.

Mille-Lindblom, C., Fischer, H., and J. Tranvik, L. (2006). Antagonism between bacteria and fungi: substrate competition and a possible trade-off between fungal growth and tolerance towards bacteria. Oikos 113, 233–242.

Miller, J.S., Nolte, P., Olsen, N., Miller, T., Bohl, B., and Thornton, M. (2006). Late blight management action plan for potatoes. (University of Idaho College Extension, United states).

Miller, R.N.G., Alves, C., Sergio, G., and Van Sluys, M.A. (2017). Plant immunity: unravelling the complexity of plant responses to biotic stresses. Annals of Botany 119, 681–687.

Mingora, C., Ewer, J., and Ospina-Giraldo, M. (2014). Comparative structural and functional analysis of genes encoding pectin methylesterases in *Phytophthora spp.* Gene 538, 74–83.

Minogue, K., Fry, W. (1981). Effect of temperature, relative humidity, and rehydration rate on germination of dried sporangia of *Phytophthora infestans*. Phytopathology 71, 1181–1184.

Mitani, S., Araki, S., Takii, Y., Ohshima, T., Matsuo, N., and Miyoshi, H. (2001). The Biochemical Mode of Action of the Novel Selective Fungicide Cyazofamid: Specific Inhibition of Mitochondrial Complex III in *Phythium spinosum*. Pesticide Biochemistry and Physiology 71, 107–115.

Mitani, S., Araki, S., Yamaguchi, T., Takii, Y., Ohshima, T., and Matsuo, N. (2002). Biological properties of the novel fungicide cyazofamid against *Phytophthora infestans* on tomato and *Pseudoperonospora cubensis* on cucumber. Pest Management Science 58, 139–145.

Mitani, S., Kamachi, K., and Sugimoto, K. (2005). Control of Potato Late Blight by Cyazofamid. Journal of Pesticide Science 30, 116–119.

Miyake, Y., Sakai, J., Shibata, M., Yonekura, N., Miura, I., Kumakura, K., and Nagayama, K. (2005). Fungicidial activity of benthiavalicarb-isopropyl against *Phytophthora infestans* and its controlling activity against late blight diseases. Journal of Pesticide Science 30, 390–396.

Mizubuti, E.S.G., and Fry, W.E. (1998). Temperature Effects on Developmental Stages of Isolates from Three Clonal Lineages of *Phytophthora infestans*. Phytopathology 88, 837–843.

Mizubuti, E.S., Aylor, D.E., and Fry, W.E. (2000). Survival of *Phytophthora infestans* sporangia exposed to solar radiation. Phytopathology 90, 78–84.

Möller, K., Habermeyer, J., Zinkernagel, V., and Reents, H.-J. (2006). Impact and Interaction of Nitrogen and *Phytophthora infestans* as Yield-limiting and Yield-reducing Factors in Organic Potato (*Solanum tuberosum* L.) Crops. Potato Research 49, 281–301.

Monie, C., Reay, G., and Wardlaw, J. (2016). Pesticide Usage in Scotland: Arable Crops and Potato Stores 2016 (SASA, United Kingdom).

Montagnes, D.J.S., Kimmance, S.A., and Atkinson, D. (2003). Using Q10: Can growth rates increase linearly with temperature? Aquatic Microbial Ecology 32, 307–313.

Montarry, J., Corbiere, R., and Andrivon, D. (2007). Is there a trade-off between aggressiveness and overwinter survival in *Phytophthora infestans*? Functional Ecology 21, 603–610.

Moons, K.G.M., Harrell, F.E., and Steyerberg, E.W. (2002). Should scoring rules be based on odds ratios or regression coefficients? Journal of Clinical Epidemiology 55, 1054–1055.

Moore, J.L. (2009). The application of degree-day models to study current and future organism development: cautions, limitations, and recommendations. Masters Thesis, Emory University, 90 pages.

Moore, J.L., and Remais, J.V. (2014). Developmental models for estimating ecological responses to environmental variability: structural, parametric, and experimental issues. Acta Biotheoretica 62, 69–90.

Moorman, G.W., and Kim, S.H. (2004). Species of *Pythium* from Greenhouses in Pennsylvania Exhibit Resistance to Propamocarb and Mefenoxam. Plant Disease 88, 630–632.

Moot, D.J., Scott, W.R., Roy, A.M., and Nicholls, A.C. (2000). Base temperature and thermal time requirements for germination and emergence of temperate pasture species. New Zealand Journal of Agricultural Research 43, 15–25.

Moretti, A., Pascale, M., and Logrieco, A.F. (2019). Mycotoxin risks under a climate change scenario in Europe. Trends in Food Science & Technology 84, 38–40.

Moschini, G., and Hennessy, D. (1999). Uncertainty, Risk Aversion and Risk Management for Agricultural Producers. Handbook of Agricultural Economics 1, 87–153.

Mundt, C.C. (2014). Durable resistance: A key to sustainable management of pathogens and pests. Infection, Genetics and Evolution 27, 446–455.

Nærstad, R., Hermansen, A., and Bjor, T. (2007). Exploiting host resistance to reduce the use of fungicides to control potato late blight. Plant Pathology 56, 156–166.

Nauta, M.J. (2000). Separation of uncertainty and variability in quantitative microbial risk assessment models. International Journal of Food Microbiology 57, 9–18.

Nelson, R. (1978). Genetics of horizontal resistance to plant diseases. Annual Review of Phytopathology 16, 359–378.

Nguyen, M.T., and Devarajan, P. (2008). Biomarkers for the early detection of acute kidney injury. Pediatric Nephrology 23, 2151−2157.

Nick, C., Aguilera, J.G., Laurindo, B.S., de Souza Almeida, V., de Freitas, R.D., Cruz, C.D., and da Silva, D.J.H. (2014). Genetic parameters and correlation between tomato late blight field resistance and fruit quality. American Journal of Plant Sciences 5, 2418−2425.

Niederhauser, J.S. (1991). *Phytophthora infestans*: the Mexican connection. In: J. A. Lucas, R. C. Shat-tock, Shaw, D.S., and Cooke, L. R. (Eds.) Phytophthora, (Cambridge University Press, United Kingdom), 25−45

Nielsen, B.J., and Bødker, L. (2002). Field Experiments with preventive and curative control of potato late blight. PPO-Special Report No. 8, 211.

Nielsen, B.J., Cooke, D.E., and Hansen, J.G. (2014). Monitoring the Danish population of potato late blight pathogen, *Phytophthora infestans* in 2011-2012 and occurrence of 13_A2. PPO-Special Report 16, 159–162.

Niks, R.E., and Marcel, T.C. (2009). Nonhost and basal resistance: how to explain specificity? New Phytologist 182, 817–828.

Niks, R.E., and Rubiales, D. (2002). Potentially durable resistance mechanisms in plants to specialised fungal pathogens. Euphytica 124, 201–216.

Niks, R.E., Qi, X., and Marcel, T.C. (2015). Quantitative Resistance to Biotrophic Filamentous Plant Pathogens: Concepts, Misconceptions, and Mechanisms. Annual Review of Phytopathology 53, 445–470.

Nishimura, M.T., and Dangl, J.L. (2010). *Arabidopsis* and the Plant Immune System. The Plant Journal 61, 1053–1066.

Njoroge, A.W., Tusiime, G., Forbes, G.A., and Yuen, J.E. (2016). Displacement of US-1 clonal lineage by a new lineage of *Phytophthora infestans* on potato in Kenya and Uganda. Plant Pathology 65, 587–592.

Noel, Z.A., Wang, J., and Chilvers, M.I. (2017). Significant Influence of EC50 Estimation by Model Choice and EC50 Type. Plant Disease 102, 708–714.

Norton, G.A. (1976). Analysis of decision making in crop protection. Agro-Ecosystems 3, 27–44.

Nowicki, M., Foolad, M.R., Nowakowska, M., and Kozik, E.U. (2011). Potato and Tomato Late Blight Caused by *Phytophthora infestans*: An Overview of Pathology and Resistance Breeding. Plant Disease 96, 4–17.

Oberhagemann, P., Chatot-Balandras, C., Schäfer-Pregl, R., Wegener, D., Palomino, C., Salamini, F., Bonnel, E., and Gebhardt, C. (1999). A genetic analysis of quantitative resistance to late blight in potato: towards marker-assisted selection. Molecular Breeding 5, 399–415.

Oerke, E.C., and Dehne, H.-W. (2004). Safeguarding production—losses in major crops and the role of crop protection. Crop Protection 23, 275–285.

Oijen, M.V. (1995). Simulation models of potato late blight. In Potato Ecology And Modelling of Crops under Conditions Limiting Growth, (Springer, Dordrecht), 237–250.

Ojika, M., Molli, S.D., Kanazawa, H., Yajima, A., Toda, K., Nukada, T., Mao, H., Murata, R., Asano, T., Qi, J., and Sakagami, Y. (2011). The second *Phytophthora* mating hormone defines interspecies biosynthetic crosstalk. Nature Chemical Biology 7, 591–593.

Okada, S. (1967). A Simple Graphic Method of Computing the Parameters of the Life Cycle of Cultured Mammalian Cells in the Exponential Growth Phase. The Journal of Cell Biology 34, 915–916.

Olanya, O.M., Honeycutt, C.W., Tschöepe, B., Kleinhenz, B., Lambert, D.H., and Johnson, S.B. (2012). Effectiveness of SIMBLIGHT1 and SIMPHYT1 models for predicting *Phytophthora infestans* in north-eastern United States. Archives of Phytopathology and Plant Protection 45, 1558–1569.

Olanya Ocen M., Plant Andrew B., Larkin Robert P., and Honeycutt Charles W. (2009). Infection Potential of Hairy Nightshade (*Solanum sarrachoides*) by *Phytophthora infestans* and Late Blight Implications of the Alternate Host. Journal of Phytopathology 157, 427–437.

Oliver, F.R. (1964). Methods of Estimating the Logistic Growth Function. Journal of the Royal Statistical Society. Series C (Applied Statistics) 13, 57–66.

Olsson, T., Terent, A., and Lind, L. (2004). Rapid Emergency Medicine score: a new prognostic tool for in-hospital mortality in nonsurgical emergency department patients. Journal of Internal Medicine 255, 579–587.

Ordoñez, M.E., Forbes, G.A., and Trognitz, B. (1998). Relationship between ineffective R-genes and expansion rate of lesions on potato leaves, caused by *Phytophthora infestans*. Plant Pathology 47, 130–136.

Oreskes, N., Shrader-Frechette, K., and Belitz, K. (1994). Verification, Validation, and Confirmation of Numerical Models in the Earth Sciences. Science 263, 641–646.

Orłowska, E., Basile, A., Kandzia, I., Llorente, B., Kirk, H.G., and Cvitanich, C. (2012). Revealing the importance of meristems and roots for the development of hypersensitive responses and full foliar resistance to *Phytophthora infestans* in the resistant potato cultivar Sarpo Mira. Journal of Experimental Botany. 63, 4765–4779.

Ortiz, O., Garrett, K.A., Health, J.J., Orrego, R., and Nelson, R.J. (2004). Management of potato late blight in the Peruvian highlands: Evaluating the benefits of farmer field schools and farmer participatory research. Plant Disease 88, 565–571.

Osbourn, A. (1996). Preformed Antimicrobial Compounds and Plant Defence against Fungal Attack. Plant Cell 8, 1821–1831.

Özgökçe, M.S., Bayındır, A., and Karaca, İ. (2016). Temperature-dependent development of the tomato leaf miner, *Tuta absoluta* (Meyrick)(Lepidoptera: Gelechiidae) on tomato plant *Lycopersicon esculentum* Mill.(Solanaceae). Turkish Journal of Entomology 40, 51−59.

Pacilly, F.C.A., Hofstede, G.J., Lammerts van Bueren, E.T., Kessel, G.J.T., and Groot, J.C.J. (2018). Simulating crop-disease interactions in agricultural landscapes to analyse the effectiveness of host resistance in disease control: The case of potato late blight. Ecological Modelling 378, 1–12.

Pais, M., Yoshida, K., Giannakopoulou, A., Pel, M.A., Cano, L.M., Oliva, R.F., Witek, K., Lindqvist-Kreuze, H., Vleeshouwers, V.G.A.A., and Kamoun, S. (2017). Gene expression polymorphism underpins evasion of host immunity in an asexual lineage of the Irish potato famine pathogen. BioRxiv 116012.

Papavizas, G., O'Neill, N., and Lewis, J. (1978). Fungistatic activity of propyl-N-(γ-dimethylaminopropyl) carbamate on *Pythium* spp. and its reversal by sterols. Phytopathology 68, 1667–1671.

Pariaud, B., Ravigné, V., Halkett, F., Goyeau, H., Carlier, J., and Lannou, C. (2009). Aggressiveness and its role in the adaptation of plant pathogens. Plant Pathology 58, 409–424.

Parlevliet, J.E., and Zadoks, J.C. (1977). The integrated concept of disease resistance: a new view including horizontal and vertical resistance in plants. Euphytica 26, 5–21.

Pasteris, R.J., Hanagan, M.A., Bisaha, J.J., Finkelstein, B.L., Hoffman, L.E., Gregory, V., Andreassi, J.L., Sweigard, J.A., Klyashchitsky, B.A., Henry, Y.T., and Berger, R. (2016). Discovery of oxathiapiprolin, a new oomycete fungicide that targets an oxysterol binding protein. Bioorganic & Medicinal Chemistry 24, 354–361.

Patro, S.G.K., and Sahu, K.K. (2015). Normalization: A Preprocessing Stage. ArXiv:1503.06462

Pavan, S., Jacobsen, E., Visser, R.G.F., and Bai, Y. (2010). Loss of susceptibility as a novel breeding strategy for durable and broad-spectrum resistance. Molecular Breeding 25, 1–12.

