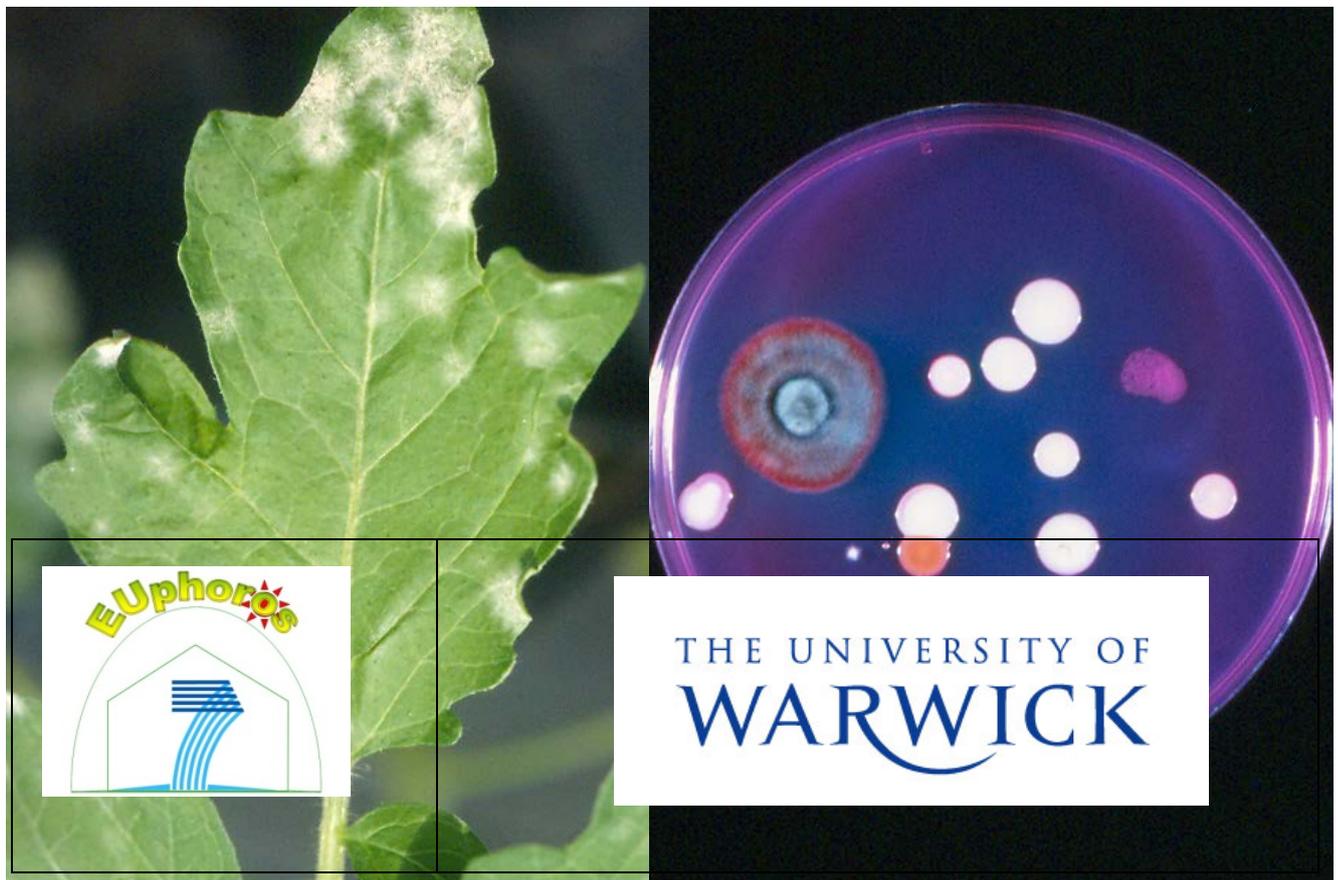


# Title: Guidelines for the Use of Biological Control Agents vs. Chemical Control for Specific Pests and Diseases in Novel Greenhouse Structures

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# Guidelines for the Use of Biological Control Agents vs. Chemical Control for Specific Pests and Diseases in Novel Greenhouse Structures

## 1. Introduction

This report presents recommendations on pest and disease control strategies in novel greenhouse structures. The implementation of innovative technology and climate management systems in novel greenhouses is likely to result in changes to the greenhouse growing environment, with changes in how and when ventilation is used particularly influential. These potential changes in greenhouse environment could affect pest and disease pressures as well as control strategies. With the move towards using biological control agents in place of plant protective chemicals all changes in greenhouse environment must take into account not only the productivity of the crop, but also the competitiveness of all three trophic levels, plant, pest/disease and control agent. Within The EUphoros project we have focussed upon two model systems in order to model how control might vary with changes in commercial protected crops:

- 1) The biological control of *Tetranychus urticae* (two-spotted spider mite) on rose by the predatory mite, *Phytoseiulus persimilis* as a representative pest system.
- 2) The growth of *Oidium neolycopersici* (tomato powdery mildew) on tomatoes a model disease, with biological control by *Bacillus subtilis*.

This report will assess the impact of changes to climatic conditions anticipated in energy efficient greenhouses, on the control options for the model pest and disease, with a primary focus on the impacts on control by the two primary biological control agents.

## 2. *Tetranychus urticae*

*Tetranychus urticae* is a generalist herbivore and a major pest of many agricultural, horticultural and ornamental crops. It has a high reproductive rate and short development time allowing it quickly to reach large populations within a crop if uncontrolled. It feeds on the cell contents of the surface tissue (usually leaves) resulting in extensive areas of pale mottling, which can greatly reduce the photosynthetic capability of the host plant. This in turn decreases nutrient production and hinders the formation of fruit and storage bodies, resulting in reduced crop yields.

Crop infestations also affect yield returns through reduction in produce quality. *T. urticae* produce webbing to protect themselves and their offspring and also to assist in dispersal. Both this webbing and the mottling damage caused by the feeding are unsightly and reduce the quality and the value of the produce. This is especially costly in ornamental crops.

### 2.1 Chemical Control

Acaricides were once widely used against *T. urticae*. However the species has demonstrated a propensity to develop resistance to chemical treatments rapidly unless the spray program is carefully managed. This has meant that *T. urticae* is now resistant to many previously effective chemicals. Additionally, many acaricidal chemicals have been de-registered in the EU, further reducing the number available to growers. The compatibility of many acaricides with integrated pest management (IPM) programs is also important and the widespread use of biological control agents in EU protected horticulture means that chemical sprays must be selected with great care. Such concerns are compounded by regulatory and consumer pressures to reduce crop residues. In consequence, only a limited number of chemicals are available which are both effective and IPM-compatible. As such growers tend to use them sparingly and in a targeted manner or when *T. urticae* infestations are severe.

In the UK abamectin (Dynamec®), etoxazole (Borneo®) and bifenthrin (Talstar®) are all commonly used treatments, although the latter is not considered IPM compatible. Abamectin is considered the most effective and is the first resort when chemical control is needed. When abamectin is unsatisfactory then etoxazole or bifenthrin sprays are applied. However, regardless of efficacy, fear of resistant strains developing means that chemical treatments are always rotated. Even with such precautions, resistance to abamectin has recently been observed (Peter Watson, Warwick, UK, personal communication).

### 2.2 Non-chemical Spray Control

A number of non-chemical treatments are marketed for control of *T. urticae* with varying degrees of effectiveness. Their mode of action is usually by impairing respiration through blocking the tracheae. A product used in the UK is SB Plant Invigorator®. It is IPM compatible and has a wide range of targets.

### 2.3 Biological Control Agents

Due to resistance, IPM and residue issues associated with chemical controls, biological control is the predominant means of controlling *T. urticae* (van Lenteren & Woets, 1988; Griffiths, 1999). This is generally effective and affordable biological control agents are widely available to growers.

*Phytoseiulus persimilis* is a very mobile predatory phytoseiid mite that feeds on all stages of *T. urticae*, particularly the egg stage, and is very effective at reducing populations rapidly. It has been used in commercial greenhouses for several decades and is still the mostly widely used biological control agent in the UK and the Netherlands. For most rose and tomato growers it is the principal biological control agent for *T. urticae*, even though these predatory mites do not always establish well on tomato crops. Some biological control companies now supply “tomato-reared” varieties, although any improved effectiveness is questionable.

The major drawback of using *P. persimilis* is that their narrow dietary range can result in rapid population decline in the absence of prey populations. This means that their use in preventative management is limited and that control can require repeated introductions of *P. persimilis*. Alternative control agents are available to growers. These include *Amblyseius californicus*, *A. andersoni* and *Neoseiulus californicus*, all of which can feed on other mites or pollen, allowing them to persist in the crop and provide a measure of preventative treatment. Other commercially available non-mite biological control agents are *Feltiella acarisuga*, *Stethorus punctillum* and *Macrolophus caliginosus*.

#### 2.4 Environmental Factors Affecting Control Agents

The effectiveness of *P. persimilis* is reduced by low relative humidity (RH), with increased adult and egg mortality rates, decreased development rate and lower predation rate. Rising temperatures are beneficial, affecting development time, population increase, dispersal behaviour and predation rate. For example, an increase in temperature from 20°C to 30°C more than halved development time from egg to adult and increased prey consumption rate (Everson, 1980; Kazak, 2008). The highest rate of population increase was found to be 30°C (Badii & McMurtry, 1984). The optimal rate of dispersal for *P. persimilis* was found to be at 25°C with significant reductions above and below this temperature (Skirvin & Fenlon, 2003). However, effective control of *T. urticae* can be achieved between 15-27°C (Stenseth; 1979) and above 23°C eradication of this pest is achieved directly without further transient increases in *T. urticae* populations. The rate at which control was achieved is twice as fast at 27°C than at 24°C, although control is lost at low humidity (e.g. 27°C/40% RH).

Effective envelopes of temperature and humidity are also known for the other biological control agents for *T. urticae*, providing growers an opportunity to select agents to suit their growing

environments. For instance, *N. californicus* and *S. punctillum* are tolerant to lower humidity levels than *P. persimilis*. *F. acarisuga* is a gall-midge whose non-predacious adult stage is able to disperse over wide distances. Its larvae are voracious predators and very effective at treating closely aggregated *T. urticae* colonies. However, its efficacy is severely reduced if humidity levels are low as this discourages gall-midge emergence. *A. andersoni* suffers increased mortality at low humidity levels while *A. californicus* is tolerant to a wide range of temperature and humidity conditions. *M. caliginosis*, a mirid bug predator that can be used against a number of important crop pests, suffers slow development rates at sub-optimal temperatures with the additional disadvantage that it can feed on the crop itself when prey is scarce.

There is no doubt that biological control agents are essential aids to pest control in protected cropping environments and assist in the goal of minimising chemical control. Clearly, there are options but, in order to make appropriate choices, prevailing environmental conditions need to be taken into account. Assistance in making knowledge-led decisions will assist growers and agronomist. Thus, models of pest and control agent behaviours should prove valuable tools.

### 3. *Oidium neolycopersici*

*O. neolycopersici* is a major disease of tomato worldwide and it can infect other crops (Whipps *et al.*, 1998; Kiss *et al.*, 2001). It is the most important of three powdery mildew diseases of tomato, the others being (*Leveillula taurica* and *O. lycopersici*). Current control is primarily through breeding programmes for improved plant resistance (Jones *et al.*, 2001; Matsuda *et al.* 2005) and fungicides (Jones *et al.*, 2001). Strains resistant to fungicides (Matsuda *et al.*, 2005) and resistant-tolerant isolates have been found (Kashimoto *et al.*, 2003). Research has shown that infection, development and sporulation are all significantly affected by temperature and humidity.

#### 3.1 Chemical Control

In the UK bupirimate, fenarimol, sulphur and quinoxifen have been shown to be effective chemical treatments for *O. neolycopersici*. However, grower feedback has shown that Thiovit Jet<sup>®</sup>, a sulphur-based fungicide is the most commonly used. Thiovit Jet<sup>®</sup> is applied through a burner. However, it has been reported to interfere with *Encarsia Formosa*, a parasitoid wasp used almost ubiquitously to control whitefly.

#### 3.2 Non-chemical Control

Spray applications of a formulated plant extract from the giant knotweed, *Reynoutria sachalinensis* (Milsana®), have been reported to give effective control of *O. neolycopersici*, although these need weekly drench applications.

### 3.3 Biological Control Agents

No biological control agents had been described for use against *O. neolycopersici* prior to this project. However, *Bacillus subtilis* (QST 713) (Serenade® ASO) has been shown to be effective as a biofungicide spray against a number of other foliar plant pathogens. In the US it has been registered for use against *L. taurica*, one of the other powdery mildews of tomato, and in the UK it has been registered for use against *Botrytis cinerea*. Due to its effectiveness against other powdery mildews it was decided to trial it for use against *O. neolycopersici*. Its mode of action is thought to be through a combination of competition for nutrients and space, physical prevention of attachment and penetration, and the production of metabolites that destroy pathogen membranes and germ tubes (Edgecomb & Manker, 2006). Its effectiveness envelope will be described later in this document.

## 4. Novel Greenhouse Structures

The EUPHOROS project has developed novel greenhouse covers and climate management systems in order to minimise fossil fuel consumption, reduce water loss and decrease the use of plant protective chemicals in crop production. Two means of achieving these aims are being investigated; innovative climate control measures and novel greenhouse covering materials.

Modified climate control systems are being trialled in the Almeria region of Spain. These aim to minimise venting of the greenhouse and produce closed or semi-closed greenhouses. This is achieved by the installation of thermal storage facilities, which allow cool water to lower the temperature during the day and for night-time temperature reductions to be ameliorated by day-warmed water.

Novel covering materials have been trialled in Bleiswijk, the Netherlands. These are a novel type of glass, which maximises the diffusion of sunlight light entering the greenhouse and increases the penetration of light into the crop, and an anti-reflective coating, which reduces light loss.

One of the objectives of EUPHORUS was to assess how new GH structures and materials might affect pest and disease pressures and the effectiveness of their biological control agents. In order to do this we used the partnership to gather climatic data from experimental GH environments so that

these data could then be used with our novel pest, disease and control agent models to assess likely pest and disease management strategies.

### 5. Impacts on the Growing Climate

The modified climate control systems were trialled in Almeria, Spain at the Estación Experimental de la Fundacion Cajamar from 2009 to 2010. A tomato crop was grown in a Spanish Tunnel type greenhouse with minimal venting. A reference tomato crop grown in a neighbouring Spanish Tunnel was grown using a standard venting program. This produced semi-closed (SC) and open or passively ventilated (PV) greenhouse systems respectively, for which climate data and yield results were collected. One of the aims of SC systems is to allow for CO<sub>2</sub> enrichment strategies to increase productivity. However, some periods of venting during very hot weather still proved necessary.

