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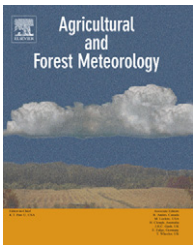

Botrytis cinerea spore balance of a greenhouse rose crop

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ARTICLE INFO

Article history:
Received 15 November 2006
Received in revised form
11 October 2007
Accepted 21 November 2007

Keywords:
Greenhouse
Air exchange rate
Spore
Balance
Botrytis cinerea
Ventilation
Rose crop

ABSTRACT

Fungal pathogens are among the most virulent bioagressors of protected crops. For sustainable plant production and to protect the crop against these airborne organisms one must determine their origin, i.e. whether they come from outside the greenhouse or are produced inside it.

We considered *Botrytis cinerea* spore concentration as a particular physical species which is transported by air in the same way as heat, CO_2, water vapour or any tracer gas. We constructed a mass balance of the viable *Botrytis* spores on the whole-greenhouse volume to assess the inside production of spores and their exchanges.

The different elements in the spore balance are considered and are determined experimentally:

- spore transfer into or out of the greenhouse is deduced from the difference between the inside and outside spore concentrations, measured by spore traps, multiplied by the whole-greenhouse ventilation rate;
- spore deposition on soil and crop and spore impaction on the plants is deduced from observations made using Petri dishes;
- inside spore production is the differential term deduced from the whole-greenhouse volume balance.

The first measurements and calculations have shown that the presence of insect-proof nets across the greenhouse openings strongly influences the spore balance, as the nets intercept the *B. cinerea* spores proportionally to the nets' solidity. Based on wind tunnel experiments, the interception capacity of the nets was quantified and the whole model completed in consequence.

These results show the diurnal *Botrytis* spore balance over 3–4-week periods in the autumn and spring season for a young rose plantation. In agreement with visual observations of *B. cinerea* sporulation within the greenhouse, it is shown that the origin of the inocula is predominantly internal (2/3 in quantity). However, due to outside climate and greenhouse opening, it can vary widely from one period to another, with a tendency to become predominantly internal as the crop ages.

1. Introduction

While biological control agents for most insect pests of greenhouse crops have been successfully applied to greenhouse crops in recent years, biological control of diseases is much less developed. Recent studies on the toxicological impact of greenhouse products (Anton et al., 2004) show that disease management still largely relies on chemicals. Most

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doi:10.1016/j.agrformet.2007.11.014

airborne fungal pathogens, particularly grey mould caused by *Botrytis cinerea*, are considered a major problem for greenhouse crop production.

Microclimatic parameters have long been recognised as key factors in the spread of fungal diseases and for greenhouse climate management for disease control. For example, open vents coupled with heating that renew the air and evacuate excess water vapour released by crop transpiration inside the greenhouse are an efficient way of controlling pests (Nicot and Baille, 1996; Jewett and Jarvis, 2001; Tantau and Lange, 2003; Körner and Challa, 2003).

However, while the prevailing climatic conditions are crucial for fungal development, it is also very important to control the level of fungal inocula. This aspect is generally poorly known and documented. To protect crops efficiently from airborne fungal spores, the origin of the inocula must be known – i.e., whether the spores come from outside the greenhouse or have been produced inside – and also their rate of production.

We have therefore adapted a classical physical method which has hitherto been chiefly used for performing mass or heat balances in finite volumes such as buildings and greenhouses, to perform a *B. cinerea* spore balance of the protected crop and determine the origins of the inocula.

In this paper we present the study designed to perform this determination, and its experimental application to a real-scale rose greenhouse in the South of France, near Nice.

2. Theory

B. cinerea spore concentration can be regarded as a particular physical species which is transported by air in the same way as heat, carbon dioxide or water vapour concentration. It can also be compared to any tracer gas, such as N_2O, which is traditionally used for measuring large-scale greenhouse air exchange rates (Boulard and Draoui, 1995).