Peters, R.D., Al-Mughrabi, K.I., Kalischuk, M.L., Dobinson, K.F., Conn, K.L., Alkher, H., Islam, M.R., Daayf, F., Lynn, J., Bizimungu, B., Koeyer, D., Levesque, C. and Kawchuck, L. (2014). Characterization of *Phytophthora infestans* population diversity in Canada reveals increased migration and genotype recombination. Canadian Journal of Plant Pathology 36, 73–82.

Pettengill, J., and Vertrees, J. (1982). Reliability and Validity in Hospital Case-Mix Measurement. Health Care Financing Review 4, 101–128.

Pilet-Nayel, M.-L., Moury, B., Caffier, V., Montarry, J., Kerlan, M.-C., Fournet, S., Durel, C.-E., and Delourme, R. (2017). Quantitative Resistance to Plant Pathogens in Pyramiding Strategies for Durable Crop Protection. Frontiers in Plant Science 8, 1838.

Pirondi, A., Brunelli, A., Muzzi, E., and Collina, M. (2017). Post-infection activity of fungicides against *Phytophthora infestans* on tomato (*Solanum lycopersicum* L.). Journal of General Plant Pathology 83, 244–252.

Pittis, J.E., and Shattock, R.C. (1994). Viability, germination and infection potential of oospores of *Phytophthora infestans*. Plant Pathology 43, 387–396.

Plassard, C.S., Mousain, D.G., and Salsac, L.E. (1982). Estimation of mycelial growth of basidiomycetes by means of chitin determination. Phytochemistry 21, 345–348.

Poland, J.A., Balint-Kurti, P.J., Wisser, R.J., Pratt, R.C., and Nelson, R.J. (2009). Shades of gray: the world of quantitative disease resistance. Trends in Plant Science 14, 21–29.

Pollard, S.J.T., Kemp, R.V., Crawford, M., Duarte-Davidson, R., Irwin, J.G., and Yearsley, R. (2004). Characterizing Environmental Harm: Developments in an Approach to Strategic Risk Assessment and Risk Management. Risk Analysis 24, 1551–1560.

Poloni, A., Schirawski, J., Poloni, A., and Schirawski, J. (2014). Red Card for Pathogens: Phytoalexins in Sorghum and Maize. Molecules 19, 9114–9133.

Powelson, M.L., Ludy, R., Heather, H., Inglis, D.A., Gundersen, B., and Derie, M. (2002). Seed Borne Late Blight of Potato. Plant Health Progress 3, 1535-1025.

Preisler, H.K., Brillinger, D.R., Burgan, R.E., and Benoit, J.W. (2004). Probability based models for estimation of wildfire risk. Intnational Journal of Wildland Fire 13, 133–142.

Pule, B.B., Meitz, J.C., Thompson, A.H., Linde, C.C., Fry, W.E., Langenhoven, S.D., Meyers, K.L., Kandolo, D.S., van Rij, N.S., McLeod, A. (2013). Phytophthora infestans populations in central, eastern and southern African countries consist of two major clonal lineages. Plant Pathology 62, 154–165.

Qin, C.-F., He, M.-H., Chen, F.P., Zhu, W., Yang, L.N., Wu, E.J., Guo, Z.L., Shang, L.P., and Zhan, J. (2016). Comparative analyses of fungicide sensitivity and SSR marker variations indicate a low risk of developing azoxystrobin resistance in *Phytophthora infestans*. Scientific Reports 6, 20483.

Quinn, P.J. (1988). Effects of temperature on cell membranes. Symposia of the Society for Experimental Biology 42, 237–258.

R Core Team (2013). R: A Language and Environment for Statistical Computing (R Foundation for Statistical Computing, Austria).

Ratkowsky, D.A., Olley, J., McMeekin, T.A., and Ball, A. (1982). Relationship between temperature and growth rate of bacterial cultures. Journal of Bacteriology 149, 1–5.

Rebaudo, F., Struelens, Q., Dangles, O., and Price, S. (2017). Modelling temperature-dependent development rate and phenology in arthropods: The devRate package for R. Methods in Ecology and Evolution 9. 1144–1150

Reis, E.M., Zanatta, T., Zanatta, M., Reis, E.M., Zanatta, T., and Zanatta, M. (2016). Curative and eradicant action of fungicides to control *Phakopsora pachyrhizi* in soybean plants. Summa Phytopathologica 42, 295–302.

Reuveni, M. (2003). Activity of the New Fungicide Benthiavalicarb Against *Plasmopara viticola* and its Efficacy in Controlling Downy Mildew in Grapevines. European Journal of Plant Pathology 109, 243–251.

Richardson, L.T., and Munnecke, D.E. (1964). Effective Fungicide Dosage in Relation to Inoculum Concentration in Soil. Canadian Journal of Botany 42, 301–306.

Riederer, M., and Friedmann, A. (2008). Transport of lipophilic non-electrolytes across the cuticle. Annual Plant Reviews 23, 250–279.

Rietman, H., Bijsterbosch, G., Cano, L.M., Lee, H.-R., Vossen, J.H., Jacobsen, E., Visser, R.G.F., Kamoun, S., and Vleeshouwers, V.G.A.A. (2012). Qualitative and quantitative late blight resistance in the potato cultivar Sarpo Mira is determined by the perception of five distinct RXLR effectors. Molecular Plant-Microbe Interactions 25, 910–919.

Riisberg, I., Orr, R.J.S., Kluge, R., Shalchian-Tabrizi, K., Bowers, H.A., Patil, V., Edvardsen, B., and Jakobsen, K.S. (2009). Seven Gene Phylogeny of Heterokonts. Protist 160, 191–204.

Ristaino, J.B., Groves, C.T., and Parra, G.R. (2001). PCR amplification of the Irish potato famine pathogen from historic specimens. Nature 411, 695–697.

Ritchie, F., Bain, R., Lees, A., Boor, T., and Paveley, N. (2017). Predicting the combined efficacy of host resistance and fungicides. In Proceedings of the Sixteenth EuroBlight Workshop, 14-17 May 2017, Aarhus, Denmark, (Applied Arable and Vegetable Research (Praktijkonderzoek AGV)), 139–145.

Ritchie, F., Bain, R., Paveley, N., and Maloney, K. (2018a). Integrated Crop Management (ICM) for potato late blight. (AHDB, United Kingdom).

Ritchie, F., Bain, R.A., Lees, A.K., Boor, T.R.W., and Paveley, N.D. (2018b). Integrated control of potato late blight: predicting the combined efficacy of host resistance and fungicides. Plant Pathology 67, 1784–1791.

Ritz, C. (2010). Toward a unified approach to dose–response modeling in ecotoxicology. Environmental Toxicology and Chemistry 29, 220–229.

Ritz, C., and Streibig, J.C. (2008). Nonlinear regression with R (Springer, United States).

Ritz, C., Baty, F., Streibig, J.C., and Gerhard, D. (2015). Dose-Response Analysis Using R. PLOS ONE 10, e0146021.

Roden, L.C., and 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, 2546–2552.

Rodewald, J., and Trognitz, B. (2013). *Solanum* resistance genes against *Phytophthora infestans* and their corresponding avirulence genes. Molecular Plant Pathology 14, 740–757.

Rohwer, F., Fritzemeier, K.-H., Scheel, D., and Hahlbrock, K. (1987). Biochemical reactions of different tissues of potato (*Solanum tuberosum*) to zoospores or elicitors from *Phytophthora infestans*. Planta 170, 556–561.

Roltsch, W.J., Zalom, F.G., Strawn, A.J., Strand, J.F., and Pitcairn, M.J. (1999). Evaluation of several degree-day estimation methods in California climates. International Journal of Biometeorology 42, 169–176.

Roman, M.L., Izarra, M., Lindqvist-Kreuze, H., Rivera, C., Gamboa, S., Tovar, J.C., Forbes, G.A., Kreuze, J.F., and Ghislain, M. (2017). R/Avr gene expression study of Rpi-vnt1.1 transgenic potato resistant to the *Phytophthora infestans* clonal lineage EC-1. Plant Cell, Tissue and Organ Culture 131, 259–268.

Roscher, R., Behmann, J., Mahlein, A.-K., Dupuis, J., Kuhlmann, H., and Plümer, L. (2016). Detection of Disease Symptoms On Hyperspectral 3D Plant Models. ISPRS Annals of Photogrammetry, Remote Sensing & Spatial Information Sciences 3, 89–96.

Rossi, V., Caffi, T., Bugiani, R., Spanna, F., and Valle, D.D. (2008). Estimating the germination dynamics of *Plasmopara viticola* oospores using hydro-thermal time. Plant Pathology 57, 216–226.

Rossing, W., Skelsey, P., Kessel, G., and van der Werf, W. (2006). Sensitivity of potato late blight epidemics and potato yield to variation in initial inoculum density: a model-based analysis with implications for predicting atmospheric transport of spores. PPO-Special Report No. 11 35.

Rotem, J., Bashi, E., and Kranz, J. (1983). Studies of crop loss in potato blight caused by *Phytophthora infestans*. Plant Pathology 32, 117–122.

Roux, F., Voisin, D., Badet, T., Balagué, C., Barlet, X., Huard-Chauveau, C., Roby, D., and Raffaele, S. (2014). Resistance to phytopathogens e tutti quanti: placing plant quantitative disease resistance on the map. Molecular Plant Pathology 15, 427–432.

Roy, M., Brodeur, J., and Cloutier, C. (2002). Relationship Between Temperature and Developmental Rate of *Stethorus punctillum* (Coleoptera: Coccinellidae) and Its Prey *Tetranychus mcdanieli* (Acarina: Tetranychidae). Environmental Entomolgy 31, 177–187.

Royston, P., and Sauerbrei, W. (2005). Building Multivariable Regression Models with Continuous Covariates in Clinical Epidemiology. Methods of Information in Medicine 44, 561–571.

Runno-Paurson, E., Williams, I.H., Metspalu, L., Kaart, T., and Mänd, M. (2013). Current potato varieties are too susceptible to late blight to be grown without chemical control under North-East European conditions. Acta Agriculturae Scandinavica, Section B — Soil & Plant Science 63, 80–88.

Russell, P.E. (2005). A century of fungicide evolution. The Journal of Agricultural Science 143, 11–25.

Samoucha, Y., and Cohen, Y. (1990). Toxicity of propamocarb to the late blight fungus on potato. Phytoparasitica 18, 27–40.

Sarcina, M., Tobin, M.J., and Mullineaux, C.W. (2001). Diffusion of Phycobilisomes on the Thylakoid Membranes of the Cyanobacterium *Synechococcus* 7942 Effects of Phycobilisome Size, Temperature and Membrane Lipid Composition. Journal of Biological Chemistry 276, 46830–46834.

Satchivi, N.M., Stoller, E.W., Wax, L.M., and Briskin, D.P. (2006). A nonlinear, dynamic, simulation model for transport, and whole plant allocation of systemic xenobiotics following

foliar application. IV: Physicochemical properties requirements for optimum absorption and translocation. Pesticide Biochemistry and Physiology 84, 83–97.

Saubeau, G., Perrin, F., Marnet, N., Andrivon, D., and Val, F. (2016). Hormone signalling pathways are differentially involved in quantitative resistance of potato to *Phytophthora infestans*. Plant Pathology 65, 342–352.

Savazzini, F., and Galletti, S. (2015). Phenotypic and genotypic characterization of Italian *Phytophthora infestans* isolates. Phytopathologia Mediterranea 54, 524–530.

Saville, A., Graham, K., Grünwald, N.J., Myers, K., Fry, W.E., and Ristaino, J.B. (2014). Fungicide Sensitivity of U.S. Genotypes of *Phytophthora infestans* to Six Oomycete-Targeted Compounds. Plant Disease 99, 659–666.

Schabenberger, O., and Pierce, F. (2002). Contemporary Statistical Models for the Plant and Soil Science (CRC Press, United States).

Schepers, H. (1998). Compilation of questionaires on practical characteristics of fungicides used to control potato late blight. PAV-Special Report No. 5. 1999 60.

Schepers, H. (2000). The development and control of *Phytophthora infestans* in Europe in 1999. PAV-Special Report 6, 10–18.

Schepers, H., and Cooke, L.R. (2015). Potato Pathogens in Northern and Western Europe. In Fungicide Resistance in Plant Pathogens, (Springer, Tokyo), 355–378.

Schepers, H., Andrivon, D., Gaucher, D., Kapsa, J., Lebecka, R., Nielsen, B., Evenhuis, B., and Ruocco, M. (2009). Results of the potato case study in the EU-network of excellence, ENDURE. In Proceedings of Eleventh Euro Blight Workshop. Pro-Special Report, 31–36.

Schepers, H., Hausladen, H., Hansen, J.G., Nielsen, B.J., Abuley, I.K., Andersson, B., Liljeroth, E., Edin, E., Bain, R., and Ritchie, F., (2017). Epidemics and control of early & late blight, 2015 & 2016 in Europe. In Proceedings of the Sixteenth EuroBlight Workshop, (Aarhus, Denmark), 11–32.

Schisterman, E.F., Perkins, N.J., Liu, A., and Bondell, H. (2005). Optimal cut-point and its corresponding Youden Index to discriminate individuals using pooled blood samples. Epidemiology 16, 73–81.

Schneider, C.A., Rasband, W.S., and Eliceiri, K.W. (2012). NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9, 671–675.

Schoina, C., Bouwmeester, K., and Govers, F. (2017). Infection of a tomato cell culture by *Phytophthora infestans*; a versatile tool to study *Phytophthora*-host interactions. Plant Methods 13, 88.