EUPHORUS partners collected climate data between December 2009 and December 2010. The results show that in the SC greenhouse the climate was smoothed (extremes were avoided) both diurnally and across the year. Still more marked were effects on RH. In SC mean daily humidity levels were similar in both winter and summer (mainly between 85-95% RH) (Fig. 1). In PV mean humidity levels fluctuated between 50% and 80% in June and 70% and 100% in December. The differences in the peak maximum and minimum values were more significant. In SC RH rarely rose above 90% RH, whereas in PV 100% RH was recorded regularly, especially in winter. Minimum relative humidity rarely dropped lower than 60% in SC whereas humidities of 50% RH and lower were often recorded in PV in the summer. Extremes in RH at either end of the scale will affect pest and predator behaviours and will affect disease prominence.

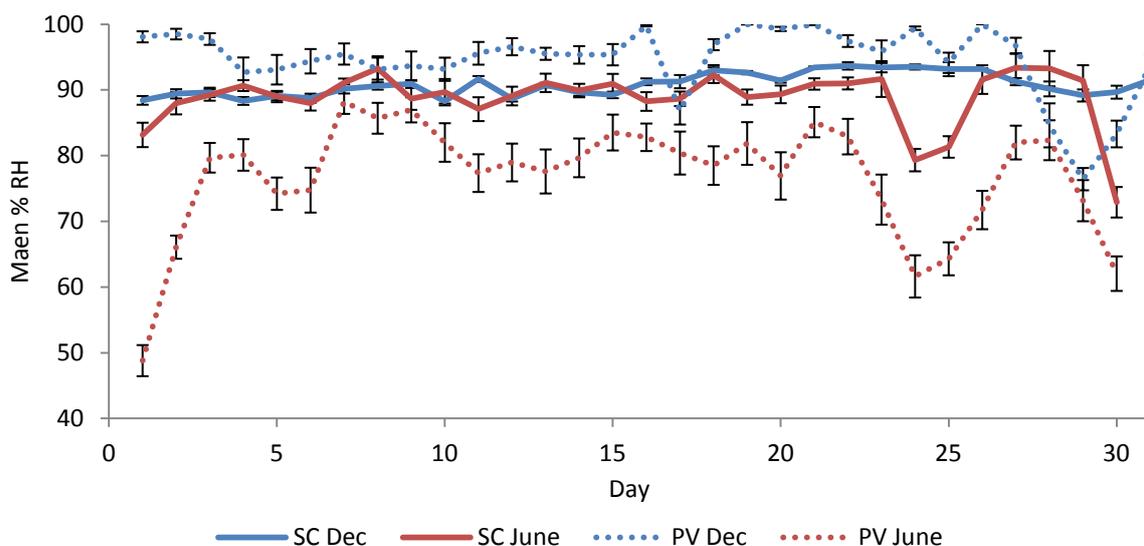


Fig. 1 Comparison of mean relative humidity (% RH) between semi-closed (SC) and passively ventilated (PV) greenhouses in December to June in Almeria, Spain. It is clear that the SC system smoothed out extremes of RH throughout the year.

In terms of mean diurnal patterns (Fig. 2), night time humidity levels in the PV greenhouse were consistently higher than those in the SC greenhouse and often close to saturation point (100% RH) in winter and spring. During the day in the PV greenhouse RH drops with the magnitude of this drop increasing from winter to summer. Conversely, in the SC greenhouse RH is more consistent, with a range of 78-88% RH in December and March and 68-75% RH in June.

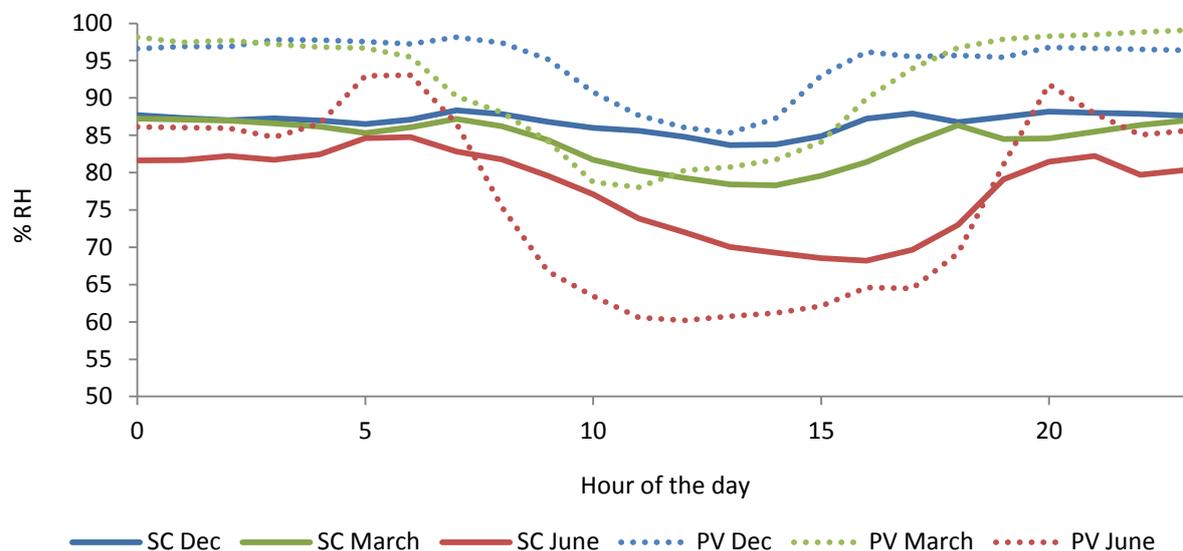


Fig. 2 Comparison of hourly mean humidity levels (% RH) between semi-closed (SC) and passively ventilated (PV) greenhouses in December, March and June in Almeria, Spain. Again, the SC system smoothed out fluctuations and provided a far more consistent environment.

Mean daily temperatures in SC and PV were similar (Fig. 3), fluctuating narrowly around 15°C in winter and more widely around 25°C in summer. When the maximum and minimum temperatures are considered, it was observed that in the cooler months temperatures dropped lower in the PV greenhouse and in the summer the minimal venting in the SC greenhouse saw temperatures rise higher than in the PV greenhouse.

In terms of mean diurnal patterns (Fig. 4), night temperatures in the SC greenhouse are 2-3°C higher than the PV greenhouse in winter and spring. This night time temperature difference is lost during the summer. Day time temperatures are similar in the two greenhouses during the winter months

but in spring and summer the SC greenhouse experienced temperatures between 2-3°C higher than the PV greenhouse.

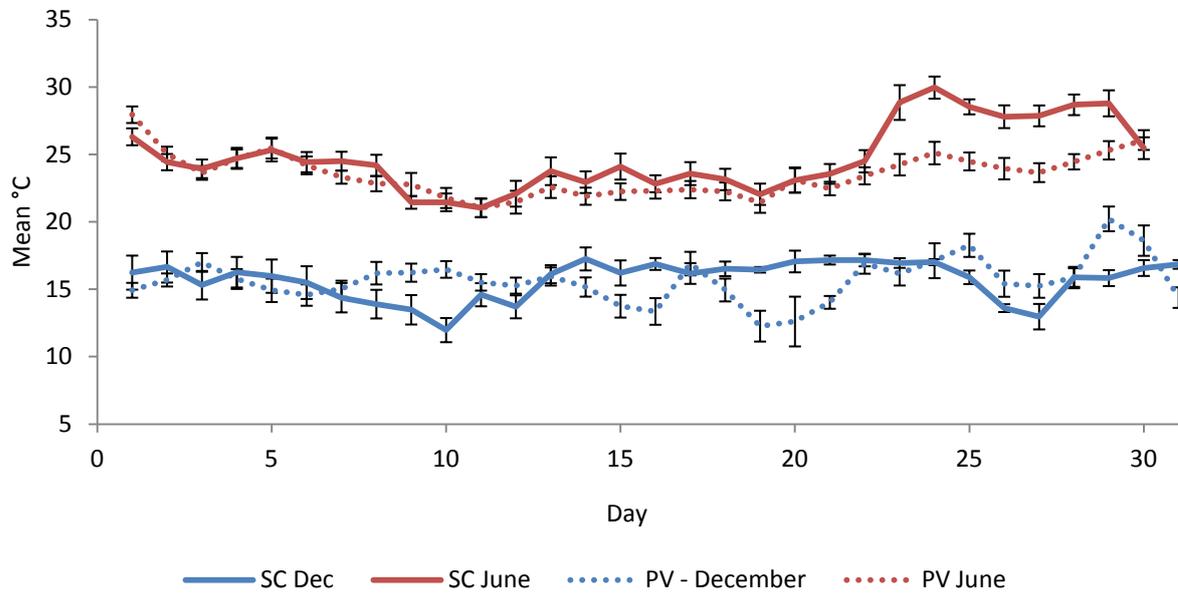


Fig. 3 Comparison of mean daily temperatures (°C) between semi-closed (SC) and passively ventilated (PV) greenhouses in December and June in Almeria, Spain.

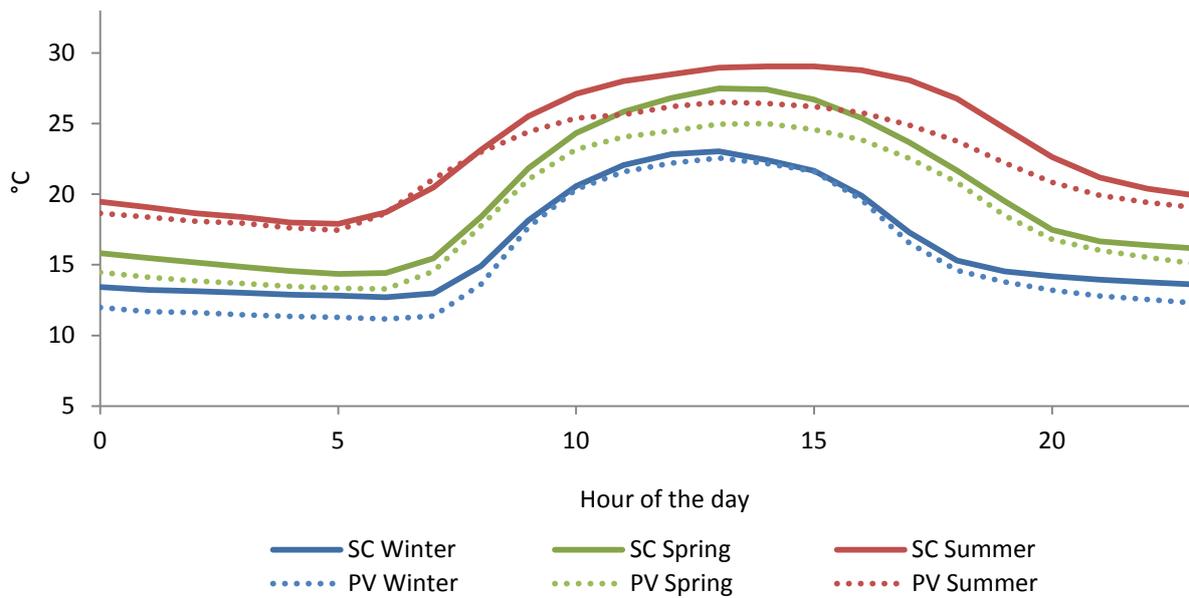


Fig. 4 Comparison of mean hourly temperatures (°C) between semi-closed (SC) and passively ventilated (PV) greenhouses in winter (December-February), spring (March-April) and summer (May-June) in Almeria, Spain.

The novel greenhouse covers employed to produce more light diffusion in the Netherlands were not found to have any significant effect on the greenhouse temperature or humidity levels. However, it was found that these covers transmitted 50% more UV light.

## 6. Assessing Impacts of Novel Greenhouse Systems on Control Agents

As part of EUPHORUS work package 4, Partner Warwick developed three separate mathematical models to forecast the effects the novel greenhouse systems may have on conditions inside. The first was a climate model that simulated the conditions within Dutch Venlo and Spanish SC and PV greenhouses with a suitable degree of stochasticity. The second was an individual-based *T. urticae* population model and the third was an *O. neolycopersici* disease model. The pest and disease models used outputs from the climate model to simulate the impacts of changes in greenhouse climate, country and environmental control strategy, on the ability of *P. persimilis* and *B. subtilis* to regulate populations of *T. urticae* and *O. neolycopersici*, respectively.

### 6.1 Greenhouse Climate Model

The greenhouse climate model simulated the seasonal and diurnal changes in temperature and humidity conditions within each greenhouse, generating hourly values across a year. Separate versions modelled the climate in each of the three greenhouses. The models were parameterised using datasets from partners.

The model for the Spanish PV used data recorded over a four year period from a commercial-standard greenhouse at the Estación Experimental de la Fundacion Cajamar

The model for the Spanish semi-closed greenhouse used data recorded in the experimental greenhouse over one year (also at the Estación Experimental de la Fundacion Cajamar).

The model for the Dutch Venlo greenhouse used data recorded over a year from twelve commercial greenhouses near Bleiswijk.

Each climate model consisted of two parts; Part 1: the pattern of variation in daily maximum and minimum temperatures and humidity. Part 2 used the maximum and minimum temperature and humidity values to model the change in these parameters across each day. This produced an accurate simulation of the greenhouse conditions in the respective countries and production systems. Stochastic elements were incorporated into these models allowing ten different data sets to be generated for each model.

To investigate the effect of greenhouse climate on pests and disease in Venlo-type glasshouses in northern Europe, the conditions were simulated to produce simulations of the ‘standard’ system as well as a ‘cool’ system (‘standard’ minus 2°C) and a ‘warm’ system (‘standard’ plus 2°C).

## 6.2 *Phytoseiulus persimilis* Biological Control Model

To assess the impacts of novel greenhouse systems on the efficacy of *P. persimilis* as a biological control agent for *T. urticae*, a model was developed based on the model of Skirvin *et al* (2002). This simulated important life-stage parameters and agent movement in order to predict the success or failure of different biological control strategies. The model was amended to include the effects of temperature and relative humidity as the original model assumed fixed temperature and humidity conditions. We used EUPHORUS and published datasets (Sabelis, 1981; Bancroft & Margolies, 1999; Skirvin & Fenlon, 2003).

