



**HAL**  
open science

## **Botrytis cinerea spore balance of a greenhouse rose crop**

Thierry Boulard, Marie Chave, Hicham Fatnassi, Christine Poncet,  
Jean-Claude Roy

### ► **To cite this version:**

Thierry Boulard, Marie Chave, Hicham Fatnassi, Christine Poncet, Jean-Claude Roy. Botrytis cinerea spore balance of a greenhouse rose crop. Agricultural and Forest Meteorology, 2008, 148 (3), pp.504-511. 10.1016/j.agrformet.2007.11.014 . hal-02655374

**HAL Id: hal-02655374**

**<https://hal.inrae.fr/hal-02655374>**

Submitted on 29 May 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# BOTRYTIS CINEREA SPORE BALANCE OF A GREENHOUSE ROSE CROP

\*T. Boulard<sup>1</sup>, M. Chave<sup>1</sup>, H. Fatnassi<sup>1</sup>, C. Poncet<sup>1</sup>, J.C. Roy<sup>2</sup>

<sup>1</sup>INRA –URIH, 400, route des Chappes, BP 167, 06903 Sophia Antipolis, France

<sup>2</sup>Institut FEMTO-ST, UMR 6174, Université de Franche-Comté, 2, Avenue Jean Moulin,  
90000 Belfort, France

\*Corresponding author: [boulard@sophia.inra.fr](mailto:boulard@sophia.inra.fr)

## Abstract

Fungal pathogens are among the most virulent bioaggressors 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<sub>2</sub>, 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 *Botrytis 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

1 of *Botrytis cinerea* sporulation within the greenhouse, it is shown that the origin of the inocula  
2 is predominantly internal (2/3 in quantity). However, due to outside climate and greenhouse  
3 opening, it can vary widely from one period to another, with a tendency to become  
4 predominantly internal as the crop ages.

5 **Keywords:** greenhouse, air exchange rate, spore, balance, *Botrytis cinerea*, ventilation, rose  
6 crop

## 8 1-Introduction

9 While biological control agents for most insect pests of greenhouse crops have been  
10 successfully applied to greenhouse crops in recent years, biological control of diseases is  
11 much less developed. Recent studies on the toxicological impact of greenhouse products  
12 (Anton *et al.*, 2004) show that disease management still largely relies on chemicals. Most  
13 airborne fungal pathogens, particularly grey mould caused by *Botrytis cinerea*, are considered  
14 a major problem for greenhouse crop production.

15 Microclimatic parameters have long been recognised as key factors in the spread of fungal  
16 diseases and for greenhouse climate management for disease control. For example, open vents  
17 coupled with heating that renew the air and evacuate excess water vapour released by crop  
18 transpiration inside the greenhouse; greenhouses are an efficient way of controlling pests  
19 (Nicot and Baille, 1996; Jewett and Jarvis, 2001; Tantau and Lange, 2003; Körner and Challa,  
20 2003).

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

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

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

## 33 2-Theory

1 *Botrytis cinerea* spore concentration can be regarded as a particular physical species  
 2 which is transported by air in the same way as heat, carbon dioxide or water vapour  
 3 concentration. It can also be compared to any tracer gas, such as N<sub>2</sub>O, which is traditionally  
 4 used for measuring large-scale greenhouse air exchange rates (Boulard & Draoui, 1995).

## 6 2-1- Whole-greenhouse spore mass balance

7 The tracer gas techniques used to measure ventilation flow rates are based on the mass  
 8 balance of natural or artificial constituents of the whole-greenhouse air. For performing a  
 9 greenhouse air mass balance, the choice of tracer gas is important: the gas should be inert,  
 10 non-toxic and non-flammable, with a molecular weight close to the average weight of air  
 11 components, and easy to measure at low concentrations. Many gases have been used,  
 12 including SF<sub>6</sub>, CH<sub>4</sub>, CO<sub>2</sub>, H<sub>2</sub>, N<sub>2</sub>O, Argon 41 and Krypton 85. The two most frequently used  
 13 for greenhouses are CO<sub>2</sub> and N<sub>2</sub>O, the latter being influenced neither by photosynthesis nor  
 14 by plant respiration. However, water vapour (H<sub>2</sub>O) has also been used as a “natural” tracer  
 15 gas (Demrati *et al.*, 2001). Sherman (1990) has analysed tracer gas techniques for measuring  
 16 ventilation in a single zone such as a greenhouse; he has shown that mixing problems can  
 17 present a major source of potential error because the tracer gas must be uniformly mixed  
 18 throughout the greenhouse. In these conditions Ducarme *et al.* (1994) estimate that the  
 19 accuracy of an analysis by tracer gas is about 30% and that additional errors can also be  
 20 caused by exfiltration air re-entering the measurement space at another location, or by  
 21 changes in external conditions (wind speed, air temperature) during the measurement process.

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

$$25 \quad V \frac{dc_{in}}{dt} = -G(t)(c_{in}(t) - c_{out}(t)) - D_{in}(t) + P_{in}(t) \quad (1)$$

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

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

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

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

8 As the time scale,  $\Delta t$ , for experimental spore counting is long (from 8h to 24h) the  
9 numerical value of  $e^{-G(t)\Delta t/V}$  attached to the capacitive effect in equation (2) tends to 0 and  
10 becomes very small in relation to the other terms. Equation (2) then simplifies into equation  
11 (3):

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

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

18 However, *Botrytis* spores are solid particles with an average 10  $\mu\text{m}$  diameter, and can be  
19 intercepted by the solid matrix, i.e. the wires of the insect proof nets deployed on all the  
20 greenhouse vent openings, according to an equation which will be presented in section (4).  
21 The spore balance (equation (3)) must be modified in consequence to take into account a  
22 dimensionless spore interception value,  $F$  (-):

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

### 3-Experimental setup

27 The experiments were carried out (Fig. 2) in three-spans of a four-span compartmented  
28 plastic greenhouse (Multclair 9600, Filclair, France). The main compartment (576  $\text{m}^2$ ) was

1 occupied by a soilless rose plantation (3240 rose plants, cv Magnum, Milva, Suella) planted in  
 2 spring (May 2003) in a new greenhouse erected just before, from December to March 2003.  
 3 *Botrytis* spore balances were systematically constructed in spring and autumn, for 2 to 4  
 4 weeks each time, from 2003 to 2005 (April 2003, October 2003, April 2004, November 2004  
 5 and May 2005), the first spore balance (April 2003) being performed before the rose crop was  
 6 planted and all the others after.

7 In the greenhouse, natural ventilation was provided by means of roof vents and a side  
 8 opening, both equipped with insect-proof nets (anti-*Bemisia* 10x20 meshes/cm, with a 0.2 mm  
 9 thread diameter and 0.52 mesh solidity).

### 11 3-1-Physical measurements

12 The main exchange parameter  $G$  was experimentally determined with the help of a  
 13 general ventilation model given by equation (5), which considers both wind and stack effects  
 14 together with the resistance of the insect-proof nets (which unfold on opening the vents) to air  
 15 penetration (Boulard and Baille, 1995):

$$G = A_l ((S_o + S_f)/2)(2gH_c/4|T_{out} - T_{in}|/(T_{out} + 273) + C_w V^2)^{0.5} \quad (5)$$

16 where  $A_l$  (-) is the overall discharge coefficient of the vent and insect-proof net,  $C_w$  (-)  
 17 is a wind effect coefficient on ventilation,  $S_o$  and  $S_f$  (m<sup>2</sup>) are vent opening area and the  
 18 corresponding leakage area,  $H_c$  (m) is an equivalent chimney height,  $T_{in}$  and  $T_{out}$  (°C) are  
 19 inside and outside temperatures, and  $V$  (m s<sup>-1</sup>) is wind speed.