Schoolfield, R.M., Sharpe, P.J.H., and Magnuson, C.E. (1981). Non-linear regression of biological temperature-dependent rate models based on absolute reaction-rate theory. Journal of Theoretical Biology 88, 719–731.

Schulze-Lefert, P., and Panstruga, R. (2011). A molecular evolutionary concept connecting nonhost resistance, pathogen host range, and pathogen speciation. Trends in Plant Science 16, 117–125.

Schwabe, W.F.S., Jones, A.L., and Jonker, J.P. (1984). Greenhouse evaluation of the curative and protective action of sterol-inhibiting fungicides against apple scab. Phytopathology 74, 249–252.

Schwinn, F., and Staub, T. (1995). Oomycetes fungicides. Modern Selective Fungicides. Jena: Gustav Fischer 323–346.

Seidl Johnson, A.C., Frost, K.E., Rouse, D.I., and Gevens, A.J. (2015). Effect of Temperature on Growth and Sporulation of US-22, US-23, and US-24 Clonal Lineages of *Phytophthora infestans* and Implications for Late Blight Epidemiology. Phytopathology 105, 449–459.

Serey, R.A., Torres, R., and Latorre, B.A. (2007). Pre- and post-infection activity of new fungicides against *Botrytis cinérea* and other fungi causing decay of table grapes. Ciencia e Investigación Agraria 34, 215–224.

Shah, S.R.A., Tao, L., Haijuan, C., Lei, L., Zheng, Z., Junming, L., and Yongchen, D. (2015). Age-related Resistance and the Defense Signaling Pathway of Ph-3 Gene Against *Phytophthora infestans* in Tomatoes. Horticultural Plant Journal 1, 70–76.

Shakya, S.K., Goss, E.M., Dufault, N.S., and van Bruggen, A.H.C. (2015). Potential effects of diurnal temperature oscillations on potato late blight with special reference to climate change. Phytopathology 105, 230–238.

Shandil, R.K., Chakrabarti, S.K., Singh, B.P., Sharma, S., Sundaresha, S., Kaushik, S.K., Bhatt, A.K., and Sharma, N.N. (2017). Genotypic background of the recipient plant is crucial for conferring RB gene mediated late blight resistance in potato. BMC Genetics 18, 22.

Sharpe, P.J., and DeMichele, D.W. (1977). Reaction kinetics of poikilotherm development. Journal of Theoretical Biology 64, 649–670.

Sharpe, S.M., Boyd, N.S., Dittmar, P.J., MacDonald, G.E., Darnell, R.L., and Ferrell, J.A. (2018). Spray Penetration into a Strawberry Canopy as Affected by Canopy Structure, Nozzle Type, and Application Volume. Weed Technology 32, 80–84.

Sharratt, B.S., Sheaffer, C.C., and Baker, D.G. (1989). Base temperature for the application of the growing-degree-day model to field-grown alfalfa. Field Crops Research 21, 95–102.

Shattock, R.C. (2002). *Phytophthora infestans*: populations, pathogenicity and phenylamides. Pest Management Science 58, 944–950.

Shepard, J.F., and Claflin, L.E. (1975). Critical Analyses of the Principles of Seed Potato Certification. Annual Review of Phytopathology 13, 271–293.

Shi, P.J., Reddy, G.V.P., Chen, L., and Ge, F. (2017). Comparison of Thermal Performance Equations in Describing Temperature-Dependent Developmental Rates of Insects: (II) Two Thermodynamic Models. Annals of the Entomological Society of America 110, 113–120.

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

Shtienberg, D., Doster, M.A., Pelletier, J.R., and Fry, W.E. (1989). Use of simulation models to develop a low-risk strategy to suppress early and late blight in potato foliage. Phytopathology 79, 590–595.

Sicbaldi, F., Sacchi, G.A., Trevisan, M., and Re, A.A.M.D. (1997). Root Uptake and Xylem Translocation of Pesticides from Different Chemical Classes. Pesticide Science 50, 111–119.

Siddique, N., Aktar, M., and Swapon, N. (2016). Comparative Efficacy of Different Fungicides against Late Blight Diseases of Potato incited by *Phytophthora infestans* (Mont.) de Bary and its Management. Journal of Plant Pathology & Microbiology. 7, 1000364.

Silver, R.M. (2012). Implications of the First Cesarean: Perinatal and Future Reproductive Health and Subsequent Cesareans, Placentation Issues, Uterine Rupture Risk, Morbidity, and Mortality. Seminars in Perinatology 36, 315–323.

Singh, B.P., Roy, S., and Bhattacharyya, S.K. (1994). Occurrence of the A2 mating type of *Phytophthora infestans* in India. Potato Research 37, 227–231.

Singh, V.K., Shailbala, and Pundhir, V.S. (2013). Forecasting Models for Potato Late Blight Management - a Review. Agricultural Reviews 34, 87–96.

Skaltsa, K., Jover, L., and Carrasco, J.L. (2010). Estimation of the diagnostic threshold accounting for decision costs and sampling uncertainty. Biometrical Journal 52, 676–697.

Skelsey, P., Kessel, G.J.T., Holtslag, A.A.M., Moene, A.F., and van der Werf, W. (2009). Regional spore dispersal as a factor in disease risk warnings for potato late blight: A proof of concept. Agricultural and Forest Meteorology 149, 419–430.

Skelsey, P., Cooke, D.E.L., Lynott, J.S., and Lees, A.K. (2016). Crop connectivity under climate change: future environmental and geographic risks of potato late blight in Scotland. Global Change Biology 22, 3724–3738.

Skelsey, P., Dancey, S., and Cooke, D. (2017). Forecasting the risk of late blight spread. In Proceedings of the Sixteenth EuroBlight Workshop, (Aarhus, Denmark), 61–66.

Skelsey, P., Dancey, S.R., Preedy, K., Lees, A.K., and Cooke, D.E.L. (2018). Forecasting the spread of aerially transmitted crop diseases with a binary classifier for inoculum survival. Plant Pathology 67, 920–928.

Skylakakis, G. (1983). Theory and Strategy of Chemical Control. Annual Review of Phytopathology 21, 117–136.

Slafer, G.A., and Savin, R. (1991). Developmental Base Temperature in Different Phenological Phases of Wheat (*Triticum aestivum*). Journal of Experimental Botany 42, 1077–1082.

Slovic, P., Fischhoff, B., and Lichtenstein, S. (1980). Facts and Fears: Understanding Perceived Risk. In R.C. Schwing, and W.A. Albers, (Eds.) Societal Risk Assessment: How Safe Is Safe Enough? (Springer, United States), 181–216.

Small, I.M., Joseph, L., and Fry, W.E. (2015). Development and implementation of the BlightPro decision support system for potato and tomato late blight management. Computers and Electronics in Agriculture 115, 57–65.

Smith, L.P. (1956). Potato Blight Forecasting by 90 per cent Humidity Criteria. Plant Pathology 5, 83–87.

Solomon-Blackburn, R.M., Stewart, H.E., and Bradshaw, J.E. (2007). Distinguishing major-gene from field resistance to late blight (*Phytophthora infestans*) of potato (*Solanum tuberosum*) and selecting for high levels of field resistance. Theoretical and Applied Genetics 115, 141–149.

Somero, G.N. (2004). Adaptation of enzymes to temperature: searching for basic "strategies." Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 139, 321–333.

Song, J., Bradeen, J.M., Naess, S.K., Raasch, J.A., Wielgus, S.M., Haberlach, G.T., Liu, J., Kuang, H., Austin-Phillips, S., Buell, C.R., Helgeson, J., and Jiang, J. (2003). Gene RB cloned from *Solanum bulbocastanum* confers broad spectrum resistance to potato late blight. Proceedings of the National Academy of Sciences 100, 9128–9133.

Song, W.Y., Wang, G.L., Chen, L.L., Kim, H.S., Pi, L.Y., Holsten, T., Gardner, J., Wang, B., Zhai, W.X., Zhu, L.H., Fauquet, C., and Ronald, P. (1995). A receptor kinase-like protein encoded by the rice disease resistance gene, Xa21. Science 270, 1804–1806.

Sparks, A.H., Forbes, G.A., Hijmans, R.J., and Garrett, K.A. (2014). Climate change may have limited effect on global risk of potato late blight. Global Change Biology 20, 3621–3631.

Spits, H.G., and Wander, J.G.N. (2001). Field evaluation of four decission support systems for potato late blight in the Netherlandes in 2000. PAV-Special Report 77–90.

Srivastava, M.P. (2003). Transfer of plant pathology knowledge for rural prosperity—an Asian perspective. Australasian Plant Pathology 32, 187–194.

Statsyuk, N., Semina, Y.V., Perez, F., Larsen, M., Kuznetsova, M., Kozlovskaya, I., Morozova, E., Deahl, K., and Grünwald, N. (2014). Characterization of Russian *Phytophthora infestans* populations: DNA fingerprinting and SSR analysis. PPO Special Report 16, 255–266.

Staub, T. (1991). Fungicide Resistance: Practical Experience with Antiresistance Strategies and the Role of Integrated Use. Annual Review of Phytopathology 29, 421–442.

Staves, P.A., and Knell, R.J. (2010). Virulence and Competitiveness: Testing the Relationship During Inter- and Intraspecific Mixed Infections. Evolution 64, 2643–2652.

St.Clair, D.A. (2010). Quantitative Disease Resistance and Quantitative Resistance Loci in Breeding. Annual Review of Phytopathology 48, 247–268.

Stein, J.M., and Kirk, W.W. (2002). Containment of existing potato late blight (*Phytophthora infestans*) foliar epidemics with fungicides. Crop Protection 21, 575–582.

Stephan, D., Schmitt, A., Carvalho, S.M., Seddon, B., and Koch, E. (2005). Evaluation of biocontrol preparations and plant extracts for the control of *Phytophthora infestans* on potato leaves. European Journal of Plant Pathology 112, 235–246.

Stern, V., Smith, R., Van den Bosch, R., Hagen, K. (1959). The integration of chemical and biological control of the spotted alfalfa aphid: the integrated control concept. Hilgardia 29, 81–101.

Steurbaut, W. (1993). Adjuvants for use with foliar fungicides. Pesticide Science 38, 85–91.

Stevens, P.J.G., Baker, E.A., and Anderson, N.H. (1988). Factors affecting the foliar absorption and redistribution of pesticides. 2. Physicochemical properties of the active ingredient and the role of surfactant. Pesticide Science 24, 265–282.

Stewart, H.E. (1990). Effect of plant age and inoculum concentration on expression of major gene resistance to *Phytophthora infestans* in detached potato leaflets. Mycological Research 94, 823–826.

Stewart, H.E., Bradshaw, J.E., and Pande, B. (2003). The effect of the presence of R-genes for resistance to late blight (*Phytophthora infestans*) of potato (*Solanum tuberosum*) on the underlying level of field resistance. Plant Pathology 52, 193–198.

van Straalen, N.M. (1983). Physiological time and time-invariance. Journal of Theoretical Biology 104, 349–357.

Streck, N.A., de Paula, F.L.M., Bisognin, D.A., Heldwein, A.B., and Dellai, J. (2007). Simulating the development of field grown potato (*Solanum tuberosum* L.). Agricultural and Forest Meteorology 142, 1–11.

Stroud, J.A., Shaw, D.S., Hale, M.D., and Steele, K.A. (2016). SSR assessment of *Phytophthora infestans* populations on tomato and potato in British gardens demonstrates high diversity but no evidence for host specialization. Plant Pathology 65, 334–341.

Suassuna, N.D., Maffia, L.A., and Mizubuti, E.S.G. (2004). Aggressiveness and host specificity of Brazilian isolates of *Phytophthora infestans*. Plant Pathology 53, 405–413.

Sujkowski, L.S. (1987). The Influence of Temperature on *Phytophthora infestans* (Mont.) de Bary. Journal of Phytopathology 120, 271–275.

Sujkowski, L.S., Goodwin, S.B., Dyer, A.T., Fry, W.E. (1994). Increased genotypic diversity via migration and possible occurrence of sexual reproduction of *Phytophthora infestans* in Poland. Phytopathology 84, 201–201.

Sukul, P., Spiteller, M. (2000). Metalaxyl: persistence, degradation, metabolism, and analytical methods. Reviews of Environmental Contamination and Toxicology 164, 1–26.

Sun, H., Wang, H., Stammler, G., Ma, J., and Zhou, M. (2010). Baseline Sensitivity of Populations of *Phytophthora capsici* from China to Three Carboxylic Acid Amide (CAA) Fungicides and Sequence Analysis of Cholinephosphotranferases from a CAA-sensitive Isolate and CAA-resistant Laboratory Mutants. Journal of Phytopathology 158, 244–252.

Sun, K., Wolters, A.-M.A., Vossen, J.H., Rouwet, M.E., Loonen, A.E.H.M., Jacobsen, E., Visser, R.G.F., and Bai, Y. (2016). Silencing of six susceptibility genes results in potato late blight resistance. Transgenic Research 25, 731–742.

Sunseri, M.A., Johnson, D.A., and Dasgupta, N. (2002). Survival of detached sporangia of *Phytophthora infestans* exposed to ambient, relatively dry atmospheric conditions. American Journal of Potato Research 79, 443–450.

Symonds, M.R.E., and Moussalli, A. (2011). A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike's information criterion. Behavioral Ecology and Sociobiology 65, 13–21.

Tafforeau, S., Bardsley, E., Latorse, M., Fabrèges, C., Schirrings, A., and Wegmann, T. (2006). Infinito: Profile of a novel potato late blight fungicides. Summary of three years of development trials in Europe. PPO-Special Report 11, 77–88.

Tang, S., Xiao, Y., Chen, L., and Cheke, R.A. (2005). Integrated pest management models and their dynamical behaviour. Bulletin of Mathematical Biology 67, 115–135.

