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

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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 10–100 billion US dollars worldwide and instability in the food supply (Jin and Wu, 2015). Grey 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 (0.5 m) 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.

1. 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 10–100 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 °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 et al., 1987). However, germination and infection is much more rapid in the presence of free water (6–8 h) (Elad and

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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.

2. Materials and methods

2.1. 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 5 mS cm^{-1}, and gradually reduced every fourteen days until it reached an EC of 2.8 mS 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 2 g per litre Thiovit (a.i. sulphur) [Syngenta Crop Protection UK Ltd., Whittlesford, Cambridge, CB2 4QT, UK] against powdery mildew and 1 g 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 1 m^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.

2.2. Production of Botrytis cinerea spore suspensions

Potato dextrose agar growth medium was prepared by suspending 39 g PDA (Oxoid Ltd., Hampshire, England) in 1 L of distilled water. The medium was boiled to dissolve completely, and sterilised at 121 °C for 15 min s. The medium was mixed and poured into Petri dishes (20 ml per plate) in a flow hood (BH 12R, Labcaire Systems Ltd., Somerset England) on cooling. A sterile PN6026 Supor 450 90 mm 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 5 ml 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.

2.3. Bio-aerosol sampling

2.3.1. 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 L min^{-1} (www.burkard.co.uk). Particulates in the airstream are channelled through 48 delivery trumpet nozzles and directed across the base of each 4 × 8 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, 2.2 m high; another trap positioned within the canopy, 1.5 m high; while the lowest was positioned close to the tomato stem bundles at a height of 0.5 m. All traps were on the same vertical axis.

Microtiter wells (4 × 8 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 (100 μl per well). The strips were incubated for 1 h at 20 °C. An inverted binocular microscope (Nikon model TMS) was used to check that the well coatings had been applied evenly. The 4 × 8 well coated microstrips were exposed to glasshouse bio-aerosols for a 24hr periods. After this the strips were removed and stored at −20 °C.

2.3.2. 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).

2.4. Measurement of Botrytis cinerea concentration by immunoassay

2.4.1. 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 100 μl per well of *B. cinerea* adjusted to 1 × 10^4 condia ml^{-1} in PBS. A further fourteen paired wells received 100 μl 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 (100 μl per well) for 1 min after which the wells were blocked with 200 μl 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 min. Residual blocking buffer was removed and wells were washed once for 1 min with 200 μl 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 100 μl per well and incubated in a Wellwarm shaker incubator (Denley Instruments Ltd, Sussex, UK) at 30 °C for 45 min. To determine endogenous phosphatase activity, 14 of the paired wells which had been coated with *B. cinerea* conidial suspension received PBS 0.05%