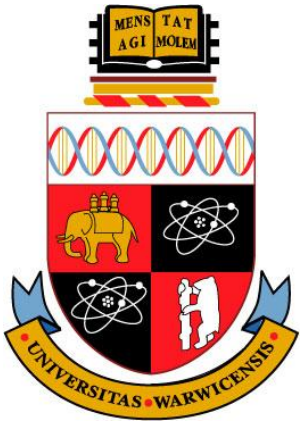
Implications of new sustainable greenhouse systems for pests, diseases and biological control: A modelling approach using *Oidium neolycopersici* and *Tetranychus urticae*

Sacha Daniel White



A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Plant and Environmental Sciences

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Acknowledgements

R M Pirsig once said that quality is something you see out of the corner of your eye. In which case, I suggest reading this thesis at a distinct angle. Whether or not this work contains quality is for others to decide, but what there is here is in no small part due to the contributions of a number of people.

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Abstract

Concerns regarding carbon emissions, increasing demands on water supplies and environmental pollution have meant that the European protected horticulture industry is being challenged to develop more sustainable greenhouse climate management systems. These new systems can however potentially impact on pest and disease (P & D) pressures and the efficacy of biological control agents (BCAs). This thesis aimed to use a combination of experimental work and simulation models to compare novel and traditional greenhouse climate management scenarios in Spain and the Netherlands using two model P & D systems. These were *Oidium neolycopersici* (powdery mildew) and its BCA, *Bacillus subtilis*, on tomato, and *Tetranychus urticae* (the two-spotted spider mite) and its BCA, *Phytoseiulus persimilis*, on ornamentals.

Experiments showed that latent period, disease development and sporulation of *Oidium neolycopersici* were strongly influenced by temperatures between 10-33°C and that the control efficacy of *B. subtilis* was significantly influenced by temperature and humidity in the ranges 10-33°C and 50-95% RH. The functional response of *P. persimilis* was found to be significantly affected by ambient humidities of 57-99% RH, with predation highest at 85% RH and lowest below 76% RH. These results, in combination with existing data, were used to construct dynamic P & D models.

A greenhouse climate model, based on observed temperatures in European greenhouses, was constructed to provide data on the diurnal and seasonal variation in temperature and humidity for different climate management scenarios. The predictions from the P & D models allowed climate control regimes in different greenhouses in Spain and the Netherlands to be identified, which minimised P & D pressures and maximised the efficacy of the BCAs. The implications of these findings for greenhouse climate management are discussed.

1. Introduction

1.1 Challenges for protected horticulture

Concerns over the environmental impacts and increasing energy costs of protected horticulture have meant that the development of more sustainable production systems has become a research priority. The European greenhouse industry produces a wide range of high quality vegetables, fruit and ornamentals, making important contributions to healthy diets and producer economies. Greenhouse production allows crops to be grown in areas that would normally be impractical and makes use of land that would otherwise be unproductive for other forms of agriculture (Wittwer and Castilla, 1995). Greenhouse structures allow year round cultivation, high yields per hectare and protection from, and improvement of, environmental conditions (Wittwer and Castilla, 1995), as well as conferring improved protection from pests and diseases, decreased pesticide residues on the product and increased production reliability (Bakker and Challa, 1995; EUphoros, 2007). Furthermore, they facilitate more efficient use of resources such as water, fertilisers and soil than open-field production (Jensen, 2002; EUphoros, 2007).

For all the benefits of greenhouse production, environmental impacts are apparent, especially in terms of high fossil energy inputs, chemical application and water usage. Limited water resources are a major problem for protected horticulture in the Mediterranean (Zaragoza *et al.*, 2007). For instance, although the Almeria region of Spain experiences an average of just 226 mm of annual rainfall (Climatemp.info, 2011), it has the largest concentration of greenhouse production in the world (27000 ha; Aznar-Sanchez *et al.*, 2011), an industry that obtains 89% of the water needed for tomato production from groundwater sources (Chapagain and Orr, 2009). This reliance on shared water resources has important impacts on aquifers, river flow, aquatic ecosystems and building infrastructure (MacMillan *et al.*, 2011).

Energy demands vary regionally, with the highest being in Northern Europe, e.g., 1900 MJ m^{-2} in Finland and 1500 MJ m^{-2} in the Netherlands (Bakker, 2009). However, usage can still be substantial in warm winter areas, e.g., 127 GJ ha^{-1} for tomato production in the Antalya region of Turkey, an area that accounts for just

5.8% of total greenhouse production in the Mediterranean (Ozkan *et al.*, 2004). High energy use has consequences for the profitability of the industry especially when energy costs are high (Bakker *et al.*, 2008; Bakker, 2009) but also has wider environmental implications in regards CO_2 emissions, being the most important greenhouse gas contributing to global warming (Lashof and Ahuja, 1990; Hofmann *et al.*, 2006). Governmental and inter-governmental emission targets (e.g., EC, 2001; EC, 2011) have added impetus to the development of energy-saving measures.

The greenhouse industry has also faced a number of drivers to reduce pesticide usage including target resistance (Hajek, 2004), interference with other control agents (Blümel *et al.*, 1999), product residues (Hamilton *et al.*, 2004), environmental impacts (Leistra and Boesten, 1989) and worker exposure (Maroni and Fait, 1993; Bassil *et al.*, 2007). A result of these issues, and a further challenge to growers, is the de-registration of a number of plant protective chemicals and the regulated use of others (Gullino and Kuijpers, 1994; Finizio and Villa, 2002; Hillocks, 2012) due to governmental and inter-governmental regulations (e.g., EU Directive 91/414 and Regulation 396/2005). Nevertheless, it is estimated that 31 kg/ha of plant-protection chemicals are applied each year to greenhouses in north-western Europe, with those in Mediterranean countries at least as high (EUphoros, 2007).

The area of crop grown under protected horticulture worldwide is estimated at approximately 2 million ha with China accounting for 1.6 million ha (Van Lenteren, 2006; Jiang and Yu, 2008), representing a rapid increase in greenhouse production (Gullino *et al.*, 1999). Europe has 150000 ha of greenhouse (EFSA, 2010) and has also experienced recent increases in coverage, particularly in the Mediterranean (Sigrimis, 2009) and Eastern EU countries (Sas Paszt, 2009). These have mainly been of plastic-covered high tunnel greenhouses, which in themselves add to environmental pollution with dumping of used plastics common (Aznar-Sanchez *et al.*, 2011).

In consideration of both the increasing size of the industry and its environmental impacts it is pertinent to develop more sustainable production systems. Sustainable development has been defined as "that which meets the needs of the present generation without compromising the ability of the future generations to meet their own needs" (WCED, 1987). To this end a number of studies have been undertaken

to improve the situation in Europe (Stanghellini *et al.*, 2003; de Pascale and Maggio, 2004; Giacomelli *et al.*, 2008; Korner *et al.*, 2008). EUphoros is an EU funded project that is a continuation of this research direction. It aims to develop sustainable, low-input greenhouse systems that minimise fossil fuel, water and chemical use through the design of novel technologies, structures and pest control strategies. A key component of the project is the development of novel climate control strategies, such as adapting closed-system climate control for mild winter areas. In northern Europe, this climate control approach has been shown to be capable of 30-35% reductions in energy use and pesticide reductions while increasing yields (Opdam *et al.*, 2005; Heuvelink *et al.*, 2008; UN, 2008).

While the development of greenhouse systems that make more efficient use of energy and water inputs is important, it is also judicious to consider the consequences novel systems may have for pest and disease pressures. In fact, it has long been recognised that the design of greenhouse structures and systems should take into account the requirements for non-chemical pest and disease control (Jarvis, 1989) and that climate control in particular presents an important tool in this regard (van Lenteren and Woets, 1988; van Straten and Challa, 1995; van Lenteren, 2000; Hanafi, 2003; Pilkington *et al.*, 2010). With this in mind, the work described in this thesis aimed to evaluate the impacts of these novel climate control systems on pest and disease pressures and biological control through a combination of experimental work and simulation modelling. Simulation models are a useful tool for "fine-tuning" integrated pest management (IPM) programs, allowing optimal control strategies and the effects of climate to be investigated (van Lenteren and Woets, 1988).

IPM and biological control are vital components of greenhouse production across Europe (Parrella *et al.*, 1999; Pilkington *et al.*, 2010; Chandler *et al.*, 2011) with 90% of Dutch tomatoes produced under IPM (van Lenteren, 2000) and 20000 ha of Spanish greenhouses regularly under biological control (Pilkington *et al.*, 2010). Implementing biological control has resulted in marked reductions in pesticide applications (Parrella, 2008) and allows growers access to high volume markets, such as supermarkets (Hillocks, 2012), and premium 'eco-label' prices (Vannoppen *et al.*, 2001; Stanghellini *et al.*, 2003). Furthermore, the continuing withdrawal of

pesticides from use in the EU has increased the importance for crop protection of IPM (Birch and Begg, 2010; Hillocks, 2012).

However, biological control faces a number of challenges. For instance, the lack of financial incentives and increasing unease regarding the use of non-indigenous organisms is hindering the development of new biological control agents (Parrella, 2008). The suppression of pest and disease populations can taken longer with biological control than chemical control, although criticisms that biological control is less effective or reliable have been rejected (Bale *et al.*, 2008). Furthermore, while biological control is widely used in vegetable and fruit production, it is yet to be commonly employed in the floriculture industry due to very low tolerance to pest presence or damage (Sadof and Alexander, 1993; Alatawi *et al.*, 2007; Marsh and Gallardo, 2009). Therefore, optimising the efficacy of currently available biological control agents is a priority; both in terms of application strategies and providing conditions ideal for activity. Climate control has been shown to be an important tool in IPM programs; minimising pest and disease pressures (Tantau and Lange, 2003; Korner and Jakobsen, 2006) as well as improving biological control performance (Shtienberg and Elad, 1997; Romero *et al.*, 2007).

Two crop systems were evaluated in the project; Dutch and Spanish production of tomato (*Lycopersicon esculentum*) and ornamentals such as rose (*Rosa* sp.). Spain has 66000 ha of predominantly plastic-covered high tunnel greenhouses (EFSA, 2010) while the Netherlands has 10000 ha of high technology glasshouses (EFSA, 2010) that generated €58 billion in agricultural exports in 2007 or 17% of the country's total exported goods and services but also 80% of the energy used in the agricultural sector (UN, 2008). As a comparison, the UK had 691 ha of protected vegetable production in 2010, although this was worth £116 million (DEFRA, 2011a; DEFRA, 2011b).

The European tomato export market is disproportionately large, commanding 68% of total global tomato exports between 1993 and 1995 while producing only 24% of the total global crop (Wijnands, 2003). In 2010 Spain, the Netherlands and the UK produced 4.3 million, 764000 and 90000 tonnes respectively (EC, 2012; FAO, 2012) with the different production types all contributing significant though differing environmental impacts (Boulard *et al.*, 2011). The European protected ornamental

market is also significant with over 300 potted plant and 100 cut flower species grown (van Lenteren, 2000). In the Netherlands exports were worth $6.28 billion in 2006 (Dons and Bino, 2008) while the UK industry was worth £340 million in 2010 (DEFRA, 2011a). *Rosa* species make up an important part of this market, which is characterised, particularly in northern Europe, by high value and productivity levels with extensive research and technological investment (Zieslin, 1996).

Two representative pest/disease and biological control systems were chosen for this study. The first was the powdery mildew disease caused by *Oidium neolycopersici* L. Kiss and the biological control agent, *Bacillus subtilis*, on tomato. The second was the mite pest, *Tetranychus urticae* Koch, and the predatory mite, *Phytoseiulus persimilis* Athias Henriot, on ornamentals.

O. neolycopersici was selected as it is a relatively new tomato disease that causes reductions in fruit quality and size, and poses a significant threat to the greenhouse tomato industry (Jones *et al.*, 2001). Studying this pathogen also provides an opportunity to investigate a newly registered biological control agent, *B. subtilis*, with reported activity against powdery mildew in various crops (Edgecomb and Manker, 2006).

T. urticae is the most important mite pest in many ornamental crops, especially rose (Van de Vrie, 1985; Landeros *et al.*, 2004), and their presence and feeding damage causes significant economic losses to growers (Sadof and Alexander, 1993; Alatawi *et al.*, 2007; Marsh and Gallardo, 2009). *P. persimilis* is probably the most commonly used biological control for *T. urticae* (McMurtry and Croft, 1997) and, as the first biological control agent trialled in greenhouses (Gerson and Weintraub, 2012), a considerable amount of information is available on its efficacy and behaviour.

1. 2 The powdery mildews

Powdery mildew diseases are caused by a number of different plant pathogenic fungi and affect nearly 10000 hosts. They are considered to be "some of the world's most frequently encountered plant pathogens" (Glawe, 2008) and along with *Botrytis*

cinerea, cause some of the most important diseases in greenhouses (Elad *et al.*, 1996). The fungi causing powdery mildews are obligate biotrophs and are defined by Yarwood (1978) as having "white superficial hyphae on the aerial parts of living plants, with large one-celled conidia produced terminally on isolated aerial unbranched conidiophores and with haustoria in the epidermal cells of their hosts". Belonging to the order Erysiphales of the Ascomycota phylum they are notable for an ability to infect hosts without the need for surface water (Yarwood, 1978; Jarvis *et al.*, 2002), unlike other plant diseases, although this is not the case for all species (Mieslerova and Lebeda, 1999). It has been reported that in some species germination can occur at extremely low humidities, including over concentrated sulphuric acid (Yarwood, 1978), and they are generally considered to be more virulent in dry conditions (Butt, 1978). The capacity to germinate in dry conditions is thought to be due to the conidia either having a high osmotic pressure (allowing the absorption of atmospheric moisture), having high water and lipid contents (respectively providing the moisture necessary for germination and preventing desiccation) or respiring oils to yield water (Yarwood, 1978). Control of powdery mildews is the primary reason for fungicide application in Western Europe (Hewitt, 1998) and annual expenditures on the application and development of fungicides and resistant varieties are "immense" (Glawe, 2008).

1. 2. 1 *Oidium neolycopersici*: taxonomy and distribution

O. neolycopersici (tribe Erysiphaceae) is one of three pathogens that cause powdery mildew disease of tomato, the others being *Leveillula taurica* and *O. lycopersici*. It can be easily differentiated from the sub-tropically occurring *L. taurica* by its morphological characteristics (Bai, 2004) and from its growth on the adaxial leaf surface. After some confusion over the emergence of a new tomato powdery mildew species at the end of the last century, *O. neolycopersici* was distinguished morphologically and phylogenetically from the superficially similar *O. lycopersici* by Kiss *et al.* (2001). The latter species was shown to have smaller, cylindrical conidia that are produced in chains, be closely related to *O. longipes* (also a powdery mildew of the Solanaceae) and be confined to Australia. *O. neolycopersici* meanwhile, has doliform or ellipsoid-ovoid conidia that are produced singularly or in

'pseudo-chains' at high humidities (Kiss *et al.*, 2001) or low wind conditions (Oichi *et al.*, 2006). It has a worldwide distribution (Whipps *et al.*, 1998; Huang *et al.*, 2000; Kiss *et al.*, 2001; Kiss *et al.*, 2005) and is considered economically more important than *O. lycopersici* (Jankovics *et al.*, 2008) . The differentiation of the two species means that prior to 2001 the majority of work was probably actually conducted on *O. neolycopersici* rather than *O. lycopersici*.

O. neolycopersici infects more than 60 hosts, predominantly in the Solanaceae and Curcubitaceae (Jones *et al.*, 2001; Kashimoto *et al.*, 2003a; Jankovics *et al.*, 2008) and it represents a particularly serious disease of tomato with almost all commercial cultivars susceptible (Jankovics *et al.*, 2008). It has been widespread in Asia since at least 1947 (Kiss *et al.*, 2001) but only became a serious and rapidly spreading disease relatively recently (Jones *et al.*, 2001). The earliest confirmed report of the pathogen outside of Asia is 1986 in the United Kingdom (Fletcher *et al.*, 1988) and though earlier reports of a novel tomato powdery mildew in Europe and South America are known herbarium specimens are absent (Kiss *et al.*, 2001). It has since spread to the rest of Europe, Russia, the Middle-East and North America (Arredonado *et al.*, 1996; Mieslerova and Lebeda, 1999; Kiss *et al.*, 2001; Marois *et al.*, 2001; Kiss *et al.*, 2005; Jacob *et al.*, 2008).

1. 2. 2 *Oidium neolycopersici*: life cycle

O. neolycopersici is not known to reproduce sexually (Jankovics *et al.*, 2008) and formation of the teleomorph has not been observed either naturally (Jones *et al.*, 2001; Kiss *et al.*, 2001; Jankovics *et al.*, 2008) or experimentally (Kiss *et al.*, 2001). However, analysis of amplified fragment length polymorphism (AFLP) patterns from Japanese, European and North American isolates has revealed variability that suggest that either "as yet unrevealed sexual reproduction or other genetic mechanisms" are occurring (Jankovics *et al.*, 2008).

Infection begins when a conidium lands on the leaf surface and germinates forming a germ tube, a process which takes between three and nine hours to occur (Jones *et al.*, 2001; Kashimoto *et al.*, 2003a; Takikawa *et al.*, 2011) and is induced by contact with a hard surface (Takikawa *et al.*, 2011). The smooth-surfaced germ tube elongates

and locates a suitable penetration site, often at the intersection of three epidermal cells, and forms a lobed or 'clover-leaf' appressorium (Jones *et al.*, 2001). This structure then forms a peg, which penetrates the cuticle and epidermal cell wall of the host, most probably through a combination of enzymatic and mechanical action (Jones *et al.*, 2001). Once completed, a haustorium is formed within the host cell, a lobed structure from which the pathogen takes up nutrients. Secondary hyphae emerge from the conidium and appressorium to produce further appressoria, resulting in rapid colonisation of the host (Jones *et al.*, 2001; Kashimoto *et al.*, 2003a). The cycle is complete when after a latent period of around five days (Oichi *et al.*, 2006; Jacob *et al.*, 2008) conidia are produced on conidiophores positioned perpendicular to the leaf surface (Fig. 1.1) and are dispersed by wind (Oichi *et al.*, 2006).

Leaf and stem tissue of tomato are affected by the disease, resulting in reduced photosynthesis and chlorosis of underlying tissue. This leads to senescence and considerable defoliation when the plant is heavily mildewed, with the effect of reducing fruit size, quality and yield (Mieslerova *et al.*, 2004).

Figure 1.1: A heavy infestation of *O. neolycopersici* on *L. esculentum*. The white powder on the upper surface of the tomato branch is the pathogen's sporulating structures.

1. 2. 3 *Oidium neolycopersici*: control methods

In the decade following the appearance of tomato powdery mildew disease in Europe, control was primarily through the use of chemical fungicides, although with

some variability in efficacy (Mieslerova and Lebeda, 1999; Jones *et al.*, 2001). Following the emergence of fungicide-resistant isolates (Matsuda *et al.*, 2005), considerable effort was then devoted to identifying resistance genes in tomato (Lindhout *et al.*, 1993; Mieslerova and Lebeda, 1999; Mieslerova *et al.*, 2000; Bai *et al.*, 2003; Bai, 2004; De Giovanni *et al.*, 2004; Matsuda *et al.*, 2005), which resulted in the successful release of resistant cultivars (Lindhout *et al.*, 1994; Kashimoto *et al.*, 2003b; Li *et al.*, 2006). However, these have been shown to confer only isolate-specific resistance (Lebeda and Mieslerova, 2002; Kashimoto *et al.*, 2003b; Bai *et al.*, 2005). Recent studies have identified a recessive gene that provides broad-spectrum (non-isolate specific) resistance and breeding programs for this are currently underway (Bai *et al.*, 2005; Bai *et al.*, 2008; Pavan *et al.*, 2008; Li *et al.*, 2012).

The need for more sustainable control methods has been recognised (Jones *et al.*, 2001) and a number of alternative control approaches have been investigated, including cultural and physical controls such as electrostatic spore precipitators (Matsuda *et al.*, 2006; Shimizu *et al.*, 2007), electrostatic discharge generators (Nonomura *et al.*, 2008), climate management (Elad *et al.*, 2009), chitosan (Borkowski and Szwonek, 2004), sunflower oil (Ko *et al.*, 2003) and the addition of silicates to hydroponic systems (Garibaldi *et al.*, 2011). Biological control approaches include the induction of host resistance through the application of non-infectious powdery mildews such as *Blumeria graminis* (Sameshima *et al.*, 2004), the application of Milsana®, an extract of the giant knotweed *Reynoutria sachalinensis* (Bardin *et al.*, 2008), the fungi *Paecilomyces farinosus* (Szentivanyi *et al.*, 2006), rhizobacteria (Silva *et al.*, 2004), a *Pseudomonas* bacteria (Agra *et al.*, 2011) and a *Rhodotorula* yeast (Agra *et al.*, 2011). However, the efficacy of all these has been variable at best.

1. 2. 4 *Bacillus subtilis* QST 713

B. subtilis is a motile, aerobic, gram-positive, rod-shaped bacterium common in soil, water and air (Edgecomb and Manker, 2006). Various strains have been used as microbial control agents in crop systems, including against soil-borne pathogens such as *Pythium* and *Phytophthora* (Berger *et al.*, 1996; Grosch *et al.*, 1999; Ongena *et al.*,

2005) and post-harvest pathogens such as *B. cinerea* and *Peronophythora litchi* (Jiang *et al.*, 2001; Toure *et al.*, 2004). QST 713 is a naturally occurring strain, shown to be effective against several bacterial and fungal foliar pathogens, including powdery mildews such as *L. taurica* on tomato (Edgecomb and Manker, 2006). The mode of action is a combination of competition for nutrients and space, the physical prevention of attachment and penetration, and the production of a number of fungicidal metabolites. These metabolites are collectively known as lipopeptides and inhibit germination and appressoria formation (Edgecomb and Manker, 2006; Kuck *et al.*, 2012). Provisional trials of this agent against *O. neolycopersici* were promising enough to include in this thesis.

1. 3 *Tetranychus urticae* Koch

1. 3. 1 Pest status, taxonomy and distribution

T. urticae is a member of the family Tetranychidae (subclass Acari, phylum Arthropoda) and is commonly known as the two-spotted or red spider mite. There are nearly 1200 species of spider mite with *T. urticae* having a worldwide distribution, particularly in temperate and sub-tropical regions (CABI, 1996; Bolland *et al.*, 1997). Spider mites are generalist herbivores and major pests of many crops, particularly those grown in protected horticulture (Rabbinge, 1985) and reportedly feed on more than 180 plant species (Chakraborty *et al.*, 2009) with *T. urticae* considered the most polyphagous (Van de Vrie, 1985).

Feeding by *T. urticae* reduces photosynthetic area and increases water stress resulting in reduced yields and even the loss of the whole crop (de Angelis *et al.*, 1982; Hussey and Scopes, 1985; Drukker *et al.*, 1997; Reddall *et al.*, 2007). The unsightly effects of its presence (leaf mottling, webbing and the mite itself) reduces crop quality (Fig. 1.2), which means that tolerance to this pest is particularly low for ornamental crops and even small infestations can bring about economically harmful aesthetic damage (Van de Vrie, 1985; Sadof and Alexander, 1993; Alatawi *et al.*, 2007; Marsh and Gallardo, 2009). As a result this family of mites are considered the "most important invertebrate pests of the roses" in some areas (Karlik *et al.*, 1995),

with *T. urticae* being the foremost (Van de Vrie, 1985; Gough, 1991; Landeros *et al.*, 2004).

Figure 1.2: Typical mottling damage on rose, caused by *T. urticae* feeding. Webbing can also be seen at the leaf edges. Damage and quantity of webbing shown here is relatively moderate.

1. 3. 2 Life cycle and biology

T. urticae have a five-stage life cycle; egg, larvae, protonymph, deutonymph and adult. Egg to adult development time depends on a number of factors but takes 10-11 days at 25°C, although this is slightly shorter for males (Sabelis, 1981; Wermelinger *et al.*, 1990). All three immature stages undergo a period of quiescence prior to forming the next stage, each of which is of approximately equal duration to the active period (Sabelis, 1981; Wermelinger *et al.*, 1990). After a pre-oviposition period of 1-2 days (Sabelis, 1981) adult females lay up to 48 eggs over approximately 22 days (Sabelis, 1981), which contributes to producing one of the highest intrinsic rates of natural increase in the Tetranychidae (Saito, 1979; Gutierrez and Helle, 1985).

T. urticae undergo arrhenotokous parthenogenesis, in which unfertilised haploid eggs develop into males, and a population sex ratio of approximately three females to one male is common (Helle and Pijnacker, 1985). Adult females are the largest stage,

having fused bodies approximately 0.5 mm long and are yellow-green with two large black spots on the back (Fig. 1.3). All active stages have four pairs of legs except the larval, which has three pairs and they feed on the mesophyll tissue of fruits, flowers and leaves by penetrating cells with their stylet and sucking up the contents, causing leaves to become mottled and yellow (Tomaczyk and Kropczynska, 1985). Copious amounts of webbing are spun that assists in dispersal and competition with other herbivores as well as providing protection against predators, inclement climatic conditions and even pesticides (Gerson, 1985).

Figure 1.3: Adult female *T. urticae* and egg (image courtesy of L. Kravar-Garde).

Dispersal is usually in an ambulatory manner from plant to plant or across the ground, although aerial dispersal in the presence of wind can occur (Smitley and Kennedy, 1985), and is related to plant damage and density of conspecifics (Bernstein, 1984; Skirvin and De Courcy Williams, 1999). *T. urticae* enter a state of suppressed development (diapause) under short-day conditions although temperature and food quality can modify this (Veerman, 1985). During diapause adult females develop a red pigmentation and migrate to sheltered sites to overwinter. Diapause is terminated in response to longer day length and increased temperature (Veerman, 1985). The conditions maintained in many greenhouses often result in no diapause occurring (Van de Vrie, 1985).

1. 3. 3 Control methods

Traditionally *T. urticae* were controlled through the application of synthetic acaricides. However, an over-reliance on these resulted in the widespread development of resistance in the 1950-60s (Helle, 1965). This propensity for the development of resistance continued for new acaricides introduced over the following years (Croft and Van De Baan, 1988; Gorman *et al.*, 2002; Kim and Yoo, 2002; Ahn *et al.*, 2004; Gerson and Weintraub, 2007) and is one of the main reasons that biological control is currently the primary means of managing *T. urticae* in Europe (Gerson and Weintraub, 2007; Gerson and Weintraub, 2012). Nevertheless, effective acaricides are available including some that are compatible with biological control (Gorman *et al.*, 2002; Kim and Yoo, 2002; Ahn *et al.*, 2004).

Biological control agents that have demonstrated good efficacy against *T. urticae* include arthropods, such as *Feltiella acarisuga* (Opit *et al.*, 1997), *Stethorus punctillum* (Rott and Ponsonby, 2000) and predatory mites, such as *Neoseiulus californicus*, *Amblyseius swirskii* (Gerson and Weintraub, 2007; Gerson and Weintraub, 2012) and *A. californicus* (Garcia-Mari and Gonzalez-Zamora, 1999). However, the most widely used is the predatory mite *P. persimilis* (Gerson and Weintraub, 2012). A number of entomopathogenic fungi have also been shown to be capable of reducing *T. urticae* populations including, *Hirsutella thompsonii*, *Metarhizium anisopliae*, *Verticillium lecanii* and in particular *Beauveria bassiana* (Chandler *et al.*, 2005). Indeed, in European greenhouse production the latter is now the recommended supplementary treatment to *P. persimilis* (Chandler *et al.*, 2011).

1. 3. 4 *Phytoseiulus persimilis* Athias Henriot

P. persimilis is a specialist predatory mite that was the first to be mass-produced for biological control purposes (Gerson and Weintraub, 2012). Investigations into its use began in the 1960s (Gerson and Weintraub, 2012) and within a decade it had been shown to be effective on a number of crops in England (Simmonds, 1972; French *et al.*, 1976). It was soon adopted into control programs in the greenhouse industry of North-West Europe (van Lenteren and Woets, 1988) and has since been used in crop protection worldwide (Gough, 1991; Trumble and Morse, 1993; Nicetic

et al., 2001; Pringle, 2001; Rhodes *et al.*, 2006; Abad-Moyano *et al.*, 2010; Mansour *et al.*, 2010).

Depending on the source, *P. persimilis* is reported to originate from Chile or Algeria and now has a worldwide distribution, due largely to its introduction for pest control purposes (Takafuji and Chant, 1976; Takahashi and Chant, 1993; De Moraes *et al.*, 2004). It is a member of the Phytoseiidae family (subclass Acari, phylum Arthropoda) and has five developmental stages; egg, larvae, protonymph, deutonymph and adult. All the active stages have eight legs, except for the larvae, which have six, and all but the larval stage consume prey (Takafuji and Chant, 1976). They have red fused bodies and adult females are approximately 0.5 mm long, with smaller males. Egg to reproductive adult development is rapid (approximately one week at 25°C, Gerson and Weintraub, 2007) and oviposition rate is high (approximately 80 per female, Gerson and Weintraub, 2007), although this is affected by factors such as temperature (Sabelis, 1981).

P. persimilis are fast-moving and are unhindered by *T. urticae* webbing (McMurtry and Croft, 1997), and in fact intensive searching behaviour is only instigated once contact with the web is made (Ryoo, 1986). Dispersal is ambulatory and long-distance prey location occurs through the detection of herbivore-induced plant volatiles (Dicke *et al.*, 1998; Mayland *et al.*, 2000; de Boer and Dicke, 2005). Its control efficacy is due to a high rate of predation and population increase as well as a tendency to aggregate in areas of high prey density (Laing and Huffaker, 1969; Takafuji and Chant, 1976; Amano and Chant, 1977). Indeed it is so effective that predator populations can die out due to over-exploitation of the prey (McMurtry and Croft, 1997).

1. 4 Modelling the pest and disease systems

The primary aim of the work described in this thesis was to quantify and understand the implications of introducing new sustainable greenhouse systems by developing simulation models to describe the dynamics of the selected pest, disease and biological control agents for different climate management scenarios. For the purposes of this work, sustainable greenhouse systems are defined as those that

minimise the need for fossil fuel, water and chemical inputs, make efficient use those that are added and reduce their escape from the system. These models would predict the pest and disease pressures and the extent to which they can be ameliorated by biological control in response to greenhouse temperature and humidity changes throughout the year. Models such as these can be defined as being "a representation in mathematical terms of the behaviour of real devices and objects" (Dym, 2007) and, as the system or device in question is a living system, they are more precisely described here as ecological models (Gillman and Hails, 1997; Hill and Coquillard, 2007). Such models contribute to the understanding of the complex interactions between organisms and the environment (Hill and Coquillard, 2007; Breckling *et al.*, 2011; Jorgensen and DeAngelis, 2011) and their uses include discerning the properties underpinning a system, testing hypotheses and assumptions, and suggesting future research priorities by indicating areas where important information is lacking (Hill and Coquillard, 2007; Breckling *et al.*, 2011). However, ecological systems are usually complex and so the ultimate aim of an ecological model is to suitably simplify a system whilst simultaneously capturing its essence (Breckling *et al.*, 2011).

Ecological models are playing an increasing role in many aspects of crop production (France and Thornley, 1984; van Ittersum *et al.*, 2003; Cao *et al.*, 2009), including for pest and disease control (de Wolf and Isard, 2007; Nietschke *et al.*, 2007; UCIPM, 2012), and provide a tool to economically and expeditiously predict the behaviour of a production system where the experimental alternative may be expensive, onerous and impractical (Nachman, 2001). They can be used to accurately identify areas where further experimentation is needed and, in the case of pest and disease management, where it would be most rewarding in terms of providing control solutions (France and Thornley, 1984). They can also be adapted for use in decision support systems that assist growers in considering when, where and how to apply control strategies (Xia *et al.*, 2007). In this thesis the models provide a means of comparing the impacts of novel climate management systems on pest and disease pressures and their control with more traditional climate management approaches.

There are a number of characteristics that define a model in a mathematical sense (Haefner, 2005). The first is the manner in which the system processes described in

the model are mathematically represented. In this regard, models largely fall into two categories; empirical (or phenomenological) and mechanistic models. The former is a relatively simple approach, whereby the system is represented purely through the use of statistical models with few of the underlying processes described and is based entirely on the data. These models may simplify systems but they are still capable of describing complex dynamic processes (Breckling *et al.*, 2011). Mechanistic models describe systems in terms of their underlying mechanisms (though they still largely rely on experimentally-derived empirical data) and can provide clearer insights into the dynamics and processes driving the system that may not be possible with empirical models (Haefner, 2005).

The second characteristic is the temporal aspects of the model. A dynamic model is one in which future states or conditions are explicitly represented. If they are not then the model is described as static. The temporal aspect can be further classified based on whether time can take any value (continuous) or an integer only, e.g., an hour or day (discrete).

The third defining aspect of a model is its representation of space. A spatially homogenous model is one in which space is not represented and a spatially explicit model is one in which it is, be it discretely, in which areas have categorical descriptions (e.g., land, water, plant *x*, plant *y*), or continuously, in which each point has its own characteristics.

The final characteristic of a model is the way in which it deals with random events. They can be either stochastic, in which probabilistic events are included, or deterministic, in which they are not. The former imparts a degree of realism to the model, allowing natural variation and uncertainty in parameter values to be accounted for, while in the latter the output will always be the same for a given set of inputs. The inclusion of stochasticity is important if the variability of a system is of interest (Grant *et al.*, 2000) as it "provides a more realistic representation of the population dynamics" and is "a natural way to incorporate process noise" (Skirvin *et al.*, 2002).

The choice between these model types largely depends on the purposes of the model, the level of complexity needed (bearing the parsimony principle in mind), the data available and the current understanding of the system. Three simulation models

were constructed in this thesis; a mechanistic, spatially homogenous and deterministic disease epidemiology model for the *O. neolycopersici* - *B. subtilis* system, a mechanistic, spatially explicit and stochastic population dynamics model for the *T. urticae* - *P. persimilis* system and an empirical, stochastic greenhouse environment model. All models were dynamic, with discrete time steps. The greenhouse environment model simulated the diurnal and seasonal variation in temperature and humidity in traditional and novel greenhouse systems in Spain and the Netherlands, providing stochastically varied climate conditions, which could be used as driving variables in the pest and disease models. This allowed the sustainable, novel greenhouses to be compared to traditional systems in terms of their impacts on pest and disease pressures and their implications for biological control. Literature sources were reviewed for data to parameterise the models and where this was not available or unsuitable, experimental work was conducted. Details of model choice and construction are given in the respective chapter for each model.

1. 5 Thesis aims and chapter outline

The thesis describes the development of models to describe the response of a disease, pest and biological control agents to greenhouse environmental conditions. Two pest and disease systems were examined: the first was *O. neolycopersici* disease causing powdery mildew of tomato and its biological control, *B. subtilis*; the second was the phytophagous mite, *T. urticae* and its biological control, *P. persimilis* on ornamentals. These pest and disease models were applied to two greenhouse climate control scenarios in Spain and three greenhouse climate control scenarios in the Netherlands. To provide the diurnal and seasonal changes in temperature and humidity for the pest and disease models a dynamic greenhouse climate model was used to generate representative data sets for each of the climate control scenarios in both countries. The aims of the thesis were:

1. To identify and address knowledge gaps regarding the response of *O. neolycopersici*, *B. subtilis*, *T. urticae* and *P. persimilis* to temperature and humidity.

2. To predict the impact of novel and traditional greenhouse climate control scenarios on pest (*T. urticae*) and disease (*O. neolycopersici*) pressures in the Netherlands and Spain using simulation models.
3. To predict the impact of novel and traditional greenhouse climate control scenarios on biological control efficacy (*P. persimilis* and *B. subtilis*) in the Netherlands and Spain using simulation models.

Chapter 2 describes experiments investigating the influence of climatic conditions on *O. neolycopersici* disease development and *B. subtilis* control efficacy as there was little quantitative information on these responses in the literature. Important epidemiological parameters for *O. neolycopersici* were quantified under a range of temperature and humidity conditions with and without the biological control agent in order to model the disease system (Chapter 5).

Chapter 3 describes experiments investigating the influence of ambient humidity and host plant on the predation behaviour of *P. persimilis* on *T. urticae* eggs for use in developing the pest system model (Chapter 6). The long-standing importance of this pest and biological control means that an abundance of literature data is available for other model parameters.

Chapter 4 describes a stochastic model developed to simulate the greenhouse environmental conditions in different climate control systems. Seasonal and daily variation in temperature and humidity is modelled in plastic-covered high tunnel greenhouses in Spain and in Venlo glasshouses in the Netherlands. In Spain both traditional, passively ventilated and sustainable, closed-system climate management is modelled and in the Netherlands minor set-point adjustments are made to the traditional climate management. The data generated by this model was then used as driving variables in the pest and disease models (Chapters 5 and 6).

Chapter 5 describes the construction of a model to simulate *O. neolycopersici* disease development with and without the biological control agent, *B. subtilis*. Using data generated by the greenhouse environment model (Chapter 4) the impacts of the different climate management systems on the development of the disease and biological control efficacy are assessed for Spain and the Netherlands.

Chapter 6 describes the construction of a model to simulate the population dynamics of *T. urticae* and *P. persimilis*. The effect of the different greenhouse environments and *P. persimilis* control methods (in terms of introduction frequency and rate) on *T. urticae* numbers and outcomes are simulated.

Chapter 7 concludes the thesis by integrating and discussing the results of the experimental work and simulation models. The potential impacts of the sustainable greenhouse systems are reviewed in light of their pest and disease pressures and their conduciveness to biological control.

Chapters describing experimental work (2 and 3) follow the format of introduction, materials and methods, results and discussion. The chapters describing modelling work (4-6) contain an introduction, model description, results and discussion.

2. The Effect of environmental factors on the development and sporulation of tomato powdery mildew caused by *Oidium neolycopersici* and its biological control with *Bacillus subtilis*

2. 1 Introduction

Tomato powdery mildew caused by *Oidium neolycopersici* is an important disease of tomato and other crops worldwide (Jones *et al.*, 2001). Development of isolates tolerant to resistant cultivars (Kashimoto *et al.*, 2003b) and fungicides (Jones *et al.*, 2001) coupled with pressures to reduce the use of plant protection chemicals has meant that the development of cultural and biological control techniques for this pathogen are now a priority.

One such approach is the management of the environmental conditions within the greenhouse so that disease pressures are reduced (van Lenteren and Woets, 1988; van Lenteren, 2000; Pilkington *et al.*, 2010). This can be achieved either through disease escape, which involves recognising and avoiding conditions optimal to the development of a pathogen (Jarvis, 1989), or by generating conditions in which the survival of the pathogen is greatly reduced (Jacob *et al.*, 2008). In general powdery mildews are considered to produce more severe epidemics in dry atmospheres (Jarvis, 1989) making them good candidates for such cultural control approaches.

The effects of the greenhouse climate on the development of different stages in the life cycle of *O. neolycopersici* have begun to be investigated (Whipps and Budge, 2000; Kashimoto *et al.*, 2003a; Jacob *et al.*, 2008; Mieslerova and Lebeda, 2010). Important infection processes of the disease are known to be affected by climatic conditions with conidial germination, germ tube extension and appressoria formation having been investigated for a wide range of temperatures and humidities (Kashimoto *et al.*, 2003a; Jacob *et al.*, 2008; Mieslerova and Lebeda, 2010). Conidial production, spore survival and disease development have also been studied, although at a less extensive range of conditions (Whipps and Budge, 2000; Jacob *et al.*, 2008; Mieslerova and Lebeda, 2010). The conclusion of these experiments has

been that manipulating both temperature (Jacob *et al.*, 2008) and humidity (Whipps and Budge, 2000) has the potential to minimise the risk of disease caused by *O. neolycopersici*.

The use of biological control agents is an increasingly popular pest and disease management tool in protected horticulture. A number of these agents have been described for use against the powdery mildews, including fungi (Dik *et al.*, 1998; Szentivanyi and Kiss, 2003; Szentivanyi *et al.*, 2006; Romero *et al.*, 2007), bacteria (Romero *et al.*, 2004; Romero *et al.*, 2007) and arthropods (English-Loeb *et al.*, 1999). Other IPM compatible treatments are compounds with physical modes of action such as salts, oils and ionised water (Reuveni *et al.*, 1995; Ko *et al.*, 2003; Fujiwara *et al.*, 2009). However, very few have been found to be effective against *O. neolycopersici*-caused tomato powdery mildew (Bardin *et al.*, 2008; Agra *et al.*, 2011). *Bacillus subtilis* QST 713, the active agent in Serenade® ASO, is a preventative biofungicide marketed for use against a number of crop diseases, and in preliminary work showed promise for effectiveness against *O. neolycopersici*.

The aim of this chapter is to better understand the effects of temperature, humidity and the novel control agent *B. subtilis* on *O. neolycopersici* disease development as well as their interactions. To achieve this, controlled environment experiments were carried out a range of temperature and humidity conditions representative of those experienced in important greenhouse production areas across Europe. To provide a good understanding of the effect of environmental conditions on *O. neolycopersici* on tomato, three important epidemiological parameters were assessed; disease development (the percentage of the leaflet affected by the disease), sporulation rate and the time from inoculation until production of new inoculum (latent period). These were chosen as *O. neolycopersici* latent period has not been studied and disease development and sporulation rate complement and develop the work from previous studies (Whipps and Budge, 2000; Kashimoto *et al.*, 2003a; Jacob *et al.*, 2008), using a greater range of temperature and humidity conditions, representative of those experienced in European greenhouse production. Further, this is the first time the influence of the interaction of these factors have been investigated.

The results of this work would give an indication of the potential that cultural and biological controls have for managing this pathogen. They would also provide the

basis for the parameterisation of a predictive disease severity model, allowing the effects of differing greenhouse climatic regimes to be assessed. In the context of this thesis such a model could be used to predict the implications for disease pressures of novel greenhouse production systems. For example, those that reduce heat inputs or adopt closed or semi-closed venting management programs.

2.2 Materials and methods

2. 2. 1 Pathogen isolate and maintenance

O. neolycopersici is an obligate pathogen and therefore has to be maintained on whole tomato plants. The isolate of *O. neolycopersici* used in all experiments was the same as that used in previous work (Whipps and Budge, 2000; Jones *et al.*, 2001). It was stored as spores at -70°C before being used to infect tomato plants in 2008. Since then, the pathogen has been maintained on young tomato plants (cv. Espero, Pinetree de Ruiter Seeds Ltd., UK) in a Venlo-type greenhouse at the University of Warwick (Wellesbourne campus). Heating was programmed to begin when temperatures dropped below 18°C and vents opened when temperatures rose above 20°C. Average conditions ranged from 21-55% RH (relative humidity) and 12-24°C. A 16:8 hour lighting regime was maintained with supplementary lighting (400 W high pressure sodium lamps) used between November and March and thermal screens were used in the summer. Tomato plants were inoculated on a weekly basis when approximately 30 cm high. This was done by tapping heavily infested leaves over the tomato plant for approximately 20 seconds. Inoculum was two to three weeks old when applied.

2. 2. 2 Experimental design

Experiments were set up to assess the effect of temperature, humidity and the application of *B. subtilis* on the latent period, disease development and sporulation rate of *O. neolycopersici*. Tomato plants cv. Espero were inoculated with the pathogen when they were approximately three weeks old, 10-20cm tall and at the two-leaf stage with the third leaf forming. They were randomly allocated to one of two treatments; *O. neolycopersici* only (ON) or *O. neolycopersici* with the *B. subtilis* treatment (BS). All were inoculated with a spore suspension ($1x10^5$ spores/ml) and sprayed until run-off (approximately 7-8 ml per plant) using a handheld trigger spray (Arco, UK). The spore solution was prepared by adding heavily infested leaflets to reverse osmosis (RO) water and Tween 20 (two drops/ litre; BDH Laboratory Supplies, UK). Spore concentration was checked using an Improved Neubauer haemocytometer and the concentration was adjusted as needed. The suspension was

then drained through muslin and the concentration checked, with further leaflets added if necessary and the process repeated. Once prepared, the spore suspension was used within two hours.

Inoculated plants were left for two hours to dry in a greenhouse at approximately 20-22°C and 50-70% RH before being transferred to Sanyo SGC970 CE cabinets (1.2 x 0.6 m). Plants receiving the *B. subtilis* treatment were sprayed prophylactically until run-off (approximately 7-8 ml per plant) one day prior to the *O. neolycopersici* spore application as per the label recommendation (spray mix 1:40 Serenade® ASO: RO water, Fargro, UK). Serenade® ASO is a formulation that contains 14g/l of *B. subtilis* and a minimum of $1x10^{12}$ colony forming units (cfu) per litre, meaning that each plant received approximately $1.8\text{-}2x10^{8}$ cfu per ml.

The CE cabinet was divided into four quarters to control for any within cabinet position effects and ten plants were placed in each quarter. Treatments (ON or BS) were randomly allocated to each quarter with the same treatment always occupying diagonally opposite positions, i.e. never next to the same treatment. Day length was set to 16:8 hours (day: night) and irradiance at the canopy level to 200 umol m^{-2} s^{-1} across the cabinet. Irradiance was measured using a quantum sensor (Skye Instruments, Wales, UK). This irradiance was chosen as it reproduced light conditions measured in the greenhouse. Lighting in the cabinet was provided by TL-D Super 80 Triphosphor 4' T8 32w fluorescent tubes (Philips, UK).

Six separate runs were carried out on different occasions to assess a total of 18 different temperature and humidity combinations with 20 plants per treatment. Three repetitions were carried out of one of the 'standard' optimum temperature and humidity combinations (25°C and 70% RH, Table 2.1). The *B. subtilis* treatment was applied in 15 of the temperature/humidity combinations. These environmental combinations were chosen to provide information on the effect of a range of temperatures and humidities previously reported to be most conducive to disease development (20-25°C and 70% RH; Jacob *et al.*, 2008). The temperature and humidity range was also chosen to reflect the breadth of conditions recorded in commercial greenhouses across Europe.

Table 2.1: Temperature, humidity and treatment combinations assessed in the experiment. ON = *O. neolycopersici* only applied. BS = *O. neolycopersici* and *B. subtilis* applied. Actual measured temperature and humidity conditions also shown as mean across duration of experiment.

Run	Temperature (°C)	Humidity (% RH)	Mean °C	Mean %RH	Treatment
1	10	70	10.7	69.4	ON + BS
1	25	70	24.9	78.9	ON + BS
1	20	70	20.7	68.0	ON + BS
2	29	70	28.9	74.3	ON + BS
2	20	80	20.4	83.3	ON + BS
2	25	80	25.8	79.6	ON + BS
3	20	50	20.5	52.7	ON
3	25	50	25.3	51.6	ON
3	15	70	15.5	76.2	ON
3	25	70	26.1	68.0	ON
3	33	70	33.5	72.5	ON
4	27	70	26.5	70.1	ON + BS
4	25	70	25.0	71.8	ON + BS
4	15	80	14.3	84.7	ON + BS
4	12.5	70	11.8	71.0	ON + BS
5	20	90	19.6	90.0	ON + BS
5	25	90	25.9	95.9	ON + BS
6	27	50	26.9	61.8	ON + BS
6	15	90	14.2	91.5	ON + BS
6	15	70	14.9	71.7	ON + BS

Once placed in CE cabinets each plant was inspected daily for disease symptoms which were first seen as a powdery silver sporulation giving a white sheen on the surface of the leaflet (Fig. 2.1). The date was recorded when any one leaf of the plant first exhibited symptoms (latent period). Tomato has compound leaves that are made of a number of leaflets (Fig. 2.1). To assess disease development leaflets were carefully cut from the plant and digitally photographed for image analysis of the diseased leaf area (Section 2. 2. 3). To assess sporulation the leaflets were then washed to create a spore suspension for subsequent spore counting (Section 2. 2. 4).

Figure 2.1: Compound tomato leaf with three leaflets growing from a central rachis (stalk). Sporulation of *O. neolycopersici* (powdery mildew disease) visible as white patches.

Diseased leaf area assessment and spore counts were initiated when disease symptoms (sporulation) were visible on five out of 20 plants in any treatment. On each sampling occasion five of the 10 plants in each quarter of the CE cabinet were sampled at random. From each plant one leaflet was randomly sampled from the two leaves that were fully formed when inoculated as well as one from the newly developed third leaf. This allowed for effects of leaf age to be considered (Jacob *et al.*, 2008). Distal and proximal leaflet position was also accounted for in the sampling regime as leaflet position appeared to have an effect on disease development in preliminary work. The total number of sampling occasions that occurred in each experiment varied from six to eight occasions, depending on leaf availability. Sampling interval was generally every two days but depended on latent period. A latent period greater than 10 days was deemed to indicate a slower rate of disease development, which would therefore need a longer overall sampling period and hence for these treatments a three day sampling interval was used.

2. 2. 3 Assessment of diseased leaf area using image analysis

Diseased leaf area has traditionally been assessed visually (e.g. James, 1974; Correa *et al.*, 2009), including for powdery mildews (e.g., Leath & Bowen, 1989; Falloon *et al.*, 1995), but such methods can be inaccurate (Sherwood *et al.*, 1983). To determine

diseased leaf area, leaflets were digitally photographed and analysed using image processing software. Each sampled leaflet was placed on a square of black velvet in a light box and photographed using a mounted Coolpix E4500 four megapixel digital camera (V1.2, Nikon, UK). Lighting was provided by four 15w Activa 172 Professional Sylvania fluorescent tubes (Philips, UK). Camera settings, distance from subject and focal area were identical for each shot allowing actual leaflet area and disease area measurements to be calculated consistently. Camera settings for the photographs were a shutter speed 1/30s, aperture F5.8, focal length 32mm, image dimensions 2272x1704 pixels with no flash and matrix metering. These were chosen to produce images with the greatest clarity, contrast and exposure for the lighting conditions.

Image analysis of the digital photographs was carried out using a script developed in collaboration with Dr Dave Skirvin using the image analysis toolbox in Matlab® (Mathworks Inc., UK) which allowed the disease area to be determined. For the script to be effective it was essential that sporulation could be accurately detected and differentiated from healthy tissue, leaf hairs, veins and senescence as well as the background.

The first step in the process was an image filter that removed senescing tissue and some other features such as leaf vein, which would otherwise be mistakenly identified as *O. neolycopersici*. This involved transforming the original red green blue (RGB) colour image (I1; Fig. 2.2) to the HSV colour space (hue, saturation and value). Pixels with values of less than 0.194 in the first dimension (hue), more than 0.09 in the second dimension (saturation) and between 0.7 and 1 in the third dimension (value) were then designated as senescing tissue and converted to 0 values. The image was then converted back to the RGB colour space creating a new image (I2), which was essentially the same as I1 except that the senescing tissue had 0 RGB values.

In the second step a new disease detection image (DD; Fig. 2.2) was created based on I2. Each pixel in DD was calculated from the RGB values (0-255) of the corresponding pixel in I2 using the following calculation:

$$DD\ pixel\ value = R + G + 30B \qquad 2.1$$

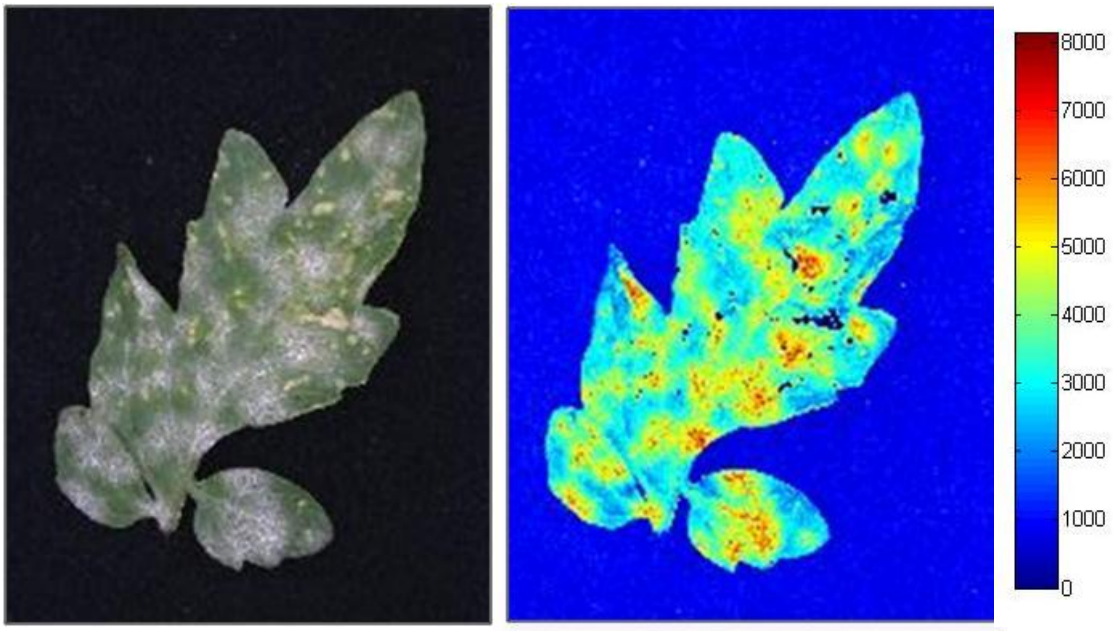

Figure 2.2: Example of a diseased leaf (I1) (left) and the disease detection image (DD) (right). The colour bar indicates the pixel values. Pixels with values above 3950 were classed as diseased. Dark blue areas indicate pixels identified by the image filter (see text) as features such as senescence.

Where *R*, *G* and *B* = the value of the red, green and blue pixels respectively in I2. A threshold value of 3950 was chosen and any pixel in the new image with a value above this was designated as *O. neolycopersici* (Fig. 2.3). This allowed effective discrimination of sporulation from healthy tissue, leaf veins and hairs.

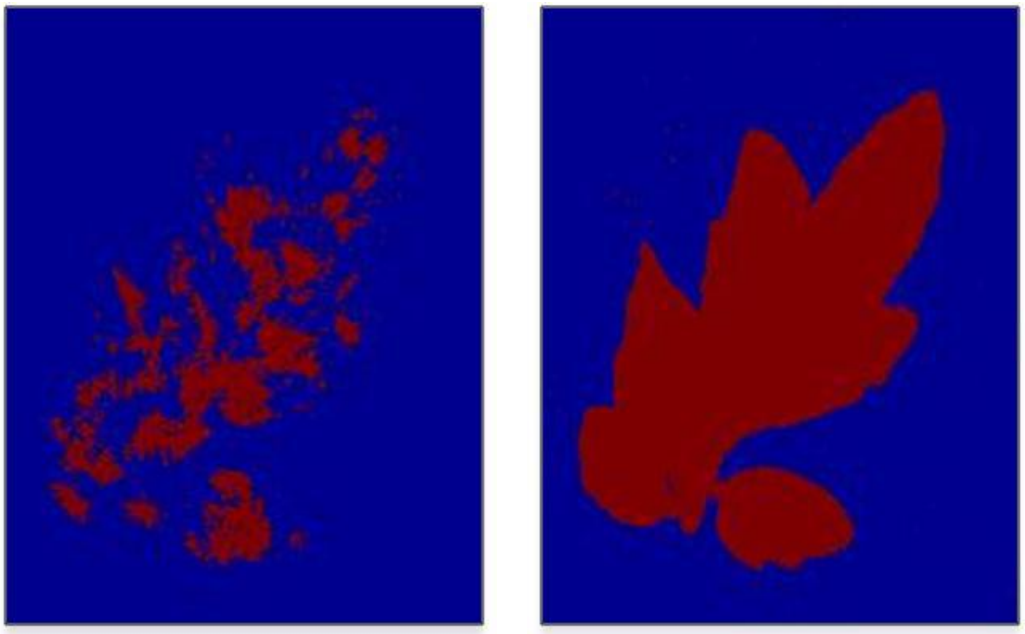

Figure 2.3: Pixels identified from the DD image as disease (shown in red in image on left) and the area identified as leaf (shown in red in image on right).

The final step identified the leaf area so that it could be discriminated from the background. It did this by filtering out all pixels in I1 with a RGB value of less than 50 and selecting the largest object (by pixel number) in the image as the leaf (to prevent light non-leaf items being included the calculation of leaf area; Fig. 2.3). This allowed the pixels identified as disease to be expressed as a percentage of the total leaf pixel area.

This process had to be modified when analysing leaflets from the 10°C cabinet. Leaflets kept at this temperature were very pale and often curled, exposing the underside of the leaflet, which is also much paler than the upper side. This resulted in the above image analysis process over estimating the disease area. To address this a term was added to the HSV image filtering process. In addition to the filter process described above this term also converted to 0 values all pixels with both a Hue value greater than 0.3 and a Saturation value greater than 0.1. The threshold value was also increased to 4500 for 10°C leaflets. For leaflets with a large degree of leaf curl (accounting for more than 2.5% of disease detection), both from the 10°C cabinet and other treatments, the paint.NET (dotPDN LLC, USA) software was used to colour underside areas black.

As black and white photographs were initially taken for the first three sampling occasions of the 20°C/70% RH and 25°C/70% RH treatments in Run 1, the image analysis process needed to be modified to cope with black and white images. The use of black and white images was discontinued after the fourth sampling occasion when colour images were taken in addition to black and white. This allowed an image analysis script to be developed for the black and white images that produced disease detection similar to that produced by the script for the colour images. For the black and white images (pixel values 0-255) no filtering process was used. A simple threshold value was chosen above which the pixel was designated as disease. This threshold varied for the two temperature treatments. At 20°C/70% RH the threshold was 135 and when this was compared to the disease area detected using the colour image script during sample occasion 4 it differed in detection by 1.1%. At 25°C/70% RH the threshold was chosen as 158 for the leaf one leaflets and 140 for leaflets from leaves two and three, resulting in a 0.5% and 2.48% mean difference respectively in detection when compared to the colour image script at sample occasion 4.

Finally, pictures were regularly taken of a ruler alongside a leaflet allowing the number of pixels per cm to be counted and the number of pixels per cm^2 to be calculated. As the focal length was kept constant for all the photographs this allowed the leaf area in cm^2 to be calculated and the disease pixel count to be converted to diseased leaf area in cm^2.