20 Wind speed and direction together with inside and outside temperature and humidity  
 21 and vent opening (Fig. 3) area were systematically measured using the sensors of the  
 22 greenhouse climate computer (L'ien, Vitrolles, 13, France) at a 1-minute frequency, stored  
 23 and processed for a minimum average time of 15 minutes.

24 The model parameters  $A_l$  and  $C_w$  together with leakage area  $S_f$ , were determined in  
 25 situ for this greenhouse by fitting the ventilation model to exchange rate measurements taken  
 26 with tracer gas techniques, using N<sub>2</sub>O as tracer gas (Roy *et al.*, 2002). A detailed description  
 27 of the experimental study conducted to determine these parameters on this greenhouse can be  
 28 found in Fatnassi *et al.* (2006) and more generally a survey of the values found in the  
 29 literature can be found in Roy *et al.* (2002).

30 Knowing the specific greenhouse ventilation parameters  $A_l$ ,  $C_w$  and  $S_f$  for the two main  
 31 wind directions observed on the site and continuously measuring the inside and outside air  
 32 temperatures  $T_{in}$  and  $T_{out}$  together with wind speed  $V$  and vent opening area  $S_o$ , one can easily

1 deduce the air exchange rate  $G$  from equation (5). In the April 2004 trial, additional inside air  
2 speed measurements for studying the determinism of spores' terminal velocity were  
3 performed, using omni-directional anemometers.

### 4 5 3-2-Biological measurements:

6 Inside ( $c_{in}$ ) and outside ( $c_{out}$ ) *Botrytis cinerea* spore concentrations were measured by  
7 means of two spore trap systems (Burkard Manufacturing Co Ltd, Rickmansworth, GB) with  
8 a  $0.6 \text{ m}^3 \text{ h}^{-1}$  flow rate, set up inside the greenhouse about 0.5 m above the crop canopy and  
9 outside, approximately 10 m away from the greenhouse. These spore traps were used  
10 continuously during the trials.

11 The total spore deposit in the greenhouse ( $D_i$ ) was estimated by means of 16 Petri dishes  
12 ( $\text{Ø } 9.5 \text{ cm}$  for a surface of  $71 \cdot 10^{-4} \text{ m}^2$ ) placed horizontally above the rose bushes ( $\approx 2 \text{ m}$ ) to  
13 measure spore sedimentation on the crop canopy, together with 20 vertical Petri dishes  
14 oriented in the 4 cardinal directions to measure vertical impaction. Figure (3) shows  
15 schematically the horizontal position of the various biological and climate measurements  
16 inside and outside the greenhouse. The *Botrytis* spores captured from the air by the spore traps  
17 and Petri dishes were placed each day on a selective medium (Kerssies, 1990), incubated for  
18 10 days and counted using a binocular microscope.

19 For comparing this indirect assessment of *Botrytis cinerea* sporulation inside the  
20 greenhouse with visual inspection, qualitative observation of *Botrytis cinerea* symptoms on  
21 rose buds and flowers and search for sporulating sources in the greenhouse were performed  
22 systematically on a weekly basis by the pathologists in charge of the experiment for all plants  
23 from 2003 to 2005.

### 24 25 3-3- Spore interception by the insect-proof net

26 Spore interception by the insect-proof net was experimentally determined by wind tunnel  
27 experiments (Fig. 4). Spore interception by the insect-proof net was regarded as the ratio of  
28 the intercepted spores to the total number of spores injected into the wind tunnel:

$$29$$
$$30 F = \text{number of spore's intercepted by the net} / \text{number of spore's injected in the vein of the wind tunnel} \quad (6)$$
$$31$$

32 The intercepted spore value (number of spores intercepted by the insect-proof net) was  
33 deduced from the measurements performed in the wind tunnel experiment, as follows:



$$\begin{aligned} & \text{Number of spores intercepted by the net} = \text{Number of spores upstream from the net} - \\ & (\text{Number of spores downstream from the net} + \text{Number of spores having sedimented in the} \\ & \text{wind tunnel}) \end{aligned} \quad (7)$$

where the number of spores upstream and downstream from the insect-proof net was measured using spore trap systems (Burkard Manufacturing Co Ltd, Rickmansworth, GB), and the number of sedimented spores was determined by means of Petri dishes.

These values were deduced from the mean of 3 wind tunnel tests lasting about 2 hours and performed with an air speed of 1.5 m s<sup>-1</sup>.

## 4-Results and discussion

Greenhouse spore balance and analysis of the principle spore characteristics are based on data recorded in 5 trials lasting between 3 and 4 weeks in April 2003, October 2003, April and November 2004 and May 2005, on a diurnal (12h from 08:00 to 20:00) and nocturnal (12h from 20:00 to 08:00) and on a 24-hour basis (from 08:00 to 08:00).

### 4-1- Determination of *Botrytis* spores' terminal velocity in greenhouse conditions

Although the determination of the spores' terminal fall velocity  $v_t$ , is not essential for performing their balance, the experimental results were also exploited for deducing this value which gives interesting insights on the dynamics of inside spores' transfer.

Theoretically, in still air, all suspended spores fall with a constant and characteristic terminal fall velocity  $v_t$ , given by Stokes' law (Seinfeld & Pandis, 1998) and depending on gravity  $g$ , spore radius  $r_p$ , density  $\rho_p$ , a slip correction factor  $C_c$  and air viscosity  $\mu$  (1.72 10<sup>-4</sup> g cm<sup>-1</sup>s<sup>-1</sup>):

$$v_t = ((2r_p)^2 \rho_p g C_c) / 18 \mu \quad (8)$$

For an average *Botrytis cinerea* spore radius  $r_p = 5 \mu\text{m}$  and density  $\rho_p = 1.1 \text{ g cm}^{-3}$ , one obtains  $v_t = 0.00368 \text{ m s}^{-1}$ .

This theoretical value must be compared with the experimental determination derived from a dimensional analysis indicating that the terminal velocity of *Botrytis* spores (m s<sup>-1</sup>) results from the ratio of inside spore deposition  $D_i$  (sp m<sup>-2</sup> s<sup>-1</sup>) to inside spore concentration  $C_i$  (sp m<sup>-3</sup>). In the experiments,  $D_i$  was measured by means of the horizontal Petri dishes and  $C_i$  by the Burkard spore traps, as described above.



1

$$v_t = D_i / C_i \tag{9}$$

2

3 For one month in April 2004 (days 104 to 134) one finds an average terminal velocity of  
 4 0.341 cm s<sup>-1</sup> with a standard deviation of 0.11 cm s<sup>-1</sup>. This value is close to the theoretical  
 5 value of 0.368 cm s<sup>-1</sup> deduced from Stokes' law (equation 8) for the average *Botrytis cinerea*  
 6 spore diameter and density given above. This value is also within the range [0.22 to 0.45 cm s<sup>-1</sup>  
 7 ] experimentally determined by Gregory (1973) for *Botrytis cinerea* spores.

8 Taking account of the dependency of the spores' terminal velocity (determined using  
 9 equation 9 on a 12-hour time basis) on the inside air speed (measured by means of an omni-  
 10 directional anemometer) suggests that an increase in inside air speed tends to accelerate  
 11 horizontal spore deposition (Fig. 5). As the mean inside air speed is also directly proportional  
 12 to the overall greenhouse air exchange rate (Wang *et al.*, 1999), it follows that increasing  
 13 ventilation tends to accelerate spore deposition as it is verified by the experimental results of  
 14 Fig.8. However, one cannot conclude if the acceleration of spore deposition is due to an  
 15 aerodynamic effect or simply to the greater exchange from outside to inside.

