

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

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15

16 **Abstract**

17

18 *Botrytis cinerea* (Grey mould) is a necrotrophic fungus infecting over 230 plant  
19 species worldwide. It can cause major pre- and post-harvest diseases of many  
20 agronomic and horticultural crops. *Botrytis cinerea* causes annual economic losses of  
21 10 to 100 billion US dollars worldwide and instability in the food supply (Jin and Wu,  
22 2015). Gray mould losses, either at the farm gate or later in the food chain, could be  
23 reduced with improved knowledge of inoculum availability during production. In this  
24 paper, we report on the ability to monitor *Botrytis* spore concentration in glasshouse  
25 tomato production ahead of symptom development on plants. Using a light weight  
26 and portable air sampler (microtitre immunospore trap) it was possible to quantify  
27 inoculum availability within hours. Also, this study investigated the spatial aspect of

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

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

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41

## 42 **Introduction**

43

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

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

57

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

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78

## 79 **Materials and Methods**

80

### 81 **1.1 Tomato plants used in glasshouse experiments**

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

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

101

## 102 **1.2 Production of *Botrytis cinerea* spore suspensions**

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

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

118

### 119 **1.3 Bio-aerosol sampling**

#### 120 **1.3.1 Positional effect of bio-aerosol samplers on *B. cinerea* collection.**

121 Microtiter immunospore trapping (MTIST) samplers (Wakeham et al. 2004) were  
122 used to monitor glasshouse bio-aerosol concentrations at locations within a  
123 glasshouse. The device was operated by a standard Burkard turbine suction unit and  
124 air was drawn through the system at a constant rate of 57 litres min<sup>-1</sup>  
125 ([www.burkard.co.uk](http://www.burkard.co.uk)). Particulates in the airstream are channelled through 48 delivery  
126 trumpet nozzles and directed across the base of each 4x8 microtiter well. The MTIST  
127 was operated by connecting to a 240-V electric supply (Kennedy et al. 2000). Three  
128 MTIST devices were placed at different levels in the tomato glasshouse to determine  
129 the positional effects of spore traps on spore numbers. One trap was positioned  
130 above the tomato canopy, 2.2m high; another trap positioned within the canopy, 1.5m  
131 high; while the lowest was positioned close to the tomato stem bundles at a height of  
132 0.5m. All traps were on the same vertical axis.

133

134 Microtiter wells (4x8 well microtitre strips: Catalogue No. 469957, Nunc  
135 Immunodiagnosics, Life Technologies Ltd. Paisley, Scotland) were coated with a  
136 mixture of petroleum jelly and paraffin wax before being inserted within an MTIST  
137 device (Wakeham et al. 2004). The mixture was dissolved in hexane in the proportion  
138 1:32 prior to coating the base of each microtiter well (100µl per well). The strips were

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

143

### 144 **1.3.2 Effect of environment on *B. cinerea* concentration in bio-aerosols**

145 A Burkard 7 day volumetric spore sampler ([www.burkard.co.uk/7dayst.htm](http://www.burkard.co.uk/7dayst.htm)) was  
146 operated continuously in the crop. The volumetric air sampler is based on the Hirst  
147 spore trap (Hirst, 1952) and can be used to determine time periods when airborne  
148 microflora are present. The measurements from this type of trap form the basis of a  
149 large amount of knowledge of aerobiological systems (Kennedy and Wakeham,  
150 2015).

151

## 152 **1.4 Measurement of *Botrytis cinerea* concentration by immunoassay**

### 153 **1.4.1 PTA ELISA working dilution of *B. cinerea* polyclonal antibody (PAb)**

154 A polyclonal antibody raised to non-germinated conidia of *Botrytis cinerea* (coded  
155 94/4/3) was titrated against its homologous antigen in an indirect plate-trapped  
156 antigen ELISA (PTA-ELISA). Twenty eight paired wells of a 96 well Nunc  
157 Immunosorbent Polysorp flat-bottomed microtiter plate (Life Technologies, Paisley,  
158 Scotland; cat. no 475094A) were coated with 100µl per well of *B. cinerea* adjusted to  
159  $1 \times 10^4$  conidia ml<sup>-1</sup> in PBS. A further fourteen paired wells received 100µl per well of  
160 PBS alone. The wells were incubated overnight under natural light and at room  
161 temperature to allow the conidia, where present, to adhere to the base of the  
162 microtitre well. To enhance this process the wells were not covered but allowed to  
163 dry (Wakeham et al. 2004). Wells were washed once with PBS (100µl per well) for 1  
164 minute after which the wells were blocked with 200µl 1% casein (1% [wt/vol] casein  
165 in PBS) and incubated in a Wellwarm shaker incubator (Denley Instruments Ltd,  
166 Sussex, UK) at 30°C for 30 minutes. Residual blocking buffer was removed and wells