2. 2. 4 Assessment of sporulation

To assess spore production, each leaflet was carefully placed in a 100ml Duran bottle containing 20ml RO water/Tween 20 solution (approximate volume 19.6:0.4 ml) and shaken briefly by hand. Three Duran bottles were used for each quarter, one for leaflets from each of the three leaves, meaning that each Duran bottle contained five leaflets (one from each of the sampled plants), although this number was occasionally less if no more leaflets were left to sample on a leaf. The spore suspensions were transferred to 20ml Universal bottles and 0.5ml of aniline blue dye solution added. The aniline blue dye solution was prepared by adding 0.03g of aniline blue dye per 100ml of a 1:1 RO water and lactic acid solution. Spores were counted using a Fuchs Rosenthal haemocytometer under a low power (x10 objective) microscope. The number of mid-sized squares (1x1 mm) in which spores were counted varied depending on the spore density (fewer spores = more squares). Three spore counts were made per Universal bottle. To calculate spores per ml the following calculation was used.

$$Spores\ per\ ml = \left(\left(\frac{c1 * c2 * c3}{3} \right) * \left(\frac{5}{Sq} \right) \right) * 1000 \qquad 2.2$$

Where *cx* = count number (e.g., *c1* = first count) and *Sq* = number of mid-sized squares counted. To calculate the total spores per Universal bottle the following calculation was used.

$$Spores\ per\ bottle = Spores\ per\ mm^3 * 1000 * 20 \qquad 2.3$$

Finally, the spores per cm^2 of leaflet were calculated by dividing the total spores per Universal bottle by the leaflet area (the combined total area of the leaflets placed in

the Duran bottle), as calculated using the image analysis. Spore counts were not taken for the last set of CE experiments (Run 6) as the image analysis assessment of disease area was considered to be a satisfactory proxy.

2. 2. 5 Statistical Analyses

Statistical analysis was carried out in Genstat® (12th edition, VSN international Ltd.). The percent diseased leaf coverage was assessed using restricted maximum likelihood (REML) variance components analyses with a fixed effects structure of environment by treatment by leaf-age by occasion (environment*treatment*leaf-age*occasion) and a random effects structure of occasion within quarter within cabinet by run ((cabinet*run)/quarter/occasion). The sporulation (spores per cm^2) in the *O. neolycopersici* only treatment was analysed using REML with a fixed effects structure of environment by treatment by leaf-age by occasion (environment*treatment*leaf-age*occasion) and a random effects structure as above. The factor "environment" was substituted for temperature and humidity to make the analysis less computationally intensive. The effect of *B. subtilis* on sporulation was analysed using analysis of variance (ANOVA) with a treatment structure of relative humidity by temperature by treatment by leaf-age by occasion (RH*temperature*treatment*leaf-age*occasion) and a blocking structure as for the REML random effects structure above. To satisfy the requirements of homogeneity of variance and reduce the influence of residuals, sporulation rates were log transformed and percentage disease coverage was logit transformed. Latent period duration data was assessed using REML variance components analysis with a fixed effects structure of relative humidity by temperature by treatment (RH*temperature*treatment) and a random effects structure of quarter within cabinet by run ((cabinet*run)/quarter).

Interpretations of the analyses and comparisons of treatment means were carried out by comparing REML or ANOVA treatment means using the approximate least significant difference values (LSD) at the 5% level. For the REML analyses the LSD values were calculated by multiplying the approximate standard error of the differences for each factor level by the relevant percentage point *t*-distribution (Lindley and Miller, 1958). Comparisons of treatment means were made between

the relevant sampling occasions. Where a comparison of sampling occasion between the same day post inoculation (dpi) was not possible the closest dpi was used as long as it was not more than one day apart. Analyses for diseased leaf area and spores per cm^2 exclude occasions 7 and 8 as not all treatment combinations had observations on these occasions. Also all Run 4 experiments were excluded from analyses as disease performance was unusually poor with low levels of diseased leaf area and spore production. In summarising the analyses all significant differences are followed by the approximate *F* statistic, the degrees of freedom (df) and the probability that the null hypotheses can be rejected (based on the approximate *F*).

2. 3 Results

2. 3. 1 Effect of temperature, humidity and *B. subtilis* on disease development

The REML variance components analysis found that environment by occasion significantly affected the percentage diseased leaflet area (F = 21.68, df = 140.8, P = <0.001). The data suggested that optimum temperatures for disease development were 20-25°C (maximum mean area of 69% at 20°C, 90% RH and 18 dpi) with a rapid reduction in diseased area above 25°C and a more gradual reduction below 20°C. The lowest disease area was recorded at 10°C (max. mean area of 2% at 40 dpi) and 29°C (max. mean area of 1% at 15 dpi). No disease was observed at 33°C.

The widest range of temperatures was investigated at 70% RH (Fig. 2.4). At this humidity the greatest rate and extent of disease development was observed at 20°C and 25°C. There was no significant difference between disease area at these temperatures except at 9-10 dpi when it was greater at 25°C than at 20°C (mean diseased area for period of 27% vs. 13%). At 25°C the disease area was significantly greater than at 29°C between 7-16 dpi (28% vs. 1%), 15°C between 7-14 dpi (26% vs. 6%) and 10°C between 5-16 dpi (23% vs. 0%). At 20°C the disease area was significantly higher than at both 29°C and 15°C between 7-16 dpi (21% vs. 1% and 5% respectively) and at 10°C between 5-16 dpi (21% vs. 0%). Diseased leaflet area at 15°C was significantly higher than at 29°C between 13-16 dpi (10% vs. 1%) and 10°C between 7-18 dpi (8% vs. 0%).

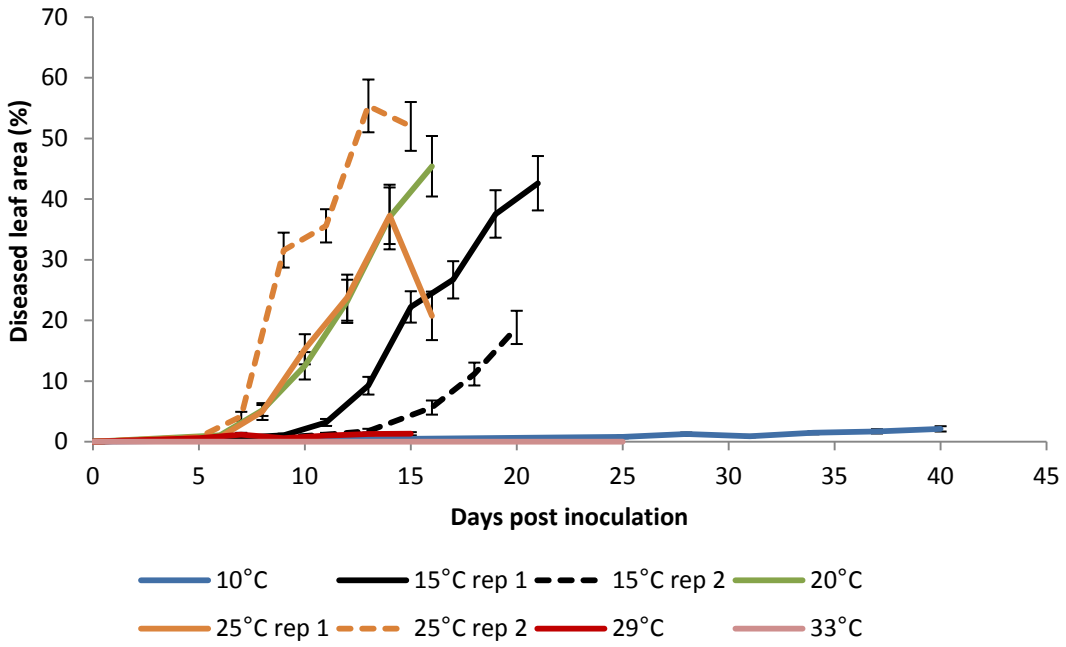

Figure 2.4: Effect of temperature on disease development of *O. neolycopersici* on tomato plants at 70% RH. Repetition of a treatment indicated by "rep". Data presented are the mean of twenty plants for each treatment. Bars denote standard error of the mean.

At the other humidity levels (50%, 80% and 90% RH), disease leaflet area at 20°C (Fig. 2.5a) and 25°C (Fig. 2.5b) was again greater than at other temperatures assessed at the same humidity (15°C and 27°C). At 50% RH disease area was significantly greater at both 20°C (11-18 dpi) and 25°C (9-16 dpi) than at 27°C (43% and 40% vs. 7 and 8% respectively). At 80% RH disease area was significantly greater at 25°C than at 20°C at 8 dpi only (9% vs. 2%). At 90% RH disease area at both 20°C and 25°C was significantly greater than 15°C between 11-18 dpi (38% and 32% vs. 8% respectively). There was no significant difference in disease area between 20°C and 25°C at 50% or 90% RH.

The effect of humidity on *O. neolycopersici* was less clear with similar disease development curves for a given temperature. At 15°C, 20°C (Fig. 2.5a) and 25°C (Fig. 2.5b) there was no significant difference between any of the humidities tested. However, at 25°C there was a strong trend for faster development at 50% and 70% RH, and the increase in disease area at both these humidities compared to 90% RH was only marginally non-significant (32% and 28% vs. 22% between 7-15 dpi respectively).

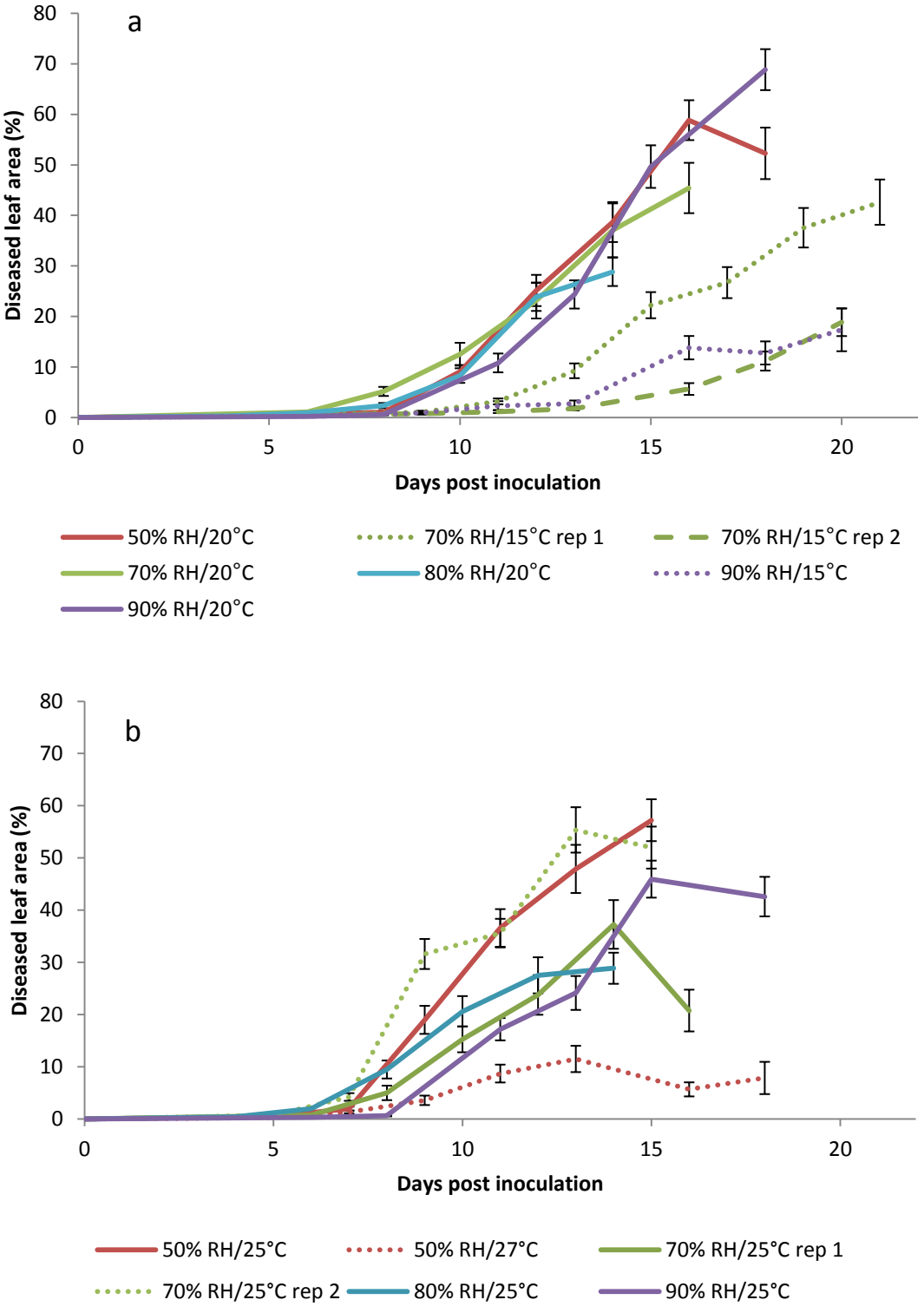

Figure 2.5: Effect of relative humidity (RH) on disease development of *O. neolycopersici* on tomato plants at a) 15-20°C and b) 25-27°C. Replication of a treatment indicated by "rep". Data presented are the mean of twenty plants for each treatment. Bars denote standard error of the mean.

B. subtilis reduced the diseased area under some conditions and hence was greatly influenced by both humidity and temperature. REML analysis found significant reductions in disease due to *B. subtilis* (F = 57.15, df = 26.3, P = <0.001), its interaction with environment (F = 21.68, df = 140.8, P = <0.001) and occasion (F = 10.19, df = 155.4, P = <0.001) and the three way interaction of environment, *B. subtilis* and occasion (F = 1.95, df = 142.5, P = 0.001). At 15°C *B. subtilis* had no significant effect on disease area at 70% RH but at 90% RH significant reductions were seen at 11 and 16 dpi (76% and 52% reductions respectively; Fig. 2.6a). At 20°C an interaction of *B. subtilis* and humidity was evident with the biological control producing significant reductions at 80% between 10-14 dpi (57% mean reduction for period and maximum reduction of 66% at 12 dpi) and 90% RH between 8-18 dpi (67% mean reduction for period and maximum reduction of 93% at 11 dpi) but not at 70% RH (Fig. 2.6a).

At 25°C *B. subtilis* reduced disease area at all humidities tested (Fig. 2.6b). At 70% RH significant reductions were found between 8-11 and 15-18 dpi (66% mean reduction for period and maximum reduction of 81% at 11 dpi). At 80% RH significant reductions occurred between 8-14 dpi (59% mean reduction for period and maximum reduction of 69% at 8 dpi). The greatest reductions were seen at 90% RH where significant differences were found between 8-18 dpi (85% mean reduction for period and maximum reduction of 90% at 11 dpi).

At 27°C *B. subtilis* was assessed at 50% RH only and significantly reduced the disease area at 7 and 11-13 dpi (65% mean reduction for period and maximum reduction of 70% at 13 dpi; Fig. 2.6b). At 10°C and 29°C *B. subtilis* did not significantly reduce disease area.

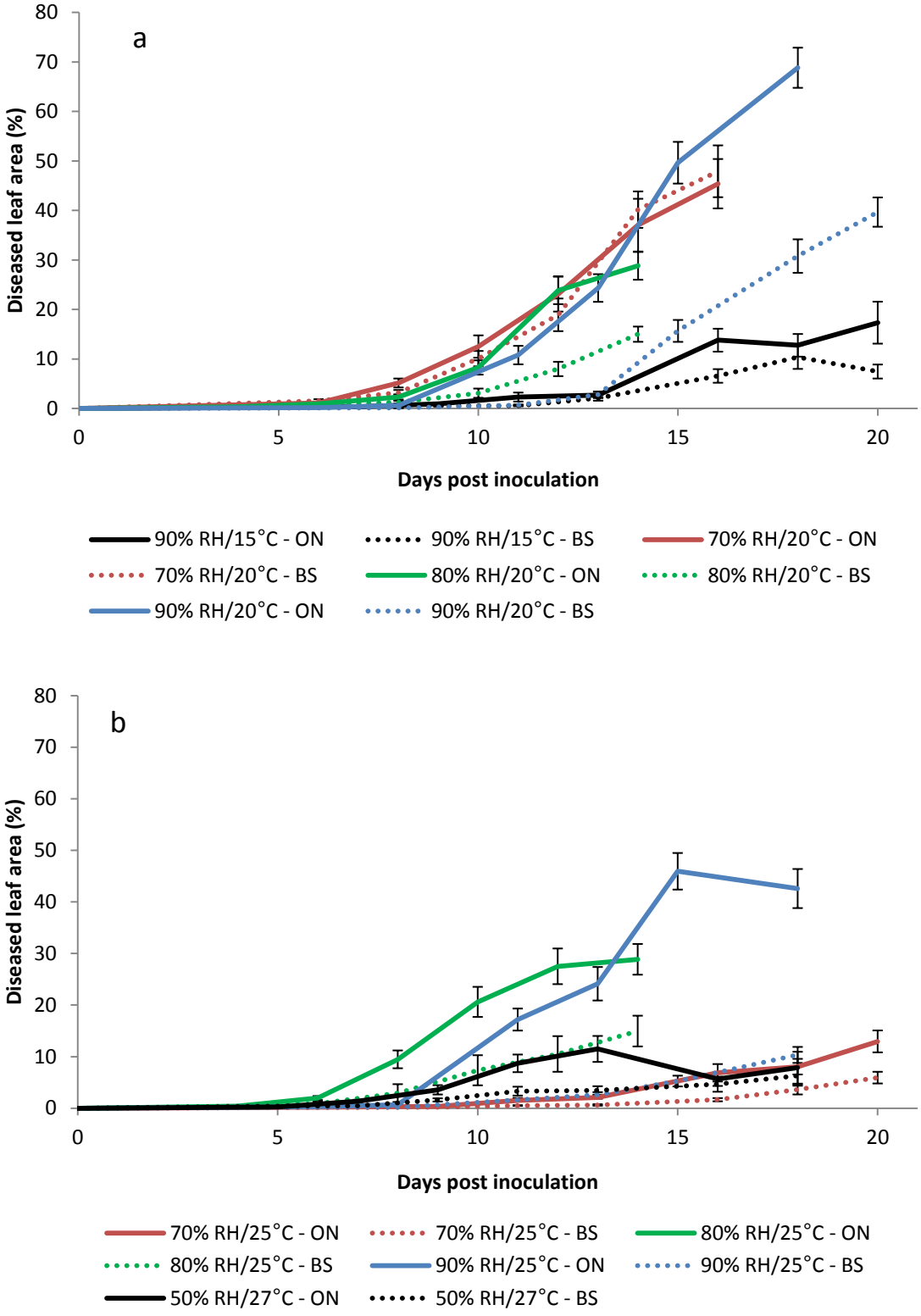

Figure 2.6: Effect of relative humidity (RH) on the ability of *B. subtilis* to reduce diseased leaf area of *O. neolycopersici* on tomato plants at a) 20°C and b) 25-27°C. ON = *O. neolycopersici* only, BS = *O. neolycopersici* pre-treated with *B. subtilis*. Data presented are the mean of twenty plants for each treatment. Bars denote standard error of the mean.

Leaf age had a significant effect on the diseased leaf area (F = 380.79, df = 336, P = <0.001) as did its interaction with occasion (F = 13.34, df = 336, P = <0.001) and *B. subtilis* (F = 17.37, df = 336, P = <0.001). Leaflets from leaf one (the oldest) were significantly more diseased than leaflets from leaf two, although the overall means were similar (13% vs. 15%) the mean estimated by the REML analysis was higher for leaf one due to the variability associated with disease area on leaf two. Both leaves were significantly more diseased than leaflets from leaf three (overall mean of 9%; Fig. 2.7). These differences increased with time. The degree of reduction in disease area due to *B. subtilis* was greater on older than younger leaves. Significant interactions of leaf age and environment (F = 25.77, df = 336, P = <0.001) and leaf age, climate and occasion (F = 1.61, df = 336, P = <0.001) were also detected with differences in disease area between leaves tending to increase with time and temperature and decrease with increasing humidity.

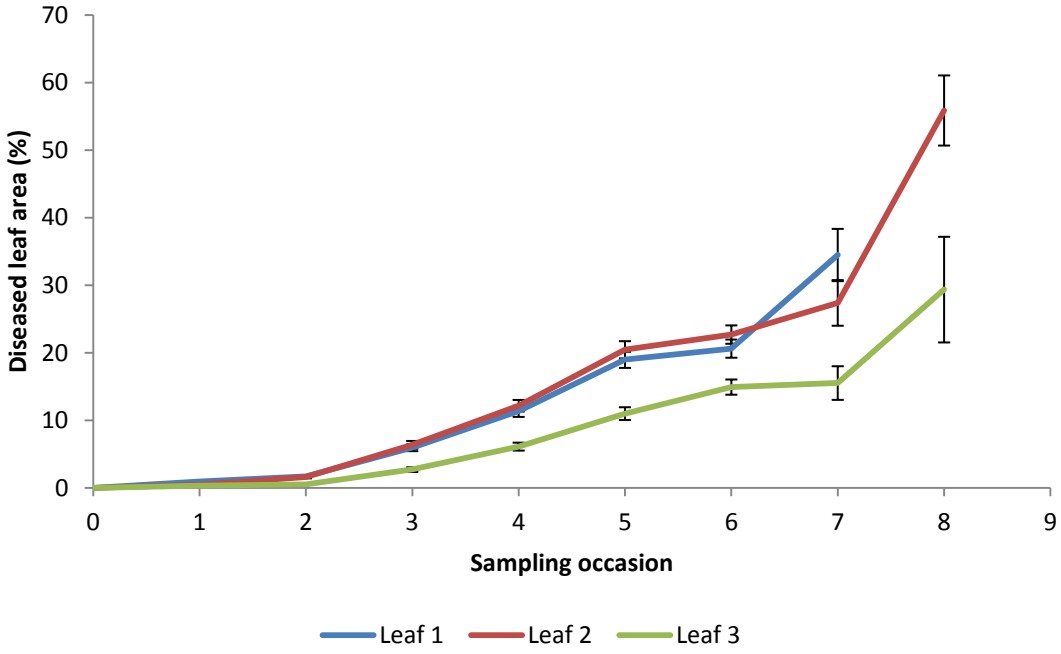

Figure 2.7: Effect of leaf age on the diseased leaf area of *O. neolycopersici* on tomato plants. Leaf one = oldest, leaf three = youngest. Data presented are the mean of 540 plants studied at each branch level. Bars denote standard error of the mean.

2. 3. 2 Effect of temperature, humidity and *B. subtilis* on sporulation

The REML variance components analysis found that environment by occasion (F = 6.14, df = 65, P = <0.001) had a significant influence on spores produced per cm² of leaf. The highest sporulation was observed at 20°C (maximum of $1.1x10^5$ per cm² at 90% RH and 18 dpi) and 25°C (maximum of $9.3x10^4$ per cm² at 50% RH and 15 dpi). Intermediate sporulation was recorded at 15°C (maximum of $4.3x10^4$ per cm² at 70% RH and 21 dpi). Reduced sporulation was observed at 10°C (maximum of $1.8x10^3$ per cm² at 70% RH and 40 dpi) and 29°C (maximum of $1.0x10^3$ per cm² at 70% RH and 15 dpi) and no sporulation was observed at 33°C.

There was no significant difference in the sporulation between 20°C and 25°C at any of the humidities investigated. At 70% RH (Fig. 2.8) sporulation at 25°C was significantly higher than at 29°C between 7-16 dpi (mean for period of $2.5x10^4$ vs. 480 per cm²), at 15°C between 7-16 dpi ($2.5x10^4$ vs. $2.1x10^3$ per cm²) and at 10°C between 5-16 dpi ($2.1x10^4$ vs. 0 per cm²). At 20°C sporulation was significantly higher than at 29°C between 11-16 dpi ($1.6x10^4$ vs. 645 per cm²) and 10°C between 6-16 dpi ($8.8x10^3$ vs. 0 per cm²). Sporulation at 15°C was significantly greater than at 29°C between 13-15 dpi ($4.9x10^3$ vs. 699 per cm²) and 10°C between 6-16 dpi ($9.8x10^3$ vs. 0 per cm²).

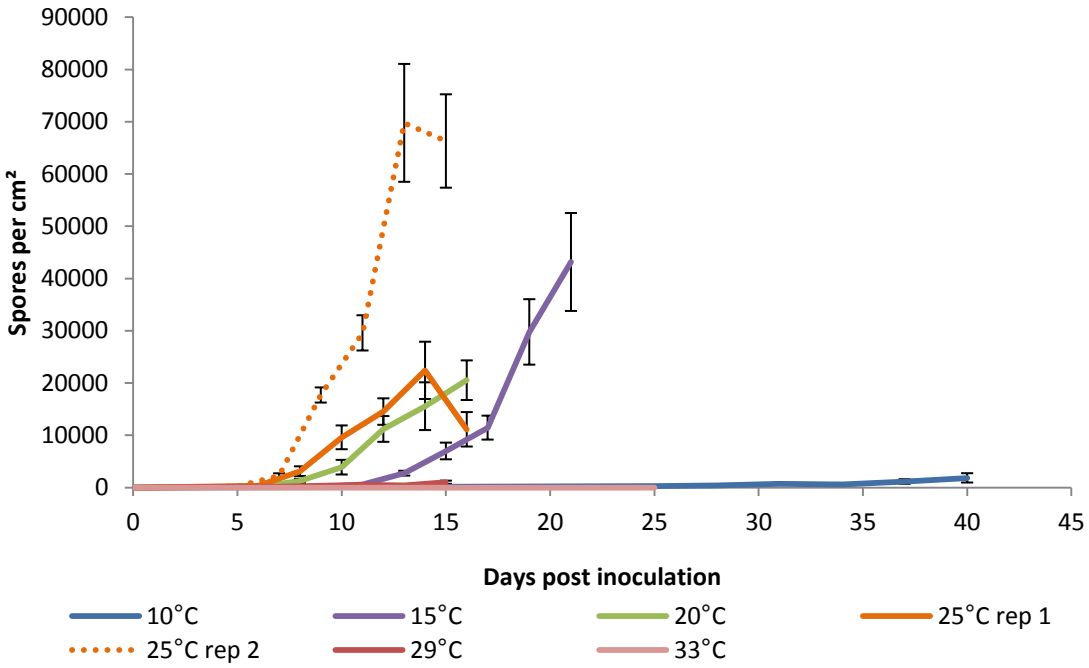

Figure 2.8: Effect of temperature on the sporulation rate of *O. neolycopersici* on tomato plants at 70% RH. Replication of a treatment indicated by "rep". Data presented are the mean of twenty plants for each treatment. Bars denote standard error of the mean.

O. neolycopersici sporulated over a wide range of humidities (50-90% RH) although it was only assessed within this range at 20°C (Fig. 2.9a) and 25°C (Fig. 2.9b). No significant difference was found between any of the humidities at either temperature, although at 25°C there was a clear trend for higher sporulation at lower humidities, especially at 50% RH.

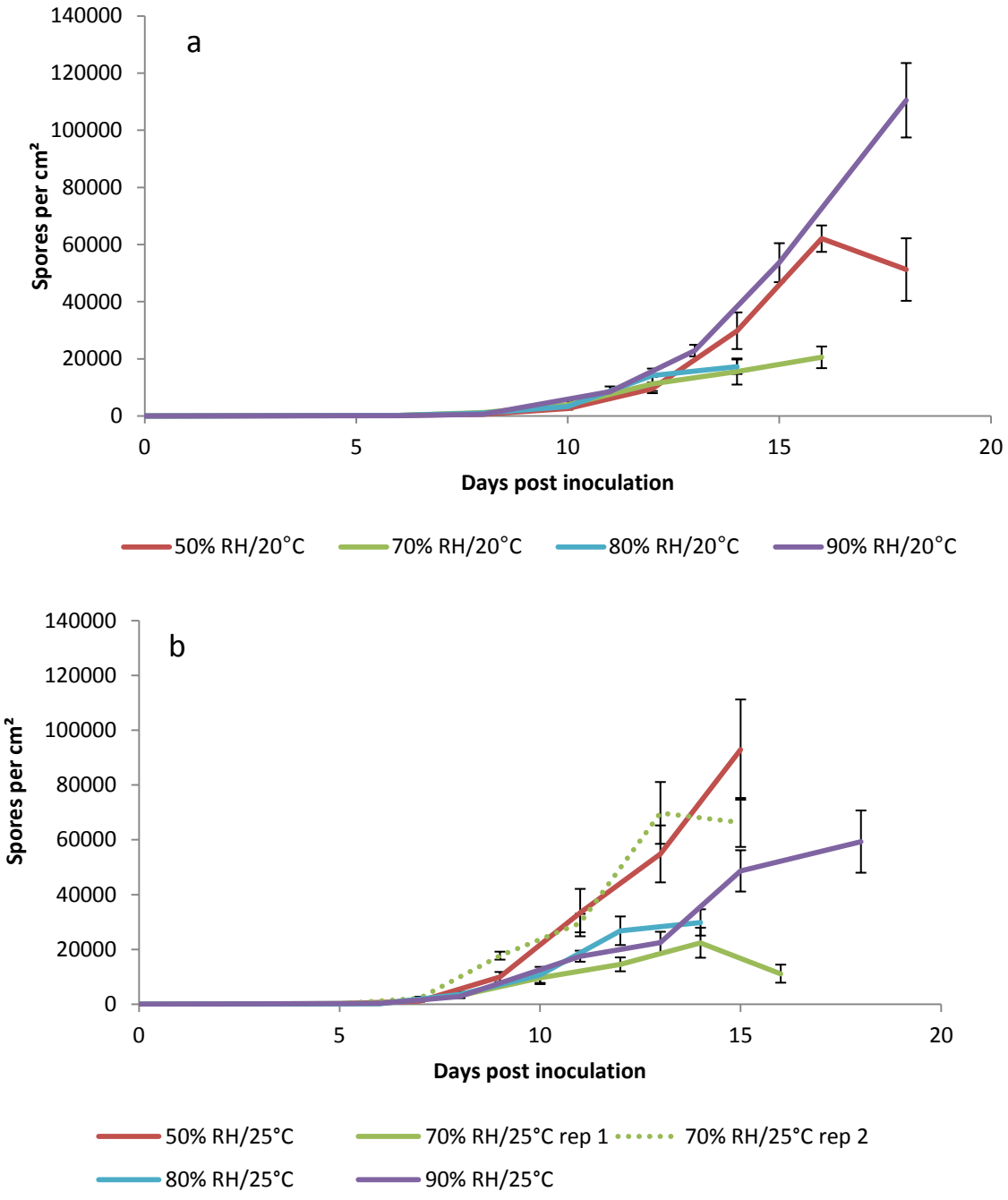

Figure 2.9: Effect of relative humidity on the sporulation rate of *O. neolycopersici* on tomato plants at a) 20°C and b) 25°C. Replication of a treatment indicated by "rep". Data presented are the mean of twenty plants for each treatment. Bars denote standard error of the mean.

The ability of *B. subtilis* to reduce *O. neolycopersici* sporulation was strongly influenced by both temperature and humidity, as well their interaction. ANOVA found significant reductions in spores per cm² due to *B. subtilis* on its own (F = 42.3, df = 1, P = <0.001) as well in terms of the magnitude of the reduction over time (F = 4.66, df = 5, P = 0.001) with greatest reductions occurring between 8-11 dpi. Humidity was found to have an impact on *B. subtilis* (F = 6.55, df = 2, P = 0.012) with significant reductions compared to the untreated at 80% RH (55% mean reduction; Fig.2.10b) and 90% RH (71% mean reduction; Fig. 2.10c) but not at 70% (32% mean reduction; Fig. 2.10a). Reductions in disease were significant at 25°C (79% mean reduction), marginally so at 20°C (56% mean reduction) and non-significant at 10℃ and 29°C (F = 11.84, df = 1, P = 0.0005). A significant interaction of temperature and humidity (F = 6.32, df = 2, P = 0.013) revealed that reductions were significant at 25°C/90% RH but only marginally so at 25°C and 70-80% RH and 20°C at 70% RH. The effect of the agent on *O. neolycopersici* was weaker for sporulation than disease area but this may be due to a smaller sample size (spores counts were pooled across quarter and were assessed in fewer environmental conditions). The greatest reductions in sporulation were seen at 90% RH with an 87-92% reduction seen at 20°C between 8-13 dpi and an 89-92% reduction at 25°C between 8-15 dpi.

Leaf age had a significant effect on sporulation (W = 260.7, df = 2, P = <0.001) as did its interactions with climate (W = 223.79, df = 20, P = <0.001) and occasion (W = 133.83, df = 10, P = <0.001). Overall, sporulation was significantly greater on leaf two than on leaf one, which in turn had significantly greater sporulation than leaf three, and these differences increased over time (Fig. 2.11). The interaction of climate and leaf age was significant (W = 133.83, df = 10, P = <0.001) with differences in spore production between the leaves increasing with decreasing humidity. Leaf age also affected the extent to which *B. subtilis* reduced sporulation (F = 4.99, df = 2, P = 0.008) with reductions in spores per cm² increasing with leaf age.

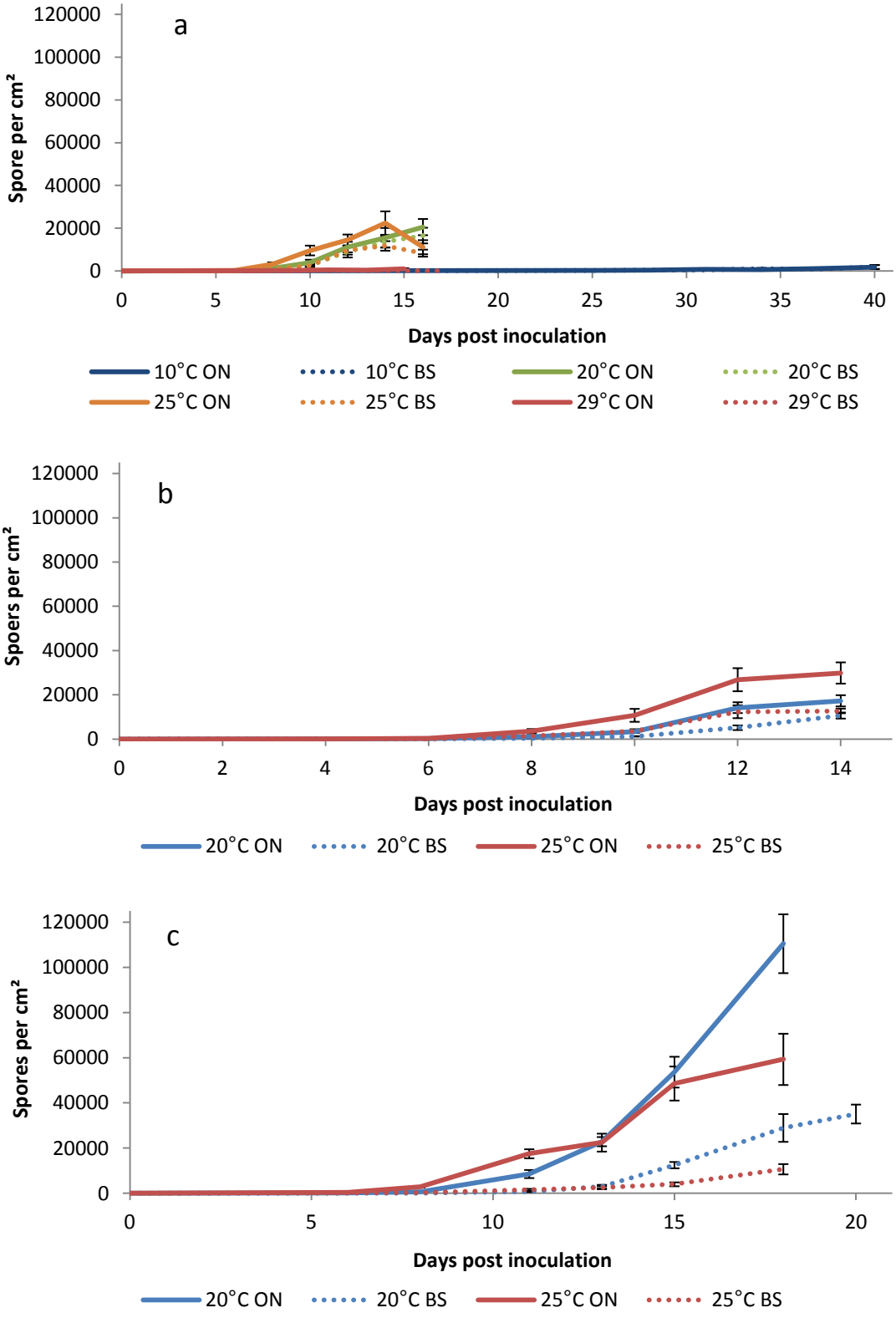

Figure 2.10: Effect of temperature on reduction of *O. neolycopersici* sporulation due to *B. subtilis* at a) 70% RH, b) 80% RH and c) 90% RH. ON = *O. neolycopersici* only, BS = *O. neolycopersici* pre-treated with *B. subtilis*. Data presented are the mean of twenty plants for each treatment. Bars denote standard error of the mean.

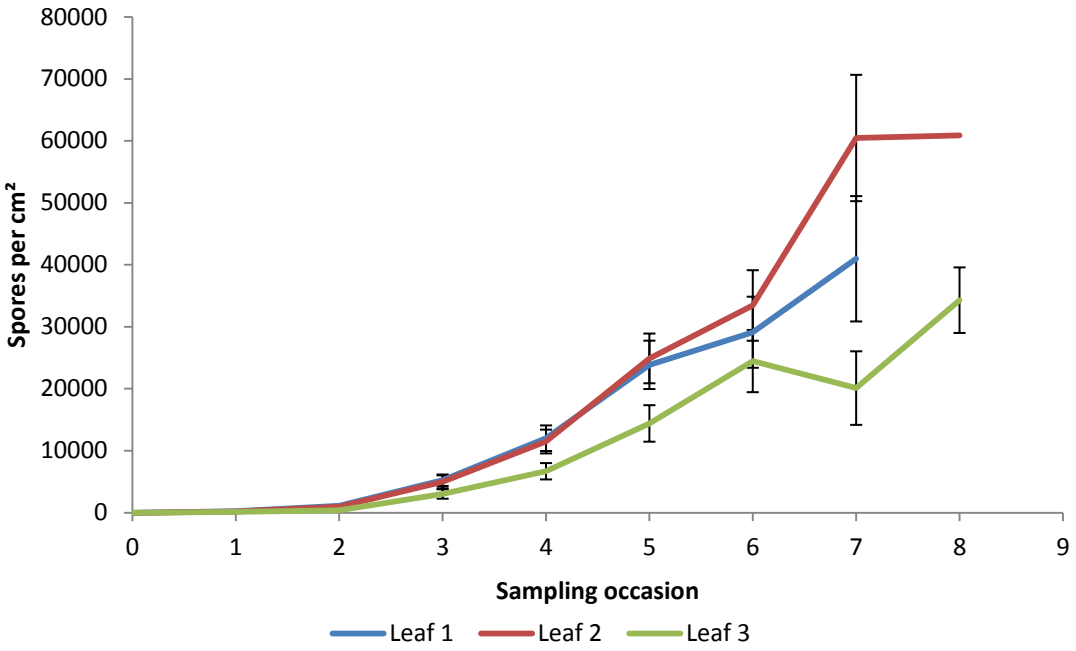

Figure 2.11: Effect of leaf age on the spore production per leaflet area of *O. neolycopersici* on tomato plants. Leaf 1 = oldest, leaf 3 = youngest. Data presented are the mean of 540 plants studied at each branch level. Bars denote standard error of the mean.

2. 3. 3 Effect of temperature, humidity and *B. subtilis* on latent period

REML variance components analysis of the full data set found that temperature (F = 103.07, df = 1.7, P = 0.019), *B. subtilis* (F = 16.36, df = 26.5, P = <0.001) and humidity (F = 34.39, df = 1.7, P = 0.042) all had a significant effect on latent period duration for *O. neolycopersici*. None of their interactions had a significant effect at the P = <0.05 level, although the interaction of humidity and *B. subtilis* was significant at the 10% level (F = 2.64, df = 26.2, P = 0.07).

Latent period was similar between 20-27°C, ranging between 4-6 days (Table 2.2). Latent period increased slightly at 15°C (8 days) and 29°C (6.5 days) and markedly at 10°C (25 days). Analysis of the effect of temperature showed that there was no significant difference between the latent periods at 20-27°C. However, latent period at 10°C (25 days) was significantly longer than at all other temperatures. At 15°C latent period was significantly longer than that at 20-27°C (5.3 days). Latent period at 29°C (6.4 days) was significantly longer than at 25°C (5.2 days).

Table 2.2 Effect of temperature, humidity (% RH) and *B. subtilis* on mean latent period (days) of *O. neolycopersici* on tomato plants for each treatment. Values in brackets denote standard error of the mean. ON = *O. neolycopersici* only treatment, BS = *O. neolycopersici* pre-treated with *B. subtilis*.

% RH	Treatment	Temperature (°C)					
		10	**15**	**20**	**25**	**27**	**29**
50	ON			6.0 (0.1)	5.6 (0.1)	5.1 (0.1)	
	BS					5.6 (0.1)	
70	ON	25 (0.0)	8.1 (0.1)	6.1 (0.1)	5.8 (0.1)		6.4 (0.5)
	BS	25 (0.0)	8.8 (0.2)	6.2 (0.1)	6.1 (0.2)		7.8 (1.7)
80	ON			4.5 (0.1)	4.1 (0.1)		
	BS			4.7 (0.1)	4.8 (0.1)		
90	ON		7.5 (0.1)	5.8(0.2)	4.8 (0.1)		
	BS		8.2 (0.1)	7.3 (0.1)	6.7 (0.1)		

There was no significant effect of humidity on latent period when this factor was analysed independently (to allow comparison of the means). This was because the residual sum of squares was doubled when temperature and *B. subtilis* were removed as factors. A similar result occurred when temperature was removed as a factor to investigate the interaction of humidity and *B. subtilis*.

The overall effect of *B. subtilis* was to significantly delay sporulation. When means were pooled across temperature and humidity the latent period (as estimated by the REML analysis) was 0.63 days longer (F = 12.55, df = 36.1, P = 0.001). The analysis that removed temperature as a factor showed that the interaction of humidity and *B. subtilis* remained marginally significant (F = 2.49, df = 33.1, P = 0.077) and when the treatment means of this interaction were inspected the effect of *B. subtilis* was only significant at 90% RH.

2. 3. 4 Validation of image analysis

To validate the image analysis process the assessments of disease area were compared to the spore counts recorded from the same leaflets. It should be that noted that as each spore count (spores per ml) represented the combined sporulation from up to five leaflets (as leaflets from the same leaf number in a cabinet quarter were placed in a single Duran bottle) the comparison was made against the total

disease area for those leaflets as calculated by the image analysis. There was a strong relationship between spore counts and disease area as calculated through the image analysis with a second order polynomial regression producing a good fit (R^2 = 0.9; Fig. 2.12). Spore counts increased linearly with disease area at low levels of disease coverage but increased more quickly at higher levels of disease coverage. The equation for this relationship can be written as:

$$Spores\ per\ ml = 60.7x^2 + 2330.7x + 1588.1 \qquad 2.4$$

Where x = leaf area covered in disease (cm^2).

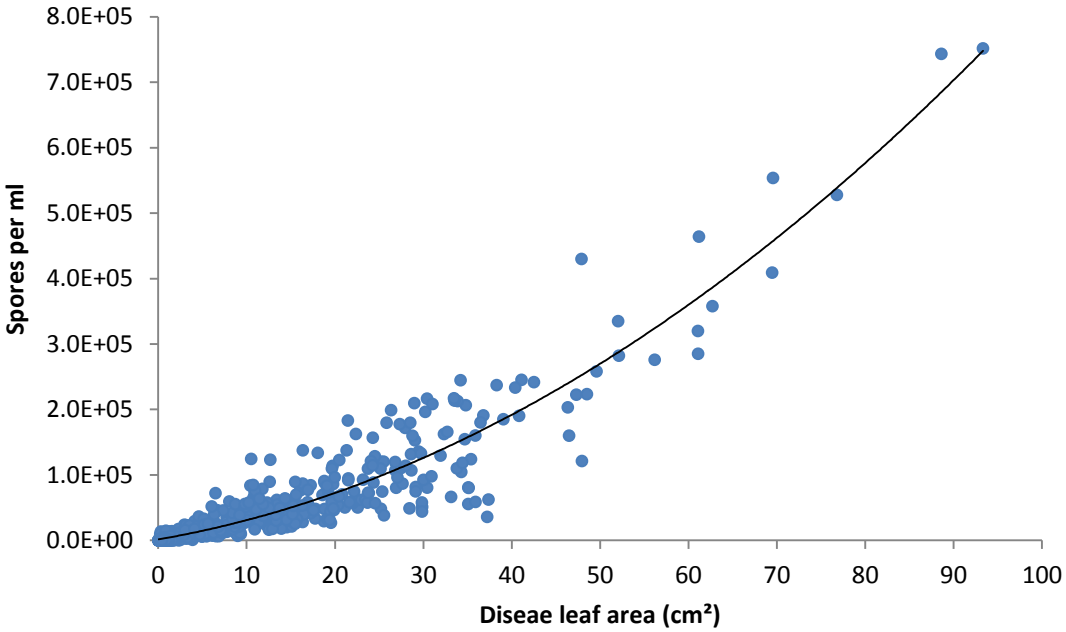

Figure 2.12: Spore counts (spores per millilitre) plotted against the respective total area of leaflet covered in disease (cm^2) as calculated by the image analysis (•). A second order polynomial regression of the two measures is also shown (—).

2. 4 Discussion

Modifying the greenhouse climate has been suggested both as a means of reducing disease pressure (Jarvis, 1989; Jacob *et al.*, 2008) and as way of developing growing systems that are more sustainable in terms of reducing energy, water and plant protection chemical usage (Opdam *et al.*, 2005). The results from these experiments show that climatic conditions have a strong influence on *O. neolycopersici* disease epidemiology. They also show for the first time that *B. subtilis* can produce appreciable reductions in disease severity under certain climatic conditions.

2. 4. 1 Effects of environmental conditions on *O. neolycopersici*

Disease caused by *O. neolycopersici* developed between 10.7-28.9°C and 51.6-95.9% RH (actual mean conditions), but was optimum between 14.2-26.9°C and 51.6-95.9% RH. The overall rate of sporulation and disease development (both in terms of the duration of the latent period and the rate with which the disease spreads across the leaflet) was greatest at 25°C. However, the differences between 20°C and 25°C were non-significant for latent period and sporulation and largely non-significant for disease development. These findings are generally in line with those of other studies (Whipps and Budge, 2000; Jacob *et al.*, 2008), although greater development at high humidity and a higher temperature was recorded.

The highest rates of sporulation were observed at 20°C/90% RH and 25°C/50% RH. While humidity had no clear effect on sporulation at 20°C, at 25°C it tended to decrease with increasing humidity, although this pattern was not significant. At 15°C sporulation was less than half that recorded at 20°C and although the maximum sporulation at 15°C was comparable to that recorded at some of the 20-25°C treatments the time taken to reach this peak was appreciably longer (21 dpi compared to 13-18 dpi). While sporulation was recorded at 10°C and 29°C it only occurred at very low levels. Jacob *et al.* (2008) found that at 75-80% RH the optimal temperature for sporulation (referred to in their paper as conidiation) occurred at 20°C (they also investigated 16°C and 26°C), with a peak spore production of approximately $5.9x10^4$ per cm^2 of leaf at 16 dpi. This is slightly higher than the

peaks recorded in these experiments at 20°C and 70% and 80% RH ($2.1x10^4$ per cm² at 16 dpi and $1.7x10^4$ per cm² 14 dpi respectively).

Jacob *et al.* (2008) also observed a significant reduction in *O. neolycopersici* sporulation between 20°C and 26°C, a trend that was not observed in this work. Indeed sporulation was higher at 25°C and 70-80% RH, although none of the differences were significant between this and 20°C. Jacob *et al.* (2008) found that at 26°C/75-80% RH sporulation peaked at approximately $1.2x10^4$ spores per cm² at 16 dpi, whereas in these experiments maximum sporulation in the 25°C/70-80% RH bracket was $6.98x10^4$ per cm² (70% RH, 13 dpi, rep 2). However, although no spore counts were taken, percentage disease area at 27°C/50% RH was observed to be considerably lower than that seen at 25°C. This suggests that the pathogen is especially sensitive to temperatures higher than 26°C. This is also supported by the extremely low spores counts and disease area observed at 29°C. The spore counts recorded in this work at 15°C ($1.15x10^4$ per cm² at 17 dpi) closely match those recorded by Jacob *et al.* (2008) at 16°C ($1.2x10^4$ spores per cm² at 16 dpi).

Humidity was shown to have no clear effect on sporulation in this work (although there was a suggestion that at 25°C spore production increased at low humidities), which is in contrast to other studies that found that humidity had a large effect on sporulation. For instance, Jacob *et al.* (2008) found that at 22°C, sporulation at 70% RH was significantly greater than at 85% RH and, although the maximum rate of sporulation (approx. $5.8x10^4$ per cm²) was similar at both humidities, the time taken to reach this peak was much shorter at the lower humidity. Moreover they recorded very significant reductions in sporulation at 99% RH. These trends were not observed in this work as relatively similar spore counts were recorded between 70-90% RH. In fact, at 20°C the greatest sporulation was recorded at 90% RH ($1.1x10^5$ per cm²), far greater than that recorded at 99% RH in their work ($1x10^4$ per cm²).

The visible signs of *O. neolycopersici* are the conidiophores and pseudo-chains of spores (Whipps *et al.*, 1998; Kiss *et al.*, 2001). These appear as white patches on the leaflet surface and it is the characteristics of these patches, in terms of the red, green and blue pixel values of the digital images, which the image analysis software used to identify the disease. As would be expected this meant that the trends in the development of the disease in terms of disease area closely reflect those seen for the

spore production. The disease developed across the leaflet surface fastest at 25°C, although the differences in disease area between this and 20°C were for the most part non-significant. At 27°C the initial development of the disease was comparable to that at 20-25°C however maximum leaflet coverage was soon reached after which disease area was significantly lower than at 20-25°C. This may be due to a detrimental effect of the temperature on the disease itself but may also be due to an effect of the temperature on the host and, indirectly, the light conditions. Fungi that cause powdery mildew diseases have been characterised as "high-sugar" pathogens (Horsfall and Dimond, 1957). It has also been shown that sporulation and disease area for *O. neolycopersici* increased with light intensity (Jacob *et al.*, 2008; Mieslerova and Lebeda, 2010). At 27°C the tomato plants grew quickly and the leaves that were sampled from in this experiment were soon shaded at the bottom of a bushy plant. As tomato plants grow the lower leaves drop, possibly due to their reduced contribution to photosynthesis. This was observed to occur more quickly at higher temperatures suggesting that at 27°C the reduced light levels and photosynthate available to the disease on the lower leaves may have limited the development of the disease. This may also explain the early plateaux in disease area at 25°C and 70% RH.

Visible disease was recorded at 29°C however only minimal development was observed. No disease was observed above this temperature. At 15°C disease was observed to reach similar maximum leaflet coverage as at 20°C and 25°C but did so at a considerably slower rate. The general trends in disease leaflet coverage in response to temperature compare well with those seen in Jacob *et al.* (2008), however comparisons of the disease area do differ with lower percent leaf coverage recorded at the equivalent dpi at all conditions in their work.

The effect of humidity on *O. neolycopersici* development was largely similar to that seen for sporulation. No significant effect of humidity was seen at 15°C to 25°C; although at 25°C there was a trend for more rapid disease development at lower humidities, especially 50% RH. Both Whipps and Budge (2000) and Jacob *et al.* (2008) observed a decrease in disease development with increasing humidity. At 24°C Jacob *et al.* (2008) reported the percentage disease area at 70% RH (the lowest humidity studied) to be significantly greater than that at 80% RH followed by a highly significant drop to less than 10% disease area at 99% RH. In this work,

although there was a slight trend for a reduction in disease between 70% and 80% RH at 25°C this was not significant. Moreover, no sharp reduction in disease area at very high humidity was seen with up to 46% disease area recorded at 95% RH, suggesting that it is humidities above 95% RH are particularly detrimental. Whipps and Budge (2000) conducted their work at 19°C and a range of humidities between 80-95% RH, finding low humidity to be more conducive to disease development but these differences were only significant five weeks after inoculation.

The latent period of *O. neolycopersici* was found to be significantly affected by temperature but not by humidity. At 25°C the latent period was shortest, although it was not significantly shorter than at 20-27°C. At low temperatures (10°C) the latent period was considerably longer that at other temperatures with the appearance of the disease delayed by over three weeks in some instances. As the development of the disease between infection and sporulation occurs within the plant the influence of temperature but not of humidity is unsurprising.

Leaf age was found to influence disease severity with older leaves suffering from higher levels of disease coverage and spore production than younger leaves. A similar effect of leaf age on disease coverage has been found in a number of pathogens causing powdery mildew (Jarvis *et al.*, 2002) including *O. neolycopersici* (Jacob *et al.*, 2008). As mentioned above, powdery mildew species are considered to have high requirements for sugars (Horsfall and Dimond, 1957) and as plants translocate metabolites toward developing fruits this may result in leaves close to these having higher metabolite content (Jarvis *et al.*, 2002), making them more conducive to the development of the disease. A further possibility is that the younger leaves experienced increased light levels, which promote the disease (Jacob *et al.*, 2008). However, it should be noted that when inoculated the youngest leaf was newly formed meaning that it would have received less total inoculum than the two other, larger, leaves. The youngest leaf grew more rapidly than the older leaves and therefore would soon have had a lower concentration of inoculum.

Overall, a number of differences were found between these results and those obtained by Jacob *et al.* (2008) and (Whipps and Budge, 2000) on *O. neolycopersici*, particularly in the response of the disease to high humidity and temperature around 25-26°C. The differences in behaviour at high humidity may be explained by

differences in the humidities tested. Due to equipment constraints in this work the maximum humidity attainable without creating free water (something both this work and Jacob *et al.* (2008) avoided) was 95.9% RH at 25°C, while Jacob *et al.* (2008) were able to investigate disease development at 99% RH. This suggests that either there is a large decrease in sporulation at humidities close to saturation or the different isolates used in these experiments respond very differently to high humidity.

A high degree of genetic (Jankovics *et al.*, 2008) and pathogenic (Lebeda and Mieslerova, 2002) diversity has been found between *O. neolycopersici* isolates. The *O. neolycopersici* isolate used in this work was identical to that used by Whipps and Budge (2000). The isolate in the Jacob *et al.* (2008) study, however, was obtained from a commercial nursery in Ashkelon, Israel. This difference may be important as it could be expected that an isolate would be adapted to the climate from which it originates, as has been shown to be the case in other fungal crop diseases (Howell and Erwin, 1995; Legreve *et al.*, 1998; Mboup *et al.*, 2011; Zhan and McDonald, 2011). Considering that Israel has a drier, warmer climate, this may explain why the isolate used in Jacob *et al.* (2008) is more vigorous at low rather than high humidities. Likewise, an isolate adapted to the wetter climates prevalent in the UK may be expected to exhibit an improved performance under high humidity conditions. The UK sourced isolate used in this study showed a high rate of development and sporulation at 90%+ RH but was equally adapted to conditions at 50% RH (the lowest humidity assessed). Similarly, one might also expect an Israeli isolate to be adapted to higher temperatures than the isolate used in these experiments. However, this does not appear to be the case with a lower sporulation rate and disease development recorded in their work at 26°C than at 25°C in ours. However, as mentioned above this may be due to a high sensitivity of the disease to temperatures around 26-27°C, regardless of the isolate. Sporulation and disease development was found to be very low at 29°C in this work and disease area at 27°C was comparable to that recorded at 26°C by Jacob *et al.* (2008).

Other differences with previous work may be due to experimental protocol, for instance Jacob *et al.* (2008) sprayed plants with a $1x10^4$ per ml spore suspension (a lower concentration than used in this study) and used older plants (6-10 weeks old). (Whipps and Budge, 2000) inoculated plants by simply shaking infected leaves over

the experimental plants. The tomato cultivar used also varied between these experiments. As cultivar and plant age can affect susceptibility to the disease (Jacob *et al.*, 2008), this may further explain differences between studies.

The manner in which results were taken also varied. For instance, in the experiments in which they assessed diseased leaf area, Jacob *et al.* (2008) sampled leaves from three different heights of older and, presumably, considerably larger plants before averaging the values to give a mean disease coverage for the whole plant. Much as was found in this work, they observed that young leaves were less affected than mature leaves and the inclusion of these young leaves may explain the lower disease areas observed in their study. Furthermore, both Whipps and Budge (2000) and Jacob *et al.* (2008) used standard (or pictorial) keys to assess disease area rather than image analysis. Visual estimates of disease severity, be they standard keys, scales (such as Horsfall & Barratt, 1945) or estimated percentages, have long been a source of controversy with numerous studies highlighting the possible inaccuracies associated with such methods (Sherwood *et al.*, 1983; Forbes and Korva, 1994; Newton and Hackett, 1994; Stonehouse, 1994; Parker *et al.*, 1995; Lorenzini *et al.*, 2000; Ferris *et al.*, 2001; Nita *et al.*, 2003; Bock *et al.*, 2008b; Bock *et al.*, 2008a; Bock *et al.*, 2009) and the consequences for hypothesis testing (Todd and Kommedahl, 1994; Bock *et al.*, 2010).