16

17 4-2- Spore balance calculations

18

19 *Spore interception by the insect-proof net*

20 Using the wind tunnel we were able to determine an average value for spore  
 21 interception by our insect-proof net of  $F=0.57$ . This value is very close to the nets' mesh  
 22 solidity (i.e. 0.52) and suggests that, as a first approximation, spore interception is directly  
 23 proportional to the solidity of the net. However, this experimental value can also be compared  
 24 with an empirical expression of  $F$  calculated by Aylor and Ferrandino (1985) and pertaining  
 25 to cylinders and ribbons, the threads of the insect-proof nets being regarded as cylinders:

26

$$F = 0.86 / (1 + 0.442 S_t^{-1.967}) \tag{10}$$

27 With  $S_t = 2.085$ , the Stokes number of the *Botrytis* spores computed for a 10 µm spore  
 28 diameter (Jarvis, 1977) and an average thread Ø of 200 µm for the insect-proof net, one finds  
 29  $F = 0.78$ . Although this value is higher than the experimental one (0.57), the two values are  
 30 not contradictory because the calculated value (0.78) assumes that all intercepted spores

1 remain on the net threads, whereas the measured value takes into account only those  
2 intercepted spores that actually do remain and are not re-circulated. Finding an experimental  
3 value lower than the theoretical one is consistent with reality. We therefore used the  
4 experimental value of  $F$  (0.57) for the spore balance calculation.

#### 6 *Whole-greenhouse Botrytis spore balance*

7 Figures 6 (a and b) show examples of the daily (24-hours) time series of the main  
8 terms of the spore balance – exchange with the outside ( $FG(C_e-C_i)$ ), spore deposition ( $D_i\Delta t$ ),  
9 and spore production ( $P_i\Delta t$ ) – for the April 2004 and May 2005 trials.

10 For the April 2004 trial, which lasted 29 days, the spore production was always  
11 positive or nil, except during 3 days when a small negative value was observed. As already  
12 said, a negative spore production makes no sense either biologically or physically and is  
13 probably due to errors or imprecision in the balance setup. It is the case for example, when the  
14 difference ( $C_e-C_i$ ) is weak, as in April 2003 and when consequently the sign of this difference  
15 can change because of small imprecision's in the measurements of  $C_e$  or  $C_i$ .

16 As the rose crop gets older, as for the May 2005 trial (Figure 6 b), spore production  
17 increases substantially and becomes systematically positive (export of spores due to an  
18 internal production), as indicated by the negative value of the transfer term  $FG(C_e-C_i) \Delta t$   
19 during about half that period. For the same trials, Table 1 gives the average values of the  
20 different terms of the balance for measurement periods both before the rose crop was planted  
21 (April 2003) and after (April 2004 and May 2005) and Table 2 summarizes the statistical  
22 values for the different terms of the balance corresponding to measurements performed after  
23 the rose crop was planted (October 2003, April 2004, November 2004 and May 2005).

24 The spore concentration difference between inside and outside ( $C_e-C_i$ ) can vary widely  
25 from one period to another with, for example, an inside concentration three times lower than  
26 outside during the April 2004 trial ( $0.8 \text{ sp m}^{-3}$  vs  $2.7 \text{ sp m}^{-3}$ ), whereas inside and outside  
27 concentrations are approximately the same during the April 2003 and May 2005 trials.  
28 Statistical analysis of the components of the *Botrytis cinerea* spore balance on a daily basis  
29 for the four trials performed after the rose crop was planted (Table 2) together with the time  
30 trend in spore concentrations (Fig. 7) show that, on average, the inside spore's concentration  
31 remains fairly stable ( $0.8 \text{ sp m}^{-3}$ , with a standard deviation= $0.05 \text{ sp m}^{-3}$ ) throughout the trials,  
32 whereas the outside concentration varies widely (from  $0.8 \text{ sp m}^{-3}$  to  $3 \text{ sp m}^{-3}$ , with a standard  
33 deviation= $1 \text{ sp m}^{-3}$ ). On a daily basis, spore production ( $P_i\Delta t$ ) is equal to 11,176 sp before

1 rose crop planting in April 2003 (Table 1) and reaches an average of 83,939 sp for the trials  
2 performed after planting (Table 2). Overall, increasing inside spore production is observed as  
3 the crop ages (Table 1).

4 Table 1 also shows that total spore deposition  $D_i$  ( $\text{sp s}^{-1}$ ) (impaction plus sedimentation)  
5 can vary widely, from  $0.65 \text{ sp s}^{-1}$  in April 2003, before the rose crop was planted, to  $2.9 \text{ sp s}^{-1}$   
6 in May 2005. The caption of Table 2 indicates that impaction represents only 20% of the total  
7 spore deposit, the major deposition being on horizontal surfaces (80% of the total deposition).  
8 With these new figures in mind, one can discuss one of the major assumptions of this study  
9 which is recall in section 2: “all spores deposited on the surfaces of soil or leaves remain there  
10 and do not return to the air”. One can then distinguish between the deposition on soil, ultimate  
11 horizontal surface, for which this assumption is true and the vertical deposition on leaves,  
12 buds and stems, which concerns only 20% of the total spore’s deposition, and for which this  
13 assumption can still be discussed.

14 Total spore deposition depends not only on inside spore concentration  $C_i$  but also on the  
15 inside air speed and leaf area index values for the part corresponding to impaction on the crop  
16 canopy. Figure 8 presents the measured time trends for spore deposition (impaction plus  
17 sedimentation) and for the whole-greenhouse air exchange rate. For the various trials, one can  
18 observe the similarity between the courses of spore deposition and greenhouse ventilation  
19 intensity. As shown by Wang *et al.* (1999) greenhouse ventilation is also proportional to  
20 inside air speed, consequently, on average we also have a fairly close correlation between  
21 inside spore deposition and inside air speed. A similar trend was already observed on a daily  
22 time scale for only sedimentation (Fig. 5) and is also confirmed for a period lasting between 3  
23 and 4 weeks for sedimentation and impaction together.

24 On average, taking into account all four trials performed after the rose crop was planted  
25 (October 2003, April 2004, November 2004 and May 2005), two thirds of the spores  
26 deposited inside were also produced inside, one-third coming from outside. However, very  
27 wide variations can occur from one period to another, as between the April 2004 and May  
28 2005 trials (Fig. 9), with a general tendency for an increasing inside production as the crop  
29 grows old.

### 31 **4-3- Comparison of spore balance results with observations of sporulation**

32 The crucial task of independently verifying spore production is hard to perform  
33 because quantitative sampling is almost impossible in a rose crop. Because of its saprophytic  
34 capacity *Botrytis c.* produces spores on small crop debris (detached petals, leaves, stems),

1 which are difficult to detect, and petal spotting, which appears post-harvest on rose flowers.  
2 Consequently, there are few symptoms of infection that can be detected in the greenhouse.

3 Based on systematic observations performed weekly, the first *Botrytis c.* sporulation  
4 was observed in July 2004 on plant debris but we cannot be sure that there was no  
5 fructification before that time, as it is very difficult to detect spore production on crop debris  
6 in a production scale greenhouse. Similarly, the first symptoms of petal flecking on rose  
7 flowers in the greenhouse were observed some time later, during the November 2004 trial.  
8 These roses were sampled and the samples (33) were placed in humid chambers; 100% of  
9 them gave fructifications.

10 Figure 10 summarizes the results of spore production (P) calculated from equation (4) with  
11 the qualitative observations of spore production. It shows the increase in inside sporulation,  
12 whereas the term of spore exchange with the outside can vary widely from one period to  
13 another. When the level of inside sporulation is high the spore exchange term can even be  
14 negative, indicating spore exportation, as in May 2005. More generally, there is an observable  
15 coherence between the general tendency for increasing inside spore production as the crop  
16 ages, as revealed by the spore balance, and the observation of *Botrytis c.* sporulation and petal  
17 spotting which occur respectively three and seven months after the first detection of a  
18 substantial increase in inside spore production.