167 were washed once for 1 minute with 200µl per well with PBS 0.05% Tween 20 0.1%  
168 casein. The polyclonal antibody (Warwick HRI 94/4/3) was diluted 1:10 in PBS 0.05%  
169 Tween 20 0.1% casein, 1:50 and subsequent doubling dilutions made to 1:102400.  
170 The serum dilutions were applied to paired wells at 100µl per well and incubated in a  
171 Wellwarm shaker incubator (Denley Instruments Ltd, Sussex, UK) at 30°C for 45  
172 minutes. To determine endogenous phosphatase activity, 14 of the paired wells  
173 which had been coated with *B. cinerea* conidial suspension received PBS 0.05%  
174 Tween 20 0.1% casein alone. Following incubation unbound material was removed  
175 and wells washed three times for 1 min each with PBS 0.05% Tween 20 0.1%  
176 casein. Aliquots of 100µl of goat anti-rabbit IgG (whole molecule) alkaline  
177 phosphatase (Sigma A-3687) diluted in PBS 0.05% Tween 20 0.1% casein (5µl in  
178 30ml PBS Tween casein) were added to each well and incubated as above. After  
179 three washes, 100µl per well of 1mg per ml p-nitrophenyl phosphate (pNPP) (Sigma  
180 N-2770), freshly dissolved in deionised water was added. The plates were incubated  
181 at room temperature in darkness for 40 min and absorbance values were read at  
182 filter wavelengths of 405nm and 630nm in a Biohit BP 800 ELISA plate reader (Alpha  
183 Laboratories, 40, Parham Drive, Eastleigh, Hampshire, UK). Mean values were  
184 calculated for each of the paired wells.

185

#### 186 **1.4.2 Polyclonal antiserum reactivity tests**

187 The polyclonal antiserum (94/4/3) was screened for reactivity with a range of plant  
188 pathogenic fungi and oomycetes (Table 1). Spores were collected in PBS, and the  
189 concentration was adjusted to about  $1 \times 10^4$  spores per ml and a PTA-ELISA was  
190 carried out. 100µl of each fungal spore test suspension was pipetted into wells of an  
191 8-well microtiter strip. Six of the eight wells had fungal spore suspension and the  
192 remaining two wells received PBS alone to serve as negative control. The microtiter  
193 strips were incubated overnight at room temperature, before removal of the unbound  
194 material and washing with 100µl PBS per well. Wells were blocked with 200µl 1%

195 casein (1% [wt/vol] casein in PBS) and incubated in a Wellwarm shaker incubator at  
196 30°C for 30 minutes. Residual blocking buffer was removed and wells were washed  
197 once for 1 minute with 200µl per well with PBS 0.05% Tween 20 0.1% casein. Four  
198 wells of each microtiter strip received 100µl of PAb 94/4/3 in PBS 0.05% Tween 20  
199 0.1% casein (1:400). The remaining four wells of each strip received 100µl PBS  
200 0.05% Tween 20 0.1% casein alone. The strips were incubated as described  
201 previously for 45 minutes. The remainder of the PTA ELISA was carried out as  
202 described above.

203

#### 204 **1.4.3 Antiserum detection sensitivity for *B. cinerea***

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

218

#### 219 **1.4.4 Immunoquantification of *B. cinerea* conidia trapped by the MTIST air** 220 **sampler.**

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



223 determined using an Olympus CK2 binocular microscope (x 200) (Kennedy et al.,  
224 2000). A PTA-ELISA was carried out using the polyclonal antiserum (94/4/3) as  
225 previously described. To accommodate the potential of endogenous phosphatase  
226 activity within the collected bio-aerosol an internal control for each 24hr period was  
227 included. Of the four microtitre strips (4x8 wells) one strip received all stages of the  
228 ELISA process but in the absence of the primary antibody (94/4/3). The mean value  
229 derived from this was removed from the mean absorbance value of each microtitre  
230 well strip that was processed for that sampling period. The results of the *B. cinerea*  
231 microtitre well microscopic conidial counts and the corresponding MTIST ELISA  
232 absorbance values were compared.