2. 4. 2 Control efficacy of *B. subtilis*

The application of *B. subtilis* was shown to result in significant reductions in *O. neolycopersici* disease severity, both in terms of disease development and sporulation. However, its efficacy was strongly influenced by ambient conditions with low humidities being especially detrimental. At 15°C and 20°C a clear interaction with humidity was evident. At 15°C, *B. subtilis* did not reduce disease area or sporulation at 70% RH but significantly reduced diseased area at 90% RH. At 20°C, significant reductions in disease were found at 80-90% RH (with greater reductions at the higher humidity) but control was lost at 70% RH. However, at 25°C *B. subtilis* significantly reduced disease area and sporulation at all the humidities tested (70-90% RH), although performance improved with increasing humidity, for instance reductions of over 90% in disease area and sporulation were

observed at 90% RH. Furthermore, significant reductions were recorded at 50% RH, when assessed at 27°C, suggesting that the detrimental influence of low humidity is related to low temperatures. No significant reductions in the disease were observed at 10°C or 29°C, although disease levels were already low. *B. subtilis* also had an effect on latent period, working to delay the appearance of the disease by almost two days in some cases. There was also a suggestion that the impact on this disease parameter was improved at high humidities.

The strong influence of humidity on this agent reflects results from other work that show that the performance of bacterial biological control agents improves in high humidity conditions (Romero *et al.*, 2007). Other *B. subtilis* isolates have been shown to be most effective at high humidities (90-100% RH; Rytter *et al*., 1989; Li *et al*., 1998; Leclere *et al.*, 2005) and 25-30°C (Fiddaman and Rossall, 1993; Wilhelm *et al*., 1998; Leclere *et al.*, 2005). The findings may not convince growers that *B. subtilis* can provide the reliable control they seek. However, it should be noted that these results were achieved with a single application of *B. subtilis* one day prior to inoculation with *O. neolycopersici*. Maximum reductions occurred between 8-13 dpi and decreased thereafter, suggesting that reapplication at approximately 10 dpi may be beneficial. Furthermore, the use of *B. subtilis* had not previously been recorded against *O. neolycopersici* and so the application concentration used was that recommended for another pathogen (*B. cinerea*). It could be expected that control efficacy would improve with repeated, regular applications of the agent and with an adjusted application concentration.

2. 4. 3 Image analysis

Digital image analysis techniques have previously been developed for assessment of other plant diseases (Shane and Lowney, 1992; Tucker and Chakraborty, 1997; Martin and Rybicki, 1998; Diaz-Lago *et al.*, 2003; Jackson *et al.*, 2007; Bock *et al.*, 2008b; Bock *et al.*, 2009), pest damage (O'Neal *et al.*, 2002; Sena *et al.*, 2003; Bakr, 2005; Mirik *et al.*, 2006) and nutritional and physical stress (Michaels, 1988; Lang *et al.*, 2000; Grant *et al.*, 2006). However, such techniques have not been developed for the symptoms of tomato powdery mildew, although they have been for other powdery mildews (Kampmann and Hansen, 1994; Parker *et al.*, 1995). Such

techniques improve precision and reproducibility of assessments, and reduce the potential for evaluation bias, although they do not always provide a better estimate of symptoms than visual assessments (Olmstead *et al.*, 2001; Moya *et al.*, 2005).

The results obtained through the image analysis process in this work were validated by comparing the spore counts data to the disease area data derived from the image analysis. Analysis of this relationship found a good fit using a second order polynomial regression, accounting for 90% of the variation in sporulation. However, as the visual symptoms are essentially sporulation, a linear relationship between the image analysis results and sporulation would be expected. The fit showed that at higher levels of disease coverage spore production per unit area of leaf also tended to increase. Examining spores per cm^2 of disease area, instead of leaf area, (data not shown) revealed that spore density increased as conditions for the disease became more optimal, e.g., at 25°C. In images of leaves at these conditions it was noticeable that the RGB values of diseased areas were closer to saturation, i.e., they were whiter, suggesting that the higher the density of spores the more white the area. The script developed in this work analysed pixels and designated them simply as either diseased, non-diseased or background (not leaf). If the whiteness of a patch can give a reliable indication of the concentration of spores within it then it is possible that an altered version of the script could use the saturation of a pixel to give an indication of the spore density and therefore an improved measure of disease severity.

There were some deficiencies identified with the image analysis approach. For instance, the initial threshold used (3950) resulted in overestimations of disease area in cool conditions (<15°C). This was due to the paler leaved plants produced at low temperatures. A higher threshold was chosen, which largely resolved this issue. However, it would be preferable to have a single image analysis calculation that is equally effective in all conditions. It may not have unduly affected the results in this work but there is the potential for issues in future work when choosing the point at which thresholds are changed or in deciding whether further thresholds are needed. Presumably there is a gradual change in leaf colouration with temperature and so it would be expected that the accuracy of the image analysis may decrease at temperatures approaching the point at which the threshold is changed. Features such as leaf veins were also occasionally misidentified as disease, a problem which other image analysis programs have also experienced (Kampmann and Hansen, 1994;

Olmstead *et al.*, 2001; Moya *et al.*, 2005). Although a filter was written into the program to minimise this it was not able to do so without compromising the program's ability to detect disease. Senescing tissue also presented problems for the software and again the filter was not infallible at removing such tissue from the analysis. A trade-off needed to be made in ensuring that, while non-diseased areas of leaf were not misidentified as disease, actual disease was detected.

Overall the image analysis used in this work allowed the disease area of thousands of leaflets to be objectively and quickly assessed and the images stored until a more suitable time for their analysis. Often long periods of time occurred between experiments and the process ensured that there was no change in evaluator criteria or that other sources of human error such as evaluator fatigue, inter-evaluator reliability and visual accuracy (Nutter *et al.*, 1993; Tucker and Chakraborty, 1997; Moya *et al.*, 2005) did not occur. Furthermore, one of the primary objectives of these experiments was to provide data for the development of a predictive disease model and it has been noted that "disease intensity needs to be quantified with a high degree of accuracy and precision if meaningful predictive models are to be developed" (Nutter *et al.*, 2006). Disease area assessments produced using the image analysis program therefore fulfilled these criteria. The approach could be used for other disease applications, however if it was desirable to assess disease development non-destructively care would need to be taken to ensure leaves are photographed from a constant distance, perpendicular to the lens and light source, and that the illumination remains constant.

2. 4. 4 Conclusion

Experiments showed that *O. neolycopersici* disease development and sporulation is influenced primarily by temperature and to a much lesser extent by humidity. The results provide a detailed representation of the optimal conditions for the pathogen and a clear indication of the climatic extremes in which it can function. Altering the greenhouse climate to avoid ideal conditions for disease severity has been proposed as an important tool in integrated disease management (Jarvis, 1989) and the results of other work suggest that manipulating humidity may be rewarding (Whipps and Budge, 2000; Jacob *et al.*, 2008). However, these results suggest that *O.*

neolycopersici was little affected by humidity in the range of 50-95% RH. Humidity above 95% RH may be more effective at reducing the disease but maintaining such conditions in the greenhouse may encourage the development of other pathogens, such as *B. cinerea*.

Altering greenhouse temperature may however have greater potential for limiting the severity of this disease. Disease development was reduced rapidly above 25°C and it has been reported that raising greenhouse temperatures above this value has been effective against *O. neolycopersici* in Israel (Jacob *et al.*, 2008; Elad *et al.*, 2009) and against *L. taurica* on sweet pepper (Elad *et al.*, 2007). However, such temperatures may negatively affect fruit set and pollination (Peet *et al.*, 1997; Jacob *et al.*, 2008). It should also be noted that increasing greenhouse temperatures to control the disease may not be economical or environmentally sustainable (if the energy source is carbon-based fossil fuels) in parts of Europe. Disease was also reduced at temperatures below 20°C, although temperatures at which acceptable reductions in disease would occur are probably unsuitable for crop productivity.

This work also showed for the first time that *B. subtilis* may be a useful biological control option for this pathogen. It could also form part of an integrated management program, combined with cultural, chemical and other biological controls, an approach that has shown to be effective against other diseases (Elad *et al.*, 1993; Elad *et al.*, 1998; Guetsky *et al.*, 2001; Guetsky *et al.*, 2002; Shishkoff and McGrath, 2002; Gilardi *et al.*, 2008). It has been noted that care ought to be exercised in interpreting the results of humidity experiments on leaf surface microorganisms as humidity at the leaf surface is likely to be substantially different from that even a short distance away (Ferro and Southwick, 1984). It could be argued that the humidity conditions maintained in the CE cabinets in these experiments had only a minimal effect on the humidity at the leaf surface. This could explain the lack of response of the disease to humidity. However, *B. subtilis* control efficacy was affected by humidity suggesting that this did have an effect at the leaf surface. Furthermore, air movement within the cabinets was low (less than 0.5m/second; S. Robertson pers. comm.), which would increase the influence of ambient humidity on leaf surface humidity (Nobel, 1974).

Finally, this data set provides a good basis for the modelling of *O. neolycopersici* disease outbreaks (Chapter 6). An issue common to the construction of many such models is finding suitable, reliable and compatible data. The model developed needed to assess disease development at a range of conditions relevant to European greenhouse production. Some data was available from literature sources but differences in experimental protocol can make the inclusion of different data sets in the same model problematic (as will be discussed further in Chapter 6). This data allows the construction of a model that can quantitatively predict the development of the disease in response to changing greenhouse climatic conditions. The process by which this data will be used to parameterise the model will be discussed in Chapter 5.

3. The functional response of *Phytoseiulus persimilis* to prey availability, relative humidity and host plant

3. 1 Introduction

Phytoseiulus persimilis is a widely used biological control of *Tetranychus urticae* that has been shown to be effective on both ornamental and vegetable glasshouse crops (Hussey and Scopes, 1985; Van de Vrie, 1985; Gough, 1991; Nicetic *et al.*, 2001). It is a specialist predator capable of quickly and drastically reducing *T. urticae* populations (Takafuji and Chant, 1976).

Figure 3.1: *P. persimilis* (lower) searching for *T. urticae* on a bean leaf. A *T. urticae* adult female (upper) and eggs are also visible.

Many of the factors governing the control efficacy of *P. persimilis* have been studied, particularly those relevant to greenhouse control. The effects of temperature have been investigated in terms of development, fecundity, activity, dispersal and functional response (Stenseth, 1979; Everson, 1980; Skirvin and Fenlon, 2003b; Skirvin and Fenlon, 2003a; Kazak, 2008). The effect of food availability has been investigated in terms of survival, dietary expansion, egg hatch, development, movement behaviour, functional response and emigration (Takafuji and Chant, 1976; Bernstein, 1983; Bernstein, 1984; De Courcy Williams *et al.*, 2004b; De Courcy Williams *et al.*, 2004a). Also, the effect of host plant and canopy structure has been investigated in terms of impacts on dispersal, functional response and control efficacy (Skirvin and De Courcy Williams, 1999; Skirvin and Fenlon, 2001; Skirvin and Fenlon, 2003b).

Functional response is defined as: "The number of prey that an individual predator kills (or the number of hosts a parasitoid attacks) as a function of prey density"

(Juliano, 2001), and it is considered to be the "essential starting point for quantitative studies of predator–prey interactions" (Fenlon and Faddy, 2006). One important factor that may affect the functional response of *P. persimilis* to prey availability is that of ambient humidity conditions. Humidity has been shown to influence the egg hatch, development, adult life span and activity of *P. persimilis* (Mori and Chant, 1966a; Stenseth, 1979; Perring and Lackey, 1989; De Courcy Williams *et al.*, 2004b; Ferrero *et al.*, 2010), and it is considered to be one of the least dry-tolerant Phytoseiid species (De Courcy Williams *et al.*, 2004b; Ferrero *et al.*, 2010). Stenseth (1979) and Mori and Chant (1966b) found that humidity affected their predation rate and ability to regulate populations of *T. urticae*. However, the range of humidities considered in these experiments was limited and their results were somewhat contradictory. Additionally, the Stenseth (1979) study did not vary prey numbers and the arena used in the Mori and Chant (1966b) study was very unrealistic. In other work the effect of vapour pressure deficit (a derivation of temperature and relative humidity) was shown to have a strong effect on the functional response of the related Phytoseiidae, *Amblyseius cucumeris* and was even considered more influential than temperature (Shipp *et al.*, 1996).

Greenhouse humidity conditions are extremely variable even in high-technology Dutch glasshouses where climate is carefully maintained. Diurnal greenhouse humidity extremes can vary by as much as 55% in Holland and 75% in Spain (see Chapter 4). Seasonal variation is also a factor although more so in Spain. The control efficacy of *P. persimilis* can be unreliable (Stenseth, 1979; Osborne and Oetting, 1989; Pringle, 2001; Mansour *et al.*, 2010) and this is an issue for growers as it increases their perception of risk (Trumble, 1998; Gutierrez *et al.*, 1999; Wawrzynski *et al.*, 2001). Greenhouse environmental conditions are thought to be an important contributory factor to this (Stenseth, 1979; Nihoul, 1993; Nihoul and Hance, 1993) and control breakdown has been associated with hot, dry conditions (Osborne and Oetting, 1989; Nihoul, 1993; Mansour *et al.*, 2010). For this reason it is essential to understand the effect of ambient humidity on the predation behaviour of this mite. In fact, given the popularity of this biological control agent it is remarkable that so little work has been carried out in this area as, even taking into account the mitigating influence of the leaf boundary layer (Ferro and Southwick, 1984), ambient humidity appears to have important impacts on *P. persimilis*.

To accurately predict the effect of novel greenhouse climate conditions on the ability of *P. persimilis* to regulate *T. urticae* populations it is clear that detailed information regarding the affect of humidity on functional response is needed. This experiment considered the effect on functional response of a range of humidities that reflect conditions observed in greenhouse production across Europe. It also investigated the effect of plant host on functional response and assessed whether any interactions with humidity occur.

3. 2 Materials and methods

The experiment assessed the effect on functional response of five levels of humidity (57%, 65%, 76%, 85% and 99% RH), four levels of prey availability (10, 20, 40 and 80 eggs) and two hosts (*Lycopersicon esculentum* and *Choisya ternata*). Data from sixteen commercial greenhouses in the Netherlands (Garcia, pers. comm.), Spain (Romero, pers. comm.) and Hungary (Bálint, pers. comm.) suggest that the humidity range chosen would cover most of the observed variation in glasshouse humidities. Preliminary results also showed very poor levels of control by *P. persimilis* below 50% RH, a finding supported by Nihoul (1992).

The range of prey availabilities chosen was based on other functional response work with *P. persimilis* that suggested that most of the variation in predation rate would fall within it (Mori and Chant, 1966b; Takafuji and Chant, 1976; Everson, 1980; Ryoo, 1986; Skirvin and Fenlon, 2001; Skirvin and Fenlon, 2003a). The *T. urticae* life stage chosen was the egg stage since this is the preferred food stage for *P. persimilis* (Takafuji and Chant, 1976).

L. esculentum (tomato) and *C. ternata* were selected as hosts due to the morphological differences of their leaf structure and because they represent two types of horticulture production; fruit and ornamental. Tomato leaves are pubescent with abundant glandular hairs, whereas *C. ternata* leaves are glabrous and glossy. Furthermore, *C. ternata* was the host plant used in related studies investigating the influence on *P. persimilis* biological control efficacy of morphology (Skirvin and Fenlon, 2001), canopy structure (Skirvin and Fenlon, 2003b) and temperature (Skirvin and Fenlon, 2003b; Skirvin and Fenlon, 2003a) and was also the host used in a model examining the effectiveness of biological control strategies (Skirvin *et al.*, 2002). Using *C. ternata* would allow better comparisons of results with those studies as well as a better integration of the findings into a population dynamics model (as will be discussed in Chapter 6).

P. persimilis are known to use webbing (Ryoo, 1986; Mayland *et al.*, 2000) and herbivore-induced plant volatiles (Sabelis and van de Baan, 1983; Dicke *et al.*, 1998; Mayland *et al.*, 2000; van den Boom *et al.*, 2002; Sznajder *et al.*, 2011) to search for prey. With few exceptions (Skirvin and De Courcy Williams, 1999; Skirvin and

Fenlon, 2001; Skirvin and Fenlon, 2003b) the majority of functional response work for this mite has neglected this important aspect of prey searching behaviour, which if not considered may affect the reliability of results. To avoid this, the plant material used in this experiment was pre-infested with *T. urticae* to ensure that webbing and olfactory cues were present in the experimental arenas.

3. 2. 1 Preliminary work

The arenas used for *P. persimilis* functional response experiments are varied, ranging from paper (Mori and Chant, 1966b; Takafuji and Chant, 1976) and leaf discs (Ryoo, 1986) to plant stems (Skirvin and De Courcy Williams, 1999; Skirvin and Fenlon, 2001; Skirvin and Fenlon, 2003b). However, as artificial substrates are known to affect functional response (Everson, 1980) it was felt that a realistic arena would be needed to ensure results are an accurate reflection of behaviour in production conditions. Initially plant stem arenas (Skirvin and De Courcy Williams, 1999; Skirvin and Fenlon, 2001; Skirvin and Fenlon, 2003a) were used as it was felt they would most closely replicate the searching options the predator would be presented with in normal conditions.

For this assay, 20 cm cut stems of tomato or *C. ternata* were placed individually in 50 ml flasks. These were then pre-infested with *T. urticae* for 2-3 days in ventilated plant propagators. The propagators were then sealed and carbon dioxide added to kill the active *T. urticae* stages. Eggs were then added or removed to reach the desired availabilities. The flasks containing the cut stems were then placed in individual Petri dishes filled with water and detergent (this broke the surface tension of the water, preventing the mites from walking across) in a controlled environment cabinet controlled for humidity and temperature. A single gravid female *P. persimilis* was placed on each cutting and left for 24 hours after which the remaining eggs were counted.

A number of issues with this method meant that it was not a feasible approach for this work. For instance, previous work had suggested that carbon dioxide exposure of three hours would kill all active *T. urticae* stages (Skirvin and Fenlon, 2001) however this could not be achieved even after 24 hours exposure, a duration which

also damaged the host plants. Inundation with *P. persimilis* followed by carbon dioxide exposure was not an effective method of killing all active stages either. There was an additional issue of high experimental mortality and loss of *P. persimilis* with large proportions dying in the water at the base of the conical flask. This presented an insurmountable problem as results are discounted from arenas in which *P. persimilis* are either dead or not recovered. Gaede (1992) found strong positive hygrotactic behaviour in *P. persimilis* subjected to low relative humidities and that survival was greatly affected by the absence of water sources, which may explain this phenomena. A number of variations were attempted including using wet absorbent cotton wool, barriers such Vaseline and Fluon® to prevent the mite from leaving the stem and the use of floating leaf arenas. However, none prevented the high mortality rates of the *P. persimilis*.

3. 2. 2 Experimental protocol

A final protocol was devised that utilised a compromise arena system that maintained a degree of realism and reduced *P. persimilis* mortality to acceptable levels. Infested leaves were placed in individual, sealed plastic boxes (12x8x2 cm) with two 5x5 cm fine mesh covered windows, which would allow external humidity conditions to enter but prevent the mites from leaving.

Relative humidity (RH) was maintained by placing the cage on a wire grill above a salt solution in a sealed box. At any given temperature specific salt solutions are able to maintain the water content of the air above it at a constant as long as the salt is in excess and the vapour and water phases are in equilibrium (Winston and Bate, 1960). This can be achieved by maintaining a sealed system and such methods have been used in similar experiments (Shipp *et al.*, 1996; De Courcy Williams *et al.*, 2004b). The salts used in solution were $Mg(NO_3)_2$ (57% RH and 65% RH), NaCl (76% RH), KCl (85% RH) and KNO_3 (99% RH).

Plants were pre-infested for one day; thus allowing *T. urticae* to produce webbing on the leaves and for herbivore-induced plant volatiles to be produced. The relatively short infestation period also prevented prey populations from getting too large. Leaves (or leaflets in the case of tomato) were then excised, active stages killed

manually and eggs added or removed to the desired prey availability (10, 20, 40 or 80). Care was taken to ensure that all leaves were of comparable size (approximately 5x3 cm). Each leaf was then placed in a cage (Fig. 3.2a) with one gravid female *P. persimilis* and the cage placed inside a humidity controlled box (Fig. 3.2b). The boxes were kept in a controlled environment cabinet for 24 hours at 24°C and a 16:8 light: dark cycle (Fig. 3.2c). After 24 hours the eggs were counted and the *P. persimilis* was located to ensure survival. Results were discarded from assays in which the predator was not recovered. Conditions within the boxes were recorded using data loggers (HydroLog, NT Series, Rotronic). Probes from the data loggers entered each box through a small notch cut where the lid meets the box. The space around the notch was sealed with Blu-Tack®. Control mortality was established by including control arenas, containing infested leaves (at the specific egg availabilities) but without *P. persimilis*, in the boxes. Eggs were counted after 24 hours to establish a baseline egg mortality/loss.

Figure 3.2: a) Experiment arena containing a *C. ternata* leaf infested with *T. urticae* eggs; b) Box containing arenas and salt solution to maintain humidity; c) Controlled environment cabinet containing boxes and data loggers.

T. urticae used for the infestation process were taken from a culture kept at approximately 20°C and 50-90% RH on dwarf French bean (*Phaseolus vulgaris* cv. Masterpiece). This culture had been maintained at the University of Warwick Wellesbourne campus continuously for a number of years. *P. persimilis* (Fargro, UK) were maintained on platforms as described in Overmeer (1985) and fed on either *T. urticae* infested *P. vulgaris* leaves or tomato leaves. *P. persimilis* were taken from the *P. vulgaris* culture if they were to be used in functional response experiments on *C. ternata* leaves. If they were to be used in functional response experiments on tomato leaflets *P. persimilis* were taken from the tomato culture. *P. persimilis* are known to adapt to their host type over generations (Drukker *et al.*, 1997; Croft *et al.*, 1999) and this approach allowed the predators to be somewhat pre-adapted to the leaf structures encountered on tomato.

3. 2. 3 Statistical analysis

Mortality/loss rates in some of the control conditions were higher than expected so a generalised linear model was used to identify those observations that were markedly higher than expectation. This was done in GenStat® (v12) by highlighting high value residuals in a regression fitted to the control data on each host. Residuals higher than 4 were removed as these represented extreme points ($P > 0.9999$) under a standard normal distribution. This analysis removed from further analysis five control values on *C. ternata* and none on tomato. Residuals with markedly low values were ignored as minimising control mortality is desirable. It is possible that the controls with higher than expected egg losses were due to eggs simply falling off the leaf. In setting up the experiments eggs were placed manually to reach the desired egg availability and in some cases these may not have been sufficiently secured to the leaf (especially on the glossy *C. ternata* leaves).

To assess whether humidity, egg availability or their interaction had a significant effect on the egg mortality analysis of deviance was carried out in GenStat® (v12). Following this the functional response curves were modelled. The Holling's 'disc equation' (1959) was the first to describe functional response curves using non-linear equations and, along with its derivative (Rogers, 1972), has been widely used (e.g., Hassell *et al.*, 1977; Chesson , 1989; De Clerq *et al.*, 2000). However, these models

are deterministic and do not allow for stochastic variation and as this data was to be used in a stochastic population dynamics model (see Chapter 6), a different approach was needed. Binomial mixture models do allow for stochastic variation and have been used in other functional response work (Skirvin and Fenlon, 2001; Skirvin and Fenlon, 2003b). They are also the approach recommended by Juliano (2001). However, as is the case with many functional response experiments (Casas and Hulliger, 1994; Fenlon and Faddy, 2006), the variance of the predation rate in this work increased dramatically with increasing prey availabilities. A beta-binomial distribution has been recommended as a remedy for over-dispersion such as this (Fenlon and Faddy, 2006; Faddy *et al.*, 2010) as it provides "a more realistic interpretation of the experimental data" (Fenlon and Faddy, 2006). This involves fitting separate beta-binomial distributions to each level of prey availability thus ensuring that variation is not underestimated.

Modelling of the functional response was then carried out in Matlab® using maximum likelihood estimation, which attempts to optimise the parameters given the data as proportions. Modelling predation rates is complicated by the fact that egg loss in the predator treatments is due to a combination of predation by *P. persimilis* and natural (or control) egg mortality. Therefore, to assess predation rates it is necessary to understand the control mortality at each availability and humidity. For the control treatments egg loss is simply assumed to be proportional to egg availability in the following manner:

$$Control\ egg\ loss = \lambda * availability \qquad 3.1$$

Where λ = is the proportion of eggs dying of natural causes. The first step was to assess whether the egg loss in the control treatments was consistent across all humidities or whether separate models for each humidity would be needed.

Next, the egg losses in the predator treatments were modelled. Mixture models were used that contained two components; a term for control mortality and one for predation (Eq. 3.2). The first component relates to the proportion of insects lost to control (or natural) mortality (availability.λ). Secondly, the predator component has the proportion (1 – λ), which is subject to a similar proportionality process (the multiplier 'availability'), which is moderated by the function a + b.log(availability), a declining value function with a negative slope.

$$Combined\ egg\ loss\ =\ availability * [\lambda + (1-\lambda) * \{a + b * \log(availability)\}] \quad 3.2$$

Once the parameters for the mixed (combined) model (Eq. 3.2) have been determined the functional response of predator can be plotted using Equation 3.3.

$$Predator\ egg\ consumption\ =\ availability * \{a + b * \log(availability)\} \quad 3.3$$

A number of mixture models were assessed with each varying in the number of parameters used. The most complex model had separate control (λ) and slope and intercept (a and b) parameters for each humidity. The simplest model had common parameter values across all humidities. Starting with the most complex, the models assessed were:

Model 1: Separate mixture models for each humidity.

Model 2: Separate models for the predator component with a common control.

Model 3: Separate slopes for the predator component with a common control and intercept.

Model 4: A single model for all the responses (common control and predator components).

Model 4 is the most parsimonious with only four parameters, while Model 3 has eight, Model 2, twelve and Model 1, twenty (4 parameters for each humidity). Each Model is a simplification of the previous Model.

The process of modelling was hierarchical. The most complicated model was developed first and compared to the next most complex, which in turn was compared to the next and so on. Models with different numbers of parameters can be compared as long as they have the same form. The likelihood ratio test (see, e.g., Fenlon & Faddy, 2006) was used to compare the models. This assesses and compares the likelihood of the data under each model. Twice the difference in the log-likelihood values is distributed according to the chi-squared distribution and can be tested using the tails of the distribution. Models with greater numbers of parameters always have a greater log-likelihood (but note that log-likelihoods are always negative as they represent the logarithm of probabilities between 0 and 1) and they represent a better fit to the data. When compared with simpler models, model selection depends on

whether this reduction in log-likelihood is significant. When the difference is judged not significant the more parsimonious model, i.e. the one with fewer parameters, is chosen. This process was applied to the modelling of egg loss in both the control and predator treatments. In summarising the analyses all significant differences are followed by the approximate chi-square (X) or F statistic, the degrees of freedom (df) and the probability that the null hypotheses can be rejected (based on the approximate X or F).

3. 3 Results

3. 3. 1 Functional response on *C. ternata*

Egg loss/consumption in the predator treatment increased significantly with egg availability (F = 4.29, df = 3, P = 0.008), ranging from a mean of 8 lost at an availability of 10 eggs to a mean of 42 lost at an availability of 80 eggs (Table. 3.1). Humidity significantly affected egg loss varied (F = 3.49, df = 4, P = 0.012; Table 3.1) and this effect was especially evident at the high egg availabilities, with 45, 35, 32, 30 and 25 lost at 85%, 99%, 76%, 65% and 57% RH respectively (averaged across availabilities of 40 and 80 eggs). However, the interaction of humidity and egg availability was not significant. In the control treatment (Table 3.1) an increase in egg loss with availability was evident however, analysis of deviance found the effect of this factor, humidity and their interaction to be non-significant.

Table 3.1: *T. urticae* egg loss on *C. ternata* in *P. persimilis* (predator) and control treatments at varying egg availabilities and humidities (% RH). Standard errors are shown in brackets.

		Egg availability			
Treatment	**RH (%)**	**10**	**20**	**40**	**80**
Predator	57	8.5 (0.9)	15.3 (1.7)	20.8 (4.1)	29.3 (2.4)
	65	7.8 (0.8)	12.8 (2.3)	22.0 (2.7)	38.3 (7.5)
	76	9.0 (0.5)	15.4 (1.3)	22.4 (6.0)	41.8 (6.9)
	85	7.0 (1.5)	16.5 (2.2)	34.3 (2.6)	56.0 (9.1)
	99	6.8 (1.4)	11.8 (2.7)	25.0 (5.2)	45.0 (10.1)
Control	57	2.3 (0.9)	4.0 (2.5)	11.0 (2.6)	22.3 (6.1)
	65	1.8 (0.9)	2.8 (1.1)	16.8 (5.1)	20.3 (7.4)
	76	1.5 (0.3)	3.2 (1.4)	5.8 (1.0)	13.8 (2.6)
	85	4.7 (0.9)	5.2 (2.6)	8.3 (2.0)	15.7 (3.8)
	99	3 .0 (0.6)	2.0 (0.9)	6.4 (2.1)	17.7 (4.7)

Analysis of the models for the control mortality (Table 3.2) found that there was no significant difference between a model that fitted a common mortality rate for all humidities and a model that fitted separate mortality rates for each humidity. As the common model had fewer parameters it was chosen.

Table 3.2: Proportional loss (*lambda*) and variability (*theta*) parameter values of models fitted to the egg loss in the control treatments on *C. ternata*. Models were fitted at each humidity separately and to all humidities combined. Model comparisons were made using the negative log-likelihood value. The lack of significance meant that the combined model was chosen.

RH (%)	- log-likelihood	*lambda*	*theta*
57	38.82	0.24	0.07
65	31.26	0.18	0.06
76	38.01	0.16	0.01
85	38.01	0.22	0.05
99	42.87	0.17	0.06
Separate	188.97		
Combined	192.73	0.19	0.06
chi-sq	7.51		
d.f.	8		
P	0.48		

Analysis of the models for the mortality in the predator treatment (Table 3.3) found that Model 3 was the most descriptive and parsimonious. This model had a common control mortality (supporting the findings of the previous analysis) and intercept value but with separate values for the slope at each humidity. The parameters for this model can be seen in Table 3.4. Parameter values for the other models can be seen in Appendix 1.

Table 3.3: Comparison of the four models fitted to the egg loss in the predator treatments on *C. ternata*. Model comparisons were made using two times the negative log-likelihood value. Model 3 was the model with the least parameters and most descriptive power.

Model no.	- log-likelihood	Comparison	- 2 × log-likelihood	d.f.	prob
1	467.81				
2	473.88	2 - 1	12.14	8	0.15
3	475.83	3 - 2	3.89	4	0.42
4	481.90	4 - 3	12.14	4	0.02

Table 3.4: Parameter values for the model describing functional response of *P. persimilis* on *T. urticae* eggs on *C. ternata* (Model 3). *Lambda* represents the egg loss due to natural mortality.

lambda	**Intercept (*b*)**	**Slope (*a*) at humidity (% RH)**				
		57	**65**	**76**	**85**	**99**
0.21	1.10	-0.18	-0.18	-0.15	-0.11	-0.16

The functional response (Eq. 3.3), the control mortality (Eq. 3.1) and the raw data can be seen in Figure 3.3. Lower predation rates are predicted at low humidities (57-65% RH) and at very high humidity (99% RH). The greatest rate of predation is predicted at 85% RH.

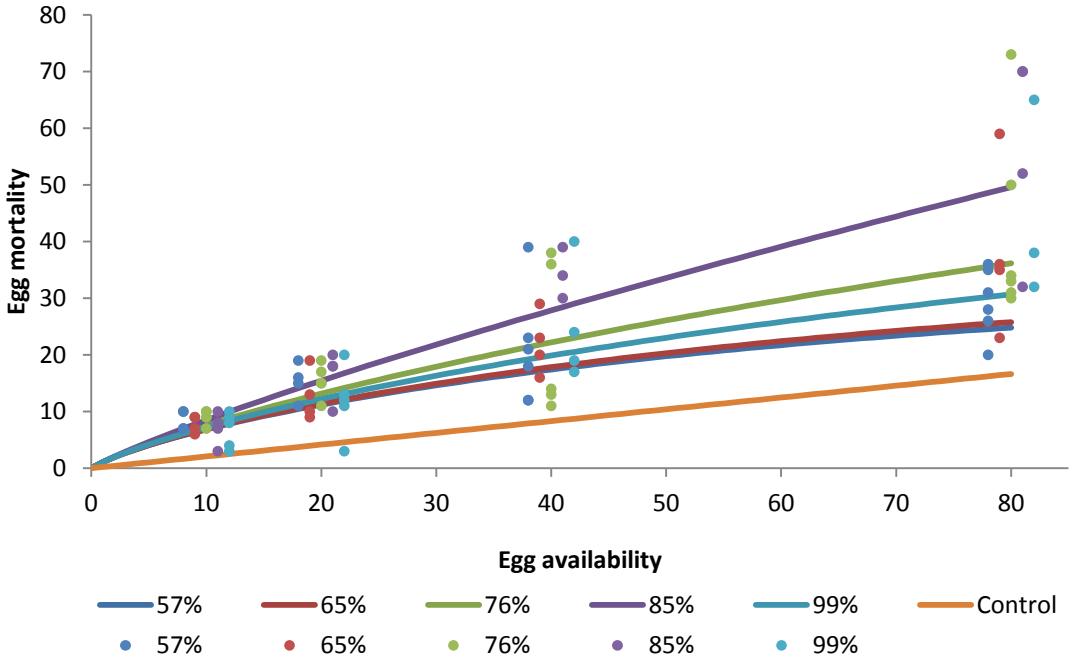

Figure 3.3: Functional response of *P. persimilis* on *T. urticae* eggs on *C. ternata* at five humidity levels (% RH). Fitted equations (—) and observed values (•) are given for each humidity. For control mortality the fitted equation only is given. The observed values are jittered slightly around the egg density for clarity.

3. 3. 2 Functional response on tomato

Egg availability had a significant effect on egg loss in the *P. persimilis* treatment ($F = 8.32$, df = 3, $P = <0.0001$) with a mean of 7 lost at an availability of 10 eggs and 36 lost at an availability of 80 eggs (Table 3.5). Humidity was not found to have a significant impact on egg loss in the *P. persimilis* treatment with average egg loss ranging from 18 at 99% RH to 21 at 85% RH (Table 3.5). The interaction of humidity and egg availability was also non-significant. The egg loss in the control treatments tended to increase with availability but this affect was not significant and nor was the influence of humidity or their interaction (Table 3.5).

Table 3.5: *T. urticae* egg loss on tomato in *P. persimilis* (predator) and control treatments at varying egg availabilities and humidities (% RH). Standard errors are shown in brackets.

		Egg availability			
Treatment	**RH (%)**	**10**	**20**	**40**	**80**
Predator	57	8.0 (0.4)	13.5 (1.4)	30.2 (2.9)	32.2 (3.9)
	65	7.4 (0.7)	14.3 (1.8)	23.6 (5.5)	37.3 (2.5)
	76	5.3 (1.3)	17.2 (0.7)	22.1 (3.1)	32.8 (4.9)
	85	7.8 (1.7)	16.3 (1.9)	24.0 (5.1)	37.6 (1.3)
	99	6.0 (1.6)	12.0 (2.5)	18.2 (5.2)	37.8 (7.0)
Control	57	2.3 (0.9)	5.0 (0.7)	9.3 (3.0)	7.7 (7.2)
	65	0.0 (0.0)	4.0 (1.0)	7.8 (3.1)	14.0 (6.2)
	76	0.7 (0.3)	5.5 (0.5)	8.7 (4.3)	9.0 (4.0)
	85	0.5 (0.5)	4.0 (1.7)	7.0 (2.1)	14.7 (3.0)
	99	0.7 (0.3)	3.3 (1.3)	2.3 (1.2)	14.5 (2.5)

Analysis of the models for the control mortality (Table 3.6) found that there was no significant difference between a model that fitted a common mortality rate for all humidities and a model that fitted separate mortality rates for each humidity. As the common model had fewer parameters it was chosen.

Table 3.6: Proportional loss (*lambda*) and variability (*theta*) parameter values of models fitted to the egg loss in the control treatments on tomato. Models were fitted at each humidity separately and to all humidities combined. Model comparisons were made using the negative log-likelihood value. The lack of significance meant that the combined model was chosen.

RH (%)	- log-likelihood	*lambda*	*theta*
57	35.5493	0.194	0.1472
65	30.788	0.155	0.0981
76	28.8376	0.1578	0.1424
85	29.7239	0.1644	0.0226
99	23.5102	0.1191	0.0384
Separate	148.409		
Combined	151.3683	0.1573	0.0971
chi-sq	5.9186		
d.f.	8		
P	0.65635		

As the analysis of deviance found no effect of humidity on egg loss on tomato the most complex mixed model (Model 1) was first compared to the simplest mixed model (Model 4). Analysis of these models found that the complex model was not significantly more descriptive than the simple model (Table 3.7). As the simplest model was most parsimonious it was chosen. This model had common parameter values at each humidity for control mortality, slope and intercept (Table 3.8). Models 2 and 3 were not constructed as they would not have been more descriptive than Model 4. Parameter values for the Model 1 can be seen in Appendix 1.

Table 3.7: Comparison of the two models fitted to the egg loss in the predator treatments on tomato. Model comparisons were made using the negative log-likelihood value. Model 4 was chosen as it had the least parameters and most descriptive power.

Model no.	- log-likelihood	Comparison	- 2 × log-likelihood	d.f.	prob
1	486.11				
4	494.23	4 - 1	16.25	16	0.44

Table 3.8: Parameter values for the model describing functional response of *P. persimilis* on *T. urticae* eggs on tomato. *Lambda* represents the egg loss due to natural mortality.

lambda	Intercept (*b*)	Slope (*a*)
0.17	1.06	-0.16

The functional response (Eq. 3.3), the control mortality (Eq. 3.1) and the raw data can be seen in Figure 3.4. Predation increases with prey availability forming a type II response curve (Holling, 1959). There was no clear pattern in mortality in relation to humidity.

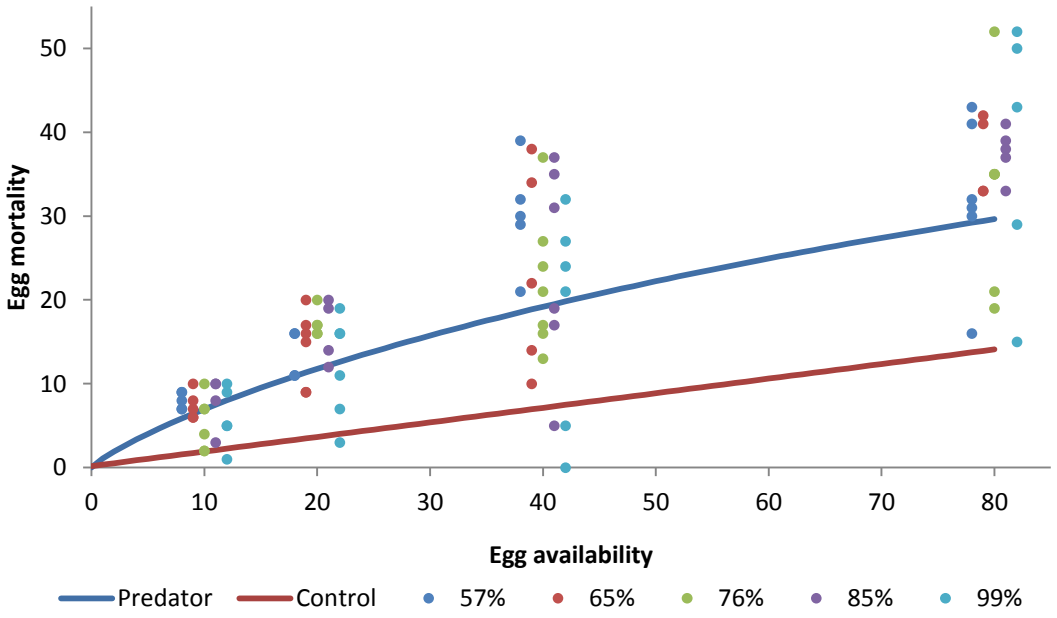

Figure 3.4: Functional response of *P. persimilis* on *T. urticae* eggs on tomato at five humidity levels (% RH). The fitted predator equation is a combined one for all humidities (—) (Model 4). The observed values (•) are given for each humidity. For control mortality the fitted equation only is given. The observed values are jittered slightly around the egg density for clarity.

3. 3. 3 Comparative host effects on functional response

When the egg loss from both hosts were included together, analysis of deviance found that only egg availability had a significant effect on predation (F = 12.1, df = 3, P = <0.0001). Humidity (F = 2.1, df = 4, P = 0.083) and its interaction with host

(F = 2.01, df = 4, P = 0.096) had a marginally significant effect on predation. The effect of host plant was not found to be significant. The loss of a significant effect of humidity in this analysis is likely due to an effect on *C. ternata* but not tomato. Analysis of deviance of the control mortality including both hosts found no significant effects, except for a marginal effect of the interaction of host and egg availability (F = 2.43, df = 3, P = 0.07).

To further investigate the predation behaviour on these two hosts, comparisons of the most complex models for each humidity (Model 1) were made between the hosts. Analysis of these models showed that there was no significant difference between any of the models, except for a marginally significant difference in the models at 85% RH (the optimal humidity for predation on *C. ternata*; X = 7.81, df = 4, P = 0.099; Table 3.9).

Table 3.9: Comparison of the models at each humidity between *C. ternata* and tomato. The negative log-likelihood of fitting separate models (Sep) for the hosts at each humidity was compared with that of fitting a combined model (Com) for the two hosts.

RH (%)	Model	- log-likelihood	Comparison	- 2 × log-likelihood	d.f.	prob
57	Separate	196.17				
57	Combined	197.74	Sep57 - Com57	3.13	4	0.536
65	Separate	169.48				
65	Combined	170.67	Sep65 - Com65	2.39	4	0.665
76	Separate	214.10				
76	Combined	216.49	Sep76 - Com76	4.78	4	0.311
85	Separate	174.35				
85	Combined	178.26	Sep85 - Com85	7.81	4	0.099
99	Separate	199.81				
99	Combined	201.54	Sep99 - Com99	3.46	4	0.484

The fitted models for each host at each humidity reveal a wide range of response to humidity on *C. ternata* compared to the far narrower response to humidity on tomato (Fig. 3.5). These models differ to those shown above for *C. ternata* (Fig. 3.3) and tomato (Fig. 3.4) as these have separate parameters for control mortality, intercept and slope.

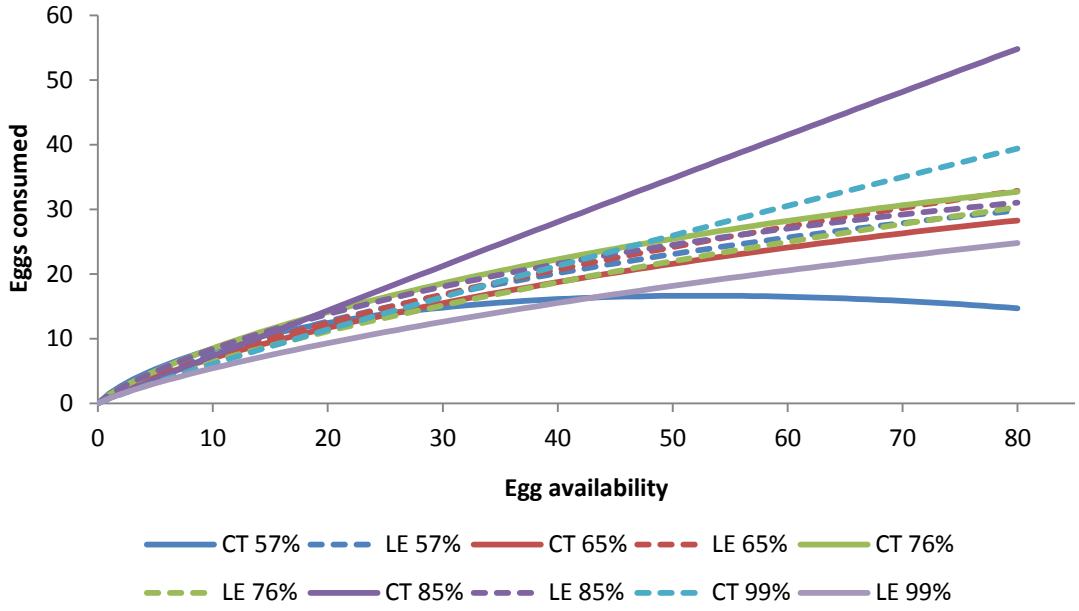

Figure 3.5: Fitted functional response curves of *P. persimilis* on *T. urticae* eggs on *C. ternata* and tomato at five humidity levels (% RH). Solid lines = *C ternata* (CT); broken lines = *L. esculentum* (CT).

A comparison of the best fitting models selected for each host, Model 3 for *C. ternata* (Section 3. 3. 1) and Model 4 for tomato (Section 3. 3. 2), can be seen in Fig. 3.6 and shows that predation is higher at 76-85% RH on *C. ternata* and higher at 57-65% RH on tomato.

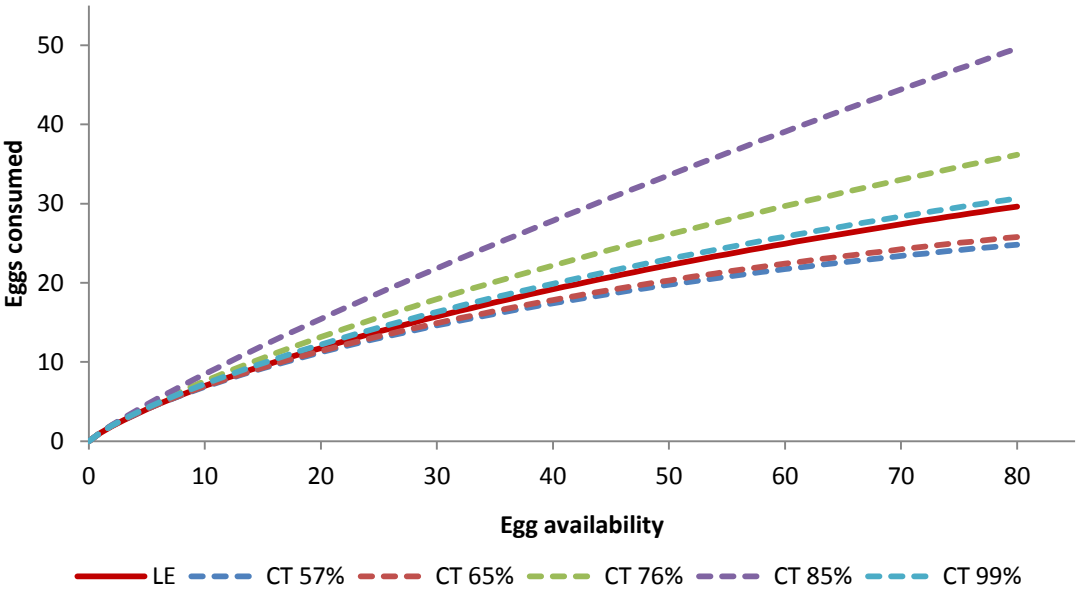

Figure 3.6: Comparison of the selected models for the functional response of *P. persimilis* on *T. urticae* eggs on *C. ternata* (CT) and tomato (LE) at five humidity levels (% RH). Solid lines = *L. esculentum* (Model 4); broken lines = *C. ternata* (Model 3).

3. 4 Discussion

P. persimilis can be very effective at reducing populations of *T. urticae*. Its high rates of predation (Laing and Huffaker, 1969), development and fecundity (Amano and Chant, 1977) have made it the most widely used biological control agent of *T. urticae* on protected crops (McMurtry and Croft, 1997). Its importance in this regard has meant that the influences of a wide range of biotic and abiotic factors, that directly or indirectly affect its control efficacy, have been well studied. A factor that has received scant attention is the role of humidity.

This work showed that the predation rate of *P. persimilis* is affected by ambient humidity conditions in the range of 57-99% RH. The data suggested that on *C. ternata* the highest consumption rate was around 85% RH with reductions in consumption at higher and lower humidities. However, this effect appeared to be mediated by the host plant as on tomato the influence of humidity was lost. When models were fitted to the egg losses at different egg availabilities on *C. ternata* the best fit was found with a model that had a different slope for each humidity with a shared intercept and control mortality. This showed that, while humidity had a minor effect at low prey availabilities, predation at 85% RH was clearly greater than at other humidities as prey became more abundant. Predation dropped slightly at 76% RH and again at 99% RH. The model predicted that the largest reductions in predation were found at 65% and 57% RH. Regardless of humidity, the fitted response was a classic Type II curve; a decelerating increase in consumption with availability. At high prey numbers the predator is limited in the number of prey items it can kill by the time taken to process each kill, eventually producing a plateaux in the predation rate where increases in prey availability produce no or little increase in predation.

When models were fitted to the predation on tomato the data was best described by a model with a single slope, intercept and control mortality, regardless of humidity. Just as for *C. ternata* the pattern of predation formed a Type II response curve. Comparison between the fitted functional response curves on the two hosts shows that the maximum predation rate was seen on *C. ternata* (at 85% RH). The response curve for tomato was similar to that at 99% RH for *C. ternata* and lower than that at

75% RH. However, predation rate at 57-65% RH was higher on tomato than on *C. ternata*.

The response curve type and the consumption at low prey densities on *C. ternata* compare well with other work carried out on the same host (Skirvin and De Courcy Williams, 1999; Skirvin and Fenlon, 2001; Skirvin and Fenlon, 2003b). All these papers studied functional response on this plant at 23.2°C and 57.5% RH so comparison of the predation behaviour is best made at the 57% RH level in this work. Such a comparison shows that the fitted model curve at 57% RH is similar to the model fitted in Skirvin and De Courcy Williams (1999) up to an availability of around 50 eggs. Above this the model at 57% RH in this work predicts a lower consumption rate. The model differs more from those fitted in Skirvin and Fenlon (2001) and Skirvin and Fenlon (2003b) estimating a lower level of predation above an availability of 20 eggs.

The effect of prey availability on the functional response of *P. persimilis* on tomato has not previously been studied so direct comparison of results is not possible. However, the control efficacy of *P. persimilis* on tomato is known to be less effective than on other crops (Drukker *et al.*, 1997) and in this work maximum predation rate on tomato was lower than that observed on *C. ternata*. This reduced level of predation may be due to the contrasting morphological structures of the leaves. While *C. ternata* leaves are waxy, smooth and glabrous, tomato leaves are pubescent with many glandular trichomes. High *P. persimilis* mortality rates on tomato have been attributed to the poisonous and sticky trichome exudates (Van Haren *et al.*, 1987; Nihoul, 1993; Nihoul, 1994).

These structures are also thought to hamper movement and foraging and when this was studied by varying trichome density on chrysanthemums (Stavrinides and Skirvin, 2003) and *Gerbera jamesonii* (Krips *et al.*, 1999) it was shown that lower densities led to higher predation by *P. persimilis*. Functional response studies on other biological control agents have also shown that these structures on tomato reduce predation rates (De Clercq *et al.*, 2000; Cedola *et al.*, 2001; Sato *et al.*, 2011). Differences in *P. persimilis* functional response between ornamentals were also attributed largely to leaf morphology (Skirvin and Fenlon, 2001). *P. persimilis* are known to be less effective on tomato when reared on *P. vulgaris* and this effect can

to some extent be counteracted if reared on tomato (Drukker *et al.*, 1997; Croft *et al.*, 1999). It was for this reason that the *P. persimilis* used in the experiments on tomato were purposely reared on tomato in an attempt to avoid the detrimental effects of non-conditioning.

It is possible that the reductions in predation on tomato were also due to an indirect effect of the host through an accumulation of plant toxins in the *T. urticae* eggs. In this case the eggs came from spider mites reared on *P. vulgaris*. Pre-infestation of the plant material used in the experiment occurred by placing infested *P. vulgaris* leaves on the clean plants. The *T. urticae* were then left for 24 hours to oviposit on the clean plants. This does present a possibility that during this period the mites absorbed plant toxins and passed them onto their eggs. However, it was rarely the case that sufficient oviposition had occurred after this period so eggs were added manually and these came directly from the *P. vulgaris* culture. As these eggs often outnumbered those actually oviposited on the experimental leaves it is felt that any indirect effects of host plant would have had a minimal impact on the results.

For all the detrimental impacts leaf structures may have on predatory mites there may be some that are beneficial. For instance, Roda *et al.* (2000) suggest that predatory mites are more abundant on plants with pubescent leaves or domatia (leaf structures often formed from leaf hairs) because they offer protection from intraguild predation, while Grostal and O'Dowd (1994) suggest that domatia offer protection from arid conditions. It is this latter role of leaf structures that may explain the influence of ambient humidity in these experiments. Humidity is known to affect *P. persimilis*, for instance the longevity of starving adults increase with humidity (Mori and Chant, 1966a; Bernstein, 1983; Gaede, 1992). Egg stage duration and mortality are also known to increase with decreasing humidity (Stenseth, 1979; Perring and Lackey, 1989; De Courcy Williams *et al.*, 2004b). In fact Ferrero *et al.* (2010) found that at 51.5% RH 100% egg mortality occurred and that the minimum humidity at which 50% egg hatch occurred was 70% RH.

This work found that humidities below 77% RH were sub-optimal for *P. persimilis* on *C. ternata*, with humidity below 66% RH particularly detrimental. This is in agreement with other work that found that *P. persimilis* control efficacy was reduced at low humidities (Stenseth, 1979; Palevsky *et al.*, 2008; Mansour *et al.*, 2010).

However, Mori and Chant's (1966a) functional response work found that predation was greatest at 33% RH. This markedly different result is difficult to explain; especially in light of the detrimental effects other work has shown humidities as low as this can impart. A possible reason is the differing arenas used; Mori and Chant (1966b) conducted their experiments on 2-inch square paper sheets, which contained up to 32 adult *T. urticae*. As the activity of *P. persimilis* increases with decreasing humidity (Mori and Chant, 1966a) it may that the increased concentration of prey in their work made locating prey easier, resulting in increased consumption rates. It seems likely that low humidities are physiologically stressful for *P. persimilis*, and it has been remarked that they are the least low-humidity tolerant of a number of Phytoseiids commonly used in biological control (De Courcy Williams *et al.*, 2004b; Ferrero *et al.*, 2010). Very high humidity (99%) was also found to reduce predation rate in this work. Mori and Chant (1966a) found that at 100% RH activity of these mites declines rapidly and "more than 90% of the mites ceased activity after 4 hours" (Mori and Chant, 1966b).

The responses to humidity observed on *C. ternata* may be due to the effect of ambient humidity on the water balance of the mites. In Gaede's (1992) comprehensive paper on the maintenance of water balance in *P. persimilis* it was shown that transpiration rate is linearly dependent on relative humidity, increasing as humidity decreases. Transpiration was described as "a (purely physical) diffusion process" and it was suggested that there was no mechanism to limit water loss at low humidities. The same paper also describes the sorption capability of this mite to maintain its water balance; that between 15-25°C it is able to absorb water from the environment when humidity is above 89% RH. However, Gaede (1992) calculated that under conditions of still air and ambient humidity as low as 20-30% RH, humidity at the leaf surface would still be high enough for *P. persimilis* to use its sorption capabilities to maintain water balance, i.e., around 90% RH. It should be noted though that this calculation was based on a cucumber leaf (which is pubescent) 15 cm in diameter while the *C. ternata* leaves used in this work were smooth and had a maximum diameter of 6 cm. This would have reduced the leaf laminar layer considerably (Nobel, 1974). Furthermore, *C. ternata* are considered drought resistant plants with waxy leaves that reduce water loss and they may also have stomatal adaptations that increase the diffusion resistance of water vapour. This

would reduce the water vapour activity at the surface of the leaf, further reducing the leaf laminar layer (Nobel, 1974).

It is therefore possible that the dehydration caused by increased transpiration at low humidity levels may have compromised the ability of the mite to function and hindered or altered their searching behaviour, resulting in reduced predation. Even though Gaede (1992) found negligible reductions in the activity of water-deficient mites it was suggested that such mites in low leaf-surface humidity conditions may prefer to spend time in areas of the leaf where water loss is lower. Indeed Bernstein (1983) found that under low humidity conditions these mites preferably seek out shade, where conditions would be cooler and more humid. Such areas could still be found on *C. ternata* leaves, by the mid-rib for instance. An alternative explanation is that their high levels of activity at low humidities meant that they simply became lost in an arena in which the plant material represented a relatively small proportion of the area. Conversely, the lack of water loss experienced by the mite at high humidities may explain the reduced predation at 99% RH. Food intake was calculated to be 75% water when feeding on adult *T. urticae* (Gaede, 1992), which suggests that restoring water balance may be as important a motivating factor in searching for food as nutritional intake. At high humidities the need to restore water balance may be lower and the mite may be more quickly satiated when feeding does occur.

The lack of humidity effects on tomato may be due to the presence of trichomes. While these structures may have reduced the effectiveness of searching in the mites they may also have increased the leaf laminar layer, helping trap moisture at the leaf surface and effectively negating the impact of ambient humidity conditions. In fact Roda *et al.* (2000) found that both *P. persimilis* and *Typhlodromus pyri*, another predatory mite, actively seek out webbing and leaf-surface structures and suggest that by doing this they are "more likely to survive predation and abiotic hazards such as wind, rain, and low humidity than on glabrous leaf surfaces." It was noticed that retrieval rates of *P. persimilis* were far lower in the *C. ternata* treatments than the tomato treatments (data not shown), especially in the low humidity treatments, which supports this contention.

The absence of refuges on excised *C. ternata* leaves may also explain the lower predation rates observed compared to those recorded in other work on the same host (Skirvin and De Courcy Williams, 1999; Skirvin and Fenlon, 2001; Skirvin and Fenlon, 2003b). These papers used cut stems trimmed to four trifoliate leaves placed in conical flasks, an approach which, as mentioned in the methodology above, was attempted but could not be achieved without an extremely low level of predator retrieval. The presence in their work of plant structures such as the petiole and (hairy) stem may have provided the mite with refuges in which the humidity conditions are more amenable than on the more exposed leaf surface. This would allow them to foray out from such locations to hunt, behaviour observed in other predatory mites (Grostal and O'Dowd, 1994; Onzo *et al.*, 2001; Romero and Benson, 2005).