## 19 5 - Conclusion

20 Although some uncertainty still attaches to our results as applied on a daily basis, this first  
21 attempt to perform a *Botrytis cinerea* spore balance inside a rose greenhouse gives plausible  
22 results if we take the average values for the different terms of the balance for an entire  
23 measurement period, i.e. between 15 and 30 days, and compare these quantitative results with  
24 more qualitative results on *Botrytis c.* sporulation. On this time scale, the main value of the  
25 method is that it enables us to determine the origin and evolution of the inocula, and in  
26 particular to state whether they came from inside or outside the greenhouse. It also enables us  
27 to identify and analyse, even sometimes on a time scale shorter than several weeks, the factors  
28 influencing spore concentration inside the greenhouse, such as ventilation rate, outside  
29 concentration, spore production and spore deposition on the rose crop canopy, with other  
30 factors such as inside spore concentration, crop surface area and air speed. The study also  
31 stresses the possibility of analysing the evolution of *Botrytis cinerea* establishment in a  
32 perennial crop such as roses.  
33

1 However, this method is based on one major assumption, i.e. that “all the *Botrytis cinerea*  
2 spores that grow on the Kerssies medium and are counted can also potentially grow on the  
3 rose crop and contribute to the rose crop’s *Botrytis* balance”. Of course this assumption  
4 requires further validation by complementary studies assessing the biodiversity of *Botrytis*  
5 *cinerea* strains (Chave *et al.*, 2004). It will then be possible to exploit this approach for  
6 studying more systematically the determination by biotic and non-biotic factors of *Botrytis*  
7 *cinerea* spore production within the greenhouse, and to confirm our first assessment that the  
8 spores in the greenhouse come predominantly from the outside, at least when the rose crop is  
9 young. It would then be possible to analyse the consequences of these findings for fungal  
10 development and the crop’s agronomic performance.

11  
12 **Acknowledgements:** This study was partly supported by the 2004-2006 Franco-Korean  
13 specific agreement on the “Development of an aerodynamic model to study microclimate of  
14 tomato canopy and predict pathogenic spore transfer” conducted by INRA (France) and  
15 NIAE-RDA (Republic of Korea). Thanks are also addressed to the trainee’s engineer or  
16 teacher Ms Fanny Girardot and Christine Nieto who have actively participated to the project.

## 17 18 **Literature cited**

- 19  
20 Anton A., Castell F., Montero JI., Huijbregts M., 2004. Comparison of toxicological impacts  
21 of integrated and chemical pest management in Mediterranean greenhouses. *Chemosphere* 54,  
22 1225-1235.
- 23 Aylor, D.E., Ferrandino, F.J., 1985. Rebound of pollen and spores during deposition on  
24 cylinders by inertial impaction. *Atmos. Environ.*, 19 (5), 803-806.
- 25 Boulard T., Baille A., 1995. Modelling of air exchange rate in a greenhouse equipped with  
26 continuous roof vents. *J. agri. Engng Res.*, 61, 37-48.
- 27 Boulard T., Draoui B., 1995. Natural ventilation of a greenhouse with continuous roof vents:  
28 measurements and data analysis. *J. agri. Engng Res.*, 61, 27-36.
- 29 Chave M., Boulard T., Thomas C., Fatnassi, H.; Julien P., Poncet C., 2004. Integrated  
30 protection in a greenhouse rose crop: origins of contamination –an approach & preliminary  
31 results. *Acta Hort.* 659, 309-313.
- 32 Demrati H., Boulard T., Bekkaoui A., Bouirden L., 2001. Natural ventilation and climatic  
33 performance of a large-scale banana greenhouse. *J. agri. Engng Res.* 80 (3), 261-271.

- 1 Ducarme D., Vandaele L., Wouters P., 1994. Single sided ventilation: a comparison of the  
2 measured air change rates with tracer gas and with the heat balance approach. Documentation  
3 for BAG meeting on ventilation related aspects in Buildings. 26-35.
- 4 Fatnassi H., Boulard T., Poncet C., Chave M., 2006. Optimisation of Greenhouse Insect  
5 Screening with Computational Fluid Dynamics, Biosystem Engineering, 93 (33), 301-312.
- 6 Gregory P.H., 1973. The microbiology of the Atmosphere. Leonard Hill Books, London,  
7 377pp.
- 8 Jarvis W.R., 1977. Botrytinia and *Botrytis* Species: Taxonomy, physiology and  
9 pathogenicity. Monograph n° 15, Agriculture Canada.
- 10 Jewett T., Jarvis W., 2001. Management of the greenhouse climate in relation to disease  
11 control: a review. Agronomie 21, 351-366.
- 12 Körner O., Challa H., 2003. Process based humidity control regime for greenhouse crops.  
13 Computers & electronics in Agriculture 39, 173-192.
- 14 Kerssies A., 1990. A selective medium for *Botrytis cinerea* in a spore-trap. Neth. J. Pl. Path.  
15 96, 247-250.
- 16 Nicot P., Baille A., 1996. Integrated control of *Botrytis cinerea* on greenhouse tomatoes.  
17 Aerial surface microbiology, edited by Morris et al., Plenum press, New York, 169-189.
- 18 Roy J.C., Boulard T., Kittas C., Wang S., 2002. Convective and ventilation transfers in  
19 greenhouses, Part 1: The greenhouse considered as a perfectly stirred tank. Biosystem  
20 Engineering 83 (1), 1-20.
- 21 Sherman M. H., 1990. Tracer-gas techniques for measuring ventilation in a single zone.  
22 Building and Environment, 25(4), 365-374.
- 23 Seinfeld J., Pandis S., 1998. Atmospheric chemistry & physics: from air pollution to climate  
24 change. John Willey & sons, New York, 1326pp.
- 25 Tantau H.G., Lange D., 2003. Greenhouse climate control: an approach for integrated pest  
26 management. Computers & Electronics in Agriculture, 00, 1-12.
- 27 Wang S., Boulard T., Haxaire R., 1999. Air speed profiles in a greenhouse. Agric. For.  
28 Meteorol., 96, 181-188.
- 29  
30



1 Tables:  
2  
3

Variables	April 2003 trial Mean value	April 2004 trial Mean value	May 2005 trial Mean value
$G$ ( $\text{m}^3 \text{s}^{-1}$ )	4.1	1.7	7.9
$C_e$ ( $\text{sp m}^{-3}$ )	0.8	2.7	0.8
$C_i$ ( $\text{sp m}^{-3}$ )	0.6	0.8	0.9
$D_i$ ( $\text{sp s}^{-1}$ )	0.6	1.9	2.9
$FG(C_e - C_i)\Delta t$ (sp)	30,017	104,197	-14,615
$D_i\Delta t$ (sp)	43,418	160,231	249,713
$P_i\Delta t$ (sp)	11,176	54,891	266,329

4  
5 Table 1: Components of the *Botrytis cinerea* spore balance on an average daily basis for  
6 the April 2003 (10 days), April 2004 (30 days) and May 2005 (14 days) trials.  
7