233

## 234 **1.5 Glasshouse Data Analysis**

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

240

241

## 242 **Results**

243

### 244 **2.1 Measurement of *Botrytis cinerea* concentration by immunoassay**

#### 245 **2.1.1 Specificity and sensitivity of *B. cinerea* polyclonal antibody (PAb) by**

#### 246 **ELISA**

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

251 region of 100 per microtitre well. Across the concentration range tested confidence  
252 intervals were fitted to the means at the  $p=0.05$  level (Figure 1). With the exception  
253 of *Botrytis narcissicola* and *Sclerotinia sclerotiorum* the antiserum 94/4/3 (IgG fraction  
254 tested) showed limited reactivity with the fungal and oomycete spore types tested  
255 (<20 % recognition compared to *B. cinerea*). For each spore type a standardised  
256 concentration of  $1 \times 10^4 \text{ ml}^{-1}$  had been tested by PTA ELISA. Interestingly, although  
257 the antiserum reacted with both *B. allii* and *B. narcissicola* the greatest interaction  
258 was to *S. sclerotiorum* (Figure 2).

259

## 260 **2.2 Measurement of MTIST trapped *B. cinerea* conidia in glasshouse grown** 261 **crop bio-aerosols.**

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

276

## 277 **2.3 Positional effect of spore traps within glasshouses.** A positional effect in 278 the concentration of *B. cinerea* conidia trapped in microtiter wells strips was observed

279 when the MTIST sampling devices were placed at three different heights in the  
280 glasshouse (Figure 5). A polynomial function was fitted to the time series of trap  
281 catches at different heights. This gave  $r^2$  values of 0.3511 (High), 0.3724 (Medium)  
282 and 0.4382 (Low) at different trapping heights. A positional effect in the concentration  
283 of *B. cinerea* was also observed with the corresponding ELISA absorbance. The  
284 mean spore counts at the three different heights was analysed using ANOVA and the  
285 result showed significant differences in the mean spore counts were present (p-  
286 value <0.001). The spore trap placed at a height of 0.5m (low level) collected the  
287 highest concentration of *B.cinerea* conidia for most trapping days. At a height of 1.5m  
288 (medium level) the concentration was reduced. The lowest conidial numbers were  
289 recorded for a sampling height of 2.2m (high level). On 04/11/05, mean spore counts  
290 were 60.29 when trapping was carried out at a height of 0.5m, 51.14 spores at height  
291 1.5m while 38.71 spores were trapped at height 2.2m.

292

#### 293 **2.4 Effect of environment on *B. cinerea* concentration in bio-aerosols**

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

303

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

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

309

310

## 311 **Discussion**

312

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

330

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

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

352

353 Measurement of pathogenic inoculum in bio-aerosols would assist in the prediction of  
354 disease occurrence. Traditionally, plant disease forecasting systems have relied  
355 upon environmental data alone to predict the risk of pathogenic inoculum in crops  
356 (Magarey *et al.* 2005, Scherm and van Bruggen, 1995). Mathematical models  
357 describing the effect of temperature and wetness on pathogen infection have been  
358 developed for many types of plant disease (Magarey *et al.* 2005). The ability  
359 however to detect and quantify pathogen inoculum at a time period when  
360 environmental risk for a disease is high would enable protective disease control  
361 strategies to be implemented more effectively (Wakeham and Kennedy, 2010).  
362 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 microtitre 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 microtitre 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 ( $0.5\text{mg ml}^{-1}$ ) 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