These results may be important in informing biological control strategies. If plant morphology has an impact on the potential effectiveness of *P. persimilis* by increasing their exposure to sub-optimal humidity conditions then it may be worthwhile for growers to consider more drought-tolerant mites, of which there are a number (De Courcy Williams *et al.*, 2004b; Ferrero *et al.*, 2010). Equally it may be worthwhile investigating the relative drought-tolerance of other strains of *P. persimilis*, for instance Perring and Lackey (1989) found that an Israeli strain was more tolerant of low humidity than a Californian strain. The findings also suggest that greenhouse ambient humidity conditions will affect the control performance of the mite and this has implications for the climate control approaches. If, as has been suggested (van Lenteren and Woets, 1988; van Lenteren, 2000; Pilkington *et al.*, 2010), growers decide to optimise the climatic conditions for biological control agents (as well as for the crop) then these results may assist them in doing so by deterring them from climate control that produces a preponderance of low or very high humidity conditions.

These results can be used to compare the impacts that novel greenhouse climate management may have on the performance of *P. persimilis*. Comparison with temperature and humidity data from Spanish and Dutch greenhouses (see Chapter 4 for details of the greenhouse conditions in the different country and climate management scenarios) suggests it would be likely that, in Spain, the drier conditions prevalent during the day in passively ventilated greenhouses would be less conducive

to *P. persimilis* control than in closed system greenhouses. Similarly, in the Netherlands the humidity conditions would be progressively more detrimental the higher the temperature set-points are adjusted. The more stable humidity conditions in Dutch greenhouses may also be more conducive to the predator than in Spain. However, these generalisations take no account of the influence of important factors such as temperature (Skirvin and Fenlon, 2003b). The interactions of these factors as well as number of other population demographic parameters are explored further in the mite population model (Chapter 6).

Future work would investigate whether the same response to humidity is observed on more realistic arenas. If whole plants, as suggested by Palevsky *et al.* (2008), or cut stems (Skirvin and De Courcy Williams, 1999; Skirvin and Fenlon, 2001; Skirvin and Fenlon, 2003b) could be used in *C. ternata* experiments it may be found that the detrimental effects of low humidity would be reduced as the predatory mites may have opportunities to hide in plant structures not found on the leaf alone. The study of the impacts of humidity on cultivars with differing densities of trichomes would also go some way to confirming whether these structures do offer protection against low humidities. Investigations into the impacts of variable humidity conditions would also be of interest as these may have different effects than constant humidity conditions. For instance, short episodes of high humidity in a 24 hour period of otherwise arid conditions may mitigate the detrimental impacts on predation. Further, this affect may be non-linear in that the increased performance of the predator may be greater than that predicted by taking the 24 hour mean humidity. Such work would assist in predicting the effects of differing greenhouse climate management on biological control efficacy.

Finally, this work also provides valuable data for inclusion in a *T. urticae* and *P. persimilis* population dynamics model (Chapter 6). Many aspects of the predation behaviour of *P. persimilis* have been well studied and can be used for model parameterisation (e.g., Zemek and Nachman, 1998; Skirvin and Williams, 1999; Skirvin and Fenlon, 2003a; De Courcy Williams *et al*., 2004a). However, this work fills a gap in the knowledge regarding the effects of humidity. The modelling approach used in this chapter provided a reasonable description of the functional response in the conditions investigated and took account of the processes occurring while allowing for stochasticity and over-dispersion of the data. The use of beta-

binomial distributions is particularly important as the implications of underestimating variance for modelling biological control have been noted to be potentially serious (Fenlon and Faddy, 2006). Additionally, the inclusion of a stochastic element allows the fitted functional response to be included in a stochastic population dynamics model.

4. Modelling the greenhouse climate

4. 1 Introduction

Understanding and controlling the physical climate within the greenhouse is vital to maintaining high quality and yields in protected horticulture. Temperature, light, humidity and CO_2 are carefully regulated in most production systems as these factors have an impact on a number of important aspects of crop production. The principal concern for most growers is providing conditions that are optimal to crop development or biomass partitioning (Kempkes *et al.*, 2000; Korner and Challa, 2003). Secondary to this is the economical need to efficiently use inputs such as temperature and CO_2, which can be expensive, and hence minimising their loss to the outside is important (Korner *et al.*, 2008). There is also a growing pressure to improve the environmental sustainability of greenhouse production. Climate management techniques that allow energy and water inputs to escape the system, such as venting, can mean that carbon-based fossil fuels are used inefficiently and water is lost to the atmosphere through vapour. Pest and disease management or risk minimisation is a further objective of climate control (van Lenteren and Woets, 1988; van Lenteren, 2000; Jewett and Jarvis, 2001; Pilkington *et al.*, 2010) and represents a "significant tool" in this regard (van Straten and Challa, 1995). High humidities provide ideal conditions for disease development (Kofoet and Fink, 2007) and, although growers cover openings with mesh to deter pests, vents still provide a means of ingress (Berlinger *et al.*, 2002). For these reasons simulating the growing conditions within the greenhouse has become a much studied subject, allowing environmental management to be refined and the impacts of alternative approaches to be assessed.

This chapter describes the modelling of the seasonal and diurnal variation in temperature and humidity within different greenhouse systems in the Netherlands and Spain. This was necessary as a means of providing input data for the models used to evaluate the effects of novel greenhouse systems on the pest, disease and biological control systems studied (Chapters 5 and 6). Environmental data from real greenhouses over a number of years could have been used for this purpose but as this

was lacking (and this is a particular problem where there are few novel, prototype greenhouse systems available) a greenhouse climate modelling approach provided an alternative approach. This allowed multiple stochastically different data sets to be generated for different greenhouse structures and climate regimes representing seasonal variation over the year. In each country growing conditions were simulated in greenhouses using climate management typical to the commercial practices in that country, e.g., passive ventilation in Spain and Venlo-type greenhouses in the Netherlands. Conditions were also simulated in greenhouses with modified climate control techniques, e.g. closed-system climate management in Spain and novel greenhouse cladding in the Netherlands. A further benefit of using the climate modelling approach is that the data generated can be used to "artificially" create novel climate regimes by making simple adjustments to the temperature and humidity. Such an approach was used here to create additional 'warm' and 'cool' climate regimes for the Dutch greenhouse. Greenhouse climate modelling also allows the variation in climate stability inherent to each climate control system to be explored which is important for reliably simulating pest and disease pressures and biological control efficacy.

The degree to which the greenhouse climate can be regulated differs with the climate management used. For instance, Dutch Venlo greenhouse production is characterised by the presence of year-round optimal conditions that maximise yields (Wittwer and Castilla, 1995). Maintaining these conditions is achieved through the use of sophisticated climate management systems that provide accurate and reliable control resulting in a relatively stable environment both diurnally and seasonally (Hanan, 1998). Passively ventilated greenhouse production, the traditional approach in Spain (Castilla and Montero, 2008), is typified by relatively low-technology climate control, primarily consisting of side and/or roof vents and minimal energy inputs, and provides economic yields at sub-optimal conditions (Wittwer and Castilla, 1995). The primary aim of such climate control changes seasonally, with the minimisation of energy loss the main concern in winter and the reduction of high temperatures a priority in the summer (de Pascale and Maggio, 2004). While passive ventilation climate control is often effective it can be complicated by contradictory objectives; for instance closing vents in the winter maintains temperature but often results in night time humidities close to saturation (Polycarpou, 2005), which

encourages pathogens such as *B. cinerea* (Elad *et al.*, 1996). Although this greenhouse management provides an affordable means of climate control it promotes the loss of energy and CO_2 (Bakker, 2009; Montero *et al.*, 2009) and has a poor water use efficiency compared to northern European greenhouse production (Stanghellini *et al.*, 2003). Efficient water usage is an especially important issue in Almeria, an important greenhouse production area in Spain, due to low rainfall and the high demand from horticulture (Chapagain and Orr, 2009). Important consequences of passive ventilation for the growing environment are that conditions can vary considerably both diurnally and seasonally with changing external conditions (Arellano *et al.*, 2006; Coelho *et al.*, 2006).

Closed-system climate control is a novel form of management in the Spanish industry that is intermediate between Dutch Venlo and Spanish passive ventilation climate management. It requires increased investment compared to passive ventilation, involving the installation of high efficiency fine wire heat exchangers and short-term heat storage in water tanks. The heat exchangers absorb high temperatures and excess water vapour, transferring these to a closed water system (Bakker *et al.*, 2006). The warmed water is then transferred to a water tank which is used to maintain greenhouse temperatures at night. Water cooled at night is then used during the day to lower greenhouse temperatures. The aim of such a system is to eliminate the need to open vents in response to high temperatures and humidities, thus reducing energy and water use and the emission of pesticides to the open air and providing considerably more climate stability than passive ventilation (Opdam *et al.*, 2005; Heuvelink *et al.*, 2008).

The greenhouse environment is a complex interaction of many factors. Foremost among these are external factors such as sunlight, temperature and wind. The physical structure of the greenhouse also impacts considerably on the dynamics of the growing environment. For instance size, position, construction materials, height, etc, can affect processes such as air movement and thermal radiation (Castilla and Montero, 2008). Positions, size and numbers of vents, their frequency and degree of opening, their relation to wind direction and the covers used to limit ingress of pests can all affect the internal temperature, humidity and air movement (Ould Khaoua *et al.*, 2005; Arellano *et al.*, 2006; Atarassi *et al.*, 2006; Sase, 2006). Finally, the crop

itself impacts on the environment with transpiration varying with temperature, humidity, air movement and light, and so influences humidity levels.

Many models have been developed to simulate the greenhouse environment and range from simple, deterministic models (Kimball and Bellamy, 1986) to complex, dynamic models (Stanghellini and de Jong, 1995; Baptista *et al.*, 2010; Fitz-Rodriguez *et al.*, 2010). The complexity of modelling such a system has meant simulation models tend to focus on a subset of the processes described above, including the impacts of greenhouse structure (Cooper and Fuller, 1983; Kittas *et al.*, 2003), vents (Katsoulas *et al.*, 2006; Nebbali *et al.*, 2006), sunlight transmission (Heuvelink *et al.*, 1995) and insect screens (Coelho *et al.*, 2006; Fatnassi *et al.*, 2006).

The modelling approach taken depends on the requirements of the model, for instance there are those that are designed to inform on the impacts of climate management on crop transpiration (Medrano *et al.*, 2005), pest and disease control (Korner and Holst, 2005; Korner and Jakobsen, 2006; Roy *et al.*, 2006), energy saving (Bakker, 2006; Korner *et al.*, 2007) and reducing chemical inputs (Korner and Van Straten, 2008). Model construction is also dependent on the data available, which for the work described here was limited in scope to location and internal temperature and humidity conditions, with other factors such as light and plant temperature inconsistently provided. Within this framework it was decided to construct a relatively simple stochastic, empirical model based on Parton & Logan's (1981) sine-exponential model of diurnal temperature trends as empirical models are generally more suitable for purposes such as this, where an accurate description of the system is desired rather than an understanding of the underlying processes *per se*. Empirical models are more parsimonious and can often provide a better fit to the data as they have fewer constraints but they also rely more intensely on the quality and amount of data (France and Thornley, 1984). Furthermore, Reicosky *et al.* (1989) suggested that the sine-exponential model may yield better results where site-specific calibration can occur, an aspect suited to the data available in this work. The model is spatially homogenous, simulating conditions immediately above the canopy (as this was where the raw climate data provided was recorded), and assumes that conditions are uniform across the greenhouse at this level. It is also temporally dynamic accounting for seasonal and diurnal changes in temperature and humidity in

discrete hourly intervals. Empirical models of the greenhouse temperature and humidity conditions are rare, being generally restricted to sub-models of specific processes (e.g., Stanghellini and van Meurs, 1992; Roy *et al.*, 2008), and this is the first time such a model has been used to compare climate control in different countries across a year.

4. 2 Model description

To parameterise the greenhouse simulation models, five sets of temperature and humidity data were provided by EUphoros project partners in Spain and the Netherlands. The Fundación Cajamar in Spain donated two sets recorded in commercial-standard, Spanish-style plastic clad greenhouses at their experimental station in Almeria. The first set was four years (2004-2007) of data recorded every minute in a greenhouse managed using passive ventilation. The second data set was nine months of observations (December 2009-August 2010) recorded every five minutes from a greenhouse whose climate was controlled using the novel closed-system management. In both the Spanish greenhouses, tomato crops were grown in commercial-like conditions with equivalent set points. Recordings were taken from approximately one metre above the crop canopy. A full year of data from the closed system greenhouse was initially intended; however a storm and lightening strike destroyed the greenhouse and the economic conditions in the country prevented the system from being rebuilt. It should also be noted that while good climate control was achieved in the closed system greenhouse for the majority of the year, extreme temperatures in the summer meant that vents were opened on occasion.

The three Dutch data sets were provided by the Wageningen University and Research centre in Bleiswijk. The first was a collection of 12 data sets recorded every five minutes for one year (2005-2006) in commercial Venlo greenhouses in Bleiswijk using standard industry practices. The second set was of 13 months of data (2010-2011) recorded every five minutes in a compartment of an experimental Venlo greenhouse using novel light diffusing glass. This glass increases the scatter of light entering the greenhouse and is designed to improve light penetration within the crop canopy. The third data set was taken from a compartment within the same greenhouse with standard glass and which was otherwise identical to that with diffuse glass, including climate management, and acted as a reference. All data was recorded from above the canopy. Rose crops were grown in all the greenhouses from which Dutch data was obtained.

The model for simulating the diurnal trends in temperature was based on the sine-exponential model (Parton and Logan, 1981), which accurately simulated outside air and soil temperature using only daily maximum and minimum temperatures and day

length as inputs. This model splits the daily variation into two parts; the first describes daylight temperature with a sine wave that starts around the time of daily minimum temperature and passes through the daily maximum temperature. The second describes night time temperature with an exponential decline toward the minimum temperature (occurring the following morning). The model assumes that the daily maximum temperature occurs in the daylight hours prior to sunset, that the minimum temperature occurs in the hours around sunrise and that the trend in temperature can be described by a truncated sine wave. The mathematical descriptions for day and night time temperature are written respectively as:

$$T_i = (T_{max} - T_{min}) \sin\left(\frac{\pi m}{(y+2a)}\right) + T_{min} \qquad \{(t_{rise} + c) < i < t_{set}\} \qquad 4.1$$

$$T_i = T_{min} + (T_{set} - T_{min}) exp - \left(\frac{bn}{Z}\right) \qquad \{0 < i \leq (t_{rise} + c) \; or \; t_{set} < i < 24\} \qquad 4.2$$

Where T_i = temperature at the *i*th hour, T_{max} = daily maximum temperature, T_{min} = daily minimum temperature, m = the number of hours between the time of T_{min} and sunset, y = the day length (in hours), a = the lag coefficient for maximum temperature, T_{set} = the temperature at sunset, b = the night time temperature coefficient, n = the number of hours from sunset to T_{min} and Z = night length. A further parameter in the model, c, is the lag coefficient of T_{min} from sunrise and is only used for calculating the time of changeover between Equation 4.2 and 4.1 in the morning. The temperature at sunset is calculated using Equation 4.1. The *a*, *b*, and *c* parameters are site specific and could be optimised using Genstat's® "fitnonlinear" directive. Day length was calculated using Genstat's® "daylength" procedure and the latitude of the greenhouse (36.8 N and 52.03 N for Almeria and Bleiswijk respectively).

Fitting this model to the data sets showed that it was necessary for some adjustments to be made to allow it to be computationally achievable and for Genstat's® fitnonlinear process to optimise the parameters fully (e.g., with standard errors). Morning night time temperature was also linked to the temperature at sunset the previous day. The final model used for this work could be described with the following equations for any given day. The time of sunset (t_{set}) and sunrise (t_{rise}) was described using Equations 4.3 and 4.4 respectively:

$$t_{set} = 12 + \frac{y}{2} \quad 4.3$$

$$t_{rise} = t_{set} - y \quad 4.4$$

The temperature at sunset:

$$T_{set} = (T_{max} - T_{min}) \sin\left(\frac{\pi(t_{set} - t_{min})}{(y+2a)}\right) + T_{min} \quad 4.5$$

The temperature during any given night time hour in the morning:

$$T_{1i} = T_{min} + \left(T_{prevset} - T_{min}\right) exp\left(\frac{-b(t_i + 24 - t_{prevset})}{(24 - y + c)}\right) \qquad \{0 < i \leq (t_{rise+c} + d)\} \quad 4.6$$

The temperature during any given day light hour:

$$T_{2i} = (T_{max} - T_{min}) \sin\left(\frac{\pi(t_i - t_{min})}{(y+2a)}\right) + T_{min} \qquad \{(t_{rise} + d) < i < (t_{set} + a)\} \quad 4.7$$

Finally, the temperature during any given night time hour in the evening:

$$T_{3i} = T_{min} + (T_{set} - T_{min}) exp\left(\frac{-b(t_i + 24 - t_{set})}{(24 - y + c)}\right) \qquad \{(t_{set} + a) \leq i < 24\} \quad 4.8$$

Where t_{min} (time of minimum temperature) = $t_{rise} + c$, T_{1i} = the temperature at the *i*th hour of night in the morning, $T_{prevset}$ = the temperature at the sunset of the previous day, t_i = the *i*th hour, $t_{prevset}$ = time of the previous sunset, T_{2i} = the temperature at the *i*th hour of day light, t_{set} = time of sunset, T_{3i} = the temperature at the *i*th hour of night in the evening. All other parameters remain the same.

The change over between Equations 4.6 – 4.8 was also adjusted slightly from Parton and Logan (1981) whereby Equation 4.6 was used if $t_i \leq t_{rise} + d$, Equation 4.7 was used if t_i was > $t_{rise} + d$ and < $t_{set} + a$, and Equation 4.8 was used if $t_i \geq t_{set} + a$. Thereby parameter *a* had the dual role of modifying the shape of the sine wave (Eq. 4.6 & 4.8) and the switch between evening equations (Eq. 4.7 to 4.8) and *d* was a new parameter modifying the transition time between cooling and warming phases in the morning, and was optimised as described for the other parameters. The addition of the role of these parameters assisted in adapting the Parton and Logan (1981) model to greenhouses where the effects of outside conditions are delayed and somewhat buffered.

Modelling the diurnal trends in greenhouse humidity is generally considered more complex with consideration of a greater number of interactions needed (Jolliet, 1994; Stanghellini and de Jong, 1995). Due to the limited data available a simple representation of humidity was essential and in this vein coupling humidity directly to the trends in air temperature (Sigrimis and Rerras, 1996) was originally considered. However, this failed to adequately describe the differences observed in the climate management approaches, especially in Spain. This was likely due to the effect of venting in the passively ventilated greenhouse. Eventually it was decided to modify the model used for diurnal temperature. Humidity does respond in a similar, although inverse, manner to temperature and the ability to optimise the parameters for country/climate management system would allow differences to be accounted for.

The model developed was essentially an inversed version of that used for temperature, except that maximum humidity was assumed to occur sometime in the early hours of the morning and minimum humidity was assumed to occur sometime in the daylight hours. The model for humidity at sunset can be written as:

$$RH_{set} = (RH_{max} - RH_{min}) \sin\left(\frac{\pi+\pi(t_{set}-t_{min})}{(y+2a)}\right) + RH_{min} \quad 4.9$$

For humidity during any given night time hour in the morning:

$$RH_{1i} = RH_{prevset} + \frac{(RH_{max}-RH_{prevset})}{\left(1+exp\left(-b\left(\frac{(t_i+24-t_{prevset})}{(24-y+c)}\right)\right)\right)} \quad \{0 < i \leq (t_{rise+c} + d)\} \quad 4.10$$

For humidity during any given day light hour:

$$RH_{2i} = (RH_{max} - RH_{min}) \sin\left(\frac{\pi+\pi(t_i-t_{min})}{(y+2a)}\right) + RH_{max} \qquad \{(t_{rise} + d) < i < (t_{set} + a)\} \quad 4.11$$

For humidity during any given night time hour in the evening:

$$RH_{3i} = RH_{set} + \frac{(RH_{max}-RH_{set})}{\left(1+exp\left(-b\left(\frac{(t_i-t_{set})}{(24-y+c)}\right)\right)\right)} \qquad \{(t_{set} + a) \leq i < 24\} \quad 4.12$$

All parameters are the same as, or the humidity equivalent of, those used in the temperature diurnal model. The diurnal models for both temperature and humidity were fitted to the data sets from each country/climate management combination.

The choice of the adjusted Parton and Logan (1981) model for simulating the diurnal trends meant that maximum and minimum temperature and humidity values were needed for each day of the year. This necessitated the modelling of seasonal changes in these maximum and minimum values. To do this a number curves built into Genstat's® "fitcurve" directive were fitted to the data and the best was selected based on the coefficient of determination, its description of the physical processes occurring and on its consistency with curves selected for other data sets. The curves were fitted to 'crop day' rather than the day of year. Crop day was an arbitrary date measure that began at the beginning of the cropping season for each country. This was done because all the data sets provided by the project partners had substantial gaps, usually during the non-growing season in the summer. Fitting curves based on day of year was problematic as the modelling process had to cope with groups of data separated by the gap in the summer. Using crop day meant that curves could be fitted to continuous data sets and improved the resulting model. Once selected, the curve was then adjusted to fit to 365 day cycle. The process of selecting the curve and adjusting to a 365 day cycle is described further in the Results (Section 4. 3).

An element of stochasticity was built into the seasonal model whereby daily maximum and minimum values were selected from a normal distribution around the fitted line. This allowed the inter-annual (in the case of passively ventilated Spanish greenhouses) and between-site (in the case industry-standard Dutch glasshouses) variation to be accounted for. Hence, this reflected the inherent stability of each climate management system, from the most stable in the Dutch glasshouses to the least in the Spanish passively ventilated greenhouses. The range either side of the fitted line was described by:

$$Stochastic\ range = \left(\sqrt{SE^2 + rms}\right) * normal\ distribution \qquad 4.13$$

Where *SE* = the standard error of the prediction and *rms* = the root mean square error of the fitted model. The normal distribution was derived using Genstat's® grnormal procedure, which generates pseudo-random numbers from a normal distribution with a mean of 0 and a variance of 1. The seed for this procedure was set using an online random number generator (www.random.org), which selects numbers from a discrete uniform distribution. The algorithm this site uses is not available, however the site has been evaluated by a non-profit regulatory body, eCOGRA, and has been

used in a number of peer-reviewed publications (e.g., Fankhauser and Maser, 2005; Biggar *et al.*, 2008). Some limits to the values chosen by the Equation 4.13 were made. For instance, values could not exceed the extreme values recorded for each country/climate management combination and humidity could not exceed 100% RH. The model was then used to generate ten different one year sets of hourly temperature and humidity data. Ten different seeds were used for this with the same set of ten seeds used for each country/climate management combination.

4. 3 Results

4. 3. 1. Greenhouse data trends

The data from the two greenhouse climate control systems investigated in Almeria, Spain show clear differences in both temperature and humidity profiles seasonally and diurnally. Generally speaking the closed system climate management produced greater levels of climate stability across the year and diurnally. The impacts of the closed system were strongest when considering greenhouse humidity (Table 4.1). In the closed system mean monthly humidity was relatively stable across the year at around 75-85% RH with a slight drop in the summer, whereas in the passively ventilated greenhouses the mean monthly humidity was generally lower and fluctuated widely. The humidity extremes were also smaller in the closed system, especially in the winter and spring. In the passively ventilated system maximum humidity was often close to saturation.

Table 4.1: Monthly average, minimum and maximum humidities (% RH) recorded in passively ventilated (PV) and closed system (CS) greenhouses in Almeria, Spain. Asterisks indicate occasions when no measurements were taken.

		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
PV	Mean	84.1	65.8	66.7	73.2	73.0	64.5	72.6	55.3	61.6	69.1	76.9	82.6
	Max	99.2	91.3	97.6	98.8	97.5	95.8	95.6	90.6	98.5	99.5	100.0	99.9
	Min	45.2	22.7	14.9	24.6	28.3	25.6	32.7	21.9	22.2	24.0	38.5	43.9
CS	Mean	85.9	84.0	84.2	85.7	78.6	77.8	68.0	76.3	*	*	*	86.8
	Max	93.8	92.3	93.1	93.8	92.7	93.4	91.4	89.9	*	*	*	93.7
	Min	52.3	53.6	51.8	69.3	27.0	36.8	19.9	58.5	*	*	*	72.2

The diurnal pattern of humidity differed markedly between the two management systems. In the passively ventilated greenhouses mean hourly overnight and early morning humidity was between 80-90% RH in winter and spring and approximately 75% in the summer. This was followed by a rapid drop in humidity in the late morning, which increased from winter to summer. In the closed system greenhouse the late morning drop in humidity was largely lost in winter and spring and much reduced in the summer (Fig. 4.1).

Scrutiny of the diurnal pattern in the maximum and minimum humidity profile (averaged for each season; winter, spring and summer) also revealed important

differences in the greenhouse climate produced by each system (Fig. 4.2). In the passively ventilated greenhouses humidity was often close to saturation in the winter and spring and lower than 30% RH in the summer. In the closed system greenhouse maximum humidity was around 90% RH across the day in winter and spring while minimum humidity dropped to approximately 50% RH in the summer.

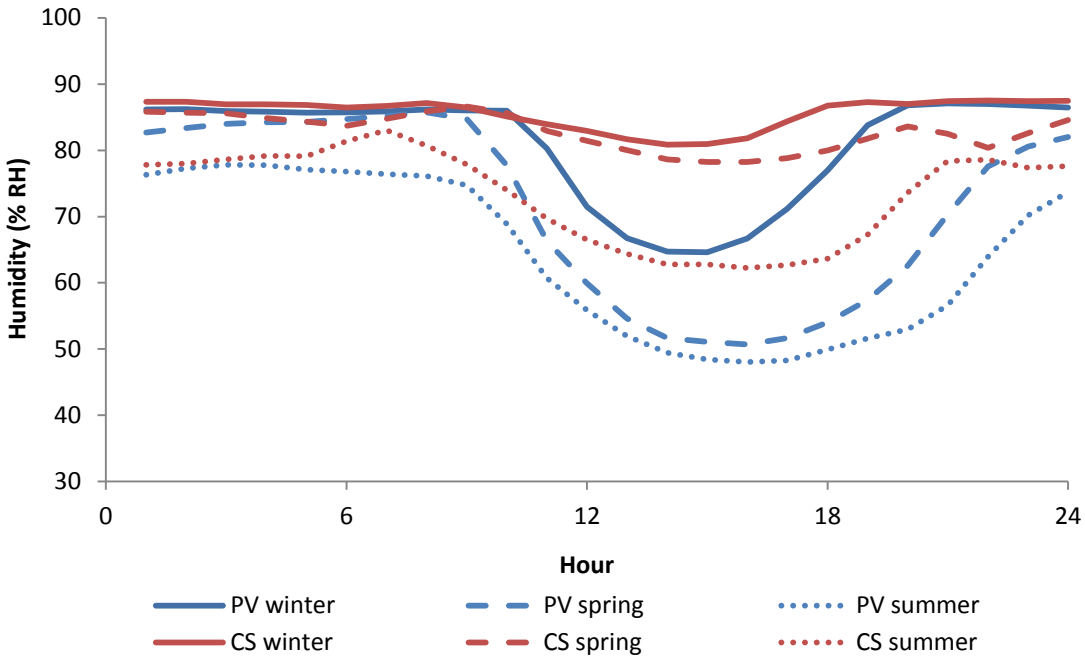

Figure 4.1: Seasonal change in mean diurnal humidity (% RH) recorded in passively ventilated (PV) and closed system (CS) greenhouses in Almeria, Spain. Winter = December – February; spring = March – May; summer = June – August.

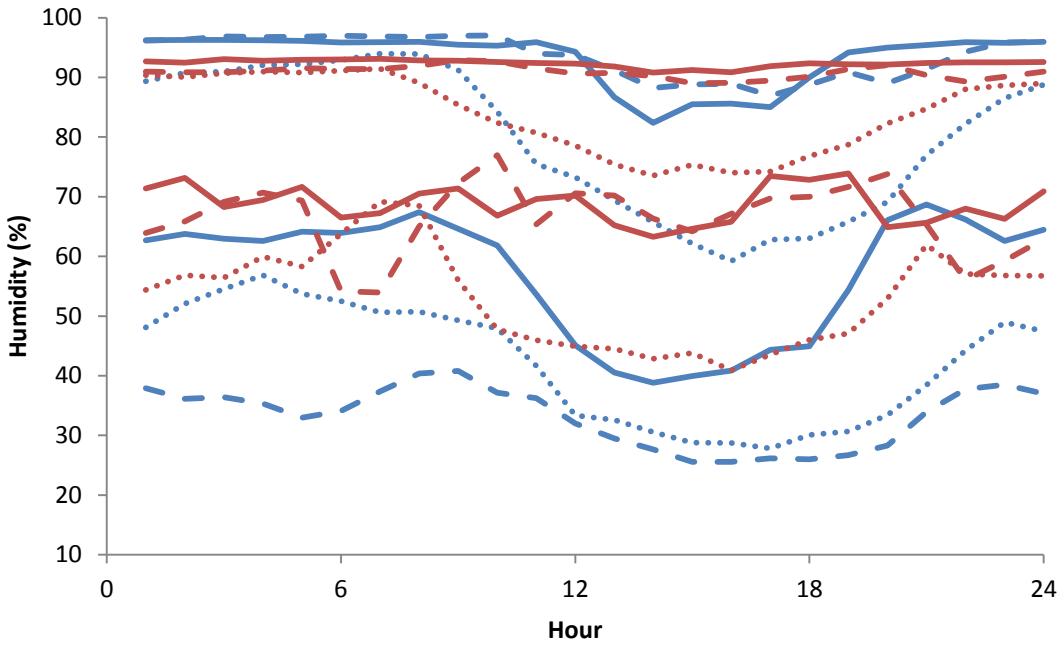

Figure 4.2: Mean maximum and minimum diurnal humidity (% RH) measurements recorded in passively ventilated (blue) and closed system (red) greenhouses in Almeria, Spain. Average taken of values in each season; winter = December – February (—); spring = March – May (- - -); summer = June – August (···).

Comparison of the seasonal temperature profiles between the two systems showed that mean monthly temperatures were similar in the winter but slightly lower in the closed system greenhouse in the summer (Table 4.2). Minimum and maximum temperature extremes during the winter and summer respectively were smaller in the semi-closed system.

Table 4.2: Monthly average, minimum and maximum temperatures (°C) recorded in passively ventilated (PV) and closed system (CS) greenhouses in Almeria, Spain. Asterisks indicate occasions when no measurements were taken.

		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
PV	Mean	15.1	17.3	19.3	20.2	22.7	26.7	27.6	32.7	27.4	24.2	18.7	15.8
	Max	25.8	31.7	37.8	34.7	40.2	44.4	42.3	48.8	46.3	43.6	33.7	29.2
	Min	6.1	9.3	9.0	10.4	13.1	16.7	20.0	23.7	17.3	12.6	8.1	7.9
CS	Mean	15.7	16.9	18.7	21.0	22.2	24.8	27.0	27.2	*	*	*	16.5
	Max	30.0	30.2	31.2	33.4	35.1	36.3	36.7	32.5	*	*	*	26.3
	Min	8.0	9.4	11.7	11.7	13.1	15.8	20.9	22.1	*	*	*	8.7

The seasonal trends (winter, spring and summer) in mean diurnal temperature variation showed a reduction in summer daytime temperature in the closed system greenhouse compared to the passively ventilated greenhouse (Fig. 4.3). However, few differences in the diurnal temperature profiles in winter and spring were recorded between the systems. Seasonal trends in the diurnal maximum and minimum temperatures (averaged for winter, spring and summer) show smaller extremes in the closed system greenhouse, with generally lower maximums and similar minimums compared to the passively ventilated greenhouse (Fig. 4.4).

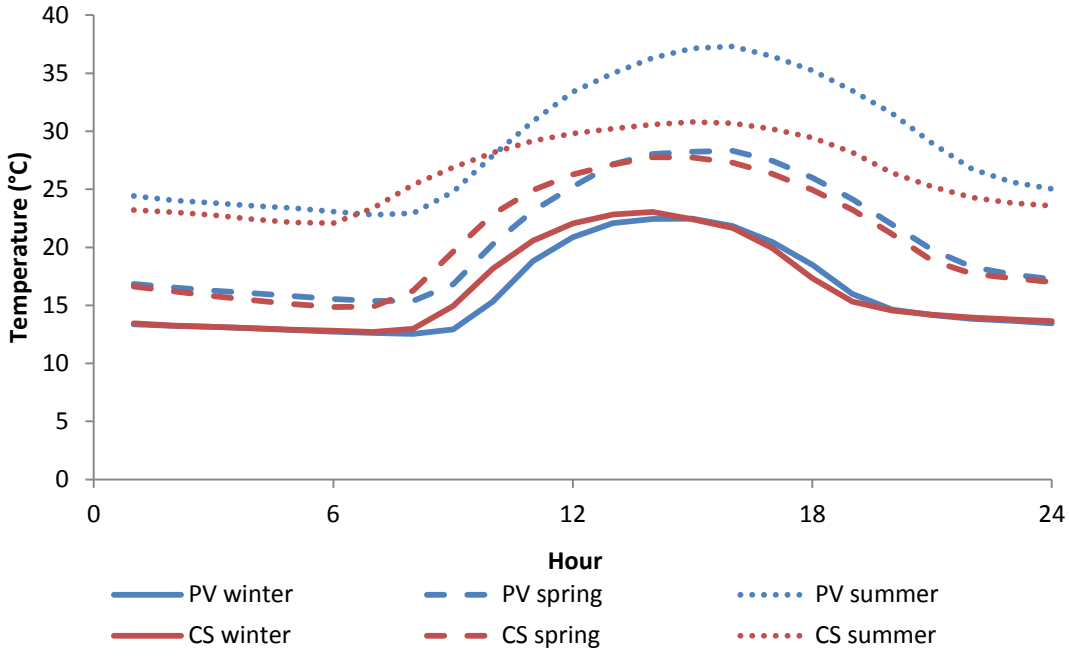

Figure 4.3: Seasonal change in mean diurnal temperature (°C) recorded in passively ventilated (PV) and closed system (CS) greenhouses in Almeria, Spain. Winter = December – February; spring = March – May; summer = June – August.

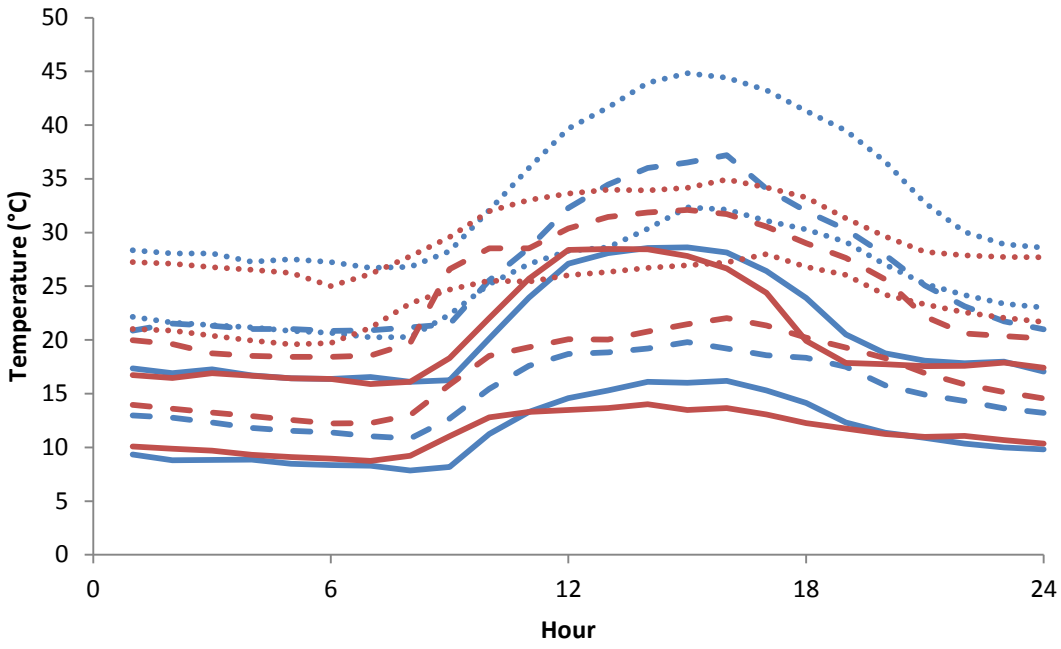

Figure 4.4: Mean maximum and minimum diurnal temperature (°C) measurements recorded in passively ventilated (blue) and closed system (red) greenhouses in Almeria, Spain. Average taken of values in each season; winter = December – February (—); spring = March – May (- - -); summer = June – August (···).

Comparison of the experimental greenhouse in Bleiswijk and the reference greenhouse revealed few differences between climates. Analysis of variance showed that the differences in temperature (F = 0.45, df = 1, P = 0.501) and humidity (F = 3.13, df = 1, P = 0.077) were non-significant between the diffuse glass greenhouse and the reference greenhouse using standard glass. The lack of effect on the greenhouse climate of the diffuse glass meant there was little to be gained from modelling the temperature and humidity in this system. Instead it was decided to model the climate in traditional greenhouse production in the Netherlands using data from the commercial greenhouses. The output of this model would then be adjusted to assess the impact of small changes in the temperature profile (plus or minus 2°C and concurrent changes in humidity) on pest and disease pressures.

As would be expected from modern high-technology Dutch greenhouses the climate in the commercial greenhouses in Bleiswijk was closely controlled and the seasonal fluctuations were relatively stable (Table 4.3). Mean temperature and humidity was 20-22°C and 73-81% RH respectively across the year and maximum and minimum measurements of both factors were also consistent throughout the year.

Table 4.3: Monthly average, minimum and maximum temperatures (°C) and humidities (& RH) recorded in twelve commercial greenhouses in Bleiswijk, the Netherlands in 2005-2006. Asterisks indicate occasions when no measurements were taken.

		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Temp. (°C)	Mean	20.6	20.9	21.0	21.3	21.9	*	*	21.8	21.5	21.0	20.6	20.9
	Max	29.3	29.3	31.6	31.4	31.7	*	*	32.1	32.0	28.5	27.6	27.3
	Min	13.2	13.2	10.7	11.2	13.5	*	*	13.9	10.1	13.6	11.4	12.4
Humidity (% RH)	Mean	80.7	81.0	79.9	77.7	73.9	*	*	74.8	77.4	79.0	79.8	79.9
	Max	97.8	96.7	97.0	96.3	95.9	*	*	93.7	98.8	97.1	98.1	96.4
	Min	65.8	66.1	53.5	42.3	32.8	*	*	49.4	41.2	53.1	59.3	63.4

The diurnal variation in humidity (Fig. 4.5) and temperature (Fig. 4.6) was also closely controlled with little difference in the daily pattern of both factors across the year. The extremes of temperature and humidity, meanwhile, occupied a relatively short range of between 13-31°C and 45-95% RH respectively.

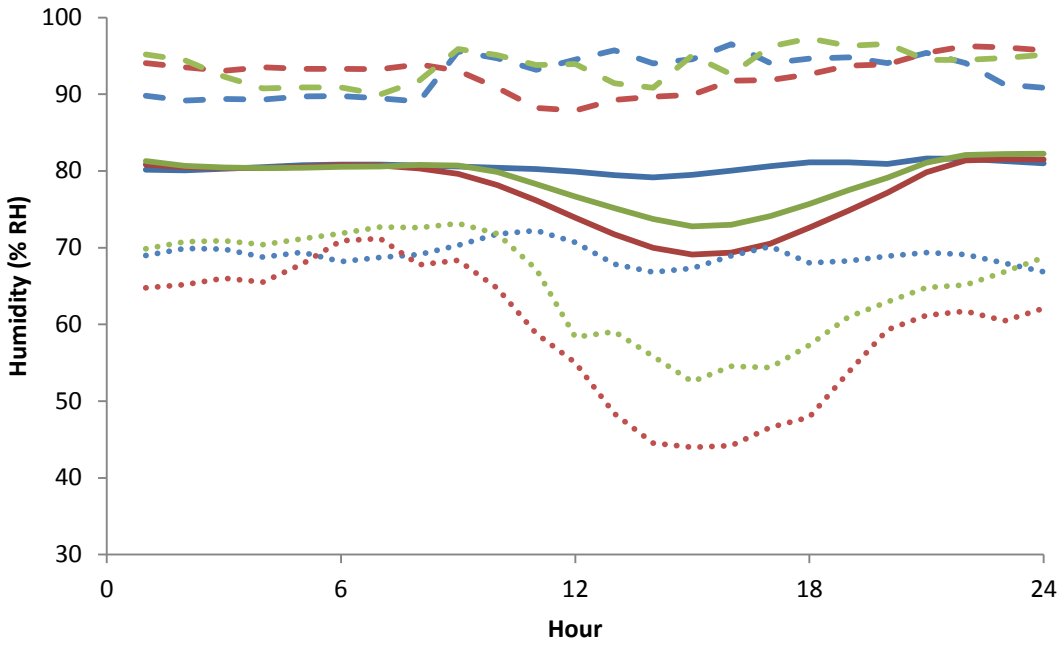

Figure 4.5: Mean diurnal humidity (—) recorded in twelve commercial greenhouses in Bleiswijk, the Netherlands in 2005-2006. Mean maximum (- - -) and minimum (···) humidity also shown. Average taken of values in each season; winter = December – February (blue); spring = March – May (red); autumn = September – November (green).

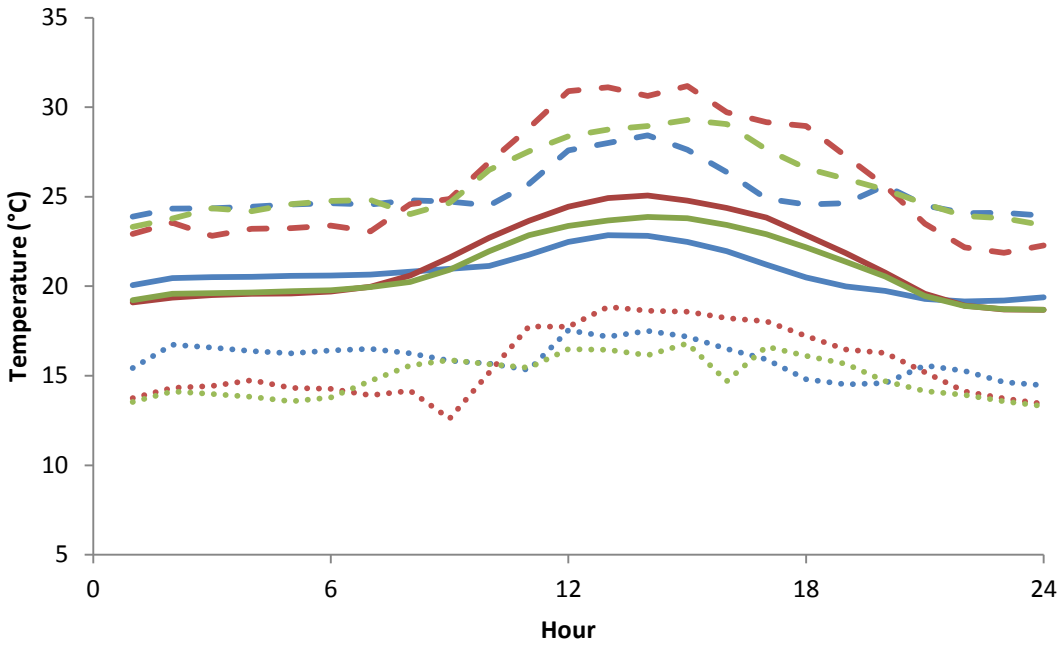

Figure 4.6: Mean diurnal temperature (—) recorded in twelve commercial greenhouses in Bleiswijk, the Netherlands in 2005-2006. Mean maximum (- - -) and minimum (···) temperature also shown. Average taken of values in each season; winter = December – February (blue); spring = March – May (red); autumn = September – November (green).

4. 3. 2 Modelling seasonal variation

To simulate daily maximum and minimum temperature and humidity across the year for each country/climate management combination various curves were fitted to each data set. Doing this showed that the coefficient of determination was generally highest for the double Fourier (dFourier) curve (Table 4.4), a trigonometric function that describes a periodic signal containing a compound of two sine waves, one having half the cyclic period of the other. Although there were some instances when this was not the case it was decided that using a dFourier curve for all country/management combinations would provide an acceptably good fit and a consistent approach.

Table 4.4: Coefficients of determination (R^2) for curves fitted to the daily maximum and minimum temperature (°C) and humidity (% RH) parameters (Param.) recorded in greenhouses in Spain (ES) and the Netherlands (NL) and under different climate management (PV = passively ventilated, CS = closed system, Trad = standard commercial). dG = double Gaussian, dF =double Fourier, qdq and qdl = rational functions. As explained in the text no curves were fitted to the maximum humidity and minimum temperature in the NL greenhouses.

Country	Mgmt.	Param.	Max / Min	R^2 of curve					
				Gaussian	Fourier	dG	dF	qdq	qdl
ES	PV	% RH	Min	0.35	0.3	0.36	0.44	0.33	0.21
ES	PV	% RH	Max	0.15	0.15	0.16	0.18	0.16	0.16
ES	CS	% RH	Min	0.42	0.42	0.44	0.46	0.46	0.42
ES	CS	% RH	Max	0.2	0.2	0.22	0.23	0.2	0.19
NL	Trad	% RH	Min	0.43	0.43	0.46	0.48	0.43	0.42
NL	Trad	% RH	Max	n/a	n/a	n/a	n/a	n/a	n/a
ES	PV	Temp (°C)	Min	0.7	0.7	0.71	0.71	0.71	0.71
ES	PV	Temp (°C)	Max	0.53	0.51	0.67	0.69	0.67	0.59
ES	CS	Temp (°C)	Min	0.81	0.8	0.82	0.82	0.82	0.8
ES	CS	Temp (°C)	Max	0.52	0.52	0.57	0.54	0.53	0.51
NL	Trad	Temp (°C)	Min	n/a	n/a	n/a	n/a	n/a	n/a
NL	Trad	Temp (°C)	Max	0.28	0.29	0.29	0.3*	0.28	0.25

Using the dFourier curve the calculation of the minimum or maximum temperature or humidity can be written as:

$$\alpha + \beta \sin\left(\frac{(2\pi(X-\mu))}{\omega}\right) + \gamma \sin\left(\frac{(4\pi(X-\nu))}{\omega}\right) \quad 4.14$$

Where X = crop day, α = the approximate inflection point of the signal, β and γ = the amplitude of their respective sine waves, μ and ν = the wavelengths of their respective sine waves, ω = the frequency of the signal. α, β, γ, μ, ν and ω are parameters fitted using Genstat®. As the data for the maximum humidity and the minimum temperature in the Dutch greenhouses were suitably constant throughout the year it was felt a straight line would suffice and no curve was fitted. The dFourier curve (Eq. 4.14) was then altered to constrain it to a 365 day cycle by remodelling the temporal aspect of the first and second sine waves as in Equations 4.15 and 4.16 respectively:

$$\sin\left(\frac{(2\pi(X-\mu 1))}{365}\right) \quad 4.15$$

$$\sin\left(\frac{(4\pi(X-\nu 1))}{365}\right) \quad 4.16$$

This allowed $\mu 1$ and ν1 to be optimised for inclusion in the 365 day dFourier, which could then be written as:

$$\alpha + \beta \sin\left(\frac{(2\pi(X-\mu 1))}{365}\right) + \gamma \sin\left(\frac{(4\pi(X-\nu 1))}{365}\right) \quad 4.17$$

The resulting parameter values and coefficient of determination for the curves for each country and climate management provided a relatively good fit, especially in regard to temperature in the Spanish greenhouses (Table 4.5). The annual pattern of humidity was more complicated and this was reflected in the fit. The coefficient of determination for both factors in the Netherlands was lower and was partly due to the more subtle annual trends evident in this country.

Table 4.5: Coefficients of determination (R^2) and parameter values for the 365 dFourier curve (Equation 4.17) fitted to the daily maximum and minimum temperature (°C) and humidity (% RH) recorded in greenhouses in Spain (ES) and the Netherlands (NL) and under different climate management (PV = passively ventilated, CS = closed system, Trad. = standard commercial).

Country	Mgmt.	Parameter	Max/Min	α	β	γ	μ	ν	R^2
ES	PV	% RH	Min	48.34	9.47	-10.82	43.56	-32.91	0.419
ES	PV	% RH	Max	85.39	6.46	-3.37	78.62	-45.59	0.177
ES	CS	% RH	Min	71.08	11.67	6.68	46.00	19.91	0.457
ES	CS	% RH	Max	89.31	2.37	1.15	40.70	6.90	0.211
NL	Trad.	% RH	Min	67.61	11.11	-0.80	64.48	43.40	0.436
NL	Trad.	% RH	Max	85.04	-0.80	0.43	62.61	-47.75	0.015
ES	PV	Temp (°C)	Min	16.37	-5.60	1.19	71.21	-48.34	0.706
ES	PV	Temp (°C)	Max	29.05	-0.67	3.44	59.73	-35.76	0.684
ES	CS	Temp (°C)	Min	16.80	-5.69	-1.72	71.43	42.41	0.842
ES	CS	Temp (°C)	Max	28.25	-4.14	1.39	58.52	-12.97	0.537
NL	Trad.	Temp (°C)	Max	25.15	-1.73	0.55	30.48	-14.67	0.285

As mentioned above, minimum temperature in the Dutch commercial greenhouses could be described with a reasonably straight line fitted to a constant:

$$\alpha + (\beta * X) \quad 4.18$$

Where α = 18.02, β = -0.0004 and X = crop day. However, it was felt that the prediction of maximum humidity in the Netherlands was better described with the

365 dFourier than a similar straight line fit as this captured more of the slight annual trend evident in this factor. The output of these models show a good fit to the observed values for both temperature (Fig. 4.7) and humidity (Fig. 4.8) in each country/climate management combination. As described in the Methodology (Section 4. 2) each run of the model randomly selected a value from a normal distribution within a specific range (Eq. 4.13) around the fitted curve (Fig. 4.7 and 4.8). As is clear in these Figures the trend in these factors during the summer is very much speculation due to the absence of data. The same comment applies to the curves fitted for the closed system greenhouse during the autumn, however this was deemed acceptable as it had a similar behaviour to that in the passively ventilated greenhouse. Both types of Spanish greenhouse system have only limited environmental control and the conditions within the greenhouse are largely defined by the outside conditions, which would be cooling at this time of year.

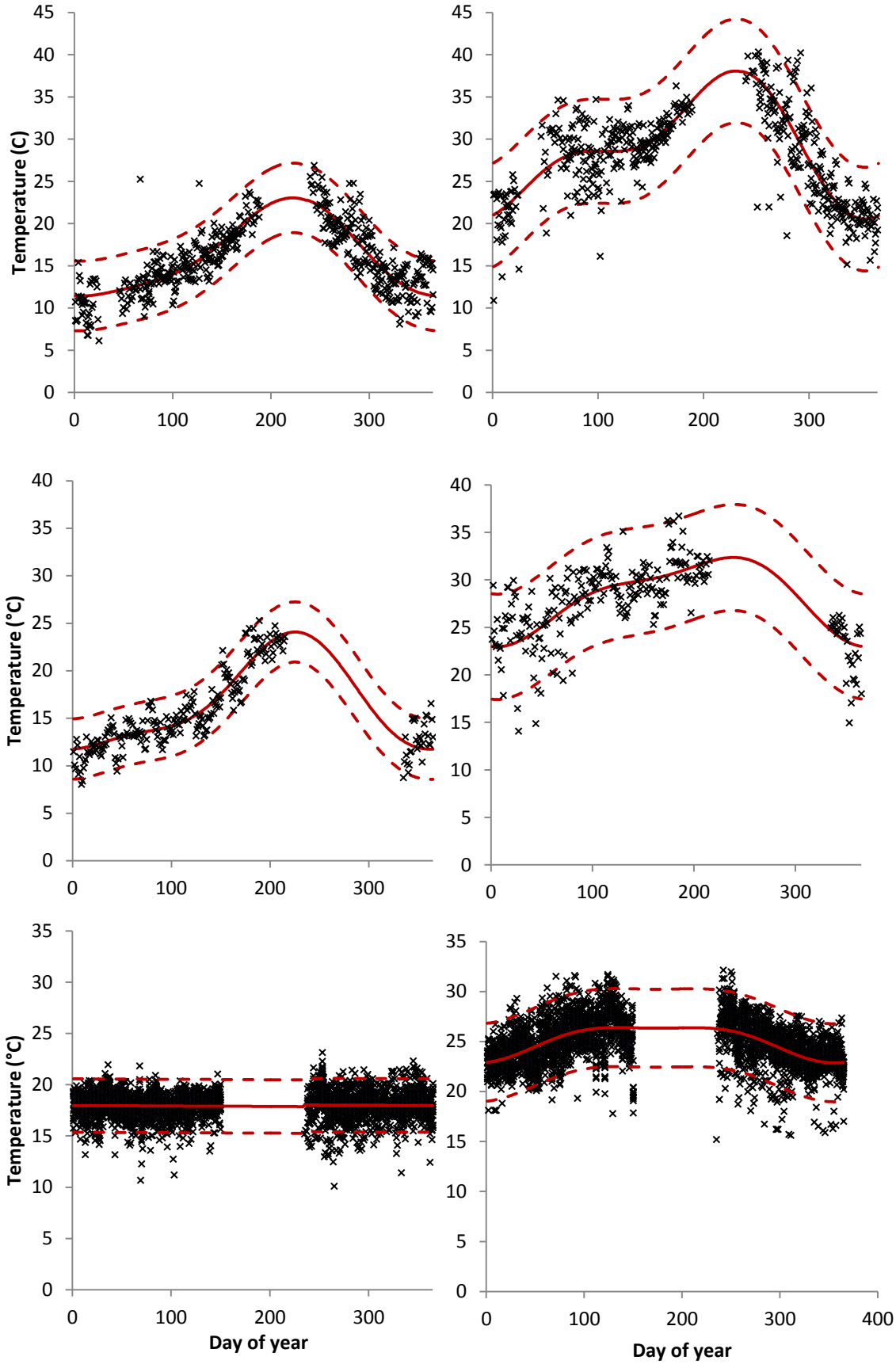

Figure 4.7: Curve fitted to annual minimum (left) and maximum (right) temperature (red line) in passively ventilated (top) and closed system greenhouses (middle) in Spain and commercial greenhouses in the Netherlands (bottom). Broken red line = range from which value is selected for diurnal model. X = observed values.

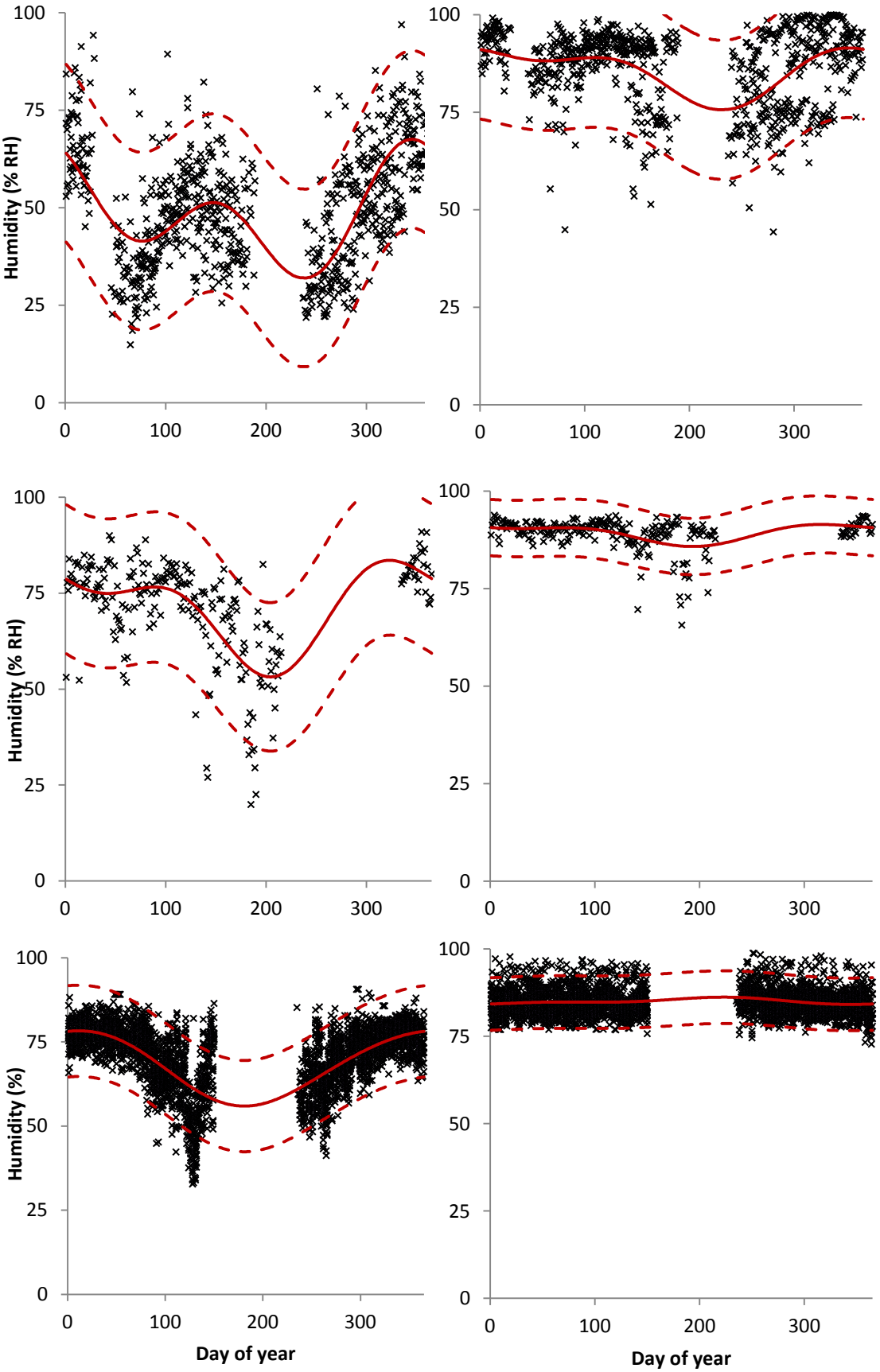

Figure 4.8: Curve fitted to annual minimum (left) and maximum (right) humidity (red line) in passively ventilated (top) and closed system greenhouses (middle) in Spain and commercial greenhouses in the Netherlands (bottom). Broken red line = range from which value is selected for diurnal model. X = observed values.