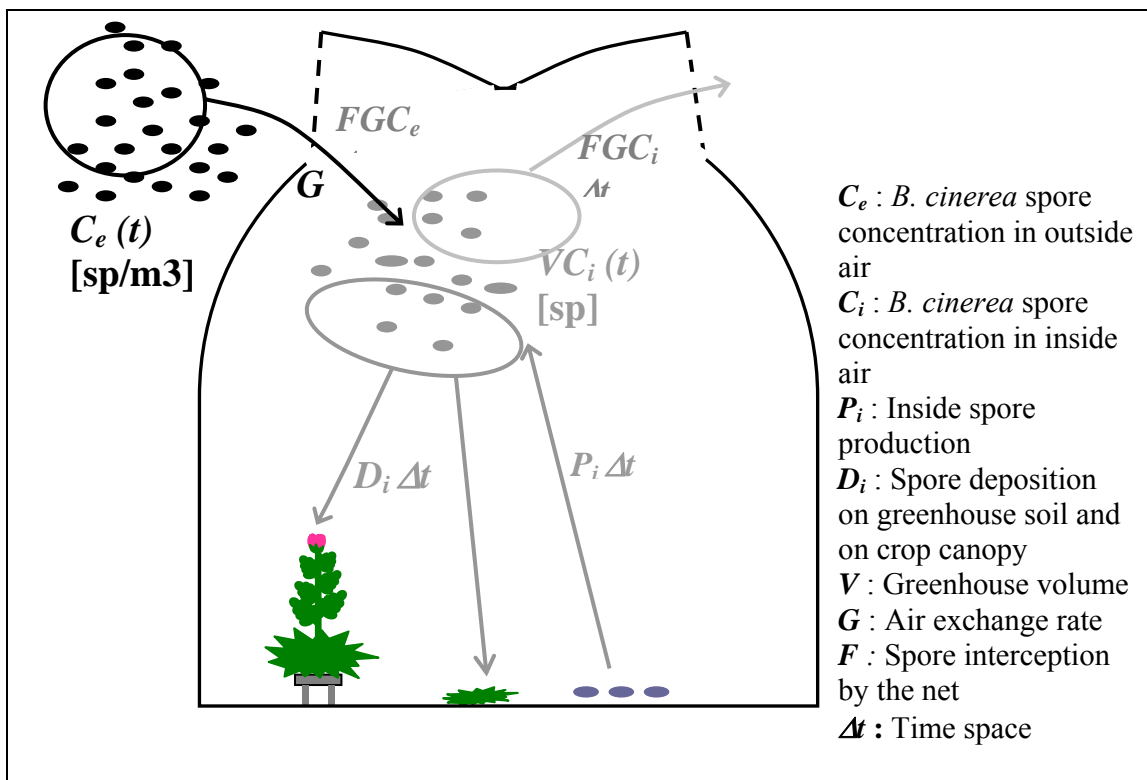
1  
2  
3

Variables	Average of the 4 trials			
	Mean value	Minimum	Maximum	Standard deviation
$G$ ( $m^3 s^{-1}$ )	2.4	0.4	7.9	3.1
$C_e$ ( $sp m^{-3}$ )	1.8	0.8	3.0	1.0
$C_i$ ( $sp m^{-3}$ )	0.85	0.8	0.9	0.05
$D_i$ ( $sp s^{-1}$ )	1.4	0.4	2.9	0.95
$FG(C_e-C_i)\Delta t$ (sp)	35,853	-14,615	22,359	45,000
$D_i\Delta t$ (sp)	122,083	37,857	249,713	80,876
$P_i\Delta t$ (sp)	85,939	14,308	266,329	96,776

4  
5  
6  
7  
8  
9

Table 2: Components of the *Botrytis cinerea* spore balance on an average daily basis for the four trials performed after the rose crop was planted (October 2003, April 2004, November 2004 and May 2005). On average the deposition on vertical surfaces due to impaction represents only 20% of the total horizontal ( $D_h$ ) plus vertical ( $D_v$ ) deposit.

1 **Figures**  
2

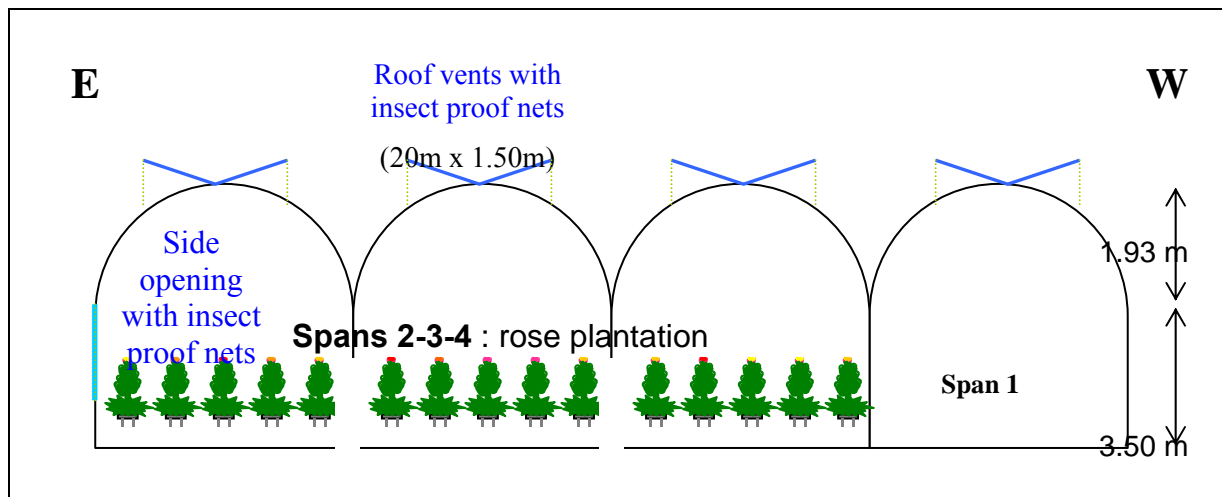


3 Figure 1: Method for identifying the origin of *Botrytis cinerea* inocula using a spore balance  
4

# Postprint

Version définitive du manuscrit publié dans / Final version of the manuscript published in : Agricultural and Forest Meteorology, 2008, vol.148, no.3, DOI : 10.1016/j.agrformet.2007.11.014:

1



2 Figure 2: Transverse cross section of the 576 m<sup>2</sup> experimental greenhouse.

3

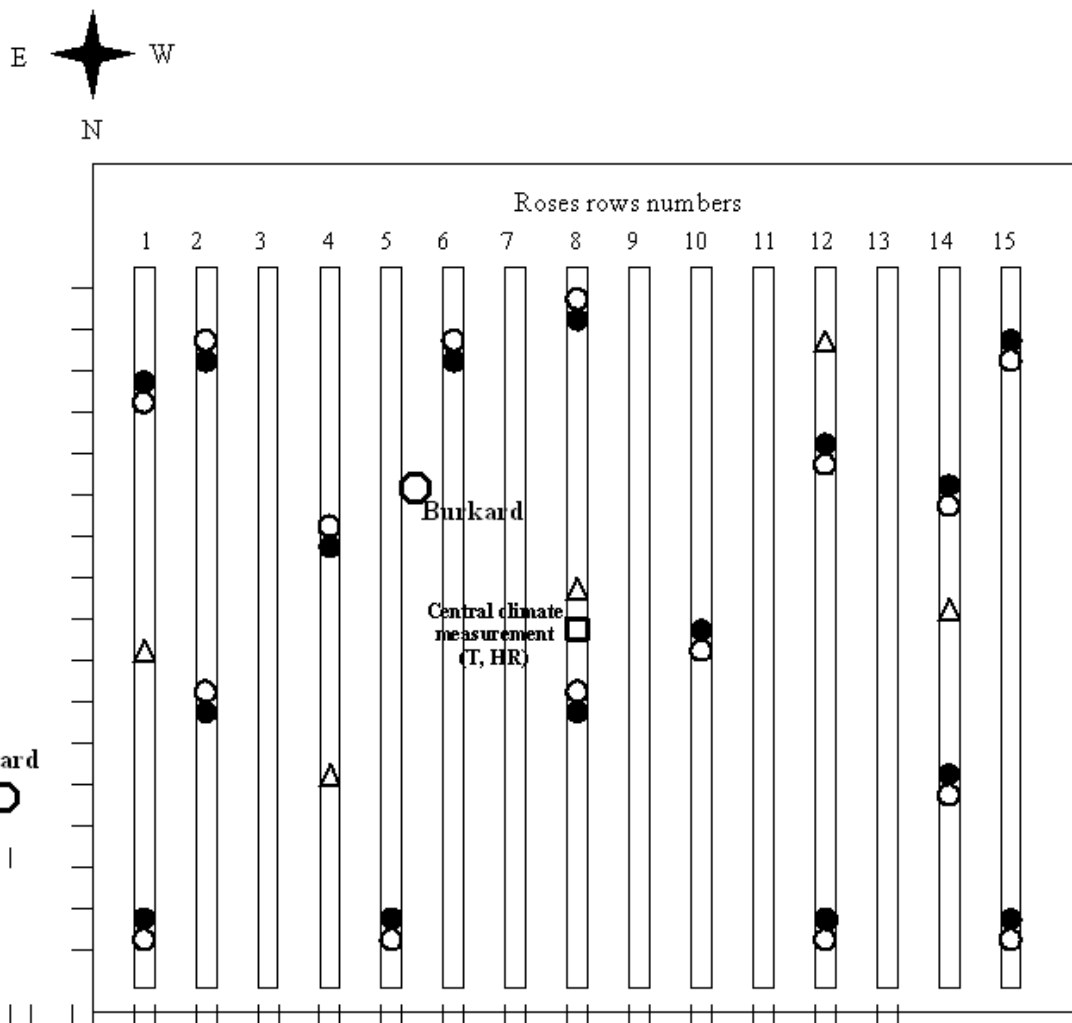
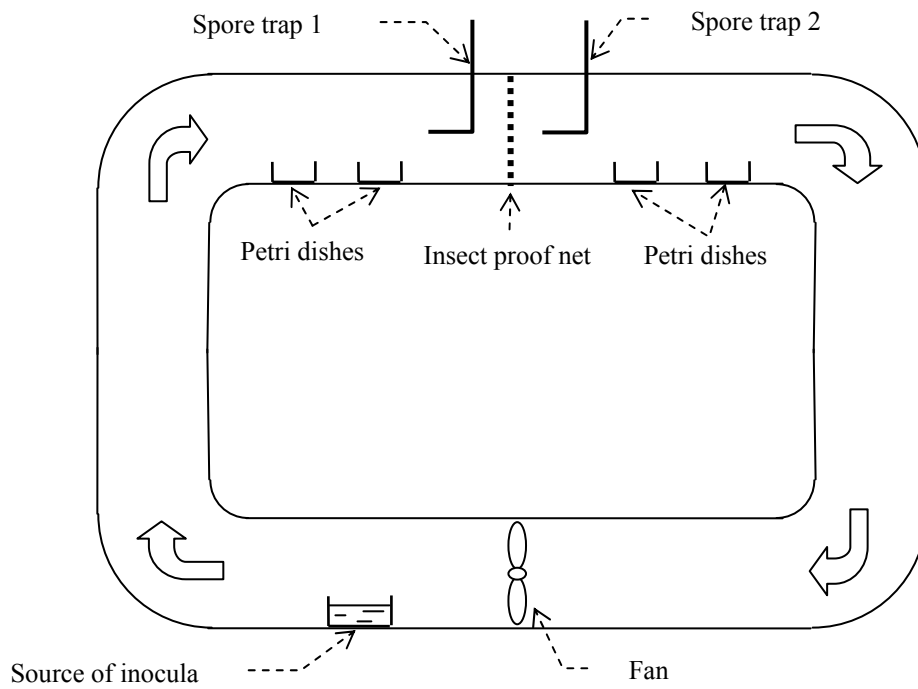


Figure 3: Plan of the greenhouse with positioning of the biological and climate measurements.

- Horizontal spore deposition traps using 90 mm ø Petri dishes with a Kerssies medium, placed at a height of 2.5 m.
  - Horizontal spore deposition traps using 90 mm ø Petri dishes with a Kerssies medium, placed just above soil level.
  - △ Vertical spore impactation traps using 90 mm ø Petri dishes with a Kerssies medium, placed at a height of 2.5 m.)
  - ⬡ Suction volumetric spore traps (Burkard)
  - Central greenhouse climate sensors (T, RH) using the greenhouse's climate computer devices (L'ien, Vitrolles, France)
- Wind speed and direction were measured outside the greenhouse at 5.5 m high about 15 meters apart West of the greenhouse.

1

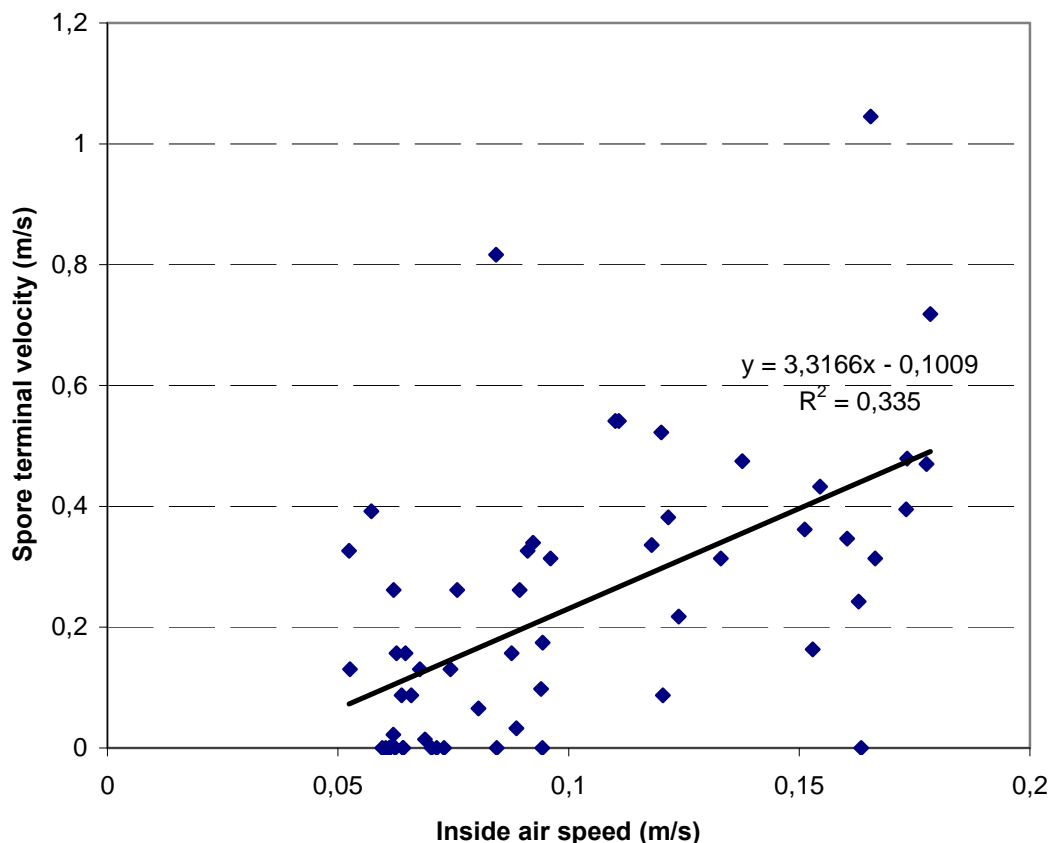


2

3

4 Figure 4: Sketch of the closed-loop wind tunnel experiment designed to determine spore  
5 interception by the insect-proof net.

