Interface of the environment and occurrence of *Botrytis cinerea* in pre-symptomatic tomato crops.

Alison Wakeham*, Allen Langton†, Steve Adams†† and Roy Kennedy†††

*Institute of Science and the Environment, University of Worcester, WR2 6AJ UK.

† Warwick Crop Centre, The University of Warwick, Wellesbourne, Warwick, CV35 9EF

††, Plant Impact plc, Rothamsted, West Common, Harpenden, Hertfordshire, AL5 2JQ

†††

Abstract

Botrytis cinerea (Grey mould) is a necrotrophic fungus infecting over 230 plant species worldwide. It can cause major pre- and post-harvest diseases of many agronomic and horticultural crops. *Botrytis cinerea* causes annual economic losses of 10 to 100 billion US dollars worldwide and instability in the food supply (Jin and Wu, 2015). Gray mould losses, either at the farm gate or later in the food chain, could be reduced with improved knowledge of inoculum availability during production. In this paper, we report on the ability to monitor *Botrytis* spore concentration in glasshouse tomato production ahead of symptom development on plants. Using a light weight and portable air sampler (microtitre immunospore trap) it was possible to quantify inoculum availability within hours. Also, this study investigated the spatial aspect of

the pathogen with an increase of *B. cinerea* concentration in bio-aerosols collected in the lower part of the glasshouse (0.5m) and adjacent to the trained stems of the tomato plants. No obvious relationship was observed between *B. cinerea* concentration and the internal glasshouse environmental parameters of temperature and relative humidity. However the occurrence of higher outside wind speeds did increase the prevalence of *B. cinerea* conidia in the cropping environment of a vented glasshouse. Knowledge of inoculum availability at time periods when the environmental risk of pathogen infection is high should improve the targeted use and effectiveness of control inputs.

Key words: bio-aerosol, immunoassay, disease, environment, integrated disease management

Introduction

Botrytis cinerea (Grey mould) is a ubiquitous, necrotrophic fungus infecting over 230 plant species worldwide. It causes major pre- and post-harvest diseases of many agronomic and horticultural crops, resulting in annual economic losses of 10 to 100 billion US dollars worldwide and instability of food supply (Jin and Wu, 2015). Tomato can be particularly badly affected, with significant pre and post-harvest losses worldwide (Dik and Wubben, 2007; Eden *et al*., 1996; O'Neill *et al*., 1997). *Botrytis* can infect all parts of the tomato plant, but the infection of tomato stems in long-season, high-wire crops can be particularly damaging. The fungus invades stems via petioles or wounds resulting from normal pruning and harvesting, and this can lead to stem girdling, wilting and ultimate plant death (O'Neill *et al*., 1997). Infection of the fruit stalk often leads to rots and premature fruit fall, and ghost-

spotting on the fruit (thought to be caused by *Botrytis spore germination*) adversely affects marketability.

Worldwide, *Botrytis* is probably the most difficult tomato disease to control. Correct identification of the disease is vital, as it is easily confused with gummy stem blight (*Mycosphaerella melonis*). No varietal resistance to *Botrytis* exists although, the physiological age of the host plant tissues is a major factor determining the incidence and severity of *Botrytis* infection (Coertze and Holz,1999) and varieties do appear to differ in susceptibility (Dik and Wubben, 2007). Current best practice is to minimise pesticide use and control of *Botrytis* is by the manipulation of environmental conditions and good husbandry. The large amount of literature on the effect of environment on *B. cinerea* suggests that infection by the pathogen is little affected by temperature within the range 5-26^{o}C, but is greatly affected by atmospheric humidity (Jarvis, 1980; O'Neill *et al*., 1997). The minimum period required for infection in "saturated air" is 15 h (Bulger, 1987). However, germination and infection is much more rapid in the presence of free water (6 – 8 h) (Elad and Yumis, 1993). To date it is not clear if the presence of free water plays a significant role in the epidemiology of *Botrytis* on tomato crops in the UK. Additionally the spatial variation in the pathogen within the crop cannot be easily ascertained in relation to the environmental conditions necessary for epidemic development. In this study we investigate the occurrence of *Botrytis* in pre symptomatic tomato crops in relation to environmental conditions.