4. 3. 3 Modelling diurnal variation

With the seasonal model completed integration with the diurnal model was carried out. The optimised parameter values for the diurnal model and the coefficient of determination for the full model can be seen in Table 4.6. Examples of the diurnal model (and the stochastic line) fitted to the observed data can be seen in Fig. 4.9. The variation in humidity is illustrated here, especially in the Spanish passively ventilated greenhouses, as is the far smaller degree of variation evident in the Dutch greenhouses.

Table 4.6: Parameter values for the diurnal model (Eq. 4.5-4.8) and coefficients of determination (R^2) for the integrated model of annual and diurnal temperature (°C) and humidity (% RH) in Spanish (ES) and Dutch (NL) greenhouses under different climate management (PV = passively ventilated, CS = closed system, Trad. = standard commercial).

Country	Mgmt.	Parameter	*a*	*b*	*c*	*d*	R^2
ES	PV	% RH	1.57	0.92	0.90	2.99	0.4976
ES	CS	% RH	0.92	-0.78	1.34	2.96	0.3474
NL	Trad.	% RH	0.37	-0.58	1.93	4.10	0.2717
ES	PV	Temp (°C)	0.49	3.73	1.49	1.52	0.7988
ES	CS	Temp (°C)	0.78	3.29	0.50	0.34	0.8195
NL	Trad.	Temp (°C)	0.64	0.02	0.36	2.33	0.4558

The data generated by the model for the Dutch greenhouse (traditional) was then used to construct 'warm' and 'cool' scenarios for Dutch greenhouse production. This was done by simply adding or subtracting 2°C to each data point generated by the model for the traditional greenhouse, producing the warm and cool versions respectively. Humidity was also altered by first calculating the vapour pressure saturation point from the temperature in the traditional model:

$$VP_{sat} = 6.11 * 10^{\left(\frac{(7.5*T)}{(237.7+T)}\right)} \quad 4.19$$

Where T = temperature (°C). The actual vapour pressure could then be calculated from the humidity (% RH) in the traditional model:

$$VP_{air} = \frac{(VP_{sat}*RH)}{100} \quad 4.20$$

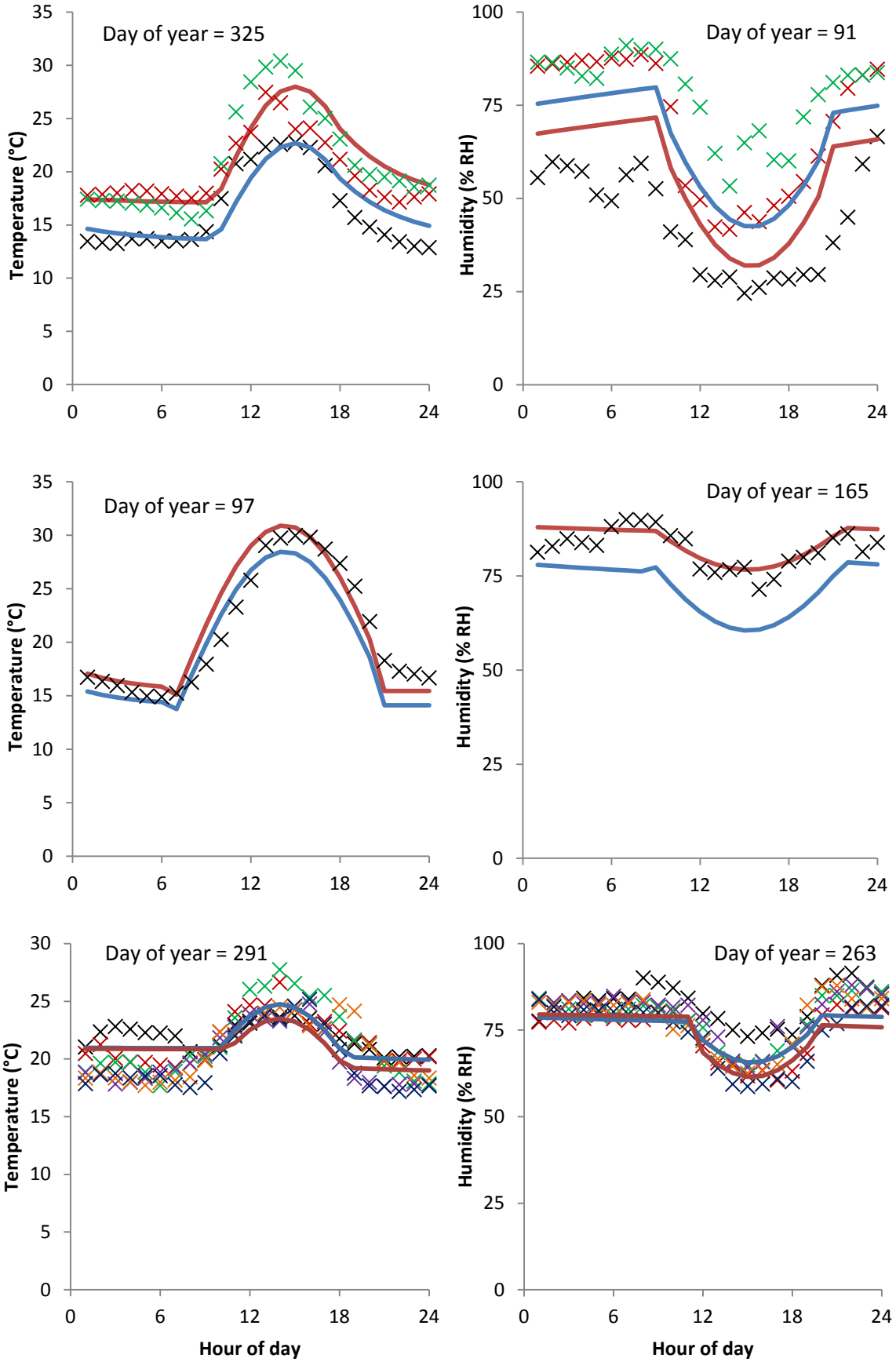

Figure 4.9 Curve fitted to the diurnal trends in temperature (left) and humidity (right) in passively ventilated (top) and closed system greenhouses (middle) in Spain and commercial greenhouses in the Netherlands (bottom). Blue line = fitted model; red line = example of stochastic fit; X = observed values with different colours for different years/greenhouses (max. 6 shown).

The humidity in the new scenario (warm or cool) was then calculated using the VP_{air}, the new temperature and the VP_{sat} for that temperature:

$$RH = \frac{(VP_{air}*100)}{VP_{sat}} \quad 4.21$$

Admittedly this is a simple approach to calculating the humidity under different temperature regimes, for instance it does not take account for changes in crop transpiration. However, it provides an indication of the humidity profile that could be expected and is sufficient for the needs of the modelling work. Although these adjustments to temperature set points may seem minor, they were chosen as they represented realistic options for growers in the Netherlands, the temperature differences would accumulate over time and they would produce considerable differences in the humidity profiles.

4. 4 Discussion

The model developed to simulate ambient temperature and humidity changes within the greenhouse produced a good description of the observed trends in these factors, taking account of both seasonal and diurnal variation and the differences between country and climate management. The model was particularly accurate at describing the temperature profile for the Spanish greenhouses under the two types of climate management. The fit of the model was reduced when simulating humidity and was also reduced when simulating either factor in the Netherlands. However, the qualitative fit was good for both humidity and the Netherlands with trends that matched those in the data.

As the model was based on the sine-exponential model for simulating temperature, (Parton and Logan, 1981), it is unsurprising that its accuracy was better for this factor. The pattern of temperature described by their model is one that increases with solar radiation, reaching a maximum in the late afternoon (when energy from sunlight combines with thermal radiation from features such the ground and vegetation) before declining exponentially to a minimum the following morning. The model describing humidity was essentially the reverse of this and described the diurnal trend in humidity fairly accurately despite greenhouse humidity being a result of a complex interaction of several different processes, e.g., crop transpiration, ventilation and condensation (Stanghellini and de Jong, 1995). Furthermore, the observed trends in humidity were more irregular than for temperature and this lack of smoothness has been observed to reduce the accuracy of the model (Reicosky *et al.*, 1989). This irregularity was likely due to factors not accounted for in the model such as plant transpiration (Baille *et al.*, 1994), venting (Arbel *et al.*, 1999), outside wind speed (Boulard and Draoui, 1995) or greenhouse events, e.g., watering or misting (Katsoulas *et al.*, 2001) and their inclusion would undoubtedly have improved the simulation of humidity.

The Parton and Logan (1981) sine-exponential model was also designed to predict outdoor temperatures rather than the more artificial conditions expected in greenhouses. Although factors found to reduce the accuracy of the original model such as wind would not be as important for this model, other factors such as heat inputs and the insulating properties of the greenhouse itself could be expected to

affect its accuracy. Indeed, the more artificial environment maintained in Dutch greenhouses may explain the reduced accuracy of the model for this country. Dutch greenhouses involve regular heating, sophisticated ventilation and materials with improved insulation properties (Bakker *et al.*, 2006), factors that may all affect trends in temperature and humidity. A further explanation for the reduced fit in the Netherlands is that it has been observed that the sine-exponential model is less accurate in cloudy conditions (Reicosky *et al.*, 1989) and where the differences between maximum and minimum values are small (Parton and Logan, 1981; Reicosky *et al.*, 1989), both of which apply more to Dutch greenhouse production than Spanish production.

The optimal parameter values chosen for the simulation model are an indication of the processes occurring in each country/climate management combination. For instance, the smaller lag coefficient for maximum temperature (*a*), in the passively ventilated Spanish greenhouse reflects the faster rate at which temperature increases in such production. Correspondingly the larger value for the night time temperature coefficient (*b*) in Spain indicates the more rapid decrease in temperature in the evening. A further example is the parameter modifying the changeover between the night and day time equations in the morning (*d*). This was larger for the Netherlands, resulting in greenhouse warming occurring longer after sunrise than in Spain, likely due to the difference in incident sunlight between the two latitudes.

There is scope for improvement of the model, for instance for the passively ventilated Spanish greenhouses maximum humidity was selected from a normal distribution around the fitted line. The distribution around the line was actually slightly skewed toward saturation meaning that the incidence of high humidities was probably under-predicted and an alternative to a normal distribution such as a beta distribution would have been a better choice. As the model was constructed for use in models predicting pest and disease pressures it would be preferable to simulate conditions within the canopy rather than above it. However, the data provided were recorded above the canopy (approximately one metre) and differences between these two levels are likely to be minimal, especially where ventilation is minimal (Boulard *et al.*, 1998; Lamrani *et al.*, 2001; Boulard *et al.*, 2002).

There were also a number of potentially important factors that were not included in the simulation model that have been used in others, such as external conditions (Boulard and Baille, 1993), solar radiation (Cunha *et al.*, 2000), crop transpiration (Fatnassi *et al.*, 2004), venting (Katsoulas *et al.*, 2006; Nebbali *et al.*, 2006) and greenhouse structure and dimensions (Cooper and Fuller, 1983; Kittas *et al.*, 2003). However, such models require data for a greater range of inputs and given that data was only available for temperature and humidity for this work a similar level of complexity was not possible. In addition, this model was not designed to predict the climate in any greenhouses other than those from which the data was collected. The greenhouses used were considered representative of those used in commercial tomato and rose production in Spain and the Netherlands and of an alternative, novel closed-system greenhouse in Spain, with the empirically fitted parameters essentially describe the differences between them. Nonetheless they assume climate management in each country/climate management combination is identical, which is unrealistic and means that applying the model to other greenhouses is likely to be unsuccessful, unless it was reparameterised.

The accuracy with which a stochastic empirical model such as this represents the trends and variability in a given system depends largely on the data on which it is based. In this case the model used collections of data ranging in size from the large sets used for passively ventilated Spanish conditions (four years of observations at one greenhouse) and Dutch conditions (one year of observations at twelve greenhouses) to a much smaller set used for the closed system conditions in Spain. It would obviously be preferable to have had access to more data for the latter, especially as it meant that no between year variation could be captured in the model. The same criticism can be applied to the Dutch data set, although it could be expected that the more closely controlled environment would be less effected by variations in outside conditions. The model would also be considered more representative of Spanish greenhouses (both passively ventilated and closed system) if data from other greenhouses under the same management were used in its construction.

While the model was a relatively simplistic representation of greenhouse conditions, it provided qualitatively and, to a lesser extent, quantitatively accurate and stochastically varied data sets. These are ideal for driving greenhouse pest and

disease models that take account of climatic conditions and allows the pest and disease pressures to be predicted in novel production scenarios and compared to those in the production scenarios currently in use as demonstrated in Chapters 5 and 6.

5. Modelling Oidium *neolycopersici* disease pressure and biological control in response to greenhouse environment

5. 1 Introduction

In Chapter 2 controlled environment (CE) experiments showed that powdery mildew disease development on tomato plants caused by *Oidium neolycopersici* was affected by ambient temperature and humidity conditions. The same experiments showed that *Bacillus subtilis* QST 713 could reduce powdery mildew diseased area by over 90% but its efficacy was dependent on (ambient) temperature and humidity. These results, along with those from other work (Whipps and Budge, 2000; Kashimoto *et al.*, 2003a; Jacob *et al.*, 2008; Mieslerova and Lebeda, 2010), suggest that control of this pathogen through manipulation of greenhouse temperature and humidity conditions may have potential for reducing disease pressure. Such an approach has been shown to be effective in reducing epidemics in commercial greenhouses in Israel (Elad *et al.*, 2009). The overall aim of this chapter was to use the data from Chapter 2 to develop a disease progress model in order to evaluate disease pressure and biological control efficacy under different greenhouse climate regimes at different times of year.

The influence of the environment on powdery mildew and its control has implications for the development of novel greenhouse climate regimes. Demands for more sustainable production systems have led to the development of novel greenhouse technologies and climate control methods that impact on the seasonal and diurnal patterns in temperature and humidity (Chapter 4). The primary aim of these systems is to minimise environmental impacts but they can also reduce expenditure on energy (Opdam *et al.*, 2005; Heuvelink *et al.*, 2008; UN, 2008). However, it is important to consider the consequences of novel climate management on pest and disease pressures. This chapter describes a predictive model for *O. neolycopersici* disease development and *B. subtilis*-mediated control in five greenhouse climate management scenarios. These scenarios are passively ventilated and closed-system greenhouse climate control in Spanish plastic-clad greenhouses and traditional, cool (traditional -2°C) and warm (traditional +2°C) Dutch Venlo greenhouses (Chapter 4).

Plant disease models have been developed both as research tools, where they can be used to improve the understanding of system processes (France and Thornley, 1984) and assess the relative effectiveness of resistance in different cultivars (de Vallavieille-Pope *et al.*, 2000), and also as forecasting models for use in crop production, where they can assist in reducing losses by providing information for disease management strategies such as the application of fungicides (Dik and Albajes, 1999). Due to the importance of water in the infection stage of many aerial plant diseases, forecast models have often used the duration of leaf wetness as the primary predictive parameter (Huber and Gillespie, 1992) but other parameters such as temperature, humidity, rainfall and periods of dryness have also been included (Hyre, 1954; Shtienberg and Elad, 1997; Korner and Holst, 2005). This has resulted in models ranging in complexity. At one end of this spectrum are models that simply rely on the coincidence of particular climatic events or conditions to identify high risk periods (Hyre, 1954; Aegerter *et al.*, 2003) and inform the timing of fungicide applications (Madden *et al.*, 1978; Berrie and Xu, 2003). More complex are dynamic models that mechanistically represent separate disease developmental stages to fully or partially simulate the disease cycle (Xu, 1999; Rossi and Giosue, 2003; Calonnec *et al.*, 2008), allowing better understanding of the interactions with environment.

The modelling of powdery mildew diseases is complicated by the reduced reliance on leaf wetness of many of the causal pathogens for infection and the involvement of more complex relationships with factors such as relative humidity (Jarvis *et al.*, 2002). Nevertheless, a number of powdery mildew disease models have been developed, including for *Erysiphe*/*Uncinula necator* on grape (Chellemi and Marois, 1991; Burie *et al.*, 2008; Calonnec *et al.*, 2008), *Podosphaera leucotricha* on apple (Xu, 1996; Xu, 1999; Berrie and Xu, 2003), *Blumeria graminis* on wheat (Rossi and Giosue, 2003) and *Leveillula taurica* on tomato (Guzman-Plazola *et al.*, 2011). However, no models have been developed for *O. neolycopersici* or its control with *B. subtilis*. Furthermore, the majority of powdery mildew disease simulators (including all of the above) have been developed for field crops. One of the limitations of such models is the difficulty in reliably forecasting weather more than a few days in advance. An advantage of modelling greenhouse diseases is that the seasonal and diurnal environmental conditions can be simulated relatively accurately (as in Chapter 4), allowing year round disease development to be predicted and integrated

pest management strategies to be developed. Such an integration of greenhouse climate and disease pressure modelling has been used in formulating novel control strategies for *Botrytis cinerea* (Tantau and Lange, 2003) and *Peronospora parasitica* (Kofoet and Fink, 2007).

The model developed here simulates the progress of powdery mildew disease caused by the *O. neolycopersici*. The factors affecting the disease in the model are ambient greenhouse temperature and humidity and the presence or absence of the biological control agent *B. subtilis* as quantified in Chapter 2. It comprises two sub-models, which focus on key stages of the pathogen disease cycle; latent period and disease development, and hence simulates a single disease cycle. The effect of seasonal and diurnal variation in greenhouse temperature and humidity conditions on the model outcomes can then be investigated by simply varying the inoculum arrival/infection times between model runs. The latent period, defined as the time from infection to first production of new inoculum, is a recognised key factor in forecasting disease epidemics (de Vallavieille-Pope *et al.*, 2000), while understanding the rate of disease progression is considered the most important epidemiological parameter in developing integrated disease management programs (Nutter, 2007). Neither of these stages have been studied in *O. neolycopersici* in terms of their response to the interaction of temperature and humidity or the application of *B. subtilis*.

The infection process for *O. neolycopersici* is also affected by temperature and humidity (Kashimoto *et al.*, 2003a; Jacob *et al.*, 2008; Mieslerova and Lebeda, 2010) and occurs over a wide range of conditions (10-35°C and 33-99% RH; Kashimoto *et al.*, 2003a; Jacob *et al.*, 2008; Mieslerova and Lebeda, 2010). However, this stage of the pathogen lifecycle was not included in the model as infection conditions regularly occur in greenhouses and incorporating this phase would have greatly increased the complexity of the model. Furthermore, while the studies investigating infection processes (Kashimoto *et al.*, 2003a; Jacob *et al.*, 2008; Mieslerova and Lebeda, 2010) generally found similar trends, some large differences in the results were evident. For instance, both percentage germination and appressorial formation at optimal conditions were over twice as high in the study by Kashimoto *et al.* (2003a) compared to that of Jacob *et al.* (2008), suggesting differences in *O. neolycopersici* isolate response. A high degree of *O. neolycopersici* inter-isolate

variation has been shown previously (Lebeda and Mieslerova, 2002; Jankovics *et al.*, 2008).

Initial inoculum load and the time period over which the pathogen and the crop interact are also regarded as key epidemiological parameters (Nutter, 2007). In a greenhouse system where the production of a single crop is continuous, the pathogen and crop can theoretically interact year round and for the purposes of applying the model to different greenhouse management scenarios, this and the constant availability of inoculum are assumed. Interactions of initial inoculum density and subsequent disease severity have also been reported to be important for a number of pathogens (Landa *et al.*, 2001; Berbegal *et al.*, 2007) but this has not yet been shown for *O. neolycopersici* and as such was not included in the model. Spore production and dispersal were also not included, although percentage disease area represented a good proxy for spore production (Section 2. 3. 4). Simulating spore dispersal in greenhouses is very complex (Frinking and Scholte, 1983), with critical factors including spore size, release height and mechanisms, crop structure and density, greenhouse layout and air movement (Legg, 1983). The latter factor alone is a product of a wide range of variables such as greenhouse dimensions, temperature and ventilation (Atarassi *et al.*, 2006; Sase, 2006; Castilla and Montero, 2008) and computational fluid dynamics modelling is the general approach to simulating its influence on spore deposition (Roy *et al.*, 2006). For simplicity, the model presented in this chapter assumes a uniform distribution of the disease across the greenhouse. While the model described here does not take into account all stages of the *O. neolycopersici* lifecycle, it was felt to be an appropriate tool for assessing seasonal and climate management effects on disease pressure and biological control efficacy.

5. 2 Model description

5. 2. 1 General

An integrated powdery mildew disease progress model was constructed in Microsoft Excel® and comprised two sub-models representing the two epidemiological stages; latent period and disease development (Fig. 5.1). The former models the length of the latent period and the latter simulates the increase in percentage diseased leaf area over time. The effect of greenhouse climate management (in terms of temperature and humidity) on these stages was modelled either with or without the biological control agent, *B. subtilis*. Five greenhouse management scenarios were investigated; passively ventilated and closed-system greenhouses in Spain and traditional, cool (traditional -2°C) and warm (traditional +2°C) greenhouses in the Netherlands.

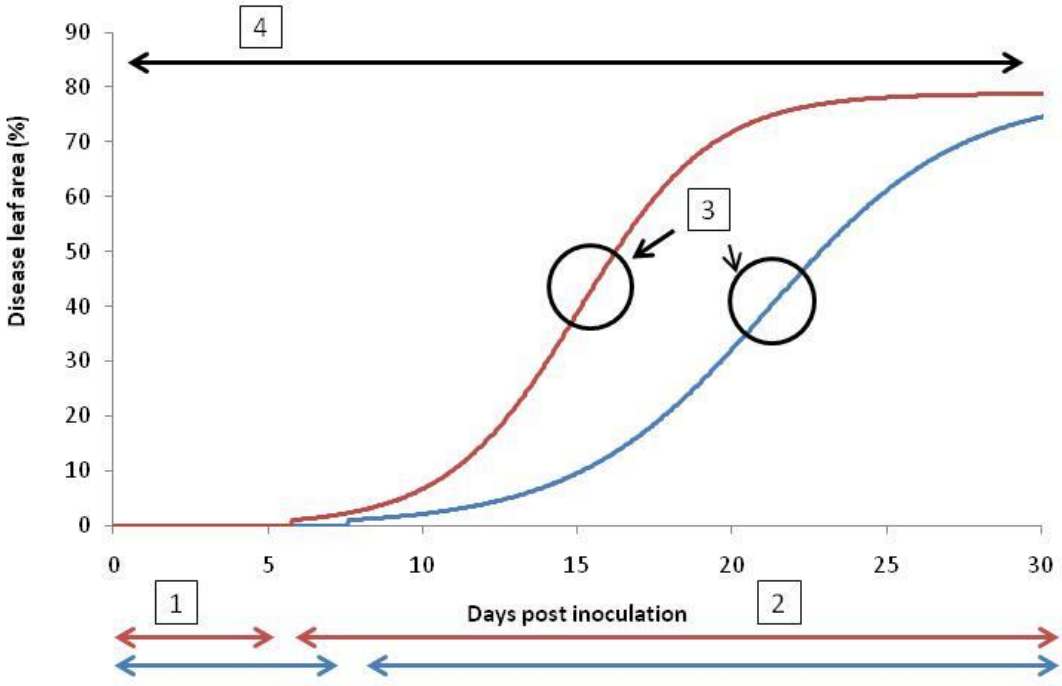

Figure 5.1: Diagram showing approach taken to modelling *O. neolycopersici* disease progress, using 16°C (blue line) and 24°C (red line) as examples. The latent period sub-model simulates the duration in days of the latent period (1). The disease development sub-model simulates the increase diseased leaf area over time (2) and, for the purposes of assessing the impacts of greenhouse environment and biological control, specifically assesses the daily maximum rate of development (3). The sub-models are combined to form the integrated disease progress model (4), which simulates the time until a threshold disease level is reached.

The greenhouses are spatially homogenous and time is dynamically modelled in discrete hourly intervals for a year. The model is deterministic but for each of the five greenhouse climate scenarios ten stochastically differing sets of one year data were used as generated in Chapter 4. To assess the influence of seasonal differences in the greenhouse environmental conditions, the model simulates the infection occurring at every hour of the year. Data for constructing the model were taken from controlled environment experiments described in Chapter 2. Mathematical models were fitted to these data using Genstat®.

5. 2. 2 Latent period sub-model

Experimental work showed that the latent period of *O. neolycopersici* varied primarily with temperature. However, humidity was also shown to have an influence (Chapter 2), especially in the presence of *B. subtilis*. As small effects can accumulate in simulation models it was decided to include the influence of humidity. Hence, the latent period of *O. neolycopersici* with and without biological control was fitted to a surface plot of humidity and temperature.

For *O. neolycopersici* without *B. subtilis* the surface plot was fitted to experimental data in the range of 70-90% RH and 15-25°C, explaining 81% of the variance. Although 50% RH was investigated at 20-25°C, these data were not included as the effect of *B. subtilis* was not investigated at this humidity/temperature(s) combination (which would have made comparison problematic). The surface plot can be described with the following equation:

$$LP = \left((0.02813 * t^2) + (0.01323 * rh^2) + (-1.226 * t) + (-2.114 * rh) + (-0.00187 * t * rh) + 105.24\right) \quad 5.1$$

Where *LP* = the latent period (days), *t* = the temperature (°C) for that hour and *rh* = the humidity (% RH) for that hour. These parameter designations also apply for all the following equations in this Chapter. For *O. neolycopersici* with *B. subtilis* a surface plot was fitted to the same data range, accounting for 84% of the variation:

$$LP = \left((0.0325 * t^2) + (0.018 * rh^2) + (-1.966 * t) + (-2.976 * rh) + (0.00575 * t * rh) + 144.82\right) \quad 5.2$$

The equations 5.1 and 5.2 were used to predict the latent period in response to hourly humidity and temperature within the 15-25°C range. To prevent extrapolation beyond the data range investigated, *LP* values for RH> 90% and <70% were set to the same values for 90% and 70% RH respectively.

To predict the latent period when temperatures were above 25°C or below 15°C quartic regressions were fitted to experimental data between 10-29°C at 70% RH for *O. neolycopersici* both with and without *B. subtilis*. These accounted for 98% and 99% of the variation respectively. The regression for *O. neolycopersici* without *B. subtilis* can be described as:

$$\mathrm{LP} = \left((0.00089 * t^4) + (-0.07981 * t^3) + (2.665 * t^2) + (-39.32 * t) + 222.6\right) \quad 5.3$$

For *O. neolycopersici* treated with *B. subtilis* the regression can be written as:

$$LP = \left((0.000789 * t^4) + (-0.0701 * t^3) + (2.343 * t^2) + (-34.93 * t) + 202.2\right) \quad 5.4$$

To ensure that the model did not extrapolate beyond the data range, *LP* values for temperatures <10°C and >29°C were set to the same values as for 10°C and 29°C respectively. For each hour of the year the model calculates the duration of the latent period at the prevailing temperature and humidity using one of the two equations for each treatment (5.1 and 5.3 for *O. neolycopersici* only or 5.2 and 5.4 when treated with *B. subtilis*), with the choice of equation dependent on the temperature. This is done assuming the conditions for that hour remain constant for the entire latent period, resulting in a latent period (days) based on the temperature and humidity for that hour, e.g., at 03.00, 18°C and 87% RH without *B. subtilis LP* would be 5.6 days. A mean *LP* value for each day and month of year was calculated to assess the impact of diurnal and seasonal changes in conditions under different climate management regimes. A rate of latent period progress can also be calculated using this model, by taking the inverse of the time, and is used in the integrated disease model as described in Section 5. 2. 4. Example output of the sub-model for *O. neolycopersici* only and for *O. neolycopersici* and *B. subtilis* can be seen in Tables 5.1 and 5.2 respectively.

Table 5.1: Example output (days) of the latent period sub-model of *O. neolycopersici* in response to humidity and temperature (Equations 5.1 and 5.3).

°C	Humidity (% RH)							
	50	**60**	**70**	**75**	**85**	**90**	**95**	**100**
10.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0
12.5	13.4	13.4	13.4	13.4	13.4	13.4	13.4	13.4
15.0	8.1	8.1	8.1	6.9	6.7	7.6	7.6	7.6
17.5	7.0	7.0	7.0	5.8	5.5	6.4	6.4	6.4
20.0	6.2	6.2	6.2	5.0	4.7	5.5	5.5	5.5
22.5	5.8	5.8	5.8	4.6	4.2	5.0	5.0	5.0
25.0	5.7	5.7	5.7	4.5	4.1	4.9	4.9	4.9
27.5	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9
30.0	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6
32.5	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6

Table 5.2: Example output (days) of the latent period sub-model of *B. subtilis*-treated *O. neolycopersici* in response to humidity and temperature (Equations 5.2 and 5.4).

°C	Humidity (% RH)							
	50	**60**	**70**	**75**	**85**	**90**	**95**	**100**
10.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0
12.5	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0
15.0	8.6	8.6	8.6	7.2	7.1	8.4	8.4	8.4
17.5	7.3	7.3	7.3	6.0	6.0	7.4	7.4	7.4
20.0	6.4	6.4	6.4	5.2	5.4	6.8	6.8	6.8
22.5	6.0	6.0	6.0	4.8	5.1	6.6	6.6	6.6
25.0	5.9	5.9	5.9	4.8	5.3	6.9	6.9	6.9
27.5	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9
30.0	8.1	8.1	8.1	8.1	8.1	8.1	8.1	8.1
32.5	8.1	8.1	8.1	8.1	8.1	8.1	8.1	8.1

5. 2. 3 Disease development sub-model

The disease development sub-model simulates the increase of leaf area (%) affected by *O. neolycopersici* over time as measured through image analysis (Section 2. 2. 2). Modelling disease development in terms of percentage disease leaf area is useful as this variable was shown to be a good proxy for spore production (Section 2. 3. 4) and hence also allows inoculum production levels to be potentially estimated. This parameter also relates directly to yield reductions, as it reflects a reduction in photosynthetic area (de Vallavieille-Pope *et al.*, 2000; Bushnell, 2002; Mieslerova *et al.*, 2004). Experimental work (Section 2. 3. 1) found that disease development exhibits a sigmoidal pattern over time that was well described by a logistic model. Logistic models have been used to describe population growth in a wide range of species (Gillman and Hails, 1997) and are probably the most commonly used for describing the development of plant diseases (Nutter, 2007). The logistic equation can be written as:

$$DA = \frac{C}{\left(1+e^{(-B*(X-M))}\right)} \qquad 5.5$$

Where *DA* = the disease area (%), *C* = the maximum possible disease area, *B* relates to the rate of change, *X* = time (days) from completion of the latent period, and *M* = the inflection point of the curve (changes to *M* are most clearly seen as changes to the lag phase of the disease development, e.g., increases in *M* increase the duration of the lag phase). Using Genstat's® "fitnonlinear" procedure, logistic curves were separately fitted to the disease development data for each temperature, humidity and biological control treatment combination as described in Chapter 2. As this sub-model was simulating the disease development post-latent period, the time when disease was first observed was set to 0 for each data set. The fitnonlinear procedure optimises the *C*, *B* and *M* parameters for Equation 5.5 and the output can be seen in Appendix 2. This initial fitting process allowed trends in the parameters in response to temperature and humidity to be examined and revealed that in the absence of *B. subtilis*, *O. neolycopersici* disease development was only affected by temperature, reinforcing the statistical analysis in Section 2. 3. 1. As trends in the parameters were evident in response to humidity for *B. subtilis* treatments, this factor was included in the model where the biological control response was simulated.

To allow *B* (the rate) and *M* (the length of the lag phase) to be directly comparable between greenhouse conditions and biological control it was necessary to select a shared *C* (maximum disease area) value for all conditions. This seemed a sensible approach as this parameter is likely to be primarily influenced by the quality and availability of remaining host tissue rather than environmental factors, and while *B. subtilis* affected the rate and shape of the disease development curves it did not appear to affect maximum disease area. The disease did not cover 100% of leaf area in any of the experiments described in Chapter 2. It was therefore decided to fix *C* at an appropriate percentage leaf cover that represented the maximum achievable percentage. The value of *C* was set to 78.8%, as fitted to the 20°C/90% RH *O. neolycopersici* only treatment data. This combination of temperature and humidity was chosen as it gave the highest value calculated using the fitnonlinear procedure and was the experiment treatment in which the highest disease area was recorded. While for the majority of the experiments % disease area continued to increase, for some, such as at 25-27°C, disease development plateaued at less than 78.8% leaf area. However, it is possible that this was the result of increased canopy growth at these temperatures, resulting in increased shading and senescence of lower leaves, which may have limited the available leaf area for disease development (Bushnell, 2002; Jacob *et al.*, 2008; Mieslerova and Lebeda, 2010). In this way, the use of a consistent value for *C* allowed greenhouse climate and biological control to be accounted for as factors determining progress toward a theoretical maximum disease area.

Following fixing the *C* parameter at 78.8, the logistic curves (Eq. 5.5) were re-fitted to the data, this time optimising *B* and *M* only (Table 5.3). For the experiments in which a plateau was reached, the equation was fitted only to data prior to the plateau. Relationships between the parameter values for different environmental conditions were then explored. As mentioned above, in the absence of the *B. subtilis* treatment *O. neolycopersici* disease development was only investigated in response to temperature while in the presence of *B. subtilis*, both humidity and temperature were considered.

Table 5.3: Parameters of logistic curves (Eq. 5.5) fitted to *O. neolycopersici* disease development data at different temperature and humidity conditions (Chapter 2). Treatment indicates whether experiments involved *O. neolycopersici* only (ON) or with *B. subtilis* (BS) and 'Pooled' indicates that data were combined for the two treatments.

Treatment	Temperature (°C)	Humidity (% RH)	*B*	*M*	*C*
ON	10.69	69.42	0.06	74.00	78.8
ON	15.53	76.21	0.28	13.60	78.8
ON	14.90	71.68	0.35	15.31	78.8
ON	14.16	91.48	0.22	19.18	78.8
ON	20.52	52.69	0.50	8.88	78.8
ON	20.73	68.00	0.35	9.72	78.8
ON	20.42	83.84	0.39	12.07	78.8
ON	19.58	90.00	0.58	9.25	78.8
ON	25.33	51.56	0.42	7.98	78.8
ON	24.86	78.92	0.39	9.19	78.8
ON	26.10	67.97	0.46	7.05	78.8
ON	25.78	79.63	0.38	10.39	78.8
ON	25.85	95.83	0.48	9.38	78.8
ON	26.93	61.80	0.32	14.37	78.8
ON	28.89	74.25	0.07	73.10	78.8
BS	10.69	69.42	0.10	48.90	78.8
BS	14.16	91.48	0.18	25.01	78.8
BS	14.90	71.68	0.31	14.80	78.8
BS	19.58	90.00	0.36	14.63	78.8
BS	20.42	83.34	0.41	14.47	78.8
BS	20.73	68.00	0.42	9.51	78.8
BS	24.86	78.92	0.38	11.70	78.8
BS	25.78	79.63	0.28	16.07	78.8
BS	25.85	95.83	0.29	19.58	78.8
BS	26.93	61.80	0.15	29.67	78.8
BS	28.89	74.25	0.03	170.00	78.8
Pooled	10.69	69.42	0.08	58.2	78.8
Pooled	28.89	74.25	0.04	123.1	78.8

For the *O. neolycopersici* only treatment a cubic regression accounting for 71% of the variation was fitted to the distribution of the *B* parameter values in response to temperature (F = 12.13, df = 3, P = <0.01; Fig. 5.2):

$$B_o = \left((-0.000275339 * t^3) + (0.0119119 * t^2) + (-0.123132 * t) + 0.370684\right) \quad 5.6$$

For the *M* parameter values in the *O. neolycopersici* only treatment a quartic regression was fitted (F = 146.74, df = 4, P = <0.01), accounting for 98% of the variation with temperature (Fig. 5.3):

$$M_o = \left((0.0141376 * t^4) + (-1.10243 * t^3) + (31.7948 * t^2) + (-403.041 * t) + 1911.6\right) \quad 5.7$$

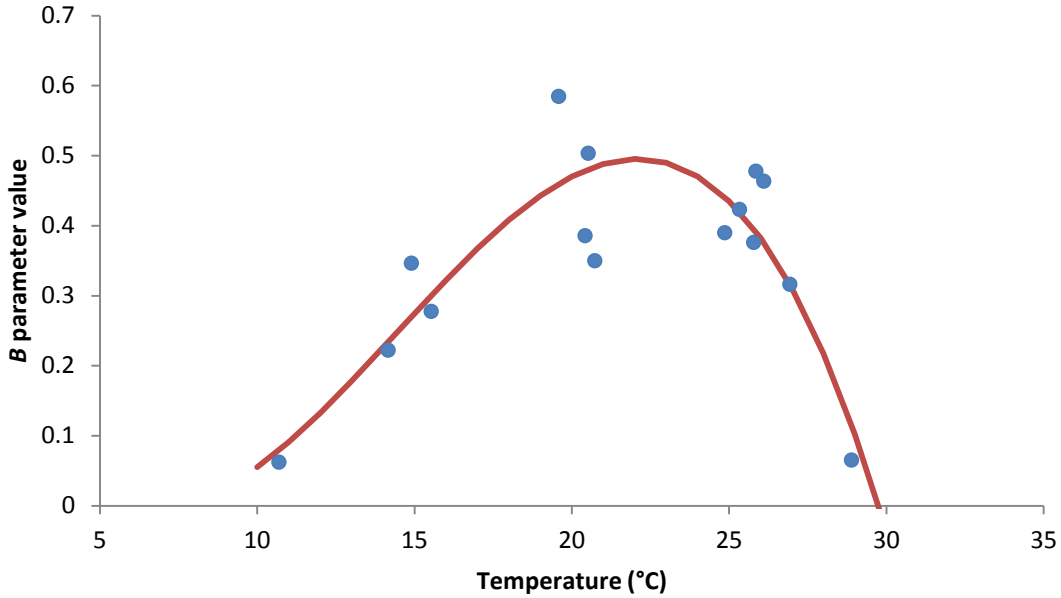

Figure 5.2: *B* parameter values (•) for logistic curves fitted to the *O. neolycopersici* disease development data at different temperatures in the absence of *B. subtilis* treatment. Fitted cubic regression (—; Eq. 5.6) is shown.

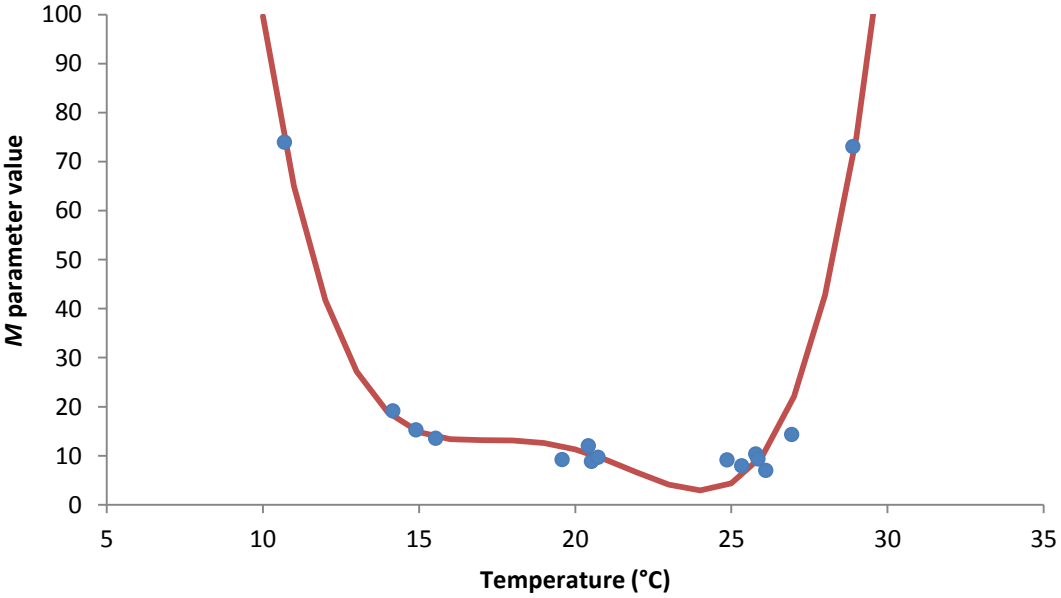

Figure 5.3: *M* parameter values (•) for logistic curves fitted to the *O. neolycopersici* disease development data at different temperatures in the absence of *B. subtilis* treatment. Fitted quartic regression (—; Eq. 5.7) is shown.

For the parameters for *O. neolycopersici* disease development following treatment with *B. subtilis*, the influence of humidity as well as temperature was included. Significant reductions in disease area due to *B. subtilis* were found between 15-27°C and 50-90% RH with a trend for increasing efficacy with temperature and humidity in Chapter 2. However, the interaction of temperature and humidity were only investigated between 15-25°C and 70-90% RH. For this reason the trends of *B* and *M* in response to environment were modelled in two ways. The first modelled the response to temperatures above 25°C and below 15°C while the second modelled the response to the interaction of temperature and humidity between 15°C and 25°C.

To model the parameter values above 25°C and below 15°C regressions of *B* and *M* were calculated for temperatures between 10.7°C and 28.9°C at 70% RH. Initial regressions for the disease development data for the *B. subtilis* treatment showed that the values for both parameters at 10.7°C and 28.9°C (*M* = 48.9 and 170 and *B* = 0.1 and 0.03 for 10.7°C and 28.9°C respectively) were having a disproportional influence on the shape of the regression curves. To remedy this, logistic curves (Eq. 5.5) were fitted to the combined data for both the *O. neolycopersici* only treatment and the *O. neolycopersici* with *B. subtilis* treatment at 10.7°C and 28.9°C, which produced *M* values of 58.2 and 123.1 and *B* values of 0.08 and 0.04 for 10.7°C and 28.9°C respectively (Table 5.3). This improved the shape of the regression curves (Fig. 5.4-5.5) and was justifiable as *B. subtilis* had a negligible effect on the disease at 10.7°C and 28.9°C.

A cubic regression was fitted to the *B* parameter values (including the pooled values) with temperature, describing 90.3% of the variation (F = 31.86, df = 3, P = <0.01; Fig. 5.4):

$$B_{b1} = \left((-0.000209511 * t^3) + (0.00821887 * t^2) + (-0.0650449 * t) + 0.0902389\right) \quad 5.8$$

The *M* parameter values (including the pooled values) were described by a quartic regression (F = 94.46, df = 4, P = <0.01; Fig. 5.5), which accounted for 97.4% of the variation:

$$M_{b1} = \left((0.0173082 * t^4) + (-1.28884 * t^3) + (35.3127 * t^2) + (-423.31 * t) + 1897.01\right) \quad 5.9$$

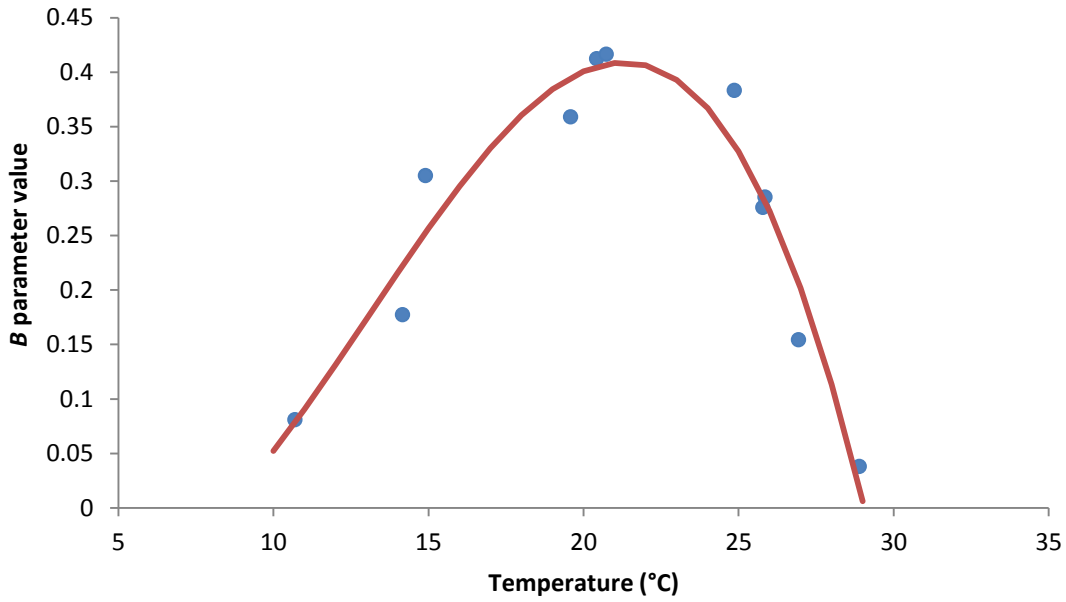

Figure 5.4: *B* parameter values (•) for logistic curves fitted to the *O. neolycopersici* disease development data at different temperatures following *B. subtilis* treatment. Fitted cubic regression (—; Eq. 5.8) is shown.

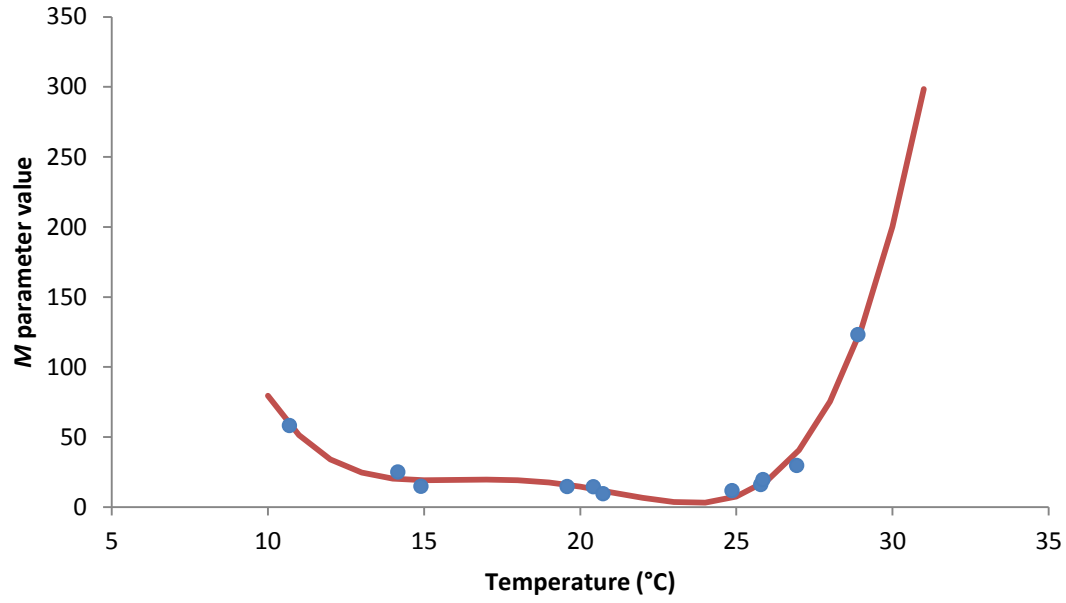

Figure 5.5: *M* parameter values (•) for logistic curves fitted to the *O. neolycopersici* disease development data at different temperatures following *B. subtilis* treatment. Fitted quartic regression (—; Eq. 5.9) is shown.

To model the response of the *B* and *M* parameter values between 15°C and 25°C and the interaction with humidity a series of linear regressions against humidity were first carried out at 15, 20 and 25°C (Table 5.4). At each of these temperatures only two or

three humidities were investigated, which makes these regressions rather simplistic and none were significant. However, while more data points would have made quantifying the trends more accurate, it was felt important to include them to describe the strong influence of humidity on *B. subtilis* control efficacy.

Table 5.4: Values for the intercept (α) and slope (β) parameters of linear regressions of the logistic growth curve parameters, *B* and *M*, against humidity at 15-25°C.

Temperature (°C)	Parameter	α	β
15	*B*	0.7674	-0.00645
20	*B*	0.573314	-0.0022
25	*B*	0.558613	-0.00287
15	*M*	-22.153	0.515556
20	*M*	-7.12044	0.248485
25	*M*	-14.0729	0.352095

Quadratic regressions were then fitted to the intercept (α) and slope (β) parameters of these regressions and integrated to allow the *B* (Eq. 5.10) and *M* (Eq. 5.11) parameters to be calculated at any humidity between 15-25°C (Fig. 5.6 and 5.7 respectively):

$$B_{b2} = \left((0.0035877 * t^2) + (-0.164387 * t) + 2.42597\right) + \left(\left((-0.0000983006 * t^2) + (0.00428951 * t) \pm 0.0486745\right) * rh\right) \quad 5.10$$

$$M_{b2} = \left((-0.4397 * t^2) + (18.396 * t) + -199.161\right) + \left(\left((0.00741362 * t^2) + (-0.312891 * t) \pm 3.54085\right) * rh\right) \quad 5.11$$

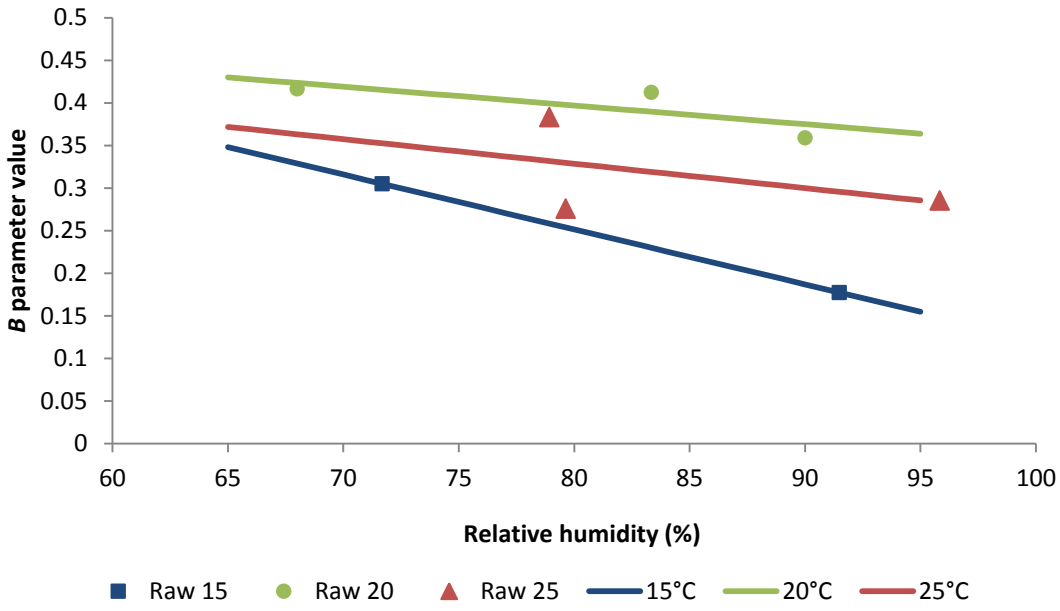

Figure 5.6: Variation in *B* parameter values (•) with humidity of logistic curves fitted to the *O. neolycopersici* disease development data at three temperatures (15, 20 and 25°C) following *B. subtilis* treatment. Lines show quadratic equation (Eq. 5.10) describing the relationship.

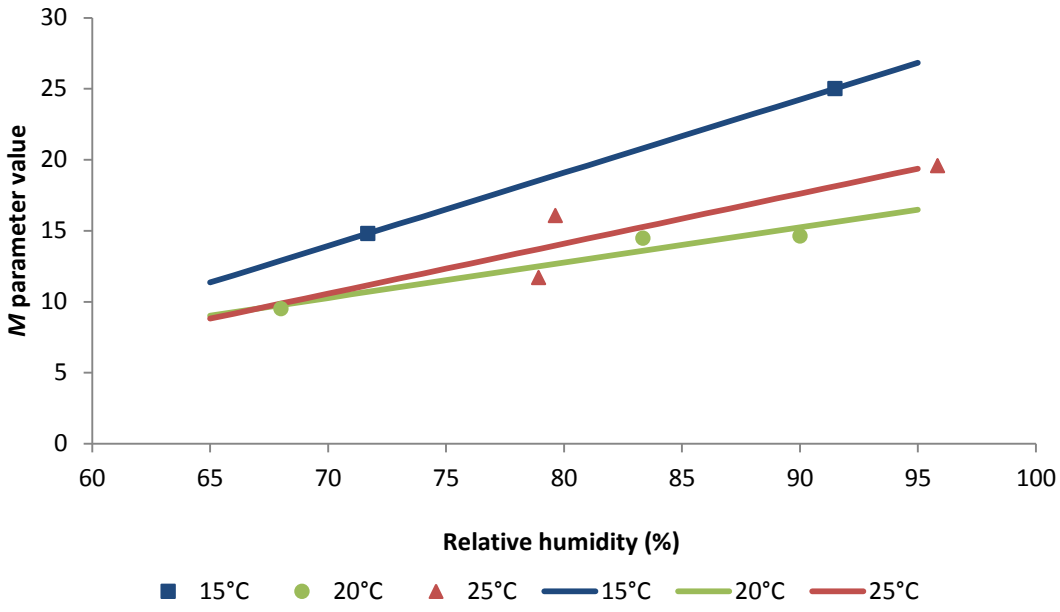

Figure 5.7: Variation in *M* parameter values (•) with humidity of logistic curves fitted to the *O. neolycopersici* disease development data at three temperatures (15, 20 and 25°C) following *B. subtilis* treatment. Lines show quadratic equation (Eq. 5.10) describing the relationship.

In this way the *B* and *M* parameters of the logistic equation (5.5) were modelled in response to temperature and humidity with or without the *B subtilis* biological control. To constrain the disease area at the intercept of the logistic equation ($X = 0$) to less than 2%, the *M* parameter was restricted to no less than 9.3 for the *O. neolycopersici* only treatment and 11.9 for the *O. neolycopersici* with *B. subtilis* treatment. To constrain powdery mildew disease development to that observed in the experiments, *B* was set to 0 at <10°C and >31°C, with the latter chosen as a half way point between the highest temperature at which development was recorded (29°C) and that at which none was recorded (33°C). Finally, although pooled data with and without *B. subtilis* treatment were used for fitting the 10°C and 29°C logistic curves for the *B. subtilis* treatment, it was decided not to do this for the *O. neolycopersici* only treatment. The reason for this was that the resulting disease curves had an improved fit to the data between 15-20°C. Overall this modelling approach provided a good fit to the observed data in both the *O. neolycopersici* only treatment (Fig. 5.8) and the *O. neolycopersici* and *B. subtilis* treatment (Fig. 5.9a and b).

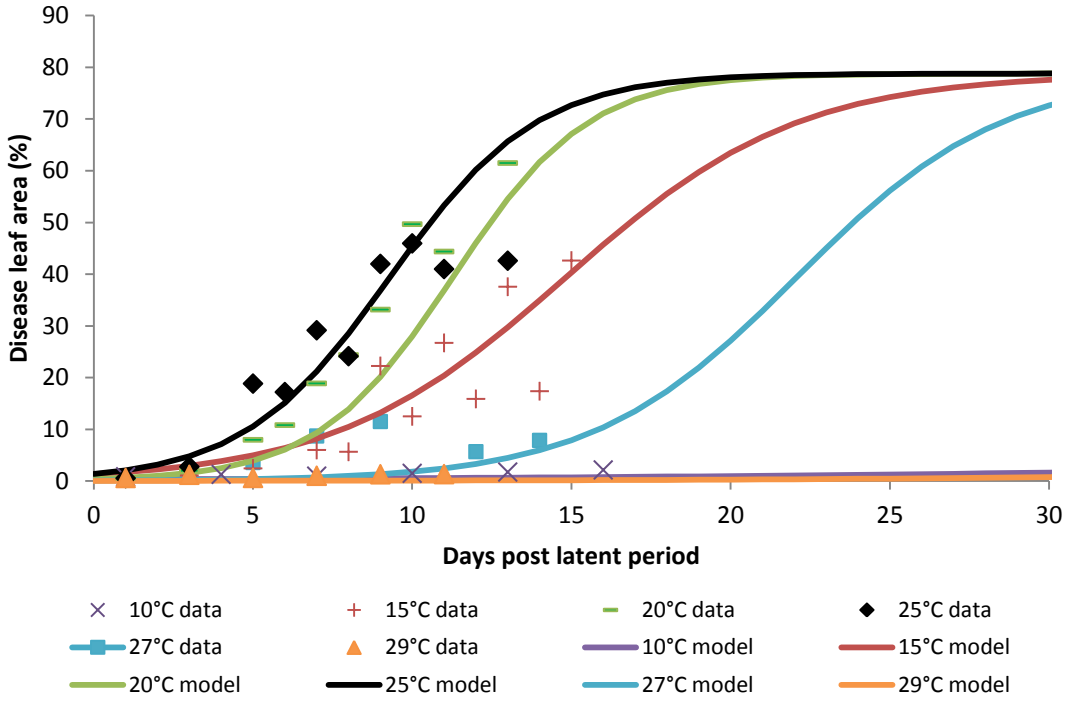

Figure 5.8: Example modelled disease development curves of *O. neolycopersici* in the absence of *B. subtilis* at different temperatures compared to experimental observations. *B* and *M* parameter values for the model were calculated using Equations 5.6-5.7.