EUPHORUS investigated the predatory response of *P. persimilis* to varying densities of *T. urticae* eggs on two host plants and under a range of humidities (55-95% RH). On the ornamental shrub, *Choisya ternata*, functional response was significantly affected by humidity ( $d = 29.27$ ,  $df = 4$ ,  $p = 0.012$ ) with the highest number of eggs eaten at 85% RH (Fig. 7). Low RHs (below 65%) were particularly detrimental to predation rate. These datasets were applied as modifiers to existing models (Skirvin *et al.*, 2002; Skirvin & Fenlon, 2003).

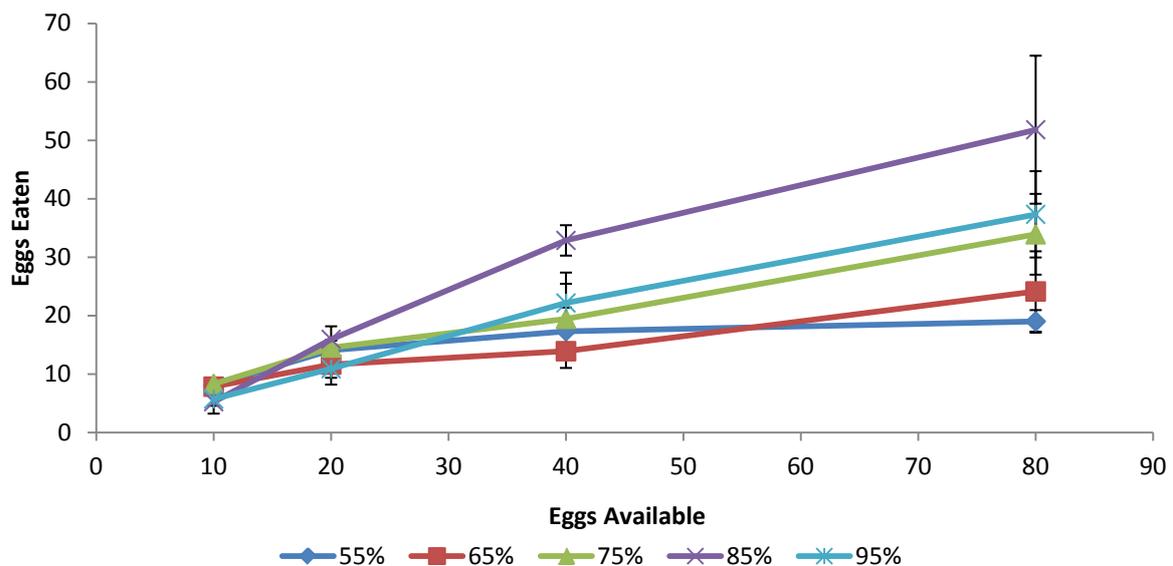


Fig. 7 Number of *T. urticae* eggs eaten in a 24 hour period by *P. persimilis* at four egg densities (10, 20, 40, 80) and five levels of RH (55, 65, 75, 85, 95% RH) on *C. ternata*. Values have been adjusted for control mortality.

On tomato (*Lycopersicon esculentum*), humidity was found to have no significant effect on the predation efficacy of *P. persimilis* (Fig. 8). *C. ternata* leaves are smooth and waxy while *L. esculentum* leaves have abundant trichomes, which may increase the thickness of the leaf boundary layer producing a microclimate that protects the mite from low ambient humidity. The maximum predation rate was also lower on tomato than on *C. ternata* and this may be due to the trichomes hindering the movement of the predatory mite.

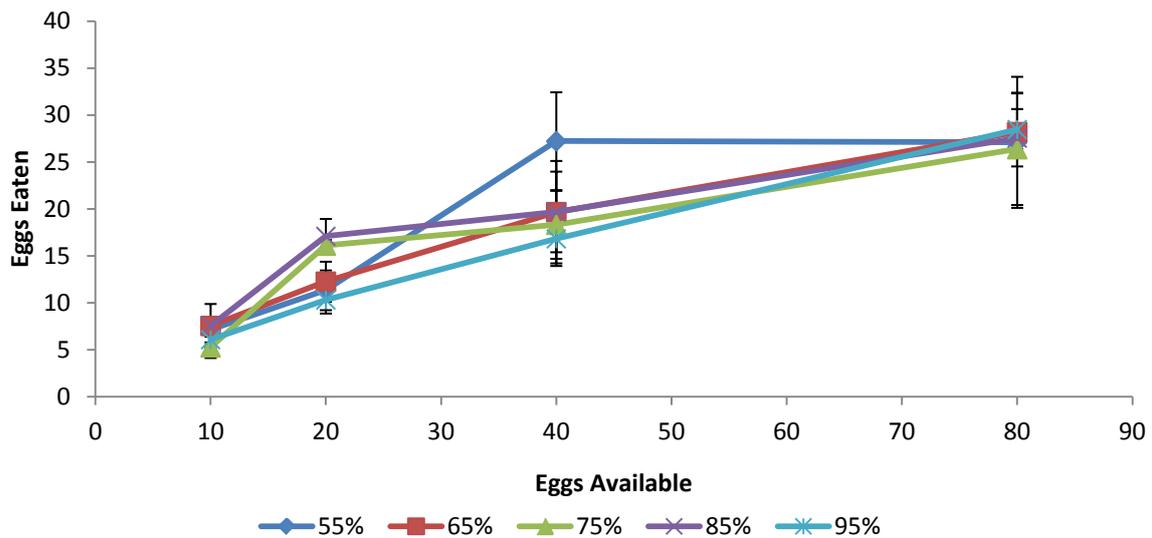


Fig. 8 Number of *T. urticae* eggs eaten in a 24 hour period by *P. persimilis* at four egg densities (10, 20, 40, 80) and five levels of RH (55, 65, 75, 85, 95% RH) on *L. esculentum*. Values adjusted for control mortality.

#### Development of a predictive model for control of *T. urticae*

To predict the success of biological control under different climate management systems, a number of control scenarios were investigated. This involved introducing *P. persimilis* into a simulated greenhouse of 100 plants at either weekly or fortnightly intervals and at densities of 10, 100 or 1000 individuals per occasion. The level of pest pressure was also varied with populations of *T. urticae* adult females beginning at 10, 100 or 1000. A population of *T. urticae* was programmed to arrive once a year. The week was systematically varied with each run so that simulations could be made across the year. No significant differences were found between the novel and traditional greenhouses in the Netherlands, so we focussed on simulations for the Spanish semi-closed and passively ventilated greenhouses.

Three different outcomes were generated by each run of the model; eradication of *T. urticae* ('control'), *T. urticae* numbers reaching 1000000 individuals (approximately 10000 per plant)

(‘uncontrolled’), and coexistence where some *P. persimilis* and less than 1000000 *T. urticae* remain at the end of the year (day 365) (‘coexistence’).

The model showed that control was rarely achieved by introducing *P. persimilis* at a density of 10 per occasion in either the PV greenhouse (Fig. 9) or the SC greenhouse (Fig. 10). Regardless of the introduction frequency *P. persimilis* or introduction density of *T. urticae*, uncontrolled *T. urticae* populations occurred more than 90% of the time from week 1 to approximately week 31, with coexistence occurring most frequently after this. Outcomes were similar between the two climate scenarios, although the results suggest less optimal conditions for *T. urticae* increase at the beginning of the year in the PV greenhouse.

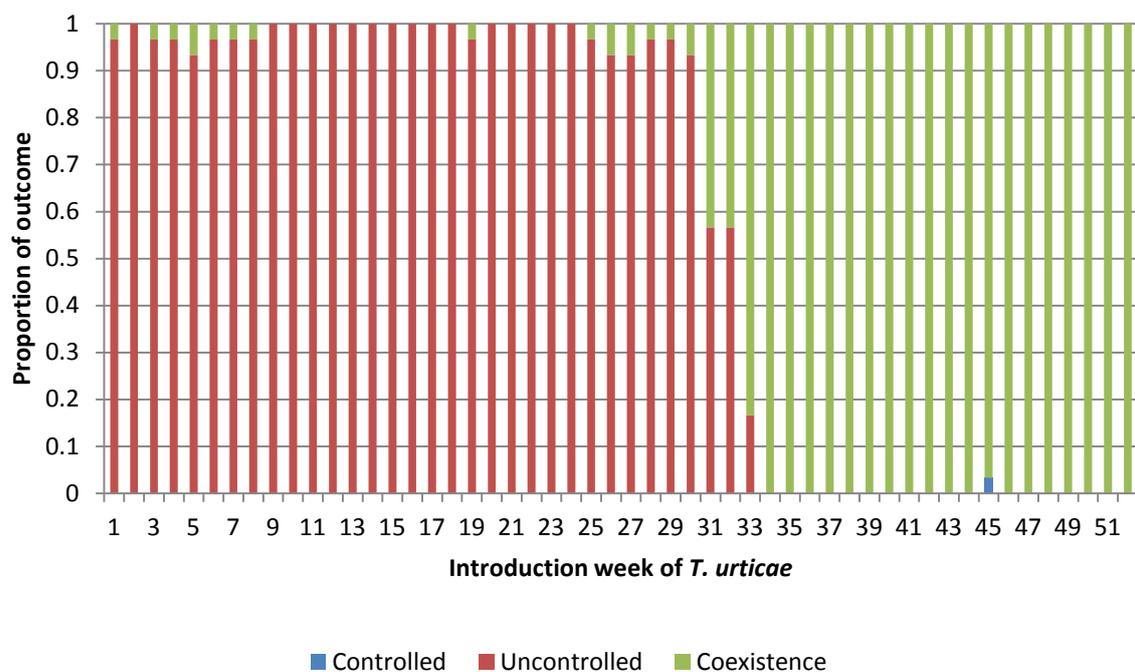


Fig 9 Modelled outcome of *T. urticae* pressures in a passively ventilated greenhouse in Almeria, Spain with 10 *T. urticae* arriving at the beginning of each calendar week and 10 *P. persimilis* arriving weekly.

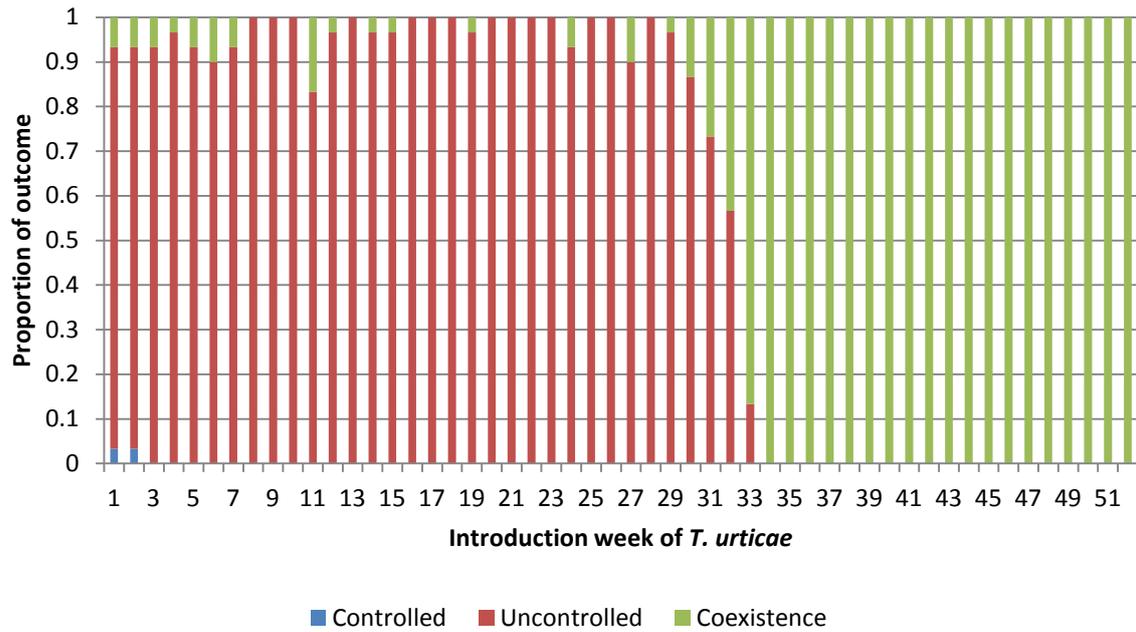


Fig 10 Modelled outcome of *T. urticae* pressures in a semi-closed greenhouse in Almeria, Spain with 10 *T. urticae* arriving at the beginning of each calendar week and 10 *P. persimilis* arriving weekly.

Introducing 100 *P. persimilis* per occasion gave improved control, regardless of *T. urticae* density. Interestingly, control was more often seen at a *T. urticae* introduction density of 1000 than 100. This may be due to increased spatial coincidence. The frequency of *P. persimilis* introduction was also important with control far less common when *P. persimilis* were introduced fortnightly. Comparison of the two climate scenarios show that control was more likely in the PV greenhouse (Fig. 11) than in the semi-closed greenhouse (Fig. 12). However, coexistence was the most common outcome at a *P. persimilis* introduction density of 100 per occasion.

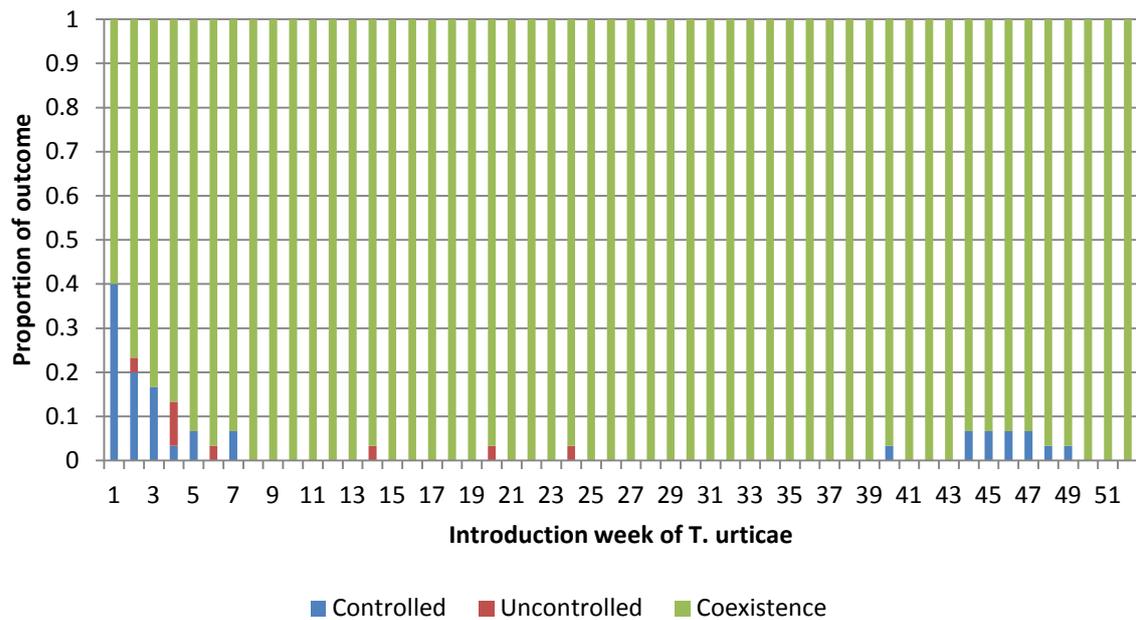


Fig 12 Modelled outcome of *T. urticae* pressures in a semi-closed greenhouse in Almeria, Spain with 10 *T. urticae* arriving at the beginning of each calendar week and 100 *P. persimilis* arriving weekly.