Materials and Methods

1.1 Tomato plants used in glasshouse experiments

Tomato plants, (cv. Encore and Elegance) were grown in a long-season, high-wire,

rockwool growing system following standard UK glasshouse growing methods. Plants were irrigated daily with a standard nutrient solution delivered using a Priva glasshouse control system (www.priva.co.uk) set to provide a feed pH of 5.2. The input EC was initially set to 5mS.cm^{-1}, and gradually reduced every fourteen days until it reached an EC of 2.8mS.cm^{-1}. The average day temperature in the glasshouse was 21°C while night temperature was maintained at 16°C. Canopy management (including leaf removal) was carried out as for a commercial tomato crop. Plants were fumigated with 2g per litre Thiovit (a.i. sulphur) [Syngenta Crop Protection UK Ltd., Whittlesford, Cambridge, CB2 4QT, UK] against powdery mildew and 1g per litre Rovral (a.i. Iprodione) [BASF Plc., Agric Division, Cheshire, UK] was sprayed three times for *Botrytis cinerea* control. Biological pest control [Fargo Ltd., Littlehampton, UK] was used: *Encarcia*,a parasitic wasp which lays eggs into whitefly scales (3 wasps per 1m^2); *Phytoseilus*, a red predatory mite which attacks all stages of spider mites (5-10 mites per m^2) and *Amblyseius*, a predatory mite which feeds on thrip larvae (50-250 mites per m^2). Plants were not artificially inoculated with spores of *B. cinerea*. The crop was managed as a long season commercial crop. Air sampling was conducted in the glasshouse when the plants were 10 months old and prior to *Botrytis* symptom development.

1.2 Production of *Botrytis cinerea* spore suspensions

Potato dextrose agar growth medium was prepared by suspending 39g PDA (Oxoid Ltd., Hampshire, England) in 1litre of distilled water. The medium was boiled to dissolve completely, and sterilised at 121°C for 15mins. The medium was mixed and poured into Petri dishes (20ml per plate) in a flow hood (BH 12R, Labcaire Systems Ltd., Somerset England) on cooling. A sterile PN6026 Supor 450 90mm diameter membrane disc was added to each agar plate. A stock culture of *B. cinerea* was obtained from a culture collection at Warwick HRI. From this a 5 mm plug was taken and sub cultured on to the membrane of each coated potato dextrose agar Petri dish.

Plates were sealed with parafilm and incubated in the dark at 20°C. Membranes were removed and 5ml of phosphate buffered saline (PBS) was added. Conidial surface washings were taken by gently stroking the surface of the membrane with a sterile glass rod. The resulting spore suspensions were combined and a conidial count made using a haemocytometer and bright field microscopy. This suspension was used in ELISA protocols for the estimation of *Botrytis cinerea* in air samples taken from glasshouses.

1.3 Bio-aerosol sampling

1.3.1 Positional effect of bio-aerosol samplers on *B. cinerea* collection.

Microtiter immunospore trapping (MTIST) samplers (Wakeham et al. 2004) were used to monitor glasshouse bio-aerosol concentrations at locations within a glasshouse. The device was operated by a standard Burkard turbine suction unit and air was drawn through the system at a constant rate of 57 litres min^{-1} (www.burkard.co.uk). Particulates in the airstream are channelled through 48 delivery trumpet nozzles and directed across the base of each 4x8 microtiter well. The MTIST was operated by connecting to a 240-V electric supply (Kennedy et al. 2000). Three MTIST devices were placed at different levels in the tomato glasshouse to determine the positional effects of spore traps on spore numbers. One trap was positioned above the tomato canopy, 2.2m high; another trap positioned within the canopy, 1.5m high; while the lowest was positioned close to the tomato stem bundles at a height of 0.5m. All traps were on the same vertical axis.