Tang, X., Cao, X., Xu, X., Jiang, Y., Luo, Y., Ma, Z., Fan, J., and Zhou, Y. (2017). Effects of Climate Change on Epidemics of Powdery Mildew in Winter Wheat in China. Plant Disease 101, 1753–1760.

Tantius, P.H., Fyfe, A.M., Shaw, D.S., and Shattock, R.C. (1986). Occurrence of the A2 mating type and self-fertile isolates of *Phytophthora infestans* in England and Wales. Plant Pathology 35, 578–581.

Taylor, F. (1981). Ecology and Evolution of Physiological Time in Insects. The American Naturalist 117, 1–23.

Taylor, J., Paterson, L., and Havis, N. (2010). A quantitative real-time PCR assay for the detection of *Ramularia collo-cygni* from barley (*Hordeum vulgare*). Letters in Applied Microbiology 50, 493–499.

Taylor, M.C., Hardwick, N.V., Bradshaw, N.J., and Hall, A.M. (2003). Relative performance of five forecasting schemes for potato late blight (*Phytophthora infestans*) I. Accuracy of infection warnings and reduction of unnecessary, theoretical, fungicide applications. Crop Protection 22, 275–283.

Tegg, R.S., Corkrey, R., Herdina, H., McKay, A.C., Crump, N.S., de Boer, R.F., Wiechel, T.J., and Wilson, C.R. (2014). Modeling Pathogen DNA Content and Visual Disease Assessment in Seed Tubers to Inform Disease in Potato Progeny Root, Stolon, and Tubers. Plant Disease 99, 50–57.

Thomas, M.B., and Blanford, S. (2003). Thermal biology in insect-parasite interactions. Trends in Ecology & Evolution 18, 344–350.

Thorburn, P.J., Jakku, E., Webster, A.J., and Everingham, Y.L. (2011). Agricultural decision support systems facilitating co-learning: a case study on environmental impacts of sugarcane production. International Journal of Agricultural Sustainability 9, 322–333.

Thrall, P.H., Barrett, L.G., Dodds, P.N., and Burdon, J.J. (2016). Epidemiological and Evolutionary Outcomes in Gene-for-Gene and Matching Allele Models. Frontiers in Plant Science 6. 1084

Thurston, H.D. (1971). Relationship of General Resistance: Late Blight of Potato. Phytopathology, 61, 620–626.

Tjørve, K.M.C., and Tjørve, E. (2017). The use of Gompertz models in growth analyses, and new Gompertz-model approach: An addition to the Unified-Richards family. PLOS One 12, e0178691.

Toffolatti, S.L., Maffi, D., Serrati, L., and Vercesi, A. (2011). Histological and Ultrastructural Studies on the Curative Effects of Mandipropamid on *Plasmopara viticola*. Journal of Phytopathology 159, 201–207.

Töfoli, J.G., Melo, P.C.T., and Domingues, R.J. (2012). Protective, residual, curative and antisporulating action of fungicides in the control of early and late blight of potatoes under controlled conditions. Arquivos Do Instituto Biológico 79, 209–221.

Tooley, P.W., Fry, W.E., and Gonzalez, M.J.V. (1985). Isozyme characterization of sexual and asexual *Phytophthora infestans* populations. Journal of Heredity 76, 431–435.

Toquin, V., Barja, F., Sirven, C., and Bffa, R. (2007). Fluopicolide, a new Anti-oomycetes Fungicide with a New Mode of Action inducing Perturbation of a Spectrin-like Protein. In W. Krämer, and U. Schirmer, (Eds.) Modern Crop Protection Compounds, (Wiley, United States), 675–682.

Toquin, V., Barja, F., Sirven, C., Gamet, S., Mauprivez, L., Peret, P., Latorse, M.-P., Zundel, J.-L., Schmitt, F., and Lebrun, M.H. (2010). Novel Tools to Identify the Mode of Action of Fungicides as Exemplified with Fluopicolide. In U. Gisi, I. Chet, and M.L. Gullino, (Eds.) Recent Developments in Management of Plant Diseases, (Springer, Netherlands), 19–36.

Toxopeus, H.J. (1956). Reflections on the origin of new physiologic races in P*hytophthora infestans* and the breeding for resistance in potatoes. Euphytica 5, 221–237.

Tracy, C.R., and Christian, K.A. (1986). Ecological relations among space, time, and thermal niche axes. Ecology 67, 609–615.

Trinci, A.P.J. (1971). Exponential Growth of the Germ Tubes of Fungal Spores. Microbiology 67, 345–348.

Trudgill, D.L. (1995). An assessment of the relevance of thermal time relationships to nematology. Fundamental and Applied Nematology 18, 407–417.

Turkensteen, L.J., Flier, W.G., Wanningen, R., and Mulder, A. (2000). Production, survival and infectivity of oospores of *Phytophthora infestans*. Plant Pathology 49, 688–696.

Turner, R.S. (2005). After the famine: Plant pathology, *Phytophthora infestans*, and the late blight of potatoes, 1845–1960. Historical Studies in the Physical and Biological Sciences 35, 341–370.

Twinin, S., Clarke, J., Cook, S., Ellis, S., Gladders, P., Ritchie, F., and Wynn, S. (2009). Research Report: Pesticide availability for potatoes following revision of Directive 91/414/EEC: Impact assessment and identification of research priorities. (AHDB, United Kingdom).

Usall, J., Smilanick, J., Palou, L., Denis-Arrue, N., Teixidó, N., Torres, R., and Viñas, I. (2008). Preventive and curative activity of combined treatments of sodium carbonates and Pantoea agglomerans CPA-2 to control postharvest green mold of citrus fruit. Postharvest Biology and Technology 50, 1–7.

Utami, D.W., and Ambarwati, D. (2017). Genotype and Phenotype Characterization of Indonesian *Phytophthora infestans* Isolates Collected From Java and Outside Java Island. HAYATI Journal of Biosciences 24, 168–175.

van den Bosch, F., Paveley, N., Shaw, M., Hobbelen, P., and Oliver, R. (2011). The dose rate debate: does the risk of fungicide resistance increase or decrease with dose? Plant Pathology 60, 597–606.

van den Bosch, F. van den, Oliver, R., Berg, F. van den, and Paveley, N. (2014). Governing Principles Can Guide Fungicide-Resistance Management Tactics. Annual Review of Phytopathology 52, 175–195.

van Maanen, A., and Xu, X.M. (2003). Modelling plant disease epidemics. European Journal of Plant Pathology 109, 669–682.

van Oijen, M. (1991). Identification of the major characteristics of potato cultivars which affect yield loss caused by late blight. PhD Thesis, Wageningen University, 108 pages.

van Schie, C., and Takken, F. (2014). Susceptibility Genes 101: How to Be a Good Host. Annual Review of Phytopathology 52, 551–581.

Vicent, A., Armengol, J., and García-Jiménez, J. (2007). Rain Fastness and Persistence of Fungicides for Control of *Alternaria* Brown Spot of Citrus. Plant Disease 91, 393–399.

Vincelli, P., and Dixon, E. (2007). Does spray coverage influence fungicide efficacy against dollar spot? Applied Turfgrass Science 4, 1–4.

Visker, M.H.P.W., Keizer, L.C.P., Budding, D.J., Van Loon, L.C., Colon, L.T., and Struik, P.C. (2003). Leaf Position Prevails Over Plant Age and Leaf Age in Reflecting Resistance to Late Blight in Potato. Phytopathology 93, 666–674.

Visker, M.H.P.W., Raaij, H.M.G. van, Keizer, L.C.P., Struik, P.C., and Colon, L.T. (2004). Correlation between late blight resistance and foliage maturity type in potato. Euphytica 137, 311–323.

Vleeshouwers, V.G.A.A., Dooijeweert, W. van, Keizer, L.C.P., Sijpkes, L., Govers, F., and Colon, L.T. (1999). A Laboratory Assay for *Phytophthora infestans* Resistance in Various *Solanum* Species Reflects the Field Situation. European Journal of Plant Pathology 105, 241–250.

Vleeshouwers, V.G.A.A., Van Dooijeweert, W., Govers, F., Kamoun, S., and Colon, L.T. (2000a). Does basal PR gene expression in *Solanum* species contribute to non-specific resistance to *Phytophthora infestans*? Physiological and Molecular Plant Pathology 57, 35–42.

Vleeshouwers, V.G.A.A., Dooijeweert, W. van, Govers, F., Kamoun, S., and Colon, L.T. (2000b). The hypersensitive response is associated with host and nonhost resistance to *Phytophthora infestans*. Planta 210, 853–864.

Vleeshouwers, V.G.A.A., Raffaele, S., Vossen, J.H., Champouret, N., Oliva, R., Segretin, M.E., Rietman, H., Cano, L.M., Lokossou, A., Kessel, G., Pel, M., and Kamoun, S. (2011). Understanding and exploiting late blight resistance in the age of effectors. Annual Review of Phytopathology 49, 507–531.

Vossen, E., Voort, J., Kanyuka, K., Bendahmane, A., Sandbrink, H., Baulcombe, D.C., Bakker, J., Stiekema, W.J., and Klein-Lankhorst, R.M. (2000). Homologues of a single resistance-gene cluster in potato confer resistance to distinct pathogens: a virus and a nematode. The Plant Journal 23, 567–576.

Waard, M.A., Georgopoulos, S.G., Hollomon, D.W., Ishii, H., Leroux, P., Ragsdale, N.N., and Schwinn, F.J. (1993). Chemical Control of Plant Diseases: Problems and Prospects. Annual Review of Phytopathology 31, 403–421.

Wang, J.Y. (1960). A Critique of the Heat Unit Approach to Plant Response Studies. Ecology 41, 785–790.

Wang, W., Zhang, P., Meng, R., Zhao, J., Huang, Q., Han, X., Ma, Z., and Zhang, X. (2014). Fungitoxicity and synergism of mixtures of fluopicolide and pyraclostrobin against *Phytophthora infestans*. Crop Protection 57, 48–56.

Wang, W.-Q., Han, X.-Y., Zhang, X.-F., Kang, L.-J., and Chen, S.-L. (2002). Synergism in mixtures of cymoxanil and mancozeb on *Phytophthora infestans* in vivo. Meded Rijksuniv Gent Fak Landbouwkd Toegep Biol Wet 67, 299–305.

Wangsomboondee, T., and Ristaino, J.B. (2002). Optimization of Sample Size and DNA Extraction Methods to Improve PCR Detection of Different Propagules of *Phytophthora infestans*. Plant Disease 86, 247–253.

Warren, R.C., and Colhoun, J. (1975). Viability of sporangia of *Phytophthora infestans* in relation to drying. Transactions of the British Mycological Society 64, 73–78.

Weir, B., Paderes, E., Anand, N., Uchida, J., Pennycook, S., Bellgard, S., and Beever, R. (2015). A taxonomic revision of *Phytophthora* Clade 5 including two new species, *Phytophthora agathidicida* and *P. cocois*. Phytotaxa 205, 21–38.

Werner, H.O. (1942). Relative response of several varieties of potatoes to progressively changing temperatures and photoperiods controlled to simulate “northern” and “southern” conditions. American Potato Journal 19, 30–40.

Weyman, P.D., Pan, Z., Feng, Q., Gilchrist, D.G., and Bostock, R.M. (2006). A Circadian Rhythm-Regulated Tomato Gene Is Induced by Arachidonic Acid and *Phythophthora infestans* Infection. Plant Physiology 140, 235–248.

Wharton, P.S., Kirk, W.W., Baker, K.M., and Duynslager, L. (2008). A web-based interactive system for risk management of potato late blight in Michigan. Computers and Electronics in Agriculture 61, 136–148.

Whisson, S.C., Boevink, P.C., Wang, S., and Birch, P.R. (2016). The cell biology of late blight disease. Current Opinion in Microbiology 34, 127–135.

Widmer, T.L. (2009). Infective Potential of Sporangia and Zoospores of *Phytophthora ramorum*. Plant Disease: An International Journal of Applied Plant Pathology 93, 30–35.

Williams, J.C. (2012). New EU pesticide legislation–The view of a manufacturer. In The Dundee Conference. Crop Protection in Northern Britain, 2012, (Dundee, United Kingdom), 7–14.

Willyerd, K., Li, C., Madden, L., Bradley, C., Bergstrom, G., Sweets, L., McMullen, M., Ransom, J., Grybauskas, A., Osborne, L., Wegulo, S., Hershman, D., Wise, K., Bockus, W., Grith, D., Dill-Macky, R., Milus, E., Esker, P., Waxman, K., Adee, E., Ebelhar, S., Young, B., and Paul, P. (2012). Efficacy and stability of integrating fungicide and cultivar resistance to manage *Fusarium* head blight and deoxynivalenol in wheat. Plant Disease 96, 957–967.

Wilson, C., and Tisdell, C. (2001). Why farmers continue to use pesticides despite environmental, health and sustainability costs. Ecological Economics 39, 449–462.

Wojtaszek, P. (1997). Oxidative burst: an early plant response to pathogen infection. Biochemical Journal 322, 681–692.

Wolfe, M.S. (1981). Integrated use of fungicides and host resistance for stable disease control. Philosophical Transactions of the Royal Society B 295, 175–184.

Worner, S.P. (1992). Performance of phenological models under variable temperature regimes: consequences of the Kaufmann or rate summation effect. Environmental Entomology 21, 689–699.

Wustman, R. (2007). The Canon of Potato Science: 21. Storage Diseases and Pests. Potato Research 50, 289–292.

Xu, X.M. (2003). On estimating non-linear response of fungal development under fluctuating temperatures. Plant Pathology 45, 163–171.

Xu, X.M. (1999). Effects of Temperature on the Length of the Incubation Period of Rose Powdery Mildew (*Sphaerotheca pannosa* var. *rosae*). European Journal of Plant Pathology 105, 13–21.