At a *P. persimilis* introduction density of 1000 per occasion control was far more likely than the other outcomes for the majority of the year, regardless of *T. urticae* introduction density, introduction frequency or climate management. With a weekly introduction of 1000 *P. persimilis* control was achieved 100% of the time from week 1 to approximately week 40, regardless of *T. urticae* introduction density or climate management. From week 40 coexistence became more likely. Fortnightly introductions of *P. persimilis* decrease the rate of control with coexistence appearing earlier in the year.

It is with fortnightly introductions that important differences between the two greenhouse designs become evident. In PV, control was predicted 100% of the time up until week 34, a striking contrast to the semi-closed system in which coexistence is predicted as early as week 6 and forms 20% of the outcomes from week 32 (Fig. 14).

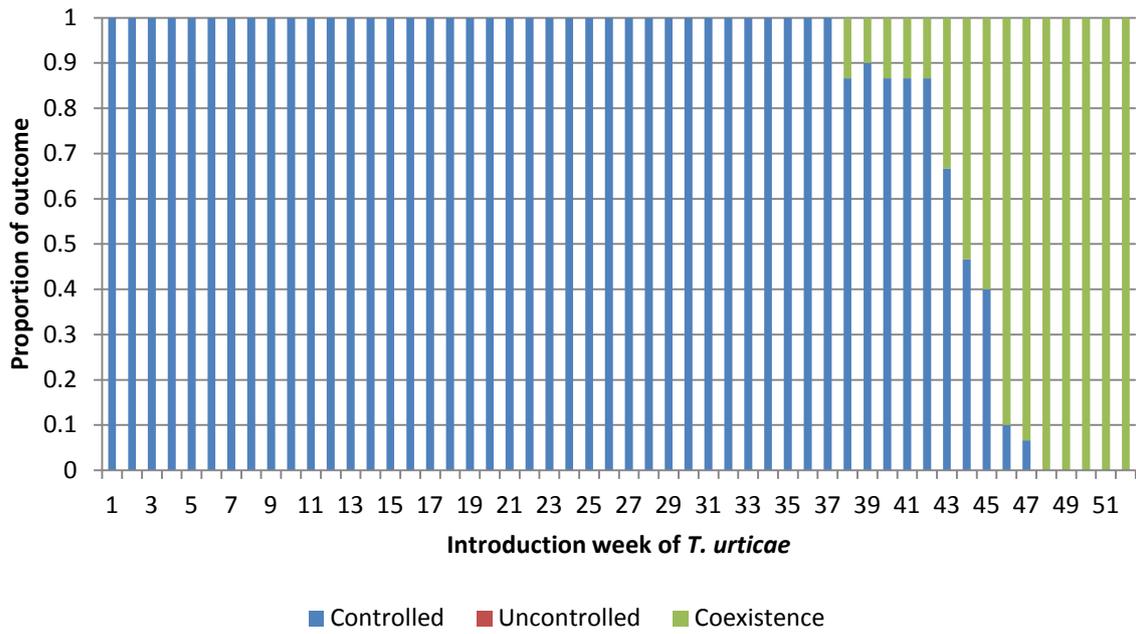


Fig 13 Modelled outcome of *T. urticae* pressures in a passively ventilated greenhouse in Almeria, Spain with 1000 *T. urticae* arriving at the beginning of each calendar week and 1000 *P. persimilis* arriving fortnightly.

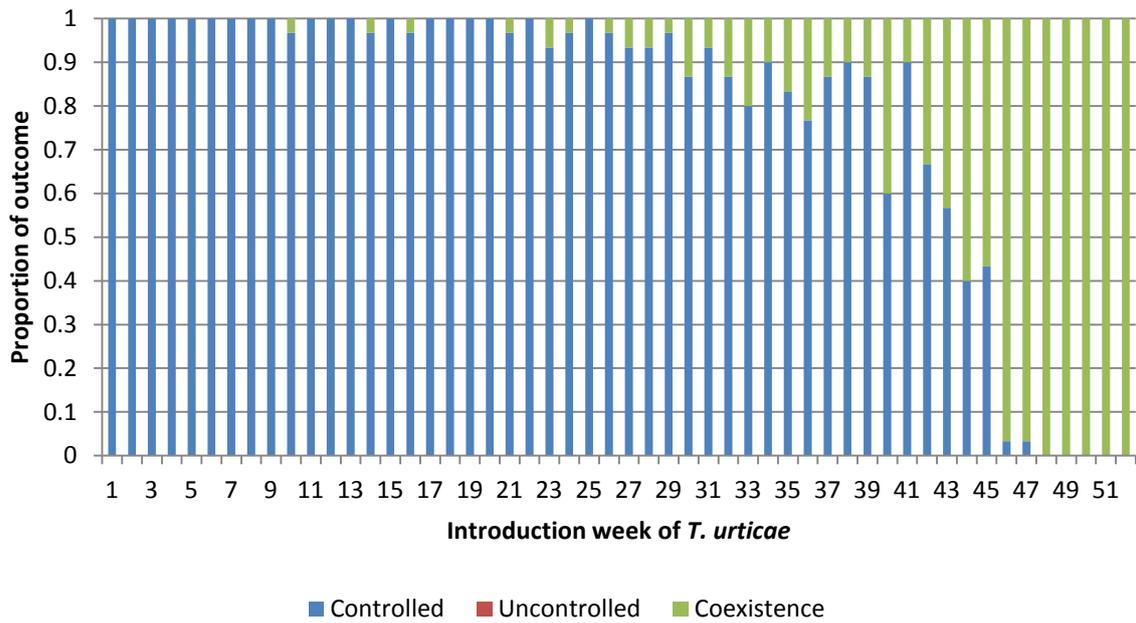


Fig 14 Modelled outcome of *T. urticae* pressures in a semi-closed greenhouse in Almeria, Spain with 1000 *T. urticae* arriving at the beginning of each calendar week and 1000 *P. persimilis* arriving fortnightly.

Northern Europe:

The novel glass employed in Bleiswijk did not significantly affect temperature and humidity in the greenhouse. However, the increased UV may be detrimental to *P. persimilis*. Other work, also carried out as part of EUPHORUS, investigated the effects of UV light on the population dynamics of *P. persimilis*-mediated control of *T. urticae*. It found that control efficacy was significantly reduced in the presence of UV-B light.

### 6.3 *Bacillus subtilis* Biological Control Model

To evaluate the impact of novel greenhouse systems on biological control of *O. neolycopersici* by *B. subtilis*, a series of controlled environment experiments were conducted to record the effectiveness of *B. subtilis* at a range of temperatures and RHs. Young tomato plants were treated with *B. subtilis* one day prior to inoculation with *O. neolycopersici* and were then placed in controlled environment cabinets at set temperature and humidity levels. Data from these experiments were used to parameterise a disease development model.

The model consisted of two components:

The first simulated the effect of temperature, humidity and *B. subtilis* on the latent period of the disease, where latent period is defined as the time between inoculation and first sporulation of the pathogen.

The second part of the model simulated the development of the disease across the leaf surface in response to temperature, humidity and how this was altered by the presence of *B. subtilis*.

The EUPHORUS work found that *B. subtilis* was effective in reducing *O. neolycopersici*, particularly between 20-27°C and 80%+ RH. The latent period of *O. neolycopersici* was significantly affected by temperature ( $F = 93.91$ ,  $ddf = 2$ ,  $P = 0.011$ ), but not by humidity (Fig 15), with an increased latent period at temperatures above 25°C and below 20°C. The increase was especially large between 15°C and 10°C. No disease was recorded at 33°C.

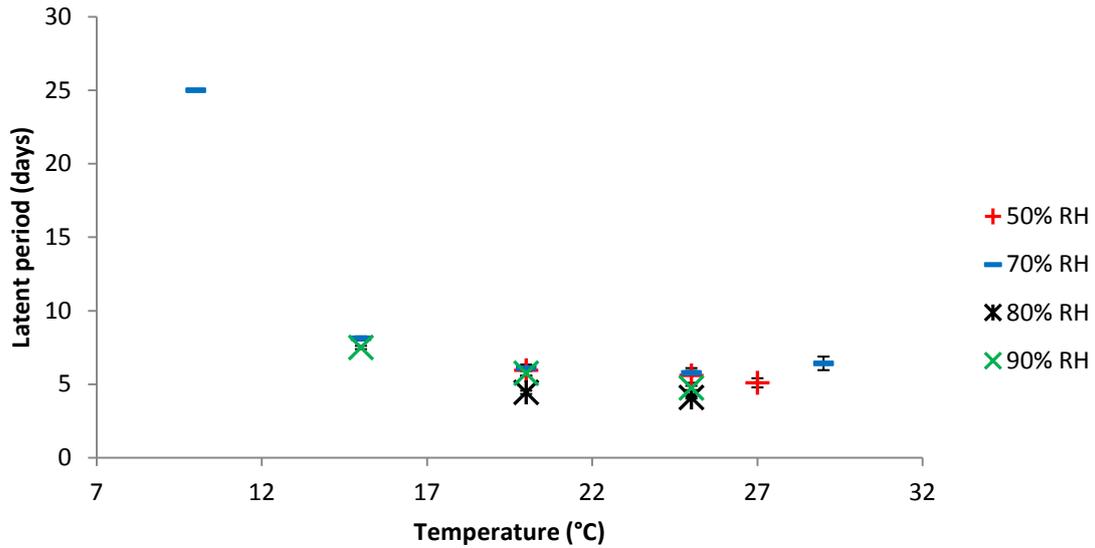


Fig15 Effect of temperature and relative humidity on the latent period of *O. neolycoerpsici*. Bars show standard error at the 5% level.

When treated with *B. subtilis* the latent period of *O. neolycoerpsici* was increased under some conditions (Fig 16), increasing with humidity and temperature, with the greatest increase in latent period being 1.9 days at 90% RH and 25°C. Therefore, both temperature ( $F = 403.7$ ,  $ddf = 11$ ,  $P < 0.001$ ) and relative humidity ( $F = 138.15$ ,  $ddf = 11$ ,  $P < 0.01$ ) were found to have a significant effect on the latent period of *O. neolycoerpsici* when treated with *B. subtilis*.

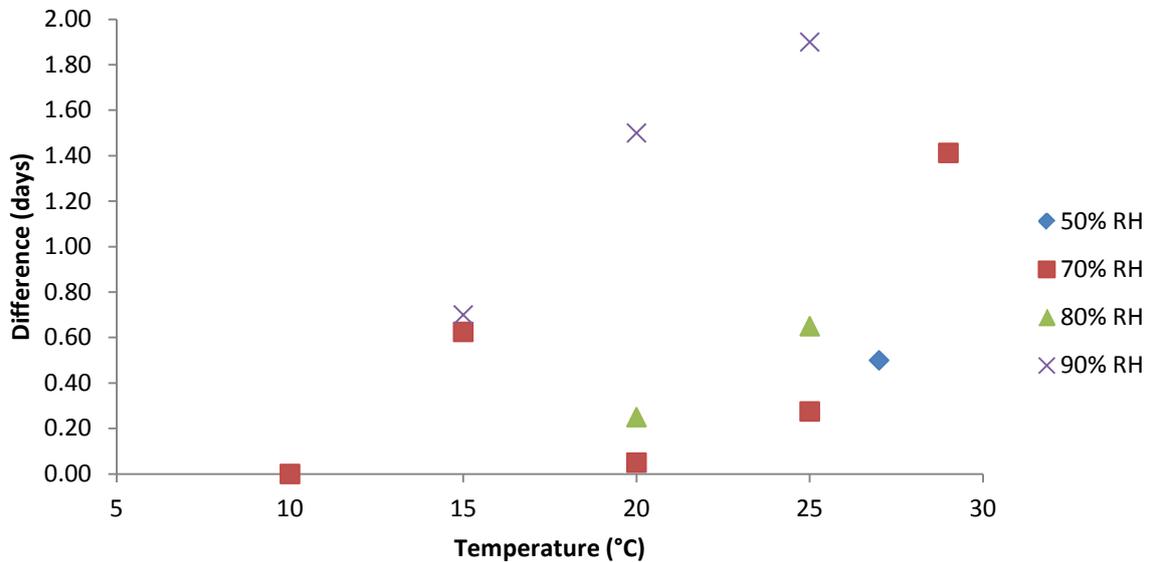


Fig 16 Effect of temperature and relative humidity on the ability of *B. subtilis* to increase the latent period of *O. neolycoerpsici*.

*B. subtilis* was also able to reduce the rate of disease development (% leaf coverage of the disease) and this improved with RH (Fig 17). Temperature ( $F = 2.14$ ,  $df = 120$ ,  $P = <0.01$ ) and relative humidity ( $F = 3.22$ ,  $df = 120$ ,  $P = <0.01$ ).