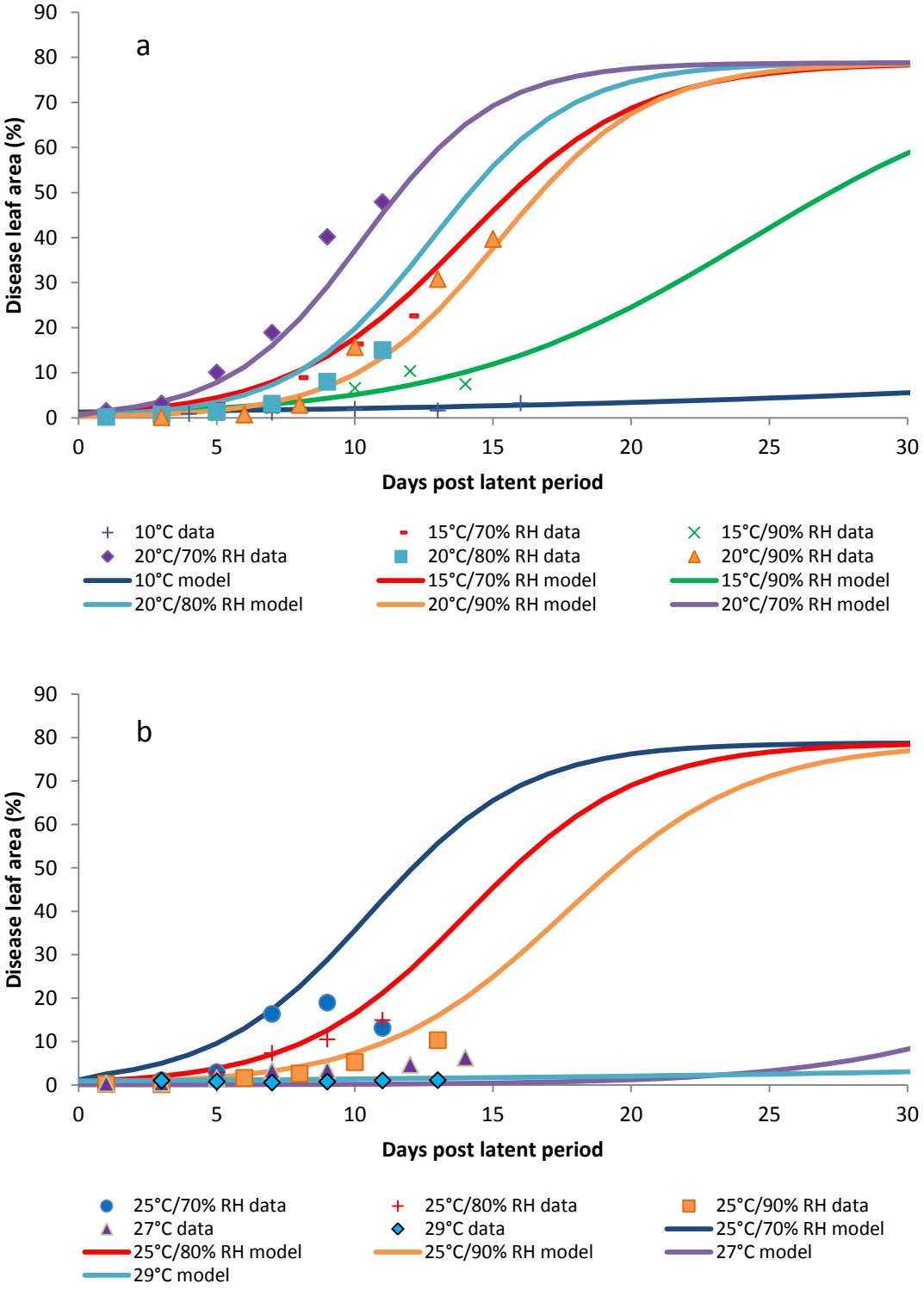

Figure 5.9: Example modelled disease development curves of *O. neolycopersici* following *B. subtilis* treatment at different humidities at a) 10-20°C and b) 25-29°C compared to experimental observations. *B* and *M* parameter values for the model were calculated using Equations 5.8-5.11.

This disease development sub-model was combined with the latent period sub-model in the integrated disease model (Section 5. 2. 4) to give an overall prediction of disease progress. However, to assess the impact of greenhouse environment on disease development alone, the maximum daily increase in diseased leaf area was assessed (Fig. 5.1). This was calculated using:

$$MG = B * \frac{C}{4} \quad 5.12$$

Where *MG* = the mean % daily increase in disease area and *B* and *C* are the logistic growth curve parameters. *MG* was calculated for every hour of the year and for analysis purposes was averaged across days and months to give a mean maximum daily increase in disease area for each day and month of the year. This allowed the seasonal trends in disease development to be examined in response to climate management and biological control.

5. 2. 4 Integrated disease progress model

The integrated powdery mildew disease model simulated disease progress, here defined as the time taken (T_{50}) for the disease area to reach 50% of the theoretical maximum of 78.8% (to distinguish it from disease development). This model combines the two sub-models representing completion of the latent period and disease development.

In this disease progress model the latent period (*LP*) is calculated as a rate. To convert the *LP* calculated in Section 5. 2. 2. (Eq. 5.1-5.4 and given in days) into an hourly rate the following calculation was used:

$$PD_i = \frac{\left(\frac{1}{LP_i}\right)}{24} \quad 5.13$$

Where PD_i = a value between 0 and 1 for the *i*th hour and LP_i = the latent period calculated for that hour (based on equations 5.1, 5.2, 5.3 or 5.4). The PD_i values accumulate over time and once $PD_i \geq 1$ the latent period is deemed to be complete.

Once the latent period is complete the development of disease across the leaf tissue is then simulated. This was modelled using logistic curves (Eq. 5.5) as described in the

previous Section where the *B* and *M* parameter values vary depending on temperature, humidity and control treatment. To simulate the change in disease area over time the logistic equation was rearranged to give:

$$\frac{d^{DA}}{d^{T}} = \frac{\left(-C*-B*\frac{e^{-B*\left(\left(\frac{\left(B*M*-\ln\left(\frac{(C-A)}{A}\right)\right)}{B}\right)-M\right)}}{1+e^{-B\left(\left(\frac{\left(B*M*-\ln\left(\frac{(C-A)}{A}\right)\right)}{B}\right)-M\right)}} \right)^{2}}{24} \qquad 5.14$$

Where dDA/dT = the % increase in disease area in one hour at any given initial disease leaf area. In this way the disease area increase for each hour was calculated in response to the greenhouse environment and the current disease area. For this part of the model, temperatures between 28.7°C and 31°C were set to 28.7°C. This was to prevent a mismatch between the *M* and *B* parameters in the *B. subtilis* treatment that would otherwise predict very high disease development. Very little difference in disease development was predicted between 28.7-31°C in the *O. neolycopersici* only treatment meaning that this adjustment had little effect otherwise. Above 31°C disease development remained set to zero.

Beginning at the start of every hour of the year, disease progress was simulated to develop until 50% of the theoretical maximum (78.8%) diseased leaf area (39.4%) was reached. The time taken (days) to reach this (T_{50}) value was calculated by the model. For analysis purposes T_{50} was averaged for every day and month allowing seasonal changes in disease development to be evaluated.

The disease progress model simulated the disease in a more sophisticated manner to the disease development sub-model, which assessed the impacts of greenhouse conditions and biological control in terms of *MG* (the maximum daily increase in disease area), a simplification of disease development that only accounted for changes in *B* (the rate of growth). The integrated model however, related disease progress to the current disease area and changes in *M* (the inflection point of the curve and the factor determining the end of the lag phase). This is important because (as detailed in Section 5. 2. 3) greenhouse environment and biological control have a strong influence on *M* and hence a strong influence on the disease progress.

5. 2. 5 Statistical analysis

The output from each model was analysed in Genstat® using analysis of variance with a treatment structure of greenhouse climate management by treatment (with or without *B. subtilis*) by month (climate*treatment*month). Day number was divided into 13 "months" of four weeks. No blocking structure was necessary. In summarising the analyses, all significant differences are followed by the approximate *F* statistic, the degrees of freedom (df) and the probability that the null hypotheses can be rejected (based on the approximate *F* value).

5. 3 Results

5. 3. 1 Latent period sub-model

5. 3. 1. 1 Spanish greenhouses

Climate management had a minor, though significant, impact on the *LP* (latent period) of *O. neolycopersici* with *LP* significantly longer in the closed-system greenhouse for months 1-5 and 12-13 (F = 132.02, df = 48, P = <0.001; Table 5.5). The largest difference was found in month 13 when *LP* was 12.8 days in the closed-system greenhouse and 11.2 days in the passively ventilated greenhouse. Seasonal impacts had a stronger influence on *LP* (F = 299.91, df = 12, P = <0.001), with the duration in the winter (months 12-2) approximately double that in the summer (months 6-8) in both greenhouses (Table 5.5).

Table 5.5: Latent period (days) of *O. neolycopersici* in Spanish closed-system (CS) and passively ventilated (PV) greenhouses over time (W = winter, Sp = spring, Su = summer and A = autumn). Trt. indicates treatment with (BS) or without (ON) the biological control agent, *B. subtilis*.

Climate Mgmt.	Trt.	Month												
		W	W	Sp	Sp	Sp	Su	Su	Su	A	A	A	W	W
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
CS	ON	12.5	10.4	9.1	8.4	7.1	6.0	5.7	5.9	5.8	5.5	6.2	9.0	12.8
PV	ON	11.1	9.7	8.4	7.3	6.4	5.9	5.9	6.1	6.2	6.0	6.1	7.9	11.2
CS	BS	11.7	10.0	8.9	8.2	7.3	6.4	6.4	6.6	6.5	6.2	6.7	9.1	12.0
PV	BS	11.6	10.2	8.9	7.9	7.0	6.5	6.7	7.1	7.0	6.7	6.6	8.5	11.7

B. subtilis application significantly increased *LP* year round in the passively ventilated greenhouse (F = 1.72, df = 48, P = <0.002; Table 5.5) with the greatest increase in month 8 (0.9 days or 15% longer). No significant increase in *LP* due to *B. subtilis* was found in the closed-system greenhouse. The greatest difference in *LP* was between the closed-system greenhouse without *B. subtilis* (5.5 days) and the passively ventilated greenhouse with *B. subtilis* (6.7 days) in month 10, an increase of 23%.

5. 3. 1. 2 Dutch greenhouses

The powdery mildew disease *LP* was significantly longer in both the cool and warm Dutch greenhouses (overall mean of 5.6 and 5.7 days respectively) than the traditional greenhouse (5.0 days; F = 1924.76, df = 4, P = <0.001) but they were not significantly different from one another. The latent period in the warm greenhouse was significantly longer than in the traditional greenhouse year round but in the cool greenhouse was longer in months 1-3 and 11-13 only. As in Spain, seasonal differences in latent period were evident (F = 299.91, df = 12, P = <0.001; Table 5.6), although the differences were smaller (≤0.5 days variation across the year).

Table 5.6: Latent period (days) of *O. neolycopersici* in Spanish closed-system (CS) and passively ventilated (PV) greenhouses over time (W = winter, Sp = spring, Su = summer and A = autumn). Trt. indicates treatment with (BS) or without (ON) the biological control agent, *B. subtilis*.

Climate Mgmt.	Trt.	Month												
		W **1**	W **2**	Sp **3**	Sp **4**	Sp **5**	Su **6**	Su **7**	Su **8**	A **9**	A **10**	A **11**	W **12**	W **13**
Cool	ON	5.9	5.7	5.5	5.4	5.5	5.5	5.5	5.5	5.4	5.4	5.5	5.6	5.8
Trad.	ON	4.8	4.8	4.9	5.1	5.3	5.4	5.3	5.3	5.1	5.0	4.9	4.7	4.8
Warm	ON	5.4	5.5	5.6	5.7	5.8	5.9	5.9	5.9	5.8	5.7	5.7	5.5	5.4
Cool	BS	6.9	6.7	6.5	6.2	6.2	6.1	6.1	6.2	6.2	6.2	6.4	6.7	6.8
Trad.	BS	5.3	5.3	5.4	5.5	5.7	5.8	5.7	5.7	5.5	5.4	5.3	5.2	5.3
Warm	BS	5.7	5.8	6.0	6.2	6.3	6.4	6.4	6.4	6.3	6.1	6.0	5.8	5.7

B. subtilis application had a significant impact on the *LP* in all the Dutch greenhouse systems (F = 20.06, df = 4, P = <0.001), causing an overall increase of 15% (0.8 days), 9% (0.4 days) and 7% (0.4 days) in the cool, traditional and warm systems respectively (Table 5.6). In the traditional system, significant increases in *LP* were only evident in the winter and months 3-4 (maximum being 11% or 0.5 days in month one). In the cool system, *B. subtilis* significantly increased the *LP* throughout the year, although its impact was greatest in the winter (e.g., 18% or 1 day longer in month two). In the warm greenhouse the opposite trend was apparent, with the greatest increase in *LP* due to *B. subtilis* in the summer (e.g., 9% or 0.5 days longer in month seven; significant differences were evident from months 2-13). The greatest difference in *LP* was between the traditional greenhouse without *B. subtilis*

(4.8 days) and the cool greenhouse with *B. subtilis* (6.7 days) in month 10, an increase of 44%.

5. 3. 1. 3 Country comparisons

Comparison of the *LP* between the two countries shows that in all three Dutch greenhouse climates, particularly the traditional greenhouse, the *LP* was significantly shorter throughout the year than in either Spanish greenhouse (F = 20.06, df = 4, P = <0.001; Tables 5.5-5.6). The differences were particularly large during the winter where the *LP* in the closed-system Spanish greenhouse was 83% longer than in the Dutch traditional greenhouse in month 1.

5. 3. 2 Disease development sub-model

5. 3. 2. 1 Spanish greenhouses

The *O. neolycopersici* disease development sub-model showed that the overall maximum rate of daily disease development (*MG*; % increase in diseased leaf area) differed significantly between the climate control scenarios with a strong seasonal influence evident (F = 145.64, df = 48, P = <0.001). In both greenhouse systems the greatest *MG* occurred in the late spring (months 3-5; *MG* = 6%) and autumn (months 9-11; *MG* = 6.1%) with the smallest in the summer (months 6-8; *MG* = 5.8%) and winter (months 12-2; *MG* = 5.5%; Fig. 5.10). However, *MG* was significantly greater in the passively ventilated greenhouse in months 4-6 and 11-12 (6.6% vs. 6.2% in the closed-system greenhouse), while during months 7-10 it was significantly greater in the closed-system greenhouse (6% vs. 5.1% in the passively ventilated greenhouse). These differences may appear minor but become important when accumulated over consecutive days, e.g. after just two weeks in month 7, 12.6% more leaf area would be affected by the sporulating stage of the disease in the passively ventilated greenhouse.

B. subtilis application resulted in significant reductions in *MG* in both greenhouse systems (F = 237.17, df = 4, P = <0.001) with overall average reductions of 1.1% in the closed-system greenhouse and 1% in the passively ventilated greenhouse.

Reductions in *MG* due to *B. subtilis* were significant throughout the year in both greenhouses but seasonal differences were also evident (F = 3.78, df = 48, P = <0.001; Fig. 5.10) with the greatest reductions in the passively ventilated greenhouse in month seven (4.2% compared to 5.4% without *B. subtilis*) and in the closed-system greenhouse in month ten (5.1% compared to 6.6% without *B. subtilis*). Comparison of *MG* between the *B. subtilis* treatments showed that *MG* was significantly greater in the passively ventilated greenhouse in months one, 4-5 and 11-13 (0.2%, 0.5% and 0.5% greater respectively) and significantly greater in closed-system greenhouse in months 7-10 (0.6% greater). Overall, the greatest reduction in *MG* was achieved when using passive ventilation climate management with *B. subtilis* compared to closed-system climate management without *B. subtilis*, with a reduction in *MG* of 2.2% in month eight (3.5% vs. 5.7%). This would result in a difference of more than 30% diseased leaf area in a fortnight.

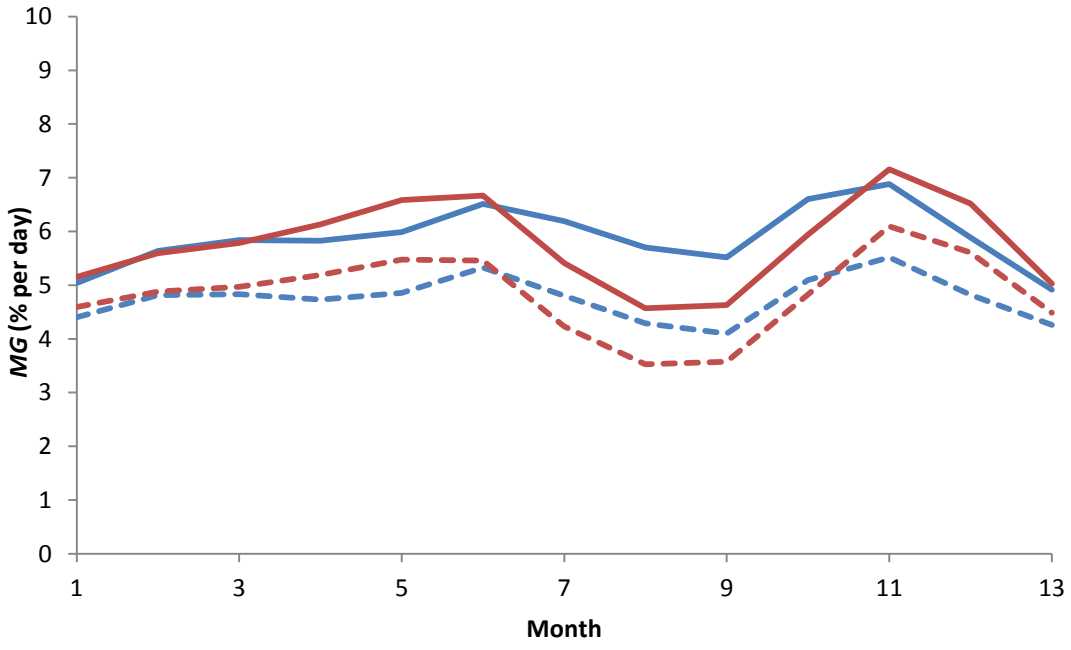

Figure 5.10: Maximum daily disease development rate (*MG*; % increase in diseased leaf area) of *O. neolycopersici* in Spanish closed-system (blue line) and passively-ventilated greenhouses (red line) over time with (broken line) and without (solid line) *B. subtilis.* Months 12-2 = winter, 3-5 = spring, 6-8 = summer, 9-11 = autumn.

5. 3. 2. 2 Dutch greenhouses

In the Dutch traditional and cool greenhouses the overall *MG* of the powdery mildew disease was similar (8.7% and 8.6% respectively) but both were significantly greater than that in the warm greenhouse (overall average of 7.8%; F = 237.17, df = 4, P = <0.001). A significant interaction of climate management and month was also evident (F = 145.64, df = 48, P = <0.001; Fig. 5.11) with *MG* significantly greater in the traditional system than in both the cool system in months 1-3 and 10-13 (average of 0.6% faster during both periods) and the warm system in months 2-12 (average of 0.7% faster). Comparison of the *MG* in the cool and warm systems showed that from spring to autumn *MG* was significantly greater in the cool greenhouse (months 4-10, average of 0.8% faster) and in the late autumn and winter it was significantly greater in the warm greenhouse (months 1-2 and 11-13, average of 0.6% faster). Seasonal variation in *MG* was relatively minor in the cool and traditional greenhouses with a marginally faster rate in the summer compared to the winter in the cool greenhouse and a reverse of this trend in the traditional greenhouse. In the warm greenhouse, the seasonal differences were greater, ranging from 7.4% in month seven to 9.2% in month 11.

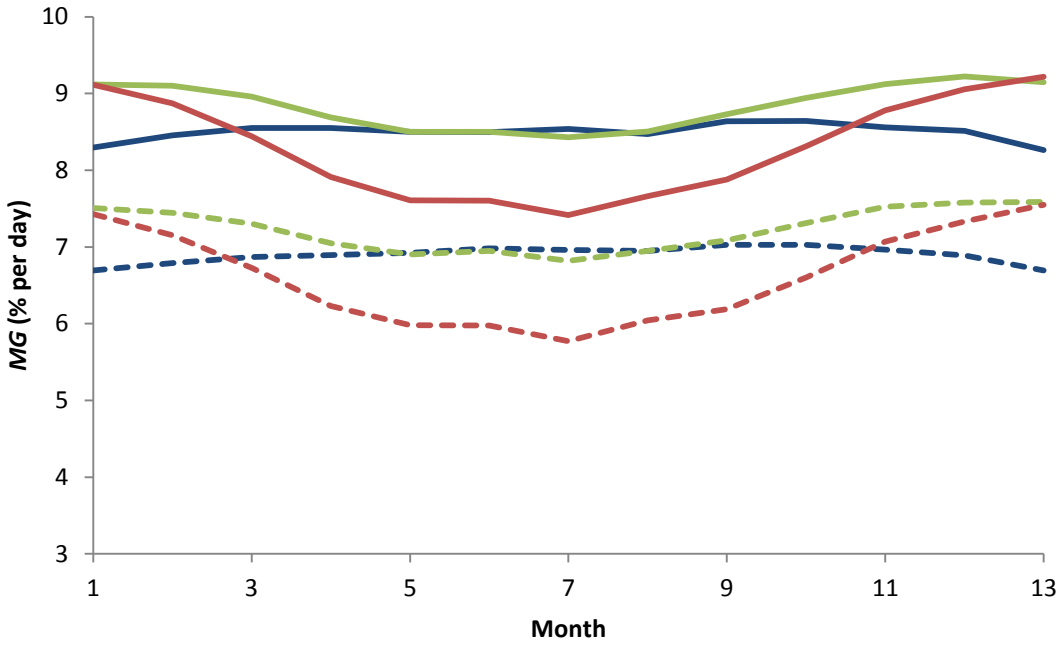

Figure 5.11: Maximum daily disease development rate (*MG*; % increase in diseased leaf area) of *O. neolycopersici* in Dutch cool (blue line), traditional (green line) and warm (red line) greenhouses over time with (broken line) and without (solid line) *B. subtilis.* Months 12-2 = winter, 3-5 = spring, 6-8 = summer, 9-11 = autumn.

B. subtilis application resulted in significant year round reductions in *MG* in all the Dutch greenhouse systems (F = 237.17, df = 4, P = <0.001), with overall reductions of 1.6%, 1.6% and 1.7% in the cool, traditional and warm greenhouses respectively. The effect of *B. subtilis* was remarkably consistent across the year (Fig. 5.11) with reductions ranging from 1.5% in month 6 to 1.6% in month 12 in the cool greenhouse, 1.6% in month 6 to 1.7% in month 2 in traditional greenhouse, and 1.7% in month 8 to 1.8% in month 12 in the warm greenhouse.

Comparison of *MG* between the *B. subtilis* treatments showed that *MG* in the cool greenhouse was significantly smaller than in the traditional greenhouse between months 1-4 and 10-13 (average of 0.5% and 0.6% lower during the respective periods). In the warm greenhouse, *MG* was significantly smaller than in the traditional greenhouse in months 2-12 (average of 0.7% lower for period), although the reduction was greater in late spring and summer (1% lower in months 5-7). Comparison of *MG* between the cool and warm greenhouses revealed a clear seasonal switch whereby *B. subtilis* limited the development of *O. neolycopersici* more in the cool greenhouse in the winter (average of 0.6% lower for months 12-2) and in the warm greenhouse from months 4-10 (average of 0.9% lower). Overall, the greatest reduction in *MG* was observed in month seven when using warm climate management with *B. subtilis* (*MG* = 5.8%) compared to traditional climate management without *B. subtilis* (*MG* = 8.4%). This means that in a fortnight the diseased area would be 36.4% less with warm climate control and *B. subtilis*.

5. 3. 2. 3 Country comparisons

Comparison of the *MG* between the two countries (Fig. 5.10-5.11) showed that in all three Dutch greenhouses the *MG* was significantly faster throughout the year than in both Spanish greenhouses (F = 237.17, df = 4, P = <0.001). The largest difference was a greater than two-fold increase in *MG* between the Spanish closed-system greenhouse and the Dutch warm greenhouse in month 13 (4.3% vs. 9.2%).

5. 3. 3 Integrated disease progress model

5. 3. 3. 1 Spanish greenhouses

The integrated disease progress model incorporating both the latent period and disease development sub-models of *O. neolycopersici* showed that the time until 50% of the theoretical maximum (78.8%) diseased area (T_{50}, Section 5. 2. 4) was significantly affected by (the interaction of) climate management and season (F = 12.85, df = 48, P = <0.001). T_{50} was significantly shorter in the closed-system greenhouse than the passively ventilated greenhouse in months two and 6-10 (17.3 vs. 19.4 days; Fig. 5.12). For months 4-5 and 11-12 the trend was reversed, with T_{50} significantly shorter in the passively ventilated greenhouse (17.6 vs. 18.2 days). The greatest difference was in month 8 when T_{50} was reduced by an average of 3.9 days (21.9%) in the closed-system greenhouse.

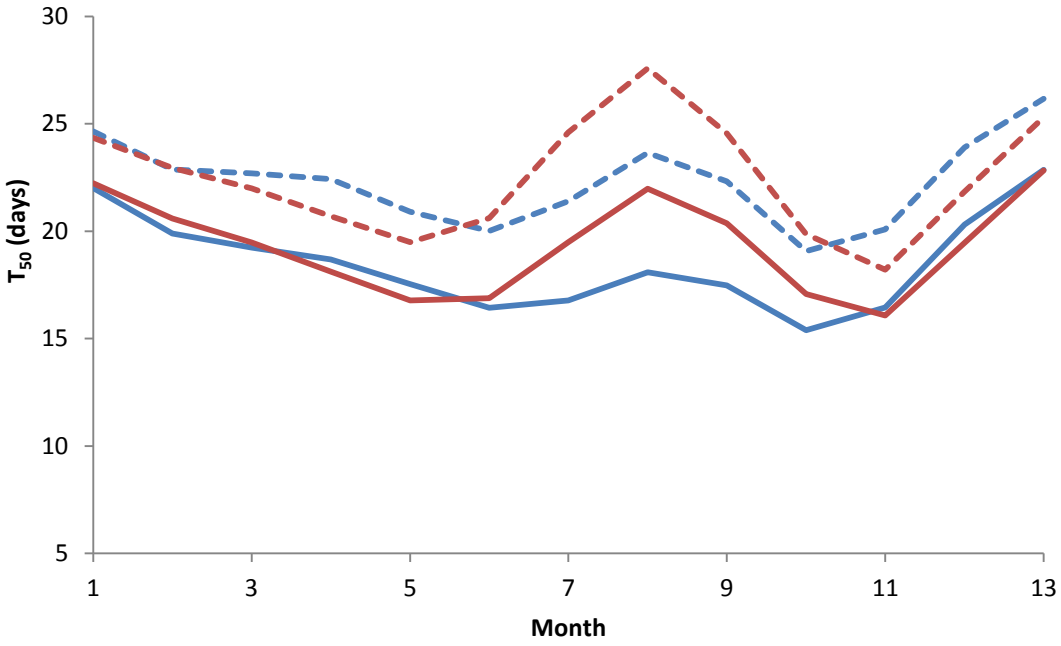

Figure 5.12: The seasonal variation in the time taken for *O. neolycopersici* disease levels to reach 50% of maximum theoretical (78.8%) diseased leaf area (T_{50}) in Spanish closed-system (blue line) and passively-ventilated greenhouses (red line), with (broken line) and without (solid line) *B. subtilis.* Months 12-2 = winter, 3-5 = spring, 6-8 = summer, 9-11 = autumn.

Application of *B. subtilis* resulted in a significant overall increase in T_{50} in both the passively ventilated (3.2 days or 16.5% longer) and the closed-system greenhouse systems (3.8 days or 20.7% longer; F = 147.89, df = 4, P = <0.001) compared to the *O. neolycopersici* only treatment. The effect of *B. subtilis* had a significant interaction with time of year (F = 12.85, df = 48, P = <0.001; Fig. 5.12). Although increases in T_{50} were highly significant throughout the year in both greenhouse systems, *B. subtilis* was most effective in the summer with a maximum increase in month eight in both the passively ventilated (5.6 days or 25.4% longer) and the closed-system greenhouses (5.6 days or 30.7% longer). The smallest increases were in month one in both greenhouse systems (2.1 days or 9.5% longer in the passively ventilated and 2.6 days or 11.9% longer in closed-system greenhouse).

Comparison of T_{50} between the *B. subtilis* treatments found a significant increase in T_{50} in the closed-system greenhouse from months 3-5 and 11-13 (average of 1.5 days longer), while from months 6-10 T_{50} was significantly longer in the passively ventilated greenhouse (average of 2.3 days). The greatest T_{50} was in month eight in the passively ventilated greenhouse with the *B. subtilis* treatment (27.6 days), 52.3% longer than in closed-system greenhouse without *B. subtilis* (18.1 days).

5. 3. 3. 2 Dutch greenhouses

The overall T_{50} was significantly greater in the warm greenhouse (average of 14.3 days) than the traditional greenhouse (average of 13.1 days; F = 147.89, df = 4, P = <0.001) but neither were significantly different from the cool greenhouse (average of 14.0 days). A significant interaction of climate management with month was also evident (Fig. 5.13; F = 12.85, df = 48, P = <0.001). The T_{50} was longer throughout the year in the cool greenhouse than in the traditional greenhouse and significantly so in months 1-4 (1.2 days or 9.1% longer) and 9-13 (1.1 days or 8.6% longer). In the warm greenhouse, the T_{50} was significantly longer than in the traditional greenhouse throughout the year, ranging from an average of 0.6 days (or 4.9%) longer in month 13 to 1.5 days (or 10.6%) longer in month 6. Comparison of the cool and warm greenhouses showed that T_{50} was significantly longer in the cool greenhouse in the winter (0.9 days or 6.6% longer) and significantly longer in the warm greenhouse from months 4-10 (1 day or 7.4% longer).

B. subtilis application resulted in significant increases in T_{50} compared to the *O. neolycopersici* only treatment in all three greenhouse systems (F = 147.89, df = 4, P = <0.001) with an overall average increase of 2.8 days (19.9%) in the cool greenhouse, 2.3 days (17.2%) in the traditional greenhouse and 2.7 days (19.1%) in the warm greenhouse. While increases due to *B. subtilis* were significant throughout the year in all the Dutch greenhouses, seasonal differences in control efficacy were evident (Fig. 5.13), although to a lesser degree than in Spain. In the cool greenhouse, increases varied from 2.5 days (17.9%) in month eight to 3.2 days (22.3%) in month two, while in the traditional greenhouse they varied from 2.1 days (16.2%) in month ten to 2.4 days (18.1%) in month four. The greatest seasonal differences in *B. subtilis* efficacy were found in the warm greenhouse, ranging from 2.0 days longer (14.9%) in month 13 to 3.4 days longer (22.3%) in month six.

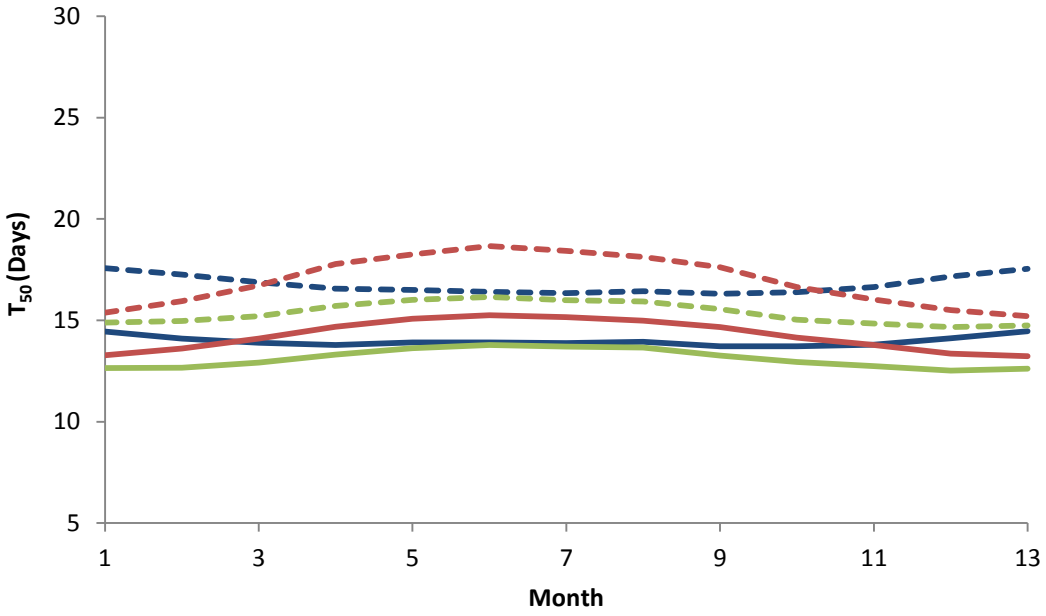

Figure 5.13: The seasonal variation in the time taken for *O. neolycopersici* disease levels to reach 50% of maximum theoretical (78.8%) diseased leaf area (T_{50}) in Dutch cool (blue line), traditional (green line) and warm (red line) greenhouses, with (broken line) and without (solid line) *B. subtilis.* Months 12-2 = winter, 3-5 = spring, 6-8 = summer, 9-11 = autumn.

Comparison of T_{50} between the *B. subtilis* treatments showed that the T_{50} was significantly greater in both the warm and cool greenhouses than the traditional greenhouse, throughout the year in the former (overall average 1.6 days or 10.6%

longer) and all but month six in the latter (overall average of 1.5 days or 9.6% longer excluding month six). Comparison of the cool and warm greenhouses revealed a clear seasonal switch in the ability of *B. subtilis* to slow the development of the disease, with T_{50} significantly longer in the cool greenhouse from months 11-2 (average of 1.6 days or 9.9% longer) and significantly longer in the warm greenhouse from months 4-10 (average of 1.5 days or 9.2% longer). The greatest monthly difference in T_{50} was between the traditional greenhouse without *B. subtilis* and the cool greenhouse with *B. subtilis* in month one with the latter being 4.9 days (or 39.1%) longer.

5. 3. 3. 3 Country comparisons

Comparison of the T_{50} for powdery mildew disease between the two countries showed that T_{50} was significantly reduced in all the Dutch greenhouses compared to the Spanish greenhouses (F = 83.34, df = 4, P = <0.001; Fig. 5.12-5.13). The largest difference was between the Dutch traditional greenhouse and the Spanish closed-system greenhouse, where T_{50} of the latter was 10.2 days (or 81%) greater than the former in month 13.

5. 3. 4. Preliminary model validation

Preliminary validation of the model was carried out using data from experiments in another project investigating the impacts of photoselective greenhouse cladding plastics on the development of *O. neolycopersici* and the control efficacy of *B. subtilis*. The experimental protocols were the same as those for the experiments used to parameterise this model (Section 2. 2), except that the plants were kept outside, under plastic-covered frames. Minor modifications to the image analysis program were also necessary due to different lighting conditions (the leaves were photographed under sunlight). The data used for model validation was taken from plants under a UV blocking plastic as this is standard for the greenhouse industry and is also similar to the UV deficient lighting conditions within the CE cabinets used in Chapter 2. Temperature and humidity recorded under the plastic (taken at ten minute intervals and averaged for each hour) were used as model inputs and predictions

compared with observations of powdery mildew disease development recorded every two days using image analysis as described previously (Section 2. 2. 3). The model predictions generated by both the latent period and disease development sub-models of powdery mildew disease progression, both with and without *B. subtilis*, underestimated that observed in the experiments. The observed latent period was 2-3 days longer than predicted (Table 5.7) and while the disease development model (corrected to start when the observed latent period was complete) had an improved fit to the observed data for the first 18 days, it underestimated diseased leaf area considerably thereafter (Fig. 5.14).

Table 5.7: Latent period (days) of *O. neolycopersici* with (BS) and without (ON) *B. subtilis* as predicted by the latent-period sub-model and observed experiments not used to construct the model.

	Treatment	Latent period
Predicted	ON	7.75
	BS	8.5
Observed	ON	10.7
	BS	10.6

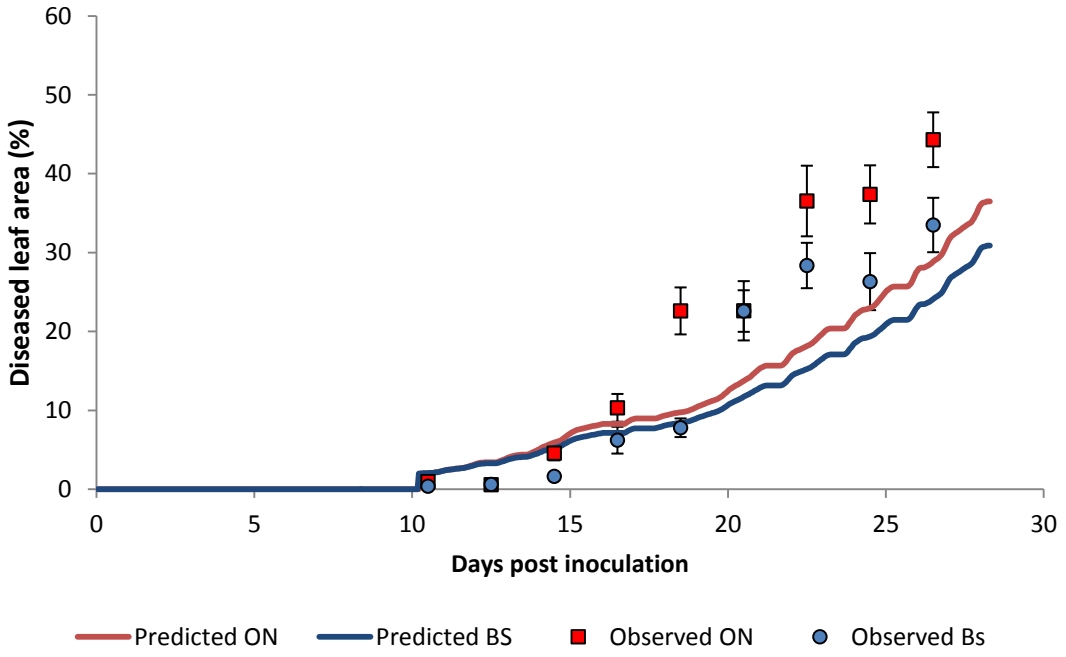

Figure 5.14: Comparison of model predictions with observations of *O. neolycopersici* disease development (% diseased leaf area) with (BS) and without (ON) *B. subtilis*. Observations are the mean of 60 plants for each treatment. Bars denote standard error of the mean. Modelled disease development is corrected to begin at the end of the observed latent period.

5. 4 Discussion

The integrated disease progress model and the latent period and disease development sub-models for *O. neolycopersici* predicted that greenhouse climate management and *B. subtilis* have significant impacts on disease pressure in both the Netherlands and Spain. The models also predicted significant time of year differences in disease pressure, although seasonal influences were predicted to be stronger in Spain. A particularly conspicuous finding was the far greater progress of the disease (in all the parameters assessed) in the Dutch greenhouses relative to the Spanish greenhouses. In fact, the conditions maintained in the traditional Dutch greenhouse could be considered to be optimal for the pathogen.

5. 4. 1 Spanish greenhouses

Climate management in Spain was found to have a significant influence on *O. neolycopersici* disease pressure using all of the disease models but of more importance were the impacts of seasonal differences in temperature and biological control. Regardless of the specific management approach used, the primary aim of climate control in Spanish plastic-clad greenhouses is to prevent environmental extremes; high temperature in the summer and high humidity in the winter (de Pascale and Maggio, 2004; Polycarpou, 2005). Hence, as long as economic yields are achieved, sub-optimal growth conditions are considered acceptable (Wittwer and Castilla, 1995). The result of this is that greenhouse conditions in Spain, especially temperature, vary considerably with the seasonal variation in external conditions, resulting in low temperatures in the winter and very high temperatures in the summer. In contrast, closed-system climate control moderates this seasonal (and diurnal) variation meaning that seasonal impacts on disease pressure were reduced in this greenhouse.

The main impact of seasonal changes in greenhouse conditions on powdery mildew disease was that, regardless of climate management, the latent period (*LP*) in the winter could be more than double that in the summer and autumn. *O. neolycopersici* latent period was particularly sensitive to low temperature (but less so to high temperature; Section 2. 3. 3) meaning that high summer temperatures had less

influence. However, for the maximum rate of disease development (*MG*), seasonal influences resulted in a greater *MG* in the late spring, early summer and late autumn in the passively ventilated greenhouse, and the early summer and late autumn in the closed-system greenhouse. In both greenhouses the smallest *MG* values were in the winter and the summer. This pattern demonstrates the detrimental effects of low and high temperature on disease development (Section 2. 3. 1). These conditions which were particularly frequent in the passively ventilated greenhouse, where winter temperatures dropped as low as 5.4°C (lowest monthly average of 15°C) and summer temperatures reached 46.9°C (highest monthly average of 28.8°C). In fact, experimental work (Section 2. 3. 1) showed that at 28.9°C disease development was negligible and certainly far less than predicted by the model. That the model predicted otherwise is because while extremely high, and detrimental, temperatures occurred in the afternoon and evening, more favourable conditions were prevalent overnight and in the morning (e.g., 18-25°C in month eight). This demonstrates the benefits of using hourly temperature data that captures diurnal variation in conditions, as opposed to daily mean values.

For the integrated powdery mildew disease model, seasonal trends in T_{50} (the time until 50% of the theoretical maximum, or 39.4% diseased area) were similar to those for *MG* in both greenhouses, particularly in that the greatest disease pressure occurred in the autumn. The similarity in patterns between T_{50} and *MG* demonstrates the relative importance of disease development over latent period in determining the severity of *O. neolycopersici* epidemics. Latent period is fairly unresponsive to temperature in the range 20-29°C (Section 2. 3. 3) whereas disease development is far more variable over the same range. As this is the range that predominates in both greenhouse systems (particularly spring to autumn), disease development becomes the key factor in determining disease severity. However, latent period has more influence in the winter with long *LP* and temperatures <20°C associated with the longest T_{50}.

B. subtilis application had a significant effect on reducing disease pressure. For both the disease development sub-model and integrated disease progress model, *B. subtilis* application resulted in year round (significant) reductions in disease in both greenhouse systems but was most effective in closed-system conditions. However, large seasonal differences in efficacy were evident; in both greenhouses reductions in

MG in the summer due to *B. subtilis* were generally double those in the winter, while increases in T_{50} in the summer were almost triple those in the winter. Seasonal differences in temperature are the primary factor explaining this seasonal variation in control efficacy. The overall improved performance of *B. subtilis* in the closed-system greenhouse is likely due to the higher humidity conditions (yearly variation in monthly mean humidity ranged from 68-87% RH compared to 54-79% RH in the passively ventilated greenhouse), which was found to improve its efficacy (Sections 2. 3. 1 and 5. 2. 3). *B. subtilis* had a minor, although significant effect of increasing *LP* in the passively ventilated greenhouse with the greatest effect in the summer. This was due to the predominance of temperatures between 25-30°C at this time of year under this climate management system, which is the range at which *B. subtilis* had the most influence on the latent period (Section 2. 3. 3).

Greenhouse climate management in Spain had important impacts on powdery mildew disease pressure, although not as great as the effect of *B. subtilis* or time of year. The integrated disease progress model suggested that while T_{50} was similar between the two greenhouse systems for the majority of the year, in the late summer and early autumn it was significantly longer in the passively ventilated greenhouse. The same late summer-early autumn trend was also found when considering *MG* but here an additional difference was evident, with *MG* in the spring and late autumn/early winter significantly greater in the passively ventilated greenhouse. These trends are due to the higher temperatures in the passively ventilated greenhouse, which, while generally conducive to disease progress, produced periods of extreme temperature in the late summer and early autumn that constrained development of the powdery mildew. The latent period sub-model also predicted some differences in *LP* (although these were comparatively small), with *LP* significantly shorter in winter and spring in the passively ventilated greenhouse. This is somewhat surprising as the mean temperature for these months was lower than in the closed-system greenhouse. However, scrutiny of the diurnal temperature reveals that this effect is probably due to the rapid decrease in temperature in the evening in the closed-system greenhouse. Here a drop of more than 6°C was observed (and subsequently modelled in Chapter 4) in the hour or so after sunset, rapidly reducing the temperature close to the overnight low. This compares to a more gradual decline in the passively ventilated greenhouse, which although reaches

a lower temperature, only does so for a comparatively shorter time. Such a finding again highlights the benefits of modelling the diurnal trends in environmental conditions.

The optimal *O. neolycopersici* management strategy in Spain suggested by the model is the use of passive ventilation climate control and the application of *B. subtilis*, with the control agent most effective from spring to autumn. This approach minimises disease pressure for much of the year, with T_{50} delayed by over 52% compared to that in the passively ventilated greenhouse without *B. subtilis* in month eight (27.6 vs. 18.1 days). However, if tomato is not being grown during the summer, which is sometimes the case in Spain (Cantliffe and Vansickle, 2003), closed-system management may be preferable due to the improved efficacy of *B. subtilis* during the spring with this climate management system. Even with *B. subtilis* application, *O. neolycopersici* disease pressure is predicted to be most severe during the spring and autumn, and if control fails, then greenhouse managers are recommended to use IPM compatible alternatives such as sulphur (Jones *et al.*, 2001; Gazquez *et al.*, 2011).

5. 4. 2 Dutch greenhouses

The powdery mildew disease models showed that the overall *O. neolycopersici* disease risk was considerably greater in the Dutch greenhouses than in Spanish greenhouses with shorter *LP* and T_{50}, and faster *MG*. However, even relatively small changes in temperature set-points can have considerable impacts on disease severity and control efficacy in Dutch greenhouses. Using the integrated model and both sub-models, disease pressure was greater in the traditional greenhouse compared to both the warm and cool greenhouses. In fact with temperatures consistently between 20-23°C and humidity predominantly between 70-81% RH, conditions in the traditional greenhouse appeared to be almost optimal for the development of *O. neolycopersici* epidemics.

Comparisons of the novel climate regimes (cool and warm) with the traditional climate management showed that significant reductions in disease pressure (for all three disease measures) were found almost year round (with the occasional exception

of a month in winter) in the warm greenhouse and from late autumn to early spring in the cool greenhouse. The conditions in the warm greenhouse ranged from 22-25°C and 62-72% RH and although these temperatures would be considered optimal for the completion of latent period when compared to those in the traditional greenhouse (Sections 2. 3. 3 and 5. 2. 2), the lower humidities in the warm greenhouse resulted in an increased *LP*. Similarly, a cursory inspection of the disease development model (Fig. 5.8) would suggest that the warm greenhouse temperatures would also be optimal for *MG* and T_{50}. The reason they are not is due to the different effects of temperature on the *M* and *B* parameters. While the *M* parameter (which relates to the shape of the progress curve and more specifically the length of the lag phase) decreases up to 24°C (Fig. 5.3), the *B* parameter (directly related to the rate of disease development) is greatest at 22°C (Fig. 5.2). This means that while the lag phase may be shorter in the warm greenhouse, *MG* is smaller. The fact it also resulted in longer T_{50} reflects the relative importance of *B* in determining this outcome (although it should be noted that low values of *M* were constrained). The reduced disease pressure in the cool greenhouse is due to lower temperatures being sub-optimal for both the completion of the latent period and disease development.

While seasonal differences in greenhouse conditions within a climate management regime were smaller in the Netherlands than in Spain, they nevertheless resulted in seasonal variation in disease pressure. This presents growers with an opportunity to exploit these seasonal differences in disease pressure by switching between climate regimes. For example, disease pressure was lowest in the summer in the warm greenhouse, due to the detrimental effects of excessive temperatures, and lowest in the winter in the cool greenhouse, due to the low temperature (18-19°C). Maximal reductions in disease pressure can therefore be achieved by employing the cool climate system in winter and the warm system from spring to autumn.

B. subtilis application produced year round significant reductions in *MG* and increases in T_{50} in all the Dutch greenhouses. For *LP*, significant increases were year round and greatest in the cool greenhouse but limited to winter and spring in the traditional greenhouse and spring to autumn in the warm greenhouse. Low humidities (approximately 60-70% RH) were responsible for the reduced efficacy in the traditional and warm greenhouses. For *MG*, *B. subtilis* efficacy was greatest in the warm greenhouse, primarily due to the effect of the increased temperature on the

B parameter. For T_{50}, the best and most consistent increases overall were found in the cool greenhouse. The reason for the improved efficacy in this greenhouse is partly due to the effect on the latent period but more importantly due to the increased humidity, which ranged from 80-90% RH. The efficacy of *B. subtilis* would be greater in the traditional and warm greenhouses if a higher humidity could be maintained, although this could encourage other pathogens such as *B. cinerea* (Elad *et al.*, 1996).

The models of *O. neolycopersici* progress in the Dutch greenhouses show that careful greenhouse control alone can provide considerable benefits for powdery mildew disease reduction in the Netherlands and highlights the importance of this tool as part of integrated pest management programs. When combined with *B. subtilis*, modifications to temperature set points (-2°C or +2°C) can produce even greater reductions in disease pressure compared to the traditional greenhouse without *B. subtilis*. For instance, *LP* and T_{50} can be increased by up to 44% (1.9 days) and 39.1% (4.9 days) respectively through the application of *B. subtilis* in the cool greenhouse and daily *MG* reduced by 1.8% with the use of *B. subtilis* in the warm greenhouse (equivalent to a reduction in disease area of over 25% in a fortnight). The model suggests that the optimal strategy to minimise *O. neolycopersici* disease pressure is the application of *B. subtilis* combined with warm climate control from month three to ten and cool climate control for the rest of the year.

5. 4. 3 Model development and further work

Although the modelling work presented here was useful in quantifying differences between greenhouse climate management systems, further improvements could be made. For instance, a more consistent approach to model construction would be to apply temperature thresholds in the latent period sub-model. For the disease development sub-model, rates were set to zero for temperatures below 10°C and above 31°C but this was not applied to the latent period sub-model. While this may have minor consequences for the modelling of the Dutch greenhouses, it may have artificially increased the predicted disease pressure in the Spanish greenhouses, especially under passive ventilation, where very low and very high temperatures were more common. Some assumptions of the model could also be challenged such

as that the response of *O. neolycopersici* to fluctuating environmental conditions are the same as at constant conditions. For instance, a model simulating the development of *P. leucotricha* and based on constant temperature conditions was found to overestimate disease development in fluctuating conditions with a mean temperature above 20°C (Xu, 1996). Further work could investigate this potential problem by carrying out small-scale greenhouse experiments on *O. neolycopersici* disease development and comparing observed disease progress with model predictions.

Currently the model is deterministic but further repetitions of the experiments carried out in Chapter 2 would provide more information on the variability of disease development allowing elements of stochasticity to be built into the model. These might also clarify the effect of humidity, where an increase in disease severity was suggested at 50% RH. Other studies have also demonstrated a similar influence of low humidity, both on the same (Whipps and Budge, 2000) and different *O. neolycopersici* isolate (Jacob *et al.*, 2008). Furthermore, two studies (Whipps and Budge, 2000; Jacob *et al.*, 2008) found that humidity close to saturation (99% RH) had a highly detrimental effect on disease development and sporulation. This was not observed in the experimental work in this thesis (Chapter 2), although the highest humidity investigated was 95.9% RH. If a humidity response could be better quantified and included in the model then the increased disease development at low humidity and the potential reduction close to saturation could have important consequences for the model outcome. For instance, disease severity could be greater in the Dutch warm and Spanish passively ventilated greenhouses where low humidity conditions are more prevalent, although the latter also experiences more high humidity conditions at night.

Although it was decided not to include the infection stage in the model, the addition of an infection sub-model may provide a clearer picture of the impacts of different climate management systems. By examining the data collected on the response of *O. neolycopersici* infection processes in other studies (Kashimoto *et al.*, 2003a; Jacob *et al.*, 2008; Mieslerova and Lebeda, 2010) such a model could be constructed allowing the rate of this process to be calculated under different greenhouse conditions. Further work could also investigate the effect of inoculum concentration on disease progress so that different initial spore loads could be simulated. Spore load has been

shown to be important in other pathosystems, for instance disease severity increased and latent period decreased with increasing inoculum spore density in *Puccinia allii* (Gilles and Kennedy, 2003), *P. hordei* (Teng and Close, 1978; Baart *et al.*, 1991), *Colletotrichum truncatum* (Chongo and Bernier, 2000) and *Rhynchosporium secalis* (Xue and Hall, 1992).

An important difference between passively vented and closed-system greenhouses is likely to be air movement and including predictions of spore dispersal would allow this to be assessed. It is likely that a passively ventilated system would result in an increased spread of the disease around the greenhouse from a localised starting point (a more realistic situation) due to greater air movement, as was found in a *B. cinerea* spore deposition model (Roy *et al.*, 2006). Furthermore, passive ventilation is also likely to result in an increased likelihood of spores entering the greenhouse from the outside due the increased number and size of openings (Leyronas *et al.*, 2011).

O. neolycopersici has also been shown to be sensitive to light conditions (Kashimoto *et al.*, 2003a; Jacob *et al.*, 2008; Mieslerova and Lebeda, 2010) with germination and sporulation increasing with light intensity and, while it may not affect within country comparisons, including this in the model may affect between country comparisons, with potentially an increased disease pressure in Spain compared to the Netherlands.

A caveat in interpreting the results of the models presented here is that variability in *O. neolycopersici* isolates have been found (Lebeda and Mieslerova, 2002) and it has been suggested that different races of this pathogen occur (Lebeda and Mieslerova, 2002; Bai *et al.*, 2005). This means that the modelling approach may need to be adapted to the locally prevalent *O. neolycopersici* isolates, as some important differences have been shown in the response of the pathogen to the environment (Chapter 2; Whipps and Budge, 2000; Kashimoto *et al.*, 2003a; Jacob *et al.*, 2008).

Finally, and most importantly in any simulation model, validation studies need to be conducted. Preliminary validation showed that latent period was underestimated by the model. The mean conditions for the duration of the latent period in the experiment were 14.7°C and 81.8% RH. However, the latent period predicted by the model was close to that recorded at a constant 15°C in CE experiments (7.5-8.1 days; Section 2. 3. 3) suggesting that fluctuating conditions experienced in the validation experiments may be affecting this. The model prediction of disease development (%

diseased leaf area) was fairly accurately until day 18. However, after this observed diseased area was greater than predicted by the model. This may be due to the occurrence of secondary disease cycles. Minor differences due to modifications to the image analysis program may also have occurred. It should also be noted that the mean conditions during the disease development period of the experiment were 13.3°C and 85.8% RH. The data used to parameterise the simulated behaviour of the disease under these conditions were taken at a constant 10°C and 15°C (Section 2. 2. 2), meaning that the accuracy of the model predictions would likely be improved by conducting experiments at further temperatures within this range. Additional small-scale greenhouse experiments using the same image analysis program would improve validation.

5. 4. 4 Conclusions

The disease models developed here are useful tools for exploring the effect of climate control regimes and selection of greenhouse structures on disease development. This is also the first time this approach has been used to predict *O. neolycopersici* disease pressure and the efficacy of a bacterial biological control agent in protected horticulture.

The models predicted that *O. neolycopersici* disease pressure is likely to be increased by the use of closed-system climate control in Spain, showing that while decreased energy and water usage can be achieved with these systems (Opdam *et al.*, 2005; Heuvelink *et al.*, 2008; UN, 2008) there may be negative consequences for pest and disease pressure, with resultant increases in crop losses and control costs. The models also predicted that switching between marginally warmer and cooler climate systems in the Netherlands may have highly beneficial consequences for reducing powdery mildew. Significant reductions in disease progress were found when temperatures were high, e.g., during the summer in both the passively ventilated greenhouse in Spain and the warm greenhouse in the Netherlands. Raising temperatures to 27-32°C by closing greenhouse doors and vents during the day has been successfully used to reduce *O. neolycopersici* disease severity in commercial greenhouses in Israel (Elad *et al.*, 2009).

The application of the bacterial biological control agent, *B. subtilis*, was predicted to result in decreased disease severity in both countries, especially when used in combination with altered greenhouse climate management. Its efficacy was relatively stable year round in all the greenhouse scenarios; however improved performance was predicted in the summer in the Spanish passively ventilated greenhouse. This biological control has potential for a role in IPM programs, and could be combined with other novel control strategies such as the addition of silicates to hydroponic systems (Garibaldi *et al.*, 2011).

An economic threshold does not appear to have been defined for *O. neolycopersici* but were it to be established then the model could be further modified to assess whether these control measures would be worthwhile. Similar modelling approaches have been used to assess climate control of *P. parasitica* (Kofoet and Fink, 2007) and *B. cinerea* (Tantau and Lange, 2003), although neither appears to have been tested. Shtienberg and Elad (1997) developed a decision support model for *B. cinerea* (BOTMAN), which used temperature and climatic events (e.g., rain) as indicators of increased disease risk periods and provided recommendations on the use of either chemical sprays or the application of the biological control, *Trichoderma harzianum*. When compared with a standard spray program the control program recommended by the model was as effective and involved less chemical applications.

Disease simulation models can provide useful information regarding the impact of management strategies for reducing disease pressure in crop production, as outlined above, but they also present a means to better understand the important processes and dynamics underpinning the pathosystem. An example of this would be the impact on the *O. neolycopersici* latent period of the rapid decrease in evening temperature in the Spanish closed-system greenhouse described earlier and the relatively larger contribution of the disease development stage to disease progress compared to the latent period. The latter illustrates an important role for these models in identifying the key biological stages that influence crop loss (de Wolf and Isard, 2007). Greater knowledge of the disease dynamics and processes in commercial-like conditions in turn would provide a sound basis for the development of new management strategies (de Wolf and Isard, 2007).

6. Modelling the population dynamics of *Tetranychus urticae* and *Phytoseiulus persimilis* in response to greenhouse environment

6. 1 Introduction

The spider mite, *Tetranychus urticae*, remains a persistent problem in protected horticulture. They are polyphagous, exhibit rapid population growth and have developed resistance to a number of acaricides (Cranham and Helle, 1985; Gerson and Weintraub, 2007). Current control on vegetable crops in Holland and Spain is primarily through the use of biological control agents such as the predatory mite, *Phytoseiulus persimilis* (van Lenteren, 2000; Pilkington *et al.*, 2010). This agent is considered to be one of the most effective against *T. urticae* due to its high predation rate and numerical and aggregation responses (Laing and Huffaker, 1969; Takafuji and Chant, 1976; Amano and Chant, 1977; Sabelis and Dicke, 1985). However, breakdown of control has been reported (Osborne and Oetting, 1989; El-Laithy, 1996; Weintraub *et al.*, 2006; Mansour *et al.*, 2010), partly due to environmental conditions (Mori and Chant, 1966b; Stenseth, 1979; Osborne and Oetting, 1989; Skirvin and Fenlon, 2003b; Mansour *et al.*, 2010) and starvation due to depletion food resources (Sabelis, 1981; De Courcy Williams *et al.*, 2004b). Additionally, optimisation of biological control for maximum effectiveness is particularly important in ornamental crops (Opit *et al.*, 2004; Schumacher *et al.*, 2006; Holt *et al.*, 2007; Alatawi *et al.*, 2011), because of the very low tolerance to pest presence or damage (Sadof and Alexander, 1993; Alatawi *et al.*, 2007; Marsh and Gallardo, 2009).