Xu, X., and Butt, D.J. (1996). Tests of fungicides for post-germination activity against *Nectria galligena*, causal agent of canker and fruit rot of apple. Crop Protection 15, 513–519.

Xue, L., Zhu, Y., and Xue, Y. (2013). RAEDSS: An integrated decision support system for regional agricultural economy in China. Mathematical and Computer Modelling 58, 480–488.

Yang, L., Wang, D., Xu, Y., Zhao, H., Wang, L., Cao, X., Chen, Y., and Chen, Q. (2017). A New Resistance Gene against Potato Late Blight Originating from *Solanum pinnatisectum* Located on Potato Chromosome 7. Frontiers in Plant Science 8, 1729.

Yang, L.N., Zhu, W., Wu, E.J., Yang, C., Thrall, P.H., Burdon, J.J., Jin, L.P., Shang, L.P., and Zhan, J. (2016). Trade-offs and evolution of thermal adaptation in the Irish potato famine pathogen *Phytophthora infestans*. Molecular Ecology 25, 4047–4058.

Yang, X., Royer, M., Tschanz, A., Tsai, B. (1990). Analysis and quantification of soybean rust epidemics from seventy-three sequential planting experiments. Phytopathology 80, 1421–1427.

Yin, X., and Struik, P.C. (2010). Modelling the crop: from system dynamics to systems biology. Journal of Experimental Botany 61, 2171–2183.

Yin, X., Kropff, M.J., McLaren, G., and Visperas, R.M. (1995). A nonlinear model for crop development as a function of temperature. Agricultural and Forest Meteorology 77, 1–16.

Yin, X., Goudriaan, J., Lantinga, E.A., Vos, J., and Spiertz, H.J. (2003). A Flexible Sigmoid Function of Determinate Growth. Annals of Botany 91, 361–371.

Yogendra, K.N., Pushpa, D., Mosa, K.A., Kushalappa, A.C., Murphy, A., and Mosquera, T. (2014). Quantitative resistance in potato leaves to late blight associated with induced hydroxycinnamic acid amides. Functional & Integrative Genomics 14, 285–298.

Yoshida, K., Schuenemann, V.J., Cano, L.M., Pais, M., Mishra, B., Sharma, R., Lanz, C., Martin, F.N., Kamoun, S., Krause, J., Thines, M., Weigel, D., Burbano, H. (2013). The rise and fall of the *Phytophthora infestans* lineage that triggered the Irish potato famine. eLife Sciences 2, e00731.

Young, G.K., Cooke, L.R., Watson, S., Kirk, W.W., Perez, F.M., and Deahl, K.L. (2018). The role of aggressiveness and competition in the selection of *Phytophthora infestans* populations. Plant Pathology 67, 1539–1551.

Yuen, J. (2006). Deriving decision rules. Plant Health Instructor 10, 1094.

Yuen, J., and Mila, A. (2004). Are Bayesian approaches useful in plant pathology? Frontis 3, 95–103.

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

Zabkiewicz, J.A. (2007). Spray formulation efficacy—holistic and futuristic perspectives. Crop Protection 26, 312–319.

Zan, K. (1962). Activity of *Phytophthora infestans* in soil in relation to tuber infection. Transactions of the British Mycological Society 45, 205–221.

Zhang, H., Wang, X., Wang, X., Qian, M., Xu, M., Xu, H., Qi, P., Wang, Q., and Zhuang, S. (2014). Enantioselective determination of carboxyl acid amide fungicide mandipropamid in

vegetables and fruits by chiral LC coupled with MS/MS. Journal of Separation Science 37, 211–218.

Zhu, S., Li, Y., Vossen, J.H., Visser, R.G.F., and Jacobsen, E. (2012). Functional stacking of three resistance genes against *Phytophthora infestans* in potato. Transgenic Research 21, 89–99.

Zhu, W., Yang, L.N., Wu, E.J., Qin, C.-F., Shang, L.P., Wang, Z.H., and Zhan, J. (2015). Limited Sexual Reproduction and Quick Turnover in the Population Genetic Structure of *Phytophthora infestans* in Fujian, China. Scientific Reports 5, 10094.

Ziogas, B., and Davidse, L. (1987). Studies on the mechanism of action of cymoxanil in *Phytophthora infestans*. Pesticide Biochemistry and Physiology 29, 89–96.

Zipfel, C. (2009). Early molecular events in PAMP-triggered immunity. Current Opinion in Plant Biology 12, 414–420.

Zuluaga, A.P., Vega-Arreguín, J.C., Fei, Z., Ponnala, L., Lee, S.J., Matas, A.J., Patev, S., Fry, W.E., and Rose, J.K.C. (2016a). Transcriptional dynamics of *Phytophthora infestans* during sequential stages of hemibiotrophic infection of tomato. Molecular Plant Pathology 17, 29–41.

Zuluaga, A.P., Vega-Arreguín, J.C., Fei, Z., Matas, A.J., Patev, S., Fry, W.E., and Rose, J.K.C. (2016b). Analysis of the tomato leaf transcriptome during successive hemibiotrophic stages of a compatible interaction with the oomycete pathogen *Phytophthora infestans*. Molecular Plant Pathology 17, 42–54.

(2009). 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 (Text with EEA relevance).

Appendix 1. Table of mathematical symbols used in this thesis

Table 0.1 List of mathematical symbols used within this thesis

Symbol	Units	Description
AIC	Dimensionless	Akaike information criterion
AICc	Dimensionless	Corrected Akaike information criterion
ci	As accompanying metric	Confidence interval, unless otherwise stated 95%
C_q	Dimensionless	Quantification cycle
Di_s	Dimensionless	Infected leaf discs scaled to control count.
d_{ac}	ln(pg)	Expected development
d_1	mm	Ellipse diameter 1
d_2	mm	Ellipse diameter 2
e	Dimensionless	Euler's number
EC_{50}	As accompanying metric	Effective dose 50%
F	Dimensionless	Fungicide treatment index
g	Dimensionless	Relative centrifugal force
I	Dimensionless	Inoculation time index
J	Dimensionless	Youden's J
K	as $°D_a$	Thermal constant
LA	mm^2	Lesion Area
LI	Dimensionless	Number of infected leaflets
LC	Dimensionless	Lesion Count
LC_{50}	As accompanying metric	Lethal concentration 50%
P_{best}	Dimensionless	Probability of harmful outcome for the group with the best prognosis
P_{cur}	Dimensionless	Probability of curative control failure
P_{worst}	Dimensionless	Probability of harmful outcome for the group with the worst prognosis
PSEP	Dimensionless	Index of separation
Pi_{DNA}	pg	Initial target template quantity of *P. infestans* DNA
RH	%	Relative humidity
R	$J\,K^{-1}\,mol^{-1}$	Gas constant
RSS	Dimensionless	Residual Sum of Squares

Table 0.1 (table continued from previous page)

Symbol	Units	Description
RSS	Dimensionless	Residual Sum of Squares
R^2	Dimensionless	Coefficient of determination
adj-R^2	Dimensionless	Adjusted R^2
pseudo-R^2	Dimensionless	Pseudo R^2
se	As accompanying metric	Standard error
t	hr	Chronological time
T	°C	Temperature
T_b	°C	Base temperature for biological growth/development
T_m	°C	Maximum temperature for biological growth/development
T_o	°C	Optimum temperature for biological growth/development
β_{ba0}	$\ln(pg)$	Biomass accumulation Parameter (intercept)
β_{ba1}	$\ln(pg)\cdot hr^{-1}$	Biomass accumulation parameter (slope coefficient)
β_{vL0}	$mm\cdot hr^{-1}$	Visible lesion growth parameter (intercept)
β_{vL1}	$mm\cdot hr^{-2}$	Visible lesion growth parameter (slope coefficient)
β_{vL2}	$mm\cdot hr^{-3}$	Visible lesion growth parameter (quadratic coefficient)
β_{vL3}	$mm\cdot hr^{-4}$	Visible lesion growth parameter (cubic coefficient)
β_{bL0}	$\ln(pg)\cdot hr^{-1}$	Biomass accumulation parameter (intercept)
β_{bL1}	$\ln(pg)\cdot hr^{-2}$	Biomass accumulation growth parameter (slope coefficient)
β_{bvL2}	$\ln(pg)\cdot hr^{-3}$	Biomass accumulation growth parameter (quadratic coefficient)
β_{bvL3}	$\ln(pg)\cdot hr^{-4}$	Biomass accumulation growth parameter (cubic coefficient)
β_{di1}	$\ln(pg)^{-1}$	Logistic Slope Parameter
β_{di2}	$\ln(pg)$	Logistic 50% Response Parameter
β_{lg0}	mm	Lesion Growth Parameter (incerecpt)
β_{lg1}	$mm\cdot hr^{-1}$	Lesion Growth Parameter (slope coefficient)

Table 0.1 (table continued from previous page)

Symbol	Units	Description
β_{li0}	Dimensionless	Lesion Infection Parameter (intercept)
β_{li1}	$°C^{-1} \cdot hr^{-1}$	Lesion Infection Parameter (slope coefficient)
${}^{o}D_a$	°C·hr	Accumulated Degree-Time
Γ	h	Physiological time
$\boldsymbol{\rho}$	Dimensionless	Spearman's Rank Correlation Coefficient

Appendix 2. List of abbreviations and acronyms used in thesis

AHDB:	Agriculture and Horticulture Development Board
a.i.s:	Active Ingredients
ANCOVA:	Analysis of Covariance
ANOVA:	Analysis of Variance
ATP:	Adenosine Triphosphate
CAAs:	Carboxylic Acid Amides
DNA:	Deoxyribonucleic Acid
DSS:	Decision Support System
ELISA:	Enzyme-Linked Immunosorbent Assay
ETI:	Effector Triggered Immunity
FRAC:	Fungicide Resistance Action Committee
FRAG-UK:	Fungicide Resistance Action Group – UK
GB:	Great Britain
GIS:	Geographic Information System
HR:	Hypersensitive Response
IPM:	Integrated Pest Management
JHI:	James Hutton Institute
LED:	Light Emitting Diode
MOA:	Modes Of Action
mtDNA:	Mitochondrial DNA
PAMP:	Pathogen Associated Molecular Pattern
PCR:	Polymerase Chain Reaction
PTI:	PAMP Triggered Immunity
qPCR:	Quantitative Polymerase Chain Reaction
RFLP:	Restricted Fragment Length Polymorphism
RH:	Relative Humidity
ROC:	Receiver Operating Characteristic Curve
RNA:	Ribonucleic acid
rRNA:	Ribosomal RNA

(List of abbreviations continued from previous page)

SNP:	Single Nucleotide Polymorphism
SPCS:	Seed Potato Classification Scheme
SRUC:	Scotland's Rural College
SSR:	Simple Sequence Repeat

Appendix 3. The EuroBlight table

Reproduced from:
http://agro.au.dk/forskning/internationale-platforme/euroblight/control-strategies/late-blight-fungicide-table/ (accessed Oct. 2019)

Product (Dose rate [litre or kg/ha])	Leaf blight	Tuber blight	New growth	Stem blight	Protec-tant	Curative	Anti sporulant	Rain-fastness	Mobility	Year
copper				●	●◖	0	0	●	C	1900
dithiocarbamates (2.0)[1]	2.0	0.0		●	●●	0	0	●◖	C	1961
chlorothalonil				◖	●●	0	0	●●◖	C	1964
cyazofamid (0.5)	3.8	3.8	●●	●	●●●	0	0	●●●	C	2001
fluazinam (0.4)	2.9			●	●●●	0	0	●●◖	C	1992
zoxamide + mancozeb (1.8)	2.8			●[5]	●●●	0	0	●●◖	C + C	2001
amisulbrom + mancozeb (0.5+2.0)	4.5	3.7		●	●●◖	0	?	●●●	C + C	2007
ametoctradin + mancozeb (2.5)	3.7		?[8]	?[8]	●●◖	0	0	●●●	C + C	2011
fluazinam + azoxystrobin (0.5)	3.6								C + C	2016
famoxadone + cymoxanil				●◖	●●	●●	●	●●◖	C + T	1996
(zoxamide + mancozeb) + cymoxanil (1.8+0.2)	3.4								C + T	2001
mandipropamid (0.6)	4.0		●●	●◖	●●●	●[6]	●◖	●●●	C/T	2005
mandipropamid + difenoconazole (0.6)	4.0		●●	●◖	●●●	●[6]	●◖	●●●	C/T + C	2005
benthiavalicarb (0.5)	4.2								T	2018
benthiavalicarb + mancozeb (2.0)	3.7			●◖[5]	●●●	●◖	●	●●◖	T + C	2003
cymoxanil + metiram				●◖	●●	●●	●	●●	T + C	1976
cymoxanil + copper				●◖	●●	●●	●	●●	T + C	1976
cymoxanil + mancozeb				●◖	●●	●●	●	●●	T + C	1976
dimethomorph + mancozeb (2.4)	3.0			●◖	●●◖	●	●●	●●◖	T + C	1988
dimethomorph + fluazinam (1.0)	3.7	3.3	●	●	●●◖	●	●●	●●◖	T + C	2012
fenamidone + mancozeb (1.5)	2.6			●◖[5]	●●◖	0	●◖[5]	●●	T + C	1998
(zoxamide + cymoxanil) + fluazinam (0.45+0.4)	4.0								C/T + C	2013
(zoxamide + dimethomorph) + fluazinam (1.0+0.4)	4.2								C/T + C	2015
mandipropamid + cymoxanil (0.6)	4.4		●●	●◖	●●●	●●	●◖	●●●	C/T + T	2013
(pyraclostrobin + dimethomorph) + adjuvant (2.5+1.0)	4.0[7]								C/T + T	2012
benalaxyl-M + mancozeb[2]	3.0		●●	●●	●●◖	●●◖	●●◖	●●●	S + C	1981
metalaxyl-M + mancozeb[2]			●●	●●	●●◖	●●◖	●●◖	●●●	S + C	1977
metalaxyl-M + fluazinam[2]			●●	●●	●●◖	●●◖	●●◖	●●●	S + C	
propamocarb + cymoxanil + cyazofamid ((2.0)+0.5)		4.6							S + T + C	2012
propamocarb + cymoxanil (2.0)					●◖	●●◖[9]	●●◖		S + T	2011
propamocarb-HCl + fenamidone (2.0)	2.5		●◖	●●	●●◖	●●	●●	●●●	S + T	1998
propamocarb-HCl + fluopicolide (1.6)	3.8	3.9	●●	●●	●●●	●●	●●◖	●●◖	S + C/T	2006
oxathiapiprolin (0.15)			●●◖	●●◖	●●●	●●	●●◖	●●●	S	2017
oxathiapiprolin + famoxadone (0.5)	4.9	4.1	●●◖	●●◖	●●●	●●	●●◖	●●●	S + C	2018
oxathiapiprolin + amisulbrom (0.15+0.3)	4.9								S + C	2018