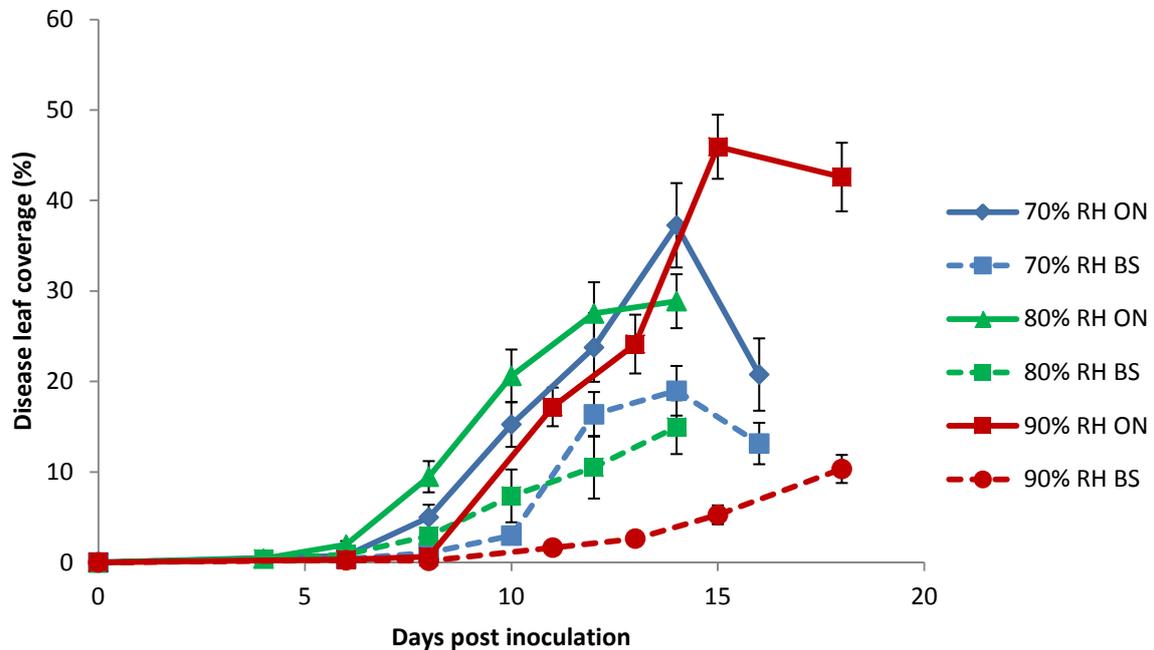


Fig 17 Effect of *B. subtilis* and relative humidity on *O. neolycoopersici* disease development at 25°C. Solid line = *O. neolycoopersici* only. Broken line = *O. neolycoopersici* and *B. subtilis*. Bars show standard error (at 5% level). In all cases, treatment with *B. subtilis* reduced disease severity.

*B. subtilis* was able to produce significant reductions in disease spread between 15°C and 27°C, and the success increased with temperature. However, a significant interaction of humidity and temperature was evident ( $F = 4.44$ ,  $df = 40$ ,  $P = <0.01$ ) with a reduction in *B. subtilis* efficacy at <90% RH and lower temperature. For instance, while *B. subtilis* was able to significantly reduce disease area at 70% RH and 25°C, it was unable to do so at 70% RH and either 15°C or 20°C (Fig 18).

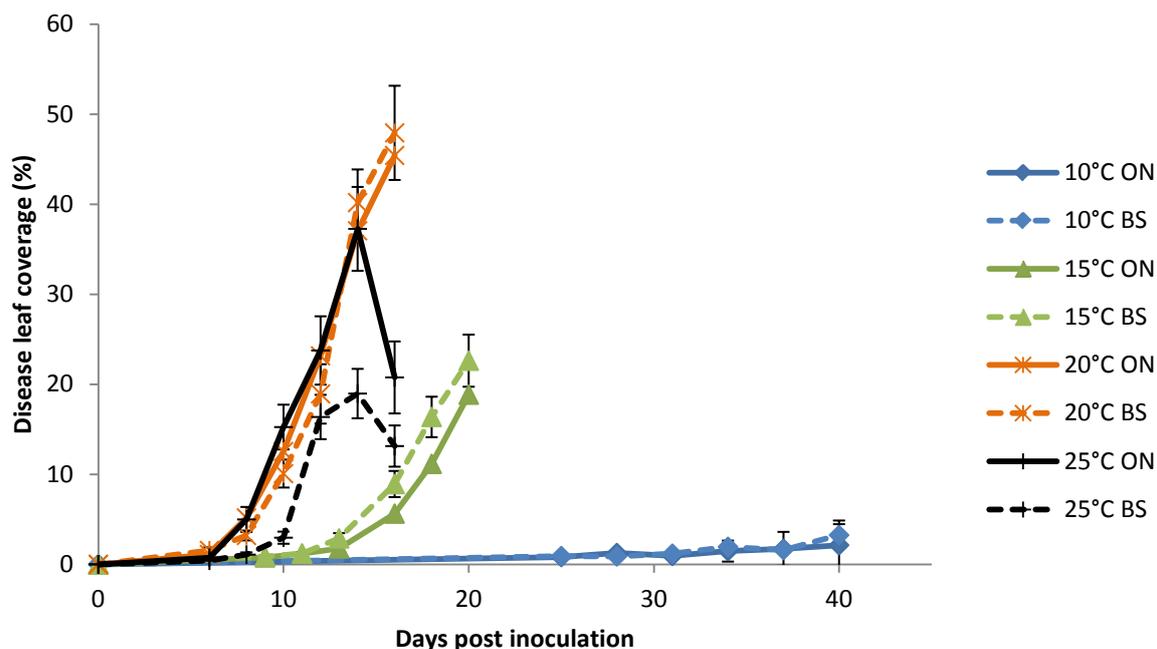


Fig 18. Effect of *B. subtilis* and temperature on *O. neolycopersici* disease development at 70% RH. Solid line = *O. neolycopersici* only. Broken line = *O. neolycopersici* and *B. subtilis*. Bars show standard error (at 5% level).

To model the effect of temperature, humidity and biological control on the latent period of the disease a quadric surface model was developed to describe the response of the disease between 70% and 90% RH and 15°C to 25°C. Within this temperature range humidities above or below 70% and 90% RH were constrained to 70% and 90%, ensuring that no extrapolation of the data occurred as the model did not accurately describe the experimental results. This model described 80.5% of the variation in respect of *O. neolycopersici* on its own and 84.1% of the variation in respect of *O. neolycopersici* treated with *B. subtilis*. Above 25°C and below 15°C a quartic regression was used to describe the latent period. This described 97.7% and 98.6% of the variation for *O. neolycopersici* only and *O. neolycopersici* treated with *B. subtilis* respectively.

When this model was run against the greenhouse climate models it was evident that the increases in latent period due to *B. subtilis* varied with both country and the climate management system employed. In Spanish tunnels *B. subtilis* is predicted to increase the latent period by approximately 0.4 to 0.8 days. This increase is greater under semi-closed climate management than passively ventilated climate management from the beginning of the year until early June and from mid September until the end of the year (Fig 19).

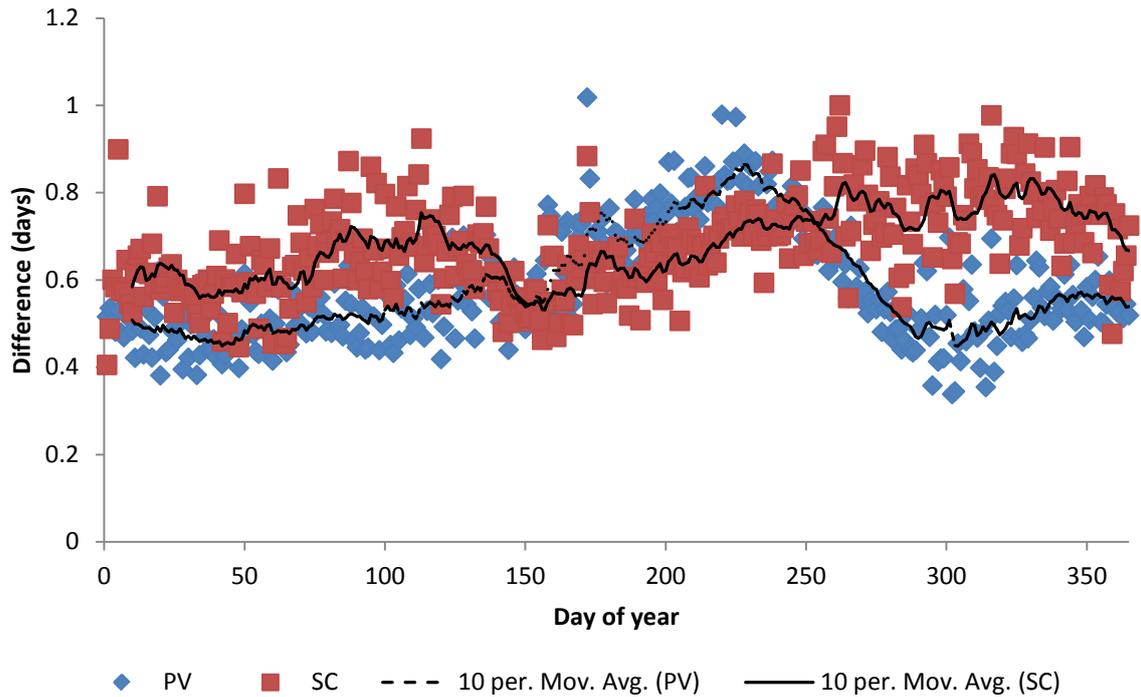


Fig 15 Modelled increase in *O. neolycopersici* latent period due to *B. subtilis* across the year in Almeria, Spain. SC = Semi-closed, PV = Passively ventilated. A moving average (Mov. Avg.) is included that plots the mean of the neighbouring ten data points.

In Dutch Venlo greenhouses the ability of *B. subtilis* to increase the latent period also varied across the year and with climate management system (Fig 20). The current 'standard' climate was more favourable than (mildew latency was longer) than the simulated 'warm' climate (2°C warmer than 'standard'). *B. subtilis* was most effective under the 'cool' climate management system (2°C cooler than 'standard') with increases in latent period of more than a one day from November to early March. Between these dates the increase drops gradually to approximately 0.6 days in the summer.

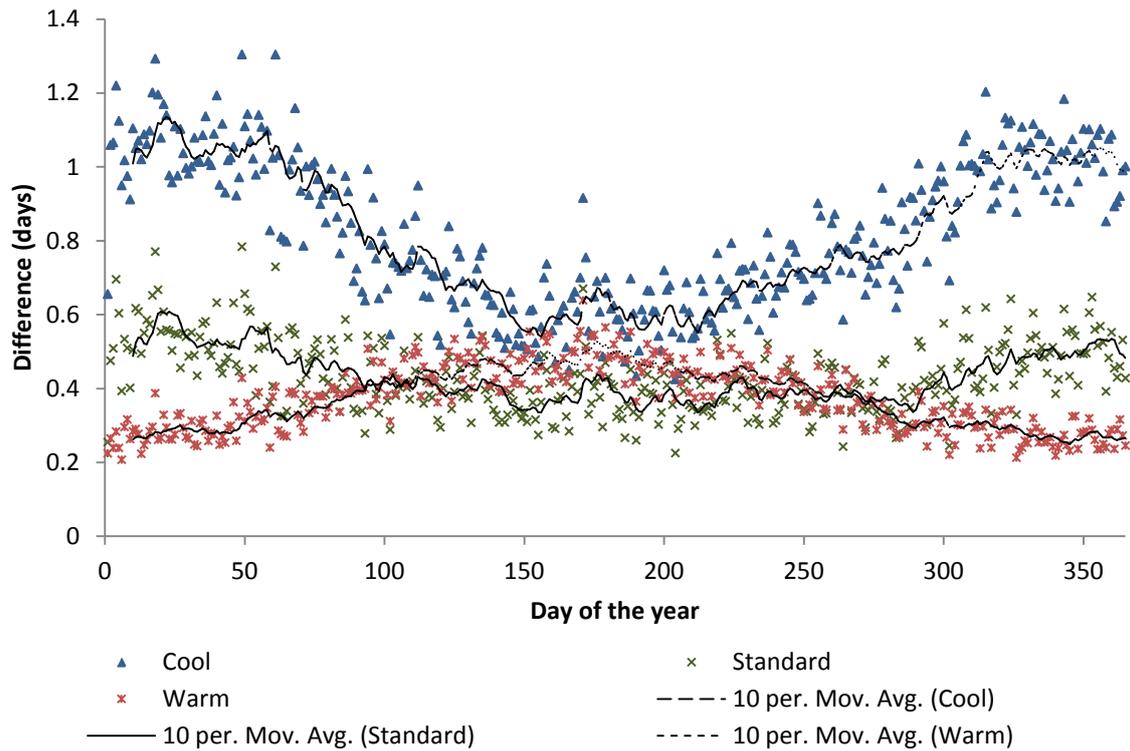


Fig 20 Modelled increase in *O. neolycopersici* latent period due to *B. subtilis* across the year in Bleiswijk, Netherlands. Cool = Standard-2°C, Warm = Standard+2°C. 10per. Mov. Avg. = 10 point moving mean

Logistic growth curves were fitted in order to model the development of *O. neolycopersici* in terms of percentage infected leaf area. The parameters of the logistic equation were treated as functions of temperature and, where necessary, relative humidity. For the *O. neolycopersici*-only treatment, analysis of the data showed that the development of the disease was significantly affected by temperature but not by humidity, meaning that only temperature was used to describe the behaviour of the logistic parameters. However, the development of the disease when treated with *B. subtilis* was significantly affected by both temperature and humidity and both of these variables were used to describe the behaviour of the logistic parameters.

For the *O. neolycopersici*-only treatment a cubic regression against temperature was fitted to a B parameter that modifies the growth rate of the disease and describes 70.5% of the variation. For the M parameter, a parameter that modifies the time of maximum growth (inflection point) of the logistic curve, a quartic regression against temperature was used. This explained 97.7% of the variation. A comparison of the model outputs to the experimental data can be seen in Fig 21.