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

393

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

406

407

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409

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413

414

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513

## 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

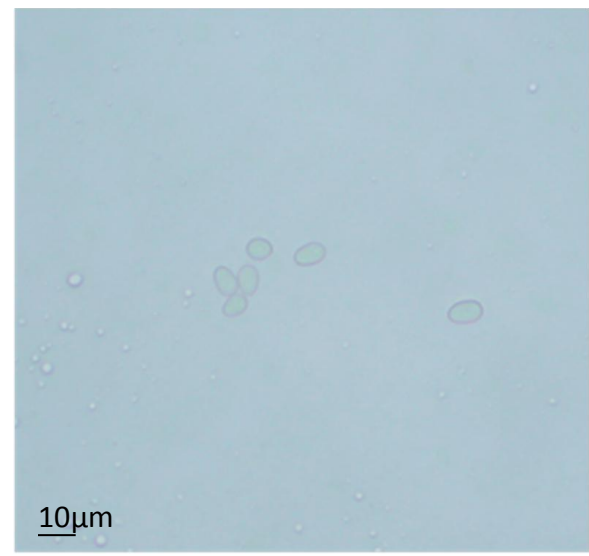
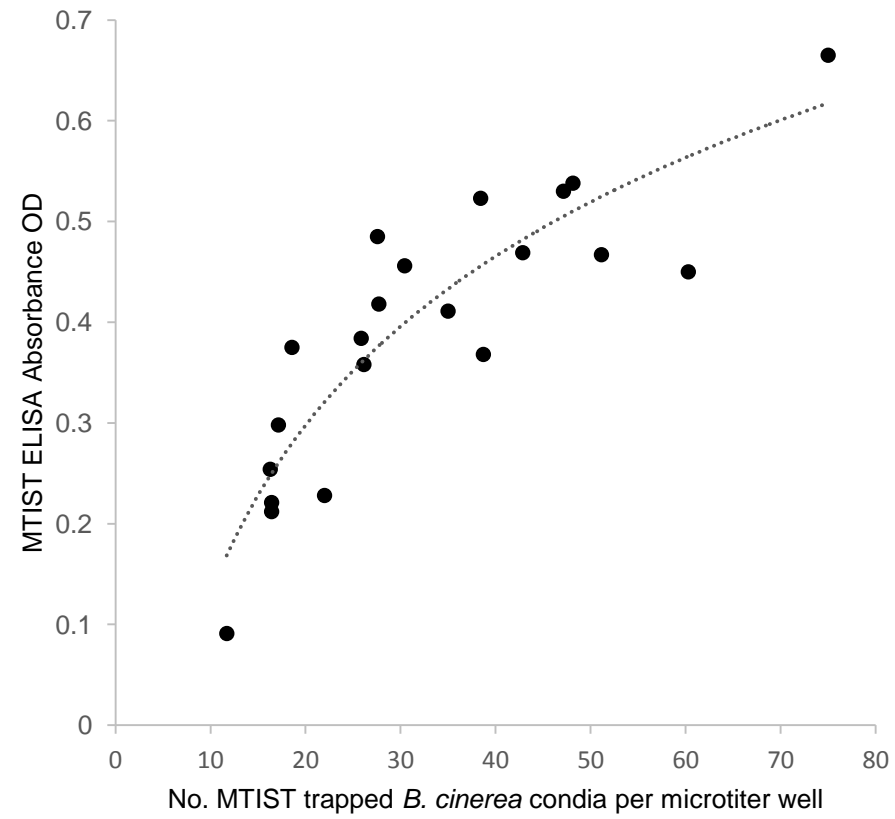
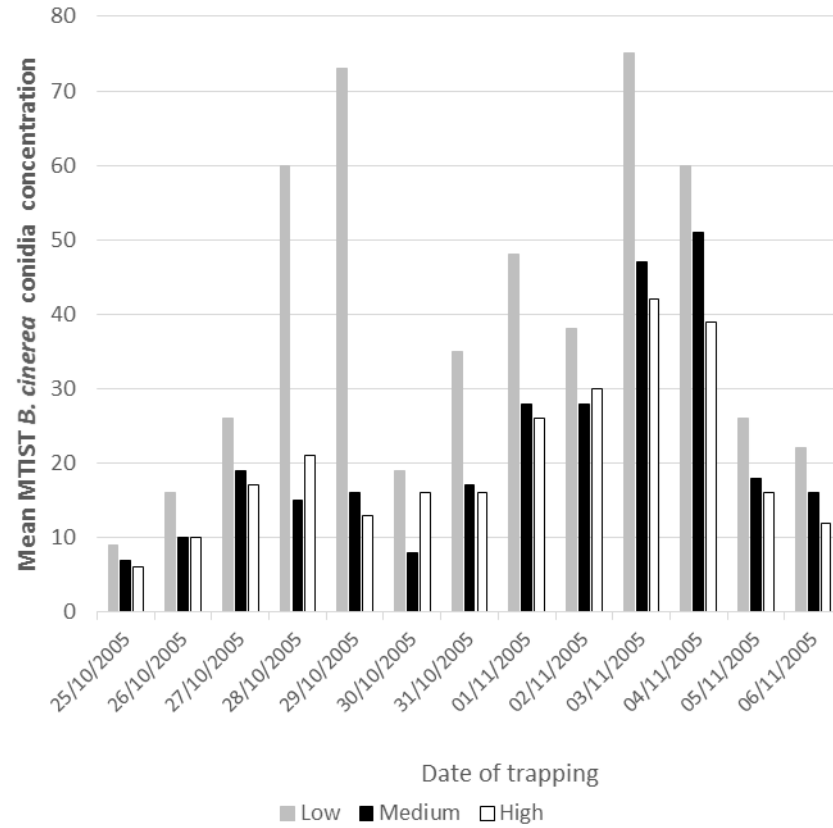


Figure 4. Relationship between the number of MTIST trapped *B. cinerea* conidia and PTA ELISA.



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