Microtiter wells (4x8 well microtitre strips: Catalogue No. 469957, Nunc Immunodiagnostics, Life Technologies Ltd. Paisley, Scotland) were coated with a mixture of petroleum jelly and paraffin wax before being inserted within an MTIST device (Wakeham et al. 2004). The mixture was dissolved in hexane in the proportion 1:32 prior to coating the base of each microtiter well (100µl per well). The strips were

incubated for 1 hour at 20ºC. An inverted binocular microscope (Nikon model TMS) was used to check that the well coatings had been applied evenly. The 4x8 well coated microstrips were exposed to glasshouse bio-aerosols for a 24hr periods. After this the strips were removed and stored at -20°C.

1.3.2 Effect of environment on *B. cinerea* concentration in bio-aerosols

A Burkard 7 day volumetric spore sampler (www.burkard.co.uk/7dayst.htm) was operated continuously in the crop. The volumetric air sampler is based on the Hirst spore trap (Hirst, 1952) and can be used to determine time periods when airborne microflora are present. The measurements from this type of trap form the basis of a large amount of knowledge of aerobiological systems (Kennedy and Wakeham, 2015).

1.4 Measurement of *Botrytis cinerea* concentration by immunoassay

1.4.1 PTA ELISA working dilution of *B. cinerea* polyclonal antibody (PAb)

A polyclonal antibody raised to non-germinated conidia of *Botrytis cinerea* (coded 94/4/3) was titrated against its homologous antigen in an indirect plate-trapped antigen ELISA (PTA-ELISA). Twenty eight paired wells of a 96 well Nunc Immunosorbent Polysorp flat-bottomed microtiter plate (Life Technologies, Paisley, Scotland; cat. no 475094A) were coated with 100µl per well of *B. cinerea* adjusted to 1x 10^4 condia ml^{-1} in PBS. A further fourteen paired wells received 100µl per well of PBS alone. The wells were incubated overnight under natural light and at room temperature to allow the conidia, where present, to adhere to the base of the microtitre well. To enhance this process the wells were not covered but allowed to dry (Wakeham et al. 2004). Wells were washed once with PBS (100µl per well) for 1 minute after which the wells were blocked with 200µl 1% casein (1% [wt/vol] casein in PBS) and incubated in a Wellwarm shaker incubator (Denley Instruments Ltd, Sussex, UK) at 30°C for 30 minutes. Residual blocking buffer was removed and wells

were washed once for 1 minute with 200µl per well with PBS 0.05% Tween 20 0.1% casein. The polyclonal antibody (Warwick HRI 94/4/3) was diluted 1:10 in PBS 0.05% Tween 20 0.1% casein, 1:50 and subsequent doubling dilutions made to 1:102400. The serum dilutions were applied to paired wells at 100µl per well and incubated in a Wellwarm shaker incubator (Denley Instruments Ltd, Sussex, UK) at 30°C for 45 minutes. To determine endogenous phosphatase activity, 14 of the paired wells which had been coated with *B. cinerea* conidial suspension received PBS 0.05% Tween 20 0.1% casein alone. Following incubation unbound material was removed and wells washed three times for 1 min each with PBS 0.05% Tween 20 0.1% casein. Aliquots of 100µl of goat anti-rabbit IgG (whole molecule) alkaline phosphatase (Sigma A-3687) diluted in PBS 0.05% Tween 20 0.1% casein (5µl in 30ml PBS Tween casein) were added to each well and incubated as above. After three washes, 100µl per well of 1mg per ml p-nitrophenyl phosphate (pNPP) (Sigma N-2770), freshly dissolved in deionised water was added. The plates were incubated at room temperature in darkness for 40 min and absorbance values were read at filter wavelengths of 405nm and 630nm in a Biohit BP 800 ELISA plate reader (Alpha Laboratories, 40, Parham Drive, Eastleigh, Hampshire, UK). Mean values were calculated for each of the paired wells.