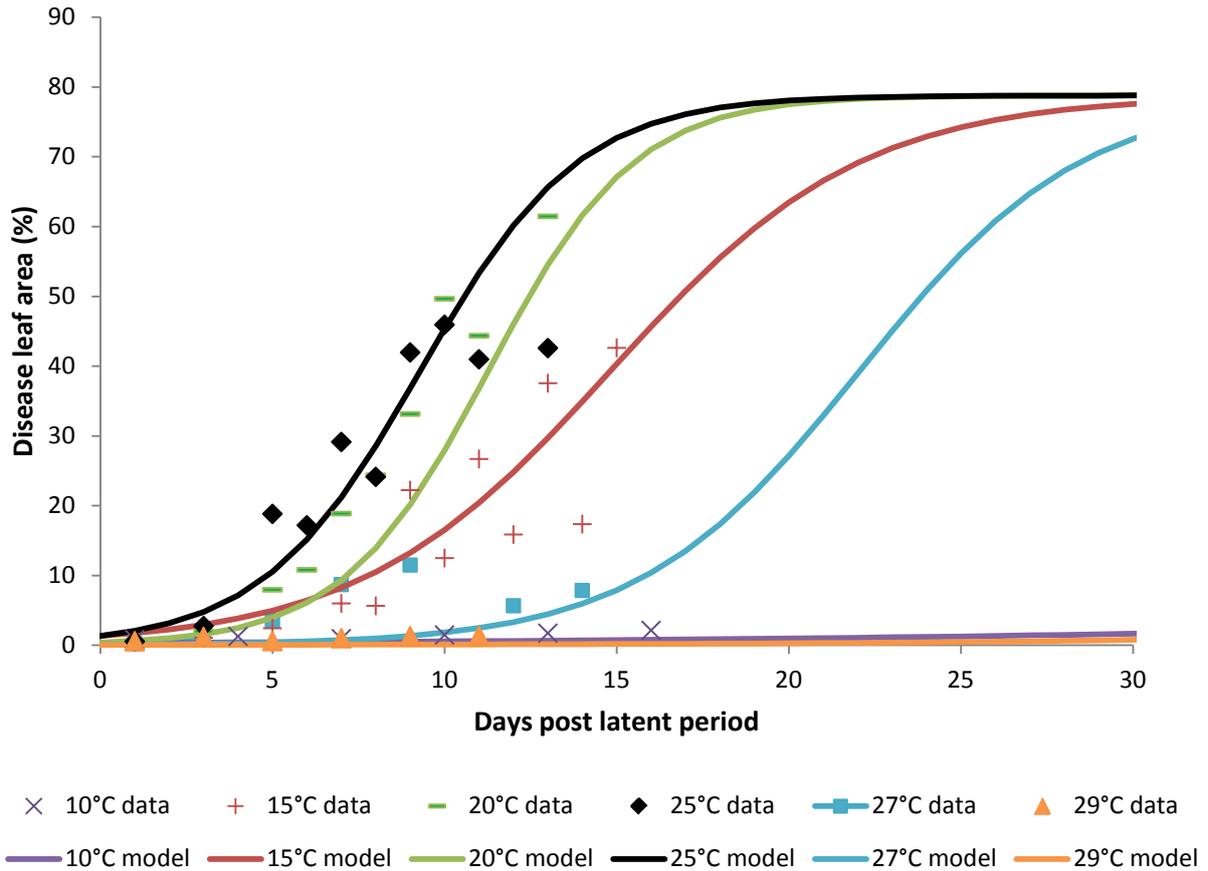


Fig 21 Disease leaf area as described by logistic growth curve model (lines) and observed data (markers). *O. neolycopersici*-only treatment on tomato leaves

For the data from plants treated with *B. subtilis*, a cubic regression against temperature was fitted to the B parameter, describing 90.3% of the variation. A quartic regression against temperature was fitted to the M parameter, describing 97.4 % of the variation. These regressions were used when temperatures were below 15°C or above 25°C. Between these temperatures the experimental work showed that increasing humidity had an additional effect of increasing the ability of *B. subtilis* to reduce disease area. This effect was described using linear regressions on the B and M parameters on the growth curves between 15°C and 25°C and 70-90% RH and subsequently fitting quadratic regressions to the *a* and *b* parameters of the linear regressions. Due to the limited number of replicates at these conditions this aspect of the model lacks a statistical summary. However the model outputs closely match the experimental data (Fig 22 and 23). A minimum value was set for the M parameter in both the *O. neolycopersici*-only treatment and the *O. neolycopersici* with *B. subtilis* treatment to constrain the intercept of the curves to <2% disease coverage.

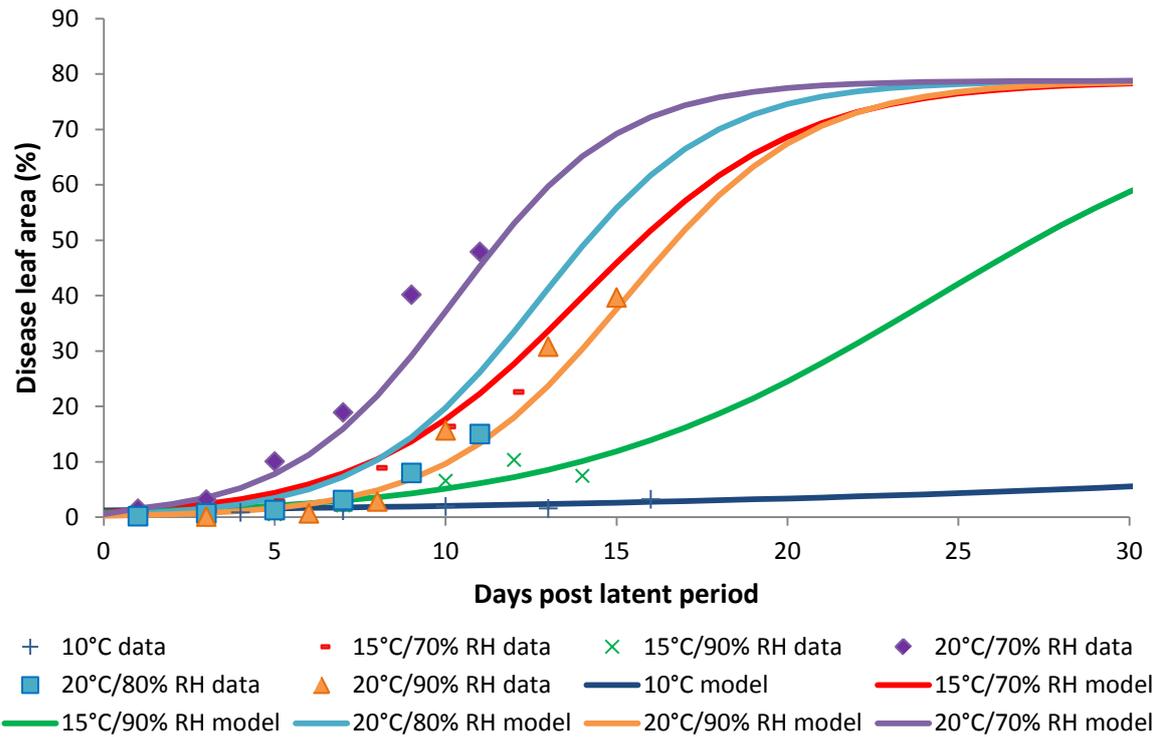


Fig 22. Disease leaf area post *B. subtilis* treatment between 10°C and 20°C as described by logistic growth curve model (lines) and observed data (markers).

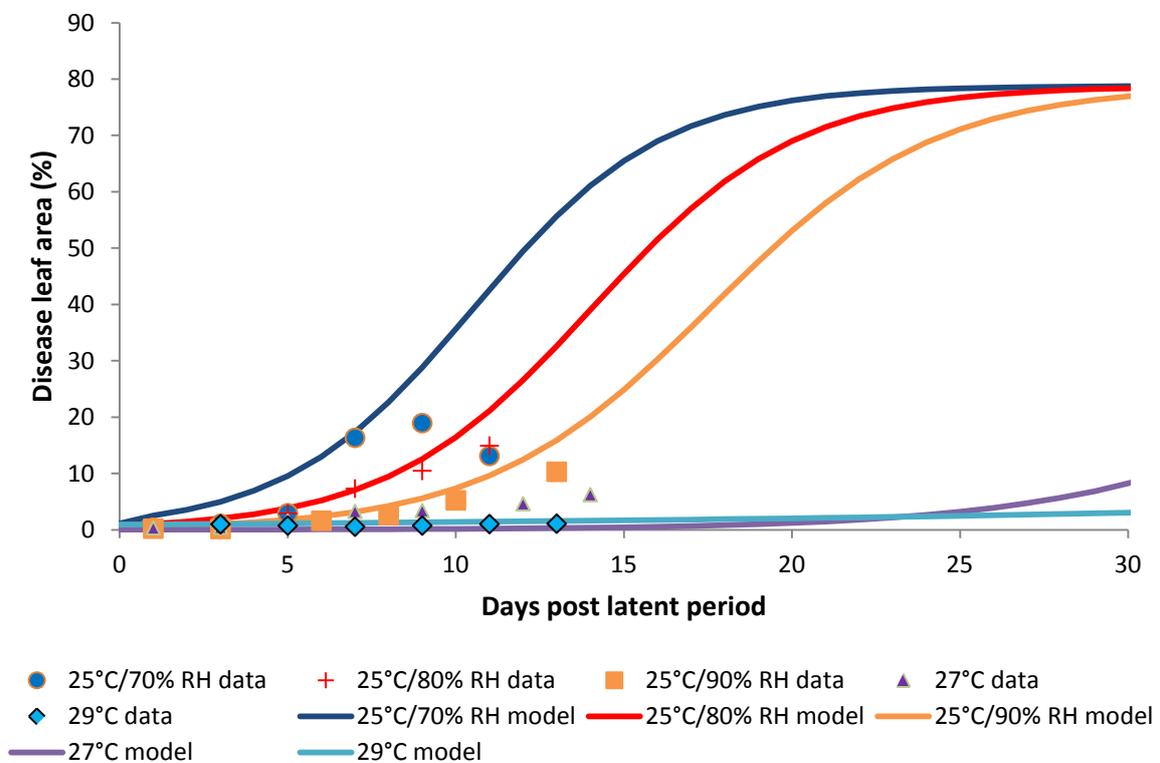


Fig 23. Disease leaf area post *B. subtilis* treatment between 25°C and 29°C as described by logistic growth curve model (lines) and observed data (markers).

This model was used to calculate the maximum rate of growth at the temperature and humidity conditions generated from each climate model, for hourly data summarised to provide the daily average maximum growth rates and for each day in the year in both Spain and the Netherlands.

In Spain *B. subtilis* is predicted to reduce the growth rate of *O. neolycopersici* throughout the year (Fig. 24). Through most of the year control was marginally better in the SC than PV, although the improvement did vary through the year. The greatest potential benefit was during the hot (non-cropping) summer months, but *B. subtilis* does offer improved control throughout the growing year.

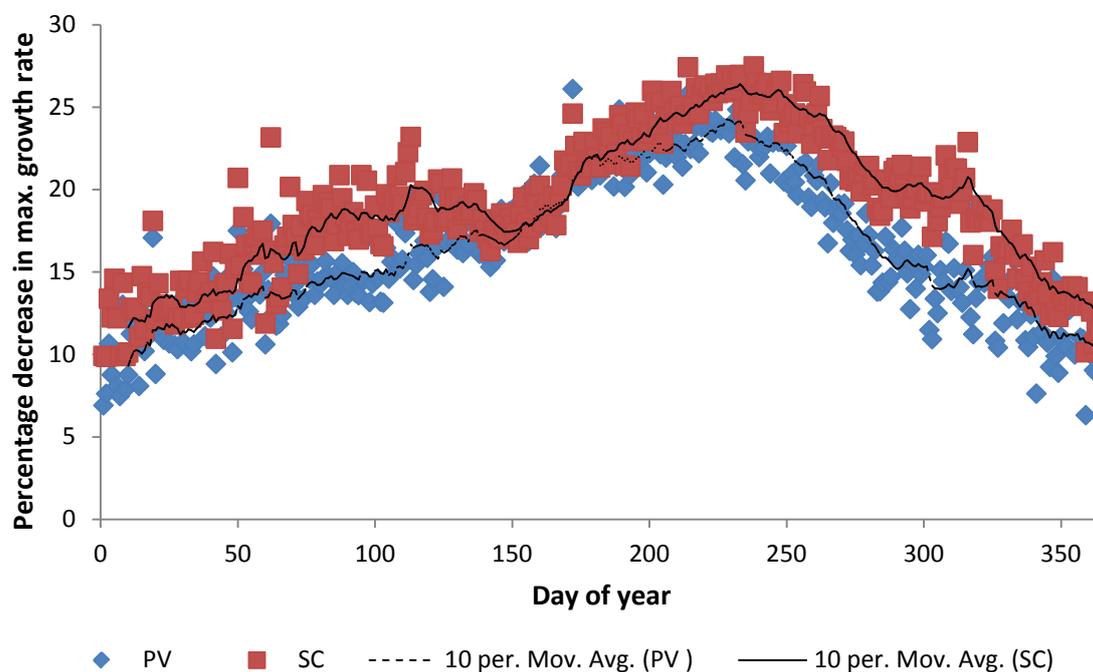


Fig 24 Modelled percentage decrease in maximum growth rate of *O. neolycopersici* due to *B. subtilis* across the year in Almeria, Spain. SC = Semi-closed, PV = Passively ventilated.

In the Netherlands *B. subtilis* could provide 15-20% improved control across the year, regardless of climate model (Fig. 25).

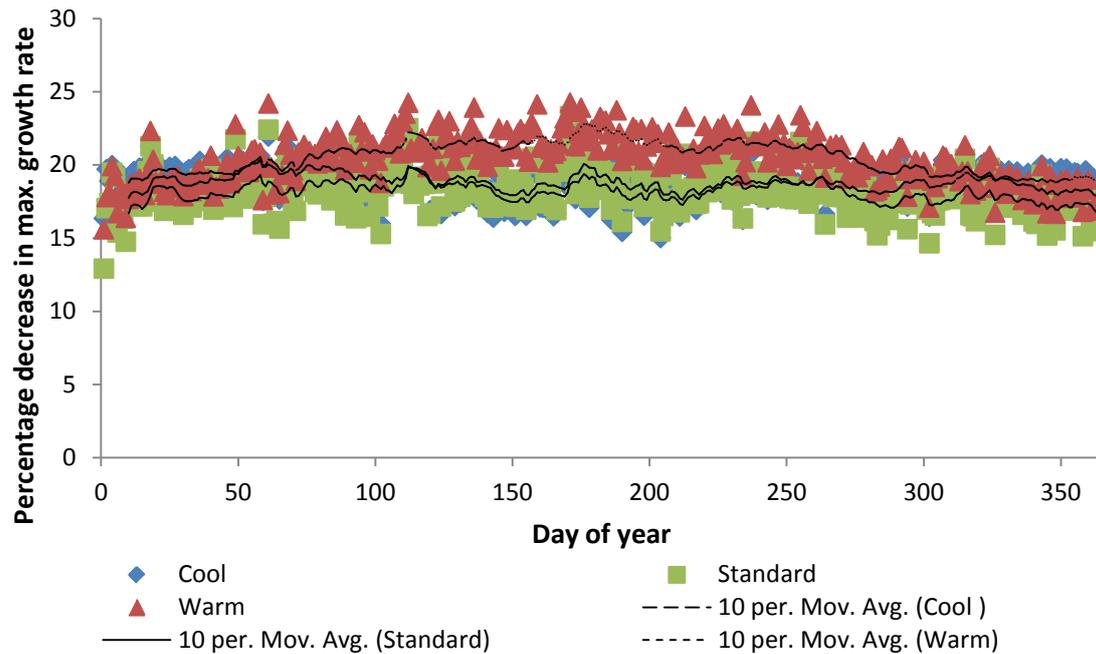


Fig 25 Modelled percentage decrease in maximum growth rate of *O. neolycopersici* due to *B. subtilis* across the year in Bleiswijk, Netherlands. Cool = Standard-2°C, Warm = Standard+2°C.