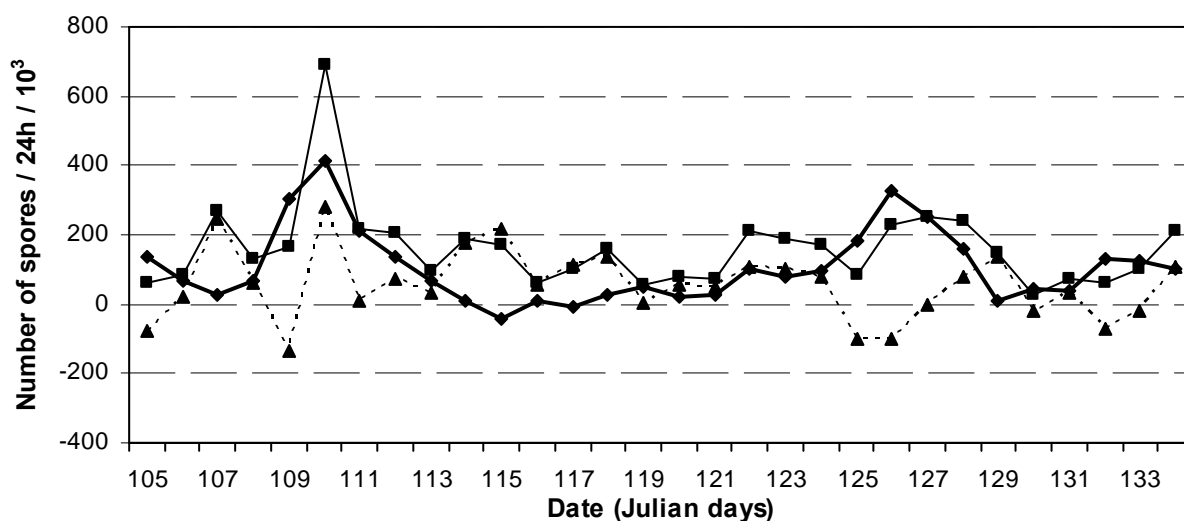
5



1  
2 Figure 5: Experimental linear relation between average inside air speed recorded over a 12  
3 hour period and calculated inside terminal velocity of spores computed for the same time span  
4 (April 2004 data).  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14

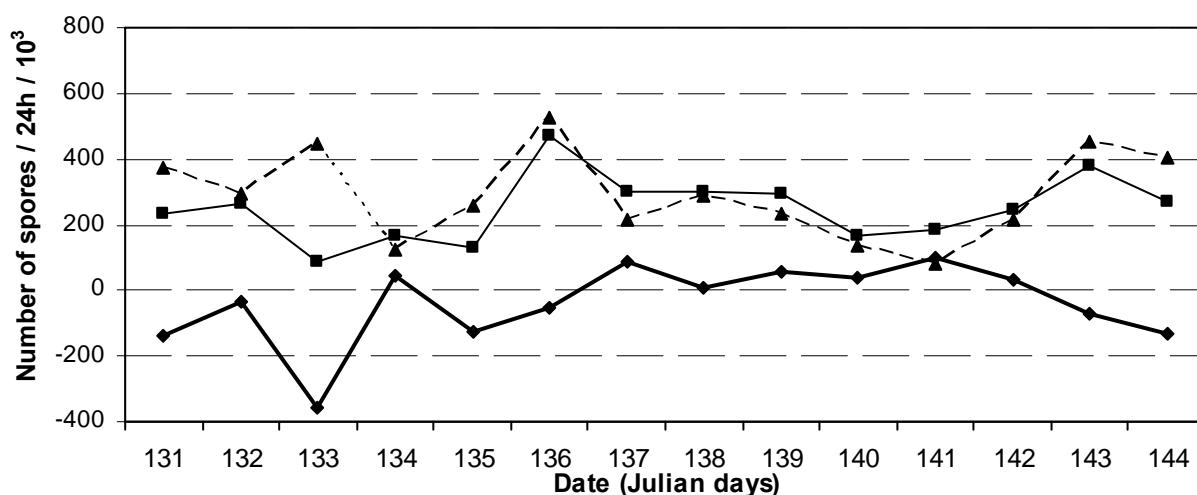
1

a)



2  
3

b)



4  
5  
6  
7  
8  
9  
10  
11

Figure 6: Time trends of the different components of the greenhouse rose *Botrytis cinerea* spore balance corrected for spore interception by the insect proof net [ $FG(Ce-Ci) \Delta t$ :  $\text{—}\blacklozenge\text{—}$ ] represents the exchange between inside and outside, [ $VsdCi + Di \Delta t$ :  $\text{---}\blacksquare\text{---}$ ] represents the inside variation due to deposit and air content variation, [ $Pi \Delta t$ :  $\text{---}\blacktriangle\text{---}$ ] represents the inside spores production; (a) data from the April 2004 trial; (b) data from the May 2005 trial.



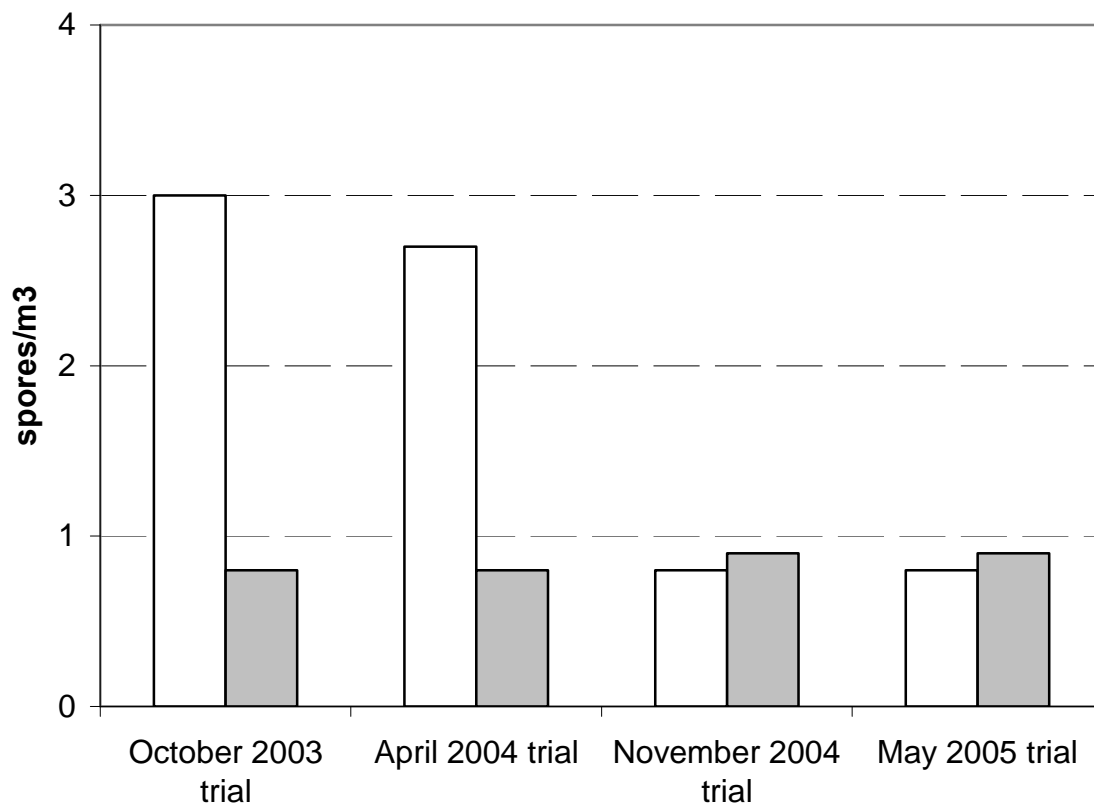
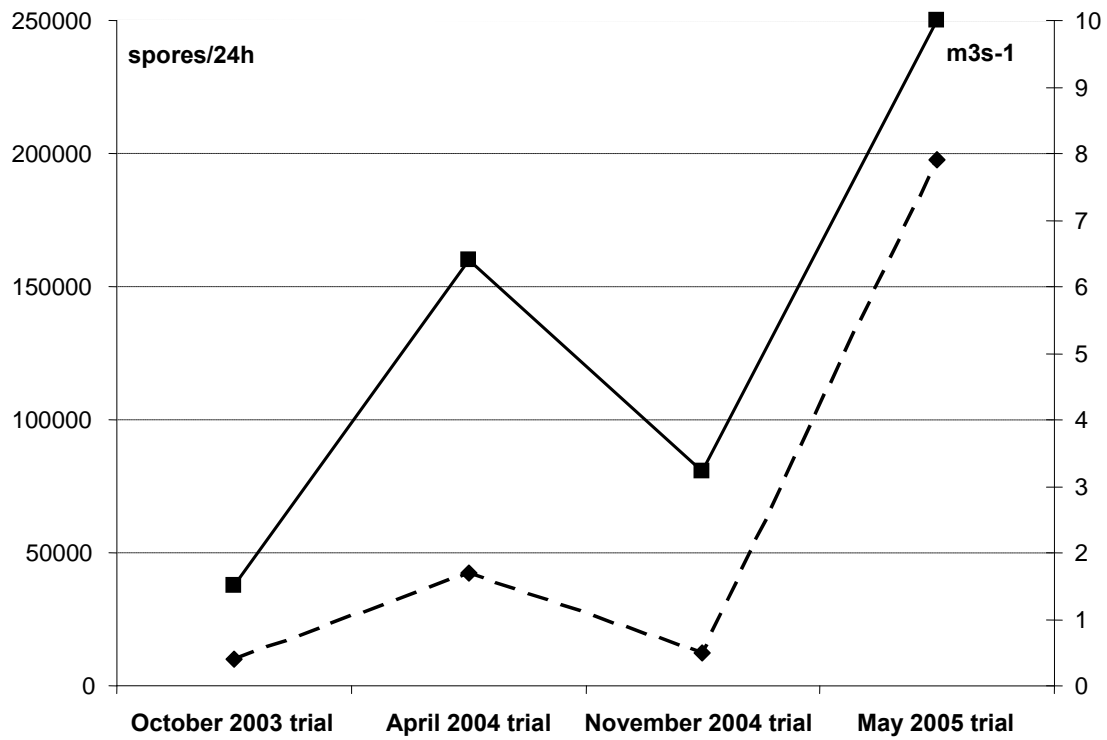