1.4.2 Polyclonal antiserum reactivity tests

The polyclonal antiserum (94/4/3) was screened for reactivity with a range of plant pathogenic fungi and oomycetes (Table 1). Spores were collected in PBS, and the concentration was adjusted to about 1x 10^4 spores per ml and a PTA-ELISA was carried out. 100µl of each fungal spore test suspension was pipetted into wells of an 8-well microtiter strip. Six of the eight wells had fungal spore suspension and the remaining two wells received PBS alone to serve as negative control. The microtiter strips were incubated overnight at room temperature, before removal of the unbound material and washing with 100µl PBS per well. Wells were blocked with 200µl 1%

casein (1% [wt/vol] casein in PBS) and incubated in a Wellwarm shaker incubator at 30°C for 30 minutes. Residual blocking buffer was removed and wells were washed once for 1 minute with 200µl per well with PBS 0.05% Tween 20 0.1% casein. Four wells of each microtiter strip received 100µl of PAb 94/4/3 in PBS 0.05% Tween 20 0.1% casein (1:400). The remaining four wells of each strip received 100µl PBS 0.05% Tween 20 0.1% casein alone. The strips were incubated as described previously for 45 minutes. The remainder of the PTA ELISA was carried out as described above.

1.4.3 Antiserum detection sensitivity for *B. cinerea*

The polyclonal antibody (coded: 94/4/3) was titrated against different concentrations of *B. cinerea* conidia in an indirect PTA-ELISA. The conidial suspension was prepared and this time adjusted to 1×10^5 conidia ml^{-1}. Ten-fold dilutions were made to a concentration of 10 conidia ml^{-1}. For each conidial dilution eight microtiter wells were each coated with 100µl per well of the conidial suspension. An 8 well control strip of PBS alone was included. Wells were incubated overnight and air dried as previous. Wells were washed with PBS (100µl per well) for 1 minute before blocking with 200µl 1% casein (1% [wt/vol] casein in PBS) and incubated in a Wellwarm shaker incubator at 30°C for 30 minutes. The remainder of the PTA indirect ELISA process was as described above and with PAb 94/4/3 at a working dilution of 1:400. As a negative control, endogenous phosphatase activity of *B. cinerea* conidia was checked by the omission of the polyclonal antibody (94/4/3) stage at each conidial concentration.

1.4.4 Immunoquantification of *B. cinerea* conidia trapped by the MTIST air sampler.

The 4x8 well microtiter strips which were removed after each glasshouse sampling period (24 hrs) and the total number of *B. cinerea* conidia in each well was

determined using an Olympus CK2 binocular microscope (x 200) (Kennedy et al., 2000). A PTA-ELISA was carried out using the polyclonal antiserum (94/4/3) as previously described. To accommodate the potential of endogenous phosphatase activity within the collected bio-aerosol an internal control for each 24hr period was included. Of the four microtitre strips (4x8 wells) one strip received all stages of the ELISA process but in the absence of the primary antibody (94/4/3). The mean value derived from this was removed from the mean absorbance value of each microtitre well strip that was processed for that sampling period. The results of the *B. cinerea* microtitre well microscopic conidial counts and the corresponding MTIST ELISA absorbance values were compared.

1.5 Glasshouse Data Analysis

Glasshouse experiments were analysed using a generalised linear model with a Poisson distribution and log link function (GenStat statistical package)The effects of internal temperature and relative humidity, and outside wind speed on the number of spores counted on tapes (Hirst style bio-aerosol sampler) and the MTIST wells were investigated.

Results

2.1 Measurement of *Botrytis cinerea* concentration by immunoassay

2.1.1 Specificity and sensitivity of *B. cinerea* polyclonal antibody (PAb) by ELISA

A working dilution of 1:400 PAb 94/4/3 was used in PTA ELISA. At this dilution the PTA ELISA value for a *B. cinerea* spore concentration fell on the linear part of a curve and achieved an absorbance value of approximately 1 OD. The detection limit of the assay for non-germinated *B. cinerea* conidia applied in solution was in the

region of 100 per microtitre well. Across the concentration range tested confidence intervals were fitted to the means at the p=0.05 level (Figure 1). With the exception of *Botrytis narcissicola* and *Sclerotinia sclerotiorum* the antiserum 94/4/3 (IgG fraction tested) showed limited reactivity with the fungal and oomycete spore types tested (<20 % recognition compared to *B. cinerea*). For each spore type a standardised concentration of $1x10^4$ ml^{-1} had been tested by PTA ELISA. Interestingly, although the antiserum reacted with both *B. allii* and *B. narcissicola* the greatest interaction was to *S. sclerotiorum* (Figure 2).