The latent period model and the disease development model were then integrated to predict the number of days until disease coverage reaches 50%. The model assumes spores arrive at the beginning of each week to produce an even infection across the greenhouse and that *B. subtilis* treatments precede arrival of the inoculum by one day.

For Spain the model predicted that tomatoes treated with *B. subtilis* benefit from a 10-25% increase in the time until 50% disease leaf coverage (Fig. 26). The model also shows that the increase in time until 50% disease leaf coverage (protection) is greater in semi-closed greenhouses than passively ventilated greenhouses, especially in the spring and autumn.

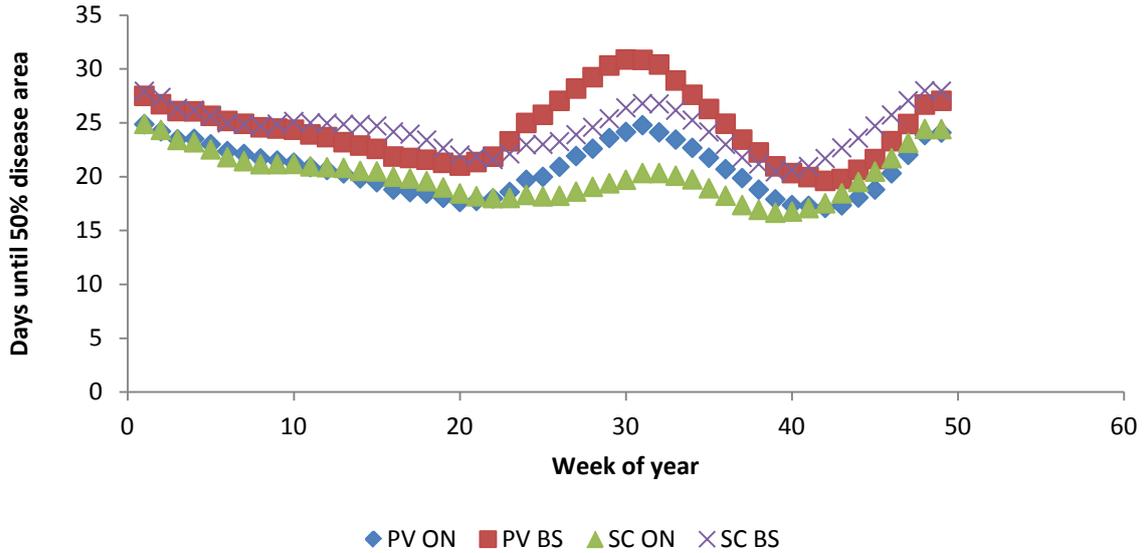


Fig 26 Time until 50% disease leaf area coverage of *O. neolyopersici* only (ON) and treated with *B. subtilis*(BS) when introduced at weekly introductions across the year in Almeria, Spain (as predicted by model). SC = Semi-closed, PV = Passively ventilated.

In the Netherlands the time until 50% disease leaf coverage varied little across the year and little with warmer and cooler climate simulations (Fig. 27). In the standard greenhouse conditions, the time until 50% disease leaf coverage is 16 to 18 days across the year, being slightly longer in the summer than the winter. When compared to the time periods in the absence of control agent they represent a 15% increase in the time until 50% disease coverage (Fig. 27).

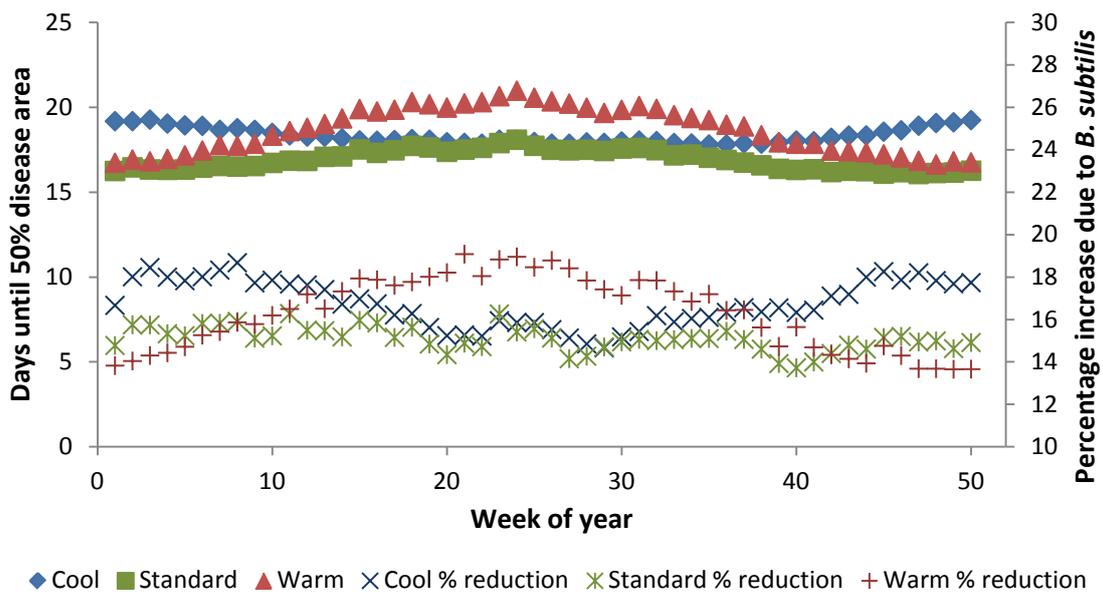


Fig 27 Time until 50% disease leaf area coverage of *O. neolyopersici* treated with *B. subtilis* at weekly introductions across the year in a Venlo glasshouse at Bleiswijk, Netherlands (as predicted by model). Secondary y axis: Percentage increase in time until 50% disease leaf coverage compared to *O. neolyopersici* only. Cool = Standard-2°C, Warm = Standard+2°C.

In separate, but linked, work the effect of increased UV irradiation on *O. neolycopersici* and *B. subtilis* were investigated in a 45 day long semi-field trial using UV-selective plastic covers to differentially filter specific regions of the UV spectrum. Results show that +/- UV had no clear effect on *O. neolycopersici*, with the area of leaf covered in disease similar under each of the plastics for the duration of the experiment (Fig. 28). UV did appear have an effect on the ability of *B. subtilis* to impair the spread of the disease with greatest reductions in the disease observed under the UV-blocking and UV-B transmitting plastics (Fig. 28). However, measurements of ambient UV irradiance found negligible levels of UV-B meaning that light conditions under the UV-B transmitting plastic would essentially have been lacking any UV (similar to UV-). These results suggest that UV-A may be detrimental to the efficacy of *B. subtilis* although the effect may not be strong. As such it is unlikely that the increased incidence of UV measured greenhouse trial in the Netherlands will significantly alter the efficacy of *B. subtilis*.

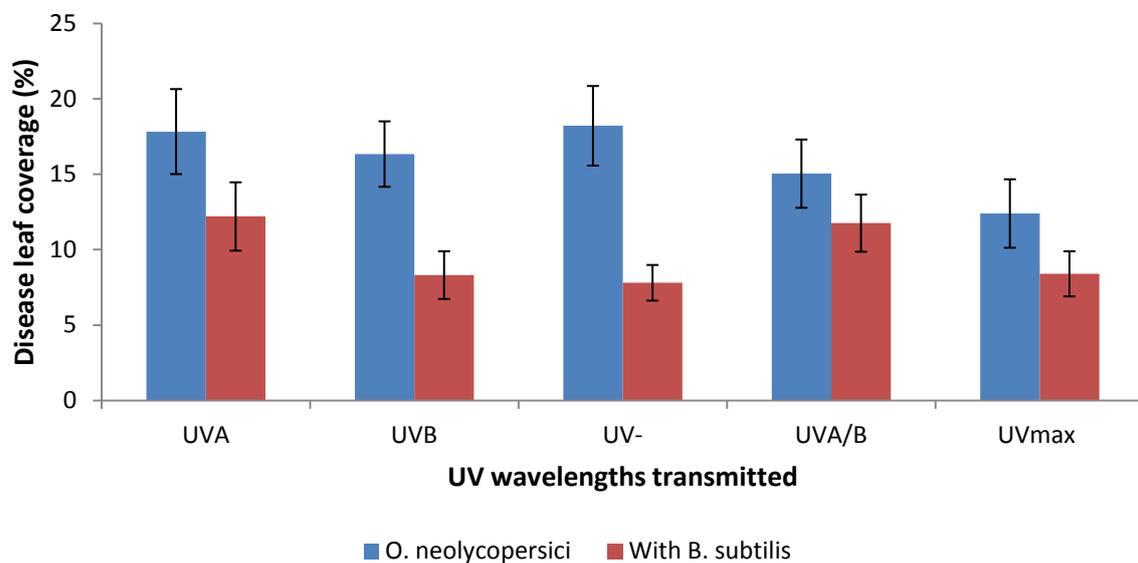


Fig. 28 Percentage disease leaf area coverage of *O. neolycopersici* on tomato leaves approximately 21 days after inoculation. Plants were kept under plastics transmitting differing sections of the UV spectrum as well as 90% of PAR. Plants were either treated with *O. neolycopersici* (ON) only or *O. neolycopersici* and a *B. subtilis* (BS) pre-treatment. Bars show standard error at 5% level.

## 7. Conclusions

### 7.1 Control of *T. urticae*

*P. persimilis* is currently the most widely used control agent of *T. urticae*. Experimental work as part of the project found that its predation behaviour was strongly influenced by humidity. When modelled in semi-closed and passively ventilated Spanish greenhouses its control efficacy was found unchanged by the different climatic conditions. However, introduction density and frequency of *P. persimilis* had large effects on control success. At an introduction density of just 10 per plant (1000 per occasion) *P. persimilis* was able to provide very good control in both climate scenarios. When *P. persimilis* was introduced at one per plant per occasion in the greenhouse it was able to prevent *T. urticae* from reaching infestation levels (10000 per plant) most of the time but rarely controlled the pest. At lower introduction rates *T. urticae* populations consistently remained uncontrolled. Coexistence was predicted for the final weeks of the year, but this was in part constrained by the model. For purposes of interpretation it is likely that outcomes for the final weeks of the year would be similar to those at the beginning of the year, which were for good control. Weekly introductions gave the best control, but fortnightly are adequate.

Overall, the model shows that the new design for semi-contained greenhouses is certainly not detrimental to P&D control. Indeed, it is likely to be more favourable for pest control using biological control agents than the current passive designs, although some differences were minor. However, if the grower wishes to introduce 10 *P. persimilis* per plant each week then control was good in both climate scenarios/greenhouse designs. Such an introduction density and frequency should not represent an excessive financial outlay for growers, approx £11.40 per week per 100 plants in the UK (Biowise Ltd., UK).

Better efficacy can be expected if *P. persimilis* were placed in pest 'hot spots' (they were randomly allocated to plants in the model). Early pest and disease detection technology such as e-nose, gas chromatography-mass spectrometry, infra-red, etc., may help identify such infection foci (EUPHORUS WP 5). Furthermore, *T. urticae* aggregations are familiar occurrences in the greenhouse (Skirvin *et al* 2002), making both local detection and the success of local treatments far more likely. In this model we assumed that *T. urticae* were distributed randomly across the plants on arrival so, given that, it is likely that control was underestimated.

Further analysis will be needed to implement a fully integrated biological and environmental control system with agents not studied or specified here. For instance, in the cooler months temperatures may drop to a level at which *P. persimilis* are not active enough to exert satisfactory control,

especially in the passively ventilated greenhouse. In these situations it would be advisable to introduce alternative biological control agents with greater tolerance to low temperatures, such as *A. californicus*. Nevertheless, the availability of pest and control agent models can now be offered for integration into greenhouse computer management information systems to alarm growers when conditions become likely to support pest population explosions and advise them of suitable biological control agents and their doses.

### 7.2 Control of *Oidium neolycopersici*

The work on *B. subtilis* shows that this agent does limit the growth of *O. neolycopersici*; however temperature and humidity influence its efficacy. The greatest disease control was seen at high (around 90% RH) humidity levels, but maintaining these conditions in the greenhouse could risk problems with other diseases.

Model predictions of *B. subtilis* control show that changing Tunnel designs in Spain from passively ventilated to a semi-closed system would improve its efficacy by increasing its latency period and decreasing the rate of disease development. In both systems *B. subtilis* is most effective from spring to autumn.

Model predictions for the Netherlands show that the conditions in the standard Venlo climate system are also conducive to the control of by *B. subtilis*, although the benefit is less marked than in Spain under polythene.

Currently *O. neolycopersici* outbreaks are predominantly controlled with chemicals and there are few alternatives available to growers. The EUPHORUS results show that *B. subtilis* may provide a suitable option to growers seeking an IPM-compatible control agent for *O. neolycopersici*. However, ideal conditions for the activity of the control agent were found to occur infrequently in any of the greenhouse systems studied. Unfortunately these ideal conditions also present ideal environments for other diseases.

The reductions in disease pressures provided by *B. subtilis*, as predicted by the model, may not be sufficient for growers to rely on. However, the model is based on data from experiments where a single application of *B. subtilis* was made one day prior to inoculation with *O. neolycopersici*. It is possible that applying regular (weekly or fortnightly) applications of *B. subtilis* could improve control. Furthermore, as *B. Subtilis* had not previously been used for the control of *O. neolycopersici* the application concentration used was that recommended for another pathogen (*B. cinerea*). Therefore

further work is required to fully optimise the application of *B. subtilis* and its efficacy as a control agent of *O. neolycopersici* (and other pathogens). At times when *B. subtilis* proves inadequate it is recommended that growers use sulphur-based chemical controls, which are more IPM compatible than other chemical alternatives.

The models we have developed confirm that novel greenhouse systems, such as semi-closed climate management in Spain and novel greenhouse covers in the Netherlands, are likely to have consequences for the efficacy of biological control agents. Clearly, in Spain greenhouse conditions vary widely across the year and we have shown that the effectiveness of control agents can also vary. The models allow periods of good and poor performance to be identified, helping identify times when close crop monitoring and other control options may be necessary. In the Netherlands, climate control is highly effective so that changes in design appear to have little impact on temperature and RH. Hence the efficacy of biological control agents varies little and control solutions will be selected on the basis of other market pressures. Overall, modelling approaches allow the effectiveness of control strategies to be forecast, tailored to the greenhouse conditions and for advising greenhouse management systems of incipient problems with pests, diseases and their biological control agents. By supporting greenhouse management systems in this way the models promote sustainable pest and disease management and the reduced use of plant protective chemicals. In order for maximum benefit, management data need to be supplemented with sensor readings so that the widest set of information is presented to the grower.