[1] *Includes maneb, mancozeb, propineb and metiram.* [2] *See proceedings for comments on phenylamide resistance.* [3] *Based on EuroBlight field test in 2006-2015.* [4] *Based on EuroBlight field trials 2009-2012.* [5] *Based on limited data.* [6] *In some trials there were indications that the rating was 1½.* [7] *A provisional rating based on 5 EuroBlight experiments.* [8] *Observations from several trials indicated that both New growth and Stem blight were ++.* [9] *In some trials the curative activity was +++.*

EuroBlight
A potato late blight network for Europe

Appendix 4. List of registered fungicide formulations

Table 0.2 Fungicide formulations which are approved for use against late blight in the United Kingdom as of 2018, according to the Health and Safety Executive's Pesticides Register (https://secure.pesticides.gov.uk/pestreg/, accessed Mar. 2019).

Trade name	Active Ingredients	Active Ingredient concentration	Formulation type	Maximum individual dose	Manufacturer
Gachinko	amisulbrom	200 g L^{-1}	Soluble concentrate	0.5 L ha^{-1}	Du Pont
Leimay	amisulbrom	200 g L^{-1}	Suspension concentrate	0.5 L ha^{-1}	Syngenta
Shinkon	amisulbrom	200 g L^{-1}	Soluble concentrate	0.5 L ha^{-1}	Nissan
Resplend Percos Zampro DM	ametoctradin + dimethomorph	300 g L^{-1} 225 g L^{-1}	Suspension concentrate	0.8 L ha^{-1}	BASF
Vendetta	azoxystrobin + fluazinam	150 g L^{-1} 375 g L^{-1}	Suspension concentrate	0.5 L ha^{-1}	Headland
Galben M Intro Plus Tairel	benalaxyl + mancozeb	80 g kg^{-1} 650 g kg^{-1}	Wettable powder	2.5 kg ha^{-1}	Headland
En−garde Valbon	benthiavalicarb + mancozeb	17.5 g kg^{-1} 700 g kg^{-1}	Water dispersible granules	1.6 kg ha^{-1}	Certis
Mixanil	chlorothalonil + cymoxanil	375 g L^{-1} 50 g L^{-1}	Suspension concentrate	2 L ha^{-1}	Sipcam
Linford Swallow	cyazofamid	400 g L^{-1}	Suspension concentrate	0.35 L ha^{-1}	AgChemAccess
Ranman Top	cyazofamid	160 g L^{-1}	Suspension concentrate	0.5 L ha^{-1}	Belchim
Rithfir	cyazofamid	400 g L^{-1}	Suspension concentrate	0.35 L ha^{-1}	Du Pont
RouteOne Roazafod	cyazofamid	400 g L^{-1}	Suspension concentrate	0.35 L ha^{-1}	Albaugh Europe
Cymbal 45 Drum	cymoxanil	450 g kg^{-1}	Water dispersible granules	0.25 kg ha^{-1}	Belchim
Cymbal Flow Danso Flow Drum Flow Krug Flow	cymoxanil	225 g L^{-1}	Suspension concentrate	0.5 L ha^{-1}	Belchim
Cymostraight 45	cymoxanil	450 g kg^{-1}	Water dispersible granules	0.25 kg ha^{-1}	Belchim
Cymozeb Profilux	cymoxanil + mancozeb	45 g kg^{-1} 680 g kg^{-1}	Water dispersible granules	2.5 kg ha^{-1}	Belchim
Dauphin 45	cymoxanil	450 g kg^{-1}	Water dispersible granules	0.22 kg ha^{-1}	S.F.P.
Option	cymoxanil	600 g kg^{-1}	Water dispersible granules	0.15 kg ha^{-1}	Du Pont
Sipcam C50 WG	cymoxanil	500 g kg^{-1}	Water dispersible granules	0.24 kg ha^{-1}	Sipcam
Tanos	cymoxanil + famoxadone	250 g kg^{-1} 250 g kg^{-1}	Water dispersible granules	0.7 kg ha^{-1}	Du Pont

Table 0.2 (continued from previous page)

Trade name	Active Ingredients	Active Ingredient concentration	Formulation type	Maximum individual dose	Manufacturer
Grecale	cymoxanil + fluazinam	200 g L^{-1} 300 g L^{-1}	Suspension concentrate	0.6 L ha^{-1}	Sipcam
Kunshi Tezuma	cymoxanil + fluazinam	250 g kg^{-1} 375 g kg^{-1}	Water dispersible granules	0.5 L ha^{-1}	Belchim
Plexus	cymoxanil + fluazinam	200 g L^{-1} 300 g L^{-1}	Suspension concentrate	0.6 L ha^{-1}	Headland
Shirlan Forte	cymoxanil + fluazinam	250 g kg^{-1} 375 g kg^{-1}	Water dispersible granules	0.5 kg ha^{-1}	Syngenta
Globe Zetanil	cymoxanil + mancozeb	60 g kg^{-1} 700 g kg^{-1}	Wettable powder	1.5 kg ha^{-1}	Sipcam
Master Cyman	cymoxanil + mancozeb	50 g kg^{-1} 800 g kg^{-1}	Water dispersible granules	2 kg ha^{-1}	Generica Europa
Nautile WP Solace Max	cymoxanil + mancozeb	45.9 g kg^{-1} 650 g kg^{-1}	Wettable powder	2.25 kg ha^{-1}	U.P.L.
Palmas WP	cymoxanil + mancozeb	45 g kg^{-1} 650 g kg^{-1}	Wettable powder	2.25 kg ha^{-1}	S.F.P.
Zetanil WG	cymoxanil + mancozeb	45 g kg^{-1} 650 g kg^{-1}	Water dispersible granule	2.4 kg ha^{-1}	Sipcam
Carial Flex	cymoxanil + mandipropamid	180 g kg^{-1} 250 g kg^{-1}	Water dispersible granules	0.6 kg ha^{-1}	Syngenta
Axidor Proxanil	cymoxanil + propamocarb	50 g L^{-1} 400 g L^{-1}	Suspension concentrate	2.5 L ha^{-1}	Arysta
Lieto	cymoxanil + zoxamide	333 g kg^{-1} 333 g kg^{-1}	Water dispersible granules	0.45 kg ha^{-1}	Sipcam
Reboot	cymoxanil + zoxamide	333.3 g kg^{-1} 333.3 g kg^{-1}	Water dispersible granules	0.45 kg ha^{-1}	Gowan
Morph	dimethomorph	500 g L^{-1}	Suspension concentrate	0.3 L ha^{-1}	Adama
Murphy 500 SC	dimethomorph	500 g L^{-1}	Suspension concentrate	0.3 L ha^{-1}	Aako BV
Navio	dimethomorph	500 g kg^{-1}	Water dispersible granules	0.3 kg ha^{-1}	Headland
Hubble	dimethomorph + fluazinam	200 g L^{-1} 200 g L^{-1}	Suspension concentrate	0.75 L ha^{-1}	Adama
Saracen	dimethomorph + mancozeb	75 g kg^{-1} 667 g kg^{-1}	Water dispersible granules	2.4 kg ha^{-1}	BASF
Consento Prompto	fenamidone + propamocarb	75 g L^{-1} 375 g L^{-1}	Suspension concentrate	2 L ha^{-1}	Bayer
Boyano Ibiza 500	fluazinam	500 g L^{-1}	Suspension concentrate	0.4 L ha^{-1}	Belchim
Deltic Pro Float Tizca	fluazinam	500 g L^{-1}	Suspension concentrate	0.4 L ha^{-1}	Headland
Fluazinova	fluazinam	500 g L^{-1}	Suspension concentrate	0.4 L ha^{-1}	Barclay

Table 0.2 (continued from previous page)

Trade name	Active Ingredients	Active Ingredient concentration	Formulation type	Maximum individual dose	Manufacturer/ distributor
Frowncide Ohayo Winby	fluazinam	500 g L^{-1}	Suspension concentrate	0.4 L ha^{-1}	ISK Biosciences
Gando	fluazinam	500 g L^{-1}	Suspension concentrate	0.4 L ha^{-1}	Harvest
Legacy Shirlan	fluazinam	500 g L^{-1}	Suspension concentrate	0.4 L ha^{-1}	Syngenta
Nando 500 SC	fluazinam	500 g L^{-1}	Suspension concentrate	0.4 L ha^{-1}	Nufarm
Smash	fluazinam	500 g L^{-1}	Suspension concentrate	0.4 L ha^{-1}	RAAT
Volley	fluazinam	500 g L^{-1}	Suspension concentrate	0.4 L ha^{-1}	Adama
Zinam 500 Zinam II	fluazinam	500 g L^{-1}	Suspension concentrate	0.4 L ha^{-1}	Euro Chemicals
Infinito	fluopicolide + propamocarb	62.5 g L^{-1} 625 g L^{-1}	Suspension concentrate	1.6 L ha^{-1}	Bayer
AUK 75 WG	mancozeb	750 g kg^{-1}	Water dispersible granules	1.7 kg ha^{-1}	Goldengrass
Cleancrop Mandrake	mancozeb	750 g kg^{-1}	Water dispersible granules	2.13 kg ha^{-1}	Agrii
Clearcrop Feudal	mancozeb	750 g kg^{-1}	Water dispersible granules	2 kg ha^{-1}	Agrii
Dithane 945	mancozeb	800 g kg^{-1}	Wettable powder	2 kg ha^{-1}	Indofil
Emzeb 80 WP	mancozeb	800 g kg^{-1}	Wettable powder	2 kg ha^{-1}	Sabero
Laminator 75 WG	mancozeb	750 g kg^{-1}	Water dispersible granule	2.13 kg ha^{-1}	Interfarm
Laminator Flo	mancozeb	455 g kg^{-1}	Suspension concentrate	3.3 L ha^{-1}	Interfarm
Malvi	mancozeb	752 g kg^{-1}	Water dispersible granules	2.13 kg ha^{-1}	AgChemAccess
Manfil 75 WG	mancozeb	750 g kg^{-1}	Water dispersible granules	2.13 kg ha^{-1}	Indofil
Manfil 80 WP	mancozeb	800 g kg^{-1}	Wettable powder	2 kg ha^{-1}	Indofil
Mewati	mancozeb	800 g kg^{-1}	Wettable powder	2 kg ha^{-1}	AgChemAccess
Penncozeb 80 WP Trimanzone	mancozeb	800 g kg^{-1}	Wettable powder	1.7 kg ha^{-1}	U.P.L.
Penncozeb WDG	mancozeb	750 g kg	Water dispersible granules	1.7 kg ha^{-1}	U.P.L.
Quell Flo	mancozeb	455 g L^{-1}	Suspension concentrate	3.3 L ha^{-1}	Interfarm
Zebra WDG	mancozeb	750 g kg^{-1}	Water dispersible granules	2.13 kg ha^{-1}	Headland
Clayton Mohawk	mancozeb + metalaxyl−M	640 g kg^{-1} 38.8 g kg^{-1}	Water dispersible granules	1.9 kg ha^{-1}	Clayton
Ensis	mancozeb + metalaxyl−M	640 g kg^{-1} 38.8 g kg^{-1}	Water dispersible granules	1.9 kg ha^{-1}	AgChemAccess

Table 0.2 (continued from previous page)

Trade name	Active Ingredients	Active Ingredient concentration	Formulation type	Maximum individual dose	Manufacturer /distributor
Fubol Gold WG	mancozeb + metalaxyl−M	640 g kg^{-1} 38.8 g kg^{-1}	Water dispersible granules	1.9 kg ha^{-1}	Syngenta
Electis 75 WG Roxam 75 WG Unikat 75 WG	mancozeb + zoxamide	666 g kg^{-1} 83 g kg^{-1}	Water dispersible granules	1.8 kg ha^{-1}	Gowan
Evagio	mandipropamid	250 g L^{-1}	Suspension concentrate	0.6 L ha^{-1}	Syngenta
Mandimid	mandipropamid	250 g L^{-1}	Suspension concentrate	0.6 L ha^{-1}	Euro Chemicals
Mandoprid	mandipropamid	250 g L^{-1}	Suspension concentrate	0.6 L ha^{-1}	Generica Europa
Revus	mandipropamid	250 g L^{-1}	Suspension concentrate	0.6 L ha^{-1}	Syngenta
Standon Mandor	mandipropamid	250 g L^{-1}	Suspension concentrate	0.6 L ha^{-1}	Standon Chemicals
Amphore Plus Carial Star	mandipropamid + difenoconazole	250 g L^{-1} 250 g L^{-1}	Suspension concentrate	0.6 L ha^{-1}	Syngenta
Zorvec Enicade	oxathiapiprolin	100 g L^{-1}	Oil Dispersion		Du Pont

Appendix 5. Table of curative fungicide studies targeting *P. infestans*

Table 0.3 Summary of studies which have assessed the curative effects of fungicides against *P. infestans*.