2.2 Measurement of MTIST trapped *B. cinerea* conida in glasshouse grown crop bio-aerosols.

Examination of the base of the microtitre wells using a Nikon model TMS inverted binocular microscope at a magnification of X200 with bright field illumination identified *B. cinerea* conidia present in all the microtiter wells (Figure 3). The conidia were distributed throughout the base of the microtiter wells, but the greatest numbers occurred in the centre of each well. The distribution effect within strips was not enumerated but it has been reported (Kennedy, 2000). When the collected 24hr bio-aerosols (31/10 to the 6/11/2006) were processed by PTA ELISA a correlation (polynomial, r^2 =0.7883) was recorded to the corresponding *B. cinerea* microcscopic well counts (Figure 4). However, using this approach the fitted relationship should not be extrapolated beyond the observed range of the data. An improved detection threshold was observed (20 *B. cinerea* conidia / microtitre well) by ELISA when the conidia were collected directly from a bio-aerosol on to the base of the microtitre well, rather than applied in solution (Section 2.1.1). This may be a result of the microtitre well coating material used in the glasshouse study (Section 1.3.1).

2.3 Positional effect of spore traps within glasshouses. A positional effect in the concentration of *B. cinerea* conidia trapped in microtiter wells strips was observed

when the MTIST sampling devices were placed at three different heights in the glasshouse (Figure 5). A polynomial function was fitted to the time series of trap catches at different heights. This gave r^2 values of 0.3511 (High), 0.3724 (Medium) and 0.4382 (Low) at different trapping heights. A positional effect in the concentration of *B. cinerea* was also observed with the corresponding ELISA absorbance. The mean spore counts at the three different heights was analysed using ANOVA and the result showed significant differences in the mean spore counts were present (p-value <0.001). The spore trap placed at a height of 0.5m (low level) collected the highest concentration of *B.cinerea* conidia for most trapping days. At a height of 1.5m (medium level) the concentration was reduced. The lowest conidial numbers were recorded for a sampling height of 2.2m (high level). On 04/11/05, mean spore counts were 60.29 when trapping was carried out at a height of 0.5m, 51.14 spores at height 1.5m while 38.71 spores were trapped at height 2.2m.

2.4 Effect of environment on *B. cinerea* concentration in bio-aerosols

Relative humidity, temperature within the glasshouse and outside wind speed of the site were averaged during the period of trapping and related to the number of spores trapped using the 24H Burkard volumetric air sampler. A regular pattern was observed for the glasshouse temperature, the average day temperature was 22°C and night temperature was 16°C throughout the bio-aerosol sampling period. No obvious relationship was observed between the spore counts and the environmental parameters (temperature, relative humidity and wind speed) considered (Figure 6). Correlation coefficients (r^2) of 0.383, and 0.042 were obtained when spore count was compared with temperature, and relative humidity.

However, a generalised linear model with a Poisson distribution and log link function did show a significant relationship between the numbers of trapped *Botrytis* conidia and the wind speed measured outside the glasshouse. This is shown in the

accumulated analysis of deviance table with the F. probability for wind speed <0.001 (Table 2).