Figure 7: Average values for measured inside ■ and outside □ spore concentrations for the 4 different trials (October 2003, April and November 2004 and May 2005).

1  
2  
3  
4  
5  
6

1  
2



3  
4  
5  
6  
7

Figure 8: Evolution of average spore deposition,  $VsdCi+Di \Delta t$  (—■—, left axis), and greenhouse ventilation rate,  $G$  (-◆-, right axis) (October 2003, April and November 2004 and May 2005).

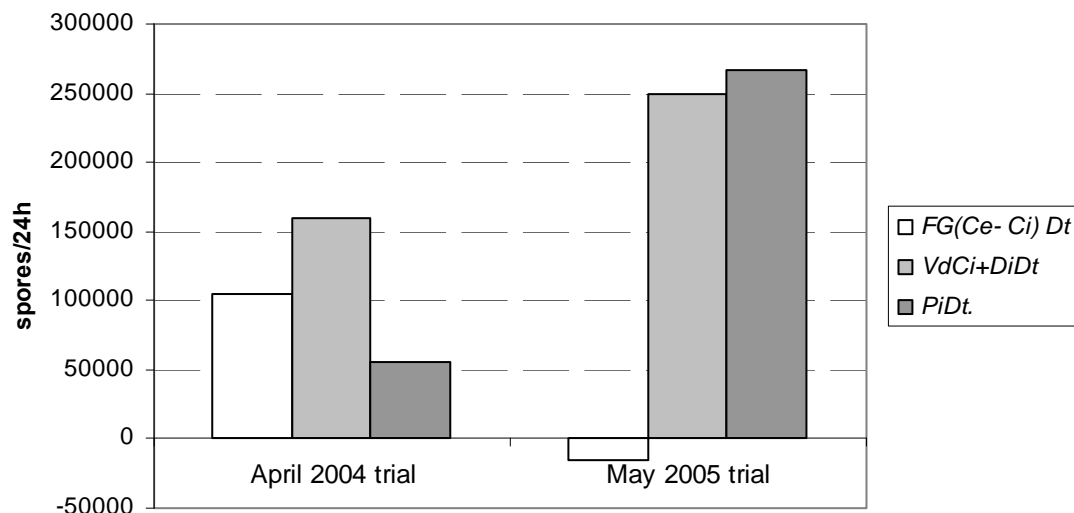


Figure 9: Average values of the different terms of the *Botrytis cinerea* spore balance for April 2004 and May 2005 trials.

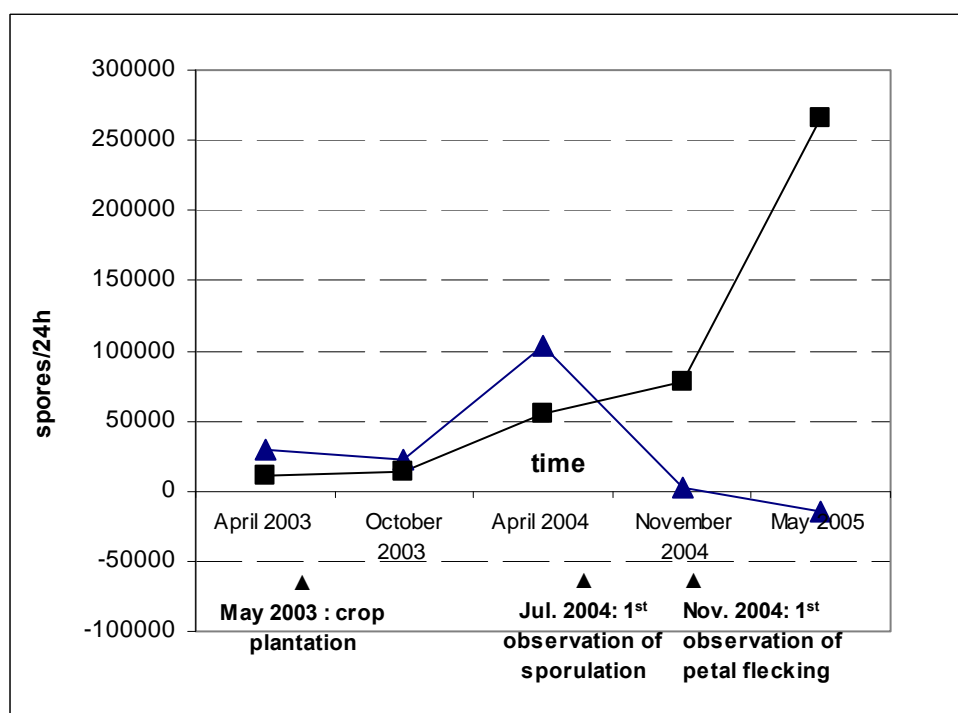


Figure 10: Time course of spore production [ $Pi \Delta t$  :  $-\blacksquare-$ ] and exchange between inside and outside [ $FG(Ce-Ci) \Delta t$  :  $-\blacktriangle-$ ] terms from April 2003 to May 2005 together with a recapitulative time schedule of the visual observations of *Botrytis c.* infestation in the greenhouse (in bold characters in the figure).