Study	Scale	Inoculum challenge	Incubation conditions	Host (cv.)	Incubation time (hours)	Compound	Dose	Score	Control quantification
(Klopping and Delp, 1980)	Whole plants	not described	20 °C 100% RH	Solanum lycopercicum	18	cymoxanil	80 mg L^{-1} 16 mg L^{-1}	100 % [a] 79 % [a]	% control
(Cohen et al., 1995)	Whole plants	2.5 x 10^3 sporangia mL^{-1} 10 mL $plant^{-1}$	18 °C Dew chamber	*Solanum tuberosum* (cv. Alpha)	24	dimethomorph	0 µg mL^{-1} 250 µg mL^{-1} 500 µg mL^{-1} 1000 µg mL^{-1}	no effect no effect no effect no effect	Proportion of tissue showing visible symptoms
							0 µg mL^{-1} 250 µg mL^{-1} 500 µg mL^{-1} 1000 µg mL^{-1}	2.0 x 10^4 sporangia $leaflet^{-1}$ 2.8 x 10^3 sporangia $leaflet^{-1}$ 7.0 x 10^2 sporangia $leaflet^{-1}$ 4.0 x 10^2 sporangia $leaflet^{-1}$	Sporangial production after 5 days
(Komyoji et al., 1995)	Whole plants	Zoosporangia Concentraion and volume not given	20 °C moist chamber	Solanum lycopercicum (cv. Ponderosa)	6	fluazinam	500 ppm	28 %	Disease incidence as a % of control
						chlorothalonil	500 ppm	20 %	
						metalaxyl	500 ppm 125 ppm 31 ppm 8 ppm 2 ppm	100 % 100 % 84 % 84 % 12 %	
(Grayson et al., 1996) [b]	Whole plants	3.5 x 10^4 sporania mL^{-1} Volume not specified	17 °C dark	*Solanum tuberosum* (cv. Desiree)	18 – 22	dimethomorph	12.5 g ha^{-1} 25 g ha^{-1} 50 g ha^{-1} 100 g ha^{-1} 200 g ha^{-1}	29.3 % [c] 20 % [d] 21.8 % [d] 25.6 % [d] 26.8 % [d]	effect %, derived from lesion area compared with untreated controls
				`	48? 'two days'		12.5 g ha^{-1} 25 g ha^{-1} 50 g ha^{-1} 100 g ha^{-1}	59 % 4 % 13 % 17 %	

Table 0.3 (continued from previous page)

Study	Scale	Inoculum challenge	Incubation conditions	Host (cv.)	Incubation time (hours)	Compound	Dose	Score	Control quantification
					24? 'one day'	dimethomorph + mancozeb [e]	25 g ha^{-1} 200 g ha^{-1} *	28 %	
							50 g ha^{-1} 400 g ha^{-1} *	20 %	
							100 g ha^{-1} 800 g ha^{-1} *	66 %	
							200 g ha^{-1} 1600 g ha^{-1} *	19 %	
					48? 'two days'	dimethomorph + mancozeb [e]	25 g ha^{-1} 200 g ha^{-1} *	0 %	
							50 g ha^{-1} 400 g ha^{-1} *	47 %	
							100 g ha^{-1} 800 g ha^{-1} *	81 %	
							200 g ha^{-1} 1600 g ha^{-1} *	23 %	
(Johnson et al., 2000)	Detached leaves	1 x 10^4 sporangia mL^{-1} 50 µL droplet	12 h @ 18 – 20 °C mist chamber followed by glasshouse @ 23 – 27 °C day 18 – 21 °C night	*Solanum tuberosum* (cv. Russet Rurbank)	12	control	no a.i.	83 % [a]	Incidence of disease (percentage leaves infected)
						chlorothalonil [e]	2.4 g L^{-1}	75 % [a]	
						propamocarb + chlorothalonil [e]	2.5 g L^{-1} 2.5 g L^{-1}	50 % [a]	
						cymoxanil + mancozeb [e]	0.37 g L^{-1} 3.7 g L^{-1}	8 % [a]	
						dimethomorph + mancozeb [e]	0.6 g L^{-1} 4 g L^{-1}	0 % [a]	

Table 0.3 (continued from previous page)

Study	Scale	Inoculum challenge	Incubation conditions	Host (cv.)	Incubation time (hours)	Compound	Dose	Score	Control quantification
						control	no a.i.	36 % [a]	Disease severity (Proportion of tissue showing visible symptoms)
						chlorothalonil [e]	2.4 g L^{-1}	28 % [a]	
						propamocarb + chlorothalonil [e]	2.5 g L^{-1} 2.5 g L^{-1}	2 % [a]	
						cymoxanil + mancozeb [e]	0.37 g L^{-1} 3.7 g L^{-1}	0 % [a]	
						dimethomorph + mancozeb [e]	0.6 g L^{-1} 4 g L^{-1}	0 % [a] 35 % [a]	
						control	no a.i.	1.9 x 10^4 sporangia cm^{-2} [a]	Sporangial production
						chlorothalonil [e]	2.4 g L^{-1}	2.2 x 10^4 sporangia cm^{-2} [a]	
						propamocarb + chlorothalonil [e]	2.5 g L^{-1} 2.5 g L^{-1}	0 sporangia cm^{-2} [a]	
						cymoxanil + mancozeb [e]	0.37 g L^{-1} 3.7 g L^{-1}	0 sporangia cm^{-2} [a]	
						dimethomorph + mancozeb [e]	0.6 g L^{-1} 4 g L^{-1}	0 sporangia cm^{-2} [a]	
					24	chlorothalonil [e]	2.4 g L^{-1}	92 % [a]	Incidence of disease (percentage leaves infected)
						propamocarb + chlorothalonil [e]	2.5 g L^{-1} 2.5 g L^{-1}	26 % [a]	
						cymoxanil + mancozeb [e]	0.37 g L^{-1} 3.7 g L^{-1}	0 % [a]	
						dimethomorph + mancozeb [e]	0.6 g L^{-1} 4 g L^{-1}	68 % [a]	

Table 0.3 (continued from previous page)

Study	Scale	Inoculum challenge	Incubation conditions	Host (cv.)	Incubation time (hours)	Compound	Dose	Score	Control quantification
						chlorothalonil [e]	2.4 g L^{-1}	37 % [a]	Disease severity (Proportion of tissue showing visible symptoms)
						propamocarb + chlorothalonil [e]	2.5 g L^{-1} 2.5 g L^{-1}	2 % [a]	
						cymoxanil + mancozeb [e]	0.37 g L^{-1} 3.7 g L^{-1}	0 % [a]	
						dimethomorph + mancozeb [e]	0.6 g L^{-1} 4 g L^{-1}	26 % [a]	
						chlorothalonil [e]	2.4 g L^{-1}	1.6 x 10^4 sporangia cm^{-2} [a]	Sporangial production
						propamocarb + chlorothalonil [e]	2.5 g L^{-1} 2.5 g L^{-1}	0 sporangia cm^{-2} [a]	
						cymoxanil + mancozeb [e]	0.37 g L^{-1} 3.7 g L^{-1}	0 sporangia cm^{-2} [a]	
						dimethomorph + mancozeb [e]	0.6 g L^{-1} 4 g L^{-1}	1.1 x 10^4 sporangia cm^{-2} [a]	
					36	chlorothalonil [e]	2.4 g L^{-1}	100 % [a]	Incidence of disease (percentage leaves infected)
						propamocarb + chlorothalonil [e]	2.5 g L^{-1} 2.5 g L^{-1}	68 % [a]	
						cymoxanil + mancozeb [e]	0.37 g L^{-1} 3.7 g L^{-1}	18 % [a]	
						dimethomorph + mancozeb [e]	0.6 g L^{-1} 4 g L^{-1}	76 % [a]	
						chlorothalonil [e]	2.4 g L^{-1}	48 % [a]	Disease severity (Proportion of tissue showing visible symptoms)
						propamocarb + chlorothalonil [e]	2.5 g L^{-1} 2.5 g L^{-1}	14 % [a]	
						cymoxanil + mancozeb [e]	0.37 g L^{-1} 3.7 g L^{-1}	3 % [a]	
						dimethomorph + mancozeb [e]	0.6 g L^{-1} 4 g L^{-1}	25 % [a]	

Table 0.3 (continued from previous page)

Study	Scale	Inoculum challenge	Incubation conditions	Host (cv.)	Incubation time (hours)	Compound	Dose	Score	Control quantification
						chlorothalonil [e]	2.4 g L^{-1}	1.2 x 10^4 sporangia cm^{-2} [a]	Sporangial production
						propamocarb + chlorothalonil [e]	2.5 g L^{-1} 2.5 g L^{-1}	1.0 x 10^3 sporangia cm^{-2} [a]	
						cymoxanil + mancozeb [e]	0.37 g L^{-1} 3.7 g L^{-1}	1.5 x 10^3 sporangia cm^{-2} [a]	
						dimethomorph + mancozeb [e]	0.6 g L^{-1} 4 g L^{-1}	7.0 x 10^3 sporangia cm^{-2} [a]	
					48	chlorothalonil [e]	2.4 g L^{-1}	78 % [a]	Incidence of disease (percentage leaves infected)
						propamocarb + chlorothalonil [e]	2.5 g L^{-1} 2.5 g L^{-1}	42 % [a]	
						cymoxanil + mancozeb [e]	0.37 g L^{-1} 3.7 g L^{-1}	94 % [a]	
						dimethomorph + mancozeb [e]	0.6 g L^{-1} 4 g L^{-1}	68 % [a]	
						chlorothalonil [e]	2.4 g L^{-1}	25 % [a]	Disease severity (Proportion of tissue showing visible symptoms)
						propamocarb + chlorothalonil [e]	2.5 g L^{-1} 2.5 g L^{-1}	5 % [a]	
						cymoxanil + mancozeb [e]	0.37 g L^{-1} 3.7 g L^{-1}	25 % [a]	
						dimethomorph + mancozeb [e]	0.6 g L^{-1} 4 g L^{-1}	31 % [a]	
						chlorothalonil [e]	2.4 g L^{-1}	2.7 x 10^4 sporangia cm^{-2} [a]	Sporangial production
						propamocarb + chlorothalonil [e]	2.5 g L^{-1} 2.5 g L^{-1}	4.5 x 10^3 sporangia cm^{-2} [a]	
						cymoxanil + mancozeb [e]	0.37 g L^{-1} 3.7 g L^{-1}	1.1 x 10^4 sporangia cm^{-2} [a]	
						dimethomorph + mancozeb [e]	0.6 g L^{-1} 4 g L^{-1}	1.65 x 10^4 sporangia cm^{-2} [a]	

Table 0.3 (continued from previous page)

Study	Scale	Inoculum challenge	Incubation conditions	Host (cv.)	Incubation time (hours)	Compound	Dose	Score	Control quantification
(Genet et al., 2001)	Leaf discs	2 x 10^4 sporangia mL^{-1} volume not specified	17 °C 'moist chamber'	*Solanum tuberosum* (cv. Bintje)	6	cymoxanil	300 mg L^{-1}	100 % [a]	% disease control (proportion of discs on which lesion develop)
					24			86 % [a]	
					36			82 % [a]	
					48			24 % [a]	
			20 °C		6			100 % [a]	
					24			72 % [a]	
					36			48 % [a]	
					48			12 % [a]	
	Whole plants		16 °C 18 h photoperiod 'dew room'		24? ('1 day')	cymoxanil	0.36 g L^{-1}	2 % [a]	% infected leaf area after seven days.
						propamocarb + chlorothalonil [e]	3.375 g L^{-1} 3.375 g L^{-1}	30 % [a]	
						dimethomorph + mancozeb [e]	0.6 g L^{-1} 4 g L^{-1}	96 % [a] 38 % [a]	
					48? ('2 days')	cymoxanil	0.36 g L^{-1}	38 % [a]	
						propamocarb + chlorothalonil [e]	3.375 g L^{-1} 3.375 g L^{-1}	82 % [a]	
						dimethomorph + mancozeb [e]	0.6 g L^{-1} 4 g L^{-1}	98 % [a]	
					72? ('3 days')	cymoxanil	0.36 g L^{-1}	100 % [a]	
						propamocarb + chlorothalonil [e]	3.375 g L^{-1} 3.375 g L^{-1}	100 % [a]	
						dimethomorph + mancozeb [e]	0.6 g L^{-1} 4 g L^{-1}	100 % [a]	

Table 0.3 (continued from previous page)

Study	Scale	Inoculum challenge	Incubation conditions	Host (cv.)	Incubation time (hours)	Compound	Dose	Score	Control quantification
			20 °C		24? ('1 day')	cymoxanil	0.36 g L^{-1}	40 % [a]	
						propamocarb + chlorothalonil	3.375 g L^{-1} 3.375 g L^{-1}	100 % [a]	
						dimethomorph + mancozeb [e]	0.6 g L^{-1} 4 g L^{-1}	100 % [a]	
					48? ('2days')	cymoxanil	0.36 g L^{-1}	60 % [a]	
						propamocarb + chlorothalonil [e]	3.375 g L^{-1} 3.375 g L^{-1}	100 % [a]	
						dimethomorph + mancozeb [e]	0.6 g L^{-1} 4 g L^{-1}	100 % [a]	
					72? ('3 days')	cymoxanil	0.36 g L^{-1}	100 % [a]	
						propamocarb + chlorothalonil [e]	3.375 g L^{-1} 3.375 g L^{-1}	100 % [a]	
						dimethomorph + mancozeb [e]	0.6 g L^{-1} 4 g L^{-1}	100 % [a]	
	Detached leaves	1 x 10^3 per inoculation	14.4°C (mean)	*Solanum tuberosum* (King Edward)	48? ('2 days')	control	no a.i.	100 % [a]	% infection based on the presence of sporulating lesions.
						cymoxanil + mancozeb [e]	0.3 g L^{-1} 4.53 g L^{-1}	62 % [a]	
						dimethomorph + mancozeb [e]	0.5 g L^{-1} 4.53 g L^{-1}	88 % [a]	
					72? ('3 days')	control	no a.i.	68 % [a]	
						cymoxanil + mancozeb [e]	0.3 g L^{-1} 4.53 g L^{-1}	12 % [a]	
						dimethomorph + mancozeb [e]	0.5 g L^{-1} 4.53 g L^{-1}	56 % [a]	