Discussion

The results show that there was a significant amount of *Botrytis cinerea* present within asymptomatic glasshouse grown tomato crops. This occured in the form of airborne conidia, mainly in the lower part of the glasshouse (0.5m) adjacent to the trained stem bundles of the tomato plants. *Botrytis* lesions are often observed on tomato stem tissues where they can be very damaging to the crop. When lesions become extensive on the stem this leads to wilting and plant death. Plants at this stage in their development are particularly susceptible to the disease since their stems have many sites of injury caused by leaf removal and fruit harvesting. The source of the *Botrytis* spores in this trial is not known but it is usual to regard the pathogen as ubiquitous, entering the glasshouse from outside and surviving for long periods, often as quiescent stem infections (Neill *et al*., 1997). It may be that the higher spore levels low down in the glasshouse reflect proximity to sources of inoculum and/or the presence of significant air circulation promoted by the presence of low-level heating pipes (Kamp and Timmerman, 2002) and unhindered by “walls” of tomato foliage at higher levels. This increased air turbulence might well be expected to raise the aerial presence of conidial spores (Wakeham et al., 2004; Wakeham and Kennedy, 2010)

The occurrence of higher outside wind speeds increased the prevalence of *B. cinerea* conidia in the air. It has long been known that wind speed affects ventilation rates and, as wind speed increases, so does air exchange between inside and outside (Kamp and Timmerman, 2002). This, in turn, can be expected to increase

aerial turbulence within the glasshouse and may increase the number of spores borne aloft on air currents and able to be trapped. Another likely effect of increased air exchange due to wind speed is a reduction in internal glasshouse air temperature. This is because outside temperatures can be expected to be lower than the set-point glasshouse temperature. The heating system will counter this but whilst internal air temperature can increase rapidly, plant temperature tends to change more slowly. It is not uncommon, therefore, for the RH close to the stems (and based on stem temperature) to be much higher (and conducive to *Botrytis* spore germination) than appears to be the case when monitoring RH based on glasshouse air temperature in the aspirated screen (Adams *et al*., 2011). It appears, therefore, that high outside wind speeds could both give elevated numbers of conidial spores and the conditions around the stem bundles conducive to *Botrytis* infection. However the occurrence of moisture will vary spatially and be dependent on factors such as proximity to heating pipes etc. It may also be that *Botrytis* inoculum is lower early in the season but this was not tested. Younger plants generally have a lower susceptibility to infection by pathogenic organisms (Holderness & Pegg, 1989) but the effect of this was also not tested.

Measurement of pathogenic inoculum in bio-aerosols would assist in the prediction of disease occurrence. Traditionally, plant disease forecasting systems have relied upon environmental data alone to predict the risk of pathogenic inoculum in crops (Magarey et al. 2005, Scherm and van Bruggen, 1995). Mathematical models describing the effect of temperature and wetness on pathogen infection have been developed for many types of plant disease (Magarey et al. 2005). The ability however to detect and quantify pathogen inoculum at a time period when environmental risk for a disease is high would enable protective disease control strategies to be implemented more effectively (Wakeham and Kennedy, 2010). Integrating bio-aerosol sampling with immunological methods offers advantages in

363 the detection and quantification of target particles (Kennedy et al. 1999, Kennedy &
364 Wakeham, 2008). For example, it can improve the speed and reliability of detection
365 of targets in comparison to microscopic analysis (Kennedy and Wakeham, 2015).
366
367 The small lightweight portable MTIST air sampler demonstrates a use for this in
368 protected cropping systems. With appropriate antiserum the four microtitire strips
369 (4x8 wells) provides a capability to measure multiple pathogen types within a single
370 bio-aerosol sample. However, from an earlier study it is important that for each spore
371 type the well coating used is assessed for optimal trapping efficiency. Improved
372 MTIST collection for *Botrytis* spores and retention of these during the ELISA process
373 was observed when the microtitier wells were pre coated with a paraffin and hexane
374 base (Wakeham et al. 2004). This may explain why an improved detection sensitivity
375 of the ELISA assay was observed when *B. cinerea* spores were collected by
376 impaction on to a paraffin and hexane base rather than when aliquoted in a liquid
377 phase. Although, the PAb used in this study includes antibodies which recognise
378 epitopes produced at the mycelial stage and material exuded by the spore following
379 impact on the base of uncoated microtitre wells (Wakeham et al. 2004). In this study,
380 no germination of MTIST trapped conidia was observed when microtitre wells were
381 observed by bright field microscopy. If required, the pre-treatment of microtitre wells
382 with sodium azide (0.5mg ml^{-1}) has been shown useful to prevent germination of
383 some fungal spores (Wakeham and Kennedy, 2010).
384
385 For commercial activities an antibody probe with improved specificity would be
386 required. In reactivity studies, the *B. cinerera* PAb was observed to bind to antigenic
387 material of *S. sclerotiorum* when tested by ELISA. This is not surprising as they are
388 closely related necrotrophic plant pathogenic fungi with wide host ranges and
389 environmental persistence. The genomes also show high sequence identity and a