**Annex 1:** Table of biocontrol products available for protected crops from Syngenta Bioline ICM (Integrated Crop Management products):

<http://www.syngenta.com/global/Bioline/en/about-us/Pages/About-us.aspx>

Products:

Control agent	Controls	Useful in crops	Also controls
<p><i>Amblyseius andersoni</i> (predatory mite)</p> <p><b>(Anderline aa formulation)</b> <b>(Bugline andersoni)</b></p>	spider mite	<ul style="list-style-type: none"> <li>• Aubergine</li> <li>• Cucumber</li> <li>• Cut Flowers</li> <li>• Melons</li> <li>• Ornamental pot plants</li> <li>• Pepper</li> <li>• Tomato</li> </ul>	<p>Broad Mite and Cyclamen Mite (<i>Polyphagotarsonemus latus</i> and <i>Phytonemus pallidus</i>)</p> <p>Spider Mite, Two Spot or Red - (<i>Tetranychus urticae</i> and <i>Tetranychus cinnabarinus</i>)</p> <p>Tomato Russet/Rust mite (<i>Aculops lycopersici</i>/<i>Eriophyidae</i>)</p>
<p><i>Amblyseius (Neoseiulus) californicus</i> (predatory mite)</p> <p><b>(Amblyline cal)</b></p>	Spider mite (and other arthropods and pollen)	<ul style="list-style-type: none"> <li>• Aubergine</li> <li>• Cucumber</li> <li>• Cut Flowers</li> <li>• Melons</li> <li>• Ornamental pot plants</li> <li>• Pepper</li> <li>• Tomato</li> </ul>	Remains active at high temp and low humidity
<p><i>Amblyseius cucumeris</i> <b>Amblyline cu CRS</b></p>	Mites and Thrips	<ul style="list-style-type: none"> <li>• Aubergines</li> <li>• Cucumber</li> <li>• Cut Flowers</li> <li>• Melons</li> <li>• Ornamental pot plants</li> <li>• Pepper</li> </ul>	
<p><i>Amblyseius (Typhlodromips) montdorensis</i> (predatory mite)</p> <p><b>(Montyline am)</b></p>	thrips and whitefly	<ul style="list-style-type: none"> <li>• Aubergine</li> <li>• Cucumber</li> <li>• Cut Flowers</li> <li>• Melons</li> <li>• Ornamental pot plants</li> <li>• Pepper</li> <li>• Tomato</li> </ul>	<p>Broad Mite and Cyclamen Mite (<i>Polyphagotarsonemus latus</i> and <i>Phytonemus pallidus</i>)</p> <p>Thrips - WFT (<i>Frankliniella occidentalis</i>) and Onion thrip (<i>Thrips tabaci</i>)</p> <p>Whitefly, Glasshouse (<i>Trialeurodes vaporariorum</i>)</p> <p>Whitefly, Tobacco or Silverleaf Whitefly (<i>Bemisia</i>)</p>

			<i>tabaci</i> )
<p><i>Amblyseius</i> (<i>Typhlodromips</i>) <i>swirskii</i> (predatory mite)</p> <p>Including formulation <b>(Amblyline cu Flo)</b> <b>(Bugline swirskii)</b></p>	<p>Whitefly</p> <p>Thrips</p>	<ul style="list-style-type: none"> <li>• Aubergine</li> <li>• Cucumber</li> <li>• Cut Flowers</li> <li>• Melons</li> <li>• Ornamental pot plants</li> <li>• Pepper</li> </ul>	<p>Thrips - WFT (<i>Frankliniella occidentalis</i>) and Onion thrip (<i>Thrips tabaci</i>)</p> <p>Whitefly, Glasshouse (<i>Trialeurodes vaporariorum</i>)</p> <p>Whitefly, Tobacco or Silverleaf Whitefly (<i>Bemisia tabaci</i>)</p>
<p><i>Aphelinus</i> <i>abdominalis</i> (aphid parasitoid)</p> <p><b>(Apheline ab)</b> <b>(Aphiline Ace Mix)</b></p>	Aphids	<ul style="list-style-type: none"> <li>• Aubergine</li> <li>• Cucumber</li> <li>• Cut Flowers</li> <li>• Melons</li> <li>• Ornamental pot plants</li> <li>• Pepper</li> <li>• Tomato</li> </ul>	
<p><i>Aphidius colemani</i> (aphid parasitoid)</p> <p><b>(Aphiline Ace Mix)</b> <b>(Aphiline c)</b></p>	Aphids	<ul style="list-style-type: none"> <li>• Aubergine</li> <li>• Cucumber</li> <li>• Cut Flowers</li> <li>• Melons</li> <li>• Ornamental pot plants</li> <li>• Pepper</li> </ul>	<i>Aphis gossypii</i> , <i>Myzus persicae</i> and <i>Myzus nicotianae</i>
<p><i>Aphidius ervi</i> (parasite)</p> <p><b>(Aphiline Ace Mix)</b> <b>(Aphiline e)</b></p>	Aphids	<ul style="list-style-type: none"> <li>• Aubergine</li> <li>• Cucumber</li> <li>• Cut Flowers</li> <li>• Melons</li> <li>• Ornamental pot plants</li> <li>• Pepper</li> </ul>	
<p><i>Aphidoletes</i> <i>aphidimyza</i> (predator)</p> <p><b>(Aphidoline aa)</b></p>	Aphids	<ul style="list-style-type: none"> <li>• Aubergine</li> <li>• Cucumber</li> <li>• Cut Flowers</li> <li>• Melons</li> <li>• Ornamental pot plants</li> <li>• Pepper</li> </ul>	
<p><i>Atheta (Taxicera)</i> <i>coriaria</i> (predator)</p>	<p>sciarid fly (<i>Bradysia paupera</i> /<i>spp</i>) and shore fly (<i>Scatella spp</i>)</p>	<ul style="list-style-type: none"> <li>• Cut Flowers</li> <li>• Ornamental pot plants</li> </ul>	
Chrysoperla carnea	Aphids	<ul style="list-style-type: none"> <li>• Cut Flowers</li> <li>• Ornamental pot</li> </ul>	Mealy bug

(aphid predator as larva) <b>(Chrysoline c)</b>		<ul style="list-style-type: none"> <li>plants</li> <li>• Pepper</li> <li>• Tomato</li> </ul>	
Encarsia Formosa (whitefly parasitoid)	Whitefly ( <i>Trialeurodes vaporariorum</i> )	<ul style="list-style-type: none"> <li>• Aubergine</li> <li>• Cucumber</li> <li>• Cut Flowers</li> <li>• Melons</li> <li>• Ornamental pot plants</li> <li>• Pepper</li> <li>• Tomato</li> </ul>	
Eretmocerus eremicus (pupae of the whitefly parasitoid)	Whitefly ( <i>Trialeurodes vaporariorum</i> )	<ul style="list-style-type: none"> <li>• Aubergine</li> <li>• Cucumber</li> <li>• Cut Flowers</li> <li>• Melons</li> <li>• Ornamental pot plants</li> <li>• Pepper</li> <li>• Tomato</li> </ul>	( <i>Bemisia tabaci</i> ) Can tolerate higher temperatures than <i>Encarsia</i>
Dacnusa sibirica (leafminer parasitoid)	Leafminer ( <i>Liriomyza spp/Phytomyza syngensia</i> )	<ul style="list-style-type: none"> <li>• Tomato</li> </ul>	
Diglyphus isaea (parasitoid)	Leafminer ( <i>Liriomyza spp/Phytomyza syngensia</i> )	<ul style="list-style-type: none"> <li>• Cut Flowers</li> <li>• Ornamental pot plants</li> <li>• Tomato</li> </ul>	Aphids Mealy bugs
<i>Eretmocerus eremicus</i> Parasitic wasp <b>(Eretline e)</b>	Whitefly <i>Trialeurodes vaporariorum</i> and <i>Bemisia tabaci</i>	<ul style="list-style-type: none"> <li>• Aubergine</li> <li>• Cucumber</li> <li>• Cut Flowers</li> <li>• Melons</li> <li>• Ornamental pot plants</li> <li>• Pepper</li> <li>• Tomato</li> </ul>	
<i>Eretmocerus mundus</i> Parasitic wasp <b>(Eretline m)</b>	Whitefly <i>Bemisia tabaci</i>	<ul style="list-style-type: none"> <li>• Aubergine</li> <li>• Cucumber</li> <li>• Melons</li> <li>• Ornamental pot plants</li> <li>• Pepper</li> <li>• Tomato</li> </ul>	
Feltiella acarisuga (predator) (Cecidomyid midge with predatory larvae) <b>(Feltiline a)</b>	Spider Mite Two Spot or Red ( <i>Tetranychus urticae</i> and <i>Tetranychus cinnabarinus</i> )	<ul style="list-style-type: none"> <li>• Aubergine</li> <li>• Cucumber</li> <li>• Cut Flowers</li> <li>• Ornamental pot plants</li> <li>• Pepper</li> <li>• Tomato</li> </ul>	

Hypoaspis miles (predatory mite)	Sciarids/Fungus gnats <i>(Bradysia paupera /spp)</i>	<ul style="list-style-type: none"> <li>• Cut Flowers</li> <li>• Ornamental pot plants</li> </ul>	Thrips - WFT ( <i>Frankliniella occidentalis</i> ) and Onion thrip ( <i>Thrips tabaci</i> )
Macrolophus caliginosus (predatory mired bug – generalist feeder)  <b>(Macroline c)</b>	Whitefly ( <i>Trialeurodes vaporariorum</i> )	<ul style="list-style-type: none"> <li>• Aubergine</li> <li>• Tomato</li> <li>• Pepper</li> </ul>	Whitefly, Tobacco or Silverleaf Whitefly ( <i>Bemisia tabaci</i> )  South American Tomato Leaf Miner ( <i>Tuta absoluta</i> )  Caterpillars ( <i>Lepidoptera spp</i> )
Nesidiocoris tenuis (predatory mired bug – generalist feeder)  <b>(Nesiline t)</b>	Whitefly ( <i>Trialeurodes vaporariorum</i> )	<ul style="list-style-type: none"> <li>• Aubergine</li> <li>• Tomato</li> <li>• Pepper</li> </ul>	Whitefly, Tobacco or Silverleaf Whitefly ( <i>Bemisia tabaci</i> )  South American Tomato Leaf Miner ( <i>Tuta absoluta</i> )  Caterpillars ( <i>Lepidoptera spp</i> )
<i>Orius majusculus</i> <b>(Oriline m)</b> <i>Orius laevigatus</i> <b>(Oriline l)</b> <i>Orius insidiosus</i> <b>(Oriline i)</b>	Thrips - WFT ( <i>Frankliniella occidentalis</i> ) and Onion thrip ( <i>Thrips tabaci</i> )	<ul style="list-style-type: none"> <li>• Pepper</li> <li>• Strawberry</li> <li>• Ornamental pot plants</li> </ul>	Often follow-up treatment to <i>Amblyseius</i>
Phytoseiulus persimilis (spider mite predator)  <b>(Phytoline p)</b>	Spider Mite Two Spot or Red - ( <i>Tetranychus urticae</i> and <i>Tetranychus cinnabarinus</i> )	<ul style="list-style-type: none"> <li>• Aubergine</li> <li>• Cucumber</li> <li>• Cut Flowers</li> <li>• Melons</li> <li>• Ornamental pot plants</li> <li>• Pepper</li> <li>• Tomato</li> </ul>	
Tricholine b (parasite - wasp)	Caterpillars ( <i>Lepidoptera spp</i> )	<ul style="list-style-type: none"> <li>• Aubergine</li> <li>• Blueberries</li> <li>• Cucumber</li> </ul>	

		<ul style="list-style-type: none"> <li>• Cut Flowers</li> <li>• Melons</li> <li>• Ornamental pot plants</li> <li>• Pepper</li> <li>• Tomato</li> </ul>	
Trichogramma brassicae (wasp parasitoid of Lepidopteran eggs)	Thrips - WFT ( <i>Frankliniella occidentalis</i> ) and Onion thrip ( <i>Thrips tabaci</i> )	<ul style="list-style-type: none"> <li>• Aubergine</li> <li>• Cucumber</li> <li>• Cut Flowers</li> <li>• Melons</li> <li>• Ornamental pot plants</li> <li>• Pepper</li> <li>• Tomato</li> </ul>	
Adaline b (ladybird larvae - predators)	Aphids	<ul style="list-style-type: none"> <li>• Aubergines</li> <li>• Cut Flowers</li> <li>• Melons</li> <li>• Ornamental pot plants</li> <li>• Pepper</li> </ul>	
<i>Atheta coriaria</i> (Staphylinid beetle that is a predator of soil and compost pests) <b>Staphyline c</b>	sciarid flies ( <i>Bradysia spp</i> , <i>Lycoriella spp</i> , and <i>Sciara spp</i> ) and shore flies ( <i>Scatella spp</i> ).	<ul style="list-style-type: none"> <li>• Cut Flowers</li> <li>• Ornamental pot plants</li> </ul>	
<i>Anthocoris nemoralis</i> (predatory bug) <b>(Antholine n)</b>	Pear Psylla ( <i>Cacopsylla pyri</i> )		Orchard crops outdoors
Nematodes <b>(Exhibitline h)</b>	Vine weevil ( <i>Otiorhyncus sulcatus</i> )	<ul style="list-style-type: none"> <li>• Ornamental pot plants</li> </ul>	Chafer grubs
<i>Diglyphus isaea</i> (ectoparasitic wasp) <b>(Digline i)</b>	Leafminers <i>Liriomyza sp. Phytomyza sp</i>	<ul style="list-style-type: none"> <li>• Cut Flowers</li> <li>• Ornamental pot plants</li> <li>• Tomato</li> </ul>	
<i>Steinernema feltiae</i> (insect pathogenic nematode) <b>(Exhibitline sf)</b>	Sciarid Fly	<ul style="list-style-type: none"> <li>• Cut Flowers</li> <li>• Ornamental pot plants</li> </ul>	

Annex 2:

Details of the bio-fungicide Serenade (*Bacillus subtilis*) are available from the BASF website [http://www.agro.basf.com/agr/AP-Internet/en/content/solutions/solution\\_highlights/serenade/bio-fungicide](http://www.agro.basf.com/agr/AP-Internet/en/content/solutions/solution_highlights/serenade/bio-fungicide)