The importance of this pest, and the challenges in controlling it, has meant that understanding and predicting its population dynamics and the efficacy of management strategies has been well studied. However, investigating their population dynamics experimentally is complicated due to their size, the difficulty in tracking individuals and the number of, often complex, interactions. Coupled with the expense, prolonged nature and logistical issues of greenhouse-scale experiments,

this has meant that these mites are usually only studied using small-scale experiments. This is an issue as populations often do not have an opportunity to reach equilibrium and therefore such experiments have been criticised as being unrepresentative of greenhouse systems, especially on long-growing crops such as ornamentals (Gerson and Weintraub, 2007). However, in ornamental production plants are frequently relocated within the greenhouse meaning that small-scale experiments (although clearly not on the same physical scale) should provide reasonably representative data for developing simulation models of population dynamics in commercial greenhouses.

Simulation models provide a relatively inexpensive means of bridging the gap between the findings of small-scale experiments and population dynamics at larger scales, as well as an improved understanding of a system, insights into further research directions and, especially important in this case, means of testing cultural and biological control hypotheses (France and Thornley, 1984; Hill and Coquillard, 2007; Breckling *et al.*, 2011). To this end a number of simulation models have been developed to predict *T. urticae* and *P. persimilis* population dynamics. Their scope, complexity and focus vary; from population-level (Shaw, 1984; Nachman, 2001) to individual based models (Bancroft and Margolies, 1999; Skirvin *et al.*, 2002), and single plant (Bernstein, 1985; Bancroft and Margolies, 1999) to spatially complex models (Nachman, 1987b; Nachman, 2001; Skirvin *et al.*, 2002). From reductionist empirical models (Buffoni and Gilioli, 2003; Kozlova *et al.*, 2005; Chakraborty *et al.*, 2009) to complex mechanistic models (Bancroft and Margolies, 1999; Skirvin *et al.*, 2002), and from deterministic (Bernstein, 1985) to stochastic models (Nachman, 1987a; Nachman and Zemek, 2003). The purpose of such models also differ, from those designed to explore the mechanisms underpinning specific behaviours (Nachman, 2001) to more applied models for optimising control programs on specific crops (Skirvin *et al.*, 2002; Schumacher *et al.*, 2006).

Most simulation models have neglected the impact of greenhouse climate even though ambient conditions have been shown to have important effects on the mechanisms described (e.g., Skirvin & Fenlon, 2003a; Skirvin & Fenlon, 2003b). Where it has been included it has been at the expense of other important factors such as spatial complexity (Bancroft and Margolies, 1999), individual complexity (Shaw, 1984) or stochasticity (Buffoni and Gilioli, 2003). Financial, governmental and

environmental pressures to reduce energy and water use have necessitated the development of novel greenhouse climate control approaches (Stanghellini *et al.*, 2003; Opdam *et al.*, 2005). Such approaches have been investigated as part of the EUphoros project in two greenhouse systems; tomato production in Spanish-style, plastic clad greenhouses and rose production in Dutch Venlo greenhouses. In the former a minimally vented, closed-system was developed and in the latter altered temperature set-points were investigated. The impacts these changes have on the diurnal and seasonal fluctuations in temperature and humidity within the greenhouse have been stochastically modelled (Chapter 4), along with those in traditional greenhouse systems for each country (passive ventilation and non-altered set-points in Spain and the Netherlands respectively).

Sabelis (1981) felt that greenhouse humidity was an unimportant consideration in modelling the populations of these mites. However, his work referred specifically to Dutch greenhouse systems where humidity remains within a relatively small range. The model in this chapter considers Spanish greenhouses as well as Dutch and the humidity in the former fluctuates far more widely, a phenomenon that has been shown to reduce the effectiveness of *P. persimilis* in similar greenhouse systems (El-Laithy, 1996; Weintraub *et al.*, 2006; Mansour *et al.*, 2010). Furthermore, work carried out as part of this thesis shows that even small variations in ambient humidity (10-15% RH) can have a significant influence on the predation rate in *P. persimilis* (Chapter 3).

The impacts of the different climate control approaches on the population dynamics of *T. urticae* and *P. persimilis*, and their interactions, are modelled in this chapter. *P. persimilis*-mediated control is varied within the model to allow optimal control strategies to be assessed in each greenhouse environment in response to differing *T. urticae* densities. The model is an individual-based mechanistic one, based on Skirvin *et al.* (2002) and includes stochasticity and spatial complexity. Individual-based models are a bottom-up approach to modelling population dynamics that can provide a better understanding of the mechanisms under-pinning a system as well insights into how behaviour at the individual level can affect emergent population properties (Werner, 1992; Bancroft and Margolies, 1999; Hill and Coquillard, 2007; Reuter *et al.*, 2011). Rather than using aggregated variables to describe a population they focus on the individuals allowing discrete events, such as emigration, to be

simulated and are particularly suited to spatially heterogeneous environments (Hill and Coquillard, 2007; Reuter *et al.*, 2011). Such an approach is especially suited to the greenhouse environment as pest populations can build up on individual plants and their locale, and allows the application of predatory mites to be simulated in a manner comparable to that occurring in commercial circumstances. Additionally, this method is appropriate to the individual-based nature of many of the experiments used to parameterise the model. Both stochasticity and spatial complexity are model components that have also been shown to help avoid the unrealistic population fluctuations that can be predicted by deterministic and single patch models; allowing local variation in extinction events whilst maintaining long-term metapopulation persistence in the system as a whole (Nachman, 1987b; Sabelis and Diekmann, 1988; Holyoak and Lawler, 1996; Nachman, 2001).

It is important to select data sources for model parameterisation carefully, based on relevance, reliability and similarity of protocols. For instance, crop species can have important effects on pests and their biological controls (Skirvin and De Courcy Williams, 1999) and so it is essential to consider these differences in model construction (Skirvin *et al.*, 2002; Gerson and Weintraub, 2007). A good example of this approach is Schumacher *et al.'s* (2006) bioeconomic pest control model on geranium. The model described here is a matrix model based on the pest population and control model of Skirvin *et al.* (2002). Matrix models are useful for such purposes as they "integrate population dynamics and population structure particularly clearly" especially for life cycles that are divided into developmental stages (Caswell, 2001). Skirvin *et al.'s* (2002) model uses the ornamental shrub *Choisya ternata*, and primarily uses data from experiments on this plant (Skirvin and De Courcy Williams, 1999). The model has been adapted to include the influence of greenhouse environmental conditions primarily by using data from experiments conducted on *C. ternata*, both as part of this thesis (Chapter 3) and from previous studies (e.g., Skirvin & Fenlon, 2003a). Where further sources were needed they were chosen so as to minimise the number of different sources used and for similarity to the system in question. An example would be Sabelis (1981), which was based on rose, an ornamental that is grown in relatively similar conditions.

The inclusion of temporally variable greenhouse environmental conditions in an individual-based, spatially complex, stochastic model is a novel approach to

simulating the population dynamics of this predator-prey system. This is also the first time that such an approach has been used to compare pest pressures and biological control efficacy over time in greenhouses under different climate management. Further, by allowing *P. persimilis* introduction frequency and rate to be varied in response to varying initial *T. urticae* populations, optimal control strategies can be assessed in each greenhouse system.

6. 2 Model description

6. 2. 1 General

The model, constructed in Matlab®, simulated the development, reproduction, movement and interactions of populations of *T. urticae* and *P. persimilis* in Spanish-style plastic clad and Dutch Venlo greenhouses under differing climate management. Five country/climate management scenarios were investigated; passive ventilation and closed system greenhouses in Spain, and traditional, cool (traditional -2°C) and warm (traditional +2°C) greenhouses in the Netherlands (for further details see Chapter 4). Each simulated greenhouse consisted of a ten by ten grid of (*C. ternata*) plants, with each plant representing a discrete patch, but with the plants being close enough to each other to enable direct plant to plant movement.

To establish the impact of seasonal differences between the greenhouse climates and the effect of initial pest population size, the arrival date into the greenhouse of differing numbers of *T. urticae* were investigated. To assess the control efficacy of *P. persimilis* in the different climate scenarios, the introduction rate and frequency of the control agent was also explored. As the majority of the data used in the model was taken from experimental work conducted over a 24 hour period, the model progressed in one day time steps. As is the case with other models (Bernstein, 1985; Skirvin *et al.*, 2002), adult males are not included in the model as they represent a relatively small proportion of the population (Skirvin *et al.*, 2002), do not disperse (Mitchell, 1973) and, as their weight gain is minor (Sabelis, 1981), they are likely to contribute less to plant damage. Their exclusion also made computation less demanding.

6. 2. 2 Mite arrival and distribution

The model was initiated on the arrival of a specified number of *T. urticae* adults (variations in arrival date are explained further in Section 6. 2. 9). Upon arrival mites were distributed randomly among the 100 plants. To ascertain the respective *x* and *y* coordinates of the destination plant for each mite separately, two values between 1 and 10 were sampled from a discrete uniform distribution.

P. persimilis adults were programmed to arrive into the greenhouse at set intervals and rates throughout the model (explained further in Section 6. 2. 9) and their distribution among the plants was randomised as for *T. urticae*.

Upon initialisation the longevity of the arriving adults of both species was calculated (see next Section). For each one day time step the model progressed through the following sub-routines (Sections 6. 2. 3-7) before moving to the next day.

6. 2. 3 Mite development

Development of the two species was simulated using a modified "boxcar train" method (de Wit and Goudriaan, 1978 ; Shaw, 1984; Skirvin *et al.*, 2002) where each mite of both species was classified into different stages (boxcars), i.e., eggs/larvae, nymphs and adults. Once they complete the development of one stage they move to the next before completing their life-span as adults. Eggs and larvae were combined as a category because they are a similar size, *T. urticae* larvae have little impact on the plant, *P. persimilis* larvae do not feed (Takafuji and Chant, 1976; Bernstein, 1985), they are relatively immobile (Bancroft and Margolies, 1999) and because it reduced the computational demands of the model.

The longevity and development of *T. urticae* were based on data derived from experiments conducted on *C. ternata* (Skirvin and De Courcy Williams, 1999). For each *T. urticae* the longevity of the combined egg and nymphal stage was determined by sampling a normal distribution with a mean (μ) of 11.1 and a standard deviation (σ) of 2.32. A ratio of 6:5 was then used to split this duration between the egg/larval stage and the nymphal stage. The life-span of the adults was determined by sampling from a uniform distribution between 1 and 34. The determination of this for the arriving adults was the first process to occur upon initialisation of the model.

Daily development was calculated as a function of temperature using a modification of the procedure employed in Bancroft and Margolies (1999). Their model had a stage-independent developmental rate that increased to a maximum at 34.33°C before declining rapidly, and was based on data from Sabelis (1981) and Carey and Bradley (1982). For the current model a simplification was made whereby the effect of temperate was described using:

$$tdev = (0.0428 * t) - 0.4715 \quad \{11 \leq t \leq 34.32\} \quad 6.1$$

$$tdev = 4.55 - (0.1034 * t) \quad \{34.32 < t \leq 44\} \quad 6.2$$

Where t = daily average temperature (°C). Below 11°C and above 44°C no development occurred. This resulted in a daily increase in age of between 0 and 1. Any mites that completed their stage duration were moved to the next stage and the duration of the new stage or life-span calculated.

Although temperature has been found to affect the duration of stages and life-span in *T. urticae* (Sabelis, 1981) the inclusion of the temperature-dependent developmental rate was felt sufficient. Additionally, temperature was not found to affect the relative duration of life-stage or –span (Sabelis, 1981). Humidity has also been shown to affect the egg-stage duration (Ferro and Chapman, 1979) however, the humidities at which duration was noticeably longer were very low (<25% RH) and Sabelis (1981) found no affect at 40-95% RH at 23°C. Furthermore, Ferro and Chapman's (1979) study was carried out on glass and as this substrate is likely to have a very different surface humidity to that on a leaf, where it is thought that the leaf boundary layer acts as a buffer to ambient humidity conditions (Ferro and Southwick, 1984; Gaede, 1992), it was decided not to include this effect.

For *P. persimilis* stage duration was 4, 2 and 36 days for egg/larvae, nymphs and adults respectively, based on data from Sabelis (1981). As for *T. urticae* daily development ranged between 0 and 1 and was based on a simplification of Bancroft and Margolies (1999):

$$tdev = (0.0582 * t) - 0.6406 \quad \{11 \leq t \leq 28.16\} \quad 6.3$$

$$tdev = 3.8657 - (0.10173 * t) \quad \{28.16 < t \leq 38\} \quad 6.4$$

Below 11°C and above 38°C no development was deemed to occur. *P. persimilis* stage duration and development has been shown to be detrimentally affected by low ambient humidity (Perring and Lackey, 1989; De Courcy Williams *et al.*, 2004b; Ferrero *et al.*, 2010), however these experiments were conducted on artificial surfaces, making translating the effects to a response on plant surfaces speculative. Adult life-span has also been shown to be affected by humidity (De Courcy Williams

et al., 2004b) but as this effect was lost when food was available it was not included in this model.

6. 2. 4 Mite reproduction

Egg production was simulated following the calculation of daily development for each mite. For *T. urticae* any adult younger than 24 days old was assumed to be reproductive. The baseline oviposition rate per adult was determined by sampling from a normal distribution ($\mu = 4.52$, $\sigma = 0.26$). Both the reproductive period and the baseline oviposition rate were derived from experimental work on *C. ternate* (Skirvin and De Courcy Williams, 1999). The baseline *T. urticae* oviposition rate was then multiplied by a temperature-dependent modifier (O_m), a quadratic equation, based on data in Sabelis (1981), which can be written as:

$$O_m = \left(\frac{-159.9+(23.9t)+\left(-0.4488\left(t^2\right)\right)}{34*4.6}\right) \quad 6.5$$

For *P. persimilis* the model considered the adults to be reproductive during their whole life-span (Skirvin *et al.*, 2002). Oviposition rate (N_{el}) was determined as a function of food consumption (Skirvin *et al.*, 2002), where oviposition can occur once a mite has met its metabolic needs by consuming adequate prey (Sabelis, 1981; Yao and Chant, 1990). The linear relationship can be written as:

$$N_{el} = \frac{(C-5.0)}{6.6} \quad 6.6$$

Where c = the amount of prey consumed (in egg equivalents). For prey a single egg/larvae, nymph and adult c is equivalent to 1, 2.3 and 5.7 respectively (Skirvin *et al.*, 2002) and a maximum of 45 egg equivalents can be consumed (Sabelis, 1981). For both species any negative oviposition rates were adjusted to 0 and the total egg production tallied, rounding to the nearest integer. The longevities of the new eggs were then calculated.

Bancroft and Margolies (1999) included a temperature coefficient in their calculation of *P. persimilis* oviposition and Sabelis (1981) found a slight affect of humidity on this parameter. However, as egg biomass production is high the most important

factor affecting reproductive rate can be considered to be prey consumption (Sabelis, 1981). Predation rate is modified by both temperature and humidity (see Section 6. 2. 5) and it was felt unnecessary to include the influence of these factors in the calculation of egg production. There is no evidence that humidity affects oviposition in *T. urticae* (Sabelis, 1981).

6. 2. 5 Mite feeding

6. 2. 5. 1 Plant damage by *T. urticae*

Feeding by *T. urticae* and the plant damage inflicted is not simulated in this model and *T. urticae* are assumed to receive the nutritional requirements to develop, breed and move. However, spider mite numbers can be used as a proxy for feeding damage (Hussey and Parr, 1963b) and vice versa (Nihoul *et al.*, 1991; Alatawi *et al.*, 2007).

6. 2. 5. 2 Predation of *T. urticae* by *P. persimilis*

For *P. persimilis*, predation by adult and nymphal stages is modelled with adult predation calculated first. Adults are assumed to feed on all *T. urticae* stages while nymphs feed on prey eggs/larvae only. Based on preferences observed in other studies (Takafuji and Chant, 1976; Fernando and Hassell, 1980) predation occurs first on eggs/larvae then nymphs and adults last, with a switch occurring when no more prey items in a specified stage were available on the plant. *P. persimilis* adults and nymphs have a maximum gut capacity of 45 and 6 egg equivalents respectively (Sabelis, 1981) and will consume prey up to this maximum.

For each *P. persimilis*, a baseline prey predation rate (P_b) was first calculated and depended on the prey density available, using the same approach as in Skirvin *et al.* (2002). This calculation differed depending on both the predator stage and the prey stage. For adults predating eggs this was based on data from functional response experiments on *C. ternata* (Skirvin and De Courcy Williams, 1999) and the consumption per mite was calculated using:

$$P_b = 0.5 + 0.546d - 0.002093d^2 \quad 6.7$$

Where d = the prey numbers available of the relevant stage on the plant. For predator nymphs preying on *T. urticae* eggs and for adult predators preying on *T. urticae* nymphs or adults P_b was calculated using a Holling Type II equation (Holling, 1959):

$$P_b = \frac{ad}{1+aT_hd} \quad 6.8$$

Where a = the attack rate and T_h = the handling time. The values for these were taken from published work (Takafuji and Chant, 1976; Fernando and Hassell, 1980; Sabelis, 1981; Bernstein, 1985; Ryoo, 1986; Nachman, 1987a; Nachman, 1987b) and were a = 0.2, 0.079 and 0.049 and T_h = 0.094, 0.062 and 0.13 for adult *P. persimilis* preying on *T. urticae* nymphs and adults, and nymphal *P. persimilis* preying on *T. urticae* eggs respectively.

Once the baseline predation was established for a predator, the influence of climate was calculated. Both temperature (Skirvin and Fenlon, 2003b) and humidity (Chapter 3) are known to affect the functional response of this mite. The temperature modifier (T_m) was calculated as being proportional to the predation rate at the temperature at which maximum predation occurs (25°C), based on data from (Skirvin and Fenlon, 2003b), and can be written as:

$$T_m = \frac{\left(-6.95+(-1.82t)+\left(-0.1119t^2\right)+\left(-0.001963t^3\right)\right)}{\left(-6.95+(-1.82*25)+\left(-0.1119*25^2\right)+\left(-0.001963*25^3\right)\right)} \quad 6.9$$

Where t = daily mean temperature (°C). The humidity modifier (RH_m) was calculated using a simplification of the work described in Chapter 3, whereby predation was assumed to be optimal at 85% RH. At humidity greater than or equal to 85% RH the modifier was calculated using:

$$RH_m = \frac{\left(d\left(1.1023+\left((-0.0054RH)+0.3489\right)\log d\right)\right)}{\left(d\left(1.1023+\left((-0.0034*65)+0.4001\right)\log d\right)\right)} \quad 6.10$$

Where d = availability of a specific prey stage and RH = the daily average relative humidity (% RH). If humidity is above 65% RH but less than 85% RH the following calculation was used:

$$RH_m = \frac{\left(d\left(1.1023+\left((-0.0034RH)+0.4001\right)\log d\right)\right)}{\left(d\left(1.1023+\left((-0.0034*65)+0.4001\right)\log d\right)\right)} \quad 6.11$$

At 65% RH or below RH_{mod} = 1. This value was chosen as the experiments from which the baseline predation rate (Eq. 6.7) was derived were conducted at 57.5% RH (Skirvin and De Courcy Williams, 1999) and a clear difference between predation at 55% and 65% RH was not found (Chapter 3). Density dependency was included in the humidity modifier as the influence of humidity was not observed at low prey availabilities (Chapter 3). Both the temperature and humidity modifiers were applied to all prey stages. For each predator the total consumption of a particular prey stage (C_t) was calculated using:

$$C_t = P_b * T_m * RH_m \quad 6.12$$

If consumption of a particular prey stage was calculated to be greater than the gut capacity or the number of the prey stage available then the total number consumed was adjusted appropriately. A mite is assumed to continue predating until its gut capacity is reached or no further prey are available.

6. 2. 6 Natural mortality

The mortality due to natural causes was calculated as a probability of survival. For *T. urticae*, the proportion of mites surviving to the next time step was calculated as a function of temperature, using a modification of the equation in Bancroft and Margolies (1999):

$$S_t = 1 - \left(\frac{\left(217-(33t)+\left(1.87t^2\right)-\left(0.047t^3\right)+\left(0.00045t^4\right)\right)}{100}\right) \quad 6.13$$

Where S_t = temperature-dependent likelihood of survival (as a proportion), t = daily mean temperature (°C). This describes a survival rate that is maximal at approximately 20°C, decreases gradually immediately above and below this before

decreasing rapidly below 13°C and above 35°C. To ensure that mortality could occur even at optimal conditions the survival rate (S_r) was adjusted to a maximum value of 0.96, 0.96 and 0.94 for eggs/larvae, nymphs and adults respectively (Skirvin *et al.*, 2002). For each mite a number was randomly sampled from 0-1 distribution, if the number was higher than the S_r value then they were deemed to have perished.

Age-dependent mortality has been included in models (Sabelis, 1981; Bancroft & Margolies, 1999) however data for this effect was not available for *T. urticae* on *C. ternata* and sensitivity analysis of the model on which this is based showed that adjustments to the probability of survival had only minor impacts (Skirvin *et al.*, 2002). Low (Ferro and Chapman, 1979) and high humidity (Harrison and Smith, 1961) have been shown to increase the mortality of *T. urticae* eggs, however because in the latter instance increased mortality only occurred after six days and in the former experiments were conducted on glass (see Section 6. 2. 3) it was decided not to include the influence of humidity here.

For *P. persimilis*, no data was available for temperature-dependent mortality on *C. ternata* and so S_r was set to 0.91, 0.91 and 0.96 for eggs/larvae, nymphs and adults respectively (Takafuji and Chant, 1976; Sabelis, 1981). However, if adults had not consumed enough prey to meet their metabolic requirements (5 egg equivalents, see Section 6. 2. 4-5) S_r was set to 0.65 (Takafuji & Chant, 1976; Sabelis, 1981). A number between 0-1 was randomly sampled for each mite and it was deemed to have died if the value was higher than S_r.

Although Bancroft and Margolies (1999) included an effect of temperature on *P. persimilis* mortality this appeared to be based on *T. urticae* data and other work has shown only a minor effect of this factor on the predator (Stenseth, 1979; Sabelis, 1981). Sabelis (1981) modelled adult mortality as a function of consumption and age class with temperature affecting the duration of each age class. A similar, if simplified, approach to adult mortality is used here.

Humidity has not been shown to have a strong effect on active stages of *P. persimilis* when food is available (De Courcy Williams *et al.*, 2004b). However, low humidity has been shown to increase egg mortality (Perring and Lackey, 1989; De Courcy Williams *et al.*, 2004b; Ferrero *et al.*, 2010), although these experiments were conducted on artificial surfaces. Nevertheless, some studies have used leaves as a

substrate to produce similar, though less severe, results but an interaction with temperature is evident (Stenseth, 1979; Sabelis, 1981) and more data is needed to fully understand this phenomena. Due to the uncertainty of this effect it was decided not to include it in the model.

6. 2. 7 Movement

The sub-routine describing the movement of mites between plants follows the same procedure as that used in Skirvin *et al.* (2002) and no influence of climatic conditions on this process was included in the model. Adults of both species are assumed to emigrate to neighbouring plants however only in *T. urticae* is the movement of nymphs simulated. This is based on the observation that *P. persimilis* nymphs remain in the vicinity of where they hatch (Takafuji, 1977). To determine whether an individual emigrates, a value was sampled from a uniform 0-1 distribution and compared to *E*, a probability value for emigration calculated for each mite individually. The mite is deemed to move if the sampled value is lower than or equal to *E*. For *T. urticae* adults *E* was calculated using:

$$E = \frac{e^{0.0048d+(-3.7)}}{1+e^{0.0048d+(-3.7)}} \qquad 6.14$$

Where *d* = the number of adult *T. urticae* on the plant. This reflects the increase in adult movement in response to increasing densities of adult conspecifics (Skirvin and De Courcy Williams, 1999). For *T. urticae* nymphs *E* was obtained by selecting from a normal distribution ($\mu = 0.07$, $\sigma = 0.05$). Bernstein (1984) found that predator presence increased emigration but it was decided not to include this in the model as it has not been investigated on *C. ternata* and because plant damage (a reflection of mite feeding intensity) was found to be a far more important factor (Bernstein, 1984).

For *P. persimilis*, the likelihood of movement was calculated using:

$$E = \frac{e^{-2.3\log d+3.6}}{1+e^{-2.3\log d+3.6}} \qquad 6.15$$

Where *d* = the number of adult *T. urticae* on the plant. This is a reflection of the tendency of the predator to remain in patches with high prey densities (Skirvin and De Courcy Williams, 1999). The parameters for all equations in this section are

derived from data in Skirvin and De Courcy Williams (1999). Once a mite was determined as emigrating, the destination plant was established. A destination plant was any neighbouring plant in the four cardinal directions and was selected randomly. If a mite was simulated to move to a non-existent destination plant (i.e., at the edge of the simulated greenhouse) it was assumed to remain on the original plant. Movement was calculated to occur once in a 24 hour time step for each mite.

6. 2. 8 Model outputs

Mite numbers of all stages of both species were recorded at the end of each one day time interval. The model had three possible outcomes. The first, control of *T. urticae,* occurred when all stages of the mite had died. The second, *T. urticae* population escape, occurred when combined *T. urticae* numbers exceeded one million. A population this size (an average of 10000 per plant) represents a catastrophic situation for a grower. The final outcome was coexistence and occurred when mites of both species remained in the system at the end of the simulation (end of the 52^{nd} week).

6. 2. 9 Model runs

To assess the impacts of climate management, pest control strategies and seasonal changes on the simulated pest pressures the following design factors were changed between runs:

1. Greenhouse climate conditions
   Five country/climate management systems were investigated; passively ventilated and closed system plastic clad greenhouses in Spain and traditional, cool (-2°C) and warm (+2°C) Venlo glasshouses in the Netherlands. Mean daily temperature and humidity values for one year were generated from the climate model in Chapter 4. For each country/climate management combination ten stochastically varied sets of a year's climate data were used.

2. Pest arrival times

   52 *T. urticae* arrival dates, one for each week of the year, were investigated in separate runs. Adults were programmed to arrive once in each run at the beginning of a specific week. Each run began when these adults arrived.

3. Initial pest population

   Three *T. urticae* starting populations were investigated. Either ten, 100 or 1000 adults were programmed to arrive once at the beginning of each run.

4. Biological control arrival frequencies

   Two *P. persimilis* application frequencies were investigated. Adult predators were programmed to be applied to the crop at either seven or 14 day intervals throughout the year.

5. Biological control introduction rate

   Three *P. persimilis* application rates were investigated. Either ten, 100 or 1000 adults were programmed to arrive with each application.

All combinations of these design factors were assessed with five replicate runs carried out of each.

6. 2. 10 Statistical analyses

The model outputs were assessed for each country separately. For the purposes of analysis the 52 *T. urticae* arrival dates were grouped into 13 "months" of four weeks and time until an outcome was rounded to the nearest week. Additionally, the five replicates for each of the design factor combinations were combined with the ten different temperature and humidity data sets generated for each of the climate management scenarios (Chapter 4) to produce 50 replicates for each of the combinations.

For ease of interpretation the analyses were conducted on the output for the Spanish and Dutch greenhouses separately. To analyse the influence of the design factors on the distribution amongst the three outcomes, a series of log-linear models were constructed. The simplest, the baseline statistical model, consisted of the terms; climate management scenario (climate) by *T. urticae* arrival month (month) by *P. persimilis* introduction rate (PP rate) by *P. persimilis* introduction frequency (PP

frequency) by initial *T. urticae* population size (initial TU), i.e., Outcome+ climate*month*PP freq.*PP rate*initial TU. The baseline model simply allows for any variability in frequencies designed into the combination of model design factors, plus the variability in frequencies between the categories of the response (outcome) factor, but does not account for any relationships between them. Essentially the R^2 of the baseline model should be close to zero (however it does deviate from this slightly due to the effect of pooling the week data into months). Terms or their interactions were sequentially added to the baseline model, increasing its complexity and improving its fit, e.g., by adding PP rate to the baseline model the variation in the outcomes due to this design factor would be analysed. As the data had a Poisson distribution, each model was assessed for significance of fit using an analysis of deviance.

To assess whether increasing the complexity of a model (by adding terms or interactions) significantly improves the fit (which always occurs when adding terms or interactions), models were compared using a contingency table analysis (CTA). CTA assesses whether the difference in the regression deviance (a measure of the fit) between a simple model and a more complex model is significant by using the one-tailed probability of a chi-squared distribution and the difference in the degrees of freedom between the two models. The formal process for the model comparison dictates that a complex model can only be compared to a related model that is simplified by one term, either through the removal of a single design factor or the removal of an interaction of design factors already included (Fig. 6.1). If the addition of a term or interaction significantly improves a model then the addition of further terms/interactions is justified and the comparison process is repeated. Informal comparison can be made with models of the same complexity by comparing their mean residual deviances, with the model with lower deviance deemed to have the better fit. Due to the number of potential factor combinations and interactions no models with greater than six terms were assessed.

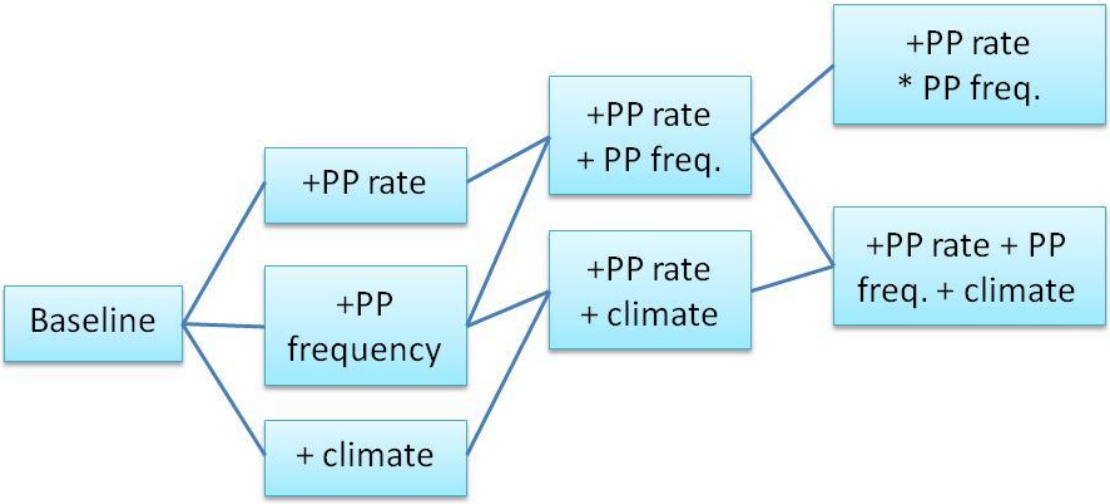

Figure 6.1: An example of the log-linear model hierarchy. Each box represents a model. The baseline model is the simplest. Models to the right of this increase in complexity in single term steps by the addition of a new term or the interaction of terms already present. Links between models indicate where formal comparison can be made (others can be made to models not shown). + = the addition of a term, * = the interaction of terms. Many other models were examined.

Restricted maximum likelihood (REML) analysis was used to assess the time until either escape or control occurred (coexistence could not be assessed as this was correlated with the end of the model, i.e. day 365). Due to the low number of escape and control outcomes under some combinations of design factors, time until control was only analysed with introduction rates of 1000 *P. persimilis* per occasion, and time until escape was only analysed with introduction rates of 10 *P. persimilis* per occasion. The REMLs had a fixed effect structure of climate by month by PP frequency by initial TU (climate*month*PP freq.*initial TU). No random effects structure was necessary. In summarising the analyses all significant differences are followed by the approximate chi-square (X) or F statistic (the former for the log-linear models and the latter for the time analysis), the degrees of freedom (df) and the probability that the null hypotheses can be rejected (based on the approximate X or F).

6. 3 Results

6. 3. 1 Spanish greenhouses

The model that best described the variation in the outcomes was made up of the interaction of *T. urticae* arrival month and initial *T. urticae* population plus climate management scenario, *P. persimilis* introduction frequency and *P. persimilis* introduction rate (month*initial TU+climate+PP freq.+ PP rate; X = 330.04, df = 553, P = <0.001; R^2 = 0.99). This model was a significant improvement on its predecessors and shows that all terms including the climate control strategy had a significant influence on the outcome of the simulation model. All the models constructed fitted the data significantly and a selection are shown in Table 6.1, which includes all the single term models, the best fitting model at each complexity level, the overall best fitting model and a selection of models connecting it to the single term models.

Table 6.1: Log-linear models fitted to the outcomes of the spider mite in the Spanish greenhouses. + = the additions of a term, * = the interaction of a term. All single term models are shown and a selection of models with 2-6 terms. The best fitting model is at the end. The degrees of freedom (df), regression deviance (Deviance), mean residual deviance (Residual), significance (*P*) and fit (R^2).

Model	df	Deviance	Residual	*P*	R^2
Baseline	469	8517	188.7	<0.001	0.046
+climate	471	8570	189.1	<0.001	0.046
+month	493	34082	165.6	<0.001	0.184
+PP frequency	471	8649	189	<0.001	0.047
+PP rate	473	129659	59.27	<0.001	0.702
+initial TU	473	8746	189.3	<0.001	0.047
+month+PP rate	497	174430	11.42	<0.001	0.944
+month*initial TU	545	37177	172	<0.001	0.201
+month*PP rate	545	175590	10.71	<0.001	0.950
+month+climate+PP rate	499	174832	11.00	<0.001	0.946
+month*initial TU+PP rate	549	179891	5.722	<0.001	0.974
+month*(PP rate+initial TU)	597	180969	4.725	<0.001	0.979
+climate+month+PP freq.+PP rate	501	176439	9.245	<0.001	0.955
+month*initial TU+climate+PP rate	551	180378	5.164	<0.001	0.976
+month*PP rate+PP freq.+initial TU	551	180265	5.296	<0.001	0.976
+climate+month+PP freq.+PP rate+initial TU	505	179447	5.936	<0.001	0.971
+month*PP rate+climate+PP freq.+intial TU	553	180769	4.716	<0.001	0.978
+month*initial TU+climate+PP freq.+PP rate	553	182511	2.666	<0.001	0.988

6. 3. 1. 1 *T. urticae* control

6. 3. 1. 1. 1 Climate management

Climate management had a significant effect on the outcome of the model in Spain (X = 18.2, df = 471, P = <0.001). However, the overall impact was relatively minor with control slightly more likely in the passively ventilated greenhouse (average across year of 30.4% of runs) than the closed-system greenhouse (average across year of 28.6% of runs; Tables 6.2-6.4). Greater differences between the climate scenarios are evident in their interactions with other design factors and are discussed in the following sections. The influence of climate control was stronger when considering the time until control (F = 5123.7, df = 1, P = <0.001). In the passively ventilated greenhouse time until control was significantly shorter than in the closed-system greenhouse from months 4-9 (average of 4.3 weeks or 90% shorter for period; F = 116.5, df = 12, P = <0.001; Tables 6.2-6.4) with a maximum reduction in month 8 of 5.4 weeks (196%).

6. 3. 1. 1. 2 *P. persimilis* introduction rate

The number of *P. persimilis* introduced per occasion had the strongest single effect of any of the design factors (X = 247.1, df = 513, P = <0.001), with an R^2 = 0.7 when fitted as a single term model. When 1000 *P. persimilis* were introduced weekly, control was achieved on every run until at least month nine in both greenhouses and regardless of the initial *T. urticae* population (Tables 6.2-6.4). From month ten the likelihood of control reduced steeply, mainly due to the reduced time available to achieve it. At either of the lower levels of *P. persimilis* introduction rate (100 or ten) control likelihood dropped drastically (<3% and <1% of runs respectively). Time until control was not analysed for any application rates other than 1000 due to the low frequency of control achieved at the other rates. However, control was observed to occur far more quickly at 1000 *P. persimilis* per occasion than at the lower rates, e.g. control took 27 days on average at 1000 *P. persimilis* compared to 116 days with 100 *P. persimilis* when applied weekly in the passively ventilated greenhouse with ten initial *T. urticae* (Table 6.2).

Table 6.2: Mean percentage of runs in which *T. urticae* control occurred in Spanish greenhouses in response to *P. persimilis* introduction rate, climate management and *P. persimilis* introduction frequency over time (month) with an initial *T. urticae* population of ten. Numbers in brackets represent mean time (days) until control. PV = passively ventilated, CS = closed-system climate management.

PP rate	Climate	PP freq.	Month												
			1	2	3	4	5	6	7	8	9	10	11	12	13
10	PV	7	1.5 (71)	-	-	-	-	-	-	-	-	-	-	1 (47)	-
		14	2 (54)	-	-	-	-	-	-	-	-	-	-	-	-
	CS	7	0.5 (57)	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	-	-	-	-
100	PV	7	42.5 (116)	13 (185)	5 (215)	3.5 (198)	3 (190)	4.5 (177)	5.5 (99)	12.5 (97)	5 (114)	3.5 (100)	8.5 (68)	22 (40)	3.5 (25)
		14	8 (40)	0.5 (80)	-	-	-	-	-	-	-	-	0.5 (56)	8 (44)	0.5 (27)
	CS	7	20.5 (53)	1.5 (60)	-	0.5 (71)	-	-	-	-	-	-	1.5 (73)	4.5 (42)	-
		14	3.5 (52)	-	-	-	-	-	-	-	-	-	-	2 (44)	-
1000	PV	7	100 (27)	100 (26)	100 (26)	100 (22)	100 (18)	100 (14)	100 (10)	100 (6)	100 (6)	100 (11)	100 (24)	93 (26)	38.5 (14)
		14	100 (32)	100 (36)	100 (41)	100 (42)	100 (44)	100 (36)	100 (19)	100 (11)	99.5 (14)	99 (29)	95.5 (41)	84 (30)	24 (19)
	CS	7	100 (31)	100 (30)	100 (29)	100 (23)	100 (21)	100 (18)	100 (14)	100 (10)	100 (13)	100 (16)	99.5 (24)	85.5 (27)	30 (17)
		14	100 (39)	100 (48)	99.5 (64)	100 (76)	99.5 (76)	98 (69)	98 (63)	97.5 (54)	95 (61)	91.5 (61)	91 (49)	77 (31)	16.5 (18)

6. 3. 1. 1. 3 *P. persimilis* introduction frequency

Whether *P. persimilis* were introduced weekly or fortnightly to the greenhouse had a significant impact on the outcome of the model in Spain (X = 18.4, df = 471, P = <0.001; Tables 6.2-6.4), although its effect was relatively minor. With 1000 *P. persimilis* applied per occasion the overall likelihood of control dropped by 3% and 8% in the Spanish passively ventilated and closed-system greenhouses respectively when fortnightly applications were used instead of weekly applications. Its main influence with 100 *P. persimilis* applied per occasion was on the control likelihood with ten initial *T. urticae* in the passively ventilated greenhouse, where control dropped from 4.5% of runs with weekly applications to 0.5% of runs with fortnightly applications. Otherwise application frequency had little effect, as very little control

occurred with other design combinations at 100 and ten *P. persimilis* application rates.

A stronger impact of introduction frequency was found on the time until control with significant interactions with climate management and month detected (F = 92.2, df = 12, P = <0.001; Tables 6.2-6.4). In both greenhouses from months 1-11 the time until control was significantly increased by using fortnightly instead of weekly applications (average of 5.8 weeks or 50% longer for period). However, the increase in time was far greater in the closed-system than the passively ventilated greenhouse with an overall average increase (months 1-11) of 147% compared to 67% (7.6 and 2.9 weeks) respectively. The time of year also affected the impact of fortnightly introductions, with the time until control a maximum of 309% longer (9.9 weeks) in month 8 in the closed-system greenhouse and 127% longer (2.7 weeks) in month 9 in the passively ventilated greenhouse.

Table 6.3: Mean percentage of runs in which *T. urticae* control occurred in Spanish greenhouses in response to *P. persimilis* introduction rate, climate management and *P. persimilis* introduction frequency over time (month) with an initial *T. urticae* population of 100. Numbers in brackets represent mean time (days) until control. PV = passively ventilated, CS = closed-system climate management.

PP rate	Climate	PP freq.	Month												
			1	2	3	4	5	6	7	8	9	10	11	12	13
10	PV	7	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	-	-	-	-
	CS	7	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	-	-	-	-
100	PV	7	3 (180)	1 (308)	0.5 (282)	0.5 (203)	1 (242)	1 (208)	1 (182)	1 (135)	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	-	-	-	-
	CS	7	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	-	-	-	-
1000	PV	7	100 (46)	100 (47)	100 (44)	100 (40)	100 (36)	100 (27)	100 (20)	100 (16)	100 (16)	100 (31)	96.5 (43)	66 (37)	3.5 (23)
		14	100 (59)	100 (68)	100 (71)	100 (77)	100 (67)	100 (53)	100 (35)	100 (30)	99.5 (42)	91.5 (68)	77.5 (60)	43.5 (41)	1.5 (28)
	CS	7	100 (49)	100 (50)	100 (49)	100 (44)	100 (40)	100 (32)	100 (29)	100 (25)	100 (29)	100 (39)	92.5 (47)	54.5 (40)	0.5 (28)
		14	99.5 (71)	100 (85)	99 (110)	99 (129)	98.5 (122)	97.5 (118)	96 (110)	93.5 (113)	78 (109)	71.5 (88)	57.5 (64)	25 (41)	-

6. 3. 1. 1. 4 Initial *T. urticae* population size

The initial *T. urticae* population size had a significant effect on the outcome of the model (X = 18.5, df = 473, P = <0.001), although its influence was restricted to specific design factor combinations. The main effect of initial *T. urticae* population size was to reduce the likelihood of control with applications of ten and 100 *P. persimilis* (with either introduction frequency) and fortnightly introductions of 1000 *P. persimilis*. For instance, with 100 *P. persimilis* applied weekly in the passively ventilated greenhouse control occurred as much as 43% of the time (month 1) with *T. urticae* starting populations of ten (Table 6.2), however this dropped to just 3% of runs with 100 *T. urticae* (Table 6.3) and 2% of runs with 1000 *T. urticae* (Table 6.4).

The size of the initial *T. urticae* population had a stronger impact on the time until control, with analysis revealing a significant interaction of climate management and month (F = 6.2, df = 23, P = <0.001; Tables 6.2-6.4). The largest differences in time until control were observed when increasing the *T. urticae* starting population from ten to 100, with time until control significantly longer in months 1-11 in the closed-system greenhouse (overall mean increase for period of 4.3 weeks or 74%) and months 1-5 and 9-11 in the passively ventilated greenhouse (overall mean increase 3.3 weeks or 85%). However, between initial starting populations of 100 and 1000 *T. urticae* the difference in time until control was relatively minor in both greenhouse systems (one week and 1.2 weeks longer in the closed-system and passively ventilated greenhouses respectively). This difference was not significant in the passively ventilated greenhouse and was significant in the closed-system greenhouse from months 1-2 only (average of 3.2 weeks or 35% longer).

6. 3. 1. 1. 5 Seasonal effects (*T. urticae* arrival month)

T. urticae arrival month had a significant effect on the outcome of the model (X = 69.1, df = 493, P = <0.001; Table 6.2-6.4) and was the second most important factor determining the outcome. In fact, the log-linear model containing just the combination of this factor and *P. persimilis* introduction rate (not their interaction) had an R^2 of 0.944 (X = 351, df = 497, P = <0.001; Table 6.1). The primary reason for its importance as a design factor is the rapid reduction in control likelihood in the

last 3-4 months of the year in all design factor combinations. However, this is largely an artefact of the model ending after 365 days. More interesting is its influence when considering weekly introduction of 100 *P. persimilis* with an initial *T. urticae* population of 10 in the Spanish passively ventilated greenhouse (Table 6.2). Here control frequency declines rapidly from a high in the first month (43%) to no more than 9% after month two, except a brief increase in month eight. The improved control efficacy in the first month was probably due to the cool Spanish conditions slowing the population growth of *T. urticae*. The peak in the summer (month eight) is likely due to the improved performance of *P. persimilis* in the hot conditions.

Table 6.4: Mean percentage of runs in which *T. urticae* control occurred in Spanish greenhouses in response to *P. persimilis* introduction rate, climate management and *P. persimilis* introduction frequency over time (month) with an initial *T. urticae* population of 1000. Numbers in brackets represent mean time (days) until control. PV = passively ventilated, CS = closed-system climate management.

Climate	PP rate	PP freq.	Month												
			1	2	3	4	5	6	7	8	9	10	11	12	13
PV	10	7	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	-	-	-	-
CS		7	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	-	-	-	-
PV	100	7	1.5 (325)	0.5 (322)	2 (278)	2 (251)	1 (230)	2 (175)	11.5 (110)	9.5 (101)	3.5 (123)	-	-	-	-
		14	-	-	-	-	-	-	-	0.5 (47)	-	-	-	-	-
CS		7	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	-	-	-	-
PV	1000	7	100 (57)	100 (58)	100 (55)	100 (49)	100 (43)	100 (32)	100 (25)	100 (21)	100 (23)	100 (41)	93 (52)	32 (43)	-
		14	100 (81)	100 (87)	100 (90)	100 (82)	100 (72)	100 (52)	100 (38)	100 (31)	99.5 (45)	93 (71)	75 (62)	17 (47)	-
CS		7	100 (64)	100 (63)	100 (60)	100 (53)	100 (48)	100 (39)	100 (33)	100 (32)	100 (36)	99 (50)	89.5 (54)	23 (46)	-
		14	100 (101)	99.5 (118)	99.5 (124)	98 (131)	99 (124)	97.5 (105)	96 (102)	95.5 (107)	88.5 (102)	73.5 (84)	49 (65)	6.5 (47)	-

T. urticae arrival month had a strong impact on the time until control (F = 407.3 df = 12, P = <0.001; Tables 6.2-6.4), with the slowest control during months 2-5 (average of 8.9 weeks) and 10-11 (average of 7 weeks) and the fastest in months 7-8 (average of 5.7 weeks). However, the seasonal effects were stronger in the passively ventilated than the closed-system greenhouse (F = 116.5, df = 12, P = <0.001; Tables

6.2-6.4) with control in month eight 184% faster than in month three with passive ventilation and 28% faster with closed-system management.

6. 3. 1. 2 *T. urticae* population escape

6. 3. 1. 2. 1 Climate management

As was the case for *T. urticae* control, the effect of climate management had a small overall effect on the probability of *T. urticae* escape (a population of one million) occurring (23.4% of runs in the passively ventilated greenhouse and 23% of runs in the closed-system greenhouse). However, important interactions with other design factors were found and are discussed in the following sections (Tables 6.5-6.7). The time until escape was consistently shorter throughout the year in the passively ventilated greenhouse and significantly so in months 1-2 (2.7 weeks or 10% shorter for period; $F = 154.3$, df = 9, $P = <0.001$, Tables 6.5-6.7).

6. 3. 1. 2. 2 *P. persimilis* introduction rate

The number of *P. persimilis* regularly applied to the greenhouse had the greatest influence on whether the *T. urticae* population reached the escape level (one million). With applications of ten *P. persimilis*, escape was very likely to occur regardless of other design factors (Tables 6.8-6.10). In fact, with this number of predators applied fortnightly escape occurred in 100% of runs until at least month seven. With applications of 100 *P. persimilis* escape had a low probability of occurring regardless of other design factors, meaning that the dominant outcome at this *P. persimilis* introduction rate was coexistence. At the highest *P. persimilis* application frequency (1000) escape did not occur in any scenario. Time until escape was not analysed at 100 or 1000 *P. persimilis* due to the low probability of escape occurring so statistical comparison is not possible. However, increasing the *P. persimilis* introduction rate from ten to 100 tended to delay escape considerably, e.g., by an overall average of 15.3 weeks in the passively ventilated greenhouse with fortnightly introductions.

Table 6.5: Mean percentage of runs in which *T. urticae* escape occurred in Spanish greenhouses in response to *P. persimilis* introduction rate, climate management and *P. persimilis* introduction frequency over time (month) with an initial *T. urticae* population of ten. Numbers in brackets represent mean time (days) until escape. PV = passively ventilated, CS = closed-system climate management.

PP rate	Climate	PP freq.	Month												
			1	2	3	4	5	6	7	8	9	10	11	12	13
10	PV	7	96.5 (184)	100 (151)	97 (131)	99.5 (112)	99.5 (98)	98 (88)	94 (87)	71.5 (91)	6 (95)	-	-	-	-
		14	98 (165)	100 (135)	100 (114)	100 (99)	100 (87)	100 (77)	100 (73)	98.5 (77)	34.5 (94)	-	-	-	-
	CS	7	94 (190)	95 (160)	95.5 (139)	97 (116)	98 (102)	99 (90)	98 (86)	70.5 (94)	4.5 (100)	-	-	-	-
		14	100 (170)	100 (141)	100 (120)	99.5 (102)	100 (89)	100 (81)	100 (76)	99 (80)	29 (92)	-	-	-	-
100	PV	7	0.5 (204)	1 (192)	-	-	-	-	-	-	-	-	-	-	-
		14	8.5 (256)	12 (219)	7.5 (171)	10 (173)	11 (136)	7.5 (118)	3 (108)	2 (117)	-	-	-	-	-
	CS	7	1.5 (259)	1.5 (254)	1.5 (228)	1 (229)	1 (162)	0.5 (134)	0.5 (120)	0.5 (110)	-	-	-	-	-
		14	9.5 (231)	11.5 (226)	10 (185)	10 (176)	10.5 (148)	8.5 (120)	3.5 (126)	1 (105)	-	-	-	-	-
1000	PV	7	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	-	-	-	-
	CS	7	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	-	-	-	-

6. 3. 1. 2. 3 *P. persimilis* introduction frequency

Reducing the frequency with which *P. persimilis* were applied to the greenhouse from weekly to fortnightly intervals had a relatively minor influence on either the probability of escape occurring or the time until escape (Tables 6.5-6.7). The notable effects were increasing the likelihood of escape when fortnightly instead of weekly introductions of 100 *P. persimilis* were made. In both greenhouses escape probability increased from <1% to > 8% in the first seven months of the year. Fortnightly applications of *P. persimilis* also significantly reduced the time until escape by an overall mean of 14% (F = 11.9, df = 9, P = <0.001) and this effect was consistent across the climate management scenarios.

Table 6.6: Mean percentage of runs in which *T. urticae* escape occurred in Spanish greenhouses in response to *P. persimilis* introduction rate, climate management and *P. persimilis* introduction frequency over time (month) with an initial *T. urticae* population of 100. Numbers in brackets represent mean time (days) until escape. PV = passively ventilated, CS = closed-system climate management.

PP rate	Climate	PP freq.	Month												
			1	2	3	4	5	6	7	8	9	10	11	12	13
10	PV	7	96.5 (172)	99 (133)	98.5 (110)	100 (93)	99.5 (78)	99.5 (70)	99 (65)	98 (70)	55 (76)	-	-	-	-
		14	100 (146)	100 (111)	100 (93)	100 (80)	100 (68)	100 (58)	100 (56)	100 (57)	92 (66)	2.5 (79)	-	-	-
	CS	7	83.5 (201)	91 (163)	96 (127)	97.5 (103)	99 (85)	100 (71)	100 (65)	97.5 (69)	48.5 (79)	-	-	-	-
		14	100 (158)	100 (125)	100 (99)	100 (85)	100 (71)	100 (60)	100 (56)	100 (56)	93.5 (68)	3 (89)	-	-	-
100	PV	7	2.5 (231)	0.5 (199)	0.5 (207)	-	2 (144)	-	0.5 (92)	-	-	-	-	-	-
		14	10 (238)	11.5 (217)	6 (198)	11 (159)	6.5 (150)	8 (126)	6.5 (107)	2.5 (103)	-	-	-	-	-
	CS	7	2 (259)	1 (249)	-	0.5 (192)	-	1 (118)	1.5 (107)	0.5 (131)	-	-	-	-	-
		14	6 (266)	4 (231)	9 (212)	5.5 (191)	8.5 (137)	11 (112)	19 (97)	7 (99)	0.5 (107)	-	-	-	-
1000	PV	7	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	-	-	-	-
	CS	7	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	-	-	-	-

6. 3. 1. 2. 4 Initial *T. urticae* population size

Increasing the initial pest population had a relatively minor effect on escape likelihood with starting populations of ten, 100 and 1000 *T. urticae* resulting in escape occurring in 12%, 23% and 25% of runs respectively (overall means; Tables 6.5-6.7). Other design factors had very little effect on this. However, this factor had an important influence on the time until escape occurring, with a significant interaction with month and climate management evident (F = 53.8, df = 17, P = <0.001; Tables 6.5-6.7). With an initial starting population of ten *T. urticae* escape took only slightly longer than with 100 *T. urticae* regardless of climate management (average of 15.9 vs. 13.3 weeks from months 1-9). However, with 1000 *P. persimilis* escape time was considerably shorter than with 100 *P. persimilis* with the reduction dependent on climate management; 3.9 weeks (or 36%, months 1-9) in the passively ventilated greenhouse and 3.1 weeks (or 22%, months 1-9) in the closed-system greenhouse.

Table 6.7: Mean percentage of runs in which *T. urticae* escape occurred in Spanish greenhouses in response to *P. persimilis* introduction rate, climate management and *P. persimilis* introduction frequency over time (month) with an initial *T. urticae* population of 1000. Numbers in brackets represent mean time (days) until escape. PV = passively ventilated, CS = closed-system climate management.

PP rate	Climate	PP freq.	Month												
			1	2	3	4	5	6	7	8	9	10	11	12	13
10	PV	7	92.5 (173)	97 (91)	100 (66)	100 (57)	100 (49)	100 (41)	100 (37)	100 (37)	100 (38)	73.5 (55)	-	-	-
		14	100 (129)	100 (82)	100 (64)	100 (55)	100 (47)	100 (40)	100 (37)	100 (36)	100 (38)	75.5 (54)	-	-	-
	CS	7	72 (227)	93 (140)	100 (79)	100 (62)	100 (52)	100 (44)	100 (39)	100 (38)	100 (41)	61 (58)	-	-	-
		14	92 (182)	97.5 (108)	100 (71)	100 (59)	100 (50)	100 (42)	100 (38)	100 (37)	100 (40)	72.5 (61)	-	-	-
100	PV	7	1.5 (239)	0.5 (276)	1 (213)	0.5 (156)	-	1 (161)	1 (87)	-	-	-	-	-	-
		14	9 (244)	9.5 (213)	10.5 (193)	7.5 (162)	9.5 (134)	4 (98)	9 (85)	4 (89)	0.5 (85)	-	-	-	-
	CS	7	1.5 (256)	1 (203)	1.5 (216)	0.5 (186)	1.5 (176)	1 (148)	-	-	-	-	-	-	-
		14	4 (277)	3.5 (204)	9.5 (194)	7.5 (178)	8.5 (153)	6 (133)	4.5 (114)	2.5 (84)	-	-	-	-	-
1000	PV	7	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	-	-	-	-
	CS	7	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	-	-	-	-

6. 3. 1. 2. 5 Seasonal effects (*T. urticae* arrival month)

As for control likelihood, the greatest effect of *T. urticae* arrival month on the outcome of the model was the reduced proportion of escape outcomes in the final months of the year, with no escape occurring after month ten. Otherwise, escape likelihood remained relatively constant across the year, except for a slight reduction in month one (Tables 6.5-6.7). *T. urticae* arrival month had a stronger impact on the time until escape (F = 11995.7, df = 9, P = <0.001; Tables 6.5-6.7) with the significant differences detected between all months. The general seasonal trends were for longer escape times during the winter (e.g., 21-30 weeks for month one) and shorter times in the summer (e.g., 5-12 weeks for month seven). Seasonal differences in the time until escape between the greenhouses were also evident, although an interaction with initial *T. urticae* population size was present (F = 53.8, df = 17, P = <0.001). With a starting population of ten *T. urticae* no significant

differences in the time until escape were found between the passively ventilated and closed-system greenhouses. However, at starting populations of both 100 and 1000 *T. urticae* time until control was significantly shorter in the passively ventilated greenhouse from months 1-3 (2.6 weeks or 14% shorter with 100 initial *T. urticae* and 4.8 weeks or 33% shorter with 1000 initial *T. urticae*).

6. 3. 2 Dutch greenhouses

In the Dutch greenhouses the model that best described the variation in the outcomes was made up of the interaction of *P. persimilis* introduction rate and the initial *T. urticae* population plus climate management scenario, *T. urticae* arrival month and *P. persimilis* introduction frequency (PP rate*initial TU+climate+month+PP freq.; $X = 364.77$, df = 749, $P = <0.001$; $R^2 = 0.99$). This model was a significant improvement on its predecessors and shows that all terms including the climate control strategy had a significant influence on the outcome of the simulation model. All the models constructed fitted the data significantly and a selection are shown in Table 6.8, which includes all the single term models, the best fitting model at each complexity level, the overall best fitting model and a selection of models connecting it to the single term models.

Table 6.8: Log-linear models fitted to the outcomes of the spider mite in the Dutch greenhouses. + = the additions of a term, * = the interaction of a term. All single term models are shown and a selection of models with 2-6 terms. The best fitting model is at the end. The degrees of freedom (df), regression deviance (Deviance), mean residual deviance (Residual), significance (*P*) and fit (R^2).