similar arrangement of genes (Amselem et al. 2011). Improved specificity of the test could be made with the use of a *B. cinerea* monoclonal antiserum (Meyer and Dewey, 2000).

Utilising environmental data and inoculum concentration should prove useful to determine disease threshold values. Positional effect should be considered with decreasing concentrations of *B. cinerea* observed with increasing height of the air sampler. Using rotating arm spore traps this vertical profile has been reported in the outside environment for the collection of the *Hymenoscyphus pseudoalbidus* (Chandelier et al. 2014, Peel et al. 2014). The MTIST bio-aerosol sampler offers a high sample volume and can be run for short or long periods e.g. hours, days, weekly (Wakeham et al. 2012). Using a twenty four hour postal service the bio-aerosol can be processed quickly by a laboratory with the PTA ELISA deliverable within 4 hours of sample receipt. Using this approach, there is the potential to apply effective disease management strategies and monitor bio-aerosols use in cultural and disease management treatments.

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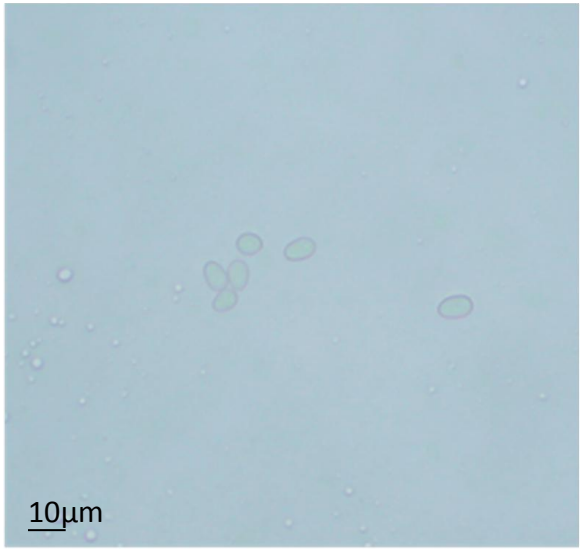
Highlights

- *Botrytis cinerea* identified in bio-aerosols of pre-symptomatic tomato crops.
- An increased spore concentration was detected in the lower part of the glasshouse (0.5m).
- Higher outside wind speeds increased detectable *B. cinerea* concentration in the glasshouse.
- Bio-aerosol analysis available within four hours by immunoassay.
- Improved timing and effectiveness of control options