2.1. Whole-greenhouse spore mass balance

The tracer gas techniques used to measure ventilation flow rates are based on the mass balance of natural or artificial constituents of the whole-greenhouse air. For performing a greenhouse air mass balance, the choice of tracer gas is important: the gas should be inert, non-toxic and non-flammable, with a molecular weight close to the average weight of air components, and easy to measure at low concentrations. Many gases have been used, including SF_6, CH_4, CO_2, H_2, N_2O, Argon 41 and Krypton 85. The two most frequently used for greenhouses are CO_2 and N_2O, the latter being influenced neither by photosynthesis nor by plant respiration. However, water vapour (H_2O) has also been used as a ''natural'' tracer gas (Demrati et al., 2001). Sherman (1990) has analysed tracer gas techniques for measuring ventilation in a single zone such as a greenhouse; he has shown that mixing problems can present a major source of potential error because the tracer gas must be uniformly mixed throughout the greenhouse. In these conditions Ducarme et al. (1994) estimate that the accuracy of an analysis by tracer gas is about 30% and that additional errors can also be caused by exfiltration air re-entering the measurement space at another location, or by changes in external conditions (wind speed, air temperature) during the measurement process.

Based on these approaches, we considered the finite volume of the greenhouse and performed its *B. cinerea* spore balance according to Eq. (1), derived from the mass balance of the constituents of the whole-greenhouse air (Roy et al., 2002):

$$V\frac{dc_{in}}{dt} = -G(t)(c_{in}(t) - c_{out}(t)) - D_{in}(t) + P_{in}(t) \quad (1)$$

where G is the ventilation flow rate, V the greenhouse volume, t the time, $c_{in}(t)$ and $c_{out}(t)$ the inside and outside concentrations of *B. cinerea* spore's, $P_{in}(t)$ (sp s^{-1}) is the spore's production rate inside and $D_{in}(t)$ (sp s^{-1}) the inside spore deposition by sedimentation and impaction. One can assess the inside production of inocula $P_{in}(t)$, measuring the inside and outside spore concentrations $c_{in}(t)$ and $c_{out}(t)$ (sp m^{-3}) together with the greenhouse air exchange rate $G(t)$ (m^3 s^{-1}), greenhouse volume V (m^3), and spore deposition on crop and soil $D_{in}(t)$ (sp s^{-1}):

In this equation we make two crucial assumptions: (i) that the variables used in Eq. (1) (i.e. $c_{in}(t)$, $c_{out}(t)$, G, D_{in} and P_{in}) are discrete values corresponding to the mean of the considered variable which vary from one time step to another and (ii) that all spores deposited on the surfaces of soil or leaves remain there and do not return to the air.

Integrating Eq. (1) on time scale ($t - \Delta t$ to t) enables us to present this equation in a continuous and recursive form (2):

$$c_{in}(t) = c_{in}(t - \Delta t)e^{-G(t)\Delta t/V} + c_{out}(t)(1 - e^{-G(t)\Delta t/V}) + ((P_{in}(t) - D_{in}(t))/G(t))(1 - e^{-G(t)\Delta t/V}) \quad (2)$$

As the time scale, Δt, for experimental spore counting is long (from 8 to 24 h) the numerical value of $e^{-G(t)\Delta t/V}$ attached to the capacitive effect in Eq. (2) tends to 0 and becomes very small in relation to the other terms. Eq. (2) then simplifies into Eq. (3):

$$G(t)(c_{in}(t) - c_{out}(t)) - D_{in}(t) + P_{in}(t) = 0 \quad (3)$$

Considering this equation and determining, for any time scale Δt between 8 and 24 h, the inside and outside spore concentrations $c_{in}(t)$ and $c_{out}(t)$ together with spore deposition $D_{in}(t)$, and determining the air exchange rate $G(t)$ by means of a general ventilation model, we can deduce the rate of inside spore production $P_{in}(t)$ for the period in question. A detailed diagram of the method is presented in Fig. 1.

However, *Botrytis* spores are solid particles with an average 10 μm diameter, and can be intercepted by the solid matrix, i.e. the wires of the insect-proof nets deployed on all the greenhouse vent openings, according to an equation which will be presented in Section 4. The spore balance (Eq. (3)) must be modified in consequence to take into account a dimensionless spore interception value, F (–):

$$FG(t)(c_{in}(t) - c_{out}(t)) - D_{in}(t) + P_{in}(t) = 0 \quad (4)$$