Model	df	Deviance	Residual	*P*	R^2
Baseline	703	15482	185.80	<0.001	0.056
+climate	707	16609	185.50	<0.001	0.060
+month	727	45507	167.20	<0.001	0.165
+PP frequency	705	16369	185.40	<0.001	0.059
+PP rate	707	195047	57.88	<0.001	0.707
+initial TU	707	16603	185.50	<0.001	0.060
+month+PP rate	731	245887	21.89	<0.001	0.981
+PP rate*initial TU	719	198563	55.84	<0.001	0.720
+month*PP rate	779	245928	22.65	<0.001	0.981
+month+PP freq.+PP rate	733	254221	15.84	<0.001	0.921
+PP rate*initial TU+month	743	255071	16.07	<0.001	0.924
+initial TU*(PP rate+month)	791	255581	15.51	<0.001	0.926
+month+PP freq.+PP rate+initial TU	737	262764	9.65	<0.001	0.952
+month*initial TU+PP freq+PP rate	785	264426	8.74	<0.001	0.958
+PP rate*initial TU+month+PP freq.	745	264656	8.31	<0.001	0.959
+climate+month+PP freq.+PP rate+initial TU	741	270124	4.28	<0.001	0.979
+month*intial TU+climate+PP freq.+PP rate	789	273028	2.23	<0.001	0.989
+PP rate*initial TU+climate+month+PP freq.	789	273211	2.03	<0.001	0.990

6. 3. 2. 1 *T. urticae* control

6. 3. 2. 1. 1 Climate management

Climate management had a significant effect on the outcome of the model (X = 23.49, df = 707, P = <0.001). However, the overall impact was relatively minor with control slightly more likely in the cool greenhouse (average across year of 27.4% of runs) than the warm (average across year of 27% of runs) or traditional greenhouses (average across year of 25.8% of runs; Tables 6.9-6.11). Greater differences between the climate scenarios were evident in their interactions with other design factors and are discussed in the following sections. The influence of climate control was stronger when considering the time until control (F = 62.17, df = 24, P = <0.001; Tables 6.9-6.11). The shortest time until control was in the warm greenhouse (overall average of 6.7 weeks), followed by cool (9.2 weeks) and traditional (10

weeks) greenhouses. The time until control was significantly shorter in the warm greenhouse than the traditional greenhouse from months 1-8 (average of 4.5 weeks or 39% shorter) and the cool greenhouse from months 3-8 (average of 3.9 weeks or 39% shorter). The maximum difference was between the warm and the traditional greenhouse in month six (5.7 weeks or 50% shorter).

Table 6.9: Mean percentage of runs in which *T. urticae* control occurred in the Dutch greenhouses in response to *P. persimilis* introduction rate, climate management and *P. persimilis* introduction frequency over time (month) with an initial *T. urticae* population of ten. Numbers in brackets represent mean time (days) until control. NLC = cool (traditional -2°C), NLT = traditional, NLW = warm (traditional +2°C) climate management.

PP rate	Climate	PP freq.	Month												
			1	2	3	4	5	6	7	8	9	10	11	12	13
10	NLC	7	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	-	-	-	-
	NLT	7	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	-	-	-	-
	NLW	7	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	-	-	-	-
100	NLC	7	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	-	-	-	-
	NLT	7	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	-	-	-	-
	NLW	7	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	-	-	-	-
1000	NLC	7	100 (28)	100 (27)	100 (27)	100 (27)	100 (24)	100 (26)	100 (24)	100 (24)	100 (24)	100 (26)	98.5 (27)	88.5 (25)	24 (15)
		14	99.5 (62)	100 (68)	100 (66)	100 (61)	99 (74)	100 (74)	96.5 (71)	98.5 (65)	94.5 (59)	88.5 (53)	74.5 (47)	42.5 (33)	7.5 (18)
	NLT	7	100 (24)	100 (23)	100 (21)	100 (21)	100 (21)	100 (20)	100 (18)	100 (22)	100 (21)	100 (21)	100 (23)	92 (19)	46.5 (13)
		14	100 (84)	99.5 (96)	97.5 (94)	98.5 (87)	95 (86)	93 (83)	92 (74)	88.5 (81)	80 (71)	78 (60)	51 (48)	28 (35)	5.5 (18)
	NLW	7	100 (21)	100 (18)	100 (17)	100 (16)	100 (13)	100 (14)	100 (13)	100 (14)	100 (15)	99.5 (16)	99 (18)	95.5 (17)	53 (11)
		14	100 (76)	100 (67)	100 (56)	100 (44)	100 (32)	99.5 (31)	99.5 (26)	96 (36)	85.5 (43)	71 (44)	41 (44)	24 (32)	4 (20)

6. 3. 2. 1. 2 *P. persimilis* introduction rate

The number of *P. persimilis* introduced per occasion was the dominant factor determining control outcome in the Dutch greenhouses, with an R^2 = 0.71 when

fitted as a single term model (X = 275.9, df = 707, P = <0.001, Table 6.8). No control occurred when ten or 100 *P. persimilis* were applied regardless of other design factors. When 1000 *P. persimilis* were applied weekly control occurred in 100% of runs until at least month eight (Tables 6.9-6.11).

6. 3. 2. 1. 3 *P. persimilis* introduction frequency

The *P. persimilis* introduction frequency had a significant influence on the control likelihood (X = 23.2, df = 705, P = <0.001; Tables 6.9-6.11). Reducing applications from weekly to fortnightly intervals reduced the likelihood of control with the degree of reduction related to climate management. For instance, with fortnightly applications of 1000 *P. persimilis* control likelihood dropped 26% and 22% in the traditional and warm greenhouses respectively but only 12% in the cool greenhouse. The introduction frequency also had a strong influence on the time until control, with an interaction with climate management and month evident (F = 40, df = 24, P = <0.001; Tables 6.9-6.11). When fortnightly instead of weekly *P. persimilis* applications were used the time until control increased with the biggest differences occurring in the traditional greenhouse (overall average of 9.9 weeks or 189% longer), followed by the warm (overall average of 5.2 weeks or 124% longer) and cool greenhouses (overall average of 6.5 weeks or 107% longer). The largest increases in each greenhouse were 256% (or 13.7 weeks) in month three in the traditional greenhouse, 197% (or 9.9 weeks) in month one in the warm greenhouse and 147% (or 8.9 weeks) in month five in the cool greenhouse. The interaction with month also showed that while the increase in time until control with fortnightly introductions was relatively stable (and significant) across the year in the cool greenhouse (5.5-7.5 weeks longer between months one and nine), clear seasonal trends were evident in the traditional and warm greenhouses. In the traditional greenhouse, these differences ranged from 12.9 weeks in month one to 7.8 weeks in month nine (all differences between months significant). In the warm greenhouse, the differences ranged from 9.9 weeks in month one to 5.8 weeks in month nine (differences significant from months 1-4 and 8-10).

Table 6.10: Mean percentage of runs in which *T. urticae* control occurred in the Dutch greenhouses in response to *P. persimilis* introduction rate, climate management and *P. persimilis* introduction frequency over time (month) with an initial *T. urticae* population of 100. Numbers in brackets represent mean time (days) until control. NLC = cool (traditional -2°C), NLT = traditional, NLW = warm (traditional +2°C) climate management.

PP rate	Climate	PP freq.	Month												
			1	2	3	4	5	6	7	8	9	10	11	12	13
10	NLC	7	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	-	-	-	-
	NLT	7	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	-	-	-	-
	NLW	7	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	-	-	-	-
100	NLC	7	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	-	-	-	-
	NLT	7	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	-	-	-	-
	NLW	7	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	-	-	-	-
1000	NLC	7	100 (53)	100 (50)	100 (48)	100 (45)	100 (47)	100 (44)	100 (49)	100 (45)	100 (46)	98.5 (47)	90.5 (46)	41.5 (39)	0.5 (23)
		14	99.5 (111)	100 (114)	98 (116)	99.5 (125)	97.5 (117)	98.5 (116)	92 (108)	85.5 (100)	80.5 (91)	61 (76)	24.5 (65)	2.5 (48)	-
	NLT	7	100 (45)	100 (43)	100 (41)	100 (41)	100 (39)	100 (39)	100 (41)	100 (43)	99.5 (42)	99.5 (43)	93.5 (42)	60.5 (36)	2 (28)
		14	96.5 (145)	96 (154)	93 (160)	90 (153)	86.5 (142)	80 (141)	69 (134)	57 (118)	37 (105)	26.5 (86)	6.5 (64)	0.5 (42)	-
	NLW	7	100 (37)	100 (36)	100 (35)	100 (31)	100 (28)	100 (28)	100 (30)	100 (31)	100 (32)	100 (37)	96.5 (38)	76 (33)	10.5 (23)
		14	100 (119)	100 (102)	100 (88)	100 (73)	99.5 (64)	99.5 (58)	91 (59)	79.5 (81)	57.5 (83)	29 (77)	6 (61)	-	-

6. 3. 2. 1. 4 Initial *T. urticae* population size

The initial *T. urticae* population size had a significant effect on the outcome of the model (X = 23.5, df = 707, P = <0.001; Tables 6.9-6.11), with increases in population size reducing the likelihood of control in the Dutch greenhouses. Large reductions in control likelihood occurred when increasing the initial *T. urticae* population from ten to 100, ranging from 14% (overall average) in the traditional greenhouse to 9% in both the cool and warm greenhouses (overall average). Increasing the initial population to 1000 resulted in minor further reduction in control likelihood (<2% lower in all greenhouses).

Table 6.11: Mean percentage of runs in which *T. urticae* control occurred in the Dutch greenhouses in response to *P. persimilis* introduction rate, climate management and *P. persimilis* introduction frequency over time (month) with an initial *T. urticae* population of 1000. Numbers in brackets represent mean time (days) until control. NLC = cool (traditional -2°C), NLT = traditional, NLW = warm (traditional +2°C) climate management.

PP rate	Climate	PP freq.	Month												
			1	2	3	4	5	6	7	8	9	10	11	12	13
10	NLC	7	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	-	-	-	-
	NLT	7	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	-	-	-	-
	NLW	7	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	-	-	-	-
100	NLC	7	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	-	-	-	-
	NLT	7	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	-	-	-	-
	NLW	7	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	-	-	-	-
1000	NLC	7	100 (62)	100 (60)	100 (59)	100 (55)	100 (56)	100 (57)	100 (59)	100 (58)	99.5 (58)	98.5 (61)	83 (57)	16 (47)	-
		14	100 (127)	100 (126)	99.5 (125)	99.5 (118)	98.5 (122)	97 (117)	91.5 (109)	90 (103)	80 (94)	55 (82)	18 (65)	0.5 (56)	-
	NLT	7	100 (53)	100 (54)	100 (50)	100 (47)	100 (49)	100 (48)	100 (50)	100 (51)	100 (50)	99.5 (54)	91.5 (52)	37.5 (41)	-
		14	96.5 (163)	93.5 (166)	93 (147)	96.5 (141)	92.5 (135)	82.5 (131)	67.5 (120)	63 (118)	41.5 (100)	32 (85)	12 (66)	1.5 (56)	-
	NLW	7	100 (48)	100 (45)	100 (41)	100 (38)	100 (35)	100 (36)	100 (36)	100 (40)	100 (41)	100 (44)	96.5 (45)	49 (38)	1 (26)
		14	100 (120)	100 (99)	100 (84)	100 (72)	99.5 (62)	99.5 (61)	93.5 (67)	79 (79)	50.5 (84)	29.5 (76)	4 (57)	-	-

The initial *T. urticae* population size also had a significant affect on the time until control, with an interaction with climate management and month evident (F = 2.6, df = 46, P = <0.001; Tables 6.9-6.11). In all greenhouses increasing the initial population size increased the time until control, with larger increases in time when the initial population increased from ten to 100 (overall average of 4.4 weeks or 77% longer) and much smaller increases when the initial population increased from 100 to 1000 (overall average of 0.8 weeks or 8% longer). However, these increases were only significant between ten and 100 initial *T. urticae* from months 1-7 in the

traditional greenhouse (average of 5.8 weeks or 43% longer for period) and months 1-5 in the cool greenhouse (average of 5.2 weeks or 44% longer for period).

6. 3. 2. 1. 5 Seasonal effects (*T. urticae* arrival month)

T. urticae arrival month was the second most important factor determining the outcome of the model in the Dutch greenhouses (X = 62.6, df = 727, P = <0.001; Table 6.8). However, as in the Spanish greenhouses the primary reason for its influence on the statistical model was the steep reduction in control likelihood toward the end of the year due to the model finishing after 365 days (Tables 6.9-6.11). A slight interaction with greenhouse management was evident with control occurring in >90% of runs for the first nine months in the cool greenhouse, the first eight months in the warm greenhouse and the first six months in the traditional greenhouse. In all greenhouses control likelihood dropped rapidly after month ten. The arrival month also had a significant effect on the time until control (F = 563.4, df = 12, P = <0.001; Tables 6.9-6.11), although the influence of this was less strong than in Spain. Time until control was generally longer in the winter than in the summer, e.g., 10.9 weeks in month one compared to 8.6 weeks in month six (overall averages). However, the seasonal influence was greater in the warm greenhouse (10 weeks in month one vs. 5.5 weeks in month six) than the cool (10.6 vs. 10.3 weeks) and traditional (12.2 vs. 11 weeks) greenhouses (F = 62.17, df = 24, P = <0.001).

6. 3. 2. 2 *T. urticae* population escape

6. 3. 2. 2. 1 Climate management

Greenhouse climate management in the Netherlands had a stronger influence on the probability of *T. urticae* reaching escape levels (one million individuals) than it had on the probability of control. The greatest overall likelihood of escape occurred in the warm greenhouse (28% of runs), followed by the traditional (24% of runs) and the cool greenhouses (19% of runs; Tables 6.12-6.14). The greenhouse climate also had an influence on the time until escape, with the time taken in the warm greenhouse significantly shorter (overall average of 10 weeks) than in the traditional

greenhouse (overall average of 12.6 weeks), which in turn was significantly shorter than in the cool greenhouse (overall average of 16.4 weeks; F = 16.4, df = 21, P = <0.001; Tables 6.12-6.14).

Table 6.12: Mean percentage of runs in which *T. urticae* escape occurred in the Dutch greenhouses in response to *P. persimilis* introduction rate, climate management and *P. persimilis* introduction frequency over time (month) with an initial *T. urticae* population of ten. Numbers in brackets represent mean time (days) until escape. NLC = cool (traditional -2°C), NLT = traditional, NLW = warm (traditional +2°C) climate management.

PP rate	Climate	PP freq.	Month												
			1	2	3	4	5	6	7	8	9	10	11	12	13
10	NLC	7	58 (180)	55.5 (176)	56 (166)	55.5 (161)	58 (162)	44.5 (161)	29.5 (161)	5.5 (147)	-	-	-	-	-
		14	94 (147)	93 (142)	95.5 (138)	96.5 (139)	93.5 (141)	78.5 (145)	50 (144)	2 (136)	-	-	-	-	
	NLT	7	93.5 (149)	90 (143)	95.5 (131)	94 (122)	92.5 (125)	87.5 (126)	77.5 (130)	63 (129)	22 (120)	0.5 (112)	-	-	-
		14	99.5 (117)	100 (112)	100 (105)	100 (103)	100 (103)	99.5 (104)	99 (109)	96 (112)	73 (114)	4 (109)	-	-	-
	NLW	7	99.5 (112)	98.5 (105)	100 (100)	100 (98)	99 (101)	97.5 (101)	96.5 (104)	93 (104)	84.5 (105)	21.5 (97)	-	-	-
		14	100 (94)	100 (90)	100 (87)	100 (85)	100 (86)	100 (86)	100 (87)	100 (90)	100 (92)	64.5 (92)	-	-	-
100	NLC	7	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	0.5 (294)	0.5 (185)	-	-	0.5 (171)	1 (205)	-	-	-	-	-	-	-
	NLT	7	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	6 (215)	3 (189)	3.5 (168)	4.5 (172)	3 (161)	2 (160)	0.5 (181)	2 (142)	-	-	-	-	-
	NLW	7	3 (260)	1.5 (149)	2 (204)	2.5 (192)	0.5 (240)	0.5 (209)	0.5 (192)	0.5 (146)	-	-	-	-	-
		14	19 (235)	14.5 (205)	18.5 (173)	12 (170)	12.5 (157)	6 (155)	6 (142)	2 (128)	0.5 (111)	-	-	-	-
1000	NLC	7	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	-	-	-	-
	NLT	7	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	-	-	-	-
	NLW	7	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	-	-	-	-

6. 3. 2. 2. 2 *P. persimilis* introduction rate

The *P. persimilis* introduction rate was the most important design factor determining the occurrence of *T. urticae* escape in the Netherlands. With ten *P. persimilis* escape occurred very frequently in all combinations of design factors (>90% of runs

between months 1-6), although climate management also had an influence (74%, 90% and >97% of runs between months 1-10 in the cool, traditional and warm greenhouses respectively). With 100 *P. persimilis* escape was very infrequent in the cool and traditional greenhouses (<1% and 2% of runs from months 1-8 respectively) but more common in the warm greenhouse (8% of runs from months 1-8), meaning that at this application rate the predominant outcome was coexistence. When 1000 *P. persimilis* were applied escape never occurred. The time until escape was not analysed for applications of 100 or 1000 *P. persimilis* due to the low occurrence of escape. However, informal comparison of escape times between fortnightly applications of ten and 100 *P. persimilis* in the warm greenhouse (design combinations at which a relatively high proportion of runs resulted in escape) show that when using 100 *P. persimilis* the time until escape from months 1-8 was 149% longer (9.3 vs. 23.1 weeks).

6. 3. 2. 2. 3 *P. persimilis* introduction frequency

Depending on climate management the frequency with which *P. persimilis* were applied to the greenhouse had an important influence on the likelihood of *T. urticae* escape occurring in the Netherlands (Tables 6.12-6.14). For instance, with applications of ten *P. persimilis* overall escape likelihood in the cool greenhouse decreased from 67% of runs with fortnightly applications to 49% with weekly applications. With the same comparison in the other greenhouses the differences were smaller (76% vs. 68% in the traditional greenhouse and 81% vs. 77% in the warm greenhouse). A further important interaction is with 100 *P. persimilis* in the warm greenhouse where escape likelihood rose from <1% with weekly application to 10% with fortnightly applications. The introduction frequency also had a significant, although relatively minor, affect on the time until control (F = 11.9, df = 9, P = <0.001; Tables 6.12-6.14). In the cool and traditional greenhouses weekly applications resulted in significant reductions in the time until escape for the majority of the year (2.7 weeks or 15% shorter for months 1-7 and 2.2 weeks or 16% shorter for months 1-8 respectively). On the other hand in the warm greenhouse significant differences were only found in months 1 and 7-8 (1.5 weeks or 14% shorter).

Table 6.13: Mean percentage of runs in which *T. urticae* escape occurred in the Dutch greenhouses in response to *P. persimilis* introduction rate, climate management and *P. persimilis* introduction frequency over time (month) with an initial *T. urticae* population of 100. Numbers in brackets represent mean time (days) until escape. NLC = cool (traditional -2°C), NLT = traditional, NLW = warm (traditional +2°C) climate management.

PP rate	Climate	PP freq.	Month												
			1	2	3	4	5	6	7	8	9	10	11	12	13
10	NLC	7	75.5 (153)	73.5 (138)	69.5 (135)	73 (135)	71 (130)	75 (135)	61.5 (134)	43 (132)	8.5 (121)	-	-	-	-
		14	100 (115)	100 (110)	100 (107)	99 (105)	98.5 (106)	99 (106)	97.5 (110)	94 (118)	61.5 (115)	6 (106)	-	-	-
	NLT	7	91.5 (116)	94.5 (114)	99 (99)	98.5 (94)	98.5 (92)	97.5 (93)	94 (97)	90 (103)	74 (103)	22.5 (97)	-	-	-
		14	100 (90)	100 (86)	100 (81)	100 (77)	100 (76)	100 (75)	100 (78)	100 (81)	99.5 (84)	76.5 (89)	1 (78)	-	-
	NLW	7	100 (85)	99.5 (80)	100 (77)	100 (73)	100 (74)	100 (76)	99.5 (78)	98.5 (79)	97.5 (80)	83 (81)	14 (77)	-	-
		14	100 (71)	100 (66)	100 (63)	100 (61)	100 (61)	100 (62)	100 (63)	100 (64)	100 (66)	100 (69)	57 (69)	-	-
100	NLC	7	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	0.5 (158)	0.5 (192)	1 (195)	-	-	-	-	-	-	-
	NLT	7	1 (222)	-	0.5 (246)	-	-	-	-	-	-	-	-	-	-
		14	1 (271)	3 (258)	2 (215)	2 (183)	2 (166)	2 (140)	2 (155)	0.5 (128)	-	-	-	-	-
	NLW	7	1.5 (197)	1.5 (240)	2 (230)	1.5 (135)	-	0.5 (187)	0.5 (180)	0.5 (149)	-	-	-	-	-
		14	20 (208)	18 (198)	18.5 (158)	21 (149)	13 (143)	13 (146)	6.5 (135)	6.5 (125)	3.5 (122)	1 (103)	-	-	-
1000	NLC	7	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	-	-	-	-
	NLT	7	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	-	-	-	-
	NLW	7	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	-	-	-	-

6. 3. 2. 2. 4 Initial *T. urticae* population size

Increasing the initial pest population size had a relatively minor influence on the escape likelihood and the time until escape in the Netherlands, although an important interaction with climate management was evident (Tables 6.12-6.14). While increasing the initial *T. urticae* number resulted in only minor increases in the likelihood of escape in the warm (33% of runs with ten *T. urticae* vs. 36% with 1000 *T. urticae* from months 1-10) and traditional greenhouses (27% of runs with ten *T. urticae* vs. 34% with 1000 *T. urticae* from months 1-10), the effects were stronger in

the cool greenhouse (18% of runs with ten *T. urticae* vs. 33% with 1000 *T. urticae* from months 1-10).

Table 6.14: Mean percentage of runs in which *T. urticae* escape occurred in the Dutch greenhouses in response to *P. persimilis* introduction rate, climate management and *P. persimilis* introduction frequency over time (month) with an initial *T. urticae* population of 1000. Numbers in brackets represent mean time (days) until escape. NLC = cool (traditional -2°C), NLT = traditional, NLW = warm (traditional +2°C) climate management.

PP rate	Climate	PP freq.	Month												
			1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
10	NLC	7	100 (77)	100 (73)	100 (70)	100 (68)	100 (66)	100 (66)	100 (67)	100 (69)	100 (77)	90.5 (80)	20 (74)	-	-
		14	100 (71)	100 (68)	100 (65)	100 (64)	100 (62)	100 (62)	100 (63)	100 (65)	100 (69)	100 (73)	42.5 (73)	-	-
	NLT	7	100 (61)	100 (58)	100 (55)	100 (53)	100 (51)	100 (50)	100 (51)	100 (52)	100 (55)	100 (58)	91.5 (61)	-	-
		14	100 (57)	100 (56)	100 (53)	100 (51)	100 (49)	100 (49)	100 (50)	100 (50)	100 (53)	100 (56)	100 (58)	2 (56)	-
	NLW	7	100 (49)	100 (47)	100 (45)	100 (43)	100 (42)	100 (41)	100 (42)	100 (42)	100 (43)	100 (46)	100 (48)	31.5 (49)	-
		14	100 (46)	100 (45)	100 (43)	100 (42)	100 (41)	100 (40)	100 (41)	100 (41)	100 (43)	100 (45)	100 (47)	45 (48)	-
100	NLC	7	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	4 (134)	-	1 (193)	-	-	-	-	-	-	-	-	-	-
	NLT	7	-	0.5 (204)	0.5 (186)	-	-	-	-	-	-	-	-	-	-
		14	17 (217)	3.5 (185)	1 (170)	3.5 (182)	3 (143)	2 (117)	0.5 (144)	2 (122)	1 (116)	-	-	-	-
	NLW	7	1 (298)	1.5 (238)	1.5 (230)	1.5 (141)	1 (147)	2.5 (169)	1 (108)	0.5 (128)	-	-	-	-	-
		14	37 (186)	19.5 (196)	17.5 (188)	23.5 (151)	17.5 (151)	18.5 (130)	13.5 (119)	9 (112)	3.5 (104)	0.5 (108)	-	-	-
1000	NLC	7	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	-	-	-	-
	NLT	7	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	-	-	-	-
	NLW	7	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	-	-	-	-

When the initial *T. urticae* numbers rose from ten to 100 the time until escape dropped significantly, with the decrease consistent between the greenhouses (average of 3-5 weeks for months 1-9; F = 4.6, df = 36, P = <0.001; Tables 6.12-6.14). However, when the pest numbers rose from 100 to 1000 the decrease in time until escape was affected by greenhouse climate management. In the warm and traditional

greenhouses the decrease was approximately 4-6 weeks while in the cool greenhouse it was 7.8 weeks for months 1-9.

6. 3. 2. 2. 5 Seasonal effects (*T. urticae* arrival month)

As in Spain the main impact of *T urticae* arrival month on *T. urticae* escape likelihood was a rapid drop in its occurrence toward the end of the year, as time for this outcome to be achieved ran out. Otherwise escape likelihood barely differed over time regardless of greenhouse climate management (<3% difference across months 1-6). However, in contrast to Spain the date of *T. urticae* arrival had a weaker influence on the time until control in the Netherlands, due to the more stable climate conditions, although significant differences were evident (F = 16.4, df = 21, P = <0.001; Tables 6.12-6.14). In all the Dutch greenhouses time until escape was longer in the winter than the summer but the degree of the increase varied with climate management. In the warm greenhouse time until escape was 2.7 weeks (or 16%) longer in month 1 than month 7, while in the traditional and cool greenhouses the same comparison found an increases of 5.8 weeks (or 41%) and 7.3 weeks (or 54%).

6. 4 Discussion

The spider mite model shows that greenhouse climate has an important influence on *T. urticae* pressures as well as on the control efficacy of *P. persimilis*. However, in both Spain and the Netherlands the overriding factor determining whether control is achieved or whether the pest population becomes unmanageable (one million individuals) was the number of *P. persimilis* applied to the system. In both countries control was largely guaranteed when 1000 predators were regularly applied to the system, regardless of climate management. Reducing this number to 100 mainly resulted in coexistence of the *T. urticae* and *P. persimilis* populations. With ten *P. persimilis* the *T. urticae* population consistently reached escape levels.

This shows that a critical density of the predators is needed to provide control. It is probable that the highest introduction rate provides the numerical advantages to exert control as well as an increased likelihood of spatial coincidence. The latter is likely to be especially important because at high *P. persimilis* introduction rates the time in which isolated *T. urticae* populations can increase in the absence of predators is reduced, meaning that the populations are rarely able to reach an unmanageable size. This could be tested by altering the mobility of the pest and predator in the model. Skirvin *et al.* (2002) showed that preventing the movement of *P. persimilis* between plants in their model resulted in reduced frequency of control (especially at low introduction rates) and increased time until control. While, *P. persimilis* introduction rate was the foremost determining factor, this model also showed that all the other design factors had an influence on the outcome and especially the time taken to reach it, demonstrating that potential for optimising the control of *T. urticae*. For instance, while control was all but certain with weekly introductions of 1000 *P. persimilis*, reducing this to fortnightly introductions was detrimental.

6. 4. 1 Spanish greenhouses

The probability of control or escape occurring in the passively ventilated or closed-system greenhouses in Spain was similar. However, the conditions in the passively ventilated greenhouse were more conducive to rapid *T. urticae* control if 1000 *P. persimilis* were regularly applied, but equally were also more conducive to rapid *T.*

urticae escape if applications of ten *P. persimilis* were used instead. This is likely to be due to the higher temperatures from spring to autumn in the passively ventilated greenhouse promoting *P. persimilis* predation rate and leading to better control when the predators have the numerical advantage, but when the predator numbers provide inadequate control these temperatures also promote *T. urticae* development and oviposition.

In both greenhouses control efficacy was slightly reduced when using fortnightly instead of weekly applications of *P. persimilis* with the effect stronger in the closed-system greenhouse. This was especially clear when considering the time until control, where fortnightly applications could result in more than a four-fold increase in control time in the closed-system greenhouse (compared to a maximum increase of a 127% in the passively-ventilated greenhouse). The time until control in the closed-system greenhouse was also more sensitive to increases in initial *T. urticae* population, especially when this increased from ten to 100 *T. urticae*. On the other hand, the time until escape was more sensitive to increases in the initial pest population size in the passively ventilated greenhouse.

Strong seasonal differences were also evident in Spain, and especially in the passively ventilated greenhouse where conditions are more similar to those outside the greenhouse. The higher summer temperatures meant that control occurred approximately 30% faster than in the winter/spring in both greenhouses, but with passive ventilation this could be as much as 184% faster (compared to a maximum increase of 28% in the closed-system). Similar trends were seen in the time until escape, which in the summer generally occurred three times faster than in the winter. However, these seasonal differences were far greater at the highest initial *T. urticae* population size, where the population could increase from 1000 to one million in approximately five to six weeks in both greenhouses in the summer. This represents a five-fold decrease in time on the same population increase in the winter in the closed-system greenhouse and a four-fold decrease in the passively ventilated greenhouse.

Overall, the model suggests that the optimal *T. urticae* control can be achieved in Spain with weekly applications of 1000 *P. persimilis* and this all but guarantees control regardless of the scale of the initial pest problem. However, more rapid

control can be achieved in passively ventilated greenhouses. Control in passively ventilated greenhouses is also less sensitive to alterations in the control strategy (e.g., reduced predator introduction frequencies) and increases in the initial pest population than in the closed-system greenhouse. The model also highlights the significance of taking account of seasonal differences in control efficacy. These were especially large in the passively ventilated greenhouse; control being achieved in less than two weeks in the summer but as long as ten weeks in the winter. Control could take even longer in the closed-system greenhouse (>ten weeks in the spring), meaning that if tolerance of crop damage is low, the grower may have to consider knock-down pesticides at times of the year when control is slowest.

6. 4. 2 Dutch greenhouses

Conditions were less favourable to *T. urticae* control in the Dutch greenhouses than the Spanish greenhouses, with control only achieved with applications of 1000 *P. persimilis*. The frequency of control was relatively even between the three Dutch climate scenarios, although control was considerably faster in the warm greenhouse (up to twice as fast as in the traditional greenhouse). The likelihood of escape, however, was influenced by climate control with this outcome considerably less likely in the cool greenhouse than the other greenhouses. Furthermore, just as the warm greenhouse was more conducive to rapid control of the pest (with applications of 1000 *P. persimilis*), it also favoured the rapid development of *T. urticae* populations when predator number were inadequate (ten *P. persimilis*). For instance, on average escape occurred 38% faster (15.8 weeks) than in the cool greenhouse (9.9 weeks).

The greenhouse climate also affected the impact of using fortnightly instead of weekly predator applications. In the warm and traditional greenhouses this resulted in large reductions in control likelihood but had little effect in the cool greenhouse. Applying *P. persimilis* fortnightly also substantially increased the time until control, ranging from almost three times longer in the warm greenhouse to nearly 1.5 times longer in the cool greenhouse. There was also a seasonal influence to the difference in control time when using fortnightly instead of weekly applications, with control time disproportionately longer in the winter than the summer, especially in the

traditional and warm greenhouses. In fact, in the warm greenhouse the increase in control time resulting from switching to fortnightly applications was over twice as severe in the winter as in the summer. With sub-optimal *P. persimilis* introduction rates (ten) fortnightly applications also increased the probability and speed of *T. urticae* escape occurring, particularly in the cool greenhouse.

The different greenhouse climates were also differentially affected by the size of the initial *T. urticae* population. Control was strongly affected by an increase from ten to 100 *T. urticae* with clear reductions in the likelihood of control occurring, particularly in the traditional greenhouse, and large increases in the time until control, especially in cool (increases of up to 88%) and traditional greenhouses (up to 53%). Further increases in the initial pest population had little effect on either the outcome or the time until control. In terms of the *T. urticae* population reaching escape levels, increasing the initial population size had little effect on the outcome, except for in the cool greenhouse where the escape was almost twice as likely with 1000 *T. urticae* as with ten *T. urticae.* The time until escape occurred also reduced as the initial *T. urticae* population increased, with the proportional reductions in time relatively similar between the climate scenarios. Escape occurred 20-30% faster with 100 than ten *T. urticae*, and 35-45% faster with 1000 than 100 *T. urticae*.

Seasonal differences in control and escape were not as strong as in Spain due to the greater year-round climate stability in the Dutch greenhouses. However, control generally took longer to occur in the winter than the summer and this was especially noticeable in the warm greenhouse where it could take twice as long. The reverse of this trend was evident for the time until escape occurring, with a more rapid population growth in the summer than the winter. This time the differences were greater in the traditional greenhouse (where escape occurred up to 41% slower in the winter) and cool greenhouses (escape occurring >50% slower in the winter).

Overall, the model suggests that the optimal strategy for *T. urticae* control in the Netherlands is to apply 1000 *P. persimilis* at weekly intervals. If there is a low tolerance for crop damage or pest presence, as is often the case in ornamental crops (Sadof and Alexander, 1993; Alatawi *et al.*, 2007; Marsh and Gallardo, 2009), then the recommendation is also for warm climate control as control is achieved more quickly in these conditions. However, if there is a higher tolerance for crop damage

and pest presence a grower may benefit from a cool greenhouse climate, especially if the overriding objective is to avoid the risk of pest populations reaching escape levels, as escape is less likely and takes longer to occur. In the cool greenhouse the grower also has greater flexibility in the *P. persimilis* control strategy used. For instance control is less affected by the use of fortnightly predator applications.

6. 4. 3 Model criticism and further work

As the literature on *T. urticae* and *P. persimilis* grows there is a temptation to continually add levels of complexity to the modelling of their population dynamics and their interactions with each other and their environment. However, Caswell (2001) recommends that this temptation should be avoided; models ought to be simple enough to provide insights into the important processes driving the system modelled and only as complex as is needed to realistically simulate the dynamics for applied purposes. With this in mind, outlined here are selected suggestions for improving the model, some of which may either add complexity or remove it.

Firstly, the accuracy of the model needs to be tested. To compare this model to the real dynamics in a comparable system is difficult (growers are reluctant to allow pest populations to remain and greenhouse managers at research facilities are particularly wary of *T. urticae* due to their tendency to persist), although Caswell (2001) suggests that testing components of the model can be more effective. In this case the observations from small-scale experiments could be compared to model predictions, e.g., assessing the time until control (or otherwise) on a single plant in fluctuating environmental conditions (CE cabinet or greenhouse) with a defined number of *T. urticae* and *P. persimilis*. However, the widespread use of *P. persimilis* in commercial production presents the opportunity to assess the influence of alterations to predator introduction rate and frequency in real situations. Unfortunately time constraints meant that it was not possible to validate this model. With more time, sensitivity analysis of the model results would also be carried out, allowing the identification of the demographic processes driving the model as well as those that can be eliminated without losing descriptive power.

A number of adjustments could be made to the model that would be simple and provide interesting additional data. Firstly, the end date of the model could be altered to allow a full year to be simulated for each *T. urticae* introduction date. The current model ended after 365 days regardless of when the *T. urticae* arrived, meaning that late arrival dates predominantly ended in coexistence. This simple change would give a clearer picture of the system dynamics, especially over the winter. Lowering the number of *T. urticae* that defines escape would also be of interest as it may provide better information on the dynamics when 100 *P. persimilis* are regularly applied (coexistence consistently occurred at this introduction rate). The vital role of *P. persimilis* introduction rate in determining the outcome of the model indicates that investigating further rates (especially between 100 and 1000) would be of interest. It would also be useful to investigate the effect of not introducing *P. persimilis* to gain a clearer idea of the influence of greenhouse climate control on the population dynamics of *T. urticae* alone. It has been suggested that weekly introductions of *P. persimilis* are not economically viable (Skirvin and Fenlon, 2003b), however this may not be the case with more high value ornamentals but nevertheless a wider range of introduction frequencies could be investigated. The effect on the control dynamics of repeated introductions of *T. urticae* (a more realistic situation) into the system could also be assessed.

As the inclusion of greenhouse temperature and humidity is one of the novel aspects of the model, the lack of data on the influence of temperature-induced mortality in *P. persimilis* is a glaring omission and further work investigating this would be beneficial. Experiments on the influence of humidity on egg mortality in both species would also clarify the detrimental but variable effects a number of studies have found (Harrison and Smith, 1961; Ferro and Chapman, 1979; Perring and Lackey, 1989; De Courcy Williams *et al.*, 2004b; Ferrero *et al.*, 2010). The use of hourly rather than daily time steps, as used in other *T. urticae* models (Bernstein, 1985; Nachman, 1987b; Nachman, 1987a), would allow the implications of the diurnal changes in greenhouse climate to be assessed. This could be particularly significant in modelling the population dynamics in the passively ventilated Spanish greenhouse, where the considerable diurnal variation in conditions were somewhat masked by the use of daily means. For example, humidity frequently oscillated between >90% RH to <60% RH across the day with this climate management

(Section 4. 3. 1), meaning that conditions for *P. persimilis* control efficacy (due to lower predation rates at humidity extremes; Chapter 3) are likely to be less optimal than predicted in this model.

Finally, the importance of predator dispersal is recognised as vital to biological control success (Skirvin *et al.*, 2002) and in this regard the simulation of mite movement in the model could be improved. *P. persimilis* is capable of moving across three plants in 24 hours (Zemek and Nachman, 1998; Skirvin and Fenlon, 2003a), suggesting that limiting movement to one plant per day in the model underestimated the dispersal of this highly mobile predator. This could be adjusted and include an increase in mortality probability with dispersal distance (Nachman, 2001) as well as a delay in other activities until arrival (Neubert *et al.*, 2002). To simulate the greenhouse layout more realistically, increased plant connectedness (Skirvin and Fenlon, 2003a; Skirvin, 2004) and barriers such as aisles between rows could be included with an associated increase in mortality when movement across the ground occurs (Skirvin and Fenlon, 2003a). Temperature and relative humidity have been shown to affect movement in both *T. urticae* and *P. persimilis*, with low humidity causing increased activity and emigration in both species (Hussey and Parr, 1963a; Mori and Chant, 1966a) and temperatures below 20°C reducing movement in *T. urticae* (Penman and Chapman, 1980) and inter-plant movement in *P. persimilis* (Skirvin and Fenlon, 2003a).

Further factors that have been found to influence mite movement are the avoidance behaviour observed in both *T. urticae* (Pallini *et al.*, 1999) and *P. persimilis* (Janssen *et al.*, 1997) to plants containing *P. persimilis*, the increased emigration of *T. urticae* from plants containing *P. persimilis* (Bernstein, 1984) and the use of chemical cues in the searching behaviour of *P. persimilis* (Zhang and Sanderson, 1992), with Nachman (2006) commenting that the importance of this factor could not be dismissed.

6. 4. 4 Conclusions

The mite model shows that the most important factor in achieving control of *T. urticae* is the number of *P. persimilis* regularly applied to the system. While other

design factors had a limited influence on the outcome of the model, their impact was more clearly seen in their influence on the time until either *T. urticae* control or escape (an important factor in greenhouse production). The climate management most favourable to the control of *T. urticae* is passive ventilation in Spain and warm climate management in the Netherlands. In both countries *T. urticae* control is optimal when their populations are treated at an early stage with weekly applications of 1000 *P. persimilis.*

The role of the initial *T. urticae* population in determining the time until an outcome also highlights the importance of early pest detection. The development of remote sensing technology provides some intriguing possibilities in this regard, with both the detection of mite feeding through spectral imaging (Fraulo *et al.*, 2009; Luedeling *et al.*, 2009; Herrmann *et al.*, 2011) and feeding-induced plant volatiles with electronic noses (Laothawornkitkul *et al.*, 2008) promising. If such technologies prove effective in commercial conditions their integration with simplified and effective pest models has great potential for improving IPM (Skirvin, 2011). While the ability of this model to predict pest pressures in the greenhouses simulated is yet to be evaluated, it does provide a tool to project the consequences of the different greenhouse climates (Caswell, 2001). Furthermore, this approach allowed control strategies to be investigated and optimised in the different greenhouse scenarios.

7. Conclusions

The protected horticulture industry in Europe faces a number of challenges, both environmental and economic, which are driving the development of novel greenhouse climate management systems. These challenges include the need to reduce the use of carbon fossil fuels (both to minimise costs and CO_2 emissions; Bakker *et al.*, 2008; Bakker, 2009), the reliance on groundwater sources (Zaragoza *et al.*, 2007; Chapagain and Orr, 2009) and the application of plant protection chemicals (e.g., to minimise residues and environmental impacts; Leistra and Boesten, 1989; Hamilton *et al.*, 2004).

The intention of this thesis was to evaluate the impacts novel greenhouse climate control would have on pest and disease pressures and the efficacy of biological control agents. Two representative pest and disease systems were chosen: *Oidium neolycopersici* causing tomato powdery mildew on tomato and its biological control, *Bacillus subtilis*; and the spider mite, *Tetranychus urticae*, and its biological control agent, *Phytoseiulus persimilis* on ornamentals. Simulation models were constructed to assess the response of these systems to greenhouse climate.

Three specific aims for the thesis were set down in the Introduction (Section 1. 5):

1. To identify and address knowledge gaps regarding the response of *O. neolycopersici*, *B. subtilis*, *T. urticae* and *P. persimilis* to temperature and humidity.
2. To predict the impact of novel and traditional greenhouse climate control scenarios on pest (*T. urticae*) and disease (*O. neolycopersici*) pressures in the Netherlands and Spain using simulation models.
3. To predict the impact of novel and traditional greenhouse climate control scenarios on biological control efficacy (*P. persimilis* and *B. subtilis*) in the Netherlands and Spain using simulation models.

To provide data for the parameterisation of the disease and biological control simulation model, the response of *O. neolycopersici* and its control with *B. subtilis* to temperature and humidity was investigated in Chapter 2. No data was available

regarding the effect of temperature and humidity on the control efficacy of *B. subtilis* against *O. neolycopersici* and, although some work had investigated the effects of temperature and humidity on the infection processes of *O. neolycopersici*, little data was available for the development of the disease following infection. Experimental work was conducted to provide the data needed to parameterise the *O. neolycopersici* simulation model (Chapter 5) and an image analysis technique developed to assess diseased leaf area. The conclusions of this work were that the latent period, disease development and sporulation of tomato powdery mildew caused by *O. neolycopersici* were strongly affected by temperature, and that the control efficacy of *B. subtilis* was strongly affected by temperature and humidity, with high humidities and temperatures ≥20°C optimal.

While several studies had investigated the response of *T. urticae* and *P. persimilis* demographic parameters to temperature and humidity, the influence of humidity on the *P. persimilis* predation rate was unknown. Experiments were therefore carried out to allow this important process to be quantified and included in the *T. urticae* and *P. persimilis* population dynamics model (Chapter 6). The effect of humidity on the functional response of *P. persimilis* feeding on *T. urticae* eggs was investigated using *Choisya ternata* and tomato leaves as a host (Chapter 3). Predation rate was examined using beta-binomial mixture models, showing that egg consumption was greatest at 85% RH with significant reductions above and below this humidity. Low humidity was particularly detrimental to *P. persimilis* control efficacy with the largest reductions in predation below 76% RH.

Temperature and humidity data were needed in order to use the pest and disease models to evaluate the effects of the different novel greenhouse climate control systems. One way to do this is to use data from real greenhouses but in the absence of multiple data sets and to circumvent the time-consuming process of gathering such data (particularly where novel, prototype greenhouses are being assessed) a greenhouse climate model was constructed (Chapter 4). This model generated multiple stochastically different sets of one year hourly temperature and humidity data for Spanish passively ventilated and closed-system plastic clad greenhouses and Dutch Venlo greenhouses. This process allowed the climate stability characteristics inherent to each greenhouse system to be described and permitted modifications to greenhouse climate control to be made, such as for the Dutch greenhouse where the

cool and warm greenhouse scenarios were created by adjusting temperature and humidity.

Chapters 5 and 6 described the development of the pest, disease and biological control models and their application to assessing the impacts of passive ventilation and closed-system climate management in Spain and traditional, cool and warm climate management in the Netherlands. Using the *O. neolycopersici* and *B. subtilis* models, it was predicted that the novel closed-system climate management in Spain would likely result in increased disease pressure but improved biological control efficacy. Of greater influence in Spain was the variation in seasonal conditions, with disease pressure predicted to be greatest in the autumn, while the efficacy of *B. subtilis* was predicted to be greatest in the summer. In the Netherlands, both the warm and cool greenhouse climate scenarios were predicted to result in reduced disease pressure compared to the traditional system. *B. subtilis* was predicted to produce significant reductions in disease pressure throughout the year in all greenhouse systems (both Spain and the Netherlands) but seasonal differences in efficacy were dependent on the climate control system employed. Optimal management strategies for reducing the impact of powdery mildew were therefore identified using the model. In Spain, the optimal strategy was the combination of passively ventilated climate management and the application of *B. subtilis* year-round, although reduced efficacy was predicted to be likely in winter. In the Netherlands, the models suggested that the greatest reduction in disease pressure could be achieved by employing warm greenhouse management from spring to early autumn and cool greenhouse management for the remainder of the year in combination with *B. subtilis* applications.

The *T. urticae* and *P. persimilis* model presented in Chapter 6 simulated the demographic processes and interactions important to the population dynamics of these mites. In addition to the influence of climate management, a number of *P. persimilis* application strategies and initial *T. urticae* population sizes were investigated. The model predicted that the overriding factor determining control success was the numbers of *P. persimilis* regularly applied to the system. Other factors, such as climate management and initial pest population size, primarily influenced the speed of control. The model predicted the optimal control strategy to be the weekly application of ten *P. persimilis* per plant with passive ventilation

management in Spain and warm climate management in the Netherlands. The model also highlighted the importance of detecting *T. urticae* populations early, with the likelihood of population escape and time until control increasing with increasing initial population size.

While pest and disease control make up a relatively small proportion of expenditure for growers (Gullino *et al.*, 1999), crop protection remains an important priority for the industry and one that is becoming increasingly difficult. The continuing process of regulating pesticide use in the EU (e.g., Plant Protection Products Regulation 1107/2009 and the Sustainable Use Directive 2009/128/EC) has resulted in the removal from the market of effective pesticides and the addition of barriers to the development of new active agents (Williams, 2011; Hillocks, 2012). With fewer pesticides available, the risk of resistance developing to the available pesticides has increased (Hillocks, 2012). Furthermore, according to the EU's 2010 'Thematic Strategy on the Sustainable Use of Pesticides' growers must adhere to IPM principles in crop protection by 2014 (Hillocks, 2012). These challenges emphasise the need to develop novel biological control agents and optimise the use of those currently available.

The pressures facing the protected horticulture industry to reduce energy and water use also present an opportunity to integrate considerations for crop protection with the move toward low energy, water conserving systems. This work illustrates the benefits of maintaining the greenhouse environment at conditions that are both sub-optimal to the pest or disease and optimal to the biological control agent. While it was predicted that the novel greenhouse climate in Spain would be likely to increase pest and disease pressures and reduce biological control efficacy, the models also suggested that other factors, such as the biological control strategy and seasonal variation in greenhouse conditions, could be more important. In fact, the differences in pest and disease pressures between the passively ventilated and closed-system management were marginal, so that if some of the model improvements (Sections 5. 4. 3 and 6. 4. 3) were made (e.g., the inclusion of hourly time steps in the pest model and spore dispersal in the disease model) a closed-system may be predicted to be more beneficial to pest and disease control

The challenges for crop protection are even greater in the floriculture industry due to low damage tolerance (Sadof and Alexander, 1993; Alatawi *et al.*, 2007; Marsh and Gallardo, 2009), meaning that adoption of IPM has been slow. For example, IPM is employed in only 15% of greenhouse rose production in the Netherlands (Pijnakker *et al.*, 2007). This work shows that *P. persimilis* can provide effective control of *T. urticae* in the greenhouse conditions traditionally associated with such production, although the modelling work did not simulate the crop damage. Nevertheless, in the past *P. persimilis* has been found to provide unsatisfactory control of *T. urticae* in rose production (Parrella *et al.*, 1999). However, the use of biological control to manage a pest or disease often occurs in tandem with other control options. For instance, effective *T. urticae* control has been achieved with both modifications to the rose production system ('the bent shoot method'; Casey & Parrella, 2002; Casey *et al*., 2007), which provides a more suitable microclimate for *P. persimilis*, and the application of *Neoseiulus californicus*, a generalist predator that can exert long-lasting, low-level control, in conjunction with the application of *P. persimilis* to pest 'hot spots' (Blumel *et al.*, 2002).

Greenhouse IPM programs involving the use of biological control agents for the management of pests and diseases can be disrupted by the use of broad-spectrum pesticides (Blümel *et al.*, 1999). In extreme cases this has lead to biological control being abandoned entirely, an example being the psyllid, *Bactericera cockerelli*, on tomato in California. The importance of this pest meant that, in the absence of effective alternatives, a broad spectrum pesticide was necessary, leading to the failure of other biological control agents and subsequently the need for chemical controls for all pests and diseases challenges (Parrella, 2008). In tomato production, control of *O. neolycopersici* currently relies on fungicides and resistant cultivars (Jones *et al.*, 2001), and while a number of potential biological and physical control agents have been investigated (Section 1. 2. 3) their efficacy has been variable. The reliance on fungicides for control of tomato powdery mildew presents a barrier to the adoption of biological control (Bardin *et al.*, 2008). Therefore, the identification of *B. subtilis* as an effective biological control for *O. neolycopersici* is promising, especially in production systems where conditions are optimal to its control efficacy, e.g., high humidity environments such as in the Netherlands and Spanish closed-system greenhouses. While *B. subtilis* may not always provide the level of control

needed, satisfactory control may be achievable when used in combination with cultural control such as resistant cultivars (Pavan *et al.*, 2008) and the addition of silicates to hydroponic systems (Garibaldi *et al.*, 2011), other biological control agents such as Milsana® (an extract of the giant knotweed *Reynoutria sachalinensis*; Bardin *et al.*, 2008), and climate control.

There is of course a trade-off in greenhouse climate management, in that conditions that minimise pest and disease pressures and maximise biological control efficacy may not be conducive to crop growth or yield maximisation. For instance, the development of *O. neolycopersici* has been shown to be minimal above 28°C (Chapter 2; Jacob *et al.*, 2008) and this knowledge has been used to reduce tomato powdery mildew disease pressure in commercial-like greenhouses in Israel (Elad *et al.*, 2009). However, temperatures above 26°C are detrimental to tomato yields (Van der Ploeg and Heuvelink, 2005) with the result that, in Spain, tomato crops are often not grown in the summer (Cantliffe and Vansickle, 2003), in which case closed-system climate management offers the conditions most conducive to *O. neolycopersici* control for the remainder of the year. In the Netherlands, where optimal conditions for tomato are considered to be 19-20°C (Van der Ploeg and Heuvelink, 2005), the recommended climate control for *O. neolycopersici* management (cool in late autumn and winter and warm for the rest of the year) would likely be unsuitable in the summer. Equally for rose production, stem quality is optimal at 18°C with crop growth increasing and flowering time decreasing with increasing temperature, although temperatures above 24°C can be detrimental (De Vries *et al.*, 1982; Baille *et al.*, 1994; Shin *et al.*, 2001). Although rose yields are greatest during spring in Spain, rose production occurs year round (Colomer *et al.*, 2006), in which case the recommended climate control would still be passive ventilation due to the faster rate of *T. urticae* control. In the Netherlands, where the overriding climate control objective is to maintain optimal growth conditions (Wittwer and Castilla, 1995), the recommendation of warm climate control for *T. urticae* management would likely not be acceptable for rose production. In this case, cool climate control would likely provide an acceptable trade-off in crop growth and pest control.

A final consideration for the model recommendations would be whether the climate management suggested for biological control of *O. neolycopersici* is compatible with

that for *T. urticae*. As *O. neolycopersici* does not occur on rose, such a consideration is only needed for tomato production, where *T. urticae* is also a pest (van Lenteren, 2000). In Spain, passive ventilation management was recommended for both the pest and disease. However in the Netherlands, the suggestion of cool climate control for the management of *O. neolycopersici* in the autumn and winter would result in *T. urticae* control taking longer to occur, although this difference would be relatively minor. Overall the recommendations appear to be broadly compatible were *B. subtilis* and *P. persimilis* used as part of the same IPM program.

As the most important factor determining whether growers adopt a new pest or disease control strategy is usually its cost effectiveness, consideration of the economic impacts of the recommendations in this thesis are necessary if they are to be implemented. Generally, the pest and disease control programs employed are those that reliably reduce crop damage to an acceptable level for the lowest expenditure (whilst adhering to regulatory restrictions). To assess whether the biological and climate control strategies proposed in this work are economically viable, the economic injury level (EIC) needs to be determined for each crop, taking into account the grower's target market, e.g. domestic or export, fresh or tinned. Establishing the EIC allows different control strategies to be compared in terms of the costs needed to prevent pest or disease damage causing financial loss through reduced yields or product quality. Additionally, the implications of altering the greenhouse climate for crop growth also need to be considered to ensure uneconomic reductions in yield or product quality are not experienced. The models themselves could be adapted and integrated with pest or disease early detection systems to provide growers with information regarding potential population growth, allowing timely interventions to be made with appropriate controls thus ensuring economic losses do not occur.

The potential consequences of novel climate regimes for pest and disease pressures and biological control have been demonstrated by the models in this thesis and without the need for expensive, large-scale and time-consuming experiments. However, the models are not intended to provide a definitive account of the pest and disease dynamics, rather an indication of the likely impacts. Model validation would obviously improve the reliability of the predictions. As applied models, the predictions for novel climate regimes could be better evaluated in terms of their

economic impacts if crop damage and yield loss were also quantified and related to economic injury levels, such as in Schumacher *et al.'s* (2006) model of *T. urticae* control on ornamentals. The models provide a framework that could be modified for other pest and disease systems. For example, currently the predominant pest in Dutch rose cultivation is the whitefly, *Trialeurodes vaporariorum* (N. G. Victoria, Pers. comm.); for which, along with its primary biological control agent, *Encarsia formosa*, an abundance of literature is available (see Parrella *et al.*, 1999). Furthermore, widening the range of pests and diseases investigated presents an opportunity to consider the implications of novel greenhouse systems for entire protected crop management strategies. As a number of biological control agents are often available for a specific pest (e.g., Gerling *et al.*, 2001) such an approach may also assist in identifying the agents most suitable to the prevailing conditions. The increasing use of insect pollinators in greenhouse production (Cauich *et al.*, 2006; Velthuis and van Doorn, 2006; Palma *et al.*, 2008) means it would also be relevant to consider the impacts novel greenhouse systems would have on these. A number of commonly used pollinators have been shown to be sensitive to temperature and humidity (Morandin *et al.*, 2001; Katsuhiko, 2002; Amano, 2004) and the models could be adapted to assess their activity in response to these factors.

The adoption of IPM practices in Europe should not simply be a matter of compelling growers through legislation. For instance, employing IPM can provide growers with access to high volume markets, e.g., supermarkets (Hillocks, 2012), and premium markets, e.g., 'eco-labels' (Vannoppen *et al.*, 2001; Stanghellini *et al.*, 2003). In spite of these incentives, growers have made clear the need for IPM strategies to be effective if they are to be adopted (Bailey *et al.*, 2009), which in light of increasing food security issues, such as human population growth and climate change (Royal Society, 2009), is essential if they are to provide an effective alternative to pesticides. To do this, current and potential pest and disease problems need to be identified and IPM solutions researched, in parallel with grower training (Heinrichs *et al.*, 2009). This latter point is important; growers have emphasised their requirement for information on how to integrate various IPM technologies effectively and the need for a systems approach to IPM (Hillocks, 2012). As part of the research effort toward developing sustainable production systems, simulation

models can provide a tool for assessing the combinations of IPM strategies, allowing problems to be anticipated and solutions identified.

Appendix 1

Table A1.1: Parameters values for the unused models fitted to the functional response of *P. persimilis* on *T. urticae* eggs on *C. ternata*.

Model	RH	Lambda	Intercept (*b*)	Slope (*a*)
1	55	0.2444	-0.3165	1.571
	65	0.1786	-0.1667	1.0841
	75	0.1837	-0.2135	1.3447
	85	0.2406	-0.024	0.7904
	100	0.1986	-0.0566	0.7408
2	55	0.2074	-0.2966	1.5277
	65	0.2074	-0.1677	1.0661
	75	0.2074	-0.2194	1.3529
	85	0.2074	-0.0282	0.7321
	100	0.2074	-0.1221	0.7424
4	All	0.2119	-0.1648	1.1259

Table A1.2: Parameters values for the most complex model (Model 1) fitted to the functional response of *P. persimilis* on *T. urticae* eggs on tomato.

RH	Lambda	Intercept (*b*)	Slope (*a*)
55	0.1879	-0.1881	1.1975
65	0.1624	-0.1536	1.0843
75	0.1671	-0.1289	0.944
85	0.1735	-0.2181	1.3438
100	0.1658	-0.113	0.8054

Appendix 2

Table A2.1: Parameters values of logistic curves initially fitted to *O. neolycopersici* disease development data at different temperature and humidity conditions. Treatment (Trt.) indicates whether experiments involved *O. neolycopersici* only (ON) or with *B. subtilis* (BS).

Temp. (°C)	Humidity (% RH)	Trt.	*B*	*M*	*C*
10	70	ON	0.2707	30.5	88.98
10	70	BS	0.1844	37.33	103.3
15	70	ON	0.248	15.28	95
15	70	BS	0.553	9.48	27.5
15	90	ON	0.8357	8.803	8
15	90	BS	0.949	8.43	8.3
20	50	ON	0.737	7.279	56.08
20	70	ON	0.542	7.18	51.2
20	70	BS	0.608	8.189	57.8
20	80	ON	1.187	7.813	28.38
20	80	BS	0.556	10.32	24.6
20	90	ON	0.528	9.346	78.8
20	90	BS	0.632	11.021	43.04
25	50	ON	0.647	5.912	61.25
25	70	ON	0.728	4.809	46.89
25	70	BS	1.41	5.928	17.07
25	80	ON	0.7756	5.511	30.48
25	80	BS	0.416	8.51	21.4
25	90	ON	0.749	7.101	45.82
25	90	BS	0.328	14.3	27
27	50	ON	1.177	5.204	7.84
27	50	BS	0.02978	138.9	808.5
29	70	ON	-3.4	7.4	-0.547
29	70	BS	3	10.82	0.373

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