Table 0.3 (continued from previous page)

Study	Scale	Inoculum challenge	Incubation conditions	Host (cv.)	Incubation time (hours)	Compound	Dose	Score	Control quantification
					120? ('5 days')	control	no a.i.	100 % [a]	
						cymoxanil + mancozeb [e]	0.3 g L^{-1} 4.53 g L^{-1}	90 % [a]	
						dimethomorph + mancozeb [e]	0.5 g L^{-1} 4.53 g L^{-1}	84 % [a]	
					168? (' 7 days')	control	no a.i.	100 % [a]	
						cymoxanil + mancozeb [e]	0.3 g L^{-1} 4.53 g L^{-1}	84 % [a]	
						dimethomorph + mancozeb [e]	0.5 g L^{-1} 4.53 g L^{-1}	100 % [a]	
(Mayton et al., 2001)	Detached leaves	2 x 10^2 zoospores 10 µL droplet	10 °C 14 h photoperiod sealed water-agar plates	*Solanum tuberosum* (cv. Frito-Lay-cv-1607)	8	cymoxanil	112 g ha-1	0 % [a]	lesion area as % of control
					24			0 % [a]	
					48			0 % [a]	
					96			25 % [a]	
			16 °C		8			0 % [a]	
					24			0 % [a]	
					48			30 % [a]	
					96			80 % [a]	
			24 °C		8			0 % [a]	
					24			40 % [a]	
					48			115 % [a]	
					96			120 % [a]	

Table 0.3 (continued from previous page)

Study	Scale	Inoculum challenge	Incubation conditions	Host (cv.)	Incubation time (hours)	Compound	Dose	Score	Control quantification
			10 °C		8			0 % [a]	sporulating area as % of control
					24			0 % [a]	
					48			0 % [a]	
					96			4 % [a]	
			16 °C		8			0 % [a]	
					24			0 % [a]	
					48			20 % [a]	
					96			20 % [a]	
			24 °C		8			0 % [a]	
					24			80 % [a]	
					48			30 % [a]	
					96			90 % [a]	
(Miyake et al., 2005)	Whole plants	1 x 10^4 sporangia mL^{-1} Volume not specified	20 °C Dark 100% RH	Solanum lycopercium (cv. Ponderosa)	12	benthiavalicarb	0.3 µg mL^{-1}	38 % [a]	Percentage control (disease index based on lesion size)
							1 µg mL^{-1}	58 % [a]	
							3 µg mL^{-1}	95 % [a]	
							10 µg mL^{-1}	100 % [a]	
							30 µg mL^{-1}	100 % [a]	
							100 µg mL^{-1}	100 % [a]	
					24		0.3 µg mL^{-1}	35 % [a]	
							1 µg mL^{-1}	56 % [a]	
							3 µg mL^{-1}	64 % [a]	
							10 µg mL^{-1}	62 % [a]	
							30 µg mL^{-1}	68 % [a]	
							100 µg mL^{-1}	72 % [a]	
(Cho et al., 2007)	Whole plants	not described	not described	Solanum lycopercicum	24	cymoxanil	50 mg L^{-1}	100 %	'control value'

Table 0.3 (continued from previous page)

Study	Scale	Inoculum challenge	Incubation conditions	Host (cv.)	Incubation time (hours)	Compound	Dose	Score	Control quantification
(Cohen and Gisi, 2007)	Detached leaves	Concentration not specified 10 µL droplets	20 °C 12 h photoperiod 60 – 70 % RH	*Solanum tuberosum* (cv. Bintje)	24	mandipropamid	0 µg mL^{-1}	3.7 x 10^4 sporangia leaflet^{-1} [a]	Sporangial production after 4 days
							1 µg mL^{-1}	3.2 x 10^4 sporangia leaflet^{-1} [a]	
							10 µg mL^{-1}	2.7 x 10^4 sporangia leaflet^{-1} [a]	
							100 µg mL^{-1}	2.5 x 10^4 sporangia leaflet^{-1} [a]	
							500 µg mL^{-1}	1.9 x 10^4 sporangia leaflet^{-1} [a]	
					48		0 µg mL^{-1}	no effect	
							1 µg mL^{-1}	no effect	
							10 µg mL^{-1}	no effect	
							100 µg mL^{-1}	no effect	
							500 µg mL^{-1}	no effect	
					24	dimethomorph	0 µg mL^{-1}	3.7 x 10^4 sporangia leaflet^{-1} [a]	
							1 µg mL^{-1}	3.2 x 10^4 sporangia leaflet^{-1} [a]	
							10 µg mL^{-1}	1.1 x 10^4 sporangia leaflet^{-1} [a]	
							100 µg mL^{-1}	5.0 x 10^2 sporangia leaflet^{-1} [a]	
							500 µg mL^{-1}	0.0 sporangia leaflet^{-1} [a]	
					48		0 µg mL^{-1}	no effect	
							1 µg mL^{-1}	no effect	
							10 µg mL^{-1}	no effect	
							100 µg mL^{-1}	no effect	
							500 µg mL^{-1}	no effect	
				Solanum lycopercicum (cv. Baby)	24	mandipropamid	0 µg mL^{-1}	3.5 x 10^4 sporangia leaflet^{-1} [a]	Sporangial production after 4 days
							1 µg mL^{-1}	2.9 x 10^4 sporangia leaflet^{-1} [a]	
							10 µg mL^{-1}	1.6 x 10^4 sporangia leaflet^{-1} [a]	
							100 µg mL^{-1}	1.5 x 10^4 sporangia leaflet^{-1} [a]	
							500 µg mL^{-1}	1.2 x 10^4 sporangia leaflet^{-1} [a]	
					48		0 µg mL^{-1}	no effect	
							1 µg mL^{-1}	no effect	
							10 µg mL^{-1}	no effect	
							100 µg mL^{-1}	no effect	
							500 µg mL^{-1}	no effect	
					24	dimethomorph	0 µg mL^{-1}	3.5 x 10^4 sporangia leaflet^{-1} [a]	
							1 µg mL^{-1}	1.3 x 10^4 sporangia leaflet^{-1} [a]	
							10 µg mL^{-1}	3.0 x 10^3 sporangia leaflet^{-1} [a]	
							100 µg mL^{-1}	5.0 x 10^2 sporangia leaflet^{-1} [a]	
							500 µg mL^{-1}	5.0 x 10^2 sporangia leaflet^{-1} [a]	
					48		0 µg mL^{-1}	no effect	
							1 µg mL^{-1}	no effect	
							10 µg mL^{-1}	no effect	

Study	Scale	Inoculum challenge	Incubation conditions	Host (cv.)	Incubation time (hours)	Compound	Dose	Score	Control quantification
							100 µg mL^{-1} 500 µg mL^{-1}	no effect no effect	
(Bugiani et al., 2010)	Whole plants	1.8 x 10^{3} sporangia mL^{-1}	18 °C sealed within plastic bags	Solanum lycopercicum (cv. UC82B)	24	mandipropamid + copper oxychloride [e]	0.00125 g ha^{-1} 0.06975 g ha^{-1}	40 % [a]	% of leaves on plant affected
						metalaxyl-M + copper oxychloride [e]	0.0096 g ha^{-1} 0.16 g ha^{-1}	42 % [a]	
						propamocarb	0.1624 g ha^{-1}	88 % [a]	
						fluopicolide [e] + propamocarb	0.01 g ha^{-1} 0.1 g ha^{-1}	48 % [a]	
						cyazofamid	0.008 g ha^{-1}	12 % [a]	
						dimethomorph + copper oxychloride [e]	0.00195 g ha^{-1} 0.013 g ha^{-1}	35 % [a]	
						ipovalicarb + copper oxychloride [e]	0.00147 g ha^{-1} 0.007105 g ha^{-1}	17 % [a]	
						cymoxanil + copper oxychloride [e]	0.00126 g ha^{-1} 0.011925 g ha^{-1}	22 %	
					48	mandipropamid + copper oxychloride [e]	0.00125 g ha^{-1} 0.06975 g ha^{-1}	65 % [a]	
						metalaxyl-M + copper oxychloride [e]	0.0096 g ha^{-1} 0.16 g ha^{-1}	40 % [a]	
						propamocarb	0.1624 g ha^{-1}	98 % [a]	
						fluopicolide [e] + propamocarb	0.01 g ha^{-1} 0.1 g ha^{-1}	94 % [a]	
						cyazofamid	0.008 g ha^{-1}	57 % [a]	
						dimethomorph + copper oxychloride [e]	0.00195 g ha^{-1} 0.013 g ha^{-1}	30 % [a]	
						ipovalicarb + copper oxychloride [e]	0.00147 g ha^{-1} 0.007105 g ha^{-1}	46 % [a]	
						cymoxanil + copper oxychloride [e]	0.00126 g ha^{-1} 0.011925 g ha^{-1}	42 % [a]	

Table 0.3 (continued from previous page)

Study	Scale	Inoculum challenge	Incubation conditions	Host (cv.)	Incubation time (hours)	Compound	Dose		Score	Control quantification
					72? ('3 days')	mandipropamid + copper oxychloride [e]	0.00125 0.06975	g ha^{-1} g ha^{-1}	no effect	
						metalaxyl-M + copper oxychloride [e]	0.0096 0.16	g ha^{-1} g ha^{-1}	no effect	
						propamocarb	0.1624	g ha^{-1}	no effect	
						fluopicolide [e] + propamocarb	0.01 0.1	g ha^{-1} g ha^{-1}	no effect	
						cyazofamid	0.008	g ha^{-1}	no effect	
						dimethomorph + copper oxychloride [e]	0.00195 0.013	g ha^{-1} g ha^{-1}	no effect	
						ipovalicarb + copper oxychloride [e]	0.00147 0.007105	g ha^{-1} g ha^{-1}	no effect	
						cymoxanil + copper oxychloride [e]	0.00126 0.011925	g ha^{-1} g ha^{-1}	no effect	

Table 0.3 (continued from previous page)

Study	Scale	Inoculum challenge	Incubation conditions	Host (cv.)	Incubation time (hours)	Compound	Dose	Score	Control quantification
(Pirondi et al., 2017)	Whole plants	2 x 10^4 zoosporangia 'to run off'	13 – 15 h @ 10 °C dark 100 % RH 16 – 21°C 12 h photoperiod 70 % RH	Solanum lycopercicum (cv. Marmande)	24	control	no a.i.	97.2 %	% disease severity, i.e. sporulating tissue.g
						metalaxyl-M + mancozeb [e]	0.0975 g L^{-1} 1.6 g L^{-1}	0.07 %	
						mandipropamid	0.15 g L^{-1}	44.81 %	
						dimethomorph	0.25 g L^{-1} 0.18 g L^{-1}	16.83 % 59.04 %	
						iprovalicarb + mancozeb [e]	0.15 g L^{-1} 1.5 g L^{-1}	75.79 %	
						bethiavalicarb + mancozeb [e]	0.035 g L^{-1} 1.4 g L^{-1}	44.58 %	
						dimethomorph + ametoctradin	0.18 g L^{-1} 0.24 g L^{-1}	30.02 %	
						dimethomorph + pyraclostrobin	0.18 g L^{-1} 0.1 g L^{-1}	2.92 %	
						pyraclostrobin	0.1 g L^{-1}	74.95 %	
						azoxystrobin	0.25 g L^{-1}	26.82 %	
						ametoctradin	0.24 g L^{-1}	85.88 %	
						cyazofamid	0.08 g L^{-1}	56.1 %	
						cymoxanil	0.2 g L^{-1} 0.125 g L^{-1}	0.31 % 0.27 %	
						propamocarb + cymoxanil	1 g L^{-1} 0.125 g L^{-1}	0.93 %	
						propamocarb	1 g L^{-1} 1.334 g L^{-1}	42.97 % 78.92 %	
						fosetyl-Al [h] + propamocarb	1.805 g L^{-1} 0.775 g L^{-1}	14.63 % 24.43 %	
						fosetyl-Al [h]	1.334 g L^{-1} 2 g L^{-1}	59.72 %	

Study	Scale	Inoculum challenge	Incubation conditions	Host (cv.)	Incubation time (hours)	Compound	Dose	Score	Control quantification
							0.775 g L^{-1}	71.22 %	

a Values approximate. Transcribed from graphic.
b Study also demonstrated improved curativity when fungicide was combined with an adjuvant.
c Mean of three runs at the same concentration. These were presented individually in the original report.
d Mean of five runs at the same concentration. These were presented individually in the original report.
e Not generally considered to act as a curative compound against *P. infestans*.
f Study assessed *Myristica fragrans* (Nutmeg) extract, which had no curative properties against *P. infestans*.
g Symptoms were assessed at three time-points, the central observations are included in this table.
h No current UK products approved for treatment of field potatoes contain this active.
***** Concentration not explicitly stated, inferred from materials and methods.