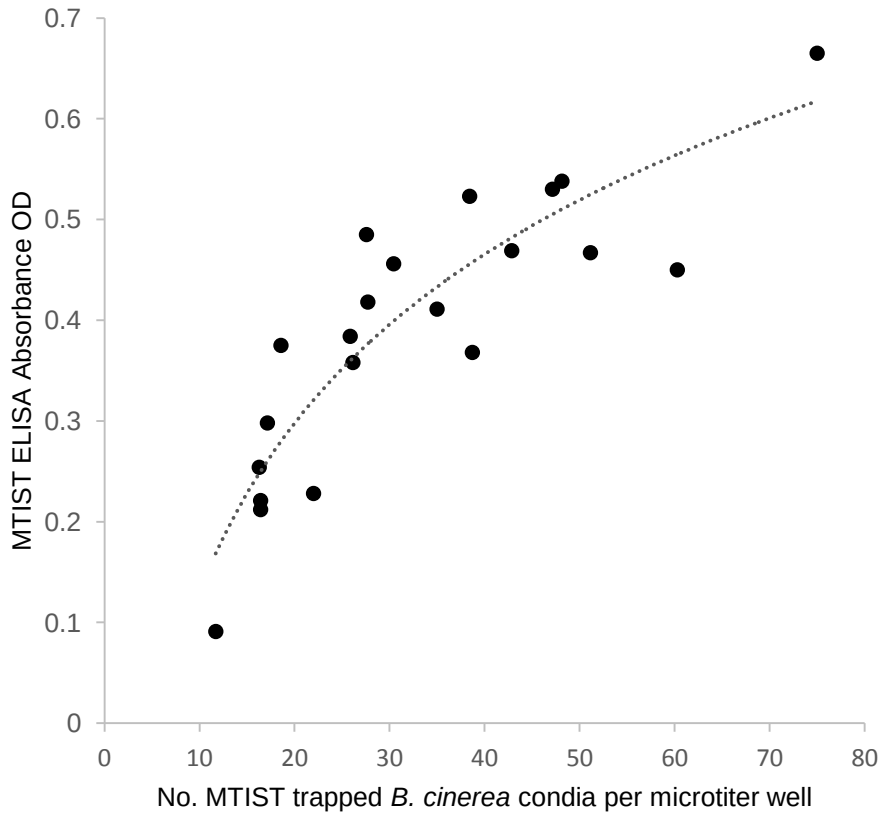
Figure 3

Figure 3. *Botrytis cinerea* conidia trapped in the base of a microtiter well of MTIST device



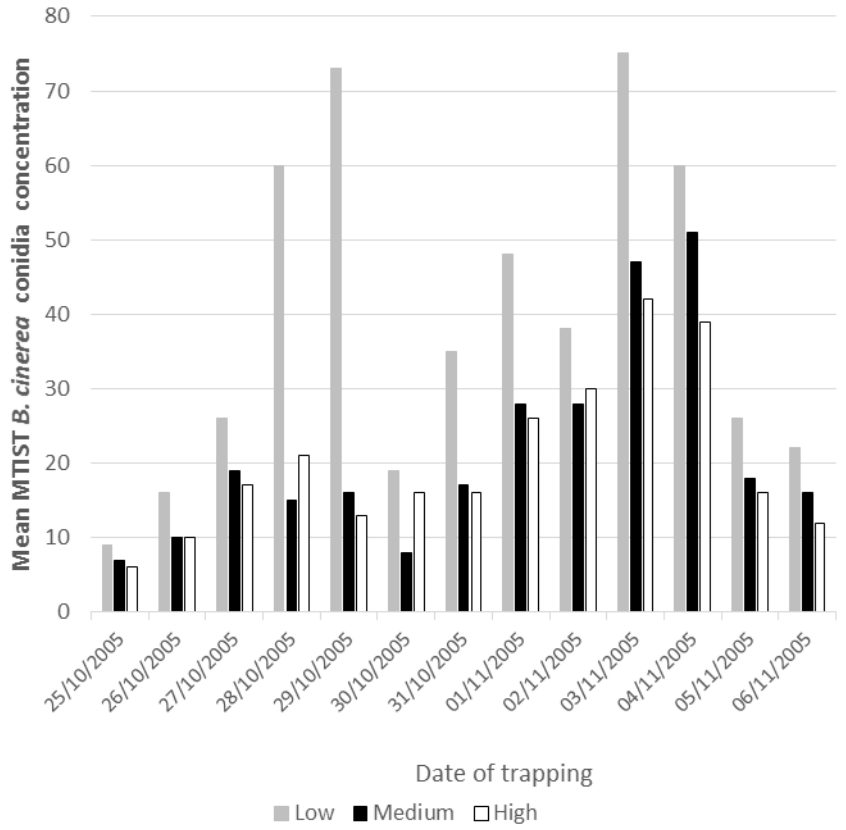
Figure(s)

Figure 4. Relationship between the number of MTIST trapped *B. cinerea* conidia and PTA ELISA.



Figure(s)

Figure 5. Positional effect of the bio-aerosol samplers in collection of *B. cinerea* conidia



Figure(s)

Figure 6. Effect of temperature, relative humidity and wind speed on spore trapping in the glasshouse